# MERCURY ACCUMULATION IN STREAM PERIPHYTON OF THE OLYMPIC PENINSULA, WA

by

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ABSTRACT

Mercury Accumulation in Stream Periphyton
of the Olympic Peninsula, WA

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Mercury is widely recognized as a priority pollutant, threatening wildlife and fish consumers globally. Despite the widespread contamination in aquatic systems, little research has measured mercury concentrations in stream periphyton. Not only does stream periphyton represent a large component of the base of stream food webs, it plays many important roles in chemical processing within streams. This research project examined stream chemistry, watershed characteristics, and mercury concentrations in stream periphyton grown on artificial substrates from three watersheds in the Olympic Experimental State Forest in Washington State, United States, during the spring of 2018. The three watersheds were chosen based on variability in factors such as wetland area, topography, and timber harvest impact in each watershed. Results show mercury areal burdens ranging from approximately 24-736 $ng/m^2$, which are relatively low compared to a nationwide survey, but comparable to results found in the region. The site with the periphyton containing the highest mercury concentrations also had the most wetland area, the most timber harvest disturbance, the lowest basin slope and reach gradient, highest periphyton biomass, and lowest pH. Results suggest that, especially in the presence of wetlands, even low impact timber harvesting can result in elevated mercury concentrations in stream periphyton relative to surrounding areas. This is likely due to the impacts of timber harvesting and topography on carbon processing and hydrology, and the role of wetlands on methylmercury production.

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# Introduction

## Mercury as an Environmental Problem

While mercury is naturally present at very low concentrations in most environmental systems (Ullrich et al., 2001), many systems are now contaminated due to human actions. Decades of industrial activity such as coal burning, waste incineration, and mining have altered the biogeochemical cycling of mercury such that atmospheric, oceanic, and terrestrial mercury concentrations are now roughly 3-5 times background levels, despite emission reductions since the 1990s (Selin, 2009; Zhang et al., 2016). Once released to the environment, mercury may spend time scales of centuries to millennia cycling between atmospheric, oceanic, and terrestrial systems before eventually accumulating in deep ocean sediments, where it may be permanently stored (i.e., time scales of millions of years; Selin, 2009).

Increased mercury pollution is especially concerning due to its tendency to be converted to an organic form that is highly toxic to animals. Under the right conditions, mercury is converted to methylmercury (MeHg), a strong neurotoxin known to bioaccumulate up aquatic food chains (Driscoll et al., 2007). This process is especially common in anoxic aquatic systems and soils. Human exposure to methylmercury through the consumption of predatory fish continues to threaten fish consumers globally. Known as Minamata disease, acute mercury poisoning results in a variety of severe health effects and can be fatal at high doses. Acute mercury poisoning via fish consumption in North America, however, is very rare (Centers for Disease Control and Prevention, 2018).

To address human health concerns related to fish consumption in Washington State, the WA Department of Ecology has a fish tissue-based human health criterion for MeHg as a water quality standard, and the WA Department of Health has determined a fish tissue mercury concentration screening level used to issue fish consumption advisories. Even in Remote lakes on the Olympic Peninsula, fish tissue collected by the Washington State Department of Ecology is commonly above both of these screening levels.

Low order streams (closer to the headwaters) drain relatively small areas of land, and more directly reflect their surrounding landscape compared to larger systems further downstream. They are also the primary pathway for mercury being transported from it's place of deposition to larger aquatic systems, such as lakes. Biota in these systems, primarily periphyton, invertebrates, amphibians, and fish may absorb some of this mercury. Stream periphyton, the mixture of algae, bacteria, detritus and microbes attached to submerged surfaces, is the base of stream food webs, making it the main source of mercury to organisms higher on the food chain. Despite its importance in stream systems, mercury accumulation in stream periphyton has received little attention in the relevant literature.

This research aims to answer the questions: Are mercury concentrations in stream periphyton on the Olympic Peninsula elevated compared to results found elsewhere, and can watershed characteristics explain differences among sites? When referring to watershed characteristics, this includes natural factors such as topography and wetland cover, as well as timber harvest influence. Timber harvest is an especially important aspect of this research, as it has been shown to significantly influence mercury cycling in

other studies, and the study sites used here are designated with the purpose of integrating revenue production in the form of logging with ecological values.

## Background Information

### The Mercury Cycle

Although mercury is relatively harmless at low concentrations (Ullrich et al., 2001), the conversion of mercury to methylmercury can potentially result in dangerously high concentrations in fish due to it's potential to bioaccumulate in aquatic food chains (Driscoll et al., 2007). What follows is a discussion of the background information necessary to briefly understand how mercury cycles and ultimately reaches high concentrations in fish tissue.

#### *Biogeochemical Cycling*

Mercury in the environment exists in three distinct oxidation states: Hg(0), Hg(I), and Hg(II). Hg(I), however, is sufficiently unstable and rare to be excluded from this discussion. The oxidation state (represented by the 0 and II), or degree of oxidation, can be defined as the number of electrons "lost" through an oxidation reaction. Because elemental Hg(0) is uncharged, Hg(II), which has "lost" two electrons, has a charge of +2 (Liu et al., 2012).

The oxidation state of mercury molecules is an important distinction, because the oxidation state and corresponding charge largely determines the reactivity of the different mercury species. Because of this distinction, Hg(II) readily forms complexes by binding

with anions and other molecules, such as methyl groups ($CH_3$). In the atmosphere, the dominant form of mercury is gaseous Hg(0), whereas in the water, soils, and sediments it is the inorganic form Hg(II), and in biota it is MeHg (A mercury molecule with a single methyl group; Liu et al., 2012).

*Sources of Mercury to the Environment*

Natural and anthropogenic sources of mercury contribute significantly to the mercury pools circulating throughout our environment. The most common pathway for mercury entering ecological systems is through the atmosphere, whether anthropogenically (primarily oxidized and particulate bound mercury) or naturally (primarily gaseous elemental mercury) emitted in the first place. While estimates of these inputs are highly uncertain, they typically range from 5,000-6,000 tons per year, and although human emissions have been decreasing since the 1990s, they still constitute approximately half of all total emissions (Liu et al., 2012).

Natural mercury inputs to the atmosphere come primarily from erupting volcanoes and geothermal emissions. Other less common sources include volatilization from aquatic environments, forest fires, and leachate from geologically enriched areas (Friedli et al., 2003). In total, over 99% of natural mercury emissions are in the form of gaseous elemental mercury, Hg(0) (Liu et al., 2012). Areas that are naturally enriched in mercury are almost exclusively found along the global "mercuriferous belts", which are found along plate tectonic boundaries with mercury deposits, and are present mostly as cinnabar ore (HgS). This mercury can be released through geothermal activity, or leached into aquatic systems, as mentioned above. Leaching, however, is generally thought to be

a negligible source to aquatic systems (Liu et al., 2012). Figure 1 shows the locations of known mercury-bearing ore in Washington State.

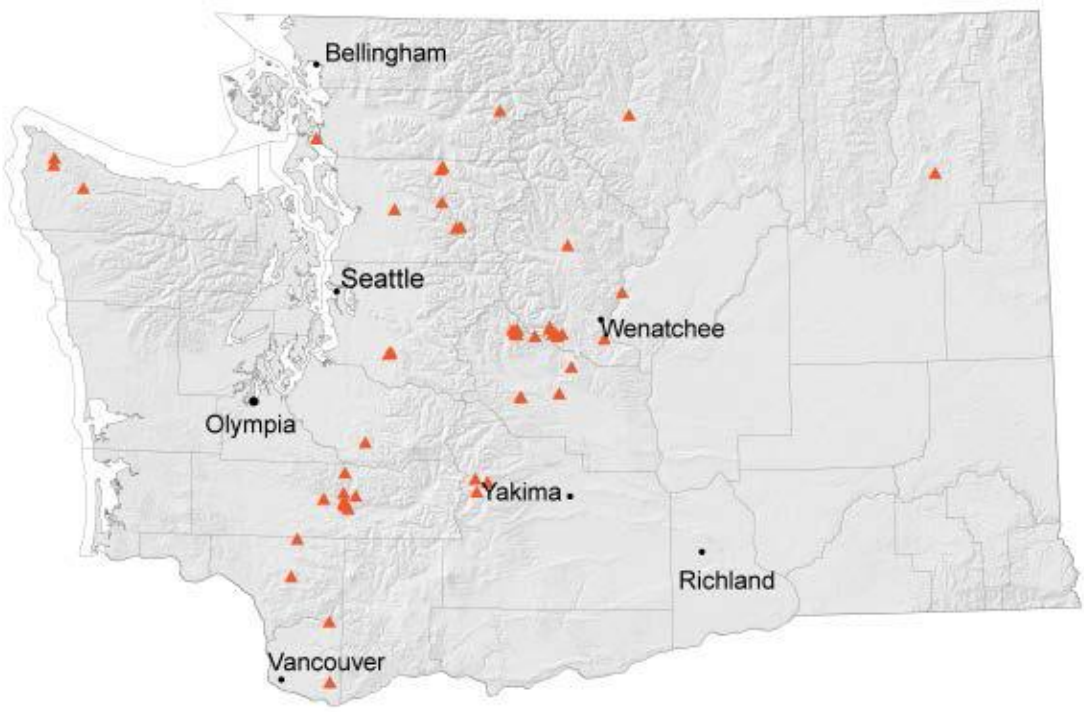


Figure 1. Distribution of reported locations of mercury-bearing ore in Washington State. From: *http://www.dnr.wa.gov/programs-and-services/geology/geologic-hazards/hazardous-minerals#mercury*

While natural emissions are almost exclusively in the form of gaseous elemental mercury, anthropogenic emissions also contain reactive gaseous mercury (Hg(II) complexes) and particulate mercury (PHg; mostly Hg(II) adsorbed onto particulates). These emissions come mostly from the combustion of fossil fuels (which contain small amounts of mercury), gold and other metal production, cement production, and waste incineration (Liu et al., 2012). Pacyna et al. (2006) estimates that coal combustion alone

contributes roughly 60% of total anthropogenic sources. Also responsible for high concentrations at local scales, while contributing little at the global scale, are diffuse sources such as landfills, sewage sludge amended fields, and mine waste (Liu et al., 2012).

*Mercury in the Atmosphere*

Once in the atmosphere, oxidation and reduction reactions take place in small water droplets, creating a heterogeneous mixture of mercury species. Primarily in the gaseous phase, Hg(0) reacts to form its oxidized form, Hg(II). Hg(II) molecules then form a range of complexes which exist mostly in the aqueous phase in water droplets, or adsorbed onto particulate matter (Liu et al., 2012).

Over 95% of mercury in the atmosphere exists as gaseous Hg(0), with an atmospheric lifetime of approximately 0.5-1.5 years. This relatively long lifetime allows for large scale mixing to spread atmospheric mercury to locations without any significant sources. Because industrial inputs of mercury are larger in the Northern Hemisphere, there is an increasing concentration gradient from the Southern to Northern Hemisphere (Liu et al., 2012).

When Hg(II) molecules in the atmosphere bind to form aqueous complexes and particulate mercury (PHg), they are deposited through wet and dry deposition. These complexes and particulates, representing less than 5% of atmospheric mercury, constitute the prominent forms of atmospherically deposited mercury, and have atmospheric lifetimes of just days to weeks. This atmospheric deposition is the main source of mercury to most aquatic and terrestrial systems (Liu et al., 2012).

*Atmospheric Deposition in Western Washington*

The National Atmospheric Deposition Program (NADP) monitors precipitation chemistry through the United States and Canada. Within this program is the Mercury Deposition Network (MDN), which provides long term data on mercury deposition through precipitation (wet deposition) in the United States. In Washington State, monitoring sites are located in Neah Bay and Seattle. As shown in Figure 2, which shows total mercury wet deposition at all MDN sites with spatial extrapolations for the year 2014, western Washington receives mercury through wet deposition at rates comparable to or higher than most of the nation. While this map only shows 2014 deposition (the most recent year with published data), previous years are very similar. In the year 2014, deposition values at the Neah Bay and Seattle stations are just 5.9 and 8.2 ug/$m^2$, respectively, but spatial extrapolations estimate rates in the range of 15-20 ug/$m^2$ in the mountainous regions of the state, including the Olympics. This trend, of higher deposition rates in mountainous regions, is true throughout western North America, as can be seen in the map.

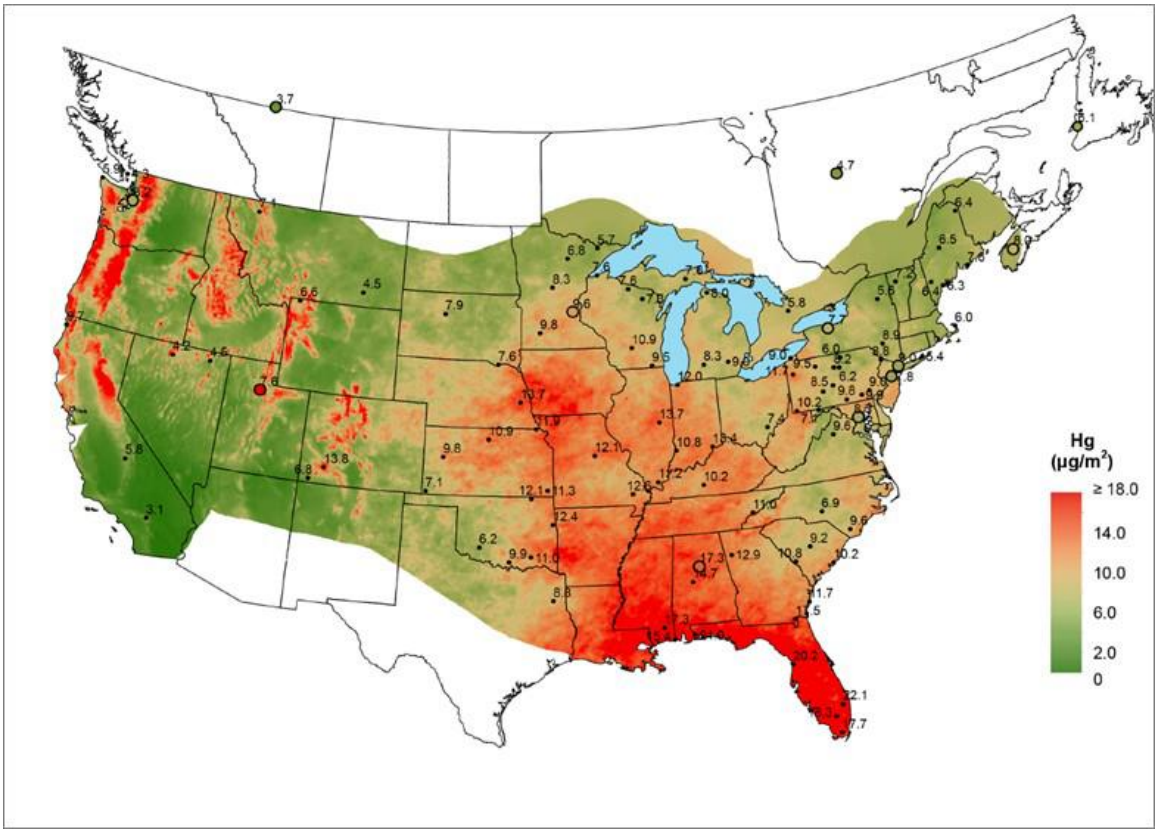


Figure 2. Total mercury wet deposition with spatial extrapolations for the year 2014. Source: the National Atmospheric Deposition Network (NADP) *http://nadp.sws.uiuc.edu/MDN/annualmdnmaps.aspx*

The wet deposition map above only considers deposition from precipitation, and does not consider dry deposition. Dry deposition refers to the process by which gaseous and particulate bound pollutants settle out of the atmosphere or are absorbed by plants. Zhang et al. (2016) found that at most of their 24 study sites throughout North America, dry deposition exceeded wet deposition by a large margin for most of the year. In forested canopies, dry deposition ranged from 5.1 to 23.8 ug/m$^2$/yr (Zhang et al., 2016). Forested canopies are known to receive significantly more dry deposition than non-forest vegetated canopies, urban land covers, or water surfaces, because they filter particulate

mercury from the air and uptake gaseous elemental mercury (Kolka et al., 1999; Miller et al., 2005; Zhang et al., 2016). As will be discussed in the literature review, subsequent logging of forested landscapes is known to expedite the transfer of deposited mercury to soils and surface waters (Porvari et al., 2003; Skyllberg et al., 2009; Eckley et al., 2018).

