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Consumption and activity decline in Northern Pike (*Esox lucius*) during and after silver nanoparticle addition to a lake

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ABSTRACT

Silver nanoparticles (AgNPs) are antimicrobial additives in many consumer products with high potential for release into aquatic ecosystems. Though AgNPs have been shown to have negative impacts on fish in laboratory experiments, these effects are rarely observed at ecologically relevant concentrations or in situ in field settings. To evaluate ecosystem-level effects of this contaminant, AgNPs were added to a lake at the IISD Experimental Lakes Area (IISD-ELA) during 2014 and 2015. Mean total silver (TAg) concentrations in the water column were 4 μg L^{-1} during additions. The growth of Northern Pike (*Esox lucius*) declined, and their primary prey, Yellow Perch (*Perca flavescens*) became less abundant after AgNP exposure. Here, we used a combined contaminant-bioenergetics modeling approach to show that individual activity and both individual and population-level consumption of Northern Pike declined significantly in the lake dosed with AgNPs, which, combined with other evidence, suggests that observed declines in body size were likely a result of indirect effects (i.e., reduced prey availability). Further, we found the contaminant-bioenergetics approach was sensitive to modelled elimination rates of mercury, overestimating consumption and activity by 43% and 55%, respectively, when using the mercury elimination rate commonly used in these models versus field-derived estimates for this species. This study contributes to the growing evidence of potentially long-term negative impacts on fish from chronic exposure to environmentally relevant concentrations of AgNPs in a natural setting.

1. Introduction

Silver nanoparticles (AgNPs) are added to a range of consumer products as an antibacterial agent, being the most common nanomaterial among 1814 consumer products evaluated in the Nanotechnology Consumer Products Inventory (Vance et al., 2015). AgNPs are added to textiles, cosmetics, health products, electronics, and water filtration products (Vance et al., 2015). The increasingly common addition of AgNPs to sports clothing, underwear, and socks results in the release of nanoparticles and their transformation products to wastewater streams from fabrics during washing (Benn and Westerhoff 2008).

Once released into the water column, AgNPs may remain in the water column in particulate or colloidal form, agglomerate into larger particles that ultimately settle, or they may be taken up by organisms like periphyton (Furtado et al., 2015). Under oxic conditions, AgNPs can oxidize to release silver ions (Ag^+; Dobias and Bernier-Latmani 2013), which are known to be more toxic than AgNPs (Bilberg et al., 2012; Martin et al., 2017). Concentrations of AgNPs in surface waters have been estimated in the low ng·L^{-1} range (Gottschalk et al., 2013) and as high as 0.3 to 1.5 μg L^{-1} (Blaser et al., 2008; Liu et al., 2009).

Negative impacts of AgNPs on aquatic organisms—particularly fish—have been demonstrated in several laboratory studies. Rainbow Trout (*Oncorhynchus mykiss*) cellular metabolism and cell membrane integrity was impaired after AgNP exposure (Farkas et al., 2010). Apoptosis, DNA damage, and oxidative stress were observed in Zebrafish (*Danio rerio*) exposed to AgNPs (Choi et al., 2010). Further, exposures of Yellow Perch (*Perca flavescens*) to both AgNPs and Ag^+ caused oxidative stress and induced expression of metallothionein (Martin et al., 2017). However, much of this previous research was conducted with high exposure concentrations over relatively short durations in a laboratory environment. By comparison, lab exposures of Rainbow Trout to AgNPs at environmentally relevant concentrations for 28 days caused an increase in cortisol levels (Murray et al., 2017a) but did not affect resting metabolic rates (Murray et al., 2017b). Recent work has also shown

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early life stages of Northern Pike (*Esox lucius*) to be more sensitive to low-level AgNP exposure than either Rainbow Trout or Lake Trout (*Salvelinus namaycush*; Schultz et al., 2021).

To assess the impacts of AgNP exposure at a whole ecosystem level, an experiment conducted at the IISD Experimental Lakes Area (IISD-ELA) dosed Lake 222 with a total of 15 kg of AgNPs over the course of two consecutive ice-free field seasons. The AgNPs were rapidly distributed throughout the water column, achieving very low concentrations of dissolved Ag (dAg; Rearick et al., 2018; Martin et al., 2018). Northern Pike and Yellow Perch rapidly accumulated Ag in their liver, gills, muscle, and kidneys (Martin et al., 2018). Multiple oxidative stress biomarkers of Yellow Perch were down-regulated, accompanied by a decrease in individual rates of total metabolism, consumption, and size-at-age in this species (Hayhurst et al., 2020). Yellow Perch density declined by approximately 40% and their gross (population-level) consumption decreased by half (Hayhurst et al., 2020). Northern Pike mass- and fork length-at-age also declined following AgNP additions (Slongo et al., 2022). While the population density of Northern Pike was stable during and after AgNP exposure, per capita prey densities declined significantly (Slongo et al., 2022). Potential direct effects of AgNP exposure include oxidative stress (Hayhurst et al., 2020), impaired olfaction (Bilberg et al., 2011), or embryotoxicity (Schulz et al. 2021), but indirect effects from changes in the food web (e.g., reduced prey fish abundance) that may have contributed to the observed declines in Northern Pike size-at-age in the dosed lake remain unresolved.

In the present study, we hypothesized that declines in active metabolism and consumption resulting from reduced prey availability could explain reduced growth of Northern Pike during and after the period of AgNP additions in Lake 222. Mercury mass balance models, when combined with bioenergetics models, can provide field estimates of individual activity and consumption in fish (e.g., Rennie et al., 2005). However, research has shown that estimates from these models are sensitive to mercury (Hg) elimination rates, and updated elimination sub-models have been proposed based on more current literature (Madenjian et al., 2021). For instance, Van Walleghem et al. (2013) observed elimination rates in Northern Pike in the field that were 2.7 times slower than those estimated by the chronic Hg exposure model reported in Trudel and Rasmussen (1997). Similar discrepancies have been observed in other fish species where Hg elimination rates have been explicitly estimated (Madenjian et al., 2021).

The primary objective of this study was to evaluate the impact of AgNP exposure on the acquisition and allocation of energy in Northern Pike using a bioenergetics framework. Specifically, we estimated changes in individual consumption, activity, growth, and conversion efficiency of Northern Pike in a lake dosed with AgNPs (Lake 222), compared to Northern Pike from a nearby reference lake (Lake 239). While Yellow Perch from Lake 222 showed evidence of oxidative stress from exposure to AgNPs, as well as decreased prey consumption, the food conversion efficiency of these fish was unchanged (Hayhurst et al., 2020). Increased stress from direct toxic effects is expected to lead to an increase in total metabolic costs (Griboff et al., 2014), which in turn is likely to lead to reduced conversion efficiency of food to soma (Sherwood et al., 2000). In contrast, previous work demonstrated *reduced* metabolic costs and *no* differences in conversion efficiency in Yellow Perch after AgNP exposure, despite evidence of oxidative stress. An indirect response to reduced food availability (with metabolic costs declining isometrically with reduced consumption) could explain this seemingly counterintuitive observation (i.e., reduced metabolic costs despite increased oxidative stress), potentially overwhelming the expected direct impacts of AgNP exposure on fish metabolic rates. As such, we sought to evaluate patterns in individual-level bioenergetics (i.e., consumption, activity, conversion efficiency) as well as population-level metrics (i.e., gross consumption) in Northern Pike populations from this same experiment to better evaluate the potential for direct versus indirect impacts of AgNP exposure. Our second objective was to compare bioenergetic models implementing different Hg elimination rates, hypothesizing that individual-level estimates of both consumption and activity would be overestimated using traditional Hg elimination rates compared to rates that better reflect Hg elimination under field conditions for this species.

