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Investigating the Role of Invasive Species in the Biomagnification of Heavy Metal Toxicants

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ABSTRACT

Presque Isle State Park located in Erie, PA is both a large tourist attraction and home to a diverse community of organisms that are interdependent upon one another to survive. Given that Presque Isle is located along Lake Erie, a large body of freshwater, it is susceptible to the introduction of invasive species, one being *Cipangopaludina chinensis*. Invasive species are known to degrade the overall quality of an ecosystem by outcompeting native species for resources, leading to their endangerment or extinction, in turn reducing the biodiversity of the ecosystem. It is not entirely known, however, the role invasive species play in the spread of pollutants within an ecosystem, such as heavy metals. The objective of this study was to gain insight on how invasive species contribute to the bioaccumulation of heavy metal toxicants within an ecosystem by reviewing current toxicity data available for other aquatic snails and analyzing heavy metal concentrations in *C. chinensis*, sediment, and water samples from Presque Isle via microwave digestion and ICP-MS. Species sensitivity distributions were constructed for cadmium, lead, copper, and zinc and illustrated toxicity parameters to the various species included. Concentrations of heavy metals, such as zinc, up to 360,000 ng/g in *C. chinensis* tissues and up to 193,000 ng/g in environmental samples were detected. This suggests that *C. chinensis* can accumulate metals in high enough quantities to elicit negative or lethal effects on native species of Presque Isle. Pollution of Presque Isle with heavy metal toxicants could not only be potentially degradative to the ecosystem, but could also pose risk to the health of visitors through biomagnification. Understanding the role of invasive species in the introduction of heavy metals is imperative in understanding how to combat the spread of these toxicants and minimize the chance of harm to the surrounding ecosystem and visitors.

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ACKNOWLEDGEMENTS

I would like to begin by acknowledging Matt Zollars, Cassidy Ulanowicz, Austin Brenner, Dr. Lynne Beaty, Dr. Adam Simpson, and Dr. Samuel Nutile collectively for collecting Chinese Mystery snails in the Summer of 2019 with which I was able to validate my methods for gathering accumulation data. These samples were used to lay the groundwork for my project and without them the entirety of the project could not have been completed. I would also like to thank Dr. Beaty, Dr. Simpson, and Dr. Nutile for taking the time to collect snail samples in the Summer of 2020 while we could not be doing on campus research due to the COVID-19 pandemic. These were the samples processed to gather my data, so they were critical to the project. I would like to thank Dr. Simpson and Dr. Nutile for allowing me to take part in this project and guiding me through its beginning stages, as well as for reviewing the thesis document itself. Last but certainly not least, I would like to specifically thank Dr. Nutile for not only allowing me to take part in this project but guiding me through its entirety. Dr. Nutile has allowed me to exercise my intellectual curiosity and challenged me to think critically throughout my project, from which I have grown both as a scientist and as an individual. In addition, Dr. Nutile helped me continue to contribute to this thesis during the remote research period by suggesting I conduct the meta-analysis described below and was immensely helpful with guiding me through it even through a virtual means of communication, which is no easy feat. Dr. Nutile has demonstrated great commitment to his students to me through all of the help and guidance he has provided me with over the past two years working on this project. I am extremely grateful for the opportunity he provided and all of the lessons he has taught me throughout this project.

Chapter 1

Introduction

Ecosystems are composed of populations of various species interacting with and influencing abiotic factors within the same geographical area. These populations of species compete within themselves and with one another for resources such as territory, water, food, and mates. Through this competition, these populations are dependent on one another as they solidify their spot in the food chain. For example, primary consumers are reliant on the presence of producers as their source of energy, and secondary consumers also rely on the presence of producers so that their prey, the primary consumers, can survive in enough abundance to where food does not become scarce. This fragile interdependency can be abruptly disturbed when anthropogenically introduced stressors, such as pollution and invasive species, alter use of resources with an ecosystem, leading to a degradation in the biodiversity and overall health of the ecosystem.

Anthropogenic pollution is ubiquitous within aquatic ecosystems, but the threat of such pollution varies depending on the chemical class being considered. One example is heavy metals. While there are natural sources of heavy metals, a significant way in which these toxicants reach aquatic ecosystems is through anthropogenic practices. One of these practices includes agriculture and the use of commercial inorganic fertilizers. In particular, phosphate fertilizers contribute to the introduction of heavy metals into aquatic environments as a byproduct of their manufacturing process (Ali et. al., 2019). In general, phosphate fertilizers are produced through the acidulation of phosphate rock, which is rich in heavy metals. These metals then reach the final fertilizer product and when they are utilized in agricultural practices they can leech into the groundwater (Ali et. al., 2019).

Another route in which heavy metals are anthropogenically introduced is through the combustion of fossil fuels in industries, homes, and transportation (Fashola et. al., 2016). One such industry that is a relatively large contributor of heavy metals to the environment is the mining industry. This industry’s contribution generally comes from their waste in the form of what is termed “tailings.” Tailings are essentially a mixture of finely ground rock and water that is left behind after retrieval of the material of interest. The issue arises when a closer look is taken at the chemical composition of these tailings as they often contain high amounts of sulfides, which contribute to acidic pH, and a large amount of heavy metals. These tailings are often dumped into aquatic ecosystems due to a poor adherence to environmental regulations and policies (Fashola et. al., 2016).

Vehicle transport is a primary way in which anthropogenically derived heavy metals are introduced into aquatic ecosystems. Vehicles mainly contribute to the introduction of heavy metals into the environment through the combustion of fossil fuels and release of the resultant emissions into the environment (Gupta, 2019). Another way in which vehicles contribute to the release of heavy metal toxicants is through non-exhaust emissions such as tire and brake lining wear as well as road surface abrasion (Adamiec et. al., 2016).

Metals are of concern in many areas as these toxicants are not always metabolized and excreted, but instead accumulate in tissues of exposed organisms. Therefore, these toxicants are more likely to persist for long periods of time in the ecosystem because they are protected from physical, chemical, and biochemical breakdown due to their accumulation within tissues of living organisms. The presence of these chemicals can directly harm exposed individuals or accumulate in the predators that consume such individuals. The buildup of toxic chemicals across trophic levels within an ecosystem is known as trophic transfer and it can negatively affect the health of an ecosystem and the ways in which humans use the impacted ecosystem (Streit, 1992). The introduction of an invasive species with a high potential to accumulate toxicants could potentially exacerbate the issue of trophic transfer already present within an ecosystem.

Invasive species can be defined as any species from a foreign environment that is introduced into a new habitat, resulting in the degradation of the ecosystem. Invasive species can be introduced to a new environment in a variety of ways. One includes anthropogenic influence in which humans can introduce an invasive species to a new ecosystem, either voluntarily or involuntarily. Once introduced, invasive species will often place stress on the balance of resource availability and competition within its new environment (Lovell & Stone, 2005). The primary way invasive species contribute to this is through a decline in the abundance of the native species in the area by outcompeting said native species for resources (Lovell & Stone, 2005). This can lead to issues in the sustainability of the ecosystem in that reducing the biodiversity within ecosystems disrupts established food webs that the species within the ecosystem directly or indirectly rely on (Lovell & Stone, 2005).

Another potential mechanism in which invasive species can degrade an ecosystem's wellbeing is through changing the ways in which toxicants such as heavy metals move throughout the ecosystem by establishing a new food web. These heavy metals can be moved throughout the ecosystem through degradation of the invasive species' tissues after death or directly passed onto predators after consumption (Ward et. al., 2010). Heavy metals are known to be toxic to nearly all organisms but their magnification within ecosystems due to introduction by invasive species accumulating them is not yet understood (Tchounwou et. al., 2012). In addition, how the duality of an invasive species and heavy metal pollution intertwine to impact ecosystem stability is currently unknown.

