Variation in platinum-group metal loads in Pennsylvania raptors: the role of diet and habitat

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April 2020



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A Senior Comprehensive Project in Partial Fulfillment of the Requirements for a Bachelor of Science Degree from Allegheny College

I hereby recognize and pledge to fulfill my responsibilities as defined in the Honor Code and to maintain the integrity of both myself and the College community as a whole.

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Pledge

Approval by the Senior Thesis Committee:

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Dr. Mumme

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Dr. Bradshaw-Wilson

Abstract

High concentrations of heavy metals from anthropogenic activities are detrimental to wildlife. Heavy metals are especially likely to cause increases in mortality in top predators such as raptors due to biomagnification. While there has been considerable research regarding toxic heavy metals such as lead and mercury in predators, no studies have examined another heavy metal group, the platinum group elements (PGEs), in raptors in Pennsylvania. Platinum, emitted from catalytic converters in vehicles, has an increased presence in the environment, but has not been studied thoroughly in top predators. Platinum-group elements have been found to have negative health effects in mice and humans who work with platinum salts. Due to the ways these metals are released into the environment, levels in raptors might change depending on environment. The goal of this study was to quantify levels of platinum-group metals in raptors that show up at wildlife centers in Pennsylvania. More specifically, it was to determine whether the area that the raptor was found in or their preferred prey have any influence on PGE concentrations in their system. I hypothesized that platinum-group metals would have higher concentrations in raptors found in urban areas than rural, and that raptors that are higher in the food chain will also have higher PGE concentrations. In order to come to conclusions, kidney and liver samples taken from multiple species of raptors admitted to wildlife centers across Pennsylvania were used to quantify concentrations of PGEs in their system. Methods involving ashing of organic tissues and analyzing via inductively coupled plasma-mass spectrometry were used to quantify levels of metals in tissues. Generally low levels of PGEs in liver and kidney tissues were observed, but a few samples were found to have higher concentrations. PGEs showed no obvious relationship with diet, and there was no relationship found between traffic volume and PGE concentrations in red-tailed hawks. My results suggest that PGEs currently do not present a major health hazard to the raptors of Pennsylvania, but more research is needed to determine its role in our environment.

Introduction

Through everyday activities, humans release toxins that have major implications on the health of wildlife (Fisk et al, 2005; Rattner, 2009). Toxins biomagnify through food webs, resulting in the loss of top predators (Frank & Lutz, 1999; Paasivirta, 2000). Top predators are the most vulnerable to biomagnification because of increased trophic levels associated with their diets (Florea & Büsselberg, 2006). Substances that biomagnify tend to be fat-soluble (Kelly et al., 2007), and when predators digest animals that have been exposed to heavy metals, those metals are released into the predator's body and stored in their fat (Kelly et al., 2007). Most heavy metals, such as lead and mercury, occur naturally in ecosystems and organisms at low levels, but are toxic when elevated (Morel, Kraepiel, & Amyot, 1998; Florea & Büsselberg, 2006; Rattner, 2009; Krone, 2018).

In the 1970s, catalytic converters were added to cars in order to reduce emissions of toxic gases and to conform to updated EPA standards (Degobert, 1995). The converters allowed the United States to reduce local air pollution by oxidizing carbon monoxide into carbon dioxide using platinum as a catalyst (Young & Finlayson, 1976; Degobert, 1995). When hot gases flow through the converter, the platinum catalyst and byproducts such as palladium (Pd) and rhodium (Rh) are ground off and released through the car's exhaust into the environment (Moldovan et al., 2001).

