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REPRODUCTIVE CHARACTERIZATION OF WALLEYE (Sander vitreus) AND LAKE WHITEFISH (Coregonus clupeaformis) IN TATHLINA LAKE, NT

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REPRODUCTIVE CHARACTERIZATION OF WALLEYE (*Sander vitreus)* AND LAKE WHITEFISH (*Coregonus clupeaformis*) IN TATHLINA LAKE, NT

By

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(HBSc, Wilfrid Laurier University, 2014)

THESIS

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Abstract

Tathlina Lake, NT is an ecologically and culturally important lake to the Ka'a'gee Tu First Nation and supports a small commercial fishery for walleye (*Sander vitreus*) and a subsistence fishery for lake whitefish (*Coregonus clupeaformis*). The community is concerned about existing fluctuations in the fish populations. They are also concerned with environmental pressures, including potential future oil and gas development in the near-by Cameron Hills and climate change, and desires to institute long-range biomonitoring in the lake. Male and female adult walleye and lake whitefish, in pre- and post-spawning conditions, were collected biannually in March and August between 2012 and 2016. General health assessment measures included: liversomatic index (LSI), gonadosomatic index (GSI), condition factor and fecundity. Additionally, gonadal and plasma hormone levels were measured to assess reproductive status and determine if seasonal steroid variations can be detected in both plasma and gonadal tissue. In March, pre-spawning female and male walleye exhibited greater LSI, GSI, fecundity and reproductive hormone levels [17β-estradiol (E_2) in females and 11-ketotestosterone (11-KT) in males], and unchanged condition factors relative to post-spawning in August. In August, pre-spawning male and female lake whitefish exhibited lower LSI, greater GSI, fecundity and E_2 and 11-KT levels, and unchanged condition factors relative to post-spawning in March. Among years, morphometric endpoints were relatively stable within months and reproductive stage and exhibited less variability than hormone levels. Critical effects sizes (CES), which represent natural variability in endpoints, were calculated in order to indicate current ranges of measured variables; fluctuations below or above CES could indicate the presence of environmental pressures on the system and can now be used by the community as the foundation of their long-term biomonitoring protocols. It is recommended that the community focus its long-term

biomonitoring on measuring fish condition factor, LSI and GSI biennially (every-other-year) during both pre- and post-spawning seasons using a CES-based approach and that it expand the number of lakes sampled in the region to better represent natural variability and, therefore, enhance the ability to detect change in the region.

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Chapter One

General Introduction

1.1 Introduction

1.2 Background to Thesis and Contributions

The contents of this thesis are the result of a multi-year partnership among community members of the Ka'a'gee Tu First Nation (KTFN), scientists of the Government of Northwest Territories (GNWT) and Fisheries and Oceans Canada (DFO), and academic researchers including those from Wilfrid Laurier University (Laurier). A multidisciplinary project was supported by the Cumulative Impact Monitoring Program (CIMP) of the GNWT with the broad scope of improving our understanding of the cumulative impacts of environmental change and human development in the Tathlina Lake, NT, watershed, a culturally and economically important area to the KTFN. Major objectives of the CIMP-funded project were to assess the current health of the aquatic system at Tathlina Lake through the implementation of a community-based regional water quality monitoring program; undertake water, sediment, and microbenthic sampling of streams in the Cameron Hills; and assess fish health. The principal role of the Laurier researchers was to support research into the status of the health and reproduction of fish in the lake. The approach taken involved the implementation of baseline fish biomonitoring protocols. The author of the thesis participated in three field sampling expeditions (August 2014, March 2015, and August 2015) and analyzed samples from these collections and March 2016, in addition to re-analyzing stored samples and comparing them with archived data from additional field expeditions to examine intra-laboratory variability. All fish collections and laboratory analyses were conducted under the guidance of Dr. Andrea Lister (Laurier) and the archived fish steroid data were generated through the efforts of various Laurier technicians for the December 2012, March 2013, June 2013, August 2013, and March 2014 sampling periods included in the study. Temperature and oxygen data were obtained from MiniDOT loggers

deployed by KTFN community members and GNWT scientists between the period of December 2014 to April 2015. Blood and gonad samples were collected by members of the Laurier group with the assistance of the KTFN and stored at – 80 °C at Laurier, as described in the materials and methods section. The author of the thesis conducted the assessment of potential fecundity and AAE Tech Services provided the data on fish age from otoliths collected by the Laurier researchers, including the thesis author. Calculations and statistical analyses were conducted by the thesis author, with the advice of Dr. Michelle Bowman (Forensecology).

1.3 General context

1.4 Biomonitoring in aquatic systems

Long-term, baseline monitoring information is useful for evaluating potential changes in freshwater ecosystems over time (Lindenmayer & Likens, 2009; Schaeffer *et al.,* 2011). Monitoring seasonal changes over long periods of time can provide important ecological insights crucial for improved management of ecosystems and natural resources (Lindenmayer & Likens, 2009). Long-term datasets are important for understanding how stressors influence aquatic ecosystems and their fish communities (Schaeffer *et al.,* 2011). Many ecotoxicological studies (McMaster *et al.,* 1991; McMaster *et al.,* 2005; Brown *et al.,* 2011; Tetreault *et al.,* 2011) have employed the techniques of the Canadian federal environmental effects monitoring (EEM) program to detect and measure changes in aquatic ecosystems, including water quality, fish habitat, and fish health (Environment Canada, 2010) using a specified set of guidelines (http://www.ec.gc.ca/esee-eem/?CFID=9825548&CFTOKEN=62758720). EEM studies provide guidelines for assessing individual indicators of energy storage, energy usage, and survival of fish (Barrett *et al.,* 2015). Basic life history information on fish species used in assessments,

which ideally are widely distributed and large enough to provide tissue for analysis, is essential for design and interpretation of data in the EEM program (Barrett *et al.,* 2015). Endpoints identified in the EEM program include liver size (liver somatic index; LSI; liver weight/ (body weight-liver weight) x 100), gonad size (gonadosomatic index GSI; gonad weight/(body weight-gonad weight) x 100) and condition (condition factor; CF; 10^5 x body weight/fork length^3) and are used to monitor the health of the aquatic system and impacts caused by stressors such as climate change and anthropogenic activities (Kilgour *et al.,* 2005; Environment Canada, 2010; Barrett *et al.,* 2015). Standardized monitoring endpoints reduce variability, allowing for their use as indicators of seasonal changes in general fish health and reproductive status (Kilgour *et al.,* 2005). Additionally, these endpoints can demonstrate reproductive cycles and possible variations in physiological condition due to environmental changes (Freitas *et al*., 2011).

1.5 Seasonality in fish as relates to monitoring

The reproductive cycles of temperate fish are strongly influenced by seasonal changes in environmental conditions. Environmental conditions provide exogenous signals, which affect the physiology, gonadal maturation, and spawning time of fish (Dahle *et al.,* 2003; Schindler & Smol, 2006; Sharma *et al.,* 2007; Prowse *et al.,* 2009). Photoperiod and temperature are the strongest signals with the potential to signal the beginning and ending of a breeding season in temperate fish (Kime, 1999; Pankhurst & Porter, 2003; Lester *et al.,* 2004; Barton, 2011). Variation in reproductive effort in fish populations is believed to reflect the optimization of a reproductive strategy for a given environment based on ecological conditions (Leggett & Carscadden, 1978; Plaza *et al.,* 2007).

The spawning season for large-bodied temperate fish is commonly described in terms of the month(s) at which the spawning starts and ends. Most freshwater fishes of the boreal region

of North America have an iteroparous life history, i.e., they are capable of spawning multiple times during their lifespans (Scott & Crossman, 1973). The number of spawning episodes during a reproductive season and the pattern of ovarian development normally determine the spawning strategy in teleosts (Plaza *et al*., 2007). Fish spawning only once during a spawning season develop their oocytes synchronously from oogonia to immature oocytes, through vitellogenesis and final maturation, while those spawning multiple times during a season develop oogonia asynchronously **(**Wallace & Selman, 1981). When addressing the spawning strategy, it is important to consider the ovarian growth as the season progresses, because reproductive cycles are coupled with pronounced changes in gonadal size. Therefore, a common measure of reproductive state is gonadal size using the GSI, which increases as gonads mature in preparation for spawning. It determines gonad size relative to body size as an index in order to standardize among fish on the assumption that larger fish will have larger gonads. CF acts as an indicator of feeding to acquire energy reserves for gonad development throughout the reproductive season (Freitas *et al*., 2011; Barrett *et al.,* 2015). Typically, changes in CF are associated with spawning activities as energy reserves are allocated towards gonadal development (Johnston *et al*., 2012); however, changes in CF are not linked to maturation per se, but factors associated with reproduction might affect the underlying annual growth cycle (Tveiten *et al*., 1998). Liver size also plays a role in gonad development in females as the liver allocates lipid reserves for gonad development, leading to decreased LSI prior to spawning as gonad size increases (Barrett *et al.,* 2015); conversely, liver size increases during vitellogenesis as the liver produces large amounts of lipoproteins in the form of vitellogenin for the developing ova (Sharpe & MacLatchy, 2007).

Because reproduction causes such large changes in morphometric endpoints, the sampling times in EEM studies are standardized to reduce potential variability in endpoints and

improve comparability of data among studies (Barrett *et al.,* 2015). Otherwise, there is a risk of identifying as "site differences" those variances which are due to normal cycling through reproductive states. EEM approaches allow for the development of predictive tools to assess natural variability and future cumulative environmental impacts. One such predictive tool is the application of critical effect sizes (CES) to the data (Environment Canada, 2010; Arciszewski & Munkittrick, 2015). These values represent natural variability in endpoints in order to indicate changes representing high risk to the population (Environment Canada, 2010). When values fluctuate below or above CES, this could indicate the presence of an ecological pressure. Biomonitoring studies must include an understanding of changes in seasonality in order to best interpret the data (Barrett & Munkittrick, 2010). To that end, patterns must be clearly identified with pre- (when gonads are developing) and post- (when gonad size is at a minimum) spawning times to reduce variability in endpoints (Barrett & Munkittrick, 2010; Barrett *et al.,* 2015).

1.6 Environmental conditions

Understanding biotic fluctuations in aquatic environments, particularly in systems with strong seasonal changes, requires understanding abiotic variability with respect to changes in environmental conditions. Many aspects of reproductive development of fishes, including spermatogenesis, oogenesis, spermiation and ovulation, are significantly influenced by abiotic factors (Van Der Kraak & Pankhurst, 1997). Temperate fishes display some degree of seasonality of reproductive activity and the amplitude of seasonal variation is thought to increase with latitude (Van Der Kraak & Pankhurst, 1997). The most important environmental conditions and cues for reproductive cycling in temperate fish are photoperiod and temperature (De Vlaming, 1972; Kime, 1999; Pankhurst & Porter, 2003; Lester *et al.,* 2004; Pankhurst, 2008; Barton, 2011).

Photoperiod is widely recognized to be a key signal for reproduction in fish. It is the only variable capable of delivering an unambiguous 'date' signal that is capable of phasing and entraining reproductive maturation (Kime, 1999). Ultimately, this can generate physiological changes in the endocrine system, which drives the reproductive process (Pankhurst & Porter, 2003).

Temperature directly influences fish physiology and behavior (Magnuson *et al.,* 1990). Each species is known to have a preferred and optimal thermal condition for survival, growth and reproduction (Sharma *et al.,* 2007; Barton, 2011). Water temperature acts as a timing cue and a modulating factor for fish reproductive cycling (De Vlaming, 1972; Pankhurst & Porter, 2003). Temperature has long been recognized to limit the range of species with respect to geographic scales and locations in particular lakes or streams (Sharma *et al.,* 2007). Air temperature plays a role in determining the composition of aquatic communities through its effects on water temperature (Lester *et al*., 2004; Sharma *et al.,* 2007). With increasing water temperature in spring, spring spawners undergo gonadal maturation and steroidogenesis, whereas in autumn with decreasing water temperatures, autumn spawners undergo gonadal maturation and steroidogenesis (Pankhurst & Porter, 2003). When water temperatures are not favorable for fish, i.e., lower for spring spawners and elevated for autumn spawners, fish may exhibit reduced weight due to reduced food intake, reduced circulating reproductive hormones, and altered gonadal development from optimal patterns (Jackson *et al.,* 2001; Pankhurst & Porter, 2003 Lester *et al.,* 2004; Schindler & Smol, 2006; Sharma *et al.,* 2007; Barton, 2011; Okuzawa & Gen, 2013). Overall, fish may experience variations in the timing of spawning with changes in temperature (Lester *et al.,* 2004; Schindler & Smol, 2006; Sharma *et al.,* 2007).

Other water quality variables of aquatic ecosystems can influence abundance, survival, and distribution of organisms, e.g., the solubility of oxygen is reduced at higher temperatures, and increased at lower temperatures (Jackson *et al.,* 2001; Mackenzie-Grieve & Post, 2006). When oxygen concentrations fall below 1 mg/L, this is considered to be lethal (Chambers *et al.,* 2000; Barton, 2011). Studies have shown that low dissolved oxygen (DO) can lead to retarded gonad growth, reduced somatic growth, reduced fertilization success, reduced reproductive output, reduced larval hatching, reduced larval success, and decreased reproductive steroid levels (McMahon *et al.,* 1984; Chambers *et al.,* 2000; Ru, 2002; Barton, 2011). There is a reduction in individual fitness as a result of reduced growth and fecundity, ultimately leading to population declines (Ru, 2002).

1.7 Climate change

Climate change, together with increasing human activities in polar regions, are altering the structure and function of northern ecosystems and the socioeconomic framework of northern communities (Brook *et al.*, 2009). Climate change has both direct and indirect implications for fish, streams and aquatic ecosystems. It is known that northern regions of Canada are especially vulnerable to large increases in air temperature compared with other regions of North America (Sharma *et al.,* 2007*)*. The impact of changes can vary with physical characteristics of the environment; e.g., shallow lakes respond rapidly to changing climatic conditions and have less resiliency than deeper lakes (Brook *et al.,* 2009; Rieman & Clayton, 2011). A climate-warming model proposed by Mackenzie-Grieve & Post (2006) predicts greater warming at increasingly northern latitudes and suggests that northern regions may experience more pronounced changes in temperature (Blumber & BiToro, 1990; Sharma *et al.,* 2007). Stewart *et al.,* (1998) reported that the Mackenzie River basin had already experienced increases in temperatures up to 2°C per

decade since the mid-1970s. Northern fish populations are also known to delay spawning when temperatures are unfavorable (Quist *et al.,* 2003). Potential increases in temperatures as a result of climate change will affect ecosystems. Alterations in aquatic ecosystem functionality, e.g., warmer and longer ice-free seasons, can result in increased growth and survival of fish (Sharma *et al.,* 2007). Warmer temperatures have the potential to alter thermal habitat resulting in altered fish physiology, e.g., increased metabolism leading to increased oxygen demands (Sharma *et al.,* 2007; Rinne & Carter, 2008; Prowse *et al.,* 2009; Rieman & Clayton, 2011).

1.8 Fish reproduction/reproductive endocrinology

Assessing fish reproductive cycles in lake populations can provide critical information regarding population recruitment and variability. Spawning in fish relies on both external and internal stimuli (Kime, 1995). Once an external stimulus, such as temperature and/or photoperiod, is experienced by the fish, an internal signal cascade follows. Initially, gonadotropin releasing hormone (GnRH) from the hypothalamus targets gonadotrophic cells of the pituitary gland. The pituitary gland synthesizes and releases luteinizing hormone (LH) and follicle-stimulating hormone (FSH) **(**Planas & Swanson, 2008; Levavi- Sivan *et al.,* 2009; Zohar *et al.,* 2010). The gonadotrophic hormones stimulate the maturation of the gonads (Planas & Swanson, 2008). The predominant circulating steroid in males is 11-ketotestosterone (11-KT), which is produced in the testes and is a main regulator of spermatogenesis (process of producing of spermatozoa) **(**Weltzien *et al*., 2004; Planas & Swanson, 2008; Levani-sivan *et al.,* 2009; Zohar *et al.,* 2010). In females, 17β-estradiol (E_2) predominates and originates in the ovaries and is a main regulator of oogenesis (process of producing mature ova), with a major role of stimulating liver synthesis of a yolk lipoprotein, vitellogenin, which is incorporated into the oocyte (King *et al.,* 2003; Weltzien *et al*., 2004; Rocha *et* al., 2008; Pankhurst, 2008). These

reproductive steroids maintain gonadal maturation as well as other physiological processes. Two MIS (maturation-inducing steroids) are common in teleosts: 17α, 20β-dihydroxy-4-pregnen-3-one (17α, 20β-P) and 17α, 20β, 21-trihydroxy-4-pregnen-3-one (17 α, 20β-21P) (Weltzien *et al*., 2004). In males, MIS is involved in germ cell final maturation and spermiation (Weltzien *et al*., 2004), while in females, MIS is involved in germ cell final maturation and ovulation.

There are a number of ways to assess steroid levels to provide an indication of reproductive state. Two common ways include measuring steroid levels extracted from gonadal tissue or plasma. Gonadal tissue levels provide an indication of seasonal variation and represent steroid production levels, while plasma levels also provide an indication of seasonal variation and represent the sum of production and clearance processes in the whole organism (Carragher & Pankhurst, 1993). Measuring terminal reproductive steroid levels in fish is a traditional and proven method to assess reproductive status (McMaster *et al.,* 1991; McMaster *et al.,* 2005; Planas & Swanson, 2008; Bosker *et al.,* 2010) and can be used to supplement morphometric data such as GSI (Ghaffari *et al.,* 2011; Kilgour *et al.,* 2005; Barrett & Munkittrick, 2010; Environment Canada, 2010; Freitas *et al*., 2011; Barrett *et al.,* 2015).

1.9 Study background: Tathlina Lake, NT

Tathlina Lake (N 60° 32'; W 117° 31'; Figure 1) is located southwest of Great Slave Lake and is ecologically and culturally important to KTFN (Kennedy, 1962). It is large (surface area = 573 km^2) and turbid, with depths ranging from 1.5 to 1.8 m (Kennedy, 1962). Tathlina Lake is part of the Tathlina Lake watershed and drains into Kakisa Lake, which in turn drains into the Mackenzie River. Lady Evelyn Falls, which is 14.63 m high, prevents any movement of fish into the Mackenzie River from Kakisa Lake (Kennedy, 1962). The river system is 496 km long, originating west of the Cameron Hills, and drains an area of 14 900 km^2 (Roberge *et al*.,

1988). While shallower cold-water lakes typically lack large-bodied fish assemblages due to a combination of both thermal stress and oxygen depletion (Jackson *et al.,* 2001), Tathlina Lake has historically contained large-bodied fish populations, albeit fluctuating levels (Kennedy 1962; Roberge *et al*., 1988; Stewart & Low, 2000; Gallagher *et al*., 2011).

In 1940, a major wildfire coated the lake with ash and killed a large number of fish in the lake (Ka'a'Gee Tu First Nation, 2002), and in 1942/1943, the local fisheries experienced a large decrease in stock abundance as a result of a large natural winterkill. Winterkill occurs when snow and ice cover decrease under-ice photosynthesis, leading to low oxygen production and limiting available DO (Stewart & Low, 2000). In the winter of 1953/1954, commercial fishing of walleye (*Sander vitreus*) began and has continued to provide important economic benefits for residents from the nearby community of Kakisa (Gallagher *et al.,* 2011). Additionally, while lake whitefish *(Coregonus clupeaformis*) are not a target for commercial fishing because their flesh is commonly infested with cysts of the parasite *Triaenophorus crassus* (Roberge *et al.,* 1988), they remain culturally important to residents (Gallagher *et al*., 2011; Stewart *et al*., 2015). There have been multiple, large-scale declines in the walleye populations (1942/1943 and 2001) and as a result, catch quotas have steadily decreased from 90 000 kg in the 1950s to 2000 kg in 2008, with closures occurring when catch-per-unit effort was deemed too low (Gallagher *et al.,* 2011; Stewart *et al.,* 2016). While Tathlina Lake has experienced fluctuating fish stocks, there is little understanding as to what drives these fluctuations. The walleye population has been studied several times since the 1940s (Kennedy, 1962; Roberge *et al*., 1988; Gallagher *et al*., 2011), although no long-term biomonitoring has been done.

