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Whole-lake nanosilver additions reduce northern pike (*Esox lucius*) growth

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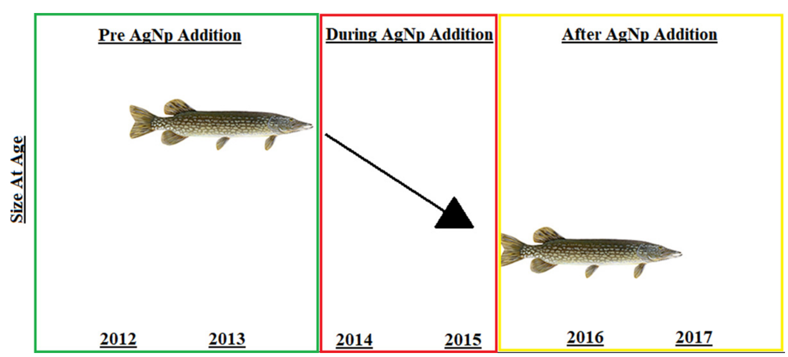
HIGHLIGHTS

- Nanosilver was added to Lake 222 for 2 years to evaluate whole-ecosystem impacts.
- Northern Pike growth declined during and after nanosilver additions.
- Per capita prey availability also declined in the study.
- This pattern was not reflected in Northern Pike from a nearby reference lake.
- Northern Pike abundance was stable over the duration of the study in all lakes.

GRAPHICAL ABSTRACT



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ABSTRACT

Nanosilver (AgNP) is an anti-microbial agent widely used in consumer products, with significant potential for these nanoparticles to be released into aquatic environments. Laboratory studies involving short-term exposures of fish to AgNP show a range of toxicological effects, but these studies do not address potential responses in long-lived organisms resulting from chronic exposures. A collaborative study involving additions of AgNP to environmentally relevant concentrations over two field seasons took place at the IISD-Experimental Lakes Area, providing an opportunity to study the impacts of chronic exposures to long-lived fish species. In the present study, we evaluated the abundance and growth of an apex predator, Northern Pike (*Esox lucius*), collected from Lake 222 before, during and after the AgNP dosing period and compared results to those from a nearby unmanipulated lake (Lake 239). While the abundance of Northern Pike from Lake 222 during the study period was essentially stable, per capita availability of their primary prey species, Yellow Perch (*Perca flavescens*) declined by over 30%. Northern Pike fork length- and weight-at-age (indices of growth rate) declined following AgNP additions, most notably in age 4 and 5 fish. No similar changes in prey availability or growth were observed in Northern Pike from the reference lake. Body condition did not change in Northern Pike collected from either Lake 222 or Lake 239. Our results indicate that declines in the growth of Northern Pike chronically exposed to AgNP likely resulted from reduced prey availability but direct sublethal effects from AgNP exposure could also have been a factor. The persistence of reduced growth in Northern Pike two years after the cessation of AgNP additions highlight the potential legacy impacts of this contaminant once released into aquatic ecosystems.

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1. Introduction

Nanosilver (AgNP) is widely used in a range of consumer products, most notably in textiles, including sports clothing, underwear and socks, where it is added to fabrics for its anti-bacterial, anti-microbial, and odour-reducing properties (Akter et al., 2018; Benn et al., 2010). As a result, AgNP is released into municipal wastewater streams when clothing items are washed (Benn et al., 2010; Benn and Westerhoff, 2008; Reidy et al., 2013). In wastewater treatment plants (WWTPs), most of the AgNP partitions into sewage sludge, with only 2–3% exiting in the effluent. Under reducing conditions in WWTPs, AgNPs can become sulfidated, which reduces the rate of dissolution into Ag^+ (Fletcher et al., 2019). However, releases of AgNP in effluent can sharply increase over short periods when sludge yield is high (Kaegi et al., 2011). Even this low percentage of AgNP release represents a potential threat to aquatic organisms, with reported measured effluent concentrations ranging from 6 ng L^{-1} to 2.89 μg L^{-1} (Johnson et al., 2014; Liu et al., 2009), and predicted concentrations in natural waterways ranging from the low ng L^{-1} range (Gottschalk et al., 2013; Sun et al., 2014) to the low (0.32 to 1.5) μg L^{-1} range (Blaser et al., 2008; Liu et al., 2009). Once in the aquatic environment, AgNP can remain as a colloidal suspension, agglomerate into larger particles and settle into the sediments, or be taken up by organisms (Furtado et al., 2015; McGillicuddy et al., 2017). Dissolution of AgNP can also result in the release of Ag^+ into solution, a highly toxic substance to aquatic organisms (Shevlin et al., 2018).

Previous laboratory studies of the effects of AgNP have shown adverse impacts on various fish species but these experiments have often been conducted at concentrations that are not environmentally relevant; that is, higher than the concentrations likely to be encountered in the environment. In studies with various life stages of Fathead Minnow (*Pimephales promelas*), AgNPs were toxic to the fish at extremely high concentrations (i.e., stirred solution: 9.4 mg L^{-1}, sonicated solution: 1.25 mg L^{-1}), causing mortalities and developmental abnormalities in hatched embryos (Laban et al., 2010). In another study with adult Fathead Minnow exposed to lower concentrations of AgNPs (50–56 μg L^{-1}), altered gene expression was observed in the gills of exposed fish and mucous production became variable, initially spiking and then becoming depressed compared to controls (Garcia-Reyero et al., 2015). Various sublethal responses, including respiratory effects have been characterized in other fish species exposed to μg L^{-1} concentrations of AgNPs (i.e., 60–300 μg L^{-1}), including juvenile Atlantic Salmon (*Salmo salar*), as reported by Farmen et al. (2012) and Eurasian Perch (*Perca fluvialitis*), as reported by Bilberg et al. (2010). In Rainbow Trout (*Oncorhynchus mykiss*) exposed to AgNPs at a concentration likely to be encountered in the environment (i.e., 0.28 μg L^{-1}) the fish accumulated Ag in tissues and showed a stress response of elevated levels of circulating cortisol (Murray et al., 2017a).

Concern over both the potential for release of AgNP into the environment and the impacts on aquatic organisms led to a whole-lake AgNP addition experiment conducted in Lake 222 at the IISD-Experimental Lakes Area (IISD-ELA) involving the additions of a total of 15 kg of AgNPs to the lake over two field seasons. (Conine et al., 2018; Hayhurst et al., 2020; Martin et al., 2018; Rearick et al., 2018). Previous to the whole-lake addition study, studies of the fate and effects of AgNP were conducted in mesocosms at the IISD-ELA. In the mesocosms, bacterioplankton and phytoplankton were not significantly impacted by exposures to AgNPs but zooplankton species richness declined, although the abundance of the remaining species increased as much as four times compared to control treatments (Vincent et al., 2017). Both phytoplankton and zooplankton have been shown to accumulate AgNP, creating a pathway for AgNP to enter the food web and transfer to higher trophic levels (Asghari et al., 2012; Conine and Frost, 2017). Juvenile Yellow Perch (*Perca flavescens*) rely heavily on diets consisting of zooplankton, which may enable AgNP to be ingested and ultimately transferred from prey species to consumers. Additionally, suspended AgNP particles can be taken up in fish via absorption through the gills (Martin et al., 2017; Scown et al., 2010).

AgNP uptake by fish through either dietary or branchial routes can result in accumulation of silver in the gill, liver, and muscle tissues, which may result in toxic effects. During additions of AgNP to Lake 222, the concentrations of total silver (tAg) in the tissues of both Yellow Perch and Northern Pike (*Esox lucius*) increased rapidly, and in the second year of additions, the tAg concentrations in the livers of Northern Pike increased to the low parts-per-million (ppm) range, which was several orders of magnitude greater than the low μg L^{-1} concentrations of tAg in the water (Martin et al., 2018). In addition, food consumption, metabolic rates and densities of Yellow Perch in Lake 222 significantly declined during and after dosing (Hayhurst et al., 2020). Yellow Perch exposed to AgNPs in laboratory settings showed increased expression of the gene for metallothionein (*mt*), as well as evidence of lipid peroxidation in gills and liver, as indicated by increased levels of thiobarbituric acid reactive substances (TBARS) and alterations to glutathione (GSH/GSSG) ratios (Martin et al., 2017). Similarly, Yellow Perch collected from Lake 222 during AgNP dosing also showed elevated TBARS and altered GSH/GSSG ratios in liver tissue (Hayhurst et al., 2020). In contrast, while Rainbow Trout (*Oncorhynchus mykiss*) in laboratory studies experienced measurable increases in their blood cortisol levels after 28 days of exposure to AgNP, no significant changes were observed in their growth or metabolism (Murray et al., 2017b, 2017a).

Northern Pike are widespread across the interior of Canada and are important to both commercial and sportfishing industries (Harvey, 2009), and to the traditional fisheries of First Nations (Kuhnlein and Humphries, 2017). Growth of Northern Pike is known to be negatively and non-linearly correlated with both prey availability and population density (Kennedy et al., 2018; Margenau et al., 1998; Pierce et al., 2003). Northern Pike demonstrate broad dietary diversity (Venturelli and Tonn, 2006) but show a foraging preference for Yellow Perch, when present, with evidence that this forage fish species provides sufficient energy for rapid growth rates in juvenile Northern Pike (Kennedy et al., 2018).

