ABSTRACT

MAYS, JASON WARREN. Bioaccumulation of Platinum Group Metals in the Freshwater Mussel *Elliptio complanata*. (Under the direction of Thomas J. Kwak and W. Gregory Cope).

The use of catalytic converters for automobile exhaust purification has led to emission and environmental contamination by the platinum group metals (PGM) platinum (Pt), palladium (Pd), and rhodium (Rh). In this study, a total of 37 sites were sampled throughout central North Carolina and were chosen based on availability of the freshwater mussel *Elliptio complanata*, varied geographic distribution, land use patterns, and vehicle traffic density. At each site, a sample of sediment and three adult *E. complanata* were collected from within 50-150 m both upstream and downstream of the road crossing. Mussel tissue and sediment samples were analyzed for concentrations of Pt, cadmium (Cd) and mercury (Hg). Related stream and local variables investigated included sediment total organic carbon, water chemistry, and estimated vehicle traffic density at the bridge. Landscape variables included human population density, land use, and density of transportation infrastructure. Pt concentrations in mussel tissue ranged 0.09-1.98 ng/g dry weight and 0.06-1.86 ng/g dry weight in sediment among sites. A biota sediment accumulation factor for Pt, calculated as the mean [tissue]/[soil], was 3.2, compared to 87 and 88 for Hg and Cd, respectively. Pt contamination of mussels and sediment at highway crossing sites were not significantly correlated with the amount of traffic crossing the specific structure. Rather, multiple regression modeling indicated a significant relation between Pt concentration in mussels at a site and the human population of the watershed.

A 28-d laboratory test was conducted with waterborne Pt and Pd to determine toxicity, bioaccumulation, and to assess several potential biomarkers of exposure to Pt and Pd. Test mussels were exposed to five concentrations of an equal mixture of Pt and Pd salts, ranging from 0.05 to 500 µg/L of each metal, in a static renewal test. The 500 µg/L concentration resulted in high mortality (4 of 9 dead by day 12) of test mussels; all individuals in each of the other test concentrations survived to the end of the test. There were nine replicate mussels per treatment concentration, allowing three mussels from each treatment to be sampled on days 7, 14 and 28. Tissue and hemolymph were assessed for concentrations of Pt, Pd, sodium (Na^+), calcium (Ca^{2+}), potassium (K^+), and chloride (Cl^-) concentrations. Na^+,K^+-ATPase activity was assessed in gill tissue as a potential biomarker of exposure. Tissue concentrations of Pt ranged from 0.10 ng/g dry weight in controls to 34,486 ng/g dry weight in the 500 µg/L treatment, and Pd ranged from 0.06 ng/g dry weight in controls to 34,404 ng/g dry weight in the 500 µg/L treatment. Concentrations of Pt in hemolymph ranged from 0.08 ng/mL in controls to 50.1 ng/mL in the 500 µg/L treatment, and Pd ranged from 0.06 ng/mL in controls to 12.2 ng/mL in the 500 µg/L treatment. On day 28, Na^+,K^+-ATPase activity displayed a logarithmic trend ($y=0.489\ln(x)+2.054$; $R^2=0.73$) of increasing activity with increasing PGM exposure concentration; however, activity was only significantly increased ($P<0.05$) at 5.0 µg/L and 50 µg/L concentrations. High variation and weak correlation of Na^+,K^+-ATPase activity with Pt and Pd exposure concentration indicate that it may not be a suitable biomarker of PGM exposure. Hemolymph Ca^{2+} concentrations were increased on day 7 at the 50 µg/L concentration. Hemolymph Na^+ levels were decreased on day 28 at the 5.0 µg/L and 50 µg/L concentrations. Cl^- and K^+ levels were

decreased at the 50 µg/L concentration. Tissue Pt and Pd concentrations in mussels exposed to the lowest test concentration (0.05 µg/L) displayed tissue concentrations of Pt that were approximately six times greater than the maximum tissue concentrations measured in stream-sampled mussels. Hemolymph ion measurement does not appear to be sensitive enough to serve as a biomarker of PGM exposure at environmentally relevant exposure concentrations.

Results from this study will provide resource managers with information on this emerging group of contaminants needed to perform risk assessments for transportation impacts to natural systems and to develop conservation, protection, and mitigation plans for this critically imperiled faunal group.

Bioaccumulation of Platinum Group Metals in the Freshwater Mussel *Elliptio Complanata*

by
Jason Warren Mays

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APPROVED BY:

Thomas J. Kwak Committee Co-Chair	W. Gregory Cope Committee Co-Chair

Damian Shea

DEDICATION

To my wife Megan Elizabeth Mays, who encouraged me to continue with my education.

BIOGRAPHY

Jason W. Mays was born October 30, 1979, in western North Carolina. An avid outdoor enthusiast and nature lover, he graduated from University of North Carolina Chapel Hill in 2002 with a degree in Biology. Jason has worked for the North Carolina Wildlife Resources Commission and the North Carolina Department of Transportation as an aquatic resource specialist and has a particular interest in the conservation and management of rare species. Since fall 2005, he has worked part time on a Master's degree and enjoys spending time with his family. He has a daughter, Avery, and a wife, Megan.

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INTRODUCTION

The introduction of catalytic converters into automobiles in the United States in the mid 1970s and in Europe in the 1980s coincided with new laws intended to reduce air pollution. However, the use of catalytic converters for automobile exhaust purification has led to the emission and environmental contamination by the platinum group metals (PGM) platinum (Pt), palladium (Pd), and rhodium (Rh). The PGM are the active components in catalytic converters that help to reduce emissions of hydrocarbons, carbon monoxide, and nitrogen oxides. Consequently, concentrations of PGM are increasing in dust and soils along highways and are being transported into aquatic habitats through surface runoff, where they are accumulating in the sediments of streams and in the tissues of aquatic organisms (Sures et al. 2001; Zimmermann et al. 2002, 2005). Due to this phenomenon, PGM are an emerging class of contaminants with potential human and environmental health implications, due to suspected mutagenic and carcinogenic activity (Ravindra et al. 2004). Also, because the influx of PGM to the environment can be almost entirely attributed to their use in automobile catalytic converters, they have potential to serve as excellent indicators of highway and transportation impacts to aquatic and terrestrial ecosystems.

Freshwater mussels have unique life history characteristics that make them especially sensitive to the impact of anthropogenic influence in the aquatic environment. Mussels have a unique, complex multistage life history. Each of the stages has differential susceptibility to disruption, with the early life stages (glochidia, juvenile) being particularly sensitive (Cope et al. 2008). Mussels have long lives compared to

other invertebrates, some greater than 100 years and most not reaching sexual maturity until 5-10 years (McMahon and Bogan 2001). Mussels are filter and deposit feeders and are primarily sessile throughout their adult life. These traits make them highly vulnerable to changes in habitat suitability and unable to temporarily flee, as other more mobile species may. Mussels throughout North America are in severe decline (Williams et al. 1993) and in need of protection from anthropogenic influences, such as transportation related impacts to aquatic systems by PGM.

Chemical Properties of Platinum Group Metals

Platinum group metals are a family of similar transition metals belonging to Group VIII of the periodic table. PGM include the elements ruthenium (Ru), rhodium (Rh), palladium (Pd), osmium (Os), iridium (Ir) and platinum (Pt). They are rare in the earth's crust, and ores containing PGM are only found in a small number of places. The most productive mines are found in Russia and South Africa, with smaller deposits being exploited in Montana (USA), Canada, Zimbabwe, and Australia (Mineral Information Institute 2000).

PGM have outstanding catalytic properties, high resistance to wear and tarnish, resistance to chemical attack, excellent high temperature characteristics, and stable electrical properties. These physical and chemical properties make them especially useful in the industrial production of chemicals by catalysis and in the electronics industry as conductive components. Other uses include alloys of PGM used in dental prosthetics and jewelry (USGS 2007). Platinum is also used in various pharmaceuticals,

particularly in anti-cancer drugs. However, automobile catalytic converters represent the largest use of PGM (Ravindra et al. 2004).

Use in Automobiles

In response to the 1970 Clean Air Act, catalytic converters containing PGM began to be added to the exhaust system of automobiles to catalyze the combustion of fuels and reduce the concentrations of nitric oxides, carbon monoxide and residual hydrocarbons present in automobile exhaust. Beginning in the mid-1970s catalytic converters were added to all new automobiles sold in the United States and Japan. Catalytic converters were made mandatory for all new European Union vehicles in 1993 (Sutherland 2003). Since their introduction, the concentration of PGM in the roadside environment has increased from background levels that were among the lowest of any element in the earth's crust (0.4 ng/g Pt and Pd, 0.06 ng/g Rh, Whedepol 1995) to concentrations of Pt in urban tunnel dust as high as 730 ng/g (Helmers and Mergel 1998). The global emission of transportation derived Pt to the environment has been estimated between 0.8 and 6.0 metric tons per year, assuming 500 million catalyst equipped vehicles with an average mileage of 15,000 km/yr (Rauch et al. 2005).

Autocatalysts represent a large fraction of the world wide demand for PGM. In 2000, Pt, Pd and Rh used in auto catalysts accounted for 31%, 61% and 99%, respectively, of worldwide demand (Ravindra et al. 2004). Due to the high cost of these metals—$39,324, $7,342, and $39,712 U.S. per kg of Pt, Pd and Rh, respectively, as of March 23, 2009 (Johnson Matthey)—they tend to be highly conserved and recycled in

their other uses (Tuit et al. 2000). Another source of Pt to the environment is its use in anti-cancer drugs, primarily cisplatin and carboplatin. These drugs are a component of hospital effluent and are estimated to account for 3.3-12.3% of Pt in municipal sewage, based on a study of five European hospitals (Kummerer et al. 1999). The majority of PGM found in the environment is directly attributable to automobile emission (Tuit et al. 2000).

Emission

The emission of Pt from automobile catalytic converters can be mainly attributed to thermal sintering, evaporation and mechanical erosion (Palacios 2000). Emission rates found in the literature have a wide range. Rates of speed, engine size, catalyst type, and the age of the catalyst appear to play a major role in the emission rates (Ravindra et al. 2003). There is high variation in emission rates reported in the literature from studies that used various methods of direct determination by exhaust collection under laboratory conditions. These reported rates range 2-1,900 ng/km. Emission rates estimated from lab tests are often conducted under the best conditions, with well maintained engines operating at a small number of operating speeds. Methods of indirect determination, those that assessed PGM in the roadside environment or in organisms, generally reported a greater mean emission rate (Palacios 2000). Emission rates of vehicles under real world driving conditions are likely to emit larger amounts of PGM. A synthesis of results from several approaches including bench testing and data collected from roadside soils and grasses indicate a Pt emission rate between 500 and 800 ng/km (Helmers 1997).

Emission of PGM from automobile exhaust is primarily in particulate form in the (0) oxidation state or as oxides (Moldovan et al. 2002). The size of the emitted particles fall primarily into the (>10 μm) class, representing 62-67% of particulate emission. Particles in the (3.1-10 μm) and (<3.1 μm) represent ~21% and ~13%, respectively (Ravindra et al. 2004). Direct methods of emissions testing have estimated the soluble fraction of platinum at 1% (Artlet et al. 2000) and 10% (Konig el al. 1992). Moldovan et al. (2000) confirmed that the soluble fraction of Pt in both diesel and gasoline exhaust in most cases represented less than 10% of the total emission; however, the soluble fraction of Pd and Rh were significantly greater, ranging between 20% and 40%.

The emission of PGM from automobiles has caused significant increases in the concentrations found in road-deposited sediment and roadside soils (Cicchella et al 2003, Sutherland 2003, Lesniewska et al. 2004, Zereini et al. 2007). PGM is also being transported to aquatic systems by runoff and waste water discharges and can be found in sediment (Laschka et al. 1996, Tuit et al. 2000, Rauch et al. 2005, Turner et al. 2006). PGM is detectible in urban air as particulate matter of widely varying size (Gomez et al. 2002) and can be transported long distances by the wind. Ice cores taken from the Greenland ice sheet indicate global anthropogenic deposition of these metals, but this likely represents significant contributions from Russian smelters (Rauch et al. 2005).

Bioavailability and Toxicity

The bioavailability of PGM is primarily determined by the solubility of the metals under environmentally relevant conditions (Sutherland 2003). Zerenini et al. (1997)

tested the solubility of catalyst material in direct contact with soil of varying pH, salt, and sulfur concentrations. They found that metallic PGM particles are largely immobile over short periods of time (Zereini et al. 1997). However, it has been found that organic acid solutions can mobilize metallic PGM over an experimental period of one year. These conditions are comparable to roadside drainage ditches, where decaying vegetation and automobile emitted catalyst are deposited (Bowles and Gize 2005). Additionally, siderophores (organic ligands produced by bacteria, fungi and plants) that increase the bioavailability of iron in the soil, are also effective at increasing the solubility of PGM (Dahlheimer et al. 2007).

Bioaccumulation has been demonstrated in a variety of different taxa. Plants grown on soil treated with soluble forms of PGM may accumulate the metals, especially Pd (Ek et al. 2004). A variety of species of roadside plants have been shown to accumulate PGM as well as other traffic related metals (Schafer et al. 1998, Djingova et al. 2003, Lesniewska et al. 2004). The sulfate-reducing bacterium, *Desulfovibrio desulfuricans*, has been shown to accumulate PGM. This bacterium is similar to bacteria naturally found in decaying detritus in aquatic systems (Yong et al. 2002). Aquatic species, European eels, *Anguilla anguilla*, and zebra mussel, *Dreissena polymorpha*, have been shown to bioaccumulate PGM when exposed to road dust under laboratory conditions (Sures et al. 2001, Zimmermann et al. 2002).

Wolterbeek and Verburg (2001) ranked 80 metals by toxicity based on the average relative toxicity from 30 literature data sets using various species and toxic endpoints. In their analysis, PGM species Pt(II), Pt(IV), Rh(I), Rh(III), and Pd(II) ranked

10th, 11th, 20th, 25th and 28th, respectively. In this ranking, the toxicity of Pt was similar to lead, Pb(IV).

PGM causes phytotoxicity and chlorosis in plants at high concentrations, and Pt complex compounds are mutagenic in bacteria (Gebel et al. 1997). PGM are known to cause allergic reactions in humans, causing asthma, conjunctivitis, dermatitis, rhinitis, and uticaria. Pt is used in cancer drugs due to its cytotoxic properties (Caroli et al. 2001). Zebra mussels exposed to PGM from road dust produce elevated levels of heat shock protein (Singer et al. 2005) and metallothionein (Frank et al. 2008), providing evidence for cellular effects in bivalves.

Freshwater Mussels as Bioindicators

Freshwater mussels (family Unionidae) may be among the groups of aquatic organisms adversely affected by persistent, low-level exposure to PGM in surface waters. Unionid mussels are filter and deposit feeding, long-lived (30-100 yr) organisms that live burrowed in sediments of streams and rivers. They are one of the most rapidly declining faunal groups in North America. About 67% of the nearly 300 freshwater mussel species found in North America are considered vulnerable to extinction or already extinct (Bogan 1993; Williams et al. 1993). The decline of mussel populations in North America has occurred steadily since the mid 1800s and has been attributed to pollution, construction of dams and impoundments, sedimentation, navigation, and habitat degradation (Fuller 1974, Bogan 1993, Neves 1997, Brim Box and Mossa 1999, Vaughn and Taylor 1999). The surface waters of North Carolina have historically supported 56 species of unionid

mussels (Bogan 2002). Today, approximately 78% of these species are listed as endangered, threatened, or of special concern by the U.S. Fish and Wildlife Service and the State of North Carolina (LeGrand et al. 2008) or are already extinct. Many of the same human-mediated and environmental stressors such as highways and associated landscape development (e.g., Wheeler et al. 2005) responsible for the declines of freshwater mussels throughout North America have also contributed to the declines in North Carolina. Principally, the stressors associated with human development and urbanization in almost all of the State's 17 river basins have hastened these declines over the past 20 to 50 years.

Freshwater mussels employ a unique reproductive strategy that involves a parasitic larva (glochidia) that must attach itself to a host fish in order to undergo a metamorphosis to its final stage as a primarily sessile juvenile. The complexity of this reproductive strategy makes freshwater mussels highly vulnerable to extirpation due to environmental alterations (Bogan 1993, Neves et al. 1997). Mussels have a unique life history that includes low trophic level, long life, and sensitive early life stages (Cope et al. 2008). These traits make them a potentially sensitive indicator species for anthropogenic PGM.

Biomarkers

Biomarkers in the ecological discipline have been described as "any biological response to an environmental chemical at the below-individual level, measured inside an organism or in its products (urine, feces, hairs, feathers, etc.) indicating a departure from

the normal status, that cannot be detected from the intact organism" (van Gestel and van Brummelen 1996). Biomarkers are restricted to biochemical, physiological, histological and morphological measurements of health. For example, the salt water mussel *Perna virdis* has been shown to be sensitive to silver and chromium contamination and display reduced oxygen uptake, filtration rates, and inhibition of Na^{+} K^{+}-ATPase, Ca^{2+} ATPase and Mg^{2+}-ATPase at sublethal exposure concentrations (Vijayavel et al. 2007), indicating ion transporters as a potential biomarker of metal contamination in bivalves.

Study Objectives

The fate of PGM in aquatic systems is poorly understood. The objective of this present study is to characterize the present tissue concentration of PGM in a common freshwater mussel and assess its correlation with vehicle traffic and other landscape scale anthropogenic activities. An accompanying laboratory study of uptake of PGM from aqueous medium is intended to elucidate the levels of environmental contamination that are necessary to attain tissue concentration at those observed in the field study and to evaluate potential biomarkers of PGM exposure.

