The Relationship between Methylation of Mercury and the Fluvial Geomorphic Variables of Streams across the Continental United States

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This thesis titled
The Relationship between Methylation of Mercury and the Fluvial Geomorphic Variables of Streams across the Continental United States

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ABSTRACT

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The Relationship between Methylation of Mercury and the Fluvial Geomorphic Variables of Streams across the Continental United States

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Methylmercury is a toxic pollutant found throughout the world in rivers and lakes. Different streams demonstrate different rates of methylmercury production and bioaccumulation from mercury pollution. This thesis compares the physical attributes of streams and watersheds to these rates. Results show that mercury methylation does relate to physical characteristics, but the nature of the relationship cannot be ascertained at this time.

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CHAPTER 1: INTRODUCTION

Many individuals consume fish as a primary component of their diet, whether it is a sustenance sport fisherman in the wilderness or a busy mother that turns to canned tuna in hard times. The media has spread the word that omega-3 fatty acids are a great health benefit; doctors recommend eating several servings of fish each week for high protein and healthy fatty acids. The expecting mother eats more fish in the form of inexpensive canned tuna, under the guidance of her doctor, and gives birth to a young healthy boy. After a year, the boy is not responding to his name and seems to have developmental difficulties. The mother, whose diet is unchanged, becomes consistently nauseous, weak, and appears to be suffering from depression. Her doctor urges her to stop eating tuna, and she feels fine in a month. However, for children the damages are permanent. Toxic levels of mercury are passed directly to their brain during pregnancy as a result of high fish consumption.

Mercury (Hg) goes largely unseen today, but can still be found in old thermometers and barometers. Atmospheric pressure is still reported in inches of mercury, even though the instruments today contain none. As little as half a century ago, children were allowed to play with mercury in schoolrooms. This liquid metal was once believed to have health benefits, such as eternal life. Well-known historical figures, Sir Isaac Newton, for example, experimented with mercury. The resulting mental health issues gave rise to interesting personalities (Waldron 1983). The expected source of
inspiration for the Madhatter in “Alice in Wonderland,” is the mercury poisoning of hat makers (Waldron 1983). Mercury was used in felting at the time that book was written, and mercury poisoning was common among hat manufacturers.

During the late 19th and early 20th century, mercury pollution was a result of gold mining. Mercury and gold share unique chemical properties that resulted in mercury being used to extract pure gold from mined material (Lecce and Pavlowsky 2008). Mercury is also found in coal, and when burned is first emitted to the atmosphere before returning to the surface in rainfall where it will eventually find its way into the ocean (USGS 2001). This is the main source of mercury pollution today and since mercury can travel around the globe before returning to earth, it is a global problem.

During recent decades, mercury contamination has become a national concern. It was common in the 1990’s to see fish consumption advisories. During that time local and state governments began routine sampling procedures to monitor pollutants in fish tissue. Mercury is not the only toxin of concern in fish tissue; PCBs, Chlordane, Dioxins, and DDT are others. Over time it was found that of these pollutants, mercury seemed to be less of a local waterway concern and more of a regional concern.

Nationally, mercury accounts for about 80% of all fish advisories. Every state in the U.S. has advisories for mercury, and at the end of 2010 there were over 4000 active advisories (US EPA 2011). In 2004, the U.S Food and Drug Administration (USFDA) along with the Environmental Protection Agency (USEPA) published a brochure outlining safety tips regarding fish consumption (USFDA 2004). The brochure states that
Sensitive populations should eat no more than 340g of store-purchased fish or 180g of locally caught fish per week. This equates to about two meals or one meal per week, respectively. These safety tips were published as a result of the growing public awareness of mercury pollution.

At about the same time that federal agencies started to warn the public about dangers of mercury contamination in fish, other federal agencies began national sampling of fish tissue. Following the National Mercury Pilot Study in 1998, the National Water Quality Assessment Program (NAWQA), run by the United States Geological Survey (USGS), sampled fish across the nation’s streams from 2002 to 2005 (Bauch 2009). In this time frame, the EPA started field-sampling fish from 500 carefully selected bodies of water. The fish were later tested for 268 pollutants (USEPA 2009).

At the start of the last decade, a cooperative of several government and academic groups formed the Northeastern Ecosystem Research Cooperative (NERC). One of the goals of this cooperative was to create a single database of all Hg sampling conducted in its region, which is comprised of New York, Maine, New England, eastern Ontario, and Quebec (Evers and Clair 2005). In the last two decades, researchers have acquired an overwhelming quantity of fish tissue data and have started to compare them to the physical landscape.
Purpose of this Thesis

Mercury must undergo a process called methylation before accumulating in fish. This process has been found to occur at varying rates across the United States independent of the amount of available mercury (Bauch 2009). These rates are referred to as methylation efficiencies. A methylation efficiency is a ratio of methylmercury to total mercury in a sample location and/or medium. It has been determined by researchers that methylation of mercury is highly influenced by local or regional variables. More recent efforts and publications have begun to consider physical characteristics, such as land use and land cover, in search of explanations for variability in methylation efficiency.

Methylation takes place primarily in sediments (Compeau and Bartha 1985). The physical characteristics of a stream are very dependent on its sediment transport regimes. Differences in methylation efficiency may relate to differences in streams. Stream and basin morphometric characteristics strongly associate with water discharge and sediment transport. For example, higher energy streams can transport both larger particles and larger quantities of sediment. Lower energy streams may create more stable environments for methylation to occur. Variables relating to stream and basin morphometry have received little attention by researchers who often focus efforts on a stream’s chemical and biological regimes.

The purpose of this thesis is to determine if there are relationships between mercury methylation efficiency and the geomorphic characteristics of the streams where fish are sampled. This is accomplished by comparing ratios calculated from data
collected nationally by the United States Geological Survey (USGS) with stream and basin properties derived using geographic information systems (GIS).
CHAPTER 2: LITERATURE REVIEW

The Mercury Cycle

Where Mercury Comes From

The source of mercury is similar to that of most metals; it is stored underground. Mercury can be found in rich deposits, often as mercuric sulfide. Although deposits of mercury tend to be rich, the element is very rare on earth. Emissions of mercury into the atmosphere and water come from geologic and anthropogenic sources. Geologic releases can come from volcanic activity both on the surface and from the ocean floor. However, these releases are orders of magnitude smaller than mercury pollution being measured today and are considered to be negligible (Fitzgerald and Engstrom 1998). Anthropogenic sources of mercury include the combustion of fossil fuels, non-ferrous metal production, cement production, pig iron and steel production, waste incineration, mercury and gold mining, and others (Pacyna et al. 2006). It was estimated by Pacyna et al. (2006) that about 65% of mercury emissions are a result of coal burning and 50% of all emissions take place in Asia, excluding Russia.

Coal has long been the suspected source of mercury pollution both in the United States and globally. A study of coal seams revealed that in the continental United States, coal has Hg concentrations between 0.1 and 0.2 parts per million (USGS 2001). The USEPA estimates that coal-fired power plants within the United States emit about 50 tons of elemental mercury each year (USEPA 2006).
Atmospheric Transport

Once in the atmosphere, Hg is found in two distinct forms, gaseous elemental (Hg\(^0\)) and reactive gaseous (Hg\(^{2+}\)) mercury. Hg\(^0\) accounts for approximately 90% of mercury in the atmosphere, and has an atmospheric lifetime on the scale of 1-2 years (Fitzgerald and Engstrom 1998). It can also be bound to a particulate, and in that form it is referred to as particulate-bound mercury (Hg\(^p\)). Hg\(^{2+}\) is capable of dissolving in water; hence it has a shorter atmospheric lifetime of a few weeks, often returning to the surface in rainfall (Lin 1999).

