# Stable isotope analyses ($\delta^{15}N$, $\delta^{13}C$) as a tool to define exposure of white sucker (*Catostomus commersonii*) to pulp mill effluent in Jackfish Bay, Lake Superior

by

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## Author's Declaration

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

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# Abstract

Effects of effluent from the pulp mill in Terrace Bay, Ontario, on fish have been extensively studied since the late 1980s. Fish collected at this site over the past several decades have shown a variety of physiological and population level responses relative to reference sites; however the patterns of stable isotopes in biota at this site have not yet been studied. The purpose of this study was to determine the spatial and temporal variability of stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in organisms exposed to pulp mill effluent from Terrace Bay, Ontario into Jackfish Bay, Lake Superior. The sentinel species, white sucker (*Catostomus commersonii*), was collected at sites with varying exposure to pulp mill effluent during late August of 2011, 2012, and 2013. In addition, white sucker were sampled at two spawning locations in May of 2011, 2012, and 2013. Incidentally, the mill closed operations after sampling in August 2011 and resumed operations after sampling in August 2012, resulting in a temporal change in effluent exposure. Population characteristics, mixed function oxygenase (MFO) induction, and stable isotopes (in liver and muscle tissues) were analyzed for white sucker during spawning in May and gonadal recrudescence in August each year. Stable isotopes were analyzed for macroinvertebrates at similar sites during the fall to track the baseline signatures to determine if there were whole ecosystem changes or only changes within trophic levels. While the mill was operating in 2013, isotopic signatures of white sucker residing in nearby Tunnel Bay (part of Jackfish Bay, Lake Superior) did not differ from Moberly Bay (also part of Jackfish Bay), the bay directly receiving the pulp mill effluent, or Mountain Bay, the reference site. However, isotopic signatures in white sucker collected in these nearshore bays of Lake Superior were significantly different from effluent-free Jackfish Lake, a small shallow inland lake through which fish must migrate to spawn in the spring (Sawmill Creek). Based on isotopic signatures it is possible that up to 23% of fish caught at Sawmill Creek were from Jackfish Lake and therefore not exposed to effluent in Jackfish Bay, Lake Superior. This may have implications for interpretation of

future monitoring of fish responses in the spawning population. Unfortunately, fish movements and other factors may limit the use of stable isotopes for separating exposure of fish within Jackfish Bay.

Isotopic signatures of liver and muscle tissue of white sucker did not respond consistently to changes in mill operation across the three years of the study. In contrast, isotopic signatures of invertebrates at the exposure sites were depleted in $\delta^{15}N$ and enriched in $\delta^{13}C$ during mill closure but after the mill reopened became enriched in $\delta^{15}N$ and depleted in $\delta^{13}C$. Stable isotopes in macroinvertebrates reflect changes in mill operation better than white sucker as the movement and migration of fish may confound exposure.

MFO activity (ethoxy-o-resorufin deethylase (EROD)), another measure of effluent exposure, was induced in fish liver at Moberly Bay and Tunnel Bay compared to the two sites that did not receive pulp mill effluent, Mountain Bay and Jackfish Lake. EROD activity was higher during 2011 when the mill was operating compared to 2012 when the mill was closed, but when the mill operation resumed in 2013 there was a much more extreme induction in EROD than both of the previous years. These results suggest that fish are exposed to chemicals in the effluent that can induce MFO activity and the fish respond quickly to effluent exposure. Spring levels of EROD activity were minimal during all three years, likely a combination of reduced effluent exposure during migration and reproductive physiology of white sucker. MFO activity remains an effective biomarker of exposure to pulp mill effluent but is only reliable in fall sampling of fish. There are indications that white sucker are exposed and respond to pulp mill effluent in this system, but differences in population endpoints (such as gonad somatic indices) are no longer evident between fish from pulp mill exposed sites and reference sites (May or August). A short term removal of continuous exposure to effluent over a year also did not affect population endpoints. Stable isotopes were able to highlight the complexity of effluent exposure in the system but were not a reliable tracer for effluent exposure in white sucker.

In conclusion, stable isotopes in macroinvertebrates reflect changes in mill operation better than white sucker, and stable isotopes are more strongly governed by differences in habitat than pulp mill effluents. The use of stable isotopes in macroinvertebrates as sentinel species is ideal since the movement and migration of fish can confound exposure. There is no longer a difference in population effects between pulp mill exposed- and reference fish, which indicates upgrades in mill operations and government regulations have improved effluent quality. MFO activity remains an effective biomarker of pulp mill effluent exposure but is only reliable in fall sampling of fish. Stable isotopes may give an indication of nutrient dynamics amongst trophic levels, but pulp-derived tracers with no confounding environmental factors are desirable to confirm and quantify pulp mill exposure.

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## 1.1 Introduction

Pulp mills are widely recognized as having detrimental impacts on the receiving environment of their effluent (Munkittrick *et al.*, 2013). Over time, federal regulations have improved the quality of pulp effluents, yet some negative impacts still occur due to the compounds that remain in the effluent (McMaster *et al.*, 2006; Munkittrick *et al.*, 1998; Parrott *et al.*, 2006). Identifying the compounds responsible for these effects has proven difficult, and the identity of the responsible chemicals remains elusive (Hewitt *et al.*, 2008; Hewitt *et al.*, 2006). Biomonitoring programs have effectively used fish as sentinel organisms to identify environmental changes associated with effluent discharges and effluent quality (Munkittrick *et al.*, 2002). Unfortunately in many situations the mobility and complex life histories of the selected species can complicate interpretation of the results (Barrett & Munkittrick, 2010; Galloway & Munkittrick, 2006; Munkittrick & Dixon, 1989). Exposure can be difficult to assess when fish are able to move in and out of effluent, as returning to clean water even briefly may allow the response of concern to recover from certain chemical exposures. Determining the relative exposure of fish that migrate during annual spawning events can be even more difficult due to potential mixing of exposed and unexposed populations (Doherty *et al.*, 2010; Walker *et al.*, 2002). Understanding movement and migration is therefore essential when selecting a sentinel species and interpreting results. Despite considerable past biomonitoring and research, the spatial and temporal exposure of fish to pulp mill effluents, especially in migratory spawning fish, remains poorly defined and understood. Understanding effluent exposure will help explain effects seen in the receiving environment at pulp mills in Canada and across the world.

## 1.2 Environmental Impacts of Pulp Mills

The Canadian pulp and paper industry is a global leader in paper products, exporting over $13 billion CDN in pulp, paper, and paperboard products in 2013 (Industry Canada, 2015). In order to manufacture paper products, wood chips or other plant fibers must be converted into pulp (consisting of cellulose fibers, lignin, hemicelluloses and water) to create thick fiberboard that is later processed in a paper mill (Bajpai, 2012). The processes used to produce pulp are water intensive and result in large quantities of effluent. Understanding how effluents interact with the ecosystem in the receiving water is important for environmental stewards working to protect the ecological integrity of the receiving environment.

In 2012, there were 74 pulp mills across Canada, primarily located in British Columbia, Ontario, and Quebec (Industry Canada, 2015). These pulp mills operated on either chemical (54 mills in Canada) or mechanical (20 mills in Canada) pulping processes (Industry Canada, 2015). Chemical pulp mills use two processes: either the kraft (sulfate) or the sulphite process, both of which use high temperatures to break down lignin. The kraft process involves treating wood chips with white liquor (sodium hydroxide and sodium sulfide) to break down and remove hemicelluloses and lignin, resulting in strong cellulose fibers. Alternatively, the sulphite process results in weaker cellulose fibers, but creates pulp that is easier to bleach. Pulp made in chemical mills is used for fine paper products, whereas mechanical pulp mills specialize in newsprint, using stone grinding rollers or pressurized sealed grinders, both of which use large amounts of electricity. Alternative methods include thermomechanical and chemi-thermomechanical pulp mills, which respectively apply steam to refine wood chips or pretreat wood chips with weak chemical solutions to refine fibers. Unfortunately, all pulping processes produce considerable pollutant loadings to land, air, and water (Bajpai, 2012).

In 2013, Canadian pulp, paper, and paperboard mills released 302 tonnes of pollution onto land, 166,613 tonnes of air emissions, and 5,955 tonnes of wastewater (Industry Canada, 2015). Wastewater

discharges from pulp mills are typically dark coloured and include solids and dissolved organic matter, which usually increase biological oxygen demand in receiving waters. Ammonia, nitrate, phosphorus, and sulfur present in effluents released by pulp mills cause nutrient enrichment. Metals such as arsenic, cadmium, hexavalent chromium, lead, manganese, selenium, and zinc can also be found in wastewater discharges from pulp mills. In addition, effluents include alcohols, chelating agents, chlorates, and organochlorine compounds (Industry Canada, 2015).

Mechanical pulp mills usually bleach with hydrogen peroxide and sodium dithionite, both of which are usually partly retained in the pulp product. Mechanical pulp mill byproducts include water and sodium sulfate, which are generally considered harmless (Smook, 1992). To limit pollution, chemical pulp mills try to recover as much sulfur as possible (except in the ammonia-based sulfite processes), but combustion of black and red liquors releases sulphur dioxide, a water-soluble compound that contributes to acid rain (Bajpai, 2012). The use of molecular chlorine for bleaching in kraft mills has historically resulted in elevated levels of organochlorine compounds in effluent and fish such as polychlorinated dibenzo-*p*-dioxins (PCDD) and dibenzofurans (PCDF) (Ali & Sreekrishnan, 2001; Owens *et al.*, 1994; Servos *et al.*, 1994). The elimination of elemental chlorine in the pulp bleaching process through new regulations in the late 1980s led to a major reduction of organochlorines and PCDD/PCDFs in effluents and in organisms in the receiving environment (Dahmer *et al.*, 2015; Servos, 1996; Solomon *et al.*, 1996; van den Heuvel *et al.*, 1996).

Pulp mills are usually built next to large water bodies such as lakes or rivers that can meet their requirement of large quantities of water for the pulp process. Wastewater effluents are discharged into these local water bodies, and contain many compounds that can have deleterious effects on the organisms that reside there. Shifts in macroinvertebrate community structure have been observed in response to pulp mill effluents where pollutant tolerant organisms dominate in eutrophic conditions (Sibley *et al.*, 1997). In effluent receiving environments, the abundance and taxon richness of macroinvertebrates can increase

with moderate levels of nutrient enrichment but then decrease with further exposure to effluent (Environment Canada, 2003). Fish populations exposed to pulp mills have demonstrated a variety of effects, including metabolic and endocrine disruption related to growth and development, and changes in gonad weight, liver weight, and condition factor (Environment Canada, 2003; McMaster *et al.*, 2006). Federal regulations have led to improvements in effluent quality and recovery of many fish population responses previously reported in these receiving bodies (McMaster *et al.*, 2006).

The Canadian pulp and paper industry is regulated through the Pulp and Paper Effluent Regulations (PPER) of the Fisheries Act (Government of Canada, 2013). Deleterious substances restricted by the PPER include acutely lethal effluent, biological oxygen demand matter, and suspended solids. However, the maximum authorized biological oxygen demand and quantity of suspended solids are based on time and the daily production of finished products of the mill, not the carrying capacity of the environment to which the effluent is released, nor the cumulative effects of multiple effluents (Government of Canada, 2013). The effectiveness of the PPER is monitored through the Environmental Effects Monitoring (EEM) program. The EEM Program is an industrial funded program that studies the potential effects of effluent on fish populations, fish tissues, and benthic invertebrate communities to ensure current regulations are sufficient (Government of Canada, 2013).

## 1.3 Use of Sentinel Species

In many studies across Canada, fish have been used as a sentinel species to detect changes associated with effluent exposure. Many species have been used within the EEM program, and Munkittrick *et al.* (2002) have discussed the selection of appropriate sentinel species for biomonitoring purposes under the EEM program based on a review of the program. The EEM program has used over 60 fish species over the first four cycles based on their residency, abundance of mature individuals, species for which permits can be obtained, species that are most exposed to effluent, and suitability of

measurements for indication of energy storage (condition and liver size), energy usage (growth and gonad size), and survival (age) (Barrett & Munkittrick, 2010; Environment Canada, 2003). Munkittrick *et al.* (2002) also recommended the use of small-bodied species as an alternative to a large bodied species due to generally higher mobility of large bodied species.

The white sucker (*Catostomus commersonii*) is a common native fish found in freshwater lakes and rivers in Canada, and has been extensively used as a sentinel species in the EEM program and other studies (Bowron *et al.*, 2009). Advantages as a sentinel species include widespread abundance, benthic nature, and tolerance to a range of environmental conditions. White sucker also have long life spans of up to 20 years (Fisheries and Oceans Canada, 2010; Mahon, 1984), making them a potentially good indicator of long-term effects. These fish grow rapidly while young, then much more slowly after sexual maturation which usually occurs between the ages of four to six years (Becker, 1983; Mahon, 1984). They can grow up to approximately 50 cm in length and two kg in weight (Fisheries and Oceans Canada, 2010). Their large biomass allows different tissues to be effectively sampled for biological responses as well as for contaminant burdens. White sucker are generally considered to be benthivores that forage for macroinvertebrates in or above the bottom of lakes and rivers. Their diet includes midge larvae, crustaceans, and gastropods (Fisheries and Oceans Canada, 2010), as well as detritus (Ahlgren, 1996). A gut content study at Duluth, Lake Superior found that near shore white sucker diet was dominated by clams, chironomids, ephippia, and Diporeia in the summer (Gamble, 2010). However, Saint-Jacques *et al.* (2000) found that some white sucker prefer to ingest zooplankton, including Holopedidae, Daphnidae, and Chaoborinae. Seasonal variation in white sucker diet has also been reported, where a switch from benthic invertebrates to zooplankton occurs in the fall, when many benthic invertebrates emerge as flying insects (Saint-Jacques *et al.*, 2000). White sucker are a good indicator of adverse environmental conditions since they often ingest macroinvertebrates in sediment, where hydrophobic toxic contaminants tend to partition.

White sucker spawn synchronously each year in rivers that have sandy to gravel substrates (Green *et al.*, 1966). They usually migrate up small streams in early spring when water temperatures reach 10ºC (Roberge *et al.*, 2002). Spawning runs can number as high as thousands of individuals, and gravid females can produce as many as 20,000 to 50,000 eggs each (Fisheries and Oceans Canada, 2010). At some sites, white sucker have been estimated to move less than 3 km during summer to late winter, suggesting low mobility, but white sucker have been reported to migrate over 40 km to reach suitable spawning habitat (Doherty *et al.*, 2010). However, this study was reported for a river system, while the study area in the current study occurs in bays and lakes along the northern coast of Lake Superior, and therefore fish may have different migration tendencies.

## 1.4 Jackfish Bay, Lake Superior

The pulp mill located in Terrace Bay, Ontario, has been discharging bleached kraft mill effluent into nearby Jackfish Bay since its opening in 1948 (Bowron *et al.*, 2009; Dahmer *et al.*, 2015). Currently the pulp mill has a softwood line and bleaches with chlorine dioxide. In 2006 the mill produced as much as 1005 air-dried metric tons of pulp per day and discharged approximately 88,541 $m^3$ of secondary-treated effluents per day (Bowron *et al.*, 2009). The mill uses a secondary treatment lagoon with an eight to ten day retention time before effluent is piped directly to Blackbird Creek, which is 65-95% effluent by volume, and little dilution occurs before the water flows 14 km into Jackfish Bay (Munkittrick *et al.*, 1992b; Stewart & Rashid, 2011) (See Figure 1.1). Effluent takes about two days to arrive at Moberly Bay via Blackbird Creek (Munkittrick *et al.*, 1992b).

The Great Lakes Water Quality Agreement between the United States and Canada identified Jackfish Bay as one of 43 "Area of Concern" sites in 1987 (Jackfish Bay RAP Team, 1991). An Area of Concern is a site in the Great Lakes that has experienced high levels of environmental harm. Changes to chemical, physical, or biological integrity of a water body called "Beneficial Use Impairments" were

identified at Jackfish Bay and included degradation of benthos, fish, and wildlife populations, loss of fish and wildlife habitat, degradation of aesthetics, restrictions on fish and wildlife consumption, bird deformities or reproduction problems, and fish tumours or other deformities (Jackfish Bay RAP Team, 1991). After major improvements in treatment of effluent and chlorine dioxide substitution for bleaching during the 1990s, it was decided that the best remedial action plan would be to allow natural recovery with monitoring for incremental improvements. In May 2011, Jackfish Bay was listed as an "Area in Recovery" instead of an "Area of Concern" (Environment Canada, 2014). Bird deformities or reproduction problems, and fish tumours or other deformities, are no longer impaired although degradation of benthos, degradation of fish and wildlife populations, and loss of fish and wildlife habitat are still impaired and degradation of aesthetics and restrictions on fish and wildlife consumption required further assessment (Environment Canada & Ontario Ministry of the Environment, 2010).

In addition to being listed as an "Area of Concern" through the Great Lakes Water Quality Agreement, Jackfish Bay has been monitored through the EEM program. In 1992 the PPER limited the amount of total suspended solids, biological oxygen demand, chlorinated dioxins and furans, and acute toxicity in effluent discharged into the environment. To ensure regulations protect the environment, EEM evaluates the effects of effluent on fish, fish habitat, and use of fisheries resources. Three year cyclical monitoring and reporting allow spatial and temporal analysis to occur. At Jackfish Bay, four cycles of EEM were conducted, where fish and benthic invertebrates were collected in the fall as indicators of overall ecosystem health (BEAK, 1995, 2000; Ecometrix, 2007; Stantec, 2004).

The effects on fish in Jackfish Bay caused by the pulp mill have been reported in a number of studies conducted by academia, government, and industry since the 1980s, making it one of the most highly studied pulp mill sites in Canada (Bowron *et al.*, 2009). While upgrades in mill operation such as secondary treatment and elemental chlorine free bleaching have reduced effects seen in fish, negative impacts are still observed compared to reference fish (Bowron *et al.*, 2009). Mountain Bay and its

tributary Little Gravel River, Lake Superior (to the west), have been the reference sites used to compare Jackfish Bay and Sawmill Creek in most studies for the pulp mill at Terrace Bay. No industrial or municipal effluents are present at Mountain Bay and few residents live there, making it an almost pristine reference site to contrast against the pulp effluent exposure site. Historically, fish exposed to pulp mill effluent in Jackfish Bay have exhibited induction of hepatic mixed-function oxygenase (MFO), increased liver sizes, and decreased reproductive abilities, as seen in delayed sexual maturity, reduced circulating levels of gonadal sex steroids, smaller gonad sizes, and reduced secondary sexual characteristics (Bowron *et al.*, 2009). Biomonitoring during intermittent disruptions in mill operation in the past has observed fast recovery of mixed-function oxygenase (MFO) induction and circulating levels of gonadal sex steroids in Jackfish Bay over a matter of days (Munkittrick *et al.*, 1992b). These results suggest that fish can respond very quickly to the removal of specific chemical exposure, and/or can alter their behaviour during mill shut downs (e.g., habitat selection) changing their exposure (Bowron *et al.*, 2009; Munkittrick *et al.*, 1992b).

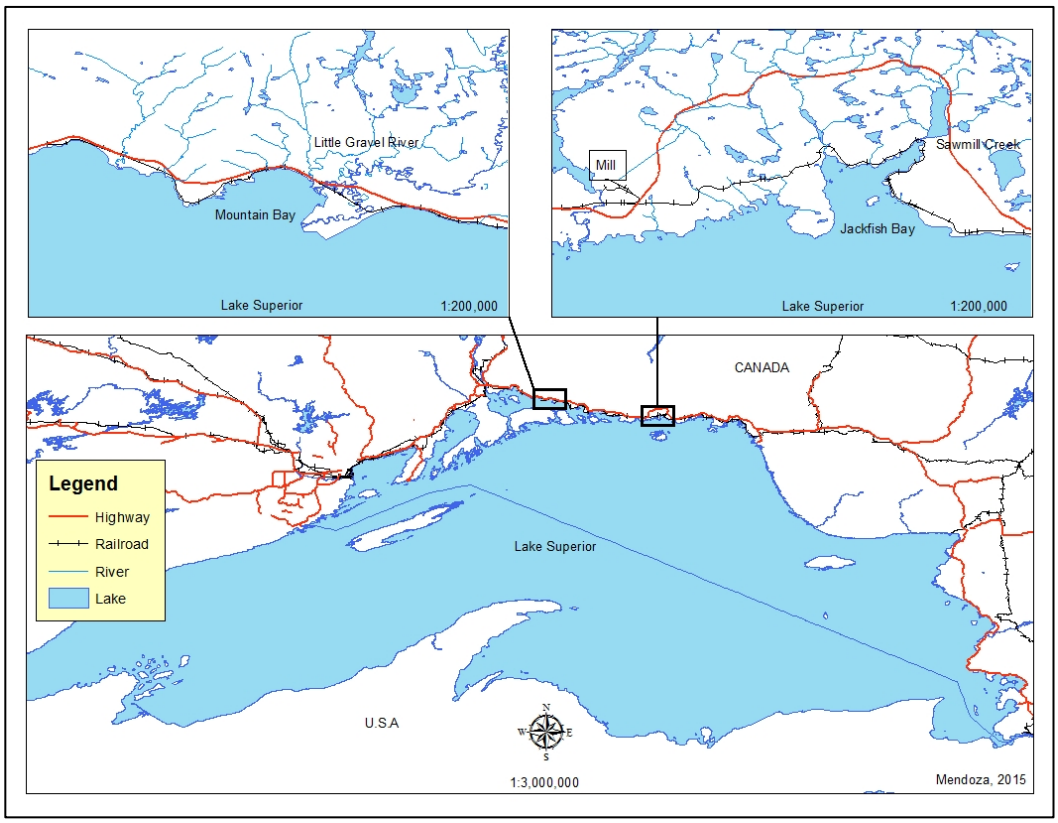


Figure 1.1: Map of Lake Superior, with Mountain Bay (Reference Site) and Jackfish Bay (Exposure Site) (Source: (DMTI CanMap Route Logistics, 2014)).

## 1.5 Determining Exposure in Fish

Previous attempts have been made to identify a suitable tracer for verifying fish exposure to pulp mill effluent (Ali *et al.*, 1997). While effluent plume studies are successful in delineating plumes and circulation in water, fish are mobile and may not constantly reside in the plume. Various contaminants and biomarkers in fish may be better indicators of exposure, especially when fish are known to be mobile and migrate during seasonal spawning. Effective effluent tracers in fish should be distinguishable between effluent and background concentrations, taken up by fish, and retained for a predictable time suitable to the research question being asked. Past tracers investigated for pulp mill effluent include polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), resin and fatty acids,

chlorinated phenols, and MFO induction (Ali *et al.*, 1997; Healey *et al.*, 1994). However, there are limitations to each of these compounds and approaches.

Although a wide variety of chemicals are present in pulp mill effluents (Suntio *et al.*, 1988) there are few, if any, that are effective tracers of fish movements and exposure. PCDDs and PCDFs are no longer produced and are a legacy of historical contamination by mills that bleached with elemental chlorine (Dahmer *et al.*, 2015). Levels of PCDD/Fs in fish associated with pulp mill effluents are now near background levels due to upgrades in mills. Resin and fatty acids, as well as chlorophenols have been detected in effluents (Robinson *et al.*, 1994) and the bile of fish exposed to pulp mill effluents (Leppanen & Oikari, 1999). However, resin acids are present only in softwood processing mills and are not representative of hardwood mill compounds. In addition, resin acids rapidly degrade during treatment (Liss *et al.*, 1997). Likewise, fatty acids are likely to be metabolized quickly and are ubiquitous in biological substrates. Chlorinated phenols and related compounds are quickly depurated from fish and likely not detectable in tissues once fish migrate out of the effluent plume (Kovacs *et al.*, 1993; Muir & Servos, 1996). Treatment upgrades and process changes, including the move away from elemental chlorine bleaching has also greatly reduced the presence of these chemicals in the receiving environment. MFO activity is related to effluent exposure but varies seasonally and can drop very quickly (i.e., days) after removal from effluent (Munkittrick *et al.*, 1991). Therefore, there are few reliable indicators of exposure in fish, especially during spawning, that can be widely applied (Ali *et al.*, 1997; Healey *et al.*, 1994). However, recent studies have suggested that stable isotope analyses may be a useful tool to define effluent exposure (Wassenaar & Culp, 1996; Wayland & Hobson, 2001).

## 1.6 Stable Isotopes

Stable isotope analyses (SIA) have been increasingly used in ecological studies. SIA have been used to determine how processes affect element cycling within the ecosystem, including metabolism,

reproduction, resource partitioning, and trophic interactions (Fry, 2006). SIA compare the amount of a heavy isotope (element with one or more additional neutron than protons) to a light isotope (element with same amount of neutrons as protons) in a sample relative to international standards. Stable isotope values are expressed in standard delta notation $\delta$ (‰), calculated as:

$$\delta X\ (‰) = \left[\left(R_{sample}/R_{standard}\right) - 1\right]\times 1000 \quad \text{Equation 1}$$

where X is the heavier isotope (e.g., $^{13}C$), $R_{sample}$ is the ratio of the heavy to light isotope in the sample, and $R_{standard}$ is the ratio of the heavy to light isotope in an internationally accepted standard. Increases in $\delta$ values indicate increases in the amount of heavy stable isotope relative to the light stable isotope, and is termed "enrichment," while decreases in $\delta$ values indicate relative decreases in the amount of heavy stable isotope to amount of light isotope and is termed "depletion."

The additional neutron in heavier stable isotopes causes the reactions in biochemical processes to occur at slightly different rates (Urey, 1947), while maintaining their chemical and physical properties. Stable isotopes are ubiquitous in the environment, and do not decay over time (i.e., they are non-radioactive) (Schimel, 1993). Stable isotopes can relay information about both source and process, based on the isotopic distribution of the sample origin and reaction conditions (Peterson & Fry, 1987). The most commonly used stable isotopes in biological studies are hydrogen, oxygen, sulfur, carbon and nitrogen (Fry, 2006), but this study will be investigating only carbon and nitrogen stable isotopes since hydrogen and oxygen are more practical for large spatial scales based on the hydrological cycle, while sulfur is relevant for marine and geological applications.

Carbon has two naturally occurring stable isotopes: $^{12}C$ and $^{13}C$, present at 98.89% and 1.11% of the carbon pool, respectively. Nitrogen stable isotopes are present in the environment as $^{14}N$ (99.64%) and $^{15}N$ (0.36%). The main reservoirs of carbon and nitrogen stable isotopes are the ocean, atmosphere, and biosphere, and cycling amongst these reservoirs has been explored by many authors including

Peterson and Fry (1987). The differential behavior (fractionation) of the isotopes results in different signatures and represents a potentially powerful tool to understand ecosystem processes.

Stable isotopes of carbon do not fractionate strongly between trophic levels in food webs, averaging 0.4‰ per trophic level, and therefore carbon signatures are generally considered to be good indicators of dietary sources (DeNiro & Epstein, 1981; Post, 2002). Alternatively, nitrogen stable isotopes are enriched in organisms by approximately 3.4‰ relative to their diet (DeNiro & Epstein, 1981; Minagawa & Wada, 1984; Post, 2002). By using nitrogen stable isotopes, inferences on trophic levels can be made. Nitrogen and carbon in effluent can have different isotope ratios than what is naturally occurring in receiving waters. Nutrients in receiving waters can also alter fractionation, especially in the lower trophic levels (Loomer *et al.*, 2015). Numerous studies have applied SIA as potential tracers of exposure to municipal and industrial effluents, such as wastewater treatment plants (Fair & Heikoop, 2006; Loomer *et al.*, 2015; Robinson, 2011; Ulseth & Hershey, 2005), oil sand operations (Farwell *et al.*, 2009), aquaculture (Salazar-Hermoso, 2007; Vizzini & Mazzola, 2004), and pulp mills (Table 1.1).

A chronological review of previous stable isotope studies at pulp mills found that pulp mill effluents tend to have carbon isotope ratios that reflect terrestrial $C_3$ plants, likely due to the intensive processing of trees. Carbon signatures of pulp mill effluents range from -28 to -24‰ (Table 1.1), which is similar to $C_3$ plant signatures of -28‰ (O'Leary, 1988). Nitrogen isotope ratios in effluent are highly variable among samples (Table 1.1). Primary producers exposed to effluent tend to have signatures similar to atmospheric air, but signatures in biota increase with trophic levels. Many aspects of the pulp process may influence isotope ratios in effluent since the types of mill, wood feed, bleaching and treatment vary considerably, making comparisons of studies difficult among different locations. In addition, fractionation of isotopes can vary among receiving environments (Table 1.1).

