THE PRESENCE OF IN-CHANNEL BEAVER IMPOUNDMENTS IN ROCKY MOUNTAIN
STREAMS: IMPLICATIONS FOR DOWNSTREAM FOOD WEBS

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By

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ABSTRACT

North American beavers (*Castor canadensis*) build dams in stream channels, thus creating impoundments that flood surrounding riparian areas. Due to the widely circulating global pool of mercury in the atmosphere, mercury is deposited onto the landscape both near and far from point sources, including areas occupied by beavers. The organic form of mercury, methylmercury, is a potent neurotoxin with potential to cause harm to both humans and wildlife due to its ability to biomagnify up food chains. Recently flooded areas, such as those resulting from beaver impoundments, create ideal environments for the methylation of mercury. These impoundments can release methylmercury to downstream food webs where there is potential for it to be transferred to higher trophic level organisms. Beaver impoundments can also boost productivity in aquatic systems, so increases in mercury may be accompanied by an increase in nutrients and algal and invertebrate biomass. The findings here describe increased concentrations of methylmercury in water, algae, and invertebrates downstream from in-channel beaver dams in the southern Canadian Rockies. There was, however, no significant increase in nutrients or algal and invertebrate biomass downstream from impoundments. An examination of trophic transfer of mercury in these stream systems reveals that uptake is enhanced at low concentrations. The uptake pathway from water to algae is especially important but is attenuated in higher trophic levels due to a small relative difference in trophic level between predators and prey. The overall rate of trophic transfer in these systems falls within the low end of the typical range, and low baseline concentrations mean that methylmercury is not biomagnifying to dangerous levels in these low-productivity mountain systems. Beavers can provide important ecosystem services such as improving landscape heterogeneity, creation of new habitat for invertebrates and fish and improved angling opportunities, but they also enhance mercury export. Therefore, in systems that are mercury-sensitive such as those with low pH or long-lived, slow-

growing predatory fish species, beaver influence should be considered as an important source of methylmercury.

ACKNOWLEDGMENTS

DEDICATION

This thesis is dedicated to my family and my husband. Thank you for your love and support.

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‰	Per mil
Δ_i	Difference between the AIC value of the best model and competing models
$\delta^{13}C$	Carbon 13 stable isotope ratio
$\delta^{15}N$	Nitrogen 15 stable isotope ratio
AIC	Akaike's Information Criterion
AIC_C	Akaike's Information Criterion adjusted for small sample size
AIR	Atmospheric nitrogen
BAF	Bioaccumulation factor
BCF	Bioconcentration factor
BMF	Biomagnification factor
Chl *a*	Chlorophyll-*a*
CRM	Certified reference material
DOC	Dissolved organic carbon
DOLT-4	Dogfish liver tissue
DORM-4	Dogfish muscle tissue
DS	Downstream
ER	Evidence ratio
MeHg	Methylmercury
n/k	Ratio of sample size to number of fitted parameters
PDB	Peedee Belemnite
ppm	Parts per million
TEF	Trophic enrichment factor (also denoted as $\Delta15N$)
THg	Total mercury
TL	Trophic level
TMF	Trophic magnification factor
TMS	Trophic magnification slope
TN	Total nitrogen
TOC	Total organic carbon

TORT-3	Lobster hepatopancreas
US	Upstream
w_i	Akaike weight

CHAPTER 1
1. GENERAL INTRODUCTION

This thesis is composed of a review of the relevant literature, two manuscript-style research chapters (Chapters 3 and 4), and a concluding synthesis chapter. The objective of these chapters is to answer the overall question: Does beaver activity alter downstream food web structure and lead to higher concentrations of methylmercury (MeHg) in biota?

In Chapter 3 (currently a manuscript in review with Environmental Science and Technology, submitted June 2014), I determined if in-channel beaver impoundments result in alteration of downstream food web-available MeHg and greater algal and invertebrate biomass. This was done by measuring MeHg concentrations in water, periphyton and benthic invertebrates above and below impoundments in Rocky Mountain foothills streams. Additionally, benthic chlorophyll a, invertebrate standing stock, and nutrients (total P, total N and dissolved organic carbon) were measured above and below impoundments. Finally, to determine if in-channel impoundments alter the flow of energy to downstream primary consumers, I used carbon stable isotope ratios ($^{13}C/^{12}C$) to determine source reliance.

Chapter 4 examined the transfer of MeHg to higher trophic levels by biomagnification (currently a draft manuscript to be submitted to Archives of Environmental Contamination and Toxicology). This was done by measuring trophic position using nitrogen stable isotope ratios ($^{15}N/^{14}N$), $^{15}N/^{14}N$ vs. bodysize, MeHg vs. $^{15}N/^{14}N$ and MeHg vs. bodysize relationships. Trophic transfer of MeHg was determined by calculating bioconcentration factors, bioaccumulation factors, biomagnification factors, and trophic magnification factors, and used to assess implications for downstream consumers.

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The concluding Synthesis chapter brings together the two research chapters, summarizes my findings, and discusses the overall implications of the research as a whole, with suggestions for future work. All literature cited throughout the thesis is presented in a References chapter immediately following the Synthesis chapter. Additional information will be presented in the Appendix at the end of the document

CHAPTER 2
2. LITERATURE REVIEW

Growth in the global population and corresponding economic activities has left freshwater ecosystems imperiled in many regions of the world due to anthropogenic resource production, overexploitation and pollution. These activities result in worldwide loss of biodiversity (Dudgeon et al. 2006), threatened fisheries (Brander 2007) and systems increasingly burdened with toxic contaminants (Kelly et al. 2010). In western Canada, in particular, resource development is on the rise putting freshwaters at risk. The western provinces have become Canada's economic engine due to resource extraction (oil, gas, and mineral mining activity). With these increased activities come modifications to hydrological systems that were once untouched. The push for development has put many waterways at risk despite the fact that little information about these systems exists. Despite their importance to downstream users (Wolfe et al. 2008), mountain stream ecosystems in western Canada are not well-studied. A growing regional population relies on these ecosystems, largely as a recreational haven. In addition, many of these streams are tributaries to larger systems that provide important freshwater resources to downstream communities across the Canadian prairies. Mountain water supplies are diminishing (Schindler and Donahue 2006); therefore, understanding the implication of human-induced modifications of these waterways and their natural analogues should be a priority to provide information for researchers and managers responsible for the protection of sensitive freshwater ecosystems.

2.1 Ecosystem engineers

Organisms within the natural environment can act as agents of change on large spatial and temporal scales, thus becoming important modifiers of freshwater ecosystems. These organisms are defined as ecosystem engineers – those that directly modulate the availability of resources to other species by causing physical state changes in biotic and abiotic materials (Jones et al. 1994). For example: dreissenid mussels (*Dreissena* sp.) in the Laurentian Great Lakes cause large-scale substrate modification and shifts in nutrient cycling (Coleman and Williams 2002; Hecky et al. 2004); hippos (*Hippopotamus amphibius*), due to their large physical size, create channels between rivers and lagoons during their daily movements, creating fish habitat and modifying the structure of riverbanks and surrounding swampland (McCarthy et al. 1998; Mosepele et al. 2009); and finally, perhaps the most well-known example of ecosystem engineering, and the focus of the current study, is the engineering of waterways by beavers (*Castor* sp.).

2.2 Beaver history and ecology

North American beavers (*C. canadensis*) have always been a source of great significance and debate, both historically during the era of the fur trade, and currently as a species often regarded with contravening opinions. Most recently, beavers have been described in a variety of ways: revered as keystone species (Naiman et al. 1986) and disdained as nuisance animals (Conover et al. 1995; Curtis and Jensen 2004). Preceding the arrival of Europeans, the population of beavers in North America was estimated at 60 to 400 million animals (Seton 1929). Following European settlement, vast overexploitation left beaver populations nearly decimated by the advent of the 20th century after beavers were heavily trapped for their valuable pelts and castoreum (Rosell et al. 2005). This continued exploitation and concomitant

conversion of many wetlands to dry land for human development resulted in near extinction of beavers in North America (Naiman et al. 1988). After coming under protective law in the early 1900s, combined with decreased natural predation (Naiman et al. 1988; Collen and Gibson 2001), beaver populations have been steadily increasing over the past century. The current population is now thought to be 6 to 12 million individuals (Naiman et al. 1988).

As populations increase so do the effects of beaver activity. Beavers impound water because they need access to water with adequate depth to allow the construction of a winter food cache and to ensure the entrance of their lodge or burrow remains underwater. These criteria make small, low gradient streams attractive for beaver to build dams and create impoundments (Collen and Gibson 2001). Landscape changes owing to beaver activity include the alteration of stream and river channels and associated riparian areas, and the creation of wetlands and new habitat for aquatic organisms (Naiman et al. 1986; Westbrook et al. 2011). In addition, dam building changes stream discharge, velocity and gradient, expands flooded soil area and increases retention of sediment and organic matter (Naiman et al. 1988). The population and distribution increase of a species which is able to significantly modify ecosystems clearly generates a considerable amount of scientific interest (Rosell et al. 2005). Where beavers remain undisturbed, their influence can be wide-reaching, affecting a large proportion of the streams in a given drainage network (Naiman et al. 1988). Beavers are generalist herbivores (Rosell et al. 2005), and have the ability to fell large trees for the purpose of impoundment creation; thus, their presence can have a profound impact on standing vegetation biomass. One study noted a 40% decrease in standing biomass around a beaver pond over the course of six years of foraging activity (Johnston and Naiman 1990). Tree-felling activity can make beavers a costly nuisance due to loss of saleable lumber in addition to the flooding of roadways and trail networks. These

changes introduce challenges for land-owners and environmental stewards that have resulted in calls to cull beaver populations and destroy dams.

In both past and recent literature, the effects of beaver activity on waterways have been of significant interest. It is known that beaver-induced stream channel alterations change the way materials flow through streams (Naiman et al. 1986; Naiman et al. 1988). Furthermore, the extent of alteration of stream ecosystem characteristics by removal of beavers long before the advent of modern limnological research remains largely unknown. Thus, much of our understanding of stream ecosystems is derived from sites that lack the influence of this ecologically significant and once-abundant animal (Naiman et al. 1988). It is important to note that there is no such thing as a typical beaver pond (Butler and Malanson 2005). Variation in pond morphology and hydrology drives effects on downstream nutrients, resources and benthic invertebrates (Fuller and Peckarsky 2011). Thus, it is difficult to make predictions about downstream effects of beaver damming activity in streams. To understand the implications of current management strategies for river systems, more information is needed about the ecological outcomes of beaver dam construction and/or removal for downstream waterways.

2.3 Mercury dynamics

Methylmercury (MeHg) has long been a contaminant of global concern, particularly because of its ability to biomagnify through food webs, often resulting in high concentrations in fish and mammals that serve as subsistence for human populations (Mergler et al. 2007). MeHg is reported to increase two- to eight-fold across trophic levels (Lavoie et al. 2013) resulting in dangerous concentrations in piscivorous fish and other wildlife that are commonly consumed (Watras et al. 1998; Ward et al. 2010). MeHg is a potent neurotoxin and can have devastating health effects on both humans and wildlife. MeHg causes central nervous system damage in

mammals, including humans, manifesting itself favorably in the fetal brain due to its ability to cross the placental barrier (Wolfe et al. 1998). This puts young children and developing fetuses particularly at risk; especially those who rely on contaminated local subsistence fisheries and marine mammal populations (Chan et al. 2003). As a result, MeHg contamination is the most frequent cause of human fish consumption advisories around the world (Ward et al. 2010). In Canada, edible portions of fish sold commercially must have concentrations less than 0.5 parts per million (ppm) wet weight. For subsistence consumers, this level is reduced to 0.2 ppm wet weight (Health Canada 2007). Indirect effects of MeHg contamination of fish are also an issue of concern: Avoidance of otherwise-healthy fish consumption by Aboriginal peoples and greater reliance on an unhealthy "Western" style diet can lead to various health problems such as diabetes and cardiovascular diseases (Chan et al. 2003).

While controls can be put in place to limit consumption of MeHg in the diet of humans, for wildlife this is not the case. Species consuming a diet consisting wholly of fish are particularly vulnerable (Ward et al. 2010). In predatory fish and piscivorous birds and mammals, MeHg has been known to cause a range of toxic effects at ecologically relevant concentrations including behavioural, neurological, hormonal and reproductive alterations (Scheuhammer et al. 2007). This has led to the development of protocols for assigning tissue reference guidelines (CCME 1998) to protect some wildlife species that are known consumers of MeHg-contaminated fish. For example, female mink (*Mustella* sp.) are expected to exhibit toxic effects when consuming fish containing more than 0.092 μg/g MeHg wet weight (Environment Canada 2003).

Beaver activity may affect downstream ecosystems through the flooding of landscapes resulting in enhanced MeHg production. Flooding forested areas promotes *in situ* formation of MeHg (Roy et al. 2009b). The increase in beaver dams and flooded forested areas may therefore

favour the production and transfer of MeHg to streams (Roy et al. 2009a). Flooding resulting

from the formation of beaver impoundments creates wetlands – sites with environmental

conditions favourable for the production of MeHg (St. Louis et al. 1994; Roy et al. 2009a).

Over the past 20 years, mercury (Hg) in lakes, reservoirs and wetlands has been

researched extensively, with streams receiving comparatively less consideration (Ward et al.

2010).   Wetland abundance and dissolved organic carbon (DOC) in stream watersheds are

strongly linked to MeHg export and availability (Driscoll et al. 1995; Brigham et al. 2009;

Chasar et al. 2009). MeHg production in hydrologically connected wetlands and transfer to

streams via run-off is likely the predominant source of MeHg to streams (Brigham et al. 2009).

Beavers play an important role in incorporating these wetlands into stream drainage networks.

Driscoll et al. (1995) investigated the role of wetlands in regulating the supply of Hg to

downstream lakes. A stream was sampled above and below a beaver impoundment and water

was found to be enriched in total Hg, MeHg and DOC after transport through the beaver-created

wetland system.

A great deal of attention has been paid to anthropogenic reservoir creation and the

subsequent influence on Hg cycling within the environment; however, little work has been done

to examine the natural version of this phenomenon – the creation of dams and resultant

impoundments by beavers. In nearly all man-made reservoirs created, MeHg concentrations in

top predatory fish have exceeded the allowable guideline for sale and human consumption (St.

Louis et al. 2004). It is known that after the initial flooding of reservoirs, Hg concentrations

increase greatly (St. Louis et al. 2004; Hall et al. 2005). Because beavers impound much smaller

waterways than most human-constructed impoundments, only limited extrapolations can be

made (Fuller and Peckarsky 2011). However, recent work suggests similarities in

biogeochemical processes but at a smaller scale. For example, higher Hg concentrations are

observed in water flowing out of ponds compared with upstream concentrations (Roy et al.

2009a), as are elevated nutrient concentrations and depleted oxygen, SO_4^{2-} and NO_2-NO_3 in

water draining from beaver impoundments, suggesting high heterotrophic microbial activity and

the presence of reducing conditions – key conditions that favor the methylation of Hg. This is

caused by the flooding of large amounts of organic matter which subsequently decompose,

increasing the activity of microorganism respiration and inducing an anoxic environment.

Inorganic Hg can also be released from inundated soil, becoming available for methylation

(Brinkmann and Rasmussen 2010). Sulfate and iron-reducing bacteria, the microbes responsible

for MeHg production (Gilmour et al. 2013), flourish in the anoxic environment created

immediately following reservoir creation (St. Louis et al. 2004). Higher proportions of total Hg

as MeHg have been noted in reservoirs (Montgomery et al. 2000), which makes for greater

potential for bioaccumulation of Hg in these types of systems.

Hg contamination in top predator fish in streams is likely dominated by the amount of

MeHg available for uptake at the base of the food web rather than by differences in fish trophic

position (Chasar et al. 2009). Biofilm and aquatic invertebrates are parts of this basal

community that transfer MeHg from the physical environment to fish; therefore, increases in

MeHg in these lower trophic levels are an important consequence of reservoir formation (Hall et

al. 1998). An experimentally flooded wetland complex studied as a component of the

Experimental Lakes Area Reservoir Project (ELARP) revealed an increase in predatory

invertebrate MeHg following flooding (Hall et al. 1998), likely a function of increased

bioaccumulation in lower trophic level organisms. During a nine year ELARP study of the same

complex, St. Louis et al. (2004) found a large initial stimulation of methylation in peat in the first

few years of flooding which receded to pre-flooding levels after approximately five years.

Methylation activity in open water areas, however, remained elevated leading to high MeHg

concentrations in biota for the entire course of the study suggesting biotic MeHg concentrations

are sustained by only a small source of Hg. Sustained MeHg production in this flooded pond is

not at the scale of that observed in large hydroelectric reservoirs but is more similar to what

would be expected in the smaller-scale case of beaver impoundments. Other studies that have

followed post-impoundment Hg levels have revealed that elevated Hg concentrations in

predatory fish remain for up to three decades before returning to normal background

concentrations (Bodaly et al. 2007). These studies of reservoirs (St. Louis et al. 2004; Bodaly et

al. 2007) along with those of beaver impoundments (Roy et al. 2009b) have indicated that

impoundment age may play an important role in the production and supply of MeHg.

All of these observations point to the potential for enhanced MeHg supply to downstream

ecosystems such as those that may be observed downstream of beaver impoundments. Upon

observing elevated MeHg in water downstream of beaver impoundments, Roy et al. (2009b)

highlighted the need for further research to address the downstream fate of MeHg in food webs.

Therefore, to better understand the consequences of the in-channel construction of beaver

impoundments on downstream fluvial environments, more information about Hg dynamics

within these small-scale systems is needed.

2.4 Dietary carbon source and invertebrate community structure

In addition to altering contaminant flow, beaver-induced stream channel alterations

change the way energy and materials flow through streams (Naiman et al. 1988) and can also

alter invertebrate community structure (Smith et al. 1989; Anderson and Rosemond 2010; Fuller

and Peckarsky 2011). Beaver ponds are believed to enhance algal growth below dams because

they foster the release of nutrients from decaying plant matter. Algae are nutritionally superior to terrestrial carbon sources (leaf litter, wood debris, etc.), so higher algal biomass may lead to higher production of invertebrates that serve as food for fish, thereby providing positive benefits for recreational fisheries. However, dams also enhance the export of DOC (Roy et al. 2009b) that can serve as food and a source of MeHg for filter-feeding insects (Harding et al. 2006). If beavers change these flows of carbon to the food web downstream of a beaver impoundment, there could be important implications for Hg entering the food web from enhanced production in the pond. Because consumers generally obtain Hg from their diet, the availability of Hg to higher predators such as fish may depend largely on the types of invertebrates present and their feeding behaviour. These kinds of interactions make it important to be able to quantify C flows in stream food webs in conjunction with analyses of contaminants (Jardine et al. 2012), something that has never been done for beaver-influenced streams.

Different foraging strategies and habitats of invertebrates influence MeHg bioaccumulation in streams even at relatively small spatial scales (Riva-Murray et al. 2013). Alterations in community structure and functional feeding groups such as algal scrapers, detrital shredders, filterers and collectors could therefore play an important role in facilitating the transfer of MeHg to higher trophic levels. Anderson and Rosemond (2007) found a taxonomically simplified but more productive (based on abundance and biomass) benthic invertebrate community in beaver ponds likely resulting from a decrease in microhabitat heterogeneity. This is because beavers transform streams from an erosional environment to a depositional environment (Butler and Malanson 2005) resulting in greater retention of sediment and organic matter. In addition, a noted shift from lotic to lentic taxa has been observed in beaver pond invertebrate communities (Naiman et al. 1986; Anderson and Rosemond 2007).

