Otoliths as Indicators of Trace Element Exposure in Freshwater Fish: A Mesocosm Experiment with Manganese and an Examination of Hydro-Impoundment on Otolith Trace Element Signatures

by

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ABSTRACT

Through biomonitoring, organisms are measured to determine levels of contamination or exposure. In freshwater, biota like fish are used to represent whole communities due to their ecological/commercial relevance. In fish, soft tissues are typically used for trace element analyses although their potential for depuration, transformation, and contaminant re-compartmentalization makes them applicable for only short-term biomonitoring. Alternatively, metabolically inert calcified tissues (e.g., otoliths) have been found useful in long-term trace element biomonitoring. Biomonitor utility was demonstrated through two studies. The first being a mesocosm study on baitfish species exposed to $MnSO_4$ in which otolith chemical signatures were compared with the ambient mesocosm environment. Under study conditions, fish otolith biomonitors were ineffective at detecting manganese. The second study utilized fish otoliths to measure the effect of impoundment by comparing water and otolith trace element concentrations between impounded and non-impounded waterbodies. Otolith signatures successfully discriminated based on impoundment status and species.

Preface (CAMP and MnOTOL)

The layout of this thesis follows a layered design (“sandwich”) by which chapters two and three are organized as separate manuscripts in preparation for publication. Thereby chapters two and three each have their own abstract, introduction, materials/methods, results and discussion sections. Each chapter was written and presented by the first author, Adam Vanderpont.

Each of the authors contributed a sizable amount of work and effort to each manuscript. Norman Halden gave expert insight into otolith-based analyses, and oversaw LA-ICP-MS operations; Feiyue Wang gave expert insight into trace element chemistry and oversaw SO-ICP-MS operations; Norman Kenkel gave expert multivariate statistical guidance concerning my biological data; Mark Hanson was my primary supervisor throughout the entirety of my research project. To be submitted in 2018.

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Table of Contents

List of Figures

List of Tables

CHAPTER 1:

General Introduction and Literature Review

1.1. Biomonitoring

The overall fitness of an aquatic organism can be adversely effected by toxicants such as metals (Barbee et al., 2014) or the portion thereof which is bioavailable (Wagner & Boman, 2003). Metal exposure can adversely affect fish behavior (e.g., avoidance, food collection, reproduction and social structure) and various metabolic functions (reviewed in Scott and Sloman, 2004). Aquatic biomonitors can be organisms collected from marine, coastal, riverine and lacustrine environments that accumulate toxicants such as trace metals (Rainbow & Phillips, 1993). Biomonitors are valued since they can be used as time-integrated measures of the bioavailable portion of contaminants providing ecotoxicological relevance to exposure assessments (Birungi et al., 2007; Rainbow & Phillips, 1993).

An effective biomonitor should meet certain requirements. These include, but are not limited to things such as being well researched, should accumulate contaminants at measureable levels, be easy to handle in lab or field, abundant within the environment, easily identified and sampled, be relatively sedentary, and tolerant to variation within the environment (Butler et al., 1971; Philips, 1980; Sanchez-Jerez et al., 2002). Results obtained from the biomonitor should also be able to be used to discriminate between locations, and reflect abiotic compartments of the environment (Linde et al., 1998). Within the aquatic environment there are various examples of common biotic biomonitors such as macroalgae, seagrasses, crustaceans, mussels, oysters, polychaetes (reviewed in Rainbow and Phillips, 1993), invertebrates (Sanchez-Jerez et al., 2002) and fish (Friedrich & Halden, 2011). In general, biota is considered better biomonitors than those abiotic. For example, sediments are more dynamic and can be

removed from study or target areas due to disruptions (e.g., flooding) unlike biota (Linde et al., 1998).

There are several important factors that need to be considered with biomonitors for trace metal abundances. To be confident that target trace elements are being identified it is recommended that a suite of biomonitors be utilized at each test location since trace elements can be taken up to differing degrees in separate biomonitors (Rainbow & Phillips, 1993; Ruelas-Inzunza & Paez-Osuna, 2000). Even if a group of biomonitors has a common feeding mechanism, the amount of detected trace element may vary. In a study with filter feeding oysters (*Crassostrea corteziensis*), mussels (*Mytella strigata*) and barnacles (*Fistulobalanus dentivarians*), trace element levels varied significantly between them indicating that a suite of biomonitors would lead to a greater likelihood of detecting all target trace elements (Ruelas-Inzunza & Paez-Osuna, 2000). Adequate monitoring durations to account for temporal patterns is also suggested (Rainbow & Phillips, 1993).

There are multiple tissues within the fish that are used for metal biomonitoring purposes. Typical fish tissues used in biomonitoring are soft tissues such as muscle, gills, kidney, and liver and hard-part tissue like otoliths (Birungi et al., 2007; Friedrich & Halden, 2011; Ranaldi & Gagnon, 2009; Wagner & Boman, 2003). Soft fish tissues such as liver has been found to accumulate more trace elements than muscle, gill and kidney making liver the most useful biomonitoring soft tissue for detection purposes (Birungi et al., 2007; Ranaldi & Gagnon, 2009; Wagner & Boman, 2003). However, body content (and in extension soft-tissue) trace element content is based upon uptake and loss of metal from the body (Birungi et al., 2007) due to soft tissue depuration, metabolic

transformation, and contaminant re-compartmentalization, resulting in soft tissues only being useable for short-term studies (Campana, 1999; Palace et al., 2007). An alternative to soft tissue is the use of otoliths, which are calcified structures which incorporate trace metals (Campana, 1999) and are considered indicators of sub-lethal contamination levels (Geffen et al., 2003). Otoliths have been used in fisheries biology to interpret migration, and age fish (Babaluk et al., 1997; Halden & Friedrich, 2008; Thorrold et al., 1998). Lastly, fish otoliths hold potential for long-term monitoring for trace element exposure within ecotoxicology since considered metabolically inert (Campana, 1999; Campana & Neilson, 1985).

1.2. Otolith: Composition and Type

The primary structure of an otolith consists of alternating protein matrix and calcium carbonate layers, forming a banding pattern of annual rings analogous to dicot plants (Degens et al., 1969), resulting in the use of otolith as a fish aging tool (Kalish, 1989; Muir et al., 2008; Sadovy & Severin, 1992). The otolith is the only fish bone composed of calcium carbonate ($CaCO_3$), while others are composed of calcium phosphate (Murayama et al., 2002). Calcium makes up ~38% of otolith weight with $CaCO_3$ comprising the majority of the otolith, leaving other elements comprising <1% (Campana, 1999). In terms of $CaCO_3$ polymorphs (crystalline configurations) there are three and they are calcite, vaterite, and aragonite (Campana, 1999). Otoliths were also found to be composed of ~96% aragonite (Campana et al., 1997). Vaterite has been attributed to occur in otoliths due to stress experienced by the fish (e.g., hatchery growth) (Halden & Friedrich, 2008; Melancon et al., 2005) or attributed to genetic and

biochemical variations (Gauldie, 1986). There is a maximum of three otolith pairs per fish, namely the sagitta, lapillus, and astericus (Campana, 1999; Melancon et al., 2009). The most used otoliths are sagittal otoliths for micro-chemical and microstructure analyses since largest and easily located (Campana, 1999; Campana & Neilson, 1985; Campana & Thorrold, 2001; Popper et al., 2005; Pracheil et al., 2014).

Some fish species seem to contain greater proportions of vaterite naturally. For example, in lake trout (*Salvelinus namaycush*) (Melancon et al., 2005) and chinook salmon (*Oncorhynchus tshawytscha*) (Gauldie, 1986) aragonite and vaterite were found to grow simultaneously in otoliths. The concentric ring patterns produced by aragonite and vaterite in the otolith differ with vaterite being less uniform and more difficult to age (Melancon et al., 2005). Each polymorph has different partition coefficients for trace elements in lake trout (*Salvelinus namaycush*) (Melancon et al., 2005). Of the measured elements: Li, Rb, Mn, Mg, Zn, Sr, and Ba the concentrations of Sr, and Ba were greater for aragonite while vaterite had greater concentrations of Mg and Mn in both otolith cores and edges (Melancon et al., 2005). Li, Rb, and Zn showed no preference between polymorphs (Melancon et al., 2005). Thereby, based on cation radius, aragonite preferred incorporation of larger cations whereas vaterite preferred the incorporation of smaller cations (Melancon et al., 2005).

1.3. Otolith: Endolymph Function and Otolith Formation

Otolith formation occurs within the ear of the fish in which three semicircular canals make up the inner ear of teleost fish species (Popper et al., 2005). Inner ears are located within the cranial cavity on either side laterally and posterior to the brain (Popper et al., 2005). Located within each of the three semicircular canals is an endolymph (fluid filled cavity (Campana, 1999)) known as the saccula, utricula, and lagena associated with the fish otolith namely, the saggita, lapillus, and astericus respectively (Melancon et al., 2009). The method by which an otolith is formed is known as "biomineralization" (Campana, 1999). Compared to other structures that grow via mineralization such as vertebrate bones, corals, and mollusk shells, the otolith does not remain in direct contact with the calcification site. Rather, it instead remains suspended within and interacts with the (Campana, 1999).

Composed of an epithelial cell wall of varying thickness, the sacculus encases endolymphatic fluid and otolith (Fig 1.1). The epithelium can be divided into three types which are the sensory (SE), transitional (TE), and squamous (SQE) epithelium (Fig 1.1) (Takagi & Takahashi, 1999). The SE contains the thickest cells, with hair cells (covered in cilia) on the interior apical surface, and basal supporting cells (Popper et al., 2005; Takagi & Takahashi, 1999). Overtop of the SE resides the otolith membrane composed of subcupular meshing (S) and a layer of gelatinous material (G) which holds the otolith in place within the sacculus (endolymph) overtop the sensory cells (Takagi & Takahashi, 1999). The ciliated hair receptors are thus embedded in the otolithic membrane facing the otolith (Popper et al., 2005). When the hair receptor cilia bundles are stimulated (inertial movement of head or sound causing a difference of motion between fish body

and otolith), calcium channels are opened as a response, creating a wave of excitation down towards the basal SE cells, which releases neurotransmitters to the brain, alerting the fish of a mechanical event taking place (e.g., sound or movement) (Popper et al., 2005). Along the SE perimeter edge, columnar cells are present (Takagi & Takahashi, 1999). Beyond the SE, the TE begins which contain cylindrical cells with large intercellular spaces (Takagi & Takahashi, 1999). These TE cells were observed to become thinner the further away from SE they were located and eventually became SQE cells (Takagi & Takahashi, 1999). Within the TE, mitochondrion rich cells are also located (MRCs) (Takagi & Takahashi, 1999). SQE are identified by their cuboidal structure, which progressively becomes flatter the further away from SE they are located (Takagi & Takahashi, 1999).

Otolith formation characteristics (e.g., size and shape) depend on an organic template matrix, housing a uniform molecular a weight of >150,000 g/mol across species (Degens et al., 1969). This proposed primary component of the organic matrix between calcified layers and template for calcification is a collagen-like substance called otolin-1 (Murayama et al., 2002). Otolin contains high amounts of aspartic and glutamic acids, low amounts of aromatic and basic amino acids (Degens et al., 1969).The sacculus exclusive otolin-1 was found to concentrate in the gelatinous layer of the otolithic membrane, and TE cells adjacent to SE (Murayama et al., 2002). It is unknown if otolin-1 is deposited onto the otolith through the gelatinous layer (Murayama et al., 2002).

It was determined that the SE < TE (not MRCs) < SQE cells produce and secrete otolith soluble matrix (OSM) material into the endolymph where it incorporates onto the

otolith of 100-200g rainbow trout (Takagi & Takahashi, 1999), while SQE < TE alone did so for rainbow trout just-hatch fry. A total of two types of secretory vesicles are excreted into the endolymph. These are the cylindrical TE which excrete small vesicles (exocytosis) whereas SQE excrete large vesicles (cytoplasm extrusion) and cuboidal type TE cells as fibrous material (Takagi, 2000). Carbohydrates such as N-acetylglucosamine (GlcNAc) and mannose (Man) are also produced in the saccular epithelial cells except MRCs (Takagi et al, 2000). The deposition pattern of the organic matrix followed a pattern of loose then tightly packed matrices (Takagi, 2000).

Growth of otolith aragonite is related to the aragonite saturation state in the endolymph (Takagi, 2002). In particular, pH (correlated to CO_3^{-2} levels in otolith) in the sacculus was found to be the significantly correlated with aragonite precipitation (supersaturation ratio > 1) onto the otolith (Takagi, 2002). Thereby, stable pH promotes aragonite precipitation onto the otolith, but as aragonite precipitation progresses, CO_3^{-2} decreases, causing more HCO_3^{-1} to produce and associated protons (H^+) leading to a likely mechanism of excess H^+ removal from the endolymph (Takagi, 2002).

1.4. Otolith: Growth Factors

Otolith growth is regulated by available material in the endolymphatic fluid (Melancon et al., 2009). The primary pathway of freshwater fish trace element incorporation into the otolith is through the gills (branchial uptake), then blood plasma, then endolymph, in which the inorganic material crystallizes on to the otolith (Campana, 1999). Comparatively, marine fish regulate incoming elements through ingestion (intestinal processes) over respiration (Melancon et al., 2009). Ingestion carries trace

elements into the blood plasma, endolymph and then otolith (Melancon et al., 2009). The primary constituents of the otolith (like calcium carbonate) are derived from the aquatic medium rather than respiration (e.g., CO_2) (Degens et al., 1969).

The size of the fish and its relation to the otolith was found to depend to a degree based on certain fish characteristics. A study of 247 marine fish species (~1% of global fish species), found that the majority of species failed to correlate with a few exceptions (Paxton, 2000). One exception was with the order Anguiliformes (true eels), in which otoliths were small, indicative of greater reliance on smell than hearing (Paxton, 2000). Also, fish from epipelagic habitats showed trends towards smaller otoliths (Paxton, 2000). Larger otoliths were somewhat associated sound producing fish (e.g., swim bladder vibrations (Veerappan et al., 2009)), low-light conditions, and luminescent properties (Paxton, 2000). Luminous counterparts of non-luminous species typically had larger otoliths (Paxton, 2000). Luminous fish develop light emitting structures (photophores) for low-light environments (Paxton, 2000).

Differences in biology and physiology within a population can change the relationship between otolith size and fish growth/length. A study with goldfish (*Carassius auratus*) determined a relationship between otolith (increments/morphology) and somatic growth (fish length) (Mugiya & Tanaka, 1992). Otolith growth (length) slowed relative to body standard length as the goldfish aged (Yasuo Mugiya & Tanaka, 1992). Growth rates also differed between the three isolated somatic groups within the same species (slow, medium, and fast) (Mugiya & Tanaka, 1992). Slow growth fish had larger otoliths than fast growth fish of equal body length (Mugiya & Tanaka, 1992). These constraints are likely due to biological and physiological means (effecting protein matrix

and calcification events) (Mugiya & Tanaka, 1992). Thereby, when considering the effect of growth rate on otolith size (Mugiya & Tanaka, 1992), growth rates should be considered and may not have been accounted for in Paxton's (2000) study.

1.5. Otolith: Aging and Banding

When an otolith is observed under reflected light, alternating light and dark bands are observed to summer and winter growth periods and/or nutrient supply (Degens et al., 1969), which, when combined form yearly banding patterns or annuli. Under high magnification, it is even possible to see monthly and daily increments (Panella, 1971). Otolith increments are connected with an endocrine driven, endogenous circadian rhythm which is established at birth by photoperiod (Campana & Neilson, 1985). Each daily growth increment is made of one thick inorganic/calcified fiber rich light band and one thinner dark band which is more dense and contains more organic materials and fibers (Panella, 1971). It has been found that environmental cues can mask the latter endogenous circadian rhythm of otolith formation, but does not affect the underlying trend (Campana & Neilson, 1985). Light and temperature were not found to effect the daily increments of juvenile starry founder (verified by tetracycline injection) otoliths in a lab setting and matched those tested in the field setting as well (maintain circadian rhythm) (Campana & Neilson, 1982). It was noted that temperature and photoperiod may still effect other species physiological cycles or processes however, and that fish age may also play a role (younger/larval fish may be more sensitive) (Campana & Neilson, 1982). Through the latter hypothesis, temperature (potentially associated with photoperiod) may have one of the strongest masking effects (Campana & Neilson,

1985). It has been found that otolith growth in the form of otolith band widths are greatest before sexual maturity (somatic or body growth), and narrow post maturity due to reallocation of resources to other processes e.g,, reproductive growth (Watkins & Spencer, 2013). In addition, anaerobic stress has been found to significantly decrease labelled calcium isotope retention in goldfish otolith and in blood plasma (mechanism hypothesized either as Ca mobilization from the otolith or inhibition of Ca deposition), but rate of Ca deposition was not significantly different (Mugiya & Uchimura, 1989). In another study with juvenile alewife (*Alosa pseudoharengus*) stress was found to decrease the otolith increment width associated with an oxygen deficiency and high temperatures (Limburg et al., 2015).

One common identifier of stress, interruptions (e.g., sexual maturity), or disturbances that can occur to the fish that affects otolith growth is known as a check. A check is an anomalous discontinuity of calcium and protein within the otolith structure (Campana & Neilson, 1985). An issue with check development is their visual similarity to actual annuli, leading to improper age determination (Beamish, 1981). Typically, checks can be differentiated from actual annuli by being less prominent, discontinuous banding, and being situated close to true annuli (Beamish, 1981). It should be noted however that checks and discontinuous zones due to anaerobic stress have yet to be linked to physiological endolymph alteration such as Ca resorption from otolith, to endolymph, then the rest of the body via acidification (Mugiya & Uchimura, 1989). Checks can thereby lead to decreased aging accuracy and trace element measurement potential across bands.

1.6. Otolith: Pathways and Incorporation

1.6.1. Trace Element Incorporation

Understanding the methods by which trace elements incorporate into the otolith and what factors influence incorporation are of paramount importance when interpreting measures within the otolith. The three main otolith incorporation methods are Ca substitution, interstitial space inclusion, and/or association with the protein matrix (Campana, 1999). Element radii and charge are most important considering trace element incorporation e.g., elements with similar radii to Ca and +2 ionic charges are more likely to substitute for calcium (+2 charge) in the otolith (such as Mn, Mg, Sr, and Ba) (Melancon et al., 2005).

Trace elements and their signatures within the otolith are not equally useful at identifying, and discriminating between groups or individuals. At least 57 elements have so far been detected in otoliths (Campana, 1999; Palace et al., 2007; Pracheil et al., 2014). Of those elements, Ca, C, and O were found to make up the majority of the otolith, with other elements in relatively low abundances (around 100 ppm for Na, Cl, K, S, N, P, and Sr), or trace element abundances (< 10 ppm) (Campana, 1999). In terms of published literature, the most commonly studied elements in hard parts (in descending order) are quite similar between marine (Sr, Ba, Mn, Mg, Pb, Zn) diadromous (Sr, Ba, Mg, Cu, Mn, Pb) and North American freshwater obligate fish studies (Sr, Ba, Mn, Mg, Zn, Pb) (Pracheil et al., 2014). Unfortunately, the otolith has been found to limit the inclusion of many abundant elements more so than other tissues (Campana, 1999). Many elements measured within the otolith seem not to reflect ambient concentrations of the water (e.g., salinity) such as Ca, Na, K, Mg, Cl, P, Cu, and S, but seemingly

reflect osmoregulation characteristics of the blood-plasma interface with the endolymph, gills and intestine due to organismal utilization (Campana, 1999). Sizable detection of elements such as Hg and Pb tend reflect anthropogenic sources, and Sr, Zn, Pb, Mn, Ba, Fe, Li, Cd, Ni have also been associated with natural sources (Campana, 1999). This is because the latter trace elements are likely less utilized and regulated within the fish (Campana, 1999). Friedrich and Halden (2010) reported elevated and overlapping Pb, Cu and Zn concentration peaks in otoliths may be attributed to exposure to mine tailings. It should be noted however that environmental availability is not the only factor that influences the uptake of elements into the otolith (Campana, 1999) and that various barriers and processes effect trace element uptake into the otolith such as gill uptake, brachial uptake, internal transport and crystallization (Melancon et al., 2009). For example, in a study by Kalish (1991), fish development was found to effect uptake and incorporation of trace elements in a seasonal fashion causing changes to feeding habits, leading to chemical partitioning into the endolymph and by extension the otolith (Kalish, 1991). These physiological changes in blood plasma and endolymph were also been associated with seasonal variation and temperature change. More specifically, seasonality was found to effect abundances of various metal binding proteins and thus metal binding capacity, which then leads to impacts on endolymph and otolith metal incorporation (Kalish, 1991). Other factors have also been found to effect trace element incorporation. Within the literature it is thought that aragonitic structures such as otoliths or bivalve shells have the ability to incorporate various trace elements but is dependent to some degree on ontogeny, metabolic rate, somatic growth and/or temperature, with the main factor argued upon within the literature (Geffen et al., 2003; Hoff & Fuiman,

1993; Javor & Dorval, 2016; Ruttenberg et al., 2005; Takesue et al., 2008). When considering growth rates specifically, changes in fish length (Geffen et al., 2003; Javor & Dorval, 2016; Sadovy & Severin, 1992), otolith weight (Javor & Dorval, 2016), age (Kalish, 1989; Papadopoulou et al., 1980; Rashed, 2001) and body weight (Hoff & Fuiman, 1993) have been some of the main fish measures attributed trace element uptake and incorporation. Although, other studies have failed to observe altered incorporation by growth (Ranaldi and Gagnon, 2008), in the form of age (with fish of similar size) (Kalish, 1989; Kingsford & Gillanders, 2000) or fish length (K. Pangle, unpublished data in Pangle et al., 2010).

The effect of temperature on otolith growth may not run in parallel with somatic growth and may instead be associated with metabolic rate as the main factor (Hoff & Fuiman, 1993). Increasing temperature was found to increase the incorporation of both organics, inorganics, and overall density but the effect has been considered small compared to other changes taking place over a fishes lifetime in juvenile red drum (*Sciaenops ocellatus*) (Hoff & Fuiman, 1993). These variations in detected trace element levels within the otolith are considered to be due to factors exclusive to individuals such as RNA/DNA ratios, growth, and kinetics (Kalish, 1989). In another example, a study with Atlantic croaker (*Micropogonias undulatus*), individuals held at lower temperatures were smaller than croaker grown at higher temperature regimes (weight and length) (Fowler et al., 1995). Lastly, larval spot (*Leiostomus xanthurus*) somatic growth rates were significantly affected by tank temperature ($F= 4.52$, $p< 0.05$, $n= 24$) and salinity ($F= 11.43$, $p< 0.05$, $n=24$) (Martin et al., 2004). In contrast, in a study

with Atlantic croaker (*M. undulatus*) salinity had no apparent effect on growth (Fowler et al., 1995) indicating the complexity of the temperature relationship.

Differences in incorporation can also occur between species, for example two sea species, plaice (*Pleuronectes platessa*) and whiting (*Merlangius merlangus*) were found to accumulate differing amounts of heavy metals, likely due to differences in behavior, physiology, diet, otolith formation and habitat (Geffen et al., 2003). Trace element variability also occurs between individual otoliths within the same species or location (Kalish, 1989) adding to the complexity of incorporation. Lastly, variation in trace element detection can occur throughout separate annuli within an individual (e.g., comparing core and edge, or as the fish ages) (Brophy et al., 2004; Friedrich & Halden, 2010, 2011, Limburg et al., 2011, 2015; Ruttenberg et al., 2005). Thereby the following review will identify many of the factors influencing the incorporation of trace elements pertinent to this study, namely Na, Mg, Mn, Ba, and Sr into otoliths.

1.6.2. Sodium Incorporation

Since sodium (Na) is biologically mediated (Campana, 1999), it will likely not represent the ambient external environment. Na incorporation and its relation with temperature is also contested within the literature from having an inverse non-linear relationship (Hoff & Fuiman, 1993), to no relationship (Kalish, 1989) dependent on species. Lastly, the ratio of Na:Ca within the otolith was found to decrease with increasing otolith density and decreasing organic content indicating that Na mainly incorporates into the organic otolith fraction (Hoff & Fuiman, 1993).

1.6.3. Magnesium Incorporation

Magnesium's incorporation variability can be attributed to various factors such as change in growth or temperature, upregulation within the fish (e.g., blood plasma) and instability within aragonite and has led to Mg in fresh or saltwater otoliths to infrequently correlate with water (Campana, 1999; Clarke et al., 2007; Dorval et al., 2007; Hoff & Fuiman, 1993; Javor & Dorval, 2016). In a laser ablation based study conducted on westslope cutthroat trout (*Oncorhyncus clarki lewisi*) from the Coeur d'alene River, Idaho, a significant positive correlation between water and otolith were established, due to sufficient water chemistry variation within the study system (R^2= 0.39, p= 0.0003) (Wells et al., 2003). The incorporation location of Mg within aragonite is associated with ion substitution and/or co-precipitation with Ca mainly (Campana, 1999). Of the two main fractions of calcified aragonitic tissue, incorporation occurs mainly in the inorganic fraction, with 33 ± 10% attributed to the organic component of bivalve shells (although Mg/Ca did not change in the largest shell tested after organic content removal) (Takesue et al., 2008). In the latter case, bivalve aragonite was used as a proxy for otolith aragonite. In terms of instability, in red drum (*S. ocellatus)*, otolith density increased and Mg concentration decreased with age, with density being nearly inversely proportional to organic content (Hoff & Fuiman, 1993). Also, using Pacific sardine (*S. sagax*), it was found that Mg:Ca decreased with increasing fish growth, and was thereby considered to not be conserved within the otolith (Javor & Dorval, 2016). Mg incorporation was also effected by fish length in Irish sea plaice (*P. platessa*) during a field study (Geffen et al., 2003) and correlated to otolith weight (considered somatic growth), for the spotted seatrout (*Cynoscion nebulosus*) (Dorval et al., 2007). Diet

effects were not observed for Mg otolith concentrations (fish prey had 23% more Mg content than shrimp prey, but was not considered significant) for bluefish (*Pomatomus saltatrix*) (Buckel et al., 2004). As it pertains to temperature multiple lab studies using marine fish have found temperature to have an insignificant effect on Mg/Ca otolith ratios (Elsdon & Gillanders, 2002; Javor & Dorval, 2016; Martin et al., 2005). Also, multiple lab studies using marine fish found that salinity insignificantly effected Mg/Ca otolith ratios (Dorval et al., 2007; Elsdon & Gillanders, 2002; Martin et al., 2005). In general, Mg incorporation and retention is influenced mainly by fish growth and less so by salinity or temperature.

1.6.4. Manganese Incorporation

Manganese incorporation is influenced by growth, ambient water concentration, redox potential, temperature, salinity, and diet. Within aragonitic component of bivalve shells, 78 ± 7% of manganese was found to incorporate in the organic fraction (Takesue et al., 2008). On top of the latter, due to the lack on consistent correlation between aragonitic carbonates and Mn incorporation is considered to be aligned more with interstitial apace and inclusion or association with the protein matrix of the otolith (Campana, 1999). Beginning with growth, a positive correlation was found between otolith Mn:Ca and otolith weight bulk measurements (correlated with standard fish length: R^2= 0.965) in Pacific sardine (*Sardinops sagax*) (Javor & Dorval, 2016). Also, daily growth rate corresponded with alewife (*A. pseudoharengus*) otolith Mn:Ca ratios (Limburg et al., 2015). Additionally, an increase in stress (hypoxic conditions) was found to cause a decline in growth rate and otolith Mn:Ca, indicating that growth rate mediates

Mn incorporation through bulk and spatially resolved measures (Limburg et al., 2015). Beyond growth, the incorporation of Mn into the otolith was found to reflect the ontogeny of diverse species where enrichment of Mn by 100-fold occurred in otolith cores versus outer annuli through a LA-ICP-MS bore transect experiment (Ruttenberg et al., 2005). Lastly, otoliths were found to better convey physiological history rather than the environmental exposure history of rainbow trout (*O. mykiss*) and Atlantic croaker (*M. undulatus*) through a bulk measure analyses (Gibson-Reinemer et al., 2009; Hanson & Zdanowicz, 1999).

In terms of otolith Mn correlation with water, it was found that the correlation varied from a non-linear correlation in spotted seatrout (*C. nebulosus*) (Dorval et al., 2007), to positive correlations in field studies using juvenile striped bass (*Morone saxatilis*) otolith, associated with base geology (Mohan et al., 2012), Arctic greyling (*Thymallus arcticus*) (R^2= 0.11, $P < 0.05$) (Clarke et al., 2007) and slimy sculpin (*Cottus cognatus*) (R^2= 0.353, $F_{1,5}$= 29.2, $P < 0.01$) (Clarke et al., 2015), to no correlation with Mn for rainbow trout (*Oncorhynchus mykiss*) (Gibson-Reinemer et al., 2009), arctic grayling (*T. arcticus*) again (R^2= 0.152, $F_{1,5}$= 2.64, P= 0.17) (Clarke et al., 2015) or for juvenile black bream (*Acanthopagrum butcheri*) even at Mn water concentrations 16-times ambient (Elsdon & Gillanders, 2003). Also, Forrester, (2005) found in a study of longjaw mudsucker (*Gillichthys mirabilis*), a sedentary estuary fish that otolith Mn correlated with water but not sediment indicating that otoliths have the potential to reflect the environmental medium although factors can alter the relationship. The lack of correlation in the latter cases were likely due to changes in the strength of redox reactions through time (Dorval et al., 2007), the variability in regulation of Mn between

otolith from separate fish, the change in concentration of Mn within the environment (Elsdon & Gillanders, 2003) or other factors such as water hardness (Stubblefield et al., 1997) . For example, Moreau et al., (1983) found that manganese content within the scale and opercula of brook trout (*Salvelinus fontinalis*) was 1.6 times greater in acidic lakes of pH 5.2-5.5 than neutral lakes of pH 6.8-7.0 due to redox sensitivity (Moreau et al., 1983).

Based on multiple marine fish studies and bulk measurements, temperature was found not to affect Mn:Ca otolith ratios (Elsdon & Gillanders, 2002; Martin et al., 2005) while others found a positive correlation (Pacific sardine (*S. sagax*)) (Javor & Dorval, 2016). Also, considering salinity, marine lab studies using juvenile black bream (*A. butcheri*) and larval/juvenile spot (*L. xanthurus*) respectively) found that salinity did not affect Mn:Ca otolith ratios (Elsdon & Gillanders, 2002; Martin et al., 2005).

Diet has been found to be another important incorporation factor. It was found that detritus, amphipod, polychaetes and leaf litter correlated significantly with otolith Mn content for juvenile trumpeters (*Pelates sexlineatus*) indicating the importance of trophic transfer in otolith Mn (Sanchez-Jerez et al., 2002). The latter was also found to be the case for walleye (*Sander vitreus*) and northern pike (*Esox lucius*) from Northern and Southern Manitoban locations in Canada in which Mn otolith chemical signatures associated with dietary factors and through extension, environmental background (Friedrich & Halden, 2010). Increases in otolith Mn also associated with increased temperature, summer feeding and growth periods (Limburg et al., 2015).

Using microbeam analysis, the Mn signature in otoliths has been found to display an oscillatory signature pattern, with intensity decreasing with increasing age and

decreasing growth for freshwater walleye, pike, Arctic char (Friedrich & Halden, 2010, 2011), yellow perch (*Perca flavescens*) (Limburg et al., 2015) and marine fish species Atlantic cod (*Gadus morhua*), European flounder (*Platichthys flesus*) and alewife (*A. pseudoharengus*) (Limburg et al., 2011, 2015). For LA-ICP-MS based experiments, an identified averaging effect may attribute to the decline in Mn with increasing age since bands become shorter with age, leading to less detection potentially (Limburg et al., 2011).The Mn signature spikes can also identify hypoxia or de-oxygenated environments for various fish species (Limburg et al., 2011, 2015). For example, Mn:Ca ratios in the zone corresponding to the first year of life increased within increasingly hypoxic areas ($R= 0.65$, $P < 0.001$) for Atlantic cod (*G. morhua*) (Limburg et al., 2011).

1.6.5. Barium Incorporation

Otolith Ba incorporation is controlled by many complex factors and is highly variable (Javor & Dorval, 2016). Ba is known to have a high depuration rate and low assimilation potential in organisms (Hope et al., 1996). Ba also bio-accumulates and concentrates in aquatic and terrestrial ecosystem, does not biomagnify and has low toxicity (Hope et al., 1996). The Ba incorporation location on the calcified hard part is mainly the aragonitic fraction, but may vary with age and exposure level (Takesue et al., 2008). The Ba partition coefficient from water to otolith was not found to be affected by temperature for larval spot (Bath et al., 2000), or metabolic rate and otolith precipitation in larval/juvenile spot (*L. xanthurus*) (Martin et al., 2005). Ba incorporation was effected by fish length for marine Irish sea plaice (*P. platessa*) (Geffen et al., 2003). Ba signature also displayed an oscillatory signature pattern run under LA-ICP-MS, with intensity

decreasing with increasing age (Friedrich & Halden, 2010, 2011). Otolith Ba:Ca levels were elevated as compared to Sr:Ca in early years but tracked each other in later years (Limburg et al., 2011).

The consensus reached for the water to otolith Ba relationship is that the otolith reflects ambient Ba. This was supported by numerous studies with species such as the spotted seatrout (*C. nebulosus*) in which otolith Ba increased with water in a non-linear fashion (Dorval et al., 2007), to larval spot (*L. xanthurus*) (Bath et al., 2000), juvenile Black bream (*A. butcheri*) in laboratory renewal experiments (Elsdon & Gillanders, 2003), juvenile striped bass (*M. saxatilis*) in a field experiment (Mohan et al., 2012) and freshwater field studies for Arctic grayling (*T. arcticus*: R^2= 0.76, P < 0.001) (Clarke et al., 2007), *T. arcticus*: R^2= 0.934, $F_{1,5}$= 407, p < 0.001 (Clarke et al., 2015), slimy sculpin *(C. cognatus*: R^2= 0.974, $F_{1,5}$= 1160, p < 0.001) (Clarke et al., 2015), and westslope cutthroat trout (*O. clarki lewisi*: R^2= 0.71, p= 0.0001) (Wells et al., 2003) all displayed positive linear relationships.

Temperature effect on the incorporation of Ba into the otolith is contested within the literature. Some studies found that greater temperatures led to greater ambient Ba (Elsdon & Gillanders, 2002, 2004). No interaction was found to occur between temperature and salinity for Ba:Ca in juvenile black bream (*A. butcheri*) (Elsdon & Gillanders, 2004). In regards, to ontogeny and temperature, Atlantic croaker (*M. undulatus*) otolith Ba differed between core and outer otolith annuli (F= 8.7, DF= 2, 41, p=0.0007) due to temperature change influencing ionic inclusion (Fowler et al., 1995). In other studies with Atlantic croaker (Fowler et al., 1995a, 1995b) and larval spot (*L. xanthurus*) (Bath et al., 2000), no effect was observed, but was attributed to insufficient

temperature ranges (Elsdon & Gillanders, 2002). Although similar results were observed again even with a broader test range for larval/juvenile spot in another study (*L. xanthurus*) (Martin et al., 2005).

In terms of the effect of salinity on the incorporation of Ba into the otolith, the consensus is that salinity may play a role in Ba incorporation. Studies refuting the salinity effect found that the effect of salinity was minimal as compared to ambient water and temperature effects on incorporation into juvenile black bream (*A. butcheri*) otolith (Elsdon & Gillanders, 2002, 2004). Similar results were also found for larval/juvenile spot (*L. xanthurus*) (Martin et al., 2005). Comparatively, for spotted seatrout (*C. nebulosus*) salinity was inversely linearly correlated to Ba in a seagrass estuary habitat (Dorval et al., 2007) and juvenile striped bass (*M. saxatilis*) in riverine habitats (Mohan et al., 2012).

Researchers agreed that Ba otolith incorporation is diet related. In a seagrass field study, juvenile trumpeters (*P. sexlineatus*) otolith Ba correlated with seagrass components (Sanchez-Jerez et al., 2002). Also, in a bluefish (*P. saltatrix*) lab study, otolith Ba differed significantly due to diet with the shrimp diet containing 250% more Ba than the fish diet (Buckel et al., 2004). Lastly, in an arctic char (*Salvelinus alpinus*) field study, otolith Ba oscillatory signature was attributed to seasonal diet change since the fish remained in an restricted area (Friedrich & Halden, 2011).

1.6.6. Strontium Incorporation

Strontium incorporation into the otolith may be influenced by many factors such as growth, ambient water, temperature, salinity, and diet. Within aragonitic compounds

Sr has been found to incorporate exclusively into the inorganic portion (Takesue et al., 2008) with the amount of substitution of Ca by Sr into the otolith as a function of the partition coefficient (Martin et al., 2004). The relationship between temperature and Sr:Ca is considered more complex for otoliths than coral skeletons due to the series of isolative membranes or barriers (e.g., branchial, intestinal and endolymphatic) (Martin et al., 2004). Considering fish growth rate in general, white grunt (*Haemulon plumieri*) otolith Sr:Ca was negatively correlated (Sadovy & Severin, 1992). Growth rate was also found to positively associate with otolith density and Sr:Ca with decreased organic content further identifying that Sr incorporates into the aragonitic portion of the otolith (Hoff & Fuiman, 1993). Fish length was also found to significantly affect Sr incorporation into the otolith in some species of marine fish (Geffen et al., 2003; Sadovy & Severin, 1992). Otolith Sr:Ca decreased with fish length proxied by otolith weight but remained conserved within the otolith (Javor & Dorval, 2016). Sr was found to increase with age (Limburg et al., 2015). Comparatively, little evidence was found to support Sr:Ca otolith correlation with metabolic rate (Martin et al., 2004).

In terms of ontogenic effects, in a study with Atlantic croaker (*M. undulatus*) otolith Sr levels differed between core and outer edge annuli based on temperature change influencing ionic inclusion (Fowler et al., 1995). Also, a study on pacific tarpon (*Megalops cyprinoides*) leptocephali larvae, metamporphosis phase and juveniles found that during metamorphosis an endogenous physiological effect (ontogeny) took place reducing otolith Sr:Ca while unaffected by salinity or diet (Chen et al., 2008). Sr:Ca was found greatest in SI, decreased in SII, then stabilized in SIII associated with changes in

ontogeny (Chen et al., 2008). Comparatively, Ruttenberg et al., (2005) found that Sr:Ca did not to reflect ontogeny.

Researchers have mainly determined a positive relationship between otolith Sr:Ca and water such as for studies dealing with spot (*L. xanthurus*) and juvenile black bream (*A. butcheri*) (Bath et al., 2000; Elsdon & Gillanders, 2003). Similarly, Sr water chemistry and juvenile striped bass (*M. saxatilis*) otolith Sr were significantly positively correlated per site (Mohan et al., 2012). In a freshwater field studies, westslope cutthroat trout (*O. clarki lewisi*: R^2= 0.96, p= 0.0001) (Wells et al., 2003)**,** Arctic greyling (*T. arcticus*) and slimy sculpin (*C. cognatus*) otolith Sr correlated significantly with water concentrations (*T. arcticus*: R^2= 0.81, P < 0.001) (Clarke et al., 2007), (*T. arcticus*: R^2= 0.909, $F_{1,5}$= 113, P < 0.001, *C. cognatus*: $F_{1,5}$= 557; P < 0.001, R^2= 0.979) (Clarke et al., 2015). Ambient Sr outweighed the effect of salinity and temperature on otolith Sr incorporation for juvenile black bream (*A. butcheri*) (Elsdon & Gillanders, 2004). Contrarily, spotted seatrout (*C. nebulosus*) found no correlation (Dorval et al., 2007).

Conclusions over the effect of temperatures on otolith Sr:Ca are mixed within literature from having no relation for pacific sardine (*S. sagax*) (Javor & Dorval, 2016), and spotted seatrout (*C. nebulosus*) (Dorval et al., 2007) to having a positive non-linear relation for species such as red drum (*S. ocellatus*) (Hoff & Fuiman, 1993), Atlantic salmon (*Salmo salar*) (John M. Kalish, 1989) and white grunt (*H. plumieri*) (Sadovy & Severin, 1992), and black bream (*A. butcheri*) (Elsdon & Gillanders, 2002, 2004; Ranaldi & Gagnon, 2008), or a positive linear correlation with larval spot (*L. xanthurus*) in a laboratory renewal experiment making Sr a potential temperature marker based on Sr partition coefficients (F= 42.2, p<0.0001) and otolith Sr:Ca (F= 63.6, p < 0.001)

(Martin et al., 2004). Temperature has been interpreted as a dominant variable, rather than growth or precipitation rates, and was found independent of salinity (Martin et al., 2004). Otolith Sr:Ca ratios may also be water and temperature dependent, while Sr and Ca uptake are considered species specific (Martin et al., 2004). The latter contradicting the work by Fowler et al., (1995a) which found Sr to be a poor temperature indicator.

Conflicting results about the relationship between salinity and incorporation of Sr have been found within the literature. In a field study using spotted seatrout (*C. nebulosus*) (Dorval et al., 2007) and a lab study with juvenile black bream (*A. butcheri*) no significant correlation with salinity was observed (Elsdon & Gillanders, 2002). Comparatively, a study of pacific tarpon (*M. cyprinoides*), salinity significantly affected otolith Sr:Ca during metamorphosis SII ($P<0.01$) and post metamorphosis SIII with interaction ($p<0.01$) (Chen et al., 2008). The post-metamorphosis phase of pacific tarpon otoith Sr:Ca was also positively influenced by ambient salinity (Chen et al., 2008). Salinity was found to have an additive effect on Sr:Ca ratios (Chen et al., 2008). Lastly, a significant positive linear relationship with salinity and larval spot (*L. xanthurus*) otolith Sr ($F= 7.42$, $p= 0.015$) and Sr partition coefficient ($F= 61.8$, $p< 0.0001$) were determined (Martin et al., 2004).

The consensus around diet and the incorporation of Sr into the otolith is that diet influences Sr incorporation. For example, in a study dealing with blue grenadier (*Macruronus novaezelandiae*) greater otolith Sr:Ca was attributed to summer feeding leading to faster growth (Kalish, 1989). Also, in a study dealing with bluefish (*P. saltatrix*), fish fed shrimp diets (containing 280% more Sr than the fish diet) led to greater Sr in shrimp-fed bluefish otoliths (Buckel et al., 2004). Also, a study dealing with

pacific tarpon (*M. cyprinoides*) the feeding regime significantly affected otolith Sr:Ca during metamorphosis SII ($p<0.001$) and post metamorphosis SIII with a salinity interaction ($p<0.05$) (Chen et al., 2008). However, some studies did not support the relationship such as for juvenile barramundi (*Lates calcarifer*) (Milton & Chenery, 2001). Also, in a seagrass meadow study, juvenile trumpeters (*P. sexlineatus*) otolith Sr did not correlate with seagrass meadow components (Sanchez-Jerez et al., 2002). Strontium (Sr) chemical signatures have great utility such as being used to discern between migratory and non-migratory fish (Babaluk et al., 1997).

. After analyses via LA-ICP-MS, if the resulting Sr otolith trace metal signature is found to be flat, the fish is assumed to have remained within a freshwater environment for the duration of its life, typical of non-migratory fish, whereas a drastic change otolith Sr:Ca was observed to occur upon transition from freshwater and saltwater environments, indicating a migration (Halden & Friedrich, 2008). The absolute levels of Sr have also been noted to have potential use as key identifiers for specific water bodies or for stock discrimination (Babaluk et al., 1997; Clarke et al., 2007). Contrary to the latter, the Sr otolith incorporation may be a function of many factors, with minor oscillations reflecting temperature, diet, or fish metabolism changes (Buckel et al., 2004; Chen et al., 2008).

1.7. Otoliths Versus Other Aging Structures

Of the fish aging structures, the otolith has been considered the most accurate as compared to scale, opercula, vertebrae, and fin rays (Phelps et al., 2007). In a study with walleye (*S. vitreus*), age frequency distributions were constructed by comparing

fish length to the age of the various calcified age estimate structures (Donabauer, 2010). It was found that otoliths created the most feasible age distributions (normally distributed/narrowly defined tails) for both sexes (Donabauer, 2010). Also in another study, otolith and scale shape were used to distinguish fish stocks and it was found that otoliths were more effective (Casselman et al, 1981). Other aging structures do have benefits though, such as using pectoral fin ray in common carp (*Cyprinus carpio*), which is deemed as the best non-lethal alternative to otoliths (Phelps et al., 2007). Older fish are aged best by pectoral fin ray or otoliths as noted by Muir et al., (2008).

1.8. Hard-Part Bulk Chemistry: ICP-MS

Hard part chemistry techniques with fish involve otoliths, statoliths, scales, and fin rays primarily (Pracheil et al., 2014). In the current study two main chemical techniques were employed, namely Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) and Solution Based (SO-) ICP-MS. LA-ICP-MS uses a high-powered laser to ablate a portion of a hard-part (e.g., otolith) (Pracheil et al., 2014). The ablated sample is then carried by an inert gas (e.g., argon (Ar)), ionized in a plasma torch and then passed through mass spectrometer which determines atomic mass (based on mass:charge ratios) and counts of each element over time (Pracheil et al., 2014). In preparation for LA-ICP-MS it is common for the otolith to first be sectioned, exposing the nuclei and separate annuli to allow environmental exposure history over time to be anaylzed (Pracheil et al., 2014). Comparatively, SO-ICP-MS involves dissolution of a portion or the entire hard part (Pracheil et al., 2014). SO-ICP-MS is generally used in young of year, or larval fish studies containing small or less developed

otoliths (Pracheil et al., 2014). Whole otolith (e.g., SO-ICP-MS) assays are more precise, faster and can test samples of greater weight whereas beam-based assays (e.g., LA-ICP-MS) retain the exposure timeline of the fish (Campana, 1999) allowing for use in long-term monitoring.

1.9. Impoundments

1.9.1 Impoundments: An Introduction

Impoundments are a form of water retention used for purposes such as electricity generation, water storage, recreation and fishing practices. For the many benefits of impoundments, there are also many adverse impacts on things such as the global sediment and carbon cycles, and fish assemblages. There are currently > 45,000 registered impoundments (>15 m high) worldwide, the majority of which having been constructed after 1950 (World Commission on Dams, 2000). Other unregistered dams likely exist (Vörösmarty et al., 2003). One main abiotic impoundment impact on water systems is their influence on the transport of suspended material downstream towards the ocean (Quist et al., 2005; Syvitski et al., 2005; Vörösmarty et al., 2003). An impoundment causes water velocity/discharge reduction causing relative proportions of suspended particle levels (dependent on size) to change, which causes changes in channel morphology and substrate characteristics (Eiriksdottir et al., 2015; Eiriksdottir et al., 2017; Horowitz et al., 1990; Quist et al., 2005). On a global scale, global particulate/sediment flux to the oceans has decreased 26-53% (a flux reduction of 1-5 billion metric tons per year) via impoundment construction (Syvitski et al., 2005; Vörösmarty et al., 2003). In the last 50 years its estimated that >100 billion metric tons

of sediment and 1-3 billion metric tons of carbon have become stored within impoundments (Syvitski et al., 2005). However, it should be noted that not all reservoirs retain sediment to the same extent. Much of the world's largest river basins display nearly complete sediment retention (Vörösmarty et al., 2003). Larger reservoirs (≥ 0.5 km^3 max storage capacity) were found to have greater impact on trapping sediment or flux compared to smaller reservoirs with a retention time of 0.21 years (Vörösmarty et al., 2003). Comparatively, smaller reservoirs have less storage volume, intercept less discharge and drain smaller areas which support more modest residence times than larger reservoirs with a retention time of 0.011 years (Vörösmarty et al., 2003). It is debated whether more sediment is making it to the oceans because of increased impoundment construction and erosional processes (e.g., poor land management, riverbank erosion) (Syvitski et al., 2005; Vörösmarty et al., 2003).

1.9.2. Impoundments: Suspended Materials and Trace Elements

The amount of suspended sediment within fluvial water systems has been considered supply rather than discharge limited (Horowitz, 2008). Examples of the effects of impoundments on discharge is described by Eiriksdottir et al., (2017, 2015). It was found that there was a direct relationship between suspended inorganic matter and river discharge, but an inverse relationship between most dissolved elements and river discharge (Eiriksdottir et al., 2015, 2017). Annual discharge distributions were also found to change pre-post dam construction with discharge peaks smaller and shorter in duration by up to a magnitude (Eiriksdottir et al., 2017). Suspended sediments can be divided into two separate categories, fine < 63um and coarse > 63um fractions

(Horowitz et al., 1990). Both fractions must be considered when determining trace element transport in water (Horowitz et al., 1990). Storms and precipitation both lead to spikes in suspended sediment load (Horowitz et al., 1990). During high flow events (e.g., storms causing high water discharges), suspended fine sediment levels increased while coarse grained sediment remained constant or decreased (Horowitz et al., 1990). However, both suspended sediment fractions may be controlled by more than just discharge rate (Horowitz et al., 1990). There is an exponential relationship between grain size and geometric surface area, thereby particulate which makes it downstream is of greater relative surface area (smaller particles <60 μm), while larger particulate 60-200 μm) remains contained within the impoundment (Eiriksdottir et al., 2017). Post-impoundment, the reduced mass flux of suspended particles also decreases the release of less soluble micronutrients (smaller higher surface area particulate somewhat counteracts this though) (Eiriksdottir et al., 2017). Lastly, impoundments are considered terrestrial organic material sinks and a means of trapping organic carbon upriver due to increased accumulation and sedimentation (Pondell & Canuel, 2017). Terrestrially based organic material sources predominate over aquatic sources within lake sediments due to events such as floods that increase levels of total organic carbon in waterbody sediments (Pondell & Canuel, 2017).

Elements of the < 63um sediment fraction can be heterogeneously distributed even though the sediment fraction is homogenously distributed in the water column in a flowing water setting (Horowitz, 2008). Additionally, the less soluble the element, the more its dissolved element flux is effected by runoff (Eiriksdottir et al., 2015). For example, more soluble elements Na, Mg, and Sr were less effected by runoff whereas

Mn, and Ba are less soluble, causing their flux to be more effected by a change in runoff (Eiriksdottir et al., 2015). Sparingly soluble metals (Mn, Ba) are thereby influenced by rapid dissolution and precipitation events, which can cause co-precipitation with colloids (mineral solubility) or binding to ligands/complexing dissolved anions, increasing their detection in dissolved water samples (Eiriksdottir et al., 2015). This is indeed plausible since runoff is typically associated with higher temperatures, and increases in biotic activity which leads to greater ligand abundance in water (Eiriksdottir et al., 2015). Also, impoundments may cause depth to increase upstream, decreasing light penetration, net primary production, and uptake of trace element containing suspended particles and nutrients (Eiriksdottir et al., 2017). Changes in suspended sediment levels did not significantly change chemical composition of suspended sediment (Horowitz et al., 1990).

1.9.3. Impoundments: Effects on Fish Assemblage

Impoundments can have an adverse effect on fish populations by altering spawning grounds, migration routes, fish mortality, and overfishing (Reviewed in Zhong & Power, 1996). Spawning habitats may also be impacted due to alterations of the timing and magnitude of flows (Quist et al., 2005). This is especially the case for fish species which lay surface-drift eggs, since they hatch weak swimming fry that sink to bottom if flow is reduced significantly, decreasing survival (Bonner & Wilde, 2000; Moore, 1944; Platania & Altenbach, 1998; Zhou et al., 1980).

In terms of the impact on the general fish assemblage, post impoundment, downstream regions typically experiences a reduction in water volume leading to the

replacement of larger, river species (Bonner & Wilde, 2000) with those more akin to smaller waterbodies (Cross & Moss, 1987; Lewis & Dalquest, 1955). The creation of an impoundment can also modify the hydrology of an environment from a fast flowing, unstable environment to one which is more stable, and slower flowing causing deposition, altering the fish assemblage (Quist et al., 2005; Taylor et al., 2014). Native species abundance in fish assemblages has also been found to decrease relative to exotic species (Quist et al., 2005), with more flow-dependent and lentic species found up and downstream of impoundments (Quist et al., 2005; Taylor et al., 2014). That is not to say that all species will be effected post impoundment however, it was found that some species manage to acclimate to the newly impounded environment by creating new spawning grounds above or below impoundment, or by becoming more sedentary (reviewed in Zhong & Power, 1996). In comparison to downstream impoundment effects, upstream impacts are also possible. In a study dealing with trace element analysis of the freshwater fluvial fish, Arctic greyling (*T. arcticus*), separate populations avoided the impoundment and remained upstream within their respective tributaries and watersheds (Clarke et al., 2007). The impoundment created a barrier to dispersal and migration for Arctic grayling (Clarke et al., 2007). The degree to which fish assemblage changes pre and post impoundment depends on the degree hydrological/fluvial environment alteration increases (Taylor et al., 2014), acting perhaps as a function of upstream distance from an impoundment (Pyron et al., 1998). The nearer an collection of fish are to an impoundment, the more of a change in members from pre to post impoundment there will be (Pyron et al., 1998). Fish stocking post impoundment may also cause differences in assemblages (Taylor et al., 2014).

1.10. Study Fish Species Review

1.10.1. Fathead Minnow (*Pimephales promelas*)

The fathead minnow (*P. promelas*) is the most commonly used test species in North America for regulatory ecotoxicology (Ankley & Villeneuve, 2006) and is also considered a standard test species for the acute toxicity of various chemicals (Johnson & Finley, 1980). This fish is a low to mid-level consumer which feeds on aquatic invertebrates, plant material and detritus within the benthic environment (Stewart & Watkinson, 2004). They are a hardy and common baitfish fish species found in Canada, and more importantly in southern Manitoba (Stewart & Watkinson, 2004) where the current study is situated. In terms of habitat, they are known to occupy bogs, and ponds (Stewart & Watkinson, 2004). During spring spawning season the male fathead makes a nest over top of softer mediums (e.g., sand or sediment) and on the underside of solid structures (e.g., stones, and plants) (Stewart & Watkinson, 2004). Females then lay their buoyant/adhesive eggs within the underside of the nest, which are guarded by the male until hatching (Stewart & Watkinson, 2004). To the authors knowledge, fathead minnow otoliths are currently understudied within the literature.

1.10.2. Northern Redbelly Dace (*Chrosomus eos*)

The Northern redbelly dace (*C. eos*) is a low-level consumer that feeds on algae, detritus and occasionally insects (Stewart & Watkinson, 2004). Common habitats include headwater creeks, streams and ponds that cover silty-peaty substrate (e.g., bog) throughout North-Central and Eastern North America (Cope, 1862; Stewart & Watkinson, 2004). Northern redbelly dace are linked to much of the nutrient flow in their

communities and are a common baitfish (Stewart & Watkinson, 2004). Spawning occurs from late May into early August throughout the Great Lakes (Cope, 1862). Females produce non-adhesive eggs which once fertilized by red-abdomen males (mature), are then dispersed throughout algae and aquatic plants, and are left unattended during incubation (Cooper, 1935). The larvae are assumed to have cement glands on their heads allowing them to attach to aquatic plants post-hatching while consuming their yolk sacs (Faber, 1985). Larvae become free-swimming at ~5.5-6 mm in length and can be found searching for food in the shallows for at least a week (Faber, 1985). Development from larvae to juveniles (~13mm) occurs in ~25-30 days (Faber, 1985). To the authors knowledge, northern redbelly dace otoliths are currently understudied within the literature. It should also be noted that northern redbelly dace have the potential to hybridize with the finescale dace (*Phoxinus neogaeus*) which support a mix of characteristics between the two species (Stewart & Watkinson, 2004).

1.10.3. Lake Whitefish (*Coregonus clupeaformis*)

Lake whitefish (*C. clupeaformis*) are naturally distributed throughout most of Manitoba, within the Canadian Shield region over the prairies and is the third most important commercial fish in Manitoba (behind sauger and walleye) (Stewart & Watkinson, 2004). During the spawning season, lake whitefish reach maturity in early to mid-October, at temperatures between 5.5-9.4^{o}C (Green & Derksen, 1987). It should be noted however that lake whitefish can reach maturity as early as age 2 or as late as age 11 (females can vary between 320 mm to 540 mm when mature) (Taylor et al., 1992). Lake whitefish that mature at a greater size also tend to grow larger than there smaller

at-maturity counterparts leading to the function of maturity occurring when a fish reaches two-thirds maximum length (Taylor et al., 1992). The association between maturity and growth was found to be weak with size at-maturity varying among, but not within different lake whitefish stocks (Taylor et al., 1992). Lake whitefish eggs are released in shallow zones (1 to 3 meters in depth) over mud/clay/detritus-based substrate (Green & Derksen, 1987) as well as gravel and sand substrate (Scott & Crossman, 1979). Lake whitefish can also ascend streams, leap from the water during spawning season (Scott & Crossman, 1979), become anadromous (Morin et al., 1981) and/or can remain freshwater exclusive.

On the topic of dispersal and migration, it has been suggested that separate lake whitefish stocks likely do not interbreed or stray from their original spawning and feeding areas (Casselman et al., 1981; Mavros, 1992). Distinct lake whitefish (*C. clupeaformis*) stocks have even been identified as near as two kilometers from each other (Casselman et al., 1981), although dispersal and migratory range for lake whitefish has been found to increase with an increase in factors such as resource scarcity (Rennie et al., 2012). Greater dispersal allows access to additional prime foraging zones, increasing a stocks resistance to ecosystem change, food consumption and faster growth (Rennie et al., 2012). This increase in dispersive character, and faster growth rate has also been attributed to poor fish-stock environments (e.g., resource poor and exploitation potential) (Rennie et al., 2012). Exploitation of lake whitefish was also found to increase average size at age (increased growth rate) and fish recruitment post-exploitation with the degree to which increasing with intensity (Healey, 1980). Lake whitefish migration is not just to lakes, or rivers (Casselman et al., 1981), but also

estuaries (Morin et al., 1981). For example, a tagging study in Québec found that river-spawning lake whitefish would migrate to the mouth of an estuary during early life stages (Morin et al., 1981).

Lake whitefish feeding and habitat preferences leans towards benthic and planktonic invertebrates, surface-water insects, small fish, and is considered a mid-level benthic-benthopelagic consumer (Stewart & Watkinson, 2004). Of particular note, juvenile lake whitefish are considered prey for walleye (*S. vitreus*) (Stewart & Watkinson, 2004). Typical lake whitefish habitat resides within the benthic-benthopelagic zone, near the bottom of rivers and lakes (Stewart & Watkinson, 2004). Although considered bottom dwellers, lake whitefish reside in a range of depths, but are typically < 30m below surface (Stewart & Watkinson, 2004).

Within the lake whitefish global population, there are two main morphologically distinct groups distinguished primarily by gill raker count (Bodaly, 1979). High-raker lake whitefish are smaller and shorter on average than the low-raker variety (Bodaly, 1979). In terms of presence within the water column, high-raker fish reside primarily at the surface (deep or shallow water), but found throughout the water column whereas low-raker fish reside primarily near lake-bottom (Bodaly, 1979). In terms of feeding, high-raker fish feed primarily on pelagic food (crustacean and plankton preference: cladocerans > copepods > chironomid pupae) (Bodaly, 1979). Larger low-raker lake whitefish are found to eat primarily benthos while smaller low-raker fish eat more pelagic prey (like high-raker), consisting of Chironomid larvae, gastropods and pelecypods primarily (Bodaly, 1979). Of both varieties, high-raker lake whitefish are considered rarer, since likely outcompeted in many lakes by other fish e.g., least cisco

(*Coregonus sardinella*), lake trout (*Salvelinus namaycush*), burbot (*Lota lota*), and northern pike (*E. lucius*) (Bodaly, 1979). Study sites which contained high-raker lake whitefish were found to lack the latter competing species (Bodaly, 1979). Also, Opeongo Lake alone has been found to contain a sympatric lake whitefish population in Central Canada (Bodaly, 1979). Thereby in the current study it has been assumed that lake whitefish caught and analyzed were of the low-raker variety.

Lastly in terms of otolith-based analyses, the lake whitefish otolith has been analyzed in numerous ways. By simpler measurement means, lake whitefish otoliths have been used in analyses such as comparative age structure studies (Muir et al., 2008), to discerning fish stocks by otolith shape (Casselman et al., 1981). Lake whitefish otoliths have also been employed in various analyses using high-energy instrumentation such as LA-ICP-MS analyses (Halden & Friedrich, 2008), cathodoluminescence microscopy (Halden et al., 2004), and micro-PIXE (Saquet et al., 2002).

1.10.4. Walleye (*Sander vitreus*)

Walleye (*S. vitreus*) have been found to occupy many limnologically diverse habitats (Crossman & Scott, 1973). In Manitoba, walleye are found throughout the province as well as the Seal River watershed (Stewart & Watkinson, 2004). Walleye are considered a principal commercial (2nd largest inland fishery in Canada), game and subsistence species (Stewart & Watkinson, 2004). As a standard test species, walleye have been used to test various chemicals for acute toxicity (Johnson & Finley, 1980).

Walleye migration occurs during the summer so as to feed in preparation for spawning (Dupont et al., 2007). In Manitoba, walleye typically spawn within lakes or rivers within April-May at around 4^{o}C, once the waterbody has thawed adequately (Stewart & Watkinson, 2004). Spawning walleye disperse their eggs over rocky substrate with no pre-hatch supervision (Stewart & Watkinson, 2004). Walleye eggs take about two weeks to hatch, followed by the larvae dispersing into the open water, where they develop into juveniles and travel then into deeper waters (Scott and Crossman 1973). Walleye spawning habitat preference is a heritable trait driven by environmental queues i.e., walleye spawned in lakes would spawn in lakes over rivers and vice versa (Jennings et al., 1996). They have been found to display natal philopatry and nonrandom mating (Stepien & Faber, 1998) in which walleye remain in their "home areas" for most of the year and venture out during spawning season primarily (Forney, 1963). As walleye grow and age, they feed on progressively higher trophic levels from planktonic invertebrates, to larger invertebrates and finally fish, making them one of the main apex predators in Manitoban waters (Stewart & Watkinson, 2004). Walleye habitat is situated in deeper, less turbid water bodies (Stewart & Watkinson, 2004).

In terms of dispersal and migration, some walleye were found to have moved as far as 282 km from their initial location (31-60 days post release) (Ferguson & Derksen, 1971). Dispersal trends are not sex, weight, or length biased (Dupont et al., 2007). The frequency of dispersal from a walleye population has also been found to be stock dependent (Dupont et al., 2007). For migration, it was observed that larger walleye typically migrate further than smaller walleye within a lake (Dupont et al., 2007). Walleye were found to migrate as much as 150 km away from spawning habitat (Dupont et al.,

2007) with multiple stocks found to mix during such events (Ferguson & Derksen, 1971). Walleye developmental stage also seems to dictate migration pattern. It was found that most adult walleyes in spring/summer were found to move upstream to spawn, then back downstream likely back to their natal spawning areas in fall/early spring (Ferguson & Derksen, 1971). The YOY and yearling walleyes migrated to areas different than adult counterparts (Ferguson & Derksen, 1971). In the late spring and summer specifically, walleyes one-year and up tended to move more upstream, whereas walleye less than a year tended to migrate further downstream to lower water bodies (lakes or rivers) (Ferguson & Derksen, 1971).

Lastly in terms of otolith-based analyses, the walleye otolith has been analyzed by various high-energy instruments. From LA-ICP-MS analyses (Friedrich & Halden, 2008, 2010; Halden & Friedrich, 2008), to cathodoluminescence microscopy (Halden et al., 2004), and micro-PIXE (Saquet et al., 2002), walleye otoliths have been utilized to answer various questions about the otolith and trace element signatures.

1.11. Manganese Mesocosm Study

1.11.1. Background and Study Rationale

To the author's knowledge, baitfish otolith studies dealing with northern redbelly dace or fathead minnows in an outdoor shallow wetland mesocosm setting has yet to be conducted. Field studies such as the current study also represent the variability in physical and chemical factors in the environment unlike laboratory studies. Also, due to their value of baitfish to larger game and commercial fish species, their use as potentially more sensitive biomonitors of manganese exposure is warranted. This study

will hopefully bridge the gap between lower and upper tier trophic level manganese detection, expand the use baitfish otoliths as biomonitors and assist in the protection of baitfish valued as prey to commercially valuable species.

1.11.2. Manganese Characterization

1.11.2.1. Manganese Sources and Forms

Manganese is a multivalent metallic element which hosts three oxidations states (+2, +3 and +4) and due to manganese's multivalent nature, it has the ability to form various atomic structures with other metal cations (Post, 1999). By natural means, manganese is typically found to partition into minerals during magmatic crystallization (Post, 1999), originating in crustal sources (Howe et al., 2004) such as metamorphic and sedimentary rocks, sediments and soils (Reimer, 1999). Anthropogenic sources include waste discharge, mining processes/tailings, metal production processes, and the combustion of fuel and additives (Howe et al., 2004; Salomons, 1995).

In the environment, manganese is usually found in the form of oxides, which are typically brown-black in color and form fine-grained and weak crystalline masses or coatings on other minerals (Post, 1999). Due to the multivalent nature of manganese, highly complex and multivalent structures are common for manganese containing compounds (Post, 1999) and can include phosphates, and organic ions among other things (Hem, 1963). There are at least 30 Mn-oxide or Mn-hydroxide minerals considered to have large surface areas and reactivity (Post, 1999). Although there are many types of manganese oxides, the general term of Mn-oxide is used as a blanket term (Post, 1999). On top of oxidized versions of manganese, two common dissolved

forms of manganese are Mn^{2+} and Mn^{4+} (Howe et al., 2004). Dissolved Mn sources are derived mainly from anaerobic environments (reduced Mn oxides) such as wetlands, and other soil and sediment zones (Howe et al., 2004). Whereas in aerobic environments, various oxidation and precipitation reactions involving Mn(II) occur leading to production of insoluble Mn-oxides (Howe et al., 2004). In freshwater systems, manganese concentrations have been found to be highly variable but typically range between 0.002 to >4 mg/L (Moore, 1991), <0.01mg/L to >10 mg/L (McNeely et al., 1979), 10 to >10,000 µg/L, (yet typically less than 200 µg/L) (Reimer, 1999). In Canadian natural surface water specifically, measures are commonly 0.2 mg/L or less (with a range of 0.001-0.2 mg/L in the 1980's) (CCME, 2008).

1.11.2.2. Manganese Bioavailability

The bioavailability for dissolved manganese depends on multiple factors within the aquatic environment. As demonstrated in flux chamber and incubation experiments, decreasing dissolved oxygen to sub-oxic or anoxic conditions causes additional release of dissolved manganese from sediments due to a decrease in redox potential (Eh) (Balzer, 1982; Pakhomova et al., 2007; Sundby et al., 1986; Tebo, 1991). This decrease in redox potential is usually accompanied by a decrease in dissolved oxygen and pH (Sundby et al., 1986). At pH 8 or greater, air based oxidation can greatly decrease dissolved manganese levels (Hem, 1963). pH increase leads to additional catalytic surfaces for free dissolved manganese to bind to, increasing Mn oxidation rates and reducing dissolved manganese within a system (replaced by oxidized or precipitated forms) (Hem, 1963; Huntsman & Sunda, 1980). On top of pH and/or redox potential

effecting dissolved manganese presence (Howe et al., 2004), microbial action can also determine the rate at which dissolved manganese is oxidized (Tebo, 1991). In an incubation study by Tebo, (1991), the antimicrobial agents azide, glutaraldehyde, formaldehyde, and mercuric chloride were tested to see if any would inhibit Mn oxidation (Tebo, 1991). Each antimicrobial agent inhibited particulate formation of Mn, thus making microbes a factor in Mn oxidation (Tebo, 1991).

1.11.2.3. Manganese Reduction-Oxidation in Water and Sediment

Reactions of manganese are considered to follow first order kinetics, but can be influenced by autocatalytic effects (self-catalysis by oxides cause further oxidation and precipitation of manganese) from the oxides produced (Hem, 1963). Anions such as bicarbonate (HCO_3^-) and sulfate (SO_4^{-2}) ions slows the rate at which manganese is oxidized and precipitated thereby influences redox potential (Eh) (Hem, 1963). Higher Eh leads to oxidation of Mn (e.g., Mn complexes), while lower Eh leads to reduction of Mn (e.g., dissolved) (Hem, 1963). Lastly, manganese and iron commonly occur in natural water together (Hem, 1963). Iron typically oxidizes more rapidly than manganese, and has been found to influence Eh levels (less iron, greater Eh value)(Hem, 1963). The effects of Eh, pH, and anion presence has a less of an influence of manganese than it does for iron solubility (Hem, 1963).

The method by which manganese reacts in the environment is primarily through redox reactions and interactions between bottom water, pore-water and sediment. Within the water column, as the dissolved oxygen level decreases, redox potential decreases allowing for increased flux of dissolved manganese (Mn^{2+}) out of the

sediment, into the water column (Balzer, 1982; Sundby et al., 1986). Within the water column, (especially for stagnant waters) a boundary layer forms between the upper oxygen-rich and lower oxygen-poor zones. This layer is known as the redoxcline and acts as a barrier for dissolved oxygen (Balzer, 1982; Sundby et al., 1986). The redoxcline is also associated with the thermal stratification of waterbodies. As stated in Escobar et al., (2009), deeper water bodies such as lakes have the potential to become thermally stratified throughout the seasons. Stratification is due to the difference in densities between warm (less dense) surface water, and deeper (more dense) bottom water (epilimnion and hypolimnion respectively) due to solar radiation attenuation with depth (Escobar et al., 2009). This then causes a density barrier blocking the flow of oxygen from surface water to greater depths (Escobar et al., 2009). Barrier occurrence can be indicated by black coloration at the sediment surface indicating reducing conditions (Sundby et al., 1986) and sulfides (Tebo, 1991). Thermal stratifications are seldom permanent, allowing for periods of water column overturn and mixing (e.g., decreased radiation and increased wind action) (Escobar et al., 2009). If the redoxcline exists above sediment level within the environment (e.g., bottom water), redox sensitive elements like manganese and iron may readily diffuse from sediment, into pore and bottom water at a rate greater than their oxidations (Balzer, 1982).

The amount of Mn^{2+} released (flux) has also been found to be correlated to porewater content of sediments rather than the sediment itself (Pakhomova et al., 2007). As water mixing decreases (less turbulence), the boundary layer (redoxcline) thickens, which causes a narrower (and more drastic) concentration gradient above and below the redoxcline. This then causes the dissolved oxygen level between sediment and

bottom water to become more similar, leading to increased reduction and dissolving of Mn, and Fe into the porewater (bottom water) and subsequently overlying water if the redoxcline is above sediment level (Sundby et al., 1986). The drop in dissolved oxygen below the redoxcline (hypolimnion) is caused by processes like organismal respiration, which produces CO_2 causing a pH drop (carbonic acid formation) (Balzer, 1982). If the hypolimnion persists too long, aerobic inhabitants in benthic or deep water habitats may become adversely effected due to oxygen depletion (Escobar et al., 2009). Mn can oxidize reduced sulfur and iron or act as an electron acceptor for H_2 and organic matter during oxidation (Mn reduction) and in turn, reduced Mn^{2+} can oxidize and remove O_2 from the water column (Tebo, 1991). Comparatively, in well mixed systems (e.g., shallow, flowing waters, windy weather), the redoxcline exists typically within the sediments of a waterbody leading to flux of dissolved manganese into the sediments, and oxidation of dissolved manganese within the water column into the sediment (Balzer, 1982).

In summary, especially in the case of larger water bodies, manganese flux (long term) depends on bottom water redox reactions with sediment porewater during oxic and suboxic conditions (Aller, 1994) which vary with depth and time period (Moreau et al., 1983). The exact kinetics of Mn oxidation are difficult to determine since various reactions involving particulate, colloidal and dissolved Mn types can occur (Tebo, 1991).

1.11.2.4. Manganese Function Within the Organism

Manganese is widely distributed in fish and animal tissues and in small quantities is considered an essential nutrient, e.g., fish require Mn in a range of 2-20 mg/Kg dry

diet (Watanabe, 1997). Manganese assists in proper brain function and lipid/carbohydrate metabolism while deficiency leads to growth reduction, (Reviewed in Watanabe, 1997). Within cell tissues the mitochondria contains the most Mn and acts as a cofactor for metal-enzyme complexes (Watanabe, 1997). Manganese also activates specific enzymes such as glycosyltransferase and non-specific enzymes like decarboxylases, hydrolases, transferases and kinases (Watanabe, 1997). Within the body, Mn also has been found to act as both an antioxidant combating the production of radical oxygen species as well as a pro-oxidant, assisting in oxidative damage to tissues (HaMai & Bondy, 2004). It was found that Mn^{2+} in the presence of Mn^{3+} increased Mn^{2+}'s pro-oxidant nature (redox cycling), whereas increased presence of Fe^{3+} or decreased presence of Mn^{3+} increased Mn^{2+}'s anti-oxidant nature (HaMai & Bondy, 2004). Mn^{2+} and Fe^{3+} both have lower redox potential than Mn^{3+} (less stable than Mn^{2+}) (HaMai & Bondy, 2004).

1.11.2.5. Manganese Toxicity

Mn toxicity tests have typically been conducted with ionic Mn, whereas other forms have seldom been tested since considered less toxic (reviewed in Howe et al., 2004). Due to naturally low levels within the environment, Mn has been deemed non-toxic (Moore, 1991), but due to lack water quality guidelines for Mn, concern and uncertainty are present over the potential adverse effects of Mn (CCME, 2007). In terms of the toxicity mechanism of Mn in fish, in a hematological study on tilapia (*Tilapia sparrmanii*) it was found that fish experience symptoms including anemia, blood vessel hemorrhaging (via necrosis of the intestinal mucosa and kidneys) and lower white blood

cell counts as main adverse effects to manganese exposure (Wepener et al., 2000). The latter toxicity responses were considered non-specific in regards to environmental stress (Wepener et al., 2000). Few chronic manganese toxicity studies exist. One study was conducted on brown trout (*Salmo trutta*) over 62 days to test the effect of water hardness on Mn toxicity (Stubblefield et al., 1997). Brown trout IC25 values (survival and growth as terminal weight) were found to be 4.67, 5.59 and 8.68 mg/L Mn respectively for 30, 150 and 450 mg/L $CaCO_3$ hardness. Mn sensitivity decreased with increasing hardness (Stubblefield et al., 1997). The latter was likely due to increased Ca^{2+} ion abundance increasing competition with Mn for absorption at fish gill surface, decreasing Mn bioavailability (Seymore et al., 1995). Also, in terms of acute tests, inorganic manganese salts have been commonly used (England & Cumming, 1971; Jones, 1939; Lewis, 1978; Stubblefield et al., 1997). In an acute 96-hour lab toxicity test of manganese sulphate ($MnSO_4$) on juvenile longfin dace (*Agosia chrysogaster*) an LC50 of 130 mg/L was determined (pH= 7.6, hardness= 224 mg/L $CaCO_3$, dissolved oxygen= 8.7 mg/L) (Lewis, 1978). In another lab toxicity test, the lethal concentration of manganese nitrate ($Mn(NO_3)_2$) on 30-50 mm threespine stickleback (*Gasterosteus aculeatus*) was found to 40 mg/L within 9.7 days (pH= 6-6.8) (Jones, 1939). Comparatively, using $Mn(NO_3)_2$, juvenile rainbow trout (*Oncorhynchus mykiss*) were found to have a 96-hour median tolerance limit of 16 mg/L (England & Cumming, 1971). Lastly, a chronic toxicity test of manganese chloride ($MnCl_2$) on brown trout larvae (*Salmo trutta*) found that IC25s (survival and growth based on terminal weight) that were calculated over a range of hardness levels (30, 150 and 450 mg/L $CaCO_3$) led to IC25s of 4.67, 5.59 and 8.68 mg/L respectively (Stubblefield et al., 1997).

1.12. CAMP Study

1.12.1. Background

Manitoba Hydro and the Province of Manitoba cooperate to run the Coordinated Aquatic Monitoring Program (CAMP). This initiative aids in long term, system-wide monitoring across most of Manitoba Hydro's dams and impoundments. More specifically, the assessment of the prolonged environmental quality of water bodies influenced by impoundments enhances decision-making around water management. A three-year pilot program was conducted from 2008 to 2011 to assess the long-term applicability of CAMP, and CAMP continues to this day.

In total, this program currently monitors eight major river-systems comprising 43 sub-basins underlain with varying geology, and impacted to various degrees by Manitoba Hydro operations (Fig 1.2.). Sampling frequency occurs at annual and rotational (monitoring every several years) scales. In terms of water quality monitoring, a suite of water quality measures are routinely sampled which may be effected by Manitoba Hydro development e.g., DO, pH, alkalinity, hardness, temperature, turbidity, nutrients and 35 elements including trace elements such as Na, Mg, Mn, Ba, and Sr (CAMP, 2014).

There is also biota monitoring within CAMP that includes the sampling of phytoplankton, benthic macroinvertebrates, and fish communities within target water bodies. For fish, gillnetting of both shallow and deep lacustrine and riverine areas are conducted for both small and large bodied fish species. The intention behind biota monitoring was to collect enough samples to properly represent the communities within

each water system. Sampling areas are typically kept constant and aid in year-to-year comparison of fish groups. Fish quality, size, and abundance are commonly measured. For fish of commercial and management importance e.g., Northern pike (*E. lucius*), lake whitefish (*C. clupeaformis*), sauger (*Sander Canadensis*) and walleye (*S. vitreus*) parameters such as age are also collected and analyzed through aging structures such as the cleithra or otolith. In each of the monitored water bodies lake whitefish, walleye and/or sauger were collected. Fish serve as the mid- to upper trophic levels in aquatic communities, are a strong integrated measure of ecosystem health due to dependence on lower trophic levels and are effected by water quality and hydrology (CAMP, 2014).

CAMP presents a unique opportunity to test the utility of fish otolith as a biomonitoring tool in several ways. The archived otoliths of multiple commercially valuable species can be compared via microbeam analyses to determine potential differences in biomonitoring capabilities (e.g., variations in trace element detection). Also, the ability for the otolith to detect the presence or absence of impoundment can be assessed. Lastly, due to the availability of both archived fish otolith and corresponding water quality data, a rigorous comparative analysis can be made possible between otoliths and water microchemistry.

1.12.2. Overview of CAMP Basins, Sub-Basins, Control Structures (CS), and Generating Structures (GS) by Region

1.12.2.1. The Saskatchewan River Region (Sub-Basin: Cormorant)

The Saskatchewan River Region is an area of rich soils that supports various plant communities (e.g., wetlands, grasslands, aspen parkland and boreal forest) (Jones & Armstrong, 2001). Cormorant Lake is in northern Manitoba at coordinates:

54° 15′ 0″ N, 100° 50′ 0″ W. Precipitation is low within this watershed ranging from 30-50 cm annually (CAMP, 2014) and drains a total of 41,600 km^2 (Jones & Armstrong, 2001). Within this region one of the study waterbodies exists known as Cormorant Lake, which resides as part of the Boreal Plain ecozone, and the mid-Boreal lowlands ecoregion (CAMP, 2014). The Cormorant Lake watershed drains a total of 3162 km^2, and receives inflow from Clearwater Lake tributaries. Cormorant Lake is an off-system, non-impounded waterbody containing a single outflow (Frog Creek) into North Moose Lake (CAMP, 2014). The community of Cormorant located on the east shore of the lake (CAMP, 2014). This community conducts commercial, subsistence, and recreational fishing, trapping, forestry, and tourism (CAMP, 2014). Cormorant Lake has also one active fishing lodge (CAMP, 2014). Previous to CAMP, little to no environmental monitoring was conducted on Cormorant Lake (CAMP, 2014).

1.12.2.2. Upper Churchill River Region (Sub-Basin: South Indian Lake (Area 4), GS: Missi Falls)

The Upper Churchill River Region is composed of the Churchill River watershed spanning Northern Alberta, Saskatchewan, and Manitoba (CAMP, 2014). In Manitoba, the watershed flows downstream into Southern Indian Lake (SIL) at Missi Falls and a constructed outlet at South Bay (CAMP, 2014). Mean annual precipitation in the basin is ~40 cm (Rosenberg et al., 2005). SIL serves as a storage reservoir for the CRD (e.g., Missi Falls and Notigi CS) (CAMP, 2014). The Community of South Indian Lake is located at coordinates: 57° 10′ 0″ N, 98° 30′ 0″ W and supports commercial, recreational and subsistence fishing, hunting and trapping operations (CAMP, 2014) multiple

commercial forestry operations (Rosenberg et al., 2005). Three mines are located within the Churchill River watershed, the Lynn, Ruttan, and Farley mines; these wer shut down in the early 2000s (MIEM, 2012). Prior to impoundment and the Churchill River Diversion, SIL supported Manitoba's largest commercial fishery (composed of ~85% lake whitefish) (Barnes & Bodaly, 1990). After Missi Falls CS construction, a decline in the mean lake whitefish catch occurred from 333,500 kg to ~111,167 kg (reduction by two-thirds) occurred due to the blockage of the Churchill River migration route and likely caused the collapse of the commercial fishery (Barnes & Bodaly, 1990). The opening of the CRD may also have caused lake whitefish stock migration into the Nelson River basin from SIL (Barnes & Bodaly, 1990). The Upper Churchill River region was first impounded between 1928-1930 by the Island Falls generating station in Saskatchewan (Mackay, 1992). SIL is thereby also effected by upstream impoundment by the province of Saskatchewan (CAMP, 2014). In Manitoba, the Missi Falls CS was built in 1976 at the outlet of SIL, raising water levels by three meters and diverted 75% of flow to the CRD (away from the lower Churchill river) (Barnes & Bodaly, 1990; CAMP, 2014). The majority of water now flows into the Rat and Burntwood River systems, and then the Nelson River (CAMP, 2014). Missi Falls is the primary water regulation structure in the Upper Churchill River region and contains six spillway bays, earth dams and dykes (CAMP, 2014). The forebay fluctuates between 256.9 to 258.3 m (CAMP, 2014). Minimum Licensed outflow is 42.5 m^3/s during ice cover and 14.2 m^3/s during open water conditions (CAMP, 2014). At a forebay level of 258.3 m CS discharge can get up to 3200 m^3/s (CAMP, 2014).

1.12.2.3. Lower Churchill River Region (Sub-Basin: Gauer)

Post CRD development, the discharge from the lower Churchill River decreased substantially (CAMP, 2014). Mean annual precipitation for the Churchill River basin is approximately 40 cm (Rosenberg et al., 2005). No forestry or mining activities currently take place within the Lower Churchill River Region (CAMP, 2014).

Gauer lake is an off-system, non-impounded waterbody located south of the Churchill River, obtaining inflows from Gauer River and other minor tributaries (CAMP, 2014). Gauer River outflow leads into the lower Churchill River downstream of Missi Falls (CAMP, 2014). Dominant land cover is shrub land, and commercial and subsistence fishing is present (CAMP, 2014). Hunting and trapping also likely take place around Gauer Lake (CAMP, 2014). No permanent residents are believed to be within this watershed, but a few seasonal fishing camps are present (CAMP, 2014). Sparse monitoring has been conducted at Gauer Lake prior to CAMP (CAMP, 2014).

1.12.2.4. Churchill River Diversion (Sub-Basins: Leftrook and Threepoint, CS: Notigi, GS: Wuskwatim)

The Churchill River Diversion (CRD) Region encompasses the South Bay Diversion Channel (constructed channel 9.3km long, 60m wide connecting South Indian Lake and Isset Lake at the Rat River headwater), through the Rat and Burntwood river systems which then lead into the First Rapids 20 km upstream of Split Lake (CAMP, 2014). Jackpine (*Pinus banksiana*) and black spruce (*Picea mariana*) are dominant tree species, along with forest cover comprised of sphagnum and brown moss, sedges and ericaceous shrubs (Jones & Armstrong, 2001). Rainfall occurs mostly in June and

September, with annual precipitation of 50 cm (Manitoba Hydro and Nisichawayasihk Cree Nation, 2003). Other than the City of Thompson, and to a lesser degree Nelson House (near Footprint lake) the region is sparsely populated (CAMP, 2014). Industrial operations held within the CRD include forestry, mining, commercial, subsistence and recreational fishing, trapping and hunting (Jones & Armstrong, 2001). The two nickel mines present in this region are the currently active VALE mining and smelting complex (south of Thompson) and the closed Birchtree Mine (at Birchtree lake) (10km SW of Thompson) (MIEM, 2012).

Leftrook Lake is an off-system, non-impounded waterbody, and is the headwater lake of the Footprint River (upstream of the CRD) (CAMP, 2014). Leftrook Lake receives inflows from multiple minor tributaries and drains into the Footprint River system (CAMP, 2014). Leftrook lake is also characterized by moderate topographic relief, some depressions and areas of peat accumulation (LWCNRSB, 1975). Dominant land cover is coniferous, with black spruce (*P. mariana*), and mixed forest as the dominant vegetation (CAMP, 2014). Leftrook Lake hosts subsistence fishing, trapping and hunting with no permanent residents, and no active mines or forestry present (CAMP, 2014). Threepoint Lake is an off-system, impounded waterbody located along the route of the CRD (Burntwood and Rat River systems) (CAMP, 2014). Within this lake, both commercial and subsistence fishing operations are conducted (CAMP, 2014). Threepoint Lake is influenced by the Notigi CS upstream and Wuskwtim GS downstream. The Notigi CS was constructed 1974-1975 and is located on the Rat River between Notigi and Wapisu Lakes (CAMP, 2014). Outflow is 991 m^3/s (open period) and 963 m^3/s under ice cover. The forebay is between 254.2-258.3 m (CAMP, 2014).

The Wuskwatim GS was constructed in 2012 on the Burntwood River (CAMP, 2014). Construction of this GS led to flooding of < 0.4km^2 (CAMP, 2014).

1.12.2.5. Lower Nelson River Region (Rivers and Sub-Basins Under Study: Lower Nelson River Downstream Limestone GS, Split and Assean, GS: Kelsey**)**

The Nelson River is the main outflow from Lake Winnipeg, draining approximately 1,050,000 km^2 (Saskatchewan, Winnipeg and Red River basins) (CAMP, 2014). The main water reservoir for power generation over the Nelson River is Lake Winnipeg (CAMP, 2014). At its mouth, the Nelson River drains ~1,392,500 km^2 (CAMP, 2014). Lacustrine clays underlie the majority of the drainage basin upstream of Lake Winnipeg which contributes to the high dissolved solid and sediment loads compared to other Canadian shield rivers (Jones & Armstrong, 2001).

The Lower Nelson River (LNR) region is no longer contiguous but instead broken up by a series of lakes, generating stations (GS) and reservoirs (CAMP, 2014). The Lower Nelson River portion of the Nelson River is characterized by steep banks which decrease in slope downstream (Rosenberg et al., 2005). The Lower Nelson River itself is characterized as a straight channel extending from Split Lake downstream to Hudson Bay (CAMP, 2014). Highest temperatures are in July (~17.7^{o}C) and lowest in January (~ -22.5^{o}C) (Rosenberg et al., 2005). Annual precipitation is ~50 cm, 60% of which falling May-October (Rosenberg et al., 2005).

The Lower Nelson River region was previously glaciated and is now covered by <2 m of glacial till, and peat wetlands (Rosenberg et al., 2005). This region also contains all present ecozones in MB (although primarily within the Canadian Shield),

and contains substantial dissolved solid and sediment loads as compared to other waterbodies of similar character due to lacustrine clay material present in upstream basins (e.g., Lake Winnipeg) (Jones & Armstrong, 2001). Dominant land cover is cultivated crops, but the native plant community is composed of black spruce (*P. mariana*), aspen (*Populus* sp.), and willow (*Salix* sp.) (Rosenberg et al., 2005).

No mines currently exist in the Lower Nelson River except those within the Grass River drainage basin well upstream of the Lower Nelson River (CAMP, 2014). In terms of the human population, the Lower Nelson River region has a low population density, with mainly the community of Bird just upstream of the Limestone generating station (GS) (CAMP, 2014). The Lower Nelson River region supports tourism, and subsistence/recreational fish operations but acts as a site for hydroelectric energy generation foremost (three existing, one proposed and one potential GS located along its stretch) (CAMP, 2014). The Limestone GS is the newest GS constructed on the Nelson River and is 23 km downstream of the nearest GS, Long Spruce (CAMP, 2014). A total of 10 turbine generators makes up Limestone GS which came into service 1990-1992 (CAMP, 2014). Operating head is 27.6 m, has a capacity of 1330 MW, and generates on average 7640 million kW per year (CAMP, 2014). The Limestone forebay is contained within the natural riverbanks on the Nelson River, and is a run-of-the-river operation (little to no water storage) (CAMP, 2014).

For the current study, one of the impounded on-system sampling sites is not a lake but instead is a section of the Lower Nelson River, located downstream of the Limestone GS (CAMP, 2014). The Lower Nelson River is affected by the Lake Winnipeg Region, Churchill River Diversion, and local GSs (CAMP, 2014). The Kettle GS was

completed in 1970, and was the first GS build on the LNR. Its operating head is 30 m, contains 12 turbine generators, and 1,253 MW capacity (CAMP, 2014). Kettle GS forebay (Stephens Lake) is 337 km^2, and forebay elevation of 141.1 m (CAMP, 2014). The Long Spruce GS was the second GS built on the Lower Nelson River and much like Limestone GS is a run-of-the-river operation, 16 km downstream of Kettle GS (CAMP, 2014). The Long Spruce GS operating head is 24.4 m, with a 1010 MW capacity, a forebay (Nelson river) of 36 km^2, a max forebay elevation of 110 m (open), and 110.3 m under ice cover (CAMP, 2014). Its flow is governed by Kettle GS (CAMP, 2014).

Before reservoir and generating station construction, Split Lake was the only lacustrine waterbody within the Lower Nelson River region (CAMP, 2014). Split lake hosts the Taskweyak Cree Nation, and the community of York Landing which supports commercial, subsistence and recreational fishing (CAMP, 2014). Inflows to Split Lake include the upper Nelson and Burntwood Rivers (CAMP, 2014). In terms of water regulation, Split Lake is effected by Lake Winnipeg regulation, the Churchill River Diversion, and the Kelsey GS (CAMP, 2014). In 1961, the Kelsey GS became the first CS/GS structure on the Nelson River (CAMP, 2014). The Kelsey GS has an operating head of 17.1 m, 288 MW licensed capacity, produces 1800 million kW h of electricity per year, and runs at max output continuously (CAMP, 2014). Forebay surface area is 708 km^2, with a max operating forebay elevation of 184.4 m (CAMP, 2014).

Lastly, Assean Lake is an off-system, non-impounded waterbody which discharges into the Nelson River (CAMP, 2014). This lake hosts seasonal camps, there are no permanent residents but fishing (commercial, subsistence and recreational), hunting and trapping operations do occur (CAMP, 2014).

1.12.3. Characterizing On- and Off-System Waterbodies in The CAMP Study

On-system (or impounded) waterbodies are those effected by Manitoba Hydro's various operating systems (e.g., waterbodies up or downstream of generating stations or reservoirs) (CAMP, 2014). Off-system (or non-impounded) waterbodies are lakes or rivers entirely or almost entirely unaffected by Manitoba Hydro (may still be subject to flow changes by Manitoba Hydro such as Upper Churchill, and Saskatchewan) (CAMP, 2014). Off-system waterbodies were intended as reference waterbodies that would provide additional regional information and help distinguish any potential adverse effects resulting from Hydro operations (CAMP, 2014).

1.13. Thesis Objectives and Hypothesis

1.13.1. Objectives: Manganese Mesocosm and CAMP Study

1. Determine if manganese concentrations within shallow wetland mesocosm water and sediment will positively correlate with fish otolith manganese concentrations.
2. Compare fish species (northern redbelly dace and fathead minnow) in a shallow wetland mesocosm setting to determine if there is a difference in otolith trace metal signatures when background metal exposure is the same.
3. Compare Manitoban waterbodies of varying underlying geology (which are part of the CAMP study) to examine the influence of underlying geology on water and otolith trace element signatures.

4. Determine the effect of impoundment on fish otolith trace element signature by comparing similar waterbodies that are impounded and non-impounded which are part of the CAMP study.
5. Examine archived otoliths across a range of trace element concentrations in CAMP study surface waters for lake whitefish (*Coregonus clupeaformis*) and walleye (*Sander vitreus*).
6. The variation within, and between tested fish species otolith trace element signature will be characterized within examined amongst CAMP study sites.

1.13.2. Hypothesis: Manganese Mesocosm And CAMP Study

1. Manganese concentrations within shallow wetland mesocosm water and sediment will positively correlate with fish otolith manganese concentrations.
2. Northern redbelly dace and fathead minnow will have different otolith trace metal signatures even though background shallow wetland mesocosm metal exposure is the same.
3. Underlying geology of CAMP study water bodies will be reflected in otolith trace element signatures.
4. Impoundment of a site will not modify the otolith signatures relative to non-impounded CAMP study sites.
5. Fish species from the same CAMP study site will have the same otolith trace element signatures.
6. As CAMP study surface water concentrations of trace elements increase, so will the trace element signatures in the fish otoliths.

Chapter 1 Figures

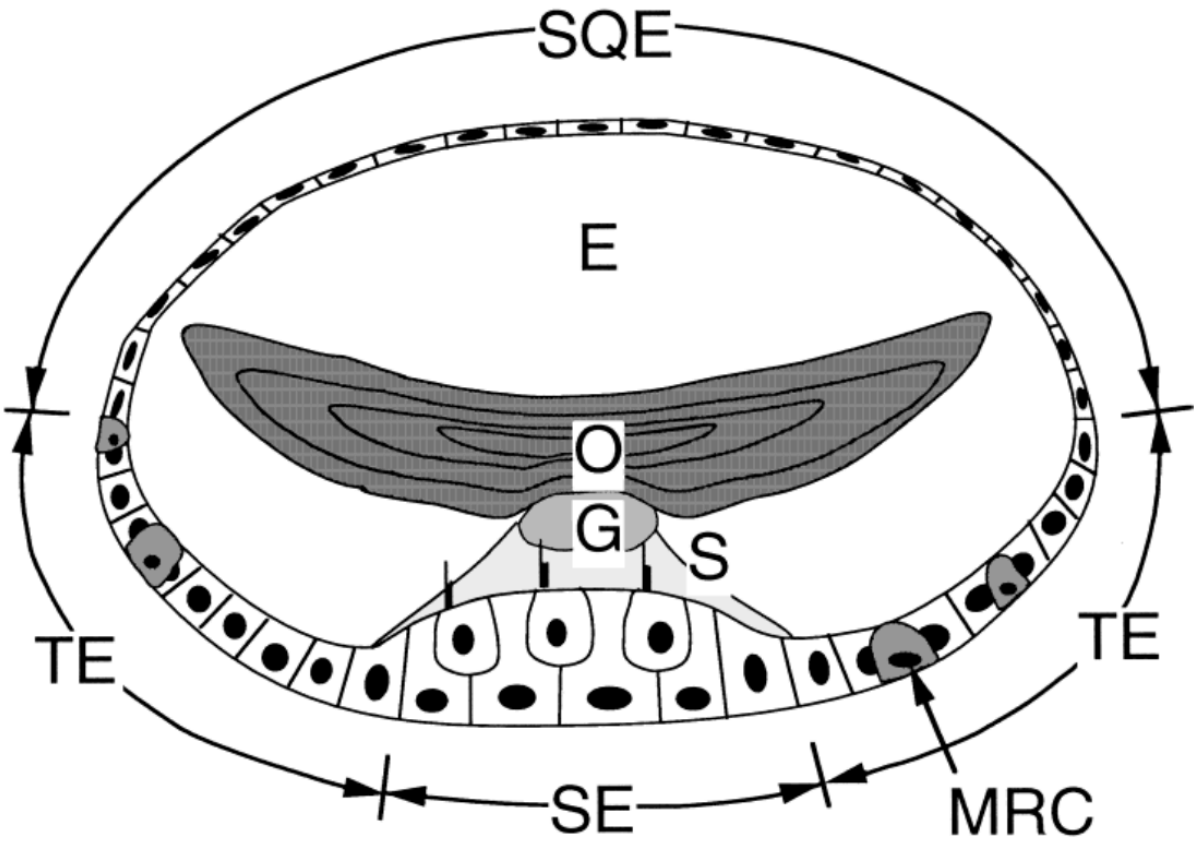

Figure 1. 1. Saccula (Endolymph) diagram depicting Sensory (SE), Transitional (TE) and Squamous (SQE) epithelial cells. Also, depicted within the diagram are Mitochondrion-rich cells (MRCs), Subcupular membrane (S), Gelatinous layer (G), Otolith (O) and Endolymph (E). Figure included with the permission of Takagi and Takahashi (1999) for thesis usage.

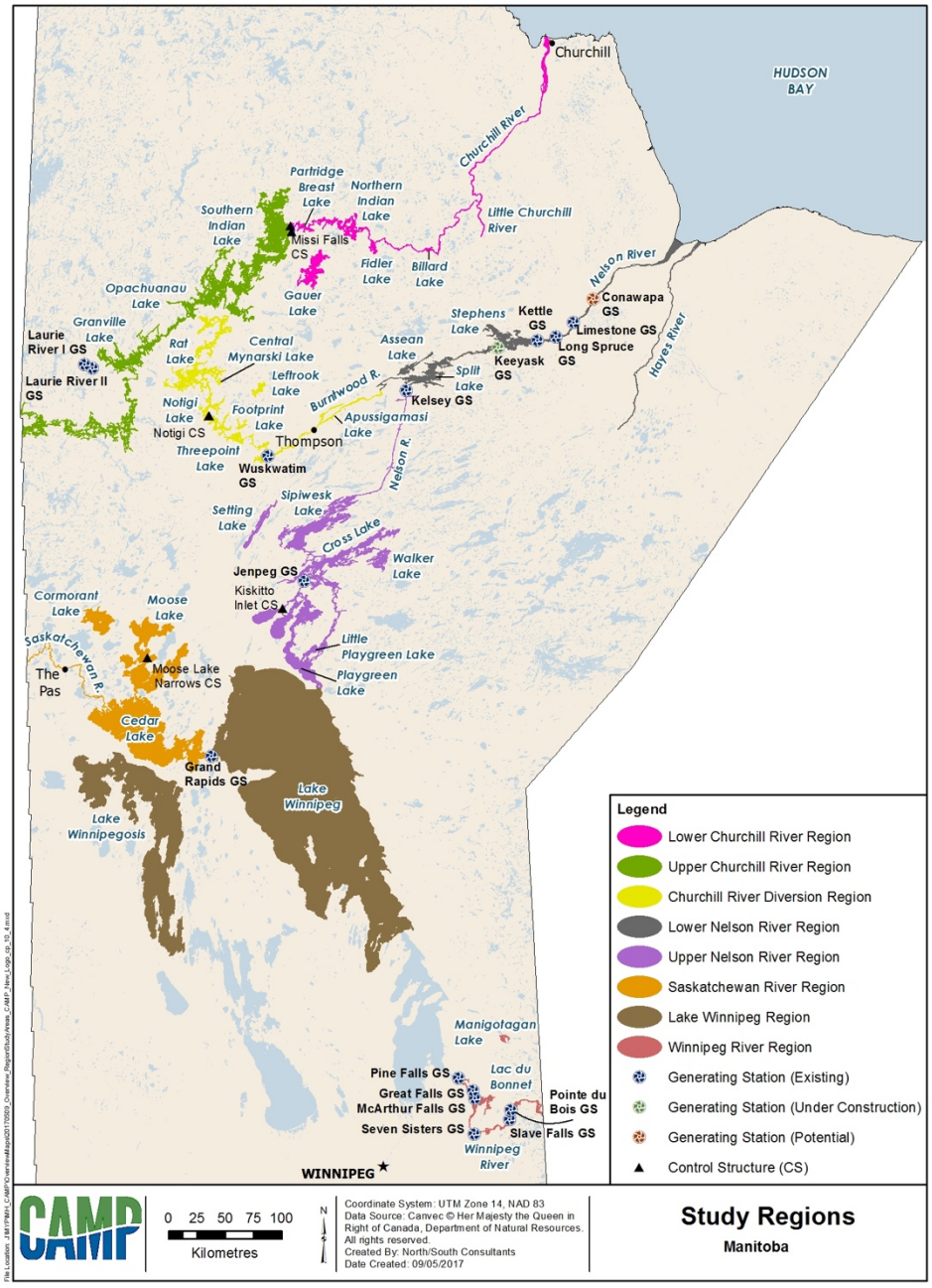

Figure 1. 2. Map of Manitoba with highlighted CAMP studied waterbodies and regions.

1.14. Chapter One References

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CHAPTER 2:

Induced otolith trace metal signature gradient by varying chronic manganese exposures in shallow wetland mesocosms

2.0. Abstract

A knowledge gap exists around freshwater fish otoliths and manganese (Mn) exposure. In the literature, otolith Mn:Ca ratios have been indicated as potentially useful biomarkers of exposure concerning scenarios such as environmental contamination and hypoxic conditions. To this end, a shallow wetland mesocosm study was conducted to determine if Mn concentrations within water and sediments would correlate with fish otolith manganese content for two baitfish species, northern redbelly dace (*Chrosomus eos*) and fathead minnow (*Pimephales promelas*). A total of nine mesocosms were used (three controls, six exposure mesocosms) and dosed with environmentally relevant concentrations of $MnSO_4$. Extracted otoliths were tested under cathodoluminescence, and inductively coupled plasma mass spectrometry. Water and sediment were sampled along with general monitoring. Measured manganese within the systems were too low to detect differences by either fish species likely due to the strongly oxidizing environment.

2.1. Introduction

Manganese (Mn) is a multivalent metallic element sourced naturally from rocks, sediment and soils in the form of fine-grained and weak crystalline oxide masses or coatings (Post, 1999; Reimer, 1999). Anthropogenic sources include waste discharge (solid or liquid), mine tailings, and fuel or additive combustion additives (Howe et al., 2004; Salomons, 1995). In Canada, surface water measures of Mn are commonly 0.2 mg/L or less (with a range of 0.001-0.2 mg/L) (CCME, 2008). Mn is also an essential nutrient for fish and acts as a cofactor for metal-enzyme complexes (Watanabe, 1997). At elevated Mn concentrations fish can experience anemic symptoms, blood vessel hemorrhaging, and lower white blood cell counts (Wepener et al., 2000). With ionic manganese (e.g., Mn^{2+}) being typically more toxic to fish than oxides (Howe et al., 2004). In an acute 96-hour lab toxicity test of manganese sulphate ($MnSO_4$) on juvenile longfin dace (*Agosia chrysogaster*) an LC50 of 130 mg/L was determined (pH of 7.6, hardness of 224 mg/L $CaCO_3$) (Lewis, 1978). Also in the lab setting, the lethal concentration of manganese nitrate ($Mn(NO_3)_2$) on 30-50 mm threespine stickleback (*Gasterosteus aculeatus*) was found to 40 mg/L within 9.7 days (pH= 6-6.8) (Jones, 1939). Comparatively, using $Mn(NO_3)_2$, juvenile rainbow trout (*Oncorhynchus mykiss*), a 96-hour median tolerance limit of 16 mg/L was determined (England & Cumming, 1971). Lastly, in a chronic toxicity test of manganese chloride ($MnCl_2$), brown trout larvae (*Salmo trutta*) IC25s (survival and growth based on terminal weight) were calculated over a range of hardness levels (30, 150 and 450 mg/L $CaCO_3$) leading to IC25s of 4.67, 5.59, and 8.68 mg/L respectively (Stubblefield et al., 1997).

The fish otolith also known as the fish ear bone, is an ageing structure found useful as a long-term biomonitoring tool for trace elements (Campana, 1999; Campana & Neilson, 1985). As the otolith is metabolically inert, and aged via annular ring pattern formations, the trends in metals can be associated with exposure events and has been used to determine fish migration patterns and other life history events. For example, strontium (Sr) chemical signatures have been used to discern between migratory and non-migratory fish (Halden & Friedrich, 2008). Also, the Mn signature has been used to identify periods of hypoxia in a fishes life (Limburg et al., 2011, 2015). Still, for many elements the association between ambient water concentration and otolith concentrations is unresolved (Elsdon & Gillanders, 2003; Forrester, 2005; Gibson-Reinemer et al., 2009). It appears otolith element signatures depend on factors such as diet, pH, and hardness as observed in northern pike (*Esox lucius*), walleye (*Sander vitreus*), brook trout (*Salvelinus fontinalis*), juvenile black bream (*Acanthopagrum butcheri*), spotted seatrout (*Cynoscion nebulosus*), banded tilapia (*Tilapia sparrmanii*), and juvenile trumpeter (*Pelates sexlineatus*) (Dorval et al., 2007; Elsdon & Gillanders, 2003; Friedrich & Halden, 2010; Moreau et al., 1983; Sanchez-Jerez et al., 2002; Wepener et al., 2000). Additionally, age and growth may also influence otolith content, as observed in walleye (*S. vitreus*), pike (*E. lucius*), arctic greyling (*Thymallus arcticus*), yellow perch (*Perca flavescens*), Atlantic cod (*Gadus morhua*), European flounder (*Platichthys flesus*), alewife (*Alosa pseudoharengus*), and juvenile trumpeter (*P. sexlineatus*) for Mn (Friedrich & Halden, 2010, 2011, Limburg et al., 2011, 2015; Sanchez-Jerez et al., 2002).

Our objective was to examine the relationship between Mn exposure and otolith signatures for small-bodied forage fish under field conditions. To this end, we exposed fathead minnow (*Pimephales promelas*) and northern redbelly dace (*Chrosomus eos*) to varying concentrations of $MnSO_4$, and then monitored for over two months at which point the baitfish were re-collected to attempt to produce and detect Mn chemical signatures. The current outdoor mesocosm study being field based incorporates a multitude of physical, chemical and biological factors that could influence Mn uptake into the otolith, thereby increasing relevance.

2.2. Materials and Methods

2.2.1. Experimental Design Summary

Two freshwater baitfish species, fathead minnow (*Pimephales promelas*) and northern redbelly dace (*Chrosomus eos*) were exposed to a range manganese sulphate ($MnSO_4$) concentrations over two-and-a-half-months in mesocosms. Water and sediment samples were collected and water quality was monitored throughout. Fish were collected at the end of the test and collected otoliths were measured for manganese content and compared with those in water and sediment.

2.2.2. The Mesocosms

The study was conducted at the Prairie Wetland Research Facility at the University of Manitoba (see Cardinal et al., 2014 for more specific details). This facility contains 18 aboveground, flat-bottomed, circular, low-density polyethylene tanks (2.7 m diameter × 0.72 m height; 3.49 m^3 total volume) that are meant to represent shallow prairie wetland setting. In 2011, each mesocosm was filled with soil to an approximate depth of 23 cm to act as wetland sediments (Anseeuw Brothers Ltd., Winnipeg, MB) (Cardinal et al., 2014). The soil used in the mesocosms is clay-dominated, and contains 50.9, 35.4, and 13.7 % clay, silt, and sand respectively. Organic carbon and organic matter content were found to be 2.6 ± 0.1 and 4.5 ± 0.2% respectively (Cardinal et al., 2014).

A total of four 7.6 L buckets were installed per mesocosm with the mouth at sediment level creating a depression and increasing water depth within the mesocosms

(Figure A1.1). The buckets were intended to act as a temperature and dissolved oxygen refugia for the fish, especially during summer months when dissolved oxygen levels were observed to fluctuate (unpublished data). The buckets were installed approximately 20 cm away from mesocosm edges evenly spaced in a square pattern. Prior to the experiments of 2016, in the 2015 experiment season the mesocosms were not drained post-season, with water removal by evaporation or addition by precipitation. It was assumed that there was no carryover of experimental materials in the mesocosms from previous works. During May 18, 2016, the mesocosms were supplemented (filled to brim) with de-chlorinated water from the City of Winnipeg water system. Mesocosm were again re-filled on June 30 (day -28), and July 21 (day -7) of 2016 due losses by evaporation. Depth measures taken pre-and-post fill. Zooplankton/other aquatic invertebrate benthos samples were collected May 5, 2016 from Oak Hammock Marsh (Stonewall, MB, 50°11′15″N, 97°7′30″W). Benthos was collected using two separate kicknets (73 and 35 µm mesh). Samples were pooled and equal aliquots were randomly added to the mesocosms. Throughout the study, the mesocosms remained accessible to natural aerial colonizers (e.g., insects). Macrophytes in the mesocosm included *Myriophyllum sibiricum*, *Lemna* spp., *Utricularia vulgaris, Stuckenia pectinata*, and *Potamogeton* spp. previously introduced from Oak Hammock Marsh.

2.2.3. Exposures

Nominal concentrations were 0.5, 1, 2, 4, 8, 16 and 32 mg/L $MnSO_4$ or 0.16, 0.32, 0.65, 1.3, 2.6, ad 5.2 mg/L Mn^{2+}. This range was based on concentrations within

freshwater and surface freshwater environments, as well as acute fish toxicity values (CCME, 2008; England & Cumming, 1971; Jones, 1939; Lewis, 1978; McNeely, Neimanis, & Dwyer, 1979; Moore, 1991; Reimer, 1999; Stubblefield et al., 1997). Manganese(II) sulphate monohydrate (99% pure) was obtained from Acros Organics (CAS: 10034-96-5). Treatments were randomly assigned to the nine mesocosms. Exposure concentrations were calculated by estimating individual mesocosm volumes at the time of dosing.

The measured $MnSO_4$ was placed into sterile falcon tubes and transported to the test site. At test site the $MnSO_4$ was mixed thoroughly with deionized water in 1 liter amber bottles. The contents were then emptied into a fertilizer sprayer, with times documented. Mesocosm tank exposure occurred on July 28, 2016 between 9:20- 10:20 am. Each mesocosm (including controls) was applied with respective treatment for three minutes (to expel all chemical) over its entire surface evenly using a broad and broadcasting spray mode while fed additional dechlorinated water from the city of Winnipeg. Mesocosms were exposed from lowest to highest $MnSO_4$ exposure treatment to avoid carryover.

2.2.4. Fish Sourcing and Ethics

Fathead minnow were sourced from Lake 114 within the International Institute for Sustainable Development – Experimental Lakes Area (IISD-ELA) and collected by IISD-ELA personnel (Lake 114 coordinates: 49.672057, -93.755753). The northern redbelly dace were acquired from Manny's Live Bait which were sourced locally in Manitoba from Reynold's Pond (coordinates: 49.7311067, -96.2704934). This was done with

approval by the Fort Gary Animal Care Committee at the University of Manitoba (Protocol reference number: F16-014 (AC11170)) and a Live Fish Handling Permit (Manitoba Conservation and Water Stewardship and Fish Culture Section) (Permit No. 24-16) and Fish Collection for Scientific Purposes License (Ontario Ministry of natural Resources and Forestry) (Licence No. 1083514).