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CHAPTER 1: Assessing PGM concentrations in freshwater mussel tissue and sediment at road crossings from streams in central North Carolina

Abstract

The aim of this study was to assess the level of platinum group metals (PGM) accumulation in the freshwater mussel *Elliptio complanata* and to assess local and landscape scale environmental variables that could be used to explain or predict exposure. A total of 37 sites were sampled throughout North Carolina and were chosen based on availability of *E. complanata*, geographic distribution, land use patterns and traffic density. At each site, samples were collected from an upstream and downstream location within 50-150 m of a highway crossing. A sample consisted of three adult *E. complanata* and sediment. Mussel tissue and sediment samples were analyzed for concentrations of rhodium (Rh), palladium (Pd), and platinum (Pt). Additional related stream, mussel, and local variables investigated included total organic carbon content, co-occurring metal contamination cadmium (Cd) and mercury (Hg), pH, conductivity, temperature, and estimated vehicle traffic density at the stream crossing. Landscape variables included human population density, land use, and density of transportation infrastructure. During development of the analytical techniques for PGM, we discovered that measurement of Pd and Rh had unresolvable interference from yttrium and strontium oxide, respectively, and were unable to be accurately quantified. Therefore, Pt was the only PGM measured and its concentrations ranged 0.09-1.98 ng/g in mussel tissue and 0.06-1.86 ng/g in sediment. A biota-sediment accumulation factor for Pt, calculated as the mean [tissue dry weight]/[soil dry weight], was 3.2, compared to 87.0 and 87.8 for Hg

and Cd, respectively. Pt contamination of mussels and sediment at the stream crossing sites was not significantly correlated with the amount of vehicle traffic crossing the structure. Multiple regression modeling indicated a significant relation between Pt concentration in mussel tissue and the human population of the watershed.

Introduction

Native freshwater mussels (family Unionidae) may be among the groups of aquatic organisms adversely affected by persistent, low-level exposure to PGM in surface waters. Unionid mussels are filter and deposit feeding, long-lived (30-100 yr) organisms that live burrowed in sediments of streams and rivers. They are one of the most rapidly declining faunal groups in North America. About 67% of the nearly 300 freshwater mussel species found in North America are considered vulnerable to extinction or already extinct (1, 2). The decline of mussel populations in North America has occurred steadily since the mid 1800s and has been attributed to pollution, construction of dams and impoundments, sedimentation, navigation, and habitat degradation (3-6). The surface waters of North Carolina have historically supported 56 species of unionid mussels (7). Today, approximately 78% of these species are listed as endangered, threatened, or of special concern by the U.S. Fish and Wildlife Service and the State of North Carolina (8) or are already extinct. Many human-mediated and environmental stressors, such as highways and associated landscape development are responsible for the declines of freshwater mussels throughout North America (9) and have also contributed to the declines in North Carolina. Mussels have a unique life history that includes low trophic

level, long life, and sensitive early life stages (10). These traits make them well-suited to serve as a sentinel or bioindicator species for anthropogenic PGM.

Presently, no information on the bioaccumulation of PGM in freshwater mussels is published in the primary literature. Laboratory investigations of PGM uptake in the distantly related zebra mussel (*Dressinia polymorpha*) indicate that PGM derived from automobile catalytic convertors is bioavailable to filter feeding bivalves and uptake is enhanced by the presence of humic substances and organic acids in the exposure water (11). The zebra mussels used in that study had a background level of PGM that was above detection limits, indicating that anthropogenic sources of PGM were being accumulated by this species in its natural environment. Freshwater mussels are known to bioaccumulate other anthropogenically derived metal pollutants, such as cadmium (Cd), copper (Cu) and zinc (Zn); however, the relationship between tissue burden and local environmental contamination is sometimes weak, indicating that complex environmental dynamics are important (12). The rarity of many unionid mussel species and the demonstrated toxicity of PGM in other species (13), make the potential of bioaccumulation of PGM in this faunal group a cause for concern.

The freshwater mussel species *Elliptio complanata* is widely distributed and abundant in North Carolina, relative to other mussel species. *E. complanata* is suited to the role of a bioindicator species due to its long life, sessile nature, and body size adequate to be used for analysis of trace metals and other contaminants (14). It has been demonstrated to be robust to nonlethal sampling techniques and a suitable bioindicator of chronic water quality conditions (15, 16).

Unpublished data collected previously by our laboratory suggested that *E. complanta* was accumulating Pt and Pd, and that there was a correlation with vehicle traffic density at the crossing site (Figure 1) (17). The present study was designed to define current levels of accumulated PGM in *E. complanata*, test its correlation with vehicle traffic at the site, and to investigate other local and watershed scale variables that might contribute to PGM concentrations in mussel tissue.

Materials and Methods

Mussel and Sediment Collection

Mussels and sediment were collected from 37 stream sites in central North Carolina (Figure 2). Sites were selected based on the presence of the target species at the site, vehicle traffic density, and geographic distribution. A sample was taken from an upstream and downstream location within an area of stream 50-150 m from the road crossing. A sample consisted of three adult *E. complanata* and a surfical (top 5 cm) single grab sample of sediment. Mussels were selected to represent roughly the average size of adult mussel from the site and were taken at random. Mussels were placed in a labeled plastic bag and stored in a cooler on ice, until they could be frozen in a standard freezer ($\geq$-20 ºC). Sediment samples were collected from within areas inhabited by *E. complanata* using a stainless steel scoop and placed in an acid washed jar. Other water quality variables measured at the site were water temperature, pH, conductivity, and dissolved oxygen (YSI Model 556 MPS, Yellow Springs Instruments, Yellow Springs, Ohio, USA). Additional large scale habitat variables were estimated using geographic

information systems (GIS) software (Environmental Systems Research Institute, ArcInfo 9.2, 2008). Landscape variables assessed included land cover, watershed size, human population, and vehicle traffic densities.

Single mussels for PGM analysis did not provide adequate tissue mass to reach detection limits for the PGMs. Therefore, homogenates from three mussels were combined to form a single composite sample for analysis of PGM; one composite sample from the upstream reach and one from the downstream reach at each site. This method produced detectable results, but an assessment of individual variation was lost for mussels from a single location. To estimate potential variation in PGM in mussels within a site, a second experimental design was employed within New Hope Creek, Orange County, North Carolina. Here, six locations were sampled at various distances upstream and downstream of the Interstate 40 (I-40) and NC Hwy 86 crossing of New Hope Creek. Vehicle crossings per day (vc/d) were estimated to be 58,000 vc/d for I-40 at its crossing of New Hope Creek, and that for NC Hwy 86 6,800 vc/d. At each sampling location, mussels were taken at random and stratified into four samples of three individuals each. A surficial sediment sample was also taken at each of these locations using the same method previously described.

Sample Analysis

Samples were analyzed at Research Triangle Institute (Durham, North Carolina, USA) for concentrations of Hg, Cd, Pt, Pd, Rh, with the PGM being the primary targets of interest. Measured concentrations of the target elements were expressed in ng/g dry

weight. At the time of processing, mussel samples were removed from the freezer, partially thawed, dissected from their shells and placed into tared, acid-cleaned 50 mL digestion tubes. The samples were then weighed to the nearest 0.01 g wet weight. The samples were then frozen for a minimum of 24 h at ≥ -20 ºC. After freezing, the samples were placed in a freeze dryer for a minimum of 36 h. Samples were lyopholized then were reweighed to determine the dry weight of the sample. All samples were coarsely ground in the plastic digestion tubes with plastic spatulas.

The samples were treated with 2.0 mL of concentrated nitric acid (Ultrex®) and 2.0 mL of concentrated hydrochloric acid (Ultrex®) and allowed to stand at room temperature for approximately 1 h. The samples were then placed in a graphite digestion block with a digital temperature control module. The samples were heated on an automated program for 1 h at 50 ºC and 1 h at 80 ºC. The samples were removed, allowed to cool, and then 0.5 mL each of concentrated nitric and hydrochloric acids and 3 mL of deionized water were added to each sample. The samples were returned to the block digestion unit and heated for 6 h at 101 ºC. Once the digestion program was completed, the samples were allowed to cool and brought to a final volume of 40 mL using deionized water. The samples were tightly capped, shaken, and a 4 mL aliquot was taken for analysis.

A nominal 4 g aliquot of sediment was transferred from the sample container to an acid washed 50 mL digestion tube. The samples were treated with 2.5 mL of concentrated nitric acid (Ultrex®) and 2.5 mL of concentrated hydrochloric acid (Ultrex®). All samples were then placed in a graphite digestion block with a digital

temperature control module. The samples were heated on an automated program for 1 h at 50 ºC, 1 h at 80 ºC, and then 6 h at 101 ºC. Once the digestion program was completed, the samples were allowed to cool and brought to a final volume of 40 mL using deionized water. The samples were tightly capped, shaken, and a 4 mL aliquot was taken for analysis.

All mussel and sediment extracts were analyzed with a Thermo X-Series II Inductively Coupled Plasma Mass Spectrometer (ICP-MS). Prior to analysis, the instrument was optimized for signal to noise and a system suitability check consisting of 10 replicates of a multi-element standard was run. The Percent Relative Standard Deviation (%RSD) of the 10 replicates was required to be <2% for the instrument to be considered ready for analysis. The ICP-MS was calibrated at the beginning of each analytical run using a series of dilutions prepared from a NIST-traceable stock solution matched to the acid matrix of the samples. The minimum acceptable correlation coefficient for the standard curve was 0.995. A calibration check was performed immediately after the calibration, at an interval of no more than every 10 samples, and at the end of the analysis so that all samples analyzed in a batch were bracketed by calibration checks. The calibration check was prepared from a separately prepared NIST-traceable stock solution than the calibration standards and at a concentration that was not used in the calibration curve.

Model Selection

An information-theoretic approach was used to develop models that best described the relationship between instream and landscape attributes (18). Instream habitat variables collected at field sites and landscape-level parameters estimated using GIS were tested for redundancy using a correlation matrix in SAS Corporation JMP7 software. Variables that were significantly correlated (Pearson product-moment correlation <0.05) were considered redundant and the number of variables included in model selection were reduced to nine as follows: the percent of the total watershed area in the forested land use category (%forested), human population of the watershed, the average number of vehicle crossings per day at the site (vc/d), water pH, water specific conductivity (μS/cm), % total organic carbon (%TOC) content of both the upstream and downstream sediment samples, the mean whole body mass (g) of the mussels collected at a site, and concentration of tissue cadmium (ng/g) in mussels at the site. The human population of the watershed and vc/d were log(base e) transformed. All-subsets regression was used to model relationships for the response variables upstream mussel tissue Pt concentration and downstream mussel tissue Pt concentration. Models were then ranked by corrected Akaike's Information Criterion (AICc) and associated statistics to identify the most parsimonious models (i.e. those that explain the most variance with fewest parameters).

Results

During development of the analytical techniques for measuring PGM in mussel tissue, we discovered that yttrium oxide interfered with the detection of Pd and that strontium oxide interfered with Rh in the mass spectrometer. At low concentrations of Pd and Rh in the samples, the interferences were unable to be resolved and thus, results for Pd and Rh were not quantified. Therefore, Pt is the only PGM reported, and its results were calculated based on the concentration of 195Pt isotope in the sample. Among all samples, Pt concentrations ranged from 0.06 to 1.98 ng/g (mean 0.58 ng/g) dry weight in mussel tissue and from 0.06 to 1.86 ng/g (mean 0.28 ng/g) dry weight in sediment (Table 1). The biota-sediment accumulation factor for Pt, calculated as the mean [tissue]/[soil], was 3.2, compared to 87.0 and 87.8 for Hg and Cd, respectively (Table 2).

For the elements Pt and Cd, mean concentrations from all sites with an upstream and downstream sampling location were greater in the downstream samples for both tissue and sediment. Mean downstream Pt concentration was elevated 9% in tissue and 4% in sediment compared to mean upstream values. Tissue Hg concentrations were greater in the upstream samples, and sediment Hg concentrations were greater in the downstream samples. Variation among sites was high and none of the site comparisons were statistically different at the $P<0.05$ level (Tables 3-6). Pt concentration in mussel tissue was not correlated with Pt concentration in sediment among sites (Figure 3.)

The relative change in Pt concentrations between upstream and downstream locations among sites was inconsistent. At some sites the concentrations in tissue or

sediment was greater in the upstream samples (17 of 37, 46% in tissue; 14 of 37, 38% in sediment; Table 6). The difference in Pt concentration was not correlated with vehicle traffic, estimated as vehicle crossings per day (vc/d), for sediment or mussel tissue concentrations (Figure 4.).

The mussel and sediment samples collected from New Hope Creek demonstrated that there is considerable variation within and among locations within a single stream. The six sites sampled within New Hope Creek had mean mussel tissue Pt concentration of 0.12 ng/g and ranged from 0.07 to 0.23 ng/g. At these sites, standard deviation (SD) ranged 0.008-0.165 ng/g, demonstrating variation among sites within the same stream. The coefficient of variation (CV) within a sampling location ranged 12%-70% (n=4 at each location), with the greatest variation occurring immediately downstream of the Interstate 40 crossing of New Hope Creek.

The variables that most parsimoniously explained variance in the Pt concentration in mussel tissue collected from upstream of the highway crossing were human population, %TOC from the upstream samples, tissue Cd concentration and mussel mass (R^2=0.72, P<0.0001, Table 7). The variables that most parsimoniously explained variance in the Pt concentration in mussel tissue collected from downstream of the highway crossing were human population, %TOC from the downstream samples, tissue Cd concentration, mussel mass, and % forested (R^2= 0.71, P<0.0001, Table 7). Relationships were positive with all explanatory variables except mussel mass, which was negative.

Discussion

The concentration of Pt in mussel tissue and sediment of central North Carolina streams was low relative to the other metal pollutants (Cd and Hg) studied. Concentrations of Pt in mussel tissue measured in this study were less than 2 ng/g dry weight and were as low as 0.07 ng/g dry weight. Sediment Pt concentrations were similarly low, ranging 0.06-1.86 ng/g dry weight. Compared to a natural background concentration of 0.4 ng/g in the earth's crust (19), this level of enrichment does not seem particularly high.

The biota-sediment accumulation factor for Pt calculated in this study (mean 3.2) was comparatively less than that for the other metals investigated, 87.0 and 87.8 for Hg and Cd, respectively. Analysis of the sediment Pt concentration involved a total digestion of the sample and may not be representative of the relatively small bioavialable fraction of Pt particles emitted from automobiles. For example, experiments with ground catalyst material in circumneutral water (pH 6-8) demonstrated that a relatively constant percentage (0.01-0.25%) of the Pt present in the catalyst matrix was dissolved after 3 months (20). The concentrations of dissolved Pt in water were not measured in the present study, however; an investigation of Pt from the Mölndalsån River in Sweden found that sediment concentrations ranged from 8.3 ng/g to 11.2 ng/g, whereas surface water Pt concentrations were <0.1 ng/L and pore water concentrations were 0.5 ng/L (21). A bioaccumulation factor calculated from Pt in water, based on a similar ratio to sediment concentrations, would be several orders of magnitude greater than that calculated from sediment.

In my study, there was little correlation between the concentration of Pt in mussel tissue and the surrounding sediment. This suggests that the route of Pt exposure for mussels is something more complex than simple physical contact with sediment bound Pt. It has been demonstrated that *Dreissena sp.*, zebra mussels, can take up particulate metals as well as dissolved metals (22). Zebra mussels can directly use dissolved organic carbon (DOC) as a food source, and complexation of the metals Cd, silver (Ag) and Hg with high molecular weight DOC significantly increased uptake by the mussels; for Cd the increase was 32-fold (23). A study investigating Cd uptake in two unionid mussel species, *Unio pictorum* and *Anodonta sp.*, in the Danube River found significant positive correlation with sediment concentrations in *Unio* and no correlation in *Anodonta*, despite the two species co-occurring and being collected from the same habitat, indicating differing routes of uptake or metabolism (12).

The amount of vehicle traffic crossing a stream in my study was not correlated with the change in tissue or sediment concentrations between upstream and downstream samples for any of the contaminants investigated. When averaged over all sites, the mean Pt concentration in tissue and sediment was slightly elevated in the downstream samples compared to the upstream group, 9% for tissue and 4% for sediments; however, due to high variation among sites these changes were not statistically significant at the $P<0.05$ level. In 46% of samples, the difference in tissue concentration between downstream samples and upstream samples was negative, indicating greater concentrations of Pt in the upstream sample. Although Pt emission into the environment from automobiles is a non-point source form of emission, its transfer from the roadside environment into the

linear stream environment was hypothesized to behave like a point source discharge, where upstream samples would be unaffected by the influence of the roadside discharge and downstream samples would be enriched. Because emission of Pt into the roadside environment is known to be correlated with vehicle traffic density (24) and runoff from highways has been shown to be enriched with Pt (25), a correlation between traffic and enrichment in the aquatic environment immediately downstream of highway crossings seemed plausible.

One possible reason for the lack of correlation is high variation of Pt concentrations within locations. A necessary tradeoff in the analytical procedure for this study required that soft tissue digestates from three mussels be combined to obtain results that were above the detection limit of the technique, which precluded estimation of the variation between individuals. Indeed, multiple samples collected from a single stream, New Hope Creek, revealed that variation among composite samples collected from a single location was high and that variation differed among locations within the stream over a 7-km distance. Among these samples, the CV ranged from 12-70%, with the greatest CV immediately downstream of a major interstate highway crossing. If this trend was common in the streams that were sampled, significantly greater variation in samples collected from downstream of a highway crossing could produce the observed results.

