LEGACY CONTAMINANTS IN BROOK TROUT FROM REMOTE NEW BRUNSWICK LAKES

BY

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A thesis submitted to the
Department of Biology
Mount Allison University
in partial fulfillment of the requirements for the
degree of Master of Science

January 3, 2023

Abstract

Dichlorodiphenyltrichloroethane (DDT) and mercury are legacy contaminants that are persistent in the environment for decades or longer. They may impact food webs through bioaccumulation, biomagnification, and chronic toxic effects in exposed biota. DDT was applied aerially on large scales to the conifer forests in north-central New Brunswick between 1952 and 1968 for pest control. I sampled seven lakes in the summers of 2020 and 2021 to investigate the impact of historical DDTs on the present day aquatic environment. I sampled brook trout, aquatic invertebrates, zooplankton, and lake surface sediments. I found DDTs were up to ~14 times higher in brook trout muscle tissues from lakes where DDTs were applied to the watershed compared to reference lakes. DDTs in brook trout exceeded CCME guidelines by up to ~22 times. Mercury is a common contaminant of concern and delivered to lakes by atmospheric transport. Most mercury originates from anthropogenic activities, including burning fossil fuels, and mining or smelting metals rather than natural geogenic sources. I sampled brook trout from seven New Brunswick lakes in 2020, and found mercury levels comparable to similar studies in eastern North America. Mercury in brook trout exceeded CCME guidelines for methylmercury by up to ~12 times. Using DDT and mercury measures, we can further our understanding of legacy contaminants in lakes of New Brunswick, Canada.

Acknowledgments

Thank you to my thesis supervisor, Dr. Joshua Kurek, for his unwavering support and encouragement. I would also like to thank my supervising committee, Dr. Andrea Morash and Dr. Justin Liefer, and my external examiner, Dr. Mike Van Den Heuvel. Thank you to Dr. Christopher Edge and Dr. Karen Kidd for their assistance, expertise, and support. I would also like to thank the past and present members of ECAB who have supported my research and made my time at Mount Allison so much fun (especially during a global pandemic).

A very special thank you to everyone who joined me in the field. Without the help of all of you, I would not have any fish to study. Emotional meltdowns, canoe flips, and road washouts couldn't stop such a great team.

I could not have done any of this without the support of my friends and family. To Alex Di Lonardo and Andrew Labaj, thank you for listening, giving great advice, and being great friends. Your encouragement has pushed me to get 'er done. To Branaavan Sivarajah, thank you for your support and your help with statistics. To Julia Campbell, thank you for being a fantastic dive buddy. Diving with you was a cure for burnout when I needed it the most. To Shae-Lyn Redmond, thank you for being a great friend, host, and running partner when I needed time away. To Sarah Waldron, thank you for being my emotional support undergrad. To Madeline Power, thank you so much for making my time at Mount A so wonderful. And finally, to my family, thank you for your support, love, and confidence in me. You have made this possible.

"They might have split up or they might have capsized
They may have broke deep and took water"
– Gordon Lightfoot

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Chapter 1

General Introduction

1.1 Legacy contaminants

Legacy contaminants are pollutants that are widespread and persist in the environment for decades or longer – even after inputs have stopped or have been greatly reduced through control measures. Many legacy contaminants are persistent, bioaccumulative and/or toxic substances, and are of interest globally because they are prone to long-range atmospheric transport (Hutchinson et al., 2013). A legacy contaminant is usually classified by its environmental persistence, ability to mobilize or volatize, and/or the harmful effects it may have on the ecosystem and biota (Matthies et al., 2016). Over the past few decades, legislative action has limited the production of and reduced exposure of legacy contaminants. However, many legacy contaminants can remain bioavailable in the environment well into the future, and still pose a risk to ecosystem health if exposure potential exists and contaminant levels are high enough to harm biota.

When considering the future consequences of persistent contaminants, climate change may have an effect on the degradation and bioavailability of contaminants. Increased temperature and precipitation due to climate change may lead to shifting environmental processes such as altered trophic structures, bioaccumulation and biomagnification, and changes to organic carbon cycling (Noyes et al., 2009). Shifting environmental conditions could lead to an increase in contaminants in water, soil, sediment, and biota (Noyes et al., 2009).

1.2 Bioaccumulation in the aquatic food web

Bioaccumulation occurs when an organism experiences an increase in the concentration of a contaminant due to uptake through diet, respiration, dermal absorption, or other exposures at a greater rate than the organism can expel the contaminant (Mackay & Fraser, 2000). Many contaminants in fishes undergo metabolic transformation and are eliminated from the gills or through feces and urine (Wang & Wang, 2005).

Contaminants with low water solubility often have low elimination rates (Wang & Wang, 2005; Hellou et al., 2013). Bioaccumulative substances will typically increase within an organism with increasing size, age, lipid content of cells, fat composition of the organism, and length of food web (Wang & Wang, 2005; Dórea, 2008).

Biomagnification is a type of bioaccumulation that occurs when a contaminant concentration increases in an organism's tissues at greater levels than expected, with increasing trophic position within a food web (Mackay & Fraser, 2000). The chemical concentrations in the predator are greater than the concentrations in the prey item (Kelly et al., 2004). Contaminants are more susceptible to biomagnification in fish when they are poorly metabolized, hydrophobic, and lipid soluble (Kelly et al., 2007). Biomagnification often negatively impacts predators at the top of the food-web, as they are accumulating more contaminants than consumers in lower trophic positions. Negative impacts in fishes are targeted on the central nervous system, which may cause behavioural abnormalities, decreased fitness, or death (Anderson & Peterson, 1969; Beckvar & Lotufo, 2011). For example, bioaccumulation and biomagnification in aquatic food webs pose potential health risks for both humans and high trophic level wildlife, as they may reach toxicologically significant concentrations in their organs and tissues (Stafford & Haines, 1997; Kelly et al., 2004, 2008).

In aquatic ecosystems, many contaminants will often preferentially bind to the organic matter in the water column or sediments, rather than dissolve in water (Morrison et al., 1996). Invertebrates are a recognized exposure pathway between contaminants and the aquatic food web. Benthic invertebrates, a key prey item for aquatic organisms, accumulate contaminants because their diet consists of primary consumers that may absorb contaminants (Augusto et al., 2022). Additionally, many benthic invertebrates live in direct contact with lake sediments for a large portion of their lifecycle, and are exposed to and can absorb contaminants because of their physiology and behavior (Morrison et al., 1996). As a result, benthic invertebrates are a primary vector between sediment-bound contaminants and predatory organisms in aquatic food webs.

Brook trout (*Salvelinus fontinalis*) are a cold-water salmonid species, native to eastern North American rivers and lakes. Their diet consists primarily of invertebrates, small fish, and other small vertebrates (Allan, 1981). Brook trout are one of the most popular sport fish in New Brunswick, Canada. They are the number one freshwater species targeted by recreational anglers in the province, giving them great cultural and economic importance (Government of Canada, 2019). The species is found in ~1,700 of the province's ~2,500 lakes, which makes it a popular resource for anglers and wildlife (NB NRED, n.d.). They prefer oligotrophic lakes with cold, clear water (Raleigh, 1982). Brook trout, like other aquatic species, are susceptible to bioaccumulation because of exposure to contaminants in the aquatic environment through their diet.

1.3 DDT and its use in New Brunswick

Dichlorodiphenyltrichloroethane (DDT) was first synthesized in 1874, and began use as a pesticide in 1939 (World Health Organization, 2004; Hellou et al., 2013). DDT has been used globally as an insecticide since World War II to manage forest and agricultural pests, biting insects, and disease-carrying insects. Use of DDT in Canada began in 1944 with a joint Canada-USA project in Algonquin Park, Ontario (Peakall et al., 1983). DDT succeeded in its ability to kill target pests, but caused toxic effects to non-target organisms in areas of application. Among the most famous example of DDT effects causing harm to wildlife was the discovery of declining bird populations, popularized by Rachel Carson's *Silent Spring* (1962), which spurred the environmental movement in North America. DDT can adversely affect the heath of humans and wildlife through endocrine disruption, neurotoxic and carcinogenic effects, decreased reproductive success, and developmental delays (Martí et al., 2010; Hellou et al., 2013; Zhang et al., 2014; G.-R. Chang, 2018). DDT has been restricted in Canada since 1970 and banned since 1985 (CCME, 1999d). Today, it is globally banned for agricultural use under the 2001 Stockholm Convention on Persistent Organic Pollutants, and is only used in countries where the benefits of reducing human illness caused by biting insects outweigh the risks of DDT exposure (CCME, 1999d; Environment and Climate Change Canada, 2020).

Despite being banned for decades, DDT still presents a risk in Canada due to its long persistence in the environment, particularly in aquatic systems. The half-life of DDT in the environment, or the time during which the concentration decreases to one half of the initial concentration, ranges from days to decades, and varies depending on environmental conditions (e.g., moisture, temperature, photochemistry) (Hellou et al., 2013). DDT will naturally degrade into its metabolites, dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD), but degradation favours DDE under aerobic conditions and DDD under anaerobic conditions (Agency for Toxic Substances and Disease Registry (ATSDR), 2019). DDE is the primary degradation product of DDT, and the terminal degradation product of DDD (Bumpus & Aust, 1987). The half-life of DDT is longer under aerobic conditions (between 45 days and >15 years) (Lichtenstein & Schulz, 1959; Stewart & Chisholm, 1971; van den Berg, 2009), and shorter under anaerobic conditions (between 7 and 100 days) (van den Berg *et al.* 2009; Castro & Yoshida 1971). The half-life for the metabolite DDD is estimated to be >190 years (OMEE, 1993).

DDT is mostly insoluble in water (van den Berg, 2009), but it adsorbs to soils, sediments, and organic matter in aquatic environments and is transferred from the water column to the benthos through sedimentation (Muir et al., 1995). Sediment-bound DDTs are bioavailable to organisms in contact with contaminated sediments, but DDT concentrations in newly deposited sediments have declined dramatically in regions with DDT restrictions. As a result, DDT exposure for aquatic organisms, which are most abundant in the uppermost sediment layers, generally decreases over time as contaminated sediments are buried under more recently deposited sediments. Hence, lake sediments are often considered as a sink for contaminants such as DDTs.

DDTs will bioaccumulate in an organism and biomagnify with increasing trophic position. DDTs are hydrophobic and lipophilic, so they are preferentially stored in tissues with high lipid content, such as the liver (Ronald et al., 1984; ATSDR, 2019). DDT will distribute throughout an organism; however, among body tissues, muscle tissue typically has the lowest concentrations of DDT (Ronald et al., 1984). Piscivorous species tend to have the highest DDT levels among terrestrial mammals (CCME, 1999). The Canadian tissue residue guideline is 14.0 µg/kg (wet weight) for wildlife consumers of aquatic biota (CCME, 1999d).

DDT was used by the New Brunswick (NB) forestry sector between 1952 and 1968 to manage outbreaks of spruce budworm (*Choristoneura fumiferana*) (Armstrong & Cook, 1993). Spruce budworm are a pest native to NB and eastern North America that cause large-scale defoliation of softwood trees during population outbreaks that occur, on average, every 35 years in NB (Royama, 1984). Damage caused by spruce budworm can result in millions of dollars in annual economic losses (up to $160 million dollars annually) to the softwood-dependent provincial forestry sector (W.-Y. Chang et al., 2012).

The introduction of DDT quickly expanded spruce budworm management across most forested land in north-central New Brunswick (Peakall *et al.* 1983). At least 5.7 million kg of DDT were applied via airplanes in NB between 1952 and 1968 – on average between 1.0 and 6.5 kg per hectare (Peakall et al., 1983)**.** Between 75,330 (in 1952) and 2.1 million hectares (in 1957) of conifer forests were treated with DDT, and many hectares were treated for multiple years (Peakall et al., 1983). In comparison, the surrounding states and provinces applied much less DDT to their conifer forests. For example, the neighboring province of Quebec reportedly applied 0.9 million kg of DDT (Peakall et al., 1983).

A recent investigation of five headwater lakes in north-central NB used dated sediment cores to analyze the DDT, DDE, and DDD (henceforth collectively referred to as DDTs) levels within lake sediments over time in response to aerial DDT application (Kurek et al., 2019). DDT concentrations in the sediments of these five lakes were some of the highest reported in North America. The maximum sedimentary DDTs in each lake were: ~139 μg/kg at Goodwin Lake, ~297 μg/kg at California Lake, ~567 μg/kg at Middle Peaked Mountain Lake, ~580 μg/kg at Sinclair Lake, and ~4500 μg/kg at Upsalquitch Lake. The DDTs in the lake sediments exceed the probable effect level (PEL) sediment guidelines for DDT (4.77 μg/kg), DDE (6.75 μg/kg), and DDD (8.51 μg/kg) by up to 452 times (CCME, 1999a; Kurek et al., 2019). Additionally, a comparison of subfossil remains of crustacean zooplankton pre- and post-DDT application showed a major shift in the zooplankton composition that corresponds to the introduction of DDT in lake sediments (Kurek et al., 2019). The elevated concentrations of DDT and its metabolites in modern sediments more than 50 years after the final aerial application is alarming and warrants further investigation. To compare these results to other Canadian studies, Nicola Lake, in the Fraser River watershed (British Columbia) had sedimentary ∑DDT at 323 μg/kg in a study from 2000. Watson Lake (Yukon) had

∑DDT at 2680 µg/kg in ~1952 (Macdonald et al., 2000; Rawn et al., 2001). The ∑DDT in other lakes of the above studies were less than 21 µg/kg (Macdonald et al., 2000; Rawn et al., 2001).

1.4 Mercury in freshwaters

Mercury is most often found in the form of an amalgam or inorganic salt when in the natural environment (CCME, 1999b). These solid forms of mercury have a much lower vapour pressure compared to elemental mercury (CCME, 1999c). Mercury is mobilized from geological storage to highly mobile atmospheric pools through both natural processes and anthropogenic activities (Kamman et al., 2005). The primary natural fluxes of mercury are atmospheric emissions from volcanic eruptions, forest fires, burning biomass, and ocean volatilization (CCME, 2000; Cooke et al., 2020). Anthropogenic sources include burning fossil fuels, processing gold ore, incinerating garbage waste, base metal mining and smelting, and biomedical waste (Health Canada and Ontario Ministry of Health, 1998; Chan et al., 2003; Cooke et al., 2020). Mercury from both anthropogenic and natural sources are equally harmful, and will continue to enter into the global pool of atmospheric mercury for worldwide deposition (Kamman et al., 2005).

Mercury is a common contaminant of concern in aquatic environments. When mercury is introduced into a lake environment through atmospheric deposition, it is mostly integrated into organic particulates and deposited to lake sediments (Cooke et al., 2020). Inorganic mercury is the most abundant form of mercury in the environment, but can be converted into its organic and more bioavailable form, methylmercury (CCME, 2000; Chan et al., 2003; Stafford & Haines, 1997; Westcott & Kalff, 1996). The Canadian freshwater guideline for the protection of aquatic life is 26 ng/L for inorganic mercury and 4 ng/L for methylmercury (CCME, 2003). Inorganic mercury is less toxic than methylmercury (CCME, 1999b).

The quantity of inorganic mercury in the physical environment is not a good indicator of mercury concentration in fish tissue, because it is not readily bioavailable for uptake by aquatic organisms (Castro & Yoshida, 1971; van den Berg, 2009). In the aquatic environment, methylation of inorganic mercury can occur in the water column or sediments but can also occur *in vivo* in the intestines or slime layer of fish (Rudd et al., 1980; Winfrey & Rudd, 1990). The biochemical transformation of inorganic mercury to methylmercury is primarily performed by sulfate reducing bacteria in the water column or sediments, and is the dominant methylation process (Westcott & Kalff, 1996; Chan et al., 2003). Abiotic mercury methylation can also occur with the acids formed during the decomposition of organic matter (Dórea, 2008). Other factors that may influence this biotransformation also include aqueous pH, availability of biodegradable carbon, temperature, and quantity of inorganic mercury (Winfrey & Rudd, 1990; Finley et al., 2016). Methylmercury is bioaccumulative and able to biomagnify through higher trophic levels. Methylation and demethylation by microorganisms occur at the same time (CCME, 2000). Microbial demethylation is the primary cause of methylmercury degradation in freshwater (CCME, 2000). Demethylation of methylmercury can also occur with photodegradation, which may encourage methylmercury degradation in aquatic systems with shallow or clear water (Seller et al., 1996; CCME, 2000).

In freshwater fish, mercury bioaccumulation varies with fish size, aqueous pH, dissolved organic carbon, algal productivity, and food web composition (Kamman et al. 2005). Total mercury composition in fish muscle is at least 90% methylmercury, regardless of the mercury composition in the animal's diet or in the water column (Bloom, 1992; CCME, 2000). Approximately 95% of methylmercury uptake in animals is absorbed into the bloodstream and distributed throughout the tissues in the body (Wolfe & Norman, 1998; CCME, 2000). Methylmercury is able to pass through the blood-brain barrier, and is therefore dangerous to the central nervous system. It damages or destroys the nerve tissue in most vertebrates (CCME, 1999b). Effects of this neurological damage may result in malnourishment, inability to evade predators, decreased reproductive success, and greater sensitivity to other environmental stressors (CCME, 2000). The Canadian methylmercury tissue residue guideline for the protection of wildlife consumers of aquatic biota is 33.0 μg/kg (CCME, 2000).

