Mercury cycling through finfish aquaculture within the lower Bay of Fundy: possibilities for control in support of the health of coastal communities

by

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ABSTRACT

Attention has been focused on food safety and the nutritional value of farmed finfish products because of increasing global demands on aquaculture. Our research has the goal of determining inputs of Hg into the aquaculture cycle and modeling resulting Hg through-puts. We present a mass-balance model to quantify biomass and Hg accumulation in farmed fish, from feed to fish. In collaboration with several fish farms in New Brunswick, fish, feed, and waste samples were collected on a regular basis and analysed for total Hg. In addition, laboratory trials were conducted to determine the rate of administered methyl Hg absorption and release from farmed Atlantic haddock under controlled tank conditions. We now have determined that Hg concentrations in locally derived fish feed are not significantly different from Hg concentrations in internationally produced diets, with values ranging from 14ppb to 56ppb (dry wt, $p<0.01$). Compared with wild Atlantic salmon, Hg concentrations in farmed fish remained consistently low with increasing fish size, but wild fish concentrations increased (respective means, 84ppb and 260 ppb (dry wt, $p<0.01$)). Hg administered to Atlantic haddock in form of a fixed dose, is distributed throughout fish from the liver, with a gradual release into muscle tissue, where Hg will reside with a half-life of between 20 and 30 days.

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APL	Allowable production limit for fish farmers in the Bay of Fundy as established by provincial authorities
BMA	Bay Management Area dividing the lower Bay of Fundy into management zones for the finfish aquaculture industry as established by provincial authorities
COMERN	Collaborative Mercury Research Network
COSEWIC	Committee on the Status of Endangered Wildlife in Canada
CVAFS	Cold vapor atomic fluorescence spectrophotometry
D.O.	Dissolved oxygen
EMG	Environmental management guidelines for the aquaculture industry as established by provincial and federal authorities
FAO	Food and Agriculture Organization of the United Nations
FWS	U.S. Fish and Wildlife Service
HMSC	Huntsman Marine Science Centre
HSI	Hepato-somatic index
K	Condition factor
LE	Lipid-extracted tissue
MeHg	Methylmercury
MMBM	Mercury mass balance model
MSW	Multiple sea-winter Atlantic salmon
NBDAFA	New Brunswick Department of Agriculture Fisheries and Aquaculture
NLE	Non lipid-extracted tissue
OC	Organo-chlorine persistent pollutant
OCAD	Office of the Commissioner of Aquaculture Development
OM	Organo-metal persistent pollutant
OP	Organochlorine pesticide
PCB	Polychlorinated biphenyl
PDBE	Polybrominated diphenyl ether
POP	Persistent organic pollutant
SGR	Specific growth rate
SRB	Sulfate reducing bacteria
UNB	University of New Brunswick
USFDA	United States Food and Drug Administration
USEPA	United States Environmental Protection Agency

Chapter 1.0-Introduction

1.1 Background

Throughout the millions of years that the earth has existed, species which have endured the most successfully have out-survived less-successful species based on their selection for specific environments which allows them to occupy niches in changing or novel environments. The ability to utilize food resources has been a determining factor in species survival: those organisms with the capabilities to exploit new resources in times of food scarcity have outlasted less-capable species. Historians and evolutionists agree that the *Homo sapien*'s ability move beyond the majority of society being "hunter and gatherer" to a majority of "food producers", distinguishes themselves from most animals who consume their food where they find it (Roberts, 1976; Diamond, 1999). Of course, the development of agriculture and the ability to produce food is dependent on favorable conditions for growing, or the ability to import food that was produced elsewhere with the latter being the case for most developed countries on the planet today. In the case of eastern North America, as early as four centuries ago, due to poor growing conditions, a lack of an ability to import food grown elsewhere, and a seemingly endless abundance of marine fish, societies relied heavily upon the capture fishery as a main diet staple. In fact, the trade of preserved fish over these centuries has dictated major trade routes and built the economic framework of most cities along the New England seaboard and Canada's eastern coast (Kurlansky, 1997). However, the recent collapse of Atlantic cod stocks has dictated changes in the way that local stocks are monitored and

commercially harvested, affecting many rural communities through loss of employment and reliance upon a dwindling food source. On a global scale, scientists now predict that commercially harvested wild fish stocks may be completely depleted within the next century (Hsieh *et al*., 2006). Because the population of the world is increasing, the demand for seafood is on the rise (FAO, 2006), and most wild-fish stocks are now being over-exploited (Folke *et al*., 1998), the culturing of wild species of fish in a domesticated environment is quickly replacing commercial wild-fishery harvest. In fact, the Food and Agriculture Organization (FAO) recognizes aquaculture as the only growing component of the fisheries sector (Ridler, 1997).