In a nationwide study, Obrist et al. (2011) investigated mercury concentrations in forest litter and soils. In a stepwise multiregression analysis and individual linear regression analyses, they found that soil carbon concentration, latitude, precipitation, and clay in soils were all positively correlated with soil Hg concentrations, and explained up to 94% of mercury concentration variability. The correlations with carbon and precipitation are unsurprising, as highly organic soils known to bind strongly to Hg, essentially sequestering Hg from air (Skyllberg et al., 2000), and precipitation is a major source of atmospheric Hg, as discussed previously. Clay content is thought to correlate positively with Hg in soils due to a combination of high carbon retention in clayey soils and strong sorption of Hg by minerals in clay (Obrist et al., 2011). Lastly, the authors speculate that at lower latitudes, increased solar and UV radiation promote photoreduction (the addition of electrons, converting Hg(II) back to gaseous Hg(0)) and subsequent volatilization of Hg directly from soils, whereas at higher latitudes these processes are less dramatic (Obrist et al., 2011).

Based on this multiregression model, they created the map shown in Figure 3. As shown, the Pacific Northwest has the highest modeled concentrations in the country. Of all sites included in the study, the litter and soil horizons from the Douglas-fir dominant portion of Thompson Forest in western Washington was highest in total mercury concentrations (Obrist et al., 2011).

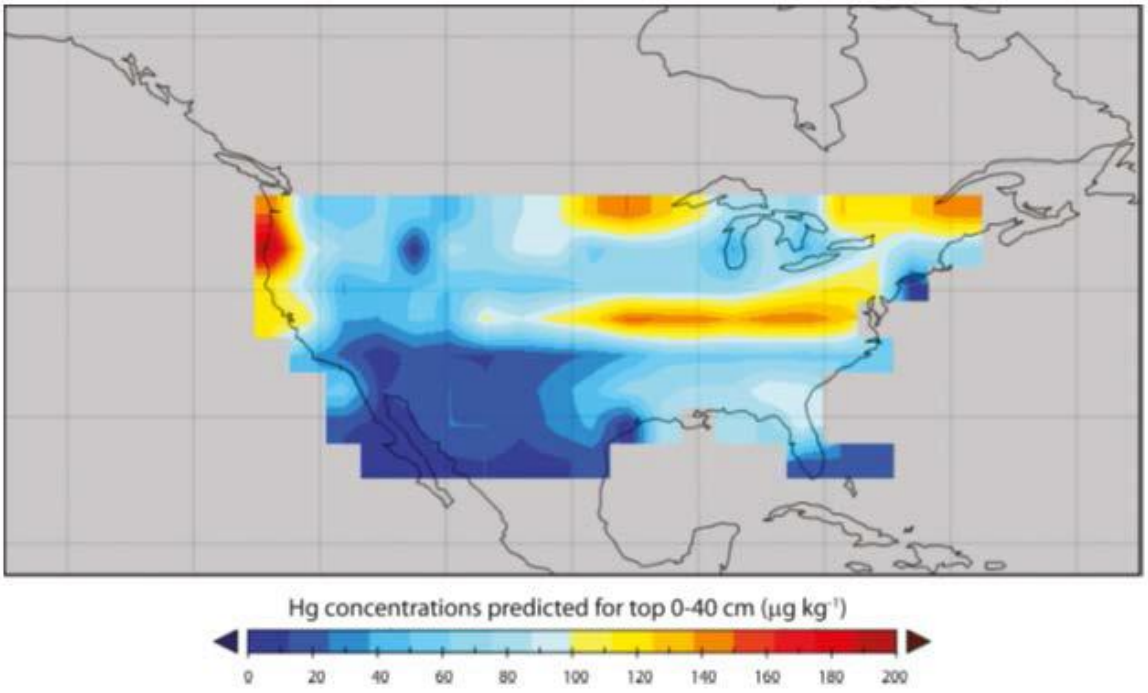


Figure 3. Spatial extrapolation of top soil (0-40 cm) mercury concentrations based on multiregression modeling using independent variables latitude, precipitation, soil carbon content, and clay content. *From Obrist et al. (2011).*

*Mercury in Aquatic and Terrestrial Systems*

Once deposited into surface waters, sediments, soils, and onto vegetation, mercury exists primarily as various organic and inorganic Hg(II) compounds. Small amounts of these Hg(II) compounds are reduced back to Hg(0) by XXX (just refer to this and explain you will discuss this further below), which readily volatilizes back to the atmosphere. The remining Hg(II) can be further complexed with various inorganic and organic ligands (Liu et al., 2012), and is typically retained in soils and vegetation for long periods of time before entering surface waters (Hintellman et al., 2002). As will be discussed in the literature review, mercury deposition rates are generally correlated to mercury concentrations on the ground only at large spatial scales, such as between

western and eastern Washington, while they fail to explain variations at smaller scales.

Of most importance is the mercury that gets methylated to form methylmercury. While methylmercury typically constitutes less than 10% of total mercury in water and less than 3% in sediments/soils, it is the main form of mercury found in biota due to it's tendency to bioaccumulate in animal tissue. This process will be discussed in much more detail next.

*Mercury Methylation*

The following section discusses mercury methylation, or the production of methylmercury, and its counterpart demethylation, or the degradation of methylmercury. Throughout the literature, the term methylation is sometimes used to represent net methylmercury production in a system, which includes both the production and degradation of methylmercury. This is because when measuring the net change in methylmercury concentrations in a system, it is difficult to tell how much production and degradation is actually occurring. In most cases, and throughout this paper, the term methylation is in reference only to the production of methylmercury.

While abiotic methylation has been demonstrated in some environments, biotic methylation by microbes is the primary source of methylmercury in most environments (Paranjape & Hall, 2017). Sulfate reducing bacteria (SRB) are widely recognized as being primarily responsible for the methylation of mercury in most environments, although the exact biochemical pathways of biotic methylation are still largely unknown (Liu et al., 2012). In short, SRB are anaerobic, so whereas aerobic organisms reduce oxygen molecules during respiration, SRB obtain energy in anaerobic environments by

oxidizing organic compounds while reducing sulfate molecules. At some point in this metabolic process, SRB passively absorb inorganic mercury molecules and methylate them to form methylmercury (Liu et al., 2012). SRB are most commonly found in anoxic sediments and inundated soils, especially where there is high carbon content.

Although SRB are broadly considered the primary methylators in most environments, not all SRB are capable of methylation, and some are more effective than others (Heyes et al., 2006). 10 of the 14 strains in the *Desulfovibrio, Desulfotomaculum*, and *Desulfobulbus* genera are known to be capable of methylation (Kaschak et al., 2014), and among these strains, those that use acetate as a carbon source are thought to exhibit higher methylation rates than those that use other carbon sources (Ekstrom et al., 2003). Furthermore, different strains of SRB and other microbes likely work in tandem to methylate elemental mercury. In some cases, certain SRB strains may oxidize mercury, at which point a second strain methylates that mercury (Hu et al., 2013). All of this is to say that although SRB are known to be mostly responsible for the production of methylmercury, the process is very complex and leaves more to be understood.

With that being said, other microbes have been shown to contribute significant methylation to some aquatic environments. Acha et al. (2011) (in periphyton in the Bolivian Amazon region) and Bravo et al. (2015) (in sediments affected by WWTP discharge) both demonstrated that SRB were not responsible for most mercury methylation, but were unable to identify primary methylators. In some cases, iron reducing bacteria (IRB) can methylate significant amounts of mercury, and may work in tandem with SRB, possibly by spatially and temporally separated processes (Yu et al., 2012). Methanogens (a broad class of microorganisms that produce methane as a

metabolic byproduct and are common in anoxic environments) have also been shown to have methylating properties (Gilmour et al., 2013), and may even be the primary methylators in some environments (Hamelin et al., 2011).

Of most importance to this study, significant methylation has been observed in periphyton in some cases. For example, studies have found periphyton to be important methylation sites in tropical lakes and other tropical freshwater environments (Coelho-Souza et al., 2006; Acha et al., 2011). In Canadian fluvial wetlands, Hamelin et al. (2015) found methylation rates in periphyton to be two orders of magnitude greater than those in sediments, which is where SRB are mostly likely to be found. The methylation observed in periphyton in these studies and others has also been attributed to SRB. Due to the importance of sulfur for SRB activity, it has been hypothesized that periphyton communities with an active microbial sulfur cycle also support Hg methylation (Cleckner et al., 1999). Mercury methylation in periphyton will be discussed again in the literature review.

The process of demethylation, or the degradation of methylmercury molecules, also occurs naturally, and may take place via metabolic activity of microbial species (Marvin-DiPasquale et al., 2000), or photodegradation (Bittrich et al., 2011). Unlike the relatively small range of microbial species capable of mercury methylation, a wide range of microbes have developed the ability to demethylate, as a form of resistance to the toxicity of methylmercury (Guadencio Dias et al., 2016). Microbial demethylation generally occurs in the subsurficial sediments and sediment-water interface of aquatic systems, and may be carried out by a variety of anaerobic and aerobic microorganisms (Zhang & Planas, 1994). Microbial demethylation occurs via two general mechanisms:

reductive and oxidative demethylation. Reductive demethylation is generally favored at high Hg concentrations in oxic conditions, and produces Hg(0) and $CH_4$ as byproducts, whereas oxidative demethylation is favored at low Hg concentrations in anoxic conditions, and produces Hg(II) and $CO_2$ as byproducts (Lu et al., 2016).

Photodegradation, or photolytic decomposition of methylmercury is still thought to be the only abiotic demethylation process significant to mercury concentrations in most surface waters (Guadencio Dias et al., 2016). Hammerschmidt and Fitzergald (2006) showed that methylmercury degradation rates are positively correlated with photosynthetically active radiation (PAR) intensity within 6 meters of the surface in the water column, and that within approximately 1 meter of the surface, ultraviolet (UV) light can significantly increase degradation rates. This is because although UV radiation degrades methylmercury faster than PAR, it penetrates less deeply through the water column (Guadencio Dias et al., 2016). The general mechanism of methylmercury photodegradation is indirect photolysis (Guadencio Dias et al., 2016). This involves the photochemical formation of aqueous free radicals (molecules with unpaired electrons), such as hydroxyl and chloride radicals, which attack the Hg-C bond in methylmercury, forming various divalent mercury products (Guadencio Dias et al., 2016). Importantly, photodegradation rates have been shown to be similar among lakes with varying water chemistry, suggesting that photodegradation is mostly controlled by environmental factors that affect light intensity and methylmercury concentrations in surface waters, as long as there are sufficient precursors for aqueous free radicals (Sellers et al., 1996).

In conclusion, elemental mercury may be converted to methylmercury, almost exclusively by sulfate reducing bacteria in anoxic sediments, and sometimes within

periphyton communities that support an active microbial sulfur cycle. The process of demethylation can be carried out by a wide variety of both aerobic and anaerobic microorganisms commonly found in sediments, or by indirect photolysis. Microbial demethylation, mostly developed as a resistance to mercury exposure in commonly contaminated environments, takes place in both oxic and anoxic sediments, either via reductive or oxidative demethylation pathways. The photodegradation of methylmercury takes place in the water column where PAR and UV radiation can penetrate (within approximately 6 and 1 meters, respectively), via the photolysis of aqueous free radicals which cleave the Hg-C bond in methylmercury.

# Literature Review

## Introduction

While decades of research have contributed to a relatively comprehensive scientific understanding of the mercury cycle and its behavior in aquatic systems, many practical questions remain. Understanding how mercury bioaccumulates in stream biota, for example, remains relatively understudied in most geographic regions, despite the global nature of mercury contamination in aquatic ecosystems. Furthermore, very few studies have examined the effects of timber harvest disturbance on mercury bioaccumulation in stream biota. The purpose of this literature review is to show the need for more research on the factors that influence mercury bioaccumulation in stream biota, while providing background information on the theoretical mechanisms behind this process, which may help elucidate results from this study.

The following review first discusses the two general mechanisms by which mercury concentrations in stream biota can become elevated. Next, it provides information on some of the natural watershed and stream characteristics that have been shown to be important drivers of the bioaccumulation of mercury in stream periphyton. Because there is a general lack of literature on this subject specifically, much of the research referenced throughout this section refers to processes associated with variation in stream water mercury concentrations and export, mercury accumulation in other stream biota such as invertebrates and fish, and mercury concentrations in lake periphyton. Although these measurements are not perfectly analogous to accumulation by stream periphyton, they serve as useful proxies. Next, the review will cover the existing literature on how logging disturbances alter the natural processes in watersheds and streams in ways that may influence the cycling and bioaccumulation of mercury in stream ecosystems. This relationship is particularly important, because the study sites are located in an area which is actively managed for forestry, with the explicit goal of maintaining the ecosystems ecological integrity. Lastly, I will address how this research project will fill gaps in the literature just discussed.

**Mechanisms of Altered Hg Cycling**

Mercury bioaccumulation in stream ecosystems can become elevated by two general mechanisms: Increased mobility and increased methylation potential. Increasing the mobility of mercury refers to the processes by which mercury bound in soils is flushed into surface waters more readily than usual. Processes that increase the solubility of soil bound mercury or increase the amount of particulates in surface waters generally

accomplish this. Increased mobilization of mercury leads to higher surface water concentrations, and therefore higher concentrations in the biota of those surface waters.

