

The Effects of Different Forest Management Practices on Mercury Contamination in  
Slimy Sculpin (*Cottus cognatus*)

By

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## Abstract

Forested catchments support an important ecological relationship between forests and streams. For example, forests filter nutrients and sediments from runoff and contribute terrestrial energy inputs (e.g. leaf litter) that support aquatic food webs. Consequently, disturbance from activities like forestry can affect the transport of matter into streams. A growing concern is the role of forestry in mobilizing mercury (Hg) from terrestrial sources into streams, which is poorly understood at catchment-sized scales. The objectives of my thesis were to evaluate the effect of forest management on spatial distributions of Hg in slimy sculpin (*Cottus cognatus*) within a catchment and between three catchments managed at different intensities in New Brunswick: minimally, extensively, and intensively harvested (NBR, NBE, and NBI, respectively). Alongside Hg analysis, stable isotopes of carbon, nitrogen, and hydrogen were measured to distinguish the relative contributions of terrestrial and aquatic energy to sculpin diet and trace the movement of Hg across trophic levels. During the fall of 2017, sculpin were sampled from five or six upstream to downstream reaches in each catchment. Where possible, 10 males and 10 females were selected for analyses ( $n = 287$ ). Total Hg (THg) was measured in dried, homogenized whole body tissue ( $n = 258$ ) using a Direct Mercury Analyzer, and  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^2\text{H}$  were measured in dried, homogenized muscle fillets ( $n = 245$ ). Stable isotope ratios were used in Bayesian mixing models to predict the contribution of terrestrial and aquatic food sources to sculpin diet. Results showed that female sculpin in the minimally disturbed catchment had significantly lower THg levels compared to sculpin from NBE ( $p = 0.0141$ ). The effect of stream order on THg concentration was significant in all three catchments (ANOVA,  $p < 0.01$ ), but there were no clear spatial trends. Stable isotope results showed that variations in THg levels could be related to differences in diet, and results in one of the mixing models found that terrestrial inputs in the diet of sculpin in NBE and NBI decreased along the upstream-downstream gradient. Overall, results suggest that variations in THg between catchments could be due to differences in diet and this could be related to forest management. This study contributes to a better understanding of how forestry affects stream food webs and Hg contamination across catchment-sized scales.

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## **1. Introduction**

In Canada, catchments are a source of many important resources, including water. Two-thirds of Canadians receive their drinking water from waterways, such as streams, that drain from headwater systems in forested catchments (Natural Resources Canada, 2011). Forests play an important role in the quality and treatability of this water, by providing aquatic ecological services including the filtering of nutrients and sediments from runoff flowing into adjacent streams (Wells et al., 2010). This movement of materials from terrestrial to aquatic systems makes streams susceptible to changes caused by forest disturbance. For example, forest management and harvesting can alter the hydrological and biogeochemical processes that occur in a forest. Along the terrestrial-aquatic linkage, these alterations can translate to poorer water quality that can impact aquatic biota and downstream communities that rely on streams to supply treatable drinking water and support recreational activities such as fishing. Of growing concern is the role of forestry in the contamination of aquatic environments and biota with mercury (Hg) and the highly toxic compound methylmercury.

### **1.1 The Terrestrial-Aquatic Linkage**

To understand the role of forest management in aquatic Hg contamination, it is important to first understand the linkages between forests and headwater systems. Headwater systems consist of low-order streams that form the head of a drainage headwork and are numerous and impactful: they drain the majority of a drainage basin and comprise most of a drainage network (Leopold, Wolman and Miller, 1964). Headwater streams are typically small in width, well-shaded, slow-flowing, and have the highest edge-to-surface ratios compared to other parts of a given drainage network (Richardson and Danehy, 2007). Consequently, these streams are closely linked to their terrestrial surroundings (Hynes, 1975) and are the most importance source of streamflow, sediments, organic matter, and nutrients for downstream systems (Macdonald and Coe, 2007). In forested catchments, forests can greatly influence the physical and chemical properties of headwater streams. For example, riparian forests can increase shading and limit in-stream productivity while supplying allochthonous basal resources, such as leaf litter, organic particulate matter, and dissolved organic and

inorganic matter to support aquatic food webs (Richardson and Danehy, 2007). In some cases, forests can be responsible for over 90% of all energy inputs in a stream ecosystem (Fisher and Likens, 1973). Forests also affect hydrological regimes and can help regulate stream water quality and provide flood control services, by enhancing soil infiltration, evapotranspiration, and nutrient and water storage (Pohjanmies et al., 2017). However, this terrestrial-aquatic linkage means that forest disturbances such as forest management can also impact headwater systems.

Forest management is a catchment-level disturbance that can impact the hydrological regimes of headwater systems, and these impacts are well-studied and understood (Hibbert, 1967; Bosch and Hewlett, 1982; Sahin and Hall, 1996; Buttle, 2011). It is generally accepted that forest management can lead to reduced interception of rainfall, evapotranspiration, and soil infiltration. In addition to increased impermeable surfaces (e.g. roads) and compacted soils, these hydrological changes can alter the relative contribution of hydrological processes to streamflow, from subsurface stormflow to overland flow, and ultimately lead to greater streamflow in streams of managed catchments (Buttle, 2011).

Forest management can also impact the biogeochemistry of headwater systems. Erdozain et al. (2018) observed that headwater streams of managed catchments are often exposed to higher dissolved inorganic nutrient and sediment loads, exhibit elevated temperatures, and contain more algae, compared to headwaters of less intensely managed catchments. Changes in the reliance of stream food webs on different energy sources – i.e. allochthonous versus autochthonous basal resources – have been documented in managed catchments. An increased reliance on allochthonous resources could be caused by hydrological changes associated with vegetation loss and more impermeable surfaces (e.g. compacted soils, roads), such as reduced soil infiltration and increased runoff, that lead to elevated loading of terrestrial material. Erdozain et al. (2019) observed a decrease in aquatic algal inputs to the diet of macroinvertebrates in headwater systems of catchments with increasing forest intensity. Alternatively, there could be an increased reliance on autochthonous resources, and this change has been more commonly observed in studies on food web effects of forest

management (England and Rosemond, 2004; Göthe, Lepori and Malmqvist, 2009). This could be explained by one or a combination of factors, such as: reductions in riparian vegetation (Bilby et al., 1992; Santiago, Molinero and Pozo, 2011), higher stream nutrient loads (Lecerf et al., 2012), or greater light penetration, the latter two of which could support increased stream productivity (Kiffney, Richardson and Bull, 2003; Clapcott and Barmuta, 2010) and the uptake of aquatic energy resources such as algae (England and Rosemond, 2004).

As headwater streams dominate drainage networks and are tightly linked to downstream systems, hydrological and biogeochemical effects caused by forest management could also be transmitted to higher-order streams, by altering the quantity and quality of constituents typically received. Of growing concern is the role of forestry in elevated Hg levels in aquatic biota and streams of managed catchments.

## **1.2 The Biogeochemical Cycle of Mercury**

Mercury is a naturally occurring metal that is present in all environmental compartments, including the atmosphere, oceans, and lands (Wiener et al., 2003). Geologic sources such as volcanoes are responsible for a large proportion of natural Hg emissions, as well as wetlands and mercuriferous belts, which contain elevated levels of Hg compared to other natural systems (Lindqvist et al., 1991; Nriagu and Becker, 2003). However, centuries of anthropogenic activities have contributed to elevated Hg levels in the environment. Mason, Fitzgerald, and Morel (1994) estimated that anthropogenic sources have tripled the levels of Hg present in the atmosphere and are ultimately responsible for two-thirds of the total Hg in global flux. Fossil fuel combustion, waste incineration, and mining contribute the greatest amount of anthropogenically emitted Hg (Lindqvist, 1986; Sunderland and Chmura, 2000). A global Hg inventory conducted by Pacyna et al. (2006) concluded that fossil fuel combustion was responsible for two-thirds of the total Hg emitted from anthropogenic sources in the year 2000. As Pacyna et al. (2006) discussed and embodied with their Hg inventory, increased awareness of Hg contamination in recent decades has motivated national and international efforts to quantify historical and current Hg fluxes and inventories in all environmental compartments, understand the movement and fate of anthropogenically emitted Hg,

investigate the impacts on receiving landscapes and biota, and ultimately, reduce anthropogenic emissions of Hg.

Currently, the cycling of Hg in the environment is well-understood, as is the significant role of anthropogenic inputs. Activities such as gold and silver mining can contaminate local terrestrial environments with Hg, and tailings can remain a significant source of Hg in the environment for decades, even after operations cease (Lacerda and Salomons, 1999). Meanwhile, waste incineration and fossil fuel combustion emit Hg into the atmosphere (Lindqvist, 1986). As a metal with relatively high volatility, as well as relative inertness in the presence of O<sub>2</sub>, atmospheric Hg is typically present as elemental Hg (Hg<sup>0</sup>) and has an atmospheric half-life of approximately one year, allowing sufficient time for Hg to be transported kilometers away from the original source (Jackson, 1997; Lindberg, 1987). Once disputed, the long-range transportation of Hg is now supported by observations of elevated Hg in sediment cores of remote locations with no record of local anthropogenic activities, including the Arctic, due to the deposition of Hg from the atmosphere (Jackson, 1997; Mason, Fitzgerald and Morel, 1994; Hermanson, 1998).

Atmospheric Hg<sup>0</sup> is oxidized to inorganic Hg(II) and undergoes dry or wet deposition onto the Earth's surface, including onto vegetation and terrestrial soils (the former can be eventually incorporated into soils via leaf litter and throughfall) (Pirrone, Glinsorn and Keeler, 1995; Grigal, 2002). In soils, Hg(II) is sorbed to organic matter in the humus layer, bound tightly to reduced sulfur groups (Lindqvist et al., 1991; Kronberg et al., 2016b). This bound Hg is relatively stable, and thus large stores of Hg can accumulate in terrestrial soils, making them an important sink. Mason, Fitzgerald, and Morel (1994) estimated that of the total amount of Hg emitted by anthropogenic sources into the atmosphere in the last century, surface soils have accumulated the largest amount – almost 18 times greater than that of the oceans and atmosphere, combined. Mercury can also be removed from the soil by processes such as revolatilization to the atmosphere or mobilization into nearby aquatic systems (Grigal, 2002). In the latter case, Hg can be converted to methylmercury (MeHg), a neurotoxin that readily crosses the blood brain barrier and can bioaccumulate in an organism and biomagnify up a food web, leading to MeHg levels many times greater than that of the surrounding water

(Wiener et al., 2003). Aquatic biota and humans are typically exposed to elevated MeHg levels through diet, and such exposure is known to impair reproductive and central nervous systems and cause death (Scheuhammer et al., 2007). These consequences have been observed in well-documented events such as Minamata and Niigata, Japan in the 1950s, where hundreds of people became sick from consuming fish and shellfish contaminated with MeHg discharged by a chemical plant, and high mortality rates in the decades following the incident were linked to acute and chronic MeHg poisoning. This and similar events have raised awareness of the role of other anthropogenic activities, such as forestry, in Hg contamination of aquatic ecosystems (Mcalpine and Araki, 1958). In Canada, tissue residue guidelines (TRGs) mark the upper limit of Hg levels in tissue for safe consumption by higher trophic level organisms. Unfortunately, Hg levels in excess of the TRGs for fish-eating predators such as kingfishers and osprey have been found in their prey (Environment Canada, 2003).

### **1.3 The Role of Forestry in Mercury Contamination**

The movement of soil Hg stores to adjacent aquatic systems is dependent on processes of remobilization and runoff, and often involves conversion between Hg species. These processes are heavily influenced by a range of factors, such as the soil microbial communities present and levels of dissolved organic carbon (DOC), which can facilitate contamination of nearby aquatic environments (Gill et al., 1999; Grigal, 2002). The hydrological and biogeochemical changes brought about by forestry can alter soil Hg stores by facilitating an increased mobilization of Hg stores and increased conversion of Hg(II) to MeHg.

Mercury stores are often mobilized by forestry via changes to hydrological processes such as increased runoff and streamflow, as previously discussed. These hydrological processes are facilitated by reduced canopy cover and evapotranspiration, from vegetation loss, as well as soil compaction from the use of heavy equipment (Buttle, 2011). These same changes can support the development of anoxic environments where Hg(II) is readily converted to MeHg, or 'methylating hotspots' as described by Eklöf et al. (2018). While Hg(II) methylation can occur through several biotic and abiotic pathways via addition of a methyl group, *in-situ* experiments such as those of Compeau



and Bartha (1985) and Gilmour, Henry, and Mitchell (1992) have helped distinguish sulfate-reducing bacteria as the dominant methylating agents, which are most active at oxic-anoxic interfaces (i.e. methylating hotspots) often created by forestry (Eklöf et al., 2018; Rudd and Winfrey, 1990). The rate of methylation is heavily influenced by abiotic factors such as temperature, pH, and particulates which increase the bioavailability of Hg for methylation, including dissolved organic carbon and sulphur (Rudd and Winfrey, 1990; Skjellberg et al., 2003).

Forestry has the potential to increase Hg levels in nearby aquatic systems via (1) increased production of MeHg and/or (2) increased Hg export from soils to surface waters, via processes such as increased runoff. Thus far, the latter process is commonly held responsible for observations of increased export of total Hg and MeHg levels into soils, groundwater, and surface waterbodies of managed catchments (Allan, Heyes and Mackereth, 2009; De Wit et al., 2014; Eklöf et al., 2014; Sørensen et al., 2009; Kronberg et al., 2016a). Meanwhile, despite some observations of increased MeHg concentrations following forest harvesting, the role of forestry in MeHg production requires further study (Porvari et al., 2003; Munthe and Hultberg, 2004).

Contamination of surface waters can eventually lead to wildlife exposure. Investigations where surface waters were 'spiked' with MeHg have observed proportional increases in MeHg in aquatic biota (Orihel et al., 2006; Harris et al., 2007). In the case of anthropogenic activities, forest harvesting has also been linked to elevated MeHg in both water and aquatic biota (Desrosiers, Planas and Mucci, 2006; Garcia and Carignan, 1999, 2005; Sampaio Da Silva et al., 2005; Van Furl, Colman and Bothner, 2010). In many of these cases, while contaminated aquatic Hg levels may be relatively low, the ability of MeHg to bioaccumulate and biomagnify up trophic levels means that MeHg levels in higher-order organisms may be many times greater than ambient water concentrations (Kim and Burggraaf, 1999; Francesconi and Lenanton, 1992).

However, as Hsu et al. (2018) notes, very different Hg responses have been observed amongst forestry impact studies, and these differences are likely the consequence of variation in forest harvesting practices, catchment traits, and climate. For example,

differences in the logging season can heavily influence the degree of soil disturbance and subsequent transport of material into adjacent waterways (Porvari et al., 2003; Kronberg et al., 2016a). Even in the case of winter logging events, differences in the presence of soil frost have the potential to influence Hg responses (Sørensen et al., 2009; De Wit et al., 2014). Meanwhile, Allan, Heyes and Mackereth (2009) posit that stark differences in MeHg response between their study catchments and that of Porvari et al. (2003) could be explained by historically greater accumulation of MeHg in the latter's Finnish catchments. These and other factors will remain a challenge in forestry impact studies on Hg contamination in aquatic systems, as well as attempts to develop a predictive understanding of such impacts.