2.2.5. Fish Acclimation and Monitoring

2.2.5.1. Fish Acclimation

A total of five to six fathead minnows (male and/or female) and seven northern redbelly daces (male and/or female) were added to each mesocosm between July 22-25th. It was assumed that the sex of the fathead minnow or northern redbelly dace would not affect manganese uptake. For fathead minnow, only one of the two sexes were placed in each mesocosm to avoid reproduction. Comparatively, northern redbelly dace sex could not be determined without dissection/gonad observation thereby a potential mix of sexes was added to each mesocosm. It was also assumed that neither species would produce offspring during the experiment and was verified at the end of test duration during collection, enumeration and with sex determination. Fish were acclimated at a rate of +2^{o}C per hour from storage to mesocosm water temperature. Acclimation was monitored using a thermometer, and temperature was manipulated through the addition of ice or mesocosm water in aerated coolers. Of the fish initially added, 6% (three fish) of the fatheads and ~5% (three fish) of the dace were found to have perished early into the study. It was assumed that acclimation stress rather than mesocosm environment caused the mortality (Table 2.1). It should be noted that the

fathead minnow and northern redbelly dace used in the study were not aged or weighed prior to addition into the mesocosms, although fish of visibly similar size were attempted to be added to each mesocosm.

2.2.5.2. Fish Monitoring

Mesocosms were observed every weekday for fish mortality. Few fish were observed throughout the test duration, likely hidden within the macrophytes or the bucket refugia. Any fish mortalities were documented and were removed without body measures or tissue collection since compromised (e.g., decay).

2.2.6. Fish Collection Process from Mesocosms

Fish were collected using standard “Gee” minnow traps with commercial cat food as bait. Collections occurred October 4-7 and October 10-14, 2016 (fish successfully caught October 4-6, or days 68-70). Minnow trap deployment occurred in the evening (between 3-4 pm). Fish collection occurred each following day between 8-10 am. Captured fish were euthanized with a clove oil-mesocosm water solution at the overdose concentration of 400 mg/L (Sladky et al., 2001). Once fish respiration visibly ceased (opercula movement), fish were kept in solution for ≥ 10 minutes before transfer to sterile, labelled plastic bags. Sample bags were placed in a cooler with ice and immediately transported to a fridge before processing to avoid tissue deterioration. If more fish were caught than could be processed within a 3 to 4-hour period, the captured minnows were left within the traps (sealed with foam plugs to prevent re-release) and processed later that day. For each subsequent collection, traps were cleaned, refreshed

with bait and re-deployed. The three-day variability in collection of both fish species was assumed to have a negligible effect on variability between measures.

2.2.7. Fish Processing and Tissue Collection

2.2.7.1. Pre-Dissection

Fish were processed individually by the author. Fish were patted dry with clean paper towel and then fork length (FL) and wet-weight (WGT) were determined. Fish collected each sample day were measured in order from control mesocosms to the highest treatment mesocosms to avoid contamination.

2.2.7.2. Fish Dissection and Tissue Removal

Dissections occurred on a gel pinning mat using a sterile stainless steel scalpel and surgical scissors. The pinning mat, and dissection tools were all sterilized between dissections by rinsing in methanol (Fisher Scientific, HPLC grade 99.9%, CAS: 67-56-1) followed by Milli-Q water and wiped clean with Kimwipes© to dry. Samples were visualized with the aid of a Zeiss Stemi 2000-C stereomicroscope. Muscle tissue was collected from above the longitudinal line but below the dorsal fin. Liver tissue was collected via longitudinal incision from the anal pore to just before the pectoral fins, followed by a vertical side-incision on either end of the initial incision creating a skin-flap which when pulled back, exposed the liver and other viscera. Each tissue sample was placed in a separate labelled, sterile cryovial and stored at -80^{o}C prior to analysis.

Saggital otolith pairs were removed after the soft tissues. Fish were first decapitated behind the operculum, followed by an incision above the eyes towards the

rear of the skull, exposing the brain and endolymphs. Both otoliths were collected from the endolymphs if possible. Extraneous fluid and tissue were removed from each otolith via Milli-Q water rinse followed by 45 second sonication (FS20 ultrasonic cleaner, Fisher Scientific). Each otolith pair was then placed in a labelled, sterile microcuvette (lid open) within a Forma Laminar Airflow Workstation (Thermo Scientific) and air dried for >12 hr before closing. Each dry otolith was then weighted on a microscale (Mettler Toledo, model XS3DU).

2.2.8. Water and Sediment Sample Collection

2.2.8.1. Water Samples

Water grab samples were collected prior to other samples to limit disruption of the water column. Sampling occurred a total of four times, July 19 (S1, day -9), July 29 (S2, day 1), August 18 (S3, day 21), October 3 (S4, day 67). Field blanks were present at each sampling session to account for QA/QC. Each field blank was filled with Milli-Q water and sealed within a class 100 clean lab prior to transport to the PWRF. During sampling, the lid of the field blank was removed and left off the blank sample bottle for the duration of water grab sampling. Sampling was conducted from least to greatest concentration treatments and separate syringes and syringe filters were used per mesocosm. If contamination was suspected during sampling, suspect sampling gear was replaced before continuing.

Sterile 250 mL Nalgene® HDPE bottles (metal analysis grade) were used to collect the water grab samples. Each sample was collected mid-depth from the same mesocosm location. Pre-acid washed 20 mL plastic syringes (Luer slip plastic syringes

by Thermo Scientific) fitted with sterile 0.2 µm syringe filter caps (Filtropur S syringe tip filters by Sarstedt) were used to transfer 50 mL of sample water from the unfiltered sample to a sterile 250 mL Nalgene bottle (filtered sample bottle was rinsed with filtered sample prior to collection). A 0.2 µm filter was used over the conventional 0.45 µm filter to further clarify that measured manganese in the test system was the dissolved fraction. It was found that 0.45 µm filters overestimate and include colloidal forms (Harford et al., 2015; Pokrovsky et al., 2010). After each water sample was collected, the filtered and unfiltered samples were re-bagged and placed in a cooler with ice packs for transfer to the lab for acid preservation. Start and end times of each sample collection were documented consistently. All equipment and filled sample bottles were double bagged in which one individual was designated to seal each sterile sample bag while the other individual was designated to collect grab samples. Acid preservation involved spiking the 50 mL filtered sample with 250 µL of 0.5% v/v HNO_3 solution while within a Forma Laminar Airflow Workstation. Preserved samples were double bagged and stored at 4^{o}C prior to processing.

2.2.8.2. Sediment Samples

Sediment jars were prepared within each test system prior to testing (Figure A1.2). Sampling occurred a total of four times, July 19 (S1, day -9), July 29 (S2, day 1), August 18 (S3, day 21), October 3 (S4, day 67). A total of five, 100 mL amber jars per mesocosm were filled during the pre-exposure period on day -23 (July 5, 2016) with sediment from the top ~8 cm of their respective mesocosm. The open-faced sediment jars were placed on cinder blocks installed at sediment-level in their respective

mesocosms. Sediment samples were collected from the jars and transferred using sterile plastic spoons to labelled whirlpacks. During sediment sample collection, one individual was designated to seal each sterile sample bag while the other individual was designated to collect the sediment sample jars and scoop out the sediment. Sediment samples were then transported in a cooler to a freezer and frozen at -20^{o}C prior to being freeze-dried. Frozen sediment samples were freeze dried in a Labconco FreeZone 12 Liter Freeze-Dry System for ~96 hours prior to acid digestion in preparation for analysis. Observations and sampling times were documented consistently.

2.2.9. Monitoring

2.2.9.1. Water Quality Monitoring

2.2.9.1.1. YSI-Sonde Measures and Hobo Temp Loggers

Using a YSI 6600 V2 Sonde (Yellow Springs, OH), temperature (^{o}C), specific conductivity (mS/cm), pH, pHmV (millivolts), chlorophyll content (µg/L), and dissolved oxygen (mg/L and % saturation) were monitored *in-situ* each weekday between 8-9:30am, and once per week between 3-4:30pm at a depth of approximately 20cm. Measures were also taken on twice within the refugia buckets pre-exposure (July 4, 2016, 8-9:30 am and July 7^{th,} 3-4 pm) to determine if the refugia promoted a more stable environment (Figure A1.3). Pre-exposure water monitoring occurred from May 24, 2016 (day -65) to July 27 (day -1), post exposure monitoring lasted from July 28 (exposure day 0) to October 3 (day 67). During a brief period (May 27 to June 24, 2016) the YSI 6600 V2 Sonde malfunctioned and was brought in for repairs. During which, a

YSI Professional Plus Sonde with quarto cable attachment (Yellow Springs, OH) was used although total chlorophyll could not be measured since lacking a chlorophyll probe. Hobo Water Temp Pro v2 (model U22-001) data loggers were deployed at sediment level, 20 cm from the edge of each mesocosm to record at 30 minute intervals (May 24-October 11, 2016). Additional temperature loggers were placed within the refugia buckets to monitor the difference between sediment and refugia (Figure A1.1 and A1.4).

2.2.9.1.2. PAR

Photosynthetically active radiation (PAR) was measured with an Apogee MQ-200 quantum sensor (in $\mu mol*m^{-2}*s^{-1}$) with AL-100 sensor levelling plate (Hoskin Scientific, Burlington, ON). Afternoon measures taken at sediment level per mesocosm from June 2 (day -56) to September 19 (day 53), 2016 on clear days.

2.2.9.1.3. Filamentous Algae

Qualitative filamentous algae assessments were conducted each week (May 27 to October 3, 2016 or day -62 to 67) by the author and two trained lab members using a scale of 1 to 3 (low to high algal density), with confidence from 1 to 10 (low to high) to approximate algal growth or productivity (Baxter et al., 2013).

2.2.9.1.4. Depth and Volume

Weekly water depth measurements (six random positions per mesocosm) were used to calculate the volume of water throughout test duration (May 27 to October 3, 2016 or day -62 to 67).

2.2.9.1.5. Hardness, Alkalinity and Ammonia Level

Measures were taken twice pre-exposure on days -34 and -13 (June 24, and July 15) and three times post exposure days 1, 21, and 67 (July 29, August 18, and October 3, 2016). Ammonia measures (in ppm) were taken using single-use test strips (Aquarium Pharmaceuticals, Chalfont, PA). General hardness and alkalinity were measured using Nutrafin® aquarium test kits (Rolf C. Hagen Inc., Montreal, QC). The latter tests were performed from integrative water samples. Integrative sampling consisted of four grab samples from different areas and depths within the respective mesocosm. The integrated sampling device was made out of white PVC pipe with an end-hose attachment described in Solomon et al., (1982). Individual grab samples were filtered through a coarse mesh funnel to remove larger debris, and poured into a clean plastic bucket. Each individual grab sample collected was approximately 900 mL in volume, creating an integrated sample of ~ 3.6 L, of which 5 mL was taken for analysis per alkalinity and hardness measures, and ammonia test strips were dipped directly into the ~3.6 L volume of integrated sample.

2.2.10. Analysis Instruments Implemented

2.2.10.1. Cathodoluminescence

The cathodoluminescence (CL) instrumentation included a Technosyn cold-CL stage and a Nikon Microscope equipped with a digital sight and display monitor using NIS-Elements F (3.0) software. The stage consisted of a sample dock within a vacuum chamber, a cathode gun and Reliotron device (Relion Industries, Bedford, MA). The

cathode gun emits electrons that bombard the sample causing the excitation of trace elements (e.g., manganese) leading to sample fluorescence of varying intensity based on composition. To provide sufficient voltage and current for luminescence beam conditions were set to ~-8.5 kv and ~0.4 mA and the vacuum was set to ~0.1 torr. CL was run on multiple otoliths from fish initially collected, control, and high concentration treatments to attempt to qualitatively identify any fluorescence.

2.2.10.2. LA-ICP-MS

The trace element concentrations in the otoliths were determined by Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS). The analysis was done on a Thermo Finnigan Element 2 ICP-MS instrument coupled to a Merchantek LUV 213 Nd:YAG laser. General laser conditions during the analysis can be found in Table 2.2. Line scans over the otolith transect surface were run from core to edge (along the clearest and most defined annuli. The internal standard used by this device was calcium (56 wt.% CaO), and the external calibration was done using NIST glass 610. Before and after each slide of otoliths the NIST 610 glass was run at least once to account for instrumental drift. During processing, the isotopes Mn^{55} and Ca^{44} were measured. Iolite software was used to process the samples collected by LA-ICP-MS to determine trace element concentration, detection limits and other variables (Iolite Team, 2016). Linescans were formed in SigmaPlot (Systat Software Inc., 2008).

2.2.10.3. SO-ICP-MS

All digested sediment, water, and otolith samples were analyzed at the Ultra Clean Trace Elements Laboratory (UCTEL) at the University of Manitoba, Winnipeg. Water samples were run on an ICP-MS (PerkinElemer ELAN® DRC II) within a Class 100 clean room (featuring class 10 enclosure) with an ambient temperature of 20^{o}C and relative humidity of 40%. Certified reference materials (CRMs) NIST-SRM 1643e, and test materials (TM) 26.3 and 28.3 were used to assure instrument performance. All whole-otolith and sediment samples were run inside the clean lab, under standard laboratory conditions on an ICP-QQQ-MS (Agilent 8900), using H_2 as the carrier gas. CRM NIST1640a and TM28.4 were used to ensure instrument performance.

2.2.11. Tissue Processing

2.2.11.1. Soft Tissue Processing

Acid digestion and processing via ICP-MS was intended however the -80^{o}C freezer malfunctioned, compromising all soft tissue samples (muscle and liver). Because of the latter, soft tissue was not analysed in this project.

2.2.11.2. Hard Tissue Processing

2.2.11.2.1. Otolith Processing for CL and LA-ICP-MS

Cleaned saggital otoliths were individually embedded in Crystalbond™ 509 sulcus side down, on 26 x 46 mm glass slides. With one otolith on the slide at a time, the otolith was hand sanded with Buehler Carbimet™ grit 600 [P1200] paper (Buehler Ltd, Lake Bluff, IL) on a roll grinder wetted with deionized water. After the annuli and

primordia were exposed, the otolith slide was briefly sonicated. Post sonication the otolith slide was hand polished on a deionized water-wetted polishing wheel (Buherler ® MeaServ 2000 Variable Speed Grinder-Polisher) with Buehler Micropolish (0.3 micro alpha alumina) (Buehler Ltd, Lake Bluff, IL). After polishing the embedded otolith, the slide was re-heated to release the otolith. The polished otolith was then transferred and embedded into a new glass analysis slide with other otoliths previously polished. All otoliths were secondarily verified by experienced personnel for nuclei exposure. Once all otoliths were transferred the analysis slide was washed with de-ionized water and allowed to air dry. Also prior to analysis, digital images of each polished otolith were taken to compare pre-and-post analysis using a Nikon SMZ 745T microscope fitted with C-W10XB/22 eyepieces and displayed using NIS-Elements F (3.0) software (Laboratory Imaging, Za Drahou, Praha, Czech Republic).

2.2.11.2.2. Otolith Processing for SO-ICP-MS

A subset of otoliths from both species was selected to represent the range of exposure concentrations for analysis. Each otolith was dissolved in 35 µL of HNO_3, and diluted to 3 mL to run for ^{55}Mn detection in µg/g (dilution factor of 85.7). Replication per sample was done three times, and results indicated that the standard deviation between each set of replicates was < 10% for each sample except one at > 15% (fathead otolith from control mesocosm 11) indicating general confidence in results.

2.2.12. Water and Sediment Processing

2.2.12.1. Water

During the processing of each water sample, no dilution was conducted after HNO_3 spiking. A 15 mL aliquot from each sample was the taken to be processed by SO-ICP-MS for the measure of ^{55}Mn. Some of the water samples had two replicate aliquots collected randomly for comparison purposes and the average was used in further analysis for said samples). Replicates were found to differ less than 4% from each other except in one case where the percent difference was 28% (control mesocosm tank 1, pre-exposure replicate). Because of the latter, error in replication was considered negligible.

2.2.12.2. Sediment

Trace elements from the freeze-dried sediment samples were extracted via microwave assisted acid digestion. The methods were adapted from EPA method 3052 (EPA, 1996). Each sample was thoroughly mixed to homogenize and then a sediment sub-sample of 0.1-0.2 g was taken and weighed to the nearest 0.001 g on an electric scale. Each sample was then mixed with 4.5 mL concentrated (70%) HNO_3 (Certified ACS Plus, Fisher Chemical) and 1.5 mL concentrated (47-51%) HF (TraceSELECT®, Sigma Aldrich). Samples were then placed within separate vessels of an XP1500Plus within a MARS microwave digestion system (Matthews, NC, CEM Corporation©). Samples pre-digested for >15 minutes before sealing. The MARS heating program for acid digestion was set to 400W (100%), 800 psi, 180^{o}C, ramp time of five minutes 30 seconds, hold time equal to nine minutes 30 seconds. Following the microwave

digestion, vessel contents were cooled back down to room temperature in preparation for HF neutralization by boric acid (H_3BO_3). A total of 30 mL of 4% w/v H_3BO_3 solution was added to each sample prior to additional microwave heating. The MARS heating program for HF neutralization was set to 400W (100%), 800 psi, 170^{o}C, ramp time 15 minutes, and a hold time10 minutes. Each run contained 10 sediment samples (two sediments from the greatest treatment mesocosm were run in duplicate), one acid blank, and one containing CRM 8704 (Buffalo River Sediment, U.S. Department of Commerce, Gathersburg, MD, 20899). Post cooling, 1.5 µL of each sediment sample was then transferred into a sample vial, then diluted with Milli-Q water, to a final volume of 15 mL (dilution factor of 50). Each sample had three separate aliquots collected and diluted for a total of three replicates to be run under ICP-QQQ-MS per sample.

2.2.13. Statistical Testing

2.2.13.1. Fish Biometric and Survivability Testing

2.2.13.1.1 Fish Biometrics

To determine if fish continued to grow throughout the test duration and that the mesocosms themselves were not adversely affecting fish health, a series of Student's *t*-tests were conducted to compare fish initially collected and control fish wet weight (in grams), otolith average mass (in micrograms) and FL (in millimeters) per species. All control mesocosms individual measures were averaged together to account for variability within mesocosms.

Student's *t*-tests were not performed against treatment groups since samples sizes were too small. Additionally, a series or correlative tests were conducted on

control and treatment fish (Spearman's and/or Pearson's). Fish measures collected from individuals per treatment were averaged to create a single value per treatment. All treatment groups (including fish initially collected and control) were also subject to a comparative graphical analysis. Parametric tests (Levene's mean tests for homogeneity of variance and Shapiro-Wilk test of normality) were conducted prior to test selections to determine if tests were deemed parametric or non-parametric. Log transformations were conducted on the dependent variables when necessary to meet normality requirements. Fish were studied for signs of poor fish growth and were noted if identified such as flat stomachs and stunted appearance (Birungi et al., 2007).

2.2.13.1.2 Fish Percent Survivability

To determine if fish survivability was impacted by $MnSO_4$ treatment concentration, Pearson's and/or Spearman's correlation tests of nominal $MnSO_4$ concentration (independent) versus percent survivability (dependent) were conducted for both fathead minnow and northern redbelly dace. Parametric tests (Levene's mean tests for homogeneity of variance and Shapiro-Wilk test of normality) were conducted prior to test selections to determine if tests were deemed parametric or non-parametric. Treatment mesocosm percent survivability was calculated as the number of fish collected at the end of study divided by the number of fish initially added to that respective mesocosm multiplied by a factor of 100. Since controls were replicated, control percent survivability was calculated as the sum of fish collected at the end of study from all three control mesocosms divided by the combined total of fish initially

added amongst the three control mesocosms and expressed as a percentage by multiplying by a factor of 100.

2.2.13.2. Cathodoluminescence Testing

A qualitative study dealing with the comparison of images taken during the CL analysis was conducted to determine if CL presence was elevated along the edge of treated otoliths versus initially collected and control otoliths per species.

2.2.13.3. LA-ICP-MS Testing: Otolith Cross Sections

A graphical analysis was first conducted between manganese signatures, focusing along the otolith edge since being the potential area impacted by $MnSO_4$ exposure. Due to the observation of Mn spikes on the laser line graphs of some of the fathead minnow and northern redbelly dace otoliths along the outer otolith edge, the outer edge (10 µm) of each otolith was averaged per individual linescan 10 µm edge segment and then averaged down by treatment to then conduct a bulk sample correlative analysis. Two types of correlative analyses were conducted, the first was between nominal water concentrations and otolith Mn concentrations, and secondly was between select day 67 sediment Mn concentrations and corresponding otolith concentrations per species if sample size is large enough. Other studies have used similarly small otolith edge distances for analysis of short time periods (e.g., Clarke et al., (2015, 2007)). Calculated standard deviations were used as error bars. Parametric tests (Levene's mean tests for homogeneity of variance and Shapiro-Wilk test of normality) were conducted prior to test selections to determine if tests were deemed

parametric or non-parametric. Log transformations were conducted on the dependent variables when necessary to meet normality requirements. The distance of 10 μm was determined by measuring the distance travelled along the otolith edge-inwards of otolith with a suspected peak before series of two Mn readings below the LOD were detected. This method was intended to determine the start point of Mn exposure per otolith due to the unreliability in visually aging a two-and-a-half-month otolith study period on the otolith or other aging structures. Since other trace element were not measured during the LA-ICP-MS, other methods of aging based of trace element signatures were not used such as zinc (Zn) in Clarke et al., (2015).

2.2.13.4. SO-ICP-MS/ICP-QQQ-MS Testing: Water, Sediment and Whole Otolith

For water and sediment samples, a series or correlative tests were conducted on control and treatment measures (Spearman's and/or Pearson's) between measured otolith or water values and nominal Mn exposure concentrations. Measures collected from individual samples per treatment were averaged together to create a single value per treatment. All control mesocosms individual measures were averaged together to account for variability. Bar graphs were additionally constructed per species to determined differences between treatments. Calculated standard deviations were used as error bars. Parametric tests (Levene's mean tests for homogeneity of variance and Shapiro-Wilk test of normality) were conducted prior to test selections to determine if tests were deemed parametric or non-parametric. Log transformations were conducted on the dependent variables when necessary to meet normality requirements.

2.2.13.5. Water Quality

A series of graphical and univariate tests were conducted on water quality measures taken throughout the course of the study. For those measures taken using the YSI-Sonde (temperature, pH, specific conductivity, chlorophyll, and dissolved oxygen) a series of repeated measure ANOVAs were conducted for both AM and PM measures and pre-and-post exposure to manganese. For other water quality measures, a series of repeated measure ANOVAs were conducted on data pooled from both pre- and post-exposure periods. If a significant difference was determined from the ANOVA, a post-hoc Dunn's (non-parametric) or Dunnett's (parametric) test was conducted to compare control and treatment groups (the three control treatments were averaged to a single value per monitoring day for use in the ANOVA tests.). Parametric tests for homogeneity of variance (Levene's) and normality (Shapiro-Wilk) were also conducted prior to test selection. All univariate statistical testing was conducted using Sigma Stat software (Systat Software Inc, 2006).

2.3. Results

2.3.1. Water Quality Monitoring

$MnSO_4$ exposure did not result in statistically significant changes in the various water quality measures from controls, when considering both AM and PM measures (Tables 2.3-2.4). However, repeated measures ANOVAs for routine water quality data indicated significant differences for each parameter between some treatments although the observed differences are likely not related to the concentration gradient but instead seasonal variaitons (Tables 2.3-2.5). It should be noted that there was relatively high variation in water quality parameters between control tanks. Overall, it was concluded that $MnSO_4$ exposure altered the water Mn concentration, but the exposure was too low and brief to cause a change in detection in otolith or sediment.

Temperature pre-exposure AM results for 0.32, 0.65, 1.3 and 2.6 mg/L Mn^{2+} treatments were significantly greater than the control average pre-exposure. Post exposure the 0.32, 1.3 and 2.6 were greater than control, and the 0.16 mg/L Mn^{2+} treatment was significantly less than control (Table 2.3). Comparatively, pre-exposure PM results indicated that the two of the lowest $MnSO_4$ exposure treatment (0.16 and 0.32 mg/L Mn^{2+}) had mesocosms temperatures significantly greater than the control average (Table 2.4). Post-exposure PM temperature results were not found to differ significantly from the control over time (Table 2.4).

Specific conductivity was significantly different between control and treatment groups pre and post exposure in both AM and PM measures (Table 2.3-2.4). Pre and post exposure AM treatment measures were significantly greater than the control

average except the 1.3 mg/L Mn^{2+} treatment (Table 2.3). Pre and post exposure PM treatment measures (0.16, and 0.32 mg/L Mn^{2+}) were significantly greater than the control average (Table 2.4). Additionally, the 5.2 mg/L Mn^{2+} treatment for pre-exposure exclusively was found to be significantly greater than the control average (Table 2.4).

pH pre-exposure AM 0.16 and 5.2 mg/L Mn^{2+} treatments had significantly lower and the 2.6 mg/L Mn^{2+} treatment mesocosms had significantly greater pH than the control average (Table 2.3). pH post-exposure AM treatments other than the 0.32 mg/L Mn^{2+} treatment were significantly less than the control average (Table 2.3). pH pre-exposure PM treatments did not differ significantly from the control average (Table 2.4). pH post-exposure PM 0.32 mg/L Mn^{2+} treatment was significantly greater and the 5.2 mg/L Mn^{2+} treatment was significantly lower than control average (Table 2.4). Post exposure AM and PM time periods found that the 5.2 mg/L Mn^{2+} treatment had significantly lower pH than control, indicating a potential treatment effect although pre-AM measures also indicated significantly lower pH than control (Tables 2.3-2.4). Background pH may be simply greater in the 5.2 mg/L Mn^{2+} treatment mesocosm instead of a treatment effect occurring (Tables 2.3-2.4).

The total chlorophyll pre-exposure AM 0.65 mg/L Mn^{2+} treatment alone had significantly different/lower total chlorophyll content than the control average (Table 2.3). Total chlorophyll post-exposure AM 0.65, 2.6 and 5.2 mg/L Mn^{2+} treatments were significantly less than the control average (Table 2.3). No significant total chlorophyll differences were found between control and treatments groups in the PM sampling period (Table 2.4).

Dissolved oxygen (DO) pre-exposure AM 0.16, 0.32, 2.6 and 5.2 mg/L Mn^{2+} treatments were significantly lower than the control average (Tables 2.3). DO post-exposure AM treatments were significantly lower than the control average (Tables 1A). DO pre-exposure PM 0.32 and 0.65 mg/L Mn^{2+} treatments were significantly greater than control (Tables 2.4). DO post-exposure PM 5.2 mg/L Mn^{2+} treatment alone was significantly less than the control average (Tables 2.4). DO post-exposure AM and PM time periods found that the 5.2mg/L Mn^{2+} treatments were significantly less than the control average although pre-AM measures also indicated significantly lower DO (Tables 2.3-2.4). Background DO may be simply lower in the 5.2 mg/L Mn^{2+} treatment mesocosm (Tables 2.3-2.4).

General hardness and alkalinity post-exposure differed significantly between treatment and control groups (Table 2.5). General hardness 0.16, 0.65 and 5.2 mg/L Mn^{2+} treatments were significantly greater than the control average (Table 2.5). Alkalinity 0.16 mg/L Mn^{2+} treatment alone was significantly greater/different than the control average (Table 2.5). Filamentous algae (confidence and assessment values) pre-exposure treatments did not significantly differ compared to the control average (Table 2.5). Photosynthetically active radiation (PAR) readings did not differ significantly between control and treatment groups (Table 2.5). Ammonia content was found to be 0 ppm in each mesocosm, thereby no significant differences were found.

As observed in tables 2.3-2.5, significant differences were established across many of the treatments for many of the water quality variables measured. Significant statistical differences were established but it must be kept in mind that this does not necessarily indicate biological significance. For example, when referring to the Table 2.3

measures variables, 1-way repeated measures ANOVA's were run, which since working with repeated measures data found significant difference between treatments even though the means of separate treatments were found to overlap within error (error as standard deviations from the mean). However, if the repeated measures were treated as individual measures and run under a 1-way ANOVA instead, many of the treatments found previously to differ significantly with control were found not to through post-hoc testing. This further supports the idea that the treatments and treatment mesocosms do not differ enough to be significantly biologically different from each other. Perhaps slight variations due to things such as some variation in macrophyte density .

Lastly, due to ORP probe issues, ORP measures were not taken over most of the study. Alternatively, archived PWRF 2014-2015 ORP measures were accessed to determine a baseline set of ORP values per tank over time for some indication of the range in these values. The minimum, maximum, average, standard deviation and range in 2014 (June to September values, mesocosms 2, 6 and 9) and 2015 (May to September/October values, mesocosms, 3, 4, 5, 10, 11 and 16) AM/PM values taken from previous control mesocosms are as follows: 2014-AM: 372, 2667, 554, 264 and 2295 mV respectively; 2014-PM: 265, 833, 505, 103 and 568 mV respectively; 2015-AM: -442, 1350, -48, 285, 1792 mV respectively; 2015-PM: -386, 585, -141, 318, and 971 mV respectively. Mesocosm results from 2014 (AM: $p= 0.58$; PM: $p= 0.257$) and 2015 (AM: $p= 0.404$) indicate that ORP fluctuated between months and years, but to a lesser degree between mesocosms leading to a lack of significant differences between mesocosms (Table 2.6).

2.3.2 Water Samples SO-ICP-MS

During the pre-exposure water sample period (day -9, July 19, 2016), Mn^{55} measures from the pooled control and treatment mesocosms were found to have a mean, median and range of 11.62, 11.71 and 17.60 µg/L Mn respectively, and were assumed to be relatively similar in content prior to treatment (Fig 2.1, Table 2.7). One-way ANOVAs of control, low and high treatment data indicated that for pre-exposure day -9 ($p = 0.5193$), 21 days' post exposure ($p = 0.7921$) and 67 days' post exposure ($p = 0.2495$) that there was no significant difference between control and treatment groups (Table 2.8). One day after treatment ($p = 0.000385$), both the low and high treatments were found to contain greater Mn than control (with the low treatment having a lower Mn concentration than the high pooled treatment) (Tables 2.8-2.9). In terms of the correlative analysis, when using the average of control mesocosm 1 and 5 (control mesocosm 11 excluded), it was observed that on day 1, day 21, and day 67 that $MnSO_4$ exposure concentration positively significantly correlated with water grab sample concentration ($p= 0.018$, 0.007 and 0.008 respectively) (Table 2.10). Comparatively, when using the average of the three control mesocosms, it was observed that on days 1, and 67 exclusively that $MnSO_4$ exposure concentration positively significantly correlated with water grab sample concentration ($p= 0.015$ and 0.003 respectively) (Table 2.10). Field blank Mn measures were within the parts per trillion (ppt) range for the entirety of the study, thereby other Mn additions or contamination events were assumed unlikely (Table 2.7). By subtracting the pre-exposure water sample concentrations (day -9) as background from each set of post exposure measured water concentrations, on post exposure study day 1 (one day post exposure) < 15% of Mn previously added was

present within the water in any of the mesocosms (Fig 2.2). On post exposure day 21, < 7% of the original Mn previously added was present. Then on post exposure day 67 the percent increased back to <11% (Fig 2.2).

2.3.3. Sediment Samples SO-ICP-MS (and/or ICP-QQQ-MS)

Only sediment samples from July 29 (day 1) and October 3 (day 67) were analyzed from mesocosms 5, 8, 6, and 3 (representing control and nominal treatment concentrations of 0, 0.65, 1.3 and 5.2 mg/L Mn^{2+}, respectively) to determine if any range in Mn^{55} detection was present (Table 2.11). If a gradient was found to be present, the rest of the sediments were to be processed from all sampling periods and treatments. Control sediment values were 223 µg/g Mn on day 1, and 242 µg/g Mn on day 67 (Table 2.11). Test blank was measured to be 0.09 µg/g for manganese and thereby contamination was deemed not to have occurred during sample preparation (Table 2.11).

It was observed that the control and 5.2 mg/L Mn^{2+} treatment increased from day 1 to day 67 and were comparable to each other and were greater than the other measured treatments over time (Fig 2.3). On day 1 detected Mn^{55} content per treatment was as follows: 0.65 mg/L Mn^{2+} (nominal) treatment > 1.3 mg/L Mn^{2+} treatment > control > 5.2 mg/L Mn^{2+} treatment (Fig 2.3) (Table 2.11). In terms of the correlative analysis, both day 1 and day 67 sampling sessions yielded a lack of significant correlation between $MnSO_4$ exposure, and sediment Mn content (Table 2.10). Thereby no relationship was observed between exposure concentration and detected Mn levels.

2.3.4. Fish Measures (length and mass)

Fish length, wet mass and otolith mass measures taken initially (time zero sacrificed specimens) and end of the study are summarized Figs 2.4-2.9. For the results of the correlative tests for each of the biometric measures, fathead minnow males (R^2= 0.741, p= 0.013), females (R^2= 0.802, p= 0.04), or both sexes combined (R^2= 0.832, p= 0.004), average otolith mass was found to be significantly positively correlated with $MnSO_4$ exposure concentration (Table 2.12). For northern redbelly dace females or both sexes combined, average otolith mass was found to be significantly positively correlated with $MnSO_4$ exposure concentration (Table 2.12). Additionally, for northern redbelly dace females, fork length and wet weight were also found to have been significantly positively correlated with $MnSO_4$ exposure concentration (Table 2.12). No other biometric combinations were found to be significantly correlated for fathead minnow or northern redbelly dace (Table 2.12).

For fathead minnows, wet mass was found to be significantly greater in control fish over those initially collected for combined male and female (p = 0.007), females (p = 0.013), but not males exclusively (p = 0.063) (Table 2.13). Fathead minnow otolith average mass did not vary significantly between fish initially collected and control fish (Male p = 0.413; female p = 0.529; male and female p = 0.542) (Table 2.13). Lastly for fathead minnow, fork length was significantly greater in control fish over fish initially collected (Male p < 0.0001; female p = 0.018; male and female p < 0.001) (Table 2.13). Comparatively for northern redbelly dace, in each case wet weight (p < 0.001), otolith average mass (p = 0.013) and fork length (p = 0.004) were each significantly greater in control fish than fish initially collected (Table 2.13).

2.3.5. Fish Survivorship

For both species tests, data was found to be parametric, and Pearson's correlation coefficient tests were conducted. For fathead minnow: R^2= 0.0499, p = 0.63, while for northern redbelly dace: R^2= 0.0607, P = 0.868 (Table 2.14). Thereby for both species, results of the survivorship correlation tests indicated that $MnSO_4$ concentration was not correlated with survivorship.

2.3.6. High Energy Analyses

2.3.6.1. Cathodoluminescence

We observed no strong detection of Mn within any portion of the otolith (lack of fluorescence) for either fathead minnow or northern redbelly dace of either sex (Figure A1.5-6).

2.3.6.2. LA-ICP-MS, Otolith Transect

Concentration versus otolith distance graphs indicate that Mn spikes at the edge of treatment otoliths were present in some of the collected otoliths of both species (Figs 2.10-2.11). The edge may not be related to treatment and instead perhaps a contamination issue since spikes were not observed to be reproducible as observed from the one max treatment exposed fathead minnow otolith (exposed to 5.2 mg/L Mn^{2+}) which was run three times separately and indicated only a single spike (Figure A1.7). LA-ICP-MS linescans indicated that other than for the core and the area adjacent to the core of the otoliths, the typical Mn signature level ranged from < 0 ppm to ~4 ppm

for both species, although most typically < 1 ppm. When comparing overall LA-ICP-MS linescan data (core to edge), neither fathead minnow or northern redbelly dace had a consistently higher or lower Mn signature across annuli even though most of the annuli for both species were made when in separate environments (IISD-ELA lake 114, ON for fatheads or Reynold's Pond, MB for dace).

For the 10 µm section control averages (sexes combined), control otoliths Mn concentrations for fathead minnow (0.317 ± 0.17 ppm) were found to be greater than northern redbelly dace (0.2003 ± 0.034 ppm), and the same was found for maximum the nominal concentration treatment otolith concentrations (5.2 mg/L Mn^{2+}), fathead minnow (1.41 ± 0.44 ppm) over that of northern redbelly dace (0.392 ± 0.12 ppm) (Table 2.15 - 2.16) (Figs 2.12-2.13). Correlative 10 µm otolith section edge bulk analysis results indicated that there was not significant correlation between $MnSO_4$ exposure and otolith edge signature, although a greater sample size and additional treatments run may change results (Table 2.17).

2.3.6.3. Whole Otolith Samples: ICP-QQQ-MS

For fathead minnow, initially collected, control (mesocosms 1 and 11), 0.65 and 2.6 mg/L Mn^{2+} nominal treatment otoliths were analyzed and for northern redbelly dace, initially collected, control, 0.65 mg/L Mn^{2+} and 5.2 mg/L Mn^{2+} nominal treatment otoliths were analyzed (Table 2.15-2.16) (Figs 2.14-2.15). Otoliths from other treatments were not selected for analysis since not collected post-study or were to be processed/included only if strongly apparent differences were found using both the latter upper and lower treatment otoliths in the current project. When combining Mn content

results from all selected treatments, for fathead minnow and northern redbelly dace, results were comparable with minimums of 35.24 and 28.85 µg/g, maximums of 191.88 and 271.04 µg/g, averages of 84.18 and 74.14 µg/g, and standard deviations of 48.77 and 78.70 µg/g, respectively (Figs 2.14 and 2.15). When combining control averages of separate treatment tanks for fathead minnow and dace separately, results were once again comparable, with northern redbelly dace having higher Mn content of 64.73 ± 41.7 µg/g versus fathead minnow having a content of 45.68 ± 23.8 µg/g (Figs 2.14 and 2.15). For both species, the greatest treatment concentration mesocosms did not produce otoliths with the greatest Mn^{55} content indicating a potential lack of relation between Mn exposure with otolith content (Figs 2.14 and 2.15). Correlation test results of the whole otolith ICP-QQQ-MS study found that Mn content did not significantly correlate with the $MnSO_4$ exposure concentration for either species (Table 2.15).

2.3.6.4. Sediment to Otolith Correlation

For the fathead minnow, correlations between sediment and both otolith measure types (LA-ICP-MS or ICP-QQQ-MS) were not possible because only two treatment concentrations were available for comparison (Tables 2.15-2.16). For northern redbelly dace, parametric assumptions were not met, causing Spearman's correlation coefficient tests to be conducted. In both LA-ICP-MS bulk average (R^2= 0.64, p= 0.333), and ICP-QQQ-MS (R^2= 0.25, p= 1) whole otolith comparisons with sediment, no significant correlation was found to occur although greater sample size is likely required to clarify (Table 2.18).

2.4. Discussion

2.4.1. Summary

Due to the brief period in which $MnSO_4$ levels differed significantly between mesocosms, significant differences observed in water quality were not attributed to the $MnSO_4$ exposure gradient, but other factors such as seasonality, and previous parameter differences between mesocosms. $MnSO_4$ content in otolith, and sediment did not correlate significantly with nominal $MnSO_4$ treatment exposure concentrations, although this may be due to low sample size and other unaccounted factors. Fish survivability and biometrics (length, mass) were generally not effected by $MnSO_4$ exposure, thereby not attributed to the observed environment to otolith correlations. Fathead minnow and northern redbelly dace were found to not consistently differ from each other regarding Mn signatures, thereby uptake was considered similar between species.

2.4.2. Water Quality Monitoring

Analysis of water quality data indicated that control and $MnSO_4$ treatment mesocosms significantly differed from each both pre and post $MnSO_4$ exposure. For example, pH and dissolved oxygen had significant differences between control and treatment both pre-and-post exposure, making it difficult to discern whether $MnSO_4$ exposure caused an effect on the parameters. These underlying differences in water across treatments may have confounded the current study due to lack of mesocosm comparability. Still, the $MnSO_4$ treatment did not seem to be associated to whether water quality parameters were significantly different from the control mesocosms or not

post-exposure. Therefore, water quality variables likely changed over the course of the study duration due to other unaccounted for factors (e.g., change in season, weather, or just stochastically). The observed variability between mesocosms throughout the day and season further displays the complexity of natural/field systems versus lab studies, and that many variables were left unaccounted for in the current study.

2.4.3. Oxidation Reduction Effect on Mesocosm Study

Early on after $MnSO_4$ exposure in the study it became evident that the $MnSO_4$ added was visibly oxidizing. For over a week after exposure the presence of a cloudy white/grey precipitate throughout treatment mesocosm water column was visible and the observed precipitate was assumed to be an oxidized manganese species (manganese oxides). This was further supported by the dramatic decline in Mn detected within the water column over the course of the experiment, and the lack of sufficient Mn detection in fish otolith caused by decrease bioavailability of Mn^{2+}. This observed precipitation occurred in most of the treatment mesocosms, but was most evident for the upper treatments of 1.3, 2.6, and 5.2 mg/L Mn^{2+}. This tendency for oxidation was likely caused by the relatively high pH, hardness, and (the likely) strong oxidizing potential within the PWRF mesocosm environment. Increased manganese availability within the environment has been found to be strongly associated with multiple factors such as decreased levels of microbial action (some microbes can oxidize Mn) (Tebo, 1991), decreased hardness (decreased Mn sensitivity) (e.g., Stubblefield et al., 1997), and lower levels of dissolved oxygen (e.g., Limburg et al., 2015, 2011) or lower pH (e.g.,

Moreau et al., 1983) levels, which also attribute to decreased redox potential (Sundby et al., 1986).

2.4.4. Water and Sediment Samples

We found that $MnSO_4$ could be used to supplement Mn present in the water column over time, but due to the mesocosm environment the desired nominal concentrations were far less than measured $MnSO_4$ concentrations. Most of the Mn was removed rapidly from the water column after addition to the mesocosms as demonstrated by the percent remaining Mn measures (although use of stable isotopes would have likely led to a more representative and accurate measure, especially due to water quality change with seasonality). The fluctuating Mn in the water through time could have also been due in part to the employed water sampling techniques not accounting for the variability within the mesocosm water environment. Perhaps additional replicates or the implementation of an integrated water sampling technique could be applied in the future to account for potential water depth or area variability, thereby making the water samples more representative. Although, for the current study integrated water sampling was used for non-trace element measures but not water samples due to concerns of metal contamination from the integrated water sampler's metallic components.

We were unable to demonstrate that $MnSO_4$ was significantly elevated in surface/near-surface sediments. The reasons for this could be the sampling method, initial low treatment concentrations (not great enough to cause a sediment Mn treatment gradient), or initial variation in Mn present within the separate mesocosm sediments

(initial differences outweighing what was added to the mesocosms). Assuming the majority of Mn added within this experiment remained at the surface or near-surface of the sediment, the volume and depth of sediment in each jar may have diluted the overall Mn collected at the surface of each sample upon the mixing and sub-sampling for analysis. The dimensions of the sediment sample jars were: 60 mm height, 57 mm diameter, 18.4 cm circumference, and 78-100 mL capacity (sediment filled to brim, thereby ~100 mL). The sediment Mn concentrations in control mesocosms were found to be comparable or greater than treatment mesocosms on day 1 and day 67 of monitoring including the max treatment concentration mesocosm (5.2 mg/L Mn^{2+}), confounding results. Additionally, the depth to which the added $MnSO_4$ target component (Mn^{2+} or oxidized versions) may have migrated down into the sediment is unknown but insight can be gathered from another wetland study. Donahoe and Liu, (1998) found that sediment Mn content decreased from the sediment-water interface (at a pH between 6-7) downwards (for at least the first 7 cm), and at the interface, deposits were noted indicating oxidative conditions (Donahoe & Liu, 1998). Comparatively, pore water Mn concentration was observed to increase with depth, attributed to decreasing Eh with sediment depth (Donahoe & Liu, 1998). It was determined that vertical depth profiles of Mn (or Fe) can be effected by reductive dissolution, oxidation, sediment porosity, dispersion coefficients and thereby the vertical advection of porewater due to redox (Donahoe & Liu, 1998). Greater dissolution rate and lower oxidation rate (greater reduction rate) were found to lead to greater concentrations of Mn (and Fe) in pore water with depth (Donahoe & Liu, 1998). Thereby, for the MnOTOL study, the depth to which exposed Mn^{2+} from $MnSO_4$ moved into the sediment depended on multiple

variables that were not all accounted in the current study. pH was much higher in the current study and from archived data had a more variable redox potential range which implied a less stable system with periods at which rates of oxidation and reduction differ from each other. Although as noted from the precipitation observed in treatment mesocosms, Eh was assumed to promote the oxidation of available Mn. Additioanlly, the dissolution rates in the PWRF mesocosms are unknown although as stated earlier, the mesocosm sediment is clay dominated indicating greater porosity greater potential for dissolution, and vertical advection of Mn to greater depths (which contains greater favorable redox potential). Because of the latter, it is assumed that most of the exposed Mn that did not remain in the water column, and that which was not taken up by biota could have remained near the water-sediment interface in pore water or bound to the sediment, but may have moved downwards via vertical advection due to the sediments higher porosity. Lastly, referring to otolith Mn responses to sediment Mn content, no significant correlation was reached. A lack of correlation between otolith and sediment was also found by Forrester, (2005). In the latter study, Mn content within otoliths from sedentary longjaw mudsuckers (*Gillichthys mirabilis*) correlated with water but not sediment indicating that otoliths have the potential to reflect the environmental medium although factors could alter the correlation. It is also recommended that in future studies requiring sediment samples that shallower and wider sampling vessels be used increasing detection potential (e.g, open-faced and submerged Petri dishes installed at sediment level), and that ORP is monitored for to better determine redox potential.

2.4.5. Fish Measures (Survivorship, Length, and Mass)

Otolith mass was found to be significantly positively correlated with $MnSO_4$ treatment concentration for both test species except for northern redbelly dace males. This increasing otolith mass could potentially be attributed to increased incorporation of Mn^{2+} in replacement of the lighter Ca^{2+} as part of the aragonite otolith protein matrix (Campana, 1999; Melancon et al., 2005) or associated with the fact that Mn is an essential micronutrient being better utilized by the fish for growth (Watanabe, 1997), which perhaps can be interpreted as otolith growth. This difference of growth between sex and species does raise concerns over the detected Mn concentrations measured in both the LA-ICP-MS otolith transect study and ICP-QQQ-MS whole otolith study as well. If males or females of either species takes up Mn at a rate that varies from their counterpart then statistical analysis would be confounded. Comparatively, fish length and mass was not significantly correlated with $MnSO_4$ exposure except for female northern redbelly dace indicating that perhaps results for the most part were not confounded by growth. Positive correlations between otolith Mn:Ca and growth have been determined for fish such as the Pacific sardine (*Sardinops sagax*) (Javor & Dorval, 2016), and alewife (*A. pseudoharengus*) (Limburg et al., 2015). The graphical comparison of determined Mn concentration and gender per treatment level reveals no strong and consistent difference in Mn uptake between sex for either species. For future studies, we recommend the use of fish that are of the same sex, preferably male to due observations made regarding change in potential growth (and implied uptake), based on sex, thereby confounding detected trace element levels within each treatment level. Additionally, through the comparison of initially collected and control otolith fish mass

and length measures, since control otolith were comparable or significantly greater than fish initially collected, and visually displayed no tell-tale signs of malnutrition or stress that health was presumed not to confound study results. This is promising because fish stress can cause a decline in growth rate and otolith Mn:Ca (Limburg et al., 2015).

During fish processing (tissue extraction and measurement) it was observed that the dace species mainly supported northern redbelly dace fish characteristics, although some individuals seemed to possess mixed features of northern redbelly dace (*P. eos*) and finescale dace (*P. neogaeus*) such as mouths which are oblique and just reach the below the front of the eye (characteristic of *P. eos*) and a silvery peritoneum with diffuse black spots (characteristic of *P. neogaeus*) (Stewart & Watkinson, 2004). Since the features in each dace indicated primarily northern redbelly dace features (e.g., dense black speckled peritoneum/black, mouth size, gut length, spawning colors), the dace used in the study in total were considered northern redbelly dace. Still, there is the potential for some hybridization in the population (Stewart & Watkinson, 2004) and this is a possible confounding factor as fish were not collected from a culture of known genetic strain. The main difference between the two-dace species are their feeding types. As mentioned previously, northern redbelly dace feed on primarily algae, detritus, and occasionally insect larvae and are thereby considered more herbivorous, whereas finescale dace feed mainly on aquatic invertebrates (Stewart & Watkinson, 2004) which based on biomagnification may contain more Mn, since Mn has been found to bioaccumulate (Rashed, 2001). This represents two distinct routes of Mn exposure. Still, fathead minnow has similar feeding characteristics to the northern redbelly dace and the overall Mn trace element contents were found comparable between the species. This is

the case despite the two species were caught in different area with presumably different background water chemistries (IISD-ELA for the fathead minnow, and Reynold's pond for the northern redbelly dace). This also further supports the idea that perhaps otoliths may better convey physiological history rather than the environmental exposure history (Gibson-Reinemer et al., 2009; Hanson & Zdanowicz, 1999). It should also be noted that all fathead minnows were clearly identified as a single species with no hybrids being identified to our knowledge (Stewart & Watkinson, 2004).

Lastly, in terms of survivorship it was presumed that fish which were not caught were still alive but evaded capture making the survivability test conducted not a true test of survivorship (but as close to a test of survivorship as possible in the current study). As stated previously in the results section, no correlation between survivorship and the $MnSO_4$ treatment exposure gradient was observed. This result does not come as a surprise due to the low and brief exposure to $MnSO_4$ that was made. Perhaps survivability would have been effected in $MnSO_4$ exposure concentrations were higher/prolonged and should be looked in to for future studies.

2.4.6. Cathodoluminescence

Cathodoluminescence can be sensitive to the presence of trace elements at the part per billion level, and Mn is a common activator element in carbonate minerals (Marshall, 1988). Neither fathead minnow nor northern redbelly dace otoliths across the $MnSO_4$ treatment range showed detectable amounts of Mn. This indicates that small baitfish species such as fathead minnow or northern redbelly dace may not be useful as biomonitors for Mn via CL analysis. When employing CL on otoliths, the most common

CL colors are yellow-green (peaks at ~540nm for aragonite) alternating with zones of dark blue (Halden et al., 2004). Dark blue coloration has been found indicative of pure to near pure aragonite (Marshall, 1988) and this is what was observed throughout all tested otoliths (Table A1.5-6). Weak luminescence was found to occur within the summer growth bands predominantly (period of rapid growth) within the literature (Halden et al., 2004). Mn^{2+} is the most common ion forming luminescence centers (Gorobets & Walker, 1995; Machel, 1985) and is known as an activator (Boggs Jr & Krinsley, 2006). In the current study the Fe:Mn ratios for whole otolith results for fathead minnow averaged as 110, while northern redbelly dace averged 74 with none of the treatments differing considerable based on initial Mn exposure in either case (Table A1.1). Mn whole otolith absolute concentrations (fathead average= 74 µg/g, and northern redbelly dace= 84 µg/g) were typically two orders of magnitude less that Fe concentrations (fathead average= 8807 µg/g, and northern redbelly dace= 5409 µg/g) (Table A1.2). It is possible that Mn was successfully quenched in the current study leading to negative results.

2.4.7. LA-ICP-MS (otolith linescan analysis) and ICP-QQQ-MS (whole otolith)

Like CL, neither otolith analyses using linescans or whole otolith dissolution methods could detect an Mn signature that followed the Mn treatment exposure gradient significantly. Whether the Mn signature spikes identified within the treatment otoliths were in fact caused by $MnSO_4$ exposure or were an artifact of the slide preparation process is still unknown. For the beam analysis of otolith concentration versus nominal treatment concentrations, although high R^2 values were determined for both species,

due to the low sample size and lack and parametric requirements, results were still considered insignificant. The selected otolith edge section to analyze may have also over or under included growth bands making results less representative, and requires additional testing. Similarly, whole otolith results found not to be correlated. This may be due to the low treatment concentrations or the duration of the study (2.5 months) being too short a period for enough new otolith to form to allow proper measurement of Mn incorporation. A total of 12 months is required to create a single annuli (annular band), with material being deposited in daily increments (Panella, 1971) since growth rate mediates Mn incorporation (Limburg et al., 2015). Also, due to the diameter of both species otoliths (around 1 mm), the thickness of each annuli is relatively small, meaning less material which can be analyzed for a given duration, thereby lower detection of trace elements.

2.4.8. The Otolith and Mn Exposure

Overall none of the three micro-chemical tests (CL, ICP-QQQ-MS, and LA-ICP-MS) produced a Mn signature that followed the exposure gradient. This could be due to several factors; perhaps the exposure concentration was too low, the environmental conditions were not conducive for bioavailable manganese (oxidizing environment, high pH and alkalinity), the study duration was too short, and/or fathead minnow and northern redbelly dace are both poor biomonitors for Mn exposure at environmental relevant, low concentrations due to their uptake/physiology among other unaccounted reasons. A lack of correlation between water and fish otolith Mn concentration is not uncommon within the literature and has been found to occur for other fresh and

saltwater species. For instance, in LA-ICP-MS based analyses for freshwater species, rainbow trout (*O. mykiss*) (Gibson-Reinemer et al., 2009) and arctic grayling (*T. arcticus*) otolith Mn contents were found not to correlate significantly with freshwater. Additionally, juvenile black bream (*A. butcheri*) otoliths in a LA-ICP-MS based analysis found that even at water Mn levels 16 times ambient, not correlation could be reached between otolith and saltwater (Elsdon & Gillanders, 2003).

In terms of other general observations made, the Mn chemical signature spike observed over top of or adjacent to the core of otoliths in the current studies fathead minnow and northern redbelly dace otoliths have also been observed in other species such as Atlantic herring (*Clupea harengus*) (Brophy et al., 2004). The latter core/near-core spikes are assumed to occur due to physiological effects during embryo development or differing core composition as compared to the rest of the otolith (Brophy et al., 2004). Additionally, no other adverse effects were observed to occur. Lastly, other tissues, if the muscle and liver tissues were not compromised, perhaps the would have shed more light over the effect or detection of $MnSO_4$ exposure.

2.4.9. Implications

Some of implications which surround this work are that small baitfish species like fathead minnow and northern redbelly dace may not be suitable biomonitors for environmentally relevant concentrations of Mn, and that other biomonitors would have to be identified to fulfill this position. Secondly, the use of redox sensitive materials in exposure studies are exceedingly difficult, and determining the difference between nominal and measured concentrations within the test environment are crucial. Thirdly,

PWRF mesocosms are highly variable based on water quality, and may no longer be usable for replication experiments. Lastly, additional testing is need with greater sample sizes, and replication to account for variability and increased the rigorousness of testing for more representative results (albeit that this was a preliminary study).

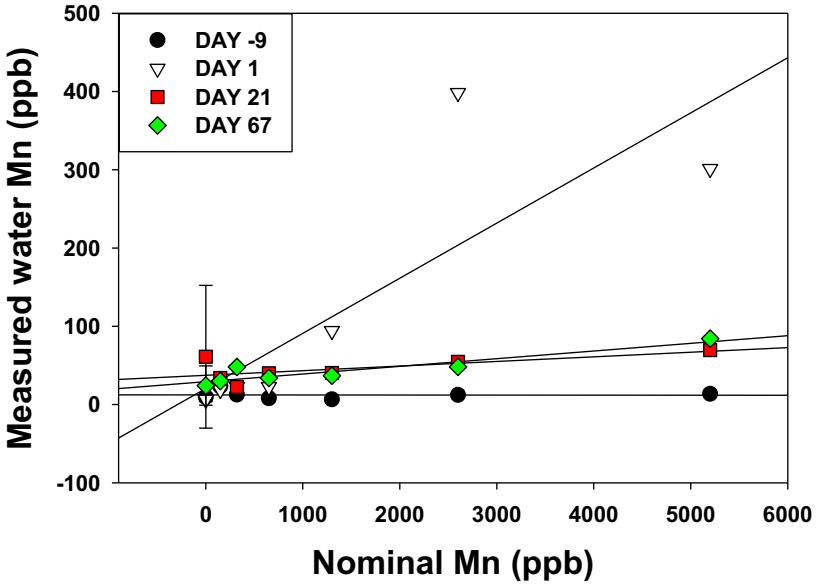

Figure 2. 2. PWRF 2016 Field study: MnOTOL. Nominal Mn^{2+} water grab-sample values versus measured 55Mn results. Field blank removed since detection of Mn considered negligible.

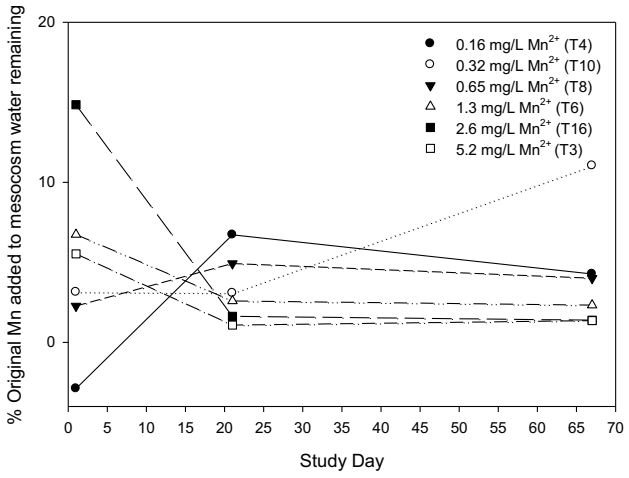

Figure 2. 3. MnOTOL 2016 scatter/line plot of the percent of Mn detected in the mesocosm if background Mn in the each mesocosm was removed.

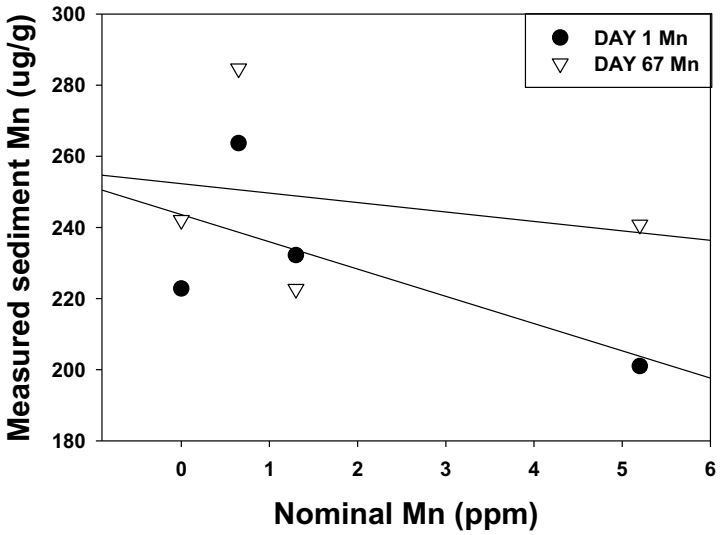

Figure 2. 4. Line/scatter plot of nominally added Mn^{2+} versus detected 55Mn in surface sediments. Sediment blank measure was found to be 0.09 µg/g, reference material percent recovery was found to be 84.4%.

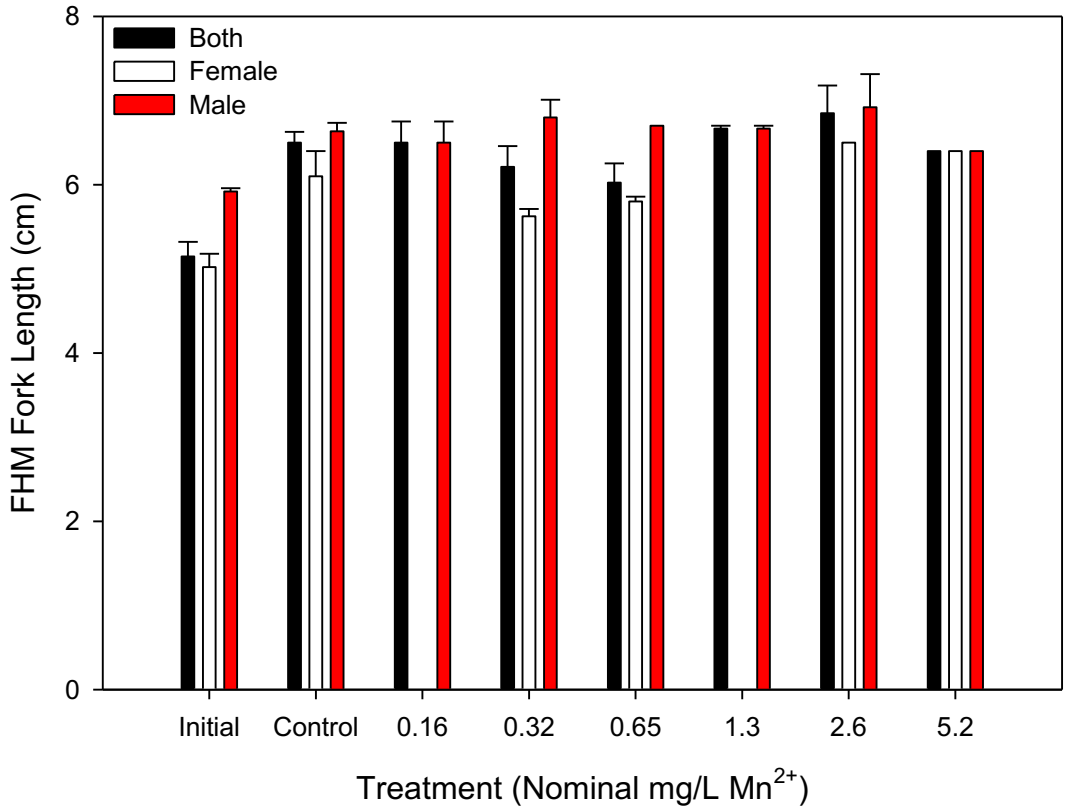

Figure 2. 5. PWRF 2016 Field study: MnOTOL. Average fork length for fathead minnow (FHM) per treatment post-study collection. Error as standard deviation.

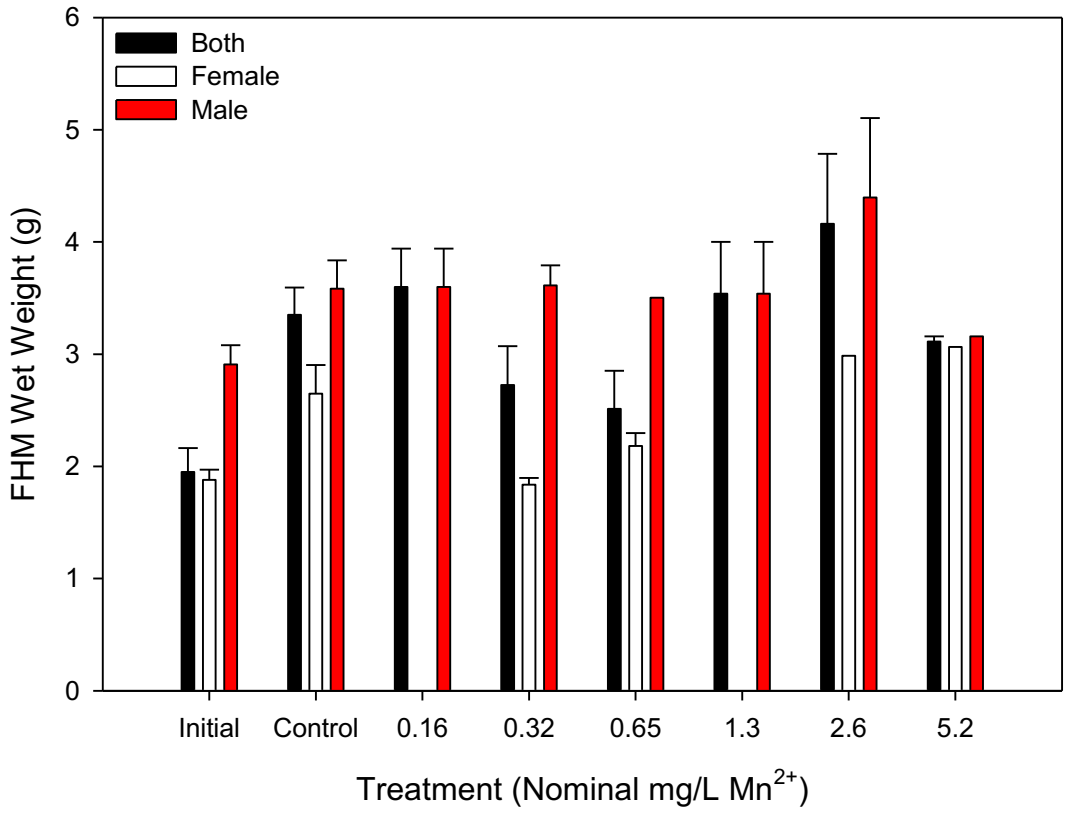

Figure 2. 6. PWRF 2016 Field study: MnOTOL. Average wet weight for fathead minnow (FHM) per treatment post-study collection. Error as standard deviation.

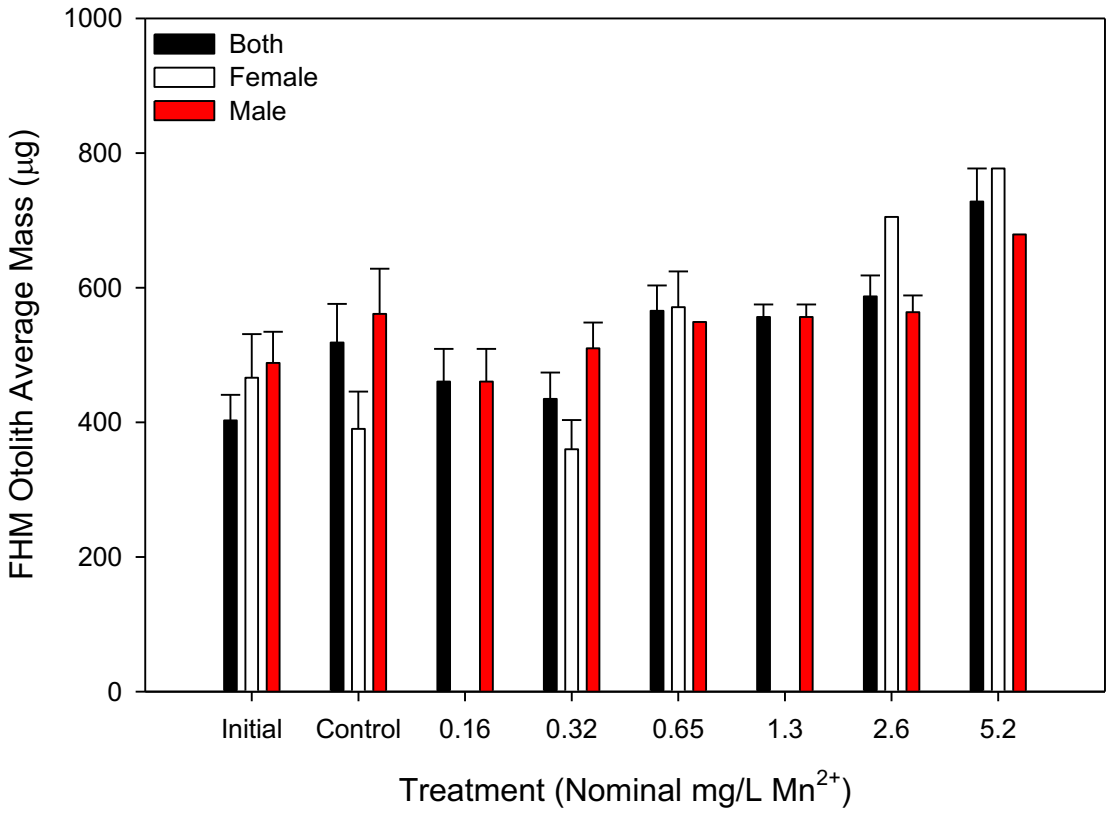

Figure 2. 7. PWRF 2016 Field study: MnOTOL. Average otolith mass for fathead minnow (FHM) per treatment post-study collection. Error as standard deviation.

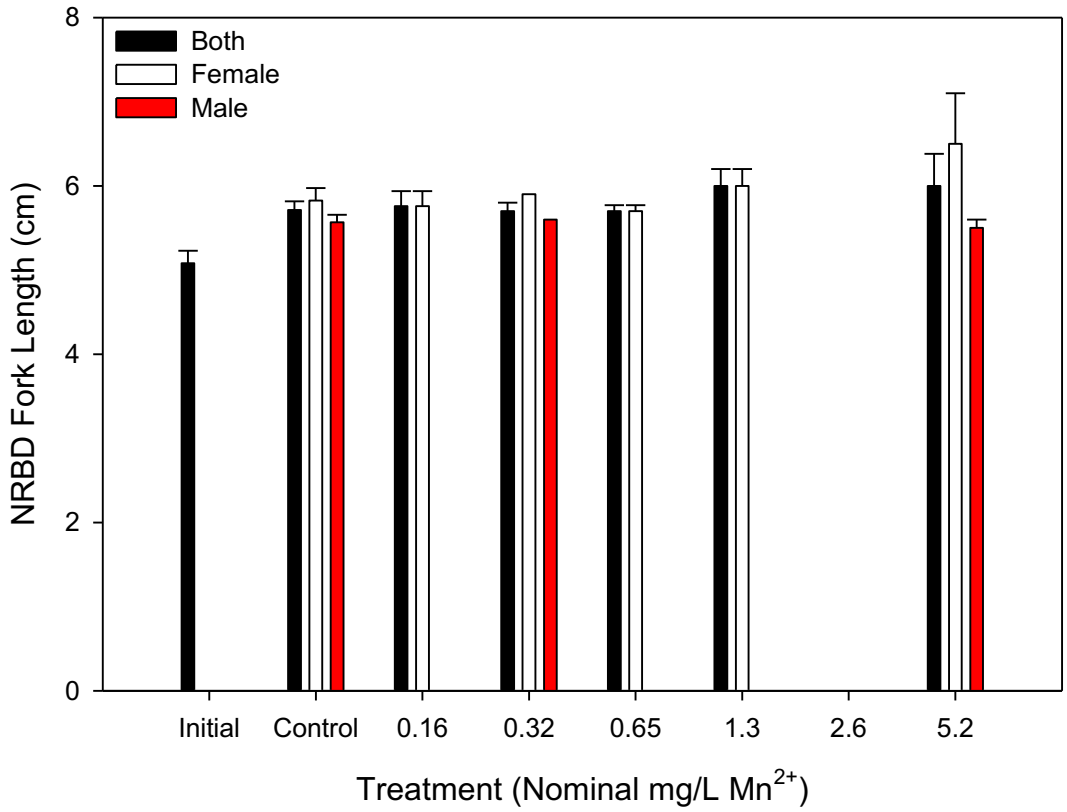

Figure 2. 8. PWRF 2016 Field study: MnOTOL. Average fork length for northern redbelly dace (NRBD) per treatment post-study collection. Error as standard deviation.

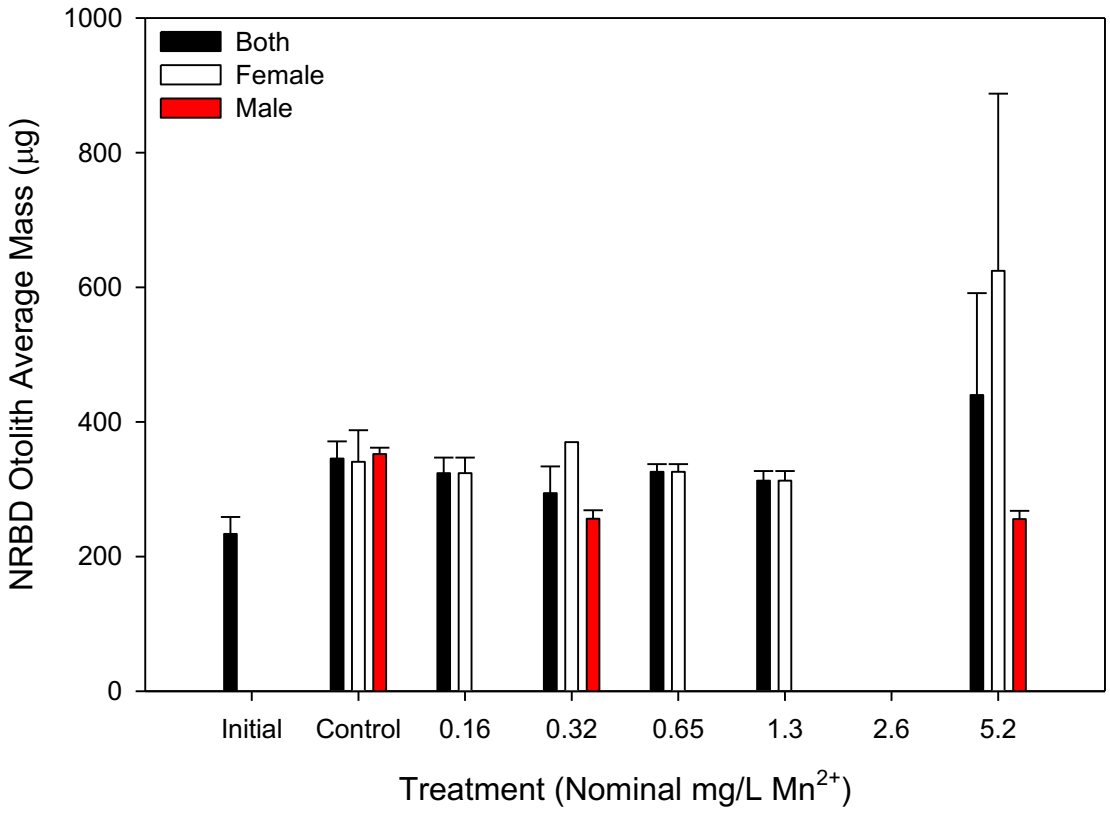

Figure 2. 9. PWRF 2016 Field study: MnOTOL. Average otolith mass for northern redbelly dace (NRBD) per treatment post-study collection. Error as standard deviation.

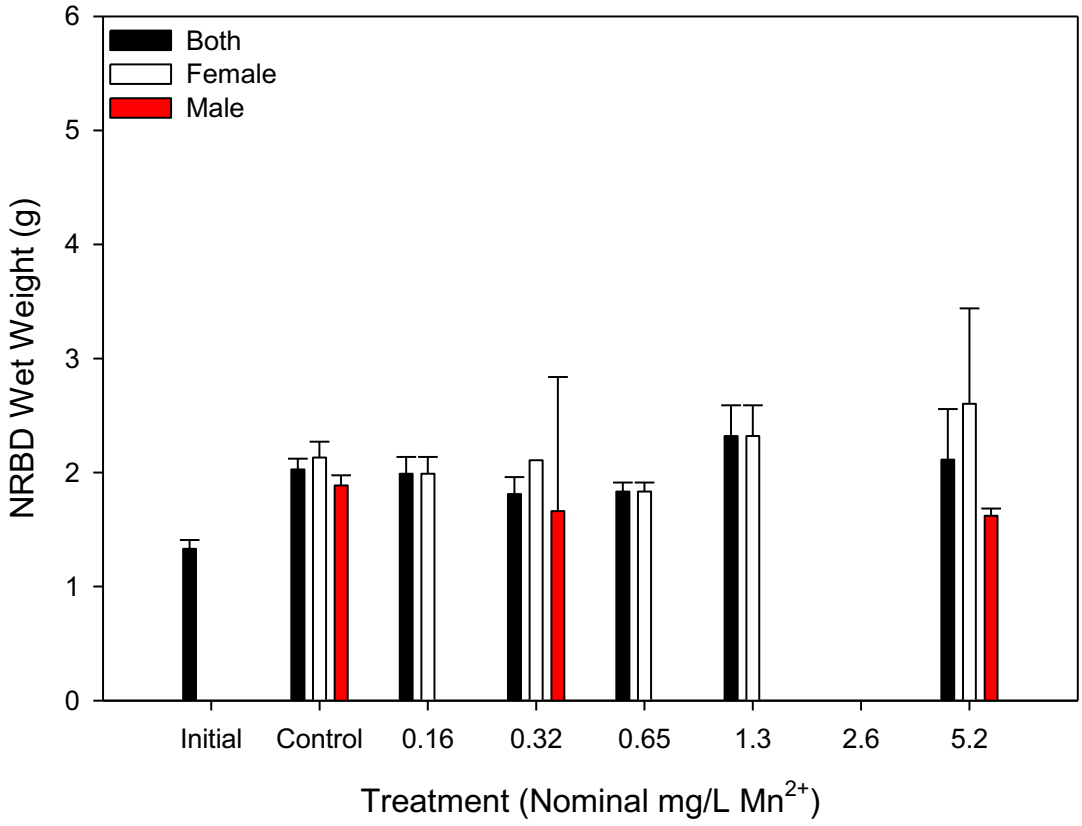

Figure 2. 10. PWRF 2016 Field study: MnOTOL. Average wet weight for northern redbelly dace (NRBD) per treatment post-study collection. Error as standard deviation.

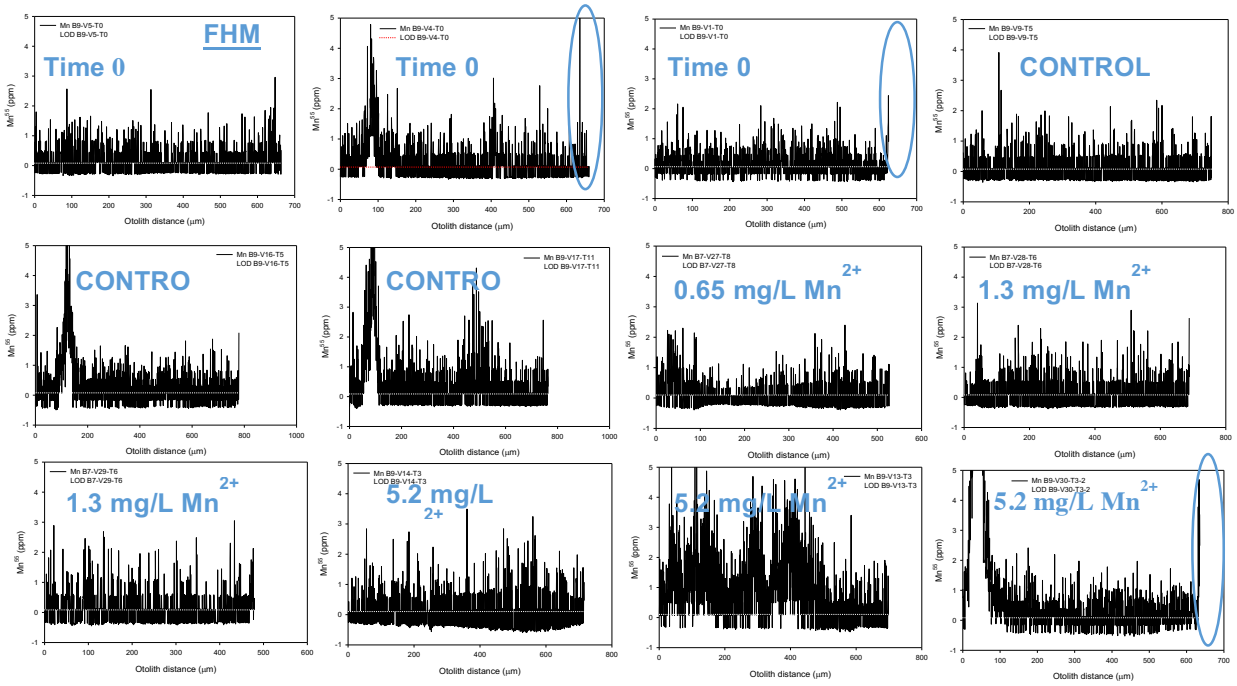

Figure 2. 11. Fathead minnow otolith laser linescans (core to edge, left to right). Circled area indicates suspect Mn edge spike associated with study treatment or background conditions.

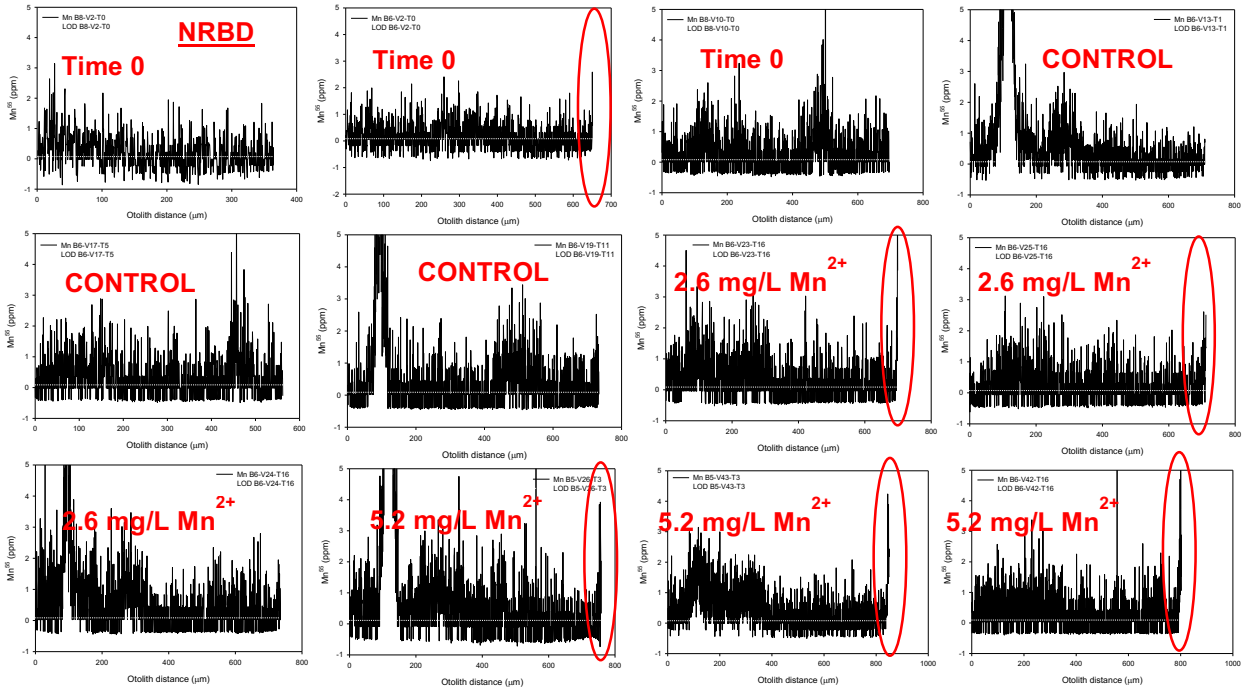

Figure 2. 12. Northern redbelly dace otolith laser linescans (core to edge, left to right). Circled area indicates suspect Mn edge spike associated with study treatment or background conditions.

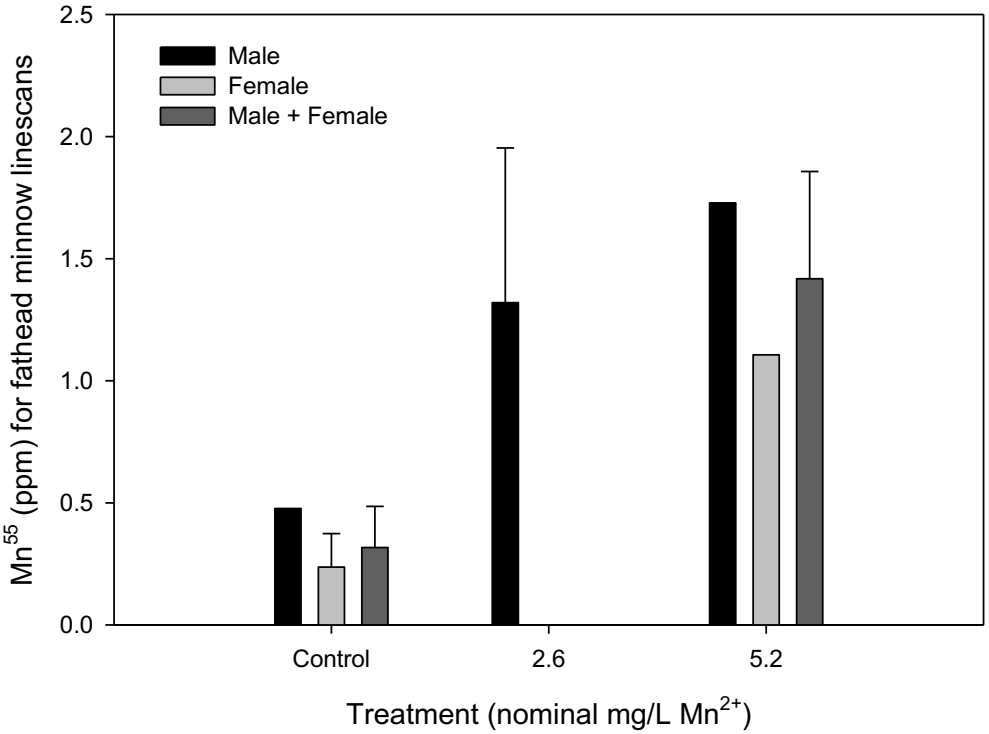

Figure 2. 13. Bar chart of fathead minnow otolith edge Mn^{55} content collected at end of test duration. Processed via LA-ICP-MS. Error bars as standard deviation.

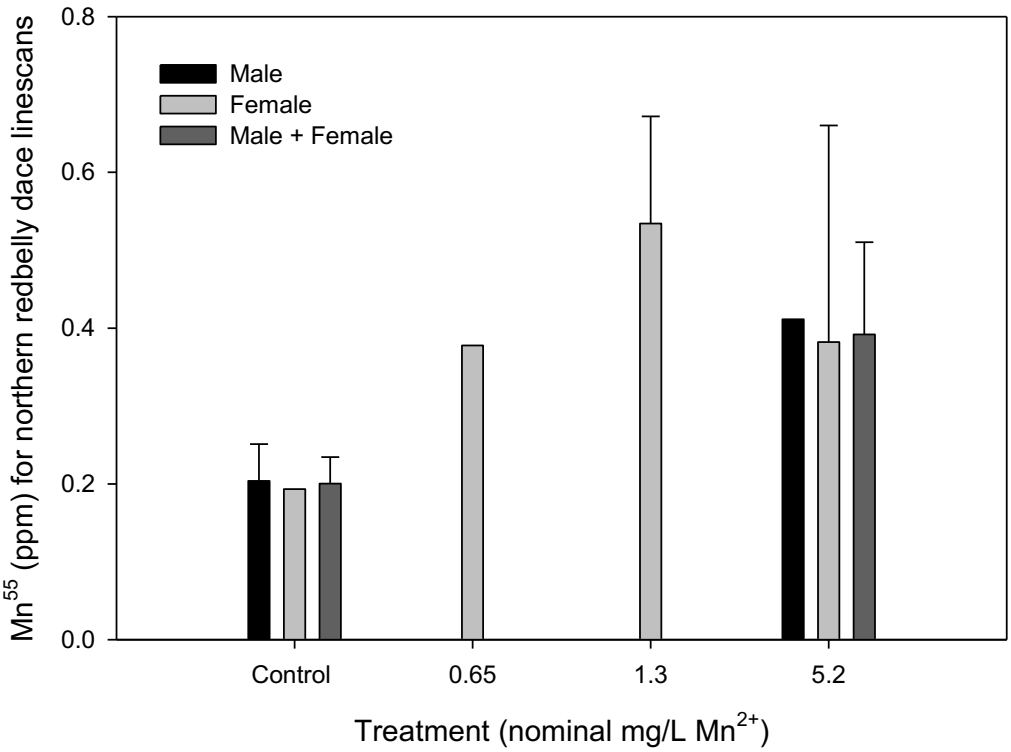

Figure 2. 14. Bar chart of northern redbelly dace Mn55 content collected at end of test duration. Processed via LA-ICP-MS. Error bars as standard deviation.

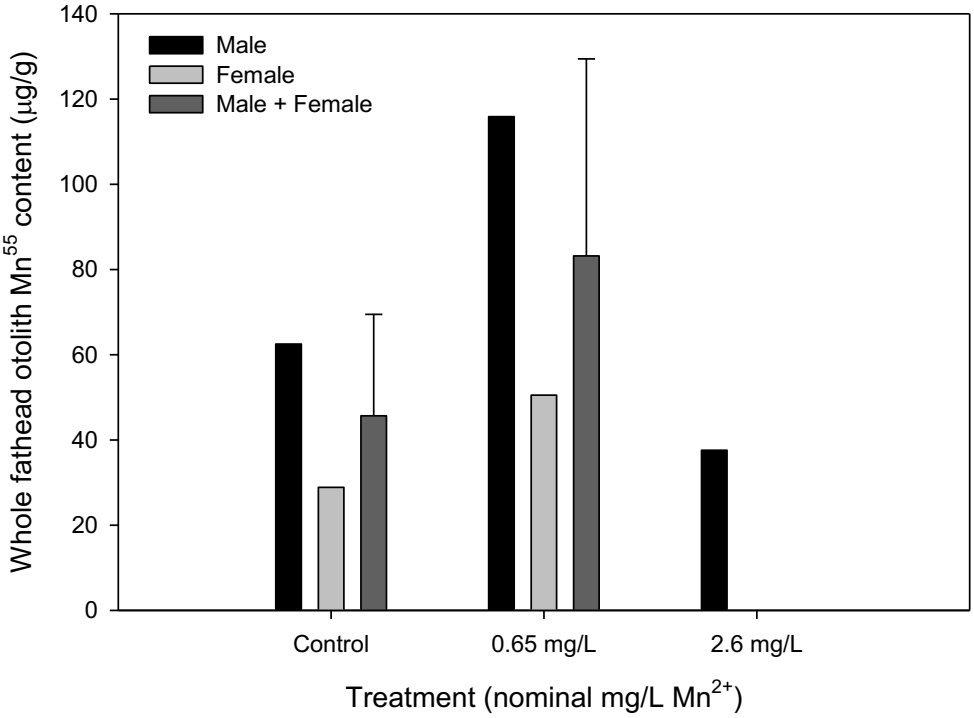

Figure 2. 15. Bar chart of fathead minnow whole otolith Mn55 content collected at end of test duration. Processed via ICP-QQQ-MS. Error bars as standard deviation. Otolith sample size of N=1 if no error bar is present, N=2 if present for control, 0.65 and 2.6 mg/L Mn^{2+} treatments.

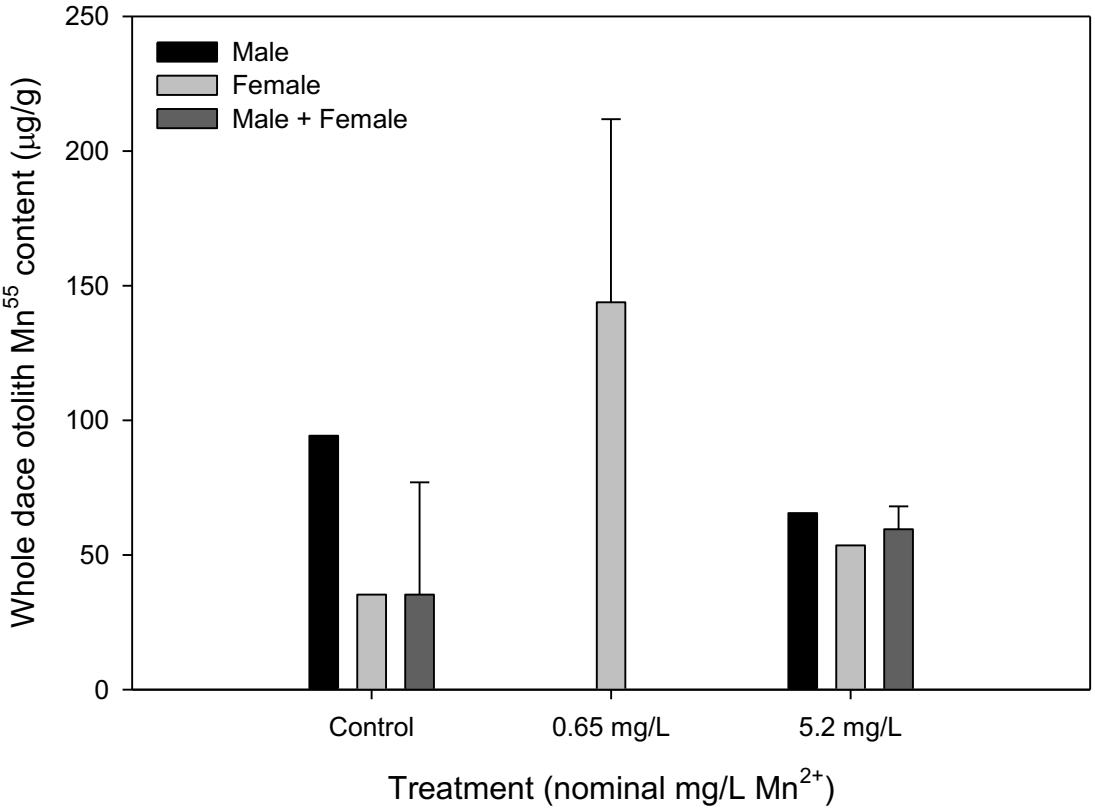

Figure 2. 16. Bar chart of northern redbelly dace whole otolith Mn55 content collected at end of test duration. Processed via ICP-QQQ-MS. Error bars as standard deviation. Otolith sample size of N = 1 if no error bar is present, N=2 if present for control, 0.65 and 5.2mg/L Mn^{2+} treatments.

Chapter 2 Tables

Table 2. 1. Fathead minnow (FHM) and northern redbelly dace (NRBD) capture/release and collection summary table. RED numbers indicate decrease in number of individuals (mortality) from those initially added to a tank. BLUE numbers indicate a greater number of samples collected than individuals within the tank.

	FHM				NRBD			
Tank	Initially Added	Presumed in Tank	Caught	# indivs. with otoliths collected	Initially Added	Presumed in Tank	Caught	# indivs. with otoliths collected
1	5	4	3	3	7	6	0	0
5	5	4	4	4	7	5	3	3
11	6	6	1	1	7	7	3	3
4	6	6	3	3	7	7	5	5
10	6	6	7	7	7	7	3	3
8	6	6	4	4	7	7	4	4
6	5	4	3	3	7	7	2	2
16	6	6	6	6	7	7	0	0
3	5	5	2	2	7	7	4	4
Sum	50	47	33	33	63	60	24	24

Table 2. 2. Typical LA-ICP-MS operating conditions and data acquisition parameters for the analysis of otoliths in MnOTOL

ICP-MS			
Forward power	1203W		
Reflected power	~3W		
Gas flows			
Plasmas (Ar)	14.8 L/min		
Auxiliary (Ar)	0.85 L/min		
Sample (Ar/He)	1.015 L/min		
He gas	0.672 L/min		
LA	Standard (spot sample)	Analysis	Pre-ablation
Repetition rate	10Hz	10Hz	10Hz
Spot size	15um	15um	25um
Power	55%	55%	45%
Incident pulse energy (mJ)	0.008	0.008	0.009
Energy density on sample (J/cm2)	4.7	4.7	1.8
Laser scan speed (um/s)	n/a	2	150
Pre-ablation warm up (s)	60	60	60
Data acquisition			
Protocol	Time resolved analysis		
Scanning mode	Bscan and Escan		
Detector mode	Analog and counting		
Isotopes determined	^{44}Ca, ^{55}Mn		
Dwell time (ms)	2 to 5		
Magnet settling time (s)	0.001 to 0.1		
Resolution	Medium		

Table 2. 3. Mean and St. Dev values for YSI water quality variable measures taken in the AM pre and post exposure. Selections with the same superscript letter as control are statistically different from control by whatever statistical test used. Superscript "a" means repeated measures ANOVA (Friedman's), post-Hoc Dunn's method while superscript "b" means Repeated measures 1-way ANOVA, post-hoc Dunnett's method.

Treatment			AM-PRE			
(mg/L Mn^{2+})	Temperature	Sp. Cond.	pH	Total chlorophyll	DO (%)	DO (mg/L)
Control	18.62 ± 2.88	0.517 ± 0.030	9.60 ± 0.43	9.83 ± 1.81	79.82 ± 17.17	7.52 ± 1.84
0.16	18.65 ± 2.87	0.607 ± 0.049[a]	9.23 ± 0.60[a]	8.80 ± 1.38	69.89 ± 20.88[a]	6.57 ± 2.11[a]
0.32	18.85 ± 2.78[a]	0.575 ± 0.037[a]	9.59 ± 0.49	9.44 ± 2.26	74.08 ± 20.18[a]	6.96 ± 2.08[a]
0.65	18.71 ± 2.84[a]	0.555 ± 0.048[a]	9.55 ± 0.57	7.53 ± 1.30[b]	80.29 ± 17.65	7.52 ± 1.81
1.3	18.74 ± 2.87[a]	0.475 ± 0.037	9.60 ± 0.53	10.15 ± 2.22	86.02 ± 18.26	8.06 ± 1.90
2.6	18.86 ± 2.85[a]	0.547 ± 0.031[a]	9.72 ± 0.43[a]	11.43 ± 3.21	74.83 ± 16.22[a]	6.99 ± 1.71[a]
5.2	18.54 ± 2.94	0.614 ± 0.033[a]	9.40 ± 0.43[a]	8.7 ± 2.14	74.93 ± 16.63[a]	7.09 ± 1.79[a]
			AM-POST			
Treatment	Temperature	Sp. Cond.	pH	Total chlorophyll	DO (%)	DO (mg/L)
Control	16.14 ± 4.40	0.557 ± 0.063	9.29 ± 0.32	12.16 ± 2.02	61.13 ± 14.46	6.10 ± 1.74
0.16	15.94 ± 4.46[a]	0.688 ± 0.083[a]	8.58 ± 0.42[a]	11.28 ± 1.60	51.60 ± 15.76[a]	5.20 ± 1.84[a]
0.32	16.32 ± 4.38[a]	0.620 ± 0.051[a]	9.36 ± 0.18	11.64 ± 3.41	52.14 ± 15.22[a]	5.22 ± 1.86[a]
0.65	16.17 ±4.44	0.626 ± 0.077[a]	9.02 ± 0.27[a]	9.44 ± 2.01[a]	56.95 ± 13.26[a]	5.68 ± 1.61[a]
1.3	16.28 ± 4.45[a]	0.538 ± 0.074	9.00 ± 0.28[a]	11.38 ± 2.06	58.28 ± 16.57[a]	5.81 ± 1.93[a]
2.6	16.44 ± 4.38[a]	0.624 ± 0.074[a]	9.16 ± 0.36[a]	10.40 ± 1.85[a]	51.40 ± 13.07[a]	5.10 ± 1.55[a]
5.2	16.03 ± 4.45	0.712 ± 0.090[a]	8.68 ± 0.56[a]	11.63 ± 4.00[a]	48.54 ± 13.55[a]	4.83 ± 1.45[a]

Table 2. 4. Mean and St. Dev values for YSI water quality variable measures taken in the PM pre and post exposure. Selections with the same superscript letter as control are statistically different from control by whatever statistical test used. Superscript "a" means repeated measures ANOVA (Friedman's), post-Hoc Dunn's method while superscript "b" means Repeated measures 1-way ANOVA, post-hoc Dunnett's method.

Treatment			PM-PRE			
(mg/L Mn^{2+})	Temperature	Sp. Cond.	pH	Total chlorophyll	DO (%)	DO (mg/L)
Control	25.17 ± 2.67	0.519 ± 0.036	10.11 ± 0.19	7.73 ± 3.01	193.89 ± 23.99	15.96 ± 1.90
0.16	25.49 ± 2.49[b]	0.581 ± 0.051[a]	9.93 ± 0.24	5.70 ± 1.33	203.46 ± 38.97	16.63 ± 3.01
0.32	25.77 ± 2.53[b]	0.572 ± 0.044[a]	10.15 ± 0.23	9.22 ± 3.60	213.16 ± 36.11[a]	17.42 ± 2.75[a]
0.65	25.43 ± 2.50	0.556 ± 0.045	10.13 ± 0.26	4.94 ± 0.65	210.21 ± 45.12[a]	17.21 ± 3.62[a]
1.3	25.31 ± 2.70	0.475 ± 0.039	10.13 ± 0.17	9.83 ± 3.64	202.44 ± 17.01	16.63 ± 1.30
2.6	25.36 ± 2.60	0.561 ± 0.036	10.18 ± 0.18	11.20 ± 7.59	198.80 ± 19.52	16.31 ± 1.63
5.2	25.09 ± 2.73	0.606 ± 0.039[a]	9.96 ± 0.17	5.14 ± 3.42	209.89 ± 19.34	17.30 ± 1.24
Treatment			PM-POST			
(mg/L Mn^{2+})	Temperature	Sp. Cond.	pH	Total chlorophyll	DO (%)	DO (mg/L)
Control	22.47 ± 4.36	0.551 ± 0.053	9.74 ± 0.35	8.52 ± 1.78	171.22 ± 32.52	14.75 ± 2.06
0.16	22.19 ± 3.92	0.658 ± 0.079[a]	9.21 ± 0.37[a]	8.69 ± 2.50	174.14 ± 53.93	14.95 ± 3.71
0.32	22.80 ± 4.28	0.609 ± 0.047	9.91 ± 0.23	8.30 ± 2.00	190.34 ± 45.59	16.37 ± 3.66
0.65	22.34 ± 4.42	0.604 ± 0.074	9.49 ± 0.35	7.04 ± 1.56	182.27 ± 38.96	15.67 ± 2.10
1.3	22.60 ± 4.31	0.520 ± 0.058	9.50 ± 0.33	8.89 ± 1.63	172.81 ± 44.00	14.74 ± 2.64
2.6	22.55 ± 4.44	0.611 ± 0.066	9.61 ± 0.41	8.31 ± 1.39	167.86 ± 38.82	14.40 ± 2.50
5.2	22.13 ± 4.32	0.691 ± 0.081[a]	9.17 ± 0.49[a]	11.01 ± 8.06	151.47 ± 35.05[a]	13.08 ± 2.22[a]

Table 2. 5. Mean and St. Dev values for water quality variables pre and post exposure non-YSI measures. Selections with the same superscript letter as control are statistically different from control by whatever statistical test used. Superscript "a" means repeated measures ANOVA (Friedman's), post-Hoc Dunn's method while superscript "b" means Repeated measures 1-way ANOVA, post-hoc Dunnett's method.

Treatment				PRE			
mg/L Mn^{2+}	Fil.alg. assess.	Fil.alg. confid.	HAR mg/L $CaCO_3$	ALK mg/L $CaCO_3$	PAR $\mu mol*m^{-2}*s^{-1}$	Depth cm	Vol. cm^3
Control	1 ± 0	9.79 ± 0.19	156.67 ± 14.14	165 ± 7.07	918 ± 67	41 ± 2.27	2084 ± 113
0.16	1 ± 0	9.93 ± 0.15	200 ± 28.28	190 ± 28.28	930 ± 97	38 ± 3.19[a]	1929 ± 158[a]
0.32	1 ± 0	9.70 ± 0.42	180 ± 28.28	175 ± 7.07	930 ± 115	39 ± 1.80 [a]	1971 ± 89[a]
0.65	1 ± 0	9.93 ± 0.15	160 ± 0	160 ± 0	910 ± 74	39 ± 1.91[a]	1960 ± 95[a]
1.3	1 ± 0	9.70 ± 0.31	140 ± 0	140 ± 0	890 ± 109	41 ± 2.06	2074 ± 102
2.6	1 ± 0	9.85 ± 0.18	170 ± 14.14	170 ± 0	900 ± 122	42 ± 2.02	2118 ± 100
5.2	1 ± 0	9.85 ± 0.24	190 ± 42.43	167.5 ± 24.75	900 ± 122	39 ± 4.07	1985 ± 202
Treatment				Post			
mg/L Mn^{2+}	Fil.alg. assess.	Fil.alg. confid.	HAR mg/L $CaCO_3$	ALK mg/L $CaCO_3$	PAR $\mu mol*m^{-2}*s^{-1}$	Depth cm	Vol. cm^3
Control	1.29 ± 0.31	9.46 ± 0.21	160 ± 30.55	171.11 ± 26.94	783 ± 87	33.9 ± 5.42	1716 ± 269
0.16	1.56 1 ± 0.5	8.67 ± 0.83	213.33 ± 23.09[b]	216.67 ± 20.82[b]	827 ± 25	28.7 ± 5.82[a]	1457 ± 289[a]
0.32	1.07 ± 0.14	9.33 ± 0.61	186.67 ± 30.55	183.33 ± 15.28	783 ± 104	32.4 ± 4.13	1641 ± 205
0.65	1 ± 0	9.63 ± 0.40	206.67 ± 46.19[b]	190 ± 26.46	817 ± 76	30.7 ± 5.54[a]	1554 ± 275[a]
1.3	1 ± 0	9.77 ± 0.22	180 ± 52.92	173.33 ± 40.41	767 ± 29	32.5 ± 5.75	1643 ± 286
2.6	1 ± 0	9.73 ± 0.31	193.33 ± 41.63	173.33 ± 32.15	750 ± 87	34.5 ± 4.67	1745 ± 232
5.2	1.03 ± 0.11	9.23 ± 0.75	226.67 ± 30.55[b]	193.33 ± 5.77	757 ± 60	30.6 ± 6.08[a]	1552 ± 302[a]

Table 2. 6. Summary table for repeated measures ANOVAs (RMAs) concerning archived 2014 and 2015 ORP (in mv) PWRF measures taken via YSI-Sonde device. 2014 data collected from June-September 2015 data collected from May-October.

Parameter	Transformation	Normal	HOV	Power	N	Test	F-stat	Chi^2	P-val
ORP 2014-AM	Raw or Log	<0.05		0.05	4	Friedman RMA		4.714	0.581
ORP 2014-PM	Raw or Log	<0.05		0.153	4	Friedman RMA		7.745	0.257
ORP 2015-AM*	Raw	0.076	0.941	0.064	5	1-way RMA	1.076		0.404
ORP 2015-PM	Could not be tested, sample size too small								

*Month of October removed, since testing required equal sample sizes per group tested

Table 2. 7. Water grab sample concentration value summary. Sample sizes indicate replicate measures of the same water sample except for "Control Avrg" in which case sample size equals the number of control mesocosms. "F. Blank" means field blank. If sample size is not indicated it is equal to one.

Treatment (mg/L Mn^{2+})	Water grab sample: SO-ICP-MS (ppb)			
	Day -9	Day 1	Day 21	Day 67
F. Blank	0.095 ± 0	0.075 ± 0	0.031 ± 0	0.043 ± 0
Control T1	5.65 ± 0	3.81 ± 0	4.73 ± 1.30 (n= 2)	9.34 ± 0
Control T5	11.11 ± 0	6.41 ± 0	12.23 ± 0	10.09 ± 0
Control T11	11.71 ± 0.26 (n= 2)	9.13 ± 0	166.24 ± 0	53.24 ± 0
Control Avrg.	9.49 ± 3.34 (*n= 3)	6.45 ± 2.66 (*n= 3)	61.07 ± 91.16 (*n= 3)	24.22 ± 25.13 (*n= 3)
0.16	23.25 ± 0	18.63 ± 0	34.0 ± 0	30.08 ± 0
0.32	12.62 ± 0	22.67 ± 0	22.47± 0	47.95 ± 0
0.65	7.91 ± 0.05 (n= 2)	22.66 ± 0.28 (n= 2)	39.92 ± 0	33.96 ± 0
1.3	6.59 ± 0	94.17 ± 0	40.25 ± 1.49 (n= 2)	36.8 ± 0
2.6	12.18 ± 0	398.18 ± 0	54.5 ± 0	48.14 ± 1.02 (n= 2)
5.2	13.58 ± 0	301.14 ± 3.86 (n= 2)	69.61 ± 0.93 (n= 2)	84.13 ± 0

Table 2. 8. Summary table, one way ANOVA on ^{55}Mn content for MnOTOL ICP-MS water samples pooled into Control (Mesocosms 1, 5, 11), Low (Mesocosms 4, 8, 10) and High (Mesocosms 3, 6, 16) treatments. **Bolded** text indicates a significant difference at an alpha of 0.05.

Day	Shapiro-Wilk W	Shapiro-Wilk p(normal)	Levene´s test HOV, from means p(same)	Levene´s test, from medians p(same)	F (ANOVA)	H (chi^2)	Kruskal-Wallis p(same)	1-way ANOVA p(same)
-9	0.952	0.7123	0.2275	0.5935	0.7323	1.867	0.3932	0.5193
1*	**0.937**	**0.5508**	**0.07542**	**0.4691**	**38.26**	**7.2**	**0.02732**	**0.000385**
21	0.8542	0.08281	**0.009236**	0.4783	0.2423	2.4	0.3012	0.7921
67	0.8583	0.09183	0.1763	0.7798	1.766	2.489	0.2881	0.2495

*log transformed

Table 2. 9. Summary matrix of post-hoc test results for one way ANOVA on ^{55}Mn content for MnOTOL ICP-MS water samples pooled into Control (Mesocosms 1, 5, 11), Low (Mesocosms 4, 8, 10) and High (Mesocosms 3, 6, 16) treatments. **Bolded** text indicates a significant difference at an alpha of 0.05.

RAW	P-val			
Dunn's Q	S2	CONTROL	LOW	HIGH
	CONTROL		> 0.05	**< 0.05**
	LOW	1.342		> 0.05
	HIGH	2.683	1.342	
LOG	P-val			
Tukey's Q	S2	CONTROL	LOW	HIGH
	CONTROL		**0.05524**	**0.0005168**
	LOW	4.228		**0.003415**
	HIGH	12.18	7.954	

Table 2. 10. MnOTOL water and sediment grab sample correlation coefficient test results. Significance at an alpha of 0.05 indicated by **bold**-text. Rows marked with an asterisk (*) excluded mesocosm 11 control from the control treatment averages. Sample size for each water and sediment grab sample tests are N= 7 and 4 respectively.

Transform	Sample	Day	R	R^2	Normal	HOV	Power	Test	F-stat	P-val
Raw*	Water grab	-9	0.00415	0.0000173	0.134	0.0341	0.025	P	0.0000863	0.993
Log*	**Water grab**	**1**	**0.841**	**0.708**	**0.874**	**0.341**	**0.689**	**P**	**12.125**	**0.018**
Raw*	**Water grab**	**21**	**0.893**	**0.797**	**0.365**	**0.073**	**0.818**	**P**	**19.6**	**0.007**
Raw*	**Water grab**	**67**	**0.888**	**0.788**	**0.61**	**0.341**	**0.805**	**P**	**18.55**	**0.008**
Raw	Water grab	-9	0.022	0.000484	0.201	0.341	0.028	P	0.00242	0.963
Log	**Water grab**	**1**	**0.851**	**0.723**	**0.732**	**0.388**	**0.711**	**P**	**13.075**	**0.015**
Raw	Water grab	21	0.671	0.451	0.237	0.096	0.369	P	4.104	0.099
Raw	**Water grab**	**67**	**0.922**	**0.85**	**0.075**	**0.66**	**0.893**	**P**	**28.367**	**0.003**
Raw/Log	Sediment grab	1	-0.4	0.16	nc.	nc.	nc.	S	nc.	0.75
Raw/Log	Sediment grab	67	-0.6	0.36	nc.	nc.	nc.	S	nc.	0.417

*P= Pearson's, S= Spearman's correlation coefficient test

Table 2. 11. Sediment grab sample concentration value summary. Control measure taken from mesocosm 5 only. "L. Blank" means Lab blank.