On the other hand, increasing the methylation potential in forest soils or surface waters refers to processes that increase the methylmercury to total mercury ratio (MeHg:THg) in the system as a whole, usually by increasing the activity of methylating bacteria. The methylation potential of a system is important, as MeHg is the form of mercury that bioaccumulates most readily. When the proportion of total mercury in a system shifts towards methylmercury, the biota in that system will uptake more of the existing mercury than they would otherwise.

**Natural factors known to influence mercury export and bioaccumulation in streams**

Although the study of mercury bioaccumulation in lake ecosystems is relatively comprehensive, there has been little research on mercury accumulation in stream ecosystems, particularly in stream periphyton (Bell and Scudder, 2005). For this reason, much of the literature referenced hereafter refers to factors that affect stream water mercury concentrations, other stream biota, and lake periphyton. Although the connection is weak due to a variety confounding variables that will be discussed, the mercury accumulated by stream periphyton is mostly derived from their surrounding surface waters (Bell and Scudder, 2007; Marvin-DiPasquale et al., 2009), so stream water Hg can be a useful proxy for stream periphyton Hg. It has also been clearly shown that accumulation of Hg by periphyton is the first step in the bioaccumulation of Hg in higher trophic levels (Bell and Scudder, 2005; 2007; Hill et al., 2011; Lowe and LaLiberte, 1996) due to intensive grazing by protozoa, benthic invertebrates, and fish (Farag et al.,

2007; Rhea et al., 2006). For these reasons, factors that have been shown to affect Hg concentrations in stream invertebrates and fish likely also affect Hg concentrations in stream periphyton. It should be noted that Hg in biota is consistently found to be almost entirely MeHg, so when referring to Hg in biota, it is usually assumed to be almost all MeHg (Ullrich et al., 2001).

Consequently, factors that increase the methylation potential in streams and surrounding forest soils are equally as important as factors that increase the delivery of total mercury to stream periphyton. The factors discussed throughout this section are generally thought to affect mercury accumulation in stream periphyton either by increasing the mobility and delivery of mercury bound in soils to streams, or by increasing methylation activity within the ecosystem.

First, the proposed mechanisms for the uptake of mercury by periphyton should be addressed. It is thought that periphyton either passively assimilate Hg from surface waters, actively assimilate it, adsorb Hg to their cell surfaces, or a combination of all three (Bell and Scudder, 2007). Some work shows that inorganic mercury and MeHg passively diffuse through the cellular membrane of diatoms at approximately the same rate, at which point the inorganic Hg binds to the cellular membrane, while the MeHg becomes associated within the soluble fraction of the cell (Moye et al., 1998; 2002). Watras et al. (1998) suggests that periphyton actively assimilate MeHg molecules because they come attached to a variety of beneficial ligands including organic carbon, chloride, sulfide, hydroxide and others. Finally, some suggest that algae and microbes in periphyton actively and passively assimilate inorganic Hg, at which point it is methylated

within the periphyton matrix, making it more likely to accumulate in microbes and algae (Cleckner et al., 1999).

*Wetlands*

Wetlands are widely considered to be the primary "hot spots" of mercury methylation within terrestrial ecosystems. They are known to retain inorganic Hg while acting as a source of MeHg (St. Louis et al., 1994). Due to favorable conditions for sulfate reducing bacteria (anoxic sediments), high concentrations of methyl mercury build up in wetlands, and can be flushed into low order streams during heavy precipitation events. This is generally understood to be the main source of methylmercury to streams, and therefore a major controlling factor of bioaccumulation rates. This relationship has been demonstrated in the literature. For example, Mierle and Ingram (1991) found that Hg export in small Ontario streams was highest in watersheds with wetlands. Likewise, in low order streams of western Maryland, Castro et al. (2007) found that brook trout from watersheds with wetlands (3-7% cover) had significantly higher mercury concentrations than brook trout from watersheds with no wetlands, and suggests that this is likely due to the Hg content of food sources in each stream. They found no correlation between brook trout Hg and stream water Hg, but their study was limited by just three water-sampling events. Finally, Munthe et al. (2001) added $^{199}$Hg isotopes to forest soil plots then measured isotope ratios of total and methylmercury, and found significantly higher methylation rates in wetlands soils as opposed to forest soils. It is important to note that rivers and stream channels are technically considered wetlands (Federal Geographic Data Committee, 2013), but do not necessarily have the

characteristics associated with mercury methylation, as do palustrine wetlands, which consist of swamps, bogs, and marshes.

While the correlation between wetlands and MeHg mobilization and bioaccumulation in stream ecosystems has been demonstrated, other processes are surely still important. Tsui et al. (2009) found that, in the absence of wetlands, the Hg concentrations in stream biota varied substantially due to variation in in-stream processes.

*Organic Matter*

Perhaps of most importance, and most commonly referenced in the literature, is the influence of organic matter in increasing both the mobilization and methylation of mercury. In a review of the interactions between mercury and dissolved organic matter, Ravichandran (2004) points out that DOC has been shown to be a major factor influencing mercury mobilization, toxicity, solubility, and speciation in watersheds. The mobilization of mercury is affected by DOC as mercury bonds strongly to ligands in DOC (Ravichandran, 2004). When this DOC is flushed into streams, it can be obtained by microbes in periphyton, increasing bioaccumulation. DOC also increases the solubility of mercury compounds such as mercuric sulfide, a highly insoluble solid, effectively increasing the amount of bioavailable mercury in the environment (Ravichandran, 2004). Finally, toxicity of mercury is affected by DOC because DOC can stimulate microbial activity in soils and aquatic sediments, including that of SRBs, which are responsible for most mercury methylation (Ravichandran, 2004).

DOC has also been shown to decrease pathways for mercury bioaccumulation through mobilization and methylation interactions. Complexation with DOC can limit the

amount of mercury available to methylation by microbes because DOC molecules are typically too large to cross the cell membranes of bacteria (Barkay et al., 1997; Kelly et al., 2003), therefore potentially limiting biotic methylation. Mercury speciation can be affected by DOC, as the transformation of oxidized mercury ($Hg^{II}$, the more reactive form) to elemental mercury ($Hg^0$, the less reactive form) is typically mediated by microorganisms, direct photolysis, or by humic substances, all of which are influenced by DOC, such that higher DOC concentrations result in higher rates of this transformation (Ravichandran, 2004). Once transformed, this mercury is more prone to evasion or redistribution in the environment via volatilization (Ravichandran, 2004). In other words, DOC increases the transformation of mercury from more reactive to less reactive species, which in turn results in less mercury methylation, and more mercury leaving the ecosystem without entering the food chain.

DOC appears to affect mercury mobilization and bioaccumulation in contrasting ways, but the correlations between DOC and Hg in stream water is well established. Some studies that have found significant positive correlations between DOC and Hg in runoff include Ekloff et al. (2012a; 2014), Porvari et al. (2003), and Skyllberg et al. (2009). Furthermore, Mierle and Ingram (1991) found that estimated Hg export was most closely correlated with water color, a rough measure of dissolved organic matter

*Sulfur*

As discussed, sulfate reducing bacteria are the primary methylators in most systems, and their metabolic activity is at least in part dependent on the abundance of available sulfate (King et al., 1999). Generally speaking, as long as organic matter is

abundant, levels of sulfur species have been found to have strong positive correlations to MeHg concentrations in aquatic environments (Paranjape & Hall, 2017).

For example, in a small boreal peatland, MeHg concentrations and %MeHg (percentage of total Hg as MeHg) in porewaters increased with atmospheric sulfate loading and declined when sulfate addition stopped (Coleman Wasik et al., 2012). Four years later, MeHg concentrations and ratios to total Hg were still higher than in control systems, even though mercury methylation decreased in the absence of sulfate addition (Coleman Wasik et al., 2012). In a different study, the addition of sulfate alone or sulfate and labile organic carbon to peat stimulated methylation, but the addition of labile organic carbon alone did not (Mitchell et al., 2008).

*pH*

The influence of pH on Hg mobilization and methylation is well documented. Many studies have demonstrated negative correlations between water column pH and Hg concentrations in lake biota (Ullrich et al., 2001). This relationship is mostly due to elevated methylation in acidic environments or lower bioaccumulation factors at high pH (Ullrich et al., 2001). It has also been demonstrated that the solubility and mobility of both THg and MeHg is dependent on pH, such that acidic waters tend to leach Hg from soils more readily than basic waters (Lee and Hultberg, 1990). Acidic waters facilitating the release of heavy metals from soils and sediments are commonly recorded in the literature (Ullrich et al., 2001). Moreover, in acidic envrionments, DOC is less negatively charged and therefore less likely to complex mercury species, resulting in more mercury being available to methylating bacteria (Barkay et al., 1997).

*Temperature*

Generally speaking, microbial activity increases with increasing temperature (Bisogni & Lawrence, 1975), which includes the activity of SRB (Ullrich et al., 2001). As mentioned in the introduction, demethylation is generally favored at cooler temperatures (Ullrich et al., 2001). Though water likely affects methylation rates, the literature does not report significant correlations between soil or stream temperature and mercury concentrations in stream water or biota. This suggests that other factors typically outweigh the effects of temperature.

*Productivity*

The effects of net primary productivity on mercury mobilization and methylation are somewhat uncertain. While some studies have shown positive correlations between Hg in lake biota and measures related to productivity, others have found the opposite relationship. This is likely due to growth dilution of Hg concentration in microorganisms. Garcia and Carignan (2000) found a positive correlation between Hg in lake biota and total N loading and light attenuation. Bell and Scudder (2007) found that both THg and MeHg in stream periphyton positively correlated with biomass, but leveled off as biomass reached approximately 100g/$m^2$. Pickhardt et al. (2002) found these same approximate results.

Finally, in a study of epiphytic communities in a large fluvial lake in Quebec, Hamelin et al. (2015) found Hg content to be negatively correlated to autotrophic index (the ratio between ash free dry weight and chlorophyll a content). In other words, this study found higher Hg concentrations when the fraction of algae in periphyton was

higher, suggesting that algae were actively taking up mercury. The authors say the reasoning for this relationship is unclear, but may be due to the algal production of small biogenic thiols, which have been shown to bind strongly to Hg, possibly resulting in higher THg concentrations in the thiol producing algae in periphyton (Hamelin et al., 2015). bioaccumulation

In contrast, shading of biofilms in a metal contaminated lab simulated stream resulted in decreased growth rates but 3X increased MeHg concentrations in periphyton (Hill & Larsen, 2005). The authors hypothesize that slow growth at the primary producer level likely contributes to higher biotic metal concentrations in cold, oligotrophic, or shaded ecosystems. These results come from a highly controlled lab experiment, so their ability to isolate the effects of primary production is high relative to field studies.

*Oxygen Availability*

It has been well established for several decades that anoxic environments are the primary location of mercury methylation, because sulfate reducing bacteria (SRB) are anaerobic (Sonke et al., 2014). Research continues to demonstrate the importance of anoxic environments for mercury methylation, especially those associated with sediments. There is, however, evidence that methylation occurs in oxic environments as well, but this research is limited to marine and tropical environments. Hintelmann et al. (2000) suggests that most methylation occurs at the geochemical interface between oxic and anoxic environments, suggesting that methylation is a process mediated by different microbial species working in tandem.

*Methylation in Stream Periphyton*

Some studies have identified methylation by periphyton as a major source of MeHg to aquatic systems. The relative importance of this source of MeHg is not well understood, but may be significant in systems with little or no other sources. For example, in low order streams in northern California, in-stream methylation accounted for a significant portion of MeHg in stream water (Tsui et al., 2009). The watersheds in this study, however, had no wetlands present. In contrast, Marvin-DiPasquale et al. (2009) found in-stream methylation to be relatively irrelevant when wetlands were present in the watershed.

Other studies to demonstrate significant methylation in periphyton include Olsen et al. (2016), which monitored methylation by stream periphyton in an industrially contaminated creek in Tennessee. The point source of Hg to this site, however, likely resulted in elevated methylation rates. Often cited in the literature as an example of methylation by periphyton, Cleckner et al. (1999) measured significant methylation by periphyton in the Florida Everglades. Other studies to demonstrate methylation in periphyton discussed in the background information section include Coelho-Souza et al. (2006), Acha et al. (2011), and Hamelin et al. (2015).

*Atmospheric Deposition*

Varying magnitudes of atmospheric deposition of Hg seems to be a logical explanation for differing Hg levels in surface waters and biota, but several studies have demonstrated that this relationship only exists at large spatial scales. For example, Hammerschmidt and Fitzgerald (2005) showed that between Florida, California,

Michigan, and Alaska, the amount of Hg in adult mosquitos was positively correlated with Hg deposition. Subsequently, Hammerschmidt and Fitzgerald (2006) found similar results when comparing largemouth bass from 25 states and each state's wet deposition of Hg. Locally, mercury in fish tissue from 30 lakes across Washington State were positively correlated with watershed precipitation, but these differences were largely between eastern and western Washington, with the west side getting much more precipitation and having higher fish Hg concentrations (Mathieu et al., 2013).

In contrast, at spatial scales of just several kilometers, variability in atmospheric deposition has been shown to be unconnected to variation in Hg in surface waters and soils. Munthe and Hultberg (2004) measured total and methylmercury runoff from a forested catchment in Sweden with a plastic roof erected over the entire catchment to eliminate wet deposition of Hg. Results found that, based on 10 years worth of data, no significant change in THg or MeHg flux occurred after erection of the roof. This suggests that the release of mercury from forest soils to aquatic systems is controlled by factors other than wet deposition input, and that mercury likely spends years to decades in terrestrial landscapes before entering aquatic systems. As a local example, atmospheric deposition records could not explain variations in sediment Hg in Lake Ozette sediment cores (Furl et al., 2010). The reason for this disconnect between atmospheric deposition and Hg in aquatic systems appears to be the relatively long term storage of mercury in soils and vegetation of terrestrial landscapes. Researchers estimated that over 80% of wet deposited Hg in a NW Ontario watershed accumulates in soils and vegetation before entering surface waters (Allan & Heyes, 1998). On the same note, Munthe et al. (2001) found that, using stable Hg isotopes, 30% of Hg added to soils was retained over long

time-periods. In a SW Sweden catchment, throughfall and litterfall was estimated to contribute 3X more Hg to surface waters than wet deposition (Hultberg et al., 1995), suggesting the high retention of Hg in vegetation.

*Summary*

The factors considered above, whether directly or indirectly, have been shown to influence certain aspects of mercury cycling and accumulation in aquatic biota. Wetlands, organic matter, suspended sediment, pH, temperature, productivity, oxygen availability, and in stream methylation all influence either the mobility of soil bound mercury, the methylation potential of the system, or both.

So where does logging activity fit into this discussion? Logically, we can assume that activities which alter the natural processes just discussed will in turn alter the cycling and bioaccumulation of mercury. The next section discusses the demonstrated linkages between logging disturbance and alterations of the mercury cycle and some of the theoretical mechanisms behind this relationship.

