Aquatic Macroinvertebrates and Metal Contamination in Forested and Urban Streams

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EXECUTIVE SUMMARY

Aquatic macroinvertebrates are a vital part of stream and riparian ecosystems. They play an important role as food sources for other aquatic or terrestrial organisms, and provide key ecosystem functions like nutrient retention and litter decomposition. Aquatic insects subsidize riparian ecosystems, moving important nutrients from the streams to the terrestrial environment. However, this also means they have the potential to move aquatic pollution into the terrestrial environment as well. Increasing urbanization and development have increased the stress and contamination of streams draining urban watersheds. The increase in impervious surface cover that accompanies urbanization facilitates a rapid accumulation and transport of urban pollutants directly into streams. Metals are a common urban pollutant, and previous research has shown that relatively low metal concentrations can negatively impact aquatic macroinvertebrates when they expend more energy towards detoxification than they put towards growth and emergence. If aquatic macroinvertebrates accumulate metal pollution through their development, they can act as vectors to carry this pollution into terrestrial environments when they emerge as adults. The two research questions studied in this masters project are:

1. What is the difference in metal concentrations between urban and forested streams in the Durham, NC area?
2. What is the effect of food material from these forested and urban aquatic ecosystems on aquatic macroinvertebrate growth, mortality, and metal body burden and accumulation?

To answer these questions, simulated leaf packs were made and left in two urban (Ellerbe Creek Glenn Stone and North Gate) and one forested (New Hope Creek) stream site in Durham, NC for 3.5 weeks. Stonefly larvae (Plecoptera Peltoperlidae) were collected from Stone Mountain State Park, NC and brought back to habitats built for the laboratory. They were fed the leaves from the Durham sewn leaf packs or leaves collected from Stone Mountain; the only difference between the habitats were the leaf food sources. Data on larvae mortality, growth, and leaf consumption were collected for about two months, at which point the larvae were frozen and analyzed for metal concentration along with the leaf samples following an acid digestion.

The reference larvae, those receiving the leaves from Stone Mountain, grew the most, shed the most casts, consumed the most leaves, and had no recorded fatalities. The most mortality was observed in the larvae receiving the North Gate (urban) sewn leaf pack leaves. There was little

difference in casts collected, larvae growth, and leaf consumption between the larvae receiving the Durham sewn leaf pack leaves. When metal concentrations were analyzed, bioconcentration was observed for zinc, copper, and silver while biodilution was observed for lead, nickel, cobalt, chromium, and arsenic across all four habitats. The reference larvae exhibited the lowest metal concentrations, while less differentiation was observed between the larvae receiving the Durham sewn leaf pack leaves. The Durham forested site generally had lower metal concentrations than the Durham urban sites as observed from the leaf samples and previously collected water sample data from Jonny Behrens.

The health of the reference larvae relative to those receiving the Durham leaf pack leaves and their observed lowest metal concentrations supports the theory that stressed larvae may put more energy towards detoxification of contaminants than towards growth and development. These results are also observed at metal concentrations well below stonefly toxicity values, showing how these negative effects on growth are being observed at relatively low metal concentrations. The accumulation of metals agrees with some previous literature, indicating which metals the stonefly larvae may potentially carry into terrestrial environments upon emergence as adults. Trends in metal concentrations from this project are also consistent with previous macroinvertebrate studies conducted in the Durham area. Combined with the general agreement between the metal concentrations of the sewn leaf pack leaves with leaves collected from the Durham stream sites, it shows this method to be relatively representative of these ecosystems.

The simple habitat and feeding design in this experiment demonstrates a way to isolate and examine the influence of a specific stressor in complex ecosystems. The success of this habitat experiment shows promise for increasing the scale of this study in the future to provide a statistical analysis on larvae growth and metal accumulation and to gather data over more molts and emergence. This experiment could be extended to other types of shredders across a range of sensitivities and life cycles, and leaves could be analyzed for additional contaminants, nutrients, or microorganisms for a more comprehensive picture of the effects of urbanization on aquatic life. Being able to better understand how anthropogenic influences on aquatic environments affect a key indicator organism and how this may influence contaminant movement through ecosystems is critical as human development continues to expand.

TABLE OF CONTENTS

INTRODUCTION

Aquatic macroinvertebrates are a vital part of stream and riparian ecosystems, and it is important to understand the effects of anthropogenic influences on their ability to play their crucial roles. Aquatic insects are food sources for many other organisms including fish, amphibians, spiders, birds, and bats (Suter & Cormier, 2015). They also act in various stream ecosystem functions such as nutrient retention, litter decomposition, stream recovery after disturbances, and stream bed stabilization (Suter & Cormier, 2015). Aquatic insects play an important role in transferring material from the aquatic environment into the terrestrial food web (subsidies) (Baxter et al., 2005). While this includes the transfer of necessary energy and nutrients, it can also include the unwanted movement of pollutants from streams into terrestrial ecosystems (Baxter et al., 2005; Walters et al., 2008). Aquatic insects acting as vectors for pollutants has been termed "the dark side of subsidies" which is important to remember when considering widespread stream pollution and the prevalence of aquatic insects as a food source to riparian predators (Walters et al., 2008).

Currently, 56% of the world population lives in urban areas, with this number projected to reach nearly 70% by 2050 (The World Bank, 2023). Urban land consumption is also outpacing urban population growth, leading to expansive urban sprawl (The World Bank, 2023). This shift in land use can have drastic impacts on water quality health. In contrast to natural land covering, which allows rainwater to slowly infiltrate the ground, the impervious surface covering that accompanies urban development facilitates rapid runoff directly into water bodies, increasing pollution loads of sediments, oils, pesticides, salts, heavy metals, thermal pollution, and more (U.S. EPA, 2003). Streams that drain watersheds dominated by urban development often exhibit changes in flow, decreased biodiversity and loss of sensitive species, and higher concentrations of nutrients and pollutants, collectively known as the "urban stream syndrome" (Ancion et al., 2013; Schoonover et al., 2006; Walsh et al., 2005). In particular, various studies have demonstrated the negative impact of urbanization on aquatic insect communities, including decreased biodiversity and colonization (Blakely et al., 2006; Lundquist & Zhu, 2019; Paul & Meyer, 2001).