Platinum (Pt) is a metal that is released into the environment because of human activities (Ek, Morrison, & Rauch, 2004). It is becoming a concern because recently it has been detected in the environment, and its long-term impacts are not yet known. These increased levels of Pt are assumed to come from the catalysts of vehicles (Rauch et al., 2005). One study looked at core samples of snow from alpine environments and found an increase of platinum elements in the samples starting around the 1970s-right at the time catalytic converters were introduced (Barbante et al., 1999). Studies have shown that once Pt is emitted from cars, it settles in the soils along the side of the road (Balcerzak, 2011). The dust can then get picked up or washed into waterways, affecting aquatic organisms and biomagnifying up the food web. One group of organisms particularly susceptible to biomagnification are raptors, birds of prey. A few studies have looked at platinum metals in raptors and found significant levels, (Jensen, Rauch, Morrison, & Lindberg, 2002; Ek, Rauch, Morrison, & Lindberg, 2004), but not much is known about its

long-term effects on raptors. It is important to monitor levels of these elements and behaviors of animals until a more definitive conclusion can be made.

Although not much research has been able to definitively pin down the negative effects that platinum-group metals (PGEs) have on organism health, trends have been identified regarding these metals. The platinum metals that are being released from catalytic converters have been found to be biologically inert themselves, but when they react with other elements found in the environment and in the body, health hazards appear (Merget, & Rosner, 2001; Ravindra, Bencs, & Van Grieken, 2004). Platinum salts are commonly inhaled, especially by employees who work around these elements. After chronic exposure to these compounds, employees were found to have developed respiratory problems and an allergy to platinum salts (Baker et al, 1990). Another study found that when mice are dosed with palladium and rhodium those animals had increased occurrence of malignant tumors, leading to the conclusion that some platinum-group metals act as carcinogens (Schroeder & Mitchener, 1971). While it is not proven that platinum metals can cause autoimmune disorders, Helm (2002) tested urine collected from students in an environmental specimen bank for palladium, a PGE, and found that the students suffering from autoimmune and thyroid disorders had increased palladium levels in their urine. This study shows that palladium may have a toxic effect on the immune system.

Raptors are indicators of imbalances in the environment which make them ideal test subjects because of their position at the top of the food chain (Cooke et al., 2018). They are also relatively long-lived, so it is easier to see the effects of environmental toxins throughout their life cycle (Cooke et al., 2018). The goal of this study is to determine the influence that diet and habitat have on concentrations of platinum group metals in raptors across Pennsylvania. In order to test these two variables, this study was done in two parts: one that focuses on habitat relationships and one that focuses on dietary tendencies of raptors. Regarding habitat, I hypothesized that there will be differences in metal concentrations based on where the raptor was found. Specifically, I predicted that PGE levels in raptors will be higher in urban areas with high traffic than rural areas. Regarding diet, I hypothesized that metal concentrations will differ based on the dietary tendencies of raptor species. I predicted that PGE levels will be higher in raptor species whose diet comes from carrion than those that come from live terrestrial prey. A summary of my predictions and rationale can be found in Table 1.

Table 1. Predictions and rationales for this study. A (+) next to the prediction indicates a predicted positive correlation and a (-) next to the prediction indicates a predicted negative correlation.

	Habitat Relationships		Dietary Relationships	
	Prediction:	Rationale:	Prediction:	Rationale:
PGE concentration	(+) urban (-) rural	Because Pt is emitted from vehicles, I would expect concentrations to be higher in raptors found in urban areas, where there are more cars and traffic.	(+) carrion (-) live prey	Because Pt settles in dust along roads, I would expect higher concentrations in species with a tendency towards carrion diets that include roadkill.

Methods and Materials

Habitat Relationships

The first half of this study focused on the relationship between where raptors are found and concentrations of PGEs in their bodies. In order to control for differences between species, only one species was used for this analysis, the red-tailed hawk (*Buteo jamaicensis*). This species was chosen because of its high abundance and presence in wildlife rehabilitation centers across Pennsylvania. Samples of kidney and liver were taken from red-tailed hawks from three wildlife centers (WLC) in Pennsylvania (Figure 1). I chose to take kidney and liver samples because they are tissues where heavy metals accumulate and where toxins are flushed out of the body.