**The Effects of logging on mercury cycling**

The following section briefly reviews literature on the effects of logging disturbance on mercury cycling and bioaccumulation. As mentioned in the introduction to this literature review, the interaction between timber harvest and mercury cycling is especially important to this study because the study site is designated with the objective of integrating revenue production in the form of timber harvest, with ecological values. As will be discussed, forestry operations have been shown to influence mercury concentrations via two main pathways. Throughout the following section I will present

the prominent theoretical mechanisms of these pathways, then walk through some examples of how timber harvest alters the export of Hg from forested watersheds and effects the biota in these systems.

*Mechanisms of Timber Harvest-Mercury Relationship*

While the previous section discussed the linkages between timber harvest disturbances and elevated mercury concentrations in surface waters and lake biota, the following section briefly discusses the mechanisms behind this relationship. As mentioned previously, these mechanisms can be categorized as affecting either the mobility or methylation of mercury. Generally speaking, landscape alterations such as forestry operations can influence both carbon processing and catchment hydrology (Schelker et al., 2012), both of which can impact Hg mobilization and methylation (Bishop et al., 2009).

*Mercury Mobility*

One of the most important ways that logging disturbances influence mercury mobility is by altering the hydrologic cycle of affected catchments, which is closely tied with the mercury cycle in a number of ways (Bosch & Hewlett, 1982). Research has clearly shown that the removal of vegetation leads to decreased water loss via evapotranspiration (Bosch & Hewlett, 1982), which often leads to rising of the water table (Ekloff et al., 2014, Sorenson et al., 2009b). One result of an elevated water table is an increased overall delivery of terrestrial Hg and MeHg to streams simply due to increased overall runoff (Ekloff et al., 2014, Porvari et al., 2003, Sorenson et al., 2009a). Furthermore, the combination of an elevated water table and increased runoff results in

more superficial flowpaths through the upper organic, Hg-rich soil horizons, resulting in elevated organic matter bound Hg in streams (Shanley & Bishop, 2012).

An elevated water table can also affect mercury mobility through chemical processes. After logging, large amounts of carbon in the form of logging slash and decaying roots are left on site, and become saturated more frequently due to the elevated water table, resulting in enhanced mobilization of DOM (Bishop et al., 2009). An elevated water table also generally results in a shift from aerobic to more anaerobic conditions in forest soils due to microbial degradation of newly added organic matter (Bishop et al., 2009). As anaerobic conditions develop, the formation of inorganic sulfides becomes more likely (Skyllberg et al., 2003). As mentioned previously, Hg and MeHg bind strongly to both inorganic sulfides and thiols associated with DOM. Consequently, timber harvest operations usually promote the mobilization of mercury species from soils to surface waters in the form of thiols or inorganic sulfide complexes.

DOC or some other measure of organic material are the only variables that consistently correlate positively with Hg concentration and export in the studies on logging disturbance and mercury cycling reviewed here (Desrosiers et al., 2006; Eklof et al., 2012; 2014; Garcia & Carignan, 1999; 2000; Porvari et al., 2003, Skyllberg et al., 2009, Sorenson et al., 2009b).

Lastly, logging disturbances are known to increase erosion and sediment delivery to streams (Chamberlin et al., 19991). The removal of vegetation and disturbance due to heavy machinery both contribute to increased sediment transport (Kozlowski, 1999), which has been shown to contribute significant amounts of particulate bound mercury to

stream ecosystems (Ekloff et al., 2014). However, when riparian buffers are left around streams, this impact is minimized.

*Mercury Methylation*

Logging disturbances have also been shown to affect mercury cycling by increasing the methylation potential in forest soils, thereby increasing the fraction of THg as MeHg. As just discussed, the removal of vegetation decreases evapotranspiration, resulting in an elevated water table, which typically leads to the development of anaerobic conditions in soil. Sulfate reducing bacteria (SRB), the main pathway for mercury methylation, thrive in anaerobic soils, so recently harvested catchments typically have high methylation potential. Increased methylation potential can also occur as a result of logging machinery depressing soils where standing water accumulates, creating "methylation hotspots" resembling wetlands (Bishop et al., 2009). Munthe and Hultberg (2004) observed this process, measuring elevated MeHg concentrations coming from a pool created by logging machinery. Depressions in the soil combined with an elevated water table make the creation of "methylation hotspots" even more likely. As discussed previously, the excess of fresh organic matter supplied by logging debris can increase methylation rates by supplying electron donors to methylating bacteria.

Lastly, it is well established that logging disturbance can increase stream and soil temperatures. Even under best management practices, timber harvest can increase stream temperatures (Macdonald et al., 2003). Similarly, Kiffney et al. (2003) found that stream temperature increased with decreasing riparian buffer width. More importantly than changes in stream temperature, logging has been shown to increase average soil

temperatures (Aust & Lee, 1991). As mentioned, increased temperatures in surface waters or soils results in elevated SRB activity (Ullrich et al., 2001).

*Global Examples*

The vast majority of research on this subject has been conducted in Scandinavia due to alarmingly elevated Hg in fish tissue concentrations across the region (Bishop et al., 2009). Several of these studies showed that both the removal of trees and preparation for the next logging cycle contribute to this relationship.

First, Porvari et al. (2003) observed increased runoff of both THg and MeHg, and increased MeHg concentrations into the third year after clear cutting, suggesting the relatively long lasting effects of the disturbance. After an initial increase during the first year, THg and TOC declined, suggesting that methylation activity in the watershed remained elevated for at least three years (Porvari et al., 2003). Subsequently, Munthe and Hultberg (2004) observed a temporary spike in MeHg runoff in response to disturbance of the study stream and surrounding soils by logging machinery. Then, Skyllberg et al. (2009) observed that in first order streams with 0-4, 4-10, and >70 year old clear-cuts, methyl and elemental mercury concentrations were both significantly elevated in the more recent clear cuts. Additionally, Eklof et al. (2012) monitored runoff in streams from 54 logged catchments all over Sweden, which had been subject to either site preparation (soil tilling and re-planting after harvest) or no treatment after timber harvest. When pooled, the two treatments were positively correlated with elevated THg and MeHg concentrations. The authors from these studies speculate that THg concentrations were elevated due to increased erosion, organic matter loading, and runoff

in response to logging, and that MeHg concentrations were elevated due to increased methylation potential in the watersheds, also in response logging. Other studies from this region with similar results, in which elevated mercury concentrations were observed in response to timber harvest include Sorenson et al. (2009b) and Eklof et al. (2014).

*Local Examples*

In 2007, the Washington State Department of Ecology (DOE) found "unprecedented" Hg concentrations in fish from Lake Ozette and Lake Dickey, two remote coastal lakes on the Olympic Peninsula (Furl et al., 2010). In assessing the sources and pathways of Hg to these two lakes, Furl et al. (2010) found that wet deposition and historic atmospheric monitoring data did not suggest elevated local or regional Hg deposition. However, sediment core data from both lakes showed elevated sediment, THg, and MeHg inputs coinciding with historic logging disturbances in each watershed. In other words, at the time of timber harvest, the lakes received increased fluxes of THg and MeHg, likely due to both increased mobility and methylation of mercury in the catchment.

Furl and Meredith (2010) then collected water samples from three streams with varying degrees of logging disturbance in and adjacent to the Lake Ozette watershed to see if logging affected mercury fluxes in the streams. THg concentration was highest in the reference stream (no logging) and elevated in all three streams when compared to a nationwide survey of 236 rivers and streams, whereas MeHg concentrations were highest in one of the treatment sites (Furl & Meredith, 2010). This same treatment site also had the highest median THg and MeHg fluxes, and yearly THg flux estimates were elevated

when compared to values found in the literature. The highest MeHg concentration recorded was collected from the other treatment site. Because THg concentrations were highest in the reference stream, but MeHg concentrations were highest in the treatment streams, these results suggest that logging in the treatment basins increased overall methylation potential and the fraction of THg as MeHg in surface waters. So, Furl et al. (2010) concluded that historic logging events in the basins surrounding Lake Ozette resulted in elevated sedimentation, as well as THg and MeHg loading, while Furl and Meredith (2010) found that streams surrounding Lake Ozette with logging in their basins had elevated MeHg concentrations, likely due to elevated methylation potential in the basins in response to logging.

In a February, 2018 study, Eckley et al. (2018) sought to evaluate how contemporary forestry operations influence mercury cycling in coastal, mountainous regions of the Pacific Northwest. After sampling eight headwater streams of the Trask River Watershed Study area in northwestern Oregon, the authors found that the harvested catchments had 42% higher discharge, 28% higher filtered Hg concentrations, 80% higher filtered Hg loads, and 40% higher DOC loads (Eckley et al., 2018). Particulate bound mercury (PHg) was not elevated in the harvested catchments, likely due to forestry practices aimed at minimizing erosion, and MeHg concentrations were not elevated in the harvested catchments due to steep slopes and well drained soils, which do not support the anoxic conditions necessary for mercury methylation (Eckley et al., 2018). Importantly, the harvested catchments were harvested using Best Management Practices (BMPs) rather than complete clear cuts, which has been the case for many other studies of this nature. The authors speculate that the logging activity resulted in elevated runoff due to

the loss of vegetation (which reduces evapotranspiration and water retention), and elevated DOC loading from the addition of slash and trimmings (Eckley et al., 2018). This combination of higher runoff and DOC loading likely resulted in the elevated THg loads observed.

*The Effects on Biota*

While the previous section demonstrated how logging disturbances can increase mercury concentrations and export in streams, this section covers the literature on the effects of logging on mercury accumulation in lake biota. Garcia and Carignan performed a series of studies comparing Hg levels in zooplankton (1999), northern pike (2000), and a variety of piscivorous fish (2005) from a set of drainage lakes with clear-cut, burnt, or undisturbed catchments. Zooplankton from lakes with recently logged watersheds had slightly higher MeHg concentrations than burnt or undisturbed watersheds (Garcia & Carignan, 1999), and in their next study, Hg in pike from the same lakes again had significantly higher concentrations in logged watersheds (Garcia & Carignan, 2000). Next, the mercury concentrations in a variety of piscivorous fish from the same lakes correlated positively with the ratio of clear-cut to lake area ratio (Garcia & Carignan, 2005). Finally, Desrosiers et al. (2006b) found lake periphyton to have elevated Hg concentrations in response to logging

**Mercury in Periphyton**

Not only have very few studies measured mercury concentrations in stream and river periphyton, those that have mostly study rivers with point sources of mercury pollution such as chlor-alkali plant effluent or mining operations (Dranguet et al., 2017; Hill and Larsen, 2005; Zizek et al., 2011). While these systems are likely at a much higher risk of elevated mercury concentrations in their food webs, they do not represent ambient concentrations in systems such as those on the Olympic Peninsula. As discussed, atmospheric deposition alone is enough of a source to result in elevated mercury concentrations even in remote regions of the globe.

At the time of writing this, it appears that the only peer reviewed research to measure mercury concentrations in stream periphyton without a point source of mercury was initiated by the United States Geological Survey (USGS) in 2003. This study monitored mercury concentrations in stream water, sediments, periphyton, macroinvertebrates, and fish. For the periphyton portion of the study, 2-3 sites were selected in the Willamette Basin in Oregon, the Western Lake Michigan Drainages in Wisconsin, and the Georgia-Florida Coastal Drainages in Florida. Results showed total mercury concentrations in periphyton ranged from 38.13 to 257,000 nanograms per square meter, or 3.62 to 12,080 nanograms per gram dry weight, with the forested sites in Oregon occupying the low end of that range (Bell and Scudder, 2004). It should be noted that these samples were collected on a variety of natural substrates (sediments, rock, or wood), which may account for some of the variation found. For reference, Hill and Larsen (2005) measured mercury concentrations in periphyton in a lab simulated, mercury contaminated stream, and found that after just 48 hours of exposure,

concentrations reached approximately 2,500 ng/g. The periphyton in this study were grown on artificial substrates in an uncontaminated, natural stream, and transferred to the lab simulated stream from just 48 hours. Likewise, Dranguet et al. (2017) measured mercury concentrations in periphyton on artificial substrates in several reservoirs in Romania contaminated with mercury from chlor-alkali plant effluents, and found concentrations of just 200 ng/g.

Bell and Scudder (2007) also found a strong positive correlation between total mercury and methylmercury in periphyton, suggesting that the percentage of methylmercury is relatively consistent across samples. These results imply that total mercury concentrations among samples can be used to estimate relative methylmercury concentrations across samples as well. Results also showed that both total and methylmercury had strong positive correlations to periphyton biomass, but this relationship eventually leveled off (Bell and Scudder, 2007), suggesting a dilution of Hg concentrations with Periphyton growth. These findings are consistent with Hill and Larsen (2005), which found that shading of periphyton in a metal contaminated stream resulted in decreased growth, but a 3X increase in concentrations of twelve metals including methylmercury. Likewise, Pickhardt et al. (2002) found that in a mesocosm experiment, as suspended algae abundance increased the mercury burden per cell decreased, resulting in a growth dilution.

**Where this research fits into the existing literature**

Although the literature discussed above demonstrates a strong understanding of the mercury cycle in aquatic systems, there is room for further research. The existing

literature rarely addresses the bioaccumulation of mercury in stream biota in response to changes in stream chemistry, it mostly takes place in regions very different from the Pacific Northwest, and it generally does not consider timber harvest techniques other than clear-cutting. For these reasons, this research project may help fill several gaps in the literature.

Very few detailed studies have examined the bioaccumulation of mercury in the periphyton of stream and river ecosystems, despite their importance in the overall mercury cycle (Bell and Scudder, 2007). Much of the research just discussed demonstrates that stream chemistry can be impacted by watershed disturbances, but the effects on the biota of these systems remains less well understood. Furthermore, while piscivorous lake fish are frequently monitored for Hg concentrations due to their high trophic level and potential for Hg bioaccumulation, streams such as those monitored in this study are the pathways for mercury that ultimately ends up in these fish. If we can gain a better understanding of how mercury bioaccumulates in stream food webs, we can better predict how it accumulates in downstream systems.

Secondly, there has been very little mercury bioaccumulation research specific to the Pacific Northwest. Not only does this region receive some of the highest mercury deposition loads in North America (NADP, MDN), it has an intensive history of logging, and the topography, vegetation, and climatic characteristics are unique to this region (Eagles-Smith et al., 2016). . The high amount of environmental variability in this region, and on the Olympic Peninsula specifically, provides a valuable opportunity to explore the various factors that influence mercury cycling.

Lastly, the timber harvesting methods in the existing literature are not consistent with contemporary methods in this region. Forest practices have evolved over the last several decades in order to reduce their environmental impacts on the land and surface waters. In most of the literature just discussed, clear-cutting large swaths of forest was the form of timber harvest disturbance investigated. In the Olympic Experimental State Forest and elsewhere in the Pacific Northwest, Best Management Practices (BMPs) such as leaving riparian buffers around streams, leaving a certain amount of standing trees and snags per acre, and leaving woody debris on-site are common. Some of these practices are aimed specifically at reducing environmental impacts such as changes in hydrology, erosion, and stream temperature, all of which may in turn influence mercury cycling in these systems. Furthermore, many of the studies on timber harvest-mercury cycle relations took place in areas that are mostly flat and harvested during periods of snow cover, while the study sites in this research are defined by mountain topography, with harvest usually occurring during periods of no snow, often on relatively steep terrain. This research will therefore contribute to a very small body of literature examining how low impact forestry operations affect mercury cycling and bioaccumulation.