The objective of this study was to contribute to the whole-lake AgNP addition project by determining the influence of long-term AgNP exposures on the abundance, size-at-age, body condition and per capita prey availability of Northern Pike in Lake 222 and in a nearby reference lake, Lake 239. We monitored the length and weight of Northern Pike as a means for evaluating fish condition and growth, which is known to scale positively with food availability (Casini et al., 2016; Rennie et al., 2019; Rennie and Verdon, 2008). Reduced body condition is often a result of increased intraspecific competition, which can be manifested as decreases in per capita prey availability (Casini et al., 2016; Rennie et al., 2019; Rennie and Verdon, 2008). We hypothesized that changes in growth and/or body condition would provide evidence that chronic exposures to AgNPs were having indirect or direct effects on Northern Pike. Indirect effects could be due to a reduction in the availability of prey (i.e., Yellow Perch). Alternatively, these responses in Northern Pike could occur as a result of direct responses to the accumulation of Ag in tissues and the metabolic costs associated with eliminating and/or coping with this substance.

2. Materials and methods

2.1. Study site

Full details of the whole-lake addition study in Lake 222 at IISD-ELA were described previously (Martin et al., 2018; Rearick et al., 2018). Briefly, the study was performed over six years (2012–2017), with the first two years of sampling dedicated to gathering baseline data. A total of 15 kg of AgNP was added to Lake 222 throughout the ice-free seasons in 2014 (9 kg) and 2015 (6 kg) and estimated concentrations of tAg in the water column during the addition phase ranged up to 11.5 μg L^{-1} (Martin et al., 2018) and averaged 4 μg L^{-1} (Rearick et al., 2018). Concentrations of dissolved Ag (e.g., Ag^+) in lake water during the AgNP addition phase were < 0.1 μg L^{-1}, and the mean size of the silver nanoparticles in suspension was 20 nm (Martin et al., 2018). The lake was also monitored in the post-addition phase in 2016 and 2017, during which time the waterborne concentrations of tAg declined rapidly, especially over the first few months after AgNP additions ceased (Martin et al., 2018). The maximum mean concentration of tAg measured in Northern Pike liver during the addition phase in Lake 222 was nearly 2.5 μg g^{-1}, and tAg was measured in the liver of one individual Northern Pike at a concentration of 5.0 μg g^{-1} (Martin et al., 2018). The half-life of Ag in liver tissue was estimated at 119 days once AgNP additions ceased (Martin et al., 2018). The reference lake for this study was Lake 239, an unmanipulated lake at IISD-ELA which has been monitored for several decades.

Both Lake 222 and Lake 239 are typical boreal lakes with low ionic strength, high organic carbon levels and they both stratify into an epilimnion and hypolimnion during the summer months. Both lakes sustain populations of Yellow Perch and Northern Pike. However, Lake 239 is a larger and deeper lake with a more oligotrophic status and a more diverse fish community (Table 1).

2.2. Fish sampling

A before-after-control-impact (BACI) study design was used to evaluate the impacts of AgNP on Northern Pike abundance and growth (Green, 1979). Fish sampling occurred each year during the pre-addition (2012 and 2013), AgNP addition (2014 and 2015), and post-addition (2016 and 2017) phases of the study. Northern Pike were captured using trap-netting, seine-netting, and angling. Non-lethal sampling was typically performed three times each year in the spring (May/June), summer (July/August), and fall (September/October). While trap-netting and angling tend to be biased towards capture of larger sizes of Northern Pike, small (YOY and yearling) Northern Pike were occasionally encountered in seine catches. However, the fork lengths of the majority (95%) of captured Northern Pike were > 259 mm in Lake 222 and > 317 mm in Lake 239, which comprises primarily pre-mature individuals, but under-represents the abundance of smaller individuals in the population.

Captured fish were held temporarily in a large cooler of lake water, after which they were taken to a shoreline sampling site and anaesthetized using a buffered solution of tricaine methanesulfonate (MS-222) prepared in lake water. Northern Pike were measured for fork and total lengths (mm) and weight (g). If possible, the sex of fish captured in spring was determined based on expression of gametes while gently applying pressure down the abdomen of the fish towards the anus. The leading 1–3 pectoral rays on one side of the fish were clipped as close to the insertion point to the body as possible for ageing analysis. To facilitate population estimates, fish ≥200 mm in fork length were then injected with a 9 mm electronic Passive Integrated Transponder (PIT) tag with a globally unique 15-digit identification number. Tags were injected just below the dorsal fin and above the lateral line in the epaxial musculature upon first capture for individual identification, and a seasonal batch-mark was applied to the dorsal fin (Northern Pike) or batch marks alternating between caudal-dorsal-pelvic fins (Yellow Perch) to allow rapid identification of the season of capture. While all sizes of both species were seasonally batch-marked, Northern Pike <200 mm in fork length did not receive PIT tags, receiving only seasonal dorsal fin batch marks to identify season of capture.

All fish were placed in bins of lake water (replenished frequently) to recover and were released when they were observed to be upright and swimming (typically within 5–10 min of handling). Capture and handling mortality of Northern Pike was minimal, as only 2% of 1043 fish handled between 2012 and 2017 died during sampling across both lakes. All fish were handled and collected under the authorization of scientific collection permits provided by the Ontario Ministry of Natural Resources and Forestry, and Animal Use Protocols issued by Fisheries and Oceans Canada (2012 − 13), the University of Manitoba (2014, AUP No. F14-007) and Lakehead University (2015–17, AUP No. 1464693).

2.3. Indices of growth

Pectoral fin rays were used in the present study to estimate the age of Northern Pike from both lakes, and these ages were referenced to morphometric data (i.e., fork length, weight) in order to generate length- and weight-at-age estimates as an index of growth. While several bony structures in Northern Pike lend themselves well to age determination (Forsman et al., 2015), analysis of fin rays for age determination in soft-rayed fish has proven accurate, as predictability between expected age and actual age is high (Glass et al., 2011; Mills and Chalanchuk, 2004; Rude et al., 2013). Additionally, Oele et al. (2015) identified strong agreement between anal fin rays with otoliths and cleithra for Northern Pike up to 5 years of age, further validating the fin ray as a reliable structure to use for age interpretation for the age range of fish examined in this study. Use of fin rays as an ageing structure is also advantageous because it does not require lethal sampling, although a certain level of experience or knowledge of interpretation is required to accurately assess ages (Campana, 2001; Rude et al., 2013). In other species, the fin ray is a structure often used to verify ages determined from other structures such as the cleithrum and otolith (Little et al., 2012).

2.4. Fin ray preparation

Fin rays collected from individual Northern Pike were placed in fin envelopes and set to dry. Dried fins were placed on squares of parafilm (a non-stick surface) and uniquely labelled. A cold-cure epoxy (System Three Resins®) was mixed in a small disposable cup using a 2:1 ratio of epoxy resin to hardener. Once stirred thoroughly, a small amount of

Table 1
Comparison of physical parameters and fish species present in Lake 222 and Lake 239. Water chemistry values are epilimnetic averages with 1 standard error.

Characteristic	Parameter	Lake 222	Lake 239
Physical	Area	16.4 ha	54.3 ha
	Inflow(s)/Outflow	1/1	3/1
	Maximum Depth	6.3 m	30.4 m
	Residence Time	1.2 years	9.3 years
	Secchi Depth	2.2 m	4.8 m
	State	Oligo/Mesotrophic	Oligotrophic
	Volume	7.2 × 10^5 m^3	5.9 × 10^6 m^3
	Conductivity	34.7 ± 0.5 μs/cm	30.5 ± 0.2 μs/cm
Chemical	pH	6.96 ± 0.02	6.98 ± 0.01
	DOC	10.0 ± 0.1 mg/L	7.0 ± 0.1 mg/L
	TP	9.8 ± 0.3 mg/L	6.6 ± 0.1 mg/L
Biological	Fish species	Northern Pike (*Esox lucius*)	Northern Pike (*Esox lucius*)
		Yellow Perch (*Perca flavescens*)	Yellow Perch (*Perca flavescens*)
		Blacknose Shiner (*Notropis heterolepis*)	Iowa Darter (*Etheostoma exile*)
			Cisco (*Coregonus artedi*)
			Lake Trout (*Salvelinus namaycush*)
			Slimy Sculpin (*Cottus cognatus*)
			White Sucker (*Catostomus commersonii*)
			Finescale Dace (*Chrosomus neogaeus*)

epoxy was poured over the fins and the epoxy was set to cure for 24-h. Five cross-sectional cuts of each epoxy-embedded fin were then taken from the proximal end of the fin ray using an Isomet low-speed jewellery saw. A pre-cut was made to the tip of each fin ray before taking sections to clear the fin of any roughness produced from field sampling. Cross-sections were approximately 0.5 mm to 0.6 mm thick. Each cross-section was rinsed with water to clear off dust and then placed on a frosted microscope slide for mounting. Slides were prepared using Cytoseal 60 and sealed with a cover slip. Mounted slides were set in a fume hood to cure for 24 h prior to age determination.

2.5. Age determination

For Northern Pike collected from 2014 to 2017, age was determined for $n = 167$ individuals from examinations of fin ray cross-sections using a compound microscope (Zeiss® Axio Lab A1). Fin ray ages were interpreted by counting annuli formed during fast summer growth and slow winter growth. For Northern Pike collected in 2012 and 2013, ages were determined for $n = 133$ individuals from fin rays using similar methods by staff with the Department of Fisheries and Oceans (DFO) Canada. To validate ages generated among all interpreters, a confirmation test was conducted. A sample of 62 fin rays collected from Northern Pike in 2012 and 2013 with a pre-existing age assigned by DFO personnel (Ager 2) were blindly re-aged independently by two readers; one with seven years of experience ageing fish for the Ontario Ministry of Northern Development, Mines, Natural Resources and Forestry (OMNDMNRF, Ager 3), and one with less than one year of experience ageing fish (Ager 1). Following this validation exercise (see results), Ager 1 aged all remaining fish collected during 2014–2017 in the current study.

