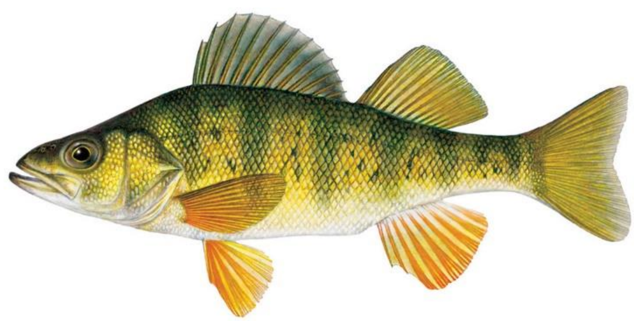
CHANGES IN YELLOW PERCH DIETS ASSOCIATED WITH NANOSILVER EXPOSURE IN FRESHWATER LAKES

by

Brennan R. DeBoer

Source: Lens Ecosystem Nanosilver Project 2012.



Source: Vermont Fish & Wildlife Department 2017.

FACULTY OF NATURAL RESOURCES MANAGEMENT
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THUNDER BAY, ONTARIO

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SCIENTIFIC ANALYSIS OF YELLOW PERCH DIETS BETWEEN A NANOSILVER MANIPULATED LAKE AND A REFERENCE LAKE AT THE EXPERIMENTAL LAKES AREA

by
Brennan R. DeBoer

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Partial Fulfillment of the Requirements for the
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Lakehead University

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Major Advisor

Second Reader

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ABSTRACT

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Keywords: diet, exposure, forage, freshwater, nanosilver, predator, prey, size.

Freshwater lake experiments on the effects of nanosilver were completed at the Experimental Lakes Area in northwestern Ontario. Since nanosilver has antibacterial properties it is expected to have negative effects on microorganisms causing indirect consequences to an aquatic food web. Diets of Yellow Perch (*Perca flavescens*) populations from a lake with nanosilver added were compared to an unmanipulated reference lake. These two study lakes were compared across the same duration period of nanosilver addition years (2014 & 2015) to lake recovery (2016 to present) during summer and fall seasons. Nanosilver was added to the lakes with a mean particle size of 40 nm with 9 kg added in 2014 and 6 kg added in 2015. The results showed no differences in diet contents due to exposure to nanosilver. There was a greater propensity for piscivory in large Yellow Perch from Lake 239, whereas Lake 222 had high levels of benthivory in both time periods and seasons but no evidence of piscivory. This study showed only natural alterations in diets, but no effects from the nanosilver added at a nearshore point source. In conclusion, ongoing monitoring is recommended, to see if future tests reveal any effects from nanosilver material entry to freshwater aquatic ecosystems.

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INTRODUCTION

Nanosilver is a material that has been engineered to 1-100 nm size, and is used in over 400 different products that are commercially-distributed, which include; health care, fitness, cosmetics, and personal hygiene due to its success as an antibacterial agent (Voelker et al. 2015). Nanosilver has also been used for water treatment processes and has properties that make it an antimicrobial (Völker et al. 2013). The extensive use of this material in commercial products has generated an increasing concern, due to its unabated release into the environment and its unknown effects on environment health (McShan et al. 2014). This material is released into aquatic ecosystems from wastewater runoff. Therefore, the antibacterial and antimicrobial properties of nanosilver may have negative effects on microorganisms in receiving waters, which could cause indirect consequences up the food chain in aquatic environments (Justice and Bernot 2014). Over time, this contaminant could alter the natural characteristics in an aquatic ecosystem. Compared to other contaminants, little is known about how nanomaterials affect aquatic ecosystems, and more research into the effects of nanomaterials on the environment is required to help develop and standardize protocols to protect aquatic ecosystems (Justice and Bernot 2014).

Nanosilver most likely enters a lake at nearshore point sources in the form of wastewater runoff. In freshwater, when a material like nanosilver enters an aquatic ecosystem, the nanosilver could be transferred from prey organisms to higher trophic levels (e.g., fish) through predation from fish species (Justice and Bernot 2014). Prey species for fish typically consist of benthic invertebrates (typically nearshore), and zooplankton (typically offshore) in the lower trophic levels of food webs. Therefore, wastewater runoff at a nearshore point source that contains nanosilver, could potentially have an impact on fish when their diets rely on nearshore prey organisms. In comparison, offshore locations may be less prone to nanosilver exposure. The result of nearshore point-additions therefore could potentially reveal a shift in fish diets away from nearshore to offshore prey sources.