1.10 Walleye (*Sander vitreus*) and lake whitefish (*Coregonus clupeaformis)*

Walleye and lake whitefish co-occur in many lakes, including Tathlina Lake, and are important components of Canada's freshwater fisheries (Brook *et al.,* 2009). Adult lake whitefish are primarily benthivorous (consume benthos), whereas adult walleye are primarily piscivorous (consume fish) (Johnston *et al*., 2012). Both species are broadcast spawners with well-defined seasonal spawning periods, though lake whitefish spawn in fall and walleye spawn in spring. Both species produce small, juvenile fish which consume planktonic foods (e.g., zooplankton) and occupy the pelagic zone (Johnston *et al*., 2012). Recently-hatched young may be particularly vulnerable to water quality conditions and predation (Malison & Held, 1996). Walleye and lake whitefish have been used in a variety of environmental monitoring programs (Johnston *et al.,* 2012; DeBoer *et al.,* 2013**)**. Because they are large, plentiful and long-lived, they are easy to collect and handle and allow for long-term incorporation of stressors (Crossman & Scott, 1998; Johnston *et al*., 2012).

Walleye, a member of the Percidae family (Crossman & Scott, 1998), are a freshwater fish distributed in temperate and subarctic North America (Johnston et al., 2012). In NT, walleye are mainly associated with lakes and rivers in the Taiga Plains terrestrial ecosystem, and have been found as far north as the MacKenzie River Delta (Crossman & Scott, 1998). They are a cool-water species which prefers turbid environments (Chu *et al.,* 2004). Adult walleye commonly range in total length from 330-500 mm with females generally larger than males. They typically live 10-12 years in the south and up to possibly 20 years in the north (Crossman & Scott, 1998). Seasonal changes in gonad condition and serum sex steroid levels demonstrate that walleye populations spawn annually in early to mid-April, shortly after ice breakup (Malison

et al., 1994; Malison & Held, 1996), depending on water temperature (3.6-6.7°C) and food availability (Crossman & Scott, 1998; Malison & Held, 1996; Jennings *et al.,* 1996).

Lake whitefish, a member of the Salmonidae family (Crossman & Scott, 1998), are widely distributed in fresh water and are found in post-glacial lakes and rivers throughout North America. They are considered to be deep-water fish occupying the cooler hypolimnetic (bottom of lake system with low DO levels) waters of lakes (Scott & Crossman, 1973). Adult lake whitefish commonly range in total length from 205-340 mm with females being larger than males. In the wild, lake whitefish mature from the age of two-four years (Beauchamp *et* al., 2004; Lu & Bernatchez, 1999). Lake whitefish spawn in the autumn or early winter, depending on water temperature (0.5°C - 10°C) and photoperiod (Healey & Nicol, 1975; Billard *et al.,* 1978; Crossman & Scott, 1998; Rinchard *et al.,* 2001).

1.11 Objectives of thesis and approach

The overall objective of this work was to initiate the development and implementation of protocols to generate baseline data indicating seasonal (pre- and post-spawning) reproductive and health endpoints in walleye and lake whitefish in Tathlina Lake, NT. The approach taken may provide guidance and tools by which a long-term community biomonitoring effort could continue, in partnership with government and academia. The assessment of environmental conditions (air and water temperature, DO and photoperiod) will help generate a baseline for environmental conditions corresponding to the biological endpoints measured. To characterize the reproductive patterns in walleye and lake whitefish in Tathlina Lake, endpoints assessed included: CF, LSI, GSI, age, plasma and gonadal hormone (E_2 and 11-KT) levels, and fecundity. Additionally, plasma hormone levels were measured in previously-analyzed samples stored from additional field expeditions to determine intra-laboratory variability. By establishing a baseline

including pre- and post-spawning data, reproductive status and general health can be monitored and assessed in the future in relation to climatic conditions and anthropogenic activities.

1.12 Research as integrative biology

This thesis demonstrates integrative biology by assessing endpoints at various levels of biological organization, such as: tissue/organ (gonad and liver size; gonadal steroid levels), physiological (plasma steroids), whole organism (body weight), and population (indirectly via fecundity) in order to assess the reproductive and health status of two fish species in a northern Canadian lake. The population level is the most important when assessing the ecological integrity of a system and fecundity directly indicates the potential of the population to be sustainable under current conditions. In relation to ecosystem health assessment, as one moves up the levels of biological organization (whole organism, population, community), the endpoints strengthen in their predictive and integrative nature. At the lower levels (molecular, physiological, tissue), the endpoints are indicators of individual health status and have the capability of providing information for understanding mechanistic pathways of higher-level endpoints.

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Chapter Two

Research Paper

2.1 Abstract

Tathlina Lake, Northwest Territories, Canada is a shallow lake (surface area = 573 km^2) of cultural and economic importance to the Ka'a'gee Tu First Nation. The present study assessed common endpoints used in the Canadian Federal Environmental Effects Monitoring (EEM) program (CF, condition factor; LSI, liversomatic index; GSI, gonadosomatic index) to assess the health of two resident large-bodied fish species (spring-spawning walleye (*Sander vitreus*) and fall-spawning lake whitefish (*Coregonus clupeaformis*). EEM endpoints were augmented by measures of fecundity and plasma and gonadal steroid levels (17β-estradiol (E_2) in females, 11-ketotestosterone (11-KT) in males). Fish were collected biannually in March and August from 2012-2016. In general, pre-spawning female and male walleye in March had greater LSI, GSI, and reproductive hormone (E_2 and 11-KT) levels, and unchanged condition factors relative to post-spawning in August. Pre-spawning female and male lake whitefish in August had lower LSI, greater GSI, and reproductive hormone (E_2 and 11-KT) levels, and unchanged condition factors relative to post-spawning in March. Fecundity remained constant throughout all pre-spawning periods for both walleye and lake whitefish. Plasma and gonadal steroids were highly correlated. Critical effects sizes (CES; ± 2 standard deviations of the means) were used to determine natural variability during the study and to set a baseline for future biomonitoring. Analysis of standardized measures of energy storage (CF, LSI) and energy use (GSI, fecundity) and reproductive steroids, taking into account normal variation due to reproductive cycling, indicate that on-going community biomonitoring using CF, LSI and GSI in March and August on a biennial (every-other-year) basis would be adequate until such a time as measured ranges exceed or deceed the calculated CES range due to environmental changes.

2.2 Introduction

A significant body of literature describes the effects of seasonality on fish reproduction and spawning (Dahle *et al.,* 2003; Pankhurst & Porter, 2003; Frick *et al.,* 2010; Schneider *et al.,* 2010; Wang *et al.,* 2010). The spawning season for large-bodied temperate fish varies widely in reproductive timing, i.e., the month(s) at which the spawning starts (pre-spawning) and ends (post-spawning). Variation in reproductive cycling is believed to reflect the optimization of a reproductive strategy for a given environment (Leggett & Carscadden, 1978**;** Plaza *et al.,* 2007). Reproductive cycling in fish is largely dependent on environmental conditions (De Vlaming, 1975; Quintana *et al.,* 2004), primarily those which favour larval growth and survival (Frick *et al.,* 2010). Population-level impacts have the potential to be more severe at latitudes where reproduction cycles are shorter and acutely phased with the season if reproduction is not synchronized with environmental conditions (Pankhurst & Porter, 2003). While the spawning season for many fish in temperate regions is the spring and summer, some species spawn in autumn and winter (De Vlaming, 1975; Dahle *et al*., 2003). For spring spawners, increasing spring water temperatures stimulate gonadal maturation, whereas, for autumn spawners, decreasing autumn water temperatures stimulate gonadal maturation (Shimizu, 2003). Spawning seasons can be indicated from changes in reproductive hormones such as 17β- estradiol (E_2), which controls ovarian development and the synthesis of vitellogenin in females, and 11-ketotestosterone (11-KT), which controls testis development in males (Dahle *et al.,* 2003). Spawning seasons can also be indicated directly by changes in gonad size and gonadosomatic index (GSI; relative gonad weight to body weight), and indirectly via liver size and liversomatic index (LSI; relative liver weight to body weight) and condition factor (CF; relative body weight to length) (Ghaffari *et al.,* 2011).

The most important environmental cues for reproductive cycling in temperate fish are photoperiod and temperature (De Vlaming, 1972; Kime, 1999; Pankhurst & Porter, 2003; Lester *et al.,* 2004; Barton, 2011). Photoperiod is the only variable capable of delivering an unambiguous 'date' signal (Kime, 1999), which is capable of phasing and entraining reproductive maturation. For spring spawning fish, under short photoperiods during late winter and spring, or for autumn spawning fish under long photoperiods, accelerated changes in light regime can stimulate the initiation of gonadal maturation altering spawning time for fish (De Vlaming, 1972). Ultimately, this can generate physiological changes in the endocrine system, which drive the reproductive process (Pankhurst & Porter, 2003).

Water temperature (via air temperature changes) acts as both a timing cue and a modulating factor for reproductive cycling (De Vlaming, 1972; Pankhurst & Porter, 2003). Changes in fish condition, gonadal maturation and steroidogenesis occur in preparation for spawning as a result of increased temperatures for spring spawners, or decreased water temperatures for autumn spawners (Pankhurst & Porter, 2003). However, when water temperatures are elevated for autumn spawners and decreased for spring spawners, fish may exhibit reduced responses, such as decreased plasma E_2 levels and 11-KT levels (Pankhurst & Porter, 2003). It is clear that the effects of small changes in temperature can generate endocrine changes (Pankhurst & Porter, 2003). Spring spawning stimulated by increases in temperature may also exhibit accelerated or delayed spawning if temperature is warmer or cooler, respectively. Whereas for autumn spawning stimulated by decrease in temperature, warm or cool autumn temperatures delay or advance respectively, the timing of spawning (Lester *et al.,* 2004; Schindler & Smol, 2006; Sharma *et al.,* 2007). Ultimately, shifts in environmental temperatures

have the potential to modify patterns of gametogenesis or the induction of gonadal regression (Van Der Kraak & Pankhurst, 1997).

Climate change, together with increasing human activities in polar regions, are dramatically altering the structure and function of northern ecosystems and the socioeconomic framework of northern communities (Schindler & Smol, 2006; Brook *et al*, 2009). Climate change has the potential to impact aquatic ecosystems by increasing air temperatures which can affect ecosystem productivity and fish biology (Sharma *et al.,* 2007; Rinne & Carter, 2008; Prowse *et al.,* 2009; Rieman & Clayton, 2011). Northern regions of Canada are especially vulnerable to large increases in air temperatures compared to other regions in North America (Sharma *et al.,* 2007). A climate-warming model purposed by Mackenzie-Grieve & Post (2006) predicts greater warming at increasingly northern latitudes. Stewart *et al.* (1998) reported that the Mackenzie River basin has already experienced increases in air temperatures up to 2°C per decade since the mid-1970s. As air temperatures increase, water temperatures are expected to increase; this change in water temperature has the potential to affect many aquatic species (Morrill *et al*., 2001; Sharma *et al.,* 2007). One reason for this is that as water temperatures increase DO content decreases (Morrill *et al*., 2001).

Baseline-monitoring information is useful for evaluating potential changes in freshwater ecosystems over time (Lindenmayer & Likens, 2009). Many ecotoxicological studies have employed Canada's federal environmental effects monitoring (EEM) program guidelines to assist in detecting changes in aquatic ecosystems, including differences between exposed and reference sites (Mills & Chichester, 2005) and changes over time (McMaster *et al.,* 1991; McMaster *et al.,* 2005; Tetreault *et al.,* 2011; Bowron *et al*., 2009). EEM studies commonly employ CES critical effect size as a predictive tool to assess natural variability and future

cumulative environmental changes (Environmental Canada, 2010). When endpoint values fluctuate below or above CES an environmental pressure could be indicated and trigger further investigation into what is causing the impact.

Hormone data have been increasingly used in biomonitoring studies and to augment the standard EEM endpoints as indicators of reproductive impairment or alteration in function (McMaster *et al*., 2001). Because of challenges related to maintaining sample integrity under field and shipping conditions, and differences in standardization of laboratory hormone assays, it is important to assess factors that could affect data interpretation and limit the value of measuring hormone levels (McMaster *et al*., 2001; Feswick *et al*., 2014). A study by McMaster *et al*. (2001) found that steroid levels examined among a number of laboratories were capable of identifying site differences in steroid hormone levels, although absolute values reported varied among laboratories. Therefore, reported steroid concentrations must be appropriately assessed for reliability if there are known factors which could affect interpretation.

Tathlina Lake, Northwest Territories (NT; Figure 1), is an ecologically, culturally and economically important lake to Ka'a'gee Tu First Nation (KTFN), due to the presence of walleye (*Sander vitreus*) and lake whitefish (*Coregonus clupeaformis*; Kennedy, 1962). In the winter of 1953/1954 commercial fishing of walleye began and has continued to provide important economic benefits for residents from the nearby community of Kakisa (Gallagher *et al.,* 2011). However, there have been multiple, large-scale declines in the walleye populations, which in some years led to the closure of the fishery (Gallagher *et al.,* 2011). Despite phylogenetic and trophic differences in walleye and lake whitefish, both share similar reproductive ecologies; i.e., they are broadcast spawners with well-defined seasonal reproductive cycles. Walleye spawn annually in early to mid-April (Malison *et al.,* 1994; Malison & Held, 1996), while lake

whitefish spawn in autumn or early winter, exhibiting seasonal changes in gonadal condition and sex hormone levels which depend on temperature and photoperiod (Crossman & Scott, 1998; Rinchard *et al.,* 2001).

The objective of this study is to create a baseline dataset that characterizes seasonal variations in condition and spawning of walleye and lake whitefish in Tathlina Lake. Tathlina Lake, the 15th largest lake in the NT, may be particularly susceptible to climate change due to its shallow depth (average depth of only 1.5 - 1.8 m) and prospective natural resource development in the region (Ka'a'Gee Tu First Nation, 2002; Gallagher *et al.,* 2011). To assess wild male and female fish health during pre- and post-spawning periods, endpoints from the Canadian federal EEM program were used and include CF, LSI, GSI, and age, enhanced by fecundity and plasma and gonadal hormone (E_2 and 11-KT) levels. Intra-laboratory variability was determined for plasma hormone levels, which were measured in previously-analyzed samples from earlier field expeditions. Given the challenges of blood sampling in remote regions, gonadal E_2 and 11-KT measurements were assessed as a potential surrogate for plasma levels. The seasonal patterns and degree of variability in walleye and lake whitefish reproductive biology determined in this study provide a baseline against which future studies can assess variation over time due to climate change or anthropogenic activities.

2.3 Materials and Methods

2.3.1 Water Quality

To measure DO and temperature of the water, MiniDOT Loggers (Precision Measurement Engineering Inc., Vista, CA) were deployed from December 2014 to April 2015 at five sites (Figure 1); three in Tathlina Lake (Tathlina Lake 1 (T1): 60°47'59.57"N, -117°93'16.47"W; Tathlina Lake 2 (T2): 60°47'59.57"N, -117°93'16.47"W; Middle Tathlina (MT): 60°51'59.50"N, -117°43'92.96"W) and two in tributaries feeding Tathlina Lake (Upper

Kakisa River (UK): 60°46’06.13”N, -118°08’91.26”W; West Cameron River (WC): 60°43’34.57”N, -117°95’43.07”W). Air temperature data (2014 and 2015) were collected by the Government of Canada’s Hay River weather station 144 km away from Tathlina Lake (60°50’20.00 ”N, -115°46’36.00 ”W) and publicly reported at http://climate.weather.gc.ca/. Photoperiod data (2014 and 2015) are a computed variable using sunrise and sunset data from the local government-owned weather stations (Government of Canada’s Hay River weather station), as calculated by taking into consideration position of earth at the time and location (http://www.timeanddate.com/astronomy/canada/hay-river).

2.3.2 Tathlina Lake fish collection

Walleye and lake whitefish were collected at four locations from Tathlina Lake (Figure 1: F1: 60.47°N 117.93°W, F2: 60.47°N -117.93°W, F3: 60.28°N 117.57°W, F4: 60.28°N 118.00°W) at multiple sampling times (December 2012, March 2013, June 2013, August 2013, March 2014, August 2014, March 2015, August 2015 and March 2016). These sample periods were chosen based on consultations with the KTFN and personnel from Fisheries and Oceans Canada (DFO) and the Government of the Northwest Territories (GNWT) while taking into consideration the pre- and post-spawning periods of the fish. In December 2012, walleye and lake whitefish were collected via index nets with a mesh size of 10.8 cm during a DFO stock assessment. During the other sampling periods, walleye and lake whitefish were collected via gillnets with a mesh size of 10.2 cm. For summer sampling, live fish were removed from the nets and placed in aerated buckets containing lake water and transported to the shore for sampling. In winter, fish were sampled at the site of fishing.

2.3.3 Morphometric endpoints

Following the collection and bleeding of walleye and lake whitefish, the fish were killed by a sharp blow to the head, and were measured and weighed to obtain fork length (to 0.1 cm)

and body weight (to 0.01g). Gonads and livers were removed and weighed to obtain liver weight and gonad weight (to 0.001g). The excised gonads and livers were frozen and shipped on dry ice to Wilfrid Laurier University (Laurier) in Waterloo, ON, where they were stored at -80°C until further analyses. To assess general fish health, morphometric endpoints of gonadosomatic, liversomatic, and condition factor were calculated. Gonad weight relative to body weight was calculated and expressed as gonadosomatic index (GSI; gonad weight/(body weight-gonad weight) x 100); liver weight relative to body weight was expressed as liversomatic index (LSI; liver weight/ (body weight-liver weight) x 100); and condition factor as body weight relative to body length (CF; 10^5 x body weight/fork length^3).

2.3.4 Chemicals and supplies

All materials were purchased from Sigma-Aldrich (Ottawa, ON) or as otherwise described.

2.3.5 Reproductive endocrine assessment

2.3.5.1 Plasma steroid extractions

The fish were bled from the caudal vasculature using heparinized 26 3/8 gauge needles on 3 mL syringes. The blood was frozen and shipped on dry ice to Laurier and stored at -80°C until further analyses. All blood samples were frozen without centrifugation and plasma collection due to the difficulty in preventing blood samples from freezing during winter sample periods. Blood samples were thawed on ice and 1 mL of blood for each sample was placed in 1.5 mL microcentrifuge tubes and centrifuged at 3000 X *g* for 10 min. Plasma (500 µL) was removed and triple ethyl-ether extracted (MacLatchy *et al.,* 2002; Gillio Meina *et al.,* 2013). The samples were dried overnight at room temperature and then reconstituted in 500 µL of enzyme immunoassay (EIA) buffer (Caymen Chemical, Ann Arbor, MI) and stored at -80°C.

2.3.5.2 Gonadal steroid extraction

Gonadal tissue was used to compare gonadal steroid levels to circulating levels of plasma E_2 and 11-KT. The steroid extraction from gonadal tissue was based on Lister & Van Der Kraak, (2009). Variations to the protocol were implemented as follows. To begin, 20 ± 2 mg of ovaries or testes were placed in 1.5 mL microcentrifuge tubes and 100 µL of pH 7.4 homogenizing buffer (Na_2HPO_4 [80mM], NaH_2PO_4 [20mM], NaCl [100mM], and ethylenediaminetetraacetic acid [1mM]). The samples were sonicated using a QSonica125 (QSonica, Newtown, CT) set at 40 Hz. Each sample was sonicated for a total of 10 s on ice. Following sonication, the homogenates underwent triple methanol extraction as per Lister & Van Der Kraak, (2009); each homogenate was treated with methanol (400 µL) and incubated at 4°C for 1 h and vortexed every 15 min during the incubation. After 1 h, homogenates were centrifuged at 3000 X *g* for 10 min at 4°C. The pellet was snap frozen on dry ice and the supernatant was decanted into 7 mL glass scintillation vials. This procedure was repeated two more times with the thawed pellets with the addition of 400 µL of methanol, which was subsequently vortexed such that the pellet was disrupted, and a shorter incubation period (30 min) was used. The methanol fractions from all three extractions were combined into the same vial and were stored at -80°C until ready to be dried under a stream of nitrogen gas.