2.6. Northern pike population abundance

Estimates of the population size of Northern Pike in both the treatment and reference lakes were determined using mark-recapture methods. Observations of unique PIT tags served as the primary record of capture in the database. Secondarily, seasonal nicks applied to the dorsal fin of Northern Pike also identified fish previously caught, handled, and released in a season that had not been previously injected with PIT tags.

The POPAN sub-module in Program MARK was used to estimate population abundance (Program MARK 2014) via the RMark package (version 2.2.7; Laake et al., 2019) in R version 4.2.1 (R Core Team, 2021). The POPAN sub-module is a modification of the Cormack-Jolly-Seber (CJS) model, where the ratios of unmarked versus marked individuals are used to estimate population size as a derived estimate (Arnason and Schwarz, 1999). 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 and Schwarz, 1999). Assumptions of POPAN models were: (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 between intervals, so animals survived between sampling midpoints; (5) survival and capture of each animal were both independent of the fate of any other animal; and (6) all emigration from the population was permanent, with no immigration or emigration or recruitment occurring during the sampling period (Amstrup et al., 2005).

For Northern Pike populations in both lakes, models representing all combinations of time-dependent and constant parameters were evaluated for each parameter in each capture period for ϕ and p and p_{ent}. The resulting model sets were compared using Akaike's Information Criteria (AIC), with the difference in AIC values between models (ΔAIC) used to determine the best fit of all candidate models evaluated. In order to correct for potential overdispersion, adjustments for goodness-of-fit (GOF) were conducted by estimating a $\hat{c}$ correction value, using Test 2 and Test 3 results of the RELEASE module in MARK. Test 2 evaluates violations of assumptions that every animal had the same probability of recapture, and Test 3 evaluates the assumption that every animal had the same probability of survival to the next capture occasion. GOF corrections were incorporated into model comparisons by adjusting the $\hat{c}$ value from 1, based on the ratio of the Chi-square and degrees of freedom of the sum of Test 2 and Test 3 results (Lake 222: $\chi2 = 76.61$, df = 71, $p = 0.30$; Lake 239: $\chi2 = 58.9$, df = 53, $p = 0.27$). The calculated $\hat{c}$ for Lake 222 was 1.08, while the calculated $\hat{c}$ for Lake 239 was 1.11, both of which were close to 1, indicating a good fit of the fully time-dependent model to the data. These $\hat{c}$ corrections were then applied to the candidate set of models to determine adjusted AIC values. Estimate of predator density from the top model are reported and used in analyses.

2.7. Prey density

The densities of Yellow Perch in both lakes reported as numbers of fish per hectare were estimated using mark-recapture methods, as described in Hayhurst et al. (2020). These data were used alongside Northern Pike abundance estimates (after conversion to numbers of fish per hectare) to estimate per capita prey density. We assumed that Yellow Perch were the main prey fish of Northern Pike in both lakes, given that they made up 64% and 95% of the total catch of forage fish during the study period from lakes 222 and 239, respectively.

The only other alternative prey species available to Northern Pike in Lake 222 is Blacknose Shiner (*Notropis heterolepis*), which made up 30% of the total catch of forage fish over the study period (less than half of the catch of Yellow Perch). Due to high mortalities resulting from extensive handling, this species does not lend itself to mark-recapture population estimates. Instead, Blacknose Shiner relative abundance was measured as catch-per-unit-effort (CPUE) in seine hauls. A 30.5 m seine net (2 m tall, 6 mm mesh opening) with 2 m cubed centre purse was deployed in a half-circle out from the shoreline using a boat across various sampling sites in Lake 222 and deployed in such a way so as to standardize the sampling area as consistently as possible across sites. Because we could estimate only relative (vs. absolute) abundance of Blacknose Shiner, and given they were numerically less than half of the total catch of Yellow Perch in Lake 222, they were not included in per capita prey density estimates of Northern Pike.

2.8. Data analysis

Mean and standard error for fork length (mm), weight (g), and body condition for each sample year among age classes were calculated. Body condition was estimated as an index of the length/weight ratio and was calculated using Fulton's Condition Factor (K):

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

where W = weight in grams, L = a measure of length (in this case fork length was used) in mm, and 10^7 was applied as a scaling constant. Analyses focused on age classes 2, 3, 4 and 5, as Northern Pike in Ontario have been reported to achieve sexual maturity by age 3 on average, though some populations have been observed to mature as old as 5 years of age (Malette and Morgan, 2005). Thus, this study focussed on the impacts of growth in age classes that were still likely investing heavily in somatic growth versus reproduction.

To evaluate whether growth of Northern Pike in Lake 222 was altered during the period of AgNP additions, a 2-way ANOVA (both factors fixed) test was conducted using the statistical program R (R Core Team, 2021). The test compared the two fixed factors of year and lake (and their interaction) against a response variable of either weight, fork length, or body condition over the four age classes evaluated in our analysis. Type III Sums of Squares were used in order to account for unequal sample sizes among groups (Quinn and Keough, 2002). In total, 12 ANOVAs (3 response variables x 4 age classes) were run to determine if there was a significant interaction between lakes and years, indicating a different response over time between the experimental Lake 222 and the reference Lake 239 (Smokorowski and Randall, 2017). Only years where data was present in both the experimental lake and the reference lake were included in the analysis (Table S1).

To control for false discovery rates, a Benjamini-Hotchberg correction was implemented to adjust alpha (α) levels to compare against the p values produced from the ANOVA tests. Under this procedure, significant p-values were ranked from highest to lowest, with the greatest p-value compared against an initial type 1 error rate of $\alpha = 0.05$ and α of sequential tests corrected to values of 0.0375, 0.025, and 0.0125. To improve model fitting where necessary, log or rank transformations were applied to the data. Assumptions of normality and heterogeneity of variance were tested using Levene's Test for Homogeneity of Variance and an Anderson-Darling Normality Test. All length, weight and body condition data passed normality tests, but four of 12 cases did not pass the test for homogeneity of variance and required data transformations to satisfy assumptions. These data were fork length for age 2 and 3 fish, weight for age 2 fish and body condition for age 3 fish.

Temporal changes in population estimates and per capita prey density in both lakes were evaluated using linear regression. Normality and variance in significant regression models were found to be sufficient as assessed with residual plots.

3. Results

3.1. Age validation among readers

The age validation exercise showed a 44% exact agreement and 95% agreement within 1 year between the ages assigned by Ager 1 and Ager 2 (Fig. S1, Table S2), and a 66% exact agreement and 100% within 1 year between Ager 1 and Ager 3 (Fig. S2, Table S2). Additionally, there was no clear bias towards under- or over-ageing between Ager 1 (novice reader) and Ager 3 (experienced OMNDMNRF reader). There was some evidence for a tendency of Ager 2 (experienced DFO reader) to consistently estimate ages that were 1 year greater relative to Ager 1 for younger fish of ages 1–3 (Fig. S1), but there was better agreement in exact age determinations between these two readers for fish estimated to be ages 4 and 5.

3.2. Population estimates

The top-ranked model describing Northern Pike abundance (those typically >250 mm, hereafter 'abundance') in both lakes modeled survival (ϕ) and probability of entry to the population (p_{ent}) as constant but capture probability (p) as time dependent (Table S3, S4). All other models, including the fully time-dependent model, were ranked much lower (20–30 ΔAIC units lower than the top ranked models; Table S3, S4). The mean abundance of Northern Pike exposed to AgNP in Lake 222 was $n = 266$ individuals. Estimates of abundance of Northern Pike in Lake 222 declined very slightly over the entire study period from 2012 to 2017 (linear regression $F_{1,19} = 30.2$, $p < 0.0001$), however the decline over the six-year study was only $n = 40$ individuals, or roughly 15% of the average lake-wide abundance (Fig. 1). Further, 95% confidence intervals of each annual estimate were on average ± 62 individuals, which encompassed any potential observed decline, indicating that the population of Northern Pike in Lake 222 was ultimately stable over the study period. Additionally, 43 Northern Pike were sacrificed to facilitate AgNP determination in organs over the study period from 2012 to 2017 (Martin et al., 2018), which very closely corresponds to the reduction in adult population size observed. No significant trend in population abundance was observed in reference Lake 239 ($F_{1,19} = 3.5$, $p = 0.08$), which averaged 86 individuals over the study period.

3.3. Per capita prey density

Per capita prey (i.e., Yellow Perch) density for Northern Pike declined significantly, as much as 40% after two years of dosing ($F_{1,13} = 259.41$, $p < 0.001$). By contrast, Lake 239 per capita prey density actually increased by as much as 129% over the same time period ($F_{1,13} = 6.0123$, $p = 0.03$; Fig. 2).

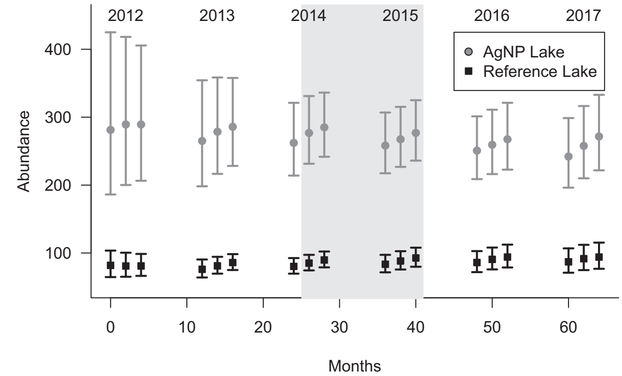

Fig. 1. Estimated abundance (number of individuals ≥250 mm fork length) of Northern Pike in the AgNP addition Lake 222 (silver circles and error bars) and in the reference Lake 239 (black squares and error bars). Error bars represent 95% confidence intervals of estimated abundance. Month on the x-axis indicates number of months from initiation of study; study year is indicated at the top of the figure. The grey polygon represents the period of AgNP additions in Lake 222.