The form of Pt emitted into the environment from automobiles is in a metallic form with relatively low solubility (26). The majority of the Pt transferred from the road-side environment to the stream would presumably remain in this form. Pt particles would

then settle and become part of the sediment where they can be transformed by geochemical and biological processes into a more bioavailable form. In this case, the deposition of Pt particles would be dispersed for some distance from the source, and enrichment of mussel tissue concentrations might be expected to increase with distance from the source. Longitudinal trends in results from New Hope Creek confirmed this hypothesis, displaying decreased concentrations in mussel tissue and sediment in the first kilometer downstream of the interstate highway crossing compared to the samples from the first kilometer upstream, but samples collected from bridge crossings approximately 2.5 and 4.5 km downstream of the Interstate were highly enriched with no obvious source of significant additional input. The concentrations from a site approximately 6.7 km downstream were slightly reduced from those at 2.5 and 4.5 km, indicating that the maximum enrichment of Pt from a road source might not occur for some distance downstream (~ 3.5 km) from the primary source (Figure 5).

An additional source of variation in Pt enrichment downstream of a highway crossing might come from attributes of the crossing structure itself. Highway bridges and culverts vary widely in their construction. Crossing structures invariably cause constriction of a stream's channel and in doing so, alter the natural hydrology and sediment dynamics. Structures that significantly constrict the flow of the stream can cause drastic changes in stream morphology and during high flow events will destabilize the substrate downstream of the constriction, potentially reducing the deposition of fine particulates and pollutants or periodically scouring them out. The degree of hydraulic influence of the structure would depend on the design of the structure and the

morphology of the stream, but would diminish some distance downstream from the structure to a point that would allow for deposition of sediment, Pt, and other substances to resume.

Pt in mussel tissue collected from upstream of the crossing structure was intended to represent a site specific background concentration for the stream that reflected human activity in the watershed upstream from the location. The variables included in the candidate model set describing both upstream and downstream concentrations (Table 7) support a watershed scale influence on the Pt concentrations at the site and that anthropogenic activity within the watershed is the overall source of Pt contamination. Candidate models for both upstream and downstream Pt displayed significant correlation with the total organic carbon content of the sediment from upstream of the crossing structure and neither was correlated with the total organic carbon content of the sediment from downstream.

This shared correlation with organic carbon content between locations could be the result of Pt transformation taking place in the sediment throughout the watershed, with the sediment TOC of the upstream sediment sample being representative of the average sediment TOC of the watershed where the substrate is less disturbed by hydraulic alterations of the road crossing. The solubility of fine Pt particles is known to increase in organic matrices and is further increased by complexation with humic acid (27, 28). Mussels are known to feed on suspended particulate organic matter and detritus, apparently preferring the living algal and bacterial fraction (29). A process of dissolution of fine Pt particles in the organic fraction of the sediment during its breakdown and

eventual assimilation into bacteria and algae would lead to bioavailable Pt being incorporated into food sources being utilized by the mussels. The production of food utilized by the mussels takes place throughout the watershed and if the primary route of Pt contamination in mussels is dietary, then significant enrichment of Pt in mussel tissue would not be expected for some distance downstream from the source. This pattern of enrichment was confirmed by the distribution of enrichment from the intensive samples collected longitudinally within New Hope Creek and could partially explain the lack of correlation with vehicle traffic density at the sites.

My results support the hypothesis that mussels uptake Pt from both waterborne and sediment routes. Waterborne Pt may be either dissolved or particulate. During the process of filtration, mussels come into contact with waterborne Pt and could absorb dissolved Pt and may ingest particulate Pt. Pt not ingested or absorbed may pass through the mussel and exit via the excurrent siphon or be incorporated with pseudofeces and biodeposited. Mussels in hydraulic refugia may increase the organic content of the sediment through biodeposition. Mussels burrowed in sediment with high organic content may be able to deposit feed and could uptake Pt via this route. Additionally, close contact with contaminated sediments may facilitate the transformation of Pt to more bioavailable forms that can be directly absorbed from the sediment. Mussels that are present in an area that does not permit the deposition of organic material, such as those downstream of a highway crossing with a scoured substrate, may not be able to deposit feed and only uptake Pt from the waterborne route and consequentially accumulate less Pt than mussels in nearby areas where biodeposition and deposit feeding occur.

Differences in the amount of biodeposition and deposit feeding could account for the measured variation between upstream and downstream locations within a single stream.

The results of this study demonstrate that *E. complanata* is accumulating Pt and that the pattern of accumulation in a stream may be affected by hydraulic factors. The spatial lag in Pt accumulation measured in New Hope Creek shows that Pt can be carried several km from its source ($\geq$4.5 km in New Hope Creek). Because Pt is only one of many pollutants associated with vehicular transportation, other pollutants may also be transported a considerable distance in aquatic systems. Because of the potential for long distance transport of pollutants from road sources, it is important for transportation planners to consider the effects of transportation infrastructure over large sections of stream.

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Tables

Table 1. Platinum (Pt), cadmium (Cd), and mercury (Hg) concentrations in mussel tissue and sediment collected from all samples.

		Metal Concentrations		
Source	n	Mean (ng/g)	Standard Deviation (ng/g)	Range (ng/g)
Pt tissue	80	0.58	0.41	0.07 - 1.98
Pt sediment	80	0.28	0.28	0.06 - 1.86
Cd tissue	74	1,002.45	678.92	33.26 – 3,065.36
Cd sediment	74	16.53	13.4	0.5 - 96.92
Hg tissue	74	470.72	240.27	28.55 – 1,147.83
Hg sediment	74	8.72	7	47.14 - 645.61

Table 2. Biota-sediment accumulation factors calculated as [tissue (ng/g dry weight)]/ [sediment (ng/g dry weight)] for platinum (Pt), cadmium (Cd), and mercury (Hg).

		Biota-sediment accumulation factor [tissue]/[sediment]			
Element	n	Mean	Standard Deviation	Median	Range
Pt	80	3.2	3.1	2.0	0.2 - 11.0
Cd	74	87.8	93.0	60.7	10.8 -582.8
Hg	74	87.0	153.4	61.5	13.4 -1313.1

Table 3. Platinum concentrations in mussels and sediment from upstream and downstream of road crossings.

	Platinum concentration in mussels and sediment (ng/g dry wt)			
Stream	Upstream Tissue (n=3)	Downstream Tissue (n=3)	Upstream Sediment (n=1)	Downstream Sediment (n=1)
Crabtree Creek	0.116	0.226	0.179	0.180
Bear Creek	0.136	0.094	0.155	0.193
Six Mile Creek	0.165	0.158	0.224	0.250
Harlands Creek	0.171	0.215	0.203	0.191
Long Creek	0.184	0.200	0.137	0.199
Tar River	0.201	0.353	0.176	0.257
Tar River	0.242	0.302	0.426	0.208
Buckhorn Creek	0.262	1.208	0.118	0.117
Little River	0.282	0.383	0.127	0.114
Densons Creek	0.292	0.414	0.308	0.247
Eno River	0.293	0.750	0.189	0.516
Terrels Creek	0.353	0.459	0.150	0.247
Stony Creek	0.397	0.438	1.390	1.861
Swift Creek	0.401	0.294	0.149	0.260
Sevenmile Creek	0.451	0.386	0.421	0.271
New Hope Creek	0.498	0.399	0.808	0.436
Little Fishing Creek	0.500	0.715	0.239	0.358
Troublesome Creek	0.508	0.500	0.117	0.152
New Hope Creek	0.513	0.321	0.751	0.891
Shocco Creek	0.523	0.516	0.119	0.268
Stony Creek	0.538	0.305	0.302	0.245
Little River	0.597	0.821	0.176	0.164
Tar River	0.603	0.666	0.143	0.233
Troublesome Creek	0.654	0.502	0.292	0.305
Contentnea Creek	0.669	0.611	0.061	0.187
Black Creek	0.689	0.669	0.075	0.069
Sevenmile Creek	0.693	0.580	0.546	0.197
Six Mile Creek	0.773	0.303	0.072	0.084
Contentnea Creek	0.829	0.805	0.114	0.074
Little Fishing Creek	0.962	1.163	0.235	0.309
Flat River	0.966	0.714	0.480	0.171
Contentnea Creek	0.998	1.173	0.154	0.207
Cane Creek	1.056	1.419	0.400	0.263
Little River	1.103	1.341	0.128	0.146
Troublesome Creek	1.126	0.988	0.177	0.219
Crains Creek	1.540	1.984	0.296	0.346
Moccasin Creek	1.595	1.505	0.146	0.137
Mean	0.591	0.645	0.275	0.286
Standard Deviation	0.374	0.436	0.257	0.302

Table 4. Cadmium concentrations in mussels and sediment from upstream and downstream of road crossings.

	Cadmium concentration in mussels and sediment (ng/g dry wt)			
Stream	Upstream Tissue (n=3)	Downstream Tissue (n=3)	Upstream Sediment (n=1)	Downstream Sediment (n=1)
Swift Creek	39	33	0.5	1.7
Bear Creek	65	36	1.6	1.6
Crabtree Creek	131	669	8.4	11.4
Tar River	377	427	21.4	39.4
Shocco Creek	382	476	17.4	7.7
Little River	391	716	11.3	30.3
Buckhorn Creek	404	1,128	9.6	6.0
Sevenmile Creek	434	387	25.8	26.7
Six Mile Creek	489	490	4.8	7.8
Harlands Creek	541	722	17.3	27.8
Eno River	575	801	11.6	16.5
Little Fishing Creek	595	1,010	22.2	16.2
Sevenmile Creek	694	843	19.2	21.8
Long Creek	696	574	10.9	11.5
Contentnea Creek	766	809	9.2	10.8
New Hope Creek	784	909	19.1	30.6
Little River	842	687	13.9	30.6
Little River	853	756	9.1	7.4
Densons Creek	874	1,032	14.4	23.0
Stony Creek	889	986	17.4	25.4
Contentnea Creek	917	1,192	11.9	17.6
Cane Creek	971	1,126	15.7	15.8
Little Fishing Creek	980	692	17.6	19.6
Black Creek	1,008	1,083	3.8	7.7
Stony Creek	1,016	657	10.1	15.0
Terrels Creek	1,034	1,025	11.2	11.5
Six Mile Creek	1,079	590	4.2	5.1
Flat River	1,182	1,646	28.6	16.5
Troublesome Creek	1,190	1,394	4.8	7.8
Tar River	1,213	454	10.2	28.8
Tar River	1,296	2,233	15.3	10.3
New Hope Creek	1,492	787	29.5	20.2
Contentnea Creek	1,663	2,500	49.3	96.9
Moccasin Creek	2,391	1,988	6.1	22.6
Crains Creek	2,451	3,055	35.5	13.9
Troublesome Creek	2,745	2,152	12.2	10.3
Troublesome Creek	3,065	1,600	5.3	13.3
Mean	987	1,018	14.5	18.6
Standard Deviation	703	663	9.9	16.1

Table 5. Mercury concentrations in mussels and sediment from upstream and downstream of road crossings.

	Mercury concentration in mussels and sediment (ng/g dry wt)			
Stream	Upstream Tissue (n=3)	Downstream Tissue (n=3)	Upstream Sediment (n=1)	Downstream Sediment (n=1)
Terrels Creek	33	29	1.2	1.1
Troublesome Creek	42	114	2.8	4.1
Harlands Creek	48	37	1.7	1.5
Densons Creek	197	206	8.1	5.8
Contentnea Creek	235	270	4.9	10.1
New Hope Creek	238	382	9.7	5.8
Six Mile Creek	272	239	8.6	17.9
Eno River	287	405	7.0	0.3
Shocco Creek	311	275	3.4	6.1
Little River	322	389	17.9	23.2
Tar River	366	349	17.3	12.4
Swift Creek	371	359	3.9	3.2
Stony Creek	373	338	5.9	8.8
Black Creek	388	328	6.4	4.8
Little Fishing Creek	397	409	2.3	2.4
Tar River	398	470	14.5	17.1
Troublesome Creek	442	326	4.8	7.8
Little River	447	329	3.6	5.5
Stony Creek	451	431	8.9	18.2
Sevenmile Creek	455	546	15.9	6.8
Contentnea Creek	500	375	3.9	4.9
Sevenmile Creek	500	555	10.8	8.1
Flat River	513	632	4.6	4.7
Crains Creek	542	570	6.1	7.0
Troublesome Creek	545	397	8.9	4.9
Cane Creek	617	652	9.1	5.8
Buckhorn Creek	630	552	6.7	6.6
Long Creek	673	899	6.7	5.3
Bear Creek	706	716	24.7	47.1
Little River	709	638	9.8	10.7
Six Mile Creek	709	550	11.4	22.1
Little Fishing Creek	749	679	4.3	12.2
Crabtree Creek	789	755	7.0	9.0
New Hope Creek	814	162	2.5	3.6
Moccasin Creek	832	568	6.1	12.8
Contentnea Creek	987	898	8.4	11.5
Tar River	1148	968	15.5	10.9
Mean	487	454	8.0	9.5
Standard Deviation	254	228	5.2	8.5

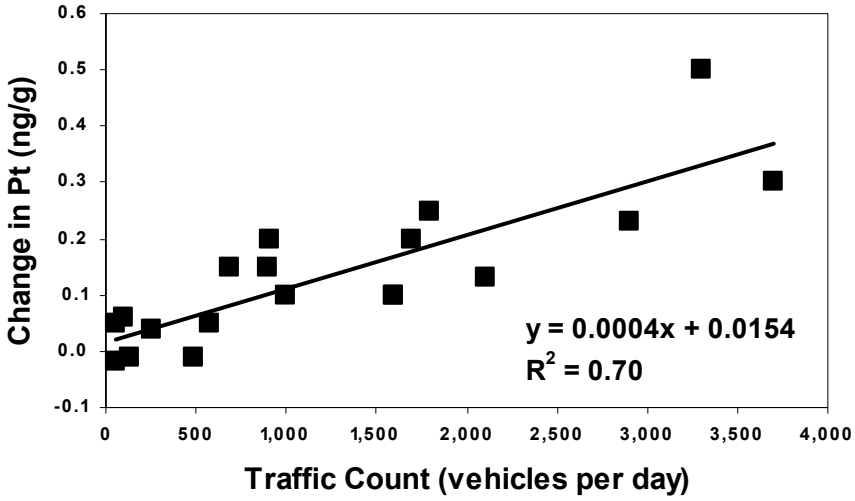
Table 6. Change in mean platinum (Pt) concentrations of mussel and sediment between upstream and downstream locations.

		Change in Pt concentration [downstream]-[upstream] ng/g	
Stream	Traffic (vc/d)	Δ[Sediment]	Δ[Tissue]
Long Creek	30	0.06	0.02
Contentnea Creek	390	0.05	0.18
Tar River	430	0.08	0.15
Cane Creek	470	-0.14	0.36
Troublesome Creek	540	0.03	-0.01
Densons Creek	610	-0.06	0.12
Little Fishing Creek	610	0.07	0.20
Little Fishing Creek	610	0.12	0.21
Tar River	680	-0.22	0.06
Bear Creek	690	0.04	-0.04
Troublesome Creek	910	0.04	-0.14
Moccasin Creek	935	-0.01	-0.09
Flat River	1,000	-0.31	-0.25
New Hope Creek	1,000	0.14	-0.19
Eno River	1,200	0.33	0.46
Buckhorn Creek	1,400	0.00	0.95
Contentnea Creek	1,600	0.13	-0.06
Sevenmile Creek	1,800	-0.15	-0.06
Shocco Creek	1,800	0.15	-0.01
Little River	2,000	0.02	0.24
New Hope Creek	2,000	-0.37	-0.10
Terrels Creek	2,200	0.10	0.11
Black Creek	2,300	-0.01	-0.02
Little River	2,400	-0.01	0.22
Stony Creek	2,800	-0.06	-0.23
Swift Creek	3,300	0.11	-0.11
Little River	5,700	-0.01	0.10
Contentnea Creek	5,850	-0.04	-0.02
Troublesome Creek	9,600	0.01	-0.15
Crains Creek	9,650	0.05	0.44
Harlands Creek	12,000	-0.01	0.04
Six Mile Creek	21,500	0.01	-0.47
Six Mile Creek	21,500	0.03	-0.01
Tar River	28,000	0.09	0.06
Stony Creek	43,000	0.47	0.04
Sevenmile Creek	87,000	-0.35	-0.11
Crabtree Creek	147,000	0.00	0.11
Mean		0.01	0.05
Standard Deviation		0.16	0.24

Table 7. Five most parsimonious models explaining variance in Pt concentration among upstream and downstream mussel tissue samples. K is the number of model parameters; ΔAICc is the difference between successive model Akaike's Information Criterion values corrected for bias; and w_i is the Akaike's weight, or probability that the model is the most informative model.

