IMPACTS OF SPRUCE BUDWORM DEFOLIATION ON STREAM FOOD WEBS AND MERCURY CYCLING

IMPACTS OF SPRUCE BUDWORM DEFOLIATION ON STREAM FOOD WEBS AND MERCURY CYCLING

By KAIYING SALLY JU, BSc.

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AUTHOR: Kaiying Sally Ju, B.Sc. (University of Waterloo)
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Lay Abstract

A recent spruce budworm outbreak is causing widespread defoliation of spruce and fir trees, but the impacts to stream environments, including primary production, its consumption, and contaminant levels, are largely unknown. Streams are sensitive to conditions in the surrounding terrestrial environment, as such changes can affect the diets of stream invertebrates and fish and are also linked to increased contaminant levels in aquatic organisms. Specifically, mercury is a metal that is transferred through diet and can reach toxic levels in fish. This study found that defoliation is contributing to increased algal production in streams in the Gaspé Peninsula, Québec. However, some stream invertebrates consumed more terrestrial material in streams that had heavier defoliation. Furthermore, defoliation and algal diets did not increase levels of mercury in aquatic organisms, but this contaminant was affected by increasing concentrations of dissolved organic carbon in the streams. These findings suggest that forest defoliation can alter organisms' diets but not mercury levels.

Abstract

Forested streams are closely linked to terrestrial catchments which affects their biogeochemical cycling and carbon inputs. Catchment disturbances alter stream water quality and food webs, including changes in productivity. Such changes in stream conditions can potentially alter consumers' reliance on autochthonous (in-stream) or allochthonous (terrestrial) sources and mercury bioaccumulation. A recent outbreak of the spruce budworm (SBW) that feeds on spruce and fir trees has provided the unique opportunity to examine stream food web responses across watersheds experiencing a range of defoliation in the Gaspé Peninsula, Québec.

This project compares streams in twelve watersheds which were selectively sprayed to control SBW and create a gradient in defoliation. Food web samples (food sources, invertebrates, fish) were analyzed for stable isotopes of carbon and nitrogen in 2019 and 2020 to characterize food web structure, and algal productivity was measured in 2019. Hierarchical partitioning models were used throughout the study to compare the contributions of various local and landscape conditions to stream responses. Models indicated that watershed defoliation contributed to increasing autochthonous production, although some invertebrates were more allochthonous in heavily defoliated watersheds, and brook diets were unaffected by defoliation.

Next, food web samples were analyzed for methylmercury (food sources, invertebrates) or total mercury (fish) and trophic magnification slopes were determined for each stream food web. Mercury levels in carnivorous invertebrates and brook trout were driven by dissolved organic carbon (DOC), but not consumer autochthony or watershed defoliation. Additionally, rates of trophic magnification were not related to defoliation severity or DOC.

This study found that defoliation contributed to increasing autochthonous production and invertebrate consumer allochthony. However, this disturbance did not increase consumer mercury levels or biomagnification in stream food webs. These findings suggest that intervention to reduce defoliation would mitigate algal responses and dietary shifts, but not mercury cycling as it is influenced by DOC levels in the streams of this region.

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Firstly, I acknowledge that this work was completed on traditional Mi’kmaq territory. The Gaspesie, or *Gespe'gewa'gi* means “the lands end” and I am grateful for the experiences I’ve gained while working in this beautiful and serene region, at the end of the land.

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List of abbreviations

Abbreviation	**Definition**
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
Btk	*Bacillus thuringiensis* var. *kurstaki*
CPOM	Coarse Particulate Organic Matter
DEM	Digital Elevation Model
DMA	Direct Mercury Analyzer
DORM	Fish protein certified reference material
dw	dry weight
EIS	Early Intervention Strategy
EVs	Explanatory Variables
FFG	Functional Feeding Groups
FPOM	Fine Particulate Organic Matter
GC-ICPMS	Gas Chromatography-Inductively Coupled Plasma Mass Spectrometry
GIS	Geographic Information Systems
HAiFLO	Inverse distance-weighted metric, based on hydrologically active flow paths
Hg	Mercury
IAEA	International Atomic Energy Agency
LDD	*Lymantra dispar dispar*
LSM	Least Square Means
MeHg	Methylmercury
MFFP	Ministère des Forêts de la Faune et des Parcs
NRCan	Natural Resources Canada
NRC-CNRC	National Research Council - Conseil National de Recherches Canada
PCA	Principal Component Analysis
RVs	Response Variables
SBW	Eastern Spruce Budworm; *Choristoneura fumiferana* (Clemens)
SOPFIM	La Société de Protection des Forêts Contre les Insectes et Maladies
SRMs	Standard Reference Materials
SUVA	Specific Ultraviolet Absorbance at 254 nm
THg	Total mercury
TMS	Trophic Magnification Slopes ($\log_{10}\delta^{15}N$ vs Hg)
US EPA	United States Environmental Protection Agency
ww	wet weight
‰	per mill (parts per thousand)
$\delta^{13}C$	Stable isotope of carbon
$\delta^{15}N$	Stable isotope of nitrogen

Chapter 1: General introduction

Chapter 1: General introduction

Canada is the third most forested country in the world, as it contains over 350 million hectares of forest that covers 40% of the total land area (Natural Resources Canada, 2021), and these forests are an important natural resource and provide a range of social, environmental, and economic benefits for Canadians. Many communities in Canada are culturally and spiritually connected to forests, and rely on their provisions for employment, recreation, and resources to support their livelihoods. Forests provide valuable ecosystem services, such as storing carbon, regulating temperature, mitigating flooding, and improving air and water quality. Additionally, healthy forests also support biodiversity, as most terrestrial animals and plants in Canada require forested habitat (Canadian Council of Forest Ministers, 2014). Finally, forestry is one of the largest sectors of the Canadian economy, which contributes $25 billion annually to the national GDP and employs over 180,000 people across the country. The vast areas of forests in Canada are important to the country's natural, social and economic well-being.

Forests also affect stream health, as streams are tightly linked to the surrounding terrestrial watershed (Hynes, 1975). The watershed influences the physical conditions, water chemistry, and energy inputs to streams. Stream food webs are supported by autochthonous (in-stream) and allochthonous (terrestrial) basal energy sources. In undisturbed systems, small streams receive high quantities of allochthonous carbon and terrestrial vegetation shades the stream and limits autochthonous production (Vannote et al., 1980). Despite their lower quantity, autochthonous carbon sources are often more nutritious (Guo et al., 2016) and can have a disproportionately large energy contributions to stream consumers (Hayden et al., 2016; McCutchan & Lewis, 2002). However, terrestrial disturbances can potentially shift these energy contributions to streams, with cascading impacts to consumers at multiple trophic levels (England & Rosemond, 2004).

Insect pest outbreaks are the leading disturbance to Canadian forests and affect a greater area than fires, logging, and deforestation combined (Boucher et al., 2018). In Canada, pest damage causes hundreds of millions of dollars in economic losses annually due to reduced timber supply (Natural Resources Canada, 2021). Many of the most destructive forest pests in Canada are defoliating insects (Kneeshaw et al., 2015) that reduce host trees' growth and productivity and can result in mortality after 5-7 years of severe damage (MacLean, 1984)**.** The Early Intervention Strategy (EIS) is a proactive approach for managing forest defoliators by targeting insecticide application at emerging insect populations to delay the onset and spread of outbreaks (Johns et al., 2019). Previously, EIS has been successful in reducing forest damage by the invasive LDD moth (*Lymantria dispar dispar*) by identifying outbreak hotspots and disrupting mating to limit population dispersal (Sharov et al., 2002). Although EIS can mitigate economic losses by reducing tree mortality, the potential trade-offs of this forest protection strategy on ecosystem services have not been widely explored. Many forest pests are endemic insect herbivores, and EIS can disrupt the natural cycle of population outbreaks that regulate forest stability and cycling of nutrients and water in the environment (Townsend et al., 2004). Furthermore, as streams are closely linked to terrestrial catchments the potential impacts (positive or negative) of EIS to these aquatic environments are unknown. While EIS can protect the health and economic value of forests, there is a knowledge gap on whether this approach compromises or protects the integrity of stream environments.

Forest disturbances can alter carbon cycling in terrestrial environments, with eventual impacts to aquatic energy sources. For example, timber harvesting removes aboveground carbon stores and alters catchment hydrology, which increases the delivery of organic carbon to streams through runoff (Likens et al., 1970). Furthermore, riparian canopy loss can alter water chemistry, productivity, and algal communities in streams (Kreutzweiser et al., 2008; Lamontagne et al., 2000). Such changes can shift the energy sources available to stream consumers, as forest harvesting has been associated with either increasing autochthony (England & Rosemond, 2004; Göthe et al., 2009) or allochthony (Erdozain et al., 2019) in macroinvertebrates and fish. These findings also suggest that stream food web responses can vary depending on the mechanism of disturbance. The impacts of defoliation on aquatic carbon cycling and energy uptake are unclear but likely, as stream food webs are influenced by changes in landscape and local conditions. In addition, defoliators are known to increase the flux of nutrients from the canopy to forest soils (Arango et al., 2019; Michalzik & Stadler, 2005; Reynolds et al., 2000), where they likely are carried to streams in runoff. Alternatively, defoliation could increase the use of allochthonous carbon by aquatic food webs through litterfall inputs, which include frass, needles, and budworm carcasses. Consequently, the overall impacts of defoliation to stream energy sources are unknown.

Stable isotopes of carbon and nitrogen are used to measure energy transfer in aquatic ecosystems because these isotopes follow predictable patterns in food webs (Vander Zanden & Rasmussen, 2001). Trophic enrichment describes the preferential assimilation of heavier isotopes during consumption, which results in different isotope ratios between consumers and their food sources (Fry, 2006). In stream food webs, stable carbon isotope ratios (expressed as $\delta^{13}C$) distinguish between basal energy sources that were derived in-stream (autochthonous) or from the terrestrial environment (allochthonous) because trophic enrichment of $\delta^{13}C$ is minor (0-1 ‰) (Post, 2002). Stable nitrogen isotope ratios (expressed as $\delta^{15}N$) represent relative trophic position as the heavier isotope is enriched by 2-4‰ in consumers, although the enrichment factor can vary depending on the consumer's diet, metabolism, and physiology (McCutchan et al., 2003). Stable isotope analyses are used in this thesis to help identify the impact of forest disturbances on the structure of stream food webs.

Mercury (Hg) is a metal contaminant of environmental concern because its levels are increasing as a result of human activities and it can accumulate to toxic concentrations in fish and fish-eating wildlife and humans. Inorganic Hg enters the atmosphere through natural processes (e.g. weathering of rocks and volcanoes) but anthropogenic activities (e.g. burning fossil fuels, mining) have increased atmospheric Hg by ~ 3-fold. This gaseous Hg travels long distances from these emission sources to contaminate remote locations. Next, atmospheric Hg can be captured by forest canopies and deposited onto the catchment from litterfall and throughfall (Driscoll et al., 2007). Finally, terrestrial Hg can enter aquatic environments through runoff, and be methylated by anaerobic microbes into methylmercury (MeHg), the neurotoxic and bioaccumulative form of this contaminant that biomagnifies in aquatic food webs. Due to the efficient trophic transfer of this contaminant, MeHg levels in fish are up to 10^7 times higher than concentrations in water (Watras et al., 1998).

Catchment disturbances can elevate MeHg in stream food webs by increasing its transport and bioavailability. Forest disturbances such as harvesting and fire can mobilize mercury stores and change water chemistry parameters (i.e. increase dissolved organic carbon, acidity, sulfate, and decrease productivity) known to increase Hg methylation and bioavailability (Kelly et al., 2006; Porvari et al., 2003; Skyllberg et al., 2009). These conditions can lead to toxic MeHg levels in fish (Garcia & Carignan, 2005), and this health risk can be transferred from aquatic consumers to terrestrial wildlife and humans. Defoliation can cause similar changes to forested catchments as forestry (Gandhi & Herms, 2010), although the impacts on mercury fate in aquatic food webs are not known. Understanding the factors that contribute to high MeHg can mitigate potential health risks and offer insights into the effects of defoliation on contaminant cycling.

An outbreak of the Eastern Spruce Budworm (*Choristoneura fumiferana [Clemens]; SBW*) has been occurring in the Gaspé Peninsula of Québec since 2016 (Ministère des Forêts de la Faune et des Parcs, 2020), which provides the opportunity to investigate different ecosystem responses of defoliation or EIS protection. This project assessed twelve watersheds experiencing a range in defoliation severity. Six watersheds were treated by the biological insecticide Btk. (*Bacillus thuringiensis* subspecies *kurstaki*) to limit severe defoliation, and six were untreated to allow defoliation to progress. This ensured a gradient of watershed defoliation, which was quantified annually using aerial, helicopter, and branch surveys. Within each watershed, one stream reach was sampled in 2019 and 2020 throughout the season for water chemistry, algal production, and hydrology. Additional intensive sampling was conducted over two years to collect food sources, invertebrates, and fish for stable isotope and mercury analyses. This study is part of a larger project including assessments of SBW defoliation to terrestrial carbon cycling, riparian bird communities, and stream microbial function by collaborators at Natural Resources Canada and the University of New Brunswick.

This thesis is structured into four chapters. The first provides a general introduction to the context of this research. The second evaluates the impact of defoliation on autochthonous carbon production and consumption in stream food webs. The third compares the drivers of mercury bioaccumulation and biomagnification in streams. The last chapter summarizes the research and discusses potential next steps. The key objectives of my thesis are to:

1) assess algal production, consumer autochthony, and stream food webs across watersheds with varying local and landscape factors, including catchment defoliation (chapter 2); and

2) understand the effect of different local and landscape factors on MeHg levels in stream consumers and mercury biomagnification in stream food webs (chapter 3).

Chapter 2 uses stable isotope analyses of food sources, macroinvertebrates and fish from streams across this gradient of impact to understand the reliance of consumers on autochthonous versus allochthonous carbon sources. These data are then used in chapter 3 to understand how a consumer's source of carbon and relative trophic position affect its mercury levels in streams with different defoliation in the catchment. Overall, this thesis will advance our understanding of the impact of forest pests on streams, which are tightly linked to watershed conditions, and this knowledge will help assess the potential ecosystem benefits of EIS for the management of spruce and fir forests in eastern Canada.

References

Arango, C., Ponette-González, A., Neziri, I., & Bailey, J. (2019). Western spruce budworm effects on throughfall N, P, and C fluxes and soil nutrient status in the Pacific Northwest. *Canadian Journal of Forest Research*, *49*(10), 1207–1218. https://doi.org/10.1139/cjfr-2018-0523

Boucher, D., Boulanger, Y., Aubin, I., Bernier, P. Y., Beaudoin, A., Guindon, L., & Gauthier, S. (2018). Current and projected cumulative impacts of fire, drought, and insects on timber volumes across Canada. *Ecological Applications*, *28*(5), 1245–1259. https://doi.org/10.1002/EAP.1724

Driscoll, C. T., Han, Y. J., Chen, C. Y., Evers, D. C., Lambert, K. F., Holsen, T. M., Kamman, N. C., & Munson, R. K. (2007). Mercury contamination in forest and freshwater ecosystems in the northeastern United States. *BioScience*, *57*(1), 17–28. https://doi.org/10.1641/B570106

England, L. E., & Rosemond, A. D. (2004). Small reductions in forest cover weaken terrestrial-aquatic linkages in headwater streams. *Freshwater Biology*, *49*(6), 721–734. https://doi.org/10.1111/j.1365-2427.2004.01219.x

Erdozain, M., Kidd, K., Kreutzweiser, D., & Sibley, P. (2019). Increased reliance of stream macroinvertebrates on terrestrial food sources linked to forest management intensity. *Ecological Applications*, *29*(4), 1–14. https://doi.org/10.1002/eap.1889

Gandhi, K. J. K., & Herms, D. A. (2010). Direct and indirect effects of alien insect herbivores on ecological processes and interactions in forests of eastern North America. *Biological Invasions*, *12*(2), 389–405. https://doi.org/10.1007/s10530-009-9627-9

Garcia, E., & Carignan, R. (2005). Mercury concentrations in fish from forest harvesting and fire-impacted Canadian Boreal lakes compared using stable isotopes of nitrogen. *Environmental Toxicology and Chemistry*, *24*(3), 685–693. https://doi.org/10.1897/04-065R.1

Göthe, E., Lepori, F., & Malmqvist, B. (2009). Forestry affects food webs in northern Swedish coastal streams. *Fundamental and Applied Limnology*, *175*(4), 281–294. https://doi.org/10.1127/1863-9135/2009/0175-0281

Guo, F., Kainz, M. J., Sheldon, F., & Bunn, S. E. (2016). The importance of high-quality algal food sources in stream food webs - current status and future perspectives. *Freshwater Biology*, *61*(6), 815–831. https://doi.org/10.1111/fwb.12755

Hayden, B., McWilliam-Hughes, S. M., & Cunjak, R. A. (2016). Evidence for limited trophic transfer of allochthonous energy in temperate river food webs. *Freshwater Science*, *35*(2), 544–558. https://doi.org/10.1086/686001

Hynes, H. B. N. (1975). The stream and its valley. *Internationale Vereinigung Für Theoretische Und Angewandte Limnologie: Verhandlungen*, *19*(1), 1–15. https://doi.org/10.1080/03680770.1974.11896033

Johns, R. C., Bowden, J. J., Carleton, D. R., Cooke, B. J., Edwards, S., Emilson, E. J. S., James, P. M. A., Kneeshaw, D., MacLean, D. A., Martel, V., Moise, E. R. D., Mott, G. D., Norfolk, C. J., Owens, E., Pureswaran, D. S., Quiring, D. T., Régnière, J., Richard, B., & Stastny, M. (2019). A conceptual framework for the spruce budworm Early Intervention Strategy: Can outbreaks be stopped? *Forests*, *10*(10), 910. https://doi.org/10.3390/f10100910

Kelly, E. N., Schindler, D. W., st. Louis, V. L., Donald, D. B., & Vladicka, K. E. (2006). Forest fire increases mercury accumulation by fishes via food web restructuring and increased mercury inputs. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(51), 19380–19385. https://doi.org/10.1073/pnas.0609798104

Kneeshaw, D., Sturtevant, B. R., Cooke, B., Work, T., Pureswaran, D., DeGrandpre, L., & MacLean, D. A. (2015). Insect disturbances in forest ecosystems. In *Routledge Handbook of Forest Ecology* (pp. 93–113).

Kreutzweiser, D. P., Hazlett, P. W., & Gunn, J. M. (2008). Logging impacts on the biogeochemistry of boreal forest soils and nutrient export to aquatic systems: A review. *Environmental Reviews*, *16*, 157–179. https://doi.org/10.1139/A08-006/ASSET/IMAGES/A08-006T3H.GIF

Lamontagne, S., Carignan, R., D'Arcy, P., Prairie, Y. T., & Paré, D. (2000). Element export in runoff from eastern Canadian Boreal Shield drainage basins following forest harvesting and wildfires. *Canadian Journal of Fisheries and Aquatic Sciences*, *57*(S2), 118–128. https://doi.org/10.1139/f00-108

Likens, G. E., Bormann, F. H., Johnson, N. M., Fisher, D. W., & Pierce, R. S. (1970). Effects of Forest Cutting and Herbicide Treatment on Nutrient Budgets in the Hubbard Brook Watershed-Ecosystem. *Ecological Monographs*, *40*(1), 23–47. https://doi.org/10.2307/1942440

MacLean, D. A. (1984). Effects of Spruce Budworm Outbreaks on the Productivity and Stability of Balsam Fir Forests. *The Forestry Chronicle*, *60*(5), 273–279. https://doi.org/10.5558/tfc60273-5

McCutchan, J. H., & Lewis, W. M. (2002). Relative importance of carbon sources for macroinvertebrates in a Rocky Mountain stream. *Limnology and Oceanography*, *47*(3), 742–752. https://doi.org/10.4319/lo.2002.47.3.0742

McCutchan, J. H., Lewis, W. M., Kendall, C., & McGrath, C. C. (2003). Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos*, *102*(2), 378–390. https://doi.org/10.1034/j.1600-0706.2003.12098.x

Michalzik, B., & Stadler, B. (2005). Importance of canopy herbivores to dissolved and particulate organic matter fluxes to the forest floor. *Geoderma*, *127*(3-4 SPEC. ISS.), 227–236. https://doi.org/10.1016/j.geoderma.2004.12.006

Natural Resources Canada. (2021). *The State of Canada's Forests: Annual Report 2021*.

Porvari, P., Verta, M., Munthe, J., & Haapanen, M. (2003). Forestry practices increase mercury and methyl mercury output from boreal forest catchments. *Environmental Science and Technology*, *37*(11), 2389–2393. https://doi.org/10.1021/es0340174

Post, D. M. (2002). Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology*, *83*(3), 703–718. https://doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2

Reynolds, B. C., Hunter, M. D., & Crossley, D. A. (2000). Effects of Canopy Herbivory on Nutrient Cycling in a Northern Hardwood Forest in Western North Carolina. *Selbyana*, *21*(1,2), 74–78. https://www.jstor.org/stable/41760056?seq=1&cid=pdf-reference#references_tab_contents

Sharov, A. A., Leonard, D., Liebhold, A. M., Roberts, E. A., & Dickerson, W. (2002). “Slow The Spread”: A National Program to Contain the Gypsy Moth. *Journal of Forestry*, *100*(5), 30–36. https://doi.org/10.1093/JOF/100.5.30

Skyllberg, U., Westin, M. B., Meili, M., & Björn, E. (2009). Elevated concentrations of methyl mercury in streams after forest clear-cut: A consequence of mobilization from soil or new methylation? *Environmental Science and Technology*, *43*(22), 8535–8541. https://doi.org/10.1021/es900996z

Townsend, P. A., Eshleman, K. N., & Welcker, C. (2004). Remote sensing of gypsy moth defoliation to assess variations in stream nitrogen concentrations. In *Ecological Applications* (Vol. 14, Issue 2, pp. 504–516). John Wiley & Sons, Ltd. https://doi.org/10.1890/02-5356

vander Zanden, M. J., & Rasmussen, J. B. (2001). Variation in δ15N and δ13C trophic fractionation: Implications for aquatic food web studies. *Limnology and Oceanography*, *46*(8), 2061–2066. https://doi.org/10.4319/lo.2001.46.8.2061

Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R., & Colbert, C. E. (1980). The River Continuum Concept. *Canadian Journal of Fisheries and Aquatic Sciences*, *37*(1), 130–137.

Watras, C. J., Back, R. C., Halvorsen, S., Hudson, R. J. M., Morrison, K. A., & Wente, S. P. (1998). Bioaccumulation of mercury in pelagic freshwater food webs. *Science of the Total Environment*, *219*(2–3), 183–208. https://doi.org/10.1016/S0048-9697(98)00228-9

Chapter 2: Effects of spruce budworm defoliation to autochthonous production and consumption in stream food webs

Abstract

Spruce budworm outbreaks cause widespread defoliation of spruce and fir trees, and this landscape disturbance can potentially alter the contribution of carbon sources to stream food webs. This study examined 12 streams in the Gaspé Peninsula, Quebec, across watersheds experiencing a range of defoliation to assess whether this disturbance alters stream algal productivity and the contribution of allochthonous (terrestrial) or autochthonous (aquatic) energy sources to consumer diets.

In 2019 and 2020, water, food sources, several macroinvertebrate taxa, and fish were collected for stable carbon and nitrogen isotope analyses. Additionally, benthic algal production was measured in 2019. Next, MixSIAR modeling was conducted for invertebrate and fish consumers to determine their dietary autochthony in the different streams and years. Finally, explanatory models were generated using hierarchical partitioning to compare the different local and landscape factors contributing to algal production and autochthonous carbon consumption.

Models explained 41% of the variation in algal production, which was greater in heavily defoliated watersheds. However, this effect was collinear with elevation and latitude, and streamside defoliation, nutrients, and water flow did not have high contributions to this model. Furthermore, models explained 41-55% of the variation for invertebrate autochthony. Defoliation contributed to increasing allochthony in Chloroperlidae and *Rhyacophila*, but decreasing allochthony in *Parapsyche*. Finally, the models explained 59% of variation for brook trout autochthony and this endpoint was not affected by catchment defoliation.

These results suggest that catchment defoliation is contributing to increased algal production in streams. However, most stream consumers had greater reliance on allochthonous carbon in catchments with greater defoliation. These findings may be caused by increased consumption of spruce budworm or litterfall produced during defoliation. This project shows that catchment disturbance can alter stream algal sources and contribute to dietary shifts in stream consumers.

Chapter 2: Effects of spruce budworm defoliation on autochthonous production and consumption in stream food webs

2.1. Introduction

Stream food webs are supported by both autochthonous and allochthonous organic materials (Polis et al., 1997), although their relative importance to consumers remains debated. Headwater streams in forested catchments receive high quantity of allochthonous inputs from the riparian area in the form of leaf litter and dissolved organic carbon (Cummins, 1974; Vannote et al., 1980). These inputs are made available to consumers through in-stream processing of the terrestrial organic matter by bacteria (Hall & Meyer, 1998), which can contribute over 99% of the energy to small headwater streams (Fisher & Likens, 1973). Although headwater streams tend to be light limited, algal growth occurs and provides an alternate energy source for consumers. Recent studies have shown that autochthonous energy provides a disproportionately greater contribution to stream consumers than originally believed (Brett et al., 2017; Hayden et al., 2016; McCutchan & Lewis, 2002; Rosi-Marshall et al., 2016), due to the higher nutrition of autochthonous biofilms (Guo et al., 2016) and lower transfer efficiencies of terrestrial carbon from one trophic level to the next (Cross et al., 2005). Understanding the relative importance of these carbon sources to stream consumers is of interest as allochthonous carbon use is linked to shorter food chains (Cross et al., 2013), lower biomass (Bilby & Bisson, 1992), and decreases in contaminant exposure (Willacker et al., 2019).

As headwater streams are tightly linked to their surroundings, terrestrial disturbances can alter carbon inputs and flow in food webs through bottom-up pathways. For example, the landscape disturbances from forestry increase canopy openings, facilitating algal production, and decrease leaf litter inputs into these small systems (England & Rosemond, 2004; Kiffney et al., 2003). Both invertebrates (Göthe et al., 2009) and fish (Bilby & Bisson, 1992) had higher autochthonous carbon use in clear-cut watersheds compared to streams in old-growth forests. Greater autochthonous production at the base of the food web was found to support higher densities of invertebrates and fish (Murphy et al., 1981). However, insect disturbances also differ from forestry because outbreaks affect a large geographic region, trees can sustain pest damage for several years, and defoliation transfers carbon from the canopy to the forest floor in the form of litterfall. Much of our understanding of how landscape disturbance affects headwater streams is based on studies of forest harvesting and little is known about other widespread disturbances such as the defoliation of trees by insects.

Pest outbreaks have damaged millions of hectares of forest in Canada, which is a greater area than fires, logging, and harvesting combined (Natural Resources Canada, 2018), and the changes in forest structure and nutrient dynamics they cause (Hunter, 2001; Mattson & Addy, 1975) may affect headwater streams. Many of the most destructive pests in Canadian forest are larval caterpillars that feed on the spring growth of new foliage (Kneeshaw et al., 2015). This defoliation creates large pulses of litterfall and frass to the forest floor, which also increases fluxes of nutrients including nitrogen, phosphorus, and potassium (Frost & Hunter, 2007; Grace, 1986). Furthermore, severe defoliation can lead to canopy gaps and tree mortality, which increase light availability and temperature in forest stands (D'Aoust et al., 2004; Gandhi & Herms, 2010; Warren et al., 2016). These conditions facilitate nutrient releases (Hart & Chen, 2006; Martínez et al., 2014) that can leach from soils into aquatic ecosystems after rainfall (Lewis & Likens, 2007; Swank et al., 1981). Finally, whereas temperate streams usually receive the greatest terrestrial subsidy during autumn leaf senescence, defoliation leads to a resource pulse that occurs early in the season. Consequently, these changes in terrestrial nutrient cycling can change the timing of nutrient delivery and increase overall nutrient inputs to stream ecosystems.

Although defoliation is a common and widespread landscape-scale disturbance, the impacts to autochthonous production and carbon use by consumers in streams have not been quantified. Changes in catchment conditions can facilitate benthic algal growth (Warren et al., 2016), which is limited by light and nutrients, and this elevated in-stream productivity can increase consumer autochthony across multiple trophic levels (Göthe et al., 2009; Heaston et al., 2018). Alternatively, consumer allochthony may increase if additional inputs of litterfall are broken down by bacteria and incorporated as a terrestrial energy source. For an example, Erdozain et al. (2019) found that streams in disturbed catchments had higher loadings of terrestrial organic matter, which stimulated heterotrophic biofilm growth and increased allochthony in macroinvertebrates. Furthermore, insect carcasses can provide an allochthonous subsidy to aquatic food webs during outbreaks due to their high abundance and nutritional value. Emergence of cicadas (Menninger et al., 2008; Nowlin et al., 2007), invasive alder sawflies (Roon et al., 2018), and hemlock woolly adelgid (Kominoski et al., 2008) were quickly assimilated as energy sources in aquatic food webs.

The Eastern Spruce Budworm (*Choristoneura fumiferana*; SBW) is a native forest pest that defoliates the needles of spruce and fir trees during outbreak cycles that last up to a decade. The Gaspé Peninsula region of Québec, Canada has been experiencing an active outbreak of SBW since 2016 which has already defoliated millions of hectares of forest (Ministère des Forêts de la Faune et des Parcs, 2019). The objective of this study is to compare stream food webs in watersheds experiencing a range of defoliation. The structure of stream food webs was assessed using stable isotopes of carbon and nitrogen. Carbon isotope ratios (expressed as $\delta^{13}C$) of consumers measure their reliance on different food sources because $\delta^{13}C$ is only enriched by 0 - 1‰ from prey to predator (Finlay, 2001; McCutchan et al., 2003) and is typically depleted in autochthonous food sources in streams compared to allochthonous vegetation (Post, 2002). Ratios of nitrogen isotopes (expressed as $\delta^{15}N$) measure relative trophic position because ^{15}N is selectively retained in consumers and their $\delta^{15}N$ is enriched by 1.4 – 3.4‰ over that of their prey (Vander Zanden & Rasmussen, 2001). Bayesian mixing models advance the application of stable isotopes by estimating contributions of multiple basal food sources to consumers (Stock et al., 2018). First, I predict that watershed defoliation will increase autochthonous biofilm growth. Next, I predict that stream consumers from watersheds with greater defoliation will have higher reliance on autochthonous food sources.

2.2. Methods

2.2.1. Study sites

The Gaspé Peninsula in Québec is located on the southern shore of the St. Lawrence River and is separated from New Brunswick by the Matapedia River and Baie des Chaleurs. The northwestern region of the Gaspé Peninsula features the Chic-Choc Mountains which contribute to its higher elevation and hilly terrain. The geology of the peninsula is dominated by sandstones and limestones that were formed during the lower and lower-middle Devonian period (Malo & Bourque, 1993). The average temperature in the region is 16.8°C in July and -11.6°C in January, and the average annual precipitation is 1135 mm, mostly as rain between the months of May to November. The dominant tree species in this region includes balsam fir (*Abies balsamea*), white spruce (*Picea glauca*), yellow birch (*Betula alleghaniensis*), and white birch (*Betula papyrifera*). The boreal forests of eastern Québec have historically experienced cycles of SBW outbreak every 30-40 years, which is facilitated by the dominant stands of balsam fir, the preferred host species for SBW, and long interval between fires in the region (Bouchard & Pothier, 2010). The current outbreak started in 2016 in the northwestern region of the Gaspé Peninsula and has moved southeast in successive years.

Twelve watersheds (Figure 2.1) were selected for this study as follows. First, all watersheds ranging from 6 to 10 km^2 in the central region of Gaspésie were delineated using a 20 m resolution digital elevation model (DEM) acquired from the Canadian DEM open dataset. Next, potential watersheds were identified if they had less than 15% of the watershed area harvested in the last 15 years, a minimum of 90% of land cover as forest (with at least 65% as spruce and fir), and an outflow within 300 m of a road. All candidate sites were visited, and the final 12 sites included only those that were easily accessible, had comparable stream substrates (pebble/rock) and catchment sizes, and would not be harvested based on discussions with the Ministère des Forêts, de la Faune et des Parcs (MFFP). Catchment size (km^2) was determined using *RSAGA* (1.3.0) on a 20 m resolution DEM. The % spruce and fir were calculated based on the forest volume in each watershed. Slope and elevation were the average measurements for each watershed. The 12 watersheds selected were from the upper (U), central (C) and lower (L) regions of the peninsula and ranged in size from 6.08 to 9.67 km^2 and in mean elevation from 280 to 520 m above sea level (Table 2.1). The watersheds drain into the Cascapédia (U01, C04), Petite Cascapédia (U02, U03, C05, C06, C07, L09, L10), or Bonaventure (L08, L11, L12) rivers. The watersheds in this study are in the boreal and hemiboreal ecozones (Brandt et al., 2013). The MFFP provided all GIS spatial layers used in the screening (roads, forest resource inventory, and land cover layers). One second- or third- order stream reach (25-60 m) was selected in each watershed, and at sites where a road crossed the stream (U01, L09, L10, L11), sampling for water quality, benthic production and food webs was conducted immediately upstream of the road.

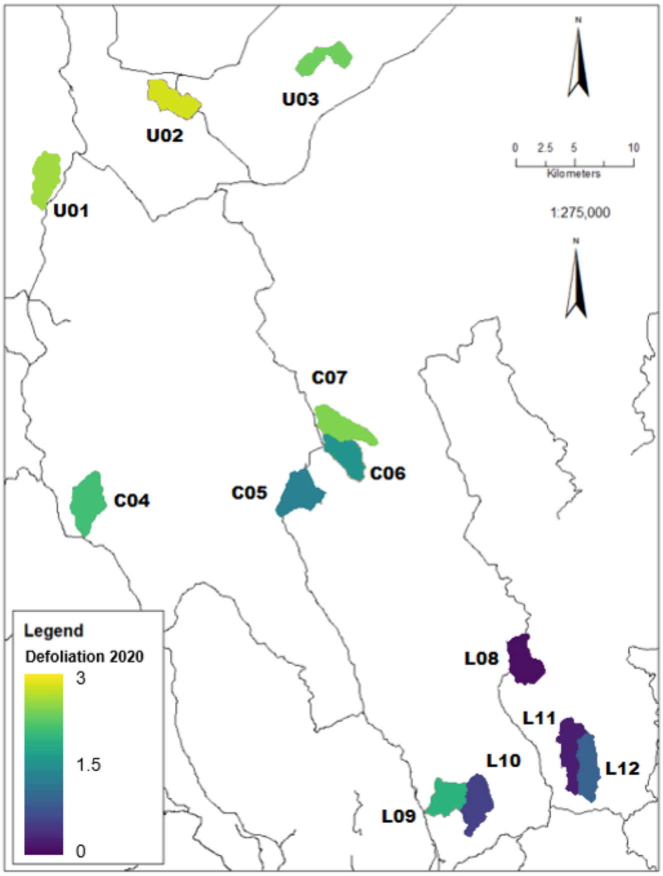

Figure 2.1. Map of twelve watersheds across the Gaspé Peninsula, Québec and their defoliation intensity from 2020. Defoliation for each watershed is calculated using an inverse distance-weighted metric (HAiFLO) for each year and ranges from 0-3 for low to high severity.

Table 2.1. Watershed characteristics of 12 sites sampled in the Gaspé Peninsula, Québec, representing a range in defoliation severity.

Site	Latitude	Longitude	Stream order	Watershed Size (km^2)	% Spruce + Fir	Slope (degrees)	Elevation (m)	Defoliation (2019)	Defoliation (2020)
U01	48.77749	-66.13444	3	7.80	90	14.8	607.2	2.54	2.64
U02	48.84763	-65.95590	2	8.11	95	11.4	774.6	2.99	2.70
U03	48.87196	-65.82987	2	6.08	96	10.9	678.1	2.68	2.03
C04	48.52692	-66.10207	2	9.69	80	17.4	462.1	2.03	1.87
C05	48.53386	-65.87085	3	8.81	83	12.8	435.4	1.91	1.27
C06	48.56082	-65.78262	2	7.87	94	8.1	489.0	2.78	1.27
C07	48.58588	-65.76319	2	8.20	91	5.7	519.5	2.74	2.06
L08	48.40672	-65.61254	3	7.84	69	10.6	332.9	0.66	0.61
L09	48.30158	-65.72190	3	7.79	65	19.2	281.0	0.76	1.50
L10	48.28294	-65.67367	2	8.03	62	17.2	281.2	0.72	0.77
L11	48.30726	-65.54561	2	8.04	77	13.9	314.8	1.04	0.72
L12	48.30666	-65.54310	3	8.46	82	11.1	274.3	0.84	0.86

Stream order and watershed size were measured using a DEM; % spruce and fir in each watershed was calculated based on forest volume; slope and elevation were watershed averages; watershed defoliation for each of 2019 and 2020 was measured using the HAiFLO inverse-distance weighted method (*hydroweight* package in R). See methods for more details.

The twelve watersheds in this study had a range in defoliation severity determined using both aerial and ground surveys (Table 2.1). In 2020, six watersheds (U02, U03, C06, L08, L10, L12) were sprayed by plane with Btk (*Bacillus thuringiensis var. kurstaki*) to limit SBW outbreaks, and six watersheds (U01, C04, C05, C07, L09, L11) were not sprayed to allow the outbreaks to progress. La Société de Protection des Forêts Contre les Insectes et Maladies (SOPFIM) sprayed the watersheds twice each year. Additionally, some of the watersheds (including those left unsprayed in 2020) may have been partially sprayed between 2016-2019 as part of the ongoing SBW control program in the region. The stand-level defoliation was measured annually at each watershed by the Province of Québec using aerial fixed-wing aircraft (2019) or helicopter (2020) surveys and supplemented with branch surveys conducted by Natural Resources Canada. Next, a defoliation metric was calculated for each watershed that combined both forest composition (proportion of spruce and fir by volume) and stand-level defoliation. Furthermore, this metric was weighted based on the distance of the flow path from the stream sampling point using the mean HAiFLO value calculated from the *hydroweight* R package (version 1.1.0; Kielstra et al., 2021). This inverse distance-weighted metric distinguishes that the areas of the watershed located closer to the sampling point have a stronger influence on in-stream conditions (Peterson et al., 2011). The HAiFLO defoliation metric ranges from 0 (low defoliation) to 3 (high defoliation) at each watershed.

2.2.2. Field sampling

Water quality and benthic production

A 500 mL water grab sample was collected from each stream reach approximately every 2-3 weeks from June – November in each of 2019 (n=8) and 2020 (n=10). Samples were frozen and then transported to the Central Water Chemistry Lab (Great Lakes Forestry Centre) where they were analyzed using standard methods for a suite of parameters including pH, conductivity, cations, anions, nutrients, carbon, and metals

(Hazlett et al., 2008). Water chemistry variables, abbreviations, detection limits, and parameters that were removed due to low detection are summarized in Table A2.

In 2019, benthic production was measured biweekly at each site (n=6/site) using a BenthoTorch (bbe Moldaenke). Five benthotorch measurements were taken along five stream transects spaced 1 m apart and then averaged before analysis. The BenthoTorch measures the fluorescence of algal cells to calculate total benthic production (biomass of green algae, cyanobacteria, diatoms). I used total benthic production to quantify biofilm density rather than individual algal groups because it is analogous to chlorophyll a, the conventional metric of primary production (Kahlert & McKie, 2014), and is correlated to the taxonomic groups (r=0.61, 0.81, 0.82 for cyanobacteria, green algae, and diatoms, respectively). Similar data were not collected in 2020 due to restrictions caused by the pandemic.

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Food sources

Within each stream reach, food source samples were collected in September of 2019 and August and September of 2020. Biofilm was collected to represent the autochthonous food source, whereas coarse particulate organic matter (CPOM) and fine particulate organic matter (FPOM) were sampled to represent allochthonous food sources. One replicate of each sample type was collected from an upstream, mid-stream, and downstream location within the stream reach. To sample biofilm, 10-15 rocks were selected from each location and scrubbed with a toothbrush. The slurry was rinsed using stream water into a Whirl-Pak® bag. CPOM was a sample of 10-15 decaying leaves (usually mountain maple, alder, birch) found at that location. Finally, a turkey baster was used to suction the top 2-3 cm from depositional areas in the stream (FPOM). Samples were kept in a cooler in the field, frozen at the end of the day and kept frozen until processed in the lab.

Benthic macroinvertebrates

In September of 2019 and 2020, benthic macroinvertebrates from different functional feeding groups (FFGs) were sampled throughout the stream reach by kick-and-sweep with a 500 mm D-net and by turning over rocks. In the field, invertebrates were identified by eye to the lowest taxonomic level (family or genus) and sorted to obtain a minimum of 5 mg (dry mass) for isotope analysis. Samples were kept in Whirl-Pak® bags and placed in a cooler during transport then frozen at the end of the day.

Fish

Fish were present in most streams and were collected by backpack electrofishing in September of 2019 and 2020. In 2019, fish were sampled using a combination of non-lethal (fin clip) and lethal methods due to the low fish numbers at the sites. Brook trout (*Salvelinus fontinalis*) were collected from all sites except for C05, C06, and C07. Slimy sculpin (*Cottus cognatus*) were collected from sites U01, L10 and U02 (2020 only at this site). At each site, lengths and weights of all fish were measured to the nearest 0.1 cm and 0.1 g. All sculpin were lethally sampled (n=3-10/site/year) in both years. In 2019, brook trout smaller than 10 cm were lethally sampled (n=2-7/site) and brook trout larger than 10 cm were non-lethally sampled (n=3-10/site) by clipping the caudal fin. In 2020, brook trout (n=6-9/site) were lethally sampled to represent a range of size classes at each site. Samples were kept in Whirl-Pak® bags and placed in coolers for transportation, then kept frozen before laboratory analysis. Samples were collected using protocols approved by McMaster's Animal Research Ethics Board (Animal Use Protocols #19-09-21 and #19-11-28) and under Québec Ministry SEG permits (#2019-05-28-009-11-SP and #2020-09-03-027-11-SP).

2.2.3. Laboratory analysis

All samples were processed in glassware cleaned in a 10% HCl bath overnight to prevent MeHg contamination (see next chapter).

Food source samples

FPOM samples were filtered through a 1 mm sieve to remove larger particles. FPOM and biofilm samples were inspected under a microscope and any debris, invertebrates, inorganic material, or other impurities were removed. CPOM was processed by rinsing leaves using deionized water to remove any clinging invertebrates.

Invertebrates

Invertebrates were identified to the lowest level possible (family or genus) and enumerated. Identification was completed using a dissection microscope and comparing morphological traits to those described in Merritt & Cummins, (1996). Invertebrates were selected to represent a range of different feeding groups. In 2019, the targeted feeding groups included predators (Chloroperlidae and *Rhyacophila*), scrapers (*Epeorus* and *Glossosoma*), and collector-filterers (*Parapsyche*). In 2020, the same taxa were collected as in the previous year along with additional taxa: collector-gatherers (Baetidae and Ephemerellidae), and shredders (Leuctridae). For each site, 1-3 replicates of each taxon were submitted for analysis based on mass availability.

Fish

In 2019, a caudal fin clip and skinless dorsal fillet were both collected from lethally-sampled fish to compare isotope values in the different tissues. In 2020, all fish were lethally collected in the field, and a skinless dorsal muscle sample was removed for isotope analyses.

Stable isotope analysis

All samples of food sources, pooled invertebrates (n=3-270/sample), and fish (individual fin clips or muscle tissue) were freeze-dried at -80°C for 48-96 hours in glass scintillation vials, then homogenized into a fine powder using acid-washed glass rods. Samples were weighed into tin capsules (1.00-1.20 mg for animal tissue, 3.00-3.20 mg for plant tissue) and submitted for carbon and nitrogen isotope analysis using continuous-flow isotope ratio mass spectrometry (Stable Isotopes in Nature Lab, University of New Brunswick). Stable isotope measurements are reported as isotope δ, which is the ratio of heavy/light isotope in the sample relative to the ratio of heavy/light isotope in a standard, in units of parts per thousand (‰). The δ notation is comparable across different elements despite varying absolute amounts of heavy/light isotope due to this double normalization (Fry, 2007). The international standards are Vienna Pee Dee Belemnite (VPDB) for carbon, and atmospheric air (AIR) for nitrogen. One replicate was submitted for every ten samples and mean relative percent differences were 0.1% for $\delta^{13}C$ and 4.6% for $\delta^{15}N$ (n=60). Analytical error was calculated as ±0.1‰ for both $\delta^{13}C$ and $\delta^{15}N$ based on repeat analyses of an in-house standard, bovine liver tissue.

2.2.4. Statistical Analysis

All analysis was completed in R version 4.0.3 with general coding and data structure following the *tidyverse* package.