The samples were reconstituted in 500 µL of pH 4.0 acetate buffer, glacial acetic acid and sodium acetate trihydrate [50mM]) and were passed through C_{18} octadecyl solid phase extraction (SPE) columns (Cleanert S C_{18}-N, 100mg, Agela Technologies, Wilmington, DE). The columns were primed as per the manufacturer's instructions, which involved a pre-wash with 1 mL each of methanol and then acetate buffer, and then the entire sample was added to the column. The columns were then washed with 1 mL each of acetate buffer and hexane. The samples were eluted with 2 mL ethyl acetate (1% methanol). A Visiprep SPE vacuum manifold was used for

the SPE procedures. The eluted sample was dried with nitrogen gas and then reconstituted in 250 µL of EIA buffer and stored at -80°C.

2.3.5.3 Enzyme immunoassay (EIA)

EIAs as per manufacturer's instructions (Cayman Chemical) were used to quantify 11-KT and E_2 levels in gonadal tissue and plasma samples. EIAs on pooled samples were conducted to determine concentration range and parallelism of steroids of extracted plasma and tissue samples, and to indicate the dilution series to use and that the samples lacked compounds that interfered in the assay. The interassay variability was 11.3% (n= 6) and 10.1% (n=7) [(standard deviation of samples/mean of samples) x 100] for plasma E_2 and 11-KT, respectively. The interassay variability was 11.9% (n= 7) and 6.9% (n=6) for gonadal E_2 and 11-KT, respectively. Validation of EIA for plasma and gonadal tissue extractions was performed as shown in Appendix 1A. All samples were diluted based on parallelism and concentration range. The samples were read at a wavelength of 420 nm using a Molecular Device SpectramaxPlus 384 microplate reader (Molecular Devices, Sunnyvale, CA).

2.3.5.4 Intra-laboratory variability in hormone levels

To examine intra-laboratory variability in plasma hormone measurements among different personnel over time, previously analyzed female and male walleye blood samples (stored at -80^{o}C) were reanalyzed for plasma E_2 and 11-KT levels, respectively, using the described methods. For females, ten samples each from December 2012, March 2013, and March 2014 sampling periods were reanalyzed. For males, ten samples each from December 2012, March 2013, June 2013, and March 2014 were reanalyzed.

2.3.6 Potential fecundity

Potential fecundity, the number of oocytes in the ovary prior to spawning, was estimated using the gravimetric method, in which fecundity is the product of the number of oocytes per gram of ovary tissue and the weight of the ovary in grams (Thorsen & Kjesbu, 2001; Fernandez *et al*., 2009). Potential fecundity was estimated for walleye collected during March 2014, March 2015, and March 2016. For lake whitefish, potential fecundity was estimated for fish collected during the August 2014 and August 2015 sampling periods. Ovarian samples were sectioned to obtain a sub-sample (2.2 ± 0.3 g) and placed in ultrapure water to thaw. The oocytes sat in ultrapure water for 4 h to aid in the separation of oocytes from connective tissue to generate a distribution pattern conducive to enumeration and measurement (Kjesbu & Holm, 1994; Chavarie *et al*., 2016). The oocytes were drained into a sieve and then placed in a plastic container, which was flooded with 10% formalin (Fisher Scientific, Ottawa) for one day until the oocytes separated from the connective tissue, followed by an additional day for fixation prior to processing. Once ready to process, connective tissue and formalin were filtered and removed, and the oocytes rinsed with water. This allowed the connective tissues to float and the oocytes to sink. The remaining water was poured off and the total number of oocytes in the sub-sample was counted with the aid of a binocular microscope (Chavarie *et al*., 2016). The number of eggs in each ovary was calculated and number of eggs in the sub-sample was multiplied by total ovary weight divided by sub-sample weight (Kjesbu & Holm, 1994). The number of eggs from each ovary sub-sample was estimated as potential fecundity for the fish.

2.3.7 Oocyte diameter

Ovary sub-samples (100 oocytes) were withdrawn from each sample using disposable Pasteur pipettes and ejected onto thin Plexiglas with ultrapure water. This aided in the separation

of oocytes to generate an even distribution of oocytes for measurement. A Leica M165 FC microscope with DMC 2900 video camera (Leica Microsystems Ltd., Richmond Hill, ON) was used to capture images of each ovarian sub-sample, using light from underneath and full aperture opening at a magnification of 7.3x.

Oocyte size measurements were performed using Sigmascan Pro 5.0 (IBM, Markham, ON), and algorithms were recorded as macros (Appendix 1B) to automate the process (Sigmascan Pro 5.0 User Manual, IBM, Markham, ON; Lukas *et al*., 2009). The image was modified and converted to gray scale and a threshold process was used to define the area of interest, the oocyte. Once the oocytes were counted, the following variables were measured: area, perimeter (length of the line drawn around the particle), shape factor (defined as perimeter^2/4 x 3.14 x area) and diameter (perimeter/3.14). For shape factor, a value of exactly one measures that the object is perfectly round, while greater and lesser values show that the particle is less round. After 100 oocytes were measured, the data were examined in order to eliminate particles that were not considered to be individual oocytes. This was done by filtering data based on shape, area and diameter threshold ranges that were estimated to be valid oocytes (Thorsen & Kjesbu, 2001). Shape threshold was set from 0.8-1.2, and area was set from 3000-10 000, which effectively removed unwanted particles. Oocyte diameter ranges were set to from 200-1900 μm to eliminate immature and hydrated oocytes based on knowledge of vitellogenic oocytes of these species (Malison *et al.,* 1994; Thorsen & Kjesbu, 2001).

2.3.8 Age determination

Otolith sections were used for determining ages of walleye and lake whitefish from Tathlina Lake. Otoliths were prepared, sectioned and aged by AAE Tech Services Inc. (La Salle, MB) according to the methods of Zhu *et al*., (2015). Otoliths were removed and placed into a storage envelope until inspected for age estimation via the crack and burn method (Zhu *et al*.,

2015). The otoliths were removed from the packages and cleaned to remove any extraneous tissue. Each otolith was embedded in an individual block of epoxy resin (ColdCure, Lee Valley Tools Ltd, Winnipeg, MB). The embedded otolith was cured as per manufacturer's specifications (ColdCure, Lee Valley Tools Ltd) and later sectioned using a low-speed saw to obtain one dorsal-ventral section. The end of the otoliths were polished then burnt to correct levels in an aluminum foil pan. Age estimations were achieved using a microscope (Lecia M60; Lecia Microsystems Ltd) with minimum 40x magnification and maximum 80x magnification.

2.8.9 Statistics

Statistical analyses were performed using SPSS v. 21 (IBM, Markham, ON) and differences were considered significant if $p < 0.05$. Analyses of fish data were conducted with species, sexes, and months separated. Analysis of covariance (ANCOVA) was used to analyze gonad and liver weight relative to body weight and body weight relative to body length. Analysis of variance (ANOVA) was used to analyze plasma and gonadal levels of E_2 and 11-KT and also age, fork length, body weight, liver weight and gonad weight, followed by Tukey's post-hoc analysis. An ANOVA was used to analyze plasma hormone levels to analyze differences in archived samples compared to re-analyzed samples, followed by Tukey's post-hoc analysis. A reduced major axis regression was carried out to determine the relationship between plasma versus gonadal tissue to assess the level of correlation between plasma (log-transformed) and gonadal steroid levels (log-transformed). Differences across time periods for potential fecundity were carried out by ANOVA and if no significant differences were found, the years were pooled and regression analyses done to compare fecundity vs weight, fecundity vs oocyte diameter, fecundity vs age, and age vs hormone level. Critical effects sizes (CES) were calculated as two standard deviations from the mean of pre-spawning and post-spawning periods, keeping the

spawning periods separate. Data were tested for normality using Shapiro-Wilk's W test, and homogeneity of variance using Levene's test ($p < 0.05$). If the data failed the normality test, they were log transformed (E_2 versus age and 11-KT versus age) to allow for a parametric comparison. A Tukey's post-hoc analysis was used in all pairwise multiple comparisons. An alternate non-parametric test (Kruskal-Wallis test) was used if parametric tests did not meet the required assumptions.

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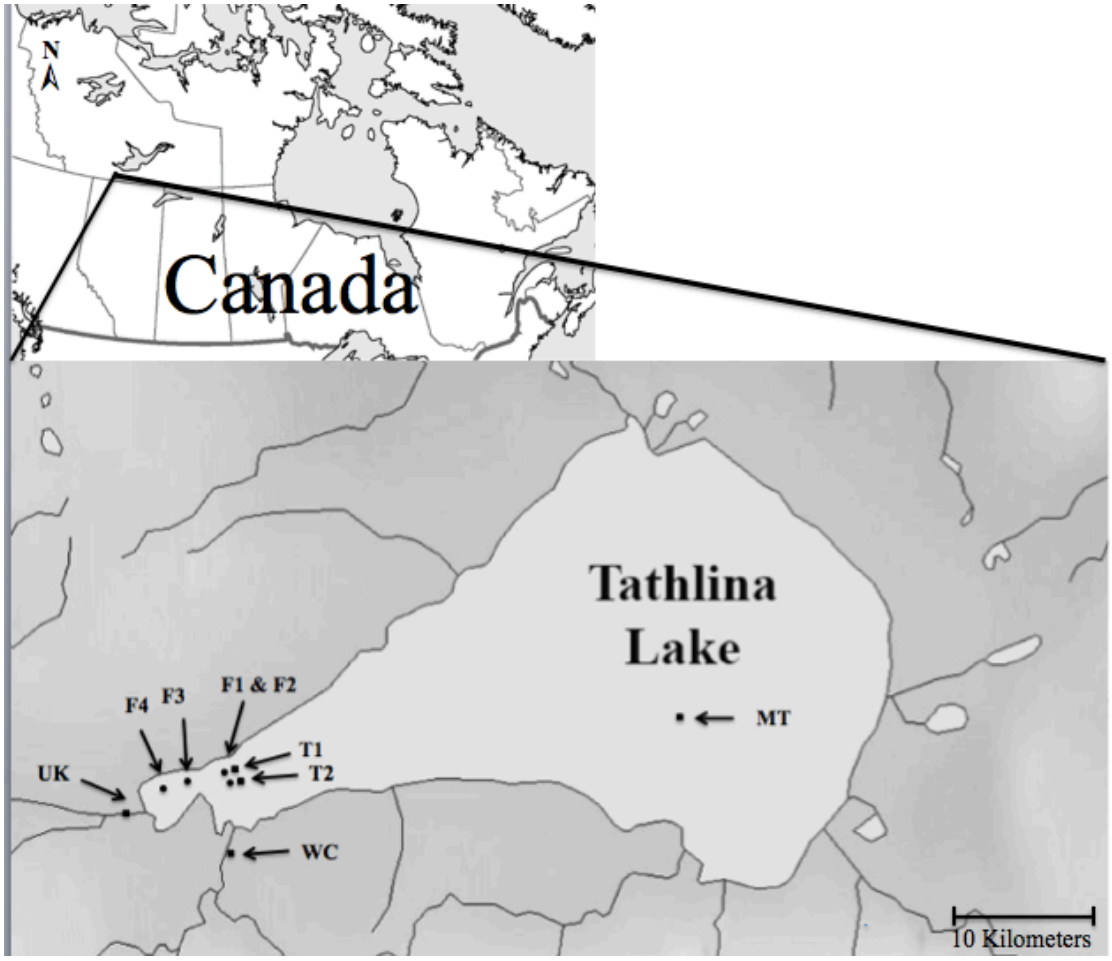

Figure 1. A map of Tathlina Lake, NT, indicating approximate fish collection sites (circles), where lake whitefish and walleye were collected in sampling periods (Fishing locations: F1, F2, F3 and F4). MiniDot loggers were placed in Tathlina Lake, NT (squares; Tathlina 1 (T1), Tathlina 2 (T2), Middle Tathlina (MT) and in two tributaries feeding Tathlina Lake (Upper Kakisa River (UK) and West Cameron River (WC)).

2.4 Results

2.4.1 Seasonal environmental conditions

Water temperature data were collected during under-ice conditions from early December 2014 to early April 2015 (Figure 2A). Water temperature ranged from 1.4°C to 2.6°C in Tathlina Lake (T1, T2, MT) and ranged from 0.1°C to 0.3°C in Kakisa River (UK) and 0.6°C to 1.1°C in the west Cameron River (WC). Dissolved oxygen (DO) concentrations (Figure 2B) in Tathlina Lake remained between 0.8 mg/L and 13.3 mg/L (T1, T2, MT) from early December 2014 to early April 2015. DO concentrations at UK remained between 4.5 mg/L to 8.6 mg/L, and were 3.7 mg/L to 8.4 mg/L at WC.

In early January 2014 to late July, air temperature was increasing from -27.9 °C to 21.6°C (Figure 3A), with light duration increasing from 5.9 h light/d to 19.1 h light/d (Figure 3B). From late July to late December, air temperature and photoperiod started declining rapidly to winter minima, from 21.6°C to -24.9°C (Figure 3A), and 19.1 h light/d to 5.7 h light/d (Figure 3B). A comparable trend was observed in 2015, in early January 2015 to late July, temperatures increased from -23.1°C to 19.3°C (Figure 3A). Similarly, after late July to late December, air temperature started decreasing rapidly to winter minima, from 19.3°C to -21.6°C (Figure 3A).

2.4.2 Morphometric endpoints in walleye

2.4.2.1 *Female and male walleye age, body weight, length, and CF*

Female walleye collected in August 2015 were the oldest (17.0 ± 1 years old; ANOVA; $p<0.001$; Table 1) compared to August 2015, and March 2016 and were larger by mass compared to younger fish (ANOVA; $p<0.017$; Table 1). Male walleye exhibited similar ages across analyzed months and exhibited no significant differences in age over March 2014, August 2015, and March 2016 (ANOVA; $p=0.327$; Table 1).

Female walleye body weight exhibited significant differences across sampling periods (ANOVA; p=0.0001; Table 1). Body weight did not differ significantly in pre-spawning periods (March 2013-2016), while body weight only differed significantly in August 2015, compared to other post-spawning periods (August 2013, August 2014). Female walleye fork length differed across the sampling periods (ANOVA; p=0.003; Table 1) with significant differences consistently observed in March to August comparisons over different years.

Male walleye body weight exhibited significant differences across sampling periods (ANOVA; p=0.035; Table 1) even though significant differences were not observed consistently in the March to August comparisons over different years. Male walleye fork length differed across the sampling periods (ANOVA; p=0.020; Table 1) even though no significant differences were consistently observed in March to August comparisons over different years.

Female walleye CF (body weight relative to body length) was significantly different across sampling times (ANCOVA; p=0.0013; Table 1) even though there were no significant differences consistently observed in March to August comparisons over different years. Male walleye also exhibited significant differences in CF across sampling times (ANCOVA; p=0.001; Table 1), similar to what was seen in females, showing no consistently observed differences in March to August comparisons over the sampling periods.

2.4.2.1 ***Female and male walleye organ weights, LSI and GSI***

Female walleye liver weight exhibited significant differences across sampling periods (ANOVA; p=0.002; Table 1). Similar to females, male walleye liver weight differed significantly across sampling periods (ANOVA; p=0.012; Table 1). Liver weights of both sexes remained unchanged when examined within either the pre- or the post-spawning sample periods.

For female walleye, LSI exhibited significant differences in liver weight relative to body weight across sampling periods (ANCOVA; p=0.002; Table 1). Similar to females, male walleye also showed significant differences in LSI across sampling periods (ANCOVA; p=0.004; Table 1). For both female and male walleye, significant differences were observed across sampling periods, with significant differences consistently observed in March to August comparisons over different years.

Female walleye gonad weight exhibited significant differences across sampling periods (ANOVA; p=0.002; Table 1) with significant differences observed consistently in March to August comparisons over different years. Male walleye also exhibited significant differences across sampling periods (ANOVA; p=0.106; Table 1), but there were no significant differences observed in March to August comparisons over different sampling years.

For GSI, female walleye exhibited significant differences in gonad weight relative to body weight across sampling periods (ANCOVA; p=0.004; Table 1) with significant differences observed consistently in March to August comparisons over different sampling periods. For males, GSI exhibited significant differences in gonad weight relative to body weight across sampling periods (ANCOVA; p=0.014; Table 1) even though significant differences were not observed in March to August comparisons over different sampling years.

2.4.3 Walleye reproductive hormone levels

2.4.3.1 *Intra-laboratory variability in hormone levels of archived vs re-analyzed samples*

For the three sample periods examined for plasma E2 there were no significant differences between the mean re-analyzed data concentrations compared to the mean archived data values (Figure 5A; December 2012, p=0.993; March 2013, p=0.938; March 2014, p=0.999). There were significant differences consistently detected between March 2013 and

both December 2012 and March 2014 whether for the archived or re-analyzed samples (ANOVA; p=0.015; Figures 5A). In the re-analysis of 11-KT levels (Figure 5B) there was a significant difference within one sample period (June 2013) in comparison to the archived data (ANOVA, p=0.006; Figure 5B); the other sample periods did not demonstrate significant differences between archived and re-analyzed data (December 2012, p=0.995; March 2013, p=0.476; March 2014, p=0.960). The general pattern among months was retained within archived and re-analyzed groups (March 2014≥March 2013≥December 2012>June 2013; ANOVA, p=0.006). Overall, the intra-laboratory differences in samples analyses was determined to be minimal irrespective of when the samples were analyzed or the age of the samples. Therefore, data are presented over the full study as combined archived (December 2012, March 2013, June 2013, August 2013, and March 2014) and current analysis (August 2014, March 2015, August 2015, and March 2016) data. The sample sizes within the sampling periods represent all the samples available from the field collections.

2.4.3.2 ***Plasma and gonadal hormone levels***

As expected, plasma E2 levels in females differed across the sample periods (ANOVA; p=0.005; Figure 6A) even though significant differences were not observed consistently in the March to August comparisons over different years. Males also showed differences in plasma 11-KT levels (ANOVA; p=0.001; Figure 6B) and showed the highest levels of steroids in March and the lowest levels in August.

Ovarian E_2 levels in female walleye sampled in August 2014, March 2015, and August 2015 were significantly different (ANOVA; p=0.013; Figure 7A) with the same statistical trend observed between ovarian tissue E_2 levels compared with plasma E_2 levels of the same fish.

There was a strong linear correlation between plasma and ovarian concentrations of E_2 (ANOVA; p=0.002; r=0.861; data not shown).

Testis 11-KT levels in male walleye samples in August 2014, March 2015, and August 2015 were significantly different (ANOVA; p=0.009; Figure 7B) with the same statistical trend observed between testis tissue 11-KT levels compared with plasma 11-KT levels of the same fish. There was a linear correlation between plasma and gonadal concentrations of 11-KT (ANOVA; p=0.014; r=0.596; data not shown) although the correlation was not as high as in females.

Plasma levels of E_2 were not correlated with age of female walleye (ANOVA; p=0.903; r=0.019; data not shown). Additionally, plasma levels of 11-KT were not correlated with age of male walleye (ANOVA; p=0.014; r=0.302; data not shown).