2. Methods

2.1. Nanosilver additions

In a multi-disciplinary study conducted at the IISD-ELA, AgNPs were added in suspension to experimental Lake 222. This lake is approximately 16 ha in surface area, 7.2×10^5 m^3 in volume, 6.3 m deep at its maximum depth, is oligotrophic and thermally stratifies in summer (Rearick et al., 2018; Slongo et al., 2022). Suspensions of AgNPs capped with polyvinylpyrrolidone (PVP) were added during the ice-free seasons of 2014 (9 kg added) and 2015 (6 kg added; Martin et al., 2018), achieving an average TAg concentration of 4 μg L^{-1} throughout the water column in the lake (Rearick et al., 2018), very close to estimated concentrations in the environment (Liu et al., 2009). Additional details on the additions of AgNPs and the fate of AgNP in the lake are provided by Rearick et al. (2018) and Martin et al. (2018). Northern Pike were monitored in experimental Lake 222 as well as in reference Lake 239, which has similar components in the fish community as Lake 222 (Slongo et al., 2022). Northern Pike were collected pre-addition in 2012 and 2013, during AgNP additions in 2014 and 2015, and post-addition during 2016 and 2017.

Fish were captured using a combination of angling and trap netting, using standard capture methods as described elsewhere (Hayhurst et al., 2020; Slongo et al., 2022). Mass and length measurements were performed in the field. Muscle biopsies were typically collected for analysis of Hg, but a small number of fish were euthanized by an overdose of tricaine methanesulfonate (TMS) for analysis of tissues. All tissues were frozen for laboratory analysis of Hg and energy density, and fin rays were taken for age determination (Hayhurst et al., 2020). 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).

2.2. Bioenergetics modelling

We used a mercury mass balance model (MMBM) in combination with a bioenergetics model to estimate the bioenergetic parameters of consumption, activity, growth, and efficiency of food conversion to growth for individual Northern Pike. The bioenergetics model and its sub-equations were as described in Fish Bioenergetics 4.0 (Deslauriers et al., 2017). Bioenergetics parameters for Northern Pike were from Bevelhimer et al. (1985), as reported by Deslauriers et al. (2017). An equation for the daily application of the bioenergetics model to individuals/cohorts of individuals is:

$$W_t = W_0 + \left[\left(C_t \cdot ED_{prey} \right) - \left(F + U + R_T \right) \right] / ED_{fish} \quad (1)$$

where W_t is the mass of the fish (g) at time t, W_0 is the mass of the fish (g) at time 0, C_t is consumption (J·day^{-1}) at time t, ED_{prey} is the prey energy density (J·g^{-1}), F is energy lost to egestion (J·day^{-1}) and is calculated as a constant multiplied by consumption, U is energy lost to excretion (J·day^{-1}) and is calculated as a constant multiplied by the difference between consumption and egestion, R_T is energy lost to metabolism (J·day^{-1}), and ED_{fish} is the fish energy density (J·g^{-1}).

The equation used in the bioenergetics model for daily rate of consumption is:

$$C_t = C_{max} \cdot p \cdot f(T) \quad (2)$$

where C_t is consumption (J·day^{-1}) at time t, C_{max} is the maximum daily consumption (J·day^{-1}) and is an empirically derived function of mass, p is the fraction of C_{max} that a fish consumes, and $f(T)$ is a temperature dependence function that describes the effects on consumption of deviations from the optimal temperature for feeding.

Energy lost to metabolism can be expressed as:

$$R_T = ACT{\cdot}R_s + R_d \tag{3}$$

where R_T is the total energy lost to metabolism (J·day^{-1}), ACT is a multiple of R_s that describes losses to active metabolism (unitless; $1 \leq ACT \leq \infty$), R_s is energy lost to standard metabolism (J·day^{-1}), and R_d is energy lost to specific dynamic action (J·day^{-1}). We adopted this formulation of metabolic costs for Northern Pike to solve for active metabolism explicitly as a multiple of standard metabolism, as opposed to the swimming speed-dependent function to describe activity which is based on several assumptions regarding Northern Pike swimming behavior that may not necessarily be globally applicable.

A mass loss to spawning was included on April 30. The mass after spawning was calculated as:

$$W_{ps} = W_{ps-1} - W_{ps-1}\left(GSI{\cdot}ED_{F(X,Y)}\right) \tag{4}$$

where W_{ps} is fish mass post spawning (g), W_{ps-1} is fish mass on April 29 (g), GSI is the gonadosomatic index (ratio of the mass of gonads to that of the whole fish), and $ED_{F(X,Y)}$ is the ratio of gonad energy density to whole fish energy density. Age 3 fish were considered mature, so spawning and reproductive losses were included at ages 3 and older (Craig and Smiley 1986).

The MMBM we used (Trudel et al., 2000) models the uptake of methylmercury (MeHg) by fish through their diet, where uptake from water through gills is considered negligible and that total mercury in fish is equal to MeHg.

The rate of change of MeHg concentration in fish tissue can be modelled as:

$$\frac{dHg}{dt} = (\alpha C_d C_t) - (E + G + K)Hg \tag{5}$$

where Hg is the concentration of MeHg in the fish (μg Hg·g^{-1} wet weight), t is time, α is the efficiency of MeHg assimilation from food, C_d is the MeHg concentration of the prey (μg Hg·g^{-1} wet weight), C_t is the mass-specific rate of consumption (day^{-1}) at time t, E is the rate of MeHg elimination (day^{-1}), G is the mass-specific rate of growth (day^{-1}), and K is the rate of MeHg loss to gonads (for mature fish only; day^{-1}). When modelled daily, any changes in parameters each day will be small enough to be assumed constant, so the equation may be integrated to give:

$$Hg_t = Hg_0 e^{-(E+G+K)t} + \frac{\alpha C_t C_d}{E+G+K}\left[1 - e^{-(E+G+K)t}\right] \tag{6}$$

where Hg_t is the concentration of MeHg (μg Hg·g^{-1} wet weight) at time t and Hg_0 is the concentrations of MeHg (μg Hg·g^{-1} wet weight) at time 0. Finally, the MMBM can be rearranged, giving:

$$C_t = \frac{Hg_t - Hg_0 e^{-(E+G+K)t}}{\alpha C_d\left[1 - e^{-(E+G+K)t}\right]}(E+G+K) \tag{7}$$

If C_t from the MMBM is multiplied by prey energy density and fish mass to convert the units from day^{-1} to J·day^{-1}, the MMBM (Eq. (7)) and the bioenergetics model (Eq. (1)) can be linked by the shared term C_t. Daily iteration of the two equations generated a unique solution of ACT (Eq. (3)) and p (Eq. (2), and therefore an estimate of C_t), which minimized the average difference between modelled W_t and Hg_t, and observed W_t and Hg_t (<0.005%) using an optimization routine.