In the atmosphere, elemental mercury can become oxidized with the assistance of other molecules and sunlight to become gaseous reactive mercury. Oxidation pathways can involve ozone (O\(_3\)), hydroxide (OH), chlorine (Cl), nitrate (NO\(_3\)), and sulfur dioxide (SO\(_2\)). Oxidation of mercury can also occur purely by exposure to UV radiation (Lin 1999).

Hg returns to the surface by wet and dry deposition. Dry deposition has only recently undergone research and it appears it may be significant for vegetated regions (Miller et al. 2005). Once mercury becomes oxidized it can quickly dissolve in water and return to the surface via rainfall, making a quick entrance into the ecosystem. Once on the surface, Hg can undergo a chemical process called methylation and begin to bioaccumulate into the food chain.
Methylation

In its elemental form (Hg), mercury does not pose a significant threat to human life at the levels found in the environment. Mercury can go through a process called methylation and become methylmercury (MeHg), which is highly toxic to life. In one case, a drop on a glove resulted in the death of a chemistry professor at Dartmouth University (Nierenberg et al. 1998). Methylmercury, an organic molecule, can bioaccumulate in organisms. Methylation is a process that takes place in water, soil, and sediments.

To obtain oxygen, certain bacteria reduce molecules into their base elements that contain oxygen. One of the byproducts of this mechanism is the methylation of mercury (CH$_3$Hg$^+$ or MeHg). Many variables are known to affect the rate of methylation. The current consensus is that most of the mercury becomes methylated by sulfate-reducing bacteria, although the exact pathway is unknown (Compeau and Bartha 1985, Dyrsen 1991, King et al. 2001). As expected, studies have demonstrated that the amount of sulfate in a water body is related to higher levels of methylmercury (Gilmour and Henry 1992, Waters et al. 1995). Nitrate-reducing bacteria also methylate mercury (Kerin 2006), but higher amounts of nitrate are found to inhibit the production of methylmercury, thus questioning the results of increased nitrate loads (Steffan 1998). Iron-reducing bacteria appear to methylate mercury as efficiently as sulfate-reducing bacteria (Fleming et al. 2006).
Hydrogen ions (pH) are catalysts for the process of mercury methylation. More acidic water, or, lower pH, in lakes relates to higher concentrations of methylmercury sampled in fish tissue, until the water becomes so acidic that necessary life forms fail to survive (Andersson and Borg 1995, Stehpans 1995, Scheuhammer and Graham 1999).

Dissolved organic carbon also plays an important role in the rate at which mercury is methylated, perhaps by increasing the levels of bacteria in a system. Methylmercury concentrations rise sharply immediately after reservoir impoundment. This is due to the sudden increase in organic material from now submerged soils and vegetation (Therriault and Schneider 1998).

**Bioaccumulation**

Methylated mercury bioaccumulates, meaning that the uptake rate into tissue far exceeds the rate at which it leaves. Species found higher in the foodchain have significant quantities of methylmercury in their tissue as a result of their diet. The concentrations continue to increase farther up the food chain, for example in the common loon. In loons, methylmercury leads to physical, behavioral, and breeding changes in the birds throughout New England. Methylmercury may be a primary stressor decreasing loon populations (Evers and Clair 2005).

Fish have received much focus in identifying mercury pollution because fish are the primary means of mercury exposure to communities and the general public. Fish greater in length, an indicator of age, are expected to have higher amounts of mercury in
their tissue compared to smaller or non-piscivorous fish in the same location (Burger 2011, Mcclain et al. 2006). In addition, the higher trophic-level fish species, such as piscivores, will have accumulated more mercury due to their diet (Lepak et al. 2009, Driscoll et al. 2007). The different levels of mercury associated with species and length have resulted in research that considers just one subset of species and size (Hammerschmidt and Fitzgerald 2006) or that normalizes tissue results by fish length (Scudder et al. 2009).

Health Concerns

Information in the following sections on mercury and health concerns is from the US Environmental Protection Agency Mercury Report to Congress in 2006 (USEPA 2006). Mercury in both the elemental and organic forms can result in major health concerns to children and adults. Elemental mercury is not easily absorbed through the intestines but is easily absorbed through the lungs when mercury vapors are inhaled. Methylmercury, however, is quickly absorbed through the digestive system. As a result of mercury’s long biological half-life, the rate of removal from the body is far exceeded by uptake in individuals with a consistent fish diet. Over time enough will accumulate to start resulting in advanced neurological damage. The time it takes for this to happen depends on how much fish is eaten, how frequently it is consumed, and the levels of methylmercury present in the fish. For those that eat fish infrequently, this time will mostly likely exceed their life-span.
Health Effects

Mercury poisoning results in neurological damage with a wide range of nervous system related symptoms. Workers exposed to industrial mercury commonly report tremors, trouble sleeping, memory issues, and in severe cases, loss of balance (Frumkin et al. 2001). Children exposed to methylmercury display impairment of vision, speech, hearing, skin sensation, walking, and neurological problems. Prenatal exposure slows development and exposed infants are often unable to walk or communicate as early as the average child and they have problems with reflexes and motor control. Mercury may play a role in the development of cerebral palsy, although statistical significance has yet to be established (Weis 2004). Sport fishers who consume fish as a major source of sustenance can develop symptoms that become more prevalent with increased fish consumption (Knobloch et al. 2006).

At Risk Populations

At risk populations are those that rely on or eat fish as a main source of sustenance. These are usually communities in coastal areas but can include active sport fishers. It has even been found that canned tuna, when eaten often, can put individuals at risk (Holloman and Newman 2010). Mercury is particularly harmful to the developing brain; it can pass through the blood brain barrier and prevent or impair key cellular events related to brain development (Holloman and Newman 2010). Children under the age of
six and in utero are at risk populations. The EPA recommends that child-bearing women
or women of child-bearing age limit fish and seafood consumption. Children started to
show symptoms ahead of the population in mercury pollution epidemics (USEPA 2006).

Relationships to the Physical World

Several studies have compared mercury in fish tissue and methylmercury
efficiency in the nation’s streams to physical properties of the watersheds from which
samples were drawn. Studies have examined drainage areas, various land use and land
cover scenarios, discharge, and soil permeability, among others. Mercury and
methylmercury concentrations have been partially associated with chemical and climatic
variables. The chemical variables are largely dependent on local watershed
characteristics.

Land Use and Land Cover

Recent research on mercury in fish tissue identified several land use and land
cover properties associated with higher rates of methylation, indicating that local factors
can play a significant role in the process. The process of methylation associates with
several chemical properties of sediment and water, such as dissolved organic carbon, loss
on ignition, and acidity.

The availability of dissolved organic carbon in streams depends on sources such
as forests, swamps, and wetlands where a supply of decaying organic material exists.
There are more bacteria to methylate mercury where there is more life. Acidity of the water is impacted by acidic soils. As a result, methylation tends to be more efficient where vegetation cover is creating acidic soils, such as in evergreen forests (Scudder et al. 2009).

Mercury in fish tissue is greater in watersheds with naturally occurring wetlands compared to those without wetlands (Castro et al. 2006). However, Castro et al. (2006) were unable to explain why levels still varied between watersheds with wetlands, which were selected based on their proximity in an effort to control climatic variables. Watersheds containing lakes do not show increased levels of mercury (Shanley et al. 2005). Reservoir impoundment, however, increases fish mercury concentrations in a watershed (Therriault and Schneider 1998) due to a fresh supply of organic material provided by the reservoir (Shanley et al. 2005).