1.5 Thesis objectives

The objective of this thesis is to investigate two legacy contaminants, DDT and mercury, in brook trout (*Salvelinus fontinalis*) from remote New Brunswick lakes.

Atmospheric deposition is a major pathway for both contaminants to enter lake ecosystems. The first data chapter of this thesis is focused on the relationship between the aquatic food web and the historical DDTs in the aquatic environment. Lake sediments and brook trout muscle tissue were analyzed from five lakes in areas impacted by DDT application and compared to two lakes in an unimpacted reference area. Benthic invertebrates were also analyzed from the seven study lakes to establish trophic positions of organisms in each lakes' food web. The second data chapter studied mercury levels in brook trout muscle tissue from several lakes in New Brunswick. These finding can be compared to other studies in Canada and the United States to gain a better understanding of New Brunswick brook trout and mercury contamination. The final concluding chapter is a summary of findings and includes recommendations for future research.

Understanding the impacts of legacy contaminants in aquatic environments can allow for better risk assessment when interacting with contaminated sites. It is important for policy makers and recreational users to be aware of the potential hazards that may arise from consuming contaminated wildlife and interacting with contaminated sediments. Chronic effects of legacy contaminants also degrade aquatic populations and harm lake ecosystems, which may impact aquatic ecosystem services.

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Chapter 2

The legacy contaminant DDT within brook trout from remote lakes in New Brunswick, Canada

2.1 Abstract

Legacy contaminants, such as organochlorine pesticides, can persist in the aquatic environment for a prolonged period of time with adverse effects to exposed biota. DDTs are persistent legacy contaminants that impact the environment through bioaccumulation, biomagnification, and chronic toxic effects in wildlife. DDT was applied aerially across most conifer forests in north-central New Brunswick between 1952 and 1968 for pest control in the forestry sector. Recent DDT concentrations from surface sediments in remote New Brunswick lakes exceeded probable effect levels by, on average, ~16 times. I sampled seven lakes in the summers of 2020 and 2021 to investigate the impact of DDT on biota in the modern aquatic environment. I sampled brook trout and lake surface sediments for DDTs. Brook trout, aquatic invertebrates, and zooplankton were also used in a stable isotope analysis to assess diet linkages. I found DDTs were up to ~14 times higher in brook trout muscle tissues from lakes impacted by DDTs, compared to reference lakes outside of the areas where DDT was applied. Brook trout sampled in the DDT impact area were above CCME guideline levels by up to ~22 times. A strong correlation between lake sediments and brook trout DDT levels suggests that lake sediments are a source, not sink, of historical DDTs to the modern environment.

2.2 Introduction

In the early 1950s, an outbreak of spruce budworm (*Choristoneura fumiferana*) in northeastern North America led to the widespread use of dichlorodiphenyltrichloroethane (DDT) and other insecticides in conifer forests of New Brunswick (NB), Canada (Royama et al., 2005). At least 5.7 million kg of DDT was applied aerially to conifer forests in NB between 1952 and 1968 (Pearce et al., 1976; Peakall et al., 1983; Armstrong & Cook, 1993). Recent investigations into DDTs in NB lake sediments recorded peak levels that ranged from ~139 µg/kg (Goodwin Lake) to ~4500 µg/kg

(Upsalquitch Lake), and recorded peak sedimentary levels from Upsalquitch Lake that exceeded probable effect levels of ∑DDD established by Canadian Council of Ministers of the Environment by 452 times (Kurek et al., 2019).

DDT has been restricted in Canada since 1970 and banned since 1985 because of its harmful impacts on humans and the environment (CCME, 1999b). Aerial spraying operations and volatilization from water, plants, and soils are two pathways DDT may enter the environment (CCME, 1999a). DDT will strongly adsorb to particulate matter, and will settle into the lake basin through sedimentation (CCME, 1999a). DDT slowly degrades into the metabolites dichlorodiphenyldichloroethane (DDD) and dichlorodiphenyldichloroethylene (DDE), which both have similar physicochemical properties as DDT (van den Berg, 2009). DDT is toxic to aquatic wildlife and with high dose exposure, aquatic organisms may experience death caused by disruption to the central nervous system (Cottam & Higgins, 1946; Beckvar & Lotufo, 2011). Of greater concern is chronic, low-dose exposure in fish, with impacts to the central nervous system that can cause behavioural abnormalities, spasms, seizures, or death (Anderson & Peterson, 1969; Beckvar & Lotufo, 2011). Negative impacts in invertebrates include lack of coordination or swimming ability in daphniids (Crosby & Tucker, 1971), crabs (Neufeld & Pritchard, 1979), and amphipods (Lotufo et al., 2000). Additionally, DDT is an endocrine disruptor that may interfere with hormone regulated systems within organisms and reduce the number and/or quality of offspring produced by fish (Beckvar & Lotufo, 2011). Metabolites of DDT such as o, p′-DDD, p, p′-DDT and o, p′-DDT are estrogenic, while other metabolites such as p, p′-DDE; p, p′-DDT are androgenic. Expression of estrogen- or androgen-responsive genes (Beckvar & Lotufo, 2011) and key organism functions controlled by these sex hormones. Piscivores have a particularly high risk of DDT exposure due to its bioaccumulation in aquatic organisms and biomagnification through aquatic food webs in contaminated ecosystems (CCME, 1999c).

Kurek et al. (2019) investigated lake sediments in five remote NB lakes and observed DDTs in modern lake sediments to be above probable effect levels more than five decades since DDTs were applied to NB conifer forests. Using dated sediment cores, they observed DDT, DDD, and DDE levels were highest about 5 to 10 years after DDT application in the 1950s and 1960s. Modern profundal sediments were still elevated in DDD and DDE with concentrations that are amongst the highest reported in eastern North America (Kurek et al., 2019). Research from Nicola Lake in British Columbia suggests the presence of DDE in fish livers indicates that contaminated lakes can recycle historical DDT that accumulated in profundal sediments and reintroducing the pesticide to modern biota (Macdonald et al., 2000). Other recent studies of DDT in fish from North American lakes have demonstrated contamination on modern populations. In Maine, skin-on brook trout muscle tissue from two locations ranged from 141 to 576 ng/g wet weight (Mierzykowski, 2013). In 125 lakes across the US, fillets from predatory fish had DDTs as high as 1481 ng/g wet weight (Stahl et al., 2009). Smallmouth bass from Fumee Lake, Michigan were as high as 587 ng/g wet weight (Henry et al., 1998). Lake trout and walleye from the Great Lakes were as high as 19,190 ng/g wet weight (De Vault et al., 1996). The persistence of DDTs in lake sediments, combined with the large-scale historical use of DDT in NB, may have long-lasting impacts on modern aquatic ecosystems of many lakes in conifer-dominated watersheds of NB.

Findings of Kurek et al. (2019) prompted questions about modern ecosystem functions and how historical DDTs may be impacting the contemporary food web of NB lakes within treated watersheds. Elevated DDTs in the modern lake sediments suggest the sediments may be acting as a source of DDT, rather than a sink, and contributing to the bioaccumulation of DDTs in the modern aquatic food web. DDTs are well-preserved in cold deep waters and sediments of thermally stratified lakes because low temperatures slow the volatilization half-life of DDTs (CCME, 1999a). DDE is also estimated to volatize 10 to 20 times slower in freshwater than in salt water (CCME, 1999a).

Brook trout (*Salvelinus fontinalis*) are among the top predators in these aquatic food webs, and so we would expect this organism to reflect the modern environmental conditions within these lakes. With this thesis, I investigate the relationship between brook trout, lake sediments, and DDTs. Are the legacy DDTs that are elevated in lake sediments now bioaccumulating in brook trout? Are brook trout in lakes without DDT application lower in comparison to lakes with watersheds that received DDT applications between 1952 and 1968? This study is the first to investigate legacy DDTs in modern NB lake fish and will help us to better understand the chronic effects of DDTs on the modern food web and long-term impacts associated with organochlorine pesticide use in Eastern Canada.

2.3 Methods

2.3.1 *Site description and study lakes*

The same five remote lakes were chosen for this project as studied in Kurek et al. (2019), as this project builds on the lake sediment results (Figure 2.1). Historical maps of spruce budworm defoliation and DDT application were used by Kurek et al. (2019) to select five headwater lakes within impacted watersheds that have similar physical characteristics (<70 ha surface area; <15 m depth), reported water quality parameters, location within historical DDT application areas and limited fish stocking history (Table 2.1). Upsalquitch, California, Middle Peaked Mountain, Sinclair, and Goodwin lakes are dimictic and oligotrophic with no major surface water inflows. These remote lakes are found within the Atlantic Maritime Ecozone. Mean summer air temperature is 16.8°C, mean winter air temperature is -10.9°C, and average annual precipitation is ~1010 mm.

Two additional headwater lakes located in southern NB were chosen as comparative reference lakes located outside of the known historical DDT application areas (Figure 2.1). These two lakes were chosen for their similar water chemistry parameters, limited stocking, and physical characteristics to the five study lakes mentioned above. Bennett Lake is located in Fundy National Park and is protected from land-use change associated with forestry activities. Bennett Lake is an artificial lake that contains a dam. Bennett Lake is also used more frequently for recreational activities than other study lakes because it is located within Fundy National Park.

All study lakes contain populations of brook trout and small-bodied fishes common to NB (Gautreau & Curry, 2020). California Lake, Goodwin Lake, and Middle Peaked Mountain Lake contain populations of brook trout and small-bodied fishes. Middle Peaked Mountain is the only lake with a history of stocked brook trout. The last stocking event occurred in 2000, in which 2000 juvenile brook trout were stocked (Kurek et al. 2019). Sinclair Lake contains populations of brook trout, Yellow Perch (*Perca flavescens*), and various small-bodied fishes. Upsalquitch Lake contains a population of brook trout, various small-bodied fishes, and may contain a remnant population of Arctic Char (*Salvelinus alpinus*). Arctic Char have not been observed at Upsalquitch Lake by the Department of Natural Resources and Energy Development in decades (*pers. comm.* D. Guitard).

2.3.2 *Sediment collection and water chemistry*

Sedimentary measurements obtained from California, Upsalquitch, Sinclair, Goodwin, and Middle Peaked Mountain lakes by Kurek et al. (2019) were used in this study to assess DDT, DDD, and DDE levels in the upper lake sediments. Sediment cores were taken from the deepest basin of each of the five lakes in north-central NB between May and June of 2016. Cores were collected with a gravity corer, and sectioned on-site with a vertical extruder (Glew, 1991). Select sediment intervals were freeze-dried and later processed for DDT analysis following the standard methods outlined in Kurek et al. (2019). I used the data from the top 2.0-3.0 cm of each dated core to assess DDTs in modern lake sediments (Table 2.1).

Sedimentary DDT measures from Anthony and Bennett lakes were obtained in the fall of 2020 following similar methodology to Kurek et al. (2019). Sediment cores were taken from the deepest basin of the lake with a 7.6-cm diameter gravity corer (Glew, 1991). Sediment intervals were sectioned with 0.5-cm resolution using a vertical extruder. The top 2.0 cm (Bennett Lake) and 2.5 cm (Lake Anthony) were used for sedimentary DDT analysis. Sediments were freeze-dried and later prepared for organochlorine analyses at the Queen's University Analytical Services Unit using similar methods as outlined in Kurek et al. (2019).

Water chemistry parameters and surface water samples were taken from Goodwin, Sinclair, and Middle Peaked Mountain lakes on September 2, 2020, California and Upsalquitch lakes on September 10, 2020, Lake Anthony on October 18, 2020, and Bennett Lake on October 28, 2020. The water chemistry parameters were measured using a YSI multiparameter probe, and measures were recorded at one-meter intervals throughout the water column in the deepest basin of each study lake near to where sediment cores were collected (Appendix B). Surface water samples were preserved on ice for no more than 48 hours before they were received by the Research & Productivity Council laboratory in Moncton, NB for surface water analyses that included a standard set of 21 analytes, 4 estimated parameters, and metals (Appendix C).

2.3.3 *Biological sampling*

Brook trout were captured primarily by angling between May 22 and June 12, 2020 in Middle Peaked Mountain, Goodwin, California, Upsalquitch, and Sinclair lakes and between September 21 and October 2, 2020 in Anthony and Bennett lakes. Fyke nets were also used as a secondary capture method at Upsalquitch, Bennett, and Anthony lakes with the assistance of staff from Parks Canada and the Department of Natural Resources and Energy Development. A size range of 25-40 cm was targeted for all brook trout retained. Retained trout were dispatched with blunt force to the head and exsanguinated immediately following guidelines approved by Mount Allison University's Animal Care Committee. Fish were stored on ice during transport to the Environmental Change & Aquatic Biomonitoring (ECAB) laboratory at Mount Allison University in Sackville, NB. Trout mass and length were recorded before samples were filleted and gut contents were removed.

Skin-on fillets were freeze dried, homogenized, and processed for organochlorine analysis before they were sent to the Queen's University Analytical Services Unit. Samples were analyzed for chlorinated pesticides by gas chromatography with a mass spectrometer as a detector (GC/MS/MS) after extraction by Soxhlet extractor and sample cleanup using solid phase extraction chromatography after gel permeation chromatography. Fish tissue (2-4 g) was mixed with sodium sulphate and Ottawa sand, and spiked with the surrogate decachlorobiphenyl prior to extraction. The samples were Soxhlet-extracted with 250 mL of dichloromethane for 6 hours (2-3 cycles per hour). Negative control samples were blank extracts that received only decachlorobiphenyl, Ottawa sand, and sodium sulphate. Positive control samples were blank samples spiked with a chlorinated pesticide mixture. Tissue samples were concentrated to 10 mL by rotoevaporation before gel permeation chromatography cleanup. The gel permeation chromatography collected both a lipid fraction and a pesticide fraction. Pesticide fractions were concentrated by rotoevaporation to approximately 1 mL, 5 mL of hexane were added, and again evaporated to 1 mL. This was repeated twice more. The extract was quantitatively transferred and applied to a Florisil extraction column. The column was rinsed with hexane into a 10 mL centrifuge tube, this was fraction 1. Fraction 1 was concentrated by a factor of 5 using nitrogen and 100 µL was transferred to a GC vial and internal standard (SS) added to each vial. In this analysis, p, p′ -DDT, o, p′ - DDT, p, p′-DDE, o, p′-DDE, p, p'-DDD, and o, p′-DDD were measured. I refer to ΣDDT, ΣDDE, and ΣDDD as the sum of their two respective isomers, and total DDTs as the sum of all six congeners. Control samples, duplicates, and blanks were extracted for 10% of the samples. DDT isomer values were reported as ng/g dry weight (ppb). The lipid fraction was analyzed gravimetrically and values were reported as percent dry weight.

Gut contents were removed from the digestive system of each brook trout and retained for identification and stable isotope analysis. Nine trout had little to no gut contents. Organisms from the same family found within a trout were pooled together for analysis. Gut contents in good condition were identified to family level, freeze dried, homogenized, and processed for stable isotope analysis in the EASIL laboratory at Mount Allison University. Gut contents included various invertebrate species, small-bodied fishes, and salamanders (Table 2.3). Unidentifiable gut contents were not included in the stable isotope analysis.

Benthic invertebrates were sampled from the littoral zone of the seven study lakes between July 15 and 22, 2021. Benthic invertebrates were captured with 400 µm kick-nets in the most common substrate at ~1-2 m deep near to where most angling occurred. Samples were sorted using 3 sieves (4.75 mm, 1.65 mm, and 500 µm), and grouped into coarse taxonomic units for identification in the laboratory. Chironomid larvae were sampled from the approximate center of the lake basin near to where coring occurred with multiple sediment grabs from the profundal zone using a Ponar sampler. Invertebrates were preserved in 95% ethanol and later viewed with a dissecting microscope, and identified to family level using dichotomous keys (Voshell, 2002; Merritt et al., 2008). Finally, zooplankton were captured from the epilimnion with an 80-µm net from the approximate center of each of the seven lakes with a horizontal tow. Zooplankton samples were stored on ice prior to transport to the ECAB laboratory for processing. The samples were sieved through 80-µm mesh to remove excess water, and later freeze dried, homogenized, and prepared for stable isotope analysis.

2.3.4 *Stable isotope analysis*

Benthic invertebrate samples, identifiable brook trout gut contents, zooplankton samples, and brook trout muscle tissue were freeze dried, homogenized, and processed for stable isotope analysis at Mount Allison University. Quantity of fish gut contents varied with each sample and were not available from every fish sample. Additionally, there was only one zooplankton sample collected from each lake. Samples were analyzed for $\delta^{13}C$ and $\delta^{15}N$ with an Elementar PyroCube Elemental Analyzer and Isoprime Precision Isotope Ratio Mass Spectrometer at the Environmental Analytics and Stable Isotope Laboratory (EASIL) at Mount Allison University. Approximately 2 mg of dry, homogenized sample was weighed in a tin capsule and encapsulated to remove air before entering the elemental analyzer for combustion. The resulting CO_2 and N_2 gases move through the elemental analyzer to the mass spectrometer. The sample peaks were verified with at least three replicates of three certified reference materials from Reston Stable Isotope Lab of the United States Geological Survey (USGS), which were interspersed with the samples for quality control/quality assurance.

