Evaluation, Assessment, and Determination of Risk to High Trophic Level Piscivores in the Mid-Atlantic: A Spatial, Biological, and Comparative Case Study of Mercury in Virginia Bald Eagle Populations.

David E. Kramar

Dissertation Submitted to the Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

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Bill Carstensen (Chair)
Jim Fraser
Steve Prisley
Jim Campbell

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Keywords: Bald Eagle, Mercury, Geographic Information Systems, Non-Linear Modeling
This research is focused on explaining the concentrations of mercury found in juvenile bald eagles (*Haliaeetus leucocephalus*) as a function of the physical and anthropogenic landscape. Due to its location in the food chain, this species is susceptible to a wide range of contaminants (xenobiotics), particularly those that bioaccumulate and biomagnify as they move through the food chain. Previous research has indicated that areas in coastal environments are less susceptible to methylation than those in freshwater environments. Sampling efforts for this research were conducted in such a manner as to obtain an equivalent number of samples from the coastal plain (expected to be low mercury) and the inland regions (expected to be statistically significantly higher). In all cases, results indicated that both feather and blood mercury concentrations were higher in the inland population (Blood: Prob > t = 0.0003, Feather: Prob > t = 0.0002).

Utilizing classification and regression tree models (CART), we were able to relate metrics such as the percent of deciduous forest, percent of mixed forest, percent of pasture, and percent of wetland to measured blood mercury concentrations. We also found that the best models were produced using the USGS HUC 12 watersheds (the smallest watershed produced by the USGS). Moreover, we found that metrics describing the amount and type of fragmentation within the watersheds exhibited a significant influence on measured blood mercury concentrations. Contrary to previous research, we found wetlands to be negatively associated with higher blood mercury, whereas the abundance of core forest and a larger patch density (PD) in the deciduous and mixed land cover classes was positively associated with higher blood mercury concentrations. We also found that a higher percentage of pasture was associated with higher blood mercury.
Dedication

I dedicate this dissertation to my mother and father, both of whom always provided support and belief in me when I was not always able to. Also, to my wonderful wife Val who did not let me give up when I became discouraged.
Acknowledgements

I would like to thank my family and friends for providing the support I needed through this process. I thank Jeff Cooper of the Virginia Department of Game and Inland Fisheries for tremendous support. I thank all of my friends in the program for listening to me when I complained or offered insight when I needed it.

In addition, I would like to offer my sincere appreciation to my committee for believing in me and supporting me throughout the (overly long) entirety of this research. My sincerest thanks go out to Dr. Carstensen, Dr. Fraser, Dr. Campbell, and Dr. Prisley. Without their guidance and support this research would not have been possible. I thank them for pushing me and not letting me give up. Lastly, I thank all the folks at the Sawyer Environmental and Chemistry Laboratory at the University of Maine for allowing me to run my samples and providing the oversight and training so I could do that myself.

I would also like to offer my sincere thanks to all of the folks that assisted in the field collection of samples, the land owners that allowed me permission to access nest sites, and the support of so many others as I worked through this process.

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Attribution

Chapter 3: Mercury Concentrations in Coastal and Inland Bald Eagles in Virginia

Bill Carstensen, PhD (Department of Geography, Virginia Tech) is currently the chair of the Department of Geography at Virginia Tech. Dr. Carstensen was a co-author on this paper and aided extensively in editorial reviews of the manuscript.

Jim Campbell, PhD (Department of Geography) is currently a full professor in geography at Virginia Tech. Dr. Campbell aided in the selection of appropriate statistical tests and offered editorial reviews of the manuscript.

Chapter 4: The Impact of the Unit of Analysis when Modeling Land Cover and Juvenile Bald Eagle Blood Mercury Concentrations

Bill Carstensen, PhD (Department of Geography, Virginia Tech) is currently the chair of the Department of Geography at Virginia Tech. Dr. Carstensen was a co-author on this paper, aided extensively in editorial reviews of the manuscript, and assisted in the experimental design used to isolate the appropriate spatial analysis techniques.

Steve Prisley, PhD (Department of Forest Resources and Environmental Conservation) is currently a full professor in forestry at Virginia Tech. Dr. Prisley was a co-author on this paper, offered extensive editorial reviews of the manuscript, and insight into the spatial modeling procedures.

Jim Campbell, PhD (Department of Geography) is currently a full professor in geography at Virginia Tech. Dr. Campbell aided in the selection of appropriate statistical tests and offered editorial reviews of the manuscript.

Jim Fraser, PhD (Department of Fish and Wildlife Conservation) is currently a full professor in wildlife at Virginia Tech. Dr. Fraser offered historical knowledge of the bald eagle population in Virginia which aided in the development of territories for analysis, and aided in editorial reviews of the manuscript.

Chapter 5: Investigating the Influence of Landscape Fragmentation on Measured Bald Eagle Blood Mercury Concentrations.

Bill Carstensen, PhD (Department of Geography, Virginia Tech) is currently the chair of the Department of Geography at Virginia Tech. Dr. Carstensen was a co-author on this paper, aided extensively in editorial reviews of the manuscript, and assisted in the experimental design used to isolate the appropriate spatial analysis techniques.

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Preface

This dissertation was written in journal style and organized into three chapters. Each individual chapter consists of an introduction, methods, results, and discussion section. Each chapter is intended for publication. As a result, repetition in some sections (i.e. Introduction, Methods, Results, Discussion, and Literature Cited) may occur. The chapters are preceded by an Introduction and followed by overall Conclusions of the research.
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Chapter 1: Narrative and Statement of Objectives

Narrative and Objectives

This research assesses current mercury (Hg) concentrations in the Virginia bald eagle
(\textit{Haliaeetus leucocephalus}) population. Samples were collected in collaboration with the Virginia
Department of Game and Inland Fisheries and the Center for Conservation Biology at the
College of William and Mary. Forty-six samples (defined as individual eaglets) from 28 nests
were collected from the Virginia population and analyzed for total Hg. The collected Hg
concentrations from the juvenile eagles were analyzed with respect to the environmental and
physical landscape characteristics within various distances of the nest locations. The results of
this research further contribute to the overall understanding of Hg in the environment and, when
coupled with previous research, will provide policy makers and citizens with additional
information regarding the distribution and effects of Hg across the landscape. This research also
serves to fill the gap in the knowledge base regarding Hg in the south east, particularly in
Virginia.

Research Rationale

This research focuses on the assessment, evaluation, and modeling of Hg in high trophic
level piscivores in the mid-Atlantic, using the Virginia bald eagle (\textit{Haliaeetus leucocephalus})
population as a case study. The bald eagle represents an excellent species for this study due to the
position it fills in the food chain, a position which allowed for a detailed assessment of Hg in the
environment using the bald eagle as the endpoint. As little was known regarding Hg in Virginia’s
eagles, the collection and analysis of samples serves to fill a gap in our current understanding of
Hg in Virginia. This also allows for regional comparisons between separate studies conducted
throughout the east coast. In addition, if a particular region exhibits substantially higher levels than another region, both between states and within, researchers may begin to infer the possible risks present to humans that consume fish from within that region. Within Virginia, the percent of sampled pairs that exhibit levels currently above thresholds known to be detrimental was identified as well as the particular regions within which they reside. Variables such as predominant land cover type, type of foraging area, and proximity to development were collected to determine the level of influence that each of these variables had on Hg in the eagle population. Whereas many researchers address the effects that Hg exhibits on a particular population, this research attempts to explain the environmental mechanisms, particularly land cover, within the state of Virginia that are responsible for the transport and availability of Hg and does not attempt to explain the biological or physiological effects that may be present in the individuals. Specifically, this research addresses the following questions:

1. What are the current levels of Hg in the Virginia bald eagle population?
2. Do the current levels exhibit a spatially significant difference between populations that reside in the coastal plain of Virginia (mesohaline, oligohaline, and polyhaline environments) and those of the piedmont and mountain regions (tidal fresh and fresh water environments)?
3. Can we estimate levels of Hg in bald eagle blood as a function of the percent of land cover types within defined areas of various size and shape?
4. What is the best unit of analysis for predicting blood Hg concentrations in bald eagles?
5. Within the context of land cover, can we use metrics that describe the shape, density, contiguity, etc. to further refine the models and thus the predictive capability?
Sampling Design

Nests within Virginia were randomly sampled, stratifying the selection by major river basin in the state to ensure adequate coverage of both coastal and inland populations, to the extent possible. Sampling efforts were restricted to breeding pairs and are therefore biased toward successful breeding pairs. As a further method of determining the areas within which to sample, a Geographic Information Systems (GIS) analysis was conducted to identify areas within each of the major river basins that will likely influence the availability of methylmercury (CH$_3$Hg$^+$). Results from the analysis provided a further way in which to stratify the samples. This approach was utilized to obtain an adequate number of samples that would allow for a comparative analysis between coastal and inland nests. Collected samples are presented in Figure 1.

Blood and Feather Sample Collection

Blood samples were collected from the brachial vein in the wing using 21-25 gauge butterfly needles (depending on the size of the juvenile) and 4cc vaccutainers. One vaccutainer was used for analysis and the second was collected for archival purposes. Blood samples were immediately packed on ice and frozen within 2 – 4 hours of collection. The samples remained frozen until they were analyzed. Samples were marked with a unique nest ID and the latitude and longitude coordinates of the nest. Feather samples included down clipped from the underside of the chest, and two contour feathers clipped from the breast. All samples were labeled in the same manner as noted above. Feathers were prepared for analysis using methods noted by Ackerman et al. (2007). It should be noted that, feather mercury represents total body burden from the time of the last molt (or development of the pin feathers), while blood mercury
represents recent dietary uptake. Current research also suggests that talon samples are an adequate predictor of Hg burden and may provide a better indication of total body burden than feathers (Hopkins et al. 2007). However, preliminary samples indicated a strong positive correlation between feather and talon such that the collection of talon samples was abandoned in favor of the less invasive collection of contour feathers.

Full metrics were collected, including but not limited to, weight, crop size, foot pad, and halix claw size. Prey remains from the nests were collected opportunistically in order to gain insight to each territorial pair’s diet. United States Geologic Survey leg bands and associated state color bands were used to mark the individuals for future identification and monitoring.

**Sample Analysis**

Sample analysis of blood and feathers followed analytical methods approved by the United States Environmental Protection Agency (EPA) (USEPA 1994, USEPA 2000, USEPA 2001). Blood and feather samples were analyzed at the University of Maine Sawyer Environmental Chemistry Research Laboratory using a Milestone DMA-80 Direct Mercury Analyzer with a resulting resolution of mg/kg wet weight for blood and mg/kg dry weight for feathers. Blood and feathers were analyzed for total Hg. Generally ~95% of the total mercury in blood is comprised of MeHg, indicating that an analysis of total Hg is adequate to explain MeHg. (Thompson 1996, BRI Unpubl. data).
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Figure 1.1: Location and distribution of currently collected samples for the proposed research within the state of Virginia. The dark line represents the fall line, which was used to differentiate between the coastal and inland populations. The area west of the fall line represents the inland, while the area east of the fall line represents the coastal plain.
Chapter 2: Review of Relevant Literature

Natural History of the Bald Eagle

The bald eagle is a symbol of national pride and has garnered both respect and dissent throughout the years. As one of the largest members of the family Accipitridae, it is also commonly known as a fish or sea eagle, in the genus Haliaeetus (Buehler 2000). Like many species, the bald eagle increases with size as a function of increasing latitude. This has led to the designation of two sub-species: *H. l. leucocephalus* in the southern portions of its range, and *H. l. alascanus* in the northern portions. Second in size only to the California Condor (*Gymnogyps californianus*), the bald eagle varies in size from approximately 3kg to over 6 kg (Palmer, 1988) and can have a wingspan reaching over 2m. Predominantly known for the majestic white head, bald eagles are one of the most well-known raptors in the United States. The white head becomes visible at approximately 4-5 years of age when sexual maturity is reached (McCullough 1989). Beyond 5 years of age, the predominant white head and white tail are fully developed (McCullough 1989). Often, juvenile bald eagles are mistaken for the only other eagle in North America, the golden eagle.

The Virginia population is part of the smaller sub-species *H.l. leucocephalus*. The largest portion of the population is concentrated in the Chesapeake Bay and coastal regions of the state. Approximately ¼ of the population resides in the piedmont and mountain regions (Kramar, Unpublished Data). It has been suggested that the Virginia population is “resident”, in that it does not migrate in the same manner that its northern and southern relatives do (Buehler et al. 1991).

Traditionally, bald eagles are known to nest in large trees, near bodies of water (Andrew and Mosher 1982). However, with the increase in development and the removal of larger trees, continued efforts to protect its nesting habitat are necessary to maintain adequate nesting habitat. Johnsgard (1990) has suggested that the preference for nesting near water is a function of their size and need for a substantial prey base. Nests range in height from approximately 12m to 30 m (40' to over 100') and
are generally located at or near the top of the tree. Territorial pairs return to the same nest year after year, maintaining and adding onto the nest structure. In Virginia, the preferred nest is in loblolly pines in the coastal areas (Andrew and Mosher 1982). In the inland portions of the state nests are often found in sycamore and oak, noted during aerial surveys conducted in 2010 by the Virginia Dept. of Game and Inland Fisheries. Abbott (1978) has suggested that the minimum distance between active nesting pairs along rivers is approximately 8km.

In Virginia, inland nests averaged 20km to 30km (12 – 18 miles) apart as noted during survey efforts. In the Chesapeake Bay (Byrd et al. 1990) an increase of nesting density can be found if adequate prey and nesting locations are available. As a result of the increasing population pressure in the coastal and Chesapeake Bay reaches of Virginia, bald eagles are now actively establishing territories inland (Watts and Therres, 2009). Due to little/no variation in the biota found in tidal fresh, oligohaline, mesohaline, and polyhaline environments, there is no differentiation in prey preference according to Watts and Markham (2009).

Breeding bald eagle pairs typically forage less than three kilometers from the nest site (Buehler et al. 1991) suggesting that during the breeding season, bald eagles are limited in the type of prey they can hunt or scavenge. This shorter range is in contrast to wintering bald eagles that may travel great distances to forage for food (Buehler et al. 1991).

*Conservation History of the Bald Eagle*

In the summer of 2007 the bald eagle was removed from the endangered species list. Currently populations are increasing however this was not always the case. In the early 1800's many counties offered bounties for eagle carcasses. For example, some counties in Maine offered upwards of $0.20 for each eagle carcass brought in. Some of the earliest published articles refer to bald eagles being “shot and left where they fell” (Brimley, H.H., 1892). As a further testament
to the persecution that bald eagles faced is the following excerpt from The Auk, dated 1902:

“It is with much pleasure that I send you the first authentic record of the taking of a specimen of the Bald Eagle in Ohio County, West Virginia. The bird was an immature female, in the second years plumage which is known as the “Gray Eagle” stage. It was killed December 27, 1901 on the farm of Mr. Ridgeley Jacob, near Clinton, W. Va., the manner of its capture being unique. Two young sons of Mr. J. S. Duvall, who lives upon the above mentioned farm, were playing in a stream, when one of the youngsters ascending the bank spied the great bird just beyond the crest of the knoll. The child -- who was only about ten years of age--instead of running away, boldly picked up a stone and threw it with such telling force and accuracy that he broke the bird's wing. Immediately the raptor faced about and ran at the boy, who fled at its approach, while his brother--two years his junior--succeeded in hitting the pursuing bird in the back of the head and fracturing its skull with another stone. The older boy stopped, upon seeing the eagle staggering about, and ran back, pounced upon the feathered enemy and held it until life became extinct. The bird weighed nine and a quarter pounds, its length was thirty-nine inches, extent seven feet eight and a half inches. The skin is now in my possession.” --Robert Baird McLain, Wheeling, W. Va. (McLain, 1902)

In another case dated 1909, Taylor et al. (1909) documented the shooting of an eagle outside of Chicago. Statements such as those above can be found extensively throughout early literature. Moreover the collection of eggs and young for specimens was widespread throughout North America. There is certainly no question that as Europeans settled the “New World” there was a substantial impact on many species, particularly the bald and golden eagles which were viewed as predators of livestock. Between 1917 and 1933 over 100,000 bald eagles were shot or killed in Alaska. In 1933, F.M. Jones from Independence, Va. documented the removal of eggs as
specimens from three nests located in James City County, Va. (Jones, 1933). As human populations increased in size, suitable nesting areas for bald eagles declined due to the clearing of land for farms and cities. Removal of many of the old growth trees for the shipping industry and other industries had the effect of limiting adequate nesting areas (Weekes, 1974). In addition, as human populations continued to increase there became increased competition for resources. There was mass removal of trees for agricultural purposes (to the extent that almost all old growth forest had been logged) which limited suitable nesting and breeding habitat (Weekes, 1974). Furthermore, many of the large human population clusters were situated along major waterways and in the Chesapeake Bay, areas that historically sustained large bald eagle populations. Simply put, as the United States grew both in population and size, and expanded to include areas west of the Mississippi suitable nest and foraging habitat decreased. This continued expansion and the growth of the industrial era continued to create a negative impact on the population.