These observations have been largely confined to the ponds with little evidence of downstream changes, though increases in abundance of suspension feeding invertebrates downstream of reservoirs and lake-outlets have been observed (Anderson and Rosemond 2010). This potential change in community composition could change carbon (C) and MeHg flows downstream of beaver ponds (Anderson and Rosemond 2010).

2.5 Stable isotopes

Stable isotope analysis is a technique that uses intrinsic chemical signatures of tissues to provide information about energy flow and the diet of organisms (Hobson 1999). Stable isotope ratios of carbon ($^{13}C/^{12}C$), nitrogen ($^{15}N/^{14}N$), oxygen ($^{18}O/^{16}O$), sulfur ($^{34}S/^{32}S$) and hydrogen (H^2/H^1) are now widely used in ecology and ecotoxicology (Jardine et al. 2006; Hobson 1999). Stable isotopes are a useful tool for ecologists because stable isotope ratios in consumers are proportional to ratios in their assumed diet, and differences in isotope ratios exist among dietary sources (Jardine et al. 2006). These characteristics make stable isotope analysis useful in tracing and characterizing sources of energy and contaminants in food webs as well as food web structure

2.5.1 Stable carbon isotopes ($^{13}C/^{12}C$)

An important consideration when studying food web accumulation of a contaminant is the source of dietary C at the base of the food web. C fixation by benthic algal photosynthesis is an important source of fixed energy to consumers occupying higher trophic levels (Hecky and Hesslein 1995). Dietary source information can be ascertained from stable isotope ratios of C, $^{13}C/^{12}C$, because values, while variable at the base of the food chain, are conserved across higher trophic levels thus providing information about the sources of energy (C) to higher consumers (Hecky and Hesslein 1995; Vander Zanden and Rasmussen 1999). This conservation of $^{13}C/^{12}C$

values from fixation at the base of the food web through to higher consumers has been relatively well-studied in lakes where $^{13}C/^{12}C$ gradients have been related to in-shore and open water food webs (Hobson 1999) due to differential fractionation of ^{13}C by benthic algae and pelagic phytoplankton (Hecky and Hesslein 1995).

Fluvial environments offer an additional layer of complexity when attempting to establish C sources. Source delineation in streams is difficult because the range of $^{13}C/^{12}C$ values resulting from photosynthesis in aquatic systems often overlaps the $\delta^{13}C$ of terrestrial plants and aquatic macrophytes (France 1995; Finlay 2001) This makes it challenging to address and quantify the reliance of consumers (i.e. benthic invertebrates) in streams on terrestrial C inputs.

2.5.2 Stable nitrogen isotopes ($^{15}N/^{14}N$)

Along with $^{13}C/^{12}C$, $^{15}N/^{14}N$ can be useful in confirming feeding linkages in aquatic food webs. The habitat-independent, step-wise enrichment of ^{15}N with trophic level (Minagawa and Wada 1984) has been widely used to characterize aquatic food webs. Trophic position estimates are calculated from $^{15}N/^{14}N$ values by interpreting the $^{15}N/^{14}N$ of higher consumers relative to a baseline $^{15}N/^{14}N$ value (Cabana and Rasmussen 1996) with an *a priori* assumption of a trophic fractionation ranging between 2 and 5‰ (Minagawa and Wada 1984; Post 2002).

The relationships that exist among ^{15}N values of primary consumers and various environmental variables have been of particular importance in developing the utility of ^{15}N analysis in aquatic systems. The relationship between ^{15}N and environmental contaminants has long been used as predictors of contaminant biomagnification in aquatic food webs (Kidd et al. 1995). Because ^{15}N increases with trophic level (TL), it can be used to compare Hg biomagnification across systems either as the slope of the regression of log-transformed Hg vs. ^{15}N or log-transformed Hg vs. trophic level (Borgå et al. 2011; Kidd et al. 2012). The use of TL,

rather than ^{15}N, when calculating this relationship is preferred (Borgå et al. 2011). The calculation of a TL value takes into consideration an enrichment factor (increase in ^{15}N from diet to consumer) and assigns discrete TLs to baseline organisms. TL can be calculated as follows:

$$TL_{consumer} = (\delta^{15}N_{consumer} - \delta^{15}N_{baseline})/ \Delta 15N + \lambda \qquad \text{(Eq. 2.1)}$$

Where λ = TL of the baseline organism: 1 for primary producers and 2 for primary consumers; and $\Delta 15N$ is the enrichment factor.

Trophic magnification factors (TMFs), also known as trophic magnification slopes (TMSs) (Lavoie et al. 2013) can be used to calculate an average change in Hg concentration per relative TL, thus representing the average bioaccumulation through the entire food web. The TMF is calculated as the antilog of Hg-TL slope (b) in the following model:

$$LogHg = TL (b) + a \qquad \text{(Eq. 2.2)}$$

$$TMF = 10^b \qquad \text{(Eq. 2.3)}$$

When TMF is equal to 1, the slope of the Hg-TL relationship is 0, leading to the conclusion that Hg does not biomagnify through the food web. When TMF is > 1, the slope is also > 1 and it can be said that Hg biomagnifies through the food web with an average factor of TMF per TL. When TMF < 1, the slope is also < 1 illustrating that Hg is decreasing in concentration with an average factor of TMF per TL (i.e. trophic dilution is occurring). TMFs for Hg are typically > 1 (Borgå et al. 2011; Kidd et al. 2012; Lavoie et al. 2013) but are highly spatially variable.

TMFs can be compared across systems to understand how biomagnification varies with the properties of the ecosystems of interest (Borgå et al. 2011; Lavoie et al. 2013). Should beaver impoundments increase productivity in downstream food webs, the resulting growth on higher quality food by benthic organisms could translate into reduced biomagnification. This is because increased algal growth can reduce the uptake of MeHg by higher trophic level organisms

as the pool of Hg is diluted by a larger amount of biomass (Pickhardt et al. 2002, Lavoie et al. 2013).

The addition of trophic links can enhance the transfer of contaminants to higher trophic levels (Rasmussen et al. 1990; Cabana and Rasmussen 1994; Vander Zanden and Rasmussen 1996). More productive sites are more likely to support additional predators (Thompson and Townsend 2005; Anderson and Cabana 2009). ^{15}N-size relationships for benthic invertebrates may be useful as tools to measure the addition of predators to food webs (Anderson and Cabana 2009) because predators are consistently larger than their prey (Riede et al. 2011). Anderson and Cabana (2009) suggest that the relationship between ^{15}N and body size can be used as an indicator of food web structure alteration by human activity, and thus beaver-induced increases in productivity may also be accompanied by altered ^{15}N-size relationships.

2.6 Summary

Chemical and biological processes at the base of the food web can have considerable influence on Hg concentrations in higher order consumers (Jardine et al. 2013). While the potential for beaver impoundments to increase waterborne Hg downstream has been demonstrated (Roy et al. 2009a,b), the current study aims to address this issue further by bringing together two key components that play a role in driving Hg contamination in fluvial systems: 1) food web availability of Hg and 2) sources of dietary carbon to primary consumers. Small changes in these two components at the base of the food web can amplify into large-scale changes in top predators. There is an increasing amount of evidence that dietary carbon source is an important control on Hg bioaccumulation in streams (Jardine et al. 2012; Riva-Murray et al. 2013). It is already known that beaver activity can substantially change the absolute carbon inputs, standing stock and outputs of C (Naiman et al. 1988). These changes in C flows owing to

beaver activity, then, could translate into a reliance on more in-stream C (autochthonous) or more terrestrially derived (allochthonous) C and result in enhanced or reduced exposure to MeHg.

Evidence suggests that autochthonous C sources accumulate higher concentrations of MeHg relative to allochthonous sources. Riva-Murray et al. (2013) studied a range of small to midsize streams to examine links between dietary C sources and bioaccumulation of MeHg. Shredding invertebrates with enriched ^{13}C associated with the consumption of terrestrial detritus, had lower MeHg concentrations than organisms with more depleted ^{13}C associated with consumption of algae. This is consistent with the findings of Jardine et al. (2012) where autochthonous carbon reliance corresponded with higher Hg in low-pH streams, and Tsui et al. (2009) who reported significantly higher MeHg concentrations in periphyton than in terrestrial organic materials in a California stream ecosystem. The findings of these studies suggest that an increased reliance on autochthonous carbon will translate into higher food web available Hg. In addition, benthic primary production across sites may be important controls on Hg bioaccumulation at small scales (Riva Murray et al. 2013), both through increased exposure but also through growth dilution by organisms feeding on the high-quality algal diet (Pickhardt et al. 2002; Karimi et al. 2007). These small-scale changes within drainage networks are what would be expected if beaver dams release water with high nutrients that stimulate algal growth.

I hypothesize that beaver ponds increase food-web available Hg but also enhance resource availability for the food web. Below beaver dams I expect higher Hg concentrations in all biological compartments, higher nutrient concentrations, more algal growth, and greater densities of invertebrates. These changes will result in more pronounced use of the aquatic food source pathway and longer food chains but lower MeHg biomagnification through the food web.

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I expect that the sum of these processes will have the potential to cause an increased risk to fish-eating wildlife downstream. This risk will be estimated from measured concentrations in predatory insects and extrapolated further up the food chain using calculated TMFs.   However, if the rate of biomagnification is low due to enhanced productivity downstream from beaver dams, then MeHg availability to top predator fish will be limited resulting in little or no risk to piscivorous wildlife and humans.

CHAPTER 3
3. IN-CHANNEL BEAVER IMPOUNDMENTS INCREASE UPTAKE OF
METHYLMERCURY BY ORGANISMS AT THE BASE OF STREAM FOOD WEBS

3.1 Preface

Supply and availability of MeHg at the base of the food web are important determinants of

bioaccumulation potential. Changes in resource availability owing to beaver activity can affect

the availability of MeHg in aquatic systems. While it is known that MeHg can be released into

water downstream from beaver impoundments, there have been no comprehensive studies

examining the availability of this MeHg to organisms. This chapter focuses on the supply of

MeHg and nutrients at the base of stream food webs by presenting an up- vs. down-stream

comparison of concentrations of nutrients, algal and invertebrate biomass and MeHg in water,

algae and invertebrates. [1]

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3.2 Abstract

Beavers (*Castor* spp.) are ecosystem engineers and important modifiers of freshwater

ecosystems. They create impoundments that flood the surrounding landscape and modify the

flow of materials through streams, thus potentially increasing nutrients, productivity and the

availability of toxic MeHg to downstream food webs. Here we quantify food web-available

MeHg in water, periphyton, and invertebrates collected from 15 streams up- and down-stream

from beaver impoundments in the Rocky Mountain foothills of Western Canada. While

nutrients, algal biomass, and total invertebrate standing stock were not significantly elevated

below ponds, MeHg concentrations (average increase of 1.7X) and percent of total Hg that was

MeHg (average increase of 1.3X) consistently increased in all compartments. This demonstrates

that beaver impoundments increase the availability and subsequent uptake of MeHg by basal

food web organisms even if their immediate influence on nutrients and resources is limited. The

findings present important implications for research and management of Hg in stream drainage

networks where beavers are present. Increasing beaver populations will have downstream

consequences for fish and wildlife and ongoing consumption advisories for Hg.

3.3 Introduction

Chemical and biological processes at the base of aquatic food webs can have

considerable influence on Hg concentrations in higher order consumers (Watras et al. 1998;

Chasar et al. 2009). This is because MeHg, a neurotoxin, biomagnifies through aquatic food

webs (Lavoie et al. 2013), resulting in high concentrations in fish and mammals that serve as

subsistence for human populations (Mergler et al. 2007). It is well established that Hg sensitivity

in freshwater aquatic systems is influenced by high DOC (Watras et al. 1998), low pH (Jardine et

al. 2012), and low productivity (Pickhardt et al. 2002). Flooding of uplands through reservoir

creation has been shown to alter many of these influencing factors and lead to higher MeHg

production (Hall et al. 2005). Beavers (*Castor canadensis* and *C. fiber*) also flood substantial

tracts of land through building dams, but how their ponds affect Hg sensitivity is not well

studied. Initial work shows that Hg concentrations in water can be elevated downstream of

beaver dams (Driscoll et al. 1995; Roy et al. 2009a; Roy et al. 2009b). At beaver pond outlets,

MeHg concentrations in stream water can be several-fold higher than at inlets, indicating that

impoundments can serve as significant sources of MeHg to downstream water bodies (Roy et al.

2009a; Roy et al. 2009b). Here we explore, for the first time, how this affects uptake by basal

food web organisms.

Beaver are ecosystem engineers (Jones et al. 1994), and their influence can be wide-

reaching, affecting most headwater streams in a given drainage network (Naiman et al. 1988).

The impoundment of streams by beaver floods local vegetation and creates wetlands (Westbrook

et al. 2006), thus potentially increasing Hg methylation, the key step leading to MeHg uptake and

trophic transfer (St. Louis et al. 2004; Roy et al. 2009a). This is because of substantial

accumulation and subsequent decay of organic matter in ponds leading to enhanced microbial

decomposition which is a precursor for Hg methylation. At the same time, impoundments often

also enhance export of DOC and other nutrients (Driscoll et al. 1995; Naiman et al. 1988; Roy et

al. 2009b), thereby altering the resource base for downstream food webs. Specifically, these

changes can alter invertebrate community structure (Anderson and Rosemond 2010; Fuller and

Peckarsky 2011) as well as food source pathways that are known to influence Hg

bioaccumulation (Jardine et al. 2012).

The predominant source of MeHg to streams is production in wetlands and subsequent

transfer during times of high hydrological connectivity (Brigham et al. 2009). While it is well

known that beaver-induced channel alterations change the way materials flow through streams (Naiman et al. 1986; Naiman et al. 1988), only recently has the enhancement of landscape hydrological connectivity by beaver damming been considered in the Hg literature. These recent findings suggest that beaver dams play an important role in creating ideal conditions for Hg methylation and thus, the subsequent export of that MeHg downstream (Driscoll et al. 1995; Roy et al. 2009b).

Our goal was to characterize the transfer of MeHg into food webs in fluvial systems affected by beaver activity. Here, we examine how elevated Hg concentrations downstream of beaver dams in fluvial systems influences food web availability of Hg and the source of available carbon at the base of the food web. We hypothesized that in-channel beaver impoundments would result in an increase of downstream food web-available Hg and nutrients, and an alteration in basal resources, specifically the flow of C, leading to increased reliance on an aquatic food source and thus greater potential for Hg to enter the food web. To test our hypothesis, we quantified Hg concentrations in biological compartments above and below beaver dams located in oligotrophic Canadian Rocky Mountain streams, as well as nutrient concentrations, algal biomass, invertebrate density, and invertebrate dietary C sources. Since the net effect of these processes ultimately dictates the risk to fish-eating wildlife downstream, this study thus provides key information about Hg bioavailability to the growing body of information regarding the role of ecosystem engineers in the movement of contaminants through fluvial systems.

3.4 Methods

3.4.1 Field sampling

From mid-July to early August 2012, 15 streams with in-channel beaver impoundments were sampled in the Canadian Rocky Mountain and Foothill Regions of Kananaskis Country,

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Alberta (Fig. A-1). Streams and impoundments were identified using aerial photographs (Alberta Parks 2007/2008), a 1:50,000 topographic map series (Natural Resources Canada 2001), through personal communications with Alberta Sustainable Resource Development staff, and by visual identification while surveying the area. Most streams selected were in conifer dominated watersheds at elevations of 1300 m to 1900 m. Sites had limited canopy cover (typically < 20%, Table A-1), were generally high gradient with cobble and gravel substrates, and had a limited accumulation of leaf litter. The flow characteristics of these mountain streams were such that a large influx of water during snowmelt (annual peak flow) compromised the structural integrity of several dams, resulting in a mix of "intact" and "breached" dams encountered during sampling (Fig. A-2). Intact beaver dams were classified as those that were actively holding back water with an intact structure (n = 11) whereas breached dams were classified as those that were damaged, allowing flow to pass through the structure (n = 4).

At each sampling location we collected water, periphyton, leaf litter and aquatic macroinvertebrates immediately above and below the impoundment (i.e. at an inflow and an outflow). Subsurface spot readings of pH, turbidity, conductivity and temperature were taken at the time of water sampling using hand-held probes (Table A-1). Unfiltered water samples were collected for MeHg in sterile flouro-carbon polymer bottles (Hall et al. 2009) using clean techniques and in polyethylene bottles for total nitrogen (TN) and total phosphorus (TP). Samples for DOC were passed through 0.45 μm syringe filters and stored in amber polyethylene bottles. All water samples were frozen at -20^0C until time of analysis. Benthic chlorophyll *a* (chl *a*) samples were obtained by scrubbing algal biomass from a known surface area of stream substrate (usually rock or cobble) and collecting algal material from the resultant slurry on 0.70 μm glass fibre filters prior to freezing. Periphyton was also collected for Hg and stable isotope

analysis by scrubbing the substrate until adequate biomass was obtained. Benthic invertebrates were collected using a 30 cm by 30 cm surber sampler in three by three replicates. At three locations within the study reach, the surber net was placed on the stream bottom at three random locations and the substrate area within the frame was disturbed by hand. All macroinvertebrates were picked from these samples and frozen immediately.

3.4.2 Laboratory analysis

Water samples were analysed for TN, TP, DOC and MeHg using standard techniques. TN and TP samples were analysed according to methods outlined in Parsons et al. (1984), Crumpton et al. (1992), and Bachmann and Canfield (1996). DOC analysis was conducted on an automated Shimadzu TOC-VCPN C and N analyzer. MeHg concentrations in water were measured following distillation (US EPA 2001) on a Tekran 2750 after addition of $C_5H_{12}N_2S_2$ and HCl. Samples were distilled at the University of Regina until ~45 mL had collected in the receiving vial, and were shipped overnight in a cooler with ice to the University of Western Ontario. Samples were analyzed for MeHg immediately upon arrival by CVAFS on a Tekran 2700 analyzer after ethylation with sodium tetraethyl borate (US EPA 2001). Duplicate analysis of 10% of samples, ultra-pure water blanks, and spike recoveries were used as quality assurance/quality control. Duplicate analyses were within 10% of each other and spike recoveries ranged from 98 to 110%.

Due to instrument availability, chl *a* analysis was conducted using both spectrophotometric and fluorometric techniques. From each site, one of the three replicates was analysed on a spectrophotometer following a 24 hour cold ethanol (EtOH) extraction (Wintermans and De Mots 1965) using methods outlined by Bergmann and Peters (1980) and Webb et al. (1992). The remaining two replicates were analysed using a Turner 10AU™

flourometer following a seven minute digestion in 90% EtOH at 80°C. Results for both methods were consistent within sites (Figure A-3).

Samples of periphyton and macroinvertebrates were dried and analysed for total Hg (THg), MeHg, and C and N stable isotopes. Invertebrates were sorted and identified to family level, counted, and weighed prior to drying. Following drying, invertebrates were weighed again and homogenized using a mortar and pestle. Invertebrate biomass was calculated by determining the average total biomass of the three replicates collected at each site. Homogenized samples of taxa dominant across all sites were analysed simultaneously for THg and MeHg. Leaf litter samples were dried and ground and analysed for stable isotopes of C and N and THg but not MeHg concentrations.