In 1998 the USGS ran a pilot study to compare variables concerning sediment, water, and fish tissue (Krabbenhoft et al. 1999). This study was then continued until 2005 and data published in the USGS DS307 report (Bauch 2009). Eventually, a statistical analysis of the entire project was published in a scientific report (Scudder et al. 2009). The findings reported that several variables relate to bioaccumulation at sites unaffected by mercury mining.

Significant correlation using Spearman’s $\rho$ was observed between mercury concentrations and both percent wetland by drainage area and percent forested land by drainage area. Evergreen forests had an even higher correlation with mercury than forests
in general. Wetlands and forests are the primary sources of dissolved organic carbon. Evergreen trees are well known to produce acidic soils that leach into the drainage system lowering pH. As previously discussed, slightly lowering pH would increase the production of methylmercury.

*Climate*

The primary source of mercury in a watershed is via wet deposition, or rainfall (Downs and Macleod 1998). Total mercury generally correlates well with annual precipitation and higher concentrations are found in watersheds with greater seasonal variability in flow (Hurley et al. 1995). This seems to indicate that watersheds with greater changes in rainfall or temperature would have larger production rates.

Efforts to link fish tissue concentrations of mercury to modeled and measured mercury wet deposition have been unsuccessful (Shanley et al. 2005, Schudder et al. 2009). Only one paper reported success in linking fish tissue to wet deposition (Hammerschmidt and Fitzgerald 2006). The methods used in the study by Hammerschmidt and Fitzgerald (2006) have limitations because their findings are a result of statewide means of measured deposition and fish tissue sampling. The comparison is limited by the spatial distributions of fish sampling and wet deposition sampling. Maps of sampling data show that fish samples are equally distributed across a state but wet deposition samples are very sparse and there are often only one or two wet deposition
sites a given state. Wet deposition is highly controlled by climate, which can vary significantly in many states.

Conclusion

It is yet uncertain whether low-energy streams or high-energy streams have greater methylation efficiencies. Higher levels of methylmercury are found in and around wetlands, which implies that a lower energy stagnant system would produce methylmercury at higher rates. However, constant high-energy events that increase water flux, such as floods, would supply more raw materials for the methylation process. Streams with cohesive sediments are expected to have higher production rates because the sulfate-reducing bacteria require an anoxic environment before they will reduce sulfate. Cohesive sediments inhibit mixing thus preventing a fresh supply of oxygen and over time lead to anoxic environments.
CHAPTER 3: SITE SELECTION

USGS Mercury Sample Sites

Fish tissue, sediment, and water sampling were performed on a national scale by
the USGS from 1998 to 2005 (Bauch 2009). These data provide the mercury and
methylation variables for this thesis. The USGS sampled 349 sites across the continental
United States (Figure 1). Of these, 90 were determined by the USGS to have been
impacted by current or historical gold or mercury mining. Mercury forms an amalgam
with gold, and was used extensively to extract gold ore in gold mines in the 19th century,
a practice that persisted until the 1960s. Watersheds containing historical gold or mercury
mines have significant differences in the mercury content of fish tissue, water, and
sediments compared to watersheds with no gold or mercury mines (Scudder et al. 2009).
Of the 259 sites not impacted by this mining activity, 208 were sampled by the USGS for
mercury in sediment, water, and fish tissue.

This thesis examines both the calculated efficiency of mercury accumulation in
fish, as well as the water and sediment methylation efficiencies. The USGS sites are
situated in a wide variety of basins, streams, land use/cover, and climate. Stream and
basin properties tend to be similar in comparable regions and climates. Sites included in
this thesis are a selection of those where fish, water, and sediment samples were recorded
and not impacted by historical gold or mercury mining.
Figure 1: Map of the locations of all USGS sample sites. USGS sample sites (points) and NAWQA study areas (gray), data from (Bauch 2009)

Site Selection Process

For each site selected, a number of variables needed to be calculated using a geographic information system (GIS). To keep the size of the project manageable, the number of potential sites was reduced using a random stratified sampling. The USGS sites in the target group are located in forty different study areas representing major drainage basins (Figure 2). Some of these basins contain many more sampling sites than others; number of sites per study area ranges from two to fifty six. In order to include all of the USGS study areas in this thesis, two sites were selected at random from each USGS study area.
Figure 2: Map of National Water Quality Assessment study areas. NAWQA study areas are reflective of major river basins. Data from http://water.usgs.gov/nawqa/

During the GIS analysis portion of this thesis, predictor variables could not be calculated for some of the initially selected sites as a result of poor quality flowlines from the National Hydrography Dataset. As a result, a number of sites were eliminated after the initial stratified random sampling. Leaving a total of 66 sites included in this thesis (Figure 3).
Figure 3: Map of sample sites used in this thesis. NAWQA sample sites used in this thesis. Data are from (Bauch 2009).
CHAPTER 4: METHODS

Variables

Criterion Variables

The three criterion variables used in this research represent how much methylmercury is being produced in a stream compared to how much mercury is available. The amount of mercury in a stream differs by several orders of magnitude depending on location and climate. Because the criterion variables are ratios of the efficiency of methylmercury production, any differences in mercury as a result of location and climate are factored out. Mercury levels could potentially be explained by physical characteristics; for example greater drainage area captures more rainfall and therefore would have more total mercury, but this thesis is purely interested in how efficient the process of methyl mercury (MeHg) production is. The data from which these variables were calculated comes from the USGS DS307 report (Bauch 2009). The variable definitions were obtained from the scientific investigation report SIR 5901 by Scudder et al. (2009) and a paper by Hurley et al. (1995). The data are freely available at mercury.usgs.gov.

Bioaccumulation Factor (BAF)

The bioaccumulation factor (BAF) is a measure that represents the amplification of mercury accumulated in fish compared to the available methylmercury sampled in water. Sites with higher BAFs would indicate that fish in this location are better able to
absorb mercury into their tissue than fish at other locations. The measure is used by the USGS in their scientific investigation report of mercury in rivers (Scudder et al. 2009).

The bioaccumulation factor is defined as:

\[ BAF = \log_{10} \left( \frac{F_{HgT}}{W_{MeHg}} \right) \]

where:

- \( F_{HgT} \) is the wet weight Hg concentration in fish tissue
- \( W_{MeHg} \) is the MeHg concentration of water

This factor is simply the log of the ratio of total mercury (Hg\(_T\)) in fish to MeHg in water. The log is used to make the dataset more linear; since mercury is concentrated in fish over time it is much greater in concentration than mercury in water. Fish tissue was not measured for both MeHg and total mercury (Hg\(_T\)) because research has shown over 98% of mercury in fish tissue is MeHg.

Water Methylation Efficiency \((E_w)\)

Recall that not all Hg\(_T\) in water is destined to become methylmercury. The process depends on many other factors of the chemical, biological, and physical systems of a stream. Water methylation efficiency \((E_w)\) indicates which locations have higher rates of MeHg production. This variable does not indicate which sites will have greater MeHg present, as that will depend on the amount of Hg\(_T\) that enters the system.
The water mercury methylation efficiency \((E_w)\) is defined as:

\[
E_w = \frac{W_{\text{MeHg}}}{W_{\text{Hg}}}
\]

where:

- \(W_{\text{MeHg}}\) is the MeHg concentration of water
- \(W_{\text{Hg}}\) is the Hg concentration of water

**Sediment Methylation Efficiency \((E_s)\)**

The efficiency of MeHg production in sediment follows the same logic as \(E_w\). Sediment is where the bacteria methylate mercury, making this an important variable to consider. It is expected that out of the three criterion variables, this one would strongly associate with a stream’s physical properties because sediment is where the methylating bacteria reside.