Beyond the impact of hunting and the associated bounties offered for them the effects of DDT and other contaminants such dieldrin and kepone in the late 1960’s and mid 1970’s had a substantial impact on the bald eagle population (Wieymeyer et al., 1984), resulting in a decline of productivity to approximately 0.2 young per pair (Taylor et al., 1982). In Virginia, the population decreased to approximately 80 breeding pairs in the early 1970's (Abbott, 1978). This was not the first time that bald eagles faced a decline in their populations. As the eagle was the National Symbol, public awareness increased as the eagle population decreased. Bald eagle populations continued to decrease through the early to mid 1900’s and by the time the effects of DDT on bald eagles were understood in the mid to late 1960’s the population was in dire straits. Because of this large population decline, the 1960’s and 1970’s marked a change in policy as
well as conservation efforts. In 1967 the Bald Eagle was officially declared an endangered species, however, it was not until 1973 that President Nixon signed into law the Endangered Species Act and officially listed the bald eagle under that act. Around that same time period, the use of DDT was banned in the early 1970's in the United States. Shortly after the eagle was placed on the list of Endangered Species, many states began implementing their own management plans, most of which exist to this day. In Virginia, the eagle was delisted as a state threatened species in August of 2013. (Virginia Dept. of Game and Inland Fisheries, Personal Communication). These plans were designed to increase the current population size to historic levels. In areas such as the Chesapeake Bay, effects of the management plans are apparent. Currently there are well over 1000 pairs that nest in the states bordering the bay. Virginia alone is home to over 800 hundred nesting pairs, with the largest concentrations occurring in the coastal plain and Chesapeake Bay regions of the state. Other areas where state management plans aided in increasing the population include states such as Maine, New York, and Florida. Unfortunately, some areas such as Vermont have not yet realized an increase in those populations. New Hampshire for example still has less than 50 nesting pairs.

The bald eagle remained listed as endangered until 1995, at which point it was reduced to threatened status. As noted above, as the population continued to increase the bald eagle was officially removed from the Endangered Species list in the summer of 2007. It should be noted that while it was removed from the Endangered Species Act, it is still protected under the Bald and Golden Eagle Protection Act as well as the Migratory Bird Act. Under the Bald and Golden Eagle Protection Act, any take, which includes disturbance, is illegal. This represents legislation that affords protection similar to the ESA. Included in the definition of take is also the concept of “disturb”.
Of all of the legislative actions taken to conserve the bald eagle as a species, there were likely two actions that truly exhibited a positive effect on population numbers. The first was the banning of DDT use in the United States. Banning of these substances marked the first step in reducing the bald eagle decline. Over time, the calcium deficiencies in eggs as a result of DDT contamination subsided. The second most important legislative action was the development and signing into law the Endangered Species Act (ESA). By placing the bald eagle on the list as endangered, and affording the species protection of the nesting and foraging habitats (e.g. reducing human disturbance during breeding season, etc.) the ESA was able to effectively manage and increase the population. Today, the bald eagle is the poster child for what the ESA can do.

Certainly, bald eagles need to continue to be afforded protection if the population is going to continue to increase. Current legislation under the Bald and Golden Eagle Protection Act should aid in the continued expansion of the species. While there are several key management issues that should be addressed over the next ten years, one of the most important management issues should be the maintenance and monitoring of suitable nesting habitat and continued protection of those habitats. As the human population continues to grow, more of the bald eagles nesting habitat will diminish. Furthermore, continued research into current contaminant issues such as mercury, lead and PCB’s should occur. There is no lack of research that suggests current populations on the east coast are subject to contaminants such as mercury. While we know that mercury poses a threat, it is likely that the long-term effects of continued exposure will not manifest themselves for the next 10-15 years. Due to the documented accumulation of mercury in species such as the bald eagle, as well as the need to further understand the methylation and transport of mercury in the environment, we focus this research on using Geographic Information
Systems (GIS) technologies to model and understand the environmental factors leading to measured mercury concentrations in Virginia eagles. The following sections review the literature associated with mercury in wildlife, mercury in the environment, and the use of GIS technologies to model and understand mercury in both wildlife and the environment.

**Mercury in Wildlife**

As awareness of environmental mercury and the associated health implications increases, states have become proactive in implementing fish advisory warnings for the protection of the general public. As of 2008, all 50 states have initiated fish consumption advisories as a direct result of mercury bioavailability and accumulation in aquatic ecosystems. The collection and assessment of samples in Virginia, can be coupled with other eagle Hg studies across the eastern seaboard to provide a regional assessment of potential Hg availability. Therefore, the health and environmental risks from Maine to Florida can be modeled, and allow for detailed risk assessments and analysis. It is not the intention of this research to address behavioral or pathological implications of mercury in individuals but rather to explain from a landscape and anthropogenic context why spatially different areas of Virginia exhibit radically different blood mercury levels of individuals.

In general, it is widely accepted that elevated mercury concentrations can cause changes in behavior, irritability, loss of cognitive function, headaches, etc. In Minamata Bay, Japan (1950's) locals that consumed mercury contaminated fish suffered from tremors, coma, seizures, and in some cases death (Gochfeld, 2003). Recently, Azevedo et al. (2012) noted that elevated mercury also affected endothelial (blood vessel) and cardiovascular function, indicating that, besides being a potent neurotoxin, it also affects other processes. Mercury is typically grouped
into three categories: Organic Mercury, Inorganic Mercury, and Elemental Mercury. For the purpose of this review and since much of what is noted in the literature, in regards to wildlife, refers to methyl-mercury ($\text{CH}_3\text{Hg}^+$) I will focus on organic mercury, the group into which $\text{CH}_3\text{Hg}^+$ falls. It should be noted however that other organomercuric compounds (ethyl and phenyl groups) are still found in some antiseptics (Clarkson, 2002), however they are not the focus of this review.

Long term studies in New England have indicated substantial risk to high trophic level piscivores such as the common loon ($\textit{Gavia immer}$), bald eagle, and osprey ($\textit{Pandion haliaetus}$), as well as various mammal populations (mink and river otter) from bioaccumulation of mercury (Evers et al. 2003, Evers et al. 2005, Evers and Clair 2005, Evers 2005, Pennuto et al. 2005, Welch 1994, Yates et al. 2004, Yates et al. 2005). While the southeast does not harbor breeding common loons due to latitudinal restrictions on breeding ground requirements, species such as the bald eagle and osprey are well established and continue to grow in numbers (Watts et al. 2009). These species, because of their position in the food web, as well as the prey upon which they forage, are particularly susceptible to mercury accumulation and the adverse effects associated with increased levels (Anthony et al., 1993; Bowerman et al., 1994, Bloom, 1992) and should represent an excellent endpoint to understanding the environmental phenomena that contribute to the methylation and transport of Hg in environmental systems. Moreover, because they are relatively long lived species, if the mechanisms that contribute to mercury availability in a system and the current levels are not monitored, detrimental effects from mercury toxicity may not become known until the population begins to decline through decreased survival of nestlings from lowered effectiveness of adult brooding and incubating as well as other neuropathological changes in behavior (Wolfe et al. 1998). Projects such as the Northeastern Ecosystem Research
Cooperative (NERC) funded mercury study in Northeastern North America have documented not only the drivers of mercury in north east North America, but have fostered the collection and analysis of detailed data for fish, crayfish, mammals, and a vast selection of avian species (Evers 2005).

In addition to the well-known aquatic dwelling species, elevated mercury levels have been documented in the Bicknell's Thrush (Rimmer et al, 2005), a high elevation montane passerine, thus indicating that mercury accumulation is not restricted to aquatic environments as previously postulated. Strong relationships have been found between adjacent land cover and common loon blood mercury levels indicating that land cover plays an important role in the production, availability, and transport of mercury to piscivores that forage in an aquatic environment (Kramar et al, 2005).

In the mid-Atlantic and southern portions of the United States, little research has been pursued (barring Florida and South Carolina) that documents and assesses the environmental mechanisms that contribute to contaminants in high trophic level piscivores such as the bald eagle. Jagoe et al. (2002) have documented elevated levels of Hg in the South Carolina bald eagle population, however little can be found regarding Hg levels in Virginia eagles baring research by Cristol et al. (2012) and Weimeyer et al. (1984) that found low levels of the toxin in the Coastal Plain. Significant research has been conducted on the Maine population of eagles where Hg concentrations are some of the highest in the nation (Desorbo et al. 2009). As research indicates that elevated levels of mercury are being found in the Virginia fish population (Virginia Dept. of Health, 2013), it is justifiable to assume that elevated levels would be found in those species foraging on the fish.

In addition to the more commonly researched avian species, much research has been
conducted on other species as well. Bergeron et al. (2007) noted elevated concentrations of total mercury and methyl-mercury in four different species of turtles, as did Hopkins et al. (2013). Additional studies found mercury in amphibians as well (Bergeron et al. 2010). Turnquist et al. (2011) noted that in New York 61% of the total tissue samples collected from snapping turtles (N = 48) had methyl-mercury concentrations that exceeded EPA consumption advisory limits of 0.3 ug. Green et al. (2010) analyzed 71 turtles from 14 different species of turtles that are commonly consumed throughout the world and also found concentrations of mercury (particularly in the carnivorous turtles) that exceeded the EPA consumption limits. In addition to freshwater turtles sampled from rivers and streams, mercury and selenium (Se) concentrations have been found in leatherback sea turtles (Perrault et al. 2013). Though liver concentrations of Se and Hg in dead leatherback hatchlings were correlated, there was no correlation between Se and Hg concentrations and reproductive success (Perrault et al. 2013). In a study conducted by Bank et al. (2005), elevated concentrations of mercury were found in two-lined salamanders, both in New England as well as Shenandoah National Park, Virginia. More recently, Huang et al. (2010) noted that Hg concentrations were correlated with both total length and body mass in eastern and ozark hellbender salamanders, with the eastern hellbender exhibiting higher Hg concentrations than the ozark.

In the aquatic environment, significant efforts have been made to model and understand the bio-accumulation factors (BAF) associated with the movement of mercury through the food chain. While significant work has been conducted on understanding bio-accumulation and biomagnification in lakes (Chen et al. 2005, Driscoll et al. 2007, Evers et al. 2007), little has been conducted on understanding the relationships between bio-accumulation and bio-magnification of Hg that exist in freshwater streams and rivers. Likely one of the largest differences between
observed variation of fish Hg in lakes versus observed variation of fish Hg in streams and rivers lies in the movement of water, and the potential reliance on both allochthonous and autochthonous inputs (St.Louis et al., 2001). Much of this will be summarized in the next section, but it is important to note that Ward et al. (2010) identified numerous factors relevant in stream ecosystems to the uptake and bio-magnification of mercury. In fish, for example, it is generally accepted that MeHg concentrations are a function of prey MeHg concentrations. Ward et al. (2010) also note that the growth efficiency of the fish, as well as changes at the base of the food-web can impact concentration of MeHg as it moves upward.

Mercury in the Environment

Substantial research has been conducted in an effort to better understand the mechanisms contributing to the methylation and transport of mercury in the environment. In elemental form, mercury does not pose a significant health risk. However, once methylated, Hg readily bioaccumulates and biomagnifies as it makes its way through the food chain. Current research suggests that the main elements required for methylation are sulfur, carbon, and hydrogen, coined the “biogeochemical axis of evil” by George Aiken of the USGS.

Within wetland environments, mercury accumulation is strongly associated with atmospheric deposition (Glooschenko W.A., 1986, and Norton S.A. 1987), however the direct discharge of pollutants due to anthropogenic sources, as well as natural sources, may also play a role in the availability of mercury. The Shenandoah River in Virginia is contaminated by a known point source of mercury. Natural sources of Hg include cinnabar and watershed runoff. Once mercury becomes available due to methylation it is readily available to the roots of plants (Huchabee J.W. 1973, Gilbert H. 1990, Czuba and Mortimer 1980), with aquatic vegetation
being one of the more efficient vectors (Gilbert, H. 1990, Maury-Brachet et al. 1990). Mosses, for example, have been found to be highly efficient at up-taking Hg (Huckabee J.W. 1973). Once Hg is in a system, the uptake by aquatic plants has the effect of making the mercury available to wildlife (Huckabee J.W. 1973). In addition to uptake by aquatic plants, Lindqvist et al (1991) have suggested that the leaves of trees have the ability to hold atmospheric mercury, thus reintroducing it once leaf fall occurs. Further methylation can then occur once the detritus is broken down and reduced to organic matter.

Numerous studies have shown that there is a strong relationship between the movement of mercury within freshwater systems and the amount of dissolved organic matter in those systems (Lee and Hultberg 1990, Mierle G. 1990, Miskimmen B.M. 1991). In the context of fish mercury, Grieb et al (1990) defined lakes as either drainage or seepage and research has shown that drainage lakes increase the availability of mercury to fish (Lee and Hultberg 1990, Miskimmen, B.M. 1991). It is generally accepted that mercury methylation occurs predominantly in sediments that exhibit anoxic conditions, as a result of sulfate reducing bacteria (Gilmour and Henry, 1991, Gilmour et al., 1992). Moreover, methylation of Hg is strongly associated with wetland environments, with much of the available mercury resulting from anthropogenic processes that release Hg into the atmosphere (Lee and Hultberg, 1990, Mierle G. 1990). Other sources of mercury can be found in industrial, medical, and municipal waste discharges. In order for mercury to be converted to the more toxic methylated form, it is necessary for Hg to be complexed with dissolved organic matter (Miller et al. 2007).

As noted in the previous section, while much research has been conducted on MeHg in lake ecosystems, little has been conducted on stream ecosystems. Stream dynamics and the surrounding land cover play a significant role in the availability of MeHg in stream ecosystems.
Once methylated mercury is present in the sediment, movement of that mercury to the water column can occur in a variety of different ways, including resuspensions of sediments, diffusion and advection, or straight bio-transfer. Ward et al. (2010) note that many of the factors that appear to contribute to Hg dynamics in streams, are the same factors commonly used in stream restoration efforts (riparian forest buffers, connectivity to floodplain, etc), and that restoration efforts may not be accomplishing the desired goals of a healthy aquatic ecosystem. Of particular note on streams is the impact that both dam construction and dam removal have on contaminant availability. Schetagne et al. (2000) note that concentrations of MeHg can remain elevated for decades after dam construction and then become a source for fish downstream. Conversely, Hart et al. (2002) and Stanley and Doyle (2003) suggest that due to the accumulation of sediments behind dam structures they may act as sinks for Hg and other contaminants which, upon removal, would have severe ecologic implications.

