Bioenergetic evaluation of a whole-lake nanosilver addition on Yellow Perch (*Perca flavescens*)

by

Lauren D. Hayhurst

A Thesis submitted to the Faculty of Graduate Studies of
Lakehead University
In partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

Department of Biology
Lakehead University
Thunder Bay, Ontario, Canada

Abstract

Nanosilver (nAg) is an antibacterial and antimicrobial agent. Its wide use in hundreds of commercial products and industrial applications suggests a high potential for release into the environment. Previous research indicates that nAg induces different physiological responses in aquatic organisms, with different toxicological thresholds than ionic silver, indicating that nAg may require a separate regulatory framework for policy on environmental release. As part of a collaborative nAg addition study (environmental concentrations = 1-15µg/L), conducted at the IISD-Experimental Lakes Area, I evaluated changes in Yellow Perch (*Perca flavescens*) bioenergetics at the individual-level, and extrapolated modelled rates to the population-level for comparison before, during, and after whole-lake nAg addition. Condition and abundance of predatory Northern Pike (*Esox lucius*) were also examined. Results were compared to a nearby unmanipulated reference lake monitored over the same period. Perch consumption and total metabolism decreased during and after nAg addition in the experimental lake. Activity levels became increasingly variable with nAg addition, but decreased on average. Growth rates and conversion efficiency appeared unaffected in both lakes. Abundance and condition of perch remained constant over the study. By contrast, survivability of pike increased after nAg addition ceased, however, condition did not improve. Gross consumption of zooplankton and benthic invertebrates by perch declined during and after nAg addition. This study evaluated fish effects in relation to the rest of the ecosystem – achievable only through whole-lake experimentation. Based on these results, nAg appears to have had significant adverse impacts on fish during the two years of exposure.

Keywords: Abundance; Activity; BACI Design; Bioenergetics Modelling; Condition; Consumption; Diet; Growth; IISD-ELA; Nanosilver; Northern Pike (*Esox lucius*); Stress; Total Metabolism; Yellow Perch (*Perca flavescens*).

Acknowledgments

Profound thanks and immense admiration to my supervisor Dr. Michael Rennie, and super committee members Dr. Joseph Carney, Dr. Robert Mackereth, and External Examiner Dr. Chris Metcalfe. Thank you to my funding sources who financed my numerous field seasons, particularly: Canada Research Chair Grant; CUPE Local 3905 Professional Development Bursary; Douglas Lindsay Memorial Graduate Bursary; Lakehead University's Graduate Student Professional Development Bursary; and Lakehead University's Water, Land, and People Award. Majorly, this work was supported by Mitacs through the Mitacs Accelerate Program.

This study would not have been possible without the world-renowned IISD-Experimental Lakes Area Inc. (IISD-ELA). Immense thanks to past and present IISD-ELA staff, for their continuous support and enthusiasm towards the study. Particularly, Executive Director Matthew McCandless, and scientists Chandra Rodgers, Lee Hrenchuk, Ken Sandilands, Michael Paterson, Scott Higgins, Sonya Havens, and Vince Palace, who are all absolute inspirations and powerhouses of knowledge in their fields. Special thanks to Marnie Potter and Sandy Chalanchuk, for their legendary passion, dedication and spirit, as well as Operations staff, including John Neall, Gerald Fitzgerald, Roger Mollot, and Mark Lyng, who were always available to remedy slimy situations.

Thanks to the Trent University researchers who were paramount in the development and orchestration of the Lake Ecosystem Nanosilver (LENs) Project at the IISD-ELA. A round of drinks and appreciation to the LENs Crew, including Andrea Conine, Daniel Rearick, Jonathan Martin, Joseph Tonin, Graham Blakelock, Katarina Cetinic, and Jennifer Vincent. These individuals permitted my piggy-backing on their whole-ecosystem experiment and were very accommodating and knowledgeable in their trophic level focuses. I wish them all the best of luck in their current endeavours and future pursuits.

IISD-ELA runs on the hard work and science-based passion of their phenomenal students and volunteers – many of whom assisted in multiple parts of my study from 2015 to 2017. These individuals provided all manners of assistance, from picking and sorting benthic invertebrates, to netting and processing fish during all hours of the day – often in inclement weather while battling incessant biting insects. I am extremely thankful for the time and effort contributed by these students and volunteers. Thanks to the upbeat attitudes and second-to-none kick-netting skills of the "Bug-Pickers" (alphabetical by first name): AJ Chapelsky, Andrea Conine, Annie Wheeler, Daniel Gilchrist, Daniel Rearick, Jonathan Martin, Joseph Tonin, Marcus Threndyle, Philip Anderson, Sherri DeGasparro, and Stefano Strapazzon. Additional thanks and immense appreciation to the rain-or-shine eagerness and quick-netting of the "Fish-Squishers" (alphabetical by first name): AJ Chapelsky, Aaron Klymasz-Swartz, Andrea Conine, Andrew Milling, Annie Wheeler, Brandon Vennell, Brendan Allan, Chandra Rodgers, Cody Jackson, Daniel Gilchrist, Daniel Rearick, Danielle Chiasson, David Callaghan, Doug Allan, Dylan Lyng, Graydon McKee, Harpreet Johal, Jamie Dearnley, Jay Siemens, Jeannie Pau, Justin Budyk, Justin Hubbard, Jonathan Martin, Joseph Tonin, Katie Chan, Kelsey Friesen, Kyle Ritchie, Lauren Timlick, Lee Hrenchuk, Marcus Threndyle, Michael Rennie, Patrick Kennedy, Patrique Bulloch, Paul Fafard, Philip Anderson, Richard Grosshans, Roger Mollot, Ryan Nykvist, Sandy Chalanchuk, Scott Bergson, Sherri DeGasparro, Stefano Strapazzon, Stephen Paterson, Steve McGovarin, and Yifan Yang.

To Lakehead University faculty, especially Technicians Daniel Brazeau and Emma Lehmberg, for their wonderful attitudes and assistance – not to mention timely advice and unwavering support. Thanks to Technicians Michael Moore and Dr. Susanne Walford, for dropping everything to help me track down random pieces of vaguely described lab equipment. Finally, to the Professors Douglas Morris, Joseph Carney, Michael Rennie and Stephen Hecnar, Researcher

Darlene Hecnar, and Administrative Assistant Eleanor Maunula for sharing their passions and knowledge, and supporting me through the entirety of my Lakehead University experience. I wish you all the best as you continue to challenge, empower, and inspire students.

Thanks to Lakehead University's Community Energetics and Ecology (CEE) Laboratory Members, particularly: Brandon Vennell, Brendan Allan, Brennan Deboer, Graydon McKee, Marissa Wegher, Patrick Kennedy, Stephen Slongo, Victoria Langen, and honourary CEE Lab member Cody Dey. Additional thanks to the graduate students I had the pleasure of learning alongside and experiencing "Superior by Nature" Thunder Bay with, including Ashley Nemec-Bakk, Brenda Huska, Cassandra Eckman, Heidi Forsyth, Joe Boyle, Lyndon Letwin, and Peter Villa. Thanks to Tyson Bouchard for endlessly rescuing me from the floor outside locked labs. Also, to my undergraduate field and laboratory assistants, Brandon Vennell and Brennan Deboer, who built so much character by grinding fish, processing rancid samples, and identifying fish guts.

Special thanks to Susan Mann, Fisheries Ageing Biologist at the Northwest Biodiversity and Monitoring Ageing Laboratory in Dryden, Ontario, for her en-route expertise and finesse of fish ageing techniques. Special thanks to Johane Joncas, Lakehead University Environmental Laboratory Manager, for her within-range standards (reference materials) and meticulous attention to detail. Extra thanks to Philip Peterson, Service Engineer at ATS Scientific for getting the Direct Mercury Analyser off the ground, and Maarit Wolfe for accommodating my weigh boat adventures in calibration curves and sample burning. Thanks to Jane Kirk, Environment and Climate Change Canada Laboratory, Burlington, Ontario, and Flett Research Ltd., Winnipeg, Manitoba, for participating in an inter-laboratory calibration for the Direct Mercury Analyser. Special thanks to Patricia Ramlal for her sample searching and shipping efforts. Additional thanks, to Brennan Deboer, Emianka Sotiri, Jackson Tsuji, and Laura Murray for contributing data to this study.

To my grandparents, Marianne and Peter Hayhurst, and my parents, Christine and Tony Hayhurst, for their endless encouragement, unconditional love, and vast support of my bush-traipsing and fish-catching. I am confident that after these past four years, they can describe my mercury-analysis-to-nanosilver-impact process as well as I can. Finally, to Erin Ward, Jamie Dearnley, Jordan Diakur, Justin Budyk, and Peter Villa – you know what you are to me and I love you for it.

Table of Contents

LIST OF TABLES 6

LIST OF APPENDICES 6

LIST OF FIGURES 7

1 Introduction 8
- 1.1 Background 8
- 1.2 Lake Ecosystem Nanosilver (LENs) Project 14
- 1.3 Fish Energetics 15
- 1.4 General Objectives 16

2 Methods 20
- 2.1 Study Site 20
- 2.2 Study Design 20
- 2.3 Nanosilver Additions 20
- 2.4 Individual-Level Mercury Mass Balance and Bioenergetics Modelling 22
- 2.5 Field Sampling Procedures 24
  - 2.5.1 Fish 24
  - 2.5.2 Mark-Recapture 26
  - 2.5.3 Benthic Invertebrates and Zooplankton 27
  - 2.5.4 Water Temperatures 28
- 2.6 Laboratory Dissections 29
- 2.7 Ageing Analysis 29
- 2.8 Gut Content Analysis 31
- 2.9 Methylmercury Analysis 32
- 2.10 Direct Mercury Analysis 35
- 2.11 Energy Density Analysis 36
- 2.12 Population Estimation 36
- 2.13 Proportion Calculations by Size Class 38
- 2.14 Population-Level Bioenergetics 39
- 2.15 Statistical Analysis 40
  - 2.15.1 Individual-Level Analysis 40
  - 2.15.2 Population-Level Analysis 41

3 Results 43
- 3.1 Individual-Level Results 43
  - 3.1.1 Yellow Perch Diet Composition 43
  - 3.1.2 Yellow Perch Length-at-Age 46
  - 3.1.3 Yellow Perch Consumption, Growth, and Conversion Efficiency 48
  - 3.1.4 Yellow Perch Activity, Standard Metabolic Rate, and Total Metabolism 52
- 3.2 Population-Level Results 55
  - 3.2.1 Northern Pike Population Estimates 55
  - 3.2.2 Yellow Perch Population Estimates 59
  - 3.2.3 Northern Pike and Yellow Perch Condition 60
  - 3.2.4 Yellow Perch Size Class Proportions 62
  - 3.2.5 Yellow Perch Gross Consumption Estimates 64

4 Discussion 67

APPENDICES 77

LITERATURE CITED 91

LIST OF TABLES

Table 1.1. Total Silver Concentrations Measured from Water Quality Samples in Canada, from 2008 to 2013. 9
Table 1.2. Predictions of Indirect and Direct Effects of a Whole-Lake Nanosilver Addition on Yellow Perch. 17
Table 2.1. Target Numbers of Yellow Perch Euthanized per Year in Lake 222 and Lake 239. 26
Table 2.2. Seasonal Fin Nicks for Yellow Perch in Lake 222 and Lake 239. 26
Table 2.3. Annual Handling Mortalities of Pike and Perch in Lake 222 and Lake 239, from 2012 to 2016. 27
Table 2.4. Growth Trends for Fish in Northwestern Ontario, from 2005 to 2016. 31
Table 2.5. Number of Perch Stomachs Analysed for Gut Contents in Lake 222 and Lake 239, in 2014 and 2016. 32
Table 2.6. Number of Prey MeHg Samples Run for Lake 222 and Lake 239, from 2012, 2015, and 2016. 33
Table 2.7. Calculated Prey MeHg Values for Lake 222 and Lake 239 Perch based on Yellow Perch Gut Contents. 34
Table 2.8. Mean Total Mercury Concentrations of SRMs and Blanks for Lake 222 and Lake 239. 35
Table 3.1. Prey Inflection Points for Lake 222 and Lake 239, in 2014 and 2016. 44
Table 3.2. BACI ANOVAs for Yellow Perch Summer 2014 and 2016 Diet Proportions in Lake 222 and Lake 239. 45
Table 3.3. BACI ANOVAs for Yellow Perch Length-at-Age Data in Lake 222 and Lake 239, from 2012 to 2016. 47
Table 3.4. ANCOVAs for Yellow Perch Consumption Rates in Lake 222 and Lake 239, from 2012 to 2016. 48
Table 3.5. ANCOVAs for Yellow Perch Growth Rates in Lake 222 and Lake 239, from 2012 to 2016. 50
Table 3.6. BACI ANOVAs for Yellow Perch Conversion Efficiency in Lake 222 and Lake 239, from 2012 to 2016. 51
Table 3.7. BACI ANOVAs for Yellow Perch Activity Levels in Lake 222 and Lake 239, from 2012 to 2016. 53
Table 3.8. ANCOVAs for Yellow Perch Standard Metabolic Rates in Lake 222 and Lake 239, from 2012 to 2016. 54
Table 3.9. ANCOVAs for Yellow Perch Total Metabolism in Lake 222 and Lake 239, from 2012 to 2016. 55
Table 3.10. BACI ANOVAs for Pike Abundance Estimates in Lake 222 and Lake 239, from 2012 to 2017. 56
Table 3.11. Breakpoint Regression Relationships for Northern Pike Abundance Estimates, from 2012 to 2017. 57
Table 3.12. BACI ANOVAs for Perch Abundance Estimates in Lake 222 and Lake 239, from 2012 to 2017. 60
Table 3.13. Tests for Heterogeneity of Slopes for Yellow Perch and Northern Pike in Lake 222 and Lake 239. 61
Table 3.14. Yellow Perch Age Assignments based on Summer Fork Lengths in Lake 222 and Lake 239. 63
Table 3.15. Annual Gross Consumption of Prey by Yellow Perch in Lake 222 and Lake 239, from 2012 to 2016. 64
Table 4.1. Results of Indirect and Direct Predictions for a Whole-Lake Nanosilver Addition on Yellow Perch. 67

LIST OF APPENDICES

Appendix A. Table 1. Limnological Comparison of Lakes Included in the Study. 77
Appendix B. Table 1. Summer to Fall MMBM Inputs for Each Age Cohort, Year, and Lake. 78
Appendix B. Table 2. MMBM Solved Consumption, Growth, and Activity Rates. 79
Appendix B. Table 3. Numbers of Sacrificed Male and Female Yellow Perch from Both Lakes, between 2012 and 2016. 79
Appendix C. Table 1. Number of Collected Fish Samples for Lake 222 and Lake 239. 80
Appendix D. Figure 1. Comparison of opercula (left) and otoliths (right) from Yellow Perch in the summer of 2015 (top) and fall of 2015 (bottom) in Lake 239. 81
Appendix D. Table 1. Selection of Blind Ageing Analysis of Yellow Perch from Lake 222 and Lake 239. 81
Appendix D. Figure 2. Cross-sections of a fin ray from Yellow Perch (#14) in Lake 222. 82
Appendix E. Table 1. Benthic Invertebrates 27-Level and Zooplankton Functional Groups for Methylmercury Analysis. 83
Appendix E. Table 2. Methylmercury Analysis of Benthic Invertebrates and Zooplankton in Lake 222 and Lake 239. 83
Appendix F. Table 1. Calculated Energy Density Based on Occurrence of Prey Items in Yellow Perch Gut Contents in Lake 222 and Lake 239 for 2014 and 2016. 84
Appendix G. Table 1. Fall Proportions of Yellow Perch in Lake 222 and Lake 239, from 2012 to 2017. 85
Appendix H. Table 1. Survivability by Study Period Arrangement of POPAN Parameters for Northern Pike in Lake 222. 86
Appendix H. Table 2. Top POPAN Model for Northern Pike in Lake 239. 87
Appendix H. Table 3. Top POPAN Model for Northern Pike in Lake 222. 87
Appendix H. Figure 1. Schnabel census estimates of Northern Pike in Lake 222 and Lake 239, from 2012 to 2017. 88
Appendix I. Table 1. Top POPAN Model for Yellow Perch in Lake 239. 89
Appendix I. Table 2. Top POPAN Model for Yellow Perch in Lake 222. 89
Appendix I. Figure 1. Yellow Perch open population estimates in Lake 222 and Lake 239, from 2012 to 2017. 90

LIST OF FIGURES

Figure 2.1. Locations of the Lake 239 processing site, centre buoy, and Yellow Perch capture sites. 24
Figure 2.2. Locations of the Lake 222 nAg addition site, processing site, centre buoy, and Yellow Perch captures. 25
Figure 3.1. Fork lengths at which Lake 222 Yellow Perch consumed a greater proportion of zooplankton (top) versus benthic invertebrates (bottom), during nAg addition in 2014 and recovery in 2016. 43
Figure 3.2. Fork lengths at which Lake 239 Yellow Perch consumed a greater proportion of zooplankton (top) versus benthic invertebrates (middle) or fish (bottom), in 2014 and 2016. 44
Figure 3.3. Seasonal diet proportions of benthos (B), fish (F), zooplankton (Z) in Lake 222 and Lake 239, during nAg addition (2014) and recovery (2016). 45
Figure 3.4. Mean summer fork lengths of Yellow Perch by age cohort in Lake 222 and Lake 239 over time. 46
Figure 3.5. Log-transformed absolute consumption rates versus mass for Yellow Perch in Lake 222 and Lake 239. 48
Figure 3.6. Annual changes in mass-specific consumption-at-age for Lake 222 (top) and Lake 239 (bottom) perch. 49
Figure 3.7. Log-transformed growth rates versus mass for Yellow Perch in Lake 222 and Lake 239. 50
Figure 3.8. Conversion efficiency for Yellow Perch in Lake 222 and Lake 239, from 2012 to 2016. 51
Figure 3.9. Annual changes in activity-at-age for Yellow Perch in Lake 222 (top) and Lake 239 (bottom). 52
Figure 3.10. Log-transformed standard metabolic rate versus mass for Yellow Perch in Lake 222 and Lake 239. 53
Figure 3.11. Log-transformed total metabolism versus mass for Yellow Perch in Lake 222 and Lake 239. 54
Figure 3.12. Top "Study Period" open estimates for Northern Pike in Lake 222 and Lake 239, from 2012 to 2017. 56
Figure 3.13. Effect of occasion with one breakpoint on Northern Pike abundance in Lake 222 and Lake 239. 57
Figure 3.14. Survivability estimates by study period for Northern Pike in Lake 222 and Lake 239. 58
Figure 3.15. Schnabel census closed estimates of Yellow Perch per unit area in Lake 222 and Lake 239, from 2012 to 2017. 59
Figure 3.16. Log-transformations of Northern Pike weight versus length in Lake 222 (left) and Lake 239 (right), from 2012 to 2017. 60
Figure 3.17. Log-transformations of summer Yellow Perch weight versus length in Lake 222 and Lake 239, from 2012 to 2017. 61
Figure 3.18. Proportions of assigned age cohorts based on perch age-length keys in Lake 222 and Lake 239, from 2012 to 2017. 62
Figure 3.19. Annual gross consumption estimates per unit area for Yellow Perch in Lake 222 and Lake 239. 64
Figure 3.20. Annual gross consumption per unit area for ages 1 and 2 Yellow Perch (top) and ages 3 to 6 Yellow Perch (bottom), in Lake 222 and Lake 239. 65

1 Introduction

1.1 BACKGROUND

Nanosilver (nAg) is the term used to describe particles of silver 1 to 100 nanometers in one or more dimensions, which are of commercial interest for their inherent antibacterial and antimicrobial properties. As a result, nAg is the most commonly used nanomaterial currently in production, occurring in over 440 products such as carpets, clothing, cosmetics, refrigerators, socks, sports performance gear, towels, underwear, and washing machines (Maillard and Hartemann 2013; Nowack *et al*. 2012; Buzea *et al*. 2007). Nanosilver is an emerging contaminant of significant concern – it is used extensively in industry, medicine, and consumer products. While ionic silver (Ag^+) has been used for centuries, society has found different broad-scale applications and uses for nAg that release the nanomaterial into the environment in greater-than-ever quantities at point sources (Colman *et al*. 2014; Maillard and Hartemann 2013; Gottschalk *et al*. 2010; Blaser *et al*. 2008). Additionally, nAg has the potential to react differently in the environment than Ag^+, due to its size and increased surface area-to-volume ratio, which make it highly reactive, and has potential to negatively affect aquatic organisms (Pulit-Prociak *et al*. 2014; Buzea *et al*. 2007).

Nanosilver is released into the environment via wastewater treatment plants, industrial discharges and run-off from agricultural sources (Nowack *et al*. 2012; Buzea *et al*. 2007). The average North American consumer contributes an estimated 470µg/L nAg per day into wastewater (Fabrega *et al*. 2011; Benn *et al*. 2010); where a maximum of 10% of the total load of nAg entering municipal sewage systems is anticipated to be released in effluent into the aquatic environment (Colman *et al*. 2014; Kaegi *et al*. 2013; Keller *et al*. 2013; Blaser *et al*. 2008). Depending on its interactions with water chemistry parameters such as dissolved organic carbon (DOC), nAg settles out of the water column to the sediment (Furtado *et al*. 2014; Liu *et al*. 2010), where it has the potential to be remobilised by benthic invertebrates (Lowry *et al*. 2012). Unsettled fractions of total silver are taken up within the water column by phytoplankton and zooplankton, and move up the food chain to fish (Pulit-Prociak *et al*. 2014; Lowry *et al*. 2012). Nanosilver has been shown to

affect a wide range of biological organisms through its toxic antimicrobial properties, but the ecosystem-level effects of this material associated with large-scale environmental release are poorly understood.

Although Canadian Water Quality Guidelines (CWQGs) exist for Ag^+, no regulations exist for nAg release into the environment (CCME 2015). The CWQGs encompass total silver, and it is currently not feasible to separate toxicity of nAg from its ionic (Ag^+) form, since a fraction of the toxicity of nAg is a result of its dissolution into silver ions (Wang *et al*. 2012; Kennedy *et al*. 2010; Laban *et al*. 2010). Environment Canada developed CWQGs for the protection of aquatic life for silver, using species sensitivity distribution (Environment Canada 2013; Table 1.1). The CWQGs for long-term total silver exposure in freshwater was assessed to be 0.25µg/L, with no recommended guideline for short-term exposure (CCME 2015). In marine systems, there were no long-term CWQGs due to insufficient data, though short-term exposure to total silver in marine systems was assessed to be 7.5µg/L (CCME 2015). However, these freshwater and marine CWQGs may not be applicable to silver nanoparticles (nAg; CCME 2015).

Table 1.1. Total Silver Concentrations Measured from Water Quality Samples in Canada, from 2008 to 2013. (Source: CCME 2015). Asterisks (*) indicate the value is below the detection limit.

Province or Territory	Range Total Silver (µg/L)	Mean Total Silver (µg/L)
BC, YK	<0.001* - 10	0.005
AB, MB, NWT, SK	<0.001* - 0.69	0.005
ON	Not listed	Not listed
QC	<0.001* - 0.085	Not listed
NB, NL, NS, PEI	<0.001* - 1.3	Majority at or <0.001*

Aquatic organisms may react differently to nAg exposure, compared with Ag^+, due to alternate modes of toxicity, where uptake occurs via respiration and digestion (nAg) versus only through respiration (Ag^+), suggesting different guidelines for environmental release are required (Murray *et al*. 2017a; Buzea *et al*. 2007). Most scientific studies suggest that nAg tends to be less toxic than Ag^+ at equivalent concentrations (Murray *et al*. 2017a; Furtado *et al*. 2016). Estimates of nAg in the environment have been reported at concentrations of 1.3 µg/L, however, with continuous use and an increase in applications within consumer products, these estimated levels are expected to rise (Massarsky *et al*. 2014).

Laboratory studies have demonstrated that nAg affects fish differently than Ag^+. Nanosilver uptake occurs in both the gills and through digestion, with the main mode of toxicity being oxidative stress (Scown *et al*. 2010). Nanoparticles of silver destabilize the electron transport chain in cell mitochondria, causing an excess of reactive oxygen species, which may result in damage to the cell DNA or lipid peroxidation and protein modification (Scown *et al*. 2010). Nanosilver has been shown to be toxic to fish through direct pathways of effect, via bioaccumulation (at exposures ranging from 10-32,000µg/L), cortisol response (20-8,000µg/L), and metabolic impairment (300µg/L; reviewed in Murray *et al*. 2017a). By contrast, the main mode of toxicity for Ag^+ occurs primarily at the gills, through the inhibition of the sodium-potassium pump in fish gill cells, which eventually leads to osmoregulatory failure with a large progressive net loss of sodium and chloride ions from the blood (Scown *et al*. 2010).

Ionic silver is a highly toxic metal to fish and aquatic organisms, lethal at low µg/L concentrations. Davies *et al.* (1978) determined the LC50 in Rainbow Trout (*Oncorhynchus mykiss*) was 6.5µg/L in soft water, and 13.0µg/L in hard water. At concentrations ≥0.17µg/L, Ag^+ caused premature egg hatching and reduced fry growth rates (Davies *et al*. 1978). Concentrations of Ag^+ ≤0.09µg/L had no observed sublethal effects on fish, compared to concentrations of nAg <0.1µg/L with no observed effects (Bilberg *et al*. 2010; Davies *et al*. 1978). Despite Ag^+ typically exhibiting greater toxicity than nAg, the multiple modes of toxicity of nAg on an organism (compared with Ag) may ultimately subject fish to greater concentrations of nAg in their organs and tissues, under conditions mimicking real-world exposure scenarios in the field.

To accurately assess the environmental impacts of this contaminant, it is crucial to examine nAg at environmentally-relevant concentrations under natural conditions (i.e. concentrations at which organisms are likely to be exposed to in field settings, either now or in the near future, compared with unrealistic laboratory conditions). By contrast, most observations of nAg toxicity to date have been based on laboratory fish exposure studies at very high concentrations (lethal response or LC50 studies: 1,000-50,000µg/L; sublethal response studies: 0.1-300µg/L), which

most frequently involve short-term, high concentration exposures using model organisms (Pham *et al*. 2012; Bilberg *et al*. 2010). Further, concentrations of nAg in exposure studies are very likely overestimated if not measured directly, since nAg settles out of the water column rapidly (Murray *et al*. 2017a). Exposed to natural media that is chemically and physically complex, nAg can agglomerate with physiochemical constituents, so final reported concentrations are often a small fraction of the nominal concentrations (Murray *et al*. 2017a; Furtado *et al*. 2016).

Responses by fish to nAg exposure have been mainly studied at the cellular and physiological level. Past studies into the impacts of nAg at environmentally-relevant levels have observed physiological effects on gene expression in fish (1µg/L, Pham *et al*. 2012), thickening of gill tissue at 10µg/L (Griffitt *et al*. 2012), and impaired osmoregulation at 20µg/L (Farmen *et al*. 2012). Physiological effects of nAg at extreme levels have been reported to: cause gill necrosis (100µg/L, Farmen *et al*. 2012); impair gas exchange (300µg/L, Bilberg *et al*. 2010); and cause embryonic abnormalities (600µg/L, Laban *et al*. 2010) in fish. However, these higher concentrations are orders of magnitude greater than current environmentally-relevant levels (i.e. 1-15µg/L), and are therefore not likely to reflect real-world responses to nAg environmental release. Further, it remains unclear how nAg tissue accumulation and cellular-level responses in fish are expressed at the whole-organism level.

Recent studies have begun to investigate the whole-organism impacts of long-term environmentally-relevant exposures of nAg on native fish species. Following a 28-day exposure, Rainbow Trout showed no significant changes in growth or metabolism, despite accumulation of nAg into muscle tissue at the highest exposure levels (50µg/L) and an increase in blood cortisol concentrations at both low (0.3µg/L) and high nAg exposures (most elevated three to seven hours after exposure, Murray *et al*. 2017a). Despite a physiological response in fish, there were no whole-organism impacts; based on these findings, it was proposed that fish may be able to adapt to and counteract the direct toxic effects of low and prolonged nAg exposure without significantly increasing metabolic costs (Murray *et al*. 2017b). These experiments provided context for

evaluating the whole-lake effects of nAg on fish populations, and suggest that whole-body effects may not occur primarily through direct routes of exposure (Murray *et al*. 2017a, b).

However, there is growing evidence that environmentally-relevant concentrations of nAg can have significant impacts at multiple aquatic trophic levels, and fish may experience indirect effects of nAg via food limitation. Nanosilver concentrations have been reported at 1.5µg/L in surface waters (Liu *et al*. 2009). Modelling attempts have estimated an expected range of between 2-18µg/L in wastewater influent (Blaser *et al*. 2008) and environmentally-relevant concentrations of 2.8µg/L in wastewater effluent (Liu *et al*. 2009). Though some studies have reported sublethal responses in fish exposed to environmentally-relevant concentrations (Murray *et al*. 2017a; Farmen *et al*. 2012; Griffitt *et al*. 2012; Pham *et al*. 2012), these low-level exposures have also been shown to have significant effects at lower trophic levels. At lower trophic levels, environmentally relevant exposures to nAg have been found to result in reduced bacteria production and enzyme activity of bacteria populations (8-66µg/L; Das *et al*. 2012; Fabrega *et al*. 2009), as well as reduced production of algae populations and growth of algal cultures (LOEC=0.92-2.4µg/L and LC50=2.21-6.83µg/L, Das *et al*. 2014).

Previous work indicated that low, environmentally-relevant concentrations of nAg (nanogram per litre range) would be unlikely to impact aquatic biogeochemical cycles: Das *et al.* (2012) demonstrated that bacterial production in the water column can be completely inhibited immediately after dosing, but this inhibition is short-lived, as bacterial production recovers by 40 to 250% after 48 hours of exposure. This suggests a potential capacity in lower trophic levels to mitigate the negative effects of nAg particles over short durations following exposure, and that dilution and fallout greatly reduces long term toxicity of the compound in surface waters (Das *et al*. 2012). Bacterial enzyme activity had a weak reaction to nAg that was not observed in Ag^{+} additions; minimal impact suggests that nAg discharges less than 100 µg/L in aquatic environments are unlikely to diminish phosphorus cycles in microbes, and therefore likely to be non-disruptive to the basal food web and nutrient retention of lower trophic organisms (Das *et al*. 2012).

Additionally, research into the effects of nAg on phytoplankton growth has revealed a strong interaction with phosphorus concentrations in laboratory settings. Phosphorus acts as both a limiting nutrient for algal production and a ligand for silver ions released from nAg, which ultimately reduces its toxicity (McTeer *et al*. 2014; Xiu *et al*. 2011). Conine and Frost (2017) determined the toxic effects of nAg on *Daphnia* growth and survival in freshwater were reduced by algae, because nAg particles agglomerate on the surface of algae or are taken up in the cell (Leclerc and Wilkinson 2014; Oukarroum *et al*. 2012). Recent studies into the trophic transfer of total silver in algae and bacteria components of the pelagic food web indicated that the highest levels of total silver were present in bacterioplankton and algae when the concentrations were normalized to organic carbon content, although these levels varied seasonally (Conine and Frost 2017; Blakelock *et al*. 2016).

A study by Das *et al*. (2014) into the interaction of phosphorus, phytoplankton, and nAg particles in natural waters demonstrated a reduction of 70 to 90% in phytoplankton volume at concentrations of 10 µg/L nAg after 72 hours of incubation. However, this effect was mitigated by higher concentrations of phosphorus, though toxicity was still apparent across bacillariophytes, chlorophytes, and chrysophytes. The near-elimination of cyanobacteria despite phosphorus supplements suggested nAg addition may shift algal community composition in aquatic environments but have a less detrimental effect to overall algal biomass (Das *et al*. 2014). Chronic exposure of aquatic organisms to environmentally-relevant low doses of nAg may produce ecosystem-level responses by affecting productivity, decomposition rates, and nutrient cycling (Das *et al*. 2012, 2014). These effects of nAg on lower trophic levels indicate nAg contamination may affect aquatic food webs.

Toxicity of nAg appears to be a combination of both its size and the rate of silver ion release. The form of nAg inside an organism is controlled by the pH and ionic strength of the individual's body fluids, affecting nAg stability (Martin *et al*. 2017a; Lapresta-Fernández *et al*. 2012; Stebounova *et al*. 2011). Similarly, environmental factors such as dissolved organic carbon,

dissolved oxygen, presence of ligands, pH, and UV radiation can alter the toxicity and fate of nAg particles in natural aquatic environments (Kennedy *et al*. 2012; Shi *et al*. 2012; Stebounova *et al*. 2011; Xiu *et al*. 2011). Nanosilver is therefore anticipated to be toxic to fish by two potential pathways of effect: (1) direct through bioaccumulation in the tissues, which may reduce their effectiveness as predators, decreasing consumption rates, and resulting in poorer growth and condition over long-term nAg addition; and (2) indirect caused by the reduction of prey items (such as benthic invertebrates and zooplankton) in the food web, which could potentially impair fish body condition and growth over long-term exposures (reviewed in Murray *et al*. 2017a; Das *et al*. 2012).

1.2 LAKE ECOSYSTEM NANOSILVER (LENS) PROJECT

To assess the impact of nAg on aquatic ecosystems at environmentally-relevant levels, the Lake Ecosystem Nanosilver (LENs) Project was initiated at the IISD-Experimental Lakes Area (IISD-ELA). The main objective of the LENs Project was to evaluate the whole-ecosystem response to a common antimicrobial agent that has high potential for entering waterways at point-sources. Lake 222 was selected as the experimental lake for the LENs Project as it was easily accessible for dosing, its small size (Appendix A Table 1) minimized the quantity of nAg required, it had sufficient depth for stratification, its water chemistry parameters (pH, DOC, phosphorus) were typical of lakes in the region, and both forage and predatory fish species were present in numbers that permitted sacrifices. Lake 222 was dosed with 15kg of nAg during the ice-free period for two years, from 2014 to 2015. Over the course of the LENs Project, nAg sedimentation, nAg fall-out from the water column, and nAg decomposition rates were monitored. Algae, bacteria, and zooplankton communities were assessed for impacts of nAg on their biomass, production, and survival; fish species were examined for biomarker response of their organs and tissues (gills, kidney, liver, and muscle) to nAg toxicity.