Most ecosystems do not face single environmental stressors; instead, populations and communities are threatened by multiple stressors at the same time. Invasive species and human-induced pollution can interact to cause devastating changes within ecosystems, altering the ways in which wildlife and humans use the landscape. Invasive species can play a large role in the spread and biomagnification of the pollutants and induce harmful effects on the surrounding ecosystem. One set of species that can play a substantial role in the sequestering and eventual spread of heavy metal toxicants are snails. Snails can be more likely to be exposed to heavy metals in their environment due to their general life history. One life history factor to consider is their diet, as many aquatic snails feed on plant matter. The plant matter they ingest has the potential to be contaminated with metals and thus has the potential to be transferred to them (Chaves-Campos et. al., 2012). Another is their typical location within water bodies and selection of water bodies. Many aquatic snail species live in relatively shallow bodies of still or slow-moving water with muddy or silty bottoms, where they tend to either graze for plant material or filter feed. Heavy metals are often sequestered within the sediment of the beds of these water bodies, although they can partition out of the sediment. This partitioning is dictated by many factors, with pH and dissolved oxygen concentration being the most impactful (Li et. al., 2013). The rate at which heavy metals move from the sediment into the water can potentially influence accumulation within organisms in that environment (Li et. al., 2013). Snails also possess some aspects within their biochemical and metabolic composition that enable them to retain, accumulate, and tolerate relatively high levels of heavy metal toxicants. One such aspect is the presence of metallothioneins, which are specialized proteins that function to bind up various metal ions (Palacios et. al., 2011). Most commonly, these proteins bind metals critical to normal physiological functioning of the snail, however, snails have evolved to additionally produce metallothioneins that bind metals frequently polluting their habitat such as cadmium (Palacios et. al., 2011).

Another reason snails are of particular concern when it comes to the accumulation of toxicants, specifically heavy metals, is their ability to incorporate them into their shells (Foster and Cravo, 2002). By partitioning heavy metals into their shells (i.e., biomineralization), snails can save their organ systems from having to undergo the physiological stress of metabolizing these toxic substances and thus are able to live among the toxicants relatively unscathed since the shell is not involved in catabolic metabolism to produce energy or biomolecules for the individual (Wilber & Yonge, 1969). Though this process is advantageous for the snails, the mechanism through which it occurs has not been outlined thoroughly other than the leeching of heavy metals into the shell mantle glands responsible for shell growth (Vermeij, 2002).

Snails not only can survive reasonable amounts of heavy metal pollution but can also develop an increased resistance to certain environmental changes as a result. In a 2015 study, it was found that snails previously exposed to heavy metal pollution where able to withstand pH changes to a more acidic range significantly more than those not exposed to the metals (Lefcort et. al., 2015). Studies in the past have suggested the mechanism for this to be alterations to pedal mucus secretion (Ballance et. al., 2001). Another mechanism by which this could occur is altered epithelium membrane permeability, though the extent to which this occurs is not well understood (Sullivan & Cheng, 1975). While this does not mean heavy metals make these snails more robust overall, it is important to note that heavy metal bioaccumulation in gastropods is more likely to occur because they are able to resist the toxicants and in addition become more resistant to changing pH conditions of their environment. This bioaccumulation can then affect other members of the ecosystem by potentially exposing them to lethal amounts of heavy metals. Given that snails are generally more tolerant to heavy metals through the various mechanisms described above, their potential to accumulate the toxicants and alter how they are distributed throughout an ecosystem makes them of great concern when they are in the role of an invasive species.

First reported in Thompson Bay in 2006, Chinese mystery snails (*Cipangopaludina chinensis)* currently have a strong foothold within waters surrounding Presque Isle. Other locations within Presque Isle have also been found to contain *C. chinensis*, including the various bays and lagoons within the park (Figure 1). Females give birth to live, crawling young that live for approximately three to five years and grow to be sixty-four millimeters (Johnson et al., 2008). These snails are typically found within slow-moving water with muddy and silty bottoms including freshwater rivers, streams, and lakes (Kipp et al., 2020). Within these waters *C. chinensis* tends to filter feed, although they have been reported to feed on microalgae as well (Jokinen, 1982). Within Presque Isle State Park some ponds that house ideal conditions for *C. chinensis* are near roadways, which are likely a vector for metal exposure for these snails. By being close to the roads, the *C. chinensis* in these ponds are exposed to heavy metal toxicants through both exhaust and non-exhaust vehicular heavy metal emission, as well as through road surface abrasion via rainwater runoff into the ponds. These snails are tolerant of toxicants, such as metals, due to the ability to sequester metal ions within their shells and tissues as seen in many other snail species. With the ability to sequester ions such as cadmium, lead, and nickel within themselves, *C. chinensis* can avoid the toxic effects associated with exposure such as disruption of heart, brain, kidney, and liver function. Conversely, this accumulation has the potential for retention, spread, bioaccumulation, and biomagnification of metals throughout the ecosystem of Presque Isle as populations of *C. chinensis* continue to grow (Kipp et al., 2020).

This investigation served to learn more about the role of the invasive *C. chinensis* in the trophic transfer and retention of heavy metal toxicants. The primary focus was to gain insight to the metal concentrations the snails can accumulate in their shells and soft tissues. Given that the investigation was done in the context of Presque Isle State Park, it was critical to also gain insight to baseline heavy metal contamination in their surrounding environment, specifically the water and sediment of the locations in which they are found. While it is well understood that heavy metals are generally toxic to all organisms, including snails, the concentrations needed for these elements to exert their effects on *C. chinensis* is currently unknown. Although there is a gap in knowledge with regards to this species, other heavy metal toxicity studies have been done using other freshwater snails. Given this, a meta-analysis on heavy metal toxicity in various freshwater snails was performed in the remote research period of the Summer of 2020 to lay the foundation for future research on the toxicity of heavy metals to *C. chinensis*. The end of the meta-analysis yielded species sensitivity distributions (SSDs) for selected heavy metals. Species Sensitivity Distributions are a tool used in ecological risk assessment, most commonly to employ toxicity test results, such as lethal concentration, to a single species and extrapolate them to paint a picture of toxicity thresholds dictating ecosystem stability and functioning (Signore et. al., 2016). Lethal concentration, commonly reported as lethal concentration 50% or LC50, refers to the concentration of a substance that causes death in 50% of exposed individuals. Species Sensitivity Distributions can also be used to compare LC50 data across similar species to predict another species' LC50 value that is not currently known (Environmental Protection Agency, 2010).

Concurrently with the conduction of the meta-analysis, *C. chinensis* were collected from Presque Isle State Park and later had their soft tissues and shells sampled to determine the amounts of lead, cadmium, and other metals present within these portions of the organisms. These values were compared to concentrations measured within sediment and water samples taken during collection. Differences in accumulation between the shell and soft tissues of the snail will allow for evaluation of potential risk of metal accumulation by predators and the ability of these invasive species to spread metal ions throughout Presque Isle and the surrounding areas. By understanding the concentrations of metal contaminants within *C. chinensis* at Presque Isle State Park, more light will be shed on how invasive species degrade the health of other ecosystems through the introduction and magnification of toxicants within native species and increase the base of knowledge regarding *C. chinensis* susceptibility to heavy metal toxicants.