Yet another factor further complicating the understanding of Hg dynamics in stream ecosystems is forest harvesting and land disturbance. At the most basic level, the removal of trees and subsequent disturbance of soil can have the effect of mobilizing DOC, as well as Hg, resulting in an increase of Hg loads to streams and downstream habitat (Porvari et al. 2003, Munthe et al. 2007). It has also been shown that a shift from deciduous forest to coniferous forest (common in many logged areas of Virginia, as replanting is often done with white pine), can result in increased mercury becoming available (Kolka et al. 1999, Witt et al. 2009). Lastly, the use of prescribed fires in forest management is widely used. There have been several studies indicating that Hg loads to streams increase after a fire event (Amirbaman et al. 2004), however others have noted a decrease of Hg in biota following a fire event (Bank et al. 2005, Allen et al. 2005).
GIS, Modeling, and Mercury Monitoring

As the need to further understand the mechanisms that drive the availability of mercury in ecosystems has grown, research studies have begun to utilize the analytical capabilities of Geographic Information Systems. Significant work has been completed at the Federal level in respect to the development of numerical and GIS based (e.g. models that can be incorporated into GIS systems) Total Maximum Daily Load (TMDL) models. Likely one of the more well-known models is EPA's WCS Mercury Loading Model, developed on the ArcView 3.x architecture. It was initially used for calculating TMDL's on the Middle and Lower Savannah Rivers basins as well as numerous other basins within EPA's Region 4 geography (U.S. EPA 2001a). Several other numerical models have also been developed by the EPA such as Rivmod, Wasp, and Merc 4, and have been used in conjunction with one another to develop mercury transport models (Carrol et al. 2000). In 2002 Wasp 6.1 was developed that included specific subroutines for mercury transport (U.S. EPA, 2002). The IEM-2m model was also developed by the U.S. EPA in 1997 (U.S EPA, 1997). Whereas many models exist for modeling mercury both in the atmosphere, as well as in aquatic environments, this review focuses primarily on the use of GIS in modeling and understanding mercury movement in aquatic environments. In that context, aquatic environments are defined as either Lacustrine, Riverine, or Coastal. In lacustrine environments Bale (2000) developed a 2D finite system to model mercury transport. Additional models used for modeling mercury in lake systems include OLMM (Henry et al. 1995), QWASI (Diamond 1999), and RMCM (Gbondo-Tubawa and Driscoll 1998). Coastal models include ECOs (Abreu et al. 1998), 2D Stattrim (Sirca et al. 1999), and the Modified PCFlow3D (Rajar et al. 2000).

In addition to the numerous numerical and GIS models developed specifically for
modeling mercury movement and transport, many studies have simply included the analytical capabilities of GIS in an effort to answer research questions. For example, Hartman et al. (2009) developed a rule based Hg flux model within the framework of a GIS system. Using a combination of classification and regression tree (CART) modeling and GIS, and integrating the CART results into the GIS, Hg flux in three distinct biomes within the United States could be estimated (Hartman et al. 2009). CART analysis, which is easily integrated into a GIS, has also been used as an effective means of modeling heavy metals in general (Vega et al. 2009).

Furthermore, Munthe et al. (2007) successfully used GIS analysis to model and measure the amount of Hg that becomes re-mobilized during large storm-fell events. Their results indicated that larger storm-fell events have the potential to increase mercury loads, particularly in areas where forest operations are occurring (Munthe et al., 2007). Lin et al. (2011) found similar results utilizing a combination of a GIS based Hydrologic model (HEC-HMS) and a simulation model for estimating mercury concentrations in water (WASP-Hg). Lin et al. (2011) also noted that an estimated 50% of the total mercury loading occurred during three large storm events, further strengthening the suggestion that storm events contribute to periodic loading of mercury in aquatic ecosystems. To address the link between deposition of mercury and fish tissue concentrations of mercury, the U.S. EPA developed the Mercury Maps project (U.S. EPA, 2001b).

In addition to the actual analysis of mercury movement using GIS techniques, many studies have exploited the use of GIS as a means of acquiring additional data from which mercury concentrations in fauna and other biota could be analyzed. Dennis et al. (2005) used GIS techniques to extract watershed based parameters from which comparisons to mercury loads could be made. Miller et al. (2005) exploited both statistical models as well as geospatial
technologies in an effort to model both wet and dry deposition of mercury in North Eastern North America.

Besides the use of GIS to model and understand the environmental movement and transport of mercury, GIS technologies have also been used to assess contaminant concentrations in numerous species of animals. Kramar et al. (2005) utilized GIS technologies in an attempt to explain mercury concentrations in loons as a function of physical landscape properties. More recently, an EPA team of scientists and collaborators developed a geospatial based model for predicting and estimating fish and loon mercury levels throughout New England (Simcox et al. 2011). In this study, researchers found that percent wetland, watershed slope, percent agricultural land, percent forest canopy, as well as several other variables were statistically related to both loon and fish mercury levels (Simcox et al. 2011). Harris et al. (2006) exploited GIS technologies to examine the relationships between mercury and DOC on Kejimkujik Lake, a lake known for exceedingly high concentrations of mercury. Additional research conducted by Evers et al. (2007) employed extensive GIS techniques to model and identify “biological hotspots” of mercury accumulation in New England and eastern Canada. They were successfully able to predict these mercury hotspots using a combination of spatial data layers in conjunction with mercury concentrations in yellow perch and common loons, two distinct species that have been extensively researched in regards to mercury accumulation.

Besides the use of GIS to model Hg, GIS technologies have been widely applied to biotic studies in an effort to delineate things such as potential habitat, migration patterns, breeding patterns, and disease dispersal. In specific regards to the use of GIS for understanding eagle habitat, Buehler at al. (1995) utilized GIS technologies to develop a model for identifying and modeling potential eagle habitat on the Melton Hill Reservoir and Clinch River system, in
eastern Tennessee. Variables that were identified as significant included distance from adequate water sources, distance from human disturbance patterns, forest cover and type, as well as several other variables.

Katzner et al. (2012) successfully used GPS telemetry tools as well as GIS technologies to assess the impact that topography, as well as potential development of wind energy, has on migrating the second species of eagle known in the eastern United States, the Golden Eagle. Moreover, Bohrer et al. (2011) utilized both GPS and spatial analysis techniques to compare the migration pattern differences between turkey vultures and golden eagles. The authors noted that turkey vultures tend to utilize thermal uplift more so than golden eagles, and golden eagles tend to use orographic uplift more.
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Chapter 3: Mercury in Virginia Bald Eagles

Mercury Concentrations in Coastal and Inland Bald Eagles in Virginia

David E. Kramar\textsuperscript{1}, Bill Carstensen\textsuperscript{1}, and Jim Campbell\textsuperscript{1}

\textsuperscript{1}Department of Geography, Virginia Polytechnic Institute and State University

Abstract

We collected blood and feather samples from juvenile bald eagles (\textit{Haliaeetus leucocephalus}) from the coastal plain of Virginia, and the Piedmont and western regions of Virginia in an effort to determine which areas of the state were more likely to contain populations “at risk” from mercury toxicity. We analyzed the samples for total mercury as it is generally accepted that >90% of the total mercury found in blood and feathers is in the methylated form. As expected, samples collected from individuals located in the Chesapeake Bay region exhibited low concentrations of mercury compared to those further inland. Mean values for the samples collected from the coastal region indicated that population was not at a particularly high risk from mercury (Hg) toxicity (0.06 mg/kg (Min = 0.012 mg/kg, Max = 0.15 mg/kg (Blood)) and 2.2 mg/kg (Min = 0.62 mg/kg, Max = 9.998 mg/kg (Feather)), supporting findings by Cristol et. al. 2012. However, samples collected from the inland population exhibited levels in some areas that are approaching what is considered to be detrimental to avian health (0.324 mg/kg (Min = 0.06 mg/kg, Max = 0.97 mg/kg (Blood)) and 8.433 mg/kg (Min = 3.811 mg/kg, Max = 21.14 mg/kg (Feather)). Inland concentrations were statistically significantly different than those of the coastal plain (Prob > |t| = < 0.0003 and Prob > |z| = < 0.0001). It should be noted that the highest concentrations of Hg were found on the Shenandoah River, which is currently under a Hg advisory due to past anthropogenic activities.

Keywords: \textit{Haliaeetus leucocephalus}, Mercury, Methyl-mercury, Virginia
Introduction

It is well known that the neurotoxic properties of mercury and methylmercury represent a substantial risk to wildlife, in particular, higher trophic level species (Hopkins et al. 2013, Scheuhammer AM. 1987, Evers et al. 2005, Yates et al. 2004, Yates et al. 2005). Wolfe et al. (1998) have suggested that the impacts of elevated mercury are likely more significant when the individuals are in the developmental stages of growth, an issue that could significantly impact all species that are susceptible to mercury accumulation. Moreover, elemental mercury readily methylates into its more toxic form given appropriate environmental conditions (sulfide and dissolved organic matter) (Miller et al. 2007), thus making it available to wildlife and humans (Evers et al. 2005, Thompson DR, 1996, Kramar et al. 2005). Furthermore, the widespread transport of mercury via atmospheric processes, and subsequent atmospheric deposition, ensures that mercury will remain globally ubiquitous (Weiner et al. 2003). Much of the available mercury in the atmosphere is due, in large part, to a myriad of industrial sources (chlor-alkali facilities, for example) as well as the continued combustion of coal and other fossil fuels (Berlin et al. 2007). Due to the extensive risks posed to humans and wildlife from the accumulation of mercury, all states within the continental US have fish consumption advisories already in place.

Prior research has indicated that wetlands, interior impoundments, and freshwater environments, in general, are more conducive to mercury methylation given an abundance of dissolved organic carbon (Grieb et al. 1990, Lee and Hultberg, 1990, and Mierle, G. 1990). Therefore the availability of mercury to wildlife, particularly in those areas that contain an abundance of dissolved organic matter (DOM) is of great significance to understanding mercury availability (Miller et al., 2007).

Substantial research has been conducted throughout the United States using numerous
species of birds, mammals and reptiles. New England in particular has exhibited levels of mercury in wildlife that represent some of the highest in the nation (Evers et al. 2005, Rimmer et al. 2005). Research on mercury in bald eagles has been conducted in numerous other eastern states including South Carolina (Jagoe et al. 2002), Florida (Wood et al. 1996), Maine (Welch 1994), and New York (Desorbo et al. 2008). Moreover, research has been conducted in the Great Lakes region of the U.S. (Bowerman et al. 1994). Although the bald eagle has been well studied in the Coastal Plain of Virginia (Buehler 1990, Buehler et al. 1991, Watts and Byrd 2011), little research on mercury in bald eagles, has been conducted in the state of Virginia. Whereas in recent years there has been an increase in interest in the State, there has been no research that has extensively examined mercury concentrations between coastal and inland populations of bald eagles. Wiemeyer et al. (1984) analyzed non-viable eggs from bald eagles in Virginia, and reported mercury concentrations between 0.03 ppm and 0.17 ppm. Cristol et al (2012) found that eagles residing near the Chesapeake Bay exhibited low concentrations of Hg as evidenced by shed feathers. Whereas the Chesapeake Bay has shown low mercury concentrations in eagles, several inland areas in the state have already been identified as hotspots for mercury availability to other species, including the Shenandoah River (Cristol et al. 2012, Hopkins et al. 2013, and Jackson et al. 2011) and the North Fork of the Holsten River (Echols et al. 2009). Jackson et al. (2011) noted that even forest songbirds were exhibiting elevated mercury concentrations over 130 kilometers downstream of the known point source on the Shenandoah River, and that downstream habitat features such as flood plain areas are likely one of the contributing factors leading to the elevated concentrations being found in their study. In that respect, we should expect mercury concentrations found in eagles nesting within the inland portions of Virginia to exhibit higher concentrations of blood and feather mercury than those nesting in the Chesapeake...
Bay and coastal regions of the state. Moreover, as mercury accumulates in the body with age (Kamman et al., 2005), juvenile eagles that are already exhibiting levels at or above those considered detrimental would continue to exhibit increases in concentrations with age. Other researchers have noted significant levels of mercury in mammals, reptiles, and amphibians (Bergeron et al., 2010, Yates et al., 2004, Hopkins et al. 2013).

Within the state of Virginia, the majority of the eagle population (N = 726 occupied territories 2011) nests within the coastal plain associated with the Chesapeake Bay and the major tributaries that feed the bay (Watts and Byrd, 2011). However, more than 25% of the total population (N > 200) nests in the Piedmont and Mountain regions (Kramar, unpublished data 2013). Prior to 2007, little to nothing was known about the inland population, and it was in many cases considered inconsequential to the overall population (Watts, B., Personal Communication).

Due to the bald eagle’s propensity to nest along rivers, lakes, and impoundments (Andrew and Mosher, 1982, Buehlar, 2000, Johnsgard, 1990, and Peterson, 1986), as well as its status as a high trophic level apex predator, it is particularly susceptible to the effects of elevated mercury concentrations, especially given the nature of mercury to methylate in freshwater environments and the bald eagles preference for a piscivorous diet taken from those environments (Johnsgard, 1990). Therefore, it is logical to expect that mercury concentrations in central and western Virginia eagles would exhibit significantly higher blood and feather mercury concentrations than those of the coastal plain.

We sampled 46 juveniles in 28 nests between 2007 and 2012. Samples were collected from both the coastal plain as well as the Piedmont (central) and mountain (western) regions. We treated the Piedmont and mountains as a single “inland” region and the coastal as the “coastal” region, stratifying between the two. The collection of inland samples proved difficult due to the
lack of knowledge regarding the distribution and location of nests. Samples collected from the coastal plain were used to establish a “baseline” concentration within the state. Samples consisted of blood, feather, and talon (initially). Given the propensity for mercury to bond with keratin (Hahn et al. 1993), the substance comprising the feathers and talons, and the knowledge that keratin acts as a known method for sequestering blood mercury concentrations (Thompson et al. 1991), the collection of feather and talon samples were used to assess total body burden. Blood was used to assess recent dietary uptake.

Specifically we wanted to answer the following questions:

1. What are the current levels of mercury in juvenile bald eagles within the state of Virginia?
2. Do the levels of mercury vary between the coastal and inland populations?
3. What are the differences in mercury concentrations among rivers?
4. Where are the highest concentrations of mercury found within Virginia?
5. Controlling for the one river on which there is a known point source, are levels between the coastal and inland populations still significantly different.

Methods

Study Area and Surveys

This research was conducted throughout the state of Virginia between the spring of 2007 and the spring of 2012. Within the state, nests have now been reported in nearly every county, from Lee County in the far southwestern portion of the state to the coast, and new nests are reported every year. We sampled 28 nests across the state with a total of 46 individuals (Figure 1)
as indicated was necessary by a power analysis. Inland nests were defined as those that fell within the Piedmont and Mountain physiographic regions. Individual nests were treated as individual samples. If more than one individual was present in the nest, the mercury concentrations were averaged such that a single value could be assigned to each nest.

Nest locations in the coastal plain of Virginia are well known and have been identified through many years of aerial surveys conducted by the University of William and Mary, and funded through the support of the Virginia Department of Game and Inland Fisheries (VDGIF). Surveys of the Chesapeake Bay and its immediate tributaries have been conducted for well over 20 years, and in 2011 the region supported 726 known occupied territories (Watts and Byrd, 2011). Due to the intense nature of the survey efforts, the Chesapeake Bay population is likely one of the best understood populations on the east coast.