3.4. Fork length-at-age

Mean values of the length-at-age of Northern Pike in the reference Lake 239 followed a variable but overall increasing trend in fork length over time across all age classes. In contrast, the length-at-age data for Northern Pike from AgNP Lake 222 followed a consistently decreasing trend in fork length (Fig. 3 A-D). These declines were especially precipitous through the latter three years of the study, which was the second year of AgNP additions (2015) and the two years of post-addition monitoring (2016 and 2017). Evaluations using ANOVA tests on rank-transformed data for age class 2, log-transformed data for age classes 3 and 4, and original data for age class 5 yielded significant interactions when compared against the corrected α levels (lake*year effect: 2 yr: $F_{3,37} = 3.57$, $p = 0.023$, $\alpha = 0.05$; 3 yr: $F_{3,56} = 4.49$, $p = 0.007$, $\alpha = 0.0375$; 4 yr: $F_{4,81} = 5.61$, $p = 0.0005$, $\alpha = 0.0125$; 5 yr: $F_{5,73} = 4.51$, $p = 0.001$, $\alpha = 0.025$). These

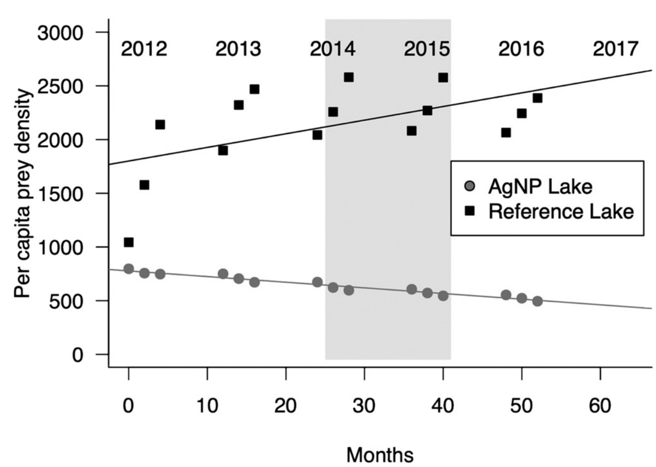

Fig. 2. Per capita prey densities (number of Yellow Perch per number of Northern Pike per ha) in a lake receiving nanosilver additions for two years (Lake 222) and an unmanipulated reference lake (Lake 239). X-axis and grey polygon are the same as in Fig. 1. Trend lines displaying the relationship of per capita prey densities over time are shown.

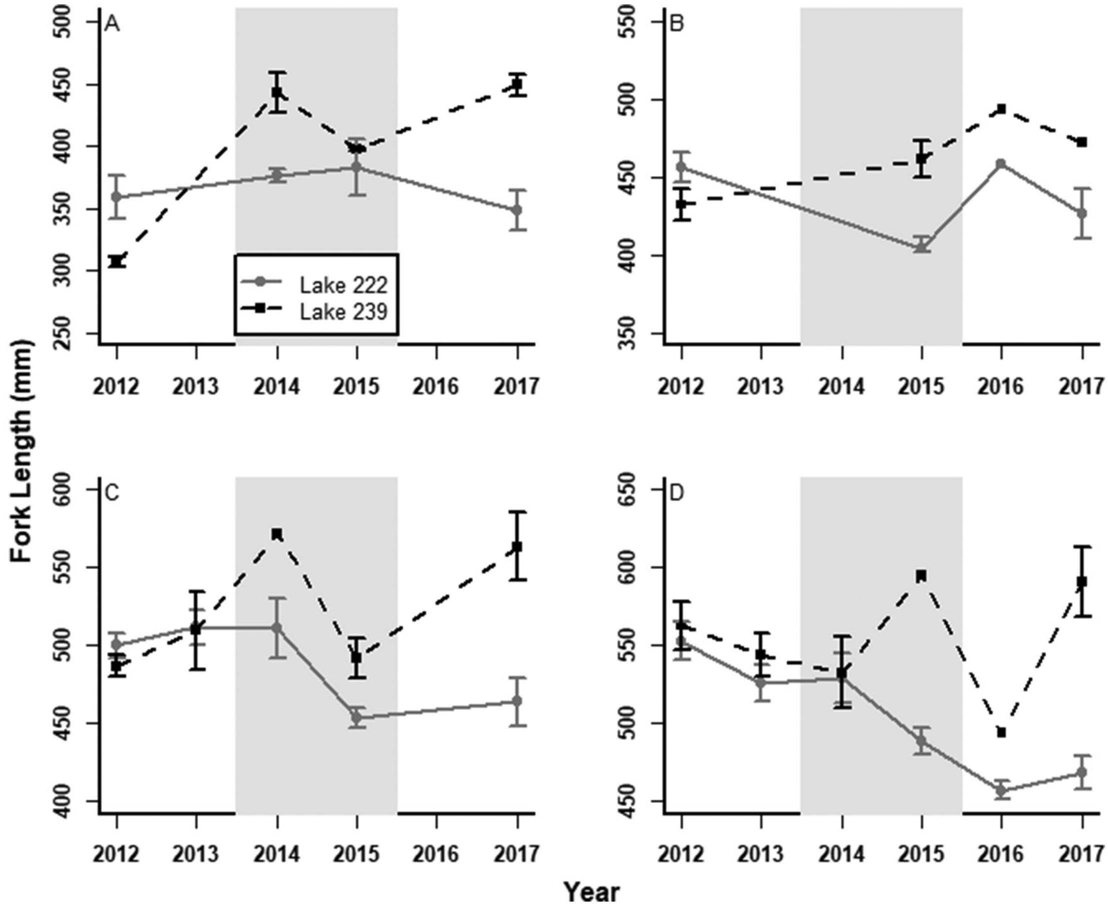

Fig. 3. Fork length (mm) of Northern Pike for age groups across the six-year study period in AgNP addition Lake 222 (black circles, solid line) and in reference Lake 239 (silver triangles, dashed line). (A) 2-year-old fish, (B) 3-year-old fish, (C) 4-year-old fish, (D) 5-year-old fish. Grey polygon represents AgNP addition period as in Fig. 1. Error bars are ± 1 SE.

interactions indicate that changes in fork length over time differed among Northern Pike collected from AgNP Lake 222 and reference Lake 239. This trend was apparent across all age classes but most notable in ages 4 and 5. Mean length of Northern Pike from AgNP Lake 222 in age class 4 decreased by a maximum of 43 mm between 2014 and 2017 (Fig. 3C), and age class 5 fish experienced a mean reduction in length of 96 mm between 2012 and 2016, with no similar observed declines in reference Lake 239 (Fig. 3D).

3.5. Weight-at-age

Fluctuations in weight within age classes over the six-year study period followed similar patterns as those observed in fork length. Northern Pike from reference Lake 239 experienced a variable but overall increase in weight over time within each age class. In contrast, Northern Pike from AgNP Lake 222 experienced an overall decrease in mean weights for all age classes over the study period (Fig. 4 A-D). Pike from age classes 4 and 5 in Lake 222 displayed the greatest reductions in mean weight, dropping by 285 g on average over 2013–2015 within age class 4, and by 445 g on average over 2012–2016 within age class 5 (Fig. 4C, D). Evaluations using ANOVA tests on rank-transformed data for age classes 2 and 5, and log-transformed data on age classes 3 and 4 yielded significant interactions when compared against the corrected α levels (lake*year effect: 2 yr: $F_{3,37} = 3.56$, $p = 0.023$, $\alpha = 0.05$; 3 yr: $F_{3,56} = 4.48$, $p = 0.007$, $\alpha = 0.0375$; 4 yr: $F_{4,81} = 5.31$, $\alpha = 0.0125$; $p = 0.0005$, 5 yr: $F_{5,70} = 4.02$, $p = 0.003$, $\alpha = 0.025$). These interactions indicate that weight-at-age patterns significantly differed over time between Northern Pike in each lake, with declines observed in the lake exposed to AgNP and no similar declines in the reference lake.

3.6. Body condition

Mean body condition across all age classes for Northern Pike collected from both Lake 222 and Lake 239 did not fluctuate dramatically over time during the study (Fig. 5 A-D). ANOVA testing on original data for age class 2 and log transformed data for age classes 3, 4 and 5 for the lake*year interaction yielded p values above $\alpha = 0.05$ for all ages examined. Body condition differed primarily between lakes, with Northern Pike in reference Lake 239 showing consistently higher body condition relative to Northern Pike from Lake 222 (lake effect: 2 yr: $F_{1,37} = 13.77$, $p = 0.0007$, $\alpha = 0.05$; 3 yr: $F_{1,56} = 15.68$, $p = 0.0002$, $\alpha = 0.0375$; 4 yr: $F_{1,81} = 16.33$, $p = 0.0001$, $\alpha = 0.025$; 5 yr: $F_{1,70} = 28.89$, $p < 0.00$, $\alpha = 0.0125$). However, neither Northern Pike from AgNP Lake 222 nor reference Lake 239 demonstrated any significant changes in body condition with time (year effect: 2 yr: $F_{3,37} = 2.25$, $p = 0.099$, 3 yr: $F_{3,56} = 1.61$, $p = 0.198$, 4 yr: $F_{4,81} = 1.21$, $p = 0.315$, 5 yr: $F_{5,70} = 0.851$, $p = 0.519$).

