Environmental Science & Technology

Article

Cite This: Environ. Sci. Technol. 2018, 52, 11114−11122

pubs.acs.org/est

Accumulation of Silver in Yellow Perch (*Perca flavescens*) and Northern Pike (*Esox lucius*) From a Lake Dosed with Nanosilver

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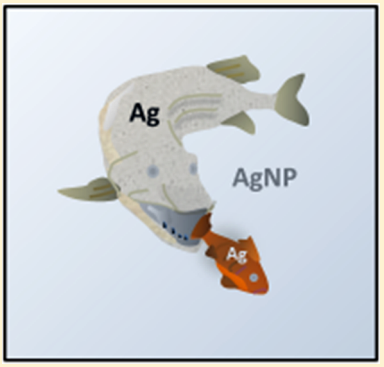
Supporting Information

ABSTRACT: A total of 15 kg of silver nanoparticles (AgNPs) was added continuously over two ice-free field seasons to a boreal lake (i.e., Lake 222) at the IISD Experimental Lakes Area in Canada. We monitored the accumulation of silver (Ag) in the tissues of yellow perch (*Perca flavescens*) and northern pike (*Esox lucius*) exposed to the AgNPs under environmentally relevant conditions. The greatest accumulation was observed in the liver tissues of pike, and a single pike sampled in the second year of additions had the highest concentration observed in liver of 5.1 micrograms per gram of wet weight. However, the Ag concentrations in gill and muscle tissue of both pike and perch did not exceed 0.35 micrograms per gram of wet weight. Following additions of AgNP, the Ag residues in fish tissues declined, with a half-life of Ag in pike liver of 119 days. Monitoring using passive sampling devices and single-particle inductively coupled plasma mass spectrometry during the AgNP addition phase confirmed that Ag nanoparticles were present in the water column and that estimated mean concentrations of Ag increased over time to a maximum of 11.5 μg/L. These data indicate that both a forage fish and a piscivorous fish accumulated Ag in a natural lake ecosystem dosed with AgNPs, leading to Ag concentrations in some tissues of the piscivorous species that were 3 orders of magnitude greater than the concentrations in the water.



INTRODUCTION

Silver nanoparticles (AgNPs) are used as additives in several hundred products, including textiles, antibacterial creams, and consumer goods.[1] Through their use in many of these products, AgNPs may be transported via domestic sewage into wastewater treatment plants (WWTP). The effluents of WWTPs may be a major point source, although AgNPs are expected to go through transformations in these systems.[2−4] Nonetheless, depending upon the degree of removal of AgNPs, there is potential for AgNPs and transformation products to be discharged from WWTPs in amounts predicted from models to yield concentrations in surface waters in the nanograms per liter range,[5] although more-recent estimates of emissions of nanomaterials into the environment are greater due to the rapid increase in production volumes.[6] In addition to inputs from WWTPs, AgNPs may enter aquatic ecosystems from industrial discharges and from diffuse sources.[7]

Once AgNPs are released into the aquatic environment, dissolution and agglomeration are the most-important transformation processes.[8] The extent of these transformations will depend on the physicochemical characteristics within the ecosystem, such as concentrations of dissolved organic carbon (DOC), pH, ionic strength, and redox as well as the particle size and surface coating.[9] There is evidence that the majority of AgNP toxicity to aquatic organisms is due to exposure to Ag^+ released from the AgNPs.[10] However, developmental effects were observed in early life stages of fish exposed to AgNPs that were not observed when fish were exposed to Ag^+.[11]

Our previous studies with yellow perch (*Perca flavescens*) exposed in the laboratory to AgNPs and Ag^+ prepared from $AgNO_3$ showed that Ag concentrations in gill, liver, and muscle tissue increased over time in all treatments.[12] In studies with rainbow trout (*Oncorhynchus mykiss*) exposed to AgNPs, the greatest Ag accumulation was observed in the liver.[13,14] In rainbow trout exposed to Ag^+ in solution, Ag rapidly accumulated in gill tissue and then was transported to the liver.[15,16] In rainbow trout exposed to different sizes of AgNP,

Received: June 9, 2018
Revised: August 8, 2018
Accepted: September 4, 2018
Published: September 4, 2018

DOI: 10.1021/acs.est.8b03146
Environ. Sci. Technol. 2018, 52, 11114−11122

the fish exposed to the smallest particles (i.e., 10 nm) accumulated the highest concentrations of Ag in the gills and liver.[17] Moreover, when rainbow trout were exposed to different types of AgNP suspensions, bioaccumulation was greatest in liver tissue for fish exposed to smaller "colloidal" AgNPs, while the highest concentrations were observed in the intestines of fish exposed to a larger "powder" form of AgNPs.[18] Therefore, bioaccumulation may depend upon the characteristics of the AgNPs to which fish are exposed. In addition, dissolution of AgNPs to Ag^+ may be an important factor controlling bioaccumulation in aquatic organisms.[19]

Adult zebrafish (*Danio rerio*) exposed to AgNPs accumulated Ag in all tissues, but after exposure ceased, Ag concentrations in the intestines remained elevated.[20] In another study with zebrafish exposed to AgNPs, tissue burdens of Ag did not decrease appreciably over a depuration period of a few days.[21] However, these bench-scale investigations may not be applicable to whole ecosystems, where exposures of fish to AgNPs and transformation products may occur through both aqueous and dietary routes and over temporal scales of months to years.