The development of a predictive framework to estimate spatial patterns in Hg contamination is also hindered by the type of systems typically studied. Small catchments (<100 km<sup>2</sup>) are most commonly studied when investigating forestry impacts on streamflow and Hg contamination because of their relative homogeneity and the logistical limitations associated with larger spatial scales (Buttle and Metcalfe, 2000; Cheng, 1989; Porvari et al., 2003; Sørensen et al., 2009). However, responses observed in headwater systems cannot be extrapolated to downstream systems because of differences in hydrological and biogeochemical processes (Creed et al., 2016; Devito et al., 2005; Coats and Miller, 1981; Sivapalan et al., 2003). Furthermore, investigations that have wider spatial considerations often study impacts in lakes, rather than streams (Garcia and Carignan, 1999; Desrosiers, Planas and Mucci, 2006; Van Furl, Colman and Bothner, 2010; Harris et al., 2007). Consequently, there is a gap in understanding of the cumulative effects of forestry on Hg levels in downstream waterbodies, including higher-order streams. These systems are equally valuable to study, as larger catchments may not be able to buffer larger disturbances as is the case with smaller or less intense disturbances, and it cannot be assumed that elevated local levels of Hg will become dilute with greater distance from the disturbance.

#### **1.4 Stable Isotope Analysis**

Stable isotope analysis can be a useful tool when investigating the role of forestry in Hg contamination of aquatic ecosystems. Stable isotope analysis of food web resources

and tissue uses natural isotopic ratios to provide insights into the diet and trophic level of an organism, and subsequently help explain the transfer of biomagnifying pollutants like MeHg (Fry, 2006; Senn et al., 2010). For example, terrestrial and aquatic plants have unique isotopic ratios of carbon ( $\delta^{13}\text{C}$ ), and thus  $\delta^{13}\text{C}$  can determine if the diet of a consumer consists of more terrestrial (e.g. leaf litter) or aquatic (e.g. algae) inputs (Finlay and Kendall, 2007). However, environmental and individual organism variations can result in similar or overlapping  $\delta^{13}\text{C}$  values, and thus stable isotope analysis of hydrogen ( $\delta^2\text{H}$ ) is becoming a popular complement to  $\delta^{13}\text{C}$  (Finlay, Doucett and McNeely, 2010). Finally, stable isotope ratios of nitrogen ( $\delta^{15}\text{N}$ ) are used to determine the trophic position of an organism, as  $^{15}\text{N}$  in organisms increases with trophic level (Finlay and Kendall, 2007). Thus, measuring  $\delta^{13}\text{C}$ ,  $\delta^2\text{H}$  and  $\delta^{15}\text{N}$  alongside MeHg levels in aquatic biota can reveal how MeHg enters a food web and how quickly the neurotoxin biomagnifies. Stable isotope analysis can be carried out with any number of model species to gain insights into food web dynamics, including slimy sculpin.

### **1.5 Slimy Sculpin (*Cottus cognatus*)**

Slimy sculpin is a benthic fish species ubiquitous throughout North America and is becoming popular in environmental monitoring studies (Scott and Crossman, 1973; Gray et al., 2018). Similar to many small fish species, slimy sculpin exhibit high home fidelity. Gray, Cunjak and Munkittrick (2004) observed site-specific  $\delta^{13}\text{C}$  values in slimy sculpin muscle tissue, while Keeler (2006) noted that tagged slimy sculpin had an annual median movement of 9 m. Thus, slimy sculpin are ideal for research on contaminant distributions and effects, as they are highly representative of the site from which they are sampled. Additionally, slimy sculpin primarily consume invertebrates, making them particularly susceptible to MeHg exposure through diet (Gray et al., 2018).

### **1.6. Objectives**

The main objective of this study was to evaluate the effect of forest management on the spatial distribution of Hg contamination and food resource contributions to sculpin diet in an upstream-downstream gradient, within and between three catchments managed at different intensities. It is hypothesized that the intensity of forestry affects Hg levels in sculpin and food resource inputs to sculpin diet along an upstream-downstream

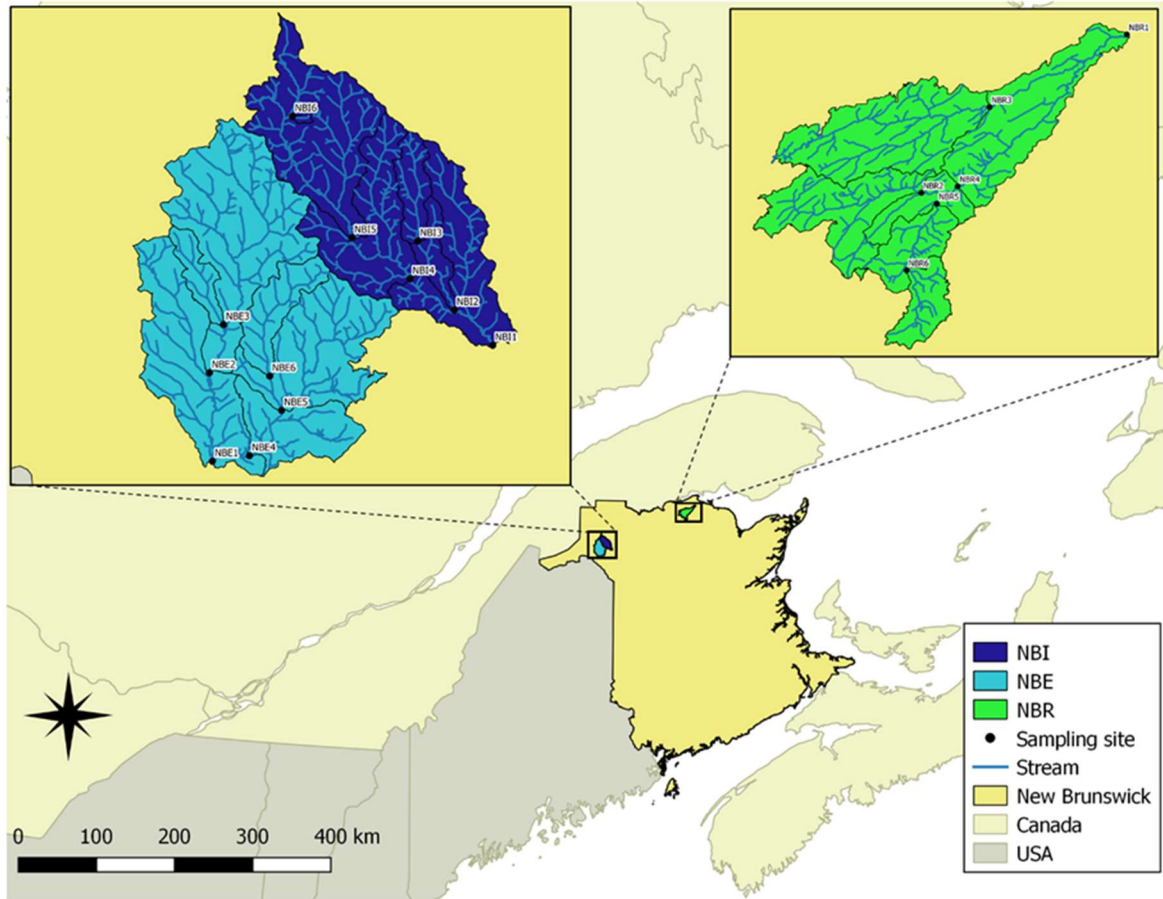
gradient. However, it is difficult to predict if this effect is cumulative, dissipative, or a pattern more complex.

## 2. Methods

### 2.1 Study Area

Slimy sculpin were sampled from three catchments located in northern New Brunswick that undergo different intensities of forest management: intensive, extensive, and minimal management (**Figure 1**). The intensively managed catchment (NBI) is a sub-basin of the Restigouche River within the Black Brook District, which is owned and operated by J.D. Irving. The district contains some of the most intensively managed forests in Canada and is dominated by softwood plantations favouring spruce (Etheridge et al., 2005). The extensively managed catchment (NBE) is a sub-basin of the Quisibis River, where management is defined by natural regeneration and longer rotation cycles (Erdozain et al., in preparation). The minimally managed catchment (NBR) is a sub-basin of the Charlo River. The Charlo River watershed is a source of drinking water to the community and is identified as a designated watershed by the Government of New Brunswick (Government of New Brunswick, 2020). Thus, it is believed to have stricter forest management guidelines, such as clear cutting that is restricted to smaller areas (Government of New Brunswick, 2020).

NBI and NBE are located in the Madawaska Uplands of the Central Uplands Ecoregion, which is underlain by Ordovician-Devonian metasedimentary rocks, some of which are calcareous (Zelazny, 2003). The region is defined by a relatively cool climate and abundant precipitation (Zelazny, 2003). NBR is located in the Northern Uplands ecoregion and underlain by calcareous Silurian-Devonian metasedimentary rocks with igneous intrusions (Zelazny, 2003). Compared to the Central Uplands Ecoregion, this region has a cooler and wetter climate (Zelazny, 2003).



**Figure 1:** NBR, NBE, and NBI catchments in northern New Brunswick, Canada (Erdozain et al., in preparation). Six stream sites were sampled in NBR (NBR1, NBR2, NBR3, NBR4, NBR5, and NBR6), which is located in a sub-basin of the Charlo River; six sites were sampled in NBE (NBE1, NBE2, NBE3, NBE4, NBE5, and NBE6), in a sub-basin of the Quisibis River; and five sites were sampled in NBI, which is located in a sub-basin of the Restigouche River (NBI1, NBI2, NBI3, NBI4, and NBI5). Darker stream network lines indicate hydrological connections between stream sites.

Five stream sites were sampled in NBI, and six sites in each of NBE and NBR and their characteristics are detailed in **Table 1**. These sites were chosen to represent an upstream to downstream gradient, and the most downstream site received water from all upstream sites. The sub-catchments represented by each of these stream sites differed in area (km<sup>2</sup>), total harvest (% of catchment harvested in the last 10 years), clearcut (% of catchment clearcut in last 10 years), crossing density (number of crossings per kilometre of stream), road density (metres of road per hectare), and depth-to-water (DTW).

**Table 1:** Location and characteristics of 17 stream sites in three catchments (NBR, NBE, NBI) experiencing different forest management intensities.

| Catchment | Site | Latitude | Longitude | Area (km <sup>2</sup> ) | Total Harvest (% 10 y) | Clearcut (% 10 y) | Crossing density (#/km) | Road density (m/ha) | DTW (m) |
|-----------|------|----------|-----------|-------------------------|------------------------|-------------------|-------------------------|---------------------|---------|
| NBR       | NBR1 | 47.94969 | -66.40167 | 167.47                  | 7.35                   | 4.07              | 0.35                    | 19.91               | 17.84   |
|           | NBR2 | 47.86021 | -66.57194 | 33.19                   | 5.54                   | 4.08              | 0.36                    | 19.47               | 16.30   |
|           | NBR3 | 47.91020 | -66.51417 | 51.05                   | 11.46                  | 3.63              | 0.37                    | 21.35               | 11.90   |
|           | NBR4 | 47.86387 | -66.54222 | 73.24                   | 6.82                   | 5.79              | 0.36                    | 19.99               | 17.46   |
|           | NBR5 | 47.85406 | -66.55833 | 28.52                   | 7.76                   | 6.82              | 0.38                    | 20.37               | 16.38   |
|           | NBR6 | 47.81680 | -66.58472 | 12.53                   | 7.25                   | 7.24              | 0.17                    | 17.02               | 12.98   |
| NBE       | NBE1 | 47.36078 | -68.07194 | 233.52                  | 14.14                  | 5.42              | 0.45                    | 24.10               | 22.86   |
|           | NBE2 | 47.41159 | -68.07556 | 85.34                   | 17.76                  | 4.36              | 0.56                    | 26.88               | 27.14   |
|           | NBE3 | 47.43935 | -68.06444 | 9.19                    | 0.00                   | 0.00              | 0.08                    | 35.82               | 27.08   |
|           | NBE4 | 47.36435 | -68.04194 | 93.16                   | 13.77                  | 7.67              | 0.34                    | 21.65               | 18.57   |
|           | NBE5 | 47.39061 | -68.01389 | 67.98                   | 16.58                  | 8.59              | 0.31                    | 23.06               | 18.69   |
|           | NBE6 | 47.41004 | -68.02500 | 18.10                   | 20.75                  | 10.13             | 0.48                    | 27.29               | 22.06   |
| NBI       | NBI1 | 47.43016 | -67.83639 | 163.03                  | 24.28                  | 2.56              | 0.78                    | 24.27               | 25.19   |
|           | NBI2 | 47.45257 | -67.87028 | 20.61                   | 22.33                  | 2.03              | 0.47                    | 21.33               | 23.50   |
|           | NBI3 | 47.48940 | -67.90139 | 11.80                   | 21.66                  | 2.61              | 0.61                    | 19.08               | 21.93   |
|           | NBI4 | 47.46766 | -67.90750 | 102.54                  | 22.48                  | 1.33              | 0.92                    | 24.85               | 26.10   |
|           | NBI5 | 47.49055 | -67.95722 | 62.01                   | 21.41                  | 1.07              | 0.83                    | 23.34               | 25.41   |

## 2.2 Sample Collection and Preparation

### 2.2.1 Slimy Sculpin

In September and October 2017, a team from the University of New Brunswick (UNB) collected approximately 10-60 slimy sculpin from each of the 17 stream sites via electrofishing, along a 100 m reach. The fish were weighed and measured for total length, sacrificed using protocols approved by the UNB Animal Care Committee, and then dissected to determine sex. The head and organs were removed, and the fish bodies were sent to McMaster University and stored at -80°C. From these fish, 8-20 were selected from each site (ideally, 10 males and 10 females) to have fish of similar

size ranges across sites where possible for this study. Fork lengths ranged from 53 – 105 mm.

One skinned muscle fillet was removed from each fish using 95% ethanol (EtOH)-washed stainless steel forceps and a stainless steel scalpel. Fillets ( $n = 258$ ) were stored in 10% hydrochloric acid (HCl)-washed 7 mL borosilicate vials and the remaining whole-body tissues ( $n = 245$ ) were stored in 10% HCl-washed 20 mL borosilicate vials. The dry weight (g) of each vial and cap pair was measured using a Sartorius Cubis analytical balance (MSA-125P-100-DI), before the wet weights (g) of fillets and whole bodies were measured in the vials. The fillets and whole bodies were freeze-dried for 48 and 96 hours respectively, then weighed to determine dry weight and calculate percent moisture. Fillets were manually homogenized using 10% HCl-washed glass rods for three to five minutes and stored at room temperature in cryoboxes in a dark space prior to analyses. The whole bodies were manually homogenized by blending for approximately 60 seconds in a coffee blender, grinding for 15 – 30 seconds with a mortar and pestle, and using dissection scissors, until different types of tissue were barely discernable. All equipment was rinsed with 95% EtOH and dried with kimwipes.

Fillet tissue was analyzed for stable isotopes of carbon, nitrogen, and hydrogen, while whole-body tissue was analyzed for total Hg (THg,  $\mu\text{g}/\text{kg}$  dry weight).

### **2.2.2 Benthic Invertebrates and Basal Food Sources**

In 2017, benthic invertebrates were sampled between June 25<sup>th</sup> to July 3<sup>rd</sup>, by electroshocking riffles along the reach site as part of a larger study. The sampling, sorting, and processing procedure followed the instructions detailed by Erdozain et al. (2019), and was completed at all stream sites of NBR, NBE, and NBI. Among the invertebrates sampled, there were *Glossosoma* (Glossosomatidae), *Ceratopsyche* (Hydropsychidae), *Epeorus* (Heptageniidae), and *Ephemerella* (Ephemerellidae). Three samples of water for H isotopes and food sources of coarse and fine particulate organic matter, CPOM and FPOM respectively, and biofilm were collected from each stream site of each catchment, also following the procedure of Erdozain et al. (2019). Samples were analyzed for stable isotopes as described below and in Erdozain et al. (2019).



### **2.3 Mercury Analysis**

Whole-body samples were analyzed for THg, which includes both inorganic and organic species of Hg. Total Hg in whole-body samples was measured using the Milestone Tri-Cell DMA-80 (Direct Mercury Analyzer) according to the Environmental Protection Agency's (EPA) Method 7473, in which samples undergo thermal decomposition, gold amalgamation, and atomic absorption spectrophotometry (U.S. EPA, 1998). The detection range of the instrument was 0.001 – 1500 ng THg. Instrument inaccuracy was minimized by measuring method blanks (acceptable range of <0.002mg/kg), DORM-4 Fish Protein Certified Reference Material (CRM) for Trace Metals (0.412 ± 0.055 mg/kg THg), and duplicates every 10-15 samples (<20% variability from original to duplicate sample) (NRCC, 2012).

