



Georgia Southern University
Digital Commons@Georgia Southern

Electronic Theses and Dissertations

Graduate Studies, Jack N. Averitt College of

Summer 2017

Silver Nanoparticle Fate and Accumulation in the Aquatic Food Web of Stream Microcosms

Stefan J. Petersen

Follow this and additional works at: <https://digitalcommons.georgiasouthern.edu/etd>

 Part of the [Terrestrial and Aquatic Ecology Commons](#)

Recommended Citation

Petersen, S.J., SILVER NANOPARTICLE FATE AND ACCUMULATION IN THE AQUATIC FOOD WEB OF STREAM MICROCOSMS, GSU, 2017

This thesis (open access) is brought to you for free and open access by the Graduate Studies, Jack N. Averitt College of at Digital Commons@Georgia Southern. It has been accepted for inclusion in Electronic Theses and Dissertations by an authorized administrator of Digital Commons@Georgia Southern. For more information, please contact digitalcommons@georgiasouthern.edu.

SILVER NANOPARTICLE FATE AND ACCUMULATION IN THE AQUATIC
FOOD WEB OF STREAM MICROCOSMS

by

STEFAN PETERSEN

(Under the Direction of Risa A. Cohen)

ABSTRACT

Silver nanoparticles (AgNPs) are used in 25% of all nano-enabled products and applied for anti-microbial properties. Silver nanoparticles are discharged into aquatic environments through wastewater discharge, runoff, and chemical spills. Once in aquatic environments silver nanoparticles have the potential to harm aquatic organisms. While the fate of silver nanoparticles in lentic systems has been investigated, limited information is available for the fate of silver nanoparticles in flowing environments. The purpose of this study was to compare the fate of AgNPs following a one-time pulsed application simulating a chemical spill, or small repetitive applications simulating effluent discharge, in artificial stream communities containing river water, sediment, periphyton, snails, and fish under realistic environmental conditions. In addition to comparing the fate of AgNPs between application types, the fate of AgNPs were also compared between 35 and 70 $\mu\text{g L}^{-1}$ concentrations of AgNPs. Water samples were collected on days 0, 7, and 14 to quantify total Ag (TAg) in the water column. Periphyton samples were taken on days 0 and 14, and sediment, snail, and fish samples were taken on day 14 for silver content. Results from this study show that AgNP concentrations applied to streams only affects the fate of AgNPs in sediment where the majority of AgNPs settled and in fish which had limited exposure to AgNPs in the water column. Additionally, application type only affected the fate of AgNPs in

periphyton samples of pulsed treated streams where snails and flowing conditions had a longer period of time to reduce Ag adsorption to periphyton compared to repetitively treated streams. In this study, silver nanoparticles rapidly settled in lotic environments placing benthic organisms at risk for Ag accumulation. Furthermore, exposure to $70 \mu\text{g L}^{-1}$ and $35 \mu\text{g L}^{-1}$ AgNPs concentrations in artificial streams was not toxic to aquatic organisms regardless of application type. This study emphasizes the importance of testing AgNP exposure under environmentally relevant conditions to assess their fate and toxicity in the environment.

INDEX WORDS: Mesocosms, Ionic strength, Sedimentation, Suspension, Dissolved organic carbon, Toxicity, Periphyton, Snails, Fish, Settling

SILVER NANOPARTICLE FATE AND ACCUMULATION IN THE AQUATIC

FOOD WEB OF STREAM MICROCOSMS

by

STEFAN PETERSEN

B.S., Buena Vista University, 2012

A Thesis Submitted to the Graduate Faculty of Georgia Southern University in Partial

Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

STATESBORO, GEORGIA

© 2017

STEFAN PETERSEN

All Rights Reserved

SILVER NANOPARTICLE FATE AND ACCUMULATION IN THE AQUATIC
FOOD WEB OF STREAM MICROCOSMS

by

STEFAN PETERSEN

Major Professor: Risa A. Cohen
Committee: John Stone
Vinoth Sittaramane
Johanne Lewis

Electronic Version Approved:
July 2017

ACKNOWLEDGEMENTS

I would like to thank my family for supporting my career goals and encouraging me to pursue a master's degree. I would like to thank Jessica Devitt for supporting me during graduate school and helping me throughout my experiment, as well as Jason Duff, Jack Eschenshroeder, Lauren Neel, Byron Collins, Garret Strickland, Becky Scott, Preston Pound, Jackson Tomlinson, Elizabeth Craddock, Allison Lutz, and Anna Wagner for collecting and processing streams samples. I would like to thank my funding sources the Georgia Southern College of Science and Mathematics, Georgia Southern Graduate Student Organization, Ogeechee River Project in addition to the Richmond Hill Fish Hatchery for providing me with the fish used in this experiment. I would also like to thank John Stone, Stephanie Canonico-May, and the Georgia Southern Chemistry department for providing the silver nanoparticles and instrumentation needed to complete my studies. I would like to thank my committee members Vinoth Sittaramane and Johanne Lewis for providing their insight and help with questions arising during this project. Lastly, I would like to thank my advisor, Risa Cohen for helping me become a better scientist and writer.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	2
LIST OF TABLES.....	4
LIST OF FIGURES.....	5
CHAPTER	
1 SILVER NANOPARTICLE FATE AND ACCUMULATION IN THE AQUATIC FOOD WEB OF STREAM MICROCOSMS.....	6
Chapter 1 Introduction.....	6
Chapter 1 Methods.....	12
Chapter 1 Results.....	20
Chapter 1 Discussion.....	22
REFERENCES.....	46
APPENDIX	
SILVER NANOPARTICLE AND SILVER ION TOXICITY TO <i>NAVICULA</i> SP. IN ARTIFICIAL STREAMS	
Appendix Introduction.....	54
Appendix Methods.....	55
Appendix Results.....	58
Appendix Discussion.....	60
REFERENCES.....	70

LIST OF TABLES

CHAPTER 1

	Page
Table 1: Total water column Ag concentration after initial AgNP exposure and one and two weeks after the start of the experiment.....	30
Table 2: Average stream pH, DO, specific conductivity, and temperature in all streams over two weeks.....	31
Table 3: Comparisons showing the overall differences between treatments in Ag accumulation Day 0 periphyton, Day 14 periphyton, snails, fish, and sediment, in addition to the corresponding Tukey-Kramer, Wilcoxon Each Pair, and Steel-Dwass All Pairs Post-Hoc tests.....	32
Table 4: Results of Ag accumulation in Day 14 periphyton, snails, fish, sediment, and water samples.....	33
Table 5: Comparisons of initial periphyton biomass, water chlorophyll <i>a</i> , periphyton chlorophyll <i>a</i> , and DOC concentration.....	35
Table 6: Analyses of the effects of AgNP concentration and application type on final periphyton biomass, water chlorophyll <i>a</i> , periphyton chlorophyll <i>a</i> , and DOC concentrations.....	36

APPENDIX

	Page
Table 1: Average stream pH, DO, specific conductivity, and temperature in artificial streams...	67
Table 2: Mean total Ag concentrations in the water column.....	63
Table 3: Comparisons of initial DOC concentrations on Day 7 and relationships between DOC concentrations and Ag concentration in the water column.....	63
Table 4: Comparisons of initial and final water chlorophyll <i>a</i> concentrations on Days 0 and 14.....	64
Table 5: Comparisons of <i>Navicula</i> sp. populations over the seven-day experiment and comparisons of initial and final stream cell counts.....	65

LIST OF FIGURES

CHAPTER 1

	Page
Figure 1: Artificial stream microcosm showing dimensions, individual microcosm setup, and experimental set up in the greenhouse.....	37
Figure 2: Citrate capped AgNPs (~50nm) used in the experiment.....	38
Figure 3: Concentration of total Ag in the water column measured on Days 0, 7, and 14 by AgNP treatment.....	39
Figure 4: Initial Ag values determined in periphyton samples comparing $\mu\text{g. of Ag. per g. of periphyton vs AgNP treatments}$	40
Figure 5: Final periphyton Ag values after two weeks comparing $\mu\text{g. of Ag. per g. of periphyton vs AgNP treatments}$	41
Figure 6: Accumulation of AgNPs by snails after two weeks comparing $\mu\text{g. of Ag. per g. of snail vs AgNP treatments}$	42
Figure 7: Accumulation of AgNPs by fish after two weeks comparing $\mu\text{g. of Ag. per g. of fish vs AgNP treatments}$	43
Figure 8: Ag accumulation in sediment after two weeks shown comparing $\mu\text{g. of Ag. per g. of sediment vs AgNP treatments}$	44
Figure 9: Diagram showing the recovery of AgNP fate and possible trophic interactions.....	45

APPENDIX

	Page
Figure 1: Stream and pond mesocosm design.....	68
Figure 2: Mean <i>Navicula</i> sp. cell counts in ponds and streams taken on day 0, 1, 3, 5, and 7.....	69

CHAPTER 1

ACCUMULATION OF SILVER NANOPARTICLES IN AQUATIC FOOD WEBS FOLLOWING PULSED VS REPEATED EXPOSURE IN ARTIFICIAL STREAMS

INTRODUCTION

Over the last decade, the biomedical, agricultural, manufacturing, textile, and pharmaceutical industries developed nanoparticles (clusters of atoms or molecules less than 100 nanometers in size) to improve human healthcare and food production (Gopal et. al, 2011; Keller et. al, 2013). Nanoparticles are created using a variety of materials including metals, metal oxides, nonmetals, carbon, polymers, and lipids, coated with acids and polymers to improve durability and prevent breakdown (Zhang et. al, 2012; Grillo et al., 2015). In 2010, an estimated 300,000 metric tons of nanomaterials were manufactured worldwide, and production volume is expected to exceed 500,000 metric tons yr⁻¹ by 2020 (Keller et al., 2013; Maurer-Jones et al., 2013). Given the rapid growth of the nanoparticle industry and increased nanomaterial discharge into the environment through wastewater discharge, runoff, and chemical spills, the potential for ecological damage must be investigated (Gottschalk et. al, 2009).

Silver nanoparticles (AgNPs) are used in 25% of all nano-enabled products because their anti-microbial properties reduce bacterial abundance and metastasizing cells (Keller et al., 2013). Biomedical and pharmaceutical products with AgNPs are used to combat bacterial infections by reducing wound healing time, and treat cancer and HIV by stimulating breakdown of tumors or infected cells (Wong et. al, 2013). In addition, AgNPs are integrated into consumer products like food packaging (De Moura et al., 2012), clothing (Liu and Hurt, 2010), cosmetics (Fabrega, et al., 2011), and oil-based paints to kill gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria (Kumar et al., 2008). Due to the versatility of AgNPs, environmental

exposure is common, although the exact amount of nanoparticles released into the environment during their manufacture, use, and disposal is difficult to determine (Keller et al., 2013). Annual production and release estimates of AgNPs in 2010 were 242 metric tons yr⁻¹ (Keller et al. 2013). Entry of AgNPs to surface waters occurs at a rate of 63 tons yr⁻¹ worldwide from wastewater treatment plants (Sohn et al., 2015), excluding AgNPs from clothes, personal care products, and medications that enter septic tanks daily from untreated domestic residences (Benn et al., 2008). Approximately 5% of the AgNPs that reach wastewater treatment facilities are discharged into rivers with the remaining 95% diverted into sewage sludge (Kaegi et al., 2011). Sludge may then be applied to agricultural fields as fertilizer, thus AgNPs can enter rivers through runoff from fields following rainfall events (Fabrega et al., 2011). While the estimated concentration of actual AgNPs in surface waters is currently below 1 µg L⁻¹, with growing rates of production and use it will become increasingly important to investigate the fate of AgNPs in the environment (Gottschalk et al., 2009; Wong et al., 2013).

The fate of AgNPs in the environment is also influenced by their capping agents (Levard et al., 2012). Two of the most commonly used capping agents are polymer based citrate and polyvinylpyrrolidone (PVP) capping agents (Thio et al., 2011). Citrate capped AgNPs possess a negative charge and are stabilized electrostatically by repulsion between charged particles, and PVP capped AgNPs are neutrally charged and are stable via steric forces and inhibiting attraction to other molecules (Levard et al., 2012). Depending on the charge of the capping agent, AgNPs may bind to molecules such as dissolved organic carbon (DOC) and stay suspended in the water column, or bind to higher molecular weight ions and settle onto the benthos (Levard et al., 2012). While high molecular weight PVP capped ions settle after seven days and low molecular weight citrate capped AgNPs remain in suspension indefinitely in laboratory tests (Jang et al., 2014), no

differences in the fate of citrate vs PVP capped AgNPs occurred under environmentally relevant conditions in mesocosms (Furtado et al., 2015). This lack of difference in fate between citrate and PVP capped AgNPs is potentially in part due to molecular transformations in natural waters.

Once AgNPs enter the environment, they commonly transform into either Ag^+ ions or silver sulfide nanoparticles (Ag_2S -NPs) following breakdown of the capping agent (Levard et al., 2012). Silver ions are typically more toxic to aquatic organisms than AgNPs, although some organisms are more vulnerable to AgNPs. Exposing *Daphnia magna* to either Ag^+ ions or AgNPs revealed that Ag^+ ions reduced lifespan and mobility (~22-fold), feeding rate (5-fold), and reproductive capacity (50%) compared to AgNP treatments (Ribeiro et al., 2014). Zebrafish embryo hatching rates were 14% lower in Ag^+ ion treatments while AgNP treatments had no effect (Ribeiro et al., 2014). Conversely, AgNPs exhibited twice the toxicity of Ag^+ ions to green algae, *Raphidocelis subcapitata* (Ribeiro et al., 2014). During wastewater treatment, most of the AgNPs and Ag^+ ions are typically transformed into more stable Ag_2S -NPs in the presence of sulfide (Kaegi et al., 2011). Findings from single-species laboratory tests on a variety of organisms suggest Ag_2S -NPs are less toxic than both AgNPs and Ag^+ ions (Levard et al., 2013). For example, mortality rates of killifish embryos and growth inhibition of duckweed were an order of magnitude greater in AgNP than Ag_2S -NPs treatments (Levard et al., 2013). In addition, AgNPs were more lethal to nematodes (5-fold) and zebrafish embryos (10-fold) than Ag_2S -NPs (Levard et al. 2013). Furthermore, the rate of transformation of AgNPs to Ag_2S -NPs decreases during rain events due to shortened wastewater processing and retention times, potentially increasing discharge of Ag^+ ions or AgNPs to surface waters (Kaegi et al., 2011). Therefore conditions that enhance AgNP discharge coupled with the release of Ag^+ ions from Ag_2S -NPs in

the presence of sunlight and Fe^+ in aquatic environments may increase contact between AgNPs and their derivatives with aquatic organisms (Li et al. 2015; Wong et al., 2013).

Suspected mechanisms of AgNP toxicity to aquatic organisms include the inhibition of Na^+/K^+ channels in cell membranes, disruption of ATP production and DNA replication, and the production of reactive oxygen species (Schultz et. al, 2012; Reidy et al., 2013; He et al., 2011). AgNPs prevent ATP enzymes from binding to cells, disrupting the active transport mechanism of Na^+/K^+ ions and blocking the uptake of Na^+ through Na^+/K^+ channels leading to cell death (Schultz et al., 2012). Once inside cells, AgNPs interact with mitochondria inducing an overproduction of reactive oxygen species (ROS), which either causes apoptosis in cells or decreases ATP production. Finally, AgNPs induce structural changes in nuclear membranes including denaturing RNA and DNA, which prevent cell replication from occurring (Reidy et al., 2013). The variety of mechanisms and severity of cellular AgNP toxicity suggests adverse effects are likely to translate to the organism level.