Prior to this work, interior nest densities and locations were not well understood. Information regarding the presence and locations of inland nests consisted predominantly of reports from the public, and no intensive surveys had been conducted. Beginning in 2007, new interior nest locations were determined through assistance from local birding clubs, private landowners, and the use of an existing VDGIF database of reported nests. In 2010 aerial surveys conducted by the VDGIF, with the assistance of Virginia Tech, identified many new nests along the major interior waterways. Continued cooperation with landowners and birding clubs has provided additional nest location information throughout the years. Through those efforts, bald eagles nesting inland in Virginia are now known to be far larger in number than initially thought. Prior estimates by biologists in the state placed the inland population at approximately 10% of the total population, a number we now know to be significantly low.
Blood, Feather, and Talon Collection

Samples from juveniles were collected using traditional climbing techniques (e.g. tree gaffs and a lanyard, or rope climbing techniques) to access the nest bowl. Once at the nest, the juvenile was placed in a large bag and lowered to the ground to facilitate the collection of samples. Juveniles were sampled between the ages of 4-7 weeks. Blood was collected from the brachial vein in the wing using 21 gauge butterfly needles and 4cc lithium heperanized vacutainers. Once collected, the blood was packed on ice and frozen within 4 hours of collection. Each vacutainer was labeled with a unique nest identification number and the federal band number. Feather samples consisted of two to three contour feathers collected from the breast of each individual. The samples were placed in a brown envelope, sealed, and labeled with the nest identification number and federal band number. Talon collection followed a modified methodology of Hopkins et al. (2007). We clipped 2 mm of talon from digit 2 on each foot. The talon samples were placed in a brown envelope, sealed, and labeled with the nest identification number and federal band number in the same manner as the feather samples. In addition to the biological samples, we collected complete morphometric measurements. Standard measurements taken included: weight, culmen, culmen with cere, culmen width, halux length, tarsus width and length, tail length, and wing chord. All activities were conducted under the appropriate state and federal permits.

Blood, Feather, and Talon Analysis

Samples were analyzed using a Milestone DMA-80 Direct Mercury Analyzer (DMA). The analysis of samples was conducted after calibrating the DMA using a certified standard mercury solution (SPEX CertiPrep) (Cell 1: $R^2 = 1.000$, Cell 2: $R^2 = 0.9998$). All calibration and
sampling consisted of measuring both the SPEX and the samples to the nearest 0.0001 g using a Metler Toledo digital scale, Model AT201). Quality assurance measures included the use of two standard reference materials (dogfish muscle tissue [DORM-2] and pine needles [1575a]). Pine needles were used due to low concentrations of mercury expected in samples collected from the coastal plain. In addition to the standard reference materials, two system and method blanks, one 10ng prepared Spex sample, one 60ng prepared Spex sample, one duplicate, and one matrix spike were run as quality assurance per every ten biological samples. Feathers and talons were mechanically washed following Ackerman et al. (2007) in a 1% Alconox solution, rinsed 6 times in deionized water, and dried for 24-48 hours at 60 degrees Celsius. The recovery of standard reference materials was near 100% and well within the accepted range of 90%-110%. QC verification was 105.6% and 103.1% during calibration. Spiked samples were based on the addition of 0.05 grams of 10ng SPEX. Once prepared, the samples were weighed out to approximately 0.02 grams, and placed in quartz boats. These were then inserted into the DMA. No more than 20 samples were run at any one time, with QC procedures conducted following every ten samples. All analysis followed U.S. Environmental Protection Agency Method 7473 (USEPA 2000).

**Statistical Analysis.**

We conducted a power analysis to determine the adequate number of samples required to test for significant differences between inland and coastal populations within the state. The analysis was conducted both prior to the collection of samples and post sampling, to insure that the a-priori estimates were sufficient. Given a standard deviation across all samples of 0.205, and 28 samples (individual nests) the power to determine differences at 0.25 was 0.874.
Many studies currently analyzing Hg data utilize a log transformation of the data in an effort to approximate linearity. In more than one instance we have noted that even given such transformations, the assumptions for linear regression, or other linear statistical tests, are often not met, indicating that a more appropriate methodology would be to utilize non-linear or non-parametric techniques, even after a transformation of the data. Given the non-linear nature often found in Hg samples we also wanted to determine if non-linear techniques could be used in an effort to by-pass the need for a transformation. Blood and feather sample means were compared using Spearman's Rank Correlation Coefficient due to the non-linear nature of the data. The comparison of mercury concentrations between coastal and inland nests utilized non-parametric equivalents of analysis of variance (ANOVA) techniques (Wilcoxon and Kruskal-Wallis tests). Prior to attempting a transformation we ran multiple goodness of fit tests to determine the current distribution. To confirm that the data did not meet normality requirements after a log-transformation, we applied the Shapiro Wilks Goodness of Fit Test. We then applied both a linear regression model and a non-linear quadratic regression model to the non-transformed data in order to determine if we could estimate blood mercury via feather mercury concentrations (or vice versa). The resulting models were then evaluated for fit using the residual distributions.

**Results**

The lowest blood Hg concentrations found (0.012 mg/kg) were located in the coastal plain of Virginia, specifically on the Pamunkey River whereas the highest concentrations were found inland on the Shenandoah River, and in particular on the South Fork of the Shenandoah River (0.974 mg/kg). Overall, the mean blood mercury concentration (0.180 mg/kg) across Virginia eagles sampled in this study was below the threshold (0.5 mg/kg) considered
detrimental to productivity (Table 1). The overall mean concentration for feather mercury was also below the current threshold (5.024 mg/kg).

When analyzed as a function of region (coastal versus inland) mean mercury concentrations begin to show significant differences. The mean blood mercury concentration in the coastal plain were 0.06 mg/kg (Min = 0.012 mg/kg, Max = 0.15 mg/kg) and feather mercury concentration was 2.2 mg/kg (Min = 0.62 mg/kg, Max = 9.998 mg/kg). Inland blood mercury concentrations had a mean of 0.324 mg/kg (Min = 0.06 mg/kg, Max = 0.97 mg/kg) and mean feather mercury concentrations of 8.433 mg/kg (Min = 3.811 mg/kg, Max = 21.14 mg/kg) (Table 2), with several nests exceeding a threshold mercury concentration of 0.5 mg/kg and several additional inland locations approaching that threshold.

Results also indicated substantial variation in blood mercury concentrations among rivers, when accounting for the difference in region (Figure 2). Feather mercury concentrations indicated a similar pattern (Figure 3).

When means were tested between inland and coastal populations, we found as expected, that inland mercury concentrations were significantly higher than samples collected from the coastal plain. We utilized both the parametric t-test as well as the non-parametric Wilcoxon Test (Tables 3&4). Lastly, we excluded the Shenandoah River because of known point source mercury issues. With the Shenandoah River excluded, mean blood and feather mercury concentrations still exhibited statistically significant differences, supporting the hypothesis that inland eagles are more susceptible to accumulation of mercury than the coastal population (Tables 5&6).

We tested the log transformed data using the Shapiro Wilks Goodness of Fit Test (W = 0.945, p = 0.0362). Prior to the transformation it was determined that the data met the Johnson
Su distribution (W = 0.976, p = 0.462). Results from the regression models indicated that traditional linear models, while adequate in their predictive capability, were not appropriate even after a log-transformation of the data (Figures 4&5). Further analysis and evaluation of the residuals supported the rationale for using non-linear models (Figures 6-7). The slight u-shaped curvature in the residuals of the linear model suggests the need for a higher order term to explain the curvature. The quadratic model addresses this problem, and consequently provides a better model fit as well as better predictive capabilities. This makes sense given that mercury in feathers accumulates over time and represents total body burden while mercury in blood represents recent dietary uptake. In fact in that respect, the relationship between feather and blood mercury, in this study, seems to represent a cumulative function.

**Discussion**

Whereas the coastal population has shown not only in this study, but also in Cristol et al. (2012), that mercury concentrations are not at a level that is currently of concern, the inland population is exposed to significantly higher concentrations than previously expected. From a management perspective, additional efforts to facilitate remediation and further understand the full distribution and potential implications of methyl-mercury within the inland portions of the state should be considered. As has been noted in prior research, the Shenandoah River and the North Fork of the Holston River (not sampled) are certainly areas where ongoing research is recommended, however, there are additional areas within the state that are approaching levels of concern, and should be further investigated. One such area is along the James River from Nelson County, Va to just north of Lynchburg, Va where concentrations, while not at the blood Hg threshold of 0.05 mg/kg, did exhibit blood Hg concentrations not far below that level. Additional
rivers that exhibited concentrations approaching a 0.5 mg/kg concentration include the inland portions of the Rappahannock River, Nottoway River, and Wolf Creek. Given that mercury is sequestered rapidly during feather growth, it is not unreasonable to expect that once feather development is complete, measured levels of blood Hg would increase. Thus an individual at 4 weeks of age, who is approaching a 0.5 mg/kg threshold, would likely exceed that threshold once the feathers have completely developed. Moreover, additional research should be conducted on the North Fork of the Shenandoah River as mercury concentrations here were also approaching the 0.5 mg/kg concentration. Given the mercury concentrations found on the rivers sampled in this research, additional efforts should be given to sample rivers that were not included such as the Powell River, the Holston River, and the New River.

**Acknowledgements**

We would like to extend our sincere gratitude to the Virginia Department of Game and Inland Fisheries: specifically Sergio Harding and Jeff Cooper. In addition, we would like to thank the staff at the Wildlife Center of Virginia, Bryan Watts and Libby Mojica from the University of William and Mary, and Justin Miller, Kari McMullen, Emily Wright, and Jessica Rich for extensive field assistance. Samples were collected under federal permits held by either the University of William and Mary (Bryan Watts) or the Virginia Dept. of Game and Inland Fisheries (Jeff Cooper), and state threatened and endangered species permits. Funding for this research was supported under the U.S. Environmental Protection Agency - Science to Achieve Results (STAR) Fellowship program and the Virginia Dept. of Game and Inland Fisheries.
Literature Cited


Figure 3.1: Study Area and sample locations. Sample locations are represented by the gray circles. The break between the Coastal Plain and the inland regions of the state is defined by the black line. Areas west of the fall line are considered “inland”, while areas east of the fall line are considered “coastal”
Table 3.1: Mean blood and feather mercury concentrations (mg/kg). Statistics are for the entire state of Virginia.

<table>
<thead>
<tr>
<th></th>
<th>Blood Hg (mg/kg)</th>
<th>Feather Hg (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.180</td>
<td>5.024</td>
</tr>
<tr>
<td>Std Dev</td>
<td>0.243</td>
<td>5.710</td>
</tr>
<tr>
<td>Std Err Mean</td>
<td>0.036</td>
<td>0.842</td>
</tr>
<tr>
<td>Upper 95% Mean</td>
<td>0.253</td>
<td>6.720</td>
</tr>
<tr>
<td>Lower 95% Mean</td>
<td>0.108</td>
<td>3.328</td>
</tr>
</tbody>
</table>
### Table 3.2: Mean blood and feather mercury concentrations by habitat type (coastal vs. inland). As expected, mean inland concentrations are higher than mean coastal concentrations.

<table>
<thead>
<tr>
<th></th>
<th>Blood (mg/kg)</th>
<th>Feather (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coastal</td>
<td>Inland</td>
</tr>
<tr>
<td>Mean</td>
<td>0.060</td>
<td>0.324</td>
</tr>
<tr>
<td>Std Dev</td>
<td>0.062</td>
<td>0.297</td>
</tr>
<tr>
<td>Std Err Mean</td>
<td>0.012</td>
<td>0.065</td>
</tr>
<tr>
<td>Upper 95% Mean</td>
<td>0.085</td>
<td>0.459</td>
</tr>
<tr>
<td>Lower 95% Mean</td>
<td>0.034</td>
<td>0.189</td>
</tr>
<tr>
<td>N</td>
<td>25</td>
<td>21</td>
</tr>
</tbody>
</table>
Figure 3.2: Mean blood mercury concentrations (mg/kg) by river and habitat type. Higher blood mercury concentrations are found on the inland rivers.
Figure 3.3: Mean feather mercury concentrations (mg/kg) by river and habitat type. Higher feather Hg concentrations are found on the inland rivers.
<table>
<thead>
<tr>
<th></th>
<th>Sample Matrix</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blood Hg (mg/kg)</td>
<td>Feather Hg (mg/kg)</td>
<td></td>
</tr>
<tr>
<td>Difference</td>
<td>0.264079</td>
<td>6.271911</td>
<td></td>
</tr>
<tr>
<td>Std Err Dif</td>
<td>0.066033</td>
<td>1.532459</td>
<td></td>
</tr>
<tr>
<td>Upper CL Difference</td>
<td>0.401221</td>
<td>9.440104</td>
<td></td>
</tr>
<tr>
<td>Lower CL Difference</td>
<td>0.126937</td>
<td>3.103719</td>
<td></td>
</tr>
<tr>
<td>Confidence</td>
<td>0.95</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>t Ratio</td>
<td>3.99920223</td>
<td>4.09270973</td>
<td></td>
</tr>
<tr>
<td>DF</td>
<td>21.4658718</td>
<td>23.2575118</td>
<td></td>
</tr>
<tr>
<td>Prob &gt;</td>
<td>t</td>
<td></td>
<td>0.0006</td>
</tr>
<tr>
<td>Prob &gt; t</td>
<td>0.0003</td>
<td>0.0002</td>
<td></td>
</tr>
<tr>
<td>Prob &lt; t</td>
<td>0.9997</td>
<td>0.9998</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.3: T-test for difference in means between coastal and inland populations. In both cases, inland mercury concentrations were statistically significantly higher in the inland population.
Table 3.4: Wilcoxon Test for Difference in means between the coastal and inland populations. Like the paired t-test, significant differences were identified.
Figure 3.4: Quadratic fit of the Blood/Feather mercury relationship - \[ \text{Blood}_\text{Hg} = 0.0185943 + 0.02095 \times \text{Feather}_\text{Hg} + 0.0017723 \times (\text{Feather}_\text{Hg} - 5.02407)^2. \] \[ R^2 = 0.83. \] The shaded area represents the confidence fit.
Figure 3.5: Linear fit of the blood/feather mercury relationship. Blood$_{Hg} = -0.008855 + 0.0376644\times$Feather$_{Hg}$. $R^2 = 0.78$. The shaded area represents the confidence fit.
Figure 3.6: Residual distribution of linear model. The slight u-shaped pattern in the residuals suggests the need for a higher order term.
Figure 3.7: Residual distribution of Quadratic model. The quadratic model exhibits a much better residual distribution.
<table>
<thead>
<tr>
<th>Sample Matrix</th>
<th>Blood Hg (mg/kg)</th>
<th>Feather Hg (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difference</td>
<td>0.117</td>
<td>3.011</td>
</tr>
<tr>
<td>Std Err Difference</td>
<td>0.032</td>
<td>0.956</td>
</tr>
<tr>
<td>Upper CL Difference</td>
<td>0.182</td>
<td>4.993</td>
</tr>
<tr>
<td>Lower CL Difference</td>
<td>0.051</td>
<td>1.029</td>
</tr>
<tr>
<td>Confidence</td>
<td>0.950</td>
<td>0.950</td>
</tr>
<tr>
<td>t Ratio</td>
<td>3.685</td>
<td>3.149</td>
</tr>
<tr>
<td>DF</td>
<td>20.526</td>
<td>22.245</td>
</tr>
<tr>
<td>Prob &gt;</td>
<td>t</td>
<td></td>
</tr>
<tr>
<td>Prob &gt; t</td>
<td>0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>Prob &lt; t</td>
<td>0.999</td>
<td>0.998</td>
</tr>
</tbody>
</table>

Table 3.5: Paired T-Test for blood and feather mercury concentrations, excluding the Shenandoah River. Values in bold indicate significance.
<table>
<thead>
<tr>
<th>Sample Matrix</th>
<th>Blood Hg (mg/kg)</th>
<th>Feather Hg (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>486</td>
<td>463</td>
</tr>
<tr>
<td>Z</td>
<td>3.99608</td>
<td>3.381</td>
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<tr>
<td>Prob &gt;</td>
<td>z</td>
<td></td>
</tr>
<tr>
<td>Chi Square</td>
<td>16.0756</td>
<td>11.5217</td>
</tr>
<tr>
<td>DF</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Prob. &gt;</td>
<td>ChiSq</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.6: Wilcoxon Test for difference in means. Conducted after excluding the Shenandoah River, means are statistically different.
Chapter 4: Spatial Modeling of Bald Eagle Mercury