Multiple effluent inputs, dilution, mixing, and environmental processes confounded interpretation of stable isotope ratios in the environment in many of the studies outlined in Table 1.1. In simple

environments, with no other point or non-point source pollution inputs, stable isotopes could be effective at isolating a single point source of pollution, although measuring both background and baseline isotopic ratios is beneficial in interpretation of the data. Difference in habitat, species present, and tissues sampled can greatly alter the pattern of isotopic signatures observed. Various tissues within an individual incorporate stable isotopes at different rates due to tissue turnover rates and therefore can reflect temporal exposure of effluents. No single pattern is therefore evident at all pulp mill exposure sites as patterns are dependent on many factors associated with the mill as well as the receiving environment. However, if applied appropriately stable isotopes offer a potentially useful tool at some sites to trace exposure and better understand biotic interactions in the receiving environment.

Table 1.1: Chronological order of pulp mill isotope studies with isotope of interest, studied sample, comparison to background level, type of mill, and author. TCF = total chlorine free.

| Isotope $\delta$ | Sample | Signature ‰ | Compared to background level | Pulp mill type | Author |
|---|---|---|---|---|---|
| $^{2}H$ | Effluent | -130.7 | enriched | bleached kraft, 2° treatment | (Wassenaar & Culp, 1994, 1996) |
| $^{13}C$ | DIC | unknown | depleted | | |
| $^{13}C$ | Biofilm | -14.5 | unknown | | |
| $^{13}C$ | Insects | -22.5 | unknown | | |
| $^{15}N$ | Biofilm | 0.8 to 3.8 | unknown | | |
| $^{15}N$ | Chironomidae | 1.8 to 2.2 | unknown | | |
| $^{15}N$ | Mayflies | 3.2 to 4.8 | unknown | | |
| $^{18}O$ | Effluent | -16.3 | enriched | | |
| $^{34}S$ | Effluent | 1.3 | no difference | | |
| $^{13}C$ | pulp mill chlorolignin | -27.1 | no difference | bleached kraft, 1° treatment | (Kukkonen *et al.*, 1996) |
| $^{15}N$ | pulp mill chlorolignin | -4.4 | depleted | | |
| $^{13}C$ | suspended sediment | -27.1 | depleted | bleached kraft, 2° treatment | (Wayland & Hobson, 2001) |
| $^{15}N$ | suspended sediment | 0.4 | depleted | | |
| $^{34}S$ | suspended sediment | 16.9 | enriched | | |
| $^{13}C$ | Algae | -27 | enriched | bleached kraft, 2° treatment | (Wayland & Hobson, 2001) |
| $^{15}N$ | Algae | 3.3 | no difference | | |
| $^{34}S$ | Algae | 0.5 | enriched | | |
| $^{13}C$ | slimy sculpin | -25 to -24 | enriched | sulphite with 2° treatment | (Galloway *et al.*, 2003) |
| $^{15}N$ | slimy sculpin | 7.75 to 8.5 | depleted | | |
| $^{13}C$ | longnose sucker gonad | -26.8 to -26 | depleted | bleached chemi-thermomechanical (TCF)2° treatment | (Dube *et al.*, 2005) |
| $^{13}C$ | longnose sucker liver | -26.9 to -27.6 | no difference | | |
| $^{13}C$ | longnose sucker muscle | -26.3 to -26.4 | no difference | | |
| $^{13}C$ | longnose sucker bone | -24.5 to -25 | no difference | | |
| $^{15}N$ | longnose sucker gonad | 7.9 to 5.7 | enriched | | |

| | | | | | |
|---|---|---|---|---|---|
| $^{15}N$ | longnose sucker liver | 7.1 to 7.5 | enriched | | |
| $^{15}N$ | longnose sucker muscle | 8.8 to 8.9 | enriched | | |
| $^{15}N$ | longnose sucker bone | 7.6 to 7.4 | enriched | | |
| $^{34}S$ | longnose sucker gonad | 6.5 to 6.9 | depleted | | |
| $^{34}S$ | longnose sucker liver | 7.2 to 7.0 | depleted | | |
| $^{34}S$ | longnose sucker muscle | 5.2 to 6.1 | depleted | | |
| $^{34}S$ | longnose sucker bone | 5.9 to 6.4 | depleted | | |
| $^{37}Cl$ | longnose sucker gonad | -0.8 to -2.5 | no difference | | |
| $^{37}Cl$ | longnose sucker liver | -2.0 to -1.8 | enriched | | |
| $^{37}Cl$ | longnose sucker muscle | -3.7 to -3.2 | depleted | | |
| $^{13}C$ | longnose sucker gonad | -26.5 to -25.2 | no difference | thermomechanical (TCF) 2° treatment | (Dube *et al.*, 2005) |
| $^{13}C$ | longnose sucker liver | -26.6 to -26.8 | no difference | | |
| $^{13}C$ | longnose sucker muscle | -26.1 to -25.8 | no difference | | |
| $^{13}C$ | longnose sucker bone | -24.4 to -24.6 | no difference | | |
| $^{15}N$ | longnose sucker gonad | 6.8 to 5.1 | no difference | | |
| $^{15}N$ | longnose sucker liver | 6.0 to 6.5 | enriched | | |
| $^{15}N$ | longnose sucker muscle | 8.3 to 8.2 | enriched | | |
| $^{15}N$ | longnose sucker bone | 6.7 to 7.8 | enriched | | |
| $^{34}S$ | longnose sucker gonad | 7.3 to 9.5 | females depleted, males enriched | | |
| $^{34}S$ | longnose sucker liver | 7.5 to 8.6 | depleted | | |
| $^{34}S$ | longnose sucker muscle | 6.6 to 7.3 | depleted | | |
| $^{34}S$ | longnose sucker bone | 6.8 to 7.7 | depleted | | |
| $^{37}Cl$ | longnose sucker gonad | -4.4 to -3 | depleted | | |
| $^{37}Cl$ | longnose sucker liver | -4.2 to -4.4 | depleted | | |
| $^{37}Cl$ | longnose sucker muscle | -1.8 to -2.3 | enriched | | |
| $^{13}C$ | DOC | -28.4 | no difference | mechanical, 1° treatment | (Oakes *et al.*, 2010) |
| $^{13}C$ | DIC | -26.21 | depleted | | |

| | | | | | |
|---|---|---|---|---|---|
| $^{13}C$ | POM | -25.9 | enriched | | |
| $^{15}N$ | Particulate nitrogen | 6.2 | depleted | | |
| $^{13}C$ | DIC | -22.2 | depleted | mechanical, 2° treatment | (Oakes *et al.*, 2010) |
| $^{13}C$ | DOC | -29.2 | no difference | | |
| $^{13}C$ | POM | -26.7 | depleted | | |
| $^{15}N$ | Particulate nitrogen | -0.1 | depleted | | |
| $^{13}C$ | Mussel | -32 to -31 | no difference | bleached kraft, 2° treatment | (Freedman *et al.*, 2012) |
| $^{13}C$ | Snail | -23 to -21 | depleted | | |
| $^{13}C$ | white sucker muscle | -29.5 to -27.5 | no difference | | |
| $^{13}C$ | yellow perch muscle | -26.5 to -25 | no difference | | |
| $^{15}N$ | Mussel | 9 to 9.75 | no difference | | |
| $^{15}N$ | Snail | 7.5 to 7.7 | no difference | | |
| $^{15}N$ | white sucker muscle | 9 to 12 | no difference | | |
| $^{15}N$ | yellow perch muscle | 12 to 14 | no difference | | |
| $^{13}C$ | mummichog muscle and bone | -20.85 | no difference | bleached kraft, 2° treatment | (Skinner *et al.*, 2012) |
| $^{15}N$ | mummichog muscle and bone | 10.18 | depleted | | |
| $^{13}C$ | mummichog muscle and bone | -20.69 | no difference | groundwood | (Skinner *et al.*, 2012) |
| $^{15}N$ | mummichog muscle and bone | 12.05 | no difference | | |
| $^{13}C$ | white sucker muscle | -27.62 to -27.2 | enriched | bleached kraft, 2° treatment | (Arciszewski *et al.*, 2014) |
| $^{13}C$ | white sucker muscle | -25.3 to -24.78 | enriched | thermomechanical, 2° treatment | (Arciszewski *et al.*, 2014) |

## 1.7 Problem Statement

White sucker have been used over several decades as a sentinel species in studies of the impact of pulp mill effluents in Jackfish Bay, Lake Superior. Movement of fish, especially in the spring during spawning, allows mixing between exposed and unexposed populations, making it difficult to associate different levels of effluent exposure. The discharge of effluent from the kraft pulp mill in Terrace Bay into Moberly Bay may result in a spatial difference in isotopic signatures in fish that may allow differentiation of effluent exposure. Altered isotopic signatures in the effluent compared to the natural ecosystem may be incorporated into the food chain and alter the stable ratios of carbon and nitrogen relative to reference conditions. It is also possible that changes in the nutrient inputs or food web structure may also alter natural processes and affect the fractionation leading to different stable isotope ratios in fish in the receiving environment. During the study period a shutdown of the mill resulted in the effluent flow being stopped for many months, creating an opportunity to examine how stable isotopes ratios in fish in this environment respond temporally to effluent inputs. Being able to separate exposed and non-exposed fish may greatly improve the reliability and sensitivity of the studies at this site.

### 1.7.1 Objectives

The objective of this thesis is to determine how stable isotopes of carbon and nitrogen change in organisms in the receiving environment of a pulp mill outfall. The following questions were addressed:

1. Do $\delta^{13}C$ and $\delta^{15}N$ signatures of white sucker and invertebrates differ among an effluent exposed site (Moberly Bay), an adjacent site (Tunnel Bay), a reference site (Mountain Bay and a small lake nearby (Jackfish Lake);
2. Can $\delta^{13}C$ and $\delta^{15}N$ signatures distinguish between effluent-exposed (Moberly Bay) and non-exposed white sucker (Jackfish Lake) during spawning; and
3. Do $\delta^{13}C$ and $\delta^{15}N$ signatures of white sucker and invertebrates reflect periods of mill operation (2011 and 2013) and non-operation (2012)?

In order to address these questions white sucker were collected from spawning creeks (Little Gravel River [reference] and Sawmill Creek [exposed]) in May, and white sucker and invertebrates were collected from lake sites (Mountain Bay [reference], Moberly Bay [exposed], Tunnel Bay [partially exposed], and Jackfish Lake [nearby and effluent-free]) in August. Samples were collected in 2011 before mill shutdown, in 2012 during mill closure, and in 2013 when the mill had reopened.

The null hypotheses tested include:

1. White sucker and invertebrates at the exposed site (Moberly Bay) do not have significantly different $\delta^{13}C$ and $\delta^{15}N$ compositions than white sucker and invertebrates at adjacent and reference sites (Tunnel Bay, Jackfish Lake, and Mountain Bay);
2. Muscle and liver of spawning white sucker have $\delta^{13}C$ and $\delta^{15}N$ signatures at Sawmill Creek associated with Jackfish Bay.
3. Short-term changes in mill operation status do not alter $\delta^{13}C$ and $\delta^{15}N$ signatures in muscle and liver tissues of white sucker and invertebrates from the exposed site (Moberly Bay).

The results of the study have been organized into two major chapters. Chapter 2 assesses the spatial differences in population endpoints, MFO activity, and stable isotopes of carbon and nitrogen to assess fish effluent exposure, movement, and migration. Chapter 3 addresses temporal shifts in stable isotope ratios of fish during mill operation and closure. The final chapter (Chapter 4) is the summary and conclusions of the major findings and their relevance for environmental monitoring.

# Chapter 2

# Spatial assessment of white sucker (*Catostomus commersonii*) exposure to pulp mill effluent at Jackfish Bay, Lake Superior using stable isotope analyses

The contributing authors of this chapter are:

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- Kelly Munkittrick: Provided general advice and background information;

- Gerald Tetreault: Provided general advice and assisted with fieldwork;

- Mark McMaster and Mark Servos: Co-supervisors of Jessica Mendoza. Assisted with fieldwork, research direction and provided general advice.

## 2.1 Summary

Jackfish Bay, Lake Superior receives effluent via Blackbird Creek from the bleached kraft pulp mill in Terrace Bay, Ontario. Extensive studies at this site have documented growth and reproductive effects of bleached kraft mill effluent (BKME) in white sucker (*Catostomus commersonii*) during both prespawning (spring) and gonadal recrudescence (fall) (Bowron *et al.*, 2009; McMaster *et al.*, 1991; Munkittrick *et al.*, 1991). Jackfish Bay is an open system with no physical barriers to the movement of fish, thus making it difficult to define fish exposure to the effluent. Fish must migrate through waterbodies of varying effluent exposure to access the nearest spawning creek, Sawmill Creek, which further complicates determining the extent of exposure. Analyses of stable isotopes of carbon and nitrogen were applied to determine whether BKME-exposed fish tissue (liver and muscle) and benthic invertebrates have distinct isotopic signatures at different sites. Population parameters of white sucker were assessed in the spring (at spawning sites) and fall (at lake sites) including age, length, weight, gondaosomatic index, liver somatic index, and condition. Mixed function oxygenase (MFO) activity was measured as ethoxyresorufin-o-deethylase (EROD) induction at each site and was used as an additional indicator of white sucker exposure to BKME. Male white sucker collected in the area of Jackfish Bay that is directly exposed to BKME (Moberly Bay) did not have a distinct isotopic signature in muscle and liver tissues compared to those collected at the adjacent, less exposed bay (Tunnel Bay), or a reference site in Lake Superior (Mountain Bay). EROD induction suggested that fish in both Moberly Bay and Tunnel Bay were more exposed to inducing compounds compared to Mountain Bay, although Tunnel Bay had lower EROD induction than Moberly Bay. White sucker collected from a warm, shallow lake through which they migrate in the spring to reach Sawmill Creek to spawn (Jackfish Lake) had depleted carbon and nitrogen stable isotope signatures compared to fish from the exposed and reference sites (Lake Superior sites). Fish caught at Sawmill Creek during the spring were divided into groups by Ward Hierarchal Clustering and revealed two distinct isotopic groups: one similar to those caught at Jackfish

Lake, and one similar to those from Moberly Bay and Tunnel Bay. Up to 23% of prespawning fish at Sawmill Creek were possibly not exposed to BKME based on these analyses, therefore potentially diluting effects endpoints in spring fish collections.

## 2.2 Introduction

Jackfish Bay, Lake Superior, has been the flagship site in Canada for understanding the impacts of bleached kraft mill effluent (BKME) on receiving environments. During the late 1980s in Sweden, researchers observed chronic effects in fish exposed to BKME (Sandstrom *et al.*, 1988; Södergren, 1989). Subsequently, Canadian researchers investigated the effects of the Terrace Bay BKME at Jackfish Bay, and their results confirmed the earlier Swedish findings (Munkittrick *et al.*, 1991; Munkittrick *et al.*, 1994). Adverse effects observed in fish exposed to BKME included reduced secondary sex characteristics, delayed sexual maturity, decreased relative gonad weight, decreased circulating and gonadal sex steroids, increased relative liver weight, and elevated activity levels of hepatic mixed function oxygenases (MFO) (McMaster *et al.*, 1991; Munkittrick *et al.*, 1991). Over the last 25 years, numerous studies have been conducted at Jackfish Bay to better understand the impact of BKME as the pulp mill in Terrace Bay has undergone mill upgrades and shutdowns in response to product demand, maintenance, and new regulations (Bowron *et al.*, 2009). While these upgrades and changes have reduced the environmental impact of the effluent on the receiving environment, adverse impacts are still observed in fish populations (Bowron *et al.*, 2009).

The white sucker (*Catostomus commersonii*) has been a preferred sentinel species for studies at multiple sites receiving pulp mill effluent across Canada. This species has widespread abundance, tolerance to a range of environmental conditions, a long life span, and young maturity rate. In addition, white sucker forage along the benthic zone of lakes, and are thus exposed to contaminants that settle and partition into sediments. The white sucker has been used extensively in previous studies at Jackfish Bay. However, there are limitations related to the white sucker life history that can make it difficult to assess

direct impacts of effluent. Some past assessments of white sucker have included fall collections after gonadal recrudescence, using gill nets in or near the effluent plume. Adult white sucker are relatively mobile, and with no physical barriers to prevent fish from swimming in or out of the effluent plume, the degree of exposure to BKME is not fully known, even when fish are caught in or adjacent to the effluent. Studies have also been conducted on prespawning white sucker as they migrate to their spawning area in early spring. Fish from Moberly Bay migrate through Tunnel Bay, then Jackfish Lake to the spawning area in Sawmill Creek. Fish in the spawning population can therefore include individuals from various parts of Jackfish Bay (exposed or unexposed areas), as well as from Jackfish Lake.

Since pulp mill-exposed fish that may have resided in the effluent plume must migrate many kilometers to spawn in spring, they may not be exposed to effluent for days to weeks before being caught. Therefore, a tracer that can define exposure, better indicate the residency and movement patterns of fish, and support assessment of the effect of industrial effluents is desirable. Stable isotope ratios might be an applicable tool for determining BKME exposure in mobile, spawning fish at this site. Stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) have been widely used to describe trophic dynamics in aquatic ecosystems. Carbon stable isotopes do not fractionate strongly between trophic levels (0-1‰ enrichment), and therefore are indicative of the source of energy on which the food web relies (Post, 2002). Nitrogen stable isotopes fractionate strongly between trophic levels (3-4‰ enrichment), and represent trophic linkages (Post, 2002). Applying these two stable isotopes in conjunction can help to define sources of carbon and the interactions of organisms such as fish with their environment. By measuring stable isotopes in fish tissues with moderate to long turnover, exposure can potentially be confirmed on the scale of weeks to months despite movement or migration.

Kraft mill effluent is predicted to have a terrestrially derived carbon signature (-30 to -25‰) due to the intensive processing of terrestrial organic matter by pulp mills (Chapter 1, Table 1.1). Organisms in the receiving environment may have depleted carbon stable isotopes if they are incorporating this

material as their energy source compared to unexposed organisms that are predicted to have a signature that reflects autochthonous organic matter. Effluent exposed organisms are likely to have a different nitrogen isotope signature compared to reference fish, due to effluent altering the food web. Effluent-derived nutrients may lengthen the food web, while toxicity of effluent may shorten the food web. Shortened food chains result in lower $\delta^{15}N$ in higher trophic organisms as less trophic interactions allow for $\delta^{15}N$ enrichment (Vander Zanden *et al.*, 1999). Therefore isotopic signatures of nitrogen may indicate length of food web at different sites.

In addition, altered physical and chemical properties in water may change isotope signatures. Changes in the relative availability of nutrients or the recycling of $CO_2$ in the environment may alter the fractionation of stable isotopes (O'Leary, 1988). Altered coloration, increased biological oxygen demand, warm temperatures and high total suspended solids of effluent may alter habitat and food web structure, creating changes in processes and food web interactions. In short, pulp mill effluent may lead to a difference in stable isotope ratios of fish that reside in the receiving environment.

The duration of fish residency in and out of the effluent plume in Jackfish Bay is unknown but is important for understanding the exposure and effects of effluent. In addition, white sucker from both exposed and unexposed populations may migrate to the same spawning sites and make it difficult to isolate the effects of the effluent during spring spawning. Pulp mill effluent entering Jackfish Bay may change the stable isotope ratios of carbon and nitrogen, which could provide a tool for identifying effluent exposed organisms. The purpose of this study was to determine whether stable isotope signatures of carbon and nitrogen in BKME-exposed white sucker differ from those of adjacent and reference populations, and whether these signatures can be used to separate exposed from unexposed individuals during gonadal recrudescence and spawning. To help determine site fidelity, isotopic signatures in white sucker tissues were contrasted to those of benthic invertebrates that are less mobile collected at each site.

## 2.3 Methods

### 2.3.1 Study Site

Jackfish Bay (48°50'N, 86°58'W) is an isolated location on the north shore of Lake Superior. It consists of two bays, Moberly Bay to the west and Tunnel Bay to the east (Figure 2.1). The pulp mill in Terrace Bay channels its effluent into Blackbird Creek, which flows into Moberly Bay. The effluent is rapidly diluted (< 4%) as it enters Moberly Bay. The effluent plume generally moves to the west side of Jackfish Bay and then into open Lake Superior. Tunnel Bay lies to the east of Moberly Bay, is separated by a protruding landmass, and receives less than 1% effluent by volume (Munkittrick *et al.*, 1991; Stewart & Rashid, 2011).

Jackfish Lake was once a part of Tunnel Bay, until it was separated by the construction of the Canadian Pacific Railroad at the turn of the century. It is now a warm, shallow lake, connected to Tunnel Bay via a small tunnel through which white sucker from Moberly Bay and Tunnel Bay must travel to reach Sawmill Creek to spawn in early May. Jackfish Lake and Sawmill Creek are free of effluent loading. The reference sites chosen for this study were located 60 km to the west of Jackfish Bay; Mountain Bay during the fall season and the Little Gravel River, which flows into Mountain Bay, during the spring season.

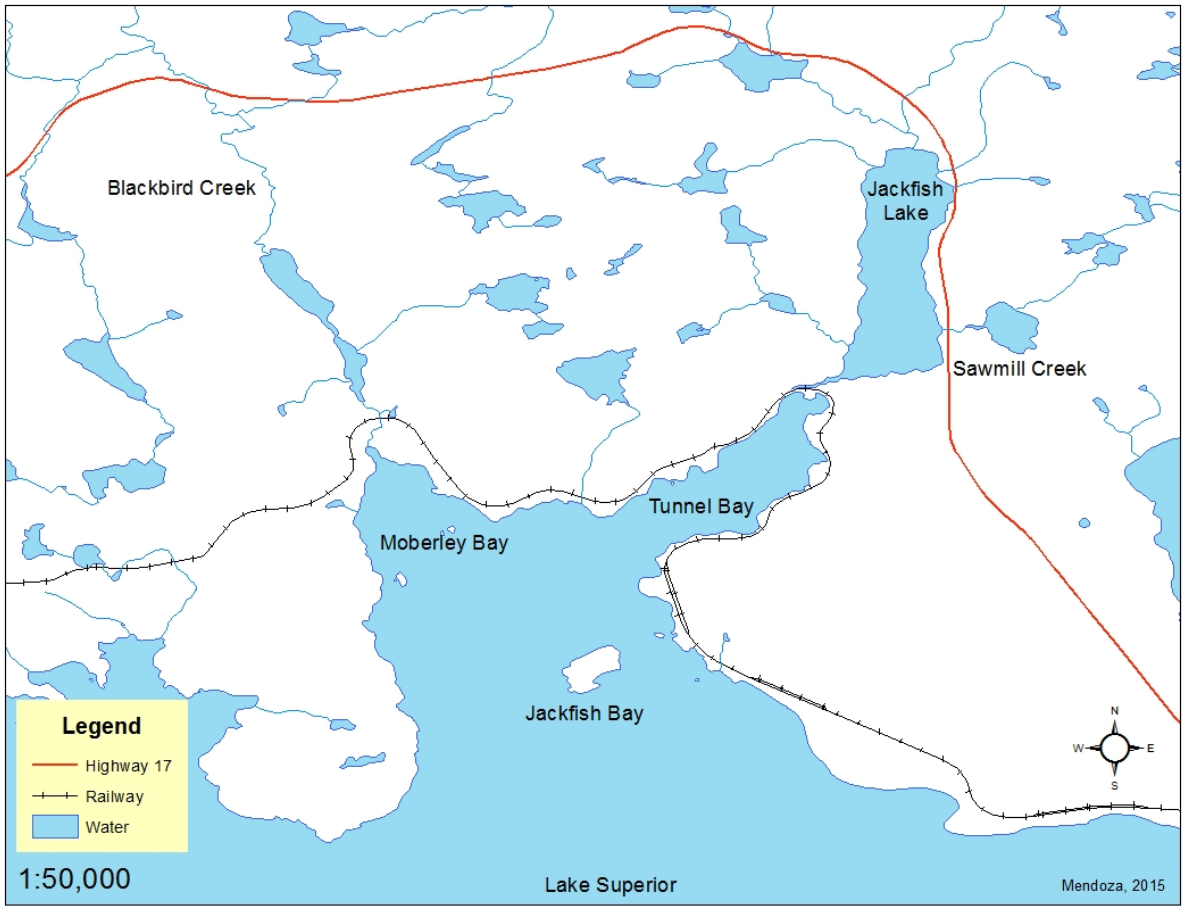


Figure 2.1: Map of Moberly Bay and Tunnel Bay, both of Jackfish Bay, and Jackfish Lake. Mountain Bay and Little Gravel River lie 60 km to the west. Effluent travels along Blackbird Creek and enters Jackfish Bay at Moberly Bay. White sucker from Jackfish Bay migrate through Jackfish Lake to Sawmill Creek to spawn in the spring (DMTI CanMap Route Logistics, 2014).

### 2.3.2 Field Collection

Prespawning white sucker were collected with hoop nets on May 14$^{th}$ to 19$^{th}$, 2013 at Sawmill Creek and Little Gravel River. Gonadal recrudescent white sucker, benthic invertebrates, plankton and particulate matter were collected on August 23$^{rd}$ to 29$^{th}$, 2013 at Moberly Bay, Tunnel Bay, Jackfish Lake, and Mountain Bay. Approximately 20 male and 20 female white sucker were collected by 10.0 and 11.3 cm gill nets set overnight and sampled immediately after being euthanized by a blunt blow to the head (University of Waterloo AUPP# 10-17). Fork length (± 0.1 cm), total body weight, liver weight, and

gonad weight (± 0.1 g) were measured on site. The right operculum was collected to evaluate age. For isotope analyses, liver and muscle tissue were kept in liquid nitrogen for transportation and transferred to a -20°C freezer for storage. Liver tissue for MFO analysis was kept in liquid nitrogen for transportation and transferred to an -80°C freezer for storage.

Benthic invertebrates were collected during the August sampling by an Ekman Dredge and washed through a sieve. These animals were sorted and kept in filtered lake water overnight to remove gut content before they were frozen at -20°C. Benthic invertebrates were identified under a dissecting microscope (0.63 to 4x magnification) to the lowest possible taxonomic level according to Thorp and Covich (2010). Plankton was collected from the water column using a 2.5 x 0.5 m, 53 μm nylon mesh plankton haul and rinsed into 1 L Nalgene bottles. Particulate organic matter (POM) was collected from 20 L surface water grabs in carboys. Plankton and POM were filtered onto Whatman 4.7 cm diameter Grade QM-A quartz filters and kept in an airtight desiccator.

### 2.3.3 Laboratory Analyses

*Stable Isotope Analyses*

White sucker muscle and liver, invertebrates, and filter samples were freeze dried for a minimum of 48 hours. After drying, muscle and liver samples were ground into a fine powder and homogenized by placing samples in a 10 mL stainless steel grinding jar with a 10 mm hardened steel grinding ball, and then shaking for 3 minutes at 3000 rpm in a Retsch MM2000. The grinding jar and ball were cleaned between samples using acetone. The fish and invertebrate samples were weighed using a high precision analytical balance (0.2 ± 0.05 mg) and placed in 5 x 3.5 mm Sercon pressed tin capsules. Invertebrates of the same lowest taxonomy were pooled if there was insufficient dry weight for stable isotope analyses. Subsamples of filters for zooplankton and particulate matter were placed into 5 x 8 mm Sercon pressed tin capsules using a Parr pellet press.

The stable isotope ratios and the percent elemental composition of carbon and nitrogen were analyzed at the University of Waterloo Environmental Isotope Lab (EIL) on a Delta Plus, Continuous Flow Stable Isotope Ratio Mass Spectrometer (Thermo Finnigan/Bremen-Germany) coupled to a Carlo Erba Elemental Analyzer (CHNS-O EA11008-Italy) (Drimmie & Heemskerk, 2005; Fry *et al.*, 1992). Standards for carbon and nitrogen were VPDB (Vienna Peedee Belemnite), carbonate (Craig, 1957), and atmospheric nitrogen (Mariotti, 1983).

