

Isotopic-based evidence for reduced benthic contributions to fish after a whole-lake addition of nanosilver

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Abstract

Silver nanoparticles (AgNPs) are added as antibacterial and anti-odor agents to a wide range of textiles, with high potential for release into aquatic environments via domestic wastewater. Previous work demonstrating the negative impacts of AgNP exposure on periphyton production suggests benthic primary production could be reduced in aquatic ecosystems impacted by AgNP discharge. To evaluate the potential for AgNPs to alter benthic–pelagic coupling in aquatic ecosystems, tissue-stable isotope ratios of carbon and nitrogen from northern pike (*Esox lucius*) and yellow perch (*Perca flavescens*) were measured before, during, and after the addition of AgNPs to a whole-lake ecosystem, and compared to those collected from a nearby reference lake. A shift in carbon isotope ratios toward more negative values was observed in both *P. flavescens* and *E. lucius* collected from the lake where AgNPs were added, with no shift in similar magnitude observed in *E. lucius* from the reference lake. Consequently, Bayesian estimates of benthic energy consumed decreased by 32% for *P. flavescens* and by 40% for *E. lucius* collected after AgNP additions relative to pre-addition estimates, greater in magnitude or opposite in direction of trends observed in our reference lake. Analyses suggest no changes in fish nitrogen isotope ratios related to AgNP additions. We hypothesize that the observed reduction in littoral energy use of fish reported here is a response to AgNP settling in littoral benthic habitats—the main habitat in lakes supporting periphyton—as AgNP has been shown elsewhere to significantly reduce the rates of periphyton production. Further, our study highlights the need to broaden the scope of risk assessments for AgNPs and other emerging contaminants prone to settling to consider habitat-specific impacts on resource utilization by organisms after their release into aquatic ecosystems.

KEYWORDS

Esocidae, food webs, habitat de-coupling, nanoparticles, Percidae, silver

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1 | INTRODUCTION

Silver nanoparticles (AgNPs), which are classified as any particle composed of elemental silver that is <100 nm in any dimension (Colman et al., 2014), are widely used in consumer products as antibacterial agents (van Aerle et al., 2010). They are found in hundreds of consumer products, including in clothing, antibacterial creams, and dental and medical equipment, and are becoming more widely used in other manufactured items (Pourzahedi et al., 2017). An estimated 400 metric tons of silver were extracted for nanosilver production in 2014, and this amount has continued to increase (Janković & Plata, 2019). In many cases, AgNPs are released from consumer items and are then carried into wastewater treatment plants (WWTPs) in domestic sewage (Kaegi et al., 2013). In WWTPs, most AgNPs are transformed into silver sulfide (Ag₂S) under reducing conditions and are deposited in sludge (Kraas et al., 2017). The resulting biosolids, which are often applied onto agricultural fields, can be toxic to microorganisms, and the silver can accumulate in plants (Schlich et al., 2018). Between 1% and 15% of AgNPs entering WWTPs are released through effluent into aquatic ecosystems (Blaser et al., 2008; Elumelu et al., 2013; Kaegi et al., 2015). Even at these low percentages, AgNP release represents a potential threat to aquatic organisms, with reported measured effluent concentrations ranging from 6 to 2.9 µg/L (Johnson et al., 2014; Liu et al., 2009) and predicted concentrations in natural waterways similarly ranging from the low nanogram per liter range (Gottschalk et al., 2013; Sun et al., 2014) to the low microgram per liter range (0.3–1.5 µg/L; Blaser et al., 2008; Liu et al., 2009). Under oxic conditions, AgNPs are subject to dissolution into silver ions (Ag⁺), which are highly soluble in water (Peijnenburg et al., 2015). Laboratory studies at the bench-scale or in microcosms have shown that both AgNPs and Ag⁺ are toxic to aquatic bacteria and algae (Das et al., 2014; Grün et al., 2018; Leonardo et al., 2016; Shrivastava et al., 2007; Stevenson et al., 2013), to invertebrates (Magesky & Pelletier, 2018; Yoo-iam et al., 2014), and to fish (Ale et al., 2018; Laban et al., 2009; Martin et al., 2017; Notter et al., 2014; Scown et al., 2010).

Although a small number of studies have been conducted in freshwater mesocosms dosed with AgNPs (Lowry et al., 2012; Norman et al., 2019), these studies have mostly reported patterns relating to AgNP fate, with marginal or inconsistent findings related to impacts on ecosystem structure and function. However, it is important to recognize that mesocosm studies do not fully replicate the complex biogeochemical processes and trophic interactions that occur in natural aquatic ecosystems. To better study these interactions, we participated in a multidisciplinary project investigating the fate and effects of AgNPs in a natural aquatic ecosystem, in which 15 kg of AgNP suspension was released over two ice-free seasons into Lake 222, a boreal lake located in the IISD Experimental Lakes Area (IISD-ELA) in northwestern Ontario, Canada. The AgNPs were rapidly distributed throughout the lake immediately after additions began, where they persisted, reaching total silver (Ag) concentrations (suspended plus dissolved Ag) in the water column of c. 4 µg/L on average but with very low concentrations of dissolved Ag, typically less than 0.4 µg/L (Rearick et al., 2018).

Previously published studies on fishes from this experiment (Martin et al., 2018) showed that during AgNP additions, Ag rapidly accumulated in the liver and gill tissues of both the yellow perch, *Perca flavescens* (Mitchill, 1814), and northern pike, *Esox lucius* (Linnaeus, 1758). Additionally, prey consumption and total metabolism of *P. flavescens* declined, and the population density of this species decreased by approximately 30% after AgNPs were added to the lake (Hayhurst et al., 2020). Though the abundance of *E. lucius* during the study period was stable, their growth declined after AgNP additions, most notably in older/larger individuals (Slongo et al., 2022), as did *E. lucius* consumption and activity rates (Geils et al., 2023).

In aquatic environments, AgNPs have been shown to readily settle from the water column into sediments (Sultana et al., 2018), which has the potential for greater impacts on nearshore primary production than offshore where settled AgNPs can negatively affect benthic microbial communities (i.e., periphyton). The diverse community of bacteria and algae that comprises periphyton (often called biofilm) is found on almost any surface in lakes but particularly in shallow, littoral zones where it is an important source of primary production (Battin et al., 2016; Gil-Allué et al., 2018). Previous work in indoor microcosms (Grün et al., 2018) and mesocosms (Gil-Allué et al., 2018) as well as controlled microcosm work in lakes (including Lake 222; Norman et al., 2015) indicates that exposures to AgNPs can alter the structure and function of periphyton communities, including changes in community composition (Grün et al., 2018) and reductions in rates of primary production by up to 50% (Gil-Allué et al., 2018), potentially modulated by the presence of nutrients (phosphorus; Norman et al., 2015). Large changes in periphyton production as a critical source of energy in lacustrine food webs have the potential for cascading effects on higher trophic levels, including fish (Vadeboncoeur et al., 2002; Vander Zanden et al., 2011).

Measurements of the ratios of stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) are widely used to understand both resource use and trophic position in aquatic organisms (Chen et al., 2012; Hecky & Hesslein, 1995; Post, 2002). Typically, nearshore/benthic resources in lakes have more positive $\delta^{13}\text{C}$ values than offshore resources (Hecky & Hesslein, 1995; Post, 2002), and isotopic enrichment (i.e., trophic discrimination factors) between prey and consumer can often be predicted (e.g., Canseco et al., 2022). Thus, changes in both the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ relative to an isotopic baseline in the ecosystem can reveal changes in the resource use and/or trophic position of consumers (Post, 2002). Further, recent advances in statistical modeling techniques (e.g., MixSIAR) can be used to identify distinct source contributions to consumers, while also incorporating error associated with these processes (Stock et al., 2018; Stock & Semmens, 2016).

Both *P. flavescens* and *E. lucius* are fish species that primarily inhabit the littoral zones of boreal lakes. *P. flavescens* are facultative consumers (Scott & Crossman, 1998) and undergo ontogenetic shifts in diet as they grow, with dominant prey items progressing in size from zooplankton to benthos to fish, confirmed from gut contents for populations included in this study (Hayhurst, 2018). *E. lucius* are voracious piscivores, and their diets frequently include *P. flavescens* (Beaudoin et al., 1999). Both species rely heavily on the littoral zones

of lakes for both feeding and spawning habitat (Scott & Crossman, 1998). Therefore, both species are good candidates for detecting potential changes in the use of littoral resources associated with exposure to chemical contaminants.

To understand the degree to which long-term exposure to AgNPs might alter the relative contributions of littoral versus pelagic carbon in fishes, we monitored $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in the tissues of *P. flavescens* and *E. lucius* before, during, and after the 2-year additions of AgNPs to Lake 222. Changes in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in *P. flavescens* and *E. lucius* exposed to AgNPs were compared to stable isotopes in these fish species from a nearby reference lake (Lake 239). We predicted that the proportion of littoral-sourced carbon in fish would decline as a consequence of the sustained impacts of settled AgNPs on nearshore benthic communities, including the periphyton associated with sediments and surfaces which supports the benthic food web. We applied a Bayesian statistical mixing model to evaluate these potential changes in source contributions to both fish consumers over time in both the experimental and reference lakes.