The Impact of the Unit of Analysis when Modeling Land Cover and Juvenile Bald Eagle Blood Mercury Concentrations

David E. Kramar\textsuperscript{1}, Bill Carstensen\textsuperscript{1}, Steve Prisley\textsuperscript{2}, Jim Fraser\textsuperscript{3}, and Jim Campbell\textsuperscript{1}
\textsuperscript{1}Department of Geography, Virginia Polytechnic Institute and State University
\textsuperscript{2}Department of Forest Resources and Environmental Conservation, Virginia Polytechnic Institute and State University
\textsuperscript{3}Department of Fish and Wildlife Conservation, Virginia Polytechnic Institute and State University

Abstract

We modeled methyl-mercury concentrations in juvenile bald eagle blood (\textit{Halieneatus leucocephalus}) using land cover percentages within estimated foraging ranges of varying size, and two watershed/catchment models of varying size. We determined that the choice of spatial scale is important when determining the impact that land cover has on juvenile eagle blood Hg concentrations. We analyzed three (3) estimated foraging territories based on 1km, 3km, and 5 km distances from the nest locations based on estimated territory sizes during breeding season (Buehler et al. 1991). Of those, we found that a distance of 5 km, that included buffers around all drainages, produced the best estimates of blood mercury when related to land cover ($R^2 = 0.62$, RMSE = 0.133, AIC = -17.415, N = 28), with the predictive capability of the models declining as distance decreased (3 km: $R^2 = 0.40$, RMSE = 0.178, AIC = -17.953, N = 28, 1 km: $R^2 = 0.47$, RMSE = 0.168, AIC = -8.106, N = 28). When we applied the same technique to the three different sized watersheds we found that the HUC 12 watersheds produced the best overall estimates of blood Hg concentrations ($R^2 = 0.63$, RMSE = 0.140, AIC = -17.953, N = 28) as compared to the larger HUC 10 watersheds ($R^2 = 0.29$, RMSE = 0.184, AIC = -1.079, N = 21). The HUC 12 level was also much better than the modeled catchments ($R^2 = 0.34$, RMSE = 0.147, AIC = -9.279, N = 20). It is important to note that one reason the larger watersheds produced a less robust model may be related to the fact that multiple nests would fall into a single drainage basin, from which we used the average to obtain a single value for each watershed, reducing the overall sample size. When all models were compared, the HUC 12 had the highest overall predictive capability, supporting research by Shanley et al. (2008) that suggested using a small watershed scale approach to understanding Hg uptake in the environment.

Keywords: Bald Eagle, Methyl-Mercury, Land Cover, Geographic Information Systems
Introduction

Mercury (Hg) concentrations in Virginia waterways have been receiving a great deal of focus over the last decade, particularly due to elevated concentrations being found in several rivers within the state. The South River (a tributary of the South Fork of the Shenandoah) has been widely studied and still remains one of the most impacted waterways in the state in regards to wildlife due to elevated Hg concentrations found in multiple different species (Cristol et al. 2008, Bergeron et al. 2007). The source of contamination on the South River is an historical point source in Waynesboro, Virginia. In addition to the South Fork of the Shenandoah River, the North Fork of the Holsten River has also been impacted by point source emissions (Echols et al. 2009). In Virginia, several rivers including the Blackwater, Nottoway, Meherrin, and the Great Dismal Swamp Canal have been designated with consumption advisories due to elevated concentrations of Hg in fish (Virginia Dept. of Health, 2013).

Whereas elemental mercury is not readily bio-available, in its methylated form it can rapidly bio-accumulate and bio-magnify as it moves through the food chain, causing severe impacts on aquatic and terrestrial biota (Heinz 1996, Bergeron et al. 2007, and Drevnick and Sandheinrich 2003). Due to environmental processes that influence methylation, as well as the associated impacts on aquatic and terrestrial biota, much research has been conducted in an effort to understand the environmental and landscape characteristics that drive the conversion of elemental Hg to methyl-mercury (CH$_3$Hg$^+$) in and around aquatic ecosystems (Brigham et al. 2009, Mason and Lawrence 1999).

Little has been done to associate blood Hg concentrations in juvenile eagles to contributing land cover classes within areas and/or drainage basins that eagles are expected to forage in during breeding season. This link is important as the methylation process occurs as a
function of chemical processes that take place within sediments and soils in the landscape 
(Ravichandran 2004, Shanley et al. 2008, Merrit and Amirbahman 2009), and has been tied in 
large part to various types of land cover (Kamman and Engstrom 2002, Rea et al. 2002, Kamman 
et al. 2004, Kramar et al. 2005, Demers et al. 2007). Moreover, numerous studies have 
adequately used land cover characteristics to estimate mercury levels in various different types of 
biota, in particular fish (Scudder et al. 2009, Brightbill et al. 2004). Several studies have 
indicated a positive relationship between Hg and forest type, with coniferous forests exhibiting 
the greatest efficiency at scavenging total and methyl Hg, followed by deciduous forests (Witt et 
inland is comprised of deciduous or mixed forest. As relationships have previously related land 
cover characteristics to blood Hg concentrations in loons (Kramar et al., 2005), it is reasonable to 
assume that if foraging areas of breeding eagles can be defined, whether it be estimated foraging 
areas or predefined drainages (USGS Hydrologic Unit Codes (HUC)), we should be able to 
establish relationships between land cover characteristics and blood Hg concentrations in 
juvenile eagles. However, one question that has not been addressed in the literature is the scale at 
which to model these relationships. If the analysis is conducted at too narrow a spatial scale, 
heterogeneity across the landscape will be lost. Conversely, at too broad of a spatial scale we lose 
the variability between samples, due to multiple samples falling within a single unit of analysis, 
and from over-aggregation of spatial variables within samples. As the previous research has 
shown relationships between blood level Hg and land cover characteristics, both in other states 
and in other species, we became concerned with the optimal scale at which to model that 
relationship. The transmission of Hg from the environment to the birds has two important 
considerations: bird territories within which they forage and interact with environmental Hg, and
the environment itself which transports the Hg to that territory. These processes operate under different spatial realms. One is distance based around the nests, and the other is watershed based around the sample location.

Buehler et al. (1991) have suggested that during the breeding season, adult eagles generally forage within 6 kilometers of the nest location, with a large abundance of that time spent between 1-3 kilometers. Due to the territorial behavior of breeding eagles we can assume that much of the prey being provided to pre-fledged eagles is likely originating from a reasonably small area surrounding the nest location. This work suggests that eagle behavior should be reflected, to the extent possible, in modeling efforts. Zones of study should reflect eagle behavior in foraging, which is largely based on distance.

In addition to exploring various size foraging areas within our study area, we explore the impact that upstream watershed/catchment landscape characteristics have on concentrations of blood Hg in juvenile eagles. Irrespective of eagle behavior, there must be mercury in the environment in order to be ingested by the eagles. Watersheds are an important vector for movement of methyl mercury (CH$_3$Hg$^+$) particularly during high flow storm events (Shanley et al. 2005). Moreover, Shanley et al (2008) have made the argument for applying a small watershed approach to understanding mercury in the environment, suggesting that it is both “well-suited and underutilized” (Shanley et al. 2008 pg 1). Zones of study may also benefit from being associated with watershed regions.

We address the following hypotheses in this research:

1. The percent of individual land cover categories, particularly wetland and forest, within each of the defined eagle foraging areas and within each of the defined watersheds/catchments is significantly related to measured blood mercury concentrations
in juvenile eagles.

2. The spatial scale of the unit of analysis will be a key factor in determining the predictive capabilities of the model.

3. The definition of the region formed for the analysis (foraging areas vs. catchment based) will also influence the predictive capabilities of the models.

Methods

Study Area

The study was conducted between the spring of 2007 and the spring of 2012, within the state of Virginia (Figure 1). Virginia is comprised of three physiographic regions including the coastal plain, the Piedmont, and the Mountains. For the purpose of this study, the Piedmont and Mountain regions were considered “inland”, and the remaining portion considered coastal. Coastal nests within the state have been well documented by the Center for Conservation Biology at The College of William and Mary by over 25 years of continuous surveys. Interior nest locations were identified via cooperation with local birding clubs, an existing Virginia Department of Game and Inland Fisheries (VaDGIF) database of inland nests, and traditional aerial surveys. Aerial surveys were conducted in the spring of 2010 by the VaDGIF, with assistance from Virginia Tech. Within the state, nests were selected to sample at least a portion of each major river basin in which eagles were present. Samples were stratified between coastal and inland nests.

Sample Collection

Biologic samples consisting of blood and feather were collected from 46 individuals, representing 25 from the coastal plain and 21 from the inland population, from a total of 28
individual nests. The nests were accessed using traditional rope and lanyard climbing techniques. A handheld GPS was used to record the geographic location of each nest. To minimize potential impact to the nest structure itself, a modified hook was used to reach the tarsus of the juvenile eaglets to “encourage” them to move closer to the climber while minimizing the risk of falling from the nest. This method allowed climbers to remain almost completely below the nest, with only the shoulders and head exposed above the nest. Once captured, the juvenile eagles were placed in a large bag and lowered to the ground to facilitate sample collection and banding efforts. Blood samples were collected from the brachial vein in the wing using sterile, 21 gauge butterfly needles and 4 cc lithium heperanized vacutainers. Two vacutainers were collected from each individual, one for analysis and the second for archive. The vacutainers were then labeled with the date, nest identification code, species, and federal band number. Upon collection, blood samples were packed on ice and frozen within four hours. Three contour feathers were collected from each individual as well. Upon collection, the feathers were placed in a small envelope and labeled in the same manner as the blood samples. The birds were then weighed, measured, banded, and placed back in the nest. In addition, we collected prey remains from the base of the nest in order to determine diet and for reference when developing the spatial models. All activities were conducted under the appropriate state and federal permits held by either The Center for Conservation Biology, or the VaDGIF.

Sample Analysis

Whole blood and feather samples were analyzed using a DMA-80 Direct Mercury Analyzer. Approximately 0.3 grams of whole blood were used and approximately 0.3 grams of feather material were used. Feathers were cleaned using a 1% Alconox solution and rinsed 6
times in deionized water, following a modified methodology of Ackerman et al. (2007). The feathers were then dried at 60° Celsius.

Prior to analysis, the DMA was calibrated using Spex Certiprep (Cell 1: \( R^2 = 1.000 \), Cell 2: \( R^2 = 0.9998 \)). The Spex Certiprep, samples, and standard reference materials (SRM) were measured to the nearest 0.0001 using an AT201 Metler Toledo digital scale. Quality assurance steps were conducted after every ten samples and consisted of two SRM's (Dogfish muscle tissue (DORM-2) and Pine needles (1575a)), two system and method blanks, one 10 ug spex sample, one 60 ug spex sample, one duplicate, and one matrix spike (+0.05g of 10ug spex solution). The SRM recovery rates were near 100% and well within the accepted range of 90%-110%. After calibration, the QC verification recovery rates were 105.6% and 103.1%. All analysis followed U.S. Environmental Protection Agency Method 7473 (USEPA, 2000).

**Spatial Data Acquisition and Processing**

Spatial data were acquired from multiple sources. Land cover data (2006 National Land Cover Database (NLCD)) were acquired from the USDA/NRCS Geospatial Data Gateway. River and waterway information was derived from the National Hydrologic Database (NHD). Eagle mercury concentration locations were geo-referenced to the North American Datum of 1983 (NAD83) for latitude/longitude. Current information regarding all known eagle nest locations within Virginia was provided by the Virginia Department of Game and Inland Fisheries (VaDGIF). All spatial data utilized in the study were projected into UTM Zone 17 North for the spatial analysis. Linear units are in meters.
Delineation of Estimated Foraging Areas

Utilizing ArcGIS extraction tools, we selected all USGS Hydrologic Code 8 (HUC 8) watersheds that contained a sampled nest. We then clipped the NHD stream features to each of the individual HUC 8 watersheds in order to reduce the data set size to manageable levels. Once the NHD data were clipped to the individual watersheds, we created 1.5 kilometer buffers around the major streams and tributaries, based on the assumption that eagles were more likely to forage nearer a water source. The buffered NHD data were then intersected with the 5 km buffers surrounding the eagle nests such that each nest was associated with a modeled foraging area that consisted of the major streams and rivers as defined in the NHD dataset (Figure 2). Point data for nest locations utilized in the study were buffered at a distance of 5 km, 3 km, and 1 km. As noted in the literature (Buehler et al. 1991), eagles generally forage within 1-5 km of the nest location during breeding season. Three separate distance classes were used to assess the impact that land cover in the estimated forage area size has on the predictive capability of the models. Because of their small areal extent, and corresponding lack of heterogeneity, the 1 km and 3 km areas were simply buffers around the nest location. An additional 5 km buffer was created in order to compare model results among the simple buffers, an analysis that could not have been done utilizing only the modeled 5 km foraging area.

Delineation and Processing of Watersheds

Watershed delineation (referred to as “catchment” from here on) was completed using the ArcGIS ArcHydro toolset, and each watershed was defined upstream from a point 5 km below the nest on the nearest waterbody. We utilized a “filled” 10 meter digital elevation model (DEM) from which we could calculate flow direction and flow accumulation. The derived accumulation
and direction data sets were then used to derive stream segments, catchments, adjoining catchments, and drainage outlets. We used the derived catchment areas as opposed to upstream HUC 8 watersheds (which limited the sample size significantly due to multiple samples along several rivers) in order to maintain a reasonable sample size (N = 20). The derived catchments were approximately 1/3 the size of the HUC 8. When multiple samples fell within a defined catchment, we took the average of the blood Hg values that occurred in the catchment. An average value was assigned due to the high level of spatial autocorrelation between blood Hg samples. In addition to the modeled catchments, we also utilized USGS HUC 12 and HUC 10 watersheds which were slightly smaller than the modeled catchments and slightly larger than the defined foraging areas based on the 5 km distance class. The HUC 12 watersheds were included based on research conducted by Shanley et al. 2008, and because they represented the smallest watersheds currently available from the USGS.

**Spatial and Statistical Analysis**

We first calculated Moran's I to determine if spatial autocorrelation existed within the collected eagle blood and feather Hg samples. A Getis-Ord Gi* hot spot analysis was also used to identify statistically “hot” areas of eagle blood and feather Hg. Significant spatial autocorrelation violates the assumptions of many parametric tests. Due to the observed spatial variability of mercury concentrations found in sediments as well as biota, it was expected that autocorrelation exists and nonparametric analysis techniques for modeling should be explored.

We applied non-parametric classification and regression tree (CART) techniques to explore the impact that land cover played in the availability of Hg to juvenile bald eagles utilizing the partition platform in JMP 10.0 (JMP 2014). CART modeling was an adequate choice
as we often reclassify mercury concentrations into categories of low, moderate, high (for example), and the resulting output from the CART models produces grouping of data based on recursive partitioning of the dependent variable against the independent variables. CART modeling has also been used to model mercury in prior studies (Cheng et al. 2009, Hartman et al. 2009, Vega et al. 2009). The National Land Cover Dataset (NLCD) was reclassified such that each of the independent land cover types was represented by its own Boolean raster file, represented by a 0 or 1, with 1 being the land cover of choice. Using the reclassified land cover data, we calculated the percent of each cover type that fell within a specified eagle foraging area, as well as the percent that fell within each defined catchment /watershed. We used nine different land cover classes including %barren, % coniferous, % deciduous, % emergent wetland, % high intensity development, % mixed forest, % row crop, and % woody wetland. These classes were chosen as they have been shown to be significant in prior research.

**Results**

*Moran's I and Getis Gi* Statistics

Moran's I statistics indicated that significant spatial autocorrelation existed within both the eagle blood and eagle feather Hg samples, indicating that the clustered pattern was not due to random chance (Table 1). The Getis Gi* statistics indicated several areas in the state with both statistically high Hg concentrations as well as statistically low Hg concentrations (Figure 3).