Model	K	ΔAICc	w_i
Upstream Mussel Tissue Platinum (ng/g)			
0.1055(ln(population))+0.2811(upstream % TOC)+0.0003(tissue Cd (ng/g))-0.0069(mass)-0.4075	6	0	0.305
0.1092(ln(population))+0.2831(upstream % TOC)+0.0003(tissue Cd (ng/g))-0.0068(mass)+0.0022(%forested)-0.5982	7	2.45	0.089
0.0963(ln(population))+0.2732(upstream % TOC)+0.0003(tissue Cd (ng/g))-0.0067(mass)-0.0732(pH)+0.2030	7	2.95	0.070
0.1085(ln(population))+0.2937(upstream % TOC)+0.0003(tissue Cd (ng/g))-0.0070(mass)-0.0337 (downstream % TOC)-0.3937	7	3.06	0.066
0.1080(ln(population))+0.2849(upstream % TOC)+0.0003(tissue Cd (ng/g))-0.0066(mass)-0.03903(specific conductivity)-0.4027	7	3.25	0.060
Downstream Mussel Tissue Platinum (ng/g)			
0.1258(ln(population))+0.3750(upstream % TOC)+0.0003(tissue Cd (ng/g))-0.0093(mass)+0.0057(%forested)-0.7480	7	0	0.274
0.1185(ln(population))+0.3748(upstream % TOC)+0.0003(tissue Cd (ng/g))-0.0084(mass)-0.3657	6	1.25	0.147
0.1374(ln(population))+0.3923(upstream % TOC)+0.0003(tissue Cd (ng/g))-0.0085(mass)+ 0.0061(%forested) -1.3844(specific conductivity)-0.7672	8	2.36	0.084
0.1146(ln(population))+0.3647(upstream % TOC)+0.0003(tissue Cd (ng/g))-0.0093(mass)+ 0.0062(%forested) -0.0972(pH)+0.0281	8	3.14	0.057
0.1318(ln(population))+0.3730(upstream % TOC)+0.0003(tissue Cd (ng/g))-0.0090(mass)+0.0060(%forested) -(ln(traffic(vc/d))-0.6862	8	3.26	0.054

Figures



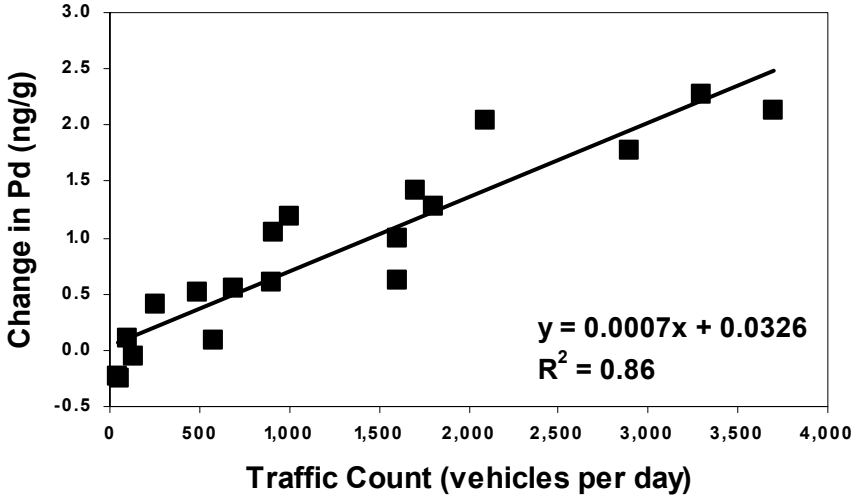


Figure 1. Preliminary data on correlation between traffic count and change in Pt and Pd in *Elliptio complanata* tissue between upstream and downstream samples at bridge crossings. Change in elemental concentration was calculated as the downstream concentration minus the upstream concentration (18).

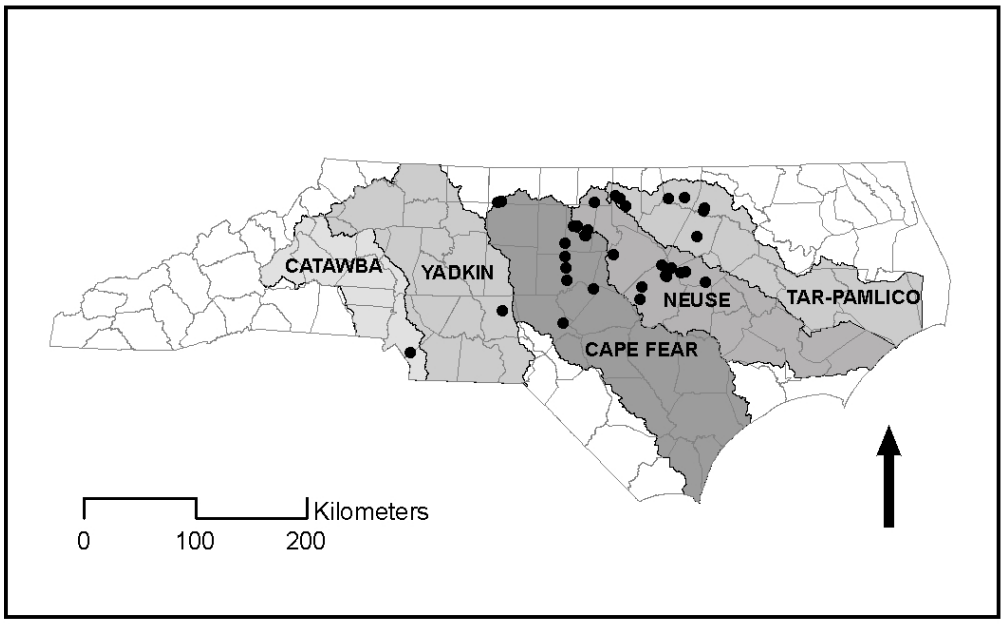


Figure 2. Site locations and river drainage basins where upstream and downstream samples of mussels and sediment were collected.

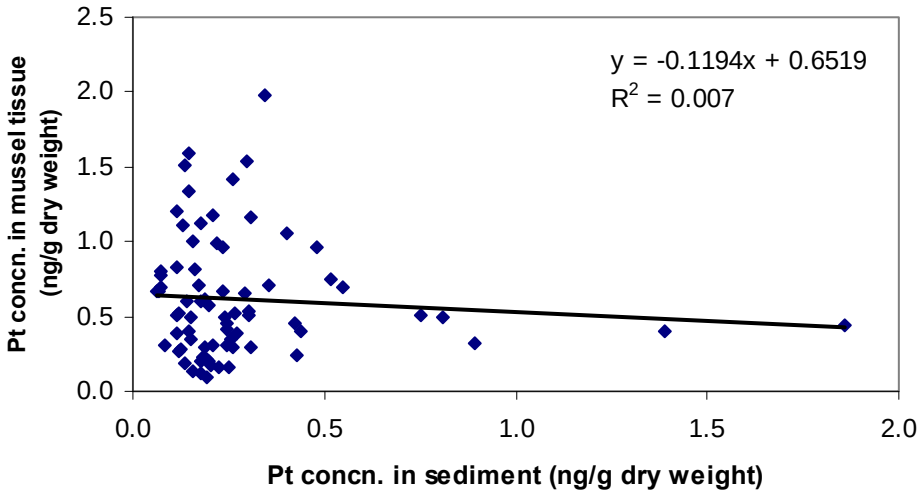
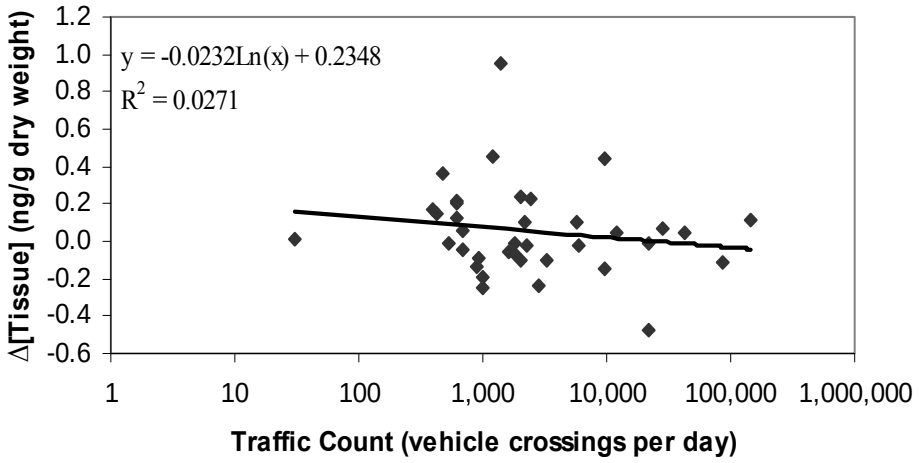


Figure 3. Correlation of tissue Pt concentration and sediment Pt concentration from all sampling locations.



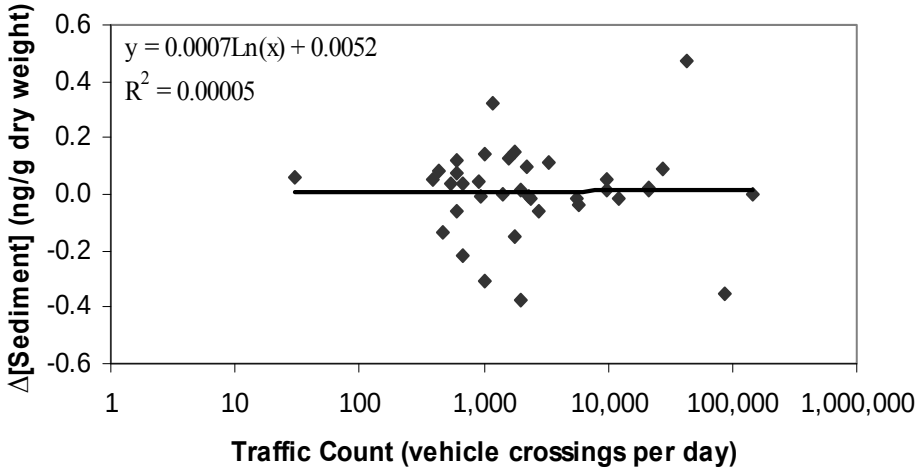


Figure 4. Correlation between traffic count at a highway crossing and the change in mussel tissue Pt and sediment Pt concentrations between upstream and downstream samples at the highway crossing.

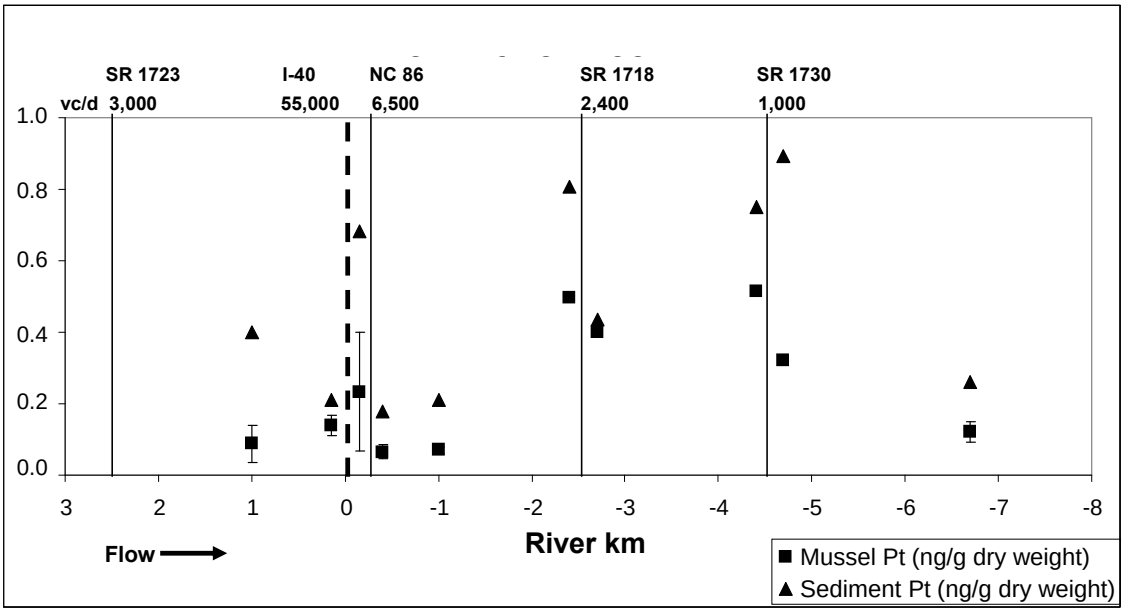


Figure 5. Longitudinal pattern of mussel tissue and sediment Pt concentrations in New Hope Creek. Vertical lines represent road crossings of the stream. Road number and traffic count in vehicle crossings per day (vc/d) are displayed across the top. The flow of the stream is from left to right with river kilometers measured from Interstate 40 crossing displayed as a broken vertical line. Where multiple samples were collected error bars display $\pm$ one standard deviation.

CHAPTER 2: Evaluation of Na^+, K^+-ATPase activity and hemolymph ion concentrations as biomarkers of Pt and Pd exposure in freshwater mussels

Abstract

A 28-d laboratory test was conducted with waterborne Pt and Pd to determine toxicity, bioaccumulation, and to assess several potential biomarkers of exposure to Pt and Pd. Test mussels were exposed to five concentrations of an equal mixture of Pt and Pd salts, ranging from 0.05 to 500 µg/L of each metal, in a static renewal test. The 500 µg/L concentration resulted in high mortality (4 of 9 dead by day 12) of test mussels; all individuals in each of the other test concentrations survived to the end of the test. There were nine replicate mussels per treatment concentration, allowing three mussels from each treatment to be sampled on days 7, 14 and 28. Tissue and hemolymph were assessed for concentrations of Pt, Pd, sodium (Na^+), calcium (Ca^{2+}), potassium (K^+), and chloride (Cl^-) concentrations. Na^+,K^+-ATPase activity was assessed in gill tissue as a potential biomarker of exposure. Tissue concentrations of Pt ranged from 0.10 ng/g dry weight in controls to 34,486 ng/g dry weight in the 500 µg/L treatment, and Pd ranged from 0.06 ng/g dry weight in controls to 34,404 ng/g dry weight in the 500 µg/L treatment. Concentrations of Pt in hemolymph ranged from 0.08 ng/mL in controls to 50.1 ng/mL in the 500 µg/L treatment, and Pd ranged from 0.06 ng/mL in controls to 12.2 ng/mL in the 500 µg/L treatment. On day 28, Na^+,K^+-ATPase activity displayed a logarithmic trend ($y=0.489\ln(x)+2.054$; $R^2=0.73$) of increasing activity with increasing PGM exposure concentration; however, activity was only significantly increased ($P<0.05$) at 5.0 µg/L and 50 µg/L concentrations. High variation and weak correlation of

Na^+,K^+-ATPase activity with Pt and Pd exposure concentration indicate that it may not be a suitable biomarker of PGM exposure. Hemolymph Ca^{2+} levels were increased on day 7 at the 50 µg/L concentration. Hemolymph Na^+ levels were decreased on day 28 at the 5.0 µg/L and 50 µg/L concentrations. Cl^- and K^+ levels were decreased at the 50 µg/L concentration. Hemolymph ion measurement does not appear to be sensitive enough to serve as a biomarker of PGM exposure at environmentally relevant exposure concentrations.

Introduction

In the United States in the mid 1970s and in Europe in the 1980s, laws were enacted to reduce air pollution that necessitated the introduction of catalytic converters for automobiles. The use of catalytic converters for automobile exhaust purification has led to the emission and environmental contamination by the platinum group metals (PGM) platinum (Pt), palladium (Pd), and rhodium (Rh). Recently, there were an estimated 500 million vehicles equipped with catalytic converters world-wide (Rauch 2005). Consequently, concentrations of PGM, formerly among the lowest of any element in the earth's crust (0.4 ng/g Pt and Pd, 0.06 ng/g Rh, Whedepol 1995), are increasing in dust and soils along highways and are being transported into aquatic habitats through surface runoff, where they are accumulating in stream sediments and tissues of organisms (Sures et al. 2001; Sutherland 2003; Zimmermann et al. 2002, 2005).

Bioaccumulation of PGM has been demonstrated in a variety of species. Plants grown on soil treated with soluble forms of PGM accumulate the metals, especially Pd

(Ek et al. 2004). A variety of roadside plant species have been shown to accumulate PGM, as well as other traffic related metals (Schafer et al. 1998; Djingova et al. 2003; Lesniewska et al. 2004). The sulfate-reducing bacterium, *Desulfovibrio desulfuricans*, has been shown to accumulate PGM, and this bacterium is similar to bacteria naturally found in decaying detritus in aquatic systems (Yong et al. 2002). European eels (*Anguilla anguilla*) have been shown to bioaccumulate PGM when exposed to road dust solutions under laboratory conditions (Sures et al. 2001, Zimmermann et al. 2002).

Laboratory investigations of PGM uptake in the zebra mussel (*Dreissena sp.*) indicate that PGM derived from automobile catalytic convertors is bioavailable to filter feeding bivalves (Zimmermann et al. 2002). The zebra mussels used in their study had a background level of PGM that was above the detectable limit, indicating that anthropogenic sources of PGM are being accumulated by this species in its natural environment (Zimmermann et al. 2002).

Presently, no information on the bioaccumulation of PGM in unionid mussels is available. Freshwater mussels are known to bioaccumulate other anthropogenically derived metal pollutants, such as cadmium (Cd), copper (Cu), and zinc (Zn); however, the relationship between tissue burden of these metals and local environmental contamination varies among species (Gundacker 2000). *Elliptio complanata* sampled from the St. Lawrence River, Canada, accumulated 2.5 times more nickel (Ni), an element similar to the PGM, and displayed less variation than a co-occurring and intermixed species, *Lampsilis radiata* (Metclafe-Smith 1995), indicating that *E. complanata* might be a good sentinel of PGM contamination.

Freshwater mussels are of particular interest in assessing effects of aquatic pollutants due to their sensitive early life stages (glochidia, juveniles) and their imperiled status world-wide (Cope et al. 2008). About 67% of the nearly 300 freshwater mussel species found in North America are considered vulnerable to extinction or are already extinct (Bogan 1993). The decline of many freshwater mussel species and the demonstrated toxicity of PGM in other species, including bivalves (Wolterbeek and Verburg 2001, Frank et al. 2008), make the potential of bioaccumulation of PGM in this faunal group cause for concern.

The purpose of this study was to investigate the toxicity and bioaccumulation of Pt and Pd in *E. complanata* from laboratory waterborne exposures. Mussels were assessed for mortality and sublethal changes in hemolymph sodium (Na^+), calcium (Ca^+), potassium (K^+) and chloride (Cl^-) ion concentrations. Additionally, the membrane bound ion transporter enzyme Na^+,K^+-ATPase was assessed as a potential sublethal biomarker of PGM exposure.