Figure 1. Map of Presque Isle State Park

Chapter 2

Meta-Analysis Methods

The conduction of the meta-analysis began with a review of the literature, which took place between June 2020 and August 2020. To gather raw data, the ECOTOX Database produced by the Environmental Protection Agency was used. The following search parameters were used: only heavy metals were selected for under the chemicals tab, effects were limited to mortality, endpoint was limited to 96-hour lethal concentration 50% (LC50), under species only mollusks were selected, and test conditions were limited to lab tests done in freshwater. The results were generated as a spreadsheet which included some major aspects of the studies such as species tested, grade of chemicals used, life stage of organisms tested, media in which the tests were performed, and more. The first analysis of the studies was done through the spreadsheet and the following criteria were used to determine whether the studies would receive further investigation: presence of reported life stage, reporting of exact values, and grade of chemicals used. If studies were found to not use analytical grade chemicals and/or reported inexact values, such as “<50 ng/mL”, they were excluded from the analysis. If the ECOTOX Database did not report life stage of tested organisms, the study was read to determine whether life stage was reported in the manuscript. The studies were also read to ensure they met various additional criteria to be deemed fit to be included in the analysis. These criteria included presence of confidence intervals or standard deviations in the reported LC50 values, monitoring of water quality throughout the study, use of field collected organisms, confirmation that the LC50 data were tested for a 96-hour time frame, and conduction of replicate toxicity tests. Based on these parameters each study received a score, and this score was used to determine whether the study was to be used in the analysis. If the studies included all or all but one of these criteria, they were deemed valid to be used for further analysis. One exception to this was the presence of confidence intervals or standard deviations. If the studies did not include confidence intervals or standard deviations in their results, the study was rejected. Following completion of the analysis of the studies, the data from studies fit for continuing the analysis were then used to construct the species-sensitivity distributions (SSD). The resultant plots showed the LC50 data for various species exposed to a particular heavy metal, allowing for comparison of toxicity of a particular metal ion to snail species with different life history characteristics. These were later used to predict LC50 values for the various metal ions to *C. chinensis.*

Following this, SSDs for nickel, copper, zinc, and cadmium were constructed based on the 96-hour LC50 data. The first step in the SSD construction process was the calculation of potentially affected fraction (PAF) rankings for each species for each metal ion of interest. To do this, the first step was to rank the LC50s of each species in order of increasing magnitude. Following ranking, PAF for a particular species could then be calculated by taking that species rank and subtracting 0.5 from it then dividing this number by the total number of species included on the SSD (European Centre for Ecotoxicology and Toxicology of Chemicals, 2014). The PAFs constituted the Y-axis of the SSDs. The X-axis was constructed by taking the log of each species’ 96-hour LC50. The resultant curve illustrated the species’ respective sensitivity to a particular heavy metal ion over a 96-hour period.

Chapter 3

Meta-Analysis Results and Discussion

Of the fifty studies gathered from the ECOTOX database, twenty-eight met the criteria to be included in the construction of the species sensitivity distributions (SSD). Eight studies were found to have reported LC50 data based on a 96-hour time frame, and it was these eight studies that were used to construct the SSDs (Figures 2-5).