2.3.5 *Statistical analysis*

All statistical analyses were conducted in R Studio version 4.1.1. An $\alpha=0.05$ was used for all statistical tests. A two-way ANOVA was used to analyze the effect of lake sediment DDTs and differences between lakes on DDTs in brook trout tissue. Lake sediment DDTs were categorized into low, medium, or high level DDTs. DDTs below 200 ng/g (Anthony, Bennett, and Goodwin lakes) were low, DDTs between 201 and 800 ng/g (Middle Peaked Mountain and Upsalquitch lakes) were medium, and DDTs above 801 ng/g (Sinclair and California lakes) were high. I tested the assumptions of the ANOVA with a Levene's test for homogeneity of variance (p=0.0.13) and a Shapiro-Wilk normality test (p<0.01). The brook trout tissue data was transformed with a log10 transformation to improve data normality and homogeneity of variance. With the log10 transformation, I repeated the Levene's test for homogeneity of variance (p=0.059) and a Shapiro-Wilk normality test (p=0.046). I predicted that lakes with the highest sedimentary DDTs have brook trout with high DDTs. The ANOVA was followed by Tukey's *post hoc* test to investigate where the differences exist.

2.4 Results

2.4.1 *DDT application to watersheds*

Historical DDT application data were provided by Natural Resources Canada (NRCAN). A map of historical DDT application to watersheds studied in this project was created by NRCAN (Figure 2.1). Cumulative DDT applied between 1952 and 1968 was highest in central NB within the watershed of the Miramichi River (Figure 2.1). Of the impact lakes in this study, cumulative spray was 9730 kg in the Upsalquitch watershed, followed by 1038 kg in Middle Peaked Mountain, 1374 kg in Sinclair, and 414 kg in California watersheds (Table 2.1). The Goodwin watershed had the lowest cumulative DDT application at 236 kg (Table 2.1). Frequency of DDT application varied between watersheds. The Middle Peaked Mountain watershed was targeted for 8 years between 1954 and 1965, and less than half of the watershed was covered for 2 of the 8 years. The Upsalquitch watershed was targeted for 7 years between 1952 and 1958, and less than half of the watershed was covered for 2 of the 7 years. The California watershed was targeted for 4 years between 1953 and 1958. The Sinclair watershed was targeted for 2 years in 1954 and 1957. The Goodwin watershed was only targeted for one year, in 1954. Reference lakes, Anthony and Bennett, had no reported DDT application in their watersheds, which is supported by the lowest sedimentary DDTs reported (Table 2.1).

2.4.2 *DDTs in brook trout and lake sediments*

Brook trout in reference lakes had DDTs below the CCME guideline for fish tissue residue of 14 ng/g wet weight (Table 2.2). All trout samples from Lake Anthony fell below the laboratory's detection level of 10 ng/g wet weight for all DDT metabolites.

In Bennett Lake, two of six samples fell below the laboratory's detection level for all DDT metabolites. Four of six trout samples were above the laboratory's detection limit for DDE, and below the laboratory's detection limit for all other metabolites. For analyses, DDT, DDD and DDE levels below detection levels were reported as 10 ng/g as a conservative estimate. Dry weight DDTs were converted to wet weight DDTs with an assumption of 80% moisture content in the fish tissue (Campbell et al., 2008). DDTs within 29 trout from impact lakes were all above the CCME guideline for fish tissue residue (Table 2.2). Brook trout total DDTs ranged from 20.75 ng/g (converted wet weight) at Goodwin Lake to 181.83 ng/g (converted wet weight) at California Lake (Table 2.2). Goodwin Lake had the lowest mean concentrations of brook trout DDTs and upper sedimentary DDTs. California Lake contained the highest mean concentrations of both brook trout DDTs and upper sedimentary DDTs (Figure 2.2).

There was a statistically significant relationship between DDTs in brook trout and DDTs in upper lake sediments ($F(2)=333.51$, $p<0.01$; Figure 2.2) and a statistically significant relationship between DDTs in brook trout and effect of lake study site ($F(4)=5.40$, $p<0.01$), though the interaction between these terms was not significant. Tukey's post-hoc testing showed significant pairwise differences ($p<0.01$) existed between sediments with low DDTs (Anthony, Bennett, and Goodwin lakes), and sediments with medium (Middle Peaked Mountain and Upsalquitch lakes) or high (Sinclair and California lakes) DDTs.

2.4.3 *Invertebrate identification and stable isotope analysis*

From the reference lakes, 14 different families were captured and identified within the gut contents and benthic invertebrate samples at Bennett Lake and 16 at Lake Anthony. From the impact lakes, 11 different families were captured and identified within the gut contents and benthic invertebrate samples at Goodwin Lake, 12 at Sinclair Lake, 14 at Upsalquitch and California lakes, and 17 at Middle Peaked Mountain Lake. Identifiable benthic invertebrates and gut contents were presented in a qualitative table, describing presence/absence at family level (Table 2.3). Chironomidae, Gammaridae, and Heptageniidae were found in benthic invertebrate samples across all seven lakes. Aeshnidae, Ameletidae, Coenagrionidae, and Corduliidae were found in benthic invertebrate samples from five of seven lakes. Baetidae and Gomphidae were found in benthic invertebrate samples from four of seven lakes. Coenagnionidae, Leuciscidae, and Phryganeidae were the most common species found in the trout gut contents. Plethodontidae were found only in the gut contents from Goodwin Lake and they were the most abundant taxon present within the gut contents. Bibionidae were found only in the gut contents from Sinclair Lake, and they were the most abundant taxon present within the gut contents.

The stable isotope analysis for this food web assessment have comparable trophic positions across each lake, even when comparing reference lakes to DDT impact lakes (Figure 2.3). The most variable isotopic measures were the benthic invertebrates and gut contents, though all were in relatively similar trophic positions across all lakes. Upsalquitch Lake had the widest range in gut content δ15N values, ranging from 0.53 to 10.03. Bennett Lake had the highest benthic invertebrate δ15N peak, at 6.8, and Lake Anthony had the lowest, at 0.92. Fish tissue measures also remained relatively similar across all seven lakes. Upsalquitch Lake had the highest δ15N peak, at 10.94, and California Lake had the lowest δ15N fish tissue value, at 4.58. California Lake had the highest zooplankton value, at 4.58, and Lake Anthony had the lowest δ15N zooplankton, at 0.92. California Lake and Goodwin Lake had the two most extreme δ13C values, -21.27 and -36.48, respectively.

2.5 Discussion

2.5.1 *DDT application to watersheds*

The map of cumulative DDT applied to the province’s watersheds shows the application efforts between 1952 and 1968 (Figure 2.1). Upsalquitch Lake watershed received the most cumulative DDT application, though it did not have the highest recorded total DDTs in modern sediments or in brook trout tissue samples (Table 2.2). California Lake has the highest modern total DDTs in brook trout tissue and second highest in modern sediments, which is in contrast to historical DDT application to the watershed. The historical sediment total DDT peaks at Upsalquitch Lake (~4500 µg/kg observed by Kurek et al. (2019)) correspond to the DDT application frequency and quantity reported by this map (Figure 2.1). Differences in historical DDT application and modern sediment and fish tissue levels show us the lake systems are processing DDTs differently. I would expect Upsalquitch Lake, the watershed with the highest cumulative DDT application, to have the highest modern DDT levels. This is not the trend observed in the modern environment, therefore the lakes must be behaving differently with respect to the breakdown of DDT and its movement within the food web.

2.5.2 *DDTs in brook trout and lake sediments*

There is a relationship between the accumulation of total DDTs in brook trout muscle tissue and the concentration of total DDTs in the lake sediments. Across all lakes except Goodwin, increasing sedimentary total DDTs corresponds to increasing total DDTs in the fish tissue. This data suggests the lake sediments are acting as a source of DDTs to the modern food web, rather than just a sink for legacy DDTs. We would expect DDTs to decrease over time and become less bioavailable to organisms living in the lakes. This data tells us that the brook trout in these study lakes are able to bioaccumulate DDTs from the upper sediments likely through their diet at levels higher than guidelines for wildlife consumers of aquatic biota (CCME, 1999b). The significant relationship between trout and sediment DDTs indicates lake sediments are a good predictor of DDTs in the aquatic food web (Figure 2.2). Trout from the DDT impact lakes exceed the CCME guides for the protection of wildlife consumers of aquatic biota by ~1.1 times (15.9 mg/kg, converted wet weight, brook trout sample from Goodwin Lake) up to ~22 times (308.4 mg/kg, converted wet weight, brook trout sample from Upsalquitch Lake) (CCME, 1999b).

Mierzykowski et al. (2013) measured DDTs in skin-on brook trout muscle tissue from two locations in northeastern Maine with DDTs ranging from 141 to 576 ng/g wet weight. In a study of 125 lakes across the United States, Stahl et al. (2009) measured the fillets of 12 predatory fish species and found DDTs ranging from 0.77 to 1481.4 ng/g wet weight. Henry et al. (1998) and De Vault et al. (1996) measured DDTs in homogenized whole fish samples. From Fumee Lake, Michigan, DDTs in smallmouth bass ranged from 138 to 587 ng/g (wet weight) (Henry et al., 1998). Samples of lake trout and walleye from the Great Lakes had DDT concentrations from 160 to 19,190 ng/g wet weight (De

Vault et al., 1996). It is difficult to compare these studies directly because of differences in fish species' metabolization of DDTs, degradation of DDT over time, and whole fish verses fillet-only measures. Mierzykowski et al. (2013) is the most directly comparable to my study, as it follows similar study parameters and was conducted in near to New Brunswick. The mean total DDTs in my skin-on fillet samples ranged from 20.7 ng/g (wet weight) to 181.8 ng/g (wet weight), which is within the range of findings observed by Mierzykowski et al. (2013).

The concentration of DDTs in brook trout sampled in the impacted DDT lakes from NB were comparable to a similar study in Maine (Mierzykowski et al., 2013), and fall well above the government recommended guideline for fish tissue residue (CCME, 1999b). Climate change is a factor to consider for contaminant research, as it is likely to change the ecosystem dynamics and modify contaminant degradation and bioavailability (Noyes et al., 2009). In aquatic environments, increased temperature and precipitation may modify trophic structure, bioaccumulation, and organic carbon cycling to increase availability of DDT in sediments and biota (Noyes et al., 2009).

Fish mass, length, and percent lipid were measured to account for the relationship between fish size and DDT accumulation. There was not a statistically significant linear relationship between trout size or lipids and total DDTs across all lakes (Figure 2.4). The single trout with the highest DDTs (Upsalquitch) also had the highest percent lipid (Figure 2.4A).

2.5.2 *Stable isotope analysis*

Food web structure is a factor that may influence the between-lake differences in the uptake of contaminants, and stable isotopes are used to investigate trophic interactions between biota (Post, 2002). In these study lakes, it does not appear the community composition of benthic invertebrates play a key factor in explaining DDT uptake in brook trout via their diet. The number of species found within each impact lake are comparable (11 to 17 families identified), and most taxa are found in more than one lake. The exceptions are Ambystomatidae found in Goodwin Lake, and Bibionidae found in Sinclair Lake. These taxa were found in high abundance within the trout gut contents, which indicated short-term availability of a food source rather than a long-term dietary niche within the lake's food web.

Availability of taxa remained fairly consistent across all seven study lakes, with few differences between impact lakes and reference lakes. Brook trout sampling for the impact lakes occurred earlier in the year (May-July) than the reference lake sampling (September-October), and is therefore difficult to compare gut contents between lakes. The number of species found across all lakes was similar, with 14 to 16 in the reference lakes and 11 to 17 in the impact lakes. Most species were found in both reference and impact lakes, and three were found across all seven lakes.

Stable isotope measures were used to determine if there is a dietary component influencing the DDTs in brook trout across the study lakes. DDTs in Goodwin Lake are lower than all other impact lakes sampled, and dietary differences were one potential reason for this. With the stable isotope results, we see some difference between the relative $\delta15N$ and $\delta13C$ position of the samples measured across all lakes sampled. Though there was isotopic variation in the benthic invertebrate samples and zooplankton samples, the fish tissue isotopes remained similar across all lakes. This suggests the fish are feeding at a similar trophic position between lakes, as we would expect more variation in the $\delta15N$ signatures in trout between lakes if they were feeding at different trophic positions (Peterson & Fry, 1987). The lake sediments are likely to play a much larger role as a source in the distribution of DDTs in the aquatic food webs when compared to invertebrate biodiversity or relative trophic position. In summary, while invertebrates are believed to be the pathway between DDTs in lake sediments and brook trout, differences in trout diet composition are not the cause of significant differences in trout DDT levels.

2.6 Conclusion

DDT was applied extensively in New Brunswick between 1952 and 1968. Its ecological effects linger at notable levels in the modern aquatic environment. This study reports brook trout sampled in 2021 within remote headwater lakes from five different watersheds in New Brunswick that contain DDTs above acceptable levels for wildlife,

~50 years after DDTs were last used in the region. Available benthic invertebrate and gut content sampling showed little difference between food web structures and did not indicate brook trout to be feeding in different trophic positions between lakes. The use of historical application rates and maps of DDTs applied may be a useful starting point to assess the relative DDT risk within dimictic lakes. However, mapping efforts may not reflect the impact on the aquatic environment because of factors that influence the transport and degradation of DDTs in an aquatic system. I conclude there is a relationship between lake sediments, brook trout, and DDTs in NB lakes. The correlation between lake sediments and trout tissue samples indicates the lake sediments are now acting as a source of DDTs for the modern food web. Uppermost lake sediments are likely a decent predictor to estimate the relative impact DDTs may have on the aquatic biota within a lake. This study investigated only five of the hundreds of lakes located in north-central NB where DDT applications were frequent and intense. The large-scale application of DDT in the province suggests this legacy contaminant warrants more research to evaluate its long-term, chronic effects on lake food webs.

2.7 References

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2.8 Tables

Table 2.1 Physical characteristeics, cumulative DDT watershed application totals (1952-1968), and select physical and chemical measures from study lakes in New Brunswick, Canada. Surface water chemistry was collected between September 2 and October 28, 2020.

	Goodwin	**Sinclair**	**Middle Peaked Mountain**	**California**	**Upsalquitch**	**Anthony**	**Bennett**
Latitude (N)	47.25	47.05	46.73	47.44	47.47	45.27	45.62
Longitude (W)	-66.35	-66.57	-66.51	-66.15	-66.49	-66.72	-65.07
Lake surface area (ha)	21.4	45.6	14.4	45	68.1	29.8	31.3
Maximum water depth (m)	7.6	8.5	9.8	11.5	15.2	12.8	11.1
Watershed area (ha)	350	950	300	600	2900	124	1280
Cumulative DDT applied to watershed (kg) (1952-1968)	236	1374	1038	414	9730	0	0
∑DDE (ng/g) (upper sediments)	24.8	87.5	120.5	94.8	69.3	7.5	6.0
∑DDD (ng/g) (upper sediments)	13.1	37.6	92.6	81.6	45.5	4.0	7.3
∑DDT (ng/g) (upper sediments)	2.3	21.3	23.7	8.5	8.2	4.0	4.0
Total ∑DDTs (ng/g) (upper sediments)	40.3	146.5	236.9	185.0	123.0	15.5	17.3
Calcium (mg/L)	3.2	2.3	3.3	2.7	7.6	1.2	3.1
Alkalinity ($CaCO_3$) (mg/L)	14	9	12	10	14	2	3
Total Nitrogen (mg/L)	< 0.2*	0.2	0.3	0.3	0.2	< 0.2*	< 0.2*
Total Phosphorus (mg/L)	0.014	0.012	0.010	0.022	0.017	0.005	0.006
Total Organic Carbon (mg/L)	2.7	4.4	4.4	2.8	2.9	2.8	2.2
Colour (TCU)	8	28	11	< 5*	8	< 5*	< 5*
Conductivity (μS/cm)	37	23	29	25	67	19	17
pH	7.5	7.3	7.2	7.7	7.5	8.0	7.9

*Below laboratory detection limits

Table 2.2 Mean (±SD) DDTs, lipid, mass, length, δ13C & δ15N of skin-on brook trout muscle tissue (n=42) from study lakes. Brook trout were collected between May 22 and October 2, 2020.