The objective of this paper was to examine stomach contents of Yellow Perch (*Perca flavescens*) from an experimental lake and a reference lake. This was to determine if there was a possible shift in fish diets from nearshore to offshore prey sources, during a period of nanosilver additions and whether the reliance on nearshore and offshore resources differed compared to a period of recovery. The main question was: does nanosilver at point source entries affect nearshore aquatic microorganisms, causing a shift diet to non-exposed offshore prey sources? The hypothesis was: there are differences in Yellow Perch diets between years when nanosilver was added to a lake versus not added, but no change over a similar time period in a unmanipulated reference lake. This study attempts to better understand how nanosilver impacts a freshwater ecosystem by examining the prey constituents of a zooplanktivorous and benthivorous fish.

LITERATURE REVIEW

TROPHIC ECOLOGY IN FRESHWATER

The bottom of the food chain, according to Covich et al. (1999), is where the highest abundance of species are present, which has a major influence on biodiversity in an ecosystem. In other words, if there are low levels of benthic invertebrates and zooplankton, there will be low biodiversity. The introduction of contaminants may contribute to low biodiversity, which can go unnoticed for some time until effects are shown in higher trophic levels (Covich et al. 1999). Therefore, the biocomplexity of a freshwater ecosystem needs to be understood in order to maintain ecosystem biodiversity.

In freshwater, the largest zooplankton and benthos are a crucial part of an aquatic ecosystem (Whitemore and Webster 2008). In natural selection, according to Brooks and Dodson (1965), predators will most likely choose the most abundant organisms. Even at the highest trophic level, if prey availability is low, predators must alternatively target other organisms, referring to planktonic prey. When predation is required in an aquatic ecosystem, fish are prone to shift to more available prey, which ultimately shapes food webs (Brooks and Dodson 1965). In other words, when a material such as nanosilver effects a nearshore prey source, another source of prey like *Bosmina* and *Daphnia* (offshore, and less likely to be influenced by high nearshore concentrations) will become the targeted prey organisms for fish. This indicates that fish diets would potentially change, resulting in more consumption of offshore prey sources, which have not been in contact with nanosilver materials.

NEARSHORE – OFFSHORE COULPING

In an aquatic ecosystem, prey sources change as fish body size increases. The energy for growth is gained when food sources are available. Primary production for energy in freshwater systems are taken from littoral (nearshore) and pelagic (offshore) zones. Furthermore, this is where predator life cycles rely on the connection of prey sources (Vadeboncoeur et al. 2003). These prey sources include benthic invertebrates at nearshore and plankton at offshore zones (Vadeboncoeur et al 2003). Top predators have ontogenetic diet shifts, which are shifts from one resource to another as they grow, such as fish, which can shift their diet from zooplanktivory to benthivory prey resources over time. The benthivorous fish rely on nearshore productivity where prey feed on algae, macrophyte, and periphyton food sources (Vadeboncoeur et al. 2003). As such, reduced production of benthos at nearshore locations can cause an impact to benthic-feeding fish. This may cause the fish to shift to offshore locations with other sources of prey. In aquatic ecosystems, whole-lake primary production relies on the coupling of nearshore and offshore environments, which include nutrient cycles and food web dynamics (Vadeboncoeur and Steinman 2002). In previous gut content and stable isotope analyses, periphyton have been shown to be key energy resources in lake food webs. Both plankton and benthic invertebrates are primary sources of prey for fish, and both contribute to whole-lake primary productivity (Vadeboncoeur et al. 2003). Fish productivity and growth will only increase when prey are available, and diets may change in correlation to prey availability; shifting to other sources of prey over time (Vadeboncoeur and Steinman 2002). As fish grow, they require larger particle sizes to counterbalance maintenance costs and permit growth (Sherwood et al. 2002).

As larvae and juvenile stages, Yellow Perch rely on planktonic sources (Brown et al. 2009). As Yellow Perch increase in size class and age, the fish switch from zooplankton to benthic invertebrates. This specific species of fish feeds on these resources, due to the amount of high energy content with low energetic cost to acquire nutrients (Brown et al. 2009). Furthermore, when Yellow Perch switch to piscivory once reaching a larger body and gape size, the ability to consume larger prey is needed for proper bodily function (Sherwood et al. 2002). A life cycle for Yellow Perch, as well as other fish species, consists of growth and maintaining a healthy diet. This involves the ability to uphold efficient energy levels which come from converting sources of prey.

NANOSILVER EFFECTS IN AQUATIC ECOSYSTEMS

Silver has been used for centuries for its elemental form, according to Maillard and Hartemann (2013). The element of silver is inherently toxic to bacteria and microbes. In addition, silver in nanoparticle form poses a threat to natural system components due to the antibacterial properties from the widely distributed commercial products it is now found in (Maillard Hartemann 2013). The high-volume of consumer related products that contain nanosilver are currently uncontrolled and unregulated by the Environmental Protection Agency (Green and Ndegwa 2011). The need to test and monitor aquatic ecosystems is required to gain knowledge about the effects of toxic materials.