Nanomaterials accumulated in lower trophic level organisms can transfer to higher-level organisms, with some evidence of biomagnification.[22] In a tropical aquatic food chain, trophic transfer of AgNPs only occurred from algae to a cladoceran, and there was no evidence of bioaccumulation in fish.[23] However, in a study with goldfish (*Carassius auratus*) exposed through waterborne or dietary routes to CuO and ZnO nanoparticles, the rapid bioaccumulation of Cu and Zn, respectively, was observed in goldfish exposed through both routes of exposure.[24] With marine species exposed to titanium dioxide nanoparticles, there was a transfer of titanium to turbot (*Scophthalmus maximus*) that were fed dosed clamworms, but tissue residues declined rapidly over 1 week of depuration.[25]

As described above, studies of the bioaccumulation and trophic transfer of nanoparticles have been primarily conducted using bench-scale systems. There have also been a small number of studies conducted in freshwater mesocosms dosed with AgNPs.[26] However, these studies do not fully replicate the complex biogeochemical processes and trophic interactions that occur in natural aquatic ecosystems.[27] We completed a study of the fate and effects of AgNPs released over two ice-free field seasons into a natural boreal lake at the International Institute of Sustainable Studies-Experimental Lakes Area (IISD-ELA) in Ontario, Canada. Here, we report the accumulation of Ag over time in tissues of yellow perch and northern pike (*Esox lucius*) that were collected from the lake.

METHODS AND MATERIALS

Whole-Lake Additions. AgNPs were added to Lake 222 at ELA over two field seasons in 2014 and 2015. Additions of 9 kg of AgNPs in 2014 (Year 1) over 18 weeks started in mid-June and ended in late October, and additions in 2015 (Year 2) of an additional 6 kg of AgNPs over 14 weeks started in early May and ended in late August. Lake 222 is a small oligotrophic lake with a maximum depth of approximately 6.5 m, a lake area of approximately 16.4 ha, and an estimated lake volume of approximately 7.2×10^5 m^3. The lake stratifies during the summer, and when the thermocline is stable, it forms at depths between 2 and 2.5 m. There is a small stream that seasonally enters the south side of the lake and a small ephemeral stream exiting through a wetland at the north end of the lake (Figure 1). However, all fish populations are resident

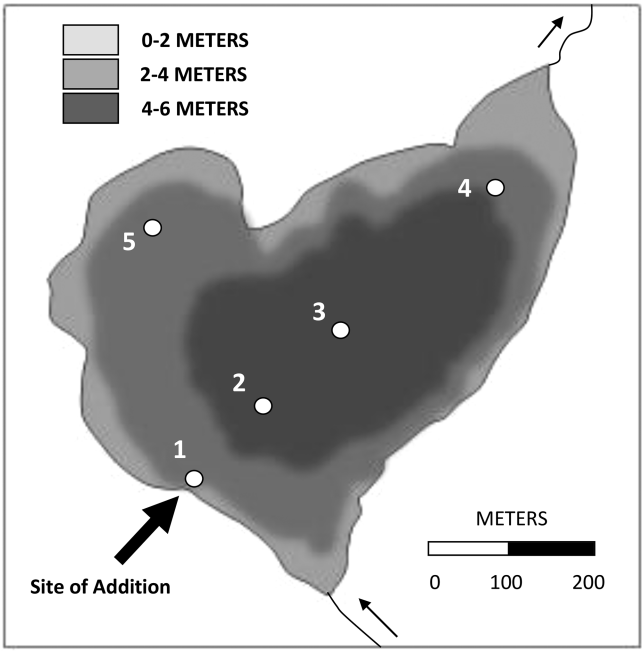


Figure 1. Map of Lake 222 showing the location of sites for deployment of passive samplers relative to the site of addition of the AgNPs. The small arrows indicate the ephemeral inlet and outlet streams.

in the lake, and there is no recruitment from other locations. Fish species in the lake include northern pike, yellow perch, and blacknose shiner (*Notropis heterolepis*). During the experiment, the mean concentration of dissolved organic carbon (DOC) was 12.1 mg/L, the mean pH was 6.6, and conductivity varied between 35 and 43 μS/cm.

The AgNPs were purchased as a powder from NanoAmor (Houston, TX). This material is capped with 0.2% (w/w) polyvinylpyrrolidone (PVP) and, according to the manufacturer, consists of 99.9% silver and has a particle size range of 30−50 nm, and the particles are spherical in shape. This material has been used previously in bench-scale toxicity studies.[12,28] AgNPs were suspended in filtered lake water with 0.025% gum arabic added as a stabilizer using a rotor-stator dispersion mill, as previously described.[29] The hydrodynamic size distribution in a suspension determined by dynamic light scattering (DLS) and a photomicrograph of the particles determined by transmission electron microscopy (TEM) are included in Figure S1. These data confirm that the hydrodynamic size of the particles in suspension were within the range of 30−50 nm, although there were larger particles in the 200 nm range in the suspension, likely a result of homoagglomeration. The mean levels of dissolved Ag in suspensions were 0.28 ± 0.08 mg/L.[29] The AgNP stock suspensions prepared every second day at a nominal Ag concentration of 5 g/L were added to the lake with a peristaltic pump from a point source along the southwestern shore of the lake. Daily discharges of AgNPs in suspension were approximately 62.5 g.