Evidence of adverse effects of AgNPs on aquatic organisms derives primarily from short-term single-species toxicity assays conducted under controlled laboratory conditions (Wong et al., 2013). Decreased growth and reproduction in *Daphnia magna* occurred after 48 hours of exposure to AgNPs ($1.1\text{--}187 \mu\text{g L}^{-1}$) (Wong et al., 2013). Adult blue mussels, *Mytilus edulis*, exposed to $0.7 \mu\text{g L}^{-1}$ AgNPs exhibited shell abnormalities after 72 hours, and 50% of snails, *Lymnaea luteola*, died after 96 hours of exposure to $48 \mu\text{g L}^{-1}$ AgNPs (Wong et al., 2013; Ali, D. et al., 2014). In fish, AgNPs accumulated in Atlantic salmon (*Salmo salar*) gills during 48-hour exposures to $20\text{--}100 \mu\text{g L}^{-1}$, and hatching rates of Japanese medaka (*Oryzias latipes*) embryos decreased in the presence of $600 \mu\text{g L}^{-1}$ (Farmen et al., 2012; Wong et al., 2013). Chronic toxicity tests over 21 days show reduced reproduction and growth in *D. magna* after exposure to $5\text{--}30 \mu\text{g}$

L⁻¹ and 19.2-50 µg L⁻¹ AgNP respectively (Mackevica et al., 2015; Sakka et al., 2016; Zhao and Wang, 2011). While acute and chronic laboratory tests on individual species are important in determining nanomaterial toxicity to aquatic organisms, it is difficult to extrapolate these responses to field conditions with multiple interacting species, natural substrates, and variable environmental factors (Lowry et al., 2012).

Environmental conditions, including presence of natural substrates, DOC, pH, and water column temperature alter the toxicity of nanoparticles to aquatic organisms (U.S. EPA, 2012). The 96-hour LC₅₀ of AgNPs for gastropods decreased from 2.18 µg L⁻¹ in the absence of sediment to >100 µg L⁻¹ when sediment was present (Bernot et al., 2010). Although increased DOC availability (particularly humic and fulvic acids) generally increases the stability and decreases the toxicity of AgNPs, responses vary by organism. Dissolved organic carbon increased AgNP toxicity to *Daphnia magna* (50 mgL⁻¹) and *Pseudomonas* sp. (10 mgL⁻¹), decreased the toxicity to *O. latipes* embryos (10 mgL⁻¹), and *Ceriodaphnia dubia* (2.3 mgL⁻¹), while the toxicity to *Escherichia coli* (5 mgL⁻¹) and *Bacillus subtilis* (5 mgL⁻¹) remained unchanged (Grillo et. al, 2015). Increasing temperature (from 25 to 31°C) combined with 1 mg L⁻¹ of AgNPs reduced chlorophyll *a* production in green algae by 40% over a 24-hour period (Oukarroum et al., 2012). Changing multiple environmental variables at once also affects AgNP toxicity; decreased pH and DOC concentration coupled with higher temperatures increased dissolution of AgNPs into Ag⁺ ions, enhancing toxicity to aquatic organisms (Liu and Hurt, 2010). Given the potential for abiotic conditions to influence AgNP toxicity and transform AgNPs to Ag₂S and Ag⁺ ions in laboratory studies, experiments under more realistic environmental conditions are required to determine AgNP toxicity and fate in aquatic ecosystems.

The fate and behavior of AgNPs under more realistic environmental conditions and exposure regimes were examined in mesocosms simulating wetland, lake, and stream environments (Lowry et al., 2012; Furtado et al., 2015; Kroll et al., 2015). In artificial wetlands receiving a single (pulse) AgNP dose of 25 mg L^{-1} , AgNPs were transformed into Ag_2S , Ag-cysteine, and Ag^0 in the water column within eight days, and after 18 months, both mosquitofish and chironomids contained silver in their tissues (Lowry et al., 2012). Lake mesocosms yielded 33% higher water column Ag concentrations in repetitive treatments than the pulsed treatment, roughly 2X higher sediment Ag concentrations in a pulsed treatment than repetitive treatments, and similar levels of accumulation in periphyton between treatments after 33 days (Furtado et al., 2015). In artificial streams treated with one AgNP pulse dose of 2 or $20 \text{ } \mu\text{g L}^{-1}$, 80-88% of AgNPs settled out of the water column after four days (Kroll et al., 2015). These studies suggest AgNPs and their transformation products are available to aquatic organisms for long periods of time after exposure, and Ag fate is likely dependent on the concentration of AgNPs, the mode of application (pulsed vs. repetitive) and the type of aquatic environment (lentic vs. lotic).

Rivers and streams are at a greater risk of AgNP pollution than lakes due to more frequent agricultural runoff and discharge from treatment plants (NOAA, 2008). Flowing water may re-suspend AgNPs, prolonging water column exposure continually exposing organisms in the water column to AgNPs (Simmons and Wallschlager, 2004). In addition, river and stream communities are more susceptible to AgNP exposure than lake or wetland communities as nearly all wastewater treatment plants discharge into rivers (Simmons and Wallschlager, 2004). The community composition of organisms differs between stream and pond systems with more benthic dwelling organisms in streams than lakes, causing greater AgNP accumulation in benthic organisms as AgNPs settle (Grabowska et al., 2014; Bunn and Arthington, 2002; Croteau et al.,

2011)). Once benthic organisms are consumed by predators, AgNPs bioaccumulate in higher trophic levels (Croteau et al., 2011). The increased likelihood of AgNP discharge, spills, and runoff to streams combined with potential for adverse effects of AgNPs to differ in stream compared to lake communities suggests examining the fate of AgNPs in streams is important.

The purpose of this study was to compare the fate and potential toxicity of two concentrations of AgNPs following one-time pulse application simulating a chemical spill, or small repetitive applications simulating effluent discharge, in artificial stream communities under realistic environmental conditions. I hypothesized that the fate of AgNPs differs depending on the mode of application and concentration applied. Specifically, I predicted that a large pulse application increases silver accumulation in sediments and bottom-dwelling stream inhabitants such as periphyton and snails (*Campeloma decisum*), while repetitive applications increase duration of AgNP exposure to fish (*Lepomis macrochirus*) in the water column. I also predicted that AgNP accumulation by organisms and sediment increases with increasing concentration regardless of application type. Results from this study provide insight into which stream components are susceptible to AgNP accumulation and should be monitored for AgNP effects.

METHODS

Artificial Stream Design

Artificial streams (N=30) were constructed in the Georgia Southern University greenhouse (32.421432, -81.790814) using 57 L, black, oval-shaped polypropylene tanks and black, oval shaped, polypropylene centerpieces (centerpiece diameter 13 - 23 cm) to create a stream channel with a depth of 11 cm and widths from 32-34 cm (Figure 1). A 1.9 cm layer of sandy sediment (Quikrete 1113, Georgia, USA) was added to each microcosm. Water collected

from the Ogeechee River near Rocky Ford, GA, USA (32.648953, -81.840798) was transported to the greenhouse and a 20 L aliquot immediately added to each microcosm. One powerhead pump (SunSun JP-024, China) was fixed to the side of each tank with adhesive tape at a depth of 2.7 cm, creating unidirectional flow with an average velocity of 0.18 m s^{-1} consistent with flow rates of the Ogeechee River during summer and fall months (USGS, 2017). To compensate for evaporation, deionized water was added daily to maintain the 20 L volume.

Unglazed ceramic tiles (5.08 cm. x 5.08 cm.) were deployed in the Ogeechee River (32.419892, -81.544509) two months prior to the start of the experiment to allow natural periphyton communities to establish (Hauer et al., 2011). One week prior to administering experimental treatments, 18 tiles were added to each artificial stream and evenly spaced ~5 cm from the tank sides, centerpiece, and neighboring tiles. Snails, *Campeloma decisum*, (N=600) were collected from the Ogeechee River near Rocky Ford, GA (32.648953, -81.840798), and juvenile fish (age 1-2 months), *Lepomis macrochirus* (N=180), 2 to 4 cm in length, were obtained from the Richmond Hill Fish Hatchery (31.955373, -81.316145). To allow time for acclimation to artificial streams, 20 snails and 6 fish were haphazardly selected and placed in each microcosm, five and four days prior to treatment respectively. Deceased or unhealthy snails and fish observed during this time period were replaced before AgNP application, accounting for <5% of all snails and fish used in the experiment. During the experiment, deceased snails (19% of population) and fish (15% of population) across all treatments were removed but not replaced. Because of the potential for food limitation within the artificial streams, fish were supplemented with 0.04g of fish food (Purina Aquamax Fry Starter 100, Missouri, USA) per day, equivalent to ~5% of their body weight (Anderson et al. 2002).

Experimental Design

Artificial streams were randomly assigned to one of four AgNP treatments or a no-addition control (n=5) for a period of two weeks from 29 July to 12 August 2016. Silver nanoparticle concentrations selected were similar to concentrations used in previous stream and lake experiments and ensured the Ag content in samples was greater than the ICP-MS detection limit of $0.25 \mu\text{g L}^{-1}$ (Kroll et al., 2015; Furtado, et al., 2015). Stock solutions of $196 \pm 1 \text{ mg L}^{-1}$ citrate capped, 50 nm diameter AgNPs were synthesized for this study (John Stone, Georgia Southern University Chemistry Department) (Figure 2). The following AgNP treatments were applied: one time pulse dose of $35 \mu\text{g L}^{-1}$ or $70 \mu\text{g L}^{-1}$ AgNPs, or repetitive (every two days) applications of $5 \mu\text{g L}^{-1}$ or $10 \mu\text{g L}^{-1}$ AgNPs totaling $35 \mu\text{g L}^{-1}$ and $70 \mu\text{g L}^{-1}$ AgNPs by the end of the experiment (Table 1).

Synthesis of AgNPs

Silver nanoparticles were synthesized in the Stone Laboratory in the Chemistry Department at Georgia Southern University by dissolving 90 mg of AgNO_3 in 500 ml of water. The solution was brought to a boil and 10 ml of 1% sodium citrate solution was added while stirring. The solution was boiled for 30 minutes, turning from transparent yellow to opaque gray. Lastly, the solution was cooled to room temperature and diluted to 420 ml creating 50 nm silver nanoparticles (Stephanie Canonico-May, pers. comm., Dieringer et al., 2007).

Sampling and Analysis

Periphyton - biomass, silver content, and chlorophyll a

Following initial AgNP treatment addition and on the final day of the experiment, nine unglazed tiles were haphazardly selected for removal from each microcosm. Tiles were brushed with a hard nylon bristled brush (Wildco 3-156-F40, Florida, USA) to collect periphyton. Ash-free dry mass (AFDM), chlorophyll *a* concentration, and silver content were each determined from periphyton collected from separate groups of three tiles (Porter et al., 1993). To measure AFDM, periphyton in pre-weighed aluminum tins was heated at 105°C and weighed followed by combustion at 500°C for one hour followed by re-wetting and drying at 105°C prior to re-weighing to correct for clay moisture (Rice et al., 2012).

For chlorophyll *a* concentration and silver content, periphyton brushed from tiles was centrifuged at 3000 rpm for ten minutes to pellet the material (EPA, 1996). To determine chlorophyll *a* concentration, the pellet was submerged in 10mL of 90% acetone for 24 hours in the dark at -20°C to extract pigments, followed by fluorescence measurement using a Trilogy Fluorometer (Turner Designs, CA, USA) according to EPA Method 445.0 (Arar and Collins, 1997). For periphyton silver content, periphyton pellets were frozen at -20°C in the dark until analysis for silver content could be performed within three months of processing (EPA, 2007). After thawing, samples were dried at 60°C and weighed (Lantry and O’Gorman, 2007) followed by digestion with 70% Trace-Metal Grade HNO₃ (9 ml) and 36% HCl (1 ml), and H₂O₂ (2 ml) and refluxing (continuously evaporating and condensing) at 120°C for six hours until any yellow/brown color disappeared (EPA, 1996; Furtado et al. 2015). Digested samples were kept at room temperature overnight to allow particulate matter to settle. A subsample (1 ml) was then removed from each sample, diluted by a factor of ten, and used to quantify silver content using

an ICP-MS (NexION 300X ICP-MS, Perkin Elmer, Massachusetts, USA) (Furtado et al. 2015). The ICP unit of the instrument aerosolized the liquid substance and converted the elemental atoms into positively charged ions. The MS unit then separated the ions by their mass-to-charge ratio and allowed for quantification of the number of ions of each element in the sample. Using a predetermined linear regression curve, the number of silver atoms in the periphyton was calculated (Wolf, R. 2005).

Snail and Fish Silver Content

Silver content in fish and snails were only determined at the end of the experiment due to the destructive nature of the sampling. All fish and snails were removed from each artificial stream and rinsed with deionized water to remove any AgNPs on the surface of each organism (Zhao et al., 2011). Fish were euthanized by cervical dislocation (AMVA, 2013). Snails and fish were frozen at -80°C until silver analysis could be performed one and four months later, respectively (EPA Method SW-846; EPA, 2007). Organisms were dried at 60°C for three days and weighed (Lantry and O' Gorman, 2007). Aqua regia (90:10, 70% trace metal-grade HNO_3 , 35% trace metal-grade HCl) was used to completely liquefy individual whole fish (3 ml) and groups of whole snails (3-4 individuals; 2 ml) at 70°C (~6 hours) (Lowry et al., 2012). Subsamples (0.5 ml) were taken from each digested fish, diluted by a factor of four, and analyzed for Ag via ICP-MS at Georgia Southern University (EPA Method 6020A; EPA 1998). Indium was added to snail samples as an internal standard to track interference from high Ca^+ ion concentration from shells during Ag content analysis using ICP-MS (NexION 300D ICP-MS, Perkin Elmer, Massachusetts, USA in the Buck Laboratory at the Skidaway Institute of Oceanography (Clifton Buck, pers. comm., EPA Method 6020A; EPA 1998).

Sediment Ag content

Sediment samples were collected only at the end of the experiment to avoid disruption from suspended sediment during the study. Three sediment cores (2.54 cm diameter, 1.9 cm depth) were haphazardly collected from each microcosm and frozen at -80°C until analysis. After thawing, sediment was dried at 80°C , weighed, and combusted at 400°C for 10 hours (Furtado et al., 2015). Three, one-gram subsamples were taken from each core, and digested with 10 ml of 70% HNO_3 . The solution was refluxed for 2 hours at 120°C , and again for two hours after adding another 5 ml of HNO_3 to the solution. Lastly, 3 ml of H_2O_2 was added prior to a final 2 hours of reflux. The solution was vortexed for five seconds and stored overnight at room temperature to settle suspended particulates. A subsample (1 ml) of the final solution was diluted by a factor of 7 and analyzed via ICP-MS for Ag content (Furtado et al., 2015).

Percent Recovery of Ag

Percent recovery of Ag added to streams was calculated by totaling the mass of Ag found in the water column, sediment, periphyton, snails, and fish and dividing the result by the mass of AgNPs added to each treatment during the experiment. Percent recovery of Ag was compared between water, sediment, and organisms to determine the fate of AgNPs in artificial streams.

Water Column Measurements

Environmental conditions (temperature, pH, conductivity, and dissolved oxygen) were measured prior to and immediately after administration of AgNP treatments, after one week, before each repetitive application, and at the end of the experiment using a hand-held multi-probe (YSI Professional Series, Yellow Springs Instruments, Ohio, USA). Snail and fish

excretion contributed ammonia to streams, consequently increasing pH. To mitigate this effect, 10% HCl was added to each stream to reduce the pH to initial levels (7.9 ± 0.1). Water temperature, pH, DO, and specific conductivity measurements did not differ across treatments, therefore measurements were pooled to calculate mean values for each day (Table 2).

Water samples were collected from the center of the water column (approx. 5 cm from the top and bottom) on days 0, 7, and 14 to quantify total Ag (TAg), chlorophyll *a*, and DOC concentrations in each artificial stream. To analyze water column total Ag concentration, water samples (10 ml) were immediately acidified in 4% HNO₃ and stored at 4°C for one week until the samples could be heated to 70°C for six hours, cooled to room temperature, and analyzed via ICP-MS (Furtado et al. 2015).