2.4.4 Potential fecundity of female walleye

Mean potential fecundity and egg size remained constant over sampling periods of March 2014, March 2015 and March 2016. Egg production was estimated for 54 walleye samples and the number of eggs increased with length (ANOVA, p=0.269; r= 0.424; data not shown) and body weight (ANOVA, p=0.15; r= 0.894; Figure 8) of the fish. In 2014, 2015, and 2016, the numbers of estimated oocytes averaged 34 084 ± 1232, 36 298 ± 1261, and 32 816 ± 1361, respectively. The examination of egg size over sampling periods (March 2014, 2015 and 2016) exhibited no significant differences, but egg size increased with potential fecundity (ANOVA, p=0.882; r= 0.01; data not shown). Egg sizes ranged from 1796 ±17.3 µm, 1656± 9.0 µm and 1691± 8.1 µm in March 2014, 2015, and 2016, respectively. Neither egg size nor GSI changed prior to spawning for walleye. The correlation between egg size versus female GSI was not

significantly different over March 2014, 2015 and 2016 (ANOVA; p=0.245; r=0.160; data not shown).

2.4.5 Morphometric endpoints in lake whitefish

2.4.5.1 Female and male lake whitefish body weight, length, and CF

Female lake whitefish body weight exhibited no significant differences across sampling periods (ANOVA; p=0.221; Table 2). Female lake whitefish fork length exhibited no significant differences across sampling periods (ANOVA; p=0.221; Table 2).

Male lake whitefish body weight exhibited no significant differences across sampling periods (ANOVA; p=0.242; Table 2). Male lake whitefish fork length exhibited significant differences across sampling periods (ANOVA; p=0.008; Table 2) even though significant differences were not observed consistently in the August to March comparisons over different years.

Female lake whitefish, CF (body weight relative to body length) exhibited significant differences across sampling periods (ANCOVA; p=0.0013; Table 2) even though no significant differences were consistently observed in August to March comparisons over sampling years. Similar to female lake whitefish, male lake whitefish exhibited significant differences in CF across sampling years (ANCOVA; p=0.001; Table 2) even though no significant differences were consistently observed in August to March comparisons over sampling years.

2.4.5.2 Female and male lake whitefish organ weight, LSI and GSI

Female lake whitefish liver weight exhibited significant differences across sampling periods (ANOVA; p=0.005; Table 2). Similar to females, male lake whitefish liver weight exhibited significant differences across sampling periods (ANOVA; p=0.001; Table 2). Liver

weights remained unchanged when examined within either the pre- or the post-spawning sample periods.

For female lake whitefish, LSI exhibited significant differences in liver weight to body weight across sampling periods (ANCOVA; p=0.001; Table 2), where LSI was significantly higher in March (post-spawning) when compared to August (pre-spawning). Similar to females, male lake whitefish LSI exhibited significant differences in liver weight to body weight across sampling periods (ANCOVA; p=0.012; Table 2) even though no significant differences were consistently observed in August to March comparisons over different years.

Female lake whitefish gonad weight exhibited significant differences across sampling periods (ANOVA; p<0.001; Table 2) with significant differences observed consistently in March to August comparisons over different years. Male lake whitefish gonad weight exhibited significant differences across sampling periods (ANOVA; p=0.004; Table 2) with significant differences observed consistently in August to March comparisons over different years.

For female lake whitefish, GSI exhibited significant differences in gonad weight relative to body weight across sampling periods (ANCOVA; p=0.001; Table 2). Similar to females, male lake whitefish exhibited significant differences in gonad weight to body weight (ANCOVA; p=0.022; Table 2). Both female and male lake whitefish consistently exhibited significant differences in August to March comparisons over different years.

2.4.6 Lake whitefish reproductive hormone levels

As expected, plasma E_2 levels in females differed across the sample periods (ANOVA; p=0.003; Figure 10A), with significant differences observed consistently in August to March comparisons over different years. Males also showed differences in plasma 11-KT levels

(ANOVA; p=0.002; Figure 10B) over the same time period and similarly to females, showed the highest levels of steroids in August and the lowest levels in March.

For gonadal tissue hormone levels, ovarian E_2 levels in female lake whitefish sampled in March 2015 and August 2015 were significantly different (ANOVA; p=0.003; Figure 11A) with the same statistical trend observed between ovarian tissue E_2 levels compared to plasma E_2 levels of the same fish. There was a strong linear correlation between plasma and ovarian concentrations of E_2 (ANOVA; p=0.025; r=0.879; data not shown). Testis 11-KT levels in male lake whitefish sampled March 2015 and August 2015 were significantly different (ANOVA; p=0.002; Figure 11B). There was a strong linear correlation between plasma and testes concentrations of 11-KT (ANOVA; p=0.001; r=0.596; data not shown), although the correlation was not as high as in females.

2.4.7 Potential fecundity of lake whitefish

Mean potential fecundity and egg size remained constant over sampling periods of August 2014 and August 2015. Egg production was estimated for 38 lake whitefish samples and the number of eggs increased with length (ANOVA, p=0.089; r= 0.454; data not shown) and body weight (ANOVA, p=0.859; r= 0.531; Figure 12) of the fish. In August 2014, and 2015, the number of estimated oocytes averaged 22 563.6 ± 2258, and 25 536 ± 2178, respectively. There were no significant differences in egg size across sampling periods, but egg size increased in potential fecundity (ANOVA, p=0.253; r= 0.196; data not shown). Egg sizes ranged from 1712.7 ± 101.3 μm in August 2014 and 1705.6 ± 36.7 μm in August 2015. Neither egg size nor GSI changed greatly in the months prior to spawning for lake whitefish. The correlation between egg

size versus female GSI was not significantly different over time (ANOVA; $p=0.301$; $r=0.177$; data not shown), suggesting that egg size was quite stable during the periods sampled.

Table 1. Mean age, body weight (g), fork length (mm), condition factor (CF), liver weight (g), gonad weight (g), liversomatic index (LSI) and gonadosomatic index (GSI) ± SEM for female and male walleye collected from Tathlina Lake at various sampling periods. All values were reported as mean ±SEM. Values showing different letters indicate statistically significant differences across time periods (ANOVA: age, body weight, fork length, liver weight and gonad weight; ANCOVA: CF, LSI, GSI).

Species	Time of Year	Sample Size (N)	Age	Body Weight (g)	Fork Length (mm)	CF (%)	Liver Weight (g)	Gonad Weight (g)	LSI (%)	GSI (%)
			p<0.001	p=0.0001	p=0.003	p=0.013	p=0.002	p=0.002	p=0.002	p=0.004
Female Walleye	2012 DEC	17		847.8 ±3.69^{ade}	418.1± 20.55^{a}	1.2±0.01^{a}	20.6 ± 0.96^{a}	59.8 ± 2.49^{a}	2.5±0.09^{a}	7.6±0.19^{a}
	2013 MARCH	11		834.5 ±15.07^{ade}	418.5 ± 3.26^{a}	1.1±0.02^{a}	19.4 ±0.99^{b}	84.4 ±3.36^{a}	2.4±0.12^{a}	11.2±0.38^{b}
	2013 JUNE	13		976.5 ± 107.89^{bde}	457.6 ± 15.45^{b}	1.0±0.03^{bc}				
	2013 AUG	17		1090.8 ± 60.07^{ce}	468.2 ± 8.05^{bc}	1.1±0.03^{ac}	16.2 ± 1.85^{b}	23.3 ± 1.79^{bc}	1.5±0.09^{b}	2.2±0.08^{c}
	2014 MARCH	16	10.1 ± 0.7^{a}	798.9 ± 22.63^{d}	415.1 ± 4.22^{a}	1.1±0.01^{a}	17.1 ± 0.68^{b}	85.4 ± 3.39^{a}	2.2±0.08^{a}	11.9±0.29^{b}
	2014 AUG	21		1025.4 ± 82.69^{e}	472.2 ± 9.47^{bc}	1.0±0.03^{c}	16.3 ± 2.16^{bc}	15.3 ± 1.39^{b}	1.5±0.12^{b}	1.5±0.08^{d}
	2015 MARCH	18		880.9 ± 39.98^{ade}	430.1 ± 5.62^{a}	1.1±0.02^{ad}	19.6 ± 0.89^{b}	68.1 ± 6.59^{a}	2.4±0.06^{a}	10.7±0.47^{b}
	2015 AUG	9	16.9 ± 1.0^{b}	1323.7 ± 107.49^{bc}	493.2 ± 16.48^{bc}	1.0±0.05^{ad}	25.9± 5.39^{ab}	32.7± 11.59^{c}	1.9±0.29^{ab}	1.7±0.13^{d}
	2016 MARCH	26	12.8 ± 0.6^{c}	866.7 ± 23.99^{ade}	427.4 ± 3.69^{a}	1.1±0.02^{a}	18.3 ± 1.09^{b}	85.2 ± 3.19^{a}	2.1±0.08^{a}	10.9±0.28^{b}
			p=0.327	p=0.035	p=0.020	p=0.001	p=0.012	p=0.106	p=0.004	p=0.014
Male Walleye	2012 DEC	24		857.3 ± 4.32^{a}	417.4 ± 46.55^{a}	1.1±0.01^{ab}	15 ± 1.01^{a}	15.5± 0.67^{a}	1.8±0.09^{a}	1.9±0.07^{a}
	2013 MARCH	23		859.8 ± 25.55^{abc}	424.9± 4.58^{ab}	1.1±0.02^{ab}	13.3± 0.78^{a}	17.8± 0.78^{a}	1.6±0.07^{ac}	2.1±0.08^{a}
	2013 JUNE	27		899.6 ± 38.51^{abc}	437.1 ± 5.42^{ab}	1.1±0.03^{ac}				
	2013 AUG	20		1014.3 ± 76.24^{b}	445.5 ± 11.23^{b}	1.1±0.03^{ab}	9.9 ± 0.55^{b}	20.1 ± 1.79^{a}	1.1±0.04^{b}	2.3±0.14^{ab}
	2014 MARCH	15	17.1 ± 0.7^{a}	801.7± 38.81^{abc}	452.3 ± 25.39^{ab}	1.1±0.02^{ac}	13.8± 0.76^{a}	17.9± 1.02^{a}	1.7±0.08^{a}	2.2±0.09^{ab}
	2014 AUG	10		917.2 ± 66.68^{abc}	453.1± 9.54^{b}	0.9±0.05^{c}	12.4 ± 1.09^{ab}	12.9 ± 1.67^{a}	1.4±0.15^{c}	1.6±0.28^{c}
	2015 MARCH	23		865.1 ± 21.91^{abc}	432.2 ± 4.37^{ab}	1.1±0.01^{ac}	14.9 ± 0.49^{a}	16.2 ± 0.8^{a}	1.8±0.05^{a}	1.9±0.07^{ab}
	2015 AUG	7	16.6 ± 1.1^{a}	911.7 ± 67.52^{abc}	433.1 ± 16.63^{ab}	1.1±0.08^{ab}	12.2 ± 3.09^{ab}	15.6 ± 2.19^{a}	1.3±0.25^{bc}	1.8±0.26^{abc}
	2016 MARCH	17	16.9 ± 0.6^{a}	828.2 ± 38.55^{c}	423.8 ± 7.67^{ab}	1.1±0.02^{ac}	13.3 ± 0.89^{ab}	15.9± 1.06^{a}	1.6±0.09^{ac}	1.9±0.09^{ab}

Table 2. Mean age, body weight (g), fork length (mm), condition factor (CF), liver weight (g), gonad weight (g), liversomatic index (LSI) and gonadosomatic index (GSI) ± SEM for female and male lake whitefish collected from Tathlina Lake at various sampling periods. All values were reported as mean ±SEM. Values showing different letters indicates statistically significant differences across time periods (ANOVA: age, body weight, fork length, liver weight and gonad weight; ANCOVA: CF, LSI, GSI).

Species	Time of Year	Sample Size (N)	Body Weight (g)	Fork Length (mm)	CF (%)	Liver Weight (g)	Gonad Weight (g)	LSI (%)	GSI (%)
			p=0.221	p=0.221	p=0.05	p=0.005	p=0.001	p=0.001	p=0.001
Female Lake Whitefish	2012 DEC	14	726.1 ±52.32^{a}	369.3 ± 7.51^{a}	1.4±0.02^{a}	13.3 ± 2.23^{ab}	8.9 ±1.15^{a}	1.8±0.18^{ab}	1.2±0.08^{a}
	2013 MARCH	14	826.8 ± 76.32^{a}	372.9 ± 9.32^{a}	1.6±0.03^{b}	18.3 ± 2.65^{ab}		2.2±0.26^{a}	
	2013 JUNE								
	2013 AUG	22	845.9±28.57^{a}	378.5 ± 4.58^{a}	1.6±0.02^{b}	13.1 ±0.63^{ab}	95.9±4.73^{b}	1.6±0.04^{bc}	12.6±0.37^{b}
	2014 MARCH	20	845.4 ± 78.13^{a}	389.8 ± 8.19^{a}	1.4±0.02^{ac}	16.2± 1.86^{ab}	8.9± 0.79^{a}	1.8±0.13^{ab}	1.1±0.08^{a}
	2014 AUG	17	835.9 ± 56.21^{a}	374.9 ± 7.47^{a}	1.5±0.03^{bc}	12.7 ± 0.99^{b}	69.1 ± 6.29^{b}	1.6±0.75^{bc}	10.2±0.55^{e}
	2015 MARCH	23	921.6 ± 81.25^{a}	393.9 ± 9.46^{a}	1.5±0.03^{a}	17.2 ± 1.89^{ab}	1.9 ± 0.19^{a}	2.0±0.14^{a}	1.0±0.07^{a}
	2015 AUG	23	1178.8± 277.29^{a}	383.8 ± 5.03^{a}	1.6±0.01^{b}	12.2± 0.63^{b}	93.7 ± 5.38^{b}	1.4±0.03^{cd}	11.7±0.02^{be}
	2016 MARCH	13	998.9 ± 102.96^{a}	395.4 ± 10.56^{a}	1.6±0.05^{ab}	19.7 ± 2.68^{ac}	11.3 ±2.25^{a}	1.9±0.12^{a}	1.1±0.14^{a}
			p=0.242	p=0.008	p=0.034	p=0.001	p=0.004	p=0.012	p=0.022
Male Lake Whitefish	2012 DEC	12	723.4 ± 42.42^{a}	364.8 ± 6.39^{a}	1.5±0.02^{ab}	12.9 ± 1.48^{a}	2.5±0.42^{a}	1.8±0.12^{a}	0.3±0.05^{a}
	2013 MARCH	16	909.9 ± 80.47^{a}	384.1 ± 10.02^{a}	1.5±0.03^{ab}	15.3± 1.55^{a}		1.7±0.17^{a}	
	2013 JUNE								
	2013 AUG	17	773.9 ± 26.22^{a}	379.2 ± 4.66^{a}	1.4±0.03^{ac}	5.3 ± 0.19^{b}	23.8 ± 0.95^{b}	0.7±0.03^{c}	3.2±0.14^{b}
	2014 MARCH	19	746.9 ± 68.42^{a}	389.9 ± 6.05^{a}	1.3±0.03^{c}	13.7 ± 1.24^{a}	1.9 ± 0.21^{a}	1.9±0.21^{a}	0.3±0.02^{a}
	2014 AUG	20	803.8 ± 52.19^{a}	377.4 ± 6.46^{b}	1.5±0.02^{ab}	6.9 ± 0.69^{b}	17.5 ± 1.19^{b}	0.9±0.03^{bc}	2.3±0.13^{c}
	2015 MARCH	16	860.9 ± 44.39^{a}	388.1 ± 6.75^{a}	1.5±0.03^{ab}	17.6 ± 1.42^{a}	8.9 ± 0.69^{a}	1.9±0.17^{a}	0.2±0.01^{a}
	2015 AUG	26	866.3 ± 65.13^{a}	391.9 ± 8.58^{a}	1.4±0.03^{ac}	6.5 ± 0.49^{b}	19.9 ± 1.39^{b}	0.8±0.04^{bc}	2.6±0.17^{d}
	2016 MARCH	15	830.9 ± 45.69^{a}	388.1 ± 5.12^{a}	1.4±0.04^{ac}	16.4 ± 1.59^{a}	2.5 ± 0.69^{a}	1.9±0.20^{a}	0.3±0.08^{a}

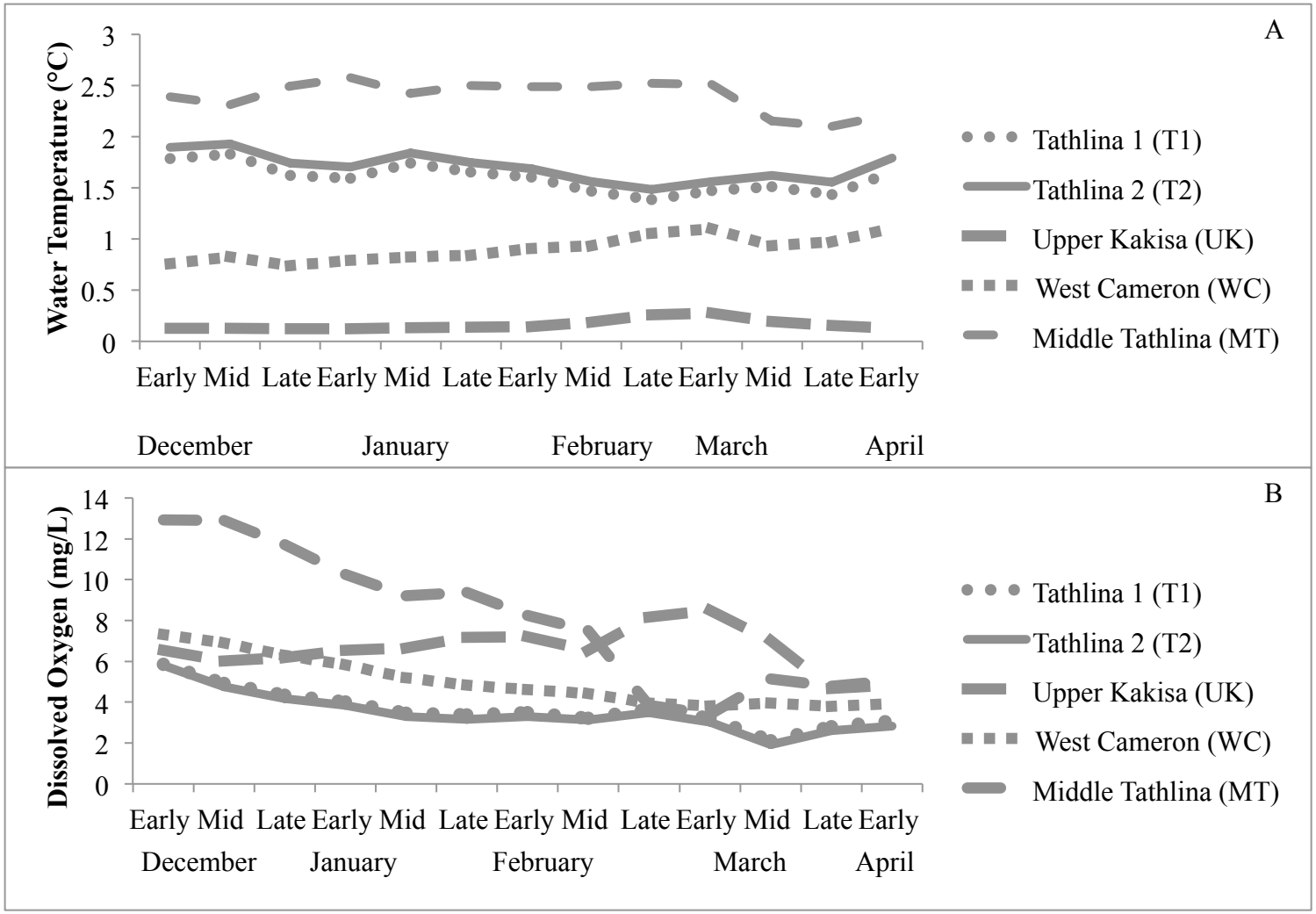

Figure 2. Annual variation in abiotic water parameters from December 2014 to April 2015. (A): Water temperature (°C) measurements collected by MiniDot loggers in Tathlina Lake, Upper Kakisa River, and West Cameron River. (B): Dissolved oxygen (mg/L) measurements collected by MiniDot loggers in Tathlina Lake, Upper Kakisa River, and West Cameron River. Data are presented as a 5-day average of photoperiod for three periods: Early (1^{st}-5^{th}), mid (12^{th}-17^{th}), and late (24^{th}-29^{th}).