The sediment mercury methylation efficiency \((E_s)\) is defined as:

\[
E_s = \frac{S_{\text{MeHg}}}{S_{\text{Hg}}}
\]

where:

- \(S_{\text{MeHg}}\) is the dry MeHg concentration of sediment
- \(S_{\text{Hg}}\) is the dry Hg concentration of sediment
Predictor Variables

Drainage Density ($D_d$)

Drainage density indicates how much linear channel exists per unit area in a drainage basin. Climate, geology, and vegetation influence drainage density. Higher densities occur where the surface is less permeable and the increased runoff creates more numerous and frequent channels to drain the basin (Langbein and Schumm 1958). Vegetation slows erosion resulting in thick permeable soils, which slows and decreases runoff thus decreasing drainage density. Precipitation events influence drainage density. Frequent high intensity precipitation events will carve more channels into the terrain, whereas infrequent low intensity precipitation events will not.

Drainage density is chosen because of its relation to surface runoff. Higher drainage densities are indicative of basins with higher and faster runoff. Runoff quickly transports organic material into the streams resulting in more dissolved organic carbon and increased methylmercury production.

Drainage density is defined as:

$$D_d = \frac{L}{D_A}$$

where:

- $L$ is the total length of all streams calculated using ArcGIS as the sum of the length of all polylines.
• $D_A$ is the drainage area for the site, provided in the auxiliary site data in the USGS DS307 report.

**Drainage Basin Relief ($D_r$)**

Drainage basin relief is the range of elevation found within a basin. The lowest elevation is the elevation of the sample site and highest elevation is defined by the topography within a basin. The difference between the highest and lowest elevation is relief. Drainage basin relief influences the available energy in a drainage system. The greater the relief, the more potential energy water will hold upstream and the more kinetic energy will be created downstream.

The greater the energy of a stream, the more work can be performed transporting sediments. This could be beneficial to methylmercury production through greater water flux or detrimental due to a lack of stability. It is possible that a relationship could be parabolic with production higher for moderate reliefs and lower at the extremes.

Drainage basin relief is defined as:

$$D_r = Z_{max} - Z_{site}$$

where:

• $Z_{max}$ is the maximum elevation in the drainage basin provided in the USGS DS307 report.
• $Z_{site}$ is the site elevation provided in the USGS DS307 report.
Stream Frequency ($S_f$)

Stream frequency is a measure used to describe how many streams per unit area are in a basin. The higher this ratio, the faster a basin drains water. High stream frequencies result when the soil or ground is impermeable, and thus there is little infiltration and groundwater storage to slow down the time it takes water to leave the basin. This often happens in rugged terrain and where there is more exposed bedrock. Stream frequency is closely related to drainage density and is included for the same reasons.

Stream frequency is defined as:

$$S_f = \frac{S_n}{D_A}$$

where:
- $S_n$ is the number of streams calculated using Rivex as the number of stream confluences in the network plus one for the mouth.
- $D_A$ is the drainage area for the site, provided in the auxiliary site data in the USGS DS307 report.
Stream Order ($S_o$)

Stream order is used to describe the hierarchical position of a stream in a stream network. This thesis uses the method of stream ordering by Strahler (1957). Perennial source streams are given a stream order of one. When two streams of the same order flow together the resulting stream is of the next higher order (Figure 4). For this thesis, stream order was calculated using RivEx, which uses a recursive algorithm developed by Gleyzer et al. (2004).

**Figure 4:** Diagram of stream ordering. Numbers indicate Strahler stream order (Strahler 1957).

Stream order is related to size; the higher a stream order the greater the size of the stream. There are other numerical ways to judge size, such as cross-sectional area, width
and depth, or stream power. The size of a stream may be a good indicator of methylmercury production. Source streams, those with low stream order, tend to transport and store less sediment and water than higher order streams. In a stream network, there is an exponential increase of flow with increasing stream order. With every increase in stream order there is an exponential increase in the number and length of streams flowing into it. While it is unknown how sediment or water methylation efficiencies will be associated with stream order, larger streams do support a diverse ecosystem with many pathways for methylation to reach fish tissue. As a result, it is expected that the bioaccumulation factor will be higher in streams of greater order.

**Stream Sinuosity ($S_s$)**

Stream sinuosity indicates the amount a stream meanders as it flows. Most streams are meandering streams but some make more turns than others as a result of the sediment load they transport (Gordon 2004). Streams with higher sinuosity also exhibit more bank erosion and deposition associated with the constantly changing channel. Sinuosity is a good indicator of increased suspended load and frequent deposition and entrainment of sediments. The constant sediment disturbance would prevent anoxic conditions from forming by introducing a continuous supply of oxygen, therefore it is expected that sediment efficiencies will decrease with increased sinuosity.
Stream sinuosity is defined as:

\[ S_s = \frac{L_s}{L_v} \]

where:

- \( L_s \) is the distance the stream flows in network space from the sample site to the nearest upstream point of confluence of same or lower stream order. This was calculated using ArcGIS by measuring the length of the polylines fitting this definition.

- \( L_v \) is the Euclidean distance from the sample site to the nearest upstream point of confluence of same or lower stream order. This was calculated using ArcGIS’s ruler tool.

Stream Width (\( S_w \))

Stream width is another variable that describes the size of a stream and often correlates well with stream order. Stream width was measured using Google Earth. For each site, six measurements of channel width were taken at equal intervals approximately 100 meters apart both upstream and downstream. This was done to reduce error caused by a stream’s changing width and to obtain a good average. Streams that meander often have wider channels in a meander than they do in straight sections as a result of erosion in high water events. Channel width is used instead of width of flow because at the time the aerial imagery was captured it is unknown if a stream was at base flow. It is unknown how stream width will relate to sediment and water efficiencies. Bioaccumulation is
expected to be higher in wider streams, for the same reasons as stream order, that there should be more biological pathways for methylmercury to accumulate.

Stream Load ($S_l$)

Different levels of energy, sediment supply, and sediment grain size result in different channel types. Characteristics of these channels are visible remotely in today’s world of high resolution aerial imagery in both space and time. Three groups of sediment grain size inferred in this method are gravel, sand, and silt. Figure 5 was used as a guide to infer sediment size from channel type.

Two other sources of information from the USGS were used to determine sediment size. In the USGS DS307 sediment data, percent of bedload less than 0.63 mm was recorded as part of several sampling projects. This was often used to help narrow down the difference between sand and silt. However, this measurement was not collected at all selected sites. In addition, some sites have attributes listed at waterdata.usgs.gov, which in some cases includes sediment size. Streams primarily transporting silt would be more prone to exhibit anoxic environments in the sediments because silt is cohesive and fine grained. Bacteria in the anoxic environment would start to break down sulfates, producing methylmercury.
Figure 5: Types of stream channels (Knighton 1998)
Sources

*United States Geological Survey DS307 Report (USGS DS307)*

The USGS report that spawned this research provided the information needed to calculate the three criterion variables as well as several of the predictor variables. The USGS dataset contains five tables, of which three were used. These are the mercury data on fish tissue, water, and sediment samples, and auxiliary site information. A relational database management system was used to aggregate and calculate the needed variables from these tables for the chosen sites. The auxiliary site information provided by the USGS gave the latitude and longitude location of each site, as well as information on drainage area and elevation.

It is important to note that fish were composite samples. A composite fish tissue sample is a measurement taken from a blended mixture of fish tissue from several individuals. The USGS often reported several samples taken at one site. Data from fish tissue samples were averaged for each site prior to calculation of the criterion variables for this thesis. The USGS study was designed to limit the effect of species between locations. It is assumed in this research that differences between samples due to species are negligible.