Fish Collections. Yellow perch and northern pike were sampled from Lake 222 and two reference lakes (i.e., Lakes 239 and 383) at IISD-ELA before AgNP addition, during the Year 1 and Year 2 addition phases, and during the post-addition phase. Pike were collected by angling and perch by beach seining. Collected fish were sacrificed on-site by an

DOI: 10.1021/acs.est.8b03146
Environ. Sci. Technol. 2018, 52, 11114−11122

overdose of tricaine methanesulfonate (i.e., MS-222) anesthetic and then weighed and measured for total length and fork length. In parallel studies of fish bioenergetics, fork length versus age relationships were determined for both species in Lake 222 (data not shown). Ages of pike were determined from cleithra bones, and the ages of perch were determined from fin rays.

Population estimates were made using mark−recapture methods, with marked perch identified by batch-marking of fins and marked pike identified by passive integrative transponder (PIT) tags. Perch population estimates were derived using a Schnabel census and pike populations were estimated using the POPAN Jolly−Seber model.[30] Survivorship and capture probabilities in the POPAN model were assumed to be constant over the pre-addition, addition, and post-addition phases. The mean number of perch of all ages in the lake during the study was estimated as 4135 ± 791 individuals. Because the pike population in Lake 222 was estimated over the 4 year course of the study to be between 194 ± 37 and 396 ± 87 individuals, there were concerns about depleting the population during the sampling campaign, so no attempt was made to select pike of a certain size or sex. Therefore, the pike included both males and females, and fork lengths varied widely from approximately 25−50 cm, which corresponds to fish between 3 and 8 years old. The numbers of fish collected at each sampling interval were 8−12 perch and 4−6 pike.

Muscle tissue was removed from both pike and perch from a location above the lateral line and below the dorsal fin, and the skin was removed. The fish were then dissected, and gill and liver tissues were removed from both pike and perch. The kidney and stomach contents (when present) were also collected from pike. All tissues were stored with cold packs and then frozen at −20 °C in a conventional freezer within 2 to 3 h of collection. All procedures for collecting and sampling fish were approved by the Animal Care Committee at Trent University, Peterborough, ON, Canada, and followed the Guidelines of the Canadian Council on Animal Care (www.ccac.ca).

Passive Samplers. Carbon nanotube integrative samplers (CNIS) and diffusive gradient in thin film (DGT) samplers were deployed in Lake 222 to monitor the distribution of suspended and dissolved Ag in the water column over the addition and post-addition phases. The CNIS passive sampler consists of a receiving phase of multiwalled carbon nanotubes functionalized with amine groups (i.e., NH_2−CNT) sandwiched between cellulose acetate (0.8 μm) membranes.[31] For the DGT samplers, the materials used to construct the samplers were purchased from DGT Research (Lancaster, UK) and consisted of a plastic housing with a surface area of 3.14 cm^2, a receiving phase of Chelex resin (25 mm diameter), and a diffusive gel (2−5 nm pore size) covered with a 0.45 μm pore size polysulfone membrane (0.14 mm thickness).[31]

For each monitoring period of 4−7 weeks, 3 of the CNIS samplers and 3 of the DGT samplers were deployed together in stainless steel cages. In the Year 1 addition phase, CNIS and DGT samplers were deployed at five sites throughout Lake 222 (Figure 1), with samplers suspended at a 1 m depth at Sites 1, 2, 4, and 5, and at depths of 1 and 4.5 m at Site 3, situated at the deepest point in the lake. In Year 2 of the addition phase and in the post-addition phase, the samplers were deployed only at Site 3 at depths of 1 and 4.5 m.

CNIS passive samplers sequester any Ag present in the water column that can pass through the 0.8 μm confining membrane and adsorb to the carbon nanotubes.[31] This can include AgNPs as well as colloidal Ag, agglomerated AgNPs with diameters of <800 nm, and dissolved Ag. DGT passive samplers sequester silver ions (Ag^+) as well as Ag associated with dissolved organic matter.[32] Because the Ag that accumulates on DGT samplers is commonly referred to as "DGT-labile Ag", the Ag that accumulates on the CNIS was similarly referred to as "CNIS-labile Ag".[31] The time-weighted average (TWA) concentrations of Ag in water over the deployment period were estimated from data on the amounts of Ag accumulated on the samplers over the time of deployment and the sampling rates determined for these devices.[31] The methods used to make these estimates are summarized in the Supporting Information. Briefly, the sampling rates for the DGT and CNIS passive samplers were determined in the laboratory by spiking water collected from a nearby reference lake (i.e., Lake 221) with $AgNO_3$ or AgNP, respectively. All concentrations were determined after the subtraction of the mean levels of Ag detected in field blank DGT and CNIS samplers (n = 3) that were carried into the field during deployment and retrieval.

Ag Analysis. Both the receiving phases and the membranes of the CNIS and DGT samplers were digested together for 1 h in 70% nitric acid of BDH Aristar Plus grade purchased from VWR (Radnor, PA) heated to 120 °C, as described previously.[31] These digests were then evaporated to 1 mL at 150 °C and filtered through a 0.45 μm membrane. Frozen fish tissues were thawed and blotted dry, and then either the whole sample or subsamples (0.2−1.0 g wet weight) were weighed on a three-decimal place balance. These samples were placed in 70% trace metal grade nitric acid of BDH Aristar Plus grade and then spiked with indium (5 ng/mL) as an internal standard. Tissue samples were digested in the nitric acid at 120 °C for 2 h, evaporated to 1 mL, and finally filtered through a 0.45 μm membrane, as described previously.[12] All digested samples were diluted with Milli-Q water to 4% nitric acid and stored at 4 °C until analyzed.