	Anthony	**Bennett**	**Goodwin**	**Middle Peaked Mountain**	**Sinclair**	**California**	**Upsalquitch**
n	6	7	6	5	6	6	6
2,4 DDE (ng/g dw)	<10*	<10*	<10*	<10*	<10*	<10*	<10*
4,4 DDE (ng/g dw)	12.4 ±2.1	<10*	53.4 ±35.0	388.5 ±109.2	637.1 ±130.1	619.0 ±194.8	645.0 ±344.9
2,4 DDD (ng/g dw)	<10*	<10*	<10*	11.3 ±1.9	10.9 ±1.4	42.4 ±18.0	11.44 ±3.5
4,4 DDD (ng/g dw)	<10*	<10*	10.2 ±0.6	99.1 ±39.2	95.9 ±43.0	198.7 ±95.5	66.1 ±53.6
2,4 DDT (ng/g dw)	<10*	<10*	<10*	<10*	11.3 ±2.1	<10*	<10*
4,4 DDT (ng/g dw)	<10*	<10*	<10*	<10*	59.1 ±32.1	28.8 ±14.2	16.1 ±7.5
Total ∑DDTs (ng/g dw)	<62.4 ±2.1	<60*	103.7 ±35.1	529.0 ±142.3	824.6 ±186.9	909.1 ±298.0	758.7 ±408.1
Total ∑DDTs (ng/g ww, converted)	<12.4 ±0.4	<12*	20.7 ±7.0	105.8 ±28.4	164.9 ±37.3	181.8 ±59.6	151.7 ±81.6
Lipid (%)	12.4 ±2.1	4.2 ±3.0	7.4 ±2.8	13.6 ±1.9	7.2 ±1.2	11.1 ±3.6	10.2 ±3.9
Mass (g)	162.0 ±43.8	109.3 ±17.1	308.3 ±172.5	502.0 ±186.4	308.3 ±86.1	310.0 ±64.8	180.6 ±102.0
Length (cm)	23.0 ±2.2	22.3 ±1.2	31.5 ±3.5	34.8 ±4.2	32.1 ±2.6	32.1 ±1.4	27.0 ±3.9
δ13C	-28.48 ±0.87	-31.86 ±3.33	-26.73 ±2.02	-29.32 ±1.48	-27.72 ±0.64	-27.47 ±0.49	-27.47 ±0.39
δ15N	7.66 ±0.22	8.63 ±0.72	8.97 ±0.26	8.79 ±0.77	8.69 ±0.38	10.0 ±0.49	9.82 ±0.72

*Below laboratory detection limits.

Table 2.3 List of taxa identified to family level from brook trout gut content samples (G) and benthic invertebrate samples (B) from each study lake. Guts contents were sampled between May 22 and October 2, 2020. Benthic invertebrates were sampled between July 15 and 22, 2021.

Family	Anthony	Bennett	California	Goodwin	Middle Peaked Mountain	Sinclair	Upsalquitch
Aeshnidae	B	B	B		B G		B
Ameletidae	B	B	B	G	B	B	
Baetidae	B			B G	B G		B
Bibionidae			G			G	G
Brachycentridae				B			
Chironomidae	B	B	B	B	B	B	B
Coenagrionidae	B	B G	B		G	B	B G
Corduliidae	B		B		B G	B	B G
Corixidae	B	B	B		G		
Elmidae	B			B		B	
Ephemeridae					B	B	B
Formicidae			G				
Gammaridae	B	B	B	B G	B	B	B
Gerridae	B						
Gomphidae	B	B				B	B
Gyrinidae	B	B	B				
Haliplidae		B	B				B
Helophoridae				G		G	
Heptageniidae	B	B	B	B	B	B	B
Leuciscidae*		G	G		G		G
Leuctridae	B				G		B G
Lymnaeidae					G		
Lumbricidae		G					
Perlidae						B	
Philopotamidae	B						
Phryganeidae	G	G		B G	B		
Physidae		G			G		
Ambystomatidae*				G			
Sialidae			B		B		B
Sphaeriidae				G			
Zygoptera					G		

*Vertebrate species

2.9 Figures

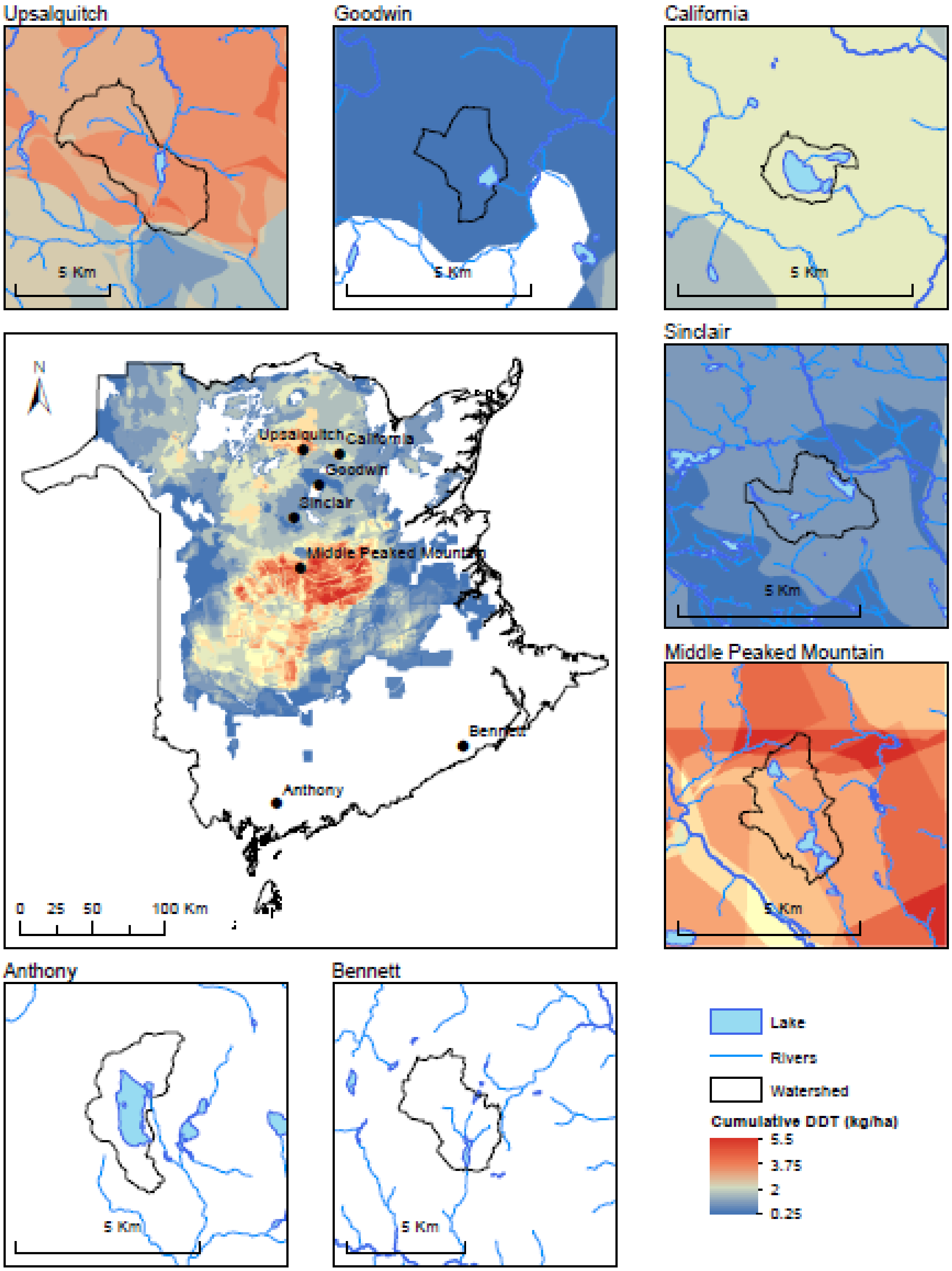

Figure 2.1 Map of study lakes and cumulative DDT applied between 1952 and 1968 in New Brunswick, Canada. Insets of cumulative DDT applied in watersheds of seven study lakes.

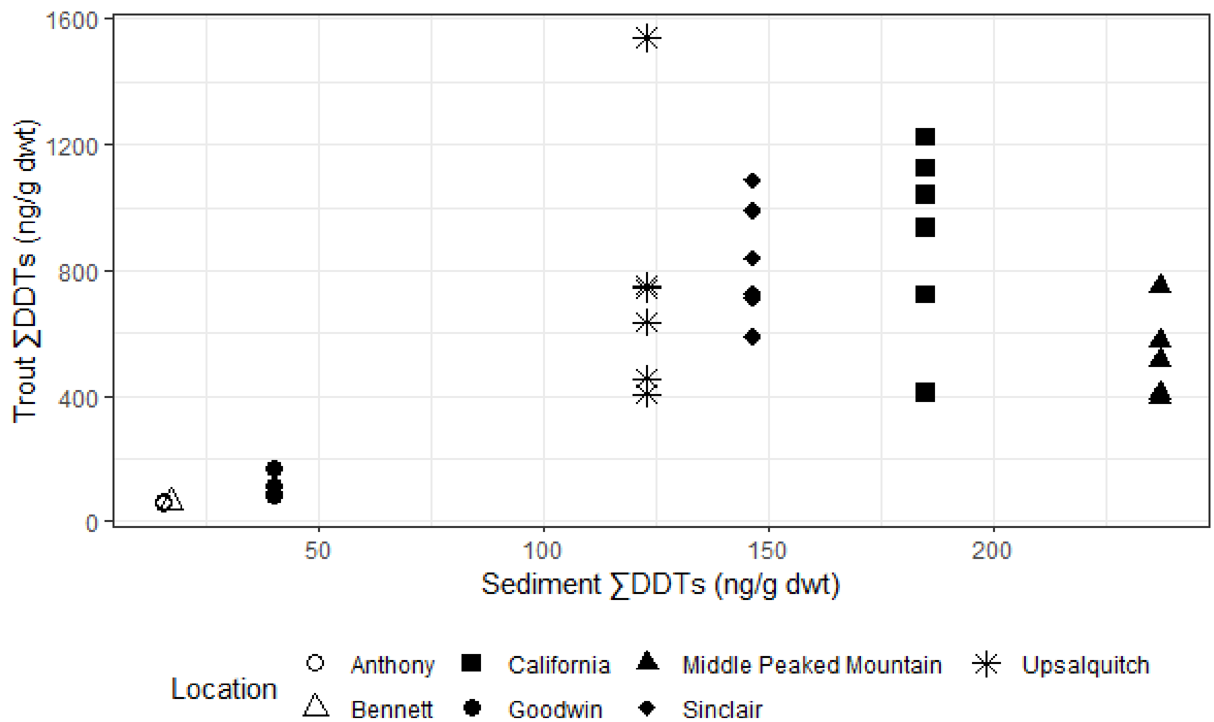

Figure 2.2 Total ∑DDTs (ng/g dry weight) in brook trout muscle tissue (n=42) compared to total ∑DDTs (ng/g dry weight) from upper sediments in seven study lakes.

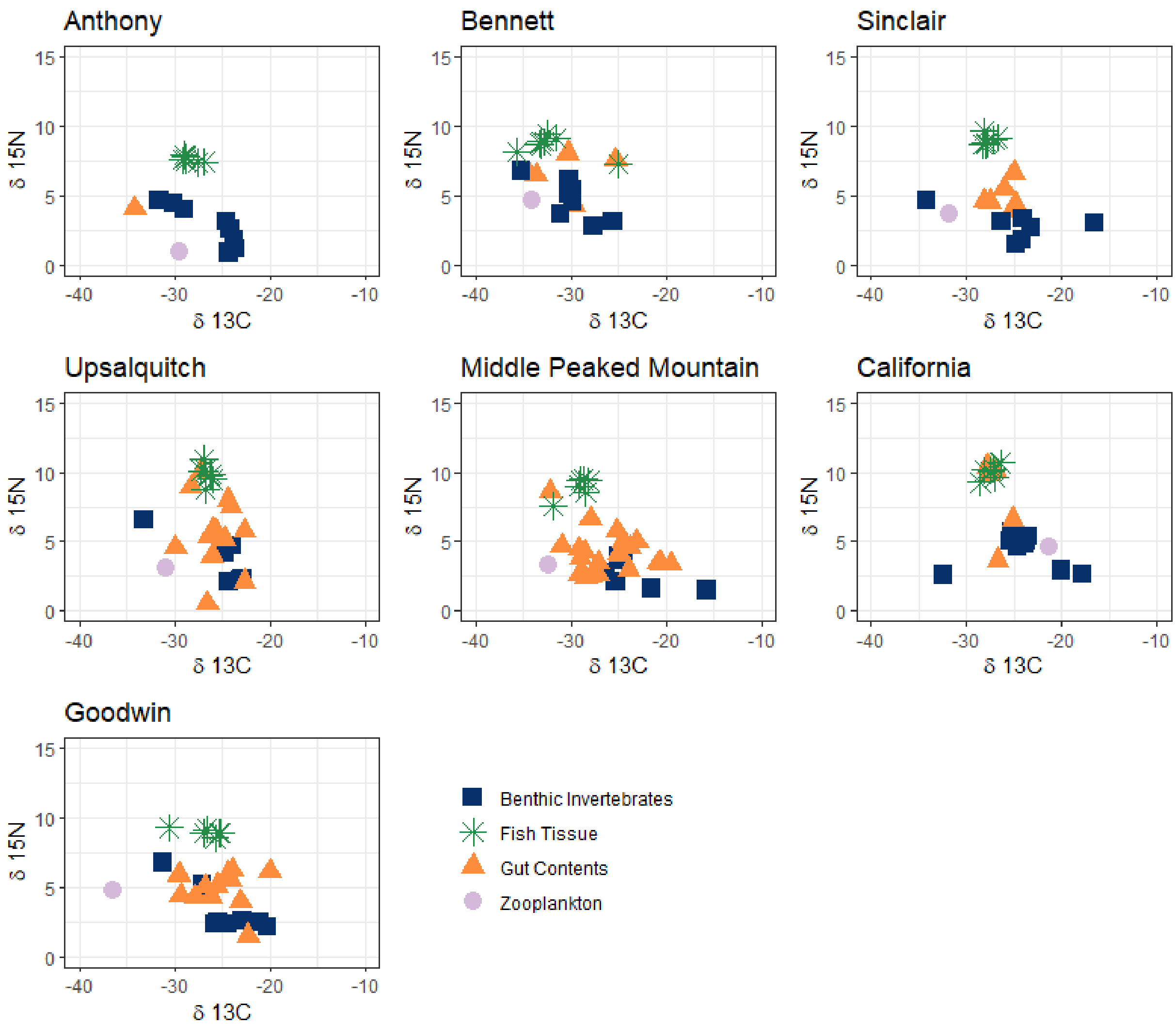

Figure 2.3 Stable isotopes of invertebrates, brook trout gut contents, zooplankton, and brook trout fillets from seven study lakes.

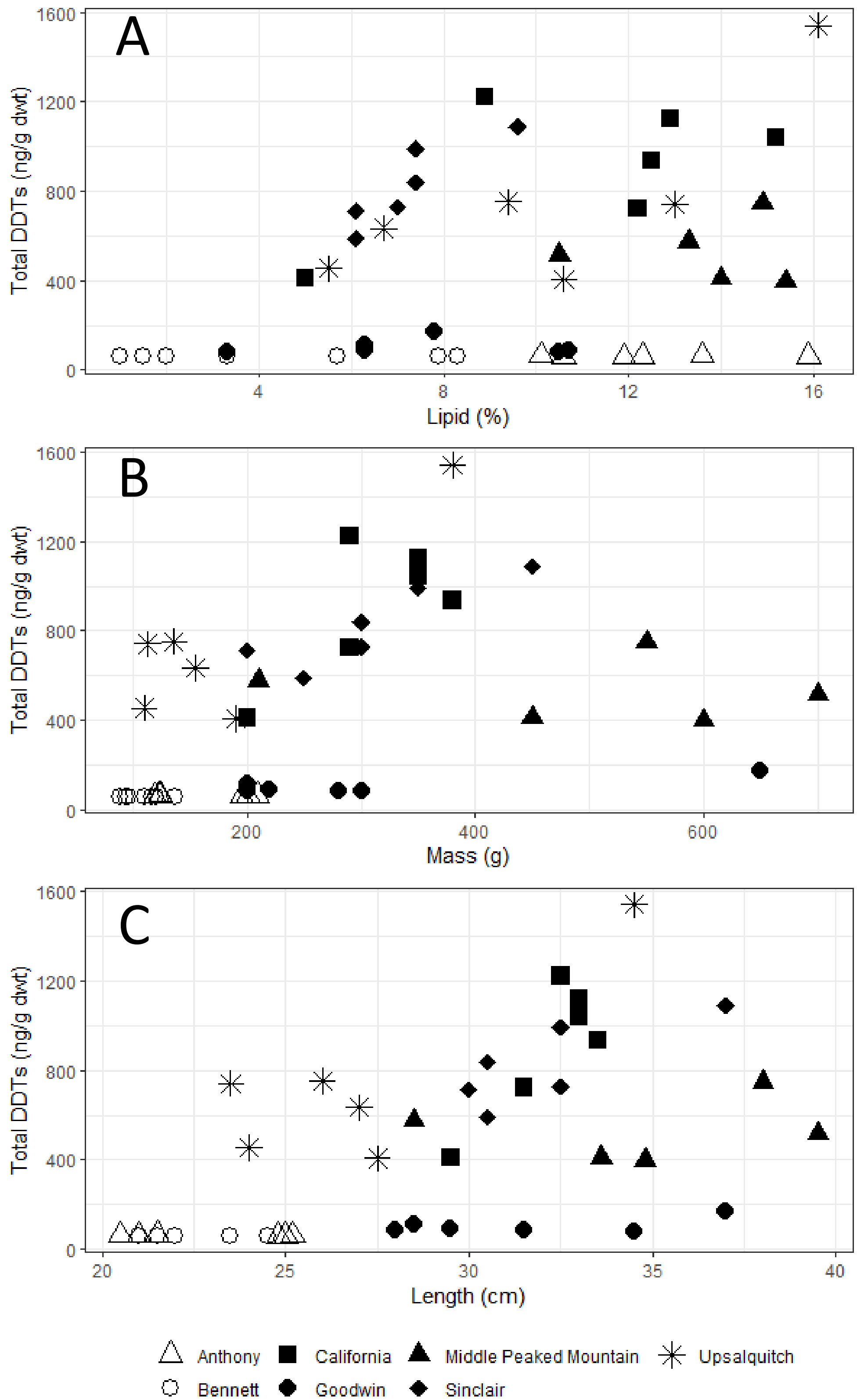

Figure 2.4 Total DDTs (ng/g dry weight) in brook trout compared to percent lipid (A), mass (B), and length (C).