2 | METHODS

2.1 | AgNP addition

This study was part of the multidisciplinary Lake Ecosystem Nanosilver (LENs) Project, conducted at the IISD-ELA, a research facility remote from the impacts of human activity. Lake 222 is a small, oligotrophic lake with a maximum depth of c. 6 m and an estimated volume of $7.2 \times 10^5 \text{ m}^3$, with one ephemeral stream entering and one ephemeral stream exiting the lake (Table 1), and no recruitment of fish from other aquatic systems (Martin et al., 2018). Lake 239 is larger, with a maximum depth of 31 m and an estimated volume of $61.7 \times 10^5 \text{ m}^3$ (Table 1), but it was selected as a reference lake because it is characteristic of other unmanipulated lakes in the area, the fish populations in the lake have been monitored for several decades, and it has a similar food web as Lake 222, with a fish community dominated by both *E. lucius* and *P. flavescens* (Slongo et al., 2022).

TABLE 1 Baseline values for both $\delta^{13}\text{C}$ in samples of benthic invertebrates and zooplankton applied in MixSIAR models to represent source values for the littoral and pelagic zones, respectively, of our study lakes.

Lake	Littoral (benthic invertebrate)		Pelagic (zooplankton)	
	$\delta^{13}\text{C}$ mean	SD (n)	$\delta^{13}\text{C}$ mean	SD
Lake 222	-21.6 (n = 4)	1.0	-35.9 (n = 4)	1.0
Lake 239	-17.9 (n = 4)	1.0	-36.0 (n = 4)	1.0

Note: Values were used to estimate littoral resource contributions for both northern pike and yellow perch. Sample sizes and standard deviation (SD) selected for model fitting are shown in parentheses.

Abbreviation: SD, standard deviation.

The dosing of Lake 222 with AgNPs has been described in detail elsewhere (Martin et al., 2018; Rearick et al., 2018). Briefly, AgNPs were added over two field seasons, with additions of 9 kg of AgNPs in 2014 over 131 days (June 14 to October 23, 2014) and an additional 6 kg in 2015 over 102 days (May 15 to August 25, 2015). The AgNPs were purchased as a powder from NanoAmor (Houston, TX, USA). This material is capped with 0.2% (w/w) polyvinylpyrrolidone (PVP), and according to the manufacturer, it consists of 99.9% total silver (Ag) and has a particle size range of 30–50 nm (measured using transmission electron microscopy and includes the PVP cap), and the particles are spherical. A stock suspension of AgNPs prepared at a nominal Ag concentration of 5 g/L was added to the lake using a peristaltic pump from a point source along the southwestern shore of the lake. Mean daily discharges of AgNPs in the suspension were c. 63.8 g during the project (68.7 g in 2014 and 58.8 g in 2015). Monitoring in the lake during the addition phase confirmed that Ag was distributed throughout the epilimnion and hypolimnion of the lake at concentrations in the range of 1–10 $\mu\text{g/L}$ (Rearick et al., 2018), and the mean size of Ag particles as determined by single-particle inductively coupled plasma mass spectrometry analysis (therefore excluding the PVP cap) was c. 20 nm (Martin et al., 2018), and the concentration of dissolved Ag in the lake was low at $<0.5 \mu\text{g/L}$ (Martin et al., 2018; Rearick et al., 2018). Importantly, these concentrations are environmentally relevant, that is, close to concentrations that have been observed in the environment (Blaser et al., 2008; Liu et al., 2009).

2.2 | Fish collections

Baseline (i.e., pre-addition) monitoring of lakes 222 and 239 began in 2012 and continued in 2013 to characterize the status of fish prior to AgNP exposure. Fish collections continued throughout the addition (2014 and 2015) and post-addition phases (2016 and 2017) during the study. To provide tissues for stable isotope analyses, fins and/or biopsy samples of muscle from live fish, as well as muscle tissues from sacrificed fish, were collected in both lakes throughout the study period. *P. flavescens* were collected by seining and trap-netting, whereas *E. lucius* were collected primarily through angling and trap-netting. Sampling occurred in spring, summer, and fall in most years (Hayhurst et al., 2020; Martin et al., 2018). All captured fish were brought to shore in coolers containing lake water. Once there, fish were slightly anaesthetized with buffered tricaine methanesulfonate (TMS) supplied by Argent Chemical Laboratories (Redmond, WA, USA) to facilitate sampling. Sampling occurred at in situ sampling sites, during which weight (g) and lengths (fork and total, mm) were determined. A subset of *P. flavescens* were euthanized using an overdose of TMS, placed in Whirl-Pak bags, and transported in a cooler with ice back to the laboratory, where they were frozen at -20°C . Anaesthetized *E. lucius* had fin clips (tips from leading rays of pectoral fins) and muscle biopsies taken for stable isotope analyses. Biopsies were taken from the left side of the fish, below the dorsal fin and above the lateral line, using a 4-mm biopsy punch (Acu-Punch, Dormer Laboratories Inc., Toronto, ON, Canada). Biopsy wounds were sealed using

Vetbond Tissue Adhesive (3M). Fish recovered in large tubs of fresh lake water and were returned to the lake. Muscle biopsies were stored in a cooler on ice in the field and returned to the laboratory where they were frozen at -20°C . A small subset of *E. lucius* were killed as described by Martin et al. (2018), and from these fish, additional muscle tissue was removed as a fillet from the left dorsal side of the carcass and frozen at -20°C . All frozen, euthanized fish were processed within 3–6 months of collection; fish were thawed, and muscle tissue for isotopic analyses was collected. Muscle was obtained below the dorsal fin, above the lateral line, on the left side of the fish. Fish for both this study and the larger project were collected and handled with approval from the Animal Care Committees at Fisheries and Oceans Canada (2012 and 2013), the University of Manitoba (2014, AUP nos. F14-007 and F14-008), and Lakehead University (2015–2017, AUP nos. 1464693, 1464399, 1454655, and 1464656; Biosafety Approval no. 1464768).

To increase the sample sizes for *E. lucius* stable isotopes, where the numbers of muscle biopsy samples were limited for both lakes, we performed a paired comparison of muscle tissue with fin tissue for $n = 19$ individuals from both lakes and across years. Additional fin tissues (leading rays of pectoral fins) were collected from all *E. lucius* upon first capture to facilitate age determination as part of broader population monitoring in these lakes (Slongo et al., 2022). This permitted the use of stable isotopes from fish for which only fin tissues (pectoral fin tips) were available to estimate isotope ratios in muscle, a common practice for estimating fish isotopic signatures in a nondestructive manner (Finzel et al., 2012; Roberts et al., 2022). Numbers of samples that relied on fin isotopes are presented in Supplementary Material A1 (Table A1.1).

Invertebrate samples were collected from both lakes during the summer (July–September) of 2012 (pre-addition), 2015 (second year of AgNP additions), and 2016 (first year post-addition). Benthic invertebrates in littoral zones were collected based on methods described by the Ontario Benthos Biomonitoring Network (OBBN, 2007), which consisted of kick-and-sweep collections using a D-net from a depth of 0.5 m. Benthos sampling was conducted at the outflows of both lakes, in the east bay inflow of Lake 239, and beside the AgNP additions site in Lake 222. Benthic invertebrates were sorted into 27 group levels (OBBN, 2007) and were frozen and stored at -20°C . Zooplankton were collected by repeated vertical tows using a 60- μm mesh net at a central buoy location (deepest point) in both lakes in the summers of 2012 and 2016 and from Lake 222 only in the summer of 2015. Zooplankton were frozen at -20°C prior to sample preparation. Unfortunately, a freezer failure reduced the availability of 2012 invertebrate samples. All remaining samples available were analysed (Table A2.1).

2.3 | Stable isotope analysis

Invertebrate samples and wet samples of fish tissue were subsampled, placed in 1.7-mL microcentrifuge tubes, and then dried at 60°C for at least 48 h until reaching a constant mass. Zooplankton samples were prepared as bulk samples across collected taxa. Although benthic taxa

were sorted in the field, the taxonomic identity was not transferred for many of the samples prepared for stable isotopes. Therefore, taxonomic identity was available only for very few benthic samples (Table A2.1). Benthic taxa consisted primarily of chironomids, followed by trichopterans, ephemeropterans, hirudinea, amphipoda, and anisopterans (Hayhurst, 2018). Zooplankton in ELA lakes are typically dominated by copepods (Kidd et al., 2014; Paterson et al., 2010). Samples were ground to a fine powder using a mortar and pestle. Dried and ground samples were then weighed in tin capsules ($2.5 \times 5\text{-mm}$ size) with a sample weight between 0.25 and 0.45 mg.

Samples were sent to Isotope Tracer Technologies Inc. (Waterloo, ON, Canada) for analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ using a DeltaPlus isotope ratio mass spectrometer (Thermo Fisher, Waltham, MA, USA) coupled with an EA1110 CHN elemental analyser (CE Instruments, Manchester, UK). Analysis of the reference material IT2-13/IT2-Beet Sugar supplied by the International Atomic Energy Agency (IAEA CH-6) showed a standard deviation (SD) of $\pm 0.15\text{‰}$ for $\delta^{13}\text{C}$ and $\pm 0.3\text{‰}$ for $\delta^{15}\text{N}$. Of 162 analyses, 9 were run in duplicate for fish tissue samples. The mean pair-wise difference among duplicates was small (i.e., 0.15‰ for $\delta^{13}\text{C}$ and 0.05‰ for $\delta^{15}\text{N}$).

2.4 | Estimation of littoral diet contribution

To estimate the changes in the contribution of littoral versus pelagic carbon during the course of our whole-lake AgNP addition experiment, we applied the MixSIAR model under a single-source application to $\delta^{13}\text{C}$ isotopes (Stock et al., 2018). All files and R-code used to generate estimates of littoral and pelagic contribution using MixSIAR are provided in Supplementary Material A4, and derivation of the values used for consumers, sources, and trophic discrimination factors, as well as model-fitting procedures, is detailed later.