# Methods

## Site Description

The study area is located in the Olympic Experimental State Forest (OESF) on the Olympic Peninsula of Washington State (Figure 4). Due to the maritime climate, snowfall in lower elevations is rare, and stream flows are mostly rain-dominated throughout the year (Minkova et al., In publication). Steep erodible terrain and heavy precipitation (200-457 cm per year) in most basins result in very high stream densities (~3.6 km/km$^2$ of mapped streams), large deposits of rocks and woody debris, and numerous wetlands (Minkova et al., In publication).

The primary designation of OESF is to facilitate research on integrating revenue production, mostly in the form of timber harvest, with ecological values, such as habitat conservation. There are 54 basins within OESF that are actively managed and monitored by The Washington State Department of Natural Resources (DNR) as part of the Status and Trends Monitoring of Riparian and Aquatic Habitat project. Within each basin is a predetermined and marked sample reach where DNR collects riparian and aquatic habitat data (Figure 5). This project utilized the same sample reaches as DNR in order to maintain data comparability. All sampling was conducted between April 1 and May 13, 2018.

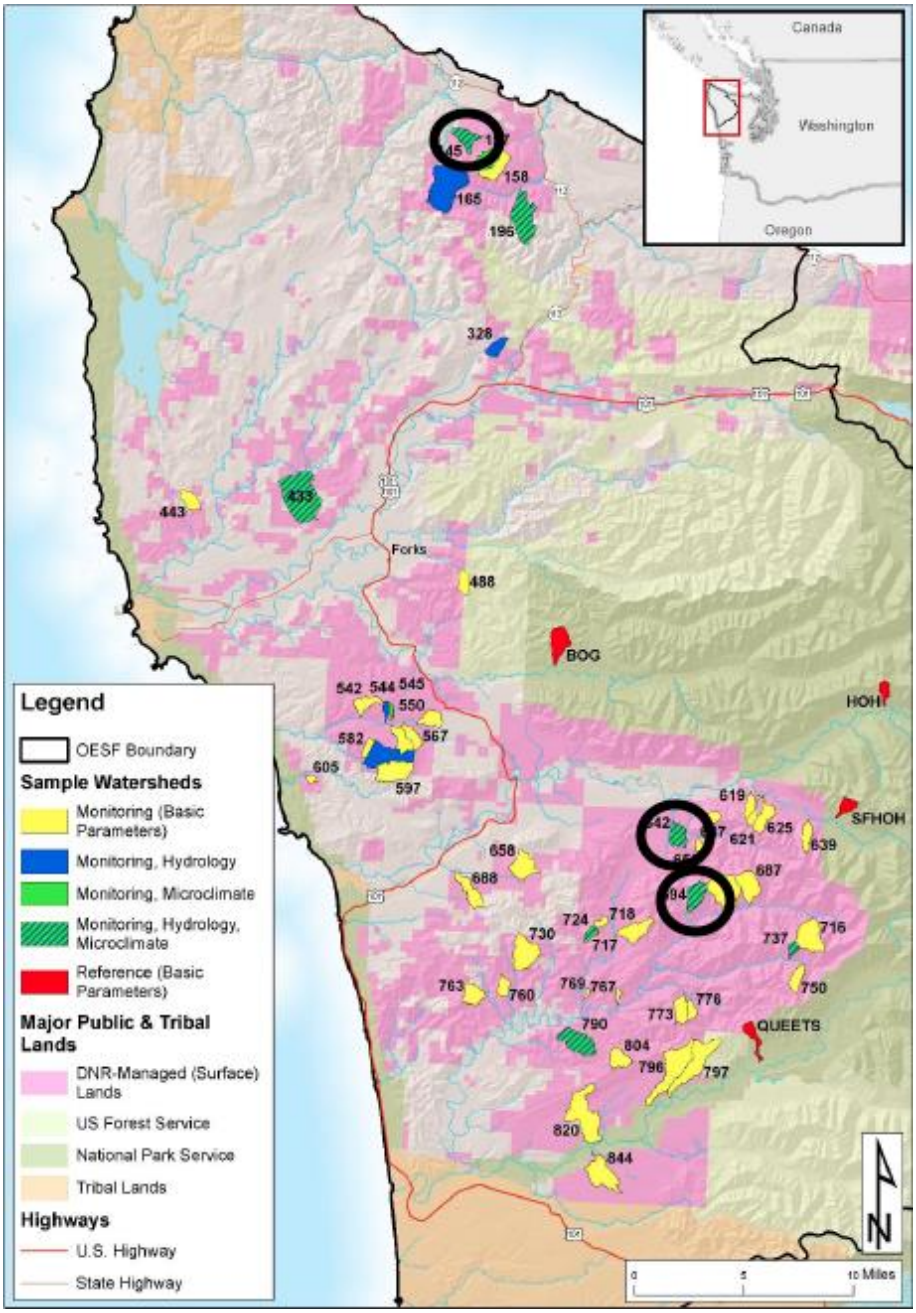


Figure 4. The 54 monitoring basins of the Olympic Experimental State Forest. The circled basins (145, 642, and 694) were used in this study.

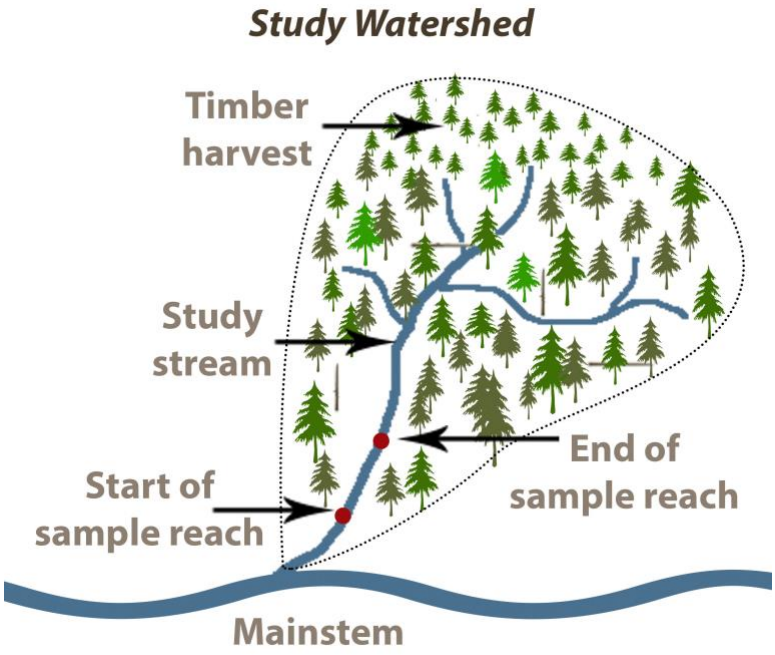


Figure 5. Study basin (watershed) and stream schematic within OESF. Within each study basin is a sample reach (section of stream), which is where all stream data for each site was collected.

Study sites within the OESF were selected based on current DNR monitoring programs, and to maximize the variability of several key variables within each basin in order to more accurately represent the entire OESF. Of the 54 basins within the OESF, three were selected to maximize the variability in important catchment scale variables such as logging disturbance history (includes time since last harvest, type of harvest, and percentage of the watershed harvested), wetland area, and median slope (ranging from 5-54%). These specific factors were selected because prior research has identified them as potentially important in influencing the cycling of mercury in forested catchments.

When discussing timber harvest, the two general methods commonly used include thinning and variable retention harvest (VRH). Thinning refers to the process of

removing small, weak, or low value (oddly shaped) trees, in order to allow the remaining trees to grow faster and stronger, resulting in a more valuable crop later on. VRH generally refers to the process of harvesting timber in ways that retain some forest structural elements, such as leaving riparian buffers, snags, and a set amount of standing trees per acre, in order to retain some of the ecological functions of the forest. So, a plot could be thinned, then harvested 15 years later, causing the same plot to be "harvested" twice over that time period.

The three study sites selected include basins 145, 642, and 694, shown in Figure 4. Basin 145 has an area of 1.82 $km^2$, a median slope of 16%, and lithology of 39% glacial deposits and 61% tertiary sediment. In terms of logging disturbances, basin 145 has undergone variable retention harvest in 24.5% of the basin's area since 2015, and an additional 29.6% of the basin undergoing some type of timber harvest (VRH or thinning) from 1999-2015. Lastly, basin 145 has an estimated total wetland area of just 7.56 acres, all of which are riverine. As discussed in the literature review, riverine wetlands are not necessarily conducive to mercury methylation, as are other, more anoxic wetlands.

Next, basin 642 has an area of 1.79 $km^2$ and a median slope of just 5%. The lithology of this catchment is 67.5% of glacial deposits and 32.5% tertiary sediment. The logging disturbance history of basin 642 includes 27.4% thinning and 33.2% VRH from 2015-2017, and 41.2% undergoing some type of timber harvest between 1999 and 2015, making this basin the most frequently and drastically disturbed by forestry operations. Importantly, this basin has an estimated total wetland area of 61.43 acres, almost twice as much as basin 694, and 8 times that of basin 145. Of these, 2.47 acres are considered palustrine (associated with swamps, marshes, or bogs). This is especially important,

because these types of wetlands are more anoxic than riverine wetlands, and more likely to be a significant source of methylmercury.

Finally, basin 694 is 2.14 $km^2$, has a median slope of 54%, and is estimated to be 100% tertiary sediment. No timber harvest has taken place within basin 694 for at least 25 years, making it the least "disturbed" site in this respect. This basin has an estimated 33.12 acres of wetlands, all of which are riverine.

| | | | | | Timber Harvest (% area of watershed) | | |
|---|---|---|---|---|---|---|---|
| **Watershed** | **Watershed area (Acres)** | **Total wetland area (Acres)** | **Non-riverine wetland area (Acres)** | **Median slope (%)** | **Completed 1999-2015** | **VRH 2015-2017** | **Thin 2015-2017** |
| 145 | 450 | 7.56 | 0 | 16 | 29.6 | 24.5 | 8.8 |
| 642 | 442 | 61.43 | 2.47 | 5 | 41.2 | 33.2 | 27.4 |
| 694 | 529 | 33.12 | 0 | 54 | 0.0 | 0 | 0 |

Table 1. Watershed characteristics for each site.

## Study Design

This study measured total mercury concentrations in periphyton from three watersheds in the OESF in order to gain an understanding of ambient concentrations in these biota, and to potentially identify stream or watershed scale variables associated with elevated concentrations. Periphyton samples were collected and analyzed for total mercury, as well as for chlorophyll a and total carbon concentration in order to see if overall growth and autotrophic index were different among basins, and could explain any differences in mercury concentrations.

Stream data including dissolved organic carbon (DOC), pH, temperature, conductivity, and dissolved oxygen were also monitored in order to assess differences between watersheds and to potentially explain differences in the periphyton data.

Periphyton samples were colonized on periphytometers (Figure 6; described below) for periods of approximately 3 and 6 weeks between April 1 and May 13, 2018. Periphytometers are artificial substrate installations, in this case constructed of glass tiles enclosed in polypropylene netting with zip ties. Glass tiles, as opposed to plastic or clay, were chosen as the artificial substrate to more thoroughly eliminate potential mercury contamination, as glass can be much more effectively cleaned of trace metals than plastic or clay. Artificial substrates were chosen because they are generally preferred for periphyton sampling in comparison-based studies such as this one in order to minimize differences in periphyton communities due to substrate variability inherent with natural substrates. Periphyton communities on natural substrates are also subject to scour during high flow events, resulting in uneven distribution throughout individual stream reaches.

Colonizing periphyton using artificial substrates helps to avoid these problems over short time periods by providing uniform colonization time, material, texture, and size (Cattaneo & Amireault, 1992).

Figure 6. A periphytometer installed in the streambed.

Each stream was sampled using four periphytometers, each with 1 $ft^2$ surface area, for a total of 4 $ft^2$ per stream. At each stream site, one periphytometer was installed in a pool (maximum depth at least 1.5X the crest depth), while the remaining three were randomly installed in riffles so that periphyton samples more accurately represented average conditions within each stream, which were mostly dominated by riffles. This also allowed comparisons between the two environments. After 3 weeks in the stream, two of

the periphytometers from basin 642 were removed for analysis and replaced. Site 642 was used for the 3 week sampling periods because it had sufficient amounts of periphyton growth after the first 3 weeks, unlike sites 145 and 694. This allowed comparison between periphyton colonized for 3 weeks and 6 weeks, and between periphyton colonized for the first 3 and second 3 weeks of the study period. At all sites, in-situ stream chemistry measurements and DOC grab samples were taken on April 1st, April 15th, April 24th, and May 13th. Samples and measurements were taken in a straight section with a relatively uniform flow in each sample reach.

**Data Collection**

*Clean Handling Techniques for Carbon and Mercury*

In order to ensure accurate sampling, the following clean handling techniques were applied for all data collection. All glassware to come in contact with samples for carbon analysis was pre-combusted at 500 ˚C for 4 hours. All plastic and glassware (including periphytometers) to come in contact with periphyton samples for mercury analysis or water samples for DOC analysis was soaked in a 10% HCl acid bath for at least 12 hours, triple rinsed with DI water, and stored in clean plastic bags until contact with samples.

*Stream Chemistry*

In situ water chemistry measurements included pH, temperature, conductivity, and dissolved oxygen. pH was measured using an *Oakton pH5+*, and conductivity and dissolved oxygen were measured using a *YSI Pro2030*. Temperature was monitored continuously by DNR. In situ measurements were completed upon arrival and just before

leaving each stream on each sampling day by averaging 5 individual measurements taken approximately 10 seconds apart.

Two DOC water samples were collected, once upon arrival and once just before leaving each stream on each sampling day, and stored on ice until lab processing. In the lab, samples were filtered through pre-combusted 0.7 um GF/F filters to remove fine and course particulate carbon. Filtered samples were frozen and stored on ice until DOC analysis by the University of Washington College of Forest Resources Analytical Services. DOC analysis was performed using a *Shimadzu Online TOC-V Analyzer*. The two DOC samples collected on each day were subsequently averaged.

*Periphyton Sample Collection*

Periphytometers were secured to the streambed using rebar. Upon removal, tiles were placed in clean zip-lock bags, placed in black garbage bags to avoid light exposure, and stored on ice until lab processing, which occurred within 48 hours (Is this true??). In the lab, periphyton was scrubbed from the tiles with a toothbrush, and rinsed with DI water into glass sample jars with PTFE (Teflon) sealed lids. Toothbrushes were acid washed and replaced between samples from different sites. Two tiles worth of periphyton sample from each periphytometer was composited to form a slurry, from which aliquots were taken for mercury, chlorophyll a, and carbon analyses.