Water chemistry and benthic algae

The water chemistry data were examined for values below detection limits and extreme outliers. First, water chemistry parameters were removed from analyses if more than 50% of the data were below detection limits (removed Al, Cd, Fe, Mn, Ni, Pb, NH4, SRP). Next, samples with values below the instrument detection limit (IDL) were given values of half the IDL for that parameter. Finally, each water chemistry parameter was plotted over time to visually check for any outliers. Extreme outliers were detected for SUVA (n=1) in the 2019 data and for TP (n=3) and TN (n=2) in the 2020 data. The extreme SUVA outlier was likely due to sample contamination, and removed from the dataset, and data analyses were run both with and without the TP and TN outliers for comparison.

Pearson's correlations were used to compare water chemistry and benthic algal production to defoliation in 2019 and 2020. Next, a Principal Component Analysis (PCA) was completed for the water chemistry data to summarize the correlated variables and identify the parameters that accounted for the most variation across sites. PCAs were completed on scaled water chemistry data using the *factomineR* and *factoExtra* packages. Any missing datapoints (due to removing outliers) were replaced with the mean value at that site.

Stable Isotope Analysis

Stable isotope data quality assurance

The carbon isotopes of food source samples were checked for any influence from inorganic carbonates, which would increase $\delta^{13}C$ values. The $\delta^{13}C$ and C:N values were compared to assess whether samples needed to be acid-treated (Figure A3). The FPOM and CPOM samples did not show signs of carbonates, but eight “candidate” biofilm samples had high δ^{13}C (-26.2 – -12.4‰) and C:N (12.4–13.8) values. These samples, along with one “reference” biofilm sample with lower δ^{13}C (-39.4 – -32.5‰) and C:N (6.3 – 9.6) values from each site, were reanalyzed after acid treatment to remove potential carbonates. After acid treatment, the decrease in δ^{13}C among candidate samples (-2.67 – -21.4 ‰) was greater than the change in reference samples (-0.3 – 1.7 ‰). Similarly, C:N ratios in candidate samples decreased by a larger magnitude (-2.16 – -7.88) than in reference samples (-0.2 – -0.29). These results indicate that excess carbonates were increasing the carbon isotope values of the eight candidate biofilm samples and not in reference samples. As such, carbonate-adjusted δ^{13}C values for were used for food webs and mixing models.

Biotic samples were evaluated for the influence of lipids, which would decrease δ^{13}C values. The average C:N was 3.5 for brook trout (range 3.2-4.1) and 3.9 for slimy sculpin (range 3.3-4.9). Fish carbon isotopes were not adjusted because plots of δ^{13}C to C:N did not indicate a strong influence of lipids. Arithmetic lipid adjustments using equation 3 of Post (2007) increased δ^{13}C by only 0.1-0.3‰ in brook trout and 0.1-0.8‰ in slimy sculpin. The C:N across invertebrate taxa ranged from 4.8-6.5, and a plot of δ^{13}C vs C:N showed an inverse-J shape which is characteristic of lipid influence on δ^{13}C. However, the invertebrate samples were not adjusted because mathematical and chemical methods can overcorrect for invertebrate chitin, which is also low in δ^{13}C. Samples were also not adjusted for lipid content because corrections can misrepresent trophic relationships and bias mixing models (Arostegui et al., 2019; Kiljunen et al., 2006).

Mixing models (as described below) were run for the 2020 data using one month (August only) and both months (August and September) and the results of diet autochthony were similar. Furthermore, I compared δ^{13}C and δ^{15}N values for each food source to determine whether isotopes differed between August and September of 2020. When pooled across sites, most food sources did not differ significantly between August and September, except δ^{13}C for CPOM was significantly lower by 0.8‰ in September ($p<0.05$, $n=36$) and δ^{15}N for biofilm was significantly lower by 0.64‰ in September ($p<0.05$, $n=36$). As the differences were small between months, the stable isotope biplots and mixing models were run using all data combined for each year.

Isotopes from fin clips vs muscle tissue

In 2019, the isotope values from the fin clips collected from non-lethally sampled fish were adjusted to represent muscle values using data from lethally sampled fish. Linear regressions for each of δ^{13}C and δ^{15}N were plotted to compare isotope values in fin clips and muscle tissue pooled across all sites. The δ^{13}C and δ^{15}N values from Brook trout fin clips were adjusted to the equivalent of muscle values using the equations $\delta^{15}N_{muscle} = 0.9059 \times \delta^{15}N_{fin} + 1.0536$ ($p<0.0001$, $R^2=0.94$, n=31; Figure A4A) and $\delta^{13}C_{muscle} = 0.9200 \times \delta^{13}C_{fin} - 2.1948$ ($p<0.0001$; $R^2=0.97$, n=31; Figure A4B).

Mixing models

Biplots of the δ^{13}C and δ^{15}N data were used to show relationships between energy sources (allochthonous or autochthonous) and trophic position within each site and year. Mixing models were run using the MixSIAR package in R (version 3.1.12; Stock et al., 2018).

Food source mixing polygon

A mixing polygon was generated to determine the most representative food sources to include in the models. It showed that biofilm samples were not suitable as the autochthonous energy source because the scraper taxa (*Epeorus* and *Glossosoma*) fell outside of the mixing polygon. Erdozain et al. (2019) also experienced the same issue with *Epeorus* and *Glossosoma* and attributed it to the scrapers' selective ingestion or assimilation of algal components in biofilm. Consequently, the pooled scraper isotope values were used as a proxy for the autochthonous energy source in the mixing models. For each site, the algal δ^{13}C and δ^{15}N (algae-proxy) were estimated by calculating the pooled mean δ^{13}C and δ^{15}N of the primary consumer (scrapers *Epeorus* and *Glossosoma*) values for each site and subtracting the trophic fractionation values of 0.4‰ in δ^{13}C and 2.3‰ in δ^{15}N (Erdozain et al., 2019; McCutchan et al., 2003).

Preliminary mixing models using all 3 food sources showed that CPOM had very low contributions to the diet of most consumer taxa (<10%) except for *Parapsyche*, where the contributions of CPOM ranged from an average of 5.8-35%. FPOM was chosen to represent the allochthonous food source because the δ^{13}C value of FPOM was higher than CPOM at most sites, making FPOM more inclusive of potential consumers. A 2-

isotope (C, N), 2-source (FPOM, algae-proxy) mixing model was generated for each site to determine the % autochthony of the different consumers. Models were run separately for the invertebrate primary consumers (Baetidae, Ephemerellidae, Leuctridae*),* invertebrate secondary consumers *(Parapsyche, Rhyacophila,* and Chloroperlidae), and fish (slimy sculpin, brook trout). Trophic enrichment factors for δ^{13}C (0.4 ±1.2‰) and δ^{15}N (2.3 ± 1.61‰) were used based on common values in freshwater food web studies (McCutchan et al., 2003) and a caging experiment for invertebrates (Erdozain et al., 2019), and multiplied based on the consumer feeding levels. I validated the enrichment factors by comparing the simulated data for the three different trophic groups to the measured isotope data (Figure A5, A6). All models were run with "long" or "very long" parameters, until the Gelman-Rubin and Geweke diagnostics reached the recommended convergence (Gelman et al., 2014; Geweke, 1991).

2.2.5. Comparison with landscape and local parameters

Two main response variables (RVs) were investigated in the study: benthic production (2019 total benthic production, ug/cm^2) and consumer % autochthony (mean MixSIAR value for each consumer) in each of 2019 and 2020. Several candidate explanatory variables (EVs) were considered to explain the patterns in the RVs. EVs included both landscape and local variables (Table 2.2) and were selected for the models based on their potential to affect aquatic algal communities or consumer feeding habits. Latitude and elevation represent variations in solar radiation, geology, and climate. Flow is a disturbance that affects biofilm accrual. Stream slope and watershed area can affect stream habitat conditions. Watershed and streamside defoliation (50 m buffer near stream area) were both included as EVs to contrast effects at different scales, and the latter was used as a substitute for riparian light availability, which was not measured during the study. Finally, the first and second principal components of the water chemistry data were included as local variables, using the corresponding year's data for each RV. Flow and streamside defoliation were not used for autochthony models as they were not expected to be ecologically relevant to consumer diet. All EVs were scaled and centered, and some variables were log-transformed when necessary to meet assumptions for linear models.

Explanatory models were created using hierarchical partitioning (Chevan & Sutherland, 1991) to compare the contributions of each EV to benthic algal production and consumer autochthony. Hierarchical partitioning was used due to the potential for high collinearity between annual defoliation and the landscape variables of latitude (r = 0.81, 0.85 in 2019 and 2020) and elevation (r = 0.84, 0.87 in 2019 and 2020). This method distinguishes between each variable's unique and shared contributions, which are calculated from all possible subsets of hierarchical models. Unique contributions isolate the effect of each variable and shared contributions account for collinear or suppressive effects among variables (Chevan & Sutherland, 1991). Individual contributions are the sum of unique and shared contributions and can be compared among variables to determine the most likely causal factors in the model (Mac Nally, 2000), and the sum of all individual contributions represents the variance explained by the model. Finally, Pearson correlation coefficients were used to determine the direction and magnitude of each EV in the model. Hierarchical partitioning models were not generated for slimy sculpin, Baetidae, and Leuctridae due to low sample sizes (n=2-11) or numbers of stream sites which made models prone to overfitting. A model was run successfully for Ephemerellidae but it had low explanatory power (R^2=0.17) and is not included in these results. Hierarchical partitioning models were generated using the *rdacca.hp* (Lai et al., 2022) and *vegan* (Oksanen et al., 2013) packages in R.

Table 2.2. Local and landscape variables used for modelling benthic production (2019) and consumer autochthony. *Year was used as a variable for consumer autochthony in models for taxa collected in both 2019 and 2020.

Response variable	Explanatory Variable
Benthic algal production	**Landscape** - Latitude - Elevation - Watershed area (km^2) - Watershed defoliation (2019) - Slope **Local** - Flow ($m^3 s^{-1}$) - Streamside defoliation - Water chemistry PC1 - Water chemistry PC2

Consumer autochthony	**Landscape** - Latitude - Elevation - Watershed area - Watershed defoliation - Slope - Year* **Local** - Water chemistry PC1 - Water chemistry PC2

2.3. Results

2.3.1. Water chemistry

Stream water chemistry was generally similar across sites, although there were some annual differences (Table 2.3). Across both years, stream nutrients ranged from an average of 0.001 - 0.009 mg/L for TP and 0.12 - 0.34 mg/L for TN. The pH of the streams were near neutral, although sites were more basic in 2019 (range 7.6 - 8.2) compared to 2020 (range 7.1 – 7.5). SUVA ranged from 2.67 - 4.87 L/mg-C m during the study. Across all sites, average SUVA values were lower in 2020 than 2019.

Table 2.3. Mean (SD) values of a subset of water chemistry parameters from samples collected biweekly from June – October in 2019 (n=8/site) and from June – November in 2020 (n=10/site) from streams in the Gaspé Peninsula, Québec (see Appendix for all parameters).

Site	TP (mg/L)		TN (mg/L)		DOC (mg/L)		pH		SUVA (L/mg-C m)	
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
U01	0.001 (0.0005)	0.009 (0.02)	0.14 (0.03)	0.16 (0.04)	0.66 (0.16)	0.61 (0.095)	8.0 (0.05)	7.3 (0.1)	4.87 (2.7)	3.02 (2.4)
U02	0.002 (0.0007)	0.007 (0.007)	0.19 (0.06)	0.34 (0.09)	1.57 (0.52)	1.37 (0.38)	7.6 (0.06)	7.1 (0.1)	4.31 (0.98)	3.98 (2.1)
U03	0.001 (0.0004)	0.004 (0.003)	0.11 (0.02)	0.15 (0.09)	1.88 (0.51)	1.69 (0.54)	8.0 (0.04)	7.3 (0.1)	3.96 (0.99)	3.31 (1.4)
C04	0.005 (0.008)	0.015 (0.02)	0.13 (0.2)	0.12 (0.1)	1.07 (0.45)	0.86 (0.18)	7.6 (0.07)	7.1 (0.1)	4.28 (1.8)	3.00 (1.3)
C05	0.001 (0.0005)	0.003 (0.003)	0.10 (0.04)	0.17 (0.2)	0.98 (0.57)	0.78 (0.13)	8.0 (0.04)	7.3 (0.2)	3.22 (1.2)	2.34 (1.7)
C06	0.001 (0.0005)	0.003 (0.002)	0.08 (0.02)	0.08 (0.03)	0.92 (0.38)	0.74 (0.17)	7.9 (0.05)	7.3 (0.1)	3.50 (1.1)	2.67 (0.67)
C07	0.001 (0.0005)	0.003 (0.001)	0.14 (0.03)	0.17 (0.06)	1.44 (0.43)	1.47 (0.75)	7.9 (0.04)	7.4 (0.2)	4.03 (1.1)	3.03 (1.3)
L08	0.001 (0.0002)	0.002 (0.001)	0.20 (0.05)	0.25 (0.1)	1.83 (0.62)	1.53 (0.44)	8.2 (0.05)	7.5 (0.2)	3.15 (0.50)	3.08 (0.70)
L09	0.001 (0.0008)	0.003 (0.003)	0.13 (0.03)	0.14 (0.1)	1.33 (0.33)	1.10 (0.31)	7.7 (0.07)	7.3 (0.1)	2.90 (0.58)	2.77 (0.87)
L10	0.001 (0.0003)	0.009 (0.02)	0.23 (0.08)	0.21 (0.1)	1.16 (0.22)	1.05 (0.28)	8.0 (0.06)	7.5 (0.2)	3.12 (0.50)	2.06 (0.81)
L11	0.001 (0.0006)	0.004 (0.005)	0.16 (0.06)	0.19 (0.1)	1.05 (0.28)	0.81 (0.26)	7.9 (0.04)	7.4 (0.2)	2.99 (0.80)	2.46 (0.94)

L12	0.001 (0.0005)	0.002 (0.002)	0.15 (0.05)	0.14 (0.04)	1.34 (0.40)	1.51 (0.51)	7.7 (0.07)	7.2 (0.1)	3.69 (0.64)	2.81 (0.58)

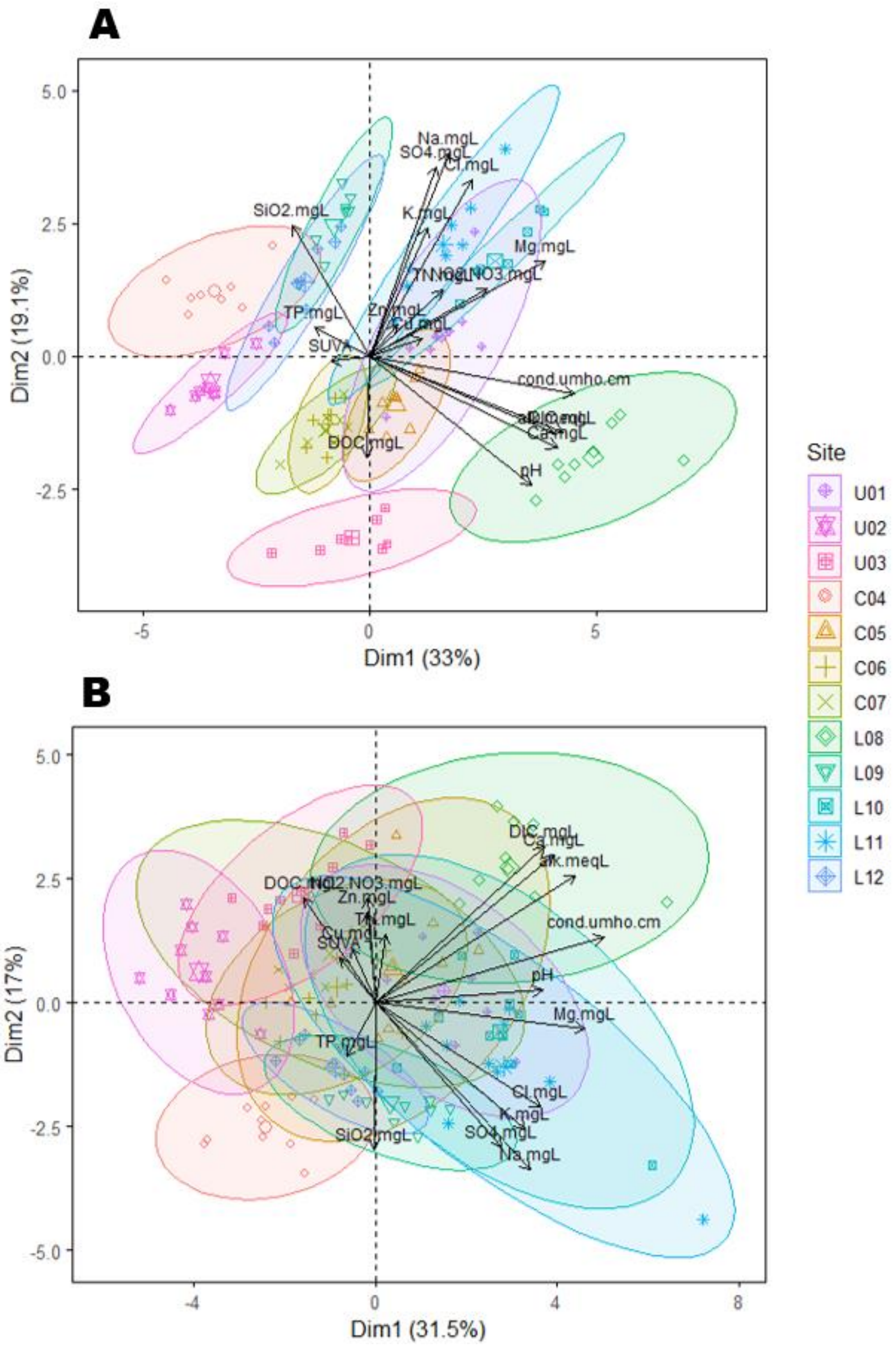

Figure 2.2. Principal component analyses of all water chemistry parameters measured in the streams in Gaspé Peninsula, Québec, in 2019 (A) and 2020 (B). Water chemistry parameters are represented by black text and

arrows. Measurements from different sites are represented by different colours and symbols, which are shown with a 95% confidence ellipse. Each measurement is represented by an individual, small symbol and the ellipse centroid is shown by the large symbol.

The variance explained by the first two axes in the water chemistry PCA were similar in 2019 and 2020, although ellipse patterns differed between years (Figure 2.2). In 2019, the first axis (33% of variation) was positively associated with conductivity, DIC, calcium, magnesium, and alkalinity, and negatively associated with TP and SUVA. The second axis (19% of variation) was positively associated with sulfate, sodium, chloride, pH, and SiO_2, and negatively associated with DOC. Sites C04, U02, L12, and L09 were clustered together due to their negative correlations with the first axis and higher SiO_2. U03 was negatively correlated with the second axis and had the highest concentration of DIC. Notably, L08 was positively correlated with the first axis and increasing conductivity, DIC, alkalinity, calcium, and pH. Water chemistry between sites were less distinct and more variable in 2020 than 2019, as the confidence ellipses were larger and overlapped more in the latter year. In 2020, the first axis (31.5% of variation) was positively associated with conductivity, pH, magnesium, and most ions. The second axis (17% of variation) was positively associated with DOC, TN, zinc, copper, and SUVA, and negatively associated with TP and SiO_2. In 2020, C04 was negatively correlated with the second axis and associated with higher SiO_2. U02 was negatively correlated with the first axis. Finally, L08 was positively associated with increasing DIC, calcium, alkalinity, and conductivity in 2020, which is consistent with results from 2019.

2.3.2. Benthic algal production

Total benthic algal production varied 3-fold across sites from 0.94-3.6 $\mu g/cm^2$ and there was a general pattern of increasing production from lower to central and upper sites. Increasing algal production was significantly related to watershed defoliation ($p=0.002$, Figure 2.3), and defoliated watersheds had higher proportions of green algae (Figure 2.4) although this trend was not statistically evaluated.

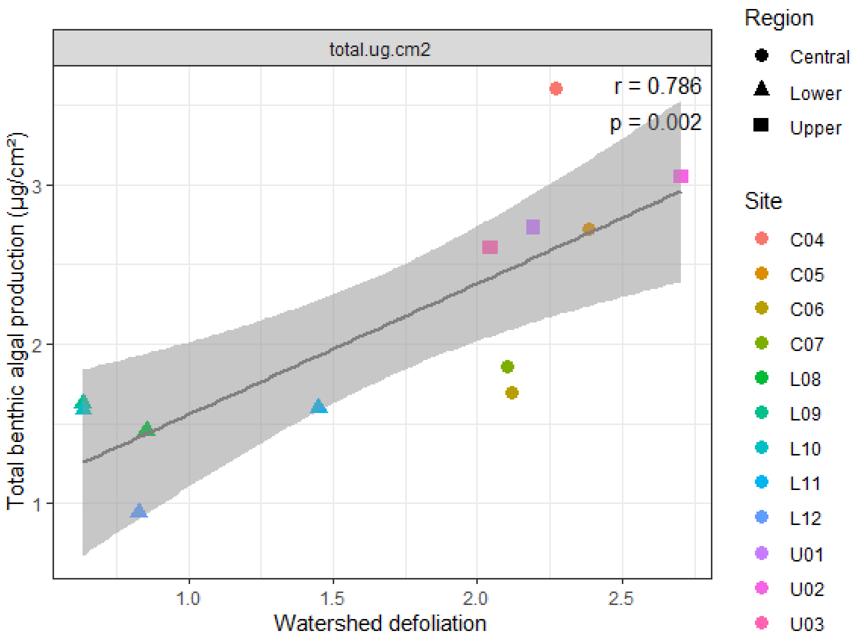

Figure 2.3. Mean total benthic algal production (µg/cm^2) versus watershed defoliation at each stream site in the Gaspé Peninsula, Québec, in 2019. Total benthic algal production was the mean of six benthotorch measurements taken at each site on each date from July – October, 2019.

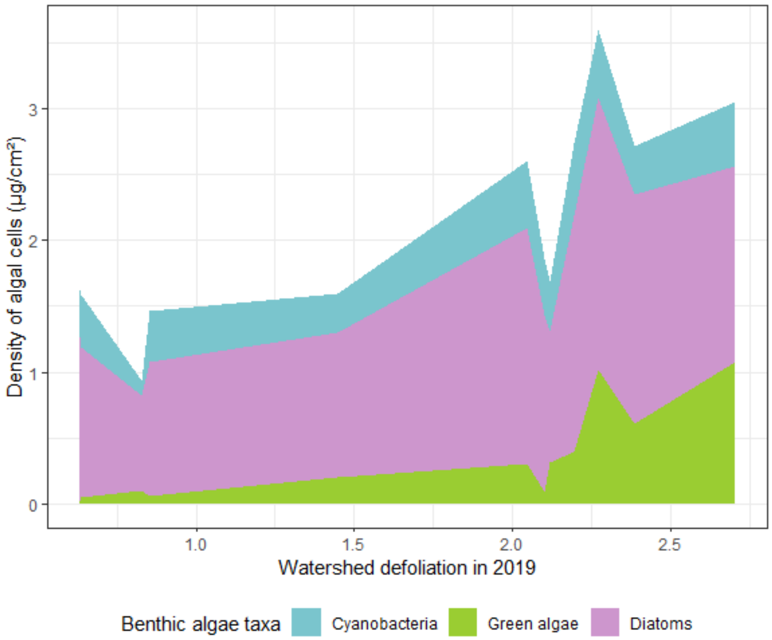

Figure 2.4. Mean density of cyanobacteria, green algae, or diatoms (µg/cm^2) versus watershed defoliation at each stream site in the Gaspé Peninsula, Québec, in 2019. The densities were calculated from the mean of six Benthotorch measurements taken at each site on each date from July – October, 2019.

Table 2.4. Hierarchical partitioning models and Pearson's correlation coefficients for total benthic production among the streams in the Gaspé Peninsula, Québec, in 2019. Individual contributions are the sum of unique and shared contributions, and the individual % is the proportion of each variable's individual contribution compared to the total explained variation. The correlation coefficients represent the direction and magnitude of each variable's effect in the model.

	Hierarchical partitioning contributions				**Correlation coefficients**
Variable	**Unique**	**Average shared**	**Individual**	**Individual %**	
Total benthic algal production					
Defoliation	0.003	0.101	0.104	25.1	0.546
Elevation	0.006	0.071	0.077	18.7	0.481
Latitude	-0.005	0.077	0.075	17.4	0.476
Slope	0.062	0.004	0.067	16.1	0.140
Watershed area	0.009	0.023	0.032	7.8	0.160
log Flow	0.002	0.018	0.019	4.6	0.345
Streamside defo.	0.003	0.014	0.017	4.1	0.134
PC2	0.006	0.009	0.015	3.6	-0.177
PC1	-0.006	0.017	0.014	2.6	-0.298
Total explained variation (model R^2)			**0.413**		

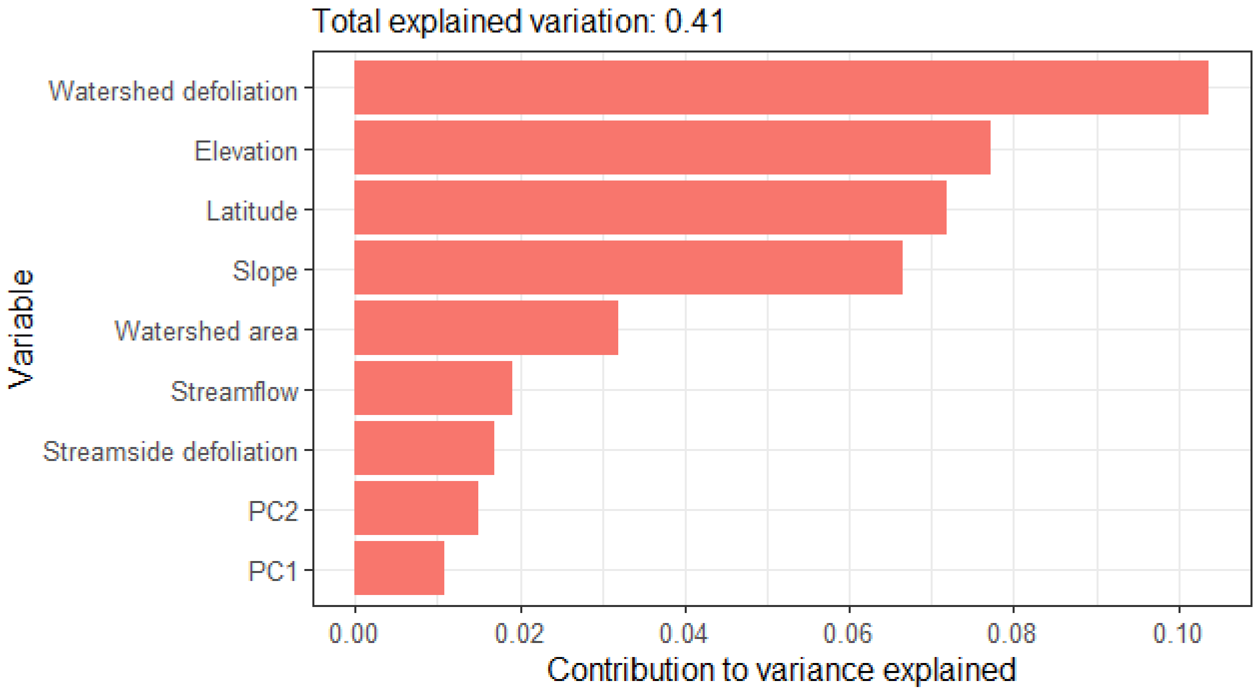

Figure 2.5. Individual contribution (proportions) of different landscape and local stream variables to benthic algal production based on hierarchical partitioning.

The landscape and local stream variables explained 41.3% of the variation in 2019 benthic algal production in the hierarchical partitioning modeling (Table 2.4, Figure 2.5). Watershed defoliation had the highest individual contribution to benthic algal production (0.1037), with a low unique contribution (0.0027). The individual contributions of elevation (0.0772) and latitude (0.0719) were similarly high, although the unique contributions were low for elevation (0.0064) and negative for latitude (-0.0049). Furthermore, shared contributions were high among defoliation (0.101), elevation (0.0708) and latitude (0.0768) due to the high multicollinearity between the variables. Slope also had a high individual contribution (0.0666), and this variable had the greatest unique contribution (0.0625) in the model. The combined contributions of watershed area, flow, streamside defoliation, and water chemistry (PC1 and PC2) explained only 9.4% of the individual variation in benthic algal production.

2.3.3. Food webs and mixing models

Biplots of the $\delta^{15}N$ and $\delta^{13}C$ data showed similar food web structures across the twelve streams in 2019 (Figure 2.6). Food sources separated out with biofilm and CPOM having lower $\delta^{15}N$ values than FPOM, and CPOM and FPOM having higher $\delta^{13}C$ values than biofilm. The two scraper taxa (*Glossosoma* and *Epeorus*) occupied the lowest trophic position of all invertebrates and had the lowest $\delta^{13}C$ values of all sample types. The two invertebrate predators (Chloroperlidae and *Rhyacophila*) and collector-filterer (*Parapsyche*) had similar $\delta^{15}N$ and $\delta^{13}C$ values and were approximately one trophic position below that of the fish. Brook trout and slimy sculpin were top predators in the streams in which they were found.

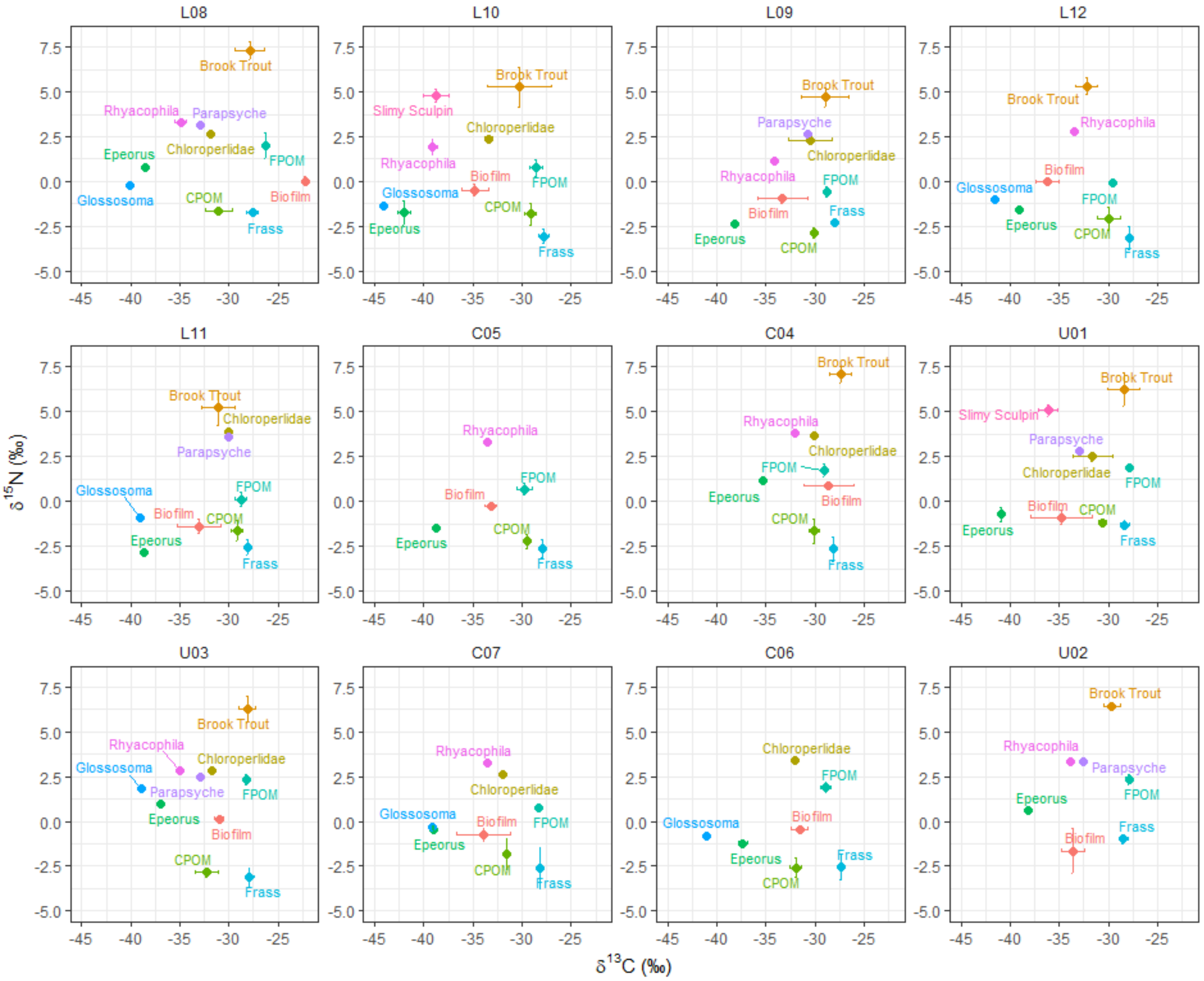

Figure 2.6. Stable isotope biplots of mean (SD) δ^{15}N vs δ^{13}C (‰) in food sources, invertebrates and fish at each site sampled in the Gaspé Peninsula, Québec, in 2019. Sites ordered from lowest to greatest watershed defoliation from the top-left to bottom-right. CPOM and FPOM are coarse and fine particulate organic matter, respectively.

Food web structure in these streams in 2020 was similar to what was found in 2019 and described above (Figure 2.7). In addition, collector-gatherers (Baetidae and Ephemerellidae) and a shredder (Leuctridae) were collected and these invertebrates had intermediate δ^{15}N values between the invertebrate scrapers and predators. Baetidae and Ephemerellidae had low δ^{13}C values, and Leuctridae had the highest δ^{13}C of all invertebrates.

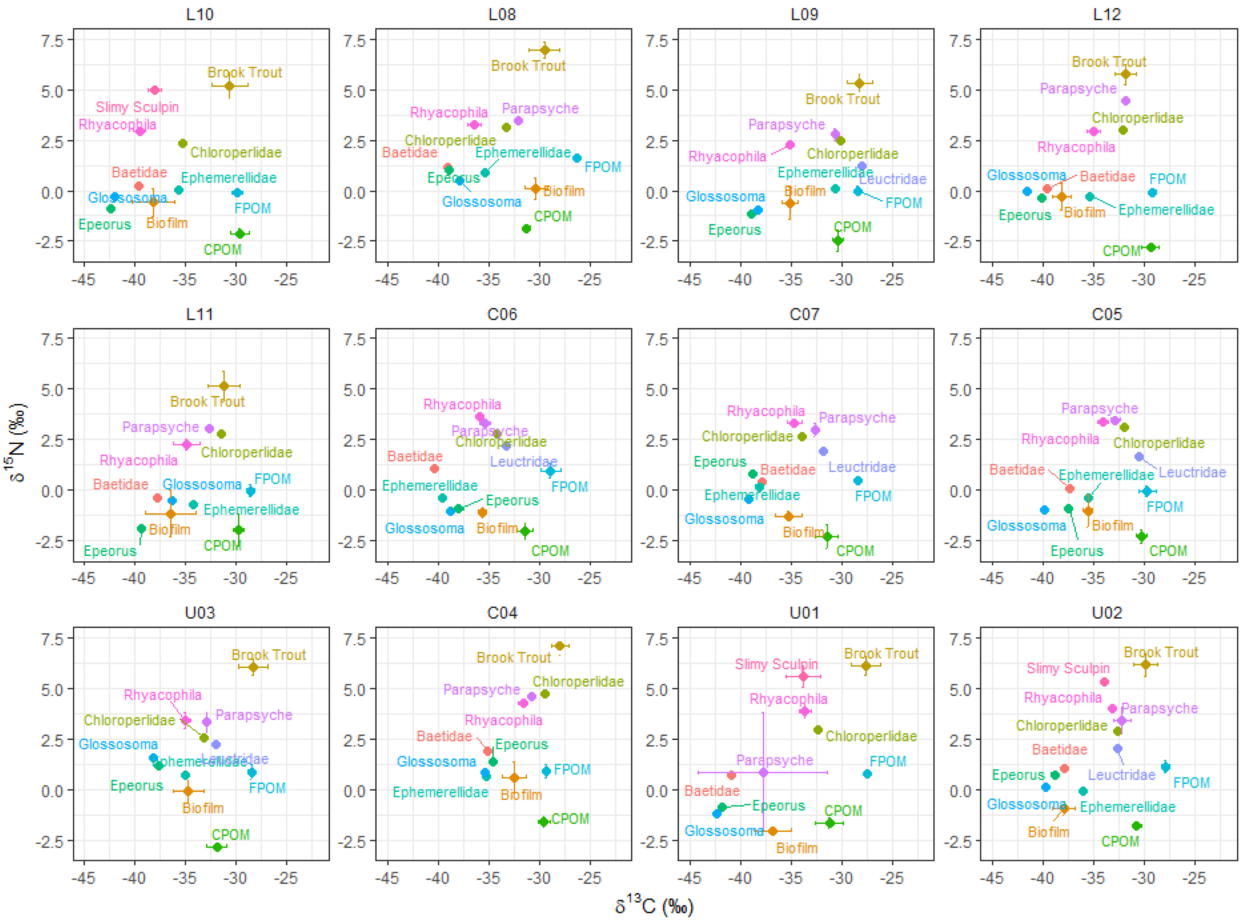

Figure 2.7. Stable isotope biplots of mean (SD) $\delta^{15}N$ vs $\delta^{13}C$ (‰) in food sources, invertebrates and fish at each site sampled in the Gaspé Peninsula, Québec, in 2020. Sites ordered from lowest to greatest watershed defoliation from the top-left to bottom-right. CPOM and FPOM are coarse and fine particulate organic matter, respectively.

2.3.4. Autochthony

Brook trout had lower reliance on autochthonous energy than allochthonous sources, and autochthony varied among sites (Figure 2.8A). Autochthonous energy contributed, on average, 29% (range 8 – 51%) in 2019 and 27% (range 12-59%) in 2020 to brook trout diets. At each site, patterns in dietary autochthony were similar between years, such as consistently low autochthonous contributions at C04, L08, and U01, and higher contributions at L10, L11 and L12. Based on the box plots, slimy sculpin had higher reliance on autochthonous energy than brook trout, with an average of 88% in 2019 and 80% in 2020, although it was only collected in two and three sites each year. Sculpin autochthony was consistently high in both years at

L10, with an average of 99% and 96% in 2019 and 2020, respectively, and autochthony was relatively lower at U01 with an average 77% and 58% in 2019 and 2020, respectively.

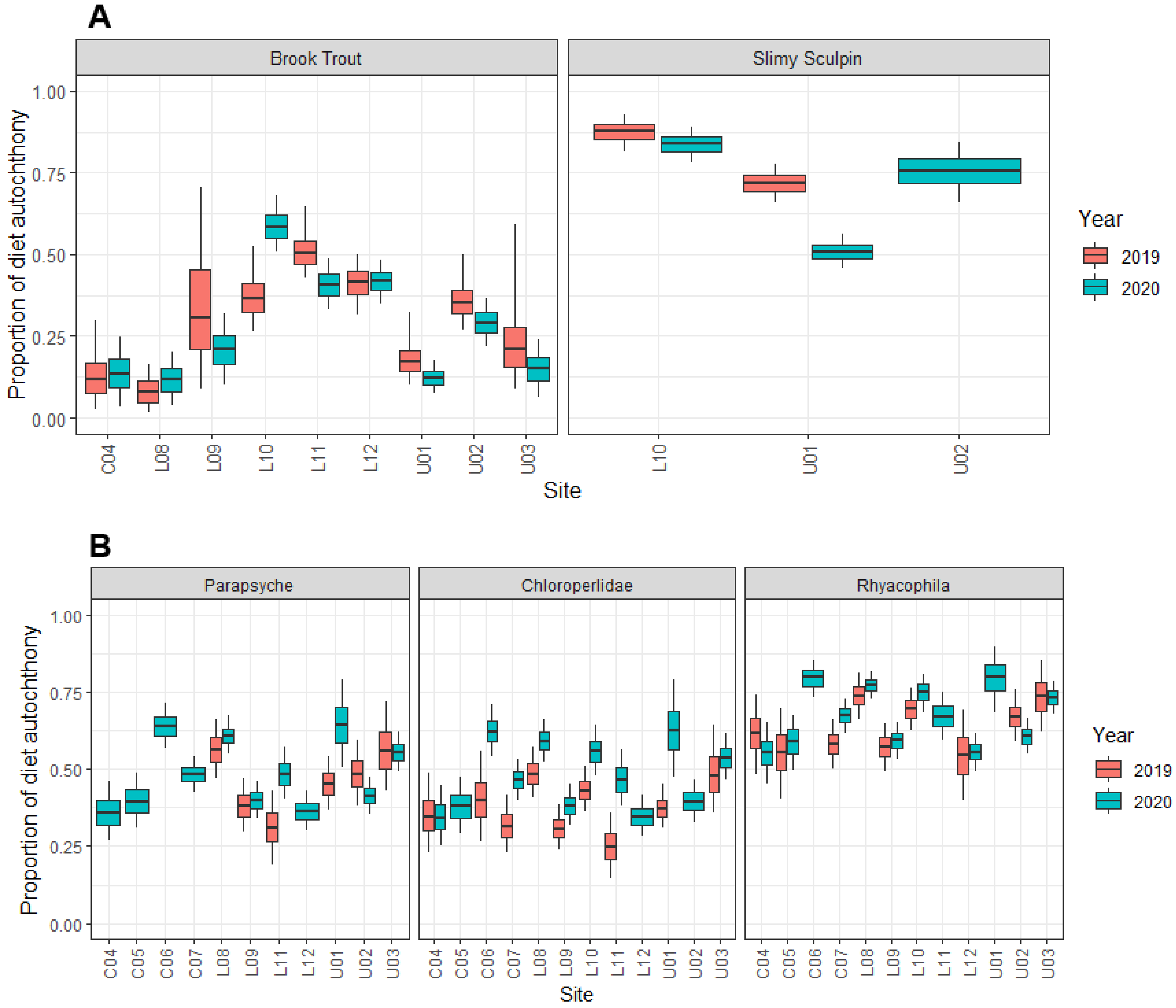

Figure 2.8. Food web autochthony of (A) brook trout and slimy sculpin and (B) Chloroperlidae, *Parapsyche*, and *Rhyachophila* collected from streams in the Gaspé Peninsula, Québec, in both 2019 and 2020. Boxplots are constructed from the median, first and third quartiles, and whiskers represent the 90% credible interval (5th and 95th percentiles of the probability distributions).

For macroinvertebrate taxa collected in both years, autochthony varied among sites and years within taxa (Figure 2.8B). Although *Parapsyche* was not as well-represented in 2019 as 2020, it did have consistent patterns in diet autochthony at sites where it was collected in both years. In 2019, the average autochthony was 46% (range 31 – 57%) and in 2020, the average was 48% (range 36-64%). Autochthony of this invertebrate was higher at U01 and L11 in 2020 than in 2019 based on the boxplot comparisons. For Chloroperlidae, mean diet autochthony was 38% (range 25 – 49%) and 48% (35 – 63%) in 2019 and 2020, respectively. For this family, the boxplots show that autochthony was higher at U01, C06, C07, L08, L09, L10, and L11 in 2020 compared to 2019. *Rhyacophila* had a greater reliance on autochthonous energy than the other predatory invertebrates at most sites, with average diet contributions of 63% (range 54-74%) in 2019, and 67% (range 55-80%) in 2020. As observed for Chloroperlidae, autochthony of *Rhyacophila* was greater at C07, L09, L10, and L12 in 2020 than 2019.

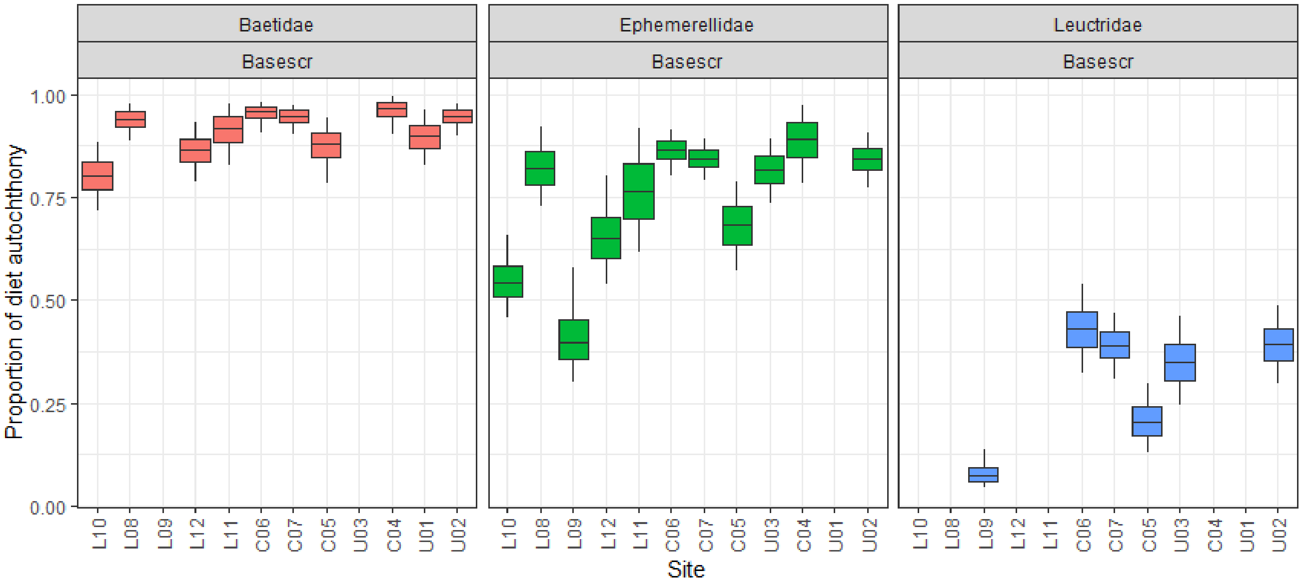

Figure 2.9. Autochthony of Baetidae, Ephemerellidae, and Leuctridae collected from streams in the Gaspé Peninsula, Québec in 2020 only. Boxplots are constructed from the median, first and third quartiles, and whiskers represent the 90% credible interval (5th and 95th percentiles of the probability distributions).