Materials and Methods

Collection, Transport, and Holding of Mussels

Laboratory toxicity testing followed the ASTM guidelines for conducting laboratory toxicity tests with freshwater mussels (ASTM 2006), as modified for use with adults. Sixty-three adult *E. complanata* were collected from a relatively uncontaminated, rural, forested section of the Eno River near Hillsborough, North Carolina. Mussels were collected by hand, placed in ice chests, and covered with damp nylon mesh bags to

prevent desiccation and reduce stress during transport (Cope et al. 2003). Mussels were transported directly to the laboratory (30 min transportation time), where they were scrubbed with a soft-bristle brush and rinsed with deionized water. Fifty-nine mussels were assigned at random to individual test aquaria containing two L of ASTM soft water (ASTM 1993). Aquaria were labeled with one of six exposure concentrations (0-500 µg/L) and day to be sampled. Four remaining mussels were placed in cold storage (-80 ºC) to serve as a baseline measurement for PGM contamination at the collection site. Test water was continuously aerated by a central aeration unit (Sweet Water Air Pump SL24 Aquatic Eco-Systems Inc., Apopka, Florida, USA). Each batch of ASTM soft water was measured for pH with a Beckman Model 240 (Beckman Instruments, Fullerton, California, USA) calibrated meter. Alkalinity was determined by titration of 0.02 M H2SO4 to pH 4.5. Hardness was determined by titration with 0.01 M ethylenediaminetetra-acetic acid (EDTA). Water in the test aquaria was periodically tested for temperature, pH, conductivity, and dissolved oxygen at 0, 48, and 72 hour time points with a calibrated multi-probe (YSI Model 556 MPS, Yellow Springs Instruments, Yellow Springs, Ohio, USA). Physiochemical characteristics of water in the test jars averaged 18.9 °C (range 18.6-19.2 °C) for temperature, dissolved oxygen 9.1 mg/L (range 8.77-9.37 mg/L), pH 7.5 (range 7.3-7.6), alkalinity 30 mg/L as CaCO3 (range 29-31 mg/L as CaCO3), and hardness 42 mg/L (range 42-44 mg/L). Composite water samples were taken, 5 mL from 3 jars per concentration, at 0, 48 and 72 hour time points for Pt and Pd concentration verification. Water was stored in 25 ml vials with 75 µL of concentrated trace metal grade nitric acid (Ultrex®) for analysis.

Experimental Procedures

Test mussels were allowed to acclimate to test conditions 5 d prior to test initiation, with a 100% water renewal of ASTM water on the third day prior to the test. Mussels were fed for the first time on the morning on the first day of the 28 d exposure and then every 48 h or 72 h prior to test water renewal and introduction of the Pt/Pd test solution. Each jar received 25 mL of a food suspension containing 2 mL of Instant Algae® Shellfish Diet and 1 mL *Nannochloropsis* concentrate (Reed Mariculture, Campbell, California, USA) in 1 L of deionized water. The test solution was a mixture of dissolved Pt and Pd, both at 1,000 ppm in 5% HCl (High-Purity Standards catalog # 100038-2 (Pd) and 100040-2 (Pt)). Test solution was added to jars by pipette to yield the appropriate test concentration.

Mussels were sampled and processed on days 0, 7, 14, and 21. During processing, mussels were weighed (to the nearest 0.1 g) and measured (to the nearest mm), gently pried open, and had 1 mL of hemolymph extracted from the anterior adductor muscle with a 25 gauge syringe (Gustafson et al. 2000). The hemolymph was divided equally into two 1.2 mL cryotubes for analysis of Pt, Pd concentration and ion concentration (Na^+, K^+, Cl^-, Ca^{2+}); samples were stored at $\geq$-20°C and -80°C, respectively. From each individual, mussel gill tissue was dissected and three samples (~15 mg each) were placed individually in 1 mL centrifuge tubes, on ice, with 100 µL of SEI buffer solution (sucrose 250 mM, disodium ethylenediaminetetraacetic acid dihydrate ($Na_2EDTA\ 2H_20$) 10 mM, imidazole 50 mM) for Na^+,K^+-ATPase activity

analysis. The remaining whole mussel was bagged and stored frozen (≥-20 °C) for tissue Pt and Pd analysis.

Na^+,K^+-ATPase Activity Assay

The Na^+,K^+-ATPase activity was determined using the method of Mosher (2008), as modified for mussels. The method used by McCormick (1993) to assess Na^+,K^+-ATPase activity in fish gill tissue relied on ouabain to inhibit Na^+,K^+-ATPase activity, however research performed by Mosher (2008) showed that ouabain was not effective at inhibiting Na^+,K^+-ATPase activity in *E. complanata*. Therefore, a K+-free salt solution was used in place of ouabain and effectively inhibited Na^+,K^+-ATPase activity for the assay.

The following procedure was adapted from Mosher (2008). An assay mixture (AM) was prepared fresh each day. Aliquots of phosphoenolpyruvate (PEP) and adenosine diphosphate (ADP) were removed from the -80 ºC freezer and thawed. Nicotinamide adenine dinucleotide (NADH) (5.45 mg) and adenosine triphosphate (ATP) (13.5 mg) were weighed and rinsed into a graduated cylinder with imidazole buffer. Lactate dehydrogenase (LDH) (12.2 µL) and pyruvate kinase (PK) (23.2 µL) were combined and centrifuged at 12,000 x g for eight min at 4ºC using an Allegra™ 25R Centrifuge, Beckman Coulter, Fullerton, CA, USA. The pellet was re-suspended with imidazole buffer, and added to the graduated cylinder along with 4.7 mL PEP. The volume was brought to 37.5 mL with imidazole buffer, and the completed AM was mixed. Two test solutions were prepared with a 3:1, AM:salt solution ratio. For solution

A, 15 mL of AM was mixed with 5 mL of a salt solution (imidazole 50mM, NaCl 189mM, MgCl 10.5mM, KCl 42mM) and stored on ice. For solution B, 15 mL of AM was mixed with 5 mL of K^+-free salt solution (imidazole 50mM, NaCl 189mM, MgCl 10.5mM) and stored on ice. Solution A is used to measure total ATPase activity and solution B is used to measure ATPase activity minus the activity of K^+ dependent Na^+,K^+-ATPase activity. Mussel gill samples were thawed and immediately homogenized with 25 µL 0.3% SEID (0.0751 g Na deoxycholic acid in 25 mL SEI) by grinding in a centrifuge tube for 20-30 s using 1.5 mL centrifuge tube pellet pestles. All samples were read within 30 min of processing. The homogenates were centrifuged at 5,000 x g for one minute at 4ºC, and 10 µL of supernatant was pipetted into each of four wells per sample on a 96-well plate. Then 200 µL of solution B (without K^+) was added to two of the four wells for each sample, and 200 µL of solution A was added to the other two wells. An ADP standard curve was measured in the first plate of each day running the assay using 4 mM ADP stock solution. ADP stock solution was diluted with imidazole buffer to concentrations of 0, 5, 10 and 20 nMoles ADP/10 µL and 10 µL were pipetted into 3 wells per concentration, and 200 µL of solution A was added to each well. Plates were analyzed at 340 nm for 10 min at 1 min intervals using a Fusion™ Universal Microplate Analyzer (A153600 Meriden, Connecticut) and results were read in milli Optical Density (mOD)/nmole ADP. ATPase activity was calculated from the depletion of ADP over the 10 minute interval as mOD/10 µL /min. The Na^+,K^+-ATPase activity was calculated as the difference between the total ATPase activity measured in the wells with solution A and the ATPase activity with Na^+,K^+-ATPase inhibited in wells with

solution B. A Bradford Protein Assay Kit (Shelton Scientific-IBI; VWR #14221-496) was used to determine protein concentration, and the final Na^+,K^+-ATPase activity was measured as μmoles ADP/mg protein/hour (Bradford 1976). A more detailed description of the necessary mixtures and calculation is included in Appendix 1.

Sample Analysis

All mussel hemolymph and tissue samples were analyzed using a Thermo X-Series II Inductively Coupled Plasma Mass Spectrometer (ICP-MS) at Research Triangle Institute (RTI, Durham, North Carolina, USA) for concentrations of Pt and Pd. Prior to analysis, the instrument was optimized for signal to noise and a system suitability check consisting of 10 replicates of a multi-element standard was run. The Percent Relative Standard Deviation (%RSD) of the 10 replicates was required to be <2% for the instrument to be considered ready for analysis. The ICP-MS was calibrated at the beginning of each analytical run using a series of dilutions prepared from a NIST-traceable stock solution matched to the acid matrix of the samples. The minimum acceptable correlation coefficient for the standard curve was 0.995. A calibration check was performed immediately after the calibration, at an interval of no more than every 10 samples, and at the end of the analysis so that all samples analyzed in a batch were bracketed by calibration checks. The calibration check was prepared from a separately prepared NIST-traceable stock solution than the calibration standards and at a concentration that was not used in the calibration curve. Measured concentrations of the

target elements in mussel tissue were expressed as ng/g dry weight and concentrations in hemolymph were expressed as ng/mL.

Mussel Tissue

At the time of processing, mussel samples were removed from the freezer, partially thawed, dissected from their shells and placed into tared, acid-cleaned 50 mL digestion tubes. The samples were then weighed to the nearest 0.1 g wet weight and were frozen for a minimum of 24 h at ≥ -20ºC. After freezing, the samples were placed in a freeze dryer for a minimum of 36 h. After lyophilizing, the samples were reweighed to determine the dry weight of the sample. All samples were coarsely ground in the plastic digestion tubes with plastic spatulas.

The samples were treated with 2.0 mL of concentrated nitric acid (Ultrex®) and 2.0 mL of concentrated hydrochloric acid (Ultrex®) and allowed to stand at room temperature for approximately 1 h. The samples were then placed in a graphite digestion block with a digital temperature control module. The samples were heated on an automated program for 1 h at 50ºC and 1 h at 80ºC. The samples were removed, allowed to cool, and then 0.5 mL each of concentrated nitric and hydrochloric acids and 3 mL of deionized water were added to each sample. The samples were returned to the block digestion unit and heated for 6 h at 101ºC. Once the digestion program was completed, the samples were allowed to cool and brought to a final volume of 40 mL using deionized water. The samples were tightly capped, shaken, and a 4 mL aliquot was taken for analysis.

Mussel Hemolymph

The mussel hemolymph samples were stored and transported to RTI in 1.2 mL cryotubes. A 0.25 mL aliquot of hemolymph was transferred to an acid washed 15 mL plastic centrifuge tube. 4.8 mL of a 2.5% HCl/ 2.5% HNO_3 acid extraction solution was added to each sample and each sample was vortex mixed. All samples were placed in a water bath at 60°C for 30 min. Samples were removed from the bath, vortex mixed, allowed to cool to room temperature, and then centrifuged for 30 min at 2,800 RPM. A 3 mL aliquot of the supernatant liquid was removed for analysis.

Statistical Analysis

Results for Na^+,K^+-ATPase activity were analyzed following a mixed-effects linear model with concentration and time considered as fixed-effect factors, and mussels and subsamples within each mussel considered as random factors. Analyses were performed with Proc MIXED procedure in SAS v9.1.3 (SAS Institute, Cary, North Carolina). Homogeneity of variances among treatments was tested with Brown Forsythe's test available in SAS (Proc GLM). Residual plots were used to visually analyze statistical assumptions for the analysis of variance: homogeneity, normality, and independence of errors. Response variables that showed increasing variability with increasing response were log transformed (log: base 10) prior to analysis to achieve homogeneity of variances. Means were compared using Dunnett's test.

Results

The relationship between exposure concentration and tissue concentration of Pt and Pd and were highly correlated with exposure concentration and were fit to a power function (Table 2, Figure 1). Accumulation of Pt and Pd from solution was linear and increased continuously throughout the experiment. The relationship between exposure concentration and hemolymph concentration for both metals was also modeled by a power function, but displayed more variation than tissue concentrations and less correlation with exposure concentration (Table 3, Figure 2). Hemolymph uptake was non- linear and for all exposure concentrations, reached a maximum concentration on day 7 or day 14 and decreased by day 28.

All mussel tissue and hemolymph concentrations from the exposed groups were greater than the tissue and hemolymph concentrations from the control groups. By day 28 the mussel tissue concentrations from the lowest exposure concentration group (0.05 µg/L) were 14.7 times greater for Pd and 16.5 times greater for Pt than. Hemolymph concentrations in the 0.05 µg/L group were 8.0 times greater for Pd and 1.9 times greater for Pt than hemolymph concentrations in controls on day 28.

Na^+,K^+-ATPase activity in gill tissue initially decreased during the 5 d acclimation period. The Na^+,K^+-ATPase activity of baseline mussels was 4.0 µmoles ADP/mg protein/hour, but after 5 d of acclimation in laboratory conditions, the Na^+,K^+-ATPase activity in mussels sampled on day 0 had decreased to a mean of 1.63 µmoles ADP/mg protein/hour. Mussels sampled on day 7, 14, 28 displayed a general trend of increasing Na^+,K^+-ATPase activity with increasing Pt and Pd exposure concentration.

On day 7 and day 28, the lowest exposure group (0.05 µg/L) was slightly less than controls, but the difference was not statistically significant. All other Na^+,K^+-ATPase activities were greater than controls and the increase was modeled by a power function (Table 4). Due to high variation within treatment groups, the only comparisons that were statistically different from the same day control, using Dunnett's adjusted probability values ($P<0.05$), were the 5.0 µg/L and 50 µg/L treatment, from the day 28 samples (Figure 3).

Ion concentrations in mussel hemolymph initially responded to increased Pt and Pd contamination by increasing Ca^{2+} and decreasing Na^+ concentration. K^+ concentrations were not decreased until day 28 of the experiment and Cl^- concentrations remained fairly stable. All hemolymph ion relationships were described by logarithmic equations (Table 5).

Hemolymph Ca^{2+} concentrations tended to increase with increasing exposure concentration during the first two weeks of exposure (Figure 4). On day 7, Ca^{2+} concentrations in the two highest exposure treatments, 50 µg/L and 500 µg/L, were statistically different from the controls. By day 14, Ca^{2+} levels in the 50 µg/L group had dropped below concentrations reflecting statistical significance, but concentrations in the 500 ug/L treatment remained significantly higher than controls. Hemolymph Na^+ concentrations tended to decrease with increasing exposure concentration throughout the exposure (Figure 5). By day 28, Na^+ concentration in the 5.0 µg/L and 50 µg/L treatments were significantly decreased compared to control. Hemolymph K^+ ion levels displayed a shifting trend during this experiment (Figure 6). On day 7, hemolymph K^+

ion levels displayed a decreasing trend with increasing exposure concentration. On day 14, K^+ ion concentrations increased with increasing exposure concentration, and by day 28, the trend was again decreasing with increasing exposure concentration, with the 50 µg/L treatment statistically different from control. Hemolymph Cl^- concentrations were highly decreased in the 500 µg/L treatments on days 7 and 14 and moderately decreased in the 50 µg/L treatment on day 28 (Figure 7).

Discussion

The present study demonstrates that *E. complanata* is able to accumulate Pt and Pd from solution. The uptake of Pt and Pd was linear and increased continuously throughout the 28 d exposure, indicating that the mussels had not reached equilibrium and may have continued to accumulate Pt and Pd for a longer period. In a similar study, zebra mussels continued to accumulate Pd from solution at a concentration of 500 µg/L for 10 weeks without reaching equilibrium and without significant mortality (Frank et al. 2008). Tissue concentration for both Pt and Pd were highly correlated with exposure concentration, indicating that *E. complanata* is a good bioindicator of PGM in solution prior to reaching equilibrium.

Hemolymph concentrations of Pt and Pd were more variable and less correlated with exposure concentration than mussel tissue concentrations. Instead, Pt and Pd concentrations in mussel hemolymph reached their peak on day 7 or day 14 for all treatments, but subsequently declined and were all decreasing by day 28 despite continuing increases in mussel tissue concentration. The mechanism responsible for this

decrease in hemolymph concentrations is unknown. However, the decreased correlation with exposure concentration makes hemolymph Pt and Pd concentrations less useful than whole tissue concentrations as a biomarker of PGM contamination.

Hemolymph ion concentrations were assessed for use as a biomarker of Pd and Pt exposure. In this study mussel Ca^{+} was increased compared to control on day 7 of the exposure in the 50 µg/L, but these concentrations were within reference levels of hemolymph Ca^{+} reported by Gustafson et al. (2005a) and had fallen back to within the control range by day 14. K^{+} was variable throughout the exposure and concentrations were only significantly decreased on day 28 in the 50 µg/L treatment. The most consistent fluctuation in hemolymph ion levels was the decrease in hemolymph Na^{+} concentrations at the higher exposure concentrations. For each sampling period, the concentration of Na^{+} in the hemolymph in the 50 µg/L treatment was less than the lowest hemolymph Na^{+} level (344 mg/L) reported by Gustafson et al. (2005b). A similar pattern of impaired osmoregulation has been described in the freshwater mussel *Anodonta cygnea* exposed to Cd, where an immediate decrease in Na^{+} was compensated for by an increase in Ca^{+} that soon stabilized and was later followed by a drop in K^{+} (Hemelraad 1990a). In a later study, it was suggested that the mechanism responsible for the changes in hemolymph ion levels was attributable to interference with oxidative metabolism within the cells of the gill and those of the kidney, affecting influx and efflux rates of ions (Hemelraad 1990b). If this were true in *E. complanata,* a switch to increased anaerobic metabolism in the cells of the gill could lead to an excess of NH^{4+} ion in the cell, which

has been shown to upregulate Na^+,K^+-ATPase activity in the freshwater shrimp *Macrobrachium olfersi* (Furriel 2004).