World production of silver, has grown since 1964 from 7.4 million kg to an estimated 14.6 million kg in 1990. The problem for both fisheries and wildlife resources, is that silver is a common toxic element that is incorporated with aquatic ecosystems (Eisler 1996). In the early 1990s, silver was recognized as a major concern by regulation agencies such as the U.S Environmental Protection Agency, who advised to better understand the impacts silver has on the environment (Eisler 1996). With the advent nanomaterial science and the incorporation of silver into nanoparticles, there is a rising concern about possible environmental changes. As a result, the issue regarding nanosilver today; is the lack of knowledge concerning the toxic material when in contact with organisms (as compared to elemental silver) and the behaviour it demonstrates in aquatic ecosystems.

Particles of nanosilver act as slow-release capsules, ejecting toxic silver ions into the water column (Gillig 2008), which has harmful effects on microbes. In addition, it is known to alter aquatic community composition of phytoplankton and bacteria (Gillig 2008). Long term exposure of nanosilver to aquatic ecosystems could lead to changes in food web structure (Maillard and Hartemann 2013). However, little research has been conducted that explores how negative effects on the base of the food chain might impact higher trophic levels (e.g., secondary consumers such as zooplankton, benthos, and fish).

A recent study observed effects of the gill and gut contents of zebrafish from short-term exposure to silver and nanosilver materials (Bacchetta et al. 2016). This study used 60 L tanks monitored at 28°C with additions of silver and nanosilver concentrations at 25, 50 and 100 µg nAg/L. These concentrations were monitored at 24 to 96 hour timelines (Bacchetta et al. 2016). The study found that ionic silver was more toxic then nanosilver to zebrafish after an exposure of 24 hours, but after for 96 hours the results were similar. The results showed negative effects on natural bodily functions (Bacchetta et al. 2016). Therefore, nanosilver in a controlled environment does have negative effects, which studies should be focused on testing and monitoring natural aquatic ecosystems exposed to nanosilver.

Short-term exposure studies using nanosilver showed that fish had negative responses, which suggest that nanosilver materials should be tested at low concentrations over longer time (Voelker et al. 2013). Long-term tests can show more a realistic effect of a material on a natural ecosystem functions (Voelker et al. 2013). This study will examine the effects of nanosilver exposure using over 2-years of addition and one year of recovery in an experimental lake (Lake 222), and will be compared to an unmanipulated lake (Lake 239). This study aims to gain knowledge on how nanosilver behaves in an ecosystem to help with future mitigation measures.

Furthermore, the specific objectives in this study are to monitor how nanosilver behaves in Lake 222 where a point-source shoreline addition was conducted (e.g., simulating release from a wastewater treatment plant). This point-source addition is predicted to have greater effects on nearshore benthic algal production (Gillig 2008), than in the offshore and might therefore have greater negative impacts on benthic versus zooplankton communities. Therefore, the expectation is to see a greater prevalence of benthic feeding in Lake 222 during nanosilver additions than when not added, in comparison to the reference lake (Lake 239) across years of additions and recovery. Furthermore, the fish samples used for this experiment were capture in both summer and fall season (Table 2).

Table 1. Number of Yellow Perch stomachs used from each lake by season and year.

Year	Experimental Stage	Season	L222 Perch (#)	L239 Perch (#)
2014	nAg Additions	Summer	13	14
		Fall	16	15
2016	Recovery	Summer	13	15
		Fall	15	14

The goal of this study was to observe what the fish were consuming and where shifts in diets were taking place.

METHODS AND MATERIALS

STUDY AREA

The IISD-Experimental Lakes Area (IISD-ELA) is a collection of 58 freshwater lakes are in northwestern Ontario, which are set aside for whole-ecosystem research due to their remote location and limited development. During this study, Lake 222 and Lake 239 (Figure 1) were used and samples of Yellow Perch were taken considering this fish species is the most common among both lakes. In addition, these lakes have been used to address environmental questions, provide research, and influence government policies and decisions; which include the most recent Lake Ecosystem Nanosilver (LENs)

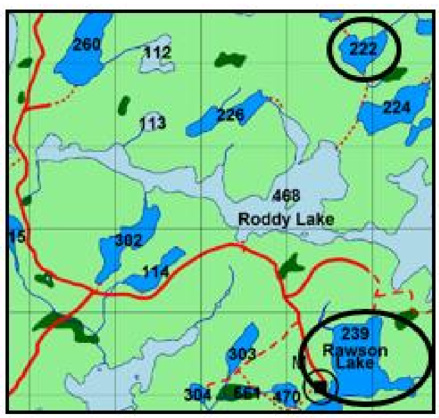

Figure 1. Lake 222 and 239 at ELA circled in yellow.