Samples for DOC analysis (50 ml) were collected and filtered through Whatman nitrocellulose filters (pore size 0.45 µm) to remove particulate carbon and analyzed for total carbon (TC) and dissolved inorganic carbon (DIC) using a TOC analyzer (Schimadzu TOC-L, Maryland, USA) in accordance with Standard Method 5310B (Rice et al., 2012). Dissolved organic carbon was then calculated by subtracting DIC from TC. Initial DOC concentration did not differ between treatments, ensuring differences in AgNP fate were not due to DOC content (Table 3).

Water column chlorophyll *a* concentration in each stream was determined from water samples (100 ml) filtered through Whatman GF/F glass microfiber filters (0.7 µm nominal pore size) to collect algal cells. Pigments were extracted from cells on the filters in 90% acetone for 22 hours at -4°C and measured using fluorometry accordance to EPA Method 445.0 (Arar and Collins, 1997).

Statistical Analysis

Data were tested for normality and homogeneity of variances using equal variance and normal distribution tests. Data not meeting assumptions were either transformed, or analyzed using nonparametric tests. Differences in Ag content between control and treated streams were analyzed using one-way ANOVA on log transformed fish and periphyton Ag concentration, and nonparametric Kruskal-Wallis tests on sediment and snail Ag concentration. To examine the effect of AgNP concentration and application type on Ag content, two-way ANOVA was conducted for sediment data and log transformed periphyton and fish Ag concentration. Water and snail Ag concentration could not be transformed, and were analyzed using Kruskal-Wallis tests followed by the Scheirer-Ray Hare extension.

Initial differences due to treatment in DOC (Log 10 transformation), periphyton biomass, and water chlorophyll *a* (square root transformation) were determined using one-way ANOVA tests and initial differences due to treatment in periphyton chlorophyll *a* were analyzed via Kruskal-Wallis tests. Differences in DOC (Log 10 transformation) on Day 14 between treatments were again analyzed using a one-way ANOVA. To determine whether AgNP application type or concentration affected periphyton biomass, periphyton chlorophyll *a*, and water chlorophyll *a* at the end of the experiment, two-way ANOVA tests (periphyton biomass and water chlorophyll *a*) or a Scheirer-Ray Hare test (periphyton chlorophyll *a*) were conducted. All statistical tests were performed using JMP statistical software (Version 12.0, SAS Institute Inc., Cary, NC).

RESULTS

Fish (*L. macrochirus*) Ag accumulation occurred in all AgNP treated streams (Table 3) and was dependent on the concentration applied not application type (Table 4). Fish tissue Ag concentration in the 70 $\mu\text{g L}^{-1}$ treatments were 2.5–3.8X those in the 35 $\mu\text{g L}^{-1}$ treatments regardless of application type (Table 4, Figure 7), with no effect on mortality (One-Way ANOVA, $F_{4, 25} = 0.9532$, $p = 0.4501$). While Ag accumulation in fish was affected by concentration applied, sediment Ag concentrations were influenced by both AgNP concentration and application type resulting in an interaction effect (Table 4, Figure 8). The interaction occurred because sediment in 35 $\mu\text{g L}^{-1}$ pulsed treatment accumulated twice as much Ag as the 35 $\mu\text{g L}^{-1}$ repetitive treatment, but there was no difference in accumulation between the two 70 $\mu\text{g L}^{-1}$ treatments.

Application type affected Ag adsorption to periphyton. Periphyton Ag concentration in repetitive treatments was 1.9-3.4X higher than the control less than one hour following initial application, indicating rapid settling of AgNPs (Table 3). Settling of AgNPs was also highly variable; periphyton in streams receiving 10 $\mu\text{g L}^{-1}$ AgNPs in the 70 $\mu\text{g L}^{-1}$ repetitive treatment accrued twice the Ag of periphyton in 35 and 70 $\mu\text{g L}^{-1}$ pulsed treated streams (Table 3, Figure 4). Final periphyton Ag concentration in repetitive treatments streams averaged 2-3X higher than pulsed AgNP treatments (Table 4, Figure 5). However, tissue Ag concentration was unrelated to periphyton pigment (chlorophyll *a*) concentration or abundance, which did not differ by AgNP concentration or application type (Table 5, Table 6).

Final water chlorophyll *a* and DOC were unaffected by AgNP concentration and application type (Table 6). Neither concentration nor application type influenced snail (*C.*

decisum) or water column Ag concentration (Table 4). Snail (*C. decisum*) tissue Ag content was 5.8 – 19.6X higher in treatments that received AgNPs than the control (Table 3, Figure 6), yet no differences in Ag concentration (Table 4, Figure 6) or mortality (One-Way ANOVA, $F_{4, 25} = 1.5402$, $p = 0.2211$) due to application type or concentration were observed. Percent error calculated between average initial total Ag (TAg) measured in the water column and nominal AgNP concentration applied was lowest in 10 $\mu\text{g L}^{-1}$ AgNP treatments (0.53%), followed by 70 $\mu\text{g L}^{-1}$ AgNP treatments (3.39%), 5 $\mu\text{g L}^{-1}$ treatments (6.07%), and 35 $\mu\text{g L}^{-1}$ treatments (19.01%) (Table 1). Silver concentrations measured among streams in each treatment were precise, having low coefficients of variations (5 $\mu\text{g L}^{-1}$ (6.63%), 10 $\mu\text{g L}^{-1}$ (1.50%), 35 $\mu\text{g L}^{-1}$ (4.24%), 70 $\mu\text{g L}^{-1}$ (5.61%). Differences determined in initial Ag concentrations were no longer detected across concentration or application treatments after one week (Table 1, Figure 3). Concentrations in 70 $\mu\text{g L}^{-1}$ and 35 $\mu\text{g L}^{-1}$ pulsed treatments decreased by 98% and 96% by day 7. Despite addition of 5 and 10 $\mu\text{g L}^{-1}$ to repetitive treatments on days 0, 2, 4 and 6, 92% and 94% of TAg settled out of the water column by day 7 (Table 1). All TAg concentrations in AgNP treatments remained below 2.32 $\mu\text{g L}^{-1}$ with no difference between treatment concentration or application type (Table 1, Table 4).

Most silver accumulation occurred in the sediment across all treatments (90-95%), while 4-8% of Ag remained in the water column, and <1% of Ag was recovered in aquatic organisms. Across organisms, snails accumulated the highest amount of Ag (10- 36 $\mu\text{g g}^{-1}$), followed by periphyton (3.5-9.6 $\mu\text{g g}^{-1}$), and fish (0.15-0.69 $\mu\text{g g}^{-1}$) (Figure 9). Percent recovery of AgNPs was between 71 and 75% for the 35 and 70 $\mu\text{g L}^{-1}$ repetitive treatments and the 70 $\mu\text{g L}^{-1}$ pulsed treatment. Streams treated with 35 $\mu\text{g L}^{-1}$ AgNPs appeared to have greater than 100%

recovery of AgNPs, but this is most likely attributed high variability in recovery of $35 \mu\text{g L}^{-1}$ treated streams and the small samples sizes of sediment.

DISCUSSION

Concentration Effects

The hypothesis that the fate of AgNPs in streams depends on the concentration and mode of application was partially supported. Specifically, I anticipated that Ag concentration in organisms, water, and sediment should increase with increasing external concentration. Silver uptake in fish was dependent only on the concentration of AgNPs applied. Fish take up silver nanoparticles via dietary consumption, water ingestion, or respiration (Bruneau et al., 2016). Though fish were observed feeding on algae in streams, their diet primarily consisted of supplementary food, suggesting Ag accumulation occurred largely via respiratory routes or ingestion of water (Bruneau et al., 2016). Furthermore, once AgNPs settled, they did not appear to become resuspended, thus potentially limiting AgNP exposure time and contact with fish. Fish ingest ~30% of their body weight per day in water, therefore fish may have quickly accumulated AgNPs from the water column prior to settling leading to a concentration effect (Bruneau et al., 2016). Fish AgNP uptake in this study ($0.20\text{-}0.69 \mu\text{g g}^{-1}$) was similar to wet weight mosquitofish body burdens ($0.5 \mu\text{g g}^{-1}$) in wetland mesocosms exposed to a pulsed treatment of 25mg L^{-1} AgNPs (3.5X greater magnitude) over 18 months, however, if dry weights were recorded for mosquitofish, body burdens would likely increase (Lowry et al., 2012). Furthermore, mosquitofish may have had higher body burdens after the initial pulsed exposure, but expelled

Ag from their tissues via depuration over the 18-month period as AgNPs settled from the water column (Jang et al., 2014).

Water column Ag concentration did not affect periphyton tissue Ag concentration. This result was surprising given that concentration influenced periphyton Ag concentration following pulsed applications of 2 and 20 $\mu\text{g L}^{-1}$ to artificial streams (Kroll et al., 2016). Final Ag adsorption to periphyton in this study was similar to adsorption in streams receiving only 2 $\mu\text{g L}^{-1}$ AgNPs after 18 days (Kroll et al., 2016). Differences in periphyton Ag adsorption between the two studies may be attributed to the presence of snails in this study (Amato et al., 2016). Benthic organisms like snails commonly change the position and chemical structure of contaminants in benthic areas by mixing sediments, disturbing periphyton, or ionizing metals through means of oxygenation in anoxic sediments (Amato et al., 2016). Bioturbation practiced by snails combined with the consumption of AgNPs on/in periphyton most likely reduced Ag concentrations adsorbed to periphyton in 70 and 35 $\mu\text{g L}^{-1}$ treatments preventing any concentration effects (Amato et al., 2016; Oliver et al., 2014).

Absence of concentration effect in snail body burdens was potentially due to dietary uptake and the variability in AgNP settling (Croteau et al., 2011; Ren et al., 2016). Concentrations of heavy metals like Ag often settle with spatial variability leading to areas with higher and lower AgNP densities in the sediment and periphyton (Tam and Wong, 1995; Ren et al., 2016). Snails feeding on periphyton may accumulate AgNPs at different rates as they travel through sediment depending on the concentration of AgNPs in the area (Oliver et al., 2014; Croteau et al., 2011). Therefore uptake of AgNPs by *C. decisum* was most likely dependent on variable AgNP densities in periphyton.

Though initial total Ag (TAg) concentrations in the water column reflected nominal concentrations applied, AgNPs rapidly settled and no differences were detected between treatment concentrations on Day 7 and 14. This finding was contradictory to water column Ag concentrations determined in previous lake and stream mesocosm studies (Furtado et al., 2015; Kroll et al., 2016). Though artificial streams in this study had similar DOC and ion concentrations as lake systems, AgNPs in lakes stayed suspended over one month longer (Furtado et al., 2015). One possible reason for this discrepancy is that flowing water in the artificial streams increased interaction with sediment and periphyton likely removing AgNPs from the water column at a faster rate than lake systems (Velzeboer et al., 2014). However, flowing water may not be the only reason for a lack of concentration effect in the water column of this study as another stream experiment also showed differences between AgNP concentrations applied (Kroll et al., 2016). Stream design potentially led to the difference in concentration effect between this experiment and Kroll et al. (2016). Though artificial streams in both experiments were recirculating, streams in this experiment were circular in shape and included sediment compared to artificial streams in Kroll et al. (2016), which lacked sediment and were straight, reducing collisions with periphyton and sediment (Kroll et al., 2016; Velzeboer et al., 2014).

Effects of Application Type

Increased Ag concentration associated with benthic organisms was expected in pulsed AgNP treatments. However, more Ag was adsorbed to periphyton in the repetitive treatments, possibly due to the presence of water flow. In the pulse treatments there was more time to move unbound AgNPs off the periphyton tiles and for bioturbation or ingestion of Ag by snails (Kroll

et al., 2016; Amato et al., 2016; Croteau et al., 2011) giving the appearance of decreased Ag concentration. Evidence of flowing water reducing periphyton Ag concentrations was indicated in Kroll et al. (2016) as periphyton Ag concentrations decreased by 80% over a two-week period following a pulsed application. Furthermore, no effect of application type on snail tissue AG concentration occurred, potentially because *C. decisum* can avoid AgNPs (Justice and Bernot, 2014). In this study, half the snails exposed to AgNPs moved away from the sediment and up the walls of artificial streams. The snail *Physa acuta* was observed climbing container walls after exposure to $0.03 \mu\text{g L}^{-1}$ of AgNP despite increased visibility to predators, suggesting contaminant avoidance (Justice and Bernot, 2014). The AgNP treatments in this experiment were three orders of magnitude greater than $0.03 \mu\text{g L}^{-1}$, therefore concentration could have been more important in eliciting avoidance behavior than application type.

Although repetitive applications of AgNPs were expected to remain suspended in the water column longer than pulsed treated streams, rapid settling occurred in both repetitive and pulsed application treatments. Citrate-capped, small, low molecular weight AgNPs may remain suspended in water indefinitely (Velzeboer et al., 2014). However, suspension time may be reduced by physicochemical conditions including ionic strength (Ca^{+2} , Mg^{+2} , Na^{+} and Cl^{-}), DOC concentration, and pH (Velzeboer et al., 2014; Fabrega et al., 2011). Silver nanoparticles in environments with DOC concentrations greater than 4 mg L^{-1} form heteroagglomerates with natural organic matter, in turn stabilizing and suspending AgNPs in the water column (Fabrega et al., 2011). Conversely, environments with high ionic strength reduce the repulsion between negatively charged citrate capped AgNPs leading to the formation of large AgNP aggregates, in turn increasing their mass and settling via gravitational forces (Hotze et al., 2014; Navaro et al., 2008). Aggregation of citrate capped AgNPs also increases in acidic environments (pH of 3) and

stabilizes AgNPs in neutral to basic environments (Badaway et al., 2010). Ogeechee River water used in this experiment was characterized by high DOC content, low ionic strength, and slightly basic conditions (Meyer et al., 1997; R. Cohen, personal communication), which would typically lead to the suspension of AgNPs, yet rapid settling occurred in artificial streams.

This rapid settling in lotic environments also seems contradictory given that higher Ag concentrations were found in lake sediment following a pulsed application compared to repetitive applications (Furtado et al., 2013). When comparing artificial streams in this study to the artificial lakes in Furtado's study, both systems were characterized by high DOC, neutral to slightly basic pH, and relatively low conductivity, suggesting flow actually decreased the suspension of AgNPs in artificial streams ([Kennedy et al., 2012](#)). In artificial streams with flow rates similar to this study (0.20 m s^{-1}), rapid settling of 80-88% of AgNPs occurred in artificial streams after four days (Kroll et al., 2016). However, the conditions in the streams used by Kroll et al. (2016) included high ionic content (conductivity 4X higher than this study) and low DOC content (6X lower), conducive to faster settling rates. In this study, it is possible that the flowing conditions increased collisions between suspended particles or phytoplankton in the water column inducing settling (Velzeboer et al., 2014). Silver nanoparticles may have also adsorbed to periphyton growing on the sides of the streams reducing suspension time of AgNPs. No periphyton was removed from the sides of streams because the majority was removed by grazing snails. Clearly more work must be conducted in order to fully determine how flow rates affect AgNP settling rates and aggregation.

Interactive Effects

Sediment responses to AgNP exposure exhibited some support for effects of concentration and application type. Concentration effects in sediment seemed to occur when comparing the 35 $\mu\text{g L}^{-1}$ and 70 $\mu\text{g L}^{-1}$ repetitive treatments, but not in pulsed treatments. Similar concentration effects occurred in lake systems where sediment Ag concentrations increased with the application of higher AgNP concentrations (Furtado et al., 2015). The unpredictably high sediment Ag concentration in the 35 $\mu\text{g L}^{-1}$ pulse treatment most likely occurred due to spatial variation in heavy metal settling and is common in aquatic environments (Tam and Wong, 1995; Ren et al., 2016). Though previous studies collected 2-3 sediments cores per mesocosm to acquire sediment Ag concentrations, this study suggests a larger sampling size could reduce variation and provide more precise results in future studies (Furtado et al., 2015; Lowry et al., 2013).