The idea of capturing and culturing wild fish in a controlled environment is not a new concept to Canadians. Aboriginal communities are believed to have been participating in proto-aquaculture activities prior to the confederation of Canada while government, dating as far back as 1850, has been recorded to have participated in the incubation and hatching of different species of shellfish and finfish (OCAD, 2003). The aquaculture industry in Canada has patterned its development over the past 30 years after the poultry and beef industry with the view that fish farming is another step towards controlled food production. This control attempts to eliminate the risk associated with reliance on natural resources subject to natural environmental and biological variability (OCAD, 2003).

In the 1970's, a modern form of cold-water finfish aquaculture in the marine environment was established on an experimental Atlantic salmon (*Salmo salar*) farm

located in a rural New Brunswick community passage. Over the span of more than two decades, the industry expanded from a relatively small number of wooden cage farms along the coastline of south eastern New Brunswick to an industry composed of 100+ farms which maintains positive growth, is currently producing three species of finfish, occupies over 1,500 hectares of coastline, and has become New Brunswick's largest agrifood industry (NBDAFA, 2004). Finfish aquaculture has brought economic prosperity to the region in the form of spin-off businesses and in drawing larger companies to the area while providing jobs to a large number of workers under the age of 40 (Stewart, 2001). The finfish aquaculture industry in New Brunswick is now the second largest aquaculture industry in Canada and it has doubled in value over the past decade to annual sales of roughly $283 million with export revenues of $150 million alone. Atlantic salmon production has been the most successful form of finfish aquaculture in New Brunswick (NBDAFA, 2004).

The start-up of a marine-based aquaculture industry in Atlantic Canada, and most marine finfish aquaculture ventures worldwide, has followed a similar framework (Figure 1.1.1). Initially, the establishment of a breeding stock (broodstock) of collected wild fish is essential to draw genetic diversity for ensuing populations. The intention is to eventually base entire farm populations on hatchery-reared broodstock in order to better control for disease which can be brought in with wild-caught fish and the assurance of a gene-pool which contains favourable traits. Wild fish captured for broodstock are typically captured shortly before their natural spawning period and are acclimated to holding facility conditions where they are switched to a commercially produced diet

based partially on wild fish and designed to maximize survival and growth. Spawning takes place within the hatchery shortly thereafter, either naturally or manually according to species' biological requirements. From this process eggs are collected and incubated, hatched and are begun being reared on commercially formulated diet. In New Brunswick, depending on the species being grown, juveniles will be raised to a size deemed tolerant of typical marine conditions at either a nursery site (for the entry of juveniles <30g, including most farmed species other than Atlantic salmon) or a grow out site (for the entry of larger fish, typically Atlantic salmon smolt of 80-120g). Species that are initially placed within nursery sites are over the following months conditioned to a size upon which they can be transferred to a grow out site. Within grow out sites, fish are grown to a marketable size and processed for market or are selected to spawn, re-contributing to the gene-pool established with the broodstock.