1.3 FISH ENERGETICS

Fish energetic models are commonly used in fish ecology studies to provide insight into the impacts of contaminant bioaccumulation or other environmental impacts on fish consumption, activity, shifts in energetic pathways among prey items, and the role of fish in cycling nutrients (Ferriss and Essington 2014; Schindler and Eby 1997). These models can help provide insights as to how physiological responses of fish interacting with their environment scale to both individual- and population-levels. By providing an estimate of consumption through contaminant modelling, linking bioenergetics to contaminant-tracer models also allows for the direct estimation of metabolic costs associated with fish activity (Ferriss and Essington 2014; Hrenchuk *et al*. 2012; Trudel *et al*. 2000). Under this framework, estimated activity rates are effectively used to balance remaining energy, after that required for growth, metabolism, and waste are allocated via allometric relationships and temperature scaling of energy expenditure in natural settings (Ferris and Essington 2014; Kitchell *et al*. 1977). This mass-balance framework allows the user to solve for unknown consumption and active metabolic costs using a set of initial and final weights, energy density and contaminant endpoints, along with similar information on diets and water temperatures.

Bioenergetics models can also be combined with population-level data to understand how sublethal effects on fish growth and consumption can scale to the ecosystem level. Multiple approaches, including size-at-age and tagging data, can be used as the basis for statistical estimation of abundance. This approach was used previously in the Great Lakes to attribute the changes in fish abundance, condition, growth, and consumption, to either top-down processes, such as predation, or bottom-up processes, such as nutrient cycling and prey limitation (Rand and Stewart 1998). Prey limitation increases stress in predatory fish, which often results in decreased fish growth and reduced survivability, as fish shift to less desired prey items and suffer disease outbreaks (Rand and Stewart 1998). As abundance declines, so too does competition, where prey resources may then become non-limiting with fewer predators in the system. Alternatively, prey increases can result in improved energy transfer and growth of predators in the food web, resulting in greater abundance.

In systems with large populations of predatory fish, top-down processes can negatively affect prey fish abundance, resulting in reduced pressure on primary and secondary producers. Alternately, a decrease in top predators would have top-down effects, causing an increase in prey fish and a reduction in producers (Rand and Stewart 1998; McQueen *et al*. 1989). Studies suggest the biological structure and energetic pathways within an ecosystem, rather than abundance of the species, is more tightly linked to whole-lake function (McCann 2007; Hilborn *et al*. 2003).

1.4 GENERAL OBJECTIVES

The general research objective of this thesis was to determine the impacts of an environmentally-relevant release of nAg on the magnitude and sources of energy flow to fishes. Yellow Perch (*Perca flavescens*) is a common prey item for Northern Pike (*Esox lucius*; Scott and Crossman 1973), and both species are present in the reference and experimental lakes for this study. This project investigated the growth, condition, and bioenergetics of Yellow Perch in a whole-lake nAg exposure, as well as population-level responses of both perch and their Northern Pike predators. Body condition in pike was also evaluated. Individual bioenergetics results for perch were scaled to the population level of total biomass consumed per year to assess the effects of nAg on energy flow within the impacted ecosystem.

Yellow Perch are known to be both metal- and acid-tolerant, but at higher ends of these exposure gradients they are sensitive to a suite of direct and indirect effects (Rasmussen *et al*. 2008). With (a) evidence of negative effects of nAg on lower trophic levels, and (b) evidence of sub-lethal physiological responses in fish (i.e. bioaccumulation, cortisol response, and metabolic impairment), all at environmentally-relevant concentrations, it was unclear whether responses at the fish level would be direct or indirect. Comparing bioenergetics and diet results from a nAg-impacted system to a nearby reference lake, I determined whether there was a greater sum of evidence for lower trophic-level impacts limiting prey availability (bottom-up, indirect effects), or if the sum of evidence indicated physiological (direct) responses of fish to nAg exposure.

Studies of Yellow Perch in metal-contaminated sites in Ontario have used bioenergetics models to understand responses of fish to indirect (food web-mediated effects of metals on perch) and direct (cellular-level, individual-level, and population-level) effects of contaminant exposure (Rasmussen *et al*. 2008; Campbell *et al*. 2003). Based on these studies, indirect consequences of nAg addition would be apparent in changes in growth and bioenergetics, as a result of reduction in prey resources (as evidenced by stomach content analysis; Rennie *et al*. 2012). Studies have indicated that Yellow Perch commonly experience stunted growth, coupled with a high dependence on smaller zooplankton prey (resulting in poorer condition), and that these responses are evidence of indirect effects of metal contamination (Rasmussen *et al*. 2008; Kaufman *et al*. 2006; Sherwood *et al*. 2002). Direct effects would also be evident in bioenergetics changes, or by total silver damage to fish tissues and organs, while prey resources remained relatively constant in the lake.

Based on studies indicating negative effects of nAg on primary and bacteriological production at environmentally-relevant concentrations, the responses of secondary producers and primary consumers exposed to nAg in the whole-lake experiment were predicted to support indirect effects (decreased prey availability and therefore diminished consumption, poorer body condition, and decreased growth rates) on secondary consumers, such as zoobenthivorous fish. If indirect effects were involved in a fish response to nAg, it was hypothesised that Yellow Perch prey consumption rates would decrease in the lake exposed to two years of nAg addition, additionally, there would be dietary changes evidenced by perch stomach contents (Table 1.2).

Table 1.2. Predictions of Indirect and Direct Effects of a Whole-Lake Nanosilver Addition on Yellow Perch.

Effect	Cause	Prey	Growth	Consumption	Growth Efficiency	Metabolism
None	No observed sublethal effects	↔	↔	↔	↔	↔
Indirect	Decrease in prey abundance, diet	↓	↓	↓	↓	↑
Direct	Oxidative or osmoregulatory stress	↔	↓	↓	↔	↓

Decreased growth and growth efficiency would result from increased activity costs that reduce the energy available for growth (Kaufman *et al*. 2006; Rennie *et al*. 2005; Sherwood *et al*. 2002). Further, a study of pollution-tolerant mummichog (*Fundulus heteroclitus*) and impoverished benthic invertebrate communities from polluted salt marshes, revealed an increase in mummichog

total metabolism, and two- to three-fold (compensatory) increase in consumption rates, compared to control populations with non-impoverished benthic communities (Goto and Wallace 2010). Based on my prediction of indirect bioenergetics responses to nAg addition, it was hypothesised that Yellow Perch activity rates, or energy expenditure, would increase in the experimental lake, which would result in increased total metabolic costs during and after nAg addition (Table 1.2).

Alternatively, at environmentally-relevant concentrations, fish could be responding to the direct negative effects of nAg on Yellow Perch tissues. In a direct nAg impact case (oxidative stress; Scown *et al*. 2010), it is expected that consumption of prey would be lower, as activity rates would be reduced to minimize the negative effects of oxidative stress; studies have found that higher activity rates and greater losses to metabolism may be associated with higher oxidative stress in fish in contaminated environments (by metal and industrial pollutants, or land-use changes; Birnie-Gauvin *et al*. 2017). Growth rates would be reduced due to negative impacts of direct toxicity, and decreased prey consumption, therefore growth (conversion) efficiency would remain constant, as fewer prey calories would be converted as efficiently as required to meet fewer metabolic costs (Table 1.2).

Beyers *et al*. (1999) integrated bioenergetics (consumption, growth, activity, and total metabolism rates) with stress to determine the physiological costs of dieldrin chemical contaminant exposure in Largemouth Bass (*Micropterus salmoides*). Reduced activity levels and total metabolism have been observed in aquatic organisms, specifically Rainbow Trout, with increased costs to osmoregulation functions (Beamish 1978). Similar to oxidative stress, osmoregulatory stress symptoms were expected as a result of direct nAg impacts (Farmen *et al*. 2012); in this case there was also no predicted change in diet, and it was expected that growth, consumption, and total metabolism would decrease in response to negative exposure effects, as was observed in contamination of Largemouth Bass that impacted their growth, consumption, and metabolic rates (Beyers *et al*. 1999).

Scaling these individual bioenergetics expectations to the population level resulted in predictions that were functions of both (a) outcomes of bioenergetics, and (b) outcomes of population density effects. Population-level hypotheses were based on McMeans *et al*. (2016), which described how ecosystem-level changes are concurrent with life-history changes at the individual level. Therefore, if nAg effects were indirect, with predicted individual-level decreases in consumption rates, it was hypothesised that Yellow Perch communities would experience a similar decrease in gross zoobenthos consumption rates. Perch condition was expected to be negatively affected by reduction of prey items (indirect), or by a combination of reduced bioenergetics and growth rates (direct). Yellow Perch abundance was only expected to decline in the lake as a result of indirect nAg-affected prey resources, since scenarios where they respond to direct effects at environmentally-relevant concentrations have demonstrated only "stress" and not mortality (Farmen *et al*. 2012; Griffitt *et al*. 2012; Scown *et al*. 2010), and thus were not expected to cause a mass decline in zoobenthivorous perch numbers. Finally, Northern Pike condition was also expected to decline with fewer perch in the system (indirect) or with the main mode of toxicity being diet in the piscivorous pike (direct). Abundance of this top predator was not expected to change during the two-year nAg addition.

Using a before-after-control-impact (BACI) study design comparing baseline (before), nAg addition (during), and recovery (after) study periods in an impact lake to a control lake, differences in Yellow Perch energetics at the individual- and population-levels, as well as Northern Pike and Yellow Perch abundance estimates and condition, were assessed. Based on the results of these objectives, I determined whether environmental release levels and duration of nAg in this experiment were detrimental to fish populations. This study of fish exposed to nAg will help inform other trophic level results from the whole-lake experiment. Collectively, the research from this whole-lake experiment will directly influence policy development regarding an evaluation of an environmentally-relevant release of nAg into freshwater, allowing provincial and federal organizations to formulate effective regulations regarding management of this nanomaterial.

2 Methods

2.1 Study Site

To determine the impacts of nAg on aquatic ecosystems, environmentally-relevant doses of nAg were applied to a Lake 222 at the International Institute for Sustainable Development – Experimental Lakes Area (IISD-ELA). The oligotrophic, dimictic Lake 222 was monitored in its natural state for two years of baseline data collection (2012 and 2013), continuously dosed with nAg particles for two years during the ice-free season (2014 and 2015), and monitored after additions for recovery (2016 and 2017; Appendix A Table 1). Lake 239 was the reference lake for this study, as it is characteristic of unmanipulated lakes in the region; fish populations in Lake 239 were monitored to rule out extrinsic environmental factors. Yellow Perch are a common zoobenthivorous fish in North America, as are the predatory Northern Pike, making them good candidate species for investigation of nAg exposures in natural settings. Both species are present in experimental Lake 222 and reference Lake 239.

2.2 Study Design

Impact assessment is used to determine the impacts of stressors in the environment, to identify changes and affected parameters, and to estimate the scale and scope of damages (Smith 2002). A general before-after-control-impact (BACI) study design was used to evaluate environmental impacts of the nAg addition on fish populations, in order to disentangle regional or temporal changes not associated with nAg addition to Lake 222. Response variables of interest were compared using a BACI design over three study periods: pre-manipulation baseline period (2012 data), nAg impact years (2014-15), and recovery (2016 data), comparing the impact site (Lake 222) with the control site (Lake 239).

2.3 Nanosilver Additions

Environmentally-relevant concentrations of nAg solutions were added to experimental Lake 222 over a period of two years. Nanosilver additions began June 14th 2014, and lasted a period

of 18 weeks, ending October 23rd 2014; a second year of nAg addition started May 15th 2015, and lasted a period of 14 weeks, ending on August 25th 2015. Nanosilver suspensions were prepared on-site using a rotor-stator dispersion Kady® mill; 125 grams of PVP-capped 30-50nm nAg powder (NanoAmor, TX, USA) was suspended in 12.5 litres of filtered water (from reference Lake 239) and stabilized with gum arabic (Martin *et al*. 2017a; Newman *et al*. 2016). Nanosilver solutions were added to the point-source dispensing unit every second day, from which a 5.2g/L nAg suspension was dispersed in Lake 222 using a peristaltic pump to mimic wastewater effluent (Newman *et al.* 2016; Daniel Rearick, unpublished data). Nine kilograms of nAg were added in 2014, and six kilograms were added in 2015 (for a total of 15kg in two years), with additions occurring at six-hour intervals, providing daily discharges of 62.5g suspended nAg (Martin *et al.* Submitted; D. Rearick, unpublished data).

An environmentally-relevant target concentration of 1-15µg/L nAg (average nAg concentration in µg/L range from North America wastewater effluent in 2014; Liu *et al*. 2009; D. Rearick, unpublished data), was largely achieved, as confirmed by monitoring with passive samplers. While measured dissolved silver concentrations were less than 1µg/L in Lake 222, total silver concentrations in Lake 222 water measured 4-18µg/L, distributed throughout the hypolimnion and epilimnion (Metcalfe 2017). Total silver concentrations measured over 20µg/L at the addition site and diffused with distance from the point-source addition, for an average of 7µg/L over the entire lake (Metcalfe 2017; Andrea Conine, unpublished data). An increase in nAg concentrations was observed over time at all sampled sites – from <0.002µg/L in 2012, to an average of 3.4µg/L after one-year of nAg additions in 2014, to 10.1µg/L in 2015 at the end of nAg additions (Newman *et al*. 2016). The size distribution in the Lake was similar to the stock solution, an average of <80nm (Rearick *et al*. Submitted; Martin *et al*. 2017a). Estimated concentrations of nAg in Lake 222 were an order of magnitude higher than the CWQGs of 0.25µg/L for Ag^+ (CCME 2015).

2.4 Individual-Level Mercury Mass Balance and Bioenergetics Modelling

A mass-balance contaminant-tracer model combined with a bioenergetics model was used to estimate the changes in consumption and energy expenditure in fish (Trudel *et al*. 2001; Trudel and Rasmussen 2001; Trudel *et al*. 2000). The mercury mass balance model (MMBM) provides an estimate of consumption by using values for daily average temperature, average initial and final fish weight, average initial and final fish methylmercury (MeHg) concentrations, and prey MeHg concentrations (Tables B1, B2). Methylmercury in Yellow Perch was estimated to be 100 percent of total mercury, with negligible MeHg uptake from water (Ciardullo *et al*. 2008; Rennie *et al*. 2005). Initial and final Yellow Perch weights and Hg concentrations ([Hg]) were averaged within age cohorts for both lakes, so energetics and mercury accumulation could be modelled from spring (initial weight) to summer (final weight), and summer to fall (Ferriss and Essington 2014; Vander Zanden and Rasmussen 1996). Diet MeHg concentrations were estimated as a mean seasonal lake-specific value for immature cohorts (<100mm, ages 1 to 2), and mature cohorts (≥100mm, ages 3-plus; Section 2.9).

Methylmercury accumulation in fish was represented as:

(1) $$dHg / dt = (\alpha \cdot C_d \cdot C) - (E + G + K) \cdot Hg$$

Over a daily time-step, one can assume losses are near constant, and the above equation can be integrated to solve for consumption (C):

(2) $$C = [Hg_t - Hg_0 \cdot e^{-(E+G+K)t}] / [\alpha \cdot C_d \cdot (1 - e^{-(E+G+K)t})] \cdot (E + G + K_s)$$

Where Hg is the amount of MeHg in the fish at time 0 and t, α is the assimilation efficiency of MeHg from food, C_d is the MeHg in food, C is the absolute ingestion rate (g_{food}/day) integrated over the time period (consumption), E is the elimination rate of MeHg, G is the mass-specific growth rate (g_{fish}/day), and K_s is losses due to spawning.

The MMBM output provided C, for use in the Wisconsin Bioenergetics Model (Hanson *et al*. 1997). The Bioenergetics Model was expressed as:

(3) $$W_t = W_0 + [C \cdot ED_{Prey} - (F + U + R_T)] / ED_{Fish}$$

Where W_t is final fish weight, W_0 is initial fish weight, ED_{Prey} is energy density of prey, F is losses due to egestion, U is losses due to excretion, R_T is losses due to metabolism, and ED_{Fish} is energy density of fish. Measured ED_{Fish} values of 4876.06±460.91J/g (Lake 222), and 4501.21±587.66J/g (Lake 239), were used in model estimates. Lake-specific ED_{Prey} was estimated for immature (age 1 to 2, <100mm) and mature (age 3-plus, ≥100mm) perch, based on gut contents analysis (Section 2.8). Prey items were assigned *ED* values from Cummins and Wuycheck (1971).

Total metabolism (R_T) was decomposed to permit a solution for the activity multiplier:

(4) $$R_T = ACT \cdot R_s + R_d$$

Where *ACT* is losses to active metabolism, R_s is standard metabolic rate, and R_d is specific dynamic action. Absolute estimates of *C* and *G* were converted to mass-specific rates by averaging values over the modelled time period and dividing by the mass of the fish.

Finally, gross growth efficiency (*K1*, or proportion of energy consumed that is converted to growth) was derived from the mass-specific rates of *C* and *G* (Kerr *et al*. 1971):

(5) $$K1 = G / C$$

It was assumed that Yellow Perch primarily occupied the epilimnion in the littoral zone of both lakes. Daily mean epilimnetic water temperatures measured in the lake were used to parameterize energetics models for basal metabolism and theoretical maximum consumption (Section 2.5.4). For juvenile Yellow Perch, modelled consumption continues up to 32°C in juvenile perch, and 28°C in adult perch, after which consumption ceases (Kitchell *et al*. 1977). The daily means recorded never exceeded these values in either lake.

Yellow Perch are sexually dimorphic (Rennie and Venturelli 2015), and allometric exponents of absolute growth are often found to vary by both population and sex (Rennie *et al*. 2010). Yellow Perch catches during this study were highly female-biased (Appendix B Table 3). I therefore combined input parameters by age cohort (overwhelmingly represented by female fish), and interpreted results as representative of populations with substantial female-bias, common among Yellow Perch populations (Rennie and Venturelli 2015).

2.5 Field Sampling Procedures

2.5.1 Fish

To permit data collection for energetic estimates, fish were captured using a variety of techniques, including trap-netting, seining, and angling. These collection methods targeted a range of fish size classes and sexes for each species, and minimized by-catch. Fish capture occurred in the spring, summer, and fall from 2012 to 2017 (Appendix C Table 1). Trap nets were deployed in Lake 239 during the spring and fall seasons and captured a range of Yellow Perch size classes, and supplemented Northern Pike angling efforts (Beamish 1972; Figure 2.1).

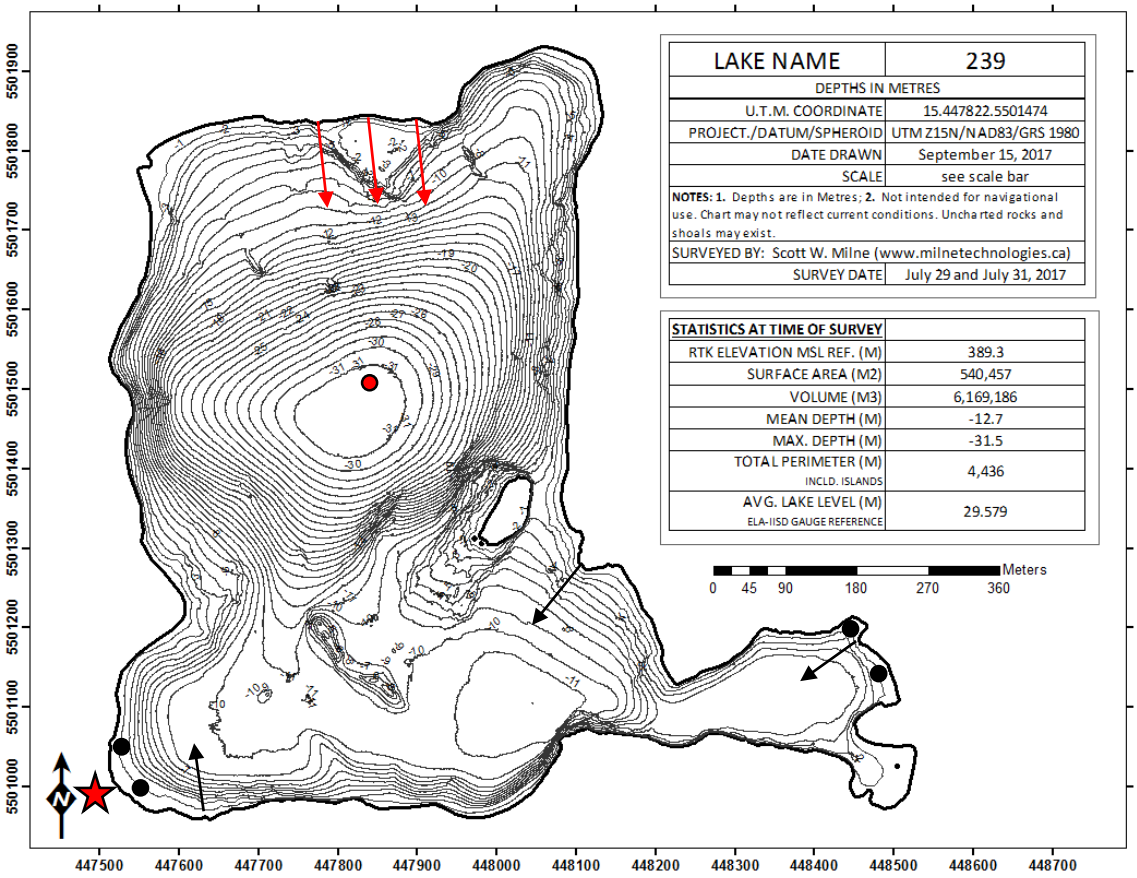


Figure 2.1. Locations of the Lake 239 processing site, centre buoy, and Yellow Perch capture sites. Where red star is the temporary sampling site, red circle is centre buoy, black circles are seine-netting sites, black arrows are spring trap-netting sites, and red arrows are fall trap-netting sites (bathymetry map source: Milne Technologies & IISD-ELA).

Seine nets were deployed along the littoral zones of both lakes and targeted perch (Figures 2.1, 2.2). Captured fish were anaesthetized using a buffered solution of tricaine methanesulfonate (TMS; Argent Chemical Laboratories Inc., Redmond, WA., U.S.A.) and lake water.

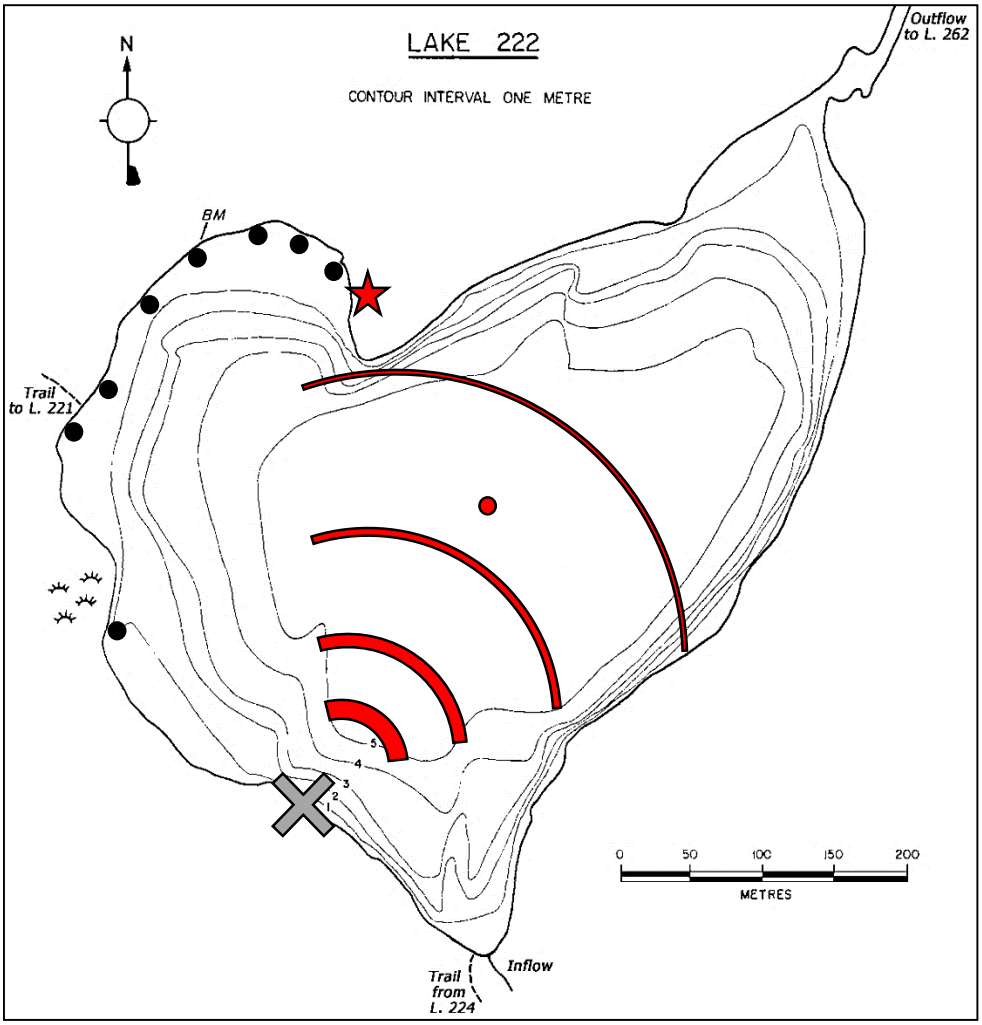


Figure 2.2. Locations of the Lake 222 nAg addition site, processing site, centre buoy, and Yellow Perch captures. Where grey "×" is the point-source nAg addition site, red star is the temporary processing site, red semi-circles represent gradient dilution, red circle is centre buoy, and black circles are seine netting sites (bathymetry map source: IISD-ELA).

Fish were measured for total and fork lengths (millimetres) and weight (grams); sex of Yellow Perch and Northern Pike released back into the lake were determined during spring by massaging the abdomen and examining expelled gonadal products, and by internal examination of sacrificed perch. For pike, the leading 1-2 pectoral fin rays, severed as close to the body as possible were taken from fish for ageing analysis. Pre-determined size classes were used to limit the number of perch sacrificed during the study, under the assumption that these size classes roughly corresponded to the age classes I targeted for bioenergetics modelling (Table 2.1; Section 2.4; Appendix D Table 1); Yellow Perch that satisfied the size class requirements were sacrificed using overdose solution of TMS.

Table 2.1. Target Numbers of Yellow Perch Euthanized per Year in Lake 222 and Lake 239.

Size Class by Fork Length (mm)	Yellow Perch (#)		
	Spring	Summer	Fall
<71	10	10	10
71-90	5	5	5
91-110	5	5	5
111-130	5	5	5
131-150	5	5	5
151-170	5	5	5
>170	5	5	5
Sub-Total	**40**	**40**	**40**
TOTAL	**120**		

After processing, non-sacrificed fish were placed in recovery bins of regularly replenished lake water, and were returned to the lake when they were upright and swimming. Sample sizes of sacrificed Yellow Perch differed on a seasonal basis, dependent largely on what was encountered during each capture period with the effort applied (Appendix C Table 1). Fish handling is detailed in Animal Use Protocols approved by Fisheries and Oceans Canada (2012-13), the University of Manitoba (2014, AUP No. F14-007), and Lakehead University (2015-17, AUP No. 1464693). Scientific collection permits were provided annually by the Ontario Ministry of Natural Resources and Forestry (OMNRF).

2.5.2 Mark-Recapture

Population estimates of Northern Pike and Yellow Perch in both lakes were determined using mark-recapture methods. Yellow Perch were captured live via seine net or trap net, given an identifying seasonal fin nick (batch mark to identify a period of capture; Table 2.2), and returned alive to the lake.

Table 2.2. Seasonal Fin Nicks for Yellow Perch in Lake 222 and Lake 239. Where "UC" is upper caudal fin, "LC" is lower caudal, "AF" is anal, "LV" is left pelvic, "RV" is right pelvic, "AD" is anterior dorsal, and "PD" is posterior dorsal.

Lakes	Season	Fin Nicks					
		2012	2013	2014	2015	2016	2017
L222	**SPR**	UC	-	AD	LC	AF	LV
	SUM	LC	LV	PD	LV	AD	RV
	FALL	AF	RV	UC	RV	PD	UC
L239	**SPR**	-	AF	AD	LC	AF	LV
	SUM	UC	LV	PD	LV	AD	RV
	FALL	LC	RV	UC	RV	PD	UC

The seasonal fin nick identified fish previously caught within a season, and allowed for recognition of fish handled, marked, and released in previous capture periods. Nicks were repeated only after sufficient time had passed to reduce the probability of detections across repeated time periods (at least four seasons between the same clips; Table 2.2).

Few Northern Pike were trap-netted (Lake 239 only), or seine-netted (juvenile pike only). The majority of pike were angled from a boat, typically via trawling. Angled pike were placed in a bin or cooler of lake water with a lid for a maximum of 30 minutes, and brought to the on-site processing location. Similar to perch, pike were weighed, measured, sexed (where possible by gamete expression in the spring), and a small sample of scales was removed from the left side of the pike, below the dorsal fin. The leading one to three pectoral fin rays were taken for ageing analysis. Additionally, pike were provided with a 9-mm electronic Passive Integrated Transponder (PIT) tag upon first capture. Tag numbers identifying recaptured pike were recorded. Fish were placed in bins of lake water to recover, and released when observed to be upright and swimming. Handling mortality of pike was minimal, with an average of one pike mortality per season per lake (<4%; Table 2.3). Perch handling mortalities contributed to sacrificed specimens in both lakes, whenever possible. Handling mortalities were greater in Lake 239, as trap nets captured substantially more young-of-year perch, which were fragile and occurred in large numbers.

Table 2.3. Annual Handling Mortalities of Pike and Perch in Lake 222 and Lake 239, from 2012 to 2016.

Lake	Species	2012		2014		2015		2016	
		Dead (#)	% of Total	Dead (#)	% of Total	Dead (#)	% of Total	Dead (#)	% of Total
222	Northern Pike	0	-	0	-	2	1.69%	4	3.41%
	Yellow Perch	150	8.35%	85	8.96%	65	7.85%	90	6.38%
239	Northern Pike	1	2.94%	1	2.04%	0	-	2	2.70%
	Yellow Perch	833	15.13%	226	7.30%	243	16.85%	112	12.21%

2.5.3 Benthic Invertebrates and Zooplankton

To determine lake-specific MeHg in prey species (Section 2.9) for use in energetics modelling (Section 2.4), benthic invertebrates and zooplankton samples were collected from both the experimental and reference lakes. Samples were collected in the summer during baseline data

collection (2012), during nAg addition (2015), and in the first year of lake recovery (2016). Benthic invertebrates were sampled with a D-net, using a kick-and-sweep method outlined by the Ontario Benthos Biomonitoring Network (OBBN 2007). Sampling efforts in experimental Lake 222 occurred at two sites, one adjacent to the nAg addition site, and one across the lake in the bay near the outflow. Sampling in Lake 239 took place in the bay at the east inflow and across the lake near the outflow. Zooplankton samples were collected during the summer of 2012 using a 60µm mesh net, deployed at Centre Buoy (CB) in Lake 222 and Lake 239. Samples were collected in three tows, put in plastic jars with lake water, and placed in a cooler with ice. Sampling occurred in Lake 222 during spring and summer 2015, and in both lakes during spring and summer 2016.

Benthic invertebrates were sorted *in situ*, placed in a Whirl-Pak® bag (4.5”×9” 532mL, Nasco, Fort Atkinson, WI., U.S.A.) of lake water in a cooler, and taken back to the laboratory for processing. There, invertebrates were separated based on a 27-group level, as set out by the OBBN. Groupings of organisms were placed in labelled Whirl-Pak® bags (3”×5” 58mL), detailing the date, lake, and location, then frozen at -20°C. Zooplankton and larval Diptera were similarly transported in coolers to the laboratory, filtered through a 60µm sieve, separated into *Chaoborus*, Cladocera, Copepoda, *Holopedium*, and Mysidacea groupings, and frozen in labelled Whirl-Pak® bags for later MeHg analysis.

2.5.4 Water Temperatures

To parameterize bioenergetics models (Section 2.4), epilimnetic temperatures were recorded using temperature loggers (HOBO Pendant® Waterproof Temperature Data Logger, Hoskin Scientific). Temperature loggers were deployed in both lakes from spring of 2014 to the end of the bioenergetics portion of the study in fall of 2016. Two littoral loggers were positioned at the inflow and outflow of each lake in 1-metre of water. Epilimnetic temperatures were also measured from a string of pelagic loggers in both lakes, attached to an anchored rope using zip-ties woven into the rope and secured with electrical tape. All loggers were labelled with their position

within the lake, and numbered, for ease of recording and downloading, and were downloaded biannually. The mean of all hourly recorded temperatures was calculated for each day. Daily mean temperatures were then averaged across the two littoral loggers (1-metre depth), and the 1- and 2-metre pelagic loggers in each lake, to obtain mean daily temperatures for the MMBM. Daily mean for Lake 222 from 2012 were obtained from the LENs Project data logger set at the surface at CB, while Lake 239 temperatures were calculated from the surface temperature logger at CB.

2.6 Laboratory Dissections

Euthanized Yellow Perch were placed in individual labelled Whirl-Pak® bags (ranging from 3”×7.25” 118mL, to 4.5”×9” 532mL), and transported from the field to the IISD-ELA Fish Laboratory. Fish were frozen at -20°C, and were dissected in the laboratory within 3-6 months of collection. For each Yellow Perch, the opercula, scales (8-12 from the left side of the fish), fin rays, and otolith structures were removed for ageing. Stomachs were removed and preserved in 95% ethanol (EtOH; Commercial Alcohols Inc., Brampton, ON. Canada) for gut content analysis. Finally, muscle tissue was taken above the lateral line and below the dorsal fin for direct mercury analysis (Section 2.10); skin was removed and the sample was placed in a plastic microcentrifuge vial and frozen again at -20°C for later analysis.

2.7 Ageing Analysis

To prepare bony structures, opercula were removed from perch specimens, placed in warm water for 10 minutes, then rubbed clean using Delicate Task Wipers (4.4”×8.4” 1-ply Kimwipes*, Kimberly Clark Professional, Roswell, GA., U.S.A.) and set to dry for 24 hours prior to ageing. Preparing fin rays for ageing required: sealing them in epoxy or resin; making slender cross-sections of the fins using a jewellery saw, as close to the insertion point to the body as possible to obtain 4-5 complete cross-sections; and then mounting cross-sections on labelled slides for examination under a microscope. The second and third cross-sections were assessed for age, since the first cross-section was often compromised from being removed from the side of the fish.