MeHg concentrations in periphyton and invertebrate samples were determined by gas chromatography combined with atomic fluorescence spectrophotometry (GC-AFS) on a Brooks Rand Model III (Brooks Rand Labs, Seattle WA, USA) following digestion in 25% KOH/MeOH and ethylation with $NaB(C_2H_5)_4$ (Florida DEP 2012). DOLT-4 (dogfish liver) was used as a certified reference material (CRM). Mean recovery of MeHg in DOLT-4 CRM was 97% (n = 14). The mean coefficient of variation (CV) for analytical replicates for MeHg was 7% (n = 6) and for method replicates was 13% (n = 4). THg was determined following the concurrent calibration for MeHg and Hg(II) and addition of these two species (Liang et al. 1994). Mean recovery of THg in DOLT-4 CRM was 97% (n = 14). The mean CV for analytical replicates for THg was 10% (n = 6) and for method replicates was 11% (n = 4). All Hg data are reported in nanograms per gram (ng/g) dry weight.

THg in leaf litter was determined by Direct Mercury Analysis on a DMA-80 (Milestone, Inc., Shelton, CT). TORT-3 (lobster hepatopancreas) and DORM-4 (dogfish muscle) were used

as CRMs. Mean recovery of THg in the TORT-3 CRM was 97% (n=4) and in the DORM-4

CRM was 95% (n=3).

3.4.3 Stable isotopes

To assess if organic carbon originating from periphyton was the dominant energy

pathway for these stream food webs, we analysed samples for stable isotopes of carbon ($^{13}C/^{12}C$)

and nitrogen ($^{15}N/^{14}N$) (Jardine et al. 2012). Stable isotope ratios are measured by isotope ratio

mass spectrometry and expressed as δ values (e.g. $\delta^{13}C$ and $\delta^{15}N$). The ratio of the raw isotopic

sample (R_{sample}, the ratio of heavy to light isotopes, e.g. $^{13}C/^{12}C$ and $^{15}N/^{14}N$) is compared to a

known standard value ($R_{standard}$) and expressed in per mil units (‰) according to:

$$\delta X = (R_{sample}/R_{standard} - 1) \times 1000 \qquad \text{(Eq. 3.1)}$$

where X is the heavy isotope (e.g. ^{13}C). The standard values are derived from heavy to light

isotope ratios of internationally accepted standards, Peedee belemnite (PDB) for $\delta^{13}C$ and

atmospheric nitrogen (AIR) for $\delta^{15}N$.

One milligram (mg) of homogenized invertebrates and three mgs of periphyton was

weighed into tin capsules and analysed using a PDZ Europa ANCA-GSL elemental analyzer

interfaced to a PDZ Europa 20-20 continuous flow isotope ratio mass spectrophotometer (Sercon

Ltd, Cheshire, UK). Accuracy and precision were measured relative to laboratory standards

considered compositionally similar to the samples analysed and the long term standard

deviations were 0.2‰ for $\delta^{13}C$ and 0.3‰ for $\delta^{15}N$. All standards were calibrated against

National Institute of Standards Technology standard reference materials. Invertebrate $\delta^{13}C$ data

were corrected for lipid content according to methods outlined in Logan et al. (2008). Upon

inspection of carbon to nitrogen ratios, periphyton samples were found to contain a high amount

of carbonate (Jacob et al. 2005). Because carbonate confounds the $\delta^{13}C$ value resulting in a

positive bias, periphyton samples were acidified by adding HCl drop-by-drop (Jacob et al. 2005) and re-analysed a second time, post-acidification, on a Costech ECS4010 elemental analyzer coupled to a Thermo Scientific Delta V mass spectrometer. Precision estimates for both isotope ratios were less than 0.2 ‰ relative to laboratory standards considered compositionally similar to the samples analysed. As expected, post-acidification periphyton $\delta^{13}C$ values were more typical of temperate stream periphyton (Jardine et al. 2012) (Fig. A-4).

3.4.4 Data analysis

Breached and intact sites were not separated in the statistical analyses because of the limited sample size (n = 15). Paired t-tests, with site as the unit of replication, were used to compare up- and down-stream concentrations of nutrients (TN, TP, DOC) and MeHg in water, chl *a*, invertebrate biomass (total and separately for dominant families), and MeHg in periphyton and invertebrates. Data were log-transformed prior to analysis to achieve normality. Statistical analyses were conducted with SYSTAT 13 (Systat Software Inc. 2009).

Invertebrates chosen for up- and down-stream comparisons included primary consumer mayflies (Heptageniidae, Ephemerellidae, Baetidae) and caddisfly and stonefly predators (Rhyacophilidae, Perlodidae), classified according to Merritt and Cummins (1996). Collectively, these five families accounted for 63.7% of biomass across sites. There were no differences in MeHg concentrations among the three families of mayflies (p > 0.05) or the two predatory families (p > 0.05); thus, a mean MeHg value was calculated for each group in each site and used for subsequent up- versus down-stream comparisons. In sites where only one or two of the taxa in each group were present, a mean value was used.

To examine variables that may explain additional variability in MeHg across sites, an information-theoretic approach was used. This analysis was carried out using upstream sites

only. Akaike's Information Criterion (AIC) was used to compare and rank competing regression models to determine the best approximating model. Model sets were developed for each biological compartment in question (water, periphyton, invertebrates) and included variables known or believed to influence MeHg (TN, TP, DOC, pH, conductivity, turbidity, chl *a* and invertebrate biomass). Due to the small sample size and small n/k, where n is the sample size and k is the number of fitted parameters, AIC_C was used. AIC_C values were used to calculate Δ_i, the difference between the AIC_C value of the best model and the value of any other given model. Δ_i was then used to calculate Akaike weights (w_i) and evidence ratios (ER) for each model (Table 3-1). The models are considered as follows: a model with $\Delta < 2$ has very good support, a model with $\Delta = 4$ to 7 has less support but should still be considered, and a model with $\Delta > 10$ has no support (Burnham and Anderson 2002; Symonds and Moussalli 2011). Following creation of model sets, it was determined that no single model was overwhelmingly supported (Akaike weight > 0.9) in three of the four sets; therefore, model averaging was carried out (Burnham and Anderson 2002) to reduce model selection uncertainty. Model-averaged parameter estimates (i.e. in this case, the regression coefficients) and corresponding standard errors were calculated according to Burnham and Anderson (2002). By examining the entire set of candidate models (all models with $\Delta i \leq 7$), it was then possible to determine the relative importance of individual parameters.

3.5 Results

3.5.1 Water chemistry and nutrients

There were no differences in water chemistry or resource availability upstream (US) and downstream (DS) from beaver ponds. All sites had moderately alkaline pH (US mean = 8.1 ± 0.2; DS mean = 8.1 ± 0.2) and low turbidity (US mean = 2.0 ± 3.4 NTU; DS mean = 2.9 ± 3.0 NTU) (Table A-1). Concentrations of nutrients, including TP (US mean = 3.1 ± 3.0 μg/L; DS

mean = 3.7 ± 3.1 µg/L), TN (US mean = 107.1 ± 69.2 µg/L; DS mean = 99.1 ± 59.4 µg/L) and

DOC (US mean = 2.9 ± 2.5 mg/L; DS mean = 3.0 ± 2.5 mg/L) were all low suggesting that these

are nutrient-limited systems (Fig. 3-1). We found no significant increase in TP ($p = 0.124$), TN

($p = 0.314$) or DOC ($p = 0.924$) downstream relative to upstream, nor was there a significant

increase in resource availability below ponds, measured as benthic chl *a* ($p=0.307$). Invertebrate

biomass, whether considered collectively ($p = 0.406$) (Fig. 3-1) or by family ($p > 0.500$ for all

families) was similar, with the exception of Perlodidae which had marginally higher biomass at

US sites ($p = 0.071$).
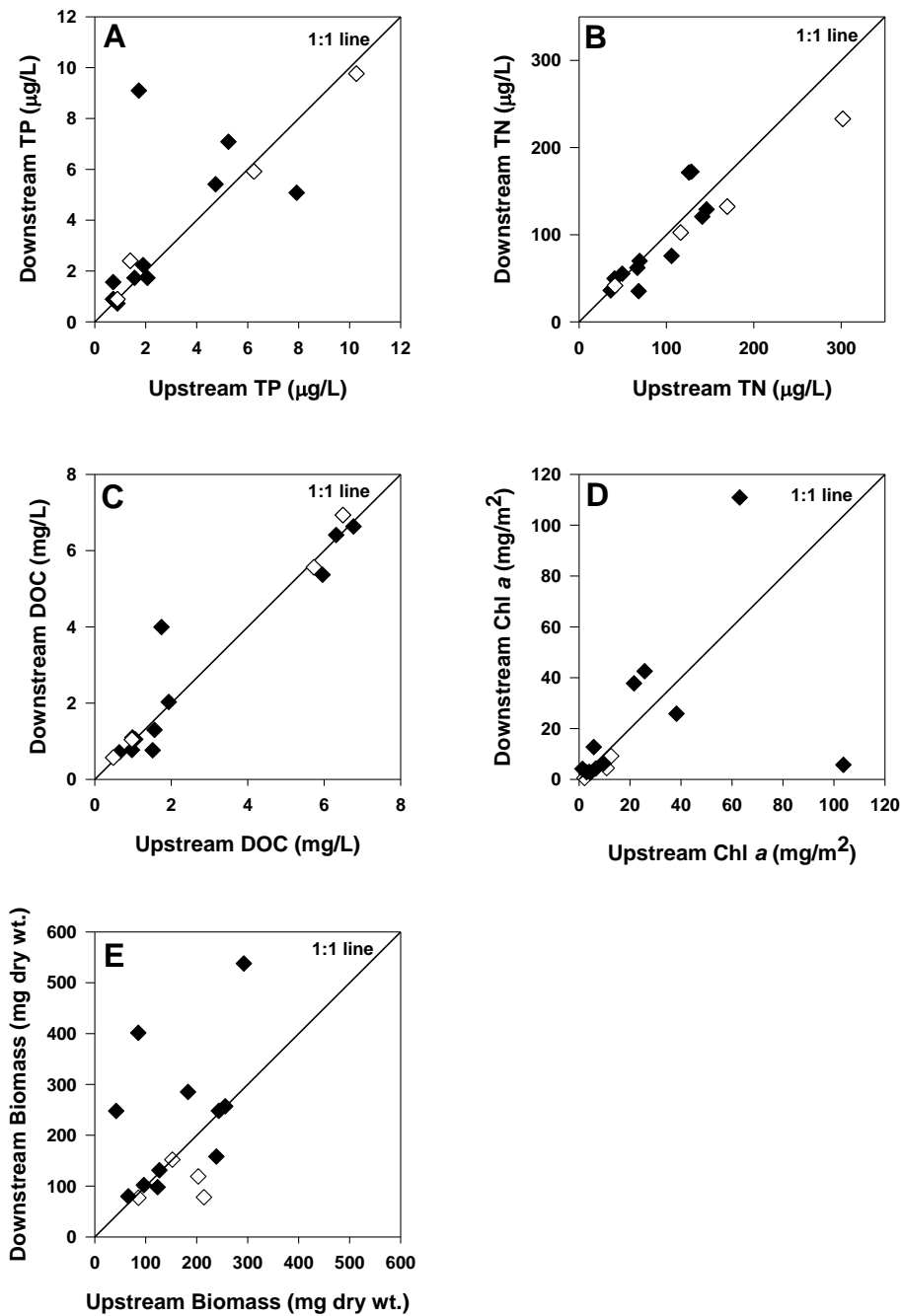
Fig. 3-1 Comparisons of A) TP, B) TN, C) DOC, D) Benthic Chl *a*, and E) Invertebrate Biomass upstream and downstream of beaver ponds in the Rocky mountain foothills, Alberta, Canada. Sites with breached dams are depicted by white symbols while sites with intact dams have black symbols. The 1:1 line represents equivalent upstream and downstream values, thus sites falling above the 1:1 line have greater downstream values and sites falling below the line have greater upstream values.

3.5.2 Mercury

Mercury concentrations were generally low but varied among food web compartments. MeHg in water was low at all sites, with several sites below detection (US n = 9 of 15, DS n = 6 of 15); mean MeHg was 0.03 ± 0.03 ng/L US and 0.04 ± 0.03 ng/L DS. Periphyton MeHg concentrations averaged 1.5 ± 1.1 ng/g US and 2.0 ± 1.7 ng/g DS. Mean herbivore MeHg concentrations were relatively high and covered the largest range of values, averaging 28.3 ± 22.2 ng/g US and 38.4 ± 38.4 ng/g DS. Mean predator MeHg concentrations were lower than herbivores, with averages of 20.4 ± 11.9 ng/g US and 34.2 ± 22.4 ng/g DS.

In contrast to nutrients and resources, beaver impoundments consistently led to increased concentrations of MeHg in water, periphyton, and herbivorous and predatory invertebrates downstream (Fig. 3-2). This increase was significant for predators (p = 0.030) and marginally non-significant for water (p = 0.068), periphyton (p = 0.134) and herbivores (p = 0.067). These values correspond to a 1.8 fold increase in water MeHg concentrations from upstream to downstream and increases of 1.4, 1.6 and 2.0 in periphyton, herbivores and predators, respectively.

The percentage of total Hg as MeHg exhibited a similar trend to overall MeHg concentrations. Mean %MeHg in periphyton was 3.3 ± 1.6 US and 4.6 ± 2.8 DS; in herbivores %MeHg was 26.3 ± 13.3 US and 35.6 ± 17.0 DS; in predators %MeHg was 27.1 ± 18.9 US and 34.1 ± 15.4 DS. As with concentration data, while not all compartments exhibited a significant increase in %MeHg (periphyton p = 0.067; herbivores p = 0.032; predators p = 0.310), all compartments were consistently higher downstream.
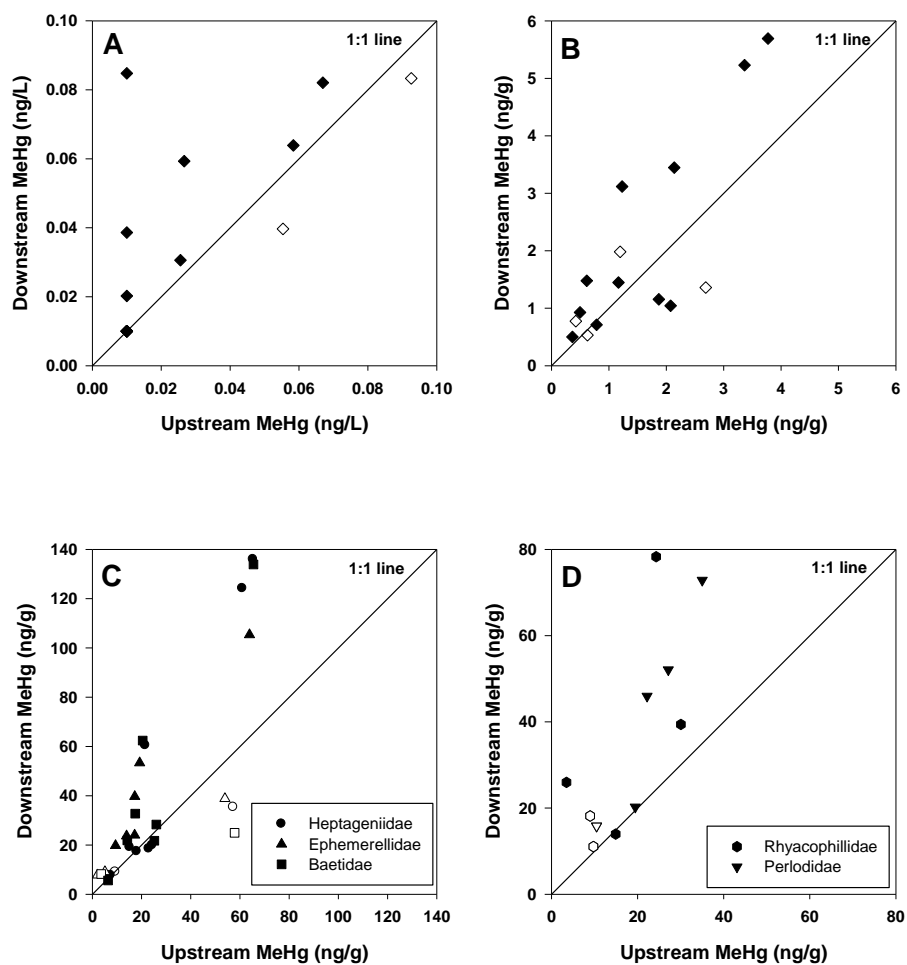
Fig. 3-2 Comparisons of MeHg concentrations in A) Water, B) Periphyton, C) Herbivores, and D) Predators upstream and downstream of beaver ponds in the Rocky Mountain foothills, Alberta, Canada. Symbols as in Fig. 3-1. To increase the number of sites considered, statistical analyses were performed using pooled means for herbivores and predators.

3.5.3 Influence of upstream variables

There was a range of MeHg concentrations observed in all compartments that was not explained by beaver ponds. Model sets derived for the four dependent variables show that higher MeHg concentrations are associated with higher DOC and nutrients (Table 3-1). The main drivers of MeHg in water were DOC and TP ($\Delta < 2$) with averaged parameter estimates of 0.529 (SE = 0.238) and 0.365 (SE = 0.318) respectively, although the intercept-only model (i.e. the "baseline") had a small enough Akaike weight to be considered. Although TN and turbidity were included in several models with $\Delta < 7$, large standard errors associated with their parameter estimates deemed them uninformative. MeHg in periphyton was influenced by TP (averaged parameter estimate = 0.589, SE = 0.220) and DOC (parameter estimate = 0.496, SE = 0.284). TN appeared in several models ranked above the intercept-only model with $\Delta < 7$; however, again the large standard error associated with its averaged parameter estimate (-0.355, SE = 0.444) deemed it uninformative. MeHg in herbivores was influenced by DOC (parameter estimate = 0.769, SE = 0.263) and TP (parameter estimate = 0.477, SE = 0.389). Again, combinations of these two parameters with TN had $\Delta < 7$ and were ranked above the intercept-only model but there was a large standard error associated with its averaged parameter estimate (0.069, SE = 0.560). MeHg in predators was explained by a combination of DOC and invertebrate biomass ($\Delta < 1$), a model which was overwhelmingly supported with the second ranked model having an ER of 1194.5; therefore model averaging was not carried out on this data set.

Table 3-1 Model sets used to account for variation in MeHg concentrations at sites upstream of beaver ponds in Rocky Mountain streams. Δi is the difference between the AIC_c value of the best model and a given competing model (due to small sample size, AIC_c values were used). w_i is the Akaike weight, with the highest ranked model in the set having the highest w_i. The evidence ratios are a measure of likelihood of the best model over competing models (i.e. the highest ranked model in set A is approx. 1.9 times more likely to be the best model than the second model).