Treatment (mg/L Mn^{2+})	Sediment sample: ICP-QQQ-MS (µg/g)	
	Day 1	Day 67
L. Blank	0.09 ± 0 (n= 1)	0.09 ± 0 (n= 1)
Control	222.79 ± 0 (n= 1)	242.06 ± 0 (n= 1)
0.16	nc.	nc.
0.32	nc.	nc.
0.65	263.69 ± 0 (n= 1)	284.67 ± 0 (n= 1)
1.3	232.21 ± 0 (n= 1)	222.71 ± 0 (n= 1)
2.6	nc.	nc.
5.2	200.99 ± 6.94 (*n= 3)	240.79 ± (*n= 3)

Table 2. 12. MnOTOL fish biometric measure correlation coefficient test results. Significance at an alpha of 0.05, significant differences indicated by **bold-text**.

Dependent Measure	Species-Sex	N	R	R^2	Normal	HOV	Power	Test	F-stat	P-value
Fork length (cm)	FHM-All	7	0.231	0.0531	0.791	0.217	0.068	P	0.281	0.619
Wet weight (g)	FHM-All	7	0.17	0.029	0.806	0.438	0.053	P	0.149	0.715
Otolith avrg, mass (µg)	**FHM-All**	**7**	**0.912**	**0.832**	**0.987**	**0.073**	**0.869**	**P**	**24.842**	**0.004**
Fork length (cm)	FHM-F	3	0.729	0.532	0.32	0.05	0.258	P	3.407	0.162
Wet weight (g)	FHM-F	3	0.742	0.55	0.713	0.05	0.271	P	3.667	0.151
Otolith avrg, mass (µg)	**FHM-F**	**3**	**0.895**	**0.802**	**0.282**	**0.05**	**0.535**	**P**	**12.132**	**0.04**
Fork length (cm)	FHM-M	7	0.317	0.1	0.776	0.66	0.096	P	0.558	0.489
Wet weight (g)	FHM-M	7	-0.286	0.081796	nc.	nc.	nc.	S	nc.	0.491
Otolith avrg, mass (µg)	**FHM-M**	**7**	**0.861**	**0.741**	**0.985**	**0.217**	**0.737**	**P**	**14.307**	**0.013**
Fork length (cm)	DACE-All	6	0.5	0.25	nc.	nc.	nc.	S	nc.	0.297
Wet weight (g)	DACE-All	6	0.38	0.144	0.398	0.06	0.103	P	0.674	0.458
Otolith avrg, mass (µg)	**DACE-All**	**6**	**0.891**	**0.794**	**0.618**	**0.06**	**0.696**	**P**	**15.446**	**0.017**
Fork length (cm)	**DACE-F**	**6**	**0.952**	**0.906**	**0.275**	**0.06**	**0.894**	**P**	**38.638**	**0.003**
Wet weight (g)	**DACE-F**	**6**	**0.836**	**0.698**	**0.487**	**0.06**	**0.552**	**P**	**9.259**	**0.038**
Otolith avrg, mass (µg)	**DACE-F**	**6**	**0.939**	**0.881**	**0.452**	**0.06**	**0.848**	**P**	**29.604**	**0.006**
Fork length (cm)	DACE-M	3	-0.5	0.25	nc.	nc.	nc.	S	nc.	1
Wet weight (g)	DACE-M	3	-1	1	nc.	nc.	nc.	S	nc.	0.333
Otolith avrg, mass	DACE-M	3	-1	1	nc.	nc.	nc.	S	nc.	0.333

*P= Pearson's, S= Spearman's correlation coefficient test

Table 2. 13. Summary table of fathead minnow (FHM) and northern redbelly dace (NRBD) comparative t-tests between time zero and control fish (post study duration) for wet mass, otolith average mass, and fork length, with species combined and separately. **Bolded** text indicates a significant difference at an alpha of 0.05.

Species	Sex	Initial "n"	Control "n"	Measure	Power	p-val Levene's	Shapiro-Wilk	t-test p-val
FHM	M	5	6	Wet mass (g)	0.377	0.946	0.055	0.063
FHM	**F**	**5**	**2**	**Wet mass (g)**	**0.836**	**0.377**	**0.281**	**0.013**
FHM	**M+F**	**10**	**8**	**Wet mass (g)**	**0.799**	**0.73**	**0.547**	**0.007**
FHM	M	5	6	Otolith average mass (µg)	0.05	0.993	0.149	0.413
FHM	F	5	2	Otolith average mass (µg)	0.05	0.167	0.983	0.529
FHM	M+F	10	8	Otolith average mass (µg)	0.05	0.73	0.162	0.542
FHM	**M**	**5**	**6**	**Fork Length (cm)**	**1**	**0.227**	**0.85**	**<0.0001**
FHM	**F**	**5**	**2**	**Fork Length (cm)**	**0.765**	**0.633**	**0.145**	**0.018**
FHM	**M+F**	**10**	**8**	**Fork Length (cm)**	**0.994**	**0.131**	**0.094**	**<0.001**
NRBD	**M+F**	**5**	**7**	**Wet mass (g)**	**0.998**	**0.36**	**0.146**	**<0.001**
NRBD	**M+F**	**5**	**7**	**Otolith average mass (µg)**	**0.739**	**0.782**	**0.581**	**0.013**
NRBD	**M+F**	**5**	**7**	**Fork Length (cm)**	**0.899**	**1**	**0.338**	**0.004**

Table 2. 14. Fish survivorship Pearson's correlation coefficient test results. Significance at an alpha of 0.05, indicated by bold text. Sample sizes of N= 7.

Species	R	R^2	Shapiro Wilk	HOV	Power	F-stat	P-value
P. promelas	0.223	0.0499	0.057	0.545	0.066	0.263	0.63
C. eos	0.0779	0.00607	0.78	0.438	0.036	0.0305	0.868

Table 2. 15. Otolith transect LA-ICP-MS and whole-otolith ICP-QQQ-MS concentration value summary for fathead minnow. Control value made up of measures taken from mesocosm tank 1 and 11.

Treatment	*P. promelas* otolith (ppm) (M+F)		*P. promelas* otolith (ppm) (Male)		*P. promelas* otolith (ppm) (Female)	
(mg/L Mn^{2+})	LA-ICP-MS	ICP-QQQ-MS	LA-ICP-MS	ICP-QQQ-MS	LA-ICP-MS	ICP-QQQ-MS
Initial	nc.	113.89 ± 271.04 (n=3)	nc.	35.31 ± 4.03 (n=2)	nc.	nc.
Control	0.317 ± 0.169 (n=3)	45.68 ± 23.80 (n=3)	0.476 ± 0 (n=1)	62.51 ± 0 (n=0)	0.237 ± 0.137 (n= 2)	28.85 ± 0 (n=1)
0.16	nc.	nc.	nc.	nc.	nc.	nc.
0.32	nc.	nc.	nc.	nc.	nc.	nc.
0.65	nc.	83.19 ± 46.22 (n=3)	nc.	115.88 ± 0 (n=1)	nc.	50.51 ± 0 (n= 1)
1.3	nc.	nc.	nc.	nc.	nc.	nc.
2.6	nc.	nc.	1.32 ± 0.634 (n=4)	33.94 ± 5.12 (n=2)	nc.	nc.
5.2	1.42 ± 0.44 (n=2)	nc.	1.72 ± 0 (n=1)	nc.	1.11 ± 0 (n=1)	nc.

Table 2. 16. Otolith transect LA-ICP-MS and whole-otolith ICP-QQQ-MS total manganese concentration summary for northern redbelly dace. Control value made up of measures taken from mesocosm tank 5 only. F= Female, M= Male.

Treatment	*C. eos* otolith (ppm ^{55}Mn) (M+F)		*C. eos* otolith (ppm ^{55}Mn) (M)		*C. eos* otolith (ppm ^{55}Mn) (F)	
(mg/L Mn^{2+})	LA-ICP-MS	ICP-QQQ-MS	LA-ICP-MS	ICP-QQQ-MS	LA-ICP-MS	ICP-QQQ-MS
Initial	nc.	68.65 ± 25.54 (n= 2)	nc.	50.59 ± 0 (n= 1)	nc.	nc.
Control	0.200 ± 0.034 (n= 3)	64.73 ± 41 (n= 2)	0.204 ± 0.047 (n= 2)	94.22 ± 0 (n= 1)	0.193 ± 0 (n= 1)	35.24 ± 0 (n= 1)
0.16	nc.	nc.	nc.	nc.	nc.	nc.
0.32	nc.	nc.	nc.	nc.	nc.	nc.
0.65	nc.	nc.	nc.	nc.	0.378 ± (n= 1)	143.79 ± 68.01 (n= 2)
1.3	nc.	nc.	nc.	nc.	0.534 ± 0.138 (n= 2)	nc.
2.6	nc.	nc.	nc.	nc.	nc.	nc.
5.2	0.392 ± 0.118 (n= 3)	59.55 ± 8.51 (n= 1)	0.411 ± 0 (n= 1)	65.57 ± 0 (n= 1)	0.382 ± 0.278 (n= 2)	53.53 ± 0 (n= 1)

Table 2. 17. MnOTOL whole otolith ICP-QQQ-MS and LA-ICP-MS measure (dependent) versus nominal exposure concentration (independent) correlation coefficient test results. Significance at an alpha of 0.05, significance indicated by bold text. Sample sizes of N=3 per ICP-QQQ-MS test for both species, N= 3 for FHM and N= 4 for NRBD for LA-ICP-MS otolith tests.

Transformation	Type	Species	R	R^2	Test	P-value
Raw/Log	ICP-QQQ-MS	NRBD	0.5	0.25	Spearman's	1
Raw/Log	ICP-QQQ-MS	FHM	-0.5	0.25	Spearman's	1
Raw/Log	LA-ICP-MS	FHM	1	1	Spearman's	0.333
Raw/Log	LA-ICP-MS	NRBD	0.8	0.64	Spearman's	0.0833

Table 2. 18. Sediment (independent), otolith (dependent) Pearson's correlation coefficient results. Sediment day -67 ICP-QQQ-MS concentrations results were compared to otolith measures. For fathead minnow, sample size was too small to conduct the correlations. For northern redbelly dace individual/averaged Mn measures from the control, 0.65, 1.3 and 5.2 mg/L Mn^{2+} treatment measures were used for the LA-ICP-MS otolith transect/bulk averages to sediment correlation, and control, 0.65 and 5.2 mg/L Mn^{2+} treatment measures were used for the ICP-QQQ-MS whole otolith to sediment correlation.

Otolith Analysis	Fish	N	R	R^2	Shapiro Wilk	HOV	Power	F-stat	P-value
LA-ICP-MS raw	NRBD	4	0.279	0.0778	0.554	<0.001	0.047	0.169	0.721
LA-ICP-MS log	NRBD	4	0.192	0.0369	0.231	<0.001	0.039	0.0765	0.808
LA-ICP-MS raw/log	NRBD	4	-0.8	0.64	nc.	nc.	nc.	nc.	0.333
LA-ICP-MS raw	FHM	2	1	1	nc.	nc.	nc.	nc.	nc.
ICP-QQQ-MS raw	NRBD	3	0.971	0.943	0.951	<0.001	<0.001	16.633	0.153
ICP-QQQ-MS log	NRBD	3	0.919	0.844	0.947	<0.001	<0001	5.419	0.258
ICP-QQQ-MS raw/log	NRBD	3	0.5	0.25	nc.	nc.	nc.	nc.	1
ICP-QQQ-MS raw	FHM	2	1	1	nc.	nc.	nc.	nc.	nc.

2.5. Chapter Two References

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CHAPTER 3:

Trace element concentrations in surface waters and their relationship to fish otolith chemistry: Evidence of signatures from hydroelectric impoundment

3.0. Abstract

Otoliths and their ability to detect changes in the aquatic environments also extends to anthropogenic activities such as hydroelectric generation and impoundment. In Manitoba, Canada, the Coordinated Aquatic Monitoring Program (CAMP) monitors impounded and non-impounded sub-basins with a focus on water quality and fish populations. We tested for correlations between water chemistry, quality, and otolith trace element concentrations, as well as the effects of impoundment, species, and geology on water and otolith trace element signatures. Water quality and trace element (Ba, Mn, Sr, Mg, and Na) data from 2008-2014 were compared to otolith chemistry for lake whitefish (*Coregonus clupeaformis*) and walleye (*Sander vitreus*) from four impounded and four non-impounded sites. No significant correlations were found between water and otolith trace elements. Geology and species-type influenced water and otolith chemistry, with sites best classified by impoundment status (Ba and Sr levels primarily) although waterbody connectivity and flow accumulation may have influenced results. Otoliths should be further tested regarding their ability to detect change resulting from hydroelectric development.

3.1. Short Introduction

Manitoba Hydro and the Province of Manitoba cooperate to run the Coordinated Aquatic Monitoring Program (CAMP). This initiative aids in long term, system-wide monitoring across most of Manitoba Hydro's impoundments. The assessment of the prolonged environmental quality of water bodies affected by impoundments is a primary focus and informs decision-making about water management. A three-year pilot program was conducted from 2008 to 2011 to assess the long-term applicability of CAMP, and CAMP monitoring continues to this day. In total, CAMP monitors eight major river regions comprising 43 sub-basins underlain with varying geology, and impacted to various degrees by Manitoba Hydro activities.

Regarding monitoring, a suite of water quality measures is routinely sampled, including trace elements. There is also biotic monitoring that includes phytoplankton, benthic macroinvertebrates, and fish communities. For fish, gillnetting of lacustrine and riverine areas is conducted for small and large bodied fish species. The intention behind biotic monitoring is to collect enough samples to represent the communities within each water system. Fish of commercial importance such as lake whitefish (*Coregonus clupeaformis*), and walleye (*Sander vitreus*) are measured for age using aging structures e.g., otolith for population age distributions. In aquatic communities, fish in the mid to upper trophic levels are an integrated measure of ecosystem health due to dependence on lower trophic levels and are effected by water quality and hydrology (CAMP, 2014).

The otolith is a useful biomonitoring tool for trace elements since metabolically inert, and is used to determine a fishes exposure timeline, making otoliths ideal for long-

term monitoring (Campana, 1999; Campana & Neilson, 1985). At least 57 elements have so far been detected in otoliths (Campana, 1999; Palace et al., 2007; Pracheil et al., 2014). Some elements measured within the otolith may not reflect ambient water concentrations (e.g., Ca, Na, K, Mg, Cl, P, Cu, S), and instead reflect osmoregulation characteristics of the blood-plasma interface with the endolymph, gills and intestine due to organismal utilization (Campana, 1999). Sizable detection of elements such as Hg and Pb tend to reflect anthropogenic sources, and Sr, Zn, Pb, Mn, Ba, Fe, Li, Cd, and Ni may be associated with natural sources since less utilized and regulated within the fish (Campana, 1999).

There are currently > 45,000 registered impoundments (> 15 m high) worldwide, with many more currently unregistered (Vörösmarty et al., 2003; World Commission on Dams, 2000). One of the main abiotic impacts of impoundments on water systems is their influence on the movement of suspended material destined for the ocean and otherwise (Quist et al., 2005; Syvitski et al., 2005; Vörösmarty et al., 2003). Suspended material retention causes water velocity/discharge reduction, changes flow stability, substrate characteristics, channel morphology, trace element levels, and fish assemblages (Eiriksdottir et al., 2015; Eiriksdottir et al., 2017; Horowitz et al., 1990; Quist et al., 2005; Taylor et al., 2014). Globally it is estimated that global particulate/sediment flux to the oceans has decreased between 26%-53% (flux reduction of 1 to 5 billion metric tons per year) via impoundment construction (Syvitski et al., 2005; Vörösmarty et al., 2003).

The CAMP program is currently based on infrequent sampling to assess water quality and biotic communities, likely causing periods of greater impact to be overlooked.

An integrative approach of water quality, and biological data of commercially relevant species may account for gaps in unaccounted exposure.

Our objective was to utilize CAMP archived otolith samples and water quality database to determine the effect background geology/impoundment on otolith and water signatures, and determine if the otolith reflects water concentration gradients. To this end, otoliths collected during the 2013-2014 season were analyzed via beam analysis and compared water quality data from 2008-2014. The results of this study will be used to better interpret the effects of background geology and impoundment on water quality, and assess the value of the otolith as a biomonitoring tool for possible impoundment impacts.

3.2. Materials and Methods

3.2.1. CAMP Waterbody Site Selection

The three-year (2008-2010) CAMP pilot study (CAMPP) report was accessed from the CAMP website (http://www.campmb.com/reports/three-year-summary-report-2008-2010). The purpose of doing so was to use the report to select a set of trace elements to analyze, narrow down potential study species for otolith analysis, and select a sub-set of currently monitored CAMP waterbodies to use in the main study. To do this, individual CAMP waterbodies were assessed and compared to determine the present fish species, and water trace element concentrations across the three years-worth of available data. Out of the original 43 sub-basins, 38 contained more than a single commercially relevant fish species at sufficient levels of abundance, and were thereby selected from. Out of the four main commercially relevant fish species monitored in CAMP, walleye and lake whitefish followed the latter requirements most closely and were thereby selected as the fish species for the main study. Of the remaining 38 waterbodies, two main monitoring regimes were run, which were either annual or rotational (sampling every couple years) in CAMP and characterized as either impounded or non-impounded. Of those 38 waterbodies, only waterbodies currently under the annual sampling program were selected from since additional water chemistry data would be available for comparison with later collected otolith trace element data unlike rotationally sampled waterbodies which lacked year-to-year measurements resulting in a total of 14 CAMP waterbodies for complete assessment.

Within the CAMPP report there was a substantial amount of summary statistics which covered various water quality parameters and of more immediate importance, trace element data associated to water samples collected from each of the CAMP waterbodies. Water samples in CAMPP were measured for a suite of 38 trace elements, namely: Ag, Al, As, B, Ba, Be, Bi, Ca, Cd, Cs, Cr, Co, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, Sb, Se, Sn, Sr Te, Ti, Th, Tl, U, V, W, Zn, and Zr. Trace element mean water concentration values were accessible and each value was found to be made of a total of one to four separate water samples, provided in the CAMPP report within the summary statistics section. Values within this section were transcribed over to an excel spreadsheet. To further filter out trace elements to be used within the main study a series of selection criteria were determined. First, potential trace elements had to be detected (above limit of detection, LOD) within the water column 100% of the time throughout the CAMPP three-year study duration. Of those trace elements which remained, only those which measured > 0.001 mg/L (arbitrary cut-off point for exclusion) or greater throughout the three-year CAMPP study period were selected from. The latter was deemed necessary since trace elements detected in greater amounts in the water column were assumed to have a greater likelihood of detectable incorporation into the otolith. Lastly, the trace elements selected should be well studied within the literature for comparative purposes. From the latter three criteria, five trace elements were selected for analysis: sodium (Na), magnesium (Mg), manganese (Mn), barium (Ba), and strontium (Sr).

Next, the 14 potential CAMP study waterbodies were evaluated. The aim was to select four impounded and four non-impounded waterbodies, for a total of eight

waterbodies. In this additional screening, two factors were considered. Factor one was that the selected waterbodies had to have a minimum of 10 archived walleye and lake whitefish otoliths available in the 2013-2014 catch range (latest available archived otolith data). The second factor was the need for a water concentration range to be present for each of the five selected trace element concentrations between selected CAMP waterbodies. The water concentration range was intended to help determine if the otolith trace element concentrations reflect the CAMP waterbody water content across a range of concentrations rather than studying the attenuation effect on trace elements, which will not be discussed in detail in this study. Factor two was completed by having each of the 14 waterbodies three-years' worth of trace element concentration data averaged to a single value per waterbody for each of the five selected trace elements with standard deviation as error. These final values were then compared between each of the 14 waterbodies. The four impounded waterbodies selected were South Indian Lake (Area 4), Threepoint Lake, Split Lake, and Lower Nelson River (downstream of Limestone generating station) and the four non-impounded waterbodies selected were Cormorant Lake, Gauer Lake, Leftrook Lake, and Assean Lake (Fig 3.1, Table 3.1).

3.2.2. CAMP 2008-2014 Water Quality Dataset

Once site selection was complete, the complete CAMP 2008-2014 water quality dataset for target waterbodies was acquired from Manitoba Sustainable Development and Manitoba Hydro (CAMP, 2016). The five selected trace elements were then re-evaluated to determine if concentrations remained similar throughout 2008-2014 prior to

further analysis. Within the complete 2008-2014 water quality dataset, multiple water quality measures and trace element measures were available. Water quality data was found to be collected from an array of depths within each waterbody ranging from a minimum of 0 m (water surface) to as deep at 24 m (below surface) (CAMP, 2016). The most consistently measured depth collected on a yearly basis was 0.3 m in each of the waterbodies and was used as the primary depth in which water quality variables and trace element concentrations were analyzed and compared with otolith measures. Additional depths were also measured but not used within the main study due to lack of sufficient data for rigorous testing (summary table is provided Table A2.1-2).

In each of the eight selected CAMP waterbodies between one and four water samples were collected by CAMP each year, with the exact number varying between waterbodies (Table 3.2.)(CAMP, 2016). To account for the lack of consistency in sampling, all water variables (trace element or water quality) were averaged per year or as a year range with sample size and standard deviation collected for presentation and analysis in this study. These data were used for comparative purposes both between CAMP waterbodies under study, and with otolith trace element data in the form of a correlative analyses (Table 3.3).

3.2.3. Otolith Selection

All walleye and lake whitefish were collected via gillnetting (mesh size range of 38 mm – 127 mm) for 16.5 to 30.2 hours per sampling session (CAMP, 2016). Gillnets were set at waterbody bottom except for during 2014 on Split Lake, here fish were caught instead using floating surface gillnets (CAMP, 2016).

Otoliths for this study were selected from fish caught during 2013-2014 only. Priority was given to fish collected from 2014 over 2013 otolith and older fish were selected over younger fish for analysis (minimum age of seven for 2013 or six for 2014 otoliths to compare with the 2008-2014 water quality dataset). Initially 10 otoliths per CAMP waterbody per species were selected by the latter criteria for an initial total of 160 otoliths for testing. Similar sample sizes were used for individual water systems in other waterbodies for arctic grayling (*Thymallus arcticus*) as well (Clarke et al., 2015, 2007). Otolith age ranged from 6 to 27 years for lake whitefish and 6 to 26 for walleye. Age was determined via the "crack and burn" method by Manitoba Sustainable Development and North/South Consultants Inc. Secondary aging was also conducted occasionally by the author for verification purposes by the counting of annuli from polished otolith transects digitally captured under high resolution imaging and compared to the previously collected age dataset by CAMP. Additional information about each archived otolith was also obtained, such as the individual fish measures (sex, age, body weight, fork length etc), and collection information (Table A2.3-4, Figure A2.1-8).

3.2.4. Otolith Preparation for LA-ICP-MS Analysis

In preparation for laser ablation, sagittal otoliths were embedded sulcus side-up in resin (Buherler® Epoxicure resin) on top of Parafilm and allowed to solidify for >24 hours, forming otolith embedded resin tabs. Each otolith was then marked by a fine tipped permanent marker over the otolith core (center) and cut transversely to expose the core as a dorso-ventral cross section (Buehler® Isomet low speed saw). The otolith cross section was then placed exposed core-side down on adhesive paper within a 25

mm lucite microprobe mount which was then filled-in with additional resin and allowed to harden for >24 hours or until solidified. A total of five otolith dorso-ventral cross sections were then placed in each individual lucite mount, with positions of each noted and documented. Each lucite disk was then ground (exposed otolith side down) with a series of Buehler Carbimet™ grit papers in the following order: 240 [P280], 320 [P400], and then 600 [P1200] (Buehler Ltd, Lake Bluff, IL) on a roll grinder wetted with deionized water. After the annuli and primordia were thought to be sufficiently exposed and smoothed for each otolith, the lucite ring was sonicated briefly for 45 seconds using a FS20 ultrasonic cleaner (Fisher Scientific) to remove excess particulate on the surface before fine-polishing. Post-sonication, the ring was then hand polished on a deionized water-wetted Buherler ® MeaServ 2000 Variable Speed Grinder-Polisher with Buehler Micropolish (0.3 micron α alumina) (Buehler Ltd, Lake Bluff, IL). After polishing, each ring was then rinsed with deionized water, verifying that the otolith core was exposed. Once verified, digital images of each polished otoliths were collected pre-and post-analysis with scale bar using a Nikon SMZ 745T microscope fitted with C-W10XB/22 eyepieces that was attached to a Nikon digital sight (DS-FI1/ DS U2/L2) and displayed using NIS-Elements F (3.0) software (Laboratory Imaging, Za Drahou, Praha, Czech Republic).

3.2.5. LA-ICP-MS

3.2.5.1. LA-ICP-MS: Test Specifications

The trace element concentrations in walleye and lake whitefish otoliths were determined by Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-

MS). The LA-ICP-MS analyses were conducted on a Thermo Finnigan Element-2 ICP-MS instrument coupled to a Merchantek LUV 213 Nd:YAG laser. General laser conditions during the analysis can be found in Table 3.4. Line scans over the otolith transect surface were run core to edge for all otoliths along the clearest and most well-defined annuli bands, typically parallel or near-parallel to the sulcus and as close to perpendicular to annuli banding as possible to remove variation due to placement and aid in comparability (Fig 3.4.). Several otoliths were also run egde-core-edge to assess whether laser line placement effected elemental signature trends in the trace elements being studied (Fig A2.9-14). The qualitative analysis consisted of otoliths from two of the selected CAMP waterbodies (Leftrook and Cormorant Lake), in which five walleye and five lake whitefish otoliths were laser-pathed via LA-ICP-MS from edge to core to opposite otolith edge. Concentration versus distance graphs were created for the comparison of otolith edges. The internal standard used in this analysis was calcium oxide (56 wt.% CaO), and the external calibration was done using NIST glass 610. Before and after each otolith ring was run (containing five otoliths), the instrument was assessed for any drift by measuring the external standard twice. The concentrations of the isotopes: Mn^{55}, Sr^{88}, Ba^{138}, Na^{23} and Mg^{25} were measured. Iolite3 software was used to process data collected by the LA-ICP-MS providing trace element concentrations, and detection limits (Iolite Team, 2016). The step backward method was applied when establishing baseline and background readings during calibrations (Iolite Team, 2016).

3.2.5.2. LA-ICP-MS: Result Overlay and Aging

Post LA-ICP-MS, line scan images of the otoliths were again taken and the annuli were to be then digitally marked to denote separate years starting from the edge and moving inwards. This edge-inwards counting and marking of annuli was done within the year range of 2008-2014 that corresponded to available CAMP water quality data. Once the otolith digital images were marked, the otolith line scan results per otolith were individually graphed over time and used as an overlay over their corresponding post-line-scan images (Fig 3.2).

The first annulus (light or summer growth and dark or winter growth band pair) at the otolith edge was omitted from measurement, which corresponded to the most recent year, and the year when the fish was captured for the fish otolith annuli determination and linescan overlay. Since the fish were caught throughout the year, rather than just at the end, the complete growth band was not available for analysis or comparison with water data (although multiple water samples were collected throughout each year from multiple locations within each selected CAMP waterbody). Since all fish in the study were caught in the summer months between July-September it was possible to determine which bands corresponded to which year (with the band at the edge being considered summer growth) (Degens et al., 1969; Halden et al., 2004). Each year marker was placed over top of the narrower winter growth band (Fig 3.2). Through this, the line-scan section (annuli corresponding to the year range between 2008-2014) could be extracted by determining the distance the laser travelled along the otolith and connect it with the annuli derived year range. The detected concentrations within the distance/year range could then be averaged per otolith for a time-integrated value per

otolith and trace element under study (i.e., bulk analysis of separate regions) (Fig 3.2). Years were averaged to remove any additional annuli positioning bias and to simplify and match the comparison of otoliths, and waterbodies concentration measures (since being a preliminary study). It should be noted that the five target trace elements being measured remained relatively stable throughout the study year range. For each of the eight CAMP waterbodies 10 otoliths concentration values (per element) per species were obtained. For all subsequent analysis, only trace element concentration data collected from otolith annuli between the years 2008-2014 were used.

3.2.6. Statistical Analysis

Data used for the analysis included water trace element concentration data, water quality data, otolith trace element data and waterbody physical characteristic/flow rate data. Each of these datasets were analyzed separately or compared with each other in a series of graphical, correlative, pairwise and/or multivariate testing. Datasets prior to testing values were typically averaged or reduced to varying degrees to allow for comparison, depending on the objective or question being asked.

Before univariate and multivariate testing was to be conducted, the data (namely the otolith trace element data) had to be tested to both clarify that bulk averaged values obtained were corrected for fish age, length or wet mass if found to influence measures and that anomalous variables or values below limit of detection (LOD) were identified and removed. Confounded datasets would require correction before testing, while anomalies or values below the LOD would be removed if considered necessary. In the univariate analysis, correlative, pairwise and ANOVA based tests were conducted

based on the desired objective. In the multivariate analysis, principal component (PCA), redundancy (RDA) and K-nearest neighbours (KNN) were to be conducted. Univariate statistical testing was conducted using PAST and SigmaStat (Hammer, Harper, & Ryan, 2001; Systat Software Inc, 2006) and multivariate analysis were conducted on R (R Core Team, 2013). Figures were developed on SigmaPlot (Systat Software Inc., 2008) and R software (package: vegan) for final presentation (Oksanen et al., 2015; R Core Team, 2013). KNN testing was conducted on R software (package: knn.cv) (Ripley, 1996; Venables & Ripley, 2002).

3.2.7. Removal of Walleye Otolith Manganese from Analysis

Manganese levels measured in walleye otoliths were frequently found to be near or below the LOD calculated by the determination of mean background signal and were thereby excluded from further univariate or multivariate statistical testing. For the dataset analysis, each individual otolith background Mn^{55} LOD (in ppm) was compared with its respective averaged otolith value obtained from the selected annuli range per otolith respectively (from within the years of 2008-2014). It was then noted if each individual otolith manganese concentration value was above or below the LOD and was tallied. It was found that out of the 79 walleye otoliths considered for the final analysis, 37 (or 47%) were below their individual respective LODs. As nearly half of the walleye manganese trace element averaged values were below the LOD, the manganese dataset was considered un-usable even though 53% of data was greater than the LOD with an average concentration of 0.109 ppm. Even though 53% of the walleye otolith line scans had Mn concentration averages greater than their respective LODs, since

each of the averages were made from line scan data ranges which were observed to occasionally detect and record measures below their respective LODs, the resulting averages could not be considered representative due to the inclusion of values below the LOD. Removal of Mn datasets was justified in other otolith based studies as well such as in Pangle et al., (2010) in which 78% of otolith samples were below the LOD. For the statistical analysis, inclusion of the walleye otolith manganese data in the univariate statistical tests yielded significantly different results between species and waterbodies compared to tests that did not include it. In multivariate analysis, manganese data inclusion skewed the modelling output of the dataset and adversely effected the interpretation of results. Therefore, it was also deemed necessary to remove walleye otolith manganese from the final analyses. Although a greater number of samples were above the LOD in the current study versus studies such as Pangle et al., (2010), the removal was still justified due to concern over the inclusion of false detects.

3.2.8. Trace Element Residuals: Fish Age, Body Weight and Fork Length Correction

Due to the potential effect of fish age, body weight and fork length on trace element concentrations in the otolith, a series of univariate tests were conducted to determine if the latter factors would influence trace element levels between species, and if the factors varied between fish sampled from separate selected CAMP waterbodies. Tests were conducted with both raw and log transformed data (to assist with normality) in each case. To test to see if a dataset was parametric, Levene's (homogeneity of

variance) and Shapiro-Wilk (normality) tests were conducted. In each case α was set to 0.05.

In terms of tests, Spearman (if non-parametric) and Pearson (if parametric) correlation coefficient tests were conducted between age, fork length and weight in a pairwise fashion for both species. This was done to determine if any relationship exists between the three variables for each species (N= 79). Also, for age, weight and fork length separately, Kruskal-Wallis and 1-way ANOVAs were conducted to compare each factor between the eight CAMP waterbodies (N= 9-10) for both walleye and lake whitefish. If a significant difference was determined for the ANOVA, a post-hoc Tukey's test (if parametric) or Dunn's test (if non-parametric) was conducted to compare waterbodies, α set at 0.05. T-test and non-parametric Mann-Whitney U tests using pooled into impounded (N= 39) and non-impounded (N= 40) waterbody data was also conducted for both species, using age, weight and/or fork length as the comparative factor, with α set at 0.05.

To examine trends between age, fish weight and fork length against the separate study trace elements, a series of Pearson (if parametric) and Spearman (if non-parametric) correlation coefficient tests were conducted per species and log-transformed trace element data. Age, weight and fork length data was also log-transformed to assist with normality. Lastly, Spearman (if non-parametric) and Pearson (if parametric) correlation coefficient tests were conducted between age, fork length and weight (independent variable) and otolith trace element concentrations (Na, Mg, Mn, Ba, Sr) in a pairwise fashion for both species (Mn excluded for walleye). Otolith trace element values were all $\log_{10}(x)$ transformed prior to testing with raw and/or $\log_{10}(x)$

transformed independent variables (fish age, body weight and fork length). A total of 79 individuals were used in each test for either walleye or lake whitefish. In each of the latter test cases, α was set at 0.05.

After testing for any significant differences and correlation from the latter tests, the AIC method was then further applied to determine which combination of factors (age, body weight and fork length) that would best explain the trend for each trace element (Na, Mg, Mn, Ba, and Sr) per species. R software and the MASS package were used to complete the AIC (R Core Team, 2013; Venables & Ripley, 2002). From the AIC output and the univariate test results, the type of standardizations deemed necessary per species and trace element were determined. Due to the notable significant correlations and the results of the AIC method for the both species and trace elements it was deemed necessary to standardize the otolith trace element dataset by fish age, weight and/or fork length. Each trace element average per individual per species was standardized by taking log residual averages, for use in the following univariate and multivariate analysis. Log residual average constant trace element (LRA) data was also used to differentiate species. To create the LRA dataset from the residual otolith trace element data, all log transformed trace element data per trace element exclusively was first averaged and then the average value was then added to each residual. Due to the non-normality of the otolith trace element data, log transformations were performed prior to residual calculation and later statistical testing. The use of residual data has also been conducted in other studies such as in a study dealing with juvenile red drum (*S. ocellatus*) in which the effect of body size was removed from the equation by taking the residual of the desired measure versus body length (Hoff & Fuiman, 1993). Additionally,

it was observed in juvenile red drum (*S. ocellatus*) that whole otolith Na and Mg decreased while Sr increased as the fish aged (Hoff & Fuiman, 1993) and decreased variability in trace element concentrations with increasing age of arctic grayling (*Thymallus arcticus*) and slimy sculpin (*Cottus cognatus*) has also observed (Clarke et al., 2015) further implying a need for correction. For all otolith based statistical testing and graphics, only LRA values were used (histograms within Figures A2.15-32).

3.2.9. Outlier Identification and Otolith Trends

Each individual otolith within the study was analyzed visually and had its trace element chemical signature patterns compared against averaged values per waterbody to identify outliers. In this study, if an otolith's average trace element value between the desired tested age section was above or below the average of the 8-9 remaining otoliths within a waterbody of its respective species by one full standard deviation of the average, the otolith was considered an outlier and removed from further univariate or multivariate testing. Digital images were taken and observed for visual anomalies, assisting in identifying potential outliers requiring removal from the study.

3.2.10 Graphical Analysis and Data Preparation

3.2.10.1. Graphical Analysis

All CAMP water quality variables were graphed by year (except those found to be consistently below the minimum detection limit (MDL) (Fig A2.33-60). Inter-lab and triplicate measured/collected samples were first averaged and then per-sample year, values were averaged and standard deviations calculated (used as error bars) (CAMP,

2016). A complete list of CAMP water quality variables monitored can be found within Table A2.5 (trace elements other than Na, Mg, Mn, Ba and Sr were excluded). Boxplots and correlation scatterplots also made for the various univariate tests conducted. Lastly, multivariate bi-plots of principal component or redundancy axes were constructed to aid in visual interpretation of the various models.

3.2.10.2. Data Preparation

For the correlation analysis, water quality and water trace element data from the depth of 0.3 m, and otolith trace element data were each averaged across multiple year ranges for comparison between waterbodies. This was done by taking each individual measure available within the 2008-2014 year-range per waterbody exclusively and averaging.

For water quality values (trace element or water quality variable both measured in situ and/or in lab), within the dataset certain samples collected throughout each sample year were collected in triplicate rather than a single sample from an individual location in the field or was analyzed twice in separate labs (inter lab comparison samples). To account for the latter, those triplicate samples or inter-lab samples were averaged before averaging each entire years-worth of data or year range depending on the test. In the case of the correlative study, all variables were averaged together within the year range between 2008-2014 for a single value per water quality variable or trace element (Na, Mg, Mn, Ba, and Sr) per waterbody.

The otolith trace element concentrations (9-10 otoliths per species per waterbody) were also averaged to single concentration values prior to comparison with

water chemistry data. For comparisons between trace element otolith concentrations, trace element water, and water quality data, the water trace element and water quality data-range averages were reduced to individual values to match the otolith year ranges (Table 3.3). Correlation tests between water trace element and water quality used full year-ranged averaged datasets (between 2008-2014) due to availability. For each correlation analysis, a total of eight values were correlated, with each pair relating to one of the eight CAMP waterbodies under study. Due to the concern of autocorrelation between the 28+ water quality variables collected in CAMP, a series of Pearson and Spearman correlation coefficient tests were run on in-situ and lab water quality variables separately to filter out significantly correlated variables ($\alpha = 0.05$). Variables found to be significantly correlated with each other were to be selected before performing the same test between the remaining in-situ and lab variables. Those that were not significantly correlated with each other after the removal were then to be used in the trace element correlation analyses and later multivariate analyses. The remaining water quality variables were dissolved organic carbon (DOC), nitrogen (N-TKN), chlorophyll-a (Chl-a), pheophytin, total dissolved solids (TDS), total suspended solids (TSS), oxidation-reduction potential (ORP), true color (T-C), and dissolved oxygen (DO).

To evaluate the influence of impoundment on otolith trace element concentrations, univariate Student's *t*-tests, ANOVAs and multivariate tests used the otolith trace element year-range averaged data. A single value per trace element was collected for each of the 79 individuals. Also, for univariate tests of the water trace element concentrations, year ranges were pooled rather than averaged for greater sample size. Lastly, individual water trace element concentrations were averaged per

year for a correlative analysis between year and water trace element concentration. In each case α was set to 0.05.

3.2.11. Correlation Analyses (Water, Otolith, and Water Quality)

3.2.11.1. Water Trace Elements Versus Water Quality Variables

A series of pairwise correlations between trace element averaged water concentrations (Na, Mg, Mn, Ba, Sr) as the dependent variables and the reduced set of water quality variables as the independent variables was conducted for pooled impounded and non-impounded waterbodies (sample size of n= 8). Each waterbody water concentration or species specific otolith value is the average of all samples within the each select waterbody exclusively. Additionally, for water Sr correlations with TDS and TSS averages, correlations were done with averaged impounded and/or non-impounded data exclusively (sample size per test of n= 4). Prior to analysis, assumptions of normality and homoscedasticity were confirmed with (Shapiro-Wilk and Levene's test respectively) to determine if a Pearson (parametric) or Spearman (non-parametric) correlation coefficient tests should be conducted. All analyses were conducted with raw and/or $\log_{10}(x)$ transformed data with α set to 0.05.

3.2.11.2. Otolith Trace Element Concentrations Versus Water Trace Element Concentrations

Trace element correlations between otolith log-age/bodyweight and/or fork length corrected values (LRA) as the dependent variable, versus water trace element concentrations (raw or $\log_{10}(x)$ transformed) as the independent variables was

conducted for walleye and lake whitefish separately. Tests were performed with pooled impounded and non-impounded waterbodies (n= 8) and with separate impounded or non-impounded groupings (n= 4 per group). Parametric assumption tests were conducted on each correlative comparison (Shapiro-Wilk's normality test and Levene's mean tests for homogeneity of variance) to determine if the Pearson or Spearman correlation coefficient tests were appropriate. The α for all significance tests was set at 0.05 and $\log_{10}(x)$ transformations were conducted on water chemistry variables and water trace element concentrations if the distributions were found non-normal to assist with meeting parametric assumptions.

3.2.11.3. Otolith Trace Element Correlations and Water Quality Variables

Trace element correlations between otolith values (LRA) as the dependent variable, versus the select water quality variables (raw or $\log_{10}(x)$ transformed) as the independent variables was conducted for walleye and lake whitefish separately. The number of comparisons/sample size was equal to the number of waterbodies (N= 8). Additionally, for otolith Sr correlations with TDS and TSS, correlations were done with averaged impounded and/or non-impounded data exclusively (sample size per test is n= 4). Parametric assumptions tests were conducted on each correlative comparison (Shapiro-Wilk's normality test and Levene's mean tests for homogeneity of variance) to determine if the parametric Pearson or non-parametric Spearman correlation coefficient test was appropriate. For all significance tests α was set at 0.05 and $\log_{10}(x)$ transformations were conducted on water chemistry variables and water trace element

concentrations if the distributions were found non-normal, to assist with meeting parametric assumptions.

3.2.11.4. Correlations Between Trace Elements

Otolith trace element correlations (LRA corrected values) were done for both walleye and lake whitefish separately, and compared between species. Averaged trace element concentrations from each individual otolith were used, for a total sample size per species equaling eight separate values (one per CAMP waterbody averaged from 9-10 bulk average otolith trace element concentrations). Both Pearson and Spearman correlation coefficient tests were conducted. In each case α was set to 0.05. Parametric assumptions tests were conducted on each significant correlative comparison (Shapiro-Wilk's normality test and Levene's mean tests for homogeneity of variance) to determine if the parametric Pearson or non-parametric Spearman correlation coefficient test was appropriate.

3.2.11.5. Water Trace Element Concentration Versus Year Sampled

To determine if there was a linear relationship between water sampling year and measured water trace element content for the five trace elements in the study, a correlative analysis was applied. A total of eight tests were done per trace element, corresponding to the eight separate selected CAMP waterbodies. Parametric assumptions tests were conducted on each significant correlative comparison (Shapiro-Wilk's normality test and Levene's mean and/or median tests for homogeneity of variance) to determine if the parametric Pearson or non-parametric Spearman

correlation coefficient test was appropriate. Where applicable, $\log_{10}(x)$ transformations were applied to assist with normality. In each case α was set to 0.05.

3.2.12. Impoundment Univariate Statistics

3.2.12.1. Water Trace Element Univariate Statistics: Impoundment

To compare the water trace element concentrations of impounded and non-impounded waterbodies, trace element water chemistry values were averaged per year within each CAMP waterbody and then the individual waterbody values were pooled into two groups, namely impounded and non-impounded waterbody values (sample sizes of N= 24-25). The concentrations were not averaged into a single value within each waterbody to account for the variability between years, because of the relative stability in concentrations between years. Student's *t*-tests for parametric and Mann-Whitney rank sum (non-parametric) tests were conducted depending on if passing Kolmogorov–Smirnov test (normality) and Levene's test (homogeneity of variance) with an α of 0.05. $\text{Log}_{10}(x)$ transformations were conducted to address normality issues with certain datasets.

3.2.12.2. Otolith Trace Element Univariate Statistics: Impoundment

Using the individual otolith trace element measures per species, a series of box plots were made per waterbody (n= 9 to 10 per plot), and pooled based on comparing impounded and non-impounded waterbodies (n= 39 to 40 per plot). To determine if waterbody impoundment would lead to differences in otolith signature, individual otoliths (n = 9-10) from impounded and non-impounded waterbodies were compared as

separate waterbodies (eight waterbodies) using a 1-way ANOVA (if parametric) or Kruskal-Wallis (in non-parametric) test followed by a post-hoc Tukey's (parametric) or Dunn's (non-parametric) test. For pooled impounded versus pooled non-impounded otolith data, Student's *t*-tests (if parametric) or Mann-Whitney Rank Sum tests (if non-parametric) were conducted. Each test was performed with α set to 0.05. Additionally, Parametric assumption tests: Kolmogorov–Smirnov test (normality) and Levene's test (homogeneity of variance) were conducted prior to determine test types to use.

3.2.13. Multivariate Statistical Testing

3.2.13.1.1. PCA and RDA characterization

PCA and RDA statistical tests assist in the simplification of complex datasets into lower dimensional data space while preserving much of the trended variation within the data (Kenkel, 2006). The relative distribution of the data point swarm remains constant, even though their coordinates in the axis change to best visualize trended variation (Kenkel, 2006). Thereby simplifying the interpretation of trends, and answering the hypothesis or questions being asked. More specifically, Principal Components Analysis (PCA) maximizes linear variation in multivariate space by re-describing the coordinated system using new and optimized, mutually perpendicular axis (Kenkel, 2006). Each new principal component axis is independent, uncorrelated and maximizes accounted variation in the data, allowing the major trends (those most likely to show the overall data trend) to be identified in fewer axes (Kenkel, 2006). PCAs are typically described in the first few axis, and visualized in two-dimensional space (scattergram) (Kenkel, 2006).

The percent each axis provides to the overall trend in the data can then be obtained. Comparatively, Redundancy Analysis (RDA) can be considered a constrained version of PCA. Unlike PCA, RDA examines two rather than a single set of data with one set being the factor variables (X) and the other being the response variables (Y) (Kenkel, 2006). In other words, and RDA is a multiple regression-based analysis followed by a PCA. To test the significance of the relationship between X and Y a Monte Carlo permutation test can be conducted per test axis and to the entire model (Kenkel, 2006). No formal statistical assumptions exist for PCA and RDA although where applicable, data transformation and standardization was utilized prior to running the tests to aid in visualization and trend interpretation. The purpose of the PCA and RDA for this study were to assist in the visualization of the data.

3.2.13.1.2. KNN and Jackknife Characterizations

Classification involves the prediction of discrete class labels for unlabeled individuals based off patterning (Kramer, 2013). When classifying it is desired to determine a function that best determines labels based off a set of individuals (e.g., patterns or points). Unlabeled individuals are assigned to groups of known labels which are most similar (e.g., similar distribution) (Kramer, 2013). K-nearest neighbors (KNN) relies upon the assumption that the nearest individuals (e.g., dimensional distance-wise) to a target individual will provide the most useful information. K is predefined at the beginning of the test, with greater K ignoring smaller or localized patterning (Kramer, 2013) and is considered a non-parametric test. The implemented KNN in R included a

leave-one-out cross validation (e.g., Jackknife) in which for each row of the training set, the k nearest (in Euclidean distance) other training set individuals are found, with classification determined by vote majority (ties broken at random) (Ripley, 1996; Venables & Ripley, 2002). This method also indicated the proportion of votes the winning class obtained for each individual tested (Ripley, 1996; Venables & Ripley, 2002). In the iterative method known as leave-one-out-cross-validation, a specific parameter is being classified through using the entire sample, and then done so again and again using less of the available sample creating partial estimates which are subtracted from the full sample values to create pseudo-values (removes linear bias) for parameter estimation (parameter error, confidence intervals, and conduct hypothesis testing) (Abdi & Williams, 2010; Kramer, 2013).

3.2.13.2. Preliminary Multivariate Analyses (pRDA)

Multiple preliminary tests were run on the CAMP trace element otolith data to determine which of the environmental factors had the greatest effect on otolith trace element content. More specifically, the trace elements Na, Mg, Mn, Br, and Sr were measured per individual otolith (n = 9-10 per waterbody). A total of 79 otoliths per set of eight CAMP selected waterbodies per species or 158 otoliths in all were tested in PCA and RDA based analyses.

A preliminary redundancy analysis (pRDA) was first run with log residual averaged (LRA) data to aid in visualizing the effect of various factors had on trace element content. Of the trace elements, walleye manganese otolith measures were excluded (see Section 3.2.7). Each point on the multivariate mapping biplot represents

an individual otolith from one of the selected CAMP waterbodies. This pRDA test included all 158 otoliths (both species) in which the trace elements Na, Mg, Ba and Sr were to be constrained by multiple factor variables (area or waterbody), region, species, sex, impoundment status, and predominant rock type) to help discern which variables best describe the trace element trends. The same pRDA was also done per species (79 otoliths per species), thereby removing species as a constraining variable. The latter tests were done to remove less important or nested variables from later refined analyses. In the end, the area (or waterbody) factor best described the trends in the data, with impoundment status found nested within. Species was also found to be a strong constraining factor variable and was also included for comparative species tests. This resulted in all following RDA comparisons being constrained by species and/or area.

3.2.13.3. Principal Component Analysis (PCA) – Otolith, Waterbody, and Water Quality

Three main PCAs of were conducted. The first PCA analysis included trace element data from both walleye and lake whitefish combined values (158 individuals in total, 79 per species) to determine trace element trends per species and if species could be differentiated based on trace element content. LRA trace element data averages per otolith were employed. Biplots of the most important axis (typically first and second) were also constructed to assist in data interpretation and visualization. All PCA tests were conducted with R-software and VEGAN package (Oksanen et al., 2015; R Core Team, 2013).

The second type of PCA was a comparison of the eight CAMP waterbody's various environmental variables excluding trace element and water quality data. This PCA analysis was conducted to help further differentiate the eight CAMP waterbodies from each other based on various terrestrial/waterbody morphometry and waterbody dynamic factors in the form of dichotomous and ratio data. Principal component biplots of the most important axis (typically first and second axis) were also constructed to assist in data visualization and interpretation.

The third PCA analysis employed the use of the CAMP water quality monitoring database variable values. Values included were those previously filtered to remove the autocorrelation issue (section 3.2.10.2). Trace element concentrations were not included in this analysis. The sample size of each treated environmental variable was equal to the number of waterbodies (n= 8). This PCA analysis was conducted to further differentiate the selected CAMP waterbodies based on water quality variables. Each water quality variable per waterbody was made from averaging entire dataset year ranges (within 2008-2014) to form single values per waterbody. Raw and $\log_{10}(x)$ transformed (if normality criterion was not met) water quality variable data were tested separately. Biplots of the most important axis (typically first and second) were also constructed to assist in data visualization and interpretation.

3.2.13.4. Redundancy Analysis (RDA) – Otolith Trace Elements

After the preliminary analysis and after unnecessary environmental factor variables were removed, a series of RDAs were conducted per species separately and, combined. In total, six separate RDA tests were conducted, all of which using area (or

waterbody) as the primary constraining variable, and various combinations of trace elements and species tested. The first test dealt with all 158 otoliths (both walleye and lake whitefish) trace element data (Na, Mg, Ba, and Sr, with Mn excluded) combined to determine if the area (or waterbody) effect or species effect influenced the observed trace element signature trend the most. The second RDA conducted was on lake whitefish exclusively in which data from all five otolith trace elements were utilized (Na, Mg, Mn, Ba, and Sr), leading to a total of 79 otoliths (9-10 per CAMP waterbody) tested, constrained by the area (or waterbody) factor. The third and fourth tests used lake whitefish and walleye respectively in which four of the five otolith trace element averages were used (Mn excluded) for comparative purposes of trace element trends constrained by area (or waterbody) factor. Lastly, the fifth and sixth tests used lake whitefish and walleye respectively again, but this time on the two non-nutrient trace elements (Ba and Sr), again constrained by AREA for comparative purposes and for the observation of potential change in the amount of trend being accounted by fewer, potentially unregulated variables.

For each of the RDA tests, at least the first three RDA axis trended variation percentages were noted along with constraining variable (e.g., AREA) centroid scores to help interpret trends in the data. Also, for each of the above tests, Monte Carlo permutation tests (999 iterations per test) were conducted on the model as a full model and per RDA axis. This was done to determine if significance was reached. Biplots of the most important axis were also constructed to assist in data interpretation. All RDA tests were conducted with R-software and VEGAN package (Oksanen et al., 2015; R Core Team, 2013).

3.2.13.5. K Nearest Neighbours (KNN)

The same combinations of otoliths (grouping of otoliths per waterbody) used for the species-specific RDAs were also used in the KNN (with cross validation) to create classification matrices (determine percent correct classification per waterbody and for the whole model). Additionally, KNNs were conducted per species to classify pooled impounded and non-impounded otolith groups for classification percent determination. For the current CAMP-O project, KNN tests were run using k= 1 to 11 (odd numbers only), for a total of 6 tests per test type (12 test types x 6 “k” value = 72 result matrices). The “k” value which determined the greatest classification accuracy is presented in this study. KNN has been employed on other otolith trace element studies as well (e.g., Carlson et al., 2017; Radigan et al., 2018).

3.2.14. Impoundment Flow Analysis

Flow rates used in this study were collected from generating stations adjacent to impounded CAMP waterbodies. These flow rates were calculated by Manitoba Hydro as the sum of the flow through powerhouse and spillway. Powerhouse flow is calculated based on the water levels up and downstream from the station, the amount of power generated, and unit efficiency curves. Spillway flow is calculated based on upstream water level and stage-discharge curves/gate openings for each individual structure. The only exception to the latter is Notigi which only has flow through the spillway at that location (no powerhouse currently exists). In the current study, flow rates were to be used as a proxy for residence times, in which the hypothesis that greater residence

times (slower flow) would lead to greater accumulation of total suspended solids, total dissolved solids and trace element detection within the selected CAMP waterbodies.

Flow rate was graphed by taking the year range averages of water flow through the seven-adjacent control or generating structures near the four impounded CAMP waterbodies under study. Averages from 2008-2014, and 2008-2017 were also graphed for comparative purposes. Pearson (parametric least squares regression), and Spearman (non-parametric) correlation coefficient tests were conducted to determine if a correlation exists between up or downstream flow rate from control and/or generating structures (independent) has on water trace element concentrations. Comparisons made on a yearly basis with samples sizes of 4-7 depending on waterbody and up or downstream water flow. Pearson (parametric), and Spearman (non-parametric) correlation coefficient tests were also conducted to determine if a relationship exists between upstream or downstream flow rate from control and/or generating structures (independent variable) and otolith average trace element concentrations per species (dependent variable). Sample size of three to four (N= 3-4) was available since only half of studies waterbodies (four) were impounded, having both upstream and or downstream adjacent impoundments and in each case α was set to 0.05.

A PCA was conducted with raw data to compare the four impounded CAMP waterbodies (Lower Nelson River, South Indian Lake (area 4) based on adjacent upstream flow adjacent to each respective waterbody (from CS or GS), latitude, longitude, altitude and drainage basin area.

3.2.15. Water Trace Element, TSS, and TDS Versus Depth

To help determine the relationship of water trace element concentration and its potential variation with depth, comprehensive table of trace element distribution with depth was constructed (Table A2.1-2) and correlative analysis was conducted against TDS and TSS. For each of the five selected trace elements, and total dissolved solid (TDS) or total suspended solid (TSS) water concentrations were averaged across year ranges versus water depth measured per waterbody. Significance was tested using either Pearson's or Spearman's correlation coefficient tests depending if the datasets were considered parametric or not. To test to see if a dataset was parametric, Levene's test of means (homogeneity of variance) and Shapiro-Wilk (normality) tests were conducted. In each case α was set to 0.05. Depth was considered the independent variables while water trace element concentration was considered the dependent variables. Of most importance, testing to see if near surface (0.3 m depth) and waterbody bottom measures are similar or differ from each other was focused upon to see if the near-surface measures would or would not be considered applicable for correlative analysis with bethic/benthopelagic fish otolith measures. Refer to tables 3.1-3.2 for known and maximum depth measures of the selected CAMP waterbodies.

3.3. Results

3.3.1. Qualitative analysis of otolith edge-core-edge LA-ICP-MS signatures

Walleye Na signatures overall were relatively uniform across the entire otolith, with a slightly greater Na concentration within proximity of the otolith core area. Walleye Mg signatures were usually of similar concentration along both edges of the otolith. Additionally, compared to the other measured trace elements, Mg signatures had greater variability across otolith linescans, although the area around the core had the greatest concentration. Some of the otoliths did display a disparity of Mg e.g., walleye otolith 123 from Cormorant Lake (Fig A2.10). Walleye Mn, Ba, and Sr followed trends in chemical signature peaks generally. Peaks for Mn, Ba, and Sr were greatest adjacent to the core, and sometimes present over the core (variation may be attributed to placement of laser line path during processing). One example of a notable edge non-uniformity was walleye otolith #121 from Cormorant Lake which displayed greater Na and Mg content on one side/edge over the other which had greater Mn, Ba, and Sr (Fig A2.11). Reasons for this are unclear but may be attributed to contamination or placement of the laser path along certain areas of the otolith that may have more concentrated annuli averaged by the beam during processing. Please refer sample edge-core-edge walleye figures in the (Figures A2.9-11).

Lake whitefish otolith Na signatures were relatively uniform across each otolith linescan from edge to core to edge. Some lake whitefish otoliths displayed a slight dip in Na signature near the core (e.g., lake whitefish otolith #24, Cormorant Lake) (Table A2.14). Lake whitefish Mg signatures were similar and comparable between edges,

displaying distinct decreasing Mg oscillations core-outwards. Some lake whitefish found to have greater otolith zone variation (e.g., Leftrook otolith 237, Cormorant otolith 24) while other trace element signatures remained similar and comparable between edges (e.g., Leftrook otolith 202) (Figs A2.12-14). Mn and Ba lake whitefish otolith signatures (like walleye signatures), contained multiple distinct oscillating peaks that decreased in concentration with distance towards the edge. The greatest peaks of Mn and Ba were found adjacent to the core. Sr lake whitefish otolith signature levels were found to be relatively uniform throughout each otolith linescan from edge to edge, and comparable between edges/sides (at times a slight dip in Sr concentration at or near-core, and towards the edges). Please refer to edge-core-edge lake whitefish in Figures A2. 12-14. Conclusions made from this analysis are that due to the similarity between otolith edges, it was deemed unnecessary to do complete edge-core-edge line scans for each otolith and instead core to edge per otolith would be sufficient.

3.3.2. Trace Element Residuals: Fish Age, Body Weight, and Fork Length Correction

Significant differences were found for all three measures between the CAMP waterbodies ($p < 0.001$), with post-hoc results found in Table 3.5, which indicated a need for correction. Additionally, for walleye, age differed significantly between pooled impounded and non-impounded waterbody datasets ($p < 0.001$), while fork length and body weight did not between datasets (log fork length t-test $p = 0.469$, log body weight t-test $p= 0.659$) (Table 3.5a). For lake whitefish, age differed significantly between impounded and non-impounded pooled datasets (Mann-Whitney $p = 0.005$), body

weight p-value was found near 0.05 (Mann Whitney p= 0.048, $\log_{10}(x)$ student *t*-test, p= 0.064), and fork length did not differ significantly (t-test p= 0.136) (Table 3.5b).
For the pairwise correlative analysis between walleye fish age, fork length, and body weight, a significant correlation was found between body weight and fork length (R^2= 0.86, p < 0.001) (Table 3.6a). For lake whitefish, both age to fork length (R^2= 0.113, p = 0.002) and body weight to fork length (R^2= 0.81, p < 0.001) were significantly correlated, although the R^2 for age and fork length was low indicating greater variability (Table 3.6a).

The correlation analysis of individual otolith trace element concentrations versus age, fork length, and/or body lengths found that for walleye: Ba with age (p < 0.001) and body weight (p = 0.026), Mg with age (p = 0.0482) and weight (p = 0.0294), Na with age (p = 0.0101), and Sr with age (p = 0.00337) were significantly correlated (Table 3.6b). For lake whitefish, Ba with age, fork length, and wet weight (p < 0.001), Mg with fork length (p = 0.0466), Mn with age (p < 0.001), Na with age (p < 0.001) and fork length (p = 0.0203), and Sr with age (p < 0.001) correlated significantly (Table 3.6b).

For the AIC method results $\text{Log}_{10}(x)$ otolith trace element results were given more consideration than raw data results since the transformed data better agreed with parametric assumptions. For walleye, Na, Mg, Ba, and Sr otolith data were suggested to undergo a correction (Table 3.7), while Mn was excluded due to LOD issues (Section 3.2.7). Na was suggested to be corrected for age, Mg for body weight, Ba for age and body weight, and Sr for all three variables. Lake whitefish otolith trace elements Na, and Sr were suggested to have an age correction, Ba for age and fork length, Mn for age and body weight, and no correction for Mg (Table 3.8).

Age was found to be the main factor influencing otolith trace element concentrations. The R^2 of age to otolith trace element correlations for each of the trace elements per species were compared to the AIC-suggested cumulative R^2 values if present (Table 3.7-3.9). If the difference between the suggested cumulative AIC R^2 was < 0.03 (conservative value chosen arbitrarily) than the age-corrected R^2, an age correction alone was employed instead of what was suggested by the AIC method. Results indicated that walleye otolith Na needed correcting for age, Mg for body weight, Ba for age and body weight, and Sr for age, body weight, and fork length. Lake whitefish Na were corrected for age, Mn for age (not body weight due to < 0.03 difference between cumulative R^2 = 0.2786 and age R^2 = 0.2611), Ba for age and fork length, Sr for age, and no correction required for Mg. Boxplots/bar graphs provided for the comparison of sex, age, fork length, and body weight between select camp waterbodies and groupings (Figs A2.1-8).

3.3.3. Outlier Identification and Otolith Trends

A total of one walleye and one lake whitefish otolith were removed from the final analysis. The walleye otolith (LNR-WAL #132) was found to have magnitude-level differences in bulk concentration averages for trace element measures Na (outlier: 716.15 ppm; non-outliers: 2174.76 ± 204.94 ppm), Mg (outlier: 628.27 ppm; non-outliers: 6.02 ± 0.96 ppm), Sr (outlier: 23.26 ppm; non-outliers: 298.82 ± 32.54 ppm), and Ba (outlier: 0.32 ppm; non-outliers: 5.88 ± 1.01 ppm) relative to the average of the remaining nine walleye otolith bulk averages collected from the waterbody (Lower Nelson River) (Table 3.10). It was assumed that an unknown species otolith was

accidentally archived as a walleye otolith or that the walleye otolith was composed of vaterite rather than the more common otolith composition of aragonite (Melancon et al., 2009). Its inclusion would have likely influenced overall trends in the data leading to less representative results. Secondly, an individual lake whitefish otolith (LNR-LKWF #56) was too young to compare fully with the CAMP water quality dataset (minimum required age of seven; #56 was six years old). Furthermore, the average of the remaining nine LKWF otolith trace element values were averaged and compared with LNR-LKWF #56 using the standard deviation as the confidence interval. It was found that the outlier otolith's Na (outlier: 2900.30 ppm; non-outliers: 2677.76 ± 197.18 ppm) and Ba (outlier: 7.02 ppm; non-outliers: 4.59 ± 2.09 ppm) average concentrations exceeded the acceptable error range, and may affect statistical results if left included (Table 3.10). Outlier and sample otoliths found in figures A2.61-68.

3.3.4. The Relation of Depth with Trace Element, TDS, and TSS Concentration in Water

Neither impounded (South Indian Lake) or non-impounded (Cormorant, Leftrook, Gauer) waterbodies were found to have a significant correlation between water trace element, total dissolved solids, or total suspended solids concentrations with depth (Table 3.11a-b). Correlation analysis was not conducted for Assean Lake, Split Lake, Threepoint Lake and the Lower Nelson River since the sample size of each was less than three. Additionally, no visually clear trend could be established with depth for Assean, Split, or Threepoint Lake and the Lower Nelson River. Please refer to the depth Tables A2.1-2.

3.3.5. Trace Element, Otolith, and Water Quality Correlations (0.3 m Depth)

3.3.5.1. Water Trace Elements Versus Water Quality Variables

Referring to the impounded and non-impounded waterbody combined correlations, out of the 45 comparisons, the nine which were significantly positively correlated were Na with TSS and ORP-L, Mg with pheophytin and TDS, Mn with N-TKN, Ba with pheophytin and TDS, and Sr with TDS and TSS (Table 3.12), other non-significant results can be found in Table A2.6. Other water quality variables removed from further analysis due to autocorrelation can be found within Appendix II. Referring to impoundment and non-impoundment exclusive correlations, all comparisons between otolith Sr with TDS or TSS failed to meet parametric requirements, leading to the use of Spearman's correlation coefficient tests which provided no significant results likely due to small sample size (Table 3.13). Although, based on Pearson's correlation coefficient tests, water Sr was found to positively correlate with TDS in impounded waterbodies (R^2= 0.994, p= 0.003) (Table 3.14). This indicated that correlations between water and TDS may be occurring, but additional sampling would be required to clarify.

3.3.5.2. Otolith Trace Element Versus Water Trace Element Concentrations

Referring to the impounded and non-impounded waterbody combined correlations, for walleye, none of the four pairwise comparisons were found to correlate significantly at an α of 0.05, namely (Na: R^2= 0.0044, p= 0.876, Mg: R^2= 0.403, p= 0.091, Ba: R^2= 0.46, p= 0.065, and Sr: R^2= 0.276, p= 0.181) (Table 3.15, Figs 3.3-3.7). For lake whitefish, none of the pairwise comparisons significantly correlated at an α of 0.05 (Na: R^2= 0.0737, p= 0.515, Mg: R^2= 0.215, p= 0.247, Mn: R^2= 0.000273, p= 0.902,

Ba: R^2= 0.102, p= 0.44, and Sr: R^2= 0.449, p= 0.069) (Table 3.18) (Figs 3.3-3.7). Referring to impoundment and non-impoundment exclusive correlations, all comparisons for either species failed to meet parametric requirements, leading to the use of Spearman's correlation coefficient tests which provided no significant results likely due to the smaller sample size (Table 3.16). Although, based on Pearson's correlation coefficient tests, walleye Mg was significantly positively correlated with water for non-impounded waterbodies (r^2= 0.928, p= 0.037), while lake whitefish Sr was positively correlated with water in impounded waterbodies (r^2= 0.967, p= 0.016), other correlations were nearly significant for both species or impoundment type (Table 3.17). This indicated that correlations between water and otolith may be occurring, but more sampling is required to clarify.

3.3.5.3. Otolith Trace Element Correlations and Water Quality Variables

Referring to the impounded and non-impounded waterbody combined correlations, for walleye, out of the 36 comparisons, five were significantly positively correlated: Mg with pheophytin ($P < 0.05$), Sr with Chl-a ($P < 0.01$), and Mg with N-TKN and DOC ($P < 0.01$) (Table 3.15, A2.7). For lake whitefish, out of the 45 comparisons two were significantly positively correlated: Ba with DOC and DO (both $P < 0.05$) (Table 3.18, A2.8). Also, the correlations of Ba with N-TKN and Mg with DO and pheophytin were both near or at the α of 0.05 (Table 3.18). Referring to impoundment and non-impoundment exclusive correlations, all comparisons between otolith Sr with TDS or TSS failed to meet parametric requirements, leading to the use of Spearman's correlation coefficient tests which provided no significant results likely due to the smaller sample size (Table 3.17). Although, based on Pearson's correlation coefficient tests

some significant results were identified. For walleye, otolith Sr positively correlated with TSS in non-impounded waterbodies (R^2= 0.922, p= 0.04), while lake whitefish otolith Sr positively correlated for both TDS and TSS for impounded waterbodies (TDS: R^2= 0.979, p= 0.01; TSS: R^2= 0.982, p= 0.009) (Table 3.16). This indicated that correlations between otolith and TDS/TSS may be occurring, but more sampling is required to clarify.

3.3.5.4. Between Separate Trace Elements

For walleye, Ba was found to significantly positively correlate with Mg (Spearman's R^2= 0.9506, p= 0.00477) and Sr (Pearson's R^2= 0.3969, p= 4.8^{-10}) (Table 3.19a-b). For lake whitefish, the correlation between Ba and Sr was significantly positively correlated (Spearman's R^2= 0.5041, p < 0.001) (Table 3.19b). The correlation between lake whitefish Na and Mg was nearly significantly positively correlated (R^2= 0.045369, p= 0.0601) (Table 3.19b). Additionally, from the walleye to lake whitefish trace element average concentration comparison of the eight CAMP waterbodies, Sr alone was found to be significantly and positively correlated (Sr: R^2= 0.726, p = 0.007), unlike the other three tested trace elements (Na: R^2= 0.0903, p = 0.469; Mg: R^2= 0.2, p = 0.266; and Ba: R^2= 0.14, p = 0.361) (Table 3.20). Mn was not tested as walleye otolith data were removed previously.

3.3.5.5. Water Trace Element Versus Year Sampled Between Waterbodies

Out of the 40 pairwise comparisons, eight were found to be significantly and positively correlated (Table 3.21). All significant positive correlations (p < 0.05) were found within four of the eight CAMP waterbodies and encompassed both impounded

and non-impounded waterbodies and from three of the five selected trace elements (Na, Mg, and Sr) (Table 3.21). They were Cormorant Lake (Mg: R^2=0.593, p =0.043), Leftrook Lake (Mg: R^2= 0.865, p = 0.007, Na: R^2= 0.946, p = 0.001, and Sr: R^2= 0.696, p = 0.039), Gauer Lake (Na: R^2= 0.802, p = 0.006, and Sr: R^2= 0.822, p = 0.005) and Threepoint Lake (Mg: R^2= 0.691, p = 0.04 and Na: R^2= 0.867, p = 0.007) (Table 3.21). All selections were deemed parametric other than Mn content in Leftrook Lake across the year range (Table 3.21). Water concentration graphs in Figures A2.69-74.

3.3.6. Impoundment Univariate Statistics

3.3.6.1. Water Trace Element Univariate Statistics: Impoundment

Due to the non-parametric nature of the datasets even after log transformation, each comparison was tested via Mann-Whitney Rank Sum test. Water Na concentration alone was found to be significantly greater in pooled impounded waterbody than non-impounded pooled waterbody values ($p < 0.001$) (Table 3.22) (Figs 3.8-3.16). Water Mg concentration was near the α of 0.05 (p= 0.051) (Table 3.22) (Figs 3.10-3.11).

An ANOVA comparing each of the eight separate waterbodies was also conducted and was followed by post Hoc Tukey's (if parametric) or Dunn's (if non-parametric) tests (Table 3.22). Both raw and $Log_{10}(x)$ transformed data were used, and in each case significant differences ($p < 0.001$) were observed in the eight selected CAMP waterbodies (Table 3.22). From the pairwise comparison (Tukey's or Dunn's) results, Na trends indicated that LNR and SPLT had the greatest measured concentrations as compared to other waterbodies (GAU, ASS, and SIL4 (for SPLT only)), while 3PT differed significantly from GAU (Table 3.22). An additional trend for Na

was that GAU had the lowest average Na concentration, but other trends were found to be unclear (Table 3.22). For Mg, most waterbodies varied significantly from each other except for three of the non-impounded waterbodies (GAU, LFT, ASS) and two of the most downstream impounded waterbodies (SPLT, LNR) exclusively (Table 3.22). Most of the non-impounded waterbodies differed significantly from the impounded waterbodies as well for Mg, except GAU and SIL4 (Table 3.22). CORM had the greatest Mg concentration, followed by SPLT and CORM, while SIL4 and 3PT had the lowest concentrations (Table 3.22). For Mn, LFT had the greatest Mn concentration, while CORM and SIL4 contained the lowest Mn concentrations (Table 3.22). No specific impoundment trends or significant differences were observed for Mn (Table 3.22). For Sr, the two impounded waterbodies LNR and SPLT were found to have significantly greater Sr concentrations than the other waterbodies but not each other (Table 3.22). Most of the non-impounded waterbodies differed significantly from the impounded waterbodies as well for Sr, except GAU with SIL4 and 3PT (Table 3.22). Impounded waterbodies SPLT and LNR contained the greatest Sr concentrations, followed by non-impounded waterbodies ASS, CORM and LFT, and then finally SIL4, GAU and 3PT (Table 3.22). Lastly, for Ba, the two impounded waterbodies LNR and SPLT and the non-impounded waterbody CORM were found to contain significantly greater Ba concentrations than the other CAMP waterbodies but not each other (Table 3.22).

3.3.6.2. Otolith Trace Element Univariate Statistics: Impoundment

Pooled walleye results indicated that both otolith Sr ($p = 0.04259$) and Ba ($p < 0.001$) were significantly greater in impounded waterbodies (Table 3.23, Figs 3.13 and

3.15). Comparatively, pooled lake whitefish indicated that both otolith Sr ($p < 0.001$) and Ba ($p < 0.001$) were significantly greater and Mg ($p = 0.0191$) was significantly less in impounded waterbodies relative to non-impounded waterbodies (Table 3.24 (Fig 3.10, 3.14, and 3.16). Other trace elements did not differ significantly for either species (Table 3.23-3.24) (Figs 3.8-3.16).

Individuals were also grouped based on their respective waterbodies (for ANOVA tests between the eight waterbodies) and each trace element tested was found to differ significantly except for walleye otolith measures for Na content ($p = 0.06792$) (Tables 3.23-3.24). From the post-hoc Tukey's or Dunn's multiple comparisons analysis it should be noted that walleye Na and Mg otolith data, and lake whitefish Na, Mg and Sr data failed to meet parametric requirements (Tables 3.23-3.24). For walleye Na otolith content, CORM had significantly less averaged Na content than GAU (Table 3.23). For walleye Mg otolith content, CORM had significantly greater averaged Mg content than the two other non-impounded waterbodies (ASS and GAU) (Table 3.23). For walleye Ba otolith content, significant differences occurred between SPLT, LNR, 3PT being significantly greater in Ba concentration than ASS, GAU, LFT, and CORM being greater than SIL4 (Table 3.23). Lastly for walleye, Sr otolith content when compared in a pairwise fashion found that three of the impounded waterbodies (LNR, SPLT, and 3PT) had significantly greater Sr average otolith contents than the four non-impounded waterbodies (Table 3.23). Also for Sr otolith content, ASS and SIL4 had the lowest Sr average concentrations while SIL4 did not differ significantly with any of the non-impounded waterbodies (Table 3.23).

For lake whitefish Na otolith content, significant differences occurred between some waterbodies (Table 3.24). GAU followed by SIL4 had the greatest Na otolith concentrations, while LFT and 3PT had the lowest (Table 3.24). For lake whitefish otolith Mg, CORM had the greatest average otolith concentration and was found to differ significantly with SIL4, LFT and LNR (Table 3.23). SIL4 was found to have the lowest Mg otolith concentration and was found to differ significantly from CORM, GAU LFT and SPLT (Table 3.24). Statistical Mg significance from the pooled impounded versus non-impounded data was likely caused by the greater variation in CORM and GAU Mg concentrations (Table 3.24). For lake whitefish otolith Mn (like walleye), no clear trend was evident (Table 3.24). SIL4 and CORM were found to have the lowest Mn average otolith concentrations, whereas GAU and 3PT were found to have the highest average otolith Mn concentrations (Table 3.24). For lake whitefish otolith Sr, SPLT and LNR (impounded) were found to be significantly greater in Sr otolith average concentration than ASS, GAU and LFT (non-impounded) waterbodies (Table 3.24). Additionally, for Sr, 3PT and SIL4 (impounded) were significantly greater in otolith Sr average otolith concentration than ASS (GAU additionally was significantly less than 3PT) (Table 3.24). All impounded waterbody Sr averages were greater than all non-impounded averages (Table 3.24). Lastly, for lake whitefish otolith Ba, impounded waterbodies were found to contain significantly greater otolith Ba concentration levels than non-impounded waterbodies except for the comparison of LNR with CORM or GAU, although all impounded waterbody averages were greater (Table 3.24). For other unspecified significant differences please refer to Tables 3.23-3.24.

3.3.7. Multivariate Statistical Testing

3.3.7.1. Preliminary Multivariate Analyses (pRDA)

The first of three pRDAs was conducted with both walleye and lake whitefish data. The entire RDA model accounted for 65.58% of the variability/trend in the data. Axis percent trend explained was found to be for RDA1= 44.75%, RDA1+2= 58.97%, and RDA1+2+3= 63.96% (Fig 3.17). Species type had the greatest influence (clear division between the species) in RDA1 as seen in the centroid scores for factor constraints, followed by AREA (Fig 3.17). Little trend was evident within RDA2 and RDA3. For the entire model ANOVA, $F_{9,148}$= 31.331, Pr(>F) = 0.001 and for each axis Pr(>F) = 0.001 (RDA1: $F_{1,153}$= 198.9372, RDA2: F= 63.1685, RDA3: F= 22.1782, and RDA4: F= 7.2244).

The purpose of the second pRDA was to filter out unnecessary environmental variables used to constrain the trace element walleye otolith data (with species type removed). The entire pRDA model accounted for 49.41% of the variability/trend in which is separated into RDA1= 33.31%, RDA1+2= 47.10%, and RDA1+2+3= 48.526% of the trend in the data (RDA1 weighted most heavily) (Fig 3.18). For the first axis RDA1, AREA (code used for CAMP waterbody) displayed the greatest consistent weights compared to the other factors tested (this trend also carried through for RDA2, thereby AREA was interpreted to contain the greatest trended variation) (Fig 3.18). The other factors had inconsistent and generally lower weighted values/centroid scores (Fig 3.18). Because of this, AREA alone was selected as the sole constraining variable for walleye otolith data used for further study. For the entire model ANOVA, $F_{8,70}$= 8.5471, Pr(>F) =

0.001 and for axis RDA1-2 Pr(>F) = 0.001 (RDA1: $F_{1,74}$= 48.7213, RDA2: F= 20.1768), RDA3: F= 2.0874, Pr(>F) = 0.113 and RDA4: F= 1.2982, Pr(>F) = 0.280.

The purpose of the third pRDA was to filter out unnecessary environmental variables used to constrain the trace element lake whitefish otolith data (species factor removed). The entire pRDA model accounts for 54.01% of the variability/trend explained in the data which is separated between RDA1= 28.09%, RDA1+2= 37.64%, and RDA1+2+3= 46.62% (RDA1 weighted most) (Fig 3.19). For the first axis RDA1, AREA (CAMP waterbody) displayed the greatest consistent weights compared to the other factors tested (Fig 3.19). The other factors had inconsistent and generally lower weighted values. Because of this, AREA alone was selected as the sole constraining variable for lake whitefish otoliths for further study (Fig 3.19). For the entire model ANOVA, $F_{8,70}$= 10.277, Pr(>F) = 0.001 and for axis RDA1-4 Pr(>F) = 0.001 (RDA1: $F_{1,74}$= 44.5924, RDA2: F= 15.1602), RDA3: F = 14.2505, and RDA4: F = 9.7687) and RDA5: F = 1.9697, Pr(>F) = 0.091.

3.3.7.2. Principal Component Analysis (PCA) – Otolith, Waterbody, and Water Quality

3.3.7.2.1. PCA – Trace Element Data and Species

Clear separation of species along axis 1 was observed, with Na, Mg, and Sr otolith content weighted more towards lake whitefish, while Ba otolith content weighed more towards walleye along PC1 (Fig 3.20). Axis 2 did not differentiate species, but shows that Ba and to a lesser degree Sr otolith content dominated the distribution (longest eigenvectors) (Fig 3.20).

3.3.7.2.2. PCA – CAMP Water Quality Variables

For the PCA of CAMP water quality database selected variables, prior to variable selection, pairwise correlations were made on the 25+ variables to determine if autocorrelation exists and to remove auto-correlated variables. Two PCAs were run: Raw data (WQDB_R) and Log_{10} transformed data (WQDB_L). All raw or log transformed data was found to be normally distributed at an alpha of 0.05 (Kolmogorov-Smirnov test) except pheophytin ("Pheophy").

Using the raw water data, nine water quality variables previously mentioned) were used to characterize the separate CAMP waterbodies and for the individual principal component axis. 30.2% of the trend was accounted by PC1, 23.33% by PC2, 21.35% by PC3, 53.54% by PC1+2, 44.58% by PC1+3 and 74.89% by PC1+2+3 (Fig 3.21, 3.22). Pheophytin (productivity) had an inverse relationship to the other variables in PC1 (Fig 3.21). Most of the impounded waterbodies (Threepoint, Lower Nelson River, Split Lake) and most of the environmental variables are located within the positive side of PC1, while non-impounded waterbodies (Cormorant, Leftrook) are in the negative axis zone (Gauer Lake is more neutral) (Fig 3.21). South Indian Lake (Area 4) did not follow the latter impoundment trend (Fig 3.21). For PC2, separation was not based on impoundment status (two of each type of waterbody found in the positive or negative zone of PC2 (Fig 3.21). DO and T.C inversely related with Pheophy, N.TKN, TSS, TDS, ORP (Fig 3.21, 3.22). Lastly, for PC3, waterbodies were not split between positive or negative regions of the axis based on impoundment (Fig 3.22). Lastly as a general observation, ORP and Chl.A were inverse with TDS, DOC, and DO (Fig 3.22).

In the second PCA, using the $\log_{10}(x)$ transformed water quality data, nine separate water quality variables used to characterize the CAMP waterbodies. The

amount of trended variation accounted for was 31.75% in PC1, 23.97% in PC2, 20.82% in PC3, 55.72% in PC1+2, 44.80% in PC1+3 and 76.54% in PC1+2+3 (Fig 3.22). Along PC1, impounded waterbodies Lower Nelson River and Split Lake were observed to have greater levels of most of the environmental variables (followed by Assean), while Cormorant Lake and South Indian Lake (Area 4) were observed to have relatively lower levels while Gauer Lake, Leftrook Lake, and Threepoint Lake were considered near neutral based on included measures (Fig 3.23). Along PC2, Cormorant Lake and South Indian Lake held the greatest weighted trends for the environmental variables (Fig 3.23-3.24). Cormorant Lake was observed to have greater pheophytin, TDS, DOS, DO, and N.TKN, while South Indian Lake was observed to have greater T.C, ORP, Chl.A, and TSS (Fig 3.23-3.24). Along PC3, waterbodies were again not divided based on any definite trend (e.g., impoundment status) (Fig 3.24). Threepoint, Gauer, and Assean lakes showing greater affinity to T.C, DO and DOC (Fig 3.24). Split, Sout Indian, Leftrook Lake, Lower Nelson River, and Cormorant Lake showing affinity to greater levels of ORP, Chl.A, TSS, Pheophy, and TDS (Fig 3.24). Overall, PC1 was considered the most trended axis. Not one environmental variable was identified specifically that strongly differentiates the CAMP waterbodies graphically. Split Lake and Lower Nelson River were found to have relatively greater amounts of each variable than the other waterbodies in PC1 that may be attributed to proximity, both being impounded and furthest downstream in location.

3.3.7.2.3. PCA – CAMP Physical Characteristics

In the third PCA, physical characteristics of the eight CAMP waterbodies were compared (refer Table 3.1). The 13 separate factors used to characterize the separate

CAMP waterbodies were found to account for 40.52% in PC1, 63.34% in PC1+2, and 80.26% of the variation/trend in PC1+2+3 (Fig 3.25). Along the first axis (PC1), Split Lake and Lower Nelson River were found in the right-hand, positive axis, whereas the other six waterbodies were situated within the left-hand, negative axis zone (Fig 3.25). This is likely due to how close geographically Split Lake and Lower Nelson River are to each other relative to the other waterbodies (Fig 3.25). Additionally, for the first axis (PC1), drainage basin size, presence of cultivated crop and longitude had the strongest positive axis influence, whereas presence or absence of coniferous land cover and altitude has the strongest negative axis influence (Fig 3.25).

3.3.7.3. Redundancy (RDA) and K Nearest Neighbours KNN) Analyses – Otolith

3.3.7.3.1. Walleye – RDA and KNN

In the case of each RDA tested for either species, redundancy axis 1 and 2 (RDA1 and RDA2) were focused upon since holding the greatest trended variations. With RDA1 given priority over RDA2 due to containing the greatest trended variation. The purpose of this RDA was to test the effect of each CAMP waterbody (AREA) has on trace element content (Na, Mg, Ba and Sr) in walleye otolith (Fig 3.26). The entire RDA constrained model accounted for 49.01% of the variability/trend in the data or based on progressive axes, RDA1= 33.31%, RDA1+2= 46.74% and RDA1+2+3= 48.16% of the trend (Fig 3.26). Biplot scores along the first axis (RDA1) displayed a division between otoliths from non-impounded individuals along the positive left hand side of the axis, and impounded waterbody otoliths along the negative, right hand side of the axis for six of the eight waterbodies observed (Fig 3.26). Cormorant Lake and South Indian Lake

carried opposite trends to their non-impounded or impounded counterparts (Fig 3.26). The impounded and non-impounded waterbodies carried either positive or negative centroid scores in RDA1. For impounded waterbody, centroid scores were: 3PT (-0.6454) < LNR (-0.4950) < SPLT (-0.2837) > SIL4 (0.4308). For non-impounded waterbody centroid scores: ASS (0.7584) > LFT (0.3272) > GAU (0.2215) > CORM (-0.3632) (Table 3.25, Fig 3.26). Based on trace element concentrations, a single diffuse cluster of waterbody centroid scores in RDA1 exist with impounded waterbodies (excluding SIL4 but including CORM), positioned nearer to Sr, followed by Ba and Mg along RDA1 whereas non-impounded waterbodies (and SIL4) were relatively less influenced (waterbody otoliths containing relatively lower concentrations of Sr and Ba), and found further away, along the positive axis (Fig 3.26). Mg and Na were less weighted on the RDA1 axis (Fig 3.26). In RDA2 Mg and Sr trace element concentrations contained the greatest biplot scores (Fig 3.26). Along RDA2, greater Ba and Mg content seems to indicate lesser relative amounts of Sr and Na within the otolith (inversely proportional) (Fig 3.26). Cormorant followed by South Indian Lake were interpreted to have relatively greater Ba and Mg relative to otoliths from the other CAMP waterbodies, whereas LNR was found to have relatively greater amounts of Sr and Na along RDA2 (Fig 3.26). For the entire model ANOVA, $F_{7,71}$= 9.7482, Pr(>F)= 0.001 and for axis RDA1+2 Pr(>F) = 0.001 (RDA1: $F_{1,74}$= 48.334, RDA2: F= 19.4893), RDA3: F= 2.0592, Pr(>F)= 0.111, RDA4: F= 1.2383, Pr(>F)= 0.285) (Table 3.26). Walleye per waterbody group KNN results further indicated that classification was correct between 34.4% (k= 5) to 45.6% (k= 1) while using Na, Mg, Ba and Sr otolith trace element measures. Correct classification per waterbody ranged from 0-90% (k= 1) (Table 3.27). Assean and

Cormorant Lakes were the most frequently classified correctly with 90% correct classification while Gauer Lake (0% correct classification) was the most misclassified (Table 3.27). Walleye impoundment grouping KNN results indicated that impounded and non-impounded waterbodies could be correctly classified between 79.7% (k= 1) to 82.2% (k= 11) of the time (k= 11, non-impounded= 85%, impounded= 79% correct classification) while using Na, Mg, Ba and Sr otolith trace element measures (Table 3.28).

A second RDA was conducted to test the effect of each CAMP waterbody (coded as “AREA”) has on barium (Ba) and strontium (Sr) trace element content in walleye otolith. Ba and Sr were isolated since noted as the only two trace elements currently under study that are not internally regulated within the fish (Campana, 1999). The entire RDA constrained model accounted for 73.88% of the variability/trend in the data or for progressive axis, RDA1= 64.01%, and RDA1+2= 73.878% of the trend (Fig 3.27). Based on trace element concentrations per individual otolith scores, a single cluster of waterbody centroids scores in RDA1 with impounded waterbodies (excluding SIL4 but including CORM), positioned nearer to Sr, followed by Ba along RDA1 whereas non-impounded waterbodies (and SIL4) were relatively less influenced (waterbody otoliths containing relatively lower concentrations of Sr and Ba), and found further away, along the positive axis (Fig 3.27). Impounded waterbody centroid scores were: 3PT (0.5504) > LNR (0.4904) > SPLT (0.2530) > SIL4 (-0.3981). For non-impounded waterbody centroid scores: ASS (-0.6240) < LFT (-0.2578) < GAU (-0.1415) < CORM (0.1767) (Table 3.25, Fig 3.27). Sr, followed by Ba trace element concentrations contained the greatest biplot scores along RDA1 (Fig 3.27). Along RDA2, Cormorant Lake was found

to have walleye otoliths with greater relative amounts of Ba content and lesser relative amounts of Sr content (also observed in the univariate statistics), while the opposite was true for Leftrook Lake, Split Lake and South Indian Lake and Lower Nelson River (Gauer Lake appeared neutral for relative Ba and Sr content) (Fig 3.27). An ANOVA permutation test with 999 iterations was conducted to the entire model and by axis. For the entire model ANOVA, $F_{7,71}$= 28.686, Pr(>F)= 0.001 and for each axis Pr(>F)= 0.001 (RDA1: $F_{1,76}$= 186.24, RDA2: F= 28.706) (Table 3.26). Walleye per waterbody group KNN results further indicated that classification was correct between 32.9% (k= 1) to 46.8% (k= 3) while using Ba and Sr otolith trace element measures (Table 3.29). Correct classification per waterbody ranged from 20 to 90% (k= 3) (Table 3.29). Assean (80% classification success) and Cormorant (80%) Lakes were most correctly classified, while Gauer and South Indian Lakes were most commonly misclassified (20% classification success) (Table 3.29). Walleye impoundment grouping KNN results indicated that impounded and non-impounded waterbodies could be correctly classified between 73.4% (k= 1) to 82.7% (k= 9/11) of the time (k= 9/11, non-impounded= 97.5%, impounded= 66.7% correct classification) while using Ba and Sr otolith trace element measures (Table 3.30).

3.3.7.3.2. Lake whitefish - RDA and KNN

In the case of each RDA tested for either species, redundancy axis 1 and 2 (RDA1 and RDA2) were focused upon since holding the greatest trended variations. RDA1 was given priority over RDA2. The third RDA was made to test the effect of each CAMP waterbody (AREA) has on trace element content (Na, Mg, Mn, Ba, and Sr) within

lake whitefish otolith (Fig 3.28). The RDA entire constrained model accounts for 52.32% of the variability/trend in the data or per progressive axis being RDA1= 27.99%, RDA1+2= 37.18%, and RDA1+2+3= 45.40% (Fig 3.28). Waterbody centroid scores along RDA1 displayed a clear division of otolith from non-impounded individuals along the positive left hand side of the axis, and otoliths collected from impounded waterbodies along the negative, right hand side of the axis (Fig 3.28). For impounded waterbody, centroid scores were: SPLT (-0.6902) < LNR (-0.5267) < SIL4 (-0.3588) < 3PT (-0.3147). For non-impounded waterbody centroid scores: ASS (0.7488) > GAU (0.5469) > LFT (0.3559) > CORM (0.1861) (Table 3.25, Fig 3.28). Along RDA1 it is also visible that Ba and Sr otolith content was weighted more heavily towards impounded waterbodies, and Na, Mg, and Mn were near neutral-to-slightly weighted towards non-impounded waterbodies (Fig 3.28). The site scores are weighted greatest for Sr, followed by Ba along RDA1 (Fig 3.28, Table 3.31). Other trace element weights were far less than the latter. Along RDA2 the impounded waterbodies were relatively clustered together while the non-impounded waterbodies were separated into two separate groups namely ASS-LFT, and CORM-GAU (Fig 3.28). Mg followed by Na had the greatest scores along RDA2, and were found situated nearest to GAU and CORM for having relatively greater Na and Mg otolith content, while other trace elements were found near the origin (Fig 3.28). For the entire model ANOVA, $F_{7,71}$= 11.131, Pr(>F) = 0.001 and for each axis Pr(>F)= 0.001 (RDA1: $F_{1,73}$= 42.8612, RDA2: F= 14.0613, RDA3: F= 12.5883, and RDA4: F= 8.8712, RDA5: F= 1.7276) (Table 3.26). Lake whitefish per waterbody group KNN results further indicated that classification was correct between 43.9% (k= 3) to 52.9% (k= 5/11) while using Na, Mg, Mn, Ba and Sr

otolith trace element measures (Table 3.31). Correct classification per waterbody ranged from 20 to 90% (k= 5 and 11 respectively) (Table 3.31). Assean, Cormorant and South Indian Lakes were most commonly correctly classified (80-90% correct classification) while Gauer, Split and Leftrook Lakes were most commonly incorrectly classified (20% correct classification) (Table 3.31). Lake whitefish impoundment grouping KNN results indicated that impounded and non-impounded waterbodies could be correctly classified between 89.9% (k= 9) to 96.2% (k= 3) of the time (k= 3, non-impounded= 97.5%, impounded= 94.9% correct classification) while using Na, Mg, Mn, Ba and Sr otolith trace element measures (Table 3.32).

The fourth constructed RDA was made to test the effect of each CAMP waterbody (AREA) has on trace element content with Mn excluded for comparative purposes with the walleye corresponding dataset (Fig 3.26), within lake whitefish otolith. The entire RDA constrained model accounts for 55.37% of the variability/trend in the data (3.05% increase with removal of Mn otolith data, Fig 3.28) or per progressive axis being RDA1= 34.83%, RDA1+2= 46.27%, and RDA1+2+3= 53.873% of the trend in the data (Fig 3.29). Biplot scores along the first axis (RDA1) displayed a clear division of otolith from non-impounded individuals along the positive left hand side of the axis, and otoliths collected from impounded waterbodies along the negative, right hand side of the axis (Fig 3.29). For the impounded waterbodies, centroid scores were: SPLT (-0.6524) < LNR (-0.4939) < 3PT (-0.3257) < SIL4 (-0.3229). For non-impounded waterbody centroid scores: ASS (0.7241) > GAU (0.4843) > LFT (0.3307) > CORM (0.2064) (Table 3.25, Fig 3.29). Along RDA1, it was also visible that Ba and Sr otolith content was found to be relatively greater in impounded waterbodies, and Na and Mg otolith content were

found to be relatively greater in non-impounded waterbodies (Fig 3.29). The site scores are weighted greatest for Sr, followed by Ba along RDA1 (Fig 3.29). Along RDA2 (like Fig 3.28), impounded waterbodies were found clustered near the origin, while non-impounded waterbodies were divided into two separate groups as explained previously (Fig 3.29). For the entire model ANOVA, $F_{7,71}$= 12.581, Pr(>F)= 0.001 and for axis RDA1-3 Pr(>F)= 0.001 (RDA1: $F_{1,74}$= 57.7377, RDA2: F= 18.9705, RDA3: F= 12.6079), and RDA4: F= 2.4742, Pr(>F)= 0.052 (Table 3.26). Lake whitefish per waterbody group KNN results further indicated that classification was correct between 46.7% (k= 1) to 55.4% (k= 9) while using Na, Mg, Ba and Sr otolith trace element measures (Table 3.34). Correct classification per waterbody ranged from 10 to 90% (k= 9) (Table 3.34). South Indian and Assean Lakes were most commonly correctly classified (90 to 100% correct classification respectively) while Gauer Lake was the most frequently misclassified (10% correct classification) (Table 3.34). Impoundment group KNN results indicated that impounded and non-impounded waterbodies could be correctly classified between 87.3%(k= 1) to 96.2% (k= 3 or 7) of the time (k= 3, 100% for non-impounded, 92.3% for impounded pooled groups) (Table 3.33).

The fifth RDA tested the effect of each CAMP waterbody has on barium and strontium trace element otolith content in lake whitefish exclusively (Fig 3.30). Ba and Sr were isolated since noted as the only two trace elements currently under study that are not presumably internally regulated within the fish (Campana, 1999). The entire RDA constrained model accounts for 72.83% of the variability/trend in the data, which was greater than the previous two lake whitefish models (Fig 3.28, 3.29, 3.30) or per progressive axis being axis, RDA1= 67.34%, and RDA1+2= 72.832% of the trend (Fig

3.30). Biplot scores along RDA1 displayed a less clear division of otolith from impounded individuals as compared to the previous two lake whitefish RDAs which contained Na, Mg (and Mn) (Fig 3.30). For impounded waterbody, centroid scores were: SPLT (0.55776) > LNR (0.40051) > 3PT (0.25374) > SIL4 (0.25218). For non-impounded waterbody centroid scores: ASS (-0.65435) < GAU (-0.32666) < LFT (-0.34903) < CORM (-0.09409) (Table 3.25, Fig 3.30). The site scores are weighted greatest for Sr, followed by Ba along RDA1 (Fig 3.30). Along RDA2, waterbodies are separated based on otolith Ba and Sr content, with most of waterbodies clustered near the origin other than SIL4 along the upper positive region near Ba, and LNR along the lower negative region near Sr (Fig 3.30). For the entire model ANOVA, $F_{7,71}$= 27.191, Pr(>F)= 0.001 and for each axis Pr(>F)= 0.001 (RDA1: $F_{1,76}$= 188.372, RDA2: F= 15.366) (Table 3.26). Ba and Sr univariate ANOVA post hoc trends followed RDA centroid scores for lake whitefish. Lake whitefish per waterbody group KNN results further indicated that classification was correct between 32.6% (k= 1) to 39.2% (k= 3) while using Ba and Sr otolith trace element measures (Table 3.35). Correct classification per waterbody ranged from 20 to 60% (k= 3) (Table 3.35). Assean and Gauer Lakes were most commonly correctly classified (60% correct classification) while Cormorant and Threepoint Lakes were most commonly misclassified (20% correct classification for each) (Table 3.35). Impoundment group KNN results indicated that impounded and non-impounded waterbodies could be correctly classified between 83.5%(k= 3) to 95% (k= 3) of the time (k= 3, 87.5% for non-impounded, 82.1% for impounded pooled groups) (Table 3.36).

3.3.7.3.3. Both Walleye and Lake Whitefish Combined – RDA and KNN

The sixth RDA was developed to determine if species type or AREA was related more so to trace element content within the otolith. The RDA entire constrained model accounts for 65.02% of the variability/trend in the data or per progressive axis being RDA1= 44.65%, RDA1+2= 58.86%, and RDA1+2+3= 63.688% of the trend in the data (Fig 3.31). Based on centroid scores for factor constraints, RDA1 indicated that the species type factor had the greatest influence (clear division between the species also seen in the PCA Fig P1), followed by AREA (Fig 3.31). Species centroid scores were weighted more than AREA centroid scores in RDA1 (Table RR1). The centroid scores along RDA1 for species type factors were -0.3910868 for lake whitefish and 0.3910868 for walleye (Fig 3.31, Table 3.39). Little trend was evident within RDA2 other than LNR followed by SPLT were situated further away from the origin, in the same direction as Ba and Sr content, whereas ASS was furthest in the opposite direction (Fig 3.31). An ANOVA permutation test with 999 iterations was conducted to the entire model and by axis. For the entire model ANOVA, $F_{8,149}$=34.615, Pr(>F)= 0.001 and for axis RDA1-3 Pr(>F)= 0.001 (RDA1: $F_{1,153}$= 195.275, RDA2: F= 62.1515, RDA3: F = 21.1136) and RDA4: F= 5.8124, Pr(>F)= 0.002 (Table 3.39). Comparing walleye and lake whitefish otolith trace element levels through KNN it was determined that when including Na, Mg, Ba and Sr measures that species could be correctly classified between 99.4 (k= 3 or 7) to 100% (k= 9 or 11) of the time (k= 9 or 11, non-impounded= 100%, impounded = 100%) (Table 3.37). Comparatively, when Ba and Sr otolith trace element measures alone were used, the two species could be correctly classified 98.1% (k= 1) to 98.7%

(k= 3 or 11) of the time (k= 3 or 11, non-impounded or impounded waterbodies correctly classified 98.7% exclusively) (Table 3.38).

3.3.8. Impoundment Flow Analysis

It was observed that the further downstream an impoundment is situated, the greater the average flow (Fig 3.32). Also, flow was found to be relatively consistent over time for each of the control or generating structure impoundments (Fig 3.33). Within error (as standard deviation), the average flow did not differ between the two time-ranges being 2008-2014 and 2008-2017 (Wuskwatim GS monitoring started in 2011 in both cases) (Fig 3.33).

Out of the 35 pairwise comparisons of water trace element content and the 14 pairwise comparisons of TSS and TDS content against adjacent (upstream or downstream) control or generating structure water flow, only three significant positive correlations were found (Table A2.9). All three were associated with control structures adjacent to SIL4 which were Missi Falls flow with Na content (R^2= 0.881, p= 0.002), Notigi with Na content (R^2= 0.617796), and Missi Falls with Mg content (R^2= 0.772, p= 0.009) (Table A2.9).

Out of the eight pairwise comparisons for walleye, or 10 pairwise comparisons for lake whitefish, none were significantly correlated with upstream adjacent impoundment water flow (Table 3.40). Greater sample sizes are required for non-parametric trst significance (none of the comparisons made parametric requirements).

Secondly, PCA using flow and other impounded waterbody characteristics was made to further differentiate CAMP waterbodies (Fig 3.34). The proportion of the trend

explained by successive axis are: PC1= 75.8%, PC2= 96.91%, and PC1+2+3= 100% (Fig 3.34). Additionally, PC1, LN and SP have greater flow, larger drainage basins, and are located at a greater longitude and lesser altitude than the other two waterbodies (Fig 3.34). Lastly, it was deemed unnecessary to conduct a flow analysis on trace elements, TDS or TSS on any other depths other than 0.3 m collected values due to the lack of significant differences determined in the previous analysis, and lack of sufficient depth data.

3.4 Discussion

3.4.1. Summary

The CAMP-O study was an attempt to characterize the effect of impoundments on freshwater chemistry and its relationship to fish otoliths. Further determining the impact of impoundments using biomonitors could help improve regulations surrounding impoundments. When referring to the objectives, it was determined that geology likely did play a role in water and otolith trace element signatures in a generalized, less distinguishable sense, but additional research is required to determine geology's role in more specific or quantitative sense (both surficial and base geology). It was also found possible to discern between individual waterbodies and groups of waterbodies pooled into impounded and non-impounded types using otolith trace element bulk averages. However, the effect of impoundment may also be confounded by other factors such as downstream accumulation and water flow connectivity. Trace element ranges in near-surface water were determined between the waterbodies tested, although no significant correlations between otolith and water trace element signatures were made, likely due to the averaging of both water (averaged measures taken across multiple years and seasons) and otolith (multiple annuli averaged) data. Lastly, walleye and lake whitefish trace element otolith signatures were successfully differentiated between each other across the separate CAMP waterbodies, likely due to differing based on habitat, physiology, trophic level, migratory distance/movement prevalence among other factors.

3.4.2. Impoundment or Non-Impoundment Influences on Water and Otolith

Walleye and lake whitefish otolith signatures were likely differentiated between impounded and non-impounded waterbodies by more than just the presence or absence of impoundment. Waterbody connectivity, and accumulation of material via flow may have also contributed (among other factors). The latter could be best explained through the comparison of ASS and SPLT Lakes. Both are in near proximity to each other, with ASS flowing into SPLT, and having similar geologies; with the difference being impoundment status and SPLT receiving inflows from the Upper Nelson and Burntwood Rivers. Based on univariate ANOVA results, for water, SPLT had greater Na, Mg, Ba and Sr concentrations than ASS, while for the otolith concentrations (walleye or lake whitefish), SPLT had greater Ba and Sr signatures than ASS only. Since similar in geology and near in proximity, the possible explanation for the difference could be the presence of impoundments adjacent to SPLT or that SPLT is acquiring water containing higher concentrations of trace elements from the Burntwood River and Upper Nelson River discharge (based on measures from Cross Lake). Eiriksdottir et al., (2017, 2015) found that less soluble trace elements like Mn (and to a lesser degree Ba) increased in water flux due to impoundment which caused saturation state changes in water, leading to changes in chemical weathering, and the dissolution of less soluble matter within the impoundment which could explain the increase of Ba in water within impounded over non-impounded waterbodies. The latter being further supported by observations made by Gibbs (1970) who found that other than atmospheric precipitation and rock dominance, that the evaporation-crystallization processes influence water chemistry. The increased accumulation of coarse grained

sediment within impoundments may also act as a source of less soluble trace elements as well to be released by the impoundment-influenced environment (Eiriksdottir et al., 2017). However, the latter does not explain the observed increase in Sr in the current study. The elevated levels of Sr in impounded waterbodies could perhaps be explained by the accumulation of weathered, or dissolved material making its way from upstream to downstream locations and accumulating, such as in the case of Eiriksdottir et al., (2017) where an increase in discharge due to a diversion from one waterbody rich in dissolved and suspended sediments into another waterbody led to increased detections of trace elements. Although in the latter example Sr was diluted because the diverted water was lower in Sr than what the original water source contained (Eiriksdottir et al., 2017), the opposite was true for the water coming from Cross Lake along the Upper Nelson, which was much higher in water trace element concentration that that which was flowing down the Burntwood River from Threepoint Lake. The effect of impoundment on increasing Ba and Sr in fish otolith with downstream position was also not observed in a study done on the Missouri River by Radigan et al., (2018). They found that for various freshwater fish species, otolith trace element variability was associated with habitats within or around the impoundments (Radigan et al., 2018). Although, based on the current study's correlation tests of flow versus water or otolith trace element content, flow may not be associated with observed trends (but additional testing is required).

Specifically, for lake whitefish it is proposed that stream connectivity/downstream influence, on top of impoundment may be causing differences in detected trace element levels. An increase in the number of trace elements incorporated into the multivariate

models also increased differentiation between impounded or non-impounded waterbodies or impoundment status indicating how each trace element differs between waterbodies. From the multivariate results, the two furthest downstream impounded waterbodies (SPLT and LNR) were nearer than the two upstream waterbodies (SIL4 and 3PT) to Ba and Sr on the RDA biplots. This ordering and proximity to the trace elements indicate that greater otolith concentrations of Ba and Sr may occur for waterbodies further downstream (which would agree with the nearly significant correlation of Sr between otolith and water, and upstream flow correlation results used as a proxy for residence time for Sr) e.g., the clustering of the impounded waterbodies versus the diffuse nature of the non-impounded waterbodies caused by impounded waterbodies being connected to the same drainage basin(s) thus perhaps sharing water chemistry, flow and suspended particulate (Eiriksdottir et al., 2017). Comparatively, when Ba and Sr alone were used as the environmental factors in the RDA2, the impounded waterbodies were more diffuse, and could again be placed in order of flow from the negative to the positive zones of the RDA2 axis. However, based on univariate statistics the separate impounded waterbodies were not significantly different from each other for both Sr and Ba otolith measures. Meaning that the downstream position (and the accumulation of weathered or suspended material) may not be causing the impoundment signature, and instead the impoundments themselves may be causing the unique signature depending on which tests results are being followed. In a study on the Missouri River, walleye Ba and Sr trace element levels in tributaries and points along the impounded Missouri river were measured (Carlson et al., 2017). Based on the k-sample nearest neighbour discriminant analysis, separate points along the impounded

water system could be successfully differentiated and significant correlations between otolith and water were established (Carlson et al., 2017). It was observed that walleye otolith Ba and Sr measures were lower in the impounded Missouri River than upstream tributary rivers (Carlson et al., 2017). This may indicate that either other tributaries are diluting Ba and Sr concentrations within the impounded Missouri River, or that downstream accumulation of material is not taking place in (Carlson et al., 2017), putting into question the effect of impoundment.

Compared to lake whitefish in the current study, walleye multivariate otolith signatures results did not indicate trends based on flow connectivity for the impounded waterbodies and the separation between impounded and non-impounded waterbodies was less apparent. Although, Walleye pooled impounded/non-impounded test results may also imply a unique chemical signature difference (although less so than lake whitefish) between impoundment types. Other factors beyond impoundment are effecting both species signatures, although walleye likely more so.

When focusing on the water chemistry of the CAMP waterbodies, none of the selected CAMP waterbodies in this study had significant known anthropogenic sources of input, although interconnected waterbodies (e.g., those impounded) were likely influenced by indirect anthropogenic sources (e.g., Split Lake and Lower Nelson River receiving inputs from the Lake Winnipeg and its associated tributaries e.g, Saskatchewan, Winnipeg and Red Rivers). One of the reasons for the nutrient rich waters of Lake Winnipeg is due to contributions from the Red River which has experienced increased flood frequency, runoff occurrences and inputs into Lake Winnipeg (Schindler et al., 2012). A method of verifying this was conducted through the

comparison of Cross Lake trace element values with that of Split Lake and the Lower Nelson River sites. Cross Lake is another annually sampled CAMP impounded waterbody which is located downstream of Lake Winnipeg, and upstream of the junction of the Nelson River with the Burntwood River at Split Lake. For this comparison, it was assumed that Cross Lakes trace element water concentrations are associated with inflow from lake Winnipeg and beyond due to a downstream waterflow accumulation effect of suspended/dissolved trace elements. For Cross Lake, average water concentrations between the years of 2008-2011 were Na= 16.27 ± 2.19, Mg= 11.33 ± 1.02, Mn= 0.0138 ± 0.0031, Ba= 0.0335 ± 0.0023, and Sr= 0.0993 ± 0.0067 mg/L (CAMP, 2014). Compared to water trace element averages, SPLT and LNR had nearly identical concentrations (within error as standard deviation). This likely indicated that waterflow from Cross Lake may have influenced the trace element water concentrations of SPLT and LNR, and demonstrate the effect water flow connectivity has on water trace element concentrations. Since the average concentrations from Cross Lake to SPLT and LNR changed little even though SPLT and LNR are receiving flow from both Burntwood and the Upper Nelson may also indicate that any strong dilution effect (from Burntwood River discharge) is not taking place or that greater discharge into SPLT comes from the Upper Nelson River, associated to sediment load from Lake Winnipeg (Jones & Armstrong, 2001). The effect of combining separate flows of water with differing chemical composition and discharge has been demonstrated in Eiriksdottir et al., (2017) in which impoundments decreased Na, Mg, Mn, Ba, DOC, TDS and TSS (slight increase in Sr downstream perhaps due to dissolution reactions) flux downstream of the impoundment, whereas water diverted from the reservoir to another river (which

increased discharge) saw an increase in flux as runoff for Na, Mg, Mn, Ba, SIM, DOC, TDS and TSS (but decrease in Sr likely due to diverted water having lower Sr concentration). Additionally concerning the effect of impoundment, SPLT and LNR did not vary greatly in water concentrations indicating that the three impoundments separating LNR and SPLT may not be limiting near-surface trace elements moving downstream which are being detected. Eiriksdottir et al., (2017) observed that impoundments with decreased water discharge had decreased fluxes of Na, Mn, Ba, TDS and TSS (likely indicating upstream collection within the reservoir), Sr flux slightly increased downstream, and Mg was relatively unchanged. Also, Eiriksdottir et al., (2017) noted the trapping of coarser grained suspended material. In the current study, water trace element concentrations typically increased downstream of successive impounded waterbodies, with the amount being held back by impoundment undetermined since trace element concentrations were found to increase further downstream. This observation of water trace element retention may be confounded by the added discharge into SPLT and LNR.

3.4.3. Geology

The ability to discern the overall contribution made by base geology was not possible with available information due to the low resolution to which each waterbodies geology was available e.g., rock type was not a strongly constraining variable in the pRDAs. Variation in the types of base and surficial geology likely play a role as suggested in Carlson et al., (2017). Due to the observation of relatively stable trace element water concentrations through time (2008-2014) in the current study, geological

stability could be considered the reason since geology is one of the three main mechanisms controlling water chemistry (Gibbs, 1970). Comparisons between primarily sedimentary based waterbodies with those of metamorphic/igneous based geology demonstrated the effect of geology the best as well as a few other select cases. Surficial geology trends were not tested, but suggested for future studies.