*Periphyton Sample Analysis*

To measure total mercury, aliquots of periphyton sample were filtered onto pre-combusted (800° C) Whatman quartz microfiber filters, frozen at -80 °C,freeze dried, and analyzed by direct thermal decomposition using a *Nippon Instrupements Corporation MA*

*3000* mercury analyzer. To measure chlorophyll a, periphyton sample aliquots were filtered onto GF/F filters, sonicated in 90% acetone for 30 minutes, soaked for 2-24 hours at approximately 4 °C, centrifuged for 20 minutes at 500g, and measured for absorbance via spectrophotometry (Lock et al., 1984; Steinman & Lamberti, 1996; Summer & McIntire, 1982; Wetzel, 1983). To measure percent carbon, sample aliquots were placed in pre-combusted glass petri dishes, dried in a drying oven at 60 °C until no longer changing weight, ground to a powder, loaded into precombusted tin capsules, and analyzed for total carbon concentration using a *PerkinElmer Series II CHNS/O Analyzer 2400.*Sample weights ranged from 1.91-3.36 mg.

Periphyton samples were then compared based on the area of substrate used to collect each sample. The concentrations of each parameter of interest (mercury, chlorophyll a, carbon) were multiplied by the volume of sample filtered and divided by the area of sampled substrate, resulting in an areal burden (weight/area) for each sample.

*Watershed Data*

All watershed scale data used for site selection and comparison purposes was compiled and provided by DNR. This data includes basin characteristics such as logging history, geologic composition, average slope, aspect, and wetland area. Most watershed scale data comes from remote sensing and operational records. For a description of methods used to collect stream and habitat data see Minkova and Foster (2017). Wetland area data comes from the United States Fish and Wildlife Service National Wetlands Inventory.

*Statistical Analysis*

Due to the exploratory nature of this methodology and some study design issues, many of the samples analyzed for mercury were not large enough to get a reliable reading of their mercury content. Specifically, many samples had much lower mass than anticipated, and ended up having less Hg than the lowest standard used in the original standard curve (<0.5 ng Hg). This caused these samples to be too low in Hg to be accurately quantified. To address this issue, a new standard curve was created using standards that bracket all samples analyzed originally (0.05, 0.1, 0.5, 1,0, and 2.0 ng). This standard curve was created on August 29$^{th}$, approximately 2 months after the original sample analysis. This new, lower standard curve was applied to all data points that were originally lower in Hg than the lowest standard in the original curve (0.5 ng Hg). The variation in Hg readings obtained using the original and new standard curves ranged from approximately 3-22%. The new standard curve was not applied to the samples that had over 0.5 ng Hg in the original analysis, because these samples can be considered quantifiably accurate. Because the new, lower standard curve was created several months after the samples were analyzed, there is less certainty regarding the accuracy of their analysis.

All statistical analyses and graphs were completed using RStudio version 1.0.153. One way ANOVAs were performed to test for significant differences between sites for all measured and calculated parameters. When significant differences were detected, Tukey's Honest Significant Difference tests were performed to determine which groups were significantly different and to determine p values.

## Results

All data was grouped by sampling location (Site 145, 642, or 694) to test for significant differences between sites. Significant differences refer to values of $p < 0.05$, unless otherwise noted.

### Periphyton

*Mercury in Periphyton*

Table 2 in the Appendix lists mercury, carbon, and chlorophyll values for all samples analyzed, expressed in mass/area, or areal burden. Samples with initial Hg readings under 0.5 ng Hg, which had the new standard curve applied to them, are shaded in grey. Samples with over 0.5 ng Hg are unshaded. In the site column, 642F and 642S refer to samples colonized and collected during the first and second three-week periods, respectively.

Considering just the 9 samples with over 0.5 ng Hg from the original analysis, site 145 had two samples with concentrations of 108.92 and 128.47 ng/m$^2$. The remaining samples were all from site 642, either from the first or second 3 week sampling period, but not the 6 week sampling period. These samples ranged from 414.00-736.48 ng/m$^2$ and 243.38-430.54 ng/m$^2$ from the first and second 3 week sampling periods, respectively.

When considering all data points, site 145 mercury areal burdens ranged from 49.87-150.81 ng/m$^2$, averaging 113.03 (s=29.8). At site 642, during the 6 week sampling period, concentrations ranged from 116.70-197.93 ng/m$^2$, averaging 161.33 (s=32.0). At

694, concentrations ranged from 23.74-54.61, averaging 39.58 (s=10.2). Mercury areal burdens at site 642 were significantly higher (F=31.73) than sites 145 (p=0.0258) and 694 (p=0.0000035; Figure 7).

At site 642, during the first 3 week sampling period, mercury areal burdens ranged from 78.09-736 ng/$m^2$, averaging 308.06 (s=239.5). During the second 3 week sampling period, burdens ranged from 243.48-430.54 ng/$m^2$, averaging 322.21 (s=67.8). Mercury areal burdens were not significantly different between the two 3 week sampling periods (Figure 8).

When considering differences between periphyton samples collected from pools and riffles, mercury concentrations from pools averaged 292.4 ng/$m^2$ (s=242.9), while samples from riffles averaged 123.0 ng/$m^2$ (s=72.9). Mercury areal burdens in pools were significantly higher than in riffles (p=0.0080, t=8.10; Figure 9).

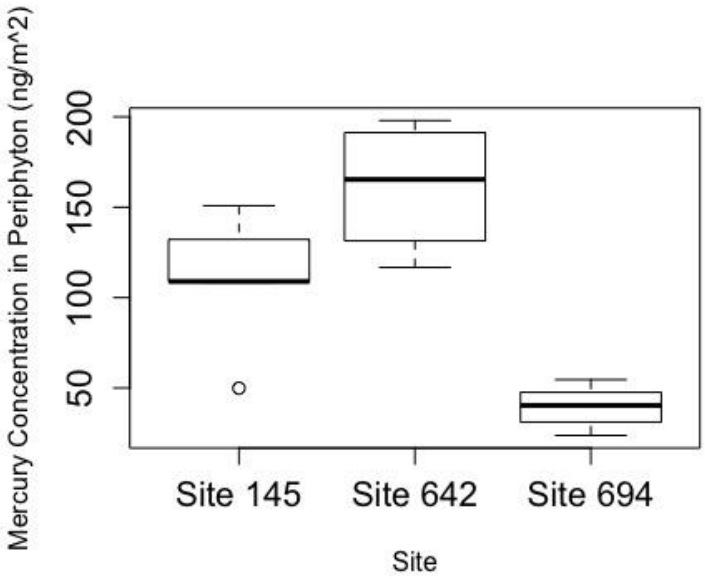


Figure 7. Mercury areal burdens at each site, expressed in ng/$m^2$

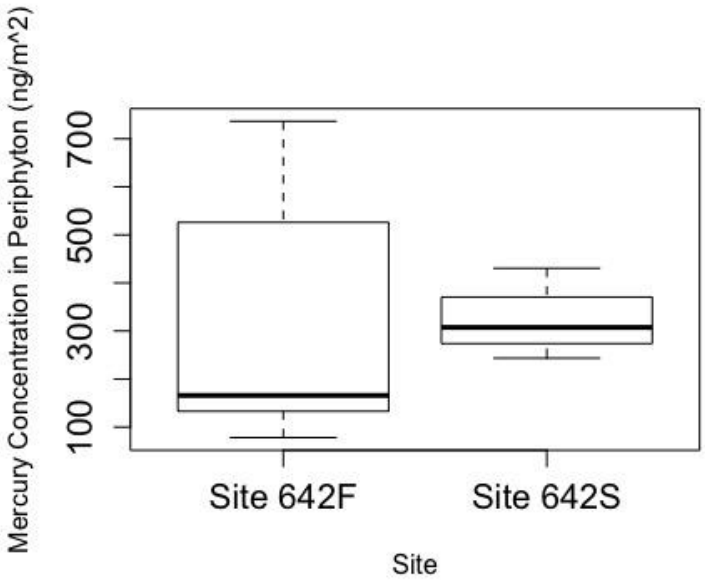


Figure 8. Mercury areal burdens during each of the two 3 week sampling periods at site 642.

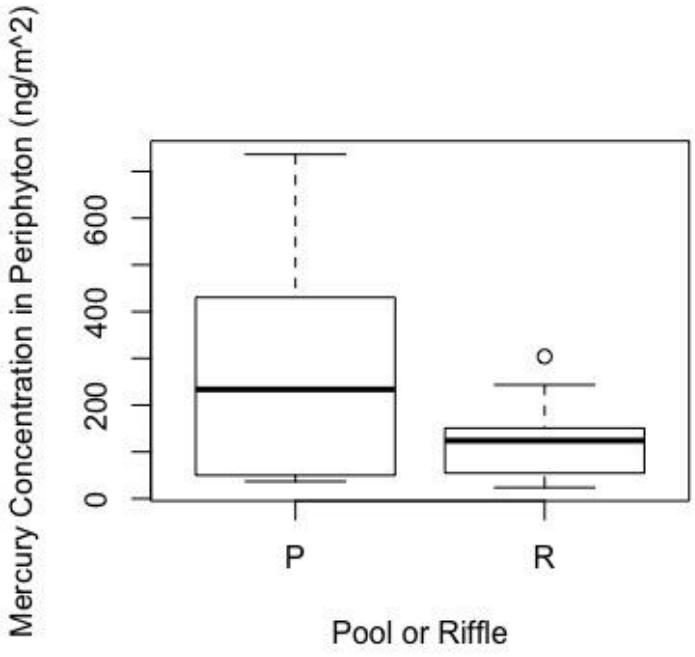


Figure 9. Mercury areal burdens from samples collected in pools and riffles.

*Carbon in Periphyton*

In this study carbon concentrations in periphyton are used as a proxy for biomass, and are presented in units of $g/m^2$. At site 145, concentrations ranged from approximately 0.02-0.39 $g/m^2$ averaging 0.207 (s=0.13). At site 642, during the 6 week sampling period, concentrations ranged from 0.50-1.23 $g/m^2$, averaging 0.662 (0.13). At site 694, concentrations ranged from 0.09 to 0.34 $g/m^2$, averaging 0.190 $g/m^2$ (s=0.07). As shown in Figure 10, biomass at site 642 was significantly higher (F=32.24) than both site 145 (p=0.0000001) and site 694 (p=0.00000001). At site 642, carbon concentrations during the first 3 week sampling period ranged from 0.10 to 1.09 $g/m^2$, and from 0.30 to 0.48 during the second 3 week sampling period, with averages of 0.464 (s=0.36) and 0.357 $g/m^2$ (s=0.02), respectively. Concentrations were not significantly different between the first and second 3 week sampling periods.

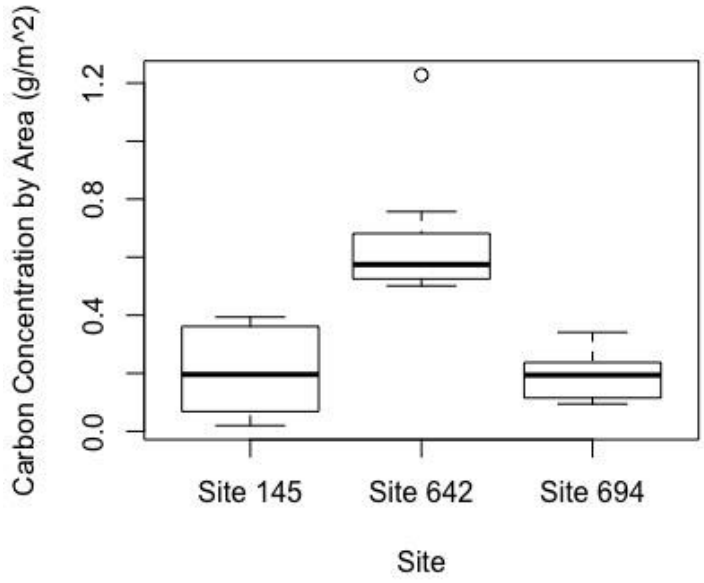


Figure 10. Carbon concentrations in periphyton from each site.

*Chlorophyll a in Periphyton*

Like the carbon data, chlorophyll a concentrations in this study are expressed in units of mass/area, in this case $mg/m^2$. At site 145, concentrations ranged from 0.05-0.85 $mg/m^2$, averaging 0.33 $mg/m^2$ (s=0.24). At site 642, during the 6 week sampling period, concentrations ranged from 0.90 to 1.75 $mg/m^2$, averaging 1.40 $mg/m^2$ (s=0.30). At site 694, concentrations ranged from 0.07-0.32 $mg/m^2$, with an average 0.19 $mg/m^2$ (s=0.08). Similarly to carbon concentrations just discussed, chlorophyll a concentrations at site 642 were also significantly higher (F=53.63) than sites 145 (p=0.00000001) and 694 (p=0.00000001). As shown in Figure 12, the ratio of chlorophyll a to carbon was relatively consistent across all sites.

At site 642, chlorophyll a concentrations ranged from 0.29-4.47 mg/m$^2$ during the first 3 week sampling period, and from 0.34-0.87 mg/m$^2$ during the second 3 week sampling period, with averages of 1.62 mg/m$^2$ (s=1.25) and 0.56 mg/m$^2$ (s=0.14), respectively. Again, concentrations from the first and second 3 week sampling periods were not significantly different from each other, although concentrations had more range and a higher average during the first sampling period.

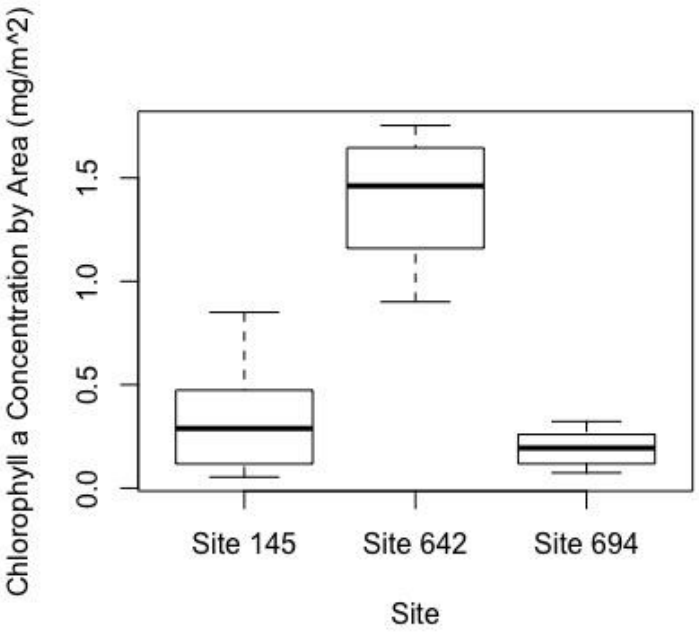


Figure 11. Chlorophyll a concentrations in periphyton at each site.