Model Set	Model	Δi	w_i	Evidence Ratios
A) Water MeHg	DOC	0.000	0.462	–
	TP	1.301	0.241	1.916
	Intercept-only	2.765	0.116	3.986
	TP, DOC	3.739	0.071	6.485
	TP, TURB	4.232	0.055	8.301
	TP, TN	4.660	0.045	10.279
B) Periphyton MeHg	TP	0.000	0.511	–
	DOC	1.904	0.197	2.592
	TP, TN	2.541	0.143	3.564
	TP, DOC	3.592	0.084	6.027
	TP, TN, DOC	5.858	0.027	18.715
	Intercept-only	6.757	0.017	29.327
C) Herbivore MeHg	DOC	0.000	0.617	–
	TP	2.731	0.157	3.917
	TP, DOC	3.935	0.086	7.155
	TN, DOC	4.044	0.081	7.554
	TP, TN	6.387	0.025	24.374
	Intercept-only	6.501	0.023	25.802
D) Predator MeHg	DOC, Invert Biomass	0.000	0.998	–
	Invert Biomass	14.171	0.000	1194.573
	Invert Biomass, TP	15.702	0.000	2568.457
	DOC	16.341	0.000	3536.605
	Intercept-only	17.387	0.000	5965.839

3.5.4 Stable Isotopes

Results of carbon and nitrogen stable isotope analysis suggest that the algal-based food web is dominant in these systems. $\delta^{13}C$ and $\delta^{15}N$ values of invertebrates aligned more closely with those of periphyton than terrestrial leaf litter, with a slight negative offset relative to both sources (Figure A-5). This is consistent with observations of little canopy cover, limited terrestrial detritus found in the study streams and the absence of shredding macroinvertebrates. Upstream and downstream $\delta^{13}C$ values were very similar, suggesting that despite the presence of beaver impoundments, there was no shift in food web energy pathways. Comparisons of $\delta^{13}C$ revealed no statistical difference up- and downstream of impoundments in the seven compartments with the exception of one of the three grazing mayfly families, Heptageniidae (p = 0.029), which exhibited a slight ^{13}C-enrichment of 1.8‰ upstream compared to downstream (Figure A-5).

3.6 Discussion

Beaver impoundments increased MeHg concentrations in water, periphyton and invertebrates downstream, likely due to increased net methylation within ponds. The changes in MeHg concentrations in higher trophic level consumers occurred despite similarities between up- and downstream TP, TN, DOC, chl a and invertebrate biomass. Our findings contrast with those of Roy et al. (2009b) who observed 2-4 fold increases in DOC, TP and TN at impoundment outlets in boreal streams. The depleted oxygen, SO_4^{2-} and NO_2-NO_3 downstream from impoundments led Roy et al. (2009b) to infer that high heterotrophic microbial activity and the presence of reducing conditions were favoring the methylation of Hg. As with Roy et al. (2009b), we observed increases in %MeHg in periphyton and invertebrates between upstream and downstream sites. However, our concentrations and %MeHg were at the low end of the

range of values reported for streams elsewhere e.g. periphyton 3 to 27% MeHg (Jardine et al 2012) and benthic insects 9 to 95% MeHg (Kidd et al. 2012).

We found no evidence of an increase in nutrient or resource availability downstream of beaver ponds. This finding is in contrast with studies that document an increase in nutrient concentrations downstream of beaver impoundments (Fuller and Peckarsky 2011). However; studies elsewhere in the Rocky Mountains suggest beaver dams enhance nutrient and C storage in these ecosystems. For example, Wohl et al. (2012) described the importance of beaver impoundments as drivers of C storage in the Colorado Rocky Mountains. They found that total and dissolved forms of organic C are preferentially deposited along floodplains, particularly when flow is forced out of the channel by beaver dams, where low-oxygen saturated soils prevent microbial degradation. Furthermore, they highlighted the importance of coarse woody debris as a form of C storage in mountain streams. Beavers incorporate a large proportion of woody debris that enters the pond into the dam structure (Naiman and Melillo 1984), while other debris is deposited into the sediment where the anoxic environment prevents further breakdown and thus nutrients are not made available (Devito et al. 1989). Sediment deposition is also a key feature of beaver impoundments (illustrated in Figure A-2b) (Westbrook et al. 2011). Strong correlations of nutrients, particularly phosphorus, with suspended sediment (Triska et al. 1994) could lead to deposition in ponds or floodplains and thus limit downstream transport. It is also important to note that we examined only total nutrient concentrations; so, while no change in nutrient concentrations were observed from up- to downstream, there may have been unmeasured changes in the dissolved vs. particulate fraction of nutrients, especially since particles are more readily retained in ponds (Naiman et al. 1988). In contrast, Hall et al. (1999) compared patterns of productivity in two reservoirs from similar locations in Saskatchewan,

Canada: Lake Diefenbaker, a large (500 km^2) reservoir for a hydroelectric dam, and Buffalo

Pound lake, a small (5 km^2) impoundment. The authors found that while the large reservoir

exhibited evidence of nutrient increase following impoundment, the small impoundment did not.

In fact, Buffalo Pound Lake showed evidence of a decline in productivity in the years

immediately following its creation which the authors suggest indicates that reservoir formation

does not necessarily lead to increases in algal production, particularly when flooding is on a

small scale and does not greatly affect the supply of nutrients to the system. Because beaver

impoundments are also small in comparison to the large reservoirs associated with hydroelectric

dams, a similar conclusion could be made about the ponds in this study.

Although MeHg concentrations in stream water are often linked to DOC and nutrients

(Chasar et al. 2009); other factors can contribute to increases in MeHg. Catchment vegetation

could drive an increase in MeHg concentrations without a corresponding change in export of

DOC and nutrients, such as was observed in our study. The dominant trees and resulting litter in

the study area are largely coniferous, which, while providing less labile organic matter to the

ponds, are still acted on by methylation processes and therefore still contribute to *in situ*

methylation (Hall et al. 2004). This is consistent with Roy et al. (2009b), where THg and MeHg

concentrations in water were positively correlated with elevations where conifer forest

dominated. Conifer-dominated streams had higher water THg and MeHg concentrations

whereas lower elevation forests dominated by deciduous trees had lower concentrations, thus

leading the authors to conclude that forest composition had an effect on Hg chemistry (Roy et al.

2009b). Further, previous findings of elevated carbon standing stock within ponds were from

work conducted in a largely deciduous-dominated landscape (Naiman et al. 1986) with high

allochthonous inputs from alder (*Alnus* spp.), birch (*Betula* spp.) and aspen (*Populus* spp.)

through both direct input and from the landscape – features that are not apparent in our study

area. These high allochthonous inputs are likely associated with the known co-transport of DOC

and Hg from the terrestrial environment into aquatic systems (Watras et al. 1998). While pH is

widely known to play a key role in determining Hg availability (Jardine et al. 2012), the high and

relatively invariant pH (mean = 8.1) indicate that it is not an important factor in our systems.

Evidence suggests that under certain chemical conditions, aquatic C sources at the base

the food web can accumulate higher concentrations of MeHg relative to terrestrial sources

(Jardine et al. 2012; Riva-Murray et al. 2013). While we did not measure MeHg in our leaf litter

samples, we found that invertebrates in these systems derive their C primarily from an aquatic

source (i.e. periphyton) rather than a terrestrial source (i.e. detritus falling into streams). Water

and periphyton MeHg concentrations were significantly positively correlated with herbivore

MeHg concentrations ($\rho = 0.637$, $p = 0.014$ Table A-2), supporting a food web linked pathway of

MeHg accumulation, and THg concentrations in periphyton were consistently higher than those

in leaf litter (Fig. A-6). The beaver ponds in our streams did not change the periphyton

contribution to invertebrate diets from upstream to downstream sites, which is supported by the

lack of a difference in nutrients and algal biomass above and below dams. In other instances

where nutrients and productivity were higher downstream of dams, such as under low flow

conditions and certain beaver pond morphologies (Fuller and Peckarsky 2011), this may not be

the case. In such instances a pronounced increase in resources at the base of the food web could

be offset by more rapid growth by primary consumers, leading to growth dilution and lower

MeHg concentrations in consumers than might be expected based on higher waterborne

concentrations (Pickhardt et al. 2002; Hill and Larsen 2005).

Generally, sites with the highest MeHg concentrations also had higher nutrient and DOC concentrations (Table 3-1). This suggests that landscape-scale factors play a role in MeHg transfer to the food web in addition to the immediate, short-term effect of the beaver ponds. Our results are consistent with previously observed correlations between DOC at inlets and Hg as well as correlations between DOC and phosphorus (Roy et al. 2009b). Watershed-derived DOC is often associated with MeHg in streams, driven by the presence of wetlands (Brigham et al. 2009; Chasar et al. 2009). Brigham et al. (2009) observed a positive correlation of MeHg with DOC and stream flow in streams with moderate wetland influence, reasoning that the conditions that cause high flows both expand flooded area and wash materials, including DOC, from wetlands into streams.

Beaver impoundments may be thought of as natural analogues to man-made reservoirs, such as those created during the construction of hydroelectric dams. Periphyton and aquatic invertebrates play an important role in the basal transfer of MeHg from the physical environment to higher consumers such as fish and piscivorous wildlife; therefore, increases in MeHg in these lower trophic levels are an important consequence of reservoir formation (Hall et al. 1998). Changes in environmental conditions, such as inundation of vegetation and increased MeHg production that occur post-impoundment are similar to those observed at beaver dams, albeit on a much larger scale. MeHg production due to the flooding of large tracts of land during hydro dam construction has been well-documented (Rosenberg et al. 1997). Downstream transport of MeHg from reservoirs has been reported in several studies, (Rosenberg et al. 1997; Bodaly et al. 2007) with the dissolved form accounting for the largest mass flux ($>64\%$) downstream (Schetagne 2000). The dominance of this dissolved form of MeHg in downstream export provides some evidence as to why we observed increases in MeHg downstream of our

impoundments that are not associated with increased nutrients, and the common mechanism of sediment trapping in both beaver impoundments and large, man-made reservoirs. This highlights the ability of MeHg to be exported from reservoirs resulting in elevated concentrations in all biological compartments as mediated by the periphyton source pathway.

   Beaver populations suffered near decimation during the fur-trade era, but after coming under protective law in the early 1900s, and decreased natural predation (Naiman et al. 1988; Collen and Gibson 2001), beaver populations have been recovering over the past century (Naiman et al. 1986). As their population increases, so do the effects of their damming activities. This has important management implications for recreational fisheries downstream as well as for the study of contaminant flows through drainage systems inhabited by beaver. For example, beaver ponds are known to create habitat for many salmonid fishes, particularly in cold mountain systems such as our study area where angling is popular (Collen and Gibson 2001). The transfer of MeHg from impoundments to downstream food webs has potentially far-reaching implications for recreational fisheries on lakes in these drainage systems. In areas that are Hg-sensitive (i.e. low pH, high DOC streams), enhanced bioaccumulation in ponds has the potential to push downstream fish MeHg concentrations above consumption guidelines with consequences for piscivorous wildlife.

CHAPTER 4
4. BIOACCUMULATION OF MERCURY IN INVERTEBRATE FOOD WEBS OF
SOUTHERN CANADIAN ROCKY MOUNTAIN STREAMS

4.1 Preface

While the previous chapter (Chapter 3) focused on supply at the base of the food web, this

chapter takes the information from Chapter 3 and applies it to trophic transfer. MeHg taken up

from the water by primary producers is transferred to higher trophic level consumers via the diet

through the process of biomagnification. It is through this process that piscivorous wildlife and

humans can become exposed to potentially dangerous MeHg concentrations. Chapter 4 aims to

determine if the presence of in-channel beaver impoundments result in enhanced trophic transfer

downstream and to estimate potential risk to higher consumers.[2]

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[2] This chapter will be submitted as a manuscript to Archives of Environmental Contamination
and Toxicology entitled "Bioaccumulation of mercury in invertebrate food webs of southern
Canadian Rocky Mountain streams" by K. J. Painter and T. D. Jardine.

4.2 Abstract

Methylmercury (MeHg) is a contaminant of concern due to its ability to biomagnify in aquatic food webs, resulting in potentially harmful concentrations in higher consumers. Beaver impoundments in the southern Canadian Rockies release bioavailable MeHg to downstream food webs. This study examined the magnitude of uptake and trophic transfer of this exported mercury to higher consumers, and controls on these transfers by site-specific (dissolved organic carbon, Hg in water, Hg in diet) and individual (body size, trophic level) variables. Bioconcentration factors (BCFs) were fairly high (mean = 79,756 ± 68, 204), a function of the low exposure concentrations present in these systems. In contrast, Biomagnification factors (BMFs) for uptake from periphyton to grazers (mean = 18.3 ± 11.7) and grazers to predators (mean = 2.1 ± 1.2) were low. Invertebrate body size had no effect on MeHg concentration; however, the relative difference in trophic level from prey to consumer was an important driver of BMFs, and MeHg in the diet was negatively associated with BMFs. While rates of uptake and transfer were greater at low concentrations relative to that at higher concentrations, overall trophic magnification through the food web (average TMF = 2.3) was on the lower end of the typical range observed worldwide. Thus, while there is little risk to wildlife and humans who consume fish from these systems, we caution that in Hg-sensitive regions where conditions for methylation and transfer are ideal, beaver activity could have important implications for the movement of Hg through food webs.

4.3 Introduction

Bioaccumulation, the process by which an organism attains a higher concentration of a chemical relative to its environment (Borgå et al. 2011), is highly variable among chemicals, organisms and ecosystems. As such, bioaccumulation is a key endpoint criterion identified

during hazard assessment, which traditionally attempts to define PBT (Persistence, Bioaccumulation and Toxicity). Bioconcentration and biomagnification are two components of bioaccumulation that are used to define rates of uptake and subsequent trophic transfer of a chemical. Bioconcentration in aquatic organisms is the uptake of a chemical across respiratory/dermal surfaces (i.e. from water), excluding the diet. Thus, bioconcentration factor (BCF) is the ratio of the concentration of a substance in a given organism to that in the water. Biomagnification is a special case of bioaccumulation that occurs when the chemical concentration is greater in an organism than in its prey due to dietary absorption occurring faster than elimination (Borgå et al. 2011). Biomagnification factor (BMF), sometimes referred to as Trophic Transfer Factor (TTF, DeForest et al. 2007), is the ratio of the concentration of a substance in a given organism to that in its diet (Gobas et al. 2009). The sum of these processes yields the Bioaccumulation factor (BAF), the ratio of the concentration of a substance in an organism to that in the ambient environment including both water and dietary sources (Gobas et al. 2009). BCF and BAF are bioconcentration and bioaccumulation endpoints that have been used in the development of environmental guidelines for risk assessment (Arnot and Gobas 2006). For example, Environment Canada identifies substances with BCF and BAF values greater than or equal to 5000 as bioaccumulative under the Canadian Environmental Protection Act (CEPA 1999).

Because some metals, such as Hg, are persistent in the environment, and their bioavailability heavily influenced by geochemical factors (DeForest et al. 2007), rates of Hg bioaccumulation are highly variable among locations (Lavoie et al. 2013) and defining critical PBT levels can be difficult (DeForest et al. 2007). For Hg, and other metals such as selenium, zinc, copper and lead, BCFs tend to be the highest (indicating hazard) at low concentrations of

exposure (low potential for toxicity) and lowest (indicating reduced hazard) at higher concentrations of exposure (high potential for toxicity). This complicates risk assessments and suggests exposure concentration may be as important a driver of BAFs as other metal- and species-specific determinants of bioaccumulation (McGeer et al. 2003; Deforest et al. 2007). MeHg is a potent neurotoxin with the potential to pose a serious health risk to both humans and wildlife (Mergler et al. 2007). Thus, it is imperative to understand the potential for MeHg to transfer from abiotic to biotic compartments and move further up the food web to higher predators. Based on a large compilation of available data from peer-reviewed literature and technical documents, DeForest et al. (2007) found that BAFs for MeHg range from 100,000 to 48,000,000 across species and trophic levels, including invertebrates, small fish and large fish. This wide range highlights the importance of understanding Hg bioaccumulation and biomagnification and their drivers in aquatic systems

Stream systems inhabited by North American beaver (*Castor canadensis*) exhibit elevated levels of methyl mercury (MeHg) in water downstream from impoundments (Roy et al. 2009b), an increase that also results in higher concentrations in algae and invertebrates (Chapter 3). However, the magnitude of change from upstream to downstream varies among these ecological compartments and among sites, ranging from a 0.5 to 2.5 times increase in algae and a 0.6 to 5.0 times increase in predatory invertebrates, suggesting that other factors may modulate responses within the food web. For example, concentrations in water and the diet are inversely related to BCFs and BMFs, respectively, in both lab- and field-based studies (DeForest et al. 2007), and low pH can also lead to higher BMFs in some organisms (Jardine et al. 2013).

DOC, an indicator of wetland influence (Chasar et al. 2009; Brigham et al. 2009), also explains additional variability in Hg concentrations across sites (Chapter 3), but relationships

between DOC and Hg transport and uptake are complex. For example, Dittman and Driscoll (2009) report that increased DOC concentrations in the water column of Adirondack lakes resulted in a negative correlation between DOC and BAF for yellow perch. They hypothesized that this was caused by DOC in the water column binding with MeHg, thus reducing the bioavailable pool. Other studies (Adams et al 2009; Brigham et al 2009) illustrate the importance of DOC in transporting MeHg into aquatic systems. Positive correlations between DOC concentrations and methyl mercury in the water column (Brigham et al. 2009) and in basal food web organisms (Adams et al. 2009) are common. Therefore, we need to understand how this variable controls both uptake and transfer of MeHg.

This study aims to examine uptake and accumulation of Hg in stream food webs with an Hg gradient (i.e. upstream to downstream of beaver impoundments) by examining the movement of this metal among environmental compartments (water, periphyton and herbivorous and predatory invertebrates). In addition to using BCFs, BAFs and BMFs as described above, this study will examine other aspects of the invertebrate food web that can influence Hg concentrations. First, trophic transfer of Hg will be assessed using nitrogen stable isotope ratios ($^{15}N/^{14}N$), denoted as $\delta^{15}N$, to establish feeding linkages in stream food webs. The habitat-independent, step-wise enrichment of ^{15}N with TL (Minagawa and Wada 1984) has been widely used to characterise aquatic food webs, and the relationship between $\delta^{15}N$ and environmental contaminants, such as MeHg, has long been used as a predictor of contaminant biomagnification (Kidd et al. 1995). Second, body size is correlated with higher Hg concentrations in fishes because larger fishes tend to be older and/or feed at higher TLs (Kidd et al. 1995; Gewurtz et al. 2011). Unlike fishes, invertebrates are rarely classified based on their size in contaminant studies because TL estimates vary widely with functional feeding group (Merritt and Cummins

1996), though we expect that ^{15}N-size relationships for benthic invertebrates may be useful as tools to measure predatory behaviour in food webs (Anderson and Cabana 2009). There is likely to be an interaction between δ^{15}N, body size and Hg because predators are consistently larger than their prey (Riede et al. 2011) and because predators tend to occupy higher TLs then their prey (Anderson and Cabana 2009). Taken together, we seek to determine the importance of physicochemical drivers, such as DOC and Hg concentrations in water and the diet, at the base of the food web and how these processes relate to bioconcentration and trophic transfer of Hg.

4.4 Methods

4.4.1 Field sampling

From mid-July to early August 2012, 15 streams with in-channel beaver impoundments were sampled in the Canadian Rocky Mountain and Foothill Regions, Kananaskis Country, Alberta, using methods described in Chapter 3. An additional six free-flowing streams assumed to have no influence from beaver activity were also sampled using identical methods. Most streams were in conifer dominated watersheds at elevations of 1300 m to 2150 m. All sites were generally clear and high gradient with cobble and gravel substrates, had moderately basic pH, and limited accumulation of leaf litter due to low canopy cover, typically < 20% (Chapter 3; Table A-1). At each sampling location we collected water, periphyton and aquatic macroinvertebrates. At dammed sites, collections were made immediately above and below the impoundment (i.e. at an inflow and an outflow). All sample collection, processing and laboratory analyses for total and methyl Hg, DOC, TP, TN and benthic chl a are described in Chapter 3.

To assess the role of body size in driving Hg concentrations, invertebrates were sorted and identified to family level (Merrit and Cummins 1996), counted and weighed prior to drying.