To verify the analytical method for Ag in tissues, subsamples (n = 5) of NIST standard reference material (SRM) 1566b (i.e., freeze-dried oyster tissue) were analyzed. The certified mass fraction value for Ag in this material is 0.666 ± 0.009 micrograms per gram of dry weight. Our analyses of the SRM were consistent with the certified value, at 0.654 ± 0.023 μg/g dry weight. An alternative digestion method that included the addition of 2 mL of 30% hydrogen peroxide after 1 h of acid digestion at 120 °C did not increase recoveries of the internal standard or the measured levels of Ag, as reported previously.[12]

Ag in digested samples of fish tissues and passive samplers was measured by inductively coupled plasma mass spectrometry (ICP-MS). The methods used for ICP-MS analysis of Ag in digests from fish samples were previously described,[12] as were the methods for analysis of digests from the CNIS and DGT passive samplers.[31] Briefly, ICP-MS analysis was conducted with an X-Series instrument purchased from Thermo Scientific, (Nepean, Ontario, Canada) operated in peak hopping scan mode with a dwell time of 25 ms for monitoring of ^{107}Ag and ^{115}In. External calibration by analysis of standard solutions over a range of Ag concentrations (0.1 to 200 μg/L) spiked with indium was the method used to generate a calibration curve. Procedural blanks (n = 5) were prepared and analyzed with each batch of samples. The masses

DOI: 10.1021/acs.est.8b03146
Environ. Sci. Technol. 2018, 52, 11114−11122

Table 1. Mean ± SD (n = 3) Estimated TWA Concentrations (μg/L) of CNIS-Labile and DGT-Labile Ag at Sites in Lake 222 Where Passive Samplers Were Deployed Every 4−7 Weeks throughout the Addition Phases (Years 1 and 2) and the Post-Addition Phase[a]

phase of project	deployment period	site 1	site 2	site 3, 1 m	site 3, 4.5 m	site 4	site 5
			DGT-labile Ag (μg/L)				
addition phase, Y1	June 24 to July 23, 2014	9.9 ± 0.5	0.33 ± 0.02	0.37 ± 0.02	0.36 ± 0.02	1.28 ± 0.06	0.17 ± 0.01
	July 23 to August 28, 2014	12.6 ± 0.1	ND	0.28 ± < 0.01	0.39 ± 0.02	0.81 ± 0.01	0.25 ± 0.01
	August 28 to October 15, 2014	31.0 ± 1.6	0.33 ± 0.02	1.14 ± 0.06	1.02 ± 0.05	ND	0.26 ± 0.01
addition phase, Y2	May 27 to June 25, 2015			0.29 ± 0.01	0.26 ± 0.01		
	June 25 to July 23, 2015			0.14 ± 0.01	0.10 ± < 0.01		
	July 23 to August 20, 2015			0.22 ± 0.01	0.75 ± 0.09		
post-addition phase	August 20 to October 7, 2015			0.10 ± 0.01	0.12 ± 0.01		
	June 3 to July 2, 2016			0.25 ± 0.08	0.44 ± 0.11		
	June 5 to July 8, 2017			ND	ND		
			CNIS-labile Ag (μg/L)				
addition phase, Y1	June 24 to July 23, 2014	48.3 ± 9.3	2.1 ± 0.8	2.4 ± 0.9	1.0 ± 0.41	2 ± 0.5	1.6 ± 0.6
	July 23 to August 28, 2014	176.6 ± 70.6	9.0 ± 3.6	2.5 ± 1.0	2.8 ± 1.1	1.0 ± 0.41	1 ± 0.4
	August 28 to October 15, 2014	850.6 ± 140.2	7.4 ± 3.0	11.5 ± 4.6	8.3 ± 3.3	1.1 ± 0.4	1.3 ± 0.5
addition phase, Y2	May 27 to June 25, 2015			2.8 ± 1.1	1.3 ± 0.5		
	June 25 to July 23, 2015			8.5 ± 4.2	5.2 ± 2.1		
	July 23 to August 20, 2015			9.5 ± 3.8	7.3 ± 2.9		
post-addition phase	August 20 to October 7, 2015			1.6 ± 0.6	1.8 ± 0.7		
	June 3 to July 2, 2016			0.2 ± 0.1	0.3 ± 0.1		
	June 5 to July 8, 2017			ND	ND		

[a]ND: not detected at levels above the field blanks.

of Ag in procedural blanks averaged 0.16 ± 0.06 ng. The method detection limits were determined as 3 times the standard deviation of the concentrations in each batch of the procedural blanks.

Single-Particle ICP-MS. Samples of surface water were collected at Site 2 in Lake 222 at the times of passive sampler deployment and retrieval throughout the addition phases in Year 1 and 2 and were flash-frozen in liquid nitrogen within a few hours of collection to preserve the particle size distribution.[33] The particle sizes (nanometers) and number concentrations (particles per liter), as well as levels of dissolved Ag (dAg) were determined by spICP-MS using a Nu AttoM magnetic sector instrument (Nu Instruments Ltd., Wrexham, UK) operated in single particle mode, as described previously.[34] Briefly, a single m/z value of 107 was monitored and data was acquired using a dwell time of 50 μs to give 8−12 points per particle peak. The peaks were differentiated from the continuous background signal from dAg by the Nu Quant software. AgNP standards with a range of sizes were used to construct a calibration of mean integrated counts per particle event as a function of particle volume. The particle number concentrations were estimated from the measured total Ag and the particle size distribution. The ionic sensitivity was determined daily using a minimum of three dissolved standards to measure the dAg concentration, and to estimate the particle size detection limit using the time-averaged background signal.

