THE EFFECTS OF NANOSILVER ON NORTHERN PIKE GROWTH

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THE EFFECTS OF NANOSILVER ON NORTHERN PIKE GROWTH

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A CAUTION TO THE READER

ABSTRACT

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Nanosilver (nAg) is an effective anti-microbial agent that is being increasingly used in consumer products. With such an expansive list of products incorporating nAg, the risk of environmental release is high. Studies testing the impacts of nAg have shown ranging effects on the physiology of fishes, but to date no significant impacts to growth in natural settings at environmentally-relevant exposure levels over long term (months to years) periods have been documented. A collaborative study was performed at the IISD – Experimental Lakes Area to evaluate whole-ecosystem changes in the presence of environmentally relevant levels of nAg. I evaluated changes in Northern Pike size-at-age before, during, and after nAg additions to a treatment lake (Lake 222) and compared the findings to a reference lake (Lake 239). Significant declines in fork length and weight at age over time were observed across all year groups, with the most notable declines occurring in the year 4 and 5 age groups. There were no similar changes observed in the reference lake. Body condition did not change with nAg additions. Despite reduced populations and no apparent changes in the availability of the prey community, growth of Northern Pike declined. The results of this study strongly suggest that nAg has a direct inhibitory effect on Northern Pike growth, likely caused by a reduced conversion efficiency

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INTRODUCTION AND OBJECTIVES

1.1 BACKGROUND

Nanosilver (nAg) is increasingly being used in consumer products for its effective anti-microbial properties (Akter et al. 2017). nAg releases small amounts of ionic silver which inhibits growth of microorganisms and bacteria (Benn et al. 2010). Textiles such as t-shirts, socks and underwear are known to incorporate nAg and release the chemical into the wastewater when washed (Reidy et al. 2013, Benn and Westerhoff 2008, Benn et al, 2010). The average household is responsible for as much as 470 µg of nAg reaching our wastewater treatment plants daily (Benn et a. 2010).

Once in the aquatic environment, nAg can remain as a colloidal solution, agglomerate into particulate silver and settle in the sediments, or be taken up by periphyton or other microorganisms (McGillicuddy et al 2017, Furtado et al. 2015,). Despite its anti-microbial capabilities, mesocosm exposure studies suggest that bacterioplankton and phytoplankton are not significantly impacted in the presence of nAg, and zooplankton species may increase in abundance. (Vincent et al. 2017, Blakelock et al. 2016). Phytoplankton and zooplankton communities have been shown to ingest and accumulate nAg, creating one pathway for nAg to enter the food web and transfer to higher trophic organisms (Conine and Frost 2016 Asghari et al. 2012). Yellow Perch (*Perca flavescens*) of small standard lengths rely heavily on diets consisting of zooplankton species, allowing nAg to transfer to and biomagnify in fishes (Vincent et al. 2017, Parke et al. 2011, Asghari et al. 2012,).

nAg is shown to affect fish in different ways. In addition to diet, nAg can enter fish via uptake through the gills, and can accumulate in the gills, liver and muscle tissues of fish (Martin et al. 2017, Scown et al, 2010). Concentrations of nAg in livers of Northern Pike (*Esox lucius*) observed in a whole lake nAg addition study, rose to levels in the low parts-per-million range, which was 3 orders of magnitude greater than the concentration of nAg in the water (Martin et al. 2018). Physiological processes have been seen to be affected by nAg exposure in lab studies and whole lake experiments. Metallothionein, a protein directly involved in the detoxification of metal cations, rapidly increased during nAg exposure (Martin et al. 2017). Lipid peroxidation in the gills increased as well, suggesting oxidative stress is imposed by nAg (Martin et al. 2017). Blood cortisol levels also experienced measurable increases in Rainbow Trout (*Oncorhynchus mykiss*) exposed to nAg over a 28-day period, experienced no change to growth, standard metabolic rate, forced maximum metabolic rate, or spontaneous maximum metabolic rate (Murray et al. 2017a, Murray et al. 2017b). A whole-lake nAg addition experiment showed decreases in consumption and metabolism of Yellow Perch, but again growth rate, was not affected, nor were conversion efficiency and abundance (Hayhurst 2018). Northern Pike survivability was reduced, noted by declines in population size (Figure 1) (Hayhurst 2018).

Apart from British Columbia and the Maritimes, Northern Pike are widespread across Canada and are important to both the commercial and sportfishing industries (Harvey 2009). Growth in Northern Pike is known to be affected by both prey availability and population density. (Kennedy et al 2018, Pierce et al. 2003, Margenau et al. 1998). Northern Pike show a foraging preference for Yellow Perch since they provide the fastest growth in juveniles (Kennedy et al 2018). As Northern Pike grow larger, they may shift their foraging effort to more energy dense offshore prey species when they are present (Kennedy et al. 2018). This allows Northern Pike to maximize their energy gain, maintain efficient growth rates and achiever larger asymptotic sizes (Kennedy et al. 2018).

Growth in Northern Pike has also been shown to be density dependent (Pierce et al. 2003). Measures of proportional stock density and growth negatively and non-linearly correlated with density (Pierce et al. 2003). Populations at lower densities were found to grow to larger sizes and populations at higher densities grew to smaller sizes.

In addition to standard growth measurements of length and weight, body condition is another useful measure that describes the body size of a fish at a given length and describes the health of a fish (Rennie and Verdon 2008). Body condition has been found to scale positively with food availability (Rennie et al. 2019, Casini et al. 2011, Rennie and Verdon 2008). When prey available to a predator increases, whether by a direct increase in abundance to the prey community or by predation release of the top predator, the predator is able to capture the surplus of prey available and increase their condition (Rennie et al. 2019, Casini et al. 2011)

Several structures in Northern Pike lend themselves well to age determination (Forsman et al. 2015). The fin ray is a structure used by scientists to verify ages determined from other structures such as the cleithrum and otolith (Little et al, 2012). Fin ray age analysis in soft rayed fish has proven accurate, as predictability between expected age and actual age is high (Mills and Chalanchuk 2004). Use of fin rays as an ageing structure is also advantageous because it does not require lethal sampling.

1.2 OBJECTIVES

The objective of this study is to determine whether nAg influences growth in Northern Pike. Hayhurst (2018) observed population reductions of Northern Pike (Figure 1) and insignificant impacts to Yellow Perch growth, condition, and abundance, when exposed to nAg, therefore ruling out the possibility that growth may be affected by a change in prey availability. When considering the effect of density on Northern Pike populations, we should expect to see an increase in growth as the population declined (Hayhurst 2018, Pierce and Tomcko 2003). Similarly, body condition should respond positively because there is more prey available to the remaining Northern Pike (Casini et al. 2011). If nAg further effects Northern Pike beyond survivability, I predict that growth and body condition of Northern Pike should hold constant or decline. By contrast, if there is no effect of nAg on Northern Pike, I predict an increase in growth and body condition.

LITERATURE REVIEW

Regarded as “the material of the 21st century”, nanomaterials have unique designs and properties which lends themselves useful in a variety of applications (Akter et al. 2017). Of all nanoproducts produced, nanosilver (nAg) is considered one of the most important (Akter et al. 2017). nAg is a highly effective anti-microbial agent, capable of controlling the growth of microorganisms on surfaces by releasing small amounts of ionic silver (Akter et al. 2017, Benn et al. 2010). nAg is defined as being a particle of silver ranging from 1 – 100nm in dimension and has a very broad application of uses (Benn et al. 2010). Nearly 30% of all products containing nanomaterials have nAg incorporated into them (Reidy et al. 2013). Items that are known to use nAg include but are not limited to: textiles (T-shirts, socks, underwear, sports clothing etc.) food packaging, medical devices (wound dressings, dental hygiene, infection treatment, surgical meshes, orthopedics, vascular prothesis, catheters), water treatment, washing machines, paints, cosmetics, sunscreens, and electronics (Reidy et al. 2013, Akter et al. 2017). With a list this extensive, concerns over its potential for environmental release have been raised. Concerns over the potential impacts of nAg have led to a significant increase in research regarding the release of nAg from products, detection of nAg in aquatic environments and potential for environmental toxicological effects (McGillicuddy et al. 2017).

Considering the ever-growing presence of nAg in consumer products, it is understandable that researchers may be wary of its impacts to the environment. Ionic silver is shown to have major toxic effects on aquatic life such as; inhibition of respiration in microorganisms, poor growth in aquatic plants, inhibition of the Na+/K+-ATPase pump at the gills in fish and ultimately death in more sensitive species (Howe & Dobson 2002). This begs two questions of the use of nAg; first, if nAg can enter our waterways, how and how much is making its way there, and second, how does nAg behave and what affects does it have on the aquatic environment? Studies have shown that nAg in consumer products do in fact leach into the wastewater. Benn and Westerhoff (2008) washed 6 types of socks to quantify the release of nAg. They discovered that each sock released some of its silver into the water, but at different rates and percentages. The amount of silver in the 500ml wash water samples ranged from 1.5 to 650 µg of silver. Some socks released just 1% after 4 washes, others released 100%. Another study by Benn et al. (2010) tested various household items (Athletic shirt, unfinished cloth fabric, medical mask, medical cloth, toothpaste, shampoo, detergent, yellow cloth/towel, and a teddy bear) to gain an understanding of their nAg releases into water, air and soil. Water testing again yielded high degrees of variability in release of nAg. The face mask released 0.01% of its silver content, the athletic shirt released 2% of its content, and the consumables (detergent, shampoo, toothpaste) were assumed to release 100% of theirs. Both studies noted that silver particulates in the wash water samples ranged in sizes, as some silver particles could pass through 20nm filters and others could not pass through 100nm filters. This suggests that once leached, the nanoparticles can agglomerate and form larger associations, or dissolve into ionic silver. It is estimated that one household will deposit 470 µg of nAg into the sewer system daily

(Benn et al. 2010). Once in the wastewater treatment plant, most nAg can be removed and settled with biosolids. The fate of these biosolids varies between incineration, landfill deposition, or agricultural fertilizer. This gives nAg three potential pathways into aquatic environments: missed removal at the wastewater plant, leaching from disposables and biosolids in landfills or surface runoff from agricultural fertilizer sources.