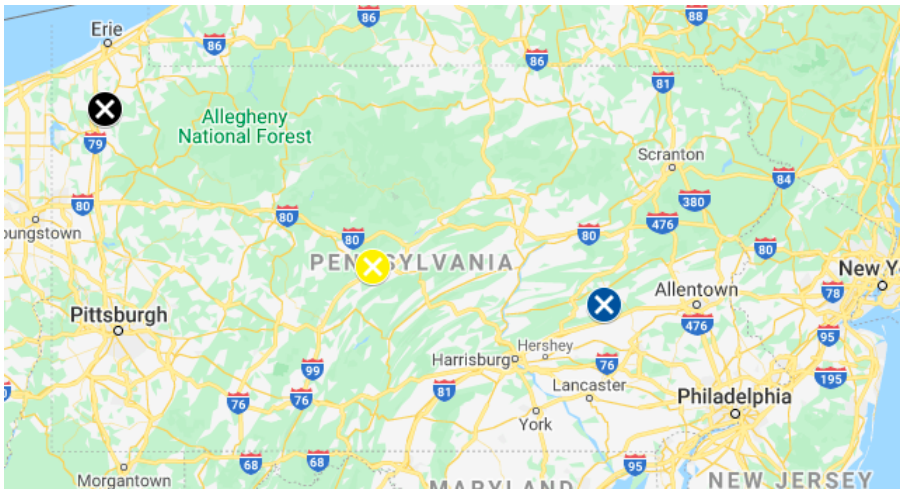


Figure 1. A map depicting the three wildlife centers that samples were taken from. Black X: Tamarack Wildlife Rehabilitation Center in Saegertown, PA, Yellow X: Centre Wildlife Care in Port Matilda, PA and, Blue X: Red Creek Wildlife Center in Schuylkill, PA.

Kits were assembled with the necessary equipment and directions for collecting samples and shipped to the wildlife centers. The kits included centrifuge tubes for storing organic tissues, scalpels, and labels. Samples were taken from deceased hawks and frozen. Each

bird sample was labelled with the date, sample type, and where the bird was found in order to assist with GIS analysis. Tissue samples from a total of 9 red-tailed hawks, those with complete location information, were used for analysis.

After collection, liver and kidney samples were prepared and tested by an inductively coupled plasma-mass spectrometer (ICP-MS) and analyzed to determine concentrations of platinum group elements-Pt, Rh, and Pd. Details on tissue preparation and analysis are provided below.

GIS analysis was used to examine the relationship between PGEs and road density and traffic volume. All GIS analysis was done in ArcGIS Pro 2.5 (ESRI, 2020). The locations of the nine red-tailed hawks were mapped from their latitude and longitudes. The average home range of a red-tailed hawk was determined to be 2.3 km^2 (Janes, 1984), which suggests that the radius of each home range will be 0.856 km. A layer with information on state-maintained roads from the PA Department of Transportation (2020) was downloaded and clipped by buffer in order to eliminate roads outside the buffer. A sum of annual average daily traffic (AADT) in vehicles per day was calculated for the roads in each buffer. Road density in km/km^2 for the roads in each buffer was also calculated. Finally, traffic volume was calculated by dividing AADT by road density for the buffer of each hawk. Traffic volume for each bird was then compared to PGE concentrations to determine if proximity to high-traffic roads causes increased PGE concentrations in liver and kidney. One assumption this study makes is that where the bird was found is in the middle of its home territory. This may not necessarily be true, but for the purposes of this study, the point where the bird was found would be considered the center of its home territory.

Dietary Relationships

The second part of my study focused on a bird's diet and the effect of diet on the concentration of PGEs in its body. Kidney and liver samples were taken from deceased raptors at Tamarack Wildlife Rehabilitation Center in addition to red-tailed hawk samples from Red Creek Wildlife Center and Centre Wildlife Care. Samples were taken as raptors came into the center and then diet preferences were determined based on species. A list of the species from which samples were collected can be found in Table 2.