Yellow Perch ages were determined from cross-sectioned and mounted fin rays in 2012, and opercula were used to age Yellow Perch collected in 2014, 2015, and 2016. While otoliths are considered to be the most precise ageing structure, dorsal spines have also been shown to be adequate in assessing ages of Yellow Perch, based on a coefficient of variation <10% (Niewinski and Ferreri 1999). Technicians at the Northwest Biodiversity and Monitoring Ageing Laboratory (NBMAL) also compared Yellow Perch dorsal spines from lakes in northwestern Ontario, to pelvic fin rays collected from Lake 222 and Lake 239; cross-sections were visually similar, and matched for slow and fast growth years (Table 2.4) when inferred ages were compared (Susan Mann, Fisheries Ageing Biologist, NBMAL, OMNRF, Pers. Comm.). I therefore assumed that ages determined using pelvic fin rays were comparable to dorsal spines, and therefore of comparable precision. Studies have also shown ageing of otoliths and opercula are comparable in Yellow Perch up to age six (Sotola *et al*. 2014; May 2005; Appendix D Figure 1). Yellow Perch opercula ageing results have also been shown to exhibit higher precision and among-reader agreement, and lower age-bias than ageing dorsal spines (Sotola *et al*. 2014).

A sample of Yellow Perch were assessed for accuracy of age assignment, by comparing 2012 otoliths (cracked-and-burned) versus 2012 fin rays (slide-mounted), and 2014-16 otoliths (cracked-and-burned) versus 2014-16 opercula (whole), from Lake 239 and Lake 222 (blind ageing analysis with ≥85.2% confidence; Appendix D Table 1). There was >95% agreement between structures with their assigned ages between zero to nine years (majority less than six years old), with high confidence levels (ranked from 0 to 9, with 9 being the most confident), upon examination using a compound microscope-and-camera setup (S. Mann, Pers. Comm.).

Ageing fish using opercula and fin rays (Appendix D Figure 2) involved analysing each structure for their large whitish bands of summer growth, followed by thin translucent bands of winter growth (Bardach 1955). This combination of summer and fall rings made up one year, whereby the end of each transparent growth made up one annulus. By counting the rings from their origin to the edge of the opercula, the age of each fish was assessed, with the exception that only

the first annulus (not the origin) was identifiable on fin rays. Ageing of opercula was achieved by alternating between holding the structure up to a consistent light source and against a black felt block, to ensure all translucent bands were counted with a degree of certainty. In occasions of uncertainty and low confidence levels, a dissecting scope was employed. All fin ray cross-sections were aged using alternating transmitting and reflective light under a dissecting scope.

Growth and resulting band thickness was directly linked to observations of "good" and "poor" growth years, as documented by the NBMAL as consistent trends across all of northwestern Ontario – based primarily on temperatures and rainfall (S. Mann, Pers. Comm.; Table 2.4).

Table 2.4. Growth Trends for Fish in Northwestern Ontario, from 2005 to 2016 (Source: S. Mann, Pers. Comm).

GROWTH	2016	2015	2014	2013	2012	2011	2010	2009	2008	2007	2006	2005
Good							×				×	×
Average	×	×		×		×				×		
Poor			×		×			×	×			

Here, "good" years have summer growth rings greater than double the size of the winter rings, "average" years have summer growth rings approximately 1.5 times as wide as the winter bands, and "poor" years have summer growth rings similar in width to winter growth bands. On this basis, it was concluded that there were no differences in the ageing technique or the assigned ages, between the two ageing structures (S. Mann, Pers. Comm.).

2.8 Gut Content Analysis

Dissected stomachs of Yellow Perch were sorted by lake, season, and size class during nAg addition (2014) and after nAg addition (2016; Table 2.5). Stomach samples from before manipulation (2012) were unavailable. A subsample of 15 stomachs were randomly selected per season for gut content analysis. Few fish were caught in the largest size classes (151-170mm, >170mm), so all specimens in this size class were analysed where possible. Otherwise, an average of three stomachs with contents from fish in each size class were analysed in each sampling period. If empty stomachs were encountered, another fish from the same size class was selected.

Table 2.5. Number of Perch Stomachs Analysed for Gut Contents in Lake 222 and Lake 239, in 2014 and 2016.

Lake	Fork Length (mm)	Yellow Perch (#)			
		2014		2016	
		SUM	FALL	SUM	FALL
222	**<71**	4	5	3	3
	71-90	3	3	4	3
	91-110	2	3	4	3
	111-130	3	3	3	3
	131-150	4	3	3	2
	151-170	0	1	1	1
	>171	0	0	0	1
	TOTAL	**34**		**28**	
239	**<71**	4	3	4	4
	71-90	3	3	3	2
	91-110	3	3	3	3
	111-130	3	3	3	3
	131-150	3	3	3	3
	151-170	2	3	3	2
	>171	0	0	0	1
	TOTAL	**36**		**37**	

Stomach contents were analyzed by dissecting and sorting preserved gut contents using a dissecting scope with both reflected and transmitted lighting. Gut contents were identified as either (1) benthic invertebrates and identified to the 27-group OBBN level, or (2) zooplankton, and sorted into orders of Cladocera, Copepoda, Mysidacea, and larval Diptera (mainly *Chaoborus*), or (3) fish (exclusively identified as conspecific Yellow Perch). Grouped clusters of the prey items were placed in tared plastic weigh boats, and excess ethanol allowed to evaporate from the dish. Diet items were then weighed (g) on a four-decimal balance and recorded. Occurrences of empty stomachs were documented; prey frequency and abundance were also determined (George and Hadley 1979). Since perch diet directly depends on fork length (Sherwood *et al*. 2002), with larger fish trending to benthivory and/or piscivory, and smaller fish exhibiting high zooplanktivory, size-specific prey type and occurrence were considered in the analyses of diets and estimates of energy density and MeHg for fish bioenergetics values.

2.9 Methylmercury Analysis

Methylmercury analysis of benthic invertebrate and zooplankton samples was conducted in Lakehead University's Environmental Laboratory (LUEL), using BROOKS-RAND and MERX MeHg Systems following EPA method 1630 (U.S. EPA. 2001). A total of 51 samples were selected

from frozen invertebrates, and nine samples from frozen bulk zooplankton among both lakes (Table 2.6). Sample selection was based on the quantity available for each sub-group or order, respectively, as well as their occurrence in the stomachs of perch specimens (Section 2.8; Appendix E Table 1).

Table 2.6. Number of Prey MeHg Samples Run for Lake 222 and Lake 239, from 2012, 2015, and 2016.

Lake 222	2012	2015		2016	
	SUM	SPR	SUM	SPR	SUM
Benthos	7	6	4	6	6
Zooplankton	1	0	0	2	2
Sub-Total	8	10		16	
TOTAL	34				
Lake 239	**2012**	**2015**		**2016**	
	SUM	SPR	SUM	SPR	SUM
Benthos	9	0	0	6	7
Zooplankton	1	0	0	1	2
Sub-Total	10	0		16	
TOTAL	26				

Samples were prepared using standard methods (Ogorek and Dewild 2010). Large invertebrates were individually transferred to tared vials and weighed, while smaller invertebrate samples and zooplankton samples were pooled to provide sufficient sample weight. Clean Teflon digestion tubes were arranged on a sample rack, and 25-150mg of sample was weighed into each tube. Under a fume-hood, 10mL of 5M HNO3 solution was dispensed into each tube and capped and vortexed. Samples were heated overnight in an oven at 50°C (minimum eight hours). After acid digestion, vials were prepared for analysis. Clean 42mL amber glass vials were placed in a sample rack, and 35mL reagent water was added to each vial. The acid-digested MeHg source (standard or sample extract) was added to each vial, with volumes dependent on mass of MeHg expected for the sample (Gastropoda sample test), from 10 to 500µL. Method blank volumes were 300 µL. The pH of each sample was tested upon cooling the next morning and adjusted to pH 4.9 with an acetate buffer, before removing the aliquot for direct analysis, since low pH interferes with the ethylation procedure (distillate with pH<3.5 must be discarded).

Within 24 hours of digestion, samples were titrated, ethylated and purged, and trapped MeHg was desorbed. Sample extracts and blanks were titrated with 4.5M KOH solution, equivalent

to the volume extract. Then 300µL of the sodium acetate buffer was added to every vial. Samples were ethylated in a closed purge vessel by the addition of 50 µL of 1% sodium tetraethyl borate. Vials were filled with reagent water until a reverse meniscus formed, to prevent air bubbles when the sample was sealed. The full rack was placed on the autosampler tray, and the MERX Program was started. MeHg was separated from the solution by purging with argon gas onto a graphitic carbon trap. Trapped MeHg was thermally desorbed from the carbon trap into an inert gas stream, which carried the released mercury through a pyrolytic decomposition column (converted organic mercury forms to elemental mercury), and then into the cell of a cold-vapor atomic fluorescence spectrometer (CVAFS) for detection. Quality assurance and quality control (QA/QC) were ensured in this process through the calibration, comparison, and testing of the distillation, ethylation, purging, and detection systems.

The MeHg System accuracy was determined to be 94%, with 6.33% system precision, calculated using DORM-3 (Environment Canada; certified value = 0.355±0.056µg/g) SRM, with measured values of 0.304µg/g and 0.308µg/g, measured in two batches from 61 samples of identified benthos and zooplankton. A sample of Mysidacea from Lake 239 was used to determine sample quantity and instrument precision, and a Gastropoda sample from Lake 222 was run twice (initial run = 0.56ng/g; duplicate run = 0.84ng/g). The detection limit for the machine was 1 picogram, and as high as 1000pg; samples were within detection range, so were not diluted.

Prey MeHg concentrations from 2012 were used in the 2012 MMBM, while prey MeHg concentrations from 2015-16 were used in the 2014-16 MMBM (Appendix E Table 2; Table 2.7).

Table 2.7. Calculated Prey MeHg Values for Lake 222 and Lake 239 Perch based on Yellow Perch Gut Contents.

Study	Fork Length (mm)	MeHg (mg/kg)	
		2012	2015-16
Lake 222	<100mm	0.0055	0.0080
	≥100mm	0.0026	0.0089
Lake 239	<100mm	0.0063	0.0095
	≥100mm	0.0679	0.0681

Some taxa in perch diets were not collected in the field, and were therefore not a part of MeHg analysis. For missing zooplankton taxa, the bulk zooplankton MeHg value was used. For

other missing taxa, or prey items encountered in gut contents but not in the MeHg measured from benthos, I assumed an average benthos value where appropriate. Seasonal differences in perch diets were assumed to be negligible based on gut content results (Section 3.1.1).

2.10 Direct Mercury Analysis

To parameterize the MMBM (Section 2.4), Yellow Perch samples from 2012 to 2016 were run on Direct Mercury Analyzer (DMA) in duplicate. The DMA was calibrated, primed, and run according to EPA method 7473 (U.S. EPA. 1998). Detection limit of the DMA was 0.001ng [Hg], with a working range of 0.01 to 1500ng [Hg]. DMA results were validated against results from two other laboratories to ensure consistency, and ongoing QA/QC standards were met.

TORT-3 lobster hepatopancreas Standard Reference Material (SRM, provided by the National Research Council of Canada or NRC) was used to evaluate ongoing precision of the DMA. This SRM was selected for its anticipated proximity to the Yellow Perch mercury values. Sample volumes of TORT-3 were targeted at 0.034±0.006g, to reflect 10ng peak absorbance, for a result within the standard range of 0.292±0.022mg/kg total [Hg] (NRC, https://www.nrc-cnrc.gc.ca/eng/solutions/advisory/crm/ certificates/tort_3.html; Table 2.8).

Table 2.8. Mean Total Mercury Concentrations of SRMs and Blanks for Lake 222 and Lake 239.

Lake 222	**2014**		**2015**			**2016**			**Average**
	SUM	**FALL**	**SPR**	**SUM**	**FALL**	**SPR**	**SUM**	**FALL**	
Blank (mg/kg Hg)	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	**0.0000**
TORT-3 (mg/kg Hg)	0.2884	0.2853	0.2814	0.2836	0.2779	0.2772	0.2757	0.2752	**0.2806**
Runs (#)	2	2	2	3	3	3	2	2	**19**
Lake 239	**2014**		**2015**			**2016**			**Average**
	SUM	**FALL**	**SPR**	**SUM**	**FALL**	**SPR**	**SUM**	**FALL**	
Blank (mg/kg Hg)	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	**0.0000**
TORT-3 (mg/kg Hg)	0.2839	0.2847	0.2776	0.2751	0.2751	0.2759	0.2754	0.2729	**0.2776**
Runs (#)	2	2	2	2	3	2	2	3	**18**

Blanks on the DMA were accepted if they were less than 0.003mg/kg total [Hg]. Samples in a given run were not accepted if the SRMs for that run fell outside the reported interval by NRC, and all samples in the run were repeated. Yellow Perch were analysed individually, and results were averaged within age cohorts per season, for each year and each lake, to provide the average initial total [Hg] (summer) and average final total [Hg] (fall) input values for the MMBM.

2.11 Energy Density Analysis

Lake-specific measured values of 4876.06±460.91J/g (Lake 222), and 4501.21±587.66J/g (Lake 239) were the ED_{Fish} values for each year. Energy densities of prey were estimated by assigning proportions of prey found in Yellow Perch gut contents (Section 3.1.1) to energy density values from Cummins and Wuycheck (1971). Since piscivory was only observed in Lake 239 (ages 3-plus, and confirmed to be largely cannibalism), lake-specific prey energy densities of fish in diets were assumed to be Yellow Perch. Examination of gut contents revealed no significant difference in prey rations between 2014 and 2016 Yellow Perch, or between summer and fall seasons, so I applied similar prey energy density for all years and seasons in each lake (Section 3.1.1). Since diet energy density values are size-specific, but gut content observations for Lake 222 and Lake 239 revealed few significant differences within immature and mature perch groupings, only two diet energy density values were estimated for each lake ($ED_{IMMATURE}$: fork length <100mm, ages 0-2; ED_{MATURE}: fork length ≥100mm, ages 3+; Appendix F Table 1).

2.12 Population Estimation

Mark-recapture batch-marking of fins (perch) and individual tag identification via PIT tags (pike) permitted population estimation. For Northern Pike populations, I used the open population POPAN method in Program MARK (Program MARK 2014; Arnason *et al*. 1998). However, few seasonal nicks for Yellow Perch were observed between capture periods, whereas captures within a given time period were more common. As a result, a closed population Schnabel census (Schnabel 1938) was used to estimate abundance of Yellow Perch in Lake 222 and Lake 239. For the Schnabel census, capture periods where sample sizes of recaptured individuals were low (below 10%), or where fewer than 10 recaptured fish were observed, were excluded from the analysis.

Assumptions of both Schnabel census and POPAN models were met, including: (1) every animal in the population at a given sampling period had an equal chance of capture; (2) every animal had an equal chance of survival until the next sampling occasion; (3) marked animals did

not lose their marks, and marks were not overlooked; (4) sampling periods were short, so animals survived between sampling midpoints; (5) survival and capture of each animal was independent of the fate of any other animal; and (6a, pike only) all emigration from the population was permanent, or (6b, perch only) the population was closed, with no immigration or emigration or recruitment occurring during the sampling period (Handbook of Capture-Recapture Analysis 2005). Handling mortalities were subtracted from the estimated total densities.

The POPAN sub-module is a modification of the Cormack-Jolly-Seber (CJS) model. Where the CJS model considers the marked cohort of animals only and follows the subsequent recaptures, the modified POPAN formulation uses ratios of unmarked versus marked individuals – to permit estimates of population size, survival, and capture probabilities (Arnason *et al*. 1998). The POPAN sub-module fits a generalized linear model to solve for survival (ϕ), capture probabilities (p), entries to the population (p_{ent}), and a single estimate for super-population size, and uses a likelihood function based on the encounter histories of individual fishes to generate a solution (Arnason *et al*. 1998).

Initially, full models (estimates in each capture period for ϕ and p) were attempted. To avoid over-parameterization of models over the short study period, we assumed p_{ent} to be constant in all models. Models were then formulated to generate parameters specific to either season (spring, summer, and fall), study period (before, during, and after), and delayed nAg effects (2012 to spring 2014, summer 2014 to spring 2016, and summer 2016 to summer 2017) combinations, to determine if these arrangements provided better fits. All models were compared using AIC and ΔAIC values were used to select the best-fitting model (where ΔAIC <2 between models indicated models with equivalent best fit).

Adjustments for goodness-of-fit (GOF) were conducted using Test 2 and Test 3 of the CAPTURE module in MARK, which were tests for violations of assumptions that (1) every animal had the same probability of recapture – Test 2; and (2) every animal had the same probability of survival to the next capture occasion – Test 3. GOF was incorporated in the model by adjusting the

$\hat{c}$ value from null, or 1.0, based on the Chi-square and degrees of freedom of the sum of Test 2 and Test 3 (Lake 222: $\chi^2 = 69.287$, $df = 56$, $p = 0.109$; Lake 239: $\chi^2 = 44.052$, $df = 38$, $p = 0.231$). The calculated $\hat{c}$ for Lake 222 was 1.237, while the calculated $\hat{c}$ for Lake 239 was 1.159, which were applied to the POPAN models to determine adjusted ΔAIC values and actual GOF of the models.

2.13 Proportion Calculations by Size Class

Since only a small number of the Yellow Perch were sacrificed each season and aged using their ageing structures, predicted ages were assigned to the remaining perch individuals via size-at-age relationships. I used summer perch from each lake, as it best represented all age cohorts, minimized range and overlap of size classes, and capture methods in each lake involved seine nets only during the summer (compared to fall populations; Appendix G Table 1).

Lake-specific fork length-at-age relationships and Yellow Perch were predicted and analysed in R using age-length keys for unequal interval age cohorts (Ogle 2016; Isermann and Knight 2005). Proportions of Yellow Perch with known ages were assessed per age cohort, as outlined in Kimura (1977), to provide an age sample against which the age-length key was run. The age-length key applied an integer-based approach to estimating fish population age structure, age frequency, and mean length-at-age, explicitly assigning ages to individual unaged perch, based on a known number of aged fish in each age cohort (Isermann and Knight 2005). The assessed fish ages and assigned length categories were summarized using a two-way contingency table, where the number of fish in the age sample was based on the conditional probability of each age given the length category (Ogle 2016). Proportion of Yellow Perch age cohorts (ages 0 to 7) in the summer populations of each study period (from 2012 to 2017) were calculated from sample size *n* (Section 3.2.4), which were multiplied by population estimates for the period when individual consumption was extrapolated to the population-level.

2.14 Population-Level Bioenergetics

Determination of perch abundance (Section 2.12) allowed for scaling the cohort-estimated energy consumption to the population level in each of the lakes, for each season, across all years of the study. Annual gross consumption at the ecosystem level was calculated using bioenergetics data (Section 2.4), population estimates (Section 2.12), and proportions of Yellow Perch size classes (Section 2.13). For each Yellow Perch age cohort, summer size distribution data for each period was used to estimate a proportion of total abundance for each age cohort. These cohort-specific abundance estimates were then applied to absolute consumption estimates (g_{food}/day) from summer-fall data, to estimate grams of food consumed per day for each cohort. These values were summed within each period, and then multiplied by the number of days from May 1st to October 31st, for each lake, to estimate annual lake-wide consumption. Feeding of perch from November 1st to April 30th was assumed to be negligible (Eckman 2004).

Gaps in the data occurred where consumption rates could not be estimated; the model failed to converge because Yellow Perch final weights and/or final [Hg] were substantially less than initial inputs, or cohorts were missing from the sampling period. Spring-summer modelling for Lake 222 only resulted in bioenergetics estimates for ages 1, 2, and 5 in 2015, and ages 1 to 4 in 2016; spring-summer modelling did not converge for Lake 239 perch data except for age 1 in 2016. As a result, spring-summer data was left out of individual- and population-level bioenergetics analyses. Missing summer-fall consumption data were calculating using an intermediate value of consumption between adjacent cohorts; where peripheral age 1 or 6 data was missing, values were calculated using the average difference in consumption between age 1 and age 2, or age 5 to age 6, in other study periods and applying the average difference to the age 2 or age 5 consumption estimate for that period. This permitted gross consumption estimates encompassing perch ages 1 to 6, for comparison between study periods and lakes. Perch proportions of age 0 and 7-plus cohorts were not analysed for bioenergetics.

2.15 Statistical Analysis

2.15.1 Individual-Level Analysis

For all analyses, data normality and variance were assessed using Anderson-Darling and Levene's tests. Tests that failed these assumptions were log-transformed. Transformations in some cases were unsuccessful in normalizing the data; results where this is the case are presented in associated tables, for evaluation by the reader.

Prey data were analysed using a generalized linear model (GLM) of size on each diet type. Since the response variable was binary (presence-absence) and the systematic component was continuous, binomial distribution and logistic regression were used. Log-likelihood ratio tests were used to evaluate model significance. To address whether there were nanosilver-related changes in Yellow Perch size-at-age, and determine if there were differences between experimental and reference lakes, BACI two-way ANOVA compared control (Lake 239) and impact (Lake 222) lakes, with baseline (2012, before), nAg addition (2014-15, during), and recovery (2016, after) study periods.

Consumption rates (*C*; J/day), growth rates (*G*; J/day) conversion efficiencies (*K1*; g_{growth}/g_{food}), activity multipliers (*ACT*; unitless), and total metabolism (R_T; J/day) derived from the MMBM, were examined in R (R Core Team 2017). In each case, A-D tests for normality and Levene's test for homogeneity of variance were performed as formal tests for assumptions, as described above. Differences in consumption rates, growth rates, standard metabolic rates, and total metabolism were assessed using test of heterogeneity of slopes and ANCOVA (Quinn and Keough 2002).

Statistical analysis of conversion efficiency and activity estimates matched the BACI study design, for before-after impacts, using two-way Analysis of Variance (ANOVA), where significant interaction terms were considered evidence of significant nAg impacts on measured response variables. Bioenergetics estimates for each age cohort were considered independent observations. Common slope from modelled consumption rates were used in conversion efficiency calculations,

along with modelled growth rates. Outliers were removed from the data analysis for one age 5 perch in 2012 in Lake 222, and one age 6 perch in 2012 in Lake 239.

Assumptions of BACI include: (1) timing of the impact or activity (nAg addition) was known, so sampling occurred at occasions before and after the impact treatment; and (2) there was independence between control (Lake 239) and treatment (Lake 222) units, as well as all combinations of sampling events and time (2012, 2014-15, and 2016). These assumptions were met for this study design, as the contaminant additions were scheduled during the ice-free season over two years, there was no hydrologic connectivity between Lake 222 and Lake 239, and fish were captured independently at sampling occasions. The model for this analysis is:

(9) $$X_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

Where μ is the overall mean, α is the effect of period (i = before or after), β_j is the effect of location (j = control or impact), $(\alpha\beta)_{ij}$ is the interaction between period and location, and ε_{ijk} is the remaining error. This approach permitted analysis of before-after impacts, reference-experimental lake impacts, interaction term, and both before-after error and reference-experimental error terms. If the interaction term was significant, it signalled an impact of the stressor – with reference and impact lakes being the same before, and potentially different after.

2.15.2 Population-Level Analysis

Similar to individual-level analysis, a BACI design approach was used to determine changes in fish abundance – comparing impact and control lakes against before, during, and after study periods. Two-way ANOVAs were performed on population estimates, with particular emphasis on interaction terms to assess impacts of nAg additions in Lake 222. Significant interaction terms were considered evidence of impact of nAg. Condition of Yellow Perch and Northern Pike individuals was assessed between study periods (before, during, and after nAg additions) in Lake 222, by comparing linear fits of logarithmic transformations of fork length and weight data. Condition of fish in Lake 239, from 2012 to 2017, was also compiled for reference.

Only perch captured and weighed in the summer were included in the condition analysis, to reduce seasonal fluctuations within each study period; all captured pike with associated weights were included in the analysis. Lines were compared between before, during, and after nAg exposure time periods, using tests for heterogeneity of slopes. Breakpoint regressions were used for pike abundance. A-D tests for normality and Levene's tests for homogeneity of variance were performed as formal tests for assumptions. Logarithmic transformations were performed on data that did not initially meet assumptions, as described above.

3 Results

3.1 Individual-Level Results

3.1.1 Yellow Perch Diet Composition

Summer and fall samples gut contents data combined for each year, and the following trends were observed: Lake 222 Yellow Perch in 2014 (nAg addition period) consumed a variety of zooplankton species, up to approximately 60mm fork length, at which point their gape size accommodated larger prey and they switched exclusively to benthivory (Figure 3.1).

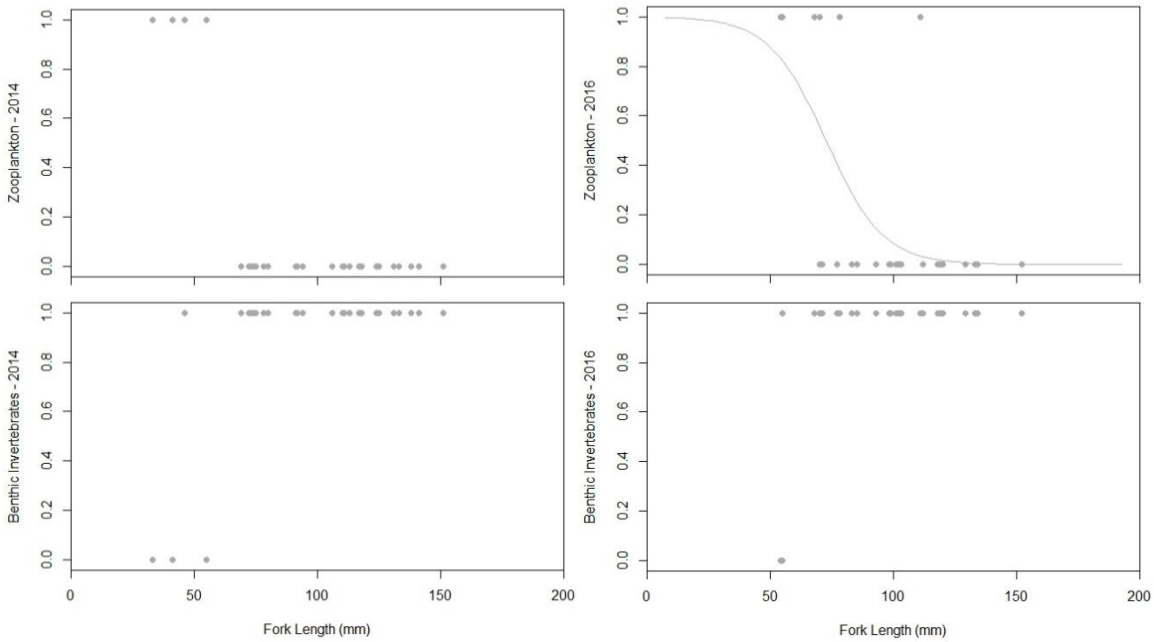


Figure 3.1. Fork lengths at which Lake 222 Yellow Perch consumed a greater proportion of zooplankton (top) versus benthic invertebrates (bottom), during nAg addition in 2014 and recovery in 2016. Where logistic regression curves indicate model convergence and results of inflection point analysis.

In 2016 (recovery period) there were occasions of zooplanktivory which overlapped with benthivory in Yellow Perch (46-112mm in fork length). In Lake 239, smaller perch consumed zooplankton, grew into a diet that included benthic invertebrates, and became piscivorous around 100mm fork length, which corresponded with sexual maturity around age 3 (Figure 3.2).

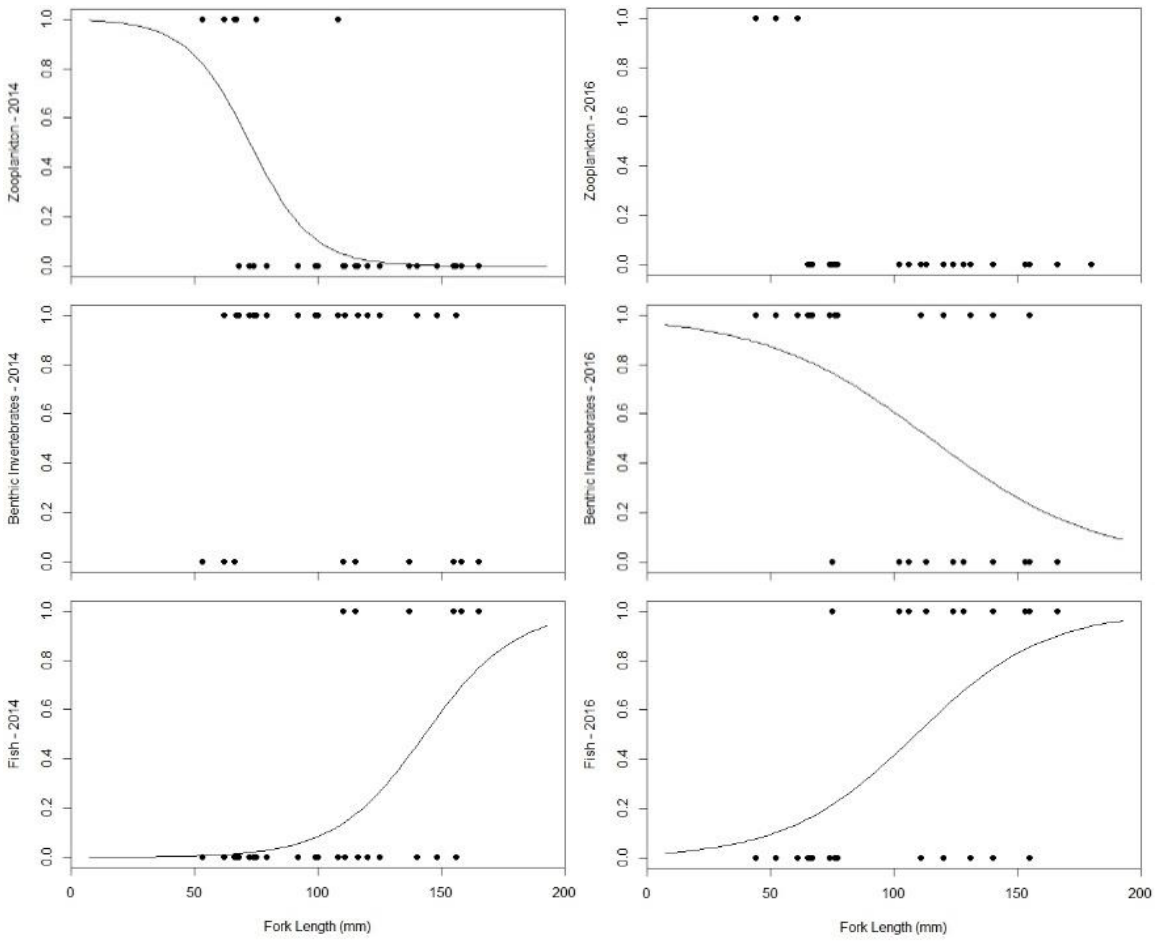


Figure 3.2. Fork lengths at which Lake 239 Yellow Perch consumed a greater proportion of zooplankton (top) versus benthic invertebrates (middle) or fish (bottom), in 2014 and 2016. Where logistic regression curves indicate model convergence and results of inflection point analysis.

I calculated inflection points (curves; Figure 3.2) for each prey type between 2014 and 2016, to determine the size at which perch transitioned from eating mostly zooplankton to mostly benthic invertebrates (50:50) in Lake 222, and all combinations of prey group transitions in Lake 239 (Table 3.1). Where the model failed to converge (Figures 3.1, 3.2), prey shifts were estimated.

Table 3.1. Prey Inflection Points for Lake 222 and Lake 239, in 2014 and 2016. Asterisks (*) indicate estimated shift.

Lake	Prey Item	Year	Inflection Point Fork Length (mm)	Assumptions and Actions
222	Zooplankton	2014	59.25*	Model did not converge
		2016	72.59	Fork length significant (p <0.05)
	Benthic Invertebrates	2014	51.25*	Fork length not significant
		2016	61.5*	Model did not converge
239	Zooplankton	2014	72.25	Fork length significant (p <0.05)
		2016	61.25*	Model did not converge
	Benthic Invertebrates	2014	109*	Fork length not significant
		2016	114.63	Fork length significant (p <0.05)
	Fish	2014	143.36	Fork length significant (p <0.05)
		2016	108.78	Fork length significant (p <0.05)

While there were clear prey transitions in Lake 222, diets of Lake 239 perch largely overlapped in prey types. Lake 222 perch in 2014 (first year of nAg addition) switched from zooplanktivory to benthivory at a smaller size than perch in 2016 (first year of recovery; Figure 3.1). The opposite was observed in Lake 239, where diet shifts from majority zooplankton to majority benthos appeared to occur at larger sizes in 2014 than 2016 (Figure 3.2).

Outside of the natural shift in diet from smaller zooplankton prey to larger benthos, the proportion of prey groups in perch stomachs changed only slightly from 2014 to 2016 (Figure 3.3).

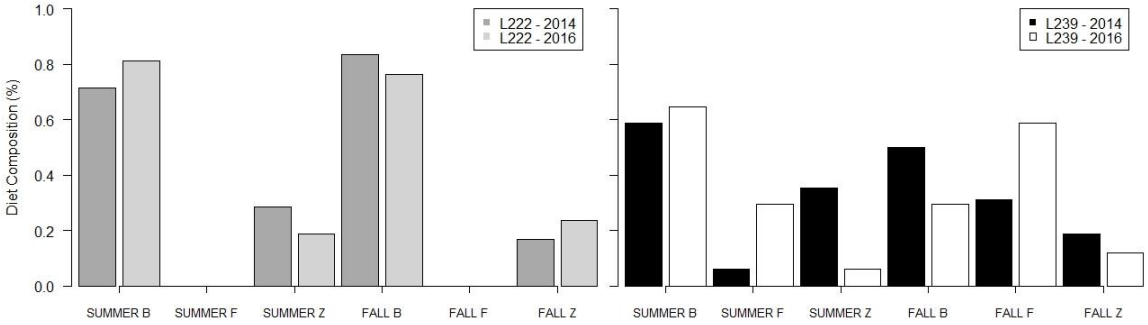


Figure 3.3. Seasonal diet proportions of benthos (B), fish (F), zooplankton (Z) in Lake 222 and Lake 239, during nAg addition (2014) and recovery (2016).

Summer benthos diet proportions were slightly lower in 2014 compared to 2016, and zooplankton were slightly higher. However, BACI ANOVAs revealed no significant interactions between lakes and time periods (Table 3.2). Assumptions were not met as a result of the zero-proportion categories; attempted square root transformations did not improve distributions.

Table 3.2. BACI ANOVAs for Yellow Perch Summer 2014 and 2016 Diet Proportions in Lake 222 and Lake 239.