### **2.4 Stable Isotope Analysis**

Isotopes are expressed as ratios of the heavy to light isotope ( $\delta$ ) in parts per thousand (‰) relative to a standard (Fry, 2006). The calculation for  $\delta$  for an atom is:

$$\delta X = \left( \frac{R_{SA}}{R_{ST}} - 1 \right) \times 1000 \quad (1)$$

where,  $R_{SA}$  is the ratio of heavy to light isotope of the sample, and  $R_{ST}$  is the ratio of heavy to light isotope of the standard, and  $X$  is  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , or  $\delta^2\text{H}$ .

Values of  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^2\text{H}$  were measured in fillet samples. For sculpin from NBR and NBE, THg,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^2\text{H}$  were measured in the same fish. For sculpin from NBI, THg,  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$  were measured in the same fish, while 50 values of  $\delta^2\text{H}$  were measured in fish different from those measured for THg because of limited availability of muscle fillet samples.

#### **2.4.1 Carbon and Nitrogen Stable Isotope Analysis**

For each fillet sample, 1.000 – 1.200 mg was weighed into 5 mm x 3.5 mm tin capsules (Elemental Microanalysis), using 95% EtOH-washed equipment and a Mettler Toledo™ Excellence Plus, XPE26 Micro-Analytical Balance. A duplicate was included approximately every 24 samples.

Samples were analyzed at the Stable Isotopes in Nature Laboratory (SINLAB) at the University of New Brunswick, using a CE NC2500 (Carlo Erba; Milan, Italy) Elemental Analyzer coupled to a Delta V Plus-Conflo IV (Thermo Finnigan; Bremen, Germany) Continuous Flow-Isotope Ratio Mass Spectrometer (CF-IRMS) (SINLAB, n.d.). Stable isotope measurements of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were normalized with the following secondary standards that have been calibrated against International Atomic Energy Agency (IAEA) primary standards: USGS61, Bovine Liver Standard, and Muskellunge Muscle Standard, where the latter two were developed by the SINLAB. The analytical accuracy of these standards was assessed using the following check standards: acetanilide, ammonium sulfate, and polyethylene foil. The precision for duplicate samples was  $\pm 0.20\text{‰}$  and  $0.17\text{‰}$  standard deviation (SD) for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  measurements respectively, and  $\pm 0.03\text{‰}$  and  $0.04\text{‰}$  on average.

Mean values of  $\delta^{15}\text{N}$  were used to calculate the trophic level of sculpin in each catchment, using mean baseline  $\delta^{15}\text{N}$  values measured in *Glossosoma* and/or *Ceratopsyche*, using the following equation:

$$TL_{consumer} = \frac{(\delta^{15}N_{consumer} - \delta^{15}N_{baseline})}{\Delta^{15}N + \lambda} \quad (2)$$

Where,  $TL_{consumer}$  is the trophic level of a given consumer,  $\delta^{15}N_{consumer}$  and  $\delta^{15}N_{baseline}$  are the  $\delta^{15}\text{N}$  values of the consumer and baseline organisms respectively,  $\lambda$  is the trophic level of the baseline organism (where  $\lambda = 1$  for primary producers, and  $\lambda = 2$  for primary consumers), and  $\Delta^{15}\text{N}$  is the trophic discrimination factor for  $\delta^{15}\text{N}$ . Based on the cage experiment in Erdozain et al. (2019),  $2.3\text{‰}$  was chosen as the discrimination factor.  $TL_{consumer}$  was calculated the mean trophic level for sculpin in each catchment (both male and female fish) using Equation 2 and *Glossosoma*, *Ceratopsyche*, or both invertebrate taxa (using the mean  $\delta^{15}\text{N}$ ) as the baseline organism.

#### **2.4.2 Hydrogen Stable Isotope Analysis**

Three fillets were randomly selected from each stream of each catchment, and 0.200 – 0.230 mg of sample was weighed into 5 mm x 3.5 mm silver capsules (Elemental

Microanalysis), using 95% EtOH-washed equipment and a Mettler Toledo™ Excellence Plus, XPE26 Micro-Analytical Balance.

Values of  $\delta^2\text{H}$  were measured by the SINLAB. Samples were loaded into a Costech Zeroblank autosampler and converted to hydrogen gas using a High Temperature Conversion EA (Thermo-Finnigan) coupled to a Delta V Plus – Conflo IV CF-IRMS system. Values of  $\delta^2\text{H}$  were normalized with the following secondary internal standards which have been calibrated against IAEA primary standards: caribou hook keratin and kudu horn keratin. The analytical accuracy of these standards was assessed using check standards of acetanilide, polyethylene foil, and keratin. The precision for duplicate samples was  $\pm 0.67\text{‰}$  (SD), on average.

### **2.4.3 Mixing Models**

Mixing models were used to predict the relative contribution of terrestrial and aquatic food sources to sculpin diet, in each stream site of each catchment. The terrestrial food sources were CPOM and FPOM, which were measured for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^2\text{H}$  in each stream site. The aquatic food source was algae. Biofilm was not used because it has been observed to be a matrix of both terrestrial and aquatic basal resources (Erdozain et al., 2019). The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of algae at stream site were estimated choosing the benthic invertebrates with the most negative  $\delta^{13}\text{C}$  values, and these were *Glossosoma*, *Epeorus* (Heptageniidae), and *Ephemerella* (Ephemerellidae) (Erdozain, 2020). The  $\delta^2\text{H}$  value of algae at each stream site was calculated in the same manner as Erdozain et al. (2019) by subtracting  $170 \pm 15\text{‰}$  from the water  $\delta^2\text{H}$ , based on studies showing that primary producers have 160-170‰ more negative  $\delta^2\text{H}$  values than surrounding waters, due to fractionation against  $^2\text{H}$  during photosynthesis (Yakir and DeNiro, 1990; Solomon et al., 2011; Hondula et al., 2014).

These stable isotope ratios of CPOM, FPOM, and algae were combined with the  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^2\text{H}$  values measured in sculpin to develop three mixing models with MixSIAR in R, for each stream site: a Bayesian 1-isotope ( $\delta^2\text{H}$ ) 2-source mixing model, a Bayesian 3-isotope ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^2\text{H}$ ) 3-source mixing model, and a Bayesian 2-isotope ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) 3-source mixing model (Stock and Semmens, 2013; Erdozain,

2020). The first model, herein called H2, predicted the proportion of algal and FPOM food inputs, while the second model CNH3 and the third model CN3 predicted the proportion of algal, FPOM, and CPOM inputs (Erdozain, 2020). These models incorporate uncertainty in both food source isotopic values and trophic fractionation values. Before accepting the MixSIAR results, Gelman-Rubin and Geweke tests were used to determine the convergence of the three models on the posterior distributions (Erdozain, 2020). Fractionation values of  $0.4 \pm 1.20\text{‰}$  and  $2.3 \pm 1.61\text{‰}$  were chosen for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively, based on published data (McCutchan et al., 2003) and the cage experiment in Erdozain et al. (2019) (Erdozain, 2020).

## **2.5 Statistics**

Analyses of variance were used to assess the variation in fork length between catchments and stream sites within a single catchment, and subsequent Tukey post-hoc tests were used to identify significant pairwise comparisons. This was done for both the entire male and female sculpin dataset, and the female sculpin subset. Analyses of variance and Tukey post-hoc tests were run to assess variations in THg measured in whole body female sculpin.

Subsequently, mean THg concentrations measured in female sculpin were calculated for each site of each catchment, and linear regression analyses were used to assess the relationship between mean THg and the six catchment characteristics.

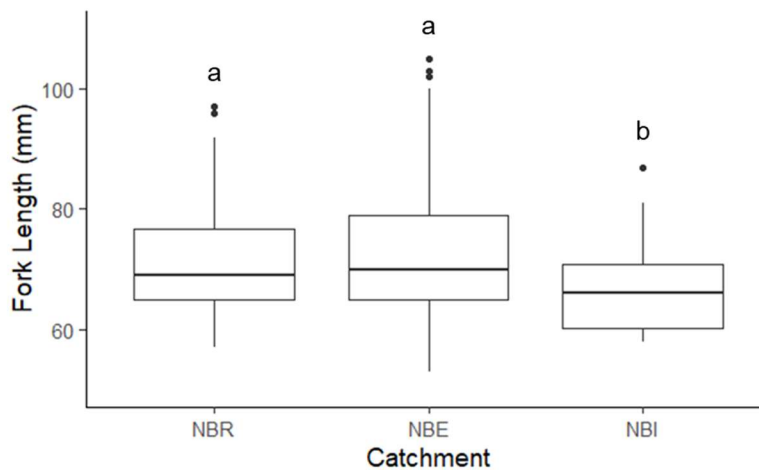
To assess the relationship between autochthony in sculpin diet and catchment characteristics, linear regression analyses were run between predicted algal inputs and each of the six characteristics, for each of the mixing models. Similarly, the relationship between THg levels measured in sculpin and autochthony in diet was assessed by running linear regression analyses between mean THg measured in each site and predicted algal inputs to diet, for each catchment and each mixing model (i.e. nine linear regression analyses, three for each mixing model).

### 3. Results

#### 3.1 Male and Female Slimy Sculpin

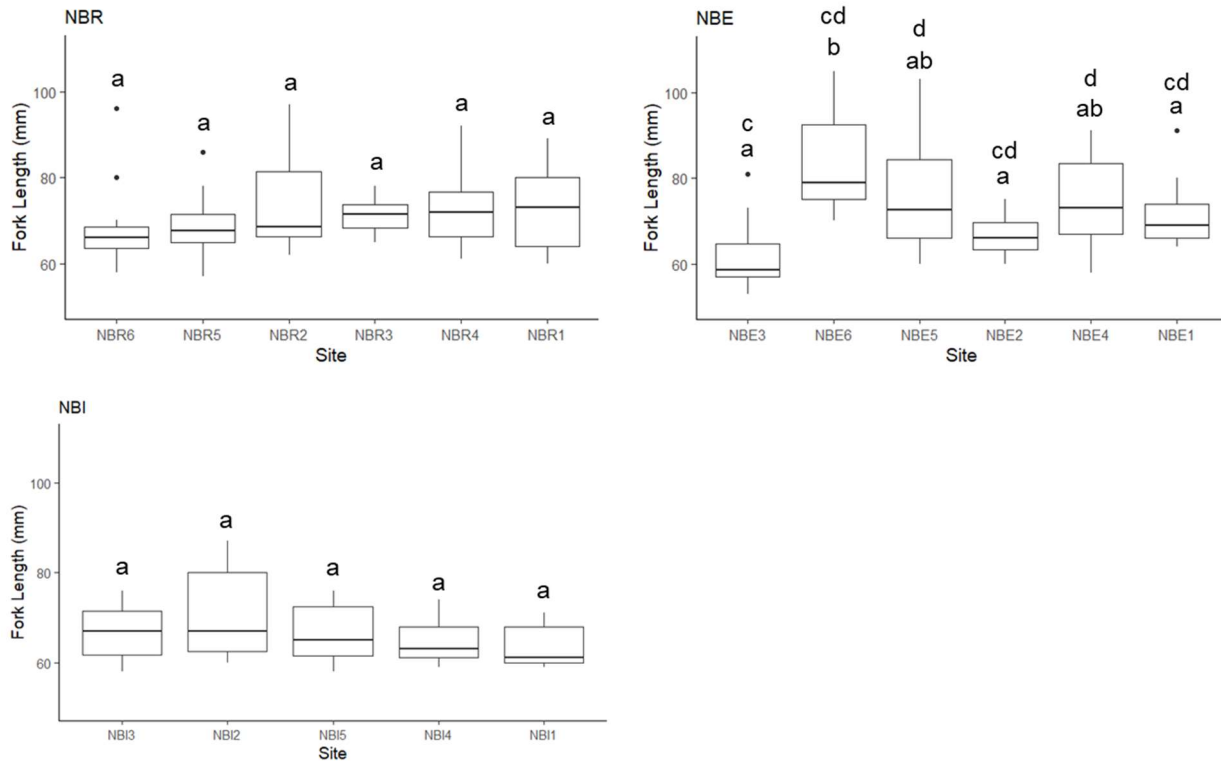
##### 3.1.1 Fork Lengths

The fork lengths of sculpin measured for THg varied between catchments (**Figure 2**). An analysis of variance showed that length significantly differed between catchments ( $F_{2,242} = 7.702$ ,  $p = 0.0006$ ). A Tukey post-hoc test showed that fork lengths in NBI were significantly shorter than lengths in NBR ( $p = 0.0135$ ) and NBE ( $p = 0.0004$ ). Fork lengths in NBR and NBE were not significantly different ( $p = 0.3622$ ).



**Figure 2:** Fork length (mm) of male and female slimy sculpin in three catchments (NBR, NBE, and NBI) and 17 streams sites in New Brunswick, in 2017. The center bolded line represents the mean. Catchments labelled with different letters are significantly different from one another.

Sculpin fork lengths also varied between sites within one catchment of the three catchments (**Figures 3a-c**). An analysis of variance showed that lengths varied significantly by site in NBE ( $F_{5,87} = 7.664$ ,  $p < 0.00001$ ), but was non-significant in NBR ( $F_{5,96} = 1.029$ ,  $p = 0.405$ ) and NBI ( $F_{4,45} = 1.274$ ,  $p = 0.294$ ). A Tukey post-hoc test showed that fish from NBE6 were significantly longer compared to NBE3 ( $p < 0.0001$ ), NBE2 ( $p < 0.0001$ ), and NBE1 ( $p = 0.0049$ ), while those from NBE3 was significantly shorter than NBE5 and NBE4 ( $p = 0.0188$  and  $0.0489$ , respectively). Note that the above analyses only included the fork lengths of sculpin analyzed for THg.

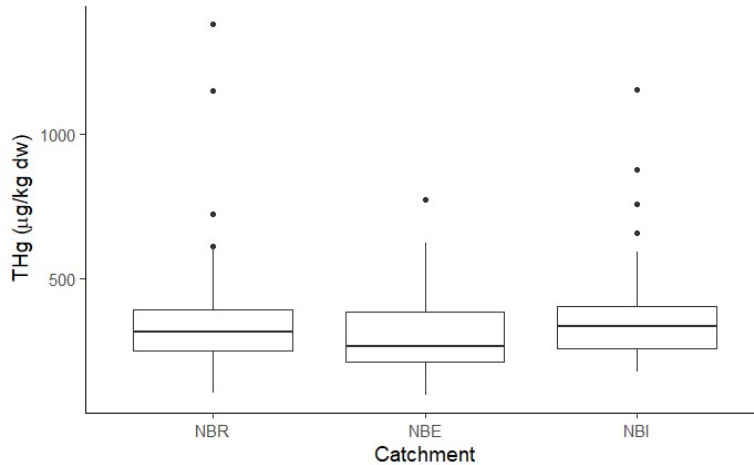


**Figures 3a-c:** Fork length (mm) of male and female slimy sculpin across 17 stream sites, in three catchments (NBR,  $n = 14-20/\text{site}$ ; NBE,  $n = 8-20/\text{site}$ ; and NBI,  $n = 7-14/\text{site}$ ) in New Brunswick and sampled in 2017. Data are ordered upstream to downstream. The center bolded line represents the mean. Within a catchment, sites labelled with different letters in a single row are significantly different from one another.