We modelled each age class of Northern Pike from July 1 of one year to June 30 of the following year using mass and Hg for fish of age x in one year (W_0 and Hg_0) and fish of age $x + 1$ in the next year (W_t and Hg_t). We performed these models to estimate consumption, activity, growth, and efficiency of food conversion to growth of Northern Pike before (2012–2013), during (2015–2016), and after (2016–2017) AgNP additions.

2.3. Model parameterization

Model parameter values are listed in Table 1. Trudel and Rasmussen (1997) modeled daily MeHg elimination (E in Eq. (7)) by fish as a function of mass and temperature:

$$E = \varphi W^{\beta} e^{\gamma T} \tag{8}$$

Where the constants φ, β, and γ were experimentally derived by (Trudel and Rasmussen 1997; Table 1). The rate of MeHg elimination from this model (either the acute or chronic formulations) has been generally shown to be too rapid (Madenjian et al., 2021). We applied the chronic elimination formulation of the (Trudel and Rasmussen 1997) model in our first run of simulations, and divided estimated values by 2.7 in our second set of simulations to scale elimination to field-based observations reported by Van Walleghem et al. (2013) for Northern Pike.

Growth in the MMBM was calculated as $W_t - W_0$ from the bioenergetics model (Eq. (1)) with units converted from J·day^{-1} to day^{-1} by dividing by ED_{fish} and mass.

Trudel et al. (2000) calculated daily losses of MeHg to gonads (K in Eq. (7)) as:

$$K = \frac{Q{\cdot}GSI}{365} \tag{9}$$

where Q is the ratio of the gonad MeHg concentration to the whole body MeHg concentration, and 365 (or 366 when appropriate) equals the number of days per year. There was insufficient data to model males and females separately, so all sex-specific parameters were averaged. Trudel et al. (2000) presented a calculation of a weighted average of Q for combining bioenergetics of a cohort applicable to both males and females (assuming equal populations of sexes):

Table 1
Values and sources for parameters used in the mercury mass balance and bioenergetics models (methylmercury, MeHg).

Symbol	Description	Value	Source
α	Assimilation efficiency of MeHg from food	0.8	1
ϕ	MeHg elimination coefficient	0.0029	2
β	MeHg elimination allometric exponent	−0.20	2
γ	MeHg elimination temperature coefficient	0.066	2
Q_m	MeHg ratio of testes to whole fish	0.59	3
Q_f	MeHg ratio of ovaries to whole fish	0.12	3
GSI_m	Gonadosomatic index of males at spawning	0.02	4
GSI_f	Gonadosomatic index of females at spawning	0.15	4
ED_{FY}	Energy density ratio of testes to whole fish	1.2	5
ED_{FX}	Energy density ratio of ovaries to whole fish	1.2	5
ED_{prey}	Energy density of prey (Yellow Perch)	4876.06 J·g^{-1} (experimental lake) 4501.21 J·g^{-1} (reference lake)	6
ED_{fish}	Energy density of consumer (Northern Pike)	4189 J·g^{-1} (experimental lake) 4396 J·g^{-1} (reference lake)	7

Sources: 1. (Trudel and Rasmussen 2006), 2. (Trudel and Rasmussen 1997), 3. (Trudel et al., 2000), 4. (Medford and Mackay 1978), 5. (Diana 1983), 6. (Hayhurst 2018). 7. This study.

$$Q = \frac{Q_m \cdot GSI_m + Q_f \cdot GSI_f}{GSI_m + GSI_f} \tag{10}$$

The remaining input parameters required for the Hg mass balance and bioenergetics models are daily mean temperature, W_0 and W_t, Hg_0 and Hg_t, fish ED, prey Hg, and prey ED (described below). Bioenergetic model results were accepted for each model where the overall percent difference between observed and predicted Hg and body mass was less than 0.005% (Table A1).

2.4. *Water temperature*

Daily mean water temperatures for each year of the study were calculated from hourly data recorded by two temperature loggers in the littoral zones of each lake (Hayhurst et al., 2020). Where logger data were missing, water temperatures were estimated from air temperatures using the enhanced multi-year model by (Matuszek and Shuter 1996). Air temperatures were obtained from the IISD-ELA meteorological station.

2.5. *Weight at age*

Because bioenergetics models in this study were applied to cohorts of fish (i.e., year classes) over one-year time steps, initial and final mass estimates were derived from a fit of a von Bertalanffy growth model applied to cohorts of fish over time (Supplementary Material).

2.6. *Mercury at age*

Concentrations of total mercury (Hg) in Northern Pike were determined using a Direct Mercury Analyzer (see Supplemental Material). Mercury at age was determined from allometric relationships with fork length, and these relationships were used to estimate Hg at a given fork length for modelled age classes in each year (see Supplementary Material).

2.7. *Energy density*

Energy density (ED) of Northern Pike was determined using a semi-micro calorimeter (see Supplementary Material). Northern Pike ED did not vary with mass of the fish collected from the experimental lake ($F_{1,26} = 0.51$, $p = 0.482$) or from the reference lake ($F_{1,6} = 5.31$, $p = 0.0608$). Northern Pike ED was therefore averaged across all individuals analyzed for each lake, and the mean value was applied to each population in bioenergetic models (Table 1).

2.8. *Prey mercury and energy density*

Yellow Perch is an important component of the diet of Northern Pike (Liao et al., 2002). For modeling purposes, we assumed that the Northern Pike diet consisted entirely of Yellow Perch in both lakes. We justified this assumption given that the densities of Yellow Perch in both the experimental lake and reference lake are high, compared to other prey species, making up 64% of the total catch of prey fish in the experimental lake and 95% in the reference lake (Slongo et al., 2022). Prey Hg was estimated as the average Hg concentration in age 1 Yellow Perch for each lake and year, as reported by (Hayhurst et al., 2020). Age 0 and 1 Yellow Perch are the most abundant size classes in the lake (Hayhurst, 2018) and based on the size of Northern Pike encountered in the lakes, match the appropriate predator:prey size ratio observed for Northern Pike (Liao et al., 2002). Prey ED for each lake was estimated as the ED for Yellow Perch from each lake as reported in Hayhurst et al. (2020).