Table 2. Summary of specimens by raptor species at Tamarack Wildlife Rehabilitation Center, Red Creek Wildlife Center, and Centre Wildlife Care listed by common name, number of specimens in parentheses, abbreviation, and scientific name.

Larger Terrestrial Diet:	Smaller Terrestrial Diet:	Carrion Diet:
Barred Owl (1) BADO *Strix varia*	Broad-winged Hawk (1) BWHA *Buteo platypterus*	Turkey Vulture (2) TUVU *Cathartes aura*
Great Horned Owl (4) GHOW *Bubo virginianus*	Eastern Screech Owl (1) EASO *Megascops asio*	
Barn Owl (1) BAOW *Tyto alba*	Merlin Falcon (1) MEFA *Falco columbarius*	
Red-tailed Hawk (23) RTHA *Buteo jamaicensis*	Cooper's Hawk (1) COHA *Accipiter cooperii*	

These samples were processed and analyzed using ICP-MS as well. The kidney and liver of 35 birds were obtained for a total of 70 samples.

Sample Preparation and Analytical Methods

Samples were stored in a freezer until ready to be prepared for ICP-MS analysis. In preparation for analysis, all glassware was soaked in a 5% solution of nitric-acid and ultra-pure water for 48 hours and then rinsed thoroughly with ultra-pure water before each use. Tissues were thawed and oven-dried in petri dishes at 60° C for 48 hours to determine biomass. They were then ashed in a muffle furnace at 500° C in porcelain crucibles for 24 hours to remove organic matter. The ash that was left over was dissolved in 10mL of a 5% solution of nitric acid and ultra-pure water. Samples were then filtered through 30mm polyvinylidene difluoride (PVDF) 0.22 μm syringe filters to collect extra ash that had not dissolved that would clog up the ICP-MS. Prepared sample solutions were stored in a refrigerator until analysis.

After filtration, prepared samples were taken to Pennsylvania State University, Behrend for analysis of metal concentration. ICP-MS analysis was done with an Agilent 7900 ICP-MS under similar conditions to Aruguete et. al (2020). Conditions that samples were tested under are outlined in Table 3. A helium chamber was used in the ICP-MS to control for extraneous interfering polyatomic ions.

Table 3. Settings on the Agilent 7900 ICP-MS used to test liver and kidney samples dissolved in 5% nitric acid solution.

Plasma gas flow	15 L/min
Auxiliary gas flow	0.9 L/min
Nebulizer gas flow	0.75-1.03 L/min
ICP RF Power	1600 W
Mode of Operation	He collision cell on
He gas flow	4.3 mL/min
Spray chamber temperature	2° Celsius
Sample uptake	0.32 mL/min
Ion optic voltages	Vary
Integration time	0.1 sec
Replicates / sample	3
Nebulizer type	Agilent MicroMist Glass U-Series
Spray Chamber Type	Agilent 7900 UHMI Spray Chamber (Scott-style quartz)
Sample Cone	Agilent 7900 Ni Sampler Cone with Cu base
Skimmer Cone	Agilent 7900/8900 Ni Skimmer Cone

Concentrations of isotopes Rh103, Pd105, and Pt194 were determined and corrected for the biomass of each sample. Statistics were run using ANOVA tests in JMP Pro 12.2 software testing species, location, tissue type, and concentrations of Pt, Rh, and Pd.

Results

Habitat Relationships

Traffic volume does not appear to be related to the concentration of Pt, Rh, or Pd in the kidneys and livers of red-tailed hawks (Figure 2). In some cases, such as the Rh103 in the kidney and the Pt194 in the liver, PGE concentrations seem to go down as traffic volume increases, but it is not a statistically significant relationship (respectively, p=0.27 and p=0.22). The Pt194 in the kidney seems to have a positive relationship with traffic volume, but again, not statistically significant (p=0.31).