4. Discussion

The size-at-age of Northern Pike collected from AgNP Lake 222 declined significantly in the second year of AgNP additions and in the post-addition phase, whereas no similar change was seen in the reference Lake 239. Changes in size at age were most obvious in age 4 and 5 fish, where age agreement between the experienced and inexperienced agers was highest. Though Northern Pike densities were stable during the study period in both lakes, per capita prey availability declined by 40% in Lake 222 during and following AgNP exposures, while per capita prey availability more than doubled (129% increase) in Lake 239 during the same time period.

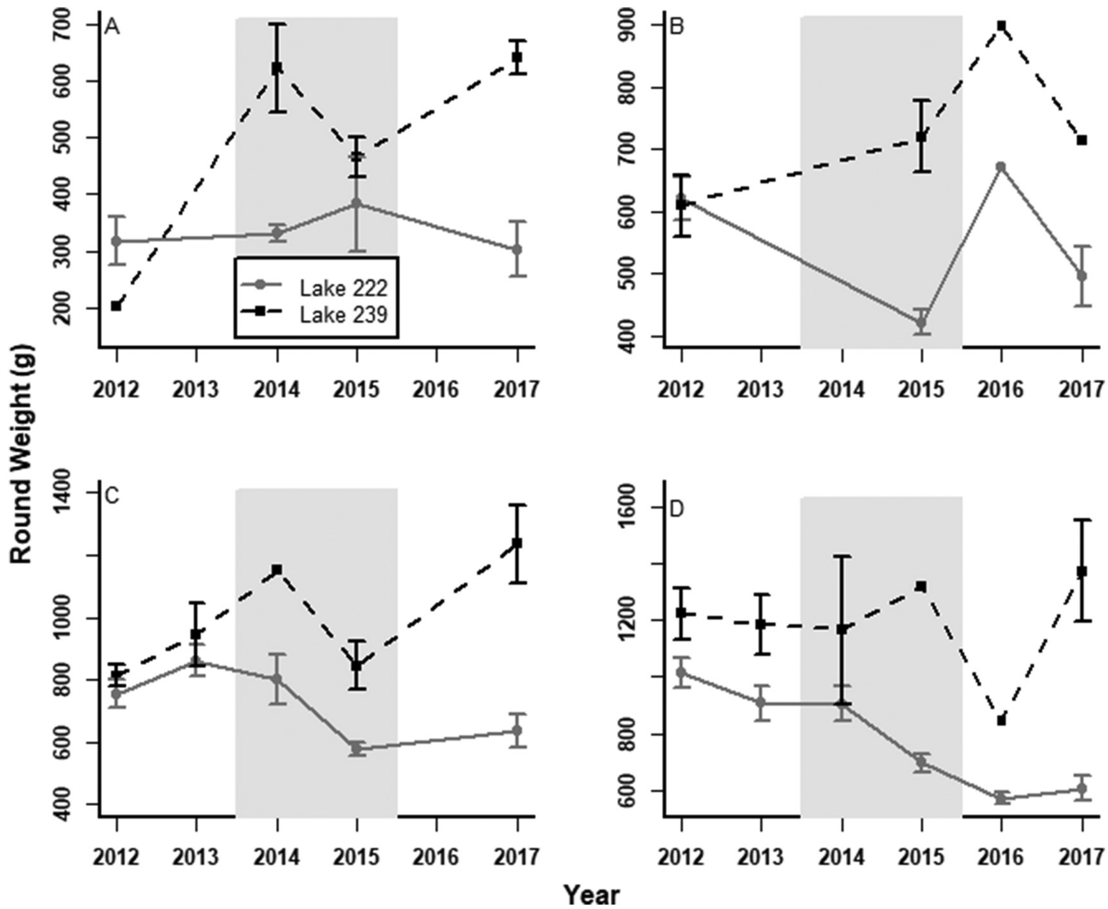

Fig. 4. Weight (g) of Northern Pike for age groups across the six-year study period in AgNP addition Lake 222 (black circles, solid line) and in reference Lake 239 (silver triangles, dashed line). (A) 2-year-old fish, (B) 3-year-old fish, (C) 4-year-old fish, (D) 5-year-old fish. Grey polygon represents AgNP addition period as in Fig. 1. Error bars are ±1 SE.

We hypothesize that these trends can be explained by a combination of: (a) a decline in the abundance of prey fish (i.e., Yellow Perch) that caused reduced growth in Northern Pike through competition among consumers, and (b) metabolic stress caused by exposure to AgNP that directed energy away from growth. Reductions in per capita prey availability are consistent with the observation of reduced growth in Lake 222 Northern Pike, indicating that growth was inhibited as a consequence of increased competition over declining prey resources (Pierce et al., 2003). The abundance of adult Yellow Perch in Lake 222 declined by about 50% between 2012 and 2017, from approximately 14,000 individuals per hectare to 7000 (Hayhurst et al., 2020). In the present study, Northern Pike abundance in Lake 222 was stable over the same time period.

The body condition of Northern Pike in Lake 222 remained stable despite an observed decline in their preferred prey, Yellow Perch. Given the reductions in observed prey availability in the lake receiving AgNP, we would expect declines in body condition, as has been observed in fish from other IISD-ELA experiments where food was dramatically limited as a consequence of experimental manipulations (Kidd et al., 2014; Mills et al., 2011; Rennie et al., 2019). Length-based body condition is known to be sensitive to rapid and large changes in prey abundance or intraspecific competition (Casini et al., 2016; Rennie and Verdon, 2008; Rennie et al., 2019). The response of growth, but not body condition to changes in prey availability indicates that declines in growth may have been a result of reduced growth efficiency due to feeding on alternative prey, the Blacknose Shiner, as a result of reduced availability of Yellow Perch in the lake. While the CPUE of Blacknose Shiner in Lake 222 also declined during the experiment (Fig. 6), it is possible that this decline was due to increased predation by Northern Pike, subsidizing the loss of Yellow Perch. A switch to the smaller prey species, the Blacknose Shiner could explain reduced growth rates in Northern Pike, since reduced growth efficiency in fish has been reported as a consequence of a reduced predator to prey size ratio (e.g., Pazzia et al., 2002; Kennedy et al., 2018).

Reductions in Northern Pike size-at-age may also be due to increased metabolic costs from the high body burdens of Ag, as was suggested for Yellow Perch from the same whole-lake study (Hayhurst et al., 2020). The elimination of metals from fish tissues is a metabolic process mediated by the cysteine-rich protein, metallothionein, which is often induced to higher levels during exposures to metals (Martin et al., 2017). In addition, oxidative stress from exposure to metals results in increased production of glutathione and other cellular antioxidant systems (Bacchetta et al., 2017; Martin et al., 2017). Respiratory stress has also been observed in fish exposed to AgNPs (Bilberg et al., 2010). Thus, physiological costs associated with reducing Ag loads or coping with stress may have limited available energy for growth in Northern Pike. While specific growth rates were not examined in the present study, our results are consistent with a slowing of growth, resulting in smaller size-at-age in fish. In short-term laboratory based exposures of Sheepshead Minnow (*Cyprinodon variegatus*) to AgNP, no reductions in growth rates or size-at-age were observed in fish exposed to a 10 μg L^{-1} nominal concentration of AgNP (Griffitt et al., 2012). Similarly, no changes in growth were observed in Rainbow Trout exposed to 0.3 and 47.6 μg L^{-1} measured concentrations of AgNP (Murray et al., 2017a). However, it was acknowledged in both laboratory studies that longer periods of exposure may be required to assess changes more thoroughly in the growth of fish exposed to environmentally relevant concentrations of AgNP.

Reduced growth in exposed Northern Pike due to direct impacts of exposure to AgNP is consistent with previous studies of the effects of metal exposure on the growth of fishes. For instance, in lakes with high