2.5 | Consumer isotopic values

All fish C:N ratios, including those measured directly or estimated from fin rays as described earlier, were evaluated for potential lipid corrections. Isotopic values with measured C:N ratios greater than 3.5 were corrected using the equation for freshwater fish muscle, reported in Logan et al. (2008). This correction was applied to only a small number (10%) of all pike values but over 90% of yellow perch values. All consumer values, including those for which lipid corrections were required, were used in our analyses and are available in Supplementary Material A4.

2.6 | Source isotopic values

Benthos and zooplankton samples were collected to help estimate source $\delta^{13}\text{C}$ values for littoral/benthic and pelagic sources; however, samples were generally limited, and those that were available were highly variable within source categories (e.g., benthic invertebrates,

zooplankton). Like fish and other organisms, benthic invertebrates and zooplankton are themselves capable of mixing carbon sources, as has been well documented (Chandra et al., 2005; Francis et al., 2011; Rennie et al., 2013). This was evident in our dataset, where both benthos and zooplankton were highly variable within and among years.

The aim of our study was not to understand the feeding pattern (i.e., diet item identification) of consumers but rather to only estimate the amount of pelagic and littoral/benthic source energy being incorporated into the two consumer species of interest. Therefore, we considered values only for invertebrates for which mean values were equal to or exceeded the minimum or maximum lipid-corrected values observed for both *E. lucius* and *P. flavescens* in our dataset (corrected for trophic discrimination factor as described later). These representative organisms most closely reflected exclusive feeding on either of the sources we were interested in evaluating (i.e., benthic/littoral, pelagic production). This criterion limited our source estimates to only the minimum and maximum values for zooplankton and benthic invertebrates, respectively, observed in each lake (Table 1; Supplementary Material).

Because samples were limited within lakes once this criterion was applied, as well as due to lack of sample availability in some years, it was not possible to generate year-specific source data. Consequently, we assumed the same source data applied to all years in our dataset. This approach assumes that baselines were stable over the duration of the experiments. Stability in isotopic baselines has been observed in other IISD-ELA reference lakes over time, such as Lake 373 (Wellman et al., 2017). The processes that maintain differences between littoral/benthic and pelagic resources are primarily physical in nature, including mixing at the lake surface for incorporation of atmospheric C into aqueous DIC and boundary layer effects resulting in greater ^{13}C incorporation in littoral/pelagic sources (Hecky & Hesslein, 1995). However, the lakes in this study are small and well mixed in the epilimnion and are unlikely to have experienced variation in physical processes that would have affected interannual variation in atmospheric C flux or boundary layer formation in the littoral zone. The flux of C is known to vary with phosphorus concentrations (Schindler, 1974); however, both dissolved (TDP) and suspended (TSP) concentrations of phosphorus in our lake were stable during the experiment (Cetinic, 2019, their Appendix 3: 2013, mean TDP = 6.0; TSP = 5.4; 2015, mean TDP = 7.8; TSP = 5.3).

To provide error estimates for our source values, we applied an SD of 1 and sample size of 4 to source values for use in the MixSIAR model.

2.7 | Trophic discrimination factor values

We assigned $\delta^{13}\text{C}$ isotope trophic discrimination factor for yellow perch of 1.7‰, derived from Canseco et al. (2022, tab. 4), which matches reported values for both freshwater fishes and fish muscle. This value was doubled for northern pike trophic discrimination factors (TDF) to reflect the increase in one trophic position for these

species. To achieve model convergence, SD for TDFs was set to zero. Although we accept that variations in TDF are expected, the mean TDF values we included in the model were from a literature survey over several species, and as a result, variation in these reported TDFs is likely to be much greater than would be directly estimated from our study system. Additionally, the inclusion of SD values reported in Canseco et al. (2022; 4.9‰ for freshwater species) prevented the convergence of our models, even when applying “extreme” numbers of Markov chain Monte Carlo (MCMC) iterations (i.e., chain length of 3 million, burn-in of 1.5 million; Stock & Semmens, 2018). Therefore, we accept that there may be more variation in TDFs in our dataset than reflected in the settings we applied but were ultimately limited by what settings would allow the model to provide solutions when applied to our data.

2.8 | Model-fitting procedure

We first attempted to run a model for each lake separately, with year as a variable factor. However, several attempts at running models using this formulation under various options for MCMC settings failed to consistently converge, such that model diagnostics could be satisfied (e.g., Gelman–Rubin statistics for all parameters <1.05; Gwerke diagnostics below 5%). We therefore adopted a different modeling strategy and ran the mixing model for each year separately, which generated estimates that would consistently converge on a solution set while consistently satisfying model diagnostics. The MCMC settings required to achieve convergence while satisfying model diagnostics ranged from “normal” to “extreme” (Stock & Semmens, 2018). Posterior probabilities from mixing models were extracted for comparison.

2.9 | Statistical analysis

To determine the degree to which fish muscle isotopic values varied with fish length, linear regressions were used to evaluate relationships between both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ using fork length for both fish species. In each case, diagnostic plots confirmed that residuals were normally distributed, and variance was homogeneous. To evaluate changes in isotopic values over time in fish from both lakes, factorial Before After Control Impact–style analysis of variance (ANOVA) was performed for both fish species, with an emphasis on the presence of a significant interaction term between year and lake, indicating a difference in isotopic values over time between lakes.

Data transformations were applied where appropriate to help normalize data and improve heterogeneity of variance. We used type III sums of squares in ANOVA models due to unequal sample sizes (Quinn & Keough, 2002). Follow-up comparisons of means were conducted using Tukey's honestly significant difference (HSD) test for unplanned comparisons. To ensure that temporal changes in isotopes over time were not a consequence of differences in fish size during the study, fork lengths of fishes over time were assessed with a Kruskal–Wallis non-parametric test. All statistical analyses were carried out using R (version 4.1.2, R Core Team, 2022). In all cases,

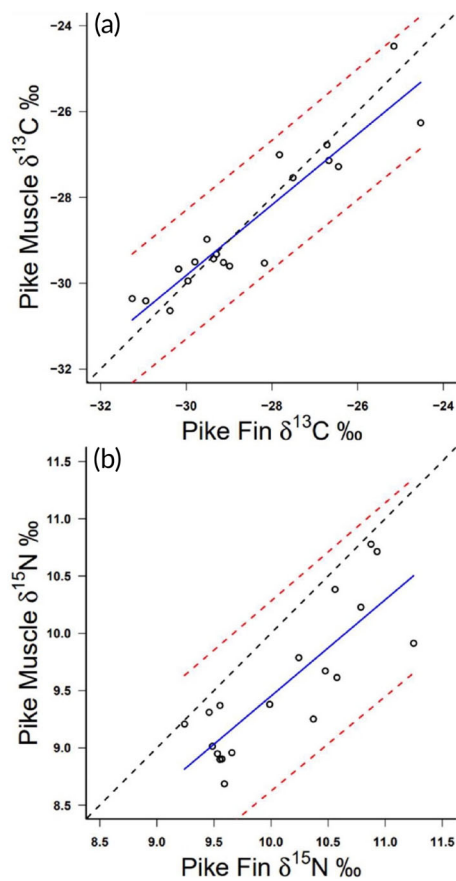


FIGURE 1 Scatterplot of the values for isotope ratios in fin and muscle samples ($n = 19$) collected from northern pike (*Esox lucius*). (a) Carbon ($\delta^{13}\text{C}$) and (b) nitrogen ($\delta^{15}\text{N}$). Dashed black line shows 1:1 line, solid line shows the line of best fit used to estimate muscle from fin isotopic values, and dashed red line indicates the 95% prediction intervals around the line of best fit.

diagnostic plots were used to evaluate normality and homogeneity of residuals; cases where assumptions were not met are indicated in the results.

3 | RESULTS

For the paired comparison of muscle tissue with fins collected from 19 *E. lucius* from both lakes and across years, a significant relationship was found between fin and muscle tissue ratios for both $\delta^{15}\text{N}$ ($r^2 = 0.69$, $F_{1,17} = 37.84$, $p < 0.0001$) and $\delta^{13}\text{C}$ ($r^2 = 0.86$, $F_{1,17} = 108.3$, $p < 0.0001$). A scatterplot of fin and muscle biopsy data indicated a linear pattern for both isotopes (Figure 1). Paired mean differences between fins and muscle showed that fin values were higher than muscle values for nitrogen isotopes (mean difference of 0.56‰) but only slightly higher for carbon isotopes (mean difference of 0.1‰).

The equations for conversion from fin to muscle isotopic values were as follows:

$$\text{Muscle } \delta^{15}\text{N} = 0.841 \times (\text{fin } \delta^{15}\text{N}) + 1.043 \quad (1)$$

and

$$\text{Muscle } \delta^{13}\text{C} = 0.822 \times (\text{fin } \delta^{13}\text{C}) - 5.158 \quad (2)$$

These equations were applied to fin isotope samples to estimate muscle isotopic values (Table A1.1).

Two-factor ANOVA revealed no significant interaction between lake and year for lipid-corrected $\delta^{13}\text{C}$ in *P. flavescens* (Figure 2a; $F_{3,129} = 0.6$, $p = 0.6$), but a significant main effect of lake ($F_{1,129} = 39.7$, $p < 0.0001$), where $\delta^{13}\text{C}$ was elevated in reference Lake 239 compared to AgNP-exposed Lake 222, as well as a significant main effect of year ($F_{3,129} = 3.8$, $p = 0.012$), where both lakes showed a general decline in $\delta^{13}\text{C}$ over time (Figure 2a). Residuals from this analysis were normal but mildly heterogeneous and could not be resolved using data transformation.