Depending on several factors, nAg can have several fates once in aquatic ecosystems (McGillicuddy et al. 2017). These include: staying in suspension, aggregating/ agglomerating, dissolving or reacting with other species present in the environment (McGillicuddy et al. 2017). To study the environmental fate of nAg in boreal lake ecosystems, mesocosm tests of low, medium, and high chronic (over time) nAg addition, and two pulse (one shot) addition tests were designed (Furtado et al. 2015). In each of the chronic additions the total silver measured was always less than total silver added to the mesocosm (Furtado et al. 2015). Silver was found in a particulate or colloidal state 99% ± 7% of the time, with the remaining trace amounts being found in periphyton or the top 2cm of the sediment horizon (Furtado et al. 2015). The results of the pulse additions show nAg concentrations drop sharply at first but then slowly dissipates from the water column (Furtado et al. 2015). Similar to the chronic additions, silver was most commonly found in a particulate or colloidal form, with small amounts occupying periphyton and sediment layers. (Furtado et al. 2015). Across all experiments, proportion of nAg was highest in the water column, followed by sediments, and lastly periphyton.

It is interesting to note that depending on the mesocosm, between 44%-77% of nAg was recovered, suggesting that some other form or function is working to sequester nAg. Working off the same mesocosm study, the effects of nAg exposure on zooplankton and phytoplankton communities were observed (Vincent et al. 2017). Zooplankton communities in chronic additions all decreased in richness (~11 sp. to ~4sp.), increased in abundance (as much as 4x greater in high chronic vs control) and ranged in biomass (increase in low chronic and control, decrease in medium and high chronic). Pulse addition testing on zooplankton lowered species richness, biomass and abundance. Despite a 98% reduction of the pigment, chlorophyll *a,* in the pulse addition after the first week, a significant difference in phytoplankton community composition between the control and pulse addition was not observed until the final week (Vincent et al. 2017). No significant results were observed on the phytoplankton community composition between the control and chronic tests. Communities of bacterioplankton also experienced no adverse effects were demonstrated (Blakelock et al. 2016).

The findings of Vincent et al. (2017) clearly show that an interaction with nAg is occurring at the microplankton level. Specific examples of this interaction have been studied more closely involving the use of *Daphnia magna*. Individuals examined showed that nAg could attach to body appendages, accumulate under the carapace, and be ingested and accumulate nAg in the gut (Asghari et al. 2012). Toxicity tests of nAg on *Daphnia magna* show that when algal food sources are present, survivability is three-fold higher than when a food source is absent, however, the increase in survivability can largely be attributed to the ability of algae to remove nAg from the water, rather than alterations to nutrition of *Daphnia magna* (Conine and Frost 2016). These studies all show that nAg can be taken up by micro-organisms living in the water, and the interaction varies between positive, negative and neutral effects based on type of microorganism, species and treatment type. This may impact ecosystem function, as phytoplankton and zooplankton are at the bottom of the food web and their ability to ingest and retain nAg particles means that they may be able to pass it along to higher trophic levels such as the fish community when consumed (Asghari et al. 2012, Vincent et al. 2017).

Various tests have also been performed in lab settings to quantify the effects of nAg on the physiological processes of fish. Yellow Perch exposed to nAg will accumulate nAg in the liver, gills and muscle tissues, with highest concentrations being found in the liver (Martin et al. 2017). Metallothionein (MT), a protein which is directly involved in detoxification of metal cations, is a sensitive biomarker and was observed to rapidly increase in production during the fish's exposure period (Martin et al. 2017). Oxidative stress was also observed in Yellow Perch, indicated by increased levels of lipid peroxidation (contributor to loss of cellular function) in the gills (Martin et al. 2017). When exposed to nAg, Rainbow Trout observed no discernable differences in metabolic rates, however showed significant stress as elevated levels of cortisol were detected in the blood and significant silver accumulation in muscle tissues was observed (Murray et al. 2017b). Despite elevated stress imposed on Rainbow Trout over a 28-day period, no significant responses in growth metrics occurred (Murray et al. 2017a). These preliminary tests have shown that nAg registers a physiological response in fish, but under short periods it is not enough to detect a response in growth or metabolism in fish at the individual level.

To better understand the impact of nAg on aquatic ecosystems, a whole-lake study was carried out at the IISD – ELA research facility. Nanosilver was added to Lake 222 at environmentally relevant concentrations over the course of the 2014 and 2015 field season (Hayhurst 2018). A bioenergetic evaluation of the Yellow Perch community in Lake 222 was performed and compared to a reference community in Lake 239 (Hayhurst 2018). Consumption and metabolism in Yellow Perch declined, however growth, body condition and abundance were not affected by nAg. Mean concentrations of silver in the livers of Yellow Perch and Northern Pike rose from ~4 nanograms per gram of wet weight (ng/g-w-wt.) to ~500 ng/g-w-wt. and ~2400 ng/g-w-wt. respectively (Martin et al. 2018). Levels of toxicity in the liver were significantly higher than gills and muscle tissues of Northern Pike, suggesting that biomagnification may be occurring. Additionally, Northern Pike in Lake 222 showed declines in population during treatment years followed by an increase in survival in the first recovery year (Figure 1).

Northern Pike (*Esox lucius*) is a species of fish with a large global distribution (Harvey 2009). In North America, its range covers the extent of Canada, save for a portion British Columbia and the Maritimes (Harvey 2009). While being reduced from what it once was, Northern Pike are still an important commercial fish to Canada and are an immensely popular sport fish due to its size, distribution, abundance and ease of accessibility to pike holding waters (Harvey 2009, Forsman et al. 2015). Northern Pike are also an emerging model organism for ecological and evolutional study purposes because they are a large, long lived, iteroparous, top-predator capable of occupying a broad range of aquatic environments (Forsman et al. 2015). Northern Pike have several different structures that lend themselves useful to age determination. Forsman et al.

(2015) lists the operculum, cleithrum and otolith as structures capable of providing ages for individual fish. An alternative structure, the fin ray, is regarded as an adequate structure to use in age determination of fishes for several reasons. First and foremost, collection of fin rays is a non-lethal way of acquiring ageing structures from the fish, making it an excellent option for studies involving mark and recapture objectives. Secondly, age determination and validation comparisons from readers of varying levels of experience and comparisons between fin rays and otoliths have produced relatively accurate and precise levels of agreement across different species, justifying its use as an acceptable ageing structure (Mills and Chalanchuk 2004, Glass et al. 2011, Rude et al. 2012). It should be mentioned however that they are considered better for use in spiny rayed fish than soft rayed fish and a certain level of experience or knowledge of interpretation is required to accurately assess ages (P. Drombolis, Pers. Comm., Alberta Environment and Sustainable Resource Development 2013). Fin rays unlike other structures do not have a point of origin, instead they form a “noodle-like” centre in which annuli begin to form around (P. Drombolis, Pers. Comm.). As the fish grows older and somatic growth slows, the bands of annuli tend to stack closely to one another, increasing the difficulty of interpretation (Rude et al. 2012, Alberta Environment and Sustainable Resource Development 2013). The first annuli of older fishes may also be resorbed, leading to under estimation in ages (Rude et al. 2013, M. Rennie in lecture BIOL 4212, March 8, 2019). In addition, false annuli may form during the summer growth period due to stress or quality of the growing season (P. Drombolis, Pers. Comm., Alberta Environment and Sustainable Resource Development 2013). When combining age data and growth metrics, we can analyze and view data in several ways, including size-at-age relationships and explore different factors that affect an individuals' growth over time (Forsman et al. 2015).

Two important factors that affect growth in Northern Pike consist of prey availability and population density. Prey availability has been shown to significantly effect the rate and efficiency in which Northern Pike can grow at. Studies suggest that Yellow Perch are the favoured forage species of juvenile Northern Pike and early growth is significantly faster when higher abundances of Yellow Perch are present in the lake (Kennedy et al 2018, Margenau et al. 1998). While Yellow Perch are beneficial to juveniles, adults of the population require larger bodied prey species to maintain efficient growth. Lake Herring (Cisco, *Coregonus artedi*), are an energy dense, pelagic prey species that can satisfy the increasing energetic costs of an adult Northern Pike (Kennedy 2017). Kennedy (2017) observed greater asymptotic lengths of Northern Pike in lakes with Cisco present, demonstrating the species ability to use generalist foraging strategies to maintain efficient growth.

The second factor influencing growth of Northern Pike is density. Northern Pike are a species in which negative density dependence occurs (Pierce et al. 2003). Individuals in populations with lower densities have the ability to grow faster and reach larger sizes due to less competition in the system. Conversely, individuals residing in populations of higher densities must deal with more competition, thus slowing growth and reaching smaller sizes.

In addition to standard growth measurements of length and weight, a third, generalized measure can be used to examine the relationship that exists between length and weight. This relationship is referred to as body condition and is a useful measure that describes the body size of a fish at a given length and describes the health of a fish (Rennie and Verdon 2008). Several experiments have shown that body condition tends to scale positively with food availability (Rennie et al. 2019, Casini et al. 2011, Rennie and Verdon 2008). When prey available to a predator increases, whether by a direct increase in abundance to the prey community or by predation release of the top predator, the predator is able to capture the surplus of prey available and increase their condition (Rennie et al. 2019, Casini et al. 2011)

MATERIALS AND METHODS

2.1 STUDY SITE

This study was performed at the International Institute for Sustainable Development – Experimental Lakes Area (Figure 2). Lake 222 was selected to be the treatment lake where environmentally-relevant doses of nAg were added, and Lake 239 to be the reference lake as it is a good representation of naturally occurring lakes in the region. Lake 239 had no additions of nAg and has not been subject to any other manipulations in years prior. Both lakes have wild populations of Yellow Perch and Northern Pike and are deep enough for stratification to occur. Sampling was performed over 6-years (2012-2017) in which 2 the first two years (2012/13) were dedicated to gathering baseline data. A total of 15kg of nAg was added to Lake 222 throughout the ice-free seasons of 2014 (9kg) and 2015 (6kg). No additional product was added in the final two years (2016/17). A general before-after-control-impact study design was implemented to evaluate the impacts of nAg on Northern Pike growth.