Project from 2012 to 2016. In this study during the summer months of 2014 and 2015 the material nanosilver was added at a nearshore location in Lake 222. While the continuation of the LENs project, whole-lake monitoring occurred prior to nanosilver additions from 2012 through to recovery in 2016. Lake 222 it has a maximum depth of 5.4 meters with a surface area of 17.5 hectares. In comparison, Lake 239 is an

unmanipulated lake and was monitored over the same time period. The unmanipulated lake has a maximum depth of 30.4 meters and a surface area of 56.1 hectares (Cleugh and Hauser 1971). The process of nanosilver manipulations began with an amount of 9 kg in 2014 at the nearshore location. In the following summer of 2015,
6 kg of nanosilver was added to Lake 222. Additions in, both in 2014 and 2015 used a mean particle size of 40 nm.

FIELD SAMPLING METHODS

Field sampling methods during this experiment, used seine and trap nets to capture Yellow Perch. Furthermore, the fork lengths of the Yellow Perch were measured; fish were assigned to length bins to obtain sufficient samples sizes (Table 1).

Table 2. Number of Yellow Perch used for stomach content analysis by fork length (mm) class, lake, year, and season.

Lake	Fork Length (mm)	2014 Yellow Perch (#)		2016 Yellow Perch (#)	
		Summer	Fall	Summer	Fall
Lake 222	<71	3	4	3	3
	71-90	3	3	3	3
	91-110	2	3	3	3
	111-130	3	3	3	3
	131-150	2	2	1	2
	151-170	0	1	0	1
	>171	0	0	0	0
	Sub-Total	13	16	13	15
	LAKE TOTAL	29		28	
Lake 239	<71	4	2	3	3
	71-90	3	2	3	1
	91-110	2	3	1	2
	111-130	3	3	3	3
	131-150	1	2	2	2
	151-170	1	3	3	2
	171>	0	0	0	1
	Sub-Total	14	15	15	14
	LAKE TOTAL	29		29	

In addition, specimens from both experimental Lakes 222 and 239 were caught during summer and fall seasons (Table 2). Yellow Perch stomachs were removed and preserved in a 95% ethanol filled vial for further analyses of gut contents.

LABORATORY SAMPLING METHODS

The preserved stomachs were removed from the ethanol filled vial and put under a dissecting microscope for identification. Stomachs were analyzed visually and identified diet items were then placed into three categories: zooplankton, benthic invertebrates (27 sub-groups, as set out by the Ontario Benthos Biomonitoring Network (OBBN)), and fish. Once contents were categorized, the excess ethanol was drained and contents were divided into separate dishes by group taxa. Afterward, the contents of the group taxa were counted and then weighed (wet weight, g) to four-decimal places. The weight of each taxa was recorded and all items were properly disposed.

RESULTS

In order to evaluate the frequency of occurrence of perch diet items, the data were summarized by examining the number of fish which had diets from the three main diet categories (zooplankton, benthos and fish; Figure 2). The number of fish that consumed either zooplankton or benthos did not change between the year of nanosilver addition and the year of recovery (Figure 2). Benthos were the dominant prey items for Yellow Perch in Lake 222 during both years and seasons. In Lake 239 benthos were similarly the most frequently found prey in fish, except when piscivory was high in fall 2016. Contrary to the expectations, there was not a higher frequency of zookplanktivory during nanosilver addition in Lake 222.

Since Yellow Perch are known to shift diets with increasing body, diets were also evaluated against fish size. The three diet categories were examined as the proportion in the diet and compared with the size (fork length, mm) of the fish. In summer months, and in both lakes, zooplanktivory was prominent at smaller size classes in both addition and recovery years in Lake 222, after which diets shifted to benthivory at approximately 70 mm (Figure 3, A-D). There was no evidence in a delay in switching to benthivory in Lake 222 in the year of nanosilver addition compared to either the reference lake or the year of recovery in Lake 222. Though there was a shift to piscivory was observed in Lake 239, Yellow Perch remained benthivorous in Lake 222 in both years.

The data from the fall diets showed a similar pattern as those in summer (Figure 4). The three diet categories were examined in the same way as the summer data to see shifts in Yellow Perch diets. Similar to fish collected in the summer, the shift from