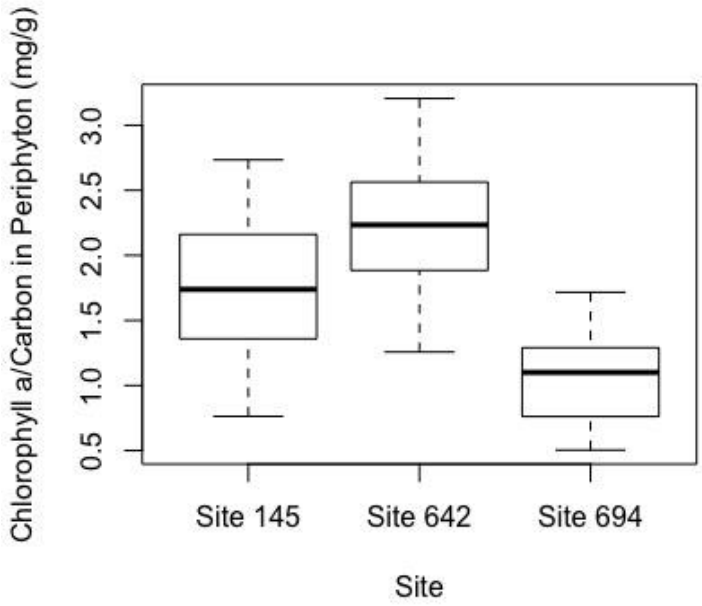


Figure 12. Chlorophyll a/Carbon in periphyton ratio at each site.

**Stream Chemistry**

Throughout the sampling period, DOC concentrations at site 694 ranged from 0.63 to 0.84 mg/L, and were significantly lower (F=36.64) than sites 145 (p=0.0000005) and 642 (p=0.0000013). DOC concentrations at sites 145 and 642 ranged from 1.71 to 2.93 mg/L, and 1.59 to 2.83 mg/L, respectively, and were not significantly different from each other (Figure 13). At site 145, DOC concentrations were elevated during the second sampling event, averaging 2.82 mg/L, whereas during the other three sampling events average concentrations ranged from just 2.0-2.1 mg/L. At site 642, DOC concentrations were elevated during the first two sampling events, ranging from 2.66 to 2.83 mg/L, then dropping to 1.59-1.76 mg/L during the last two sampling events. At site 694, DOC

concentrations did not vary temporally, with average concentrations during each sampling event ranging from 0.70 to 0.80 mg/L.

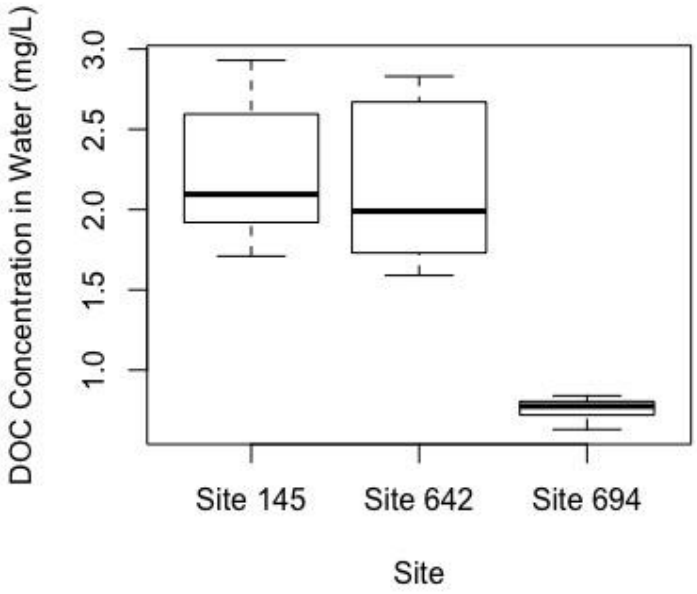


Figure 13. DOC concentrations at each site over the sampling period.

pH at site 642 ranged from 6.02 to 6.38, and was significantly lower (47.85) than sites 145 ($p=0.000065$) and 694 ($p=0.000024$). pH at sites 145 and 694 ranged from 6.68 to 6.99 and from 6.92 to 7.01, respectively, and were not significantly different from each other (Figure 14). No temporal trends in pH were observed at any of the sites; at 145 and 642, measurements oscillated above and below the overall average, and at 694, measurements were confined to a very narrow range throughout the sampling period.

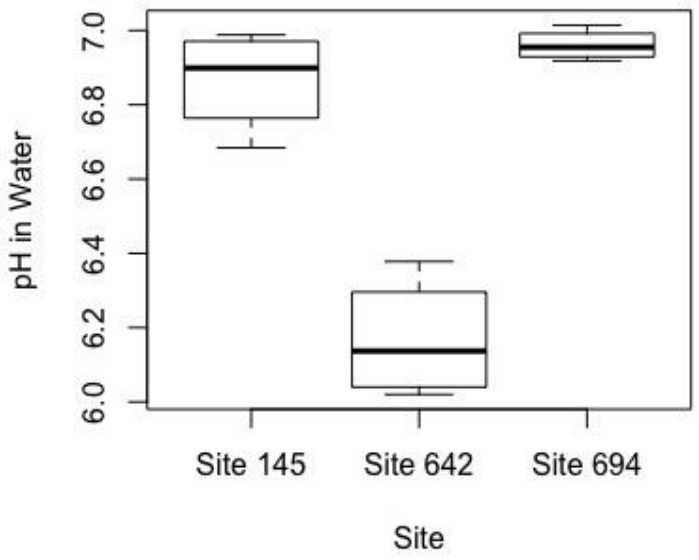


Figure 14. pH measurements at each site over the sampling period.

Throughout the sampling period, DO concentrations ranged from 10.83 to 12.51 at site 145, from 9.81 to 11.91 at site 642, and from 10.96 to 12.26 at site 694 (Figure 15). Although average DO concentrations were slightly lower at site 642 compared to the other two sites, these differences were not statistically significant. At all sites, DO concentrations were higher during the first half of the sampling period, with the second sampling event having the highest DO concentration, and the first sampling event having the next highest.

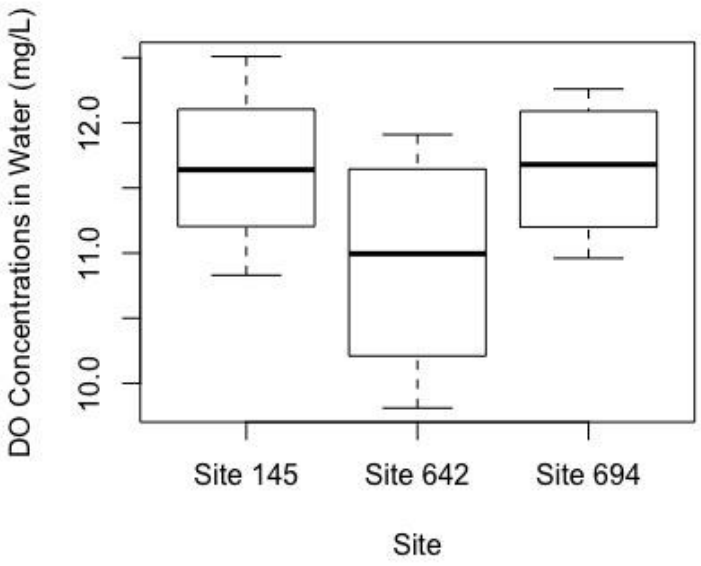


Figure 15. Dissolved oxygen concentrations at each site over the sampling period.

Temperature measurements ranged from 7.4 to 9.9 °C at site 145, from 6.5 to 9.8 °C at site 642, and from 6.4 to 9.7 at site. Overall, temperatures at each site were not significantly different from each other. At all sites, temperatures generally increased over the sampling period.

## Discussion

*Mercury in Periphyton*

Mercury concentrations at the site with the most timber harvest (site 642) were significantly higher than at sites with less timber harvest influence (145 or 694). This was true regardless of the use of low-mercury values obtained from the original analysis, or

the revised standard curve (incorporating lower mercury concentrations). This difference in mercury concentrations is likely due largely to varying biomass found across sites. As shown in Figure 16 below, mercury concentrations in periphyton across all sites and sampling periods were roughly positively correlated with the amount of carbon in periphyton. These results are consistent with Bell and Scudder (2004), which found that samples with higher biomass generally had higher mercury areal burdens, simply because at the same concentration, the sample with more biomass will also have more mercury. However, Bell and Scudder (2004) observed a leveling off of mercury areal burden as biomass reached approximately 100 $g/m^2$, which was not observed in this study, likely because the sampling periods were too short to reach this level of biomass.

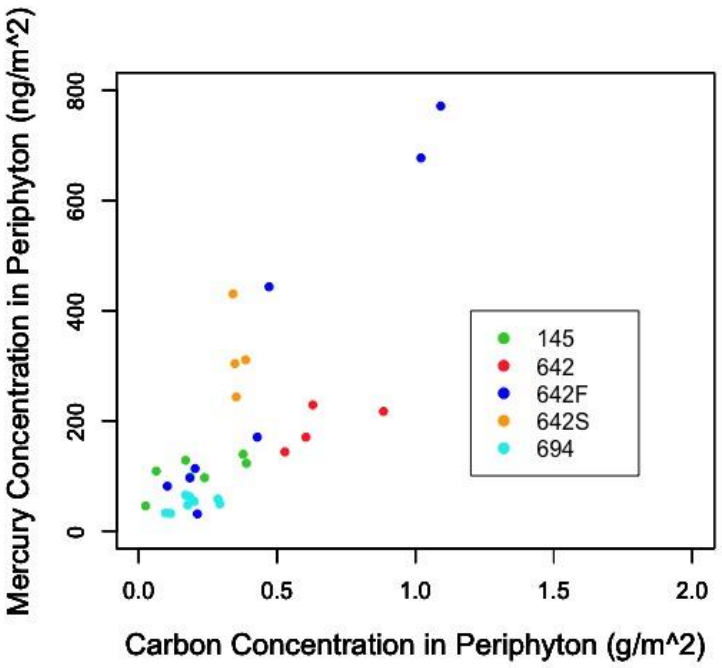


Figure 16. Mercury areal burdens plotted against carbon areal burdens in all periphyton samples. The loose positive correlation suggests that mercury areal burdens are largely, but not completely, a function of biomass.

However, biomass alone may not entirely explain the observed trends in mercury concentrations. Stream chemistry may also help explain these trends. As discussed in the literature review, DOC is widely considered to be a controlling factor in the methylation and mobilization of mercury, such that high DOC concentrations in surface waters are generally correlated with higher mercury concentrations (Ravichandran, 2004). In short, DOC facilitates the transport of elemental and methylmercury molecules from terrestrial to aquatic systems via runoff, it increases the solubility of many mercury containing compounds, and it increases overall methylation potential in soils in sediments, resulting in more methylmercury, which accumulates more readily in biota than does elemental mercury (Ravichandran, 2004). As discussed, DOC was significantly higher at sites 145 and 642 during the sampling period. Also discussed previously, pH may affect mercury mobilization and methylation, as acidic waters tend to leach mercury from soils more readily, and potentially increase methylation in soils (Ullrich et al., 2001). So, more acidic runoff may result in more total mercury and methylmercury entering downstream aquatic systems. As discussed, pH was significantly lower at site 642, potentially due to higher wetland influence in the basin, which can produce acidic waters. Lastly, DO may have influenced concentrations in the study sites. Anoxic conditions are known to be important for sulfate reducing bacteria (SRB) to thrive, which are the microbes most responsible for mercury methylation (Sonke et al., 2014). DO was slightly lower at site 642, although this difference was not statistically significant. These lower DO concentrations may hint that some runoff in basin 642 is coming from areas with elevated SRB and potentially methylmercury, such as wetlands.

Landscape characteristics and land use may also help explain differences in Hg. First, basin 642 is the only basin with palustrine wetlands detected, with approximately 2.47 acres. Palustrine wetlands are those associated with bogs, swamps, and marshes, and are widely considered to be primary hot spots for mercury methylation. These wetlands are known to produce high amounts of methylmercury, which can be flushed into streams during precipitation events, causing elevated concentrations (St. Louis et al., 1994). Second, basin 642 has been most recently and dramatically affected by timber harvest, which is known to alter hydrology and carbon cycling in ways that increase mercury runoff and methylation in forested catchments (Eckley et al., 2018; Porvari et al., 2003). These two landscape characteristics combined likely influenced the stream chemistry and mercury accumulation in periphyton at each site.

In conclusion, the site with the highest mercury concentrations (site 642), also had significantly higher biomass areal burden, significantly lower pH, lower DO, more wetlands, more timber harvest disturbance, and high DOC in water relative to site 694. It is possible that the presence of palustrine wetlands in this basin contributed to the lower pH and DO, and that historic timber harvest and relatively low slope contributed to the higher DOC concentrations. So, these variables likely interact to influence each other, and to influence the mercury areal burden found in each basin.

*Mercury at site 642*

When considering only site 642, concentrations were higher during both of the 3 week sampling periods when compared to the 6 week sampling period, and among the two 3 week sampling periods, higher in the first. The reason for this relationship is not

entirely clear, but may be due to a combination of the effect of scouring on periphyton communities, taxonomic composition, and the exact location within the stream. Once periphyton communities reach a high enough biomass, or once stream flows become strong enough, periphyton mats can be scoured from their substrates (Hondzo & Wang, 2002). It is possible that after the first three weeks of sampling, stream flows began to scour periphyton from the glass tiles, resulting in greater carbon and mercury areal burdens during the first 3 week sampling event. However, hydrological data from a nearby gauging station does not support this claim. The stream draining basin 642 enters the Hoh River approximately 1 mile downstream of the sampling station, and approximately 10 miles downstream of this confluence is a USGS maintained gauging station. Records from this station indicate significantly higher flows during the first 3 week sampling period, with an average discharge approximately 50% greater than the second 3 week sampling period. Precipitation records show the same general pattern, with approximately 50% more precipitation during the first 3 weeks of sampling. Hydrological and climatological data was provided by the Climate and Hydrology Database Projects, a partnership between the U.S. Forest Service Pacific Northwest Research Station and Long-Term Ecological Research program.

Because periphyton consists of a matrix of various algae, cyanobacteria and other microbial species that colonize submerged substrata in succession, it is possible that the periphyton assemblages were different after the six-week sampling period than the three-week sampling periods. If this is the case, it is possible that the earlier species absorb mercury from surface waters more readily than the species present at the end of the six-week sampling period. This study did not assess taxonomic composition, and I could not

find any literature discussing mercury accumulation by different periphyton assemblages. However, Bell and Scudder (2004) postulate a relationship between periphyton community composition and mercury uptake, such that communities with higher percentages of diatoms as opposed to blue-green algae may take up more mercury, but this relationship has not been clearly demonstrated.

It is also likely that the exact placement of periphytometers within the stream resulted in varying levels of carbon and mercury areal burdens. Within small streams, there can be relatively large differences in turbulence and stream flow just centimeters apart. This was observed in the streams used in this study, as evidenced by adjacent glass tiles with noticeably different levels of periphyton biomass. Therefore, it is possible that the exact placement of periphytometers within the stream are responsible for the varying levels of carbon and mercury areal burdens observed.