When considering the effect of geology for the separate trace elements within the literature, Na was been found to be derived primarily from catchment rock dissolution, and found to elevate because of the latter within impoundments (Eiriksdottir et al., 2017). Elevated Na was also observed when comparing pooled impounded versus non-impounded waterbodies (e.g., SPLT vs. ASS) although confounded with flow waterbody connectivity/accumulation in the current study as stated previously. For Mg, the effect of base geology was best demonstrated in the comparison of CORM, SPLT and LNR. CORM is the only non-impounded waterbody with base geology of primarily sedimentary rock which contains soluble, Mg-rich dolomite (Fairchild et al., 2000; Lamar and Shrode, 1953) and was likely the reason why CORM had the greatest average Mg water concentration out of the eight CAMP waterbodies (Fig A2.74). CORM walleye and lake whitefish otoliths were also observed to have the greatest average Mg signatures indicating geological influence overriding bodily regulation suggested by Campana, (1999). Referring to impounded waterbodies, LNR is also situated on dolomite, while SPLT is not, and yet still high concentrations of Mg in water were detected in both sites (second and third highest Mg water concentrations). It was thereby assumed that Mg-rich discharge was contributed from the Upper Nelson River. For Mn water concentration, LFT was found to have the greatest Mn concentration, which was likely

due to LFT's amphibolite content being a potential source of Mn. In a study done concerning soil amelioration, Jakl et al., (2014) used an amphibolite application which increased detected concentrations of Mn in soil. Additionally, more favorable dissolution and runoff events may have also attributed to the observed Mn in LFT (Eiriksdottir et al., 2015). LFT's water Mn variability was also noted as being relatively higher that the other waterbodies further indicating that available Mn in aquatic environments is unstable (Wetzel, 1983). For Ba concentrations in water, since CORM is not impounded and intersected by a major river system yet still contains a higher concentration of Ba than many of the non-impounded waterbodies it is assumed that CORM may contain mineral deposits rich in soluble Ba, leading to the greater detected Ba levels. Ba in groundwater originates from both igneous and sedimentary rocks through the dissolution of minerals that are typically of lower solubility (e.g., Ba rich feldspars, plagioclases, mica, apatite, baryte, Ba-aragonite group minerals, and rare earth minerals) (Mokrik et al., 2009). Seasonal Ba/Ca differences may be associated with land runoff and diffusion e.g. precipitation events (Coffey et al., 1997; Eiriksdottir et al., 2015) and is thereby associated to suspended sediments and clays (Li and Chan, 1979) although not strongly supported in the current study. Na, Mg and Sr are all relatively soluble and not as influenced by dissolution and runoff events (Eiriksdottir et al., 2015), making geological origin challenging to assign.

3.4.4. The Role of Water, Sediment, Diet and Ecology on Otolith Trace Element Uptake

3.4.4.1. Water

Trace element uptake within the otolith can be effected by more than just geology, impoundment and flow. Many factors both internal and external to fish which house the otoliths may also influence uptake as observed by the lack of significant (although potentially present) correlations between water and otolith signatures. Within the literature, especially for freshwater studies, positive correlations have frequently been established for trace elements Mg, Mn, Ba and Sr. For example, in a laser ablation based study conducted on westslope cutthroat trout (*Oncorhyncus clarki lewisi*) from the Coeur d'alene River, Idaho, a significant positive correlation between water and otolith Mg, Ba and Sr (Mn and Na were not measured) were established, likely due to sufficient water chemistry variation within the study system (Mg: R^2= 0.39, p= 0.0003, Ba: R^2= 0.71, p= 0.0001, Sr: R^2= 0.96, p= 0.0001) (Wells et al., 2003). Also, in studies conducted on arctic grayling (*T. arcticus*) and slimy sculpin (*C. cognatus*) from the Upper Peace River in the Williston reservoir tributaries, significant correlations were made such as in Clarke et al., (2007) where arctic grayling otoliths were measured in which Mn, Ba, and Sr (Na was not measured) correlated significantly for both species while Mg held no correlation between water and otolith (Mn: R^2= 0.11, $P < 0.05$, Ba: R^2= 0.76, $P < 0.001$, Sr: R^2= 0.81, $P < 0.001$). Additionally, from Clarke et al., (2015), grayling and sculpin otoliths were measured in which Ba and Sr correlated significantly while Mn did for sculpin only, and Mg held no remote relation between otolith and water for either species (Na was not measured) (*T. arcticus*: Ba: R^2= 0.934, $F_{1,5}$= 407, $P < 0.001$, Sr: R^2= 0.909, $F_{1,5}$= 113, $P < 0.001$, Mn: R^2= 0.152, $F_{1,5}$= 2.64, P= 0.17; *C.*

cognatus: Ba: R^2= 0.974, $F_{1,5}$= 1160, P < 0.001, Sr: R^2= 0.979, $F_{1,5}$= 557; P < 0.001, Mn: R^2= 0.353, $F_{1,5}$= 29.2, P < 0.01). Lastly, a Colorado fish hatchery study using rainbow trout (*O. mykiss*) found Ba and Sr but not Mn to correlate significantly between water and otolith (R^2 and p values not provided) (Gibson-Reinemer et al., 2009). As compared to the current study results, the lack of correlation could have also occurred due to the low sample size (sample size of 4 or 8 depending on if correlations pooled impounded and non-impounded waterbodies or not). Based on the separate impounded and non-impounded correlations, Lake whitefish otolith Sr in impounded waterbodies, and walleye otolith Mg in non-impounded waterbodies may prove to be significantly positively correlated but additional testing is required. Through the PCA and RDA analyses comparing species, a clear separation was observed between species (explaining greater than 50% of the trended variation), even though the fish were caught in the same waterbodies indicating differences in uptake. Differences in water, sediment, diet, and ecology could also be considered external influences most attributable to differences in trace element uptake in fish (Rashed, 2001). Sr and Mn transfer factors for water were found to outweigh sediment or diet for uptake into fish tissue (Rashed, 2001) which may explain why Sr levels correlated significantly between species in the current study, and imply that otolith Sr best reflects the ambient environment. Although as mentioned previously, since no trace element produced a significant correlation with water trace element concentrations in the current study it could not be concluded that walleye and lake whitefish otoliths reflect ambient water trace element concentrations exclusively, and instead associated with additional factors such as water quality, diet, sediment interactions and ecological factors discussed further in this section. Other

factors such as growth (age, mass, and length) have already been corrected for in the current study and are thereby assumed not to have effected uptake and trace element signatures.

Within the literature concerning water quality, many studies focused on the effect temperature and salinity had on trace element concentrations e.g., Mn, Ba and Sr (refer to chapter 1). Although this relationship (or lack therof) seemed species and environment specific, and is highly debated within the literature (refer to chapter 1). In the current study, near-surface conductivity (auto-correlated with TDS) or temperature (auto-correlated with dissolved oxygen) did not correlate with any of the trace element concentrations in water or otolith thereby no perceived effect. When referring to the observed lack of correlation in the current study, Na or Mg otolith signatures lacked correlation likely due to regulation (Campana, 1999) and have even been denoted as unnecessary in the otolith-based discrimination of waterbodies (Zeigler and Whitledge, 2011), although in the current study, classification increased due to the additional signatures. Mn correlations between water and otolith rely on Mn availability within the environment and has been found to be strongly associated with multiple factors such as microbial action (Tebo, 1991), hardness (decreased Mn sensitivity) (e.g., Stubblefield et al., 1997), dissolved oxygen (e.g., Limburg et al., 2015, 2011) or pH (e.g., Moreau et al., 1983), which attribute to redox potential (Sundby et al., 1986). Additional testing (impounded and non-impounded exclusive correlations) and greater sample sizes are required.

3.4.4.2. Sediment

Few pertinent sediment studies were found within the literature which discussed the relationship between sediment and otolith although a study conducted by Forrester, (2005) in southern Californian estuaries found that Ba, Sr and Mn within sediment (Ba: r= 0.1, p= 0.71, Sr: r= 0.12, p= 0.64, Mn: not reported) or water (Ba: r= -0.30, p= 0.25, Sr: r= 0.26, p= 0.32, Mn: not reported) failed to correlate with longjaw mudsucker (*Gillichthys mirabilis*) otolith concentrations. Additionally, in a lake study, Rashed, (2001) determined the transfer factor of sediment to fish tissue to be negligible as compared to water for Nile tilapia (*Tilapia nilotica*). The latter articles indicate that sediment is seldom found to infleunce fish uptake of trace elements, although the accumulation of coarser sediments due to impoundment (Eiriksdottir et al., 2017) may still influence fish uptake, as observed in the impounded water body exclusive correlations with TDS and TSS (near statistiscal significance), and may have led to the unique otolith signatures established in the impounded waterbodies versus non-impounded waterbodies. To the author's knowledge sediment sample collection for trace element analysis is not currently conducted in CAMP.

3.4.4.3. Diet

Fish diet has been considered an important factor regarding otolith trace element uptake within the literature and may be influencing both walleye and lake whitefish in the current study. In a lab study on the estuarine bluefish (*Pomatomus saltatrix*) conducted by Buckel et al., (2004) it was observed that diet effected otolith trace element uptake for Ba and Sr, but not Mg. Bluefish were fed either fish or shrimp-based

diets, with shrimp diets being richer in trace elements (23% for Mg, 250% for Ba and 280% Sr) than the fish diet (Buckel et al., 2004). Those fish fed the shrimp diet were found to have otoliths with greater concentrations of Ba and Sr but not Mg (Na and Mn did not differ between diets or within the otoliths) (Buckel et al., 2004). In a seagrass meadow field study by Sanchez-Jerez et al., (2002), juvenile trumpeter (*Pelates sexlineatus*) otolith and various seagrass dietary components were measured for Mn, Ba and Sr content. It was found that for Mn content in detritus ($r= 0.843$, $p< 0.05$), amphipod ($r= 0.693$ $p< 0.05$), polychaetes ($r= 0.591$, $p< 0.05$), young leaves ($r= 0.957$, $p< 0.001$) and old leaves ($r= 0.63$, $p< 0.05$) correlated significantly with otolith Mn, while Ba correlated with young leaves ($r= 0.963$, $p< 0.001$) only, and Sr failed to correlate with any of the seagrass components (Sanchez-Jerez et al., 2002). Comparatively to Sanchez-Jerez et al., (2002), in a study dealing with blue grenadier (*Macruronus novaezelandiae*) greater otolith Sr:Ca was attributed to summer feeding further indicating a dietary component to Sr (Kalish, 1989). The same was also observed in a field study measuring Ba signatures in a freshwater arctic char (*Salvelinus alpinus*) where otolith Ba oscillatory signatures were attributed to seasonal diet change since the fish remained in an enclosed area limiting other confounding variables (Friedrich and Halden, 2011). In the current study, Ba oscillations were observed for both walleye and lake whitefish and could also be associated with diet. Whereas Mn otolith content in walleye (*S. vitreus*) and northern pike (*Esox lucius*) from Northern and Southern Manitoban, Canada locations also had chemical signatures which varied with dietary factors, related to the background environmental signature (Friedrich and Halden, 2010). However, otolith and water Mn failed to correlate between water and otolith in the

current study, and may thereby not represent background. Seasonality was found to effect abundances of various metal binding proteins and thus metal binding capacity, which then leads to impacts on endolymph and otolith metal incorporation (Kalish, 1991). Within the current study it is difficult to determine how much diet contributed to the various trace element signatures per species although freshwater fish trace element incorporation has been assumed to be primarily through the gills (Campana, 1999), rather than ingestion which is more important for saltwater species (Melancon et al., 2009). Perhaps diet did indeed cause otolith uptake differences, but is also reflective to some degree to the background environmental water signature. For impounded waterbodies, this signature through biota may be influenced by the impoundment itself since noted to change water quality and biotic assemblages (Santucci Jr et al., 2005). Although walleye had a less consistent chemical signature between impounded waterbodies indicating that perhaps trophic feeding level may also play a role in chemical signatures (Stewart and Watkinson, 2004).

3.4.4.4. Ecology

Fish ecology has been found to affect the incorporation of trace elements. From the differential incorporation based on their level of regulation in the fish (Campana, 1999), their pathway of uptake, internal transport and crystallization (Melancon et al., 2009), metabolic rate factor (Hoff and Fuiman, 1993), RNA/DNA ratios, growth, kinetics (Kalish, 1989) or general physiology (Gibson-Reinemer et al., 2009; Hanson and Zdanowicz, 1999) have all been proposed and may be partially responsible for differences in uptake between species. Other than controlling for the effect of growth

(through age, fork length and mass corrections), many of the latter were not covered in the current study, but should be considered in future work.

Referring to development stage effects of otolith uptake, as observed in the multivariate results, developmental stage was not considered a strong constraining variable in otolith trace element content, which further supports claims made by Clarke et al., (2015) in which fish movement (or lack thereof) rather than ontogeny causes changes in the detection of trace elements though a fish's life. Thereby the inclusion both juvenile and adult fish in the current study was assumed not to effect results (especially due to the latter mentioned corrections).

3.4.5. Fish Otoliths and the Ability to Discern Between Environments

To the author's knowledge, forming correlations off bulk averaged otolith data taken via LA-ICP-MS and comparing to water over a multi-year timespan (four to six years or 100's of micrometers of otolith) has not been attempted within the literature and it is suggested that this integrated concentration approach may be effective when measuring over pre-determined time periods. Although, the averaging of data did fail to establish a correlation between water and otolith unlike the studies described below. Lab or field studies establishing said correlations typically used otolith edge segments dealt with shorter test durations (weeks/months) and were based on single water concentration measures to determine recent rather than historic exposure trends (e.g., Clarke et al., 2015, 2007, Elsdon and Gillanders, 2004, 2002; Ranaldi and Gagnon, 2008). For example, Clarke et al., (2007) analyzed the outer 10 μm of the otolith for comparison with water samples taken at the same time. Similarly for Clarke et al.,

(2015), the outer 25-32 μm of slimy sculpin otoliths (*C. cognatus*) were used. Although one study of note did compare multiple years of otolith and water chemistry in a study on the Missouri River using walleye (Carlson et al., 2017). In the latter study, walleye otolith Ba and Sr signatures were used to determine the movement and entrainment of walleye by impoundments along the Missouri River through comparison with water chemistry across multiple years (Carlson et al., 2017). Water to otolith correlations were determined from walleye otolith edge measures and corresponding water measures (removing the effect of walleye movement from confounding correlation results), while individual walleye movement was determined by conducting LA-ICP-MS spot measures across the separate annuli of adult fish to track movement by comparison with water chemistry across the Missouri River, and its reservoirs and tributaries (Carlson et al., 2017). Significant correlations between water and otolith for Ba (R^2= 0.40, p< 0.01) and Sr (R^2= 0.71, p< 0.01) were established, and walleye movement was determined to be entrained due to impoundment, and assisted by flooding events (Carlson et al., 2017). Accurate classification was made possible via K-sample nearest neighbor discriminant analysis of natal otolith edge measures (Carlson et al., 2017).

In the current study, walleye and lake whitefish otoliths could discern between the eight selected CAMP waterbodies and pooled groupings of impounded and non-impounded environments to varying degrees with Ba and Sr trace element signatures holding the greatest weight in classification. When considering per-waterbody KNN results, most of the misclassification occurred within impounded or non-impounded groupings exclusively indicating a potential impoundment signature (one of the main exceptions being South Indian Lake). For the waterbody KNN tests, lake whitefish could

be correctly classified to a greater degree than walleye, with the combinations of trace element used causing variation in classification accuracy. For example, lake whitefish classification using Na, Mg Ba and Sr was greater than when all five trace elements were used, or when Ba and Sr were used alone. Also for impoundment KNNs, lake whitefish were more correctly classified than walleye, with accuracy increasing with the number of trace elements included in the KNN tests. Of the three main KNN test types (waterbody groups, impoundment groups, species groups), waterbody classifications were least correct, while species classifications were most correct (as also indicated from PCA and RDA results). It should also be noted that certain waterbodies were found to be more accurately classified than other CAMP waterbodies. For walleye, Assean and Cormorant Lakes were most accurately classified, while Gauer was least making non-impounded waterbody classification more variable. For lake whitefish, depending on the number of elements included in the KNN, the more accurately and least accurately classified waterbody differed. South Indian, Assean and Cormorant Lakes were better classified with the inclusion of more trace elements, while Gauer Lake performed better when only Ba and Sr were included. Classification accuracy may be associated with things such as background geology, source inputs from connecting tributaries, and other factors un-identified. However, the lack of correlation between water and otolith puts into question classification results as well. Classification accuracy could have also been attributed to the species used in the current study, and their ability to classify based on their environment. In Radigan et al., (2018), separate fish species and habitats led to differences in classification accuracy due to association with geology and hydrology. Thereby, poor classification could be the result of selecting the wrong

biomonitor species for any one specific location, or a location which is highly variable and should be considered in future studies (Radigan et al., 2018).

3.4.6. CAMP Sampling Issues

3.4.6.1. Resolution and Timing

There are multiple sampling-based reasons for why significant correlations between water and otolith trace element concentrations did not occur, and why classification of impounded and non-impounded waterbodies varied in efficacy. The current study issues may be attributed to sampling resolution, the variation in the timing of sampling, and the differences in water and fish sampling locations within each waterbody. When comparing the current study to two other freshwater otolith studies done, some key differences were observed in sampling methods. One study was conducted in the Upper Peace River (BC, CAN.) by Clarke et al., (2015, 2007) in which correlations between water and otolith for both a pelagic (*T. arcticus*) and benthic (*C. cognatus*) species for trace elements Ba, Sr, and Mn were determined. The other study was by Carlson et al., (2017) on walleye in the Missouri River as mentioned in the previous section.

Beginning with resolution, the size of the study area in the current study was much greater and lower in resolution sampling-wise than Clarke et al., (2015, 2007) and Carlson et al., (2017). In the CAMP study, multiple lakes of various sizes and volume, from multiple separate drainage systems were sampled from and compared, whereas in Clarke et al., (2015, 2007) and Carlson et al., (2017) single river systems containing reservoir(s), rivers and tributaries were measured which represented a smaller area.

Clarke et al., (2015, 2007) and Carlson et al., (2017) additionally had greater numbers of water grab samples per unit area in their comparison studies, allowing for more variation to be accounted for in any one area. This likely made the grab sampling techniques employed in Clarke et al., (2015, 2007) and Carlson et al., (2017) more effective at collecting representative water samples (thereby higher resoltuion) than the current study which may have missed finer scale variation in each water system. Referring to the timing of water samples, CAMP water sampling occurred in each of the four seasons rather than specific seasons due to it being considered logistically unreasonable to complete all necessary sampling in a single season e.g., spring, summer. Comparatively, Carlson et al., (2017) water samples were collected at relatively similar times for comparison on a per-season and per-year basis. Efforts were made in CAMP to sample separate waterbodies exclusively at around the same time of the year that it was sampled previously, although sometimes it was not found possible. This made the exact comparison of water chemistry between waterbodies impossible based on water since water collection dates differed in many cases. Comparatively, fish sampling was conducted throughout the open water period only. CAMP fish sampling consisted of the use of 6-12 gill net sets (lake size dependent), distributed over all available habitat types in the CAMP waterbodies, rather than sampling at the center of the waterbody like most of the water grab samples which may have led to correlation issues due to likely differences in water chemistry within the center of the lake versus the fish sampling areas (and being unable to account for fish movement). In comparison, water and otolith sampling for both Clarke et al., (2015, 2007) and Carlson et al., (2017) were conducted within rivers, tributaries and reservoirs, and both were collected within

more specific time-frames and areas which likely led to greater likelihood of achieving correlations through specificity of time and area selection for water and otolith.

The overall intent of the CAMP sampling style was to be consistent temporally and spatially for each component (e.g., water and fish sampling) year to year within each waterbody exclusively, but not temporally or spatially comparable between CAMP waterbodies. Clarke et al., (2015) conducted tests to determine the effect of temporal variability of water trace element concentrations and found that significant variation can exist between sites, over time and with an interaction effect in their tested locations regarding water dissolved trace element signatures of Sr, Ba, Mg, and Mn ($p < 0.001$ for each). The latter placing into question the value of water quality measures taken throughout the CAMP program for comparative purposes between waterbodies. The timing of the collection of CAMP samples may have led to discrepancies or a lack of correlation with the fish otoliths. But, in the current study, averaging multiple years-worth of water and comparing it to otolith linescan measures across entire year ranges was intended to limit error introduced by determining concentration averages on a per-year basis (although much of the variation needed for waterbody classification via otoliths may have been lost by averaging multiple years together). Although, since bulk averages were used for the otolith measures, the use of averaged water data which was taken across the seasons was ideal, since water averages could thereby be interpreted to represent the averaged water chemistry across entire years (except winter to some extent). This allows water and otolith averages to better compare with each other. Comparatively, using age-0 otolith collected from natal locations, and comparing them with water samples taken from the same natal habitat may increase likelihood of

correlations being established (Radigan et al., 2018). Additionally, in the current study if the otoliths collected in each waterbody were separated by where or by what habitat they came from, habitat-specific environmental signatures would be established, leading to greater likelihood of correlation and correct classification (higher resolution) (Radigan et al., 2018).

In summary, based on the comparison with other pertinent studies (e.g., Carlson et al., 2017; Clarke et al., 2015, 2007), the CAMP has some weaknesses when being applied for otolith biomonitoring. Compared to the latter studies, CAMP's study area resolution is low, requiring additional samples, and larger sample sizes to fully account the variation in the separate drainage basins due to the observed variation. Also, the discrepancies with sampling time(s), and location(s) in CAMP or both water and fish may have led to comparison issues. Lastly, variation between waterbodies may have been lost due to the averaging of water and otolith concentrations across years. Taken together, these may have caused classification accuracy and correlation strength in the current study to be less.

3.4.6.2. CAMP Waterbody Variation in Area/Depth

It has been suggested that water quality and the distribution of dissolved or suspended material may vary with depths and that reduced flow (due to impoundment) may cause trace elements to accumulate leading to differences in trace element water chemistry with depth. Trace elements of the < 63 μm sediment fraction and their associated elements can be heterogeneously distributed even though the sediment fraction is homogenously distributed in fluvial water columns (Horowitz, 2008), such as

Mn which likely increases in concentration with depth due to decreases in DO and pH (Eiriksdottir et al., 2017). Coarse suspended sediment can also become trapped behind impoundments (Eiriksdottir et al., 2017) where it may settle. However, in the current study, accumulation with depth was not found to occur based on water quality versus water depth concerning trace elements, TDS and TSS water measures consistently. This was interpreted as being that trace elements, and many of the measured water quality variables vary little in depth within the studied CAMP waterbodies or that insufficient sampling at depth was conducted in the current study (especially at greater depths). The latter of the two assumptions seem most likely since most of the water sampling occurred at the near surface (0.3 m depth), with less consistent sampling done at greater depths.

Additionally, the correlation between water and otolith could have been influenced by the depth at which water samples were taken. Near-surface water samples (0.3 m depth) were used since other samples were less consistently taken on a yearly basis in each of the waterbodies, although both walleye and lake whitefish were thought to reside typically at greater depths as stated previously in Stewart and Watkinson, (2004). Assuming water quality/chemistry varies with depth in each of the selected waterbodies, then the current comparison of water and otolith may not have been matched as effectively as desired. The effects of vertical chemical ranges were brought up briefly in Clarke et al., (2015) although their findings could not be fully explained by said differences. Additional testing using replicated annual sampling (depth, time, and site) is required to clarify if any of the water quality variables or measured trace elements differ significantly with depth in any of the selected CAMP

waterbodies. Although it should be noted that near-surface grab samples have been used in other otolith based water studies with species including walleye and were still able to achieve correlations indicating that perhaps depth was not the issue in the current study (e.g., Carlson et al., 2017; Clarke et al., 2015, 2007; Gibson-Reinemer et al., 2009; Radigan et al., 2018; Shiller, 2003).

3.4.7. The Otolith as a Biomonitoring Tool

Through this study, it was observed that fish and their otoliths vary in their utility as biomonitoring tools, and that to classify waterbodies with confidence requires correlations between water and otolith which can likely be achieved through appropriate sampling, as clarified in the literature. As biomonitors, both walleye and lake whitefish otoliths have the potential to be effective biomonitoring tools within the CAMP program. Both species are present in lakes and rivers (Jennings et al., 1996; Morin et al., 1981), they frequently remain within their original areas (e.g., feeding and spawning) and have selective non-random mating (Casselman et al., 1981; Forney, 1963; Mavros, 1992; Stepien and Faber, 1998) which leads to the identification of spatially separate and unique populations for comparison. Although lake whitefish (Casselman et al., 1981) may be considered a slightly better biomonitor since found to migrate or disperse smaller distances than walleye (Dupont et al., 2007; Ferguson and Derksen, 1971) and residing in a more specific area of the water column than walleye (benthic-benthopelagic zone) (Stewart and Watkinson, 2004). Also, with regards to walleye movement, Carlson et al., (2017) found that walleye were able to move between and become entrained within impoundments throughout their lives in the Missouri River.

This implies that walleye in the current CAMP study may also be migrating up or downstream throughout their lives, causing bulk otolith linescan averages to perhaps be less representative of any one CAMP waterbody site and instead representative of multiple the lakes and tributaries of the Burntwood, and Nelson River systems. The same may also be true for lake whitefish and may help explain why otolith signatures seemed to differ less between impounded waterbodies than water chemistry did. The effect of levels of motility and differences in habitat and its effect on trace element signatures has also been noted in Clarke et al., (2015) (e.g., artic greyling and slimy sculpin otolith signatures, potentially associated to benthic or pelagic characteristics, and degree of movement).

To further support the latter, it was also observed that lake whitefish otolith results were easier to interpret and attribute to various factors such as impoundment, water flow/downstream accumulation, and relate findings to water Ba and Sr trace element signatures. Additionally, through the KNN analysis lake whitefish otoliths classified waterbodies and impoundment status to a greater degree than walleye (although lack of water to otolith correlations places observations into question). As discussed in earlier sections, the otoliths ability to uptake can be influenced by many internal and external factors that can either help or hinder its ability to represent ambient environmental conditions. Thereby the utility of the otolith as a biomonitoring tool depends on how many factors effecting otolith uptake can be identified, and accounted for when interpreting information from the otolith.

3.4.8. Summary Conclusion

Of the initial objectives and hypothesis of the current study, all objectives were addressed, with some of the results in disagreement with the hypotheses. Like the hypothesis for objective one, geology likely did play a role in detected trace element water and otolith concentrations, although there were limitations in the current study when quantifying impact. Unlike the hypothesis for objective two, impoundment was possibly attributed to fish otoliths display of differing trace element signatures (Ba and Sr primarily) but flow connectivity and accumulation may also have confounded with the latter (which could not be fully discerned between in the study). Classification analyses were successful in discerning between waterbodies and impoundment type to varying degrees of success. Unlike the hypothesis for objective three, differences in trace element uptake occurred between walleye and lake whitefish, associated with various physiological, habitat, and other species differences. In this study, the Lake whitefish was deemed a better biomonitor than walleye and was found to take up relatively more Sr, Mg and Na than walleye, but less Ba. Lastly, unlike the hypothesis for the fourth objective, surface water trace element concentrations did not correlate with either species otolith bulk-average otolith concentrations. Although additional testing is required since the lack of correlation may have been attributed to CAMP sampling procedures (e.g., resolution, sampling area, depth, temporality discrepancies, averaging effect).

Chapter 3 Figures

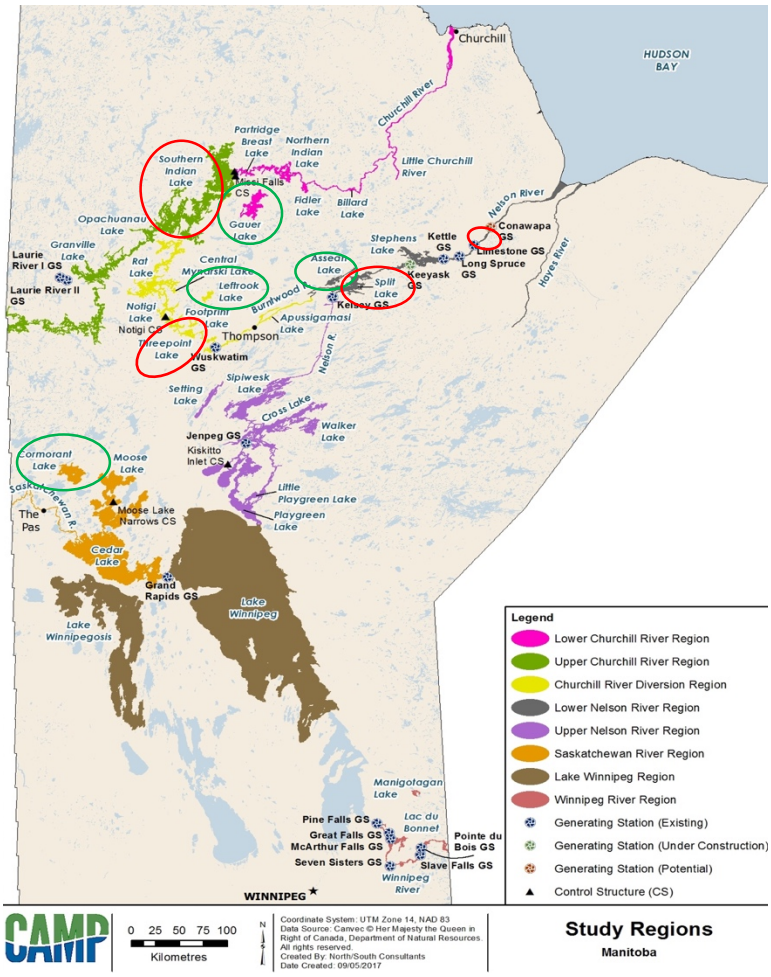

Figure 3. 1. Map of Manitoba with selected CAMP waterbodies and regions. Green circled waterbodies are non-impounded, red circled waterbodies are impounded.

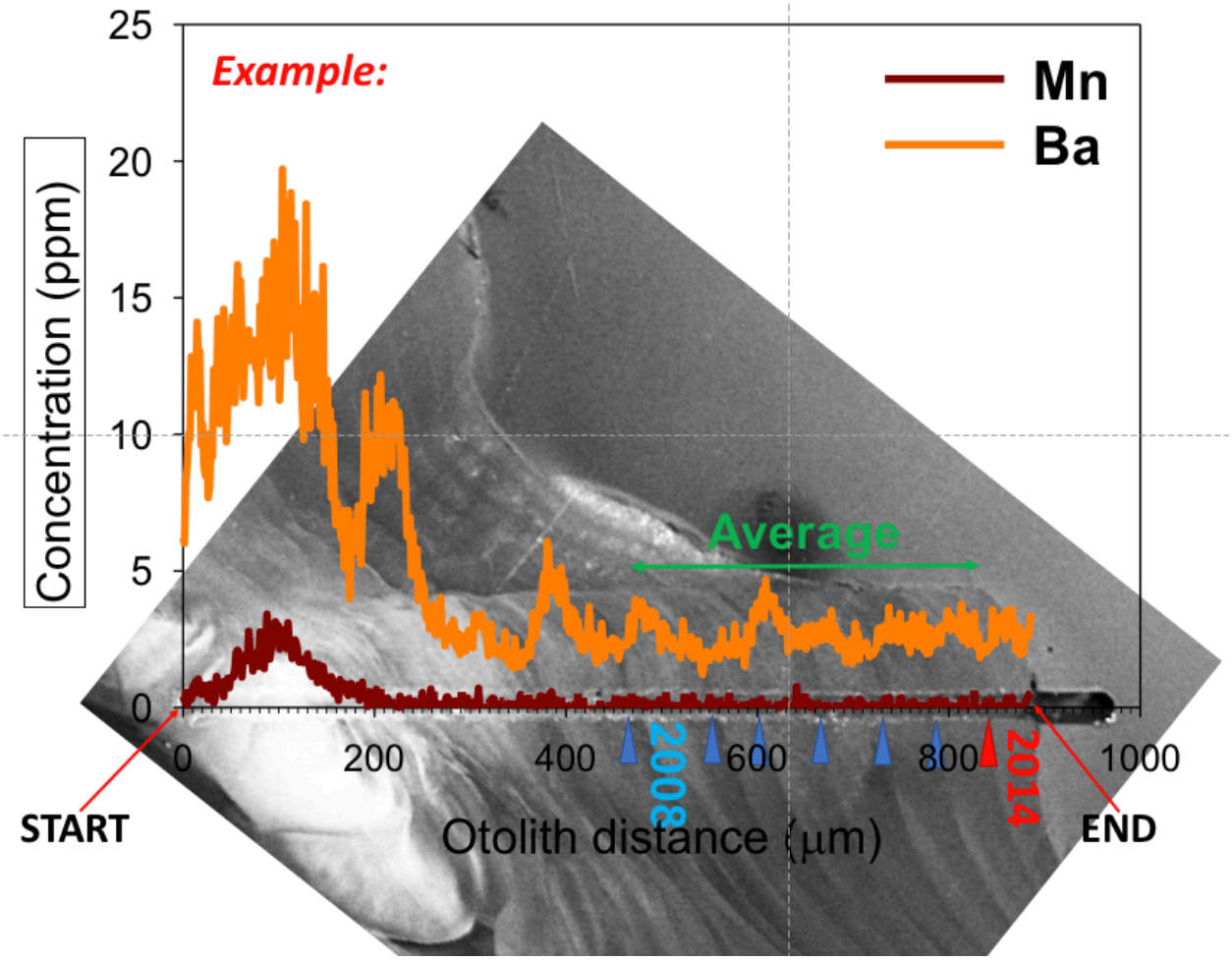

Figure 3. 2. Otolith cross section image with LA-ICP-MS linescan overlay demonstrating the determination of bulk trace element averages.

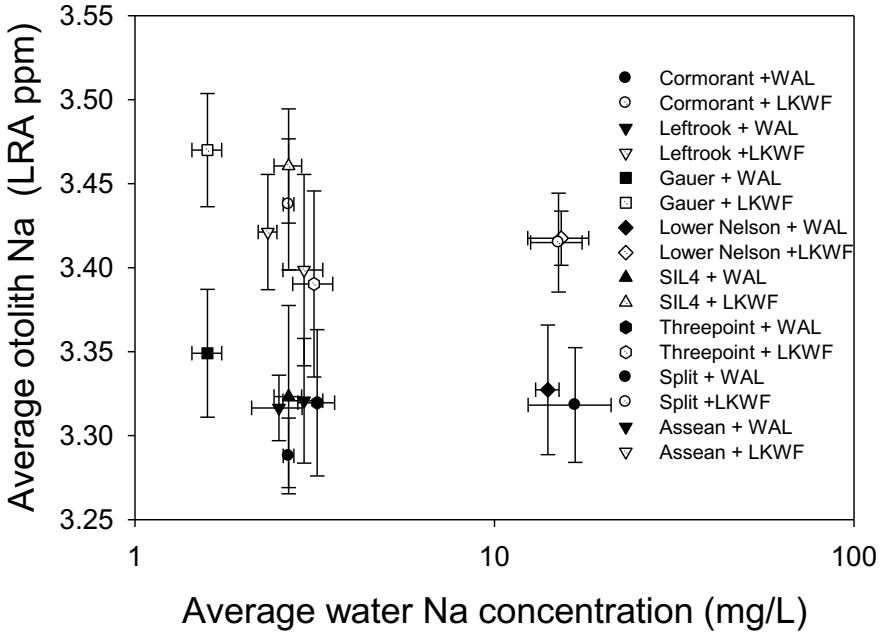

Figure 3. 3. Scatterplot of average water and fish otolith (LRA) Na concentrations. Error bars as standard deviation. WAL = walleye, LKWF= lake whitefish, CORM= Cormorant Lake, LFTRK= Leftrook Lake, GAU= Gauer Lake, LNR= Lower Nelson River, SIL4= South Indian Lake (area 4), 3PT= Threepoint Lake, SPLT= Split Lake, ASS= Assean Lake.

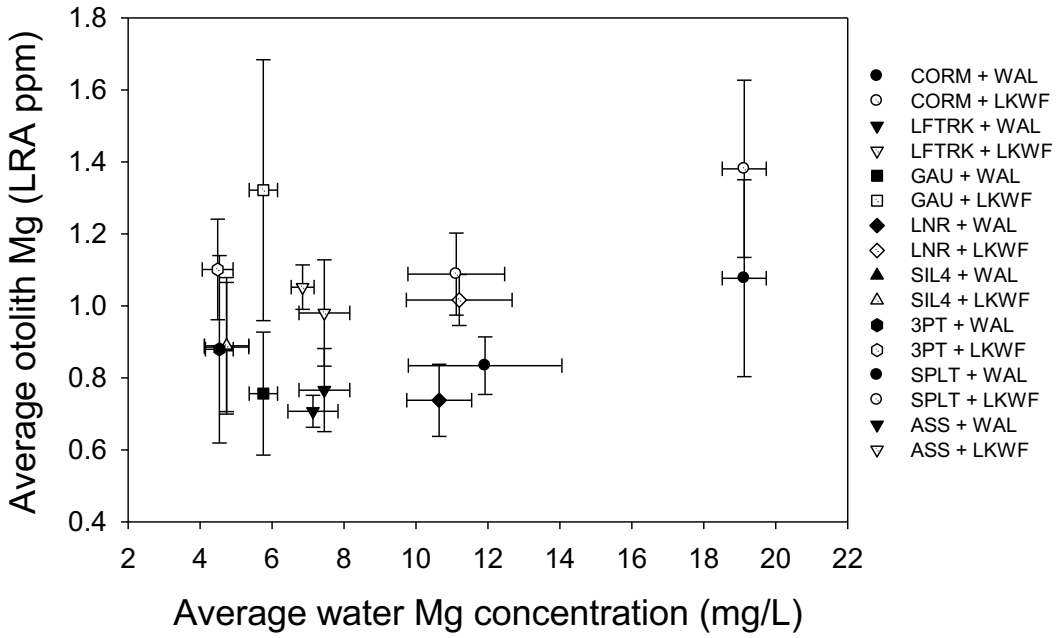

Figure 3. 4. Scatterplot of average water and fish otolith (LRA) Mg concentrations. Error bars as standard deviation. WAL = walleye, LKWF= lake whitefish, CORM= Cormorant Lake, LFTRK= Leftrook Lake, GAU= Gauer Lake, LNR= Lower Nelson River, SIL4= South Indian Lake (area 4), 3PT= Threepoint Lake, SPLT= Split Lake, ASS= Assean Lake.

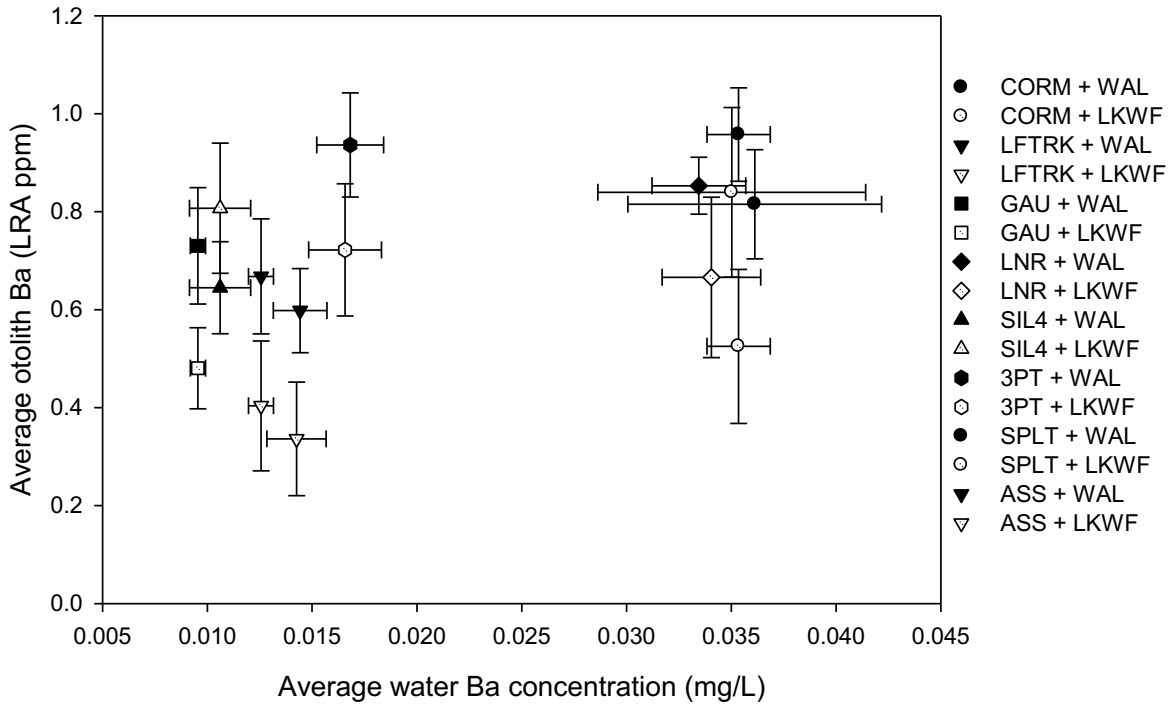

Figure 3. 5. Scatterplot of average water and fish otolith (LRA) Ba concentrations. Error bars as standard deviation. WAL = walleye, LKWF= lake whitefish, CORM= Cormorant Lake, LFTRK= Leftrook Lake, GAU= Gauer Lake, LNR= Lower Nelson River, SIL4= South Indian Lake (area 4), 3PT= Threepoint Lake, SPLT= Split Lake, ASS= Assean Lake.

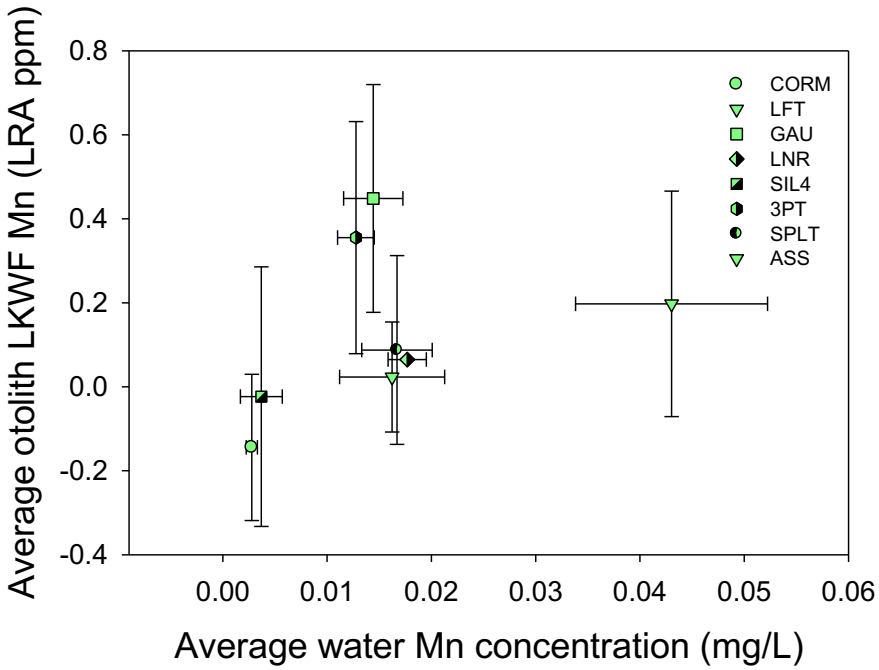

Figure 3. 6. Scatterplot of average water and lake whitefish otolith (LRA) Mn concentrations. Error bars as standard deviation. WAL = walleye, LKWF= lake whitefish, CORM= Cormorant Lake, LFTRK= Leftrook Lake, GAU= Gauer Lake, LNR= Lower Nelson River, SIL4= South Indian Lake (area 4), 3PT= Threepoint Lake, SPLT= Split Lake, ASS= Assean Lake.

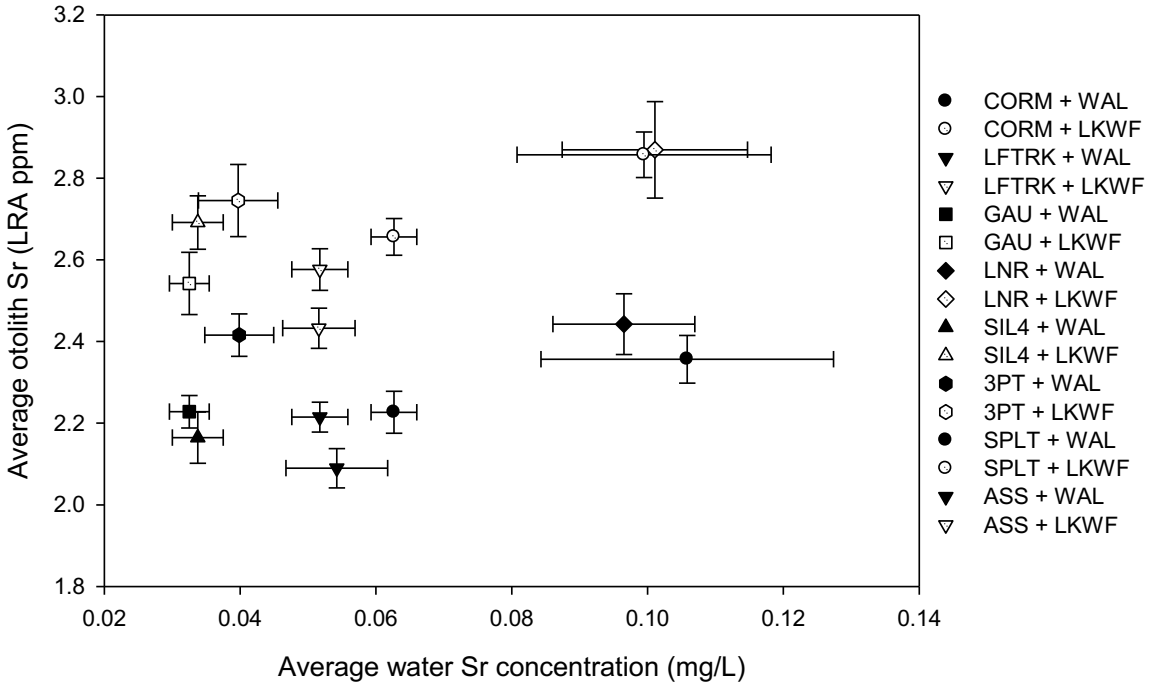

Figure 3. 7. Scatterplot of average water and fish otolith (LRA) Sr concentrations. Error bars as standard deviation. WAL = walleye, LKWF= lake whitefish, CORM= Cormorant Lake, LFTRK= Leftrook Lake, GAU= Gauer Lake, LNR= Lower Nelson River, SIL4= South Indian Lake (area 4), 3PT= Threepoint Lake, SPLT= Split Lake, ASS= Assean Lake.

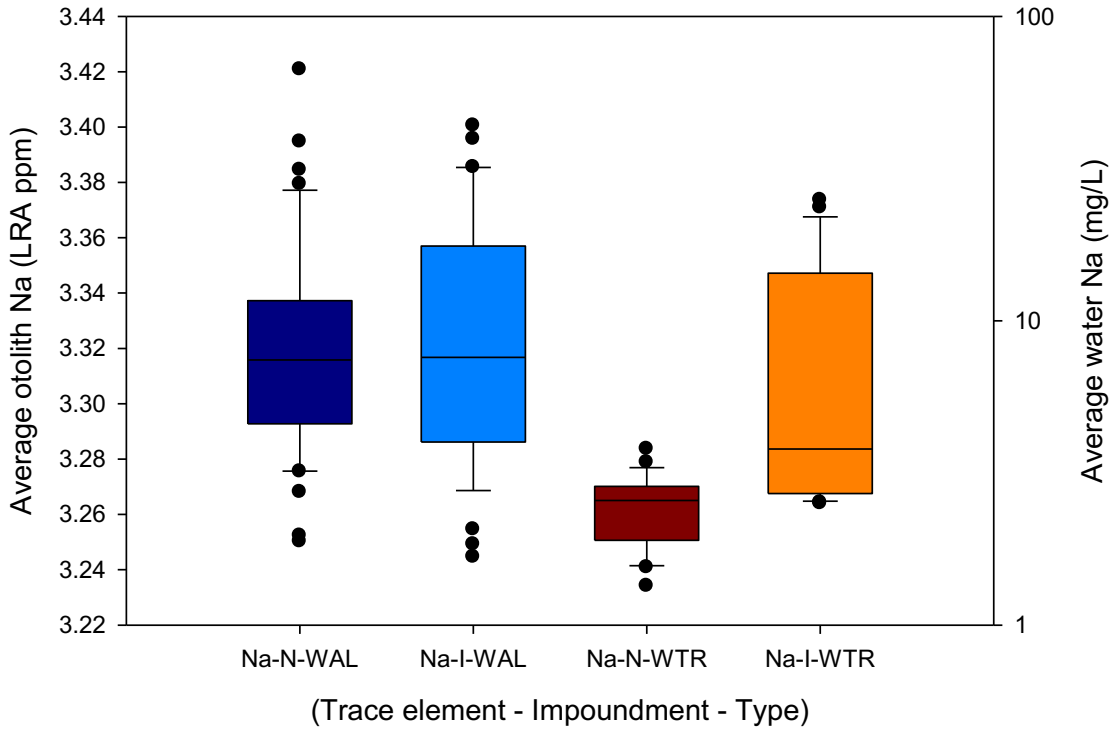

Figure 3. 8. Boxplots of water and walleye otolith Na content (LRA correction) in CAMP waterbodies pooled by impoundment status. N = non-impounded, I = impounded, WAL= walleye, WTR= water concentration.

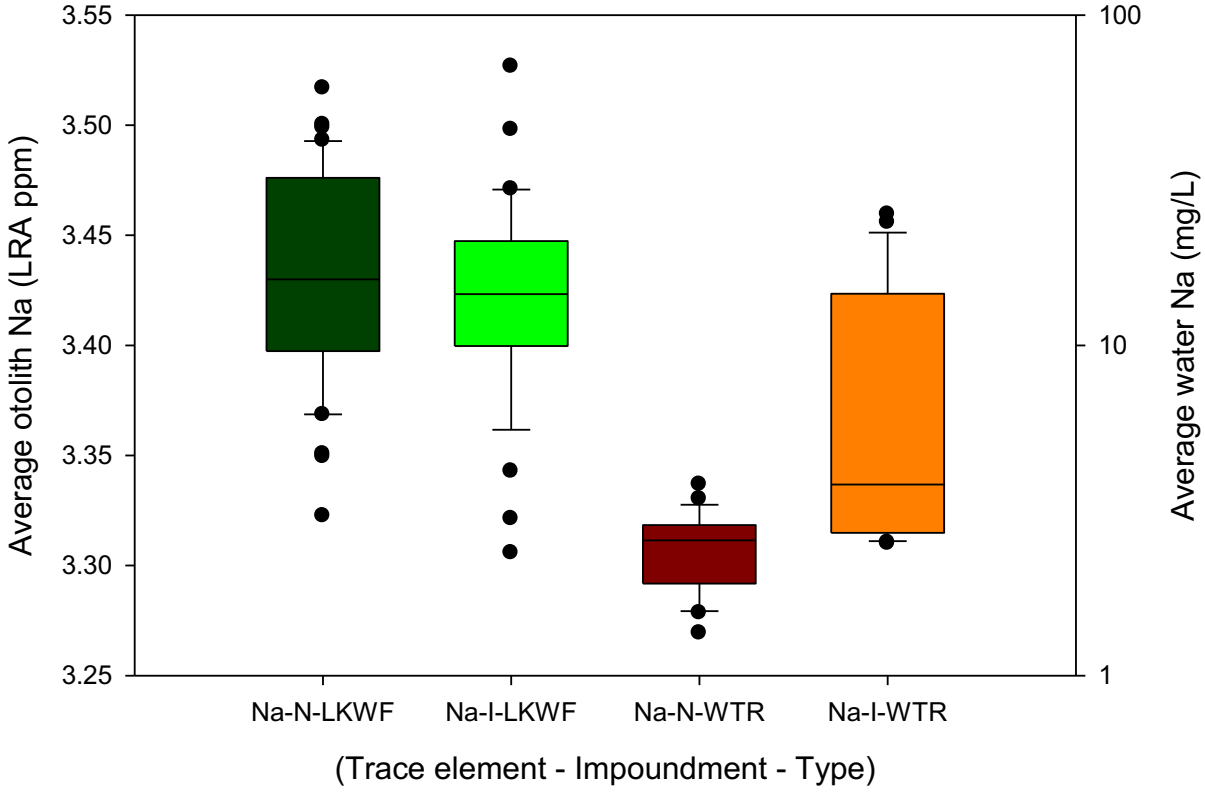

Figure 3. 9. Boxplots of water and lake whitefish otolith Na content (LRA correction) in CAMP waterbodies pooled by impoundment status. N = non-impounded, I = impounded, LKWF= lake whitefish, WTR= water concentration.

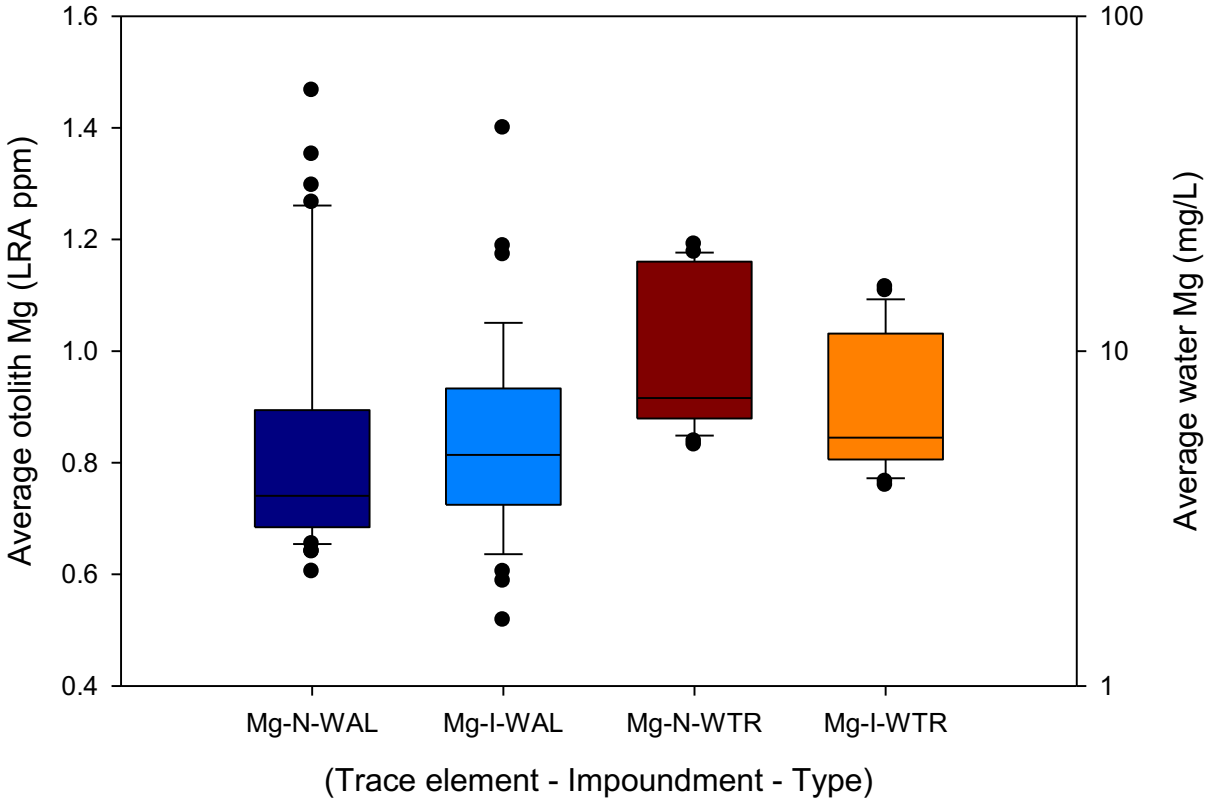

Figure 3. 10. Boxplots of water and walleye otolith Mg content (LRA correction) in CAMP waterbodies pooled by impoundment status. N = non-impounded, I = impounded, WAL= walleye, WTR= water concentration.

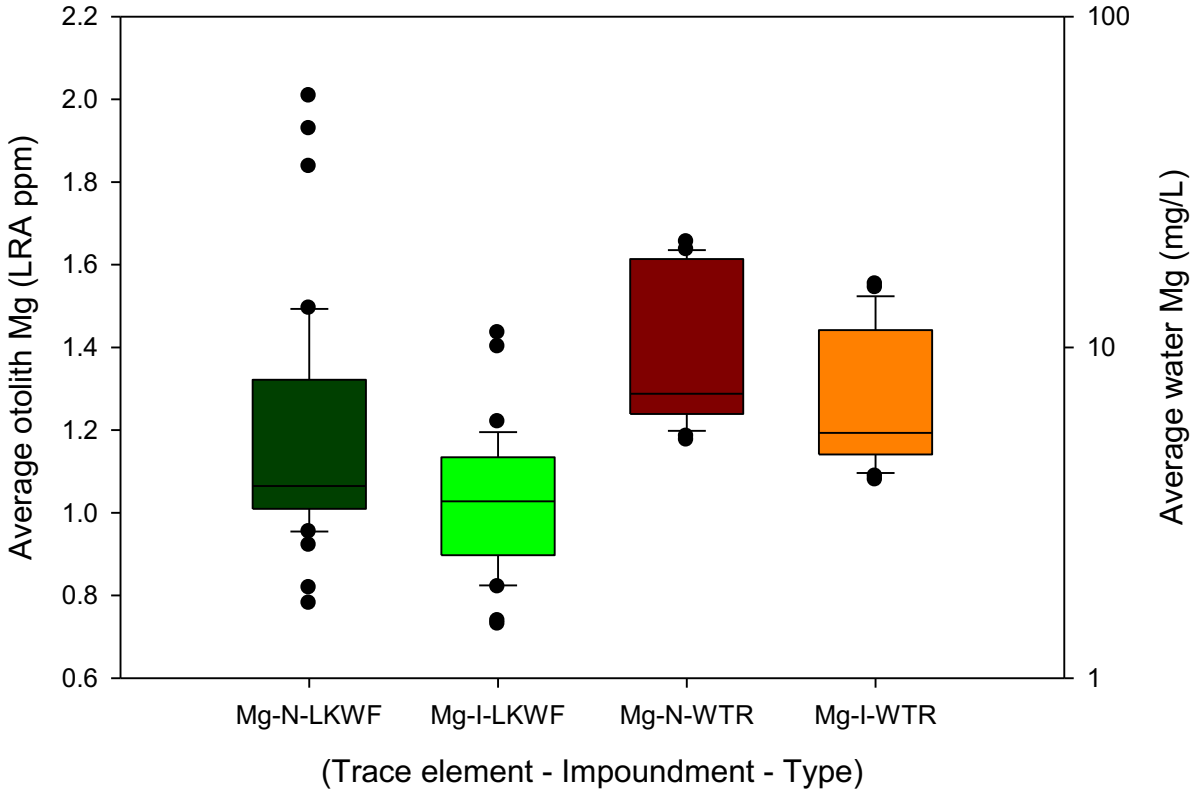

Figure 3. 11. Boxplots of water and lake whitefish otolith Mg content (LRA correction) in CAMP waterbodies pooled by impoundment status. N = non-impounded, I = impounded, LKWF= lake whitefish, WTR= water concentration.

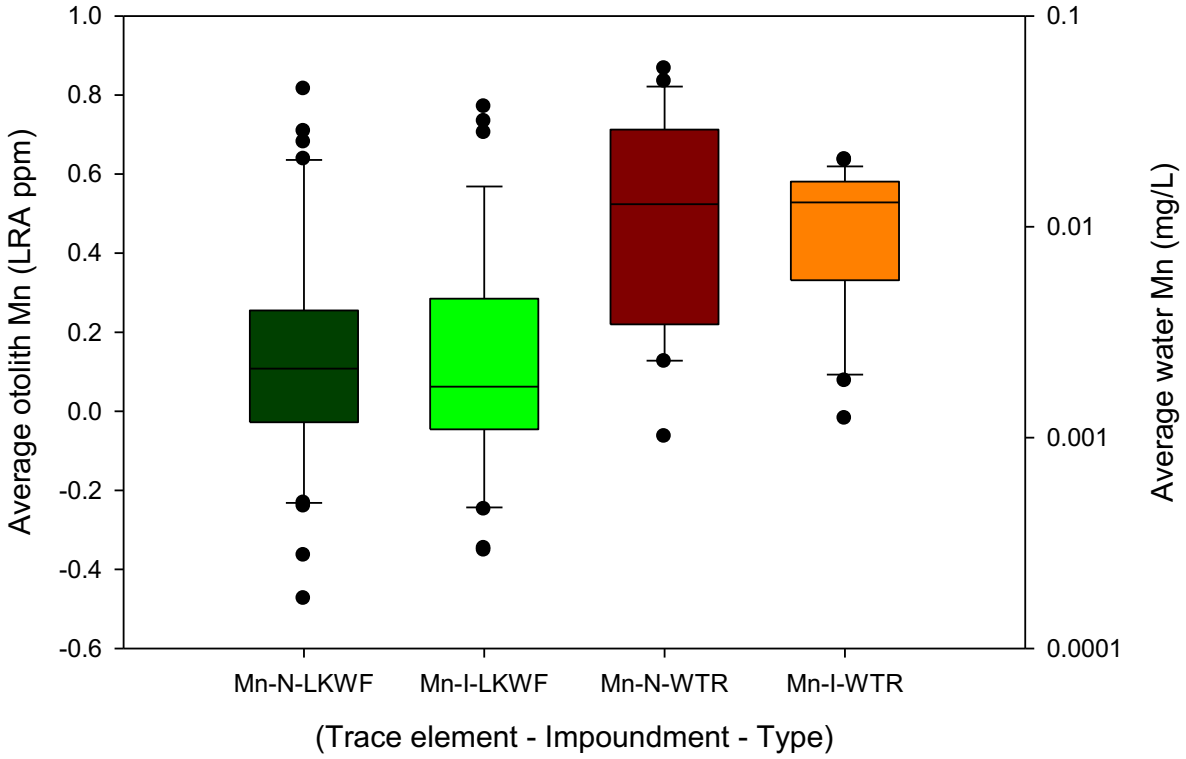

Figure 3. 12. Boxplots of water and walleye otolith Mn content (LRA correction) in CAMP waterbodies pooled by impoundment status. N = non-impounded, I = impounded, LKWF= lake whitefish, WTR= water concentration.

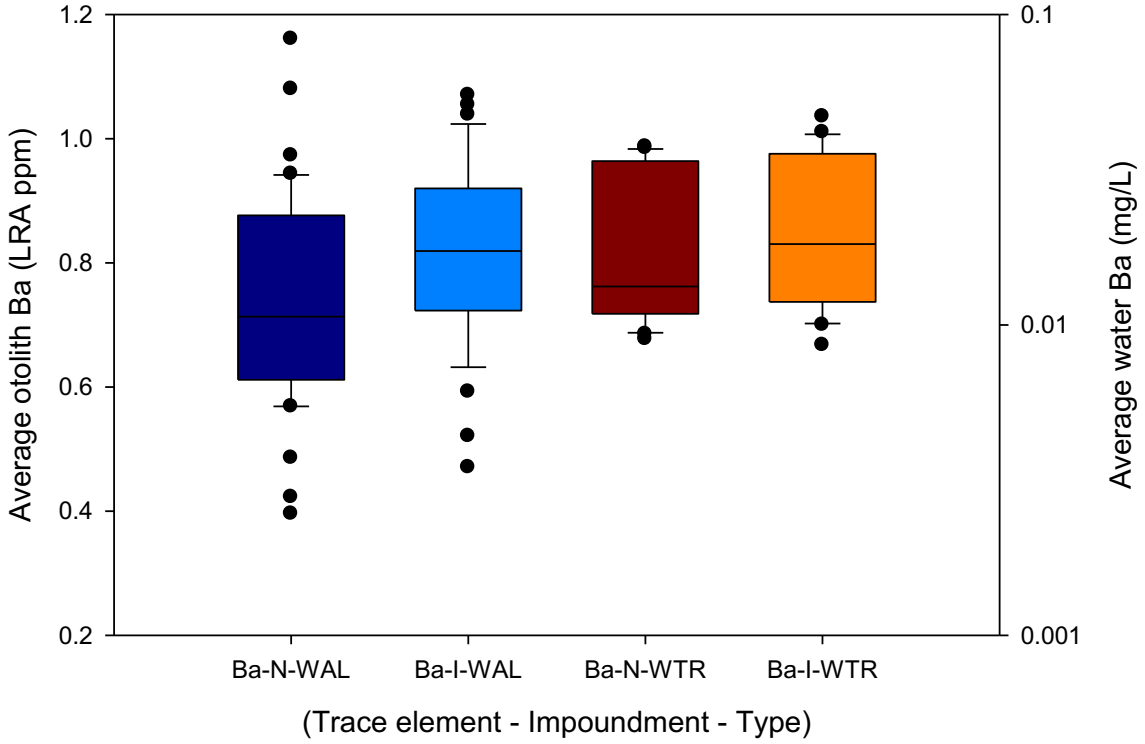

Figure 3. 13. Boxplots of water and walleye otolith Ba content (LRA correction) in CAMP waterbodies pooled by impoundment status. N = non-impounded, I = impounded, WAL= walleye, WTR= water concentration.

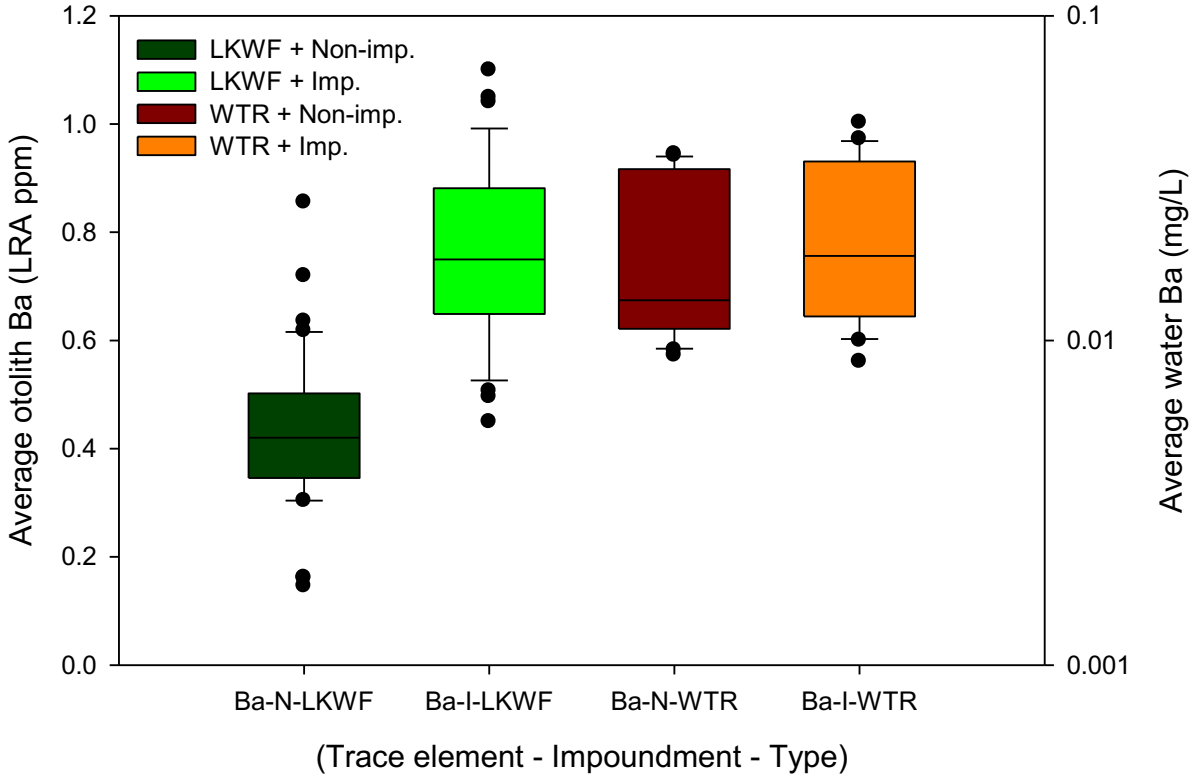

Figure 3. 14. Boxplots of water and walleye otolith Ba content (LRA correction) in CAMP waterbodies pooled by impoundment status. N = non-impounded, I = impounded, LKWF= lake whitefish, WTR= water concentration.

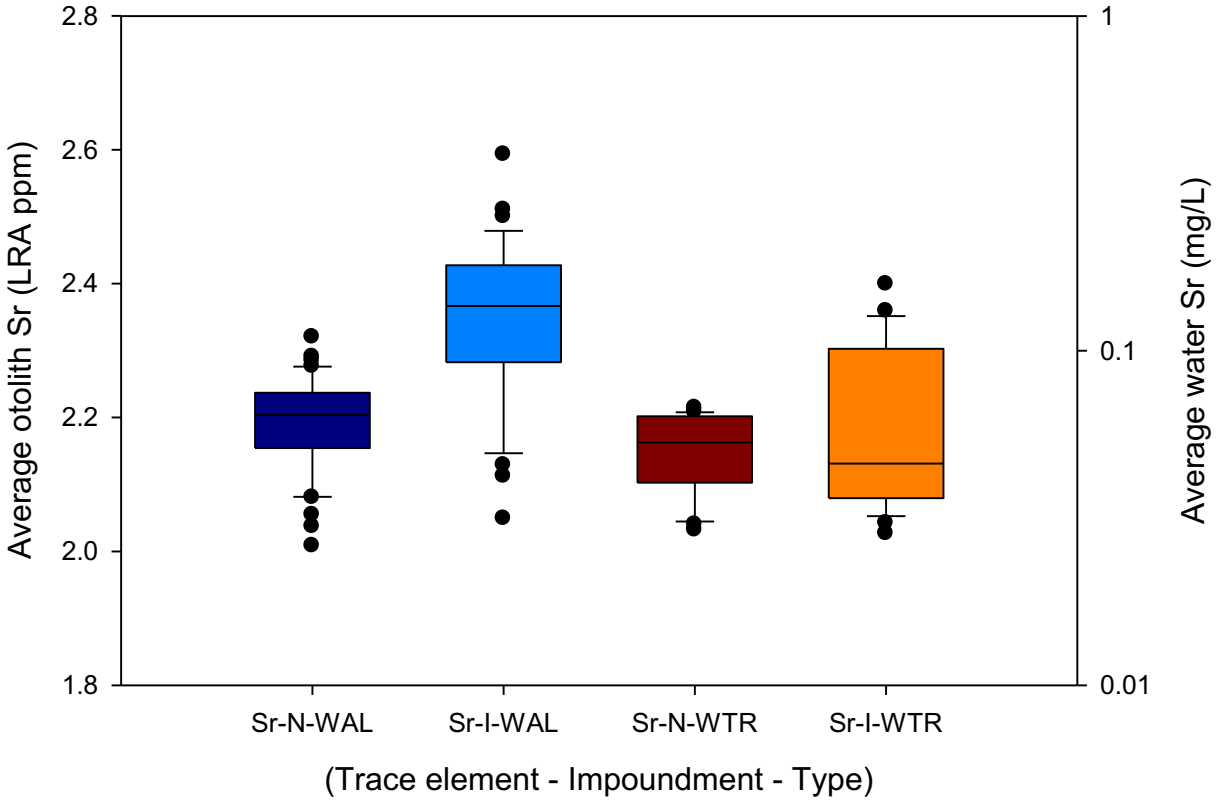

Figure 3. 15. Boxplots of water and walleye otolith Sr content (LRA correction) in CAMP waterbodies pooled by impoundment status. N = non-impounded, I = impounded, WAL= walleye, WTR= water concentration.

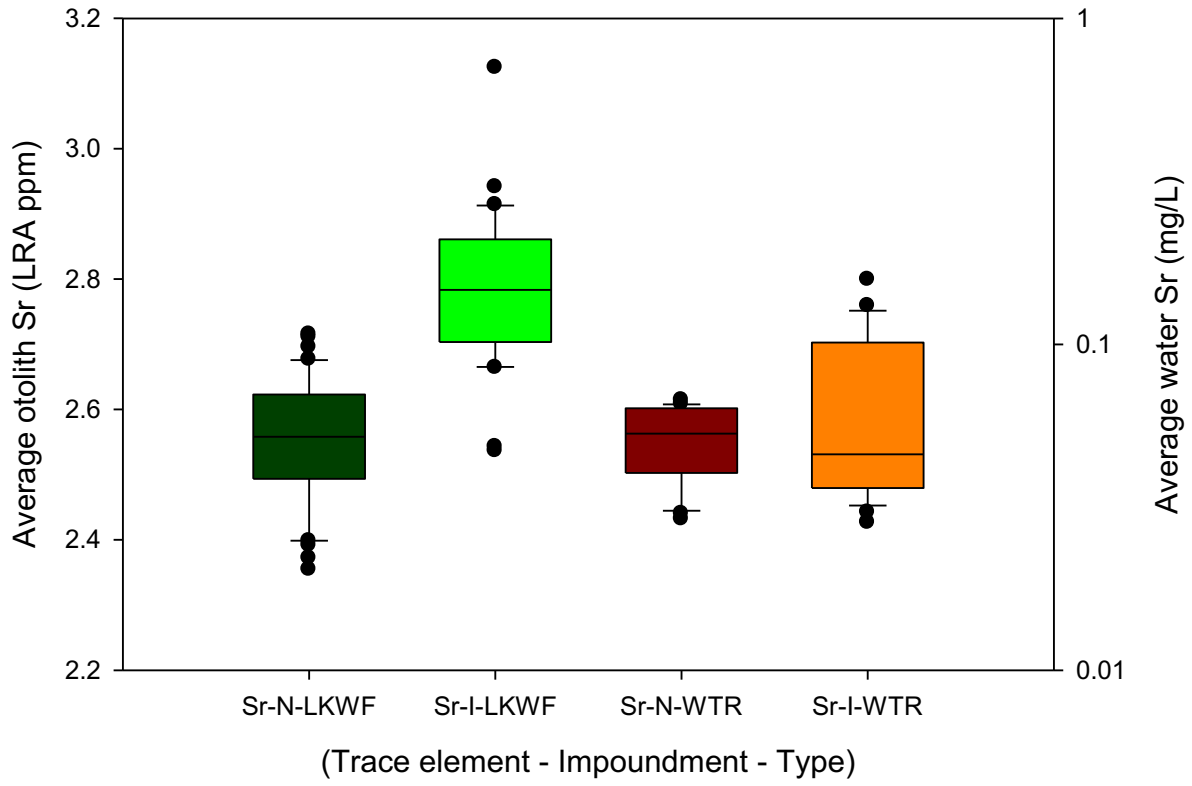

Figure 3. 16. Boxplots of water and walleye otolith Sr content (LRA correction) in CAMP waterbodies pooled by impoundment status. N = non-impounded, I = impounded, LKWF= lake whitefish, WTR= water concentration.

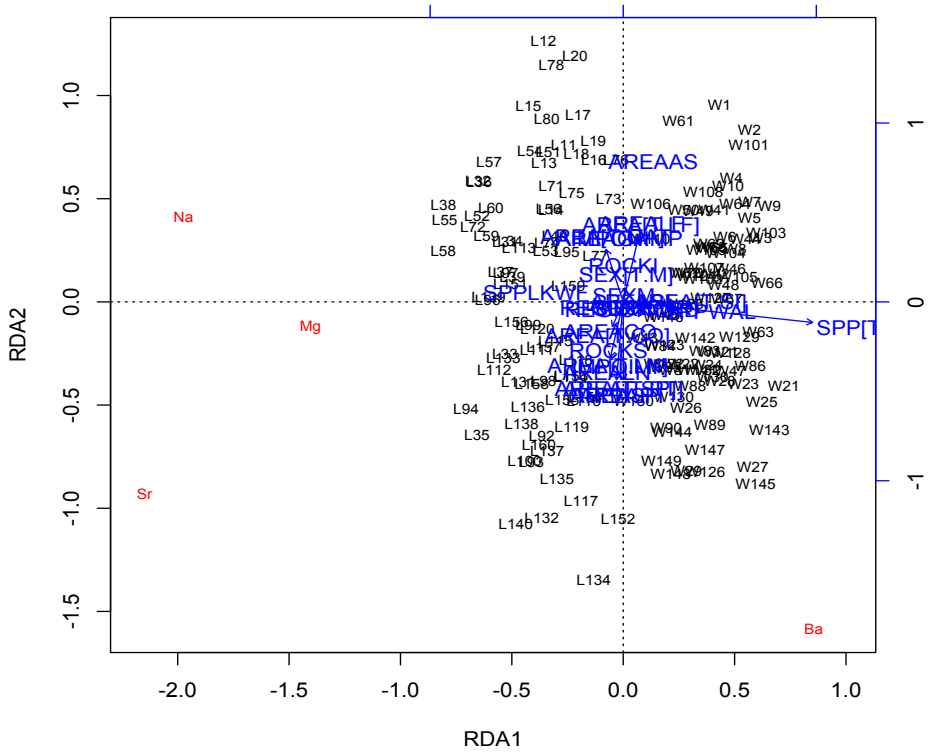

Figure 3. 17. RDA of both walleye and lake whitefish otolith trace element data constrained by multiple environmental variables. Environmental variables tested: Area (waterbody), species, sex, impoundment status, region, basin dominant rock type. 158 individuals total, 79 walleye and 79 lake whitefish. RDA1= 44.75%, $F_{1.153}$= 198.94 , Pr(>F)= 0.001, RDA2= 14.22%, $F_{1.153}$= 63.17, Pr(>F)= 0.001.

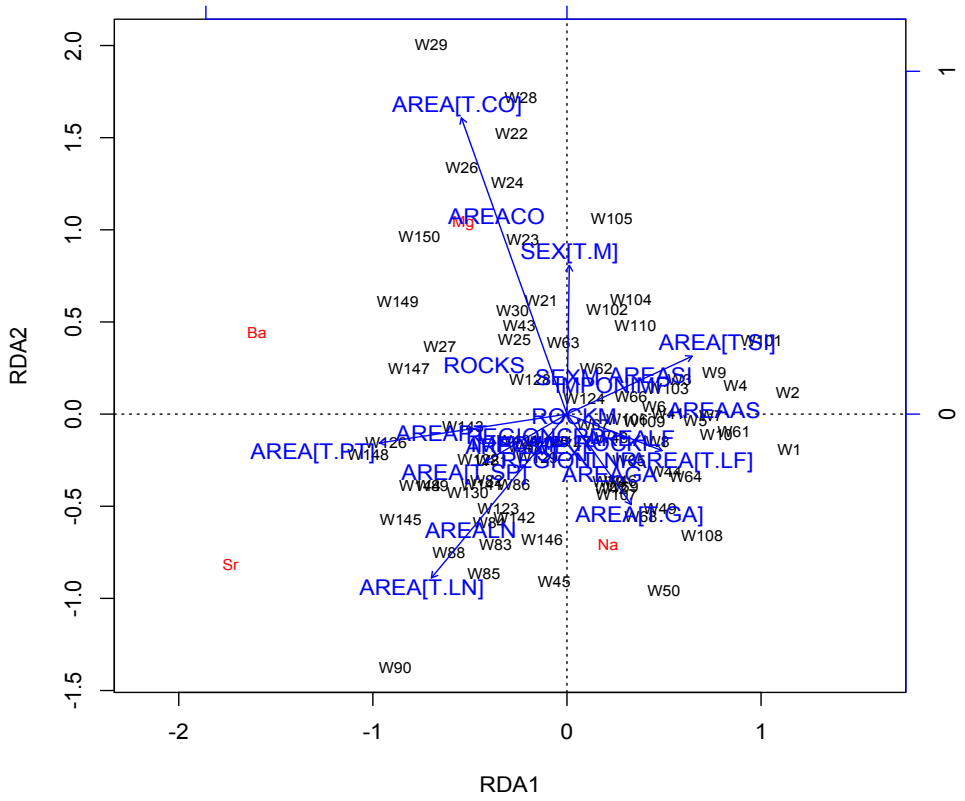

Figure 3. 18. RDA analysis of walleye otolith trace elements (Na, Mg, Ba, Sr) constrained by multiple factor variables: area rock type, fish sex, region, impoundment status and area (waterbody). RDA1= 33.31%, $F_{1,74}$= 48.72, Pr(>F)= 0.001, RDA2= 13.79%, F1,74= 20.18, Pr(>F)= 0.001.

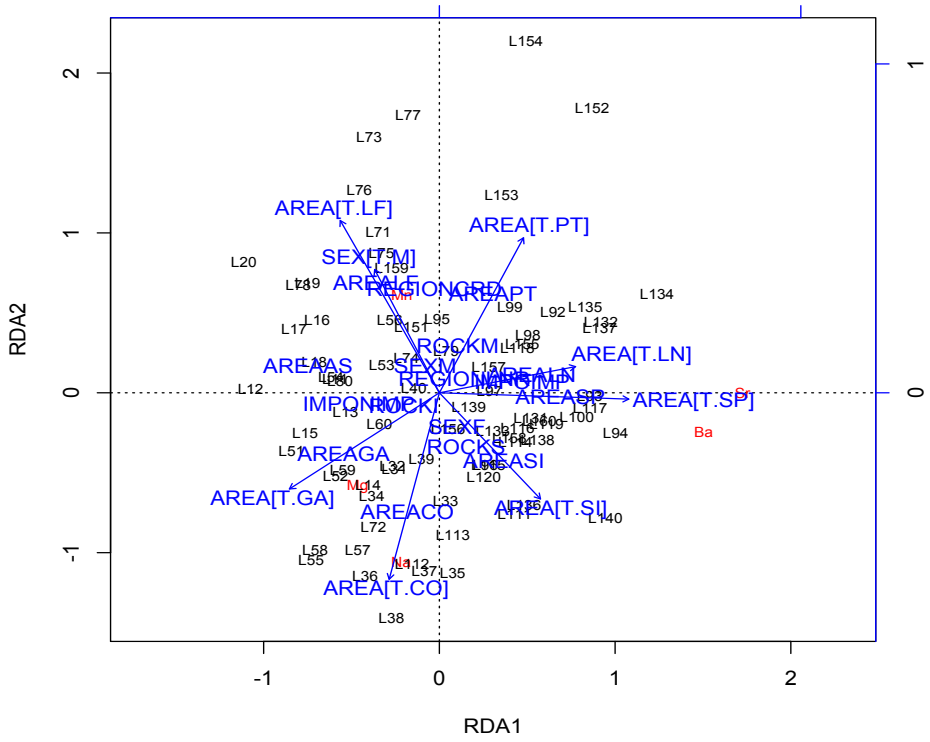

Figure 3. 19. RDA analysis of lake whitefish otolith trace elements (Na, Mg, Mn, Ba, Sr) constrained by multiple factor variables: area rock type, fish sex, region, impoundment status and area (waterbody). RDA1= 28.09%, $F_{1,73}$= 44.5924, Pr(>F)= 0.001, RDA2= 9.55%, $F_{1,73}$= 15.1602, Pr(>F)= 0.001.

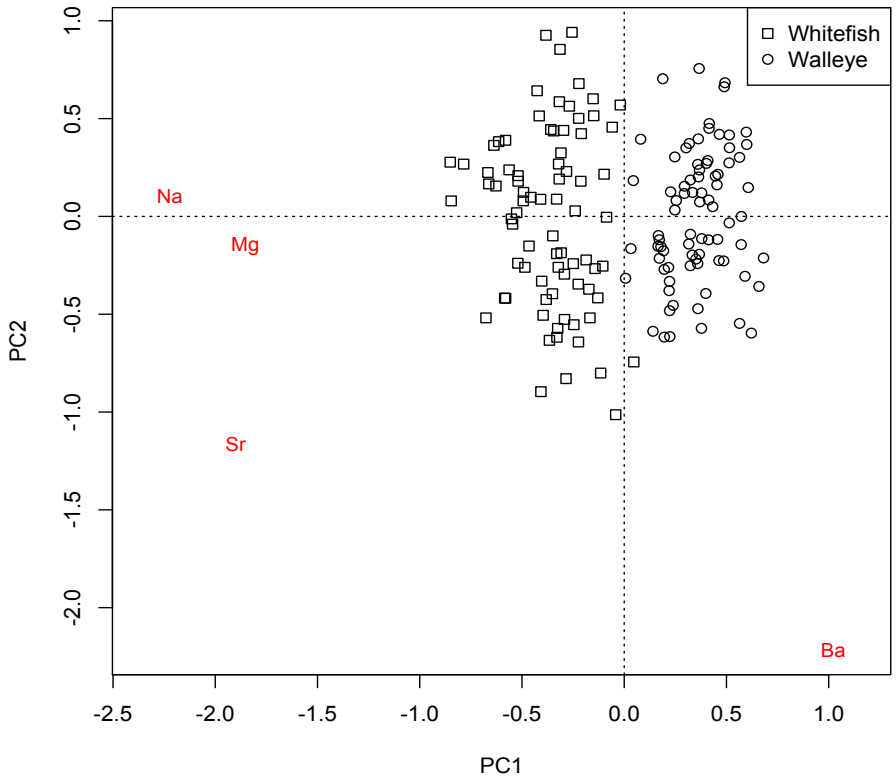

Figure 3. 20. PCA using log-residual average (LRA) trace element data (Na, Mg, Ba and Sr) for walleye and lake whitefish. PC1= 51.98%, PC2= 25.16% of variation explained.

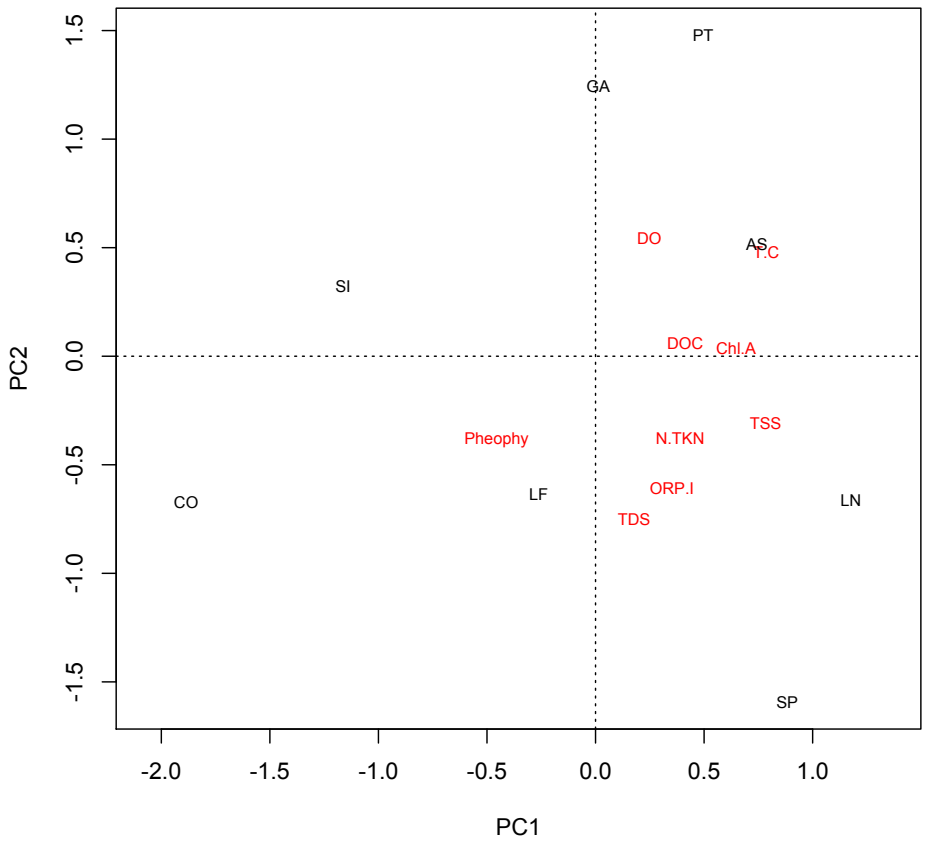

Figure 3. 21. PCA using selected raw water quality variables with respect to the eight select CAMP waterbodies. PC1, 30.2% by PC2, 21.35% variation explained.

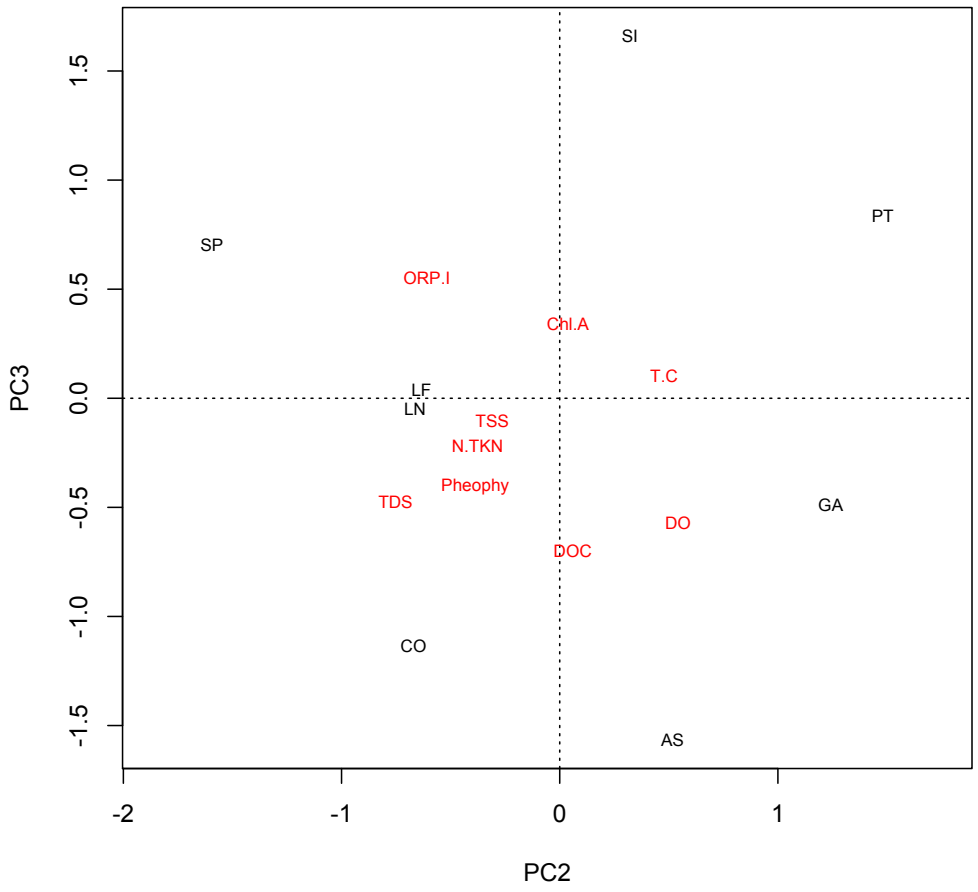

Figure 3. 22. PCA using selected raw water quality variables with respect to the eight select CAMP waterbodies. PC2= 23.33%, PC3= 21.35% variation explained.

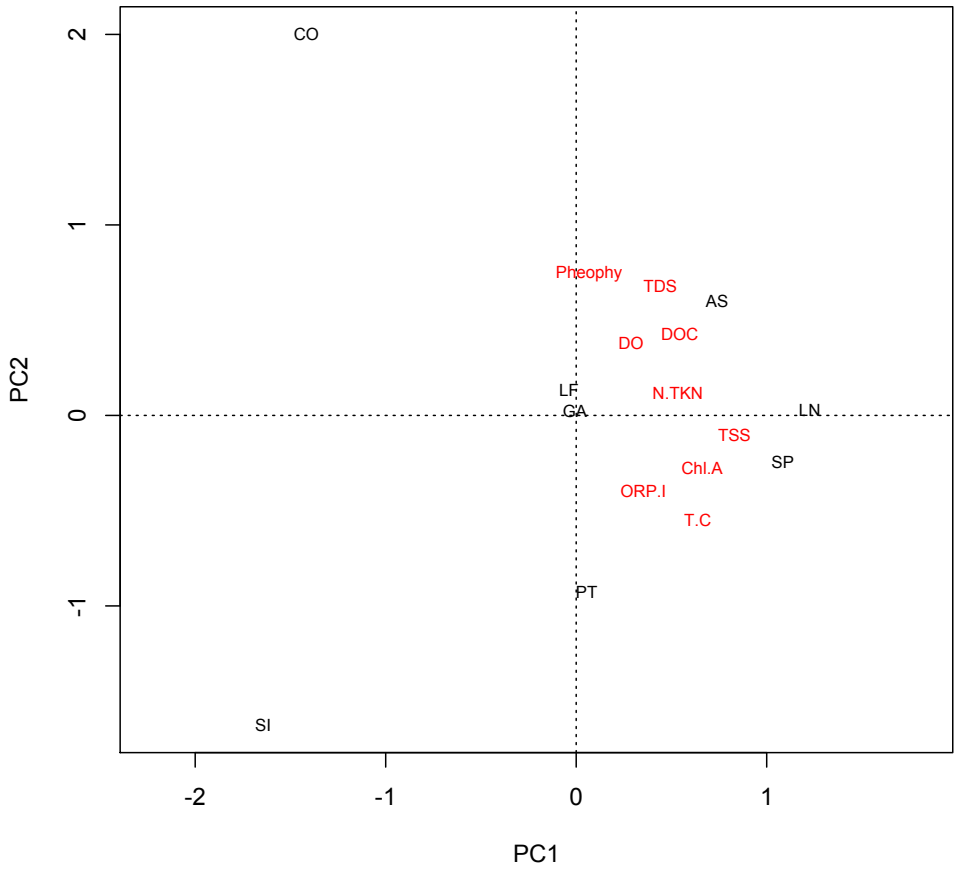

Figure 3. 23. PCA using selected log transformed water quality variables with respect to the eight select CAMP waterbodies. PC1= 31.75%, PC2= 23.97% variation explained.

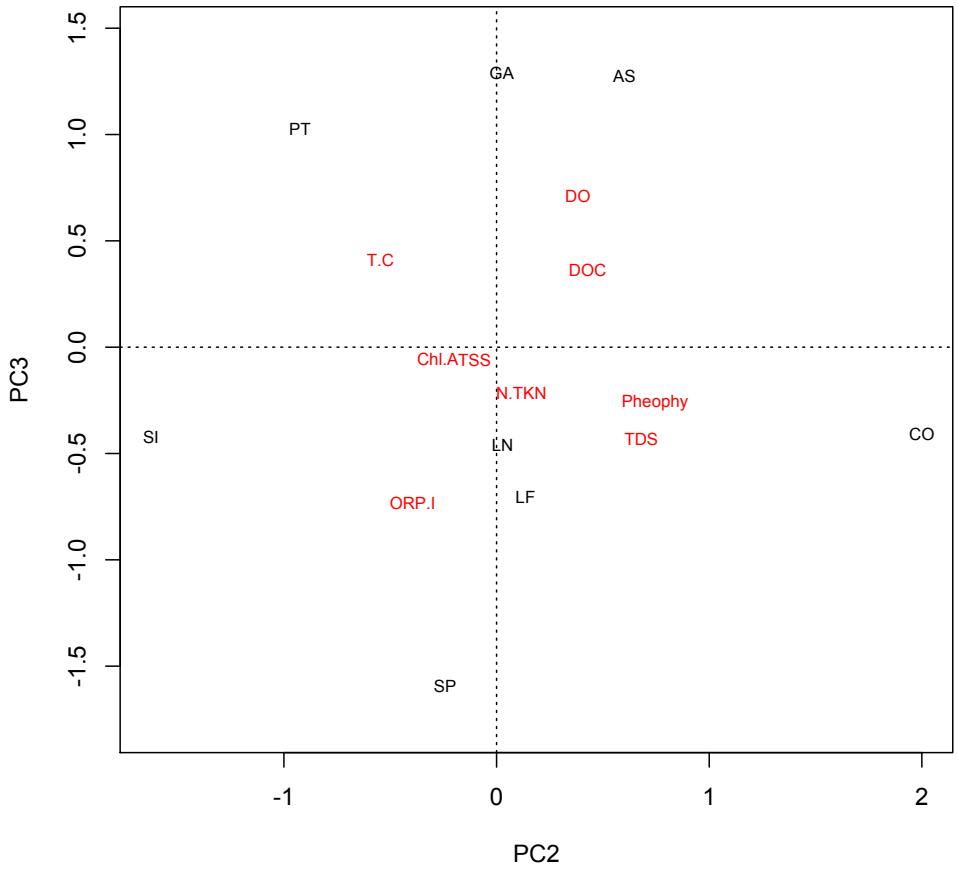

Figure 3. 24. PCA using selected log transformed water quality variables with respect to the eight select CAMP waterbodies. PC2= 23.97%, PC3= 20.82% variation explained.

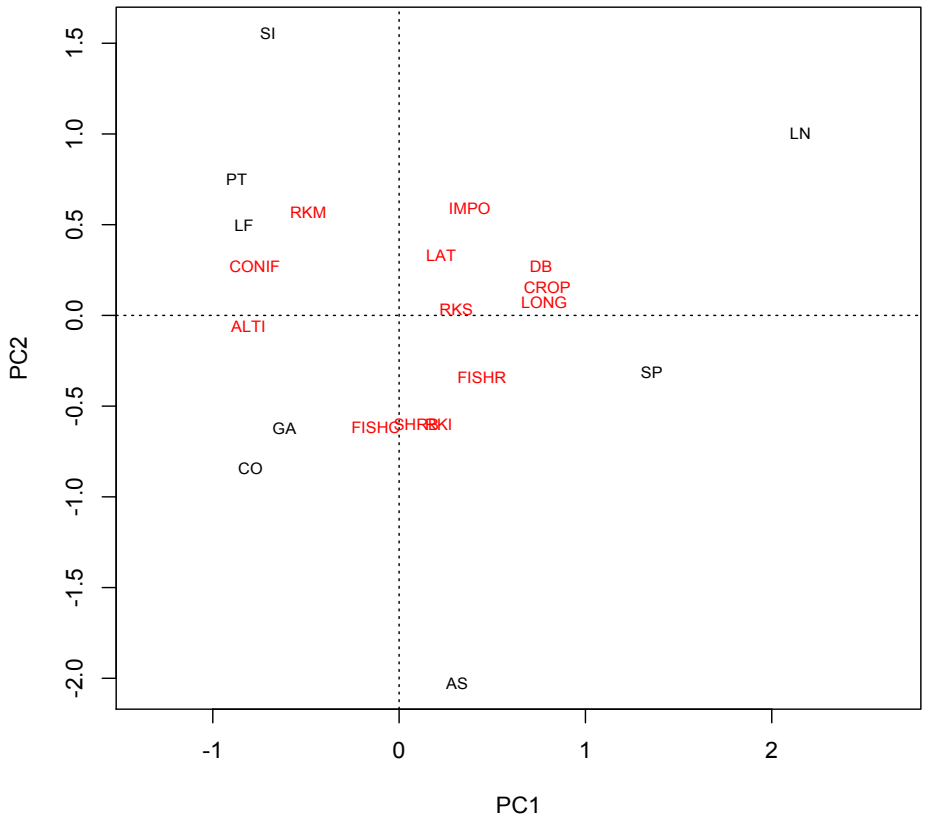

Figure 3. 25. PCA 1+2 Comparison of CAMP waterbody (eight waterbodies) characteristics (e.g., rock type, impoundment status, altitude, latitude and longitude, primary plant type, fishing practices, drainage basin designation). PC1= 40.52%, PC2= 22.82% of variation explained.

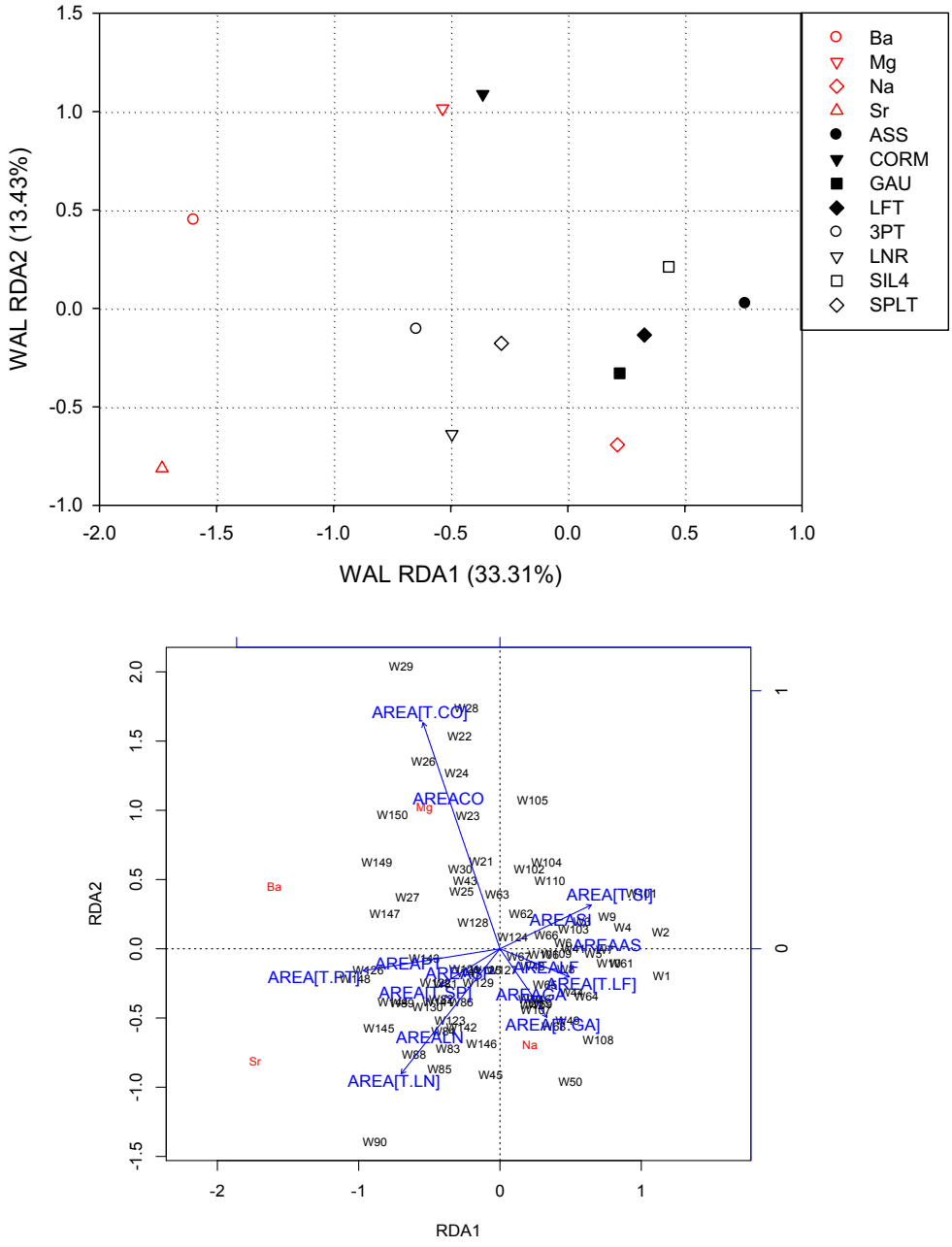

Figure 3. 26. RDA for walleye otolith log-residual averaged trace element data (Na, Mg, Ba, Sr) constrained by Area (CAMP select waterbodies). RDA1= 33.31%, $F_{1,73}$= 48.33, Pr(>F)= 0.001, RDA2= 13.43%, $F_{1,73}$= 19.49, Pr(>F)= 0.001. Upper graph displays centroid scores for CAMP waterbodies only.

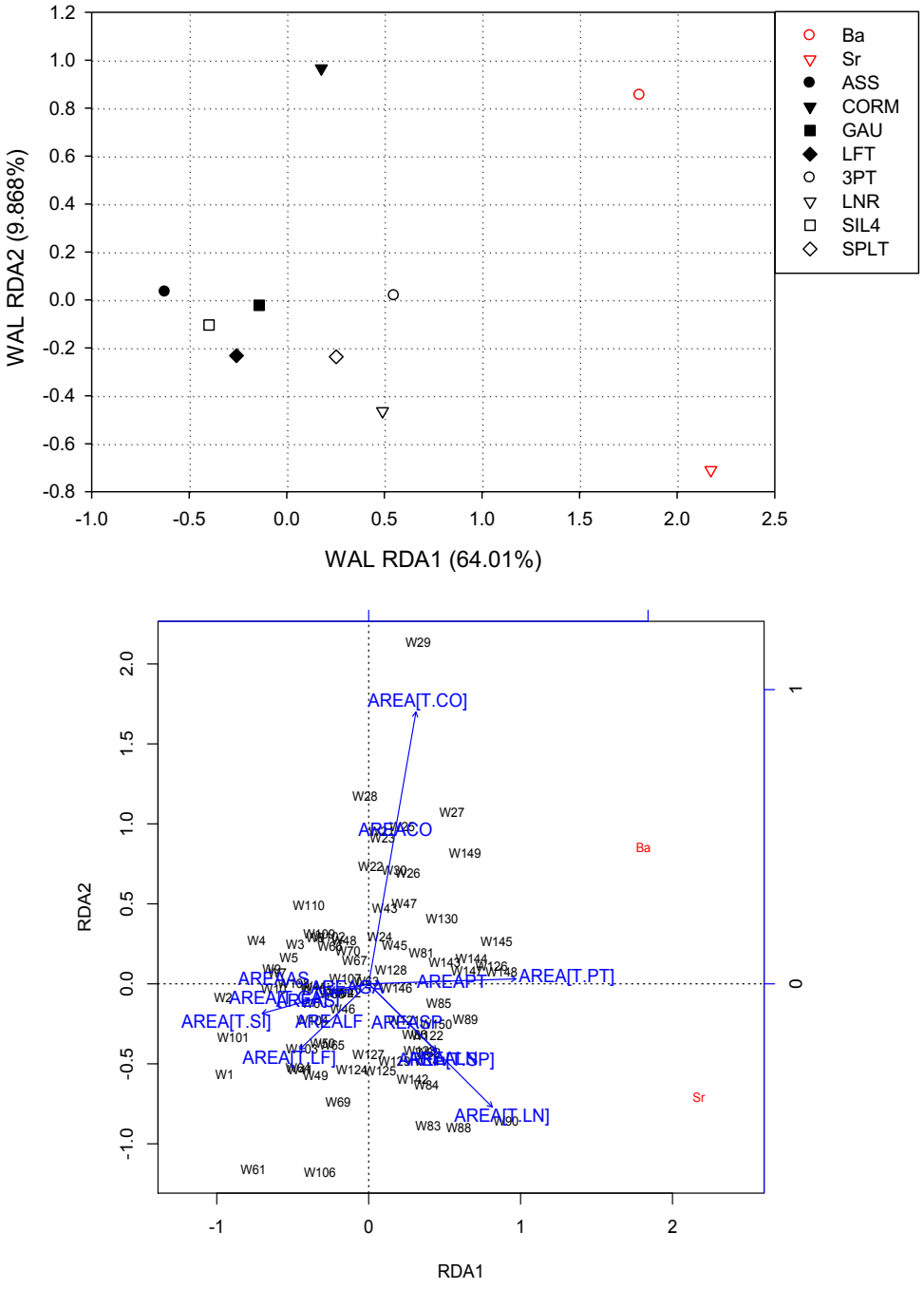

Figure 3. 27. RDA of walleye otolith Ba and Sr trace element data (LRA: log-residual average) constrained by Area (CAMP select waterbodies). RDA1= 64.01%, $F_{1,76}$= 186.24, Pr(>F)= 0.001, RDA2= 9.87%, $F_{1,76}$= 28.71, Pr(>F)= 0.001. Upper graph displays centroid scores for CAMP waterbodies only.

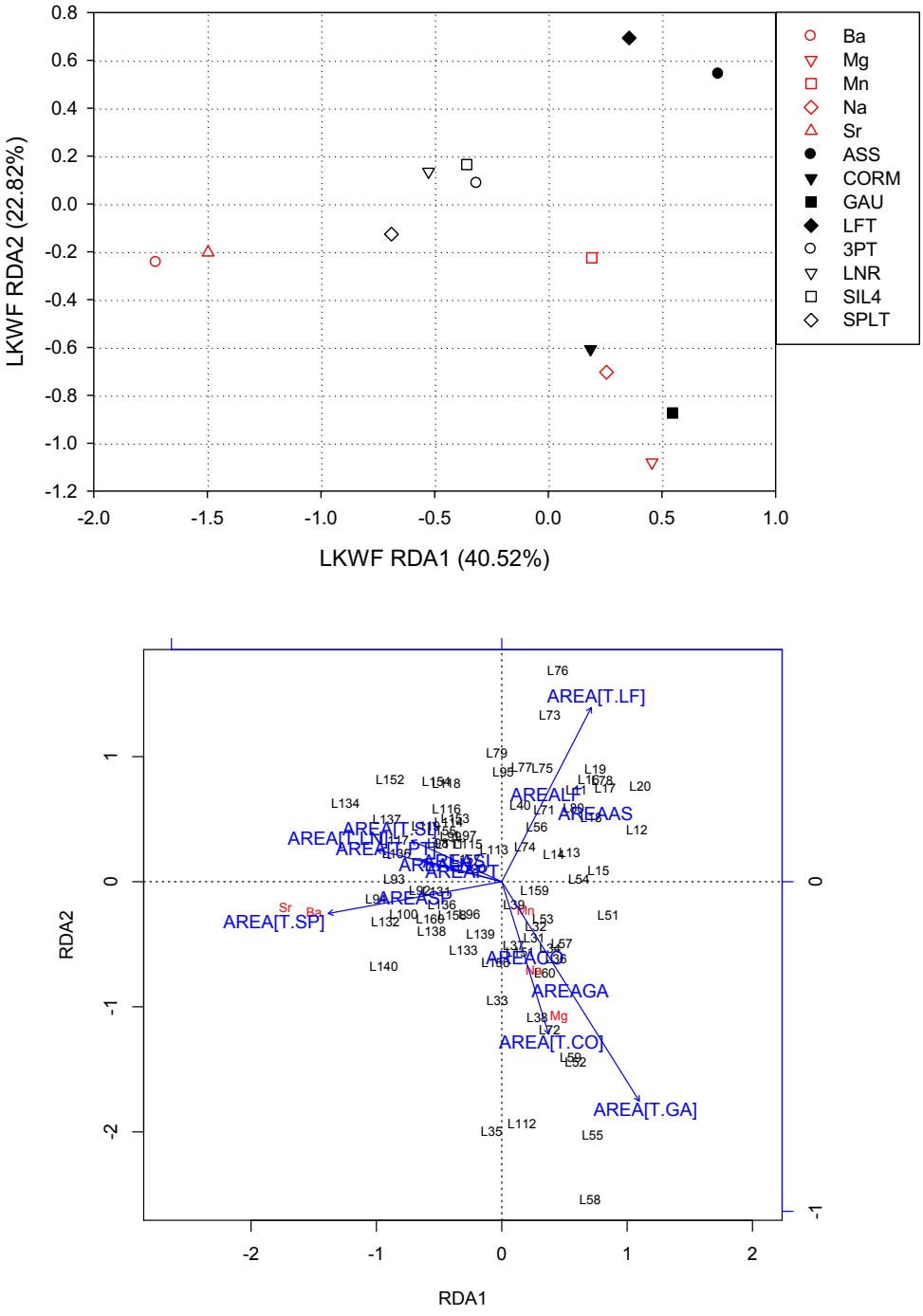

Figure 3. 28. RDA for lake whitefish otolith log-residual averaged trace element data (Na, Mg, Mn, Ba, Sr) constrained by Area (CAMP select waterbodies). RDA1= 40.52%, $F_{1,73}$= 42.86, Pr(>F)= 0.001, RDA2= 22.82%, $F_{1,73}$= 14.06, Pr(>F)= 0.001. Upper graph displays centroid scores for CAMP waterbodies only.