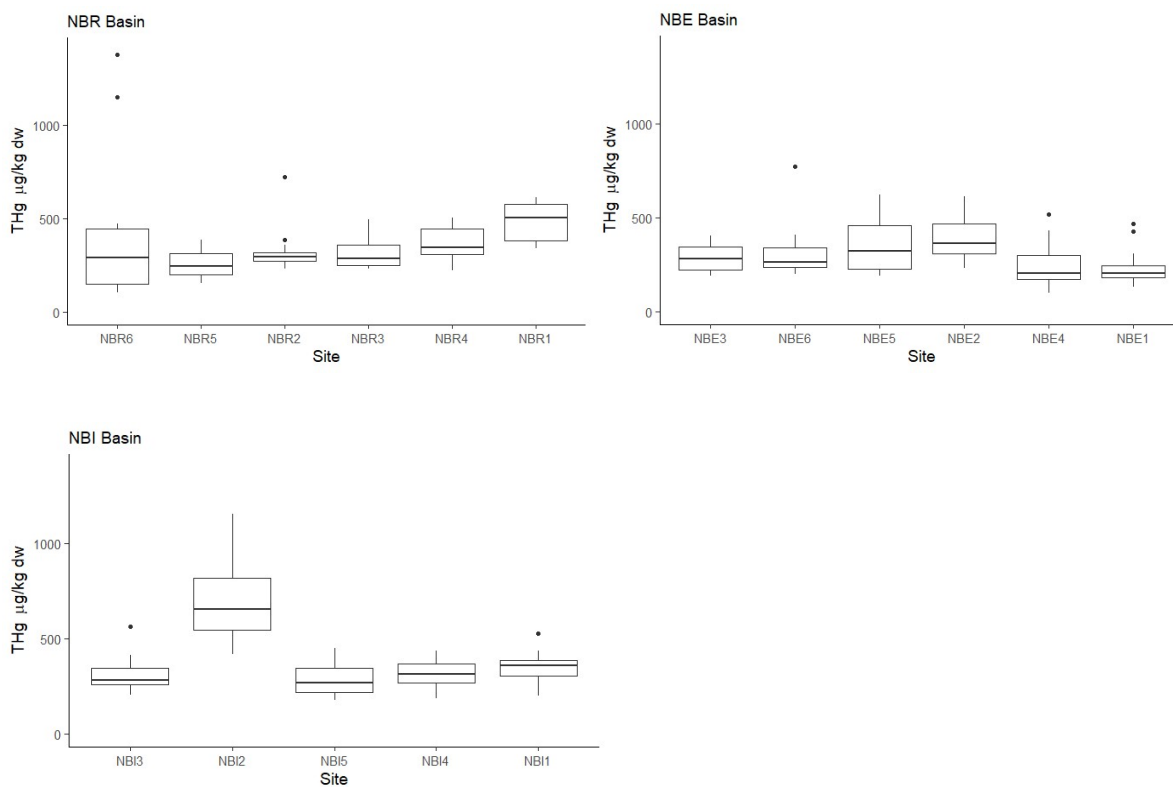
Linear regression analyses showed that THg concentrations were significantly related to fork length ( $p < 0.05$ ) in seven stream sites (NBR1, NBR2, NBR6, NBE5, NBE6, NBI2, and NBI5) (see **Appendix A** for summary of linear regression analyses of THg and fork length, for male and female sculpin).

### 3.1.2 Total Mercury

Total Hg measurements in male and female slimy sculpin varied little between catchments (**Figure 4**), but more within a catchment (**Figures 5a-c**). Slimy sculpin in NBE had the lowest overall mean THg measurement of  $308.77 \mu\text{g}/\text{kg dw}$ , while fish from NBI had the highest of  $369.96 \mu\text{g}/\text{kg dw}$ , and those from NBR were intermediate with  $354.60 \mu\text{g}/\text{kg dw}$  (ranges:  $99.03 - 772.84 \mu\text{g}/\text{kg dw}$  in NBE,  $177.44 - 1154.52 \mu\text{g}/\text{kg dw}$  in NBI, and  $104.83 - 1378.52 \mu\text{g}/\text{kg dw}$  in NBR).



**Figure 4:** THg ( $\mu\text{g}/\text{kg dw}$ ) whole-body tissue of male and female slimy sculpin collected in fall, 2017, from 17 stream sites in three catchments in New Brunswick (NBR, NBE, and NBI; see **Figure 1** for catchment locations). Mean THg is represented by the bolded line in the boxplot.

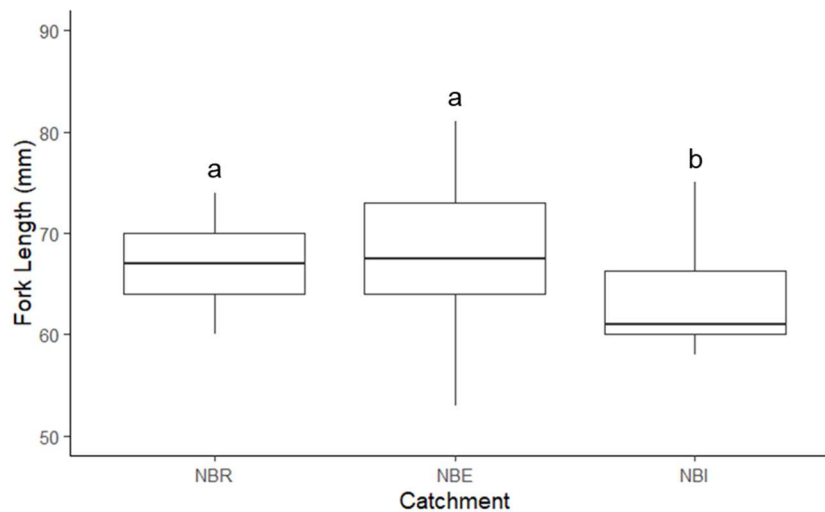


**Figures 5a-c:** THg ( $\mu\text{g}/\text{kg dw}$ ) in whole-body tissues of male and female slimy sculpin collected in fall, 2017, from 17 stream sites in three catchments in New Brunswick (NBR,  $n = 14\text{-}20/\text{site}$ ; NBE,  $n = 8\text{-}20/\text{site}$ ; and NBI,  $n = 7\text{-}14/\text{site}$ ; see **Figure 1** for locations). Data are ordered from upstream to downstream. Mean THg is represented by the bolded line in the boxplot.

## 3.2 Female Slimy Sculpin

### 3.2.1 Fork Lengths

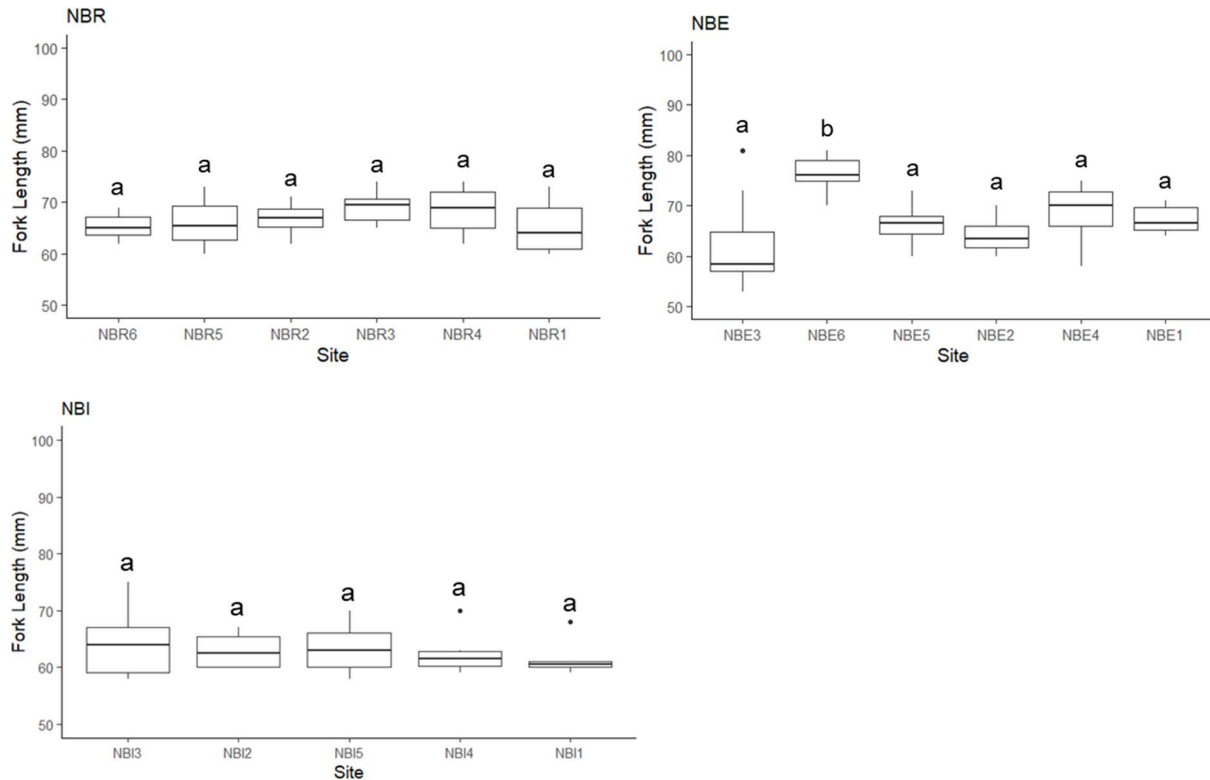
Fork lengths of female sculpin varied by catchment (**Figure 6**). An analysis of variance showed that fork length differed significantly among catchments ( $F_{2,143} = 9.512$ ,  $p = 0.0001$ ). A Tukey post-hoc test showed that fork lengths in NBI were significantly shorter compared to those measured in NBR ( $p = 0.0024$ ) and NBE ( $p = 0.0001$ ). Fork lengths in NBR and NBE were not significantly different from each other ( $p = 0.5484$ ).



**Figure 6:** Fork length (mm) of female slimy sculpin in three catchments (NBR, NBE, and NBI) and 17 streams sites in New Brunswick that were collected in 2017. The center bolded line represents the mean. Catchments labelled with different letters are significantly different from one another.

Fork lengths of female sculpin also varied by site (**Figures 7a-c**). Analyses of variance showed that fork length differed significantly among sites in only the NBE catchment ( $F_{5,50} = 8.348$ ,  $p < 0.0001$ ), while differences were nonsignificant in NBR ( $F_{5,52} = 1.811$ ,  $p = 0.127$ ) and NBI ( $F_{4,40} = 1.678$ ,  $p = 0.174$ ). A Tukey post-hoc test showed that fish lengths from NBE6 were significantly longer than those at all other sites in NBE (NBE1,  $p = 0.0029$ ; NBE2,  $p = 0.0001$ ; NBE3,  $p < 0.0001$ ; NBE4,  $p = 0.0198$ ; NBE5,  $p = 0.0010$ ). Note that the above analyses only included the fork lengths of sculpin analyzed for THg.

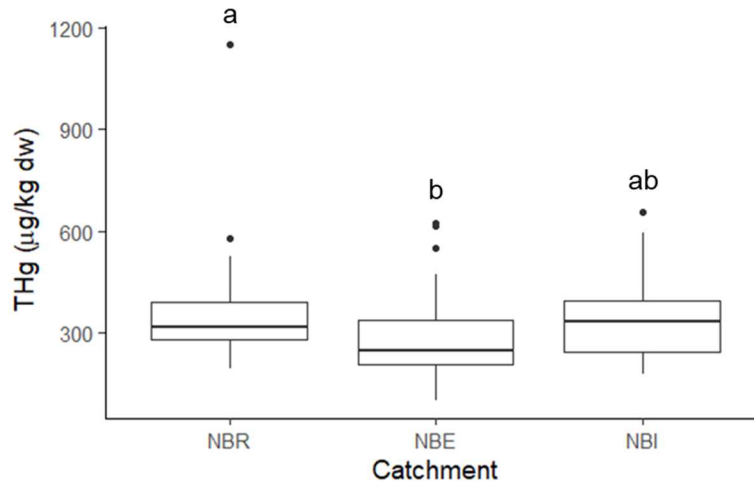




**Figures 7a-c:** Fork length (mm) of female slimy sculpin across 17 stream sites, in three catchments (NBR,  $n = 8-11/\text{site}$ ; NBE,  $n = 8-10/\text{site}$ ; and NBI,  $n = 4-9/\text{site}$ ) in New Brunswick, sampled in 2017. Data ordered upstream to downstream. The center bolded line represents the mean. Fork length of sites labelled with different letters are significantly different from one another.

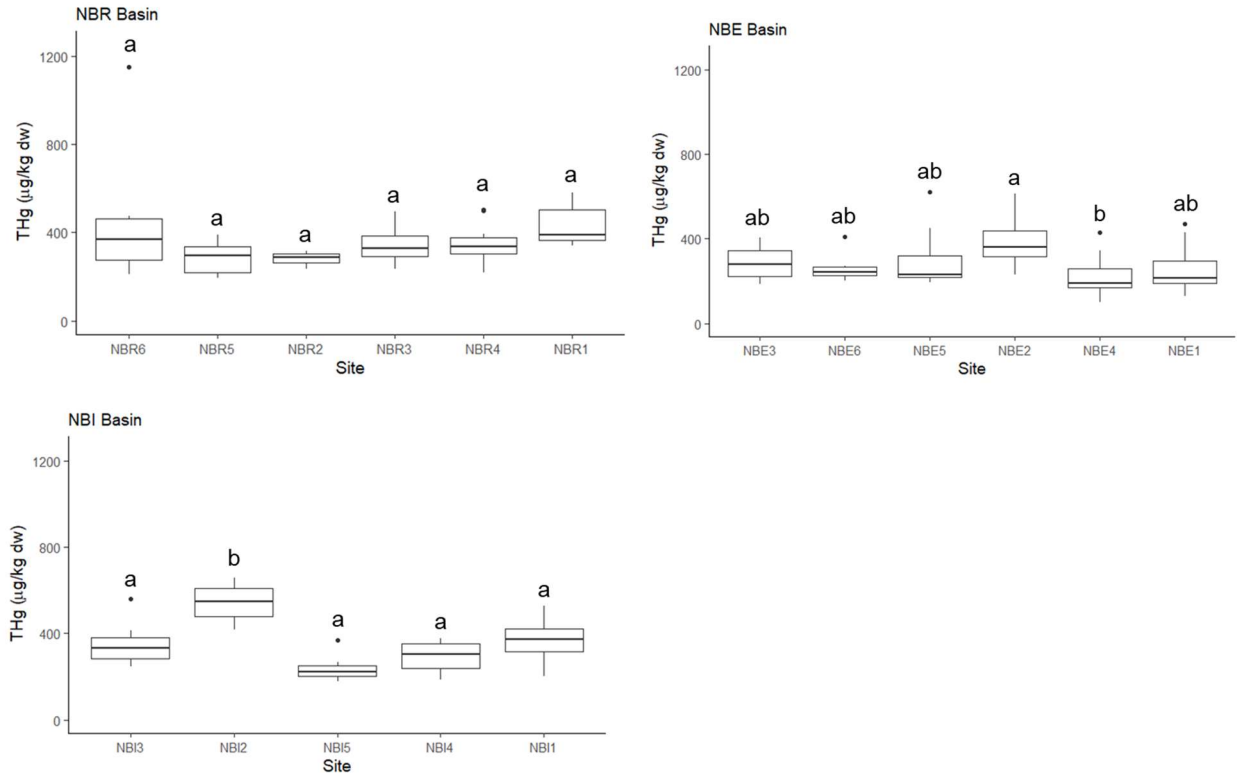
### 3.2.2 Total Mercury

Total Hg measured in whole bodies of female sculpin varied across catchments; the mean THg values were 351.72, 284.25, and 343.03  $\mu\text{g}/\text{kg dw}$  in NBR, NBE, and NBI, respectively (ranges: 194.75 – 1150.73  $\mu\text{g}/\text{kg dw}$  in NBR, 99.03 – 622.61  $\mu\text{g}/\text{kg dw}$  in NBE, and 177.43 – 656.31  $\mu\text{g}/\text{kg dw}$  in NBI) (**Figure 8**). An ANOVA showed that THg levels varied significantly between catchments ( $F_{2,143} = 4.509$ ,  $p = 0.0126$ ) and a Tukey post-hoc test showed that THg levels in NBR were significantly greater than those in NBE ( $p = 0.0141$ ), while the other two pairwise comparisons were nonsignificant (NBR-NBI:  $p = 0.9480$ ; NBE-NBI:  $p = 0.0945$ ).