For the three additional invertebrate taxa collected in 2020 only, reliance on autochthonous carbon varied among sites for some families (Figure 2.9). Autochthonous energy accounted for an average of 80% of Baetidae diets and was consistent across sites (range 76 – 83%). Similarly, mean diet autochthony contributed to 74% of Ephemerellidae diet (range 41 – 89%), with lower autochthony at C05, L09, L10, and L12. Finally, Leuctridae had the lowest reliance on autochthonous energy of all invertebrates, which averaged 31% (range 8% – 43%) over the six sites where it was collected.

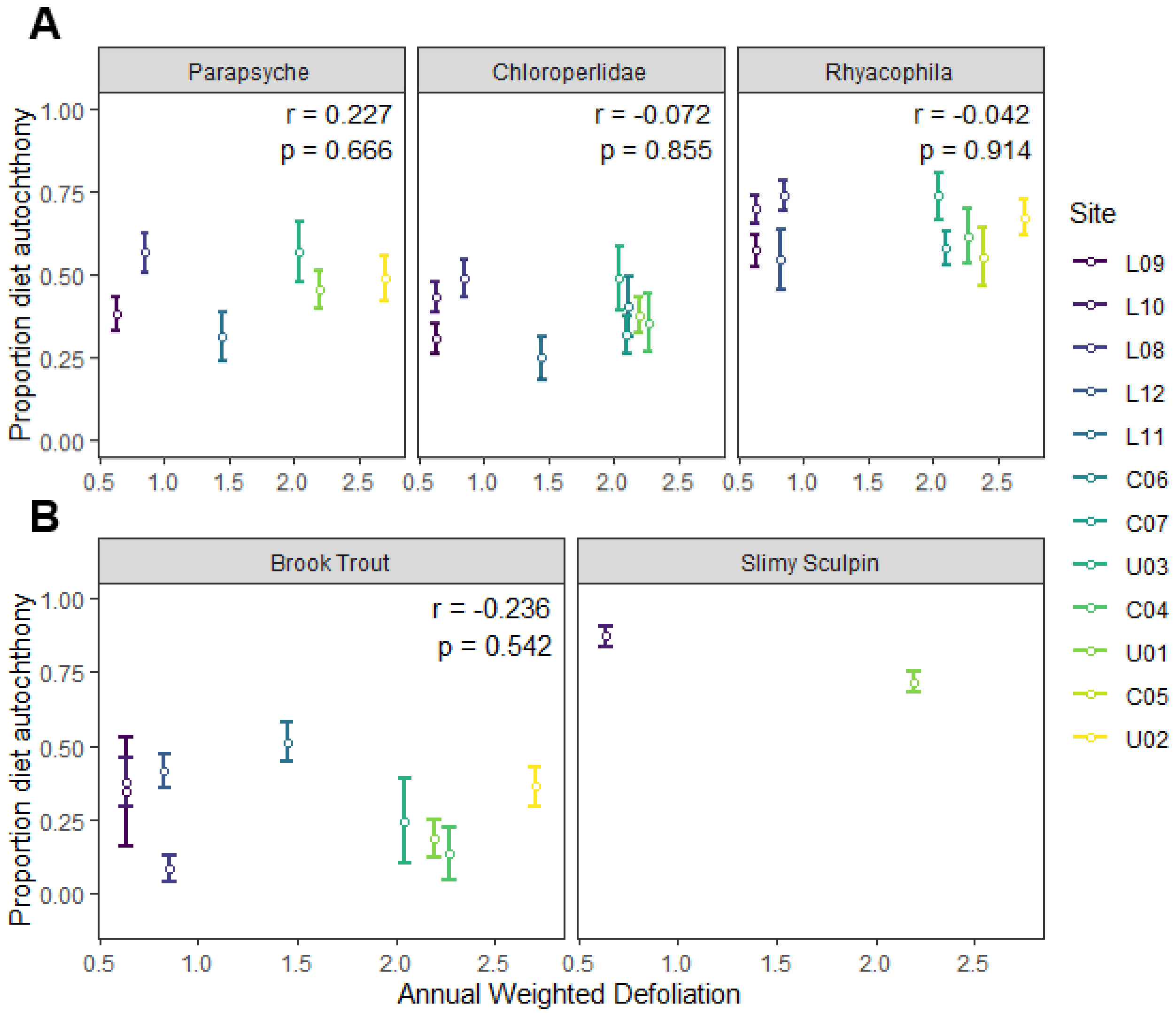

Figure 2.10. Correlations between mean autochthony of (A) *Parapsyche*, Chloroperlidae, and *Rhyacophila* or (B) brook trout and slimy sculpin and watershed defoliation in 2019. Error bars represent standard deviation.

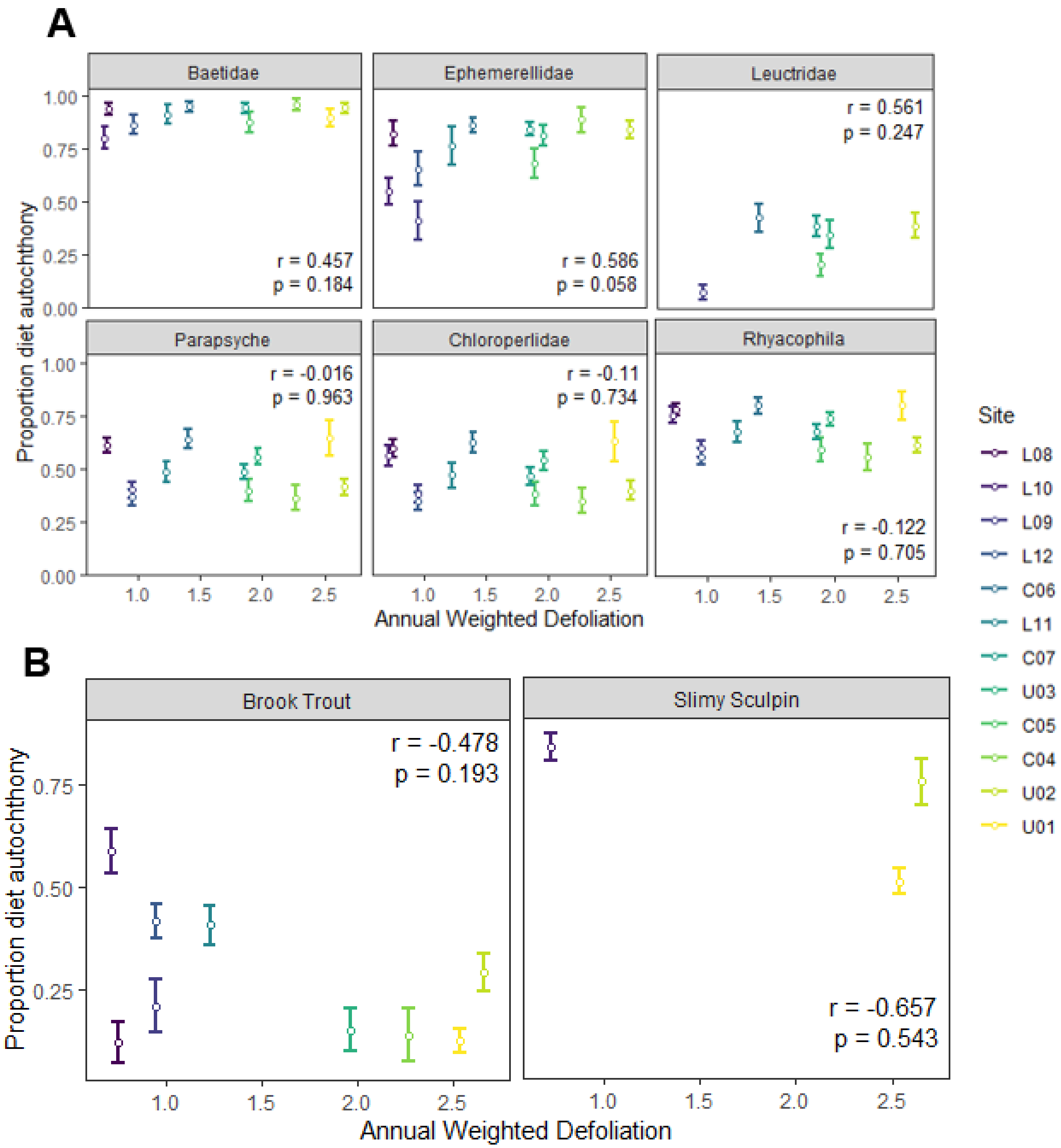

Figure 2.11. Correlations between mean autochthony in (A) Baetidae, Ephemerellidae, Leuctridae, *Parapsyche*, Chloroperlidae, *Rhyacophila* and (B) brook trout and slimy sculpin and the defoliation index in 2020. Error bars represent standard deviation.

The landscape and stream variables explained 58.8% of the variation in brook trout autochthony. The greatest individual contributions to the model were from latitude (0.187), elevation (0.150), and PC2 (0.124), and indicated that autochthony decreased with lower latitudes and elevations, and lower values of PC2. The next highest individual scores were due to annual differences (0.050) and indicated higher autochthony in 2019 than 2020. When combined, the remaining variables (stream slope, catchment defoliation, watershed area, and PC1) contributed less than 10% to the total explained variation in the model. Catchment defoliation was correlated with decreasing brook trout autochthony, although this variable had relatively low explanatory power in the model.

Hierarchical partitioning models explained between 41.3 and 54.9% of the autochthony of Chloroperlidae, *Rhyacophila* and *Parapsyche* and there were some similarities in the variables that explained their dietary habits (Figure 2.12, Table 2.5). Although year, PC1 + watershed area and slope had high individual contributions for Chloroperlidae, *Rhyacophila* and *Parapsyche*, respectively, latitude (0.1983, 0.0783, 0.1049) and defoliation (0.0691, 0.1074, 0.0643) also had high individual contributions to autochthony for all three taxa. Within Chloroperlidae, the correlations indicated that autochthony was positively affected by year (2020 was higher) and latitude, and negatively related to defoliation. For *Rhyacophila,* autochthony was positively correlated to PC1 and latitude and negatively related to watershed area and defoliation. Autochthony of *Parapsyche* was positively related to latitude and defoliation and negatively related to slope.

Table 2.5. Hierarchical partitioning models and Pearson's correlation coefficients for mean consumer autochthony from taxa sampled in both 2019 and 2020. Individual contributions are the sum of unique and shared contributions, and the individual % is the proportion of each variable's individual contribution compared to the total explained variation. The correlation coefficients represent the direction and magnitude of each variable's effect in the model.

	Hierarchical partitioning contributions				**Correlation Coefficient**
Variable	**Unique**	**Average shared**	**Individual**	**Individual %**	
Brook trout					

Latitude	0.109	0.078	0.187	31.72	-0.45
Elevation	0.265	-0.115	0.150	25.48	-0.36
PC2	0.240	-0.117	0.124	21.02	0.40
Year	0.239	-0.189	0.050	8.42	-0.07
Slope	0.177	-0.156	0.021	3.59	-0.10
Defoliation	-0.009	0.030	0.020	3.47	-0.36
Watershed area	0.066	-0.047	0.019	3.25	0.04
PC1	0.131	-0.112	0.018	3.13	-0.03
Total explained variation (model R^2)			**0.588**		
		Chloroperlidae			
Year	0.002	0.197	0.198	48.0	0.462
Latitude	0.057	0.015	0.071	17.2	0.256
Defoliation	0.068	0.002	0.069	16.7	-0.081
Watershed area	-0.030	0.068	0.038	9.3	-0.409
PC1	-0.017	0.033	0.016	3.9	0.089
Elevation	-0.037	0.046	0.009	2.2	0.195
PC2	0.007	0.000	0.007	1.6	0.104
Slope	0.010	-0.006	0.005	1.2	-0.227
Total explained variation (model R^2)			**0.413**		
		Rhyacophila			
PC1	0.202	-0.040	0.162	29.4	0.334
Watershed area	0.012	0.105	0.117	21.4	-0.555
Defoliation	0.198	-0.091	0.107	19.6	0.085
Latitude	0.072	0.007	0.078	14.3	0.267
PC2	0.104	-0.067	0.038	6.9	-0.116
Year	-0.032	0.066	0.034	6.2	0.234
Elevation	-0.031	0.058	0.027	4.8	0.215
Slope	0.011	-0.024	-0.013	-2.4	-0.191
Total explained variation (model R^2)			**0.549**		
		Parapsyche			
Latitude	0.051	0.054	0.105	25.1	0.407
Slope	0.045	0.041	0.086	20.7	-0.481
Defoliation	0.147	-0.082	0.064	15.4	0.073
PC1	0.001	0.045	0.046	11.1	0.234
Watershed area	-0.049	0.093	0.043	10.4	-0.494
PC2	0.054	-0.022	0.032	7.7	0.411
Year	-0.046	0.070	0.024	5.7	0.124
Elevation	-0.061	0.077	0.017	4.0	0.319
Total explained variation (model R^2)			**0.418**		

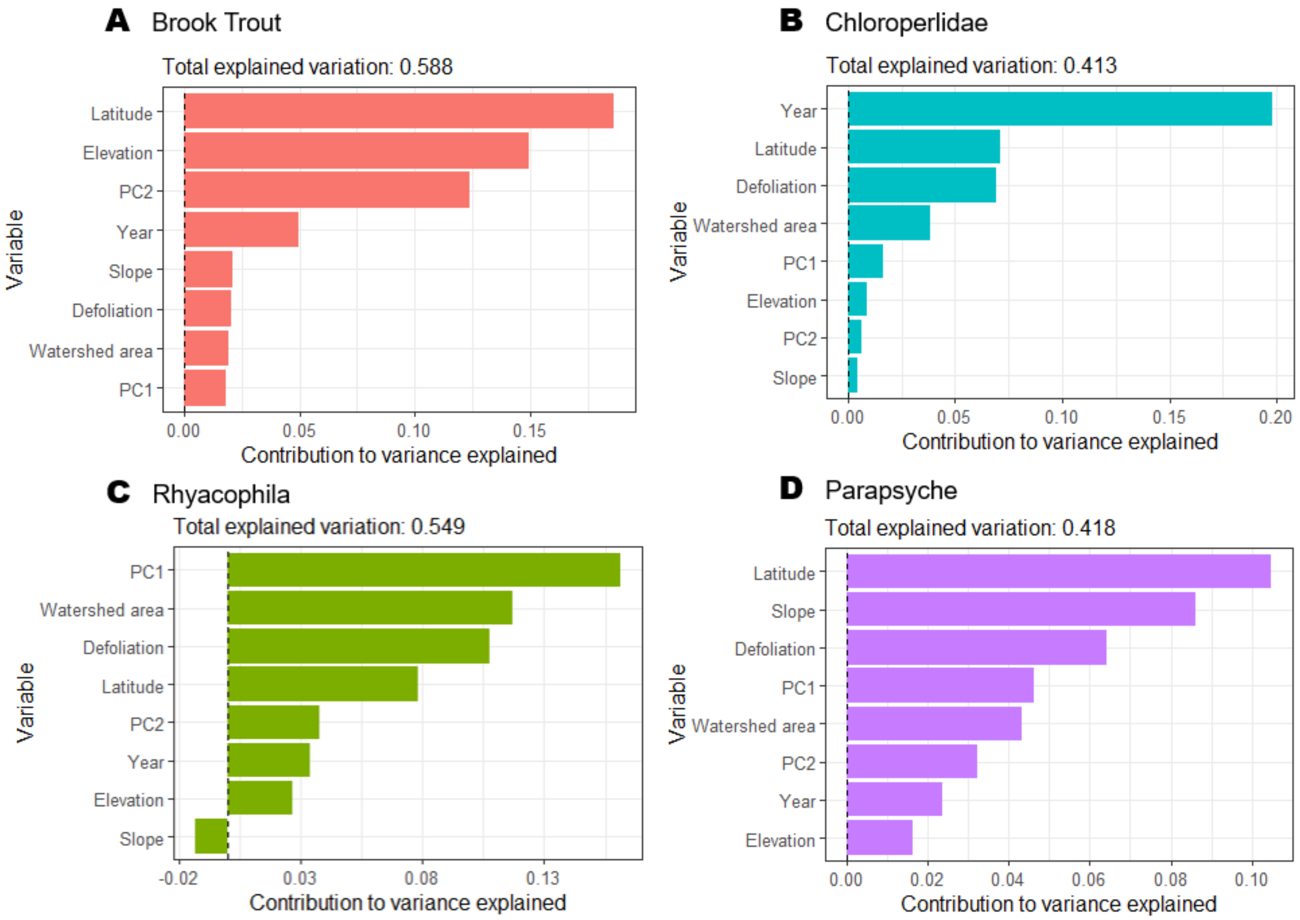

Figure 2.12. Individual contributions (proportions) of different landscape and local variables to average consumer autochthony of (A) brook trout, (B) Chloroperlidae, (C) *Rhyacophila*, and (D) *Parapsyche* using hierarchical partitioning.

2.4. Discussion

2.4.1. Drivers of benthic algal production in streams

Increasing defoliation was the most important variable driving autochthonous production, and this was expected based on studies of other terrestrial disturbances as autochthonous productivity is sensitive to changes in light and nutrients, which commonly limit algal growth (Atkinson & Cooper, 2016). Forest harvesting studies have found 6-15 times greater algal production in clear-cut streams than in unharvested sites, and these differences were attributed to the loss of canopy cover (Kiffney et al., 2003; Wilkerson et al., 2010). As well, LDD moth defoliation decreased the riparian canopy cover and increased algal growth from 35% to 80% in a headwater stream (Sheath et al., 1986). There is evidence that SBW defoliation decreases canopy cover in forests (D'Aoust et al., 2004), which can in-turn facilitate stream production as I observed herein.

In my study, increasing biofilm production was mainly associated with landscape characteristics including greater defoliation, higher elevations, northern latitudes, steeper slopes, and larger watershed areas, rather than local stream variables. Although defoliation, elevation, and latitude explained a high amount of variance in the algal production model, most of the variance was shared, and their impact on algal production cannot be considered independently. Elevation and latitudinal gradients characterize climactic differences that can predict benthic algal production (Alahuhta et al., 2017). Defoliation had a low unique contribution to the model and most of the variance was shared among landscape variables. As a result, the effect of defoliation on algal production cannot be isolated from these other factors. Watershed area and slope can represent variation in stream nutrients, habitat, and hydrologic regimes (van Nieuwenhuyse & Jones, 1996). However, my results may not be representative of general drivers of biofilm production because the landscape parameters were collinear and spanned a relatively limited range.

Local conditions in the streams explained a small amount of variance in the model for algal production. Stream flow had a small, positive effect on algal production and this is likely because increasing stream flow delivers nutrients from runoff (Biggs & Close, 1989) and temporarily displaces invertebrate scrapers (Hart & Finelli, 1999). In the models, I used streamside defoliation in lieu of stream light availability, although this parameter did not explain a large amount of model variation. This was unexpected because terrestrial canopy cover often limits light availability and biofilm growth in headwater streams (Warren et al., 2016). However, streamside defoliation may not be an accurate representation of stream light due to differences in channel morphology and stream canopy cover among the sites. Finally, the water chemistry variables were the least important in the model. Contrary to expectations, PC1 (increasing N) was negatively related to benthic algal production, suggesting that the biofilms were not limited by N. In freshwater environments, nutrients such as N and P can limit primary production. However, the variation in nutrients across streams was low herein and may not have been sufficient to cause differences in algal production among sites.

In 2019, proportions of green algae were higher and diatoms were lower in streams with severe watershed defoliation (Figure 2.4). Shifts in both density and community composition of benthic algae can affect secondary growth in stream consumers (Rosemond et al., 2000) because the various taxonomic classes of benthic algae (diatoms, green algae, and cyanobacteria) have different nutritious qualities and responses to disturbance. Although scrapers commonly consume green algae, the filamentous forms are a low-quality food source that grow rapidly with increasing light and outcompete nutritious diatoms (Davies et al., 2008). However, BenthoTorch measurements in this study did not distinguish the filamentous green algae. Diatoms are commonly ingested by invertebrate scrapers and are a source of fatty acids (Guo et al., 2016; Tamura & Kagaya, 2019). Additionally, Grady et al. (2007) associated the proliferation of stream diatoms with historic LDD moth defoliation; however, I did not observe this pattern in this study, potentially due to the short-term scope of my study. Finally, cyanobacteria are sensitive to increasing nutrients, and can be a nuisance in aquatic environments as they can contain toxins and are not an important food source for herbivorous invertebrates (Stevenson et al., 1996).

2.4.2. Stream food webs and consumer autochthony

The two fish species had distinct feeding habits as brook trout mainly relied on allochthonous energy, whereas slimy sculpin relied more on autochthonous energy. For brook trout, allochthonous energy was more important than autochthonous energy at most sites except L11 in 2019, and L10 in 2020. This is consistent with studies that found terrestrial invertebrates were the dominant food source for salmonids (Nakano & Murakami, 2001; Wipfli, 1997). In contrast, sculpin autochthony was greater than 70% in most cases and this concurs with gut content analyses as this species primarily feeds on benthic invertebrates, especially Chironomidae and Simuliidae (Mundahl et al., 2012), which were abundant in the study streams (personal obs.).

The weak effect of watershed defoliation on brook trout diets was unexpected, as brook trout are reliant on terrestrial food sources (Wipfli et al., 1997) and forest and riparian plant composition determine the terrestrial invertebrates available as prey for salmonids (Albertson et al., 2018; Allan et al., 2002). The brook trout in this study had high reliance on allochthonous energy regardless of the level of defoliation in the catchment; however, it is not known whether brook trout consumed greater proportions of budworm larvae in lieu of other terrestrial insects in the heavily-defoliated catchments, as the increased densities of SBW may subsize prey for fish. Brook trout are opportunistic predators, and lepidopterans are important prey in late summer when trout have the greatest reliance on terrestrial prey (Syrjänen et al., 2011; Utz & Hartman, 2007; Webster & Hartman, 2005); this is the same period (July – August) of peak of SBW larval populations (Natural Resources Canada, 2012). However, increased consumption of SBW by brook trout may not be detectable using stable isotopes as budworm larvae are likely to share similar isotope values as terrestrial invertebrates, and another approach such as traditional or DNA analyses of gut contents would have been helpful. The use of litterfall traps in the riparian zone would have also helped to understand the inputs of budworm carcasses to streams and their availability as prey subsidies. Finally, the results for brook trout contrasts the findings for invertebrate consumers, as defoliation was a relatively important variable in explaining increasing invertebrate allochthony. Brook trout diets may be less susceptible to defoliation as fish have a greater variety of prey sources that can potentially mitigate shifts in food source availability (Baxter et al., 2005). Furthermore, the invertebrate predators had greater reliance on autochthonous energy than brook trout in less defoliated streams and, as such, they may be more susceptible to such catchment disturbances that affect the relative inputs and production of allochthonous and authochthonous carbon in streams and more amenable to the use of carbon isotopes to detect dietary shifts.

All macroinvertebrates in this study relied on autochthonous energy sources to varying extents, consistent with other studies in shaded headwater streams (Hayden et al., 2016; Rosi-Marshall et al., 2016). In the current study, autochthony was greatest in the two scrapers, *Epeorus* and *Glossosoma* (based on raw $\delta^{13}C$), and the collector-gatherers Baetidae and Ephemerellidae (based on mixing models). In some sites, the $\delta^{13}C$ of these invertebrates were lower than biofilm; this was also observed in the same taxa collected by Erdozain et al. (2019) and may be caused by scrapers selectively ingesting certain components of biofilms (Finlay, 2001) including ^{13}C-depleted methanotrophic bacteria (Sampson et al., 2019). Although Leuctridae stoneflies are classified as shredders, autochthonous sources had some contribution (average 8-43%) to their diet. This is consistent with studies that found large amounts of fine detritus and benthic algae in Leuctridae gut contents, especially in early-instar larvae (Dangles, 2002). Next, autochthony ranged from 20-60% in the net-spinning caddisfly *Parapsyche*. This is greater than reports from studies in boreal streams where autochthonous sources were less than 25% of *Parapsyche* diets (Charbonneau et al., 2022; Erdozain et al., 2019). However, this range is within the values reported for *Hydropsyche*, a similar net-spinning caddisfly (Jonsson et al., 2018). As a filter feeder, *Parapsyche* has a non-selective diet that can be representative of catchment food availability (Jonsson et al., 2018). As expected, the two predatory invertebrates Chloroperlidae and *Rhyacophila* had similar contributions of both autochthonous and allochthonous energy, although the latter had a greater dependence on autochthonous carbon. Chloroperlidae was the only invertebrate with differences in autochthony between years, as the hierarchical partitioning models found that autochthony was greater in 2020 compared to 2019.

In most consumers (brook trout, Chloroperlidae, *Rhyacophila*) except *Parapsyche*, allochthony was higher in watersheds with higher defoliation and this positive effect was stronger for consumers at a higher trophic position. This contrasts my prediction that increasing defoliation would increase consumer autochthony. However, this prediction was based on studies that found greater invertebrate autochthony with canopy loss due to forestry activity (England & Rosemond, 2004). My results may reflect differences between defoliation and forest harvesting as landscape disturbances. Firstly, insect carcasses can become abundant during outbreaks and provide an important terrestrial food source to stream predators. This contrasts with forest harvesting where terrestrial prey inputs to streams are reduced (Eros et al., 2012). Secondly, trees can withstand multiple years of defoliation, whereas tree biomass is removed during forest harvesting. Even after defoliation, remaining riparian biomass can mitigate some physical and biogeochemical effects of the disturbance and provide habitat for terrestrial invertebrates (Broadmeadow & Nisbet, 2004). Finally, responses of consumer autochthony to forest disturbances are not consistent; for example, Göthe et al. (2009), and Bilby & Bisson, (1992) observed increased consumer autochthony following forest disturbance, but Erdozain et al. (2019) and Jonsson et al., (2018) found that forestry decreased autochthony in stream consumers.

2.5. Conclusion

This project investigated the impact of spruce budworm defoliation on algal production and autochthonous carbon consumption. Among the factors that I examined, defoliation in the watershed was the most influential factor for increasing algal production and allochthony in brook trout. However, the effect of defoliation on these responses were largely shared with site elevation and latitude. Additionally, the invertebrates *Rhyacophila* and *Chloroperlidae* had higher allochthony in the defoliated watersheds, although the opposite effect was observed for *Parapsyche*. Finally, most consumers relied on autochthonous energy to some degree, although the increase in algal production in highly-defoliated watersheds did not result in greater autochthony of their consumers and suggests a different mechanism for the shifts in dietary habits. This was the first study to describe the impact of a forest defoliator on stream food web structure, and contributes new knowledge of how terrestrial disturbances change basal energy sources and their consumption in streams. Results suggest that there are potential benefits of early intervention to reduce SBW defoliation through spraying. First, intervention can prevent benthic algae from proliferating in streams, including fast-responding filamentous green algae that are not as nutritious for stream consumers. Second, intervention can prevent the shift in stream invertebrates towards more allochthonous diets which may alter their biomass and exposure to terrestrially-derived contaminants. These results show that terrestrial defoliation affects stream food webs at multiple different trophic levels, and these responses should be considered when forests are being managed for insect outbreaks.

References

Alahuhta, J., Kosten, S., Akasaka, M., Auderset, D., Azzella, M. M., Bolpagni, R., Bove, C. P., Chambers, P. A., Chappuis, E., Clayton, J., de Winton, M., Ecke, F., Gacia, E., Gecheva, G., Grillas, P., Hauxwell, J., Hellsten, S., Hjort, J., Hoyer, M. v., … Heino, J. (2017). Global variation in the beta diversity of lake macrophytes is driven by environmental heterogeneity rather than latitude. *Journal of Biogeography, 44*(8), 1758–1769. https://doi.org/10.1111/JBI.12978

Albertson, L. K., Ouellet, V., & Daniels, M. D. (2018). Impacts of stream riparian buffer land use on water temperature and food availability for fish. Journal of Freshwater Ecology, 33(1), 195–210. https://doi.org/10.1080/02705060.2017.1422558

Allan, J. D., Wipfli, M. S., Caouette, J. P., Prussian, A., & Rodgers, J. (2003). Influence of streamside vegetation on inputs of terrestrial invertebrates to salmonid food webs. Canadian Journal of Fisheries and Aquatic Sciences, 60(3), 309–320. https://doi.org/10.1139/f03-019

Arostegui, M. C., Schindler, D. E., & Holtgrieve, G. W. (2019). Does lipid-correction introduce biases into isotopic mixing models? Implications for diet reconstruction studies. *Oecologia*, *191*(4), 745–755. https://doi.org/10.1007/s00442-019-04525-7

Atkinson, C. L., & Cooper, J. T. (2016). Benthic algal community composition across a watershed: coupling processes between land and water. *Aquatic Ecology, 50*(2), 315–326. https://doi.org/10.1007/S10452-016-9580-5/FIGURES/6

Baxter, C. V., Fausch, K. D., & Saunders, W. C. (2005). Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zones. Freshwater Biology, 50(2), 201–220. https://doi.org/10.1111/J.1365-2427.2004.01328.X

Bertini, S. C. B., Azevedo, L. C. B., de Carvalho Mendes, I., & Cardoso, E. J. B. N. (2014). Hierarchical partitioning for selection of microbial and chemical indicators of soil quality. *Pedobiologia*, *57*(4–6), 293–301. https://doi.org/10.1016/j.pedobi.2014.06.001

Biggs, B. J. F., & Close, M. E. (1989). Periphyton biomass dynamics in gravel bed rivers: the relative effects of flows and nutrients. *Freshwater Biology*, *22*(2), 209–231. https://doi.org/10.1111/j.1365-2427.1989.tb01096.x

Bilby, R. E., & Bisson, P. A. (1992). Allochthonous versus autochthonous organic matter contributions to the trophic support of fish populations in clear-cut and old-growth forested streams. *Canadian Journal of Fisheries and Aquatic Sciences*, *49*(3), 540–551. https://doi.org/10.1139/f92-064

Bouchard, M., & Pothier, D. (2010). Spatiotemporal variability in tree and stand mortality caused by spruce budworm outbreaks in eastern Quebec. *Canadian Journal of Forest Research*, *40*(1), 86–94. https://doi.org/10.1139/X09-178

Brandt, J. P., Flannigan, M. D., Maynard, D. G., Thompson, I. D., & Volney, W. J. A. (2013). An introduction to Canada's boreal zone: Ecosystem processes, health, sustainability, and environmental issues. *Environmental Reviews, 21*(4), 207–226. https://doi.org/10.1139/er-2013-0040

Brett, M. T., Bunn, S. E., Chandra, S., Galloway, A. W. E., Guo, F., Kainz, M. J., Kankaala, P., Lau, D. C. P., Moulton, T. P., Power, M. E., Rasmussen, J. B., Taipale, S. J., Thorp, J. H., & Wehr, J. D. (2017). How important are terrestrial organic carbon inputs for secondary production in freshwater ecosystems? *Freshwater Biology*, *62*(5), 833–853. https://doi.org/10.1111/fwb.12909

Broadmeadow, S., & Nisbet, T. R. (2004). The effects of riparian forest management on the freshwater environment: a literature review of best management practice. *Hydrology and Earth System Sciences*, *8*(3), 286–305.

Carlsson, N. O. L., Sarnelle, O., & Strayer, D. L. (2009). Native predators and exotic prey - An acquired taste? *Frontiers in Ecology and the Environment*, *7*(10), 525–532. https://doi.org/10.1890/080093

Charbonneau, K. L., Kidd, K. A., Kreutzweiser, D. P., Sibley, P. K., Emilson, E. J. S., O'Driscoll, N. J., & Gray, M. A. (2022). Are There Longitudinal Effects of Forest Harvesting on Carbon Quality and Flow and Methylmercury Bioaccumulation in Primary Consumers of Temperate Stream Networks? *Environmental Toxicology and Chemistry*, *41*(6), 1490–1507. https://doi.org/10.1002/etc.5330

Chevan, A., & Sutherland, M. (1991). Hierarchical partitioning. *American Statistician*, *45*(2), 90–96. https://doi.org/10.1080/00031305.1991.10475776

Cross, W. F., Benstead, J. P., Frost, P. C., & Thomas, S. A. (2005). Ecological stoichiometry in freshwater benthic systems: Recent progress and perspectives. In *Freshwater Biology* (Vol. 50, Issue 11, pp. 1895–1912). John Wiley & Sons, Ltd. https://doi.org/10.1111/j.1365-2427.2005.01458.x

Cummins, K. W. (1974). Structure and Function of Stream Ecosystems. *BioScience*, *24*(11), 631–641. https://doi.org/10.2307/1296676

Dangles, O. (2002). Functional plasticity of benthic macroinvertebrates: Implications for trophic dynamics in acid streams. *Canadian Journal of Fisheries and Aquatic Sciences*, *59*(9), 1563–1573. https://doi.org/10.1139/f02-122

D'Aoust, V., Kneeshaw, D., & Bergeron, Y. (2004). Characterization of canopy openness before and after a spruce budworm outbreak in the southern boreal forest. *Canadian Journal of Forest Research*, *34*(2), 339–352. https://doi.org/10.1139/x03-278

Davies, P. M., Bunn, S. E., & Hamilton, S. K. (2008). Primary production in tropical streams and rivers. In *Tropical Stream Ecology* (pp. 23–42). Academic Press. https://doi.org/10.1016/B978-012088449-0.50004-2

England, L. E., & Rosemond, A. D. (2004). Small reductions in forest cover weaken terrestrial-aquatic linkages in headwater streams. *Freshwater Biology*, *49*(6), 721–734. https://doi.org/10.1111/j.1365-2427.2004.01219.x

Erdozain, M., Kidd, K., Kreutzweiser, D., & Sibley, P. (2019). Increased reliance of stream macroinvertebrates on terrestrial food sources linked to forest management intensity. *Ecological Applications*, *29*(4), 1–14. https://doi.org/10.1002/eap.1889

Eros, T., Gustafsson, P., Greenberg, L. A., & Bergman, E. (2012). Forest-stream linkages: Effects of terrestrial invertebrate input and light on diet and growth of brown trout (salmo trutta) in a boreal forest stream. *PLoS ONE*, *7*(5), e36462. https://doi.org/10.1371/journal.pone.0036462

Finlay, J. C. (2001). Stable-carbon-isotope ratios of river biota: Implications for energy flow in lotic food webs. *Ecology, 82*(4), 1052–1064. https://doi.org/10.1890/0012-9658(2001)082[1052:SCIROR]2.0.CO;2

Fisher, S. G., & Likens, G. E. (1973). Energy Flow in Bear Brook, New Hampshire: An Integrative Approach to Stream Ecosystem Metabolism. *Ecological Monographs*, *43*(4), 421–439. https://doi.org/10.2307/1942301

Frost, C. J., & Hunter, M. D. (2007). Recycling of nitrogen in herbivore feces: Plant recovery, herbivore assimilation, soil retention, and leaching losses. *Oecologia*, *151*(1), 42–53. https://doi.org/10.1007/s00442-006-0579-9

Fry, B. (2007). Stable isotope ecology. In *Encyclopedia of Ecology*. https://doi.org/10.1016/B978-0-12-409548-9.10915-7

Gandhi, K. J. K., & Herms, D. A. (2010). Direct and indirect effects of alien insect herbivores on ecological processes and interactions in forests of eastern North America. *Biological Invasions, 12*(2), 389–405. https://doi.org/10.1007/s10530-009-9627-9

Gelman, A., Carlin, J. B., Stern, H. S., & Rubin, D. B. (2014). *Bayesian Data Analysis*. Chapman and Hall/CRC.

Geweke, J. F. (1991). *Evaluating the accuracy of sampling-based approaches to the calculation of posterior moments*. Federal Reserve Bank of Minneapolis.

Göthe, E., Lepori, F., & Malmqvist, B. (2009). Forestry affects food webs in northern Swedish coastal streams. *Fundamental and Applied Limnology*, *175*(4), 281–294. https://doi.org/10.1127/1863-9135/2009/0175-0281

Grace, J. R. (1986). The influence of gypsy moth on the composition and nutrient content of litter fall in a Pennsylvania oak forest. *Forest Science*, *32*(4), 855–870. https://doi.org/10.1093/forestscience/32.4.855

Grady, A. E., Scanlon, T. M., & Galloway, J. N. (2007). Declines in dissolved silica concentrations in western Virginia streams (1988–2003): Gypsy moth defoliation stimulates diatoms? *Journal of Geophysical Research: Biogeosciences*, *112*(G1), 1009. https://doi.org/10.1029/2006JG000251

Guo, F., Kainz, M. J., Sheldon, F., & Bunn, S. E. (2016). The importance of high-quality algal food sources in stream food webs - current status and future perspectives. *Freshwater Biology*, *61*(6), 815–831. https://doi.org/10.1111/fwb.12755

Hall, R. O., & Meyer, J. L. (1998). The trophic significance of bacteria in a detritus-based stream food web. *Ecology*, *79*(6), 1995–2012. https://doi.org/10.1890/0012-9658(1998)079[1995:TTSOBI]2.0.CO;2

Hart, D. D., & Finelli, C. M. (1999). Physical-biological coupling in streams: The pervasive effects of flow on benthic organisms. *Annual Review of Ecology and Systematics*, *30*, 363–395. https://doi.org/10.1146/annurev.ecolsys.30.1.363

Hart, S. A., & Chen, H. Y. H. (2006). Understory vegetation dynamics of North American boreal forests. In *Critical Reviews in Plant Sciences* (Vol. 25, Issue 4, pp. 381–397). Taylor & Francis Group. https://doi.org/10.1080/07352680600819286

Hayden, B., McWilliam-Hughes, S. M., & Cunjak, R. A. (2016). Evidence for limited trophic transfer of allochthonous energy in temperate river food webs. *Freshwater Science*, *35*(2), 544–558. https://doi.org/10.1086/686001

Heaston, E. D., Kaylor, M. J., & Warren, D. R. (2018). Aquatic food web response to patchy shading along forested headwater streams. *Canadian Journal of Fisheries and Aquatic Sciences*, *75*(12), 2211–2220. https://doi.org/10.1139/cjfas-2017-0464

Hunter, M. D. (2001). Insect population dynamics meets ecosystem ecology: Effects of herbivory on soil nutrient dynamics. *Agricultural and Forest Entomology*, *3*(2), 77–84. https://doi.org/10.1046/j.1461-9563.2001.00100.x

Jonsson, M., Polvi, L. E., Sponseller, R. A., & Stenroth, K. (2018). Catchment properties predict autochthony in stream filter feeders. *Hydrobiologia*, *815*(1), 83–95. https://doi.org/10.1007/s10750-018-3553-8

Kahlert, M., & McKie, B. G. (2014). Comparing new and conventional methods to estimate benthic algal biomass and composition in freshwaters. *Environmental Science: Processes and Impacts*, *16*(11), 2627–2634. https://doi.org/10.1039/c4em00326h

Kielstra, B. W., Mackereth, R. W., Melles, S. J., & Emilson, E. J. (2021). *hydroweight: Inverse distance-weighted rasters and landscape attributes*. https://doi.org/10.5281/zenodo.4728559

Kiffney, P. M., Richardson, J. S., & Bull, J. P. (2003). Responses of periphyton and insects to experimental manipulation of riparian buffer width along forest streams. *Journal of Applied Ecology*, *40*(6), 1060–1076. https://doi.org/10.1111/j.1365-2664.2003.00855.x

Kiljunen, M., Grey, J., Sinisalo, T., Harrod, C., Immonen, H., & Jones, R. I. (2006). A revised model for lipid-normalizing δ13C values from aquatic organisms, with implications for isotope mixing models. *Journal of Applied Ecology*, *43*(6), 1213–1222. https://doi.org/10.1111/j.1365-2664.2006.01224.x

Kneeshaw, D., Sturtevant, B. R., Cooke, B., Work, T., Pureswaran, D., DeGrandpre, L., & MacLean, D. A. (2015). Insect disturbances in forest ecosystems. In *Routledge Handbook of Forest Ecology* (pp. 93–113).

Lai, J., Zou, Y., Zhang, J., & Peres-Neto, P. R. (2022). Generalizing hierarchical and variation partitioning in multiple regression and canonical analyses using the rdacca.hp R package. *Methods in Ecology and Evolution*, *13*(4), 782–788. https://doi.org/10.1111/2041-210X.13800

Lesutiene, J., Gorokhova, E., Stankevičiene, D., Bergman, E., & Greenberg, L. (2014). Light increases energy transfer efficiency in a boreal stream. *PLoS ONE*, *9*(11), e113675. https://doi.org/10.1371/journal.pone.0113675

Lewis, G. P., & Likens, G. E. (2007). Changes in stream chemistry associated with insect defoliation in a Pennsylvania hemlock-hardwoods forest. *Forest Ecology and Management*, *238*(1–3), 199–211. https://doi.org/10.1016/j.foreco.2006.10.013

Mac Nally, R. (2000). Regression and model-building in conservation biology, biogeography and ecology: The distinction between - and reconciliation of - “predictive” and “explanatory” models. *Biodiversity and Conservation*, *9*(5), 655–671. https://doi.org/10.1023/A:1008985925162

Malo, M., & Bourque, P.-A. (1993). Timing of the deformation events from Late Ordovician to Mid-Devonian in the Gaspé Peninsula. *Geological Society of America*, *275*.

Martínez, A., Larrañaga, A., Pérez, J., Descals, E., & Pozo, J. (2014). Temperature affects leaf litter decomposition in low-order forest streams: Field and microcosm approaches. *FEMS Microbiology Ecology*, *87*(1), 257–267. https://doi.org/10.1111/1574-6941.12221

Mattson, W. J., & Addy, N. D. (1975). Phytophagous Insects as Regulators of Forest Primary Production. *Science*, *190*(4214), 515–522. http://www.jstor.org/stable/1740415

McCutchan, J. H., & Lewis, W. M. (2002). Relative importance of carbon sources for macroinvertebrates in a Rocky Mountain stream. *Limnology and Oceanography*, *47*(3), 742–752. https://doi.org/10.4319/lo.2002.47.3.0742

McCutchan, J. H., Lewis, W. M., Kendall, C., & McGrath, C. C. (2003). Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos*, *102*(2), 378–390. https://doi.org/10.1034/j.1600-0706.2003.12098.x

Merritt, R. W., & Cummins, K. W. (1996). *An introduction to the aquatic insects of North America*. Kendall/Hunt.

Mundahl, N. D., Mundahl, D. E., & Merten, E. C. (2012). Success of Slimy Sculpin Reintroductions in Minnesota Trout Streams: Influence of Feeding and Diets. *Source: The American Midland Naturalist*, *168*(1), 162–183.

Murphy, M. L., Hawkins, C. P., & Anderson, N. H. (1981). Effects of Canopy Modification and Accumulated Sediment on Stream Communities. *Transactions of the American Fisheries Society*, *110*(4), 469–478. https://doi.org/10.1577/1548-8659(1981)110<469:eocmaa>2.0.co;2

Nakano, S., & Murakami, M. (2001). Reciprocal subsidies: Dynamic interdependence between terrestrial and aquatic food webs. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(1), 166–170. https://doi.org/10.1073/pnas.98.1.166

Natural Resources Canada. (2012). *Research at the Laurentian Forestry Centre of Natural Resources Canada – Spruce budworm*. http://scf.rncan.gc.ca/publications.