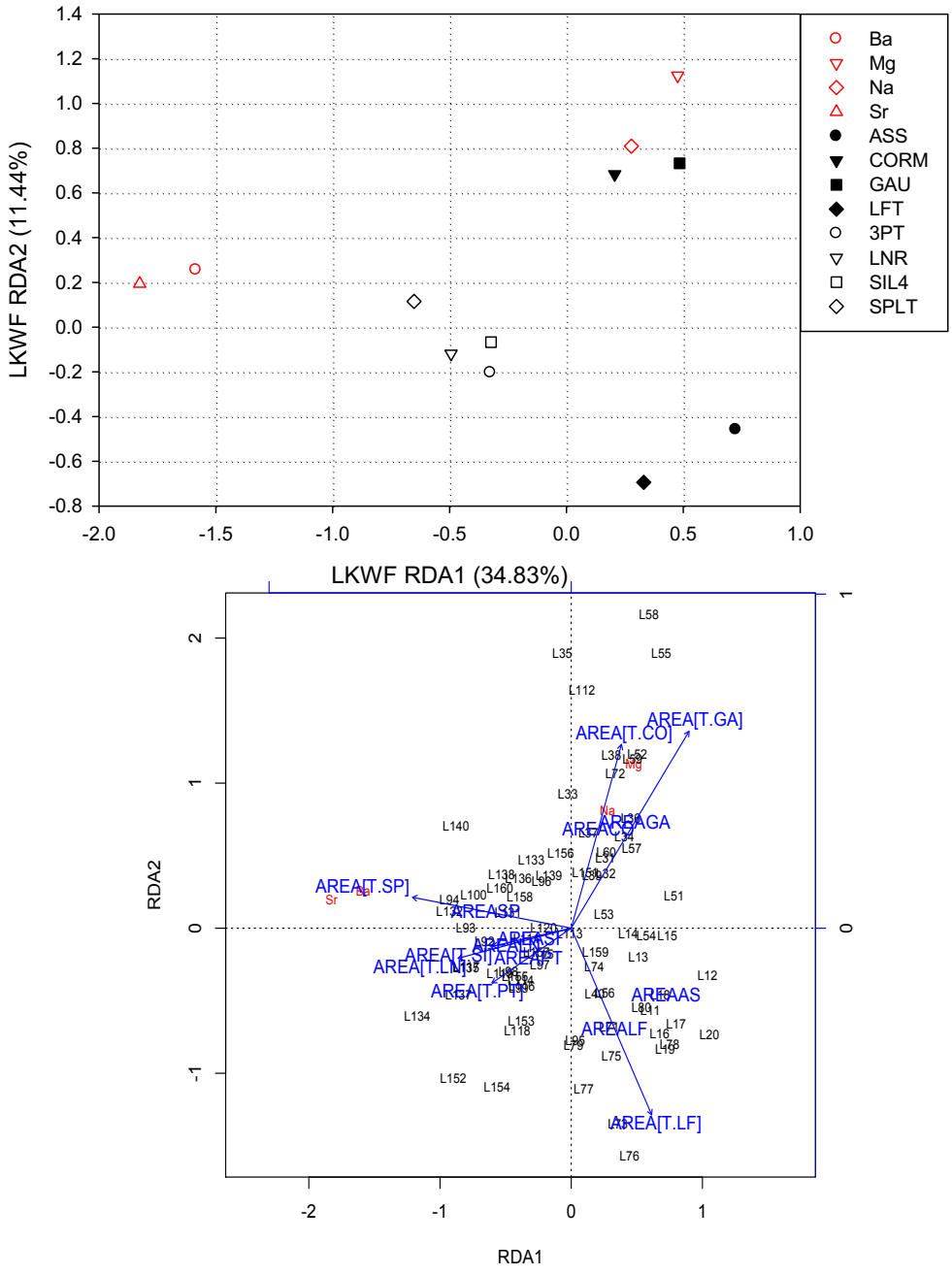

Figure 3. 29. RDA for lake whitefish otolith log-residual averaged trace element data (Na, Mg, Ba, Sr) constrained by Area (CAMP select waterbodies). RDA1= 34.83%, $F_{1,74}$= 57.74, Pr(>F)= 0.001, RDA2= 11.44%, $F_{1,74}$= 18.97, Pr(>F)= 0.001. Upper graph displays centroid scores for CAMP waterbodies only.

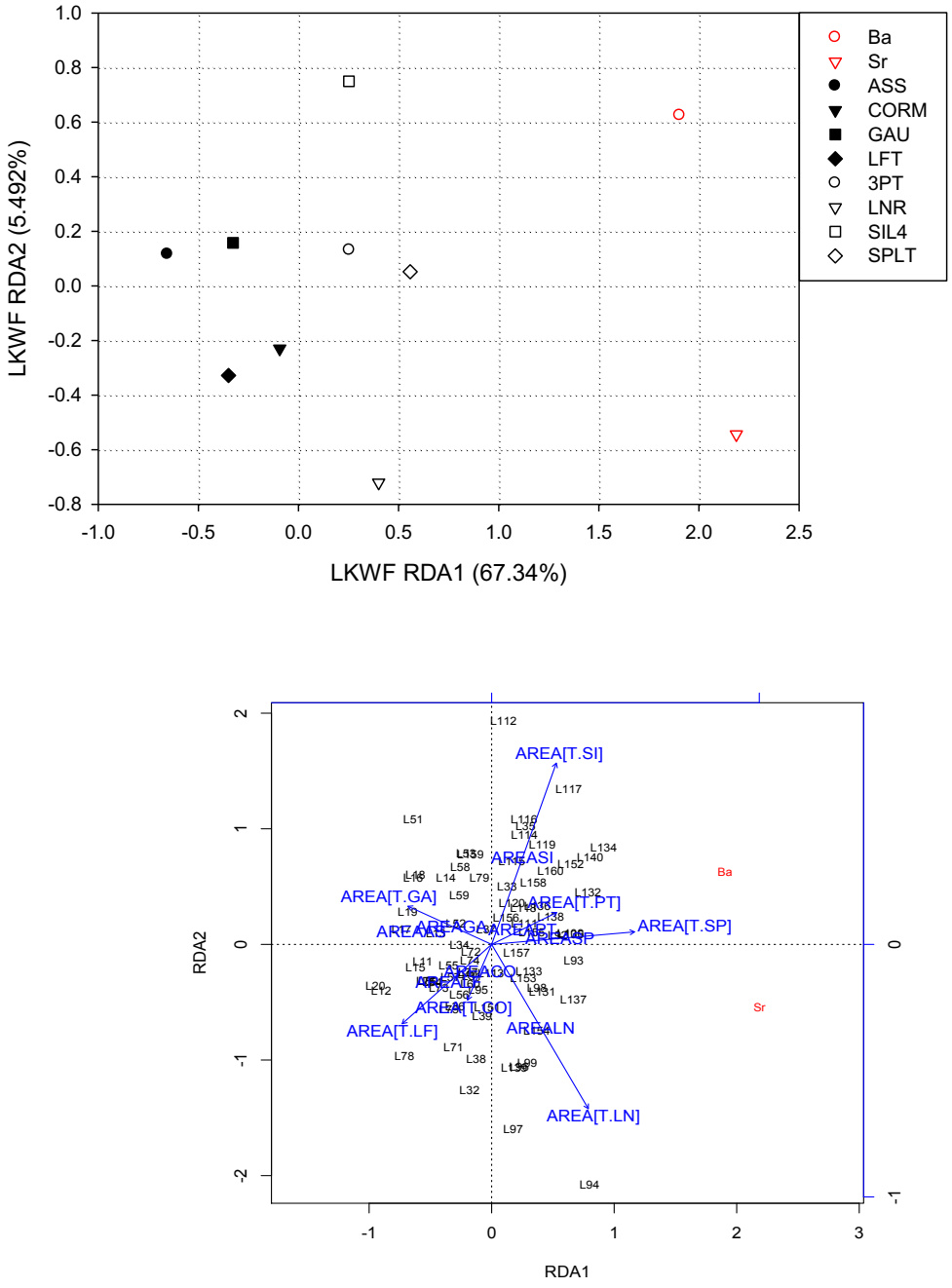

Figure 3. 30. RDA of lake whitefish otolith Ba and Sr trace element data (LRA: log-residual average) constrained by Area (CAMP select waterbodies). RDA1= 67.34%, $F_{1,76}$= 188.37, Pr(>F)= 0.001, RDA2= 5.49%, $F_{1,76}$= 15.37, Pr(>F)= 0.001. Upper graph displays centroid scores for CAMP waterbodies only.

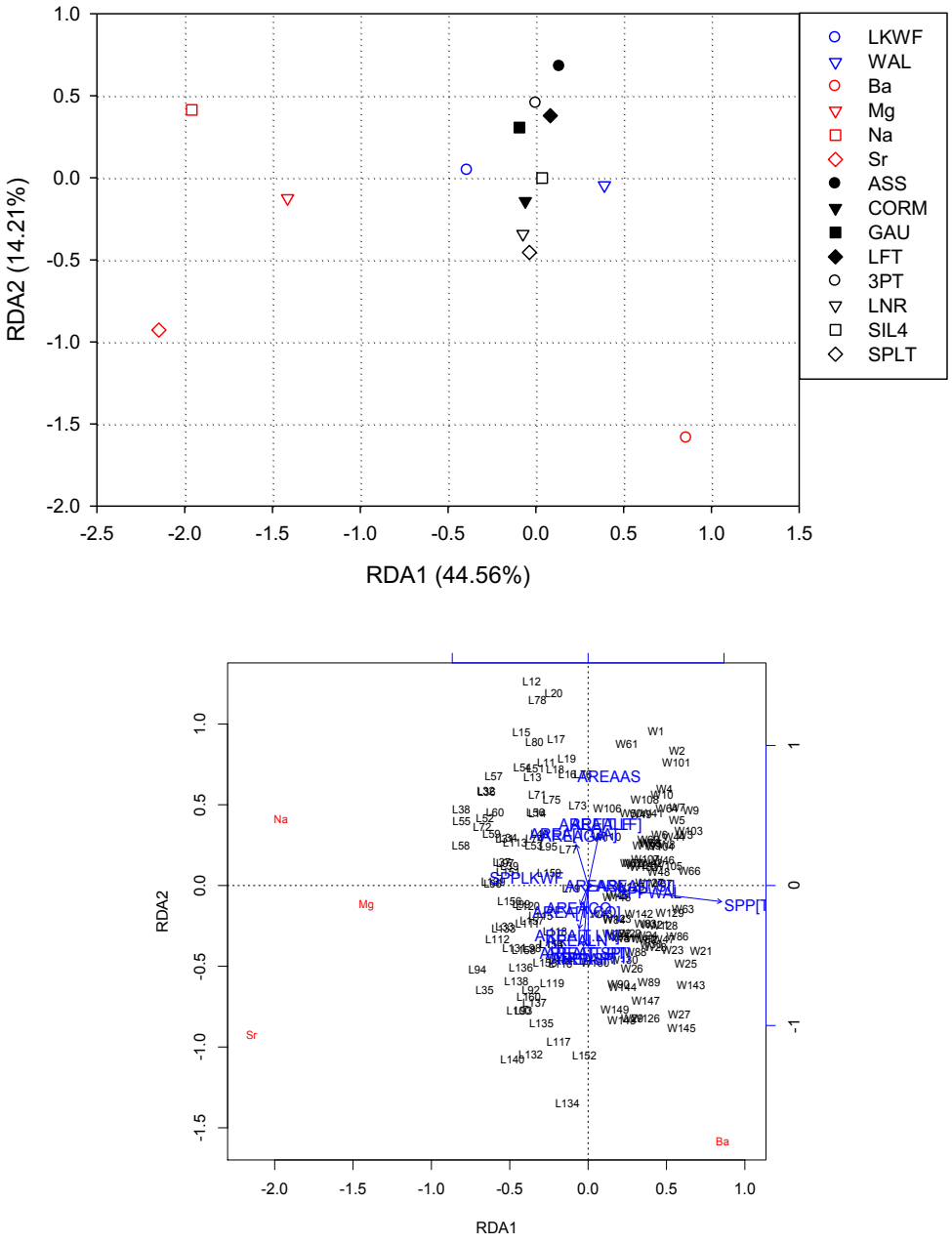

Figure 3. 31. RDA of both walleye and lake whitefish trace element data constrained by multiple environmental variables. Environmental variables tested were: Area and species. 158 individuals total, 79 walleye and 79 lake whitefish. RDA1= 44.65%, $F_{1,153}$= 195.28, Pr(>F)= 0.001, RDA2= 14.21%, $F_{1,153}$= 62.15, Pr(>F)= 0.001.Upper graph displays centroid scores for CAMP waterbodies only.

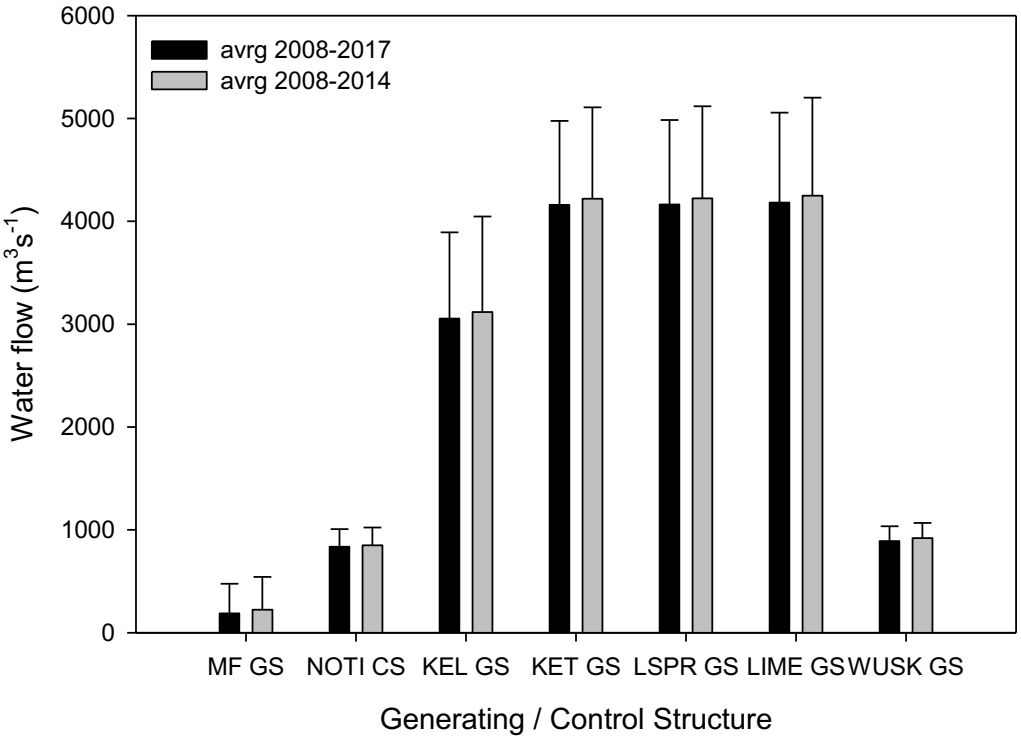

Figure 3. 32. Bar graph of control (CS) and generating (GS) structure average water flow rate found adjacent to the four selected CAMP impounded waterbodies (refer to area map). Error bars as standard deviation. "MF"= Missi Falls, "NOTI"= Notigi, "KEL"= Kelsey, "KET"= Kettle, "LSPR"= Long Spruce, "LIME"= Limestone, "WUSK"= Wuskwatim.

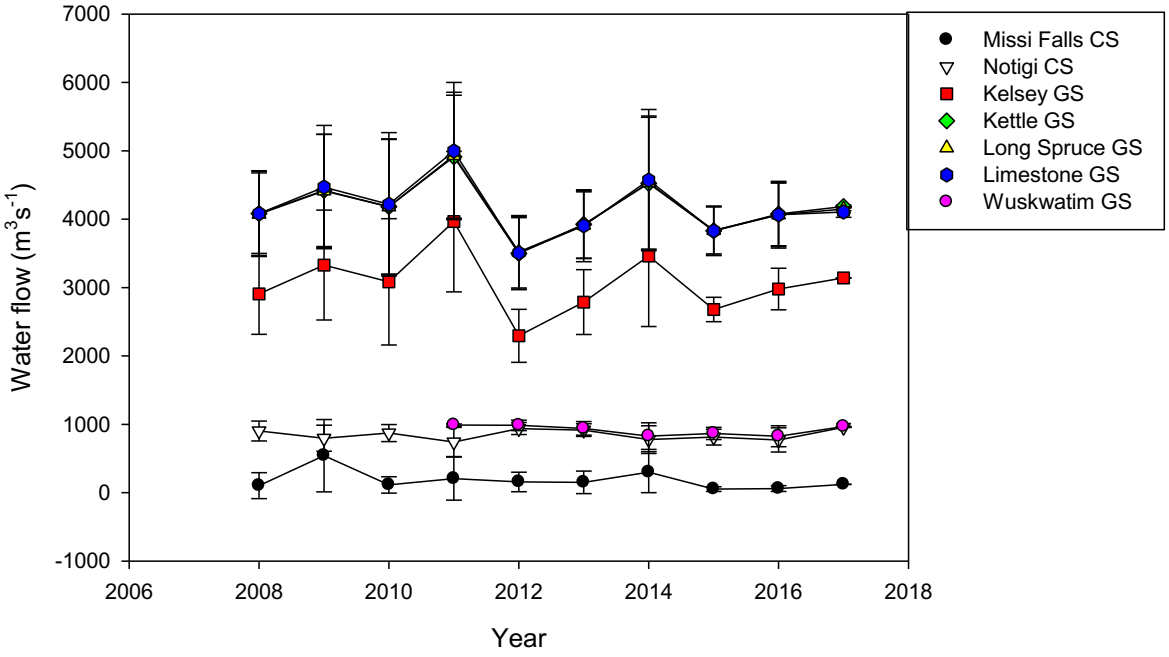

Figure 3. 33. Line/scatter plot of control and generating structure average water flow rate found adjacent to the four selected CAMP impounded waterbodies (refer to area map). Error bars as standard deviation. Year range from 2008-2017. "CS"= control structure, "GS"= generating structure.

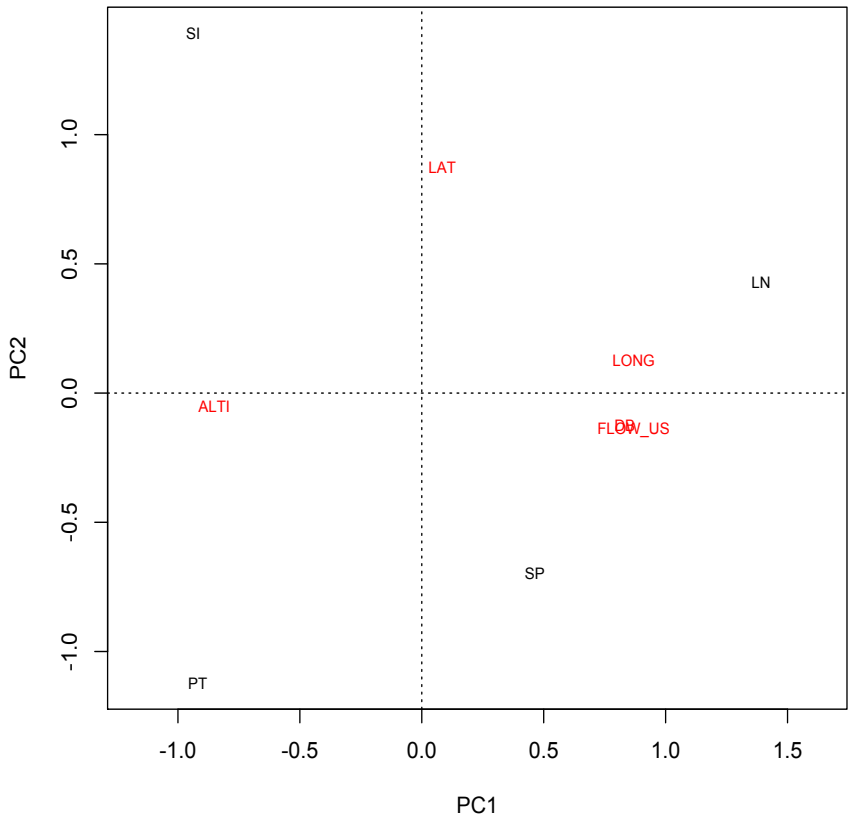

Figure 3. 34. PCA of the comparison of the four impounded waterbodies (LN, SI, SP, PT) using upstream flow (FLOW US, latitude/longitude (LAT, LONG), altitude (ALTI), and drainage basin area (DB). PC1= 75.8%, PC2= 21.11% of variation explained.

Chapter 3 Tables

Table 3. 1. CAMP waterbody background summary (Bracketed = references, unreferenced = CAMP, 2014

Waterbody	**Ecoregion**	**Drainage Basin (km^2)** (1)	**Surface Area (km^2)** (2)	**1^o Cover** (9)	**Surficial Geology** (10)	**Base Geology*** (11)
Saskatchewan River Region						
Cormorant Lake (CORM)	Mid-Boreal Lowland	3162	333	Conif. Forest	Precambrian ter. > Glaciolacust. sed.	Dolomite/Limestone [S]
Upper Churchill River Region						
South Indian lake Area 4 (SIL4)	Selwyn Lake Upland	261394	681	Conif. forest	Lacust. seds, Organic dep, Prox. glaciofluvial sed.	Pelitic schist [MS] > Granite [I] > Pelitic schist/felsic gneiss [MS]
Lower Churchill River Region						
Gauer Lake (GAU)	Churchill River Upland	4897	263	Conif. Forest	Offshore lacust. sed., Sand diamicts, Organic dep.	Granite [I] > Pelitic schist [MS]
Churchill River Diversion Region						
Leftrook Lake (LFT)	Churchill River Upland	389	46.3	Conif. Forest	Precambrian ter. > Glaciolacust. sed.	Pelitic schist/Felsic gneiss [MS] > Amphibolite [M]
Threepoint Lake (3PT)	Churchill River Upland	276853	62.2	Conif. Forest	Precambrian ter. > Glaciolacust. sed.	Pelitic schist [MS] > Granite [I]
Lower Nelson River Region						
Nelson River (LNR)	Hudson + Coastal Hudson Bay Lowland	1392453	-	Crops	Alluvial sed., Glaciomarine sed.	Dolomite/Limestone [S]
Split Lk. (SPLT)	Hayes River Upland	1374157	269	Crops	Lacust. sed., Silt diamicts	Tonalite [I] > Granite [I]

Assean Lake (ASS)	Hayes + Churchill River Upland	542	76.3	Shrub	Lacust. sed.	Tonalite [I] > Tonalite gneiss [M]

***Base geology: [square bracketed] "S" = sedimentary, "M" = metamorphic, "MS" = metasedimentary, "I" = igneous**

References associated with Table 3.1.

1 Prairie Farm Rehabilitation Administration (PFRA) Watershed Project. 2008. Version 8, 2008.03.31.

2 Natural Resources Canada (NRC). 2011. Canvec Edition 8, 2011.04.18 (www.geogratis.ca)

3 Manitoba Conservation. 2006. Cormorant Lake angling map. Manitoba

4 Manitoba Natural Resources. 1990. Gauer Lake angling map.

5 Manitoba Conservation. 2003a. Threepoint Lake angling map.

6 Manitoba Hydro Unpublished Data

7 Manitoba Conservation. 2003b. Split Lake angling map.

8 Data collected by NSC between 2010 and 2011 as part of CAMP

9 Natural Resources Land Cover (NRLC). 2000. Circa 2000 Vector (www.geobase.ca)

10 Matile, G.L.D. and Keller, G.R. 2006. Surficial geology of the Norway House map sheet (NTS 63H), Manitoba; Manitoba Science, Technology, Energy and Mines, Manitoba Geological Survey, Surficial Geology Compilation Map Series SG-54C, 63H, 63K, 63O, 64A, 64B, 64G, 64H scale 1:250 000. <http://www.gov.mb.ca/iem/info/libmin/SG-63H.pdf> [April 2017].

11 Manitoba Mineral Resources. 2013. Bedrock geology, Manitoba; in Map Gallery – Geoscientific Maps, Manitoba Mineral Resources, URL <http://web33.gov.mb.ca/mapgallery/mgg-gmm.html> [April 24, 2017]

12 Coordinated Aquatic Monitoring Program (CAMP). 2014. Three Year Summary Report (2008-2010). Report prepared for the Manitoba/Manitoba Hydro MOU Working Group by North/South Consultants Inc., Winnipeg, MB.

Table 3. 2. Depth measures taken per CAMP waterbody. Depth range was from 0m (surface) to max depth per CAMP waterbody exclusively (CAMP, 2016).

Waterbody	Max CAMP dataset depth measures	Lowest depth multiple measures	Year range
Cormorant Lake	26	24	2009-2014
South Indian Lake (Area 4)	20	16	2008-2013
Gauer Lake	13	4	2008-2013
Leftrook Lake	9		2009-2013
Threepoint Lake	6	5	2010-2014
Lower Nelson River (d/s Limestone GS)	18	15	2010-2013
Split Lake	18	15	2010-2013
Assean Lake	3	3	2009-2012

Table 3. 3. Years used for water to otolith concentration correlation and number of trace element (TE) water samples per CAMP waterbody (sampling depth: 0.3 m).

Waterbody	Species	Year Range	# Yrs.	TE Sample size (N)
Cormorant (CORM)	BOTH	2008-2013	6	23
South Indian lake (SIL4)	BOTH	2008-2013	6	18
Gauer (GAU)	BOTH	2008-2013	6	19
Leftrook (LFT)	BOTH	2009-2013	5	19
Threepoint (3PT)	WAL	2009-2013	5	19
	LKWF	2009-2012	4	15
Lower Nelson River (LNR)	WAL	2008-2012	5	11
	LKWF	2008-2013	6	14
Split (SPLT)	WAL	2009-2013	5	21
	LKWF	2009-2012	4	17
Assean (ASS)	WAL	2009-2013	5	15
	LKWF	2009-2012	4	14

Table 3. 4. Typical LA-ICP-MS operating conditions and data acquisition parameters for the analysis of otoliths in CAMP-O

ICP-MS			
Forward power	1205W		
Reflected power	~3W		
Gas flows			
Plasmas (Ar)	14.8 L/min		
Auxiliary (Ar)	0.99 L/min		
Sample (Ar/He)	1.02 L/min		
He gas	0.63 L/min		
LA	Standard (spot sample)	Pre-Ablation	Sample
Repetition rate	5	20	10
Spot size	30	40	30
Power	55%	40	55
Incident pulse energy (mJ)	0.032	0.008	0.032
Energy density on sample (J/cm2)	4.5	0.65	4.5
Laser scan speed (um/s)	n/a	150	3

Data acquisition	
Protocol	Time resolved analysis
Scanning mode	Bscan and Escan
Detector mode	Analog and counting
Isotopes determined	^{43}Ca, ^{55}Mn, ^{88}Sr, ^{23}Na, ^{138}Ba, ^{25}Mg
Dwell time (ms)	5
Magnet settling time (s)	0.001 to 0.1
Resolution	Low
laser warmup time between samples (s)	100

Table 3. 5. Part a. Walleye and lake whitefish biometric value summary (mean with standard deviation as error). Selections with the same superscript letter are not statistically different from each other by whatever statistical test was used (letters are species exclusive).

Site	Walleye (a)		
	Age (years)	Mass (g)	Fork Length (mm)
Test	Mann-Whitney	T-test (log transformed)	T-test (log transformed)
Non-Impounded	16.2 ± 3.2[a]	1107.2 ± 465.2	457.5 ± 58.3
Impounded	12 ± 3.6[a]	1055.3 ± 446.4	448.36 ± 62.3
Test	Kruskal-Wallis	1-way ANOVA	1-way ANOVA
Post-hoc test	Dunn's	Tukey's	Tukey's
Assean[b]	13 ± 1.2[b,c,d,f,g,h,i]	1097 ± 359.2[b,c,d,f,g,h,i]	451.2 ± 45.3[b,c,d,e,f,g,h,i]
Cormorant[c]	15.2 ± 2.7[b,c,d,e,f,g,h,i]	1474.5 ± 447.9[b,c,d,f,g]	509 ± 49.8[b,c,d,f,g]
Gauer[d]	17.1 ± 0.9[b,c,d,e,i]	1200 ± 428.2[b,c,d,f,g,h,i]	466.3 ± 51.7[b,c,d,e,f,g,h,i]
Leftrook[e]	19.4 ± 3.0[c,d,e,i]	658 ± 202.3[e,h,i]	403.5 ± 34.8[b,d,e,g,h,i]
Lower Nelson[f]	11.1 ± 3.6[b,c,f,g,h,i]	1388.9 ± 462.2[b,c,d,f,g]	491.8 ± 70.1[b,c,d,f,g,h]
South Indian (area 4)[g]	9.9 ± 1.5[b,c,f,g,h]	1315 ± 444.0[b,c,d,f,g]	472.4 ± 58.3[b,c,d,e,f,g,h,i]
Split[h]	10.1 ± 2.5[b,c,f,g,h]	782 ± 252.9[b,d,e,h,i]	425.4 ± 52.7[b,d,e,f,g,h,i]
Threepoint[i]	16.8 ± 1.1[b,c,d,e,f,i]	768.5 ± 172.8[b,d,e,h,i]	408.2 ± 31.0[b,d,e,g,h,i]

Table 3.5. Part b. Lake whitefish biometric value summary.

Site	Whitefish (b) Age (years)	Mass (g)	Fork Length (mm)
Test	Mann-Whitney	T-test (log transformed)	T-test
Non-Impounded	16.6 ± 5.8[a]	1323.9 ± 456.3	442.6 ± 36.8
Impounded	13.2 ± 4.9[a]	1154.1 ± 484.4	427.7 ± 50.1
Test	Kruskal-Wallis	Kruskal-Wallis	Kruskal-Wallis
Post-hoc test	Dunn's	Dunn's	Dunn's
Assean[b]	8.4 ± 1.3[b,d,f,h,i]	1190 ± 286.2[b,c,d,e,f,g,h,i]	432.7 ± [26.1b,c,d,e,f,g,h,i]
Cormorant[c]	20.2 ± 3.0[c,d,e,f,g]	849.5 ± 168.2[b,c,g,h,i]	405.3 ± 22.1[b,c,g,h,i]
Gauer[d]	16.3 ± 3.4[b,c,d,e,f,g,h,i]	1508 ± 215.0[b,d,e,f,h,i]	457.7 ± 19.3[b,d,e,f,h,i]
Leftrook[e]	21.5 ± 2.3[c,d,e,g]	1748 ± 488.3[b,d,e,f,h]	474.7 ± 35.8[b,d,e,f,h]
Lower Nelson[f]	13 ± 4.7[b,c,d,f,g,h,i]	1458.9 ± 426.5[b,d,e,f,h,i]	465.7 ± 40.5[b,d,e,f,h,i]
South Indian (area 4)[g]	17.5 ± 1.0[c,d,e,f,g,h,i]	812 ± 132.6[b,c,g,h,i]	405 ± 19.5[b,c,g,h,i]
Split[h]	11.4 ± 3.8[b,d,f,g,h,i]	1404.5 ± 664.0[b,c,d,e,f,g,h,i]	433.2 ± 66.7[b,c,d,e,f,g,h,i]
Threepoint[i]	11 ± 5.9[b,d,f,g,h,i]	971.5 ± 202.6[b,c,d,f,g,h,i]	410.8 ± 44.2[b,c,d,f,g,h,i]

Table 3. 6. Part a. Correlation analysis for fish age, fork length and body weight per species using pooled CAMP waterbodies. part b. Pearson and/or Spearman* correlation coefficient tests for walleye and lake whitefish age, fork length and body weight separate effect on trace element concentrations (Na, Mg, Mn, Sr, and Ba) per species using pooled CAMP waterbodies. **Bolded** text indicates a significant difference at an alpha of 0.05.

PART A	Comparison	Transform.	R	R^2	Normal	HOV	F-stat	Test	P-val
Walleye	Age-FL	None	0.0381	0.00145	nc.	nc.	nc.	S	0.738
Walleye	Age-Wgt	$Log_{10}(x)$	0.0397	0.00157	0.277	0.025	0.121	P	0.728
Walleye	**Wgt-FL**	**None**	**0.926**	**0.857476**	nc.	nc.	nc.	**S**	**<0.001**
Lake whitefish	**Age-FL**	**None**	**0.336**	**0.113**	**0.276**	**0.959**	**9.774**	**P**	**0.002**
Lake whitefish	**Age-Wgt**	**$Log_{10}(x)$**	**0.248**	**0.0613**	**0.377**	**0.0258**	**5.033**	**P**	**0.028**
Lake whitefish	**Wgt-FL**	**None**	**0.901**	**0.812**	**0.299**	**0.472**	**332.414**	**P**	**<0.001**

*P= Pearson's, S= Spearman's correlation coefficient test

Table 3. 6. part b. Pearson and/or Spearman* correlation coefficient tests for walleye and lake whitefish age, fork length and body weight separate effect on trace element concentrations (Na, Mg, Mn, Sr, and Ba) per species using pooled CAMP waterbodies. **Bolded** text indicates a significant difference at an alpha of 0.05.

PART B	Dep.	Indep.	R^2	Normal?	HOV	F-stat	P-val
WAL	**Log Ba**	**Age**	**0.178**	**0.971**	**0.77**	**16.729**	**<0.001**
WAL	Log Ba	FL	0.0476	0.552	0.687	3.848	0.053
WAL	**Log Ba**	**Wgt**	**0.0627**	**0.421**	**0.0627**	**5.151**	**0.026**
WAL*	**Log Mg**	**Log Age**	**0.049729**	**nc.**	**nc.**	**nc.**	**0.0482**
WAL*	Log Mg	Log FL	0.047524	**nc.**	**nc.**	**nc.**	0.0537
WAL*	**Log Mg**	**Wgt**	**0.060025**	**nc.**	**nc.**	**nc.**	**0.0294**
WAL*	**Log Na**	**Age**	**0.083521**	**nc.**	**nc.**	**nc.**	**0.0101**
WAL	Log Na	FL	0.0077	0.154	0.644	0.598	0.442
WAL	Log Na	Wgt	0.00187489	0.153	0.162	0.144	0.705
WAL*	**Log Sr**	**Log Age**	**0.106929**	**nc.**	**nc.**	**nc.**	**0.00337**
WAL*	Log Sr	Log FL	0.00851929	**nc.**	**nc.**	**nc.**	0.418
WAL*	Log Sr	Wgt	0.016384	**nc.**	**nc.**	**nc.**	0.262
LKWF	**Log Ba**	**Age**	**0.175**	**0.079**	**0.144**	**16.358**	**<0.001**
LKWF	**Log Ba**	**FL**	**0.205**	**0.201**	**0.746**	**19.853**	**<0.001**
LKWF*	**Log Ba**	**Wgt**	**0.1849**	**nc.**	**nc.**	**nc.**	**0.000088**
LKWF*	Log Mg	Log Age	0.0361	**nc.**	**nc.**	**nc.**	0.0942
LKWF*	**Log Mg**	**Log FL**	**0.050625**	**nc.**	**nc.**	**nc.**	**0.0466**
LKWF*	Log Mg	Wgt	0.014884	**nc.**	**nc.**	**nc.**	0.285
LKWF*	**Log Mn**	**Log Age**	**0.261121**	**nc.**	**nc.**	**nc.**	**0.00000184**
LKWF*	Log Mn	Log FL	0.001444	**nc.**	**nc.**	**nc.**	0.739
LKWF*	Log Mn	Wgt	0.010609	**nc.**	**nc.**	**nc.**	0.368
LKWF*	**Log Na**	**Log Age**	**0.186624**	**nc.**	**nc.**	**nc.**	**0.0000804**
LKWF*	**Log Na**	**Log FL**	**0.068121**	**nc.**	**nc.**	**nc.**	**0.0203**

LKWF*	Log Na	Wgt	0.034225	**nc.**	**nc.**	**nc.**	0.103
LKWF*	**Log Sr**	**Log Age**	**0.205209**	**nc.**	**nc.**	**nc.**	**0.0000328**
LKWF*	Log Sr	Log FL	0.039601	**nc.**	**nc.**	**nc.**	0.0782
LKWF*	**Log Sr**	**Wgt**	**0.050625**	**nc.**	**nc.**	**nc.**	**0.0467**

*Spearman correlation

Table 3. 7. AIC (Bi-directional) method analysis of the effects of FL, WT and AGE for walleye trace elements. Fork length (FL), wet weight/mass (WT) and fish age (AGE). Log transformed measures. **Bolded** text indicates a significant difference at an alpha of 0.05. *CUM = cumulative, df (I, E) = degrees of freedom (number of independent variables, remaining degrees of freedom).

TE	Cum. R^2	Cum. F stat (df 3,75)	Cum. p-val	AIC select	Post AIC cum. R^2	Pr (>|t|) AGE	Pr (>|t|) FL	Pr (>|t|) WT	AIC select F-stat	df (I,E)	Post AIC P-val
Na	**0.1182**	**3.353**	**0.02333**	**AGE**	**0.102**	**0.00412**	**nc.**	**nc.**	**8.744**	**1,77**	**0.004122**
Mg	**0.07583**	**2.051**	**0.1139**	**WT**	**0.0688**	**nc.**	**nc.**	**0.0195**	**5.689**	**1,77**	**0.01954**
Ba	**0.2258**	**7.293**	**0.0002344**	**AGE+WT**	**0.2251**	**0.000186**	**nc.**	**0.017984**	**11.04**	**2,76**	**6.17E-05**
Sr	**0.1901**	**5.867**	**0.001179**	**AGE+FL+WT**	**0.1901**	**0.000211**	**0.164556**	**0.067596**	**5.867**	**3,75**	**0.001179**

Table 3. 8. AIC (Bi-directional) method analysis of the effects of fork length (FL), wet weight/mass (WT) and fish age (AGE) for lake whitefish trace elements. Log transformed measures. *CUM = cumulative, df (I, E) = degrees of freedom (number of independent variables, remaining degrees of freedom).

TE	Cum. R^2	Cum. F stat (df 3,75)	Cum. p-val	AIC select	Post AIC cum. R^2	Pr (>|t|) AGE	Pr (>|t|) FL	Pr (>|t|) WT	AIC select F-stat	df (I,E)	Post AIC P-val
Na	**0.2002**	**6.26**	**0.0007523**	**AGE**	**0.1916**	**5.48E-5**	nc.	nc.	**18.25**	**1,77**	**5.48E-5**
Mg	0.05343	1.411	0.2461	none	nc.	nc.	nc.	nc.	nc.	nc.	nc.
Mn	**0.2824**	**9.838**	**1.51E-5**	**AGE+WT**	**0.2786**	**7.88E-7**	nc.	**0.0567**	**14.68**	**2,76**	**4.07E-6**
Ba	**0.2651**	**9.019**	**3.58E-5**	**AGE+FL**	**0.263**	**0.02968**	**0.00105**	**nc.**	**13.56**	**2,76**	**9.21E-6**
Sr	**0.2159**	**6.885**	**0.0003702**	**AGE**	**0.2087**	**2.32E-5**	**nc.**	**nc.**	**20.3**	**1,77**	**2.32E-5**

Table 3. 9. Summary table comparing R2 values of walleye or lake whitefish trace element (TE) concentrations versus AIC suggested biometric set or age alone

Species	Elements	LR-AGE R^2	LR WT R^2	LR FL R^2	LR COMBO R^2
LKWF	Na	0.1916			
LKWF	Mg				
LKWF	Mn	0.261121			0.2786
LKWF	Ba	0.175			0.263
LKWF	Sr	0.205209			
Walleye	Na	0.07897			
Walleye	Mg	0.049729	0.06797		0.07583 (ALL)
Walleye	Ba	0.178			0.2184 (AGE+WT)
Walleye	Sr	0.106929			0.1901 (ALL)

Table 3. 10. Outlier otolith average trace element concentration comparisons. **Bold** numbering indicates outside standard deviation (SD) range.

Measure	Na (ppm)	Mg (ppm)	Mn (ppm)	Sr (ppm)	Ba (ppm)
Average LNR-LKWF (N= 9)	2677.76	10.55	1.57	838.41	4.59
SD LNR-LKWF	197.18	1.74	1.16	332.76	2.09
LNR-LKWF #56	**2900.30**	12.24	1.72	855.80	**7.02**
Measure	Na (ppm)	Mg (ppm)	Mn (ppm)	Sr (ppm)	Ba (ppm)
Average LNR-WAL (N= 9)	2174.76	6.02	0.04	298.82	5.88
SD LNR-WAL	204.94	0.96	0.06	32.54	1.01
LNR-WAL #132	**716.15**	**628.27**	0.10	**23.26**	**0.32**

Table 3. 11. Part a. CAMP waterbody depth versus trace element (TE: Ba, Mg, Mn, Na, Sr), total dissolved sediment (TDS) and total suspended sediment (TSS). Cormorant and Gauer Lake presented. part b. CAMP waterbody depth versus trace element (TE: Ba, Mg, Mn, Na, Sr), total dissolved sediment (TDS) and total suspended sediment (TSS). Leftrook, South Indian presented.

PART A	Measure	N	R	R^2	Normality	HOV	Power	F-stat	Test	P-val
Cormorant	Ba	5	0.0913	0.00833	0.275	0.05	0.034	0.0252	1	0.884
Cormorant	Mn	5	0.532	0.283	0.45	0.05	0.131	1.185	1	0.356
Cormorant	Na	5	0.103	0.0106	0.058	0.05	0.035	0.0321	1	0.869
Cormorant	Sr	5	0.139	0.0192	0.309	0.05	0.039	0.0587	1	0.824
Cormorant	TDS	5	0.666	0.444	0.498	0.05	0.205	2.396	1	0.219
Cormorant	Log Mg	5	0.0667	0.00445	0.06	0.05	0.031	0.0134	1	0.915
Cormorant	Log TSS	4	0.465	0.216	0.942	0.05	0.106	0.828	1	0.43
Gauer	Ba	3	-1	1	nc.	nc.	nc.	nc.	2	0.333
Gauer	Mg	3	-0.5	0.25	nc.	nc.	nc.	nc.	2	1
Gauer	Mn	3	1	1	nc.	nc.	nc.	nc.	2	1
Gauer	Na	3	-0.5	0.25	nc.	nc.	nc.	nc.	2	1
Gauer	Sr	3	0.5	0.25	nc.	nc.	nc.	nc.	2	1
Gauer	TDS	3	0.5	0.25	nc.	nc.	nc.	nc.	2	1
Gauer	TSS	3	-1	1	nc.	nc.	nc.	nc.	2	0.333

*1= Pearson's correlation coefficient test, 2= Spearman's correlation coefficient test

Table 3.11. part b. CAMP waterbody depth versus trace element (TE: Ba, Mg, Mn, Na, Sr), total dissolved sediment (TDS) and total suspended sediment (TSS). Leftrook, South Indian presented.

PART B	Measure	N	R	R^2	Normality	HOV	Power	F-stat	Test	P-val
Leftrook	Ba	6	0.368	0.136	0.341	0.06	0.098	0.627	1	0.473
Leftrook	Mg	6	0.322	0.104	0.629	0.06	0.084	0.463	1	0.533
Leftrook	Na	6	0.307	0.0942	0.618	0.06	0.079	0.416	1	0.554
Leftrook	Sr	6	0.378	0.143	0.209	0.06	0.102	0.668	1	0.46
Leftrook	Log Mn	6	0.34	0.116	0.212	0.06	0.089	0.524	1	0.509
Leftrook	TDS	6	-0.0857	0.00734449	nc.	nc.	nc.	nc.	2	0.919
Leftrook	TSS	4	0	0	nc.	nc.	nc.	nc.	2	1
Sout Indian (a4)	Ba	4	0.2	0.04	nc.	nc.	nc.	nc.	2	0.917
Sout Indian (a4)	Mg	4	-0.4	0.16	nc.	nc.	nc.	nc.	2	0.75
Sout Indian (a4)	Mn	4	-0.2	0.04	nc.	nc.	nc.	nc.	2	0.917
Sout Indian (a4)	Na	4	-0.4	0.16	nc.	nc.	nc.	nc.	2	0.75
Sout Indian (a4)	Sr	4	0	0	nc.	nc.	nc.	nc.	2	1
Sout Indian (a4)	TDS	4	0.6	0.36	nc.	nc.	nc.	nc.	2	0.417
Sout Indian (a4)	TSS	4	-0.632	0.399424	nc.	nc.	nc.	nc.	2	0.333

*1= Pearson's correlation coefficient test, 2= Spearman's correlation coefficient test
a4* = area 4

Table 3. 12. Water trace elements (dependent) water quality variables as independent variables. Alpha = 0.05, "n"= 8. All tests conducted are least squares regression (Pearson's correlation coefficient). **Bolded** text indicates a significant difference at an alpha of 0.05.

Dependent Measure	Independent Measure	R	R^2	Shapiro Wilk	HOV	Power	F-stat	P-value
Na	**TSS**	**0.781**	**0.611**	**0.315**	**0.16**	**0.65**	**9.413**	**0.022**
Na	**ORP-L**	**0.821**	**0.674**	**0.391**	**0.662**	**0.737**	**12.431**	**0.012**
Mg	**Pheophytin**	**0.933**	**0.871**	**0.994**	**0.353**	**0.964**	**40.353**	**<0.001**
Mg	**TDS**	**0.815**	**0.664**	**0.197**	**0.102**	**0.724**	**11.881**	**0.014**
Mn	**N-TKN**	**0.881**	**0.776**	**0.079**	**0.749**	**0.87**	**20.762**	**0.004**
Ba	**TDS**	**0.832**	**0.692**	**0.214**	**0.885**	**0.762**	**13.507**	**0.01**
Sr	**TDS**	**0.837**	**0.701**	**0.802**	**0.047**	**0.773**	**14.035**	**0.01**
Sr	**TSS**	**0.761**	**0.578**	**0.606**	**0.885**	**0.607**	**8.231**	**0.028**
log Ba	**log Pheophy.**	**0.784**	**0.615**	**0.978**	**0.705**	**0.657**	**9.601**	**0.021**
log Sr	**log TDS**	**0.868**	**0.754**	**0.168**	**0.26**	**0.842**	**18.352**	**0.005**

Table 3. 13. Summary of impounded (IMP) and non-impounded (NIMP) CAMP select correlation (Spearman's) results between otolith (bulk average) Sr concentration or average water Sr concentration, versus averaged total dissolved (TDS) or total suspended sediment (TSS) trace element concentration. Sample size n= 4 per test, alpha set to 0.05.

Type	Dependent Measure	Indep. Measure	R	R^2	P-value
Nimp otolith	Wal-Sr	TDS	0	0	1
Nimp otolith	Wal-Sr	TSS	-0.8	0.64	0.333
Imp otolith	Wal-Sr	TDS	0.4	0.16	0.75
Imp otolith	Wal-Sr	TSS	0.8	0.64	0.333
Nimp otolith	LKWF-Sr	TDS	0.4	0.16	0.75
Nimp otolith	LKWF-Sr	TSS	-1	1	0.0833
Imp otolith	LKWF-Sr	TDS	1	1	0.0833
Imp otolith	LKWF-Sr	TSS	1	1	0.0833
Nimp water	Water-Sr	TDS	1	1	0.0833
Nimp water	Water-Sr	TSS	-0.4	0.16	0.75
Imp water	Water-Sr	TDS	1	1	0.0833
Imp water	Water-Sr	TSS	0.8	0.64	0.333

Table 3. 14. Summary of impounded (IMP) and non-impounded (NIMP) CAMP select waterbody correlation (Pearson's) results between otolith (bulk average) Sr concentration or average water Sr concentration, versus averaged total dissolved (TDS) or total suspended sediment (TSS) trace element concentration Sample size n= 4 per test, alpha set to 0.05. **Bolded** text indicates a significant difference at an alpha of 0.05.

Type	Dependent Measure	Indep. Measure	R	R^2	Shapiro Wilk	HOV	Power	F-stat	P-val
NIMP Otolith	Wal-Sr	TDS	0.284	0.0806	0.189	<0.001	0.048	0.175	0.716
NIMP Otolith	**Wal-Sr**	**TSS**	**0.96**	**0.922**	**0.742**	**<0.001**	**0.497**	**23.78**	**0.04**
IMP Otolith	Wal-Sr	TDS	0.61	0.372	0.993	<0.001	0.106	1.187	0.39
IMP Otolith	Wal-Sr	TSS	0.768	0.589	0.406	<0.001	0.172	2.869	0.232
NIMP Otolith	LKWF-Sr	TDS	0.438	0.192	0.017	<0.001	0.068	0.475	0.562
NIMP Otolith	LKWF-Sr	TSS	0.921	0.849	0.96	<0.001	0.359	11.228	0.079
IMP Otolith	**LKWF-Sr**	**TDS**	**0.99**	**0.979**	**0.851**	**<0.001**	**0.747**	**94.287**	**0.01**
IMP Otolith	**LKWF-Sr**	**TSS**	**0.991**	**0.982**	**0.915**	**<0.001**	**0.767**	**107.5**	**0.009**
NIMP Water	Water-Sr	TDS	0.943	0.889	0.137	<0.001	0.423	16.092	0.057
NIMP Water	Water-Sr	TSS	0.00474	0.0000224	0.427	<0.001	0.025	0.0000449	0.995
IMP Water	**Water-Sr**	**TDS**	**0.997**	**0.994**	**0.942**	**<0.001**	**0.896**	**311.186**	**0.003**
IMP Water	Water-Sr	TSS	0.935	0.875	0.254	<0.001	0.397	13.948	0.065

Table 3. 15. Walleye (dependent) LRA trace element correlations, with water quality variables and trace element concentrations. Alpha = 0.05, "n"= 8. **Bolded** text indicates a significant difference at an alpha of 0.05.

Dependent Measure	Independent Measure	R	R^2	Shapiro Wilk	HOV	Power	Test	F-stat	P-value
Na	**Pheopytin**	**0.747**	**0.558**	**0.06**	**0.139**	**0.579**	Pearson's	**7.573**	**0.033**
Mg	**DOC**	**-0.833**	**0.693889**	**nc.**	**nc.**	**nc.**	**Spearman's**	**nc.**	**0.00526**
Mg	**N-TKN**	**-0.857**	**0.734449**	**nc.**	**nc.**	**nc.**	**Spearman's**	**nc.**	**0.00178**
Mg	**Pheopytin**	**0.781**	**0.61**	**0.171**	**0.387**	**0.65**	Pearson's	**9.391**	**0.022**
Sr	**Chl-A**	**0.893**	**0.798**	**0.676**	**0.977**	**0.895**	Pearson's	**23.664**	**0.003**
Sr	**Log Chl-A**	**0.85**	**0.722**	**0.246**	**0.321**	**0.801**	Pearson's	**15.56**	**0.008**
Na	Na	0.0663	0.0044	0.179	0.931	0.035	Pearson's	0.0265	0.876
Mg	Mg	0.635	0.403	0.275	0.794	0.388	Pearson's	4.053	0.091
Ba	Ba	0.678	0.46	0.565	0.387	0.455	Pearson's	5.11	0.065
Sr	Sr	0.526	0.276	0.609	0.839	0.256	Pearson's	2.289	0.181

Table 3. 16. Summary of impounded and non-impounded CAMP select waterbody correlation (Spearman's) results between otolith (bulk average) trace element concentration (Dependent measure), versus averaged water trace element concentration (independent measure). Sample size n= 4 per test, alpha set to 0.05. IMP= impounded, NIMP= non-impounded waterbodies, TE= trace element.

Species	Type	te	R	R^2	P-value	Species	Type	TE	R	R^2	P-value
Walleye	NIMP	Na	0	0	1	Lake whitefish	NIMP	Na	-0.4	0.16	0.75
	NIMP	Mg	0.8	0.64	0.333		NIMP	Mg	0.2	0.04	0.917
	NIMP	Sr	-0.4	0.16	0.75		NIMP	Mn	0.4	0.16	0.75
	NIMP	Ba	0.2	0.04	0.917		NIMP	Sr	0.8	0.64	0.333
	IMP	Na	-0.4	0.16	0.75		NIMP	Ba	0.2	0.04	0.917
	IMP	Mg	-0.6	0.36	0.417		IMP	Na	-0.2	0.04	0.917
	IMP	Sr	0.4	0.16	0.75		IMP	Mg	-0.4	0.16	0.75
	IMP	Ba	0.2	0.04	0.917		IMP	Mn	-0.4	0.16	0.75
							IMP	Sr	1	1	0.0833
							IMP	Ba	0.2	0.04	0.917

Table 3. 17. Summary of impounded and non-impounded CAMP select waterbody correlation (Pearson's) results between otolith (bulk average) trace element concentration (Dependent measure), versus averaged water trace element concentration (independent measure). Sample size n= 4 per test, alpha set to 0.05. IMP= impounded, NIMP= non-impounded waterbodies

Species	Type	TE	R	R^2	Normal?	HOV	Power	F-stat	P-val
Walleye	NIMP	Na	0.0638	0.00407	0.701	<0.001	0.029	0.00818	0.936
	NIMP	**Mg**	**0.963**	**0.928**	**0.954**	**<0.001**	**0.511**	**25.695**	**0.037**
	NIMP	Sr	0.218	0.0476	0.143	<0.001	0.041	0.0999	0.782
	NIMP	Ba	0.883	0.779	0.863	<0.001	0.283	7.044	0.117
	IMP	Na	0.0203	0.000414	0.723	<0.001	0.026	0.000828	0.98
	IMP	Mg	0.714	0.51	0.84	<0.001	0.144	2.083	0.286
	IMP	Sr	0.537	0.288	0.843	<0.001	0.087	0.809	0.463
	IMP	Ba	0.412	0.17	0.726	<0.001	0.064	0.409	0.588
Lake whitefish	NIMP	Na	0.258	0.0664	0.405	<0.001	0.045	0.142	0.742
	NIMP	Mg	0.523	0.273	0.83	<0.001	0.084	0.751	0.477
	NIMP	Mn	0.106	0.0112	0.821	<0.001	0.032	0.0227	0.894
	NIMP	Sr	0.398	0.158	0.154	<0.001	0.062	0.376	0.602
	NIMP	Ba	0.609	0.371	0.939	<0.001	0.105	1.18	0.391
	IMP	Na	0.264	0.0696	0.751	<0.001	0.046	0.15	0.736
	IMP	Mg	0.202	0.0407	0.861	<0.001	0.04	0.0848	0.798
	IMP	Mn	0.587	0.0344	0.385	<0.001	0.099	1.05	0.413
	IMP	**Sr**	**0.984**	**0.967**	**0.973**	**<0.001**	**0.669**	**59.485**	**0.016**
	IMP	Ba	0.155	0.024	0.795	<0.001	0.036	0.0491	0.845

Table 3. 18. Lake whitefish (dependent) LRA trace element correlations, with water quality variables and trace element concentrations. Alpha = 0.05, "n"= 8. **Bolded** text indicates a significant difference at an alpha of 0.05.

Dependent Measure	Independent Measure	R	R^2	Shapiro Wilk	HOV	Power	Test	F-stat	P-value
Ba	**DOC**	**0.768**	**0.59**	**0.473**	**0.46**	**0.622**	**Pearson's**	**8.617**	**0.026**
Ba	**DO**	**-0.707**	**0.499849**	**nc.**	**nc.**	**nc.**	**Spearman's**	**nc.**	**0.0374**
Na	Na	0.272	0.0737	0.821	0.26	0.091	Pearson's	0.478	0.515
Mg	Mg	0.464	0.215	0.513	0.794	0.201	Pearson's	1.647	0.247
Mn	Mn	0.119	nc.	nc.	nc.	nc.	Spearman's	nc.	0.749
Ba	Ba	0.32	0.102	0.574	0.353	0.111	Pearson's	0.684	0.44
Sr	Sr	0.67	0.449	0.731	0.353	0.442	Pearson's	4.887	0.069

Table 3. 19. Part a. Summary table of correlation (Pearson's coefficient test) outputs for the comparison of otolith trace element concentrations for both walleye and lake whitefish. Sample size for both species equal to 79, with an alpha of 0.05. Part b. Summary table of correlation (Spearman's coefficient test) outputs. **Bolded** text indicates a significant difference at an alpha of 0.05.

Part A Species	TE1	TE2	R	R^2	Shapiro-Wilk W	Shapiro-Wilk p(normal)	Levene´s means p(same)	Levene´s medians p(same)	P-val
WAL	Na	Mg	0.0804	0.00646416	nc.	nc.	nc.	nc.	0.481
WAL	Na	Sr	0.0283	0.00080089	nc.	nc.	nc.	nc.	0.804
WAL	Na	Ba	-0.05	0.0025	nc.	nc.	nc.	nc.	0.662
WAL	Mg	Sr	-0.0057	3.27184E-05	nc.	nc.	nc.	nc.	0.96
WAL	**Mg**	**Ba**	**0.323**	**0.104329**	**0.9506**	**2.29E-05**	**0.235**	**0.5866**	**0.00366**
WAL	**Ba**	**Sr**	**0.63**	**0.3969**	**0.9938**	**0.7379**	**0.06363**	**0.6339**	**4.8E-10**
LKWF	**Sr**	**Ba**	**0.685**	**0.469225**	**0.989**	**0.2516**	**0.0006978**	**0.001446**	**3.35E-12**
LKWF	Sr	Na	-0.191	0.036481	nc.	nc.	nc.	nc.	0.0922
LKWF	Sr	Mg	-0.139	0.019321	nc.	nc.	nc.	nc.	0.222
LKWF	Sr	Mn	-0.102	0.010404	nc.	nc.	nc.	nc.	0.37
LKWF	Ba	Na	-0.0333	0.00110889	nc.	nc.	nc.	nc.	0.771
LKWF	Ba	Mg	-0.108	0.011664	nc.	nc.	nc.	nc.	0.341
LKWF	Ba	Mn	0.0209	0.00043681	nc.	nc.	nc.	nc.	0.855
LKWF	Na	Mg	0.178	0.031684	nc.	nc.	nc.	nc.	0.117
LKWF	Na	Mn	0.0615	0.00378225	nc.	nc.	nc.	nc.	0.59
LKWF	Mg	Mn	0.153	0.023409	nc.	nc.	nc.	nc.	0.177

Table 3.19. part b. Summary table of correlation (Spearman's coefficient test) outputs for the comparison of otolith trace element concentrations for both walleye and lake whitefish. Sample size for both species equal to 79, with an alpha of 0.05. **Bolded** text indicates a significant difference at an alpha of 0.05.

Part B Species	TE1	TE2	R	R^2	Shapiro-Wilk W	Shapiro-Wilk p(normal)	Levene´s test HOV, from means p(same)	Levene´s test, from medians p(same)	P-val
WAL	Na	Mg	0.135	0.018225	nc.	nc.	nc.	nc.	0.235
WAL	Na	Sr	0.013	0.000169	nc.	nc.	nc.	nc.	0.909
WAL	Na	Ba	-0.092	0.008464	nc.	nc.	nc.	nc.	0.419
WAL	Mg	Sr	0.0999	0.00998001	nc.	nc.	nc.	nc.	0.38
WAL	**Mg**	**Ba**	**0.315**	**0.099225**	**0.9506**	**2.29E-05**	**0.235**	**0.5866**	**0.00477**
WAL	**Ba**	**Sr**	**0.652**	**0.425104**	**0.9938**	**0.7379**	**0.06363**	**0.6339**	**0.0000002**
LKWF	**Sr**	**Ba**	**0.71**	**0.5041**	**0.989**	**0.2516**	**0.0006978**	**0.001446**	**0.0000002**
LKWF	Sr	Na	-0.197	0.038809	nc.	nc.	nc.	nc.	0.082
LKWF	Sr	Mg	-0.090	0.00801025	nc.	nc.	nc.	nc.	0.432
LKWF	Sr	Mn	-0.109	0.011881	nc.	nc.	nc.	nc.	0.338
LKWF	Ba	Na	-0.0129	0.00016641	nc.	nc.	nc.	nc.	0.91
LKWF	Ba	Mg	-0.109	0.011881	nc.	nc.	nc.	nc.	0.339
LKWF	Ba	Mn	-0.0252	0.00063504	nc.	nc.	nc.	nc.	0.825
LKWF	Na	Mg	0.213	0.045369	nc.	nc.	nc.	nc.	0.0601
LKWF	Na	Mn	0.0681	0.00463761	nc.	nc.	nc.	nc.	0.55
LKWF	Mg	Mn	0.129	0.016641	nc.	nc.	nc.	nc.	0.255

Table 3. 20. Correlation analysis Pearson correlation coefficient) of Walleye and lake whitefish LRA trace element average concentrations per CAMP waterbodies (n=8).

Dependent Measure	Independent Measure	R	R^2	Shapiro Wilk	HOV	Power	F-stat	P-value
WAL-Na	LKWF-Na	0.301	0.0903	0.112	0.423	0.103	0.596	0.469
WAL-Mg	LKWF-Mg	0.447	0.2	0.86	0.321	0.188	1.501	0.266
WAL-Sr	LKWF-Sr	0.852	0.726	0.977	0.26	0.806	15.874	0.007
WAL-Ba	LKWF-Ba	0.374	0.14	0.98	0.749	0.14	0.978	0.361
LKWF-Na	WAL-Na	0.301	0.0903	0.244	0.26	0.103	0.596	0.469
LKWF-Mg	WAL-Mg	0.447	0.2	0.75	0.321	0.188	1.501	0.266
LKWF-Sr	WAL-Sr	0.852	0.726	0.428	0.537	0.806	15.874	0.007
LKWF-Ba	WAL-Ba	0.374	0.14	0.166	0.182	0.14	0.978	0.361

Table 3. 21. Water trace element (TE) concentration (dependent) versus year range (independent) per selected CAMP waterbody (N= 8) for correlation coefficient test (Pearson's correlation coefficient) table. Year range is within the range of 2008-2014. **Bolded** text indicates a significant difference at an alpha of 0.05.

TE	Waterbody	N	R^2	Normal (SW)	HOV	Power	F-stat	P-val
Ba	Assean	5	0.0025	0.876	0.05	0.029	0.00766	0.936
Ba	Cormorant	7	0.0564	0.211	0.388	0.07	0.299	0.608
Ba	Gauer	7	0.47	0.839	0.096	0.389	4.426	0.089
Ba	Leftrook	6	0.53	0.814	0.06	0.36	4.503	0.101
Ba	Lower Nelson	5	0.00954	0.313	0.05	0.034	0.0289	0.876
Ba	South Indian	7	0.0612	0.712	0.491	0.073	0.326	0.593
Ba	Split	6	0.24	0.972	0.06	0.151	1.264	0.324
Ba	Threepoint	6	0.307	0.723	0.06	0.19	1.771	0.254
Mg	Assean	5	0.391	0.679	0.05	0.178	1.924	0.26
Mg	**Cormorant**	**7**	**0.593**	**0.423**	**0.255**	**0.532**	**7.278**	**0.043**
Mg	Gauer	7	0.537	0.537	0.217	0.464	5.799	0.061
Mg	**Leftrook**	**6**	**0.865**	**0.145**	**0.06**	**0.819**	**25.528**	**0.007**
Mg	Lower Nelson	5	0.649	0.556	0.05	0.35	5.535	0.1
Mg	South Indian	7	0.095	0.097	0.217	0.093	0.525	0.501
Mg	Split	6	0.421	0.538	0.06	0.268	2.914	0.163
Mg	**Threepoint**	**6**	**0.691**	**0.252**	**0.06**	**0.542**	**8.948**	**0.04**
Mn	Assean	5	0.109	0.518	0.05	0.07	0.368	0.587
Mn	Cormorant	7	0.312	0.679	0.217	0.242	2.263	0.193
Mn	Gauer	7	0.178	0.774	0.438	0.145	1.084	0.345
Mn	Lower Nelson	5	0.0000145	0.308	0.05	0.025	0.0000436	0.995
Mn	South Indian	7	0.00361	0.461	0.073	0.033	0.0181	0.898
Mn	Split	6	0.00365	0.943	0.06	0.032	0.0147	0.909

Mn	Threepoint	6	0.392	0.815	0.06	0.246	2.582	0.183
Mn*	Leftrook	6	0.294849	nc.	nc.	nc.	nc.	0.297
Na	Assean	5	0.491	0.909	0.05	0.232	2.895	0.187
Na	Cormorant	7	0.201	0.667	0.491	0.16	1.255	0.313
Na	**Gauer**	**7**	**0.802**	**0.585**	**0.72**	**0.826**	**20.269**	**0.006**
Na	**Leftrook**	**6**	**0.946**	**0.257**	**0.06**	**0.96**	**70.436**	**0.001**
Na	Lower Nelson	5	0.519	0.215	0.05	0.25	3.235	0.17
Na	South Indian	7	0.0889	0.241	0.096	0.089	0.488	0.516
Na	Split	6	0.431	0.463	0.06	0.275	3.035	0.156
Na	**Threepoint**	**6**	**0.867**	**0.063**	**0.06**	**0.823**	**26.076**	**0.007**
Sr	Assean	5	0.582	0.058	0.05	0.294	4.173	0.134
Sr	Cormorant	7	0.438	0.553	0.388	0.357	3.9	0.105
Sr	**Gauer**	**7**	**0.822**	**0.962**	**0.438**	**0.855**	**23.109**	**0.005**
Sr	**Leftrook**	**6**	**0.696**	**0.709**	**0.06**	**0.549**	**9.166**	**0.039**
Sr	Lower Nelson	5	0.218	0.505	0.05	0.107	0.834	0.428
Sr	South Indian	7	0.461	0.129	0.096	0.38	4.28	0.093
Sr	Split	6	0.403	0.584	0.06	0.254	2.702	0.176
Sr	Threepoint	6	0.577	0.346	0.06	0.406	5.446	0.08

*Spearman's correlation coefficient

Table 3. 22. Mean and St. Dev values for trace element water concentrations from pooled impounded and non-impounded t-tests (a), and post-hoc ANOVA individual CAMP waterbody trace element water concentration tests (b-i) with selections with the same letter being not statistically different from each other by whatever statistical test was used.

Site	Water (mg/L)				
	Na	Mg	Mn	Ba	Sr
Test	Kruskal-Wallis	1-Way ANOVA	1-Way ANOVA	1-Way ANOVA	1-Way ANOVA
Post-hoc test	Dunn's	Tukey's	Tukey's	Tukey's	Tukey's
Assean[b]	2.42 ± 0.42[b,c,d,e,f,g,i]	6.98 ± 1.04[b,d,e]	0.016 ± 0.0096[b,d,h,i]	0.014 ± 0.0025[b,e,i]	0.053 ± 0.0089[b,c,e]
Cormorant[c]	2.69 ± 0.20[b,c,d,e,f,g,h,i]	19.24 ± 1.23[c]	0.0027 ± 0.0012[c,g]	0.035 ± 0.0025[c,f,h]	0.063 ± 0.0042[b,c,e]
Gauer[d]	1.61 ± 0.25[b,c,d,e,f,g]	5.80 ± 0.84[b,d,g]	0.014 ± 0.0092[b,d,h,i]	0.0097 ± 0.0013[d,g]	0.033 ± 0.0053[d,g,i]
Leftrook[e]	3.02 ± 0.48[b,c,d,e,f,g,h,i]	7.59 ± 1.05[b,e]	0.044 ± 0.028[e]	0.013 ± 0.0028[b,e]	0.053 ± 0.0069[b,c,e]
Lower Nelson[f]	15.49 ± 3.64[c,e,f,g,h,i]	11.32 ± 1.65[f,h]	0.018 ± 0.0031[b,d,f,h,i]	0.034 ± 0.0040[c,f,h]	0.10 ± 0.017[f,h]
S. Indian[g]	2.64 ± 0.24[b,c,d,e,f,g,i]	4.75 ± 0.60[d,g,i]	0.004 ± 0.0034[c,g]	0.011 ± 0.0015[d,g]	0.034 ± 0.0040[d,g,i]
Split[h]	17.17 ± 5.49[c,e,f,h,i]	12.03 ± 2.84[f,h]	0.016 ± 0.0048[b,d,f,h,i]	0.036 ± 0.0077[c,f,h]	0.11 ± 0.028[f,h]
Threepoint[i]	3.27 ± 0.44[b,c,e,f,g,h,i]	4.58 ± 0.53[g,i]	0.013 ± 0.0031[b,d,f,h,i]	0.017 ± 0.0023[b,i]	0.040 ± 0.0049[d,g,i]
Test			Mann-Whitney U		
Non-Imp.[a]	2.46 ± 0.63	10.37 ± 5.81[a]	0.018 ± 0.016[a]	0.019 ± 0.011[a]	0.051 ± 0.013[a]
Impounded[a]	9.34 ± 7.62	8.03 ± 3.92[a]	0.012 ± 0.0062[a]	0.024 ± 0.012[a]	0.070 ± 0.039[a]

Table 3. 23. Mean and St. Dev values for walleye otolith trace elements. Superscripted letters are for pooled impounded and non-impounded otolith trace element concentration t-test (a), post-hoc ANOVA individual CAMP waterbody otolith trace element tests (b-i) and trace element-trace element correlations (j-n) with selections with the same letter being not statistically different from each other by whatever statistical test used.

Site	Walleye Otolith (LRA) Na [j,k,l,m]	Mg [j,k,m]	Ba [j,l]	Sr [j,k,m]
Test	1-Way ANOVA	Kruskal-Wallis	1-Way ANOVA	1-Way ANOVA
Post-hoc test	Tukey's	Dunn's	Tukey's	Tukey's
Assean[b]	3.32 ± 0.0019[b,c,d,e,f,g,h,i]	0.71 ± 0.044[b,d,e,f,g,h,i]	0.60 ± 0.086[b,d,e,g]	2.09 ± 0.048[b,g]
Cormorant[c]	3.29 ± 0.023[b,c,e,f,g,h,i]	1.08 ± 0.27[c,e,f,g,h,i]	0.96 ± 0.095[c,f,i]	2.23 ± 0.051[c,d,e,g]
Gauer[d]	3.35 ± 0.038[b,d,e,f,g,h,i]	0.76 ± 0.17[b,d,e,f,g,h,i]	0.73 ± 0.12[b,e,f,g,h]	2.23 ± 0.040[c,d,e,g]
Leftrook[e]	3.32 ± 0.037[b,c,d,e,f,g,h,i]	0.77 ± 0.12[b,c,d,e,f,g,h,i]	0.67 ± 0.12[b,d,e,g]	2.21 ± 0.040[c,d,e,g]
Lower Nelson[f]	3.33 ± 0.039[b,c,d,e,f,g,h,i]	0.74 ± 0.10[b,c,d,e,f,g,h,i]	0.85 ±0.058[c,d,f,h,i]	2.44 ± 0.037[f,i]
South Indian[g]	3.32 ± 0.054[b,c,d,e,f,g,h,i]	0.89 ± 0.18[b,c,d,e,f,g,h,i]	0.64 ± 0.094[b,d,e,g]	2.16 ± 0.063[b,c,d,e]
Split[h]	3.32 ± 0.034[b,c,d,e,f,g,h,i]	0.83 ± 0.080[b,c,d,e,f,g,h,i]	0.82 ± 0.11[d,f,h,i]	2.36 ± 0.058[h,i]
Threepoint[i]	3.32 ± 0.044[b,c,d,e,f,g,h,i]	0.88 ± 0.26[b,c,d,e,f,g,h,i]	0.94 ± 0.11[c,f,h,i]	2.42 ± 0.052[f,h,i]
Test	1-Way ANOVA	Kruskal-Wallis	Kruskal-Wallis	1-Way ANOVA
Non-Imp.[a]	3.32 ± 0.037[a]	0.83 ± 0.22[a]	0.74 ± 0.17	2.19 ± 0.073
Impounded[a]	3.32 ± 0.042[a]	0.84 ± 0.18[a]	0.81 ± 0.14	2.34 ± 0.13

Table 3. 24. Mean and St. Dev values for lake whitefish otolith trace elements. Superscripted letters are for pooled impounded and non-impounded otolith trace element concentration t-test (a), post-hoc ANOVA individual CAMP waterbody otolith trace element tests (b-i) and trace element-trace element correlations (j-n) with selections with the same letter being not statistically different from each other by whatever statistical test used.

Site	Lake whitefish Otolith (LRA)				
	Na [j,k,l,m,n]	Mg [j,k,l,m,n]	Mn [j,k,l,m,n]	Ba [j,k,l,n]	Sr [j,k,m,n]
Test	1-Way ANOVA	Kruskal-Wallis	1-Way ANOVA	Kruskal-Wallis	1-Way ANOVA
Post-hoc	Tukey's	Dunn's	Tukey's	Dunn's	Tukey's
Assean[b]	3.42 ± 0.034[b,c,d,e,f,g,h,i]	1.05 ± 0.062[b,c,d,e,f,g,h,i]	0.023 ± 0.13[b,c,e,f,g,h,i]	0.34 ± 0.12[b,c,d,e]	2.43 ± 0.049[b,c,d,e]
Cormorant[c]	3.44 ± 0.039[b,c,d,e,f,g,h,i]	1.38 ± 0.25[b,c,d,h,i]	(-)0.14 ± 0.17[b,c,f,g,h]	0.52 ± 0.16[b,c,d,e,f]	2.66 ± 0.045[b,c,d,e,f,g,h,i]
Gauer[d]	3.47 ± 0.034[b,c,d,f,g,h]	1.32 ± 0.36[b,c,d,e,f,h,i]	0.45 ± 0.27[d,e,i]	0.48 ± 0.083[b,c,d,e,f]	2.54 ± 0.076[b,c,d,e,g]
Leftrook[e]	3.40 ± 0.057[b,c,e,f,h,i]	0.98 ± 0.15[b,d,e,f,g,h,i]	0.20 ± 0.27[b,d,e,f,g,h,i]	0.40 ± 0.13[b,c,d,e]	2.58 ± 0.051[b,c,d,e,g,i]
L. Nelson[f]	3.42 ± 0.016[b,c,d,e,f,g,h,i]	1.02 ± 0.071[b,d,e,f,g,h,i]	0.065 ± 0.020[b,c,e,f,g,h,i]	0.67 ± 0.16[c,d,f,g,h,i]	2.87 ± 0.12[c,f,g,h,i]
S. Indian[g]	3.46 ± 0.034[b,c,d,f,g,h]	0.89 ± 0.19[b,e,f,g,h,i]	(-)0.024 ± 0.31[b,c,e,f,g,h]	0.81 ± 0.13[f,g,h,i]	2.69 ± 0.065[c,d,e,f,g,h,i]
Split[h]	3.41 ± 0.029[b,c,d,e,f,g,h,i]	1.09 ± 0.11[b,c,d,e,f,h,i]	0.087 ± 0.23[b,c,e,f,g,h,i]	0.84 ± 0.17[f,g,h,i]	2.86 ± 0.056[c,f,g,h,i]
Threepoint[i]	3.39 ± 0.055[b,c,e,f,h,i]	1.10 ± 0.14[b,c,d,e,f,g,h,i]	0.36 ± 0.28[b,d,e,f,h,i]	0.72 ± 0.14[f,g,h,i]	2.75 ± 0.088[c,e,f,g,h,i]
Test	1-Way ANOVA	Kruskal-Wallis	Kruskal-Wallis	1-Way ANOVA	1-Way ANOVA
Non-Imp.[a]	3.43 ± 0.048[a]	1.18 ± 0.28	0.13 ± 0.31[a]	0.44 ± 0.14	2.55 ± 0.098
Impounded[a]	3.42 ± 0.044[a]	1.02 ± 0.16	0.12 ± 0.29[a]	0.76 ± 0.16	2.79 ± 0.11

Table 3. 25. Centroid/biplot scores from the redundancy analyses (RDA) with walleye and lake whitefish otoliths exclusively using AREA as the species variables, and trace elements (TE) as the environmental variables.

	Lake whitefish						Walleye			
	Na,Mg,Mn,Ba,Sr		Na,Mg,Ba,Sr		Ba,Sr		Na,Mg,Ba,Sr		Ba,Sr	
Waterbody	RDA1	RDA2	RDA1	RDA2	RDA1	RDA2	RDA1	RDA2	RDA1	RDA2
ASS	0.749	0.543	0.724	-0.458	-0.654	0.116	0.758	0.024	-0.624	0.033
CORM	0.186	-0.607	0.206	0.683	-0.094	-0.230	-0.363	1.088	0.177	0.965
GAU	0.547	-0.874	0.484	0.732	-0.327	0.158	0.222	-0.330	-0.142	-0.023
LFT	0.356	0.694	0.331	-0.694	-0.349	-0.328	0.327	-0.135	-0.258	-0.233
LNR	-0.527	0.134	-0.494	-0.119	0.401	-0.721	-0.495	-0.639	0.490	-0.464
3PT	-0.315	0.086	-0.326	-0.204	0.254	0.132	-0.645	-0.106	0.550	0.018
SIL4	-0.359	0.164	-0.323	-0.067	0.252	0.750	0.431	0.211	-0.398	-0.105
SPLT	-0.690	-0.127	-0.652	0.115	0.558	0.052	-0.284	-0.178	0.253	-0.238
TE										
Na	0.257	-0.704	0.278	0.809	nc.	nc.	0.212	-0.693	nc.	nc.
Mg	0.456	-1.080	0.476	1.124	nc.	nc.	-0.534	1.016	nc.	nc.
Mn	0.192	-0.225	nc.	nc.	nc.	nc.	nc.	nc.	nc.	nc.
Sr	-1.497	-0.203	-1.826	0.194	2.187	-0.544	-1.733	-0.812	2.173	-0.710
Ba	-1.725	-0.245	-1.586	0.255	1.904	0.625	-1.597	0.449	1.809	0.853

Table 3. 26. RDA analyses permutation test results for walleye and lake whitefish otoliths exclusively using area as the species variables and trace elements (TE) as the environmental variables.

		RDA1			RDA2			RDA3		
Species	TE	df	F	P-val	df	F	P-val	df	F	P-val
LKWF	Na, Mg, Mn, Ba, Sr	1,73	42.8612	0.001	1,73	14.0613	0.001	1,73	12.5883	0.001
LKWF	Na, Mg, Ba, Sr	1,74	57.7377	0.001	1,74	18.9705	0.001	1,74	12.6079	0.001
LKWF	Ba, Sr	1,76	188.372	0.001	1,76	15.366	0.001	1,76	nc.	nc.
WAL	Na, Mg, Ba, Sr	1,74	48.334	0.001	1,74	19.4893	0.001	1,74	2.0592	0.111
WAL	Ba, Sr	1,76	186.24	0.001	1,76	28.706	0.001	nc.	nc.	nc.
		RDA4			RDA5			FULL MODEL		
Species	Trace Elements	df	F	P-val	df	F	P-val	df	F	P-val
LKWF	Na, Mg, Mn, Ba, Sr	1,73	8.8712	0.001	1,73	1.7276	0.001	7,71	11.131	0.001
LKWF	Na, Mg, Ba, Sr	1,74	2.4742	0.052	nc.	nc.	nc.	7,71	12.581	0.001
LKWF	Ba, Sr	nc.	nc.	nc.	nc.	nc.	nc.	7,71	27.191	0.001
WAL	Na, Mg, Ba, Sr	1,74	1.2383	0.285	nc.	nc.	nc.	7,71	9.7482	0.001
WAL	Ba, Sr	nc.	nc.	nc.	nc.	nc.	nc.	7,71	28.686	0.001

Table 3. 27. Summary matrix of KNN (k=1) for walleye otolith Na, Mg, Ba and Sr trace element concentrations and classified based on waterbody. Jackknife percent correct classification = 45.6%. **Bolded** numbers are the number of correct classifications.

	ASS	CORM	GAU	LFT	LNR	SIL4	SPLT	3PT	Total	% correct
ASS	**9**	0	0	1	0	0	0	0	10	90
CORM	0	**9**	1	0	0	0	0	0	10	90
GAU	1	2	**0**	5	0	1	0	1	10	0
LFT	0	0	3	**3**	0	4	0	0	10	30
LNR	0	0	0	0	**4**	0	3	2	9	44.4
SIL4	2	0	1	3	0	**4**	0	0	10	40
SPLT	0	0	0	1	3	0	**4**	2	10	40
3PT	0	2	1	0	3	0	1	**3**	10	30
Total	12	13	6	13	10	9	8	8	79	

Table 3. 28. Summary matrix of KNN (k= 11) for walleye otolith Na, Mg, Ba and Sr trace element concentrations and classified based on impoundment status. Jackknife percent correct classification = 82.2%. **Bolded** numbers are the number of correct classifications.

	NIMP	IMP	Total	% correct
NIMP	**34**	6	40	85
IMP	8	**31**	39	79.5
Total	42	37	79	

Table 3. 29. Summary matrix of KNN (k= 5) for walleye otolith Ba and Sr trace element concentrations and classified based on waterbody. Jackknife percent correct classification = 46.8%. **Bolded** numbers are the number of correct classifications.

	ASS	CORM	GAU	LFT	LNR	SIL4	SPLT	3PT	Total	% correct
ASS	**8**	0	0	0	0	2	0	0	10	80
CORM	0	**9**	1	0	0	0	0	0	10	90
GAU	1	2	**2**	3	0	2	0	0	10	20
LFT	0	0	4	**3**	0	3	0	0	10	30
LNR	0	0	1	0	**4**	0	2	2	9	44.4
SIL4	3	0	1	3	0	**3**	0	0	10	30
SPLT	0	0	1	1	2	0	**4**	2	10	40
3PT	0	0	0	0	3	0	3	**4**	10	40
Total	12	11	10	10	9	10	9	8	79	

Table 3. 30. Summary matrix of KNN (k= 11) for walleye otolith Ba and Sr trace element concentrations and classified based on impoundment status. Jackknife percent correct classification = 82.1%. **Bolded** numbers are the number of correct classifications.

	NIMP	IMP	Total	% correct
NIMP	**39**	1	40	97.5
IMP	13	**26**	39	66.7
Total	52	27	79	

Table 3. 31. Summary matrix of KNN (k= 5) for lake whitefish otolith Na, Mg, Mn, Ba and Sr trace element concentrations and classified based on waterbody. Jackknife percent correct classification = 52.9%. **Bolded** numbers are the number of correct classifications.

	ASS	CORM	GAU	LFT	LNR	SIL4	SPLT	3PT	Total	% correct
ASS	**8**	1	1	0	0	0	0	0	10	80
CORM	1	**8**	1	0	0	0	0	0	10	80
GAU	2	1	**2**	2	0	0	0	3	10	20
LFT	1	0	3	**5**	0	1	0	0	10	50
LNR	0	0	0	0	**3**	1	2	3	9	33.3
SIL4	0	0	1	0	0	**9**	0	0	10	90
SPLT	0	0	0	0	4	1	**2**	3	10	20
3PT	0	0	1	0	2	0	2	**5**	10	50
Total	12	10	9	7	9	12	6	14	79	

Table 3. 32. Summary matrix of KNN (k= 3) for lake whitefish otolith Na, Mg, Mn, Ba and Sr trace element concentrations and classified based on impoundment status. Jackknife percent correct classification = 96.2%. **Bolded** numbers are the number of correct classifications.

	NIMP	IMP	Total	% correct
NIMP	**39**	1	40	97.5
IMP	2	**37**	39	94.9
Total	41	38	79	

Table 3. 33. Summary matrix of KNN (k= 3) for lake whitefish otolith Na, Mg, Ba and Sr trace element concentrations and classified based on impoundment status. Jackknife percent correct classification = 96.2%. **Bolded** numbers are the number of correct classifications.

	NIMP	IMP	Total	% correct
NIMP	**40**	0	40	100
IMP	3	**36**	39	92.3
Total	43	36	79	

Table 3. 34. Summary matrix of KNN (k= 9) for lake whitefish otolith Na, Mg, Ba and Sr trace element concentrations and classified based on waterbody. Jackknife percent correct classification = 55.42%. **Bolded** numbers are the number of correct classifications.

	ASS	CORM	GAU	LFT	LNR	SIL4	SPLT	3PT	Total	% correct
ASS	**10**	0	0	0	0	0	0	0	10	100
CORM	0	**6**	2	1	0	0	0	1	10	60
GAU	3	4	**1**	2	0	0	0	0	10	10
LFT	3	1	1	**5**	0	0	0	0	10	50
LNR	0	0	0	1	**3**	4	0	1	9	33.3
SIL4	0	0	0	0	0	**9**	1	0	10	90
SPLT	0	1	0	0	1	0	**5**	3	10	50
3PT	0	1	0	0	0	1	2	**5**	10	50
Total	16	13	4	9	4	14	8	10	79	

Table 3. 35. Summary matrix of KNN (k= 3) for lake whitefish otolith Ba and Sr trace element concentrations and classified based on waterbody. Jackknife percent correct classification = 39.2%. **Bolded** numbers are the number of correct classifications.

	ASS	CORM	GAU	LFT	LNR	SIL4	SPLT	3PT	Total	% correct
ASS	**6**	2	0	2	0	0	0	0	10	60
CORM	0	**2**	2	3	1	2	0	0	10	20
GAU	1	1	**6**	2	0	0	0	0	10	60
LFT	1	3	1	**4**	0	0	0	1	10	40
LNR	0	1	0	0	**3**	0	4	1	9	33.3
SIL4	0	2	0	0	0	**5**	1	2	10	50
SPLT	0	0	0	0	4	0	**3**	3	10	30
3PT	0	1	1	0	3	2	1	**2**	10	20
Total	8	12	10	11	11	9	9	9	79	

Table 3. 36. Summary matrix of KNN (k= 3) for lake whitefish otolith Ba and Sr trace element concentrations and classified based on impoundment status. Jackknife percent correct classification = 95%. **Bolded** numbers are the number of correct classifications.

	NIMP	IMP	Total	% correct
NIMP	**37**	3	40	92.5
IMP	1	**38**	39	97.4
Total	38	41	79	

Table 3. 37. Summary matrix of KNN (k= 9) for walleye and lake whitefish species comparison via trace element concentrations: Na, Mg, Ba and Sr. Jackknife percent correct classification = 100%. **Bolded** numbers are the number of correct classifications.

	WAL	LKWF	Total	% correct
WAL	**79**	0	79	100
LKWF	0	**79**	79	100
Total	79	79	158	

Table 3. 38. Summary matrix of KNN (k= 9) for walleye and lake whitefish species comparison via trace element concentrations: Ba and Sr. Jackknife percent correct classification = 98.7%. **Bolded** numbers are the number of correct classifications.

	WAL	LKWF	Total	% correct
WAL	**78**	1	79	98.7
LKWF	1	**78**	79	98.7
Total	79	79	158	

Table 3. 39. Centroid/biplot scores from the redundancy analyses (RDA) with walleye and lake whitefish otoliths using AREA and SPP as the species variables, and trace elements as the environmental variables.

Waterbody	RDA1	RDA2
ASS	0.1351422	0.678215
CORM	-0.0598429	-0.143018
GAU	-0.0928139	0.305843
LFT	0.0825693	0.378244
LNR	-0.0729714	-0.342871
3PT	0.0004778	0.454578
SIL4	0.0363392	-0.001287
SPLT	-0.0361974	-0.454835
Species		
LKWF	-0.3910868	0.045889
WAL	0.3910868	-0.045889
Trace Element		
Na	-1.96	0.4132
Mg	-1.414	-0.1252
Sr	-2.147	-0.9281
Ba	0.858	-1.5854

Table 3. 40. Otolith trace element averages (dependent) versus upstream or downstream flow rate averages (m*s-3) as independent variables for adjacent control or generating structures. Alpha = 0.05, Parametric requirements not met, Spearman's correlation tests conducted for each comparison.

Dependent Measure	Species	US or DS	"n"	R	R^2	P-value
Na	WAL	US	4	0.2	0.04	0.917
Na	WAL	DS	3	-1	1	0.333
Na	LKWF	US	4	-0.2	0.04	0.917
Na	LKWF	DS	3	-0.5	0.25	1
Mg	WAL	US	4	-1	1	0.0833
Mg	WAL	DS	3	-1	1	0.333
Mg	LKWF	US	4	0.2	0.04	0.917
Mg	LKWF	DS	3	0.5	0.25	1
Mn	LKWF	US	4	0.2	0.04	0.917
Mn	LKWF	DS	3	0.5	0.25	1
Sr	WAL	US	4	0.8	0.64	0.333
Sr	WAL	DS	3	0.5	0.25	1
Sr	LKWF	US	4	1	1	0.0833
Sr	LKWF	DS	3	1	1	0.333
Ba	WAL	US	4	0.2	0.04	0.917
Ba	WAL	DS	3	0.5	0.25	1
Ba	LKWF	US	4	-0.8	0.64	0.333
Ba	LKWF	DS	3	-0.5	0.25	1

3.5. Chapter Three References

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CHAPTER 4:

Study Synthesis: Otoliths as Biomonitors (Conclusions and Future Work)

4.1. Thesis Objectives and Main Findings

4.1.1. MnOTOL Study

The main objectives of the MnOTOL mesocosm manganese exposure study were to first determine if manganese (Mn) concentrations (exposure as $MnSO_4$) within shallow wetland mesocosm water and sediment will positively correlate with fish otolith Mn concentrations and secondly to compare separate fish species (northern redbelly dace and fathead minnow) in the shallow wetland mesocosm setting to determine if there is a difference in otolith trace metal signatures when background metal exposure is the same. It was hypothesized that Mn concentrations within shallow wetland mesocosm water and sediment would positively correlate with fish otolith Mn concentrations and that northern redbelly dace and fathead minnow otolith trace metal signatures will differ even though background shallow wetland mesocosm metal exposure is the same. After conducting the study, results indicated that first, Mn concentrations within shallow wetland mesocosm water and sediment did not significantly correlate with fish otolith Mn concentrations. This was likely due to the initial water Mn exposure concentrations in treatment mesocosms having decreased too rapidly through time due to oxidizing conditions (greater eH), and additionally that exposure concentrations were too low once measured versus nominal concentrations. Because of the latter, sediment Mn did not follow the Mn exposure gradient, otolith signatures were unaffected by treatment and no correlation was established for either species. Comparing the trace element signatures between species indicated that northern redbelly dace and fathead minnow did not show a consistent difference in

otolith trace metal content between each other per treatment. Pre-exposure annuli also did not differentiate species consistently (low detected Mn levels in each). Additional testing is required in all cases to clarify results, especially due to the low exposure concentration and sample sizes for the various correlations tests.

4.1.2. CAMP-O Study

The main objectives of the CAMP-O study were to first compare Manitoban waterbodies of varying underlying geology to examine the influence of underlying geology on otolith trace element signatures, secondly to determine the effect of impoundment of fish otolith and water trace element signature by comparing CAMP waterbodies that are impounded and non-impounded, thirdly to examine archived otoliths across a gradient of trace element concentrations in the CAMP-O study near-surface waters for lake whitefish (*C. clupeaformis*) (benthic) and walleye (*S. vitreus*) (bentho-pelagic) and lastly to characterize the variation within, and between tested fish species otolith trace element signatures within and amongst CAMP study sites. It was hypothesized that the underlying geologies of CAMP study water bodies will be reflected in otolith trace element signatures, that the impoundment of a site will not modify the otolith signature relative to non-impounded CAMP study sites, that the fish species from the same CAMP study site will have the same otolith trace element signatures and that as CAMP study surface water concentrations of trace elements increase, so too will the trace element signatures in the fish otoliths. After conducting the study, results indicated that first, underlying geologies of CAMP study sites reflected otolith trace element signatures in a generalized sense e.g., waterbodies underlain with

more soluble sedimentary geology or abundant sediments contained greater concentrations of certain trace elements than those underlain with igneous or metamorphic. Secondly, Impounded waterbodies and non-impounded waterbodies were found to contain differing trace element concentrations (mostly for otolith Ba and Sr signatures). Differences between impounded waterbodies were assumed to be associated to some degree as well with upstream influences/accumulation, and flow alteration (effect of impoundment may be secondary to upstream influences). Both species and their otoliths successfully classify waterbodies and impoundment type to varying degrees. Thirdly, that separate fish species otoliths from the same CAMP study site did not contain the same trace element concentrations; being species specific more so than area specific. Lastly, no correlation was reached between near-surface water and otolith trace element concentrations for either species. Water trace element and water quality variable tests that were conducted at different depths and against each other failed to show any consistent correlation with otolith or water trace element content. Additional testing is required in all cases to clarify results.

4.2. Study Implications

4.2.1. MnOTOL Implications

Some of implications of this study are that small baitfish species like fathead minnow and northern redbelly dace may not be suitable biomonitors for environmentally relevant concentrations of Mn, and that other biomonitors would have to be identified to fulfill this position (although insufficient exposure concentration may be to blame for this) due to the lack of correlation established in each case. Secondly, the use of redox

sensitive materials in exposure studies are exceedingly difficult, and determining the difference between nominal and measured concentrations within the test environment are crucial. Thirdly, PWRF mesocosms are highly variable based on water quality, and sediment measures indicating that the PWRF mesocosms may no longer be usable for replication based experiments. Lastly, additional testing with greater sample sizes, and replication to account for variability and increased the rigorousness of testing for more representative results (albeit that this was a preliminary study).

4.2.2. CAMP Implications

Geology likely played a role in detected trace element water concentrations and otolith signatures although there were limitations in the current study as to determining quantifiable amounts of impact. Dissolution, and weathering of rock (surficial and base geology) likely varied across the CAMP study sites leading to some of the detected variation. Impoundment was possibly attributed to fish otoliths display of greater trace element signatures (Ba and Sr primarily) but may instead have been due to impoundment flow order, or flow connectivity. Regardless fish otoliths could correctly classify between impounded and non-impounded and between CAMP waterbodies to varying degrees. This implies that fish otoliths can be used to differentiate between waterbodies, and that elevated Ba and Sr signatures may be used as an indicator of impoundment. Collecting and testing a gradient of trace element levels between waterbodies was achieved to various degrees for each of the five studied trace elements although the lack of correlation between near-surface water trace element concentrations with either species otolith bulk-average concentrations may indicate that

otolith chemical signatures may not reflect ambient conditions. CAMP sampling methods and the authors decision to average otolith linescan segments (and water concentrations) may have led to the lack of correlation. This implies that CAMP sampling method may need to be modified to have greater utility for the comparison to otoliths. Lastly, differences in trace element uptake and ability to classify waterbodies differed between walleye and lake whitefish, and indicated that walleye utility for detecting differences in trace element levels between waterbodies is less than that of lake whitefish, making lake whitefish a better biomonitor. This implies that further work should focus more on lake whitefish regarding use as a biomonitor within the freshwater environment due to its representative nature.

4.3. Otoliths as Biomonitors

The value of otoliths as biomonitors lies in the sheer amount of information the otolith as an exposure timeline can obtain. To be able to make use of the otolith as a biomonitoring tool, a substantial amount of background information on the fish and the system must be acquired depending on the question being asked. Things such as fully understanding the test system in which the otoliths are exposed to (e.g., location or types of inputs and outputs, present biotic community, geology), fish background information to determine where within a given environment the fish (and its otoliths) is encountering the exposure and what the chemical signatures are likely based on (e.g, habitat, migration patterns, physiology). For example, in the CAMP-O study, lake whitefish otoliths were considered a more useful biomonitoring tool to measure the impact of impoundment and/or downstream water flow accumulation between

waterbodies than walleye since their otolith signatures allowed for more precise classification of separate waterbodies. Lake whitefish reside mainly in the benthic-benthopelagic zone and feed at the bottom of waterbodies environment (Stewart & Watkinson, 2004), and through the literature were found to travel lesser distances (Casselman et al., 1981) than the more mobile walleye as mentioned earlier (Carlson et al., 2017; Dupont et al., 2007; Ferguson & Derksen, 1971). It was thereby assumed that walleye were less able to account for the accumulation of materials (e.g., feeding) at greater depths or a particular area effected by impoundment and other factors made lake whitefish a better biomonitor in the current study. One cannot control exactly what a fish does within a given environment (e.g., do the fish migrate in and out of the target test environment? What habitats does the fish preside in and for how long?). The latter can cause variation to increase, and may make a biomonitor less effective depending on how specific a zone one is trying to monitor using said species. To account for the latter variation in the field setting, sufficient replication of treatments, and/or sufficient sample sizes are important to account for it. With greater sample size, comes a greater number of signatures which can be gathered which represent the fish's unique movement and life through the otolith, thereby acquiring a more representative set of data for each study area. In both the MnOTOL and CAMP-O projects, it was mentioned that low sample sizes (among other factors) may have led to the insignificant results obtained by the multitude of tests and comparisons including those dealing with otolith, water and sediment t-tests, ANOVAs and correlations. Through conducting power analyses "a posteriori", the required sample size estimates were determined. To conduct a power analysis, you need three of four measures or determined values and

they are: sample size (“n”), effect size (quantification of the magnitude of effect a test contains), significance level (the probability of type 1 error, which is typically denoted as 0.05), and power (1 - P(type 1 error)). Having three of the quantities lets you solve for the fourth.

The statistical software package G*power 3.1 was used to determine required sample sizes (Faul et al., 2007; Faul et al., 2009). The power analysis labelled “A priori: Compute required sample size – given alpha, power, and effect size” was used a posteriori due to study needs. For the CAMP study, t-test comparisons using pooled (impounded and non-impounded waterbody) water or otolith data, F-test (ANOVA) comparisons using water and otolith measures per CAMP-O selected waterbody (group standard deviation as an average of each of the eight waterbody averages combined), and otolith to water correlation tests selected were analyzed. For MnOTOL, Otolith to water and sediment correlations run under LA-ICP-MS and ICP-QQQ-MS were analyzed. In G*power 3.1, t-tests were run under the “Means: Wilcoxon-Mann-Whitney test (two groups)” if non-parametric, and “Means: Difference between two independent means (two groups)” if parametric. ANOVA tests were run in G*power 3.1 under “ANOVA: Fixed effects, omnibus, one-way”. Lastly, in in G*power 3.1, all correlation tests were run under the “Correlation: Bivariate normal model”. Although many of the datasets run were non-parametric, non-parametric equivalent tests were unavailable. The results presented here are merely mean to demonstrate preliminary estimates of required samples sizes depending off calculated effect sizes at a power of 0.95 and an alpha of 0.05. Referring to the summary tables, most of the t-test and ANOVA datasets for the CAMP study were of appropriate or near-appropriate sample size (Table 4.1). Of

note, Ba and Sr most consistently contained the appropriate sample sizes, and carried most of the trends observed in the CAMP-O study giving more confidence in results. Most of the CAMP otolith to water correlations were of insufficient sample size (at times multiple orders of magnitude) which likely led to the lack of effect detection (Table 4.2). Like the CAMP correlations, the MnOTOL correlations were also of insufficient sample size (Table 4.3). Lastly, the Water and otolith Sr concentrations versus TDS and TSS concentrations indicated that approximately half the tests were near the required sample size, although since the data used was non-parametric, results must be interpreted with caution (Table 4.4). The latter results indicate that for those tackling similar projects in the future will require greater sample sizes to determine within statistical confidence.

Using the otolith as a biomonitoring tool in natural systems such as in the CAMP-O study, study area selection is crucial. If waterbodies are too similar (e.g., Split Lake and Lower Nelson River trace element concentrations) misclassification, can occur. It was found that study areas/waterbodies must differ enough from each other chemically (e.g., take representative water samples), and are present at detectable levels to have any hope of detecting differences within the otolith, or to establish water to otolith correlations. Additionally, due to the concern over movement of both walleye and lake whitefish in the CAMP-O study (e.g., in and out of the specified study areas over time), perhaps using contained/caged individuals in each CAMP waterbody would be desirable? The latter was accomplished in another study (Mohan et al., 2012).

One otolith limitation is that they can be used to monitor for trace elements, but not for other whole compounds or molecules, which may be better monitored for in other fish tissues (e.g, pharmaceuticals, pesticides). On top of the latter, as observed in the CAMP study and throughout the literature, not every trace element was found to be equally detectable, or taken up by fish due to regulation, presence in the water, diet or other environmental factors. In the CAMP-O study, Ba, and Sr

were found to be the most useful measured trace elements since non-nutritive, and detectable in the otolith and water column at sufficient levels, which varied between areas. Comparatively, if you are trying to measure certain trace elements that are near or below the detection limit (e.g., manganese), otoliths will be unable to discern between environments. Although otoliths may be metabolically inert, various factors can impact the trace element signatures such as growth which were identified and controlled for in the current study. A simple correction (such as taking the residual average as conducted in the CAMP-O study) can help account for this, and will lead to more representative and less biased results. Also, bulk-average sample analyses used in both studies were observed to have caused loss of variability (e.g., seasonal). The bulk-average sample analysis for CAMP-O is a starting point for overall uptake, and paired well with water quality measures since also taken year-round allowing otolith bulk-sample measures to discern differences between impounded and non-impounded waterbodies which water chemistry alone was not able to account for, further proving the utility of the otolith as a biomonitoring tool. Future work should make use of the seasonal variation within the otolith to better interpret trend in impounded and non-impounded waterbodies through time.

Referring to the MnOTOL study specifically, if one wishes to experimentally induce trace element exposures that are then to be detected using otoliths as biomonitoring tools, it is critical that adequate time is given for fish annuli to develop in the test medium to have enough of an otolith bandwidth to measure. As stated previously in the MnOTOL discussion the 2.5-month period in which the experiment exposure was made may not have been substantial enough to determine with confidence new otolith growth from old, and if a signature was made by $MnSO_4$ addition or not. A greater and more stable Mn exposure concentration through time would have assisted with the latter detection. A range-finding study in future studies would help with the latter. Determining an appropriate range of nominal concentrations that would translate to predicted measured concentrations would be an asset in future studies

since some trace elements can be highly sensitive e.g., manganese to redox potential in the MnOtol study.

4.4. Next Study Steps

4.4.1. MnOTOL Next Steps

Much was learned from the MnOTOL experiment at the PWRF that can be used towards improving future experiments. Naturally the next step for MnOTOL otolith study at the PWRF would be to improve Mn exposure within mesocosms to allow signature development in baitfish (Future study: MnOTOL2). Before conducting MnOTOL2, a more rigorous range-finding study is suggested, which may include the construction of species sensitivity distributions, and the use of a biotic ligand model (BLM) (or other models) to both determine the appropriate range of Mn exposure concentrations, and the true amounts of Mn required to add to the system respectively. Due to the multiple water quality variables measured within the MnOTOL study, and the available archived background data available on the mesocosms at the PWRF, the BLM produced would have been an asset in determining the nominal concentration required for the desired dissolved (bioavailable) concentration for appropriate fish exposure in the current study. After determining the desired range of concentrations, the study could be initiated over a longer study duration to allow for the construction of a larger otolith growth band, thereby making it easier to detect Mn exposure signatures, and to be able to discern anomalous contamination peaks from true Mn incorporation peaks on the otolith. Collecting and using fish of similar age and size would also help eliminate any bias or confounding variables surrounding uptake of target compounds among other things.

More specifically for beam analysis, marking the fish with a fluorescent injection e.g., tetracycline would help identify the region of otolith growth which occurred during the course of the study, aiding in the otolith edge measures for bulk sample analysis (e.g., Babaluk and Campbell, 1987; Campana and Neilson, 1982; Mugiya et al., 1991; Ranaldi and Gagnon, 2009, 2008). Additionally, for test species, using only a single sex in the study would avoid the risk of progeny being produced, and sex specific uptake/mannerism differences (although not observed or presumed to have occurred in the current MnOTOL). Both fathead minnows and northern redbelly dace were found to be poorly visible in the mesocosms, and were assumed dead for most of the study minus introduction and collection, making it difficult to visually determine health and survivorship throughout the experiment. It is suggested that on future studies that fish are periodically trapped, weighed/measured, and then re-released into their respective mesocosms (assisted with GEE minnow traps and sedated with clove oil for measurement). Trap catch efficiency varied little between traps with or without food. Future studies should use traps without bait since the bait may release additional material into the system, potentially detracting test species from ingesting and accumulating Mn exposed material. Beyond that, the use of less redox-sensitive metals (e.g, Pb) for testing would be another approach, as well as more rigorous water and sediment sample measures (as mentioned in the MnOTOL discussion). Lastly, when considering micro chemical techniques (e.g., LA-ICP-MS, SO-ICP-MS, ICP-QQQ-MS), it is suggested that greater quantities of individual fish be processed to fully account for the variability of readings, and allow for more rigorous or complex univariate or multivariate statistical approaches to be undertaken. Having replicate mesocosm of both

control and treatment mesocosm would also help account for variability observed in the current study, although the current study was intended to be a preliminary study.

4.4.2. CAMP-O Next Steps

Having been given the opportunity to work with CAMP and its extensive dataset, much has been learned both in terms of how CAMP can be improved for use in otolith based biomonitoring, and the efficacy of otoliths as trace element biomonitors. To further test the effect of impoundments on water and otolith trace element levels, additional factors must be accounted for. Perhaps generating structures and control structures should be treated differently or separately in terms of their overall impact on water bodies. Various factors such as how recent an impoundment was developed, impoundment size, number of turbines, change in elevation, and average discharge among other variables may impact the level to which trace elements are detected in water and otolith. Additionally, the effect of multiple consecutive impoundments may also affect how a single impoundment effects trace element detections levels in water or otolith and should be considered.

Considering univariate and multivariate test results of otolith impounded waterbodies, the observed impoundment trend may be due to impounded waterbodies being interconnected by flow and being part of the same drainage system thereby, sharing the same water and chemical signatures rather than the impoundment itself. It is suggested that to prove If the impoundment effect is real or not is to test sets of impounded waterbodies not connected by water flow/drainage system, and to test non-impounded waterbodies that are interconnected by water flow (although the multivariate

RDA analysis did still separate impounded and non-impounded waterbodies, and classification was found possible to some degree through the KNN analyses). Additionally, it is recommended that the same multivariate and univariate analysis should be conducted with the extended 2008-present water quality dataset, and compare with the most recently caught fish otolith bulk-sample averages to acquire an even more representative measure. Also, for comparative purposes, instead of averaging entire year ranges along the otolith, measuring the average trace element concentrations per year may be another method of more closely comparing water chemistry over time (although it may also increase human error in otolith aging/annuli determination). This way, year-to-year variation within the otolith and test systems are not lost, and may pave the way for using the otolith to track movement from waterbody to waterbody.

The discrepancy between water quality and water trace element data with otolith trace element concentrations may have been associated with the collection depth, waterbodies selected, and area sampled. Sampling depth used for comparison was 0.3 m while the both tested fish species typically reside in the benthic/ bentho-pelagic regions. However, results did indicate that the selected CAMP waterbodies are relatively well mixed (based on the available depth data), or that the results may be confounded by variations in the time of year samples were taken throughout the season. Although Thorrold et al., (1998) in a study conducted on freshwater rivers found that that the relationship between ambient water and otolith (American shad (*Alosa sapidissima*) trace elements Mg, Mn, Sr, and Ba did not vary with season and may not have caused correlation issues for the latter at least. Regardless, additional testing is

required to clarify if any of the water quality variables or measured trace elements differ significantly with depth in any of the selected CAMP waterbodies. It is recommended that additional depths should be measured more frequently in CAMP, especially bottom or near-bottom depths for comparison with both species that reside in the benthic/bentho-pelagic region primarily. Perhaps this near-bottom zone is the zone most impacted by impoundment? Or alternatively, a sampling program should be developed to obtain average measures (measures representative of depth ranges) of each of the parameters, or those which focus on certain areas of the water column to better compare with target fish species (e.g., benthic depths for lake whitefish, or comparison of epilimnion or hypolimnion stratifications). Max depth varied in each lake so comparing water chemistries at different depths would have to be done when dealing with near sediment level concentrations. Comparatively, in the marine environment, it was found that Mn and Ba concentrations in whole otoliths varied significantly with depth (Kingsford and Gillanders, 2000). Mn and Ba concentrations were found to be greatest in shallower depths (Kingsford and Gillanders, 2000). The magnitude to which Ba varied with depth was dependent on the estuary sampled from (shallow, mid deep, or just shallow-deep comparisons) unlike Mn which was considered more constant (Kingsford and Gillanders, 2000). Difference in collected trace element with depth was likely due to varying oceanographic regimes, and chemical inputs which varied between estuaries (Kingsford and Gillanders, 2000). If a fish is less specific-depth exclusive, a depth-averaged record may be more effective (Kingsford and Gillanders, 2000).

Referring to sampling resolution, the low-resolution and lack of otolith to water correlation issues apparent in CAMP could be addressed in multiple ways. For sampling,

water and otolith programs can be coordinated to have sampling overlap specific sampling areas thereby increasing the comparative nature between water and otolith like Clarke et al., (2015, 2007). Additional water samples can also be collected across each CAMP waterbody surface to obtain more representative or integrated measures for comparison with archived otoliths. Even though it was not determined to what extent walleye of lake whitefish migrated or moved throughout their lives, there is potential that some may have left their respective waterbodies and resided perhaps for most of their lives within tributaries. It is because of this that it is suggested that later CAMP studies also collect water samples from their respective tributaries of CAMP waterbodies as well. Lastly, out of the eight CAMP waterbodies studied, seven were lakes and one was a river. Seeing how similar Split Lake and the Lower Nelson River (downstream of Limestone GS), it was assumed that its inclusion was justified.

Other things that should be considered in future studies include defining the limnological characteristics of the selected CAMP waterbodies in further detail (to account for lake tendency for stratification or mixing events). This would help determine the movement of dissolved/suspended sediments within the water column, along with trace elements. Also, the simple geological analysis done in the current study should be further worked upon in later CAMP studies. It is proposed that CAMP waterbody geological profiles should be created, which contain information about the geological formations (surficial and base) therein to then determine to a better degree the contributions base or surficial geology give to the water, and in extension the biota. Once a better connection is made between geology and chemical signature, perhaps fish otoliths could prove useful in detecting things such as mineral deposits in water

systems. The collection of a series of sediment core samples from each of the CAMP waterbodies may help better determine the concentrations of trace elements located within the benthic environment, thus the exposure regime of benthic fish species (e.g., lake whitefish) and the effect of impoundment. Lastly, regarding trace element analysis, additional trace metals should also be included in later otolith microchemistry studies using the CAMP dataset. Namely copper (Cu), zinc (Zn), nickel (Ni), cadmium (Cd), and/or lead (Pb) due to their frequent detection within the CAMP water quality dataset, and prevalence in the literature. In particular, Zn may have utility as an aging tool (Halden et al., 2000) and could assist in aging accuracy when determining bulk average concentrations in the current study.