Fate of AgNPs in Artificial Streams

The overall goal of this study was to determine if the fate and effects of AgNPs differ with concentration and application type in lotic environments under environmentally relevant conditions. Nearly all (90-95%) of the Ag recovered from artificial streams was in the sediment regardless of treatment, suggesting benthic organisms are at greater risk of AgNP exposure than pelagic organisms. In addition, this risk may be long-term and spread to new areas. Once AgNPs and their derivatives settle, they often remain in the sediment and bioavailable to aquatic organisms (US EPA, 2017). Silver bound to sediments may also become resuspended and contaminate aquatic communities downstream (US EPA, 2017). Recovery of Ag in organisms of artificial streams was in the following order: benthic snails > periphyton > fish. When comparing

biotic uptake of Ag in artificial stream and wetland mesocosms, benthic organisms also accumulated more Ag silver than pelagic organisms: riparian plants (primarily roots) > benthic macroinvertebrates (chironomids, odonates) > fish (*Gambusia* sp.) (Lowry et al., 2012). As AgNPs accumulate in benthic organisms, there is potential for trophic transfer to pelagic organisms in both lotic and lentic systems (Wang et al., 2014). Despite Ag accumulation in aquatic organisms, neither mortality (fish or snail) nor periphyton abundance was affected by AgNP exposure. While AgNP concentrations below $70 \mu\text{g L}^{-1}$ were lethal to algae and snails under controlled laboratory settings (Wong et al., 2013), the presence of multiple species, sulfide ions, and natural organic matter (NOM) in artificial streams may have reduced toxic effects (Wu et al., 2017; Levard et al., 2012). It is also possible that the AgNPs accumulated in benthic organisms undergo trophic transfer to pelagic organisms in lotic systems (Wang et al., 2014) but this may require longer time frames than investigated in the present study.

Conclusion

This study shows that both concentration and application type have a role in the fate of AgNPs in artificial streams. Concentration affected water column (albeit briefly) and fish tissue Ag concentration, likely via respiratory contact and water ingestion. Application type influenced Ag adsorption to periphyton, possibly because AgNPs in pulse treatments had more time to be removed from tiles as a result of water movement and snail grazing. Sediment Ag concentration appeared to be affected by concentration in the repetitive treatments, but not the pulsed treatments due to the variability in AgNP settling. Overall, rapid settlement of AgNPs regardless of application type suggests sediment as a sink for Ag, placing benthic organisms at particular risk of exposure. In addition, absence of mortality following exposure to concentrations

previously shown to be lethal to snails and periphyton under laboratory settings indicates the importance of testing AgNP exposure under ecologically and environmentally relevant conditions to properly assess their toxicity in aquatic environment.

Table 1: Measured total water column Ag concentration \pm one standard error of the mean (SEM) initially and one and two weeks after treatment application (n=6).

AgNP Treatments	Day 0	Day 7	Day 14
Ctrl	0 ± 0	0 ± 0	0 ± 0
$35 \mu\text{g L}^{-1}$ repetitive	5.30 ± 0.16	1.69 ± 0.02	2.16 ± 0.05
$70 \mu\text{g L}^{-1}$ repetitive	9.95 ± 0.07	2.33 ± 0.28	2.32 ± 0.04
$35 \mu\text{g L}^{-1}$ pulsed	28.35 ± 0.54	1.15 ± 0.03	1.95 ± 0.02
$70 \mu\text{g L}^{-1}$ pulsed	67.63 ± 1.70	1.17 ± 0.02	2.00 ± 0.02

Table 2: Average stream pH, DO, specific conductivity, and temperature in all streams over two weeks (N=30). Numbers in parentheses represents \pm one SEM.

Day	pH	DO (mg L ⁻¹)	Specific Cond (μ s)	Temperature
0 (before dose)	8.05 (0.02)	7.31 (0.03)	92.20 (1.67)	29.12 (0.11)
0 (after dose)	8.35 (0.02)	6.73 (0.07)	93.49 (1.34)	30.61 (0.09)
2	9.00 (0.04)	7.83 (0.07)	91.69 (1.23)	32.06 (0.20)
4	8.96 (0.03)	8.39 (0.14)	97.78 (1.28)	32.11 (0.16)
6	8.80 (0.03)	7.36 (0.10)	102.54 (1.70)	30.79 (0.06)
7	8.51 (0.06)	7.38 (0.06)	107.35 (2.17)	30.66 (0.17)
8	8.56 (0.04)	7.48 (0.07)	104.49 (1.76)	29.64 (0.08)
10	8.33 (0.03)	7.38 (0.07)	107.84 (1.94)	29.20 (0.08)
12	8.32 (0.04)	7.29 (0.07)	108.66 (2.22)	29.45 (0.13)
14	7.81 (0.05)	7.31 (0.04)	110.68 (2.39)	28.93 (0.12)

Table 3: Comparisons showing overall differences between treatments in Ag accumulation Day 0 periphyton, Day 14 periphyton, snails, fish, and sediment on day 14, in addition to the corresponding Tukey-Kramer, Wilcoxon Each Pair, and Steel-Dwass All Pairs Post-Hoc tests (n=6). Significant values are denoted with an asterisk.

	Test	Df	Test Statistic	p-value
Periphyton Ag Day 0	Kruskal-Wallis	4, 23	$\chi^2 = 14.4906$	<0.0059*
	Wilcoxon Each Pair Comparison			
	10 $\mu\text{g L}^{-1}$ vs all treatments			<0.05*
	ctrl vs. 35 $\mu\text{g L}^{-1}$ Repetitive			0.0216*
	ctrl vs. 35 $\mu\text{g L}^{-1}$ Pulsed			0.0552
Periphyton Ag Day 14	One-Way ANOVA	4, 24	F = 13.4084	< 0.0001*
	Tukey-Kramer post hoc Comparisons			
	ctrl vs. all treatments			< 0.05*
Snails Ag	Kruskal-Wallis	4, 25	$\chi^2 = 14.4172$	0.0061*
	Wilcoxon Each Pair Comparisons			
	ctrl vs. all treatments			< 0.05*
Fish Ag	One-Way ANOVA	4,25	F = 29.3845	< 0.0001*
	Tukey-Kramer post hoc Comparisons			
	ctrl vs. all treatments			< 0.05*
Sediment Ag	Kruskal-Wallis	4, 25	$\chi^2 = 21.2000$	0.0003*
	Steel-Dwass All Pairs Comparisons			
	ctrl vs. all treatments			< 0.05*

Table 4: Comparisons of final Ag concentration in periphyton, snails, fish, sediment, and water (n=6) across application type and concentration treatments. Asterisks denote significant differences.

	Analysis	Df	Test Statistic	p-value
Periphyton Ag Day 14	Two-Way ANOVA			
	Treatment	1, 19	F = 0.00350	0.9532
	Application Type	1, 19	F = 15.7322	0.0008*
	Interaction	1, 19	F = 0.0883	0.7695
Snails Ag	Scheirer-Ray Hare			
	Treatment	1, 20	H = 1.270	> 0.10
	Application Type	1, 20	H = 0.0013	> 0.10
	Interaction	1, 20	H = 0.6413	> 0.10
Fish Ag	Two-Way ANOVA			
	Treatment	1, 20	F = 22.0049	0.0001*
	Application Type	1, 20	F = 0.0132	0.9096
	Interaction	1, 20	F = 22.0049	0.4484
Sediment Ag	Two-Way ANOVA			
	Treatment	1, 20	F = 7.7080	0.0117*
	Application Type	1, 20	F = 3.3913	0.0804
	Interaction	1, 20	F = 5.6000	0.0282*
Table continued on next page.				

Table continued from previous page.				
Water TAg Day 14	Scheirer-Ray Hare			
	Treatment	1, 20	H = 0.3925	> 0.05
	Application Type	1, 20	H = 2.3789	> 0.05
	Interaction	1, 20	H = 0.1252	> 0.05

Table 5: Comparisons of initial periphyton biomass, water chlorophyll *a*, periphyton chlorophyll *a*, and DOC concentration across treatments (n=6).

	Test	Df	Test Statistic	p-value
Periphyton Chl- <i>a</i>	Kruskal-Wallis	4	$\chi^2 = 1.9140$	0.7516
Periphyton biomass	One-Way ANOVA	4,25	F = 1.7525	0.1701
Water Chl- <i>a</i>	One-Way ANOVA	4,25	F = 1.5342	0.2228
DOC	One-Way ANOVA	4,25	F = 0.2162	0.9270

Table 6: Comparisons of the effects of AgNP concentration and application type on final periphyton biomass, water chlorophyll *a*, periphyton chlorophyll *a*, and DOC concentrations (n=6).

Periphyton Biomass	Two-Way ANOVA	Df	Test Statistic	p-value
	Treatment	1, 20	F = 0.3239	0.5756
	Dosing Regimen	1, 20	F = 0.0351	0.8533
	Interaction	1, 20	F = 0.0149	0.9042
Water Chl <i>a</i>	Two-Way ANOVA			
	Treatment	1, 20	F = 0.4239	0.5224
	Dosing Regimen	1, 20	F = 0.9416	0.3434
	Interaction	1, 20	F = 2.474	0.1314
Periphyton Chl <i>a</i>	Scheirer-Ray Hare			
	Treatment	1, 20	H = 0.0213	> 0.05
	Dosing Regimen	1, 20	H = 0.6482	> 0.05
	Interaction	1, 20	H = 1.822	> 0.05
DOC	One-Way ANOVA			
		4, 25	F = 0.1460	0.9631

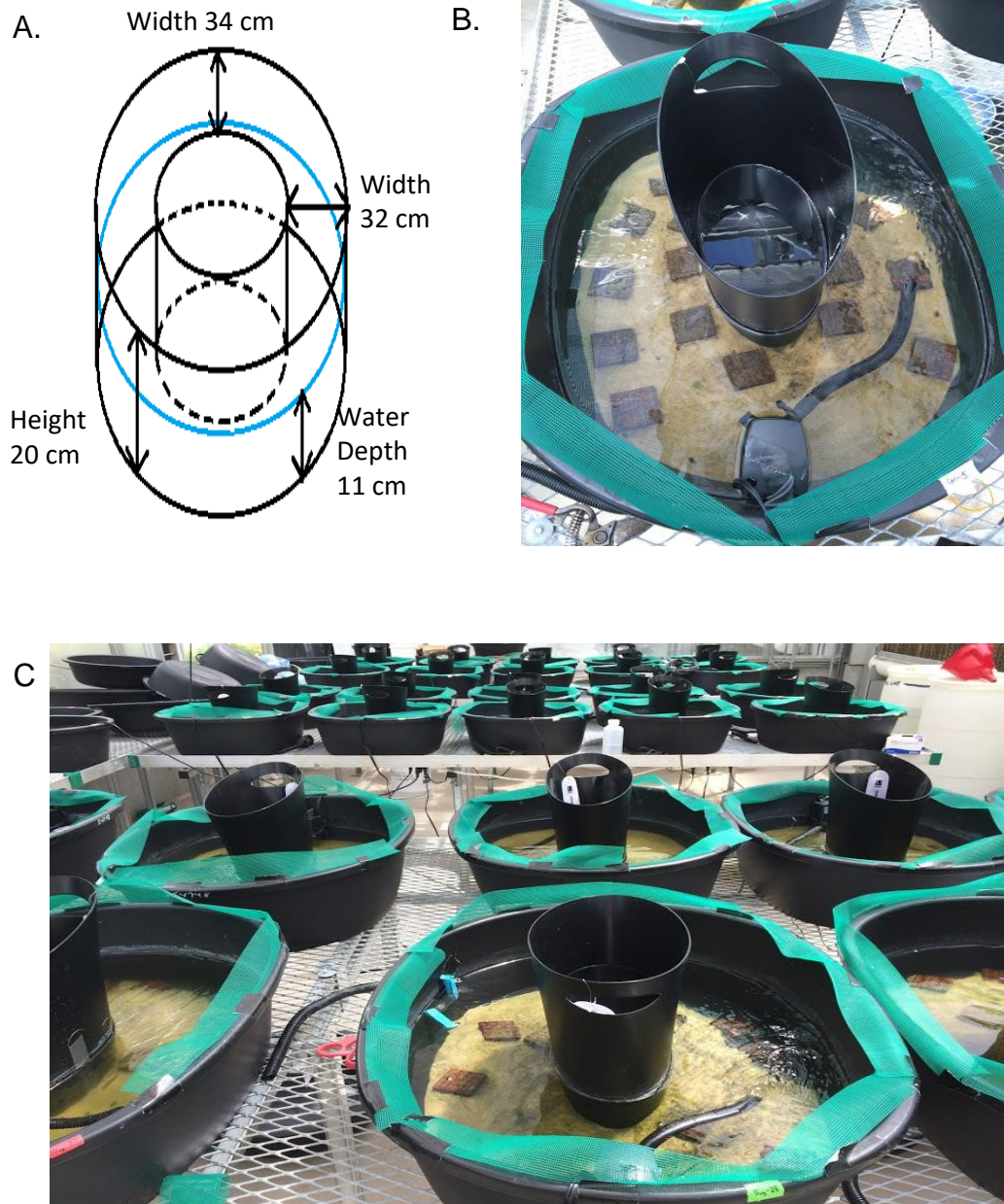


Figure 1: Artificial stream microcosm A) dimensions, B) set up with water, sediment and tiles, and C) experimental set up in the Georgia Southern University greenhouse.

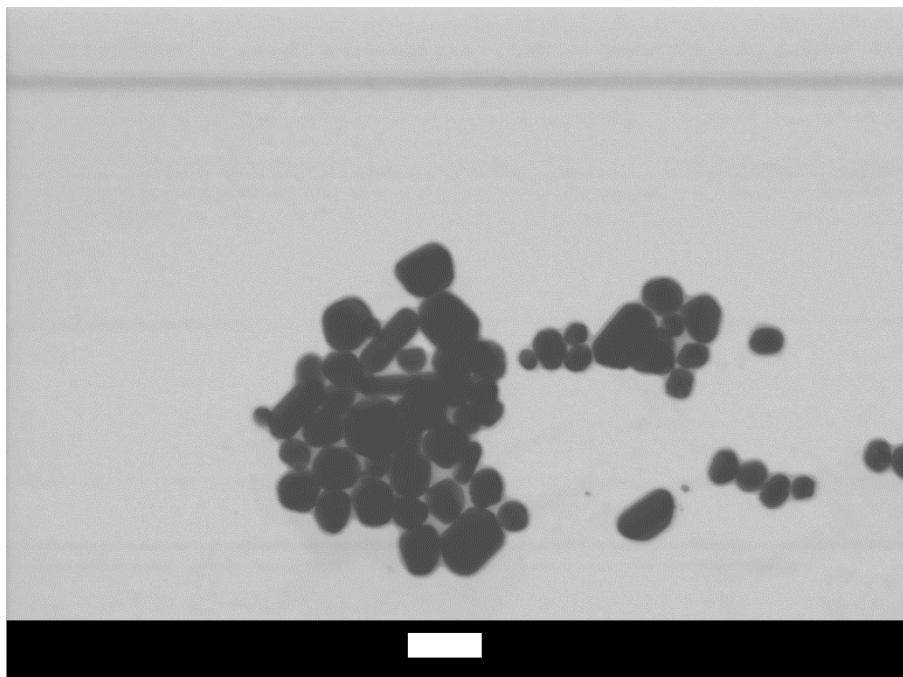


Figure 2: Citrate capped AgNPs (~50nm) used in the experiment.