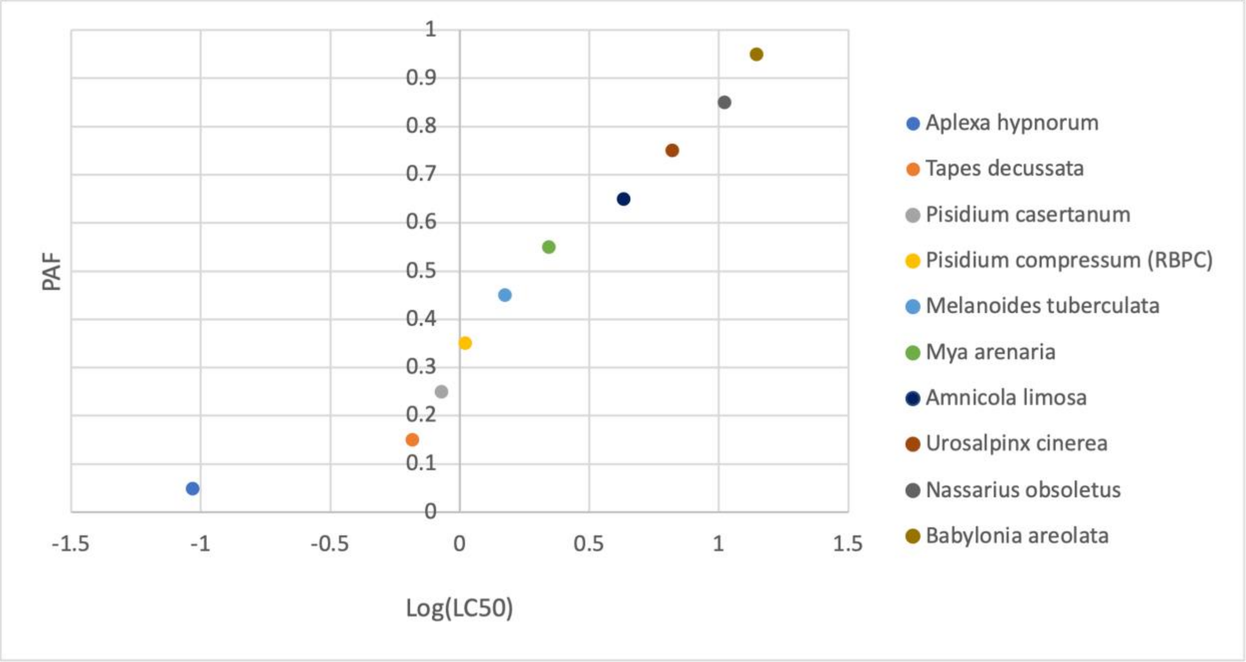

Figure 2. 96-hour SSD for Cadmium

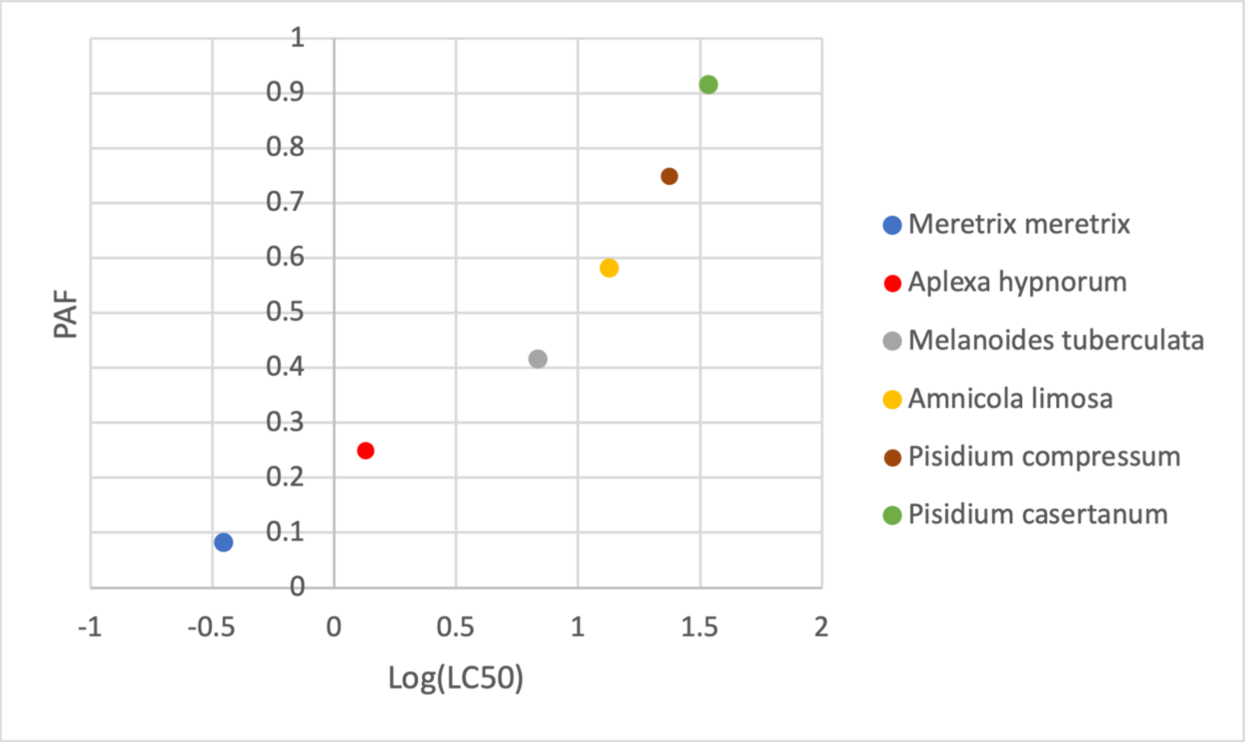

Figure 3. SSD for Lead

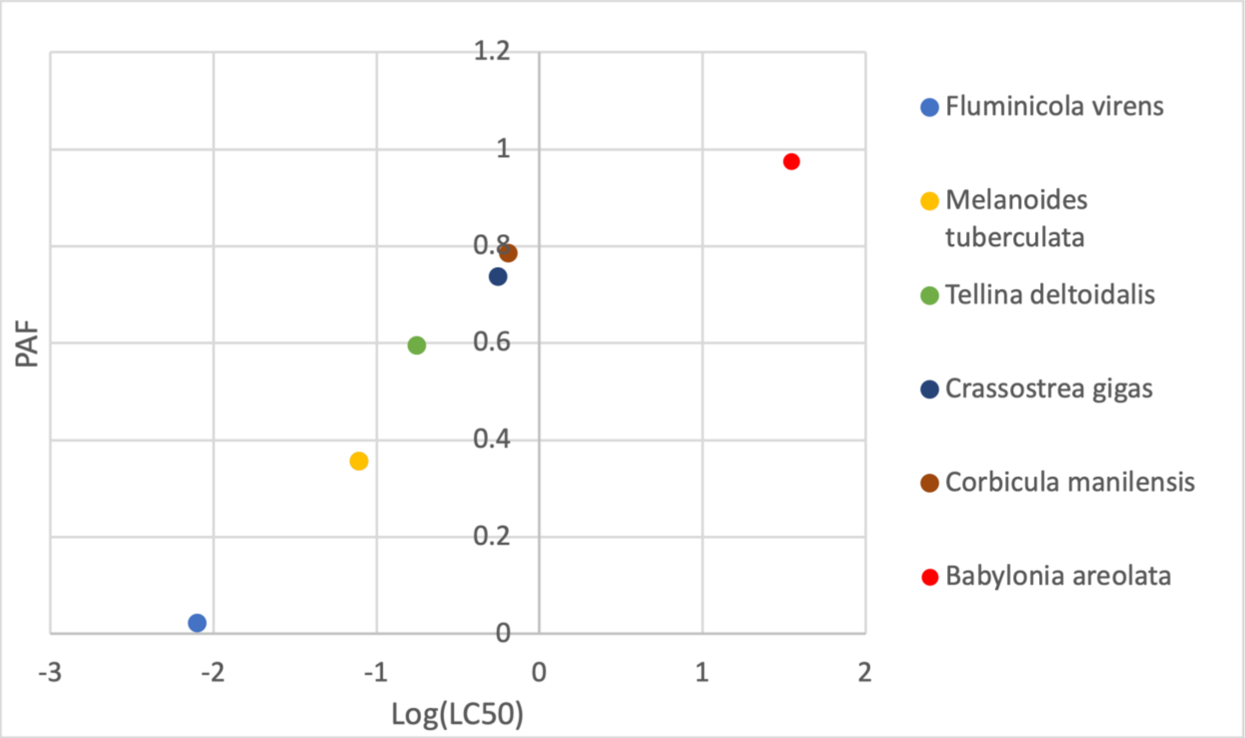

Figure 4. SSD for Copper

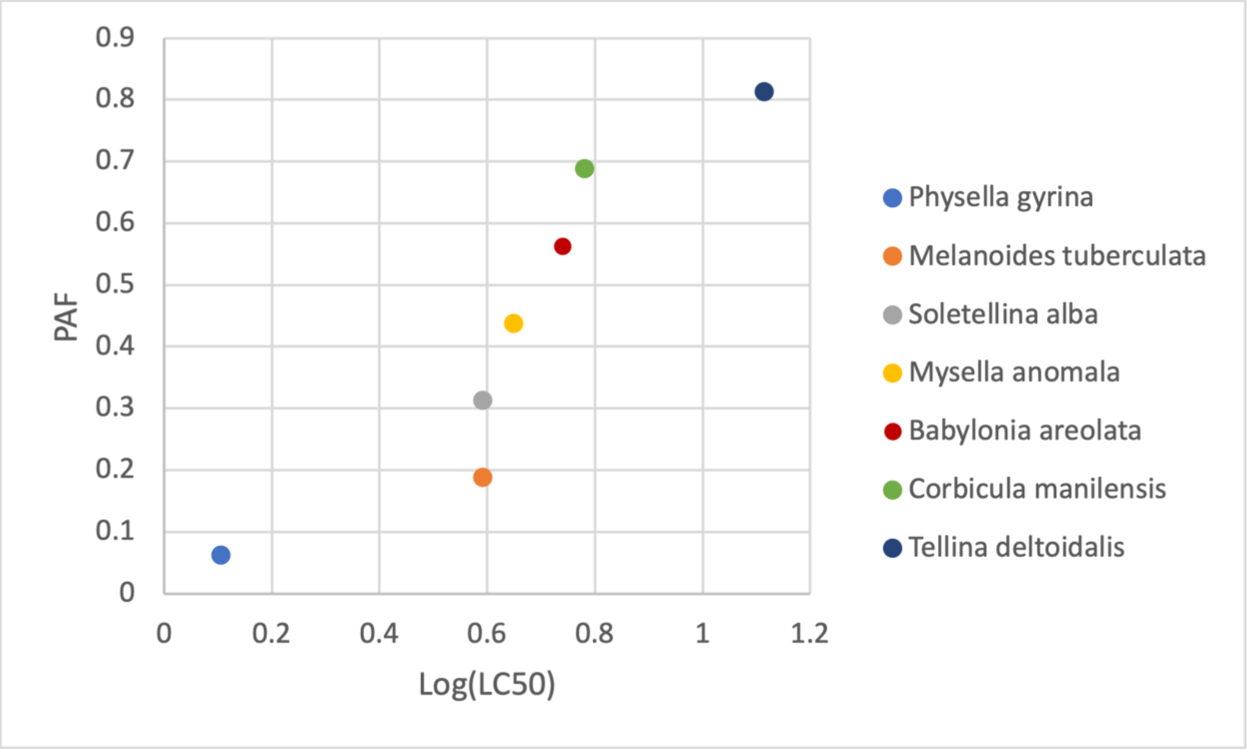

Figure 5. SSD for Zinc

The SSDs suggest that out of the metals looked at in the analysis lead exhibits the strongest toxicity to the aquatic snail species tested in the selected studies. The SSDs produced allow us to take the now compiled data and gain insight as to how life history serves to influence toxicity. Life history characteristics to consider are organism size, metabolic physiology, life stage of the organism, presence or absence of an operculum, the organism's environment, and a particular toxicant's ability to be absorbed and excreted. From this, we are also able to gain insight as to what else may be driving toxicity if two species share similar life history characteristics. In addition, from here it is possible to speculate what possible LC50 values for *C. chinensis* may be for these heavy metals.