Numerous studies show that metal contamination is observed in aquatic macroinvertebrates collected from the field (Arnold et al., 2021; Chiba et al., 2011; De Jonge et al., 2014). It has been shown that metal concentrations below aquatic life criteria can have minimal effects on larval density but may greatly reduce the emergence of adult insects (Schmidt et al., 2013). These lower concentrations of metals can negatively affect aquatic insects when more energy is expended to regulate and detoxify the consumed metals than is put towards growth and development (Rainbow, 2007; Schmidt et al., 2013). If metal contamination inhibits aquatic larval growth, increases the time to emergence, or prevents adult emergence at all, it can have effects across the aquatic and terrestrial ecosystems and the organisms that depend on aquatic insects.

This project focused on two research questions: 1) What is the difference in metal concentrations between urban and forested streams in the Durham, North Carolina area? 2) What is the effect of food material from these forested and urban aquatic ecosystems on aquatic macroinvertebrate growth, mortality, and metal body burden and bioconcentration? The hypothesis was that urban ecosystems would display a greater metal loading than forested ecosystems, and that macroinvertebrates consuming food from urban ecosystems would exhibit increased mortality, decreased growth and development, and higher metal body burdens.

METHODS

Stream sites

Three stream sites around the Durham, NC area were selected for this study (Table 1). These are sites that the Bernhardt lab has been studying for years, and their water quality has been characterized (see Appendix I). New Hope Creek drains an area mostly occupied by the Duke research forest, making it the forested site for this study. Ellerbe Creek Glenn Stone and Ellerbe Creek North Gate are both urban sites located in Durham, but Glenn Stone is located just downstream of the North Durham Water Reclamation Facility (a wastewater treatment plant) while North Gate is heavily influenced by stormwater runoff. Information on watershed basin land coverage was found through the USGS StreamStats application; NLCD 2011 classes 41-43 was the statistic used for forested land coverage and NLCD 2011 classes 21-24 was the statistic used for developed (urban) land coverage.

Table 1. The Durham, NC stream sites evaluated in this project, their locations, and the percentage of forested and developed land coverage in the site's watershed drainage basin.

Stream Site	Coordinates	Forested Land Coverage (%)	Developed (urban) Land Coverage (%)
New Hope Creek	35.983430, -79.006250	73.9	8.2
Ellerbe Creek Glenn Stone	36.056319, -78.841159	14.4	76.4
Ellerbe Creek North Gate	36.019144, -78.894612	12.2	85.9

Leaf packs

A large bin of fallen leaves was collected from the Duke Forest in early September 2023 and brought back to the laboratory to be spread out and dried. About 30 g of leaves were sewn into 0.25 cm plastic mesh netting with fishing line to make six leaf packs. Two leaf packs were placed in each of the stream sites mentioned above on September 21, 2023. Leaf packs were either secured to concrete blocks which were placed on the bottom of the stream, or they were secured to tree roots extending into the water using zip ties (Figure 1). Leaf packs were oriented such that the largest surface area was facing the direction of water flow. Leaf packs were left in the streams until collection on October 15, 2023 (roughly 3.5 weeks). Upon collection, the contents of the leaf packs were emptied into acid-washed plastic containers with water from their respective stream sites (for six total collection containers). A subsample was taken from each leaf pack to be dried and stored for future analysis. Each container was stored in a cold room at 4 degrees C with tubing connected to an aquarium pump for the duration of the study.

Figure 1. The leaf packs were left in streams either secured to a concrete block (a) or to submerged tree roots (b). Thin knotted rope and zip ties were used for securing the leaf packs.

Macroinvertebrate collection

Aquatic larvae were collected from Bullhead Creek in Stone Mountain State Park, NC (coordinates: (36.398311, -81.061874); 94.2% forested; 2.3% developed (urban)) on October 13, 2023. Leaf packs were pulled from the stream and vigorously agitated in a bucket with water to dislodge any macroinvertebrates. The contents of the bucket were poured over a sieve and onto a white tray. Moving macroinvertebrates on the white tray were easy to spot, and the sieve and leaves were closely inspected for remaining insects. It became obvious that "roach-like stoneflies" (Plecoptera *Peltoperlidae*) were an abundant insect in this creek, and the decision was made to collect these for transport back to Durham. When found, stonefly larvae were pipetted (not tweezed) into a separate bucket with water, some leaf matter, and a battery-powered aquarium pump with an air stone. After about three hours of leaf pack sampling, 42 stonefly larvae were collected. About five liters of water (some filtered and unfiltered) and leaf pack samples were also collected for transport back to Durham. Upon arrival in Durham, the bucket of stonefly larvae was left in the lab covered with mesh netting for about 36 hours for acclimation, one leaf pack sample was stored in the cold room in Stone Mountain water as described above, and the remaining leaves and water samples were frozen.

Larvae habitats

Four larvae habitats were constructed using plastic containers, disposable plastic cups, and fine mesh netting (Figure 2) following a previously outlined procedure for simple larvae rearing chambers (Keiper & Foote, 1996). This included cutting a hole in each container lid onto which the cup was taped. The bottom of each cup was cut off and covered with the mesh netting. Each habitat had a small opening cut into the lid for an air stone to be connected to an aquarium pump, and habitats were kept at ambient room temperature (70 – 72 degrees F). Habitats were placed on a white background to easily spot and photograph larvae.