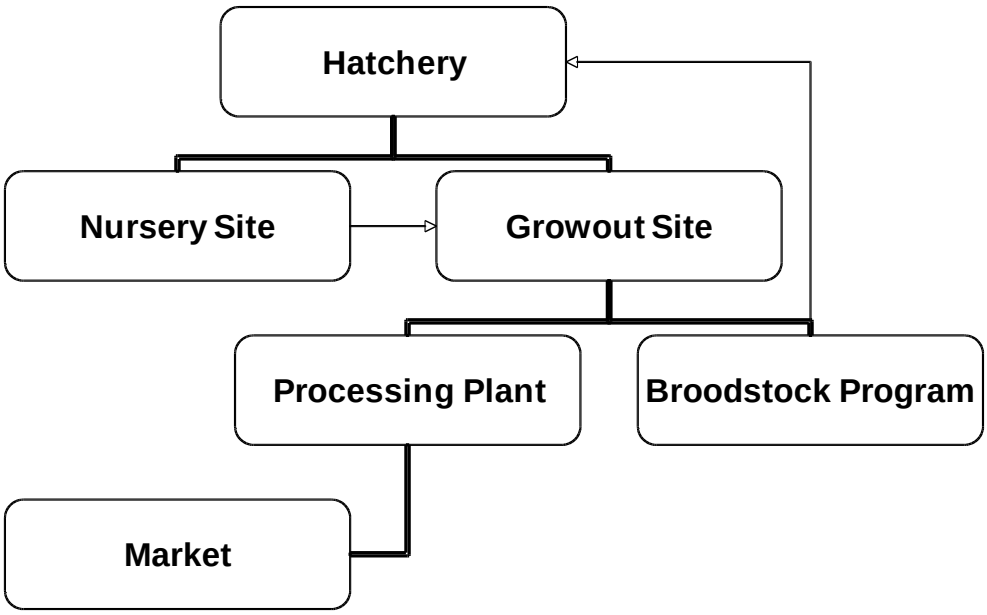

Figure 1.1.1 An overview of the requisite components of a renewable marine finfish operation.

The type of modern marine-based aquaculture described and practiced in south western New Brunswick and worldwide is currently reliant on wild-stock populations for both the production of juveniles and more importantly, the partial composition of commercially produced diet although there has been recent research in shifting fish feed composition to a non-fish based source (Folke *et al*., 1998; Ogunji *et al*., 2003; Seierstad *et al*., 2005). In Atlantic Canada, commercially produced fish feed is currently partially composed (30-90%) of fish-base of wild fish taken from various locations worldwide according to availability and price of the nutrient-base desired (Easton *et al*., 2002). Compounded by environmental pollution in select locations, contaminants present in wild fish used to formulate fish feed can contribute directly to contaminant loading within the diet and consequentially be incorporated into marketable fish (Choi & Cech, 1998).

As a result, since modern marine finfish aquaculture's inception in the mid 1970's, aquaculture contaminant loading concerns have been raised regarding the health-safety impacts of farmed fish consumption (Easton *et al*., 2002). In 2004, Hites *et al*. reported that farmed Atlantic salmon were unfit for regular consumption due to high levels of organic pollutants (Hites *et al*., 2004). In response to the well-publicized study, Canadian fish farmers realized significant losses despite Health Canada intervention and declaration that organic contaminant levels along with mercury levels in both farmed and wild Atlantic salmon are fit for regular human consumption (Health Canada, 2004).

However, mercury uptake in farmed fish has not been well studied, nor has its potential to add alternate pathways for mercury into its surrounding ecosystem through

fish feed and waste. Statistically valid comparisons between contaminant levels in "alternative" species (farmed species other than Atlantic salmon) and their wild counterparts have also not been examined. Such comparisons are important to make because currently the majority of seafood consumption of "alternative" species is of commercially caught wild fish of which no control over contaminant loading exists. Food safety and environmental sustainability are major concerns for aquaculture producers in taking responsibility for the safety of the product to be consumed and in ensuring that there will be places to farm fish in the future. Consumer concerns are similar and thus whether aquaculture product is a safer and overall better alternative to commercial product consumption is examined within this thesis.

1.2 Objectives

The general goal of this project is to determine and model mercury inputs into and relationships within the finfish aquaculture cycle in southwestern New Brunswick in order to recommend solutions to further minimize mercury inputs and outputs in connection with this food chain. Previous studies have shown that mercury most likely enters the finfish aquaculture production cycle by means of fishmeal-based diet and is reflected almost immediately in blood, gill and muscle tissue (Berntssen *et al*., 2004; Choi & Cech, 1998). Using feed and growth information collected from growers in addition to controlled laboratory dosage administration, a mass-balance model will be developed to quantify biomass and mercury accumulation and concentrations in finfish aquaculture. This model will trace mercury from fish feed to muscle tissue, to waste and

to invertebrates within the lease boundaries of aquaculture sites and surrounding areas for participating aquaculture farms in southwestern New Brunswick. Practical outcomes deal with modifying, monitoring, and modeling mercury in fish feed, marketed fish and fish waste, in collaboration with participating farms. In developing this model, the following questions will be examined:

- Are there significant differences between mercury concentrations in wild fish compared to aquaculture-reared fish?
- How long after a specific dose of mercury is consumed will levels drop to control group levels in farmed Atlantic haddock (*Melanogrammus aeglefinus*) muscle, liver, blood and gut tissue?
- Does mercury move from fish feed to flesh and to depositional sediment (waste)?