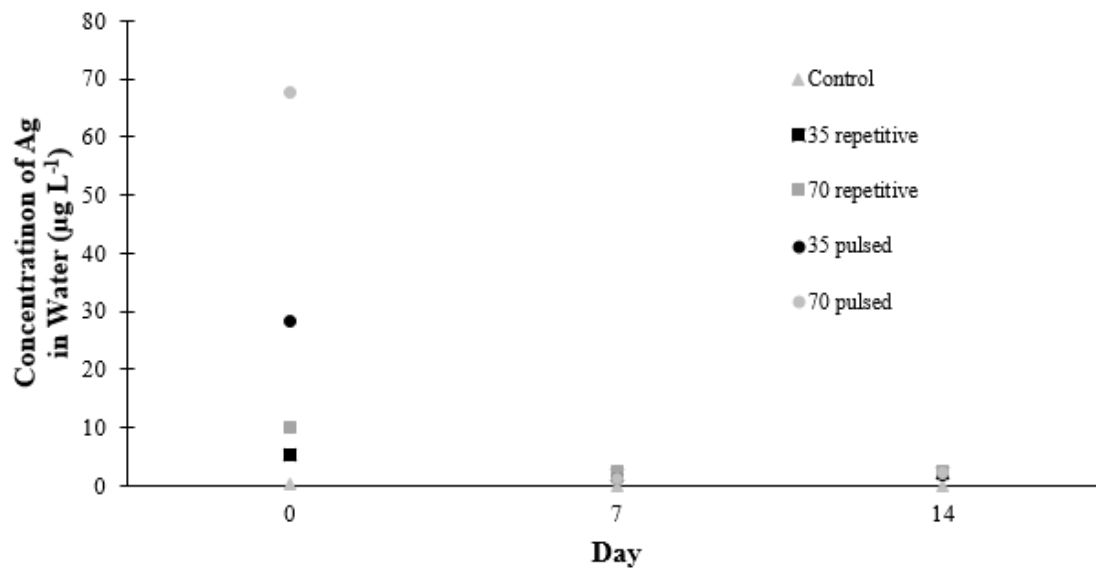


Figure 3: Average water column total Ag concentration ($\mu\text{g L}^{-1}$) following 0, 7, and 14 days of AgNP exposure ($n=6$). Standard error bars are not visible due to low variation among total Ag measurements.

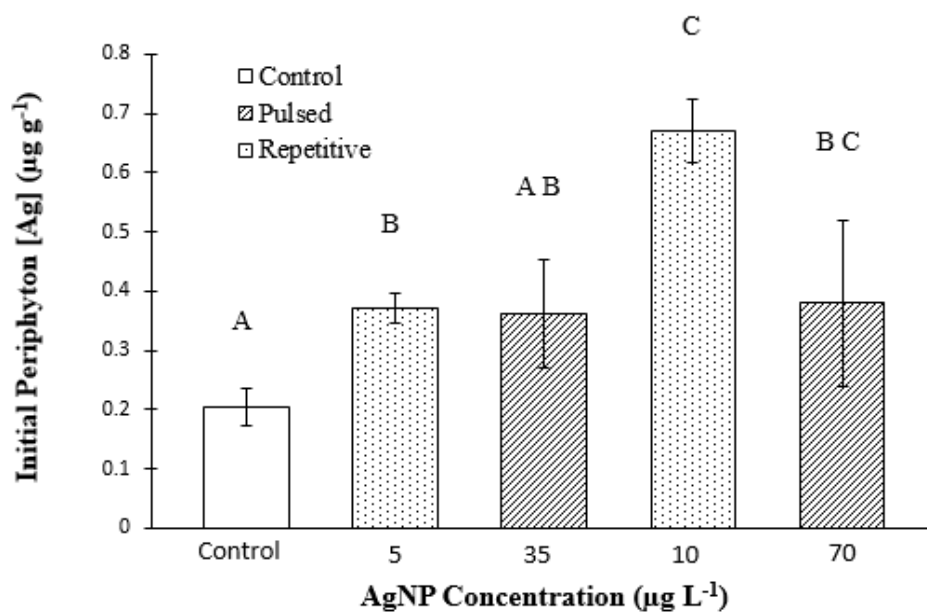


Figure 4: Initial periphyton tissue Ag concentration across AgNP treatments (n=6). Treatments not sharing the same letters are significantly different from one another. Error bars represent \pm one SEM.

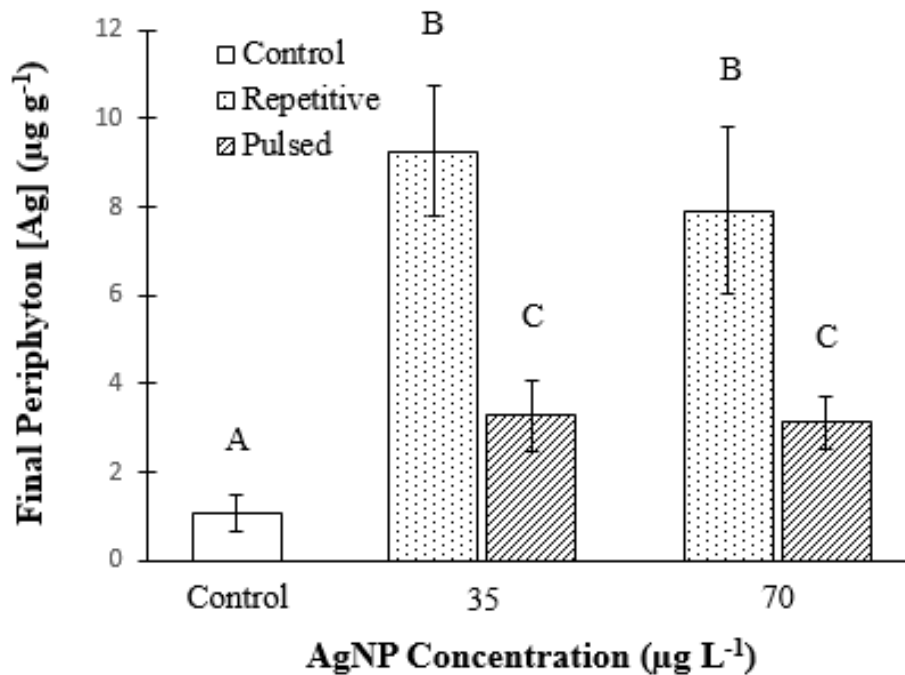


Figure 5: Final periphyton tissue Ag concentration across AgNP treatments (n=6). Error bars represent \pm one SEM and treatments not sharing the same letters are significantly different from one another.

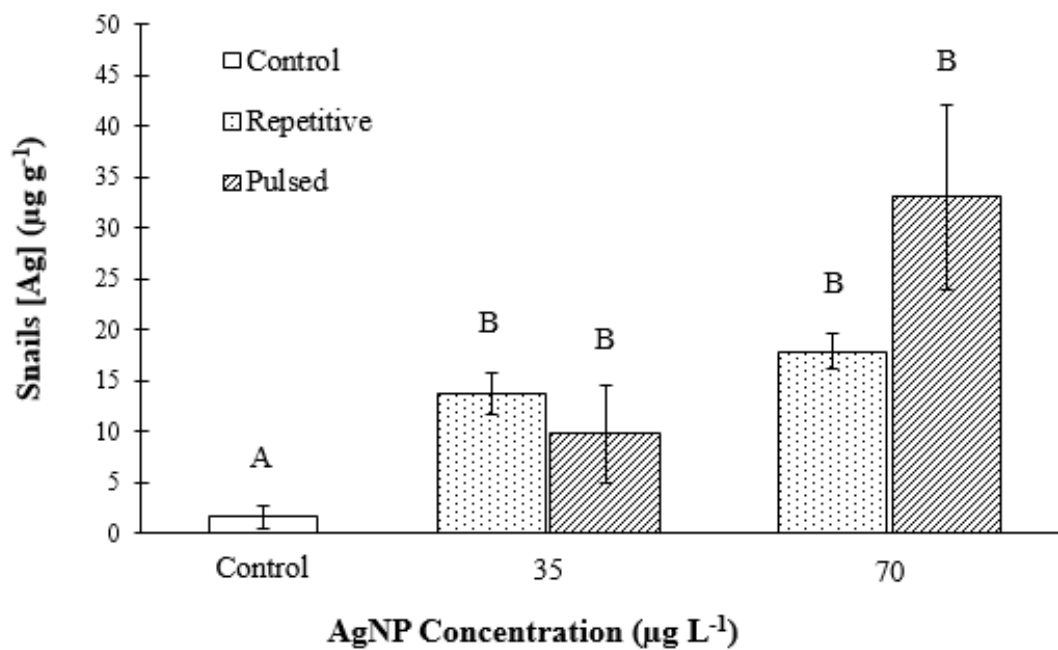


Figure 6: Mean snail Ag concentration after 14 days of exposure to AgNP treatments (n=6).

Error bars represent \pm one SEM and treatments not sharing the same letters are significantly different from one another.

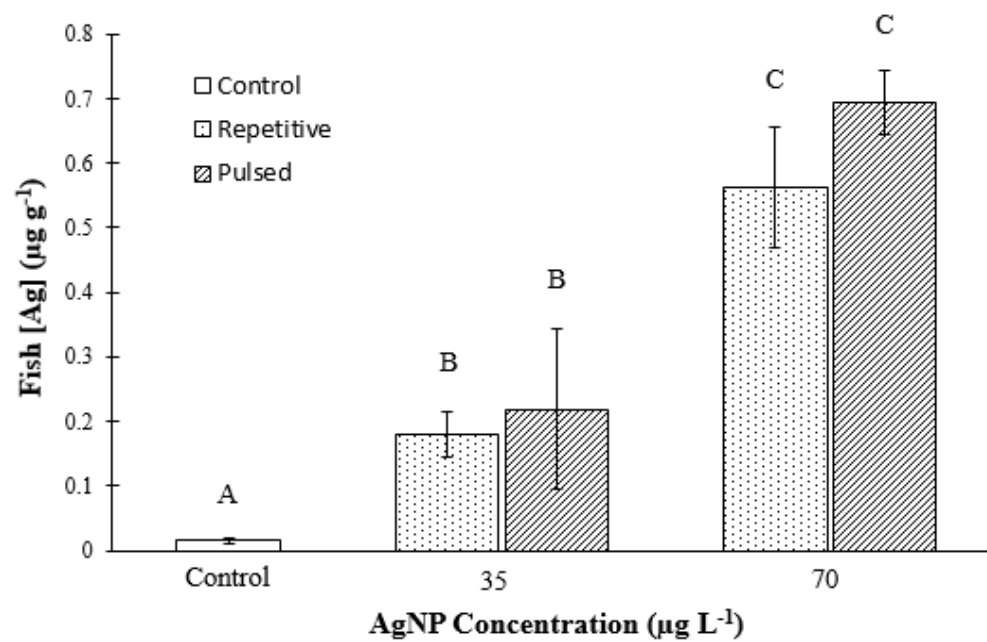


Figure 7: Average fish tissue Ag concentration after 14 days of exposure to AgNP treatments (n=6). Error bars represent \pm one SEM and treatments not sharing the same letters are significantly different from one another.

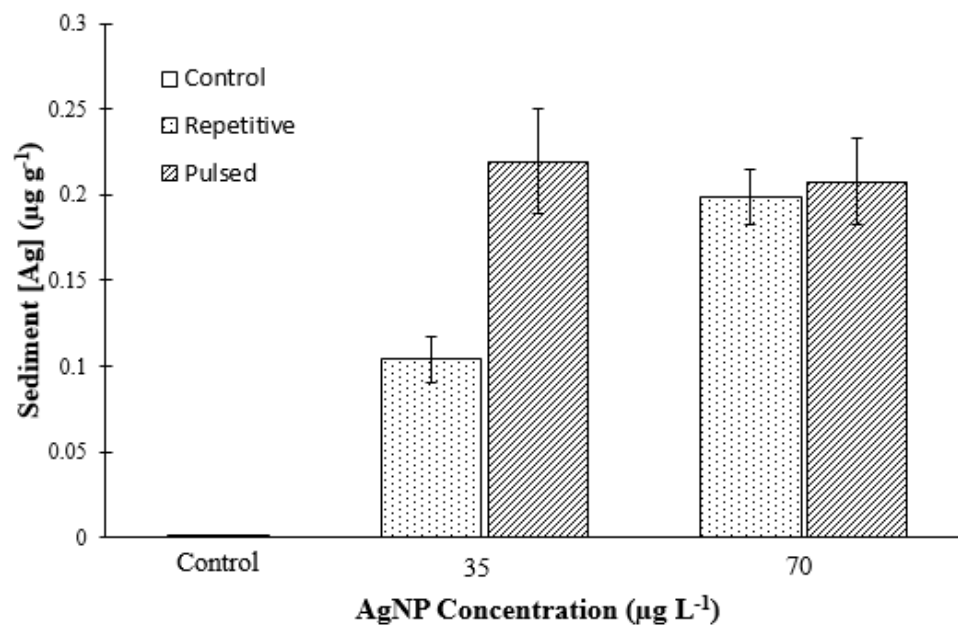


Figure 8: Mean sediment Ag g⁻¹ concentration after 14 days of exposure to AgNP treatments (n=6). Error bars represent ± one SEM and treatments not sharing the same letters are significantly different from one another.

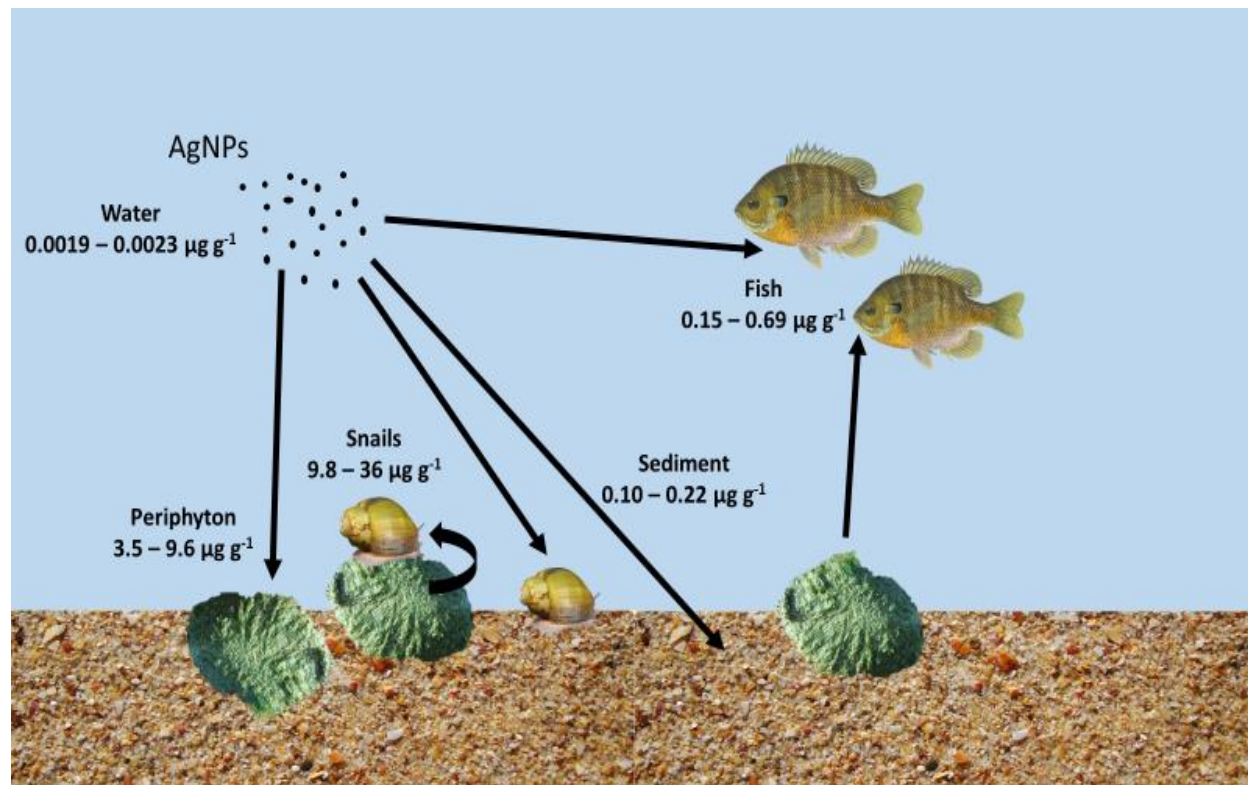


Figure 9: The concentration of Ag in, and possible trophic interactions among periphyton, fish and snails in artificial stream microcosms. Concentration ranges include Ag recovered from pulsed and repetitive AgNP treatments.