Statistical Analysis. The data on concentrations of Ag in fish tissues did not conform to the assumptions for parametric analysis, even when $\log_{10}$-transformed. Therefore, the non-parametric Kruskal−Wallis test was used to analyze whether there were significant differences over time in Ag concentrations in the individual tissues (e,g, gill and liver) of pike and perch. Where significant temporal differences were observed, pairwise comparisons were made between Ag concentrations in the individual tissues of pike and perch sampled at different dates using a Dunn's method post-hoc test. These statistical analyses were conducted with the SigmaStat add-on to SigmaPlot version 12 (Systat Software, San Jose, CA).

Data on the concentrations of Ag in liver tissues from individual pike collected at different dates during the addition phase were plotted as dependent variables versus the length of the fish and versus the Fulton's condition factor (i.e., 100 × [weight/length] × 10^3) and linear relationships calculated by least-squares linear regression analysis. The natural log (ln) of the concentration of Ag in liver tissue of individual northern pike during the post-addition phase of the study were plotted against the number of days post-addition to generate a regression line describing the first-order kinetics of loss of Ag in the pike population over time. All regression analyses were conducted using Microsoft Excel 2016.

RESULTS

Ag in Water. The mean concentrations of CNIS-labile Ag and DGT-labile Ag in the water column varied over time and location in Lake 222. Mean concentrations of DGT-labile Ag were <1.5 μg/L except for higher concentrations at Site 1 adjacent to the point source of AgNPs. The concentrations of CNIS-labile Ag in the lake during the addition phases were in the range of 1−11.5 μg/L except for higher concentrations adjacent to Site 1. The analysis of the passive samplers deployed at five sites in Year 1 of the addition phase showed that Ag was detected in surface waters throughout the lake.

Ag was detected in samplers deployed at both 1 and 4.5 m in the water column at Site 3 over both Year 1 and Year 2 of addition. In the post-addition phase at Site 3, the mean estimated TWA concentrations of CNIS-labile Ag declined

DOI: 10.1021/acs.est.8b03146
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after the end of the addition phase (i.e., August 2015) and by the end of the post-addition monitoring phase in July 2017, Ag was not detected in CNIS or DGT samplers retrieved at Site 3 (Table 1). Data forthcoming in subsequent publications show that a large proportion of the Ag added to the lake was eventually deposited in the bottom sediments.

AgNPs were detected by spICP-MS analysis of surface water samples collected at Site 2 throughout the addition phase of the experiment. As illustrated for samples collected in August 2014 and August 2015 (Figure 2), the mean particle sizes were

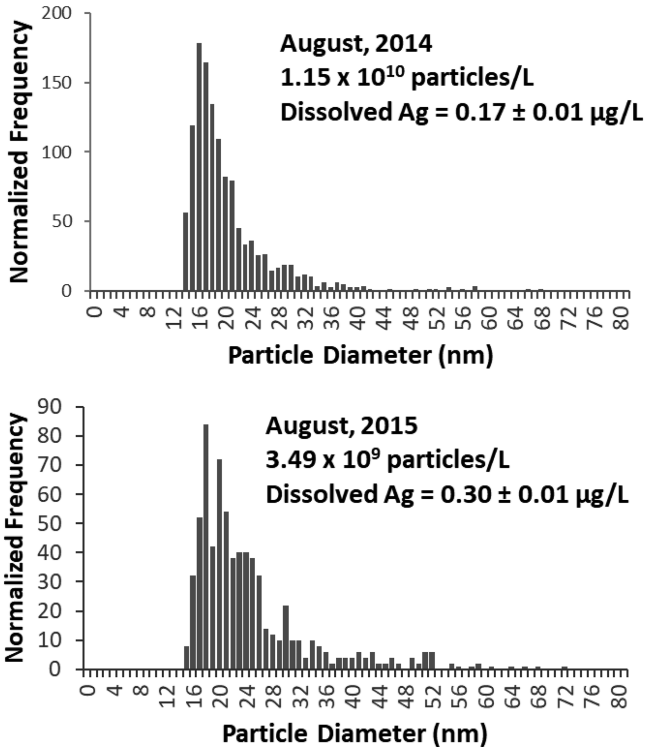

Figure 2. Particle-size histograms, mean particle sizes, and particle concentrations for AgNPs and dissolved Ag detected in surface water samples collected at Site 2 in August of 2014 and 2015. Data are from single aliquots diluted by a factor of 50 prior to analysis. The estimated size detection limit was 12 nm.

around 20 nm, and the size ranges were approximately 14−70 nm. Concentrations of dissolved Ag (dAg) measured as the background signal during spICP-MS analysis did not exceed 0.34 μg/L in any of the samples.