By contrast, a two-factor ANOVA revealed a significant interaction between the lake sampled and the year of sampling for lipid-corrected $\delta^{13}\text{C}$ in *E. lucius* ($F_{5,144} = 8.3$, $p < 0.0001$; Figure 2b). Due to limited *E. lucius* sampling in 2014, observations for Lake 239 *E. lucius* in this year were represented only by two tissue samples, whereas all other lake years were represented by at least seven samples (Figure 2b). A Tukey's test revealed that $\delta^{13}\text{C}$ for *E. lucius* collected from Lake 222 pre-AgNP addition (2012, 2013) were grouped together with data from the first year of AgNP additions in 2014 (Figure 2b), whereas mean values for *E. lucius* collected from the second year of additions in 2015 and post-addition to 2017 were grouped together. Additionally, values from the last year of observation were significantly lower compared to the first 3 years of the study. By contrast, significant differences among years for the carbon isotope ratios in *E. lucius* collected from reference Lake 239 were in the opposite direction and of lesser magnitude to those observed in Lake 222 (Figure 2b). As with *P. flavescens*, we observed a significant main effect of lake on *E. lucius* $\delta^{13}\text{C}$ ($F_{1,144} = 790.8$, $p < 0.0001$), where $\delta^{13}\text{C}$ values from Lake 239 were consistently and significantly elevated compared to those observed in Lake 222 (Figure 2b).

Two-factor ANOVA demonstrated a significant interaction between lake and year on log-transformed *P. flavescens* $\delta^{15}\text{N}$ ($F_{3,129} = 5.7$, $p = 0.0009$) but not in a pattern that was consistent with a response to AgNP additions to the lake (Figure 2c). Tukey's HSD test showed that $\delta^{15}\text{N}$ for *P. flavescens* from Lake 222 did not change over time. In contrast, $\delta^{15}\text{N}$ for *P. flavescens* from Lake 239 was lowest in 2012 but then increased and did not change significantly between 2014 and 2016. A significant main effect of the lake on *P. flavescens* $\delta^{15}\text{N}$ was observed ($F_{1,129} = 37.9$, $p < 0.0001$), where $\delta^{15}\text{N}$ in *P. flavescens* from Lake 222 was consistently greater than those observed in Lake 239.

We found no significant interaction between lake and year for $\delta^{15}\text{N}$ of *E. lucius* ($F_{5,144} = 0.7$, $p = 0.6$). *E. lucius* $\delta^{15}\text{N}$ was clearly elevated in Lake 222 relative to Lake 239 (significant main effect of lake,

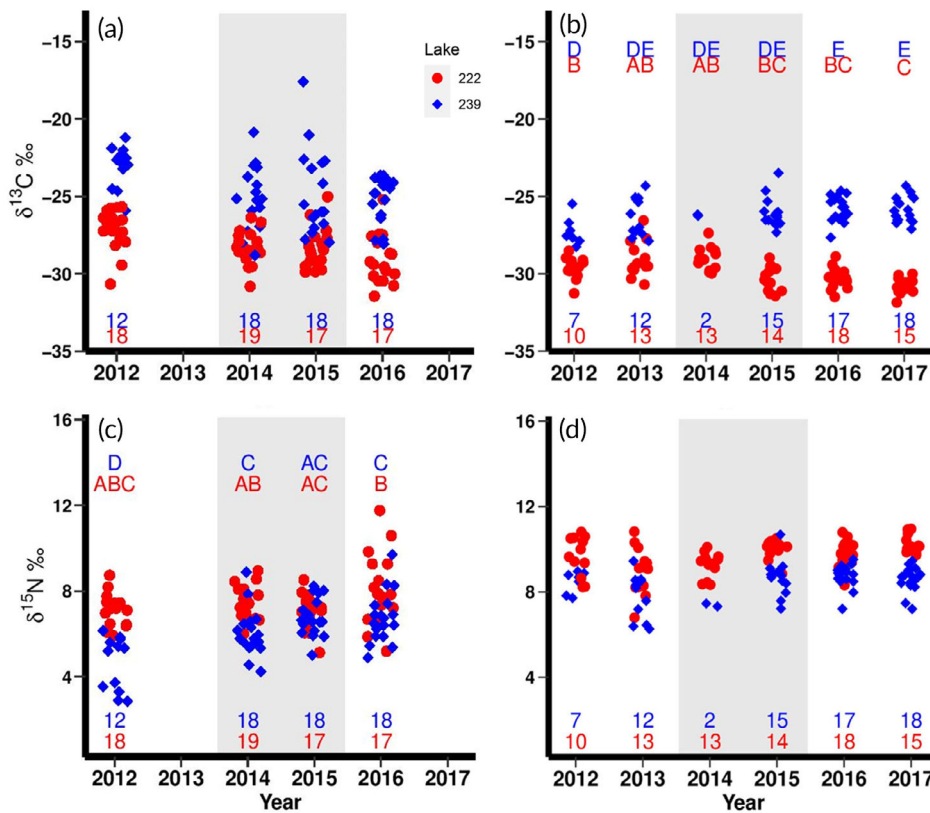


FIGURE 2 Interaction plot of mean lipid-corrected $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ muscle isotopic signatures of (b, d) northern pike (*Esox Lucius*) and (a, c) yellow perch (*Perca flavescens*) from a lake experiencing AgNP (silver nanoparticle) additions (Lake 222, red circles) and an unmanipulated reference lake (Lake 239, blue diamonds), 2012–2017. Gray shading indicates years of AgNP additions in Lake 222. Numbers along the x-axis indicate sample size for each year corresponding to each lake by color (222 = red, 239 = blue). Where significant year by lake interactions are present, uppercase letters indicate group associations both within and among lakes for each panel based on a post hoc Tukey's HSD (honestly significant difference) test from two-factor ANOVA (analysis of variance). Panels (a) and (c) have significant main effects of both year and lake.

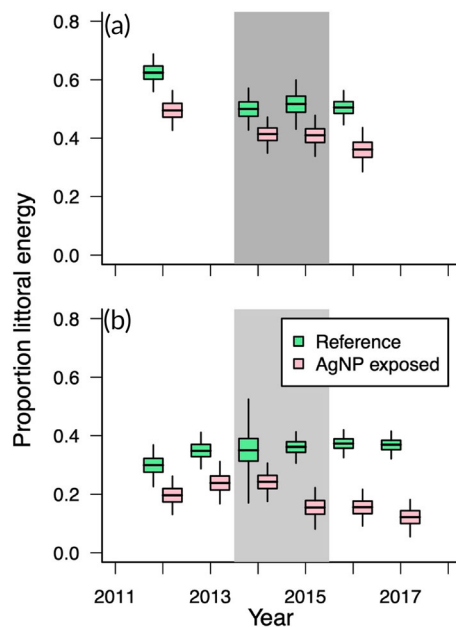


FIGURE 3 Posterior distributions of percentage littoral contribution estimates generated from Bayesian mixing models applied to (a) yellow perch (*Perca flavescens*) and (b) northern pike (*Esox lucius*) across lakes (reference Lake 239 and AgNP [silver nanoparticle] exposed Lake 222). Boxes show the interquartile range of posterior distributions, vertical lines are the 95% confidence intervals, and horizontal lines show the 50th percentile. Gray shading indicates years of AgNP additions in Lake 222.

$F_{1,144} = 134.4$, $p < 0.0001$; Figure 2d), and there was a significant main effect of year ($F_{5,144} = 9.4$, $p < 0.0001$), with $\delta^{15}\text{N}$ in both lakes generally increasing over time (Figure 2d). Diagnostic plots revealed that whereas variance was homogeneous, residuals were nonnormal and could not be corrected using log transformation.

We found no significant relationship between *P. flavescens* lipid-corrected $\delta^{13}\text{C}$ and body size for either lake but a significant relationship between $\delta^{15}\text{N}$ and body size in both fish species from both lakes (Supplementary Material A3). For *E. lucius*, there was a significant relationship between lipid-corrected $\delta^{13}\text{C}$ and body size in Lake 222 only and a significant relationship between $\delta^{15}\text{N}$ and body size in Lake 239 only. Although the mean size of *E. lucius* declined during the course of the experiment, changes in body size alone were not sufficient to explain temporal changes in isotopic values of fish in our lakes (Supplementary Material A3).

Estimates of percentage littoral contributions to the diet of both *P. flavescens* and *E. lucius* showed changes in fish from Lake 222 after the addition of AgNPs, which were different in either magnitude or direction from fish in reference Lake 239 (Figure 3). Posterior median estimates of *P. flavescens* absolute littoral resource use in Lake 222 declined from 50% before AgNP additions to 41% in the first year of AgNP addition (2014), and declined further to 36% in the post-addition year of 2016 (Figure 3a), nearly one third of littoral contributions prior to AgNP additions. The 95% credible intervals of posterior probabilities just barely overlapped between 2012 before AgNP addition (2.5% quantile, 0.427) and 2016 (97.5% quantile, 0.436).