The following three chapters give an outline of specific questions examined, methods utilized to answer the questions, results from experimental trials and discussion of findings.

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Chapter 2.0-Mercury comparisons between farmed and wild Atlantic salmon (*Salmo salar*) and Atlantic cod (*Gadus morhua*)

2.1 Introduction

Intensive cold-water marine finish aquaculture, mainly of Atlantic salmon (*Salmo salar*), has existed globally in its most modern form since the mid-1970's in Norway (Saunders, 1995). Since then, farmed salmon has grown to represent the predominant species of aquaculture production in Norway, Chile, the United Kingdom and Canada and annual global production of this species currently exceeds 1,000,000 tonnes (FAO, 2006). At present, Norway is the leading producer, providing 55.6% of the world's farmed salmon (FAO, 2006). In Canada, Atlantic salmon culture came about when various growers followed the Norwegian initiative in the late 1970's and began growing native species of finfish experimentally in marine cages. They met with moderate success after many trials. However, through the early pioneering of such ventures, initial obstacles were overcome and heightened profits along with increased government contributions were made leading to the rapid expansion of the aquaculture industry in a relatively short period (~20 years). This expansion now accounts for over 5,000 new jobs in rural eastern Canada and has become the largest agrifood producer in the province of New Brunswick (NBDAFA, 2004). The finfish aquaculture industry in eastern Canada today is based primarily in the southwestern region of New Brunswick and is also taking place in the nearby provinces of Nova Scotia, Quebec, and Newfoundland.

The growth of the aquaculture industry has been met with strong criticism from various groups mainly for its role in potentially irreversible degradation to the marine environment (Hargrave *et al*., 1997; Sather *et al*., 2005), negative effects on current wild fisheries (Gross, 1998; Folke *et al*., 1998) and potentially harmful human health impacts through the consumption of farmed fish products (Easton *et al*., 2002; Hites *et al.*, 2004; Foran *et al.*, 2005). These criticisms have been met with skepticism from industry although comparatively few studies have been undertaken by industry itself to reveal otherwise; most industry studies focus on the positive socio-economic impacts of aquaculture development (Ridler, 1997; Stewart, 2001). Therefore, improving upon current aquaculture practices through applied research has become a popular venue for scientists and industry to share expertise in collaboration. Currently, a major focus of aquaculture research is on how to diversify from the culture of a single species (monoculture) which has potentially far-reaching effects on the environment that it occupies (Folke *et al*., 1998), to an integrated aquaculture approach (polyculture) where compatible species are cultured together for a more environmentally and economically sustainable venture (Chopin *et al*., 2001). Along with scoping out the possibilities associated with polyculture, government and scientific authorities have promoted diversification from the culture of salmonid species such as Atlantic salmon to "alternative" species, which include Atlantic cod (*Gadus morhua*), Atlantic haddock (*Melanogrammus aeglefinus*), halibut (*Hippoglossus hippoglossus*) and Atlantic sturgeon (*Acipenser fulvescens*) (FAO, 2006). Currently, both Atlantic salmon and Atlantic cod are in commercial production in southwestern New Brunswick.