Throughout the run, a minimum of 20% of the samples were international reference materials (IAEA, USGS) or in-house EIL standards used to normalize data, correct for precision and accuracy, and assess linearity and drift issues. The error for $\delta^{13}C$ and $\delta^{15}N$ were ± 0.2‰ and ± 0.3‰, respectively. The precision (mean ±1SD) within runs was +0.09 ± 0.05‰ $\delta^{13}C$ and -0.03 ± 0.04‰ $\delta^{15}N$ for duplicates of muscle (N = 57), and +0.17 ± 0.17‰ $\delta^{13}C$ and -0.06 ± 0.06‰ $\delta^{15}N$ for duplicates of liver (N = 54).

The relationship between $\delta^{13}C$ and percent C:N were plotted to determine if there was a relationship between $\delta^{13}C$ and the lipid content of liver and muscle tissues in fish. Few sites inconsistently had C:N to $\delta^{13}C$ relationships greater than $r^2 = 0.5$, and on average muscle C:N ratios were approximately 3.5. However, liver had high C:N ratios (Appendix B). Therefore, $\delta^{13}C$ fish and invertebrate tissues were lipid-corrected using the formula from Post *et al.* (2007):

$$\delta^{13}C_{normalized} = \delta^{13}C_{untreated} - 3.32 + (0.99) \times (C:N) \qquad \text{Equation 2.1}$$

*MFO Analysis*

MFO activity was measured by the induction of ethoxyresorufin-o-deethylase (EROD) (Elfer *et al.*, 1997). Liver samples were retrieved from the -80°C freezer, and placed on ice. Samples were weighed (± 0.001 g), then homogenized with HEPES grinding buffer and a motor-driven Teflon pestle. Homogenates were placed into a microcentrifuge at 9600 rpm at 4°C for 20 minutes. A Pasteur pipette

was used to aspirate the pellet and extract the microsomal fraction within the supernatant. Samples were then stored in an -80°C freezer.

Microsomal fractions were thawed and analyzed for EROD standardized to protein. Samples were plated on a 96-well plate in triplicate. For EROD analysis, 7-ethoxy-resorufin solution and NADPH were added to each sample, and the plate was read in a Biotek Synergy 4 plate reader at wavelength 530/590. For protein analysis, microsomal fractions were first diluted with 750 µL $dH_2O$, gently vortexed, and then plated with a pipette. Bio-Rad 1:5 protein reagent was added to each well, and then read. The inter- and intra-assay coefficients of variability for EROD in liver samples were respectively 30.8% (n = 25) and 7.8% (n = 540), using the following equations:

$$Inter - assay\ \%\ CV = \frac{standard\ deviation\ of\ plate\ means \times 100}{mean\ of\ plate\ means} \quad \text{Equation 2.2}$$

$$Intra - assay\ \%\ CV = \frac{standard\ deviation\ of\ duplicates \times 100}{duplicate\ mean} \quad \text{Equation 2.3}$$

### 2.3.4 Statistical Analyses

Statistical analyses were conducted in R version 2.15.1 (R Core Team, 2012). All statistical tests considered significance level $\alpha = 0.05$ (95% confidence). Genders were separated before the comparison of both population parameters and stable isotopes among sites. Population parameters were log-transformed to create a normal distribution, and Q-Q Plots, Shapiro-Wilk Test, and Levene's Test were used to test ANOVA assumptions.

To evaluate age, length, weight, and EROD activity differences among sites, analysis of variance (ANOVA) tests were conducted, and pairwise t-test with Bonferroni correction was used to determine differences among multiple sites during the fall. Analysis of co-variance (ANCOVA) was used to compare gondaosomatic index (GSI), liver somatic index (LSI), and condition factor (*k*). If there was an interaction, a type III ANCOVA was used, but if there was no interaction a type II ANCOVA was

conducted. A general linear hypothesis test was used to make multiple comparisons for sites during the fall.

Fish samples from Sawmill Creek had a non-normal distribution of stable isotopes, which was not corrected by transformation. Therefore, a non-parametric Kruskal-Wallis Test was applied with a non-parametric comparison test (outlined in Siegel and Castellan (1988)) to determine differences of carbon and nitrogen stable isotopes in both muscle and liver among sites. To compare chironomids with white sucker liver an ANOVA on logged isotopic data was conducted with a pairwise t-test with Bonferroni correction to determine differences among sites.

Cluster analysis of carbon and nitrogen isotopic values in the liver of spawning white sucker was conducted on a dual isotope representation of $\delta^{13}C$ by $\delta^{15}N$ determined by Ward Hierarchical Clustering and cutting the dendogram in two by the first two groupings. Age, length and weight between the two groups were then analyzed using ANOVA, while LSI, GSI and condition were analyzed using ANCOVA.

## 2.4 Results

Prespawning male white sucker collected at Sawmill Creek were heavier than those collected at Little Gravel River (ANOVA, $p = 0.006$), and also had reduced relative liver weight (ANCOVA, $p = 0.0002$) and increased condition (ANCOVA, $p = 0.0003$) (Table 2.1). Prespawning females at Sawmill Creek were also heavier (ANOVA, $p = 0.0003$), had less relative liver weight (ANCOVA, $p < 0.0001$), and increased condition (ANCOVA, $p < 0.001$) compared to reference females, but were also older (ANOVA, $p = 0.003$) and longer (ANOVA, $p = 0.01$) (Table 2.2). There was no difference in relative gonad weight between reference and exposed fish for both males (ANCOVA, $p = 0.1$) and females (ANCOVA, $p = 0.5$).

During recrudescence, exposed males were younger than reference fish (ANOVA, $p = 0.009$) and had increased condition (ANCOVA, $p < 0.001$) compared to reference males. Exposed females were also

younger (ANOVA, $p = 0.009$) than reference females during recrudescence but condition was not statistically distinguishable (ANCOVA, $p = 0.1$). Tunnel Bay males and females did not differ from Moberly Bay fish in any of the population parameters (Appendix A), and males were also younger (ANOVA, $p = 0.03$) with increased condition (ANCOVA, $p < 0.001$) relative to males from Mountain Bay, while females were only younger (ANOVA, $p = 0.02$) than reference females and had the same condition as females from Mountain Bay (Appendix A). Males from Jackfish Lake were shorter, weighed less, and had lower relative liver weight than all other sites, but had similar age and condition as all other sites (Appendix A). Females from Jackfish Lake also weighed less than all other sites, but there were no differences in length or age (Appendix A). Jackfish Lake females had decreased relative liver weight compared to Mountain Bay (ANCOVA, $p < 0.001$) and Moberly Bay (ANCOVA, $p < 0.001$), but were not different than Tunnel Bay females (ANCOVA, $p = 0.06$). Jackfish Lake females had decreased condition compared to females from Moberly Bay (ANCOVA, $p = 0.01$) and Tunnel Bay (ANCOVA, $p = 0.04$) but not females from Mountain Bay (ANCOVA, $p = 0.3$). There was no significant difference in relative gonad weight among all sites for males (ANCOVA, $p = 0.6$) and females (ANCOVA, $p = 0.2$) during gonadal recrudescence.

During prespawning, there was no difference in EROD activity in males (ANOVA, $p = 0.8$) and females (ANOVA, $p = 0.2$) between Little Gravel River and Sawmill Creek (Table 2.3). However, during gonadal recrudescence, EROD activity was higher at Moberly Bay for males and females than at all other sites (Appendix A). Tunnel Bay males and females were also elevated compared to Mountain Bay males (ANOVA, $p < 0.001$) and females (ANOVA, $p < 0.001$), but less than Moberly Bay males (ANOVA, $p = 0.02$) and females (ANOVA, $p < 0.001$). Jackfish Lake fish were not significantly different from Tunnel Bay or Mountain Bay males and females (Appendix A).

Across sites, males did not differ in muscle $\delta^{13}C$ values (Fig. 2.3). This is likely due to the low sample number of males from Jackfish Lake ($n = 4$). Females did not differ in muscle $\delta^{13}C$ values either,

except for Jackfish Lake females, which had depleted $^{13}C$ compared to the other sites (Fig 2.2). Liver tissue of males did not differ in $\delta^{13}C$ except for Jackfish Lake, which was depleted. However, male livers in Jackfish Lake were not statistically depleted compared to Mountain Bay and Moberly Bay male liver. Prespawning females did not have different $\delta^{13}C$ values in their livers between Little Gravel River and Sawmill Creek (Fig. 2.4). During gonadal recrudescence, there was no difference in liver $\delta^{13}C$ between Moberly Bay and Mountain Bay. Tunnel Bay females had liver $\delta^{13}C$ signatures similar to Mountain Bay females, while Jackfish Lake females were highly depleted compared to all other sites.

Prespawning males and females caught at Sawmill Creek were enriched in muscle $\delta^{15}N$ compared to those caught at Little Gravel River (Fig. 2.6 and Fig. 2.7). During gonadal recrudescence, Moberly Bay and Tunnel Bay females were enriched in muscle $\delta^{15}N$ compared to females from Mountain Bay while males from the three sites had the same signature. Jackfish Lake males and females were highly enriched compared to all other sites during gonadal recrudescence and similar only to prespawning fish caught at Sawmill Creek. Prespawning males and females caught at Sawmill Creek were enriched in liver $\delta^{15}N$ compared to those caught at Little Gravel River (Fig. 2.8 and Fig. 2.9). There was no difference in liver $\delta^{15}N$ among sites during gonadal recrudescence except Jackfish Lake males and females, who were highly enriched, like Sawmill Creek fish. During gonadal recrudescence, males and females only differed within sites at Moberly Bay, where females were depleted by 2.5‰ $\delta^{13}C$ in livers compared to males (Table 2.4). At all other sites there were no difference between sexes for liver $\delta^{13}C$ or liver $\delta^{15}N$.

Chironomids were the only invertebrate found in high enough abundance at all sites to conduct statistical tests with white sucker. Chironomids did not differ in $\delta^{13}C$ compared to female and male livers within sites except Moberly Bay. Moberly Bay chironomids were depleted by 8 to 11‰ $\delta^{13}C$ compared to livers of white sucker males and females. Compared to white sucker sampled at each site, Mountain Bay chironomids were depleted by 1.7 to 1.9‰ $\delta^{15}N$, Moberly Bay were depleted by 4.2 to 4.4‰ $\delta^{15}N$, Tunnel Bay chironomids were depleted by 3.1‰ $\delta^{15}N$, and Jackfish Lake chironomids were depleted by

2.1 to 2.8‰ $\delta^{15}N$. Other invertebrates (*Hyalella*, *Caecidotea*, *Pisidium*, *Valvata*) and zooplankton and particulate matter were collected to supplement chironomids data, but not enough samples were collected at all sites to conduct statistical analyses. Invertebrates tended to have similar ranges of chironomids isotope signatures, while zooplankton and particulate matter had highly depleted $\delta^{13}C$ and enriched $\delta^{15}N$ (Table 2.4).

Ward Hierarchal Clustering distinguished two distinct groups of fish collected at Sawmill Creek (Fig. 2.10). Separation into two groups was done for each sex, with five males (16%) and ten females (30%) in group one, and 27 males (84%) and 23 females (70%) in group two. Overall 23% of fish collected at Sawmill Creek were in group one, while 77% were in group two. The first group was more enriched in $\delta^{15}N$ (+6.5 to +9‰) and depleted in $\delta^{13}C$ (-24 to -30‰) than the second group (+5 to +7.5‰ $\delta^{15}N$ and -10 to -22‰ $\delta^{13}C$). The first group was approximately three years younger, 5 cm shorter, and 400 g less than the second group for males and females (Table 2.5). However, there was no difference for relative gonad weight, relative liver weight or condition (Appendix A).

Table 2.1: Age, length, weight, condition factor (k), gonad somatic index (GSI), and liver somatic index (LSI) of male white sucker (*Catostomus commersonii*) from prespawning sites (spring): Little Gravel River (LGR) and Sawmill Creek (SMC); and gonadal recrudescent sites (fall): Mountain Bay (MTB), Moberly Bay (MOB), Tunnel Bay (TB), and Jackfish Lake (JFL) during 2013. Values are reported as mean ± SE (n). Letters signify statistically similar sites within each variable ($p < 0.05$).

| Season | Site | Age (year) | Length (cm) | Weight (g) | GSI[a] | LSI[b] | $k$[c] |
|---|---|---|---|---|---|---|---|
| spring | LGR | 6.1 ± 0.3 (19) a | 37.5 ± 0.5 (19) a | 677.4 ± 27.4 (19) a | 4.55 ± 0.13 (19) a | 1.81 ± 0.07 (19) a | 1.28 ± 0.04 (19) a |
| | SMC | 7.6 ± 0.5 (32) a | 39.0 ± 0.6 (32) a | 848.6 ± 42.6 (32) b | 5.00 ± 0.13 (32) a | 1.52 ± 0.04 (32) b | 1.40 ± 0.01 (32) b |
| fall | MTB | 9.4 ± 0.4 (27) A | 41.4 ± 0.3 (27) A | 970.0 ± 23.7 (27) A | 4.82 ± 0.29 (27) A | 1.20 ± 0.03 (27) A | 1.36 ± 0.02 (27) A |
| | MOB | 7.2 ± 0.6 (20) B | 41.2 ± 0.6 (20) A | 1115.8 ± 49.2 (20) A | 4.67 ± 0.41 (20) A | 1.40 ± 0.07 (20) A | 1.58 ± 0.04 (20) B |
| | TB | 7.5 ± 0.6 (19) AB | 40.5 ± 0.4 (19) A | 1015.5 ± 29.4 (19) A | 5.14 ± 0.46 (19) A | 1.35 ± 0.09 (19) A | 1.53 ± 0.03 (19) C |
| | JFL | 6.8 ± 0.9 (4) AB | 35.3 ± 1.3 (4) B | 653.4 ± 72.7 (4) B | 5.64 ± 0.84 (4) A | 0.82 ± 0.09 (4) B | 1.47 ± 0.02 (4) ABC |

[a]GSI = (gonad weight/body weight) x 100.

[b]LSI = (liver weight/body weight) x 100.

[c]$k$ = (weight/length$^3$) x 100.

Table 2.2: Age, length, weight, condition factor (k), gonad somatic index (GSI), and liver somatic index (LSI) of female white sucker (*Catostomus commersonii*) from prespawning sites (spring): Little Gravel River (LGR) and Sawmill Creek (SMC); and gonadal recrudescent sites (fall): Mountain Bay (MTB), Moberly Bay (MOB), Tunnel Bay (TB), and Jackfish Lake (JFL) during 2013. Values are reported as mean ± SE (n). Letters signify statistically similar sites within each variable ($p < 0.05$).

| Season | Site | Age (year) | Length (cm) | Weight (g) | GSI[a] | LSI[b] | *k*[c] |
|---|---|---|---|---|---|---|---|
| Spring | LGR | 6.9 ± 0.5 (20) a | 41.0 ± 0.6 (20) a | 950.7 ± 45.8 (20) a | 12.51 ± 0.44 (20) a | 2.23 ± 0.05 (19) a | 1.36 ± 0.02 (20) a |
| | SMC | 9.1 ± 0.5 (33) a | 43.5 ± 0.6 (33) a | 1275.4 ± 58.9 (33) b | 13.77 ± 0.36 (33) a | 1.83 ± 0.05 (33) b | 1.51 ± 0.02 (33) b |
| Fall | MTB | 10.1 ± 0.6 (34) A | 43.2 ± 0.7 (34) A | 1118.5 ± 32.8 (34) AB | 2.67 ± 0.11 (34) A | 1.58 ± 0.05 (34) A | 1.51 ± 0.17 (34) AB |
| | MOB | 7.4 ± 0.7 (20) B | 43.2 ± 0.8 (20) A | 1267.5 ± 70.6 (20) A | 2.49 ± 0.13 (20) A | 1.60 ± 0.09 (20) AB | 1.55 ± 0.03 (20) A |
| | TB | 7.5 ± 0.7 (20) B | 42.5 ± 0.9 (20) A | 1191.0 ± 64.6 (20) AB | 2.48 ± 0.20 (20) A | 1.38 ± 0.05 (20) B | 1.52 ± 0.02 (20) AB |
| | JFL | 7.7 ± 0.7 (12) AB | 40.1 ± 1.2 (12) A | 927.3 ± 89.3 (12) B | 2.48 ± 0.11 (12) A | 1.07 ± 0.06 (12) C | 1.39 ± 0.03 (12) B |

[a]GSI = (gonad weight/body weight) x 100.

[b]LSI = (liver weight/body weight) x 100.

[c]$k$ = (weight/length$^3$) x 100.

Table 2.3: Mean ± standard error (n) of mixed function oxygenase (MFO) activity measured as ethoxy-o-resorufin deethylase (EROD) activity of male and female white sucker (*Catostomus* commersonii) during spring and fall at Little Gravel River (LGR), Sawmill Creek (SMC), Mountain Bay (MTB), Moberly Bay (MOB), Tunnel Bay (TB), and Jackfish Lake. Letters signify statistically similar sites ($p < 0.05$). Lowercase letters compare differences among male fish while uppercase letters compare differences among female fish.

| Season | Site | EROD Activity (pmol/min/mg) in male white sucker | EROD Activity (pmol/min/mg) in female white sucker |
|---|---|---|---|
| Spring | LGR | 1.2 ± 0.1 (19) a | 0.5 ± 0.2 (20) a |
| | SMC | 1.4 ± 0.1 (32) a | 0.5 ± 0.0 (33) a |
| Fall | MTB | 0.9 ± 0.1 (20) A | 0.5 ± 0.1 (20) A |
| | MOB | 6.9 ± 1.2 (17) B | 5.8 ± 1.1 (20) B |
| | TB | 3.5 ± 0.8 (15) C | 1.9 ± 0.5 (16) C |
| | JFL | 0.9 ± 0.1 (3) AC | 0.8 ± 0.1 (6) AC |

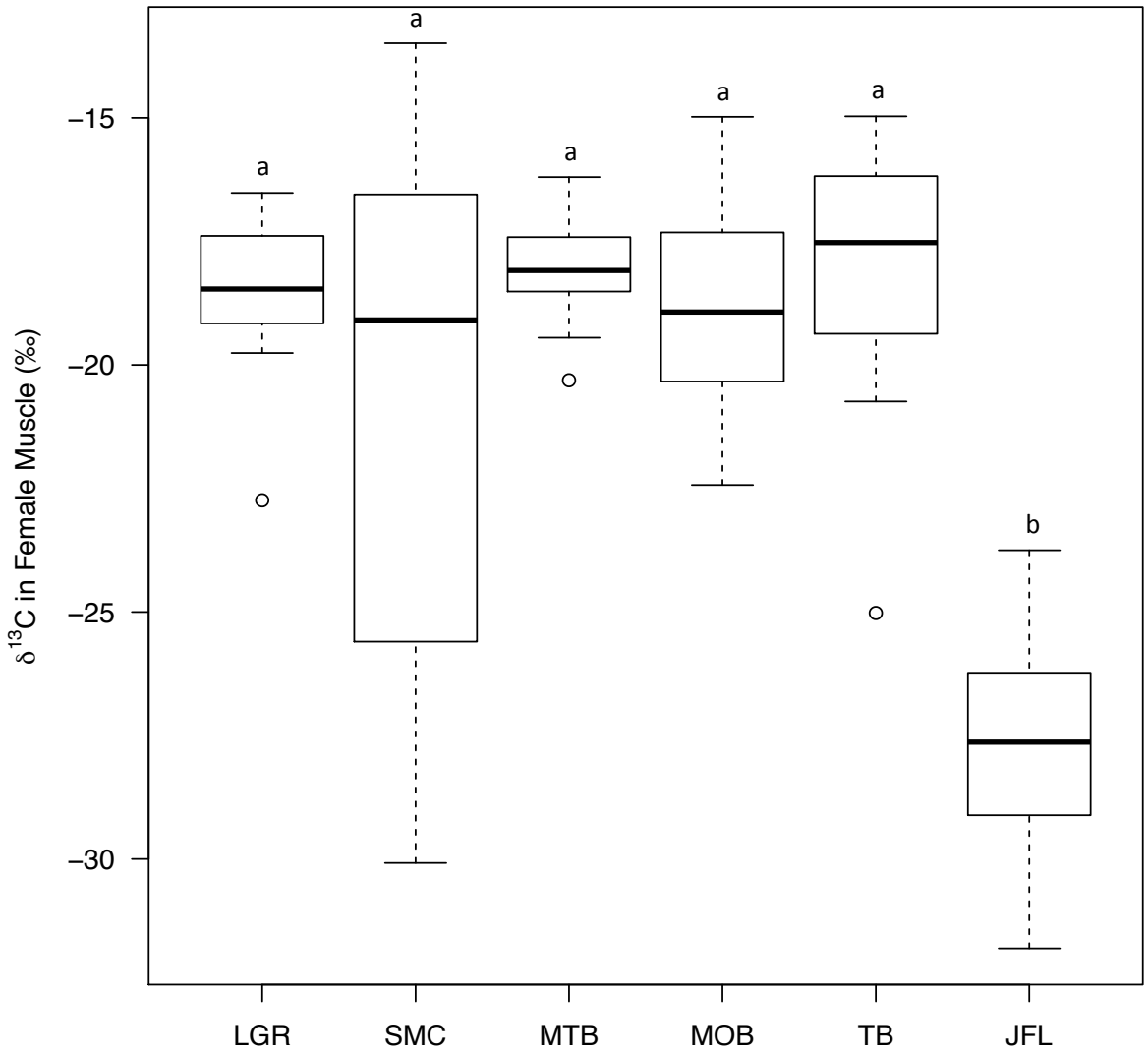


Figure 2.2: Box-plot of $\delta^{13}C$ in muscle tissue of female white sucker by site. LGR = Little Gravel River, SMC = Sawmill Creek, MTB = Mountain Bay, MOB = Moberly Bay, TB = Tunnel Bay, and JFL = Jackfish Lake. Letters signify statistically similar sites ($p < 0.05$).

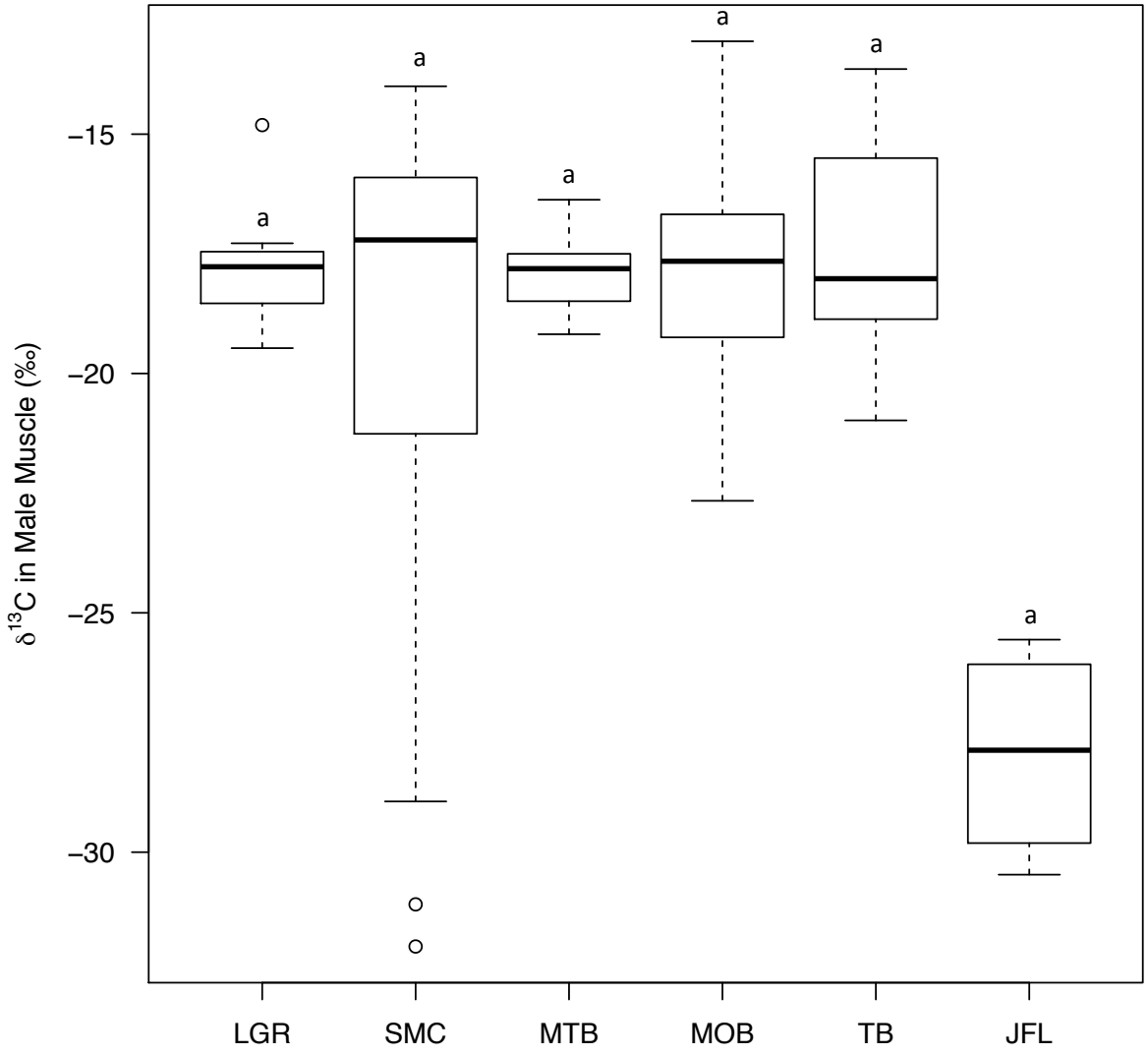


Figure 2.3: Box-plot of $\delta^{13}C$ in muscle tissue of male white sucker by site. LGR = Little Gravel River, SMC = Sawmill Creek, MTB = Mountain Bay, MOB = Moberly Bay, TB = Tunnel Bay, and JFL = Jackfish Lake. Letters signify statistically similar sites ($p < 0.05$).

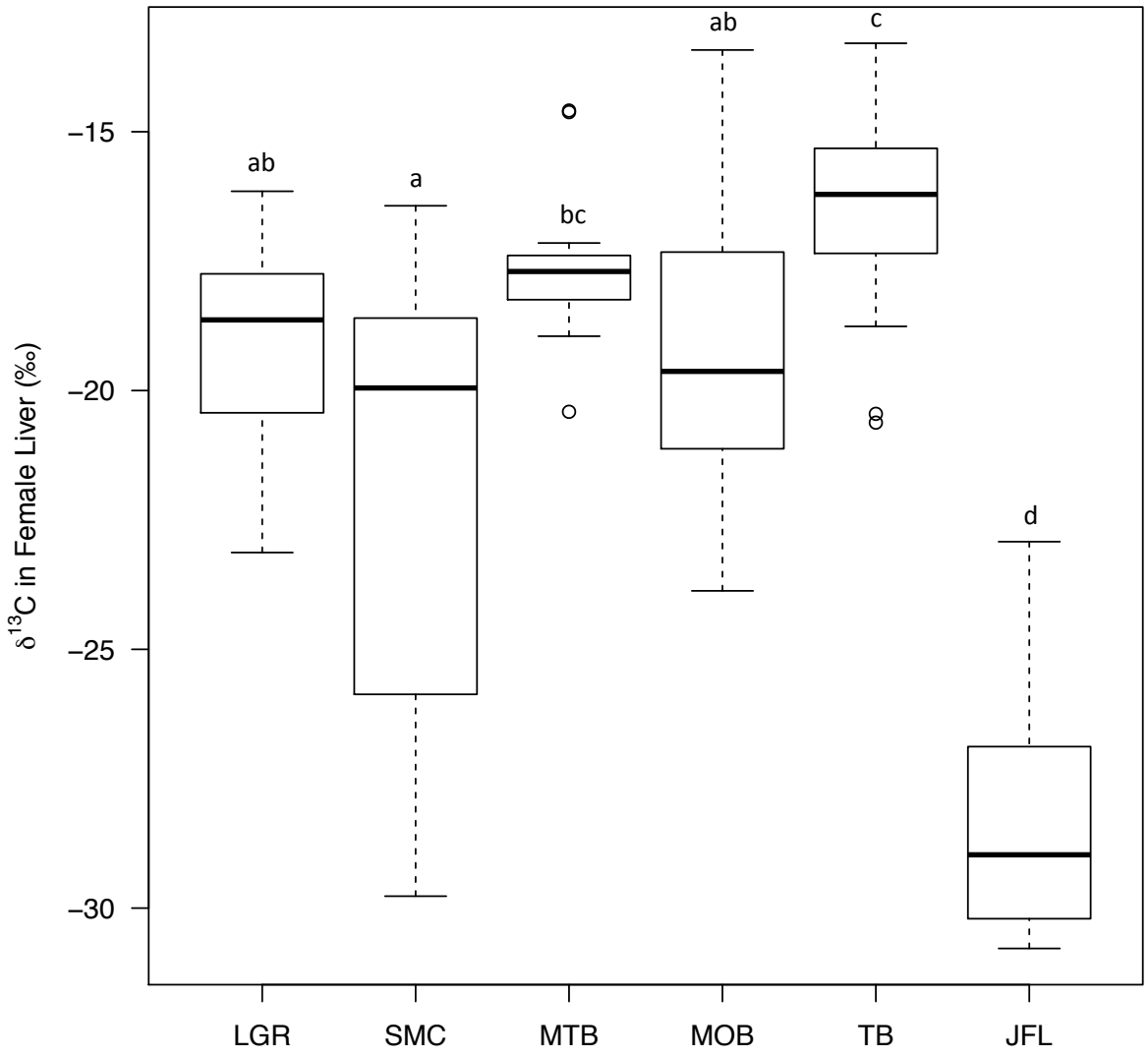


Figure 2.4: Box-plot of $\delta^{13}C$ in liver tissue of female white sucker by site. LGR = Little Gravel River, SMC = Sawmill Creek, MTB = Mountain Bay, MOB = Moberly Bay, TB = Tunnel Bay, and JFL = Jackfish Lake. Letters signify statistically similar sites ($p < 0.05$).