**Figure 8:** THg ( $\mu\text{g}/\text{kg dw}$ ) in whole-body tissues of female slimy sculpin collected in fall, 2017, from 17 stream sites in three catchments in New Brunswick (NBR, NBE, and NBI). Mean THg is represented by the bolded line in the boxplot. Pairs of boxplots that do not share the same letter are significantly different.

Total Hg measurements also varied by site (**Figures 9a-c**). A one-way ANOVA demonstrated that stream site had a significant effect on THg concentration within all three catchments (NBR:  $F_{5,52} = 2.534$ ,  $p = 0.0398$ ; NBE:  $F_{5,50} = 2.572$ ,  $p = 0.038$ ; NBI:  $F_{4,27} = 7.723$ ,  $p = 0.0003$ ). A subsequent Tukey post-hoc indicated that there were no significant pairwise comparisons in NBR, while fish in NBE4 were significantly lower in THg compared with those from NBE2 ( $p = 0.0200$ ), and THg of sculpin from NBI2 were significantly higher than values in fish from each of the four other NBI sites (NBI1,  $p = 0.0448$ ; NBI3,  $p = 0.0130$ ; NBI4,  $p = 0.0019$ ; NBI5,  $p = 0.0001$ ).



**Figures 9a-c:** THg ( $\mu\text{g}/\text{kg dw}$ ) whole-body tissue of female slimy sculpin collected in fall, 2017, from 17 stream sites in three catchments in New Brunswick (NBR,  $n = 8-11/\text{site}$ ; NBE,  $n = 8-10/\text{site}$ ; and NBI,  $n = 4-9/\text{site}$ ). Data are ordered from upstream to downstream. Mean THg is represented by the bolded line in the boxplot. Letters above boxplots indicate significant pairwise comparisons.

Linear regression analyses showed that THg was not significantly related to fork length in any of the catchments, and within stream site, NBE2 was the only location where THg was significantly related to fork length ( $p = 0.0171$ ) (see **Appendix B** for summary of linear regression analyses of THg and fork length, for female sculpin). Because there was only one site that was significantly different from others in terms of fork length, only one site where fork length and THg had a significant relationship (compared to many sites in the male and female dataset, see **Appendix A**), and more consistent sample sizes across sites, the following analyses on the effects of catchment characteristics focussed on data for female sculpin only.

Overall, catchment and forest harvesting variables were not significantly related to THg measurements in female sculpin pooled from all three catchments. Linear regression analyses demonstrated that catchment area, total harvest, clear cut, crossing density,

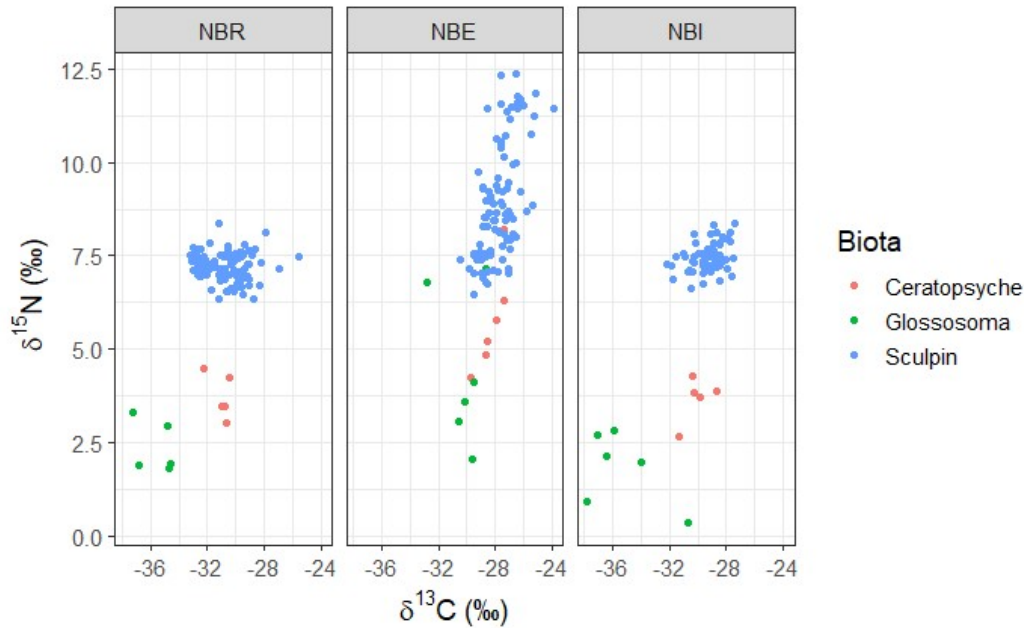
road density, and depth-to-water did not have a significant effect on mean THg (**Table 2**).

**Table 2:** Linear regressions analyses of the effect of five catchment characteristics on log-transformed mean THg ( $\mu\text{g}/\text{kg dw}$ ) measured in whole-body female sculpin sampled in fall, 2017, pooled across all three catchments (NBR, NBE, NBI) in New Brunswick.

| Catchment Variable       | df | F-value | p-value |
|--------------------------|----|---------|---------|
| Area ( $\text{km}^2$ )   | 1  | 0.122   | 0.7316  |
| Total harvest (% , 10 y) | 1  | 0.015   | 0.9036  |
| Clear cut (% , 10 y)     | 1  | 0.643   | 0.435   |
| Crossing density (#/km)  | 1  | 0.208   | 0.655   |
| Road density (m/ha)      | 1  | 1.523   | 0.2362  |
| Depth-to-water (m)       | 1  | 0.183   | 0.6751  |
| Total                    | 15 |         |         |

### 3.3 Stable Isotopes

Similar to THg measurements, stable isotope values measured in male and female slimy sculpin varied between catchments. NBR had the lowest mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values and highest mean  $\delta^2\text{H}$  value ( $-30.71\text{‰}$ ,  $7.19\text{‰}$ , and  $-189.27\text{‰}$ , respectively), while sculpin from NBE had the highest mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values and lowest mean  $\delta^2\text{H}$  value ( $-27.68\text{‰}$ ,  $9.00\text{‰}$  and  $-200.76\text{‰}$ , respectively), and sculpin from NBI had mean  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^2\text{H}$  values of  $-29.28\text{‰}$ ,  $7.46\text{‰}$ , and  $-190.32\text{‰}$ , respectively ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values displayed in **Figure 10**). The invertebrates *Glossosoma* and *Ceratopsyche* had mean  $\delta^{15}\text{N}$  values of  $2.39\text{‰}$  and  $3.75\text{‰}$  respectively in NBR,  $4.46\text{‰}$  and  $5.77\text{‰}$  in NBE, and  $1.84\text{‰}$  and  $3.69\text{‰}$  in NBI (**Figure 10**). Stable isotope ratios of nitrogen and carbon for female sculpin follow a similar pattern between catchments (see **Appendix C** for scatterplot of relationship between  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  for female sculpin).



**Figure 10:** Values of  $\delta^{15}\text{N}$  (‰) and  $\delta^{13}\text{C}$  (‰) measured in male and female slimy sculpin muscle fillets (NBR:  $n = 14\text{-}20/\text{site}$ ; NBE:  $n = 8\text{-}20/\text{site}$ ; NBI:  $n = 11\text{-}15/\text{site}$ ), *Glossosoma* invertebrates ( $n = 1/\text{site}$ , in each catchment), and *Ceratopsyche* invertebrates ( $n = 1/\text{site}$ , in each catchment) sampled in the fall of 2017 from 17 stream sites in three managed catchments (NBR, NBE, and NBI) in New Brunswick.

For all three calculations of trophic level, sculpin from NBE had the lowest calculated trophic level, while those from NBI had the highest (**Table 3**).

**Table 3:** Trophic level of male and female slimy sculpin calculated using mean  $\delta^{15}\text{N}$  of *Glossosoma*, *Ceratopsyche*, or the mean  $\delta^{15}\text{N}$  of both invertebrates combined as the baseline organism. Samples were collected in the fall of 2017 from 17 stream sites in three managed catchments (NBR, NBE, and NBI) in New Brunswick.

| Catchment | Baseline Organism   | Trophic Level of Sculpin |
|-----------|---------------------|--------------------------|
| NBR       | <i>Glossosoma</i>   | 4.08                     |
|           | <i>Ceratopsyche</i> | 3.49                     |
|           | Both                | 3.79                     |
| NBE       | <i>Glossosoma</i>   | 3.96                     |
|           | <i>Ceratopsyche</i> | 3.39                     |
|           | Both                | 3.68                     |
| NBI       | <i>Glossosoma</i>   | 4.44                     |
|           | <i>Ceratopsyche</i> | 3.64                     |
|           | Both                | 4.04                     |

\*See Equation 2 for how to calculator the trophic level of consumers.

### 3.4 Food Web Mixing Models

The predicted mean median inputs to sculpin diet varied between mixing models and catchments (**Table 4; Figures 11a-i**). See **Appendix C** for quantile boxplots of percent food inputs predicted by the mixing models for the three catchments.

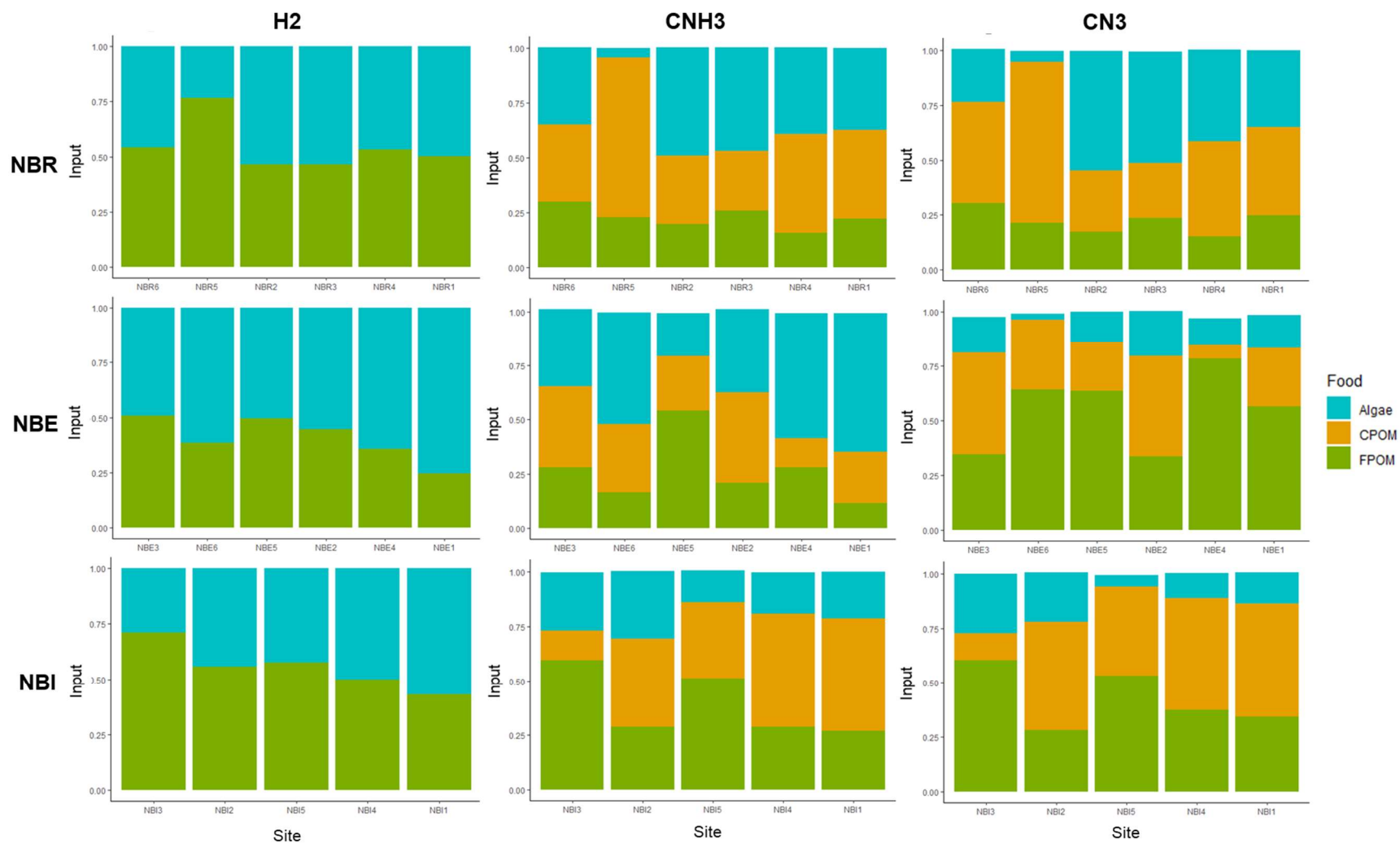
**Table 4:** Mean median food contributions to sculpin diet predicted by three Bayesian mixing models for three managed catchments (NBR, NBE, NBI), using values of  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^2\text{H}$ .

| Model | Catchment | Mean Predicted Median Food Input |       |       |
|-------|-----------|----------------------------------|-------|-------|
|       |           | Algae                            | FPOM  | CPOM  |
| H2    | NBR       | 0.454                            | 0.546 | -     |
|       | NBE       | 0.594                            | 0.406 | -     |
|       | NBI       | 0.445                            | 0.555 | -     |
| CNH3  | NBR       | 0.354                            | 0.227 | 0.421 |
|       | NBE       | 0.447                            | 0.265 | 0.288 |
|       | NBI       | 0.225                            | 0.390 | 0.386 |
| CN3   | NBR       | 0.354                            | 0.220 | 0.427 |
|       | NBE       | 0.136                            | 0.551 | 0.300 |
|       | NBI       | 0.161                            | 0.426 | 0.414 |

In the H2 model, NBR had mostly consistent proportions of predicted algal and FPOM inputs across all stream sites. Meanwhile, fish from NBE and NBI had increasing FPOM inputs and decreasing algal inputs from upstream to downstream.

In comparison, the CNH3 model also considered CPOM as a terrestrial input and predicted a larger terrestrial component in the diets of sculpin, compared to two of the three catchments (NBE and NBI) in the H2 model. In NBR, the proportion of contribution between the three food sources, and between terrestrial versus aquatic sources, was similar across sites. In NBE and NBI, proportions fluctuated greatly from site to site – although in NBI, consistently large terrestrial contributions were defined by a shift from dominant FPOM to CPOM inputs, partly at the expense of algal inputs.

Finally, the CN3 model, which does not use  $\delta^2\text{H}$  values, predicted similar proportions of dietary inputs for the sculpin as those from the CNH3 model. One difference was the smaller contribution of algae in NBE and NBI that is predicted by the CN3 model. Thus, without  $\delta^2\text{H}$  values (compared to with, in the H2 and CNH3 models), the CN3 model predicts greater terrestrial contributions in the diet of sculpin from NBE and NBI.



**Figures 11a-i:** The median proportional input of algae, CPOM, and FPOM in male and female slimy sculpin sampled from NBR, NBE, and NBI, predicted by three Bayesian mixing models (H2, CNH3, and CN3), for each of 17 stream sites in New Brunswick ordered from upstream to downstream.



### 3.4.1 Relationship Between Catchment Characteristics and Algal Inputs

Linear regression analyses showed that of the six catchment characteristics (when pooled across all stream sites of all three catchments), only catchment area had a significant effect on the median proportional algal input to sculpin diet predicted by the H2 mixing model (**Table 5**).