2.2 FISH SAMPLING

Northern Pike were captured in this study using 3 methods: trap-netting, seine-netting and angling. Capture effort was performed three times each season, occurring in spring, summer, and fall. Captured fish were anaesthetized using a buffered solution of tricaine methanesulfonate (TMS; Argent Chemical Laboratories Inc., Redmond, WA., U.S.A.) and lake water. Field sampling procedures included measuring fork and total lengths (in millimetres) and weight (in grams). If possible, sex of spring caught fish were determined. The leading 1-3 fin rays of the pectoral fin were clipped, and Northern Pike were fixed with a 9-mm electronic Passive Integrated Transponder (PIT) tag upon first capture to allow for identification in subsequent recaptures.

2.3 FIN RAY PREPARATION

Fins rays collected from individual Northern Pike were placed in scale envelopes and set to dry. Upon removal from the envelope, a portion was trimmed off the distal end of the fin ray for stable isotope analyses. Cut and dried fins were placed on squares of parafilm (a non-stick surface) and uniquely labelled to distinguish one another. A cold cure epoxy (System Three Resins ®) was mixed in a small Dixie cup using a 2:1 ratio of epoxy resin to hardener. Once stirred thoroughly the epoxy was poured over the fins and then fins were set to cure for 24 hours. 5 cross-sectional cuts of each fin were taken using an Isomet low speed saw. A pre-cut was made to the tip of each fin ray before taking sections to clear the fin of any roughness produced from the field sampling process. Cross sections were approximately 0.5mm – 0.6mm thick, a value achieved by rotating the saws' dial 95mm (1 full rotation plus 35mm). Each cross section was rinsed to clear any dust off and then placed on a frosted microscope slide (Thermo Fisher Scientific®). Slides were prepared using Cytoseal 60 (Thermo Fisher Scientific ®) and sealed with a cover slip. The final step was to set the fins in a fume hood to cure for an additional 24 hours.

2.4 AGE DETERMINATION

An initial run through of age determination was completed with a Motic® SMZ-168 series microscope which had a Canon® Rebel T5 Camera attached to allow for images of each fin to be captured. A second ageing event using a higher magnification microscope (Zeiss® Axio Lab A1) was performed to double check ages assigned in the first pass. Fin ray ages were interpreted by examining annuli formed during two periods of growth in the fin, summer growth and winter growth. Under reflected, incandescent light, the periods of fast summer growth appear white and the slow winter growth appears dark (Alberta Environment and Sustainable Resource Development 2013). The combination of these two growth periods would constitute 1 full year of growth/life, therefore, counting annuli to determine an age was done so by reading the outermost edge of the winter growth band. To help determine a level of accuracy and validity of ages assigned, an age validation test was conducted. A sample size of 20 fins with a pre-existing age assigned (aged by Ken Mills from the Department of Fisheries and Oceans) were blindly re-aged independently by myself and Paul Drombolis, an experienced ager with the Ontario Ministry of Natural Resources Upper Great Lakes Management Unit (UGLMU). This validation test sought to calibrate my ability of producing accurate ages for the study.

2.5 DATA ANALYSIS

Once fins were assigned an age, the data was separated into their respective age class to evaluate changes in size at a specific age over time. Descriptive statistics of sample size, mean, standard deviation and standard error were performed on fork length (mm), weight (g), and body condition for each sample year among the age class represented. Body condition was estimated as an index of the length/weight ratio and was calculated using Fulton's Condition Factor:

$$K = W * L^{-3} * 10^{7} \tag{1}$$

where W = weight in grams, L = a measure of length (in this case fork length was used) in mm, and 10^{7} was applied as a scaling constant. Initial observations showed that age classes 2-5 were best represented across the 6 years, so further analyses would be focused on these four age groups (Appendix A).

To determine if nAg had a significant impact on the growth of Northern Pike in Lake 222, A 2-way ANOVA (both factors fixed) test was selected. ANOVA's were run using IBM's® Statistical Package for Social Sciences (SPSS) program. The tests compared the two fixed factors of year and lake against a response variable of either weight, fork length or body condition over the four age classes. In total, 12 ANOVA's would be run to determine if a significant interaction was observed between lakes*years, between years or between lakes, indicating a different response over time between the experimental system and the reference system (Smokoroski & Randall 2017). Significance (*p*) values were compared against a confidence level of 95% ($\alpha = 0.05$). if $p > \alpha = 0.05$, no significant interaction was observed (fail to reject H_0). Conversely, if $p < \alpha = 0.05$, then a significant interaction between factors was observed (reject H_0). Full statistical output tables are listed in (Appendix B).

RESULTS

3.1 AGE TEST

Across a sample size of 20 fins, age validation testing showed a 60% exact agreement and 90% agreement within one year between the age I assigned a sample and the pre-determined age of the sample (Figure C 1), and 55% exact agreement and 100% agreement within one year between my assigned age and Paul's assigned age (Figure C2). Paul's assigned age compared to the pre-determined age agreed exactly only 35% of samples but was 100% agreeance within one year. Agreement tends to falter slightly as the fish get older, but enough consistency remains to validate a level of confidence in the ages I assign throughout the remainder of the dataset. When agreement was not exact, the ages both Paul and I produced were consistently below the previously determined age (Figure C 1, Figure C3).

3.2 FORK LENGTH

Mean values of fork length in Lake 239 tend to follow an overall increasing trend over time across all age classes, whereas Lake 222 follows a decreasing trend (Figure 3 W, X, Y, Z). Lake 222 appears to have similar means throughout the first 3 years of the study before experiencing precipitous declines in fork length. This trend is viewable across all age classes but is best exemplified in age class four and age class five (Figure 3 Y & Z). Mean size in age class four Northern Pike decreases 58.15mm between 2013 and 2015, and 85.28mm between 2013 and 2016 in Lake 222, whereas mean length in Lake 239 from 2012 to 2015 holds steady (Figure 3 Y). Similarly, the mean fork length in Lake 222's age class five decreases by 64.0mm and 82.56mm between 2012-2015 & 2012-2017 respectively (Figure 3 Z). Between 2012 – 2015/17 mean fork length of Lake 239's age class five is observed to gain ~30mm (+32.25mm to 2015, +27.92mm to 2017). ANOVA tests for each year class yield significant interactions (2yr: $F_{3,40} = 3.503$, $p = 0.005$, 3yr: $F_{3,61} = 3.965$, $p = 0.012$, 4yr: $F_{4,84} = 5.549$, $p = 0.00$, 5yr: $F_{5,73} = 4.512$, $p = 0.031$) , indicating that fork length responds differently over time between the experimental and reference lakes (Appendix B: Table B 1- Table B4).

3.3 WEIGHT

Fluctuations in weight of age classes over the 6-year study period follow the same patterns observed in fork length. Lake 239 experiences an overall increase in weight over time at each age class whereas Lake 222 experiences declines (Figure 4 W, X, Y, Z). Northern Pike at age class three from 2012-17 in Lake 222 decrease 126.14g in mean weight, while Lake 239's age class three gains 103.55g in mean weight (Figure 4 X). Age classes four and five in Lake 222 display the greatest reductions in mean weight, dropping 350g between 2013 to 2016 for age class four and 444.50g between 2012 to 2016 for age class five (Figure 4 Y & Z). Lake 239 Northern Pike increased in mean weight by 290g in age class four and 190g in age class five from 2013 to 2017 (Figure 4 .Y & Z) ANOVA tests for each year class yield significant interactions (2yr: $F_{3,39} = 4.955$, $p = 0.024$, 3yr: $F_{3,61} = 3.931$, $p = 0.012$, 4yr: $F_{4,84} = 6.176$, $p = 0.001$, 5yr:

$F_{5,70} = 2.62$, $p = 0.001$), indicating that weight responds differently over time between the experimental and reference lakes (Appendix B: Table B5 - Table B8).

3.4 BODY CONDITION

Mean body condition within all age classes over time did not fluctuate significantly across the study period (Figure 5 W, X, Y, Z). The lake factor played the most significant role in variation of body condition as different values were seen between the lakes, but consistent values were seen across years. As a result, ANOVA tests showed that body condition responded differently between the experimental and reference lakes but did not respond differently over time (2yr: $F_{1,39} = 13.631$, $p = 0.001$, 3yr: $F_{1,61} = 9.048$, $p = 0.004$, 4yr: $F_{1,84} = 14.990$, $p = 0.00$, 5yr: $F_{1,70} = 22.290$, $p = 0.00$).

DISCUSSION

I observed clear reductions in the size at age of Northern Pike exposed to nAg and no similar response in our reference lake. This finding is consistent with my hypothesis that if nAg were to have further effects beyond survivability of Northern Pike, growth would be reduced or held constant. Both fork length and weight experienced significant declines from their pre-addition levels in all age classes examined. Body condition did not decline across the years. Fish aged at four and five years old across the study period exhibited the greatest declines in growth. Four-year-old fish after additions had a mean size 85mm smaller than the mean size before addition, and mean weight declined 350g from pre-addition to post-addition. Mean fork length of five-year-old fish declined 96mm during the study period and weight declined 445g from mean weights observed before nAg additions took place. These results are consistent with previous studies examining metal exposure to growth of fishes (Sherwood et al. 1999), where Yellow Perch grew almost three times slower in lakes exposed to heavy metals of copper, cadmium and zinc than reference lakes without exposure to these heavy metals. While specific growth rates were not examined in the present study, our result conveys similar information from Sherwood et al (1999), in that metal exposure slows growth, resulting in smaller size-at-ages in fish. Studies looking directly at impacts to growth from exposure to nAg have so far been focused on shorter time periods and have found no effect of nAg on growth. Griffitt et al. (2012) and Murray et al. (2017a) both examined effects of nAg on growth across a 28-day study period, using Sheepshead Minnows

(*Cyprinodon variegatus*) and Rainbow Trout respectively. Juvenile Sheepshead Minnows were seen to increase slightly in growth while Rainbow Trout declined slightly. Both studies however had insignificant statistical results when examining this relationship. It was acknowledged that longer time periods of exposure should be used to obtain more thorough assessments on changes to growth rates. Our study examined size-at-age over time across two years of nAg exposure throughout the open water seasons. We observed suppressions to growth during this period and in the following two years after nAg additions were discontinued. Notably, the decline in growth was observed despite a 20% reduction to population size (Figure 1) (Hayhurst 2018). This long-term exposure experiment provides the most thorough assessment to date, evaluating the long-term impacts of nAg exposure at environmentally relevant concentrations in an ecosystem setting.