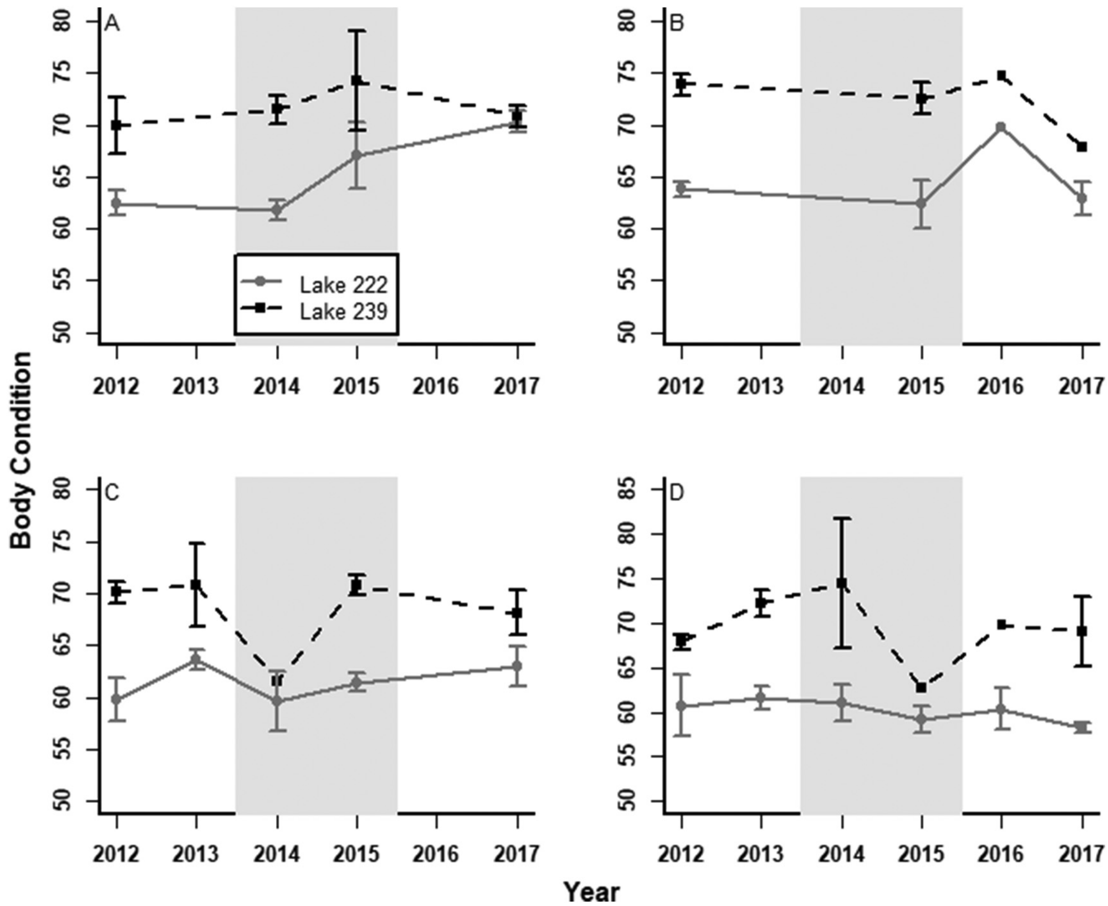

Fig. 5. Body condition of Northern Pike in age groups across the six-year study period in AgNP addition Lake 222 (black circles, solid line) and in reference Lake 239 (silver triangles, dashed line). (A) 2-year-old fish, (B) 3-year-old fish, (C) 4-year-old fish, (D) 5-year-old fish. Grey polygon represents AgNP addition period as in Fig. 1. Error bars are ±1 SE.

concentrations of copper, cadmium and zinc, Yellow Perch grew almost three times slower relative to the growth of Yellow Perch from reference lakes without heavy metal contamination (Sherwood et al., 2000). These authors demonstrated that the rates of food consumption by Yellow Perch from metal contaminated lakes were comparable to those in reference lakes, yet the overall growth in metal-exposed fish was slower, indicating a reduction in conversion efficiency (Sherwood et al., 2000). Part of this

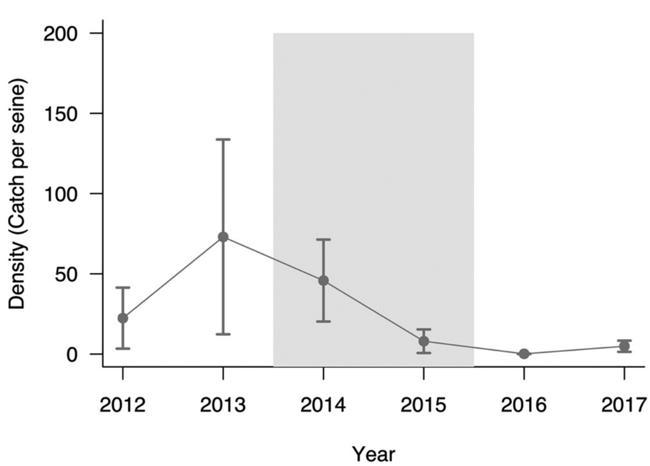

Fig. 6. Summer (June–August) Blacknose Shiner catch per unit effort from seine hauls taken in AgNP addition Lake 222 during a nanosilver exposure experiment. Grey polygon as in previous figures. Error bars are ±1 SE.

reduction was attributed to increased activity costs due to "trophic bottlenecks", where the Yellow Perch failed to switch from benthic to piscivorous diets until much later in life (Sherwood et al., 2000). However, further analysis showed that direct effects of metal exposure also occurred, causing increased production of metallothionein as a detoxification response and decreasing the capacity of the Yellow Perch to secrete cortisol and thyroid hormones, which are key hormones for metabolic regulation (Campbell et al., 2003). In these studies with Yellow Perch, the cellular-level physiological responses reduced the metabolic activity of the fish and likely also impaired conversion efficiency (Campbell et al., 2003; Sherwood et al., 2000).

The data generated in the present study and the available literature indicate that both reduced growth efficiency through reductions in Yellow Perch prey and potentially switching to smaller-sized prey (i.e., Blacknose Shiner), as well as increased metabolic costs associated with coping with high tissue levels of Ag are likely both factors that reduced available energy for growth of Northern Pike. Previous investigations where environmental contaminants have been added using a whole-lake study design have similarly shown that perturbations to the ecosystem will result in both direct and indirect impacts on the ecosystems biological occupants (Kidd et al., 2014; Mills et al., 2011; Rennie et al., 2019).

Observed between-lake differences in body condition are likely due to differences in prey communities between lakes (Casini et al., 2016; Rennie et al., 2019). In Lake 222, there is no high-energy, offshore prey species present, whereas in reference Lake 239, Cisco (*Coregonus artedi*) are present (Table 1). Previous research has indicated that when Cisco are available, Northern Pike and other top predators will shift their diet to target this larger and more energy-dense species (compared to Yellow Perch), providing predatory fish that can make this dietary switch the benefits of reduced foraging costs and maximized energy gain (Kaufman et al., 2006; Kennedy et al., 2018).

Our results reflect the potential impact of AgNP exposures to fish populations in a whole ecosystem at concentrations likely to be encountered in waterways currently or in the near future. The mean measured concentrations of Ag in the lake were 4 μg L^{-1} (Rearick et al., 2018), which is similar to measured values in surface waters of 1.5 μg L^{-1} (Liu et al., 2009) and slightly greater than the high ng L^{-1} concentrations predicted by modelling for surface waters (Blaser et al., 2008; Gottschalk et al., 2009; Sun et al., 2014). The speciation of Ag in the lake was not assessed beyond determining that the average size of particles in the water column was 20 nm and the levels of Ag^+ were very low (Rearick et al., 2018). The oligotrophic/mesotrophic status of Lake 222 means that ionic strength, pH and amounts of suspended particulates are low, while levels of DOC are high, which are all conditions that favor AgNPs remaining in suspension without hetero- or homo-aggregation and with low rates of dissolution to Ag^+ (Unrine et al., 2012). These conditions do not reflect parameters in all surface waters, but the results of the present study provide an indicator of the potential for impacts resulting from long-term exposure of fish populations to AgNP.

Comparisons of ages determined from fin rays of Northern Pike showed that exact agreement between readers was fair and agreement between all readers within-one-year was very strong. There was some evidence of age overestimation in age classes 2 and 3 fish from previously assigned DFO ages. However, bias in this direction should reduce our ability to determine significant declines in size-at-age (as a similarly sized fish is potentially assigned to an earlier age class during AgNP exposure compared with pre-exposure). Despite this potential bias, we still observed significant declines in size-at-age for these age classes. Furthermore, in age classes 4 and 5, where overall agreement was better between the novice and DFO-assigned ages, we observed our clearest trends in growth declines, again suggesting our results were not subject to potential between-reader variation. Using a similar sample size, the ageing ability of the novice ager in our study was comparable to or better than reported assessments for other species (Rude et al., 2013).

The previous observations of impacts on the population of Yellow Perch in Lake 222 reported by Hayhurst et al. (2020) are consistent with evidence of impacts observed in Northern Pike in the present study. Results from the current study indicate the potential for indirect effects in Northern Pike related to changes in lower trophic levels, and the numbers of Yellow Perch specifically. However, direct effects from exposures to AgNPs may also have been a factor in the reduced growth of Northern Pike. Continued suppression of the growth of Northern Pike up to two years after cessation of AgNP additions indicates that the impacts extended well beyond the period of AgNP exposure. Similarly, other whole-lake experiments have demonstrated significant lags in terms of the duration of impacts, extending years to decades beyond the period of manipulation (Kidd et al., 2014; Mills et al., 2000; Rennie et al., 2019). This may be particularly true in the case of AgNP contamination because the material that settles to the sediments may be an ongoing source of “in place” contamination (Lowry et al., 2012). The capacity of AgNP to slowly release Ag^+, which is the same attribute that makes it desirable as an antimicrobial agent, highlights the potential for prolonged impacts in aquatic ecosystems.

Author contributions

The manuscript was written through contributions of all authors. CDM and MDR were part of the team that secured funding conceptualized and designed the whole-lake experiment; MDR supervised this aspect of the project. LDH and MDR conducted field sampling efforts and collected data; LDH and BDS curated data; BDS aged fish, and PCTD served as a second ager for validation testing and provided training and expertise in age structure preparation and age determination. BDS, LDH, and MDR analyzed data and prepared figures. BDS prepared the original draft of the manuscript; LDH and MDR wrote subsequent sections of the manuscript; BDS, MDR, LDH and CDM edited the manuscript. All authors have reviewed and given approval to the final version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.156219.

References

Akter, M., Sikder, M.T., Rahman, M.M., Ullah, A.K.M.A., Hossain, K.F.B., Banik, S., Hosokawa, T., Saito, T., Kurasaki, M., 2018. A systematic review on silver nanoparticles-induced cytotoxicity: physicochemical properties and perspectives. J. Adv. Res. https://doi.org/10.1016/j.jare.2017.10.008.