Other recommendations for future CAMP studies are to include the other two currently monitored commercial fish species in CAMP namely, sauger (*Sander canadensis*) and northern pike (*Esox lucius*), and their associated aging structures. This will allow for further comparison of trace element signatures for detection of impoundments, and help determine additional useful species for biomonitoring purposes (not all fish species are found in any one CAMP waterbody). Additionally, incorporating genetic analyses of the separate fish species per waterbody would help determine and discern between populations in each waterbody (Clarke et al., 2007), further clarifying how representative fish samples are per waterbody. On the topic of correction for intra-species differences in later studies, fish of closer age, weight, fork length and level of development (adult vs. juvenile) will continued to be tested and accounted for in future studies.

4.5. Implementation of $^{87}Sr/^{86}Sr$ into Future CAMP Analyses

Through the current analysis, a few shortcomings were evident. Since the exact origin of the walleye and lake whitefish were unknown, as well as where exactly they migrated of resided throughout their lives, it is difficult to say how comparable water quality and otolith microchemistry was over time. Insufficient geological knowledge of the CAMP waterbodies made it a challenge to attribute trace element levels and the geological influence on water and otolith trace element concentrations. The lack of resolution in the collected base geological data and determining overall contributions and origin of trace elements from surficial deposits (Faure et al., 1963) added to difficulty interpreting the effect of geology on the otolith. Additionally, discerning between waterbodies based on impoundment status, and flow order/accumulation alone was deemed insufficient to properly interpret trends in the data, and the true effect of impoundment. It is thereby recommended that additional tests or measures such as measuring $^{87}Sr/^{86}Sr$ isotopic ratio as part of the suite of ratios measured in the otolith during LA-ICP-MS may assist with determinations (Faure et al., 1963; Gibson-Reinemer et al., 2009). The $^{87}Sr/^{86}Sr$ ratio collected from organismal hard-parts can help determine drainage patterns of waterbodies by determining geologic age from the ratio. In one example the $^{87}Sr/^{86}Sr$ ratio was measured from shells of freshwater mollusks collected from geologically unique environments to discern between sites and identify source geology. Faure et al., (1963) attempted discern between the Cambrian shield, Paleozoic rocks (e.g., limestone), and modern-day basalt clusters. Freshwater mollusk shells were used since found to reflect Sr within the water column, and the isotopic composition in rocks. One noted issue with this method was that differential weathering

of rocks can occur due to difference in rock solubility and may affect observed $^{87}Sr/^{86}Sr$ in mollusk shells (Faure et al., 1963). Another issue was that the transported mantle (due to glacial advance) and retreat can cover landscapes with material which may not hold any resemblance to the original bedrock underneath, thereby causing the release of Sr boasting a different ratio than the actual area. In a second example, Gibson-Reinemer et al., (2009) reported that the $^{87}Sr/^{86}Sr$ ratio reflected ambient water levels (various hatchery water sources) in rainbow trout (*Oncorhynchus mykiss*). The rainbow trout otolith $^{87}Sr/^{86}Sr$ detected changes through otolith linescans more effectively than Sr/Ca ratio measures. The $^{87}Sr/^{86}Sr$ ratio did so by helping to discern dietary and waterborne Sr influence. It was determined that Sr between otolith and water was not a 1:1 relationship with water accounting for 66% and diet accounting for 34% making the $^{87}Sr/^{86}Sr$ ratio useful in classifying fish environmental histories, especially fish origin. Ba/Ca, Sr/Ca, and $^{87}Sr/^{86}Sr$ best classified fish from their hatchery of origin and patterns in otolith Sr were best explained by both water and food, but varies by species (Gibson-Reinemer et al., 2009). In the CAMP-O study, food or diet was not specifically studied and may also have accounted for the otolith lacking correlation with water, and other observed trended difference between species. Adding $^{87}Sr/^{86}Sr$ to models helped discern between sites when low variation in Ba and Sr/Ca occur with the predictive power of Sr, Ba, and others dependent to some degree by how broad or narrow a range of water chemistry was available (Gibson-Reinemer et al., 2009). Smaller chemical gradients and unknown origin can lead to difficulty tracking fish movements with Sr/Ca or Ba/Ca, although $^{87}Sr/^{86}Sr$ helps offset both (Gibson-Reinemer et al., 2009). Variation between sites in the current CAMP-O study were detectable by both univariate and

multivariate tests, although each waterbody did have its own trace element similarities amongst themselves (regardless if impounded or non-impounded), adding to the complexity. Perhaps a wider range of water chemistry was required to detect more specific difference between separate CAMP waterbodies. Although pooled impounded, non-impounded results were quite clear. It was also noted that $^{87}Sr/^{86}Sr$ can be used to discern between surface and groundwater samples, allowing a person to discern between two geologically similar sites unlike Sr/Ca (Gibson-Reinemer et al., 2009). This would have been useful since the most available CAMP-O water chemistry data was taken at a depth of 0.3 m and had to be compared to otolith trace element averages from fish who resided in more benthic – benthopelagic locations. Lastly for Sr, $^{87}Sr/^{86}Sr$ analysis may help determine the main sources of the Sr, although like Na, Mg, and Ba, the greater Sr concentrations in SPLT and LNR are likely associated to downstream accumulation, although additional measures are needed due to flow and depth result discrepancies in the current study. In summary, the implementation of $^{87}Sr/^{86}Sr$ into the LA-ICP-MS trace element analysis suite may assist in clearing up many of the uncertainties and challenges faced within the CAMP-O study. Namely, concerns over fish movement and migration causing signature not to represent a target study area may be solved by determining fish origin and history of movement using the $^{87}Sr/^{86}Sr$ ratio. Secondly, geologies of separate CAMP waterbodies may be further discerned by measuring the $^{87}Sr/^{86}Sr$ ratio, thus assisting in differentiating environments, and the origin of Sr in the otolith, versus other things such as fish food/prey. Lastly, determining differences in water chemistry with depth was found unsuccessful in the current study, and perhaps measuring the $^{87}Sr/^{86}Sr$ ratio would lead to the determination of Sr

differences with depth since able to discern between surface and groundwater samples as stated previously.

Table 4. 2. CAMP-O summary table of otolith and water t-test and ANOVA test sample sizes ("n") required to achieve an alpha of 0.05 and a power of 0.95. Estimates based off the determined effect size of the previously collected data. **Bolded text** indicates sample sizes used in the current study were near those or were of appropriate sample size determined as required.

Measure	Grouping	TE	n per group	n total
Water (mg/L)	IMP/NIMP pooled (2 groups)	**Na**	**15**	**30**
		Mg	103	206
		Mn	94	188
		Ba	**21**	**42**
		Sr	**54**	**108**
	Per Waterbody (8 groups)	**Na**	**2**	**16**
		Mg	**2**	**16**
		Mn	**3**	**24**
		Ba	**2**	**16**
		Sr	**2**	**16**
Walleye otolith (LRA ppm)	IMP/NIMP pooled (2 groups)	Na	nc.	nc.
		Mg	9158	18316
		Ba	113	226
		Sr	12	24
	Per Waterbody (8 groups)	Na	15	120
		Mg	**7**	**56**
		Ba	**3**	**24**
		Sr	**2**	**16**
Lake whitefish (LRA ppm)	IMP/NIMP pooled (2 groups)	Na	460	920
		Mg	47	94
		Mn	20423	40846
		Ba	**6**	**12**
		Sr	**5**	**10**
	Per Waterbody (8 groups)	**Na**	**7**	**56**
		Mg	**5**	**40**
		Mn	**5**	**40**
		Ba	**3**	**24**
		Sr	**2**	**16**

Table 4. 3. CAMP-O summary table of otolith to water correlation test sample size ("n") requirements to achieve an alpha of 0.05 and a power of 0.95. Estimates based off the determined effect size of the previously collected data. Bolded text indicates sample sizes used in the current study were near those or were of appropriate sample size determined as required. "Points" refer to the number of scatterplot points.

Measure	Grouping	TE	n total
Walleye otolith:water correlation	IMP/NIMP waterbodies (8 points)	Na	2455
		Mg	22
		Ba	18
		Sr	34
	NIMP waterbodies (4 points)	Na	2635
		Mg	**5**
		Ba	223
		Sr	8
	IMP waterbodies (4 points)	Na	27052
		Mg	16
		Ba	33
		Sr	59
Lake whitefish otolith:water correlation	IMP/NIMP waterbodies (8 points)	Na	142
		Mg	45
		Mn	759
		Ba	101
		Sr	19
	NIMP waterbodies (4 points)	Na	158
		Mg	35
		Mn	961
		Ba	64
		Sr	**5**
	IMP waterbodies (4 points)	Na	151
		Mg	261
		Mn	310
		Ba	**5**
		Sr	446

Table 4. 4. MnOTOL summary table of otolith to water or sediment correlation test sample size ("n") requirements to achieve an alpha of 0.05 and a power of 0.95. Estimates based off the determined effect size of the previously collected data. Bolded text indicates sample sizes used in the current study were near those determined as required. The term "points" refer to the number of scatterplot points.

Measure	Instrument	Species	Raw data "n"	Log data "n"
Otolith:water	ICP-QQQ-MS both	NRBD (used n = 3)	49	43
		FHM (used n= 3)	40	28
	LA-ICP-MS otolith, ICP-QQQ-MS water	NRBD (used n= 4)	114	71
		FHM (used n= 3)	**7**	**8**
Otolith:sediment	ICP-QQQ-MS both	NRBD (used n= 4)	138	288
		FHM (used n= 2)	nc.	nc.
	LA-ICP-MS otolith, ICP-QQQ-MS sediment	**NRBD (used n= 4)**	**5**	**5**
		FHM (used n= 2)	nc.	nc.

Table 4. 5. CAMP-O summary table of otolith and water versus total dissolved (TDS) or total suspended (TSS) solids required sample size ("n") to achieve an alpha of 0.05 and a power of 0.95. Estimates based off the determined effect size of the previously collected data. Bolded text indicates sample sizes used in the current study were near those or were of appropriate sample size determined as required. (Study used sample sizes of n= 4)

Type	Dependent Measure	Independent Measure	n total
NIMP Otolith	Wal-Sr	TDS	129
NIMP Otolith	**Wal-Sr**	**TSS**	**5**
IMP Otolith	Wal-Sr	TDS	24
IMP Otolith	Wal-Sr	TSS	13
NIMP Otolith	LKWF-Sr	TDS	51
NIMP Otolith	LKWF-Sr	TSS	7
IMP Otolith	**LKWF-Sr**	**TDS**	**4**
IMP Otolith	**LKWF-Sr**	**TSS**	**4**
NIMP Water	**Water-Sr**	**TDS**	**6**
NIMP Water	Water-Sr	TSS	483129
IMP Water	**Water-Sr**	**TDS**	**4**
IMP Water	**Water-Sr**	**TSS**	**6**

4.6. Chapter Four References

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Ranaldi, M. M., & Gagnon, M. M. (2009). Accumulation of cadmium in the otoliths and tissues of jevenile pink snapper (*Pargus auratus* Forster) following dietary and waterborne exposure. *Journal of Experimental Marine Biology and Ecology*, *150*,

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Appendices

Appendix I – MnOTOL Study Chapter

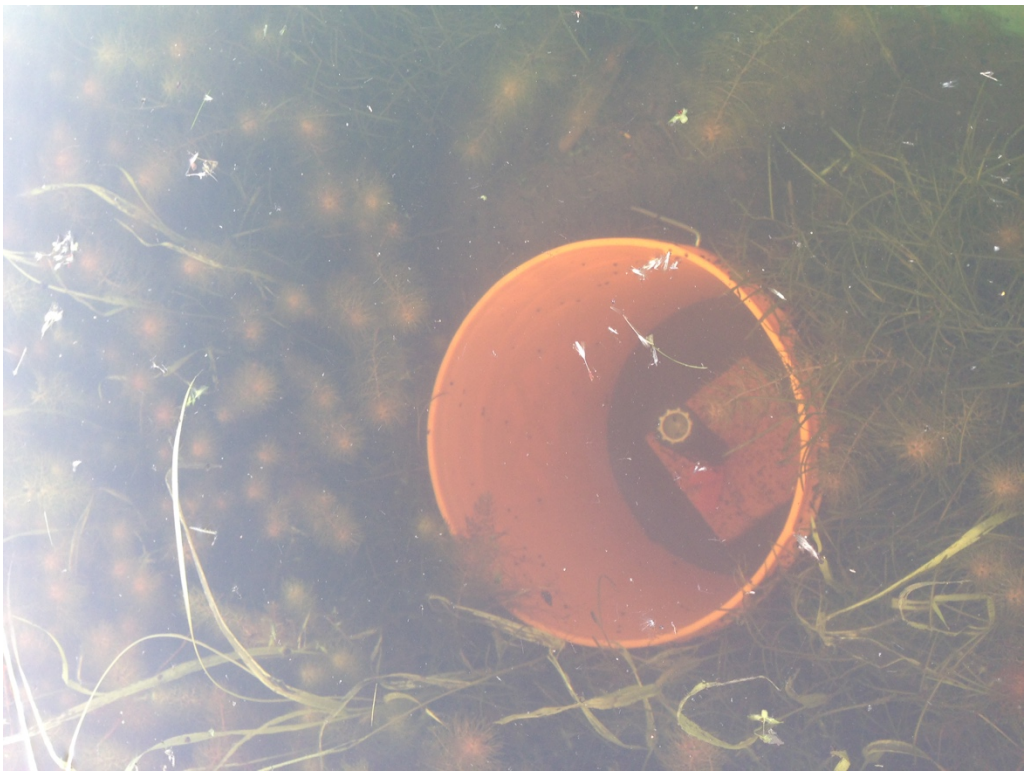


Figure A1. 1. Temperature refugia bucket installed at sediment level within one of the PWRF mesocosms, with deployed temperature logger at its base.

Figure A1. 2. Amber glass sample jars filled with sediment for MnOTOL study sediment sample collection.

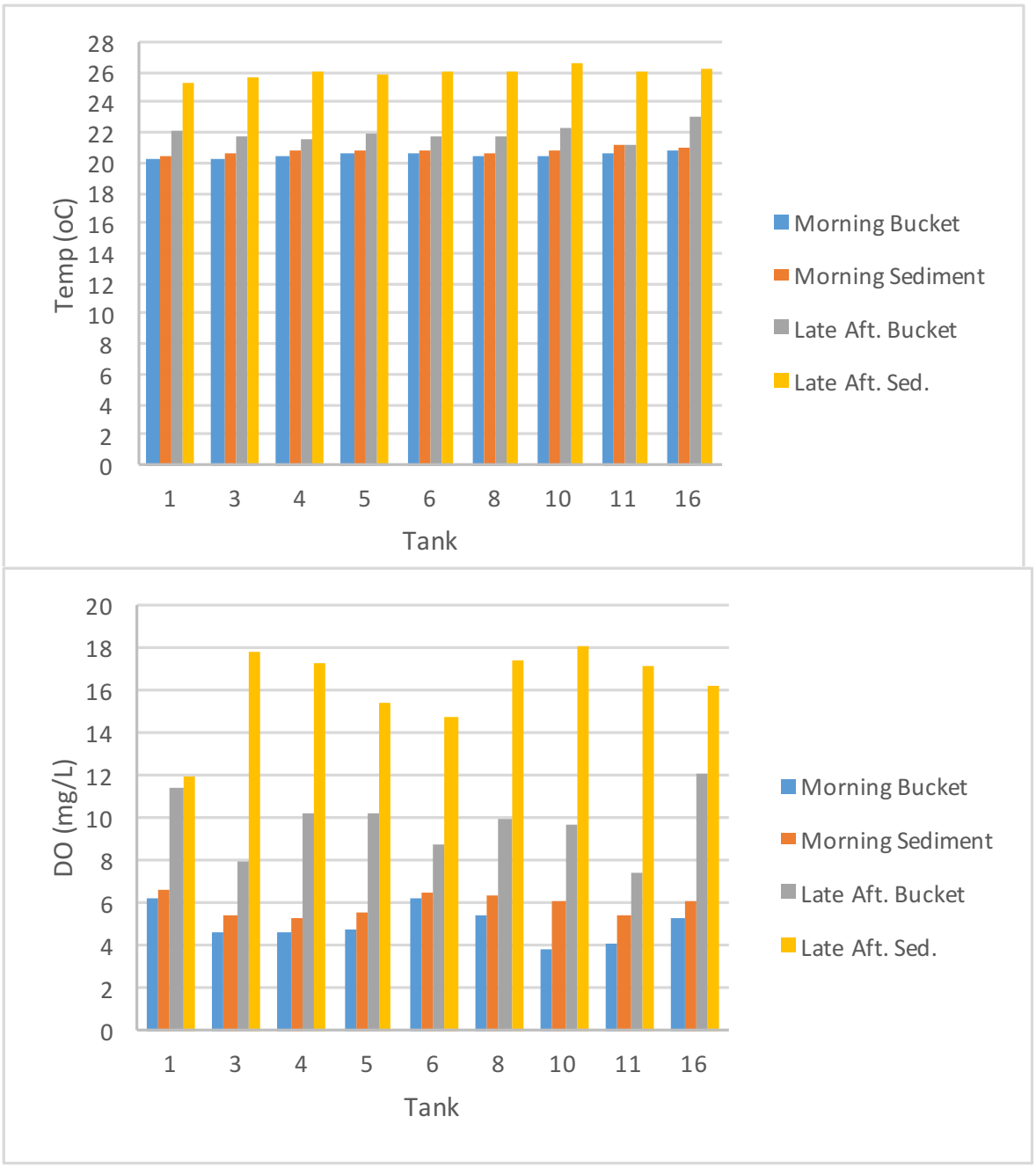


Figure A1. 3. Comparative bar-graphs of mesocosm sediment-level and refugia bucket water temperature and dissolved oxygen in both the morning and afternoon taken by YSI-Sonde device. Morning measures on day -24 (July 4, 2016), afternoon measures on day -22 (July 6, 2016). Sample size equal to one (N = 1) for each mesocosm tank.

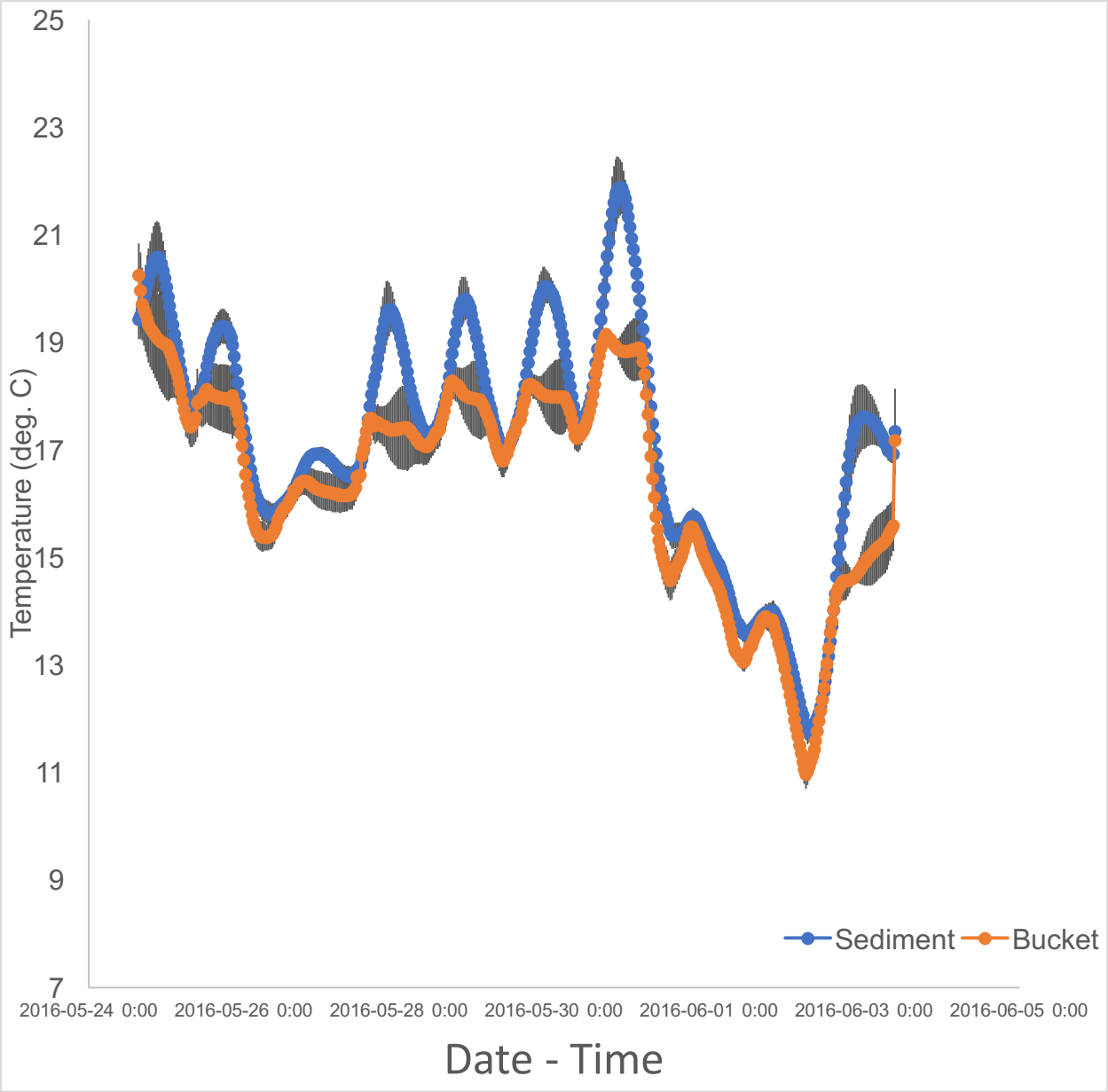


Figure A1. 4. Hobo temperature logger results for sediment level (blue) and within the bucket refugia (orange). Values taken as the average of mesocosms 1, 10 and 16, with grey error bars as standard deviation.

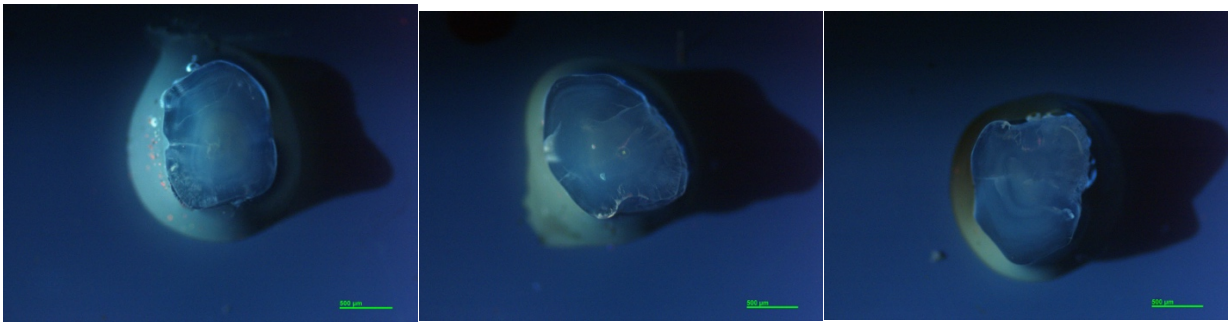


Figure A1. 5. Fathead minnow otoliths under CL analysis. Left to right: Control tank 1, 5.2 mg/L Mn^{2+}, and initial/time zero otolith. Control and treatment otoliths collected at end of test duration. Treatment and control fish added to the mesocosms July 22-25, mesocosms exposed to $MnSO_4$ treatments on July 28 (Day 0) and re-collected October 4-6 (Day 68-70), 2016.

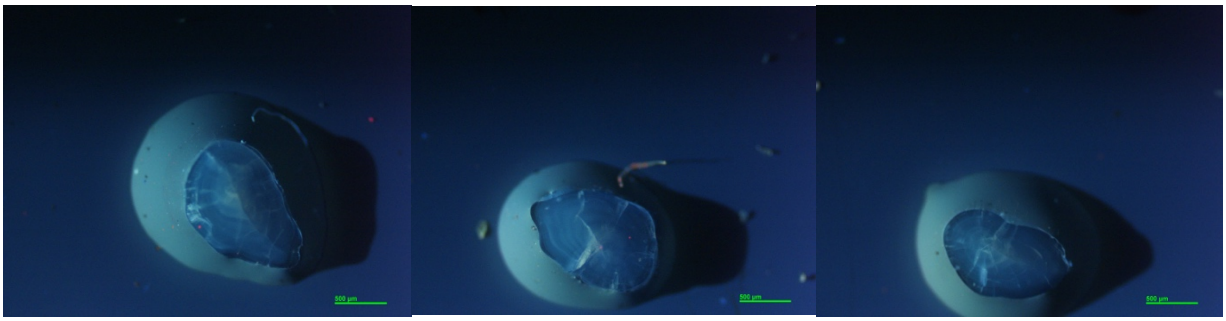


Figure A1. 6. Northern redbelly dace otoliths under CL analysis. Left to right: Control tank 5, 5.2 mg/L Mn^{2+}, and initial/time zero otolith. Control and treatment otoliths collected at end of test duration. Treatment and control fish added to the mesocosms July 22-25, mesocosms exposed to $MnSO_4$ treatments on July 28 (Day 0) and re-collected October 4-6 (Day 68-70), 2016.

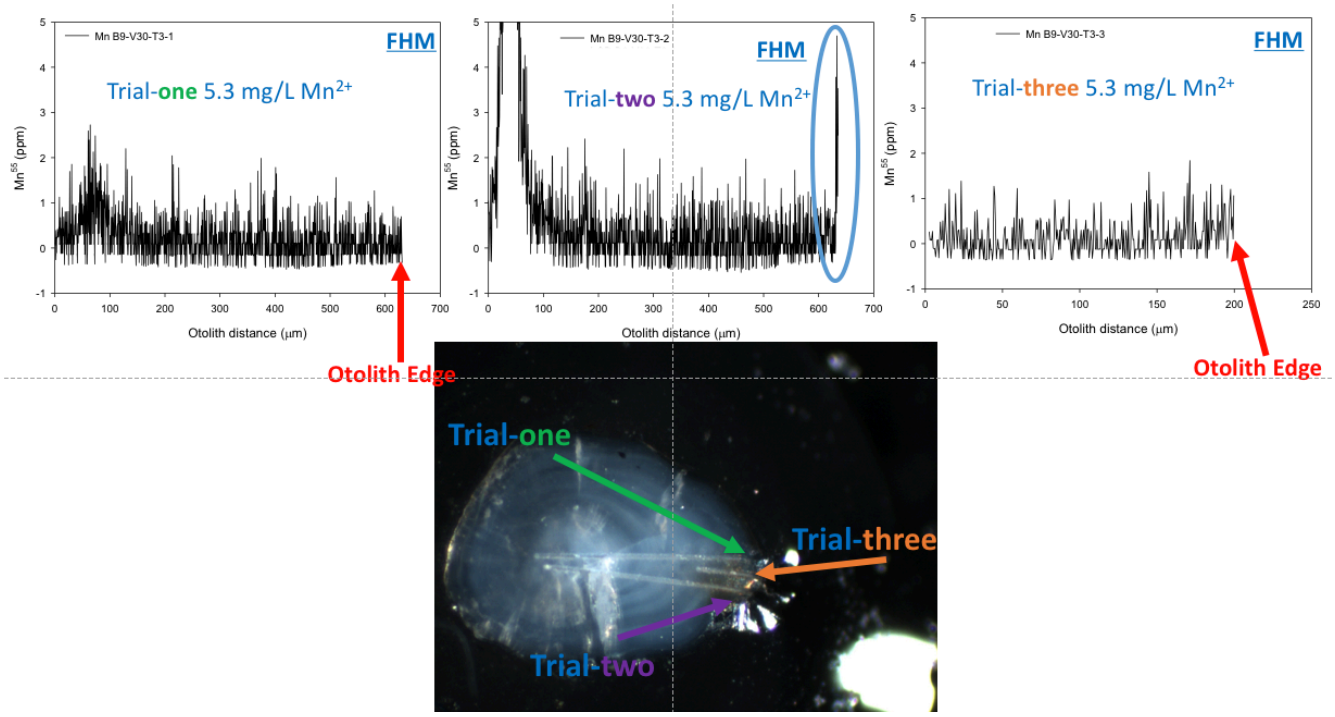


Figure A1. 7. Fathead minnow otolith with three separate laser line trials run. Evident Mn peak at the edge of the otolith for only trial two of three trials.

Table A1. 1. Northern redbelly dace, Fe/Mn values. Absolute concentration in micrograms per gram. Mesocosm “0” is an initial or time zero fish.

Mesocosm	fish	Fe/Mn	56 -> 72 Fe [O2]	55 -> 55 Mn [H2]
11	D17	87.63	3087.83	35.24
0	D5	130.05	6579.07	50.59
3	D13	42.86	2294.34	53.53
3	D14	63.86	4187.36	65.57
0	D4	61.70	5350.08	86.71
5	D9	82.86	7807.26	94.22
8	D27	98.82	9457.12	95.70
8	D26	23.52	4513.21	191.88

Table A1. 2. Fathead minnow, Fe/Mn values. Absolute concentration in micrograms per gram. Mesocosm “0” is an initial or time zero fish.

Mesocosm	fish	Fe/Mn	56 -> 72 Fe [O2]	55 -> 55 Mn [H2]
1	F13	114.04	3290.38	28.85
16	F23	110.60	3353.45	30.32
0	F4	110.00	3571.01	32.46
16	F24	90.31	3392.33	37.56
0	F2	136.19	5196.27	38.16
8	F37	131.44	6639.32	50.51
11	F17	104.99	6562.45	62.51
8	F36	34.07	3947.73	115.88
0	F1	159.81	43314.47	271.04

Appendix II – CAMP-O Study Chapter

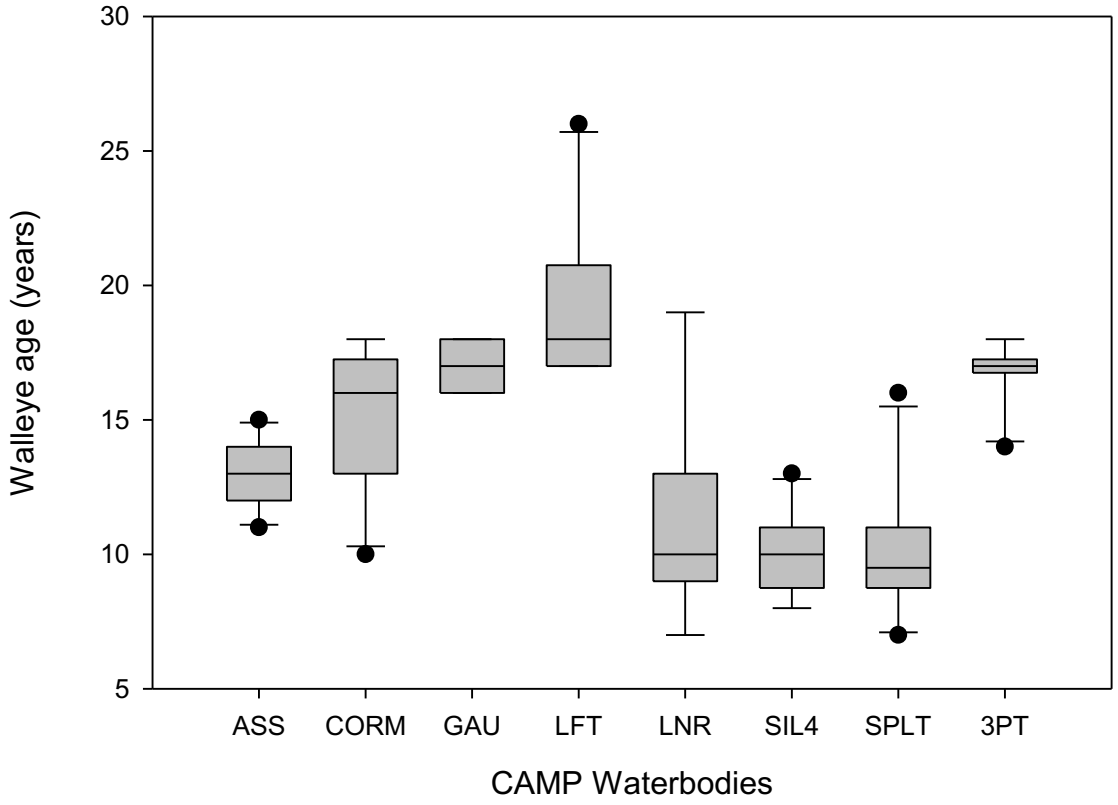


Figure A2. 1. Boxplots of separate CAMP waterbodies walleye otolith age distributions.

N= 9 for LNR, 10 each for each other waterbody.

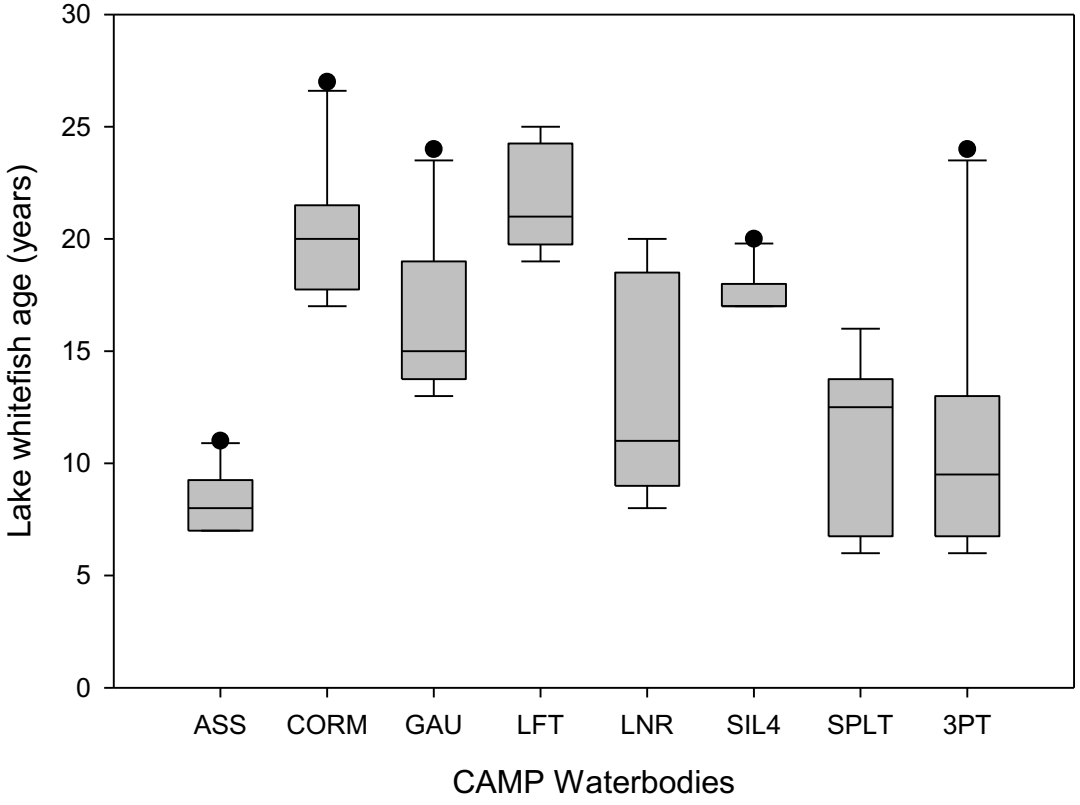


Figure A2. 2. Boxplots of separate CAMP waterbodies lake whitefish otolith age distributions. N= 9 for LNR, 10 each for each other waterbody.

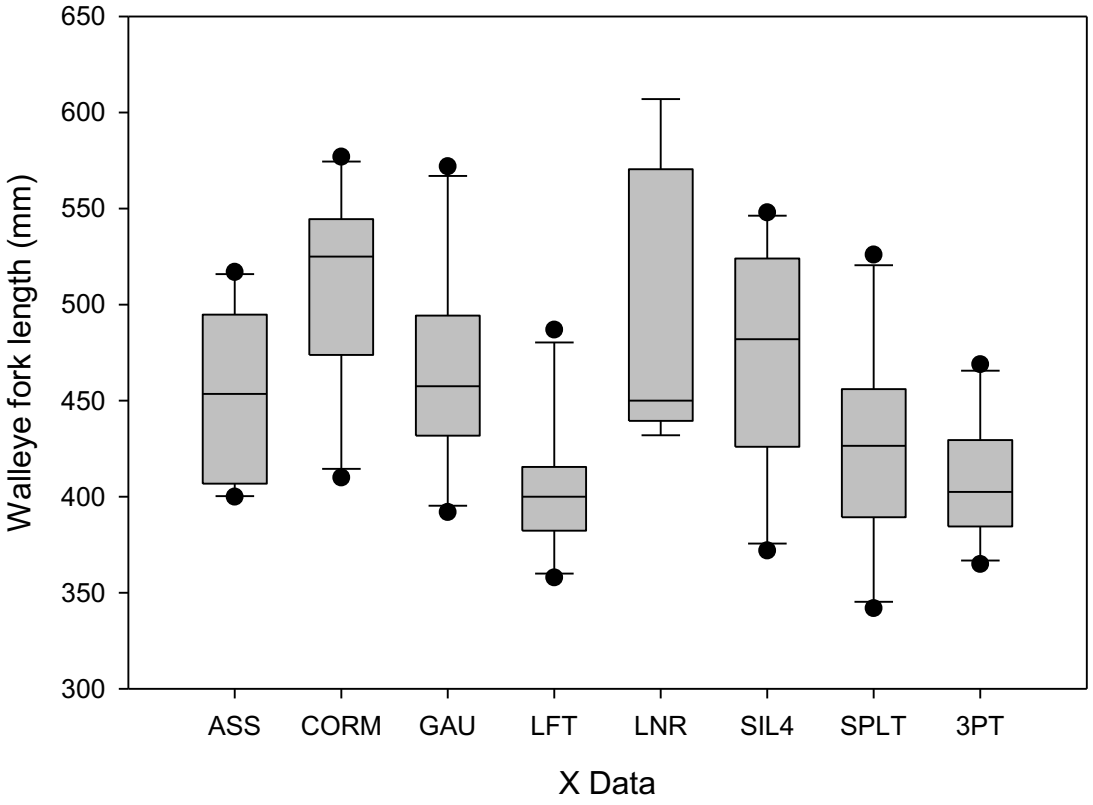


Figure A2. 3. Boxplots of separate CAMP waterbodies walleye fork length distributions.

N= 9 for LNR, 10 each for each other waterbody.

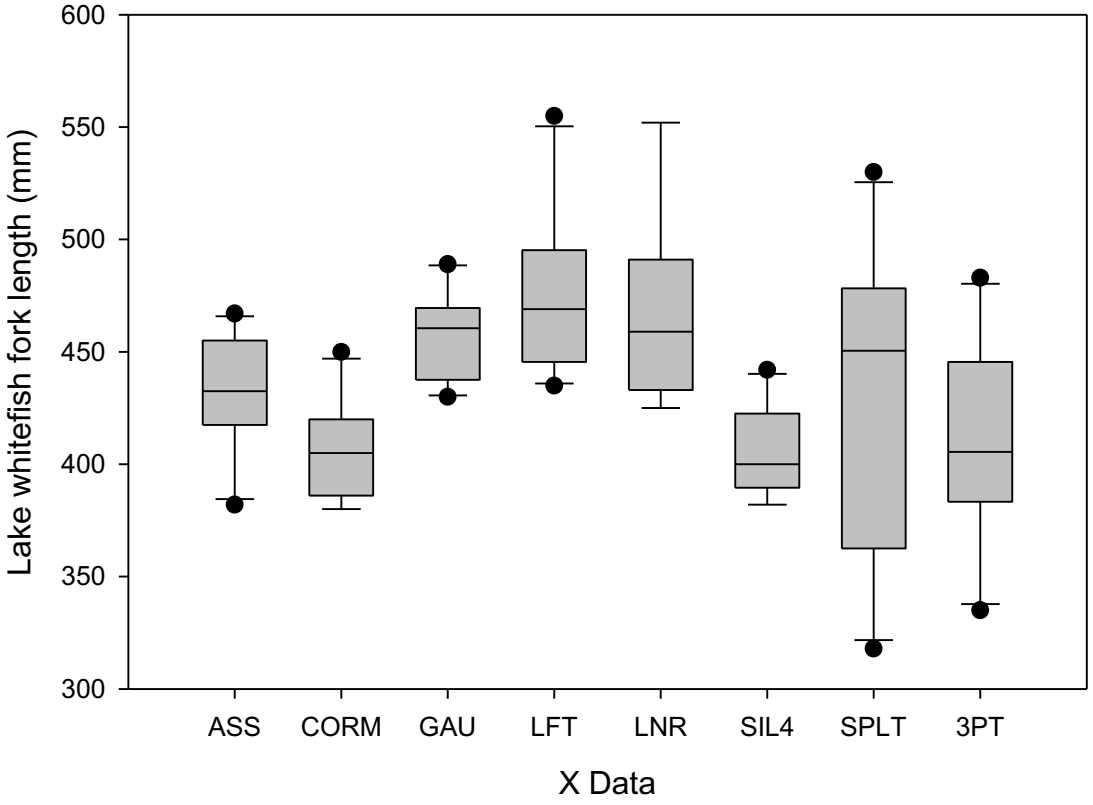


Figure A2. 4. Boxplots of separate CAMP waterbodies lake whitefish fork length distributions. N= 9 for LNR, 10 for each other waterbody.

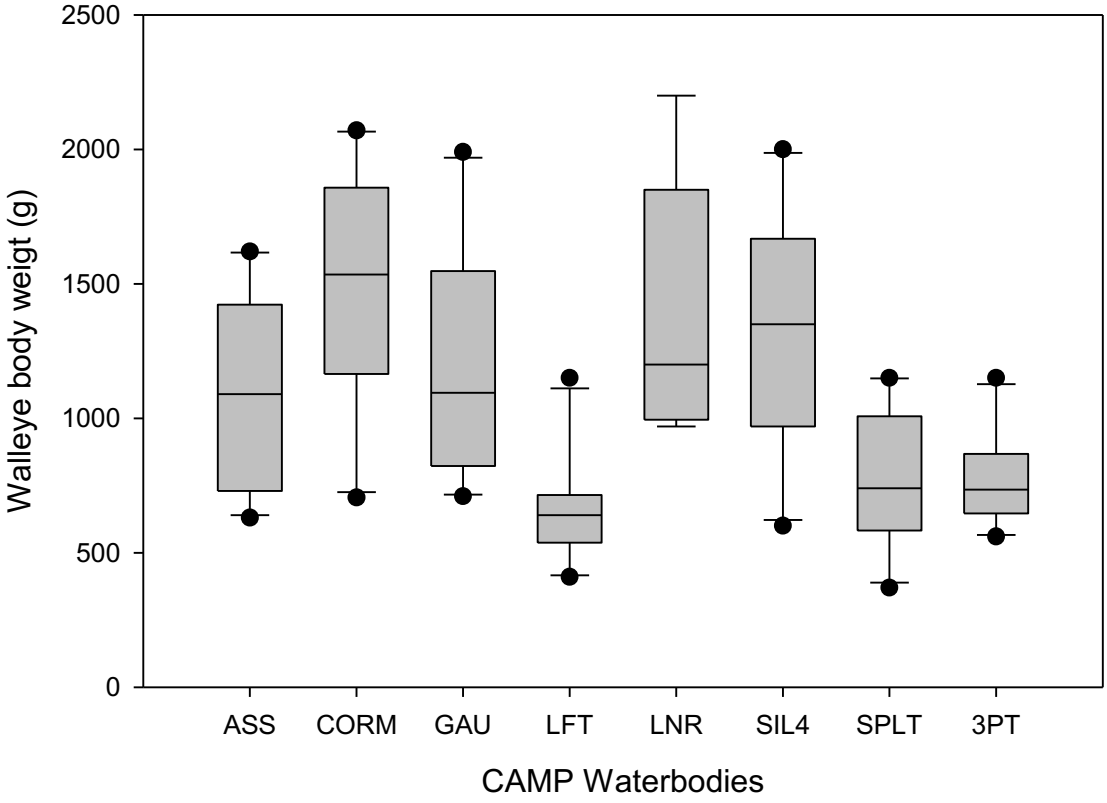


Figure A2. 5. Boxplots of separate CAMP waterbodies walleye body weight (grams) distributions. N= 9 for LNR, 10 for each other waterbody.

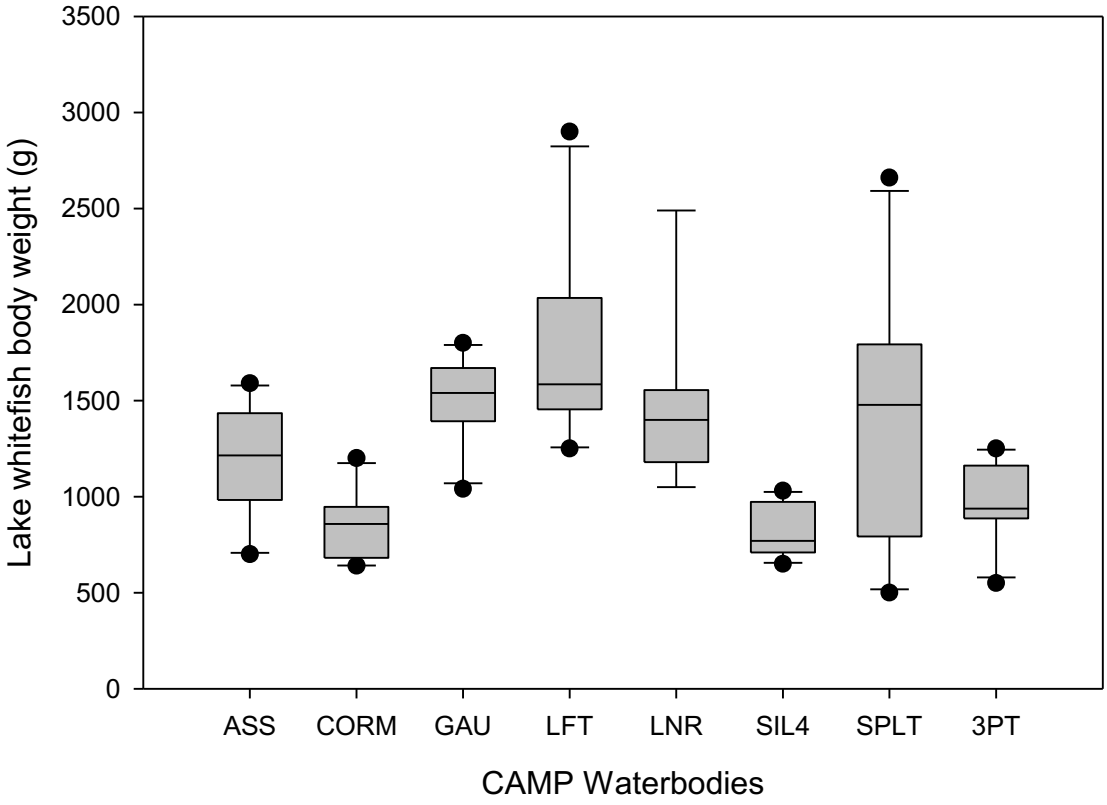


Figure A2. 6. Boxplots of separate CAMP waterbodies lake whitefish body weight (g) distributions. N= 9 for LNR, 10 for each other waterbody.

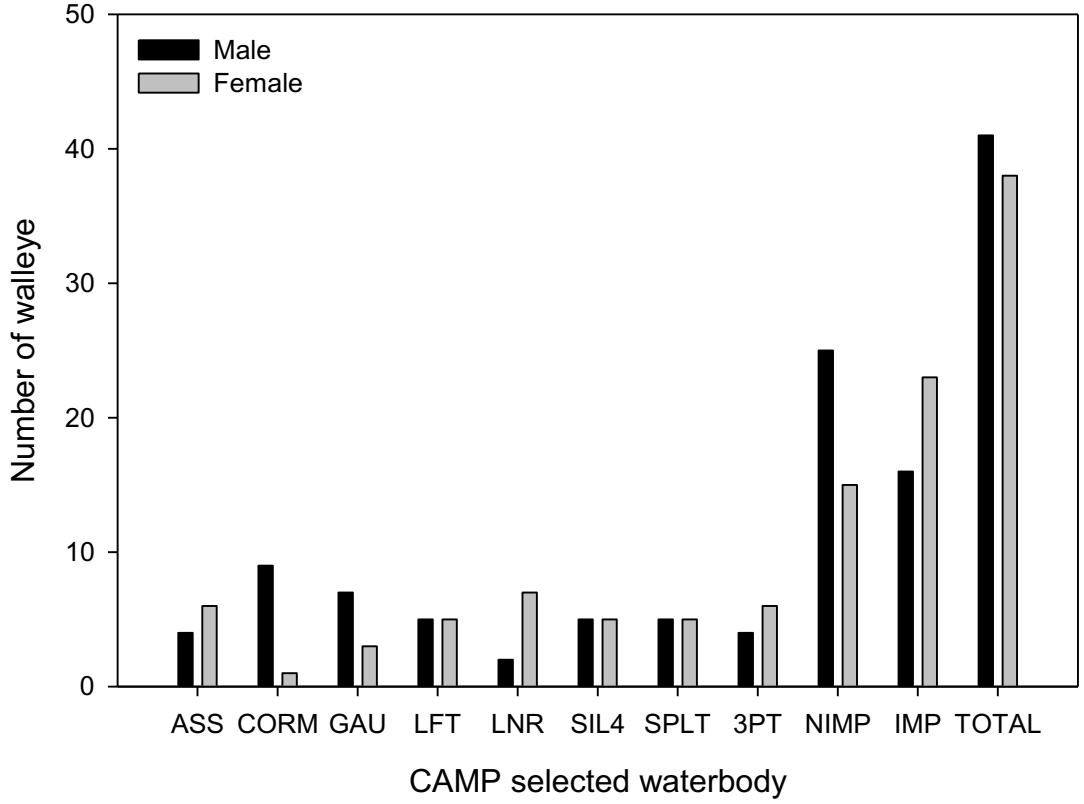


Figure A2. 7. Distribution of walleye sex across each of the select CAMP waterbodies, pooled impoundment groups (IMP = impounded, NIMP = Non-impounded) of CAMP waterbodies, and individuals in total.

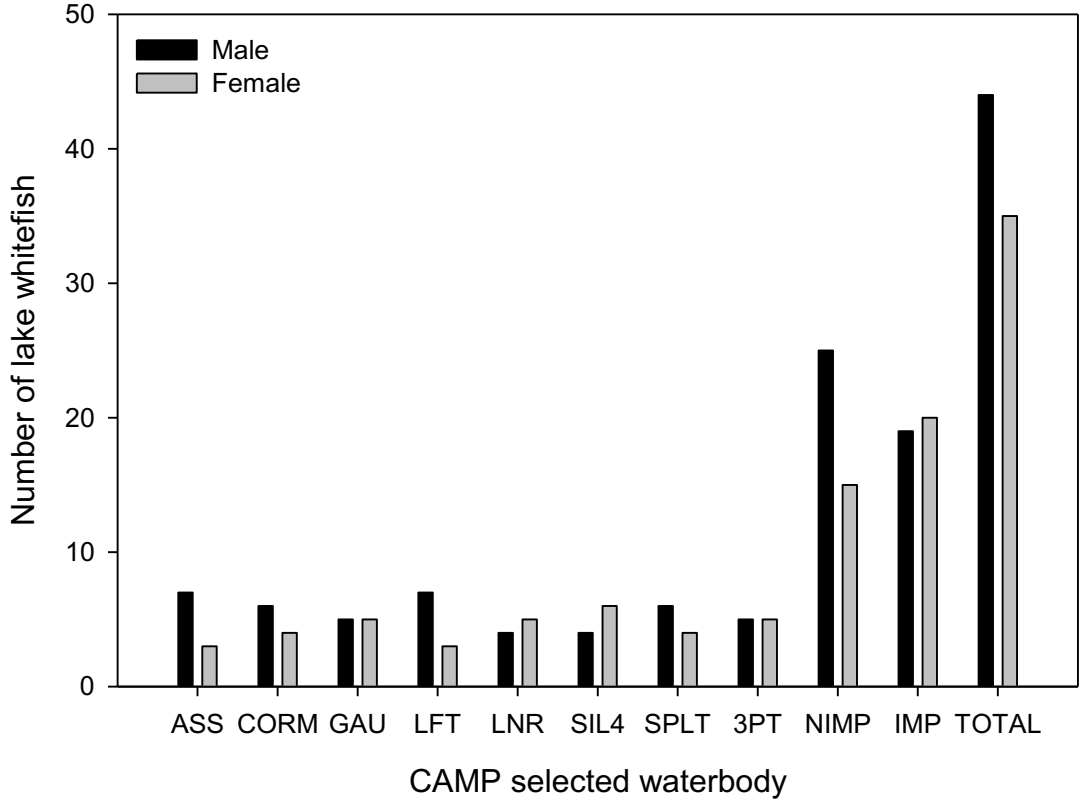
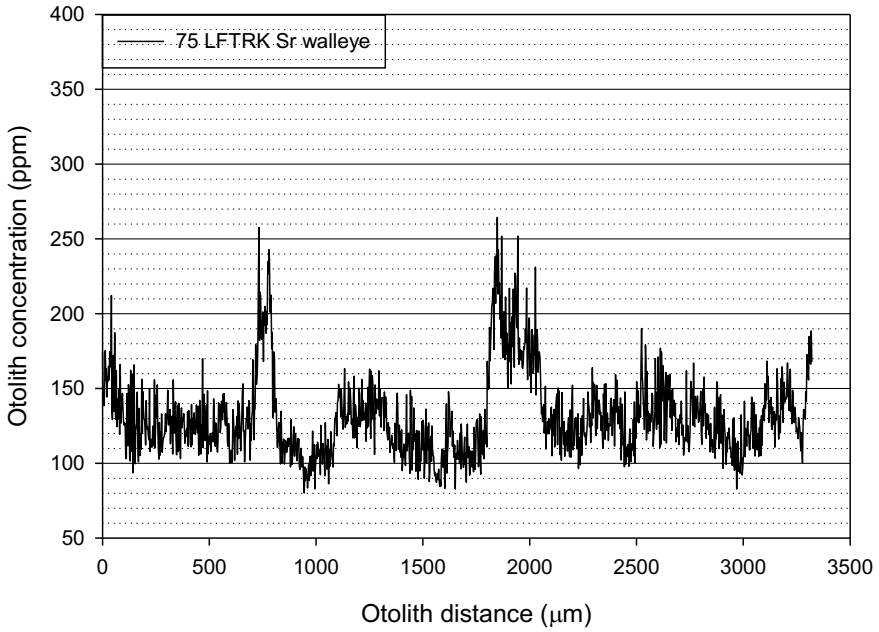
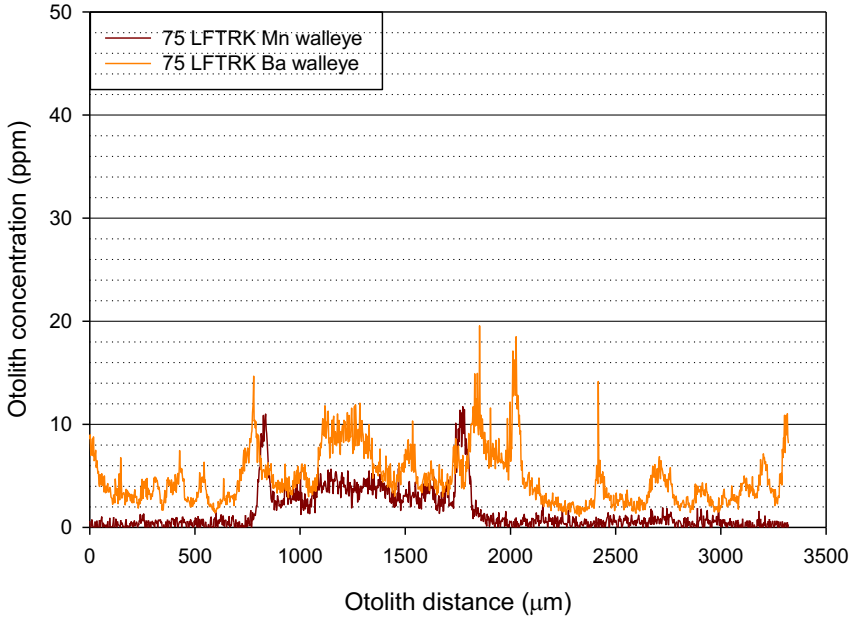


Figure A2. 8. Distribution of lake whitefish sex across each of the select CAMP waterbodies, pooled impoundment groups (IMP = impounded, NIMP = Non-impounded) of CAMP waterbodies, and individuals in total.

EDGE-CORE-EDGE





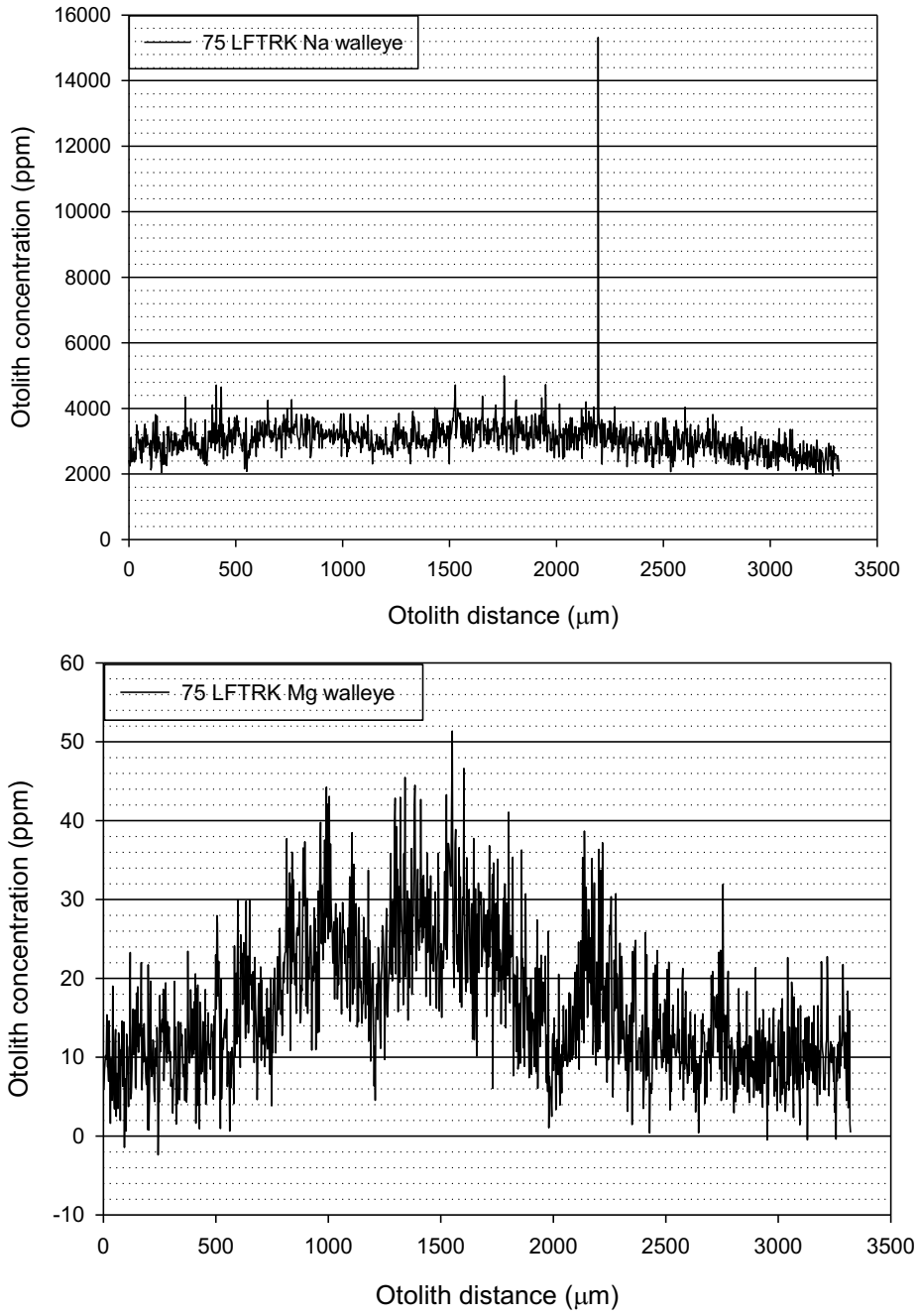
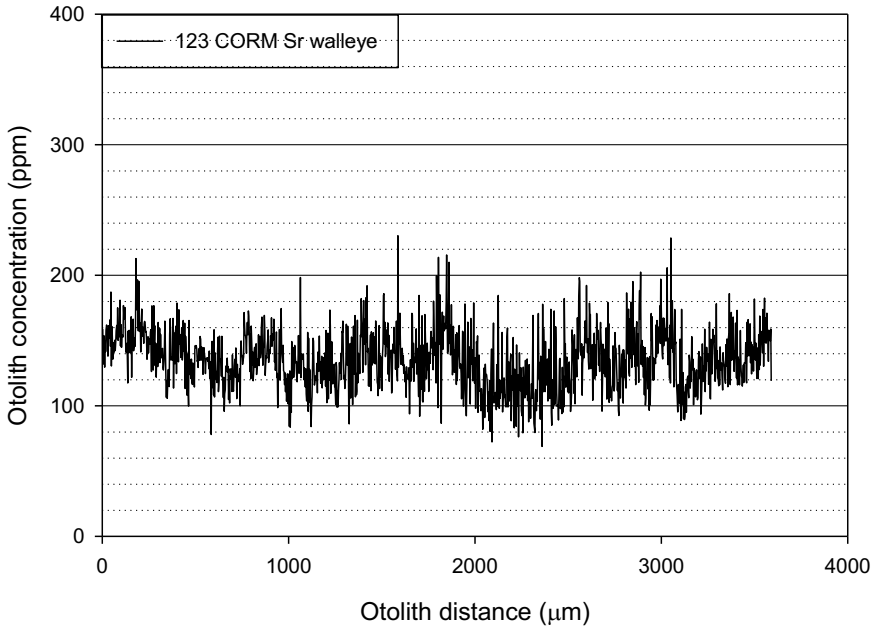
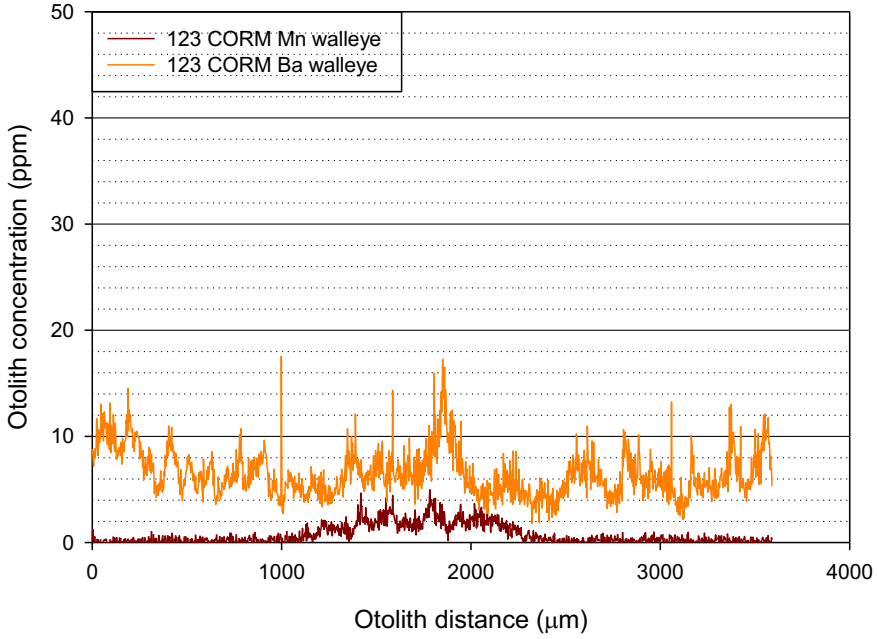


Figure A2. 9. Sample LA-ICP-MS linescan (edge-core-edge, left to right) of walleye (*Sander vitreus*). Elements graphed: Na, Mg, Mn, Ba, and Sr. CODE: WAL-LFT-75


123 CORM Sr walleye
Otolith concentration (ppm)
400
300
200
100
0
0
1000
2000
3000
4000
Otolith distance (μm)


123 CORM Mn walleye
123 CORM Ba walleye
Otolith concentration (ppm)
50
40
30
20
10
0
0
1000
2000
3000
4000
Otolith distance (μm)

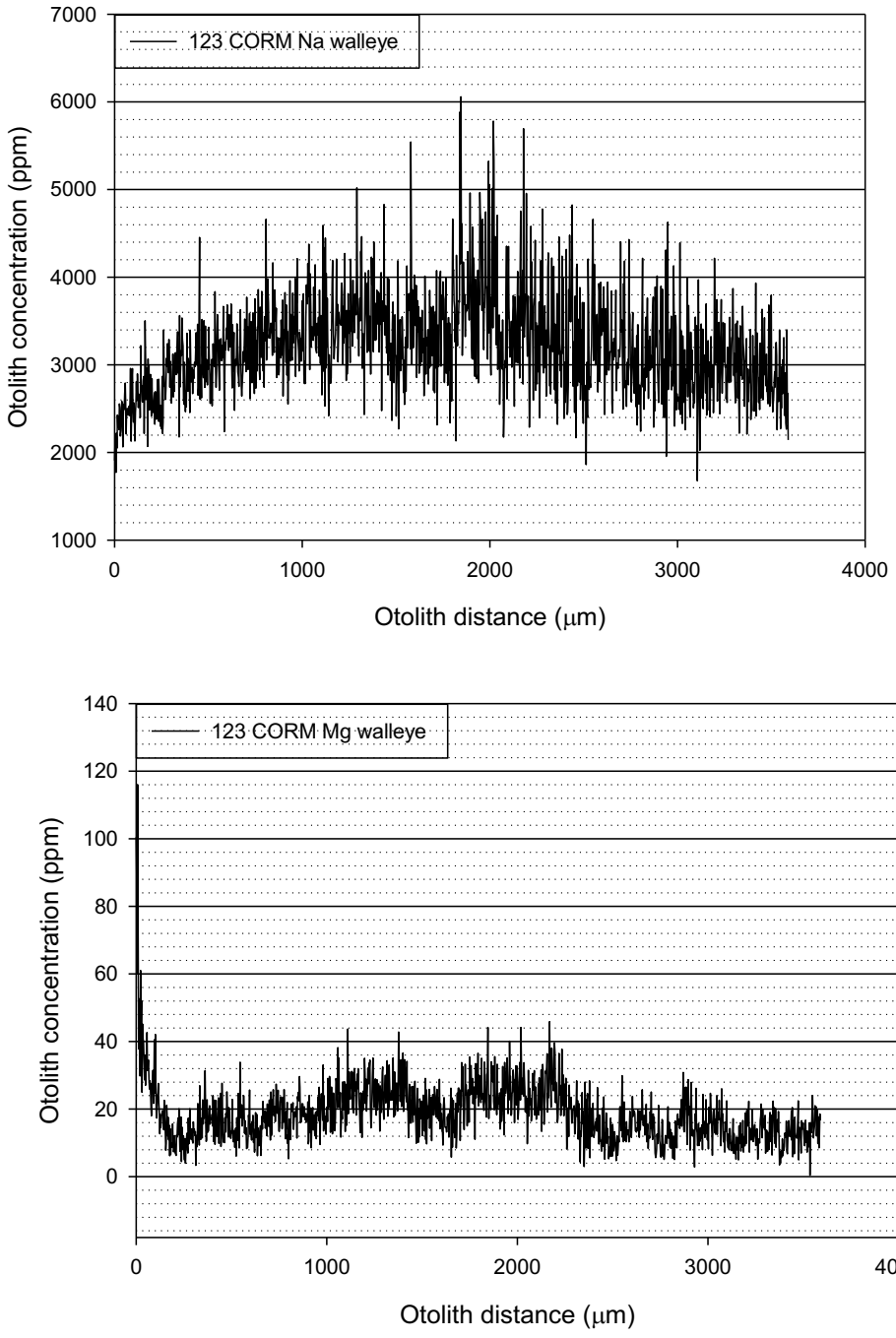
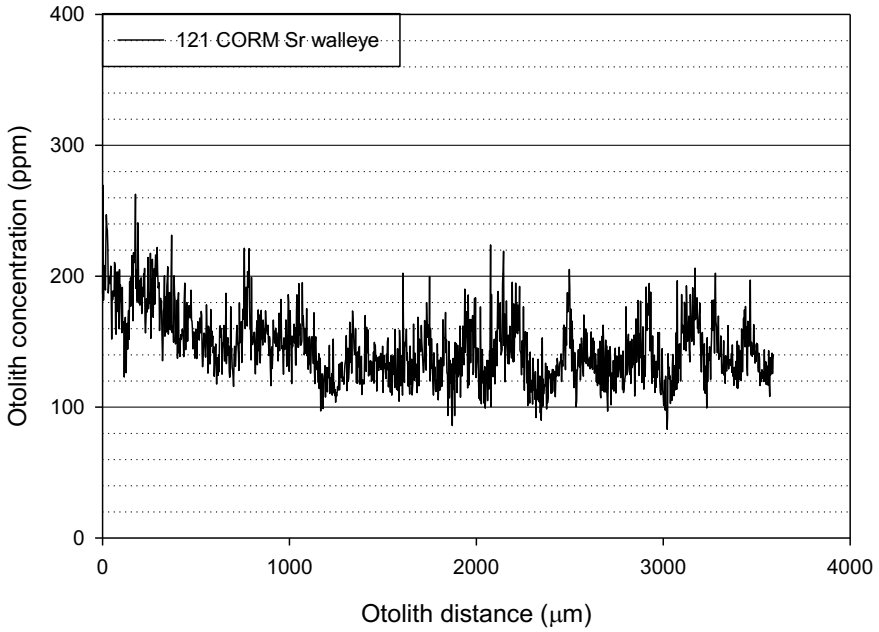


Figure A2. 10. Sample LA-ICP-MS linescan (edge-core-edge, left to right) of walleye (*Sander vitreus*). Elements graphed: Na, Mg, Mn, Ba, and Sr. CODE: WAL-CORM-123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Otolith concentration (ppm)
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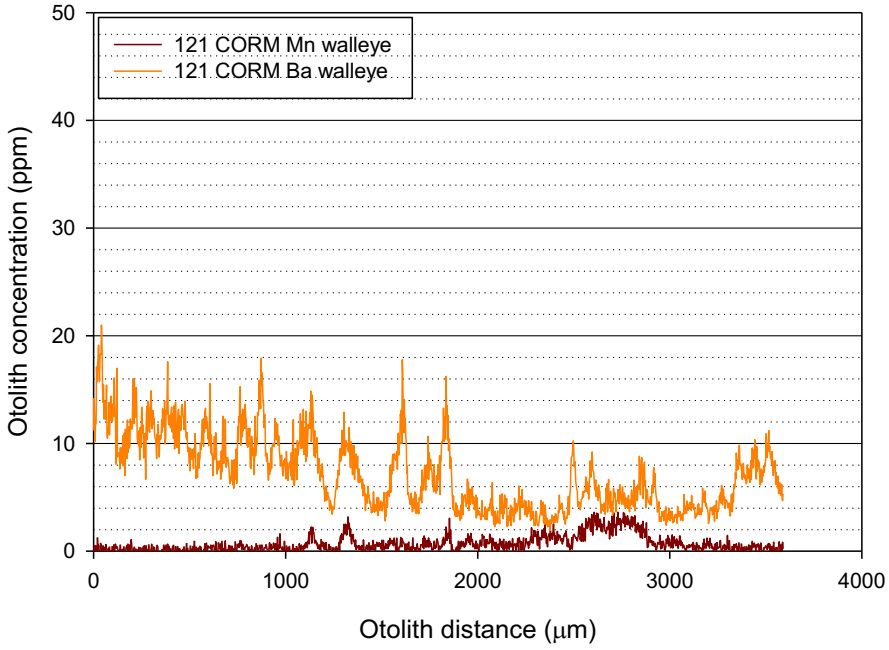
100
200
300
400
0
1000
2000
3000
4000
Otolith distance (μm)


121 CORM Mn walleye
121 CORM Ba walleye
Otolith concentration (ppm)
0
10
20
30
40
50
0
1000
2000
3000
4000
Otolith distance (μm)

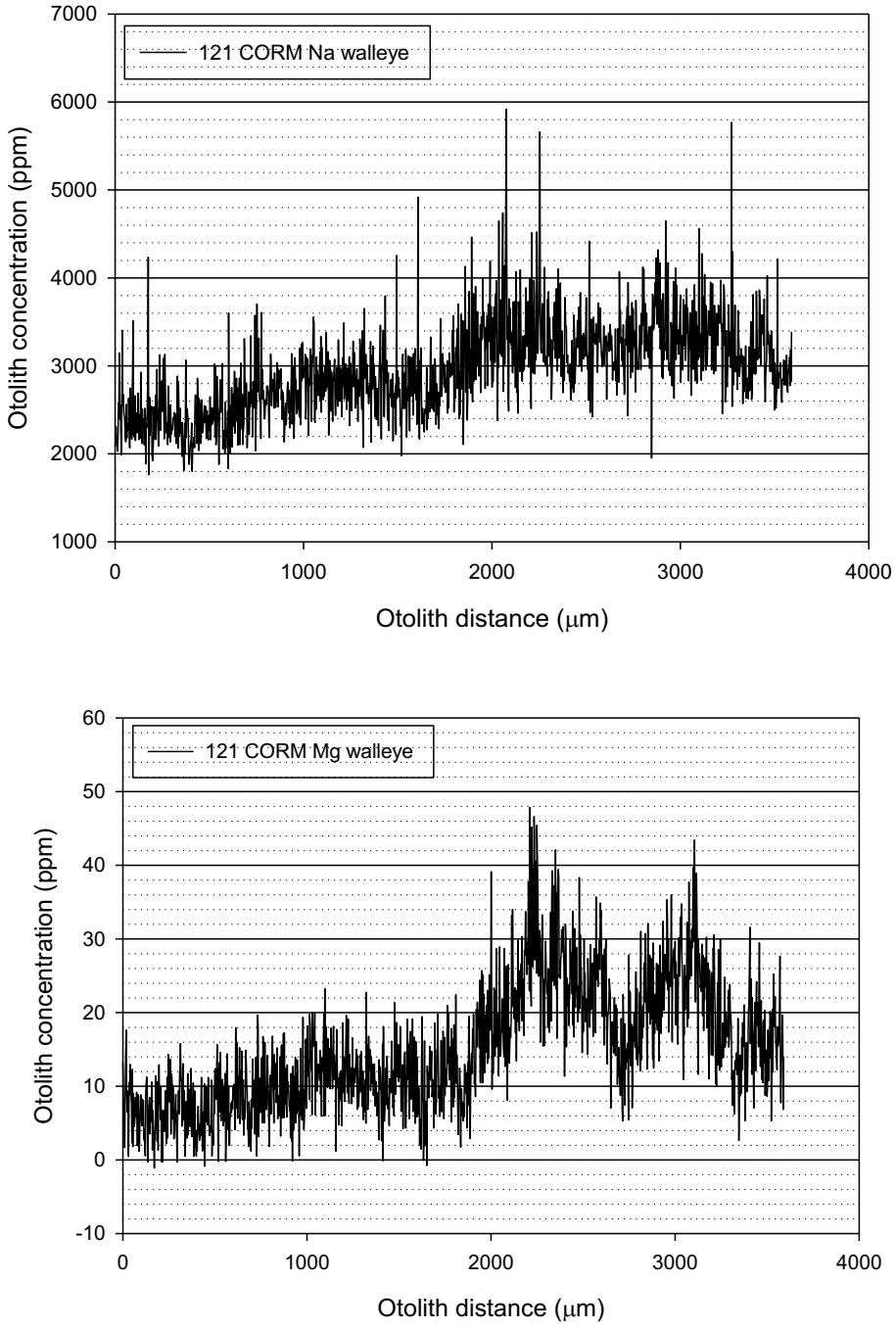
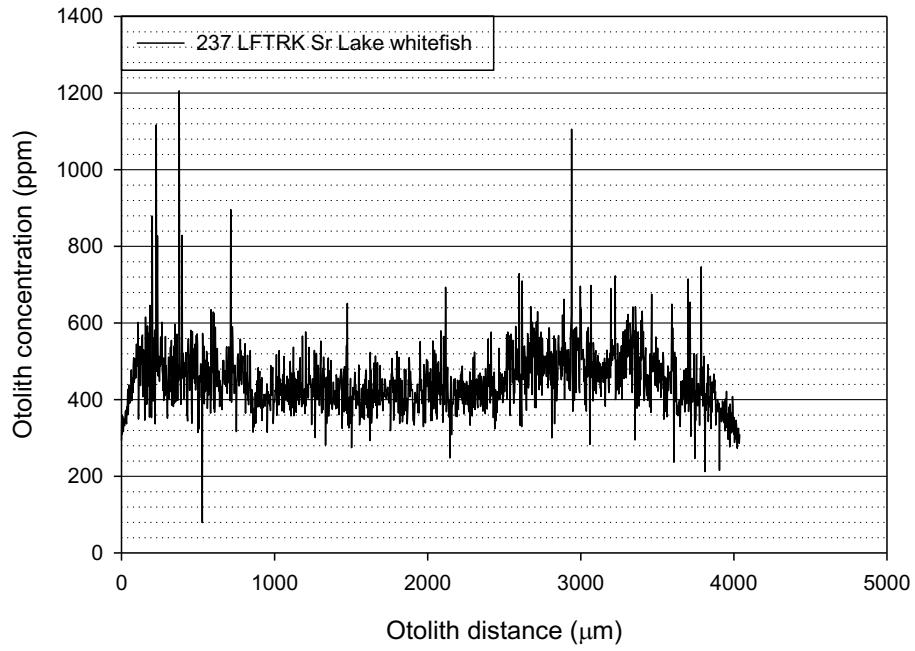
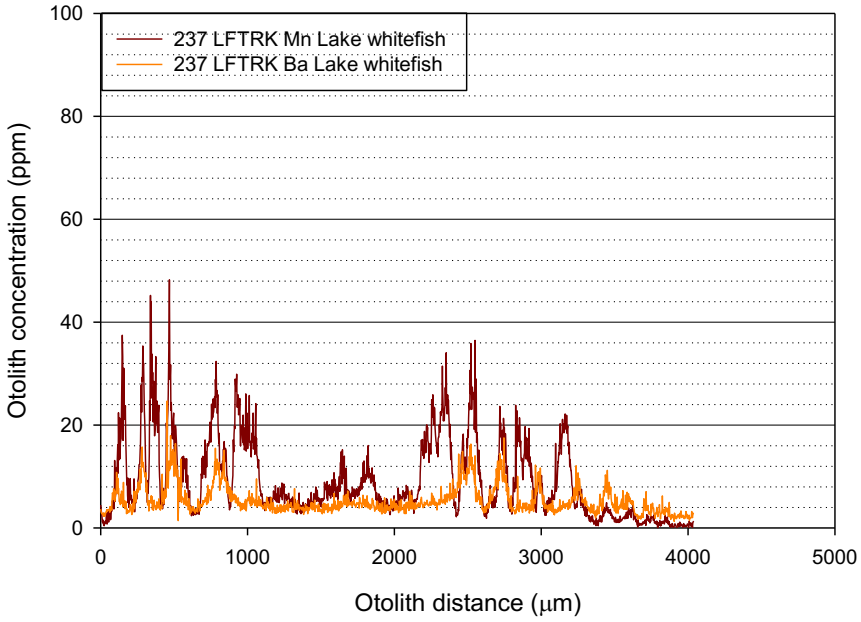


Figure A2. 11. Sample LA-ICP-MS linescan (edge-core-edge, left to right) of walleye (*Sander vitreus*). Elements graphed: Na, Mg, Mn, Ba, and Sr. CODE: WAL-CORM-121


237 LFTRK Sr Lake whitefish
Otolith concentration (ppm)
Otolith distance (μm)
0
200
400
600
800
1000
1200
1400
0
1000
2000
3000
4000
5000


237 LFTRK Mn Lake whitefish
237 LFTRK Ba Lake whitefish
Otolith concentration (ppm)
Otolith distance (μm)
0
20
40
60
80
100
0
1000
2000
3000
4000
5000

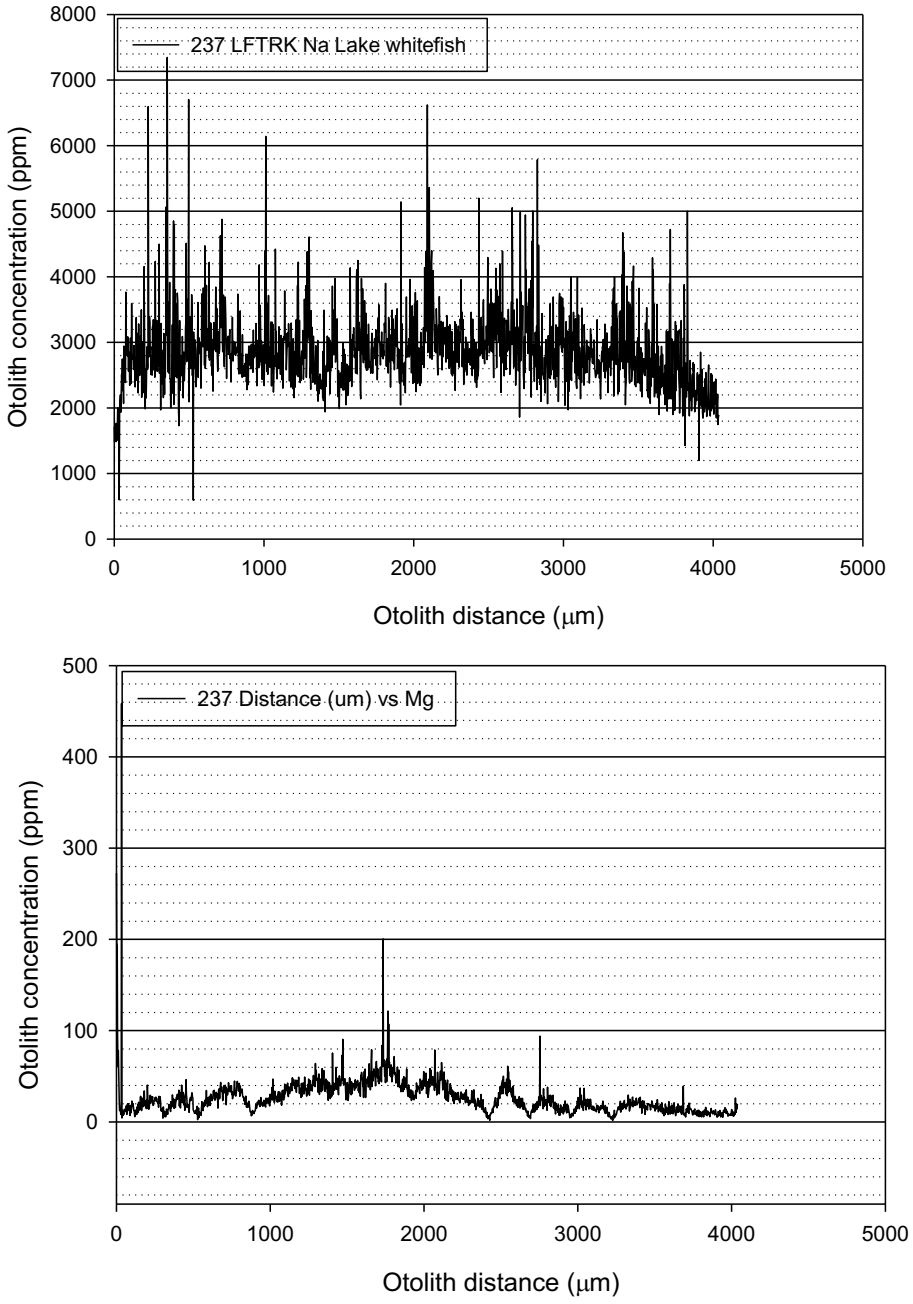
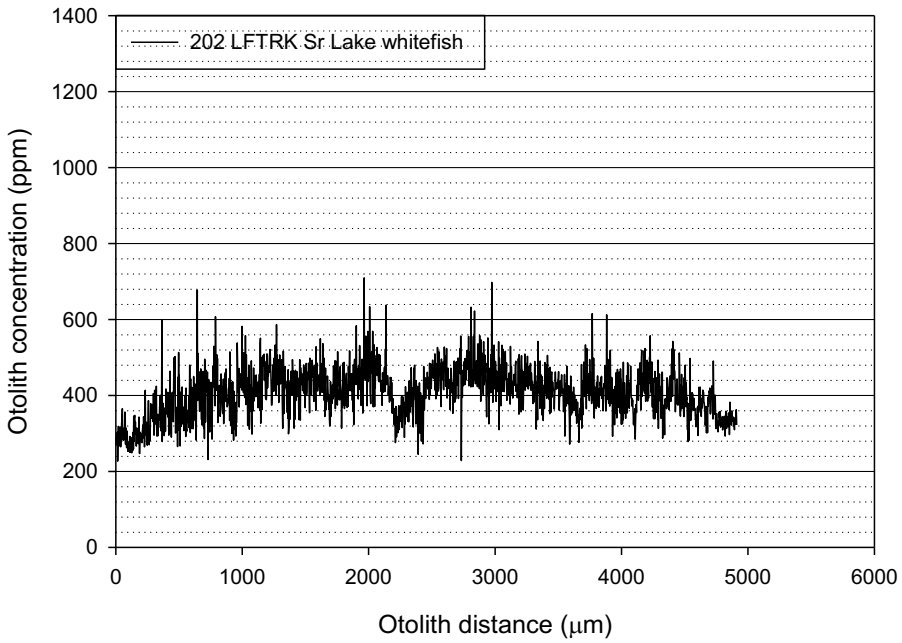
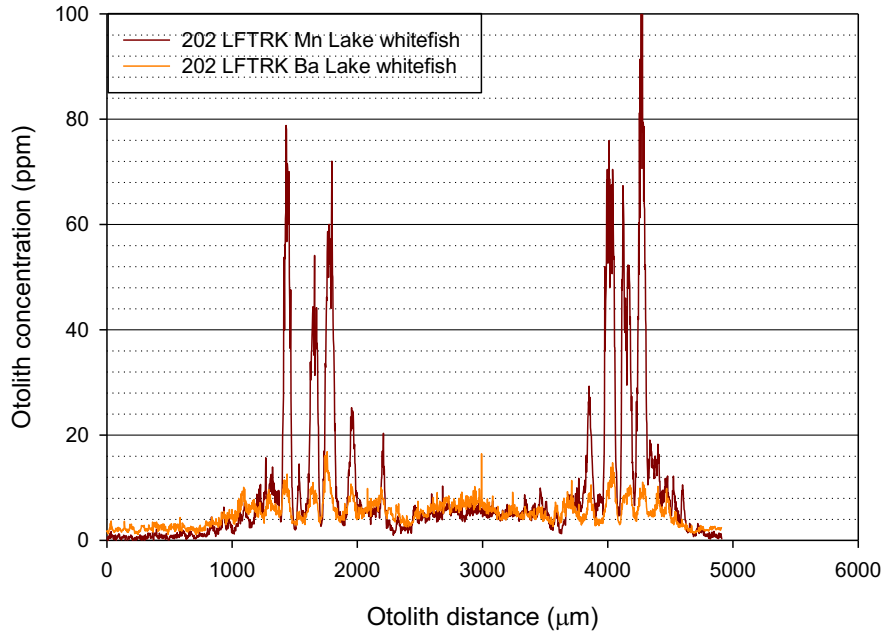
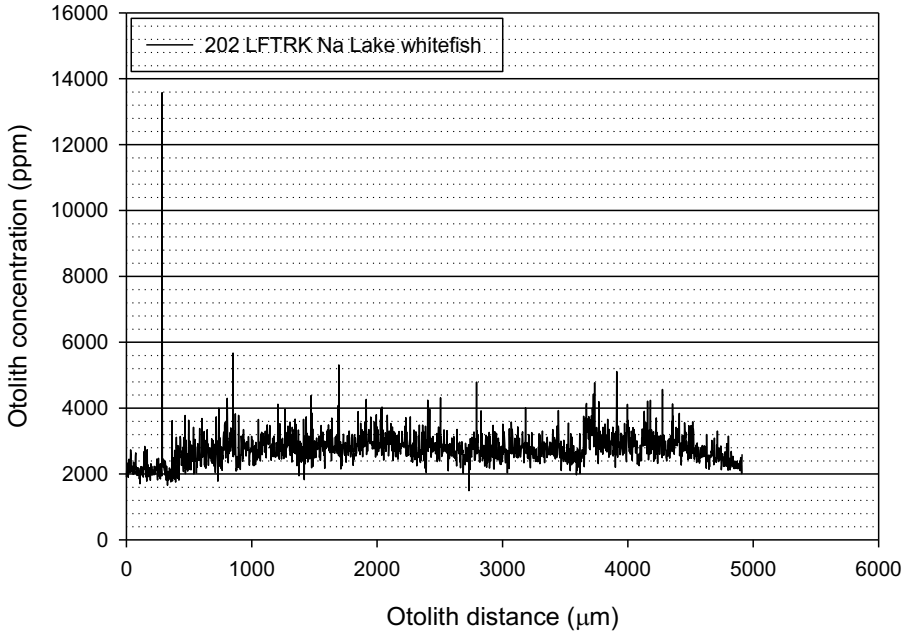


Figure A2. 12. Sample LA-ICP-MS linescan (edge-core-edge, left to right) of lake whitefish (*Coregonus clupeaformis*). Elements graphed: Na, Mg, Mn, Ba, and Sr. CODE: LKWF-LFT-121


202 LFTRK Sr Lake whitefish
Otolith concentration (ppm)
Otolith distance (μm)
0
200
400
600
800
1000
1200
1400
0
1000
2000
3000
4000
5000
6000


202 LFTRK Mn Lake whitefish
202 LFTRK Ba Lake whitefish
Otolith concentration (ppm)
Otolith distance (μm)
0
20
40
60
80
100
0
1000
2000
3000
4000
5000
6000



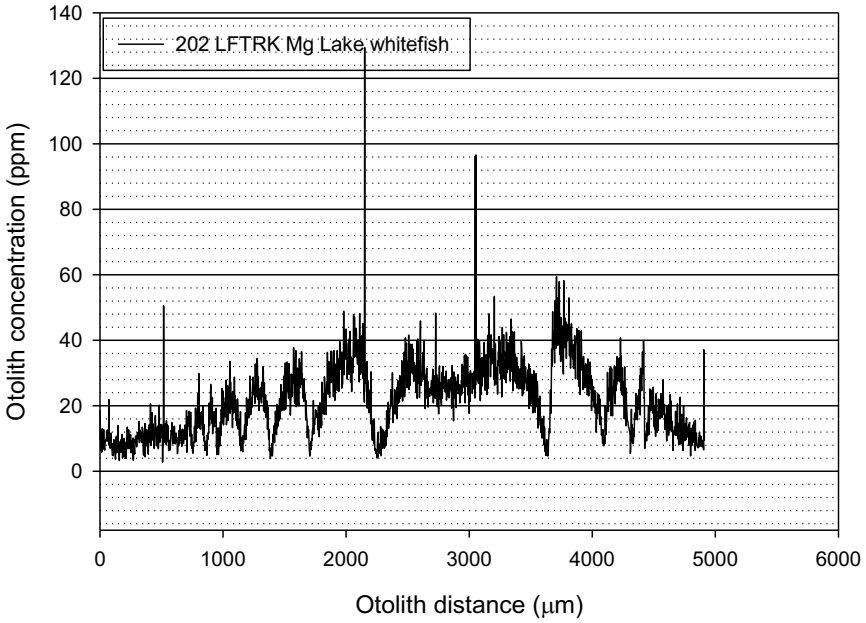
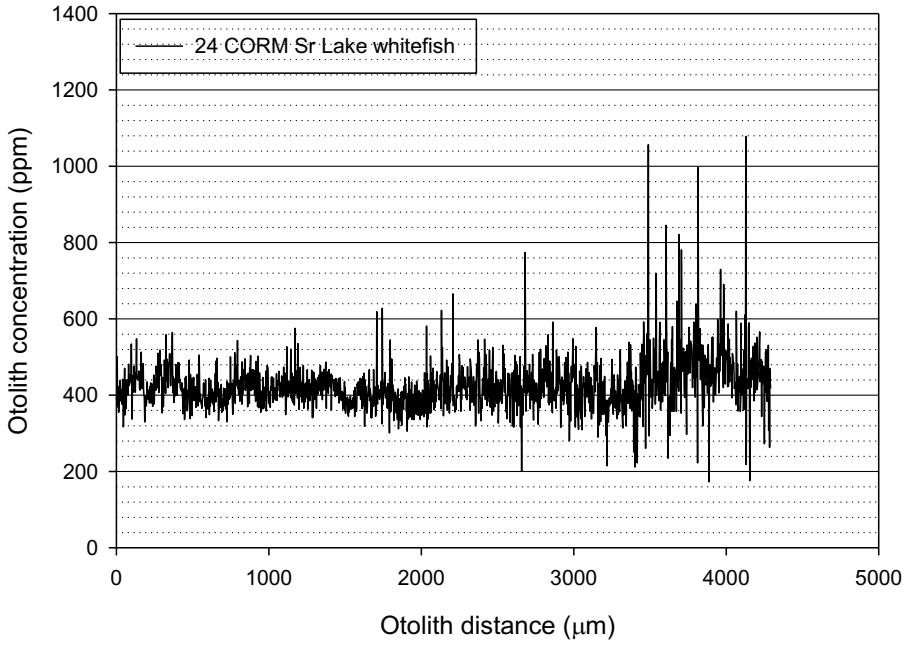
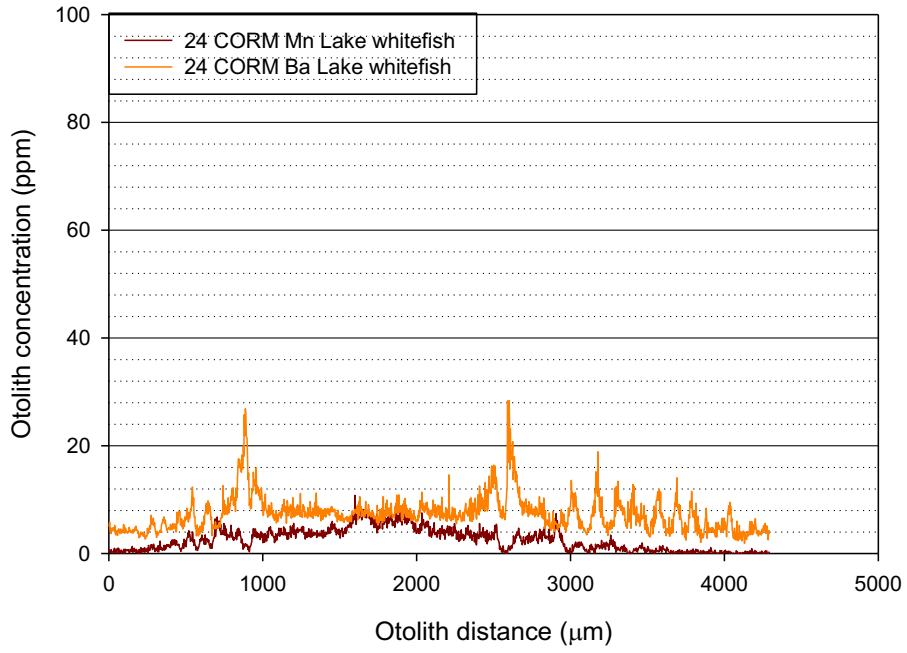
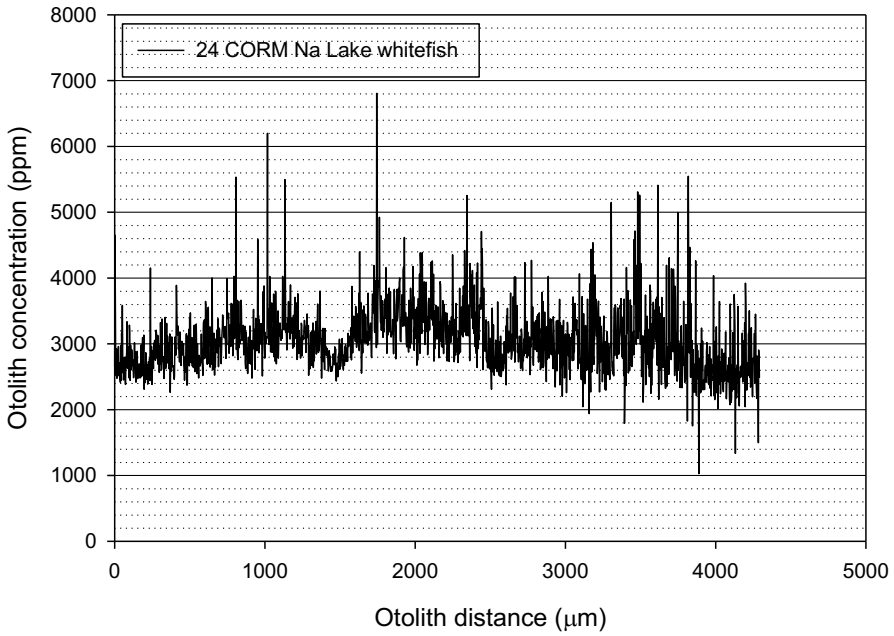


Figure A2. 13. Sample LA-ICP-MS linescan (edge-core-edge, left to right) of lake whitefish (*Coregonus clupeaformis*). Elements graphed: Na, Mg, Mn, Ba, and Sr. CODE: LKWF-LFT-202


24 CORM Sr Lake whitefish
Otolith concentration (ppm)
Otolith distance (μm)
0
200
400
600
800
1000
1200
1400
0
1000
2000
3000
4000
5000


24 CORM Mn Lake whitefish
24 CORM Ba Lake whitefish
Otolith concentration (ppm)
Otolith distance (μm)
0
20
40
60
80
100
0
1000
2000
3000
4000
5000



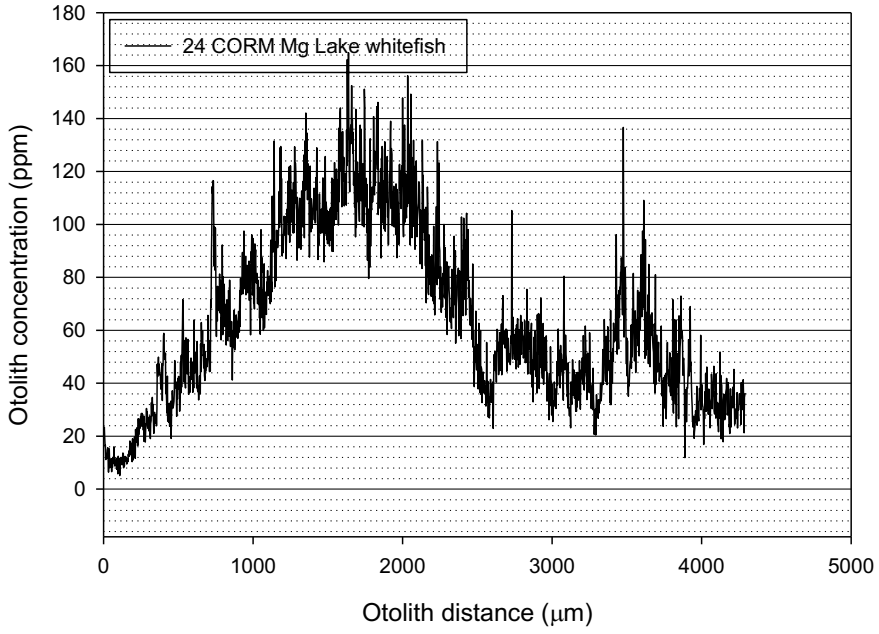


Figure A2. 14. Sample LA-ICP-MS linescan (edge-core-edge, left to right) of lake whitefish (*Coregonus clupeaformis*). Elements graphed: Na, Mg, Mn, Ba, and Sr. CODE: LKWF-CORM-24

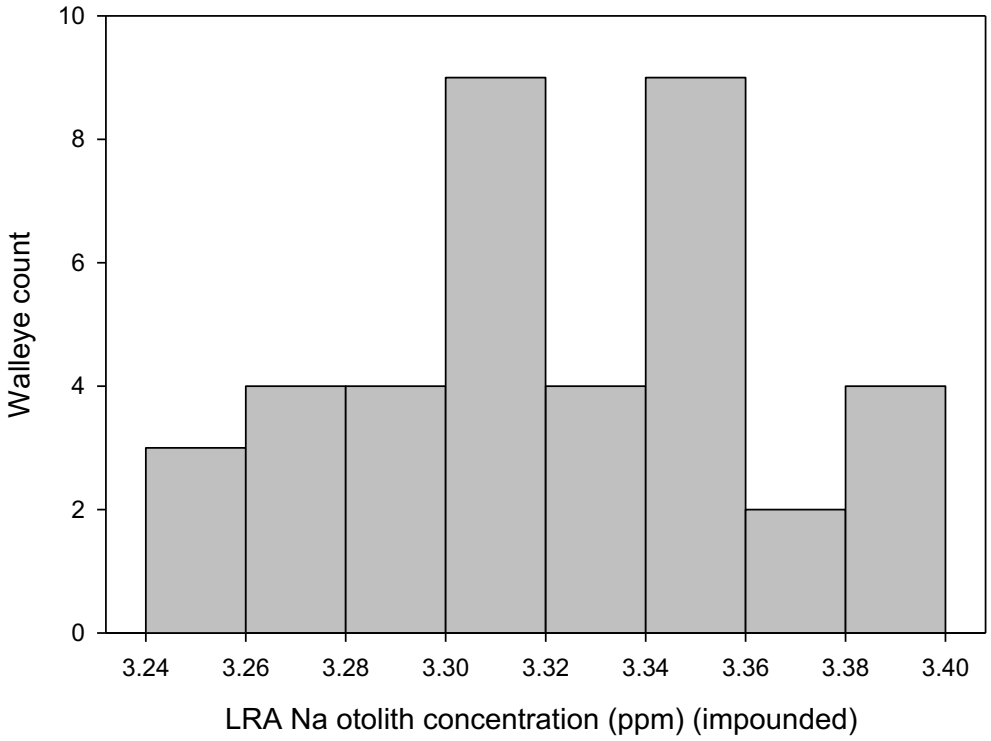


Figure A2. 15. Histogram of walleye otolith LRA sodium (Na) concentration across all four selected impounded CAMP waterbodies. 39 individual walleye tested, 9-10 otoliths per waterbody.

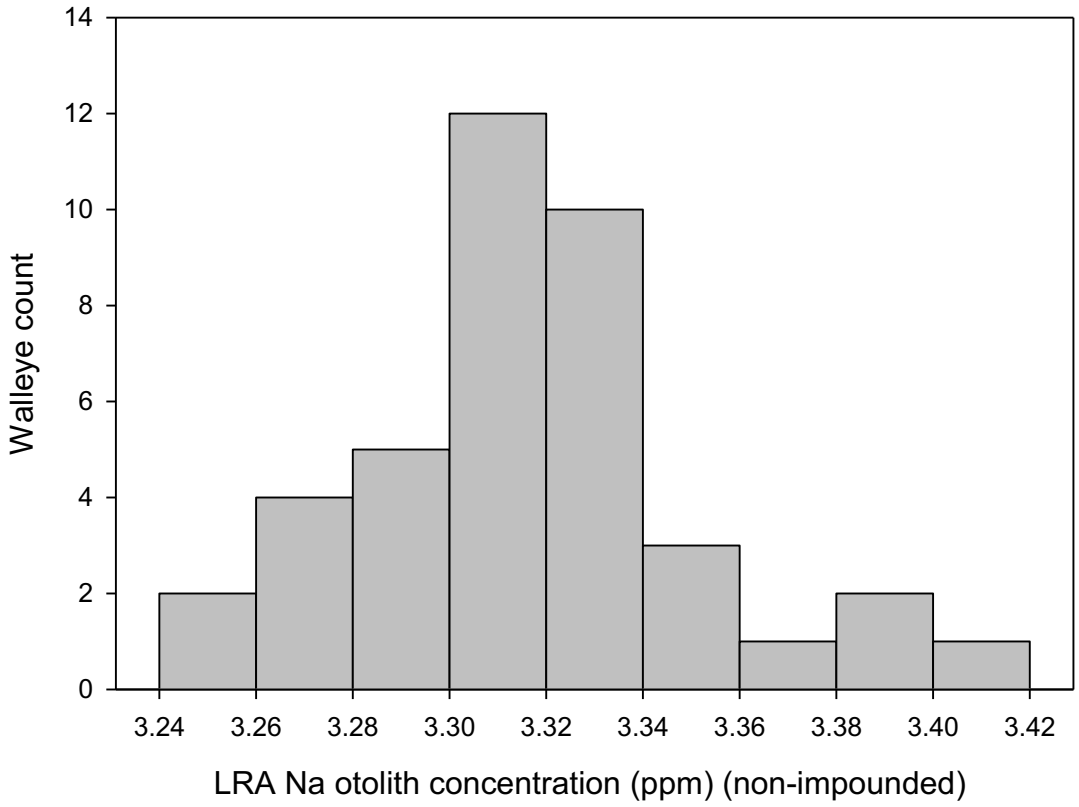


Figure A2. 16. Histogram of walleye otolith LRA sodium (Na) concentration across all four selected non-impounded CAMP waterbodies. 40 individual walleye tested,10 otoliths per waterbody.

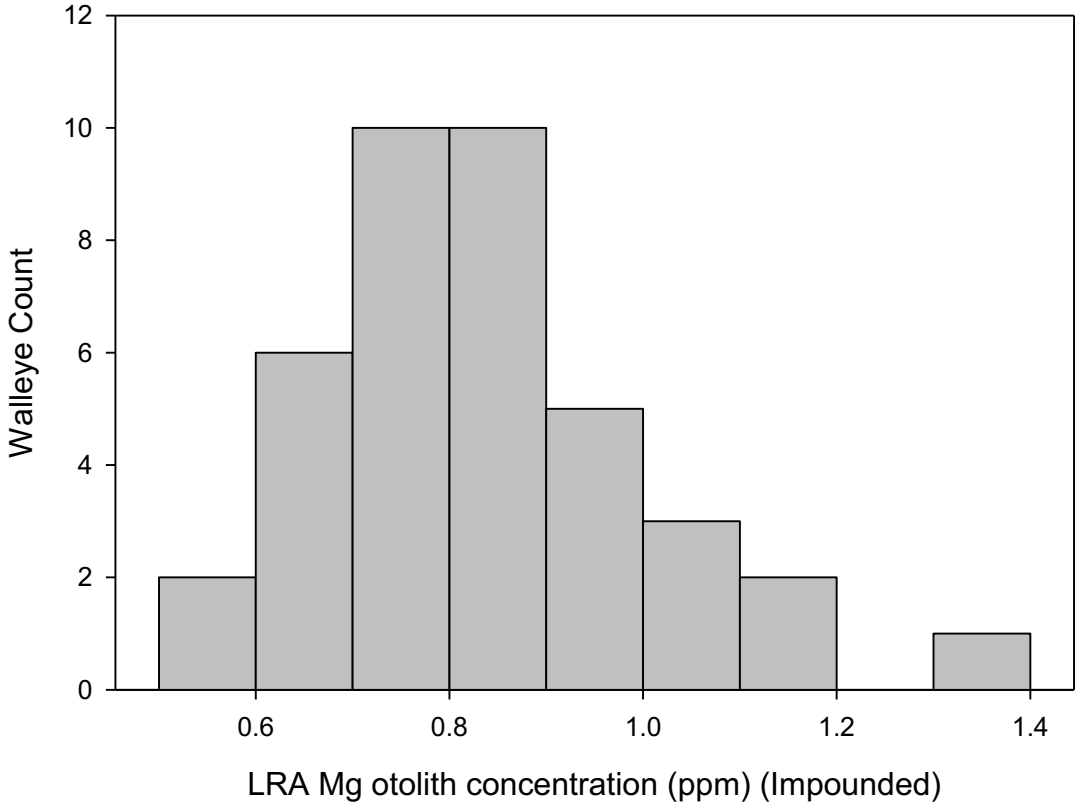


Figure A2. 17. Histogram of walleye otolith LRA sodium (Na) concentration across the four impounded CAMP waterbodies. 39 individual walleye tested, 9-10 otoliths per waterbody.

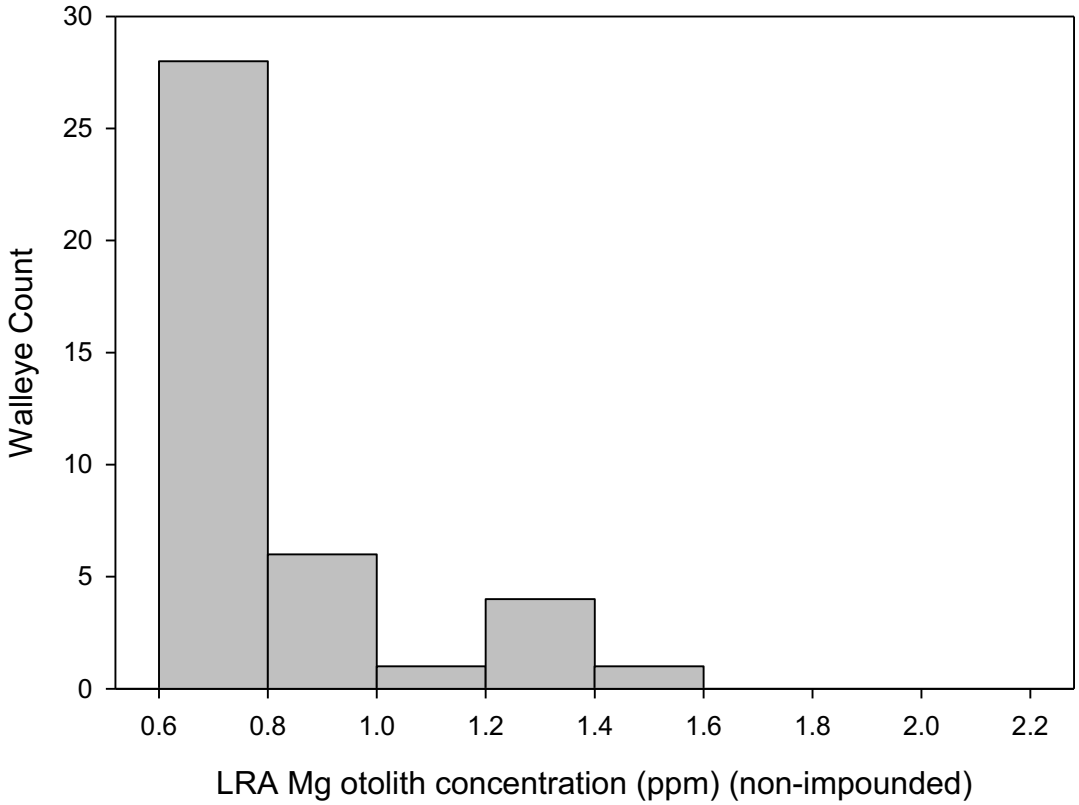


Figure A2. 18. Histogram of walleye otolith LRA sodium (Na) concentration across the four non-impounded CAMP waterbodies. 40 individual walleye tested, 10 otoliths per waterbody.