Chapter 3

Mercury in brook trout from remote lakes in New Brunswick, Canada

3.1 Abstract

Mercury (Hg) is a legacy contaminant of global concern because it is easily moved through long-range atmospheric transport and global change may facilitate increased methylation in certain situations. Though mercury is a widespread contaminant, there are few studies investigating freshwater fish in New Brunswick, Canada (NB). I sampled brook trout from seven NB lakes in 2020, and found mercury levels within the same range as similar studies in eastern North America. Brook trout from my study lakes exceeded MeHg guideline levels by up to ~12 times the limit for consumption of aquatic biota. My study indicates Hg levels in NB brook trout were elevated to levels not safe for consumption, which implies chronic effects of this legacy contaminant are possible for piscivorous biota.

3.2 Introduction

Mercury (Hg) is a naturally occurring element and a global pollutant that often enters aquatic ecosystems through atmospheric deposition and long-range transport from emission sources. Elemental Hg is highly volatile and prone to global atmospheric transportation (Chan et al., 2003). After deposition, elemental Hg is oxidized to inorganic Hg, and may then undergo the biochemical transformation to methylmercury (Chan et al., 2003).The Maritime region is called the “tailpipe of North America” because of the influence of west-to-east upper-atmosphere circulation, where contaminants from other regions are often concentrated and deposited (Dunnington et al., 2020). Human activities, such as mining and metal extraction, combustion of fossil fuels, and Hg use in industrial products, results in anthropogenic emission sources of Hg that are much greater than geogenic sources (Driscoll et al. 2013).

Methylmercury (MeHg), which is believed to account for at least 90% of total mercury (THg) in fish muscle, is a legacy contaminant and the only form of Hg able to bioaccumulate and biomagnify in aquatic food webs (Bloom, 1992; Chan et al., 2003; Campbell et al., 2008). However, some studies report this estimation may be less than the expected 90% MeHg, and the percentage of MeHg in THg may vary with fish species, size, or age (Lescord et al., 2018). Inorganic Hg may be assimilated and stored in the body of a fish, however this occurs at less than MeHg accumulation, and the majority is stored in the intestines and liver (Lescord et al., 2018). Canadian tissue residue guidelines for MeHg for wildlife consumers of aquatic biota is 33.0 µg/kg (wet weight), set by the Canadian Council of Ministers of the Environment (CCME, 2000). Unlike other legacy contaminants, MeHg is not very lipid soluble, but is bioaccumulated in biota because it binds strongly with sulfhydryl groups (Clarkson, 1994; CCME, 2000). Aquatic biota are the primary source of MeHg exposure for piscivorous animals. The proportion of MeHg to THg increases with increasing trophic position, typically being lowest in aquatic primary producers, and increasing through invertebrates, and highest in piscivorous fish, mammals, and birds (USEPA, 1997; CCME, 2000). Factors that may influence Hg methylation include aqueous pH, availability of organic carbon, water temperature, and availability of inorganic mercury (Winfrey & Rudd, 1990; Chan et al., 2003; Finley et al., 2016). In fish, MeHg exposure is associated with reduced reproductive success, and suppressed sex hormones (Drevnick & Sandheinrich, 2003; Sandheinrich & Miller, 2006).

Researchers have investigated mercury in freshwater fish in Canada and the United States for decades. Studies of hundreds of lakes and reservoirs in the United States have found mean THg levels in fish samples were up to 0.79 mg/kg wet weight (Stafford & Haines, 1997) and 6.60 mg/kg wet weight (Stahl et al., 2009). Studies from Eastern North America measured mean Hg in brook trout muscle tissue up to 0.23 mg/kg wet weight in Maine (Mierzykowski, 2013) and up to 1.2 mg/kg dry weight in New Brunswick (Finley et al., 2016).

The objective of this research chapter is to investigate THg levels in brook trout from remote New Brunswick lakes, to better understand environmental contaminants in the aquatic environment. Brook trout are one of the most abundant and popular sport fish in NB, and they are also amongst the top aquatic predators in these study lakes. These factors make it an ideal study species to examine for a bioaccumulative legacy contaminant.

3.3 Methods

3.3.1 *Study sites*

Seven headwater lakes with similar physical characteristics (<70 ha surface area; <15 m depth), water chemistry parameters, and limited fish stocking history were chosen for use in this study (Table 3.1). Five lakes are located in north-central NB, one lake located in south-western NB, and one located within Fundy National Park in south-eastern NB (Figure 3.1). These lakes are dimictic and oligotrophic to mesotrophic with no major surface water inflows. Lakes are located within the Atlantic Maritime Ecozone where mean summer air temperature is 16.8°C, mean winter air temperature is -10.9°C, and average annual precipitation is ~1010 mm.

All lakes contain wild populations of Brook Trout (*Salvelinus fontinalis)* and small-bodied fishes. Upsalquitch Lake may contain a remnant population of Arctic Char (*Salvelinus alpinus)*, and Sinclair Lake contains a population of Yellow Perch (*Perca flavescens).* Middle Peaked Mountain was last stocked with 2000 juvenile Brook Trout in 2000, and is the only lake with a reported history of fish stocking (Kurek et al., 2019). Bennett, California, Goodwin, and Middle Peaked Mountain lakes are restricted recreational brook trout fisheries with limited angler access and special license and angling requirements.

Water chemistry parameters and surface water samples were collected for water chemistry analysis from Goodwin, Sinclair, and Middle Peaked Mountain lakes on September 2, 2020, California and Upsalquitch lakes on September 10, 2020, Lake Anthony on October 18, 2020, and Bennett Lake on October 28, 2020. Relative water clarity and *secchi* depth was measured at each lake. A YSI multiparameter probe was used to measure water chemistry parameters at one-meter intervals in the deepest basin of each lake. Water samples were stored on ice for up to 48 hours before they were received by the Research & Productivity Council (RCP) laboratory in Moncton, New Brunswick. RPC analyzed surface water samples for a standard set of 21 analytes, 4 estimated parameters, and metals (Table 3.1).

3.3.2 *Sample collection and tissue processing*

Brook trout from seven study lakes (total n=42) between 25 and 40 cm were targeted for this study. Trout were captured between May 22 and June 12, 2020 at Middle Peaked Mountain, Goodwin, California, Upsalquitch, and Sinclair lakes and between September 21 and October 2, 2020 at Anthony and Bennett lakes. Primary capture method was angling with a single, barbless hook, and the secondary capture method was fyke netting. Fyke nets were only deployed at Upsalquitch, Bennett, and Anthony lakes with the assistance of Parks Canada and the New Brunswick Department of Natural Resources and Energy Development. Fish were immediately dispatched with blunt force to the head and exsanguinated upon capture following procedures approved by Mount Allison University Animal Care Committee. Samples were stored on ice for transport to the Environmental Change & Aquatic Biomonitoring (ECAB) laboratory at Mount Allison University. Trout length and mass were recorded before fish were processed. Skin-on fillets were freeze-dried, homogenized, and prepared for total mercury analysis.

3.3.3 *Mercury analysis and stable isotopes of processed muscle tissue*

Processed muscle tissues were sent to the Kidd Laboratory at McMaster University in Hamilton, Ontario for total mercury analysis with a Milestone Tri-Cell DMA-80 direct mercury analyzer. Quality control/ quality assurance for each run included certified reference materials (DORM-4 fish protein), blank samples, intra-lab system standard, and duplicate samples. The certified reference materials were used at the start of each run and after every 10th sample. The mean percent recovery of DORM was 91.4 ± 2.6% (range 87.6–95.3%, n = 7). Lab standards and duplicates were run after every 10th sample. Blanks were run after every 10th sample.

Processed muscle tissue measured was measured in dry weight. For comparison to guideline levels and other studies reported with wet weight measures, dry weight THg was converted to wet weight THg with an assumption of 80% moisture content in the fish tissue.

Processed skin-on muscle tissue samples were analyzed for $\delta^{13}C$ and $\delta^{15}N$ with an Elementar PyroCube Elemental Analyzer and Isoprime Precision Isotope Ratio Mass Spectrometer at the Environmental Analytics and Stable Isotope Laboratory (EASIL) at Mount Allison University. Muscle tissue samples were weighed to approximately 2 mg, weighed in a tin capsule, and encapsulated to remove air before combustion in the elemental analyzer. As a result of combustion, the CO_2 and N_2 gases move through the elemental analyzer to the mass spectrometer. The sample peaks were verified with at least three replicates of three certified reference materials from Reston Stable Isotope Lab of the United States Geological Survey (USGS), which were interspersed with the samples for quality control/quality assurance.

3.3.4 *Statistical analysis*

All statistical analyses were conducted in R Studio version 4.1.1. An $\alpha=0.05$ was used for all statistical tests. A one-way ANOVA was used to analyze the effect different lakes have on the total mercury levels found in brook trout tissue samples. I used a Levene's test for homogeneity of variance ($p=0.07$) and a Shapiro-Wilk normality test ($p=0.085$) to test the assumptions of an ANOVA. The ANOVA was followed by a Tukey's *post hoc* test to investigate where the differences lie between the study lakes.

A principal component analysis (PCA) is an ordination approach used to summarize patterns in multivariate data. In this study, eight physical and chemical metrics were obtained from brook trout samples and a PCA was conducted to explore patterns in measured variables of 42 samples from seven lakes. Total mercury (THg), mass, length, lipid content, percent nitrogen, percent carbon, δ15N, and δ13C were included in the PCA.

3.4 Results

Lake Anthony had the lowest mean THg levels amongst brook trout from study lakes at 0.252 (±0.019) mg/kg dry weight, followed by California Lake (0.327 ± 0.060 mg/kg), Goodwin Lake (0.490 ± 0.171 mg/kg), Upsalquitch Lake (0.511 ± 0.182 mg/kg), and Middle Peaked Mountain Lake (0.748 ±0.284 mg/kg) (Table 3.2; Figure 3.2). Bennett Lake (0.832 ± 0.245 mg/kg) and Sinclair Lake (1.748 ±0.289 mg/kg) were the lakes with the highest mean THg levels in skin-on brook trout muscle tissue samples in this study (Table 3.2; Figure 3.2).

There was a significant between-lake difference in THg levels (ANOVA, $F(6)=42.06$, $p<0.01$; Figure 3.2). Significant differences between lakes were determined using a Tukey post-hoc test. THg in Sinclair Lake was significantly higher than all other lakes ($p<0.001$), Bennett Lake was significantly higher than all lakes except Sinclair Lake ($p<0.05$), and Middle Peaked Mountain Lake was significantly higher than California and Anthony lakes ($p<0.05$).

Dry weight THg was converted to wet weight THg with an assumption of 80% moisture content in the fish tissue. This conversion is for comparison to similar studies and for comparison to guideline levels most commonly reported in wet weight. The CCME tissue residue guideline for MeHg for the protection of wildlife consumers of aquatic biota is 33 µg/kg or 0.033 mg/kg wet weight (CCME, 2000). Assuming THg in the muscle tissue samples is primarily MeHg, all fish samples (n=42) are likely above this guideline level by approximately 6 to 61 times (Bloom, 1992).

PCA axis 1 and 2 captured 34.4% and 19.7%, respectively, of the variation in the measured variables from brook trout samples from the seven study lakes (Figure 3.3). Axis 1 of the PCA was influenced by measurements related to brook trout size (mass, length, lipid content) and percent nitrogen. Axis 2 of the PCA was influenced by THg and percent carbon. Brook trout samples from Sinclair Lake where samples had elevated THg levels, grouped together along axis 2 in the top quadrant. Similarly, samples that had high THg concentrations from Bennett Lake also plotted in the top right quadrant. Brook trout samples from California and Middle Peaked Mountain lakes were larger (mass) and had greater lipid content, and plotted in the bottom left quadrant. Brook trout samples from Anthony had some of the lowest THg concentrations and plotted in the bottom right quadrant (opposite to samples from Sinclair).

3.5 Discussion

The PCA support the findings from *post-hoc* testing in the one-way ANOVA, in which Sinclair and Bennett lakes have the highest THg levels amongst all study sites. The PCA shows Sinclair and Bennett lakes align with Axis 2, which is influenced by THg. Sinclair Lake had the highest THg levels and the darkest water colour among the study lakes (Table 3.1). Water colour is one environmental factor that may influence the methylation of inorganic mercury in freshwater systems. Bennett Lake is a manufactured lake with a dam, which may account for the elevated THg levels from this site. The artificial creation of a lake intentionally floods a terrestrial area, and the submerged vegetation and soils promote microbial methylation of inorganic mercury (Hall et al., 2005). Other factors are likely to contribute to THg levels in study lakes, such as microorganism population composition and availability of inorganic mercury in the lake system, wetland size in lake catchment area, atmospheric transportation, and deposition patterns of Hg (CCME, 1999, 2000, 2003). Other water chemistry parameters that may influence methylation, such as pH and organic carbon, are similar across all lakes and do not appear to be associated with THg levels in trout between lakes.

My study found THg in skin-on fillets ranged from 0.25 mg/kg dry weight (or 0.05 mg/kg converted wet weight) at Lake Anthony to 1.75 mg/kg dry weight (or 0.35 mg/kg converted wet weight) at Sinclair Lake. In a study of 125 lakes and reservoirs across the United States, Hg in fish fillets ranged from 0.023 to 6.605 mg/kg wet weight (Stahl et al., 2009). From up to 125 lakes in Maine, fillets ranged from 0.03 to 0.79 mg/kg wet weight (Stafford & Haines, 1997). Skin-off muscle tissue samples from Lake Diefenbaker in Saskatchewan ranged from 0.303 to 0.593 mg/kg wet weight in piscivorous fish and from 0.114 to 0.255 mg/kg wet weight in prey fish (Donald et al., 2015). Homogenized smallmouth bass from Fumee Lake in Michigan ranged from 0.100 to 0.490 mg/kg wet weight (Henry et al., 1998). My findings fall within the range reported mean THg by the aforementioned Hg studies, and below the highest reported mean THg value by Stahl et al. (2009).

Studies most similar to my research were Finley et al. (2016) and Mierzykowski et al. (2013). Skin-on brook trout fillets from the Aroostook National Wildlife Refuge in Maine had Hg that ranged from 0.091 to 0.234 mg/kg wet weight (Mierzykowski, 2013). THg in dried, skinless dorsal muscle tissue from New Brunswick (A, Bathurst, Moose, Nictau, and Tahoe lakes) ranged from 0.33 to 1.2 mg/kg dry weight. Mean Hg levels in Sinclair Lake brook trout were up to 1.7 times higher than Mierzykowski et al. (2013) and up to 1.6 times higher than Finley et al. (2016). The other 6 study lakes were within the mean THg range measured by Mierzykowski et al. (2013) and Finley et al. (2016). Brook trout from my study are comparable to brook trout from other lakes in the region, which suggests my results are typical for New Brunswick and north-eastern North America.

MeHg guidelines are used as a conservative estimate, with the assumption that almost all THg in brook trout tissue is MeHg (Bloom, 1992). All fish sampled (n=42) fell above the CCME tissue residue guideline for methylmercury for the protection of wildlife consumers of aquatic biota is 33 µg/kg or 0.033 mg/kg wet weight (CCME, 2000). Brook trout exceeded guidelines by ~1.4 times (0.045 mg/kg converted wet weight, Lake Anthony) up to ~12.8 times (0.40 mg/kg converted wet weight, Sinclair Lake). The brook trout caught in these seven lakes are above safe consumption levels for wildlife consumers of aquatic biota. These findings may have implications for human health, as some of study lakes are popular with recreational anglers.