Previous knowledge of Northern Pike population size and growth trends suggest that growth is slower in more dense populations, and growth is faster in less dense populations (Pierce et al. 2003). When considering this, if a population were reduced from an equilibrium, in theory, the remaining Northern Pike should experience an increased growth rate from the release of competition. Hayhurst et al. (2018) showed that survivability in Lake 222 decreased during the addition period, and therefore population size of Northern Pike also decreased (mid 200s in 2014 down to 190s in 2016). if nAg had no further effects, one would expect the growth rates of the remaining individuals to increase throughout this period. Since we observed reduced fork length and weight-at-age over time, this study suggests that nAg has further effects on Northern Pike as the trend in growth was opposite to what would be expected.

An interesting result produced from the study showed that body condition in Northern Pike did not decline across years, but instead differed significantly between lakes. Studies have shown that body condition in fishes positively correlates with prey availability (Rennie et al. 2019, Casini et al. 2016).When the amount of available prey to a predator increases, whether by a direct increase in prey abundance or by reduced predator populations (predation release), the predator is able to take advantage of the surplus prey and improve their body condition (Rennie et al. 2019, Casini et al. 2016). Hayhurst et al. (2018) reported that growth, condition, and importantly abundance of Yellow Perch was unaffected by nAg additions, indicating that the main forage species for Northern Pike was not a limiting factor, particularly considering the declines in Northern Pike abundance. The lack of change in body condition throughout the nAg exposure period suggests that Northern Pike are utilizing the surplus prey available to maintain their condition with pre-exposure levels but are not able to increase their body condition as previous literature suggests they would be able to. This likely means that by some direct internal energetic process, nAg is limiting the conversion efficiency of Northern Pike.

Sherwood et al. (1999) noted that consumption rates of Yellow Perch in the presence of heavy metals were similar to consumption rates of Yellow Perch in reference lakes, yet the overall growth slowed, again, indicating a reduction in conversion efficiency. Part of this reduction can be attributed to increased activity costs because Yellow Perch did not switch from benthic to piscivorous diets until much later in life (Sherwood et al. 1999). However, further analysis showed that direct effects of metal exposure also occurred, causing increased metallothionein production as a detoxification response and decreased their capacity to secrete cortisol and thyroid hormones, which are key hormones to metabolic regulation (Campbell et al. 2003). In the case of Yellow Perch, the reduced ability to control metabolism likely caused impairments to their conversion efficiency. Comparatively, Murray et al. (2017b) reported that nAg increased cortisol levels but did not alter the metabolic rate of Rainbow Trout, a finding they acknowledge disagrees with literature suggesting that cortisol impacts metabolism. Metabolic impacts to fish mediated by nAg exposure have been observed when additional stresses such as gill damage are imposed on the fish. The literature suggests that metal exposure elicits a range of physiological effects in fishes which can have direct effects on metabolism, resulting in reduced conversion efficiencies and ultimately suppressed growth (Campbell et al. 2003, Sherwood et al. 1999). When evaluating effects of nAg on fishes, we see similar physiological responses that are also induced by other metals, and these responses have the same ability to alter metabolism and conversion efficiency (Murray et al. 2017a, Murray et al. 2017b, Martin et al. 2018 Martin et al. 2017, Griffitt et al. 2012, Farmen et al. 2011). The results of the study show that when faced with a declining population and increased prey availability to Northern Pike, growth is limited, and body condition does not improve, suggesting that nAg directly impairs the conversion efficiency of Northern Pike by affecting internal energetic processes such as metabolism.

The significant difference observed in body condition between waterbodies can be explained by food web availability, as Lake 222 only has three fish species present; Northern Pike, Yellow Perch and Blacknose Shiner (Table 1). In comparison, Lake 239 has seven different fish species present, most notably, Northern Pike, Yellow Perch and

Lake Herring (Table 1). The greater mean body condition seen in Lake 239 across age classes is likely a result of the greater fish community, and specifically the presence of Lake Herring (Kennedy et al. 2018). When Lake Herring are available, Northern Pike will shift their diet to target this species. In comparison to Yellow Perch, Lake Herring are a larger and more energy dense prey, providing Northern Pike that can make the dietary switch the benefits of reduced foraging costs and maximized energy gain (Kennedy et al. 2018). Northern Pike in Lake 239 are able to switch their foraging effort to Lake Herring as they grow, whereas Northern Pike in Lake 222 cannot make this shift.

A potential issue that may be identified with the results of this study is the sample size. While adequate sampling of the lakes was performed across the study years, once the ages were assigned, only age classes two – five were considered for the study, leaving out the one and six plus age classes. Additionally, the number of samples at a specific age in a specific year are variable, a quick look through appendix A confirms this. Consider the data collected for Lake 222. We see more total samples in age class four and five compared to age class two and three and likely this is why the trends are more pronounced in these two age classes. Sample numbers within an age class are variable from year to year, with the greatest number of samples occurring in 2012, 2015, and 2017. While lack of data is usually problematic, it is important to note the significance of these years as 2012 represents pre-addition, 2015 represents the second year of addition, and 2017 represents the second year after additions were discontinued. Age class three in Lake 222 is a good example of this year to year sample size variability. 2012, 2015, and 2017 are relatively well represented, however 2013, 2014 and 2016 are not, but this is ok because when looking at 2012, 2015, and 2017 we are able to see that general decreasing size-at-age trend persist over time. cohort analysis was not performed because the study window was deemed too short to accurately analyze the cohorts of Northern Pike in Lake 222. Compared to other studies, the sample size of our age validation test was smaller, which resulted in higher levels of exact and within one year's agreement between agers.

Across a sample size of 20 fins, age validation testing showed a 60% exact agreement and 90% agreement within one year between the age I assigned a sample and the pre-determined age of the sample, and 55% exact agreement and 100% agreement within one year between my assigned age and Paul's assigned age. Interestingly enough, Paul's assigned age compared to the pre-determined age agreed exactly only 35% of samples but was 100% agreeance within one year. Age validation testing using pectoral fins of smallmouth bass have relayed similar, but different results across larger sample sizes (n=72) (Rude et al. 2013). Between arbitrarily designated reader experience levels, high reader experience (3-4 years) and low experience (< 1 year) exact agreement was 26% and within one-year agreement was 58%, and high to moderate agreement was 54% exact and 92% within one year (Rude et al. 2013). Despite the lack of exact agreeance between Paul's age and the predetermined age, I have a relatively adequate agreeance between both, suggesting my results lie somewhere between the two more experienced agers. Under small sample sizes the result of our validation testing suggests my ability to provide a relatively accurate age is adequate to confer a level of trust in the ages that I have determined for the remainder of the samples

CONCLUSION

In summary, my research concludes that the presence of nAg impacted Northern Pike beyond a reduction in population size as previously reported. Mean fork length size at age over time and mean weight at age over time were seen to reduce significantly after nAg was added to Lake 222 and no similar change was observed in the reference lake. Mean body condition held constant over the study period, indicating that food availability was likely not the driving factor behind the declines in growth, but rather an effect on the Northern Pike's conversion efficiency likely brought about by increased energetic costs such as metabolism. It is important to note that ages determined in this study were validated by independent readers, which helps to confer a degree of confidence to the ages reported in the study. Growth following the addition of nAg did not immediately rebound, indicating further monitoring of the system may be necessary to determine ecosystem recovery from nAg additions.

Table 1. Comparison of physical parameters between Lake 222 and Lake 239

Characteristic	Parameter	Lake 222	Lake 239
Physical	Area	16.39 hectares	54.28 hectares
	Inflow(s)/Outflow	1/1	3/1
	Maximum Depth	6.3 metres	30.4 metres
	Residence Time	1.2 years	9.3 years
	Secchi Depth	2.2 metres	4.8 metres
	State	Oligo/Mesotrophic	Oligotrophic
	Volume	7.2×10^5 m^3	5.9 x 106 m3
Biological	Fish Species	Northern Pike (*Esox lucius*)	Northern Pike (*Esox lucius*)
		Yellow Perch (*Perca flavescens*)	Yellow Perch (*Perca flavescens*)
		Blacknose Shiner (*Notropis heterolepis*)	Iowa Darter (*Etheostoma exile*)
			Lake Herring (*Coregonus artedi*)
			Lake Trout (*Salvelinus fontinalus*)
			Slimy Sculpin (*Cottus cognatus*)
			White Sucker (*Catostomus commersoni*)