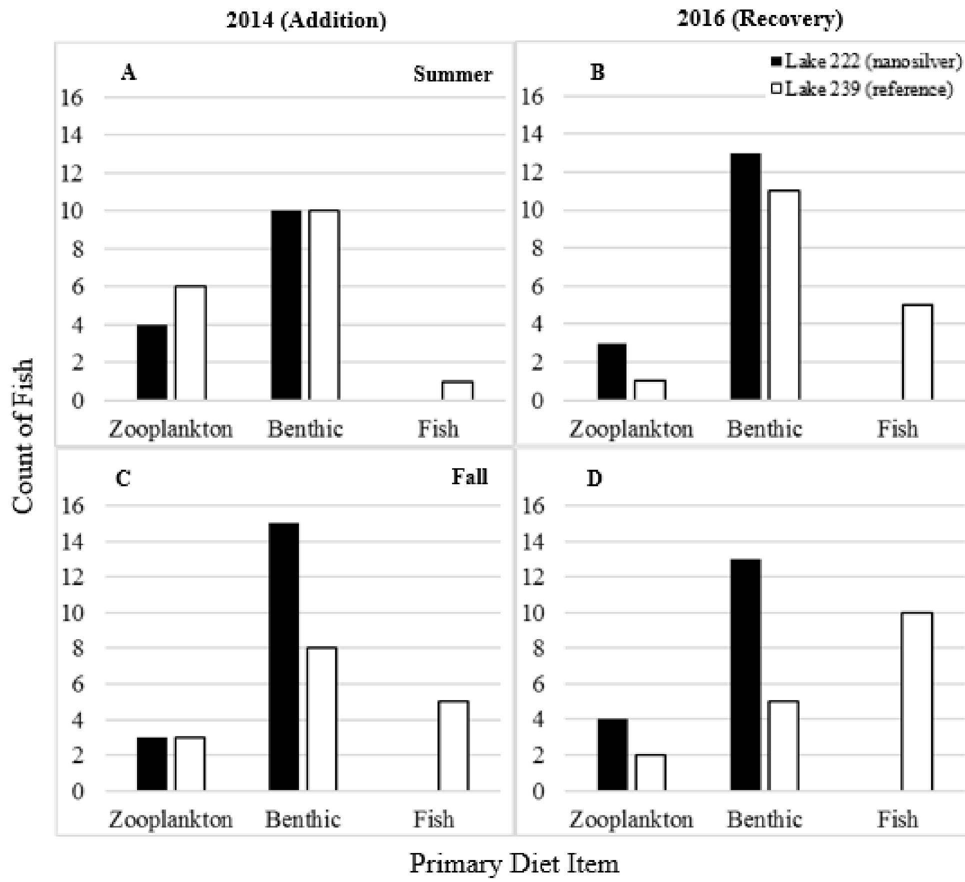

Figure 2. Frequency of occurrence of Yellow Perch diet items in a lake with nanosilver added (Lake 222) and a reference lake (Lake 239), comparing a year where nanosilver was added (2014, Panels A, C) with a year free of nanosilver additions (2016; Panels B, D). Solid (black) bars are Lake 222 and open (white) bars are Lake 239. Panels A, B = summer diets, Panels C, D = fall diets.

zooplanktivory to benthivory happened at approximately 60 mm. Unlike the summer diets, most fish of smaller sizes consumed both zooplankton and benthos at smaller sizes during fall. This was observed in both addition and recovery years (Figure 4 A-B) Fish greater than 60 mm in Lake 222 were 100% benthivorous (Figure 4 C-D). The shift from benthivory to piscivory was again only observed in Lake 239 fish, in both years, where fish over a fork length of 100 mm began switching to piscivory, almost exclusively so in 2016 (Figure 4 F). There was no evidence to show a delay in switching to benthivory in Lake 222 in fall diets.