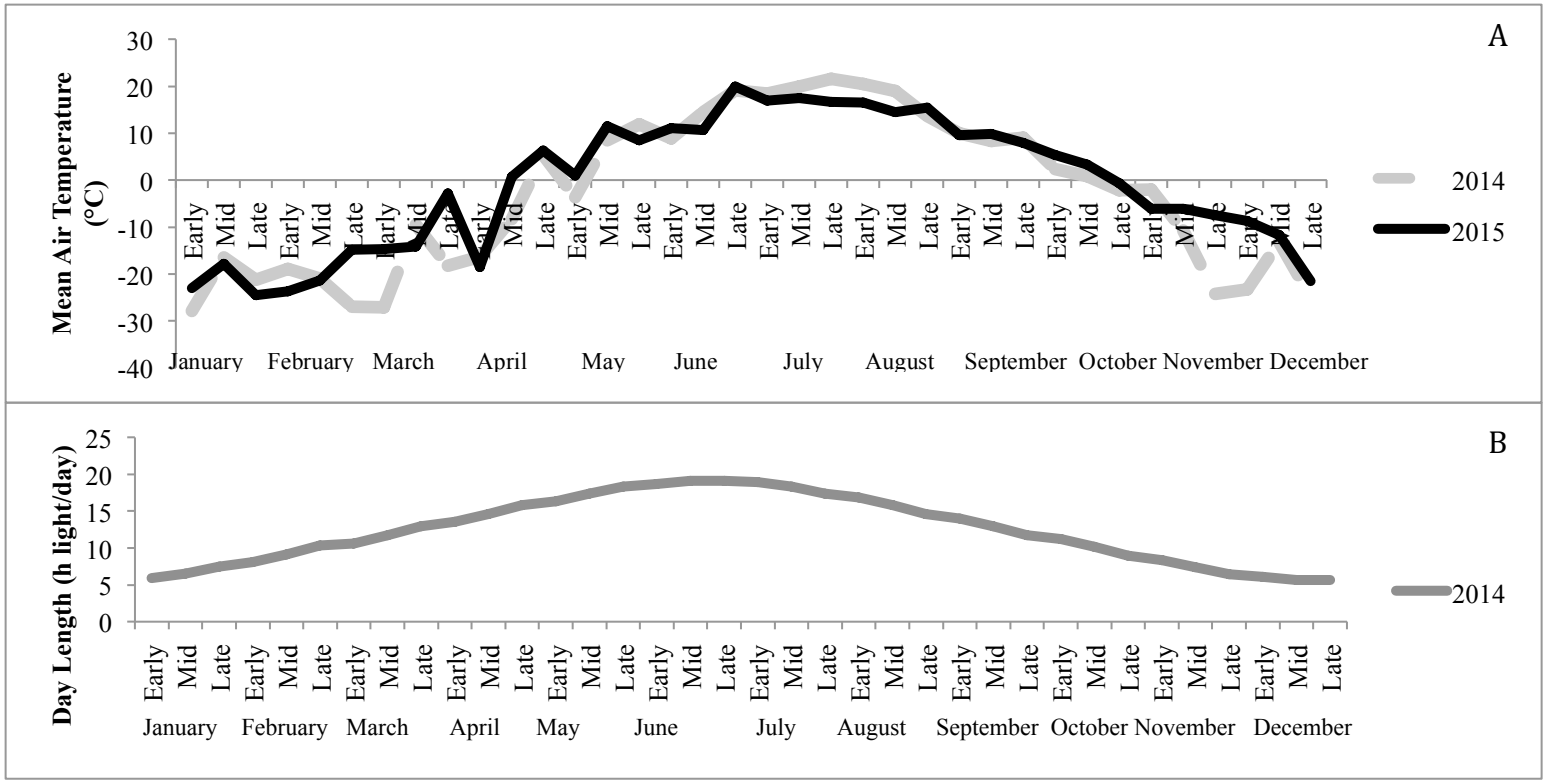

Figure 3. Air temperature and photoperiod data (2014) were collected by the Government of Canada's Hay River weather station 144 km away from Tathlina Lake (60°50'20.00 "N, -115°46'36.00 "W; http://climate.weather.gc.ca/). (A): Annual variation in air temperature (°C; http://climate.weather.gc.ca/). (B): Photoperiod (day length; h light/d; http://www.timeanddate.com/astronomy/canada/hay-river) 2014. Data are presented as a 5-day average of photoperiod for three periods: Early (1^{st}-5^{th}), mid (12^{th}-17^{th}), and late (24^{th}-29^{th}).

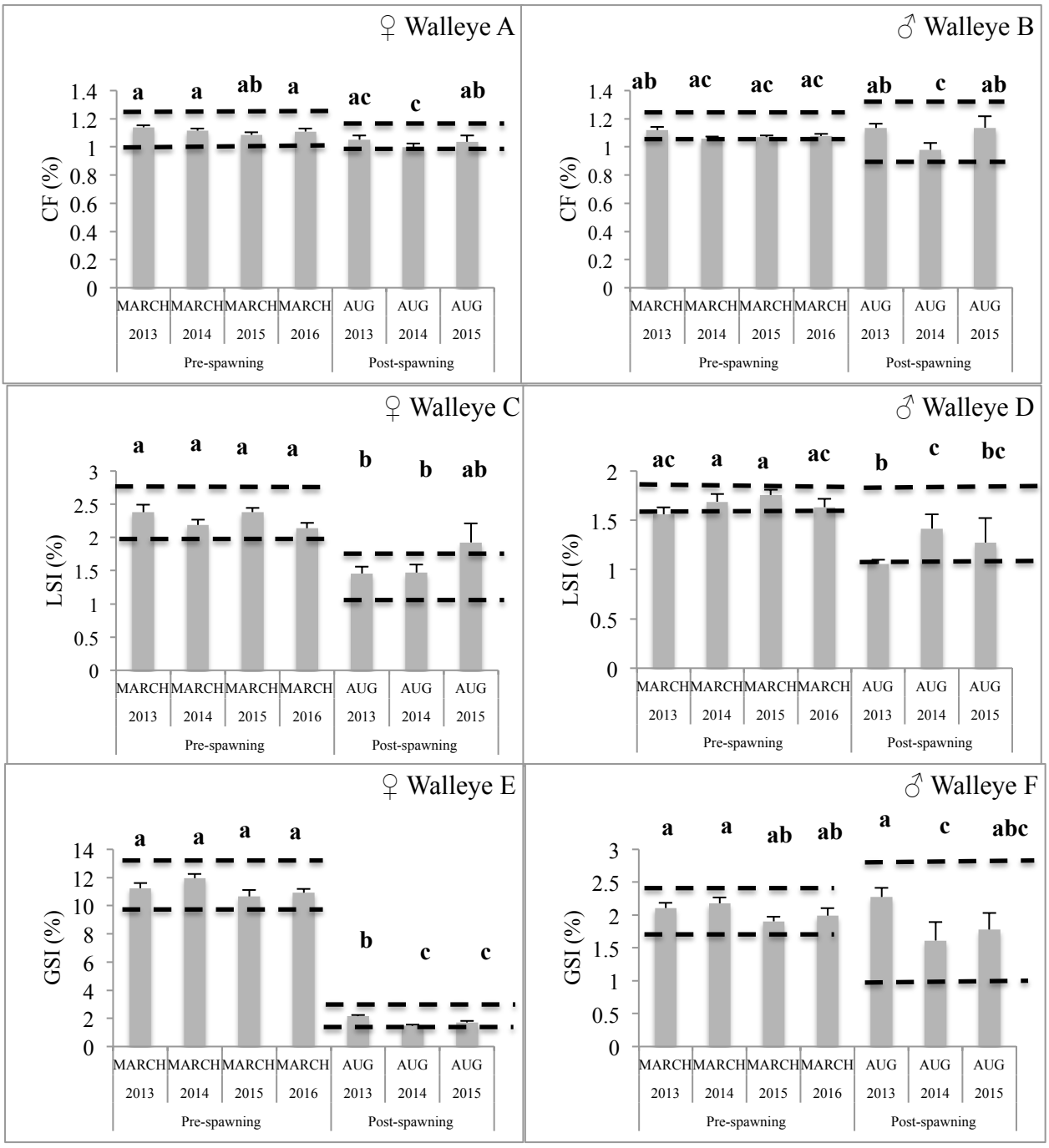

Figure 4. Mean condition factor (CF; A: male, B: female), liversomatic index (LSI; C: male, D: female) and gonadosomatic index (GSI; E: male, F: female) ± SEM for female and male walleye collected in Tathlina Lake at various sampling periods. Dotted lines indicate CES (+/- 2 standard deviations of the mean).

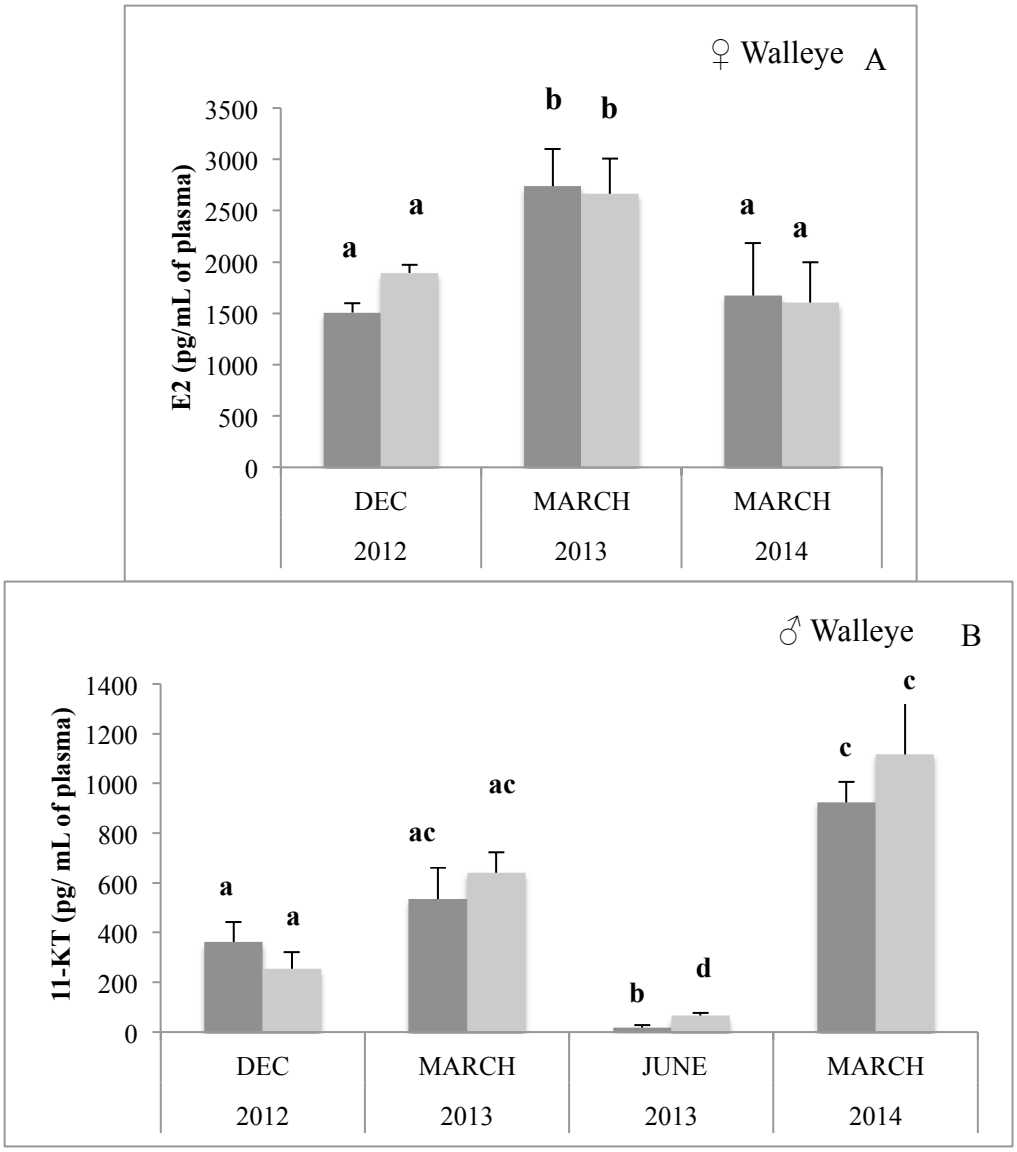

Figure 5. Female plasma E_2 (n=10) (A: ANOVA; p=0.015) and male plasma 11-KT (n=10) (B: ANOVA; p <0.001) of previously analyzed samples (archived data; dark bars) and the same samples that were re-analyzed (re-analyzed data; light bars) for walleye sampled from Tathlina Lake in Dec 2012, March 2013, June 2013, and March 2014. All values are reported as mean ± SEM. Bars showing different letters indicate statistically significant differences across time periods

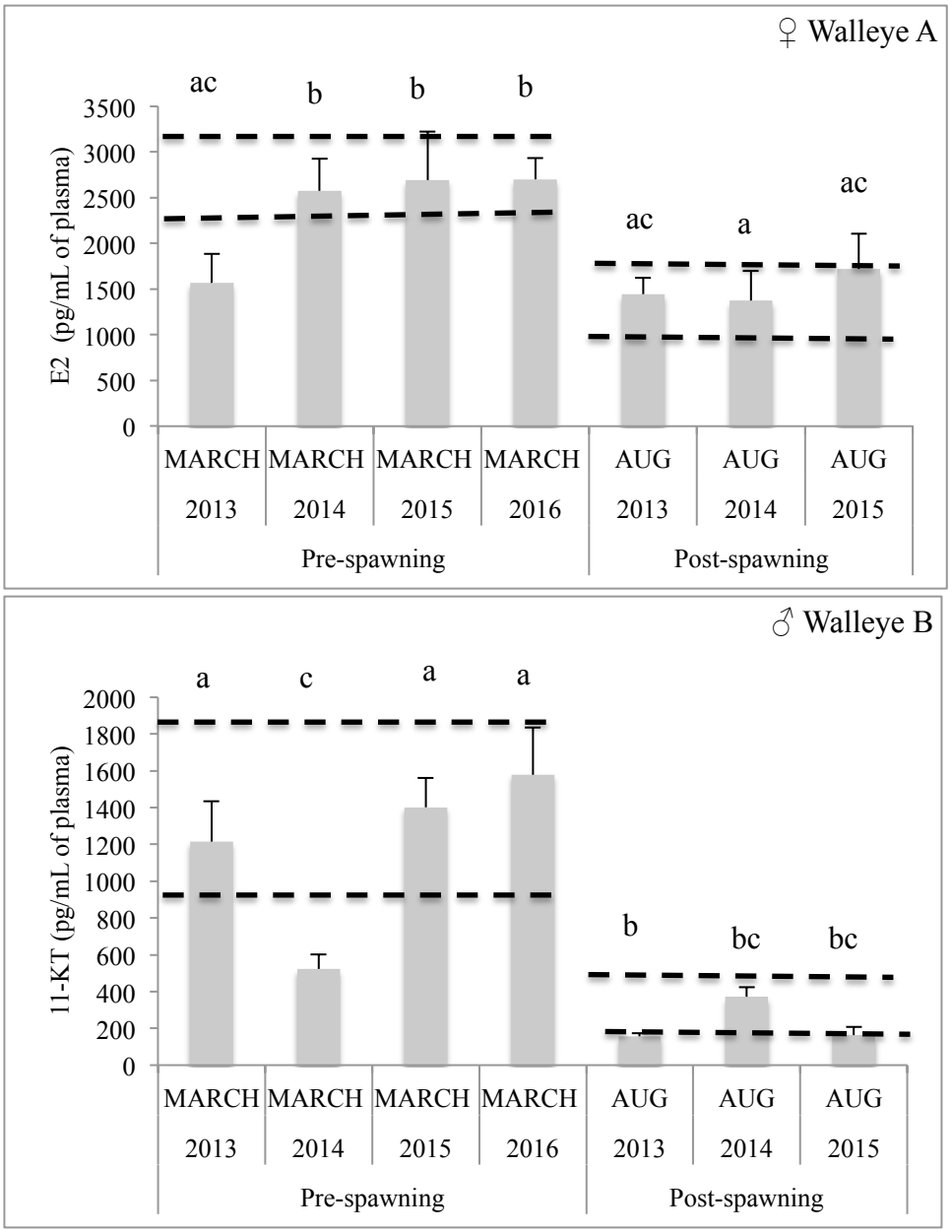

Figure 6. Hormone levels in female and male walleye collected at various time periods in Tathlina Lake (archived samples: March 2013 and August 2014). (A): Plasma E_2 levels in walleye (ANOVA; p=0.001). (B): Plasma 11-KT levels in walleye (ANOVA; p=0.002). All values are reported as mean ±SEM. Bars showing different letters indicate statistically significant differences across time periods (ANOVA; $p<0.05$). Dotted lines indicate CES (+/- 2 standard deviations of the mean).

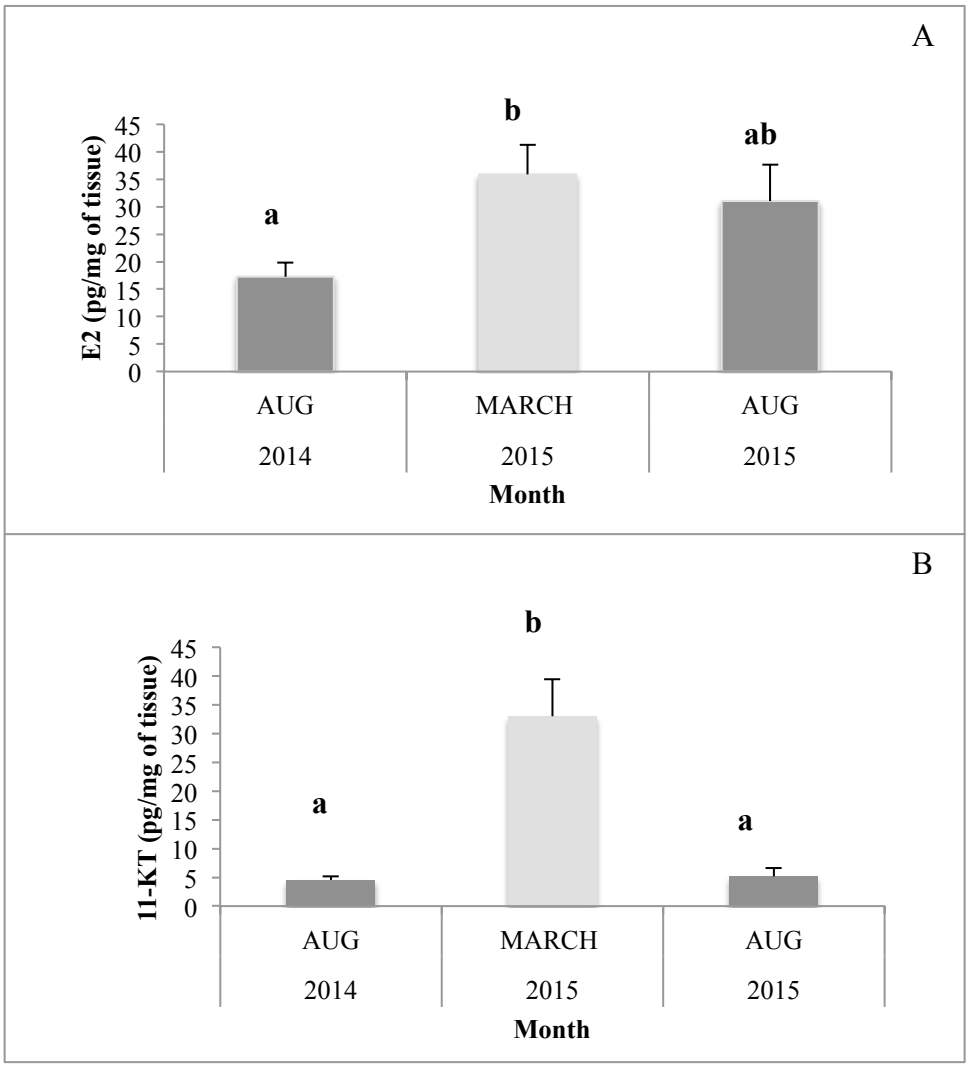

Figure 7. Tissue hormone levels in female and male walleye (A): Gonadal E_2 levels in female walleye (ANOVA; p=0.013). (B): Gonadal 11-KT levels in male walleye (ANOVA; p=0.01). All values are reported as mean ±SEM. Bars showing different letters indicate statistically significant differences across time periods (ANOVA; $p<0.05$). Dark bars indicate post-spawning, light bars indicate pre-spawning.