LITERATURE CITED

- Ali, D.; Yadav, P. G.; Kumar, S.; Ali, H.; Alarifi, S.; Harrath, A. H., Sensitivity of freshwater pulmonate snail *Lymnaea luteola* L., to silver nanoparticles. *Chemosphere* **2014**, *104*, 134-140.
- Amato, E. D.; Simpson, S. L.; Remaili, T. M.; Spadaro, D. A.; Jarolimek, C. V.; Jolley, D. F., Assessing the effects of bioturbation on metal bioavailability in contaminated sediments by diffusive gradients in thin films (DGT). *Environmental science & technology* **2016**, *50*, (6), 3055-3064.
- Anderson, D.; Saoud, I. P.; Davis, D. A., The effects of stocking density on survival, growth, condition, and feed efficiency of bluegill juveniles. *North American Journal of Aquaculture* **2002**, *64*, (4), 297-300.
- AVMA, Guidelines for the Euthanasia of Animals *American Veterinary Medical Association* **2013**.
- Arar, E. and Collins, G., In Vitro Determination of Chlorophyll a and Pheophytin a in Marine and Freshwater Algae by Fluorescence Revision 1.2. *USEPA* **1997**, 1-23.
- Badawy, A. M. E.; Luxton, T. P.; Silva, R. G.; Scheckel, K. G.; Suidan, M. T.; Tolaymat, T. M., Impact of environmental conditions (pH, ionic strength, and electrolyte type) on the surface charge and aggregation of silver nanoparticles suspensions. *Environmental science & technology* **2010**, *44*, (4), 1260-1266.
- Benn, T. M.; Westerhoff, P., Nanoparticle silver released into water from commercially available sock fabrics. *Environmental science & technology* **2008**, *42*, (11), 4133-4139.
- Bernot, R. J.; Brandenburg, M., Freshwater snail vital rates affected by non-lethal concentrations of silver nanoparticles. *Hydrobiologia* **2013**, *714*, (1), 25-34.
- Bruneau, A.; Turcotte, P.; Pilote, M.; Gagné, F.; Gagnon, C., Fate of silver nanoparticles in wastewater and immunotoxic effects on rainbow trout. *Aquatic Toxicology* **2016**, *174*, 70-81.
- Bunn, S. E.; Arthington, A. H., Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. *Environmental management* **2002**, *30*, (4), 492-507.
- Choi, J. E.; Kim, S.; Ahn, J. H.; Youn, P.; Kang, J. S.; Park, K.; Yi, J.; Ryu, D.-Y., Induction of oxidative stress and apoptosis by silver nanoparticles in the liver of adult zebrafish. *Aquatic Toxicology* **2010**, *100*, (2), 151-159.

Croteau, M.-N.; Misra, S. K.; Luoma, S. N.; Valsami-Jones, E., Silver bioaccumulation dynamics in a freshwater invertebrate after aqueous and dietary exposures to nanosized and ionic Ag. *Environmental science & technology* **2011**, *45*, (15), 6600-6607.

De Moura, M. R.; Mattoso, L. H.; Zucolotto, V., Development of cellulose-based bactericidal nanocomposites containing silver nanoparticles and their use as active food packaging. *Journal of Food Engineering* **2012**, *109*, (3), 520-524.

Dieringer, J. A.; Lettan, R. B.; Scheidt, K. A.; Van Duyne, R. P., A frequency domain existence proof of single-molecule surface-enhanced Raman spectroscopy. *Journal of the American Chemical Society* **2007**, *129*, (51), 16249-16256.

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

Fabrega, J.; Fawcett, S. R.; Renshaw, J. C.; Lead, J. R., Silver nanoparticle impact on bacterial growth: effect of pH, concentration, and organic matter. *Environmental science & technology* **2009**, *43*, (19), 7285-7290.

Fabrega, J.; Luoma, S. N.; Tyler, C. R.; Galloway, T. S.; Lead, J. R., Silver nanoparticles: behaviour and effects in the aquatic environment. *Environment international* **2011**, *37*, (2), 517-531.

Farkas, J.; Christian, P.; Gallego-Urrea, J. A.; Roos, N.; Hassellöv, M.; Tollefsen, K. E.; Thomas, K. V., Uptake and effects of manufactured silver nanoparticles in rainbow trout (*Oncorhynchus mykiss*) gill cells. *Aquatic Toxicology* **2011**, *101*, (1), 117-125.

Furtado, L. M.; Norman, B. C.; Xenopoulos, M. A.; Frost, P. C.; Metcalfe, C. D.; Hintelmann, H., Environmental Fate of Silver Nanoparticles in Boreal Lake Ecosystems. *Environmental Science & Technology* **2015**, *49*, (14), 8441-8450.

Gopal, M.; Gogoi, R.; Srivastava, C.; Kumar, R.; Singh, P. K.; Nair, K. K.; Yadav, S.; Goswami, A., Nanotechnology and its application in plant protection. *Plant pathology in India: vision* **2011**, *2030*, 224-232.

Gottschalk, F.; Sonderer, T.; Scholz, R. W.; Nowack, B., Modeled environmental concentrations of engineered nanomaterials (TiO₂, ZnO, Ag, CNT, fullerenes) for different regions. *Environmental science & technology* **2009**, *43*, (24), 9216-9222.

Grabowska, M.; Glińska-Lewczuk, K.; Obolewski, K.; Burandt, P.; Kobus, S.; Dunalska, J.; Kujawa, R.; Goździewska, A.; Skrzypczak, A., Effects of hydrological and

physicochemical factors on phytoplankton communities in floodplain lakes. *Polish Journal of Environmental Studies* **2014**, *23*, (3).

Grillo, R.; Rosa, A. H.; Fraceto, L. F., Engineered nanoparticles and organic matter: A review of the state-of-the-art. *Chemosphere* **2015**, *119*, 608-619.

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

Gunsolus, I. L.; Mousavi, M. P.; Hussein, K.; Bühlmann, P.; Haynes, C. L., Effects of humic and fulvic acids on silver nanoparticle stability, dissolution, and toxicity. *Environmental science & technology* **2015**, *49*, (13), 8078-8086.

Hall, C. M.; Rhind, S. M.; Wilson, M. J., The potential for use of gastropod molluscs as bioindicators of endocrine disrupting compounds in the terrestrial environment. *Journal of Environmental Monitoring* **2009**, *11*, (3), 491-497.

Hauer, F. R.; Lamberti, G. A., *Methods in stream ecology*. Academic Press: **2011**.

He, D.; Jones, A. M.; Garg, S.; Pham, A. N.; Waite, T. D., Silver nanoparticle– reactive oxygen species interactions: application of a charging– discharging model. *The Journal of Physical Chemistry C* **2011**, *115*, (13), 5461-5468.

Hotze, E. M.; Phenrat, T.; Lowry, G. V., Nanoparticle aggregation: challenges to understanding transport and reactivity in the environment. *Journal of environmental quality* **2010**, *39*, (6), 1909-1924.

Iowa DNR. "Fishing." *Fish Details*. N.p., n.d. Web. 19 Apr. **2017**.

Jang, M.-H.; Kim, W.-K.; Lee, S.-K.; Henry, T. B.; Park, J.-W., Uptake, tissue distribution, and depuration of total silver in common carp (*Cyprinus carpio*) after aqueous exposure to silver nanoparticles. *Environmental science & technology* **2014**, *48*, (19), 11568-11574.

Justice, J. R.; Bernot, R. J., Nanosilver inhibits freshwater gastropod (*Physa acuta*) ability to assess predation risk. *The American Midland Naturalist* **2014**, *171*, (2), 340-349.

Kaegi, R., et al., *Behavior of Metallic Silver Nanoparticles in a Pilot Wastewater Treatment Plant*. *Environmental Science & Technology*, **2011**. 45(9): p. 3902-3908.

- Keller, A. A.; McFerran, S.; Lazareva, A.; Suh, S., Global life cycle releases of engineered nanomaterials. *Journal of Nanoparticle Research* **2013**, *15*, (6), 1-17.
- Kennedy, A. J.; Chappell, M. A.; Bednar, A. J.; Ryan, A. C.; Laird, J. G.; Stanley, J. K.; Steevens, J. A., Impact of organic carbon on the stability and toxicity of fresh and stored silver nanoparticles. *Environmental science & technology* **2012**, *46*, (19), 10772-10780.
- Kroll, A.; Matzke, M.; Rybicki, M.; Obert-Rausser, P.; Burkart, C.; Jurkschat, K.; Verweij, R.; Sgier, L.; Jungmann, D.; Backhaus, T., Mixed messages from benthic microbial communities exposed to nanoparticulate and ionic silver: 3D structure picks up nano-specific effects, while EPS and traditional endpoints indicate a concentration-dependent impact of silver ions. *Environmental Science and Pollution Research* **2016**, *23*, (5), 4218-4234.
- Kumar, A.; Vemula, P. K.; Ajayan, P. M.; John, G., Silver-nanoparticle-embedded antimicrobial paints based on vegetable oil. *Nature materials* **2008**, *7*, (3), 236-241.
- Lantry, B. F.; O'Gorman, R., Drying temperature effects on fish dry mass measurements. *Journal of Great Lakes Research* **2007**, *33*, (3), 606-616.
- Levard, C.; Hotze, E. M.; Lowry, G. V.; Brown Jr, G. E., Environmental transformations of silver nanoparticles: impact on stability and toxicity. *Environmental science & technology* **2012**, *46*, (13), 6900-6914.
- Levard, C.; Hotze, E. M.; Colman, B. P.; Dale, A. L.; Truong, L.; Yang, X.; Bone, A. J.; Brown Jr, G. E.; Tanguay, R. L.; Di Giulio, R. T., Sulfidation of silver nanoparticles: natural antidote to their toxicity. *Environmental science & technology* **2013**, *47*, (23), 13440-13448.
- Liu, J.; Hurt, R. H., Ion Release Kinetics and Particle Persistence in Aqueous Nano-Silver Colloids. *Environmental Science & Technology* **2010**, *44*, (6), 2169-2175.
- Li, L.; Wang, Y.; Liu, Q.; Jiang, G., Rethinking Stability of Silver Sulfide Nanoparticles (Ag₂S-NPs) in the Aquatic Environment: Photoinduced Transformation of Ag₂S-NPs in the Presence of Fe (III). *Environmental science & technology* **2015**, *50*, (1), 188-196.
- Lowry, G. V.; Espinasse, B. P.; Badireddy, A. R.; Richardson, C. J.; Reinsch, B. C.; Bryant, L. D.; Bone, A. J.; Deonarine, A.; Chae, S.; Therezien, M., Long-term transformation and fate of manufactured Ag nanoparticles in a simulated large scale freshwater emergent wetland. *Environmental science & technology* **2012**, *46*, (13), 7027-7036.
- Lukhaup, Chris. "Campeloma Decisum". N.p., 2017. Web. 19 Apr. **2017**.
- Mackevica, A.; Skjolding, L. M.; Gergs, A.; Palmqvist, A.; Baun, A., Chronic toxicity of silver nanoparticles to *Daphnia magna* under different feeding conditions. *Aquatic Toxicology* **2015**, *161*, 10-16.

Maurer-Jones, M. A.; Gunsolus, I. L.; Murphy, C. J.; Haynes, C. L., Toxicity of engineered nanoparticles in the environment. *Analytical chemistry* **2013**, *85*, (6), 3036-3049.

Meyer, J. L.; Benke, A. C.; Edwards, R. T.; Wallace, J. B., Organic matter dynamics in the Ogeechee River, a blackwater river in Georgia, USA. *Journal of the North American Benthological Society* **1997**, 82-87.

Munawar, M.; Dermott, R.; McCarthy, L.; Munawar, S.; Van Stam, H., A comparative bioassessment of sediment toxicity in lentic and lotic ecosystems of the North American Great Lakes. *Aquatic Ecosystem Health & Management* **1999**, *2*, (4), 367-378.

NOAA. *Nonpoint Source Pollution. NOAA's National Ocean Service Education.*, 25 Mar. 2008. Accessed: Web. 20 Mar. 2016.

Oliver, A. L.-S.; Croteau, M.-N.; Stoiber, T. L.; Tejamaya, M.; Römer, I.; Lead, J. R.; Luoma, S. N., Does water chemistry affect the dietary uptake and toxicity of silver nanoparticles by the freshwater snail *Lymnaea stagnalis*? *Environmental Pollution* **2014**, *189*, 87-91.

Oukarroum, A.; Polchtchikov, S.; Perreault, F.; Popovic, R., Temperature influence on silver nanoparticles inhibitory effect on photosystem II photochemistry in two green algae, *Chlorella vulgaris* and *Dunaliella tertiolecta*. *Environmental Science and Pollution Research* **2012**, *19*, (5), 1755-1762.

Padhi, B. K.; Rath, J.; Padhy, P. K., Diatoms for assessing the ecological condition of inland freshwater bodies. *World Review of Science, Technology and Sustainable Development* **2010**, *7*, (4), 352-359.

Porter, S. D.; Cuffney, T. F.; Gurtz, M. E.; Meador, M. R. *Methods for collecting algal samples as part of the National Water-Quality Assessment Program*; **1993**, 93-409.

Regional Aquatics Monitoring Program (RAMP). "Aquatic Organisms: Microorganisms." *Aquatic Organisms: Microorganisms*. Hatfield Consultants, n.d. Web. 19 Apr. **2017**.

Reidy, B.; Haase, A.; Luch, A.; Dawson, K. A.; Lynch, I., Mechanisms of silver nanoparticle release, transformation and toxicity: a critical review of current knowledge and recommendations for future studies and applications. *Materials* **2013**, *6*, (6), 2295-2350.

- Ren, B.; Chen, Y.; Zhu, G.; Wang, Z.; Zheng, X., Spatial Variability and Distribution of the Metals in Surface Runoff in a Nonferrous Metal Mine. *Journal of Analytical Methods in Chemistry* **2016**, 2016, 11.
- Relyea, R.; Hoverman, J., Assessing the ecology in ecotoxicology: a review and synthesis in freshwater systems. *Ecology Letters* **2006**, 9, (10), 1157-1171.
- Ribeiro, F.; Gallego-Urrea, J. A.; Jurkschat, K.; Crossley, A.; Hassellöv, M.; Taylor, C.; Soares, A. M.; Loureiro, S., Silver nanoparticles and silver nitrate induce high toxicity to *Pseudokirchneriella subcapitata*, *Daphnia magna* and *Danio rerio*. *Science of the Total Environment* **2014**, 466, 232-241.
- Rice, E.W.; Baird, R.B.; Eaton, A.D.; Clesceri, L.S., Standard Methods: For the Examination of Water and Wastewater 22nd Addition. *American Public Health Association, American Water Works Association, Water Environment Federation*. **2012**. 10: 38-40
- Sakka, Y.; Skjolding, L. M.; Mackevica, A.; Filser, J.; Baun, A., Behavior and chronic toxicity of two differently stabilized silver nanoparticles to *Daphnia magna*. *Aquatic Toxicology* **2016**, 177, 526-535.
- Schultz, A. G.; Ong, K. J.; MacCormack, T.; Ma, G.; Veinot, J. G.; Goss, G. G., Silver nanoparticles inhibit sodium uptake in juvenile rainbow trout (*Oncorhynchus mykiss*). *Environmental science & technology* **2012**, 46, (18), 10295-10301.
- 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., Effects of aqueous exposure to silver nanoparticles of different sizes in rainbow trout. *Toxicological Sciences* **2010**, 115, (2), 521-534.
- Silk, N.; Ciruna, K., *A practitioner's guide to freshwater biodiversity conservation*. Island Press: **2013**.
- Simmons, D. B.; Wallschläger, D., A critical review of the biogeochemistry and ecotoxicology of selenium in lotic and lentic environments. *Environmental Toxicology and Chemistry* **2005**, 24, (6), 1331-1343.
- Sohn, E. K.; Johari, S. A.; Kim, T. G.; Kim, J. K.; Kim, E.; Lee, J. H.; Chung, Y. S.; Yu, I. J., Aquatic Toxicity Comparison of Silver Nanoparticles and Silver Nanowires. *BioMed Research International* **2015**.