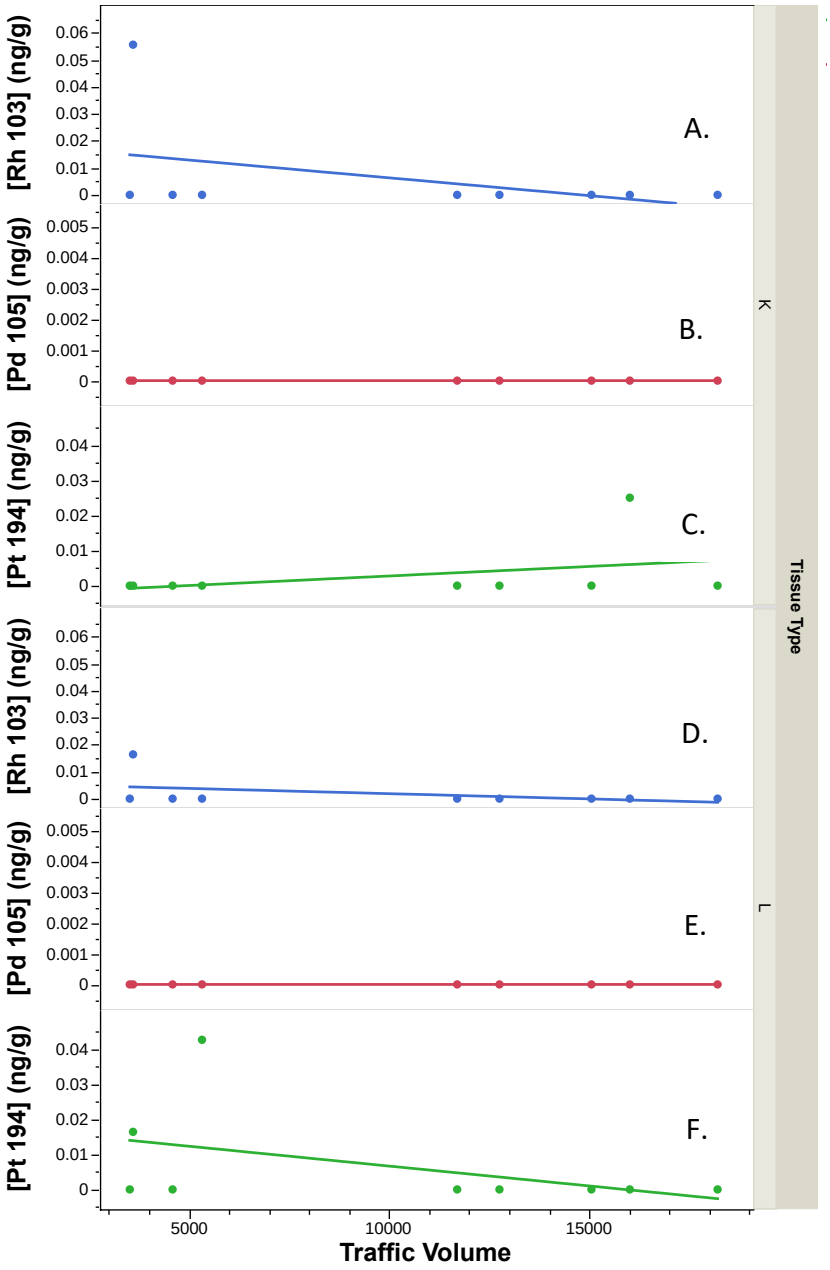


Figure 2. Comparing the relationship between Traffic Volume (vehicles/day *km2/km) and PGE concentrations with respect to tissue type. A, B, and C show the relationship between the kidney samples, and D, E, and F show the relationship between the liver samples.

Dietary Relationships

After controlling for the dry biomass of each sample, the concentrations of Pt194, Rh103, and Pd105 can be compared between species. Although there are not a lot of PGEs in the samples across all species, a couple of the birds have increased PGEs (Figure 3). The Cooper's hawk (COHA) samples have high Rh103 in both the liver and the kidney and

elevated Pd105 in the liver. Higher levels of Pd194 are also present in the liver, but not as elevated as the other PGEs.