Amstrup, S.C., McDonald, T.L., Manly, B.F.J., 2005. Handbook of Capture-recapture Analysis. Princeton University Press, Princeton, New Jersey.

Arnason, N.A., Schwarz, C.J., 1999. Using popan-5 to analyse banding data. Bird Study 46, S157–S168. https://doi.org/10.1080/00063659909477242.

Asghari, S., Johari, S.A., Moon, M.C., Choi, H.J., Lee, J.H., Yu, I.J., Jeon, Y.B., Kim, Y.S., 2012. Toxicity of various silver nanoparticles compared to silver ions in *Daphnia magna*. J. Nanobiotechnol. 10, 14. https://doi.org/10.1186/1477-3155-10-14.

Bacchetta, C., Ale, A., Simoniello, M.F., Gervasio, S., Davico, C., Rossi, A.S., Desimone, M.F., Poletta, G., López, G., Monserrat, J.M., Cazenave, J., 2017. Genotoxicity and oxidative stress in fish after a short-term exposure to silver nanoparticles. Ecol. Indic. 76, 230–239. https://doi.org/10.1016/J.ECOLIND.2017.01.018.

Benn, T.M., Westerhoff, P., 2008. Nanoparticle silver released into water from commercially available sock fabrics. Environ. Sci. Technol. 42, 7025–7026. https://doi.org/10.1021/es801501j.

Benn, T., Cavanagh, B., Hristovski, K., Posner, J.D., Westerhoff, P., 2010. The release of nanosilver from consumer products used in the home. J. Environ. Qual. 39, 1875–1882. https://doi.org/10.2134/jeq2009.0363.

Bilberg, K., Malte, H., Wang, T., Baatrup, E., 2010. Silver nanoparticles and silver nitrate cause respiratory stress in Eurasian Perch (*Perca fluviatilis*). Aquat. Toxicol. 96, 159–165. https://doi.org/10.1016/j.aquatox.2009.10.019.

Blaser, S.A., Scheringer, M., MacLeod, M., Hungerbühler, K., 2008. Estimation of cumulative aquatic exposure and risk due to silver: contribution of nano-functionalized plastics and textiles. Sci. Total Environ. 390, 396–409. https://doi.org/10.1016/j.scitotenv.2007.10.010.

Campana, S.E., 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. J. Fish Biol. 59, 197–242. https://doi.org/10.1006/jfbi.2001.1668.

Campbell, P.G.C., Hontela, A., Rasmussen, J.B., Giguère, A., Kraemer, L., Kovesces, J., Lacroix, A., Levesque, H., Gravel, A., 2003. Human and ecological risk assessment: differentiating between direct (physiological) and food-chain mediated (bioenergetic) effects on fish in metal-impacted lakes. Hum. Ecol. Risk. Assess. 9, 37–41. https://doi.org/10.1080/713610012.

Casini, M., Käll, F., Hansson, M., Plikshs, M., Baranova, T., Karlsson, O., Lundström, K., Neuenfeldt, S., Gårdmark, A., Hjelm, J., 2016. Hypoxic areas, density-dependence and food limitation drive the body condition of a heavily exploited marine fish predator. R. Soc. Open Sci. 3. https://doi.org/10.1098/rsos.160416.

Conine, A.L., Frost, P.C., 2017. Variable toxicity of silver nanoparticles to Daphnia magna: effects of algal particles and animal nutrition. Ecotoxicology 26, 118–126. https://doi.org/10.1007/s10646-016-1747-2.

Conine, A.L., Rearick, D.C., Paterson, M.J., Xenopoulos, M.A., Frost, P.C., 2018. Addition of silver nanoparticles has no long-term effects on natural phytoplankton. Limnol. Oceanogr. 3, 311–319.

Farmen, E., Mikkelsen, H.N., Evensen, Einset, J., Heier, L.S., Rosseland, B.O., Salbu, B., Tollefsen, K.E., Oughton, D.H., 2012. Acute and sub-lethal effects in juvenile Atlantic Salmon exposed to low μg/L concentrations of Ag nanoparticles. Aquat. Toxicol. 108, 78–84. https://doi.org/10.1016/j.aquatox.2011.07.007.

Fletcher, N.D., Lieb, H.C., Mullaugh, K.M., 2019. Stability of silver nanoparticle sulfidation products. Sci. Total Environ. 648, 854–860. https://doi.org/10.1016/j.scitotenv.2018.08.239.

Forsman, A., Tibblin, P., Berggren, H., Nordahl, O., Koch-Schmidt, P., Larsson, P., 2015. Pike (Esox lucius) as an emerging model organism for studies in ecology and evolutionary biology: a review. J. Fish Biol. 87, 472–479. https://doi.org/10.1111/jfb.12712.

Furtado, L.M., Norman, B.C., Xenopoulos, M.A., Frost, P.C., Metcalfe, C.D., Hintelmann, H., 2015. Environmental *Fate of Silver Nanoparticles in Boreal Lake Ecosystems*. Environ. Sci. Technol. 49 (14), 8441–8450. https://doi.org/10.1021/acs.est.5b01116.

Garcia-Reyero, N., Thornton, C., Hawkins, A.D., Escalon, L., Kennedy, A.J., Steevens, J.A., Willett, K.L., 2015. Assessing the exposure to nanosilver and silver nitrate on fathead minnow gill gene expression and mucus production. Environ. Nanotechnol.Monit. Manag. 4, 58–66. https://doi.org/10.1016/j.enmm.2015.06.001.

Glass, W.R., Corkum, L.D., Mandrak, N.E., 2011. Pectoral fin ray aging: an evaluation of a non-lethal method for aging gars and its application to a population of the threatened Spotted Gar. Environ. Biol. Fish 90, 235–242. https://doi.org/10.1007/s10641-010-9735-5.

Gottschalk, F., Sonderer, T., Scholz, R.W., Nowack, B., 2009. Modeled environmental concentrations of engineered nanomaterials (TiO2, ZnO, Ag, CNT, fullerenes) for different regions. Environ. Sci. Technol. 43, 9216–9222. https://doi.org/10.1021/ES9015553/SUPPL_FILE/ES9015553_SI_001.PDF.

Gottschalk, F., Sun, T., Nowack, B., 2013. Environmental concentrations of engineered nanomaterials: review of modeling and analytical studies. Environ. Pollut. 181, 287–300. https://doi.org/10.1016/j.envpol.2013.06.003.

Green, R., 1979. Sampling Design and Statistical Methods for Environmental Biologists. Wiley Interscience, Chichester UK.

Griffitt, R.J., Brown-Peterson, N.J., Savin, D.A., Manning, C.S., Boube, I., Ryan, R.A., Brouwer, M., 2012. Effects of chronic nanoparticulate silver exposure to adult and juvenile Sheepshead Minnows (*Cyprinodon variegatus*). Environ. Toxicol. Chem. 31, 160–167. https://doi.org/10.1002/etc.709.

Harvey, B., 2009. A biological synopsis of Northern Pike (*Esox lucius*). Can. Manuscr. Rep. Fish. Aquat. Sci. 2885 31p.

Hayhurst, L.D., Martin, J.D., Wallace, S.J., Langlois, V.S., Xenopoulos, M.A., Metcalfe, C.D., Rennie, M.D., 2020. Multi-level responses of Yellow Perch (*Perca flavescens*) to a whole-lake nanosilver addition study. Arch. Environ. Contam. Toxicol. 79, 283–297. https://doi.org/10.1007/s00244-020-00764-5.

Johnson, A.C., Jürgens, M.D., Lawlor, A.J., Cisowska, I., Williams, R.J., 2014. Particulate and colloidal silver in sewage effluent and sludge discharged from British wastewater treatment plants. Chemosphere 112, 49–55. https://doi.org/10.1016/j.chemosphere.2014.03.039.

Kaegi, R., Voegelin, A., Sinnet, B., Zuleeg, S., Hagendorfer, H., Burkhardt, M., Siegrist, H., 2011. Behavior of metallic silver nanoparticles in a pilot wastewater treatment plant. Environ. Sci. Technol. 45, 3902–3908. https://doi.org/10.1021/es1041892.

Kaufman, S.D., Gunn, J.M., Morgan, G.E., Couture, P., 2006. Muscle enzymes reveal Walleye (Sander vitreus) are less active when larger prey (Cisco, *Coregonus artedi*) are present. Can. J. Fish. Aquat. Sci. 63, 970–979. https://doi.org/10.1139/F06-004.

Kennedy, P.J., Bartley, T.J., Gillis, D.M., McCann, K.S., Rennie, M.D., 2018. Offshore prey densities facilitate similar life history and behavioral patterns in two distinct aquatic apex predators, Northern Pike and Lake Trout. Trans. Am. Fish. Soc. 147, 972–995. https://doi.org/10.1002/tafs.10090.

Kidd, K.A., Paterson, M.J., Rennie, M.D., Podemski, C.L., Findlay, D.L., Blanchfield, P.J., Liber, K., 2014. Direct and indirect responses of a freshwater food web to a potent synthetic oestrogen. Philos. Trans. R. Soc. B Biol. Sci. 369, 20130578. https://doi.org/10.1098/rstb.2013.0578.

Kuhnlein, H.V., Humphries, M.M., 2017. Traditional Animal Foods of Indigenous Peoples of Northern North America: http://traditionalanimalfoods.org/ [WWW Document]. URLCent. Indig. Peoples' Nutr. Environ. McGill Univ. Montr (accessed 1.30.22). http://traditionalanimalfoods.org/fish/freshwater/page.aspx?id=6404.