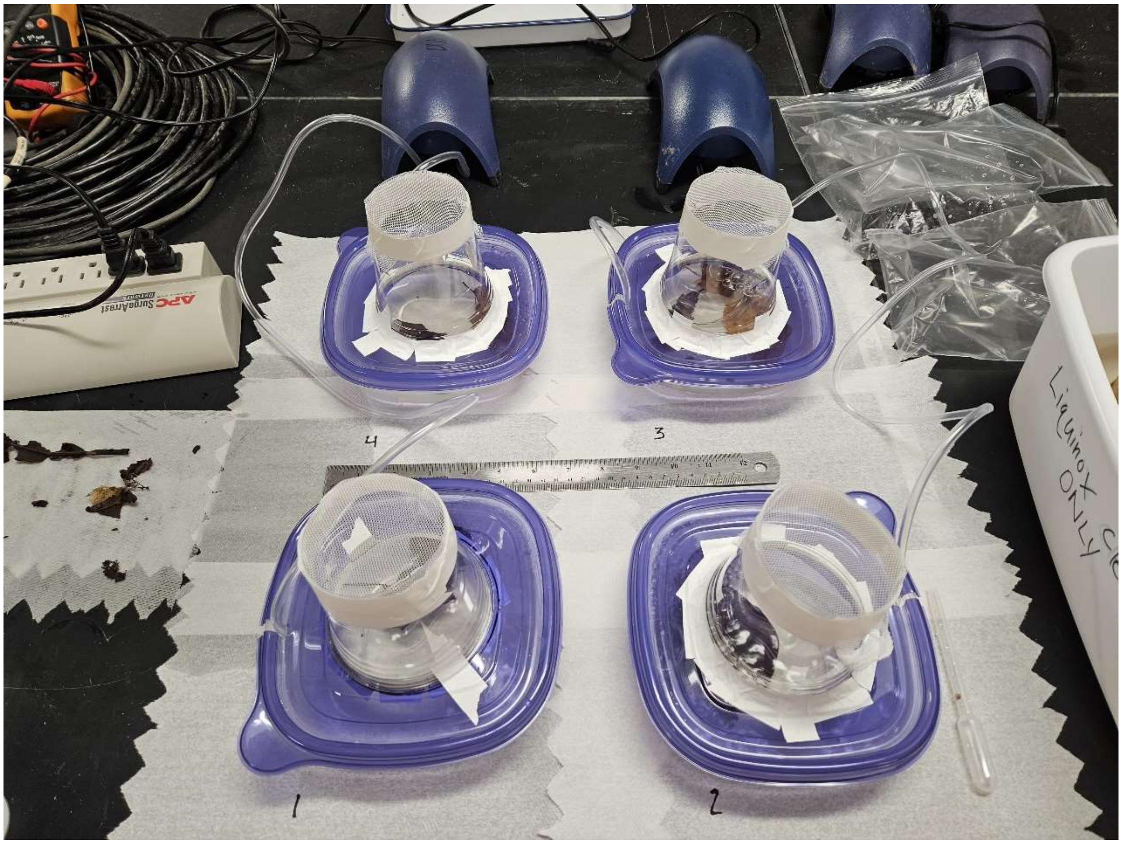

Figure 2. An image of the habitat set up for the stonefly larvae in the laboratory.

Each habitat was filled with roughly 400 mL of water from New Hope Creek (or about 1 inch of water depth) and was given one or two beech leaves from either the New Hope Creek, Glenn Stone, or North Gate sewn leaf packs or the leaf sample collected from Stone Mountain. Following the acclimation period, 40 stonefly larvae had survived the travel back to Durham and were divided equally among the habitats on October 15, 2023. The breakdown of habitat contents is outlined in Table 2.

Table 2. The contents of the larvae habitats.

Habitat	Initial Larvae Count	Water Source	Leaf Food Source
1	10	New Hope Creek	New Hope Creek leaf pack
2	10	New Hope Creek	Glenn Stone leaf pack
3	10	New Hope Creek	North Gate leaf pack
4	10	New Hope Creek	Stone Mountain leaf sample

About half the water was exchanged roughly twice per week (or was topped off to roughly 400 mL with New Hope Creek water after evaporation) and beech leaves were replaced when obvious leaf matter consumption had occurred. During the duration of the habitat experiment, the number of fatalities and the casts collected were recorded, pictures were taken to measure growth rates, and leaf consumption was recorded by massing leaves (wet) before and after being in the habitats. Any larvae that died during the habitat experiment were collected and frozen, and on December 7, 2023, the larvae remaining were frozen and added to the collected larvae to end with four frozen samples of ten larvae each.

Metals analysis

Leaf samples for analysis included the leaf pack subsamples saved when the sewn leaf packs were brought in from the Durham stream sites, leaf pack subsamples that were taken from the sewn leaf packs and the Stone Mountain leaf pack after they were stored for the duration of the habitat experiment, and leaf pack samples that were collected from each of the four stream sites (not part of the sewn leaf pack experiment). All samples were freeze-dried for about 24 hours. Leaf samples were acid digested on a CEM MARS 6 microwave digestor with MARSXpress disposable liners following EPA microwave assisted digestion Method 3052 (U.S. EPA, 1996b). Larvae samples were acid digested on a hot block using a modified EPA acid block digestion Method 3050B (U.S. EPA, 1996a): each sample was spiked with 1 mL each trace grade concentrated nitric acid and ultra-pure di-ionized water, left in the hot block at 85 degrees C for two hours, spiked with 0.5 mL of trace grade hydrogen peroxide, then placed back in the hot block at 85 degrees C for another two hours until all solid material was dissolved. Metal concentrations were analyzed via inductively coupled plasma mass spectrometry (Agilent 7900 ICP-MS) following EPA Method 6020B using standard reference material DORM-4 (fish protein) (U.S. EPA, 2014). Concentrations for zinc (Zn), copper (Cu), silver (Ag), lead (Pb), nickel (Ni), cobalt (Co), chromium (Cr), arsenic (As), and cadmium (Cd) were determined; limits of quantification for the leaf analysis were 0.099, 0.024, 0.024, 0.024, 0.099, 0.024, 0.048, 0.024, 0.024 ppb, respectively and for the larvae analysis were 0.054, 0.054, 0.014, 0.014, 0.014, 0.014, 0.014, 0.014, 0.014 ppb, respectively.