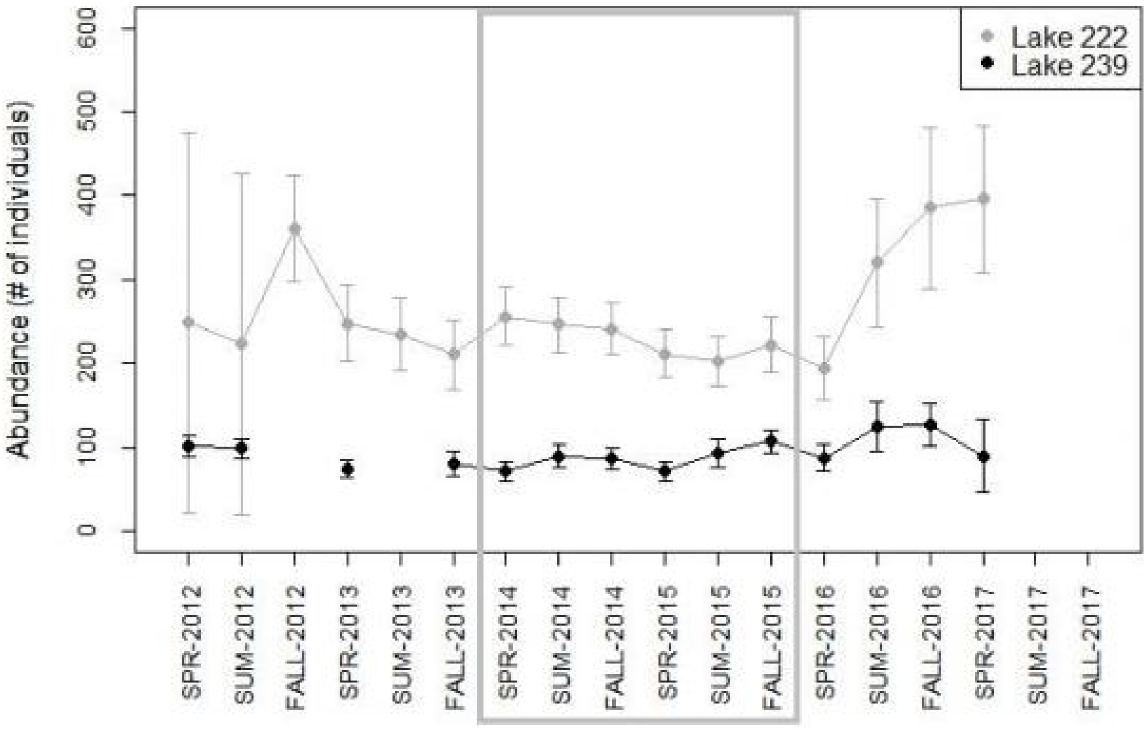

Figure 1. Top “Study Period” open estimates for Northern Pike in Lake 222 and Lake 239, from 2012 to 2017 as reported in Hayhurst (2018)

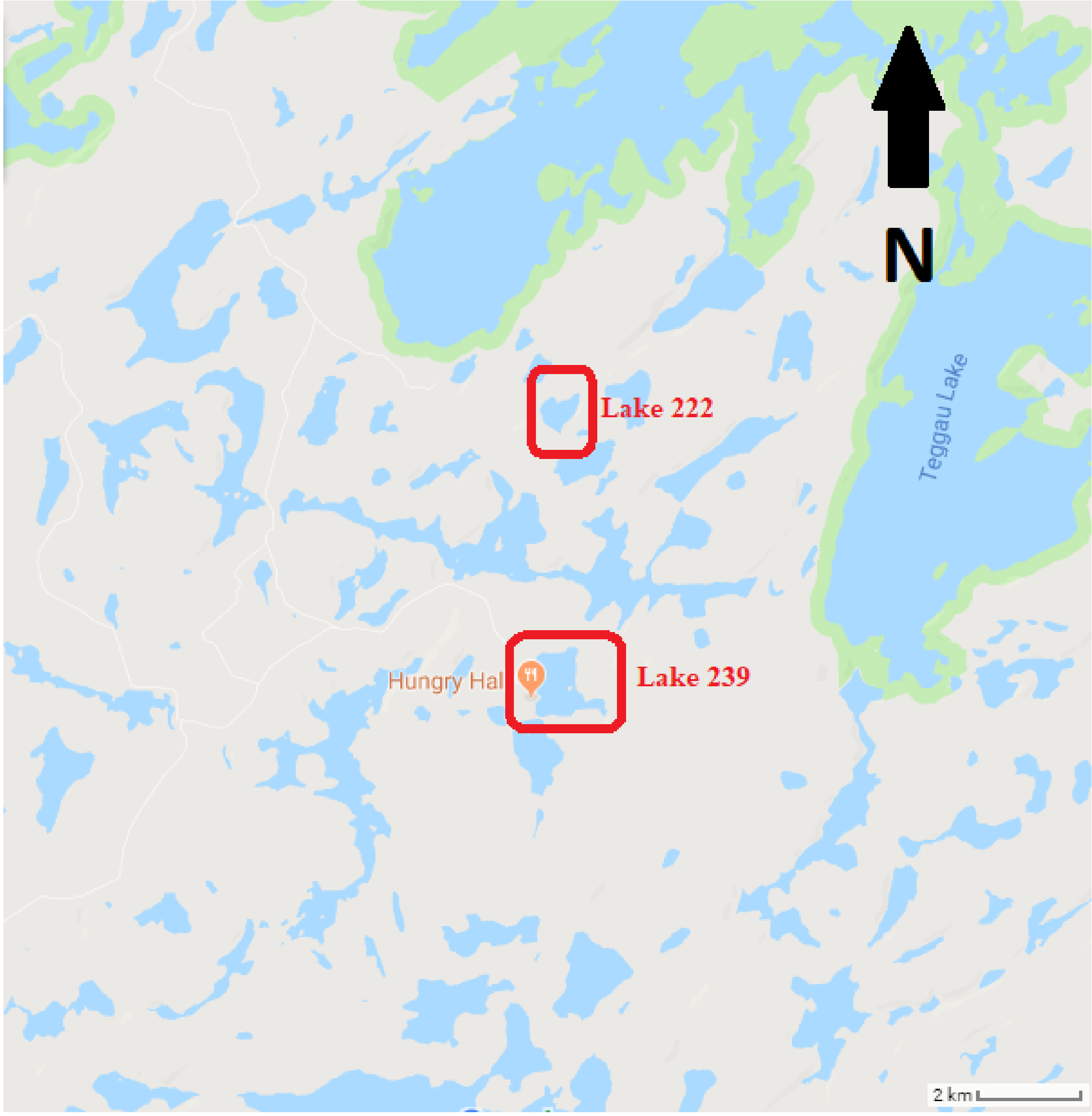

Figure 2. Map of Study Area (IISD – ELA). Study lakes, Lake 222 and Lake 239, are highlighted

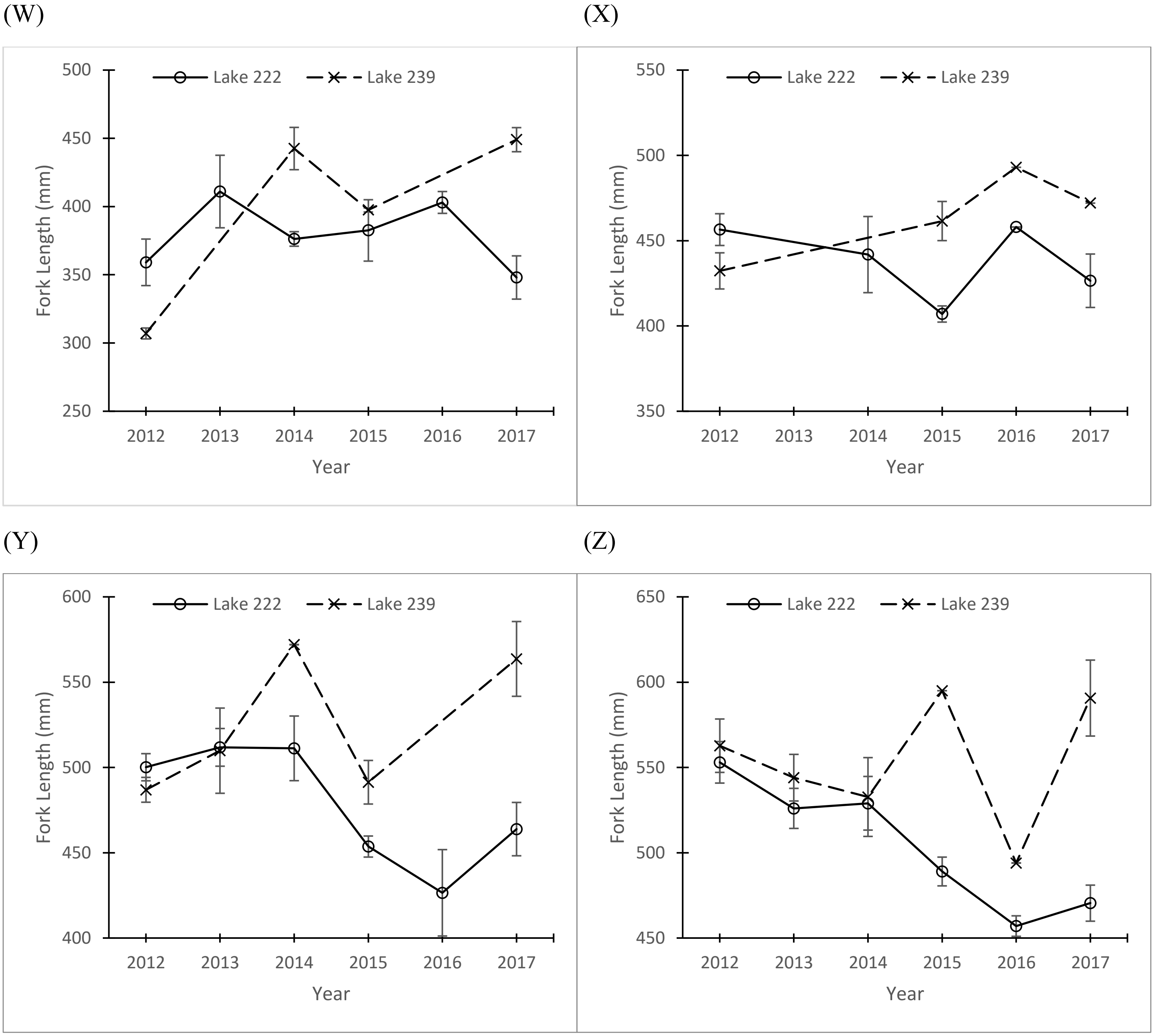

Figure 3. Fork length (mm) of Northern Pike age groups across the 6-year study period. (W) 2-year-old fish, (X) 3-year old fish, (Y) 4-year old fish, (Z) 5-year old fish