Natural Resources Canada. (2018). *The State of Canada's Forests: Annual Report 2018*. http://cfs.nrcan.gc.ca/publications?id=39336

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., & Wagner, H. (2013). Vegan: community ecology package. R package version 2.4-0. http://cran.r–project.org/package=vegan

Peterson, E. E., Sheldon, F., Darnell, R., Bunn, S. E., & Harch, B. D. (2011). A comparison of spatially explicit landscape representation methods and their relationship to stream condition. *Freshwater Biology*, *56*(3), 590–610. https://doi.org/10.1111/j.1365-2427.2010.02507.x

Polis, G. A., Anderson, W. B., & Holt, R. D. (1997). Toward an integration of landscape and food web ecology: The dynamics of spatially subsidized food webs. In *Annual Review of Ecology and Systematics* (Vol. 28, pp. 289–316). https://doi.org/10.1146/annurev.ecolsys.28.1.289

Post, D. M. (2002). Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology*, *83*(3), 703–718. https://doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2

Post, D. M., Layman, C. A., Arrington, D. A., Takimoto, G., Quattrochi, J., & Montaña, C. G. (2007). Getting to the fat of the matter: Models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia, 152(1), 179–189. https://doi.org/10.1007/s00442-006-0630-x

Roon, D. A., Wipfli, M. S., & Kruse, J. J. (2018). Riparian defoliation by the invasive green alder sawfly influences terrestrial prey subsidies to salmon streams. *Ecology of Freshwater Fish*, *27*(4), 963–975. https://doi.org/10.1111/eff.12407

Rosemond, A. D., Mulholland, P. J., & Brawley, S. H. (2000). Seasonally shifting limitation of stream periphyton: Response of algal populations and assemblage biomass and productivity to variation in light, nutrients, and herbivores. *Canadian Journal of Fisheries and Aquatic Sciences*, *57*(1), 66–75. https://doi.org/10.1139/f99-181

Rosi-Marshall, E. J., Vallis, K. L., Baxter, C. v, & Davis, J. M. (2016). Retesting a prediction of the River Continuum Concept: Autochthonous versus allochthonous resources in the diets of invertebrates. *Freshwater Science*, *35*(2), 534–543. https://doi.org/10.1086/686302

Sampson, A., Ings, N., Shelley, F., Tuffin, S., Grey, J., Trimmer, M., Woodward, G., & Hildrew, A. G. (2019). Geographically widespread 13 C-depletion of grazing caddis larvae: A third way of fuelling stream food webs? *Freshwater Biology*, *64*(4), 787–798. https://doi.org/10.1111/fwb.13262

Sheath, R. G., Burkholder, J. A. M., Morison, M. O., Steinman, A. D., & VanAlstyne, K. L. (1986). Effect of Tree Canopy Removal by Gypsy Moth Larvae on the Macroalgae of a Rhode Island Headwater Stream. *Journal of Phycology*, *22*(4), 567–570. https://doi.org/10.1111/j.1529-8817.1986.tb02506.x

Stock, B. C., Jackson, A. L., Ward, E. J., Parnell, A. C., Phillips, D. L., & Semmens, B. X. (2018). Analyzing mixing systems using a new generation of Bayesian tracer mixing models. *PeerJ*, *2018*(6), e5096. https://doi.org/10.7717/peerj.5096

Swank, W. T., Waide, J. B., Crossley, D. A., & Todd, R. L. (1981). Insect defoliation enhances nitrate export from forest ecosystems. *Oecologia*, *51*(3), 297–299. https://doi.org/10.1007/BF00540897

Syrjänen, J., Korsu, K., Louhi, P., Paavola, R., & Muotka, T. (2011). Stream salmonids as opportunistic foragers: The importance of terrestrial invertebrates along a stream-size gradient. *Canadian Journal of Fisheries and Aquatic Sciences*, *68*(12), 2146–2156. https://doi.org/10.1139/F2011-118

Tamura, S., & Kagaya, T. (2019). Food habits of invertebrate grazers in a forested stream: variations according to taxonomic affiliation, flow habitat, and body size. *Hydrobiologia*, *841*(1), 109–120. https://doi.org/10.1007/s10750-019-04010-5

Utz, R. M., & Hartman, K. J. (2007). Identification of critical prey items to Appalachian brook trout (Salvelinus fontinalis) with emphasis on terrestrial organisms. *Hydrobiologia*, *575*(1), 259–270. https://doi.org/10.1007/S10750-006-0372-0/TABLES/6

van Nieuwenhuyse, E. E., & Jones, J. R. (1996). Phosphorus-chlorophyll relationship in temperate streams and its variation with stream catchment area. *Canadian Journal of Fisheries and Aquatic Sciences*, *53*(1), 99–105. https://doi.org/10.1139/f95-166 vander Zanden, M. J., & Rasmussen, J. B. (2001). Variation in δ15N and δ13C trophic fractionation: Implications for aquatic food web studies. *Limnology and Oceanography*, *46*(8), 2061–2066. https://doi.org/10.4319/lo.2001.46.8.2061

Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R., & Colbert, C. E. (1980). The River Continuum Concept. *Canadian Journal of Fisheries and Aquatic Sciences*, *37*(1), 130–137.

Warren, D. R., Keeton, W. S., Kiffney, P. M., Kaylor, M. J., Bechtold, H. A., & Magee, J. (2016). Changing forests-changing streams: Riparian forest stand development and ecosystem function in temperate headwaters. *Ecosphere*, *7*(8). https://doi.org/10.1002/ecs2.1435

Webster, J. J., & Hartman, K. J. (2005). The role of terrestrial invertebrates in allopatric brook trout headwater streams in the Central appalachian mountains. *Journal of Freshwater Ecology*, *20*(1), 101–107. https://doi.org/10.1080/02705060.2005.9664942

Wilkerson, E., Hagan, J. M., & Whitman, A. A. (2010). The effectiveness of different buffer widths for protecting water quality and macroinvertebrate and periphyton assemblages of headwater streams in Maine, USA. *Canadian Journal of Fisheries and Aquatic Sciences*, *67*(1), 177–190. https://doi.org/10.1139/F09-162

Willacker, J. J., Eagles-Smith, C. A., Kowalski, B. M., Danehy, R. J., Jackson, A. K., Adams, E. M., Evers, D. C., Eckley, C. S., Tate, M. T., & Krabbenhoft, D. P. (2019). Timber harvest alters mercury bioaccumulation and food web structure in headwater streams. *Environmental Pollution*, *253*, 636–645. https://doi.org/10.1016/j.envpol.2019.07.025

Wipfli, M. S. (1997). Terrestrial invertebrates as salmonid prey and nitrogen sources in streams: Contrasting old-growth and young-growth riparian forests in southeastern Alaska, U.S.A. *Canadian Journal of Fisheries and Aquatic Sciences*, *54*(6), 1259–1269. https://doi.org/10.1139/f97-034

Chapter 3: Drivers of methylmercury bioaccumulation and biomagnification in streams draining watersheds defoliated by spruce budworm

Abstract

Methylmercury (MeHg) is a potent contaminant that can be elevated to toxic levels in aquatic environments. Forests can efficiently capture atmospheric mercury (Hg) but terrestrial disturbances can increase MeHg bioaccumulation in aquatic consumers and its biomagnification through food webs. A recent spruce budworm outbreak is causing widespread terrestrial defoliation that can potentially alter stream environments and MeHg concentrations in stream biota.

Streams were sampled in watersheds experiencing a range of defoliation. Basal food sources (coarse and fine particulate organic matter, biofilm, seston), and several macroinvertebrate taxa were sampled in 2019 and analyzed for MeHg. Additionally, fish (brook trout, slimy sculpin) were sampled in 2019 and 2020 and analyzed for total mercury (THg). Trophic magnification slopes were measured by comparing $\log_{10}$Hg to $\delta^{15}N$ in each stream food web, and these slopes were compared to catchment defoliation and streamwater DOC levels. Hierarchical partitioning was used to model Hg levels in pooled carnivorous invertebrate taxa (Chloroperlidae, *Rhyacophila*, *Parapsyche*) and brook trout using various local and landscape parameters that are known to affect MeHg production and uptake.

Models explained 76 and 65% of variation in brook trout THg and carnivorous invertebrate MeHg levels, respectively. Both models included DOC as the main driver of increasing biotic mercury levels, and mercury levels were not affected by reliance on algal carbon sources or severity of catchment defoliation. Trophic biomagnification slopes ranged from 0.27-0.38 across all watersheds but were not related to defoliation or DOC concentrations.

These findings support previous studies that have found DOC as a driver of biotic mercury concentrations in aquatic environments and offers new insights into the impacts of catchment disturbance from a forest pest on MeHg bioaccumulation and biomagnification in stream food webs.

Chapter 3: Drivers of methylmercury bioaccumulation and biomagnification in streams draining watersheds defoliated by spruce budworm.

3.1. Introduction

Mercury is a ubiquitous metal that can reach toxic concentrations in aquatic environments through both natural and anthropogenic disturbances. Inorganic mercury (Hg) emitted by volcanic eruptions or coal-fired generating stations, e.g., is transported atmospherically, captured by forest canopies, and then deposited on land in litterfall where it can be carried to aquatic systems in runoff (Rea et al., 1996; St. Louis et al., 2001). In aquatic environments, this Hg is transformed by anaerobic microbes into methylmercury (MeHg), which is the neurotoxic and bioaccumulative form that biomagnifies up the food web. Consequently, predatory consumers in aquatic ecosystems draining forested watersheds are susceptible to elevated levels of MeHg (Eagles-Smith et al., 2016). Terrestrial disturbances such as fire and forest harvesting increase MeHg in aquatic biota due to increased transport, methylation, and trophic transfer of Hg (Kelly et al., 2006; Skyllberg et al., 2009). Streams may be especially sensitive to these landscape disturbances as they are closely linked to watershed conditions. However, the landscape drivers of MeHg accumulation in streams are not as well understood as in lakes, where catchment conditions play an important role in biotic MeHg uptake (Ward et al., 2010).

Landscape disturbances may affect stream water parameters, including those known to influence MeHg bioaccumulation in aquatic organisms. First, dissolved organic carbon (DOC) is the primary ligand that transports inorganic Hg from the terrestrial to the aquatic environment (Grigal, 2002). However, different studies have found both positive (Burns & Riva-Murray, 2018; Driscoll et al., 2007), or negative (Chaves-Ulloa et al., 2016; Luengen et al., 2012), relationships between DOC and MeHg levels in biota. Additionally, MeHg availability to biota depends on both the levels of DOC and measures of its quality, such as aromaticity, as both have a stronger relationship to tissue MeHg than quantity alone (Baken et al., 2011; Ravichandran, 2004). MeHg levels in biofilms and consumers can also be lower in streams with low phosphorus, as this nutrient can limit productivity and growth dilution of mercury, respectively (Pickhardt et al., 2002; Ward et al., 2010). Furthermore, MeHg becomes more bioavailable for uptake into autochthonous food sources in aquatic environments that have lower pH (Kelly et al., 2003) and conductivity levels (Daguené et al., 2012). Finally, increasing concentrations of sulfate (SO_4^{-2}) can stimulate MeHg production because sulfate is an energy source for anaerobic bacteria that methylate inorganic Hg (Marvin-Dipasquale et al., 2009). Forest disturbances have been observed to affect these water quality parameters, and indirectly alter mercury uptake in stream food webs (Eklöf et al., 2012; Shah et al., 2022).

The variation in MeHg among stream consumers can also be caused by dietary differences, such as trophic position and reliance on in-stream or terrestrially derived energy sources. Predatory species have elevated MeHg levels because this contaminant is primarily transferred through consumption (Hall et al., 1997) and it biomagnifies efficiently in the food web (Cabana & Rasmussen, 1994). Although autochthonous food sources like biofilms commonly support the diets of many aquatic consumers from various feeding groups (chapter 2), such energy sources bioconcentrate MeHg from water and act as a site of Hg methylation (Tsui et al., 2009) resulting in higher MeHg levels in biofilms than those in terrestrial food sources (Jardine et al., 2012). In streams, MeHg levels at the base of the food web can have a strong influence on MeHg levels at higher trophic levels (de Wit et al., 2014). As such, consumers with greater reliance on autochthonous energy often have elevated MeHg levels compared to consumers that rely on terrestrial energy (Riva-Murray et al., 2013).

The Gaspé Peninsula region of Québec is experiencing an outbreak of spruce budworm (SBW) that can potentially impact mercury cycling in stream food webs via changes in water quality and food web structure. SBW outbreaks cause widespread spruce and fir defoliation and increased terrestrial litterfall inputs to the forest floor (Grandpré et al., 2022). Litterfall is a dominant source of inorganic Hg to terrestrial soils (Grigal, 2002), and these inputs can alter organic carbon fluxes and increase DOC in the soil water (Michalzik & Stadler, 2005). Furthermore, different defoliators have been associated with increasing stream acidity, sulfate (Webb et al., 1995), DOC (Lewis & Likens, 2007), nutrients (Woodman et al., 2021), and benthic algal growth (Grady et al., 2007; Sheath et al., 1986; my chapter 2 results), which are factors known to affect mercury cycling in aquatic systems and food webs. However, stream responses may vary with the type of forest defoliator, and the impact of SBW defoliation on mercury bioaccumulation is unknown. My research will help bridge a knowledge gap on the effects of SBW defoliation on MeHg cycling in streams. I will investigate this by comparing the influence of different landscape and local factors on MeHg levels in macroinvertebrates and fish from catchments with a range in defoliation. This chapter includes stable isotope and autochthony modelling results that were presented in chapter 2. I predict that 1) defoliation and DOC will be positively related to biotic MeHg, and 2) MeHg will be greater in consumers that have greater autochthony among sites.

3.2. Methods

3.2.1. Field sampling

This study was completed in the Gaspé Peninsula of Québec, which experiences SBW outbreaks on a 30-40 year cycle. The terrain in this region is mountainous and hilly, and the northwestern region of the peninsula has a higher elevation and greater density of spruce and fir forest. Population density in this region is low, and the major land uses are forest harvesting, hunting, fishing, and nature tourism. In 2019 and 2020, twelve streams were sampled in watersheds experiencing a gradient of SBW defoliation. Stream sites were selected based on a minimum 90% forest cover (>65% as spruce and fir), no disturbance from beaver dams or road crossings, and minimal harvest in the past 15 years. Detailed descriptions of the study region, rationale for site selection, and defoliation calculations are included in chapter 2, section 2.2.1.

3.2.2. Water and seston

Water (125 mL) and seston samples for MeHg analyses were collected concurrently at the upstream location of each stream reach. Samples were collected in duplicate in August and in triplicate in September of 2020. However, it was not possible to process these samples in a timely manner because of the pandemic. As such, additional water samples were collected in September 2021 for a general assessment of aqueous MeHg concentrations. To remove contamination, 1.2 um VWR glass-fibre filters were ashed in a muffle furnace, and polytetrafluoroethylene (PTFE) tubing and 47 mm single-stage Savillex filter units were acid washed overnight before their use. Before sampling, acid washed-equipment were rinsed with distilled water and flushed with stream water at each site. Next, a 125 mL filtered stream water sample was collected into a polycarbonate sample bottle using the “clean hands, dirty hands” method as described in Negrazis et al., (2022). After collecting water, 15 L of streamwater was pumped through the pre-weighed filter to collect seston. Filters were folded into a clean plastic petri dish, transported in a cooler, then frozen at the end of the day. A field blank of distilled water was brought each day and opened briefly at the site. Water samples were transported in a cooler, preserved with 0.5% volume of OmniTrace HCl at the end of the day, then kept in the dark and refrigerated until analysis.

3.2.3. Food web samples

Food sources and invertebrates were collected for both stable isotope (chapter 2) and MeHg analyses in 2020, and fish were sampled for stable isotope and Hg analyses in both 2019 and 2020. Food sources (biofilm, CPOM, FPOM) were collected in triplicate in both August and September. Each biofilm sample was a composite of 10-20 rocks which were scraped with a toothbrush and rinsed into a Whirlpak bag. Approximately 10 conditioned leaves (unidentified, but usually poplar, birch, alder, mountain maple) were collected in stream for CPOM samples. FPOM was collected using a turkey baster by suctioning the top layer of organic matter in depositional areas. Invertebrates were collected by overturning rocks or kick-netting riffle sections and coarsely sorted live on site and then confirmed to family- or genus-level in the lab. Invertebrate taxa were targeted to represent different functional feeding groups: scrapers (*Epeorus* and *Glossosoma*), a collector-filterer (*Parapsyche*), and predators (*Rhyacophila* and Chloroperlidae*)*. Fish were collected using backpack electrofishing and were handled and processed in accordance with protocols approved by McMaster’s Animal Research Ethics Board (Animal Use Protocols #19-09-21 and #19-11-28) and under Québec Ministry SEG permits (#2019-05-28-009-11-SP and #2020-09-03-027-11-SP). Brook trout (*Salvelinus fontinalis*) were present in sites U01, U02, U03, C04, L08, L09, L10, L11, and L12. Slimy sculpin (*Cottus cognatus*) were present in sites U01, U02, and L10. All fish were measured for lengths and weights, then a maximum of nine samples of each species were lethally collected to represent a range in length distributions at each site, and the remaining fish were released. In 2019, only fish smaller than 10 cm were lethally sampled for mercury measurements. All samples were transported on ice in coolers, then frozen the same day.

3.2.4. Lab analysis

All samples were processed in the lab using acid-washed glassware. CPOM samples were rinsed with distilled water to remove debris. FPOM samples were sieved with a 1 mm mesh to remove large particles. Both FPOM and biofilm samples were examined under a dissection microscope to discard any invertebrates or macrophytes. Invertebrates were identified and pooled into a maximum of three replicates to obtain a minimum of 15 mg dry mass each (2-142 individuals per sample). Samples were freeze-dried at -80°C for 12 (seston filters), 48 (food sources, invertebrates, fish tissue), or 96 hours (whole body fish). After freeze-drying, seston filters and fish were re-weighed to determine dry sample mass and % moisture, and invertebrates and fish were homogenized to a powder using glass rods. Biofilm, CPOM, FPOM, invertebrates, and fish were measured for stable isotopes of carbon and nitrogen as described in chapter 2, section 2.3.3. Average (SD) moisture content was 81 ± 2.3% in brook trout tissue and 80 ± 2.5% in slimy sculpin tissue.

MeHg analysis

MeHg analyses were completed at the University of Toronto Scarborough using the isotope-dilution method (Hintelmann & Evans, 1997). MeHg in water samples from 2020 could not be reliably quantified due to laboratory access restrictions during the COVID-19 pandemic. Water samples collected in 2021 were analyzed in lieu. Additionally, a subset of fish fillet samples from 2020 (23 brook trout, 2 slimy sculpin) were analyzed to compare the proportion of THg as MeHg.

To prepare samples for analysis, water, food source and scraper invertebrates were distilled, and predatory invertebrates and fish were extracted. For biofilm, FPOM, CPOM, and seston, I analyzed all samples (n=2-3/site) from August, but only one sample per site from September was analyzed due to time constraints. For distillations, sample masses were 40 mL for water, 50 mg for biofilm, 100 mg for FPOM, 200mg for CPOM, 0.127-7.7 mg on seston filters, and 30 mg for scrapers and some collector-filterer invertebrate taxa. Extractions were run with samples masses of 30 mg for predatory invertebrates and 50 mg of fish. Scrapers were analyzed by distillation due to low sample mass and MeHg content below detection limit using extractions. A subset of brook trout and slimy sculpin (n=1-2/site) were analyzed to compare the ratio of MeHg:THg in fish muscle samples.

For distillations, I weighed samples into Teflon vessels, added 40 mL of distilled water (except for water samples), added $CuSO_4$, KCl, and H_2SO_4 to improve recovery, and steam distilled samples. Biotic samples were extracted using KOH in methanol and gently heated in Erlenmeyer flasks for 4 hours. All samples were spiked with a known amount of enriched $MeHg^{199}$ for the isotope dilution method. Next, distilled or extracted samples were buffered using sodium tetraethylborate and amalgamated with nitrogen gas onto Tenax traps. Samples were analyzed using gas chromatography-inductively coupled plasma mass spectrometry (GC-ICPMS) and isotope ratios were calculated to measure $MeHg^{202}$.

Quality assurance measures include regular intervals (5-10% of samples) of blanks and replicate samples. Standard reference materials (SRMs) included fish protein (DORM3; NRC-CNRC, 2007) for fish and invertebrates, and marine sediment (IAEA158; IAEA, 2008) for food sources. MeHg recovery from SRMs were 87.4% (n=7) for fish and invertebrate extractions, 87.4% (n=2) for invertebrate distillations, and 116% (n=14) for food source samples. The relative percent difference between duplicate samples (mean ± standard deviation) were 10.7 ± 7.3% (n=9 pairs) for CPOM and FPOM, 9.8 ± 7.3% (n=4 pairs) for biofilm, and 10.7 ± 12.5% (n=14 pairs) for invertebrates and fish. Method detection limits were calculated as 3 times the standard deviation of blanks, and detection limits were 0.01 ng/L for water and seston, 0.016 ng/g for CPOM and FPOM, 0.019 ng/g for biofilm, 0.32 ng/g for distilled invertebrates, and 4.9 ng/g for extracted invertebrates and fish.

Fish THg analysis

A skinless dorsal muscle fillet was dissected from all fish greater than 4.5 cm in length for isotope (chapter 2) and total mercury analyses. In 2020, four sculpin were collected across three sites were too small to fillet (size range 3.8-4.5 cm) and were processed as whole-body samples. To facilitate comparisons between tissue types, I measured THg in muscle and whole-body samples from 16 other sculpin representing a range of sizes > 4.5 cm.

Fish were measured for total mercury (THg) at McMaster University using a Milestone Tri-Cell Direct Mercury Analyzer-80 (DMA) using the United States Environmental Protection Agency method 7473 (US EPA, 1998). Quality assurance measures include blanks, replicate samples, and SRMs to compare recovery rates. THg recovery from the SRM (fish muscle tissue, DORM4; NRC-CNRC, 2012) was 94%, and the relative percent difference (mean ± standard deviation) was 1.45 ± 1.7% (n=19 pairs) for fish tissue and 4.3 ± 3.4% (n=6, in duplicate or triplicate) for whole-body samples.

3.2.5. Statistical analysis

One- or two- way analyses of variance (ANOVAs) were run to compare differences in MeHg between groups (site and sample type or year). Type III sum of squares were used due to unbalanced data and Tukey post-hoc contrasts were compared at $\alpha=0.05$. Analysis was completed in R version 4.1.3 using the packages *tidyverse* for data manipulation, *car* for ANOVA with type III sum of squares, and *emmeans* for Tukey pairwise comparisons and for size-adjusting fish THg data.

Food source samples

Water samples from 2021 were not statistically compared because 22 of 54 total samples were below detection limits. Food source samples from August and September were pooled for analyses because MeHg concentrations from the September sample typically fell within the range of August samples for most sites (Figure B1). A two-way ANOVA was used to compare differences in MeHg among the sample types (biofilm, seston, CPOM, FPOM) and twelve stream sites.

Invertebrates

Invertebrate MeHg concentrations were compared based on dietary habits, as they affect MeHg uptake (Riva-Murray et al., 2013). First, a two-sample t-test was used to compare MeHg between the two scraper taxa, using data pooled within taxa across sites, to assess whether scraper data could be pooled for subsequent analyses. As MeHg concentrations were significantly different between these taxa, correlations were run for each of MeHg in *Epeorus* and *Glossosoma* and average stream DOC or watershed defoliation. I used DOC because it has a positive relationship with MeHg bioaccumulation in some systems (Burns & Riva-Murray, 2018; Driscoll et al., 2007). Additionally, sites were sampled from watersheds experiencing a range of defoliation, and this disturbance can potentially alter biotic MeHg. However, I did not compare MeHg to autochthony for *Epeorus* or *Glossosoma,* as these taxa were used as endmembers in the mixing models (chapter 2). Next, the predatory and collector-filterer taxa were pooled (n=3-7 across sites) as "carnivorous invertebrates" because the three invertebrates had similar trophic niches as determined by stable isotopes (chapter 2, Figures 2.6 and 2.7). A one-way ANOVA was run for pooled carnivorous invertebrates to assess variation in MeHg among sites, and this was followed by hierarchical partitioning modelling to compare the factors that contributed to that variation.

Hierarchical partitioning was used to generate explanatory models for MeHg in carnivorous invertebrates only, as scrapers had low sample sizes across sites. Both landscape and local variables can affect biotic mercury concentrations in aquatic food webs (Kozak et al., 2021; Ward et al., 2010). I included latitude, watershed area, and defoliation as landscape variables because these parameters can indirectly affect mercury transfer to aquatic food webs. Local variables included annual averages of DOC, pH, SO_4^{-2}, total phosphorous (TP), and conductivity because they can affect MeHg biomagnification or bioaccumulation (Clayden et al., 2013; Ullrich et al., 2001). Autochthony (chapter 2) was included to represent diet as a source of MeHg, and invertebrate taxa was an additional variable in the hierarchical partitioning model to account for the pooled data. Finally, all variables were z-score standardized to remove scale differences. Hierarchical partitioning was used due to multicollinearity among the parameters. In the hierarchical partitioning framework, the variance explained by the individual parameters are calculated from unique and shared contributions to the model. Unique contributions represent relationships after accounting for multicollinearity whereas shared contributions represent joint correlation between variables. Finally, Pearson correlations were calculated to determine the direction of effects. Hierarchical partitioning models were completed using the *rdacca.hp* package (Lai et al., 2022).

Fish

I first verified the use of THg as a proxy of MeHg by comparing results from the two different analyses. MeHg was an average of 100% (range 72-130%) of THg in brook trout (n=23) and 94 and 121% for two slimy sculpin (Figure B3). Since THg concentrations were representative of MeHg, the fish THg values were used in all subsequent analyses. Next, I created a regression using comparisons of muscle and whole body sculpin THg values (n=21). I adjusted the whole body sculpin THg concentrations (n=4) to muscle equivalents using the equation $[\text{THg}]_{\text{muscle}} = [\text{THg}]_{\text{WholeBody}} \times 0.723 - 0.115$ ($p<0.001$, $R^2 = 0.96$; Figure B4).

To account for potential covariates in the data, I checked for relationships between 1) fish lengths and sites, and 2) fish THg and lengths across sites. Analyses were run separately for each species (brook trout and slimy sculpin) and year (2019 and 2020), and I $\log_{10}$-transformed THg concentrations and lengths to meet assumptions of heteroscedasticity and normality among residuals for the models described in this section. First, I ran Welch's t-tests to compare fish lengths between years and used one-way ANOVAs to check the size distributions of fish across sites within each year. Brook trout lengths differed between years (p=0.015) and between sites in 2019 (p<0.0001) but not in 2020 (p=0.99), and slimy sculpin lengths did not differ between years (p=0.103) or among sites in either 2019 or 2020 (p=0.35 and 0.34, respectively). Next, I compared THg as a function of length and site using ANCOVAs and found that THg concentrations were significantly different for fish at different lengths and sites in all cases except site-wise for sculpin in 2020 (Table B1). As I found some differences between fish lengths and THg at different sites, I least-square means (LSM) adjusted all fish THg data to facilitate comparisons across sites and years. I adjusted THg data to LSM values at median lengths that were common across all sites and years (8.35 cm for brook trout, 5.8 cm for slimy sculpin). The size-adjusted THg data (herein THg_{size}) were used for statistical analyses for brook trout but only for visual comparisons for slimy sculpin, as sculpin had low representation across sites.

First, a two-way ANOVA was run to test whether brook trout THg_{size} differed between years and sites. Next, brook trout THg_{size} was modelled using hierarchical partitioning to identify the factors that contributed to differences between sites. A full model was generated using the same local and landscape variables as described for carnivorous invertebrates. Year was also included in the model as trout were collected in 2019 and 2020. Parameters were removed if they had negative unique and individual contributions and positive shared contributions, as these variables are unimportant factors in the model (Lai et al., 2022).

Food web

Trophic magnification slopes (TMS) were calculated by comparing $\log_{10}$ Hg (in ng/g dw; MeHg for food sources and invertebrates, THg for fish) to $\delta^{15}N$ of each sample across all 12 streams sampled in 2020. Slopes were compared between the watersheds using an ANCOVA with site as a covariate. Next, linear models were run to compare whether TMS varied as a function of watershed defoliation or aqueous DOC. Comparisons were first completed using all stream sites, and then using only the nine streams with fish present, because TMSs were higher in the three sites without fish (C05, C06, C07).

3.3. Results

3.3.1. MeHg in water and food sources

The MeHg concentrations in 2021 water samples ranged from 0.012-0.088 ng/L in samples that could be reliably quantified, although 22 out of 54 samples were below the detection limit of 0.01 ng/L (Figure B2). Among food source samples (Figure 3.1A), the range in MeHg levels was lowest in CPOM (range 0.04-0.4 ng/g dw), higher in biofilm (range 0.12-1.4 ng/g dw) and FPOM (range 0.19-4.3 ng/g dw), and highest in seston (range 0.56-23.7 ng/g dw). The two-way ANOVA indicated that MeHg concentrations differed among food sources ($p<0.0001$; Table B2), but not between sites ($p=0.13$). Post-hoc comparisons demonstrated that MeHg was significantly higher in seston than biofilm, CPOM, or FPOM ($p<0.0001$ for all; Table B3) but there were no differences between biofilm and CPOM ($p=0.79$), biofilm and FPOM ($p=0.38$) or CPOM and FPOM ($p=0.06$).

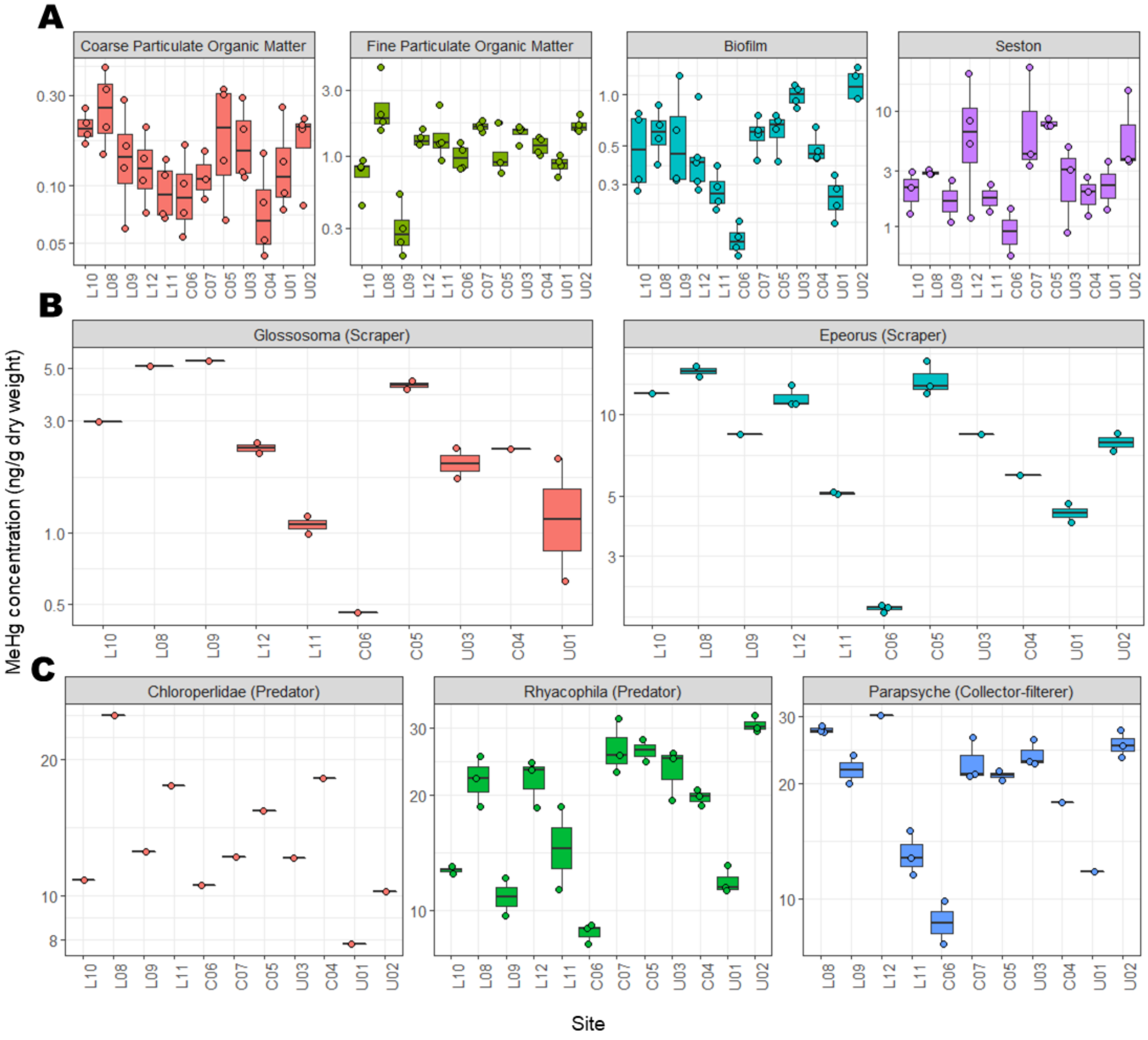

Figure 3.13. Methylmercury concentrations (ng/g dry weight) of (A) food sources (B,C) and invertebrates collected in 2020 from twelve streams draining watersheds experiencing a range of defoliation. Points represent individual composite samples. Food sources were collected in August (n=2-3/site) and September (n=1/site), and invertebrates were collected from September 2020 (n=1-3/site/taxa).

3.3.2. MeHg in macroinvertebrates

For the scraper taxa, the range in MeHg concentrations was lower in *Glossosoma* (0.46-5.4 ng/g dw) than *Epeorus* (1.9-15.8 ng/g dw) (Figure 3.1B). There were significant differences in MeHg between scraper taxa ($p<0.0001$) when site data were pooled, which justified their subsequent analysis as separate taxa. *Epeorus* MeHg levels were positively correlated to DOC but not defoliation, and *Glossosoma* MeHg was not correlated to either factor across all sites (Table 3.6). No among-site comparisons were made within each scraper taxa because of low sample sizes within sites.

Table 3.6. Pearson's correlations between MeHg in *Epeorus* (n=21) or *Glossosoma* (n=16) and either defoliation or average stream DOC in 2020.

	Epeorus		*Glossosoma*	
	Correlation coefficient	**p-value**	**Correlation coefficient**	**p-value**
Defoliation	-0.32	0.16	-0.38	0.15
DOC	0.53	0.015	0.21	0.43

The range in MeHg was similar among the three carnivorous invertebrates (Figure 3.1C). MeHg concentrations differed significantly across all sites when these three taxa were pooled ($p<0.0001$; Figure B6). The hierarchical partitioning model explained 65% of the variation in MeHg among the pooled carnivorous invertebrates (Table 3.7). Overall, DOC, taxa, and SUVA had high individual contributions to the model, and increasing DOC and SUVA were positively related to MeHg. The different taxa had high unique contributions to the model (0.069) as THg concentrations were higher in *Parapsyche* and *Rhyacophila* than Chloroperlidae. Both autochthony and SO_{4-}^{2} were negatively related to MeHg in carnivorous invertebrates. The landscape variables (watershed area, latitude, and defoliation) also had lower individual contributions to the model, and these parameters all had a positive effect on MeHg. However, the negative unique contributions and positive shared contributions signify high collinearity among the landscape variables. The remaining local parameters (conductivity, pH, and TP) did not have high individual contributions to the model, although the biplot scores indicate that these parameters all had a negative relationship with MeHg in carnivorous invertebrates.

Table 3.7. Hierarchical partitioning model for MeHg in carnivorous invertebrates (*Parapsyche*, Chloroperlidae, *Rhyacophila*) collected in 2020.

	Hierarchical partitioning contributions				
Variable	**Unique**	**Average shared**	**Individual**	**Individual %**	**Correlation coefficient**
Carnivorous Invertebrate MeHg					
DOC	0.022	0.212	0.234	36.1	0.619

Taxa	0.069	0.050	0.119	18.4	NA
SUVA	-0.005	0.110	0.105	16.3	0.474
Autochthony	0.002	0.045	0.047	7.2	-0.177
SO4	0.002	0.037	0.039	6.0	-0.319
Watershed area	-0.006	0.034	0.028	4.3	0.010
Latitude	-0.006	0.026	0.019	3.0	0.174
Conductivity	0.011	0.007	0.019	2.9	-0.169
pH	-0.001	0.016	0.015	2.3	-0.153
Defoliation	-0.005	0.018	0.013	2.1	0.110
TP	-0.006	0.016	0.010	1.5	-0.150
Total explained variation (model R^2)			**0.65**		

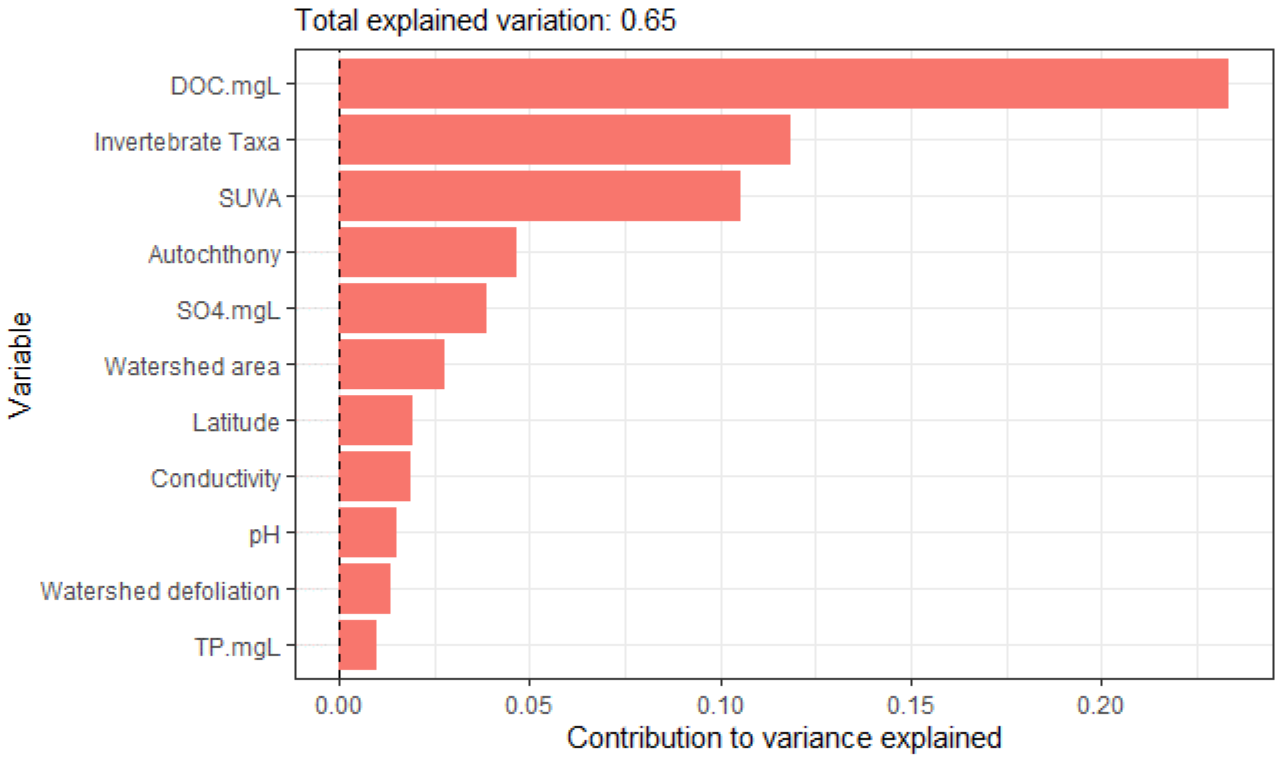

Figure 3.14. Individual contributions (proportions) of different landscape and local variables to MeHg in pooled carnivorous invertebrates (Chloroperlidae, *Rhyacophila*, *Parapsyche*) collected from twelve streams draining watersheds experiencing a range of catchment defoliation in the Gaspé Peninsula, Québec.

3.3.3. THg in fish

The average brook trout THg (raw) was 69.8 (range 19.8-260 ng/g dw; 8 sites) and 68.1 (range 21.8-195 ng/g dw; 9 sites) in 2019 and 2020, respectively (Figure 3.3). Total Hg in slimy sculpin was also similar among years, with averages of 27.9 (range 16.6-70 ng/g dw; 2 sites) and 30.9 (range 12.1-76.7 ng/g dw; 3 sites) in 2019 and 2020, respectively.

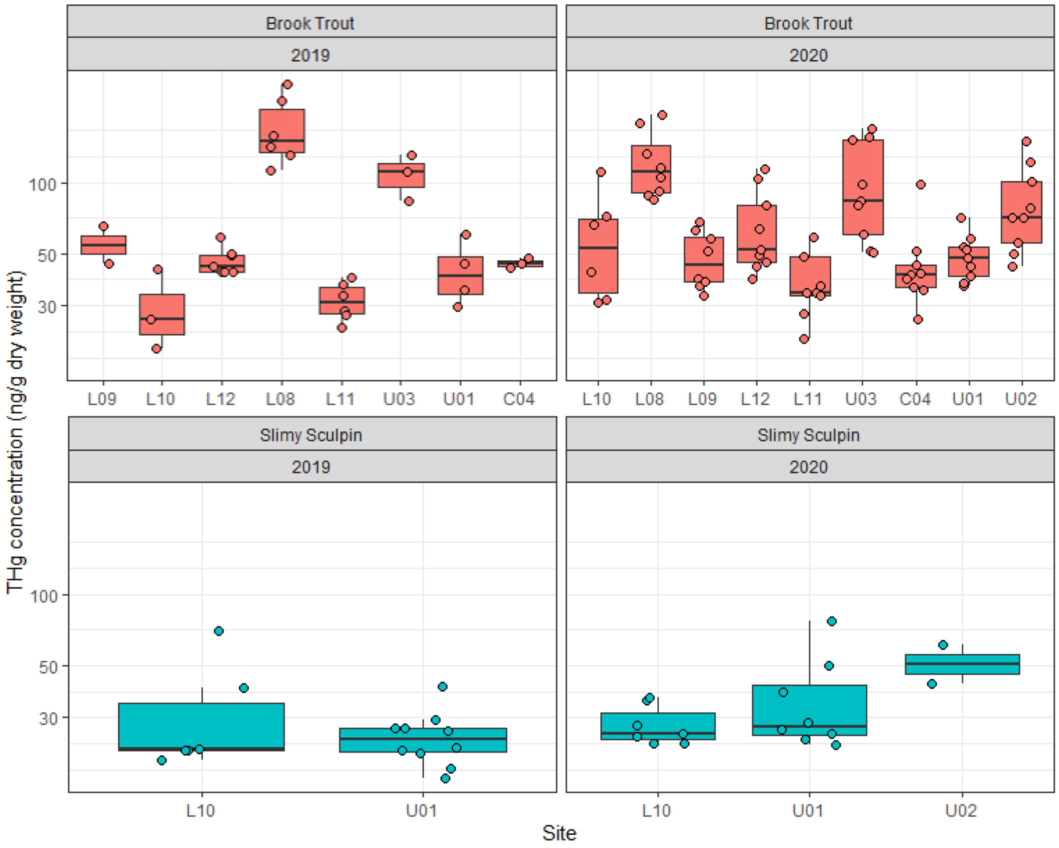

Figure 3.15. Total mercury concentrations (ng/g dry weight, no size adjustments) of brook trout and slimy sculpin collected in 2019 and 2020 from twelve streams draining watersheds experiencing a range of catchment defoliation in the Gaspé Peninsula, Québec.

The average THg_{size} for brook trout was similar between 2019 (range 27.8-155.3 ng/g dw) and 2020 (range 39.7-124.5 ng/g dw; Figure 3.16; p=0.61), but there were significant differences between sites ($p<0.0001$). In both years, the Tukey post-hoc tests indicated that THg_{size} in brook trout was significantly higher in L08 than every site except U03 and U02 (collected at this site in 2020 only). The variability in average sculpin THg_{size} was low and ranged from 25.1-31.2 ng/g dw across sites when data from both years were combined.