Factor	BACI ANOVA	*F*-value	*p*-value	Assumptions and Actions
Benthos	During – After	$F_{1,53} = 0.712$	$p = 0.4030$	Period effect is not significantly different
	Control – Impact	$F_{1,53} = 1.466$	$p = 0.2310$	Lake effect is not significantly different
	D-A:C-I Interaction	$F_{1,53} = 1.204$	$p = 0.2770$	Interaction is not significantly different
	Test for Normality	$A\text{-}D = 5.850$	$p < 0.0001$	Data are not normally distributed
	Test for Homogeneity	$F_{3,53} = 1.128$	$p = 0.3463$	Variance is homogeneous
Zooplankton	**During - After**	$\mathbf{F_{1,53} = 9.590}$	$\mathbf{p = 0.0031}$	**Period effect is significantly different**
	Control - Impact	$F_{1,53} = 0.123$	$p = 0.7275$	Lake effect is not significantly different
	D-A:C-I Interaction	$F_{1,53} = 0.280$	$p = 0.5990$	Interaction is not significantly different
	Test for Normality	$A\text{-}D = 3.721$	$p < 0.0001$	Data are not normally distributed
	Test for Homogeneity	$F_{3,53} = 3.331$	$p = 0.0263$	Variance is not homogeneous

Diet proportions of fish prey were significantly different by lake ($F_{1,53} = 7.054$, $p = 0.0104$), as there were no documented occasions of piscivory in Lake 222, despite presence of perch and Blacknose Shiner (*Notropis heterolepis*). Overall, perch diets appeared unaffected by nAg addition.

3.1.2 Yellow Perch Length-at-Age

To determine whether perch growth was affected by nAg addition, summer fork lengths for all aged perch were plotted for each year and analysed (Figure 3.4).

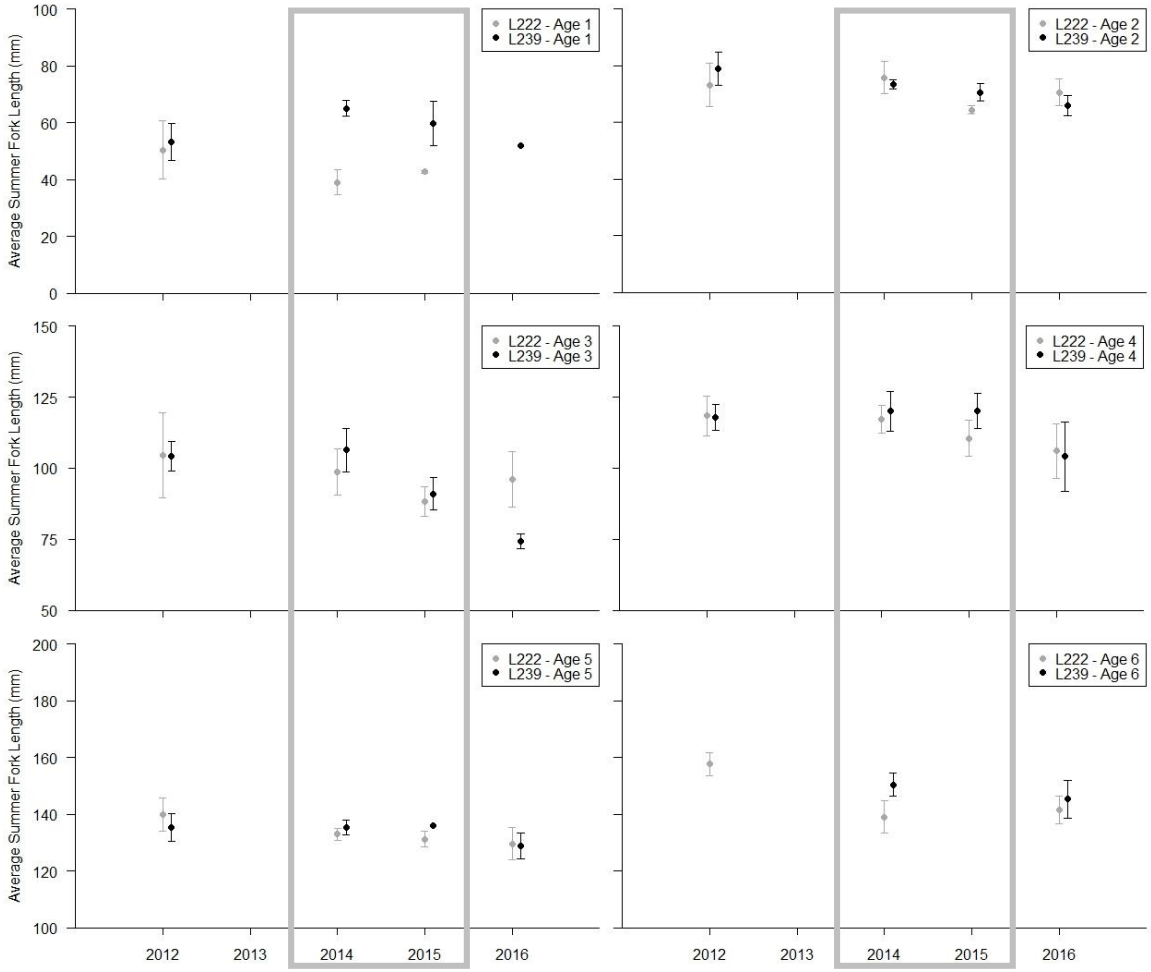


Figure 3.4. Mean summer fork lengths of Yellow Perch by age cohort in Lake 222 and Lake 239 over time. Where the period of nAg addition is outlined in grey from 2014 to 2015; error bars are the standard error of the mean.

The greatest disparity in fork lengths occurred with age 1 perch during nAg addition in Lake 222. There was overlap between experimental and reference lakes for all length-at-age cohorts during the baseline (2012) period, then fork lengths deviated between systems on an age-dependent basis that corresponded with the start of nAg addition. Fork lengths were lower in the second year of nAg addition (2015) for perch ages 2-plus. BACI ANOVAs were used to determine significant differences for length-at-ages, using Tukey's tests to identify significant periods and interactions (Table 3.3). Data were normally distributed, except for ages 1 to 3, which were log-transformed.

Table 3.3. BACI ANOVAs for Yellow Perch Length-at-Age Data in Lake 222 and Lake 239, from 2012 to 2016. Where "Base" is Baseline-Before (2012), "nAg" is Addition-During (2014-15), and "Rec" is Recovery-After (2016). Asterisks (*) indicate log-transformed response variables.

Factor	Age	BACI ANOVA	*F*-value	*p*-value	Assumptions and Actions	TukeyHSD (p_{ADJ})
Fork Length	1*	Before - After	$F_{2,82}$ = 0.155	p = 0.8570	Periods not significant	L239-L222: <0.0001
		Control - Impact	**$F_{1,82}$ = 23.015**	**p < 0.0001**	**Lake effect is significant**	$L222_{nAg}$-$L239_{nAg}$: <0.0001
		B-A:C-I Interaction	**$F_{1,82}$ = 23.317**	**p < 0.0001**	**Interaction is significant**	$L222_{BASE}$-$L222_{nAg}$: 0.0079 $L222_{BASE}$-$L239_{nAg}$: <0.0001
		Test for Normality	*A-D* =0.351	p = 0.4624	Data are normally distr.	$L222_{nAg}$-$L239_{BASE}$: 0.0006
		Test for Homogeneity	$F_{4,82}$ = 2.019	p = 0.0994	Variance is homogeneous	$L239_{BASE}$-$L239_{nAg}$: 0.0179
	2*	**Before - After**	**$F_{2,81}$ = 16.745**	**p < 0.0001**	**Period effect is significant**	Baseline-nAg: 0.0012
		Control - Impact	$F_{1,81}$ = 3.216	p = 0.0766	Lake effect not significant	Baseline-Recovery: <0.0001
		B-A:C-I Interaction	**$F_{2,81}$ = 7.248**	**p = 0.0013**	**Interaction is significant**	$L222_{nAg}$-$L239_{BASE}$: <0.0001 $L239_{BASE}$-$L239_{nAg}$: 0.0332 $L222_{BASE}$-$L239_{BASE}$: 0.0264 $L222_{BASE}$-$L239_{REC}$: 0.0065
		Test for Normality	*A-D* = 0.580	p = 0.1276	Data are normally distr.	$L222_{REC}$-$L239_{BASE}$: 0.0008
		Test for Homogeneity	$F_{5,81}$ = 1.585	p = 0.1737	Variance is homogeneous	$L239_{BASE}$-$L239_{REC}$: <0.0001
	3*	**Before - After**	**$F_{2,60}$ = 28.524**	**p < 0.0001**	**Period effect is significant**	Baseline-nAg: 0.0205
		Control - Impact	$F_{1,60}$ = 1.869	p = 0.1767	Lake effect not significant	nAg-Recovery: <0.0001
		B-A:C-I Interaction	**$F_{2,60}$ = 9.620**	**p = 0.0002**	**Interaction is significant**	Baseline-Recovery: <0.0001 $L222_{BASE}$-$L222_{nAg}$: 0.0377 $L222_{nAg}$-$L239_{BASE}$: 0.0348 $L222_{nAg}$-$L239_{REC}$: <0.0001 $L239_{nAg}$-$L239_{REC}$: <0.0001 $L222_{BASE}$-$L239_{REC}$: <0.0001
		Test for Normality	*A-D* = 0.583	p = 0.1243	Data are normally distr.	$L239_{BASE}$-$L239_{REC}$: <0.0001
		Test for Homogeneity	$F_{5,60}$ = 2.008	p = 0.0903	Variance is homogeneous	$L222_{REC}$-$L239_{REC}$: 0.0029
	4	**Before - After**	**$F_{2,41}$ = 11.439**	**p = 0.0001**	**Period effect significant**	nAg-Recovery: 0.0024
		Control - Impact	$F_{1,41}$ = 0.027	p = 0.8711	Lake effect not significant	Baseline-Recovery: 0.0002
		B-A:C-I Interaction	$F_{2,41}$ = 1.427	p = 0.2518	Interaction not significant	
		Test for Normality	*A-D* = 0.446	p = 0.2708	Data are normally distr.	
		Test for Homogeneity	$F_{5,41}$ = 0.958	p = 0.4546	Variance is homogeneous	
	5	**Before - After**	**$F_{2,32}$ = 12.456**	**p < 0.0001**	**Period effect significant**	Baseline-nAg: 0.0166
		Control - Impact	$F_{1,32}$ = 1.254	p = 0.2710	Lake effect not significant	Baseline-Recovery: <0.0001
		B-A:C-I Interaction	$F_{2,32}$ = 1.984	p = 0.1540	Interaction not significant	
		Test for Normality	*A-D* = 0.591	p = 0.1172	Data are normally distr.	
		Test for Homogeneity	$F_{5,32}$ = 0.881	p = 0.5052	Variance is homogeneous	
	6	**Before - After**	**$F_{2,14}$ = 15.241**	**p = 0.0003**	**Period effect significant**	Baseline-nAg: 0.0187
		Control - Impact	$F_{1,14}$ = 1.285	p = 0.2759	Lake effect not significant	Baseline-Recovery: 0.0026
		B-A:C-I Interaction	$F_{1,14}$ = 1.634	p = 0.2219	Interaction not significant	
		Test for Normality	*A-D* = 0.443	p = 0.2563	Data are normally distr.	
		Test for Homogeneity	$F_{4,14}$ = 0.700	p = 0.6044	Variance is homogeneous	

Interactions for length-at-ages 1 to 3 were significant, indicating a differential response over time in Lake 222 Yellow Perch compared with those in Lake 239. Only age 1 provides clear evidence of nAg impact; lower size-at-age after nAg addition in Lake 222, but higher in Lake 239 (Figure 3.5; Table 3.3). Ages 2 and 3 had potential differences in Lake 222 over time, but were relatively stable compared with much larger declines in reference Lake 239. Length-at-ages 2 to 6 were significantly different between study periods.

3.1.3 Yellow Perch Consumption, Growth, and Conversion Efficiency

The MMBM converged for a majority of sampling occasions, providing consumption (*C*), growth (*G*), and conversion efficiency (*K1*; Appendix B Tables 1, 2) outputs. Log-transformed consumption rates versus mass were plotted for each period, in each lake (as per Pazzia *et al*. 2002; Figure 3.5).

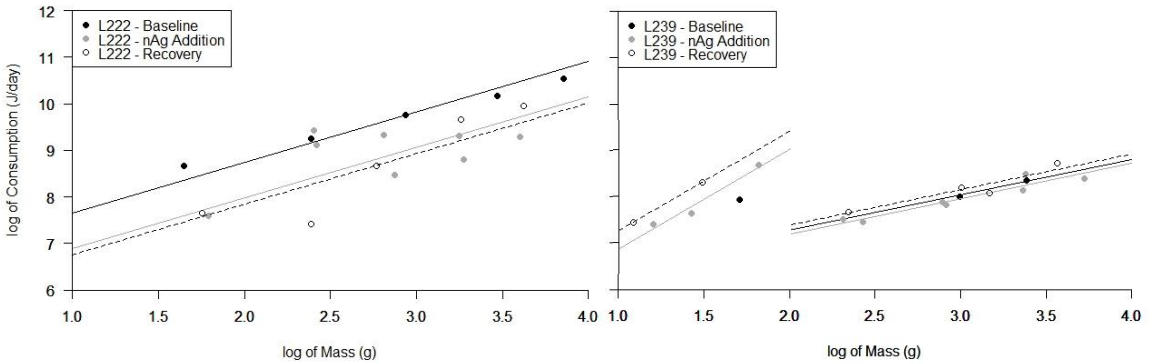


Figure 3.5. Log-transformed absolute consumption rates versus mass for Yellow Perch in Lake 222 and Lake 239.

Size-specific differences in slopes were apparent in Lake 239; diet analysis revealed all age cohorts of perch in Lake 222 are zoobenthivorous, but only ages 1 and 2 perch in Lake 239 are zoobenthivorous. Therefore, consumption rates were separated based on diet type for perch in Lake 239. Perch consumption was lower during and after nAg addition in Lake 222, compared to the baseline period. By contrast, Lake 239 consumption rates for zoobenthivorous perch were lowest in 2012 (baseline), increased in 2014-15 (during), and were highest in 2016 (after; Table 3.4).

Table 3.4. ANCOVAs for Yellow Perch Consumption Rates in Lake 222 and Lake 239, from 2012 to 2016. Results are the trends across all age cohorts of perch in Lake 239 (compared to Fig. 3.5, which displays young vs. old perch).

Lake	Consumption Rate Analysis	*F*-value	*p*-value	Assumptions and Actions
222	Test for Heterogeneity of Slopes	$F_{2,13}$ = 0.804	p = 0.4686	Slopes not significant
	ANCOVA: log(C)~log(M)+PRD	**$F_{2,15}$ = 4.629**	**p = 0.0272**	**Intercepts significant**
	Test for Normality	*A-D* = 0.155	p = 0.9463	Data are normally distr.
	Test of Homogeneity	$F_{2,16}$ = 0.283	p = 0.7575	Variance is homogeneous
239	Test for Heterogeneity of Slopes	$F_{2,13}$ = 0.037	p = 0.9635	Slopes not significant
	ANCOVA: log(C)~log(M)+PRD	$F_{2,15}$ = 0.366	p = 0.6996	Intercepts not significant
	Test for Normality	*A-D* = 0.495	p = 0.1888	Data are normally distr.
	Test of Homogeneity	$F_{2,16}$ = 1.009	p = 0.3866	Variance is homogeneous

ANCOVA results for Lake 222 revealed significant differences; equations of lines were: y_{BASE}=1.0866x+6.5704; y_{nAg}=1.0866x+5.7955; and y_{REC}=1.0866x+5.6697. Consumption-at-age was plotted for each lake, to depict annual variation in age cohorts (Figure 3.6).

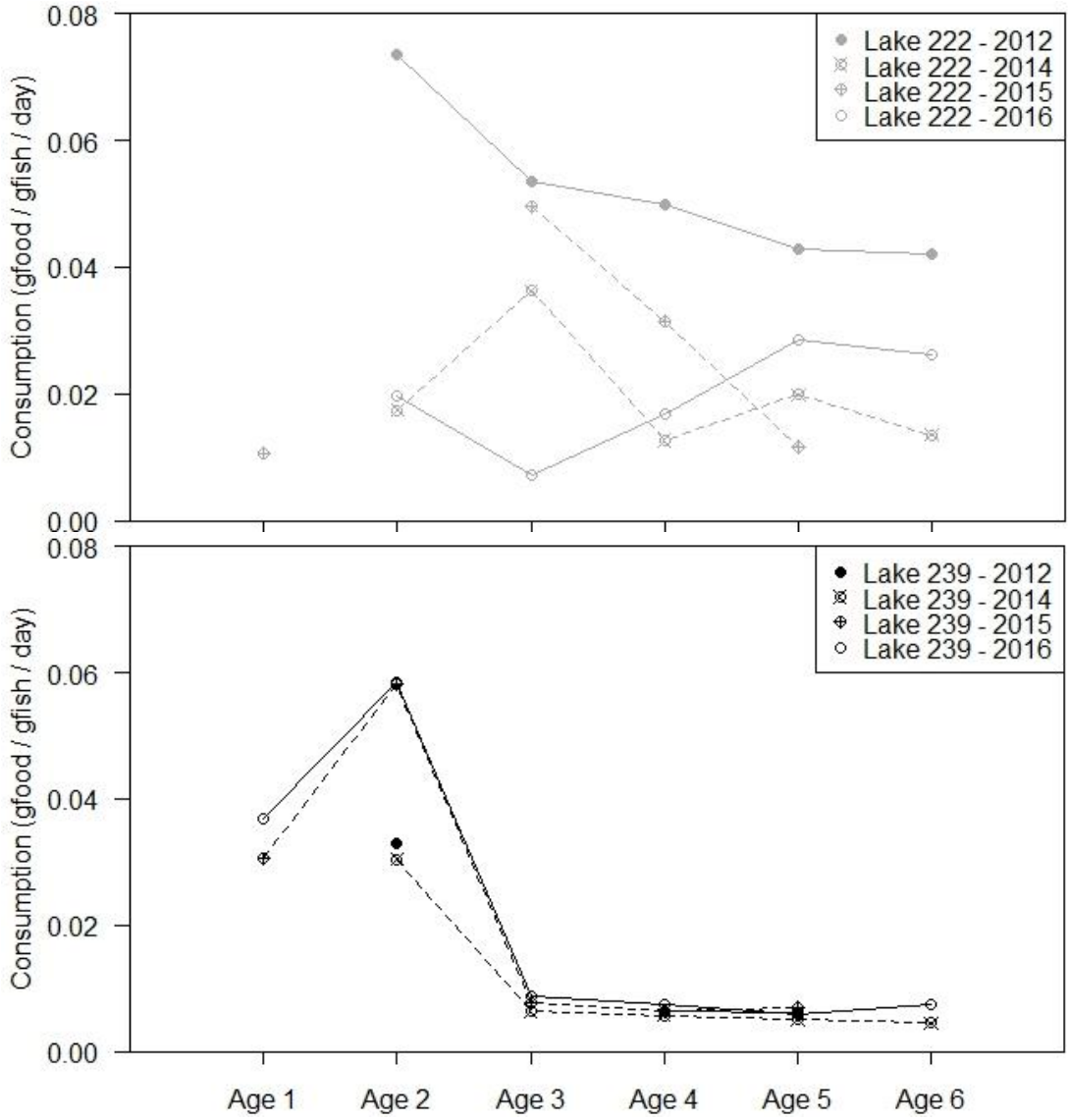


Figure 3.6. Annual changes in mass-specific consumption-at-age for Lake 222 (top) and Lake 239 (bottom) perch.

Mass-specific consumption rates decreased and appeared highly variable for all age cohorts during and after nAg addition in Lake 222, compared to baseline levels. By contrast, reference Lake 239 had very consistent consumption rates – especially across the mature (ages 3 to 6) cohorts. By recovery, consumption rates were slightly higher, with the exception of age 3 perch (these were the age 1 perch in 2014 and age 2 in 2015), which experienced two years of nAg addition at vulnerable stages in their growth and development.

Similar to consumption rates, log-log relationship of growth rates and mass for each period were plotted (Figure 3.7).

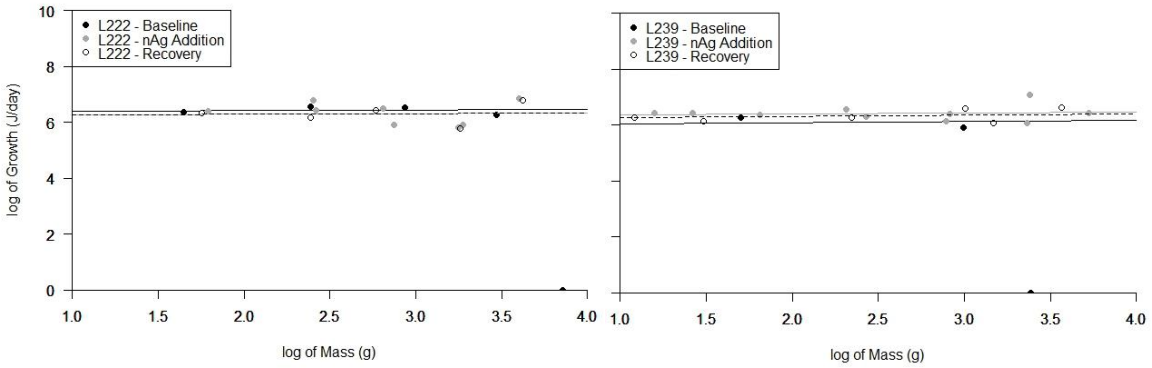


Figure 3.7. Log-transformed growth rates versus mass for Yellow Perch in Lake 222 and Lake 239.

Yellow Perch growth rates were constant between study periods in Lake 222, and did not appear to increase or decrease with increasing mass. Growth rates of perch in reference Lake 239 appeared slightly higher in 2014-15 and 2016 time periods, compared to 2012. To determine significant differences between periods, tests for heterogeneity of slopes and ANCOVAs were conducted within each lake (Table 3.5).

Table 3.5. ANCOVAs for Yellow Perch Growth Rates in Lake 222 and Lake 239, from 2012 to 2016.

Lake	Growth Rate Analysis	*F*-value	*p*-value	Assumptions and Actions
222	Test for Heterogeneity of Slopes	$F_{2,12} = 0.047$	$p = 0.9540$	Slopes not significantly different
	ANCOVA: log(G)~log(M)+PRD	$F_{2,14} = 0.217$	$p = 0.8077$	Intercepts not significantly different
	Test for Normality	$A\text{-}D = 0.410$	$p = 0.3081$	Data are normally distributed
	Test of Homogeneity	$F_{2,15} = 1.253$	$p = 0.3139$	Variance is homogeneous
239	Test for Heterogeneity of Slopes	$F_{2,12} = 0.785$	$p = 0.4782$	Slopes not significantly different
	ANCOVA: log(G)~log(M)+PRD	$F_{2,14} = 1.111$	$p = 0.3565$	Intercepts not significantly different
	Test for Normality	$A\text{-}D = 0.711$	$p = 0.0523$	Data are normally distributed
	Test of Homogeneity	$F_{2,15} = 0.004$	$p = 0.9962$	Variance is homogeneous

Unlike consumption rates, and contrary to both indirect and direct hypotheses of decreased growth rates, relationships between log-transformed growth and mass were not significantly different between study periods in either lake.

Annual growth – or conversion – efficiency (*K1*; quotient of growth and consumption rates), was calculated to determine how efficiently prey consumption was converted into perch growth in Lake 222 and Lake 239 (Figure 3.8).

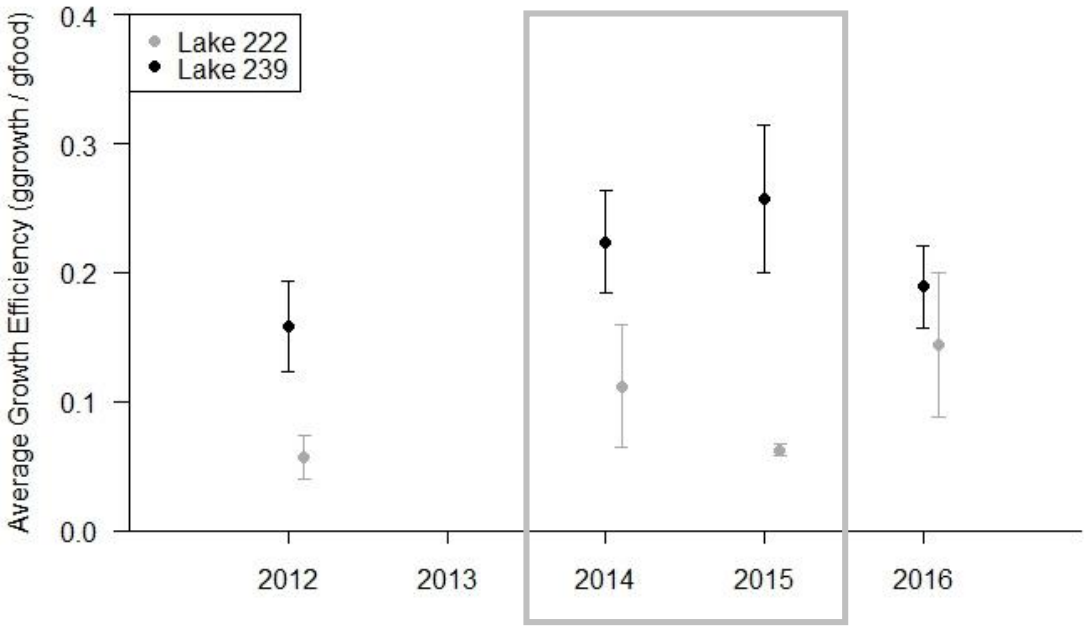


Figure 3.8. Conversion efficiency for Yellow Perch in Lake 222 and Lake 239, from 2012 to 2016. Where the period of nAg addition is outlined in grey from 2014 to 2015; error bars are the standard error of the mean.

Conversion efficiency appeared to increase from baseline (2012) for the first year of nAg addition (2014) in Lake 222, and decline during the second year of nAg addition (2015). Growth efficiency was greater than the 2012 rate in Lake 239 for all other years. Lake-specific growth efficiency only overlapped for in the recovery-2016 period. BACI ANOVAs indicated a significant difference in conversion efficiency between lakes but not periods, and the interaction was not significant (Table 3.6).

Table 3.6. BACI ANOVAs for Yellow Perch Conversion Efficiency in Lake 222 and Lake 239, from 2012 to 2016.

Factor	ANOVA	*F*-value	*p*-value	Assumptions and Actions
Conversion Efficiency	Before - After	$F_{2,29} = 1.963$	$p = 0.1586$	Periods are not significantly different
	Control - Impact	$\mathbf{F_{1,29} = 11.321}$	$\mathbf{p = 0.0022}$	**Lakes are significantly different**
	B-A:C-I Interaction	$F_{2,29} = 1.036$	$p = 0.3676$	Interaction is not significant
	Test for Normality	$A\text{-}D = 0.599$	$p = 0.111$	Data are normally distributed
	Test for Homogeneity	$F_{5,29} = 1.325$	$p = 0.2812$	Variance is homogeneous

Perch in Lake 222 converted consumed energy to growth at greater rates during both years of nAg addition (2014-15) and recovery (2016), compared to baseline (2012). Growth rates (based on weight) were relatively constant before, during, and after nAg addition in Lake 222, and for similar time periods in Lake 239. Only juvenile age 1 perch grew less (length-at-age; Figure 3.4) during nAg and recovery periods, compared to baseline and Lake 239 rates.

3.1.4 Yellow Perch Activity, Standard Metabolic Rate, and Total Metabolism

Activity rates from the WBM are unitless, but describe a range of activity levels (1 to ∞), where 1 represents no movement, and higher values are increased activity. Activity-at-age was modelled for Yellow Perch over the course of this study in Lake 222 and Lake 239 (Figure 3.9).

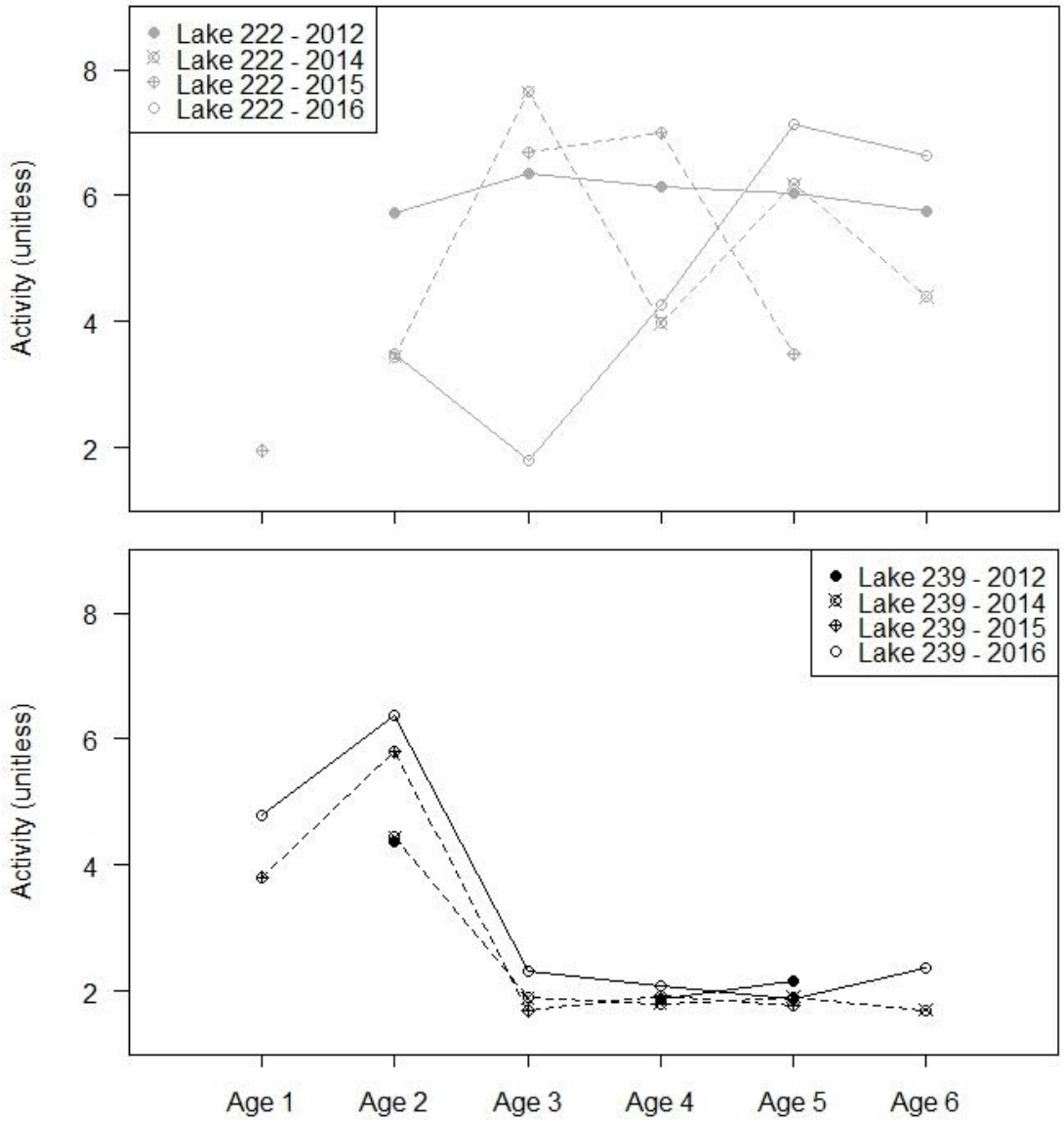


Figure 3.9. Annual changes in activity-at-age for Yellow Perch in Lake 222 (top) and Lake 239 (bottom).

Average activity levels in Lake 222 were around 6 for all age cohorts at the start of the study, then declined for most age cohorts, encompassing a range of activity from 2 to less than 8. Notably, variability increased substantially during (2014-15) and after (2016) nAg addition. In Lake 239, ages 1 and 2 perch were the most active cohorts, with activity levels of 3 to 7, while

mature perch (ages 3 to 6) maintained activity levels around 2 over the duration of the study. Additionally, ages 1 and 2 perch appeared to experience an increase in activity 2015 and 2016 in reference Lake 239, though activity could not be modelled for age 1 perch prior to 2015. BACI ANOVAs were performed on activity-at-age estimates (Table 3.7). Activity rates were significantly different between lakes only, though activity levels in Lake 222 had greater variance during and after nAg addition. Assumptions of data normality were not met, despite log-transformations.

Table 3.7. BACI ANOVAs for Yellow Perch Activity Levels in Lake 222 and Lake 239, from 2012 to 2016.

Factor	ANOVA	*F*-value	*p*-value	Assumptions and Actions
Log(Activity)	Before - After	$F_{2,32} = 1.251$	$p = 0.2998$	Period effect is not significant
	Control - Impact	$\mathbf{F_{1,32} = 16.613}$	$\mathbf{p = 0.0003}$	**Lake effect is significantly different**
	B-A:C-I Interaction	$F_{2,32} = 0.701$	$p = 0.5035$	Interaction is not significant
	Test for Normality	$A\text{-}D = 1.111$	$p = 0.0057$	Data are not normally distributed
	Test for Homogeneity	$F_{L222} = 3.713$	$p = 0.0474$	Variance is not homogeneous in Lake 222
		$F_{L239} = 0.033$	$p = 0.9675$	Variance is homogeneous in Lake 239

Standard metabolic rate (R_s; J/day) is the allometric function of water temperature and body mass (Kitchell *et al*. 1977). Changes in perch respiration were provided by standard metabolic rates (Figure 3.10) for each lake.

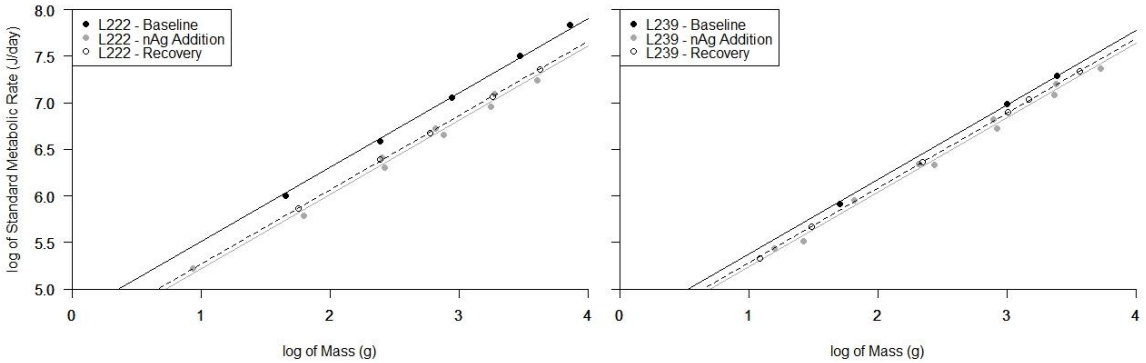


Figure 3.10. Log-transformed standard metabolic rate versus mass for Yellow Perch in Lake 222 and Lake 239.