RESULTS

Larvae habitats

The larvae receiving the Stone Mountain leaf diet generally seemed to thrive more than those receiving the diets of Durham urban or forested sewn leaf pack leaves (Figure 3).

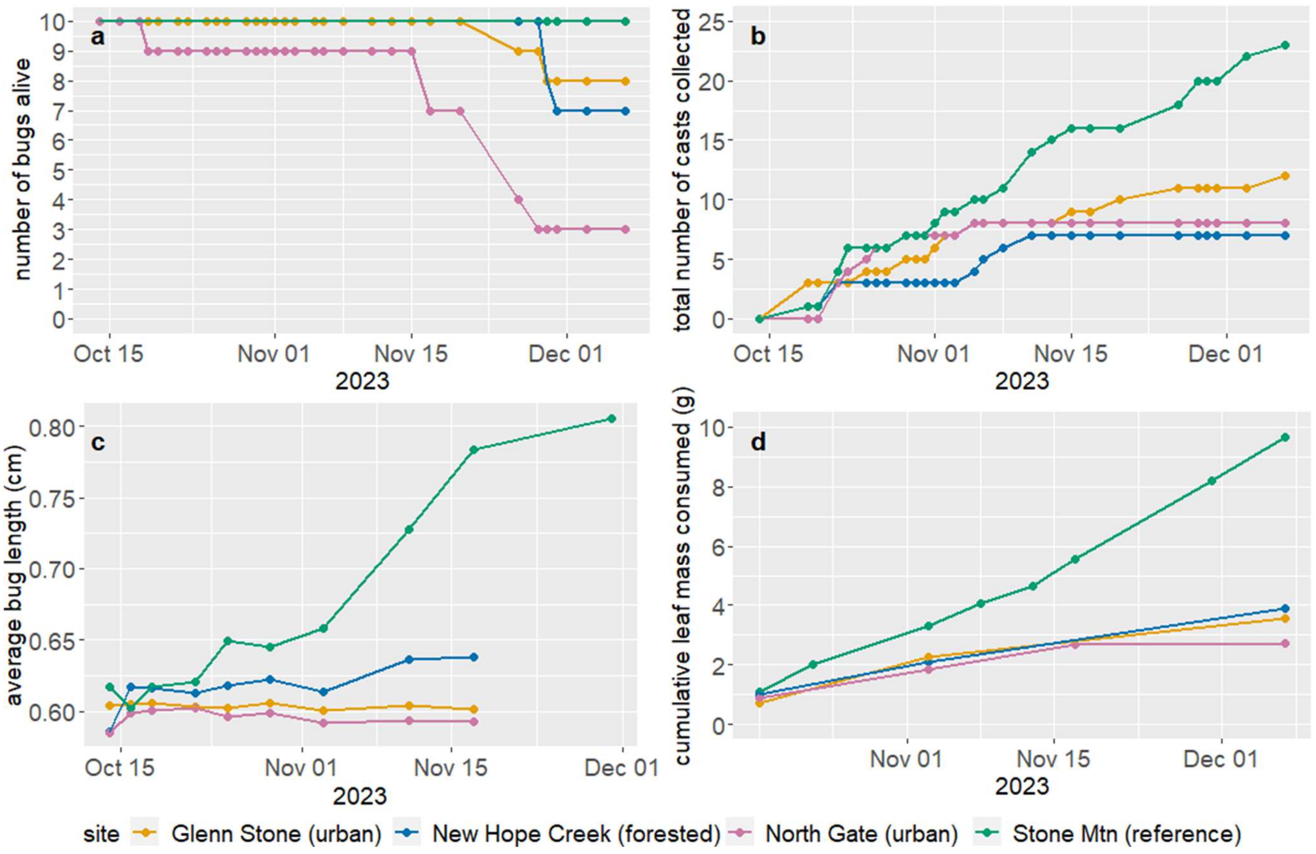

Figure 3. Stonefly larvae mortality (a), molts (b), growth (c), and leaf consumption (d) over the duration of the habitat experiment. Average bug length (c) is calculated as the average length of all the larvae alive in the habitat; this was no longer calculated after larvae started to die to avoid skewing the average if a larger or smaller than average larva died.

Based on the number of casts collected, each of the larvae receiving the Stone Mountain leaf diet likely molted about twice during the duration of habitat study. The larvae receiving leaves from the Durham area sewn leaf packs (urban and forested) likely each molted about once over the course of the study. This means that the larvae receiving the Stone Mountain leaf diet likely grew through one instar stage while living in the laboratory habitats, while the larvae receiving Durham sewn leaf pack leaves completed the instar stage they had started while in their natural Stone Mountain habitat and did not develop through a further instar stage while in the laboratory habitats.

The average length of the larvae receiving the Stone Mountain leaf diet increased more than those receiving the Durham sewn leaf pack leaves. Essentially no growth was observed in the larvae receiving the Durham urban sewn leaf pack leaves, while some possible growth was observed in the larvae receiving the Durham forested sewn leaf pack leaves. The Stone Mountain leaves were consumed the most, while there was minimal difference in the consumption of the Durham sewn leaf pack leaves.

Metals analysis

The concentrations of metals in the leaves after they had been stored through the duration of the habitat experiment are displayed in Figure 4a. These were the leaves from the Durham sewn leaf packs and the leaf pack sample brought back from Stone Mountain after they had been stored in a cold room for roughly two months and used as the food sources for the larvae habitats. Generally, these metal concentrations agree with the metal concentrations of leaf subsamples taken from the Durham sewn leaf packs the day of their collection from the streams, and with metal concentrations of leaf pack samples taken from the four stream sites (not associated with the sewn leaf pack experiment) (see Appendix II). This shows that the use of sewn leaf packs as a food source for the larvae habitat experiment is relatively representative of leaf matter found in the streams, and that storage during the duration of the habitat experiment has little effect on the metal concentrations. Generally, the leaves from the forest sites (New Hope Creek and Stone Mountain) exhibited lower metal concentrations than leaves from the Durham urban sites.