3.6 Conclusion

My findings suggest mercury levels in brook trout collected from the seven remote study lakes are comparable to Finley et al. (2016). Brook trout (n=42) from New Brunswick study lakes were presumed to be above tissue residue guideline levels of methylmercury for the protection of aquatic biota, under the assumption most THg is MeHg in fish muscle tissue (Bloom, 1992). Though the brook trout sampled were within range of what has been reported in NB and Maine, mercury levels in the brook trout fillet samples are higher than acceptable for wildlife consumption. Aquatic biota are susceptible to bioaccumulation and biomagnification of mercury, and consumption of brook trout from these lakes may have chronic impacts on piscivorous wildlife.

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3.8 Tables

Table 3.1 Physical characteristics, and select physical and chemical measures from study lakes in New Brunswick, Canada. Surface water chemistry was collected between September 2 and October 28, 2020.

	Goodwin	**Sinclair**	**Middle Peaked Mountain**	**California**	**Upsalquitch**	**Anthony**	**Bennett**
Latitude (N)	47.25	47.05	46.73	47.44	47.47	45.27	45.62
Longitude (W)	-66.35	-66.57	-66.51	-66.15	-66.49	-66.72	-65.07
Surface area (ha)	21.4	45.6	14.4	45	68.1	29.8	31.3
Maximum depth (m)	7.6	8.5	9.8	11.5	15.2	12.8	11.1
Watershed area (ha)	350	950	300	600	2900	124	1280
Calcium (mg/L)	3.29	2.35	3.35	2.73	7.65	1.20	3.14
Alkalinity ($CaCO_3$) (mg/L)	14	9	12	10	14	2	3
Sulfate (SO_4) (mg/L)	2	2	< 1*	1	16	2	< 1*
Total Nitrogen (mg/L)	< 0.2*	0.2	0.3	0.3	0.2	< 0.2*	< 0.2*
Total Phosphorus (mg/L)	0.014	0.012	0.010	0.022	0.017	0.005	0.006
Total Organic Carbon (mg/L)	2.7	4.4	4.4	2.8	2.9	2.8	2.2
Colour (TCU)	8	28	11	< 5*	8	< 5*	< 5*
Conductivity (µS/cm)	37	23	29	25	67	19	17
pH	7.5	7.3	7.2	7.7	7.5	8.0	7.9
Secchi depth	5.5	5.8	5.9	5.5	6.6	11.3	5.9

*Below laboratory detection limits.

Table 3.2 Mean (±SD) total mercury (THg), lipid, mass, length, δ13C & δ15N of skin-on brook trout muscle tissue samples (n=42) from study lakes. Brook trout were collected between May 22 and October 2, 2020.

	Anthony	**Bennett**	**Goodwin**	**Middle Peaked Mountain**	**Sinclair**	**California**	**Upsalquitch**
n	6	7	6	5	6	6	6
THg (mg/kg dw)	0.252 ±0.0199	0.832 ±0.245	0.490 ±0.171	0.748 ±0.284	1.748 ±0.289	0.327 ±0.0604	0.511 ±0.182
THg (mg/kg ww converted)	0.0504 ±0.003	0.166 ±0.049	0.098 ±0.034	0.149 ±0.056	0.349 ±0.057	0.0654 ±0.012	0.102 ±0.036
Lipid (%)	12.41 ±2.13	4.25 ±3.07	7.48 ±2.84	13.66 ±1.93	7.27 ±1.29	11.13 ±3.60	10.22 ±3.93
Weight (g)	162.07 ±43.87	109.31 ±17.14	308.33 ±172.56	502 ±186.47	308.33 ±86.12	310 ±64.81	180.67 ±102.03
Length (cm)	23.0 ±2.22	22.36 ±1.22	31.5 ±3.59	34.88 ±4.28	32.17 ±2.60	32.17 ±1.47	27.08 ±3.97
δ13C	-28.48 ±0.87	-31.86 ±3.33	-26.73 ±2.02	-29.32 ±1.48	-27.72 ±0.64	-27.47 ±0.77	-27.47 ±0.39
δ15N	7.66 ±0.22	8.63 ±0.72	8.97 ±0.26	8.79 ±0.77	8.69 ±0.38	10.0 ±0.49	9.82 ±0.72

3.9 Figures

Figure 3.1. Map of study lakes located in New Brunswick, Canada.

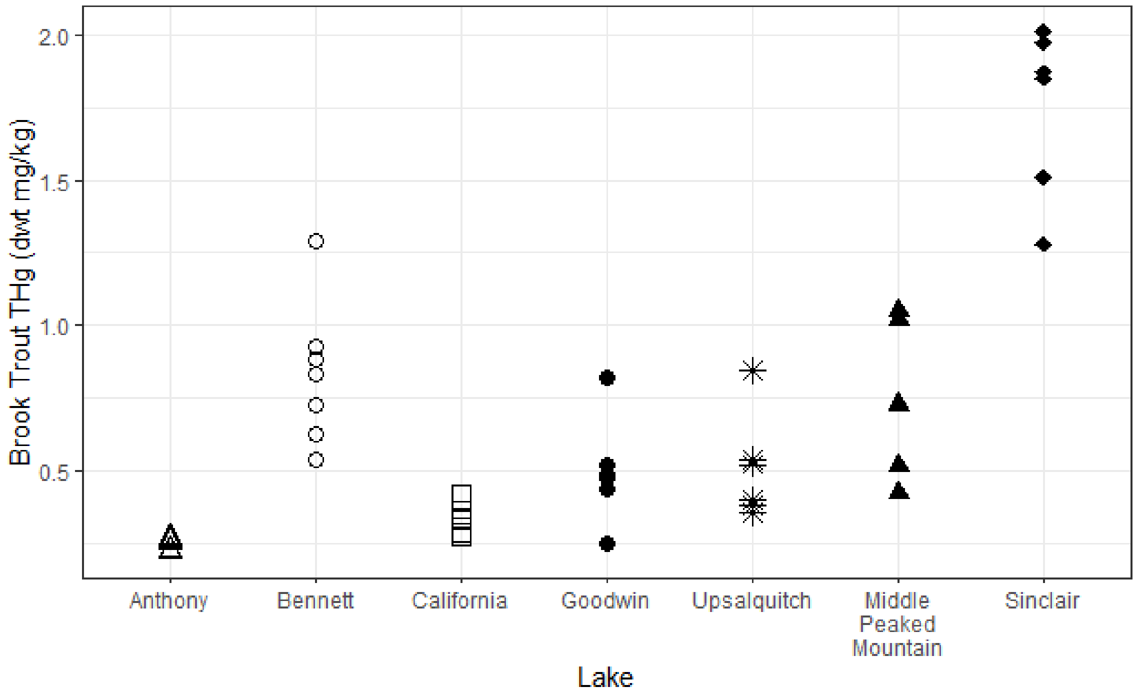

Figure 3.2. Total mercury (mg/kg, dry weight) from skin-on brook trout muscle tissue samples (n=42) collected from seven study lakes in NB, listed in order of lake color (lightest to darkest).

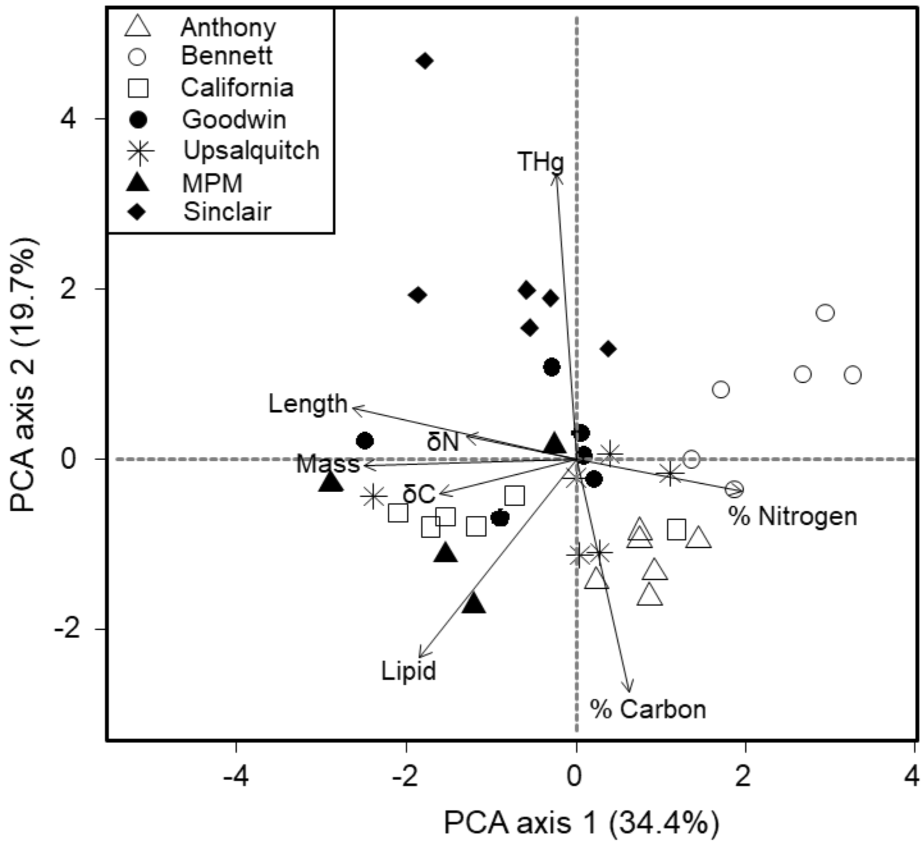

Figure 3.3. A principal component analysis biplot comparing six variables (total mercury, length, mass, δ13C, δ15N, %N, %C, and lipid) from skin-on brook trout muscle samples from seven study lakes in NB.

Chapter 4
General Discussion and Conclusions

4.1 Main Findings

The main objective of this thesis was to investigate the legacy contaminants, DDT and mercury, in brook trout (*Salvelinus fontinalis*) from seven headwater lakes in New Brunswick, Canada (NB).

In chapter 2, DDTs from skin-on brook trout muscle tissue were compared against historical DDT application rates and sedimentary DDTs. DDT levels in reference lakes Anthony and Bennett were much lower than DDT levels in the DDT impact lakes, and fell below the CCME guidelines for wildlife consumption of aquatic biota (CCME, 1999). DDT levels in brook trout from the DDT impact lakes were up to ~22 times higher than the CCME guidelines (Upsalquitch Lake) (CCME, 1999). There was a significant positive correlation between the lake sediment DDTs and brook trout DDTs, which indicates a relationship between the lake sediments as a source of DDT, rather than a sink. My findings indicate historical DDT is leaving the lake sediments and entering the aquatic food web. Additionally, a stable isotope analysis was conducted to investigate the potential that differences in brook trout diets between lakes may influence DDT levels in brook trout samples. There were no consistent isotopic signals between the measures taken from each lake that indicated a trout diet was a contributing factor to the between lake differences observed during data analysis of brook trout and sediment measures.

In chapter 3, total mercury (THg) was analyzed from skin-on brook trout muscle tissue samples. THg from these study lakes are comparable to others in the region, however they are likely above methylmercury CCME guidelines for wildlife consumption of aquatic biota (CCME, 2000). Sinclair Lake had the highest mean THg levels in brook trout samples, with samples exceeding the CCME guideline by up to ~12 times.

4.2 Implications

Legacy contaminants DDT and mercury (Hg) in brook trout from NB lakes were elevated beyond safe consumption guidelines for wildlife (CCME, 1999), as demonstrated in chapters 2 and 3. Middle Peaked Mountain, California, and Goodwin lakes are Crown Reserve Waters with access controlled by NB Natural Resources and Energy Development. They are advertised as prime fishing locations for local anglers. Upsalquitch, Anthony, and Sinclair lakes are known to be used by anglers with varying frequency. Bennett Lake is controlled by Parks Canada and is not fished by the general public. Consuming fish above guideline levels may have implications for the long-term health and well-being of piscivorous wildlife in north-central NB. There are also likely risks present to humans that frequently consume brook trout from NB lakes.

DDT and Hg will bioaccumulate in organisms and biomagnify with increasing food web trophic position. As a result, biomagnification disproportionately impacts top predators in aquatic food webs. Generally, DDTs affect cellular metabolism, neural activity, and liver function (USEPA 1980). DDTs are mutagenic and carcinogenic, and may reduce life longevity. In fish, DDT impacts the central nervous system, which may influence behavioural abnormalities, survival fitness, and reproductive success (Anderson & Peterson, 1969; Beckvar & Lotufo, 2011). In mammals, exposure to DDT may result in reduced reproductive success, limited growth, or a compromised immune system (CCME, 1999). The effect of historical DDT on modern wildlife in NB has not yet been studied.

MeHg is a toxic form of Hg, as it is able to pass through the blood-brain barrier and nuclear membranes of most vertebrates (Sloss, 1995; CCME, 2000). In the neurological system, MeHg is able to damage or destroy nerve tissue (CCME, 2000). Resulting impacts may include ataxia, staggering, paralysis, and anorexia (CCME, 2000). Exposure to these legacy contaminants may reduce an animal's ability to evade predation, hunt, reproduce, and increase susceptibility to other diseases. The results reported in this thesis were given for skin-on muscle tissue from medium to large brook trout (25-40 cm). The average wildlife consumer of brook trout is ingesting the entire fish, including intestines and liver, which often contain the highest concentrations of DDT and/or Hg.

Though this thesis did not include any study to evaluate the effects of DDTs in humans, it is important to note that recreational anglers frequent the study lakes researched in this thesis and consume brook trout with levels of DDT and Hg likely similar to those reported in chapters 2 and 3. Frequent consumption of wild brook trout from some NB lakes may present risks to humans.

4.3 Future research

This project only investigated two contaminants in seven of the province's ~2,500 lakes. The findings from this thesis warrant questions about the contaminant levels in other NB lakes. Are there other lakes with higher levels of DDTs or Hg? It is likely that other lakes in the DDT application area will have higher levels of DDTs. We have not sampled any lakes in the area (Main Southwest Miramichi River watershed) with the highest DDT application rates between 1952 and 1968. Further sampling will offer a more widespread understanding of DDT in NB. Sedimentary DDT analyses at other dimictic and polymictic lakes would likely act as a good indicator of relative DDT concentration within an aquatic ecosystem. Collecting sediment cores is more efficient than sampling aquatic organisms, and does not require organism mortality.

Lakes in the region are warming and growing season is extended as climate change impacts Atlantic Canada. An investigation into the impact warming water temperatures and greater thermal stratification may have on DDT and Hg degradation would be beneficial to understand how the contaminants may volatize or degrade under changing lake conditions, especially near the sediment-water interface. Warming water may shift the bacterial communities within a lake, change macrophyte coverage, increased algal blooms, and impact other factors that can influence contaminant degradation in lake sediments as a result of climate change.

Finally, recreational anglers frequent these study lakes and consume brook trout unaware of the potential impacts chronic DDT or Hg exposure may have in the long-term. A human health research project to develop accurate guidelines for human consumption of fish contaminated with DDT and/or Hg would be beneficial for local anglers. Current risk guidelines are unclear and not necessarily indicative of actual exposure risks, given the elevated levels of DDT and Hg found in lakes studied in chapters 2 and 3.

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Appendix A

Brook trout measures

Lake Anthony						
Date Captured	02-Oct-2020	02-Oct-2020	02-Oct-2020	02-Oct-2020	02-Oct-2020	02-Oct-2020
Capture Method	Fyke netted	Fyke netted	Fyke netted	Fyke netted	Fyke netted	Fyke netted
Trout ID	ANT1	ANT2	ANT3	ANT4	ANT5	ANT6
Mass (g)	200.99	195.33	209.31	119.06	124.21	123.5
Length (cm)	25	24.8	25.2	21	21.5	20.5
Lipid %	10.6	11.9	13.6	15.9	10.1	12.3
2,4 DDE	<10*	<10*	<10*	<10*	<10*	<10*
4,4 DDE	<10*	<10*	13.4	12.6	15.6	13.2
2,4 DDD	<10*	<10*	<10*	<10*	<10*	<10*
4,4 DDD	<10*	<10*	<10*	<10*	<10*	<10*
2,4 DDT	<10*	<10*	<10*	<10*	<10*	<10*
4,4 DDT	<10*	<10*	<10*	<10*	<10*	<10*
∑DDT (ng/g dwt)	60	60	63.4	62.6	65.6	63.2
THg (mg/kg dwt)	0.272	0.270	0.256	0.224	0.259	0.232
$\delta^{15}N$	7.91	7.59	7.58	7.93	7.40	7.45
$\delta^{13}C$	-28.94	-29.19	-28.81	-29.07	-27.64	-26.90

*Below laboratory detection limits.