Ag in Fish Tissues. The mean concentrations of Ag in liver tissue of pike and perch sampled from Lake 222 throughout the experiment are illustrated in Figure 3. The mean "baseline" concentrations determined for fish sampled in Lakes 222, 239, and 383 in 2013 and 2014 were 4 ± 1 nanograms per gram of wet weight in the livers of pike and 4 ± 2 nanograms per gram of wet weight in the livers of perch. There was no significant change in the mean concentrations of Ag in pike and perch collected from the reference lakes (i.e., Lakes 239 and 383) over the study period. Once additions started in Lake 222, the concentrations of Ag in liver tissue of both species increased rapidly (Figure 3). Concentrations in pike liver in Year 2 (i.e., 2015) were significantly different from concentrations in Year 1 of AgNP additions (i.e., 2014). These Ag concentrations increased to the low parts-per-million range, with the highest

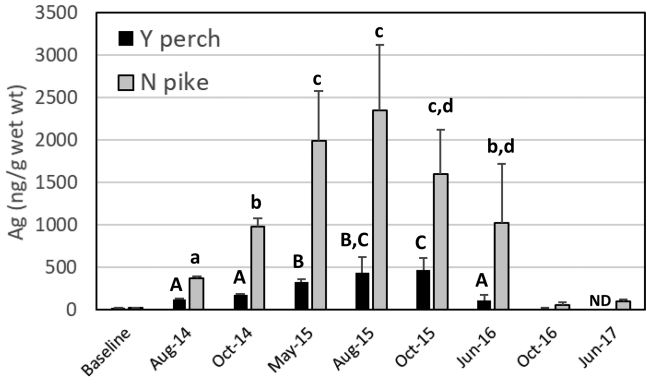

Figure 3. Mean (plus or minus standard deviation) concentrations of Ag in liver (nanograms per gram of wet weight) of yellow perch (n = 8−12 per sampling time) and northern pike (n = 4−6 per sampling time) throughout the pre-addition phase (Baseline), Years 1 (2014) and 2 (2015) of the addition phase, and the post-addition phase (2016 and 2017). ND: not detected. Uppercase letters for perch and lowercase letters for pike indicate that mean concentrations were significantly different from the baseline concentrations, and bars with the same letter indicate data in which concentrations were not significantly different from each other.

concentration of 5074 ng/g detected in the liver of an individual northern pike sampled in May 2015.

The variability in Ag concentrations in the livers of northern pike was quite high. This could be because of variations in exposure or, alternatively, due to the wide variations in size or condition of the pike that were sampled. However, there were no relationships between liver Ag concentrations and the weight of individual fish sampled at each time during the addition phase except for the relationship between weight and concentration for 6 northern pike collected in October 2015, with a linear regression slope of 4.1 (r^2 = 0.78), as illustrated in Figure S2. No relationships were observed between liver Ag concentrations and the condition of the northern pike (Figure S3).

The mean Ag concentrations in the liver tissues of perch were much lower relative to pike (Figure 3). However, as with pike, the concentrations of Ag in perch livers increased throughout the addition phase of the experiment and declined in the post-addition phase. The highest concentration detected in the liver tissue of perch was 762 ng/g in an individual fish sampled in August 2015. In the final samples collected in the post-addition phase in June 2017, the Ag concentrations in the livers of perch were below the limits of detection. However, the mean Ag concentrations in livers of pike were still above detection limits, at 97 ± 51 nanograms per gram of wet weight (Figure 3). The decline in levels of Ag in livers of pike over the post-addition phase from October 2015 to June 2017 (t = 656 days), illustrated in Figure S4, conformed to a first-order relationship yielding an estimated half-life of 119 days:

$$\text{Concentration}(t) = 6.98e^{(-0..0058\times t)},\ r^2 = 0.97$$

Mean Ag concentrations in the gill tissue of perch and pike were lower than in liver, but the concentrations were also elevated throughout the addition phase relative to the baseline concentrations and declined in the post-addition phase (Figure 4). However, the mean concentrations in the gills of pike collected at all dates during the addition phase were not significantly different (Figure 4). In contrast to the data on liver concentrations, levels of Ag in the gills of perch were

DOI: 10.1021/acs.est.8b03146
Environ. Sci. Technol. 2018, 52, 11114−11122

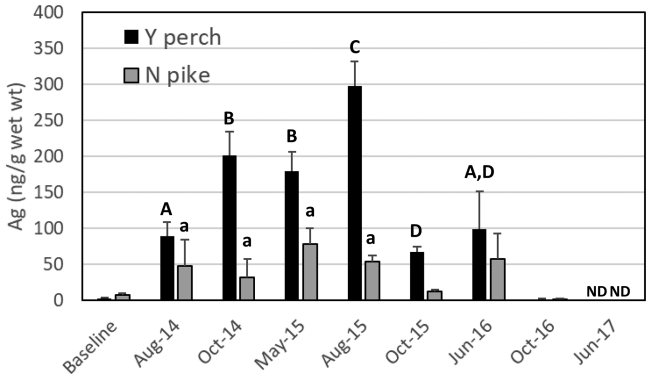

Figure 4. Mean (plus or minus standard deviation) concentrations of Ag in gills (nanograms per gram of wet weight) of yellow perch (n = 8−12 per sampling time) and northern pike (n = 4−6 per sampling time) throughout the pre-addition phase (baseline), Years 1 (2014) and 2 (2015) of the addition phase, and the post-addition phase (2016 and 2017). ND: not detected. Uppercase letters for perch and lowercase letters for pike indicate that mean concentrations were significantly different from the baseline concentrations, and bars with the same letter indicate data in which mean concentrations were not significantly different from each other.

approximately 2 to 3 times higher than the concentrations in the gills of pike. During the post-addition monitoring phase in October 2016, mean concentrations of Ag in gill tissue declined to 1.7 ± 1.2 and 1.5 ± 0.8 ng/g in perch and pike, respectively. By June of 2017, the concentrations of Ag in the gills of both pike and perch were not above detection limits.