The concentrations of metals in the larvae from the habitat experiment are shown in Figure 4b. The larvae receiving the Stone Mountain leaf diet displayed the lowest concentrations of metals compared to the larvae receiving the Durham sewn leaf pack leaves. There is not as clear a trend when comparing metal concentrations between the larvae receiving the Durham forested sewn leaf pack leaves and the Durham urban sewn leaf pack leaves.

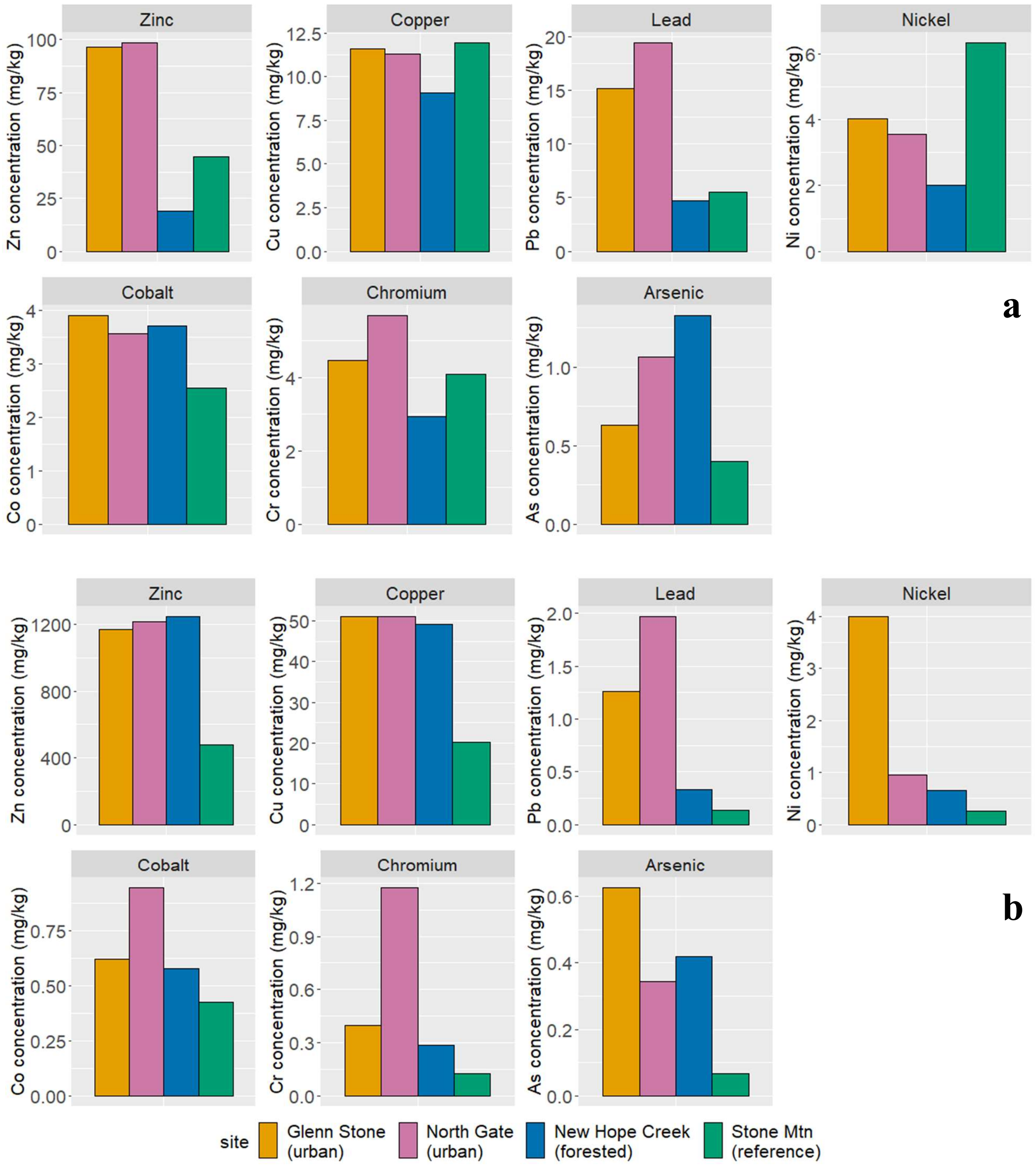

Figure 4. (a) The metal concentration of the leaves used as the larvae food source after cold room storage. The Glenn Stone, North Gate, and New Hope Creek leaves were from the sewn leaf packs. The Stone Mtn leaves were leaf pack samples brought back from Stone Mountain. (b) The metal concentrations of the stonefly larvae after the habitat experiment.

The bioconcentration factors (BCF) of the metals in the stonefly larvae are displayed in Table 3. Across all four habitats, zinc, copper, and silver were observed to bioconcentrate (BCF > 1) while lead, nickel, cobalt, chromium, and arsenic were observed to biodilute (BCF < 1). Generally, the larvae receiving the Stone Mountain leaf diet had the lowest BCFs indicating the least amount of metal accumulation.

Table 3. The bioconcentration factors of the metals in the stonefly larvae from each habitat, calculated as mg/kg metals in the larvae divided by the mg/kg metals in the leaf food source (the leaves stored in the cold room for the duration of the habitat experiment).