P. flavescens in Lake 239 demonstrated a similar trend, though lesser in magnitude relative to pre-manipulation conditions, as posterior median estimates declined from 63% to approximately 50% during 2014–2016 or one-fifth of littoral contributions relative to pre-manipulation estimates. Whereas the 95% credible intervals of posterior probabilities just barely overlapped between 2012 (2.5% quantile, 0.560) and 2016 (97.5% quantile, 0.563) in Lake 239 *P. flavescens*, no directional, consistent decline in littoral energy was observed between 2014 and 2016, as was observed in fish exposed to AgNPs.

Percentage littoral contributions to *E. lucius* exposed to AgNPs were distinct from those observed in the reference Lake 239, as posterior median estimates of absolute littoral resource use in *E. lucius* from Lake 222 decreased from approximately 21% during 2012–2013 to 15% in both 2015 (second year of AgNP additions) and 2016 (first year after additions) and decreased further to only 12% in 2017 (second year after additions; Figure 3b). Although there was overlap in 95% credible intervals of posterior probabilities for *E. lucius* prior to AgNP exposure during 2012–2013, compared with AgNP-exposed fish during 2015–2017, differences were apparent at the interquartile range (Figure 3b). No such decline was observed in *E. lucius* from Lake 239.

4 | DISCUSSION

We observed significant changes toward more negative carbon isotope values in the tissues of both *P. flavescens* and *E. lucius* collected from Lake 222 during and after additions of nanosilver particles, though only the pattern for *E. lucius* was clearly distinguishable from the pattern in reference Lake 239. For *E. lucius*, this shift toward more negative $\delta^{13}\text{C}$ is consistent with a shift away from benthic-sourced carbon produced in the littoral zone toward pelagic-sourced carbon that was not reflected in an unmanipulated lake. Applying a Bayesian mixing model to these results, *P. flavescens* littoral resource use declined by one third of what it was before AgNP exposure in Lake 222 (change in the 50th percentile posterior probabilities between 2012 and 2016 relative to the estimate in 2012), compared to a reduction by only one fifth in *P. flavescens* littoral use in Lake 239 over the same period. Similarly, we observed a relative decline in *E. lucius* littoral resource use in Lake 222 after AgNP exposure by two-fifths (comparing the change in the mean 50th percentiles in 2012–2013 to that observed in 2017, relative to pre-addition conditions in 2012–2013), compared with a relative increase in Lake 239 by one-tenth over a similar period.

Supporting our observations of reduced littoral carbon consumed by fish in Lake 222 after AgNP exposure, reduced periphyton productivity due to AgNP exposure has been observed in controlled field-based experiments at IISD-ELA conducted in both our experimental Lake 222 and in reference Lake 239 (Norman et al., 2015). Others have reported changes in periphyton communities after AgNP exposure, where metal-sensitive species declined and were replaced by metal-tolerant species of bacteria (Grün et al., 2018). As littoral production via periphyton is associated with surface-attached algae,

deposition of AgNPs in sediments within the photic zone (e.g., top 2 m of the littoral zone of the lake) has the greatest potential for negative impacts on periphytic primary production. By accumulating in surface sediments, AgNPs provide a continual source of toxic Ag^+ through dissolution that we hypothesize suppressed littoral primary production in Lake 222, as reflected in reduced littoral energy use in *P. flavescens* and *E. lucius* after AgNP additions to the lake.

The measured pattern of AgNP deposition in lake sediments across the lake, both spatially and temporally, supports our proposed mechanism of periphyton production limiting littoral carbon usage in fish, as well as the persistent effects of AgNP exposure observed 2 years after additions ceased. In Lake 222, sedimentation rates of Ag during AgNP additions averaged 31 g per day across the entire lake (Conine, 2017). Sediment concentrations of total Ag at nearshore sites in Lake 222 were typically about 20% of those at the deepest point in the lake (Rearick, 2017), indicating that deposition in littoral regions of the lake was measurable and available to potentially suppress primary production by periphyton. Additionally, accumulation of Ag in sediments was observed over the course of the experiment; total Ag in the top 0–2 cm from sediment core samples collected from the deepest point of Lake 222 (where sampling was consistently performed across all years) increased 10-fold from 3 to 5 $\mu\text{g/g}$ dry weight on average in 2014 and 2015, respectively, to 50 $\mu\text{g/g}$ dry weight on average in the winter of 2017 (Rearick, 2017). This increase in surface sediment concentrations by an order of magnitude 2 years after the cessation of additions in 2015 also helps explain the continued reduction in littoral energy observed in the fish occupying this lake after AgNP additions, and is consistent with other recent studies (Geils et al., 2023; Slongo et al., 2022) indicating that impacts on the *E. lucius* population in this lake likely persisted after 2017.

No changes were observed in pelagic phytoplankton in Lake 222 as a result of AgNP additions (Conine et al., 2018), indicating that the concentrations of AgNP and dissolved Ag in the water column were not sufficient to alter pelagic productivity or dynamics. Unfortunately, we did not collect data for direct measurements of changes in benthic productivity in Lake 222 to be able to corroborate the results of our isotopic analyses, but we believe that the sum of evidence showing accumulation of Ag in sediment, a lack of change in pelagic phytoplankton, and changes in fish $\delta^{13}\text{C}$ values support the hypothesis that benthic productivity declined due to the impacts of AgNP accumulation in the sediments and that this had a significant impact on the carbon source supporting fish higher up the food chain.

Given the limitations of our dataset regarding poor availability of invertebrates to better characterize annual baselines in our study, we assumed that source carbon representing primary production in both the littoral and pelagic regions of the lake was relatively stable over the years where fish were collected from both lakes. The physical processes that can drive differences between littoral and pelagic source carbon (i.e., boundary layer effects resulting in higher $\delta^{13}\text{C}$ in littoral regions, wind mixing maintaining lower $\delta^{13}\text{C}$ in DIC fraction in pelagic zones) were unlikely to have changed dramatically in either the experimental or the reference lakes during the course of this study. Further, there was no clear evidence of a change in the trophic state of Lake

222 during the study that might influence baseline $\delta^{13}\text{C}$, as mean annual total phosphorus (TP) in Lake 222 ranged from 11.4 $\mu\text{g/L}$ in 2013 to 13.4 $\mu\text{g/L}$ in 2015. Based on other studies that have shown changes in $\delta^{13}\text{C}$ as a consequence of increased TP (Perga & Gerdeaux, 2006), a change on the scale of 50–70 $\mu\text{g/L}$ in TP would be required to change fish $\delta^{13}\text{C}$ by the 2‰–3‰ that we observed in Lake 222. However, consistent and regular sampling of the lower trophic levels would help remove uncertainty regarding our assumption of baseline stability in our lakes.

A reduction in littoral energy use by fish in Lake 222 is also consistent with the patterns of changes in gross food consumption by *P. flavescens* observed during this experiment (Hayhurst et al., 2020). *P. flavescens* in Lake 222 are primarily zooplanktivorous in their first and second years but mostly benthivorous later in life (Hayhurst, 2018). Correspondingly, the gross food consumption by *P. flavescens* during benthivorous life stages in Lake 222 (i.e., age 3–6) declined from c. 600 kg/ha before AgNP additions to only 100 kg/ha post-addition in 2016 (Hayhurst et al., 2020). Across the entire *P. flavescens* population, total gross food consumption estimates declined by 50% from c. 1200 kg/ha in 2012 before AgNP additions to only c. 600 kg/ha post-addition in 2016. Combining these total gross consumption declines with our estimates of changes in carbon source utilization over the course of the experiment suggests that 74% of the observed decline in gross consumption was associated with reductions in littoral-sourced carbon. Similarly, gross consumption in *E. lucius* declined by nearly 60% (Geils et al., 2023). Considering changes in littoral carbon use reported here, 46% of the total decline in *E. lucius* gross consumption in 2017 relative to pre-manipulation (2012–2013) is associated with reductions in littoral carbon. This value closely reflects the reduction in the abundance of *P. flavescens* over the same time period, whose density declined by nearly half in Lake 222 (44%; Geils et al., 2023). The relative abundance of the only other prey fish in the lake, the blacknose shiner *Notropis heterolepis* (Eigenmann & Eigenmann, 1893), declined to 10% of pre-addition levels (Slongo et al., 2022).

The shift in resource use from benthic production by *E. lucius* was relatively large compared with that observed in *P. flavescens*, indicating that this response was not simply a consequence of changes in the isotopic composition of the preferred prey of *E. lucius* (i.e., *P. flavescens*) but also potentially an additional consequence of diet switching in this species. The dramatic declines in the primarily zooplanktivorous *N. heterolepis* (Roberts et al., 2006) may have been a result of increased predation by *E. lucius* as the abundance of *P. flavescens* declined (Slongo et al., 2022). Therefore, the greater change in *E. lucius* after AgNP additions was likely due to the combined effects of a shift toward greater consumption of a pelagic zooplankton specialist, in addition to the reduction in littoral-sourced carbon observed in *P. flavescens*. This is also supported by the relative stability of $\delta^{15}\text{N}$ values of *E. lucius*, indicating that any potential diet switching by *E. lucius* was at an equivalent trophic position (e.g., among prey fish), as opposed to shifting toward invertebrates, such as reported by Venturelli and Tonn (2006).

It is possible (but not necessary) that changes in littoral carbon sources observed in Lake 222 were also associated with resource limitation (e.g., reduced littoral resource availability in the form of benthic invertebrates). Although resource limitation can potentially alter TDFs of carbon isotopic signatures (Barnes et al., 2007), the reported magnitude of these impacts is small (0.2‰), comparable to instrument measurement error and repeatability for this isotope (i.e., 0.15‰, see “Methods” section). However, such changes would be in the direction we observed here, toward more negative $\delta^{13}\text{C}$ values. Under the assumption that large reductions in food availability might be capable of causing greater changes in TDF than are currently reported in the literature, to generate changes to the degree observed in our study (2‰–3‰ $\delta^{13}\text{C}$), the magnitude of TDF changes would have to be quite large, effectively causing TDF to shift from the values currently applied to negative values for *P. flavescens* and effectively becoming zero for *E. lucius* over the course of the study.