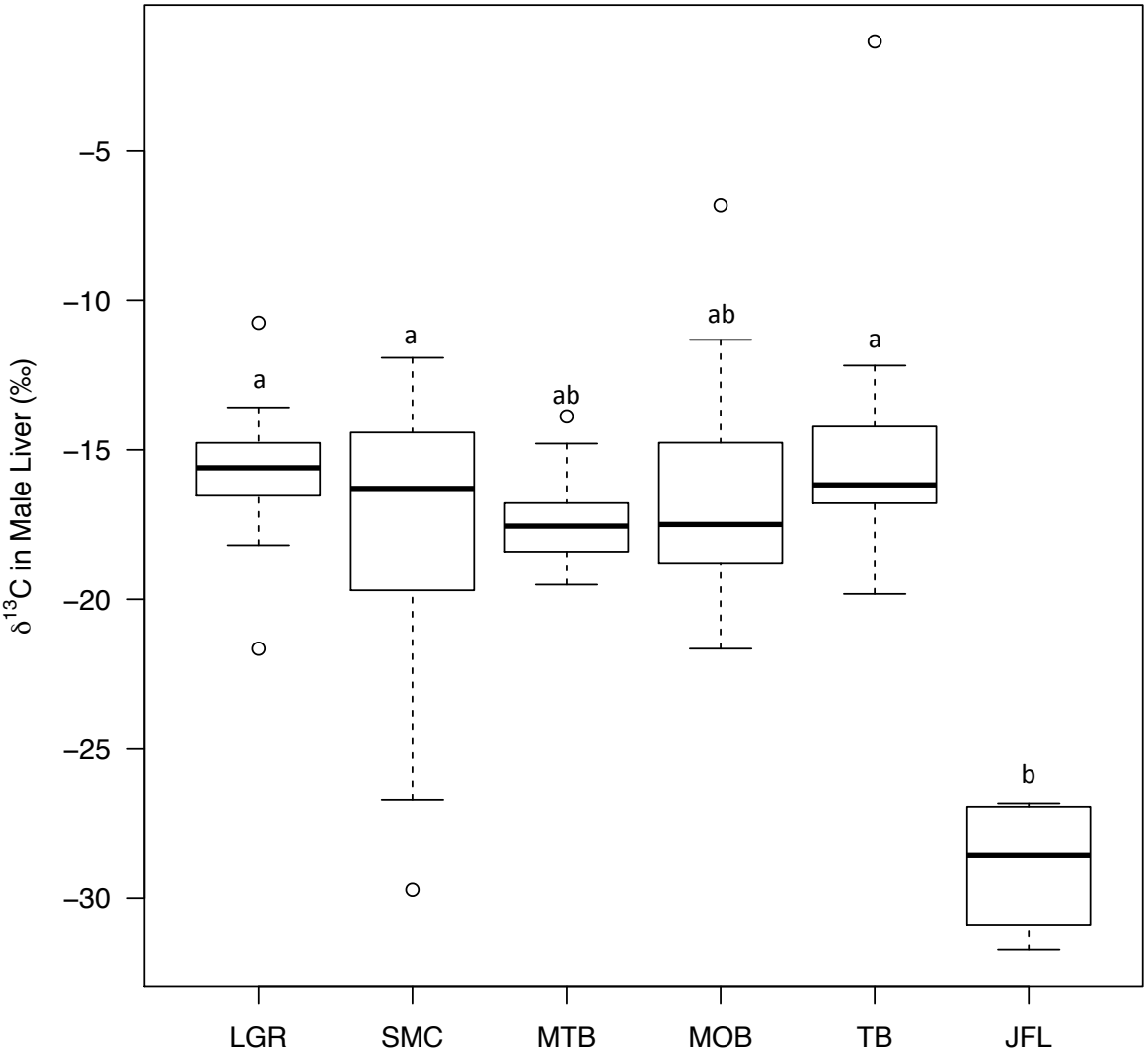


Figure 2.5: Box-plot of $\delta^{13}C$ in liver tissue of male white sucker by site. LGR = Little Gravel River, SMC = Sawmill Creek, MTB = Mountain Bay, MOB = Moberly Bay, TB = Tunnel Bay, and JFL = Jackfish Lake. Letters signify statistically similar sites ($p < 0.05$).

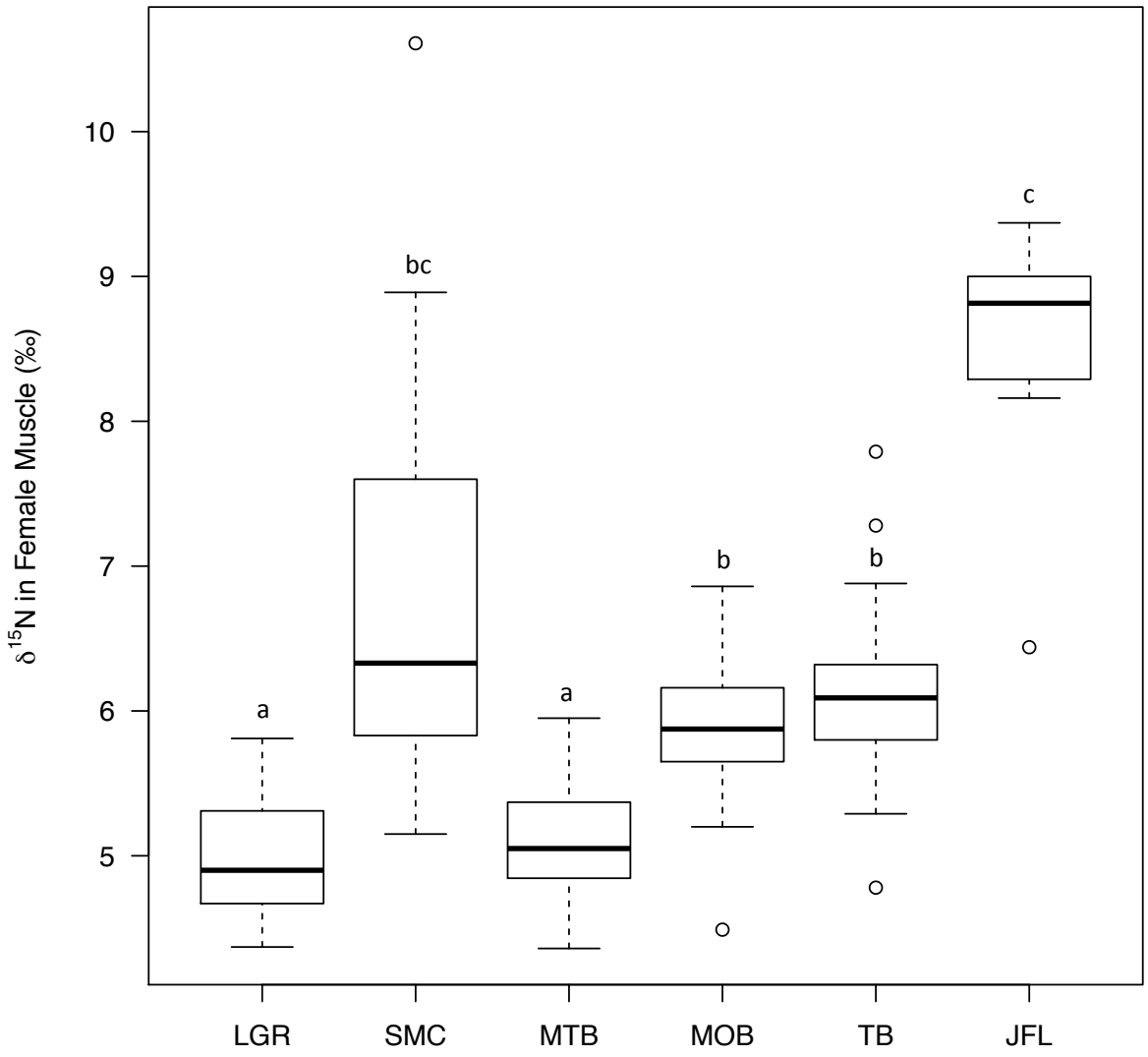


Figure 2.6: Box-plot of $\delta^{15}N$ in muscle tissue of female white sucker by site. LGR = Little Gravel River, SMC = Sawmill Creek, MTB = Mountain Bay, MOB = Moberly Bay, TB = Tunnel Bay, and JFL = Jackfish Lake. Letters signify statistically similar sites ($p < 0.05$).

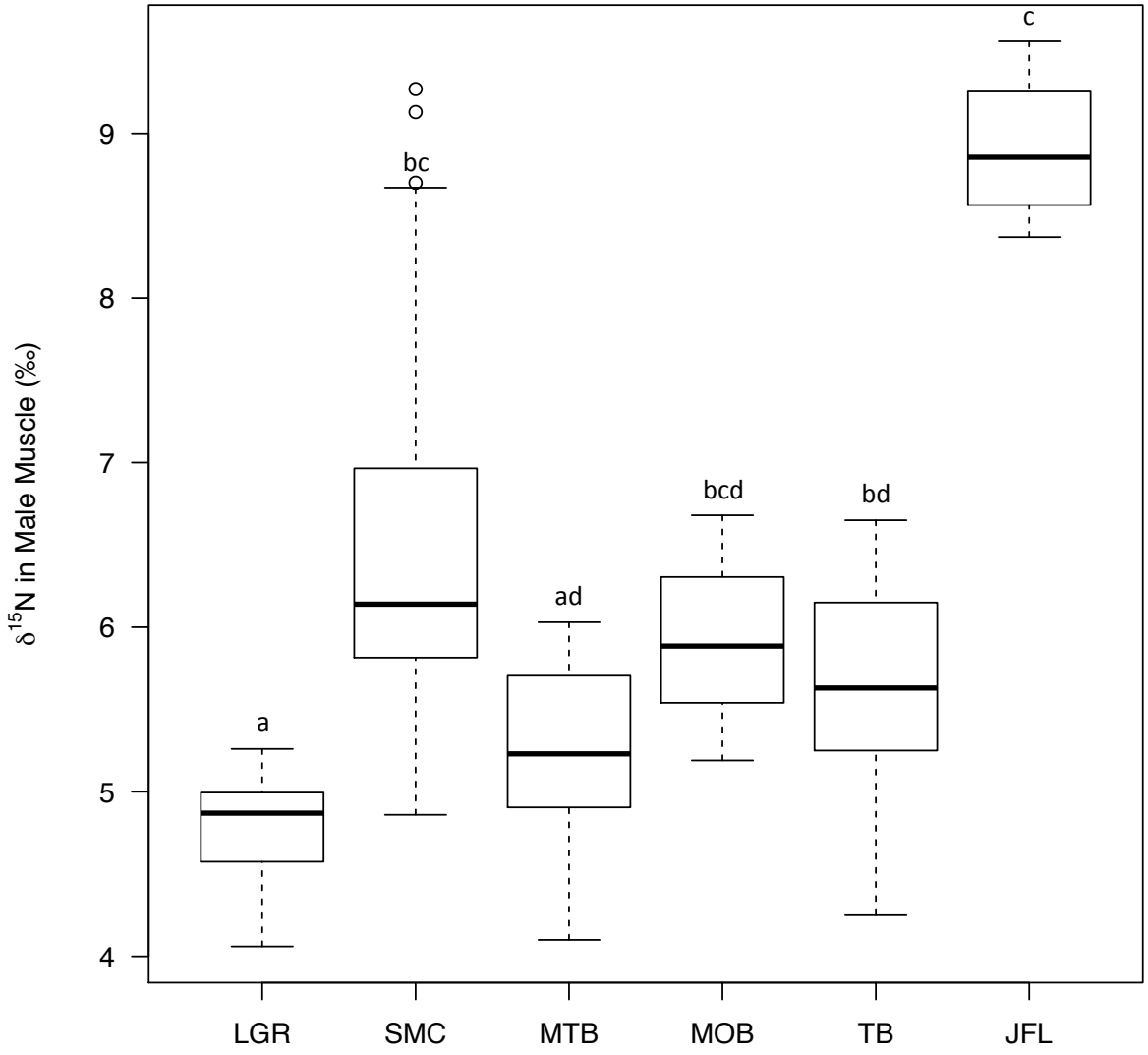


Figure 2.7: Box-plot of $\delta^{15}N$ in muscle tissue of male white sucker by site. LGR = Little Gravel River, SMC = Sawmill Creek, MTB = Mountain Bay, MOB = Moberly Bay, TB = Tunnel Bay, and JFL = Jackfish Lake. Letters signify statistically similar sites ($p < 0.05$).

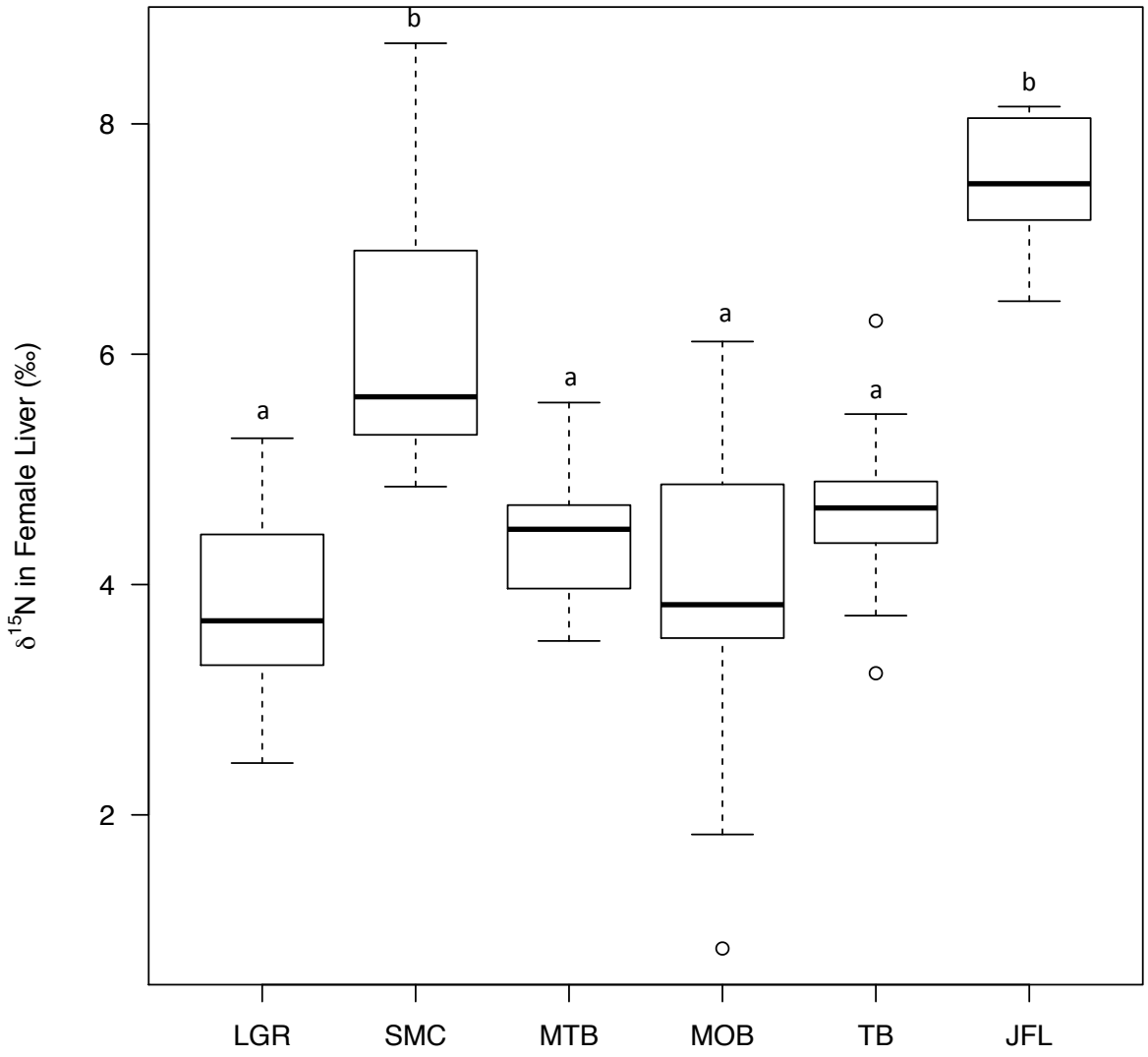


Figure 2.8: Box-plot of $\delta^{15}N$ in liver tissue of female white sucker by site. LGR = Little Gravel River, SMC = Sawmill Creek, MTB = Mountain Bay, MOB = Moberly Bay, TB = Tunnel Bay, and JFL = Jackfish Lake. Letters signify statistically similar sites ($p < 0.05$).

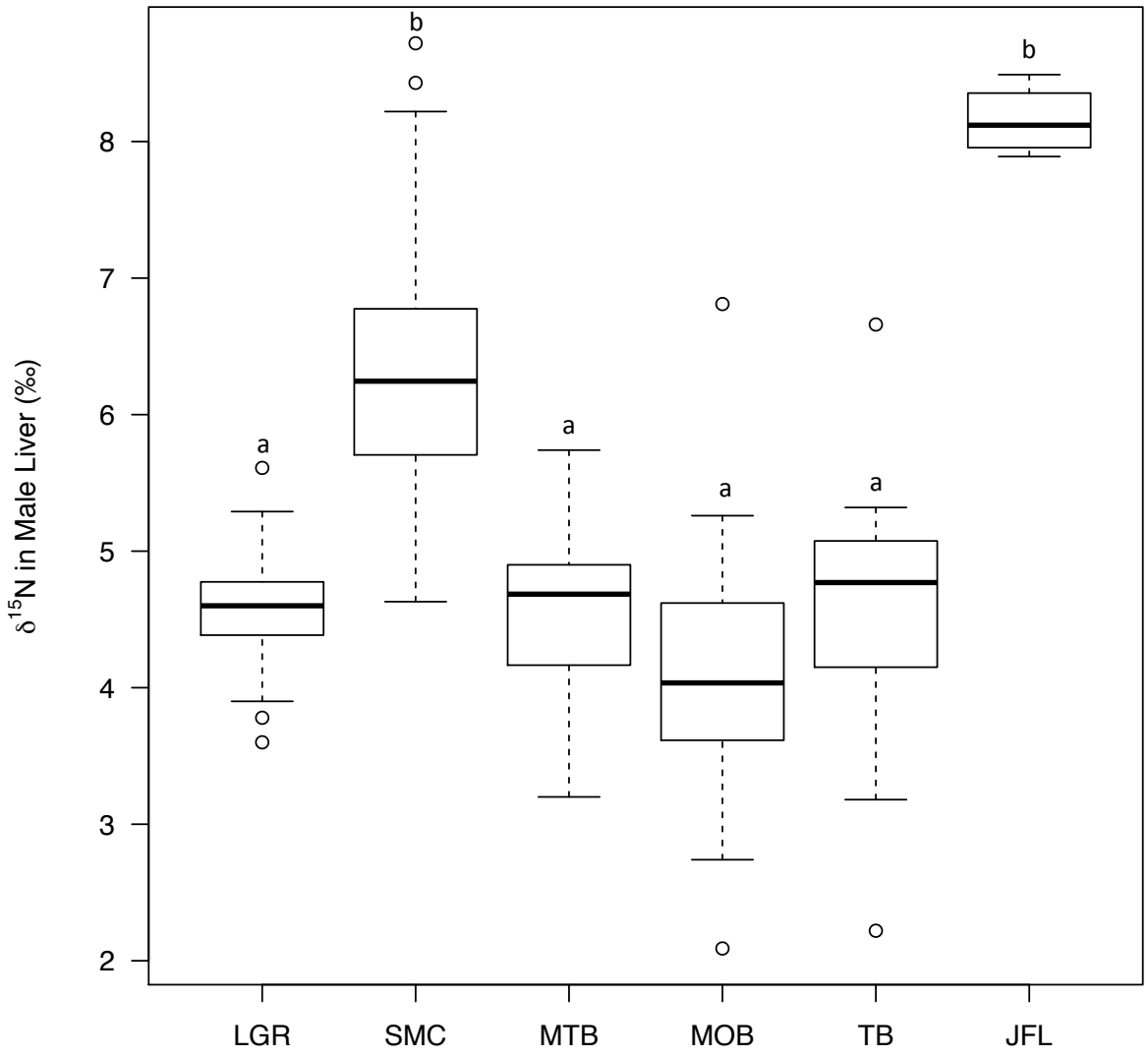


Figure 2.9: Box-plot of $\delta^{15}N$ in liver tissue of male white sucker by site. LGR = Little Gravel River, SMC = Sawmill Creek, MTB = Mountain Bay, MOB = Moberly Bay, TB = Tunnel Bay, and JFL = Jackfish Lake. Letters signify statistically similar sites ($p < 0.05$).

Table 2.4: $\delta^{13}C$ and $\delta^{15}N$ stable isotope average ± standard errors (n) of different samples at different sites during 2013. Comparisons were made among male and female white sucker liver and chironomini $\delta^{13}C$ and $\delta^{15}N$ stable isotope ratios. Capital letters signify similar $\delta^{13}C$ signatures among samples and sites while lower case letters signify similar $\delta^{15}N$ signatures among samples and sites.

| Sample | Stable Isotope | Mountain Bay (‰) | Moberly Bay (‰) | Tunnel Bay (‰) | Jackfish Lake (‰) |
|---|---|---|---|---|---|
| White sucker male liver | $\delta^{13}C$ | -17.39 ± 0.31 (20) AB | -16.67 ± 0.81 (20) A | -15.85 ± 0.46 (18) A | -28.92 ± 1.19 (4) C |
| | $\delta^{15}N$ | +4.61 ± 0.15 (20) ab | +4.14 ± 0.23 (20) ab | +4.62 ± 0.22 (18) ab | +8.15 ± 0.13 (4) f |
| White sucker female liver | $\delta^{13}C$ | -17.67 ± 0.29 (20) AB | -19.16 ± 0.61 (20) B | -16.48 ± 0.43 (20) A | -28.32 ± 0.70 (12) C |
| | $\delta^{15}N$ | +4.42 ± 0.11 (20) ab | +3.92 ± 0.30 (20) ac | +4.62 ± 0.15 (20) ab | +7.52 ± 0.15 (12) f |
| Chironomini | $\delta^{13}C$ | -17.73 ± 1.02 (8) AB | -27.61 ± 0.06 (9) C | -17.02 ± 0.46 (7) AB | -26.33 ± 0.69 (9) C |
| | $\delta^{15}N$ | +2.73 ± 0.59 (8) cd | -0.27 ± 0.08 (9) e | +1.52 ± 0.08 (7) d | +5.39 ± 0.10 (9) b |
| *Hyalella* spp. | $\delta^{13}C$ | -16.05 ± 0.53 (9) | N/A | -18.82 ± 0.31 (9) | -26.19 ± 0.24 (9) |
| | $\delta^{15}N$ | +1.59 ± 0.14 (9) | N/A | +0.70 ± 0.14 (9) | +3.34 ± 0.07 (9) |
| *Caecidotea* spp. | $\delta^{13}C$ | N/A | -27.21 ± 0.21 (4) | -16.49 ± 0.57 (3) | N/A |
| | $\delta^{15}N$ | N/A | -2.97 ± 0.31 (4) | +1.05 ± 0.48 (3) | N/A |
| *Pisidium* spp. | $\delta^{13}C$ | -17.39 ± 1.67 (8) | -24.34 ± 2.57 (2) | -25.71 ± 0.78 (9) | N/A |
| | $\delta^{15}N$ | +1.45 ± 0.31 (5) | -1.09 ± 0.90 (2) | +1.55 ± 0.11 (9) | N/A |
| *Valvata* spp. | $\delta^{13}C$ | -15.41 ± 0.78 (9) | -26.74 (1) | -16.81 ± 0.45 (9) | -26.41 ± 0.38 (9) |
| | $\delta^{15}N$ | +2.16 ± 0.17 (9) | -2.29 (1) | +0.85 ± 0.26 (9) | +4.16 ± 0.05 (9) |
| Zooplankton | $\delta^{13}C$ | -26.55 (1) | -24.69 ± 1.17 (3) | -24.52 ± 1.06 (3) | -29.82 ± 1.86 (3) |
| | $\delta^{15}N$ | +1.79 (1) | +11.90 ± 0.13 (3) | +4.08 ± 0.38 (3) | +8.22 ± 0.72 (3) |
| Particulate Matter | $\delta^{13}C$ | -19.93 ± 0.34 (3) | -26.91 (1) | -28.06 ± 0.31 (3) | -33.61 ± 0.25 (3) |
| | $\delta^{15}N$ | +4.84 ± 1.04 (3) | +1.98 (1) | +2.56 ± 0.72 (3) | +4.61 ± 1.14 (3) |

*N/A indicates data not available.