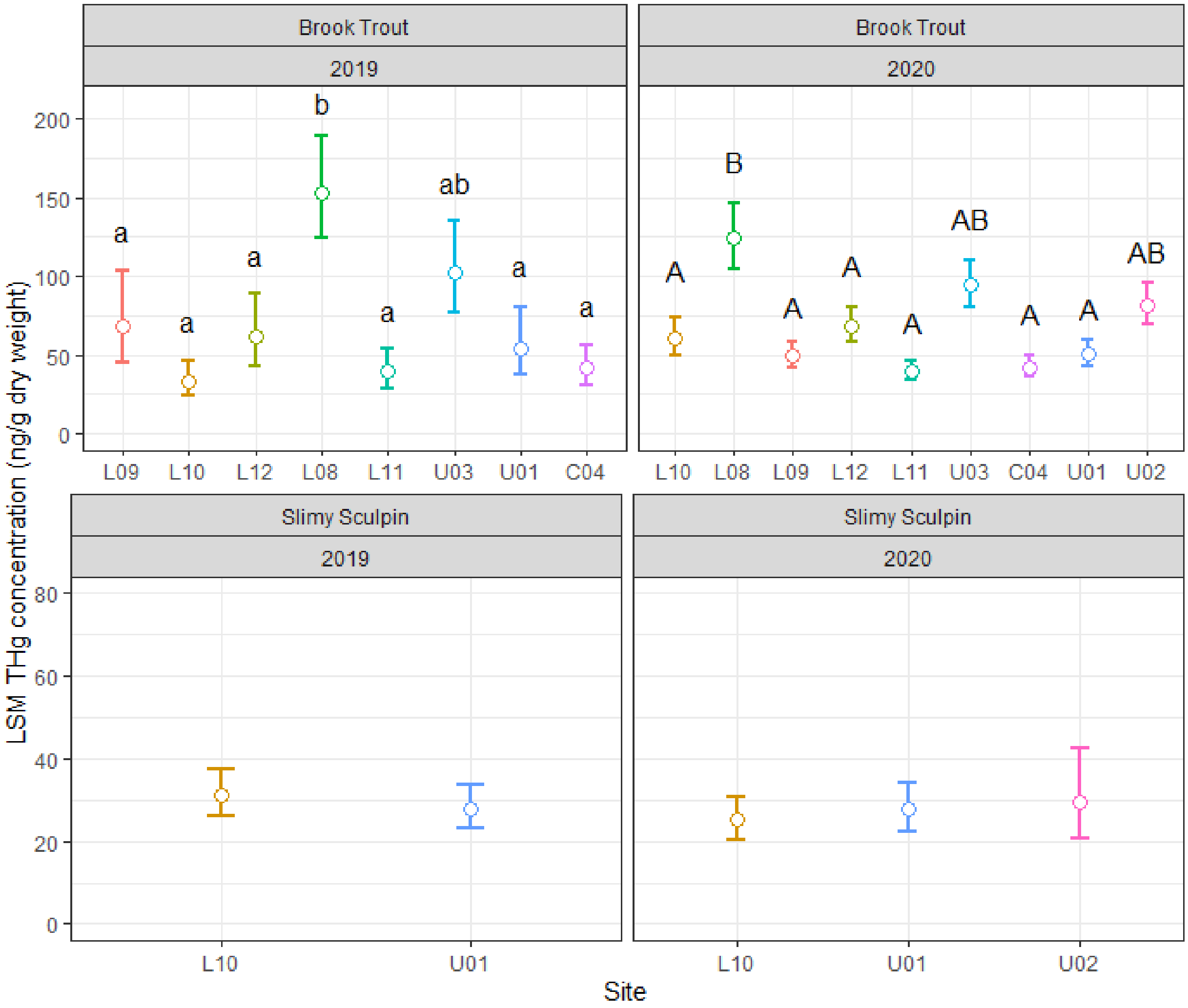

Figure 3.16. Size-adjusted total mercury concentrations (ng/g dry weight) of brook trout and slimy sculpin collected in 2019 and 2020 from twelve streams draining watersheds experiencing a range of catchment defoliation in the Gaspé Peninsula, Québec. Letters denote significant differences between sites based on pairwise Tukey comparisons; lowercase letters were used for 2019 and capital letters were used for 2020.

The landscape and local parameters explained 76% of the variation in brook trout THg_{size} (Table 3.3). DOC had the highest individual contribution to the model (0.402) and was positively correlated to brook trout THg_{size}. This parameter also had the highest unique (0.295) and shared (0.108) contribution among all variables. The landscape variables also had high individual contributions to the model and brook trout THg_{size} was positively correlated to sites with higher latitudes, smaller watershed areas, and lower defoliation. Several local variables (SUVA, TP, and pH) and autochthony did not contribute to the variation in trout THg_{size} and were excluded from the final model. Between the remaining the local variables, SO_4^{-2} had a higher individual contribution (0.081) and it was negatively related to trout THg_{size}, whereas conductivity had a small individual contribution (0.04) and a positive relationship with THg_{size}.

Table 3.8. Hierarchical partitioning model for brook trout THg_{size} concentrations from 2019 and 2020.

	Hierarchical partitioning contributions				**Correlation coefficient**
Variable	**Unique**	**Average shared**	**Individual**	**Individual %**	
		Brook Trout THg			
DOC	0.295	0.108	0.402	53.02	0.790
Latitude	0.146	-0.050	0.096	12.64	0.301
SO4	0.008	0.074	0.081	10.72	-0.476
Watershed area	0.097	-0.019	0.078	10.3	-0.499
Defoliation	0.046	0.013	0.058	7.68	-0.103
Conductivity	0.101	-0.062	0.040	5.2	0.288
Year	0.114	-0.110	0.004	0.46	0.041
Total explained variation (model R^2)			**0.76**		

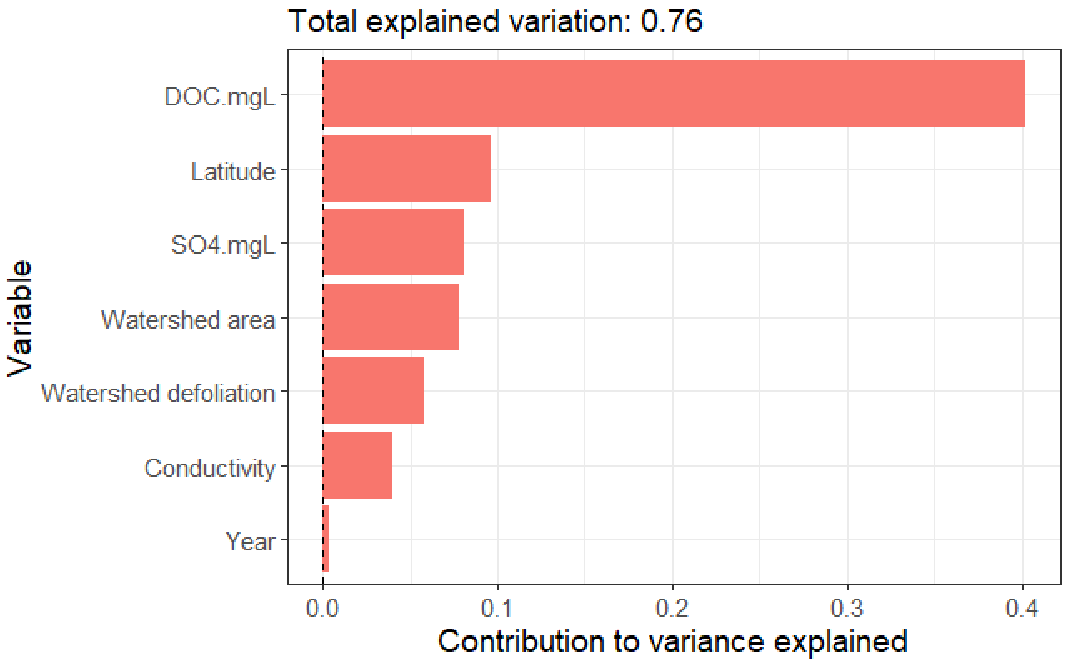

Figure 3.17. Individual contributions (proportions) of different landscape and local variables to brook trout size-adjusted THg concentrations. In 2019 and 2020, Brook trout were collected from twelve streams draining watersheds experiencing a range of catchment defoliation in the Gaspé Peninsula, Québec.

3.3.4. Trophic magnification among watersheds

The trophic magnification slopes (TMS) of $\log_{10}$ Hg vs δ^{15}N ranged from 0.27-0.38 and all regressions were significant ($p<0.001$) across all 12 watersheds (Figure 3.18). The TMS were higher among the three streams without fish (C05=0.30, C06=0.33, C07=0.38) although these differences were not significant (independent Welch's t-test, p=0.23). Furthermore, the TMS did not differ among all twelve watersheds (p=0.61), nor in the nine sites with fish present (p=0.83). Finally, correlations between TMS and defoliation or DOC were not significant, even when comparisons were limited to sites with fish present (Table 3.5).

Table 3.4. ANCOVA p-values: $\log_{10}$Hg as a function of δ^{15}N, site as a covariate

	All twelve sites	**Nine sites with fish present**
δ^{15}N	< 2.2e-16	< 2.2e-16
Site	8.5E-06	9.5e-07
δ^{15}N:Site	0.61	0.83

Table 3.5. Pearson's correlations of TMS (from all twelve sites or fish-present sites only) to average watershed defoliation and DOC in 2020.

	All Sites		***Fish-present sites only***	
	Correlation coefficient	**p-value**	**Correlation coefficient**	**p-value**
Defoliation	-0.04	0.90	-0.27	0.48
DOC	0.34	0.28	0.54	0.13

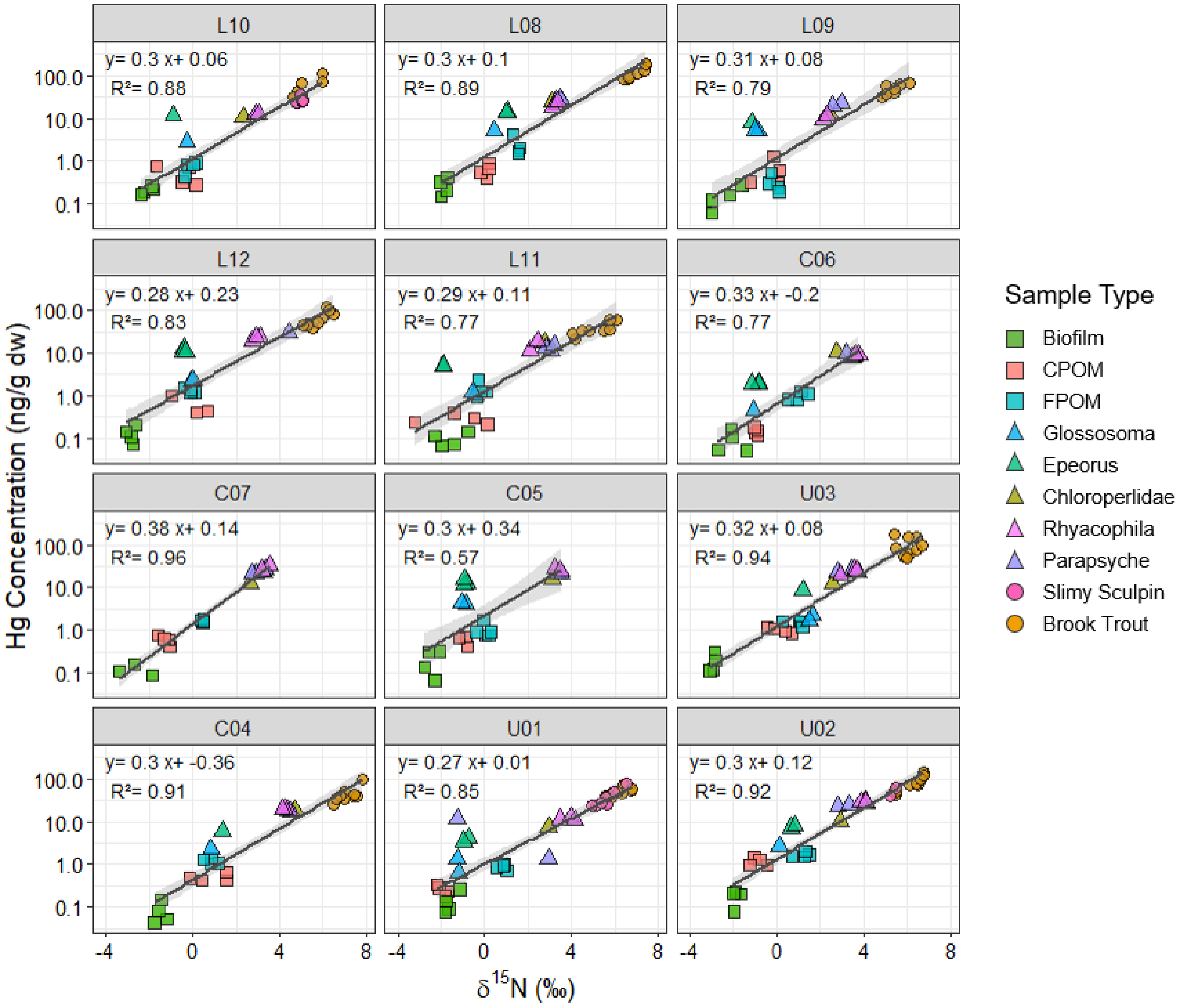

Figure 3.18. Regressions between $\log_{10}$Hg (MeHg for food sources and invertebrates, THg for fish) and δ^{15}N (‰) in food webs of twelve streams experiencing a range in catchment defoliation severity in the Gaspé Peninsula, Québec. Sites are arranged from in order of increasing defoliation from the top-left to the bottom-right.

3.4. Discussion

3.4.1. Mercury in basal food sources

In this study, MeHg concentrations in water and most food sources were similar to or lower than findings from forested stream systems elsewhere. Aqueous MeHg levels in the Gaspé streams were all below 0.1 ng/L in 2021, and these concentrations were consistent with those of streams in New Brunswick (Negrazis et al. 2022), although lower than levels from 337 streams across the United States (Scudder et al., 2009).

Biofilm MeHg levels from forested streams in New Brunswick ranged from 1-100 ng/g dw (Jardine et al., 2013; Negrazis et al., 2022), which is nearly two orders of magnitude higher than samples from the Gaspé Peninsula. Seston MeHg levels in my study were in the range of published values in forested streams (Negrazis et al., 2022; Tsui & Finlay, 2011). Finally, MeHg in both CPOM and FPOM in my study were below the range of values in low-order, reference streams in New Brunswick (Negrazis et al. 2022).

Basal food sources did not follow expected patterns in MeHg based on their origin in their environment, as seston was the only food source that differed from the rest. Autochthonous sources often have higher levels of MeHg than allochthonous sources because mercury methylation occurs in aquatic environments and in-stream primary producers can bioconcentrate MeHg from the water (Chasar et al., 2009; Jardine et al., 2012). Although seston isotopes were not measured in this project, previous studies have found it to be a mixture of algae, plankton, and bacteria (Atkinson et al., 2009; Kondratieff & Simmons, 1985) which can bioaccumulate MeHg. Seston had the highest MeHg levels among food sources in the Gaspé Peninsula, which is consistent with other studies that have found elevated MeHg in this food source (le Faucheur et al., 2014; Watras et al., 1998). Furthermore, I expected biofilms to have higher levels of MeHg than allochthonous sources because biofilms can act a site of mercury methylation and absorption (Mauro et al., 2002; Painter et al., 2016). Furthermore, biofilms can have elevated MeHg in in cold, unproductive systems that prevent growth dilution (Hill & Larsen, 2005; Pickhardt et al., 2002), and these conditions were found in the Gaspé streams. Although the difference in MeHg between FPOM and CPOM was not significant, MeHg was almost always higher in FPOM than CPOM within the same site (Figure B5). This is consistent with previous findings (Negrazis et al., 2022) and the higher δ^{15}N in FPOM (chapter 2) and may be caused by heterotrophic fractions in FPOM (Cummins, 1974; Richardson et al., 2005) that can accumulate MeHg.

3.4.2. MeHg in macroinvertebrates

MeHg was significantly higher in the *Epeorus* than *Glossosoma* despite having similar diets (chapter 2, Figures 2.6 and 2.7). Tsui et al. (2014) also observed higher MeHg in Heptageniid mayflies than in *Glossosoma*, despite overlapping δ^{13}C ranges between these two scrapers. Other food web studies have found relatively high MeHg levels in Heptageniid mayflies, despite their low trophic position (Riva-Murray et al., 2013; Willacker et al., 2019). The elevated MeHg in *Epeorus* from the Gaspé streams may be caused by its larger body size or lower lipid content (C:N of 5.2 in *Epeorus*, 6.3 in *Glossosoma*) than *Glossosoma*, as MeHg binds more effectively to proteins than lipids (Amlund et al., 2007).

There were some unexpected trends in scraper stable isotope values and MeHg in this study that suggest a distinct exposure pathway for these taxa. I assumed that scrapers were ingesting MeHg from biofilm based on studies of gut contents and feeding groups (Minshall, 1978). However, the isotope data suggest that scrapers were not obtaining MeHg from bulk biofilm as their δ^{13}C was lower than biofilm (chapter 2, Figures 2.6 and 2.7), which may be due to scrapers' selective consumption of biofilm components (McNeely et al., 2006). Additionally, the two scrapers were often above the best-fit line formed by the plot of $\log_{10}$ Hg vs δ^{15}N (Figure 3.18), which indicates that their MeHg concentrations were greater than predicted by their trophic position. These patterns suggest the high MeHg in the scraper taxa was from a low-δ^{13}C and high-MeHg food source not sampled herein. Sampson et al. (2019) attributed low δ^{13}C in scraper caddisflies to their ingestion of methanotrophic bacteria in streams. Furthermore, methanotrophs include anaerobic bacteria which are associated with elevated MeHg in some systems (Lu et al., 2017; Regnell & Watras, 2019). Although scrapers' consumption of methanogenic carbon was not specifically examined in the current study, methanotrophic bacteria may be an unexpected source of MeHg to scraper invertebrates in the Gaspé streams.

Among the carnivorous invertebrates, it was surprising that MeHg was lower in the predatory taxa (*Rhyacophila,* Chloroperlidae) than in the filter feeder (*Parapsyche*). I expected similar patterns in MeHg among taxa that had similar isotopic values (chapter 2) because the dietary exposure is the main source of MeHg to consumers (Mason et al., 2000; Tsui & Wang, 2004). The hydropsychid caddisfly *Parapsyche* was highest in MeHg despite its feeding habit as a collector-filterer, rather than a predator. This is consistent with food web studies that have observed elevated MeHg in filter feeders relative to other invertebrates (Walters et al, 2015; Riva-Murray et al., 2013), which may be explained by filter feeders' non-selective consumption of high-MeHg seston particles. The high MeHg in seston and *Parapsyche* relative to other food sources and invertebrates in my study were similar to findings of Tsui & Finlay (2009). Finally, MeHg was lower in the engulfing predators *Rhyacophila* and Chloroperlidae, which may be due to their consumption of invertebrate prey items with lower levels of MeHg.

The hierarchical partitioning model showed that several parameters affected MeHg bioaccumulation in carnivorous invertebrates. First, both increasing DOC and SUVA had high individual contributions in the model, and these parameters are commonly identified as drivers of biotic MeHg in stream invertebrates (Chaves-Ulloa et al., 2016; Jeremiason et al., 2016; Mason et al., 2000). DOC is a dominant source of Hg to aquatic environments and is associated with elevated MeHg concentrations in food sources and consumers (Grigal, 2002). Additionally, SUVA represents aromaticity of organic molecules, which can bind and deliver bioavailable MeHg to streams (Haitzer et al., 2002; Tsui & Finlay, 2011). Autochthony had a negative relationship to the MeHg of carnivorous invertebrates, which was unexpected, as consumption of autochthonous carbon is often associated with higher invertebrate MeHg (Jardine et al., 2012; Riva-Murray et al., 2013). However, this is dependent on autochthonous sources having higher MeHg concentrations. In contrast, I found that MeHg levels were similar between biofilms and allochthonous food sources, which explains the lack of relationship between invertebrate mercury levels and diet herbivory. Next, the negative effect of sulfate was unexpected, as sulfate is an energy source for microbes that methylate mercury (Benoit et al., 2002). Jeremiason et al. (2016) found a similar lack of relationship between MeHg in aquatic predators and aqueous sulfate concentrations and attributed this finding to the absence of reduced sulfate in lotic systems, as this form of sulfate is necessary for microbial methylation (Hsu-Kim et al., 2013). Furthermore, MeHg of carnivorous invertebrates increased in streams that were more acidic, and had lower conductivity and TP, which is consistent with other studies on similar invertebrate (Clayden et al., 2014; de Wit et al., 2014; Jardine et al., 2013). However, these parameters had low explanatory power in the model of invertebrates in the Gaspé Peninsula, whereas pH can be a strong driver of invertebrate MeHg levels in some systems (Malcata Martins et al., 2021; Rennie et al., 2005). Lower pH and conductivity can facilitate MeHg uptake into basal food sources by increasing membrane permeability, and low TP can limit growth dilution

(Daguené et al., 2012; Herendeen & Hill, 2004). In addition, MeHg in carnivorous invertebrates was associated with larger watershed area, higher latitudes, and higher defoliation, although their individual contributions to the model were low. Although I expected this terrestrial disturbance to increase MeHg among invertebrate consumers, de Wit et al. (2014) also observed no effect of forest harvesting on invertebrate MeHg levels.

3.4.3. THg in fish

Both fish species from the Gaspé Peninsula were low in THg relative to similar studies in unimpacted sites. Brook trout in Adirondack streams had mean size-adjusted THg concentrations of 52 and 96 ng/g ww at 65 and 100 mm, respectively (Burns & Riva-Murray, 2018), which are higher than the maximum THg of brook trout from in this study (54 ng/g ww at 97 mm). Similarly, THg_{size} in sculpin collected by Negrazis et al. (2022) ranged from 228-772 ng/g dw at 66 mm, which was an order of magnitude higher than the range in THg_{size} from sculpin in this study (20-42 ng/g dw at 58 mm). Low THg from fish in the Gaspé Peninsula may be due to low DOC (range 0.6-1.8 mg/L across both years and all sites), as DOC is commonly associated with transporting bioavailable Hg to fish (Driscoll et al., 1995; Ward et al., 2010).

Both local and landscape parameters contributed to the hierarchical partitioning model that explained 76% of the variance in brook trout THg_{size}. Brook trout THg_{size} was greater in streams with increasing DOC and conductivity and decreasing SO_4^{-2}. This positive relationship between stream DOC and fish THg has been commonly reported as higher DOC facilitates MeHg transfer and bioavailability (Finley et al., 2016; Riva-Murray et al., 2011; Scudder et al., 2009). However, the increasing relationship with conductivity and decreasing relationship with SO_4^{-2} contrast previous findings on factors that contribute to elevated fish THg (Benoit et al., 1999). In the model, trout THg_{size} was positively associated with streams draining smaller catchments and sites at higher latitudes. These factors can contribute to lower fish growth rates because lower productivity can facilitate greater biotic MeHg accumulation. However, TP was not an important variable for the final explanatory model, which suggests that climactic factors such as temperature may be limiting productivity. Although the explanatory power was relatively small, brook trout THg_{size} was negatively related to catchment defoliation. Finally, diet autochthony was not an important variable and was not retained in the final explanatory model of brook trout THg_{size}. This was unexpected as lower autochthonous carbon consumption has been associated with decreasing THg in brook trout (Ward et al., 2012). Brook trout are opportunistic feeders (Syrjänen et al., 2011) and their THg levels may be due to consumption of prey that haven't been considered in this study, such as terrestrial invertebrates. In chapter 2, I found that brook trout allochthony was greater in more heavily defoliated watersheds and speculated that trout may be obtaining energy from budworm carcasses or litterfall. The results found herein suggest that increasing defoliation and diet shifts were not explanatory factors for increasing brook trout THg.

3.4.4. Drivers of Hg bioaccumulation

The effects of DOC, defoliation, and autochthony were consistent between both models for carnivorous invertebrates and fish, which suggests that there are common mechanisms for biotic Hg exposure for between these taxa. First, DOC was the most important factor for both consumers, and DOC is commonly identified as a factor that drives MeHg bioaccumulation in aquatic environments (Ward et al., 2003). Recent food web studies have supported a parabolic relationship between DOC and MeHg, where low concentrations of DOC can be a source of MeHg, but higher DOC (4-8 mg/L) concentrations inhibit the bioavailability of MeHg for biotic uptake (Broadley et al., 2018; Taylor et al., 2019). The Gaspé Peninsula streams had low DOC levels and likely follow the pattern of DOC being a Hg source when DOC is limited in the environment. Next, Hg in carnivorous invertebrates and fish were both relatively unaffected by defoliation, which indicates that this terrestrial disturbance is not facilitating increased Hg transfer or availability to stream consumers. Finally, increasing diet autochthony did not increase Hg concentrations in the consumers. This is likely explained by the similar MeHg concentrations between biofilm, CPOM and FPOM, as MeHg levels in basal food sources are a strong determinant for biotic Hg at upper trophic levels (Chasar et al., 2009; de Wit et al., 2012). Overall, these results suggests that Hg concentrations in aquatic invertebrate carnivores and brook trout were mainly controlled by DOC sources that increase mercury bioavailability rather than by differences in dietary habits.

3.4.5. Trophic magnification slopes

Mercury biomagnified in all twelve stream food webs, and TMS were higher in streams without fish, although the difference was not significant. The TMS (0.27-0.38) across the 12 catchments in my study are within the higher range of published values (0.24±0.07) for temperate freshwater streams and lakes (Lavoie et al., 2013). The relatively high TMS in these streams are consistent with studies that observed higher biomagnification rates in aquatic food webs at higher latitudes (Lavoie et al., 2013) and that are less productive (Clayden et al., 2014). These environments can facilitate higher MeHg transfer because bioaccumulation is greater when growth is slow (Herendeen & Hill, 2004) and animals need to consume higher quantities of food due to greater energetic costs and lower nutrients in prey items (Ward et al., 2011). Finally, TMS were higher at the sites where fish were absent (C05, C06, C07) although the difference was not significant. Brook trout were the top predator in streams where fish were present, and this species has been commonly observed to have low THg levels compared to other fish, including salmonids (Kamman et al., 2005; Ward et al., 2012). Low THg levels in brook trout may be due to their habitat requirements that limit this species to cold, well-oxygenated environments (Petty et al., 2003), which are usually low in Hg.

Correlations were not significant between TMS and watershed defoliation or DOC among the twelve sites ($p>0.05$ for both). This result in the Gaspé Peninsula is consistent with similar stream food web studies, which found that TMS were not higher in catchments with greater forest disturbance. In a study comparing forest buffer strips, trophic magnification of mercury was lowest in streams draining clear-cut watersheds, and this was attributed to lower abundance of shredder invertebrates in these watersheds, which were influential to slope calculations (Willacker et al., 2019). Furthermore, Negrazis et al. (2022) found higher TMS in watersheds with extensive harvest than reference or intensively-managed sites. Although DOC is commonly associated with elevated MeHg in water and biota across different trophic levels, a global meta-analysis found that DOC does not have a strong relationship with increasing trophic biomagnification (Lavoie et al., 2013).

3.5. Conclusion

In this chapter, I found that Hg levels in carnivorous invertebrates and fish were explained mainly by DOC. However, increasing autochthony was not associated with increasing Hg in consumers, and may be due to the comparable MeHg levels in biofilms and terrestrial food sources. Finally, trophic biomagnification rates did not differ based on catchment defoliation or stream DOC concentrations. The congruence between the models for carnivorous invertebrates and brook trout suggests that Hg bioaccumulation in these consumers may have shared exposure mechanisms that are affected mainly by aquatic DOC concentrations, rather than dietary exposure or defoliation. There are many interactive factors that affect MeHg levels in aquatic biota, and my project contributes new knowledge on the relative importance of landscape, water chemistry, or dietary sources of this contaminant. As DOC levels were low in the Gaspé streams, disturbances that increase this organic material to the stream can potentially increase mercury levels in aquatic biota, including brook trout, a sensitive coldwater sport fish.

References

Alahuhta, J., Kosten, S., Akasaka, M., Auderset, D., Azzella, M. M., Bolpagni, R., Bove, C. P., Chambers, P. A., Chappuis, E., Clayton, J., de Winton, M., Ecke, F., Gacia, E., Gecheva, G., Grillas, P., Hauxwell, J., Hellsten, S., Hjort, J., Hoyer, M. v., … Heino, J. (2017). Global variation in the beta diversity of lake macrophytes is driven by environmental heterogeneity rather than latitude. *Journal of Biogeography*, *44*(8), 1758–1769. https://doi.org/10.1111/JBI.12978

Amlund, H., Lundebye, A. K., & Berntssen, M. H. G. (2007). Accumulation and elimination of methylmercury in Atlantic cod (Gadus morhua L.) following dietary exposure. *Aquatic Toxicology*, *83*(4), 323–330. https://doi.org/10.1016/j.aquatox.2007.05.008

Arango, C., Ponette-González, A., Neziri, I., & Bailey, J. (2019). Western spruce budworm effects on throughfall N, P, and C fluxes and soil nutrient status in the Pacific Northwest. *Canadian Journal of Forest Research*, *49*(10), 1207–1218. https://doi.org/10.1139/cjfr-2018-0523

Arostegui, M. C., Schindler, D. E., & Holtgrieve, G. W. (2019). Does lipid-correction introduce biases into isotopic mixing models? Implications for diet reconstruction studies. *Oecologia*, *191*(4), 745–755. https://doi.org/10.1007/s00442-019-04525-7

Atkinson, C. L., & Cooper, J. T. (2016). Benthic algal community composition across a watershed: coupling processes between land and water. *Aquatic Ecology*, *50*(2), 315–326. https://doi.org/10.1007/S10452-016-9580-5/FIGURES/6

Atkinson, C. L., Golladay, S. W., Opsahl, S. P., & Covich, A. P. (2009). Stream discharge and floodplain connections affect seston quality and stable isotopic signatures in a coastal plain stream. *Journal of the North American Benthological Society*, *28*(2), 360–370. https://doi.org/10.1899/08-102.1

Baken, S., Degryse, F., Verheyen, L., Merckx, R., & Smolders, E. (2011). Metal complexation properties of freshwater dissolved organic matter are explained by its aromaticity and by anthropogenic ligands. *Environmental Science and Technology*, *45*(7), 2584–2590. https://doi.org/10.1021/es103532a

Benoit, J. M., Gilmour, C. C., Mason, R. P., & Heyes, A. (1999). Sulfide controls on mercury speciation and bioavailability to methylating bacteria in sediment pore waters. In Environmental Science and Technology (Vol. 33, Issue 10, p. 1780). ACS. https://doi.org/10.1021/es992007q

Bertini, S. C. B., Azevedo, L. C. B., de Carvalho Mendes, I., & Cardoso, E. J. B. N. (2014). Hierarchical partitioning for selection of microbial and chemical indicators of soil quality. *Pedobiologia*, *57*(4–6), 293–301. https://doi.org/10.1016/j.pedobi.2014.06.001

Biggs, B. J. F., & Close, M. E. (1989). Periphyton biomass dynamics in gravel bed rivers: the relative effects of flows and nutrients. *Freshwater Biology*, *22*(2), 209–231. https://doi.org/10.1111/j.1365-2427.1989.tb01096.x

Bilby, R. E., & Bisson, P. A. (1992). Allochthonous versus autochthonous organic matter contributions to the trophic support of fish populations in clear-cut and old-growth forested streams. *Canadian Journal of Fisheries and Aquatic Sciences*, *49*(3), 540–551. https://doi.org/10.1139/f92-064

Bloom, N. S. (1992). On the Chemical Form of Mercury in Edible Fish and Marine Invertebrate Tissue. *Canadian Journal of Fisheries and Aquatic Sciences*, *49*(5), 1010–1017. https://doi.org/10.1139/f92-113

Bouchard, M., & Pothier, D. (2010). Spatiotemporal variability in tree and stand mortality caused by spruce budworm outbreaks in eastern Quebec. *Canadian Journal of Forest Research*, *40*(1), 86–94. https://doi.org/10.1139/X09-178

Boucher, D., Boulanger, Y., Aubin, I., Bernier, P. Y., Beaudoin, A., Guindon, L., & Gauthier, S. (2018). Current and projected cumulative impacts of fire, drought, and insects on timber volumes across Canada. *Ecological Applications*, *28*(5), 1245–1259. https://doi.org/10.1002/EAP.1724

Brett, M. T., Bunn, S. E., Chandra, S., Galloway, A. W. E., Guo, F., Kainz, M. J., Kankaala, P., Lau, D. C. P., Moulton, T. P., Power, M. E., Rasmussen, J. B., Taipale, S. J., Thorp, J. H., & Wehr, J. D. (2017). How important are terrestrial organic carbon inputs for secondary production in freshwater ecosystems? *Freshwater Biology*, *62*(5), 833–853. https://doi.org/10.1111/fwb.12909

Broadmeadow, S., & Nisbet, T. R. (2004). The effects of riparian forest management on the freshwater environment: a literature review of best management practice. *Hydrology and Earth System Sciences*, *8*(3), 286–305.

Burns, D. A., & Riva-Murray, K. (2018). Variation in fish mercury concentrations in streams of the Adirondack region, New York: A simplified screening approach using chemical metrics. *Ecological Indicators*, *84*, 648–661. https://doi.org/10.1016/j.ecolind.2017.09.031

Canadian Council of Ministers of the Environment. (2000). *Canadian Tissue Residue Guidelines for the Protection of Wildlife Consumers of Aquatic Biota: Methylmercury*. Canadian environmental quality guidelines.

Carlsson, N. O. L., Sarnelle, O., & Strayer, D. L. (2009). Native predators and exotic prey - An acquired taste? *Frontiers in Ecology and the Environment*, *7*(10), 525–532. https://doi.org/10.1890/080093

Charbonneau, K. L., Kidd, K. A., Kreutzweiser, D. P., Sibley, P. K., Emilson, E. J. S., O'Driscoll, N. J., & Gray, M. A. (2022). Are There Longitudinal Effects of Forest Harvesting on Carbon Quality and Flow and Methylmercury Bioaccumulation in Primary Consumers of Temperate Stream Networks? *Environmental Toxicology and Chemistry*, *41*(6), 1490–1507. https://doi.org/10.1002/etc.5330

Chasar, L. C., Scudder, B. C., Stewart, A. R., Bell, A. H., & Aiken, G. R. (2009). Mercury cycling in stream ecosystems. 3. Trophic dynamics and methylmercury bioaccumulation. *Environmental Science and Technology*, *43*(8), 2733–2739. https://doi.org/10.1021/es8027567

Chaves-Ulloa, R., Taylor, B. W., Broadley, H. J., Cottingham, K. L., Baer, N. A., Weathers, K. C., Ewing, H. A., & Chen, C. Y. (2016). Dissolved organic carbon modulates mercury concentrations in insect subsidies from streams to terrestrial consumers. *Ecological Applications*, *26*(6), 1771–1784. https://doi.org/10.1890/15-0025.1

Chevan, A., & Sutherland, M. (1991). Hierarchical partitioning. *American Statistician*, *45*(2), 90–96. https://doi.org/10.1080/00031305.1991.10475776

Clayden, M. G., Kidd, K. A., Chételat, J., Hall, B. D., & Garcia, E. (2014). Environmental, geographic and trophic influences on methylmercury concentrations in macroinvertebrates from lakes and wetlands across Canada. *Ecotoxicology*, *23*(2), 273–284. https://doi.org/10.1007/s10646-013-1171-9

Clayden, M. G., Kidd, K. A., Wyn, B., Kirk, J. L., G Muir, D. C., & O, N. J. (2013). Mercury Biomagnification through Food Webs Is Affected by Physical and Chemical Characteristics of Lakes. *Environmental Science and Technology*, *47*(21), 12047–12053. https://doi.org/10.1021/es4022975

Cross, W. F., Benstead, J. P., Frost, P. C., & Thomas, S. A. (2005). Ecological stoichiometry in freshwater benthic systems: Recent progress and perspectives. In *Freshwater Biology* (Vol. 50, Issue 11, pp. 1895–1912). John Wiley & Sons, Ltd. https://doi.org/10.1111/j.1365-2427.2005.01458.x

Cummins, K. W. (1974). Structure and Function of Stream Ecosystems. *BioScience*, *24*(11), 631–641. https://doi.org/10.2307/1296676

Daguené, V., McFall, E., Yumvihoze, E., Xiang, S., Amyot, M., & Poulain, A. J. (2012). Divalent base cations hamper Hg II uptake. *Environmental Science and Technology*, *46*(12), 6645–6653. https://doi.org/10.1021/es300760e

Dangles, O. (2002). Functional plasticity of benthic macroinvertebrates: Implications for trophic dynamics in acid streams. *Canadian Journal of Fisheries and Aquatic Sciences*, *59*(9), 1563–1573. https://doi.org/10.1139/f02-122

D'Aoust, V., Kneeshaw, D., & Bergeron, Y. (2004). Characterization of canopy openness before and after a spruce budworm outbreak in the southern boreal forest. *Canadian Journal of Forest Research*, *34*(2), 339–352. https://doi.org/10.1139/x03-278

Davies, P. M., Bunn, S. E., & Hamilton, S. K. (2008). Primary production in tropical streams and rivers. In *Tropical Stream Ecology* (pp. 23–42). Academic Press. https://doi.org/10.1016/B978-012088449-0.50004-2

de Wit, H. A., Granhus, A., Lindholm, M., Kainz, M. J., Lin, Y., Braaten, H. F. V., & Blaszczak, J. (2014). Forest harvest effects on mercury in streams and biota in Norwegian boreal catchments. *Forest Ecology and Management*, *324*, 52–63. https://doi.org/10.1016/j.foreco.2014.03.044

de Wit, H. A., Kainz, M. J., & Lindholm, M. (2012). Methylmercury bioaccumulation in invertebrates of boreal streams in Norway: Effects of aqueous methylmercury and diet retention. *Environmental Pollution*, *164*, 235–241. https://doi.org/10.1016/j.envpol.2012.01.041

Driscoll, C. T., Blette, V., Yan, C., Schofield, C. L., Munson, R., & Holsapple, J. (1995). The role of dissolved organic carbon in the chemistry and bioavailability of mercury in remote Adirondack lakes. Water, Air, & Soil Pollution, 80(1–4), 499–508. https://doi.org/10.1007/BF01189700

Driscoll, C. T., Han, Y. J., Chen, C. Y., Evers, D. C., Lambert, K. F., Holsen, T. M., Kamman, N. C., & Munson, R. K. (2007). Mercury contamination in forest and freshwater ecosystems in the northeastern United States. *BioScience*, *57*(1), 17–28. https://doi.org/10.1641/B570106

Eagles-Smith, C. A., Herring, G., Johnson, B., & Graw, R. (2016). Conifer density within lake catchments predicts fish mercury concentrations in remote subalpine lakes. *Environmental Pollution*, *212*, 279–289. https://doi.org/10.1016/j.envpol.2016.01.049

Eklöf, K., Kraus, A., Weyhenmeyer, G. A., Meili, M., & Bishop, K. (2012). Forestry Influence by Stump Harvest and Site Preparation on Methylmercury, Total Mercury and Other Stream Water Chemistry Parameters Across a Boreal Landscape. *Ecosystems*, *15*(8), 1308–1320. https://doi.org/10.1007/s10021-012-9586-3

England, L. E., & Rosemond, A. D. (2004). Small reductions in forest cover weaken terrestrial-aquatic linkages in headwater streams. *Freshwater Biology*, *49*(6), 721–734. https://doi.org/10.1111/j.1365-2427.2004.01219.x

Erdozain, M., Kidd, K., Kreutzweiser, D., & Sibley, P. (2019). Increased reliance of stream macroinvertebrates on terrestrial food sources linked to forest management intensity. *Ecological Applications*, *29*(4), 1–14. https://doi.org/10.1002/eap.1889

Eros, T., Gustafsson, P., Greenberg, L. A., & Bergman, E. (2012). Forest-stream linkages: Effects of terrestrial invertebrate input and light on diet and growth of brown trout (salmo trutta) in a boreal forest stream. *PLoS ONE*, *7*(5), e36462. https://doi.org/10.1371/journal.pone.0036462

Eshleman, K. N., Morgan, R. P., Webb, J. R., Deviney, F. A., & Galloway, J. N. (1998). Temporal patterns of nitrogen leakage from mid-Appalachian forested watersheds: Role of insect defoliation. *Water Resources Research*, *34*(8), 2005–2016. https://doi.org/10.1029/98wr01198

Finlay, J. C. (2001). Stable-carbon-isotope ratios of river biota: Implications for energy flow in lotic food webs. *Ecology*, *82*(4), 1052–1064. https://doi.org/10.1890/0012-9658(2001)082[1052:SCIROR]2.0.CO;2

Finley, M. L. D., Kidd, K. A., Curry, R. A., Lescord, G. L., Clayden, M. G., & O'Driscoll, N. J. (2016). A comparison of mercury biomagnification through lacustrine food webs supporting brook trout (Salvelinus fontinalis) and other salmonid fishes. *Frontiers in Environmental Science*, *4*(APR), 23. https://doi.org/10.3389/fenvs.2016.00023

Fisher, S. G., & Likens, G. E. (1973). Energy Flow in Bear Brook, New Hampshire: An Integrative Approach to Stream Ecosystem Metabolism. *Ecological Monographs*, *43*(4), 421–439. https://doi.org/10.2307/1942301

Frost, C. J., & Hunter, M. D. (2007). Recycling of nitrogen in herbivore feces: Plant recovery, herbivore assimilation, soil retention, and leaching losses. *Oecologia*, *151*(1), 42–53. https://doi.org/10.1007/s00442-006-0579-9

Fry, B. (2007). Stable isotope ecology. In *Encyclopedia of Ecology*. https://doi.org/10.1016/B978-0-12-409548-9.10915-7

Gandhi, K. J. K., & Herms, D. A. (2010). Direct and indirect effects of alien insect herbivores on ecological processes and interactions in forests of eastern North America. *Biological Invasions*, *12*(2), 389–405. https://doi.org/10.1007/s10530-009-9627-9

Garcia, E., & Carignan, R. (2005). Mercury concentrations in fish from forest harvesting and fire-impacted Canadian Boreal lakes compared using stable isotopes of nitrogen. *Environmental Toxicology and Chemistry*, *24*(3), 685–693. https://doi.org/10.1897/04-065R.1

Gelman, A., Carlin, J. B., Stern, H. S., & Rubin, D. B. (2014). *Bayesian Data Analysis*. Chapman and Hall/CRC.

Geweke, J. F. (1991). *Evaluating the accuracy of sampling-based approaches to the calculation of posterior moments*. Federal Reserve Bank of Minneapolis.