Laake, J., Rakhimberdiev, E., Augustine, B., Turek, D., McClintock, B., Collier, B., Rotella, J., Pavlacky, D., Paul, A., Eberhart-Phillips, L., Ivan, J., Wood, C., 2019. R Code for Mark Analysis. https://cran.r-project.org/web/packages/RMark/RMark.pdf.

Laban, G., Nies, L.F., Turco, R.F., Bickham, J.W., Sepúlveda, M.S., 2010. The effects of silver nanoparticles on fathead minnow (*Pimephales promelas*) embryos. Ecotoxicology 19, 185–195. https://doi.org/10.1007/s10646-009-0404-4.

Little, D., Maclellan, S.E., Charles, K., 2012. A guide to processing fin-rays for age determination. Can. Tech. Rep. Fish. Aquat. Sci. 3002 19p.

Liu, J.F., Chao, J.B., Liu, R., Tan, Z.Q., Yin, Y.G., Wu, Y., Jiang, G.Bin, 2009. Cloud point extraction as an advantageous preconcentration approach for analysis of trace silver nanoparticles in environmental waters. Anal. Chem. 81, 6496–6502. https://doi.org/10.1021/ac900918e.

Lowry, G.V., Espinasse, B.P., Badireddy, A.R., Richardson, C.J., Reinsch, B.C., Bryant, L.D., Wiesner, M.R., 2012. Long-term transformation and fate of manufactured Ag nanoparticles in a simulated large scale freshwater emergent wetland. Environ. Sci. Technol. 46, 7027–7036. https://doi.org/10.1021/es204608d.

Malette, M.D., Morgan, G.E., 2005. Provincial summary of northern pike life history characteristics based on Ontario's fall walleye index netting (FWIN) program 1993 to 2002. Coop. Freshw. Ecol. Unit Dep. Biol. Laurentian Univ., Sudbury, Ontario P3E 2C6 141.

Margenau, T.L., Rasmussen, P.W., Kampa, J.M., 1998. Factors affecting growth of Northern Pike in small northern Wisconsin lakes. North Am. J. Fish. Manag. 18, 625–639. https://doi.org/10.1577/1548-8675(1998)018<0625:fagonp>2.0.co;2.

Martin, J.D., Colson, T.L.L., Langlois, V.S., Metcalfe, C.D., 2017. Biomarkers of exposure to nanosilver and silver accumulation in Yellow Perch (*Perca flavescens*). Environ. Toxicol. Chem. 36, 1211–1220. https://doi.org/10.1002/etc.3644.

Martin, J.D., Frost, P.C., Hintelmann, H., Newman, K., Paterson, M.J., Hayhurst, L., Rennie, M.D., Xenopoulos, M.A., Yargeau, V., Metcalfe, C.D., 2018. Accumulation of silver in Yellow Perch (*Perca flavescens*) and Northern Pike (*Esox lucius*) from a lake dosed with nanosilver. Environ. Sci. Technol. 52, 11114–11122. https://doi.org/10.1021/acs.est.8b03146.

McGillicuddy, E., Murray, I., Kavanagh, S., Morrison, L., Fogarty, A., Cormican, M., Dockery, P., Prendergast, M., Rowan, N., Morris, D., 2017. Silver nanoparticles in the environment: sources, detection and ecotoxicology. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2016.10.041.

Mills, K.H., Chalanchuk, S.M., 2004. The fin-ray method of aging Lake Whitefish. Ann. Zool. Fennici 41, 215–223.

Mills, K.H., Chalanchuk, S.M., Allan, D.J., 2000. Recovery of fish populations in Lake 223 from experimental acidification. Can. J. Fish. Aquat. Sci. 57, 192–204. https://doi.org/10.1139/f99-186.

Mills, K.H., Chalanchuk, S.M., Allan, D.J., 2011. Recovery of fish populations in Lake 223 from experimental acidification. Can. J. Fish. Aquat. Sci. 57, 192–204. https://doi.org/10.1139/f99-186.

Murray, L., Rennie, M.D., Enders, E.C., Pleskach, K., Martin, J.D., 2017a. Effect of nanosilver on cortisol release and morphometrics in rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. 36, 1606–1613. https://doi.org/10.1002/etc.3691.

Murray, L., Rennie, M.D., Svendsen, J.C., Enders, E.C., 2017b. Effect of nanosilver on metabolism in rainbow trout (*Oncorhynchus mykiss*): an investigation using different respirometric approaches. Environ. Toxicol. Chem. 36, 2722–2729. https://doi.org/10.1002/etc.3827.

Oele, D.L., Lawson, Z.J., McIntyre, P.B., 2015. Precision and bias in aging northern pike: comparisons among four calcified structures. North Am. J. Fish. Manag. 35, 1177–1184. https://doi.org/10.1080/02755947.2015.1099579.

Pazzia, I., Trudel, M., Ridgway, M., Rasmussen, J.B., 2002. Influence of food web structure on the growth and bioenergetics of lake trout (*Salvelinus namaycush*). Can. J. Fish. Aquat. Sci. 59, 1593–1605. https://doi.org/10.1139/F02-128.

Pierce, R.B., Tomcko, C.M., Margenau, T.L., 2003. Density dependence in growth and size structure of Northern Pike populations. North Am. J. Fish. Manag. 8675, 331–339. https://doi.org/10.1577/1548-8675(2003)023<0331.

Quinn, G.P., Keough, M.J., 2002. Experimental Design and Data Analysis for Biologists. Cambridge University Press, New York.

R Core Team, 2021. R: a language and environment for statistical computing. URLR Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Rearick, D.C., Telgmann, L., Hintelmann, H., Frost, P.C., Xenopoulos, M.A., 2018. Spatial and temporal trends in the fate of silver nanoparticles in a whole-lake addition study. PLoS One 13, 1–18. https://doi.org/10.1371/journal.pone.0201412.

Reidy, B., Haase, A., Luch, A., Dawson, K.A., Lynch, I., 2013. Mechanisms of silver nanoparticle release, transformation and toxicity: a critical review of current knowledge and recommendations for future studies and applications. Materials (Basel) 6, 2295–2350. https://doi.org/10.3390/ma6062295.

Rennie, M.D., Verdon, R., 2008. Development and evaluation of condition indices for the Lake Whitefish. North Am. J. Fish. Manag. 28, 1270–1293. https://doi.org/10.1577/m06-258.1.

Rennie, M.D., Kennedy, P.J., Mills, K.H., Rodgers, C.M.C., Charles, C., Hrenchuk, L.E., Chalanchuk, S., Blanchfield, P.J., Paterson, M.J., Podemski, C.L., 2019. Impacts of freshwater aquaculture on fish communities: a whole-ecosystem experimental approach. Freshw. Biol. 64, 870–885. https://doi.org/10.1111/fwb.13269.

Rude, N.P., Hintz, W.D., Norman, J.D., Kanczuzewski, K.L., Yung, A.J., Hofer, K.D., Whitledge, G.W., 2013. Using pectoral fin rays as a non-lethal aging structure for Smallmouth Bass: precision with otolith age estimates and the importance of reader experience. J. Freshw. Ecol. 28, 199–210. https://doi.org/10.1080/02705060.2012.738253.

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., van Aerle, R., Tyler, C.R., 2010. Effects of aqueous exposure to silver nanoparticles of different sizes in rainbow trout. Toxicol. Sci. 115, 521–534. https://doi.org/10.1093/toxsci/kfq076.

Sherwood, G.D., Rasmussen, J.B., Rowan, D.J., Brodeur, J., Hontela, A., 2000. Bioenergetic costs of heavy metal exposure in Yellow Perch (*Perca flavescens*): in situ estimates with a radiotracer (137Cs) technique. Can. J. Fish. Aquat. Sci. 57, 441–450. https://doi.org/10.1139/f99-268.

Shevlin, D., O'Brien, N., Cummins, E., 2018. Silver engineered nanoparticles in freshwater systems – likely fate and behaviour through natural attenuation processes. Sci. Total Environ. 621, 1033–1046. https://doi.org/10.1016/j.scitotenv.2017.10.123.

Smokorowski, K.E., Randall, R.G., 2017. Cautions on using the before-after-control-impact design in environmental effects monitoring programs. FACETS 2, 212–232. https://doi.org/10.1139/facets-2016-0058.

Sun, T.Y., Gottschalk, F., Hungerbühler, K., Nowack, B., 2014. Comprehensive probabilistic modelling of environmental emissions of engineered nanomaterials. Environ. Pollut. 185, 69–76. https://doi.org/10.1016/j.envpol.2013.10.004.

Unrine, J.M., Colman, B.P., Bone, A.J., Gondikas, A.P., Matson, C.W., 2012. Biotic and abiotic interactions in aquatic microcosms determine fate and toxicity of Ag nanoparticles. Part

1.Aggregation and dissolution. Environ. Sci. Technol. 46, 6915–6924. https://doi.org/10.1021/ES204682Q/SUPPL_FILE/ES204682Q_SI_002.PDF.

Venturelli, P.A., Tonn, W.M., 2006. Diet and growth of Northern Pike in the absence of prey fishes: initial consequences for persisting in disturbance-prone lakes. Trans. Am. Fish. Soc. 135, 1512–1522. https://doi.org/10.1577/t05-228.1.

Vincent, J.L., Paterson, M.J., Norman, B.C., Gray, E.P., Ranville, J.F., Scott, A.B., Frost, P.C., Xenopoulos, M.A., 2017. Chronic and pulse exposure effects of silver nanoparticles on natural lake phytoplankton and zooplankton. Ecotoxicology 26, 502–515. https://doi.org/10.1007/s10646-017-1781-8.