**Table 5:** Summary of linear regression analyses of median % algae inputs to sculpin diets from Bayesian modeling verses six catchment characteristics, pooled across five to six stream sites within each of three managed catchments (NBR, NBE, NBI) in New Brunswick.

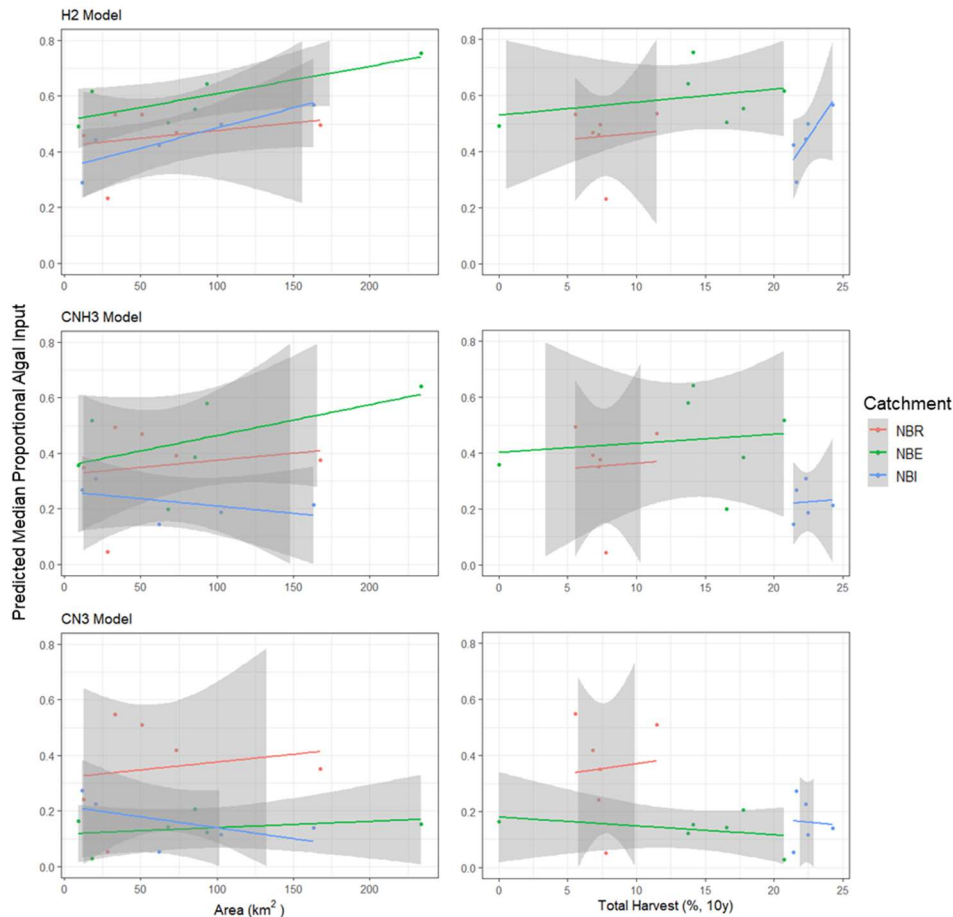
| Model | Variable                | Df | F-value | p-value |
|-------|-------------------------|----|---------|---------|
| H2    | Area (km <sup>2</sup> ) | 1  | 7.977   | 0.0128* |
|       | Total harvest (%)       | 1  | 0.072   | 0.7919  |
|       | Clearcut (%)            | 1  | 0.7816  | 0.391   |
|       | Crossing density (#/km) | 1  | 0.0241  | 0.879   |
|       | Road density (m/ha)     | 1  | 1.471   | 0.2440  |
|       | DTW (m)                 | 1  | 0.315   | 0.5829  |
|       | Total                   | 15 |         |         |
| CNH3  | Area                    | 1  | 1.096   | 0.3117  |
|       | Total harvest           | 1  | 0.916   | 0.3537  |
|       | Clearcut                | 1  | 1.457   | 0.2461  |
|       | Crossing density        | 1  | 2.52    | 0.133   |
|       | Road density            | 1  | 0.0566  | 0.815   |
|       | DTW                     | 1  | 0.5251  | 0.4798  |
|       | Total                   | 15 |         |         |
| CN3   | Area                    | 1  | 0.074   | 0.7893  |
|       | Total harvest           | 1  | 3.665   | 0.0748  |
|       | Clearcut                | 1  | 0.3612  | 0.5568  |
|       | Crossing density        | 1  | 1.629   | 0.2213  |
|       | Road density            | 1  | 3.058   | 0.1008  |
|       | DTW                     | 1  | 6.473   | 0.0225* |
|       | Total                   | 15 |         |         |

\*significant p-value ( $\alpha = 0.05$ ).

When considering algal inputs in each catchment separately, in the H2 model, predicted algal inputs in all three catchments shared a positive relationship with catchment area (**Figures 12a-f**). In the CNH3 model, algal reliance for sculpin in NBE and NBI shared a positive linear relationship with area, but there was a negative relationship in NBR.

Finally, in the CN3 model, algal inputs for sculpin NBR and NBE shared a positive relationship with area, and a negative relationship in NBI.

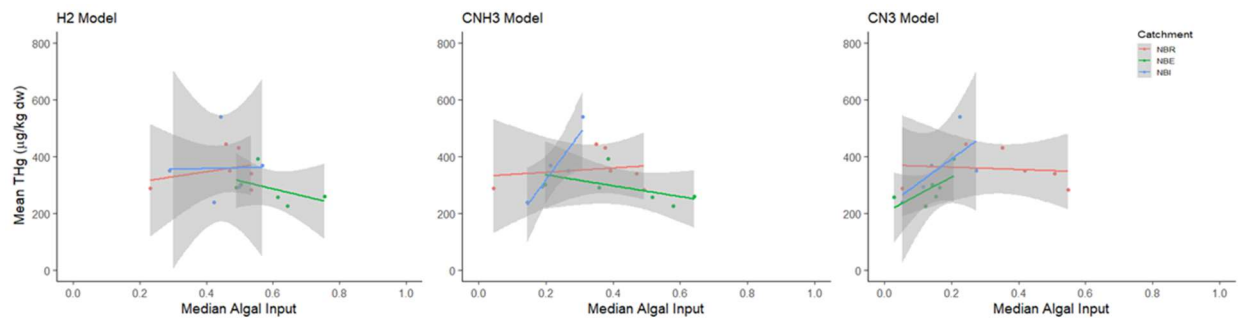
In both the H2 and CNH3 models, proportional algal inputs shared a positive relationship with total harvest in all catchments (**Figures 12a-f**). Meanwhile, in the CN3 model, proportional algal inputs in NBR shared a positive relationship with total harvest, and a negative relationship in NBE and NBI.



**Figures 12a-f:** Relationship between median proportional algal input to sculpin diet predicted by three Bayesian mixing models (H2, CNH3, and CN3) and catchment area (km<sup>2</sup>) or total harvest (% 10 y), for sculpin sampled in fall, 2017, from 17 stream sites in three catchments (NBR, NBE, and NBI) in New Brunswick. Lines represent lines of best fit.

### 3.4.1 Relationship Between THg and Algal Inputs

Simple linear regression analyses showed that mean THg values measured in female sculpin were positively and significantly related to their algal inputs as predicted by the CNH3 model, for fish from NBI ( $F_{1,3} = 14.92$ ,  $p = 0.0307$ ) (**Figure 13a-c**). Mean THg levels were not significantly related to algal inputs in any other catchment of any of the three mixing models ( $p > 0.05$ ). See **Appendix E** for summary of linear regression analyses.



**Figure 13a-c:** Relationship between mean THg ( $\mu\text{g}/\text{kg dw}$ ) measured in female sculpin and median proportional algal input to sculpin diet predicted by three Bayesian models (H2, CNH3, and CN3) for sculpin sampled in fall, 2017, from 17 stream sites in three catchments (NBR, NBE, and NBI) in New Brunswick. Lines represent lines of best fit.

## **4. Discussion**

### **4.1 Total Mercury Trends in Female Sculpin**

The results showed that THg levels measured in female sculpin did vary between catchments and, in NBE and NBI, between some stream sites. The significantly high THg levels in sculpin of NBR, relative to those in NBE, cannot be attributed to fork length, as sculpin in NBR and NBE were not significantly different in length and THg was not significantly related to fork length in any of the catchments. Differences between stream sites also cannot be attributed to fork length, as there was only one site (in NBE) that had significantly longer sculpin, and THg levels in these sculpin were not significantly different from any other NBE site. In addition, THg was not significantly related to fork length in any of the sites.

Out of the 245 slimy sculpin measured for whole body THg, 185 fish (82 in NBR, 62 in NBE, and 42 in NBI) had wet weight concentrations greater than 62  $\mu\text{g}/\text{kg}$  ww (41 fish had more than two times this concentration). This value represents the Canadian tissue residue guideline (TRG) for kingfishers and is the maximum MeHg concentration in prey that can be safely consumed by these birds, which prefer relatively small fish (10-15 cm) (Environment Canada, 2003). However, THg measurements include both organic and inorganic species. Although it is often assumed that MeHg is the predominant form of Hg in fish tissue, Hg speciation between inorganic and organic forms varies greatly depending on the type of sample (e.g. inclusion of organs such as the liver), trophic level, and species (Lescord et al., 2018; Barst et al., 2013; Harris, Pickering and George, 2003), and MeHg does not necessarily reflect the majority of Hg present in a sample. Regardless, if we assumed a lower proportion of MeHg as observed in smaller species and smaller, younger fish, such as 70% of THg (Lescord et al., 2018; note MeHg was measured in muscle tissue), 100 fish would still exceed the TRG for kingfishers.

#### 4.2 Variations in Sculpin Diet and Trophic Level

Results showed that *Ceratopsyche* invertebrates, *Glossosoma* invertebrates, and sculpin from NBE had the highest mean  $\delta^{13}\text{C}$  values, and these sculpin also had the lowest trophic levels calculated (regardless of the baseline organism used).

Greater terrestrial matter in NBE streams could help explain the more enriched  $\delta^{13}\text{C}$  values. The  $\delta^{13}\text{C}$  values of NBE invertebrates and sculpin are close to or at  $-28\text{‰}$ , which is an established value for terrestrial plant matter (Finlay, 2001). While *Glossosoma* (i.e. scrapers) have been observed to preferentially consume the algal components of biofilm (Erdozain et al., 2018), higher levels of CPOM, FPOM, and allochthonous DOM (likely to be characterized by a more humic composition that is preferentially decomposed by soil microbes and is  $^{13}\text{C}$ -enriched compared to less decomposed terrestrial matter) (Cleveland et al., 2004)), could lead to the incorporation of more terrestrial matter into the diet. Terrestrial matter could also be incorporated into diet by consuming heterotrophic bacteria, which have been observed to benefit from highly humic inputs (Erdozain et al., 2018). In addition, the greater input of terrestrial matter in harvested catchments could alter the composition and energy sources of the benthic invertebrate community. For example, decreases in scraper abundance and increases in the abundance of shredders, whose diet is more terrestrial-dominated, have been documented in streams of managed catchments and are often positively, indirectly, related to road development and logging, which facilitate the delivery of more inorganic and organic sediments and terrestrial debris into the streams (Stone and Wallace, 1998; Erdozain et al., 2018). In turn, sculpin diet could be comprised of a greater proportion of benthic invertebrates that benefit from more terrestrial debris, such as primary consumer shredders, and this would result in enriched  $\delta^{13}\text{C}$  values and a lower calculated trophic level despite enriched  $\delta^{15}\text{N}$  values for invertebrates. Without samples of shredders, however, this cannot be said more conclusively.

There are several potential explanations for the enrichment of  $\delta^{15}\text{N}$  for *Ceratopsyche* invertebrates, *Glossosoma* invertebrates, and sculpin from NBE. If the invertebrates were consuming more heterotrophic bacteria, theoretically this would enrich their  $\delta^{15}\text{N}$  values (compared to consuming primary producers). Another potential explanation for

the  $\delta^{15}\text{N}$  enrichment is that NBE streams are receiving external inputs of nitrogen, such as human waste from septic systems in the catchment. Human waste can be a source of nitrogen, phosphorus, and several other nutrients and organic compounds (Lusk et al., 2017). Algae would also benefit from this input, but with low light availability in smaller streams, this benefit could be limited (Greenwood and Rosemond, 2005), thus preventing higher autochthony in invertebrate and sculpin diets.

Higher levels of decomposed allochthonous materials could help to explain the significantly lower THg levels measured in sculpin in NBE, compared to those in NBR. There is evidence that high levels of terrestrial-derived DOM, which tends to be more humic and complex compared to stream-derived DOM, in water is related to lower levels of THg (Bravo et al., 2018) and MeHg in stream water (Soerensen et al., 2017; Bravo et al., 2018), and reduced MeHg bioaccumulation in algae (Leugen, Fisher, and Bergamaschi, 2012), possibly through mechanisms that reduce Hg (II) bioavailability to methylating microbes and enhance MeHg photodemethylation. Similarly, relatively lower levels of terrestrial-derived DOM in NBR streams could help explain the higher THg levels measured in sculpin in NBR. Sculpin in NBR had the most depleted mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values ( $-30.71\text{‰}$  and  $7.19\text{‰}$ , respectively). These values could reflect a greater algal reliance in sculpin diet, potentially due to relatively low levels of allochthonous material, such as CPOM, FPOM, and terrestrial-derived DOM that can help reduce THg and MeHg levels in water and biota.

However, the reason for potentially greater terrestrial debris in NBE streams is unclear. Streams in managed catchments have been observed to have greater biomass of biofilm, DOM humification, and allochthony in benthic invertebrate diets, compared to streams in less intensely managed catchments (Erdozain et al., 2018; Erdozain et al., 2019). However, similar patterns of allochthony in benthic invertebrates diet and sculpin diet and THg were not observed in NBI streams, and contrary to predictions, no catchment characteristics – including those related to forestry – were significantly related to sculpin THg levels in any of the catchments. Thus, it is assumed that NBE streams naturally have higher levels of terrestrial debris.

In addition, if there were indeed higher levels of DOM in NBE streams that were also contributing to lower THg levels (and in contrast, lower levels in NBR streams that were related to higher THg levels), one might expect to see diet comprised largely of CPOM and FPOM in sculpin from NBE and NBI, as predicted by the Bayesian mixing models. Results from the H2 mixing model showed that there was an increase in algae and decrease in FPOM contributions to diet in sculpin from NBE and NBI, from upstream to downstream. Meanwhile, algal and FPOM inputs to sculpin diets in NBR remained relatively consistent across sites. An increase in allochthonous contributions to stream food webs has been observed with greater forest management intensity (Erdozain et al., 2019). In general, however, both the H2 and CNH3 models predicted higher FPOM inputs in NBR and NBI, than NBE. In addition, mean THg measured in female sculpin was only significantly (positively) related to algal inputs predicted by the CNH3 model for the NBI catchment. Thus, other factors might be more important in explaining the variations in THg levels in sculpin from NBR and NBE.

Results from the mixing models also showed that mixing models that included  $\delta^2\text{H}$  values (H2 and CNH3 models) predicted higher levels of algae in sculpin diet, compared to the CN3 model which did not use  $\delta^2\text{H}$  values, and this is most evident for sculpin in NBE. This bias has been observed in several other studies using mixing models and it has been proposed that this could be due to the relatively low  $\delta^2\text{H}$  values of lipids, which would imply algal inputs and lead to an underestimation of terrestrial-derived resources (Erdozain et al., 2019; Syväranta et al., 2016).

## **5. Conclusions**

The purpose of this study was to investigate the effects of forest management and harvesting on spatial trends of Hg contamination and food resource contributions to sculpin diet, in stream sites in three catchments managed at different intensities. It was hypothesized that disturbance caused by forestry activity affects the spatial distribution of Hg contamination in an upstream-downstream gradient, and that forestry activity affects the contribution of food sources to sculpin diet. The results suggest that forest management does affect Hg contamination in sculpin and resource contributions to sculpin diet.