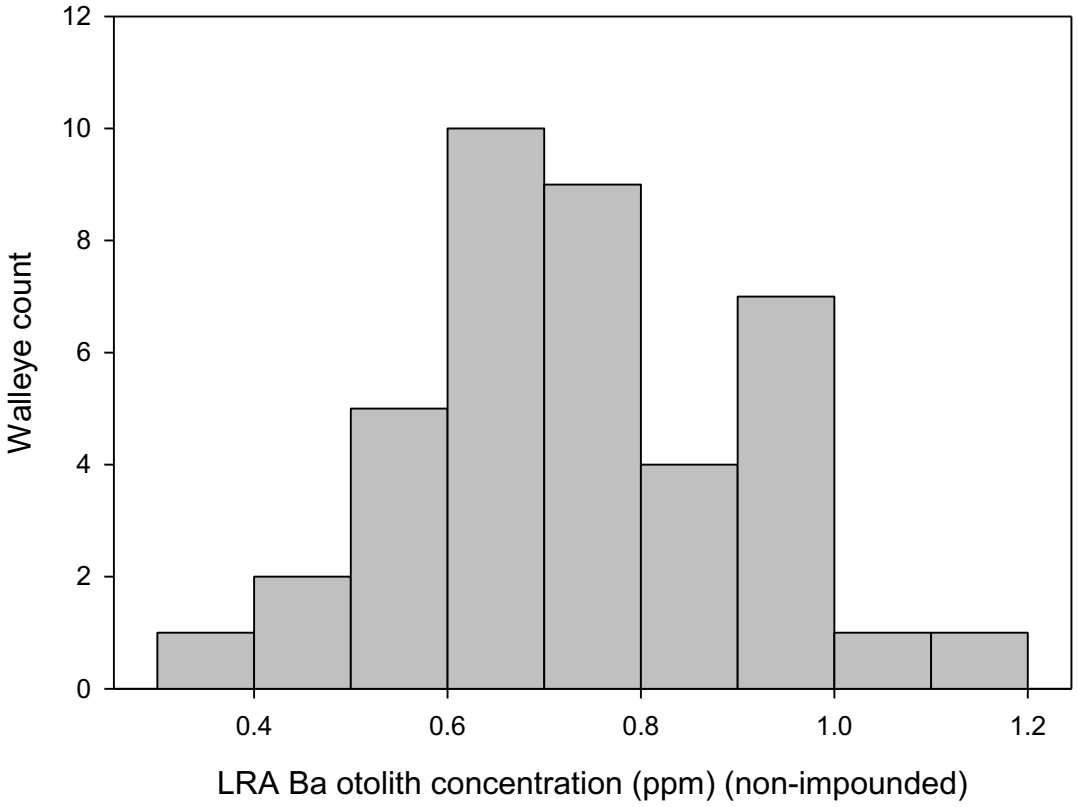


Figure A2. 19. Histogram of walleye otolith LRA barium (Ba) concentration across all four selected non-impounded CAMP waterbodies. 40 individual walleye tested,10 otoliths per waterbody.

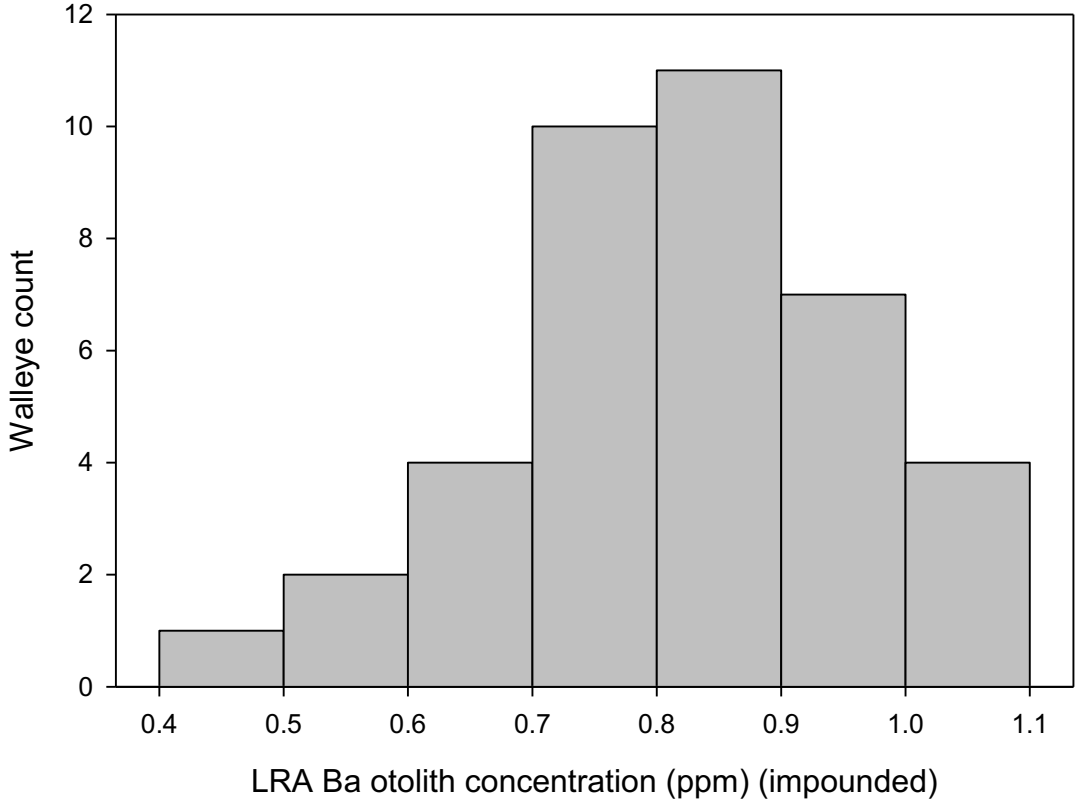


Figure A2. 20. Histogram of walleye otolith LRA barium (Ba) concentration across all four selected impounded CAMP waterbodies. 39 individual walleye tested, 9-10 otoliths per waterbody.

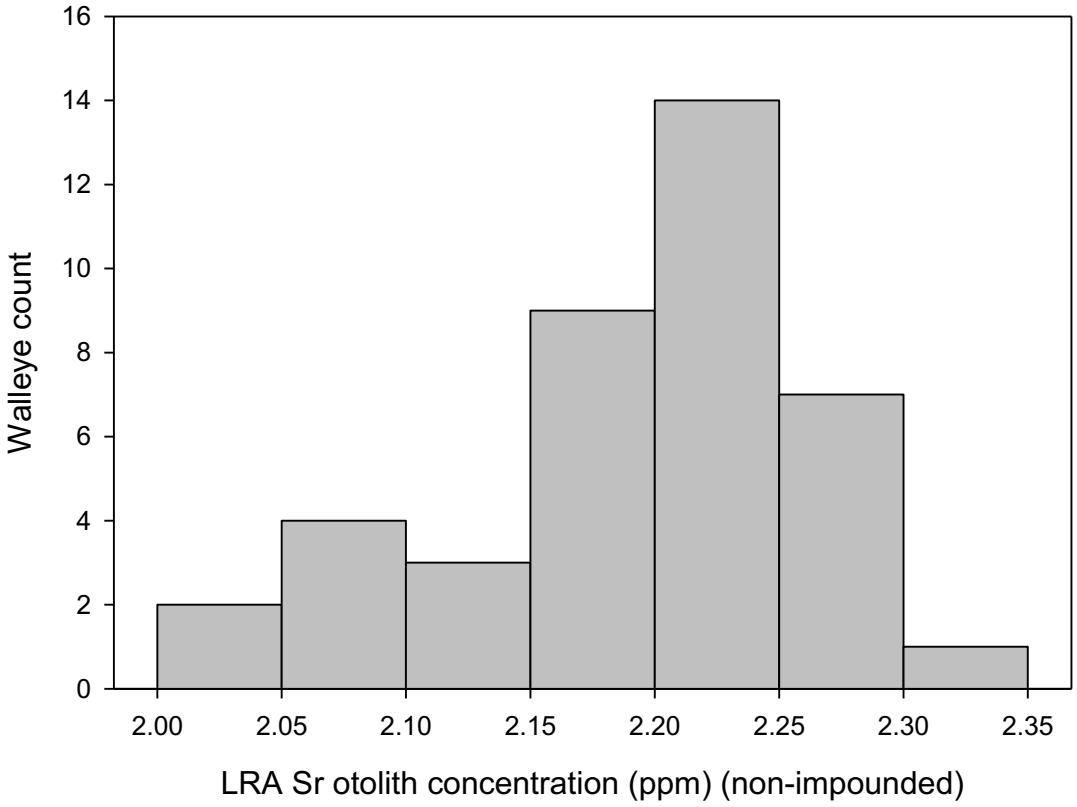


Figure A2. 21. Histogram of walleye otolith LRA strontium (Sr) concentration across all four selected non-impounded CAMP waterbodies. 40 individual walleye tested,10 otoliths per waterbody.

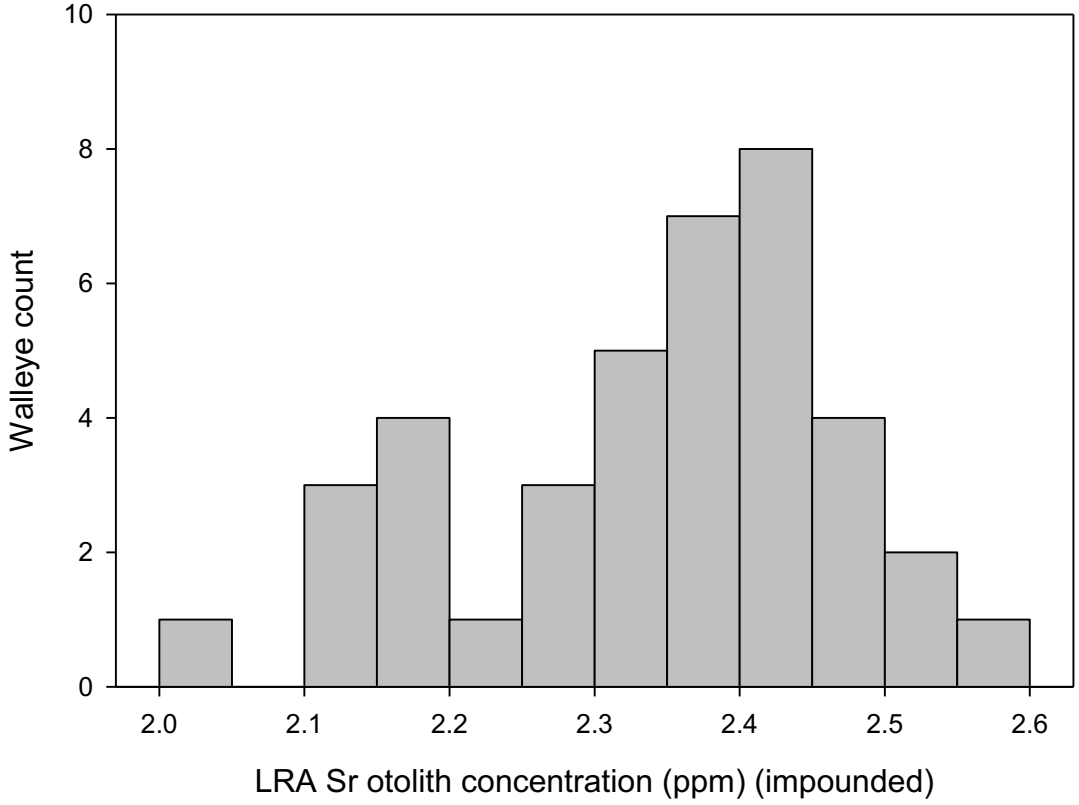


Figure A2. 22. Histogram of walleye otolith LRA barium (Ba) concentration across all four selected impounded CAMP waterbodies. 39 individual walleye tested, 9-10 otoliths per waterbody.

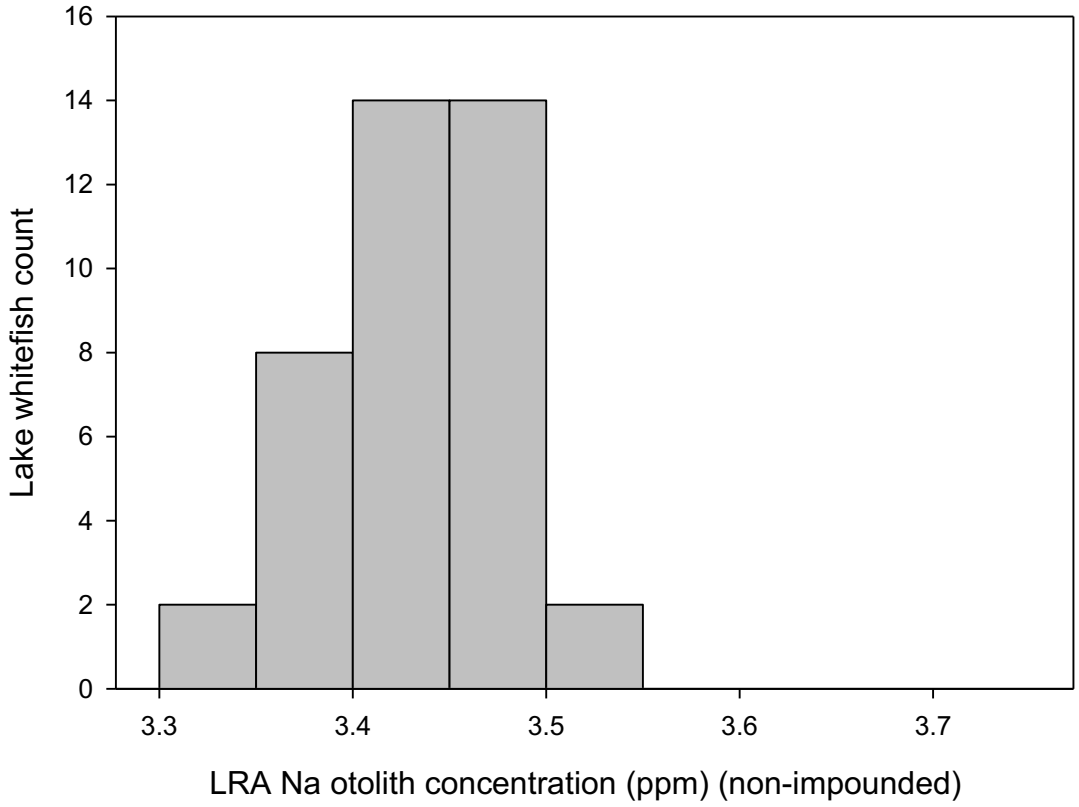


Figure A2. 23. Histogram of lake whitefish otolith LRA sodium (Na) concentration across all four selected non-impounded CAMP waterbodies. 40 individual walleye tested, 10 otoliths per waterbody.

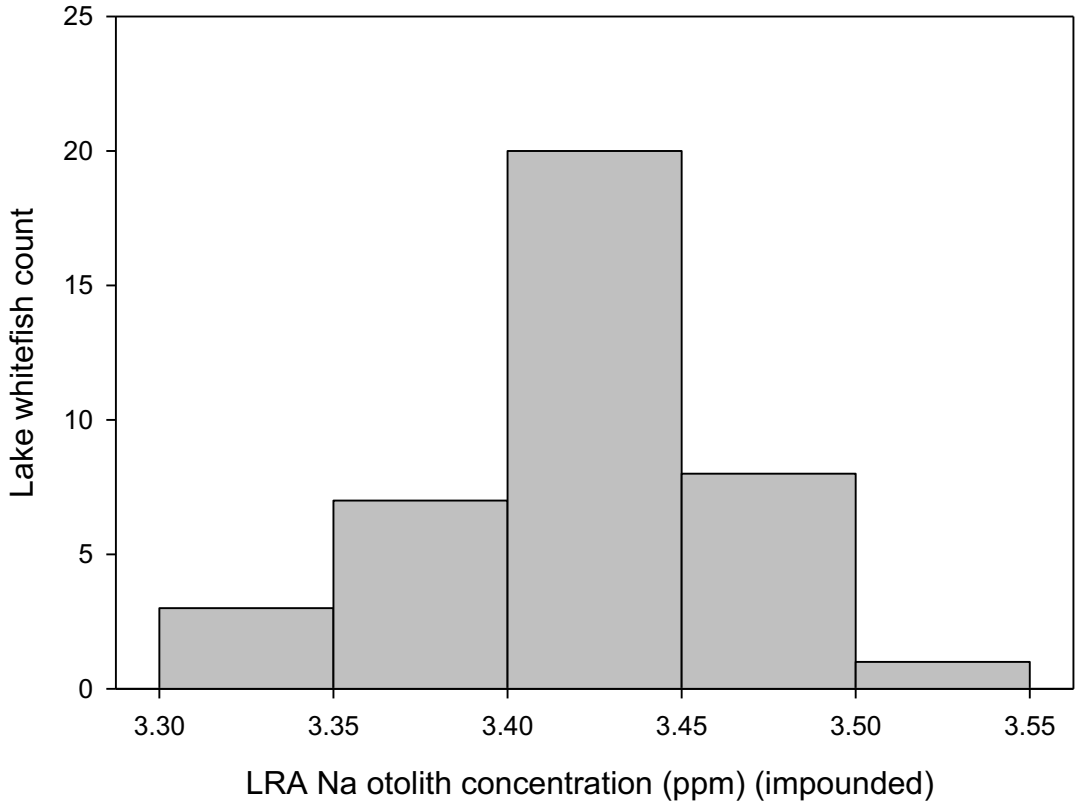


Figure A2. 24. Histogram of lake whitefish otolith LRA sodium (Na) concentration across all four selected impounded CAMP waterbodies. 39 individual walleye tested, 9-10 otoliths per waterbody.

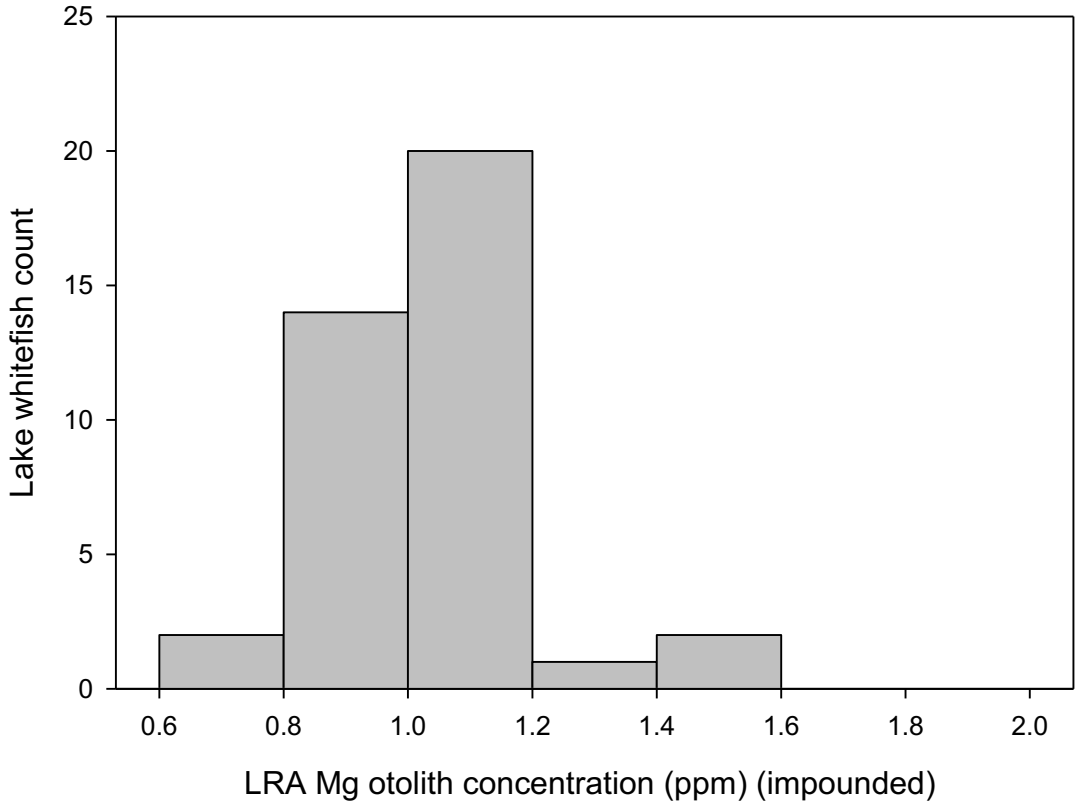


Figure A2. 25. Histogram of lake whitefish otolith LRA magnesium (Mg) concentration across all four selected impounded CAMP waterbodies. 39 individual walleye tested, 9-10 otoliths per waterbody.

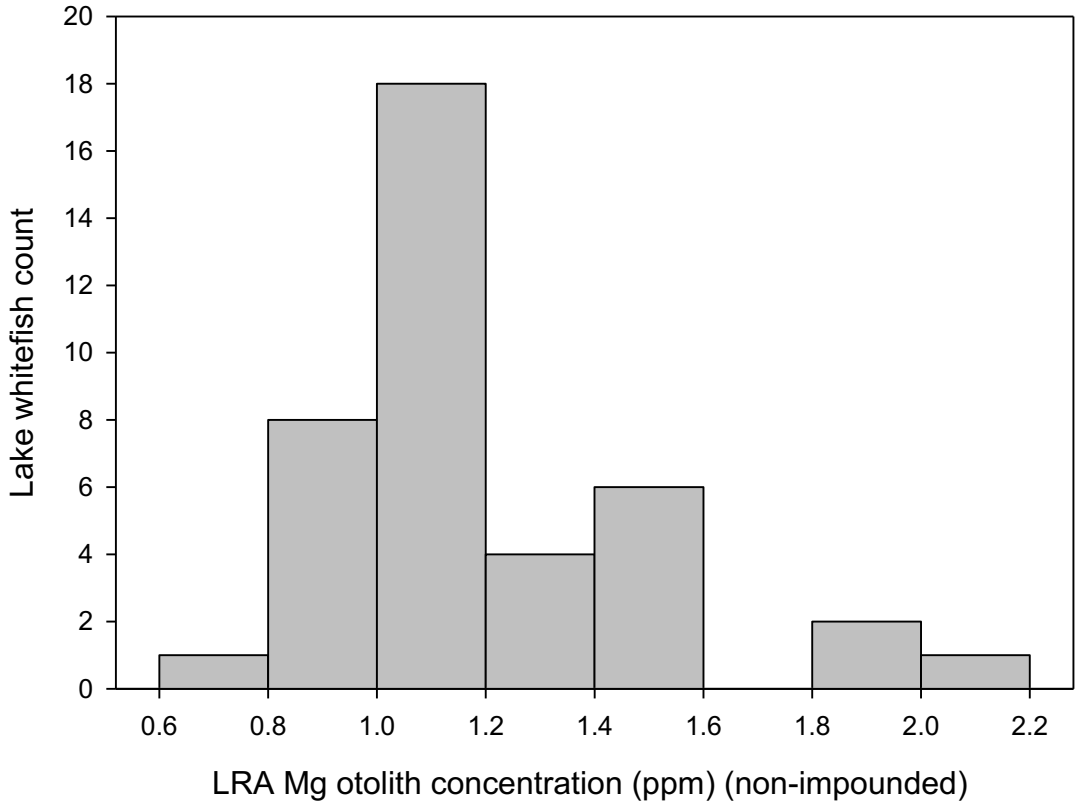


Figure A2. 26. Histogram of lake whitefish otolith LRA magnesium (Mg) concentration across all four selected non-impounded CAMP waterbodies. 40 individual walleye tested, 10 otoliths per waterbody.

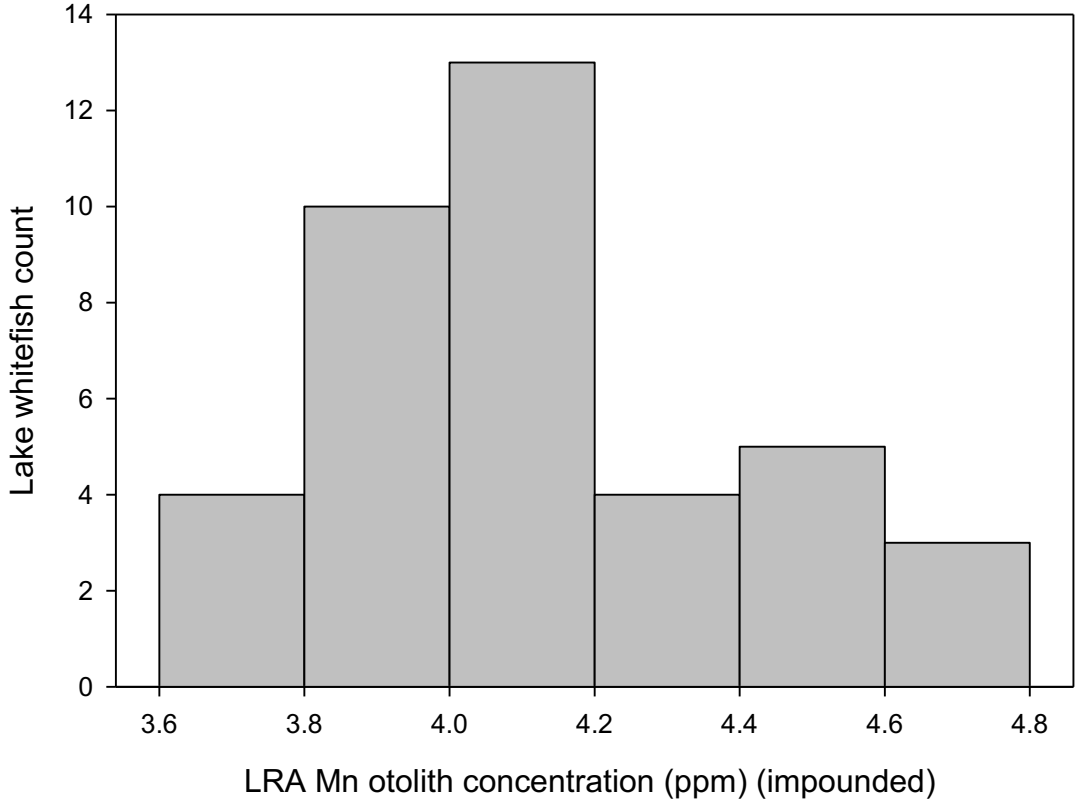


Figure A2. 27. Histogram of lake whitefish otolith LRA manganese (Mn) concentration across all four selected impounded CAMP waterbodies. 39 individual walleye tested, 9-10 otoliths per waterbody.

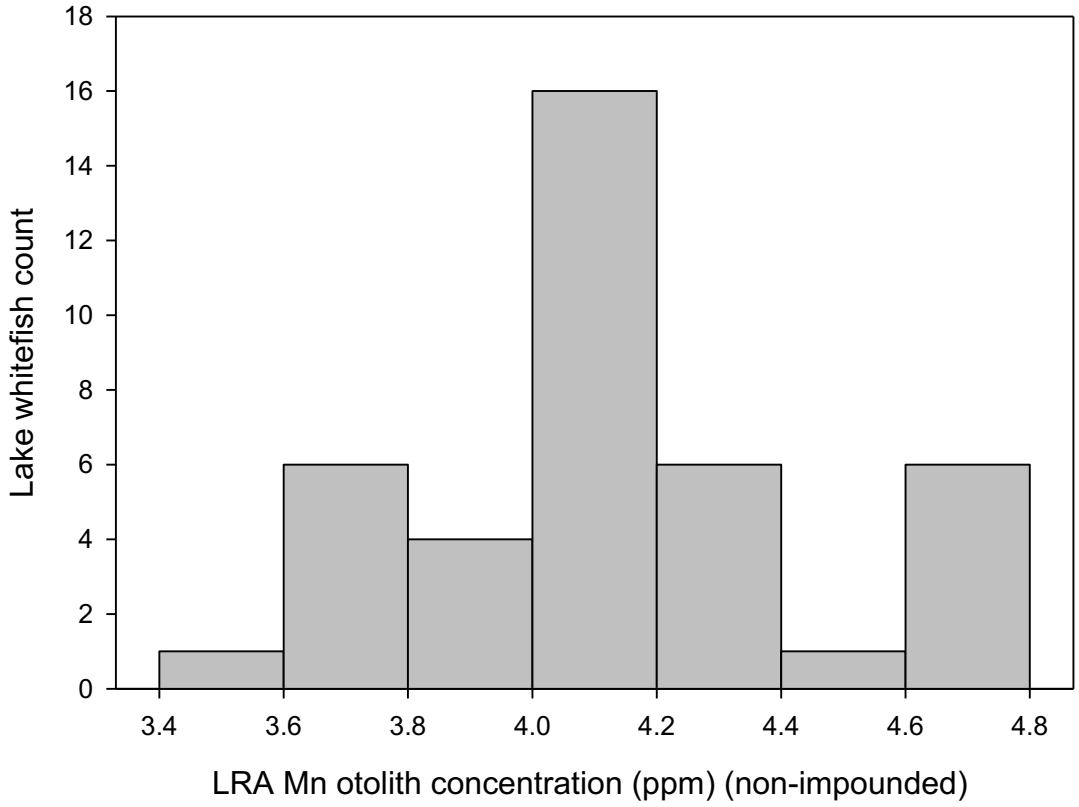


Figure A2. 28. Histogram of lake whitefish otolith LRA manganese (Mn) concentration across all four selected non-impounded CAMP waterbodies. 40 individual walleye tested,10 otoliths per waterbody.

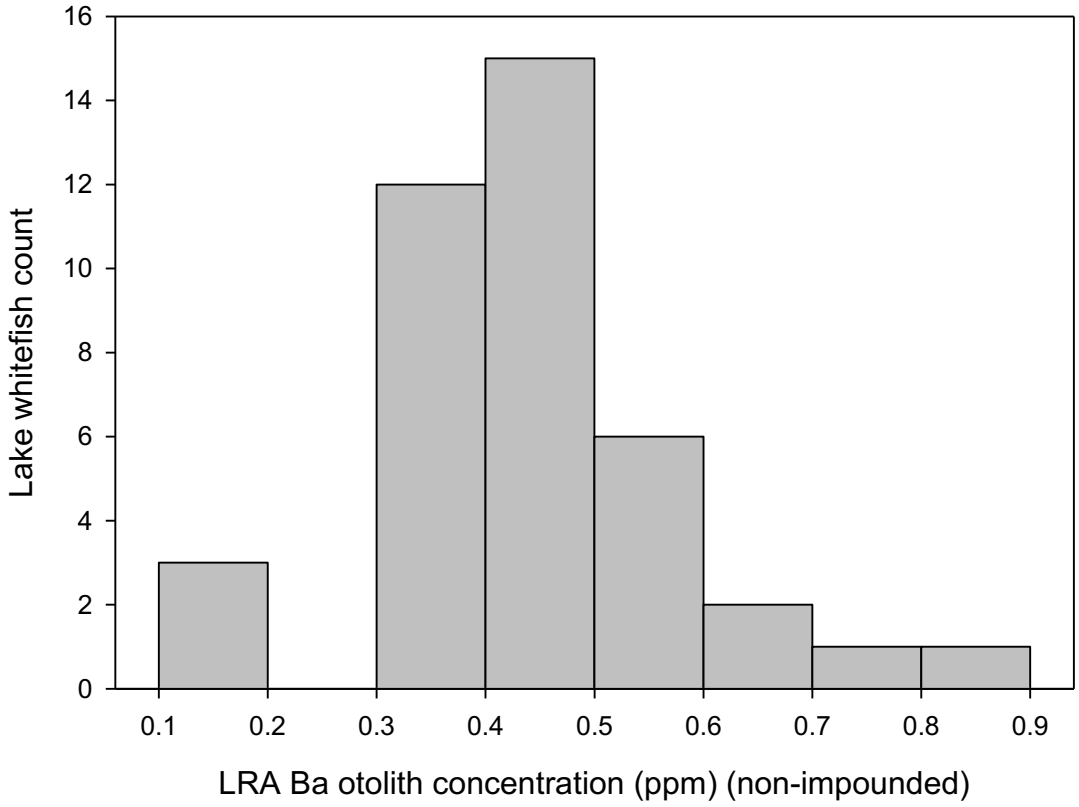


Figure A2. 29. Histogram of lake whitefish otolith LRA barium (Ba) concentration across all four selected non-impounded CAMP waterbodies. 40 individual walleye tested,10 otoliths per waterbody.

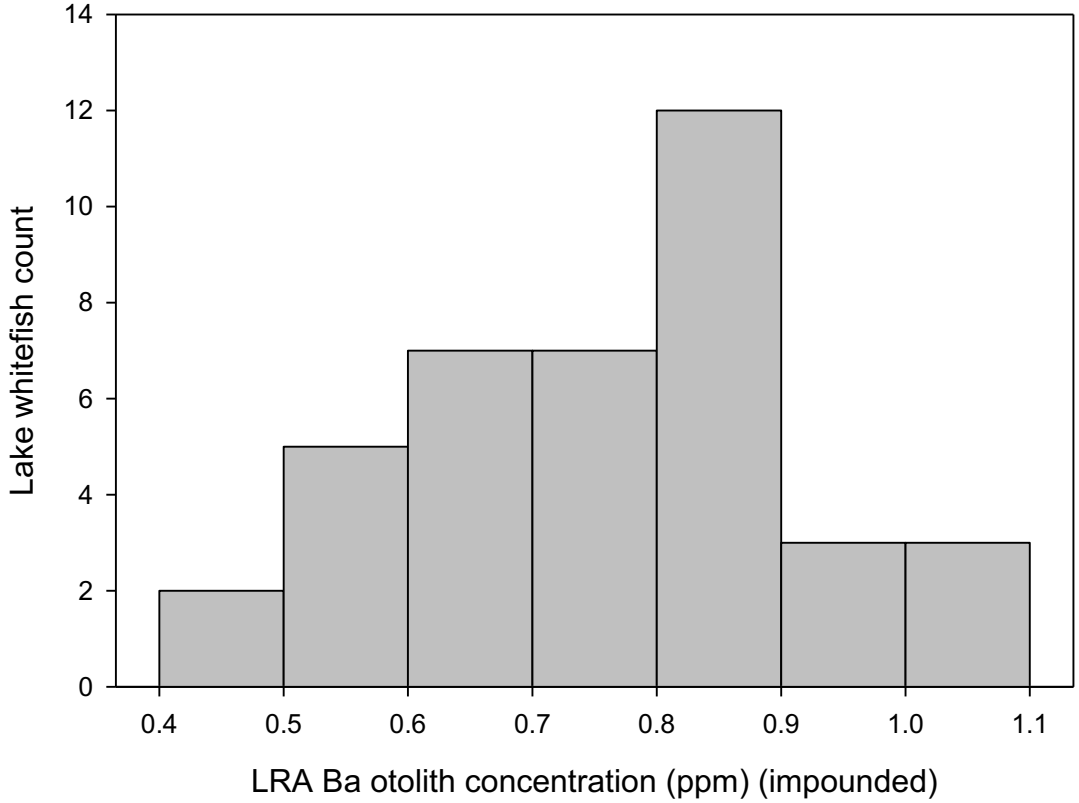


Figure A2. 30. Histogram of lake whitefish otolith LRA barium (Ba) concentration across all four selected impounded CAMP waterbodies. 39 individual walleye tested, 9-10 otoliths per waterbody.

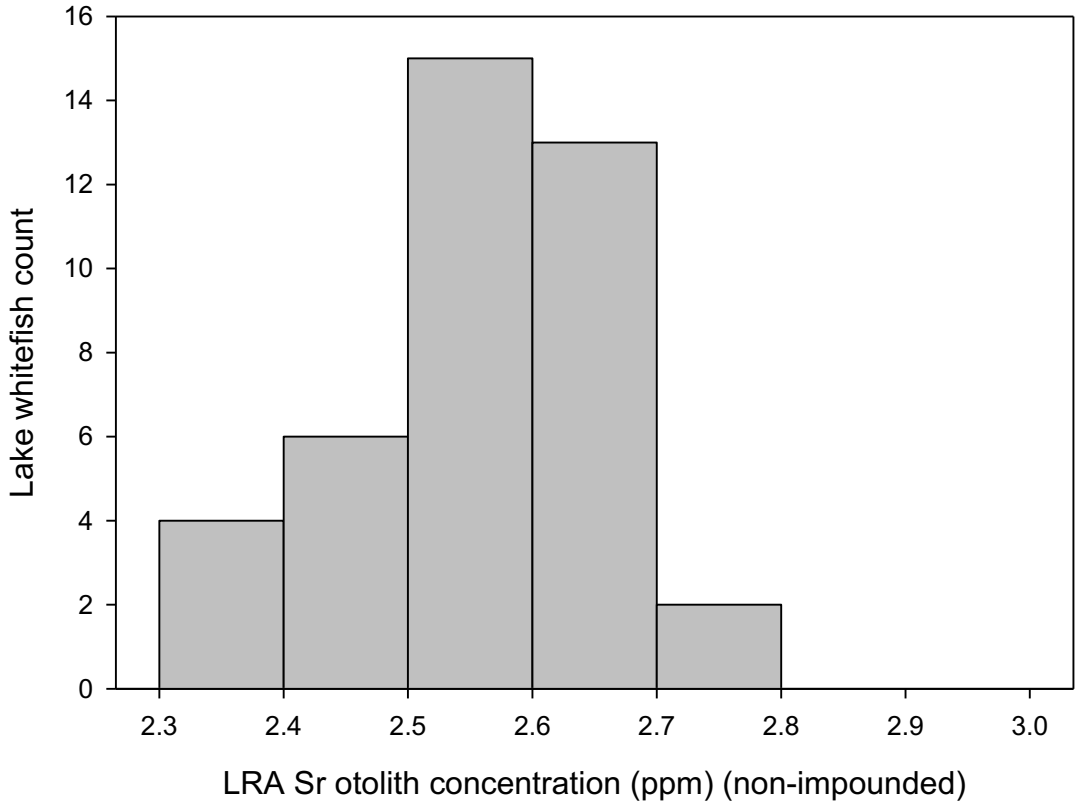


Figure A2. 31. Histogram of lake whitefish otolith LRA strontium (Sr) concentration across all four selected non-impounded CAMP waterbodies. 40 individual walleye tested,10 otoliths per waterbody.

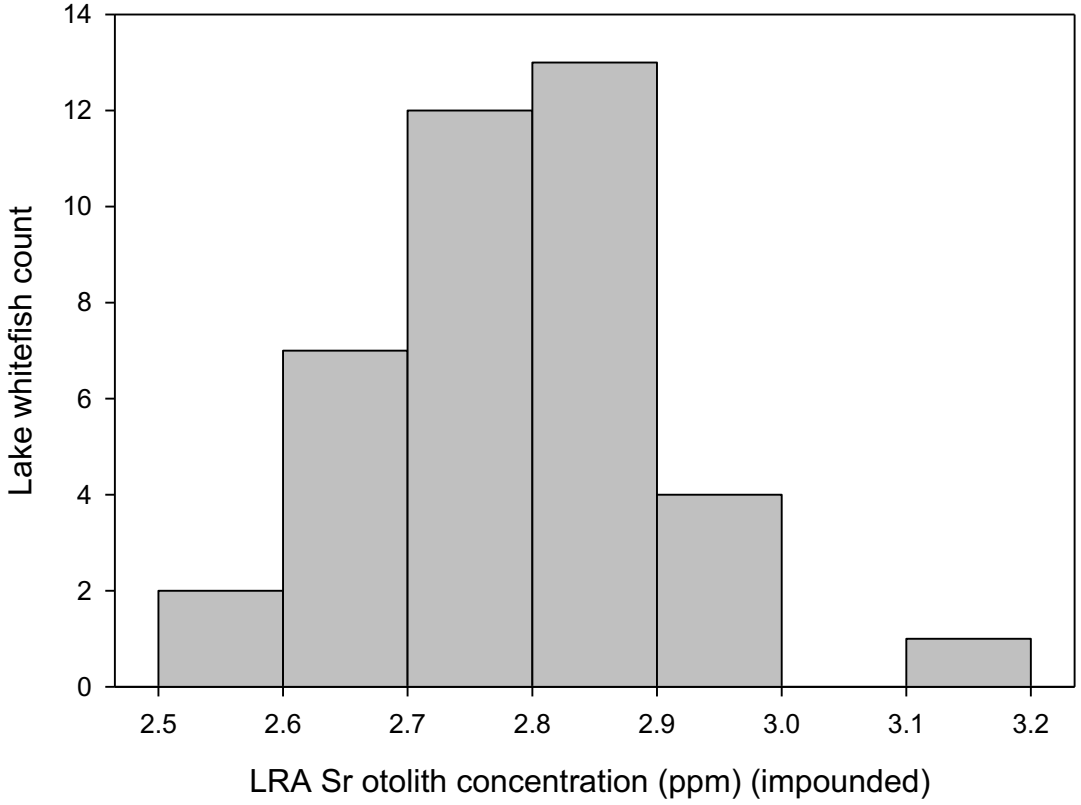


Figure A2. 32. Histogram of lake whitefish otolith LRA strontium (Sr) concentration across all four selected impounded CAMP waterbodies. 39 individual walleye tested, 9-10 otoliths per waterbody.

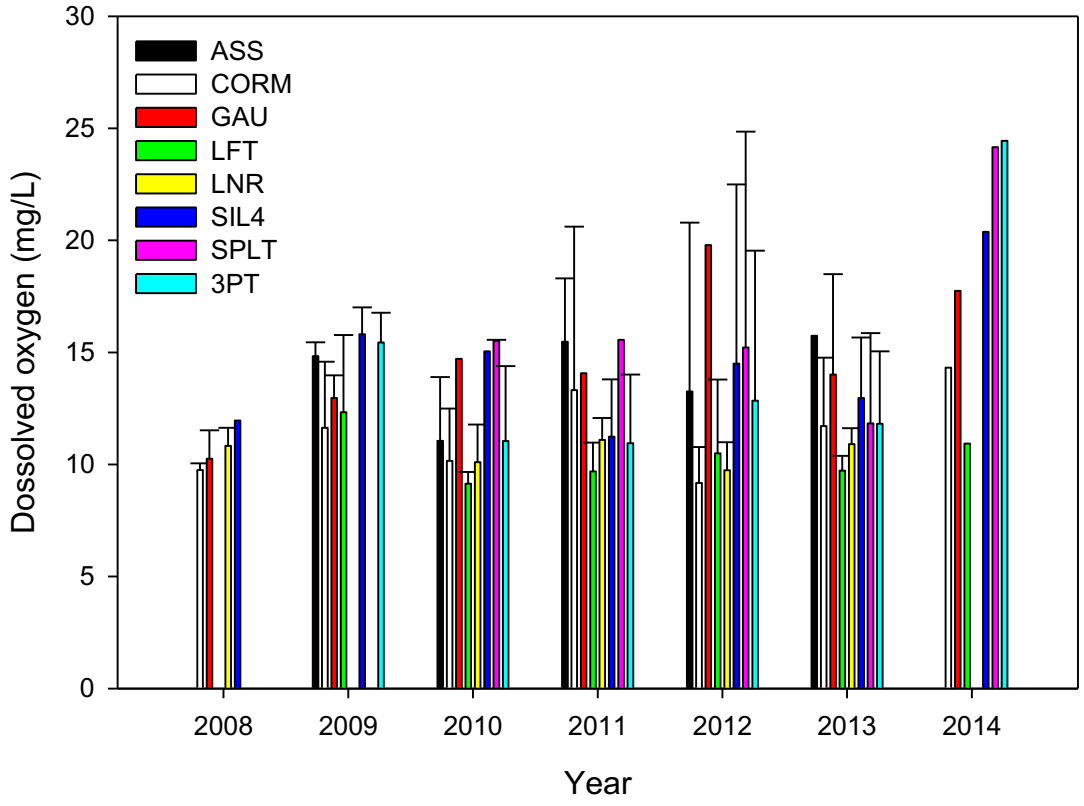


Figure A2. 33. Bar graph of CAMP water quality database in-situ dissolved oxygen concentration averages per year between 2008-2014 for the selected eight CAMP waterbodies. Error bars as standard deviation. Water samples collected at a depth of 0.3 m.

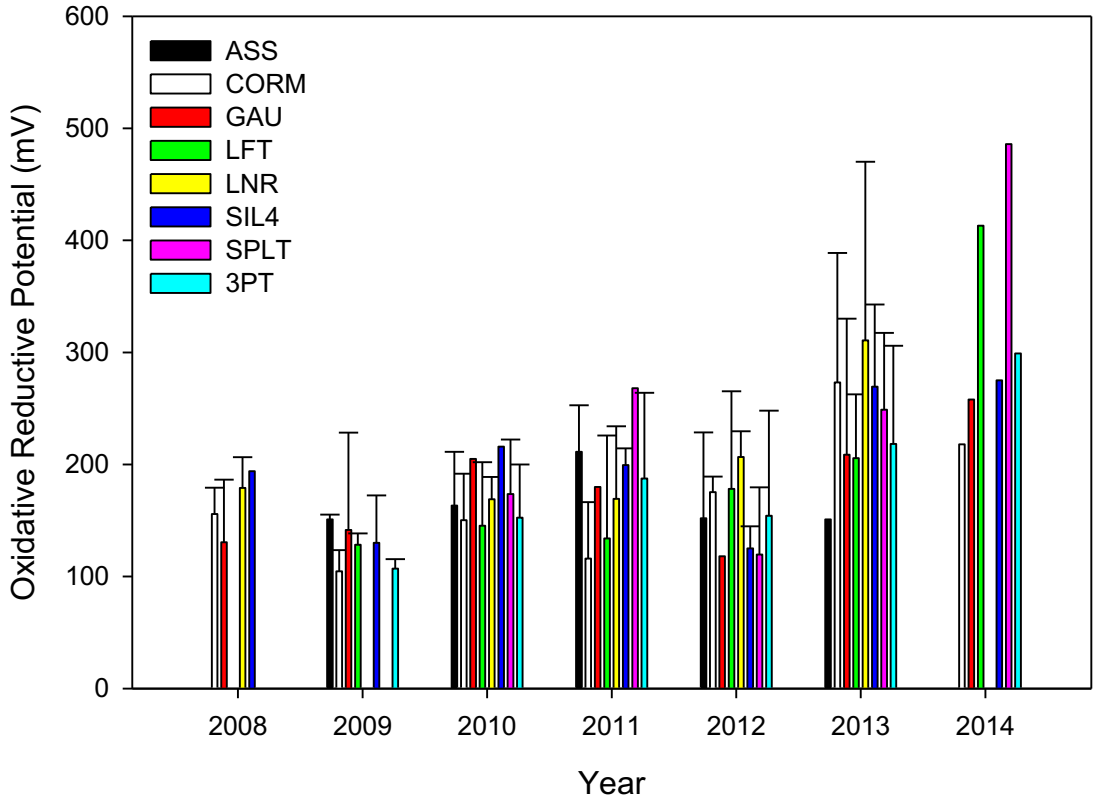


Figure A2. 34. Bar graph of CAMP water quality database in-situ oxidative reduction potential averages per year between 2008-2014 for the selected eight CAMP waterbodies. Error bars as standard deviation. Water samples collected at a depth of 0.3 m.

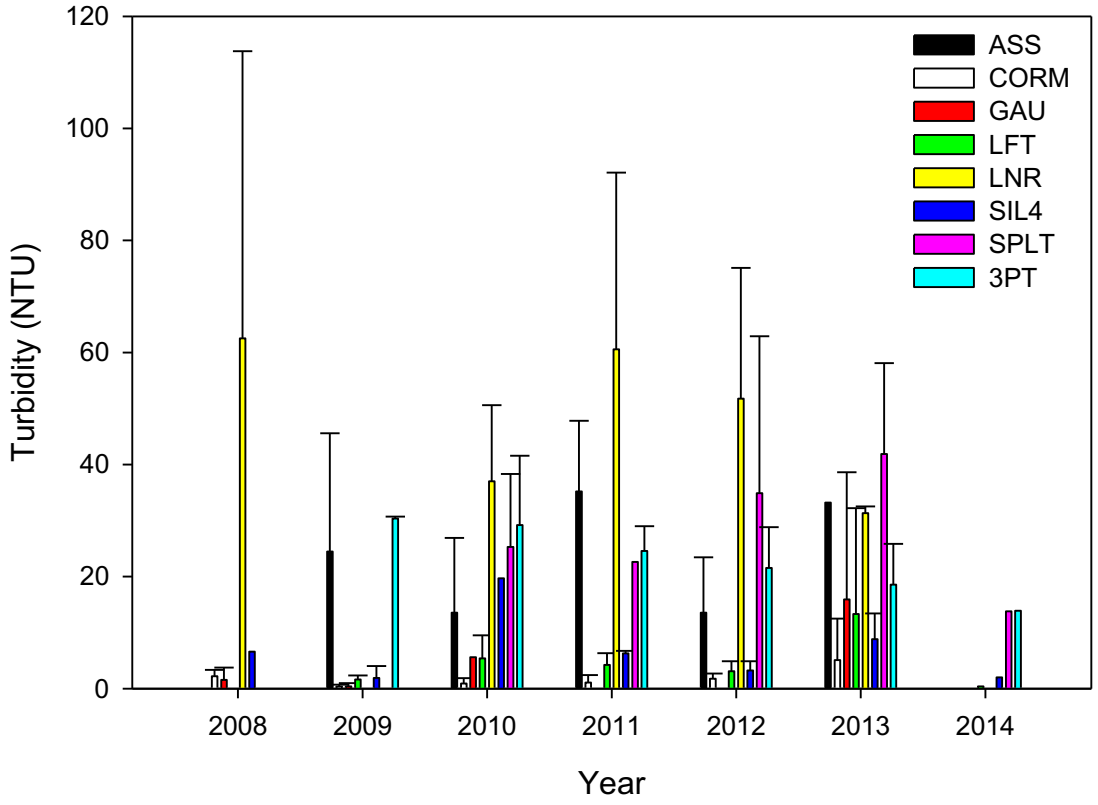


Figure A2. 35. Bar graph of CAMP water quality database in-situ turbidity averages per year between 2008-2014 for the selected eight CAMP waterbodies. Error bars as standard deviation. Water samples collected at a depth of 0.3 m.

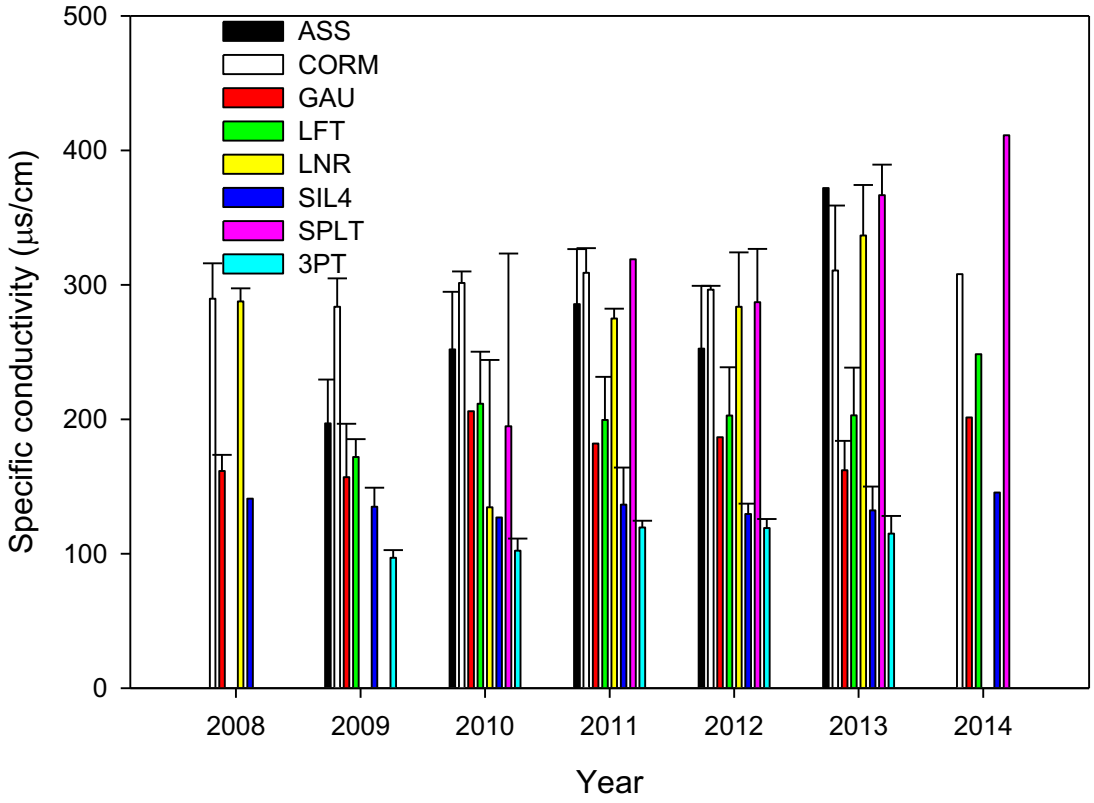


Figure A2. 36. Bar graph of CAMP water quality database in-situ specific conductivity measure averages per year between 2008-2014 for the selected eight CAMP waterbodies. Error bars as standard deviation. Water samples collected at a depth of 0.3 m.

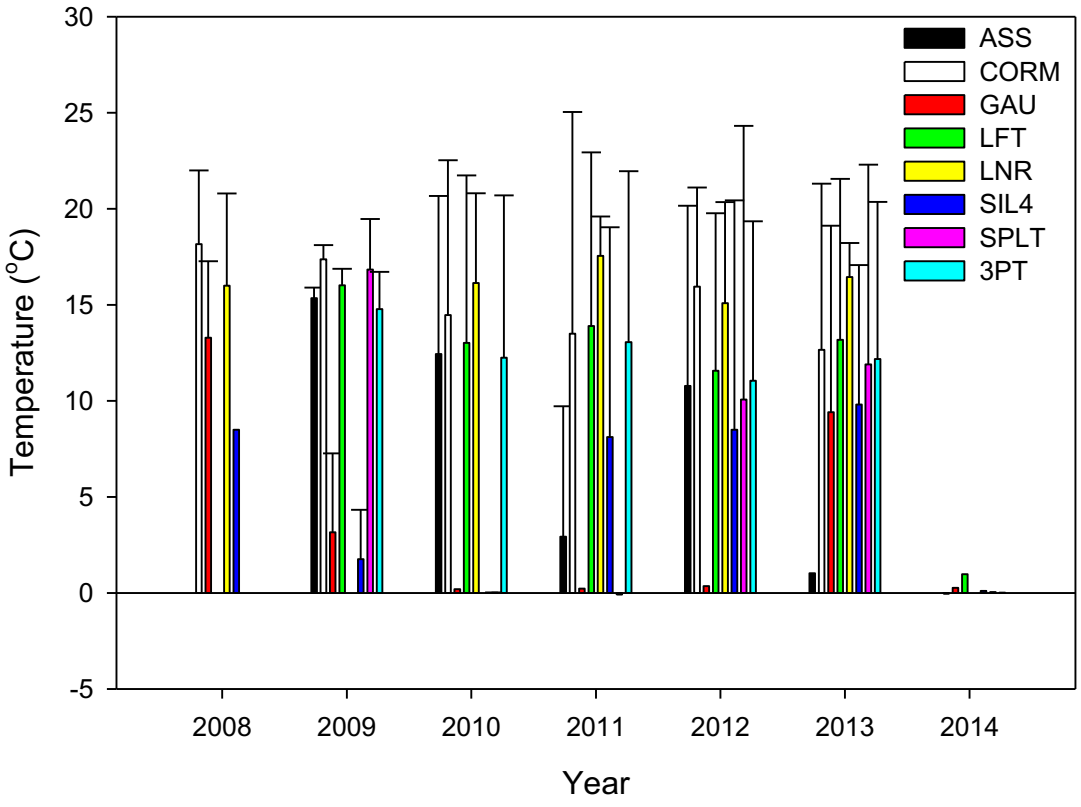


Figure A2. 37. Bar graph of CAMP water quality database in-situ temperature averages per year between 2008-2014 for the selected eight CAMP waterbodies. Error bars as standard deviation. Water samples collected at a depth of 0.3 m.

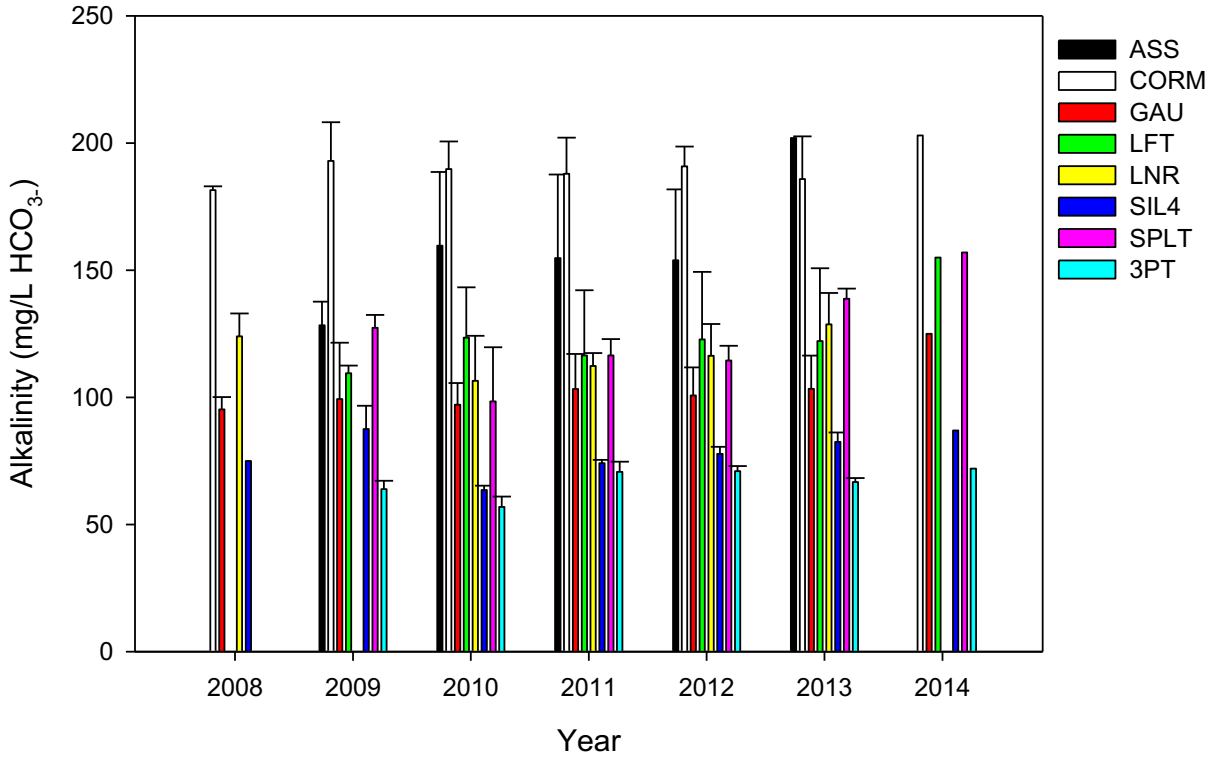


Figure A2. 38. Bar graph of CAMP water quality database lab-determined alkalinity (bicarbonate) averages per year between 2008-2014 for the selected eight CAMP waterbodies. Error bars as standard deviation. Water samples collected at a depth of 0.3 m.

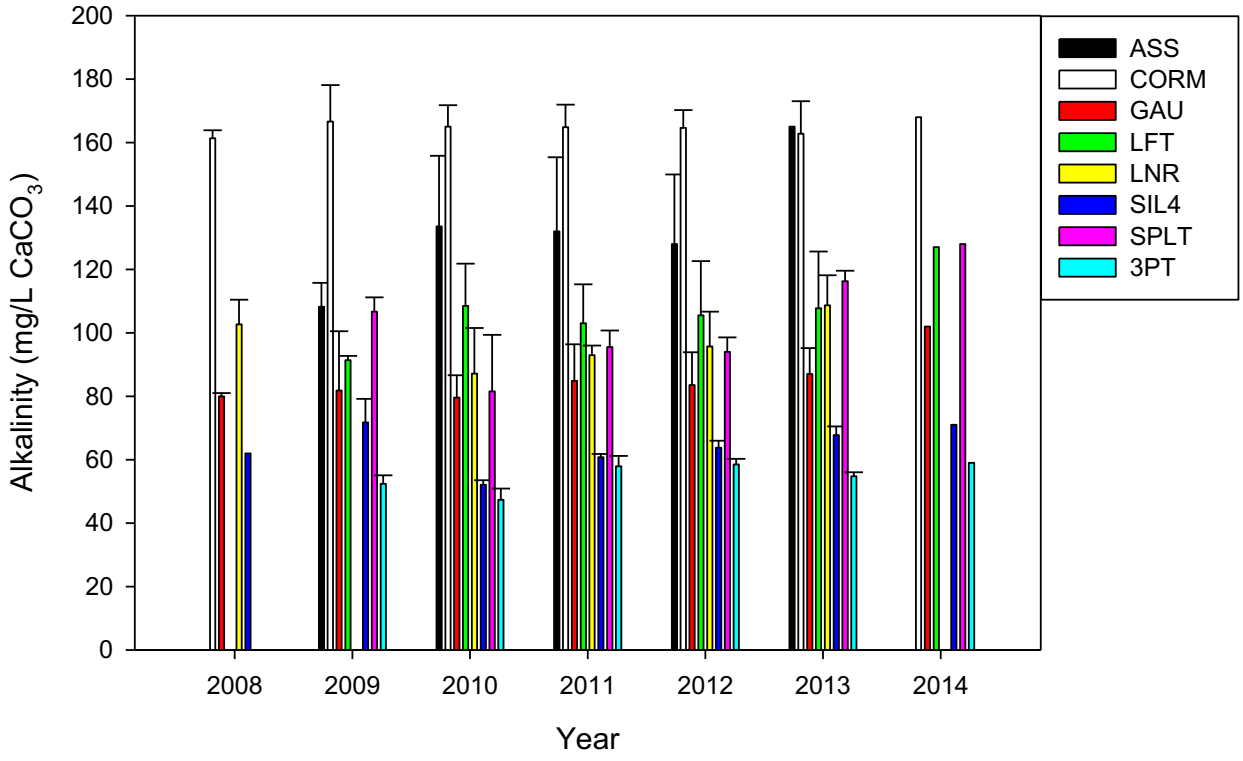


Figure A2. 39. Bar graph of CAMP water quality database lab-determined alkalinity (mg/L $CaCO_3$) averages per year between 2008-2014 for the selected eight CAMP waterbodies. Error bars as standard deviation. Water samples collected at a depth of 0.3 m.

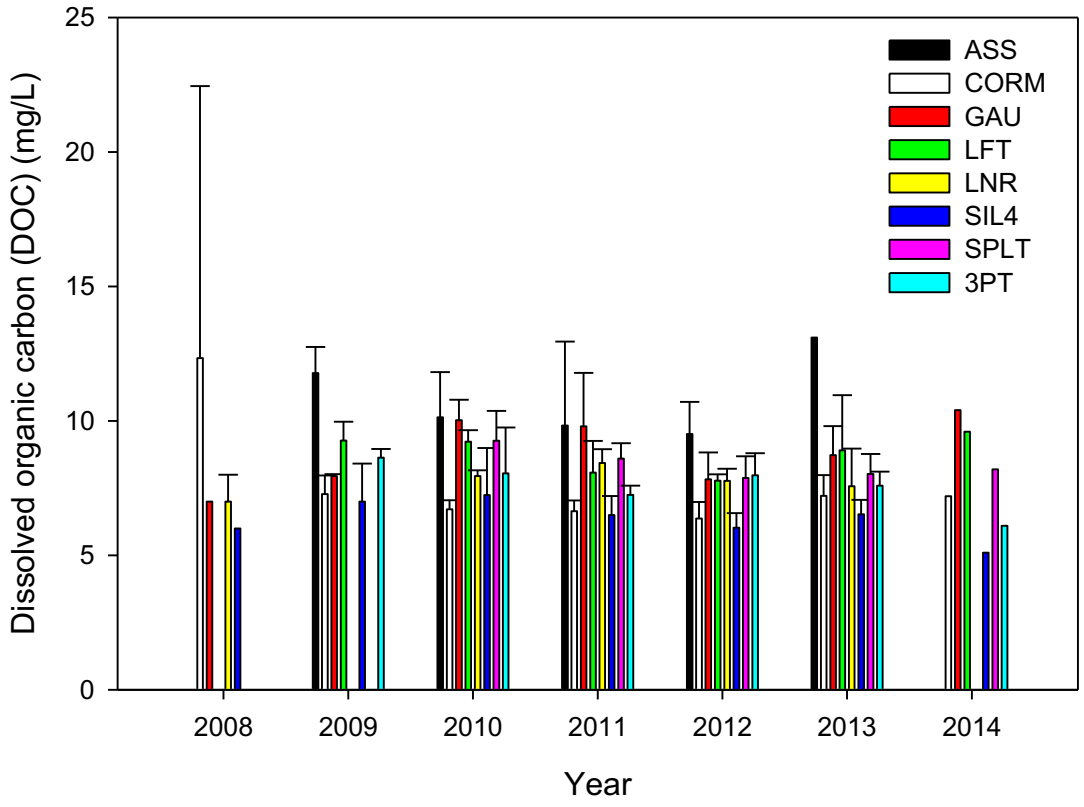


Figure A2. 40. Bar graph of CAMP water quality database lab-determined dissolved organic carbon (DOC) averages per year between 2008-2014 for the selected eight CAMP waterbodies. Error bars as standard deviation. Water samples collected at a depth of 0.3 m.

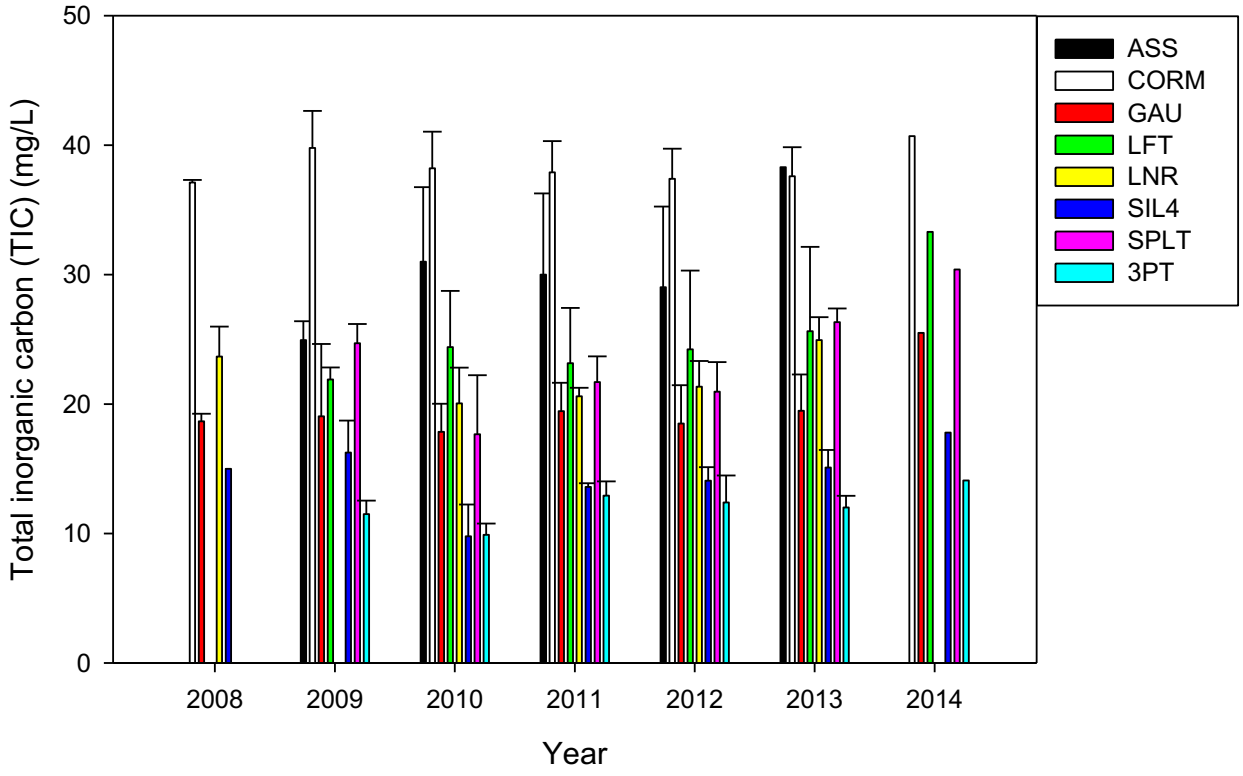


Figure A2. 41. Bar graph of CAMP water quality database lab-determined total inorganic carbon (TIC) averages per year between 2008-2014 for the selected eight CAMP waterbodies. Error bars as standard deviation. Water samples collected at a depth of 0.3 m.

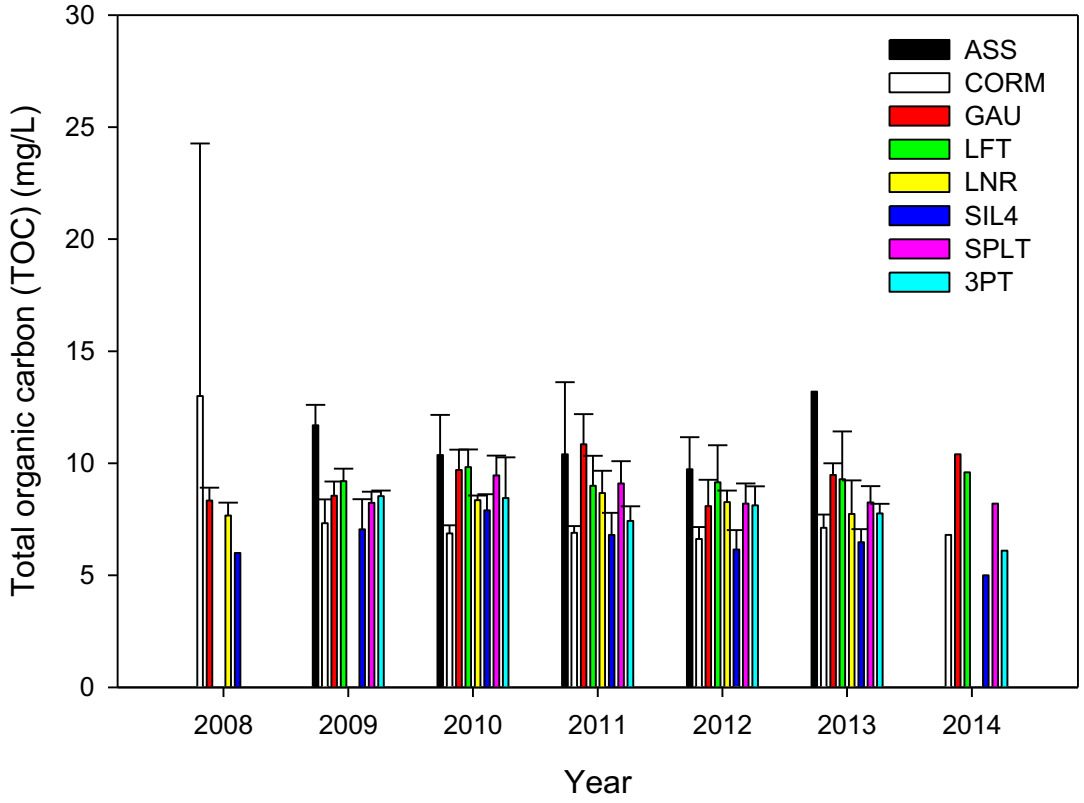


Figure A2. 42. Bar graph of CAMP water quality database lab-determined total organic carbon (TOC) averages per year between 2008-2014 for the selected eight CAMP waterbodies. Error bars as standard deviation. Water samples collected at a depth of 0.3 m.

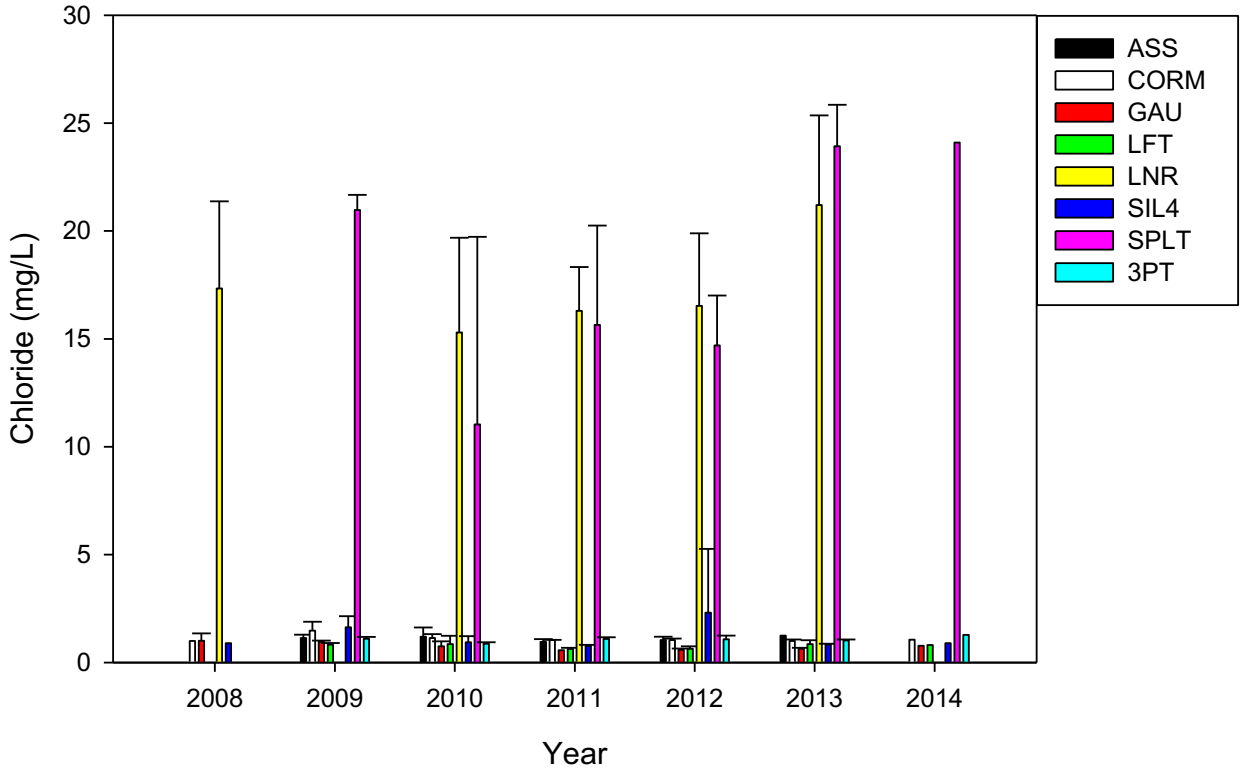


Figure A2. 43. Bar graph of CAMP water quality database lab-determined chloride content averages per year between 2008-2014 for the selected eight CAMP waterbodies. Error bars as standard deviation. Water samples collected at a depth of 0.3 m.

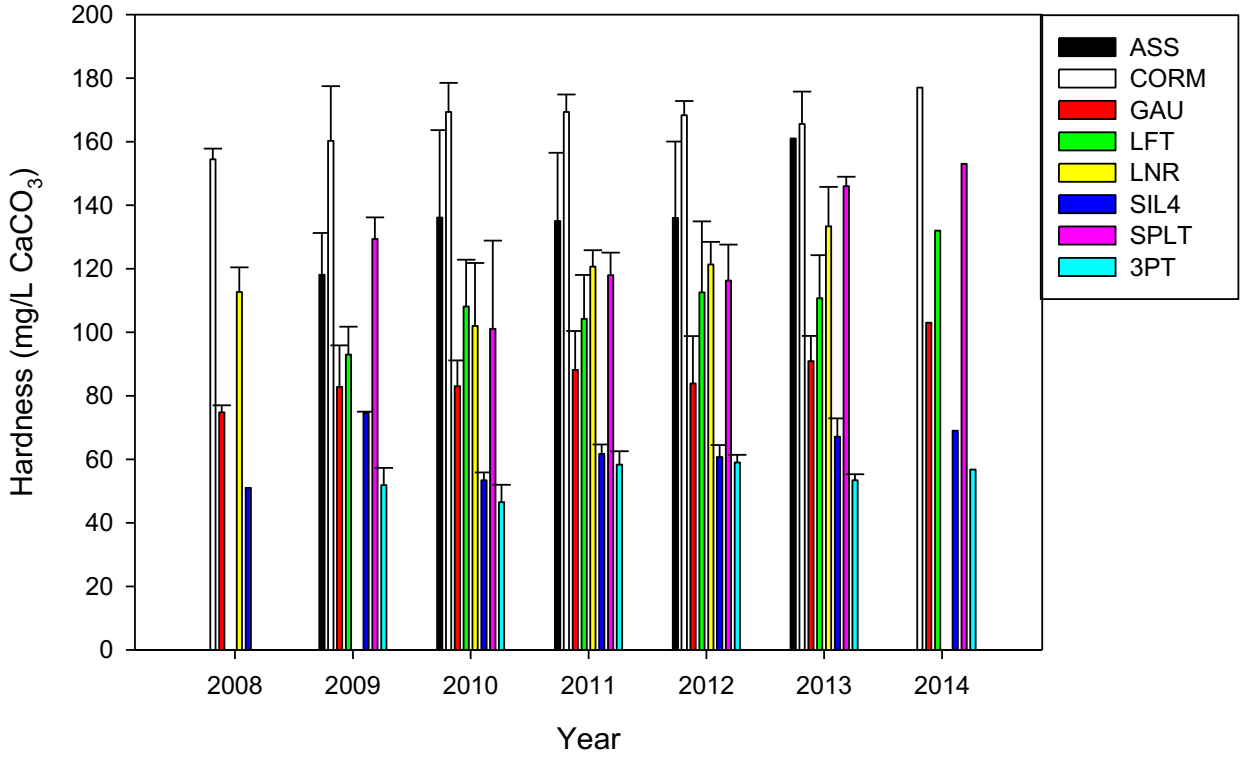


Figure A2. 44. Bar graph of CAMP water quality database lab-determined hardness (mg/L $CaCO_3$) averages per year between 2008-2014 for the selected eight CAMP waterbodies. Error bars as standard deviation. Water samples collected at a depth of 0.3 m.

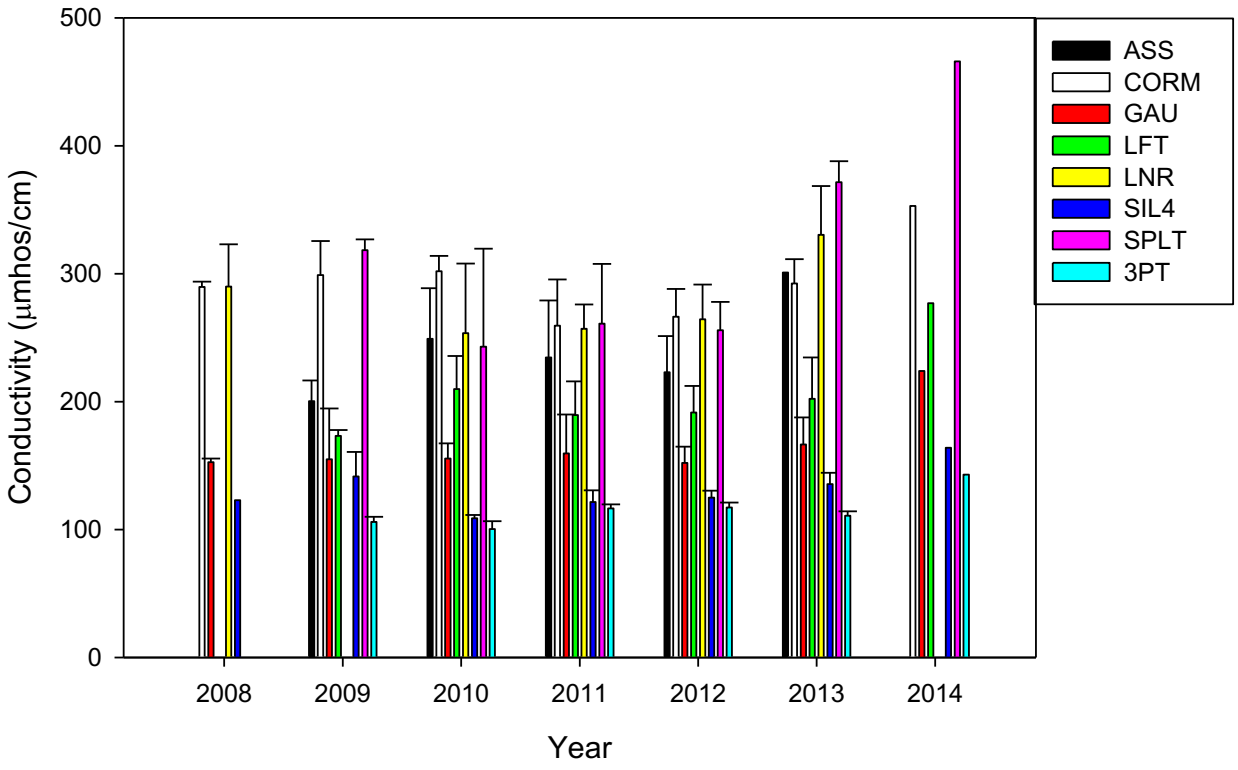


Figure A2. 45. Bar graph of CAMP water quality database lab-determined conductivity measure averages per year between 2008-2014 for the selected eight CAMP waterbodies. Error bars as standard deviation. Water samples collected at a depth of 0.3 m.

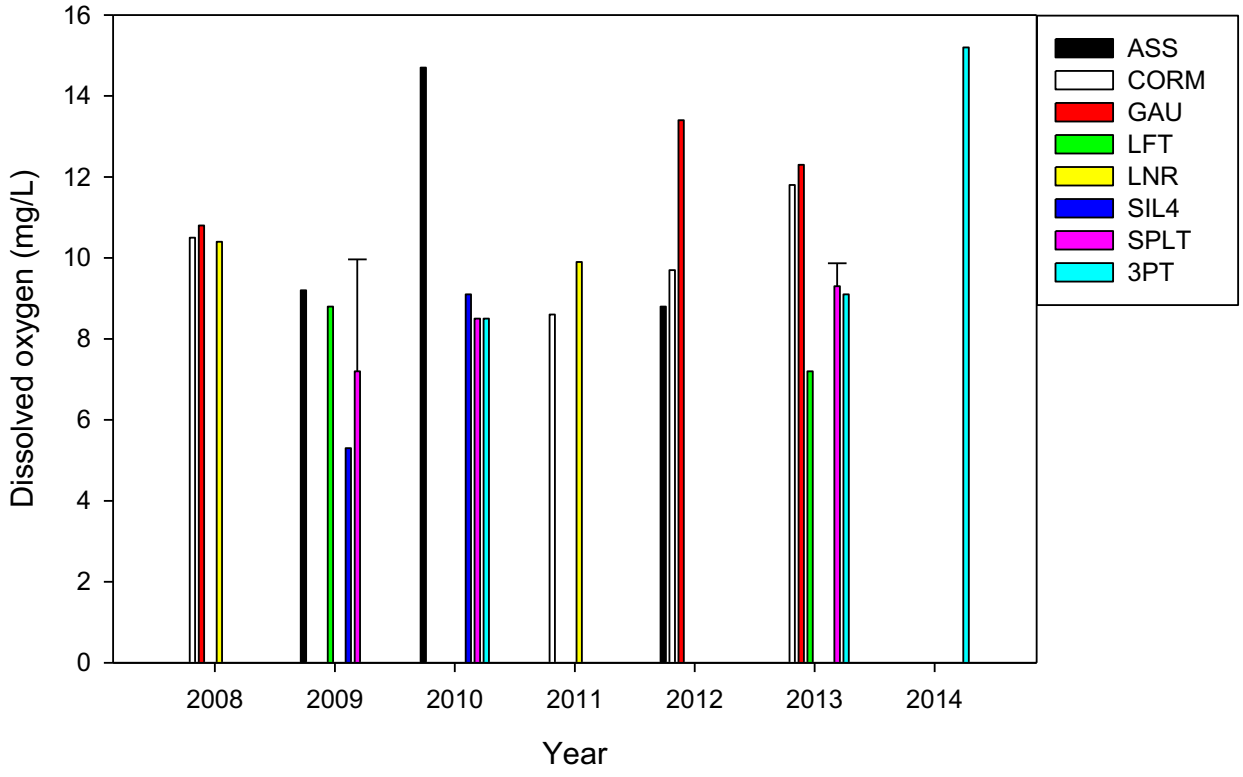


Figure A2. 46. Bar graph of CAMP water quality database lab-determined dissolved oxygen concentration averages per year between 2008-2014 for the selected eight CAMP waterbodies. Error bars as standard deviation. Water samples collected at a depth of 0.3 m.

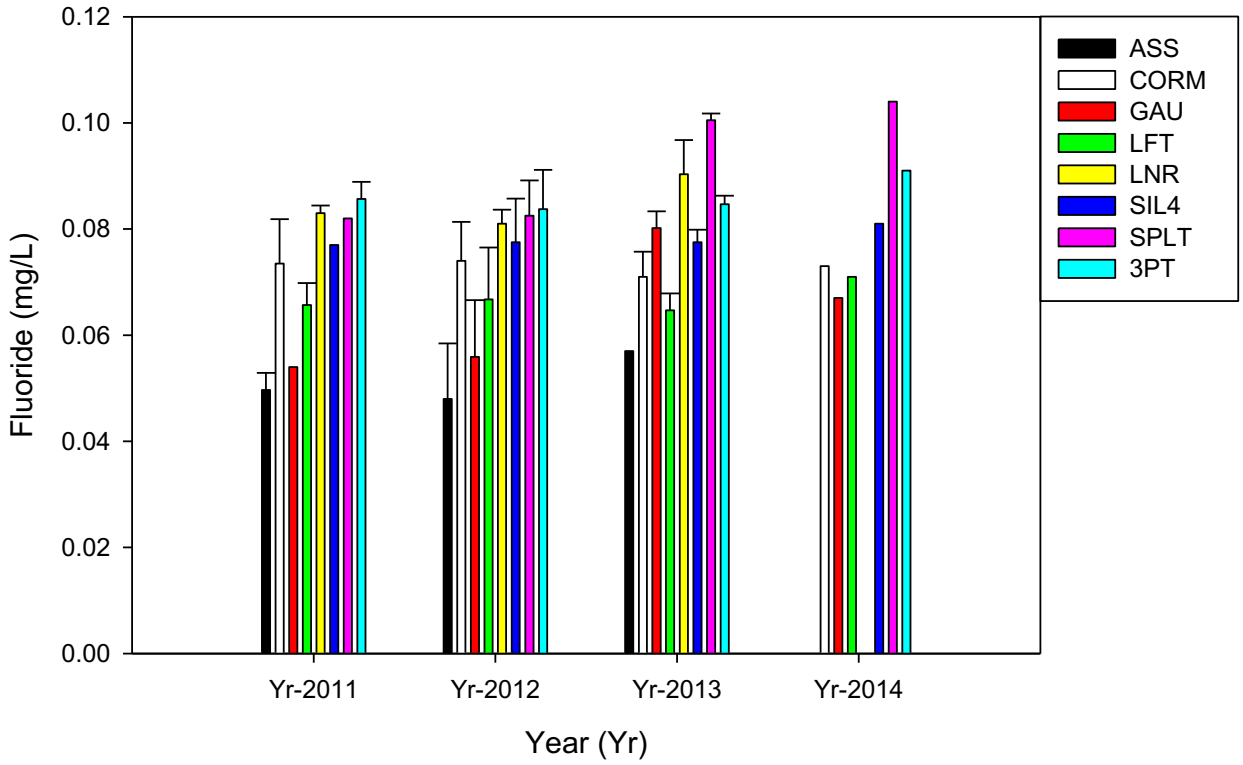


Figure A2. 47. Bar graph of CAMP water quality database lab-determined fluoride concentration averages per year between 2011-2014 for the selected eight CAMP waterbodies. Error bars as standard deviation. Water samples collected at a depth of 0.3 m.

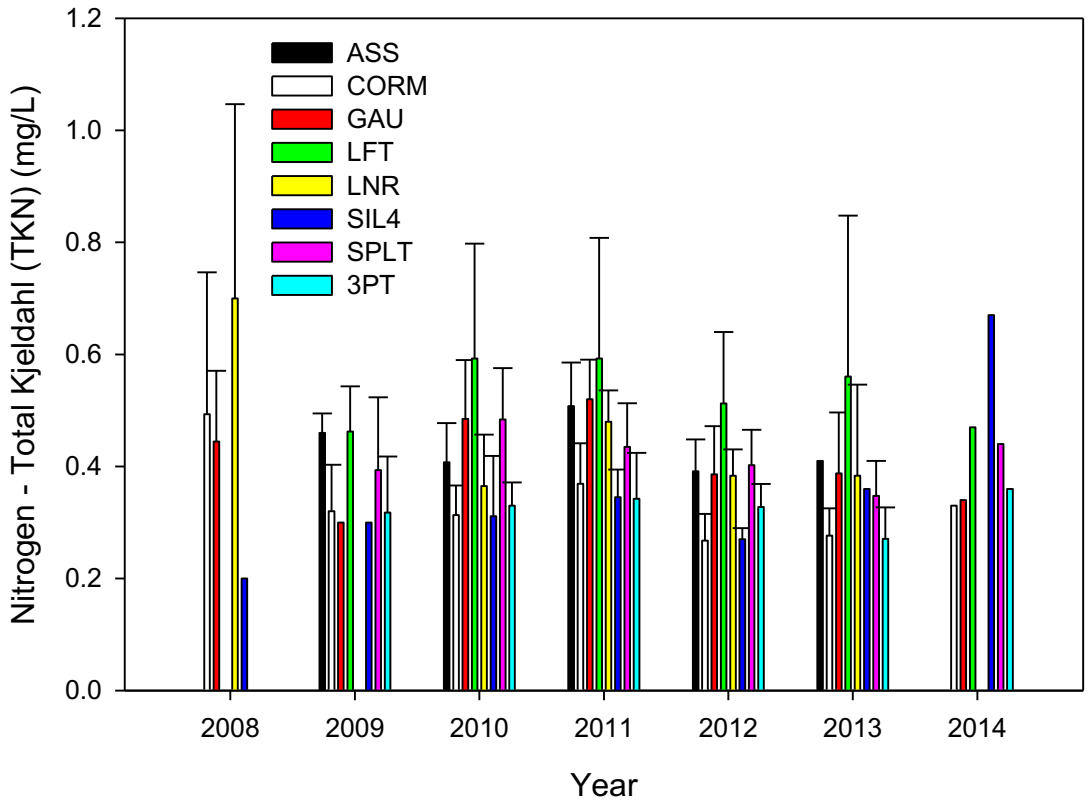


Figure A2. 48. Bar graph of CAMP water quality database lab-determined nitrogen (as total Kjeldahl units) averages per year between 2008-2014 for the selected eight CAMP waterbodies. Error bars as standard deviation. Water samples collected at a depth of 0.3 m.

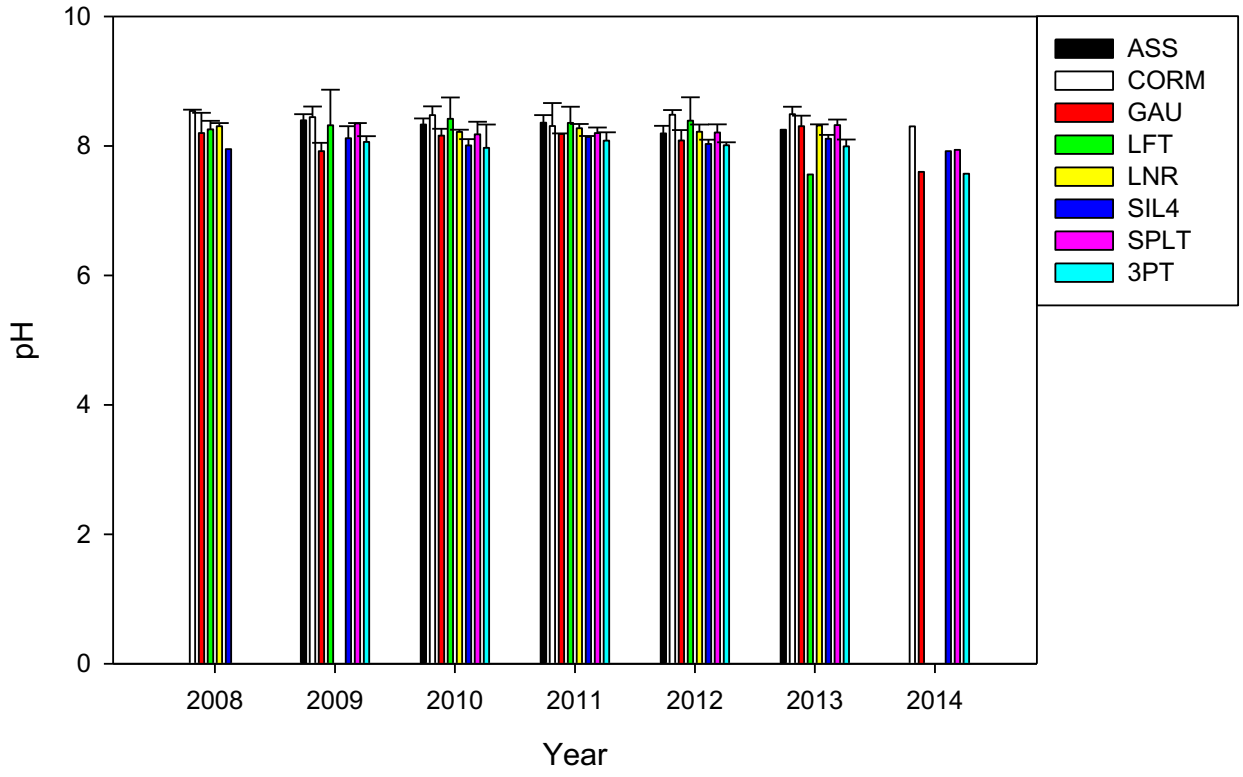


Figure A2. 49. Bar graph of CAMP water quality database lab-determined pH level averages per year between 2008-2014 for the selected eight CAMP waterbodies. Error bars as standard deviation. Water samples collected at a depth of 0.3 m.

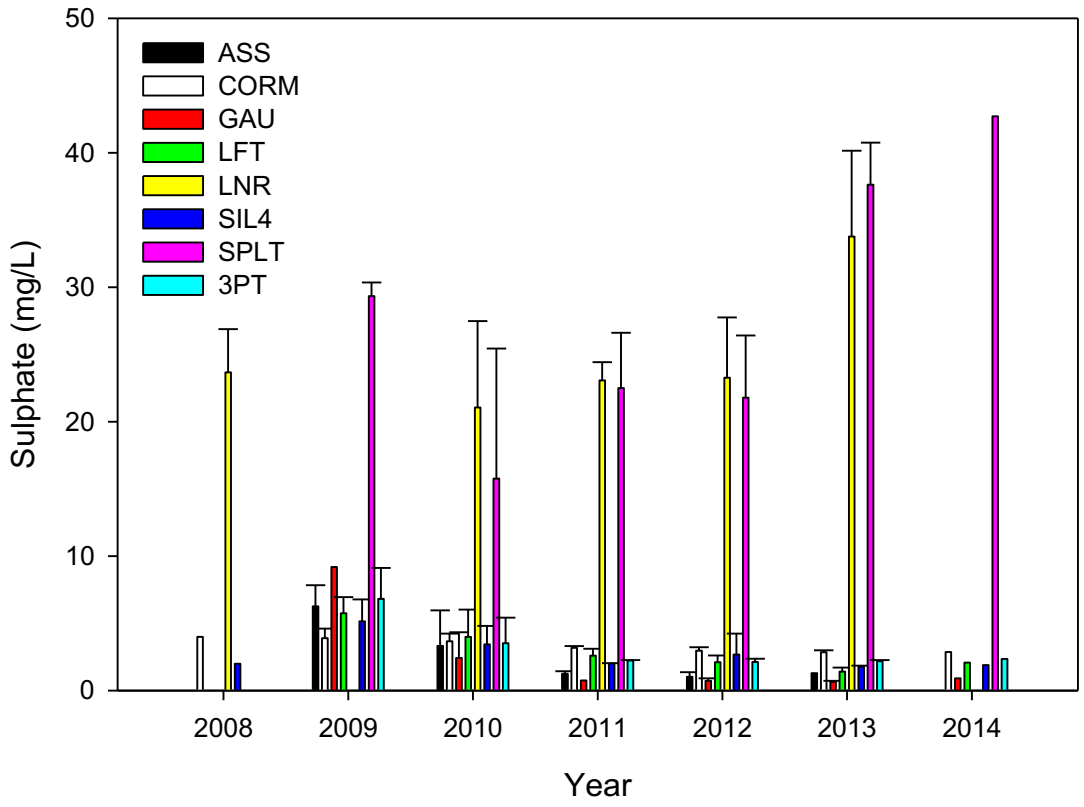


Figure A2. 50. Bar graph of CAMP water quality database lab-determined sulphate concentration averages per year between 2008-2014 for the selected eight CAMP waterbodies. Error bars as standard deviation. Water samples collected at a depth of 0.3 m.

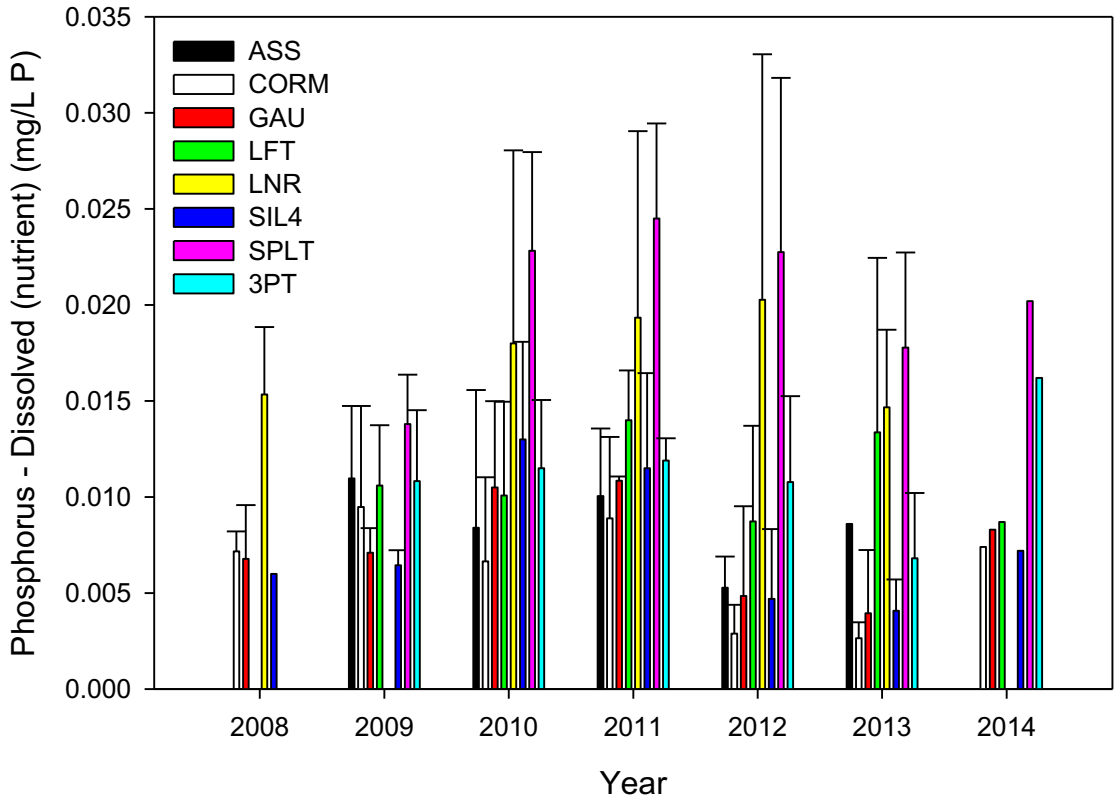


Figure A2. 51. Bar graph of CAMP water quality database lab-determined dissolved phosphorus (nutrient) averages per year between 2008-2014 for the selected eight CAMP waterbodies. Error bars as standard deviation. Water samples collected at a depth of 0.3 m.

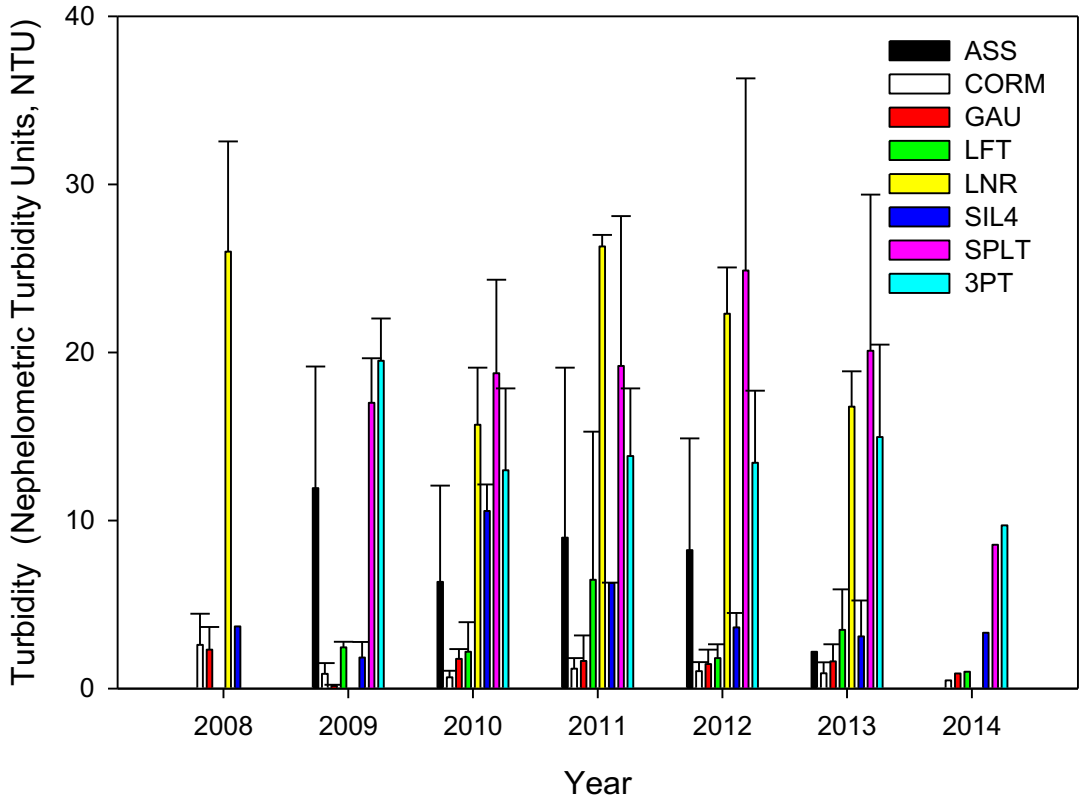


Figure A2. 52. Bar graph of CAMP water quality database lab-determined turbidity level averages per year between 2008-2014 for the selected eight CAMP waterbodies. Error bars as standard deviation. Water samples collected at a depth of 0.3 m.

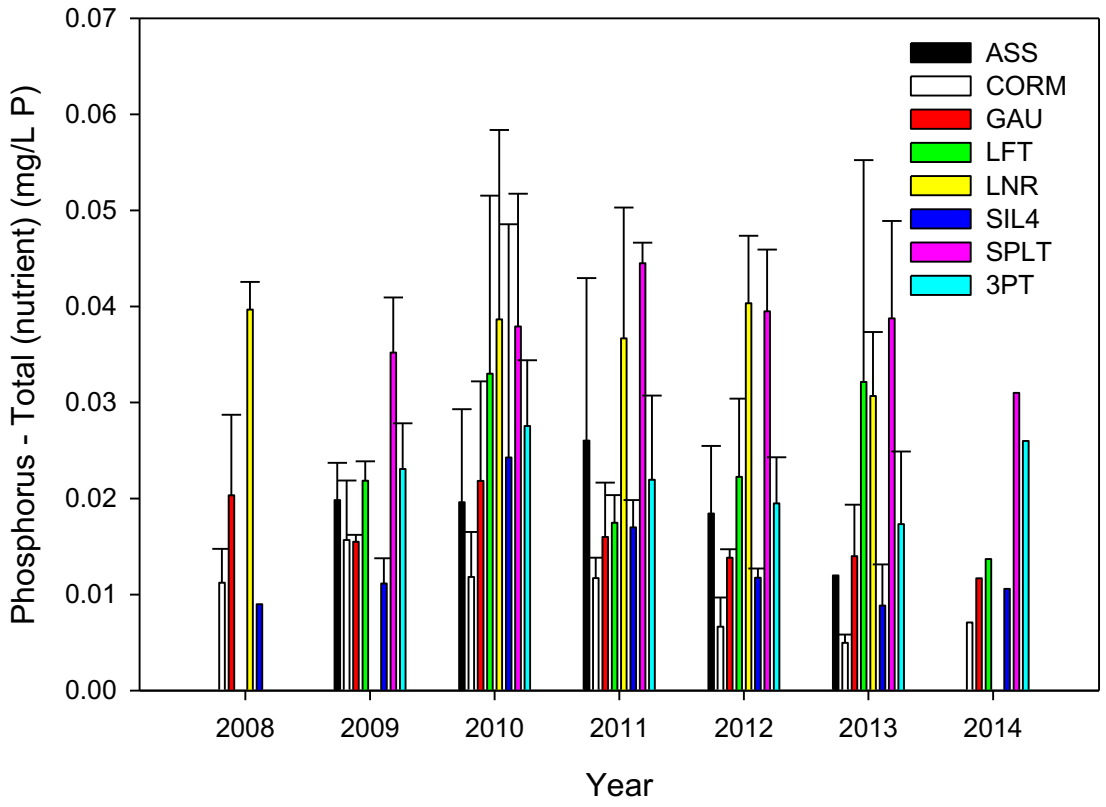


Figure A2. 53. Bar graph of CAMP water quality database lab-determined total phosphorus (nutrient) averages per year between 2008-2014 for the selected eight CAMP waterbodies. Error bars as standard deviation. Water samples collected at a depth of 0.3 m.

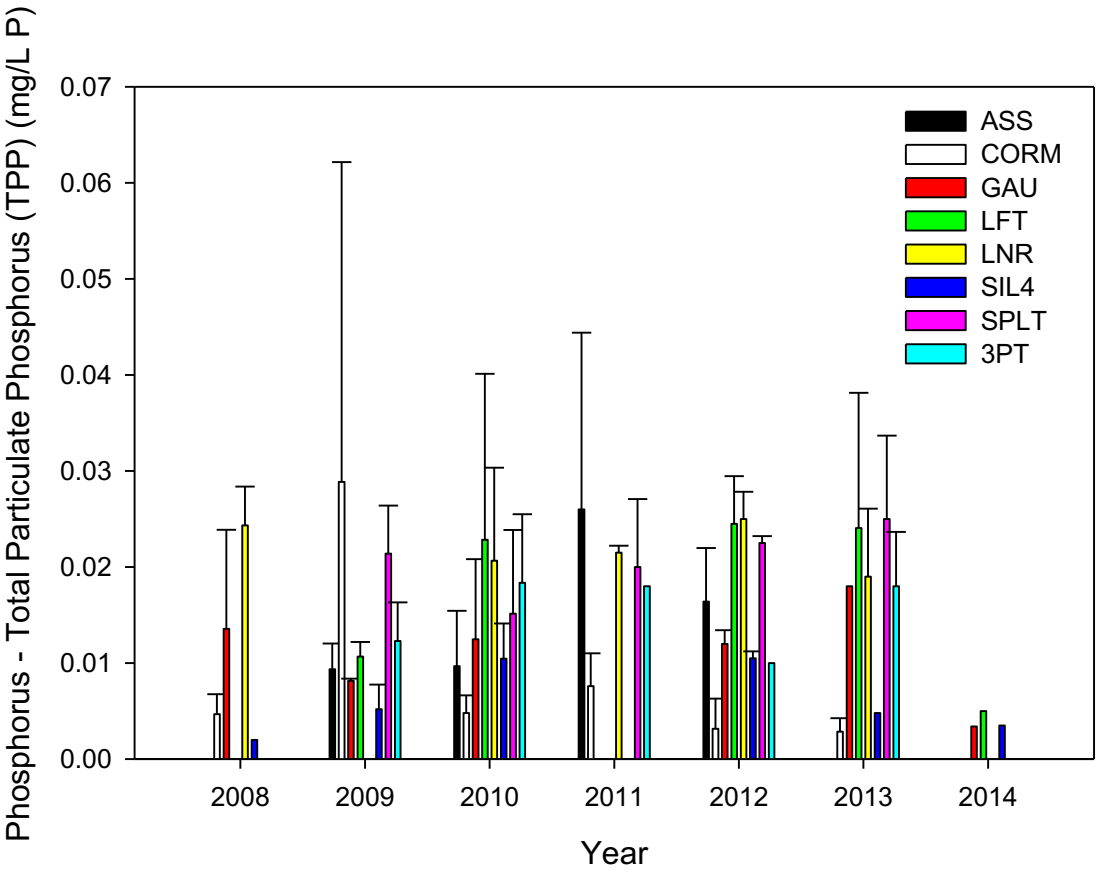


Figure A2. 54. Bar graph of CAMP water quality database lab-determined total particulate phosphorus averages per year between 2008-2014 for the selected eight CAMP waterbodies. Error bars as standard deviation. Water samples collected at a depth of 0.3 m.