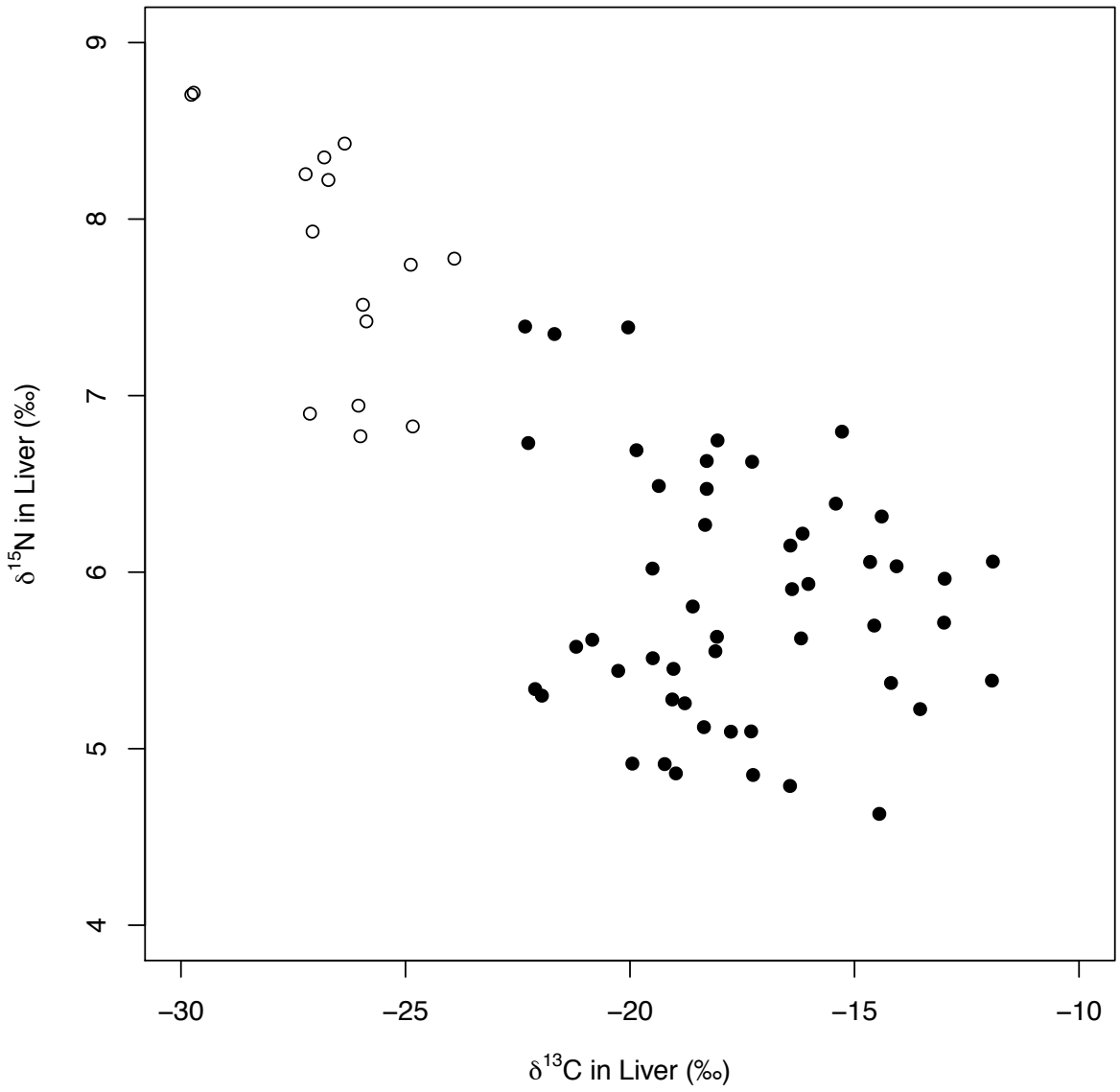


Figure 2.10: Dual isotope plot of $\delta^{13}C$ by $\delta^{15}N$ in liver of white sucker at Sawmill Creek during May 2013. Ward Hierarchal Clustering differentiated groups. Open circles represent group 1, while solid circles represent Group 2.

Table 2.5: Age, length, weight, condition factor (k), gonadic somatic index (GSI), and liver somatic index (LSI) of male and female white sucker (*Catostomus commersonii*) from groups created by Ward Hierarchal Clustering at Sawmill Creek. Values are reported as mean ± SE (n). Uppercase letters signify similarities among groups for males while lowercase letters signify similarities between groups for females ($p < 0.05$).

| Sex | Group | Age (year) | Length (cm) | Weight (g) | GSI[a] | LSI[b] | $k$[c] |
|---|---|---|---|---|---|---|---|
| Male | Group 1 | 5.0 ± 1.3 (5) A | 34.0 ± 0.4 (5) A | 547.1 ± 22.6 (5) A | 4.76 ± 0.20 (5) A | 1.38 ± 0.08 (5) A | 1.39 ± 0.01 (5) A |
| | Group 2 | 8.1 ± 0.6 (27) B | 39.9 ± 0.5 (27) B | 904.4 ± 37.2 (27) B | 5.05 ± 0.15 (27) A | 1.54 ± 0.04 (27) A | 1.40 ± 0.02 (27) A |
| Female | Group 1 | 7.1 ± 0.7 (10) a | 40.1 ± 1.1 (10) a | 963.4 ± 96.4 (10) a | 12.26 ± 0.84 (10) a | 1.61 ± 0.09 (10) a | 1.46 ± 0.04 (10) a |
| | Group 2 | 10.0 ± 0.5 (23) b | 45.0 ± 0.5 (23) b | 1411.0 ± 51.0 (23) b | 14.43 ± 0.29 (23) a | 1.93 ± 0.05 (23) a | 1.54 ± 0.02 (23) a |

[a]GSI = (gonad weight/body weight) x 100.

[b]LSI = (liver weight/body weight) x 100.

[c]$k$ = (weight/length$^3$) x 100.

## 2.5 Discussion

The combination of fish population endpoints, MFO activity, and stable isotopes in muscle and liver tissue (along with supporting benthic invertebrate data) distinguish groups of fish from Mountain Bay, Moberly Bay, Tunnel Bay, and Jackfish Lake. Only minor differences in fish physiological endpoints could be determined (age, condition) between Moberly Bay and the reference site Mountain Bay despite large differences being reported historically in several endpoints including relative gonad and liver size (Bowron *et al.*, 2009). Although fish in Moberly Bay had higher MFO activity than fish in Tunnel Bay, suggesting greater exposure to effluent, physiological effects could not be distinguished, possibly due to their mobility and mixing of the population. Stable isotopes of either carbon or nitrogen in muscle tissue were also unable to distinguish between fish collected from Moberly Bay and Tunnel Bay. Differences in fish endpoints during spring migration at Sawmill Creek may be confounded by the mixing of fish from Jackfish Bay and Jackfish Lake, which had markedly different isotopic signatures in fish during the fall collections. Isotopic signatures may allow the fish from these populations to be separated and aid in the interpretation of effects data associated with specific fish populations. Although white sucker collected during the spawning run in Sawmill Creek could be separated into two groups by stable isotope signatures, they had similar population endpoints to Jackfish Lake white sucker, and were younger and smaller than Lake Superior fish.

*Population Characteristics*

In the current study, white sucker collected at the Moberly Bay site which directly received effluent had differences in age and condition but not length, weight, and gonad and liver somatic indices relative to the reference site in Lake Superior (Mountain Bay). Fish collected at the pulp mill effluent site (Moberly Bay) were younger and males had higher condition than sites not exposed to effluent (Mountain Bay). Gibbons and Munkittrick (1994) suggested that fish with these trends are possibly undergoing exploitation or metabolic redistribution. In past studies in Jackfish Bay, male white sucker were younger

than or of a similar age to males at Mountain Bay since 1988, despite changes and upgrades to mill operations. Until 1995, female white sucker at Moberly Bay were older than or similar in age to those at Mountain Bay. , After 1995, female white sucker from Moberly Bay were younger than or the same age as female white sucker from Mountain Bay, which coincides with the updated chlorine dioxide generator and prevention of bypassed untreated effluent from the aerated stabilization basin (Bowron *et al.*, 2009). In 2006, after upgrades to the mill, including secondary treatment and a shift to chlorine dioxide bleaching, exposed fish exhibited younger ages and higher condition in both genders, with males being heavier and females being shorter and having smaller ovaries than their counterparts at Mountain Bay (Bowron *et al.*, 2009).

Populations with young fish can indicate chronic mortality (Gibbons & Munkittrick, 1994; Munkittrick & Dixon, 1989), which may be a result of effluent exposure and historical contamination in sediments at Jackfish Bay. Increased condition has been observed in fish collected at Moberly Bay relative to Mountain Bay since studies began in 1988 (Bowron *et al.*, 2009). Increased condition could be due to increased nutrients, higher food availability, or changed habitat (Gibbons & Munkittrick, 1994).

There were no physiological differences (age, length, weight, LSI, GSI, *k*) between white sucker from Moberly Bay and those from Tunnel Bay, a site that was assumed to have much lower effluent exposure. Fish could be moving between Moberly Bay and Tunnel Bay, or the decreased amount of effluent at Tunnel Bay that the fish are exposed to may result in the same degree of physiological impacts (or lack of response) as those in Moberly Bay. The extent of temporal and spatial movements of white sucker at these sites is unknown, and therefore exposure to effluent is unclear. White sucker are large bodied and known to move considerable distances, especially during migration for spawning purposes (Doherty *et al.*, 2010; Freedman, 2005; Logan *et al.*, 1991). There is no major barrier for movement of white sucker between Moberly Bay and the adjacent Tunnel Bay, therefore fish could be moving freely between the exposed and relative unexposed sites within these two bays. Tunnel Bay is known to have at

least partial exposure to the pulp mill effluent even though the effluent plume has been shown to predominately extend along the northwest shore of Jackfish Bay (Comba *et al.*, 1994; Kovacs *et al.*, 2013). Therefore it is unclear if Moberly Bay and Tunnel Bay fish are mixing together as a single population, or if they are two different populations that share a similar profile despite differing effluent exposure.

Decreased length, weight, and relative liver weight of white sucker were observed in fish collected from Jackfish Lake relative to the sites in Lake Superior. It is not known if fish in Jackfish Lake are resident to the lake or move freely into Lake Superior (through the very shallow railway tunnel). These fish are likely to use Jackfish Lake as a long-term residence, rather than a temporary nursery, since they are the same age and have similar relative gonad size as fish collected in Lake Superior, despite being shorter and lighter. Chalanchuk (1998) found there was no relationship between fish length and size of lake (surface area, volume, depth), although white sucker grow faster in lakes with predators present than in lakes without predators. Decreased relative liver weight was not due to lack of exposure to effluent, since fish from Mountain Bay had similar liver sizes to Jackfish Bay fish. Decreased relative liver size could be a function of habitat and food availability, where fish deplete glycogen stores in the liver when there is decreased food availability (Gibbons & Munkittrick, 1994). Benthic collection efforts support decreased food availability at Moberly Bay, and is supported by previous research (Sibley *et al.*, 2001).

The optimal temperature for white sucker growth is 19 to 26°C (Koenst &Smith Jr., 1982), which would infer that Jackfish Lake, which is warm and shallow, would have the fastest growing fish. However, Jackfish Lake fish were smaller at age than fish in Lake Superior. This may be supported by the fact that white sucker exposed to lower light intensities grow faster (Koenst & Smith Jr., 1982). This may be an important factor for white sucker growing in the effluent plume where light intensity is lowest.

The effort to catch white sucker was much higher in Jackfish Lake than at the Lake Superior sites. This is likely due to better habitat in Lake Superior as white sucker prefer depths of 2 to 15 m, while Jackfish Lake was at a maximum of 4 m deep. In addition, a high density of piscivores in Jackfish Lake, and competition with other fish species for food and habitat may have increased competition in white sucker, which seem to have matured at a younger and smaller life stage than its Lake Superior counterparts.

*MFO activity*

MFO activity is a useful biomarker of effluent exposure since it gives a rapid response to inducing agents, is contaminant concentration dependent, and is not strongly affected by capturing methods. MFO in fish has been shown to be induced by a variety of environmental contaminants, including polycyclic aromatic hydrocarbons, polychlorinated biphenyls, dibenzo-*p*-dioxins and dibenzofurans and many others (Hewitt *et al.*, 2000). MFOs ultimately add polar groups to the contaminants, increasing the water solubility of the aromatic and relatively lipophilic compounds, therefore increasing their elimination. This study confirms that MFO activity, measured as EROD induction, is still elevated in exposed fish relative to reference fish during gonadal recrudescence, as observed in past studies from 1990 to 2000 despite upgrades and changes to mill operation (Bowron *et al.*, 2009; McMaster *et al.*, 1991; Munkittrick & McCarty, 1995; Munkittrick *et al.*, 1991; Munkittrick *et al.*, 1992a; Oakes *et al.*, 2003; Parrott *et al.*, 2000). EROD induction was elevated at sites exposed to effluent relative to sites not receiving pulp mill effluent loadings during the fall period. EROD activity in fish at Moberly Bay was elevated compared to sites without effluent exposure (7 to 11.5 fold), and Tunnel Bay with less exposure (2 to 3 fold). A survey of pulp mills across Canada found that MFO was induced in kraft mills that had either bleached or unbleached effluents, and that replacement of chlorine for bleaching did not eliminate EROD activity in exposed fish (Martel *et al.*, 1994). There is a suggestion that the kraft process may be responsible for creating MFO-inducing compounds. Ongoing research is

attempting to isolate MFO-inducing compounds in effluent, but causative agents remain elusive at Jackfish Bay and other sites (Hewitt *et al.*, 2006; Hodson *et al.*, 1996; Parrott *et al.*, 2006). Recent papers have shown that EROD induction has declined in many pulp mill effluents (Chiang *et al.*, 2011; Ratia *et al.*, 2014). Although the elevation of EROD activity in fish is not large in Moberly Bay (<11.5 fold) and Tunnel Bay (<3 fold), this change suggests that MFO inducing compounds still remain bioavailable in effluent or the environment. Mountain Bay and Jackfish Lake, both of which are not exposed to effluent, shared similar baseline levels of EROD activity. EROD induction in white sucker could occur due to effluent as well as historical contamination in sediments. However these levels decline rapidly (within days) once they move away from the effluent and during changes in seasonal physiology (i.e., spawning versus gonadal recrudescence (Munkittrick *et al.*, 1992b).

It is possible that the fish in the two main bays of Jackfish Bay are two distinct groups during the summer. Tunnel Bay fish may experience low exposure to effluent which slightly elevates their EROD activity relative to the highly exposed reference site (Mountain Bay). Tunnel Bay has been reported to receive 1% effluent when water and wind currents move the effluent plume away from the west shore of Moberly Bay (Comba *et al.*, 1994; Kovacs *et al.*, 2013). Dahmer *et al.* (2015) found that both surface sediment and livers of white sucker from both Moberly Bay and Tunnel Bay were contaminated with polychlorinated dibenzo dioxins and furans. Milani and Grapentine (2009) also found that benthic invertebrates in Tunnel Bay were contaminated with dioxins and furans and dioxin-like polychlorinated biphenyls. The lack of difference between the Jackfish Bay sites may be a result of minimal exposure to the effluent due to movement of the fish, or a lack of difference in the isotopic signatures caused by the effluent.

*Stable Isotope Analyses*

Carbon stable isotope analyses were not able to detect a difference in liver and muscle of white sucker of both genders between Moberly Bay and Mountain Bay. This suggests that either the effluent does not have a distinct signature from background levels, the two ecosystems have different baseline signatures and effluent-induced fractionation makes them similar, or that white sucker at Moberly Bay are not incorporating effluent-derived carbon. Nitrogen stable isotope analyses detected a difference between Moberly Bay and Mountain Bay only in the muscle of females.

Stable isotope signatures in muscle also did not differ between Moberly Bay and the immediately adjacent Tunnel Bay. Enriched carbon signatures were observed in female livers at Tunnel Bay in comparison to Moberly Bay. This supports that there are minimal changes in signatures of stable isotopes of white sucker at these sites compared to reference condition in Lake Superior. Fish from Tunnel Bay and Moberly Bay might move easily across Jackfish Bay and may represent the same population.

Stable isotopes can be an effective indicator of diet due to long-term integration of stable isotope ratios in fish tissue. Stable isotope ratios from the environment are incorporated over time in fish tissue depending on the rate of growth in the tissue and metabolism of the fish. In temperate lakes, ectotherms usually exhibit discontinuous growth over the years (Perga & Gerdeaux, 2005). Muscle tissue has a slower turnover rate than liver, and reflects only somatic growth during months that have warmer temperatures and higher food availability. Constant protein turnover in liver would reflect somatic growth as well as isotopic routing into gonadic growth and routine metabolism (Perga & Gerdeaux, 2005). Turnover rates in tissues of various fish have been previously studied (Hesslein *et al.*, 1993; Sweeting *et al.*, 2005), but rates of tissue turnover in specifically white sucker have yet to be quantified. Differences in stable isotope ratios in muscle tissue are more likely to represent long-term ecological difference over months and years while difference in stable isotope ratios in liver tissue are more likely to represent short term changes over a matter of weeks.

There was minimal seasonal variation in carbon and nitrogen isotopes in reference fish, so fasting during migration and resource-partitioning of metabolically active liver to gonad tissues was not picked up by isotopes. Therefore differences between spawning fish at Sawmill Creek and gonad recrudescent fish at Moberly is possibly explained by the mixing of Jackfish Bay fish and the carbon-depleted and nitrogen-enriched Jackfish Lake fish.

Particulate matter levels of $^{13}C$ and $^{15}N$ were very depleted at sites exposed to effluent compared to the reference site. The isotopic signature at exposed sites is more similar to terrestrial signatures, and may be caused by effluent contributions. Jackfish Lake had highly depleted $^{13}C$ and enriched $^{15}N$ compared to Moberly Bay and Tunnel Bay, likely due to the high cycling of nutrients in shallow, warm, small Jackfish Lake with more allochthonous inputs. However, isotopic signatures of particulate matter can be highly variable due to the high turnover of bacteria and plankton communities and the sample size of this study was too small to conduct statistical analyses.

Chironomids had depleted carbon isotope ratios at Moberly Bay and Jackfish Lake relative to Mountain Bay, while chironomids from Tunnel Bay had similar signatures to those from Mountain Bay. The depletion in $^{13}C$ in chironomids from Moberly Bay is likely due to depleted $^{13}C$ in pulp mill effluent or anoxic conditions. Therefore it would be expected that white sucker liver tissue would be similar to chironomid carbon ratios. Carbon isotope ratios in liver of white sucker reflected carbon isotope ratios in chironomids at each site except at Moberly Bay. This suggests the white sucker collected in Moberly Bay have been consuming a different food source than the invertebrates found in Moberly Bay. White sucker may feed differently at this site or may not have fed at this site long enough to retain the isotopic signature of its invertebrates. Therefore, carbon isotope ratios in white sucker at Moberly Bay may not be indicative of effluent exposure, and chironomids and other invertebrates may be better sentinels of ecosystem exposure. Due to their low mobility and fast growth, chironomids and other benthic

invertebrates are more likely to have a more distinctive stable isotope signature that reflects the effluent exposure than white sucker.

Depletion of $^{13}C$ in chironomids from Jackfish Lake is likely due to changes in the nutrient cycling in the warm shallow environment. Stable isotopes of nitrogen were depleted in chironomids from Moberly Bay and enriched in chironomids from Jackfish Lake relative to Mountain Bay and Tunnel Bay chironomids. Baseline isotope ratios in Jackfish Lake are depleted in $^{13}C$ and enriched in $^{15}N$, which is reflected by ratios in white sucker liver. Post (2002) found a positive relationship between lake area and $\delta^{13}C$. Jackfish Lake is considerably smaller and shallower than Jackfish Bay.

Sampling efforts were higher and invertebrate diversity was lower at Moberly Bay compared to all other sites. More substrate samples were collected to obtain invertebrates at Moberly than other sites, and the majority of invertebrates were highly pollution tolerant blood chironomids. This was likely due to the steep bathymetry of Moberly Bay, presence of sandy substrate, and the presence of a thick, tar-like organic deposition in the deeper water past the sandy substrate at the mouth of Blackbird Creek. The continued presence of pulp mill effluent, or historical sediment contamination could potentially be affecting the invertebrate community at Moberly Bay. Food may be more limited in Moberly Bay and suckers may therefore forage in other less contaminated locations (e.g., Tunnel Bay).

*Implications for collections during prespawning at Sawmill Creek*

The only physiological response that was consistent throughout prespawning and gonadal recrudescence was condition in males, which was higher at the exposed site than the reference site. The inconsistency in observed effects between prespawning and gonadal recrudescent fish may be a combination of reproductive stage, season, sampling method (hoop net versus size-selective gill net) and the addition of fish with varying exposure at Sawmill Creek. Previously significant differences between Little Gravel River and Sawmill Creek have been reported in age, length, weight, LSI, GSI and condition

in male prespawning fish (Bowron *et al.*, 2009). However, condition was the only population endpoint at Sawmill Creek that was consistently significantly different than Little Gravel River every year (Bowron *et al.*, 2009). It would appear that there is considerable recovery of physiological endpoints in the spring collections in 2007 (Bowron et al., 2009). As the level of effect has declined, subtle differences may be masked by the mixing of the Jackfish Lake fish with those of Jackfish Bay (Moberly Bay and Tunnel Bay).

White sucker at Mountain Bay maintained a similar stable isotope signature to the spawning fish at Little Gravel River. White sucker collected from Sawmill Creek had an enriched nitrogen signature in liver of both males and females compared to fish collected from Moberly Bay in the fall. This difference may be explained by fish from multiple sites mixing at Sawmill Creek during the spawning run. Indeed, Jackfish Lake males and females also have an enriched liver nitrogen signature. Using Ward Hierarchical analysis to examine the stable isotope ratios in white sucker spawning in Sawmill Creek, two different groups were distinguished. Group one had fish that were younger, shorter, and lighter in both males and females relative to group two.. $^{15}N$ enriched and $^{13}C$ depleted white sucker from Jackfish Lake may be included in the spring samples collected at Sawmill Creek. Based on the isotopic analyses, up to 23% of the fish caught at Sawmill Creek are not exposed to pulp mill effluent (from Jackfish Lake). This finding is similar to van den Heuvel *et al.* (1995) who reported that 15 to 20% of white sucker at Sawmill Creek were not exposed to pulp mill effluent based on extractable organic halogen (EOX) in muscle of white sucker.

*Conclusions*

In conclusion, the most consistent response in white sucker to exposure to effluent was increased condition relative to fish caught at the reference site (Mountain Bay). Moberly Bay fish were expected to be a distinct population due to the exposure to effluent in the bay but did not have distinct population

responses (e.g., somatic indices) or stable isotope ratios. MFO activity was highest in fish collected at Moberly Bay and decreased in fish at the adjacent Tunnel Bay site possibly due to fish movements and low current or historical (sediment) exposure to effluents. Stable isotope ratios of carbon in benthic invertebrates were associated with signatures observed in white sucker liver at all sites except Moberly Bay where fish may feed differently or travel throughout all of Jackfish Bay. Determining effects in fish collected at Sawmill Creek during spring may be confounded by mixing of populations from various sites, with two groups of fish with population and isotopic characteristics that are different. Up to 23% of fish may be from the unexposed but ecologically different Jackfish Lake. Although these fish may skew the results from the main group of fish that are assumed to reside in Jackfish Bay (Moberly Bay and Tunnel Bay) for some endpoints (e.g., age, weight), they did not differ in the somatic indices. Inclusion of the potentially unexposed fish with exposed fish during the spawning in Sawmill Creek has the capacity to partially mask the effects of effluent at this site or falsely suggest changes that are not associated with effluent exposure. Future studies that incorporate spawning fish in the spring should carefully consider sample size and confirmation techniques due to the mixing of fish populations from different locations in the spawning location.

# Chapter 3

# Stable isotope variation in white sucker (*Catostomus commersonii)* during a short term closure of the pulp mill discharging into Jackfish Bay, Lake Superior

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- Mark McMaster: Co-supervisor of Jessica Mendoza. Assisted with fieldwork, research direction, and provided general advice;
- Mark Servos: Co-supervisor of Jessica Mendoza. Assisted with fieldwork, research direction, and providing general advice.

## 3.1 Summary

The pulp mill in Terrace Bay, Ontario that discharges into Jackfish Bay, Lake Superior, had an unscheduled shutdown for almost a year between October 2011 and September 2012. This created a unique opportunity to study the influence of pulp mill effluent on stable isotope signatures ($\delta^{13}C$, $\delta^{15}N$) in the receiving environment. White sucker (*Catostomus commersonii*) were sampled before, during, and after the shutdown during the fall and spring at exposed and reference sites. A suite of population endpoints, and MFO activity were also measured in white sucker at each site. In addition, macroinvertebrates were sampled for stable isotope analyses during August 2012 and August 2013 to understand pulp mill exposure at lower levels of the white sucker food chain. During the shutdown period isotopic signatures in invertebrates were similar or depleted compared to reference invertebrates but when the mill resumed operation, invertebrates became much more depleted in $^{13}C$. $\delta^{15}N$ in Chironomini and other invertebrates was enriched in 2012 during the shutdown but depleted in 2013. Muscle of both male and female white sucker were consistently enriched in $^{15}N$ compared to muscle of reference fish, except males in 2013. Liver showed a similar response in 2011 (pre-shutdown) but in fall 2012 (shutdown) and 2013 (operation resumed), liver $\delta^{15}N$ in exposed males was depleted to levels more similar to that of reference male livers. The $^{13}C$ signature difference in exposed invertebrates did not reflect exposed fish, while reference fish and invertebrates had similar signatures, and this difference was further amplified during mill operation in 2013. This suggests that exposed fish are not feeding directly on the invertebrates at this site and may move elsewhere to obtain food. MFO activity measured as EROD showed weak but detectable changes in response to effluent exposure. However, population level changes in white sucker, such as relative gonad size, did not change during the period of mill shutdown. Stable isotopes may be a very useful tool to detect subtle changes in the environment in response to pulp mill effluents but careful consideration of tissue turnover rates, organism life-cycle and movement needs to be considered.

## 3.2 Introduction

The reproductive and metabolic impacts on white sucker (*Catostomus commersonii*) exposed to the pulp mill discharging into Jackfish Bay, Lake Superior, have been well studied since the late 1980s, and include smaller gonad sizes, reduced secondary sex characteristics, lower circulating reproductive steroid hormones, and elevated mixed function oxygenase (MFO) induction (McMaster *et al.*, 1991; Munkittrick *et al.*, 1991). Process changes and treatment upgrades at the pulp mill have greatly improved the quality of effluent discharging into Jackfish Bay over the past several decades. There has been at least a partial recovery in many biological endpoints including MFO activity and relative gonad sizes of white sucker compared to reference sites (Bowron *et al.*, 2009; Munkittrick *et al.*, 1992b). Studies conducted during short-term shutdowns of the mill, or removal experiments, also demonstrated rapid recovery of many of the biological endpoints, such as MFO activity, supporting the conclusion that the responsible chemicals in later years were derived primarily from continuous effluent inputs rather than historical contamination of sediments (Munkittrick *et al.*, 1992b). In addition, historical contamination of fish associated with polychlorinated dibenzo-*p*-dioxins is approaching background levels even though there is localized sediment contamination (Dahmer *et al.*, 2015). Despite this apparent recovery, current mill operation and potential future changes in the mill processes continue to raise concerns for the receiving environment. An operational issue at Terrace Bay pulp mill caused the mill to be shut down between October 2011 and September 2012, creating an opportunity to examine the potential of the pulp mill effluent to continue to cause changes in the receiving environment and recovery.

Studies of stable isotopes of carbon and nitrogen have not yet been applied to Jackfish Bay even though this technique has the potential to detect subtle changes in nutrient and energy cycling in the environment (Peterson & Fry, 1987). The current study takes advantage of the unique opportunity of a mill shutdown and restart to contrast the responses in stable isotope ratios in the fish population to the removal of continuous effluent inputs. The input of effluent into an isolated bay of oligotrophic Lake

Superior has the potential to alter the nutrient and food web dynamics in the nearshore and therefore the stable isotope signatures of carbon and nitrogen present there. Stable isotopes in the receiving environment are likely to be altered as effluent is primarily derived from terrestrial sources of production (i.e., trees), undergoes considerable treatment, and has a potential to alter isotopic fractionation. Distinct isotopic signatures in biota in the receiving environment can therefore be used to trace subtle changes and exposure to effluent. Stable isotopes were analyzed in white sucker and invertebrates in Moberly Bay, and a reference site, Mountain Bay, from 2011 to 2013. Since tissues in organisms can turn over at different rates, both muscle and liver were examined in white sucker tissues. In addition, MFO induction and population endpoints in white sucker were collected to determine changes in exposure and effects from pulp mill effluent over a three year period that included the year long mill closure.

## 3.3 Methods

### 3.3.1 Study Site

The pulp mill in Terrace Bay directly channels its effluent into Blackbird Creek, which can consist of 65 to 95% effluent by volume during the summer to winter periods (Munkittrick *et al.*, 1992b). With a drainage area of 62 $km^2$, the creek runs for 14 km before flowing into Jackfish Bay, an isolated bay on the north shore of Lake Superior (Stewart & Rashid, 2011). Exposed fish were collected at Moberly Bay (48°50'N, 86°58'W), which has no domestic or industrial effluent except the effluent it receives via Blackbird Creek from 2011 to 2013. Reference fish were collected from Mountain Bay (48°56'N, 87°50'W), 60 km west of Jackfish Bay, which has no industrial or municipal effluent inputs. During spring, prespawning exposed and reference fish were collected at Sawmill Creek and Little Gravel River, respectively.