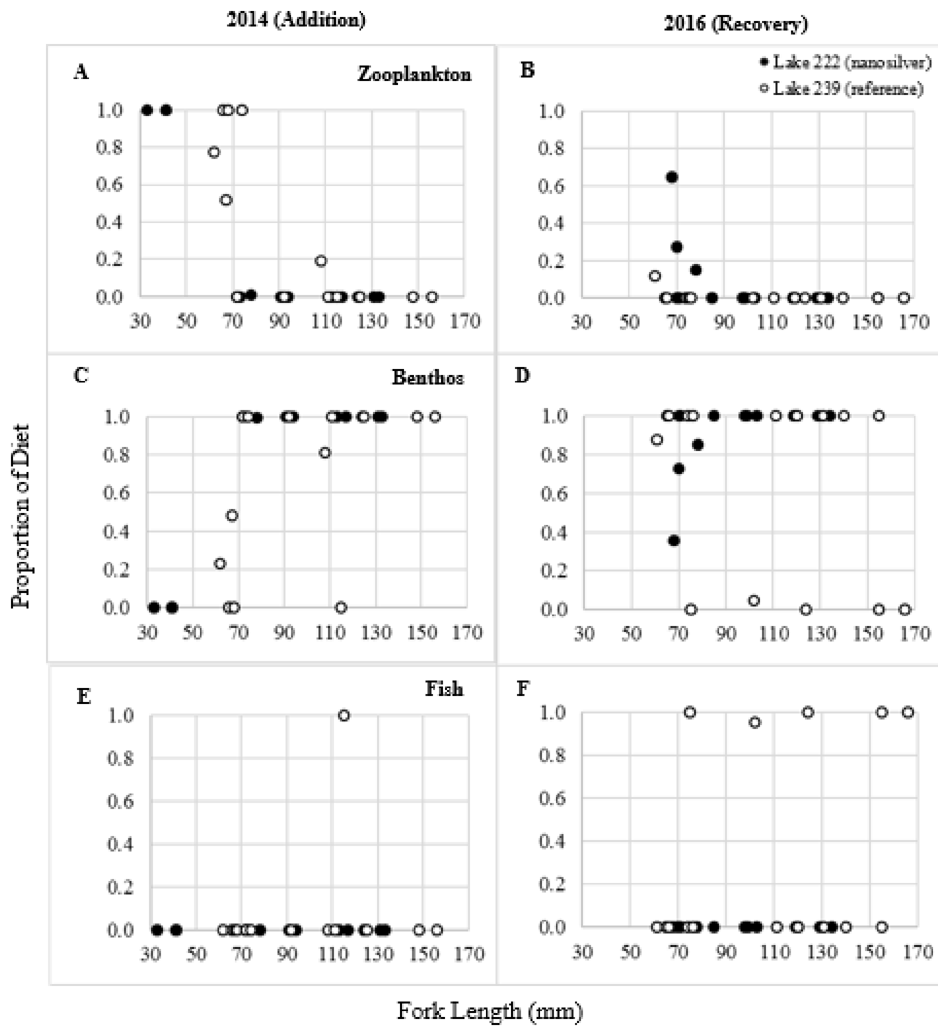

Figure 3. Proportion of Yellow Perch summer diet items with increasing body size in a lake with nanosilver added (Lake 222) and a reference lake (Lake 239) comparing a year where nanosilver was added (2014, Panels A, C, E) with a year free of nanosilver additions (2016, Panels B, D, F). Lake 222 (closed symbols) and Lake 239 (open symbols) are the proportion of diet from three food item categories for summer seasons across addition and recovery years by fork length size of fish. Panels A, B = proportion of zooplankton in diets; Panels C, D = proportion of benthos in diets; and Panels E, F = proportion of fish in diets.

Since most of the fish captured were feeding 100% on benthos prey sources, the benthic taxa were examined at finer resolution to see if particular groups of benthic taxa were available being consumed by captured Yellow Perch. In Lake 222 during summer and there was a