Concentrations of Ag in muscle tissue were low relative to Ag concentrations in liver tissue in both pike and perch sampled from Lake 222. Pike sampled in August 2015 had a mean Ag concentration of 78 ± 41 nanograms per gram of wet weight in dorsal muscle, with the highest concentration of 133 nanograms per gram of wet weight in an individual pike. By the end of the post-addition sampling in June 2017, Ag was still detectable in the muscle tissue of pike at a mean concentration of 13 ± 6 nanograms per gram of wet weight, significantly elevated above baseline levels. For perch muscle, the highest mean Ag concentration of 121 ± 15 nanograms per gram of wet weight was also observed in fish sampled in August 2015. Ag concentrations in the muscle of perch were not above detection limits in the fish collected in June 2017. The concentrations of Ag in muscle and kidney tissues of northern pike sampled throughout the addition phase and in October 2015 and 2016 of the post-addition phase are illustrated in Figure S5. The Ag concentrations in the stomach contents of pike are also illustrated in Figure S5. The range of Ag concentrations from 22 to 75 nanograms per gram of wet weight in the stomach contents during the addition phase was consistent with the levels of Ag detected in the muscle of yellow perch during this period. Yellow perch are a major forage fish for northern pike living in Lake 222. Some of the stomach contents were recognizable as perch that had been consumed recently by the pike.

DISCUSSION

Ag was distributed throughout the lake at concentrations in the low microgram per liter range, with 11.5 μg/L as the highest TWA concentration estimated from CNIS deployed at Site 3. These estimates from the passive samplers are consistent with data from a concurrent study in Lake 222 where mean total Ag concentrations measured in the epilimnion throughout the lake varied between 0 and 17.4 μg/L.[35] Lake stratification was not a barrier to the mobility of the AgNPs, as CNIS-labile Ag was detected below the thermocline at 4.5 m, consistent with a concurrent study of the distribution of total and dissolved Ag in Lake 222.[36] The concentrations of total Ag are about an order of magnitude higher than the Canadian water-quality guideline for silver for the protection of aquatic life of 0.25 μg/L.[37] We found low estimates of TWA concentrations of dissolved Ag in the water column during the addition phase. This is consistent with low dissolved Ag levels previously observed in mesocosms spiked with AgNPs that were deployed in a nearby high DOC lake at IISD-ELA.[33] It is likely that any Ag^+ released into the lake or generated in situ from the dissolution of AgNPs was rapidly bound to dissolved organic matter.

The distribution of particle sizes was skewed to mean diameters in the 20 nm range, which is lower than the 30−50 nm size range of the AgNP stock material reported by the manufacturer. However, particle sizes estimated from spICP-MS analysis refer to the particle diameter of the Ag core and do not include the dimensions of the particle coating. Previous analysis of the stock suspensions by DLS, which includes the coating, indicated that the mean hydrodynamic diameter of the AgNPs was 39.3 ± 3.6 nm, but there were also some larger particles in the 200 nm range.[29] It is possible that a reduction in the size of AgNPs in the lake relative to the size range in the stock suspension was due to agglomeration and sedimentation of larger particles, leaving only smaller particles suspended in the water column. However, humic and fulvic acids can reduce Ag^+ to form stable AgNPs, so in situ production of AgNPs could also have been a source of the smaller-sized AgNPs.[38,39]

Both yellow perch and northern pike accumulated Ag in their tissues during the Years 1 and 2 addition phases, with the highest concentrations observed in liver tissue. The highest Ag concentration of 5.1 micrograms per gram of wet weight was detected in the liver of an individual pike. This concentration is 3 orders of magnitude higher than the estimated concentrations of Ag in the water column. The degree of accumulation in liver was not related to the weight or the condition of the fish, so pike that were heavier or were in better condition did not accumulate more Ag. Once AgNP additions ceased, the liver residues began to decline, with a half-life of 119 days for total Ag in liver tissue over the entire pike population. In bench-scale studies with fish,[20,21] Ag concentrations declined to baseline levels within a few days of cessation of exposure. However, these Ag depuration studies are not directly comparable to the whole-ecosystem study because in Lake 222 the concentrations in fish tissues were declining concurrently with dropping levels of Ag in the water column. The rates of decline of Ag concentrations in the livers of yellow perch were not calculated because young fish (i.e., 1−2 years old) were monitored and the post-addition trends would have been influenced by population recruitment.

In perch, the Ag concentrations in gill tissue were almost as high as concentrations in liver. Previous bench-scale tests with various fish species exposed to suspensions of AgNPs have shown variable results, with the site of greatest accumulation usually being either the liver[14,15,19] or the gill.[15−17,21] Our previous laboratory studies with yellow perch exposed for 10 d to the same suspensions of AgNPs that were used in the experiment at Lake 222 at a nominal concentration of 100 μg/L showed that Ag accumulated to mean concentrations of 478

DOI: 10.1021/acs.est.8b03146
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ng/g in the gill.[12] These levels are comparable to the concentrations of Ag observed in gills of perch collected from Lake 222 at the end of Year 2 additions.

The differences in the Ag tissue distribution in pike and perch indicate that the kinetics of accumulation of Ag were different in the two species. Comparative studies on the kinetics of uptake and depuration of Ag in the tissues of rainbow trout (*O. mykiss*) and European eel (*A. anguilla*) exposed to Ag free ion and AgCl showed that the two fish species showed different patterns of accumulation in gill tissue[40] as well as different rates of Ag depuration from liver and kidney.[41] Therefore, differences in Ag accumulation patterns in perch and pike from Lake 222 could have resulted from species-specific differences in Ag kinetics.