*Mercury in Pools and Riffles*

Mercury concentrations in periphyton were likely higher in pools than riffles for several reasons. First, water typically moves much more slowly in pools than in riffles, resulting in higher deposition of fine sediment in pools. As discussed in the literature review, mercury and methylmercury molecules bind strongly to particulates (Citation needed) , so depositional areas such as pools likely receive higher amounts of particulate bound mercury relative to areas with faster flowing water such as riffles. These particulates may become a part of the periphyton matrix, contributing mercury to the sample.

Second, water in pools, especially just above the streambed, likely has less dissolved oxygen than surrounding surface waters, especially in riffles. This could potentially result in mercury methylation taking place in the sediments or even periphyton in pools (Hamelin et al., 2015; Tsui et al., 2009), resulting in higher concentrations in periphyton. Differences in stream chemistry between these two environments was not measured.

Lastly, it seems logical that the slowing moving waters in pools would cause less scouring of periphyton, and therefore higher biomass, which could largely control mercury concentrations among these samples. Surprisingly, biomass differences between pools and riffles cannot explain differences in mercury concentrations, as carbon concentrations were not significantly different between the two environments.

*Mercury Concentrations In Relation to Other Studies*

In relation to other studies, periphyton concentrations at the sites measured here are somewhat low. Bell and Scudder (2004) measured mercury in periphyton at multiple streams in the Georgia-Florida Coastal Plain Drainages, the Western Lake Michigan Drainages, and the Willamette Basin in Oregon, and found total mercury concentrations ranging from 38.13-257,500 ng/$m^2$, whereas in this study, concentrations ranged from approximately 24-736 ng/$m^2$. The sites from the Willamette Basin, which are geographically closest to the sites from this study, had both the lowest and highest concentrations among all sites.

The relatively low concentrations found in this study compared to those found by Bell and Scudder (2004) are very likely due to the difference in sampling methods used.

First, Bell and Scudder (2004) collected periphyton samples from natural substrates only, including cobbles, sediments, and wood. These natural substrates may have been accumulating periphyton biomass and mercury for much longer periods than 6 weeks. This would be especially important in areas with relatively slow-moving water, where periphyton is not at risk of being scoured, and could accumulate to relatively high levels.

Secondly, the samples with the highest mercury concentrations in this study were consistently found on periphyton collected on sediments, likely for a combination of reasons. Metals such as mercury are commonly bound to particulate matter, which settle in depositional areas, potentially contributing Hg to the mercury found in these periphyton mats. Furthermore, periphyton samples collected from surface sediments must be decanted before processing to remove sediments from the sample. Bell and Scudder (2004) note that despite decanting, some fine sediments may be inadvertently left in the sample, contributing extra mercury. Lastly, the periphyton found on different substrates in this study had different compositions of diatoms vs. blue-green algae, which may have resulted in higher mercury accumulation rates by periphyton communities on sediments (Bell and Scudder, 2004). This relationship, however, has not been clearly demonstrated.

When considering only the samples colonized on cobble substrates by Bell and Scudder (2004), which more closely resemble the artificial substrates used in this study, concentrations ranged from approximately 38-3,163 ng/m$^2$. When considering only the Willamette Basin sites, concentrations on cobble substrates ranged from 38-1,024 ng/m$^2$. This range of concentrations is only slightly higher than the range of concentrations found in this study. Under this context, considering geography and substrate material, the

mercury concentrations found in this study are comparable to those found by Bell and Scudder (2004).

*Carbon and Chlorophyll a in Periphyton*

The higher biomass concentrations at site 642 are likely due to a combination of factors. First, the high DOC concentrations in stream 642 likely contributed to the higher biomass concentrations when compared to site 694. Frost et al. (2007) demonstrated that increased dissolved organic matter (DOM) significantly increases periphyton biomass, total carbon, and chlorophyll a concentration in stream periphyton. As will be discussed, these higher DOC concentrations are likely due to the lower overall slope in basin 642. DOC concentrations, however, cannot fully explain differences in carbon concentration, as site 145 also has high DOC concentrations. Site 642 also has a considerably lower reach gradient (2.06%, compared to 4.13% and 4.53% at sites 145 and 694, respectively). Reach gradient is known to influence periphyton biomass, as steeper reaches experience faster, more turbulent stream flows, which make periphyton establishment difficult, and can scour already established periphyton from substrates (Hondzo & Wang, 2002). This difference in turbulence among different sites was visually observed during sample collection. Lastly, the pH at site 642 may have influenced the periphyton biomass. In general, carbon dioxide dissolves in water to form carbonic acid, resulting in a more acidic solution. At equilibrium, the amount of carbonic acid will be proportional to the amount of carbon dioxide. So, in more acidic waters (lower pH), there will be more carbon dioxide available for primary production, resulting in higher biomass than there would be otherwise. Thus, the combination of higher DOC concentrations at site 642

compared to site 694, the considerably lower reach gradient compared to both sites, and more acidic waters may have contributed the higher biomass/area at site 642.

The patterns in chlorophyll a concentrations are likely just a function of the higher overall biomass at site 642 just mentioned. As discussed, and shown in Figure 12, the ratio of chlorophyll a to carbon concentrations are relatively constant across sites.

*Stream Chemistry*

The trends in DOC concentrations at site 694 are likely due to multiple factors including a steeper median slope in the watershed at site 694 (54% compared to 5% and 16% in basins 642 and 145, respectively), which causes more surface flow and less leaching of organic matter from soils (Clair et al., 1994; Clair & Ehrman, 1996). Secondly, timber in basin 694 has not been harvested since at least before 1999, whereas the other two basins were partially harvested over that same time period (**Table_**). As discussed, timber harvesting is known to contribute large amounts of organic material to soils, which subsequently results in elevated DOC concentrations in surface waters, even after several years, or decades in extreme cases from the leaching of this material (Skyllberg et al., 2009). It is difficult to say whether the relatively low impact logging operations in these basins significantly contribute to the DOC concentrations found in their surface waters, although recent, local research has suggested that timber harvest conducted under Best Management Practices (BMPs) still results in elevated DOC loads (Eckley et al., 2018).

The slightly lower DO measurements at site 642 are possibly due to the lower reach gradient (2.06%, compared to 4.13% and 4.53% at sites 145 and 694, respectively),

which results in less turbulence and atmospheric reaeration of surface waters. Also, the higher presence of wetlands in basin 642, which may be sources of very lowly oxygenated water, could potentially lower DO concentrations in the stream if seepage from these wetlands is occurring.

Basin 642's relatively low slope likely results in more subsurface flow than the other two sites, which may help explain the lower pH values at site 642. Although many factors influence the pH of ground and surface waters, subsurface flow may spend more time in contact with decaying organic matter, resulting in higher carbonic acid concentrations and lower pH. Furthermore, pH in the study stream may be affected by wetlands in the basin. Basin 642 has an estimated 2.47 acres of palustrine wetlands (associated with swamps, marshes, or bogs), compared to 0 acres in the other two basins. Wetlands of this type are generally known to be more acidic than surface waters, and could be seeping relatively acidic waters into the study stream.

## Conclusions

Results from this study agree with the theoretical mechanisms behind logging disturbance, wetland presence, and topography's influence on mercury accumulation by stream periphyton. DOC was lowest in the site with no logging disturbance and highest median slope, while pH and DO were lowest in the site with the most wetland area. More recent and drastic timber harvest and greater wetland presence in basin 642 likely resulted in elevated DOC and mercury concentrations and reduced pH and DO concentrations in surface waters, as compared to sites with less timber harvest and wetland area. Elevated mercury concentrations in periphyton at site 642 were associated

with high DOC, low pH, low DO, and high biomass relative to the other two sites. Mercury concentrations in periphyton found in this research are comparable to concentrations found by other studies in the region, but are much lower than the range of concentrations found nationwide. These results suggest that in forested catchments with significant wetland presence, even low impact logging can result in elevated mercury concentrations in stream biota relative to surrounding areas.

# Appendix

| Site | Hg mass (ng) | Area sampled ($m^2$) | Mercury areal burden ($ng/m^2$) | Carbon areal burden ($g/m^2$) | Chlorophyll a areal burden ($mg/m^2$) |
|---|---|---|---|---|---|
| 145 | 0.623 | 0.00485 | 128.47 | 0.1695 | 0.3967 |
| 145 | 0.321 | 0.00296 | 108.36 | 0.1853 | 0.2863 |
| 145 | 0.513 | 0.00471 | 108.92 | 0.06354 | 0.1093 |
| 145 | 0.350 | 0.00701 | 49.87 | 0.02502 | 0.05762 |
| 145 | 0.294 | 0.00269 | 108.92 | 0.2381 | 0.2171 |
| 145 | 0.388 | 0.00257 | 150.81 | 0.3775 | 0.6030 |
| 145 | 0.326 | 0.00239 | 135.92 | 0.3891 | 0.6636 |
| 642F | 0.529 | 0.00290 | 156.67 | 0.4284 | 0.8889 |
| 642F | 0.566 | 0.00137 | 414.00 | 0.4710 | 1.458 |
| 642F | 0.847 | 0.00115 | 736.48 | 1.091 | 2.607 |
| 642F | 0.656 | 0.00103 | 638.26 | 1.020 | 4.477 |
| 642F | 0.152 | 0.00122 | 124.10 | 0.1034 | 0.2909 |
| 642F | 0.162 | 0.00114 | 141.80 | 0.1850 | 0.9430 |
| 642F | 0.149 | 0.00085 | 175.13 | 0.2038 | 1.508 |
| 642F | 0.092 | 0.00117 | 78.09 | 0.2122 | 0.8186 |
| 642S | 1.099 | 0.00255 | 430.54 | 0.3407 | 0.5398 |
| 642S | 0.936 | 0.00301 | 310.75 | 0.3863 | 0.4569 |
| 642S | 0.639 | 0.00262 | 243.48 | 0.3525 | 0.5727 |
| 642S | 0.854 | 0.00281 | 304.06 | 0.3477 | 0.6788 |
| 642 | 0.418 | 0.00211 | 197.93 | 0.6291 | 1.320 |
| 642 | 0.363 | 0.00197 | 184.60 | 0.8846 | 1.462 |
| 642 | 0.264 | 0.00226 | 116.70 | 0.5280 | 1.378 |
| 642 | 0.386 | 0.00264 | 146.09 | 0.6043 | 1.434 |
| 694 | 0.212 | 0.00508 | 41.71 | 0.2006 | 0.2596 |
| 694 | 0.212 | 0.00574 | 36.87 | 0.1771 | 0.2614 |
| 694 | 0.168 | 0.00708 | 23.74 | 0.1149 | 0.09029 |
| 694 | 0.208 | 0.00813 | 25.46 | 0.09705 | 0.08646 |
| 694 | 0.320 | 0.00584 | 54.61 | 0.1693 | 0.2276 |
| 694 | 0.250 | 0.00497 | 50.16 | 0.1838 | 0.2165 |
| 694 | 0.204 | 0.00452 | 45.10 | 0.2861 | 0.2441 |
| 694 | 0.227 | 0.00581 | 38.99 | 0.2932 | 0.1545 |

Table 2. Corrected mercury, carbon, and chlorophyll a values for all periphyton samples analyzed. The unshaded mercury values were obtained during the original analysis, and the shaded values were obtained by applying the new standard curve to the data. The unshaded values can be considered quantifiably accurate, whereas there is less confidence in the shaded values because the standard curve was run after the samples were.

| Site | Hg mass (ng) | Area sampled ($m^2$) | Mercury areal burden ($ng/m^2$) | Carbon areal burden ($g/m^2$) | Chlorophyll a areal burden ($mg/m^2$) |
|---|---|---|---|---|---|
| 145 | 0.623 | 0.00485 | 128.47 | 0.1695 | 0.3967 |
| 145 | 0.290 | 0.00296 | 98.01 | 0.1853 | 0.2863 |
| 145 | 0.513 | 0.00471 | 108.92 | 0.06354 | 0.1093 |
| 145 | 0.320 | 0.00701 | 45.62 | 0.02502 | 0.05762 |
| 145 | 0.261 | 0.00269 | 96.94 | 0.2381 | 0.2171 |
| 145 | 0.358 | 0.00257 | 139.44 | 0.3775 | 0.6030 |
| 145 | 0.295 | 0.00239 | 123.19 | 0.3891 | 0.6636 |
| 642F | 0.495 | 0.00290 | 170.52 | 0.4284 | 0.8889 |
| 642F | 0.566 | 0.00137 | 414.00 | 0.4710 | 1.458 |
| 642F | 0.847 | 0.00115 | 736.48 | 1.091 | 2.607 |
| 642F | 0.656 | 0.00103 | 638.26 | 1.020 | 4.477 |
| 642F | 0.100 | 0.00122 | 81.55 | 0.1034 | 0.2909 |
| 642F | 0.110 | 0.00114 | 96.54 | 0.1850 | 0.9430 |
| 642F | 0.096 | 0.00085 | 113.58 | 0.2038 | 1.508 |
| 642F | 0.037 | 0.00117 | 31.23 | 0.2122 | 0.8186 |
| 642S | 1.099 | 0.00255 | 430.54 | 0.3407 | 0.5398 |
| 642S | 0.936 | 0.00301 | 310.75 | 0.3863 | 0.4569 |
| 642S | 0.639 | 0.00262 | 243.48 | 0.3525 | 0.5727 |
| 642S | 0.854 | 0.00281 | 304.06 | 0.3477 | 0.6788 |
| 642 | 0.484 | 0.00211 | 229.15 | 0.6291 | 1.320 |
| 642 | 0.427 | 0.00197 | 217.24 | 0.8846 | 1.462 |
| 642 | 0.325 | 0.00226 | 143.79 | 0.5280 | 1.378 |
| 642 | 0.450 | 0.00264 | 170.50 | 0.6043 | 1.434 |
| 694 | 0.272 | 0.00508 | 53.57 | 0.2006 | 0.2596 |
| 694 | 0.272 | 0.00574 | 47.35 | 0.1771 | 0.2614 |
| 694 | 0.227 | 0.00708 | 32.08 | 0.1149 | 0.09029 |
| 694 | 0.267 | 0.00813 | 32.85 | 0.09705 | 0.08646 |
| 694 | 0.382 | 0.00584 | 65.39 | 0.1693 | 0.2276 |
| 694 | 0.310 | 0.00497 | 62.41 | 0.1838 | 0.2165 |
| 694 | 0.264 | 0.00452 | 58.46 | 0.2861 | 0.2441 |
| 694 | 0.287 | 0.00581 | 49.43 | 0.2932 | 0.1545 |

Table 3. Uncorrected mercury, carbon, and chlorophyll a values for all periphyton samples analyzed. All mercury values were obtained during the original analysis. The shaded values were not used in the analysis, because their concentrations fell outside the range of standards used when creating the original standard curve.

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