The initial decrease in Na^+,K^+-ATPase activity observed between the baseline mussels and the day 0 mussels could be a natural response to acclimation to the laboratory environment. The ASTM soft water used to contain mussels in the laboratory had a greater average specific conductivity (165 µS/cm) compared to the specific conductivity of the water from the Eno River, North Carolina (88 µS/cm), where the source mussels were collected. Na^+,K^+-ATPase activity in mussels from control treatments increased slightly throughout the experiments, but remained within the 95% confidence interval of the day 0 mussels throughout the experiment, and Na^+,K^+-ATPase activity increased in mussels exposed to Pt and Pd. By day 28, the Na^+,K^+-ATPase activity in mussels from the 5 µg/L and the 50 µg/L had increased to the levels observed in the baseline mussels. The increase in Na^+,K^+-ATPase activity observed in this study could represent a compensatory mechanism used by the gill cells to compensate for the negative effects of reduced oxidative metabolism and impaired gill and renal function.

The combination of high variability in Na^+,K^+-ATPase and the lack statistical significant difference in activity levels at environmentally relevant concentrations make the use of Na^+,K^+-ATPase activity questionable as a biomarker of Pt and Pd exposure. The additional confounding issue of activity fluctuation due to fluxes in ambient solute concentrations would make it unusable as a biomarker in the field.

Significant changes in Na^+,K^+-ATPase activity and in ion concentrations were only observed at the highest exposure concentrations, despite significantly elevated levels

of tissue Pt and Pd in all of the exposure groups. Based on the responses measured in this study, the environmentally relevant concentrations of Pt (<2.0 ng/g in mussel tissue) and Pd may not pose an urgent concern. However, this study constitutes only a first step in elucidating the dynamics and effects of PGM in freshwater mussels. The response to PGM exposure should be evaluated in other species and in additional life stages in mussels to ensure that chronic low level PGM contamination does not have an adverse impact on mussel species density and richness at environmentally relevant concentrations.

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Tables

Table 1. Average daily exposure of Pt and Pd (µg/L) in test water sampled from aquaria throughout the 28 d exposure period. Three 5 mL samples were collected from three aquaria per concentration at random and combined for analysis. Time (T) is represented in hours after addition of the Pt/Pd test solution and values are an average of the sample concentrations collected at the corresponding time point. % of Target is the percent of the intended exposure concentration of Pt or Pd that was recovered at each time point. The average daily exposure is the calculated by averaging three T 0, three T 48, and one T 72 time points.

Target Pt	Mean Measured Concentration (ug/L)						
Concentration (ug/L)	T 0 (*n=4)	% of Target	T 48 (*n=3)	% of Target	T 72 (*n=4)	% of Target	Average Daily Exposure
Control 0	0.03	n/a	0.01	n/a	0.01	n/a	0.00
0.05	0.04	80.0	0.04	87.3	0.04	77.0	0.04
0.5	0.38	75.3	0.34	68.2	0.32	63.8	0.35
5	4.50	89.9	3.77	75.5	3.55	71.1	4.05
50	51.77	103.5	36.34	72.7	29.37	58.7	41.96
500	454.25	90.9	382.39	76.5	129.77	26.0	377.10

* Each sample is a composite sample from three aquaria

Target Pd	Mean Measured Concentration (ug/L)						
Concentration (ug/L)	T 0 (*n=4)	% of Target	T 48 (*n=3)	% of Target	T 72 (*n=4)	% of Target	Average Daily Exposure
Control 0	0.00	n/a	0.00	n/a	0.00	n/a	0.00
0.05	0.00	-1.0	0.01	10.0	0.01	13.0	0.00
0.5	0.04	8.0	0.09	18.8	0.09	18.8	0.07
5	0.60	12.1	1.02	20.3	1.07	21.4	0.85
50	12.83	25.7	5.72	11.4	7.38	14.8	9.01
500	427.26	85.5	125.94	25.2	95.80	19.2	250.77

* Each sample is a composite sample from three aquaria

Table 2. Regression of Pt and Pd concentrations (ng/g) in mussel tissue as a power function of dissolved Pt and Pd exposure concentration at 7, 14 and 28 d of exposure (x= exposure concentration (ng/L)+1, y= tissue concentration (ng/g)).

Element	Source	Day	Equation	R^2
Platinum	Tissue	7	$y=0.53x^{0.76}$	0.99
Platinum	Tissue	14	$y=0.63x^{0.79}$	0.99
Platinum	Tissue	28	$y=0.82x^{0.79}$	0.99
Palladium	Tissue	7	$y=1.07x^{0.62}$	0.99
Palladium	Tissue	14	$y=2.02x^{0.61}$	0.92
Palladium	Tissue	28	$y=0.79x^{0.82}$	0.92

Table 3. Regression of Pt and Pd concentrations in mussel hemolymph as a power function of dissolved Pt and Pd exposure concentration at 7, 14 and 28 d of exposure (x= exposure concentration (ng/L)+1, y= hemolymph concentration (ng/g)).

Element	Source	Day	Equation	R^2
Platinum	Hemolymph	7	$y=0.19x^{0.20}$	0.83
Platinum	Hemolymph	14	$y=0.29x^{0.20}$	0.84
Platinum	Hemolymph	28	$y=0.15x^{0.24}$	0.89
Palladium	Hemolymph	7	$y=0.25x^{0.16}$	0.54
Palladium	Hemolymph	14	$y=0.44x^{0.18}$	0.59
Palladium	Hemolymph	28	$y=0.65x^{0.35}$	0.87

Table 4. Regression of Na^+,K^+-ATPase activity in mussel gill tissue as a power function of dissolved Pt and Pd exposure concentration at 7, 14 and 28 d of exposure (x= exposure concentration (ng/L)+1, y= Na^+,K^+-ATPase activity (μmoles ADP/mg protein/hour)).

Day	Equation	R^2
7	$y=1.10x^{0.02}$	0.05
14	$y=2.46x^{0.02}$	0.78
28	$y=2.13x^{0.05}$	0.72

Table 5. Regression of Pt and Pd concentrations in mussel hemolymph as a logarithmic function of dissolved Pt and Pd exposure concentration at 7, 14 and 28 d of exposure (x= exposure concentration (ng/L)+1, y=hemolymph ion (mg/L)).

Ion	Day	Equation	R^2
Na^+	7	y= -6.29 ln(x)+416.45	0.34
Na^+	14	y= -5.77 ln(x)+363.34	0.36
Na^+	28	y= -11.20 ln(x)+366.68	0.73
Ca^+	7	y= 6.80 ln(x)+117.24	0.43
Ca^+	14	y= 3.57 ln(x)+133.60	0.25
Ca^+	28	y= 4.63 ln(x)+104.76	0.45
K^+	7	y= -0.72 ln(x)+22.27	0.35
K^+	14	y= 0.44 ln(x)+17.55	0.64
K^+	28	y= -0.94 ln(x)+24.99	0.67
Cl^-	7	y= 2.33 ln(x)+324.27	0.08
Cl^-	14	y= -5.81 ln(x)+334.91	0.79
Cl^-	28	y= -4.03 ln(x)+255.08	0.12

Figures

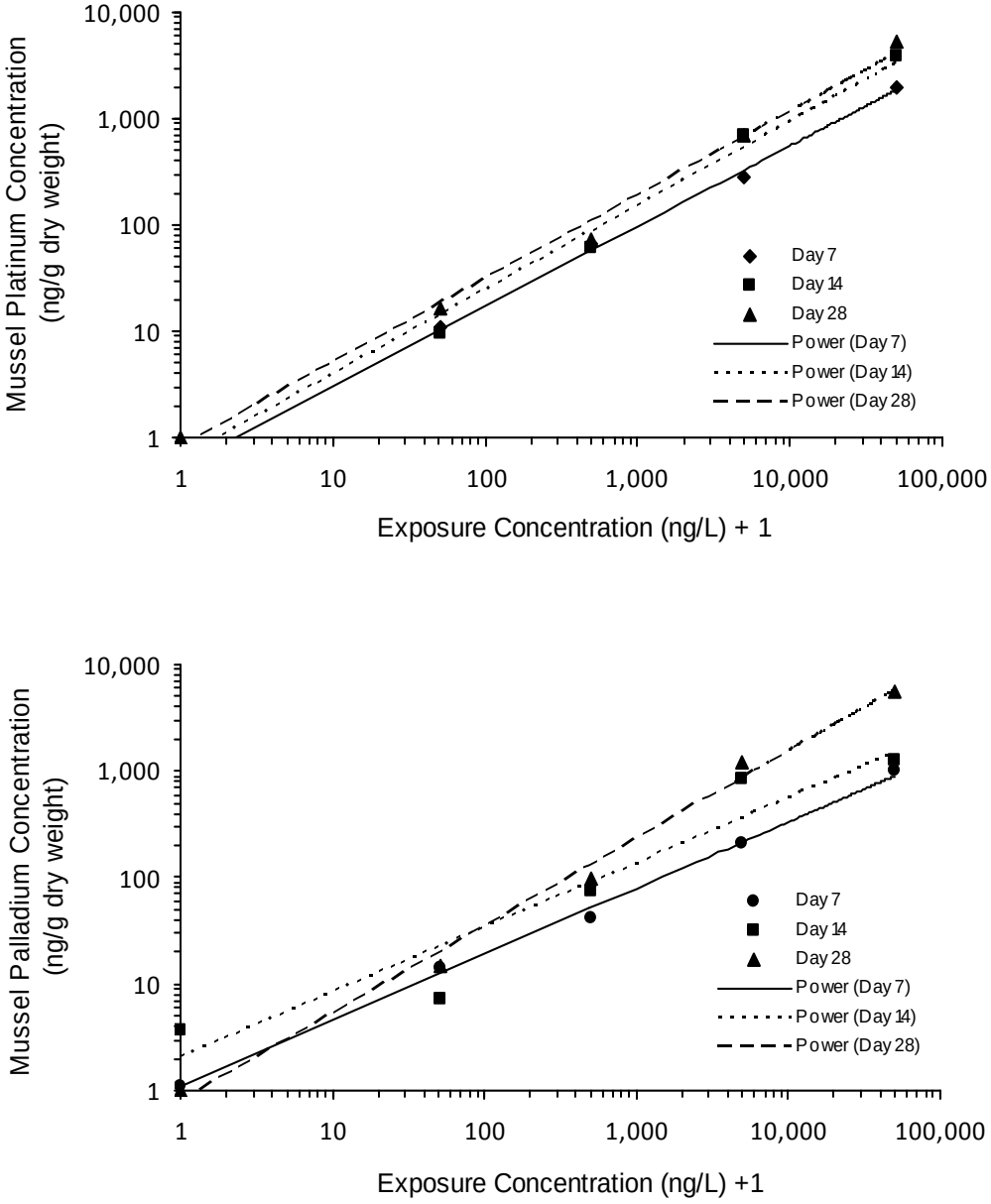

Figure 1. Relationship between Pt and Pd concentrations (ng/g) in mussel tissue and dissolved Pt and Pd exposure concentration (ng/g) +1 at 7, 14 and 28 d of exposure.

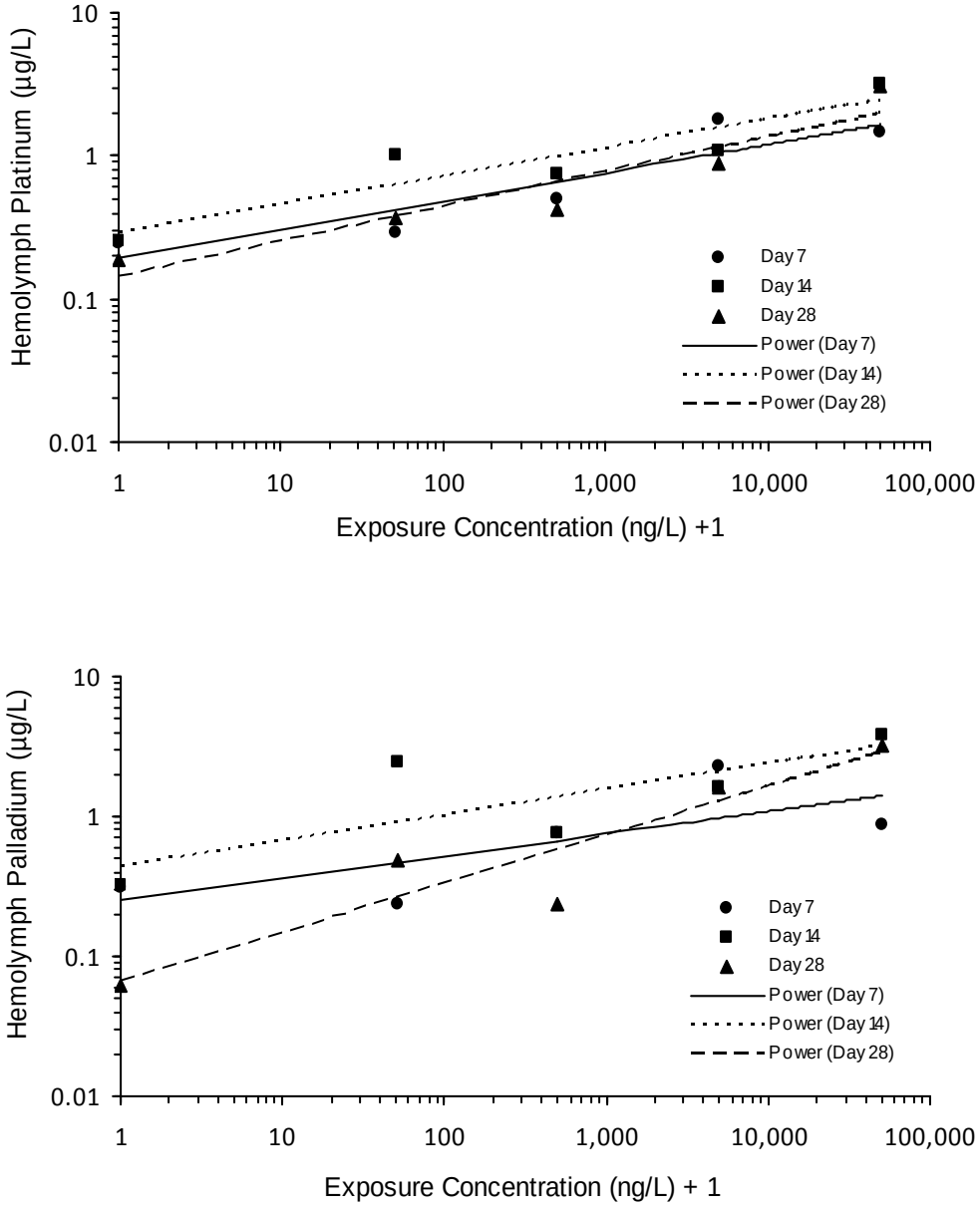

Figure 2. Relationship between Pt and Pd concentrations (µg/L) in mussel hemolymph and dissolved Pt and Pd exposure concentration (ng/L) +1 at 7, 14 and 28 d of exposure.

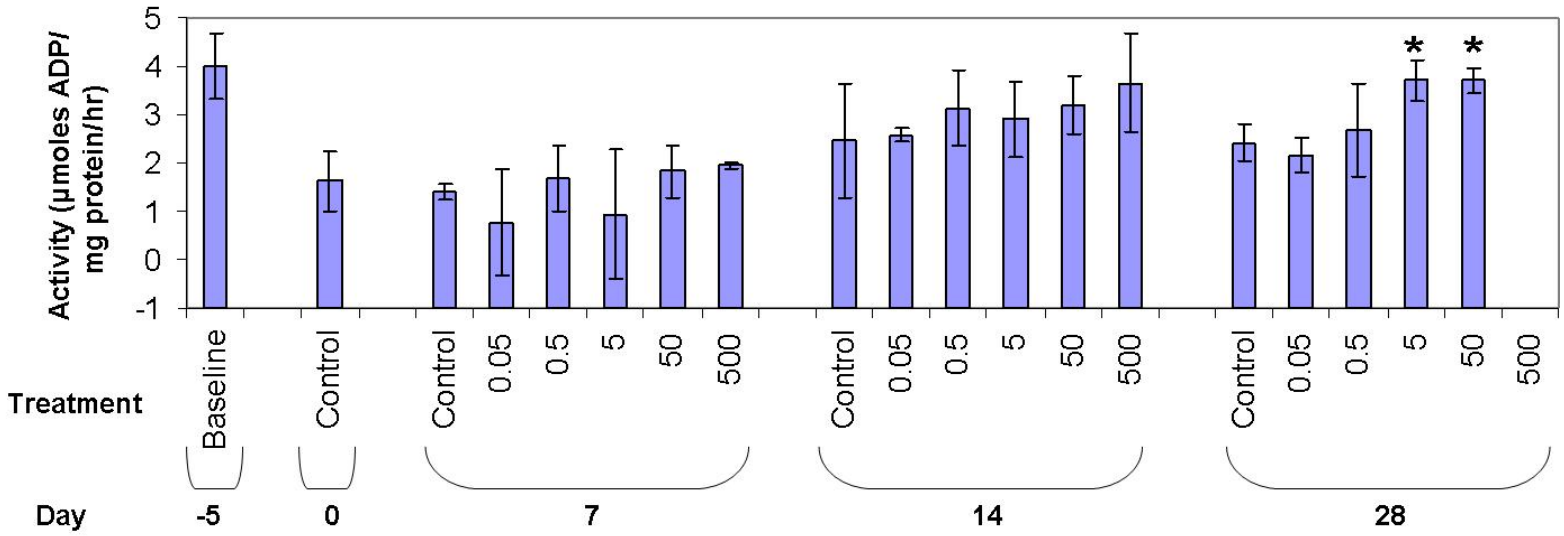

Figure 3. Na^{+},K^{+}-ATPase activity (µmoles ADP/mg protein/hr) measured in mussel gill tissue collected on day -5 from the source location (baseline), day 0 pre-exposure control mussels, and from exposed mussels on days 7, 14, and 28. Error bars represent $\pm$1 standard deviation. Asterisks indicate results are statistically different from control sampled on the same day.