There were large differences in standard metabolic rates in Lake 222 during and after nAg addition, compared to the baseline study period. Lake 239 appeared to have slight differences in baseline standard metabolic rate, compared to recovery and nAg addition time periods. Tests for heterogeneity of slopes and ANCOVAs were performed on the output for each lake, to determine differences in slopes and intercepts (Table 3.8).

Table 3.8. ANCOVAs for Yellow Perch Standard Metabolic Rates in Lake 222 and Lake 239, from 2012 to 2016.

Lake	Standard Metabolic Rate Analysis	*F*-value	*p*-value	Assumptions and Actions
222	Test for Heterogeneity of Slopes	$F_{2,13}$ = 1.206	p = 0.3308	Slopes not significantly different
	ANCOVA: log(Rs)~log(M)+PRD	$\mathbf{F_{2,15}}$ **= 58.533**	$\mathbf{p < 0.0001}$	**Intercepts significantly different**
	A-D Test for Normality	A-D = 0.500	p = 0.1834	Data are normally distributed
	Test of Homogeneity	$F_{2,16}$ = 0.103	p = 0.9030	Variance is homogeneous
239	Test for Heterogeneity of Slopes	$F_{2,13}$ = 0.534	p = 0.5987	Slopes not significantly different
	ANCOVA: log(Rs)~log(M)+PRD	$\mathbf{F_{2,15}}$ **= 9.780**	$\mathbf{p = 0.0019}$	**Intercepts significantly different**
	Test for Normality	A-D = 0.529	p = 0.1542	Data are normally distributed
	Test of Homogeneity	$F_{2,16}$ = 0.240	p = 0.7895	Variance is homogeneous

Slopes were not different for either lake (Table 3.8). Intercepts varied between baseline (L222: $y_{BASE} = 0.7972x + 4.7312$; L239: $y_{BASE} = 0.7998x + 4.5810$) and nAg addition (L222: $y_{nAg} = 0.7972x + 4.4199$; L239: $y_{nAg} = 0.7998x + 4.4443$) periods in both lakes, as well as baseline and recovery (L222: $y_{REC} = 0.7972x + 4.4739$; L239: $y_{REC} = 0.7998x + 4.4888$) periods. Intercepts were significantly different between periods in both lakes, however, the greatest decrease in standard metabolic rates occurred between Lake 222 baseline and nAg addition periods.

Losses to metabolism (R_T; J/day), which encompassed activity, standard metabolic rate, and specific dynamic action, were also investigated for each lake. Log-transformed total metabolism was plotted against log-transformed mass, and differences in slopes were assessed (Figure 3.11). Similar to consumption rates, total metabolism between Lake 239 zoobenthivorous and piscivorous perch had different slopes, so data were plotted by diet type.

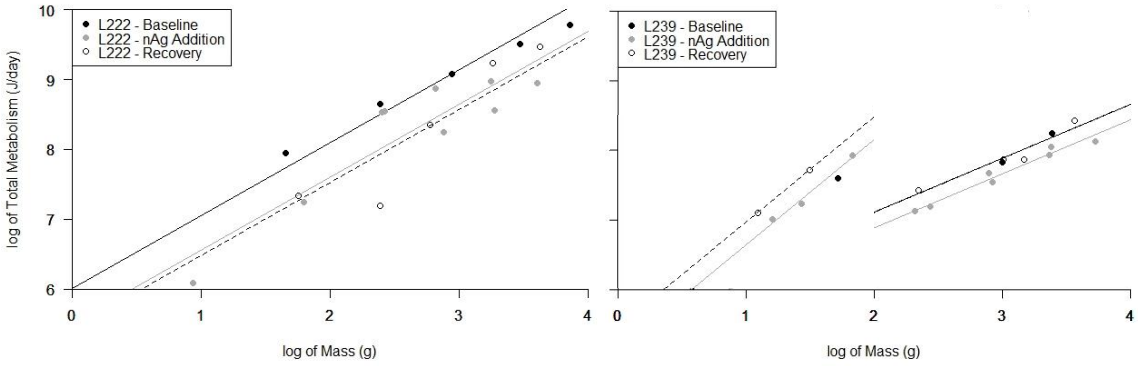


Figure 3.11. Log-transformed total metabolism versus mass for Yellow Perch in Lake 222 and Lake 239.

Perch in Lake 222 experienced fewer losses to metabolism during and after nAg addition – the opposite was observed in Lake 239 zoobenthivorous perch. This supports the declines in consumption in Lake 222 during and after nAg addition, as well as the increases in Lake 239

consumption. Further, baseline data points in Lake 222 appeared shallower (less activity and fewer losses to metabolism with increased mass) compared to steeper data points during nAg addition and in recovery (more activity and greater losses to metabolism with increased mass). Tests for heterogeneity of slopes and ANCOVAs were performed on the total metabolism data (Table 3.9).

Table 3.9. ANCOVAs for Yellow Perch Total Metabolism in Lake 222 and Lake 239, from 2012 to 2016. Results are the trends across all age cohorts of perch in Lake 239 (compared to Fig. 3.11, which displays young vs. old perch).

Lake	Total Metabolism Analysis	*F*-value	*p*-value	Assumptions and Actions
222	Test for Heterogeneity of Slopes	$F_{2,13} = 1.167$	$p = 0.3420$	Slopes not significantly different
	ANCOVA: log(R_T)~log(M)+PRD	$F_{2,15} = 3.572$	$p = 0.0539$	Intercepts not significantly different
	A-D Test for Normality	$A\text{-}D = 0.209$	$p = 0.8386$	Data are normally distributed
	Test of Homogeneity	$F_{2,16} = 0.322$	$p = 0.7294$	Variance is homogeneous
239	Test for Heterogeneity of Slopes	$F_{2,13} = 0.015$	$p = 0.9852$	Slopes not significantly different
	ANCOVA: log(R_T)~log(M)+PRD	$F_{2,15} = 1.761$	$p = 0.2403$	Intercepts not significantly different
	Test for Normality	$A\text{-}D = 0.231$	$p = 0.7718$	Data are normally distributed
	Test of Homogeneity	$F_{2,16} = 0.483$	$p = 0.6259$	Variance is homogeneous

At alpha = 0.05 significance level, and Lake 222 ED_{Fish} value of 4876.06J/g, intercepts were not significantly different by period for either lake ($p = 0.0539$). However, incorporating a higher energy density value for Yellow Perch in Lake 222 (mean + standard error; ED_{Fish} = 4876.06+460.91J/g; ED_{Fish} = 5336.97J/g) provided significant differences between intercepts (ANCOVA: $F_{2,15} = 3.846$, $p = 0.0448$). Equations of Lake 222 lines were: $y_{BASE} = 1.0438x + 6.0248$; $y_{nAg} = 1.0438x + 5.5119$; and $y_{REC} = 1.0438x + 5.4345$.

3.2 Population-Level Results

3.2.1 Northern Pike Population Estimates

Northern Pike abundance was estimated using open population POPAN sub-module, as there is documented immigration and emigration of pike from the lake adjacent to reference Lake 239. The top-ranked model for pike in Lake 239 factored in differences in survival between spring, summer, and fall seasons. The "Study Period" model was similarly ranked ($\hat{c} = 1.159$, $\Delta AIC_{ADJ} = 0.247$), and estimated pike abundance with a consideration of differences in survivability between 2012-13, 2014-15, and 2016-17, corresponding with baseline, nAg addition, and recovery periods in Lake 222 (Appendix H Table 1). The "Study Period" model for pike in Lake 239 was compared to the top "Study Period" model in Lake 222 (Figure 3.12; Appendix H Tables 2 and 3).

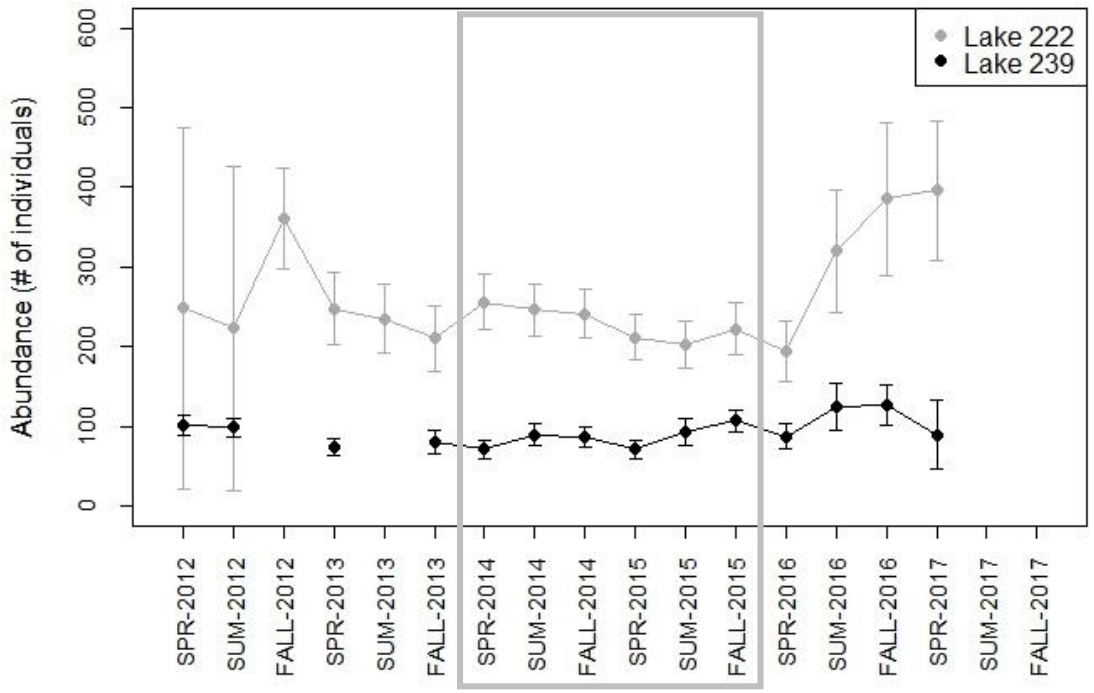


Figure 3.12. Top "Study Period" open estimates for Northern Pike in Lake 222 and Lake 239, from 2012 to 2017. Where the period of nAg addition is outlined in grey from 2014 to 2015**;** error bars are the standard error of the mean.

Open population estimates for Lake 239 produced the lowest number of Northern Pike in the spring of 2014 and 2015 (71±11 individuals), with the highest number in the fall of 2016 (127±24 individuals). The lowest number of pike in Lake 222 occurred in the first year of recovery (spring 2016, 194±37 individuals), and the highest number in the second year of recovery (spring 2017, 396±87 individuals). BACI ANOVAs were performed on the pike population estimates, and there were significant site and time period effects, but no significant interaction (Table 3.10).

Table 3.10. BACI ANOVAs for Pike Abundance Estimates in Lake 222 and Lake 239, from 2012 to 2017.

Factor	ANOVA	*F*-value	*p*-value	Assumptions and Actions
POPAN	**Before - After** **Control - Impact**	**$F_{2,24}$ = 4.255** **$F_{1,24}$ = 113.764**	**p = 0.0262** **p < 0.0001**	**Period effect is significant** **Lake effect is significantly different**
	B-A:C-I Interaction	$F_{2,24}$ = 1.731	p = 0.1985	Interaction is not significant
	Test for Normality	$A\text{-}D_{L222}$ = 0.556 $A\text{-}D_{L239}$ = 0.527	p = 0.1265 p = 0.1467	Data are normally distributed for each lake
	Test for Homogeneity	F_{L222} = 1.800 F_{L239} = 2.787	p = 0.2042 p = 0.1049	Variance is homogeneous for each lake

Segmented regression analysis of the open population estimates for pike in Lake 222 fit a single breakpoint at the start of whole-lake recovery (spring 2016; Figure 3.13), then fit two breakpoints at the start of nAg addition (spring 2014) and recovery (spring 2016) study periods.

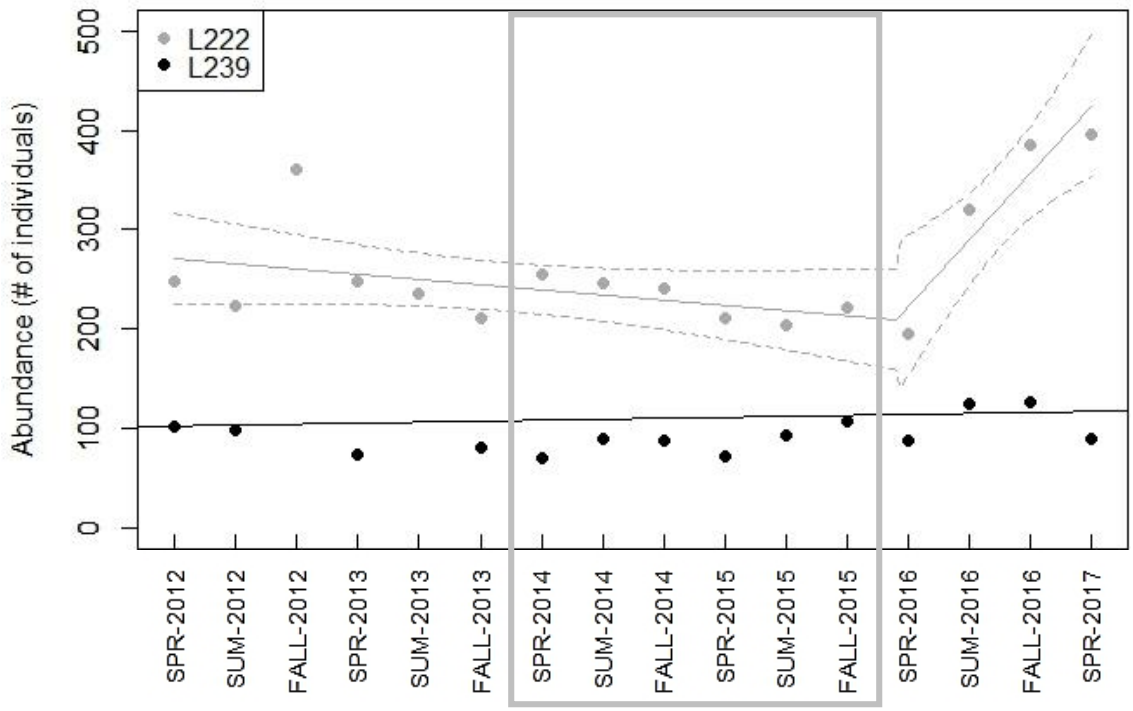


Figure 3.13. Effect of occasion with one breakpoint on Northern Pike abundance in Lake 222 and Lake 239, with 95% confidence interval. Where the period of nAg addition is outlined in grey from 2014 to 2015.

Breakpoints were analysed with ANOVAs to determine the best number of breaks to fit to the model with the least variation (Table 3.11); models provided equivalent fits for pike abundance.

Table 3.11. Breakpoint Regression Relationships for Northern Pike Abundance Estimates, from 2012 to 2017.

Factor	Lake	ANOVA	*F*-value	*p*-value	Assumptions and Actions
Breakpoint	**222**	ANOVA: 1 Break vs. 2 Breaks	$F_{2,10} = 0.644$	$p = 0.5458$	Number of breaks is not significant
		ANOVA: 0 Breaks vs. 1 Break	$\mathbf{F_{2,12} = 12.955}$	$\mathbf{p = 0.0010}$	**1 break is significant from none**
		Test for Normality	$A\text{-}D = 0.442$	$p = 0.2520$	Data are normally distributed
		Test for Homogeneity	$F_{2,13} = 1.800$	$p = 0.2042$	Variance is homogeneous
	239	ANOVA: 1 Break vs. 2 Breaks	$F_{2,8} = 2.676$	$p = 0.1289$	Number of breaks is not significant
		ANOVA: 0 Breaks vs. 1 Break	$F_{2,10} = 3.478$	$p = 0.0714$	1 break is not significant from none
		Test for Normality	$A\text{-}D = 0.466$	$p = 0.2127$	Data are normally distributed
		Test for Homogeneity	$F_{2,11} = 2.787$	$p = 0.1049$	Variance is homogeneous

Davies' test for change in the slope significantly fit the best breakpoint at spring 2016 (two-sided, $p = 0.0032$, reject H_0: no breakpoints in regression model), and pseudo-score test for more than one change in the slope significantly fit a second best breakpoint at fall 2012 (two-sided, $p = 0.0306$, reject H_0: no breakpoints in regression model). I focused on the one breakpoint model that indicated the switch-point or rebound in pike abundance occurred during spring 2016 (Lake 222 recovery), which deviated from Lake 239 abundance trends.

Lake 239 was not fit with any breakpoints, as ANOVA and Davies' test for change in the slope fit were not significant, despite a "best" fit at occasion 4.889 (summer 2013; two-sided, p = 0.1079, accept H_0: no breakpoints in regression model). Pseudo-score test for more than one change in the slope fit a second best breakpoint at summer 2012 (two-sided, p = 0.0814, accept H_0: no breakpoints in regression model), but the breakpoint was similarly not significant. Schnabel census closed population estimates generally corroborated the POPAN open population estimates for Northern Pike abundance in both lakes (Appendix H Figure 1).

Abundance estimates reflected an overall decrease in pike during nAg addition and a substantial increase once nAg addition was terminated, which was significantly different between periods and lakes (Table 3.10), suggesting Northern Pike survivability was impacted during nAg addition in Lake 222 (Figure 3.14).

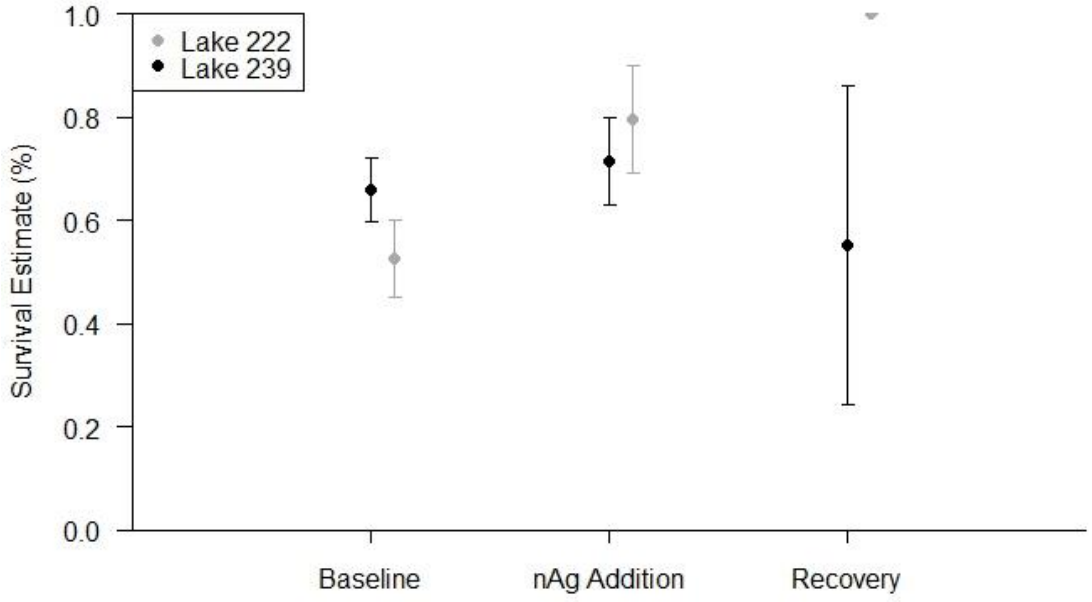


Figure 3.14. Survivability estimates by study period for Northern Pike in Lake 222 and Lake 239. Where error bars are the standard error of the mean.

Based on the POPAN top "Study Period" models, there appeared to be a significant difference in pike survivability estimates, attributable to the dosing of nAg particles in Lake 222.

3.2.2 Yellow Perch Population Estimates

Despite seasonal sampling efforts to capture Yellow Perch in Lake 239 and Lake 222, there was limited data for open population estimation as few recaptured perch featured previous seasonal nicks (Appendix I Tables 1 and 2, respectively). Alternately, estimation using the POPAN sub-module reflected only long-term abundance of perch hanging around the few seine-able sites in the large reference Lake 239. As a result, there was a gross under-estimation of Yellow Perch in Lake 239 (54.28 ha), of fewer than 3400 perch in the entirety of the lake, while the estimated abundance of perch in Lake 222 (16.39 ha) was between 121761 and 224144 (Appendix I Figure 1).

Changes in Yellow Perch abundance over the course of the study were therefore investigated using closed population Schnabel census (Figure 3.15), which assumes no immigration or emigration in the population. This approach investigated the occurrences of recaptures within seasons only, so fewer observations were available (especially in Lake 239), as some sampling occasions encountered no multiple seasonal fin nicks and therefore no value could be provided within occasions (as opposed to POPAN estimating across occasions).

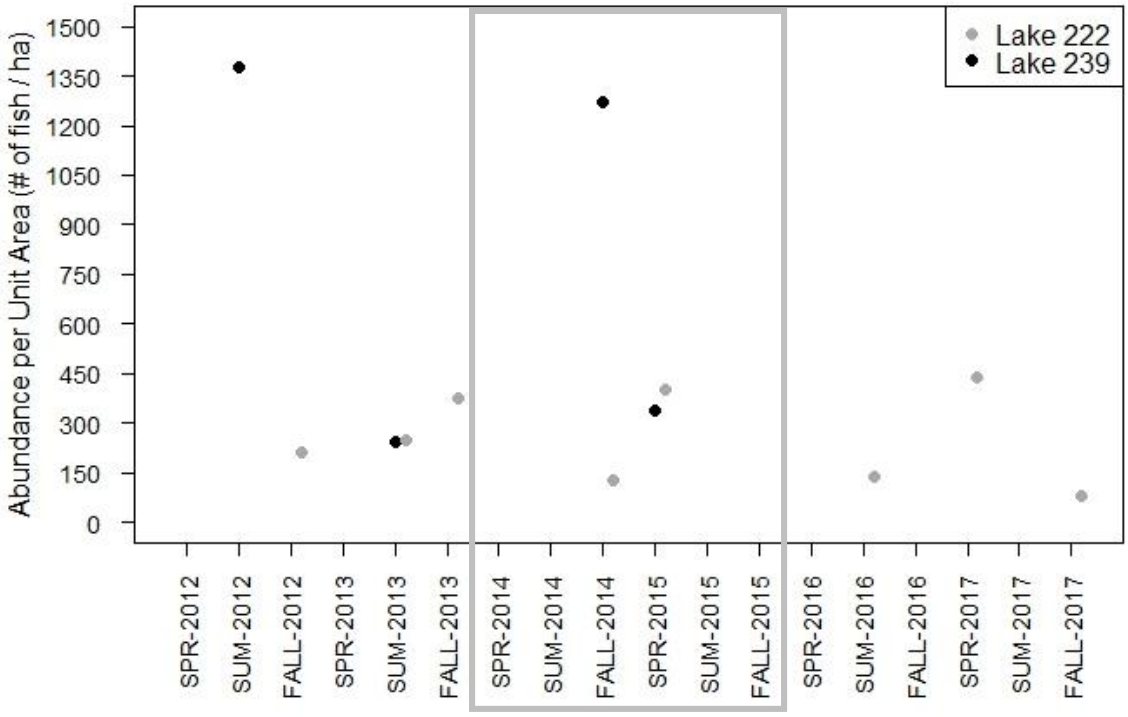


Figure 3.15. Schnabel census closed estimates of Yellow Perch per unit area in Lake 222 and Lake 239, from 2012 to 2017. Where the period of nAg addition is outlined in grey from 2014 to 2015**.**

Yellow Perch closed population estimates averaged 4555 (277 perch per hectare) during baseline, 4325 (263 per ha) during nAg addition, and 3589 (218 per ha) during recovery period in Lake 222, for a mean and standard error of 4135±791 over the course of the study. Yellow Perch in Lake 239 were more abundant, with an average of 44028 from 2012-13 (811 perch per hectare), and 43686 from 2014-15 (804 per ha), for a mean and standard error of 43857±16291. BACI ANOVA results revealed significant lake effect on Yellow Perch abundance, however, study periods and interaction were not significant in Lake 222 and Lake 239 (Table 3.12).

Table 3.12. BACI ANOVAs for Perch Abundance Estimates in Lake 222 and Lake 239, from 2012 to 2017.

Factor	ANOVA	*F*-value	*p*-value	Assumptions and Actions
Schnabel Census	Before - After	$F_{2,7} = 0.859$	$p = 0.4638$	Period effect is not significant
	Control - Impact	$\mathbf{F_{1,7} = 7.436}$	$\mathbf{p = 0.0295}$	**Lake effect significantly different**
	B-A:C-I Interaction	$F_{1,7} = 0.000$	$p = 0.9970$	Interaction not significant
	Test for Normality	$A\text{-}D = 0.369$	$p = 0.3664$	Data are normally distributed
	Test for Homogeneity	$F_{2,9} = 0.494$	$p = 0.6261$	Variance is homogeneous

Despite the few sampling occasions in which multiples were encountered in a decent number ($n \geq 10$), Schnabel census provided more reasonable estimates of abundance of perch in Lake 222 and Lake 239, as it reflected lake-specific morphometry (differences in lake size).

3.2.3 Northern Pike and Yellow Perch Condition

Logarithmic transformations were performed on individual Northern Pike weights and fork lengths (Figure 3.16).

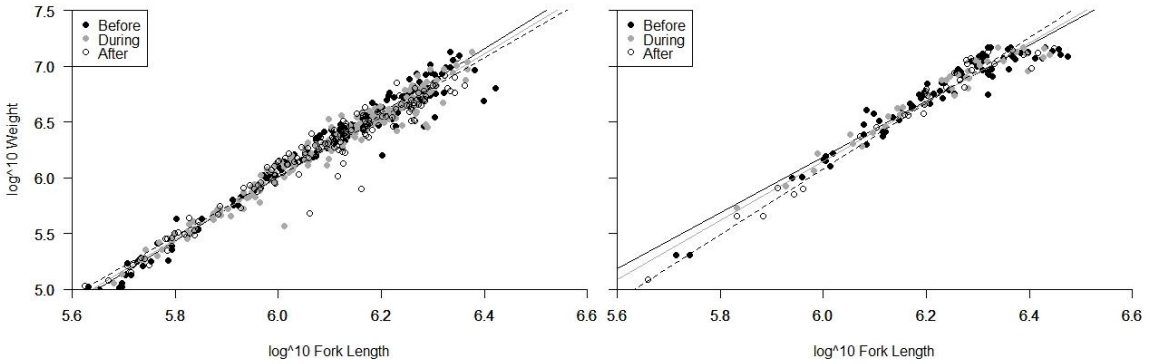


Figure 3.16. Log-transformations of Northern Pike weight versus length in Lake 222 (left) and Lake 239 (right), from 2012 to 2017. L222 line equations: $y_{Before} = (2.867)x + (-11.195)$; $y_{During} = (2.778)x + (-10.667)$; $y_{After} = (2.661)x + (-9.959)$. L239 line equations: $y_{Before} = (2.506)x + (-8.849)$; $y_{During} = (2.657)x + (-9.793)$; $y_{After} = (2.948)x + (-11.615)$.

Slopes appeared different for baseline, nAg addition, and recovery pike in Lake 222 and Lake 239. The steepest slope in Lake 222 was prior to nAg addition (baseline), which indicated the heavier pike at the largest sizes compared with other time periods, and relatively skinnier large pike during nAg additions and after. The opposite pattern was apparent in Lake 239, where the larger pike increased in weight over time (increasing slopes with time). Logarithmic transformations were similarly performed on perch weight and fork length data in Lake 222 and Lake 239 (Figure 3.17).

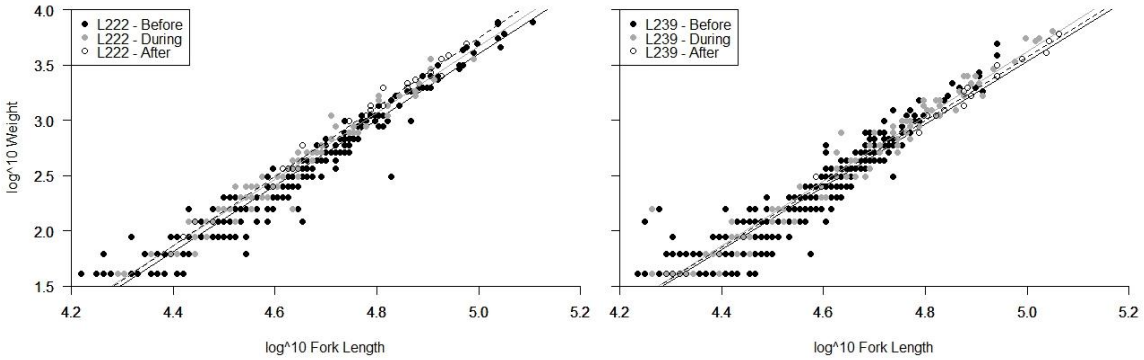


Figure 3.17. Log-transformations of summer Yellow Perch weight versus length in Lake 222 and Lake 239, from 2012 to 2017. L222 line equations: y_{Before} = (2.985)x + (-11.327); y_{During} = (3.012)x + (-11.388); y_{After} = (3.133)x + (-11.924). L239 line equations: y_{Before} = (2.844)x + (-10.686); y_{During} = (2.931)x + (-11.034); y_{After} = (2.864)x + (-10.755).

Log-weight versus log-length for Yellow Perch in Lake 222 appeared different between periods, while Lake 239 before, during, and after slopes were relatively similar. The shallowest condition slope in Lake 222 occurred during baseline, suggesting that larger perch were slimmer while shorter perch were fatter; larger perch appeared to weigh more during and after nAg addition, with the steepest slope in recovery (fatter large fish and thinner small fish). While baseline and recovery periods in L239 had similarly shallow slopes, the same time period as nAg addition was slightly steeper. Tests for heterogeneity of slopes were used to determine significant differences in perch and pike condition between study periods in each lake (Table 3.13).

Table 3.13. Tests for Heterogeneity of Slopes for Yellow Perch and Northern Pike in Lake 222 and Lake 239.

Lake	Species	Growth Rate Analysis	*F*-value	*p*-value	Assumptions and Actions
222	Perch	Test for Heterogeneity of Slopes	$F_{2,468}$ = 1.448	p = 0.2360	Slopes not significantly different
		ANCOVA: log(WT)~log(FL)+PRD	**$F_{2,470}$ = 20.336**	**p < 0.0001**	**Intercepts are significantly different**
	Pike	**Test for Heterogeneity of Slopes**	**$F_{2,487}$ = 6.398**	**p = 0.0018**	**Slopes are significantly different**
239	Perch	Test for Heterogeneity of Slopes	$F_{2,1133}$ = 0.933	p = 0.3936	Slopes not significantly different
		ANCOVA: log(WT)~log(FL)+PRD	**$F_{2,1135}$ = 8.925**	**p = 0.0001**	**Intercepts are significantly different**
	Pike	**Test for Heterogeneity of Slopes**	**$F_{2,150}$ = 10.230**	**p < 0.0001**	**Slopes are significantly different**

Tests for heterogeneity of slopes revealed no significant differences between study periods for Yellow Perch in Lake 222 and Lake 239, however, ANCOVA intercepts were significantly different between periods in each lake. While changes in condition of Northern Pike were statistically significant between periods in both lakes, the differences were not biologically significant (<10g). Further, assumptions of normality were not met, despite log-transformations.

3.2.4 Yellow Perch Size Class Proportions

Yellow Perch individuals were provided age assignments within each study period, using age-validated sacrificed perch data. Perch captured in the summer (from 2012 to 2017) were assigned ages from algorithms that factored in the frequencies and values of their fork lengths, via age-length keys (Ogle 2016; Section 2.13; Figure 3.18).

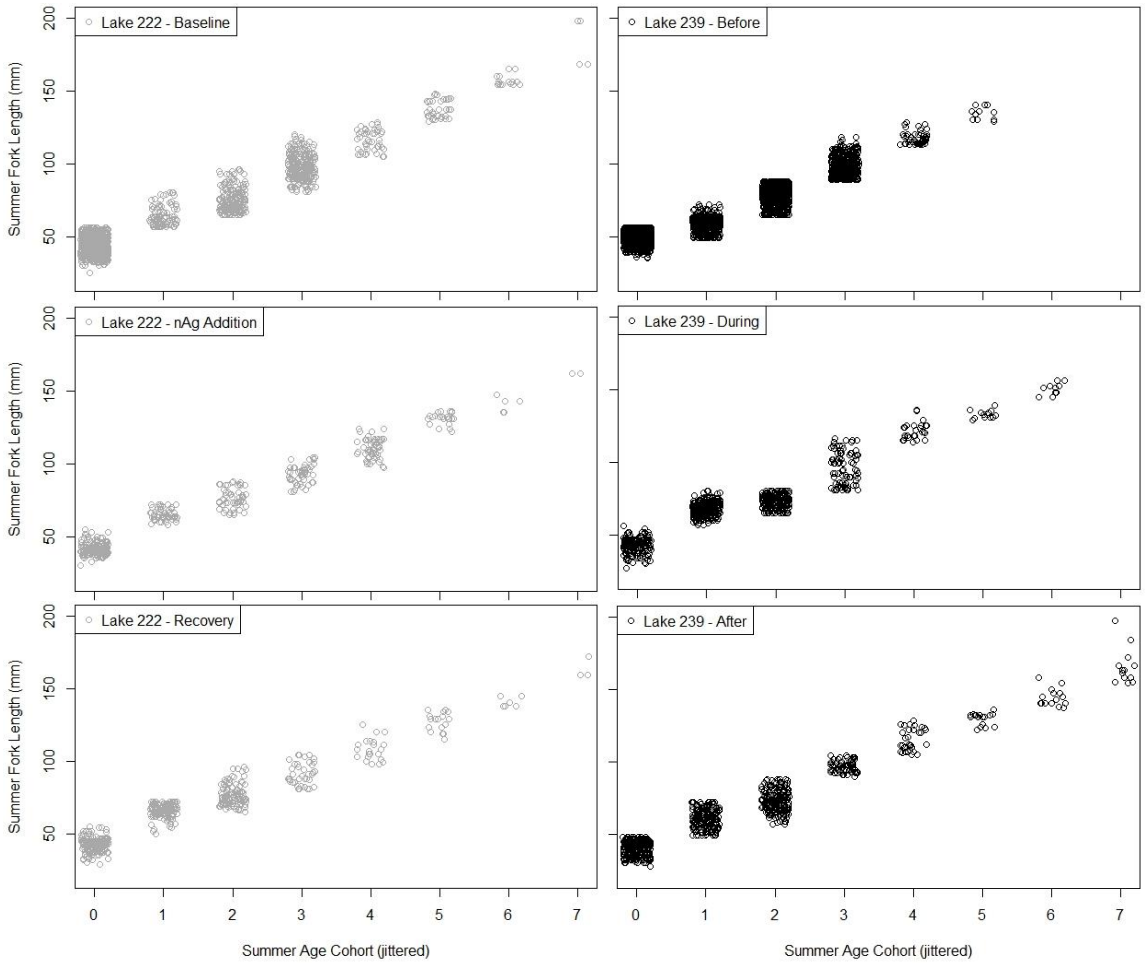


Figure 3.18. Proportions of assigned age cohorts based on perch age-length keys in Lake 222 and Lake 239, from 2012 to 2017. Where age 7 is 7-plus.