Another alternative explanation for the changes in $\delta^{13}\text{C}$ values in fish exposed to AgNPs is a possible increase in littoral heterotrophy in Lake 222, resulting in a carbon pool that is increasingly depleted in ^{13}C isotopes for autotrophs, but we do not believe this to be the case for several reasons. In studies of periphyton exposed to AgNPs, Gil-Allué et al. (2018) observed a large, dose-dependent reduction in total autotrophic production, which was also correlated with a small increase in bacterial production. An increase in carbon sourced through bacterial production would be expected to generate more negative $\delta^{13}\text{C}$ in the available DIC pool, because respired CO_2 produced by bacteria is ^{13}C depleted (Rau, 1978). However, the decline in periphyton (autotrophic) production (i.e., a measured decrease in 300–400 $\mu\text{g g/C/day}$) reported by Gil-Allué et al. (2018) was two orders of magnitude greater than the increase observed in bacterial (heterotrophic) production in their study (i.e., a measured increase in 2–3 $\mu\text{g g/C/day}$), making it unlikely that such a small increase in bacterial production relative to a comparatively massive decrease in autotrophic production could contribute substantially to more negative $\delta^{13}\text{C}$ values at the base of the benthic food chain. Alternatively, although an increased direct utilization of bacterial production by primary consumers would act to make $\delta^{13}\text{C}$ more positive, this same study indicates that impacts of bacterial production in either direction would be small, given that our observations were toward more pelagic resources and shift toward potential direct utilization of bacterial production is overwhelmed by shifts toward pelagic carbon. Further, an increase in bacterial production (i.e., increase in direct utilization of heterotrophic resources) would be expected to increase basal $\delta^{15}\text{N}$ values (Hoch et al., 1996) that would then be expressed up the food chain, which was not observed in the current study. Based on these findings, we conclude that it is unlikely that such relatively small increases in heterotrophy would influence littoral baselines.

Though water level changes were observed in Lake 222, we do not believe they are responsible for the isotopic changes we observed in fishes. Starting in the spring of 2015 and continuing through the post-addition phase, beaver activity at the Lake 222 outflow increased water level by c. 0.25–0.5 m, submerging c. 1 m of shoreline around the lake. Based on several lines of evidence, we believe this

modest increase in water level had little impact on fish isotopic values reported here. First, the flooding experienced in this experiment was small in scale (less than 1% increase in surface area, 5%–9% by lake volume) compared to studies that have reported the impacts of flooding on fish isotopic values involving changes in water level and volumetric increases that were orders of magnitude greater than those observed in Lake 222 (Paterson et al., 2019; Perga et al., 2005; Wang et al., 2011). The changes reported in these studies were also not consistent in direction, with more positive organismal values observed by Perga et al. (2005) and Wang et al. (2011), more negative values observed by Paterson et al. (2019) and Ponton et al. (2021), and both positive and negative changes observed by Tadoléké et al. (2012).

The prolonged whole-lake response to environmentally relevant levels of AgNPs reported here indicates that long-term exposure to these nanoparticles represents a potential threat to ecosystem stability and function. The coupling between benthic and pelagic food webs for freshwater fishes occurs extensively in aquatic ecosystems (Vadeboncoeur et al., 2002). Although the littoral benthic community is an important component of the ecosystem in most lakes, lake size and biotic and abiotic factors in the lake govern the significance of the littoral community to overall production (Fee & Hecky, 1992; Lodge et al., 1998). In relatively shallow and small lakes, such as Lake 222, littoral benthic production would be expected to have greater importance and therefore reflect greater impacts from exposures to AgNPs. Further, the coupling of benthic and pelagic resources contributes significantly to ecosystem stability in the face of disturbances. In aquatic ecosystems supported primarily by pelagic resources, energy is transferred relatively quickly, leading to cyclic patterns of resource availability to consumers that can quickly become destabilized by disturbances (McCann et al., 1998; Rooney & McCann, 2012). In contrast, benthic resources represent a “slow” channel of energy production that can help buffer consumer populations from fluctuations in pelagic energy streams and large perturbations (Rooney et al., 2006). Thus, a reduction in benthic–pelagic coupling in response to AgNP exposure has the potential to make ecosystems less resilient to other disturbances, such as climate change or invasive species.

The results reported here highlight the need to evaluate directly how AgNP deposition and subsequent accumulation in sediments can impact benthic communities, which would provide a better understanding of the fate and effects of AgNPs and other materials prone to settling in lake ecosystems. Previous studies have focused mainly on the toxic effects of AgNPs in suspension and have not evaluated the potential for effects on benthic organisms once nanoparticles are deposited to sediments. Whereas there are guidelines in Canada for the protection of freshwater organisms from long-term exposure to dissolved silver (i.e., 0.25 µg/L), no sediment quality guidelines for nanosilver currently exist (CCME, 2015). Although the concentrations of total Ag observed in Lake 222 sediments are one order of magnitude greater than the maximum concentration of c. 0.5 µg/g dry weight reported in sediments near WWTPs in western Lake Ontario (Sultana et al., 2018), they may represent future contamination scenarios if Ag accumulations continue near wastewater discharges. Based on the results of the present study, we recommend more

research effort to directly measure the toxic effects of nanoparticles to benthic organisms and, in particular, changes to benthic production in response to AgNPs in sediments. Studies such as these will hopefully broaden the scope of risk assessments related to the release of AgNPs into aquatic environments.

AUTHOR CONTRIBUTIONS

The whole-lake experiment was conceived by Chris Metcalfe, along with several other researchers at Trent University who secured funding for the whole-lake experiment. Isotopic analyses were funded by Michael Rennie. Michael Rennie and Lauren Hayhurst conceived the isotope study. Lauren Hayhurst and Michael Rennie collected the samples in the field. Fish were dissected and invertebrates were identified in the lab by Lauren Hayhurst, and fish length data were compiled by Lauren Hayhurst. Tyler Ripku and Lauren Hayhurst prepared samples for stable isotopes analysis. Tyler Ripku and Michael Rennie analysed data and prepared figures. Tyler Ripku wrote the first draft of the manuscript, and Michael Rennie and Chris Metcalfe contributed subsequent sections of the manuscript. All authors contributed to manuscript edits and approved the final submitted version.

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REFERENCES

- Ale, A., Rossi, A. S., Bacchetta, C., Gervasio, S., de la Torre, F. R., & Cazenave, J. (2018). Integrative assessment of silver nanoparticles toxicity in *Prochilodus lineatus* fish. *Ecological Indicators*, 93, 1190–1198. <https://doi.org/10.1016/j.ecolind.2018.06.023>
- Barnes, C., Sweeting, C., Jennings, S., Barry, J., & Polunin, N. (2007). Effect of temperature and ration size on carbon and nitrogen stable isotope trophic fractionation. *Functional Ecology*, 21(2), 356–362.