2.9. *Gross consumption*

To estimate the annual biomass of prey consumed by Northern Pike in both lakes at the population level, we combined individual-scale bioenergetics results from the current study with population estimates of Northern Pike reported elsewhere (Slongo et al., 2022). To estimate the number of Northern Pike in each modelled age class, the percentage of captured Northern Pike belonging to each age class for every year was estimated. These percentages were then multiplied by the corresponding estimated abundance of Northern Pike (Slongo et al., 2022) in each year, and averaged over the model period for all cohorts (e.g. age x in one year to age $x + 1$ in the following year). Biomass of each age class of Northern Pike was then calculated by multiplying the total estimated number of fish in each age class by the average mass of fish in that age class. The average mass of fish in age classes for which a Hg mass balance-bioenergetics model was performed was the mean mass over the modelled time period (Supplementary Material, Table A4). For age classes for which a Hg mass balance-bioenergetics model was not performed, mass was interpolated between adjacent age classes across the appropriate time period. If there were no fish of a particular age captured in a given year to facilitate interpolation, the average mass for that age across all years was used. In Lake 239, from 2016 to 2017, age 19 fish were represented by the mass of age 18 fish, as there were no data for age 19 fish from any year. As fish in this older age category made up only about 1.6% of the fish in that model period, and are likely at or near asymptotic size, any error in the mass estimate would have very little effect on our gross consumption estimates. Northern Pike of ages 0–1 over the model period were excluded due to a lack of data for age 0 individuals.

The total mass of prey consumed by each Northern Pike age class was then calculated by multiplying the biomass of the age class by the estimated individual consumption (in $g \cdot g^{-1} \cdot day^{-1}$) for each age class (as estimated from the Hg mass balance-bioenergetics model). For age classes where data were not available to support bioenergetics modeling, the consumption value for the nearest age class that was modelled was used. The total mass of prey consumed by each age class in each modelled period were then summed to determine the gross (population-level) consumption of Northern Pike in the lake for each period. Gross consumption estimates were expressed as $kg \cdot ha^{-1} \cdot year^{-1}$ using the estimated areas of each lake; that is, 16 ha for Lake 222 and 54 ha for Lake 239 (Slongo et al., 2022).

To permit comparison to estimates of gross consumption, Yellow Perch population biomass was calculated from annual estimates of Yellow Perch abundance per hectare (Hayhurst et al., 2020), the percent of Yellow Perch present in each age class (Hayhurst et al. 2018), and the average estimated mass of Yellow Perch in each age class. For age 0 Yellow Perch, mass was determined from fork length using linear relationships between log of mass and log of fork length for before, during, or after AgNP additions for each lake (Hayhurst 2018). For ages 1 to 6, the average of the initial (summer) and final (fall) mass of each age class was used (Hayhurst et al., 2020); no mass data were available for ages 7 and up, but these ages made up less than 1.5% of the population in all cases, so they were excluded from calculations. As with Northern Pike, in the case of a missing initial or final mass, the average from all years in the lake was used. Yellow Perch data were for a single year, not a over a period of two years (as with Northern Pike), so Yellow Perch data were matched to the first year of each Northern Pike model period. Size-at-age data were not available for Yellow Perch in 2013.

2.10. *Statistical analyses*

To determine the effect of disparities in literature estimates of Hg elimination rates on bioenergetics model outputs, estimates of individual consumption ($kJ \cdot day^{-1}$) and activity ($kJ \cdot day^{-1}$) using the chronic Hg elimination rate from Trudel and Rasmussen (1997) were compared to those scaled to reflect field-derived elimination rates (e.g., estimates from the chronic equation divided by 2.7; van Walleghem et al. 2013). Model outputs were compared using paired *t*-tests.

To determine if individual consumption (kJ·day^{-1}) and activity (kJ·day^{-1}) scaled differently with body size between years of study for a given lake, we first compared slopes of log-transformed variables using a test of heterogeneity of slopes. Where slopes were found not to be significantly different, we conducted an ANCOVA to test for differences in intercepts between years. Despite the slopes being significantly different for activity in the reference lake, we performed an ANCOVA test for simplicity of comparison with the experimental lake. In the reference lake, only younger age classes showed linear relationships between log of consumption or activity and log of mass; older age classes were clear outliers from the relationship. Thus, only younger age classes that followed the relationship were used in model fits. Estimates for older age classes are presented in figures for comparison, but not included in model fits (Fig. 1). Models were constructed using contrasts that specifically tested the differences between the pre-addition period (2012–13) with all other years (Crawley, 2013).

Efficiency of food conversion to growth (conversion efficiency) for individual Northern Pike was calculated as the growth (J·year^{-1}) divided by consumption (J·year^{-1}) from bioenergetic models. We used a two-way ANOVA to determine if conversion efficiency (log-transformed) differed between years or lakes. In addition, we used a two-way ANOVA to determine if mass-specific growth (g·g^{-1}·day^{-1}, log-transformed) differed between years or lakes. The outliers that were removed for analysis of consumption and activity were also removed from these analyses. We also compared temporal trends in predicted values from von Bertalanffy growth metrics; specifically, maximum cohort size, as well as size-at-age 4 and 5, the latter two being chosen as having demonstrated changes from empirically observed size-at-age in published work elsewhere (Slongo et al., 2022). All statistics were performed in R version 4.0.5 (R Core Team 2021).

3. Results

3.1. Hg elimination rates

Applying differential elimination rates had a significant impact on bioenergetic results; individual consumption (as g·g^{-1}·day^{-1}) was significantly lower using the slower, field-based estimate of Hg elimination rate compared to the chronic elimination model (paired *t*-test, t_{35} $= -26.8$, $p < 0.0001$), with a mean difference of 0.006 g·g^{-1}·day^{-1} or 0.6% of body mass in daily consumption estimates. Mean individual consumption estimated using the Hg elimination rate from the Trudel and Rasmussen (1997) model was 0.020 g·g^{-1}·day^{-1}, which is much higher compared with those from the elimination rate adjusted to reflect field conditions (mean = 0.014 g·g^{-1}·day^{-1}). Similarly, activity (*ACT*), expressed as a multiple of basal metabolism, was significantly lower using the adjusted elimination rate ($t_{35} = -23.2$, $p < 0.0001$), with a mean difference of 0.98 between activity values. Mean *ACT* obtained