Most farmed fish are fed on products derived from wild fish (Folke *et al*., 1998). In Atlantic Canada, and globally, commercially-produced fish feed is composed partially of wild fish taken from various locations, worldwide and locally, according to availability and price of the nutrient base desired (Easton *et al*., 2002). It has been shown that contaminants present in wild fish used to formulate fish feed, can directly contribute to contaminant loading within marketable fish. Both Easton (2002) and Hites (2004) have revealed contaminant loadings of a suite of persistent organic pollutants (POPs) within the fillet portion of farmed fish: polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (OPs). These POPs were gleaned primarily from the diet of farmed fish and both Easton and Hites claimed that farmed loadings were higher than similar contaminant loadings within wild fish that were compared. They did not however, compare wild and farmed fish of the same species and therefore, conclusions drawn from the study are not accurate without further investigation into same species differences. Choi and Cech (1998) reported unexpectedly high concentrations of total (organic and inorganic fractions) mercury in pelleted commercial fish feed, which corresponded to elevated concentrations of total mercury in various organ tissues from aquaculture-reared fish. Furthermore, Berntssen *et al*. (2004) show that the fraction of organic and inorganic mercury within aquaculture feed is reflected in the fillet of fish reared on a mercury-laden diet. This information has led to a recent shift in aquaculture research for the protein composition of aquaculture diet to be switched to a non-fish-based protein source (Ogunji *et al*., 2003; Seierstad *et al*., 2005). Meanwhile, this information has also brought into question whether benefits of farmed fish consumption, particularly polyunsaturated fatty acids, outweigh the potentially negative

implications associated with increased contaminant loading (Sather, 2005; Foran *et al*. 2006; Huang *et al*., 2006).

The theory of bio-dilution proposed by Jensen (1982) (herein referred to as "growth dilution") states that fast-growing fish assimilate lower concentrations of persistent pollutants than do slow growing, due to the dilution of pollutants by growth within tissue. For the individual fish this means that the concentrations of pollutants increase slowly when growth rate is rapid (such as in early life stages), and when the growth rate declines (proceeds towards an asymptote as in later life stages) the concentrations of pollutants increase more quickly (Jenson *et al*. 1982). In 1993, Hammar *et al*. studied a sympatric Arctic char (normal and dwarf) population within Lake Blasjon and found that slower-growing dwarf fish tended to accumulate higher concentrations of organic pollutants. This further confirmed that growth rate is an important factor in explaining differing contaminant concentrations in fish of similar species with dissimilar growth rates (Hammar *et al*., 1993). However, growth dilution may also be obscured by the variability of diet between individuals within field studies (Stafford *et al*., 2001).

Within the field of contaminant studies in fish, the title of 'persistent pollutant' can include both highly lipid-soluble contaminants, like organochlorines, and contaminants that are less lipid-soluble, like mercury. In comparing organo-metal (OM) and organo-chlorine (OC) persistent pollutants like mercury with PCB and PBDE, it is important to consider that although they share some general characteristics, i.e. both

types of pollutants may be transported atmospherically to the same location sink (Clarkson, 1995), their behavior within organisms can be quite different. Within fish, mercury has a high affinity for covalent binding with sulfur present in protein rich tissue (i.e. muscle) (Harris *et al*., 2003) while OC persistent pollutants tend to associate more strongly with lipid-rich tissue (i.e. fatty deposits within muscle tissue) (Clarkson, 1995; Easton *et al*., 2002).

From Jensen (1982) we predicted that mercury concentrations in aquaculture-reared fish fed on a consistent low-mercury diet should not readily accumulate mercury within edible muscle tissue due to their consistently enhanced growth rate throughout their shortened lifespan. Moreover, we predict that slower-growing, longer-lived wild fish of the same species and size as aquaculture-reared counterparts will carry higher individual mercury concentrations, based on lower growth rates. However, we also predict that lipid loading within fillets will be an important factor determining mercury uptake. We know that fillets from Atlantic cod contain <1% of the lipid stored within the fish (wild, mature; Schwalmn & Chouinard, 1999), while Atlantic salmon fillets may contain >12% of the lipid load (farmed, immature; Johnston *et al*., 2006). Therefore, lipid-rich fillet should be more likely to carry higher lipid-soluble contaminant loadings based on high OP-lipid interaction. This prediction was addressed by Hites (2004) and concluded that OP contaminant loads were higher in farmed as compared to wild fish. However, because the study used OP concentrations in farmed fish of a different species than wild fish compared, the accuracy of stated results was diminished. Therefore, this idea remains unverified. We predict that lipid-rich fillet will carry lower mercury

concentrations based on decreased association of mercury with lipids and the "lipid dilution" of mercury by lipids within fillets. We also predict that differences in mercury concentration between farmed and wild fish of the same species will be a result of the influence of lipid loading within fillets. We expect that farmed fish will contain higher fillet lipid loads based on differences in diet, feed availability and lifespan.