- Battin, T. J., Besemer, K., Bengtsson, M. M., Romani, A. M., & Packmann, A. I. (2016). The ecology and biogeochemistry of stream biofilms. *Nature Reviews Microbiology*, 14(4), 251–263. <https://doi.org/10.1038/nrmicro.2016.15>
- Beaudoin, C. P., Tonn, W. M., Prepas, E. E., & Wassenaar, L. I. (1999). Individual specialization and trophic adaptability of northern pike (*Esox lucius*): An isotope and dietary analysis. *Oecologia*, 120(3), 386–396. <https://doi.org/10.1007/s004420050871>
- 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. *Science of the Total Environment*, 390(2–3), 396–409. <https://doi.org/10.1016/j.scitotenv.2007.10.010>
- Canseco, J., Niklitschek, E., & Harrod, C. (2022). Variability in δC and δN trophic discrimination factors for teleost fishes: A meta-analysis of temperature and dietary effects. *Review in Fish Biology and Fisheries*, 32(2), 1–17. <https://doi.org/10.1007/s11160-021-09689-1>
- CCME. (2015). *Canadian water quality guidelines for the protection of aquatic life: Silver*. Canadian Council of Ministers of the Environment.
- Cetinic, K. A. (2019). The effects of silver nanoparticles on lower trophic levels in aquatic ecosystems, Canada. Doctoral thesis, Trent University, Peterborough, ON.
- Chandra, S., Vander Zanden, M., Heyvaert, A., Richards, B., & Goldman, C. (2005). The effects of cultural eutrophication on the coupling between pelagic primary producers and benthic consumers. *Limnology and Oceanography*, 50(5), 1368–1376.
- Chen, G., Zhou, H., Ji, D., & Gu, B. (2012). Stable isotope enrichment in muscle, liver, and whole fish tissues of brown-marbled groupers (*Epinephelus fuscoguttatus*). *Ecological Processes*, 1(1), 1–5. <https://doi.org/10.1186/2192-1709-1-7>
- Colman, B. P., Espinasse, B., Richardson, C. J., Matson, C. W., Lowry, G. V., Hunt, D. E., & Bernhardt, E. S. (2014). Emerging contaminant or an old toxin in disguise? Silver nanoparticle impacts on ecosystems. *Environmental Science and Technology*, 48, 5229–5236. <https://doi.org/10.1021/es405454v>
- Conine, A. L. (2017). The effects of particulate matter on the fate and toxicity of silver nanoparticles. (Doctoral thesis, Trent University, Peterborough, ON). Available at <http://digitalcollections.trentu.ca/objects/etd-540>
- 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. *Association for the Science of Limnology and Oceanography*, 3, 311–319. <https://doi.org/10.1002/ol.2.10071>
- Das, P., Metcalfe, C. D., & Xenopoulos, M. (2014). Interactive effects of silver nanoparticles and phosphorus on phytoplankton growth in natural waters. *Environmental Science and Technology*, 48(8), 4573–4580. <https://doi.org/10.1021/es405039w>
- Elumelu, M., Ort, C., Mueller, E., Voegelin, A., Thalmann, B., Kaegi, R., & Hagendorfer, H. (2013). Fate and transformation of silver nanoparticles in urban wastewater systems. *Water Research*, 47, 3866–3877. <https://doi.org/10.1016/j.watres.2012.11.060>
- Fee, E. J., & Hecky, R. E. (1992). Introduction to the Northwest Ontario lake size series (NOLSS). *Canadian Journal of Fisheries and Aquatic Sciences*, 49(12), 2434–2444. <https://doi.org/10.1139/f92-269>
- Fincel, M. J., Vandehey, J. A., & Chipps, S. R. (2012). Non-lethal sampling of walleye for stable isotope analysis: A comparison of three tissues. *Fisheries Management and Ecology*, 19(4), 283–292.
- Francis, T., Schindler, D., Holtgrieve, G., Larson, E., Scheurell, M., Semmens, B., & Ward, E. (2011). Habitat structure determines resource use by zooplankton in temperate lakes. *Ecology Letters*, 14(4), 364–372.
- Geils, K. M., Slongo, B. D., Hayhurst, L. D., Ripku, T., Metcalfe, C. D., & Rennie, M. D. (2023). Consumption and activity decline in Northern Pike (*Esox lucius*) during and after silver nanoparticle addition to a lake. *Aquatic Toxicology*, 257, 106458. <https://doi.org/10.1016/j.aquatox.2023.106458>
- Gil-Allué, C., Tlili, A., Schirmer, K., Gessner, M. O., & Behra, R. (2018). Long-term exposure to silver nanoparticles affects periphyton community structure and function. *Environmental Science: Nano*, 5, 1397–1407. <https://doi.org/10.1039/c8en00132d>
- Gottschalk, F., Kost, E., & Nowack, B. (2013). Engineered nanomaterials in water and soils: A risk quantification based on probabilistic exposure and effect modeling. *Environmental Toxicology and Chemistry*, 32(6), 1278–1287.
- Grün, A. Y., App, C. B., Breidenbach, A., Meier, J., Metreveli, G., Schaumann, G. E., & Manz, W. (2018). Effects of low dose silver nanoparticle treatment on the structure and community composition of bacterial freshwater biofilms. *PLoS One*, 13(6), e0199132. <https://doi.org/10.1371/journal.pone.0199132>
- Hayhurst, L. D. (2018). Bioenergetic evaluation of a whole-lake nanosilver addition on Yellow Perch (*Perca flavescens*). Master's Thesis. Available at <http://knowledgecommons.lakeheadu.ca/handle/2453/4204>
- 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. *Archives of Environmental Contamination and Toxicology*, 79, 283–297. <https://doi.org/10.1007/s00244-020-00764-5>
- Hecky, R. E., & Hesslein, R. A. (1995). The importance of benthic algal carbon to food webs in tropical, temperate and arctic lakes. *Journal of the North American Benthological Society*, 14, 631–653.
- Hoch, M. P., Snyder, R. A., Cifuentes, L. A., & Coffin, R. B. (1996). Stable isotope dynamics of nitrogen recycled during interactions among marine bacteria and protists. *Marine Ecology Progress Series*, 132(1–3), 229–239. <https://doi.org/10.3354/meps132229>
- Janković, N. Z., & Plata, D. L. (2019). Engineered nanomaterials in the context of global element cycles. *Environmental Science: Nano*, 6(9), 2697–2711. <https://doi.org/10.1039/c9en00322c>
- 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., Ort, C., Sinnet, B., Thalmann, B., Krimer, J., Hagendorfer, H., Elumelu, M., & Mueller, E. (2013). Fate and transformation of silver nanoparticles in urban wastewater systems. *Water Research*, 47, 3866–3877. <https://doi.org/10.1016/j.watres.2012.11.060>
- Kaegi, R., Voegelin, A., Sinnet, B., Zuleeg, S., Siegrist, H., & Burkhardt, M. (2015). Transformation of AgCl nanoparticles in a sewer system—a field study. *Science of the Total Environment*, 535, 20–27. <https://doi.org/10.1016/j.scitotenv.2014.12.075>
- Kidd, K., Patterson, M., Rennie, M., Podemski, C., Findlay, D., Blanchfield, P., & Liber, K. (2014). Direct and indirect responses of a freshwater food web to a potent synthetic oestrogen. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 369(1656), 20130578.
- Kraas, M., Schlich, K., Knopf, B., Wege, F., Kägi, R., Terytze, K., & Hundrinke, K. (2017). Long-term effects of sulfidized silver nanoparticles in sewage sludge on soil microflora. *Environmental Toxicology and Chemistry*, 36, 3305–3313. <https://doi.org/10.1002/etc.3904>
- Laban, G., Nies, L. F., Turco, R. F., Bickham, J. W., & Sepúlveda, M. S. (2009). The effects of silver nanoparticles on fathead minnow (*Pimephales promelas*) embryos. *Ecotoxicology*, 19, 185–195. <https://doi.org/10.1007/s10646-009-0404-4>
- Leonardo, T., Farhi, E., Pouget, S., Motellier, S., Boisson, A. M., Banerjee, D., Rebeille, F., Den Auwer, C., & Rivasseau, C. (2016). Silver accumulation in the green microalga *Coccomyxa actinobiotis*: Toxicity, *in situ* speciation and localization investigated using synchrotron XAS, XRD and TEM. *Environmental Science and Technology*, 50, 359–367. <https://doi.org/10.1021/acs.est.5b03306>