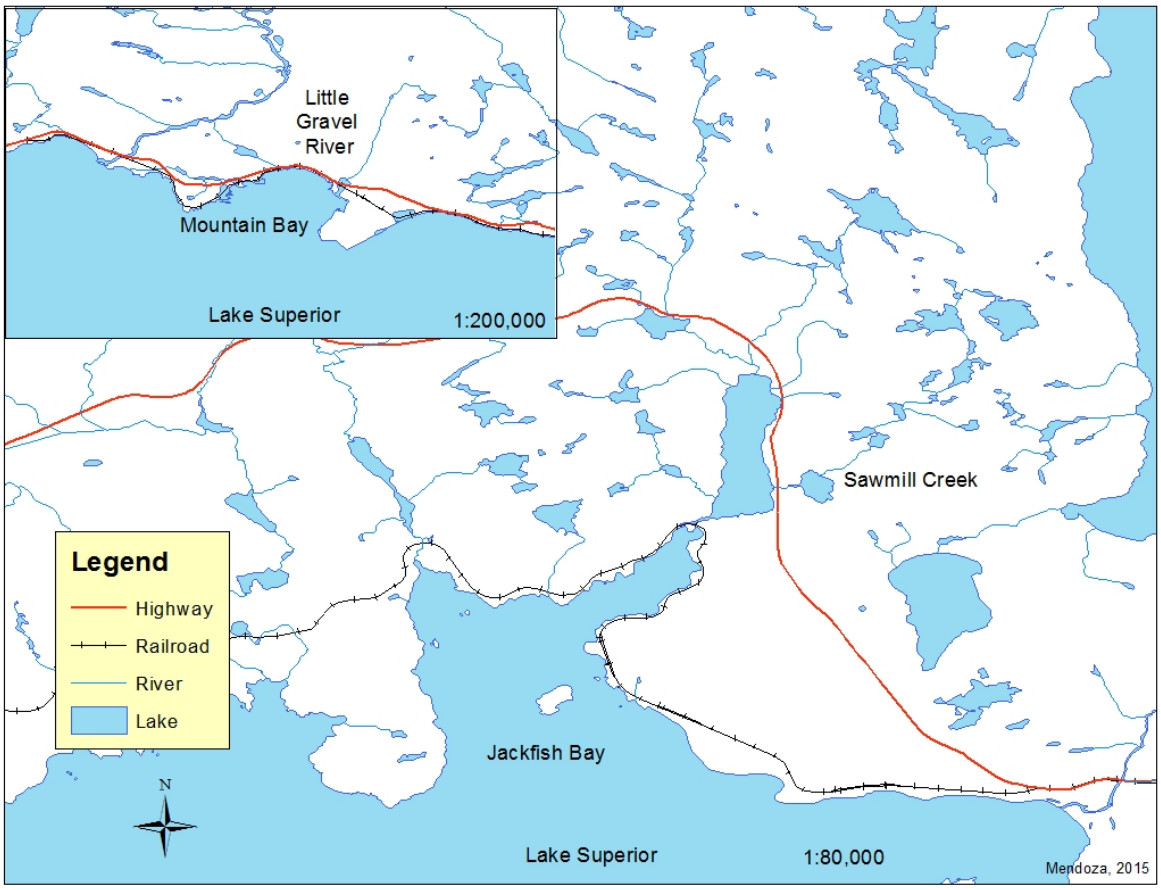


Figure 3.1: Map of Jackfish Bay (fall) and Sawmill Creek (spring). Inset map of reference site: Mountain Bay (fall) and Little Gravel River (spring) (DMTI CanMap Route Logistics, 2014).

### 3.3.2 Field Collection

Twenty male and twenty female white sucker were collected at each site during 2011 to 2013. In May, fish were collected in hoop nets from spawning streams, while in August fish were collected at lake sites by 10.0 and 11.3 cm gill nets to target mature adults. Each fish was rendered unconscious by a sharp blow to the head. The right operculum from each fish was collected for aging by annuli count. Skinless, epaxial white muscle tissue from the left side of the fish and liver tissue were stored in -20°C freezers or kept in liquid nitrogen for stable isotope analyses. Another sample of liver from each fish was stored in

liquid nitrogen for ethoxyresorufin-O-deethylase (EROD) analysis. Fish were collected and sampled under University of Waterloo AUPP# 10-17. Fork length (mm), total weight (g), gonad weight (g), and liver weight (g) were recorded. Relative gonad weight was calculated using the gonadosomatic index (GSI) as:

$$GSI = \frac{gonad\ weight}{total\ weight} \times 100 \qquad \text{Equation 3.1}$$

Relative liver weight was calculated using the hepatic somatic index (LSI) as:

$$LSI = \frac{liver\ weight}{total\ weight} \times 100 \qquad \text{Equation 3.2}$$

Condition factor was calculated using Fulton's condition (k) as:

$$k = \frac{total\ weight}{fork\ length^3} \qquad \text{Equation 3.3}$$

As the mill shutdown was unplanned, archived samples were used for some analysis, such that some sample times are missing for fish collections (e.g., muscle spring 2012, female liver fall 2012). Benthic invertebrates were sampled at Moberly Bay and Mountain Bay during the fall of 2012 during mill closure and 2013 during mill operation (no samples were available in 2011). The animals were collected by an Ekman dredge, washed through a sieve, and sorted in the field before being placed in filtered lake water for 24 hours to remove gut content. The animals were then frozen at -20°C for transport and storage. Using a dissecting microscope (0.63 to 4x magnification), the animals were identified according to Thorp and Covich (2010) to the lowest possible taxonomic level.

### 3.3.3 Laboratory Analyses

To prepare samples for carbon and nitrogen stable isotope analyses, the white sucker muscle and liver samples were freeze dried for a minimum of 48 h before being ground and homogenized by a ball mill grinder, then weighed into tin capsules (0.2 ± 0.05 mg). Carbon and nitrogen isotopes signatures ($\delta^{13}C$ and $\delta^{15}N$) and per cent elemental composition were analyzed using a Delta Plus, Continuous Flow Stable Isotope Ratio Mass Spectrometer (Thermo Finnigan/Bremen-Germany) coupled to a Carlo Erba

Elemental Analyzer (CHNS-O EA1108-Italy), as outlined in Fry *et al.* (1992). Stable isotope analyses were performed at the Environmental Isotope Lab at the University of Waterloo (Drimmie & Heemskerk, 2005). Standards for carbon and nitrogen were VPDB (Vienna Peedee Belemnite) carbonate and nitrogen gas in the atmosphere.

At least 20% of samples analyzed were international reference materials or in-house EIL standards (IAEA, USGS). These were used for data normalization, precision and accuracy, and assessment of linearity issues and drift throughout the duration of the run. Error for $\delta^{13}C$ and $\delta^{15}N$ were 0.2‰ and 0.3‰, respectively. Duplicates for muscle and liver were run every 10 samples, with a precision (mean ± 1SD) within runs of 0.09 ± 0.05‰ $\delta^{13}C$ and -0.03 ± 0.04‰ $\delta^{15}N$ for muscle (N = 57) and 0.17 ± 0.17‰ $\delta^{13}C$ and -0.06 ± 0.06‰ $\delta^{15}N$ for liver (N = 54).

Based on the relationship between percent C:N and percent lipid, aquatic animal samples with C:N < 3.5 do not need to be corrected for lipid content (Post 2007). While white sucker tissue had low C:N ratios for muscle, while C:N ratios in liver were high. Lipid extraction can cause protein degradation which can alter the integrity of $\delta^{15}N$ (Sotiropoulos *et al.*, 2004). There were only weak relationships between C:N and $\delta^{13}C$ in muscle and liver tissues (see Appendix B). The $\delta^{13}C$ values of muscle and liver were lipid therefore corrected according to Post *et al.* (2007):

$$\delta^{13}C_{normalized} = \delta^{13}C_{untreated} - 3.32 + (0.99)\times(C:N) \qquad \text{Equation 3.4}$$

Liver samples from white sucker collected during 2011 to 2013 were analyzed for hepatic cytochrome P450 IA-dependent MFO activity, based on the catabolism of 7-ethoxyresorufin. The bioassay for the induction of EROD in fish liver was adapted from Elfer *et al.* (1997). Liver samples were thawed from -80°C, weighed (± 0.001 g), and homogenized with grinding buffer (0.15 M KCl, 0.02 HEPES sodium salt, pH 7.5) (HEPES = N-2-Hydroxyethylpiperazine-N'-2-ethanesulphonic acid) and a motor-driven Teflon pestle on ice. Homogenates were spun in a microcentrifuge at 9600 rpm at 4°C for 20 minutes. The pellet was aspirated with a Pasteur pipette, and supernatant was collected to extract the

microsomal fraction. Microsomal fractions were stored at -80°C until analysis was conducted. To analyze for EROD, microsomal fractions were thawed, and 50 µL were dispensed in triplicate on 96-well plates. Fifty µL of 7-ethoxy-resorufin solution (resorufin ethyl ether and dimethyl sulfoxide, absorbance = 1.60 to 1.70 units) was added to each well, and the plates were kept in the dark. After 10 min, 10 µL NADPH was dispensed into each well, and then analyzed immediately on a Biotek Synergy 4 plate reader at a wavelength of 530/590. Samples on each 96-well plate were compared to a standard curve (0, 0.2, 0.4, 0.6, 0.8, and 1.0 µg/mL resorufin (resorufin sodium salt and dimethyl sulfoxide). To standardize EROD activity to protein, protein concentrations were read for each sample in triplicate. Fifty µL of microsomal fraction was diluted with 750 µL $ddH_2O$, and gently vortexed. In triplicate, 10 µL of each protein dilution was transferred into the wells. Within two minutes, 200 µL of Bio-Rad 1:5 protein reagent (Bio-Rad protein assay dye reagent concentrate and $ddH_2O$) was added to each plate. After five minutes, but before 7.5 minutes, the samples were analyzed with a protein standard curve (0, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.50 mg/mL BSA (Albumin, bovine serum). Positive and negative controls were included in each plate. The inter- and intra-assay coefficients of variability were respectively 30.8% (n = 25) and 7.8% (n = 540), using the following equations:

$$Inter - assay\ \%\ CV = \frac{standard\ deviation\ of\ plate\ means \times 100}{mean\ of\ plate\ means} \quad \text{Equation 3.5}$$

$$Intra - assay\ \%\ CV = \frac{standard\ deviation\ of\ duplicates \times 100}{duplicate\ mean} \quad \text{Equation 3.6}$$

### 3.3.4 Statistical Analyses

Statistical analysis was conducted on R version 2.15.1. Q-Q plots and Levene's Test confirmed the normality and homogeneity of variance in data. Population parameters were separated into males and females and log transformed to ensure normality before analyses. Age, length, weight, and EROD activity were analyzed with ANOVA to compare amongst sites within years. Analysis of co-variance (ANCOVA) was conducted to compare the gonadal somatic index (GSI), liver somatic index (LSI), and

condition factor. Type II ANCOVA was conducted when there was no interaction between variables, and Type III ANCOVA was conducted when there was an interaction. Stable isotope analyses at Sawmill Creek during the spring were non-normal and did not become normally distributed after transformation; therefore, the Kruskal-Wallis Rank Sum Test and then the multiple comparison test outlined in Siegel and Castellan (1988) was conducted to compare sites and years. Male white sucker and chironomids were compared using ANOVA and pairwise t-test with Bonferroni correction on log transformed isotope data. All tests used an alpha value ($\alpha$) of 0.05 for significance.

## 3.4 Results

### 3.4.1 Population Parameters

Measured population parameters of white sucker include age, length, weight, condition (*k*), relative liver weight (LSI), and relative gonad weight (GSI) and are separated into males (Table 3.1) and females (Table 3.2) with supporting statistical information in Appendix B.

Regardless of mill operation, exposed (Moberly Bay) male white sucker did not differ significantly in age, length, weight, and GSI compared to reference (Mountain Bay) male white sucker (Table 3.1). Pulp mill effluent exposed males collected during the fall had significantly higher condition compared to reference males during all years, including during mill shutdown. Exposed males collected during the spring (Sawmill Creek) had significantly greater condition during the spring of 2011, but were otherwise similar in condition to reference (Little Gravel River) male white sucker. This difference may have been observed because of the large sample size in 2011. Spring liver size (i.e., LSI) was lower at the exposed site but only significantly different in the spring of 2011 (ANCOVA, $p < 0.01$) and 2013 (ANCOVA, $p = 0.047$). During fall collections, exposed males had similar relative liver size across the three years.

Exposed female white sucker were not different from the reference site in length across the three years (Table 3.2). Exposed females had similar ages to reference female white sucker, except in fall 2013, when exposed females were significantly younger (ANOVA, $p = 0.003$). Exposed females were heavier during spring 2011 (ANOVA, $p = 0.04$), fall 2012 (ANOVA, $p = 0.004$), and spring 2013 (ANOVA, $p = 0.0006$), but were otherwise similar in weight to reference female white sucker. Exposed female white sucker consistently had greater condition than reference female white sucker, except spring 2012 (ANCOVA, $p = 0.06$). Exposed female white sucker had similar relative liver and gonad weights, except in spring 2011 when exposed females had smaller relative gonad weight (ANCOVA, $p = 0.02$) and in spring 2013 when exposed females had smaller relative liver weight (ANCOVA, $p < 0.01$).

### 3.4.2 MFO Activity

MFO activity was measured as average EROD activity for each gender throughout the three years with variable mill operation (Table 3.3). Exposed white sucker of both sexes consistently had greater EROD activity than their counterparts at reference sites. EROD activity was relatively low (< 2 pmol/min/mg) in spring fish from both sexes at both sites. Only female white sucker had significant differences in EROD that correlated with mill operation, that is, exposed female white sucker had significantly higher levels of EROD induction during mill operation than reference fish, and when the mill shutdown, there was not a significant difference. Exposed male white sucker had significantly higher EROD induction only in 2013.

### 3.4.3 Stable Isotope Signatures in White Sucker

Stable isotopes of nitrogen and carbon in muscle and liver of white sucker males and females during spring and fall were compared between exposed and reference sites across years of variable mill operation (Fig. 3.2-3.9).

Muscle of exposed male and female white sucker were consistently enriched in $^{15}N$ compared to reference fish across seasons and years (Fig. 3.2 and Fig 3.3). During mill operation, livers of exposed males and females were consistently enriched in $^{15}N$ compared to reference fish, but in fall 2012, when the mill was not operating, liver $\delta^{15}N$ in exposed males was depleted to levels more similar to that of reference male livers (Fig. 3.7). In spring 2013 exposed fish livers were again enriched in $^{15}N$ compared to reference fish livers, but in fall 2013 both exposed female and male livers were similar to reference fish liver $\delta^{15}N$ (depleted)(Fig 3.6 and Fig. 3.7). In fall 2011 reference male liver $\delta^{15}N$ was significantly depleted compared to reference males from spring 2011, but this trend was not exhibited in exposed fish or reference fish during any other seasons in 2012 and 2013.

There were no differences in $\delta^{13}C$ in muscle of both males and females among all seasons and years (Fig. 3.4 and Fig. 3.5) and there were no differences in $\delta^{13}C$ between exposed and reference fish livers during the three year study (Fig. 3.8 and Fig. 3.9). In 2012 fall reference males had depleted $\delta^{13}C$ in livers compared to males collected in spring. In spring 2013, reference females were depleted in $\delta^{13}C$ in their livers compared to previous years, but returned to an enriched condition in fall 2013. In spring 2013, exposed female livers were depleted in $^{13}C$ compared to spring 2011.

### 3.4.4 Stable Isotope Baseline

During 2012 and 2013, $\delta^{13}C$ and $\delta^{15}N$ signatures in livers of male white sucker were similar between exposed (Moberly Bay) and reference (Mountain Bay) sites (Table 3.4). Similarly, $\delta^{13}C$ signatures in chironomids were similar in 2012 between sites. However, in fall of 2013, $\delta^{13}C$ signatures of exposed chironomids were more depleted relative to the reference site (the reference site maintained similar $\delta^{13}C$ between years). During 2012 and 2013, $\delta^{13}C$ signatures in liver of male white sucker at reference sites reflected $\delta^{13}C$ signatures of chironomids. Liver of exposed fish was enriched in $\delta^{13}C$ compared to chironomids during both 2012 and 2013. Other benthic invertebrates (*Valvata spp.*, *Hyalella pp.*, *Caecidotea spp.*, *Pisidium spp.*) had $\delta^{13}C$ signatures that were enriched compared to chironomids at

Mountain Bay, but were depleted in $\delta^{13}C$ values compared to chironomids at Moberly Bay. $\delta^{15}N$ signatures in liver of both reference and exposed male white sucker were enriched compared to $\delta^{15}N$ signatures of chironomids. Additional benthic invertebrates collected were depleted compared to male white sucker livers, similar to the findings for chironomids. Although chironomid at the exposed site has different $\delta^{15}N$ values compared to the reference, it was in a different direction in each year sampled.

Table 3.1: Age, length, weight, condition factor (k), gonad somatic index (GSI), and liver somatic index (LSI) of male white sucker (*Catostomus commersonii*) from prespawning sites (spring): Little Gravel River (LGR) and Sawmill Creek (SMC); and gonadal recrudescent sites (fall): Mountain Bay (MTB), Moberly Bay (MOB), Tunnel Bay (TB), and Jackfish Lake (JFL) during 2011 (mill operational), 2012 (mill shutdown), and 2013 (mill operational). Values are reported as mean ± SE (n). Asterisks (*) signify statistically different sites within each variable ($p < 0.05$).

| Season | Year | Site | Age (year) | Length (cm) | Weight (g) | k | LSI | GSI |
|---|---|---|---|---|---|---|---|---|
| Spring | 2011 | LGR | 9.0 ± 0.4 (100) | 38.2 ± 0.3 (100) | 761.6 ± 19.8 (100) | 1.34 ± 0.02 (100) | 1.89 ± 0.04 (100) | 4.67 ± 0.09 (100) |
| | | SMC | 8.0 ± 0.3 (98) | 37.7 ± 0.4 (98) | 806.1 ± 29.0 (98) | 1.43 ± 0.01 (98) * | 1.43 ± 0.03 (98) * | 4.45 ± 0.11 (98) |
| | 2012 | LGR | 9.5 ± 0.9 (20) | 39.6 ± 0.7 (20) | 824.1 ± 45.2 (20) | 1.31 ± 0.02 (20) | 1.69 ± 0.06 (20) | 4.92 ± 0.17 (20) |
| | | SMC | 9.2 ± 0.5 (20) | 39.4 ± 0.5 (20) | 888.9 ± 35.2 (20) | 1.44 ± 0.02 (20) | 1.43 ± 0.06 (20) | 4.89 ± 0.20 (20) |
| | 2013 | LGR | 6.1 ± 0.3 (19) | 37.5 ± 0.5 (19) | 677.4 ± 27.4 (19) | 1.28 ± 0.04 (19) | 1.81 ± 0.07 (19) | 4.55 ± 0.13 (19) |
| | | SMC | 7.6 ± 0.5 (32) | 39.0 ± 0.6 (32) | 848.6 ± 39.1 (32) | 1.40 ± 0.01 (32) | 1.52 ± 0.04 (32) * | 5.00 ± 0.73 (32) |
| Fall | 2011 | MTB | 8.2 ± 0.6 (13) | 42.2 ± 1.0 (18) | 971.4 ± 19.7 (18) | 1.34 ± 0.06 (18) | 1.11 ± 0.04 (18) | 6.46 ± 0.23 (18) |
| | | MOB | 7.5 ± 0.6 (17) | 40.3 ± 0.5 (20) | 1056.7 ± 37.9 (20) | 1.60 ± 0.02 (20) * | 1.23 ± 0.06 (20) | 6.45 ± 0.33 (20) |
| | 2012 | MTB | 11.5 ± 0.3 (20) | 41.6 ± 0.4 (20) | 952.1 ± 30.1 (20) | 1.32 ± 0.02 (20) | 1.13 ± 0.04 (20) | 6.06 ± 0.32 (20) |
| | | MOB | 10.1 ± 0.6 (20) | 41.4 ± 0.5 (20) | 1104.9 ± 37.6 (20) | 1.55 ± 0.02 (20) * | 1.30 ± 0.06 (20) | 5.28 ± 0.43 (20) |
| | 2013 | MTB | 9.4 ± 0.4 (27) | 41.4 ± 0.3 (27) | 970.0 ± 23.7 (27) | 1.36 ± 0.02 (27) | 1.20 ± 0.03 (27) | 4.82 ± 0.29 (27) |
| | | MOB | 7.2 ± 0.6 (20) | 41.2 ± 0.6 (20) | 1115.8 ± 49.2 (20) | 1.58 ± 0.04 (20) * | 1.40 ± 0.08 (20) | 4.67 ± 0.41 (20) |

Table 3.2: Age, length, weight, condition factor (k), gonad somatic index (GSI), and (liver somatic index (LSI) of female white sucker (*Catostomus commersonii*) from prespawning sites (spring): Little Gravel River (LGR) and Sawmill Creek (SMC); and gonadal recrudescent sites (fall): Mountain Bay (MTB), Moberly Bay (MOB), Tunnel Bay (TB), and Jackfish Lake (JFL) during 2011 (mill operational), 2012 (mill shutdown), and 2013 (mill operational). Values are reported as mean ± SE (n). Asterisks (*) signify statistically different sites within each variable ($p < 0.05$).

| Season | Year | Site | Age (year) | Length (cm) | Weight (g) | k | LSI | GSI |
|---|---|---|---|---|---|---|---|---|
| Spring | 2011 | LGR | 11.7 ± 0.4 (100) | 43.9 ± 0.3 (100) | 1231.7 ± 28.6 (100) | 1.43 ± 0.01 (100) | 1.92 ± 0.03 (100) | 15.10 ± 0.30 (100) |
| | | SMC | 10.2 ± 0.2 (103) | 43.8 ± 0.3 (103) | 1355.9 ± 25.1 (103) * | 1.59 ± 0.01 (103) * | 1.93 ± 0.02 (103) | 14.45 ± 0.21 (103) |
| | 2012 | LGR | 13.4 ± 1.1 (19) | 45.1 ± 0.7 (20) | 1281.7 ± 56.2 (20) | 1.38 ± 0.02 (20) | 1.63 ± 0.06 (20) | 15.38 ± 0.48 (20) |
| | | SMC | 11.0 ± 0.4 (20) | 44.0 ± 0.4 (20) | 1339.2 ± 44.1 (20) | 1.56 ± 0.02 (20) | 1.87 ± 0.04 (20) | 13.59 ± 0.27 (20) |
| | 2013 | LGR | 6.9 ± 0.5 (20) | 41.0 ± 0.6 (20) | 950.7 ± 45.8 (20) | 1.36 ± 0.02 (20) | 2.23 ± 0.05 (19) | 12.51 ± 0.44 (20) |
| | | SMC | 9.1 ± 0.5 (33) | 43.5 ± 0.6 (33) | 1275.4 ± 58.0 (33) * | 1.51 ± 0.02 (33) * | 1.83 ± 0.05 (33) * | 13.77 ± 0.36 (33) |
| Fall | 2011 | MTB | 9.5 ± 0.7 (16) | 44.3 ± 0.5 (20) | 1163.4 ± 32.0 (20) | 1.34 ± 0.02 (20) | 1.49 ± 0.05 (20) | 4.12 ± 0.10 (20) |
| | | MOB | 8.2 ± 0.6 (18) | 43.6 ± 0.7 (20) | 1284.3 ± 65.7 (20) | 1.53 ± 0.05 (20) * | 1.48 ± 0.07 (20) | 3.46 ± 0.39 (20) * |
| | 2012 | MTB | 9.1 ± 0.6 (20) | 42.3 ± 0.6 (20) | 1003.9 ± 33.6 (20) | 1.32 ± 0.02 (20) | 1.35 ± 0.03 (20) | 3.01 ± 0.09 (20) |
| | | MOB | 10.1 ± 0.7 (19) | 44.1 ± 0.7 (20) | 1332.8 ± 45.5 (20) * | 1.55 ± 0.03 (20) * | 1.65 ± 0.07 (20) | 3.49 ± 0.16 (20) |
| | 2013 | MTB | 10.1 ± 0.6 (34) | 43.2 ± 0.7 (34) | 1118.5 ± 32.8 (34) | 1.51 ± 0.17 (34) | 1.58 ± 0.05 (34) | 2.67 ± 0.11 (34) |
| | | MOB | 7.4 ± 0.7 (20) * | 43.2 ± 0.8 (20) | 1267.5 ± 70.6 (20) | 1.55 ± 0.03 (20) * | 1.60 ± 0.09 (20) | 2.49 ± 0.13 (20) |

Table 3.3: Mean ± standard error (n) of mixed function oxygenase (MFO) activity measured as ethoxy-o-resorufin deethylase (EROD) activity of male and female white sucker (*Catostomus* commersonii) during spring at Little Gravel River (LGR) and Sawmill Creek (SMC), and fall at Mountain Bay (MTB) and Moberly Bay (MOB). Asterisks (*) signify statistically different sites ($p < 0.05$).

| Season | Year | Site | EROD Activity (pmol/min/mg) in males | EROD Activity (pmol/min/mg) in females |
|---|---|---|---|---|
| Spring | 2011 | LGR | 0.64 ± 0.07 (20) | 0.14 ± 0.01 (21) |
| | | SMC | 1.28 ± 0.15 (20) | 0.21 ± 0.03 (21) |
| | 2012 | LGR | 0.59 ± 0.05 (20) | 0.37 ± 0.02 (20) |
| | | SMC | 1.74 ± 0.13 (20) * | 0.62 ± 0.03 (20) |
| | 2013 | LGR | 1.20 ± 0.10 (19) | 0.45 ± 0.04 (20) |
| | | SMC | 1.36 ± 0.13 (32) | 0.48 ± 0.02 (33) |
| Fall | 2011 | MTB | 0.97 ± 0.11 (18) | 0.49 ± 0.04 (19) |
| | | MOB | 2.05 ± 0.38 (20) | 1.26 ± 0.19 (20) * |
| | 2012 | MTB | 0.82 ± 0.06 (20) | 0.57 ± 0.03 (20) |
| | | MOB | 1.43 ± 0.23 (20) | 1.01 ± 0.13 (20) |
| | 2013 | MTB | 0.93 ± 0.08 (20) | 0.50 ± 0.05 (20) |
| | | MOB | 6.93 ± 1.24 (17) * | 5.84 ± 1.13 (20) * |

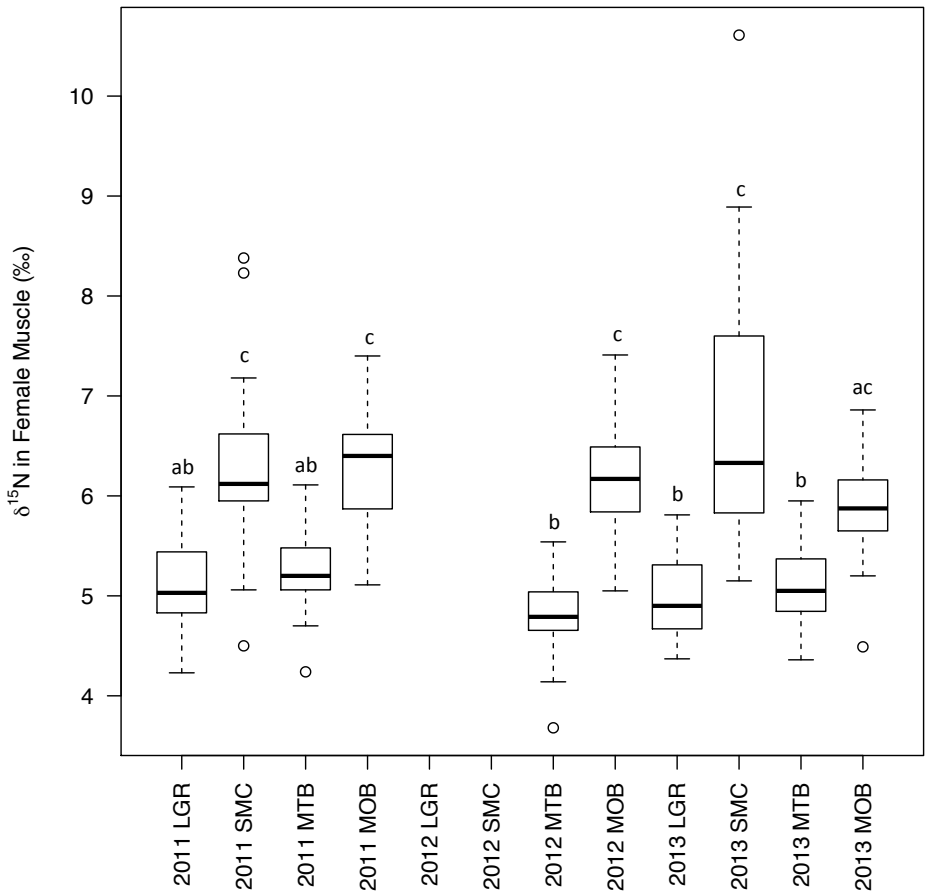


Figure 3.2: Box-plot of $\delta^{15}N$ in muscle of female white sucker by site over time. LGR = Little Gravel River, SMC = Sawmill Creek, MTB = Mountain Bay, MOB = Moberly Bay. The mill in Terrace Bay was operating during 2011 and 2013. Letters signify statistically similar sites ($p < 0.05$). Note: no data was collected for 2012 fish muscle at Little Gravel River or Sawmill Creek.