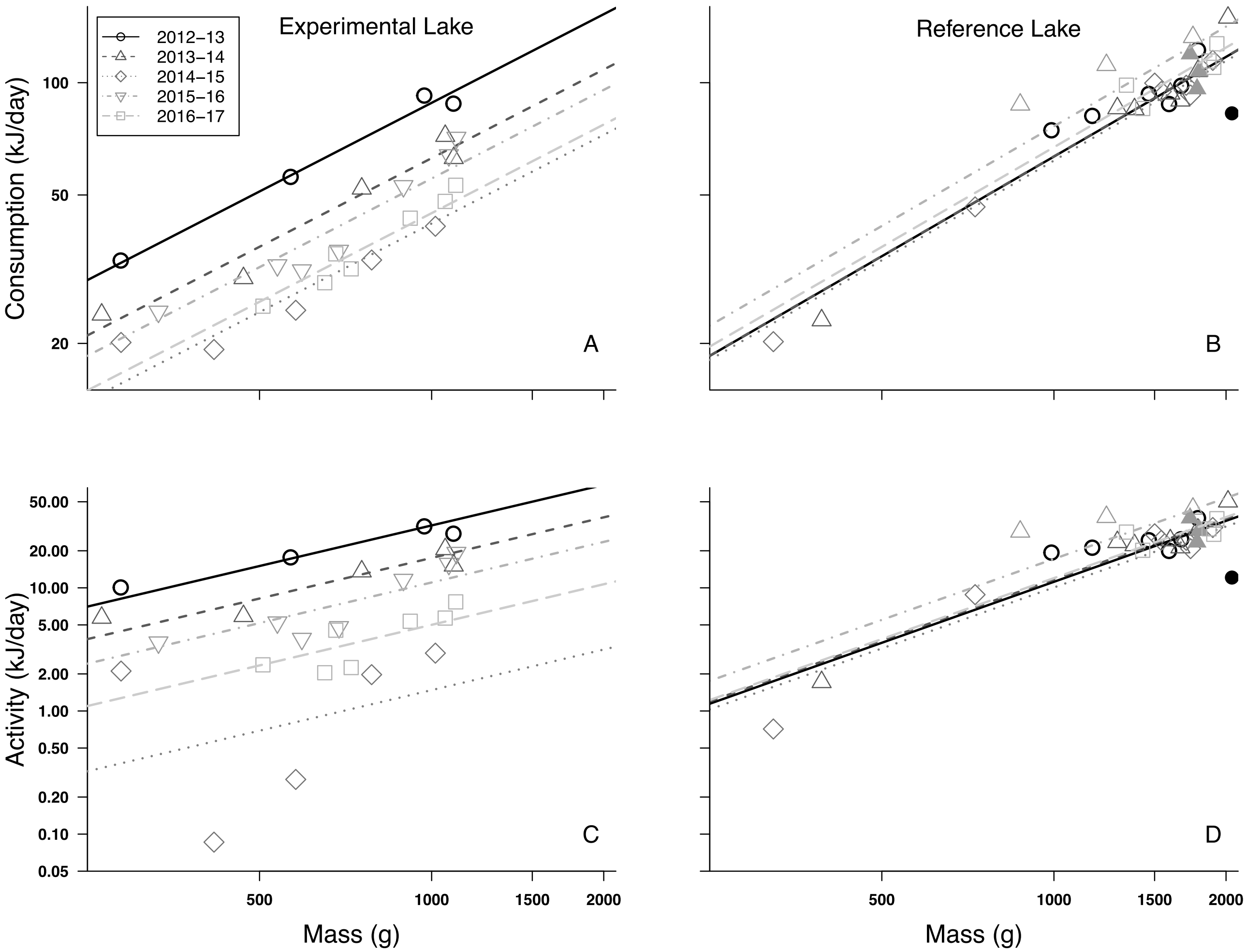

Fig. 1. Individual (cohort-based) consumption (A and B) and activity (C and D) of Northern Pike estimated from a mercury mass balance-bioenergetics model for a lake which received silver nanoparticle additions in 2014 and 2015 (experimental lake; A and C) and a lake which did not receive silver nanoparticle additions (reference lake; B and D). Four points in the reference lake data (filled circles, triangles) were not included in analysis because they were clear outliers. Data are plotted as the log of x and y values on arithmetic axes.

using the original elimination rate was 2.71 times R_s, which was much higher compared to those from field-derived elimination rates (mean = 1.75). Put another way, individual consumption determined using the Hg elimination rate reported by Trudel and Rasmussen (1997) was 43% higher and activity was 55% higher compared to estimates of elimination rate that more closely match those observed in the field for this species. As such, we report bioenergetic rates using corrected elimination rates (chronic/2.7) for further analyses.

3.2. *Consumption and activity*

Slopes of the relationship between individual consumption (kJ·day) and mass (g) on a log-log scale were not significantly different among years for Northern Pike from the experimental lake (Test for homogeneity of slopes, $F_{4,18} = 1.38$, $p = 0.280$). An ANCOVA subsequently revealed differences in elevation of the consumption-body mass relationship among years ($F_{4,22} = 37.0$, $p = < 0.0001$). Northern Pike consumption in the experimental lake was significantly lower in all years compared to 2012–2013, which represents baseline measurements prior to AgNP additions (Table 2; Fig. 1A).

For Northern Pike from the reference lake, the slopes of log individual-level consumption (kJ·day^{-1}) versus log mass (g) were not significantly different between years (Homogeneity of slopes, $F_{4,18} = 2.29$, $p = 0.0991$). An ANCOVA indicated significant differences in intercepts between years ($F_{4,22} = 16.6$, $p = < 0.0001$), but in a different direction/pattern than for the Northern Pike in the experimental lake. The intercept for 2015–2016 was significantly higher than the intercept for all other years, but there were no significant differences in intercepts between any other combination of years (Table 2; Fig. 1B).

Slopes of relationships between activity expressed in units of energy (kJ·day^{-1}) and mass (g) on a log-log scale were also not significantly different between years for Northern Pike from the experimental lake ($F_{4,18} = 0.182$, $p = 0.9$). An ANCOVA indicated a significant difference in intercepts between years ($F_{4,22} = 15.9$, $p < 0.0001$). Activity of Northern Pike in the experimental lake was significantly lower in all years compared to 2012–2013, which represents baseline measurements prior to AgNP additions, except in 2013–2014 (Table 3; Fig. 1C).

For Northern Pike from the reference lake, the slopes of log of activity (kJ·day^{-1}) versus log of average mass (g) were significantly different between years ($F_{4,18} = 3.73$, $p = 0.02$); 2013–2014 and 2014–2015 appeared to have similar slopes to one another, while the remaining years had shallower slopes compared with those in 2013–2014 and 2014–2015. For simplicity of comparison with data from the experimental lake, we proceeded with ANCOVA, assuming a common slope among years. Doing so, we found there was a significant difference in intercepts between years ($F_{4,22} = 8.28$, $p = 0.0003$), though in a pattern that was different from Northern Pike from the experimental lake, as the intercept for 2015–2016 was significantly higher than all other years. There were no significant differences in intercepts between any other combination of years (Table 3; Fig. 1D).

Table 2
ANCOVA results for comparison of intercepts of log consumption (kJ·day^{-1}) versus log mass (g) between baseline years (2012–13) and subsequent years for an experimental lake for Northern Pike estimated from a mercury mass balance-bioenergetics model for a lake which received silver nanoparticle additions in 2014 and 2015 (experimental lake) and a lake which did not receive silver nanoparticle additions (reference lake). Bold signifies significant differences between years; italics signifies that the intercept is significantly higher than the comparison year.