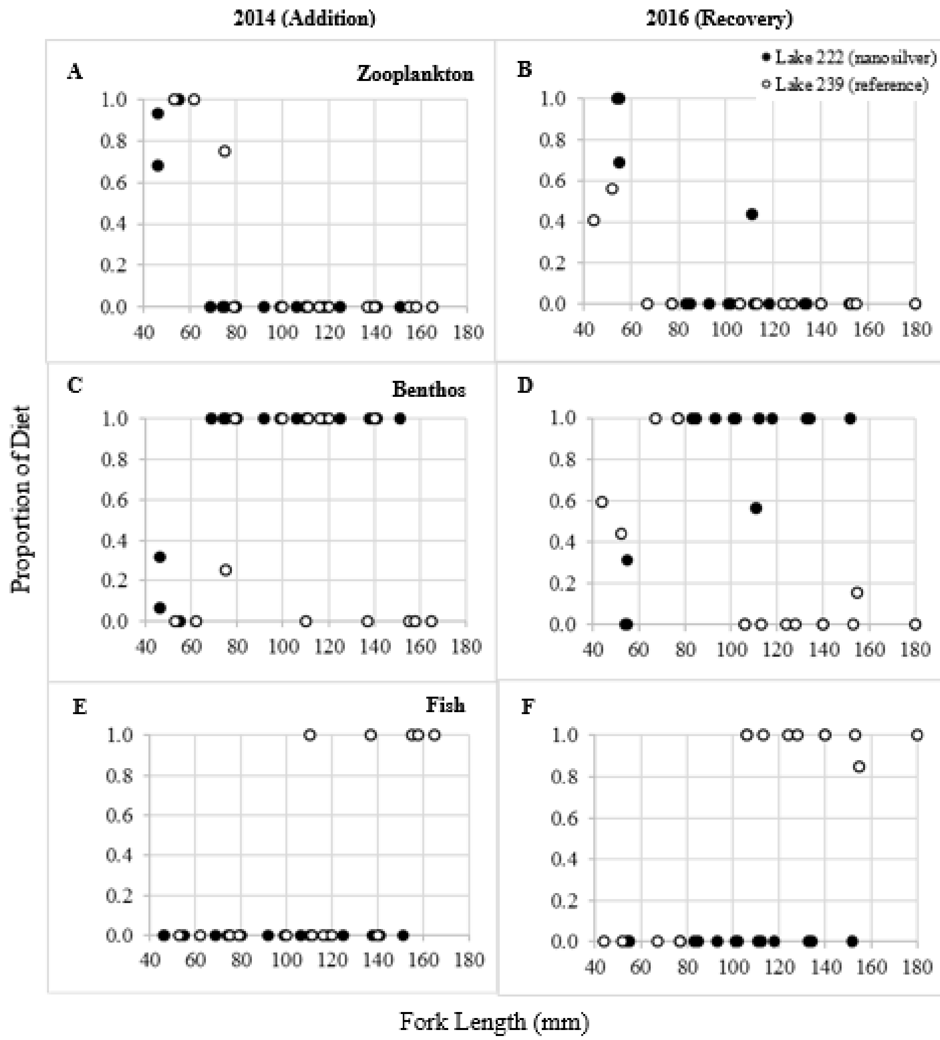

Figure 4. Proportion of Yellow Perch fall diet items with increasing body size in a lake with nanosilver added (Lake 222) and a reference lake (Lake 239) comparing a year where nanosilver was added (2014, Panels A, C, E) with a year free of nanosilver additions (2016, Panels B, D, F). Lake 222 (closed symbols) and Lake 239 (open symbols) are the proportion of diet from three food item categories for fall seasons across addition and recovery years by fork length size of fish. Panels A, B = proportion of zooplankton in diets; Panels C, D = proportion of benthos in diets; and Panels E, F = proportion of fish in diets.

decrease in dragonflies consumed after nanosilver additions were stopped. There were no additional clear changes in benthos in diets from the year nanosilver was added to the year of recovery during either summer (Figure 5 A-B) or fall (Figure C-D). In Lake 239, a similar decline in dragonflies in diets was observed in summer diets, but not in fall

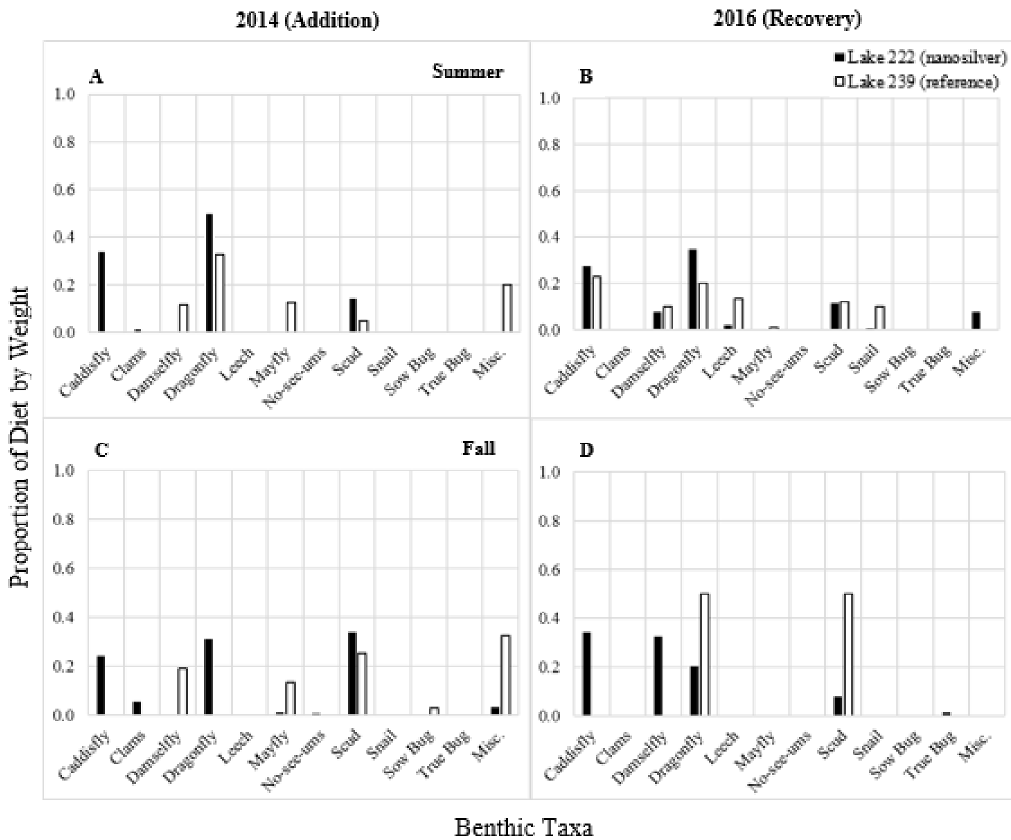

Figure 5. Proportion of benthic invertebrates consumed by Yellow Perch in lake with nanosilver added (Lake 222, black bars) and a reference lake (Lake 239, white bars), comparing a year where nanosilver was added (2014; Panels A, C) with a year free o nanosilver additions (2016; Panels B, D). Panels A, B = summer diets, Panels C, D = fall diets.

diets when they became more prevalent in 2016. Furthermore, 2014 shows some seasonal changes in both lakes; In Lake 222 fewer caddisfly and dragonfly groups were consumed and scuds were more commonly consumed from summer to fall. In Lake 239, larger seasonal changes are shown with dragonflies being consumed the most in the summer and then not being consumed at all in the fall. Also, in Lake 239, scuds and damselfly groups increased in perch diets from summer to fall in 2014. The recovery year in 2016 also shows some seasonal changes in both lakes.