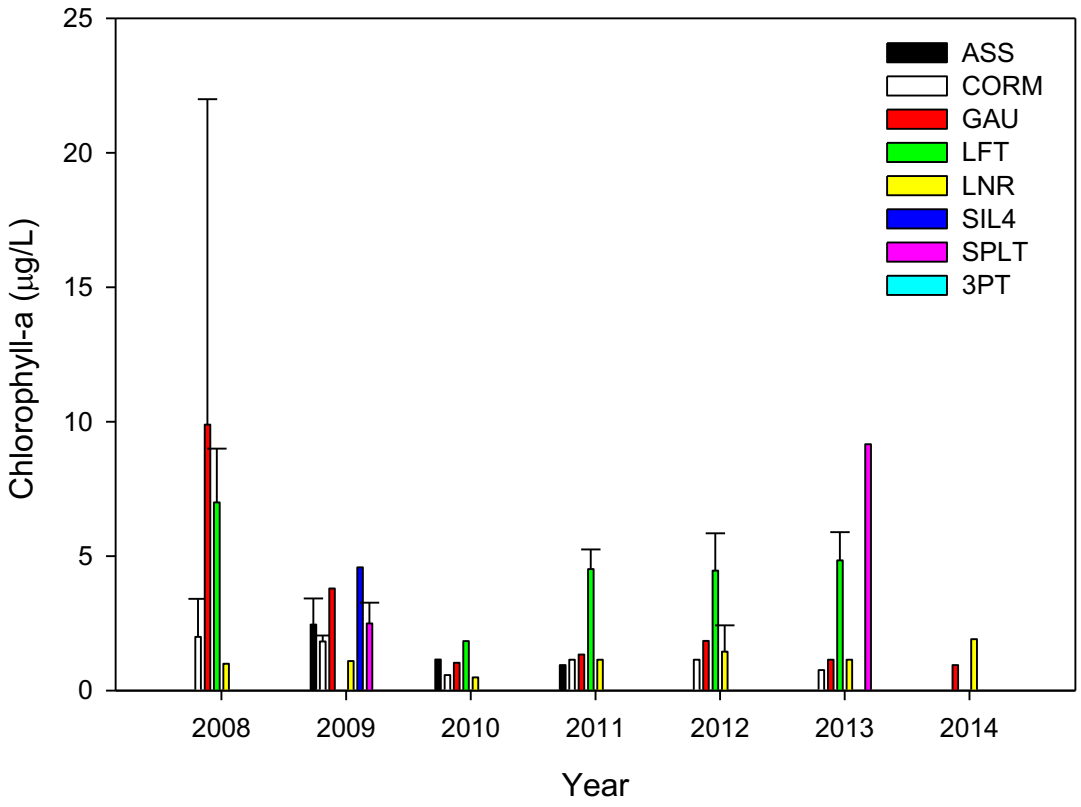


Figure A2. 55. Bar graph of CAMP water quality database lab-determined chlorophyll-a concentration averages per year between 2008-2014 for the selected eight CAMP waterbodies. Error bars as standard deviation. Water samples collected at a depth of 0.3 m.

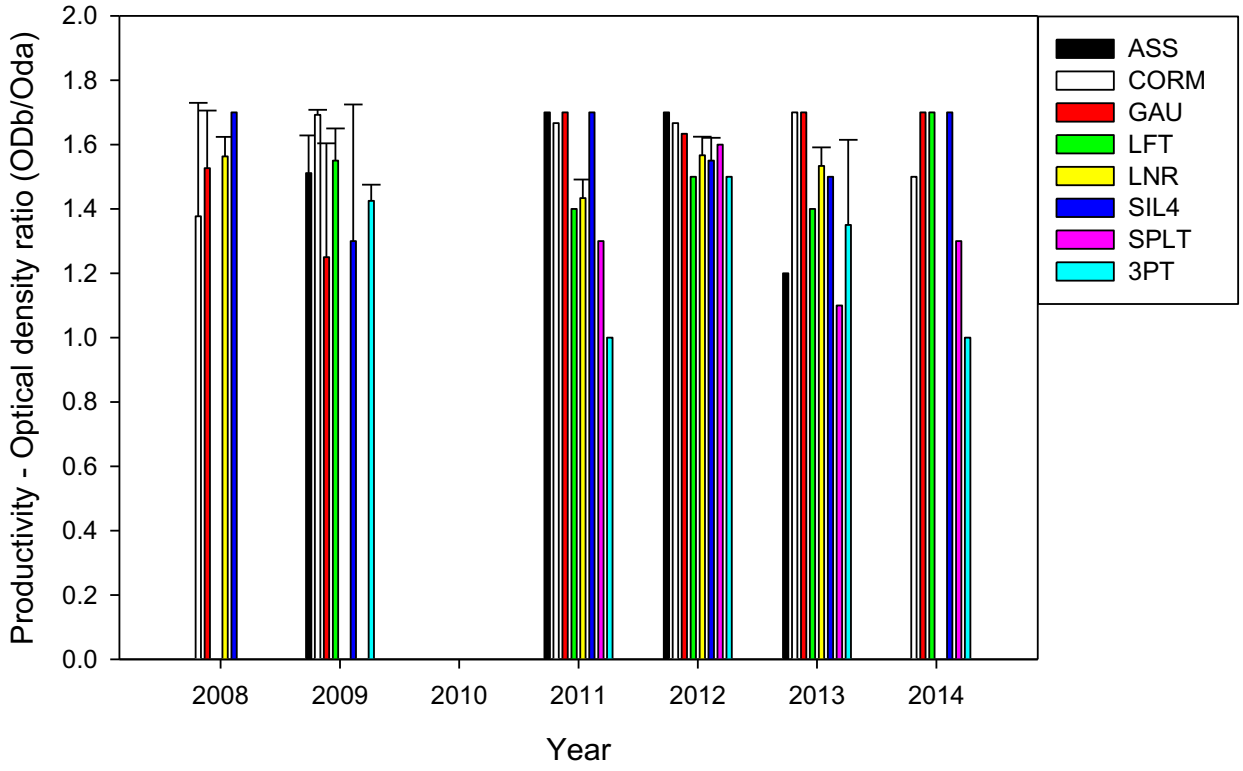


Figure A2. 56. Bar graph of CAMP water quality database lab-determined productivity (optical density ratio) averages per year between 2008-2014 for the selected eight CAMP waterbodies. Error bars as standard deviation. Water samples collected at a depth of 0.3 m.

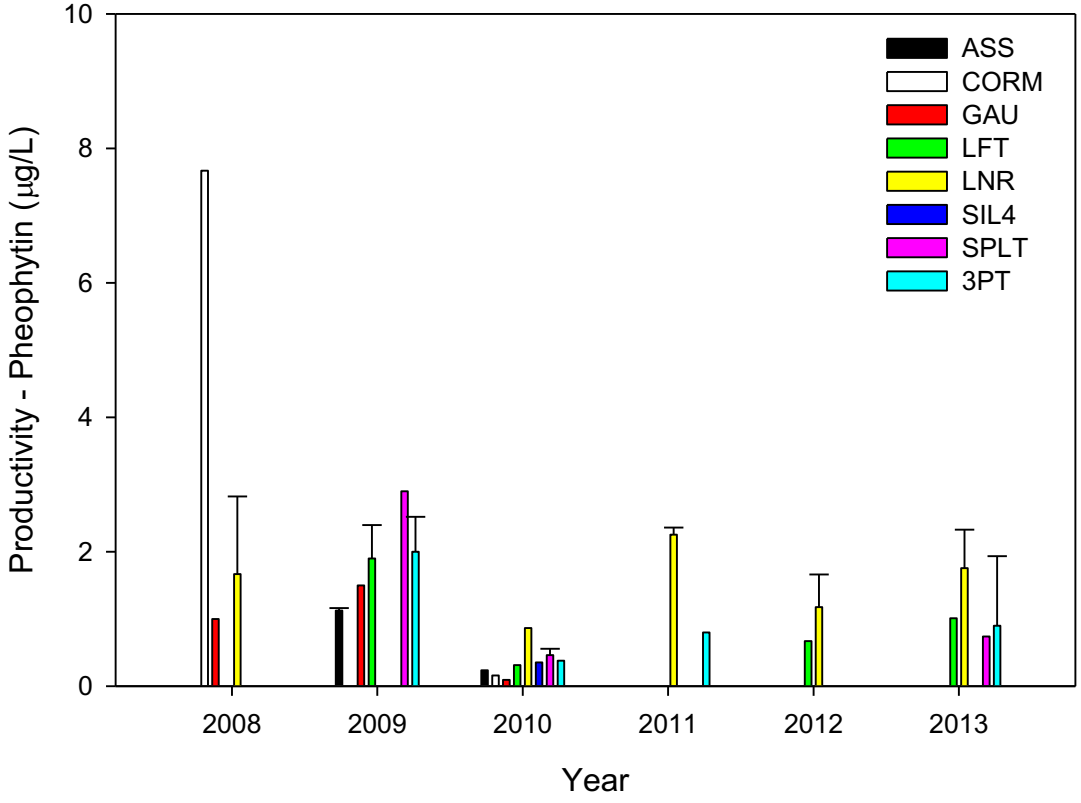


Figure A2. 57. Bar graph of CAMP water quality database lab-determined pheophytin (productivity) concentration averages per year between 2008-2014 for the selected eight CAMP waterbodies. Error bars as standard deviation. Water samples collected at a depth of 0.3 m.

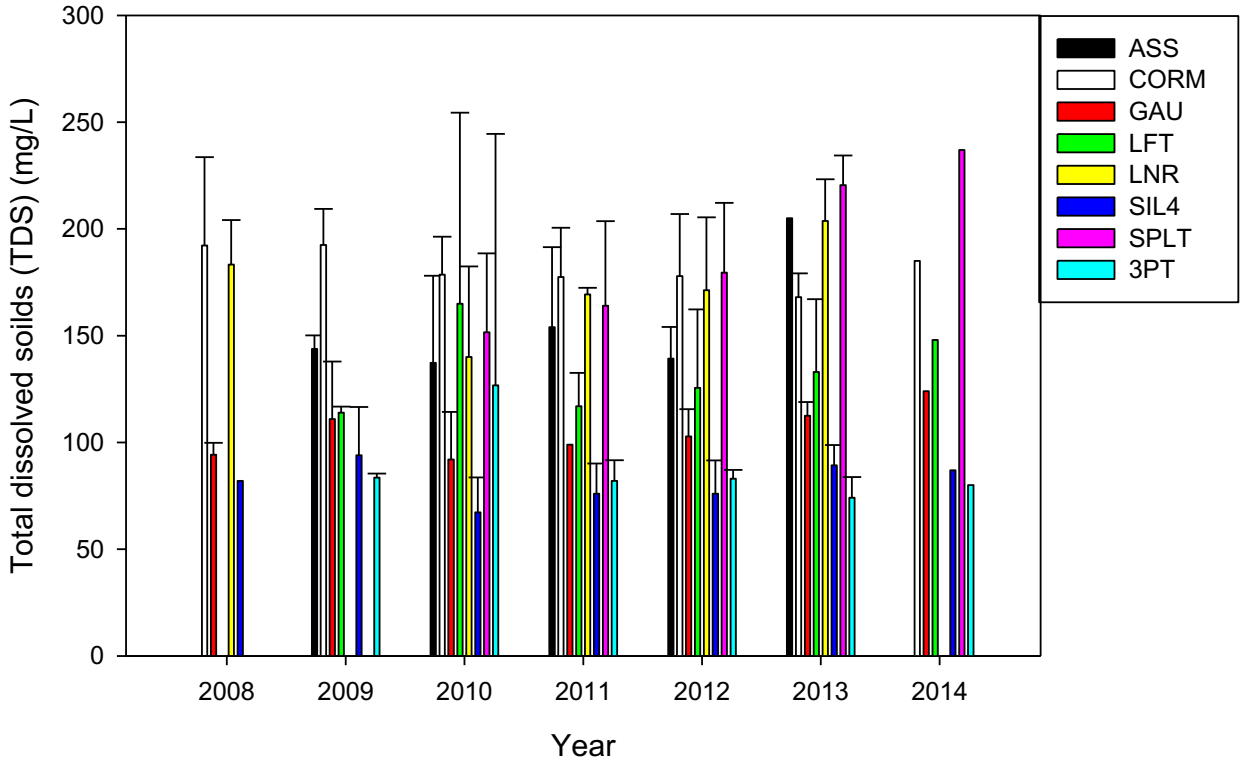


Figure A2. 58. Bar graph of CAMP water quality database lab-determined total dissolved solid averages per year between 2008-2014 for the selected eight CAMP waterbodies. Error bars as standard deviation. Water samples collected at a depth of 0.3 m.

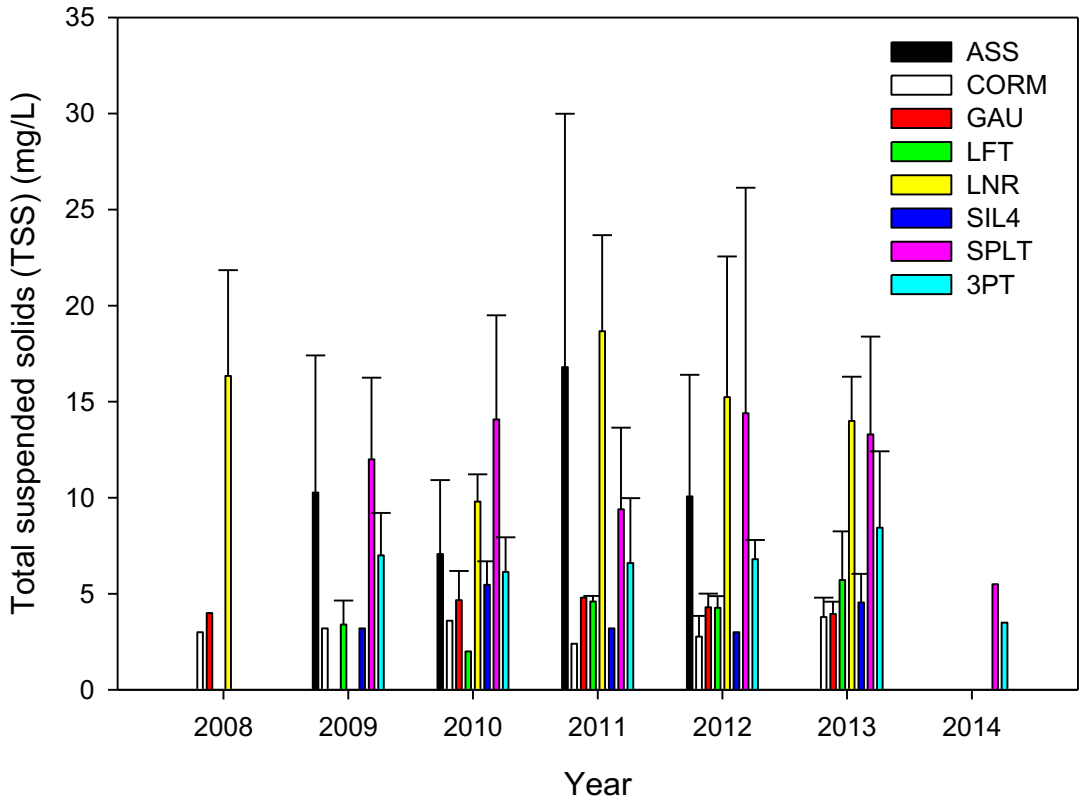


Figure A2. 59. Bar graph of CAMP water quality database lab-determined total suspended solid averages per year between 2008-2014 for the selected eight CAMP waterbodies. Error bars as standard deviation. Water samples collected at a depth of 0.3 m.

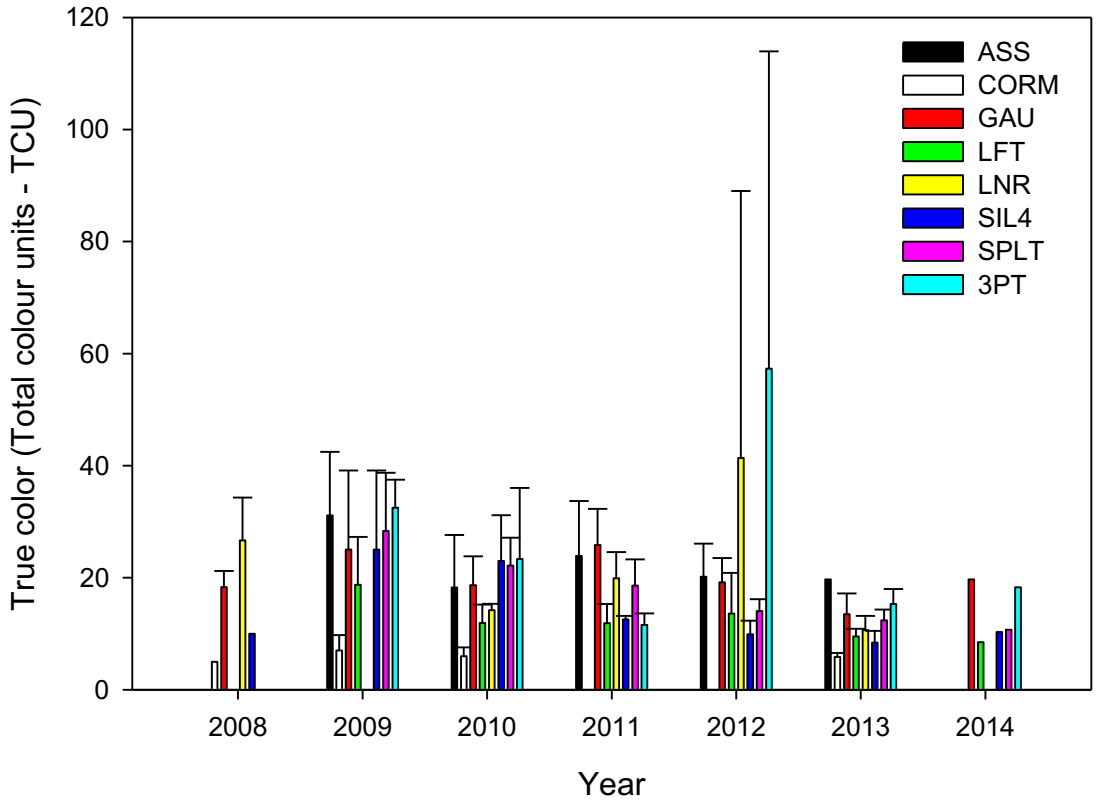


Figure A2. 60. Bar graph of CAMP water quality database lab-determined true color (true color units) averages per year between 2008-2014 for the selected eight CAMP waterbodies. Error bars as standard deviation. Water samples collected at a depth of 0.3 m.

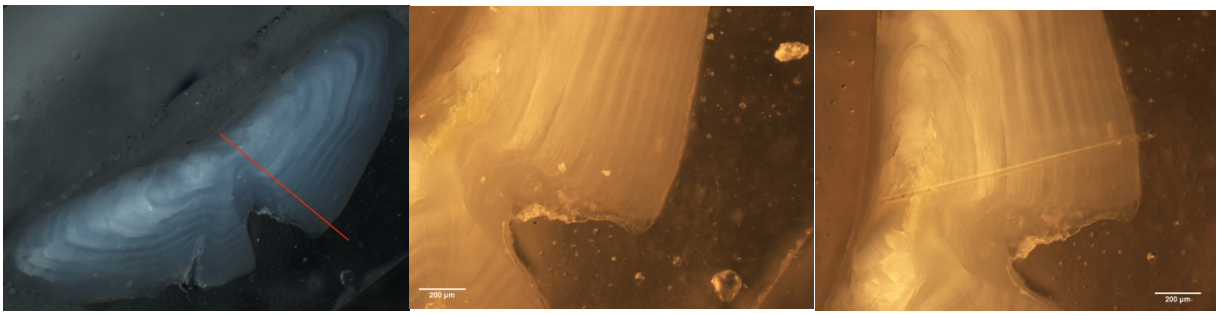


Figure A2. 61. Example of lake whitefish. Line path, pre-ablation and post ablation (left to right).
CODE: LKWF-3PT-5

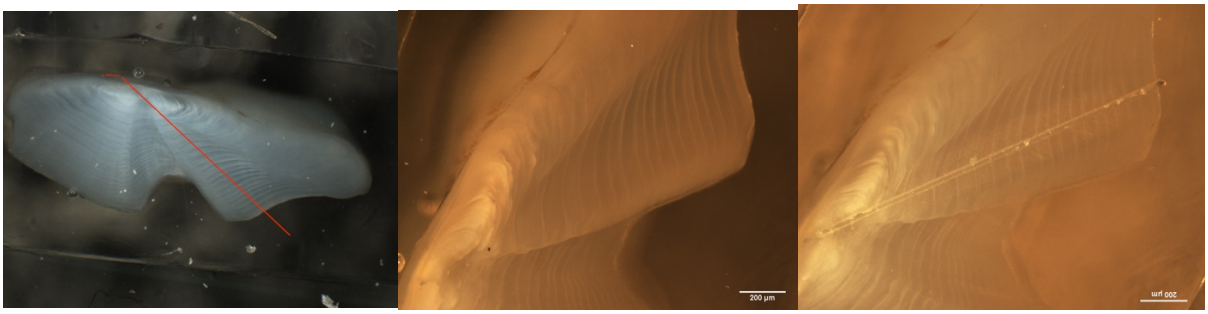


Figure A2. 62. Example of walleye LA-ICP-MS. Line path, pre-ablation and post ablation (left to right).
CODE: WAL-3PT-148

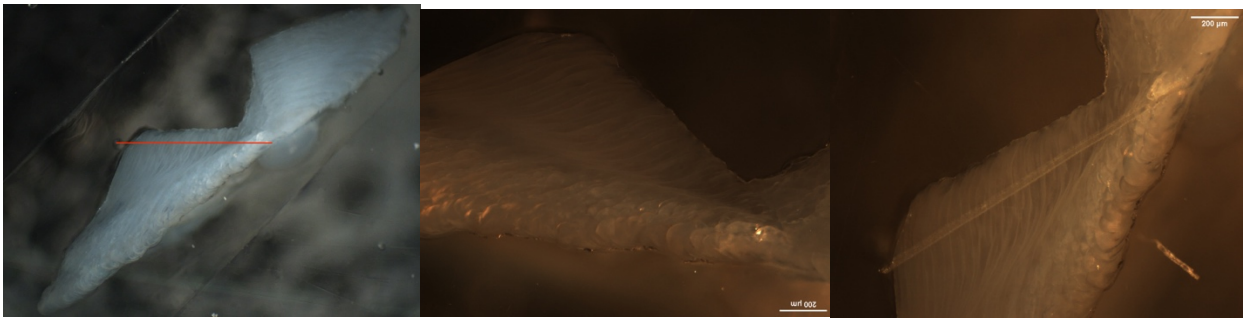


Figure A2. 63. Anomalous outlier walleye otolith. Line path, pre-ablation and post ablation (left to right).
CODE: WAL-LNR-132

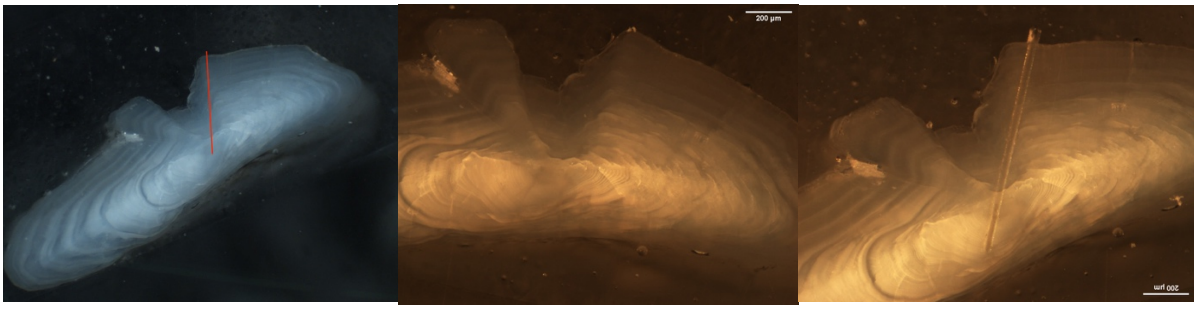
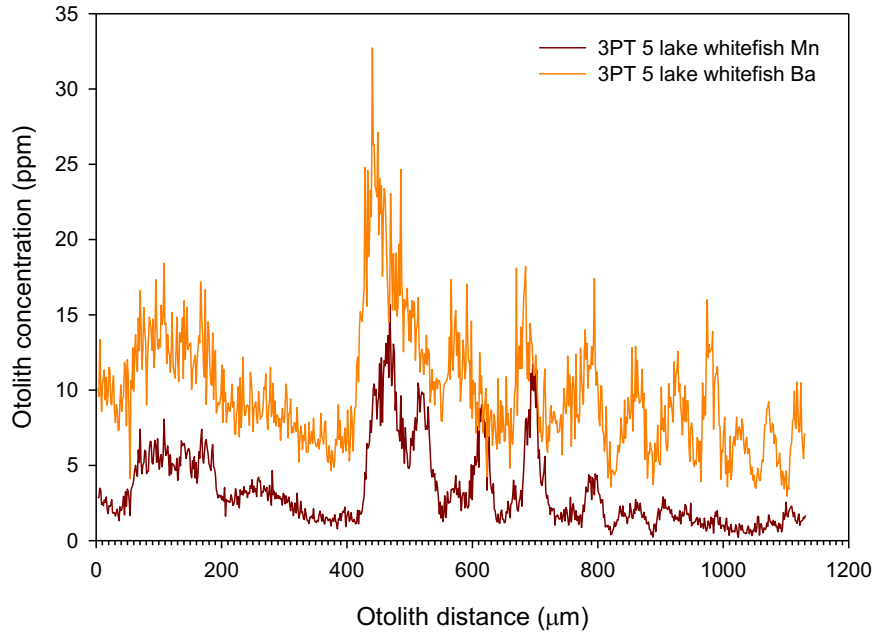
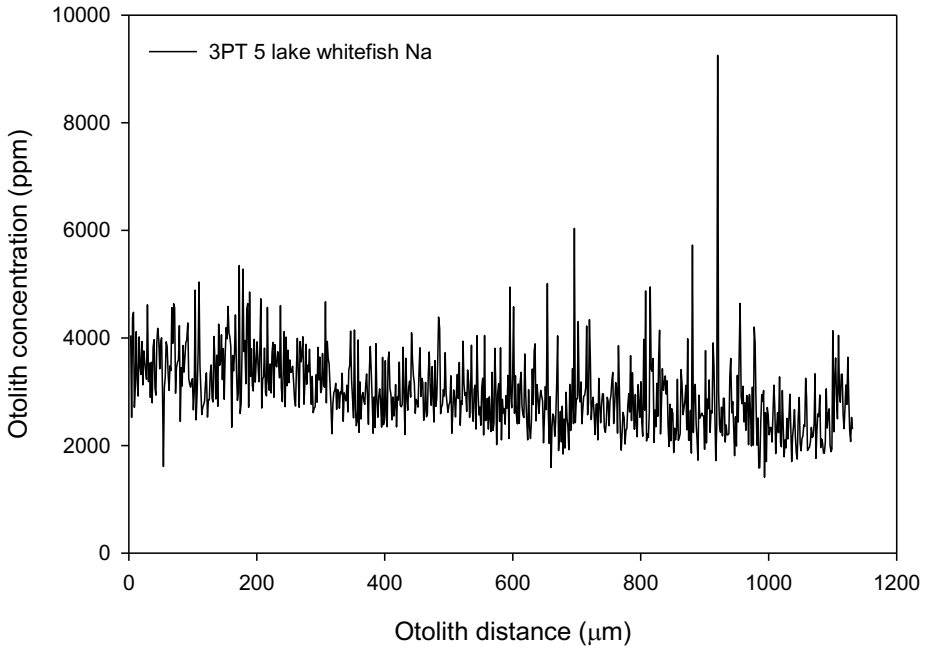


Figure A2. 64. Lake whitefish otolith that was too young for inclusion in main study. Line path, pre-ablation and post ablation (left to right).
CODE: LKWF-LNR-56

LKWF-3PT-5


3PT 5 lake whitefish Mn
3PT 5 lake whitefish Ba
Otolith concentration (ppm)
Otolith distance (μm)
0
5
10
15
20
25
30
35
0
200
400
600
800
1000
1200


3PT 5 lake whitefish Na
Otolith concentration (ppm)
Otolith distance (μm)
0
2000
4000
6000
8000
10000
0
200
400
600
800
1000
1200

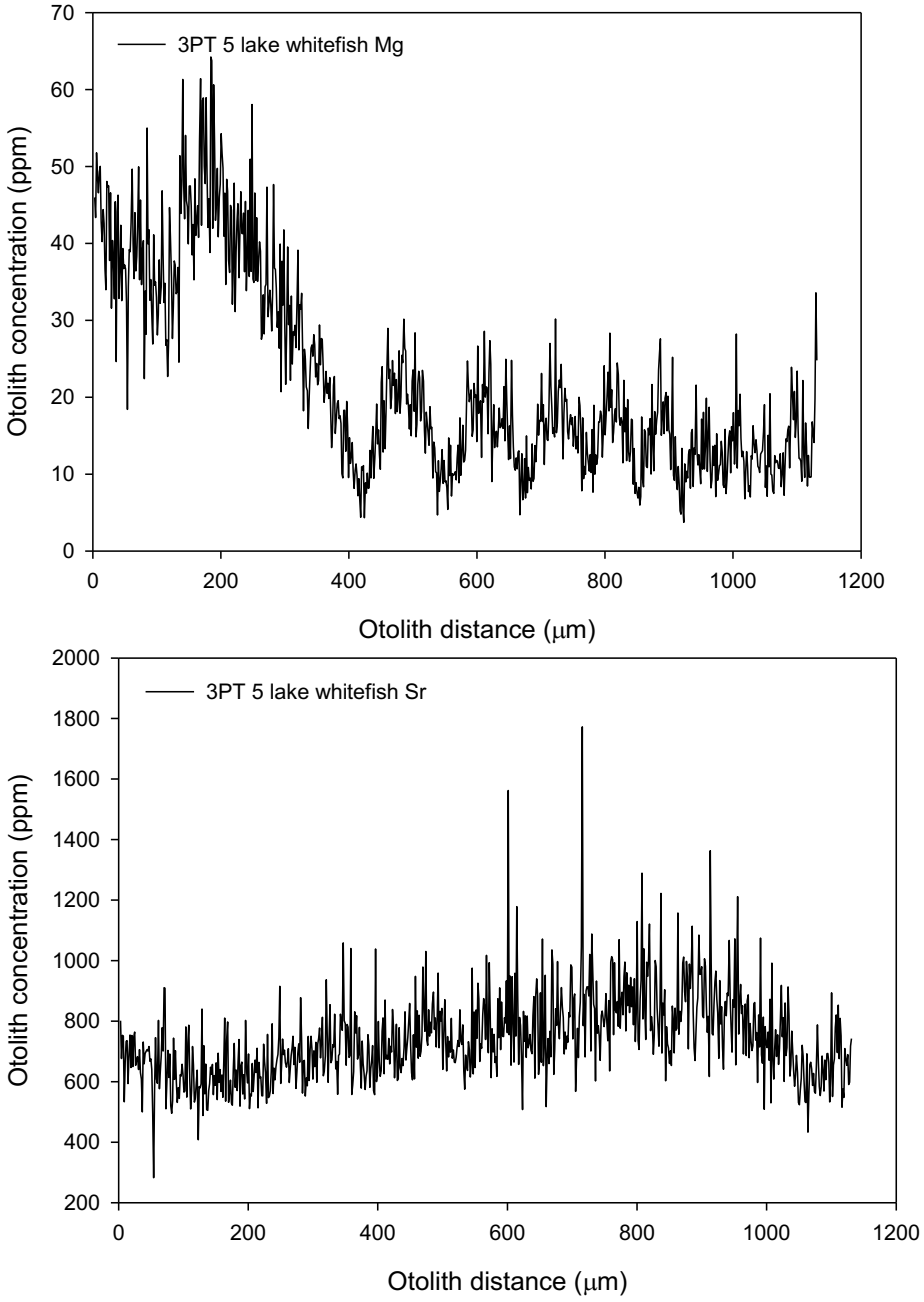
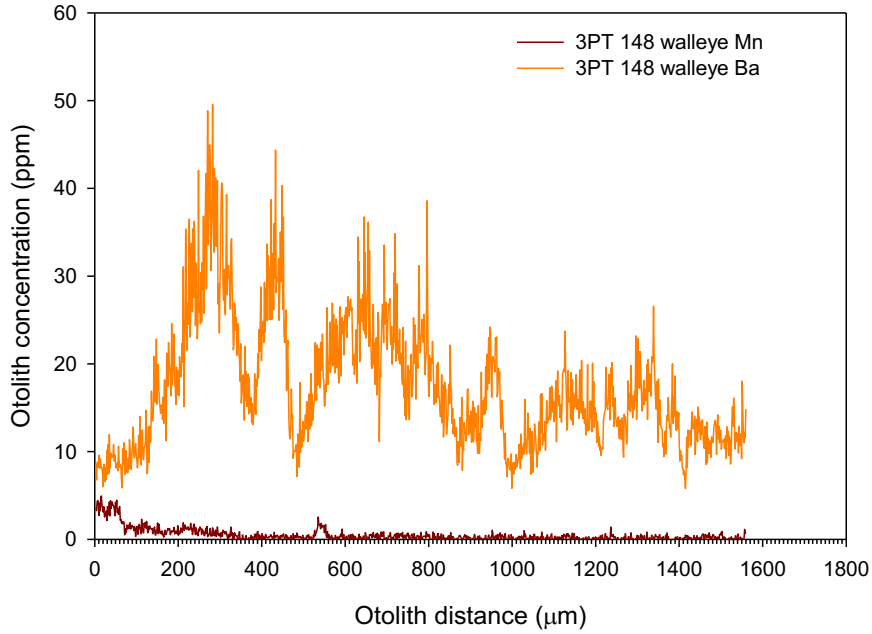
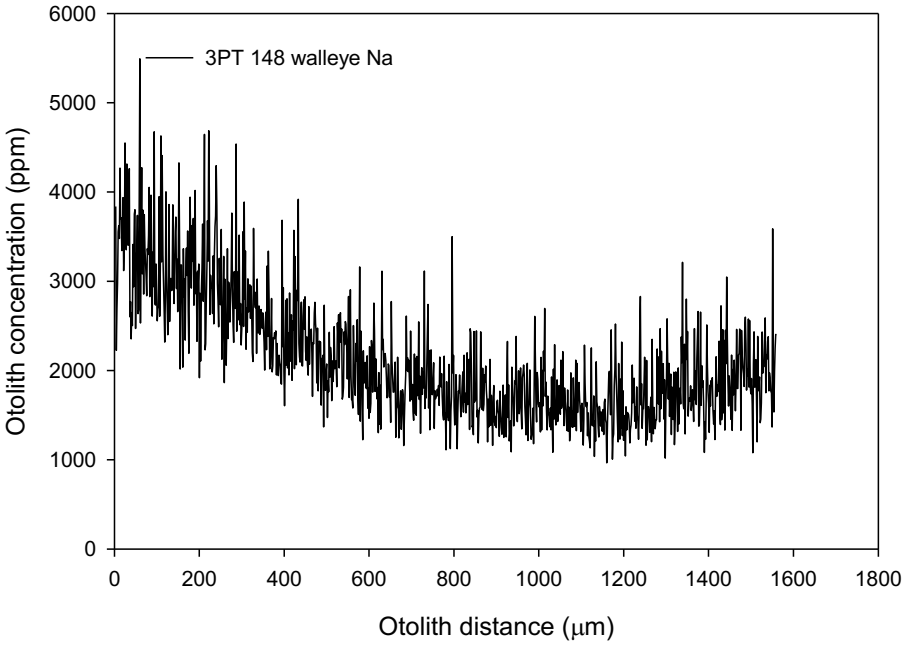


Figure A2. 65. Sample otolith with core (origin) to edge (max otolith distance) lake whitefish laser line graphs. Elements Na, Mg, Mn, Ba and Sr graphed.

CODE: LKWF-3PT-5

WAL-3PT-148


3PT 148 walleye Mn
3PT 148 walleye Ba
Otolith concentration (ppm)
Otolith distance (μm)
0
10
20
30
40
50
60
0
200
400
600
800
1000
1200
1400
1600
1800


3PT 148 walleye Na
Otolith concentration (ppm)
Otolith distance (μm)
0
1000
2000
3000
4000
5000
6000
0
200
400
600
800
1000
1200
1400
1600
1800

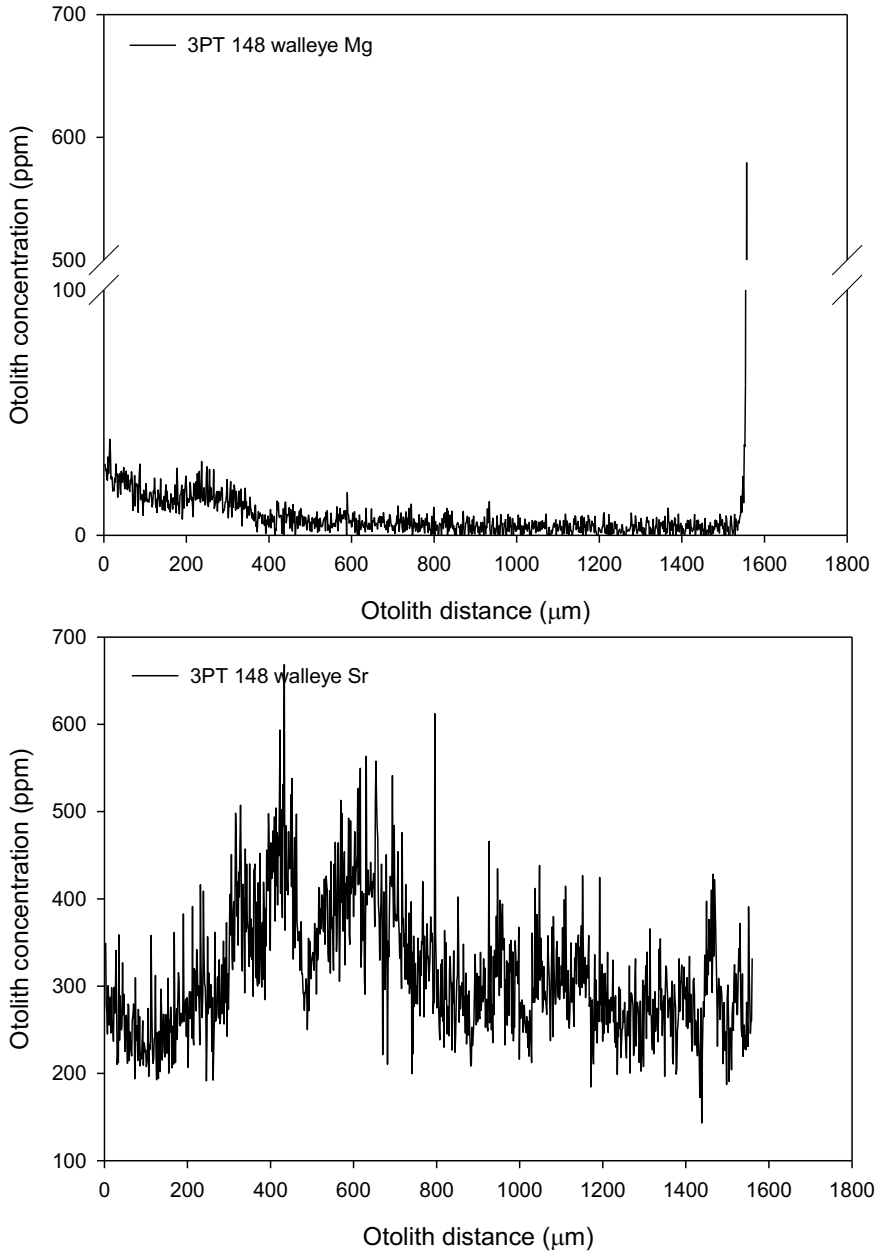
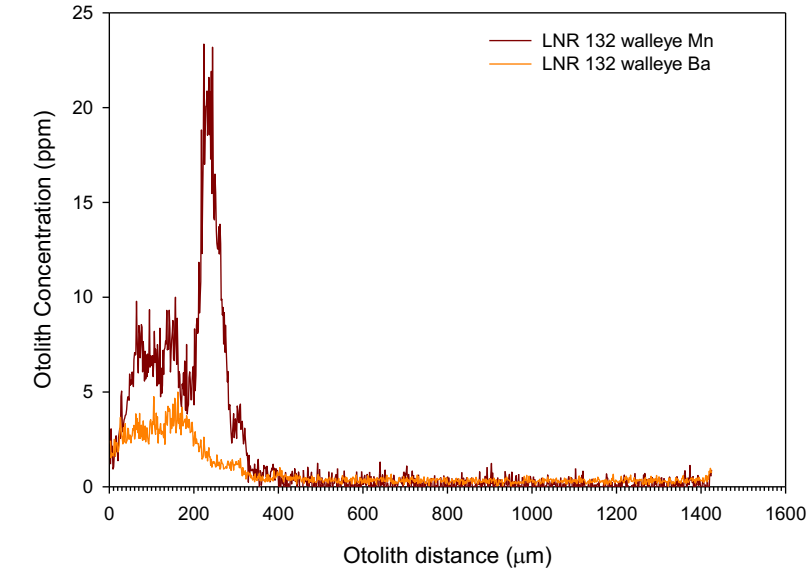
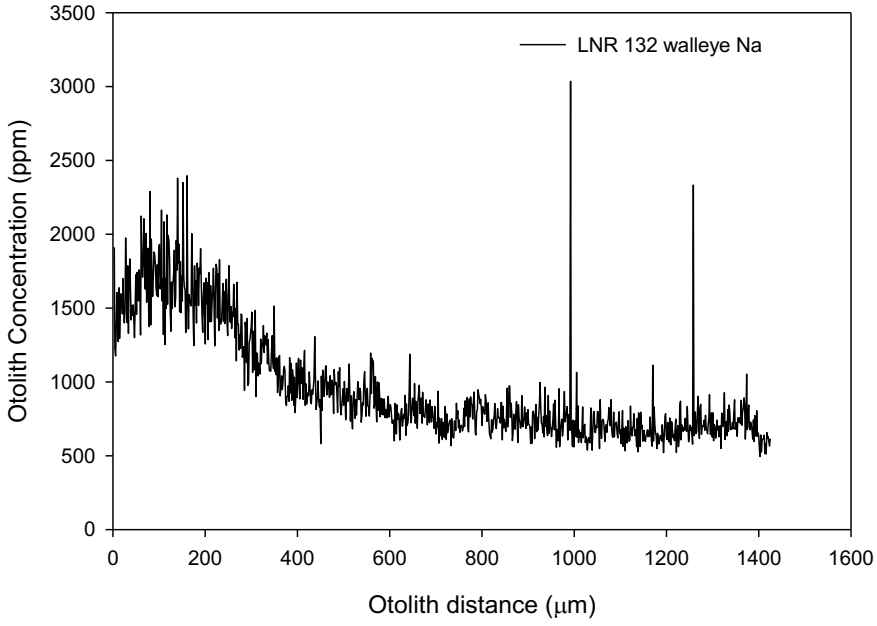


Figure A2. 66. Sample otolith with core (origin) to edge (max otolith distance) walleye laser line graphs. Elements Na, Mg, Mn, Ba and Sr graphed.

CODE: WAL-3PT-148

WAL-LNR-132


LNR 132 walleye Mn
LNR 132 walleye Ba
Otolith Concentration (ppm)
Otolith distance (μm)
0
5
10
15
20
25
0
200
400
600
800
1000
1200
1400
1600


LNR 132 walleye Na
Otolith Concentration (ppm)
Otolith distance (μm)
0
500
1000
1500
2000
2500
3000
3500
0
200
400
600
800
1000
1200
1400
1600

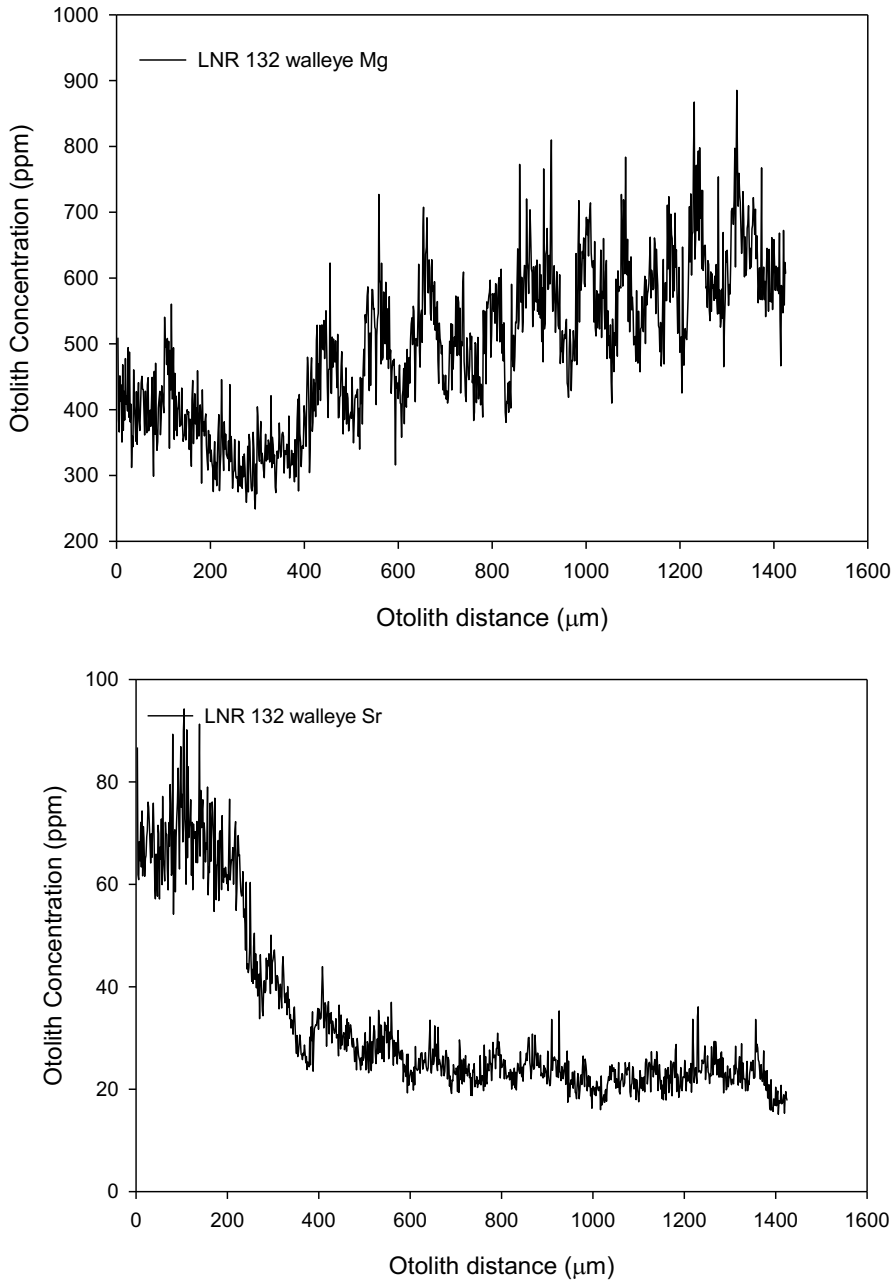
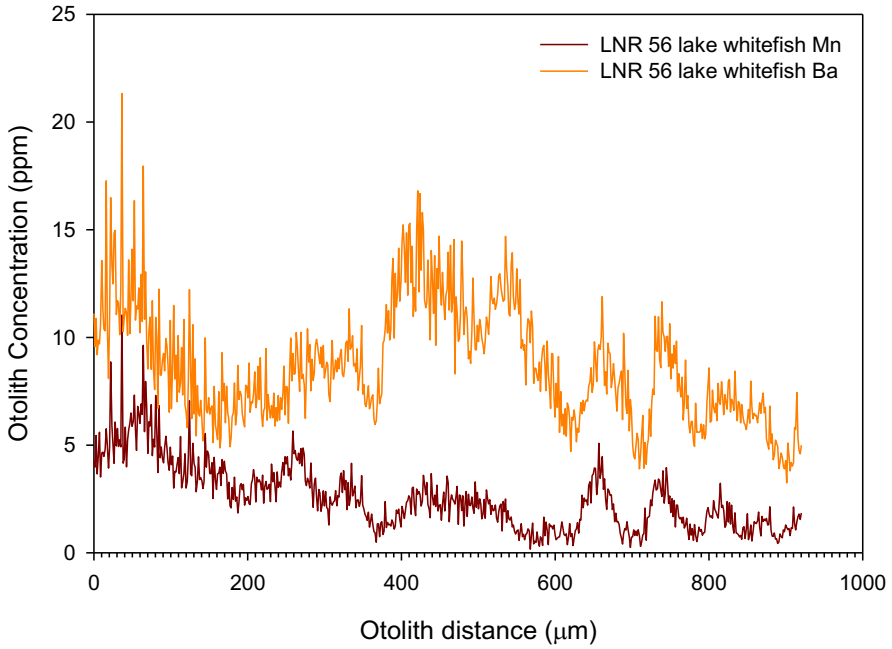
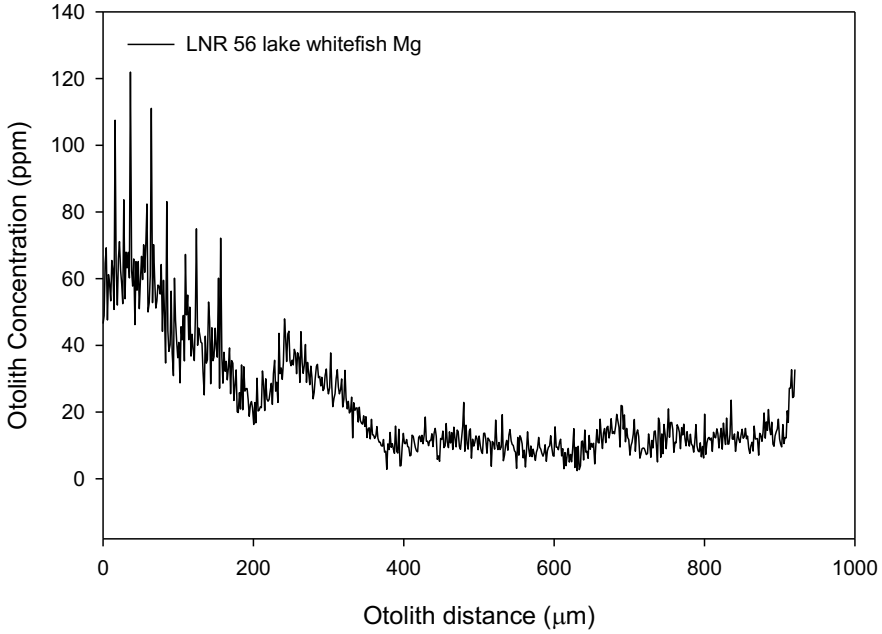


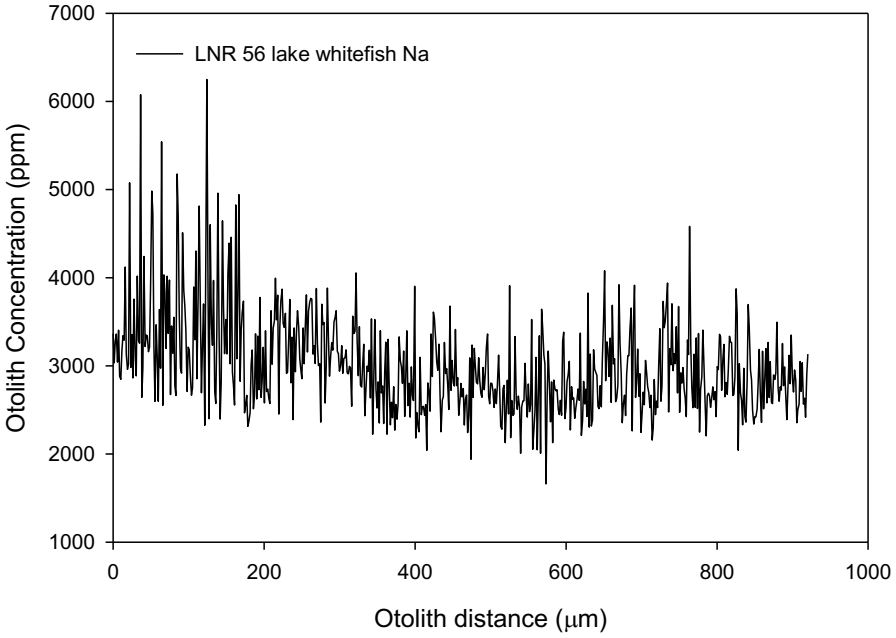
Figure A2. 67. Core (origin) to edge (max otolith distance) walleye laser line graphs. Elements Na, Mg, Mn, Ba and Sr graphed. *Anomalous walleye otolith removed from study

CODE: WAL-LNR-132

LKWF-LNR-56


LNR 56 lake whitefish Mn
LNR 56 lake whitefish Ba
Otolith Concentration (ppm)
Otolith distance (μm)
0
5
10
15
20
25
0
200
400
600
800
1000


LNR 56 lake whitefish Mg
Otolith Concentration (ppm)
Otolith distance (μm)
0
20
40
60
80
100
120
140
0
200
400
600
800
1000



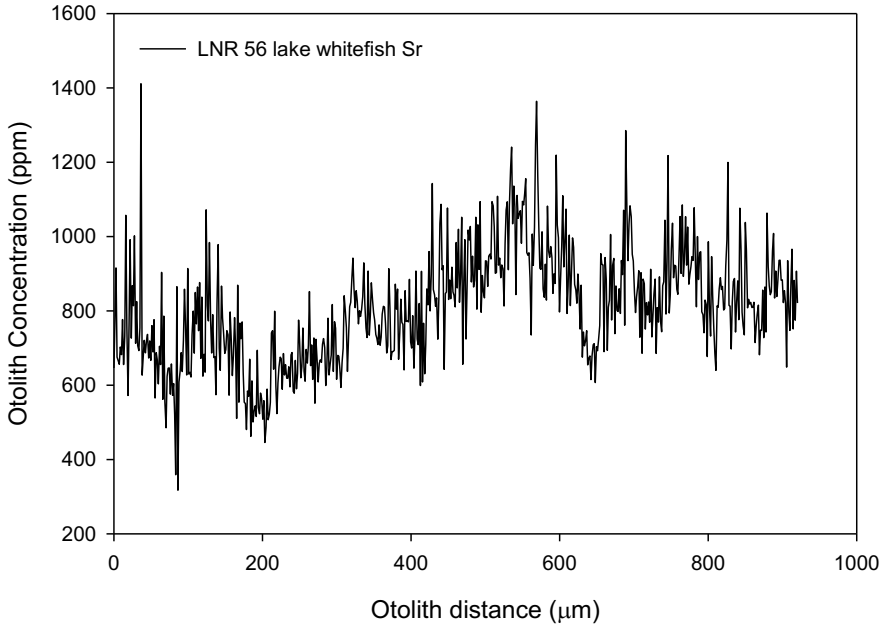


Figure A2. 68. Core (origin) to edge (max otolith distance) walleye laser line graphs. Elements Na, Mg, Mn, Ba and Sr graphed. *Otolith found to be too young for the study

CODE: LKWF-LNR-132

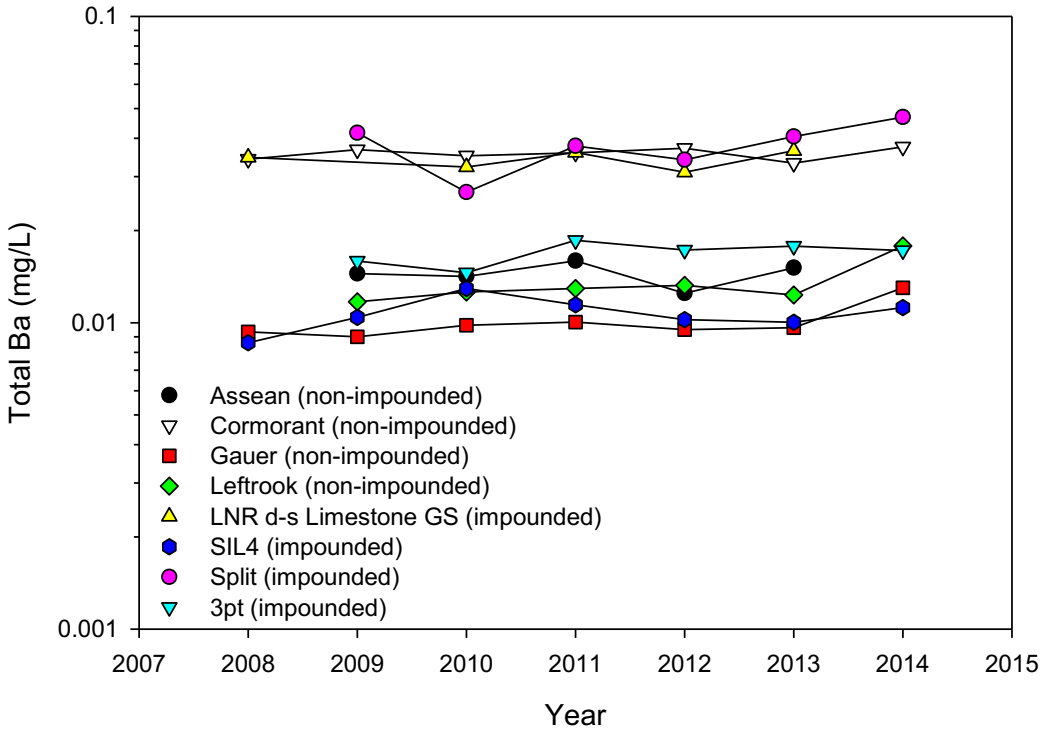


Figure A2. 69. Line/scatter plot of total barium (Ba) average water concentration per year between 2008-2014 per CAMP waterbody. Averages based on samples collected at 0.3 m depth.

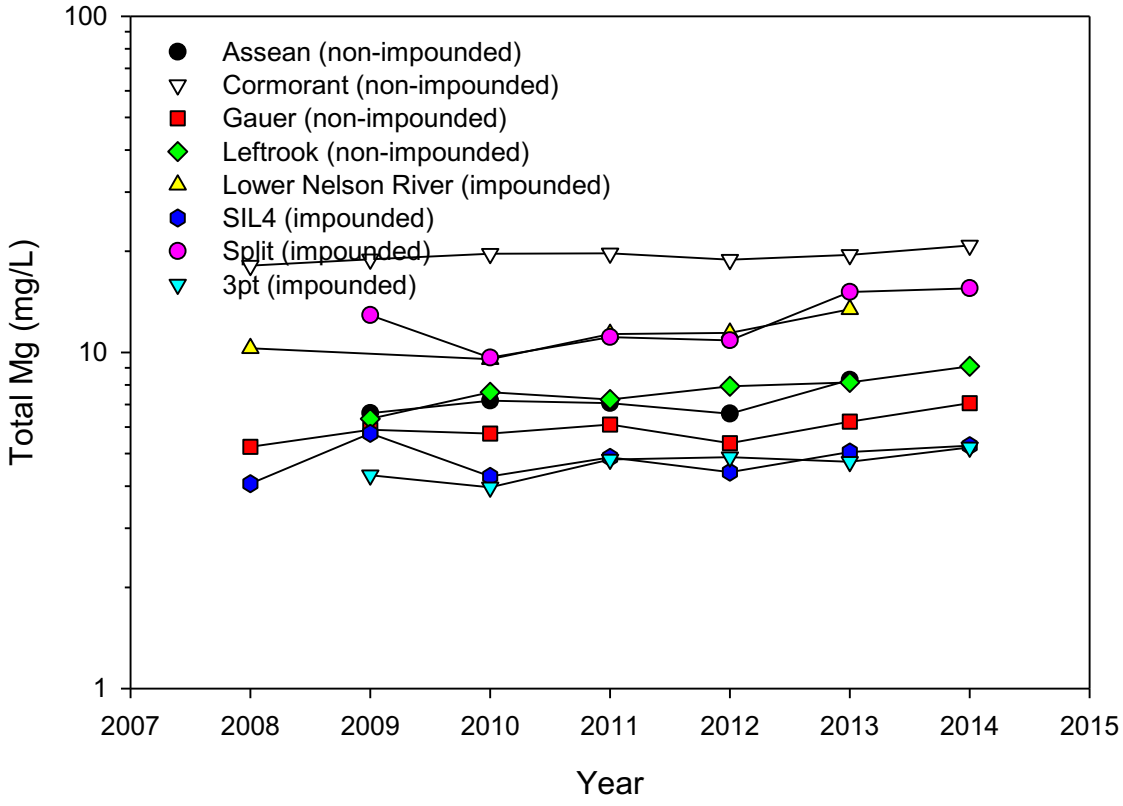


Figure A2. 70. Line/scatter plot of total magnesium (Mg) average water concentration per year between 2008-2014 per CAMP waterbody. Averages based on samples collected at 0.3 m depth.

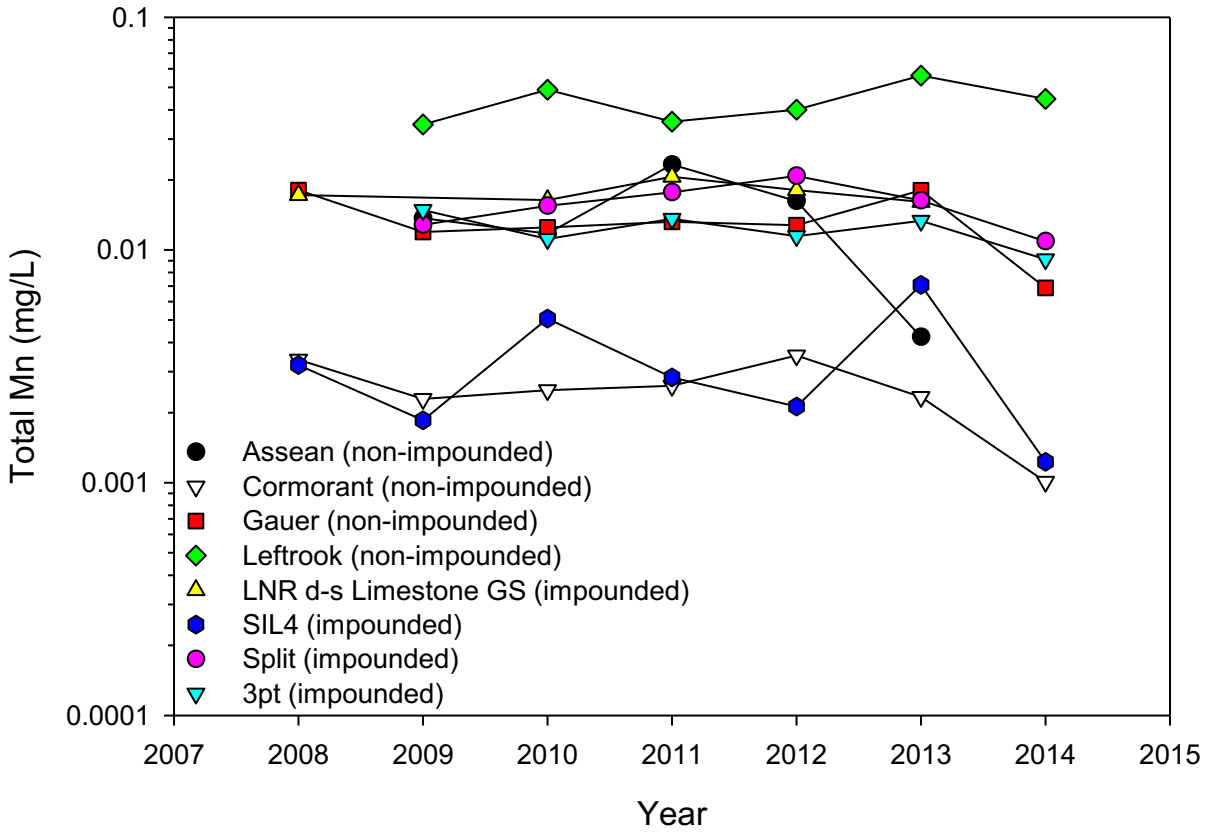


Figure A2. 71. Line/scatter plot of total manganese (Mn) average water concentration per year between 2008-2014 per CAMP waterbody. Averages based on samples collected at 0.3 m depth.

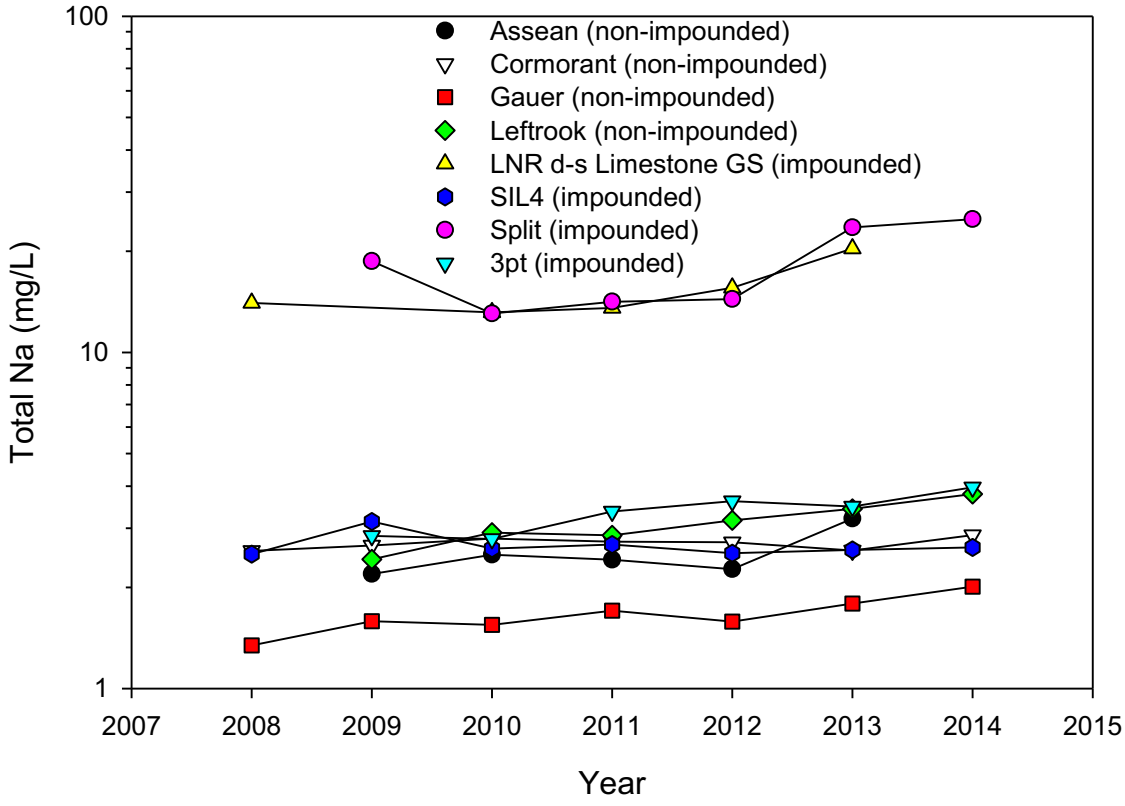


Figure A2. 72. Line/scatter plot of total sodium (Na) average water concentration per year between 2008-2014 per CAMP waterbody. Averages based on samples collected at 0.3 m depth.

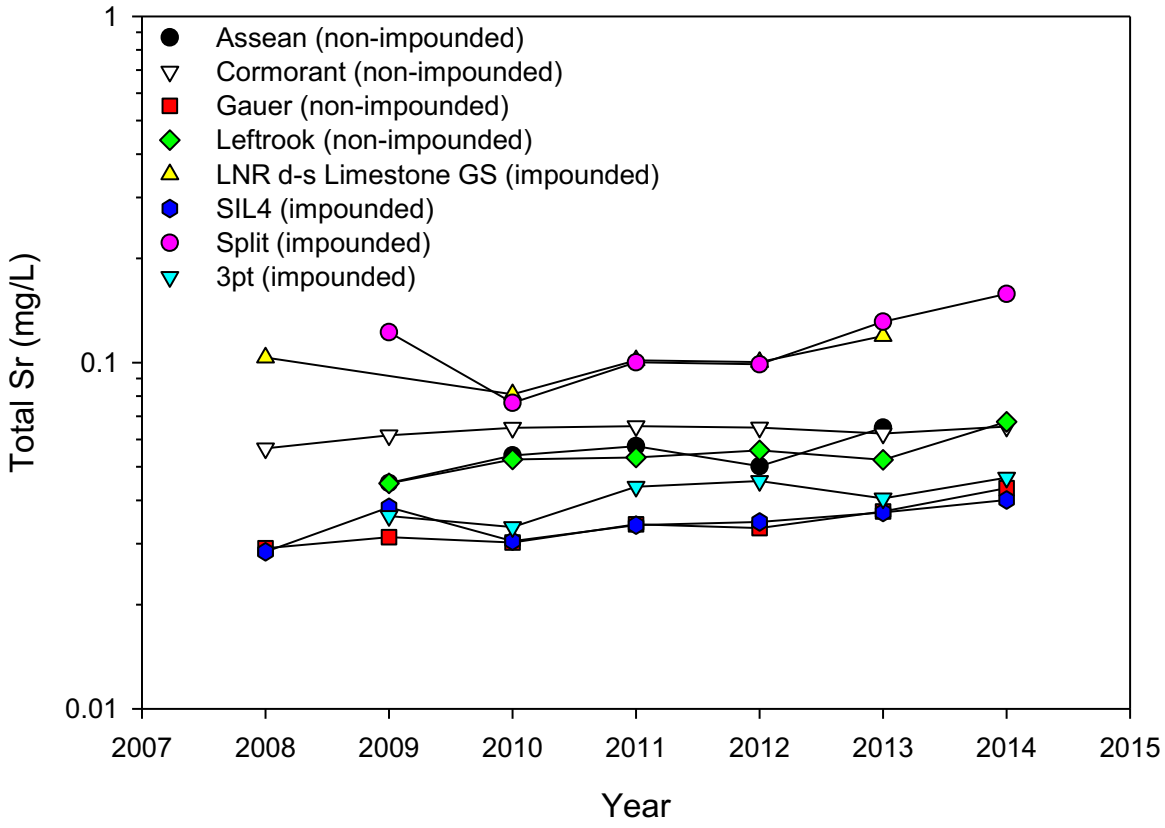


Figure A2. 73. Line/scatter plot of total strontium (Sr) average water concentration per year between 2008-2014 per CAMP waterbody/ Averages based off samples collected at 0.3 m depth.

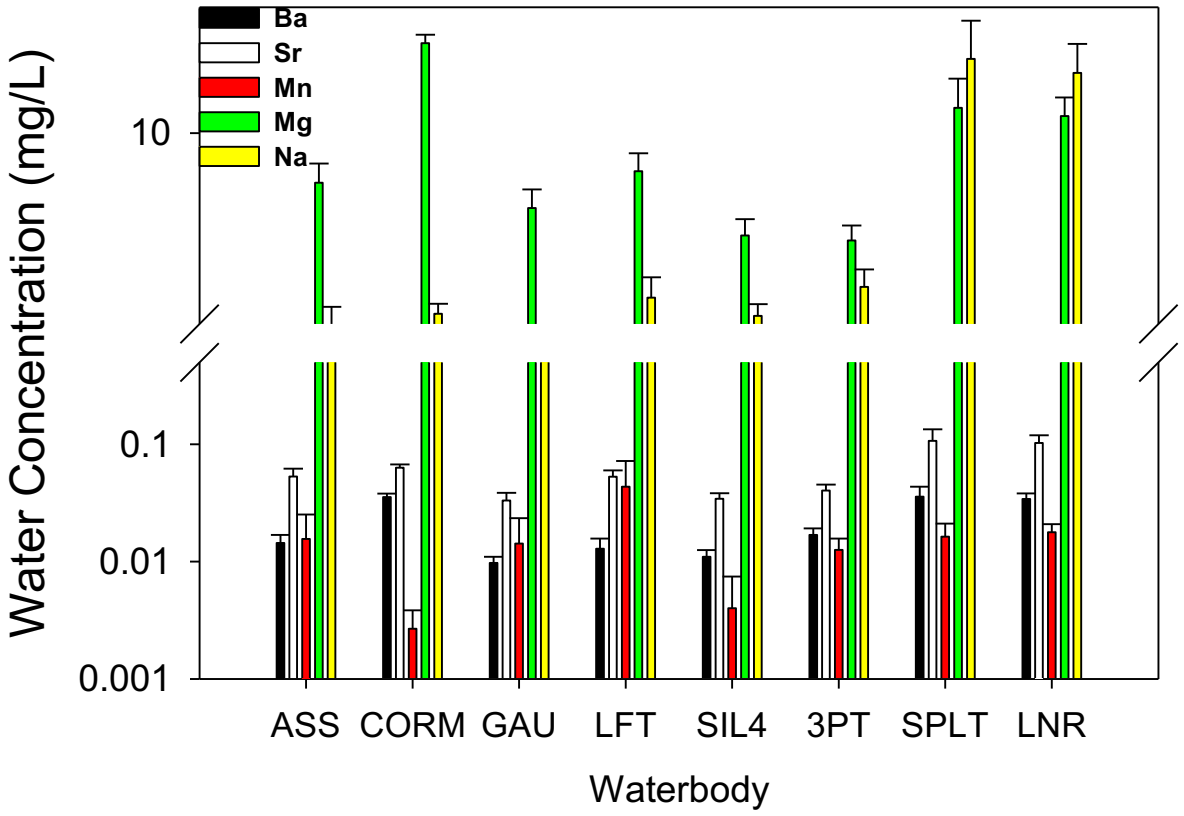


Figure A2. 74. Bar graph of total selected trace element average water concentrations between 2008-2014 for selected CAMP waterbodies. Averages based off samples collected at 0.3 m depth, error bars as standard deviation.

Table A2. 1. Summary table of select CAMP waterbody average trace elements Ba, Mg, and Mn, in mg/L with depth in meters. Error represented as standard deviations from the mean. "nc." indicates a sample size of 1 or that the value was never measured if an error term, or not measured if an average term.

	Depth (m)	Ba avrg.	Ba err.	Mg avrg.	Mg err.	Mn avrg.	Mn err.
Assean	0.3	0.0144	0.00249	6.98	1.04	0.0156	0.00957
	1.3	0.0136	nc.	9.67	nc.	0.00304	nc.
Cormorant	0.3	0.0355	0.00251	19.2	1.23	0.00267	0.00117
	17	0.0325	nc.	16.6	nc.	0.00220	nc.
	22	0.0359	nc.	20.0	nc.	0.0102	nc.
	24	0.0335	0.000849	19.3	1.56	0.00364	0.000318
	25	0.0359	0.00250	19.5	0.902	0.00768	0.00545
Gauer	0.3	0.0097	0.00126	5.80	0.844	0.0142	0.00919
	0.5	0.0090	0.0000990	5.02	0.141	0.0145	0.00382
	2	0.0089	nc.	5.76	nc.	0.0285	nc.
Leftrook	0.3	0.0129	0.00283	7.59	1.05	0.0435	0.0284
	8.4	0.0165	nc.	8.57	nc.	0.0455	nc.
	8.5	0.0234	nc.	7.65	nc.	1.26	nc.
	8.8	0.0177	nc.	10.9	nc.	0.0570	nc.
	9	0.0114	0.00127	7.07	0.474	0.0449	0.0344
	9.5	0.0166	0.000566	9.09	0.658	0.452	0.4568
Lower Nelson	0.3	0.0342	0.00399	11.3	1.65	0.0178	0.003119524
South Indian	0.3	0.0110	0.00153	4.75	0.603	0.00400	0.003445181
	0.5	0.0103	0.00125	4.11	0.262	0.00195	0.000622254
	14.5	0.00961	nc.	4.72	nc.	0.00317	nc.
	15.5	0.0111	nc.	4.40	nc.	0.00355	nc.
Split	0.3	0.0357	0.00770	12.0	2.84	0.0163	0.00476
	0.5	0.0362	0.000849	10.6	0.071	0.0238	0.004949747
Threepoint	0.3	0.0169	0.00231	4.58	0.526	0.0126	0.00315
	3.5	nc.	nc.	nc.	nc.	nc.	nc.

Table A2. 2. Summary table of select CAMP waterbody average trace elements Na and Sr, total dissolved solids (TDS) and total suspended solids (TSS) in mg/L with depth in meters. Error represented as standard deviations from the mean. "nc." indicates a sample size of 1 or that the value was never measured if an error term, or not measured if an average term.

	Depth (m)	Na avrg.	Na err.	Sr avrg.	Sr err.	TDS avrg.	TDS err.	TSS avrg.	TSS err.
ASS	0.3	2.42	0.415	0.0530	0.00889	147	30.7	11.1	8.04
	1.3	3.31	nc.	0.0663	nc.	184	nc.	nc.	nc.
CORM	0.3	2.69	0.205	0.0630	0.00420	181	22.3	3.11	0.774
	17	2.40	nc.	0.0549	nc.	170	nc.	nc.	nc.
	22	2.77	nc.	0.0668	nc.	174	nc.	4.80	nc.
	24	2.73	2.73	0.0619	0.0619	177	1.41	2.70	0.424
	25	2.67	0.159	0.0645	0.00310	168	9.07	3.43	2.653
GAU	0.3	1.61	0.249	0.0332	0.00532	103	16.3	4.33	0.854
	0.5	1.45	0.00707	0.0319	0.000707	97.0	1.41	3.60	nc.
	2	1.57	nc.	0.0342	nc.	111	nc.	3.00	nc.
LFT	0.3	3.02	0.484	0.0529	0.00693	132	44.3	4.21	1.66
	8.4	4.01	nc.	0.0594	nc.	139	nc.	nc.	nc.
	8.5	3.05	nc.	0.0565	nc.	125	nc.	6.80	0.566
	8.8	4.54	nc.	0.0735	nc.	118	nc.	nc.	nc.
	9	2.91	0.276	0.0515	0.00177	115	18.4	4.00	nc.
	9.5	3.33	0.163	0.0605	0.00792	168	26.2	nc.	nc.
LNR	0.3	15.5	3.64	0.103	0.0169	176	29.1	15.2	5.06
SIL4	0.3	2.65	0.237	0.0343	0.00403	79.9	15.4	4.36	1.39
	0.5	2.34	0.0919	0.0318	0.00071	76.0	nc.	2.40	2.40
	14.5	2.50	nc.	0.0361	nc.	85.0	nc.	2.50	nc.
	15.5	2.36	nc.	0.0333	nc.	82.0	nc.	2.40	nc.
SPLT	0.3	17.2	5.49	0.107	0.0275	183	40.9	13.3	6.98
	0.5	11.7	0.636	0.0941	0.0169	155	4.24	16.6	1.34
3PT	0.3	3.27	0.441	0.0404	0.00493	89.4	49.7	7.84	3.37
	3.5	nc.	nc.	nc.	nc.	78.0	nc.	9.60	nc.

Table A2. 3. Walleye summary table of year of capture, ID Code, and fish characteristics/measures for the selected eight CAMP waterbodies (Assean (ASS), Cormorant (CORM), Gauer (GAU), Leftrook (LFT), Lower Nelson (LNR), South Indian (Area 4)(SIL4), Split (SPLT), and Threepoint (3PT)).

Yr. Captured	ID Code			Fish characteristics and measures				
	Species	ID	CAMP#	SEX	Fish stage	Age (years)	Fork length (mm)	Wet weight (g)
2014	WAL	ASS	200	F	Adult	14	491	1370
2014	WAL	ASS	38	M	Adult	13	403	630
2014	WAL	ASS	19	F	Adult	12	470	1210
2014	WAL	ASS	11	F	Juvenile	11	408	730
2014	WAL	ASS	8	F	Adult	12	471	1240
2014	WAL	ASS	620	M	Adult	13	409	890
2014	WAL	ASS	429	M	Adult	15	437	970
2014	WAL	ASS	281	F	Adult	14	517	1620
2014	WAL	ASS	259	M	Adult	13	400	730
2014	WAL	ASS	258	F	Adult	13	506	1580
2014	WAL	CORM	129	M	Adult	13	455	910
2014	WAL	CORM	273	F	Adult	17	552	2030
2014	WAL	CORM	125	M	Adult	13	494	1290
2014	WAL	CORM	231	M	Adult	18	577	2070
2014	WAL	CORM	123	M	Adult	17	520	1455
2014	WAL	CORM	203	M	Adult	17	530	1800
2014	WAL	CORM	121	M	Adult	15	530	1620
2014	WAL	CORM	197	M	Adult	14	480	1250
2014	WAL	CORM	86	M	Adult	10	410	705
2014	WAL	CORM	187	M	Adult	18	542	1615
2014	WAL	GAU	275	M	Adult	16	477	1470
2014	WAL	GAU	51	M	Adult	17	485	1240
2014	WAL	GAU	50	M	Adult	17	447	1060
2014	WAL	GAU	11	M	Adult	18	434	840
2014	WAL	GAU	9	M	Adult	16	441	1010
2014	WAL	GAU	569	F	Adult	18	468	1130
2014	WAL	GAU	535	M	Adult	18	425	770
2014	WAL	GAU	526	M	Adult	17	392	710
2014	WAL	GAU	432	F	Adult	18	522	1780
2014	WAL	GAU	431	F	Adult	16	572	1990
2014	WAL	LFT	152	M	Adult	17	410	660
2014	WAL	LFT	617	M	Adult	26	384	580
2014	WAL	LFT	75	M	Adult	18	377	470

2014	WAL	LFT	481	F	Adult	20	414	760
2014	WAL	LFT	69	F	Adult	18	385	560
2014	WAL	LFT	407	F	Adult	18	400	670
2014	WAL	LFT	68	M	Adult	17	358	410
2014	WAL	LFT	267	F	Adult	20	400	700
2014	WAL	LFT	11	F	Adult	17	487	1150
2014	WAL	LFT	275	M	Adult	23	420	620
2014	WAL	LNR	79	F	Adult	7	441	980
2014	WAL	LNR	64	F	Adult	9	441	1010
2014	WAL	LNR	51	F	Adult	13	568	1770
2014	WAL	LNR	49	F	Adult	11	432	970
2014	WAL	LNR	3	F	Adult	9	450	1200
2013	WAL	LNR	138	M	Adult	13	573	1930
2013	WAL	LNR	132	F	Adult	18	562	1700
2014	WAL	LNR	118	F	Adult	10	476	1350
2014	WAL	LNR	117	M	Adult	9	438	1090
2014	WAL	LNR	82	F	Adult	19	607	2200
2014	WAL	SIL4	1138	M	Juvenile	9	372	600
2014	WAL	SIL5	1116	F	Juvenile	8	436	1020
2014	WAL	SIL6	112	F	Adult	11	474	1370
2014	WAL	SIL7	111	M	Adult	10	548	2000
2014	WAL	SIL8	2	M	Adult	11	490	1330
2014	WAL	SIL9	1281	F	Adult	13	512	1870
2014	WAL	SIL10	1257	M	Adult	10	522	1450
2014	WAL	SIL11	1253	M	Adult	10	530	1600
2014	WAL	SIL12	1232	F	Adult	9	432	1090
2014	WAL	SIL13	1141	F	Juvenile	8	408	820
2014	WAL	SPLT	67	F	Adult	9	441	965
2014	WAL	SPLT	52	F	Adult	10	375	590
2014	WAL	SPLT	5	M	Adult	9	397	720
2014	WAL	SPLT	4	M	Adult	11	471	560
2014	WAL	SPLT	3	M	Adult	9	342	370
2014	WAL	SPLT	403	M	Adult	11	445	1150
2014	WAL	SPLT	302	M	Adult	11	526	760
2014	WAL	SPLT	133	F	Adult	8	394	680
2014	WAL	SPLT	121	F	Adult	16	412	890
2014	WAL	SPLT	71	F	Adult	7	451	1135
2014	WAL	3PT	326	M	Unkown	18	385	655
2014	WAL	3PT	216	F	Adult	18	469	1150
2014	WAL	3PT	179	F	Adult	14	428	850

2014	WAL	3PT	153	F	Adult	16	365	560
2014	WAL	3PT	148	F	Adult	17	425	800
2014	WAL	3PT	124	M	Adult	17	395	730
2014	WAL	3PT	99	F	Adult	17	434	920
2014	WAL	3PT	98	F	Adult	17	388	660
2014	WAL	3PT	97	M	Adult	17	410	740
2014	WAL	3PT	85	M	Adult	17	383	620

Table A2. 4. Lake whitefish summary table of year of capture, ID Code, and fish characteristics/measures for the selected eight CAMP waterbodies (Assean (ASS), Cormorant (CORM), Gauer (GAU), Leftrook (LFT), Lower Nelson (LNR), South Indian (Area 4)(SIL4), Split (SPLT), and Threepoint (3PT)).

Yr. Captured	ID Code			Fish characteristics and measures				
	Species	ID	CAMP#	SEX	Fish stage	Age (years)	Fork length (mm)	Wet weight (g)
2014	LKWF	ASS	267	M	Juvenile	8	421	1210
2013	LKWF	ASS	120	M	Adult	7	407	700
2014	LKWF	ASS	3	F	Juvenile	11	455	1420
2014	LKWF	ASS	2	M	Juvenile	9	421	1160
2014	LKWF	ASS	1	M	Juvenile	8	430	1220
2014	LKWF	ASS	570	F	N/A	10	454	1480
2014	LKWF	ASS	562	M	Juvenile	8	435	1290
2013	LKWF	ASS	415	M	Adult	9	455	1050
2014	LKWF	ASS	340	F	juvenile	7	467	1590
2013	LKWF	ASS	339	M	Juvenile	7	382	780
2014	LKWF	CORM	151	M	Adult	17	420	945
2014	LKWF	CORM	401	M	Adult	27	380	640
2014	LKWF	CORM	68	M	Juvenile	19	390	850
2014	LKWF	CORM	394	F	Adult	18	380	800
2014	LKWF	CORM	56	M	Adult	20	410	900
2014	LKWF	CORM	192	F	Adult	17	420	950
2014	LKWF	CORM	35	F	Adult	20	415	865
2014	LKWF	CORM	190	F	Adult	21	400	655
2014	LKWF	CORM	24	M	Adult	20	388	690
2014	LKWF	CORM	152	M	Adult	23	450	1200
2014	LKWF	GAU	192	M	Adult	16	461	1600
2014	LKWF	GAU	173	M	Adult	15	436	1340
2014	LKWF	GAU	172	F	Adult	13	461	1550
2014	LKWF	GAU	158	M	Adult	13	438	1450
2014	LKWF	GAU	62	M	Adult	19	454	1660
2014	LKWF	GAU	539	M	Adult	15	483	1700
2014	LKWF	GAU	450	F	Adult	19	489	1530
2014	LKWF	GAU	313	F	Adult	14	430	1040
2014	LKWF	GAU	305	F	Adult	15	465	1800
2014	LKWF	GAU	304	F	Adult	24	460	1410
2014	LKWF	LFT	510	M	Adult	25	446	1320
2014	LKWF	LFT	694	F	Adult	21	508	2140

2014	LKWF	LFT	486	M	Adult	25	470	1530
2014	LKWF	LFT	692	M	Adult	20	555	2900
2014	LKWF	LFT	237	M	Adult	19	435	1250
2014	LKWF	LFT	677	M	Adult	20	468	1670
2014	LKWF	LFT	202	M	Adult	24	472	1550
2014	LKWF	LFT	531	F	Adult	21	458	1500
2014	LKWF	LFT	136	F	Adult	19	491	2000
2014	LKWF	LFT	518	M	Adult	21	444	1620
2014	LKWF	LNR	56	F	Juvenile	6	407	990
2014	LKWF	LNR	55	M	Adult	9	435	1150
2014	LKWF	LNR	53	M	Adult	18	552	2490
2014	LKWF	LNR	37	F	Adult	9	446	1500
2014	LKWF	LNR	25	F	Adult	13	459	1210
2014	LKWF	LNR	101	F	Adult	8	431	1050
2014	LKWF	LNR	97	F	Adult	19	500	1460
2014	LKWF	LNR	60	M	Adult	11	425	1260
2014	LKWF	LNR	59	M	Adult	20	482	1610
2014	LKWF	LNR	58	F	Adult	10	461	1400
2014	LKWF	SIL4	1020	F	Juvenile	17	396	760
2014	LKWF	SIL5	114	M	Adult	17	422	1030
2014	LKWF	SIL6	39	F	Juvenile	18	396	780
2014	LKWF	SIL7	38	F	Adult	20	424	970
2014	LKWF	SIL8	32	M	Adult	17	382	710
2014	LKWF	SIL9	1242	F	Adult	17	404	980
2014	LKWF	SIL10	1095	F	Juvenile	18	410	650
2014	LKWF	SIL11	1091	F	Adult	17	442	800
2014	LKWF	SIL12	1089	M	Juvenile	17	392	710
2014	LKWF	SIL13	1084	M	Adult	17	382	730
2014	LKWF	SPLT	91	F	Adult	6	355	680
2014	LKWF	SPLT	90	M	Adult	16	444	1560
2014	LKWF	SPLT	85	F	Juvenile	7	365	830
2014	LKWF	SPLT	82	F	Adult	16	485	1980
2014	LKWF	SPLT	65	M	Adult	13	476	1710
2014	LKWF	SPLT	344	M	Adult	13	457	1730
2013	LKWF	SPLT	330	M	Adult	13	431	1000
2013	LKWF	SPLT	210	M	Adult	12	471	1395
2014	LKWF	SPLT	205	M	Juvenile	6	318	500
2013	LKWF	SPLT	161	F	Adult	12	530	2660
2014	LKWF	3PT	72	M	Adult	10	400	910
2013	LKWF	3PT	54	F	Adult	19	456	1250

2013	LKWF	3PT	53	M	Juvenile	6	335	550
2013	LKWF	3PT	25	M	Adult	24	483	1150
2013	LKWF	3PT	5	M	Adult	9	411	950
2014	LKWF	3PT	360	F	N/A	6	363	845
2014	LKWF	3PT	359	F	N/A	10	442	1195
2013	LKWF	3PT	258	F	Adult	8	390	900
2013	LKWF	3PT	158	M	Juvenile	7	395	925
2013	LKWF	3PT	80	F	Adult	11	433	1040

Table A2. 5. CAMP water quality database (in-situ and lab based) variables under study

Variable	Measure	Units	Frequently above MDL and graphed	Correlation Study
Total Na	Lab	ppm	Yes	Yes
Total Mg	Lab	ppm	Yes	Yes
Total Mn	Lab	ppm	Yes	Yes
Total Ba	Lab	ppm	Yes	Yes
Total Sr	Lab	ppm	Yes	Yes
Carbon (DOC)	Lab	mg/L	Yes	Yes
Dissolved oxygen	Lab	mg/L	Yes	Yes
Nitrogen	Lab	Tot. Kjeldahl (TKN)	Yes	Yes
Productivity (Chlor-a)	Lab	ug/L	Yes	Yes
Productivity (pheophytin)	Lab	ug/L	Yes	Yes
Total dissolved solids	Lab	mg/L	Yes	Yes
Total suspended solids	Lab	mg/L	Yes	Yes
ORP	In-situ	mV	Yes	Yes
True color	Lab	TCU	Yes	Yes
Dissolved oxygen	In-situ	mg/L	Yes	No
Turbidity	In-situ	NTU	Yes	No
Specific conductivity	In-situ	uS/cm	Yes	No
Temperature	In-situ	oC	Yes	No
Alkalinity (bicarbonate)	Lab	mg/L	Yes	No
Alkalinity (CaCO3)	Lab	mg/L	Yes	No
Carbon (TIC)	Lab	mg/L	Yes	No
Carbon (TOC)	Lab	mg/L	Yes	No
Chloride	Lab	mg/L	Yes	No
Hardness (CaCO3)	Lab	mg/L	Yes	No
Conductivity	Lab	umhos/cm	Yes	No
Fluoride	Lab	mg/L	Yes	No
pH	Lab	pH units	Yes	No
Sulphate	Lab	mg/L	Yes	No
Phosphorus (dissolved)	Lab	mg/L P	Yes	No
Turbidity	Lab	NTU	Yes	No
Phosphorus (total nutrient)	Lab	mg/L P	Yes	No
Phosphorus (TPP)	Lab	mg/L P	Yes	No
Productivity (ODb/Oda)	Lab	Optical density ratio	Yes	No

Alkalinity (carbonate)	Lab	mg/L	No	No
Microbiology (E. coli) (CFU)	Lab	CFU/100mL	No	No
Ammonia	Lab	mg/L N	No	No
Nitrate	Lab	mg/L N	No	No
Nitrite	Lab	mg/L N	No	No
P - Total (metal scan)	Lab	mg/L	No	No
Alkalinity (hydroxide)	Lab	mg/L	No	No

Table A2. 6. Water trace elements (dependent) water quality variables as independent variables. Sample size "n"= 8. **Bolded** text indicates a significant difference at an alpha of 0.05. P = Pearson's and S= Spearman's correlation coefficient test.

Dependent Measure	Independent Measure	R	Shapiro Wilk	HOV	Test	F-stat	P-value
Na	**TSS**	**0.781**	**0.315**	**0.16**	**P**	**9.413**	**0.022**
Na	**ORP-L**	**0.821**	**0.391**	**0.662**	**P**	**12.431**	**0.012**
Mg	DO	0.0207	0.101	0.885	P	0.00257	0.961
Mg	Chl-a	0.172	0.183	0.46	P	0.182	0.685
Mg	**Pheophytin**	**0.933**	**0.994**	**0.353**	**P**	**40.353**	**<0.001**
Mg	**TDS**	**0.815**	**0.197**	**0.102**	**P**	**11.881**	**0.014**
Mg	TSS	0.0792	0.071	0.16	P	0.0279	0.852
Mg	T-C	0.616	0.66	0.029	P	3.665	0.104
Mg	ORP-L	0.0906	0.064	0.705	P	0.0496	0.831
Mn	DO	0.21	0.163	0.037	P	0.277	0.618
Mn	**N-TKN**	**0.881**	**0.079**	**0.749**	**P**	**20.762**	**0.004**
Ba	DOC	0.18	0.11	0.233	P	0.201	0.669
Ba	Chl-a	0.269	0.081	0.46	P	0.47	0.519
Ba	**TDS**	**0.832**	**0.214**	**0.885**	**P**	**13.507**	**0.01**
Ba	TSS	0.478	0.358	0.46	P	1.772	0.231
Ba	T-C	0.225	0.091	0.537	P	0.319	0.592
Ba	ORP-L	0.449	0.41	0.619	P	1.518	0.264
Sr	DOC	0.0281	0.069	0.977	P	0.00474	0.947
Sr	DO	0.19	0.182	0.578	P	0.225	0.652
Sr	N-TKN	0.32	0.174	0.16	P	0.684	0.44
Sr	**TDS**	**0.837**	**0.802**	**0.047**	**P**	**14.035**	**0.01**
Sr	**TSS**	**0.761**	**0.606**	**0.885**	**P**	**8.231**	**0.028**
Sr	T-C	0.0247	0.085	0.353	P	0.00367	0.954
Sr	ORP-L	0.682	0.925	0.977	P	5.223	0.062
Na	log T-C	0.218	0.149	0.139	P	0.3	0.604
Na	log DOC	-0.333	nc.	nc.	S	nc.	0.387
Na	log DO	-0.323	nc.	nc.	S	nc.	0.387
Na	log N-TKN	0.143	nc.	nc.	S	nc.	0.705
Na	log Chl-a	0.619	nc.	nc.	S	nc.	0.086
Na	log Pheophy.	0.643	nc.	nc.	S	nc.	0.0716
log Mg	log T-C	0.611	0.748	0.794	P	3.582	0.107
log Mg	log DOC	0.19	nc.	nc.	S	nc.	0.619
log Mg	log N-TKN	0.333	nc.	nc.	S	nc.	0.387
log Mn	log DOC	0.561	0.987	0.977	P	2.758	0.148
log Mn	log Chl-a	0.595	0.65	0.29	P	3.291	0.12

log Mn	log Pheophy.	0.183	0.273	0.885	P	0.208	0.664
log Mn	log TDS	0.131	0.129	0.839	P	0.105	0.757
log Mn	log T-C	0.543	0.192	0.086	P	2.504	0.165
log Mn	log ORP-I	0.267	0.471	0.29	P	0.459	0.523
log Mn	log DO	-0.168	nc.	nc.	S	nc.	0.662
log Mn	log TSS	0.619	nc.	nc.	S	nc.	0.086
log Ba	log DO	0.00388	0.08	0.839	P	0.00009	0.993
log Ba	log N-TKN	0.0684	0.079	0.662	P	0.0282	0.872
log Ba	**log Pheophy**	**0.784**	**0.978**	**0.705**	**P**	**9.601**	**0.021**
log Sr	log Chl-a	0.363	0.355	0.072	P	0.909	0.377
log Sr	log Pheopht.	0.564	0.42	0.102	P	2.8	0.145
log Sr	**log TDS**	**0.868**	**0.168**	**0.26**	**P**	**18.352**	**0.005**

Table A2. 7. Walleye (dependent) LRA trace element correlations, with water quality variables and trace element concentrations. Alpha = 0.05, "n"= 8.

Dep.	Indep.	R	R^2	Shapiro Wilk	HOV	Test	F-stat	P-val.
Na	ORP	0.0768	0.0059	0.188	0.885	P	0.0356	0.857
Na	DOC	0.0454	0.00206	0.279	0.46	P	0.0124	0.915
Na	DO	0.26	0.0674	0.174	0.26	P	0.433	0.535
Na	N-TKN	0.28	0.0784	0.73	0.578	P	0.51	0.502
Na	Chl-A	0.324	0.105	0.784	0.705	P	0.705	0.433
Na	**Pheo.**	**0.747**	**0.558**	**0.06**	**0.139**	**P**	**7.573**	**0.033**
Na	TDS	0.533	0.284	0.99	0.662	P	2.383	0.174
Na	TSS	0.124	0.0153	0.234	0.29	P	0.0934	0.77
Na	T-C	0.554	0.306	0.495	0.794	P	2.651	0.155
Na	Na	0.0663	0.0044	0.179	0.931	P	0.0265	0.876
Mg	ORP	0.0533	0.00284	0.076	0.353	P	0.0171	0.9
Mg	**DOC**	**-0.833**	**0.693889**	**nc.**	**nc.**	**S**	**nc.**	**0.00526**
Mg	DO	-0.299	0.089401	nc.	nc.	S	nc.	0.423
Mg	**N**	**-0.857**	**0.734449**	**nc.**	**nc.**	**S**	**nc.**	**0.00178**
Mg	Chl-A	0.362	0.131	0.675	0.387	P	0.907	0.378
Mg	**Pheo.**	**0.781**	**0.61**	**0.171**	**0.387**	**P**	**9.391**	**0.022**
Mg	TDS	0.109	0.012	0.131	0.26	P	0.0726	0.797
Mg	TSS	0.504	0.254	0.452	0.29	P	2.039	0.203
Mg	T-C	0.629	0.395	0.757	0.29	P	3.925	0.095
Mg	Mg	0.635	0.403	0.275	0.794	P	4.053	0.091
Ba	ORP	0.00144	0.00000207	0.592	0.578	P	0.0000124	0.997
Ba	DOC	0.335	0.112	0.876	0.26	P	0.757	0.418
Ba	DO	0.0171	0.000293	0.566	0.578	P	0.0018	0.968
Ba	N-TKN	0.284	0.0809	0.439	0.072	P	0.528	0.495
Ba	Chl-A	0.597	0.356	0.052	0.233	P	3.317	0.118
Ba	Pheo.	0.624	0.39	0.312	0.353	P	3.831	0.098
Ba	TDS	0.256	0.0654	0.983	0.578	P	0.42	0.541
Ba	TSS	0.0848	0.00719	0.738	0.353	P	0.0434	0.842
Ba	T-C	0.0716	0.00513	0.512	0.794	P	0.0309	0.866
Ba	Ba	0.678	0.46	0.565	0.387	P	5.11	0.065
Sr	ORP	0.317	0.1	0.606	0.207	P	0.67	0.444
Sr	DOC	0.363	0.132	0.286	0.072	P	0.909	0.377
Sr	DO	0.196	0.0386	0.467	0.839	P	0.241	0.641

Sr	N-TKN	0.0908	0.00825	0.24	0.749	P	0.0499	0.831
Sr	**Chl-A**	**0.893**	**0.798**	**0.676**	**0.977**	**P**	**23.664**	**0.003**
Sr	***Chl-A**	**0.85**	**0.722**	**0.246**	**0.321**	**P**	**15.56**	**0.008**
Sr	Pheo.	0.11	0.0121	0.32	0.931	P	0.0734	0.796
Sr	TDS	0.149	0.0223	0.542	0.931	P	0.137	0.724
Sr	TSS	0.481	0.231	0.384	0.102	P	1.803	0.228
Sr	T-C	0.455	0.207	0.312	0.16	P	1.567	0.257
Sr	Sr	0.526	0.276	0.609	0.839	P	2.289	0.181

*Log transformed

P= Pearson's, S= Spearman's correlation coefficient test

Table A2. 8. Lake whitefish (dependent) LRA trace element correlations, with water quality variables and trace element concentrations. Alpha = 0.05, "n"= 8.

Dep.	Indep.	R	R^2	Shapiro Wilk	HOV	Test	F-stat	P-val.
Na	ORP	0.241	0.058	0.257	0.794	P	0.37	0.565
Na	DOC	0.292	0.0852	0.981	0.977	P	0.559	0.483
Na	DO	0.379	0.143	0.499	0.749	P	1.004	0.355
Na	N-TKN	0.365	0.134	0.626	0.139	P	0.925	0.373
Na	Chl-A	0.317	0.101	0.867	0.705	P	0.672	0.444
Na	Pheo.	0.0336	0.00113	0.645	0.537	P	0.00678	0.937
Na	TDS	-0.0476	0.00227	nc.	nc.	S	nc.	0.885
Na	TSS	0.359	0.129	0.36	0.662	P	0.886	0.383
Na	T-C	0.397	0.158	0.603	0.321	P	1.124	0.33
Na	Na	0.272	0.0737	0.821	0.26	P	0.478	0.515
Mg	ORP	0.393	0.154	0.535	0.12	P	1.094	0.336
Mg	DOC	0.142	0.0202	0.18	0.619	P	0.123	0.737
Mg	DO	0.659	0.434	0.667	0.578	P	4.61	0.075
Mg	N-TKN	0.167	0.0278	0.237	0.46	P	0.172	0.693
Mg	Chl-A	0.0743	0.00553	0.402	0.102	P	0.0333	0.861
Mg	*Pheo.	0.66	0.436	0.387	0.662	P	4.64	0.075
Mg	TDS	0.301	0.0909	0.076	0.794	P	0.6	0.468
Mg	TSS	0.297	0.0881	0.656	0.002	P	0.579	0.475
Mg	*TSS	0.323	0.104	0.607	0.029	P	0.697	0.436

Mg	TSS	-0.119	0.014161	nc.	nc.	S	nc.	0.749
Mg	T-C	0.263	0.0692	0.804	0.102	P	0.446	0.529
Mg	Mg	0.464	0.215	0.513	0.794	P	1.647	0.247
Mn	ORP	0.417	0.174	0.993	0.46	P	1.261	0.304
Mn	DOC	0.174	0.0303	0.623	0.885	P	0.188	0.68
Mn	DO	0.239	0.0569	0.645	0.182	P	0.362	0.569
Mn	N-TKN	0.205	0.0421	0.147	0.102	P	0.264	0.626
Mn	Chl-A	0.37	0.137	0.224	0.578	P	0.952	0.367
Mn	Pheo.	0.489	0.239	0.75	0.086	P	1.886	0.219
Mn	TDS	0.549	0.302	0.739	0.139	P	2.592	0.159
Mn	TSS	0.118	0.014	0.917	0.072	P	0.0852	0.78
Mn	T-C	0.583	0.34	0.18	0.705	P	3.091	0.129
Mn	Mn	0.119	nc.	nc.	nc.	S	nc.	0.749
Ba	ORP	0.336	0.113	0.235	0.977	P	0.763	0.416
Ba	**DOC**	**0.768**	**0.59**	**0.473**	**0.46**	**P**	**8.617**	**0.026**
Ba	**DO**	**-0.707**	**0.499849**	**nc.**	**nc.**	**S**	**nc.**	**0.0374**
Ba	N-TKN	0.686	0.47	0.962	0.977	P	5.328	0.06
Ba	Chl-A	0.0186	0.000346	0.795	0.498	P	0.00208	0.965
Ba	Pheo.	0.121	0.0147	0.776	0.072	P	0.0894	0.775
Ba	TDS	0.181	0.0328	0.347	0.423	P	0.203	0.668
Ba	TSS	0.0277	0.000768	0.702	0.885	P	0.00461	0.948
Ba	T-C	0.0186	0.000347	0.815	0.839	P	0.00298	0.965
Ba	Ba	0.32	0.102	0.574	0.353	P	0.684	0.44
Sr	ORP	0.573	0.328	0.967	0.931	P	2.929	0.138
Sr	DOC	0.572	0.327	0.091	0.705	P	2.92	0.138
Sr	DO	0.457	0.208	0.903	0.794	P	1.58	0.255
Sr	N-TKN	-0.0952	0.00906304	nc.	nc.	S	nc.	0.794
Sr	Chl-A	0.405	0.164025	nc.	nc.	S	nc.	0.29
Sr	Pheo.	0.235	0.055	0.521	0.578	P	0.349	0.576
Sr	TDS	0.268	0.0718	0.272	0.662	P	0.464	0.521
Sr	TSS	0.429	0.184041	nc.	nc.	S	nc.	0.26
Sr	T-C	0.176	0.0309	0.572	0.207	P	0.191	0.677
Sr	Sr	0.67	0.449	0.731	0.353	P	4.887	0.069

*Log transformed

P= Pearson's, S= Spearman's correlation coefficient test

Table A2. 9. Water trace element averages (dependent) versus individual control or generating structures flow rate averages (m^*s^{-3}) as independent variables per year. Alpha = 0.05.

TE	WB	CS or GS	Pos.	"n"	R^2	S. W. Norm.	HOV	Test	F	P-val
Na	LNR	Limestone GS	DS	5	0.2070	0.08	0.05	P	0.78	0.442
Na	**SIL4**	**Missi Falls CS**	**DS**	**7**	**0.8810**	**0.56**	**0.18**	**P**	**37**	**0.002**
Na	3pt	Notigi CS	DS	6	0.0001	0.61	0.06	P	0.00	0.985
Na	SPLT	Kelsey GS	DS	6	0.0012	0.20	0.06	P	0.00	0.948
Na	SPLT	Kettle GS	US	6	0.0030	0.20	0.06	P	0.01	0.918
Na	**SIL4**	**Notigi CS**	**US**	**7**	**0.6178**	**nc.**	**nc.**	**S**	**nc.**	**0.025**
Na	3pt	Wuskwatim GS	US	4	0.6400	**nc.**	**nc.**	S	nc.	0.333
Mg	LNR	Limestone GS	DS	5	0.0390	0.91	0.05	P	0.12	0.75
Mg	**SIL4**	**Missi Falls CS**	**DS**	**7**	**0.7720**	**0.43**	**0.22**	**P**	**17**	**0.009**
Mg	SIL4	Notigi CS	US	7	0.3430	0.24	0.97	P	2.61	0.167
Mg	3pt	Notigi CS	DS	6	0.0288	0.71	0.06	P	0.12	0.748
Mg	SPLT	Kelsey GS	DS	6	0.0054	0.35	0.06	P	0.02	0.89
Mg	SPLT	Kettle GS	US	6	0.0083	0.35	0.06	P	0.03	0.863
Mg	3pt	Wuskwatim GS	US	4	0.1600	nc.	nc.	S	nc.	0.75
Mn	LNR	Limestone GS	DS	5	0.4220	0.52	0.05	P	2.19	0.236
Mn	SIL4	Missi Falls CS	DS	7	0.2670	0.28	0.49	P	1.82	0.235
Mn	SIL4	Notigi CS	US	7	0.2360	0.74	0.15	P	1.54	0.269
Mn	3pt	Notigi CS	DS	6	0.0083	0.79	0.06	P	0.03	0.864
Mn	SPLT	Kelsey GS	DS	6	0.2380	0.81	0.06	P	1.25	0.326
Mn	SPLT	Kettle GS	US	6	0.2690	0.83	0.27	P	1.47	0.292
Mn	3pt	Wuskwatim GS	US	4	0.6400	nc.	nc.	S	nc.	0.333
Sr	LNR	Limestone GS	DS	5	0.0298	0.87	0.05	P	0.09	0.781
Sr	SIL4	Missi Falls CS	DS	7	0.4540	0.58	0.84	P	4.16	0.097
Sr	SIL4	Notigi CS	US	7	0.1670	0.11	0.78	P	1.00	0.363
Sr	3pt	Notigi CS	DS	6	0.0142	0.36	0.06	P	0.06	0.822
Sr	SPLT	Kelsey GS	DS	6	0.0248	0.99	0.06	P	0.10	0.766
Sr	SPLT	Kettle GS	US	6	0.0318	0.99	0.06	P	0.13	0.735
Sr	3pt	Wuskwatim GS	US	4	0.1600	nc.	nc.	S	nc.	0.75
Ba	LNR	Limestone GS	DS	5	0.2970	0.66	0.05	P	1.27	0.342
Ba	SIL4	Notigi CS	US	7	0.1570	0.21	0.39	P	0.93	0.379

Ba	3pt	Notigi CS	DS	6	0.0391	0.39	0.06	P	0.16	0.707
Ba	SPLT	Kelsey GS	DS	6	0.1030	0.80	0.06	P	0.46	0.535
Ba	SPLT	Kettle GS	US	6	0.1150	0.79	0.06	P	0.52	0.511
Ba	3pt	Wuskwatim GS	US	4	0.6400	nc.	nc.	S	nc.	0.333
Ba	SIL4	Missi Falls CS	DS	7	0.1030	nc.	nc.	S	nc.	0.438
TDS	LNR	Limestone GS	DS	5	0.0605	0.93	0.05	P	0.19	0.69
TDS	SIL4	Notigi CS	US	7	0.0306	0.88	0.55	P	0.16	0.707
TDS	SPLT	Kettle GS	US	5	0.0021	0.52	0.05	P	0.01	0.941
TDS	SIL4	Missi Falls CS	DS	7	0.2372	nc.	nc.	S	nc.	0.217
TDS	3PT	Notigi CS	DS	6	0.0073	nc.	nc.	S	nc.	0.919
TDS	3PT	Wuskwatim GS	US	4	0.3600	nc.	nc.	S	nc.	0.417
TSS	SIL4	Missi Falls CS	DS	5	0.2391	0.74	0.05	P	0.94	0.403
TSS	SIL4	Notigi CS	US	5	0.0999	0.92	0.05	P	0.33	0.605
TSS	3pt	Notigi CS	DS	6	0.2480	0.18	0.06	P	1.32	0.315
TSS	SPLT	Kelsey GS	DS	6	0.4706	0.13	0.06	P	3.56	0.132
TSS	SPLT	Kettle GS	US	6	0.4844	0.18	0.06	P	3.75	0.125
TSS	LNR	Limestone GS	DS	5	0.0900	nc.	nc.	S	nc.	0.683
TSS	3pt	Wuskwatim GS	US	4	0.0400	nc.	nc.	S	nc.	0.917

POS = WB position versus CS/GS (Upstream or Downstream)

P= Pearson, S= Spearman correlation coefficient test