	Stone Mtn (reference)	**New Hope Creek (forested)**	**Glenn Stone (urban)**	**North Gate (urban)**
Zinc	10.72	65.76	12.14	12.36
Copper	1.69	5.43	4.40	4.50
Silver	1.19	10.75	6.71	4.50
Lead	0.02	0.07	0.08	0.10
Nickel	0.04	0.33	0.99	0.27
Cobalt	0.17	0.16	0.16	0.27
Chromium	0.03	0.10	0.09	0.21
Arsenic	0.16	0.32	0.99	0.32

DISCUSSION

The stonefly larvae receiving the Stone Mountain leaf diet appeared to be the healthiest larvae in the habitat experiment. These larvae grew the most, molted the most, consumed the most food, and had no fatalities for the duration of the habitat experiment. They were also observed to have the lowest metal body burdens. Of the metals that bioconcentrated in the stonefly larvae, those receiving the Stone Mountain leaf diet had the lowest BCF values. This is consistent with the theory that the larvae that are the least stressed have more energy to put towards detoxification and regulation of ingested metal pollution as well as towards growth and development (Schmidt et al., 2013). The larvae receiving the Durham sewn leaf pack diet exhibited greater stress during the habitat experiment and greater metal body burdens and BCF values.

Cadmium, lead, cobalt, and chromium have been observed to biodilute in stonefly larvae in previous studies (Cain et al., 1995; Smock, 1983). Other studies demonstrate that copper has been observed to accumulate in stonefly larvae while zinc has been observed to accumulate or undergo a simple transfer (BCF of approximately 1) (Sjcbakk et al., 1997; Soucek et al., 2002). In another study where caddisfly larvae were transferred from a reference to a contaminated stream site, larvae were observed to have increased accumulation of copper, zinc, nickel, and lead (Tochimoto et al., 2003). This is like the increased metal body burdens observed in the stonefly larvae transferred from the Stone Mountain creek into the habitats receiving the Durham sewn leaf pack leaves. Metal accumulation literature for stonefly larvae specifically is rather limited; however, metal accumulation across different taxa can vary significantly (Aydoğan et al., 2017; De Jonge et al., 2014; Rainbow, 2007). In stonefly survival toxicity studies, lead was observed to have an EC50 over 200 ug/L, zinc had an EC50 over 1500 ug/L, and copper had an EC50 value of about 16 ug/L (Clements et al., 2013; Mebane et al., 2012). These are concentrations well above what was observed in the waters of the Durham stream sites. However, laboratory single species toxicity testing for aquatic macroinvertebrates may be limited by lack of organisms in the earliest life stages and a study duration not sufficiently long enough to observe toxic effects (Clements et al., 2013). While the mortality and growth observed in the stonefly larvae during this habitat study cannot be solely attributed to metal contamination, these results could indicate that relatively low metal concentrations could still negatively impact larval growth and development. Combined with previous literature, this study also indicates which metals stonefly larvae may be able to accumulate and carry with them into terrestrial ecosystems upon adult emergence.

While the stonefly larvae receiving the Stone Mountain leaf diet exhibited the lowest metal concentrations, less difference was observed when comparing the larvae receiving the Durham urban and forested sewn leaf pack diets. The only difference between the habitats was the leaf food source, and while the leaves were only tested for metal concentrations, there are various other factors that could influence the effects of the leaf consumption on the larval health. For example, while each habitat received beech leaves, their incubation in different streams could have influenced the microbial colonies that developed on the leaves. Microbial colonies can be highly dependent on flow rate, water temperature, and dissolved oxygen content, and these factors could have differed among the stream sites of this study (Arias-Real et al., 2018). Aquatic microbes help link leaf packs to aquatic macroinvertebrates by improving the nutritional quality of the leaves through increased nitrogen and phosphorous concentrations and more bioavailable fats, lipids, and proteins that aquatic macroinvertebrates cannot always make for themselves (Bärlocher, 2016; Kuehn, 2016). Considering the nutrient composition of the leaves could give a possible explanation behind why the stonefly larvae receiving the Glenn Stone (urban) sewn leaf pack diet demonstrated survival similar to those receiving the New Hope Creek (forested) sewn leaf pack diet while the larvae receiving the North Gate (urban) sewn leaf pack diet displayed the most mortality. The Glenn Stone location is just downstream of a wastewater treatment effluent site; wastewater effluent can have high nitrogen and phosphorous concentrations, and increases in the density of some macroinvertebrates have been observed in stream sites downstream of wastewater effluent locations (Carey & Migliaccio, 2009; Sánchez-Morales et al., 2018). Aside from nutrients, the leaves from the Durham sewn leaf packs visually appeared to be more decomposed than the leaf pack samples brought back from Stone Mountain. However, studies have shown that the optimal stage in decomposition for aquatic shredder macroinvertebrates to colonize leaf packs is around 28 to 65 days into decomposition (Abelho, 2008). This covers the time frame in which the Durham sewn leaf packs were being used as a food source for the Stonefly larvae, suggesting that the stage of decomposition would not affect larval success in this experiment.

Previous work has studied metal concentrations in aquatic macroinvertebrates from similar stream sites in the Durham, NC area (Baruch et al., 2018). Over a range of forested and impervious surface land coverage in the watershed drainage basins at these various sites, concentrations of zinc, copper, and lead were determined for collected tipulidae larvae (Baruch et al., 2018). Tipulidae are another type of shredder, so while metal concentrations of stonefly larvae from the Durham area are not available for comparison, data from the tipulidae larvae could indicate if the habitat study in this experiment is representative of metal concentrations for shredder macroinvertebrates in Durham streams. Over a similar range of forested land coverage for the watershed basins, the tipulidae and stonefly larvae exhibited similarly low lead concentrations, followed by a comparable range of copper concentrations, and the highest ranges of zinc concentrations, though the stonefly larvae had zinc concentrations about an order of magnitude higher than the tipulidae larvae (Baruch et al., 2018). Concentrations can be seen in Appendix III. Given the variation of metal accumulation across macroinvertebrate taxa, it is still promising to see similar trends in metal concentrations across two types of shredder macroinvertebrates.