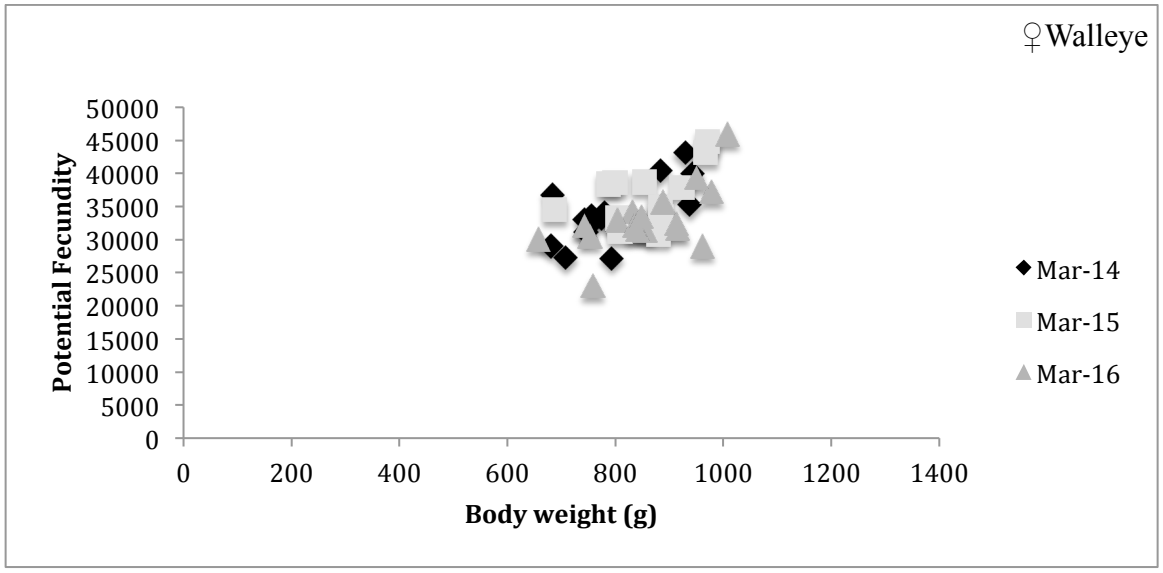

Figure 8. Scatter plot of female walleye potential fecundity versus body weight across sampling years (diamond, square, triangle) in pre-spawning in Tathlina Lake, NT, 2014-2016 (ANOVA; p=0.15; r=0.894).

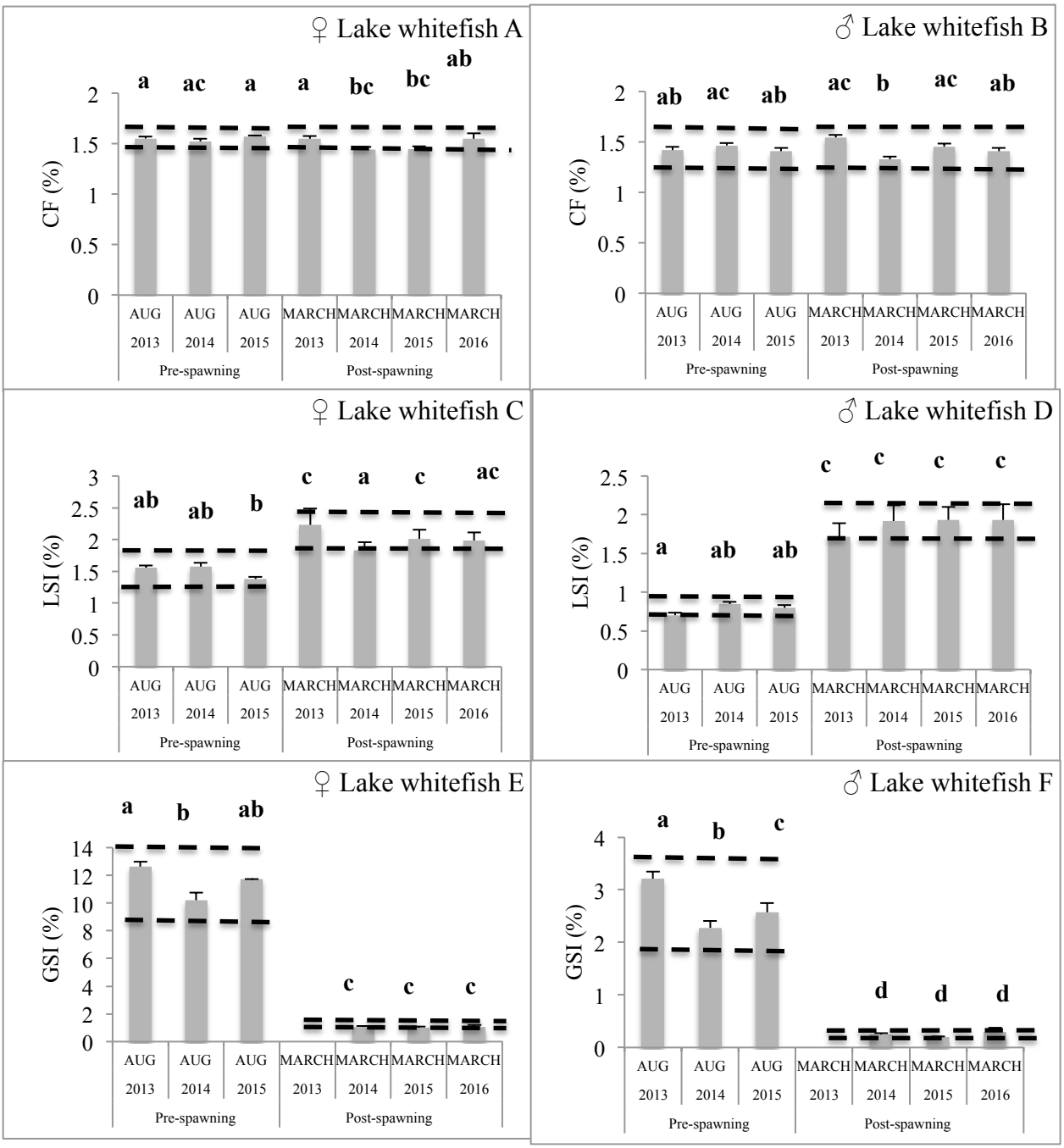

Figure 9. Mean condition factor (CF; A: male, B: female), liversomatic index (LSI; C: male, D: female) and gonadosomatic index (GSI; E: male, F: female) ± SEM for female and male lake whitefish collected in Tathlina Lake at various sampling periods. Dotted lines indicate CES (+/- standard deviations of the mean).

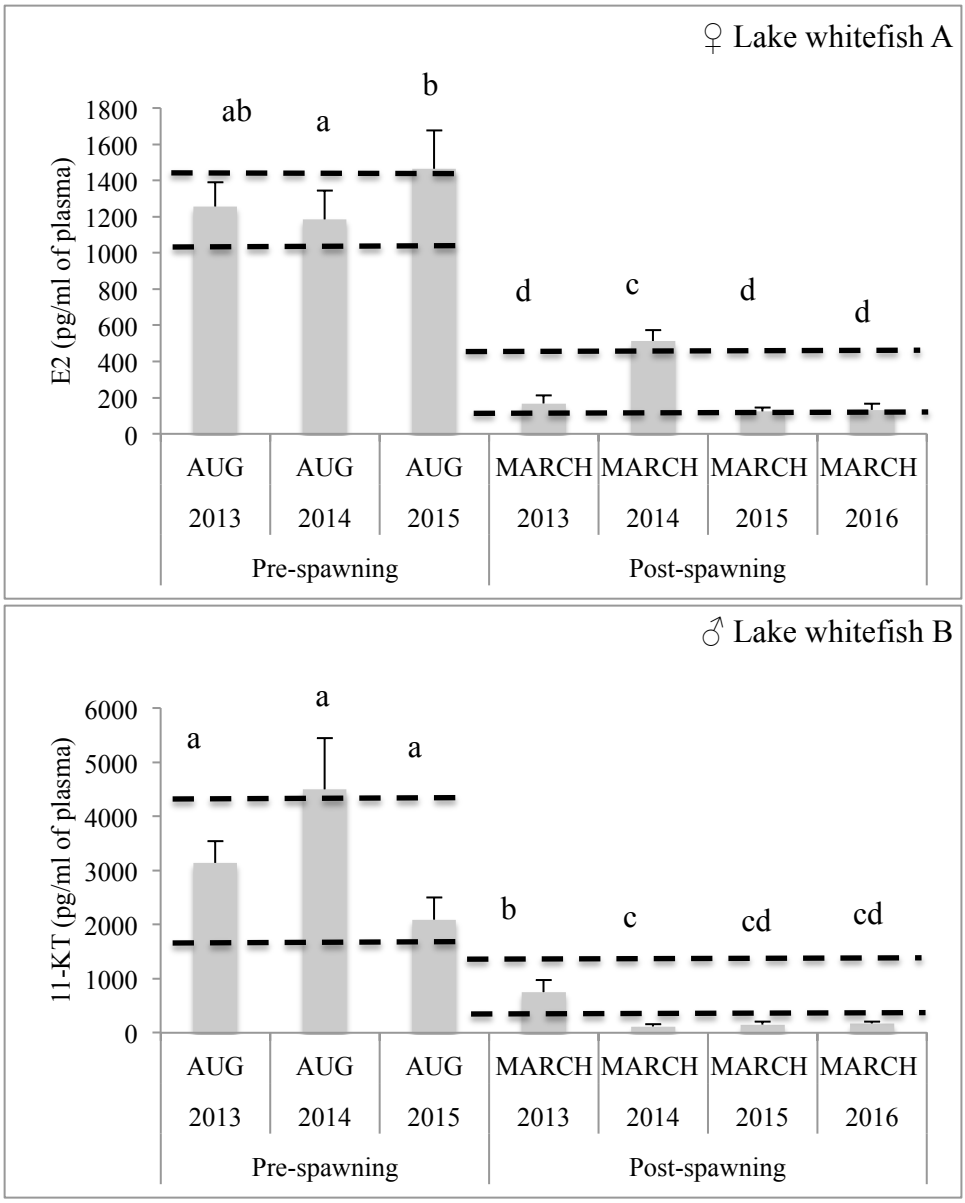

Figure 10. Hormone levels in female and male lake whitefish collected at various times periods in Tathlina Lake (archived samples: March 2013, and August 2014). (A): Plasma E_2 levels in lake whitefish (ANOVA; p=0.001). (B): Plasma 11-KT levels in lake whitefish (ANOVA; p=0.001). All values are reported as mean ± SEM. Bars showing different letters indicate statistically significant differences across time periods (ANOVA ; $p<0.05$). Dotted lines indicate CES (+/- 2 standard deviations of the mean).

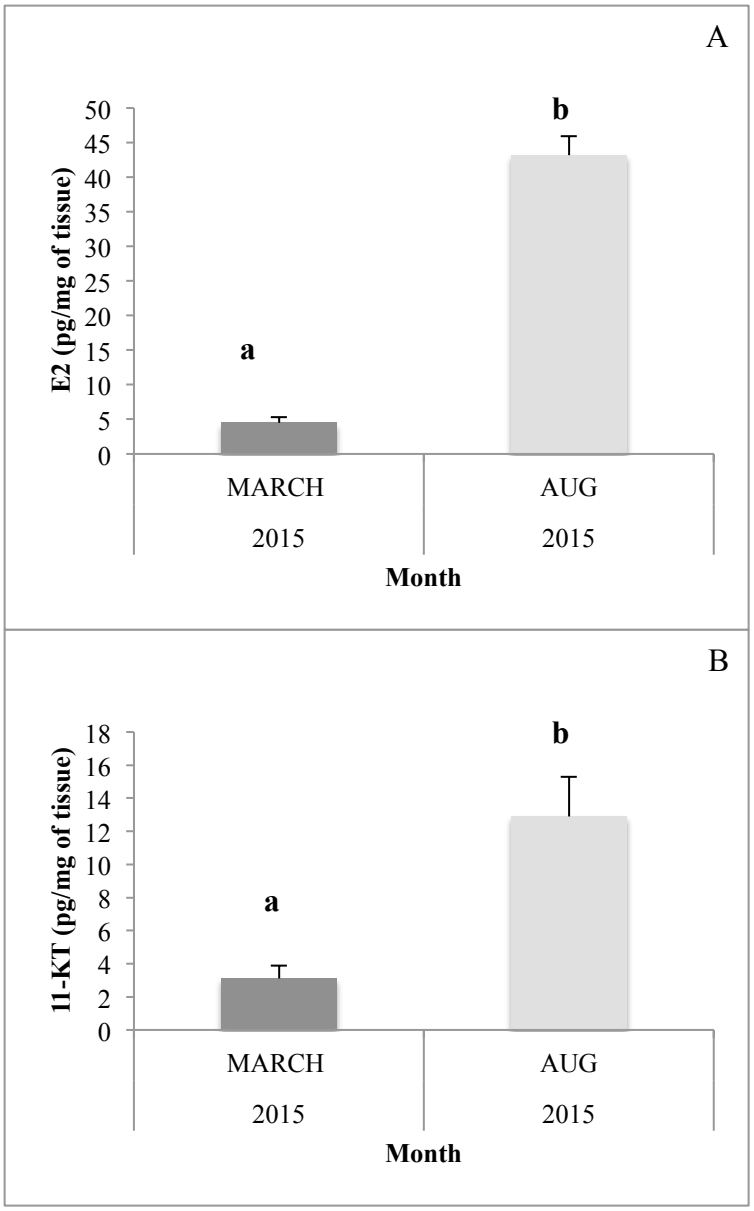

Figure 11. Tissue hormone levels in female and male lake whitefish (A): Gonadal E_2 levels in female lake whitefish (ANOVA; p=0.001). (B): Gonadal 11-KT levels in male lake whitefish (ANOVA; p=0.001). All values are reported as mean ± SEM. Bars showing different letters indicate statistically significant differences across time periods (ANOVA ; $p<0.05$). Dark shadow indicates post-spawning, light shadow indicates pre-spawning.

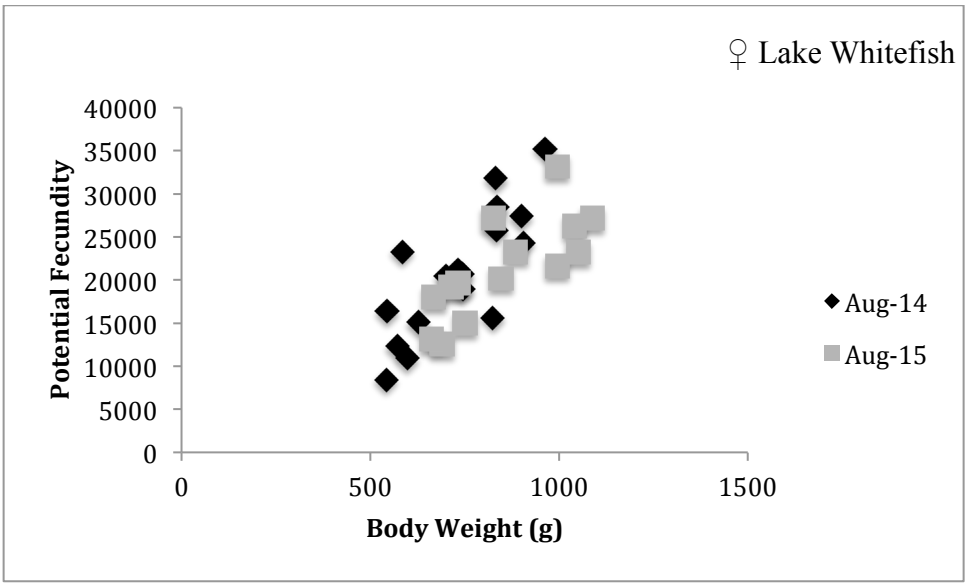

Figure 12. Scatter plot of female lake whitefish potential fecundity versus body weight across sampling years (diamond, square, triangle) during pre-spawning in Tathlina Lake, NT, 2014-2016 (ANOVA; p=0.859.; r=0.531).

2.5 Discussion

This is the first dataset developed to characterize seasonal variation in condition and reproduction for large-bodied fish species (walleye and lake whitefish) in Tathlina Lake, NT. Tathlina Lake is culturally and commercially important to the KTFN peoples of the Kakisa region; these data provide the critical baseline information to support ongoing community monitoring due to concerns regarding the sustainability of the system, which is potentially vulnerable to climate change and future resource extraction. For walleye and lake whitefish, seasonally-appropriate significant differences in CF, LSI, GSI and reproductive hormone levels associated with the natural pre-spawning and post-spawning times were confirmed. These biologically-relevant indicators of health were similar to patterns in these species in more southerly parts of their ranges. Additionally, the study provides evidence on which to recommend additional guidance regarding endpoint selection and study designs for a fish monitoring framework for the Lake and the region.

Typically, EEM studies are used to detect and measure changes in aquatic ecosystems after being exposed to a single stressor (i.e., a point source contamination; Environment Canada, 2010). This approach has helped develop our understanding of single stressor effects, but ignores impacts and potential interaction among multiple stressors over time (Culp *et al*., 2000). In the context of this study, there are potential concerns of impacts from future local resource extraction as well as ecosystem degradation originating from outside the watershed, such as atmospheric transport of contaminants and climate change. Large river systems in northern regions of Canada present a particular challenge for cumulative effects assessments because the ecology of these ecosystems is poorly understood in the context of increasing pressures of anthropogenic and environmental stressors (Culp *et al*., 2000). Using EEM approaches in the

current study on fish in Tathlina Lake has resulted in the collection of data, which can assist in the development of predictive tools to assess both natural variability and future cumulative environmental impacts. One such predictive tool is the application of critical effects sizes (CES) to the data. CES are threshold values commonly used to identify natural variability in endpoints in order to indicate changes representing high risk to the population (Environment Canada, 2010). Typically for EEM programs, CES for fish GSI, LSI, age, and weight at age have been set at 25%, while condition has been set at 10% (Environment Canada, 2010). In the current study, CES was set at +/- 2 standard deviations from the mean of pre-spawning and post-spawning periods, keeping spawning periods separate as per Arciszewski & Munkittrick (2015). When values exceed or deceed the normal range of variability (CES) it could indicate the presence of a pressure. Because fish exhibit high variability in morphometric endpoints during and after the spawning season and in relation to the availability of resources (e.g., food sources) and environmental conditions, seasonal changes in EEM endpoints, the time periods when these endpoints are changing rapidly, and the periods when they are stable for lengths of time need to be established (Dahle *et al*., 2003; Barrett *et al.,* 2015) to appropriately use CES to assess impacts within the context of seasonality. Having a basic understanding of the reproductive strategy of fishes in northern Canada is important for the development of an effective study that can be used in future community environmental effects monitoring programs (Barrett *et al.,* 2015), including at Tathlina Lake.