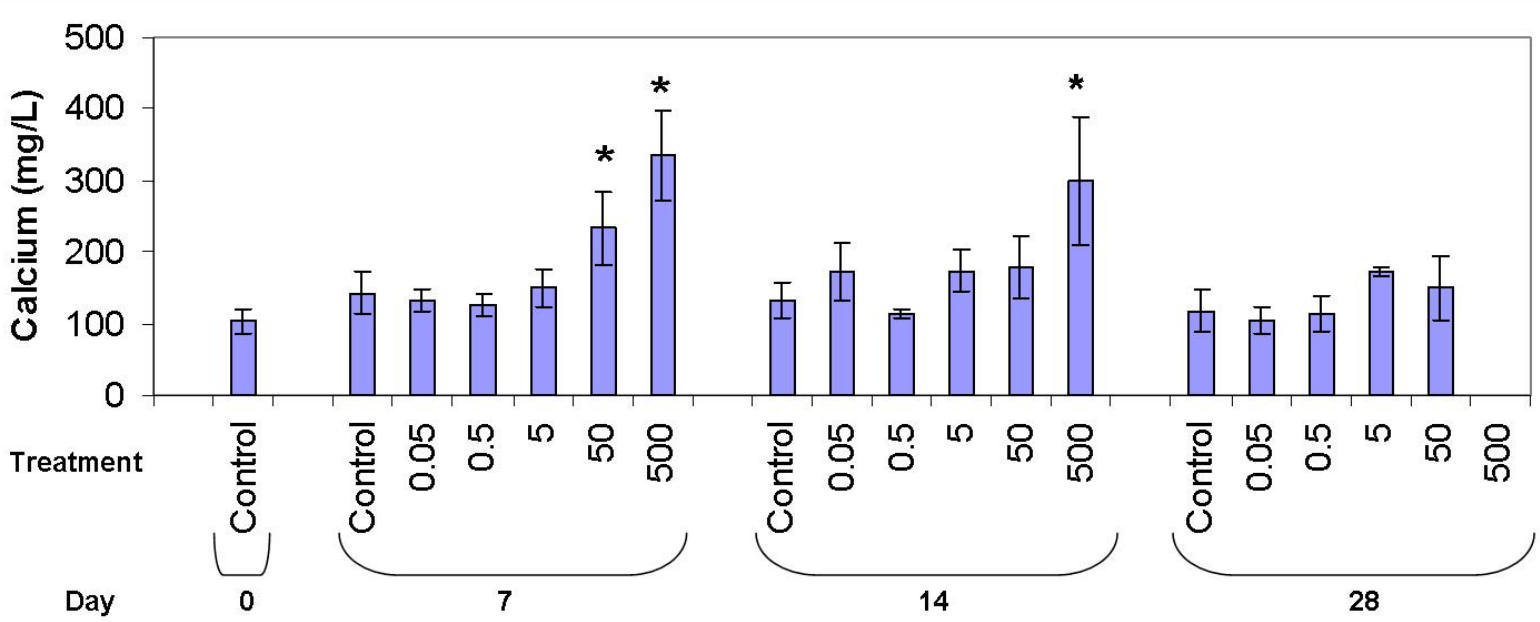

Figure 4. Calcium (Ca^{2+}) concentration measured in mussel hemolymph collected from day 0 pre-exposure control mussels, and from exposed mussels on days 7, 14, and 28. Error bars represent $\pm$1 standard deviation. Asterisks indicate results are statistically different ($P<0.05$) from control sampled on the same day.

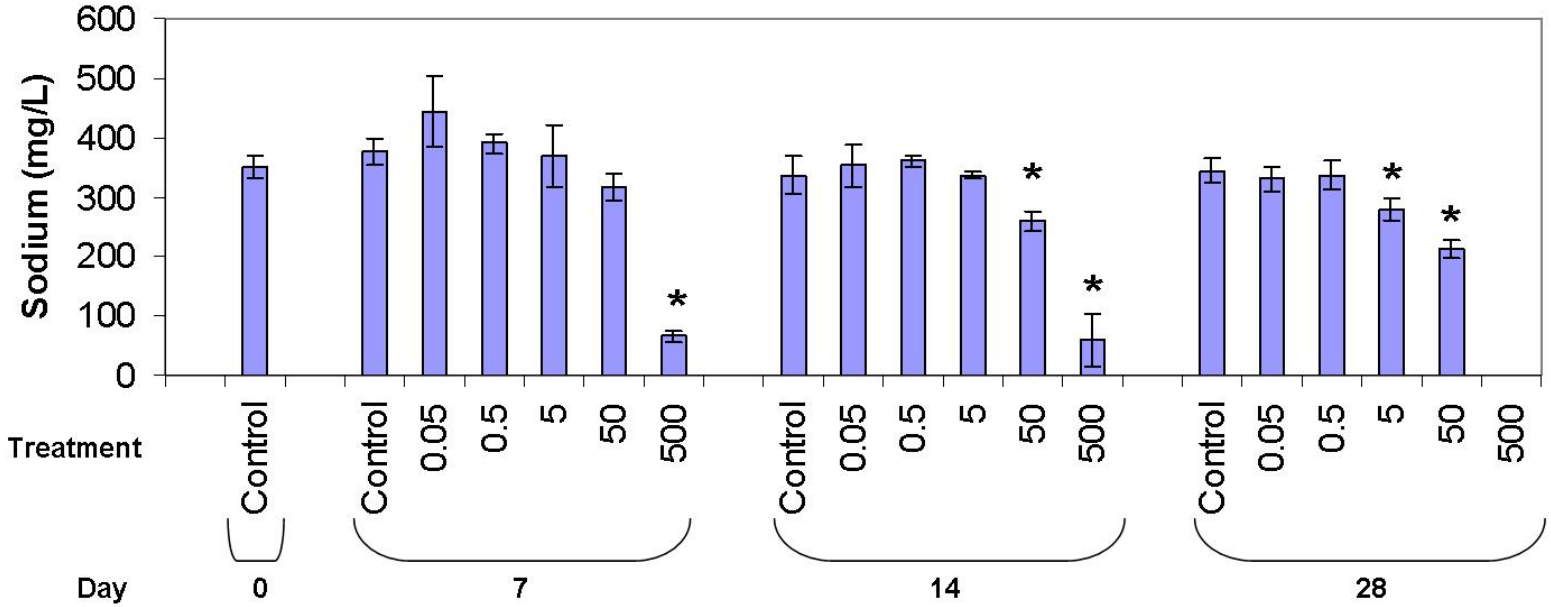

Figure 5. Sodium (Na^+) concentration measured in mussel hemolymph collected from day 0 pre-exposure control mussels, and from exposed mussels on days 7, 14, and 28. Error bars represent $\pm$1 standard deviation. Asterisks indicate results are statistically different ($P<0.05$) from control sampled on the same day.

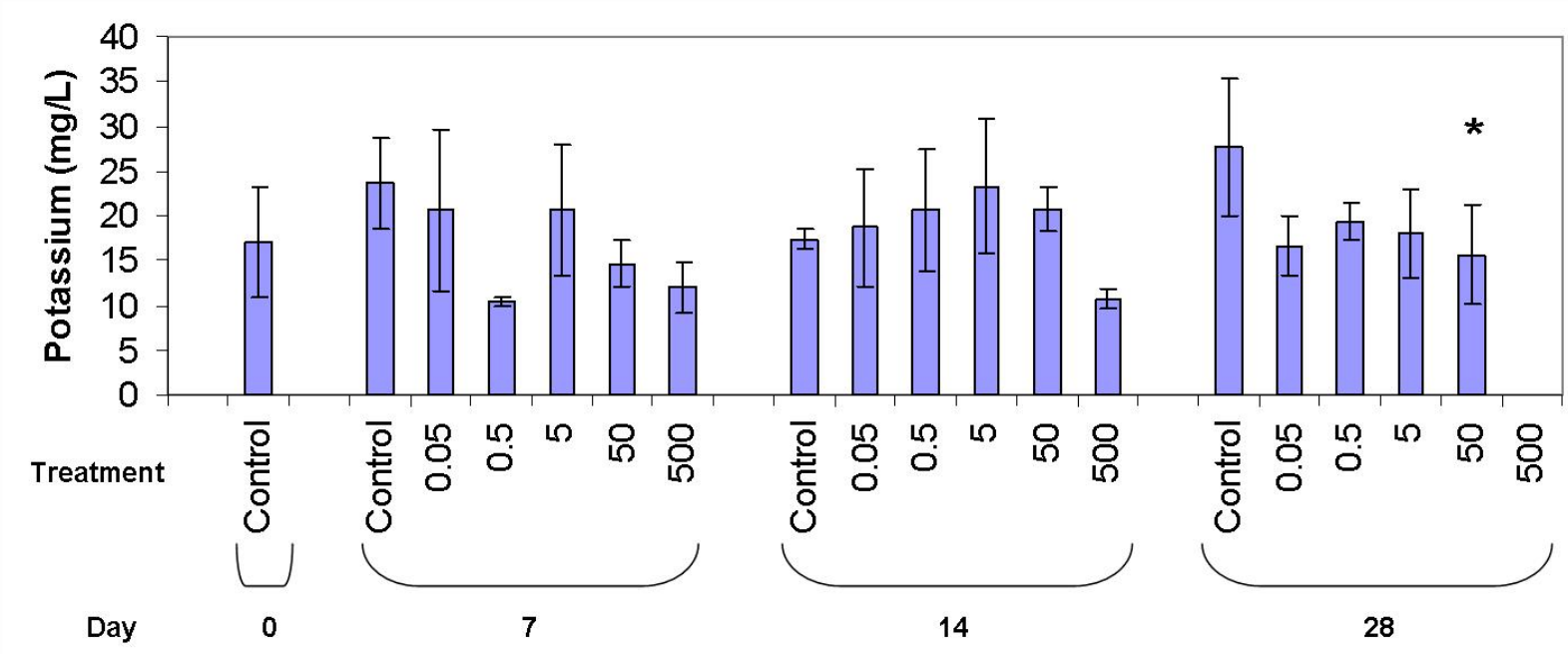

Figure 6. Potassium (K^+) concentration measured in mussel hemolymph collected from day 0 pre-exposure control mussels, and from exposed mussels on days 7, 14, and 28. Error bars represent $\pm$1 standard deviation. Asterisks indicate results are statistically different ($P<0.05$) from control sampled on the same day.

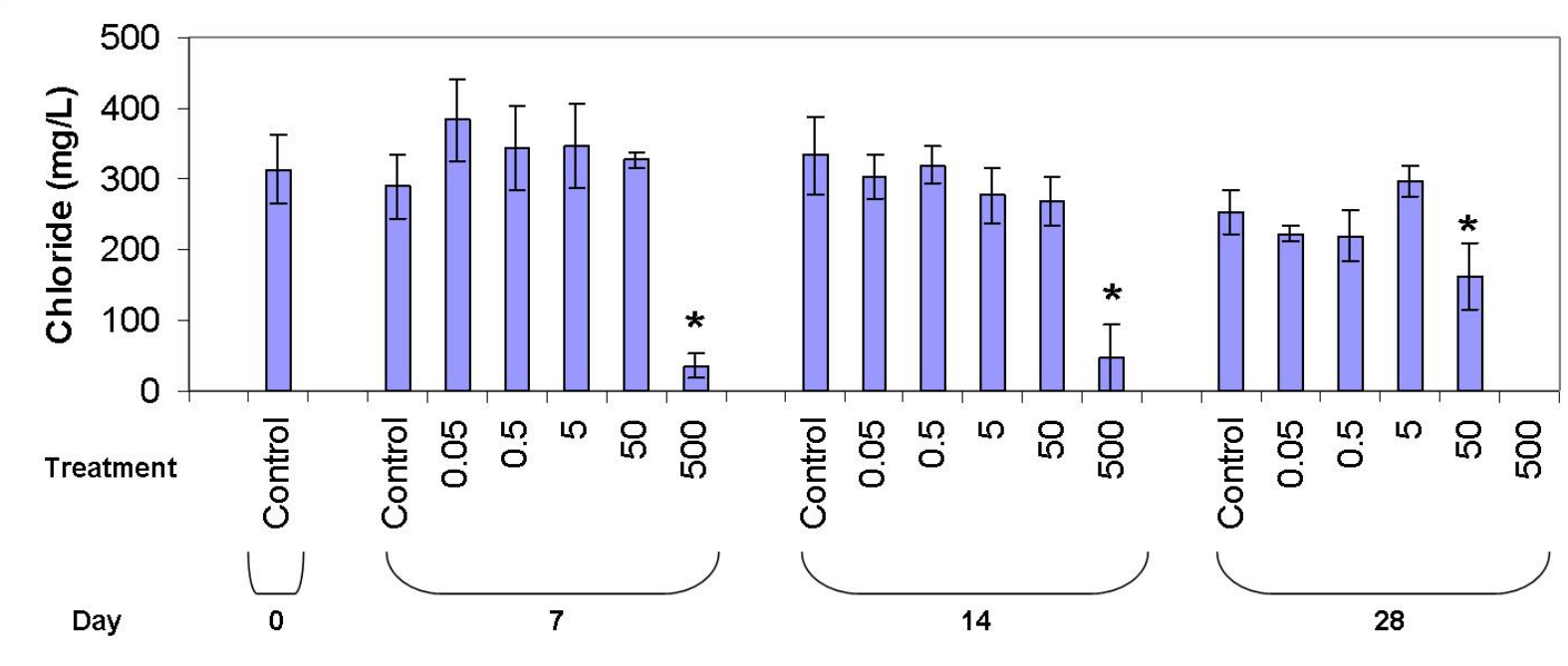

Figure 7. Chloride (Cl^-) concentration measured in mussel hemolymph collected from day 0 pre-exposure control mussels, and from exposed mussels on days 7, 14, and 28. Error bars represent $\pm$1 standard deviation. Asterisks indicate results are statistically different ($P<0.05$) from control sampled on the same day.

APPENDIX

Na^+, K^+-ATPase Assay – Microplate Assay

I. Buffer preparation and reaction mixtures

Salt Solution

Imidazole (50 mM)	1.702 g
NaCl (189 mM)	5.52 g
$MgCl_2 \cdot 6H_2O$ (10.5 mM)	1.02 g
KCl (42 mM)	3.14 g

Add 450 mL deionized water, adjust to pH 7.5 with HCl, qs to 500 mL. Store up to 3 months at 4°C.

Salt Solution (-K^+)

Imidazole (50 mM)	1.702 g
NaCl (189 mM)	5.52 g
$MgCl_2 \cdot 6H_2O$ (10.5 mM)	1.02 g

Add 450 mL deionized water, adjust to pH 7.5 with HCl, qs to 500 mL. Store up to 3 months at 4°C.

SEI Buffer

Sucrose (250 mM)	42.79 g
Na2EDTA (10 mM)$\cdot 2H_2O$	1.86 g
Imidazole (50 mM)	1.70 g

Add 450 ml deionized water, adjust to pH 7.3 with HCl, qs to 500 mL. Store up to 1 month at 4°C.

0.3% SEID (3X concentrate)

0.1 g Na deoxycholic acid in 33.3 mL SEI, (0.0751g in 25 mL). Store up to 1 week at 25°C.

Assay Mixture (AM) Reagents

Sigma #	Buffer, Abbreviation, Molecular Weight	Stock Conc.	Final Conc.	Conc. in AM
I-2399	**Imidazole Buffer (IB; MW = 68.08)** 1.702 g Imidazole in 475 mL DiH_2O, adjust to pH 7.5 with HCl, qs to 500 mL, store up to 3 months at 4°C	50 mM	50 mM	50 mM
P-7252	**Phosphoenolpyruvate (PEP; MW = 465.6)** 0.978 g in 100 mL IB, divide into 10 or 20 mL aliquots, store up to 6 months at -80°C	21 mM	2 mM	2.8 mM
N-6005	**NADH- reduced (MW = 709.4)** Add to assay mixture, make fresh with each batch of AM	–	0.16 mM	0.22 mM
A-3377	**Adenosine Triphosphate (ATP; MW = 551.1)** Add to assay mixture, make fresh with each batch of AM	–	0.5 mM	0.7 mM
L-2500	**Lactic Dehydrogenase (LDH)** Spin for 8 minutes at 12,000g at 4°C; a distinct pellet should result, remove supernatant, suspend pellet in several mL IB; add to assay mixture, make fresh with each batch of AM	–	3.3 U/ml	4.6 U/ml
P-1506	**Pyruvate Kinase (PK)** Spin for 8 minutes at 12,000g at 4°C; a distinct pellet should result, remove supernatant, suspend pellet in several mL IB; add to assay mixture, make fresh with each batch of AM (centrifuge LDH and PK together)	–	3.6 U/ml	5.1 U/ml

The final concentration calculations include the salt solution and homogenate volumes (210 µL)

II. Assay Standard

ADP Standard (MW = 427.2)
4mM Stock Solution:
0.0427g in 25 mL Na Acetate (57 mM)
(0.4627g Na Acetate in 100 mL deionized water, pH 6.8)
Store in 500 μL aliquots at –80°C. On day of assay, thaw aliquot and dilute:

nmoles/10 μL	IB	4 mM ADP Stock
0	200 μL	0 μL
5	175 μL	25 μL
10	150 μL	50 μL
20	100 μL	100 μL

Each standard is added to the plate in quadruplicate of 10 μL, after which, 200 μL of AM/Salt Solution is added. Standard curve slope should be in the range of 17-19 mOD/nmole.