Stoneflies are typically only found in relatively clean or forested streams in the Piedmont region of North Carolina, and the Plecoptera Peltoperlidae specifically used for this experiment are found in the clean streams of the western mountain region of North Carolina as they are relatively intolerant to pollution and changes in their habitats (Beaty, 2015; Tierno De Figueroa et al., 2024). Most stonefly larvae have a long nymph stage, lasting one to two years for most species, and they can molt up to 20 or more times (DeWalt et al., 2015; Patrick, 2013). Rearing stoneflies in the laboratory is not well documented in the literature, but aquatic macroinvertebrate rearing chambers range from complicated aquarium set ups to simple habitats (DeWalt et al., 2015; Keiper & Foote, 1996; Tsuruishi, 2003; Yokoyama et al., 2009). The stonefly's sensitivity likely contributed to the growth and mortality data that was able to be gathered in this experiment, but their long life cycle means it may take more time to collect sufficient data to observe more differences in growth, emergence, and the ability to accumulate or shed ingested contaminants. The relatively simple habitat and feeding design outlined in this experiment demonstrates a way to isolate and examine the influence of a specific stressor in complex ecosystems.

CONCLUSION

The important role aquatic macroinvertebrates play across ecosystems and the rapid changes happening in aquatic environments due to urbanization make it important to study how contamination differs between forested and urban stream ecosystems and how the food materials from these ecosystems affect macroinvertebrate growth and mortality. This study demonstrated how metal loading is generally higher in urban than in forested aquatic environments and provides a useful method to isolate one stressor, food, to study its effects on macroinvertebrate growth and potential accumulation of contaminants. Demonstrating the success of this relatively simple habitat experiment shows promise for increasing the scale of this study in the future to be able to provide a statistical analysis on macroinvertebrate growth and metal bioconcentration and investigate what information could be captured over more insect molts or emergence. This leaf pack and habitat experiment could be expanded to other types of shredder macroinvertebrates as well to explore how contaminant accumulation may differ across taxa. Being able to better understand how anthropogenic influences on aquatic environments affect a key indicator organism and how this may influence contaminant movement across ecosystems is critical as human development continues to expand.

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APPENDIX I

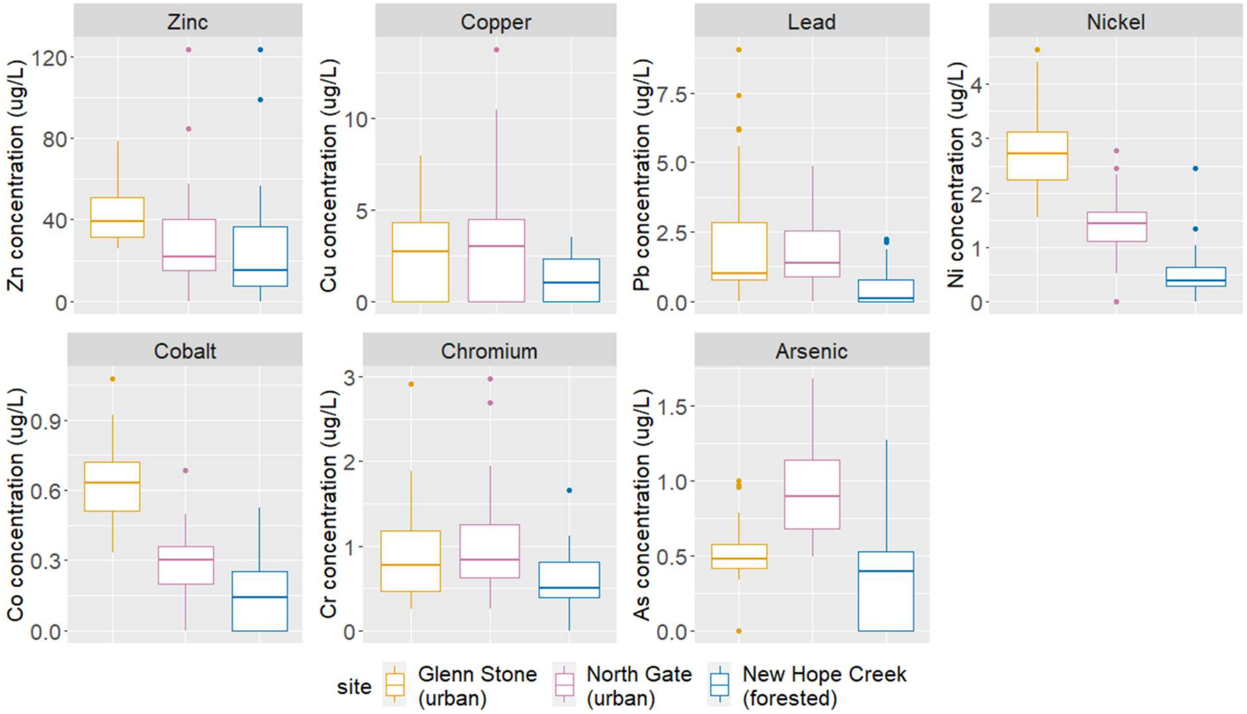

Figure Ia. Metal concentrations in water samples from the Durham stream sites collected and analyzed by Jonny Behrens during a biweekly sample study from May 2021 through May 2022.