The SSDs constructed illustrate the idea that aquatic invertebrate sensitivities to heavy metals vary widely. This point should serve as encouragement to conduct further research in this area. These SSDs could serve as a way of attempting to predict LC50 data for *C. chinensis*, who's data regarding heavy metal toxicity is currently not found in scientific literature. It is likely that *C. chinensis* LC50 values will be on the higher range of the values presented in the SSDs above due to their large size, relatively thick shells, and possession of an operculum. The prediction of potential LC50 values for these metals will allow for easier determination of actual LC50 values for the *C. chinensis* by serving as a starting point and potentially eliminating the need to scan a very wide range of concentrations. Furthermore, understanding the toxicity thresholds of heavy metal toxicants to the *C. chinensis* will aid in their management as an invasive species and help to gain insight as to how this species, and other invasive snail species, play a role in the bioaccumulation and biomagnification of heavy metal toxicants within their new ecosystems.

Chapter 4

C. Chinensis Metal Accumulation Materials and Methods

C. chinensis, sediment, and water samples were collected from Presque Isle State Park in the summer of 2020 by investigating selected areas with still or slow-moving waters with silty or muddy bottoms. Sampling occurred in the following locations: Thompson Bay, Graveyard Pond, Lily Pond, and Feather Platform. Sampling within these locations was organized into quadrats of 1 m by 1 m in size. Snail samples, along with water and sediment samples, were collected from each quadrat. Water and sediment samples were stored at in plastic containers to prevent metal leeching that can occur in glass containers. Following collection, the samples were held at 4° C until analysis.

Following collection and storage, the samples were then ready for processing. *C. chinensis* samples from all four sites were processed with the methods described below. This process began with crude dissection. Prior to dissection the snails were thawed at room temperature for ease of handling. Tools used in the dissections included scalpels, blunt metal probes, and metal spatulas. The most practical method of separation was determined to be cracking the shell by penetrating it with a blunt metal probe in one of the nooks in the curves of the shells. After penetration of the shell, the shell and tissue were separated manually. Following dissection, the shell of the snails and the tissue were homogenized manually, ensuring that data would not be skewed by localized accumulation of metals within one region of either the shell or the tissue. For the shells this was done with a blunt metal probe and for tissue samples this was done via scalpel. Dissected samples were then placed into 50 mL plastic test tubes and placed in a freezer at -20° C.

The next step in the analysis process was microwave digestion. To begin, two replicates of both shell and tissue samples of the 5 snails were weighed into 0.5 g aliquots and placed into separate microwave digester tubes. The remaining four tubes were used for blanks and matrix spikes (n=2). Each of the blank and matrix spike tubes utilized 0.5 g of chicken breast to mimic the matrix of snail tissue, and the matrix spikes were spiked with 37.5 μg of Agilent Technologies Initial Calibration Verification Standard. Following addition of snail shell and tissue samples, along with the blanks and matrix spikes, to the available 24 microwave digester tubes, trace metal grade nitric acid was added to each tube at a volume of 10 mL. The tubes were then loaded into a Mars 6^{TM} Microwave Digester (CEM Corporation, Matthews, NC, USA). Environmental Protection Agency (EPA) protocol 3051a was used for digestion of the tissue and shell samples.

Water and sediment samples were handled differently in terms of their processing prior to microwave digestion. For the water samples, 45 mL of the water was poured into the microwave digester tubes and 5 mL of trace metal grade nitric acid was added. For sediment, 0.5 g samples were added to the tubes along with 10 mL of trace metal grade nitric acid. The water samples were run in the microwave digester using EPA protocol 3015, while the sediment samples were digested using EPA protocol 3051a.

All samples were diluted to 5% nitric acid following microwave digestion. This was done with ultrapure water to avoid any ionic contamination or interferences with ions that may be present in tap water. Calibration standards were made with Agilent Technologies Initial Calibration Verification Standard at concentrations of 1, 5, 10, 25, 50, 100, 250, and 500 parts per billion (ppb). A spike check consisting of ultrapure water and 37.5 μg of Agilent Technologies Initial Calibration Verification Standard was made to ensure percent recovery from the matrix spikes was sufficient (80-120%). The samples were then loaded into the Agilent 7900 Inductively Coupled Plasma Mass Spectrometer (ICP-MS) for analysis.

Following the run on the ICP-MS the standard curves were checked to ensure that they were sufficient to give accurate, reliable data. This determination was made by seeing if the R^2 of the standard curves for each ion was equal to or greater than 0.995. Following analysis on the ICP-MS the data were exported as an Excel file for analysis. In Excel, the measurements were corrected for the dilutions made prior to being measured by the ICP-MS. These corrections included taking the values provided by the ICP-MS and back calculating to get the original concentration in the sample by accounting for the 15X dilution factor and volumes pipetted during sample preparation for the ICP-MS. The values were reported as metal mass divided by the mass of the matrix that was microwave digested.

The values, now corrected for dilution, were used to construct box-and-whisker plots. Separate plots were made for nickel, cadmium, lead, copper, and zinc. The plots were also made according to matrix, including sediment, water, tissue, and shell. Data from each of the locations sampled for a particular toxicant in each matrix were included on the same plot to allow for comparison of said data.