Tam, N.; Wong, Y. S., Spatial and temporal variations of heavy metal contamination in sediments of a mangrove swamp in Hong Kong. *Marine Pollution Bulletin* **1995**, *31*, (4-12), 254-261.

U.S. EPA. *Analysis of the Transport and Fate of Metals Released from the Gold King Mine in the Animas and San Juan Rivers*. U.S. Environmental Protection Agency, Washington, DC. **2017**. EPA/600/R-16/296.

U.S. EPA. Nanomaterial Case Study: *Nanoscale Silver in Disinfectant Spray (Final Report)*. U.S. Environmental Protection Agency, Washington, DC. **2012**. EPA/600/R-10/081F.

U.S. EPA. EPA Method 6020A. Induced Coupled Plasma – Mass Spectrometry. **1998**.

U.S. EPA. EPA Method 3052. Microwave assisted acid digestion of siliceous and organically based matrices. **1996**.

U.S. EPA. EPA Method SW-846. **2007**.

USGS. Stream Flow Measurements for the Nation. Ogeechee River at GA, 24, Near Oliver, GA. <https://nwis.waterdata.usgs.gov/nwis/measurements?> **2017**.

Velzeboer, I.; Quik, J. T.; van de Meent, D.; Koelmans, A. A., Rapid settling of nanoparticles due to heteroaggregation with suspended sediment. *Environmental toxicology and chemistry* **2014**, *33*, (8), 1766-1773.

Wang, J. and W.-X. Wang, *Low Bioavailability of Silver Nanoparticles Presents Trophic Toxicity to Marine Medaka (Oryzias melastigma)*. *Environmental Science & Technology*, **2014**. 48(14): p. 8152-8161.

Wolf, R.E., What is ICP-MS?... and more importantly, what can it do? *USGS*. **2005**

Wong, S. W.; Leung, K. M.; Djurišić, A., A comprehensive review on the aquatic toxicity of engineered nanomaterials. *Reviews in Nanoscience and Nanotechnology* **2013**, *2*, (2), 79-105.

Wu, F.; Harper, B. J.; Harper, S. L., Differential dissolution and toxicity of surface functionalized silver nanoparticles in small-scale microcosms: impacts of community complexity. *Environmental Science: Nano* **2017**.

Zhao, C. M.; Wang, W. X., Comparison of acute and chronic toxicity of silver nanoparticles and silver nitrate to *Daphnia magna*. *Environmental Toxicology and Chemistry* **2011**, *30*, (4), 885-892.

Zhang, H.; Smith, J. A.; Oyanedel-Craver, V., The effect of natural water conditions on the anti-bacterial performance and stability of silver nanoparticles capped with different polymers. *Water research* **2012**, *46*, (3), 691-699.

Zheng, Z.; Lv, J.; Lu, K.; Jin, C.; Zhu, J.; Liu, X., The impact of snail (*Bellamya aeruginosa*) bioturbation on sediment characteristics and organic carbon fluxes in an eutrophic pond. *Clean-Soil, Air, Water* **2011**, *39*, (6), 566-571.

APPENDIX

SILVER NANOPARTICLE AND SILVER ION TOXICITY TO *NAVICULA* SP. IN ARTIFICIAL STREAMS

INTRODUCTION

As the base of aquatic food webs, phytoplankton communities, composed of diatoms, green algae, and cyanobacteria, play an integral part in energy to transfer to primary consumers like zooplankton, macroinvertebrates, and filter feeding fish (Sandgren, C.D., 1988; Carpenter et al., 1996). Fluctuations in phytoplankton communities due to environmental changes drastically influence the composition of aquatic food webs by limiting or increasing food availability (Richardson et al., 2004). As the production of contaminants like silver nanoparticles increase and enter aquatic environments, it is crucial to understand how AgNPs impact phytoplankton communities.

To date laboratory studies conducted have focused on the acute toxicity of AgNPs compared to silver ions in green algae (*Chlorella vulgaris*, *Chlamydomonas reinhardtii*, *Dunaliella tertiolecta*), marine diatoms (*Thalassiosira pseudonana*), and cyanobacteria (*Synechococcus* sp.). Silver nanoparticle exposure to *Chlorella vulgaris* and *Dunaliella tertiolecta* over 24 hours increased cell aggregation at 0.1 mg L⁻¹, reduced chlorophyll *a* content at 1 mg L⁻¹, and amplified reactive oxygen species (common mode of AgNP toxicity) at 1 mg L⁻¹ (Oukarroum et al., 2012). When comparing 72 hour growth inhibition caused by Ag⁺ ions and AgNPs, cell growth was 50% lower in *Thalassiosira pseudonana* at 1.2 μM and 10μM concentrations respectively. Ag⁺ ion and AgNP exposure to *Synechococcus* sp. caused a 50% reduction in growth at 0.9 μM and 3.5 μM respectively (Burchardt et al., 2010). Toxicity in AgNP treatments in this study were attributed to both the dissolution and release of Ag⁺ ions

from AgNPs over the 72 hour period and AgNPs themselves (Burchardt et al., 2010). Comparing changes in photosynthetic production of *Chlamydomonas reinhardtii* after two hours, Ag⁺ ions exhibited an 18X greater toxicity than AgNPs, however, similar to work conducted by Burchardt et al. (2010), AgNP toxicity was determined to be a combination of AgNP releasing Ag⁺ ions and a AgNP/cell interaction (Navarro et al., 2008). While single species toxicity studies demonstrate Ag⁺ ions to be more toxic than AgNPs to phytoplankton, no work has been conducted comparing the impacts AgNP and Ag⁺ ions have on phytoplankton under environmentally relevant conditions or for chronic periods of time longer than three days.

The purpose of this study was to compare the toxicity of AgNP and Ag⁺ ions to the freshwater diatom, *Navicula* sp., under both environmentally relevant flowing and non-flowing conditions. I hypothesized that AgNPs and Ag⁺ ions will negatively impact diatom communities in lentic and lotic environments. Specifically I predicted Ag⁺ ions to exhibit greater lethality to diatom populations than AgNP treatments. Results from this study will provide insight on AgNP toxicity relative to Ag⁺ ions and their impact on primary producers.

METHODS

Stream and pond microcosm design

Individual stream microcosms (N=28) were constructed in the Georgia Southern University greenhouse (32.421432, -81.790814) using a 27 L polyethylene circular tank and pond microcosms were created using an 11.4 L white, polyethylene circular bucket, also serving as the stream's centerpiece (Figure 10). Stream bottoms were coated with clear polyethylene plastic lining and pond centerpieces were encased in 11 L plastic bags to prevent AgNP contamination in future experiments. Ogeechee River water collected near Rocky Ford, GA,

USA, (32.648953, -81.840798) was transported to the greenhouse. Aliquots of 7.5 L were immediately added to each stream microcosm creating stream channels with a width of 12.7 cm and depth of 7.9 cm. Additionally, four liters of Ogeechee River water were added to each pond with a width of 22.9 cm and depth of 11.4 cm. One powerhead pump (SunSun JP-022, Zhejiang, China) was attached to the outside of each bucket (5.8 cm below water) with adhesive tape creating a unidirectional flow with an average of 0.1 m s^{-1} (Figure 10). To compensate for evaporation, deionized water was added daily to streams and ponds to maintain a 7.5 and four liter volumes respectively. Artificial streams and ponds were inoculated with nominal concentrations of 1000 cells per ml of *Navicula* sp. To ensure *Navicula* sp. cell growth was not limited by deficient nutrient levels, supplemental nominal additions of 0.25 mg L^{-1} nitrate and 0.015 mg L^{-1} phosphorus were added to each stream from stock solutions of 1925 mg L^{-1} nitrate and 230 mg L^{-1} phosphorus at the start of the experiment.

Streams and ponds (n=7) were randomly assigned to either one of the four following treatments over a one week period from July, 7 to July, 14 2016: one time pulse dose of $35 \text{ } \mu\text{g L}^{-1}$, one time pulse dose of $70 \text{ } \mu\text{g L}^{-1}$ AgNPs, one-time pulse dose of $35 \text{ } \mu\text{g L}^{-1}$ Ag^+ ion, or no addition control treatment. Nominal AgNP concentrations were added to assigned streams from stock concentrations of 20 mg L^{-1} citrate capped 50nm diameter AgNPs (nanoComposix, California, USA). Nominal Ag^+ ion concentrations were added to corresponding streams from a 20 mg L^{-1} Ag^+ ion stock solution created by dissolving AgNO_3 in deionized water. Although AgNP concentrations selected were higher than current estimates for surface water, they were chosen to ensure Ag content in samples were greater than ICP-MS detection limits of $0.25 \mu\text{g L}^{-1}$.

Sample collection and analysis

Three 0.5 ml water samples were collected on days 0, 1, 3, 5, and 7 from the bottom, middle, and surface of stream and pond water columns and pooled to reduce variance in sampling. Samples were analyzed via BD Accuri C6 flow cytometer (Becton-Dickinson, CA, USA) to determine *Navicula* sp. populations. Water quality measurements (temperature, pH, DO, conductivity) were taken on days 0, 1, 3, 5, and 7 using a hand-held multi-probe meter (YSI Professional Series, Yellow Springs Instruments, Ohio, USA) to ensure environmental measurements taken during the experiment were within the relevant stream conditions (Table 7). Water samples were collected after the initial addition of *Navicula* sp. and on the final day of the experiment to be analyzed for Ag content (100 ml) and chlorophyll *a* fluorescence (100 ml). Water samples for DOC content (50 ml) were also collected from a subset of streams and ponds (n=4) on Day 0. DOC samples were processed in accordance to Standard Method 5310B and analyzed via Shimadzu TOC analyzer (Rice et al., 2012). Water samples for Ag content were filtered through Whatman GF/F glass fiber filters (0.7 μm pore size), immediately acidified to a concentration of 4% HNO_3 and stored at 4°C for one week until the samples could be analyzed. Samples were then heated to 70°C for six hours, cooled to room temperature, and analyzed via ICP-MS (Furtado et al., 2013). Filtered particulate matter was frozen at -20°C and intended to be analyzed for Ag accumulation in phytoplankton, however, due to complications with digestion instrumentation the process was not completed (Furtado et al., 2013). Chlorophyll *a* concentration in each stream was determined from algal cells collected from water samples (100 ml) filtered through Whatman GF/F (0.7 μm nominal pore size). Pigments were extracted from cells on the filters with 90% acetone in the dark at -20°C and measured using a Trilogy

Fluorometer (Turner Designs, CA, USA) in accordance to EPA Method 445.0 (Arar and Collins, 1997).

Statistical Analysis

Normal distribution and equal variance assumptions were tested prior to performing parametric one way ANOVA analyses. If assumptions failed to be met, transformations were performed and assumptions tested again. Normal distribution was also tested before parametric Pearson's correlation tests were conducted. If the assumption failed to be met before and after data transformation, a non-parametric Spearman's ranks test was performed to analyze the data. Due to the non-normal distribution of data, Friedman's tests were carried out to analyze repeated measures datasets.

To determine if AgNP or Ag ion⁺ treatments affected diatom populations in ponds or streams over the one week experiment, cells counts were analyzed using Friedman's tests. Initial and final differences in water chlorophyll *a* in both pond and stream due to treatments were determined using one-way ANOVA tests. Initial differences in DOC between stream treatments and pond treatments were also tested using one-way ANOVA tests. To establish relationships between Ag concentration in the water column and column DOC concentration on Day 0, Pearson's correlation coefficient tests were run with stream Ag data and Spearman's rank correlations were applied with pond Ag data.

RESULTS

Initial Ag concentrations detected in the water column were roughly 50% of nominal AgNP applications and 20% of Ag⁺ ion applications in treated ponds and streams (Table 8).

Silver concentrations in control streams and ponds were near or below detection limits. Initial DOC concentrations were not significantly different between treatments in streams or ponds (Table 9). No relationship between initial DOC and Ag concentrations occurred in streams, however, a trend emerged showing a positive correlation between DOC concentrations and Ag concentrations in ponds (Table 9). Final stream Ag concentrations in the water column did not differ between all AgNP and Ag⁺ ion treatments (One-Way ANOVA, $F_{2, 18} = 1.1545$, p-value = 0.3375). Final silver concentrations in the water column of 35 $\mu\text{g L}^{-1}$ Ag⁺ ion treated ponds were significantly greater than both 70 $\mu\text{g L}^{-1}$ AgNP and 35 $\mu\text{g L}^{-1}$ AgNP treated streams (Kruskal-Wallis, $\chi^2_4 = 13.9221$, p-value < 0.0009), however, final mean Ag concentrations in ponds only ranged between 1-2.5 $\mu\text{g L}^{-1}$.

Initial chlorophyll *a* values were 3.2X lower in 35 $\mu\text{g L}^{-1}$ Ag⁺ ion treated streams and 2.3-2.5X lower in 35 $\mu\text{g L}^{-1}$ Ag⁺ ion treated ponds when compared to all other treatments (Table 10). Final chlorophyll *a* content did not differ between stream treatments, whereas final pond chlorophyll *a* content did vary between treatments (Table 10). Pond chlorophyll *a* was significantly greater in 35 $\mu\text{g L}^{-1}$ Ag⁺ ion treated streams than control and 35 $\mu\text{g L}^{-1}$ AgNPs treated streams. Furthermore, 70 $\mu\text{g L}^{-1}$ AgNP treated streams possess more chlorophyll *a* than control streams (Table 10).

Corresponding to initial chlorophyll *a* values, diatom populations in ponds were 3.1-3.6X lower in 35 $\mu\text{g L}^{-1}$ Ag⁺ ion treated ponds (Table 11). Overall diatom populations in ponds did not vary between treatments throughout the experiment as populations in 35 $\mu\text{g L}^{-1}$ Ag⁺ ion treated ponds started to recover after three days (Friedman's Test, Table 11). Although differences in Day 7 pond cell counts were not statistically significant between treatments, populations in 35 μg

L^{-1} Ag^+ ion treated streams were trending towards significantly higher totals than control streams (Table 11).

Diatom populations were 3.7-4.3X lower in $35 \mu\text{g L}^{-1}$ Ag^+ ion treated streams than all other treatments (Table 11, Figure 11). Overall, differences in diatom populations in ponds continued to be seen during the seven day experiment as cell growth in $35 \mu\text{g L}^{-1}$ Ag^+ ion treated streams was slow to recover (Friedman's Test, Table 11, Figure 11). After seven days, final cell counts in streams showed ~2.1X greater cell densities in control and $70 \mu\text{g L}^{-1}$ AgNP treated streams than $35 \mu\text{g L}^{-1}$ Ag^+ ion treated streams, as well as, $35 \mu\text{g L}^{-1}$ AgNP treated streams trending towards this pattern with 1.6X more cells than $35 \mu\text{g L}^{-1}$ Ag^+ ion treated streams (Table 11, Figure 11).