APPENDIX II

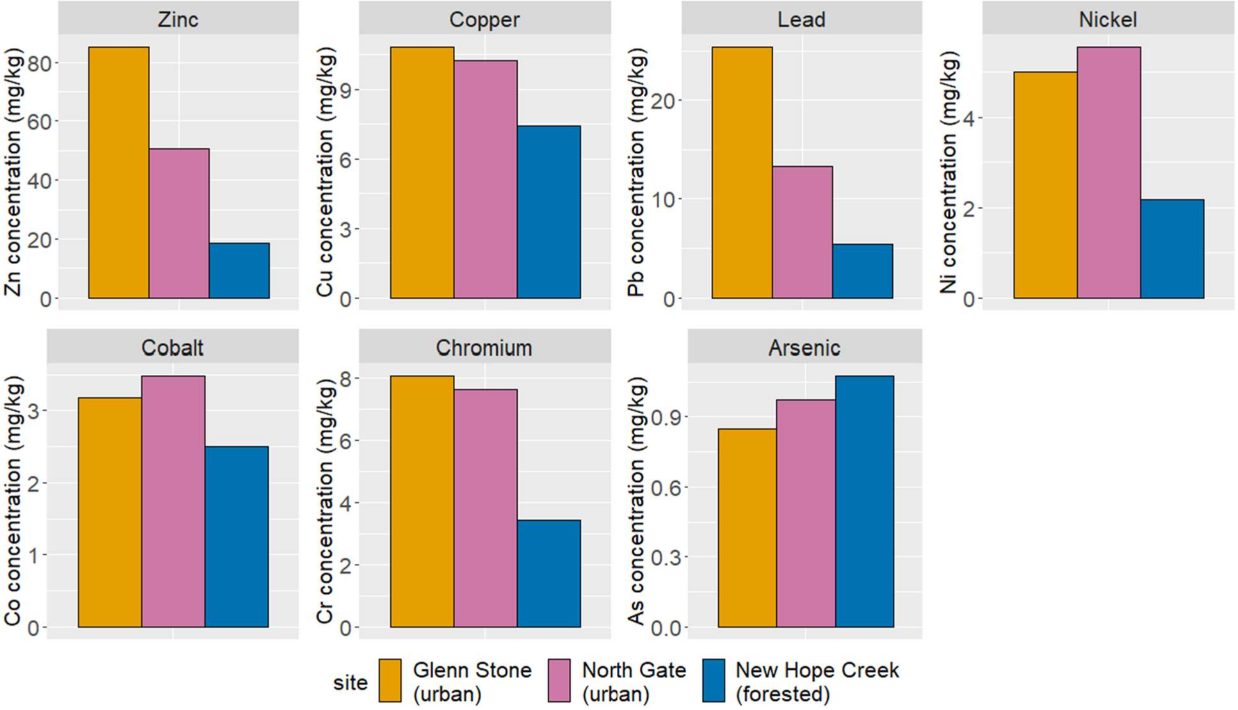

Figure IIa. Metal concentrations in leaves taken as a subsample from the Durham stream site sewn leaf packs immediately upon their collection. Leaves were dried and stored until analysis.

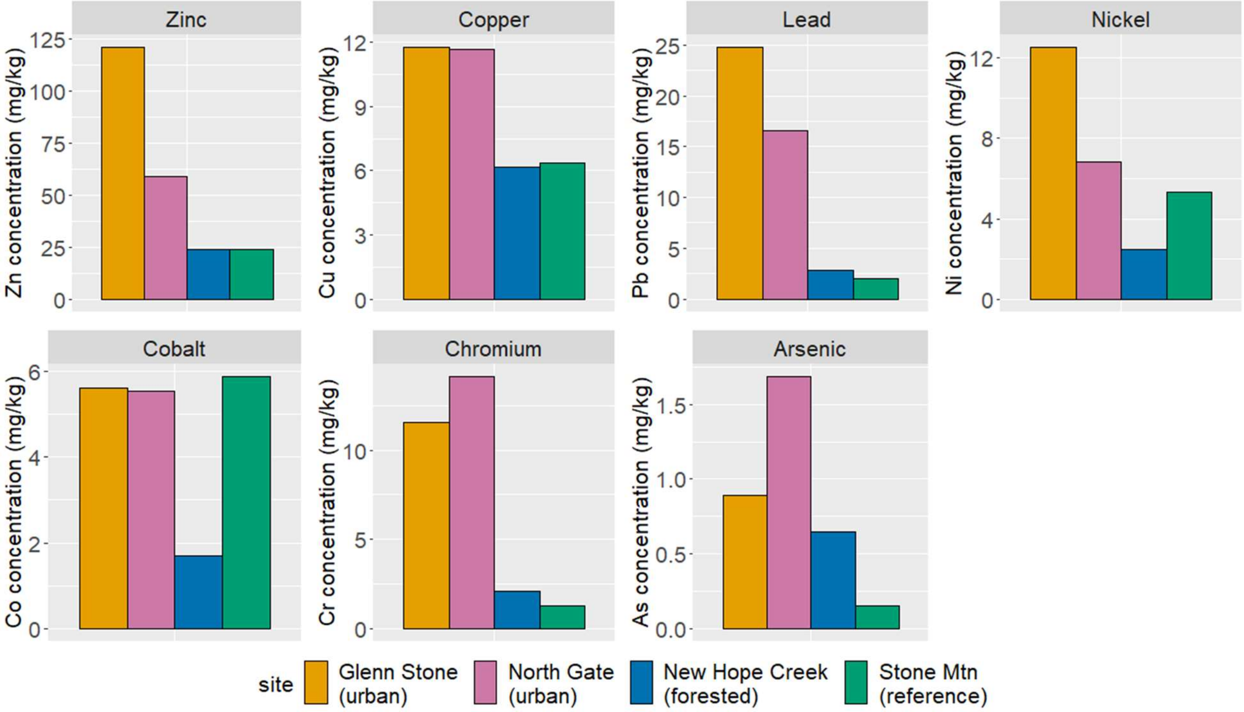

Figure IIb. Metal concentrations in leaves from leaf packs collected from the stream sites, not associated with the sewn leaf pack experiment.

APPENDIX III

Table IIIa. A comparison of forested land coverage and larvae metal concentrations between the present study and Baruch et al. (2018), which analyzed metal concentrations in tipulidae larvae (another type of shredder macroinvertebrate) collected from similar stream sites around the Durham area.

Source	Forest Land Coverage (%) range	Cu (mg/kg) range	Zn (mg/kg) range	Pb (mg/kg) range
Baruch et al. (2018)	3.13 – 78.18	22.3 – 90.0	109.5 – 340.4	2.2 – 21.6
Present study	12.20 – 73.90	49.3 – 51.1	1172.6 – 1250.3	0.33 – 1.97