The greatest proportions of perch in both lakes occurred in the immature age cohorts 0, 1, and 2. Lake 222 had 83, 56, and 54 sacrificed summer perch with ages for the baseline, nAg addition and recovery periods, respectively, out of 1334, 396, and 538 perch with fork lengths (proportions 6.22%, 14.14%, and 10.04%), for a total of 2268 summer perch captured between 2012 and 2017. Lake 239 had 89, 69, and 59 sacrificed summer perch with ages for the baseline, nAg addition and recovery periods, out of 5071, 791, and 824 with fork lengths (proportions 1.76%, 8.72%, and 7.16%), for a total of 6686 perch captured between 2012 and 2017 summer seasons.

Proportions of perch were multiplied by mean Schnabel population estimates (Section 3.2.2) for each period and lake ($L222_{BASE}$ = 4555.53, $L222_{nAg}$ = 4325.38, $L222_{REC}$ = 3589.96; and $L239_{BEF}$ = 44028.61, $L239_{DUR}$ = 43686.75, average $L239_{BEF}$ and $L239_{DUR}$ values for $L239_{AFT}$ = 43857.68), to derive numbers of perch within each age cohort (Table 3.14).

Table 3.14. Yellow Perch Age Assignments based on Summer Fork Lengths in Lake 222 and Lake 239. Where "FL" is fork length, "Prop" is proportion, and "Popn" is the number of individuals.

Lake	Age Cohort	Baseline				nAg Addition				Recovery			
		n	**Mean FL (mm)**	**Prop (%)**	**Popn (#)**	***n***	**Mean FL (mm)**	**Prop (%)**	**Popn (#)**	***n***	**Mean FL (mm)**	**Prop (%)**	**Popn (#)**
222	**0**	695	44.20	**52.10**	**2373**	153	41.41	**38.64**	**1671**	214	42.79	**39.78**	**1428**
	1	103	63.86	**7.72**	**352**	57	64.89	**14.39**	**622**	129	65.91	**23.98**	**861**
	2	188	75.23	**14.09**	**642**	52	76.15	**13.13**	**568**	108	76.80	**20.07**	**721**
	3	245	98.37	**18.37**	**837**	53	92.72	**13.38**	**579**	38	91.21	**7.06**	**253**
	4	49	116.49	**3.67**	**167**	52	110.37	**13.13**	**568**	23	108.26	**4.28**	**154**
	5	35	137.49	**2.62**	**119**	22	131.00	**5.56**	**240**	17	127.06	**3.16**	**113**
	6	15	156.93	**1.12**	**51**	5	140.60	**1.26**	**54**	6	140.67	**1.12**	**400**
	7+	4	183.00	**0.30**	**14**	2	162.00	**0.51**	**22**	3	163.33	**0.56**	**20**
Lake	**Age Cohort**	**Before**				**During**				**After**			
		n	**Mean FL (mm)**	**Prop (%)**	**Popn (#)**	***n***	**Mean FL (mm)**	**Prop (%)**	**Popn (#)**	***n***	**Mean FL (mm)**	**Prop (%)**	**Popn (#)**
239	**0**	2712	48.72	**53.48**	**23546**	168	42.63	**21.24**	**9279**	284	40.79	**34.47**	**15118**
	1	1112	59.05	**21.93**	**9655**	316	67.40	**39.95**	**17453**	186	60.75	**22.57**	**9899**
	2	763	78.58	**15.05**	**6626**	175	72.63	**22.12**	**9664**	214	72.77	**25.97**	**11390**
	3	422	98.53	**8.32**	**3663**	81	96.72	**10.24**	**4474**	64	96.55	**7.77**	**3408**
	4	48	117.46	**0.95**	**418**	27	120.93	**3.41**	**1490**	31	115.10	**3.76**	**1649**
	5	12	134.58	**0.24**	**106**	14	133.79	**1.77**	**773**	17	130.18	**2.06**	**903**
	6	0	-	**0**	**0**	10	150.40	**1.26**	**550**	15	144.27	**1.82**	**798**
	7+	2	221.00	**0.04**	**18**	0	-	**0**	**0**	13	165.54	**1.58**	**693**

Considering only the age cohorts that were analysed for bioenergetics (ages 1 to 6): there were fewer age 1 and 2 perch in Lake 222 than Lake 239; and more age 3 to 6 in Lake 222 than Lake 239. The "Popn (#)" values were used in annual gross consumption estimates (Section 3.2.5).

3.2.5 Yellow Perch Gross Consumption Estimates

Annual gross consumption was calculated from absolute consumption rates (g_{food}/day), multiplied by period-specific proportions of each age cohort, summed across age cohorts, and multiplied by the number of expected feeding days per year (May 1st to October 31st; Table 3.15).

Table 3.15. Annual Gross Consumption of Prey by Yellow Perch in Lake 222 and Lake 239, from 2012 to 2016. Asterisks (*) refer to missing values that were replaced with averages from other period(s) for the same age cohort.

Lake	Age	Baseline			nAg Addition					Recovery		
		Popn (#)	2012		Popn (#)	2014		2015		Popn (#)	2016	
			Cons (g/day)	Gross C (kg)		Cons (g/day)	Gross C (kg)	Cons (g/day)	Gross C (kg)		Cons (g/day)	Gross C (kg)
222	1	352	0.04*	2.76	622	0.13*	15.09	0.16	17.85	861	0.05*	7.43
	2	642	1.84	216.66	568	0.62	64.68	2.54*	264.49	721	0.66	87.39
	3	837	2.82	432.35	579	2.48	262.26	3.38	357.49	253	0.45	20.93
	4	167	4.71	144.20	568	1.31	135.79	3.07	318.56	154	1.56	43.94
	5	119	7.04	153.82	240	2.99	131.58	1.79	78.75	113	4.25	88.19
	6	51	10.36	96.70	54	2.96	29.56	2.29*	22.84	400	5.72	418.76
TOTALS		**4555**	**26.82**	**1046.49**	**4324**	**10.49**	**638.96**	**13.22**	**1059.98**	**3950**	**12.69**	**666.64**
Lake	**Age**	**Before**			**During**					**After**		
		Popn (#)	**2012**		**Popn (#)**	**2014**		**2015**		**Popn (#)**	**2016**	
			Cons (g/day)	**Gross C (kg)**		**Cons (g/day)**	**Gross C (kg)**	**Cons (g/day)**	**Gross C (kg)**		**Cons (g/day)**	**Gross C (kg)**
239	1	9655	0.35*	610.78	17453	0.26*	841.93	0.60	1904.22	9899	0.62	1125.98
	2	6626	1.01	1226.57	9664	0.75	1327.34	2.13	3771.83	11390	1.47	3068.91
	3	3663	4.08*	2734.88	4474	0.43	352.82	0.46	375.96	3408	0.54	336.40
	4	418	0.75	57.58	1490	0.62	169.27	0.67	182.09	1649	0.90	272.50
	5	106	1.06	20.53	773	0.86	121.71	1.21	171.57	903	0.80	132.98
	6	0	1.97*	0	550	1.10	111.16	1.77*	178.24	798	1.54	225.21
TOTALS		**44032**	**9.22**	**4650.33**	**43713**	**4.03**	**2924.24**	**6.84**	**6583.90**	**43858**	**5.88**	**5161.97**

Gross consumption rates (summed totals per unit area) were plotted by year (Figure 3.19).

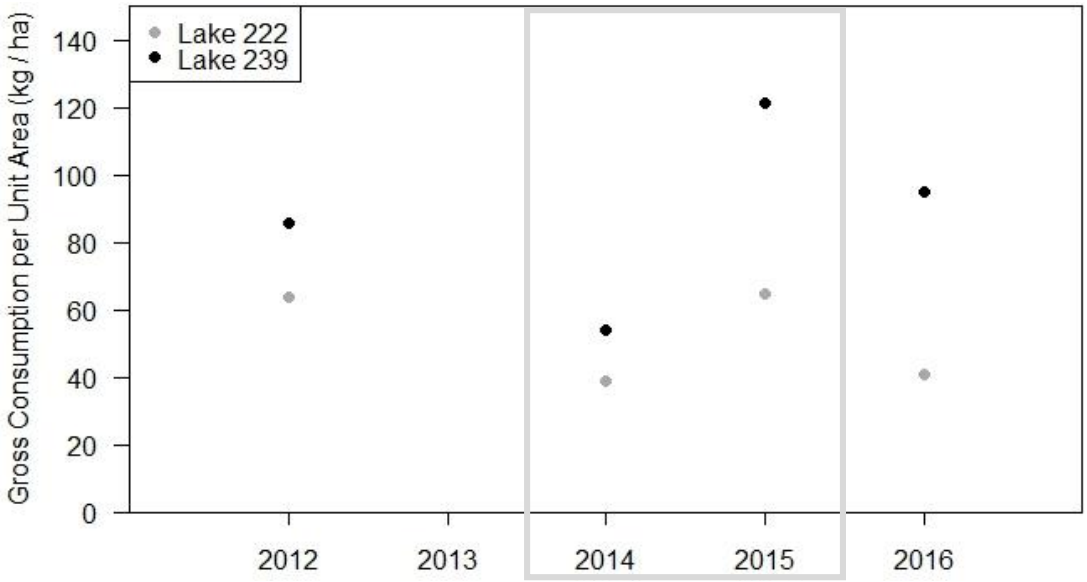


Figure 3.19. Annual gross consumption estimates per unit area for Yellow Perch in Lake 222 and Lake 239. Where the period of nAg addition is outlined in grey from 2014 to 2015.

In Lake 222, baseline gross consumption represented 48% of the population, nAg addition was 61%, and recovery was 70%, as the rest was made up of age 0 and age 7-plus perch that did not have consumption estimates associated with them. In Lake 239, gross consumption was 46%, 79%, and 64%, respectively. Grossest consumption in Lake 222 was during the second year of nAg addition (2015), but it was suppressed compared to Lake 239, and remained low in recovery (2016).

Due to lake-specific differences in dietary habits of age 1 and 2 zoobenthivorous perch versus ages 3 to 6 mixed prey types, gross consumption rates were separated by diet (Figure 3.20).

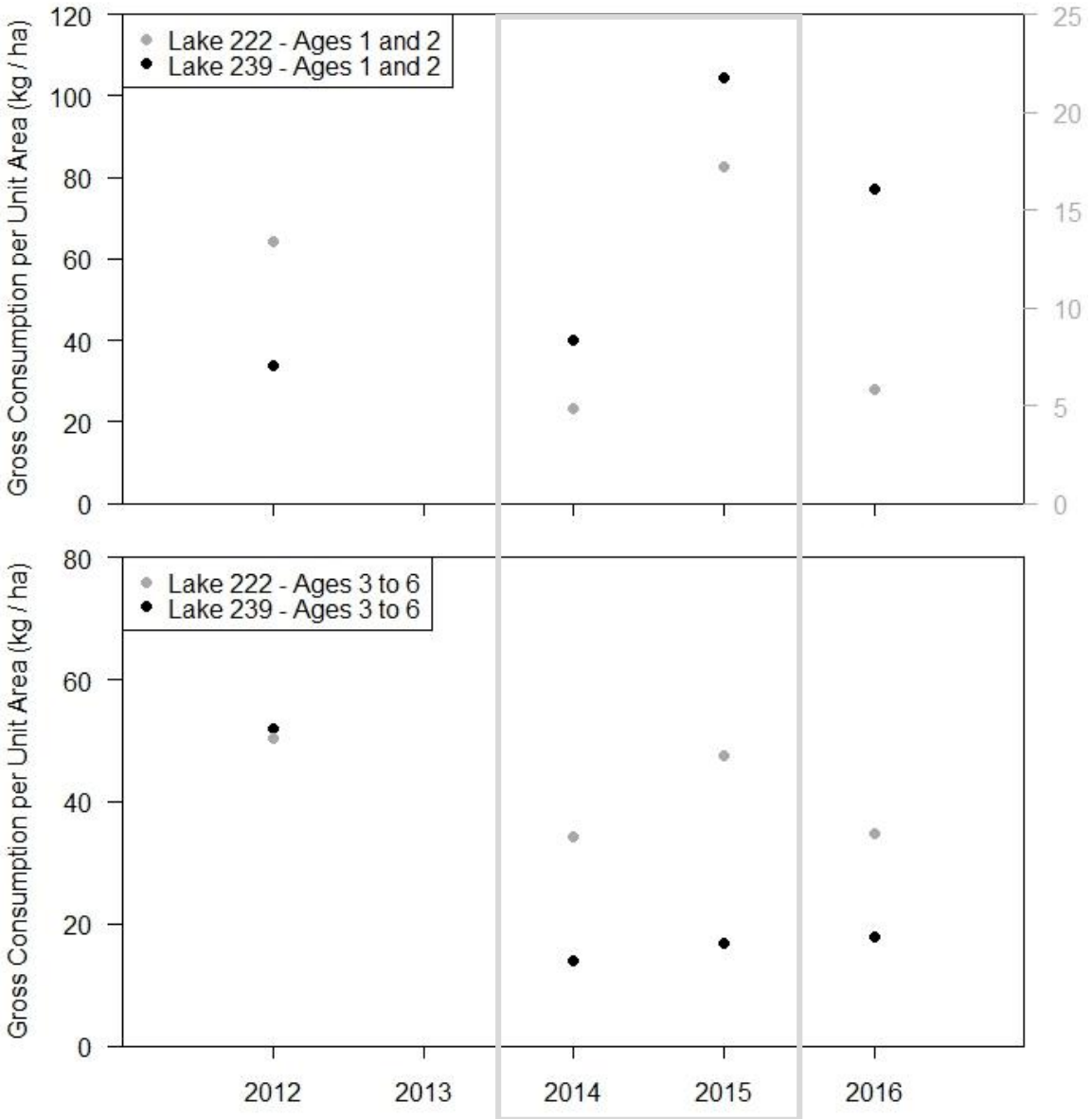


Figure 3.20. Annual gross consumption per unit area for ages 1 and 2 perch (top) and ages 3 to 6 (bottom), in Lake 222 and Lake 239. Where the period of nAg addition is outlined in grey from 2014 to 2015; Lake 222 axis in grey (top).

There were lesser proportions of ages 1 and 2 perch in Lake 222 versus Lake 239 populations over the course of the study. Gross consumption of zoobenthos by perch in Lake 222 was almost an order of magnitude less than zoobenthos consumption in Lake 239. Annual gross consumption per unit area for zoobenthivorous perch had the most similar proportions in recovery, but also the most different gross consumption estimates (2016). Zoobenthivorous ages 1 and 2 perch in 2016 had been exposed to nAg particles their entire lives, and gross consumption was most suppressed relative to zoobenthivorous perch in reference Lake 239 fort the same time period. Similar to the decline in individual-level Yellow Perch consumption rates during and after nAg addition, there also appeared to be declines in population-level consumption per unit area in Lake 222 for ages 1 and 2 cohorts.

There were greater proportions of mature perch in experimental Lake 222 compared to reference Lake 239, for all years. Gross consumption estimates for benthivorous perch (ages 3 to 6 in Lake 222) were highest in 2012, as were estimates for benthos- and fish-feeding perch in Lake 239. While direct inter-lake comparisons were not possible for the mature age cohorts (as a result of their dietary differences; Pazzia *et al*. 2012), Lake 239 estimates suggest extrinsic environmental factors that may have resulted in depressed gross consumption in the reference system. Despite declines in benthivory and piscivory estimates in Lake 239, population-level consumption of benthos in Lake 222 appeared relatively constant over the duration of the study.

4 Discussion

Based on predictions, the overall sum of evidence seemed to suggest a response of fish to direct effects of nAg in Lake 222 (Table 4.1). Nanosilver-induced individual-level effects on Yellow Perch were apparent in significantly reduced consumption, reduced total metabolism during and after nAg addition, lower conversion efficiency, and higher variability of activity rates in Lake 222. At the population-level, pike had lower survivability, and perch community bioenergetics indicated reduced gross consumption by immature age cohorts, compared to reference Lake 239.

Table 4.1. Results of Indirect and Direct Predictions for a Whole-Lake Nanosilver Addition on Yellow Perch. Where red arrows indicate that the predicted response was not observed, and black arrows are prediction agreements.

Effect	Cause	Prey	Growth	Consumption	Growth Efficiency	Metabolism
None	No observed sublethal effects	↔	↔	↔	↔	↔
Indirect	Decrease in prey abundance, diet	↓	↓	↓	↓	↑
Direct	Oxidative or osmoregulatory stress	↔	↓	↓	↔	↓

At the individual-level, changes in diet occurred in Lake 222 as Yellow Perch switched from consuming zooplankton to benthic invertebrates at a smaller fork length during the first year of nAg addition (2014), compared to recovery (2016). The opposite was observed for the reference Lake 239. Studies suggest it is necessary for Yellow Perch to switch to larger prey items as they grow, to maintain high growth efficiency (Pazzia *et al*. 2002; Boisclair and Leggett 1989). With little evidence to suggest that zooplankton populations in Lake 222 were affected by the release of nAg particles (indirect), perch may have made the switch to larger prey at a smaller size to mitigate the direct effects of nAg on the age 1 cohort (in an effort to maintain growth rates and conversion efficiency), or some other environmental factor(s) influenced the change.

In Lake 222, nAg particles were present in lower trophic level organisms, though anticipated toxic effects of this contaminant, such as population declines, changes in composition, or diminished species' richness or diversity, were not observed. Direct analysis of nAg particles by LENs Project researchers, found that *Daphnia magna*, benthic invertebrates, and bacteria and algae, were able to incorporate total silver within their systems and were not significantly affected by this contaminant at low (environmentally-relevant) levels (Rearick *et al*. Submitted; Conine and Frost

2017; Katarina Cetinic, unpublished data). Studies have determined that hetero-agglomeration is the primary factor in the reduction of nAg in a whole-lake ecosystem; nAg agglomerates with particulates and algae, which reduces its toxicity in the system, and therefore its negative effects on benthic invertebrates and fish (Conine and Frost 2017; Furtado *et al*. 2016; Lowry *et al*. 2012). Additionally, bacteria, detritus, protists, and sediments all act as binding sites within freshwater environments, which represent a significant nAg sink, thereby mitigating nAg toxicity via water chemistry parameters (Conine *et al*. 2018; Conine and Frost 2017; Das *et al*. 2014, 2012). As it pertains to the validity of the MMBM (specifically, concentrations of MeHg): the microbial community composition and abundance of sulfur-reducers in the anoxic hypolimnion of Lake 222 were similar to other lakes at the IISD-ELA, during the LENs Project (Jackson Tsuji, Pers. Comm.).

Studies on metal-impacted lakes revealed that populations of zoobenthos are most sensitive to contaminant exposure (Rasmussen *et al*. 2008; Gunn and Mills 1998). Elsewhere, ecosystem contamination resulted in a decline in energy transfer efficiency in the ecosystem – with fewer preferred prey items for mature perch, coupled with reduced ability of larger fish to feed on smaller zooplankton prey (Sherwood *et al*. 2002; Persson 1987). However, these effects were not apparent during nAg addition in Lake 222. In a nAg-treated mesocosm study, nAg accumulated in phytoplankton with no significant change in community structure or biomass (Vincent *et al*. 2017). However, as nAg exposure rate increased (from low to medium to high chronic exposures), zooplankton abundance and size increased, while both biomass and species richness decreased (Vincent *et al*. 2017). These subtle effects were not observed in the whole-lake experiment, where LENs Project results revealed zooplankton had the lowest biomass-specific total silver concentrations (<0.15µgAg/mgC; A. Conine, unpublished data) in the lake, when compared to bacterioplankton (0.1-18µgAg/mgC; A. Conine, unpublished data), and algae (<4µgAg/mgC; A. Conine, unpublished data). Additionally, benthic invertebrates in Lake 222 maintained an abundant and diverse population over the entirety of the LENs Project (K. Cetinic, unpublished data).

The smaller size that perch shifted from consuming zooplankton to benthos in 2014 in Lake 222 was supported by shorter age 1 perch during the nAg addition period compared to recovery (Section 3.2.3). Yellow Perch often experience stunted growth when subjected to high exposures of metal toxicity; when stunted growth is coupled with a high dependence on smaller zooplankton prey, perch experience poorer condition, which is seen as evidence of indirect effects of metal contamination (Rasmussen *et al*. 2008; Kaufman *et al*. 2006; Sherwood *et al*. 2002). However, with few significant nAg impacts on lower trophic level abundance, it is unlikely that perch were indirectly affected by the two-year nAg addition.

Despite lower trophic groups appearing to be largely unaffected by nAg addition (Rearick *et al*. Submitted; Conine *et al*. 2018; Conine and Frost 2017), direct effects of nAg on individual fish were observed as early as the first month of nAg addition (Martin *et al*. Submitted). In Lake 222, total silver was detected in significant quantities in Yellow Perch and Northern Pike gills, liver, kidneys, and later, muscle tissue, as early as one month after introduction of this contaminant into the system (Martin *et al*. 2017a; Metcalfe 2017). Long-term exposure results indicated that total silver accumulated in the gills and liver tissues of perch and pike, to ppm levels compared to the ppb levels in the water column, suggesting the potential for impacts on fish at the whole-organism and population levels (Metcalfe 2017). This was a surprising result, as Ag^{+} is likely more toxic than nAg, and nAg agglomerates with other compounds in water, such as algae and dissolved organic carbon (DOC), and settles out of the system quickly (Conine and Frost 2017; Furtado *et al*. 2014; Liu *et al*. 2010). The high concentrations of total silver observed in pike and perch, and responses at the cellular-level, most likely explain observed responses in fish bioenergetics (impacted growth, consumption, and metabolism) at the individual-level.

Yellow Perch fork lengths for all age cohorts overlapped for both lakes prior to the start of nAg addition in Lake 222. Inter-lake variability increased with the addition of nAg particles in 2014, and ages 1, 2, and 3 exhibited significant interactions of lake and study period effects on size-at-age. Juvenile perch (ages 1 to 2) and newly mature perch (age 3) experienced reduced sizes for

one or both years of nAg addition, and rebounded in recovery, compared to reference Lake 239. In conjunction with diminished size-at-age of younger perch, consumption rates in Lake 222 were significantly depressed during and after nAg addition. This was also seen in Largemouth Bass, where prey consumption decreased as a result of contaminant exposure (Beyers *et al.* 1999). Reduced consumption rates were not reflected in Lake 239 before, during, or after the study. Lake 239 had shallower consumption slopes, possibly as a result of perch shifting from zooplankton to benthos to fish prey, with a high degree of overlap. Lake 222 indicated steeper slopes, though rates decreased during and after nAg addition, as perch consumed less zoobenthos prey.

Mass-specific growth rates of perch remained constant over the course of the study in both lakes. Decreased consumption and constant growth rates should result in an overall increase in conversion efficiency after nAg additions (less food consumed but still maintaining growth). Growth efficiency appeared to increase during and after nAg addition, however, this observation was not significant by lake and study period interaction. Individual-level decreased consumption rates and constant growth efficiency during nAg addition agreed with hypothesised direct effects. While size-at-age for ages 1 to 3 perch experienced a significant interaction, and declined for one or both years of nAg addition, there was no significant change in growth rate between study periods. It was unlikely that these changes to perch bioenergetics were caused indirectly, as prey were not limiting in experimental Lake 222. Instead, there is evidence that the addition of nAg particles had a direct impact on fish organs and tissues; despite constant weight-based growth rates in both lakes, Yellow Perch consumed less during and after nAg addition in Lake 222, and perch ages 1 to 3 experienced stunted length-based growth, compared to the baseline period and reference Lake 239.

Activity levels of Yellow Perch in Lake 222 were similar for all modelled age cohorts during the baseline period. Activity showed increased variability during and after nAg addition, while decreasing on average over time. In contrast, activity levels in Lake 239 were constant for mature age cohorts, and appeared to increase in ages 1 and 2 perch. Standard metabolic rate is the physiological requirement of a species (Trudel *et al*. 2000), represented by the allometric function

of water temperature and body mass (Rennie *et al*. 2010). A higher standard metabolic rate indicates fish must consume more to meet their bioenergetic requirements, and therefore exert more energy in acquiring their prey (Trudel *et al*. 2001). Standard metabolic rates were relatively constant for perch in both lakes during the baseline period, however, they were lower in Lake 222 during and after nAg addition to a greater extent than reference Lake 239, which suggests perch required less prey to meet their bioenergetic requirements, as they were exerting less energy to acquire prey, or metabolic impairment due to nAg exposure. Leadley *et al*. (2016) detailed how exposure to toxic contaminants (i.e. metals, pesticides, persistent organic pollutants, etc.) directly influenced the metabolic rates of fishes – either as a result of a stressor response in energy allocation or a toxic interaction between the contaminant and the biochemical pathway regulating fish metabolism.

Losses to total metabolism encompass activity levels, standard metabolic rates, and specific dynamic action (heat increment, varies proportionally with *C*; Rennie *et al*. 2010). Similar to trends in Lake 222 consumption rates, total metabolism declined during nAg addition and recovery periods, while reference Lake 239 changed less over the course of the study. This was similarly reflected in the reduction of total metabolism in contaminant-exposed Largemouth Bass (Beyers *et al*. 1999). Bioenergetics inputs of consumption are balanced against outputs of egestion, excretion, metabolism, reproduction, and respiration, with the remainder going to growth. Reduced total metabolic costs could contribute to reduced consumption, or vice-versa, as perch use less energy and consume less prey, resulting in reduced growth during and after nAg addition in Lake 222.

Body size is a major structuring factor in food webs: energy flow in aquatic ecosystems is partly a result of predator size, as larger sizes result in increased range of movement (McMeans *et al*. 2016; Petchey *et al*. 2008). In an unmanipulated system, these reduced consumption and activity levels might be expected of perch during winter months, however, summer and fall rates should represent peak growth, consumption, and activity. Contrary to the (indirect) hypothesised increase in activity, but in agreement with direct hypotheses, perch experienced a decrease in activity and total metabolism (Table 4.1), which may be attributable to direct effects of nAg addition.

Significant individual-level fish effects occurred from low doses of nAg particles over a sustained period of time (June 14th to October 23rd 2014, and May 15th to August 25th 2015). Nanosilver was rapidly transported across Lake 222 immediately following the first addition, and nAg persisted, suspended within the water column for the duration of the study (D. Rearick, unpublished data). Total silver concentrations were estimated in the range of 4-18µg/L, across the lake, and concentrations of total silver in perch and pike tissues from Lake 222 were significantly higher than baseline levels (<0.005µg/g), by the end of the first season of nAg addition (Martin *et al*. Submitted).

Nanosilver has the capacity to affect fish via two routes – respiration and digestion. Impacts of nAg at environmentally-relevant levels have been observed to have sublethal effects on fish between 1-20µg/L (Farmen *et al*. 2012; Griffitt *et al*. 2012; Pham *et al*. 2012). The study by Scown *et al*. (2010) determined the main mode of nAg toxicity to be oxidative stress, with nAg particles affecting cell mitochondria, damaging cell DNA or affecting lipid peroxidation and protein modification. Bilberg *et al*. (2010) studied gills of Eurasian Perch (*Perca fluviatilis*) exposed to suspended nAg particles and provided evidence of respiratory impairment, citing reduced oxygen diffusion, with nAg affecting the gills externally. Biomarker response of Yellow Perch exposed to low (1µg/L) or high (100µg/L) concentrations of Ag^{+} or nAg for either 96 hours or 10 days, revealed indications of oxidative stress at high exposures to nAg after a period of 10 days, primarily in livers versus gills (Martin *et al*. 2017b).

Results of the LENs Project by Martin *et al*. (Submitted) revealed total silver concentrations in some Northern Pike tissues bioaccumulated to three orders of magnitude greater than concentrations measured in the water column. Total silver concentrations in pike were highest in the liver (maximum mean <2.4µg/g, August 2015), compared to the gills (maximum mean <0.08µg/g, May 2015; Martin *et al*. Submitted). With the majority of total silver in pike bioaccumulating in their livers, the primary mode of toxicity appeared to be their diet of perch (Metcalfe 2017). Fortunately, concentrations of total silver in pike declined sharply upon

termination of nAg addition – likely due to excretion of total silver from pike tissues, as a result of slower growth of this top predator (Metcalfe 2017). Compared to their pike predators, concentrations of total silver in perch livers increased to a lesser extent (maximum mean <0.5µg/g, October 2015), however, perch experienced the higher concentration of total silver in their gills (maximum mean <0.3µg/g, August 2015), suggesting predominant respiratory sources of total silver (Martin *et al*. Submitted), especially considering the reduced consumption by perch of nAg-exposed prey. Similar to their Northern Pike predators, at the termination of nAg addition in Lake 222, Yellow Perch experienced a rapid decline in total silver concentration. Based on LENs Project direct analysis of perch (age 2 comprised the majority), growth dilution likely factored into the decline of total silver in perch tissues after nAg addition ceased (Metcalfe 2017).

This study revealed a significant reduction in Yellow Perch consumption rates, and declines in total metabolism during and after nAg addition in Lake 222 consistent with direct effects of nAg exposure, while bioenergetics of perch in reference Lake 239 remained constant over time. Increased basal osmoregulatory costs have been observed to reduce the scope of fish activity at the individual-level (Beamish 1978). A study of Atlantic Salmon (*Salmo salar*) indicated osmoregulatory stress could be a direct effect of nAg toxicity, and not only a response to Ag^+, as osmoregulation was impaired at exposures of 20µg/L nAg, as well as 20µg/L Ag^+ (Farmen *et al*. 2012). Golden Shiners (*Notemigonus crysoleucas*) exposed to different levels of cadmium (up to maximum sublethal concentrations), metabolic rates and osmoregulatory sodium-potassium pump activity were strongly correlated, and significantly lower than the control (Peles *et al*. 2012).

Symptoms of osmoregulatory stress were described in a study by Cardeilhac *et al*. (1979), whereby Sheepshead (*Archosargus probatocephalus*) were exposed to toxic copper concentrations in sea water, resulting in five stages (based on body posturing and behaviour) of increasing osmoregulatory failure: (1) lethargy, (2) indifference, (3) incoordination, (4) moribundity, and (5) death. While Sheepshead livers were similar to controls, the serum electrolytes, uric acid levels, gill and liver copper concentrations, kidney weights, and respiratory rates increased in copper-

poisoned fish, and were correlated to the five stages described above (Cardeilhac *et al*. 1979). Similar to the effects of Ag^+ (and nAg) toxicity in fish (Farmen *et al*. 2012; Scown *et al*. 2010), copper toxicity caused potassium intoxication, as a result of cell damage and failure of the gills and kidneys to osmoregulate (Cardeilhac *et al*. 1979). Bioenergetics results of this study indicate diminished consumption and activity levels that parallel the first stage in osmoregulatory failure (lethargy; Cardeilhac *et al*. 1979). With changes in gill and liver total silver concentrations, and modelled respiration rates, the presentation of lethargic behaviour is surmised for Lake 222 perch exposed to two years of nAg addition.

In addition to individual-level bioenergetics, abundance and condition of fish in both lakes were monitored to determine whether nAg addition affected the growth and survival of these zoobenthivorous and piscivorous species. Nanosilver addition appeared to have a direct effect on the survivability of pike in Lake 222. The POPAN sub-module rated the top model as having different survivability by study period, with the greatest survival estimated in Lake 222 recovery. Breakpoint analysis revealed Northern Pike experienced declining survival rates during the nAg addition, and a sharp increase in survival at the start of whole-lake recovery (spring 2016), which corresponded with changes in total silver concentrations in pike organs and tissue during and after nAg addition (Martin *et al*. Submitted). There was also a significant difference in survival and resulting population estimates between lakes, with Lake 239 remaining relatively constant over the course of the study.

There were significant differences in Yellow Perch population estimates between lakes only, even though average study period estimates within lakes declined. Condition (based on slopes of log-transformed length versus weight by study period) was not significantly different between periods, however, intercepts were significantly different between periods for Yellow Perch captured in the summer from 2012 to 2016. Changes in condition for Northern Pike revealed large pike were slimmer during and after nAg addition in Lake 222; the opposite was observed in Lake 239, as large pike became heavier over the course of the study. While Murray *et al*. (2017)

concluded Rainbow Trout were able to mitigate the physiological toxicity of nAg such that expression of effects at the whole-organism level were not detectable, and were unaffected by low exposures in laboratory studies over the 28-day study, results of this field study of two-year (spring to fall) environmentally-relevant nAg addition revealed significant effects on Northern Pike abundance and condition, as well as Yellow Perch consumption and total metabolism.

Overall gross consumption was suppressed in Lake 222 during and after nAg addition, compared to Lake 239. Separating gross consumption by age cohorts provided estimates of gross zoobenthivory and gross benthivory (Lake 222), or zoobenthivory and gross piscivory (Lake 239); ages 1 and 2 perch in 2016 (exposed to nAg their entire lives) experienced reduced consumption at the population-level. Reduced gross consumption of zoobenthos by ages 1 and 2 perch is likely a population-level manifestation of the direct effects of nAg toxicity on immature perch cohorts – with symptoms of oxidative or osmoregulatory stress decreasing consumption rates, activity levels, and total metabolism at the individual-level. Mature perch appeared to maintain their rates of benthic invertebrate consumption in the system. Research indicates that managing biological structure, functions, and species interactions is necessary to sustain energy flow within an ecosystem (McMeans *et al*. 2016; McCann 2007; Hillborn *et al*. 2003). The constant gross consumption of benthos by perch ages 3 to 6 additionally suggests there were few to no negative impacts on benthos communities, and therefore less evidence to support indirect effects.

With the results of this bioenergetics study, and collaborators' research into direct measures of nAg particle addition, there is evidence that Yellow Perch may have experienced sublethal total silver effects at the individual-level, via accumulation in their gills (also through diet sources, though to a lesser extent than pike, as consumption rates decreased with nAg addition). While the two-year nAg addition significantly impacted consumption and total metabolism of perch at the individual-level, anticipated adverse effects (decreased abundance, impaired condition, and reduced gross consumption) were mitigated for Yellow Perch at the population-level. Analysis of top predator, Northern Pike, indicated only subtle population-level effects.