The other two species with higher PGE concentrations are the barred owl (BADO) and broad-winged hawk (BWHA). The barred owl only has increased Pt194 concentrations in the kidney. The broad-winged hawk shows slightly elevated PGE concentrations in Pt194 in the kidney and liver, and Rh103 in the liver. PGE concentrations in the other samples are minimal (Figure 3). It can be observed that the turkey vulture (TUVU), the species with a fully carrion diet, has little to no PGEs in its tissues.

The PGE concentration data from only the red-tailed hawk samples are provided because the larger sample size will make it easier to come to conclusions about the range of variation within a single species (Figure 4). Concentrations did not vary much between the two tissue types or the PGEs and are generally quite low.

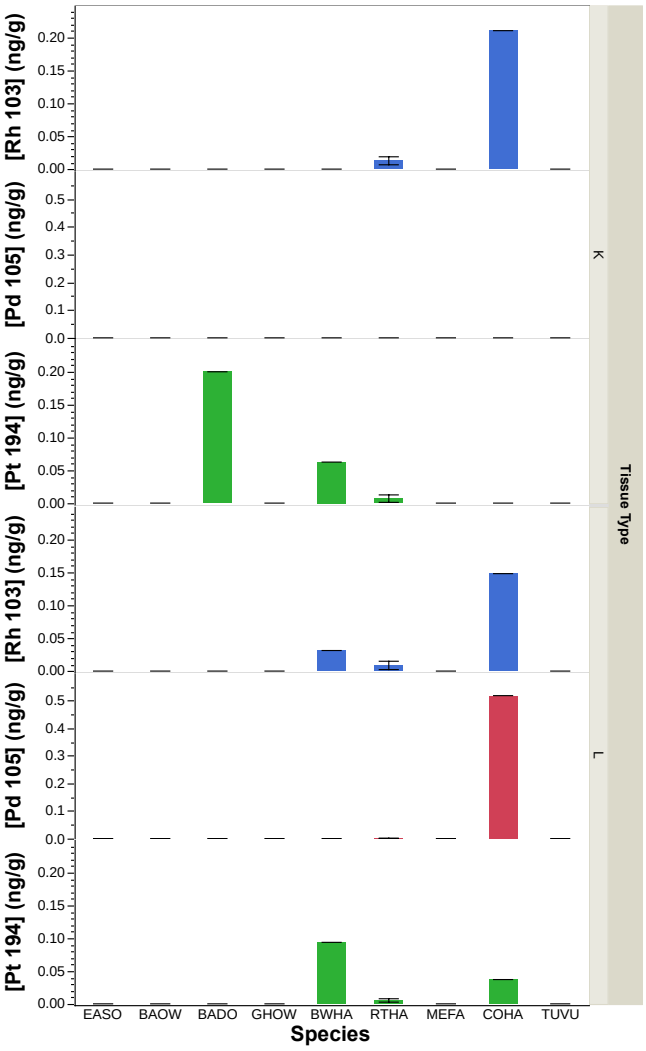


Figure 3. Comparing the PGE concentrations (ng/g) of all species of birds in the liver and kidney. Due to small sample size, not much can be taken away from this figure. Increased levels of PGEs can be observed in the Cooper's Hawk (COHA), Broad-winged Hawk (BWHA), and Barred Owl (BADO), but conclusions cannot be drawn because samples were only taken from one bird in each species.