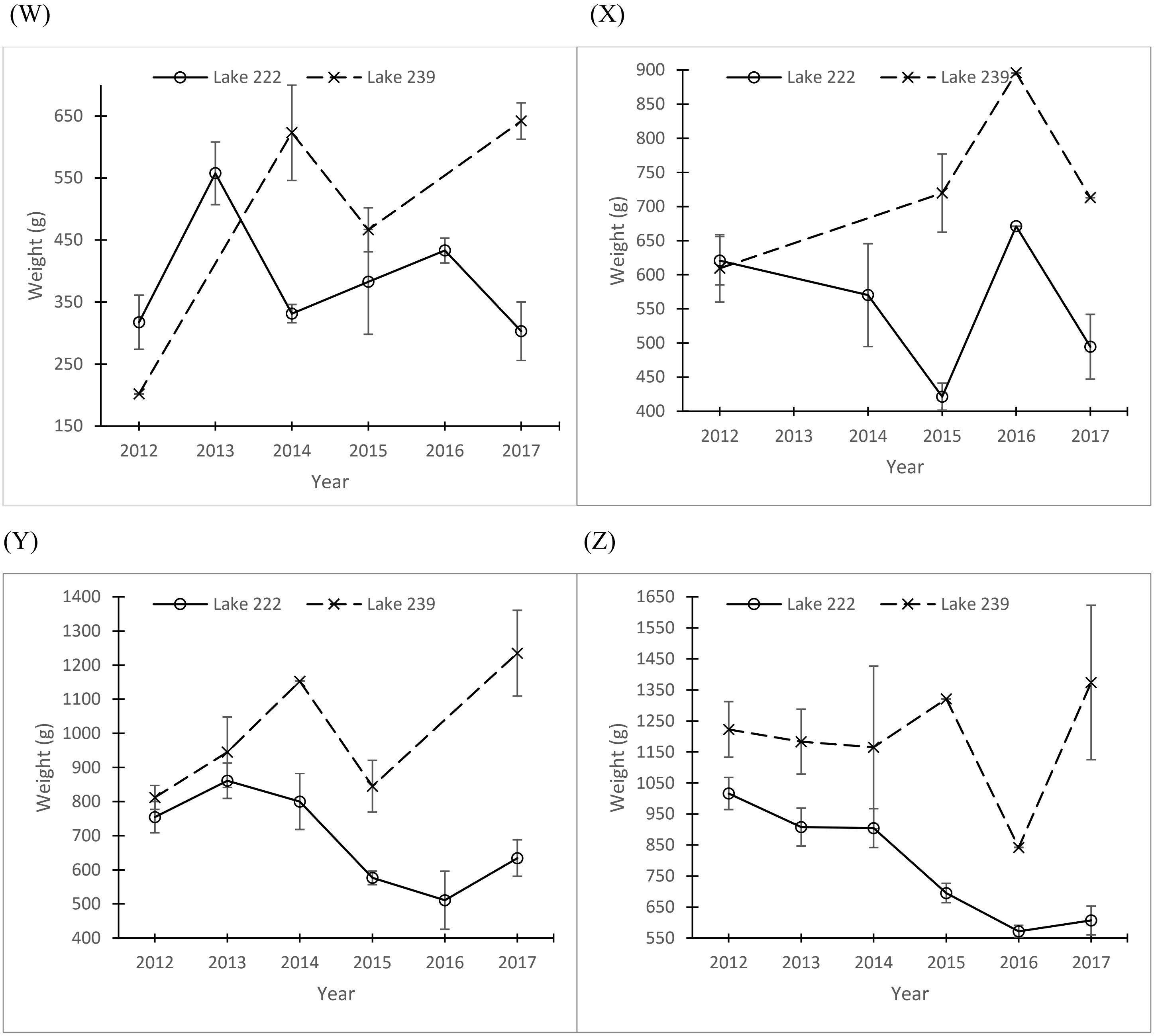

Figure 4. Weight (g) of Northern Pike age groups across the 6-year study period. (W) 2-year-old fish, (X) 3-year old fish, (Y) 4-year old fish, (Z) 5-year old fish

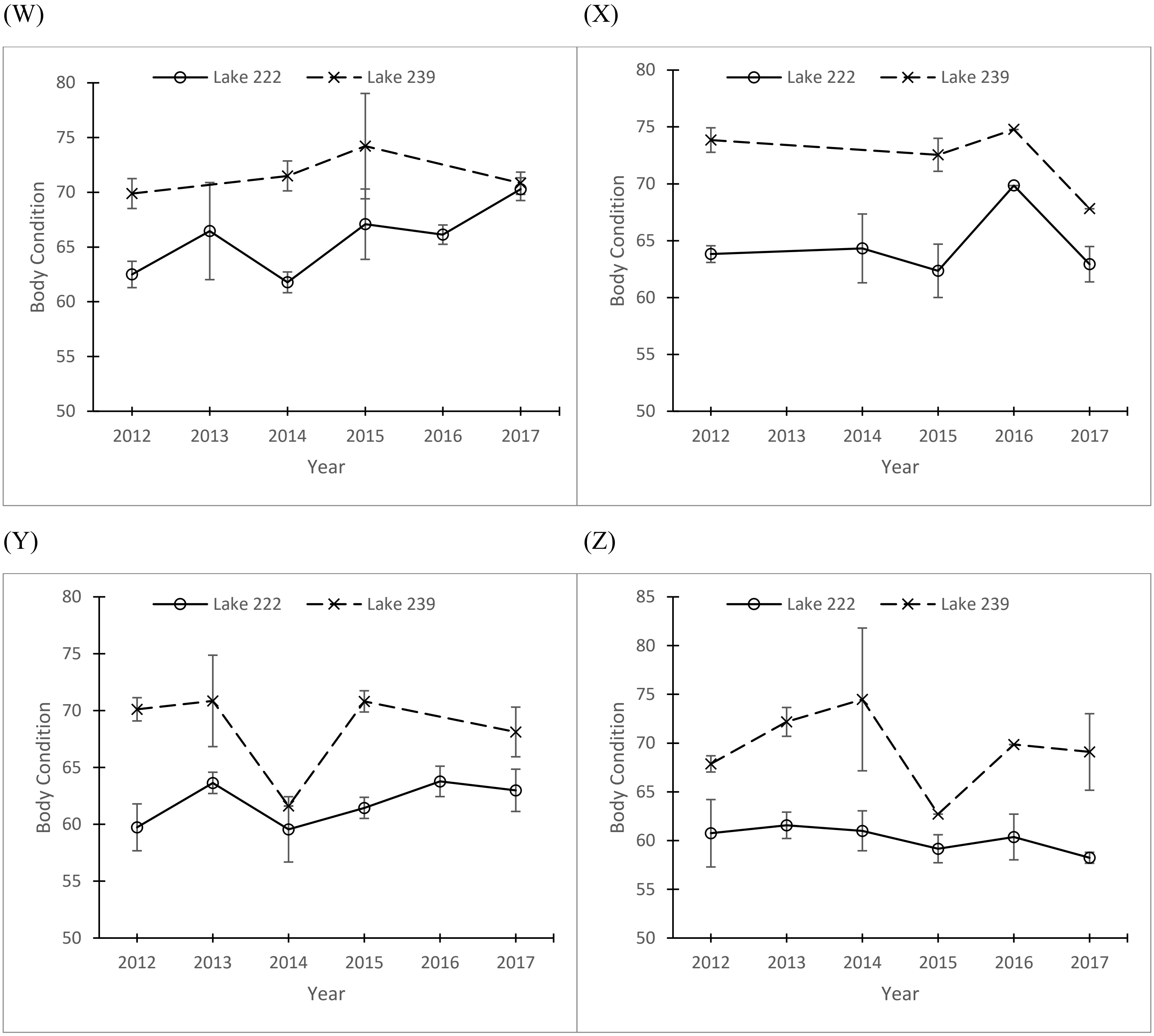

Figure 5. Body condition of Northern Pike age groups across the 6-year study period. (W) 2-year-old fish, (X) 3-year old fish, (Y) 4-year old fish, (Z) 5-year old fish

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APPENDICES

APPENDIX A

Appendix A displays the descriptive statistics performed in the study. All data within a table is representative of one age across the study period (age remains constant as year increases) Mean values were calculated for fork length (FLEN), weight (RWT) and body condition (BCD). Additionally, standard deviation and standard error were calculated. Finally, a count of samples was performed. These calculations were executed for each year of the study.

Table A1. Descriptive statistics of year 2 age group from Lake 222

	Lake 222					
	2012	2013	2014	2015	2016	2017
mean FLEN	359.12	411.00	376.25	382.50	403.00	348.00
standard dev	70.31	46.03	18.44	31.82	11.31	31.70
standard error	17.05	26.58	5.32	22.50	8.00	15.85
# of samples	17.00	3.00	12.00	2.00	2.00	4.00
Mean RWT	317.47	557.50	331.33	382.50	433.00	303.00
standard dev	179.70	71.42	51.09	119.50	28.28	94.39
standard error	43.58	50.50	14.75	84.50	20.00	47.19
# of samples	17.00	2.00	12.00	2.00	2.00	4.00
mean BCD	62.49	66.46	61.78	67.09	66.13	70.29
standard dev	4.97	6.27	3.28	4.55	1.25	2.08
standard error	1.20	4.43	0.95	3.21	0.88	1.04
# of samples	17.00	2.00	12.00	2.00	2.00	4.00

Table A2. Descriptive statistics of year 3 age group from Lake 222

Lake 222						
	2012	2013	2014	2015	2016	2017
mean FLEN	456.48		441.83	407.00	458.00	426.50
standard dev	46.57		54.68	17.09	#DIV/0!	38.35
standard error	9.31		22.32	4.74	#DIV/0!	15.66
# of samples	25.00		6.00	13.00	1.00	6.00
Mean RWT	620.64		570.17	421.31	671.00	494.50
standard dev	177.29		184.72	71.56	#DIV/0!	116.25
standard error	35.46		75.41	19.85	#DIV/0!	47.46
# of samples	25.00		6.00	13.00	1.00	6.00
mean BCD	63.82		64.32	62.35	69.84	62.93
standard dev	3.67		7.41	8.46	#DIV/0!	3.81
standard error	0.73		3.03	2.35	#DIV/0!	1.56
# of samples	25.00		6.00	13.00	1.00	6.00