Following drying, invertebrates were weighed again and homogenized. Invertebrate body size was calculated by determining the average wet weight of individuals from the three replicates collected at each site. Homogenized samples of taxa dominant across all sites, including grazing mayflies from the families Heptageniidae (flat-headed mayflies), Ephemerellidae (spiny crawler mayflies) and Baetidae (small minnow mayflies) and predatory invertebrates from the families Perlodidae (perlodid stoneflies) and Rhyacophilidae (free-living caddisflies), were analysed simultaneously for Hg and isotopes. In addition, we analysed all collected organisms from upstream and downstream at two beaver-impacted sites, to develop an understanding of Hg behaviour in more comprehensive invertebrate food webs in these systems. Additional taxa analyzed at those sites were Hydropsychidae (net-spinning caddisflies), Limnephilidae (case-constructing caddisflies), Tipulidae (crane flies), Chironomidae (nonbiting midges), Siphlonuridae (primitive minnow mayflies), Polycentropodidae (tube maker caddisflies), Peltoperlidae (roach-like stoneflies) and Phyrygancidae (giant case-making caddisflies).

4.4.2 Calculations

Uptake and trophic transfer is calculated by three factors: the BCF, the BMF and the BAF. BCF for animals can only be measured under controlled laboratory conditions (i.e. steady state) in which dietary uptake is deliberately excluded (Arnot and Gobas 2006; Gobas et al. 2009), but for autotrophs such as periphyton with a single route of uptake it can be calculated from field data as the ratio of the chemical concentration in the organism (C_B) to the chemical concentration in the water (C_W) according to:

$$BCF = C_B/C_W \qquad\qquad\qquad\qquad (Eq. 4.1)$$

Biomagnification is the process in which the thermodynamic activity of the chemical in an organism exceeds that of its diet (Arnot and Gobas 2006; Gobas and Morrison 2000). BMF

for metals such as mercury that are almost exclusively derived from the diet (Hall et al. 1997) is

expressed as the ratio of the chemical concentration in an organism (C_B) to that in its diet (C_D)

according to:

BMF = C_B/C_D (Eq. 4.2)

A BMF > 1 indicates that the chemical is a probable bioaccumulative substance (Gobas et al.

2009). For these analyses, we classified Heptageniidae, Ephemerellidae and Baetidae as

herbivorous grazers and Perlodidae and Rhyacophilidae as predatory taxa and assumed each

trophic level fed 100% on the trophic level below it. Because their Hg concentrations were not

statistically different, the three mayfly taxa were pooled as were the two predator taxa (see

Chapter 3). To account for potential omnivory, we used stable N isotope data to confirm feeding

linkages (see below).

Bioaccumulation, the multiplier of all exposure routes (Arnot and Gobas 2006), is

expressed as the BAF, according to:

BAF = C_B/C_W (Eq. 4.3)

BAFs, while calculated similarly to BCFs, are used when the sample organism is exposed to a

given chemical through both water and their diet and is usually inferred from field data (Gobas et

al. 2009). For chemicals with a logKOW (octanol-water partitioning coefficient) ≤5, BCFs and

BAFs ≤ 5000 L/kg wet weight indicate a lack of biomagnification potential in water-respiring

organisms (Gobas et al. 2009).

4.4.3 Stable isotopes

Because δ^{15}N increases with TL, it can be used to compare Hg biomagnification across

systems as the slope of the regression of log-transformed Hg vs. δ^{15}N or TL, (Borgå et al. 2011;

Lavoie et al. 2013). The calculation of a TL value takes into consideration a trophic enrichment

factor (TEF, increase in δ^{15}N from diet to consumer, denoted as Δ15N) and assigns discrete TLs

to a baseline organism (e.g. periphyton TL = 1) because δ^{15}N can vary widely among sites

(Cabana and Rasmussen 1996). δ^{15}N was converted to TL as follows:

$$TL_{consumer} = (\delta^{15}N_{consumer} - \delta^{15}N_{baseline})/\Delta15N + TL_{baseline} \qquad \text{(Eq. 4.4)}$$

where $TL_{baseline}$ = TL of the baseline organism and Δ15N is the TEF. We used periphyton as the

baseline organism with a TL=1. We chose a TEF of 2.0‰ based on McCutchan et al. (2003), a

value suitable for stream food webs (Bunn et al. 2013; Jardine et al. 2013).

      Trophic magnification factors (TMFs) represent the average biomagnification per TL

through the entire food web and are calculated as the antilog of the logHg vs. TL slope (b) as

follows (Borgå et al. 2011):

$$TMF=10^{b} \qquad \text{(Eq. 4.5)}$$

      When TMF = 1, (b = 0), Hg does not biomagnify on average through the food web.

When TMF > 1 (b > 0), Hg biomagnifies through the food web by an average of TMF per TL

and when TMF < 1 (b < 0), Hg decreases by an average of TMF per TL (Borgå et al. 2011).

Trophic magnification slopes (TMSs) can also be derived directly from the logHg vs. δ^{15}N

regression, and can be compared across systems without standardizing to a baseline (Lavoie et al.

2013).   The TMS is calculated as the slope (b) in the following model:

$$LogHg=\delta^{15}N \, (b) + a \qquad \text{(Eq. 4.6)}$$

No change of the chemical concentration with increasing δ^{15}N produces a TMS of 0, indicating

no biomagnification. An increase in logHg concentration with increasing δ^{15}N produces a TMS

> 0, which indicates biomagnification. A decrease of logHg concentration with increasing δ^{15}N

produces a TMS < 0 and would indicate trophic dilution, i.e. the opposite of biomagnification (Gobas et al. 2009).

For this study we were able to calculate BCFs, BAFs and BMFs for each of our 36 sites (hereafter expressed as log-transformed values unless otherwise specified); however, TMSs and TMFs require a regression model within each site which we were unable to calculate due to limited sample sizes within individual sites, with the exception of the two comprehensive food web sites (both upstream and downstream). Instead, we calculated overall TMF values for all sites combined into a single regression, and then back-calculated TMS to compare to other studies (Lavoie et al. 2013) by dividing the logHg vs. TL slopes by the TEF.

4.4.4 Data analysis

BCFs, BAFs and BMFs were compared between up- (US) and down-stream (DS) for all beaver-impacted sites using paired t-tests. The relative strength of associations between MeHg and invertebrate body size, TL and body size, and MeHg and TL were measured using linear regression analysis. To account for differences in MeHg between sites, MeHg was normalized to periphyton by dividing invertebrate MeHg values by their site-specific periphyton MeHg values before regressions with body size and TL were carried out. Linear regressions were also used to measure the MeHg vs. δ^{15}N relationship for two sites where the food web was more comprehensively sampled.

To examine variables that may explain among-site variability in uptake and trophic transfer, an information-theoretic approach was used. Akaike's Information Criterion (AIC) was used to compare and rank competing regression models to determine the best approximating model or set of models. Prior to AIC analysis, initial screening of data suggested upstream and

downstream values at beaver impacted sites to be similar, therefore we only used upstream data to conduct the AIC analysis. We combined data for upstream locations of beaver-impacted streams with free flowing streams (model sets A and B). Then, to expand our analysis on a broader scale, we used data from a set of New Brunswick streams to increase the range of variation in our variables (model sets C and D). Details on collection and analysis for the New Brunswick streams can be found in Jardine et al. (2012), and include similar taxa (Ephemeroptera, Plecoptera) as our Alberta streams. The New Brunswick data did not include water MeHg concentrations; therefore, these data are only included when examining BMFs. New Brunswick data also included total organic carbon (TOC) in place of DOC; however, previous studies have shown that $\geq 95\%$ of TOC exists as DOC in these systems (Clair et al 1994).   AIC analysis was not performed on BCFs because many of the sites had water MeHg concentrations that were below detection limits.

Model sets were developed for $BMF_{(grazers/periphyton)}$ and $BMF_{(predators/grazers)}$ and included variables selected *a priori* because they were likely to influence BMF. These include $MeHg_{diet}$ (DeForest et al. 2007, Jardine et al. 2013), DOC (Chasar et al. 2009), TP (Lavoie et al. 2013) and benthic chl *a* (Lavoie et al. 2013) and the relative difference in TL from prey to consumer, calculated as $TL_{(consumer/prey)}$ (Fisk et al. 2001). We could not test for the effects of pH (Jardine et al. 2013) because of the limited range of values for this variable at our study sites (7.8 to 8.4). Due to the small sample size and small n/k, where n is the sample size and k is the number of fitted parameters, AIC_C was used as described in Chapter 3. Following creation of model sets, it was determined that no single model was overwhelmingly supported (Akaike weight > 0.9); therefore, model averaging was carried out (Burnham and Anderson 2002) to reduce model selection uncertainty.   Model-averaged parameter estimates (i.e. in this case, regression

coefficients) and corresponding standard errors were calculated according to Burnham and

Anderson (2002). By examining the entire set of candidate models (all models with $\Delta_i \leq 7$), it

was then possible to determine the relative importance of individual parameters. All data except

TL were log-transformed prior to analysis. Statistical analyses were carried out using SYSTAT

13 (Systat Software Inc. 2009).

4.5 Results

4.5.1 General bioaccumulation trends

BCFs were fairly high while BMFs and BAFs were generally low (Table 4-1).

Untransformed BCFs ranged from approx. 12,000 to 344,000 while untransformed BMFs ranged

from approximately 3 to 48 for $BMF_{(grazers/periphyton)}$ and < 1 to < 10 for $BMF_{(predators/grazers)}$. At

beaver-affected sites, there was no difference between up- (US) and down-stream (DS) log-

transformed BCFs (US mean $logBCF_{(periphyton/water)} = 4.79 \pm 0.40SD$; DS mean

$logBCF_{(periphyton/water)} = 4.74 \pm 0.35SD$), BAFs (US mean $logBAF_{(grazers/water)} = 6.02 \pm 0.35SD$; DS

mean $logBAF_{(grazers/water)} = 6.00 \pm 0.33SD$; US mean $logBAF_{(predators/water)} = 6.09 \pm 0.44SD$; DS

mean $logBAF_{(predators/water)} = 6.21 \pm 0.42SD$), or BMFs (US mean $logBMF_{(grazers/periphyton)} = 1.18 \pm$

$0.34SD$; DS mean $logBMF_{(grazers/periphyton)} = 1.22 \pm 0.26SD$; US mean $logBMF_{(predators/grazers)} = 0.27$

$\pm 0.22SD$; DS mean $logBMF_{(predators/grazers)} = 0.25 \pm 0.15SD$) ($p > 0.05$ for all comparisons).

Table 4-1 MeHg concentrations and trophic levels (TLs) for all biological compartments in invertebrate food webs in Rocky Mountain Foothills streams, and corresponding Bioconcentration Factors (BCFs), Bioaccumulation Factors (BAFs) and Biomagnification Factors (BMFs). BCFs, BAFs and BMFs are log transformed. Periphyton TL is assumed to be 1. U indicates sites upstream from beaver impoundments. D indicates sites downstream from beaver impoundments. No suffix indicates sites without beaver activity.

Site	Water MeHg (ng/L)	Periphyton MeHg (ng/g)	Herbivore MeHg (ng/g)	Predator MeHg (ng/g)	Grazer TL	Predator TL	BCF (periphyton/ water)	BAF (grazers/ water)	BAF (predators/ water)	BMF (grazers/ periphyton)	BMF (predators/ grazers)
BEV012U	0.01	0.63	6.00	10.10	0.85	2.09	4.80	5.78	6.00	0.98	0.23
BEV002U	0.09	1.20	57.83	-	1.42		4.11	5.80	-	1.68	-
BEV004U	0.06	1.17	17.55	36.36	1.80	2.45	4.30	5.48	5.79	1.18	0.32
BEV015U	0.01	0.61	24.42	-	1.83	2.83	4.79	6.39	-	1.60	-
BEV028U	0.01	2.08	6.94	17.19	1.35	1.96	5.32	5.84	6.24	0.52	0.39
BEV020U	0.01	1.87	19.73	35.02	0.89	1.63	5.27	6.30	6.54	1.02	0.25
BEV021U	0.01	2.14	25.99	25.72	1.35	1.96	5.33	6.41	6.41	1.08	0.00
BEV019U	0.01	0.42	2.04	8.98	0.18	1.04	4.63	5.31	5.95	0.68	0.64
BEV024U	0.06	2.69	55.58	-	0.35	-	4.69	6.00	-	1.32	-
BEV025U	0.01	1.23	12.32	30.04	1.88	2.94	5.09	6.09	6.48	1.00	0.39
BEV027U	0.07	3.36	62.23	-	1.55	-	4.70	5.97	-	1.27	-
BEV030U	0.03	0.36	-	3.48	-	1.97	4.15	-	5.13	-	-
BEV026U	0.01	0.50	20.36	-	1.74	2.50	4.70	6.31	-	1.61	-
BEV022U	0.03	3.77	65.44	-	1.46	2.13	5.15	6.39	-	1.24	-
BEV010U	0.01	0.79	19.12	16.92	1.75	2.37	4.90	6.28	6.23	1.39	-0.05
BEV012D	0.01	0.53	8.81	13.45	1.31	2.70	4.73	5.95	6.13	1.22	0.18
BEV002D	0.08	1.98	24.98	-	2.78	-	4.38	5.48	-	1.10	-
BEV004D	0.06	1.45	24.73	22.85	0.71	1.21	4.36	5.59	5.55	1.23	-0.03
BEV015D	0.08	1.48	37.56	-	1.11	-	4.24	5.65	-	1.40	-
BEV028D	0.01	1.04	6.67	17.10	0.82	2.16	5.02	5.82	6.23	0.81	0.41
BEV020D	0.02	1.16	54.19	72.87	0.77	0.87	4.76	6.43	6.56	1.67	0.13
BEV021D	0.01	3.45	28.68	65.19	1.59	2.52	5.54	6.46	6.81	0.92	0.36

BEV019D	0.01	0.78	8.54	18.15	1.51	2.34	4.89	5.93	6.26	1.04	0.33
BEV024D	0.04	1.36	37.12	-	0.74	1.31	4.54	5.97	-	1.44	-
BEV025D	0.04	3.12	19.47	34.52	1.34	1.84	4.91	5.70	5.95	0.80	0.25
BEV027D	0.08	5.23	114.76	-	1.60	3.05	4.80	6.15	-	1.34	-
BEV030D	0.03	0.50	-	17.43	1.83	2.71	4.21	-	5.76	-	-
BEV026D	0.01	0.93	20.21	-	0.83	2.02	4.97	6.31	-	1.34	-
BEV022D	0.06	5.69	132.35	-	0.88	1.84	4.98	6.35	-	1.37	-
BEV010D	0.01	0.71	19.49	45.95	1.14	2.22	4.85	6.29	6.66	1.44	0.37
BEV016	0.01	0.87	8.87	-	1.36	-	4.94	5.95	-	1.01	-
BEV017	0.01	0.49	4.68	27.89	0.88	2.12	4.69	5.67	6.45	0.98	0.33
BEV018	0.01	0.41	4.84	13.34	0.72	1.26	4.61	5.68	6.13	1.08	0.22
BEV031	0.01	0.79	16.98	17.26	1.23	-	4.90	6.23	6.24	1.33	0.00
BEV032	0.01	1.08	20.32	-	1.25	-	5.03	6.31	-	1.27	-
BEV033	0.01	0.38	1.46	9.12	0.24	-	4.58	5.17	5.96	0.59	0.76

The slope of the logHg vs. TL regression for all 21 Alberta sites combined was 0.34 for MeHg and 0.08 for THg (Fig. 4-1), resulting in TMFs of 2.3 for MeHg and 1.2 for THg. These values correspond to TMSs of 0.18 for MeHg and 0.03 for THg. At the two locations where the food web was more comprehensively sampled, the MeHg TMSs were higher upstream of in-channel beaver impoundments (BEV004 US TMS = 0.327, r^2 = 0.61, p = 0.022; BEV028 US TMS = 0.267, r^2 < 0.01, p = 0.957) than downstream (BEV004 DS TMS = -0.007, r^2 = 0.39, p = 0.099; BEV028 DS TMS = 0.136, r^2 = 0.21, p = 0.182) despite higher concentrations overall downstream (Fig. 4-2), though this relationship was not significant for three of the four relationships and confidence intervals around slope estimates overlapped due to high variability and low sample size.
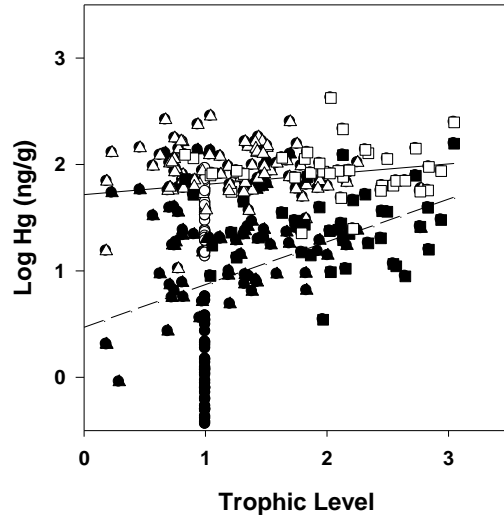
Fig 4-1 LogHg vs. trophic level for MeHg (black symbols, dashed line) and THg (white symbols, solid line) for Rocky Mountain stream food webs (circle = periphyton, triangle = grazers, square = predators).
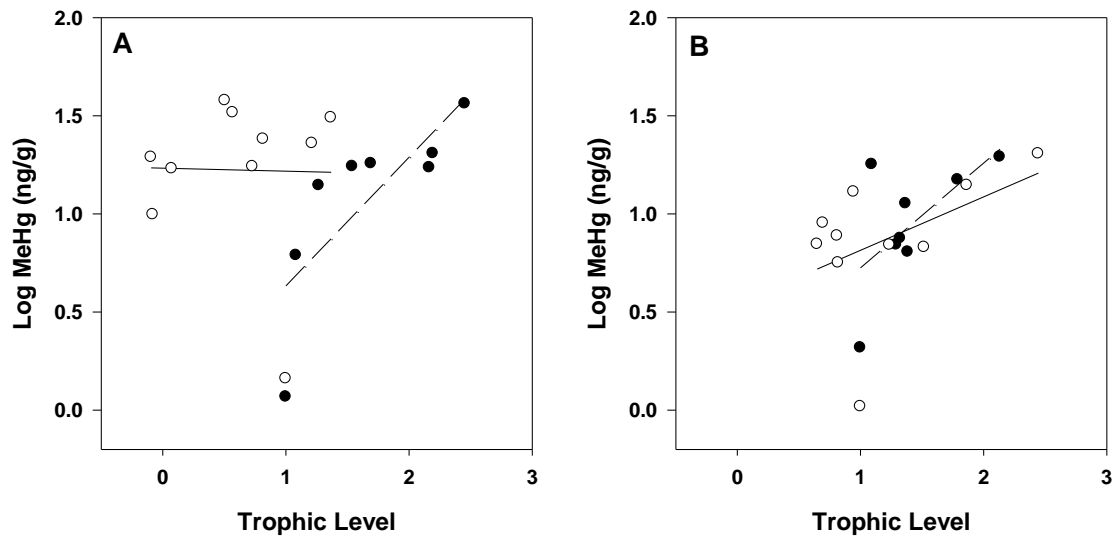
Fig. 4-2 Two comprehensive invertebrate food web sites comparing up- (black symbols, dashed line) and downstream (white symbols, solid line) slopes. Panel A refers to site BEV004 and panel B refers to site BEV028.