*Analysis of Land Cover on Juvenile Bald Eagle Blood Hg Concentrations in Defined Foraging Areas (1, 3, 5 Kilometer)*

In all of the CART analyses, we found that the significant land cover types included
deciduous forest, mixed forest, pasture land, and wetland, supporting previous literature that has also found these cover types to influence the availability of Hg within a system. Results from the land cover analysis indicated that the proportion of contributing land cover types within each of the defined foraging areas at the 5 km scale (Figures 4-5) exhibited the greatest influence in blood mercury concentrations ($R^2 = 0.62$, RMSE = 0.133, $N = 28$, AIC = -17.415) compared to the 3 km and 1 km buffers. (Figure 6-7). It should also be noted that the more sophisticated “foraging territories” developed at the 5 km range produced much better results than those from the simple 5 km circular buffer ($R^2 = 0.62$ vs. $R^2 = 0.54$). At 1 km and 3 km the predictive capability of the CART models dropped significantly (Table 2), (Figures 6-7). Of importance to note is that Virginia, as opposed to the New England states in which a great deal of previous research has been done, does not have the extent of freshwater wetlands which are known sources of Hg methylation. In Virginia many of the wetlands fall in the oligohaline, mesohaline, and polyhaline environments and are characterized by higher salinity concentrations, which is why we likely see lower mercury associated with wetland abundance. Second in importance was the percent of pasture, followed by deciduous and mixed forest types.

We did not include sediment Hg, fish Hg, and habitat (inland vs. coastal), as the intent was to explore the relationship between land cover and blood Hg concentrations. As blood Hg represents recent dietary uptake, and feather Hg represents total body burden since feather growth (the rate at which Hg is sequestered from blood to feather varies among individuals), we opted not to analyze the influence of land cover on feather Hg concentrations and focus only on blood Hg concentrations for the purpose of this analysis.
When we analyzed the data utilizing the HUC 12 watersheds we obtained slightly better predictive results than any previous model with a generally good fit overall ( $R^2 = 0.63$, RMSE = 0.140, N = 28, AIC = -17.953), indicating that a smaller drainage is a better predictor of blood Hg concentrations in juvenile eagles than either larger drainage basins, or estimated foraging areas (Figure 8). This idea is further supported by Shanley et al. (2008) who noted that a small watershed approach is generally “well-suited but under-utilized” in Hg research. It should be noted that while the HUC 12 produced the best estimates, it was closely followed by the 5 kilometer foraging area which, in this sample, was only slightly smaller in size.

Results from defined drainage basins (catchments delineated at roughly 1/3rd the size of a HUC 8 level and those at the HUC 12 and HUC 10 levels) further indicated the significance of identifying the correct spatial scale at which to analyze juvenile blood Hg concentrations. At the larger catchment sizes (HUC 10 and the modeled larger catchments), the predictive capability of the CART model provided less robust estimates than those calculated within the previously defined 5 km, 3km, and 1 km foraging ranges, and provided the least robust results overall. (Catchment: $R^2 = 0.34$, RMSE = 0.147, N = 20, AIC = -9.279, HUC 10: $R^2 = 0.29$, RMSE = 0.0.184, N = 21, AIC = -1.07873) (Figure 9-10).

These results further suggest that much of the mercury found in juvenile eagle blood is likely becoming methylated within a fairly small geographic area around the nest and therefore looking at a smaller contributing area will likely produce better estimates. It is important to note that at the larger spatial scale, multiple nests were located in individual catchments such that the resulting sample size was reduced by eight (N = 20). When we graphed the $R^2$ values, the variation between unit size and the predictive capabilities of the individual models is clear.
(Figure 11). Furthermore, when we plot the $R^2$ value versus the area (hectares), we find that the average area of the HUC 12 at approximately 10100 hectares produces the highest $R^2$ value (Figure 12).

**Discussion**

The land cover types identified as significant in this study have also been found as significant in numerous other studies (Kramar et al. 2005, Demers et al. 2007, Selvendiran et al. 2008), however the most interesting aspect of this research has been identifying the most appropriate spatial scale at which predictive models should be developed to estimate blood Hg concentrations in juvenile bald eagles. As such, future investigations of mercury, whether in sediment, water, fish, or birds should explore the relationships at varying scales in order to determine the most appropriate unit of analysis for the current question. In our research, both the HUC 12 watersheds as well as the 5 Km estimated foraging areas produced the best results, with the slightly larger HUC 12 watersheds providing slightly better models. We also found that as the area of the watershed increased above the HUC 12 level, the predictive capability of the CART models decreased. These results were apparent in the decline of the predictive capabilities at both the HUC 10 level as well as at the modeled catchment level. A similar, though not as drastic pattern, was seen with the smaller 3km and 1 km areas. Therefore, we conclude that there is clearly a relationship between the predictive capabilities of statistical models and spatial scale when trying to understand blood Hg concentrations in juvenile eagles. Even the models with the least predictive capabilities still exhibited statistically significant results, albeit not as strong. One of the likely reasons for the HUC 12 watersheds better estimates of Hg is that they represent smaller drainage basins, yet still hold characteristics of actual watersheds and while it is likely
that the eagles are foraging within these watersheds, the models do not attempt reflect the behavioral aspect of eagle foraging. Moreover, the buffered territories, as well as the more sophisticated 5 km territories did not account for the drainage patterns within a basin. Further investigation is warranted, particularly in applying this method to other species.
Literature Cited


Hartman, J.S., Weisberg, P.J., Kuiken, T., Lindberg, S.E., Zhang, H., Rytuba, J.J., Gustin, M.S.


Figure 4.1: Study area and nest locations. Nests were located throughout the state, generally along major waterways. The solid black line represents the divide between the inland and coastal regions of the state, while the black dots represent the nest locations. Areas that are west of the fall line represent “inland” samples, while areas east represent “coastal samples.”
Figure 4.2: Example of an eagle “foraging area” as defined by NHD data. Streams were clipped at the 5 kilometer buffer around the nest and then buffered to create a “territory” that was based off the NHD drainage network.
Figure 3: Getis Gi* Hot Spot Analysis of blood Hg concentrations (mg/kg) in Virginia. In general, areas west of the fall line (inland) exhibited hot spots of elevated blood Hg, while areas east of the fall line exhibited low concentrations of blood Hg.
Table 4.1: Moran's I for mercury data used. Both blood and feather Hg data in eagles were significantly auto-correlated, indicating that the Hg concentrations were distributed randomly. Interpreted the same as a correlation index, spatial autocorrelation was positive.

<table>
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<td>Moran's I</td>
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<tr>
<td>P-Value</td>
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<td>0.000029</td>
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</table>
Figure 4.4: Land cover influence on blood Hg concentrations in sampled juvenile bald eagles using a custom 5km territory. ($R^2 = 0.61$, RMSE = 0.134, $N = 28$, AIC = -15.552). Contrary to numerous studies, mean blood Hg concentrations are higher when the percentage of wetlands is lower. However, we find a higher mean blood Hg concentration as the percent of pasture increases.
Figure 4.5: CART analysis of land cover influence on blood Hg levels using a simple 5 km circular buffer. ($R^2 = 0.54$, RMSE = 0.156, $N = 28$, AIC = -11.949). At the first terminal node we find high Hg when low wetlands are present. Conversely, at the lower terminal nodes, where blood Hg concentrations are low in general we find the inverse relationship. These samples are located in the coastal plain and follow literature suggesting wetlands positively influence Hg availability. Mean blood Hg concentrations are mg/kg.
<table>
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<tr>
<th></th>
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<th>RMSE</th>
<th>N</th>
<th># of Splits</th>
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<td>-17.414598</td>
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Table 4.2: Regression results for all units of analysis incorporated in the study. The 1 Km buffer is presented with 2 splits, and three splits. Little was gained from three splits and we notice an increase (from -10.643 to -8.106) in the AICc values. Mean blood Hg concentrations are mg/kg.
Figure 4.6: CART model utilizing 3 km range ($R^2 = 0.398$, RMSE = 0.177, AIC = -4.605). Again we find higher blood Hg associated with a lower percentage of wetlands. As the percentage of pasture increase, blood Hg concentrations are found to be higher. A similar, though not as strong of a pattern is found with deciduous forest cover. Mean blood Hg concentrations are mg/kg.
Figure 4.7: CART model utilizing 1 km area. ($R^2 = 0.46$, RMSE = 0.168, AIC = -10.643). Based on two splits, wetlands are associated with lower blood Hg concentrations. Mean blood Hg concentrations are mg/kg.
Figure 4.8: CART model utilizing modeled catchment ($R^2 = 0.34$, RMSE = 0.147, AIC = -9.279). As the amount of mixed forest decreases, the model shows blood Hg concentrations to be lower. Conversely, they are higher with an increase in deciduous forest. Mean blood Hg concentrations are mg/kg.
Figure 4.9: CART model utilizing a HUC 12 size catchment ($R^2 = 0.63$, RMSE = 0.140, AIC = -17.953). Mean blood Hg concentrations were found to be higher as wetland percentage decreased. Conversely, pasture presence has a positive influence on mean blood Hg concentrations (mg/kg). Mean blood Hg concentrations are mg/kg.
Figure 4.10: CART model of blood Hg at a HUC 10 level ($R^2 = 0.29$, RMSE = 0.1184, AIC = -1079). Higher mean blood Hg concentrations were associated with a higher percentage of deciduous forest. Mean blood Hg concentrations are mg/kg.
Figure 4.11: $R^2$ values versus the Unit of analysis. Note that the best model was isolated at the HUC 12 level.
Figure 4.12: Scatter plot of $R^2$ values versus area (hectares). There is a clear peak at the mean HUC 12 watershed level, with the $R^2$ values decreasing with both an increase in watershed size, as well as the smaller buffered areas around the nest location.
Chapter 5: Landscape Fragmentation

Investigating the Influence of Landscape Fragmentation on Measured Bald Eagle Blood Mercury Concentrations.

David E. Kramar¹, Bill Carstensen¹, Steve Prisley², Jim Fraser³, and Jim Campbell¹

¹Department of Geography, Virginia Polytechnic Institute and State University
²Department of Forest Resources and Environmental Conservation, Virginia Polytechnic Institute and State University
³Department of Fish and Wildlife Conservation, Virginia Polytechnic Institute and State University

Abstract

We conducted a landscape fragmentation analysis using Fragstats (McGarigal et al. 2012) and ArcGIS 10.1 (ESRI, 2012) to determine if landscape fragmentation influenced the methyl-mercury (CH₃Hg⁺) concentrations found in fledgling chicks in 28 sampled bald eagle nests within the state of Virginia, USA. Our analysis focused on measured blood mercury concentrations, as blood samples best represent recent dietary uptake. We used classification and regression tree (CART) modeling techniques to construct the models. Our analysis indicates that several landscape metrics not conventionally used in Hg analysis have significant relationships to blood Hg concentrations in bald eagles. When we modified a previous approach using only the percent land cover by incorporating a distance weighted analysis of the same variables we found that there were negligible differences between the models. However the incorporation of metrics such as patch density, Euclidean nearest neighbor distance between patches, percent of core area, and mean disjunct core area all influenced measured Hg concentrations. Overall we found that the best predictive model (R² = 0.76, RMSE = 0.113, AIC = -26.693) was strongly tied to metrics associated with wetlands, albeit in a negative manner (more wetland = less Hg). This is likely due, in part, to the salinity levels in most of Virginia’s wetlands. When we combined metrics from each of the models and excluded several of the wetland metrics we found that metrics associated with pasture and mixed land cover types exhibited strong relationships with measured Hg concentrations (R² = 0.65, RMSE = 0.136, AIC = -16.451).

Keywords: Bald Eagle, Geographic Information Systems, Fragstats, Landscape Fragmentation
**Introduction**

Many studies have addressed the impact that land cover type and landscape alteration have on the methylation and subsequent availability of methyl-mercury (CH$_3$Hg$^+$) to biota, particularly in aquatic environments (Bonzongo and Lyons 2004, Shanley et al. 2005, Fostier et al. 2000, Veiga et al. 1994). Of particular interest is the impact that activities such as logging have on the availability of CH$_3$Hg$^+$ in aquatic systems. Several studies have indicated that the removal of trees in forest logging operations can (at least initially) increase the transport and subsequent loads of CH$_3$Hg$^+$ and Hg to riverine and lacustrine systems (Fostier et al. 2000, Veiga et al. 1994), particularly due to the mobilization of dissolved organic carbon (DOC) and mercury (Hg) (Porvari et al., 2003, Munthe et al. 2007). It should be noted that the effect of logging or other forest removal operations, aside from the obvious change in land use/land cover, has the effect of fragmenting otherwise intact landscapes. In fact, Garcia and Carignan (2000) found that pike in logged watersheds within their study area exhibited levels of Hg that were higher than the World Health Organization safe consumption limit. Moreover, the conversion of deciduous to coniferous forest appears to increase Hg availability to aquatic systems, due to the Hg scavenging ability of conifers (Kolka et al. 1999, Witt et al. 2009). It should be noted that many of these results are from areas with abundant coniferous forest whereas much of the inland areas of Virginia are mixed/deciduous.

Another activity that increases forest fragmentation and has been shown to contribute to elevated loads of Hg in aquatic ecosystems has been the prescribed management of fires (Amirbaman et al., 2004). Although several studies have noted a decrease in Hg concentrations in biota following natural fire events, research suggests this is not always the case for prescribed events (Bank et al. 2005, Allen et al. 2005).
Hg concentrations in streams are positively associated with forest cover, wetland cover, and in particular, connectivity of associated cover types (St. Louis et al. 1994, Rypel et al. 2008, Scudder et al. 2009, Ward et al. 2010). Krabbenhoft et al. (1999) showed strong relationships between high methylation rates and the abundance of both forested and agricultural land. However, Kamman et al. (2004) has noted that in many cases, while methylation rates are high in agricultural lands, subsequent bioaccumulation of mercury by fish is low, possibility due to phosphorous loading and the type of DOC present in agricultural environments.

Moreover, Shanley et al. (2005) has noted that much of the variation found in surface water mercury concentrations is a factor of both large storm events as well as heterogeneity in watershed characteristics, furthering the argument that fragmentation and habitat characteristics should be investigated in more detail. In fact, Donald et al. (2001) noted that relatively “patchy” areas are more likely to become impacted by erosion processes because of vegetation and soil disturbance. In that respect, Donald et al. (2001) also noted that they are likely to become pollution sources to both coastal and inland waters. Salminen et al. (2001) noted that pollutants distributed in patchy environments have the potential to produce source-sink behavior in exposed biota. Moreover, the total percent of certain classes of land cover has already been shown to be a major driver in the production and availability of methymercury (Kramar et al. 2005, St. Louis et al. 2001, Shanley et al. 2008).