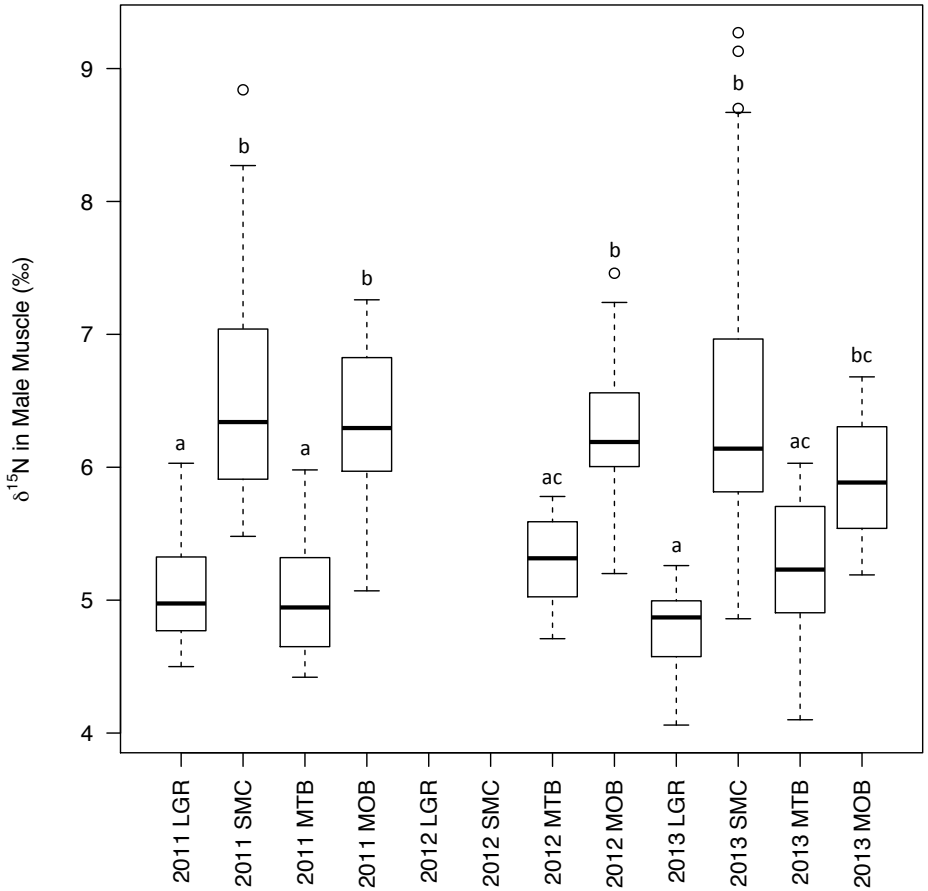


Figure 3.3: Box-plot of $\delta^{15}N$ in muscle of male white sucker by site over time. LGR = Little Gravel River, SMC = Sawmill Creek, MTB = Mountain Bay, MOB = Moberly Bay. The mill in Terrace Bay was operating during 2011 and 2013. Letters signify statistically similar sites ($p < 0.05$). Note: no data was collected for 2012 fish muscle at Little Gravel River or Sawmill Creek.

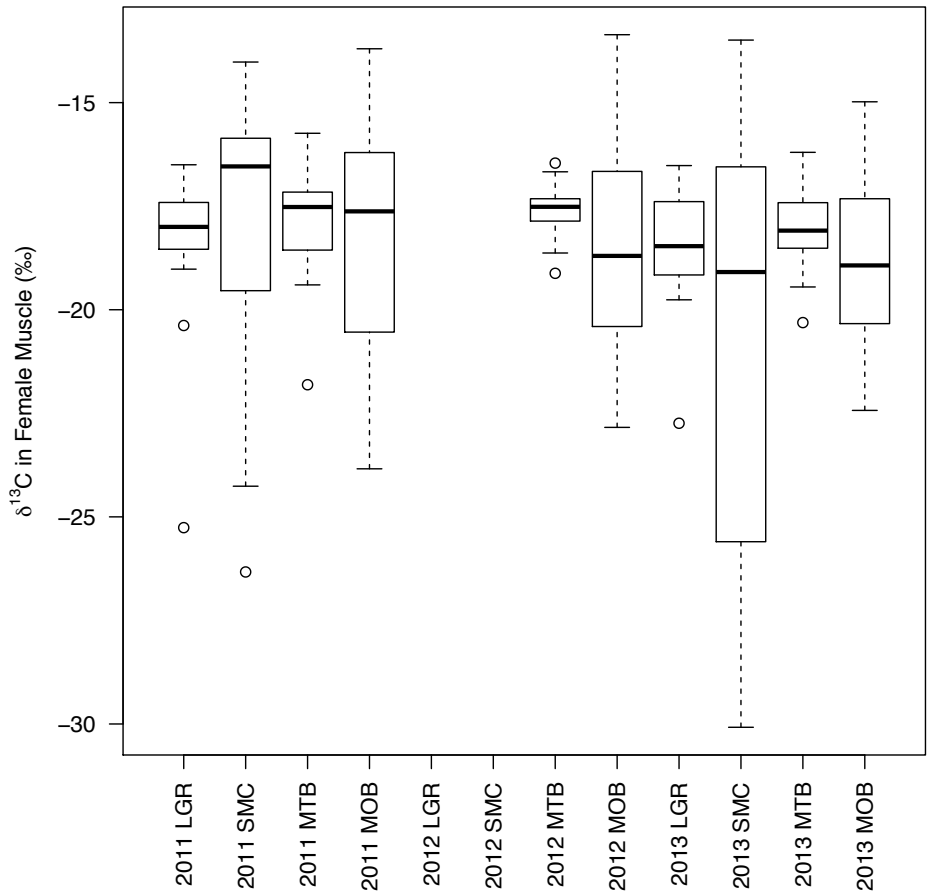


Figure 3.4: Box plot of $\delta^{13}C$ in muscle of female white sucker by site over time. LGR = Little Gravel River, SMC = Sawmill Creek, MTB = Mountain Bay, MOB = Moberly Bay. The mill in Terrace Bay was operating during 2011 and 2013. Note: no data was collected for 2012 fish muscle at Little Gravel River or Sawmill Creek. No significant differences were identified by Kruskal-Wallis test.

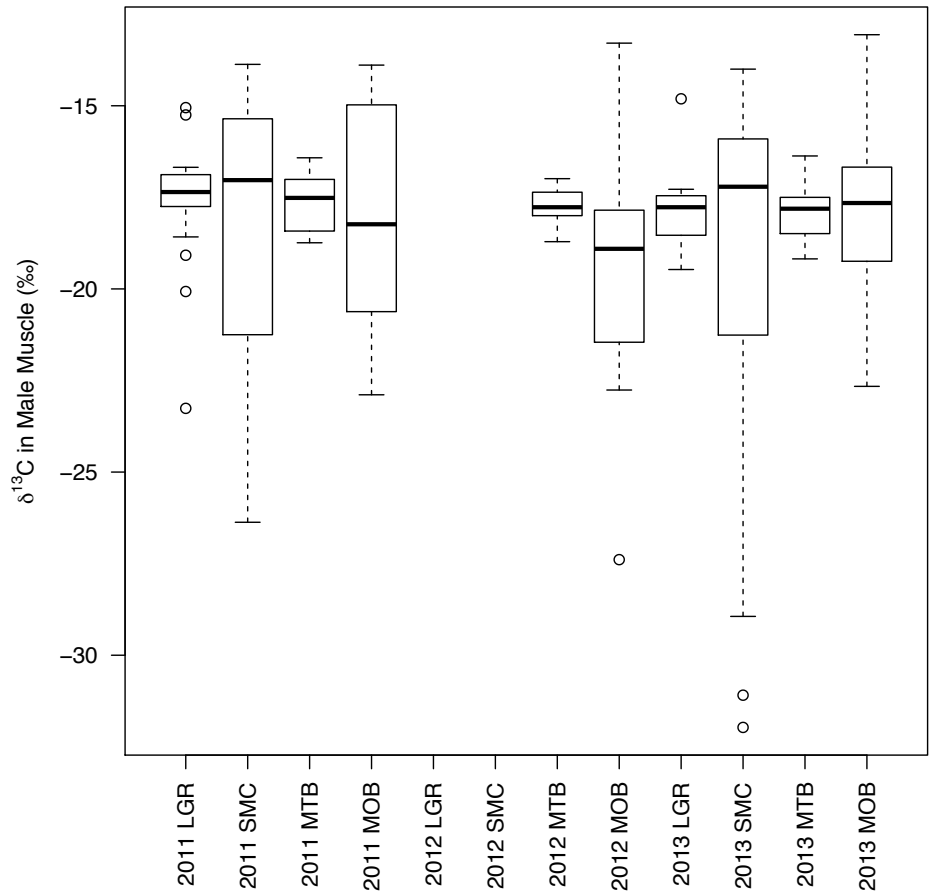


Figure 3.5: Box-plot of $\delta^{13}C$ in muscle of male white sucker by site over time. LGR = Little Gravel River, SMC = Sawmill Creek, MTB = Mountain Bay, MOB = Moberly Bay. The mill in Terrace Bay was operating during 2011 and 2013. Note: no data was collected for 2012 fish muscle at Little Gravel River or Sawmill Creek. No significant differences were identified by Kruskal-Wallis test.

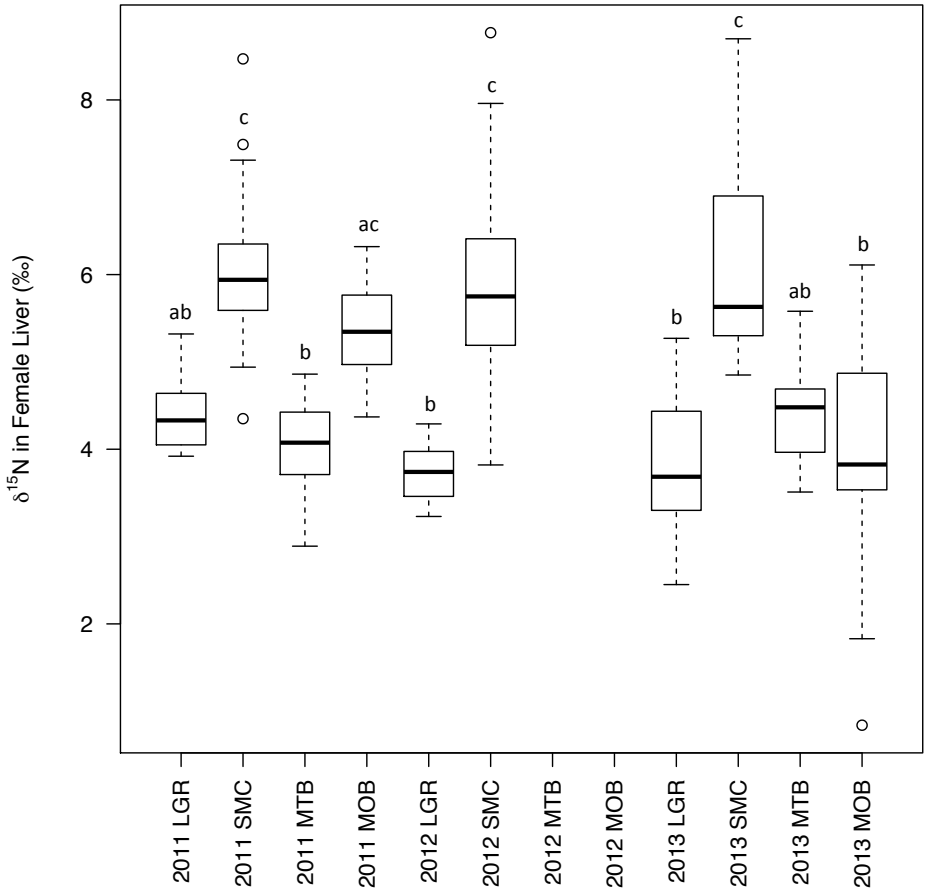


Figure 3.6: Box-plot of $\delta^{15}N$ in liver of female white sucker by site over time. LGR = Little Gravel River, SMC = Sawmill Creek, MTB = Mountain Bay, MOB = Moberly Bay. The mill in Terrace Bay was operating during 2011 and 2013. Letters signify statistically similar sites ($p < 0.05$). Note: no data was collected for 2012 female fish liver at Mountain Bay or Moberly Bay.

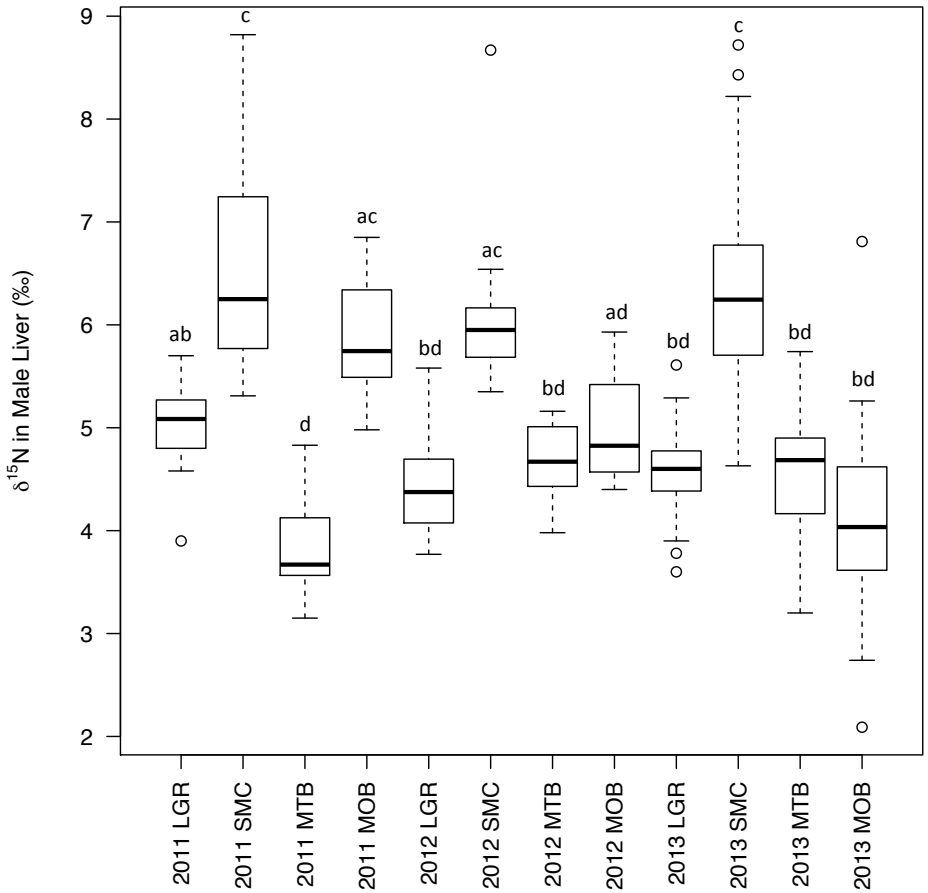


Figure 3.7: Box-plot of $\delta^{15}N$ in liver of male white sucker by site over time. LGR = Little Gravel River, SMC = Sawmill Creek, MTB = Mountain Bay, MOB = Moberly Bay. The mill in Terrace Bay was operating during 2011 and 2013. Letters signify statistically similar sites ($p < 0.05$).

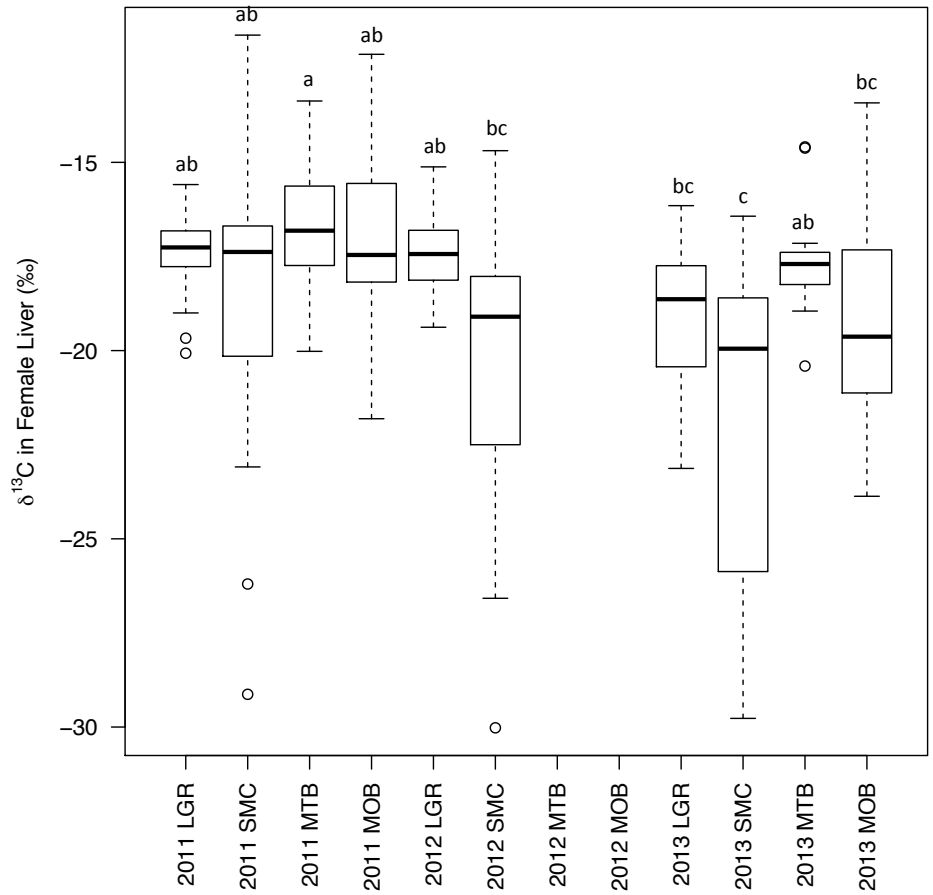


Figure 3.8: Box-plot of δ $^{13}$C in liver of female white sucker by site over time. LGR = Little Gravel River, SMC = Sawmill Creek, MTB = Mountain Bay, MOB = Moberly Bay. The mill in Terrace Bay was operating during 2011 and 2013. Letters signify statistically similar sites ($p < 0.05$). Note: no data was collected for 2012 female fish liver at Mountain Bay or Moberly Bay.

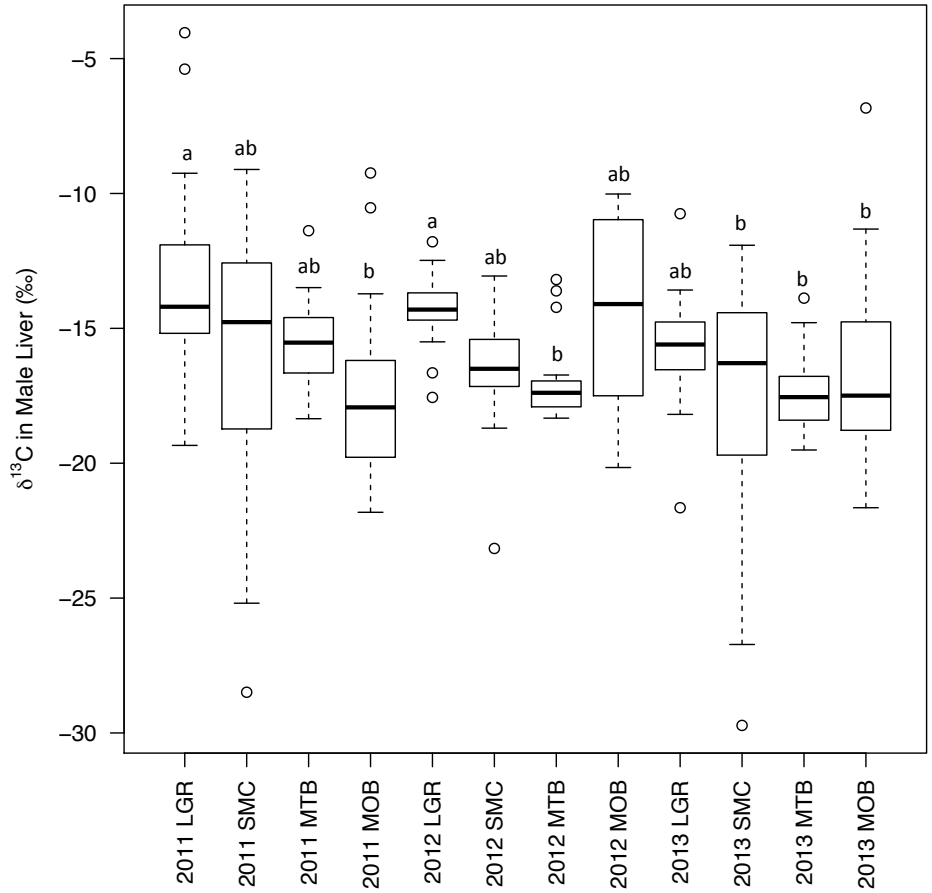


Figure 3.9: Box-plot of $\delta^{13}C$ in liver of male white sucker by site over time. LGR = Little Gravel River, SMC = Sawmill Creek, MTB = Mountain Bay, MOB = Moberly Bay. The mill in Terrace Bay was operating during 2011 and 2013. Letters signify statistically similar sites ($p < 0.05$).

Table 3.4: $\delta^{13}C$ and $\delta^{15}N$ stable isotope average ± standard errors (n) of different environmental compartments at Mountain Bay and Moberly Bay during mill closure (2012) and mill operation (2013). Comparisons were made between male white sucker liver and chironomini $\delta^{13}C$ and $\delta^{15}N$ stable isotope ratios. Capital letters signify similar $\delta^{13}C$ signatures among samples for sites and years, while lower case letters signify similar $\delta^{15}N$ signatures among samples for sites and years.

| Sample | Stable Isotope | 2012 | | 2013 | |
|---|---|---|---|---|---|
| | | Mountain Bay | Moberly Bay | Mountain Bay | Moberly Bay |
| White sucker male liver | $\delta^{13}C$ | -16.86 ± 0.39 (17) AB | -14.31 ± 0.93 (14) B | -17.39 ± 0.31 (20) AD | -16.67 ± 0.81 (20) AB |
| | $\delta^{15}N$ | +4.68 ± 0.09(17) a | +5.00 ± 0.14 (14) a | +4.61 ± 0.15 (20) a | +4.14 ± 0.23 (20) a |
| White sucker female liver | $\delta^{13}C$ | N/A | N/A | -17.67±0.29 (20) | -19.16 ± 0.61 (20) |
| | $\delta^{15}N$ | N/A | N/A | +4.42 ± 0.11 (20) | +3.92 ± 0.30 (20) |
| Chironomini | $\delta^{13}C$ | -18.24±0.39 (6) ABC | -20.64±1.16 (5) CD | -17.73±1.02 (8) ABC | -27.61±0.06 (9) E |
| | $\delta^{15}N$ | +0.49±0.21 (6) b | +1.84±0.19 (5) c | +2.73±0.59 (8) c | -0.27±0.08 (9) b |
| *Valvata spp.* | $\delta^{13}C$ | -17.49±0.18 (7) | -23.24±0.31 (5) | -15.41±0.78 (9) | -26.74 (1) |
| | $\delta^{15}N$ | -0.13± 0.13 (7) | +4.73±0.18 (5) | +2.16±0.17 (9) | -2.29 (1) |
| *Hyalella spp.* | $\delta^{13}C$ | -16.01±0.04 (4) | N/A | -16.05±0.53 (9) | N/A |
| | $\delta^{15}N$ | -0.56±0.03 (4) | N/A | +1.59±0.14 (9) | N/A |
| *Caecidotea spp.* | $\delta^{13}C$ | -16.80 (1) | -22.05±0.72 (6) | N/A | -27.21±0.21 (4) |
| | $\delta^{15}N$ | -0.07 (1) | +2.66±0.36 (6) | N/A | -2.97±0.31 (4) |
| *Pisidium spp.* | $\delta^{13}C$ | N/A | -23.46±0.59 (2) | -17.39±1.67 (8) | -24.34±2.57 (2) |
| | $\delta^{15}N$ | N/A | +0.18±0.53 (2) | +1.45±0.31 (5) | -1.09±0.90 (2) |

## 3.5 Discussion

The temporary shutdown of the pulp mill in Terrace Bay may have resulted in a change in the stable isotope ratios in the bottom of the food web (e.g., invertebrates). Chironomini and other invertebrates had similar (or slightly depleted) $\delta^{13}C$ in exposed sites relative to the reference site in 2012 during the shutdown, but became much more depleted the following year after the mill resumed operation (fall 2013). The liver of exposed fish became more depleted in $^{15}N$ after effluent release was halted for almost a year, and this change was still evident the following fall. The mechanism for this change is not known but could be related to a number of factors. The effluent could have depleted carbon and enriched nitrogen signatures that were assimilated into the biota through the food chain. Invertebrates in Moberly Bay did not have depleted $^{15}N$ in 2012 during the shutdown, although they were depleted the following fall (2013). This depleted $^{15}N$ signature in fish could also be due to a change in the source of nitrogen or degree of fractionation. In spring 2013, only eight months after the mill had reopened, fish appeared to have returned to an enriched $^{15}N$ signature, although the fish in the fall collections (2013) remained depleted in $^{15}N$ relative to the reference site. The spring fish collections are likely also affected by the presence of non-exposed fish from Jackfish Lake or unexposed areas of Jackfish Bay that mix with fish during spawning in Sawmill Creek (see Chapter 2), which could have partially masked a possible change. The results could also have been confounded by four power outages the mill experienced between September 2012 to October 2013, but the total days of mill shutdown during this period is unknown (Ross, 2013).

Isotopic ratios in benthic invertebrates indicate that there was a change at the base of the food web when the mill resumed operation. Unfortunately pre-shutdown samples were not available, but Chironominae, *Valvata* spp., and *Caecidotea* spp. were all enriched in $^{13}C$ in Moberly Bay during fall of 2012 compared to fall 2013. Enrichment of $\delta^{13}C$ in invertebrates during the absence of effluent in 2012 is similar to the reference site, where invertebrates have enriched $\delta^{13}C$ compared to the exposure site during both years. $\delta^{13}C$ in chironomids were similar in the reference site (Mountain Bay) in both years, as well

as Moberly Bay during the shutdown while the following year they became much more depleted after mill operation restarted. The Chironomidae family is known to have a diversity of feeding behaviours, and therefore trophic levels, within all taxonomic levels even to the species level (Berg, 1995). Since this study only identified chironomids to the subfamily level, high variability in stable nitrogen isotopes is likely to occur due to the multiple trophic levels of different species. However, all exposed benthic invertebrates, when comparisons could be made, had depletion in nitrogen from 2012 to 2013, while reference invertebrates had enrichment in $\delta^{15}N$. Determining the baseline stable isotope signature of an ecosystem is best done using long-lived primary producers such as mussels and snails (Post, 2002). Unfortunately these benthic invertebrates had limited availability at these sites and few species comparisons could be made. It is also difficult to make firm conclusions based on only two years of data and a longer term data set is needed to define annual variability.