CF represents the relationship between the length and weight of fish and is often used as an indicator of overall fish health (van der Oost *et al.,* 2003). Female and male walleye exhibited significant differences across sampling periods, with no consistent differences between March and August. To identify natural variability within CF, the CES threshold established for female

walleye in pre-spawning condition (March) ranged from 1.0-1.2%, while for post-spawning (August) CES ranged from 0.9-1.1%. For males, CES for pre-spawning ranged from 1.0-1.2% and post-spawning ranged from 0.8-1.3%. Female and male lake whitefish exhibited significant differences across sampling periods, with no consistent differences between August and March. To identify natural variability within CF, the CES threshold established for female lake whitefish in pre-spawning condition (August) ranged from 1.5-1.6%, while for post-spawning (August) CES ranged from 1.4-1.6%. For males, CES for pre-spawning ranges from 1.3-1.5% and post-spawning ranged from 1.2-1.6%. Natural variability exists in CF as it represents energy or nutrient reserves and provides an indirect indicator of reproductive potential of fish (Lambert & Dutil, 2000; Van der Oost *et al.,* 2003). Any value of CF fluctuating below or above the threshold CES for pre- and post-spawning fish could indicate that availability of food is limited or food consumption of the fish is impaired due to stress factors, as there might be changes in metabolic function or impairment of transfer of energy reserves (van der Oost *et al.,* 2003). Typically, seasonal changes in CF are expected as fish are allocating energy towards gonad growth and maturation prior to spawning, whereas, after spawning, fish are actively feeding and directing energy towards somatic growth (Lambert & Dutil, 2000; Kaufman *et al*., 2007). Consistent values in CF are reflective of liver and gonad weight because as liver decreases in weight, gonad increases in weight and vice versa (Lambert & Dutil, 2000). Generally, heavy individuals will have greater energy reserves (Kaufman *et al*., 2007), while lower conditions would reduce reproductive investment in order to limit somatic energy (Lambert & Dutil, 2000). CF for walleye and lake whitefish aligned with other studies where CF remained constant in pre- and post-spawning conditions (Barnes *et al.,* 1984; Quist *et al*., 2003; Gallagher *et al*., 2011**)**. Increases in CF are known to be associated with fish actively feeding prior to spawning, or an

increase in gonad weight during pre-spawning times leading to a large CF prior to spawning (Kjesbu *et al*., 1991; Lambert & Dutil, 1997). For Atlantic cod *(Gadus morhua*) and Argentinian silverside (*Odontesthes bonariensis*), during gonadal maturation, female fish exhibit cessation of feeding and the energy is allocated for reproduction at the expense of somatic growth (Lambert & Dutil, 2000; Freyre *et al.,* 2009), whereas, males constantly feed investing energy into gonadal development earlier than females (Lambert & Dutil, 2000). Thus, changes in CF could be attributed to feeding or towards gonad growth. These differences could partly be due to the productivity of Tathlina Lake, as Stewart *et al*., (2015) found an increase in productivity attributed to climate warming can lead to increased food supply and increased growth.

In addition to CF, LSI (ratio of liver weight to body weight) provides an indication of overall fish health and energy storage (van der Oost *et al.,* 2003; Barrett *et al.,* 2015). Female and male walleye exhibited significant differences across sampling periods, showing no consistent differences in March and August comparisons. Pre-spawning female walleye had a threshold CES for LSI from 1.9-2.6%, while for post-spawning the range was from 0.9-1.4%. For males, CES for pre-spawning fish ranged from 1.7-1.9% for LSI, while post-spawning ranged from 0.7-1.8%. Female and male lake whitefish exhibited significant differences across sampling periods, showing no consistent differences in August and March comparisons. Pre-spawning female lake whitefish had a threshold CES for LSI from 1.3-1.7%, while for post-spawning the range was from 1.7-2.3%. For males, CES for pre-spawning fish ranged from 0.6-1.0% for LSI, while post-spawning ranged from 1.7-2.1%. Seasonal increases in LSI are associated with actively feeding fish that are building up liver stores that play a role in female gonad development prior to spawning. Ultimately, this leads to an increase in liver size and LSI, while decreased LSI indicates that energy reserves are being allocated towards somatic growth (Scott & Pankhurst,

1992; Lambert & Dutil, 1997; Kjesbu *et al.,* 1998; Plaza *et al.,* 2007; Moles *et al.,* 2008). For females, the liver is important for food processing and contributes to the production of energy-rich eggs (Casselman & Schulte, 2004). The liver produces vitellogenin, which is further processed into yolk proteins and larger livers are associated with higher production of vitellogenin and higher energy content in pre-spawning condition. Following vitellogenesis, LSI will decrease once the yolk proteins (vitellogenin) have been allocated to the ova (Barrett *et al*., 2015). In the current study, female walleye LSI cycles with GSI, which coincides with the accumulation of liver stores (vitellogenin) for reproduction prior to spawning. For males, spawning might be a fairly energy-consuming event and may be reflected in both CF and LSI declines during or just after the spawning season (Dahle *et al.,* 2003). Any mean value of LSI fluctuating below or above the threshold CES for pre- and post-spawning fish could indicate changes in metabolic function, and rate and availability of feeding (including starvation), and is thus an important indicator for EEM studies. For walleye CF, increases observed in another study were attributed to increasing liver weight and gonad weight (Johnston *et al*., 2012). This is largely due to actively-feeding fish storing energy in whole body reserves and liver lipid reserves. LSI of female and male northern walleye in Tathlina Lake compare favourably to patterns recorded in southern walleye in Lake Erie (Henderson *et al*., 1996) as LSI are greater in pre-spawning than post-spawning conditions. In Lake Erie females, LSI pre-spawning and post-spawning levels were 3.4% and 1.1%, respectively, compared to female walleye in Lake Tathlina (2.3% and 1.0%, respectively). In Lake Erie males, LSI pre-spawning and post-spawning levels were 1.7% and 1.1%, respectively, compared to male walleye in Lake Tathlina (1.6% and 1.1%, respectively).

However, female lake whitefish exhibit a different LSI trend, where liver size was larger in March (post-spawning) and smaller in August (pre-spawning). This could be attributed to liver lipids (vitellogenin) being allocated to the ova earlier on in the reproductive cycle and cessation of feeding prior to spawning (Dahle *et al*., 2003; Ghaffari *et al*., 2011; Barrett *et al*., 2015). LSI of female northern lake whitefish in Tathlina Lake exhibited similar values of pre-spawning LSI when compared to a study by Barnes *et al*. (1984) where lake whitefish LSI in pre-spawning condition collected in Ten Mile Lake, Labrador exhibited a 9.0% LSI, and a study by Johnston *et al.,* (2012), where pre- spawning LSI from lake whitefish collected in Lake Winnipeg, Manitoba was 1.5%, while in Lake Ontario it was 1.5%. While limited data are available on post-spawn female lake whitefish LSI, fish in the same family, Salmonidae; *Micropterus salmoides* from Aquilla Lake, TX, exhibited LSI of 1.0% (Brown *et al*., 2004). When compared to female lake whitefish in Lake Tathlina pre-spawning LSI was 1.5%, while post-spawning LSI was 2.0%. For males, Munkittrick *et al.,* (1991) LSI in pre-spawning lake whitefish ranged from 1.3-1.5% in northern Lake Superior, while Johnston *et al.,* (2012) found a similar result in which LSI in pre-spawning lake whitefish collected in Lake Winnipeg, Manitoba was 1.0%, and in Lake Ontario was 1.2%, compared to male lake whitefish in Lake Tathlina (0.8%). *Micropterus salmoides* (Salmonidae) in Aquilla Lake, Texas, exhibited LSI of 1.3% (Brown *et al*., 2004), compared to LSI from Tathlina Lake (1.9%). For both male walleye and lake whitefish, spawning appears to be energy consuming and results in smaller liver sizes; thus, male walleye and lake whitefish may in post-spawning condition be actively feeding (Dahle *et al.,* 2003). Differences in LSI have been correlated with metabolic function, rate of feeding, starvation and gonad development (Lambert & Dutil, 1997). In EEM point-source studies, liver size or LSI is found to increase in the presence of contaminants as changes in liver size can be attributed to the activation of

detoxification systems and increased metabolic function (Bowron *et al*., 2009). The increases in LSI observed in the present study are most likely a result of seasonal changes associated with feeding and liver stores accumulating in the liver for female gonad development prior to spawning, while lower LSI indicates energy reserves being allocated towards somatic growth.

GSI provides an approximate assessment of reproductive maturity, and permits a gross measure of reproductive capabilities in fish and may indicate reproductive stimulation (Anderson, 2013). Female and male walleye exhibit significant differences across sampling periods, with GSI being the highest in March and lowest in August, consistent with pre-spawning and post-spawning states. Threshold GSI CES were established: female pre-spawning fish ranged from 9.5-13%, while for post-spawning GSI CES ranged from 0.7-2.8%; for males, pre-spawning CES ranged from 1.7-2.4%, while post-spawning fish ranged from 0.9-3.0%. Female and male lake whitefish exhibit significant differences across sampling periods, with GSI being the highest in August and lowest in March, consistent with pre-spawning and post-spawning periods. Threshold GSI CES were established: female pre-spawning fish ranged from 9.0-14%, while for post-spawning GSI CES ranged from 0.9-1.1%; for males, pre-spawning CES ranged from 1.8-3.4%, while post-spawning fish range from 0.1-0.3%. Any value of GSI fluctuating below or above the threshold CES for pre- and post-spawning fish could indicate differences in gonad size related to reproductive state or less energy having been available for gametogenesis and ultimately egg production (Lumb *et al.,* 2007). Seasonal changes in GSI are normally seen with high GSI prior to spawning, associated with body lipids being allocated for gonad development, whereas lower GSI prior to spawning is indicative of impaired spawning. For GSI, increases observed are attributed to increasing gonad weight and decreased body weight (Johnston *et al*., 2012). Typically, fish allocate whole body lipids towards gonadal development

and maturation leading to increased gonad size (DeVlaming *et al.,* 1972; Johnston *et al*., 2012). For EEM studies, slow gonad growth indicates environmental factors that may influence investment into gonadal development. Factors influencing gonadal investment are more plastic and responsive to short-term environmental or physiological cues than egg size and fecundity (Johnston *et al*., 2012). The large GSI for female walleye is related to fecundity and is not surprising; it is a species that develops and spawns its gametes in a single annual spawning event (Dahle *et al* 2003; Barrett *et al.,* 2015). GSI of female northern lake whitefish in Tathlina Lake exhibited similar values of pre-spawning GSI when compared to a study by Rosch (2001), in Lake Constance, Europe, in which female GSI varied from 3-11%, while post-spawned females ranged from 0.8-1.2%. A study by Johnston *et al.,* (2012), had pre- spawning GSI for lake whitefish collected in Lake Winnipeg, MB was 19%, while in Lake Ontario it was 20%. *Micropterus salmoides* (Salmonidae) from Aquilla Lake, TX, had a GSI of 1.0% (Brown *et al*., 2004). When compared to female lake whitefish in Lake Tathlina, pre-spawning GSI was 11.5%, while post-spawning GSI was 1.0%. For males, Johnston *et al.,* (2012) found a similar result where GSI in pre-spawning fish collected in Lake Winnipeg, MB was 2.5%, while in Lake Ontario was 1.3%, compared to male lake whitefish in Lake Tathlina (2.7 %). *Micropterus salmoides* (Salmonidae) from Aquilla Lake, TX, had a GSI of 0.3% (Brown *et al*., 2004) compared to the GSI from Tathlina Lake (0.2%).

Fecundity is a factor regulating reproductive success and recruitment (Muth & Ickes, 1993). Fecundity and egg size for female walleye in Tathlina Lake exhibited no significant differences among pre-spawning periods across years and the range of eggs produced for walleye fit within ranges previously reported for walleye egg number (40 000 to 612 000; Muth & Ickes, 1993) and size (1680 to 1720 μm; Wolfert, 1968; Scott & Crossman, 1973; Muth & Ickes, 1993;

Hartman, 2009; Johnston *et al.,* 2012). For female lake whitefish, fecundity and egg size from Tathlina Lake exhibited no significant differences across pre-spawning periods and the range of eggs produced for lake whitefish fit within ranges previously reported for lake whitefish egg numbers (8000 to 36 000; Lawler, 1961) eggs and size (1706 to 1713 µm; Lawler, 1961).

Fecundity is a non-EEM endpoint, and was measured to enhance the standard EEM assessment suite. Changes in fecundity and egg size can be directly linked to alterations in fish size, population, exploitation rate and latitude (Muth & Ickes, 1993). Variations in fecundity may be caused by a variety of factors associated with changes in environmental conditions (Muth & Ickes, 1993). Typically, walleye exhibit a relationship between fecundity and egg size in which they develop small eggs and higher fecundity (Johnston *et al*., 2012). Walleye are determinate spawners (i.e., capital breeders), meaning increases in gonadal growth and fecundity in females are associated with the acquisition and storage of food resources in advance of offspring production (Armstrong & Witthames, 2010). This is in contrast to indeterminate fecundity or income breeding, in which food intake is adjusted concurrently with offspring production, without reliance on stores (Armstrong & Witthames, 2010). Typically, when food availability is low, spawning stocks exhibit lower fecundity (Muth & Ickes, 1993).

While GSI and fecundity indicate reproductive potential, sex hormone levels, another non-EEM endpoint, are used to indicate sexual maturation and specific stages of reproductive processes (McMaster *et al*., 2001; Dahle *et* al., 2003). Sex hormone levels can provide a reliable indicator of reproductive status in fish (McMaster *et al*., 2001), linking physiological and whole-organism levels of biological organization. In the current study, male and female walleye hormone levels experienced significant differences among sampling periods, typically experiencing higher reproductive steroid levels pre-spawning (indicating that the gonads may

still be undergoing maturation and development) and lower values post-spawning (indicating gonadal regression). For female walleye in pre-spawning condition, threshold CES ranged from 2146-3239 pg E_2/mL of plasma, while for post-spawning it ranged from 1262-2628 pg/mL of plasma. For male walleye, CES for pre-spawning 11-KT ranged from 939-1863 pg/mL of plasma, while post-spawning 11-KT ranged from 248-492 pg/mL of plasma. Similar to walleye, male and female lake whitefish hormone levels experienced significant differences among sampling periods, typically experiencing higher reproductive hormone levels in pre-spawning (August; indicating that the gonads may still be undergoing maturation and development) and lower values in the post-spawning period (March; indicating gonadal regression). For female lake whitefish E_2 in pre-spawning condition, threshold CES ranged from 1088-1422 pg/mL of plasma, while for post-spawning it ranged from 22-312 pg/mL of plasma. For male lake whitefish, CES for pre-spawning 11-KT ranged from 1930-4343 pg/mL of plasma, while post-spawning 11-KT ranged from 449-1055 pg/mL of plasma. Seasonal changes in walleye and lake whitefish are similar to seasonal endocrine changes associated with spawning times described in most freshwater teleosts studied, e.g., river catfish (*Hemibagrus nemurus*: Adebiyi *et al*., 2013) which experienced a surge of E_2 prior to spawning associated with final oocyte maturation and ovulation and had decreased E_2 following a spawning event while males generally exhibit increased 11-KT prior to spawning and decreased 11-KT following spawning in a variety of species (Prat *et al.,* 1990; Mailson *et al.,* 1994; Pavlidis *et al.,* 2000; Dahle *et al.,* 2003; Pankhurst, 2008). A study by Rinchard *et al.* (2001) (lake whitefish) found similar findings to the current study, in which 11-KT reached its maximum levels during pre-spawning and started declining prior to or soon after the initiation of spermiation or post-spawning. Typically, reductions in circulating levels of sex hormones are related to lower gonad size (Bowron *et al*.,

2009). Fish in Tathlina Lake may become vulnerable to climate changes and future resource extraction, thus changes outside the CES for gonad size or fecundity may warrant further investigation of hormone levels to determine if hormone signaling has been affected, including where the steroidogenic pathway may be affected by environmental impacts (McMaster *et al*., 2001). The baseline data provided here may be of value to those future, mechanistically-focused studies if such a situation arises.

Gonadal steroid levels represent local steroid production levels, whereas, plasma levels represent the sum of production and clearance process in whole organisms (Carragher & Pankhurst, 1993). For the purpose of biomonitoring in remote regions, given the challenges of bleeding fish in the field, it is useful to determine whether gonadal tissue steroid values relate to those in plasma. In this study, there was a similar trend between steroids extracted from gonadal tissue and from plasma, i.e., changes in plasma hormones were also identified as changes in gonadal tissue hormone levels. There is limited information on the correlation between ovarian and plasma levels of steroids for other species. Bradford & Taylor (1987) reported parallel changes in plasma and ovarian E_2 levels during cycles of semilunar spawning in killifish (*Fundulus heteroclitus*). Hobby & Pankhurst (1996) investigated the relationship between plasma and ovarian levels in gonadal steroids in the repeat-spawning marine fishes *Pagrus auratus* (Sparidae) and *Chromis dispilus* (Pomacentridae). They found that in the correlations between plasma and ovarian E_2 and testosterone in *Chromis dispilus* and *Pagrus auratus* that plasma steroid levels give an accurate picture of concurrent gonadal production in *Chromis dispilu* but less clear in *Pagrus auratus*, perhaps due to difference in spawning cycle lengths (Hobby & Pankhurst, 1996). Additionally, changes in E_2 were the same in plasma and ovarian extracts during vitellogenesis in the catfish, *Clarias batrachus* (Singh & Singh, 1987). In walleye

and lake whitefish in Tathlina Lake, both methods of determining hormone profiles represent a correlated measure of steroids and appear to provide valid steroid values as aligned with reproductive state and gonadal stage. However, because freezing gonads for future extraction is easier under field conditions and for community-based monitoring approaches than bleeding fish and retaining blood samples, it is recommended that measuring gonadal steroid levels is a more practical method.

A number of potential modifying factors must be accounted for when using sex hormones in reproductive evaluations (McMaster *et al*., 2001). Significant differences observed in pre-spawning condition over sampling periods could be attributed to differences in sampling times, when sampling did not occur at the exact same part of the cycle as the previous year. The reproductive cycles of fish are largely influenced by abiotic parameters: water and air temperature and photoperiod (Van Der Kraak & Pankhurst, 1997). Differences in yearly temperature may alter the timing of the fish reproductive cycle. Also, there is stress associated with capture, handling, and sampling of fish, which are known to alter circulating hormone levels (McMaster *et al*., 2001). These factors may contribute to differences observed when comparing within pre-spawning periods across years as well as when comparing to post-spawning periods. To provide consistent, reliable and reproducible information with respect to sex hormone levels for biomonitoring purposes, difficulties in field conditions and in maintaining sample integrity during storage and transportation, as well as variations in laboratory protocols, require methods to be standardized for collection, storage and analysis (McMaster *et al*., 2001). To that end, the current study indicates that previously analyzed archived samples in long-term studies can be re-analyzed, reproducing similar results with little variability. There were no significant differences observed when comparing archived samples to re-analyzed

samples for E_2. While there was a significant difference observed for one sampling month for 11-KT levels between archived and re-analyzed samples, the pattern of differences among sampling periods within one analysis (i.e., for either archived or re-analyzed samples) held. Intra-assay variability for all steroids analyzed remained below the acceptable level (Feswick *et al*., 2014) of 15%. Because intra-assay variability was low, and there was little variability between archived and re-analyzed samples, there is confidence in the steroid extraction and EIA methods used in the analysis.