Bennett Lake							
Date Captured	21-Sep-2020	21-Sep-2020	12-Nov-2020	12-Nov-2020	12-Nov-2020	12-Nov-2020	12-Nov-2020
Capture Method	Angled	Angled	Fyke netted	Fyke netted	Fyke netted	Fyke netted	Fyke netted
Trout ID	BEN1	BEN2	BEN3	BEN4	BEN5	BEN6	BEN7
Mass (g)	120	110	89.3	137.6	117.24	96.71	94.32
Length (cm)	22	23.5	21.5	24.5	22	21	22
Lipid %	8.3	5.7	1.5	1.0	7.9	3.3	2.0
2,4 DDE	<10*	<10*	<10*	<10*	<10*	<10*	<10*
4,4 DDE	<10*	<10*	<10*	<10*	<10*	<10*	<10*
2,4 DDD	<10*	<10*	<10*	<10*	<10*	<10*	<10*
4,4 DDD	<10*	<10*	<10*	<10*	<10*	<10*	<10*
2,4 DDT	<10*	<10*	<10*	<10*	<10*	<10*	<10*
4,4 DDT	<10*	<10*	<10*	<10*	<10*	<10*	<10*
∑DDT (ng/g dwt)	60	60	60	60	60	60	60
THg (mg/kg dwt)	0.540	0.832	1.29	0.885	0.626	0.727	0.927
$\delta^{15}N$	8.92	9.45	9.18	8.16	7.29	8.75	8.66
$\delta^{13}C$	-33.16	-32.49	-31.52	-35.66	-25.04	-33.29	-33.16

*Below laboratory detection limits.

California Lake						
Date Captured	29-May-2020	29-May-2020	29-May-2020	29-May-2020	30-May-2020	30-May-2020
Capture Method	Angled	Angled	Angled	Angled	Angled	Angled
Trout ID	CAL1	CAL2	CAL3	CAL4	CAL5	CAL6
Mass (g)	350	380	200	290	350	290
Length (cm)	33	33.5	29.5	31.5	33	32.5
Lipid %	15.2	12.5	5.0	12.2	12.9	8.9
2,4 DDE	<10*	<10*	<10*	<10*	<10*	<10*
4,4 DDE	760.4	628.4	285.7	505.6	798.3	736.1
2,4 DDD	39.0	49.3	18.6	35.7	39.1	73.1
4,4 DDD	175.0	196.8	75.2	148.4	238.1	359.1
2,4 DDT	<10*	<10*	<10*	<10*	<10*	<10*
4,4 DDT	46.7	40.4	11.5	13.6	27.0	33.9
∑DDT (ng/g dwt)	1041.1	934.9	411.0	723.3	1122.5	1222.2
THg (mg/kg dwt)	0.338	0.272	0.422	0.289	0.367	0.272
$\delta^{15}N$	10.68	10.10	9.64	10.13	10.22	9.26
$\delta^{13}C$	-26.44	-27.47	-27.03	-28.10	-27.18	-28.57

*Below laboratory detection limits.

Goodwin Lake						
Date Captured	26-May-2020	26-May-2020	26-May-2020	26-May-2020	26-May-2020	26-May-2020
Capture Method	Angled	Angled	Angled	Angled	Angled	Angled
Trout ID	GDW1	GDW2	GDW3	GDW4	GDW5	GDW6
Mass (g)	650	280	300	220	200	200
Length (cm)	37	31.5	34.5	29.5	28	28.5
Lipid %	7.8	10.5	3.3	6.3	10.7	6.3
2,4 DDE	<10*	<10*	<10*	<10*	<10*	<10*
4,4 DDE	121.3	33.5	30.0	39.4	35.1	61.6
2,4 DDD	<10*	<10*	<10*	<10*	<10*	<10*
4,4 DDD	<10*	<10*	<10*	<10*	<10*	11.6
2,4 DDT	<10*	<10*	<10*	<10*	<10*	<10*
4,4 DDT	<10*	<10*	<10*	<10*	<10*	<10*
∑DDT (ng/g dwt)	171.3	83.5	80.0	89.4	85.1	113.2
THg (mg/kg dwt)	0.489	0.251	0.824	0.475	0.519	0.436
$\delta^{15}N$	8.95	8.90	8.91	8.55	9.31	9.18
$\delta^{13}C$	-25.21	-25.26	-26.94	-25.74	-30.60	-26.61

*Below laboratory detection limits.

Middle Peaked Mountain Lake					
Date Captured	22-May-2020	22-May-2020	22-May-2020	22-May-2020	23-May-2020
Capture Method	Angled	Angled	Angled	Angled	Angled
Trout ID	MPM1	MPM2	MPM3	MPM4	MPM5
Mass (g)	700	550	210	450	600
Length (cm)	39.5	38	28.5	33.6	34.8
Lipid %	10.5	14.9	13.3	14.0	15.4
2,4 DDE	<10*	<10*	<10*	<10*	<10*
4,4 DDE	407.4	551.2	409.4	282.4	292.4
2,4 DDD	14.2	12.5	<10	<10	<10
4,4 DDD	63.7	153.1	125.8	88.0	65.3
2,4 DDT	<10*	<10*	<10*	<10*	<10*
4,4 DDT	<10*	<10*	<10*	<10*	<10*
∑DDT (ng/g dwt)	515.3	746.8	575.2	410.4	397.6
THg (mg/kg dwt)	0.728	1.02	1.05	0.520	0.424
$\delta^{15}N$	9.44	9.37	8.56	9.02	7.56
$\delta^{13}C$	-28.96	-28.13	-28.45	-29.19	-31.85

*Below laboratory detection limits.

Upsalquitch Lake						
Date Captured	31-May-2020	08-Jun-2020	09-Jun-2020	09-Jun-2020	09-Jun-2020	09-Jun-2020
Capture Method	Fyke netted	Angled	Fyke netted	Fyke netted	Fyke netted	Fyke netted
Trout ID	UPS1	UPS2	UPS3	UPS4	UPS5	UPS6
Mass (g)	190	380	155	110	136	113
Length (cm)	27.5	34.5	27	24	26	23.5
Lipid %	10.6	16.1	6.7	5.5	9.4	13.0
2,4 DDE	<10*	<10*	<10*	<10*	<10*	<10*
4,4 DDE	338.8	1300	542.6	396.8	650.2	641.7
2,4 DDD	<10*	18.7	<10*	<10*	<10*	<10*
4,4 DDD	37.8	173.6	49.9	28.3	55.4	52.0
2,4 DDT	<10*	<10*	<10*	<10*	<10*	<10*
4,4 DDT	<10*	29.8	11.8	<10*	17.1	18.0
∑DDT (ng/g dwt)	416.6	1542.1	634.3	465.1	752.6	741.8
THg (mg/kg dwt)	0.402	0.846	0.539	0.358	0.539	0.382
$\delta^{15}N$	10.12	10.94	9.77	8.75	9.86	9.47
$\delta^{13}C$	-26.84	-26.98	-26.38	-26.77	-26.15	-26.03

*Below laboratory detection limits.

Sinclair Lake						
Date Captured	04-Jun-2020	11-Jun-2020	11-Jun-2020	11-Jun-2020	11-Jun-2020	12-Jun-2020
Capture Method	Angled	Angled	Angled	Angled	Angled	Angled
Trout ID	SIN1	SIN2	SIN3	SIN4	SIN5	SIN6
Mass (g)	350	300	450	300	250	200
Length (cm)	32.5	30.5	37	32.5	30.5	30
Lipid %	7.4	7.4	9.6	7.0	6.1	6.1
2,4 DDE	<10*	<10*	<10*	<10*	<10*	<10*
4,4 DDE	759.5	680.1	808.6	533.1	503.8	538.1
2,4 DDD	10	12.2	13.2	<10*	<10*	<10*
4,4 DDD	121.2	70.7	162.5	67.2	45.3	108.8
2,4 DDT	10.3	12.9	<10*	15.1	<10*	<10*
4,4 DDT	80.1	52.7	84.0	92.6	<10*	35.8
∑DDT (ng/g dwt)	991.1	838.6	1088.3	728.0	589.0	712.7
THg (mg/kg dwt)	1.85	1.87	1.97	1.51	2.01	1.28
$\delta^{15}N$	8.69	8.78	9.66	9.19	9.02	8.69
$\delta^{13}C$	-28.02	-28.00	-28.14	-26.74	-27.13	-28.34

*Below laboratory detection limits.

Appendix B

Water chemistry parameters measured with multiparameter sonde at one meter depth increments in the deepest basin of the lake.

Middle Peaked Mountain Lake (02/09/2020)							
Depth (m)	Water temperature (°C)	Dissolved oxygen (%)	Specific conductance (µS/cm)	Conductivity (µS/cm)	Total dissolved solids (mg/L)	Salinity (g/kg)	pH
0	16.9	N/A*	29.9	0.025	18.9	0.01	7.58
1	16.9	N/A*	28.8	0.024	18.9	0.01	7.31
2	16.9	N/A*	28.7	0.024	18.9	0.01	7.18
3	16.9	N/A*	28.7	0.024	18.9	0.01	7.12
4	16.8	N/A*	28.7	0.024	18.9	0.01	7.13
5	13.9	N/A*	33.0	0.026	20.8	0.01	6.36
6	8.4	N/A*	38.3	0.026	24.7	0.02	6.15
7	6.6	N/A*	37.0	0.024	24.1	0.02	6.08
8	5.7	N/A*	57.2	0.036	36.4	0.03	6.33
9	5.7	N/A*	61.1	0.039	39.7	0.03	6.40
10	5.7	N/A*	63.7	0.040	41.6	0.03	6.45

*Dissolved oxygen probe was not working properly and needed recalibration.

Sinclair Lake (02/09/2020)							
Depth (m)	Water temperature (°C)	Dissolved oxygen (%)	Specific conductance (μS/cm)	Conductivity (μS/cm)	Total dissolved solids (mg/L)	Salinity (g/kg)	pH
1	16.2	N/A*	22.7	0.019	15.0	0.01	6.91
2	16.2	N/A*	22.8	0.019	15.0	0.01	6.88
3	16.1	N/A*	22.8	0.019	15.0	0.01	6.88
4	15.9	N/A*	22.8	0.019	15.0	0.01	6.90
5	15.0	N/A*	22.7	0.018	15.0	0.01	6.79
6	14.6	N/A*	22.9	0.018	15.0	0.01	6.71
7	14.2	N/A*	23.0	0.018	15.0	0.01	6.57
8	10.2	N/A*	30.2	0.021	19.5	0.01	6.07
<9	9.9	N/A*	24.8	0.025	26.0	0.02	6.26

*Dissolved oxygen probe was not working properly and needed recalibration.

Goodwin Lake (02/09/2020)							
Depth (m)	Water temperature (°C)	Dissolved oxygen (%)	Specific conductance (µS/cm)	Conductivity (µS/cm)	Total dissolved solids (mg/L)	Salinity (g/kg)	pH
0	16.2	N/A*	36.2	0.030	23.4	0.02	7.28
1	16.2	N/A*	36.1	0.030	23.4	0.02	7.26
2	16.2	N/A*	36.0	0.030	23.4	0.02	7.24
3	16.0	N/A*	36.1	0.030	23.4	0.02	7.25
4	15.4	N/A*	36.1	0.029	23.4	0.02	7.23
5	14.6	N/A*	36.4	0.029	23.4	0.02	7.15
6	12.5	N/A*	36.4	0.027	23.4	0.02	6.70
7	8.4	N/A*	37.7	0.026	24.1	0.02	6.32
8	8.1	N/A*	39.6	0.027	26.0	0.02	6.30

*Dissolved oxygen probe was not working properly and needed recalibration.

Upsalquitch Lake (10/09/2020)							
Depth (m)	Water temperature (°C)	Dissolved oxygen (%)	Specific conductance (µS/cm)	Conductivity (µS/cm)	Total dissolved solids (mg/L)	Salinity (g/kg)	pH
0	16.3	84.30	68.2	0.057	44.2	0.03	8.35
1	16.3	85.00	67.9	0.057	44.2	0.03	8.23
2	16.3	83.50	67.9	0.057	44.2	0.03	8.21
3	16.3	83.50	67.9	0.057	44.2	0.03	8.18
4	16.3	82.40	67.9	0.057	44.2	0.03	8.16
5	16.3	83.00	67.8	0.057	44.2	0.03	8.13
6	16.1	82.80	67.6	0.056	44.2	0.03	8.09
7	15.1	76.80	66.1	0.054	42.9	0.03	7.95
8	11.7	60.10	57.5	0.042	37.7	0.03	7.90
9	7.2	39.80	55.4	0.036	35.8	0.03	8.49
10	6.7	32.50	55.6	0.036	35.8	0.03	8.79
11	6.4	25.10	58.0	0.039	37.1	0.03	9.01

California Lake (10/09/2020)							
Depth (m)	Water temperature (°C)	Dissolved oxygen (%)	Specific conductance (µS/cm)	Conductivity (µS/cm)	Total dissolved solids (mg/L)	Salinity (g/kg)	pH
0	16.4	83.20	26.6	0.022	17.6	0.02	8.32
1	16.4	79.70	26.5	0.022	16.9	0.01	8.30
2	16.4	80.10	38.3	0.032	21.5	0.01	8.25
3	16.4	79.60	26.3	0.022	16.9	0.01	8.23
4	16.4	79.10	26.4	0.022	17.6	0.01	8.22
5	16.4	76.50	32.0	0.027	20.2	0.01	8.18
6	16.4	77.90	26.4	0.022	18.2	0.01	8.15
7	14.7	21.50	40.8	0.032	23.4	0.01	7.63
8	13.4	5.90	39.2	0.030	25.4	0.02	7.85
9	10.2	1.60	70.1	0.050	49.4	0.04	8.36

Lake Anthony (18/10/2020)							
Depth (m)	Water temperature (°C)	Dissolved oxygen (%)	Specific conductance (µS/cm)	Conductivity (µS/cm)	Total dissolved solids (mg/L)	Salinity (g/kg)	pH
0	12.8	87.30	20.0	0.015	12.4	0.01	7.48
1	12.7	85.60	18.8	0.014	12.4	0.01	7.15
2	12.6	85.00	19.2	0.015	12.4	0.01	6.87
3	12.5	86.20	19.2	0.015	12.4	0.01	6.73
4	12.4	86.00	19.2	0.015	12.4	0.01	6.59
5	12.4	84.30	19.2	0.015	12.4	0.01	6.56
6	12.4	83.80	19.2	0.015	12.4	0.01	6.55
7	12.3	82.40	19.2	0.015	12.4	0.01	6.53
8	12.3	82.20	19.5	0.015	13.0	0.01	6.52
9	12.3	82.00	19.2	0.015	12.4	0.01	6.52
10	12.2	80.10	19.2	0.014	12.4	0.01	6.51
11	12.1	79.40	20.0	0.015	13.0	0.01	6.48
12	12.2	78.40	21.3	0.016	13.7	0.01	6.40
13	12.3	6.60	24.4	0.018	15.6	0.01	6.26
14	12.3	4.60	24.3	0.018	15.6	0.01	6.15

Bennett Lake (28/10/2020)							
Depth (m)	Water temperature (°C)	Dissolved oxygen (%)	Specific conductance (μS/cm)	Conductivity (μS/cm)	Total dissolved solids (mg/L)	Salinity (g/kg)	pH
0	8.0	78.40	34.0	0.023	22.1	0.01	6.68
1	8.0	78.30	34.0	0.023	22.1	0.01	6.57
2	8.0	79.10	34.0	0.023	22.1	0.01	6.52
3	7.9	78.00	34.0	0.023	22.1	0.01	6.52
4	7.9	77.20	34.0	0.023	22.1	0.01	6.50
5	7.8	77.20	34.1	0.023	22.1	0.01	6.51
6	7.8	77.30	34.1	0.023	22.1	0.01	6.50
7	7.8	76.20	34.1	0.023	22.1	0.01	6.49
8	7.7	76.60	34.1	0.023	22.1	0.01	6.50
9	7.7	76.50	34.1	0.023	22.1	0.01	6.50
10	7.7	75.20	34.1	0.023	22.1	0.01	6.48
11	7.7	75.50	35.4	0.024	22.8	0.02	6.45

Appendix C

Water chemistry parameters sampled at the lake surface and analyzed in-laboratory.

Analytes	**Unit**	**RL**	Goodwin	Sinclair	Middle Peaked Mountain	California	Upsalquitch	Anthony	Bennett
Sodium	mg/L	0.05	1.35	1.39	1.05	0.82	1.18	1.59	2.41
Potassium	mg/L	0.02	0.45	0.38	0.23	0.29	0.37	0.24	0.11
Calcium	mg/L	0.05	3.29	2.35	3.35	2.73	7.65	1.20	3.14
Magnesium	mg/L	0.01	1.25	0.35	0.63	0.72	1.63	0.29	0.41
Alkalinity (as $CaCO_3$)	mg/L	2	14	9	12	10	14	2	3
Chloride	mg/L	0.5	< 0.5	0.6	0.6	0.6	0.7	2.3	2
Fluoride	mg/L	0.05	< 0.05	0.10	0.20	< 0.05	< 0.05	0.19	< 0.05
Sulfate	mg/L	1	2	2	< 1	1	16	2	< 1
Bromine	mg/L	0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.01
Ammonia (as N)	mg/L	0.05	0.16	0.37	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Un-ionized @ 20ºC	mg/L	-	0.002	0.003	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Nitrate + Nitrite (as N)	mg/L	0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Nitrite (as N)	mg/L	0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Nitrate (as N)	mg/L	0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Nitrogen - Total	mg/L	0.2	< 0.2	0.2	0.3	0.3	0.2	< 0.2	< 0.2
Phosphorus - Total	mg/L	0.002	0.014	0.012	0.010	0.022	0.017	0.005	0.006
Carbon - Total Organic	mg/L	0.5	2.7	4.4	4.4	2.8	2.9	2.8	2.2
Colour	TCU	5	8	28	11	< 5	8	< 5	< 5
Conductivity	µS/cm	1	37	23	29	25	67	19	17
pH	units	-	7.5	7.3	7.2	7.7	7.5	8.0	7.9
Turbidity	NTU	0.1	0.5	0.6	0.7	1.0	0.6	0.2	0.4

Calculated Parameters	**Unit**	**RL**	Goodwin	Sinclair	Middle Peaked Mountain	California	Upsalquitch	Anthony	Bennett
Bicarbonate (as $CaCO_3$)	mg/L	-	13.9	9.0	12.0	9.9	13.9	1.9	2.9
Carbonate (as $CaCO_3$)	mg/L	-	0.041	0.017	0.018	0.047	0.041	0.018	0.022

Hardness (as $CaCO_3$)	mg/L	0.2	13.4	7.3	11.0	9.8	25.8	4.2	9.5
TDS (calc)	mg/L	-	20	18	18	15	39	12	12
Saturation pH (20°C)	units	-	9.6	10.0	9.7	9.8	9.3	10.9	10.3
Langelier Index (20°C)	-	-	-2.12	-2.65	-2.48	-2.14	-1.78	-2.90	-2.41

RL = Reporting limit

Appendix D

Upper lake sediment DDT measures.