Lake	Comparison Year	Year	Difference	p-value
Experimental Lake	**2012–13**	**2013–14**	**Significantly lower**	**0.00011**
Experimental Lake	**2012–13**	**2014–15**	**Significantly lower**	**<0.0001**
Experimental Lake	**2012–13**	**2015–16**	**Significantly lower**	**<0.0001**
Experimental Lake	**2012–13**	**2016–17**	**Significantly lower**	**<0.0001**
Reference Lake	2012–13	2013–14	No significant difference	0.34
Reference Lake	2012–13	2014–15	No significant difference	0.21
Reference Lake	***2012–13***	***2015–16***	***Significantly higher***	***0.0004***
Reference Lake	2012–13	2016–17	No significant difference	0.82

Table 3
ANCOVA results for comparison of intercepts of log activity (kJ·day^{-1}) versus log mass (g) between baseline years (2012–13) and subsequent years for an experimental lake for Northern Pike estimated from a mercury mass balance-bioenergetics model for a lake which received silver nanoparticle additions in 2014 and 2015 (experimental lake) and a lake which did not receive silver nanoparticle additions (reference lake). Bold signifies significant differences between years; italics signifies that the intercept is significantly higher than the comparison year.

Lake	Comparison Year	Year	Difference	p-value
Experimental Lake	2012–13	2013–14	No significant difference	0.20
Experimental Lake	**2012–13**	**2014–15**	**Significantly lower**	**<0.0001**
Experimental Lake	**2012–13**	**2015–16**	**Significantly lower**	**0.020**
Experimental Lake	**2012–13**	**2016–17**	**Significantly lower**	**0.0003**
Reference Lake	2012–13	2013–14	No significant difference	0.47
Reference Lake	2012–13	2014–15	No significant difference	0.15
Reference Lake	***2012–13***	***2015–16***	***Significantly higher***	***0.0057***
Reference Lake	2012–13	2016–17	No significant difference	0.45

3.3. *Conversion efficiency and growth*

A two-way ANOVA showed no significant interaction between lake and year on log of conversion efficiency ($F_{4,46} = 0.350$, $p = 0.843$), and Northern Pike from both the experimental and reference lakes showed similar conversion efficiencies throughout the study (Fig. 2A). There were also no significant effects of lake ($F_{1,46} = 0.176$, $p = 0.7$) or year ($F_{4,46} = 2.119$, $p = 0.094$). A two-way ANOVA also showed that there was also no significant interaction between lake and year on the log of mass-specific growth (g·g^{-1}·day^{-1}; $F_{4,46} = 1.56$, $p = 0.200$; Fig. 2B), as well as no significant effects of lake ($F_{1,46} = 0.024$, $p = 0.9$) or year ($F_{4,46} = 1.482$, $p = 0.2$). However, growth of Northern Pike in the experimental lake appeared to decline over the course of the study (Fig. 2B). In contrast to mean inter-cohort growth estimates from bioenergetic models, the size of Northern Pike did decline over time; size-at-age 4 and 5 declined precipitously after AgNP additions in the experimental lake, but not in the reference lake (Figure 2C; Table A4). Similarly, maximum size of Northern Pike from the experimental lake declined, but not in the reference lake (Figure 2D; Table A1).

3.4. *Gross consumption*

Gross (population-level) consumption of Northern Pike in the experimental lake decreased by approximately 57% from before AgNP additions to after AgNP additions. However, in the Northern Pike from the reference lake, gross consumption stayed relatively constant, with only a slight increase (Fig. 3A). In addition, in the experimental lake, Yellow Perch biomass stayed relatively constant throughout the first part of the study, but decreased sharply by 42% from 2015 to 2016,