This study provides observations that the forest management could be related to the origin and level of organic matter in streams, which can subsequently affect fish diet and Hg accumulated in fish, and demonstrates some of the challenges that come with larger spatial considerations. The results of this study contribute to a better understanding of how forest management and harvesting affect Hg contamination across catchment-sized scales. This can be used by government and industry leaders to evaluate the efficacy of best management practices that aim to reduce forestry impacts on streams, and thus protect aquatic biota and human consumers.



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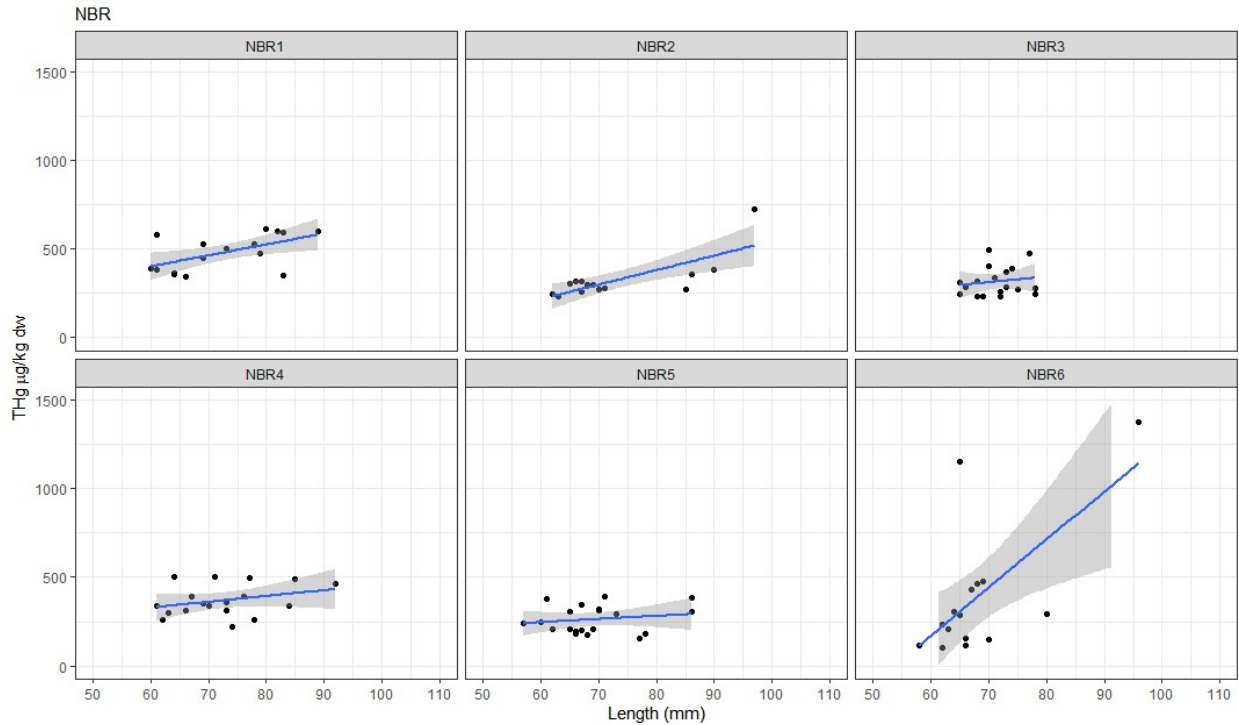
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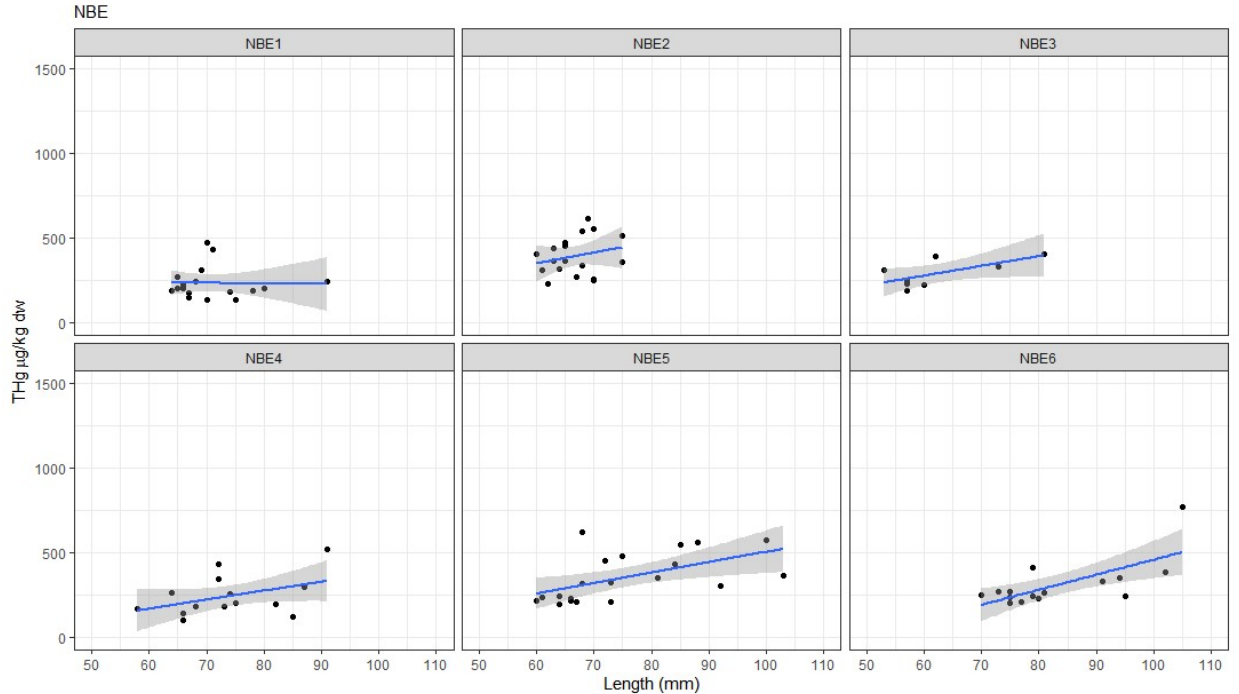
**Appendices****Appendix A: Relationship between THg and fork length, male and female fish****Table 6:** Summary of linear regression analyses of relationship between THg ( $\mu\text{g}/\text{kg}$  dw) and fork length (mm) of male and female slimy sculpin collected from 17 stream sites in three catchments (NBR, NBE, and NBI) in New Brunswick in 2017

| <b>Catchment</b> | <b>Site</b> | <b><i>F</i><sub>#, #</sub></b> | <b><i>F</i>-value</b>  | <b><i>p</i>-value</b> |
|------------------|-------------|--------------------------------|------------------------|-----------------------|
| NBR              | NBR1        | 1, 15                          | 7.243                  | 0.0168*               |
|                  | NBR2        | 1, 12                          | 15.87                  | 0.0018*               |
|                  | NBR3        | 1, 16                          | 0.413                  | 0.5294                |
|                  | NBR4        | 1, 16                          | 1.698                  | 0.2109                |
|                  | NBR5        | 1, 18                          | 0.595                  | 0.4506                |
|                  | NBR6        | 1, 13                          | 9.833                  | 0.0079*               |
| NBE              | NBE1        | 1, 15                          | 0.008                  | 0.9279                |
|                  | NBE2        | 1, 16                          | 1.005                  | 0.3311                |
|                  | NBE3        | 1, 6                           | 5.065                  | 0.0654                |
|                  | NBE4        | 1, 13                          | 3.212                  | 0.0964                |
|                  | NBE5        | 1, 18                          | 7.849                  | 0.0118*               |
|                  | NBE6        | 1, 13                          | 11.75                  | 0.0045*               |
| NBI              | NBI1        | 1, 7                           | 0.444                  | 0.5264                |
|                  | NBI2        | 1, 5                           | 31.21                  | 0.0025*               |
|                  | NBI3        | 1, 12                          | $2.958 \times 10^{-5}$ | 0.9957                |
|                  | NBI4        | 1, 7                           | 0.735                  | 0.4198                |
|                  | NBI5        | 1, 9                           | 9.446                  | 0.0133*               |

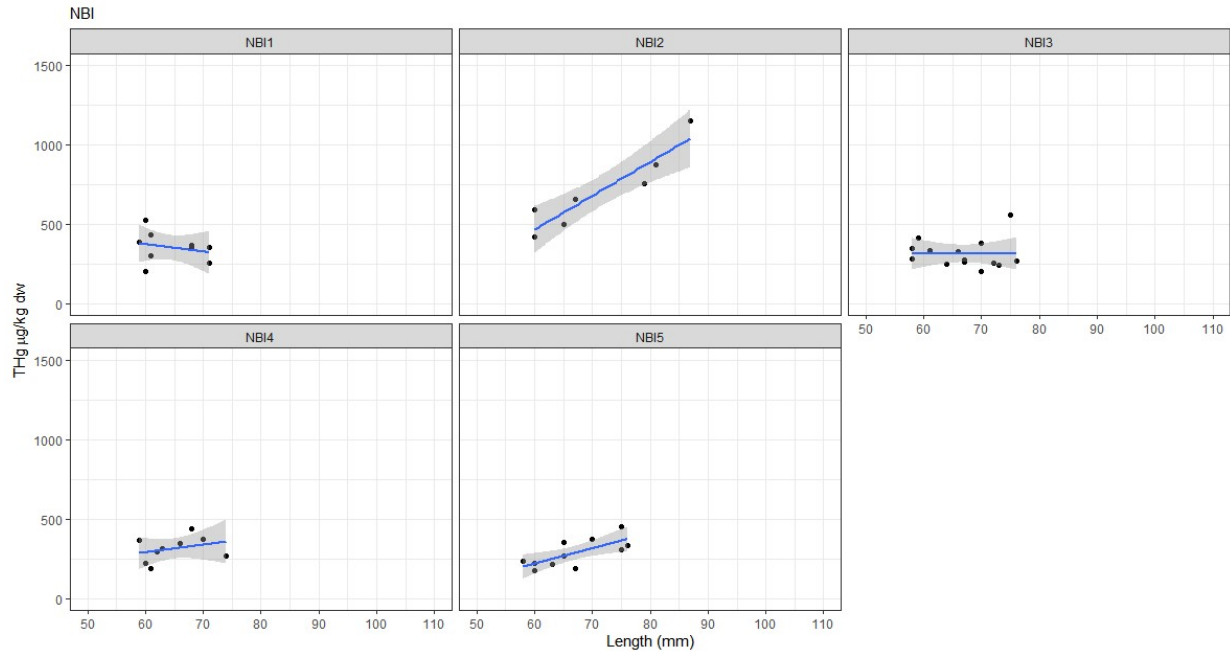
\*Relationship is significant,  $p < 0.05$ .



**Figures 14a-f:** The relationship between THg ( $\mu\text{g/kg dw}$ ) and fork length (mm) for male and female sculpin sampled from six NBR stream sites, located in New Brunswick. Line represents line of best fit.



**Figures 15a-f:** The relationship between THg ( $\mu\text{g/kg dw}$ ) and fork length (mm) for male and female sculpin sampled from six NBE stream sites, located in New Brunswick. Line represents line of best fit.



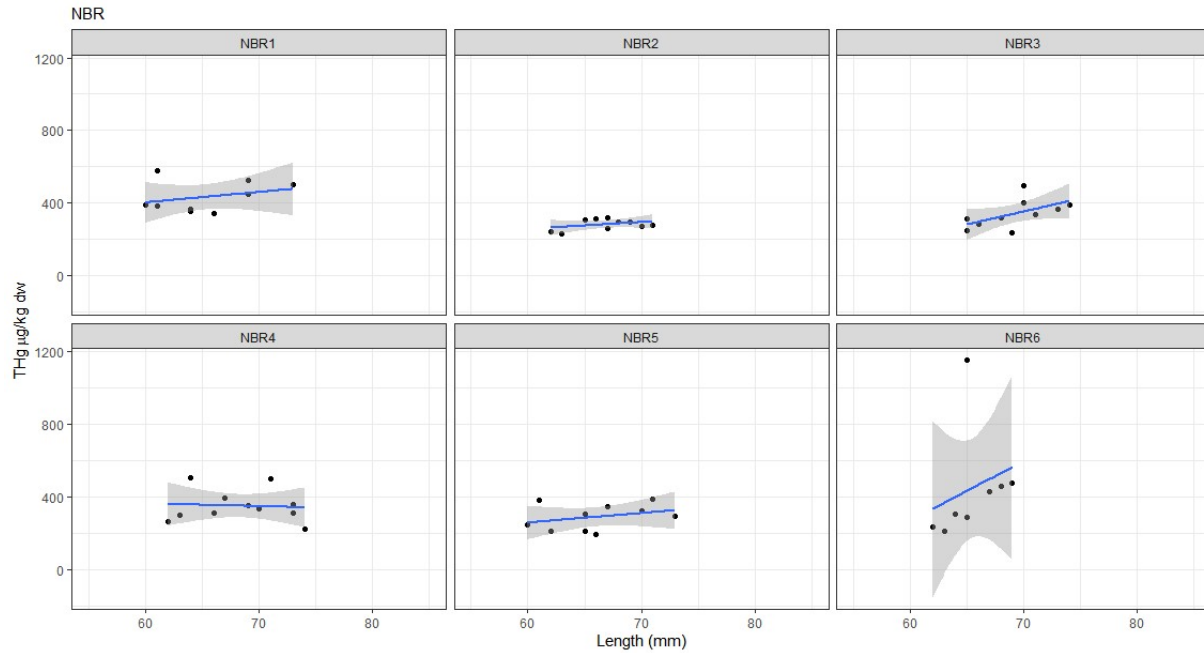
**Figures 16a-e:** The relationship between THg ( $\mu\text{g}/\text{kg dw}$ ) and fork length (mm) for male and female sculpin sampled from five NBI stream sites, located in New Brunswick. Line represents line of best fit.