DISCUSSION

The hypothesis that AgNPs and Ag ions will negatively impact diatom populations in lentic and lotic environments was partially supported. Specifically I predicted $35 \mu\text{g L}^{-1}$ Ag^+ ions to yield greater toxicity to diatom populations in ponds and streams than both $35 \mu\text{g L}^{-1}$ and $70 \mu\text{g L}^{-1}$ AgNP treatments. While Ag^+ ions reduced diatom cell counts in both streams and ponds, neither AgNP treatment impacted diatom populations compared to controls streams. These results also support previous laboratory studies indicating significantly greater phytoplankton toxicity to Ag^+ ions than AgNPs (Oukarroum et al., 2012; Burchardt et al., 2010; Navarro et al., 2008), and the idea that Ag^+ ions are taken up rapidly by freshwater algae increasing their toxicity potential to aquatic organisms (U.S. EPA, 2012). While pond diatom populations in Ag^+ ion treated streams recovered after five days, stream populations were slower to recover never reaching populations in control and AgNP treated streams. This may be attributed to

phytoplankton reproducing less effectively in turbid, flowing environments than non-flowing environments (Zhang et al., 2015).

The reduction of diatom cells in Ag⁺ ions treated ponds and streams was confirmed using chlorophyll *a* values indicating the same trend in toxicity. However, significant differences were detected in final chlorophyll *a* values of ponds relative to flow cytometry cell counts, and vice versa in stream results (Table 10, Table 11). While chlorophyll *a* is a good proxy for determining phytoplankton abundance, variation can occur due to differences in cell size and chlorophyll *a* production during the time of sampling (Jakob et al., 2005; White and Payne, 1977). Whereas, flow cytometry methods are able analyze individual cells increasing the accuracy of population analyses (Veldhuis and Kraay, 200). Results from both methods do agree on the order of mean diatom abundance between treatments in ponds and streams.

Initial silver concentrations in the water column below nominal concentrations applied is most likely due to Ag⁺ ions and AgNPs collecting on diatom cells during the filtration process (Furtado et al., 2015). Furthermore, Ag analysis was limited due to filters not being rinsed to remove unbound Ag without diluting water samples. Though final water samples suggest Ag falling out of the water column in ponds and streams over the one week period, the analysis was again limited due to filtering procedures. Previous lentic mesocosm studies have shown contradicting results on suspension time of AgNPs with settling occurring in wetlands ~8 days compared to AgNP settling estimated at 66 days in boreal lakes. However, the AgNP concentrations applied in wetlands were three orders of magnitude larger than AgNP concentrations applied in boreal lakes and the present study (Furtado et al., 2015; Lowry et al., 2012). High concentrations of AgNPs may lead to aggregation of AgNPs and shorter suspension times than low AgNP concentrations (Furtado et al., 2015) Differences in suspension time may

be increasing by site specific factors like higher DOC content, ionic content, and biotic particles described in Chapter 1 (Furtado et al., 2015).

This study confirms laboratory findings suggesting Ag^+ ions are more toxic to phytoplankton communities than AgNPs in environmentally relevant conditions. Findings also suggest contaminants like heavy metals may have a greater impact on diatom communities in flowing vs non-flowing environments due to slower recovery times in flowing systems. Slower recovery times of diatom communities in streams may have far reaching implications in the aquatic food web by limiting the growth of primary and secondary consumers.

Table 1: Average stream pH, DO, specific conductivity, and temperature in all streams over one week (N=28). Numbers in parentheses represents \pm one standard error of the mean.

Day	Temp		Spec. Cond		pH		DO	
	pond	stream	pond	stream	pond	stream	pond	stream
0	32.21 (0.27)	33.02 (0.24)	134.18 (0.28)	134.95 (0.48)	7.78 (0.02)	7.94 (0.02)	6.69 (0.07)	6.34 (0.09)
1	32.89 (0.41)	33.44 (0.41)	133.06 (0.98)	132.96 (1.37)	7.88 (0.02)	7.99 (0.03)	5.82 (0.13)	6.22 (0.10)
3	32.15 (0.28)	32.83 (0.28)	133.94 (0.74)	134.90 (1.75)	8.11 (0.08)	8.12 (0.03)	6.59 (0.29)	6.39 (0.08)
5	31.08 (0.27)	31.90 (0.32)	133.04 (0.98)	132.79 (1.88)	8.17 (0.08)	8.15 (0.04)	6.67 (0.32)	6.51 (0.08)
7	30.87 (0.24)	31.66 (0.25)	133.56 (0.83)	133.66 (1.77)	8.10 (0.07)	8.13 (0.04)	6.60 (0.26)	6.54 (0.09)

Table 2: Mean total Ag concentrations in the water column \pm one standard error of the mean (SEM) on Days 0 and 7 (n=7).

	Treatment	Day 0	Day 7
Ponds			
	control	0.40 \pm 0.04	0.07 \pm 0.07
	35 $\mu\text{g L}^{-1}$ AgNP	16.77 \pm 0.70	1.01 \pm 0.03
	35 $\mu\text{g L}^{-1}$ Ag ⁺ ion	7.56 \pm 0.49	2.55 \pm 0.28
	70 $\mu\text{g L}^{-1}$ AgNP	36.96 \pm 1.29	1.37 \pm 0.16
Streams			
	control	0.17 \pm 0.06	0 \pm 0
	35 $\mu\text{g L}^{-1}$ AgNP	17.81 \pm 0.53	0.93 \pm 0.07
	35 $\mu\text{g L}^{-1}$ Ag ⁺ ion	9.95 \pm 0.60	1.59 \pm 0.40
	70 $\mu\text{g L}^{-1}$ AgNP	33.74 \pm 2.47	1.52 \pm 0.29

Table 3: One-Way ANOVA comparisons of initial DOC concentrations on Day 0 (n=7) and relationships between DOC concentrations and Ag concentrations in the water column using parametric Pearson's Correlations tests and non-parametric Spearman's Ranks tests (n=7).

	Test	Df	Test Statistic	p-value
Stream DOC Day 0	One-Way ANOVA	3, 12	F = 2.0667	0.1582
Pond DOC Day 0	One-Way ANOVA	3, 12	F = 2.2429	0.1358
Stream DOC vs Ag Day 0	Pearson's Correlation	12	r = - 0.0111	0.9727
Pond DOC vs Ag Day 0	Spearman's Ranks	12	rho = 0.5664	0.0548

Table 4: One-Way ANOVA comparisons of initial and final water chlorophyll *a* concentrations on Days 0 and 14 (n=7). Asterisks denote significant differences between treatments.

	Test	Df	Test Statistic	p-value
Stream Chl-a Day 0	One-Way ANOVA	3, 24	F = 88.3842	<0.0001*
	Tukey-Kramer post hoc comparison			
	35 $\mu\text{g L}^{-1}$ Ag ⁺ ions vs all treatments			<0.05*
Pond Chl-a Day 0	One-Way ANOVA	3, 23	F = 101.0486	<0.0001*
	Tukey-Kramer post hoc comparison			
	35 $\mu\text{g L}^{-1}$ Ag ⁺ ions vs all treatments			<0.05*
Stream Chl-a Day 14	One-Way ANOVA	3, 23	F = 0.8414	0.4852
Pond Chl-a Day 14	One-Way ANOVA	3, 24	F = 8.6924	0.0004*
	Tukey-Kramer post hoc comparison letters report			
			35 $\mu\text{g L}^{-1}$ Ag ⁺ ions	A
			70 $\mu\text{g L}^{-1}$ NP	A B
			35 $\mu\text{g L}^{-1}$ NP	B C
			Ctrl	C

Table 5: Non-parametric Friedman's tests comparing *Navicula* sp. populations over the seven day experiment (n=7). One-Way ANOVA comparisons of initial and final stream cell counts (n=7). Asterisks denote significant differences between treatments.

	Test	Df	Test Statistic	p-value
7 Day Stream Cell Counts	Friedman's Test	3	2.04	>0.05*
7 Day Pond Cell Counts	Friedman's Test	3	9.24	<0.05*
Initial Stream Cell Counts	One-Way ANOVA	3, 24	F = 92.6115	<0.0001*
Initial Pond Cell Counts	One-Way ANOVA	3, 24	F = 66.9865	<0.0001*
Final Stream Cell Counts	One-Way ANOVA	3, 24	F = 8.4244	0.0005*
	Tukey-Kramer post hoc comparison letters report		Ctrl	A
			70 $\mu\text{g L}^{-1}$ NP	A
			35 $\mu\text{g L}^{-1}$ NP	A B
			35 $\mu\text{g L}^{-1}$ Ag ⁺ ions	B
Final Pond Cell Counts	One-Way ANOVA	3, 24	F = 2.8037	0.0614



Figure 1: Stream mesocosm with a powerhead pump attached to the side of the bucket generating a recirculating flow of 0.1 m s^{-1} . The water inside the bucket serves as a lentic system.

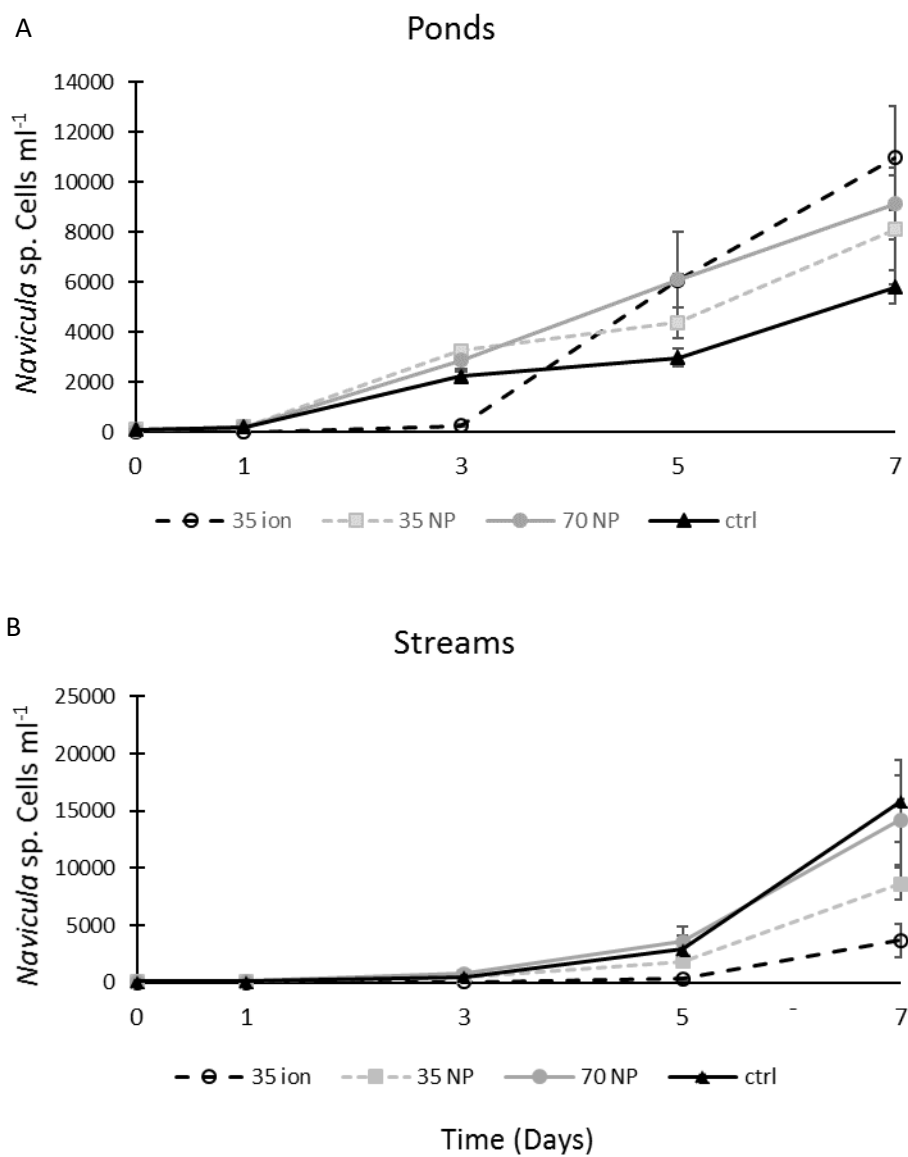


Figure 2: Mean *Navicula* sp. cell counts in ponds and streams taken on day 0, 1, 3, 5, and 7.

Error bars represent ± 1 standard error of the mean (SEM) ($n=7$).

LITERATURE CITED

Arar, E. and Collins, G., In Vitro Determination of Chlorophyll a and Pheophytin a in Marine and Freshwater Algae by Fluorescence Revision 1.2. *USEPA* **1997**, 1-23.

Burchardt, A. D.; Carvalho, R. N.; Valente, A.; Nativo, P.; Gilliland, D.; Garcia, C. P.; Passarella, R.; Pedroni, V.; Rossi, F. o.; Lettieri, T., Effects of silver nanoparticles in diatom *Thalassiosira pseudonana* and *Cyanobacterium synechococcus* sp. *Environmental science & technology* **2012**, *46*, (20), 11336-11344.

Carpenter, S. R.; Kitchell, J. F., *The trophic cascade in lakes*. Cambridge University Press: **1996**.

Furtado, L. M.; Norman, B. C.; Xenopoulos, M. A.; Frost, P. C.; Metcalfe, C. D.; Hintelmann, H., Environmental Fate of Silver Nanoparticles in Boreal Lake Ecosystems. *Environmental Science & Technology* **2015**, *49*, (14), 8441-8450.

Jakob, T.; Schreiber, U.; Kirchesch, V.; Langner, U.; Wilhelm, C., Estimation of chlorophyll content and daily primary production of the major algal groups by means of multiwavelength-excitation PAM chlorophyll fluorometry: performance and methodological limits. *Photosynthesis Research* **2005**, *83*, (3), 343-361.

Lowry, G. V.; Espinasse, B. P.; Badireddy, A. R.; Richardson, C. J.; Reinsch, B. C.; Bryant, L. D.; Bone, A. J.; Deonaraine, A.; Chae, S.; Therezien, M., Long-term transformation and fate of manufactured Ag nanoparticles in a simulated large scale freshwater emergent wetland. *Environmental science & technology* **2012**, *46*, (13), 7027-7036.

Navarro, E.; Piccapietra, F.; Wagner, B.; Marconi, F.; Kaegi, R.; Odzak, N.; Sigg, L.; Behra, R., Toxicity of silver nanoparticles to *Chlamydomonas reinhardtii*. *Environmental science & technology* **2008**, *42*, (23), 8959-8964.

Oukarroum, A.; Polchtchikov, S.; Perreault, F.; Popovic, R., Temperature influence on silver nanoparticles inhibitory effect on photosystem II photochemistry in two green algae, *Chlorella vulgaris* and *Dunaliella tertiolecta*. *Environmental Science and Pollution Research* **2012**, *19*, (5), 1755-1762.

Rice, E.W.; Baird, R.B.; Eaton, A.D.; Clesceri, L.S., Standard Methods: For the Examination of Water and Wastewater 22nd Addition. *American Public Health Association, American Water Works Association, Water Environment Federation*. **2012**. 10: 38-40

Richardson, A. J.; Schoeman, D. S., Climate impact on plankton ecosystems in the Northeast Atlantic. *Science* **2004**, *305*, (5690), 1609-1612.

Sandgren, C. D., *Growth and reproductive strategies of freshwater phytoplankton*. Cambridge University Press: 1988.

Veldhuis, M. J.; Kraay, G. W., Application of flow cytometry in marine phytoplankton research: current applications and future perspectives. *Scientia Marina* **2000**, *64*, (2), 121-134.

White, E.; Payne, G., Chlorophyll production, in response to nutrient additions, by the algae in Lake Taupo water. *New Zealand journal of marine and freshwater research* **1977**, *11*, (3), 501-507.

Zhang, H.; Chen, R.; Li, F.; Chen, L., Effect of flow rate on environmental variables and phytoplankton dynamics: results from field enclosures. *Chinese Journal of Oceanology and Limnology* **2015**, *33*, (2), 430-438.