*National Hydrography Dataset (NHD)*

The NHD is a product from the USGS and has been incorporated into the United States National Map. The dataset uses vector-based geometry to map cataloged watershed
boundaries, stream flowlines, and other areal and linear features depicting the flow of the nation’s streams. Designated stewards throughout the country are continuously updating the data. The intention of the datasets is to be a digital representation of the stream network that allows for tracing and modeling of flow.

For every site, NHD data were used to calculate the variables that make up several of the morphometric parameters used in this research. This was done using geographic information systems (GIS).

Freely Available Aerial Imagery

While the NHD provided the context in which to measure linear network-based parameters, two variables required data in two dimensions. For these, freely available aerial imagery provided by Google Inc. and their partners was used. This allowed an estimation of both channel type and width.

Software Used

Rivex

Rivex is a tool that runs as a plugin to ESRI’s ArcGIS 9 for the purpose of deriving attributes of river network. For input, Rivex requires a shapefile or feature class of polylines representing a river network. The start and end point of each line must be a source, mouth, or intersection of a stream. Rivex is used in this thesis to calculate stream order, number of streams, and total upstream length (Hornby 2009).
Polylines representing the stream network for each selected site were obtained from the National Hydrography Dataset (NHD). Stream networks were downloaded for each NHD sub-basin that contains a study site, and then they were put through the following process:

1. Data were projected to the USA Contiguous Lambert Conformal Conic Projection (European Petroleum Survey Group (EPSG) Registry Code: 102004).
2. All arcs were removed downstream of the selected USGS sample site to ensure that the network consists of only upstream features.
3. Arcs that did not connect were connected manually to ensure network topology. This was done iteratively until Rivex reported seeing only one mouth in the network.
4. Rivex was then used to calculate the needed values.
5. Results were recorded in a spreadsheet, tied to the site “Station_ID” to be later joined in a relational database.

In some cases, NHD data were in too poor of a condition for analysis and these sites were then dropped from the study. Stream network data were deemed too poor when it would have taken extensive digitizing from other sources to create a workable network.
Google Earth

Google Earth was used to populate variables for stream load and stream width. This software was chosen because of its ability to plot several years of freely available aerial photography for many locations (Google). Access to several years was necessary because streams were occasionally photographed during non-base flow events. The higher water obscured depositional features, and floods obscured channel width.

Open Source Software

The USGS DS307 data and values calculated using Rivex and Google Earth were imported and stored in a PostGIS enabled PostgreSQL relational database. Maps were created using an open source GIS software named uDig, which has the ability to connect to and map spatial data stored in PostGIS. SQL queries were used to join the different tables prior to statistical analysis. Statistical analyses were performed and products created using R, a popular command-based statistical computing software.

Statistical Methods

Data analysis relied on the use of several statistical methods. The workflow was as follows:

1. Calculation of summary and descriptive statistics.
2. Visualization of the distributions for each variable.
3. Correlation and scatter plots for variables to test for associations.
4. Multiple linear regressions to explore the potential effects of the predictor variables on the criterion variables.

**Test for Normality**

In order to ensure the correct test for correlation is used, all ratio or interval variables were tested to see if they are normally distributed using the Shapiro-Wilkes Test (Shapiro and Wilk 1964). This tests the null hypothesis that data come from a normal distribution. If the tests p-value is less than 0.1 then the null hypothesis is rejected, and it is assumed the data are not normally distributed. To visually supplement this test, histograms were produced.

**Correlation**

Correlation is used to describe if an association may exist, which is a similar but more limited statistical test than linear regression. The choice of test for each comparison depended on whether or not both variables have normal distributions. If they are normally distributed, then Pearson’s R was used, otherwise Spearman’s $\rho$ was used. Scatterplots were used as a visual aid, as well as to determine the effects of outliers on any test results. Correlation testing was done prior to linear regression to understand the limit and presence of any colinearity in the predictor variables.
**Linear Regression**

Linear regression is a technique used to explore and answer several questions about relationships between a criterion variable and one or more predictor variables. These are:

1. Does a relationship exist?
2. What is the nature of this relationship?
3. How well does the model perform?
4. What is the relative importance of the predictor variables?

(Kachigan 1986)

The three criterion variables could possibly be explained as a function of the predictor variables in the form:

\[ y = a + \sum_{i=1}^{n} \beta_n x_n \]

The process used is typically referred to as multivariate linear regression. Linear regression can only include ordinal predictor variables, only the variables that are ordinal in this study were used. These are drainage density, drainage basin relief, stream frequency, stream order, and stream sinuosity. The model will then be in the form:

\[ y = a + B_1 D_d + B_2 D_r + B_3 S_f + B_4 S_s + B_5 S_w + B_6 S_o \]

Two variables that are exponential in nature a priori had to be transformed prior to using them in a linear regression model. These are drainage basin relief and stream width. If all locations along streams on the planet are sampled for stream width and drainage
basin relief the result would be a high frequency of smaller values and a low frequency of larger values because there happen to be exponentially many more relatively smaller streams draining less relief and area than large streams. Stream width and drainage basin relief were log-transformed prior to model inclusion so that they are closer to being normally distributed. Revising the previous equation yields:

\[
y = a + B_1D_d + B_2 \log(D_r) + B_3S_f + B_4(S - 1) + B_5\log(S_w) + B_6(S_o - 1)
\]

### Choosing a Model

For each criterion variable there are \(2^n\) possible models; where \(n\) is the number of predictor variables. Since six variables were selected to include in the selection process there are 64 possible models that need to be considered. All possible models were considered and compared to each other. The one with the highest adjusted R\(^2\) was selected as the best model, as the one with the most explanatory power. Adjusted R\(^2\) was used over other goodness of fit statistics because the highest adjusted R\(^2\) value would indicate the best model including only terms that perform better than random chance and not just the model that has the best fit. The model with the best fit does not necessarily contain only predictor variables that are performing better than random. Since linear regression in this situation is being used in an exploratory approach and not built from a pre-existing theory, it is desirable to restrict the model to the better than chance predictors.
Assumptions

Four assumptions are made when using linear regression. These are:

1. The relationship being modeled is linear.
2. The errors are independent (residuals do not exhibit autocorrelation)
3. The errors are homoscedastic and have constant variance, meaning variance is not a function of any of the predictor variables.
4. The errors are normally distributed.

The procedures developed by Pena and Slate (2006), available in R from the package ‘gvlma’, are used to test whether or not these assumptions are violated by a chosen model. If the assumptions are violated then the model can be considered misleading.
CHAPTER 5: RESULTS

Description of Variables

Data on the nine variables were described and analyzed from all 66 selected sites (Table 1). None of the six predictor variables are normally distributed. Drainage basin relief and stream width are normally distributed when considered logarithmically, confirming the original assumption. This was to be expected when using sites from various stream sizes, as there are exponentially more small streams than large ones.