4.5.2 Drivers of Hg transfer within food webs

There were weak relationships between log normalized MeHg and log-transformed mean body size (slope = -0.011, $r^2 < 0.01$, p = 0.789) and body size and trophic level (slope = 0.174, r^2 = 0.01, p=0.109) (Fig. 4-3). Though the log normalized MeHg-TL relationship was also weak (slope = 0.133, $r^2 = 0.10$, p = 0.0001), TL accounted for more variation in invertebrate Hg across sites than did body size.
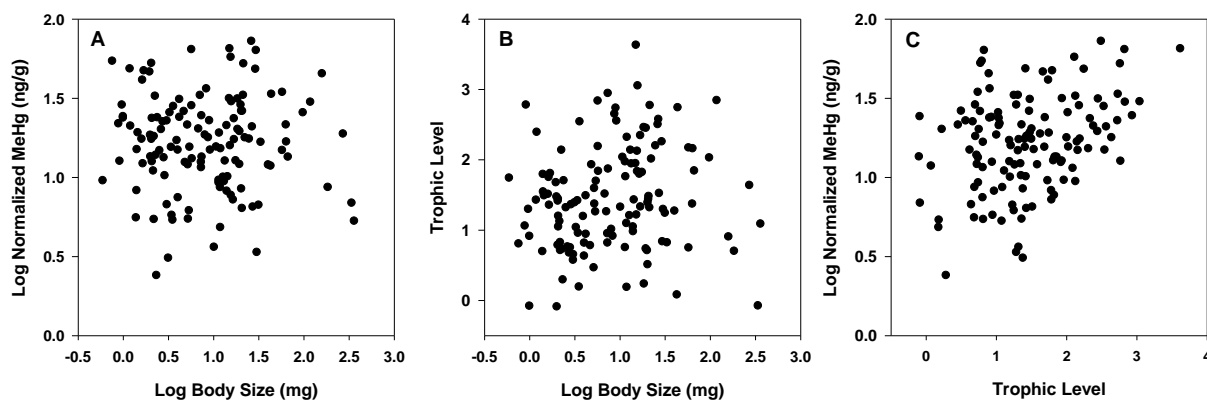
Fig. 4-3 A) Log periphyton-normalized MeHg concentrations vs. log-transformed body size, B) Log body size vs. trophic level, and C) Log periphyton normalized MeHg concentrations vs. trophic level for invertebrates from 21 Alberta sites (n=131)

BCFs and BMFs were negatively associated with Hg concentrations in water and the diet, respectively (Fig. 4-4), and DOC had a relatively weak influence on both of these bioaccumulation endpoints (Fig. 4-5). The most important variables used to explain BMFs were $TL_{(consumer/prey)}$, MeHg in the diet and DOC (Table 4-2). For model set A ($BMF_{(grazers/periphyton)}$ for Alberta sites), the top four models had $\Delta \leq 2$, with $TL_{(grazers/periphyton)}$ alone being the top model and a combination of $TL_{(grazers/periphyton)}$ and DOC being the second-best model with an ER of only 1.034, suggesting almost equal weight between the two top models (Table 4-2). After model averaging, the strongest parameter estimates for model set A were $TL_{(grazers/periphyton)}$ and DOC with averaged parameter estimates of 0.287 (SE = 0.121) and 0.381 (SE = 0.199) respectively. MeHg in the diet, in this case periphyton MeHg concentration, TP and chl a had averaged parameter estimates of -0.317 (SE = 0.379), -0.296 (SE = 0.250) and 0.047 (SE = 0.116), respectively, but the large standard error makes them weak predictors of $BMF_{(grazers/periphyton)}$. The intercept-only (i.e. null) model had a Δ value of 3.9, making it a plausible model, however the top 4 models had a summed weight of .809, or accounted for 80.9% of the weight in the set, compared to only 3.7% for the intercept-only model.   For model set B ($BMF_{(predators/grazers)}$ for Alberta sites), the top two models had $\Delta \leq 2$ with MeHg in the diet (grazer MeHg concentration) and DOC being the best-ranked model and the second best model containing MeHg in the diet, DOC and benthic chl a (Table 4-2). $MeHg_{diet}$ and DOC had the strongest averaged parameter estimates and were -0.631 (SE = 0.107) and 0.379 (SE = 0.119), respectively. The averaged parameter estimates for chl a and $TL_{(predators/grazers)}$ were 0.120 (SE = 0.055) and 0.084 (SE = 0.035), respectively, while TP had no support whatsoever.

While there was a positive association between DOC and BMFs in the Alberta sites, the addition of the New Brunswick sites weakened the relationship. Model set C

(BMF$_{(grazers/periphyton)}$ for combined AB and NB sites) had similar results to model set A with the top 4 models having $\Delta \leq 2$ (Table 4-2). The strongest averaged parameter estimates for model set C were: TL$_{(grazers/periphyton)}$ (0.250, SE = 0.110), MeHg$_{diet}$ (-0.382, SE = 0.149), and TP (0.358, SE = 0.263). DOC had an averaged parameter estimate of 0.167 with a high standard error (SE = 0.205) making it uninformative in set C despite appearing in three of the four top models. For model set D, (BMF$_{(predators/grazers)}$ for AB and NB sites), the top three models had $\Delta \leq 2$ including TL$_{(predators/grazers)}$ as the top ranked model and TL$_{(predators/grazers)}$ and MeHg$_{diet}$ as the second-best model. The averaged parameter estimates were: TL$_{(predators/grazers)}$, 0.115 (SE = 0.059); Hg$_{diet}$, -0.143 (SE = 0.102); and TP, 0.355 (SE = 0.259). DOC had an averaged parameter estimate of 0.031 (SE = 0.154), again with a high standard error making it uninformative in set D also.
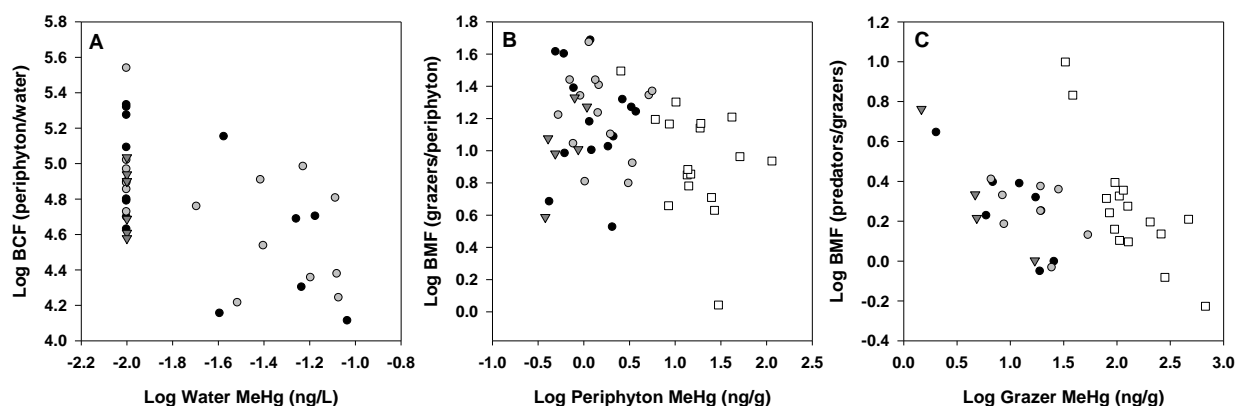
Fig 4-4 A) LogBCF vs. LogMeHg in water, B) LogBMF$_{(grazers/periphyton)}$ vs. LogMeHg in periphyton , and C) LogBMF$_{(predators/grazers)}$ vs. LogMeHg in grazers. Circles are up- (black) and down- (grey) stream beaver impacted sites, triangles are free-flowing sites and squares are New Brunswick sites.
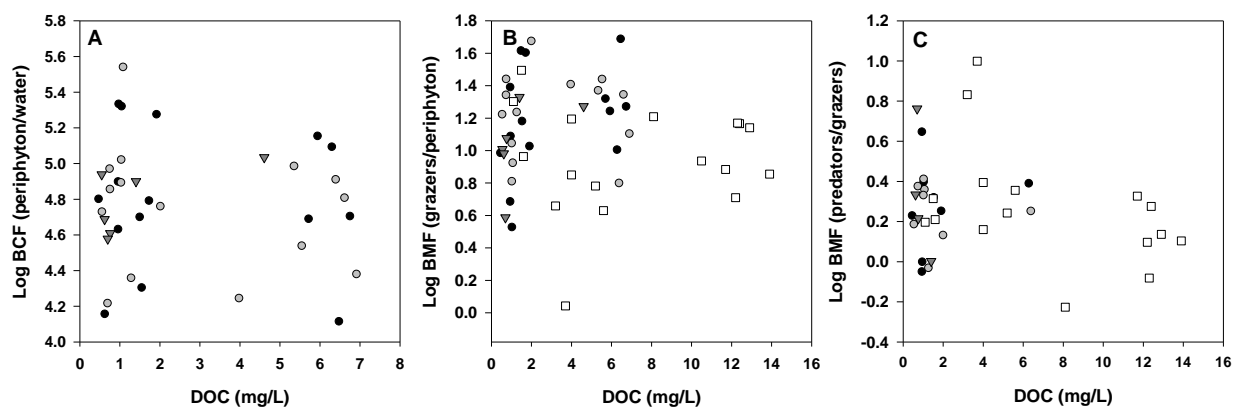
Fig. 4-5 A) LogBCF vs. DOC, B) LogBMF$_{(grazers/periphyton)}$ vs. DOC, and C) LogBMF$_{(predators/grazers)}$ vs. DOC. Circles are up- (black) and downstream (grey) beaver impacted sites, triangles are free-flowing sites and squares are New Brunswick sites.

Table 4-2 Model sets used to account for variation in BMFs at Alberta sites (A and B), and Alberta sites and New Brunswick sites combined (C and D). Δi is the difference between the AICc value of the best model and a given competing model. wi is the Akaike weight, with the highest ranked model in the set having the highest wi. Evidence ratios are a measure of likelihood of the best model over competing models. Models shown are those which were used for model averaging with $\Delta \leq 7$.

Model Set	Model	Δi	w_i	Evidence Ratios
A) $BMF_{(grazers/periphyton)}$	$TL_{(grazers/periphyton)}$	0.000	0.259	-
	$TL_{(grazers/periphyton)}$, DOC	0.066	0.251	1.034
	$MeHg_{diet}$, $TL_{(grazers/periphyton)}$, DOC	0.588	0.193	1.342
	DOC	1.791	0.106	2.449
	$MeHg_{diet}$, $TL_{(grazers/periphyton)}$	3.138	0.054	4.803
	$MeHg_{diet}$, DOC	3.317	0.049	5.252
	Intercept Only	3.909	0.037	7.059
	$MeHg_{diet}$, $TL_{(grazers/periphyton)}$, DOC, Chl *a*	4.402	0.029	9.032
	$MeHg_{diet}$	6.421	0.010	24.792
	DOC, TP, Chl *a*	6.765	0.009	29.442
B) $BMF_{(predators/grazers)}$	$MeHg_{diet}$, DOC	0.000	0.456	-
	$MeHg_{diet}$, DOC, Chl *a*	0.257	0.401	1.137
	$MeHg_{diet}$	3.120	0.096	4.759
	$MeHg_{diet}$, Chl *a*	5.796	0.025	18.136
	$TL_{(predator/grazer)}$	7.107	0.013	34.932
	$MeHg_{diet}$, DOC, TP, Chl *a*	8.988	0.005	89.483
	$MeHg_{diet}$, $TL_{(predator/grazer)}$	11.274	0.002	280.630
	$MeHg_{diet}$, $TL_{(predator/grazer)}$, DOC	11.876	0.001	379.108
	Intercept Only	12.995	0.001	663.349
C) $BMF_{(grazers/periphyton)}$ incl. NB sites	$MeHg_{diet}$, $TL_{(grazers/periphyton)}$	0.000	0.297	-
	$MeHg_{diet}$, $TL_{(grazers/periphyton)}$, DOC	0.114	0.281	1.059
	$MeHg_{diet}$, $TL_{(grazers/periphyton)}$, DOC, TP	0.571	0.223	1.330
	$MeHg_{diet}$, DOC	2.068	0.106	2.812
	$MeHg_{diet}$	3.421	0.054	5.532
	Intercept Only	5.309	0.021	14.217
	TP	7.141	0.008	35.539
D) $BMF_{(predators/grazers)}$ incl. NB sites	$TL_{(predators/grazers)}$	0.000	0.424	-
	$MeHg_{diet}$, $TL_{(predators/grazers)}$	1.647	0.186	2.279
	$MeHg_{diet}$	2.079	0.150	2.828
	$TL_{(predators/grazers)}$, DOC	2.793	0.105	4.041
	$MeHg_{diet}$, $TL_{(predators/grazers)}$, DOC	3.699	0.067	6.357
	$MeHg_{diet}$, $TL_{(predators/grazers)}$, DOC, TP	4.903	0.036	11.609
	Intercept Only	5.886	0.022	18.977
	DOC	7.387	0.011	40.193

4.6 Discussion

We found high Hg uptake from abiotic to biotic compartments but low rates of transfer within the invertebrate food webs of these Rocky Mountain streams. Mean untransformed BCFs were $79,756 \pm 68,204$, which are high compared to literature values for MeHg reported by McGeer et al. (2003) for a range of organisms including algae, invertebrates and fish of $8952 \pm 24,675$ (n = 53). These high values are likely not indicative of a greater hazard of toxicity but rather reflect natural conditions where uptake is high when ambient concentrations are low (DeForest et al. 2007). Bioaccumulation factors (mean $BAF_{(grazers/water)} = 1,259,951 \pm 838,812$, mean $BAF_{(predators/water)}$ $1,969,288 \pm 1,541,130$) were within the 100,000 to 48,000,000 range of literature BAF values for MeHg reported by DeForest et al. (2007). Within the food web, we found rates of trophic transfer to be low in these systems. Our calculated mean MeHg TMS (0.18) was near the global average TMS value for MeHg in freshwater systems (0.24 ± 0.08), but the THg TMS (0.03) was well below the average THg TMS (0.15 ± 0.11) (Lavoie et al. 2013). Accordingly, TMFs (2.3 for MeHg and 1.2 for THg) were also at the lower end of the range for freshwater systems (8.3 ± 7.5 for MeHg and 4.3 ± 4.8 for THg, Lavoie et al. 2013).

The far larger BCF values compared to BMFs illustrate that processes at the base of the food web will have a greater influence on overall Hg concentrations of higher consumers than trophic transfer of Hg through the food web. At every one of the 35 sites in our study, the magnitude of change in MeHg was greatest from water to organisms. It declined from primary producers to consumers and further again from primary consumers to predators. This is consistent with other studies that have shown that bioconcentration from water to algae is several orders of magnitude larger than biomagnification from algae to successively higher trophic levels

(Weiner et al. 2007; Chasar et al. 2009). This places a renewed emphasis on understanding controls on the delivery and uptake of Hg from water to periphyton in these systems.

Based on the two beaver-impacted sites where a more comprehensive food web analysis was conducted, our findings suggest that MeHg is being taken up by periphyton and invertebrates more efficiently at the lower concentrations upstream than at the higher concentrations downstream (Chapter 3). This is consistent with the inverse relationship between concentration and uptake observed for lab-reared organisms (Tsui and Wang 2004; DeForest et al. 2007) and further demonstrated for grazers and predators in New Brunswick streams (Jardine et al. 2013) that may be explained by a saturation of binding sites for Hg at high concentrations combined with slow turnover (Tsui and Wang 2004). Across all sites in the current study, MeHg was readily taken up into the food web even when water concentrations were below detection, resulting in highest BCFs when concentrations were low. This is further supported by the results of our AIC analysis where $MeHg_{diet}$ had a negative effect on BMFs from periphyton to grazers and from grazers to predators. Similarly, Lavoie et al. (2013) found that MeHg TMSs were lowest when Hg at baseline trophic level and GIS-derived atmospheric Hg deposition were highest.

DOC leads to higher baseline MeHg in the study streams (Chapter 3), an effect that has been well described for MeHg in aquatic systems (Driscoll et al. 1995). Particularly in streams, DOC is associated with the presence of wetlands, major sources of Hg methylation, in the surrounding watershed as well as DOC-Hg complexes washing in from the terrestrial environment (Brigham et al. 2009; Chasar et al. 2009). DOC is positively associated with BMFs for the Alberta streams (Table 4-2, Model Set A and B), however at the higher concentrations observed in the New Brunswick streams (Table 4-2, Model Set C and D), the effect is dampened.

This suggests that DOC enhances the biomagnification of MeHg through the food web at low concentrations but at higher concentrations may inhibit trophic transfer. This is in agreement with Driscoll et al. (1995) who reported that fish MeHg correlated positively with DOC at low concentrations (<8 mg/L) but then declined when DOC was very high (24 mg/L).

This study highlights the relative difference in trophic level from prey to consumer as an important variable driving BMFs. The addition of stable isotope information helps to refine BMFs: for example, Dietz et al. (2000) examined contaminant biomagnification in an arctic ecosystem and found that some concentrations were unexplained by BMF alone, speculating that trophic linkages likely played a role. The stepwise increase in TL from prey to predator has long been associated with increased contaminant concentrations i.e. the TL-Hg relationship is significant overall (Kidd et al. 1995). Thus, it is expected that trophic transfer of Hg will be greater when the "step up" in TL is greater for a given predator/prey pair. When calculating trophic transfer, we caution that good judgement should be used when selecting the TEF because TMFs are sensitive to changes in the TEF value used (Lavoie et al. 2013). For example, the use of a commonly applied TEF of 3.4‰ (Minagawa and Wada 1984, Post 2002) would result in TMFs of 4.06 for MeHg and 1.27 for THg in our food webs, effectively doubling the rate of biomagnification of MeHg from what we have calculated using a TEF of 2.0‰. In an analysis of 144 records across arid, tropical, subtropical and temperate sites, Bunn et al. (2013) found that many TEF estimates for invertebrate food webs were lower than the commonly reported 3.4‰. We chose the 2.0‰ value based on similar TEFs from the literature for stream invertebrate food webs (McCutchan et al. 2003, Bunn et al. 2013).

Hg bioaccumulation models that were largely developed from pelagic freshwater systems (Watras et al. 1998), where there is strong size structuring due to gape limitation in fishes, do not

necessarily apply to stream invertebrate food webs. Instead, invertebrates in streams have high

diversity and occupy many different niches, resulting in weak size structuring and short food

chains (Vander Zanden and Fetzer 2007, Riede et al. 2011). For example, a study by Jardine

(2014) found that body size significantly increased with trophic position in stream invertebrate

food webs in tropical, subtropical and temperate regions, but the relationship was weak,

especially compared to strongly size-structured food webs commonly found in the pelagic zone

of temperate lakes (Hairston and Hairston 1993). The implication of this weak size structuring is

that larger animals will not necessarily have the highest Hg, nor might they occupy the highest

trophic levels. An example of this is seen in the two sites that were analysed more

comprehensively – Tipulidae spp. occur in these food webs and have the largest body sizes

despite having low MeHg concentrations and low TLs. Instead, smaller invertebrates,

particularly grazing mayflies, have the highest Hg suggesting that other factors drive

accumulation of Hg in these organisms. Mason et al. (2000) found that smaller-sized

invertebrates can have higher concentrations of arsenic and selenium due to their high surface

area to volume ratio, resulting in a greater absorptive area. Though findings for Hg were

inconsistent in that study (Mason et al. 2000), the relative contribution of dermal absorption

versus the diet in dictating uptake in small invertebrates is less well understood relative to fishes

(Hall et al. 1997).