The intestinal epithelium in fish may be a significant ligand for dietary silver,[42] so dietary uptake may have been an important source of the Ag that accumulated in perch and pike from Lake 222. Data on the concentrations of Ag in the lower trophic levels of Lake 222 during additions of AgNPs are forthcoming in future publications. Hou et al.[22] concluded in a review article that there is evidence of biomagnification of nanoparticles in food chains. However, it may be misleading to define the high concentrations of Ag observed in the liver tissue of pike as evidence of "biomagnification" from yellow perch to this piscivorous species. Only the liver contained elevated concentrations of Ag because the mean concentrations of Ag in the gill and muscle tissue of pike were lower than the mean concentrations of Ag in these tissues in perch. The pike and perch were not homogenized and analyzed for total body burdens of Ag, so there are no data to compare the levels of Ag in the whole bodies of these two species. Bench-scale studies with element-based nanoparticles (i.e., CuO, ZnO, and TiO_2) indicate that fish can accumulate these elements through the diet.[24,25] Further studies are needed to determine whether accumulation of Ag from the diet in piscivorous fish such as northern pike results in elevated concentrations in the liver relative to other tissues.

The concentrations of Ag in the livers of pike and perch during the addition phases were within the range of concentrations that have been associated with sublethal biological responses in fish exposed to AgNPs in the laboratory.[12,13,43] Data are forthcoming in future publications on biological responses in both pike and perch collected during the addition and post-addition phases in Lake 222. The tissues of pike and perch were analyzed for total Ag, so no information is available on the forms or speciation. While advances have been made in techniques for analyzing AgNPs in aquatic matrixes, there are significant challenges to overcome in analyzing the accumulation of nanoparticles in biological tissues.[44] If there are sufficiently high concentrations of Ag, it is possible to generate information on speciation of Ag in biological tissues using X-ray spectroscopy techniques.[45]

Surprisingly, there are few recent data on the concentrations of Ag in the tissues of wild fish. However, in a recent study of the concentrations of several metals in the tissues of American eels (A. *rostrata*) collected in Quebec, Canada and European eels collected in France, Ag was detected in liver, kidney and muscle tissue, with the highest concentrations in liver in the range 2−3 micrograms per gram of dry weight, with much-lower concentrations in muscle (<0.2 micrograms per gram of dry weight).[46] Assuming that the eel tissues are 75% water, a dry weight concentration of 3 μg/g Ag in eel liver would correspond to a wet weight concentration of approximately 0.7 μg/g Ag, an order of magnitude lower than the highest concentration of Ag detected in the livers of pike from Lake 222 (i.e., 5.1 μg/g). In the wild eels, there were no significant relationships observed between the weights of the fish and the concentrations of Ag in the tissues.[46] The highest concentration of Ag detected in a sample of muscle from a northern pike in Lake 222 was 133 nanograms per gram of wet weight (i.e., 133 μg/kg). The U.S. Environmental Protection Agency reference dose for human consumption of silver is 5 μg/kg/day.[47] To reach this reference dose, a 70 kg person would have to consume approximately 2.6 kg per day of the pike tissue contaminated with Ag at this level.

This whole-lake experiment was conducted with Ag concentrations in water greater than the Ag nanoparticle levels expected in the aquatic environment[5,6] and greater than the 0.25 μg/L guideline for Ag recommended in Canada for protection of aquatic life.[36] In addition, these nanoparticles had not gone through an aging process or the transformations that are typical of nanoparticles released into the environment,[8] including the transformations of AgNPs that occur in municipal wastewater.[2−4] However, in a recent study of TiO_2 nanoparticles in municipal wastewater, the majority of these materials were removed to activated sludge in two WWTPs, but Ti levels were still elevated in fish collected from a river impacted by discharges from the WWTPs.[48] More work is needed to evaluate the fate and effects of aged and transformed AgNPs, but this unique whole-lake experiment showed that releases of AgNPs at parts-per-billion concentrations in water can result in the accumulation of Ag to parts-per-million levels in the liver tissues of a piscivorous fish species at the top of the aquatic food chain.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b03146.

A description of time-weighted average concentrations; figures showing the distribution of particles in a stock suspension, the relationships among silver concentrations in the livers of northern pike and fish characteristics during the AgNP addition phase of the study, and the mean concentrations of silver in muscle and kidney tissues and the stomach contents of northern pike during the study (PDF)

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The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Funding for the Lake Ecosystem Nanosilver (LENS) project was provided by the Natural Sciences and Engineering Research Council (NSERC) of Canada through the Strategic Grants Program (SGP413230-2011) and matching grants from Environment and Climate Change Canada. IISD-ELA made a significant in-kind contribution to the project. Daniel Rearick,

DOI: 10.1021/acs.est.8b03146
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Andrea Conine, Joey Tonin, and Dan Gilchrist assisted with lake dosing and with sample collection. Andrew Scott helped with project management and logistics. Many thanks go to Li Shen for preparing some of the passive samplers and to Brenda Seaborn for her help with preparation of samples for analysis. We are grateful for the help and cooperation of the research staff at IISD-ELA, especially Chandra Rogers. Thanks go to Alistair Boxall, Mark Chappell, Steve Diamond, Yasir Sultan, and Kevin Wilkinson of the LENS Research Advisory Committee for their valuable advice.

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