Within watersheds as a unit of analysis, land cover can have a direct effect on the movement of mercury into streams and rivers. Miller et al. (2005) have shown that forest cover can effectively capture atmospheric mercury and subsequently impact availability due to litterfall. The movement of DOC, particularly following a disturbance which increases the transport of DOC within forested watersheds, can potentially increase methylation (Goodale et
Whereas the amount of wetland found within freshwater forested catchments has long been shown to be a major contributor to the production of $\text{CH}_3\text{Hg}^+$ (Chaser et al. 2009, Grigal, D.F. 2002, St. Louis et. 1994), many of these studies were conducted in areas where there is an abundance of freshwater wetland systems, a phenomena not found in Virginia. Given the lack of a significant proportion (< 0.3 % in several study watersheds) of freshwater wetlands within the piedmont and mountains of Virginia, it is apparent that other factors are driving the variability of $\text{CH}_3\text{Hg}^+$ within the state. While the research was not conducted within Virginia, Tsui et al (2009) found highly variable concentrations of MeHg within watersheds that have no wetlands present. Furthermore, within individual watersheds, much of the available Hg is retained within terrestrial soils, effectively binding with DOC (Shanley et al. 2008) which is then transported via in-watershed processes (Grigal D.F. 2002). If left undisturbed and intact, forested and terrestrial soils likely act as a sink for MeHg until anthropogenic processes increase the potential for sediment transport. Within the Southeast, Rypel et al. (2008) noted that much of the $\text{CH}_3\text{Hg}^+$ found in fish was in large part due to the type of waterbody, with unregulated streams exhibiting higher concentrations than regulated streams. Lastly, of particular interest, is that watersheds exhibiting a lower density of anthropogenic development, and lower population densities, have been shown to have lower concentrations of MeHg in fish (Scudder et al. 2008, Chen et al. 2005). Given the limited information regarding MeHg in eagles, as well as other species as it relates to land cover, as well as limited information regarding fragmentation statistics, we examine the following hypotheses:

1. The distance of the contributing land cover types from the nest are related to measured blood Hg concentrations and can be analyzed based on weighting land cover closer to the nest more heavily than land cover further from the nest.
2. Adding landscape fragmentation measures to the commonly used measures of land cover percent to model blood Hg concentrations in juvenile eagles provides a better predictive capability than simple land cover percent.

The notion that land cover will influence juvenile eagle blood mercury concentrations is based on three facts: 1. Adult eagles generally forage close to the nest location during breeding season suggesting that juvenile blood Hg concentrations are a reflection of prey items from within a fairly small geographic area, 2. Juvenile eagles that were sampled in the study had not yet reached fledging age, and therefore had no means to secure prey other than that which the adults provided, and 3. Land cover has been shown to be one of the major components that influence the conversion of mercury to methylmercury. Certain cover types increase mercury retention, and if disturbed, also increase movement within watersheds. It should also be noted that in many cases, while prey collected at the nests did include various fish species, there were a large amount of remains that were indicative of a diet heavy on aquatic turtles (Kramar 2014 unpublished data), which do not exhibit as large of a home range as fish.

Methods

Study Area

Data for this research were collected throughout the state of Virginia (Figure 1), and consisted of blood and feather samples from juvenile eagles spanning roughly 4-7 weeks in age. We stratified the sample collection between coastal areas and inland areas (following a similar methodology to Jagoe et al. 2002). Inland areas were defined as those that fell within the Piedmont and Mountain physiographic provinces. A combination of aerial surveys, historic data,
and crowd-sourcing was used to identify nests that fell in one of the two defined locations (coastal vs. inland), as well as to locate nests along the major inland waterways. Once a nest was identified, a field visit was made to secure property owner permission and assess the condition of the tree and nest. If the tree was deemed climbable, and the property owners provided the necessary access, we ascended the tree to collect samples from the juvenile eagles. Samples were collected along many of the major rivers within the state including the Nottoway, James, Shenandoah, Appomattox, Rappahannock, Pamunkey/York, and Roanoke Rivers. We also collected samples along several smaller creeks and inland water bodies.

**Sample Analysis and Collection**

Blood samples were collected from the brachial vein in the wing using 21 gauge butterfly needles and 4cc heperanized vaccutainers. Feathers were collected from the breast and consisted of three feathers per individual. Upon collection, all samples were labeled with the unique nest identification code, species, and federal band number. Samples were then packed on ice and frozen within 4-8 hours of collection, and remained frozen until the Hg analysis was conducted. In addition to the blood samples, we also collected full morphometric data on each of the individuals.

Sample analysis was conducted at the Sawyer Environmental Chemistry Laboratory using a Milestone Direct Mercury Analysis (DMA-80) and standard reference materials (SRM). SRM's were used both to calibrate the DMA-80 and to provide quality assurance and quality control (QA/QC) during the actual analysis of biological samples. Blood Hg analysis followed Environmental Protection Agency Method 7473 (USEPA, 2000). Feather preparation followed Ackerman et al. (2007).
As found optimal in Kramar et.al, 2014, the unit of analysis for this study was the United States Geologic Survey (USGS) Hydrologic Unit Code Level 12 (HUC 12) watershed.

Data Acquisition and Processing

Data for this research were collected from multiple different sources. Blood samples were collected from live juvenile eagles and geo-referenced to the nest location in the field (N = 28). When a nest had more than one individual, we averaged the blood Hg concentrations to obtain a single value for each nest. Samples were collected from a total of 46 individuals. Land cover data (2006 NLCD) were acquired from the USDA Geo-spatial Data Gateway and projected to the Universal Transverse Mercator (UTM) projection for Zone 17N. The USGS HUC 12 watersheds were also acquired from the USDA Geo-Spatial Data Gateway and projected to UTM Zone 17N. A projected coordinate system was necessary to facilitate accurate measurements of area and land cover.

The land cover information was extracted for each watershed using the spatial analyst tools in ArcGIS 10.1. For the distance weighted analysis, one ArcGIS 10.1 grid file was created for each land cover class (N = 4). Zonal statistics were then computed using the nest code as the unique value for joining the resulting tables to the original Hg data. For the fragmentation analysis (discussed in detail below), land cover data were extracted for each of the 4 land cover classes for each of the 28 watersheds. These were then converted to GeoTiff format to facilitate implementation in the Fragstats software package (N = 112).
**Distance Analysis using Distance Weighting**

We masked the NLCD data in order to extract only those cells that fell within the HUC 12 watersheds. Using the raster calculator, and the watersheds as a mask, we multiplied the NLCD classes by 1 to extract those cells. As research has been conducted by (Buehler et al 1991) that indicates eagles generally forage within 1-3 km of their nest location (and up to 5-6 km) during breeding season, we wanted to determine if there was a noticeable influence on mercury concentrations when we weighted the land cover cells that were closer to the nest location more heavily. Using the ArcGIS Euclidian Distance tool, we calculated a raster layer to provide a distance for each cell from the nest location to the edge of each watershed. We then inverted the distances to obtain a raster that represented the inverse distance from the nest to the edge of the watershed. For example, if the maximum distance from the nest to the edge of the watershed was 5000 meters, and the distance at the nest was 0 meters, we inverted the distances such that the value at the nest location was 5000 and the value at the watershed edge furthest from the nest was 0. In order to obtain distance-weighted land cover classes we summed the inverse distances of all cells corresponding to the land cover types of interest (deciduous, mixed, pasture, and wetland), and then divided by the sum of the inverse distances of all cells within the basin following methods described by Zampella et al. (2007). This procedure effectively weights the individual land cover types that are closer to the nest location higher than those further away from the nest location. The land cover variables were expressed as percentages.

**Fragmentation Analysis**

Landscape fragmentation within each of the HUC 12 watersheds was evaluated using the Fragstats program (McGarigal et al. 2012). The extraction and conversion of the land cover rasters was completed using a previously developed custom tool in ArcGIS Model Builder, and
modified to suit the needs of this analysis. We iterated through the HUC 12 watersheds that had
nests within them, extracted only the land cover data that fell within the HUC 12, and then
converted them to a GeoTiff. In order to minimize edge effects that could negatively affect the
landscape evaluation, we opted not to count any background or boundary interfaces as edge,
effectively restricting the roving window from analyzing any data that the window extended
beyond. A number of landscape metrics were chosen for this analysis in an effort to tease out
those that may have the most significant influence. As the percent of cover type in each
watershed was analyzed in a previous study of these data (Kramar et al. 2014), we opted not to
include it initially in this particular analysis and focus only on landscape shape, contiguity, and a
number of other available metrics. However, we did include the percentage of “core area” for
each of the cover types at the individual level. The final analysis including ALL cover types
incorporated the total percent, as those were found significant in a previous study (Kramar et al.
2014).

Statistical Analysis

The statistical analysis for this research was conducted using the JMP Statistical software
package (JMP, 2014). We utilized the Partition platform to grow and prune the classification and
regression trees (CART) recursively until a suitable model was acquired. We evaluated the fit of
the model using a combination of the $R^2$, AIC, and RMSE. We opted to use CART analysis as it
offers several alternatives to traditional parametric statistical analysis. First, we generally
categorize CH$_3$Hg+ concentrations into ordinals representing low, moderate, high, etc. As the
output from a CART model effectively does that categorization, it was a reasonable approach.
Secondly, our data simply did not meet the requirements for traditional parametric analysis, and

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CART analysis allows for a robust analysis while not assuming any specific distribution in the data. Moreover, the lack of independence (covariance) among independent variables, as well as the spatial-autocorrelation of the dependent variable violated traditional assumptions of parametric tests. It should be noted that many landscape metrics co-vary. Lastly, given the nature of CART modeling, it seemed underutilized for analysis of Hg in systems even though several papers have indicated it is an excellent approach (Greenfield et al. 2001, Qian et al. 2001, Hartman et al. 2009, Vega et al. 2009). Additional exploratory analysis was also conducted to visualize trends and variation in each of the individual cover types prior to the fragmentation analysis.

Results

Distance Analysis of Land cover percentages within the HUC 12 drainages

Analysis of land cover, weighted by distance, again indicated that the two land cover classes that most influenced juvenile bald eagle blood Hg levels were woody wetlands and pasture land ($R^2 = 0.627$, RMSE = 0.139, $\text{AIC} = -17.997$) (Figure 2). However, the results from this analysis were not significantly different than the analysis using only the percent of land cover types conducted previously (Kramar et al. 2014). Whereas the predictive capability was comparable to that of the model utilizing only the raw percent of land cover, there does appear to be an association between blood Hg concentrations and the distance that wetlands and pasture lands fall from the actual nest location. It is not clear however, whether or not the additional analysis necessary to create the distance weighted classes is worthwhile. In order to address potential bias due to a known point source impacting the territories of four nests located on the South Fork of the Shenandoah, we re-ran the analysis and excluded those nests. We found that,
while the land cover variables remained the same as the first analysis, the predictive capability increased by 3%. These results further support the analysis of land cover and suggest that even when including territories that contain known point sources, robust models can be built to model Hg in eagles.

**Analysis of Landscape Patterns and Fragmentation**

We found strong relationships between the fragmentation statistics for each of the 4 land cover types (deciduous, mixed, pasture, and wetland), as they relate to Hg in juvenile bald eagle blood (summary in table 1).

The strongest relationship was found between wetlands and blood Hg concentrations, however, it is likely that the limited number of freshwater wetlands located in the Piedmont and Mountain regions of Virginia drove much of the predictive capabilities, and we acknowledge that such results may be spurious ($R^2 = 0.76$, RMSE = 0.113, AIC = -26.693.) (Figure 3). Because the vast majority of Virginia’s wetlands are in the coastal plain province, and in many cases tidal, they likely exhibit higher salinity. The result is actually counter to that of previous studies; increased wetland presence was negatively associated with blood mercury.

The landscape characteristics that influenced blood Hg concentrations as identified by the CART model for wetlands included (in order of importance) the median proximity index (PROX_MD), Euclidean nearest neighbor distance distribution (ENN_MD), and patch shape (SHAPE_MD). We found that as the proximity index (between patches) increased above 0.0755, Hg concentrations decreased.

The deciduous land cover analysis also indicated a strong relationship to bald eagle blood Hg concentrations. Of particular interest, was that both patch density (PD), and the percent of
core forest area (CPLAND) had the strongest overall influence when compared to the other variables. The highest concentrations of blood Hg in eagles were found when the percent of core forest area increased above 24.32 percent. We also found that watersheds exhibiting a patch density greater than 11.739, blood Hg concentrations were higher than at lower patch density. In addition, we found that the area weighted mean (AREA_MN), and total edge (TE) exhibited an influence, although at that level of the CART tree blood Hg concentrations were not high enough to be of concern. (Figure 4). In all, the predictive capability was reasonably good ($R^2 = 0.50$, RMSE = 0.162, AIC = -.543).

The mixed forest (Figure 5) and pasture (Figure 6) land cover types also exhibited an overall good relationship to the measured blood Hg concentrations. For the mixed forest types, we found that PD had the overall greatest influence, followed by disjunct core area density (DCAD) and the Largest Patch Index (LPI). The highest Hg concentrations were associated with a higher patch density (PD >=19.6224), as well as a larger amount of disjunct core areas (DCAD >= 6.5933). For pasture we found that the Euclidean distance between patches (ENN_AM), as well as the median area (AREA_MD) had the largest influence (Figure 6). The highest concentrations of blood Hg were strongly related to the Euclidean distance between patches when distance (meters) decreased (ENN_AM <= 60.3611), indicating that the more contiguous/closer together the patches were, the more likely we were to find elevated blood Hg concentrations. The last metric identified as significant was the circumscribing circle (CIRCLE_MN) where 0 = a square and 1 is the result when land cover patches take an elongated linear form. Results indicated that higher Hg concentrations tended to be associated with pasture patches that were more rectangular in form, although at that particular level of the CART tree concentrations of blood Hg were not at levels of concern.
When we combined the variables that were found significant in the land cover classes defined above, and included the total percent of each land cover (PLAND) for each of the four land cover classes, we found that besides the (already known) wetland percent, several of the metrics generated from Fragstats were also important (Figure 7). In an effort to address the potential bias from the limited wetland extent that exists in the Piedmont and Mountains, we created two different CART models. One of the models included ALL of the wetland variables identified as significant and the second included only the wetland percent (PLAND) and shape (SHAPE_MD). The model that included all variables produced the same results as the wetland only model, so we opted not to put that CART tree in this paper. When we eliminated all of the wetland metrics except shape (SHAPE_MD) and total percent (PLAND) we found that the disjunct core area (DCAD) for the mixed cover type and the median area (AREA_MD) for the pasture cover type were significant, and still offered a fairly robust model ($R^2 = 0.65$, RMSE = 0.136, AIC = -16.451), improving slightly on the previous models generated using only the percent of each cover type, indicating that fragmentation of the landscape, and associated metrics are significant to understanding Hg in both the environment, as well as the biota.

Discussion

Results from this research indicate a clear link between watershed land cover characteristics beyond the traditionally used metric of total land cover percent of the contributing land cover types. However, similarly to Zampella et al. (2007) we did not find that weighting the land cover classes by distance from the nest significantly impacted the predictive capabilities of the models. We did find that, in addition to simple measures commonly used, more complex
metrics regarding the shape, size, contiguity, linearity, patch density, and core areas (for example) exhibited statistically strong influences on measured bald eagle blood Hg concentrations. These results support the hypothesis that landscape fragmentation may increase Hg availability, likely due to increased mobility of Hg laden sediments as a direct result from the fragmented landscapes. Moreover, several studies have indicated that undisturbed watersheds can store up to 80% of the total Hg deposited, hence the likely reason we find that fragmentation of certain cover types in watersheds matters (Hurley et al. 1995, Allen and Hayes, 1998).

The results from this study further support prior research suggesting that landscape alteration, which results in fragmentation in many cases, influences Hg bioavailability (Kelly et al 2006, Garcia and Carigan 2005, Allen et al. 2005), particularly to high trophic level piscivorous species that are more susceptible to bio-magnification processes. Moreover, much research has indicated that Hg flux in aquatic systems is strongly influenced by overland flow within the contributing watershed (Hewlett 1982), as well as in locations where point sources are present. As research has indicated that intact forests don't commonly exhibit the large amounts of overland flow (Chorley 1978, Hewlett 1982), found in areas of high agricultural/pasture use, it is not surprising that metrics describing the density of patches, as well as other characteristics show strong predictive relationships.

Clearly further investigation is warranted given the relationships identified by the procedures used in this research. As there is clearly a link between landscape characteristics and mercury, additional efforts should be made to expand this research to additional locations, both within Virginia as well as outside of Virginia, as well as extend the research to additional species. While some previous research utilizes CART methods to explain Hg concentrations (Qian et al. 2001, Hartman et al. 2009), there is certainly evidence that these techniques could be applied
beyond the traditional analysis of modeling fish mercury concentrations via land cover characteristics, as well as modeling mercury concentrations in various birds, as evidenced by the results from this analysis. Further research should certainly include expanding these analysis techniques to, not only, other avian species but mammalian species as well. One of the most interesting aspects of this research has been the ability to tie landscape fragmentation at a watershed level to blood Hg concentrations in juvenile bald eagles, something that has not been done prior to this research.