Ecosystems are constantly changing – they are capable of adapting individual traits or food web structure in response to environmental conditions, and human-mediated impacts (McMeans *et al*. 2016). In moderated applications, the human health benefits of incorporating nAg may outweigh environmental costs. However, these results are based on two years of nAg addition, which were completely terminated in the late summer of 2015. Total silver moved from the water column to the sediment at an estimated rate of 31g/day (1.3 years for the bulk nAg addition to settle to the sediment without remobilisation), and the concentrations of total silver in fish tissues were almost back to baseline levels by October 2016 (Metcalfe 2017). This is not the case globally, as nanosilver continues to enter the environment in greater concentrations than ng/L, as a result of the drastic increase in nanomaterial production and application volumes (Massarsky *et al*. 2014; Sun *et al*. 2014). Ongoing environmental studies are critical; laboratory trials of nAg cannot adequately evaluate population-level effects or the complexities of food webs in natural settings (Das *et al*. 2012). Continued research into the prolonged effects of nAg on fish at current levels is encouraged.

These results aim to contribute to the development of federal risk assessment guidelines for nanomaterials – an identified need by the Canadian Government. Based on the results of these objectives, I submit that the 2014 environmentally-relevant release levels and two-year duration of nAg addition in this experiment were directly detrimental to fish. Yellow Perch experienced negative individual-level effects, with potential for population-level impacts with continued long-term exposure. This has implications for energy flow and nutrient cycling in aquatic ecosystems. Nanosilver should therefore be regulated to the same extent and regulations as use and release of Ag^{+} in the environment (0.25µg/L; CCME 2015), as nAg appears to have the same oxidative or osmoregulatory stress effects on fish at concentrations of 4-18µg/L. In my recommendation, I considered that separation of total silver into Ag^{+} and nAg forms is not possible with current technologies, thus environmental monitoring can only assess total silver concentrations (CCME 2015). This study fills a significant gap in the current understanding of the non-lethal response of Yellow Perch to nAg exposure, through analysis of diet, bioenergetics, growth, and condition.

APPENDICES

Appendix A. Table 1. Limnological Comparison of Lakes Included in the Study. Data are means from four (2012-16) years of monitoring for Lake 222 and Lake 239.

Constituent	Parameter	Experimental Lake 222	Reference Lake 239
Physical	**Area**	16.39 hectares	54.28 hectares
	Basin	Bedrock	Bedrock
	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
	Substrate	Silt and few boulders	Sand and boulders
	Thermocline	2.0 to 2.5 metres	5.1 to 8.7 metres
	Volume	7.2×10^5 m^3	5.9×10^6 m^3
Chemical	**Conductivity**	35-43 µS/cm	28 µS/cm
	DOC	High (12.1mg/L)	Moderate (6.8 mg/L)
	DOM	High	High (50 mg/L)
	pH	6.6	<7
	Phosphorus	High (9.8mg/L)	Moderate (6.3mg/L)
Biological	**Fish**	Northern Pike (*Esox lucius*) Yellow Perch (*Perca flavescens*) Blacknose Shiner (*Notropis heterolepis*)	Northern Pike (*Esox lucius*) Yellow Perch (*Perca flavescens*) Iowa Darter (*Etheostoma exile*) Lake Herring (*Coregonus artedi*) Lake Trout (*Salvelinus namaycush*) Slimy Sculpin (*Cottus cognatus*) White Sucker (*Catostomus commersoni*)
	Misc. Fauna	Beaver dam and lodge (active in 2014)	Resident loons
	Vegetation	Reedy with lots of submerged brush and submersed vegetation	Patches of submersed vegetation

Appendix B. Table 1. Summer to Fall MMBM Inputs for Each Age Cohort, Year, and Lake. Where grey cells indicate missing values, and red font indicates the model could not converge (either due to missing values or declines in final weight and/or [Hg] values, relative to initial weight and/or [Hg] values).

Lake	Year	Age	Sex	Maturity	W_0	W_t	Hg_0	Hg_t	$MeHg_{Prey}$	ED_{Prey}	ED_{Fish}
222	2012	1	2	0	3.4	2.1	0.1588	0.1389	0.0055	3175	4876.06
		2	2	0	4.8	5.7	0.1949	0.1928	0.0055	3175	4876.06
		3	2	1	9.7	12.1	0.1782	0.2665	0.0160	3682	4876.06
		4	2	1	17.8	20.0	0.2505	0.3361	0.0160	3682	4876.06
		5	2	1	32.2	32.3	0.3288	0.3155	0.0160	3682	4876.06
		6	2	1	50.5	44.0	0.2909	0.3571	0.0160	3682	4876.06
222	2014	1	2	0		4.0		0.2123	0.0080	3175	4876.06
		2	2	0	5.4	6.6	0.1951	0.1673	0.0080	3175	4876.06
		3	2	1	10.5	12.0	0.1451	0.1911	0.0089	3682	4876.06
		4	2	1	18.5	17.0	0.1849	0.2052	0.0089	3682	4876.06
		5	2	1	26.8	24.8	0.1764	0.2157	0.0089	3682	4876.06
		6	2	1	34.0	39.5	0.2604	0.2286	0.0089	3682	4876.06
222	2015	1	2	0	2.9	2.3	0.1841	0.1967	0.0040	3175	4876.06
		2	2	0	6.0	5.0	0.2351	0.2131	0.0080	3175	4876.06
		3	2	1	8.6	13.5	0.1490	0.2249	0.0130	3682	4876.06
		4	2	1	15.6	17.7	0.1679	0.1953	0.0089	3682	4876.06
		5	2	1	27.2	25.7	0.2134	0.2230	0.0089	3682	4876.06
		6	2	1		40.0		0.2434	0.0089	3682	4876.06
222	2016	1	2	0	3.5	3.0	0.2204		0.0080	3175	4876.06
		2	2	0	5.3	6.2	0.1947	0.1745	0.0080	3175	4876.06
		3	2	1	11.0	10.8	0.1915	0.1702	0.0032	3682	4876.06
		4	2	1	15.3	16.7	0.2052	0.1966	0.0089	3682	4876.06
		5	2	1	27.2	25.0	0.1417	0.2006	0.0089	3682	4876.06
		6	2	1	35.0	40.0	0.1454	0.1683	0.0089	3682	4876.06
Lake	**Year**	**Age**	**Sex**	**Maturity**	W_0	W_t	Hg_0	Hg_t	$MeHg_{Prey}$	ED_{Prey}	ED_{Fish}
239	2012	1	2	0		2.44		0.1204	0.0063	2737	4501.21
		2	2	0	5.38	5.57	0.1112	0.1351	0.0063	2737	4501.21
		3	2	1	13.50	11.40	0.1417		0.0679	4003	4501.21
		4	2	1	21.00	19.00	0.1419	0.1403	0.0049	4003	4501.21
		5	2	1	33.00	26.00	0.1504	0.1896	0.0150	4003	4501.21
		6	2	1					0.0679	4003	4501.21
239	2014	1	2	0	2.50	1.00	0.1159	0.1613	0.0095	2737	4501.21
		2	2	0	3.50	4.80	0.0952	0.1226	0.0095	2737	4501.21
		3	2	1	11.20	11.57	0.1209	0.1729	0.0440	4003	4501.21
		4	2	1	18.00	19.00	0.1319	0.1463	0.0260	4003	4501.21
		5	2	1	29.50	28.25	0.1085	0.1364	0.0270	4003	4501.21
		6	2	1	40.80	42.00	0.1354	0.1832	0.0560	4003	4501.21
239	2015	1	2	0	2.63	4.00	0.0885	0.1080	0.0095	2737	4501.21
		2	2	0	5.75	6.50	0.0838	0.1978	0.0095	2737	4501.21
		3	2	1	8.80	11.40	0.0838	0.1758	0.0681	4003	4501.21
		4	2	1	18.50	17.67	0.1279	0.1866	0.0430	4003	4501.21
		5	2	1	25.00	33.86	0.1173	0.1849	0.0681	4003	4501.21
		6	2	1		48.20		0.1808	0.0681	4003	4501.21
239	2016	1	2	0	2.92	3.00	0.1023	0.1640	0.0095	2737	4501.21
		2	2	0	4.75	4.08	0.0941	0.2371	0.0095	2737	4501.21
		3	2	1	10.33	10.50	0.1085	0.2441	0.0681	4003	4501.21
		4	2	1	18.75	21.67	0.1292	0.2229	0.0681	4003	4501.21
		5	2	1	24.40	23.25	0.1264	0.2163	0.0660	4003	4501.21
		6	2	1	33.75	36.83	0.1125	0.2184	0.0681	4003	4501.21

Appendix B. Table 2. MMBM Solved Consumption, Growth, and Activity Rates. Where "×" indicates model convergence, grey cells indicate initial and/or final weight and/or mercury data were missing, and "–" indicates the MMBM was unable to reach a solution that satisfied all criteria as a result of lower final weight and/or mercury data.

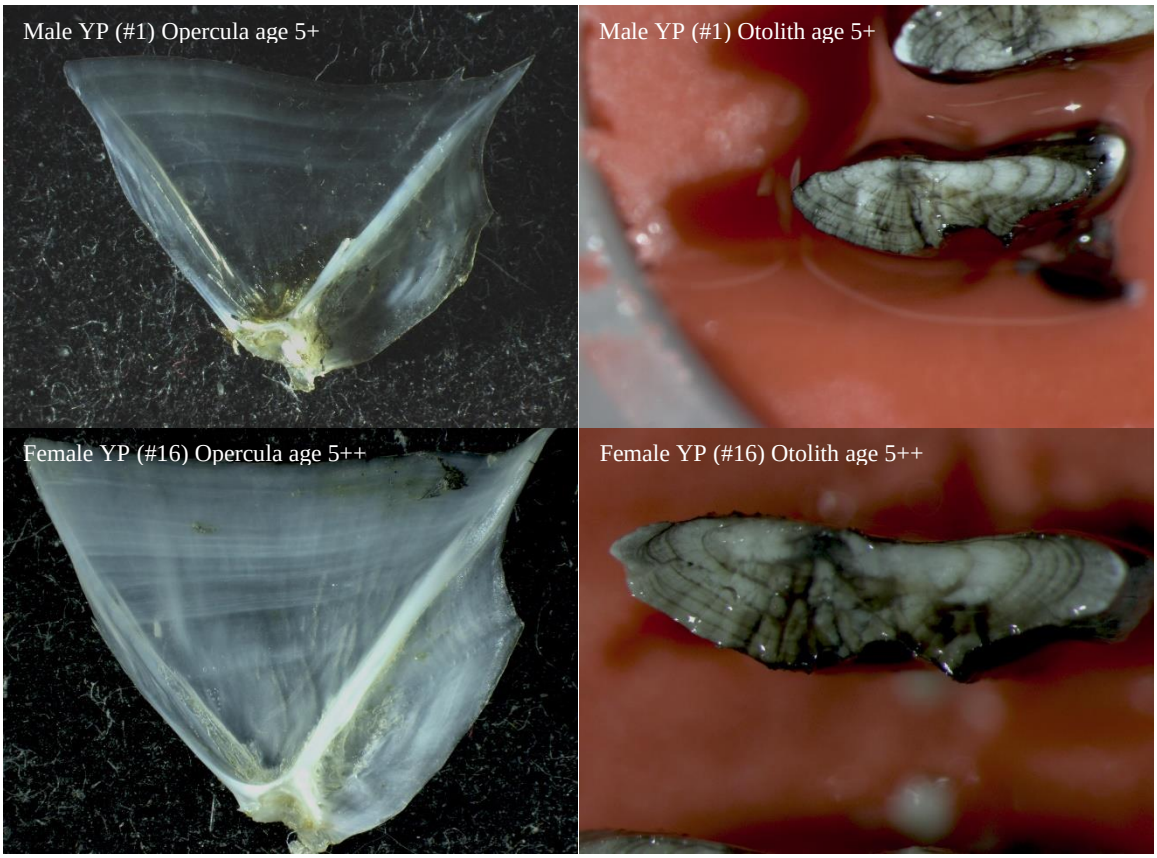
Year	Period	Age Cohort											
		1		2		3		4		5		6	
		L222	L239	L222	L239	L222	L239	L222	L239	L222	L239	L222	L239
2012	SUM-FALL	–		×	×	×		×	×	×	×	×	
2014	SUM-FALL		–	×	×	×	×	×	×	×	×	×	×
2015	SPR-SUM	×		×	–	–	–	–	–	×	–		
	SUM-FALL	×	×	–	×	×	×	×	×	×	×		
2016	SPR-SUM	×	×	×	–	×	–	×	–	–	–		–
	SUM-FALL		×	×	×	×	×	×	×	×	×	×	×

Appendix B. Table 3. Numbers of Sacrificed Male and Female Yellow Perch from Both Lakes, between 2012 and 2016.

Lake	Year	Season	Sex (#)		Total	Mature Males (#)	Percent of Total Mature M (%)	Mature Females (#)	Percent of Total Mature F (%)
			M	F					
222	2012	SUM	4	16	**20**	3	**15**	12	**60**
		FALL	10	19	**29**	5	**17.2**	12	**41.3**
	2014	SUM	3	18	**21**	1	**4.8**	15	**71.4**
		FALL	7	17	**24**	4	**16.7**	16	**66.7**
	2015	SPR	6	17	**23**	3	**13**	14	**60.8**
		SUM	11	15	**26**	8	**30.7**	10	**38.5**
		FALL	7	15	**22**	3	**13.6**	9	**40.9**
	2016	SPR	6	16	**22**	3	**13.6**	11	**36.4**
		SUM	6	18	**24**	3	**12.5**	14	**58.3**
		FALL	5	16	**21**	4	**19**	10	**47.6**
Lake	**Year**	**Season**	**Sex (#)**		**Total**	**Mature Males (#)**	**Percent of Total Mature M (%)**	**Mature Females (#)**	**Percent of Total Mature F (%)**
			M	**F**					
239	2012	SUM	2	14	**16**	1	**6.3**	11	**68.8**
		FALL	1	25	**26**	0	**0**	10	**38.5**
	2014	SUM	5	22	**27**	4	**14.8**	14	**51.9**
		FALL	4	19	**23**	1	**1.1**	17	**73.9**
	2015	SPR	5	16	**21**	3	**14.3**	13	**61.9**
		SUM	3	19	**22**	1	**4.5**	9	**40.9**
		FALL	12	17	**29**	8	**27.6**	15	**51.7**
	2016	SPR	22	7	**29**	14	**48.2**	5	**17.2**
		SUM	7	24	**31**	4	**12.9**	19	**61.3**
		FALL	11	9	**20**	11	**55**	7	**35**

Appendix C. Table 1. Number of Collected Fish Samples for Lake 222 and Lake 239. Asterisks (*) indicate population data only; "×" indicates when benthos and zooplankton were collected; and "-" indicates no data was collected.

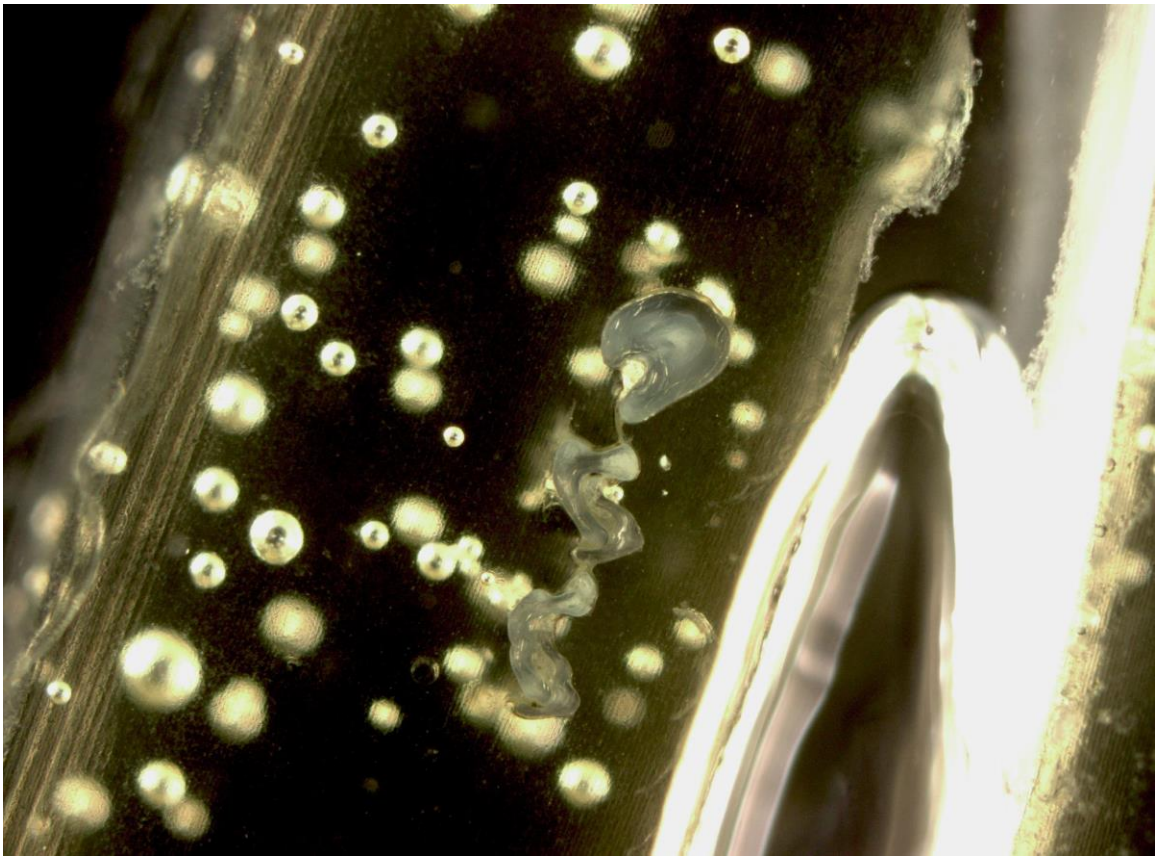
STUDY PERIOD	YEAR	LAKE	Yellow Perch			Northern Pike			Benthos and Zoopl.		
			SPR	SUM	FALL	SPR	SUM	FALL	SPR	SUM	FALL
Baseline	2012	222	-	39	44	6	29	*	-	×	-
		239	-	44	44	12	24	*	-	×	-
	2013	222	-	-	-	-	7	-	-	-	-
		239	-	-	-	-	-	-	-	-	-
Additions	2014	222	-	26	28	-	*	*	-	-	-
		239	-	29	28	-	*	*	-	-	-
	2015	222	25	29	28	44	34	30	×	×	-
		239	26	23	40	31	30	18	-	-	-
Recovery	2016	222	28	32	28	31	30	31	×	×	-
		239	31	44	29	31	18	18	×	×	-
	2017	222	*	*	-	31	31	-	-	-	-
		239	*	*	-	33	14	-	-	-	-



Appendix D. Figure 1. Comparison of opercula (left) and otoliths (right) from Yellow Perch in the summer of 2015 (top) and fall of 2015 (bottom) in Lake 239 (Age 5+ and 5++, with 100% agreement; S. Mann, Pers. Comm).

Appendix D. Table 1. Selection of Blind Ageing Analysis of Yellow Perch from Lake 222 and Lake 239 (S. Mann, Pers. Comm). Asterisks (*) indicate blind information.

Date	Species	Lake	ID	FL (mm)*	Size Class*	Sex*	Age	C.I.	Structure(s)
2012-07-18	Y. Perch	222	6	38	<71	-	**0+**	6	Fin Ray
2012-07-18	Y. Perch	222	14	145	131-150	M	**5+**	9	Fin Ray
2012-08-21	Y. Perch	222	21	114	111-130	M	**3+**	9	Fin Ray
2012-08-21	Y. Perch	222	22	71	71-90	M	**1+**	6	Fin Ray
2012-08-21	Y. Perch	222	24	79	71-90	F	**1+**	9	Fin Ray (Photo)
2014-09-18	Y. Perch	239	5	55	<71	-	**1++**	9	Opercula
2014-09-18	Y. Perch	239	11	78	71-90	M	**2++**	9	Opercula
2014-09-18	Y. Perch	239	13	107	91-110	F	**3++**	9	Opercula
2014-09-22	Y. Perch	239	2	122	111-130	F	**4++**	9	Opercula
2014-10-10	Y. Perch	239	2	138	131-150	F	**5++**	9	Opercula
2015-05-22	Y. Perch	239	2	52	<71	-	**1**	9	Opercula
2015-05-14	Y. Perch	239	3	77	71-90	M	**2**	9	Opercula
2015-05-22	Y. Perch	239	12	97	91-110	M	**3**	9	Opercula
2015-05-28	Y. Perch	239	3	111	111-130	F	**3**	9	Opercula
2015-05-07	Y. Perch	239	1	136	131-150	M	**5**	9	Opercula and Otolith (Photos)
2015-05-07	Y. Perch	239	3	153	151-170	F	**6**	9	Opercula
2015-10-01	Y. Perch	239	16	160	151-170	F	**5++**	9	Opercula and Otolith (Photos)



Appendix D. Figure 2. Cross-sections of a fin ray from Yellow Perch (#14) in Lake 222 (Age 1+, S. Mann, Pers. Comm).

Appendix E. Table 1. Benthic Invertebrates 27-Level and Zooplankton Functional Groups for Methylmercury Analysis. Where "AS" is Addition Site, "CB" is Centre Buoy, "OF" is Outflow. Asterisks (*) refer to the Mysis sample used to calibrate the MeHg System, and Gastropoda were analysed in duplicate.

Lake 222	**2012**	**2015**		**2016**	
	SUM	**SPR**	**SUM**	**SPR**	**SUM**
Benthos	- Amphipoda - Annelida - Bivalvia - Chironomidae - Gastropoda* - Trichoptera - Odonata	- Caddisfly (AS) - Dragonfly (OF) - Dragonfly (AS) - Mayfly (OF) - Scud (OF) - Scud (AS)	- Damselfly (OF) - Dragonfly (OF) - Dragonfly (AS) - Scud (OF)	- Caddisfly (AS) - Damselfly (OF) - Dragonfly (OF) - Dragonfly (AS) - Mayfly (OF) - Scud (OF)	- Clams (OF) - Clams (AS) - Dragonfly (OF) - Dragonfly (AS) - Leech (OF) - Scud (OF)
Larval Dipt. & Zooplank.	- Bulk Zoopl. (CB)	N/A	N/A	- *Chaoborus* (CB) - *Daphnia* (CB)	- Bulk Zoopl. (CB) - *Chaoborus* (CB)
Lake 239	**2012**	**2015**		**2016**	
	SUM	**SPR**	**SUM**	**SPR**	**SUM**
Benthos	- Amphipoda - Annelida - Chironomidae - Coleoptera (beetle) - Coleoptera (larvae) - Ephemeroptera - Gastropoda - Notonectidae - Odonata	N/A	N/A	- Beetle (OF) - Damselfly (OF) - Dragonfly (OF) - Dragonfly (Bay) - Scud (OF) - Scud (Bay)	- Caddisfly (Bay) - Chironomid (Bay) - Dragonfly (Bay) - Leech (OF) - Mayfly (OF) - Scud (OF) - Scud (Bay)
Zooplankton	- Bulk Zoopl. (CB)	N/A	N/A	- Bulk Zoopl. (CB)	- Copepoda (CB) - Mysidacea* (CB)

Appendix E. Table 2. Methylmercury Analysis of Benthic Invertebrates and Zooplankton in Lake 222 and Lake 239. Asterisks (*) refer to prey items used in calculating prey MeHg concentrations; "a" is an average concentration from two values (prey collected from two sites within the lake; Appendix E. Table 1).

Samples	**Prey Items**	**MeHg Analysis (mg/kg)**							
		2012		**2015**		**2016**			
		L222	**L239**	**L222**		**L222**		**L239**	
		SUM	**SUM**	**SPR**	**SUM**	**SPR**	**SUM**	**SPR**	**SUM**
Benthos	Amphipoda (scud)*	0.0015	0.0032	0.0120_a	0.0164	0.0088	0.0342	0.0107_a	0.0174_a
	Anisoptera (dragonfly)*	-	-	0.0071_a	0.0062_a	0.0027_a	0.0022_a	0.0182_a	0.0165
	Annelida (worm)	0.0034	0.0058	-	-	-	-	-	-
	Bivalvia (clam)*	0.0009	-	-	-	-	0.0035_a	-	-
	Chironomidae (midge)	0.0039	0.0014	-	-	-	-	-	0.0056
	Coleoptera (beetle)	-	0.0093	-	-	-	-	0.0037	-
	Coleoptera (larvae)	-	0.0153	-	-	-	-	-	-
	Ephemeroptera (mayfly)*	-	0.0030	0.0165	-	0.0011	-	-	0.0087
	Gastropoda (snail)*	0.0007_a	0.0017	-	-	-	-	-	-
	Hirudinea (leech)*	-	-	-	-	-	0.0014	-	0.0215
	Notonectidae (true bug)*	-	0.0165	-	-	-	-	-	-
	Odonata (dragon/damselfly)*	0.0025	0.0056	-	-	-	-	-	-
	Trichoptera (caddisfly)*	0.0039	-	0.0042	-	0.0018	-	-	0.0109
	Zygoptera (damselfly)*	-	-	-	0.0609	0.0115	-	0.0081	-
Larval Dipt.	*Chaoborus**	-	-	-	-	0.0051	0.0088	-	-
Zooplankton	BULK Zooplankton*	0.0150	0.0032	-	-	-	0.0003	0.0003	-
	Copepoda*	-	-	-	-	-	-	-	0.0003
	*Daphnia**	-	-	-	-	0.0010	-	-	-
	Mysidacea	-	-	-	-	-	-	-	0.0111

Appendix F. Table 1. Calculated Energy Density Based on Occurrence of Prey Items in Yellow Perch Gut Contents in Lake 222 (n_{TOTAL}=112, $n_{<100mm}$=68, $n_{\geq 100mm}$=44) and Lake 239 (n_{TOTAL}=73, $n_{<100mm}$=36, $n_{\geq 100mm}$=37) for 2014 and 2016. Asterisks (*) indicate energy density values are from Cummins and Wuychek (1971), unless otherwise stated.

Lake	Prey Items	Percent Occurrence by Fork Length (%)		Energy Density Values* (J/g wet weight)	Average Energy Density (J/g wet weight)	
		<100mm	≥100mm		<100mm	≥100mm
222	*Bosmina*	1.47	0	1218	17.9	0
	Chaoborus	2.94	0	1763 (Hanson *et al*. 1997)	51.83	0
	Copepoda	-	-	-	-	-
	Daphnia	19.12	2.27	1314	251.24	29.83
	Caddisfly	32.35	22.73	3848	1244.83	874.65
	Clam	2.94	2.27	2113	62.12	47.97
	Crayfish	-	-	-	-	-
	Damselfly	5.88	9.09	4220	248.14	383.6
	Dragonfly	7.35	34.09	3139	230.72	1070.09
	Leech	0	4.55	5795 (Driver *et al*. 1974)	0	263.67
	Mayfly	1.47	0	3791	55.73	0
	Mite	1.47	0	4099	60.26	0
	No-see-um	1.47	0	4392 (Driver *et al*. 1974)	64.56	0
	Scud	22.06	18.18	3908	862.1	710.47
	Snail	1.47	4.55	1799	26.45	81.85
	Sow Bug	-	-	-	-	-
	True Bug	0	2.27	9686	0	219.87
	Yellow Perch	-	-	-	-	-
				TOTAL	**3175**	**3682**

Lake	Prey Items	Percent Occurrence by Fork Length (%)		Energy Density Values* (J/g wet weight)	Average Energy Density (J/g wet weight)	
		<100mm	≥100mm		<100mm	≥100mm
239	*Bosmina*	11.11	0	1218	135.32	0
	Chaoborus	-	-	-	-	-
	Copepoda	0	2.7	2653 (Hanson *et al*. 1997)	0	71.63
	Daphnia	30.56	0	1314	401.56	0
	Caddisfly	11.11	5.41	3848	427.51	208.18
	Clam	-	-	-	-	-
	Crayfish	0	2.7	4506	0	121.66
	Damselfly	5.56	8.11	4220	234.63	342.24
	Dragonfly	8.33	13.51	3139	261.48	424.08
	Leech	2.78	2.7	5795 (Driver *et al*. 1974)	161.1	156.47
	Mayfly	5.56	5.41	3791	210.78	205.09
	Mite	-	-	-	-	-
	No-see-um	-	-	-	-	-
	Scud	16.67	5.41	3908	651.46	211.42
	Snail	2.78	0	1799	50.01	0
	Sow Bug	2.78	0	3142	87.35	0
	True Bug	-	-	-	-	-
	Yellow Perch	2.78	54.05	4186	116.37	2262.53
				TOTAL	**2737**	**4003**

Appendix G. Table 1. Fall Proportions of Yellow Perch in Lake 222 and Lake 239, from 2012 to 2017.

Lake	Age Cohort	n	Proportion of Popn.	Fork Length (mm)						
				Mean	Std. Dev	Min	Q1	Median	Q3	Max
222	0	132	0.1285	48.74	3.34	43	46.0	48.0	50.0	58
	1	303	0.2959	62.75	9.22	51	55.0	60.0	70.0	82
	2	234	0.2278	79.95	5.99	67	76.0	80.0	85.0	90
	3	234	0.228	97.42	8.83	75	92.0	98.0	104.0	114
	4	75	0.0730	115.45	7.98	99	110.0	116.0	122.0	130
	5	30	0.0292	132.10	7.57	116	130.2	132.5	136.0	147
	6	17	0.0166	153.29	5.57	141	151.0	154.0	156.0	165
	7+	2	0.0019	169.50	4.95	166	167.8	169.5	171.2	173
Lake	Age Cohort	*n*	Proportion of Popn.	Fork Length (mm)						
				Mean	Std. Dev	Min	Q1	Median	Q3	Max
239	0	7071	0.9085	44.71	3.98	21	43.0	45.0	47.00	60
	1	358	0.0460	57.90	5.28	53	54.0	56.0	60.75	75
	2	128	0.0164	79.40	6.71	69	74.0	78.5	85.00	92
	3	69	0.0089	101.35	7.50	86	95.0	103.0	106.00	116
	4	46	0.0059	120.39	6.36	109	116.0	121.0	125.00	132
	5	51	0.0066	137.08	10.05	110	131.5	138.0	140.00	164
	6	46	0.0059	153.91	6.94	142	148.0	155.0	159.00	171
	7+	14	0.0018	179.00	10.86	165	170.2	179.5	186.50	202

Appendix H. Table 1. Survivability by Study Period Arrangement of POPAN Parameters for Northern Pike in Lake 222.

Parameter	Time Period	Code																																						
Phi	1 – SPR 2012	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2 – SUM 2012	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3 – FALL 2012	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4 – SPR 2013	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	5 – SUM 2013	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	6 – FALL 2013	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	7 – SPR 2014	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	8 – SUM 2014	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	9 – FALL 2014	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	10 – SPR 2015	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	11 – SUM 2015	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	12 – FALL 2015	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	13 – SPR 2016	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	14 – SUM 2016	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	15 – FALL 2016	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	16 – SPR 2017	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	17 – SUM 2017	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	18 – FALL 2017	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
p	1 – SPR 2012	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2 – SUM 2012	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3 – FALL 2012	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4 – SPR 2013	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	5 – SUM 2013	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	6 – FALL 2013	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	7 – SPR 2014	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	8 – SUM 2014	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	9 – FALL 2014	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	10 – SPR 2015	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	11 – SUM 2015	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	12 – FALL 2015	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	13 – SPR 2016	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	14 – SUM 2016	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	15 – FALL 2016	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	16 – SPR 2017	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	17 – SUM 2017	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	18 – FALL 2017	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
pent	1 – SPR 2012	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2 – SUM 2012	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3 – FALL 2012	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4 – SPR 2013	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	5 – SUM 2013	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	6 – FALL 2013	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	7 – SPR 2014	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	8 – SUM 2014	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	9 – FALL 2014	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	10 – SPR 2015	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	11 – SUM 2015	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	12 – FALL 2015	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
	13 – SPR 2016	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
	14 – SUM 2016	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	15 – FALL 2016	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	16 – SPR 2017	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	17 – SUM 2017	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
	18 – FALL 2017	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

Appendix H. Table 2. Top POPAN Model for Northern Pike in Lake 239.

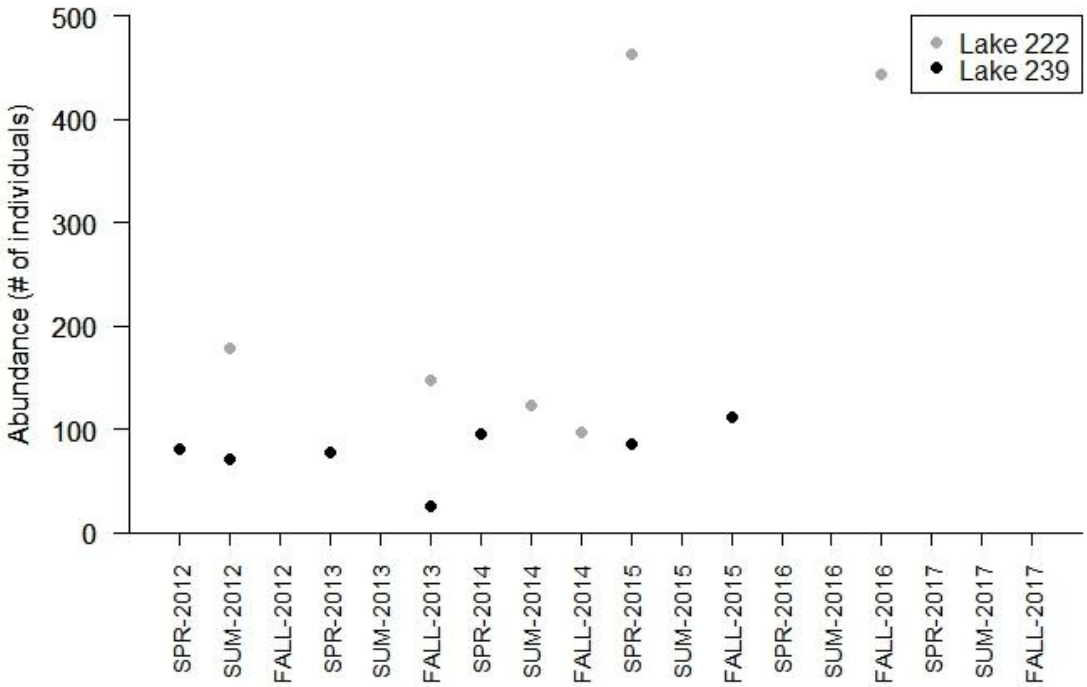
Model Parameters	$\hat{c}_{ADJ}$	AIC_{ADJ}	ΔAIC_{ADJ}	AIC $Weight_{ADJ}$	Model Likelihood	No. Parameters
Phi$_{season}$ p(g*t) pent(g*t)	1.2373	898.9361	0.0000	0.53068	1.0000	27
Phi$_{period}$ p(g*t) pent(g*t)		899.1835	0.2474	0.46893	0.8836	27
Phi(g*t) p(g*t) pent(g*t) - *FULL*		913.5982	14.6621	0.00035	0.0007	35
Phi(g*t) p$_{season}$ pent(g*t)		917.9272	18.9911	0.00004	0.0001	27
Phi(g*t) p$_{period}$ pent(g*t)		965.9047	66.9686	0.00000	0.0000	27

Phi_{season} and Phi_{period} refer to different survivability of pike across seasons or time periods, while p_{season} and p_{period} consider a season or time period effect on pike catchability.