DISCUSSION

EFFECT OF NANOSILVER ADDITION

The findings during this study show that there are no appreciable differences in Yellow Perch diets between years when nanosilver was added compared with a recovery year in Lake 222. With addition of nanosilver to Lake 222, fish were expected to shift diets from nearshore to offshore feeding sources, by negatively affecting algal and bacterial growth in nearshore areas, ultimately causing a reduction of food for benthic invertebrates (Croteau et al. 2011). Contrary to this expectation, the results of this study showed that there were no adverse effects to the presence of benthos in the contents of fish diets.

The diet contents in this study did reveal some differences between lakes, but that differences are likely the consequence of differences in light penetration or seasonal changes in diet preferences. Interestingly, seasonal changes were observed in the feeding strategies of Yellow Perch, particularly those feeding on benthic invertebrates (Figure 5). Food web relationships in a freshwater ecosystem are associated with changes in water temperature, which naturally causes alterations in predator feeding observed (Magnan et al. 1994). Therefore, the results demonstrate that the alternations in feeding strategies are solely based on natural changes in the seasons, not the additions of nanosilver material.

WATER TRANSPARENCY

The water transparency in the two study lakes were measured by using secchi depth readings. In Lake 222 the maximum secchi disc visibility is 2.2 meters, whereas Lake 239 is 4.8 meters (Cleugh and Hauser 1971). Furthermore, water transparency in aquatic ecosystems is a key mechanism for predators to visualize prey for selection. Predators are influenced by water transparency when the clarity of the water decreases so does the availability to detect piscivorous prey (Turesson and Brönmark 2007). In a recent study Robertis et al. (2003) found that an increase in turbidity caused a decrease in piscivory. Similarly, piscivory was only ever observed in Lake 239 which has water transparency more than twice that of Lake 222. Therefore, greater water transparency seen in Lake 239 may indicate why piscivorous diets were only observed in Lake 239, whereas Lake 222 had no piscivory.

In regard to food web dynamics, foraging and survival as prey or predator is crucial. Yellow Perch are visual predators most often found in high water clarity regimes of aquatic systems, which helps with visual foraging strategies (Manning et al. 2014). The selection of appropriately-sized prey for predator growth is essential to meet the needs for body functions. Recent studies have shown that water clarity influences growth and forage strategies of Yellow Perch as predators (Manning et al. 2013). Therefore, secchi depth readings from the two study lakes suggest why more piscivory occurred in Lake 239, whereas Lake 222 had a shallower secchi depth readings and no piscivory in Yellow Perch diets.

PREDATOR-PREY INERACTIONS

The predator-prey interactions are influenced by not only, water transparency, but also the capture success of piscivorous fish (Lundvall et al. 1999). In addition, prey escape in relation to capture success, and both can shape food web functions. As prey abundance increases in an aquatic food web, the escape speeds and abilities increase as well (Lundvall et al. 1999). By extension, these natural functions are shown in the results, with no effects of nanosilver on Yellow Perch diets.

STUDY DESIGN

This study design of this experiment used a small sample size of fish for an examination of diets, and this could potentially influence the ability to observe significant changes from the exposure to nanosilver. However, the proportion of diets in summer (Figure 3) and fall (Figure 4) incorporate a large number of fish from each time period and neither suggest that an increase in sample size would change conclusions. In addition, it is possible that the lack of difference between 2014 and 2016 may potentially be due to have a carry-over effect (i.e., lack of recovery from nanosilver addition). However, the consistency in patterns between the manipulated lake (Lake 222) to the unmanipulated lake (Lake 239) across years and seasons also does not suggest that carry-over effects are present, due to similar in percent zooplankton and benthic feeding fish. Therefore, the study design and sample size appear to have been robust to test nanosilver effects on the diets of Yellow Perch.

CONCLUSION

Yellow Perch diets did not show significant changes from nanosilver addition. The results instead showed a tendency for pisivory in Lake 239 fish and no piscivory perch from Lake 222, and some possible seasonal shifts in the feeding on benthic invertebrates from summer to fall. Otherwise, there were no appreciable differences in diets across lakes, years, and seasons. Differences in lake water transparency are proposed as a potential mechanism from the difference in perch feeding between lakes. Therefore, this study at present is unable to show that nanosilver exposure at a nearshore point source addition caused differences in Yellow Perch diets in Lake 222. It is possible that the recovery stage has not been long enough to show the effects of nanosilver, since recovery at present has only been approximately one-year. As a consequence, ongoing monitoring could reveal effects to diets from nanosilver material in the future. All things considered, nanosilver could potentially take longer for effects to be detected in ongoing monitoring and sampling processes on both the manipulated lake (Lake 222), and unmanipulated lake (Lake 239). In conclusion, monitoring should continue with possibility of looking at other ecosystem components or organisms, to indicate how nanosilver behaves in an aquatic ecosystem.

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