Chapter 5

C. chinensis Metal Accumulation Results and Discussion

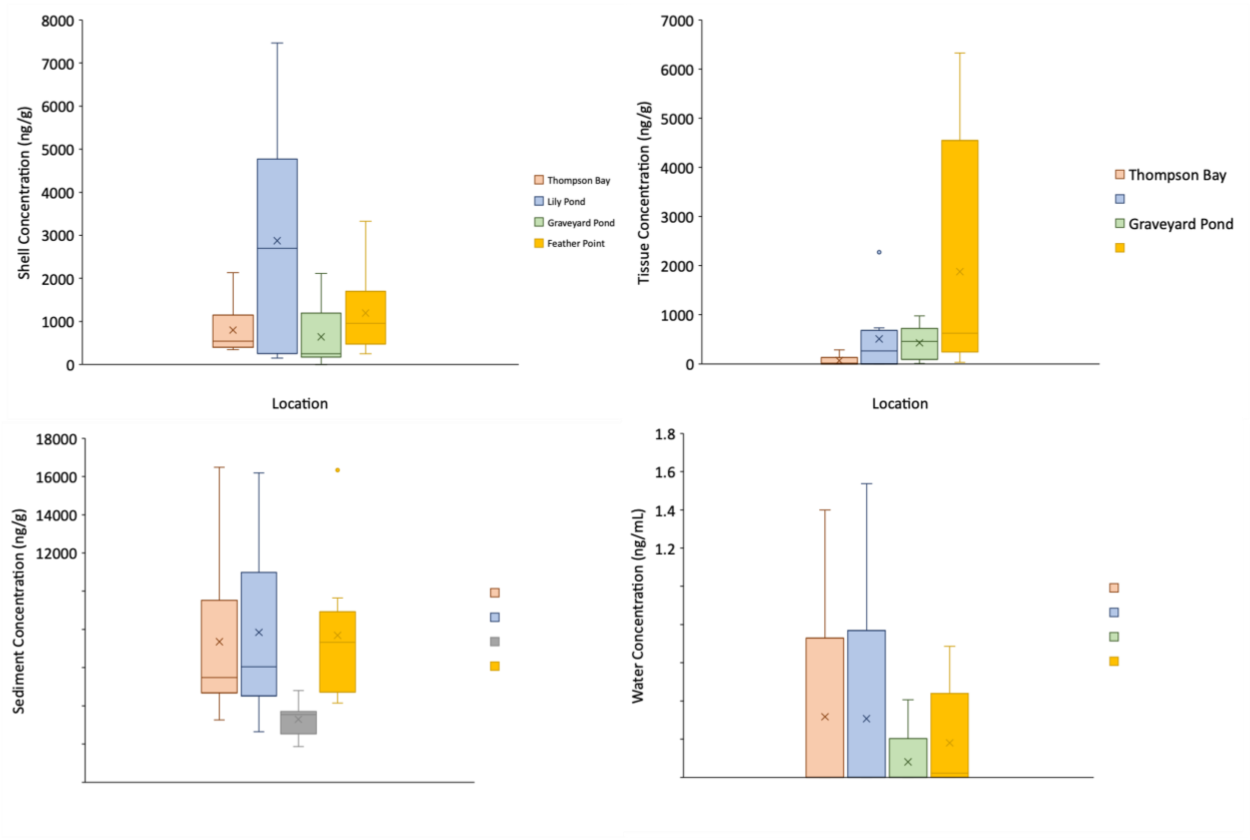

Figure 6. Concentrations of Nickel Detected in Locations and Matrices Analyzed

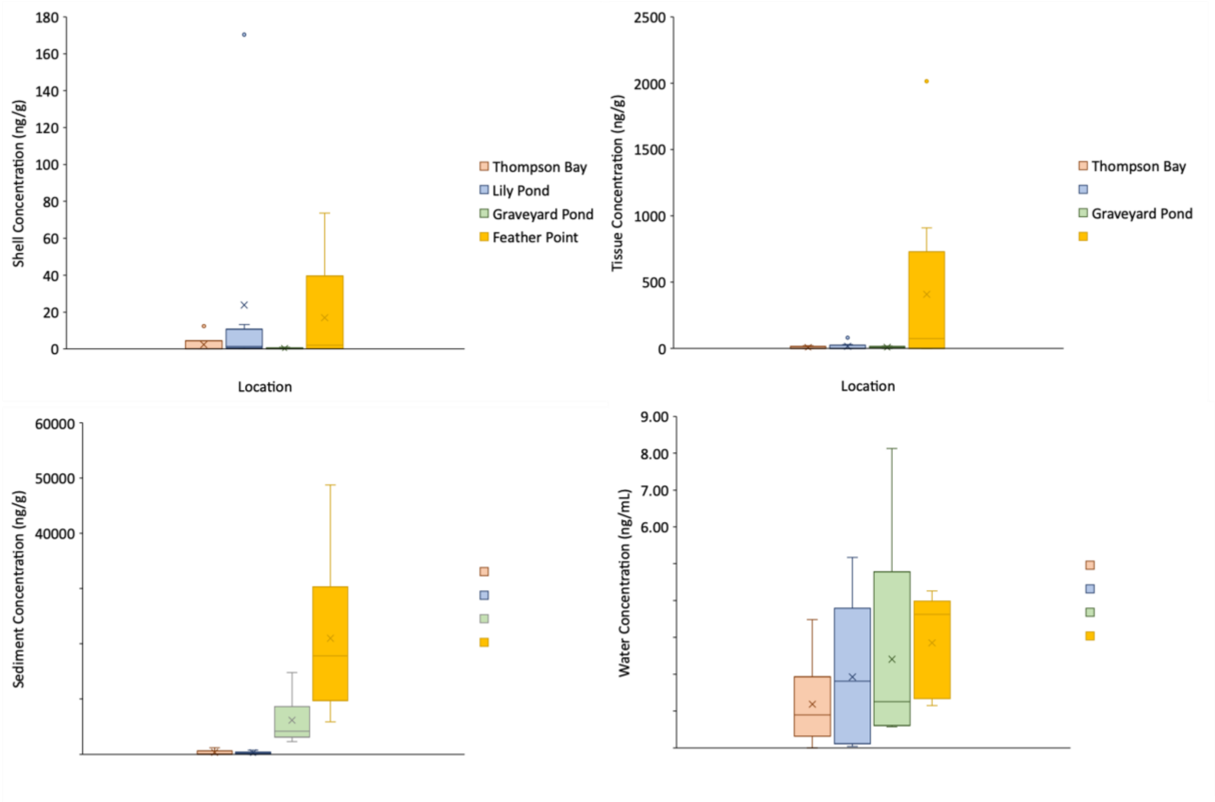

Figure 7. Concentrations of Lead Detected in Locations and Matrices Analyzed

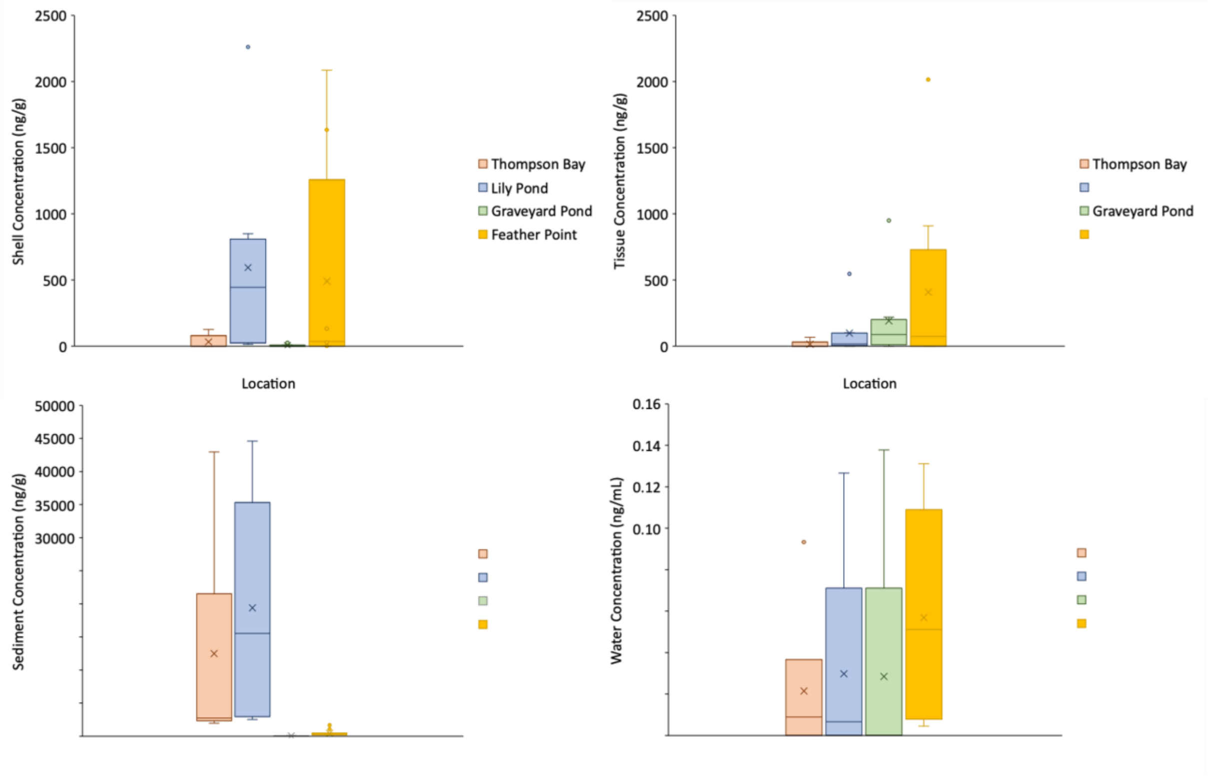

Figure 8. Concentrations of Cadmium Detected in Locations and Matrices Analyzed

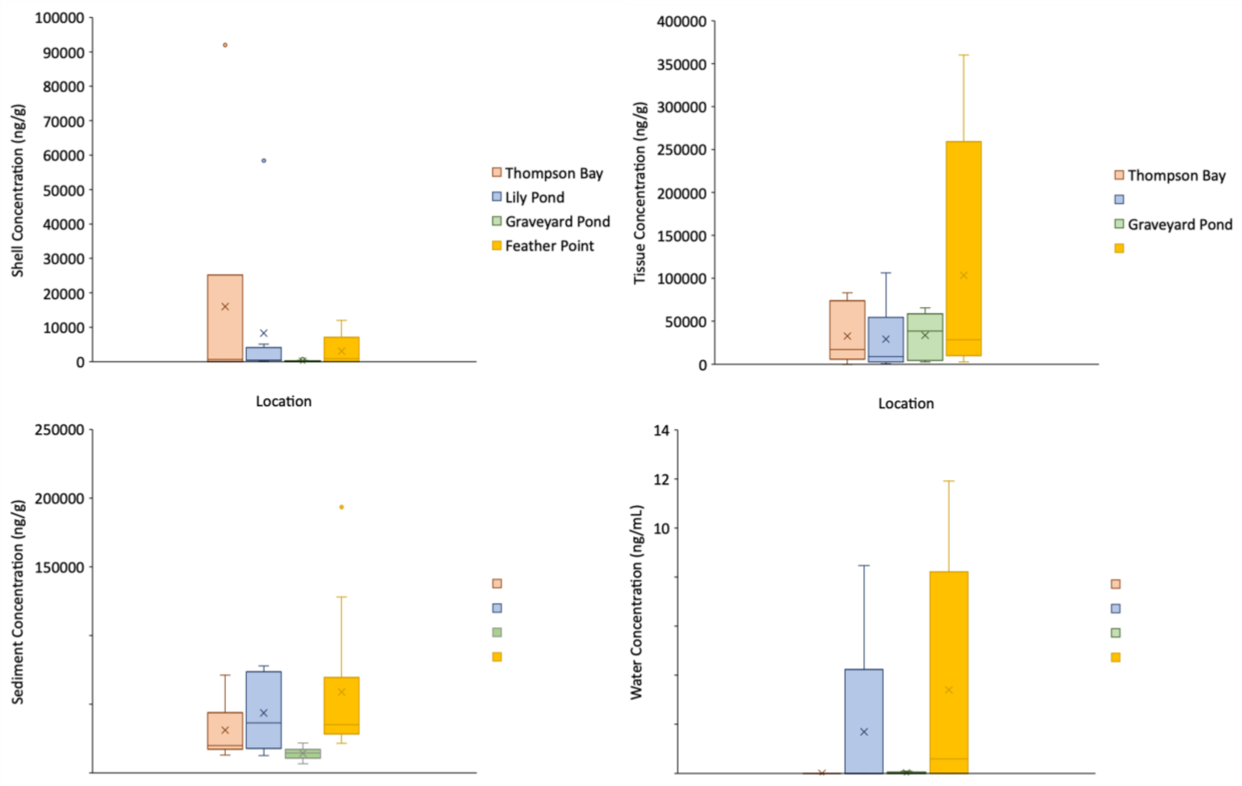

Figure 9. Concentrations of Zinc Detected in Locations and Matrices Analyzed

The highest concentrations of metals within the *C. chinensis* samples were generally seen in the shells, pointing toward a potential physiological preference to partition heavy metals into the shell rather than keeping them within the soft tissues of the organism (Figures 6-9). Metal ions of similar charge may leech into the mantle glands responsible for shell production, thus making the *C. chinensis* to move the heavy metal toxicants they are exposed to into the shell (Vermeij, 2002). A primary exception to this trend includes the snail samples collected from the Feather Point sites. In this subset of samples, higher concentrations were detected in the tissues than the shells (Figures 6-9). This observation could point to a potential genetic difference between the snails of the other locations and those of Feather Point, possibly including an increased concentration of circulating metallothioneins that could bind the metal ions before they get to the mantle gland (Palacios et. al., 2011). Localized genetic differences within the overarching population of *C. chinensis* are reasonably feasible in that an individual's mobility throughout the habitat is relatively low, which has been reported in life history studies of this species (Kipp et. al., 2020). Further study into the potential localized genetic differences of these snails could provide insight as to how to better manage these species from an invasive species control perspective.

The environmental samples showed a strong trend in that the sediment samples showed higher concentrations than the water samples for all metal ions of interest and all locations. This is likely due to heavy metals' tendency to be relatively insoluble and instead sequester into the sediment towards the bottom of water bodies. Though zinc was recorded at the highest concentration in water at Feather Point, cadmium showed a relatively strong presence in the four sites. Within the sediment samples, there was significant variation in which site showed the highest concentrations across the different metal ions evaluated (Figures 6-9). This variation could be due to proximity to roadways or other methods of anthropogenically derived introduction (Adamiec et. al., 2016).

From these data an inference can be made on how the heavy metal toxicants of interest are transferred to the *C. chinensis* within Presque Isle State Park. The data seem to suggest that the metal toxicants are transferred to the snails from the sediment as they feed or on the food they are ingesting, which is reasonable given that the amounts detected in the water are simply not high enough to correlate to the quantities of the metals detected within the snails' shells and tissues. The amount of heavy metals they partition to the shell could potentially be dictated by the amount of heavy metals they are exposed to through feeding, the particular heavy metal they are exposed to, as well as variances in metabolic physiology across individuals. For example, if a