One potential cause of higher TLs in smaller organisms that have not been classified as

predators is omnivory. Invertebrates can exhibit both obligate and facultative functional feeding

behaviour (Cummins and Klug 1979). Both Heptageniidae and Ephemerellidae mayflies are

classified as herbivorous scrapers (Merrit and Cummins 1996) but can also be facultative

collectors. Thus, they have the potential to collect particles "of animal origin" or bacteria and

may therefore occupy a higher trophic position due to omnivory (Anderson and Cabana 2007). This further reiterates the importance of using stable isotopes in bioaccumulation studies, particularly the relative difference in TL from prey to consumer ($TL_{consumer/prey}$) as it should accurately depict omnivorous behavior. For example, we observed instances where grazing mayflies had higher $\delta^{15}N$ than presumed predators, leading to lower $TL_{(consumer/prey)}$ values, and Ephemerellids occupied the same trophic level as predators at two upstream beaver sites.   As such, there are implications for trophic transfer because if fish tend to eat larger invertebrates and larger invertebrates have lower Hg, then there will be less Hg transferred up the food chain.

Although trophic transfer is more efficient at low concentrations, our results suggest that MeHg in top predators will be governed more by supply of MeHg at the base of the food web, rather than trophic transfer and trophic efficiency (Chasar et al. 2009). Hg inputs to ecosystems are largely as Hg(II), while MeHg production and subsequent uptake into algae occurs within the system (Driscoll et al. 2013) and is highly variable. The relative importance of supply versus uptake and transfer in determining Hg in higher trophic level organisms is thus of considerable interest because inputs are more readily managed than transfers through the food web. For example, following the flooding of large reservoirs, concentrations of MeHg in predatory fishes can remain elevated for years following impoundment due to release of Hg from soils and vegetation (Hall et al. 2005, St. Louis et al. 2004). Furthermore, atmospheric deposition of Hg is strongly linked to Hg in fish (Harris et al. 2007) with anthropogenic emissions contributing to two-thirds of all atmospheric deposition (Hammerschmidt and Fitzgerald 2006) and affecting fish populations that are far from any direct emissions source. Decreases in Hg emissions are predicted to result in rapid declines in fish concentrations (Harris et al. 2007).   Also, decreases in anthropogenic SO_4^{2-} deposition (i.e. acid rain) are associated with decreased MeHg

concentrations in fish (Dittman and Driscoll 2009); however, decreases in acid deposition have

been reported to increase dissolved organic matter inputs, thus increasing availability of Hg to

the food web (Hongve et al. 2012; Driscoll et al. 2013).   These examples, taken together with

our findings, further highlight the importance of processes at the base of the food web in driving

uptake and trophic transfer of Hg.

CHAPTER 5
5. SYNTHESIS

This study was the first of its kind to examine MeHg bioaccumulation at the base of

stream food webs that are influenced by beaver activity. Prior work had focused on elevated Hg

in water downstream from in-channel beaver impoundments (Driscoll et al. 1995; Roy et al.

2009b). In addition, this study contributed to our understanding of the ecology of Rocky

Mountain stream systems and their potential to be contaminated by Hg.

The global reservoir of atmospheric Hg has increased 2-5 times since the onset of the

industrial revolution (Boening 2000). Hg is rapidly circulated around the globe in its gaseous

elemental form, Hg^0, meaning that although emission sources tend to be clustered around areas

of human industrialization, Hg is problematic across all landscapes regardless of remoteness, due

to long range transport (Morel et al. 1998; Driscoll et al. 2013). Hg^0 is slowly oxidized to Hg(II),

enhancing its ability to circulate in the atmosphere for long periods of time. Hg(II) is then

deposited onto the landscape via precipitation (wet deposition) and by adhering to particulates

(i.e. aerosols, soot) where it enters terrestrial and aquatic environments (Morel et al. 1998).

However, environmental and health impacts are only indirectly related to ambient concentrations

in abiotic compartments because toxicity generally results from the net conversion of Hg(II) to

MeHg, the more bioaccumulative and toxic form (Driscoll et al. 2013).

In freshwater systems, Hg(II) undergoes methylation by sulfate-reducing microbes in the

low oxygen environments of wetlands and sediments (Gilmour et al. 1992). Human and wildlife

exposure occurs almost entirely via the consumption of MeHg contaminated organisms. In lakes,

the average proportion of total Hg as MeHg increases from about 10% in water to 15% in

phytoplankton, 30% in zooplankton, and 95% in fish (Watras and Bloom 1992); these increases

are mirrored in stream food webs (Kidd et al. 2012). Reasons for differences in the proportion

of MeHg in different biological compartments include the relative concentrations of Hg(II) and

MeHg likely to be present and lipid solubility of Hg species (Boening 2000). This makes uptake

into primary producers (periphyton) and efficiency of assimilation by grazers important (Morel

et al. 1998). Thus, the impact of Hg on ecosystems is related not only to regional and global

emissions and deposition, but also to the potential for aquatic systems to methylate Hg,

subsequent biomagnification of MeHg in food webs, and the processes that drive net methylation

and trophic transfer (Driscoll et al. 2007; Munthe et al. 2007; Driscoll et al. 2013).

This study offers an important piece of the puzzle for researchers studying MeHg in

running waters. Contaminant cycling in river systems that are naturally engineered by beavers

receives comparatively little attention to their man-made counterparts. While MeHg in aquatic

systems has been well-studied, the degree of complexity and controls on its availability are still

not entirely understood, especially in stream ecosystems (Ward et al. 2010). For perspective,

approximately 1.3 million river miles in the United States alone (or approximately 40%) are

under an Hg advisory (US EPA 2010). My study revealed that elevated MeHg concentrations in

water downstream from impoundments enters the food web and biomagnifies to higher

consumers. MeHg at the primary consumer level often dictates subsequent trophic transfer up

the food chain (Chasar et al. 2009); therefore, understanding the processes, such as ecosystem

engineering by beavers, that change the way materials flow through streams are key in increasing

our understanding of the behavior of Hg in aquatic systems. Furthermore, beavers have the

ability to create impoundments across a wide variety of climates and environments throughout

North America. As such, these impoundments can occur in areas that receive large loads of

atmospheric Hg deposition (Hammerschmidt and Fitzgerald 2006), areas near point source

emitters of Hg (Jardine et al. 2009) and areas that are naturally Hg-sensitive due to prevailing environmental conditions (Ward et al. 2010).

I hypothesized that in-channel beaver impoundments would increase food-web available Hg and enhance resource availability in these systems. Below beaver dams, I expected higher Hg concentrations in all biological compartments, higher nutrient concentrations (TP, TN and DOC), more algal growth (measured as benthic chl *a*), and greater densities of invertebrates (measured as total invertebrate biomass). In terms of food webs/trophic transfer, I hypothesized that beaver impoundments would cause a shift in the flow of energy (primary consumer derived C) from upstream to downstream. Assuming increased productivity and algal growth, I hypothesized an increased reliance on aquatically derived C (algal-based) as opposed to terrestrially derived C (terrestrial leaf litter). This was important because algal C is associated with higher available MeHg concentrations at the base of food webs (Jardine et al. 2012; Riva-Murray et al. 2013). Increased productivity also has the potential to result in increased invertebrate body size and the addition of more trophic linkages (i.e. a longer food chain with more predators); therefore, I also hypothesized that an increase in invertebrate biomass and body size would also be associated with higher MeHg concentrations and increased trophic transfer. Through the sum of all of these processes, I then expected that beaver impoundments would have the potential to cause an increased risk to fish and ultimately fish-eating wildlife downstream. However, I understood that processes acting on Hg would be complex and made a secondary hypothesis that the rate of biomagnification could also be lower due to growth dilution from enhanced productivity downstream from beaver dams (Pickhardt et al. 2002). This would limit MeHg availability to predators further up the food chain, resulting in little or no risk to piscivorous wildlife and humans, despite increases in water and at lower trophic levels.

My overall findings are summarized in a conceptual model (Fig. 5-1). I observed a consistent increase in MeHg concentrations and % MeHg downstream from in-channel beaver impoundments in all biological compartments (water, periphyton and invertebrates, Fig. 5-1). This suggests that net methylation is occurring in the ponds and the MeHg is being subsequently released downstream. However, unlike other studies on beaver impoundments (Naiman et al. 1986, 1988; Roy et al 2009ab), this was not accompanied by an increase in nutrients or algal and invertebrate biomass downstream (Fig. 5-1). This could potentially be attributed to local conditions. For example, my study streams had limited local litter input from trees compared to other studies referenced here (Roy et al. 2009b; Naiman et al. 1988) likely due to the short growing season at high elevations with snowmelt occurring relatively late in the year. For example, in sub-Antarctic streams in Patagonia, South America, where *Castor canadensis* is invasive, Anderson and Rosemond (2010) found that the greatest impact of beaver activity on food web resources is seen in the ponds themselves. There, the overall effect of in-channel impoundments on resources at the base of food webs below ponds was attenuated by local conditions at high latitude (56°S), with low nutrient concentrations similar to the streams studied here. These features of mountain systems therefore provide different perspectives on beaver impacts since hydro-climatic conditions are unlike those observed at other beaver research sites in lowlands (Naiman et al. 1986; 1988; Roy et al 2009ab). For example, in an area where vegetation is largely comprised of deciduous trees with a large annual leaf dropping event, the ponds themselves would act as a sink for the high volume of terrestrial inputs, thus increasing the amount of standing stock associated with ponds. This highlights the importance of studying the effects of impoundments in many different ecosystem types – mountains, forests and plains would likely all exhibit different responses.
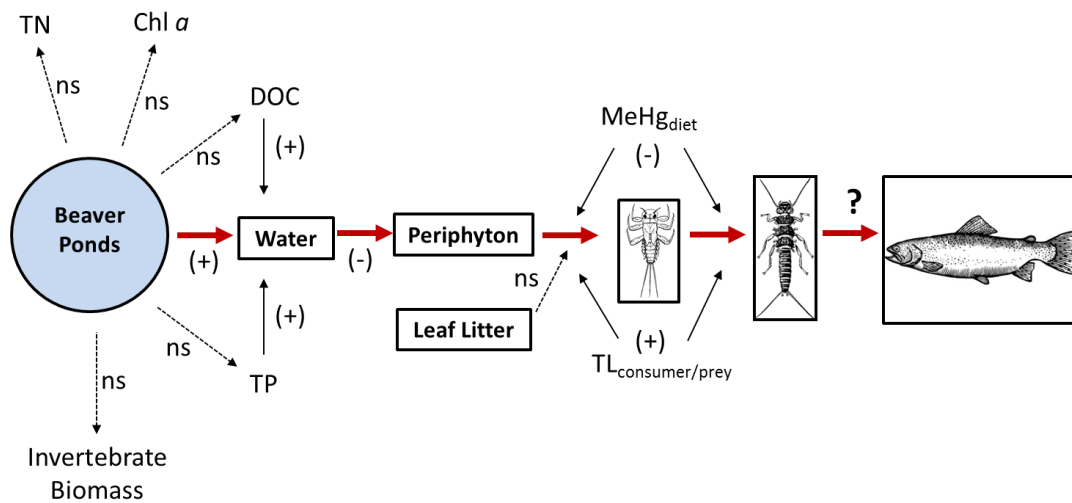
Fig. 5-1 Conceptual model showing drivers of supply and trophic transfer in the study systems. Objects enclosed in solid lines represent compartments. Dashed arrows indicate no effect (ns = not significant), solid arrows indicate a positive (+) or negative (-) effect. Red bold arrows indicate transfer of Hg.

While I did not observe an increase in DOC below impoundments (Fig. 5-1), it is apparent based on AIC analysis that DOC is an important driver of variability in MeHg concentrations in these systems. Variation in DOC could be driven by the long-term effects of beaver activity (Westbrook et al. 2011) as well as other landscape features (wetlands, peat deposits, deciduous trees) that create between-stream differences. I observed a positive association between DOC and MeHg concentrations in all compartments at upstream sites, consistent with a role for DOC in aiding in the transport of Hg into systems. For example, in lakes with DOC values below 4.0 mg/L, McMurty et al. (1989) observed that Hg concentrations in lake trout muscle tissue were strongly positively correlated with DOC, which explained 37% of the variation in Hg concentrations. This is in agreement with Driscoll et al. (1995) who observed increased concentrations of MeHg in fish tissue associated with levels of DOC up to 8 mg/L in Adirondack lakes. However, at higher concentrations DOC can interact competitively with Hg for binding sites, inhibiting uptake (Dittman and Driscoll 2009). This was evident in my work as well. The low DOC Alberta sites showed positive correlations between MeHg in all biological compartments and DOC as well between BMFs and DOC. When higher DOC New Brunswick sites were added to the analysis, that effect was no longer apparent.

Despite my predictions, I did not observe a shift in dietary carbon source downstream from impoundments. Rather, I observed complete reliance on aquatic carbon both up- and down-stream (Fig. 5-1). Stream invertebrate community structure provides clues as to why this occurred: shredding invertebrates were not present, there was limited canopy cover (Table A-1), and leaf litter was only present in small quantities. The small offset between periphyton $\delta^{13}C$ and invertebrate $\delta^{13}C$ (Fig. A-5) is likely attributed to impurities in the bulk algal sample rather than consumption of an alternative basal energy source. Hamilton et al. (2005) describe caveats

associated with bulk biofilm scrapings, stating that these samples are often comprised of algal material and detritus including bacterial cells and inorganic components. Invertebrates may selectively feed on only some of these components (Clapcott and Bunn 2003); therefore these impurities should be considered when reporting stable isotope ratios. Anderson and Rosemond's (2010) study of sub-Antarctic streams, which were largely reliant on terrestrial carbon, showed that beaver ponds increase autochthonous resources downstream, but this shift was small relative to the overall terrestrial influence. Stream food webs, such as the ones in this study that are entirely reliant on aquatic carbon (McCutchan and Lewis 2002), accumulate Hg more effectively than those with reliance on terrestrial carbon because terrestrial C sources are associated with lower Hg than aquatic C sources (Jardine et al. 2012). This suggests that when beavers impound waterways in areas where terrestrial inputs dominate stream food webs (Anderson and Rosemond 2010), Hg availability to basal food web organisms may be low even if water Hg concentrations are elevated.

I measured trophic transfer of Hg by calculating TLs, TMFs, BCFs, BAFs and BMFs. Overall TMFs of 2.3 for MeHg and 1.2 for THg were at the low end of the range for freshwater systems (8.3 ± 7.5 for MeHg and 4.3 ± 4.8 for THg, Lavoie et al. 2013). This means that MeHg is biomagnifying at a rate of approximately 2.3 times per trophic level in these systems, though this number would have been higher had I used a more conventional TEF for $\delta^{15}N$ (Post 2002). Care should thus be taken to choose a suitable TEF for a food web biomagnification study because depending on the characteristics of the food web, this value could depart greatly from the typical literature value of 3.4‰ (Bunn et al. 2013). Small changes in TEF can result in very different TMFs (Lavoie et al 2013).

MeHg BCFs are on the order of 10,000 to 100,000 (Boening 2000). I observed BCFs for periphyton ranging from 12,000 to 344,000 while BMFs for invertebrates were 3 to 48 for the grazer/periphyton linkage and <1 to 10 for the predator/grazer linkage, resulting in BAFs of 1,259,950 for grazers/water and 1,969,288 for predators/grazers, consistent with the range (100,000 to 48,000,000) reported by DeForest et al. (2007). The magnitude of change decreased with each subsequent trophic linkage, which emphasizes the importance of uptake from water to periphyton in dictating MeHg availability to higher trophic levels. This, combined with the greater uptake of Hg at low concentrations, highlights supply at the base of the food web as a key driver of subsequent biomagnification.

BMFs were particularly influenced by the relative difference in TL from prey to consumer (Fig. 5-1). TL, calculated using $\delta^{15}N$, refines BMFs and is particularly useful when some trophic linkages in a food web may be unknown. The importance of the relative difference in TL from prey to consumer in determining BMFs is intuitive because the significant positive linear relationship between Hg and TL is well documented (Kidd et al. 1995). Here, I stress the importance of accurately incorporating stable isotopes and TLs into biomagnification studies rather than making assumptions about diet (Dietz et al. 2000). There could be a small difference in TL between an omnivore and a predator which would equate to a small $TL_{prey/consumer}$ and BMF even though the two taxa might be compared as a predator and its prey. For example, at some sites in this study grazing mayflies had higher Hg concentrations than predators, resulting in lower BAFs for predators than for grazers. Some mayflies occupied similar TLs to predatory invertebrates, suggesting omnivory via the consumption of bacteria or other animal particles. Assuming strict adherence to functional feeding group classifications (Merritt and Cummins

1996) may be erroneous because invertebrates, especially facultative omnivores, likely deviate from functional feeding groups as defined in the literature.

My AIC analysis revealed a negative association between $MeHg_{diet}$ and BMF, meaning that biomagnification occurs at a greater rate when MeHg concentrations in prey organisms are low. This means that at low concentrations, MeHg is transferred more efficiently through the food web than at higher concentrations. This "uptake paradox" has been reported in other studies (Tsui and Wang 2004; DeForest et al. 2007; Lavoie et al. 2013) and may be explained by a saturation of binding sites for Hg combined with slow turnover. Once MeHg is bound, it is not easily excreted, thus adding more Hg to the system would not necessarily increase uptake. The physiological turnover rate constant for MeHg is smaller than that of other trace metals (Cd, Cr, Zn and Se) and inorganic Hg (Tsui and Wang 2004). In a laboratory-based experiment using *Daphnia magna*, Tsui and Wang (2004) found that after seven days of depuration, *D. magna* retained 57 to 66% MeHg while they only retained 37 to 46% Hg(II). The authors also observed decreasing concentration factors with increased exposure concentrations, which led them to suggest the possibility that saturation of binding sites is achieved at higher concentrations.

In addition to using $\delta^{15}N$ to calculate TLs, it was also regressed against invertebrate body size. I hypothesized that increases in nutrients below impoundments would equate to more resources for the growth of invertebrates, resulting in larger-bodied organisms (Anderson and Cabana 2009). Larger body size is associated with higher trophic levels (i.e. predators) and therefore accumulation of greater concentrations of Hg, so it was of particular interest in this study. However, I found that mean body size was the same up- and downstream of beaver ponds and overall it was not a driver of Hg concentrations (Chapter 4). Invertebrate sizes, densities and species abundance and TLs were the same above and below ponds suggesting that if beaver

activity alters invertebrate communities, the change is confined to within the ponds themselves, as was observed in the Anderson and Rosemond (2010) study. The beaver ponds studied here were likely relatively new because of recurring floods and breaching, which may limit the establishment of new communities. Beaver ponds are associated with habitat creation, particularly for salmonids in mountain regions (Collen and Gibson 2001). If ponds created fish habitat, there would be addition of predators to the food chain. A longer food chain and with animals occupying higher TLs would mean further biomagnification of Hg.

Beavers flood landscapes in a similar way to human dams. I observed net methylation occurring in ponds which likely follows the same mechanism of flooding and subsequent methylation of Hg in human-made reservoirs. MeHg tends to be exported from reservoirs in the dissolved form (Schetagne 2000) and dissolved Hg is also likely the most important form in these systems. While I did not sample both particulate and dissolved fractions of nutrients and Hg, most particulates would have probably settled out in beaver ponds (Naiman and Melillo 1984) and are unlikely to contribute to loads in the downstream channel. Similarly, coarse debris is likely entirely retained in the ponds and sediments (Wohl et al. 2012), but would still provide a surface on which methylation can occur (Hall et al. 2004).