**Appendix B: Relationship between THg and fork length, female fish**

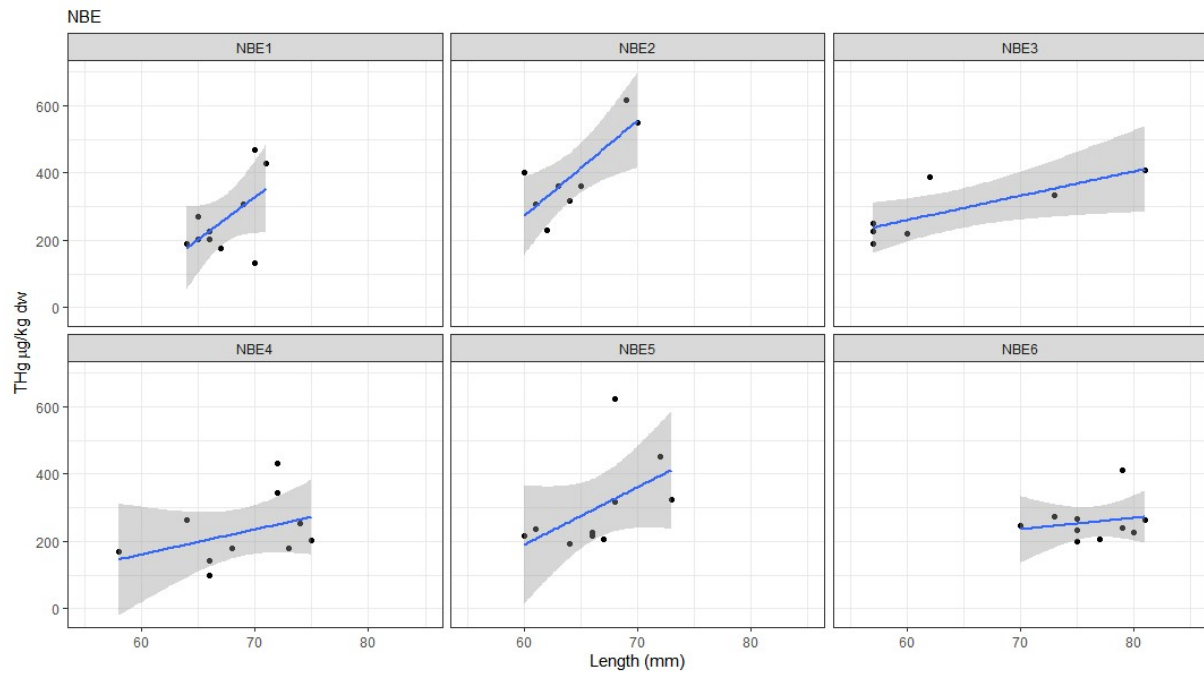
**Table 7:** Summary of linear regression analyses of relationship between THg ( $\mu\text{g}/\text{kg}$  dw) and fork length (mm) of female slimy sculpin collected from 17 stream sites in three catchments (NBR, NBE, and NBI) in New Brunswick in 2017

| <b>Catchment</b> | <b>Site</b> | <b><i>F</i><sub>#, #</sub></b> | <b><i>F</i>-value</b> | <b><i>p</i>-value</b> |
|------------------|-------------|--------------------------------|-----------------------|-----------------------|
| NBR              | -           | 1, 56                          | 0.362                 | 0.5499                |
| NBE              | -           | 1, 54                          | 1.28                  | 0.2629                |
| NBI              | -           | 1, 30                          | 2.227                 | 0.1461                |
| NBR              | NBR1        | 1, 7                           | 0.668                 | 0.4406                |
|                  | NBR2        | 1, 8                           | 1.378                 | 0.2742                |
|                  | NBR3        | 1, 8                           | 3.837                 | 0.0858                |
|                  | NBR4        | 1, 9                           | 0.049                 | 0.83                  |
|                  | NBR5        | 1, 8                           | 0.894                 | 0.3722                |
|                  | NBR6        | 1, 6                           | 0.458                 | 0.5238                |
| NBE              | NBE1        | 1, 8                           | 3.735                 | 0.0894                |
|                  | NBE2        | 1, 6                           | 10.67                 | 0.0171*               |
|                  | NBE3        | 1, 6                           | 5.065                 | 0.0654                |
|                  | NBE4        | 1, 8                           | 1.512                 | 0.2538                |
|                  | NBE5        | 1, 8                           | 2.925                 | 0.1256                |
|                  | NBE6        | 1, 8                           | 0.322                 | 0.5858                |
| NBI              | NBI1        | 1, 4                           | 0.012                 | 0.9183                |
|                  | NBI2        | 1, 2                           | 0.791                 | 0.4676                |
|                  | NBI3        | 1, 7                           | 2.727                 | 0.1426                |
|                  | NBI4        | 1, 4                           | 1.114                 | 0.3508                |
|                  | NBI5        | 1, 5                           | 2.909                 | 0.1488                |

\*Relationship is significant,  $p < 0.05$ .

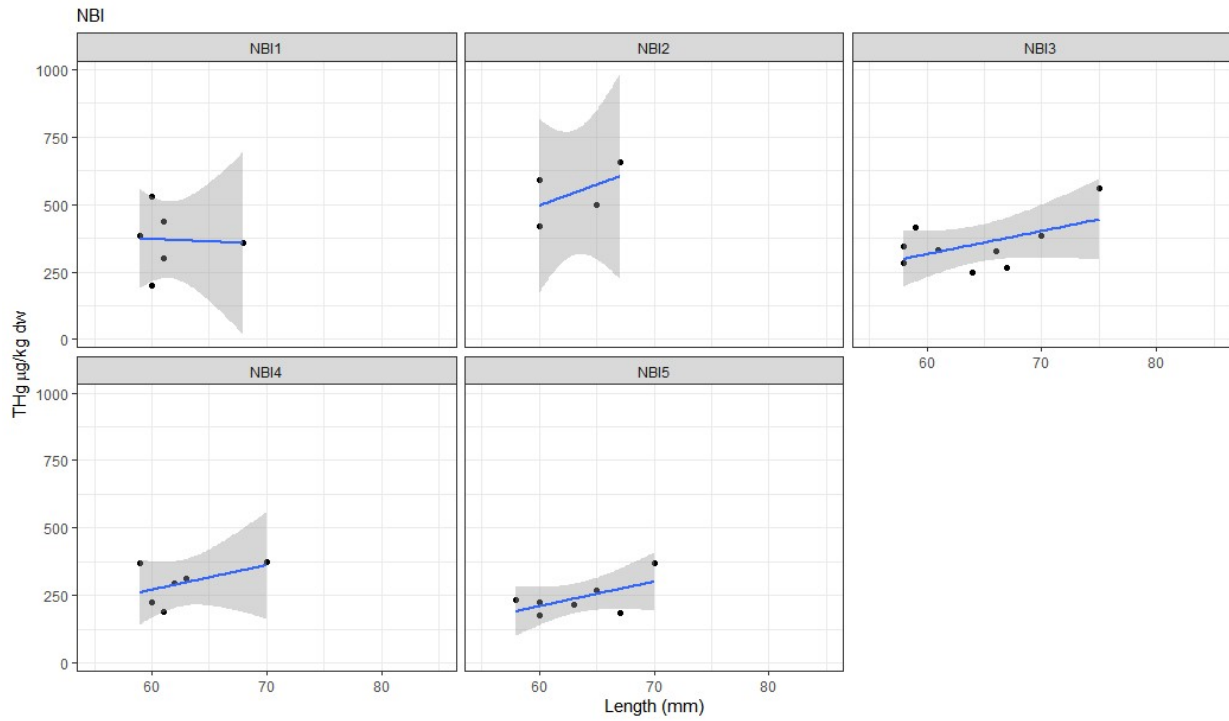


**Figures 17a-f:** The relationship between THg ( $\mu\text{g}/\text{kg dw}$ ) and fork length (mm) for female sculpin sampled from six NBR stream sites, located in New Brunswick. Line represents line of best fit.



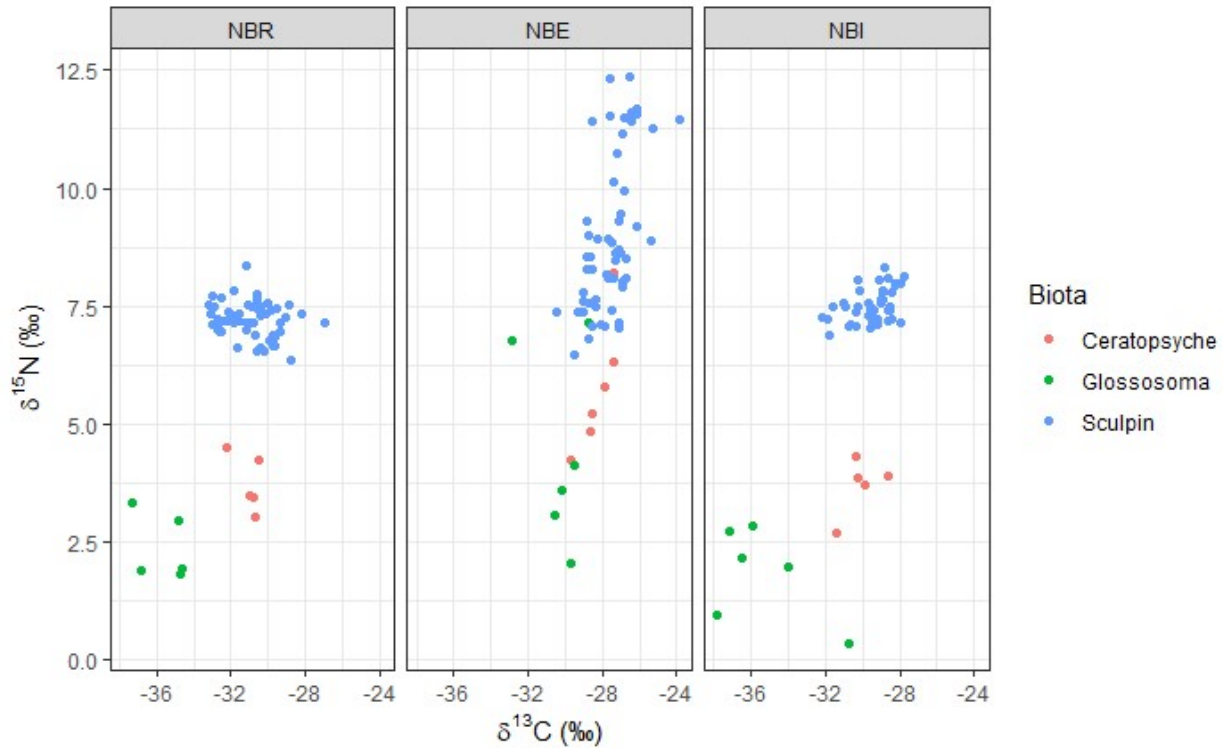
**Figures 18a-f:** The relationship between THg ( $\mu\text{g}/\text{kg dw}$ ) and fork length (mm) for female sculpin sampled from six NBE stream sites, located in New Brunswick. Line represents line of best fit.





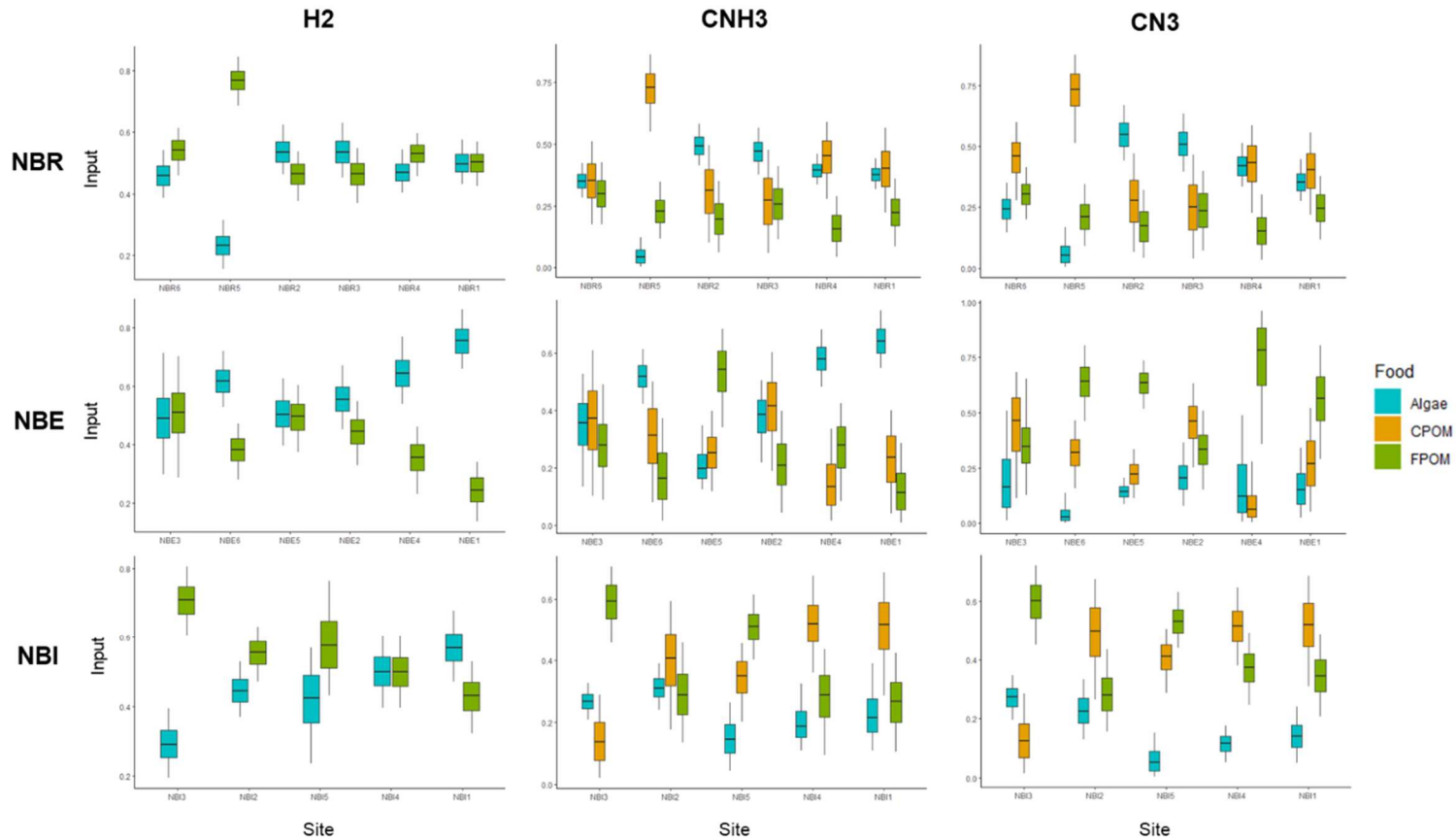
**Figures 19a-e:** The relationship between THg ( $\mu\text{g/kg dw}$ ) and fork length (mm) for female sculpin sampled from five NBI stream sites, located in New Brunswick. Line represents line of best fit.

**Appendix C: Relationship between  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  measured in female sculpin**



**Figure 20:** Values of  $\delta^{15}\text{N}$  (‰) and  $\delta^{13}\text{C}$  (‰) measured in female slimy sculpin muscle fillets (NBR:  $n = 8-11/\text{site}$ ; NBE:  $n = 8-10/\text{site}$ ; NBI:  $n = 8-10/\text{site}$ ), *Glossosoma* invertebrates ( $n = 1/\text{site}$ , in each catchment), and *Ceratopsyche* invertebrates ( $n = 1/\text{site}$ , in each catchment) sampled in the fall of 2017 from 17 streams sites in three managed catchments (NBR, NBE, and NBI) in New Brunswick.

**Appendix D: Mixing model outputs**



**Figure 21a-i:** The proportional input of algae, CPOM, and FPOM in NBR, NBE, and NBI predicted by three mixing models, for each of seventeen stream sites in three catchments (NBR, NBE, and NBI) in New Brunswick, where sites are ordered from upstream to downstream. The centre bolded horizontal line is the median, the topmost and bottommost horizontal lines are the 75% and 25% quantiles, and the top and bottom vertical lines are the 95% and 5% percentiles, respectively.

**Appendix E: Relationship between mean THg and algal inputs to sculpin diet**

**Table 8:** Summary of linear regression analyses of relationship between mean THg ( $\mu\text{g}/\text{kg dw}$ ) measured in female sculpin and algal inputs to sculpin diet predicted by three Bayesian mixing models (H2, CNH3, and CN3), for sculpin sampled in fall, 2017, from 17 stream sites in three catchments (NBR, NBE, and NBI) in New Brunswick

| <b>Model</b> | <b>Site</b> | <b><i>F</i><sub>#, #</sub></b> | <b><i>F</i>-value</b> | <b><i>p</i>-value</b> |
|--------------|-------------|--------------------------------|-----------------------|-----------------------|
| H2           | NBR         | 1, 4                           | 0.369                 | 0.5765                |
|              | NBE         | 1, 4                           | 1.19                  | 0.3367                |
|              | NBI         | 1, 3                           | 0.001                 | 0.9729                |
| CNH3         | NBR         | 1, 4                           | 0.128                 | 0.7385                |
|              | NBE         | 1, 4                           | 1.662                 | 0.2668                |
|              | NBI         | 1, 3                           | 14.92                 | 0.0307*               |
| CN3          | NBR         | 1, 4                           | 0.047                 | 0.8386                |
|              | NBE         | 1, 4                           | 2.95                  | 0.161                 |
|              | NBI         | 1, 3                           | 2.35                  | 0.2228                |