Göthe, E., Lepori, F., & Malmqvist, B. (2009). Forestry affects food webs in northern Swedish coastal streams. *Fundamental and Applied Limnology*, *175*(4), 281–294. https://doi.org/10.1127/1863-9135/2009/0175-0281

Grace, J. R. (1986). The influence of gypsy moth on the composition and nutrient content of litter fall in a Pennsylvania oak forest. *Forest Science*, *32*(4), 855–870. https://doi.org/10.1093/forestscience/32.4.855

Grady, A. E., Scanlon, T. M., & Galloway, J. N. (2007). Declines in dissolved silica concentrations in western Virginia streams (1988–2003): Gypsy moth defoliation stimulates diatoms? *Journal of Geophysical Research: Biogeosciences*, *112*(G1), 1009. https://doi.org/10.1029/2006JG000251

Grandpré, L. de, Marchand, M., Kneeshaw, D. D., Paré, D., Boucher, D., Bourassa, S., Gervais, D., Simard, M., Griffin, J. M., & Pureswaran, D. S. (2022). Defoliation-induced changes in foliage quality may trigger broad-scale insect outbreaks. *Communications Biology*, *5*(1), 1–10. https://doi.org/10.1038/s42003-022-03407-8

Grigal, D. F. (2002). Inputs and outputs of mercury from terrestrial watersheds: A review. In *Environmental Reviews* (Vol. 10, Issue 1, pp. 1–39). NRC Research Press Ottawa, Canada. https://doi.org/10.1139/a01-013

Guo, F., Kainz, M. J., Sheldon, F., & Bunn, S. E. (2016). The importance of high-quality algal food sources in stream food webs - current status and future perspectives. *Freshwater Biology*, *61*(6), 815–831. https://doi.org/10.1111/fwb.12755

Haitzer, M., Aiken, G. R., & Ryan, J. N. (2002). Binding of mercury(II) to dissolved organic matter: The role of the mercury-to-DOM concentration ratio. *Environmental Science and Technology*, *36*(16), 3564–3570. https://doi.org/10.1021/ES025699I/SUPPL_FILE/ES025699I_S1.XLS

Hall, R. O., & Meyer, J. L. (1998). The trophic significance of bacteria in a detritus-based stream food web. *Ecology*, *79*(6), 1995–2012. https://doi.org/10.1890/0012-9658(1998)079[1995:TTSOBI]2.0.CO;2

Hart, D. D., & Finelli, C. M. (1999). Physical-biological coupling in streams: The pervasive effects of flow on benthic organisms. *Annual Review of Ecology and Systematics*, *30*, 363–395. https://doi.org/10.1146/annurev.ecolsys.30.1.363

Hart, S. A., & Chen, H. Y. H. (2006). Understory vegetation dynamics of North American boreal forests. In *Critical Reviews in Plant Sciences* (Vol. 25, Issue 4, pp. 381–397). Taylor & Francis Group. https://doi.org/10.1080/07352680600819286

Hayden, B., McWilliam-Hughes, S. M., & Cunjak, R. A. (2016). Evidence for limited trophic transfer of allochthonous energy in temperate river food webs. *Freshwater Science*, *35*(2), 544–558. https://doi.org/10.1086/686001

Heaston, E. D., Kaylor, M. J., & Warren, D. R. (2018). Aquatic food web response to patchy shading along forested headwater streams. *Canadian Journal of Fisheries and Aquatic Sciences*, *75*(12), 2211–2220. https://doi.org/10.1139/cjfas-2017-0464

Herendeen, R. A., & Hill, W. R. (2004). Growth dilution in multilevel food chains. *Ecological Modelling*, *178*(3–4), 349–356. https://doi.org/10.1016/j.ecolmodel.2004.03.011

Hill, W. R., & Larsen, I. L. (2005). Growth dilution of metals in microalgal biofilms. *Environmental Science and Technology*, *39*(6), 1513–1518. https://doi.org/10.1021/es049587y

Hintelmann, H., & Evans, R. D. (1997). Application of stable isotopes in environmental tracer studies – Measurement of monomethylmercury (CH3Hg+) by isotope dilution ICP-MS and detection of species transformation. *Fresenius' Journal of Analytical Chemistry 1997 358:3*, *358*(3), 378–385. https://doi.org/10.1007/S002160050433

Hunter, M. D. (2001). Insect population dynamics meets ecosystem ecology: Effects of herbivory on soil nutrient dynamics. *Agricultural and Forest Entomology*, *3*(2), 77–84. https://doi.org/10.1046/j.1461-9563.2001.00100.x

Hynes, H. B. N. (1975). The stream and its valley. *Internationale Vereinigung Für Theoretische Und Angewandte Limnologie: Verhandlungen*, *19*(1), 1–15. https://doi.org/10.1080/03680770.1974.11896033

IAEA. (2008). *IAEA-158 Marine Sediment (Methyl Mercury)*. International Atomic Energy Agency. https://nucleus.iaea.org/sites/ReferenceMaterials/Pages/IAEA-158.aspx

Jardine, T. D., Kidd, K. A., & O' Driscoll, N. (2013). Food web analysis reveals effects of pH on mercury bioaccumulation at multiple trophic levels in streams. *Aquatic Toxicology*, *132–133*, 46–52. https://doi.org/10.1016/j.aquatox.2013.01.013

Jardine, T. D., Kidd, K. A., & Rasmussen, J. B. (2012). Aquatic and terrestrial organic matter in the diet of stream consumers: Implications for mercury bioaccumulation. *Ecological Applications*, *22*(3), 843–855. https://doi.org/10.1890/11-0874.1

Johns, R. C., Bowden, J. J., Carleton, D. R., Cooke, B. J., Edwards, S., Emilson, E. J. S., James, P. M. A., Kneeshaw, D., MacLean, D. A., Martel, V., Moise, E. R. D., Mott, G. D., Norfolk, C. J., Owens, E., Pureswaran, D. S., Quiring, D. T., Régnière, J., Richard, B., & Stastny, M. (2019). A conceptual framework for the spruce budworm Early Intervention Strategy: Can outbreaks be stopped? *Forests*, *10*(10), 910. https://doi.org/10.3390/f10100910

Jonsson, M., Polvi, L. E., Sponseller, R. A., & Stenroth, K. (2018). Catchment properties predict autochthony in stream filter feeders. *Hydrobiologia*, *815*(1), 83–95. https://doi.org/10.1007/s10750-018-3553-8

Kahlert, M., & McKie, B. G. (2014). Comparing new and conventional methods to estimate benthic algal biomass and composition in freshwaters. *Environmental Science: Processes and Impacts*, *16*(11), 2627–2634. https://doi.org/10.1039/c4em00326h

Kamman, N. C., Burgess, N. M., Driscoll, C. T., Simonin, H. A., Goodale, W., Linehan, J., Estabrook, R., Hutcheson, M., Major, A., Scheuhammer, A. M., & Scruton, D. A. (2005). Mercury in freshwater fish of northeast North America - A geographic perspective based on fish tissue monitoring databases. Ecotoxicology, 14(1–2), 163–180. https://doi.org/10.1007/s10646-004-6267-9

Kaylor, M. J., & Warren, D. R. (2018). Canopy closure after four decades of postlogging riparian forest regeneration reduces cutthroat trout biomass in headwater streams through bottom-up pathways. *Canadian Journal of Fisheries and Aquatic Sciences*, *75*(4), 513–524. https://doi.org/10.1139/cjfas-2016-0519

Kelly, C. A., Rudd, J. W. M., & Holoka, M. H. (2003). Effect of pH on mercury uptake by an aquatic bacterium: Implications for Hg cycling. *Environmental Science and Technology*, *37*(13), 2941–2946. https://doi.org/10.1021/es026366o

Kelly, E. N., Schindler, D. W., st. Louis, V. L., Donald, D. B., & Vladicka, K. E. (2006). Forest fire increases mercury accumulation by fishes via food web restructuring and increased mercury inputs. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(51), 19380–19385. https://doi.org/10.1073/pnas.0609798104

Kielstra, B. W., Mackereth, R. W., Melles, S. J., & Emilson, E. J. (2021). *hydroweight: Inverse distance-weighted rasters and landscape attributes*. https://doi.org/10.5281/zenodo.4728559

Kiffney, P. M., Richardson, J. S., & Bull, J. P. (2003). Responses of periphyton and insects to experimental manipulation of riparian buffer width along forest streams. *Journal of Applied Ecology*, *40*(6), 1060–1076. https://doi.org/10.1111/j.1365-2664.2003.00855.x

Kiljunen, M., Grey, J., Sinisalo, T., Harrod, C., Immonen, H., & Jones, R. I. (2006). A revised model for lipid-normalizing δ13C values from aquatic organisms, with implications for isotope mixing models. *Journal of Applied Ecology*, *43*(6), 1213–1222. https://doi.org/10.1111/j.1365-2664.2006.01224.x

Kneeshaw, D., Sturtevant, B. R., Cooke, B., Work, T., Pureswaran, D., DeGrandpre, L., & MacLean, D. A. (2015). Insect disturbances in forest ecosystems. In *Routledge Handbook of Forest Ecology* (pp. 93–113).

Kondratieff, P. F., & Simmons, G. M. (1985). Microbial colonization of seston and free bacteria in an impounded river. *Hydrobiologia*, *128*(2), 127–133. https://doi.org/10.1007/BF00008732

Kozak, N., Ahonen, S. A., Keva, O., Østbye, K., Taipale, S. J., Hayden, B., & Kahilainen, K. K. (2021). Environmental and biological factors are joint drivers of mercury biomagnification in subarctic lake food webs along a climate and productivity gradient. *Science of the Total Environment*, *779*, 146261. https://doi.org/10.1016/j.scitotenv.2021.146261

Kreutzweiser, D. P., Hazlett, P. W., & Gunn, J. M. (2008). Logging impacts on the biogeochemistry of boreal forest soils and nutrient export to aquatic systems: A review. *Environmental Reviews*, *16*, 157–179. https://doi.org/10.1139/A08-006/ASSET/IMAGES/A08-006T3H.GIF

Lai, J., Zou, Y., Zhang, J., & Peres-Neto, P. R. (2022). Generalizing hierarchical and variation partitioning in multiple regression and canonical analyses using the rdacca.hp R package. *Methods in Ecology and Evolution*, *13*(4), 782–788. https://doi.org/10.1111/2041-210X.13800

Lamontagne, S., Carignan, R., D’Arcy, P., Prairie, Y. T., & Paré, D. (2000). Element export in runoff from eastern Canadian Boreal Shield drainage basins following forest harvesting and wildfires. *Canadian Journal of Fisheries and Aquatic Sciences*, *57*(S2), 118–128. https://doi.org/10.1139/f00-108

Lavoie, R. A., Jardine, T. D., Chumchal, M. M., Kidd, K. A., & Campbell, L. M. (2013). Biomagnification of mercury in aquatic food webs: A worldwide meta-analysis. *Environmental Science and Technology*, *47*(23), 13385–13394. https://doi.org/10.1021/es403103t

le Faucheur, S., Campbell, P. G. C., Fortin, C., & Slaveykova, V. I. (2014). Interactions between mercury and phytoplankton: Speciation, bioavailability, and internal handling. *Environmental Toxicology and Chemistry*, *33*(6), 1211–1224. https://doi.org/10.1002/ETC.2424

Lesutiene, J., Gorokhova, E., Stankevičiene, D., Bergman, E., & Greenberg, L. (2014). Light increases energy transfer efficiency in a boreal stream. *PLoS ONE*, *9*(11), e113675. https://doi.org/10.1371/journal.pone.0113675

Lewis, G. P., & Likens, G. E. (2007). Changes in stream chemistry associated with insect defoliation in a Pennsylvania hemlock-hardwoods forest. *Forest Ecology and Management*, *238*(1–3), 199–211. https://doi.org/10.1016/j.foreco.2006.10.013

Likens, G. E., Bormann, F. H., Johnson, N. M., Fisher, D. W., & Pierce, R. S. (1970). Effects of Forest Cutting and Herbicide Treatment on Nutrient Budgets in the Hubbard Brook Watershed-Ecosystem. *Ecological Monographs*, *40*(1), 23–47. https://doi.org/10.2307/1942440

Lu, X., Gu, W., Zhao, L., Ul Haque, M. F., DiSpirito, A. A., Semrau, J. D., & Gu, B. (2017). Methylmercury uptake and degradation by methanotrophs. *Science Advances*, *3*(5). https://doi.org/10.1126/sciadv.1700041

Luengen, A. C., Fisher, N. S., & Bergamaschi, B. A. (2012). Dissolved organic matter reduces algal accumulation of methylmercury. *Environmental Toxicology and Chemistry*, *31*(8), 1712–1719. https://doi.org/10.1002/etc.1885

Mac Nally, R. (2000). Regression and model-building in conservation biology, biogeography and ecology: The distinction between - and reconciliation of - “predictive” and “explanatory” models. *Biodiversity and Conservation*, *9*(5), 655–671. https://doi.org/10.1023/A:1008985925162

MacLean, D. A. (1984). Effects of Spruce Budworm Outbreaks on the Productivity and Stability of Balsam Fir Forests. *The Forestry Chronicle*, *60*(5), 273–279. https://doi.org/10.5558/tfc60273-5

Malcata Martins, B., O’Driscoll, N. J., Mallory, M. L., & Canário, J. (2021). A Review of Freshwater Invertebrates as Biomonitors of Methylmercury: the Importance of More Complete Physical and Chemical Reporting. In *Bulletin of Environmental Contamination and Toxicology* (Vol. 107, Issue 5, pp. 801–808). Springer. https://doi.org/10.1007/s00128-021-03274-9

Malo, M., & Bourque, P.-A. (1993). Timing of the deformation events from Late Ordovician to Mid-Devonian in the Gaspé Peninsula. *Geological Society of America*, *275*.

Martínez, A., Larrañaga, A., Pérez, J., Descals, E., & Pozo, J. (2014). Temperature affects leaf litter decomposition in low-order forest streams: Field and microcosm approaches. *FEMS Microbiology Ecology*, *87*(1), 257–267. https://doi.org/10.1111/1574-6941.12221

Marvin-Dipasquale, M., Lutz, M. A., Brigham, M. E., Krabbenhoft, D. P., Aiken, G. R., Orem, W. H., & Hall, B. D. (2009). Mercury cycling in stream ecosystems. 2. Benthic methylmercury production and bed sediment - Pore water partitioning. *Environmental Science and Technology*, *43*(8), 2726–2732. https://doi.org/10.1021/es802698v

Mattson, W. J., & Addy, N. D. (1975). Phytophagous Insects as Regulators of Forest Primary Production. *Science*, *190*(4214), 515–522. http://www.jstor.org/stable/1740415

Mauro, J. B. N., Guimarães, J. R. D., Hintelmann, H., Watras, C. J., Haack, E. A., & Coelho-Souza, S. A. (2002). Mercury methylation in macrophytes, periphyton, and water - Comparative studies with stable and radio-mercury additions. *Analytical and Bioanalytical Chemistry*, *374*(6), 983–989. https://doi.org/10.1007/s00216-002-1534-1

McCutchan, J. H., & Lewis, W. M. (2002). Relative importance of carbon sources for macroinvertebrates in a Rocky Mountain stream. *Limnology and Oceanography*, *47*(3), 742–752. https://doi.org/10.4319/lo.2002.47.3.0742

McCutchan, J. H., Lewis, W. M., Kendall, C., & McGrath, C. C. (2003). Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos*, *102*(2), 378–390. https://doi.org/10.1034/j.1600-0706.2003.12098.x

McNeely, C., Clinton, S. M., & Erbe, J. M. (2006). Landscape variation in C sources of scraping primary consumers in streams. *Journal of the North American Benthological Society*, *25*(4), 787–799. https://doi.org/10.1899/0887-3593(2006)025[0787:LVICSO]2.0.CO;2

Merritt, R. W., & Cummins, K. W. (1996). *An introduction to the aquatic insects of North America*. Kendall/Hunt.

Michalzik, B., & Stadler, B. (2005). Importance of canopy herbivores to dissolved and particulate organic matter fluxes to the forest floor. *Geoderma*, *127*(3-4 SPEC. ISS.), 227–236. https://doi.org/10.1016/j.geoderma.2004.12.006

Minshall, G. W. (1978). Autotrophy in Stream Ecosystems. *BioScience*, *28*(12), 767–771. https://doi.org/10.2307/1307250

Mundahl, N. D., Mundahl, D. E., & Merten, E. C. (2012). Success of Slimy Sculpin Reintroductions in Minnesota Trout Streams: Influence of Feeding and Diets. *Source: The American Midland Naturalist*, *168*(1), 162–183.

Murphy, M. L., Hawkins, C. P., & Anderson, N. H. (1981). Effects of Canopy Modification and Accumulated Sediment on Stream Communities. *Transactions of the American Fisheries Society*, *110*(4), 469–478. https://doi.org/10.1577/1548-8659(1981)110<469:eocmaa>2.0.co;2

Nakano, S., & Murakami, M. (2001). Reciprocal subsidies: Dynamic interdependence between terrestrial and aquatic food webs. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(1), 166–170. https://doi.org/10.1073/pnas.98.1.166

Natural Resources Canada. (2012). *Research at the Laurentian Forestry Centre of Natural Resources Canada – Spruce budworm*. http://scf.rncan.gc.ca/publications.

Natural Resources Canada. (2018). *The State of Canada's Forests: Annual Report 2018*. http://cfs.nrcan.gc.ca/publications?id=39336

Natural Resources Canada. (2021). *The State of Canada's Forests: Annual Report 2021*.

Negrazis, L., Kidd, K. A., Erdozain, M., Emilson, E. J. S., Mitchell, C. P. J., & Gray, M. A. (2022). Effects of forest management on mercury bioaccumulation and biomagnification along the river continuum. *Environmental Pollution*, *310*. https://doi.org/10.1016/j.envpol.2022.119810

NRC-CNRC. (2007). *DORM-3: Fish protein certified reference material for trace metals*. National Research Council of Canada. https://doi.org/10.4224/crm.2007.dorm-3

NRC-CNRC. (2012). *DORM-4: Fish protein certified reference material for trace metals*. National Research Council Canada. https://doi.org/10.4224/crm.2012.dorm-4

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., & Wagner, H. (2013). Package 'vegan.' In *Community ecology package, version* (Vol. 2, Issue 9, pp. 1–295).

Painter, K. J., Janz, D. M., & Jardine, T. D. (2016). Bioaccumulation of mercury in invertebrate food webs of Canadian Rocky Mountain streams. *Freshwater Science*, *35*(4), 1248–1262. https://doi.org/10.1086/688897

Peterson, E. E., Sheldon, F., Darnell, R., Bunn, S. E., & Harch, B. D. (2011). A comparison of spatially explicit landscape representation methods and their relationship to stream condition. *Freshwater Biology*, *56*(3), 590–610. https://doi.org/10.1111/j.1365-2427.2010.02507.x

Petty, J. T., Lamothe, P. J., & Mazik, P. M. (2005). Spatial and Seasonal Dynamics of Brook Trout Populations Inhabiting a Central Appalachian Watershed. Transactions of the American Fisheries Society, 134(3), 572–587. https://doi.org/10.1577/t03-229.1

Pickhardt, P. C., Folt, C. L., Chen, C. Y., Klaue, B., & Blum, J. D. (2002). Algal blooms reduce the uptake of toxic methylmercury in freshwater food webs. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(7), 4419–4423. https://doi.org/10.1073/pnas.072531099

Polis, G. A., Anderson, W. B., & Holt, R. D. (1997). Toward an integration of landscape and food web ecology: The dynamics of spatially subsidized food webs. In *Annual Review of Ecology and Systematics* (Vol. 28, pp. 289–316). https://doi.org/10.1146/annurev.ecolsys.28.1.289

Porvari, P., Verta, M., Munthe, J., & Haapanen, M. (2003). Forestry practices increase mercury and methyl mercury output from boreal forest catchments. *Environmental Science and Technology*, *37*(11), 2389–2393. https://doi.org/10.1021/es0340174

Post, D. M. (2002). Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology*, *83*(3), 703–718. https://doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2

Ravichandran, M. (2004). Interactions between mercury and dissolved organic matter - A review. *Chemosphere*, *55*(3), 319–331. https://doi.org/10.1016/j.chemosphere.2003.11.011

Rea, A. W., Keeler, G. J., & Scherbatskoy, T. (1996). The deposition of mercury in throughfall and litterfall in the Lake Champlain watershed: A short-term study. *Atmospheric Environment*, *30*(19), 3257–3263. https://doi.org/10.1016/1352-2310(96)00087-8

Regnell, O., & Watras, C. J. (2019). Microbial Mercury Methylation in Aquatic Environments: A Critical Review of Published Field and Laboratory Studies. *Environmental Science and Technology*, *53*(1), 4–19. https://doi.org/10.1021/ACS.EST.8B02709/ASSET/IMAGES/LARGE/ES-2018-02709P_0004.JPEG

Rennie, M. D., Collins, N. C., Purchase, C. F., & Tremblay, A. (2005). Predictive models of benthic invertebrate methylmercury in Ontario and Quebec lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, *62*(12), 2770–2783. https://doi.org/10.1139/f05-181

Reynolds, B. C., Hunter, M. D., & Crossley, D. A. (2000). Effects of Canopy Herbivory on Nutrient Cycling in a Northern Hardwood Forest in Western North Carolina. *Selbyana*, *21*(1,2), 74–78. https://www.jstor.org/stable/41760056?seq=1&cid=pdf-reference#references_tab_contents

Richardson, J. S., Bilby, R. E., & Bondar, C. A. (2005). Organic matter dynamics in small streams of the Pacific Northwest. *Journal of the American Water Resources Association*, *41*(4), 921–934. https://doi.org/10.1111/j.1752-1688.2005.tb03777.x

Riva-Murray, K., Bradley, P. M., Chasar, L. C., Button, D. T., Brigham, M. E., Scudder Eikenberry, B. C., Journey, C. A., & Lutz, M. A. (2013). Influence of dietary carbon on mercury bioaccumulation in streams of the Adirondack Mountains of New York and the Coastal Plain of South Carolina, USA. *Ecotoxicology*, *22*(1), 60–71. https://doi.org/10.1007/s10646-012-1003-3

Riva-Murray, K., Chasar, L. C., Bradley, P. M., Burns, D. A., Brigham, M. E., Smith, M. J., & Abrahamsen, T. A. (2011). Spatial patterns of mercury in macroinvertebrates and fishes from streams of two contrasting forested landscapes in the eastern United States. *Ecotoxicology*, *20*(7), 1530–1542. https://doi.org/10.1007/s10646-011-0719-9

Roon, D. A., Wipfli, M. S., & Kruse, J. J. (2018). Riparian defoliation by the invasive green alder sawfly influences terrestrial prey subsidies to salmon streams. *Ecology of Freshwater Fish*, *27*(4), 963–975. https://doi.org/10.1111/eff.12407

Rosemond, A. D., Mulholland, P. J., & Brawley, S. H. (2000). Seasonally shifting limitation of stream periphyton: Response of algal populations and assemblage biomass and productivity to variation in light, nutrients, and herbivores. *Canadian Journal of Fisheries and Aquatic Sciences*, *57*(1), 66–75. https://doi.org/10.1139/f99-181

Rosi-Marshall, E. J., Vallis, K. L., Baxter, C. v, & Davis, J. M. (2016). Retesting a prediction of the River Continuum Concept: Autochthonous versus allochthonous resources in the diets of invertebrates. *Freshwater Science*, *35*(2), 534–543. https://doi.org/10.1086/686302

Sampson, A., Ings, N., Shelley, F., Tuffin, S., Grey, J., Trimmer, M., Woodward, G., & Hildrew, A. G. (2019). Geographically widespread 13 C-depletion of grazing caddis larvae: A third way of fuelling stream food webs? *Freshwater Biology*, *64*(4), 787–798. https://doi.org/10.1111/fwb.13262

Scudder, B. C., Chasar, L. C., Wentz, D. A., Bauch, N. J., Brigham, M. E., Moran, P. W., & Krabbenhoft, D. P. (2009). *Mercury in fish, bed sediment, and water from streams across the United States, 1998–2005*. Scientific Investigations Report 2009-5109.

Shah, N. W., Baillie, B. R., Bishop, K., Ferraz, S., Högbom, L., & Nettles, J. (2022). The effects of forest management on water quality. *Forest Ecology and Management*, *522*, 120397. https://doi.org/10.1016/J.FORECO.2022.120397

Sharov, A. A., Leonard, D., Liebhold, A. M., Roberts, E. A., & Dickerson, W. (2002). "Slow The Spread": A National Program to Contain the Gypsy Moth. *Journal of Forestry*, *100*(5), 30–36. https://doi.org/10.1093/JOF/100.5.30

Sheath, R. G., Burkholder, J. A. M., Morison, M. O., Steinman, A. D., & VanAlstyne, K. L. (1986). Effect of Tree Canopy Removal by Gypsy Moth Larvae on the Macroalgae of a Rhode Island Headwater Stream. *Journal of Phycology*, *22*(4), 567–570. https://doi.org/10.1111/j.1529-8817.1986.tb02506.x

Skyllberg, U., Westin, M. B., Meili, M., & Björn, E. (2009). Elevated concentrations of methyl mercury in streams after forest clear-cut: A consequence of mobilization from soil or new methylation? *Environmental Science and Technology*, *43*(22), 8535–8541. https://doi.org/10.1021/es900996z

st. Louis, V. L., Rudd, J. W. M., Kelly, C. A., Hall, B. D., Rolfhus, K. R., Scott, K. J., Lindberg, S. E., & Dong, W. (2001). Importance of the forest canopy to fluxes of methyl mercury and total mercury to boreal ecosystems. *Environmental Science and Technology*, *35*(15), 3089–3098. https://doi.org/10.1021/es001924p

Stock, B. C., Jackson, A. L., Ward, E. J., Parnell, A. C., Phillips, D. L., & Semmens, B. X. (2018). Analyzing mixing systems using a new generation of Bayesian tracer mixing models. *PeerJ*, *2018*(6), e5096. https://doi.org/10.7717/peerj.5096

Swank, W. T., Waide, J. B., Crossley, D. A., & Todd, R. L. (1981). Insect defoliation enhances nitrate export from forest ecosystems. *Oecologia*, *51*(3), 297–299. https://doi.org/10.1007/BF00540897

Syrjänen, J., Korsu, K., Louhi, P., Paavola, R., & Muotka, T. (2011). Stream salmonids as opportunistic foragers: The importance of terrestrial invertebrates along a stream-size gradient. *Canadian Journal of Fisheries and Aquatic Sciences*, *68*(12), 2146–2156. https://doi.org/10.1139/F2011-118

Tamura, S., & Kagaya, T. (2019). Food habits of invertebrate grazers in a forested stream: variations according to taxonomic affiliation, flow habitat, and body size. *Hydrobiologia*, *841*(1), 109–120. https://doi.org/10.1007/s10750-019-04010-5

Townsend, P. A., Eshleman, K. N., & Welcker, C. (2004). Remote sensing of gypsy moth defoliation to assess variations in stream nitrogen concentrations. In *Ecological Applications* (Vol. 14, Issue 2, pp. 504–516). John Wiley & Sons, Ltd. https://doi.org/10.1890/02-5356

Tsui, M. T. K., Blum, J. D., Finlay, J. C., Balogh, S. J., Nollet, Y. H., Palen, W. J., & Power, M. E. (2014). Variation in terrestrial and aquatic sources of methylmercury in stream predators as revealed by stable mercury isotopes. *Environmental Science and Technology*, *48*(17), 10128–10135. https://doi.org/10.1021/es500517s

Tsui, M. T. K., & Finlay, J. C. (2011). Influence of dissolved organic carbon on methylmercury bioavailability across minnesota stream ecosystems. *Environmental Science and Technology*, *45*(14), 5981–5987. https://doi.org/10.1021/es200332f

Ullrich, S. M., Tanton, T. W., & Abdrashitova, S. A. (2001). Mercury in the aquatic environment: A review of factors affecting methylation. In *Critical Reviews in Environmental Science and Technology* (Vol. 31, Issue 3, pp. 241–293). https://doi.org/10.1080/20016491089226

US EPA. (1998). *Mercury in solids and solutions by thermal decomposition, amalgamation, and atomic absorption spectrophometry-method 7473-total Mercury*.

Utz, R. M., & Hartman, K. J. (2007). Identification of critical prey items to Appalachian brook trout (Salvelinus fontinalis) with emphasis on terrestrial organisms. *Hydrobiologia*, *575*(1), 259–270. https://doi.org/10.1007/S10750-006-0372-0/TABLES/6

van Nieuwenhuyse, E. E., & Jones, J. R. (1996). Phosphorus-chlorophyll relationship in temperate streams and its variation with stream catchment area. *Canadian Journal of Fisheries and Aquatic Sciences*, *53*(1), 99–105. https://doi.org/10.1139/f95-166

vander Zanden, M. J., & Rasmussen, J. B. (2001). Variation in δ15N and δ13C trophic fractionation: Implications for aquatic food web studies. *Limnology and Oceanography*, *46*(8), 2061–2066. https://doi.org/10.4319/lo.2001.46.8.2061

Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R., & Colbert, C. E. (1980). The River Continuum Concept. *Canadian Journal of Fisheries and Aquatic Sciences*, *37*(1), 130–137.

Ward, D. M., Nislow, K. H., Chen, C. Y., & Folt, C. L. (2010). Rapid, Efficient Growth Reduces Mercury Concentrations in Stream-Dwelling Atlantic Salmon. *Transactions of the American Fisheries Society*, *139*(1), 1–10. https://doi.org/10.1577/t09-032.1

Ward, D. M., Nislow, K. H., & Folt, C. L. (2010). Bioaccumulation syndrome: Identifying factors that make some stream food webs prone to elevated mercury bioaccumulation. *Annals of the New York Academy of Sciences*, *1195*(1), 62–83. https://doi.org/10.1111/j.1749-6632.2010.05456.x

Ward, D. M., Nislow, K. H., & Folt, C. L. (2012). Do Low-Mercury Terrestrial Resources Subsidize Low-Mercury Growth of Stream Fish? Differences between Species along a Productivity Gradient. *PLoS ONE*, *7*(11), e49582. https://doi.org/10.1371/journal.pone.0049582

Warren, D. R., Keeton, W. S., Kiffney, P. M., Kaylor, M. J., Bechtold, H. A., & Magee, J. (2016). Changing forests-changing streams: Riparian forest stand development and ecosystem function in temperate headwaters. *Ecosphere*, *7*(8). https://doi.org/10.1002/ecs2.1435

Watras, C. J., Back, R. C., Halvorsen, S., Hudson, R. J. M., Morrison, K. A., & Wente, S. P. (1998). Bioaccumulation of mercury in pelagic freshwater food webs. *Science of the Total Environment*, *219*(2–3), 183–208. https://doi.org/10.1016/S0048-9697(98)00228-9

Webb, J. R., Cosby, B. J., Deviney, F. A., Eshleman, K. N., & Galloway, J. N. (1995). Change in the acid-base status of an appalachian mountain catchment following forest defoliation by the gypsy moth. *Water, Air, & Soil Pollution*, *85*(2), 535–540. https://doi.org/10.1007/BF00476884

Webster, J. J., & Hartman, K. J. (2005). The role of terrestrial invertebrates in allopatric brook trout headwater streams in the Central appalachian mountains. *Journal of Freshwater Ecology*, *20*(1), 101–107. https://doi.org/10.1080/02705060.2005.9664942

Wilkerson, E., Hagan, J. M., & Whitman, A. A. (2010). The effectiveness of different buffer widths for protecting water quality and macroinvertebrate and periphyton assemblages of headwater streams in Maine, USA. *Canadian Journal of Fisheries and Aquatic Sciences*, *67*(1), 177–190. https://doi.org/10.1139/F09-162

Willacker, J. J., Eagles-Smith, C. A., Kowalski, B. M., Danehy, R. J., Jackson, A. K., Adams, E. M., Evers, D. C., Eckley, C. S., Tate, M. T., & Krabbenhoft, D. P. (2019). Timber harvest alters mercury bioaccumulation and food web structure in headwater streams. *Environmental Pollution*, *253*, 636–645. https://doi.org/10.1016/j.envpol.2019.07.025

Wipfli, M. S. (1997). Terrestrial invertebrates as salmonid prey and nitrogen sources in streams: Contrasting old-growth and young-growth riparian forests in southeastern Alaska, U.S.A. *Canadian Journal of Fisheries and Aquatic Sciences*, *54*(6), 1259–1269. https://doi.org/10.1139/f97-034

Woodman, S. G., Khoury, S., Fournier, R. E., Emilson, E. J. S., Gunn, J. M., Rusak, J. A., & Tanentzap, A. J. (2021). Forest defoliator outbreaks alter nutrient cycling in northern waters. *Nature Communications*, *12*(1), 1–8. https://doi.org/10.1038/s41467-021-26666-1

Chapter 4: General Conclusion

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Insect outbreaks are a major disturbance to Canadian forests, although their impacts on stream food webs and contaminant cycling are not well understood. Widespread defoliation has been observed to alter carbon fluxes in terrestrial environments (Grandpré et al., 2022) and increase stream nutrients and algal productivity (Eshleman et al., 1998; Sheath et al., 1986; Webb et al., 1995). Additionally, catchment disturbances have been found to raise levels of aquatic methylmercury (MeHg), a neurotoxic metal that biomagnifies in stream food webs (Porvari et al. 2003, Garcia & Carignan., 2005). However, there is a knowledge gap on whether defoliation alters dietary carbon sources or facilitates MeHg bioaccumulation in stream consumers.

Studies on similar forest disturbances, such as harvesting, have shown that changes in terrestrial carbon cycling in can impact several trophic levels in stream food webs (England & Rosemond, 2004; Erdozain et al., 2019). Catchment disturbances can alter the contribution of carbon sources that originate from the terrestrial (allochthonous) or aquatic (autochthonous) environment and lead to consumer diet shifts. Aquatic consumers can have altered productivity and health if changes in stream energy contributions are accompanied by diet shifts to less nutritious sources, or food sources with higher contaminant levels (Broadley et al., 2019). For an example, autochthonous energy sources can affect mercury transfer in food webs and contribute to elevated levels of this contaminant in top predators (Riva-Murray et al., 2013). Furthermore, catchment disturbances can increase MeHg levels in aquatic biota by facilitating the delivery of terrestrially-derived mercury to streams, or through changes in water chemistry that increase MeHg bioavailability and uptake (Ward et al., 2010). As streams are closely linked to catchment conditions, terrestrial defoliation can have widespread implications for stream consumers and their MeHg levels.

The recent start of the spruce budworm (SBW) outbreak in the Gaspé Peninsula provided a unique opportunity to study the impacts of defoliation on stream ecosystems. I studied streams in twelve watersheds experiencing a range of defoliation severity to assess autochthonous production, consumption, and mercury bioaccumulation. Data were collected on landscape conditions of each watershed, and stream reaches were sampled for water chemistry, algal production, food sources, invertebrates, and fish in 2019 and 2020. I analyzed the food web samples for stable isotopes and mercury and used hierarchical partitioning models to determine the local and landscape drivers of autochthony and biotic mercury in these streams. I found that defoliation was associated with increasing autochthonous production, but not its consumption, as some stream invertebrates had greater reliance on allochthonous energy in heavily defoliated watersheds. The severity of watershed defoliation contributed to increasing invertebrate allochthony, but this disturbance did not have a strong impact on brook trout diets. In contrast, defoliation had a negligible impact on increasing mercury levels in invertebrates and fish and this disturbance did not increase trophic biomagnification in the stream food webs. Instead, dissolved organic carbon (DOC) was the key driver of biotic Hg levels in carnivorous invertebrates and brook trout, which is consistent with findings from similar low-DOC stream studies (Burns & Riva-Murray, 2018; Driscoll et al., 2007).

The findings from my study suggest that intervention to reduce defoliation can potentially mitigate dietary shifts in aquatic consumers, although the benefits to contaminant cycling are not as clear. Increasing defoliation was associated with increasing diet allochthony in brook trout and several invertebrate consumers, and this energy source can be less nutritious and lead to lower biomass production in stream consumers (Kaylor & Warren, 2018). Although defoliation was not a driver of biotic Hg, I found that DOC had a positive effect on increasing this contaminant in carnivorous invertebrates and brook trout. The streams in the Gaspé Peninsula have low concentrations of DOC, and disturbances that increase organic material runoff to the stream can potentially increase MeHg bioavailability to aquatic organisms. Furthermore, brook trout was the top predator in these streams and this species is culturally and ecologically important as a sport fish and food for wildlife. However, brook trout THg concentrations exceeded wildlife protection guidelines (33 ng/g ww) in some streams (Canadian Council of Ministers of the Environment, 2000), which emphasizes the importance of identifying factors that can mitigate toxic levels of this contaminant.

I encountered some limitations, such as additional data that would strengthen my findings on the impacts of defoliation to streams. Defoliation had a large proportion of shared variance in the hierarchical partitioning models because defoliation severity was collinear with elevation and latitude during the two years of my study. As defoliation progresses in the region, I expect that ongoing spraying will create more distinct differences in the watersheds. These differences in catchment defoliation severity can help determine the effect of defoliation independent of correlated landscape variables. Additional years of data would also help identify patterns due to defoliation. Furthermore, quantification of stream litterfall or budworm inputs would help determine whether increased allochthony of consumers might be due to greater consumption of either of these resources. Although defoliators can increase litterfall to forest soils (Grace, 1986), studies have not measured inputs to streams, even though this can be a direct link to consumer diet shifts. Additionally, benthic production was not measured in 2020 due to pandemic travel restrictions, and riparian light measurements were not considered during the study design. These data would also help determine whether defoliation is promoting algal growth by increasing light availability. Finally, aqueous MeHg levels could not be compared among the streams because 2020 stream water samples had likely degraded before they could be analyzed.

Defoliation is expected to continue in the Gaspé Peninsula, and tree mortality will begin as forest stands sustain repeated years of damage. Next steps should include continued assessment of the stream responses, especially as mortality can cause ongoing changes to carbon cycling and hydrology in the catchment. Additionally, continued studies of this outbreak can identify any delayed impacts of defoliation on the streams. Forests have tight internal nutrient cycling, but catchments with greater mortality may have greater nutrient leaching to streams. In the longer term, dead wood can change stream detritus processing and increase carbon inputs. These can alter consumer energy uptake, and may be especially influential to mercury dynamics, as I found that increasing DOC was a driver of biotic mercury in several stream invertebrates and brook trout. Although I observed some responses, my study was completed during the beginning stages of the outbreak, and the full scope of stream responses in the Gaspé Peninsula are likely yet to be seen as the SBW continues to defoliate these catchments.

References

Broadley, H. J., Cottingham, K. L., Baer, N. A., Weathers, K. C., Ewing, H. A., Chaves-Ulloa, R., Chickering, J., Wilson, A. M., Shrestha, J., & Chen, C. Y. (2019). Factors affecting MeHg bioaccumulation in stream biota: the role of dissolved organic carbon and diet. *Ecotoxicology*, *28*(8), 949–963. https://doi.org/10.1007/s10646-019-02086-2

Burns, D. A., & Riva-Murray, K. (2018). Variation in fish mercury concentrations in streams of the Adirondack region, New York: A simplified screening approach using chemical metrics. *Ecological Indicators*, *84*, 648–661. https://doi.org/10.1016/j.ecolind.2017.09.031

Canadian Council of Ministers of the Environment. (2000). *Canadian Tissue Residue Guidelines for the Protection of Wildlife Consumers of Aquatic Biota: Methylmercury*. Canadian environmental quality guidelines.

Driscoll, C. T., Han, Y. J., Chen, C. Y., Evers, D. C., Lambert, K. F., Holsen, T. M., Kamman, N. C., & Munson, R. K. (2007). Mercury contamination in forest and freshwater ecosystems in the northeastern United States. *BioScience*, *57*(1), 17–28. https://doi.org/10.1641/B570106

England, L. E., & Rosemond, A. D. (2004). Small reductions in forest cover weaken terrestrial-aquatic linkages in headwater streams. *Freshwater Biology*, *49*(6), 721–734. https://doi.org/10.1111/j.1365-2427.2004.01219.x

Erdozain, M., Kidd, K., Kreutzweiser, D., & Sibley, P. (2019). Increased reliance of stream macroinvertebrates on terrestrial food sources linked to forest management intensity. *Ecological Applications*, *29*(4), 1–14. https://doi.org/10.1002/eap.1889

Eshleman, K. N., Morgan, R. P., Webb, J. R., Deviney, F. A., & Galloway, J. N. (1998). Temporal patterns of nitrogen leakage from mid-Appalachian forested watersheds: Role of insect defoliation. *Water Resources Research*, *34*(8), 2005–2016. https://doi.org/10.1029/98wr01198

Garcia, E., & Carignan, R. (2005). Mercury concentrations in fish from forest harvesting and fire-impacted Canadian Boreal lakes compared using stable isotopes of nitrogen. *Environmental Toxicology and Chemistry*, *24*(3), 685–693. https://doi.org/10.1897/04-065R.1

Grace, J. R. (1986). The influence of gypsy moth on the composition and nutrient content of litter fall in a Pennsylvania oak forest. *Forest Science*, *32*(4), 855–870. https://doi.org/10.1093/forestscience/32.4.855

Grandpré, L. de, Marchand, M., Kneeshaw, D. D., Paré, D., Boucher, D., Bourassa, S., Gervais, D., Simard, M., Griffin, J. M., & Pureswaran, D. S. (2022). Defoliation-induced changes in foliage quality may trigger broad-scale insect outbreaks. *Communications Biology*, *5*(1), 1–10. https://doi.org/10.1038/s42003-022-03407-8

Porvari, P., Verta, M., Munthe, J., & Haapanen, M. (2003). Forestry practices increase mercury and methyl mercury output from boreal forest catchments. Environmental Science and Technology, 37(11), 2389–2393. https://doi.org/10.1021/es0340174

Riva-Murray, K., Bradley, P. M., Chasar, L. C., Button, D. T., Brigham, M. E., Scudder Eikenberry, B. C., Journey, C. A., & Lutz, M. A. (2013). Influence of dietary carbon on mercury bioaccumulation in streams of the Adirondack Mountains of New York and the Coastal Plain of South Carolina, USA. *Ecotoxicology*, *22*(1), 60–71. https://doi.org/10.1007/s10646-012-1003-3

Sheath, R. G., Burkholder, J. A. M., Morison, M. O., Steinman, A. D., & VanAlstyne, K. L. (1986). Effect of Tree Canopy Removal by Gypsy Moth Larvae on the Macroalgae of a Rhode Island Headwater Stream. *Journal of Phycology*, *22*(4), 567–570. https://doi.org/10.1111/j.1529-8817.1986.tb02506.x.

Ward, D. M., Nislow, K. H., & Folt, C. L. (2010). Bioaccumulation syndrome: Identifying factors that make some stream food webs prone to elevated mercury bioaccumulation. *Annals of the New York Academy of Sciences*, *1195*(1), 62–83. https://doi.org/10.1111/j.1749-6632.2010.05456.x

Webb, J. R., Cosby, B. J., Deviney, F. A., Eshleman, K. N., & Galloway, J. N. (1995). Change in the acid-base status of an appalachian mountain catchment following forest defoliation by the gypsy moth. *Water, Air, & Soil Pollution*, *85*(2), 535–540. https://doi.org/10.1007/BF00476884

Appendix A

Table A1. Average value of different water chemistry parameters in samples collected biweekly from June-Oct in 2019 (n=8/site) and June-Nov in 2020 (n=10/site) from streams in the Gaspé Peninsula, Québec.

Site	**pH**	**DOC (mg/L)**	**SUVA (L/mg-C m)**	**NO2-NO3 (mg/L)**	**DIC (mg/L)**	**TP (mg/L)**	**TN (mg/L)**	**Cond. (umho/cm)**	**Alk (meq/L)**	**Ca (mg/L)**	**K (mg/L)**	**Mg (mg/L)**	**Na (mg/L)**	**SO4 (mg/L)**	**Cl (mg/L)**	**SiO2 (mg/L)**	**Zn (mg/L)**	**Cu (mg/L)**
2019																		
C04	7.6	1.07	3.22	0.02	7.8	0.0050	0.13	75	0.62	9.1	0.5	2.5	1.9	4.4	0.7	6.7	0.0025	0.0010
C05	8.0	0.98	3.50	0.07	21.4	0.0010	0.10	178	1.76	32.2	0.4	3.0	1.8	3.5	0.9	6.0	0.0042	0.0012
C06	7.9	0.92	4.03	0.04	17.5	0.0011	0.08	145	1.43	26.1	0.4	2.3	1.4	2.8	0.7	6.0	0.0029	0.0011
C07	7.9	1.44	3.15	0.10	15.7	0.0011	0.14	135	1.31	24.0	0.4	2.2	1.4	2.5	0.6	5.8	0.0024	0.0013
L08	8.2	1.83	2.90	0.15	35.3	0.0012	0.20	278	3.18	49.7	0.4	6.3	1.8	5.8	1.1	5.4	0.0025	0.0017
L09	7.7	1.33	3.12	0.07	12.2	0.0014	0.13	128	1.00	17.8	0.4	3.7	2.5	12.5	2.3	6.7	0.0029	0.0016
L10	8.0	1.16	2.99	0.20	22.1	0.0011	0.23	200	1.82	29.1	0.4	6.8	2.6	9.2	2.4	5.9	0.0030	0.0012
L11	7.9	1.05	3.69	0.12	18.7	0.0014	0.16	178	1.51	24.2	0.5	6.7	2.1	12.3	2.5	6.1	0.0028	0.0014
L12	7.7	1.34	4.87	0.10	9.9	0.0009	0.15	113	0.92	13.9	0.4	4.2	1.8	12.6	1.3	6.1	0.0025	0.0012
U01	8.0	0.66	4.31	0.13	18.5	0.0009	0.14	177	1.67	26.6	0.4	5.0	1.6	10.2	2.6	4.0	0.0042	0.0012
U02	7.6	1.57	3.96	0.14	6.9	0.0017	0.19	67	0.65	10.0	0.2	1.5	1.1	2.1	0.7	6.9	0.0025	0.0012
U03	8.0	1.88	3.22	0.05	18.4	0.0010	0.11	152	1.62	26.5	0.3	3.1	0.9	2.9	0.6	3.5	0.0022	0.0013
2020																		
C04	7.1	0.86	2.81	0.03	6.7	0.0150	0.12	63	0.50	6.7	0.5	2.4	2.0	4.3	0.7	6.8	0.0018	0.0011
C05	7.3	0.78	2.23	0.10	14.2	0.0035	0.17	118	1.12	18.0	0.4	2.9	1.7	3.0	1.1	5.9	0.0035	0.0011
C06	7.3	0.74	2.45	0.05	12.0	0.0032	0.08	103	0.98	16.0	0.4	2.3	1.5	2.5	0.8	6.0	0.0025	0.0009
C07	7.4	1.47	2.92	0.12	11.4	0.0026	0.17	104	0.98	16.5	0.4	2.2	1.5	2.3	0.8	6.1	0.0029	0.0009
L08	7.5	1.53	2.93	0.17	19.3	0.0020	0.25	169	1.63	24.2	0.4	6.2	1.7	4.9	1.2	5.3	0.0025	0.0013
L09	7.3	1.10	2.67	0.05	9.6	0.0034	0.14	110	0.74	13.2	0.4	3.6	2.6	12.3	2.3	6.4	0.0024	0.0012
L10	7.5	1.05	1.95	0.13	14.2	0.0090	0.21	144	1.22	15.9	0.4	6.8	2.7	8.8	2.4	5.9	0.0024	0.0015
L11	7.4	0.81	2.34	0.11	13.4	0.0037	0.19	146	1.14	16.0	0.5	7.0	2.3	12.5	2.6	6.2	0.0017	0.0007
L12	7.2	1.51	2.72	0.08	8.8	0.0024	0.14	100	0.66	11.0	0.4	4.2	1.8	12.4	1.1	6.1	0.0011	0.0007
U01	7.3	0.61	2.88	0.14	13.4	0.0092	0.16	134	1.06	17.0	0.4	5.1	1.7	10.3	2.7	4.1	0.0034	0.0021
U02	7.1	1.37	3.87	0.29	6.8	0.0074	0.34	61	0.51	8.6	0.2	1.6	1.2	2.1	0.8	6.9	0.0030	0.0017
U03	7.3	1.69	3.21	0.06	12.2	0.0042	0.15	101	0.95	14.9	0.2	3.2	0.9	2.5	0.7	3.5	0.0018	0.0015

Table A2. Abbreviations and detection limits (DL) for water chemistry parameters measured from streams in the Gaspé Peninsula, Québec. A total 216 measurements were taken across all sites (96 in 2019, 120 in 2020). Parameters removed from analyses (>50% of measurements below DL) are shaded.