<table>
<thead>
<tr>
<th>Units</th>
<th>Min.</th>
<th>1st Quart</th>
<th>Mean</th>
<th>3rd Quart</th>
<th>Max</th>
<th>σ</th>
<th>Wilks(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_d$</td>
<td>km$^{-1}$</td>
<td>0.14</td>
<td>0.89</td>
<td>1.36</td>
<td>1.53</td>
<td>6.75</td>
<td>0.98</td>
</tr>
<tr>
<td>$D_r$</td>
<td>m</td>
<td>10.00</td>
<td>97.25</td>
<td>443.50</td>
<td>350.80</td>
<td>2683.00</td>
<td>625.22</td>
</tr>
<tr>
<td>$S_f$</td>
<td>km$^{-2}$</td>
<td>0.05</td>
<td>0.84</td>
<td>1.906</td>
<td>2.49</td>
<td>14.13</td>
<td>1.90</td>
</tr>
<tr>
<td>$S_s$</td>
<td>n/a</td>
<td>1.00</td>
<td>1.10</td>
<td>1.30</td>
<td>1.40</td>
<td>2.37</td>
<td>0.28</td>
</tr>
<tr>
<td>$S_w$</td>
<td>m</td>
<td>4.90</td>
<td>11.71</td>
<td>23.84</td>
<td>27.88</td>
<td>144.90</td>
<td>22.08</td>
</tr>
<tr>
<td>$S_o$</td>
<td>n/a</td>
<td>2</td>
<td>4</td>
<td>4.7</td>
<td>5</td>
<td>8</td>
<td>1.13</td>
</tr>
<tr>
<td>BAF</td>
<td>n/a</td>
<td>-0.59</td>
<td>0.06</td>
<td>0.33</td>
<td>0.60</td>
<td>1.53</td>
<td>0.48</td>
</tr>
<tr>
<td>$E_w$</td>
<td>n/a</td>
<td>0.00</td>
<td>0.03</td>
<td>0.06</td>
<td>0.07</td>
<td>0.13</td>
<td>0.03</td>
</tr>
<tr>
<td>$E_s$</td>
<td>n/a</td>
<td>0.00</td>
<td>0.01</td>
<td>0.03</td>
<td>0.04</td>
<td>0.13</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Drainage density ranges from 0.14 km$^{-1}$ in Perry, Florida, to 6.75 km$^{-1}$ in Angelus, California, with an average of 1.36 km$^{-1}$, the majority of sites having a drainage density less than 2 km$^{-1}$. The distribution of $D_d$ is positively skewed. The Wilkes Shapiro test indicates that the null hypothesis that drainage density does come from a normal distribution is rejected.
Drainage basin relief has a wide range of values in the selected study sites. Since locations range from the smooth Great Plains to the sharp peaks of the Rocky Mountains, so does the range of relief. The average elevation drop in the studied basins is 443 meters with the minimum at 10 meters for Deep Creek in Delaware and the maximum of 2,683 meters in McKenzie Bridge, Oregon. Drainage basin relief is very positively skewed, with over $\frac{3}{4}$ of the sites having a relief less than the average. However, the log transform of relief can be assumed to be normal, and is only slightly positively skewed.

Stream frequency on average is 1.9 channels per square kilometer, with the lowest at 0.05 channels per square kilometer and the greatest at 14.13 channels per square kilometer. The greatest value was found in the East Fork of Dairy Creek near Meacham Corner, Oregon. This site also had a large value for drainage density and drainage basin relief. The minimum value of 0.05 is the Calamus River in Nebraska. A majority of basins have a stream frequency between 0.5 and 2.5 channels per square kilometer. A log transformation was not sufficient to assume a normal distribution for stream frequency, which has a positive skew. Higher stream frequencies are found near or in rugged terrain and the lowest in flatter or arid regions.

Most streams in this study have a stream order of 4 or 5, with 5 most common. Stream order ranges from 2 to 8. There are no source streams, only one stream with an order of 2, and 13 above an order of 5. The largest stream is the Trinity River in Texas, with a stream order of 8.
Stream sinuosity is positively skewed since the value cannot be less than 1 and the higher the value, the more infrequent these streams become. Sinuosity ranged from 1 to 2.366. The average stream traveled 1.3 times the distance of its valley, with a majority traveling between 1.1 and 1.4 times the distance of their valleys. The most sinuous stream in the study is the Hudson River in New York. The least sinuous is a mountain stream in Washington.

Stream width is positively skewed, but is normally distributed (W=0.97, p=0.24) when log transformed. Stream width ranges from the 4.9 meter wide Umtanum Creek in Washington to the 144 meter wide Great Miami River in Ohio. The average stream width is 24 meters. Most streams have a width between 11.7 and 24 meters.

Thirteen streams were identified with gravel-dominant loads, 35 with sand, and 9 with silt. A total of 9 streams were indeterminate due to a lack of quality imagery. It is possible that some of the sand classified streams could actually be gravel or silt as they were occasionally difficult to discern.
Figure 6: Kernel density plot with histograms of predictor variables.
One of the criterion variables was normally distributed and the others are positively skewed. The bioaccumulation factor (BAF) ranged from -0.59 in Popple River, Wisconsin, to 1.58 in the Cooper River of New Jersey. The BAF is normally distributed with a mean of 0.33 and standard deviation of 0.6 (Figure 7). Sediment methylation efficiency (Es) ranged from 0 to 13%. The stream with the highest rate of methylation in sediment is Chesterville Branch near Crumpton, Maryland. The average methylation efficiency is 3%. Water methylation efficiency (Ew) occurs in identical ranges to that of sediment methylation efficiency, but with a mean of 6%.

Figure 7 - Kernel density plot with histograms of criterion variables
Associations

Bivariate correlation showed weak but statistically significant associations between the bioaccumulation factor and drainage density, the water methylation efficiency and drainage density, and the sediment methylation efficiency and drainage basin relief (Table 2). The most determinate associations among the predictor variables are between drainage density and stream frequency and between stream width and stream order. Spearman’s ρ is reported since the variables are not normally distributed.

Table 2: Correlation coefficients between all variable pairs

<table>
<thead>
<tr>
<th></th>
<th>BAF</th>
<th>Es</th>
<th>Ew</th>
<th>Dd</th>
<th>Dr</th>
<th>Sf</th>
<th>Sw</th>
<th>So</th>
<th>Ss</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAF</td>
<td>1</td>
<td>0.02</td>
<td>-0.36**</td>
<td>0.29**</td>
<td>-0.18</td>
<td>-0.01</td>
<td>0</td>
<td>0.16</td>
<td>-0.08</td>
</tr>
<tr>
<td>Es</td>
<td>1</td>
<td>0.2</td>
<td>-0.04</td>
<td>0.34**</td>
<td>-0.04</td>
<td>-0.11</td>
<td>0.07</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Ew</td>
<td>1</td>
<td></td>
<td>-0.22*</td>
<td>0.1</td>
<td>-0.21</td>
<td>0.19</td>
<td>0.03</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>Dd</td>
<td>1</td>
<td></td>
<td></td>
<td>0.37**</td>
<td>0.68**</td>
<td>-0.04</td>
<td>0.39**</td>
<td>-0.15</td>
<td></td>
</tr>
<tr>
<td>Dr</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>0.28**</td>
<td>0.04</td>
<td>0.33**</td>
<td>-0.28**</td>
<td></td>
</tr>
<tr>
<td>Sf</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.17</td>
<td>0.17</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>Sw</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.58**</td>
<td>0.21*</td>
<td></td>
</tr>
<tr>
<td>So</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.16</td>
<td></td>
</tr>
<tr>
<td>Ss</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

Correlation Matrix using Spearman’s ρ. * p<0.1, ** p<0.05. Criterion variables in bold, predictor variables in italics.
Stream Load ($S_i$)

There does not appear to be any obvious association between primary sediment size and any of the criterion variables (Figure 8). The distributions of sediment efficiency and water methylation efficiency appear to be independent of stream load, with the mean almost equal for each group ($\bar{Es} = 0.025$ and $\bar{Ew} = 0.050$). The bioaccumulation factor had different means for each group, 0.27 for gravel, 0.37 for sand, and 0.18 for silt. The Kruskal-Wallace test was used to test the null hypothesis that there is no difference in BAF between stream load groups. The results of this test were a $\chi^2$ of 1.4724, df = 3, and p-value = 0.6886. This indicates that the null hypothesis cannot be rejected and it is assumed that there is no difference in BAF between groups.