Appendix H. Table 3. Top POPAN Model for Northern Pike in Lake 222.

Model Parameters	$\hat{c}_{ADJ}$	AIC_{ADJ}	ΔAIC_{ADJ}	AIC $Weight_{ADJ}$	Model Likelihood	No. Parameters
Phi$_{period}$ p(g*t) pent(g*t)	1.1593	1039.1337	0.0000	0.74458	1.0000	28
Phi$_{season}$ p(g*t) pent(g*t)		1041.2744	2.1407	0.25531	0.3429	28
Phi(g*t) p(g*t) pent(g*t) - *FULL*		1058.0833	18.9496	0.00006	0.0001	39
Phi(g*t) p$_{period}$ pent(g*t)		1058.2773	19.1436	0.00005	0.0001	26
Phi(g*t) p$_{season}$ pent(g*t)		11063.5127	24.3790	0.00000	0.0000	30

Phi_{period} refers to different survivability of pike as different across study periods – baseline (2012-13), nAg addition (2014-15), and recovery (2016-17). Catchability (*p*) and probability of entry (*pent*) are the full models, with every parameter treated independently. Phi_{season} refers to a seasonal factor in the survivability of Northern Pike in Lake 222, while the third model represents the full model. P_{period} and p_{season} consider a study period effect or seasonal effect on Northern Pike catchability, respectively.



Appendix H. Figure 1. Schnabel census estimates of Northern Pike in Lake 222 and Lake 239, from 2012 to 2017. Where Northern Pike averaged 241±67 individuals in Lake 222, and 78±10 individuals in Lake 239.

Appendix I. Table 1. Top POPAN Model for Yellow Perch in Lake 239.

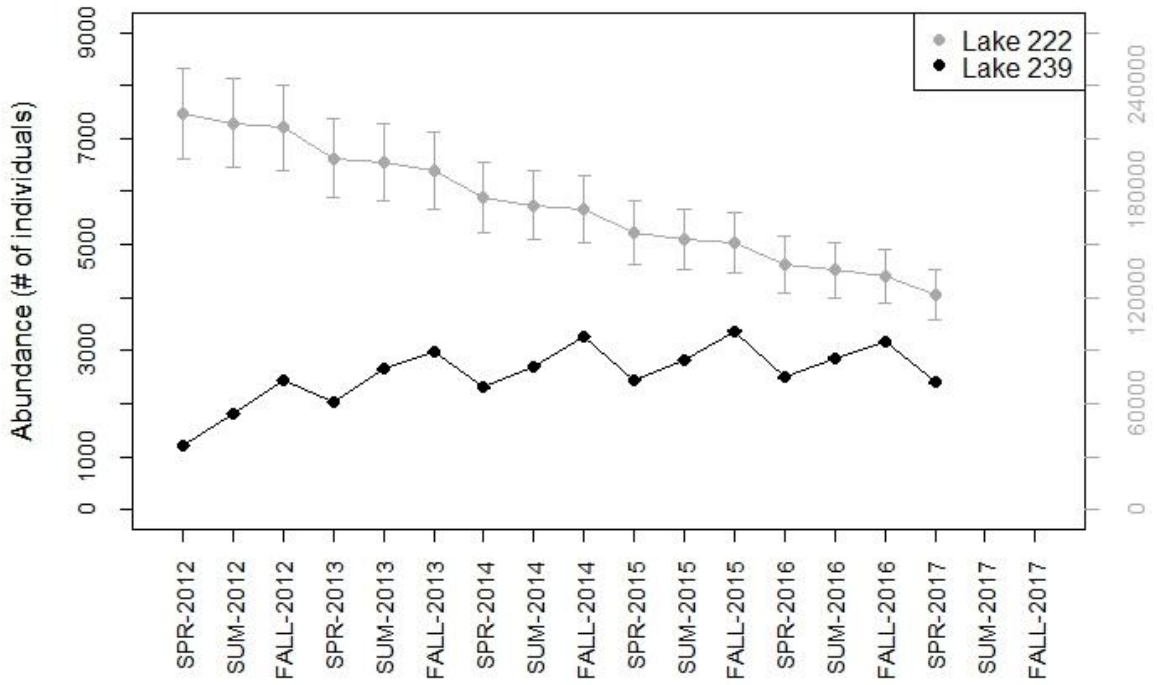
Model Parameters	AIC	Delta AIC	AIC Weight	Model Likelihood	No. Parm
Phi p pent	47400.5943	0.0000	1	1	1

The sole model for perch in Lake 239 considers all parameters and time intervals to be the same.

Appendix I. Table 2. Top POPAN Model for Yellow Perch in Lake 222.

Model Parameters	AIC	Delta AIC	AIC Weight	Model Likelihood	No. Parm
Phi $p_{period2}$ pent	4118.4156	0.0000	1	1	4
Phi p pent	4155.0138	36.5982	0	0	3
$Phi_{period2}$ p pent	5557.4481	1439.0325	0	0	2
Phi p_{season} pent	5829.4244	1711.0088	0	0	5
Phi p_{period} pent	5830.1844	1711.7688	0	0	5

The Yellow Perch models were limited by how many parameters could be considered in the POPAN estimation model. Here, *phi* encompasses all the time intervals for one parameter, $p_{period2}$ separates baseline and recovery periods from the nAg addition period (second and third parameters), and *pent* encompasses all the time intervals for the fourth parameter. *Phi*, *p*, *pent* considers each of survivability (first parameter), catchability (second parameter), and probability of entry (third parameter) as having the same effect on perch populations across all time intervals.



Appendix I. Figure 1. Yellow Perch open population estimates in Lake 222 and Lake 239, from 2012 to 2017.

LITERATURE CITED

Arnason, A.N., Schwarz, C.J. and Boyer, G. 1998. POPAN-5: A data maintenance and analysis system for mark-recapture data. Technical Report, University of Manitoba, Department of Computer Science.

Bardach, J.E. 1955. The opercular bone of the Yellow Perch, *Perca flavescens*, as a tool for age and growth studies. *Copeia* 1955(2):107-109.

Beamish, R.J. 1972. Design of a trapnet for sampling shallow-water habitats. Technical Report, *Journal of the Fisheries Research Board of Canada* 305:14p.

Beamish, F.W.H. 1978. Eds. W.S. Hoar and D.J. Randall. Swimming capacity. Fish Physiology, vol. VII, Locomotion. New York, London: Academic Press. p101-187.

Benn, T., Cavanagh, B., Hristovski, K., Posner, J.D. and Westerhoff, P. 2010. The release of nanosilver from consumer products used in the home. *Journal of Environmental Quality* 39:1875-1882.

Beyers, D.W., Rice, J.A., Clements, W.H. and Henry, C.J. 1999. Estimating physiological cost of chemical exposure: integrating energetics and stress to quantify toxic effects in fish. *Canadian Journal of Fisheries and Aquatic Sciences* 56:814-822.

Bilberg, K., Malte, H., Wang, T. and Baatrup, E. 2010. Silver nanoparticles and silver nitrate cause respiratory stress in Eurasian perch (*Perca fluviatilis*). *Aquatic Toxicology* 96:159-65.

Birnie-Gauvin, K., Constantini, D., Cooke, S.J. and Willmore, W.G. 2017. A comparative and evolutionary approach to oxidative stress in fish: A review. *Fish and Fisheries* 18:928-942.

Blakelock, G.C., Xenopoulos, M.A., Norman, B.C., Vincent, J.L. and Frost, P.C. 2016. Effects of silver nanoparticles on bacterioplankton in a boreal lake. *Freshwater Biology* 61:2211-2220.

Blaser, S., Scheringer, M., Macleod, M. and Hungerbühler, K. 2008. Estimation of cumulative aquatic exposure and risk due to silver: contribution of nanofunctionalized plastics and textiles. *Science of the Total Environment* 390:396-409.

Boisclair, D. and Leggett, W.C. 1989. Among-population variability of fish growth. 1. Influence of the quantity of food consumed. *Canadian Journal of Fisheries and Aquatic Sciences* 46:457-7.

Buzea, C., Pacheco, I. and Robbie, K. 2007. Nanomaterials and nanoparticles: Sources and toxicity. *Biointerphases* 2(4):MR17-MR71.

Campbell, P.G.C., Hontela, A., Rasmussen, J.B., Giguère, A., Gravel, A., Kövecses, J., Kraemer, L., Lacroix, A., Levesque, H. and Sherwood, G.D. 2003. Differentiating between direct (physiological) and food-chain mediated (bioenergetic) effects on fish in metal impacted lakes. *Human and Ecological Risk Assessment* 9:1-5.

Canadian Council of Ministers of the Environment. 2015. Canadian water quality guidelines for the protection of aquatic life: Silver. In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment, Winnipeg. 16p.

Canadian Water Quality Guidelines: Silver. Scientific Criteria Document. 2015. Canadian Council of Ministers of the Environment, Winnipeg. PN 1539:73p.

Cardeilhac, P.T., Simpson, C.F., Lovelock, R.F., Yosha, S.F., Calderwood, H.W. and Gudat, J.C. 1979. Failure of osmoregulation with apparent potassium intoxication in marine teleosts: A primary toxic effect of copper. *Aquaculture* 17(3):231-239.

Ciardullo, S., Aurell, F., Coni, E., Guandalini, E., Iosi, F., Raggi, A., Rufo, G. and Cubadda, F. 2008. Bioaccumulation potential of dietary arsenic, cadmium, lead, mercury, and selenium in organs and tissues of rainbow trout (*Oncorhynchus mykiss*) as a function of fish growth. *Journal of Agricultural and Food Chemistry* 56:2442-2451.

Colman, B.P., Espinasse, B., Richardson, C.J. Matson, C.W., Lowry, G.V., Hunt, D.E., Wiesner, M.R. and Bernhardt, E.S. 2014. Emerging contaminant or an old toxin in disguise? Silver nanoparticle impacts on ecosystems. *Environmental Science and Technology* 48:5229-5236.

Conine, A.L. and Frost, P.C. 2017. Variable toxicity of silver nanoparticles to *Daphnia magna*: effects of algal particles and animal nutrition. *Ecotoxicology* 26:118-126.

Conine, A.L., Rearick, D.C., Paterson, M.J., Xenopoulos, M.A. and Frost, P.C. 2018. Addition of silver nanoparticles has no long-term effects on natural phytoplankton community dynamics in a boreal lake. *Limnology and Oceanography Letters* 00:00-00.

Cummins, K.W. and Wuycheck. J.C. 1971. Caloric equivalents for investigations in ecological energetics. International Association of Theoretical and Applied Limnology 18. E. Schweizerbart, Stuttgart, Germany.

Das, P., Metcalfe, C.D. and Xenopoulos, M.A. 2014. Interactive effects of silver nanoparticles and phosphorus on phytoplankton growth in natural waters. *Environmental Science and Technology* 48:4573-4580.

Das, P., Williams, C.J., Fulthorpe, R.F., Hoque, M.E., Metcalfe, C.D. and Xenopoulos, M.A. 2012. Changes in bacterial community structure after exposure to silver nanoparticles in natural waters. *Environmental Science and Technology* 46:9120-9128.

Davies, P.H., Goettl, J.P. and Sinley, J.R. 1978. Toxicity of silver to rainbow trout (*Salmo gairdneri*). *Water Research* 12:113-117.

Driver, E.A., Sugden, L.G. and Kovach, R.J. 1974. Calorific, chemical and physical values of potential duck foods. *Freshwater Biology* 4:281-292.

Eckmann, R. 2004. Overwinter changes in mass and lipid content of *Perca fluviatilis* and *Gymnocephalus cernuus*. *Journal of Fish Biology* 65:1498-1511.

Environment Canada. 2013. Email correspondence of raw data for regional surface water quality. Fresh Water Quality Monitoring, Environment Canada. October 11th 2013.

Fabrega, J., Fawcett, S.R., Renshaw, J.C. and Lead, J.R. 2009. Silver nanoparticle impact on bacterial growth: effect of pH, concentration, and organic matter. *Environmental Science and Technology* 43(19):7285-90.

Fabrega, J., Luoma, S.N., Tyler, C.R., Galloway, T.S. and Lead, J.R. 2011. Silver nanoparticles: behavior and effects in the aquatic environment. *Environment International* 37:517-531.

Farmen, E., Mikkelsen, H.N., Evensen, O., Einset, J., Heier, L.S., Rosseland, B.O., Salbu, B., Tollefsen, K.E. and Oughton, D.H. 2012. Acute and sub-lethal effects in juvenile Atlantic salmon exposed to low μg/L concentrations of Ag nanoparticles. *Aquatic Toxicology* 108:78-84.

Ferriss, B.E. and Essington, T.E. 2014. Can fish consumption rate estimates be improved by linking bioenergetics and mercury mass balance models? Application to tunas. *Ecological Modeling* 272:232-241.

Furtado, L.M., Bundschuh, M. and Metcalfe, C.D. 2016. Monitoring the fate and transformation of silver nanoparticles in natural waters. *Bulletin of Environmental Contamination and Toxicology* 97:449-455.

Furtado, L.M., Hoque, M.E., Mitrano, D.M., Ranville, J.F., Cheever, B., Frost, P.C., Xenopoulos, M.A., Hintelmann, H. and Metcalfe, C.D. 2014. The persistence and transformation of silver nanoparticles in littoral lake mesocosms monitored using various analytical techniques. *Environmental Chemistry* 11:419-430.

George, E.L. and Hadley, W.F. 1979. Food and habitat partitioning between rock bass (*Ambloplites rupestris*) and smallmouth bass (*Micropterus dolomieui*) young of the year. *Transactions of the American Fisheries Society* 108:253-261.

Goto, D. and Wallace, W.G. 2010. Bioenergetic responses of a benthic forage fish (*Fundulus heteroclitus*) to habitat degradation and altered prey community in polluted salt marshes. *Canadian Journal of Fisheries and Aquatic Sciences* 67:1566-1584.

Gottschalk, F., Sonderer, T., Scholz, R.W. and Nowack, B. 2010. Possibilities and limitations of modeling environmental exposure to engineered nanomaterials by probabilistic material flow analysis. *Environmental Toxicology and Chemistry* 29:1036-1048.

Gottschalk, F., Sun, T.Y. and Nowack, B. 2013. Environmental concentrations of engineered nanomaterials: Review of modeling and analytical studies. *Environmental Pollution* 181:287-300.

Griffitt, R.J., Brown-Peterson, N.J., Savin, D.A., Manning, C.S., Boube, I., Ryan, R.A. and Brouwer, M. 2012. Effects of chronic nanoparticulate silver exposure to adult and juvenile sheepshead minnows (*Cyprinodon variegatus*). *Environmental Toxicology and Chemistry* 31(1):160-167.

Gunn, J.M. and Mills, K.H. 1998. The potential for restoration of acid-damaged lake trout lakes. *Restoration Ecology* 6:390-7.

Handbook of Capture-Recapture Analysis. 2005. Eds. S.C. Amstrup, T.L. McDonald and B.F.J. Manly. Princeton, New Jersey: Princeton University Press. ISBN: 0-691-08967-1.

Hanson, P.C., Johnson, T.B., Schindler, D.E. and Kitchell, J.F. 1997. Fish Bioenergetics 3.0. University of Wisconsin, Sea Grant Institute, Technical Report WISCU-T-97-001, Madison.

Hilborn, R, Quinn, T.P., Shindler, D.E. and Rogers, D.E. 2003. Biocomplexity and fisheries sustainability. *Proceedings of the National Academy of Sciences* 100(11):6564-6568.

Hrenchuk, L.E., Blanchfield, P.J., Paterson, M.J. and Hintelmann, H.H. 2012. Dietary and waterborne mercury accumulation by yellow perch: A field experiment. *Environmental Science and Technology* 46:509-516.

Isermann, D.A. and Knight, C.T. 2005. A computer program for age-length keys incorporating age assignment to individual fish. *North American Journal of Fisheries Management* 25:1153-1160.

Kaegi, R., Voegelin, A., Ort, C., Sinnet, B., Thalmann, B., Krismer, J., Hagendorfer, H., Elumelu, M. and Mueller, E. 2013. Fate and transformation of silver nanoparticles in urban wastewater systems. *Water Research* 47:3866-3877.

Kaufman, S.D., Gunn, J.M., Morgan, G.E. and Couture, P. 2006. Muscle enzymes reveal walleye (*Sander vitreus*) are less active when larger prey (cisco, *Coregonus artedi*) are present. *Canadian Journal of Fisheries and Aquatic Sciences* 63:970-979.

Keller, A.A., McFerran, S., Lazareva, A. and Suh, S. 2013. Global life cycle releases of engineered nanomaterials. *Journal of Nanoparticle Research* 15:1-17.

Kennedy, A.J., Hull, M.S., Bednar, A.J., Goss, J.D., Gunter, J.C., Bouldin, J.L., Vikesland, P.J. and Steevens, J.A. 2010. Fractionating nanosilver: Importance for determining toxicity to aquatic test organisms. *Environmental Science and Technology* 44:9571-9577.

Kennedy, A.J., Chappell, M.A., Bednar, A.J., Ryan, A.C., Laird, J.G., Stanley, J.K. and Steevens, J.A. 2012. Impact of organic carbon on the stability and toxicity of fresh and stored silver nanoparticles. *Environmental Science and Technology* 46:10772-10780.

Kerr, S.R. 1971. Prediction of fish growth efficiency in nature. *Journal of the Fisheries Research Board of Canada* 28:809-814.

Kimura, D.A. 1977. Statistical assessment of the age-length key. *Journal of the Fisheries Research Board of Canada* 34:317-324.

Kitchell, J.F., Stewart, D.J. and Weininger, D. 1977. Applications of a bioenergetics model to yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum vitreum*). *Journal of the Fisheries Research Board of Canada* 34:1922-1935.

Laban, G., Nies, L.F., Turco, R.F., Bickham, J.W. and Sepúlveda, M.S. 2010. The effects of silver nanoparticles on fathead minnow (*Pimephales promelas*) embryos. *Ecotoxicology* 19:185-95.

Lapresta-Fernández, A., Fernández, A. and Blasco, J. 2012. Nanoecotoxicity effects of engineered silver and gold nanoparticles in aquatic organisms. *Trends in Analytical Chemistry* 32:40-59.

Leadley, T.A., McLeod, A.M., Johnson, T.B., Heath, D. and Drouillard, K.G. 2016. Uncovering adaptive versus acclimatized alterations in standard metabolic rate in brown bullhead (*Ameiurus nebulosus*). *Canadian Journal of Fisheries and Aquatic Sciences* 73:973-981.

Leclerc, S. and Wilkinson, K.J. 2014. Bioaccumulation of nanosilver by *Chlamydomonas reinhardtii* – nanoparticle or the free ion? *Environmental Science and Technology* 48:358-364.

Liu, J., Chao, J., Liu, R., Tan, Z., Yin, Y. and Wu, Y. 2009. Cloud point extraction as an advantageous preconcentration approach for analysis of trace silver in environmental waters. *Analytical Chemistry* 81:6496-6502.

Liu, J. and Hurt, R.H. 2010. Ion release kinetics and particle persistence in aqueous nano-silver colloids. *Environmental Science and Technology* 44(6):2169-2175.

Lowry, G.V., Espinasse, B.P., Badireddy, A.R., Richardson, C.J., Reinsch, B.C., Bryant, L.D., Bone, A.J., Deonarine, A., Chae, S., Therezien, M., Colman, B.P., Hsu Kim, H., Bernhardt, E.S., Matson, C.W. and Wiesner, M.R. 2012. Long-term transformation and fate of manufactured Ag nanoparticles in a simulated large scale freshwater emergent wetland. *Environmental Science and Technology* 46:7027-36.

Maillard, J-Y. and Hartemann, P. 2013. Silver as an antimicrobial: facts and gaps in knowledge. *Critical Reviews in Microbiology* 39(4):373-383.

Martin, J.D., Colson, T-L., Langlois, V.S. and Metcalfe, C.D. 2017b. Biomarkers of exposure to nanosilver and silver accumulation in yellow perch (*Perca flavescens*). *Environmental Toxicology and Chemistry* 36:1211-1220.

Martin, J.D., Frost, P.C., Hintelmann, H., Newman, K., Paterson, M.J., Rennie, M.D., Xenopolous, M.A., Yargeau, V. Hayhurst, L.D. and Metcalfe, C.D. Submitted. Bioaccumulation of

silver in yellow perch (*Perca flavescens*) and northern pike (*Esox lucius*) from a lake dosed with nanosilver. *Environmental Science and Technology*

Martin, J.D., Telgmann, L. and Metcalfe, C.D. 2017a. A method for preparing silver nanoparticle suspensions in bulk for ecotoxicity testing and ecological risk assessment. *Bulletin of Environmental Contamination and Toxicology* 98:589-594.

Massarsky, A., Trudeau, V.L. and Moon, T.W. 2014. Predicting the environmental impact of nanosilver. *Environmental Toxicology and Pharmacology* 38:861-873.

May, C.J. 2005. Comparison of techniques used to age yellow perch in Southern Lake Michigan. An honours thesis submitted to Ball State University, Muncie, Indiana. Retrieved from <https://cardinalscholar.bsu.edu/bitstream/handle/handle/191529/M348_2005MayCassandraJ.pdf?sequence=1>

McCann, K. 2007. Protecting biostructure. *Nature* 446:29.

McMeans, B.C., McCann, K.S., Tunney, T.D., Fisk, A.T., Muir, A., Lester, N., Shuter, B. and Rooney, N. 2016. The adaptive capacity of lake food webs: from individuals to ecosystems. *Ecological Monographs* 86(1):4-19.

McTeer, J., Dean, A.P., White, K.N. and Pittman, J.K. 2014. Bioaccumulation of silver nanoparticles into *Daphnia magna* from a freshwater algal diet and the impact of phosphorus availability. *Nanotoxicology* 8:305-316.

McQueen, D.J., Johannes, M.R.S., Post, J.R., Stewart, T.J. and Lean, D.R.S. 1989. Bottom-up and top-down impacts on freshwater pelagic community structure. *Ecological Monographs* 59(3):289-309.

Metcalfe, C. 2017. Report to Environment and Climate Change Canada: Post-addition monitoring of Lake 222. Lake Ecosystem Nanosilver (LENs) Project. p 1-8.

Murray, L., Rennie, M.D., Enders, E., Pleskach, K. and Martin, J. 2017a. Effect of nanosilver on cortisol release and morphometrics in rainbow trout (*Oncorhynchus mykiss*). *Environmental Toxicology and Chemistry* p1-8.

Murray, L., Rennie, M.D., Svendsen, J.C. and Enders, E.C. 2017b. Effect of nanosilver on metabolism in rainbow trout (*Oncorhynchus mykiss*): An investigation using different respirometric approaches. *Environmental Toxicology and Chemistry* 36:2722-2729.

Newman, K., Metcalfe, C.D., Martin, J., Hintelmann, H., Shaw, P. and Donard, A. 2016. Improved single particle ICP-MS characterization of silver nanoparticles at environmentally relevant concentrations. *Journal of Analytical Atomic Spectrometry* 31:2069-2077.

Niewinski, B.C. and Ferreri, C.P. 1999. A comparison of three structures for estimating the age of yellow perch. *North American Journal of Fisheries Management* 19(3):872-877.

Nowack, B., Ranville, J.F., Diamond, S., Gallego-Urrea, J.A., Metcalfe, C., Rose, J., Horne, N., Koelmans, A.A. and Klaine, S.J. 2012. Potential scenarios for nanomaterial release and subsequent alteration in the environment. *Environmental Toxicology and Chemistry* 31:50.

Ogle, D.H. 2016. A computer program for age-length keys incorporating age assignment to individual fish. *North American Journal of Fisheries Management* 25:1153-1160.

Ogorek, J. and Dewild, J. 2010. Analysis of methylmercury in biological samples by cold vapor atomic fluorescence detection with the Brooks-Rand "MERX" automated methylmercury analytical system. Brooks-Rand 13p.

Ontario Benthos Biomonitoring Network: Protocol Manual. 2007. Prepared by C. Jones, K.M. Somers, B. Craig and T.B. Reynoldson. Ontario Ministry of the Environment and Climate Change. Retrieved from <https://desc.ca/sites/default/files/OBBN2007finalapril18c.pdf>

Oukarroum, A., Sebastien, B., Perrault, F., Popovic, R. 2012. Inhibitory effects of silver nanoparticles in two green algae, *Chlorella vulgaris* and *Dunaliella tertiolecta*. *Ecotoxicology and Environmental Safety* 78:80-85.

Pazzia I., Trudel, M., Ridgway, M. and Rasmussen, J.B. 2002. Influence of food web structure on the growth and bioenergetics of lake trout (*Salvelinus namaycush*). *Canadian Journal of Fisheries and Aquatic Sciences* 59:1593-605.

Peles, J.D., Pistole, D.H. and Moffe, M. 2012. Influence of cadmium concentration and length of exposure on metabolic rate and gill Na^+/K^+ ATPase activity of golden shiners (*Notemigonus crysoleucas*). *Comparative Biochemistry and Physiology* Part C.

Persson, L. 1987. The effects of resource availability and distribution on size class interactions in perch, *Perca fluviatilis*. *Oikos* 48:148-60.

Petchey, O.L., Beckerman, A.P., Riede, J.O. and Warren, P.H. 2008. Size, foraging, and food web structure. *Proceedings of the National Academy of Sciences* 105:4191-4196.

Pham, C.H., Yi, J. and Gu, M.B. 2012. Biomarker gene response in male Medaka (*Oryzias latipes*) chronically exposed to silver nanoparticle. *Ecotoxicology and Environmental Safety* 78:239-245.

Program MARK. 2014. Eds. E.G. Cooch and G.C. White. Program MARK: A Gentle Introduction Edition 13. Retrieved from <http://www.fwspubs.org/doi/suppl/10.3996/122012-JFWM-110R1/suppl_file/10.3996_122012-jfwm-110r1.s8.pdf?code=ufws-site>

Pulit-Prociak, J., Stokłosa, K. and Banach, M. 2014. Nanosilver products and toxicity. *Environmental Chemistry Letters* 13:59-68.

Quinn, G.P. and Keough, M.J. 2002. Experimental Design and Data Analysis for Biologists. Cambridge University Press, New York. ISBN 0-521-81128-7.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from <http://R-project.org>

Rand, P.S. and Stewart, D.J. 1998. Prey fish exploitation, salmonine production, and pelagic food web efficiency in Lake Ontario. *Canadian Journal of Fisheries and Aquatic Sciences* 55:318-327.

Rasmussen, J.B., Gunn, J.M., Sherwood, G.D., Iles, A., Gagnon, A., Campbell, P.G.C. and Hontela, A. 2008. Direct and indirect (food-web mediated) effects of metal exposure on the growth of yellow perch *(Perca flavescens)*: implications for ecological risk assessment. *Human and Ecological Risk Assessment* 14: 317-350.

Rearick, D.C., Telgmann, L., Hintelmann, H., Frost, P.C. and Xenopoulos, M. Submitted. Spatial and temporal trends in the fate of silver nanoparticles in a whole-lake addition study. *PlosOne*.

Rennie, M.D., Collins, N.C., Shuter, B.J., Rajotte, J.W. and Couture, P. 2005. A comparison of methods for estimating activity costs of wild fish populations: more active fish observed to grow slower. *Canadian Journal of Fisheries and Aquatic Sciences* 62:767-780.

Rennie, M.D., Johnson, T.B. and Sprules, W.G. 2012. Energy acquisition and allocation patterns of lake whitefish (*Coregonus clupeaformis*) are modified when dreissenids are present. *Canadian Journal of Fisheries and Aquatic Sciences* 69:41-59.

Rennie, M.D., Purchase, C.F., Shuter, B.J., Collins, N.C., Abrams, P.A. and Morgan, G.E. 2010. Prey life-history and bioenergetics response across a predation gradient. *Journal of Fish Biology* 77:1230-1251.

Rennie, M.D. and Venturelli, P.A. 2015. (Book chapter, invited contribution). In: Biology and Culture of Percid Fishes – Principles and Practices. Editors: P. Kestemont, K. Dabrowski and R C. Summerfelt. Springer.

Schindler, D.E. and Eby, L.A. 1997. Stoichiometry of fishes and their prey: implications from nutrient recycling. *Ecology* 78:1816-1831.

Schnabel, Z.E. 1938. The estimation of the total fish population of a lake. *American Mathematical Monthly* 45:348-352.

Scott, W.B. and Crossman, E.J. 1973. Freshwater Fishes of Canada. Bulletin 184: Fisheries Research Board of Canada, Ottawa, Ontario.

Scown, T.M., Santos, E.M., Johnston, B.D., Gaiser, B., Baalousha, M., Mitov, S., Lead, J.R., Stone, V., Fernandes, T.F., Jepson, M., Aerle, R.V. and Tyler, C.R. 2010. Effects of aqueous exposure to silver nanoparticles of different sizes in rainbow trout. *Toxicological Sciences* 115(2):521-534.

Sherwood, G.D., Pazzia, I., Moeser, A., Hontela, A. and Rasmussen, J.B. 2002. Shifting gears: enzymatic evidence for the energetic advantage of switching diet in wild-living fish. *Canadian Journal of Fisheries and Aquatic Sciences* 59:229-241.

Shi, J-P., Ma, C-Y., Xu, B., Zhang, H.W. and Yu, C.P. 2012. Effect of light on toxicity of nanosilver to *Tetrahymena pyriformis*. *Environmental Toxicology and Chemistry* 31:1630-1638.

Smith, E.P. 2002. Eds. A.H. El-Shaarawi and W.W. Piegorsch. BACI design. *Encyclopedia of Environmetrics* 1:141-148. ISBN 0471 899976.

SOP-EL109: MeHg in biological samples by acid digestion followed by aqueous ethylation purge and trap and CVAFS using the Brooks-Rand System. Environmental Lab (LUEL), Lakehead University Centre for Analytical Sciences.

Sotola, V.A., Maynard, G.A., Hayes-Pontius, E.M., Mihuc, T.B., Malchoff, M.H. and Marsden, J.E. 2014. Precision and bias of using opercles as compared to otoliths, dorsal spines, and scales to estimate ages of largemouth and smallmouth bass. *Northeastern Naturalist* 21(4):565-573.

Stebounova, L.V., Guio, E. and Grassian, V.H. 2011. Silver nanoparticles in simulated biological media: a study of aggregation, sedimentation, and dissolution. *Journal of Nanoparticle Research* 13:233-244.

Sun, T.Y., Gottschalk, F., Hungerbühler, K. and Nowack, B. 2014. Comprehensive probabilistic modelling of environmental emissions of engineered nanomaterials. *Environmental Pollution* 185:69-76.

Trudel, M. and Rasmussen, J. 2001. Predicting mercury concentration in fish using mass balance models. *Ecological Applications* 11(2):517-529.

Trudel, M., Tremblay, A., Schetagne, R. and Rasmussen, B. 2000. Estimating food consumption rates of fish using a mercury mass balance method. *Canadian Journal of Fisheries and Aquatic Sciences* 57:414-428.

Trudel, M., Tremblay, A., Schetagne, R. and Rasmussen, J.B. 2001. Why are dwarf fish so small? An energetic analysis of polymorphism in lake whitefish (*Coregonus clupeaformis*). *Canadian Journal of Fisheries and Aquatic Sciences* 58:394-405.

U.S. EPA. 1998. Method 7473 (SW-846): Mercury in solids and solutions by thermal decomposition, amalgamation, and atomic absorption spectrophotometry. Revision 0. United States Environmental Protection Agency, Washington, D.C.

U.S. EPA. 2001. Method 1630: Methyl mercury in water by distillation, aqueous ethylation, purge and trap, and CVAFS. EPA 821-R-01-020. United States Environmental Protection Agency, Washington, D.C.

Vander Zanden, M.J. and Rasmussen, J.B. 1996. A trophic position model of pelagic food webs: Impact on contaminant bioaccumulation in lake trout. *Ecological Monographs* 66:451-477.

Vincent, J.L., Paterson, M.J., Norman, B.C., Gray, E.P., Ranville, J.F., Scott, A.B., Frost, P.C. and Xenopoulos, M.A. 2017. Chronic and pulse exposure effects of silver nanoparticles on natural lake phytoplankton and zooplankton. *Ecotoxicology* 26:502-515.

Wang, Z., Chen, J., Li, X., Shao, J. and Peijenburg, W.J.G.M. 2012. Aquatic toxicity of nanosilver colloids to different trophic organisms: contributions of particles and free silver ion. *Environmental Toxicology and Chemistry* 31(10):2408-2413.

Wood, C.M., Playle, R.C. and Hogstrand, C. 1999. Physiology and modeling of mechanisms of silver uptake and toxicity in fish. *Environmental Toxicology and Chemistry* 18(1):71-83.

Xiu, Z-M., Ma, J. and Alvarez, P.J.J. 2011. Differential effect of common ligands and molecular oxygen on antimicrobial activity of silver nanoparticles versus silver ions. *Environmental Science and Technology* 45:9003-9008.

References to Animal User Protocol, Biosafety, and User Manuals:

- Animal User Protocol (AUP) No.1464693: *Effects of whole-lake nanosilver addition on fish populations* (04/05/2015 - 30/10/2015; Renewal 02/05/2016 - 30/10/2016)
- AUP Renewal – Mark-Recapture (IISD-ELA via University of Manitoba from 2012 to 2015; renewed through Lakehead University 2016 to present)
- AUP Renewal – Biopsies (IISD-ELA via University of Manitoba from 2012 to 2015; renewed through Lakehead University 2016 to present)
- Awards No. 1465260: *Recovery of fish populations from environmental nanosilver release*
- Biosafety Approval No.1464768 *Evaluating environmental release of nanosilver on native fish populations*
- Milestone DMA-80 User Manual, ATS Scientific, Burlington, ON. Canada