Parameter	**Abbreviation**	**Detection Limit (mg/L)**	**Number of samples above DL**
Dissolved organic carbon	DOC	0.4	216
Dissolved inorganic carbon	DIC	0.5	216
Nitrite/Nitrate	NO2/NO3	0.04	187
Ammonia	NH4	0.01	58
Total nitrogen	TN	0.05	211
Soluble reactive phosphorus	SRP	0.001	73
Total phosphorus	TP	0.001	194
Calcium	Ca	0.01	216
Potassium	K	0.01	216
Magnesium	Mg	0.005	216
Sodium	Na	0.01	216
Sulfate	SO4	0.2	216
Chloride	Cl	0.2	216
Silica dioxide	SiO2	0.25	216
Aluminum	Al	0.005	91
Iron	Fe	0.005	110
Manganese	Mn	0.0005	33
Zinc	Zn	0.001	192
Cadmium	Cd	0.0005	0
Copper	Cu	0.0005	201
Nickel	Ni	0.0005	13
Lead	Pb	0.0005	0

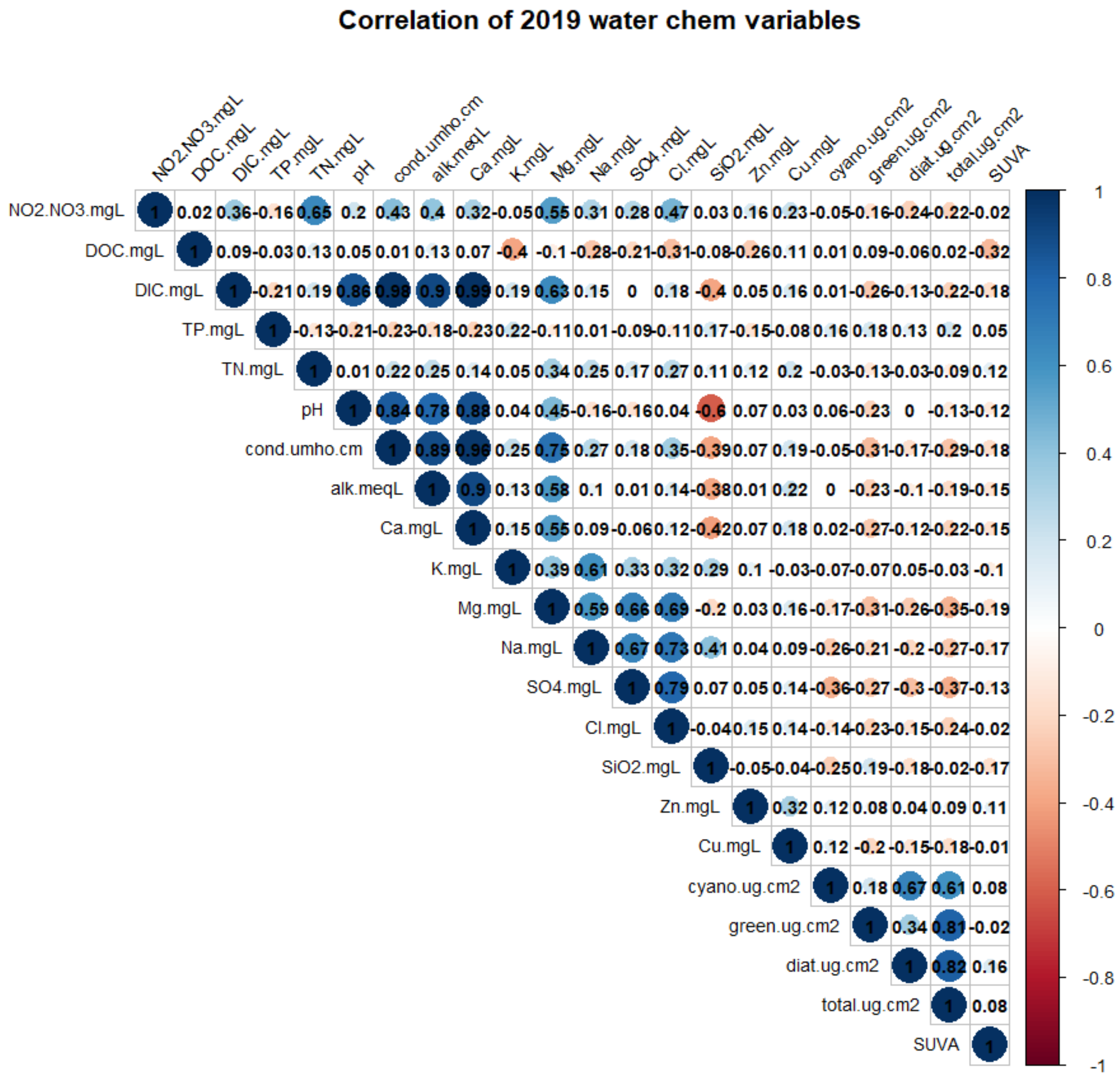
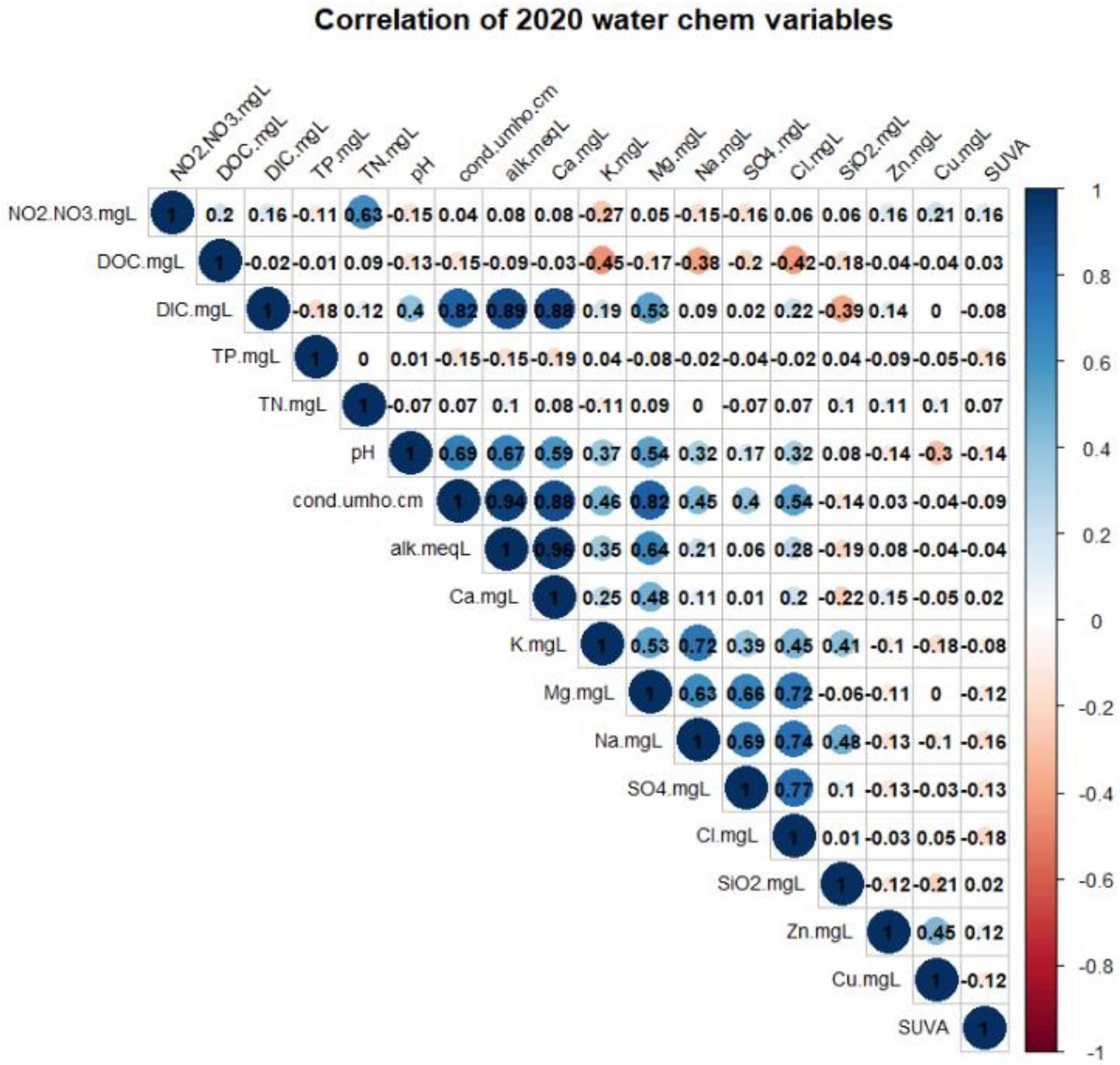


Figure A1. Correlation between water chemistry (n=8) and benthic production (n=6) measurements from twelve streams sampled from June – October 2019 in the Gaspé Peninsula



	NO2.NO3.mgL	DOC.mgL	DIC.mgL	TP.mgL	TN.mgL	pH	cond.umho.cm	alk.meqL	Ca.mgL	K.mgL	Mg.mgL	Na.mgL	SO4.mgL	Cl.mgL	SiO2.mgL	Zn.mgL	Cu.mgL	SUVA
NO2.NO3.mgL	1	0.2	0.16	-0.11	0.63	-0.15	0.04	0.08	0.08	-0.27	0.05	-0.15	-0.16	0.06	0.06	0.16	0.21	0.16
DOC.mgL		1	-0.02	-0.01	0.09	-0.13	-0.15	-0.09	-0.03	-0.45	-0.17	-0.38	-0.2	-0.42	-0.18	-0.04	-0.04	0.03
DIC.mgL			1	-0.18	0.12	0.4	0.82	0.89	0.88	0.19	0.53	0.09	0.02	0.22	-0.39	0.14	0	-0.08
TP.mgL				1	0	0.01	-0.15	-0.15	-0.19	0.04	-0.08	-0.02	-0.04	-0.02	0.04	-0.09	-0.05	-0.16
TN.mgL					1	-0.07	0.07	0.1	0.08	-0.11	0.09	0	-0.07	0.07	0.1	0.11	0.1	0.07
pH						1	0.69	0.67	0.59	0.37	0.54	0.32	0.17	0.32	0.08	-0.14	-0.3	-0.14
cond.umho.cm							1	0.94	0.88	0.46	0.82	0.45	0.4	0.54	-0.14	0.03	-0.04	-0.09
alk.meqL								1	0.96	0.35	0.64	0.21	0.06	0.28	-0.19	0.08	-0.04	-0.04
Ca.mgL									1	0.25	0.48	0.11	0.01	0.2	-0.22	0.15	-0.05	0.02
K.mgL										1	0.53	0.72	0.39	0.45	0.41	-0.1	-0.18	-0.08
Mg.mgL											1	0.63	0.66	0.72	-0.06	-0.11	0	-0.12
Na.mgL												1	0.69	0.74	0.48	-0.13	-0.1	-0.16
SO4.mgL													1	0.77	0.1	-0.13	-0.03	-0.13
Cl.mgL														1	0.01	-0.03	0.05	-0.18
SiO2.mgL															1	-0.12	-0.21	0.02
Zn.mgL																1	0.45	0.12
Cu.mgL																	1	-0.12
SUVA																		1

Figure A2. Correlation between water chemistry (n=10) measurements from twelve streams sampled from June – November 2020 in the Gaspé Peninsula

Table A3: Stable isotopes of carbon and nitrogen from stream samples collected in 2019. Standard deviation was calculated for samples with more than 1 replicate. N refers to the total number of samples measured for isotope analysis. Composite samples were measured for CPOM and invertebrates.

Site	Sample Type	N	δ^{13}C Mean	δ^{13}C SD	δ^{15}N Mean	δ^{15}N SD
U01	Biofilm	3	-34.7	3.14	-0.9	0.39
U01	Brook trout	10	-28.4	1.67	6.2	0.95
U01	Chloroperlidae	3	-31.6	2.02	2.5	0.26
U01	CPOM	3	-30.6	0.37	-1.2	0.17
U01	*Epeorus*	3	-40.9	0.10	-0.7	0.40
U01	FPOM	3	-27.8	0.16	1.9	0.07
U01	Frass	3	-28.3	0.42	-1.3	0.26
U01	*Parapsyche*	1	-32.9	-	2.8	-
U01	Slimy Sculpin	9	-36.1	1.04	5.1	0.31
U02	Biofilm	3	-33.6	1.17	-1.7	1.26
U02	Brook trout	10	-29.6	0.79	6.4	0.14
U02	*Epeorus*	3	-38.1	0.36	0.6	0.17
U02	FPOM	3	-27.9	0.35	2.3	0.30
U02	Frass	3	-28.5	0.46	-1.0	0.26
U02	*Parapsyche*	1	-32.5	-	3.3	-
U02	*Rhyacophila*	3	-33.8	0.23	3.3	0.06
U03	Biofilm	2	-31.0	0.46	0.1	0.20
U03	Brook trout	8	-28.1	0.90	6.3	0.72
U03	Chloroperlidae	1	-31.8	-	2.8	-
U03	CPOM	3	-32.2	1.19	-2.8	0.26
U03	*Epeorus*	1	-36.9	-	1.0	-
U03	FPOM	3	-28.2	0.10	2.3	0.30
U03	Frass	3	-27.9	0.47	-3.1	0.52
U03	*Glossosoma*	1	-38.9	-	1.8	-
U03	*Parapsyche*	1	-32.9	-	2.5	-
U03	*Rhyacophila*	1	-35.0	-	2.8	-
C04	Biofilm	3	-28.6	2.51	0.9	0.23
C04	Brook trout	10	-27.4	1.11	7.1	0.48
C04	Chloroperlidae	2	-30.2	0.21	3.7	0.07
C04	CPOM	3	-30.1	0.47	-1.7	0.68
C04	*Epeorus*	2	-35.4	0.18	1.1	0.06
C04	FPOM	3	-29.1	0.04	1.7	0.35
C04	Frass	3	-28.2	0.26	-2.7	0.68
C04	*Rhyacophila*	1	-32.0	-	3.8	-
C05	Biofilm	3	-33.2	0.57	-0.3	0.22
C05	CPOM	3	-29.5	0.37	-2.2	0.39
C05	*Epeorus*	3	-38.8	0.06	-1.5	0.12
C05	FPOM	3	-29.7	0.81	0.7	0.34
C05	Frass	3	-27.9	0.13	-2.6	0.49

C05	*Rhyacophila*	1	-33.5	-	3.3	-
C06	Biofilm	2	-31.6	0.81	-0.4	0.06
C06	Chloroperlidae	1	-32.1	-	3.4	-
C06	CPOM	3	-32.0	0.61	-2.6	0.52
C06	*Epeorus*	3	-37.4	0.49	-1.2	0.15
C06	FPOM	3	-28.9	0.54	1.9	0.30
C06	Frass	3	-27.3	0.19	-2.5	0.73
C06	*Glossosoma*	1	-41.1	-	-0.8	-
C07	Biofilm	3	-33.9	2.74	-0.7	0.35
C07	Chloroperlidae	1	-32.0	-	2.6	-
C07	CPOM	3	-31.5	0.28	-1.9	0.86
C07	*Epeorus*	2	-39.1	0.07	-0.5	0.21
C07	FPOM	3	-28.3	0.11	0.8	0.19
C07	Frass	3	-28.1	0.27	-2.6	1.16
C07	*Glossosoma*	1	-39.2	-	-0.3	-
C07	*Rhyacophila*	3	-33.5	0.36	3.3	0.15
L08	Biofilm	3	-22.3	1.03	0.0	0.26
L08	Brook trout	10	-27.9	1.47	7.3	0.51
L08	Chloroperlidae	3	-31.8	0.15	2.6	0.21
L08	CPOM	3	-31.0	1.43	-1.6	0.11
L08	*Epeorus*	2	-38.6	0.07	0.8	0.00
L08	FPOM	3	-26.3	0.32	2.0	0.70
L08	Frass	3	-27.6	0.59	-1.7	0.03
L08	*Glossosoma*	1	-40.1	-	-0.2	-
L08	*Parapsyche*	1	-32.9	-	3.2	-
L08	*Rhyacophila*	2	-34.9	0.57	3.3	0.14
L09	Biofilm	3	-33.3	2.54	-1.0	0.24
L09	Brook trout	3	-29.0	2.40	4.7	0.53
L09	Chloroperlidae	3	-30.5	2.26	2.3	0.10
L09	CPOM	3	-30.1	0.15	-2.9	0.28
L09	*Epeorus*	3	-38.2	0.17	-2.4	0.21
L09	FPOM	3	-28.8	0.27	-0.6	0.30
L09	Frass	3	-28.0	0.24	-2.3	0.16
L09	*Parapsyche*	1	-30.7	-	2.7	-
L09	*Rhyacophila*	2	-34.2	0.07	1.2	0.07
L10	Biofilm	3	-34.8	1.35	-0.5	0.34
L10	Brook trout	4	-30.3	3.22	5.3	1.11
L10	Chloroperlidae	3	-33.4	0.38	2.4	0.15
L10	CPOM	3	-29.1	0.61	-1.8	0.60
L10	*Epeorus*	3	-42.1	0.66	-1.8	0.70
L10	FPOM	3	-28.6	0.63	0.8	0.46
L10	Frass	3	-27.8	0.51	-3.1	0.37
L10	*Glossosoma*	1	-44.1	-	-1.4	-
L10	*Rhyacophila*	2	-39.1	0.42	1.9	0.42

L10	Slimy Sculpin	6	-38.7	1.29	4.8	0.35
L11	Biofilm	3	-33.1	2.22	-1.4	0.38
L11	Brook trout	9	-31.1	1.70	5.2	0.96
L11	Chloroperlidae	1	-30.0	-	3.9	-
L11	CPOM	3	-29.2	0.55	-1.7	0.58
L11	*Epeorus*	3	-38.7	0.06	-2.9	0.06
L11	FPOM	3	-28.8	0.61	0.1	0.37
L11	Frass	3	-28.1	0.36	-2.6	0.43
L11	*Glossosoma*	1	-39.1	-	-0.9	-
L11	*Parapsyche*	1	-30.1	-	3.6	-
L12	Biofilm	3	-36.2	1.21	0.0	0.14
L12	Brook trout	8	-32.2	1.10	5.3	0.47
L12	CPOM	3	-29.9	1.18	-2.1	0.65
L12	Epeorus	3	-39.1	0.06	-1.6	0.15
L12	FPOM	3	-29.5	0.30	-0.1	0.13
L12	Frass	3	-27.9	0.24	-3.2	0.65
L12	*Glossosoma*	1	-41.6	-	-1.0	-
L12	*Rhyacophila*	1	-33.5	-	2.8	-

Table A4: Stable isotopes of carbon and nitrogen from stream samples collected in 2020. Standard deviation was calculated for samples with more than 1 replicate. N refers to the total number of samples measured for isotope analysis. Composite samples were measured for CPOM and invertebrates.

Site	Sample Type	N	δ^{13}C Mean	δ^{13}C SD	δ^{15}N Mean	δ^{15}N SD
U01	Baetidae	2	-40.9	0.06	0.7	0.10
U01	Biofilm	6	-36.8	1.89	-2.0	0.21
U01	Brook trout	9	-27.6	1.49	6.1	0.47
U01	Chloroperlidae	1	-32.4	-	2.9	-
U01	CPOM	6	-31.2	1.37	-1.7	0.26
U01	*Epeorus*	2	-41.8	0.14	-0.9	0.14
U01	FPOM	6	-27.5	0.29	0.8	0.18
U01	*Glossosoma*	2	-42.3	0.03	-1.2	0.04
U01	*Parapsyche*	2	-37.8	6.39	0.8	2.98
U01	*Rhyacophila*	3	-33.6	0.55	3.9	0.34
U01	Slimy Sculpin	8	-33.8	1.71	5.6	0.51
U02	Baetidae	1	-37.9	-	1.0	-
U02	Biofilm	6	-37.9	1.10	-0.9	0.33
U02	Brook trout	9	-29.8	1.16	6.2	0.56
U02	Chloroperlidae	1	-32.7	-	2.9	-
U02	CPOM	6	-30.7	0.46	-1.8	0.18
U02	*Epeorus*	2	-38.9	0.37	0.8	0.12
U02	Ephemerellidae	2	-36.0	0.11	-0.1	0.13

U02	FPOM	6	-27.9	0.41	1.1	0.29
U02	*Glossosoma*	2	-39.7	0.06	0.1	0.01
U02	Leuctridae	1	-32.7	-	2.1	-
U02	*Parapsyche*	3	-32.2	0.88	3.4	0.64
U02	*Rhyacophila*	3	-33.2	0.27	4.0	0.13
U02	Slimy Sculpin	2	-34.0	0.28	5.4	0.18
U03	Biofilm	6	-34.7	1.51	-0.1	0.58
U03	Brook trout	9	-28.4	1.48	6.1	0.43
U03	Chloroperlidae	1	-33.1	-	2.6	-
U03	CPOM	6	-31.9	0.97	-2.8	0.17
U03	*Epeorus*	1	-37.7	-	1.2	-
U03	Ephemerellidae	2	-35.1	0.16	0.7	0.02
U03	FPOM	6	-28.5	0.32	0.9	0.34
U03	*Glossosoma*	2	-38.2	0.05	1.6	0.12
U03	Leuctridae	1	-32.0	-	2.2	-
U03	*Parapsyche*	3	-32.9	0.14	3.3	0.49
U03	*Rhyacophila*	3	-35.0	0.48	3.4	0.41
C04	Baetidae	1	-35.1	-	1.9	-
C04	Biofilm	6	-32.4	1.20	0.6	0.76
C04	Brook trout	9	-27.9	0.89	7.1	0.46
C04	Chloroperlidae	1	-29.5	-	4.7	-
C04	CPOM	6	-29.5	0.60	-1.6	0.27
C04	*Epeorus*	1	-34.6	-	1.4	-
C04	Ephemerellidae	1	-35.3	-	0.7	-
C04	FPOM	6	-29.3	0.17	0.9	0.32
C04	*Glossosoma*	1	-35.3	-	0.8	-
C04	*Parapsyche*	2	-30.8	0.29	4.6	0.10
C04	*Rhyacophila*	3	-31.6	0.46	4.3	0.14
C05	Baetidae	1	-37.4	-	0.1	-
C05	Biofilm	6	-35.6	0.50	-1.0	0.78
C05	Chloroperlidae	1	-32.0	-	3.1	-
C05	CPOM	6	-30.3	0.54	-2.3	0.32
C05	*Epeorus*	3	-37.5	0.25	-0.9	0.09
C05	Ephemerellidae	1	-35.6	-	-0.4	-
C05	FPOM	6	-29.7	0.85	-0.1	0.27
C05	*Glossosoma*	2	-39.9	0.13	-1.0	0.16
C05	Leuctridae	1	-30.6	-	1.6	-
C05	*Parapsyche*	2	-32.8	0.45	3.5	0.10
C05	*Rhyacophila*	2	-34.1	0.54	3.4	0.20
C06	Baetidae	1	-40.3	-	1.0	-
C06	Biofilm	6	-35.5	0.39	-1.1	0.27
C06	Chloroperlidae	1	-34.2	-	2.8	-
C06	CPOM	6	-31.4	0.76	-2.0	0.42

C06	*Epeorus*	3	-38.0	0.57	-0.9	0.18
C06	Ephemerellidae	1	-39.6	-	-0.4	-
C06	FPOM	6	-28.9	0.98	1.0	0.35
C06	*Glossosoma*	1	-38.7	-	-1.1	-
C06	Leuctridae	1	-33.2	-	2.2	-
C06	*Parapsyche*	3	-35.3	0.51	3.3	0.16
C06	*Rhyacophila*	3	-35.9	0.08	3.7	0.11
C07	Baetidae	1	-37.8	-	0.4	-
C07	Biofilm	6	-35.3	1.35	-1.3	0.23
C07	Chloroperlidae	1	-33.9	-	2.7	-
C07	CPOM	6	-31.5	1.13	-2.3	0.61
C07	*Epeorus*	1	-38.8	-	0.8	-
C07	Ephemerellidae	2	-38.2	0.42	0.2	0.12
C07	FPOM	6	-28.4	0.22	0.5	0.07
C07	*Glossosoma*	1	-39.1	-	-0.5	-
C07	Leuctridae	1	-31.9	-	1.9	-
C07	*Parapsyche*	3	-32.7	0.16	3.0	0.31
C07	*Rhyacophila*	3	-34.7	0.73	3.3	0.20
L08	Baetidae	1	-39.0	-	1.2	-
L08	Biofilm	6	-30.3	1.11	0.1	0.53
L08	Brook trout	8	-29.4	1.52	6.9	0.41
L08	Chloroperlidae	1	-33.3	-	3.1	-
L08	CPOM	6	-31.2	0.35	-1.8	0.17
L08	*Epeorus*	2	-38.9	0.05	1.0	0.03
L08	Ephemerellidae	1	-35.4	-	0.9	-
L08	FPOM	6	-26.3	0.09	1.6	0.15
L08	*Glossosoma*	2	-37.8	0.06	0.5	0.11
L08	*Parapsyche*	3	-32.1	0.28	3.4	0.13
L08	*Rhyacophila*	3	-36.4	0.68	3.3	0.11
L09	Biofilm	6	-35.1	0.77	-0.6	0.78
L09	Brook trout	8	-28.2	1.26	5.3	0.41
L09	Chloroperlidae	1	-30.1	-	2.5	-
L09	CPOM	6	-30.4	0.54	-2.4	0.53
L09	*Epeorus*	1	-38.9	-	-1.1	-
L09	Ephemerellidae	1	-30.7	-	0.1	-
L09	FPOM	6	-28.4	0.26	0.0	0.24
L09	*Glossosoma*	2	-38.3	0.14	-0.9	0.09
L09	Leuctridae	1	-28.1	-	1.2	-
L09	*Parapsyche*	2	-30.7	0.05	2.8	0.29
L09	*Rhyacophila*	2	-35.1	0.14	2.2	0.12
L10	Baetidae	1	-39.6	-	0.2	-
L10	Biofilm	6	-38.2	2.07	-0.6	0.66
L10	Brook trout	6	-30.7	1.82	5.2	0.63

L10	Chloroperlidae	1	-35.4	-	2.3	-
L10	CPOM	6	-29.7	0.93	-2.1	0.23
L10	*Epeorus*	1	-42.4	-	-0.9	-
L10	Ephemerellidae	1	-35.7	-	0.1	-
L10	FPOM	6	-29.9	0.53	-0.1	0.20
L10	*Glossosoma*	1	-42.1	-	-0.3	-
L10	*Rhyacophila*	2	-39.5	0.58	3.0	0.10
L10	Slimy Sculpin	7	-38.1	0.64	4.9	0.14
L11	Baetidae	1	-37.9	-	-0.4	-
L11	Biofilm	6	-36.5	2.46	-1.1	1.18
L11	Brook trout	9	-31.3	1.57	5.1	0.76
L11	Chloroperlidae	1	-31.6	-	2.8	-
L11	CPOM	6	-29.8	0.49	-2.0	0.76
L11	*Epeorus*	2	-39.4	0.06	-1.9	0.04
L11	Ephemerellidae	1	-34.3	-	-0.7	-
L11	FPOM	6	-28.6	0.42	0.0	0.30
L11	*Glossosoma*	2	-36.4	0.20	-0.5	0.07
L11	*Parapsyche*	3	-32.6	0.37	3.0	0.20
L11	*Rhyacophila*	2	-34.9	1.37	2.3	0.25
L12	Baetidae	1	-39.6	-	0.1	-
L12	Biofilm	6	-38.2	0.94	-0.3	0.65
L12	Brook trout	9	-31.8	1.07	5.8	0.50
L12	Chloroperlidae	1	-32.1	-	3.0	-
L12	CPOM	4	-29.4	0.83	-2.8	0.18
L12	*Epeorus*	3	-40.1	0.06	-0.4	0.06
L12	Ephemerellidae	1	-35.4	-	-0.3	-
L12	FPOM	6	-29.2	0.28	-0.1	0.17
L12	*Glossosoma*	2	-41.5	0.11	0.0	0.04
L12	*Parapsyche*	1	-31.9	-	4.4	-
L12	*Rhyacophila*	3	-35.0	0.65	2.9	0.17

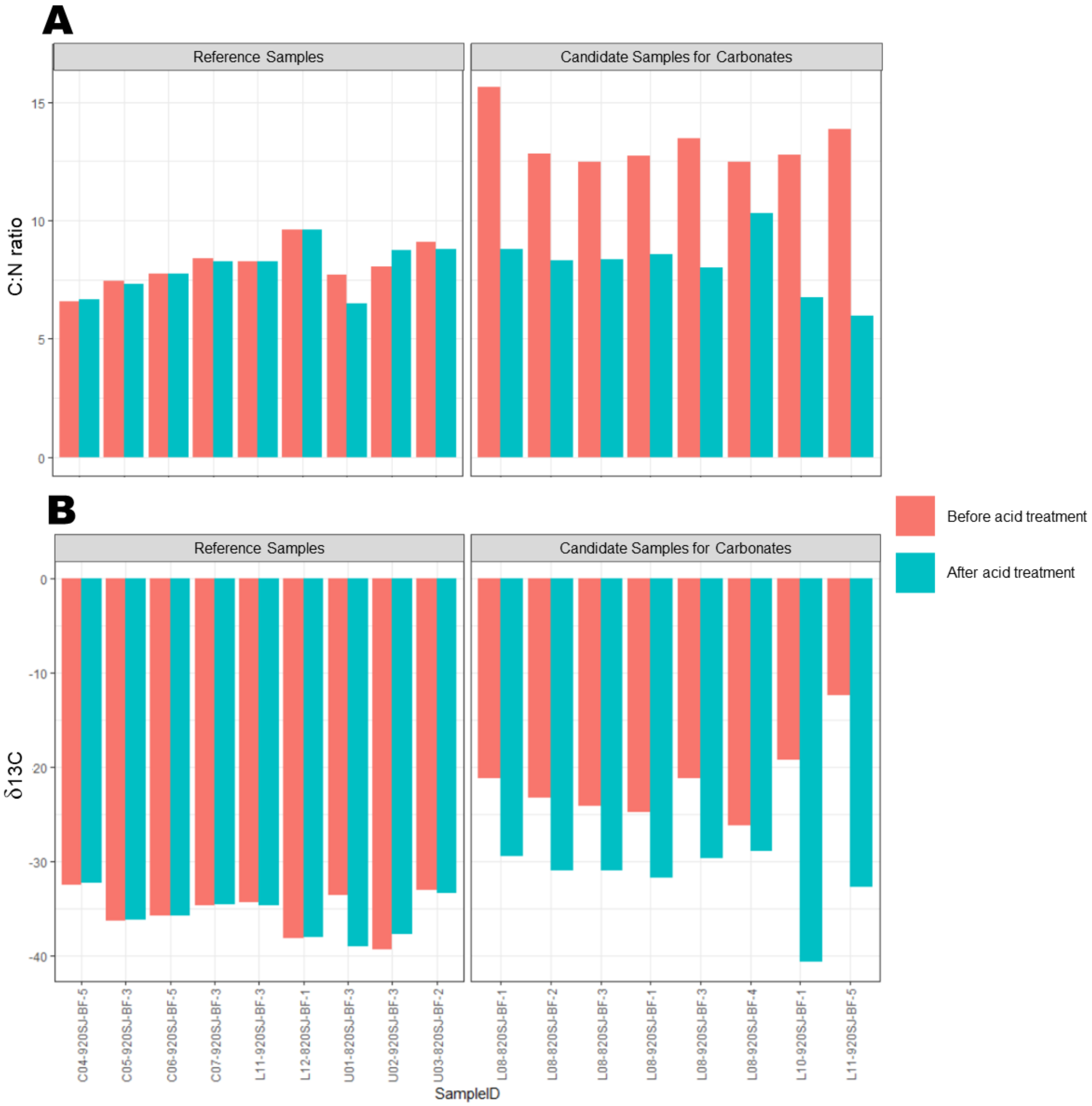


Figure A3: Boxplots showing changes in (A) C:N ratio and (B) δ^{13}C in reference and candidate biofilm samples that were submitted for acid treatment.

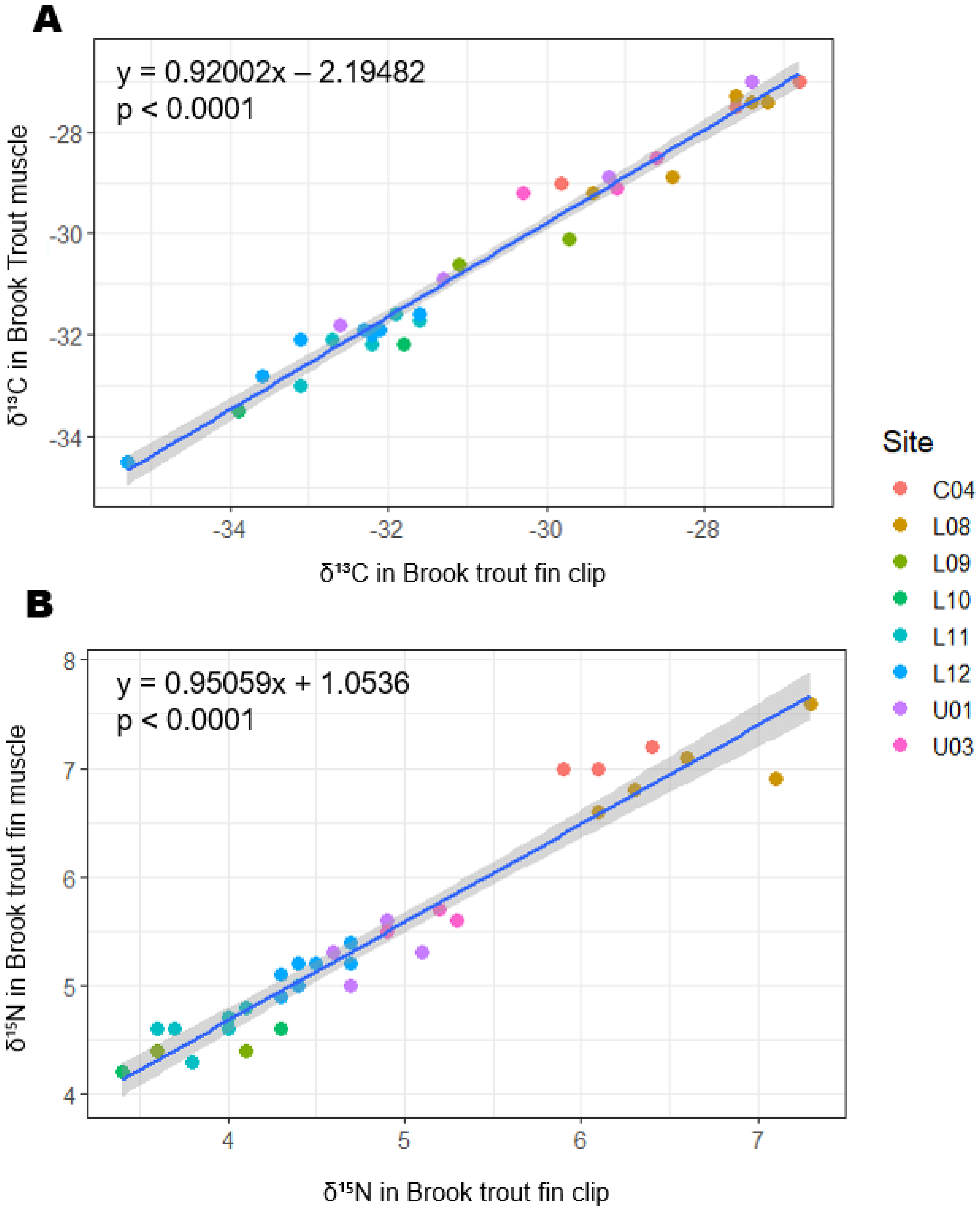


Figure A4: Linear relationships and equations to calculate brook trout muscle (A) δ^{13}C and (B) δ^{15}N values from fin clips in 2019 samples

Table A5: Measurements and isotope data from fish collected in 2019. Isotope values from muscle samples were measured and clip values were calculated based on the equations $\delta^{15}N_{muscle} = 0.9059 \times \delta^{15}N_{fin} + 1.0536$ and $\delta^{13}C_{muscle} = 0.9200 \times \delta^{13}C_{fin} - 2.1948$.

Site	Species	Sample Type	Length (cm)	Weight (g)	C:N Ratio	$\delta^{13}C$	$\delta^{15}N$
U01	Brook trout	Muscle	4.6	0.76	3.5	-30.9	5.0
U01	Brook trout	Muscle	4.7	1.33	3.4	-31.8	5.3
U01	Brook trout	Muscle	5.4	1.22	3.7	-28.9	5.3
U01	Brook trout	Muscle	8.4	4.97	3.4	-27.0	5.6
U01	Brook trout	Clip	11.4	14.35	4.1	-27.2	7.1
U01	Brook trout	Clip	12.7	16.9	4.0	-27.3	6.2
U01	Brook trout	Clip	13	19.62	3.8	-28.1	6.4
U01	Brook trout	Clip	13	15.6	4.0	-27.8	6.4
U01	Brook trout	Clip	19.6	70.71	3.5	-26.9	7.4
U01	Brook trout	Clip	19.6	70.93	3.5	-28.3	7.8
U02	Brook trout	Clip	11.4	12.08	4.0	-30.3	6.3
U02	Brook trout	Clip	11.4	11.35	4.0	-30.8	6.2
U02	Brook trout	Clip	11.4	11.9	3.9	-29.8	6.3
U02	Brook trout	Clip	12	14.19	4.0	-30.3	6.5
U02	Brook trout	Clip	12	14.73	4.0	-29.8	6.5
U02	Brook trout	Clip	14.2	28	3.5	-29.1	6.5
U02	Brook trout	Clip	14.4	24.53	3.6	-29.2	6.7
U02	Brook trout	Clip	16.1	38.4	3.6	-29.3	6.3
U02	Brook trout	Clip	16.3	37.1	3.8	-29.3	6.5
U02	Brook trout	Clip	18.2	54.38	3.4	-28.0	6.6
U03	Brook trout	Muscle	7.8	3.97	3.6	-29.1	5.6
U03	Brook trout	Muscle	9.4	6.11	3.3	-29.2	5.7
U03	Brook trout	Muscle	9.6	8.79	3.5	-28.5	5.5
U03	Brook trout	Clip	10.6	9.61	3.9	-29.0	7.1
U03	Brook trout	Clip	12.6	20.73	3.7	-27.3	6.3
U03	Brook trout	Clip	13.8	20.06	3.6	-27.2	7.5
U03	Brook trout	Clip	14.2	27.65	3.7	-27.7	6.4
U03	Brook trout	Clip	14.6	24.85	3.6	-27.1	6.1
C04	Brook trout	Muscle	9.2	7.6	3.3	-27.5	7.2
C04	Brook trout	Muscle	9.5	6.5	3.5	-29.0	7.0
C04	Brook trout	Muscle	9.5	7.74	3.4	-27.0	7.0
C04	Brook trout	Clip	10.4	9.24	4.1	-27.2	7.3
C04	Brook trout	Clip	10.6	15.8	3.9	-28.0	6.5
C04	Brook trout	Clip	10.8	11.76	3.8	-28.8	6.5
C04	Brook trout	Clip	11.9	13.55	3.9	-27.1	6.8
C04	Brook trout	Clip	14	22.9	3.8	-27.8	6.9
C04	Brook trout	Clip	15.2	31.26	3.4	-26.6	8.0
C04	Brook trout	Clip	18.5	66.4	3.3	-25.1	7.6
L08	Brook trout	Muscle	8.4	5.25	4.0	-30.0	6.9

L08	Brook trout	Muscle	8.4	4.98	3.3	-28.9	6.9
L08	Brook trout	Muscle	9.5	8.01	3.4	-29.2	6.6
L08	Brook trout	Muscle	9.5	6.55	3.6	-27.4	7.1
L08	Brook trout	Muscle	9.6	7.52	3.5	-27.4	6.8
L08	Brook trout	Muscle	9.7	8.58	3.5	-27.3	7.6
L08	Brook trout	Clip	10.8	11.24	4.0	-29.3	7.7
L08	Brook trout	Clip	11.3	15.07	3.9	-27.7	8.0
L08	Brook trout	Clip	12.6	16.55	3.5	-25.4	7.7
L08	Brook trout	Clip	13.2	23.05	3.6	-26.1	7.8
L09	Brook trout	Muscle	5.4	1.39	3.5	-30.1	4.4
L09	Brook trout	Muscle	6.8	2.24	3.3	-30.6	4.4
L09	Brook trout	Clip	16.4	42.69	3.9	-26.2	5.3
L10	Brook trout	Muscle	5.7	1.51	3.6	-33.5	4.2
L10	Brook trout	Muscle	6.5	2.18	3.4	-32.2	4.6
L10	Brook trout	Clip	12.8	15.72	3.8	-29.2	5.7
L10	Brook trout	Clip	18.6	61.28	3.5	-26.3	6.7
L11	Brook trout	Muscle	5.4	1.4	3.4	-31.6	4.7
L11	Brook trout	Muscle	6	1.92	3.5	-31.7	4.6
L11	Brook trout	Muscle	6	2.03	3.4	-31.9	4.6
L11	Brook trout	Muscle	6.1	1.59	3.4	-32.1	4.8
L11	Brook trout	Muscle	6.2	1.82	3.6	-33.0	4.6
L11	Brook trout	Muscle	6.4	2.23	3.6	-32.2	4.3
L11	Brook trout	Clip	10.8	13.88	3.7	-29.1	6.5
L11	Brook trout	Clip	12.6	19.9	3.5	-27.8	6.7
L11	Brook trout	Clip	12.8	20.95	3.7	-30.2	6.3
L12	Brook trout	Muscle	5.2	1.36	3.4	-32.8	5.2
L12	Brook trout	Muscle	5.2	1.33	3.4	-34.5	4.9
L12	Brook trout	Muscle	5.2	1.24	3.4	-32.1	5.1
L12	Brook trout	Muscle	5.4	1.28	3.5	-32.0	5.2
L12	Brook trout	Muscle	5.6	1.51	3.4	-31.9	5.2
L12	Brook trout	Muscle	5.8	1.36	3.5	-31.6	5.4
L12	Brook trout	Muscle	6.2	2.03	3.5	-31.9	5.0
L12	Brook trout	Clip	12	15.35	3.8	-30.7	6.4
U01	Slimy sculpin	Muscle	4.2	0.64	4.9	-37.5	4.8
U01	Slimy sculpin	Muscle	4.5	0.84	4.6	-36.3	5.0
U01	Slimy sculpin	Muscle	4.5	0.98	4.0	-34.7	5.4
U01	Slimy sculpin	Muscle	4.5	0.78	4.0	-35.4	5.0
U01	Slimy sculpin	Muscle	4.6	0.94	4.4	-37.3	4.6
U01	Slimy sculpin	Muscle	4.7	0.96	4.2	-35.0	5.2
U01	Slimy sculpin	Muscle	4.7	1.03	4.0	-35.4	5.2
U01	Slimy sculpin	Muscle	5.5	1.43	4.0	-37.0	4.9
U01	Slimy sculpin	Muscle	8.2	5.67	3.7	-36.6	5.6
L10	Slimy sculpin	Muscle	4.8	1.24	4.4	-39.5	4.6
L10	Slimy sculpin	Muscle	5	1.26	4.1	-39.0	4.5

L10	Slimy sculpin	Muscle	5.3	1.47	4.5	-39.6	4.4
L10	Slimy sculpin	Muscle	6	2.26	3.8	-36.8	5.1
L10	Slimy sculpin	Muscle	7.6	5.32	4.9	-40.0	4.8
L10	Slimy sculpin	Muscle	8.5	7.82	4.0	-37.5	5.3

Table A6: Measurements and isotope data from fish collected in 2020

Site	Species	Length (cm)	Weight (g)	C:N ratio	δ^{13}C	δ^{15}N
U01	Brook trout	4.5	0.7	3.4	-28.7	5.6
U01	Brook trout	4.8	1.3	3.4	-29.2	5.8
U01	Brook trout	5.4	1.6	3.6	-30.2	5.6
U01	Brook trout	5.8	1.7	3.5	-27.4	5.8
U01	Brook trout	7.2	3.6	3.4	-27.3	6.3
U01	Brook trout	7.9	5.2	3.4	-27.2	6.7
U01	Brook trout	10.8	13.1	3.3	-26.6	6.8
U01	Brook trout	12.7	20.7	3.3	-25.6	6.2
U01	Brook trout	19	62.4	3.2	-26.3	6.4
U02	Brook trout	4.7	0.9	3.5	-30.8	5.4
U02	Brook trout	4.8	0.8	3.4	-31.5	5.5
U02	Brook trout	4.8	0.9	3.4	-30.3	5.5
U02	Brook trout	6.4	2.5	3.4	-30.4	6.4
U02	Brook trout	7.8	3.6	3.4	-28.9	6.1
U02	Brook trout	8.2	4	3.5	-30.6	6.5
U02	Brook trout	10.6	11.3	3.3	-29.8	6.6
U02	Brook trout	12.7	19.3	3.3	-28.4	6.8
U02	Brook trout	13.8	21.1	3.3	-28.1	6.7
U03	Brook trout	4.2	0.6	3.5	-30.1	6.0
U03	Brook trout	4.5	0.8	3.4	-27.9	5.5
U03	Brook trout	5.5	1.5	3.6	-30.0	5.9
U03	Brook trout	6	1.5	3.5	-29.2	6.1
U03	Brook trout	7	3.5	3.4	-29.9	6.4
U03	Brook trout	8.4	4.2	3.3	-27.1	6.4
U03	Brook trout	9.8	7.2	3.3	-28.4	6.7
U03	Brook trout	16.8	37.3	3.2	-26.3	5.5
U03	Brook trout	19.8	74.4	3.3	-26.7	6.1
C04	Brook trout	4.8	1	3.3	-27.9	6.8
C04	Brook trout	4.8	0.8	3.3	-29.5	6.5
C04	Brook trout	5.8	1.6	3.4	-28.5	6.6
C04	Brook trout	6.8	2.9	3.4	-28.6	7.0
C04	Brook trout	8	4.9	3.3	-28.2	7.0
C04	Brook trout	9	6.1	3.4	-27.1	7.6
C04	Brook trout	14.8	28.2	3.3	-27.6	7.4
C04	Brook trout	15.8	40.3	3.2	-26.6	7.8
C04	Brook trout	16.8	42.3	3.4	-27.3	7.5
L08	Brook trout	4.8	1.1	3.4	-29.5	6.6