Table A3. Descriptive statistics of year 4 age group from Lake 222

Lake 222						
	2012	2013	2014	2015	2016	2017
mean FLEN	500.13	511.78	511.20	453.63	426.50	463.86
standard dev	31.85	33.14	42.37	32.07	50.67	41.36
standard error	7.96	11.05	18.95	6.17	25.33	15.63
# of samples	16.00	9.00	5.00	27.00	4.00	7.00
Mean RWT	754.38	861.00	800.20	576.19	510.75	634.43
standard dev	182.92	155.53	183.33	103.43	170.33	141.43
standard error	45.73	51.84	81.99	19.91	85.16	53.45
# of samples	16.00	9.00	5.00	27.00	4.00	7.00
mean BCD	59.73	63.64	59.55	61.45	63.77	62.99
standard dev	8.24	2.81	6.42	4.83	2.67	4.92
standard error	2.06	0.94	2.87	0.93	1.34	1.86
# of samples	16.00	9.00	5.00	27.00	4.00	7.00

Table A4. Descriptive statistics of year 5 age group from Lake 222

	Lake 222					
	2012	2013	2014	2015	2016	2017
mean FLEN	553.00	526.00	529.00	489.00	457.00	470.44
standard dev	36.50	37.11	35.16	34.77	19.00	33.40
standard error	12.17	11.73	15.72	8.43	6.01	10.56
# of samples	9.00	10.00	5.00	17.00	10.00	10.00
Mean RWT	1016.00	907.70	904.40	695.18	571.50	606.60
standard dev	155.43	193.04	140.12	128.68	54.66	146.33
standard error	51.81	61.04	62.66	31.21	19.33	46.27
# of samples	9.00	10.00	5.00	17.00	8.00	10.00
mean BCD	60.75	61.57	61.00	59.16	60.37	58.24
standard dev	10.37	4.28	4.58	5.92	6.63	1.79
standard error	3.46	1.35	2.05	1.44	2.34	0.57
# of samples	9.00	10.00	5.00	17.00	8.00	10.00

Table A5. Descriptive statistics of year 2 age group from Lake 239

	Lake 239					
	2012	2013	2014	2015	2016	2017
mean FLEN	307.00		442.50	397.50		449.00
standard dev	5.66		21.92	2.12		17.64
standard error	4.00		15.50	1.50		8.82
# of samples	2.00		2.00	2.00		4.00
Mean RWT	202.00		623.00	466.50		641.75
standard dev	0.00		108.89	50.20		58.68
standard error	0.00		77.00	35.50		29.34
# of samples	2.00		2.00	2.00		4.00
mean BCD	69.88		71.50	74.22		70.82
standard dev	3.86		1.93	6.80		2.03
standard error	1.37		1.37	4.81		1.02
# of samples	8.00		2.00	2.00		4.00

Table A6. Descriptive statistics of year 3 age group from Lake 239

	Lake 239					
	2012	2013	2014	2015	2016	2017
mean FLEN	432.27			461.50	493.00	472.00
standard dev	35.15			28.12	#DIV/0!	#DIV/0!
standard error	10.60			11.48	#DIV/0!	#DIV/0!
# of samples	11.00			6.00	1.00	1.00
Mean RWT	609.45			719.67	896.00	713.00
standard dev	163.62			140.29	#DIV/0!	#DIV/0!
standard error	49.33			57.28	#DIV/0!	#DIV/0!
# of samples	11.00			6.00	1.00	1.00
mean BCD	73.85			72.55	74.78	67.81
standard dev	3.58			3.55	#DIV/0!	#DIV/0!
standard error	1.08			1.45	#DIV/0!	#DIV/0!
# of samples	11.00			6.00	1.00	1.00

Table A7. Descriptive statistics of year 4 age group from Lake 239

	Lake 239					
	2012	2013	2014	2015	2016	2017
mean FLEN	486.89	509.83	572.00	491.33		563.63
standard dev	21.84	61.17	#DIV/0!	22.12		61.98
standard error	7.28	24.97	#DIV/0!	12.77		21.91
# of samples	9.00	6.00	1.00	3.00		8.00
Mean RWT	812.11	944.67	1153.00	845.00		1235.00
standard dev	105.37	253.26	#DIV/0!	131.41		355.19
standard error	35.12	103.39	#DIV/0!	75.87		125.58
# of samples	9.00	6.00	1.00	3.00		8.00
mean FLEN	70.11	70.85	61.61	70.81		68.12
standard dev	3.07	9.85	#DIV/0!	1.62		6.18
standard error	1.02	4.02	#DIV/0!	0.93		2.18
# of samples	9.00	6.00	1.00	3.00		8.00

Table A8. Descriptive statistics of year 5 age group from Lake 239

Lake 239						
	2012	2013	2014	2015	2016	2017
mean FLEN	562.75	544.00	532.67	595.00	494.00	590.67
standard dev	44.25	38.64	40.02	#DIV/0!	#DIV/0!	38.55
standard error	15.65	13.66	23.10	#DIV/0!	#DIV/0!	22.26
# of samples	8.00	8.00	3.00	1.00	1.00	3.00
Mean RWT	1222.63	1183.38	1165.00	1321.00	842.00	1374.00
standard dev	253.64	295.70	453.66	#DIV/0!	#DIV/0!	352.14
standard error	89.68	104.55	151.22	#DIV/0!	#DIV/0!	249.00
# of samples	8.00	8.00	9.00	1.00	1.00	2.00
mean BCD	67.86	72.17	74.48	62.71	69.84	69.09
standard dev	2.35	4.18	12.69	#DIV/0!	#DIV/0!	5.55
standard error	0.83	1.48	7.32	#DIV/0!	#DIV/0!	3.93
# of samples	8.00	8.00	3.00	1.00	1.00	2.00

APPENDIX B

Appendix B displays the results produced from running 2 factor ANOVA tests in IBM’s SPSS. Each table examines one variable of fork length, weight, or body condition of one year group compared against the fixed factors of year and location (Lake 222 or Lake 239).

Table B 1. Results of the ANOVA test examining the dependent variable *fork length* in age 2 Northern Pike

Tests of Between-Subjects Effects

Dependent Variable: FORK LENGTH

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	53581.765[a]	9	5953.529	2.569	0.020
Intercept	3645222.368	1	3645222.368	1573.157	0.000
LOCATION_ID	6409.320	1	6409.320	2.766	0.104
YEAR_CATCH	33693.383	5	6738.677	2.908	0.025
LOCATION_ID * YEAR_CATCH	24352.892	3	8117.631	3.503	0.024
Error	92685.515	40	2317.138		
Total	7295004.000	50			
Corrected Total	146267.280	49			

a. R Squared = .366 (Adjusted R Squared = .224)

Table B2. Results of the ANOVA test examining the dependent variable *fork length* in age 3 Northern Pike

Tests of Between-Subjects Effects

Dependent Variable: FORK LENGTH

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	29596.316[a]	8	3699.540	2.396	0.026
Intercept	4275803.042	1	4275803.042	2769.707	0.000
LOCATION_ID	3466.381	1	3466.381	2.245	0.139
YEAR_CATCH	4260.123	4	1065.031	0.690	0.602
LOCATION_ID * YEAR_CATCH	18363.472	3	6121.157	3.965	0.012
Error	94170.255	61	1543.775		
Total	13728618.000	70			
Corrected Total	123766.571	69			

a. R Squared = .239 (Adjusted R Squared = .139)

Table B3. Results of the ANOVA test examining the dependent variable *fork length* in age 4 Northern Pike

Tests of Between-Subjects Effects

Dependent Variable: FORK LENGTH

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	116950.467[a]	10	11695.047	7.780	0.000
Intercept	10398887.678	1	10398887.678	6917.739	0.000
LOCATION_ID	14641.019	1	14641.019	9.740	0.002
YEAR_CATCH	31255.978	5	6251.196	4.159	0.002
LOCATION_ID * YEAR_CATCH	33367.963	4	8341.991	5.549	0.001
Error	126270.523	84	1503.221		
Total	22867877.000	95			
Corrected Total	243220.989	94			

a. R Squared = .481 (Adjusted R Squared = .419)

Table B4. Results of the ANOVA test examining the dependent variable *fork length* in age 5 Northern Pike

Tests of Between-Subjects Effects

Dependent Variable: FORK LENGTH

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	131953.778[a]	11	11995.798	9.628	0.000
Intercept	11212400.661	1	11212400.661	8998.792	0.000
LOCATION_ID	24541.618	1	24541.618	19.696	0.000
YEAR_CATCH	22101.351	5	4420.270	3.548	0.006
LOCATION_ID * YEAR_CATCH	28107.093	5	5621.419	4.512	0.001
Error	90957.233	73	1245.989		
Total	22753648.000	85			
Corrected Total	222911.012	84			

a. R Squared = .592 (Adjusted R Squared = .530)

Table B5. Results of the ANOVA test examining the dependent variable *weight* in age 2 Northern Pike

Tests of Between-Subjects Effects

Dependent Variable: WEIGHT

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	657337.542[a]	9	73037.505	4.617	0.000
Intercept	4425689.433	1	4425689.433	279.743	0.000
LOCATION_ID	135774.075	1	135774.075	8.582	0.006
YEAR_CATCH	348290.733	5	69658.147	4.403	0.003
LOCATION_ID * YEAR_CATCH	235159.378	3	78386.459	4.955	0.005
Error	617001.152	39	15820.542		
Total	8244693.000	49			
Corrected Total	1274338.694	48			

a. R Squared = .516 (Adjusted R Squared = .404)

Table B6. Results of the ANOVA test examining the dependent variable *weight* in age 3 Northern Pike