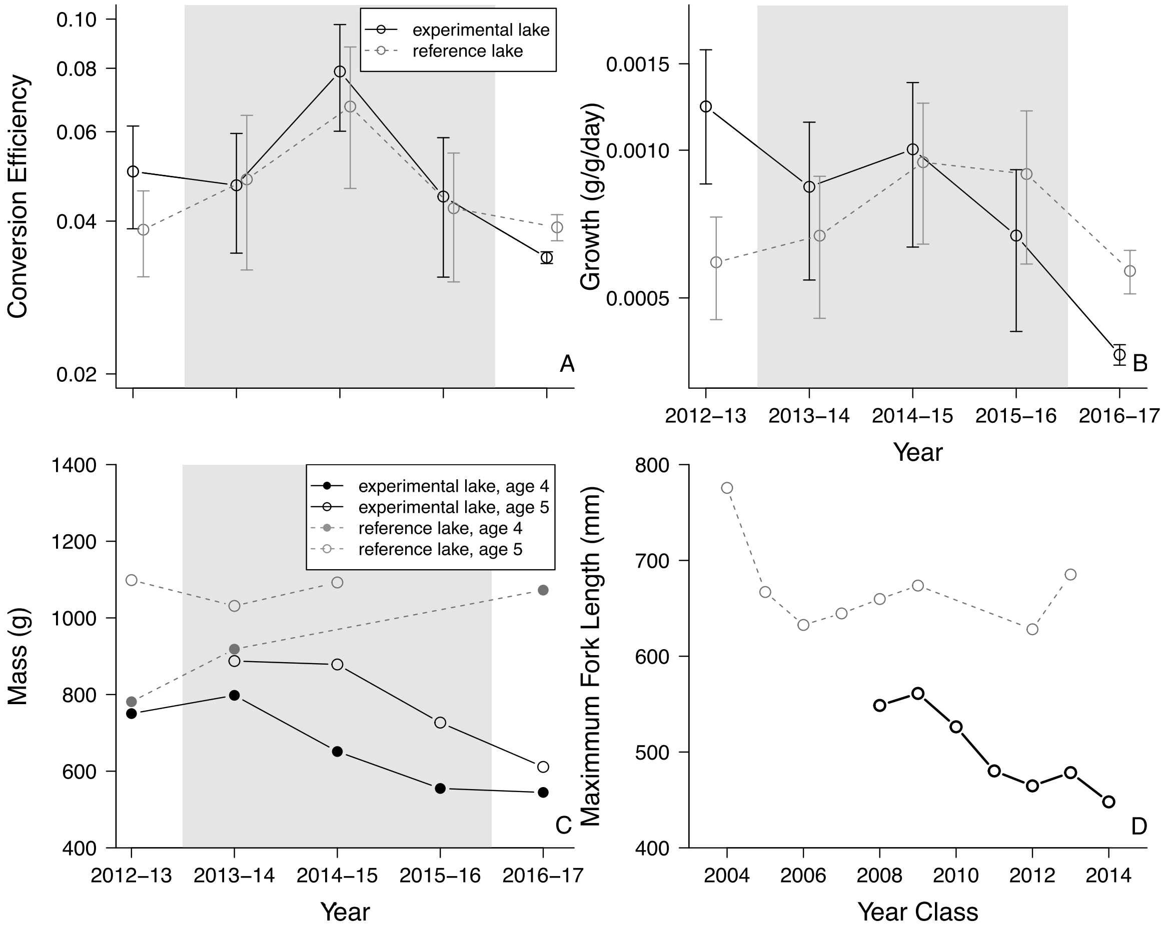

Fig. 2. Conversion efficiency of food to growth (A) and growth (B) of Northern Pike estimated from a mercury mass balance-bioenergetics model for a lake which received silver nanoparticle additions in 2014 and 2015 (experimental lake) and a lake which did not receive silver nanoparticle additions (reference lake). Points are means and error bars are standard errors. Conversion efficiency and growth are plotted on log axes. C) Mass of age 4 and 5 Northern Pike, estimated from a cohort-specific von Bertalanffy growth model (see text), also used as initial mass of Northern Pike for age classes 4 and 5 in bioenergetics models. gray shading in panels A-C indicate period of AgNP additions relevant to cohort growth being modelled. D) Maximum (asymptotic) fork lengths achieved for year class cohorts estimated from von Bertalanffy growth models; e.g., the 2008 year class would be age 7 at the time of silver nanoparticle exposure in 2014, the 2009 age class 6 years old, etc. Legend in Panel A also applies to panels B and D.

while Yellow Perch biomass in the reference lake slightly increased. Northern Pike biomass was fairly constant in both lakes (Fig. 3B).

4. Discussion

Controlling for body mass, individual-level consumption and activity of Northern Pike were significantly lower during and after AgNP exposure compared to before AgNP additions to the lake. By contrast, individual-level consumption and activity in Northern Pike from the reference lake were relatively stable or increased slightly over the same time period. Reduced consumption in Northern Pike during and following AgNP exposure is consistent with the pattern observed for Yellow Perch from the same lake (Hayhurst et al., 2020). Unlike Yellow Perch, which experienced an overall decrease in total metabolic costs (Hayhurst et al., 2020), and increased variability in activity (Hayhurst 2018), the estimated activity of Northern Pike declined precipitously after AgNP exposure.

Combined with previous observations, our study suggests that a reduction in prey availability likely played a major role in the observed reduced consumption and activity in Northern Pike. Densities (abundance) of Yellow Perch declined in the experimental lake following AgNP exposure, whether expressed as absolute density (Hayhurst et al., 2020) or per capita prey density (Slongo et al., 2022). Further, relative densities of the only other prey fish species present in Lake 222, the Blacknose Shiner (*Notropis heterolepis*), also declined significantly following AgNP exposure (Slongo et al., 2022). Assuming that activity costs in this species are tightly linked to consumption, reductions in both of these metrics would be expected to decline with decreased prey availability. Northern Pike are ambush predators, and therefore activity costs may be lower compared to more active predators (Rowan and Rasmussen 1996) but are likely also more tightly coupled with feeding events compared to other predatory species.

Further supporting this food limitation hypothesis, gross consumption of Northern Pike in the experimental lake decreased by 57% following AgNP additions, from approximately 92 kg·ha^{-1} to 50 kg·ha^{-1}, despite their stable population size (Slongo et al., 2022), while gross consumption in Northern Pike from the reference lake actually increased slightly over the same period. While similar declines in gross consumption were previously observed for Yellow Perch in the experimental lake (Hayhurst et al., 2020), Yellow Perch biomass also decreased by about 44% in the experimental lake over the period from before to after AgNP additions. The major decline in the availability and consumption of Yellow Perch biomass (and Blacknose Shiner relative abundance) further supports a bottom-up hypothesis of limited food availability contributing to Northern Pike growth declines.

Whereas mainly direct effects of AgNP exposure were identified in Yellow Perch from this lake, our evidence suggests that impacts on adult Northern Pike monitored in this study were largely due to indirect

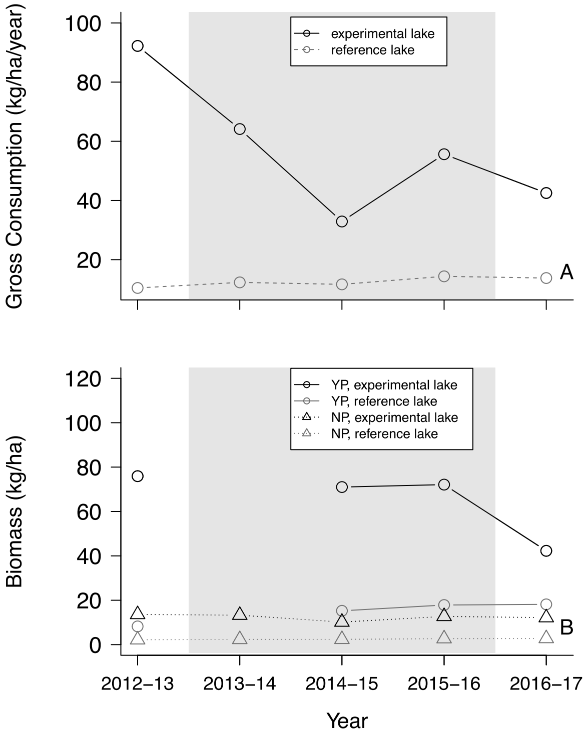

Fig. 3. Gross consumption and biomass of Northern Pike and their prey, Yellow Perch, in a lake which received silver nanoparticle additions in 2014 and 2015 (experimental lake) and a lake which did not receive silver nanoparticle additions (reference lake). Population-level estimates of consumption and biomass were derived from individual-level estimates from a mercury mass balance-bioenergetics model. A) Gross consumption of Northern Pike. B) Biomass of Northern Pike (NP) and Yellow Perch (YP). gray shading in panels A-C indicate period of AgNP additions relevant to cohort growth being modelled.

effects, though direct effects of AnNP toxicity cannot be entirely ruled out. Hayhurst et al. (2020) suggested that decreased consumption (both individual-level and gross consumption) in Yellow Perch following AgNP exposure resulted from a cascade of effects, where reduced metabolism in Yellow Perch may have reflected fatigue resulting from the energetic costs of oxidative stress, leaving less energy to search out and capture prey. While the decrease in consumption (individual and gross) and activity observed in Northern Pike is also consistent with this explanation, no biomarker data were available to evaluate potential oxidative stress changes in Northern Pike. Oxidative stress responses have been observed in fish exposed to AgNPs (Martin et al., 2017; Griboff et al., 2014), as has lower levels of glucose, which can also indicate increased energy costs of oxidative stress (Valerio-García et al., 2017). Silver concentrations in Northern Pike livers were substantially higher than in Yellow Perch (Martin et al., 2018), which might predict a greater cellular-level response in this species. Further, Northern Pike have been indicated among the most sensitive early life stage compared to two salmonid species in recent work (Schultz et al., 2021), indicating that direct effects may be more likely to manifest at earlier life stages.