![Figure 8](image_url)  
*Figure 8 – Kernel density distributions of criterion variables grouped by sediment load.*
Colinearity

Significant correlations exist between some of the predictor variables. None of the correlations, however, are strong enough to indicate that there will be a problem with colinearity when using multiple linear regression (Table 2 and Figure 9). The strongest association is between drainage density (Dd) and stream frequency (Sf) with a $\rho$ of 0.68 ($p<0.05$). Dd and Sf share a common denominator, drainage area, in their calculation, so the correlation is not surprising. Stream width (Sw) and Stream Order (So) also have a strong association, $\rho = 0.58$ ($p<0.05$).
Figure 9: Scatterplot matrix of predictor variables. Figure depicts a scatterplot matrix of predictor variables including a fitted line using least mean squares with 95% confidence intervals. The diagonal plots are the kernel density curves for each variable.
Bioaccumulation Factor

The bioaccumulation factor shows statistically significant positive correlation ($\rho = 0.29$, $p<0.05$) with drainage density. BAF also associates with water methylation efficiency; this was expected since the BAF is the log ratio of Hg in fish to MeHg in water.

**Figure 10** - Scatterplot matrix of the bioaccumulation factor (BAF) and the predictor variables.
Figure 11 shows the top six performing models for BAF in terms of adjusted $R^2$. This plot is useful not for only choosing the best performing model, but also to see the relative importance of each potential predictor variable. Drainage density is present throughout the plot, with drainage basin relief then stream frequency. The selected model for the bioaccumulation factor has an $R^2 = 0.24$, $p=0.001$, and $F=6.045$. The selected predictor variables that best explain the bioaccumulation factor are drainage density, drainage basin relief, and stream frequency.

![BAF model selection](image)

**Figure 11:** Linear regression model selected for the bioaccumulation factor
The chosen model is significant enough to reject the null hypothesis that the effects of Dd, Dr, and Sf on BAF can be explained by random chance. However, the model is not very determinate so it is possible that further data could significantly change the explanation. The model fails the assumption test for residual normality (Figure 12). The residuals are positively skewed with a skewness of 5.21. This does not affect the significance of the model, but indicates that the determinacy and estimation of the coefficients could be misleading. There is one site that comes close to being an influential outlier with a drainage density of 4.59 in Oregon.
The model demonstrates that drainage density and drainage basin relief are the most influential explanatory variables. All variables, however, enter as significant factors (Table 3). The standardized coefficients ($\beta$) indicates that a one standard deviation increase in drainage density is about twice as influential as a one standard deviation decrease in basin relief. Since drainage density is generally higher in basins with more
relief, it was expected that both of these coefficients would be positive. It seems that bioaccumulation is better explained as being higher in basins with higher drainage density and less basin relief. Stream frequency and drainage density are often considered analogous to each other, but in this model have almost opposite influences. The reason for this is unknown and suggests other unmeasured factors are in play.

**Table 3:** Linear regression summary of BAF ~ Dd + Sf + log(Dr)

<table>
<thead>
<tr>
<th></th>
<th>β</th>
<th>B</th>
<th>Std. Error</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.572</td>
<td>0.243</td>
<td>0.022</td>
<td></td>
</tr>
<tr>
<td>Dd</td>
<td>0.742</td>
<td>0.542</td>
<td>0.142</td>
<td>0.00</td>
</tr>
<tr>
<td>Sf</td>
<td>-0.47</td>
<td>-0.122</td>
<td>0.048</td>
<td>0.015</td>
</tr>
<tr>
<td>log(Dr)</td>
<td>-0.342</td>
<td>-0.304</td>
<td>0.108</td>
<td>0.00</td>
</tr>
</tbody>
</table>

It is clear from the model that the associated patterns are not random, and that the three variables directly relating to the ‘roughness’ of the planet’s surface are significant predictors of the bioaccumulation rate of mercury in fish tissue. These variables are also considered indicators of watersheds that have high-peaked short flood events and tend to generate surface runoff instead of water infiltrating into the subsurface.
Sediment Efficiency

Sediment efficiency showed a significant association with drainage basin relief ($\rho = 0.34$, $p<0.05$) (Figure 13). Sediment efficiency showed no association with any of the other criterion or predictor variables.

Figure 13- Scatterplot matrix of sediment efficiency ($Es$) and the predictor variables
All potential predictors, with the exception of stream frequency, are in the selected model for sediment methylation efficiency (Figure 14). Drainage density and drainage basin relief have a strong inclusion, similar to the model of the bioaccumulation factor. The variables closely related to base-flow sediment transport regimes are included in this model, indicating that there is potential for sediment transport to have an impact on sediment methylation efficiency.

**Figure 14** – Linear regression model selection for the sediment methylation efficiency
This model has an $R^2$ of 0.23, F-statistic of 3.269, and $p=0.01$. The results are statistically significant but not very determinate. The null hypothesis, that sediment efficiency relates to $D_d$, $\log(D_r)$, $\log(S_w)$, $S_s -1$, and $S_o -1$ by pure chance, is rejected.

According to the standardized coefficients, a standard deviation change in the $\log(D_r)$ is more influential than a standard deviation change in any of the other predictor variables (Table 4). Drainage density and stream width both negatively affect the model, and are loaded similarly to $\log(D_r)$. All the predictor variables are significant except stream sinuosity, which may or may not be an explanatory variable.

**Table 4:** Linear regression summary of $E_S \sim D_d + \log(D_r) + \log(S_w) + S_s + S_o$

<table>
<thead>
<tr>
<th></th>
<th>$\beta$</th>
<th>B</th>
<th>Std. Error</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.0153</td>
<td>0.5802</td>
<td></td>
</tr>
<tr>
<td>$D_d$</td>
<td>-0.3733</td>
<td>-0.0109</td>
<td>0.0046</td>
<td>0.0224</td>
</tr>
<tr>
<td>$\log(D_r)$</td>
<td>0.4243</td>
<td>0.0161</td>
<td>0.0049</td>
<td>0.0018</td>
</tr>
<tr>
<td>$\log(S_w)$</td>
<td>-0.3750</td>
<td>-0.0239</td>
<td>0.0123</td>
<td>0.0571</td>
</tr>
<tr>
<td>$(S_s -1)$</td>
<td>0.1600</td>
<td>0.0105</td>
<td>0.0084</td>
<td>0.2140</td>
</tr>
<tr>
<td>$(S_o -1)$</td>
<td>0.2993</td>
<td>0.0053</td>
<td>0.0035</td>
<td>0.1429</td>
</tr>
</tbody>
</table>

The model violates one assumption; the residuals are not normally distributed (Figure 15). There are three sample points on the positive extreme of the residual.
distribution that could be accounting for this. One of these three has a very high drainage basin relief, another is very wide, and the third is very sinuous.

Figure 15– Linear regression diagnostic plot of \((E_s \sim D_d + \log(D_r) + S_f + \log(S_w) + S_s + S_o)\).
Water Efficiency

Water methylation efficiency negatively associates with drainage density ($\rho = -0.22$, $p<0.1$) (Figure 16). This association is less significant, with a 10% chance they do not associate. Water methylation efficiency had no clear associations with the other predictor variables.

Figure 16- Scatterplot matrix of water efficiency (Ew) and the predictor variables
The top-performing model for water methylation efficiency is a function of drainage density, the log of drainage basin relief, and stream sinuosity. All three of these variables have a strong presence in the top performing models (Figure 17).