L08	Brook trout	5.2	1.1	3.4	-31.1	6.4
L08	Brook trout	6.4	2.6	3.4	-30.7	6.7
L08	Brook trout	7.8	3.8	3.4	-30.9	6.7
L08	Brook trout	8.4	4.2	3.3	-28.4	7.0
L08	Brook trout	9.6	6.6	3.4	-30.0	7.4
L08	Brook trout	11.5	11.7	3.3	-28.1	7.4
L08	Brook trout	11.8	12.7	3.2	-26.9	7.4
L09	Brook trout	5	1.8	3.4	-29.6	5.4
L09	Brook trout	5.4	1.8	3.4	-28.3	5.1
L09	Brook trout	6.2	2.2	3.6	-29.1	5.1
L09	Brook trout	6.6	2.1	3.4	-29.8	4.9
L09	Brook trout	7	2.7	3.4	-28.5	5.0
L09	Brook trout	11	13.6	3.3	-27.3	5.7
L09	Brook trout	11.4	14.6	3.3	-26.5	6.1
L09	Brook trout	11.8	13.3	3.3	-26.8	5.4
L10	Brook trout	4.9	1.1	3.3	-32.2	4.8
L10	Brook trout	5.5	1.4	3.5	-32.8	4.6
L10	Brook trout	5.9	1.9	3.3	-28.5	5.0
L10	Brook trout	6.9	3.2	3.5	-31.8	4.7
L10	Brook trout	8.3	6	3.3	-29.6	6.0
L10	Brook trout	10.9	14.6	3.3	-29.0	6.0
L11	Brook trout	4.9	1.4	3.3	-33.6	4.2
L11	Brook trout	5.1	1	3.4	-32.1	4.8
L11	Brook trout	5.2	2	3.3	-33.0	4.1
L11	Brook trout	6.2	2.3	3.6	-31.9	4.5
L11	Brook trout	8.2	5.9	3.3	-29.3	5.8
L11	Brook trout	8.8	5.2	3.4	-31.7	5.5
L11	Brook trout	10	7.8	3.3	-30.4	5.7
L11	Brook trout	10.2	7.5	3.3	-30.4	6.1
L11	Brook trout	14.7	27.5	3.2	-29.1	5.7
L12	Brook trout	4.5	0.7	3.4	-32.0	5.5
L12	Brook trout	4.7	1.1	3.5	-32.0	5.3
L12	Brook trout	4.9	1	3.5	-31.8	5.2
L12	Brook trout	6.3	2.3	3.5	-33.3	5.1
L12	Brook trout	8	4.2	3.4	-30.0	6.5
L12	Brook trout	8.4	6.1	3.5	-32.1	5.9
L12	Brook trout	9.3	6.2	3.4	-30.7	6.3
L12	Brook trout	9.6	7.8	3.4	-33.2	5.8
L12	Brook trout	12.1	14.7	3.4	-31.3	6.2
U01	Slimy sculpin	4.6	1	3.8	-34.1	5.6
U01	Slimy sculpin	5.3	1.2	3.5	-34.7	5.4
U01	Slimy sculpin	5.8	2	3.4	-32.1	5.6
U01	Slimy sculpin	6	1.7	3.4	-35.8	5.1
U01	Slimy sculpin	6.7	3.2	3.5	-34.3	4.9

U01	Slimy sculpin	7.2	4.3	3.6	-33.8	5.7
U01	Slimy sculpin	9.2	8.3	3.3	-30.5	6.6
U01	Slimy sculpin	9.5	8.2	3.5	-35.0	6.0
U02	Slimy sculpin	7.6	3.5	3.4	-34.2	5.2
U02	Slimy sculpin	10	10.2	3.4	-33.8	5.5
L10	Slimy sculpin	4.9	1.1	3.7	-38.6	4.7
L10	Slimy sculpin	5	1.8	3.9	-37.5	5.1
L10	Slimy sculpin	5.5	1.7	3.6	-38.2	5.0
L10	Slimy sculpin	5.8	2	3.5	-37.2	4.9
L10	Slimy sculpin	6	2.1	4.2	-39.0	5.0
L10	Slimy sculpin	6.9	2.9	3.8	-37.7	5.1
L10	Slimy sculpin	7	3.5	3.7	-38.3	4.8

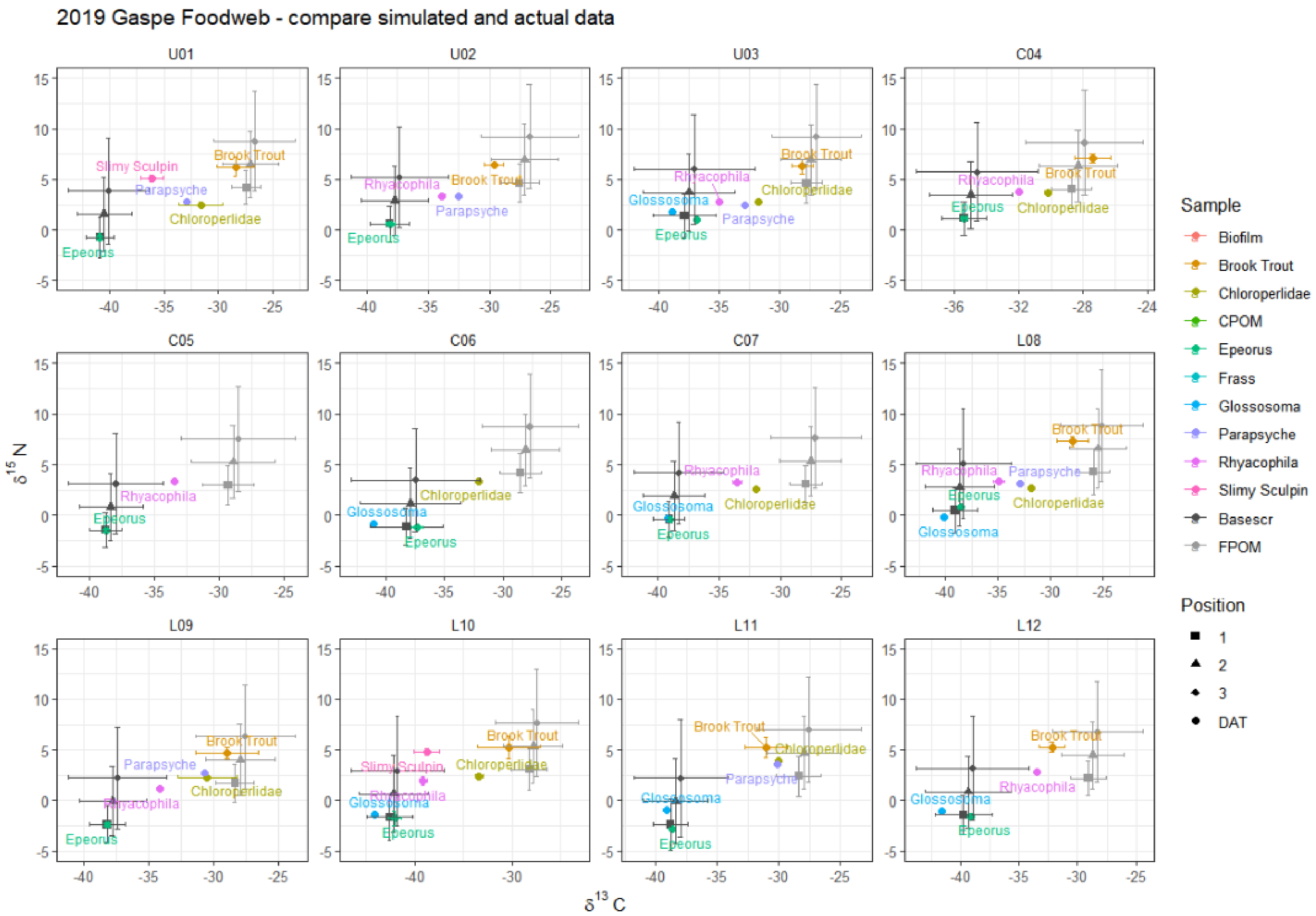


Figure A5: Compare simulated trophic enrichment factors (black: autochthonous, grey: allochthonous) and measured data for 2019 food webs

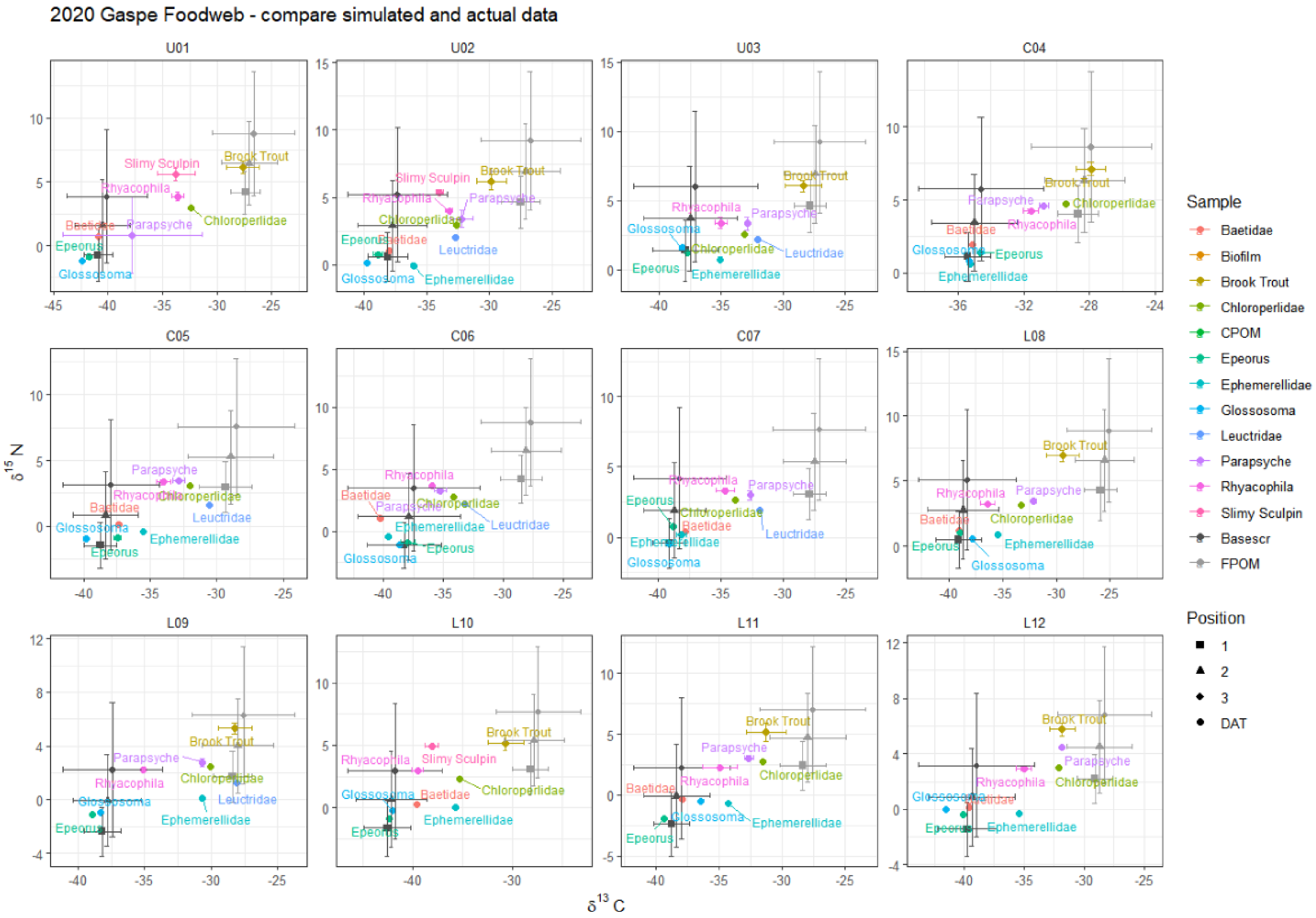


Figure A6: Compare simulated trophic enrichment factors (black: autochthonous, grey: allochthonous) and measured data for 2020 food webs

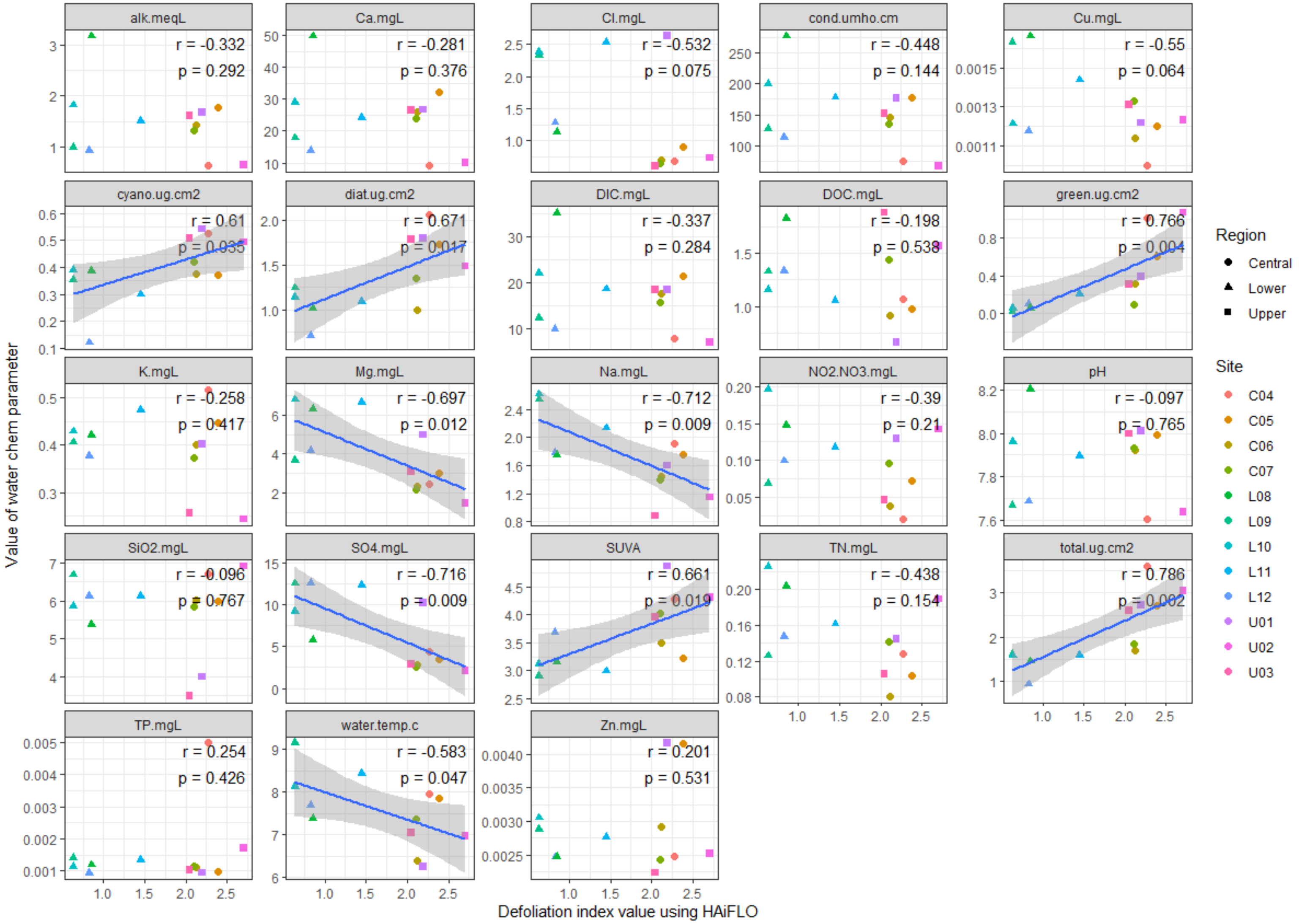


Figure A7. Correlation between water chemistry (n=8) or benthic production (n=6) and 2019 watershed defoliation levels. Each point represents the average measurement at each site from biweekly measurements in June – October

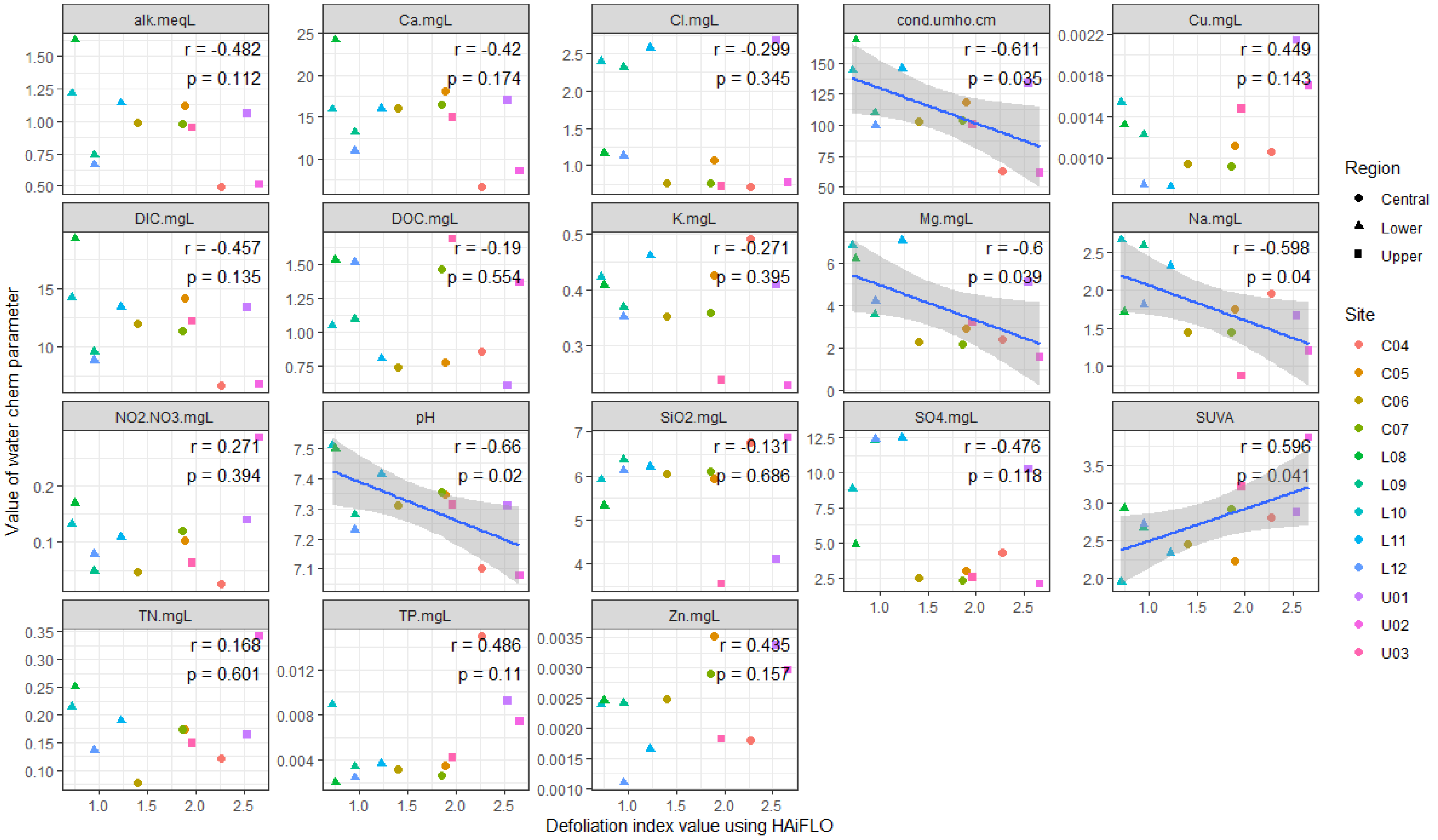


Figure A8. Correlation between water chemistry (n=10) and 2020 watershed defoliation levels. Each point represents the average measurement at each site from biweekly measurements in June – November

Appendix B

Table B1. ANCOVA results comparing slimy sculpin and brook trout THg concentrations at different lengths and sites

	Slimy sculpin				**Brook trout**			
	2019		**2020**		**2019**		**2020**	
	F-value	**p-value**	**F-value**	**p-value**	**F-value**	**p-value**	**F-value**	**p-value**
$\log_{10}$(Length)	35.73807	<0.0001	9.79761	0.007971	4.243635	0.05	142.0913	<0.0001
Site	7.370556	0.02	0.133357	0.876334	17.86665	<0.0001	21.34725	<0.0001

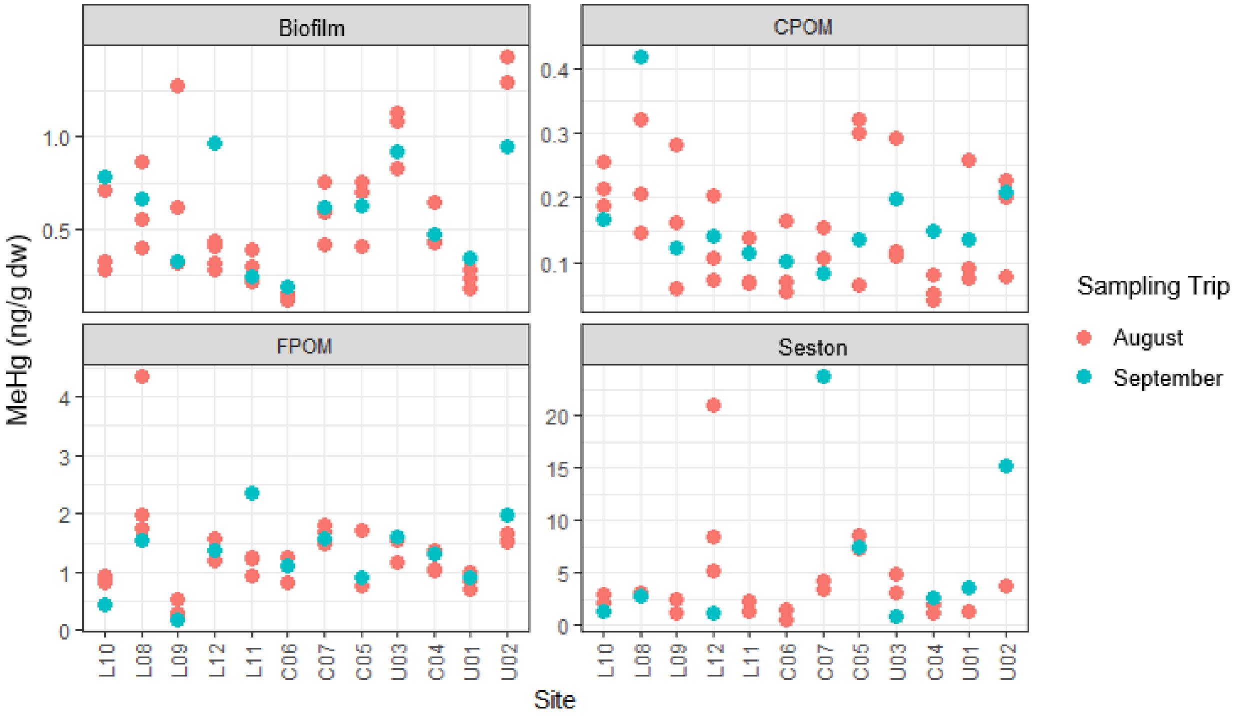


Figure B1. Comparison of MeHg concentrations in food source (biofilm, CPOM, FPOM, seston) samples collected in August (n=2-3/site) and September (n=1/site) from the Gaspé Peninsula in 2020.

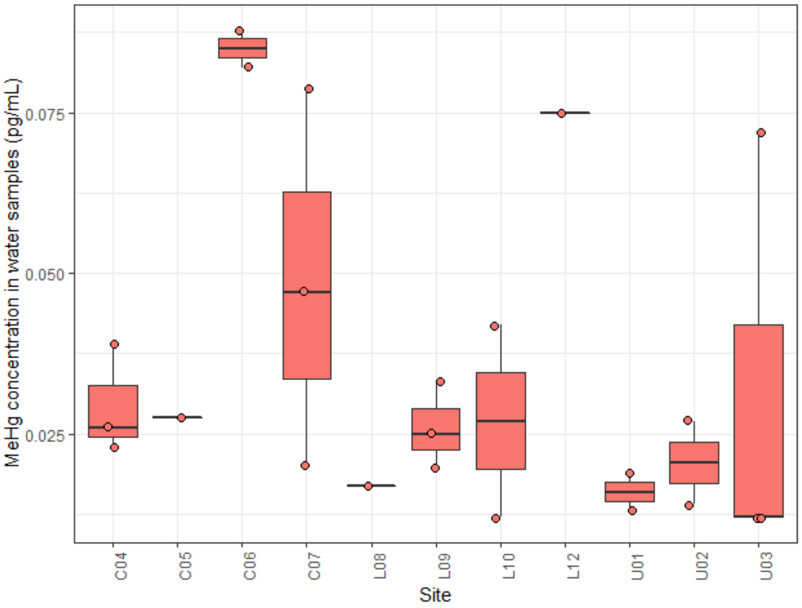


Figure B2. MeHg concentrations in water samples collected from the Gaspé Peninsula in 2021. Each point represents an individual sample. Samples below detection limit are not displayed.

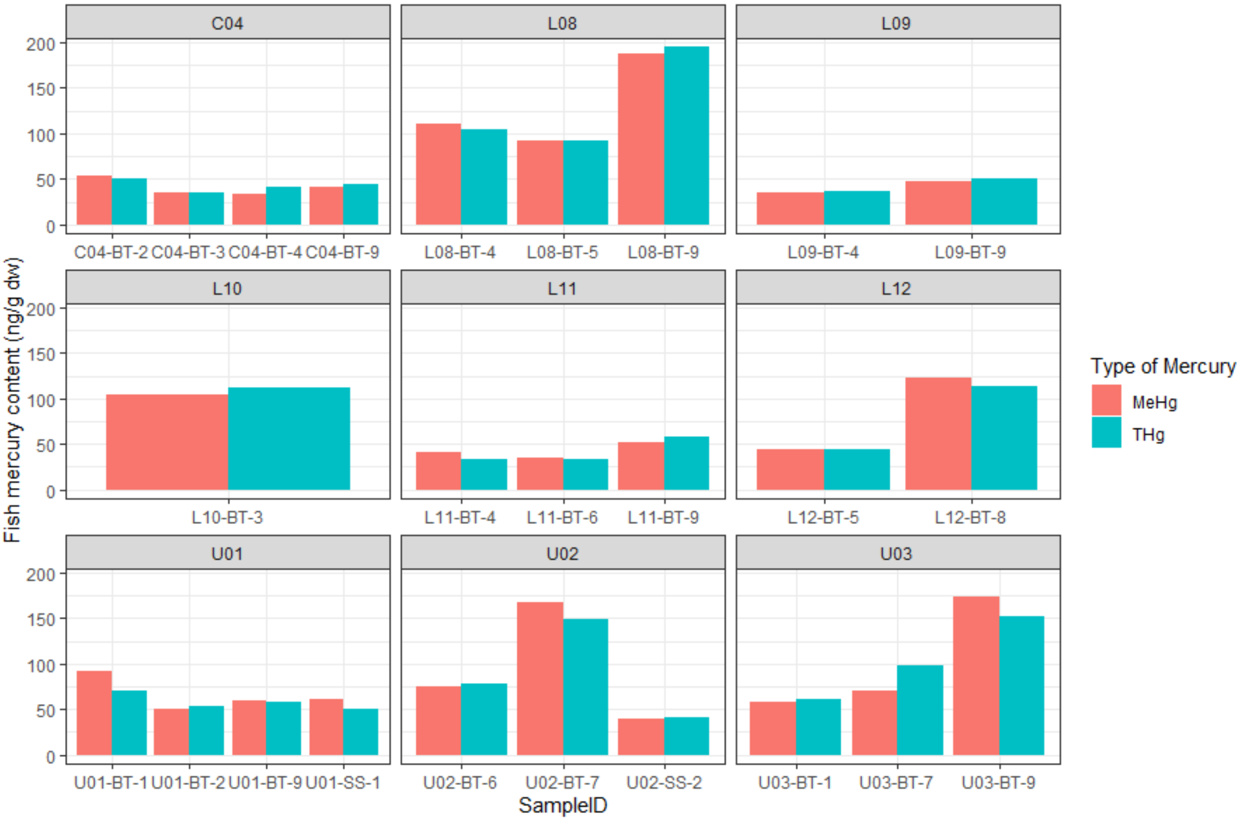


Figure B3. Comparison of MeHg and THg concentrations (ng/g dw) in muscle fillet samples from fish collected in the Gaspé Peninsula in 2020.

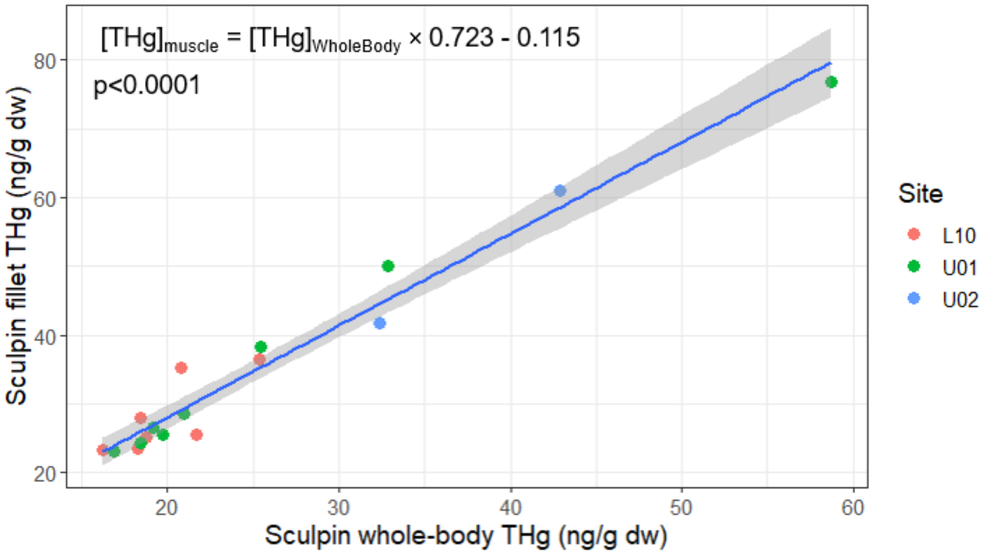
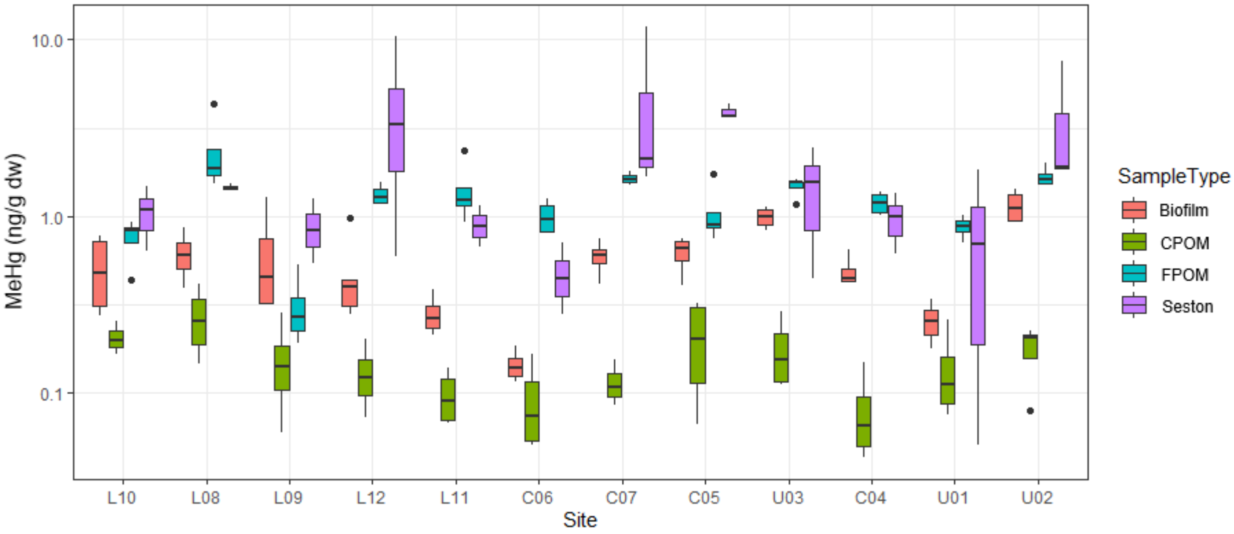
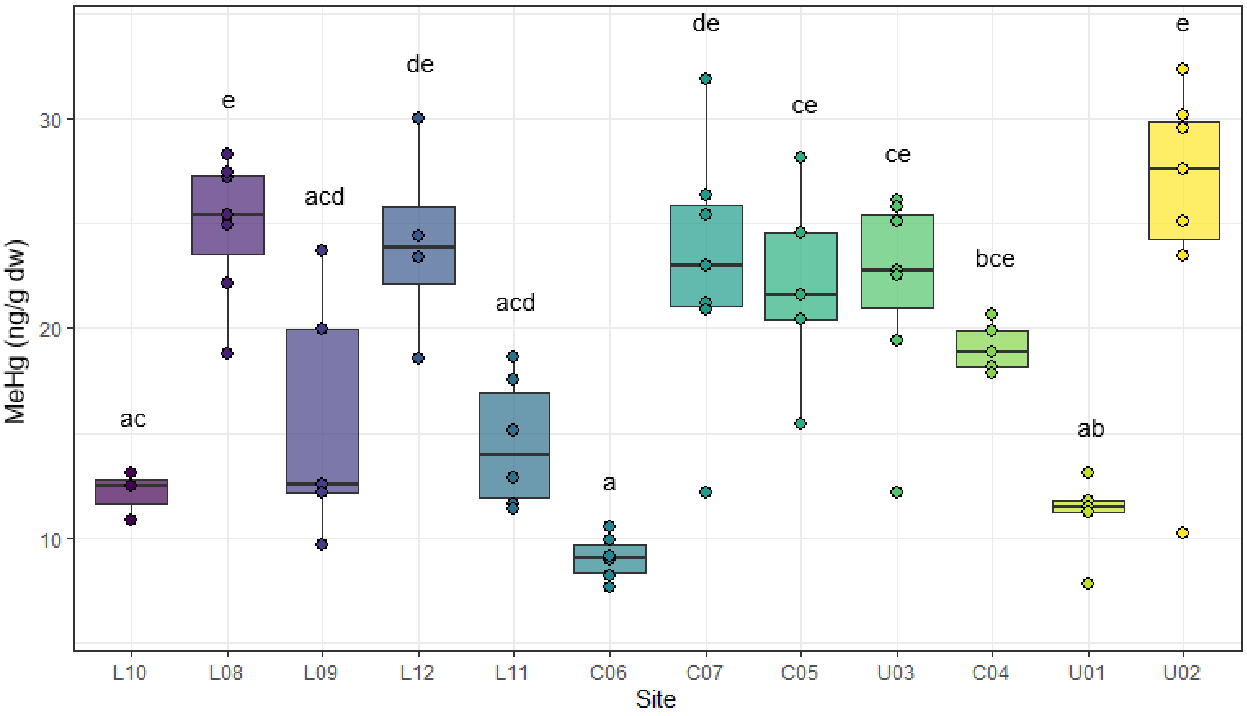


Figure B4. Linear relationships and equations to calculate slimy sculpin muscle THg concentrations from whole-body concentrartions.



Sites on x-axis are in relative order of defoliation

Figure B5. Boxplots of MeHg (ng/g dw) food source samples (biofilm, CPOM, FPOM, seston) collected in the Gaspé Peninsula in 2020.



Sites on x-axis are in relative order of defoliation

Figure B6. Boxplots of carnivorous invertebrates (*Rhyacophila*, Chloroperlidae, *Parapsyche*). Each point represents an individual sample. Letters denote significant differences between sites based on pairwise Tukey comparisons.

Table B2. Two-way ANOVA comparing MeHg concentrations from different food source samples (biofilm, CPOM, FPOM, seston) and sites

	df	**F value**	**P-value**
Site	11	0.072	0.13
Sample Type	3	27	<0.0001
Site:SampleType	33	1.8	0.00087
Residuals	129		

Table B3. Results of Tukey post-hoc contrasts to compare MeHg concentrations in different food sources

Contrast	**difference**	**Lower CI**	**Upper CI**	**Adjusted p-value**
CPOM - Biofilm	-0.41	-1.7	0.84	0.83
FPOM - Biofilm	0.70	-0.54	1.9	0.45
Seston - Biofilm	4.2	2.8	5.6	<0.0001
FPOM - CPOM	1.1	-1.5	2.3	0.11
Seston - CPOM	4.6	3.2	6.0	<0.0001
Seston - FPOM	3.5	2.1	4.9	<0.0001

Table B4. Two-way ANOVA comparing THg_{size} concentrations in Brook trout among sites and years (2019 and 2020)

	df	F value	P-value
Site	8	14	0.00098
Year	1	0.29	0.61
Residuals	7		

Table B5. Average (range) of THg (ng/g dw) and length (cm) measurements for fish caught in the Gaspé Peninsula in 2019

Species	Site	Mean (range) THg (ng/g dw)	Mean (range) length (cm)	n
Brook trout	C04	45.6 (43.3 - 48.1)	9.4 (9.2 - 9.5)	3
Brook trout	L08	171 (113 - 260.5)	9.2 (8.4 - 9.7)	6
Brook trout	L09	55.1 (45.2 - 65.1)	6.1 (5.4 - 6.8)	2
Brook trout	L10	29.6 (19.8 - 42.8)	6.6 (5.7 - 7.6)	3
Brook trout	L11	31.6 (24.2 - 39.4)	6 (5.4 - 6.4)	6
Brook trout	L12	46.6 (41.6 - 58.3)	5.5 (5.2 - 6.2)	7
Brook trout	U01	42.6 (29.7 - 60.1)	5.8 (4.6 - 8.4)	4
Brook trout	U03	108.6 (83.8 - 131.3)	8.9 (7.8 - 9.6)	3
Slimy sculpin	L10	32.6 (19.6 - 70)	5.7 (4.7 - 8.5)	6
Slimy sculpin	U01	25.1 (16.6 - 40.7)	5 (4.2 - 8.2)	10

Table B6. Average (range) of THg (ng/g dw) and length (cm) measurements for fish caught in the Gaspé Peninsula in 2020

Species	Site	Mean (range) THg (ng/g dw)	Mean (range) length (cm)	n
Brook trout	C04	45.9 (26.3 - 98.9)	9.6 (4.8 - 16.8)	9
Brook trout	L08	124 (84.7 - 195.2)	8.2 (4.8 - 11.8)	8
Brook trout	L09	48.4 (33.3 - 68.3)	8.1 (5 - 11.8)	8
Brook trout	L10	58.9 (31 - 111.5)	7.1 (4.9 - 10.9)	6
Brook trout	L11	39.2 (21.8 - 58.9)	8.1 (4.9 - 14.7)	9
Brook trout	L12	65.6 (39.1 - 113.7)	7.5 (4.5 - 12.1)	9

Brook trout	U01	48.9 (36.3 - 70.7)	8.7 (4.5 - 19)	9
Brook trout	U02	82.3 (44.3 - 148.8)	8.2 (4.7 - 13.8)	9
Brook trout	U03	100.3 (50.6 - 170.3)	9.1 (4.2 - 19.8)	9
Slimy sculpin	L10	28.2 (23.3 - 36.6)	5.9 (4.9 - 7)	7
Slimy sculpin	U01	36.6 (23.1 - 76.7)	6.8 (4.6 - 9.5)	8
Slimy sculpin	U02	51.3 (41.7 - 60.9)	8.8 (7.6 - 10)	2

Curriculum Vitae: Kaiying Sally Ju

BSc. – University of Waterloo

EDUCATION

MSc. Candidate, McMaster University – Kidd Lab (September 2020 – Present)

Completing a research-based project to investigate the effects of spruce budworm defoliation on stream food web structure and mercury cycling in Gaspé, Québec.

- Lead teams of 3-5 researchers during two intensive field seasons to sample remote streams in the Gaspe region. Collected over 500 samples each year (water, leafpacks, base food web sources, invertebrates, fish)
- Laboratory processing includes invertebrate identification using morphological features, preparing samples for stable isotope (carbon and nitrogen) analysis, methylmercury analysis using ICP-MS
- Statistical analyses in R include principal component analysis, linear mixed effects models, Bayesian mixing models, and hierarchical partitioning using data on water chemistry, stable isotopes, landscape variables, and methylmercury concentrations

BSc., University of Waterloo – (September 2013 – May 2020)

Honours Environmental Science (Ecology specialization) with a Minor in Environment and Resource Studies

PROFESSIONAL EXPERIENCE

Teaching assistant, McMaster University (September – April 2022, 3 terms)

- Teaching assistant for Ecotoxicology (65 students, 2 terms) and Introductory Biodiversity, Evolution and Humanity (50 students, 1 term).
- Mentored students on report writing, introductory statistics in Excel, current topics in ecotoxicology, and techniques for science communication

Research Technician, University of British Columbia – Richardson Lab (August – December 2019)

- Sorted and identified 15 years of invertebrate community samples to genus- and species- level classification using Merrit and Cummins (1996) to monitor the role of riparian buffers to streams following forest harvest
- Assisted in setup and collection of invertebrate drift-net samplers in remote alpine streams

Landscape Assessment Science Officer, Canadian Wildlife Service (January – April 2019)

- Conducted statistical analysis in R including t-tests, ANOVA, linear regression, and linear mixed-effects models to report on trends in Great Lakes wetland habitat integrity based on water quality and biotic communities
- Developed an interactive application using R and Shiny to allow ecologists to easily calculate an Index of Ecological Condition for wetland habitats, reducing processing time from 30+ to 2 minutes
- Deployed acoustic recording units in wetlands to monitor communities of breeding birds, which required setup of specialized equipment, and site access by canoe and motorboat using a GPS

Research Technician, University of Waterloo - Servos Lab (May – August 2018)

- Quantified estrogenic compounds in 30 wastewater treatment plants in the Grand River watershed by assisting in wastewater collection, solid phase extractions and conducting Yeast Estrogen Screening assays
- Participated in fish sampling (electrofishing, seine netting), identification, and dissection in the Grand River and tributaries. Processed up to 200 fish during sampling days

Ecotoxicology Research Technician, Environment and Climate Change Canada (September – December 2017)

- Conducted aquatic ecotoxicology tests using *Hyalella azteca*, *Hexagenia* sp., Uniodid mussels (adult and glochidia), and *Tubifex tubifex* to assess the toxicity of emerging organic contaminants
- Assisted with *Hyalella azteca* cultures and recorded mortality and juvenile production
- Worked on a team to collect and dissect freshwater mussels in the Grand River to investigate the toxicity of wastewater effluent

PRESENTATIONS

Joint Aquatic Sciences Meeting – Grand Rapids, Michigan (May 2022)

- 15- minute presentation: "Effects of spruce budworm defoliation on stream food webs and mercury cycling"

Canadian Rivers Institute Day (March 2022)

- Images of Research: "Snacking in the Stream"

Laurentian - Prairie-Northern SETAC Joint AGM (June 2021)

- Twitter poster. *Category winner (Most Engagement)*

Canadian Rivers Institute Day (March 2021)

- 5- minute presentation: "Does a hungry little caterpillar affect mercury cycling in stream food webs?"

McMaster Biology Graduate Research Day (February 2021)

- 5- minute proposal presentation: "Does a hungry little caterpillar affect mercury cycling in stream food webs?" *Category winner*

SCHOLARSHIPS & AWARDS

- NSERC CGS-M – $17,500 (2021 – 2022)
- Canadian Rivers Institute: H.B.N Hynes Scholarship – $1000 (December 2021)

CERTIFICATIONS & COURSES

- Class 2 Backpack Electrofishing (2022)
- Standard First Aid & CPR-C (2021)
- Guide to Stable Isotope Ecology (2021)
- ROM Introductory Fish Identification Workshop (2018)
- Wilderness First Aid (2018)
- Pleasure Craft Operator's Card (2015)
- Ontario Benthic Biomonitoring Network – Basic Training (2015)