Streams vary in their sensitivity to Hg contamination (Driscoll et al. 2007; Ward et al. 2010). My study systems are not Hg-sensitive but the range of beavers includes many regions of greater Hg sensitivity. On a broad-scale, some high altitude areas (i.e. Adirondacks), those that experience high atmospheric Hg loading, and those with naturally low pH would all be susceptible. While pH did not play an important role in my study systems, its significance as an overall driver of Hg in many freshwater systems means it is worth mentioning here. The near neutral pH (mean = 8.1) of my study streams likely provides some protective effect from Hg. If

systems were instead low in pH, had low buffering capacity (soft water) or local geology that lends itself to these features then beaver impoundments could become problematic. For example, McMurty et al. (1989) observed that Hg concentrations in smallmouth bass were negatively correlated with variables affecting water hardness (Mg, Ca, conductivity) and positively correlated with those affecting acidity (pH, alkalinity). Jardine et al. (2013) found a significant relationship between pH and BMFs between predatory invertebrates and blacknose dace, indicating that pH can act directly on the accumulation of Hg by higher trophic level organisms. Should beavers build impoundments in low pH waterways such as those found in the Roy et al. (2009b) study, fishes residing downstream could have higher concentrations than what we might assume based only on concentrations in lower trophic level organisms.

In contrast, Hg outputs from ponds in areas that are less likely to be Hg-sensitive, such as those that are lower gradient (i.e. prairie or flood plains) and higher nutrient systems would likely be diminished. In low gradient systems, ponds would be expected to remain intact for a longer period of time due to a lack of high-flow events such as those observed in the mountains. This longevity would be associated with a decrease in Hg methylation over time (Bodaly et al. 2007). High nutrient systems where algal biomass is abundant are likely to be associated with lower Hg concentrations in organisms due to bloom dilution – an increase in algal cells results in a decrease in Hg concentration per cell (Pickhardt et al. 2002). This would result in lower inputs at the base of the food web. High DOC systems are also associated with decreased Hg concentrations, likely due to DOC forming complexes with Hg and inhibiting uptake (Dittman and Driscoll 2009). Therefore systems with high nutrient loads are likely to exhibit little, if any, increase in Hg concentrations downstream from beaver impoundments.

An additional factor to consider when assessing potential for Hg contamination downstream from beaver impoundments is dam age (Roy et al. 2009b). While I was unable to estimate dam ages, evidence suggests most are young. These mountain systems endure occasional high flows associated with rain on snow events, meaning the dams probably are breached at regular intervals. My observations of elevated Hg concentrations downstream from ponds, and data from previous research below experimental reservoirs (St. Louis et al. 2004) suggests these ponds are likely quite new (less than five years old). The continual breaching and rebuilding of beaver impoundments, particularly those in mountain regions, could therefore result in continual pulses into the system, similar to natural wetland disturbances that can induce mobility of Hg stored on the landscape (Zillioux et al. 1993). Because the cycle of breaching and rebuilding is an important reality in these mountain systems, I chose to include four breached dams in the study. Despite not actively holding water back, the large area of exposed sediment and alteration of normal flow patterns (Fig. A-2) makes these breached dams important disrupters of stream channels related to beaver activity. While it was not logistically possible to sample additional breached sites in this study, future work comparing breached vs. intact beaver dams would be interesting. I would hypothesize that recently breached ponds would still show a consistent downstream increase in MeHg, though perhaps not to the extent observed here with intact dams. In other systems where high flow events are uncommon, dams could persist for many years and MeHg concentrations downstream from ponds likely would decline with time (Roy et al. 2009b).

Beavers are often regarded as pests, and the implementation of removals/culls may occur even when their hydrological and ecological effects are positive (Collen and Gibson 2001; Westbrook et al. 2011). Beavers are major drivers of change in ecosystem and geomorphic

function. Their ponds in North America number in the millions, with billions of m^3 of sediment retained (Butler and Malanson 2005). The ecosystem services they provide are of great importance, forming ponds and meadows that contribute to landscape heterogeneity (Westbrook et al. 2011) and creating habitat for fishes and associated angling opportunities (Collen and Gibson 2001). It is estimated that over thousands of years, beavers have aggraded valleys, forming even valley plains and the fine silt retained in the beaver ponds has contributed to the rich farm land in the valleys of northern North America (Ruedmann and Schoonmaker 1938).

All of these positive effects mean we need to balance our interactions with beavers. While dam building does have consequences, including elevated Hg in streams and flood damage to human infrastructure and property, this does not mean beavers should automatically be labelled as pests. Because we now know that in-channel beaver impoundments consistently increase MeHg levels in basal food web organisms downstream, we can advise both researchers and land managers to consider the role of beaver activity in these sensitive regions, particularly where there is potential for accumulation of high MeHg concentrations in food sources for wildlife and humans.

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APPENDIX
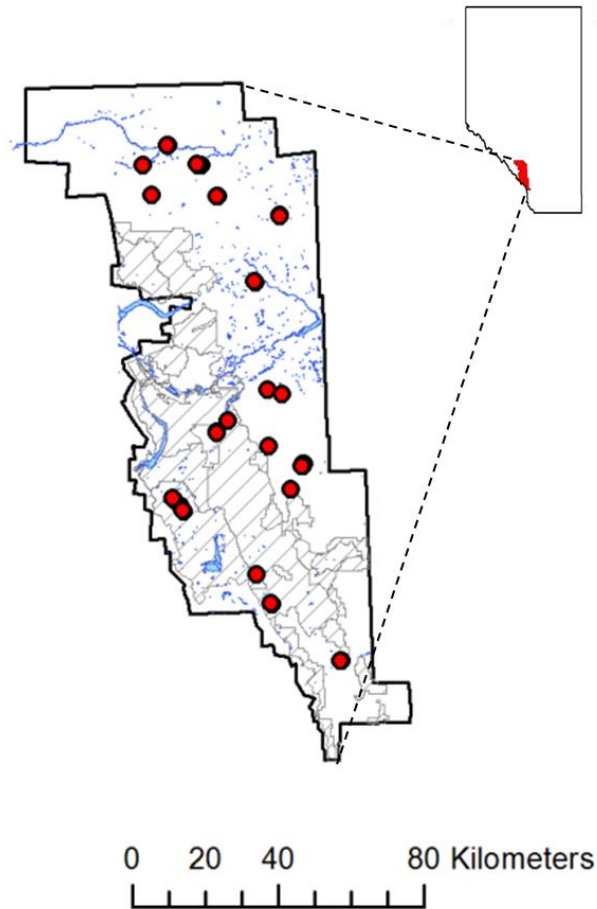
Fig. A-1 Study area comprising 7912 km^2. The hatched area represents the parks and recreational areas that make up Kananaskis Country. Major water features are highlighted in blue and sites are indicated by red circles (sites in very close proximity to one another overlap)

Fig. A-2 An intact (left) and recently breached (right) beaver impoundment
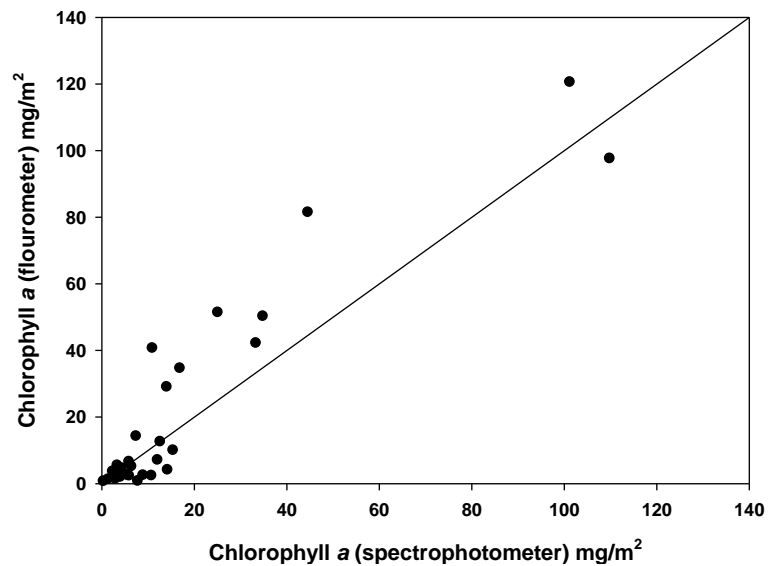
Fig. A-3 Comparison of chlorophyll *a* concentrations obtained using the two methods outlined in the text. Line represents 1:1.
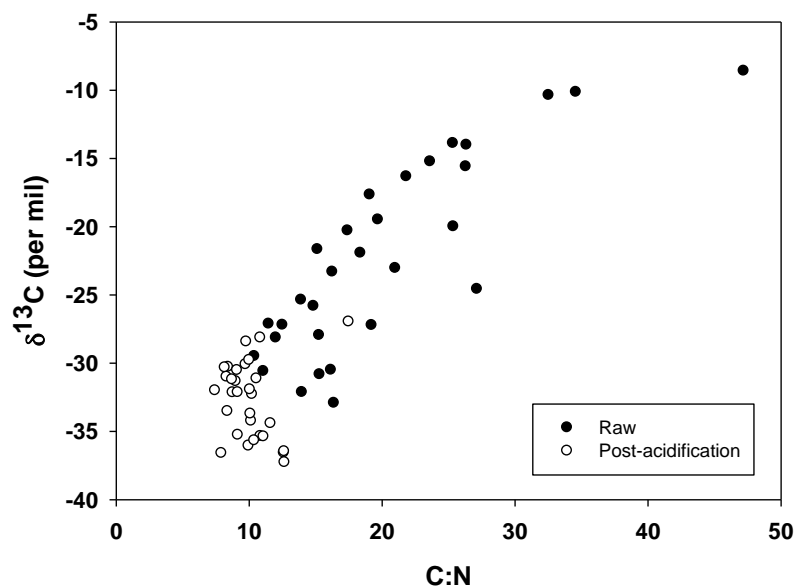
Fig. A-4 Change in C:N and $\delta^{13}C$ pre- ("Raw") and post-acidification of periphyton samples. High carbonate content caused erroneous enrichment of ^{13}C, necessitating acid treatment.
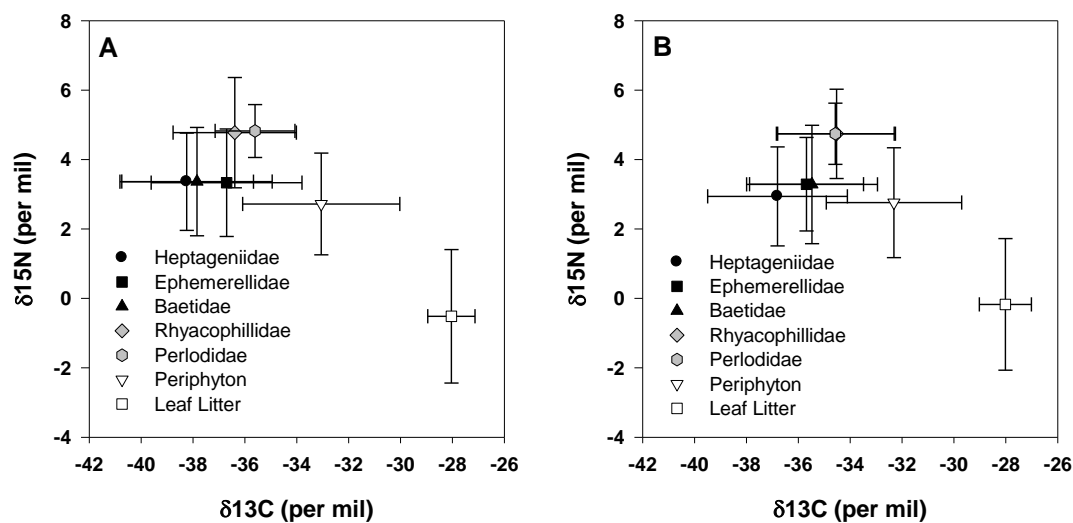
Fig. A-5 Isotope bi-plots showing $\delta^{13}C$ and $\delta^{15}N$ values for invertebrate food webs in streams A) upstream and B) downstream of beaver impoundments.

Fig. A-6 Total mercury (THg) concentrations in periphyton vs. leaf litter. Each point represents a site

Table A-1 Environmental characteristics of streams sampled upstream (US) and downstream (DS) of beaver impoundments in the study area.

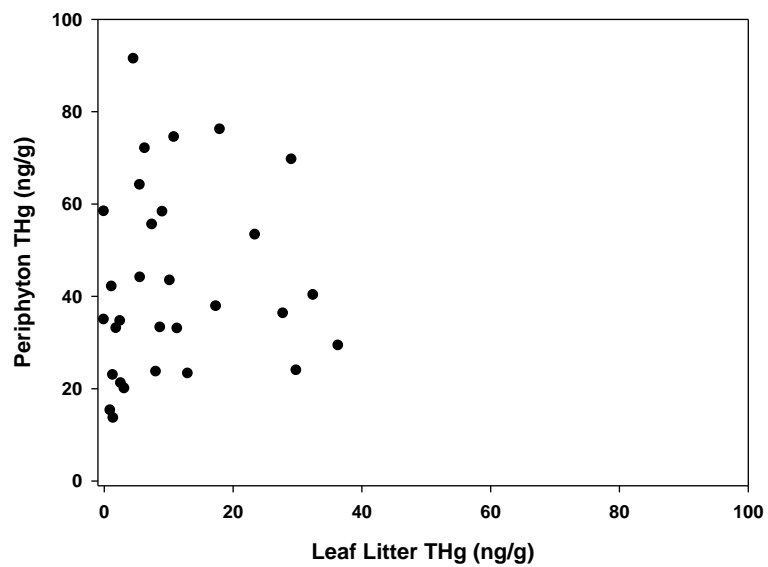| Site		Lat	Lon	Stream Width	Canopy Cover	Substrate Composition (%)							pH	Cond (µS/cm)	Turb (NTU)
						bedrock	boulder	rock	coble	gravel	sand	mud			
BEV012	US	50.759	115.264	8	0	0	0	0	40	50	10	0	8.2	203	1.51
	DS	50.758	115.263	8	0	0	0	0	40	40	10	10	8.1	206	1.27
BEV002	US	51.318	114.960	2	50	0	0	10	0	40	0	50	8.2	400	13.30
	DS	51.317	114.958	2	25	0	0	20	0	30	20	30	8.3	207	10.25
BEV004	US	50.379	114.668	3	0	0	0	10	40	10	0	40	8.0	312	0.21
	DS	50.378	114.666	3	10	0	0	40	40	0	20	0	8.0	318	0.42
BEV015	US	51.051	114.924	4	30	0	0	40	40	0	10	10	8.3	299	0.25
	DS	51.038	114.868	3	10	0	0	40	10	10	0	40	7.9	305	8.13
BEV028	US	50.526	114.930	2	10	0	0	30	30	10	10	20	8.1	244	0.10
	DS	50.523	114.927	1.5	0	0	0	30	40	20	0	10	8.2	245	1.06
BEV020	US	50.865	114.790	3	0	0	0	0	50	15	15	20	7.9	287	0.15
	DS	50.866	114.787	2.5	10	0	0	30	30	30	10	0	8.1	293	1.17
BEV021	US	50.860	114.795	5	10	0	0	20	30	30	10	10	8.1	298	0.97
	DS	50.862	114.794	3	20	0	0	20	30	20	10	10	8.2	299	0.60
BEV019	US	50.803	114.844	6	0	0	0	0	20	20	20	40	8.1	263	0.12
	DS	50.804	114.842	3	0	0	0	10	40	30	10	10	8.2	262	0.91
BEV024	US	51.609	115.161	2.5	10	0	0	10	30	30	15	15	8.3	392	2.89
	DS	51.609	115.160	2.5	10	0	0	0	50	20	0	30	8.3	390	3.72
BEV025	US	51.610	115.176	3	0	0	0	10	30	20	10	30	8.3	402	1.82
	DS	51.609	115.170	2	0	0	0	10	30	30	10	20	8.2	406	2.83
BEV027	US	51.531	115.099	3	0	0	0	10	45	35	10	0	8.1	168	5.35
	DS	51.531	115.097	2.5	0	0	0	10	30	30	10	20	8.4	166	5.98
BEV030	US	50.789	115.304	3	20	10	0	10	30	10	10	30		321	0.10
	DS	50.787	115.299	4	0	0	0	20	50	10	0	20	7.8	302	1.34
BEV026	US	51.659	115.292	3.5	0	0	5	20	30	30	10	5	7.9	354	1.06
	DS	51.659	115.290	3.5	0	0	0	10	40	20	0	30	7.9	355	1.23
BEV022	US	51.478	114.855	3	0	0	0	0	20	50	15	15	8.0	261	2.01
	DS	51.480	114.853	4	0	0	0	0	40	30	15	15	7.8	259	3.90
BEV010	US	50.949	115.126	9	0	0	0	5	60	20	10	5	7.9	326	0.13
	DS	50.951	115.126	3	0	0	0	0	60	20	10	10	7.9	325	0.14

Table A-2 Pearson correlation matrix illustrating relationships between measured variables at sites upstream from beaver impoundments. Top values are Pearson correlation coefficients (ρ) and bottom italicized values are probabilities (p-values). Variables that are significantly correlated p ≤ 0.05 are highlighted in bold text.

	Water MeHg	Periphyton MeHg	Herbivore MeHg	Predator MeHg	TP	TN	pH	Cond.	Turb.	DOC	Benthic Chl *a*	Invert. Biomass
Water MeHg	1.000											
	0.000											
Periphyton MeHg	0.378	1.000										
	0.165	*0.000*										
Herbivore MeHg	**0.637**	**0.637**	1.000									
	0.014	***0.014***	*0.000*									
Predator MeHg	-0.017	**0.823**	**0.811**	1.000								
	0.964	***0.006***	***0.014***	*0.000*								
TP	**0.532**	**0.691**	**0.631**	0.464	1.000							
	0.041	***0.004***	***0.016***	*0.209*	*0.000*							
TN	**0.504**	0.402	**0.555**	0.472	**0.772**	1.000						
	0.055	*0.137*	***0.039***	*0.200*	***0.001***	*0.000*						
pH	0.112	0.232	0.029	0.294	**0.616**	**0.569**	1.000					
	0.691	*0.406*	*0.921*	*0.442*	***0.014***	***0.027***	*0.000*					
Conductivity	0.059	-0.216	0.181	0.259	0.023	0.264	0.061	1.000				
	0.834	*0.438*	*0.535*	*0.502*	*0.934*	*0.341*	*0.830*	*0.000*				
Turbidity	**0.551**	0.443	**0.633**	0.293	**0.813**	**0.706**	0.495	0.052	1.000			
	0.033	*0.098*	***0.015***	*0.445*	***0.000***	***0.003***	*0.061*	*0.853*	*0.000*			
DOC	**0.585**	**0.638**	**0.710**	0.655	**0.857**	**0.809**	0.415	0.215	**0.706**	1.000		
	0.022	***0.011***	***0.004***	*0.055*	***0.000***	***0.000***	*0.124*	*0.442*	***0.003***	*0.000*		
Benthic Chl *a*	-0.241	-0.087	-0.156	0.227	-0.421	-0.255	-0.183	-0.028	-0.562	-0.413	1.000	
	0.387	*0.758*	*0.594*	*0.557*	*0.118*	*0.358*	*0.513*	*0.922*	*0.029*	*0.126*	*0.000*	
Invertebrate Biomass	0.073	0.297	0.136	**0.743**	0.091	0.117	0.247	-0.036	0.068	-0.024	0.322	1.000
	0.795	*0.282*	*0.643*	***0.022***	*0.746*	*0.679*	*0.374*	*0.899*	*0.810*	*0.934*	*0.242*	*0.000*