White sucker have a benthic nature and usually feed on a diversity of invertebrates including mussels, chironomids, ephippia, and Diporeia (Gamble, 2010). At the reference site the white sucker tissues reflect the invertebrate signatures but at the exposed site the invertebrates are much more depleted in $^{15}N$ and $^{13}C$ than the fish. It appears that the invertebrates responded to the change in effluent exposure but the more mobile fish do not respond in the same way. It is possible that fish are not feeding exclusively on invertebrates at the impacted area for extended periods of time so they do not change in the same way. Fish tissues will also not turn over as quickly as benthic invertebrates, especially the muscle.

The mill in Terrace Bay started operation in 1948 as an unbleached kraft mill and introduced chlorine bleaching in 1958. Secondary treatment was introduced in 1989 and in the late 1990s the mill started to implement chlorine dioxide substitution into the bleaching sequence, reaching 100% by 1999 (see Dahmer *et al.* (2015), for a summary of mill process changes). This had a dramatic effect on the composition of the final effluent, including the elimination of the release of polychlorinated dibenzo-*p*-dioxins and many other chlorinated compounds (Dahmer *et al.*, 2015). The mill has had numerous short

term closures over the last several decades including the prolonged closure in 2011 to 2012 for approximately a year, examined in the current study. The mill at the time of this study was operating as a northern bleached softwood kraft mill but there are plans to convert it to dissolving wood pulp for viscose staple fibre (rayon manufacturing) in the near future (i.e., 2016). The outcome of this study therefore has implications for current and future assessments.

Population parameters in white sucker including age, length, weight, condition (*k*), relative liver weight (LSI) and relative gonad weight (GSI) of white sucker did not exhibit clear differences or trends in response to mill operation or shutdown. The results of the current study suggest that the recovery at Jackfish Bay has continued. Historically smaller gonad sized, reduced secondary sex characteristics, and lower levels of circulating reproductive steroid hormones were observed in fish at Moberly Bay relative to fish at the reference site at Mountain Bay (Munkittrick *et al.*, 1991). There has been a gradual recovery of population endpoints over time in response to changes in mill operation and process (Bowron *et al.*, 2009). The results from 2011 to 2013 support that there are minimal effects on population level indicators such as gonad somatic index, with the only consistent response being slight altered condition in males. MFO activity (EROD) in fish shows some differences to effluent exposure, including shutdown, but the magnitude is minimal and much lower than previous observations at Moberly Bay (Bowron *et al.*, 2009). EROD is known to be influenced by a large number of abiotic and biotic factors including water temperature, age, and reproductive phase in addition to xenobiotic induction (Andersson & Förlin, 1992), which might explain why EROD levels were depressed in spring when fish are migrating in cold water to spawn in effluent-free Sawmill Creek. Munkittrick *et al.* (1992b) found that EROD activity was reduced very quickly (weeks) after the removal of effluent inputs into Moberly Bay during a previous mill shutdown and the EROD induction returned quickly once the mill resumed operation. Although indicators of exposure, such as EROD induction, are still detectable in fish exposed to the effluent, it does not cause major changes in the population endpoints as it historically did. Continued improvements in

mill effluent quality coupled with natural attenuation appear to be working effectively, and Jackfish Bay is now listed as an Area in Recovery (Environment Canada & Ontario Ministry of the Environment, 2010). The long-term dataset in Jackfish Bay, including the results of the current study, will provide a substantial baseline to which future comparisons can be made.

Stable isotopic signatures of nitrogen and carbon in macroinvertebrates, as well as the more rapidly turned over liver tissue in white sucker, were altered during mill shutdown demonstrating a shift in ecosystem function during the period of reduced effluent inputs. Changes in isotopic signatures in fish, such as white sucker, are more difficult to interpret because of their mobility, and need to be examined within the context of the ecosystem and changes in the lower trophic levels (baseline). Despite there being minimal changes in fish physiology or population parameters, stable isotopes did detect a change during mill shutdown. The specific mechanisms for the shift in isotopic signatures are not known but it represents a potential tool for detecting and exploring change in the ecosystem in response to effluent inputs. The use of stable isotope analyses can be a very powerful tool to help understand change in aquatic ecosystems.

# Chapter 4

# General Conclusions

The studies presented in this thesis characterized the influence of pulp mill exposure on white sucker in Jackfish Bay, Lake Superior, during 2011 to 2013 using stable isotopes of carbon and nitrogen. The stable isotope analyses were supported with another measure of exposure, mixed function oxygenase (MFO) activity, measured as EROD. A number of population endpoints (e.g., relative gonad size, liver size, and condition) were collected and compared to reference sites during both fall (lake collections) and spring spawning (stream collections) periods. An unplanned relatively long-term shutdown of the mill for almost a year created a unique opportunity to examine how a major reduction in effluent inputs would affect the receiving environment and these endpoints. Using sample archives, as well as new collections of fish and invertebrates, the changes spatially and temporally in this system was explored.

Stable isotopes in white sucker (liver and muscle tissues) at several sites within Jackfish Bay (Moberly Bay and Tunnel Bay), a reference site (Mountain Bay), and an adjacent connected lake (Jackfish Lake) were examined during the fall of 2013, along with samples of representative invertebrates from each site. As the white sucker migrate to small streams in early spring to spawn, collections were also made in the stream where pulp mill exposed fish are expected to spawn (Sawmill Creek) and a stream near the reference site at Mountain Bay (Little Gravel River). These studies showed that despite detectable differences in invertebrates, stable isotope signatures in white sucker did not vary spatially within Jackfish Bay (Moberly Bay, Tunnel Bay) or in the reference site (Mountain Bay). Stable isotopes in white sucker body tissues do not reflect the isotopic signature of pulp mill effluent well, likely due to the white sucker's rate of tissue turnover in liver and muscle, and annual migration out of the effluent plume to spawn each spring. Although EROD induction was much lower than in past years, it did show a gradient of response (Moberly Bay > Tunnel Bay > Mountain Bay) suggesting that fish do have different

exposure to effluent components in the different areas sampled. The major difference in isotopic signatures was for the warm shallow adjacent lake (Jackfish Lake) through which fish must migrate in spring to spawn. Fish in Jackfish Lake were enriched in $^{15}N$ and depleted in $^{13}C$. A statistical analysis of the spring results in Sawmill Creek suggest that fish from Jackfish Lake are mixed with the fish from Jackfish Bay during spawning. Although the fish separated into one of two groups by stable isotope signatures (assumed to be from Jackfish Lake) were small and young, they have similar somatic indices and condition and are likely to be the resident population of Jackfish Lake. Separation of the two populations in future may help to refine the interpretation of future studies.

The mill shutdown represented a unique opportunity to determine how stable isotopes in fish and benthic invertebrates change seasonally and annually with exposure to effluent. These studies were supported with analyses of EROD activity and population endpoints in white sucker. During a shutdown of the mill for almost a year, stable isotopes in invertebrates in Moberly Bay became depleted in carbon relative to Mountain Bay that had remained relatively similar over the two years. White sucker muscle remained enriched in $^{15}N$ at the exposed site relative to the reference site across three consecutive years. Fish liver, which has a faster turnover rate, showed a change in $\delta^{15}N$ in the fall during shutdown and the following year such that they were similar to the reference site, likely due to inconsistent mill operation. The fish did not reflect directly the patterns in the food web (i.e., invertebrates) possibly due to their mobility in the environment and different turnover rates. Although the processes controlling isotopic signatures in the environment are complex, stable isotopes may be a valuable tool for detecting and understanding changes in ecosystems.

Reduced differences between exposed and reference fish in most of the population endpoints (e.g., gonadal somatic index) has occurred since the early 1990s due to process and treatment changes. Few differences are now evident in population endpoints between exposed and reference fish in either the spring or fall collections. Subtle differences in MFO (EROD) induction can still be detected in Moberly

Bay (<11.5 fold) and Tunnel Bay (<3 fold) relative to the reference site (Mountain Bay). The only population endpoint that consistently remains altered is an increase in condition in both male and female white sucker collected at the exposed site in spring, and males collected in the fall. This suggests an increased abundance or assimilation of food resources at the exposed site. Although the effluent alters the stable isotopic ratios of invertebrates, this change is not reflected in the signatures of the stable isotopes in the white sucker which are likely much more mobile and able to integrate exposure across the bays (Moberly Bay and Tunnel Bay). It is also possible that increased condition in fish at this site is a natural difference within the scope of natural variability. The mill at Terrace Bay is expected to introduce major process changes in 2016 as it converts to a dissolving pulp process (for rayon production). Long-term monitoring will benefit from additional information on fish exposure and movements so as to be able to accurately reflect environmental change.

While it may have been preferable to capture fish at Moberly Bay in spring prior to migration to guarantee exposure to effluent, fish initiate the migration in very early spring to Sawmill Creek to spawn. Working on open Lake Superior in early spring at this time of year is possible but would be very difficult logistically and in terms of safety. A caging study may allow for fish to be exposed at Moberly Bay during spring, but caged fish would not reflect the biological processes fish undergo during migration and spawning. The present study therefore must take into account that fish migrate into effluent-free waters during annual spawning and mix with less or non-exposed fish on the spawning beds. The residence time of fish moving from Moberly Bay through Jackfish Lake on the way to spawn is unknown. Therefore a study tracking movement of fish within Jackfish Bay as well as into Jackfish Lake would be helpful to better define length of time fish are exposed to effluent.

If future studies at Jackfish Bay continue to use Sawmill Creek as a collection site of pre-spawning exposed white sucker, caution should be used and the exposure verified if possible as mixing of exposed fish with non-exposed fish may confound interpretation of impacts from effluent exposure.

Genetic variability may help to discern different populations of white sucker from Jackfish Lake, Tunnel Bay, and Moberly Bay to clarify mixing during spawning at Sawmill Creek. Although many of the effects have become reduced or eliminated at this site, future changes at the mill may result in new issues and effects that will need to be monitored accurately.

Determining the turnover rates of various tissues (e.g., muscle and liver) of white sucker in a dietary experiment would help to interpret the results so that extrapolation from other species is not needed. Understanding the time it takes for a given tissue to completely incorporate a signature (come to steady state) would clarify how long it takes to observe differences in stable isotope signatures due to environmental changes. Observing effects of varying exposure of pulp mill effluent at different sites over a longer time scale would ensure isotopes from pulp mill effluent are in steady state with the ecosystem, and give a better idea of variability. Unfortunately the environment as well as pulp mill inputs are dynamic and constantly changing, making it difficult to interpret field data. In addition, fish can be very mobile and have very intermittent exposure.

Although invertebrates are typically much less mobile than fish, they can change dramatically in their behaviour and feeding during their life cycle. Since benthic invertebrates had distinctly different stable isotopes at Moberly Bay compared to Tunnel Bay, the relatively stationary species better support interpretation of changes associated with effluent exposure better than a migratory species like the white sucker. Therefore incorporating more endpoints for assessing benthic invertebrates may be more indicative of local effluent exposure within Jackfish Bay., Monitoring programs, such as the Environmental Effects Monitoring program, incorporate both invertebrate and fish endpoints.

Stable isotopes are a useful tool as analyses can be conducted on preserved historical samples to show relevant responses to mill shutdowns and upgrades. If preserved properly (i.e., frozen), historical samples may be subjected to stable isotope analyses to determine changes of effluent quality over time. Stable isotope analyses in bone or scales may be very useful to track changes over time as they can

indicate changes over the life span of a long lived species. Since white sucker can live up to twenty years, analyses on this type of boney structures may reveal important changes concurrent with mill operation, process, and treatment changes.

Stable isotopes were a very useful tool for creating a better understanding of the changes occurring in an environment receiving pulp mill effluents. They may be applied in the future to enhance biomonitoring programs to detect and understand change. Natural variability in complex ecosystems creates considerable uncertainty and future studies should explore how these processes influence how ecosystems function and respond to environmental stressors. The studies in Jackfish Bay have advanced our understanding of ecosystems, but have also generated many new questions and avenues for future research.

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# Appendix A

# Supporting Information for Chapter 2

Table A1: Two way ANOVAs for age, length, weight, and EROD of fish with site and sex as factors.

| Variable | P-Value Site | P-Value Sex |
|---|---|---|
| Age | 3.27E-08 | 0.03 |
| Length | 6.48E-06 | 4.56E-12 |
| Weight | 5.45E-11 | 4.61E-15 |
| EROD | <2e-16 | <2E-16 |

Table A2: Results of ANCOVA tests for LSI, GSI and *k* for males and females.

| Variable and Sex | P-Value | ANOVA Type | Intercept |
|---|---|---|---|
| LSI Males | 0.000755 | III | 3.40E-13 |
| LSI Females | 0.1241 | II | |
| GSI Males | 0.443 | II | |
| GSI Females | 0.0003097 | III | <2.2e-16 |
| *k* Males | 0.06283 | II | |
| *k* Females | <2.2e-16 | III | 0.0720889 |

Table A3: P-values for ANOVAs and ANCOVAs between sites for males.

| Sites | Age (ANOVA) | Length (ANOVA) | Weight (ANOVA) | LSI (ANCOVA) | GSI (ANCOVA) | *k* (ANCOVA) |
|---|---|---|---|---|---|---|
| LGR-SMC | 0.50406 | 0.66841 | 0.0134 | 0.04427 | 0.997 | 0.00148 |
| MTB-MOB | 0.03451 | 1 | 0.0772 | 0.20999 | 0.947 | <0.001 |
| MTB-TB | 0.12063 | 1 | 1 | 0.58914 | 1 | <0.001 |
| MTB-JFL | 0.6231 | 0.00015 | 0.0133 | 0.00496 | 0.799 | 0.76746 |
| MOB-TB | 1 | 1 | 1 | 0.98836 | 0.988 | 0.67937 |
| MOB-JFL | 1 | 0.00045 | 5.30E-05 | <0.001 | 0.568 | 0.50665 |
| TB-JFL | 1 | 0.0029 | 0.0035 | <0.001 | 0.762 | 0.93103 |

Table A4: P-values for ANOVAs and ANCOVAs between sites for females.

| Site Comparison | Age (ANOVA) | Length (ANOVA) | Weight (ANOVA) | LSI (ANCOVA) | GSI (ANCOVA) | k (ANCOVA) |
|---|---|---|---|---|---|---|
| LGR-SMC | 0.1253 | 0.28 | 0.00085 | <0.001 | 1 | 0.00741 |
| MTB-MOB | 0.0118 | 1 | 0.85371 | 0.9683 | 0.502 | 0.15424 |
| MTB-TB | 0.0217 | 1 | 1 | 0.03 | 0.247 | 0.45839 |
| MTB-JFL | 0.1746 | 0.23 | 0.60842 | <0.001 | 0.999 | 0.77536 |
| MOB-TB | 1 | 1 | 1 | 0.314 | 0.999 | 0.99418 |
| MOB-JFL | 1 | 0.41 | 0.01408 | <0.001 | 0.597 | 0.0324 |
| TB-JFL | 1 | 1 | 0.14592 | 0.0472 | 0.375 | 0.10297 |

Table A5: P-Values for Kruskal-Wallis tests between sites for males and females.

| Variable | Male P-Value | Female P-Value |
|---|---|---|
| Carbon Muscle | 0.05881 | 5.82E-06 |
| Carbon Liver | 0.001251 | 7.10E-12 |
| Nitrogen Muscle | 3.57E-12 | 8.36E-16 |
| Nitrogen Liver | 3.57E-12 | 9.18E-16 |

# Appendix B

# Supporting Information for Chapter 3

Table B1: Supporting significance values for comparisons of age, length, and weight between exposed and reference sites for male and female white sucker during 2011, 2012, and 2013 using the pairwise t-test with Bonferroni correction.

| Parameter | Comparison | Male P-Value | Female P-Value |
|---|---|---|---|
| Age | 2011 SMC-LGR | 1.000 | 0.400 |
| | 2011 MOB-MTB | 1.000 | 1.000 |
| | 2012 SMC-LGR | 1.000 | 1.000 |
| | 2012 MOB-MTB | 1.000 | 1.000 |
| | 2013 SMC-LGR | 1.000 | 0.150 |
| | 2013 MOB-MTB | 0.160 | 0.003 |
| Length | 2011 SMC-LGR | 1.000 | 1.000 |
| | 2011 MOB-MTB | 1.000 | 1.000 |
| | 2012 SMC-LGR | 1.000 | 1.000 |
| | 2012 MOB-MTB | 1.000 | 1.000 |
| | 2013 SMC-LGR | 1.000 | 1.000 |
| | 2013 MOB-MTB | 1.000 | 1.000 |
| Weight | 2011 SMC-LGR | 1.000 | 0.040 |
| | 2011 MOB-MTB | 1.000 | 1.000 |
| | 2012 SMC-LGR | 1.000 | 1.000 |
| | 2012 MOB-MTB | 1.000 | 0.004 |
| | 2013 SMC-LGR | 0.790 | 0.001 |
| | 2013 MOB-MTB | 1.000 | 1.000 |

Table B2: Supporting significance values to determine interaction effects for comparisons of condition (K), relative liver weight (LSI), and relative gonad weight (GSI).

| Parameter | Sex | Interaction Present | Result |
|---|---|---|---|
| Condition | Male | <2.2E-16 | Type III test |
| | Female | <2.2E-16 | Type III test |
| GSI | Male | 0.212 | Type II test |
| | Female | 0.3544 | Type II test |
| LSI | Male | 0.004538 | Type III test |
| | Female | 4.813E-05 | Type III test |

Table B3: Supporting significance values for comparison of condition (K), relative liver weight (LSI), and relative gonad weight (GSI) between exposed and reference sites for male and female white sucker during 2011, 2012, and 2013 using simultaneous tests for general linear hypotheses with multiple comparisons of means using Tukey contrasts.

| Parameter | Comparison | Male P-Value | Female P-Value |
|---|---|---|---|
| Condition | 2011 SMC-LGR | <0.01 | <0.01 |
| | 2011 MOB-MTB | <0.01 | 0.013 |
| | 2012 SMC-LGR | 0.180 | 0.055 |
| | 2012 MOB-MTB | <0.01 | <0.01 |
| | 2013 SMC-LGR | 0.130 | <0.01 |
| | 2013 MOB-MTB | <0.01 | 0.036 |
| LSI | 2011 SMC-LGR | <0.01 | 1.000 |
| | 2011 MOB-MTB | 0.890 | 1.000 |
| | 2012 SMC-LGR | 0.134 | 0.190 |
| | 2012 MOB-MTB | 0.498 | 0.080 |
| | 2013 SMC-LGR | 0.047 | <0.01 |
| | 2013 MOB-MTB | 0.410 | 1.000 |
| GSI | 2011 SMC-LGR | 0.810 | 0.520 |
| | 2011 MOB-MTB | 1.000 | 0.021 |
| | 2012 SMC-LGR | 1.000 | 0.570 |
| | 2012 MOB-MTB | 0.072 | 1.000 |
| | 2013 SMC-LGR | 1.000 | 1.000 |
| | 2013 MOB-MTB | 0.920 | 0.695 |

Table B4: Supporting significance values for comparisons of EROD activity among sites and years using pairwise t-test with Bonferroni correction.

| Comparison | Male P-Value | Female P-Value |
|---|---|---|
| 2011 SMC-LGR | 0.070 | 1.000 |
| 2011 MOB-MTB | 1.000 | 0.004 |
| 2012 SMC-LGR | <0.0001 | 0.190 |
| 2012 MOB-MTB | 1.000 | 0.358 |
| 2013 SMC-LGR | 1.000 | 1.000 |
| 2013 MOB-MTB | <0.0001 | <0.0001 |

Table B5: Supporting significance values for comparisons of stable isotopes among sites and years using the Kruskal-Wallis Test.

| Isotope | Tissue | Male P-Value | Female P-Value |
|---|---|---|---|
| Carbon | muscle | 0.5439 | 0.2497 |
| nitrogen | muscle | <2.2E-16 | <2.2E-16 |
| carbon | liver | 7.69E-06 | 2.57E-09 |
| nitrogen | liver | <2.2E-16 | <2.2E-16 |

Table B6: Supporting significance values for comparisons of stable isotopes between male white sucker liver and chironomids between 2012 and 2013 between Moberly Bay and Mountain Bay using pairwise t-tests with Bonferroni correction.

| Stable Isotope | Degrees of Freedom | Sum Sq. | Mean Sq. | F-Value | P-Value |
|---|---|---|---|---|---|
| Carbon | 7 | 3.433 | 0.4904 | 45.22 | <2E-16 |
| Nitrogen | 7 | 5.613 | 0.8019 | 97.5 | <2E-16 |

Table B7: Supporting C:N ratios of muscle and liver for male and female white sucker at Little Gravel River, Sawmill Creek, Mountain Bay, Moberly Bay, Tunnel Bay, and Jackfish Lake.

| Year | Site | Sex | Percent C:N | |
|---|---|---|---|---|
| | | | Muscle | Liver |
| 2011 | LGR | F | 3.92 ± 0.50 (20) | 5.00 ± 0.39 (20) |
| | | M | 4.35 ± 0.84 (20) | 10.24 ± 3.86 (20) |
| | SMC | F | 3.61 ± 0.28 (21) | 4.72 ± 0.37 (20) |
| | | M | 3.68 ± 0.24 (20) | 7.85 ± 0.98 (19) |
| | MTB | F | 3.54 ± 0.41 (20) | 6.22 ± 1.81 (20) |
| | | M | 3.67 ± 0.58 (18) | 7.45 ± 2.25 (15) |
| | MOB | F | 3.31 ± 0.11 (20) | 6.62 ± 2.21 (20) |
| | | M | 3.41 ± 0.21 (20) | 7.87 ± 2.22 (14) |
| 2012 | LGR | F | ND | 4.77 ± 0.40 (19) |
| | | M | ND | 8.74 ± 1.12 (20) |
| | SMC | F | ND | 4.54 ± 0.37 (19) |
| | | M | ND | 7.81 ± 2.05 (15) |
| | MTB | F | 3.36 ± 0.10 (20) | ND |
| | | M | 3.52 ± 0.46 (20) | 6.20 ± 2.22 (17) |
| | MOB | F | 3.44 ± 0.15 (20) | ND |
| | | M | 3.44 ± 0.17 (20) | 7.55 ± 2.25 (14) |
| | TB | F | 3.36 ± 0.11 (6) | 5.12 ± 1.39 (6) |
| | | M | 3.38 ± 0.02 (3) | 6.38 ± 1.17 (3) |
| 2013 | LGR | F | 3.77 ± 0.50 (20) | 4.85 ± 0.50 (20) |
| | | M | 3.61 ± 0.40 (19) | 7.80 ± 1.46 (19) |
| | SMC | F | 3.34 ± 0.22 (33) | 4.59 ± 0.46 (33) |
| | | M | 3.61 ± 0.44 (32) | 7.92 ± 1.45 (32) |
| | MTB | F | 3.32 ± 0.08 (20) | 5.08 ± 0.96 (20) |
| | | M | 3.37 ± 0.12 (20) | 5.64 ± 1.05 (20) |
| | MOB | F | 3.26 ± 0.04 (20) | 6.21 ± 1.67 (20) |
| | | M | 3.29 ± 0.09 (20) | 8.20 ± 2.09 (20) |
| | TB | F | 3.36 ± 0.04 (20) | 5.89 ± 0.93 (20) |
| | | M | 3.38 ± 0.06 (19) | 6.91 ± 2.18 (18) |
| | JFL | F | 3.28 ± 0.05 (12) | 4.36 ± 0.35 (12) |
| | | M | 3.25 ± 0.04 (4) | 4.49 ± 0.26 (4) |

Table B8: Supporting relationship between C:N ratios and $\delta^{13}C$ in liver and muscle of male and female white sucker from Little Gravel River, Sawmill Creek, Mountain Bay, Moberly Bay, Tunnel Bay, and Jackfish Lake.

| | | | Male Formula | Female Formula | Male (n) | Female (n) | Male (R-squared) | Female (R-squared) |
|---|---|---|---|---|---|---|---|---|
| 2011 | LGR | Muscle | y=-0.4159x-16.798 | y=-1.2213x-14.095 | 20 | 20 | 0.04164 | 0.10162 |
| | LGR | Liver | y=-0.0151x-19.704 | y=-0.4587x-16.747 | 20 | 20 | 0.00102 | 0.02432 |
| | MOB | Muscle | y=4.8483x-34.622 | y=-11.16x+18.604 | 20 | 20 | 0.11162 | 0.17313 |
| | MOB | Liver | y=-0.7404x-15.722 | y=-0.8165x-14.95 | 14 | 20 | 0.17039 | 0.30462 |
| | MTB | Muscle | y=-0.8496x-15.04 | y=-0.8312x-15.023 | 18 | 20 | 0.22078 | 0.06655 |
| | MTB | Liver | y=-0.4074x-16.571 | y=-0.3353x-17.411 | 15 | 20 | 0.32854 | 0.25827 |
| | SMC | Muscle | y=0.8573x-21.885 | y=-3.433x-5.9826 | 20 | 21 | 0.00283 | 0.07202 |
| | SMC | Liver | y=-0.9506x-13.011 | y=1.6368x-28.067 | 19 | 20 | 0.03281 | 0.02641 |
| 2012 | LGR | Liver | y=-0.3954x-16.161 | y=0.0171x-19.14 | 20 | 19 | 0.13198 | 5.70E-05 |
| | MOB | Muscle | y=1.8351x-25.615 | y=5.3393x-36.871 | 20 | 20 | 0.00899 | 0.11987 |
| | MOB | Liver | y=0.1281x-19.281 | ND | 14 | ND | 0.01105 | ND |
| | MTB | Muscle | y=-0.8379x-14.962 | y=-2.3169x-9.8092 | 20 | 20 | 0.412 | 0.12796 |
| | MTB | Liver | y=-0.3407x-17.556 | ND | 17 | ND | 0.52794 | ND |
| | SMC | Liver | y=-1.4461x-9.615 | y=-2.5356x-10.062 | 15 | 19 | 0.63888 | 0.05667 |
| | TB | Muscle | y=5.3348x-34.637 | y=-21.279x+53.875 | 3 | 6 | 0.0385 | 0.85789 |
| | TB | Liver | y=0.1077x-17.16 | y=-1.1115x-11.306 | 3 | 6 | 0.6292 | 0.61219 |

Table B8 (continued): Supporting relationship between C:N ratios and $\delta^{13}C$ in liver and muscle of male and female white sucker from Little Gravel River, Sawmill Creek, Mountain Bay, Moberly Bay, Tunnel Bay, and Jackfish Lake.

| | | | Male Formula | Female Formula | Male (n) | Female (n) | Male (R-squared) | Female (R-squared) |
|---|---|---|---|---|---|---|---|---|
| 2013 | JFL | Muscle | y=-33.346x+80.446 | y=13.861x-73.174 | 4 | 12 | 0.37766 | 0.08893 |
| | JFL | Liver | y=4.6212x-50.767 | y=3.1966x-43.249 | 4 | 12 | 0.307 | 0.25836 |
| | LGR | Muscle | y=-0.6735x-15.769 | y=-1.339x-13.733 | 19 | 20 | 0.06273 | 0.19949 |
| | LGR | Liver | y=0.2366x-21.863 | y=-0.4896x-18.237 | 19 | 20 | 0.07047 | 0.01695 |
| | MOB | Muscle | y=6.7165x-40.019 | y=-6.168x+1.3706 | 20 | 20 | 0.07392 | 0.01502 |
| | MOB | Liver | y=0.251x-23.543 | y=-0.6123x-18.18 | 20 | 20 | 0.04241 | 0.13059 |
| | MTB | Muscle | y=-3.1346x-7.3191 | y=1.2585x-22.203 | 20 | 20 | 0.20676 | 0.0129 |
| | MTB | Liver | y=-0.1211x-18.982 | y=-0.0126x-19.316 | 20 | 20 | 0.01388 | 0.00019 |
| | SMC | Muscle | y=2.6871x-29.372 | y=6.4861x-42.404 | 32 | 33 | 0.05557 | 0.08595 |
| | SMC | Liver | y=-0.1939x-20.735 | y=1.7713x-30.886 | 32 | 33 | 0.00402 | 0.0514 |
| | TB | Muscle | y=-5.1009x-0.3042 | y=16.608x-73.68 | 19 | 20 | 0.02233 | 0.06457 |
| | TB | Liver | y=-0.754x-14.154 | y=-0.2004x-17.799 | 18 | 20 | 0.43452 | 0.01061 |