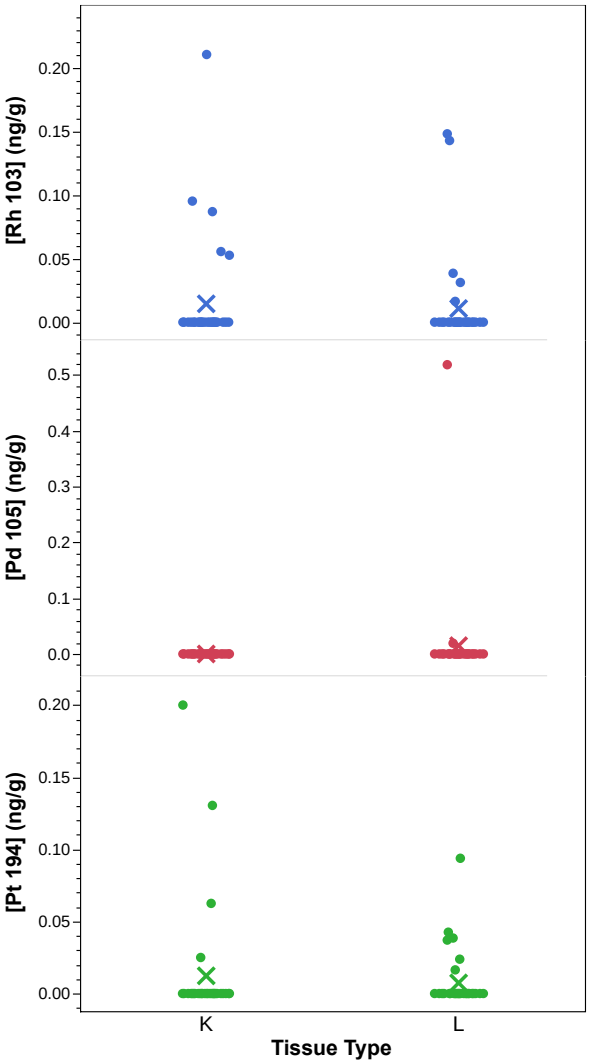


Figure 4. Expanded from only the red-tailed hawk data comparing PGE concentration to tissue type.

Discussion

Habitat relationships

It was hypothesized that differences in PGE concentrations would be observed based on where the raptor was found before it was brought into the wildlife center. PGEs have been found to be deposited by roadsides; therefore, the prediction could be made that birds that live closer to high traffic areas would come into contact with PGEs more often and have elevated concentrations. This hypothesis is not supported by the data found in my study. I found no significant positive relationships between PGE concentrations and traffic volume within a 0.856 km radius of where the bird was found (Figure 2). A similar study looking at the occurrence of PGEs in peregrine falcons from northern and southern Sweden found that there was no significant trends between where the birds came from and their PGE levels in the liver and kidney (Ek, Rauch, Morrison, & Lindberg, 2004) which supports the findings of my research. Using similar methods to this study, they tested for Rh, Pd, and Pt concentrations in the liver and kidney using ICP-MS. This study found PGE concentrations in their samples higher than what I found which may show that the levels of PGEs in Pennsylvania are not as hazardous as in Sweden. Their Pt concentrations in the peregrine falcons ranged from 0-4.5 ng/g, 0-1.25 ng/g for Pd, and 0-0.65 ng/g for Rh whereas the highest reported concentrations from my study were 0.2 ng/g of Pt in the BADO, 0.52 ng/g of Pd in the COHA, and 0.2 ng/g of Rh in the COHA. The study by Ek et al (2004) shows that the levels of PGEs found in my study are significantly lower than other places which is a positive outcome for the raptors of Pennsylvania. The absence of a relationship between traffic volume and PGEs may be due to the fact that PGEs are found in the environment in particles and, due to the widespread use of automobiles in today's society, may be scattered equally throughout the environment by air (Balcerzak, 2011).

Dietary relationships

It was hypothesized that PGE concentrations would differ based on the dietary tendencies of different raptor species, and more specifically, it was predicted that birds that eat carrion would have increased PGE concentrations than birds with a terrestrial diet. This prediction was not supported by the data in my study; the two turkey vultures that I sampled had no detectable levels of PGEs in either kidney or liver tissue (Figure 3). Although my data suggests that there may be a difference in PGE concentration by diet, it seems as though avian dietary tendencies

cause larger PGE concentrations, definitive conclusions cannot be drawn due to small sample size.