- Liu, J. F., Chao, J. B., Liu, R., Tan, Z. Q., Yin, Y. G., Wu, Y., & Jiang, G. B. (2009). Cloud point extraction as an advantageous preconcentration approach for analysis of trace silver nanoparticles in environmental waters. *Analytical Chemistry*, 81(15), 6496–6502. <https://doi.org/10.1021/ac900918e>
- Lodge, D. M., Stein, R. A., Brown, K. M., Covich, A. P., Brönmark, C., Garvey, J. E., & Klosiewskt, S. P. (1998). Predicting impact of freshwater exotic species on native biodiversity: Challenges in spatial scaling. *Australian Journal of Ecology*, 23(1), 53–67. <https://doi.org/10.1111/j.1442-9993.1998.tb00705.x>
- Logan, J., Jardine, T., Miller, T., Bunn, S. E., Cunjak, R., & Lutcavage, M. (2008). Lipid corrections in carbon and nitrogen stable isotope analysis: Comparison of chemical extraction and modelling methods. *Journal of Animal Ecology*, 77, 838–846.
- 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. *Environmental Science and Technology*, 46, 7027–7036. <https://doi.org/10.1021/es204608d>
- Magesky, A., & Pelletier, É. (2018). Cytotoxicity and physiological effects of silver nanoparticles on marine invertebrates. *Advances in Experimental Medicine and Biology*, 1048, 285–309. https://doi.org/10.1007/978-3-319-72041-8_17
- Martin, J. D., Colson, T. L., Langlois, V. S., & Metcalfe, C. D. (2017). Biomarkers of exposure to nanosilver and silver accumulation in yellow perch (*Perca flavescens*). *Environmental Toxicology and Chemistry*, 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. *Environmental Science and Technology*, 52, 11114–11122. <https://doi.org/10.1021/acs.est.8b03146>
- McCann, K., Hasting, A., & Huxel, G. R. (1998). Weak trophic interactions and the balance of nature. *Nature*, 395, 794–798. https://doi.org/10.1007/978-3-319-06257-0_9
- Norman, B. C., Frost, P. C., Blakelock, G. C., Higgins, S. N., Hoque, M. E., Vincent, J. L., Cetinic, K., & Xenopoulos, M. (2019). Muted response to Ag accumulation by plankton to chronic and pulse exposure to silver nanoparticles in a boreal lake. *Facets*, 4, 566–583. <https://doi.org/10.1139/facets-2018-0047>
- Norman, B. C., Xenopoulos, M. A., Braun, D., & Frost, P. C. (2015). Phosphorus availability alters the effects of silver nanoparticles on periphyton growth and stoichiometry. *PLoS One*, 10(6), 1–13. <https://doi.org/10.1371/journal.pone.0129328>
- Notter, D. A., Mitrano, D. M., & Nowack, B. (2014). Are nanosized or dissolved metals more toxic in the environment? A meta-analysis. *Environmental Toxicology and Chemistry*, 33(12), 2733–2739. <https://doi.org/10.1002/etc.2732>
- Paterson, M. J., Beaty, K. G., Findlay, D. L., Findlay, W. J., Schiff, S. L., St. Louis, V. L., & Venkiteswaran, J. J. (2019). Long-term changes in nutrient dynamics and plankton communities following the creation of a new reservoir. *Canadian Journal of Fisheries and Aquatic Sciences*, 76, 1459–1468. <https://doi.org/10.1139/cjfas-2018-0197>
- Paterson, M. J., Podemski, C. L., Findlay, W. J., Findlay, D. L., & Salki, A. G. (2010). The response of zooplankton in a whole-lake experiment on the effects of a cage aquaculture operation for rainbow trout (*Oncorhynchus mykiss*). *Canadian Journal of Fisheries and Aquatic Sciences*, 67(11), 1852–1861.
- Peijnenburg, W. J. G. M., Baalousha, M., Chen, J., Chaudry, Q., Von Der Kammer, F., Kuhlbusch, T. A. J., Lead, J., Nickel, C., Quik, J. T. K., Renker, M., Wang, Z., & Koelmans, A. A. (2015). A review of the properties and processes determining the fate of engineered nanomaterials in the aquatic environment. *Critical Reviews in Environmental Science and Technology*, 45(19), 2084–2134. <https://doi.org/10.1080/10643389.2015.1010430>
- Perga, M. E., Arfi, R., & Gerdeaux, D. (2005). Seasonal variations in fish $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in two West African reservoirs, Sélingué and Manatali (Mali): Modifications of trophic links in relation to water level. *Isotopes in Environmental and Health Studies*, 41(2), 109–123. <https://doi.org/10.1080/10256010500131734>
- Perga, M., & Gerdeaux, D. (2006). Seasonal variability in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the zooplankton taxa in two alpine lakes. *Acta Oecologica*, 30(1), 69–77.
- Ponton, D. E., Lavoie, R. A., Leclerc, M., Bilodeau, F., Planas, D., & Amyot, M. (2021). Understanding food web mercury accumulation through trophic transfer and carbon processing along a river affected by recent run-of-river dams. *Environmental Science and Technology*, 55, 2949–2959. <https://doi.org/10.1021/acs.est.0c07015>
- Post, D. M. (2002). Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology*, 83(3), 703–718. [https://doi.org/10.1890/0012-9658\(2002\)083\[0703:USITET\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2)
- Pourzahedi, L., Vance, M., & Eckelman, M. J. (2017). Life cycle assessment and release studies for 15 nanosilver-enabled consumer products: Investigating hotspots and patterns of contribution. *Environmental Science and Technology*, 51(12), 7148–7158. <https://doi.org/10.1021/acs.est.6b05923>
- Quinn, G., & Keough, M. (2002). *Experimental design and data analysis for biologists*. Cambridge University Press.
- R Core Team. (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. <https://www.R-project.org/>
- Rau, G. (1978). Carbon-13 depletion in a subalpine lake: Carbon flow implications. *Science*, 201(4359), 901–902. <https://doi.org/10.1126/science.201.4359.901>
- Rearick, D. C. (2017). Fate and effects of silver nanoparticle addition in a Lake ecosystem, Canada. (Doctoral thesis, Trent University, Peterborough, ON). Available at <http://digitalcollections.trentu.ca/objects/etd-577>
- 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(8), 1–18. <https://doi.org/10.1371/journal.pone.0201412>
- Rennie, M., Evans, D., & Young, J. (2013). Increased dependence on near-shore benthic resources in Lake Simcoe ecosystem after dreissenid invasion. *Inland Waters*, 3(2), 297–310.
- Roberts, K. N., Lund, T., Hayden, B., & Poesch, M. (2022). Season and species influence stable isotope ratios between lethally and nonlethally sampled tissues in freshwater fish. *Journal of Fish Biology*, 100(1), 229–241.
- Roberts, M. E., Burr, B. M., Whiles, M. R., & Santucci, V. J. (2006). Reproductive ecology and food habits of the blacknose shiner, *Notropis heterolepis*, in Northern Illinois. *American Midland Naturalist*, 155(1), 70–83. [https://doi.org/10.1674/0003-0031\(2006\)155\[0070:REAFHO\]2.0.CO;2](https://doi.org/10.1674/0003-0031(2006)155[0070:REAFHO]2.0.CO;2)
- Rooney, N., & McCann, K. S. (2012). Integrating food web diversity, structure and stability. *Trends in Ecology and Evolution*, 27(1), 40–46. <https://doi.org/10.1016/j.tree.2011.09.001>
- Rooney, N., McCann, K., Gellner, G., & Moore, J. C. (2006). Structural asymmetry and the stability of diverse food webs. *Nature*, 442(7100), 265–269. <https://doi.org/10.1038/nature04887>
- Schindler, D. (1974). Eutrophication and recovery in experimental lakes: Implications for lake management. *Science*, 195(184), 260–262. <https://doi.org/10.1126/science.195.4275.260>
- Schlich, K., Hoppe, M., Kraas, M., Schubert, J., Chanana, M., & Hundrinke, K. (2018). Long-term effects of three different silver sulfide nanomaterials, silver nitrate and bulk silver sulfide on soil microorganisms and plants. *Environmental Pollution*, 242, 1850–1859. <https://doi.org/10.1016/j.envpol.2018.07.082>

- Scott, W. B., & Crossman, E. J. (1998). *Freshwater fishes of Canada*. Galt House Publications.
- 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. *Toxicology Science*, 115, 521–534.
- Shrivastava, S., Bera, T., Roy, A., Singh, G., Ramachandrarao, P., & Dash, D. (2007). Characterization of enhanced antibacterial effects of novel silver nanoparticles. *Nanotechnology*, 18(22), 103–112.
- Slongo, B. D., Hayhurst, L. D., Drombolis, P. C. T., Metcalfe, C. D., & Rennie, M. D. (2022). Whole-lake nanosilver additions reduce northern pike (*Esox lucius*) growth. *Science of the Total Environment*, 838, 156219. <https://doi.org/10.1016/j.scitotenv.2022.156219>
- Stevenson, L. M., Dickson, H., Klanjscek, T., Keller, A. A., McCauley, E., & Nisbet, R. M. (2013). Environmental feedbacks and engineered nanoparticles: Mitigation of silver nanoparticle toxicity to *Chlamydomonas reinhardtii* by algal-produced organic compounds. *PLoS One*, 8(9), e74456. <https://doi.org/10.1371/journal.pone.0074456>
- Stock, B., Jackson, A., Ward, E., Parnell, A., Philips, D., & Semmens, B. (2018). Analyzing mixing systems using a new generation of Bayesian tracer mixing models. *PeerJ*, 6, e5096. <https://doi.org/10.7717/peerj.5096>
- Stock, B. C., & Semmens, B. X. (2016). Unifying error structures in commonly used biotracer mixing models. *Ecology*, 97(10), 2562–2569. <https://doi.org/10.1002/ecy.1517>
- Stock, B. C., & Semmens, B. X. (2018). MixSIAR GUI User Manual. Version 3.1. <https://github.com/brianstock/MixSIAR/>
- Sultana, T., Metcalfe, C. D., Newman, K., Helm, P., Martin, J., Kleywegt, S., & Shen, L. (2018). Silver near municipal wastewater discharges into western Lake Ontario. *Canada. Environmental Monitoring and Assessment*, 190(9), 1–12. <https://doi.org/10.1007/s10661-018-6922-x>
- Sun, T. Y., Gottschalk, F., Hungerbühler, K., & Nowack, B. (2014). Comprehensive probabilistic modelling of environmental emissions of engineered nanomaterials. *Environmental Pollution*, 185, 69–76. <https://doi.org/10.1016/j.envpol.2013.10.004>
- Tadonlélé, R. D., Marty, J., & Planas, D. (2012). Assessing factors underlying variation of CO₂ emissions in boreal lakes vs. reservoirs. *FEMS Microbiology Ecology*, 79, 282–297.
- Vadeboncoeur, Y., Vander Zanden, M. J., & Lodge, D. M. (2002). Putting the lake back together: Reintegrating benthic pathways into lake food web models. *Bioscience*, 52(1), 44–54. [https://doi.org/10.1641/0006-3568\(2002\)052\[0044:PTLBTR\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2002)052[0044:PTLBTR]2.0.CO;2)
- van Aerle, R., Lead, J. R., Mitov, S., Santos, E. M., Stone, V., Tyler, C. R., & Scown, T. M. (2010). Effects of aqueous exposure to silver nanoparticles of different sizes in rainbow trout. *Toxicological Sciences*, 115(2), 521–534. <https://doi.org/10.1093/toxsci/kfq076>
- Vander Zanden, M. J., Vadeboncoeur, Y., & Chandra, S. (2011). Fish reliance on littoral-benthic resources and the distribution of primary production in lakes. *Ecosystems*, 14, 894–903. <https://doi.org/10.1007/s10021-011-9454-6>
- 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. *Transactions of the American Fisheries Society*, 135, 1512–1522.
- Wang, Y. Y., Yu, X. B., Li, W. H., Xu, J., Chen, Y. W., & Fan, N. (2011). Potential influence of water level changes on energy flows in a lake food web. *Chinese Science Bulletin*, 56(26), 2794–2802. <https://doi.org/10.1007/s11434-011-4649-y>
- Wellman, S., Kidd, K., Podemski, C., Blanchfield, P., & Paterson, M. (2017). Incorporation of wastes by native species during and after an experimental aquaculture operation. *Freshwater Science*, 36(2), 387–401.
- Yoo-iam, M., Chaichana, R., & Satapanajaru, T. (2014). Toxicity, bioaccumulation and biomagnification of silver nanoparticles in green algae (*Chlorella* sp), water flea (*Moina macrocopa*), blood worm (*Chironomus* spp.) and silver barb (*Barbonymus gonionotus*). *Chemical Speciation and Bioavailability*, 26(4), 257–265. <https://doi.org/10.3184/095422914X14144332205573>

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