C. chinensis ingests more cadmium than the amount of metallothioneins they have circulating can handle, it could leech into the mantle glands. One example of how the particular metal exposed to dictates concentrations in the shell comes from the specificity of the metallothioneins. If a *C. chinensis* only possesses metallothioneins capable of binding cadmium, then other metals such as lead would be able to leech into the mantle glands more freely (Stillman et. al., 1987). Further investigation should be done to determine how metallothionein concentration and specificity dictates accumulation of heavy metals into *C. chinensis*, along with other snails, shells and tissues.

Chapter 6

Conclusion

Prior to analysis of *C. chinensis* and environmental samples, a meta-analysis of current data on heavy metal toxicity to various aquatic snails was performed. This analysis showed that LC50 values vary significantly for the different metal toxicants included between snail species. The analysis also elucidated a gap in knowledge regarding heavy metal toxicity to specifically *C. chinensis*, which is an area that requires further study. It is likely that *C. chinensis* has relatively high resistance to heavy metal toxicants given their large size and possession of an operculum. The metal accumulation data showed that the Chinese mystery snails within Presque Isle State Park are in fact accumulating the heavy metal toxicants of interest. This accumulation was shown to occur to a greater extent in the shells of the snails than the soft tissues, although there were exceptions in some cases. For environmental analysis, sediment samples showed far higher concentrations of the heavy metal toxicants than the water samples. This pattern could possibly help elucidate the mechanisms by which the metal ions are transferred to the snails and how they are able to tolerate the concentrations reported. Further research into the mechanism of transfer and toxicity thresholds of heavy metal toxicants to *C. chinensis* could produce more insight as to how to effectively manage this invasive species to minimize the potential harm to the Presque Isle State Park ecosystem.

BIBLIOGRAPHY

Adamiec, E., Jarosz-Krzemińska, E., & Wieszała, R. (2016, June). Heavy metals from non-exhaust vehicle emissions in urban and motorway road dusts. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4880625/

Ali, H., Khan, E., & Ilahi, I. (2019, March 05). Environmental chemistry and Ecotoxicology of Hazardous Heavy metals: ENVIRONMENTAL Persistence, toxicity, and Bioaccumulation. Retrieved from https://www.hindawi.com/journals/jchem/2019/6730305/

Ballance, S., Phillips, P., McCrohan, C., Powell, J., Jugdaohsingh, R., White, K., . . . K N WhiteView all articles by this author. (2001, September 01). Influence of sediment biofilm on the behaviour of aluminum and ITS BIOAVAILABILITY to the SNAIL LYMNAEA STAGNALIS in NEUTRAL FRESHWATER. Retrieved from https://cdnsciencepub.com/doi/10.1139/f01-104

Chaves-Campos, J., Coghill, L., García de León, F., & Johnson, S. (2012, September). The effect of aquatic plant abundance on shell crushing resistance in a freshwater snail. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3435308/

Environmental Protection Agency. (2010, January). Web-based Interspecies Correlation Estimation (Web-ICE) for Acute Toxicity: User Manual. Retrieved from https://www.epa.gov/sites/production/files/documents/WebICE_User_manual.pdf

European Centre for Ecotoxicology and Toxicology of Chemicals. (2014, February). *Estimating toxicity thresholds for aquatic ecological communities from sensitivity distributions 11-13 February 2014, Amsterdam* [PDF]. ECETOC.