*Research Implications*

As the collection of biologic samples from species such as the bald eagle can be both time-consuming, as well as dangerous, the results of this research help researchers to isolate areas within which to conduct studies while excluding those areas that are not likely to exhibit elevated concentrations. Moreover, as Virginia’s inland eagle population has exhibited both blood Hg and feather Hg concentrations significantly higher than their coastal counterparts, wildlife managers may use this research to direct analysis and mitigation efforts within the inland portions of the state. Furthermore the techniques utilized in this research may be applied to other avian species in an attempt to better understand the environmental characteristics within Virginia that drive the availability of mercury to animals and humans.
Literature Cited


Figure 5.1: Study area showing the locations of the HUC 12 watersheds that were sampled within Virginia. Blue represents Hg concentrations below 0.2 mg/kg while red indicates concentrations above 0.4 mg/kg. The black line represents the divide between the coastal region of the state and the inland region of the state.
Figure 5.2: CART regression model using distance weighted land cover. Smaller values indicate further distances from the nest location. Overall fit was evaluated using the $R^2$ value, RMSE values, and AIC values. ($R^2 = 0.63$, RMSE = 0.139, AIC = -17.997). Blood Hg concentrations are higher when the distance weighted wetlands are lower, conversely as the distance weighted pasture increases, we see higher blood Hg concentrations. Mixed forest cover exhibits a similar relationship to that of the pasture land cover class.
<table>
<thead>
<tr>
<th>Metric</th>
<th>Definition</th>
<th>Description</th>
<th>Land Cover</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROX_MDX</td>
<td>Median Proximity</td>
<td>Size and distance to all neighboring patches.</td>
<td>Wetland</td>
</tr>
<tr>
<td>ENN_MDX</td>
<td>Euclidean Nearest Neighbor</td>
<td>Distance to nearest neighboring patch.</td>
<td>Wetland</td>
</tr>
<tr>
<td>SHAPE_MDX</td>
<td>Shape Index</td>
<td>Equals 1 when patch is square, increases as it becomes more irregular.</td>
<td>Wetland</td>
</tr>
<tr>
<td>ENN_AM</td>
<td>Euclidean Nearest Neighbor</td>
<td>Distance to nearest neighboring patch.</td>
<td>Pasture</td>
</tr>
<tr>
<td>Area_MDX</td>
<td>Median Area</td>
<td>Median area of all patches</td>
<td>Pasture</td>
</tr>
<tr>
<td>CIRCLE_MN</td>
<td>Circumscribing Circle</td>
<td>Equals 0 for square patches and approaches 1 for elongated patches.</td>
<td>Pasture</td>
</tr>
<tr>
<td>PD</td>
<td>Patch Density</td>
<td>Density of patches in hectares.</td>
<td>Mixed</td>
</tr>
<tr>
<td>DCAD</td>
<td>Disjunct Core Area</td>
<td>Sum of the number of disjunct core areas within each patch.</td>
<td>Mixed</td>
</tr>
<tr>
<td>LPI</td>
<td>Largest Patch Index</td>
<td>Area of the largest patch in hectares.</td>
<td>Mixed</td>
</tr>
<tr>
<td>PD</td>
<td>Patch Density</td>
<td>Density of patches in hectares.</td>
<td>Deciduous</td>
</tr>
<tr>
<td>AREA_MN</td>
<td>Mean Area</td>
<td>The mean patch area in hectares.</td>
<td>Deciduous</td>
</tr>
<tr>
<td>TE</td>
<td>Total Edge</td>
<td>Sum of the length of all edge segments.</td>
<td>Deciduous</td>
</tr>
</tbody>
</table>

Table 5.1: Landscape metrics from the fragmentation analysis that were found to influence the blood Hg concentrations within the study area.
Figure 5.3: CART regression model utilizing only the fragmentation statistics for the wetland land cover class ($R^2 = 0.76$, RMSE = 0.113, AIC = -26.693). As the median proximity of wetland patches decreases, we find higher blood Hg concentrations. A similar relationship is found for the median Euclidian Nearest Neighbor distance. The median shape index exhibits slightly higher blood Hg when the index is larger.
Figure 5.4: CART regression model utilizing only the fragmentation statistics for the deciduous land cover class ($R^2 = 0.50$, RMSE = 0.162, AIC = -6.543). As the core percentage of deciduous forest cover increases, mean blood Hg concentrations are found to be higher. Similarly, as the density of patches (PD) increase we find a higher mean blood Hg concentration. The percent of core forest was the most significant variable, followed by the patch density.
Figure 5.5: Cart model using only the fragmentation statistics from the mixed forest cover type. \( R^2 = 0.51, \ RMSE = 0.161, \ AIC = -10.152 \). Patch density (PD) exhibited the strongest influence over mean blood Hg concentrations, followed by the amount of disjunct core area (DCAD). As PD increases we find higher mean blood Hg concentrations. Similarly, as the DCAD increases, mean blood Hg concentrations are found to be higher.
Figure 5.6: CART regression model utilizing only the fragmentation statistics for the pasture land cover class ($R^2 = 0.53$, RMSE = 0.157, AIC = -11.637). Mean blood Hg concentrations were found to be higher when the Euclidian Nearest Neighbor (ENN_AM) distances between patches decreased. Similarly, as the median area (AREA_MD) decreased we found a higher mean blood Hg concentration.
Figure 5.7: CART regression model utilizing the fragmentation statistics for the all land cover classes, excluding several of the wetland metrics ($R^2 = 0.65$, RMSE = 0.136, AIC = -16.451). As the percent of wetland decreases (PLAND) we found higher mean blood Hg concentrations. However, as the amount of mixed disjunct core area (DCAD) increased, we found higher mean blood Hg concentrations.
Chapter 6: Discussion

Prior to this research, little was known regarding concentrations of mercury in Virginia's bald eagle population, or even the extent of the inland population in general. Although population surveys have been conducted on the coastal plain for decades, less was known regarding Virginia's inland population. Surveys were conducted by the Virginia Department of Game and Inland Fisheries during the 2010 spring breeding season. The work concentrated on areas along the major waterways in order to identify nests located inland. Virginia Tech assisted in these efforts, and we found that a significant portion of Virginia's total eagle population resided in the inland portions of the state. This group represented a substantially higher percentage of the population than previously postulated (previous estimates were less than 10%). As of the publication of this research, new inland nests are located regularly bringing the proportion of inland nests to nearly 25% of the total population.

In 2012, Cristol et al (2012) published an article noting that feather samples collected within the coastal plain indicated no particular threat from mercury (Hg). Findings from this research support their findings in the coastal plain, although we did find that there were significantly higher concentrations of both blood and feather Hg associated with Virginia's inland population (defined as the area west of the fall line, and comprising the Piedmont and Mountains physiographic regions). This region is generally defined as the area of the state which falls west of Interstate 95.

As noted, concentrations of Hg in Virginia's inland bald eagle chick population were in some cases above currently accepted thresholds for neurological impairment from Hg (>0.5 mg/kg). While we were aware of elevated Hg being found throughout the South Fork of the Shenandoah River likely from a known point source in Waynesboro, VA, we also identified concentrations approaching concern on several other rivers, including the Nottoway River, North Fork of the Shenandoah River, Rappahannock River, and Wolf Creek. Of importance to note is that while Hg in some locations fell
within the 0.3 – 0.4 ppm range, those concentrations increase once feather development is complete
due to Hg binding with keratin, making feather growth a major vector for the sequestration of Hg in
individuals. Moreover, we found that even when we controlled for a known point source on the
Shenandoah, concentrations in inland eagles were still statistically significantly higher than those of the
coastal plain. That being the case, as noted in the literature (Welch 1994, Kannan et al. 1998, Gariboldi
et al. 2001), inland areas are generally more susceptible to mercury methylation and accumulation in
birds than coastal areas. This phenomenon is borne out in Virginia’s resident eagle population.

As conducted in many prior research studies, we wanted to find out if we could relate blood Hg
concentrations (which represent recent diet) and feather Hg concentrations (which are long term
accumulations). Given the non-linear nature of our sample, which fit a Johnson-Su distribution, we
developed a quadratic regression model that more accurately modeled the accumulation of Hg in
feathers given that feathers are one of the major methods of mercury sequestration from the body
(Blood_Hg = 0.0185943 + 0.02095 * Feather_Hg + 0.0017723 * (Feather_Hg – 5.02407)^2, R^2 = 0.83).

As the methylation of Hg occurs as a by-product of sulfate reducing bacteria in soils, we
decided to see if variations in land cover/land use could be used to predict the blood Hg concentrations
measured in Virginia's eagles. Current research has indicated that land cover properties have been used
to model Hg concentrations in fish (Simcox et al. 2011), as well as some birds (Simcox et al. 2011).
However, at the time this research was conducted, there was no research that estimated bald eagle Hg
as a function of either territory ranges or contributing watersheds, using land cover influence/percent as
explanatory variables. Like many similar studies with other species, we found that the percentage of
wetlands, deciduous, mixed, and pasture/agricultural land cover types (Kamman and Engstrom, 2002,
exhibited a strong influence over measured mercury concentrations in juvenile bald eagles. Whereas
coniferous forests have shown the greatest efficiency at scavenging mercury in the environment, they
were followed closely by deciduous and mixed forest types (Witt et al. 2009, Johnson et al. 2007, Risch et al. 2012, Frohne et al. 2012, Ward et al. 2010). Of interest, is that while much of the research that exists shows a strong correlation between the percent of wetlands and elevated mercury in various matrices, we found that our lowest mercury concentrations in eagles existed in coastal wetland areas. However, as several studies (mentioned above) have indicated higher concentrations found in freshwater prey, when compared to coastal or estuarine prey, this is not surprising as Virginia simply does not have the extent of freshwater wetlands as do many other areas. In Virginia, much of the inland areas are characterized by mixed, deciduous, and pasture/agricultural cover types, all of which have been shown to contribute to mercury methylation.

As previous research has shown relationships between blood level Hg and land cover characteristics, both in other states and in other species, we became concerned with the optimal modeling of that relationship from the perspective of spatial scale. The transmission of Hg from the environment to the birds has two important considerations: bird territories within which they forage and interact with environmental Hg, and the environment itself which transports the Hg to that territory. These processes operate under different spatial realms. One is distance based around the nests, and the other is watershed based around the nests. We constructed CART models at varying spatial scales under each of these processes. For the “eagle territory”, we looked at simple distance buffers of 1 km, 3 km, and 5 km around each nest location. Of those, the 5 km buffer provided the best predictive capability ($R^2=0.54$, RMSE = 0.156, AIC = -11.949). When we defined a more complex territory by extracting the streams from within 5 km and buffering them (assuming that eagles are more likely to forage along streams), we found that our predictive capability rose from an $R^2$ of 0.54 to an $R^2$ of 0.62. We also analyzed three different sized watersheds. These included the smallest of the available USGS watersheds (HUC 12), as well as HUC 10 watersheds, and a third modeled watershed that is approximately $\frac{1}{2} - \frac{1}{3}$ the size of the next smallest USGS watershed, the HUC 8. This allowed us to
increase the area within which we were analyzing, without significantly dropping our sample size, as would have been the case with the HUC 8 watersheds. From this we found the best predictive model overall was the HUC 12 watershed ($R^2 = 0.63$, RMSE = 0.140, $N = 28$, AIC = -17.953), which is supported by Shanley et al. (2008) who suggested a small watershed approach “is well suited and underutilized”. Lastly, given the identification for the most appropriate spatial scale within which to analyze Hg data in eagles, researchers may direct their efforts to analysis within these spatial scales.

To further understand the impact that land cover plays in juvenile bald eagle blood Hg, we took the best model from the previous analyses (the HUC 12 territory), and conducted an analysis of fragmentation as well as a distance weighted analysis for each of the four identified land cover classes (deciduous, mixed, pasture, and wetland). Results from the fragmentation analysis support prior research suggesting that landscape alteration, via natural or anthropogenic activities, influences the movement of contaminants such that they become more available for wildlife (Goodale et al. 2000, Donald et al. 2001, Kreutzweiser et al. 2008, St. Louis et al. 1994, Rypel et al. 2008, Scudder et al. 2009). In our study we found that for deciduous forest cover the most important landscape measurement was the percent of core forest (CPLAND), followed closely by the patch density (PD). Within the mixed forest cover class we found that PD was the most significant followed closely by the amount of disjunct core area (DCAD). Pasture/agricultural land was strongly associated with the mean Euclidian nearest neighbor distance (ENN_AM), and followed by the median area (AREA_MD). In each of the separate analyses, the predictive capabilities of the models ranged from an $R^2$ of 0.50 (deciduous) to an $R^2$ of 0.53 (Pasture/Agricultural). Given that the analysis was conducted using only one cover type at a time, the individual values are high. The wetland cover type exhibited the strongest overall predictive capability ($R^2 = 0.76$) although caution should be exercised while interpreting these results due to a significant lack of wetlands located within the inland portions of the state. In an attempt to address any potential bias from some of the wetland metrics, we produced a final model that
included all of the metrics identified from each land cover class, while using only the total percent of cover and the mean shape index for wetlands. The resulting model was slightly better than the previous models providing an estimate of 65% of the total variance in juvenile bald eagle blood Hg concentrations. Results from this model indicated that the percent of total wetland was still the most significant variable, and was followed by the amount of disjunct core area of the mixed land cover class.

Results from a distance analysis indicated that, while there appeared to be a link between the distance that certain land cover types were from the actual nest location within the HUC 12 ($R^2 = 0.63$, RMSE = 0.139, AIC = -17.997), it was comparable to the original model that only looked at the total percent of each cover type. We did find that the most significant variable was the distance weighted wetland land cover class, followed by the distance weighted pasture land cover class. As the results from both the traditional method of using the percent of certain land cover, and the use of the distance weighted land cover are similar, the argument cannot be made one way or another as to which is the best method. Results from this are similar to those of Zampella et al. (2007), who noted that a distance weighted approach did not add a significant improvement to the overall predictive capabilities of their models.

Suffice to say, methods utilized in this analysis should be explored in more detail, as it does appear that the inclusion of metrics other than just the percent of individual land cover types can be used to produce better model estimates. Whereas these methods are not directly found in the literature, there is substantial evidence to suggest that measurements such as patch density, distance between patches, as well as numerous others fragmentation metrics are important to understanding the movement and availability of Hg to both aquatic systems and biota. Future research should include the application of these techniques, specifically landscape fragmentation, to other species of birds, as well as fish, and other biota. Current models exist that estimate fish mercury as a function of land cover, as
well as Hg in different species of birds. However, it does not appear that watershed metrics that include measurements of fragmentation have been used in any extensive manner. Including metrics such as those utilized in this research may improve the predictive capabilities of currently developed Hg models and offer additional insight to understanding the biogeochemical processes that make Hg available.

Research Implications

This research has clearly shown that bald eagles residing in the Piedmont and Mountain physiographic regions of the state are at a much larger risk from MeHg toxicity than their coastal counterparts. As such, managers should expand the current study and focus more concerted efforts on understanding the current threats to the interior population. With nearly 25% of the total population residing in the Piedmont and Mountain regions, effects from MeHg or other contaminants could have a significant impact on the overall population. Moreover, even when known point sources were controlled for, blood Hg concentrations in the Piedmont and Mountain regions were still statistically significantly higher than those of the coastal plain. Certainly, additional research should be conducted to determine the true extent to which the interior population is susceptible to the effects of elevated blood Hg. Whereas it is known that several interior rivers are under advisories for Hg, several areas of the state that are not currently under a Hg advisory are showing levels that could potentially become a concern in the future. These areas should be monitored.
Literature Cited