Larger terrestrial birds such as the red-tailed hawk, barred owl, great-horned owl, and barn owl have a tendency to eat larger terrestrial prey such as rabbits, squirrels, and rats whereas the smaller terrestrial birds such as the eastern screech owl and broad-winged hawk would eat smaller prey such as mice and insects. Cooper's hawks and merlin falcons, on the other hand, have diets that consist mostly of other bird species (*Merlin*, 2013; Meng, 1959). This is interesting because, although the PGE concentrations in the merlin falcon tissues were near zero, the samples from the Cooper's hawk had the highest elevation in all three PGEs and in both tissues. A study by Jensen (2000) looked at PGE concentrations in the feathers of three raptor species: sparrowhawks, gyrfalcons, and peregrine falcons. Two of these species, peregrine falcon and sparrowhawks, have mostly avian diets much like the Cooper's hawk. Although they quantified concentrations from feathers and not internal tissues, the study had results much similar to my study: the species with mostly avian diets had increased PGE levels from the other species (Jensen, 2000). Similar to the study mentioned earlier, Jensen (2000) found much higher concentrations of PGEs in their specimens than I did. The peregrine falcons had Pd concentrations from about 0.5-5.5 ng/g, and the sparrowhawk had Pd concentrations that reached over 16 ng/g which is about 32 times larger than the concentrations found in my study. The differences in the studies may be due to location or species differences. Jensen (2000) was done in Sweden with three different species of birds. The air pollution and catalytic convertor regulations between Sweden and the United States may be different, leading to drastically different results.

The results of my study may be due to the fact that food chains including avian predation have the potential to have more trophic levels than other food chains, which allows for increased biomagnification. A red-tailed hawk might prey on a squirrel that was eating nuts, whereas a Cooper's hawk might prey on an insect-eating bird, and those insects ate vegetation. The addition of one more trophic level in the food chain may be the reason why increased PGE levels are observed in the Cooper's hawk samples and lowered in the other species' samples. Rimmer et al. (2010) sampled blood from organisms throughout an ecosystem in Vermont including both vertebrates and invertebrates. The study observed the mercury levels of Bicknell's thrush and a well-known predator of Bicknell's thrush, the sharp-shinned hawk. The blood mercury levels of

the sharp-shinned hawk were ten degrees higher than that of the thrush (Rimmer et al, 2010) demonstrating how prevalent bioaccumulation can be for birds with avian diets.

Conclusion

In obtaining the information in this study, I reached some roadblocks and limitations. When locations were recorded for the red-tailed hawks, some did not get recorded specifically, and only a city or town was listed. Some of the red-tailed hawk samples had to be omitted from the habitat analysis because specific addresses were not reported, and a latitude and longitude could not be derived. Although the data from these birds was still used in the dietary relationship part of this study, this led to a decreased sample size in the habitat analysis.

Due to the nature of this study, it was unknown exactly what the sample size would look like and what kinds of species would be brought into the wildlife centers. Ideally, I would have a good sample size and at least five individuals from each species. I also would have included a couple species that had an aquatic diet such as eagles or ospreys, but tissue samples cannot be taken from eagles under the Bald Eagle Protection Act of 1940, and osprey are not brought into wildlife centers very often. I think that some interesting data could come out of obtaining a larger sample size, especially regarding the Cooper's hawk data. Future studies would benefit from looking more into Cooper's hawks, or other bird species with a primarily avian diet, and obtaining a higher sample size to see if higher PGE levels are found in all bird-eating species or if the Cooper's hawk in this study was an anomaly.

More research should be done in order to closely monitor the impacts that PGEs could have on apex predators. Catalytic converters were implemented to reduce local air pollution but may be detrimental in other ways to the environment.

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