Fashola, M., Ngole-Jeme, V., & Babalola, O. (2016, October 26). Heavy metal pollution from gold Mines: Environmental effects and BACTERIAL strategies for resistance. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5129257/

Foster, P., & Cravo, A. (2002, October). MINOR ELEMENTS AND TRACE METALS IN THE SHELL OF MARINE GASTROPODS FROM A SHORE IN TROPICAL EAST AFRICA. Retrieved from https://link.springer.com/content/pdf/10.1023/A:1023631022989.pdf

Gupta, V. (2019, July 4). Vehicle-Generated Heavy Metal Pollution in an Urban Environment and Its Distribution into Various Environmental Components. Retrieved from https://link.springer.com/chapter/10.1007/978-981-13-5889-0_5

Johnson, P. T., Olden, J. D., Solomon, C. T., & Vander Zanden, M. J. (2008). Interactions among invaders: Community and ecosystem effects of multiple invasive species in an experimental aquatic system. *Oecologia, 159*(1), 161-170. doi:10.1007/s00442-008-1176-x

Jokinen, E. (1982). Cipangopaludina chinensis (Gastropoda, Viviparidae) in North America; review and update. *Natilus,96*.

Kipp, R., Benson, A., Larson, J., Fusaro, A., & Morningstar, C. (2020, January 15). Cipangopaludina chinensis. Retrieved from https://nas.er.usgs.gov/queries/factsheet.aspx?SpeciesID=1044

Lefcort, H., Cleary, D., Marble, A., Phillips, M., Stoddard, T., Tuthill, L., & Winslow, J. (2015, June 17). Snails from heavy-metal polluted environments have reduced sensitivity to carbon dioxide-induced acidity. Retrieved from https://springerplus.springeropen.com/articles/10.1186/s40064-015-1073-9

Li, H., Shi, A., Li, M., & Zhang, X. (2013, December 02). Effect of ph, temperature, dissolved oxygen, and flow rate of overlying water on heavy metals release from storm sewer sediments. Retrieved from https://www.hindawi.com/journals/jchem/2013/434012/

Lovell, S., & Stone, S. (2005, January). *The Economic Impacts of Aquatic Invasive Species: A Review of the Literatur*[PDF]. National Center for Environmental Economics.

Mackie, G. (1989). Tolerances of five benthic invertebrates to hydrogen ions and metals (Cd, Pb, Al). Retrieved April 04, 2021, from https://link.springer.com/article/10.1007/BF01056206

Nebeker, A., Stinchfield, A., Savonen, C., & Chapman, G. (2009, October 20). Effects of copper, nickel and zinc on three species of oregon freshwater snails. Retrieved April 04, 2021, from https://setac.onlinelibrary.wiley.com/doi/abs/10.1002/etc.5620050905

Palacios, O., Pagani, A., Peacuterez-Rafael, S., Egg, M., Höckner, M., Brandsaumlatter, A., . . . Dallinger, R. (2011, January 21). Shaping mechanisms of metal specificity in a family of metazoan metallothioneins: Evolutionary differentiation of mollusc metallothioneins. Retrieved from https://bmcbiol.biomedcentral.com/articles/10.1186/1741-7007-9-4

Shuhaimi-Othman, M., Nur-Amalina, R., & Nadzifah, Y. (2012, April 24). Toxicity of metals to a Freshwater SNAIL, melanoides tuberculata. Retrieved April 04, 2021, from https://www.hindawi.com/journals/tswj/2012/125785/

Signore, A., Hendriks, A., Lenders, H., Leuven, R., & Breure, A. (2016). Development and application of the Ssd approach in scientific case studies for ecological risk assessment. Retrieved from https://pubmed.ncbi.nlm.nih.gov/27144499/

Stillman, M., Cai, W., & Zelazowski, A. (1987, April). Cadmium binding to metallothioneins. domain specificity in reactions of alpha and beta fragments, apometallothionein, and zinc metallothionein with cd2+. Retrieved from https://pubmed.ncbi.nlm.nih.gov/3558354/

Streit, B. (1992). Bioaccumulation processes in ecosystems. Retrieved April, from https://link.springer.com/article/10.1007/BF01919142

Sullivan, J., & Cheng, T. (1975, December 16). HEAVY METAL TOXICITY TO BIOMPHALARIA GLABRATA (MOLLUSCA: PULMONATA). Retrieved from https://nyaspubs.onlinelibrary.wiley.com/doi/abs/10.1111/j.1749-6632.1975.tb35121.x

Tchounwou, P., Yedjou, C., Patlolla, A., & Sutton, D. (2012). Heavy metal toxicity and the environment. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4144270/

Vedamanikam, V., & Hajimad, T. (2013, December). Temperature effects on the toxicity of four trace metals to adult SPOTTED babylonia SNAILS (Babylonia areolata). Retrieved from https://www.tandfonline.com/doi/abs/10.1080/02772248.2013.864450

Vedamanikam, V., & Hayimad, T. (2014, January). Effect of mixtures of metals on the spotted Babylon snail (Babylonia areolata) under different temperature conditions. Retrieved April 04, 2021, from https://www.tandfonline.com/doi/abs/10.1080/02772248.2014.881077

Vermeij, G. (2002, March). Characters in context: Molluscan shells and the forces that mold them. Retrieved from https://bioone.org/journals/Paleobiology/volume-28/issue-1/0094-8373(2002)028%3C0041:CICMSA%3E2.0.CO;2/Characters-in-context--molluscan-shells-and-the-forces-that/10.1666/0094-8373(2002)028%3C0041:CICMSA%3E2.0.CO;2.short?tab=ArticleLink

Ward, D., Nislow, K., & Folt, C. (2010, May). Bioaccumulation syndrome: Identifying factors that make some stream food webs prone to elevated mercury bioaccumulation. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2977981/

Wilbur, K. M., & Yonge, C. M. (1969). *Physiology of Mollusca* (Vol. 2). New York: Academic Press.

ACADEMIC VITA

Education

Penn State Behrend *Aug 2017 to May 2021*

- Pursuing a Bachelor of Science degree in biology with a focus on health professions

Corry Area High School *Aug 2013 to June 2017*

- Honors Student

Work Experience.

Family Video *Jan 2017 to June 2018*

- Assistant Manager
- Composed schedules along with payroll packets with biweekly revenue and expense reports
- Gained extensive experience in customer service and interacting with customers to meet their needs

Penn State Behrend Learning Resource Center *Aug 2018 to Present*

- Lead tutor for sixteen courses primarily consisting of biology, chemistry, and psychology
- Extensive experience in one on one tutoring
- Responsible for organizing and conducting group tutoring sessions
- Assist in training new tutors how to conduct one on one and group tutoring sessions
- Office assistant aiding in the organization of scheduling software as well as ensuring students are able to obtain the help they need in a convenient and timely manner

Saint Vincent's Hospital *July 2019 to Present*

- Central Processing Technician
- Follow safety protocol precisely in order to sterilize biohazardous surgical equipment
- Utilize sharp attention to detail in order to properly assemble instrument trays prior to sterilization
- Ensure surgical equipment is working properly prior to use within the operating room

Penn State Behrend *May 2020 to Aug 2020*

- Research Student
- Conducted a meta-analysis of heavy metal toxicity to various aquatic invertebrates
- Scanned scientific literature to seek well-constructed studies
- Reorganized large amounts of data gathered from various databases

Extracurricular Activities

- Scrubs Club, Member
- Beta Beta Beta: Biology Honors Society, Member
- Science Ambassadors, Member