III. Assay Mixture Recipe

Solution	4 microplates (96 assays)	8 microplates (192 assays)
PK	30.6 μL *	61.2 μL *
LDH	24.3 μL *	48.6 μL *
NADH	10.9 mg	21.8 mg
PEP	9.33 mL	18.66 mL
ATP	27 mg	54 mg
IB	qs to 75 mL	qs to 150 mL
	Divide into 35 mL halves	Divide into 70 mL halves
	Add 11.66 mL Salt Solution to one (A)	Add 23.32 mL Salt Solution to one (A)
	Add 11.66 mL Salt Solution ($-K^+$) to the other (B)	Add 11.66 mL Salt Solution ($-K^+$) to the other (B)
	Make AM fresh every day to ensure quality	

* Swirl Sigma vial; remove indicated volume; centrifuge for 8 minutes at 12,000 g at 4°C; remove supernatant; resuspend in IB; add to assay mixture. These values change with each vial bought. Recalculate amount every time enzymes are bought.

IV. Sample Preparation and Assay Notes

1. Run standard curves for ADP

a. ADP standard curve is run to ensure that reagents for that batch of assay mixture are prepared correctly and in good condition. Additionally, this is the slope that is used to calculate ATPase activity.
b. The ADP curve is usually 13-14 mOD/nmole ADP.
c. When running the ADP standard curve there should be rapid equilibrium of ADP (within 3 – 4 minutes) and the optical density of the 0 Standard should be between 0.4 and 1.2 OD units.
d. If either of these 2 observations are not made then one or more reagents have gone bad or is not present in high enough concentrations.

2. Sample preparation and assay protocol

a. Gill tissue is stored in 100 μL SEI buffer at –20^{o}C for up to 2 months and –80^{o}C for 6 months
b. Thaw samples immediately prior to assay and add 50 μL SEID (3X concentrate).
c. Homogenize in tube (20 – 30 seconds), ensuring all tissue is homogenized.
d. Centrifuge at 5,000g for 1 minute at 4°C to remove insoluble material.
e. Pipette 10 μL of sample into 4 wells (2 for Solution A and 2 for Solution B). Uses a total of 40μL homogenate.
f. With AM/Salt solutions still in ice bath, add 200 μL of either solution A or B to respective wells.
g. Read plate at 340 nm for 10 minutes with 60-second intervals at room temperature.

V. Bradford Protein Assay

Methods

1. Prepare standards for curve in centrifuge tubes.
2. Pipette 90 µL of Salt solution into centrifuge tubes for samples.
3. Pipette 10 µL of each homogenate sample into appropriate centrifuge tubes.
4. Add 1 mL of reagent to each tube.
5. Vortex samples briefly.
6. Incubate for 2 minutes at 25^{o}C, and transfer to 1 mL microcuvetes.
7. This is an endpoint assay read at a wavelength of 595 nm

Preparation of standards:

ug/10uL	BSA standard	Salt Solution
0	--	100 µL
5	5 µL	95 µL
10	10 µL	90 µL
15	15 µL	85 µL
20	20 µL	80 µL

VI. ATPase Activity Calculation

The ATPase standard curve should be read in mOD/nmole ADP
The ATPase acitivy measurements should be read in mOD/10□L /minute

Sample calculation:

Standard curve = 20 mOD/nmole ADP
ATPase (Solution A) = 30.0 mOD/10µL /minute
ATPase (Solution B (-K^{+})) = 13.5 mOD/10µL /minute
Na+,K+-ATPase = 30.0 – 13.5 = 16.5 mOD/10µL/minute
Protein reading = 8.5 µg/10µL

$$\frac{16.5 \text{ mOD}/10\mu\text{L}/\text{minute}}{20 \text{ mOD/nmole ADP}} = 0.825 \text{ nmoles ADP}/10\mu\text{L}/\text{minute}$$

$$\frac{0.825 \text{ nmoles ADP}/10\mu\text{L}/\text{minute}}{8.5 \ \mu\text{g}/10\mu\text{L protein}} = 0.097 \ \mu\text{moles ADP/mg protein/minute}$$

(0.097)(60min) = 5.82 µmoles ADP/mg protein/hour

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CONCLUSIONS

Distribution of PGM in a Stream

This study has demonstrated for the first time that platinum (Pt) and palladium (Pd), platinum group metals (PGM), are accumulated from solution by *Elliptio complanata* and that Pt is enriched in *E. complanata* soft tissues at road crossings in North Carolina. However, the results of this study did not demonstrate a correlation between the number of daily vehicle crossings on a highway waterway crossing structure and the downstream enrichment of mussel tissue or sediment with PGM. The nonpoint source deposition of PGM from automobile catalytic converters along linear highway corridors was expected to behave like a point source discharge by concentrating road deposited sediments and allowing their transport, via stormwater, to a common point of discharge into the stream environment near highway stream crossings. The correlation between PGM in the roadside environment and the density and conditions of automobile traffic is well documented (Helmers 1997, Morcelli et al. 2005, Limbeck et al. 2007, Zereini et al. 2007). Transport from the roadside environment to the stream environment has been shown to occur where road runoff reaches waterbodies (Tuit et al. 2000, Essumang et al. 2007). In this study, we have demonstrated that enrichment of PGM in sediment and mussel tissue is occurring, and that the level of enrichment in the watershed is related to the number of people living in the watershed, and by extension the number of vehicles operating within the watershed.

My conclusions on the lack of observable correlation with traffic fall into two general categories. First, emission of PGM from automobiles is primarily in a metallic

form that is not immediately bioavailable, and chemical weathering of PGM in organic sediments to a bioavialable form favors a dietary route of exposure for mussels. Second, hydraulic alterations of the watershed and stream channel can have an influence on the location of deposition of PGM in the stream sediments, production of the mussel's primary food source, and the location and density of mussels in the stream; all three variables influence the distribution of PGM in sediment and in mussel tissue.

The emission of PGM from automobiles is primarily in a metallic form attached to an alumina substrate (Ravindra et al. 2003). The fraction of this emission that is immediately soluble is small, perhaps less than 5% (Palacios et al. 2000). Soluble ions of PGM are unstable in solution and tend to be rapidly adsorbed to organic molecules in the water column or to sediments (Turner et al. 2006). As shown in the laboratory portion of this study, mussels are able to accumulate Pt and Pd from solution, but the concentration of PGM in solution has been shown to be very low (<0.1 ng/L) even when sediments are enriched to concentrations more than 5 times the sediment concentration observed in our field study (Rauch and Morrison 1999). It has been demonstrated that particulate PGM displays increased solubility in environmental matrices through interaction with organic acids, similar to the environmental conditions encountered in soils and sediments with decaying vegetation, and that complexation with organic molecules increases its mobility (Bowles and Gize 2005). The deposition of PGM particles in organically enriched stream sediments would result in increased solubility of PGM at the site of production of food sources utilized by the mussels.

Mussels are known to feed on suspended particulate organic matter and detritus, apparently preferentially incorporating the living algal and bacterial fraction (Raikow and Hamilton 2001). The sulfate-reducing bacterium *Desulfovibrio desulfuricans* has been shown to accumulate PGM. This bacterium is similar to bacteria naturally found in decaying detritus in aquatic systems (Yong et al. 2002). Marine algae have also been shown to accumulate PGM from solution (Turner et al. 2007). Bacteria facilitating the breakdown of allocthanous organic materials that are contaminated with catalyst emitted particles would be exposed to bioavialable PGM, creating a dietary route.

Another route of exposure could be deposit feeding on biodeposited and other organic sediment components. Mussels are known to biodeposit unused nutrients in the form of pseudofeces and feces (Vaughn and Hakencamp 2001). Recent studies have shown that mussels may derive a substantial portion of their energy by deposit feeding on surrounding organic sources (Raikow and Hamilton 2001). Although it has not been demonstrated that mussels are able to feed on pseudofeces, it seems possible that the organic material in biodeposited pseudofeces may contribute to material available for deposit feeding. Deposit feeding could be accomplished by movement of food particles to the mouth by cilliary action or, more recently suggested, direct absorption of dissolved organic carbon (DOC). Studies with zebra mussels have shown that they are able to utilize dissolved organic carbon from the water column as a food source. In the presence of metals that readily complex with DOC, this form of carbon sequestration increases the metal concentration factors in exposed mussels. It was concluded that the method of

uptake was absorption rather than uptake by filtration and that this route of uptake could supply up to half of the mussels no-growth energy requirements (Roditi et al. 2000).

Complexation with dissolved organic carbon (DOC) has been shown to increase the amount of PGM that is able to remain in solution (Turner et al. 2006). Sures and Zimmermann (2007) demonstrated that the presence of humic substances increased the uptake of Pt, but hampered the uptake of Pd in zebra mussels. Observations that complexation with humic acids and bile increase the lipid solubility of PGM (Zimmermann et al. 2003) introduces the possibility that unionid freshwater mussels may accumulate PGM through deposit feeding and absorption of DOC.

Mussels in dense beds are thought behave as ecosystem engineers, by biodeopsiting nutrients and stabilization of the substrate (Vaughn et al. 2007). The pore water of carbon enriched sediments in mussel beds is likely to have more dissolved organic carbon than the overlying surface water. Additionally, the increased organic activity of sediments in mussel beds may increase the solubility of deposited catalyst derived PGM. Mussels aggregated in dense beds may facilitate increased uptake of PGM and other pollutants by sequestration and feeding on contaminated organic matter that was not incorporated in the first pass of filtration, compared to solitary counterparts.

Creation of mussel beds requires that the flow of water across the bed is adequate to provide the mussels an adequate supply of suspended food particles, but not so great that it destabilizes the bed during high flow. In places where the hydrology allows for long-term biodeposition of organic material in a mussel bed, the efficiency of PGM uptake may be enhanced. In contrast, where mussels are in a more hydraulically active

area of stream, such as a riffle, where pseudofeces and other organic materials may not accumulate, the bioaccumulation of PGM may be limited to only what is incorporated in the first pass of filtration. If the bioaccumulation of PGM is dependent on the transformation of catalyst derived PGM in the sediment and its complexation with the mussels food source, then the behavior of the deposition of these small particles will likely be important in their pattern of distribution in mussel tissues, as shown in our data collected from New Hope Creek, North Carolina.

If hydrology of the stream plays in important role in PGM deposition, speciation, and incorporation into mussels, then alterations to the natural hydrology of the stream could alter patterns of PGM abundance. The field portion of our investigation was largely limited to the areas of stream immediately surrounding a highway crossing of a stream. Bridges and culverts are known to cause alteration of the area immediately downstream and sometimes for some distance upstream of the crossing. Bridges over North Carolina streams are almost never long enough to span the entire floodplain of the stream. Where the structure does span the floodplain, bridge bents are necessarily placed in the stream or in the floodplain to achieve adequate span. A structure that constricts the natural floodplain invariably causes hydraulic alteration. The exact nature of this alteration is based on many factors and is site specific. My general observation of piedmont streams in North Carolina is that bridges commonly cause stream scour immediately downstream of the structure by increasing the maximum velocity of the water that passes under the structure during high water events. Often this is accompanied by bank destabilization and alteration of the substrate (Johnson 2006). Importantly, the

increased range of stream velocity immediately downstream of the bridge tends to scour the substrate, keeping it relatively free of organics and often leading to deposition of sand. This periodic cleaning of the substrate also causes biodeposited and other fine organics to be washed away, potentially prohibiting the establishment of dense mussel beds.

In the final phase of our field experiment, multiple samples were collected from New Hope Creek in Orange County, North Carolina, in an effort to estimate the variability of the composite samples of mussels collected at other sites. The coefficient of variation at six sites ranged from 12 to 70%. Of these samples, the coefficient of variation of the tissue concentrations of Pt were much higher immediately downstream of the I-40 crossing of New Hope Creek. The wide, multichannel box culvert under I-40 allowed for a well established and dense mussel bed approximately 150 m downstream of the crossing. Farther downstream (150 m), NC Hwy 86 crossed New Hope Creek. This bridge was a short span bridge, common on secondary roads in North Carolina, and caused considerable floodplain constriction. Downstream of NC Hwy 86 (150 m), mussel density was considerably lower and mussels were primarily confined to the more stable areas along banks. Mean tissue Pt concentrations immediately downstream of NC 86 were one-third of the concentrations immediately downstream of I-40 despite being separated by only 300 m. One kilometer downstream of NC Hwy 86, mussel densities remained low and tissue Pt was similar to concentrations observed immediately downstream of NC Hwy 86. At sites 2.5 km and 4.5 km downstream of the I-40 and NC 86 crossings, mussel densities were high and hydrologic conditions permitted a well

established mussels bed, and tissue Pt concentrations were highly elevated compared to samples from upstream of I-40 and immediately downstream of the I-40 crossing, despite no apparent source of significant additional input of PGM between sites. At a site 6.7 km downstream of the I-40 crossing, levels of Pt in mussel tissue and sediment had decreased to within the range observed upstream of I-40, which I consider to represent a background concentration for this part of the watershed.

My interpretation of the distribution of mussel tissue Pt enrichment in New Hope Creek is that PGM metal particles from I-40 and NC 86 are being unevenly distributed in the area downstream of the sources, largely dependent on hydraulic characteristics. Due to the process of transformation in the sediment and subsequent transport on food particles, the peak of PGM accumulation in mussel tissue is not observed for several km downstream from the primary source. Based on the available data, the area of maximum enrichment from the I-40 and NC 86 crossing is somewhere between the sites located between 2.5 km and 4.5 km downstream of the primary source (i.e., I-40) (Figure 5. Chapter 1).

The implication of this observation for transportation planning, in areas that support protected species of mussels, is that the impact of road derived pollutants is projected for a considerable distance from a road crossing (i.e., a longitudinal lag). A typical distance for consideration of adverse effects to protected mussels is 400 m. Based on the data gathered in this project, it may be necessary to consider impacts from transportation projects for several kilometers downstream of a crossing.

Potential Threat to Freshwater Mussels

This study represents the first information available on the concentrations of PGM in unionid freshwater mussels. Based on the data that we gathered, the concentration of PGM in freshwater mussels is low relative to other common metal pollutants (i.e., Cd and Hg), but the enrichment in mussels over background levels and the short time these metals have been appreciably emitted into the environment elevate them as an environmental concern.

Reported natural background concentrations in the earth's crust are 0.4 ng/g Pt and Pd (Whedepol 1995). In this study, the observed maximum sediment concentrations were 1.86 ng/g; approximately 4.7 times the background level. Due to the inert form of emission of PGM and slow transformation to its bioavailable form, it is possible that a considerable portion of the PGM emitted within the last three decades remains available for transformation. If this is true, we would expect to see the concentrations of PGM in the mussel tissues to increase disproportionately to its emission from automobiles. The streams included in this study were limited to streams that were able to support mussel fauna and are generally among the least impacted in the region. The enrichment of heavily degraded streams is likely to be greater than in streams that support mussels.

It has been my experience that in streams in the piedmont region of North Carolina supporting species rich mussel assemblages are restricted to relatively undisturbed watersheds. Mussels are rarely found in streams that drain semi-urban areas and never in streams that are primarily urban drainage. The decline of a healthy mussel assemblage in free flowing streams in this region generally follows the pattern of the loss

of species until only *E. complanata* remains. *E. complanata* far out numbers all other species in the Atlantic Slope of North Carolina. This common pattern suggests that *E. complanata* is more tolerant of common anthropogenic pollution than species with which it commonly co-occurs, making extrapolation of effects to other more sensitive species difficult.

In the laboratory portion of this study, observable physiological changes and mortality were limited to the highest exposure concentrations. The exposure concentrations that produced significant physiological changes were associated with tissue concentrations that are far in excess of tissue concentrations that we observed in mussels collected from the field. The potential for adverse effects to *E. complanata* adults from present ambient concentrations of PGM appear to be low, but the potential for interactive or synergistic effects may exist.

The potential that mussels aggregated in dense beds are able to incorporate more PGM could have negative implications for juveniles and other mussel species dependent on mussel bed habitat. Young mussels have been reported to be more susceptible to some pollutants than adults (Wang et al. 2007). If mussel beds are established because the engineered habitat increases recruitment, then the concentration of PGM and other pollutants in the bed sediments could lead to reduction in recruitment. Reduced recruitment would eventually lead to a reduction in mussel densities that are necessary to maintain the mussel bed, leading to the loss of function of the mussel bed habitat causing adverse effects to mussels in general.

Avenues for Future Study

Many aspects of the fate and effects of PGM in the environment remain unknown. The effects of combinations of pollutants, the effects to other life stages of *E. complanata,* and effects to other less tolerant species are unknown and should be evaluated. Even though concentrations of PGM are presently low, the long term effects of low level chronic exposure could have detrimental effects on populations of mussels.

The prospect that dense aggregation of mussels in beds may facilitate the concentration of pollutants is an interesting avenue for study. Mussel beds are often made up of multispecies communities that may have some commensal relationship. Mussel bed communities are often dominated by one or a few common species with fewer numbers of less common species. The recent loss of many of the rare species may be due to reliance on the mussel bed habitat. Reductions in recruitment of the dominant species could cause the loss of the bed habitat, reducing the opportunities for dependent species to persist in a stream.

An undesirable weakness in the field portion of this study was an inability to distinguish between sediment PGM in metallic form and that in a bioavailable form. Due to the low ambient concentrations, the technique used for analysis required complete digestion of the samples and did not allow for elucidation of the chemical species. Studies on the chemical species of PGM present in different sediment types, especially focused on sediments in dense mussel beds, would be able to test for enrichment by mussel biodeposition.

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