Another possible explanation for decreased consumption in Northern Pike from the experimental lake during and after AgNP additions may be due to sensory impairment. Johari et al. (2015) found that Rainbow Trout exposed to very high concentrations (0.5 mg·L^{-1}) of AgNPs did not feed, which they suggested may have been a result of an impact of AgNPs on the lateral line receptors as well as sight, olfaction, and taste. Though the Johari et al. (2015) study was a short-term experiment with an exposure concentration nearly 1000 times greater than that recorded in the experimental lake during the present study, other tests at lower concentrations (45 μg L^{-1}), have also shown effects of AgNP exposure on olfaction of fish (Bilberg et al., 2011). Osborne et al. (2016) also observed that AgNPs impacted expression of genes related to oxidative stress and detoxification in the sensory systems (i.e., olfactory bulbs and neuromasts) of zebrafish embryos.

Consistent with the observations of Slongo et al. (2022), both the maximum size of cohorts and size-at-age of Northern Pike within cohorts declined following AgNP exposure. However, there were no statistically significant changes in mass-specific growth modelled as growth from one year to the next within single cohorts (expressed as a proportion of body mass per day). Thus, while there were no significant changes in the mean inter-annual growth of Northern Pike, the cumulative, incremental effects over several years of otherwise small, non-detectable changes (at the 0.05 level) appear to have been sufficient to alter the accumulated size-/mass-at-age in Northern Pike from the experimental lake by the end of the experiment. As such, our expectations that the growth of Northern Pike would decline as a result of decreased consumption were partially confirmed, though only when considered over a multi-year measure of cumulative growth such as mass- and fork length-at-age (e.g., Slongo et al., 2022).

Conversion efficiency was very similar in Northern Pike from both the experimental lake and the reference lake throughout the study, suggesting that the impacts of reduced foraging efficiency or potential increased metabolic activity associated with oxidative stress were less important than large changes in prey availability on Northern Pike bioenergetics and size-at-age. However, recent AgNP exposure studies (Schultz et al., 2021) reported earlier developmental times and delayed swim-up of Northern Pike at exposures as low as 0.1 nM exposures (approx. 0.01 μg L^{-1}), which would be expected to lead to reduced growth and increased mortality in these early life stages (as was observed in their study at 0.1 to 0.3 μg L^{-1}; Schultz et al., 2021). While we did not measure the abundance or recruitment of YOY Northern Pike, this laboratory work suggests both might be expected to decline after AgNP exposure, leading to reduced population size; while we did not observe reductions in Northern Pike abundance, it is possible that these would manifest after the period of monitoring reported here, as the best model used to estimate population abundance in Northern Pike was one that assumed constant recruitment (Slongo et al., 2022).

The impacts of AgNP on Northern Pike populations persisted after addition of AgNP ceased. Individual consumption, activity, and gross consumption of Northern Pike in the experimental lake were lowest during the period representing the bulk of AgNP additions (2014–2015), and while they increased slightly in subsequent periods, they never increased to pre-addition levels. In Yellow Perch, population density, consumption (individual and gross) and metabolism also remained depressed post-addition (Hayhurst et al., 2020). Impacts of experimental manipulations on fishes from previous whole-lake manipulations at IISD-ELA, such as exposure to an aquaculture operation (Rennie et al., 2019) and exposure to the synthetic estrogen 17α-ethynyloestradiol (Kidd et al., 2014) have also been found to persist long past the manipulation period. Indeed, the TAg concentration in the sediments of the experimental lake was found to be highest post-addition at 30 μg·g^{-1} in 2017, compared to the levels in sediment during the first and second years of AgNP additions of 3 μg·g^{-1} in 2014 and 5 μg·g^{-1} in 2015; Rearick 2017). This increased concentration in sediments indicates that benthic production in the lake could have been negatively impacted well after AgNP additions stopped and could provide a potential explanation for declines in the biomass of benthic-oriented fish (e.g., Yellow Perch) and their predators (e.g., Northern Pike; Ripku et al., n.d.).

Substantial differences in bioenergetic estimates were obtained using the chronic Hg elimination rate (Trudel and Rasmussen 1997) against field-derived elimination rates (Van Walleghem et al. 2013), supporting the inclusion of accurate elimination rates in contaminant mass balance models when available. The Trudel and Rasmussen (1997) chronic Hg elimination model overestimated activity by nearly an entire multiple of standard metabolism, and the mean difference between individual-level consumption estimates using the different elimination rates was 0.006 g·g^{-1}·day^{-1} or 0.6% of body mass. For context, Rowan and Rasmussen

(1996) estimated consumption of Northern Pike using ^{137}Cs and obtained mean daily consumption rates for different lakes ranging from 0.75 to 2.66% of body mass. Thus, differences of the magnitude reported here between consumption estimates using the different elimination terms may represent 23% to 80% of previously reported daily consumption estimates for this species using the chronic elimination model. Our present work strongly suggests that the use of adjustment factors for Hg elimination rates should be considered in future applications of this model, particularly in species where Hg elimination has been estimated directly (e.g., Van Walleghem et al. 2013) to avoid overestimation of bioenergetic parameters.

Overall, we found that individual-level consumption, activity, and gross consumption of Northern Pike in the experimental lake all decreased after exposure to AgNPs, despite relative stability in their population size. While conversion efficiency and mass-specific growth were not significantly affected, small, insignificant changes in growth accumulated over age classes to reflect reduced size-at-age of Northern Pike in the experimental lake during and after AgNP additions. The results from the present study show that there was a significant impact on Northern Pike bioenergetics during and after AgNP additions in a natural ecosystem at environmentally relevant concentrations; we hypothesize (based on our findings) that these responses were due primarily to indirect effects to AgNP additions, namely, reductions in the availability of prey fish as a food resource (Slongo et al., 2022). Based on the impacts observed in Northern Pike exposed to two years of AgNPs, and that AgNPs continue to enter aquatic environments at greater concentrations, it is important that efforts are made to limit the release of AgNPs into aquatic environments.

Author contributions

CDM acquired funding and conceptualized the design of the whole-lake addition experiment in Lake 222, along with several other researchers at Trent University, and was the Lead for the NSERC funded project. KMG and MDR conceptualized the design and approach for the bioenergetics study. The investigation (sample collection) in the field was conducted by LDH, MDR and CDM. LDH and KMG were responsible for data curation. Formal analysis was conducted by KMG, MDR, BS (aged fish), TR (estimated benthic energetic contribution information for Northern Pike), and LDH (determined Yellow Perch gross consumption and abundance estimates, which facilitated the prey biomass estimates reported here). Mercury and energy density analyses were conducted by KMG and LDH. KMG compiled data, parameterized, constructed and executed bioenergetics models, analyzed data and wrote the original draft of the manuscript. CDM, LDH, MDR, TR and BS reviewed and edited 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.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.aquatox.2023.106458.

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