Tests of Between-Subjects Effects

Dependent Variable: WEIGHT

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	665425.663[a]	8	83178.208	3.573	0.002
Intercept	8690722.902	1	8690722.902	373.286	0.000
LOCATION_ID	150764.993	1	150764.993	6.476	0.013
YEAR_CATCH	101432.080	4	25358.020	1.089	0.370
LOCATION_ID * YEAR_CATCH	274537.831	3	91512.610	3.931	0.012
Error	1420182.923	61	23281.687		
Total	25729987.000	70			
Corrected Total	2085608.586	69			

a. R Squared = .319 (Adjusted R Squared = .230)

Table B7. Results of the ANOVA test examining the dependent variable *weight* in age 4 Northern Pike

Tests of Between-Subjects Effects

Dependent Variable: WEIGHT

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3581275.995[a]	10	358127.599	11.385	0.000
Intercept	28973482.716	1	28973482.716	921.097	0.000
LOCATION_ID	812090.190	1	812090.190	25.817	0.000
YEAR_CATCH	640105.717	5	128021.143	4.070	0.002
LOCATION_ID * YEAR_CATCH	777015.077	4	194253.769	6.176	0.000
Error	2642253.311	84	31455.397		
Total	61409096.000	95			
Corrected Total	6223529.305	94			

a. R Squared = .575 (Adjusted R Squared = .525)

Table B8. Results of the ANOVA test examining the dependent variable *weight* in age 5 Northern Pike

Tests of Between-Subjects Effects

Dependent Variable: WEIGHT

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4863924.384[a]	11	442174.944	11.796	0.000
Intercept	36911456.146	1	36911456.146	984.732	0.000
LOCATION_ID	1532934.364	1	1532934.364	40.896	0.000
YEAR_CATCH	521524.835	5	104304.967	2.783	0.024
LOCATION_ID * YEAR_CATCH	491007.263	5	98201.453	2.620	0.031
Error	2623861.921	70	37483.742		
Total	72214123.000	82			
Corrected Total	7487786.305	81			

a. R Squared = .650 (Adjusted R Squared = .595)

Table B9. Results of the ANOVA test examining the dependent variable *body condition* in age 2 Northern Pike

Tests of Between-Subjects Effects

Dependent Variable: BODY CONDITION

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	792.778[a]	9	88.086	5.165	0.000
Intercept	104747.821	1	104747.821	6142.084	0.000
LOCATION_ID	232.460	1	232.460	13.631	0.001
YEAR_CATCH	124.502	5	24.900	1.460	0.225
LOCATION_ID * YEAR_CATCH	87.986	3	29.329	1.720	0.179
Error	665.111	39	17.054		
Total	210263.401	49			
Corrected Total	1457.889	48			

a. R Squared = .544 (Adjusted R Squared = .439)

Table B10. Results of the ANOVA test examining the dependent variable *body condition* in age 3 Northern Pike

Tests of Between-Subjects Effects

Dependent Variable: BODY CONDITION

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1394.614[a]	8	174.327	6.183	0.000
Intercept	98032.772	1	98032.772	3477.185	0.000
LOCATION_ID	255.092	1	255.092	9.048	0.004
YEAR_CATCH	82.560	4	20.640	0.732	0.574
LOCATION_ID * YEAR_CATCH	32.818	3	10.939	0.388	0.762
Error	1719.782	61	28.193		
Total	309279.104	70			
Corrected Total	3114.396	69			

a. R Squared = .448 (Adjusted R Squared = .375)

Table B11. Results of the ANOVA test examining the dependent variable *body condition* in age 4 Northern Pike

Tests of Between-Subjects Effects

Dependent Variable: BODY CONDITION

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1467.600[a]	10	146.760	4.322	0.000
Intercept	177523.005	1	177523.005	5227.917	0.000
LOCATION_ID	509.021	1	509.021	14.990	0.000
YEAR_CATCH	153.891	5	30.778	0.906	0.481
LOCATION_ID * YEAR_CATCH	99.992	4	24.998	0.736	0.570
Error	2852.366	84	33.957		
Total	390433.955	95			
Corrected Total	4319.966	94			

a. R Squared = .340 (Adjusted R Squared = .261)

Table B12. Results of the ANOVA test examining the dependent variable *body condition* in age 5 Northern Pike

Tests of Between-Subjects Effects

Dependent Variable: BODY CONDITION

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1994.952[a]	11	181.359	5.037	0.000
Intercept	159888.529	1	159888.529	4440.822	0.000
LOCATION_ID	802.531	1	802.531	22.290	0.000
YEAR_CATCH	195.041	5	39.008	1.083	0.377
LOCATION_ID * YEAR_CATCH	95.573	5	19.115	0.531	0.752
Error	2520.299	70	36.004		
Total	328333.542	82			
Corrected Total	4515.251	81			

a. R Squared = .442 (Adjusted R Squared = .354)

APPENDIX C

Appendix C displays the agreement relationships of the blind ageing tests conducted. A total of 20 samples were considered for the validation test. X and Y values indicate the age assigned from the examiner. Values within the figure are not age values, but rather a count of how many fins were aged as X and Y. The diagonally ascending boxes indicate exact agreement between examiners.

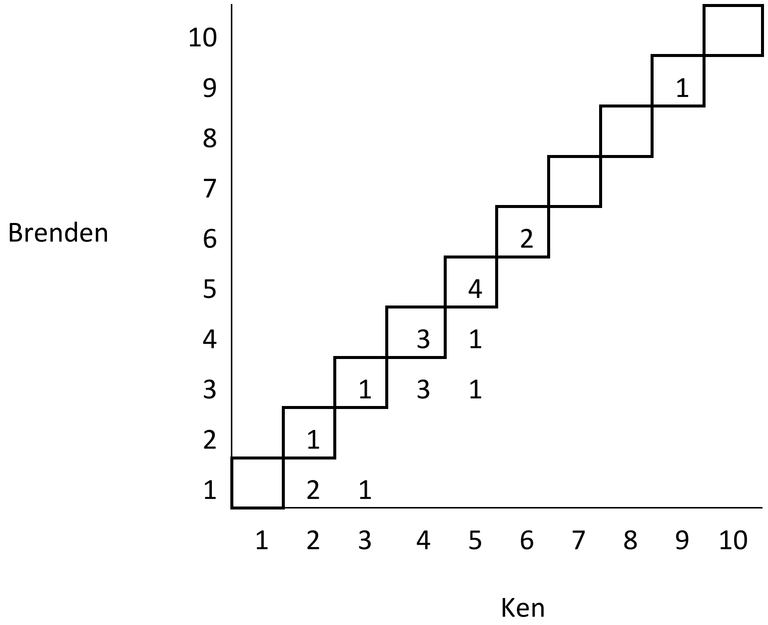

Figure C 1. Age validation test comparing readership agreement between Ken and Brenden

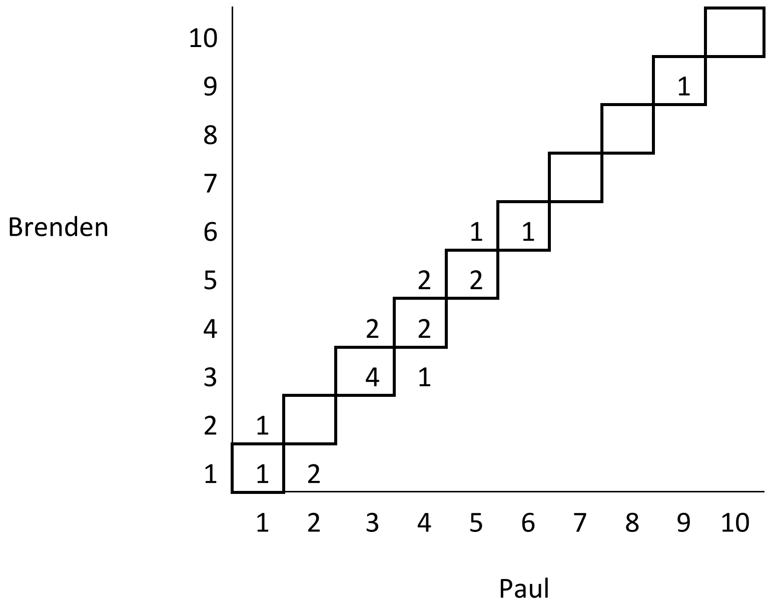

Figure C2. Age validation test comparing readership agreement between Paul and Brenden

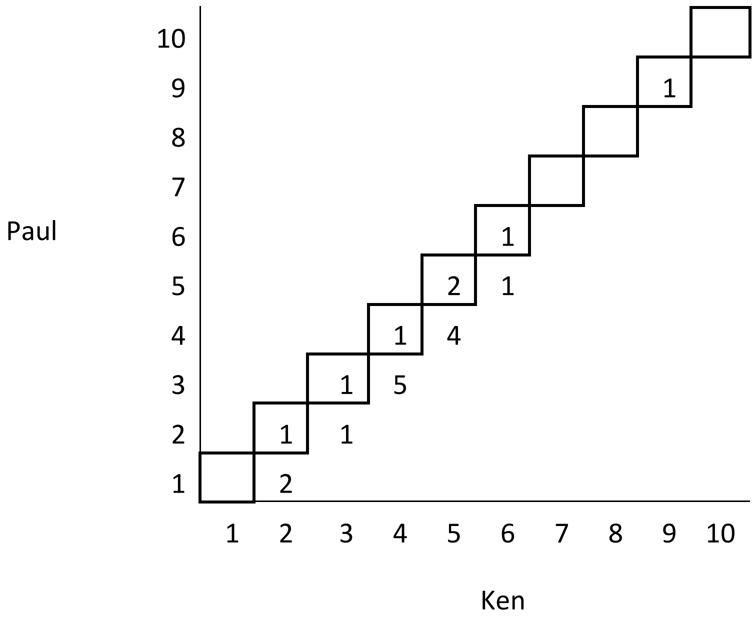

Figure C3. Age validation test comparing readership agreement between Ken and Paul