**Figure 17** – Linear regression model selection for the water methylation efficiency.

The selected model has an $R^2$ of 0.11, F-statistic of 2.33 and $p=0.08$. The model is neither as significant nor determinate as the others. The null hypothesis can be rejected, that the relationship between water methylation efficiency and the three predictor variables is not occurring by chance. There is approximately a 92% probability that the
relationship is not a random occurrence. There is a complementary 8% probability that water methylation efficiency has no relationship to the variables presented in this thesis.

**Table 5:** Linear regression summary of \( \text{Ew} \sim \text{Dd} + \log(\text{Dr}) + \text{Ss} \)

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The model violates none of the assumptions of linear regression. The predictor variables are almost equally loaded, with drainage density negatively relating to water methylation efficiency but drainage basin relief and sinuosity relating positively.
**Figure 18** – Linear regression diagnostic plot of the selected model ($E_w \sim D_d + \log(D_r) + S_s$)
CHAPTER 6: DISCUSSION

Results from both correlation and linear regression indicate that the null hypothesis of there being no relationship to fluvial geomorphic parameters can be rejected for the bioaccumulation factor and sediment efficiency. Water methylation efficiency demonstrates a less statistically significant and determinate result and its null hypothesis cannot be rejected with any certainty. The extents of any relationships are still unknown, as all correlation and regression efforts are indeterminate. What is known about individual relationships is that some are positive and some are negative.

Bioaccumulation

The bioaccumulation factor positively correlates with drainage density. Linear regression indicates that drainage density, stream frequency, and drainage basin relief are significant predictors of bioaccumulation. Linear regression reveals that the relationship between these predictors and BAF is positive for drainage density and negative for the other predictors. Watersheds with higher drainage density will flood more quickly, and have more surface runoff. In terms of fluvial systems this would equate to frequent sediment transport events. From the biological perspective, increased runoff would bring a more continuous supply of organic material from decaying plant matter in soils. The negative relationship between BAF and drainage basin relief probably reflects the fact that watersheds with higher relief have the potential to have more energy than those with less total change in elevation. It is possible that, compared with streams with lower
energy, streams with higher energy create a more unstable environment that hinders the biological accumulation of methylated mercury. The literature indicates that wetlands are ‘methylation hotspots,’ which are very low-energy fluvial systems (Castro et al. 2006, Evers and Clair 2005). Wetlands are thought to have high methylation rates due to the abundance of decaying organic material (Shanley et al. 2005, Scudder et al. 2009).

The bioaccumulation factor appears unaffected by sediment type, stream order, width, or sinuosity. This suggests that BAF can be any value regardless of a stream’s sediment load and size. However, not enough evidence exists to support this claim. Overall, the significant pattern is that the uptake rate of methylmercury into fish tissue is greater in streams with frequent flood events and less energy, and independent of a stream’s size or sediment load.

Sediment Methylation

The efficiency of methylation in sediment correlates positively with drainage basin relief. Regression indicates that sediment methylation efficiency can be explained as a function of drainage density, drainage relief, stream width, stream sinuosity, and stream order. Drainage density, drainage relief, and stream width are the significant predictors. Sediment methylation efficiency is greater in watersheds with fewer and narrower channels per unit area, and greater relief. SAF is also tends to be greater for streams of lower order. This is a somewhat contradictory result since streams of higher order also have greater width. The model is indeterminate, as indicated by the low
coefficient of determinacy ($R^2$) and near-zero model coefficients; therefore the true relationship could be different from what is observed here.

Sediment efficiency can be generalized as being greater in drainage basins exhibiting gradual and less peaked flood events, less runoff, and greater stream energy.

Water Methylation

Water methylation efficiency may or may not have any relationship to the predictor variables. Ew showed a weak negative correlation with drainage density and associated with no other predictor variables. Linear regression results are non-determinate and not significant. The water methylation efficiency is a less meaningful measure in the scope of mercury methylation, and as such may not depend on any physical factors. Mercury methylation is expected to take place primarily in sediments and then fish ingest it from water and water-bound species. Water methylation efficiency could just be a ratio of MeHg in transit from sediment to fish, and total Hg in transit from water to sediment. The methylation efficiency could just be a ratio of the time it takes these two processes to happen, which if dependent on physical factors, might not have been captured by the data. The weak negative correlation with drainage density is expected if the bioaccumulation factor has a positive correlation with drainage density. More mercury in fish compared to water would be less in water compared to fish. The results show that BAF and Ew are correlated in this manner.
Drainage Density and Forested Terrain

Drainage density is the strongest predictor variable in two of the three linear regression models, and the second strongest predictor of sediment efficiency. The USGS reported that forested areas, specifically evergreen forests, are good predictors of bioaccumulation (Scudder et al. 2009). The areas that are mostly forested in the United States happen to be areas of greater drainage densities which contribute to the difficulty of land management in these areas. This pattern is apparent on almost any map that includes land cover and relief shading. In fact, drainage density from this thesis and percent evergreen forest from the USGS research positively correlate with a Spearman’s ρ of 0.4 (p<0.01). An even stronger correlation exists between drainage density and all forested land cover with a Spearman’s ρ of 0.5 (p<0.01).

It is possible the correlation between drainage density and the bioaccumulation factor may be reflecting an association between the bioaccumulation factor and evergreen forests. To test this, drainage density was normalized by percent evergreen forests and this result compared to the bioaccumulation factor, but there is no observed association between the normalized variable and BAF (ρ = -0.025, p>>0.1).

In this thesis dataset, sediment efficiency is positively correlated with evergreen forests (ρ = 0.31, p<0.05), as is drainage basin relief. Normalizing drainage basin relief by percent evergreen forest demonstrates no correlation with sediment efficiency (ρ = 0.08, p>>0.1). It is uncertain then if any physical parameters have significant influence
on the bioaccumulation factor or sediment efficiency. The relationship to land cover may just a reflection of other physical parameters or vice versa.
CHAPTER 7: CONCLUSION AND FUTURE WORK

This research failed to find any definitive relationships between the methylation rates and a stream or basin’s physical characteristics. Results indicate that there is a relationship between the bioaccumulation factor (BAF) and drainage density and drainage basin relief. The bioaccumulation factor could be positively impacted by increased surface runoff and decreased soil infiltration. Sediment efficiency associates with drainage basin relief, a good surrogate for potential energy. Recall that the higher the energy of a stream, the larger grain size of sediment particles or larger quantity of sediment can be transported. However, it is unclear how these two might relate. Stream load did not appear to be an obvious predictor and the quantity of sediment transported cannot be easily inferred from the predictor variables. Results with the water methylation efficiency are statistically significant, but inconclusive.

The results of this study have indeterminacies, and it is likely that these relationships are either indirect or in combination with other factors that were not a part of this study. Comparing methylation to landcover and landuse has been a strong focus of recent literature and may relate to methylation rates in combination with these physical characteristics. This indeterminacy could also be a direct result of the sample site selection process, which was designed to ensure the inclusion of a wide range of physical characteristics. A more controlled study, with fewer differences between sites, may be able to discern what these relationships are.
Future work might consider carefully selecting or sampling sites with a focus on drainage density and landcover. An attempt to control for the codependence of these variables is necessary to ascertain the relationship either could have to mercury bioaccumulation. Currently the USEPA is in the process of attributing the National Hydrography Dataset with various variables describing each stream segments physical properties, which will decrease the effort and cost of using physical characteristics in future research of this nature.
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USFDA. 2004. “What you need to know about mercury in fish and shellfish.”

US Food and Drug Administration, US Environmental Protection Agency.


The United States Geological Survey.


### APPENDIX

#### Data Table

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