Lake	Upsalquitch	California	Middle Peaked Mountain	Goodwin	Sinclair	Bennett	Anthony
Sediment depth (cm)	1.5-3.0	1.5-3.0	1.0-2.0	1.5-2.5	1.5-2.5	0.0-2.0	0.0-2.5
Total DDE	69.3	94.8	120.5	24.8	87.5	6	7.5
Total DDD	45.5	81.6	92.6	13.1	37.6	7.3	4
Total DDT	8.2	8.5	23.7	2.3	21.3	4	4
∑DDTs	123.0	185.0	236.9	40.3	146.5	17.3	15.5

Appendix E

Isotopic measures from benthic invertebrates, brook trout muscle tissue, zooplankton, and fish gut contents.

Lake	Sample ID	Sample Type	C (%)	N (%)	$\delta^{15}N$	$\delta^{13}C$
Anthony	AB1	Benthic	13.61	0.52	NA	-11.01
Anthony	AB10	Benthic	48.77	12.59	4.03	-29.01
Anthony	AB2	Benthic	50.39	9.42	4.68	-31.63
Anthony	AB3	Benthic	44.12	11.86	4.42	-30.11
Anthony	AB4	Benthic	34.73	8.73	0.9	-24.33
Anthony	AB5	Benthic	46.93	13.3	1.85	-23.75
Anthony	AB6	Benthic	46.27	12.71	3.12	-24.56
Anthony	AB7	Benthic	47.44	12.17	2.57	-24.17
Anthony	AB8	Benthic	47.65	12.06	1.2	-23.61
Anthony	ANT1	Fish	50.49	14.01	7.91	-28.94
Anthony	ANT1R	Fish	51.05	14	7.79	-28.85
Anthony	ANT2	Fish	50.05	13.94	7.59	-29.19
Anthony	ANT3	Fish	51.84	13.02	7.58	-28.81
Anthony	ANT4	Fish	50.64	13.74	7.93	-29.07
Anthony	ANT5	Fish	50.29	14.26	7.4	-27.64
Anthony	ANT6	Fish	50.97	13.24	7.45	-26.9
Anthony	A4G1	Gut	45.1	8.62	4.06	-34.19
Anthony	ANTZOO	Zooplankton	50.41	11.81	0.92	-29.52
Bennett	BB1R	Benthic	43.29	10.94	5.4	-29.82
Bennett	BB1R	Benthic	47.28	11.43	5.12	-30.19
Bennett	BB2	Benthic	46.53	10.47	4.61	-29.8
Bennett	BB3	Benthic	47.86	9.96	3.71	-31.06
Bennett	BB4	Benthic	33.2	5.82	6.8	-35.18
Bennett	BB5	Benthic	34.75	7.92	2.85	-27.64
Bennett	BB7	Benthic	46.7	12.42	6.23	-30.19
Bennett	BB8	Benthic	24.53	4.71	3.15	-25.59
Bennett	BEN1	Fish	51.78	14.23	8.92	-33.16
Bennett	BEN1R	Fish	50.36	14.42	8.89	-32.82
Bennett	BEN2	Fish	49.17	14.5	9.45	-32.49
Bennett	BEN3	Fish	59.78	18.99	9.18	-31.52
Bennett	BEN4	Fish	47.61	15.33	8.16	-35.66
Bennett	BEN5	Fish	48.55	14.76	7.29	-25.04
Bennett	BEN6	Fish	48.39	15.05	8.75	-33.29
Bennett	BEN7	Fish	50.25	16.5	8.66	-33.16
Bennett	B1G2	Gut	42.91	10.68	6.46	-33.62
Bennett	B1G3	Gut	44.33	10.17	6.59	-34.41

Bennett	B3G1	Gut	41.38	10.19	8.08	-30.29
Bennett	B3G2	Gut	21.07	3.52	7.58	-25.29
Bennett	B5G1	Gut	41.2	9.55	4.27	-29.68
Bennett	BENZOO	Zooplankton	52.24	11.09	4.62	-34.12
California	CB1	Benthic	41.57	11.52	2.68	-17.84
California	CB2	Benthic	48.35	9.69	4.65	-24.62
California	CB3	Benthic	44.39	12.85	5.35	-23.59
California	CB4	Benthic	42.2	10.56	5.08	-25.36
California	CB5R	Benthic	48.4	9.67	5.66	-25.34
California	CB5R	Benthic	46.48	10.16	5.08	-23.67
California	CB6	Benthic	46.79	10.41	2.58	-32.4
California	CB7	Benthic	34.12	8.87	2.94	-20.09
California	CB8	Benthic	42.74	12.28	4.83	-23.87
California	CAL1	Fish	48.12	14.19	10.68	-26.44
California	CAL1R	Fish	76.13	21.35	10.67	-26.49
California	CAL2	Fish	50.43	13.81	10.1	-27.47
California	CAL3	Fish	54.6	16.82	9.64	-27.03
California	CAL4	Fish	50.63	13.67	10.13	-28.1
California	CAL5	Fish	49.92	14.32	10.22	-27.18
California	CAL6	Fish	50.58	13.49	9.26	-28.57
California	C1G1	Gut	20.97	3.68	10.32	-27.67
California	C3G1	Gut	39.75	7.91	10.39	-27.82
California	C3G2	Gut	52.64	5.46	3.63	-26.75
California	C4G1	Gut	43.44	9.66	6.59	-25.14
California	C5G1	Gut	37.65	8.86	10.07	-26.98
California	C6G1	Gut	38.36	8.96	9.83	-27.62
California	CALZOO	Zooplankton	49.2	10.7	4.58	-21.27
Goodwin	GB1	Benthic	45.53	10.46	2.6	-22.89
Goodwin	GB2	Benthic	51.36	10.13	2.16	-20.24
Goodwin	GB3	Benthic	42.52	6.63	2.36	-25.79
Goodwin	GB3R	Benthic	46.25	7.34	2.39	-24.43
Goodwin	GB4	Benthic	49.16	12.53	2.49	-25.45
Goodwin	GB5	Benthic	44.12	12.02	6.79	-31.26
Goodwin	GB7	Benthic	20.11	2.52	5.15	-27.07
Goodwin	GB8	Benthic	38.11	10.49	2.47	-21.15
Goodwin	GDW1	Fish	48.89	14.01	8.95	-25.21
Goodwin	GDW1R	Fish	49.74	12.96	8.92	-25.33
Goodwin	GDW2	Fish	49.92	14.06	8.9	-25.26
Goodwin	GDW3	Fish	49.23	14.64	8.91	-26.94
Goodwin	GDW4	Fish	48.29	14.43	8.55	-25.74
Goodwin	GDW5	Fish	49.91	14	9.31	-30.6

Goodwin	GDW6	Fish	49.85	14	9.18	-26.61
Goodwin	G1G1	Gut	53.05	8.81	5.56	-24.25
Goodwin	G1G2	Gut	48.05	10.64	6.25	-24.01
Goodwin	G2G2	Gut	44.4	8.02	5.94	-29.57
Goodwin	G2G3	Gut	50.75	9.27	4.27	-27.85
Goodwin	G3G1	Gut	47.9	9.39	5.07	-26.8
Goodwin	G3G2	Gut	40.15	8.1	5.12	-25.55
Goodwin	G3G3	Gut	55.77	10.35	1.5	-22.32
Goodwin	G3G5	Gut	19.87	2.73	6.14	-20.02
Goodwin	G3G6	Gut	46.07	9.98	5.84	-29.6
Goodwin	G5G2	Gut	50.18	9.24	4.36	-26.24
Goodwin	G5G3	Gut	45.12	11.18	5.51	-24.15
Goodwin	G5G4	Gut	43.2	8.43	4.4	-29.41
Goodwin	G6G2	Gut	48.79	9.56	5.99	-24.39
Goodwin	G6G7	Gut	40.91	8.88	3.98	-23.07
Goodwin	GDWZOO	Zooplankton	57.85	8.16	4.79	-36.48
Middle Peaked Mountain	MPB1	Benthic	52.26	10.03	-1.14	-22.73
Middle Peaked Mountain	MPB2	Benthic	47.33	6.82	2.07	-25.28
Middle Peaked Mountain	MPB3	Benthic	45.76	11.24	3.94	-24.99
Middle Peaked Mountain	MPB4	Benthic	46.79	8.1	3.95	-40.18
Middle Peaked Mountain	MPB5	Benthic	49.48	10.65	2.77	-26.27
Middle Peaked Mountain	MPB6	Benthic	45.67	10.48	3.68	-24.64
Middle Peaked Mountain	MPB6R	Benthic	43.95	10.91	3.71	-24.35
Middle Peaked Mountain	MPB7	Benthic	16	1.76	1.5	-15.74
Middle Peaked Mountain	MPB8	Benthic	33.11	8.14	1.63	-21.53
Middle Peaked Mountain	MPM1	Fish	53.02	13.23	9.44	-28.96
Middle Peaked Mountain	MPM1R	Fish	52.7	13.17	9.48	-28.7
Middle Peaked Mountain	MPM2	Fish	52.09	13.05	9.37	-28.13
Middle Peaked Mountain	MPM3	Fish	49.38	13.98	8.56	-28.45

Middle Peaked Mountain	MPM4	Fish	53.06	13.61	9.02	-29.19
Middle Peaked Mountain	MPM5	Fish	54.58	14.26	7.56	-31.85
Middle Peaked Mountain	M1G1	Gut	47.78	10.9	2.49	-27.88
Middle Peaked Mountain	M1G1	Gut	43.02	8.2	2.66	-29.02
Middle Peaked Mountain	M1G2	Gut	46.16	10.16	4.43	-29.09
Middle Peaked Mountain	M2G1	Gut	45.95	11.7	4.27	-28.52
Middle Peaked Mountain	M2G3	Gut	40.16	9.94	4.2	-24.91
Middle Peaked Mountain	M2G4	Gut	48.78	10.72	3.82	-29.01
Middle Peaked Mountain	M2G5	Gut	45.2	9.89	5.75	-25.1
Middle Peaked Mountain	M2G6	Gut	41.95	10.59	8.61	-32.19
Middle Peaked Mountain	M2G7	Gut	47.85	11.07	3.45	-27.05
Middle Peaked Mountain	M3G1	Gut	46.03	8.3	4.65	-30.93
Middle Peaked Mountain	M3G2	Gut	26.69	4.79	5.05	-24.51
Middle Peaked Mountain	M4G1	Gut	47.1	10.92	4.98	-23.07
Middle Peaked Mountain	M4G2	Gut	48.45	11.24	2.63	-27.07
Middle Peaked Mountain	M4G3	Gut	22.58	3.59	3.42	-20.58
Middle Peaked Mountain	M4G4	Gut	39.81	9.51	6.66	-27.95
Middle Peaked Mountain	M5G1	Gut	47.55	12	4.01	-24.84
Middle Peaked Mountain	M5G2	Gut	22.28	3.63	3.5	-20.78
Middle Peaked Mountain	M5G3	Gut	45.39	10.16	3.01	-23.92
Middle Peaked Mountain	M5G4	Gut	46.98	11.01	4.58	-23.76

Middle Peaked Mountain	M5G5	Gut	42.48	8.51	2.38	-28.46
Middle Peaked Mountain	M5G6	Gut	24.72	4.42	3.35	-19.47
Middle Peaked Mountain	MPMZOO	Zooplankton	49.88	9.93	3.36	-32.3
Sinclair	SB1	Benthic	46.53	7.33	1.54	-24.88
Sinclair	SB2	Benthic	45.98	10.38	4.64	-34.2
Sinclair	SB3	Benthic	49.77	11.33	-0.04	-25.86
Sinclair	SB4	Benthic	39.81	10.76	1.87	-24.17
Sinclair	SB5	Benthic	38.93	10.21	3.4	-24.1
Sinclair	SB6	Benthic	50.46	11.85	3.17	-26.31
Sinclair	SB7	Benthic	18.11	2.18	3.02	-16.62
Sinclair	SB8	Benthic	47.2	12.15	2.74	-23.26
Sinclair	SIN1	Fish	48.98	14.44	8.69	-28.02
Sinclair	SIN2	Fish	49.55	14.24	8.78	-28
Sinclair	SIN2R	Fish	49.85	14.54	8.8	-27.91
Sinclair	SIN3	Fish	49.68	14.2	9.66	-28.14
Sinclair	SIN4	Fish	49.12	14.75	9.19	-26.74
Sinclair	SIN5	Fish	37.18	11.14	9.02	-27.13
Sinclair	SIN6	Fish	49.29	14.49	8.69	-28.34
Sinclair	S1G1	Gut	42.63	11.27	5.53	-26.03
Sinclair	S2G1R	Gut	47.1	10.47	4.56	-27.6
Sinclair	S2G1R	Gut	46.56	10.55	4.6	-28.09
Sinclair	S3G1	Gut	46.07	12.34	3.89	-24.75
Sinclair	S4G1	Gut	46.69	12.14	3.9	-24.73
Sinclair	S5G1	Gut	45.68	12.16	4.02	-24.67
Sinclair	S5G2	Gut	46.59	11.47	6.65	-25.04
Sinclair	S6G1	Gut	44.38	11.55	4.37	-24.9
Sinclair	SINZOO	Zooplankton	42.97	9.45	3.66	-31.77
Upsalquitch	UB1	Benthic	42.73	10.17	4.23	-24.75
Upsalquitch	UB2	Benthic	41.29	11.29	2.27	-22.93
Upsalquitch	UB3	Benthic	33.34	7.91	2.22	-23.17
Upsalquitch	UB4	Benthic	42.85	10.03	2.12	-24.35
Upsalquitch	UB5	Benthic	45.08	10.33	4.82	-25.02
Upsalquitch	UB6	Benthic	40.5	8.7	6.61	-33.2
Upsalquitch	UB7	Benthic	43.64	9.96	4.76	-23.99
Upsalquitch	UPS1	Fish	53.22	14.91	10.12	-26.84
Upsalquitch	UPS1R	Fish	51.32	13.03	10.12	-27.22
Upsalquitch	UPS2	Fish	50.54	14.11	10.94	-26.98
Upsalquitch	UPS3	Fish	49.86	14.8	9.77	-26.38
Upsalquitch	UPS4	Fish	50.23	14.16	8.75	-26.77

Upsalquitch	UPS5	Fish	49.9	14.01	9.86	-26.15
Upsalquitch	UPS6	Fish	50.72	14.42	9.47	-26.03
Upsalquitch	U1G1	Gut	46.84	10.46	4.58	-29.92
Upsalquitch	U2G1	Gut	48.53	12.24	5.57	-25.78
Upsalquitch	U2G2	Gut	34.87	7.74	8.95	-28.28
Upsalquitch	U2G3	Gut	49.73	11.3	5.74	-22.66
Upsalquitch	U2G4	Gut	49.73	12.27	5.73	-25.66
Upsalquitch	U2G5	Gut	53.59	9.77	0.53	-26.57
Upsalquitch	U3G1	Gut	50.26	12.16	3.96	-26.04
Upsalquitch	U4G1	Gut	41.43	9.16	5.16	-24.73
Upsalquitch	U4G1	Gut	45.87	13.27	7.54	-24.08
Upsalquitch	U5G1	Gut	45.34	11.53	10.03	-27.3
Upsalquitch	U5G2	Gut	49.79	12.01	5.67	-26.14
Upsalquitch	U5G3	Gut	48.34	12.23	5.9	-25.99
Upsalquitch	U6G1	Gut	48.16	12.5	8.01	-24.41
Upsalquitch	U7G1	Gut	47.11	12	5.36	-26.44
Upsalquitch	UPS3R	Gut	32.1	7.67	2.13	-22.65
Upsalquitch	UPSZOO	Zooplankton	49.6	10.21	3.08	-30.87