Variations in air temperature are known to be reflected in temperature of the uppermost meter of water (Livingstone & Lotter, 1998). Air temperature from December 2014 to April 2015 fluctuated from -24.5°C to 6.35°C. The collection of under-ice water temperature data from within the Kakisa River watershed fluctuated from 0.1°C to 2.6°C. For walleye, ideal spawning temperature is 3.6-6.7°C, which is shortly after ice-breakup (Scott & Crossman, 1973). The water temperature range measured was below the ideal temperature range for pre-spawning in walleye but the temperature was increasing. This low temperature could be responsible for the delay in spawning seen in this study of northern walleye, in comparison to southern walleye populations (Malison *et al.,* 1994). DO concentrations (mg/L) from the sampled sites were measured to fluctuate between 1.9 mg/L and 12.9 mg/L from early December 2014 to early April 2015. Although the lowest midpoint was 1.9 mg/L in mid-March 2015 at T1, it was likely due to its shallow depths. Tathlina Lake experiences hypoxic (low DO levels; <2 mg/L) conditions (Ka'a'Gee Tu First Nation 2002; Gallagher *et al.,* 2011**)**, and DO levels in the present study fluctuated at T1 below the presumed lethal threshold for fish (<1mg/L; McMahon *et al.,* 1984; Chambers *et al.,* 2000; Vanderploeg *et al.,* 2009; Barton, 2011; Stewart *et al*., 2015) A lack of barriers among parts of the lake could provide access for the fish to refugia where DO levels are

higher. When DO levels were the lowest (March 2015), both walleye and lake whitefish were collected in the general region of the MiniDot loggers; the fish at that time were present in approximately 30 cm of water, similar to what was found by Stewart *et al.,* (2015). Walleye exhibit greater survival when DO levels are 3-5 mg/L, but are able to tolerate DO levels of 2 mg/L for a short period of time (McMahon *et al*., 1984; Vanderploeg *et al.,* 2009; Barton, 2011; Stewart *et al.,* 2015). Lake whitefish are tolerant to low DO levels, as they occupy hypolimnetic waters, but exhibit greater survival when DO levels are greater than 6 mg/L (Wahl & Loffler, 2009). Oxygen depletion can retard gonad growth, reduce somatic growth, reduce fertilization success, decrease reproductive output, reduce larval hatching, reduce larval success, and reduce reproductive hormone (11-KT & E_2) levels (Wu, 2003; Landry *et al.,* 2007; Wahl & Loffler*,* 2009). This suggests that while walleye and lake whitefish in Tathlina Lake may experience DO levels below optimum in the winter, the combination of refugia and physiological tolerance limits impacts because fish sampled in March 2015 sampling periods do not have impaired conditions or reproduction. With air temperatures expected to increase as a result of climate change, water temperatures are expected to increase (Morrill *et al*., 2001; Sharma *et al.,* 2007). As water becomes warmer, DO concentrations decrease, with the potential to subsequently limit the productivity and physiological health of resident biota (Morrill *et al*., 2001; Sharma *et al.,* 2007). As the depth of Tathlina Lake ranges from 1.5-1.8m, it is particularly vulnerable to increases in air temperature, as shallow lakes respond rapidly to changing climatic conditions and have less resiliency than deeper lakes (Gallagher *et al.,* 2011).

2.5.1 Future biomonitoring in Tathlina Lake

CF, LSI, GSI and fecundity are endpoints that are responsive to, and indicators of, population and ecosystem changes, while also being relatively easy to collect and standardize

(Johnston *et al*., 2012). In this study, they were effectively used to assess general health and develop baseline patterns of reproduction in walleye and lake whitefish in Tathlina Lake, NT. CF, LSI and GSI could be readily adopted in a long-term community-based biomonitoring program as standardization is relatively easy to ensure and they do not require advanced technology. GSI was demonstrated to be a reliable indicator of reproductive status and correlated well with fecundity during pre-spawning periods for both species. If changes in morphometric endpoints related to reproduction are observed, sex steroid levels can provide an indication of whether signaling in the pathway has been affected. Field studies examining seasonal variation of sex steroid levels have demonstrated that circulating levels of sex steroids can be used to indicate exposure to environmental conditions or anthropogenic stressors which affect the reproductive endocrine systems in fish (McMaster *et al.,* 1992; Malison *et al.,* 1994; Malison & Held, 1996; Plaza *et al*., 2007). In this study, it was determined that for walleye and lake whitefish, collection of gonads (followed by extraction in the laboratory) provides a reliable indicator of reproductive endocrine status. This is further supported because gonads are easier to collect in the field than blood samples which require additional training and equipment.

The baseline data collected provide an opportunity to begin to understand the natural variability in pre- and post-spawning walleye and lake whitefish in Lake Tathlina in particular and northern boreal lakes in general. Calculation of critical effects sizes or CES (Table 3) can support future biomonitoring; values that exceed or deceed these CES ranges can act as trigger warnings that system changes are occurring. Arciszewski & Munkittrick, (2015), suggest eight years of data are required before industrial development to establish the normal range of variability at the site, while EEM typically uses 3- to 4- cycles (Munkittrick *et al.,* 2002) to assess impacts. Additionally, pooled means from multiple lakes in the region will provide a

better option than using means from individual sites compared with every other site (Arciszewski & Munkittrick, 2015). There is a need to expand the number of lakes sampled in the region to determine if the natural variability in Tathlina Lake reflects a more general pattern. Additionally, sampling multiple reference lakes in the region will generate a pooled mean of sampling sites which is a better option than using means from individual sites, compared to every other site, as it eliminates site bias (Arciszewski & Munkittrick, 2015). Over time, the grand mean of references sites can be used to gauge the presence of unusual observations using CES. CES will become more precise and accurate as more sites are added; what is provided here at best demonstrates the viability of the approach.

The spawning periods used in the study are rough estimates based on monthly sampling; more frequent sampling could be conducted to obtain a more detailed overview of changes during the pre-spawning and spawning periods. The pre-spawning period can be one of particular sensitivity to contaminants, low DO, and other environmental factors (Van Der Kraak & Pankhurst, 1997). However, this may be of more academic interest than of value in a long-term biomonitoring program as collection in the current months (March and August) appears to be suitable as a monitoring protocol for both species; changes during pre-spawning periods for either species could indicate a need for more intense sampling in future years to better understand impacts. Additionally, a question remains as to how mobility of walleye and lake whitefish affects their survival and reproduction in Lake Tathlina; while refugia exist in the lake (higher DO at other locations in the lake and tributaries) the extent to which these can support the population is unknown. Various methods exist to tag and track fish, including under ice (Cooke *et al*., 2013). Future monitoring could initially reduce sampling to biennially (every-other year) in (pre- (March) and post- (August) spawning times for walleye and pre- (August)

and post- (March) spawning times for lake whitefish) as little change has occurred over the study period; however, if development activities begin in the region or biennial sampling indicates changes are occurring, reinstituting annual sampling should be considered.

Table 3. Summary of critical effects size (CES) ranges for condition factor (CF), liversomatic index (LSI) and gonadosomatic index (GSI) and hormones (E_2 and 11-KT) for female and male walleye and lake whitefish collected in Tathlina Lake during pre- and post-spawning periods. Critical effects sizes (CES) were calculated as ± 2 standard deviations around the mean.

Species	Spawning time	CF (%)	LSI (%)	GSI (%)	Hormones (E_2/11-KT; pg/mL of plasma)
♀ **Walleye**	Pre-spawning	1.0-1.2	1.9-2.6	9.5-13	2146-3239
	Post-spawning	0.9-1.1	0.9-1.4	0.7-2.8	1262-2628
♂ **Walleye**	Pre-spawning	1.0-1.2	1.7-1.9	1.7-2.4	939-1863
	Post-spawning	0.8-1.3	0.7-1.8	0.9-3.0	248-492
♀ **Lake whitefish**	Pre-spawning	1.5-1.6	1.3-1.7	9.0-14.0	1088-1422
	Post-spawning	1.4-1.6	1.7-2.3	0.9-1.1	22-312
♂ **Lake whitefish**	Pre-spawning	1.3-1.6	0.6-0.9	1.8-3.6	1933-4343
	Post-spawning	1.2-1.6	1.7-2.1	0.1-0.3	449-1055

2.6 Conclusion

This study demonstrated the reproductive pattern of walleye and lake whitefish in Tathlina Lake, NT, confirming walleye as spring spawners and lake whitefish as fall spawners in this northern Canadian lake. It provides guidance for future sampling of walleye and lake whitefish in a long-term community monitoring program and considers the need for robust standardized protocols in a remote field-sampling location while prioritizing assessment of reliable environmental indicators. Use of a modified EEM protocol resulted in a baseline dataset, to which application of CES methodology supports a longer-term monitoring program which can be applied in Lake Tathlina and across the region.

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Appendix 1

A1.1 Validation of EIA for hormone extractions

Validation of EIA for plasma and tissue extractions was performed using ten female walleye plasma samples and ovarian tissues following steroid extraction as previously noted. Variations for plasma included: 1 mL of blood from an individual fish was placed in 1.5 mL microcentrifuge tubes and centrifuged at 3000 x *g* for 5 min, the plasma (500 µL) was extracted, and pooled in a test tube with the other plasma samples. The pooled sample was divided into 10 individual samples and the steroid extraction proceeded as previously described. Following EIA, the coefficient of variation was calculated to be 10% [(standard deviation of samples/mean of samples) x 100], which indicates that the method is reproducible and reliable. Validation of EIA for tissue extractions was followed similar to the previously noted tissue extraction protocol. The minor variation was that after homogenization of the tissue, the tissue from ten fish was pooled, vortexed and subsequently divided into 10 individual samples. These samples proceeded to follow the methodology of steroid extraction from gonadal tissue, mentioned above. Following EIA, the coefficient of variation was calculated to be 13% [(standard deviation of samples/mean of samples) x 100], which indicates that the method is reproducible and reliable.

Appendix 2

A1.2 Walleye and lake whitefish MACRO for egg diameter measurements

```
Sub Main
        Dim App As Object

   Set App = CreateObject("SigmaScan.Application")
   Dim Worksheet As Object
   ' Recorded macro begins...


   Dim YWLWF As Object

   ' Open up the image you want to scan
   Set YWLWF = App.OpenImage("C:\Users\harr7420\Desktop\800 JPEG\868-4.JPG")

   ResultCode = YWLWF.SetZoomLevel(0.250)
   ResultCode = YWLWF.ConvertToGrayScale

   ResultCode = App.Combine(YWLWF, YWLWF, 768)
   ResultCode = App.Combine(YWLWF, YWLWF, 1536)


   ResultCode = YWLWF.Posterize(1, 2)
   ResultCode = YWLWF.ChangeColorResolution(8, 4)
   ResultCode = YWLWF.ConvertToGrayScale
   Dim Left0(1) As Long
   Left0(0) = 0
   Dim Right1(1) As Long
   Right1(0) = 0
   ResultCode = YWLWF.IntensityThreshold(1, 1, Left0, Right1)

   ResultCode = YWLWF.FilterOverlay(10, 1, 1, 1, 2) '* delete edge object
   ResultCode = YWLWF.FilterOverlay(8, 1, 1, 1, 2) '* delete residue
   ResultCode = YWLWF.FilterOverlay(2, 1, 2, 3, 2)  '* Erode, split objects
   ResultCode = YWLWF.FilterOverlay(5, 2, 3, 1, 2)  '* Dilate everything
   ResultCode = YWLWF.FilterOverlay(6, 1, 2, 1, 1)   '* Dilate, don't merge
   ResultCode = YWLWF.FilterOverlay(10, 2, 3, 1, 1)  '* Remove edge objects
   ResultCode = YWLWF.FilterOverlay(2, 1, 1, 2, 2) '* erode- split, preserve
   ResultCode = YWLWF.FilterOverlay(3, 1, 1, 10, 5) '* split
   ResultCode = YWLWF.FilterOverlay(3, 3, 1, 10, 5) '* preserve shape
   ResultCode = YWLWF.AndOverlays(3, 1, 3)   '* Logical AND overlays 1 and 4


Set Worksheet = App.GetWorksheet
   Worksheet.Show
   Worksheet.MakePermanent
   YWLWF.MakePermanent

   App.CollectMeasurement(M_AREA, "J") ' getting area of each sample
```

```
    App.CollectMeasurement(M_SHAPEFACTOR, "M") ' getting shape factor area of each sample
    App.Collectmeasurement (M_FERETDIAMETER, "N") ' getting feret diameter
            App.Collectmeasurement (M_PERIMETER, "O") ' getting PERIMETER

    ResultCode = YWLWF.MeasureObjects(1)

            ' Eliminate all objects not sufficiently round
    MsgBox ("Removing all non-compliant objects.  Click OK to continue.")

    ' get the number of "samples" counted
    SampleTotNum = Worksheet.GetCellValue("A", 1)
    MsgBox (SampleTotNum + " objects detected")

    Counter = 0

    ' iterate through all of the samples and delete ones that are too small
    ' or too large
    For i = 1 To SampleTotNum
       ObjArea = Worksheet.GetCellValue("B", i)
       If (ObjArea < 30000 Or ObjArea > 100000) Then
             '* Eliminate the object
             ResultCode = YWLWF.EliminateObject(i)
             Counter = Counter + 1
       End If
    Next i

    'Recorded Macro Ends

End Sub
```

Chapter Three:

Summary

3.1 Summary

Tathlina Lake is of economic and cultural importance to KTFN (Ka'a'gee Tu First Nation), whom now reside in the community of Kakisa (Kennedy, 1962; Stewart *et al*., 2015). Band members have hunted, trapped, and fished in the area for years, and in 2013, approximately 10% of the community was directly supported by the fishery (Stewart *et al*., 2015). Tathlina Lake has historically contained large-bodied fish populations, which have experienced fluctuating stock levels (Kennedy, 1962; Roberge *et al*., 1988; Stewart & Low, 2000; Gallagher *et al*., 2011). Major wildfires and large natural winterkills have threatened the fish populations by depleting stocks and increased temperature due to climate change and future oil and gas exploration and extraction are of increasing concern (Ka'a'Gee Tu First Nation, 2002); the need for long-term biomonitoring is essential to understand the effects on biota of the changing environment. This multidisciplinary project supported by the Cumulative Impact Monitoring Program (CIMP) of the Government of Northwest Territories (GNWT) helps to improve our understanding of the cumulative impacts of environmental change and human development in the Tathlina Lake, NT, watershed. The collaboration between scientists of the GNWT, Fisheries and Oceans Canada, KTFN and academic researchers including those from Wilfrid Laurier University has been supportive of the project and its goal to report baseline data for walleye and lake whitefish populations and implement guidance for future long-term community biomonitoring specific to the status of the health and reproduction of fish in the lake.

Long-term, baseline-monitoring information is useful for evaluating potential changes in freshwater ecosystems over time (Lindenmayer & Likens, 2009; Schaeffer *et al.,* 2011). Monitoring seasonal changes over long periods of time can provide important ecological insights crucial for improved management of ecosystems and natural resources (Lindenmayer & Likens, 2009). Long-term datasets are important for understanding how stressors influence aquatic

ecosystems and their fish communities (Schaeffer *et al.,* 2011). In Tathlina Lake, protocols based on the Canadian Federal Environmental Effects Monitoring (EEM) program (Environment Canada, 2010) to assess fish health were used. EEM studies provide guidelines for assessing individual indicators of energy storage (condition factor and liver somatic index) and energy use (gonadosomatic index) (Barrett *et al.,* 2015) to assess health of the aquatic system and impacts caused by stressors such as climate change and anthropogenic activities (Kilgour *et al.,* 2005; Environment Canada, 2010; Barrett *et al.,* 2015). Generally, pre-spawning female and male walleye in March had greater LSI, GSI, and reproductive hormone (17β-estradiol (E_2) and 11-ketotestosterone (11-KT)) levels, and unchanged condition factors relative to post-spawning in August. Pre-spawning female and male lake whitefish in August had lower LSI and greater GSI, and greater reproductive hormone (E_2 and 11-KT) levels, and unchanged condition factors relative to post-spawning in August. Fecundity remained constant throughout the pre-spawning periods for both walleye and lake whitefish.

Standardized monitoring endpoints help to reduce variability, allowing for their use as indicators of seasonal changes in general fish health and reproductive status (Kilgour *et al.,* 2005). Standardized and consistent endpoints, analytical methods, calculations and equipment are needed to undertake future community-based environmental monitoring to add confidence in data if changes occur (Table 4). Additionally, these endpoints have demonstrated the occurrence of reproductive cycles in fish and possible variations in physiological condition due to changes in environmental conditions (Freitas *et al*., 2011). Because reproduction causes such large changes in morphometric endpoints (CF, LSI, and GSI), the sampling times in EEM studies need to be standardized to reduce potential variability in endpoints and improve comparability of data among studies (Barrett *et al.,* 2015). Otherwise, there is a risk of identifying as "site differences"

those variances, which are due to normal cycling through reproductive states. Critical effect sizes (CES) are used as predictive tools to assess natural variability and environmental impacts (Table 3; Environment Canada, 2010; Arciszewski & Munkittrick, 2015). The values represented in the current study established natural variability over four years in endpoints that are commonly used to indicate changes to fish populations (Environment Canada, 2010). Arciszewski & Munkittrick (2015) suggest eight years of data be required before industrial development to establish the normal range of variability at a site, whereas EEM typically uses 3- to 4- cycles to assess impact (Munkittrick *et al.,* 2002). When values fluctuate below or above CES, this could indicate the presence of an ecological pressure. Biomonitoring studies must include an understanding of changes in seasonality in order to best interpret the data (Barrett & Munkittrick, 2010).

Endpoints such as fecundity and reproductive hormones remained consistent over the study years and warrant no further monitoring attention unless and until changes to CF, LSI, and GSI are noted. Walleye and lake whitefish exhibited a relationship between fecundity and egg size in which they develop small eggs and higher fecundity (Johnston *et al*., 2012). Fecundity for both walleye and lake whitefish remained constant across the sampling periods. When changes become significantly different in pre-spawning periods, further investigation is warranted because fecundity may be caused by a variety of factors associated with changes in environmental conditions (Muth & Ickes, 1993). Changes in fecundity and egg size can also be directly linked to alterations in fish size, population, exploitation rate and latitude (Muth & Ickes, 1993). Sex hormone levels can provide a reliable indicator of reproductive status in fish (McMaster *et al*., 2001), linking physiological and whole-organism levels of biological organization. Both male and female walleye and lake whitefish experienced greater reproductive hormones (female: E_2 and male: 11-KT) in pre-spawning sampling periods compared to post

spawning. CES were established for the measured steroids; future environmental changes causing values outside of the CES for morphometric endpoints are a crucial warning of change and the baseline steroid levels may be of value in investigating mechanisms of action related to the morphometric changes (McMaster *et al*., 2001).

Future studies need to expand the number of lakes sampled in the region to enhance the robustness of the CES approach. It is not known if the CES values computed for Tathlina Lake are representative of natural variability in the region. For Lake Tathlina, a biennial (every-other-year) monitoring approach (for both pre- and post-spawning periods) is recommended; for other lakes, biannual baselines (pre- and post-spawning) should be undertaken for representative lakes. The assessment of environmental conditions (air and water temperature, DO and photoperiod) will generate a baseline for environmental conditions corresponding to the biological endpoints measured and be of value in assessing the effects of climate change or other environmental perturbations.

Table 4. Proposed biological endpoints to be used in a long-term fish biomonitoring program in Lake Tathlina.

Fish	Sampling Months	Endpoints	Analytical Methods	Calculation	Fishing Equipment	Sampling Equipment
Walleye & lake whitefish	March & August	Condition Factor (CF)	ANCOVA ($p<0.05$)	10^5 x body weight/fork length^3	Gillnets with a mesh size of 10.2 cm; air for buckets when transporting fish to sampling site; boats or snowmobiles depending on months	Dissecting equipment (scales, weigh boats, knives, markers and paper); scales to 0.01g
		Liver somatic index (LSI)		Liver weight/ (body weight-liver weight) x 100	Gillnets with a mesh size of 10.2 cm; air for buckets when transporting fish to sampling site; boats or snowmobiles depending on months	Dissecting equipment (scales, weigh boats, knives, markers and paper); scales to 0.01g
		Gonado-somatic index (GSI)		Gonad weight/(body weight-gonad weight) x 100	Gillnets with a mesh size of 10.2 cm; air for buckets when transporting fish to sampling site; boats or snowmobiles depending on months	Dissecting equipment (scales, weigh boats, knives, markers and paper) ; scales to 0.01g

3.2 References

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