To test these predictions, we collected samples of wild and farmed Atlantic salmon and Atlantic cod of various sizes to assess changes in mercury with size, and differences between wild and farmed fish. We then compared concentrations in wild and farmed fish with established consumption guidelines from Health Canada and the USEPA. In order to examine differences in mercury concentration in relation to lipid content, an experiment was run to compare lipid-extracted (LE) to non-lipid-extracted (NLE) flesh samples in both farmed and wild Atlantic salmon in order to determine if lipid content of fillet was acting to influence mercury assimilation in flesh.

2.2 Methods

2.2.1 Farmed Fish

Six active marine finfish aquaculture sites were selected from 96 sites that are currently operational within the lower Bay of Fundy, New Brunswick, Canada. These sites were chosen based on site location and partnership availability. One of the sites

produces Atlantic cod, a second site contains both Atlantic salmon and Atlantic halibut, and the remaining four sites produce only Atlantic salmon. The New Brunswick Department of Agriculture, Fisheries and Aquaculture (NBDAFA) has divided the aquaculture-intensive lower Bay of Fundy area into Bay Management Areas (BMA) that determine the yearclass (even or odd) of Atlantic salmon smolt being entered in each particular zone. Three of our sites are located within even yearclass BMAs and three within odd-yearclass BMAs. Yearclass separation was introduced as a tool to prevent the spread of disease from older fish to younger fish (or vice versa) by separating them according to the year that the fish are entered into marine cages as smolt (Bay of Fundy Marine Aquaculture Site Allocation Policy). When our project began, one site contained no fish (all had been harvested prior to the Fall of 2004 and not restocked until the Spring of 2005), two contained newly entered smolt, two contained fish that had been held for over a year, and one contained Atlantic halibut (*Hippoglossus hippoglossus*) of multiple yearclasses. This spread of yearclasses provided fish samples from various stages growth and ages while giving a clear overall picture of mercury concentrations within farmed species in the lower Bay of Fundy. Samples of five fish per site were collected from a single cage every two months (if possible) from August 2004 until July 2005. Samples were labeled, placed on ice at the site and later frozen at -20°C until preparation for total mercury analysis.

All farmed Atlantic salmon in the lower Bay of Fundy originate from the Saint John River stock of wild Atlantic salmon. The Saint John River drains into the lower Bay of Fundy. Farmed Atlantic cod originate from George's Bank wild stock.

2.2.2 Wild Fish

Since 2002, wild cod were collected from the Passamaquoddy/lower Bay of Fundy region as part of a five year Collaborative Mercury Research Network (COMERN) Bay of Fundy Coastal Zone project. These fish were collected annually by trawling various transects within the Eastern Passage in the lower Bay of Fundy. Fish were labeled and kept on ice within the trawler until they could be later frozen at -20°C until preparation for total mercury analysis.

For wild Atlantic salmon data, adult and juvenile fish were collected from the Miramichi River system that drains into the southern Gulf of St. Lawrence. Because wild Atlantic salmon in Canada are listed under Species of Special Concern by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC), and are listed as Endangered under the Endangered Species Act by the United States Fish and Wildlife Service (FWS), live wild Atlantic salmon were not sacrificed for the purposes of this study. Rather, adult Atlantic salmon spawning and juvenile smolt mortalities were collected during the summers of 2004, 2005 (adult spawn mortalities) and 2006 (juvenile mortalities). Upon collection, fish were placed on ice until received at the UNB Mercury Laboratory where they were frozen at -20°C until preparation for total mercury analysis.

Preparation for analysis included fork-length measurement (tip of nose to fork of tail) and the excision of a 10-gram aliquot of muscle tissue from the dorsal region

anterior to the first dorsal fin. All tissue was homogenized and then freeze-dried in a Virtis Benchtop Freeze-dryer (Virtis, Gardiner, New York, US) until all moisture was removed and weight fluctuations of dried material ceased. All samples were analyzed for total mercury by Cold Vapour Atomic Fluorescence Spectrometry on a Tekran 2500 (Tekran; Knoxville, Tennessee, US). When possible, liver samples were taken from both wild and farmed samples and prepared for total mercury analysis in the same way.

2.2.3 Lipid Extraction Trials

We set up a laboratory experiment in which we tested the same LE and NLE Atlantic salmon flesh samples for mercury. An aliquot of leftover flesh material from samples previously prepared and run for total mercury analysis was divided into 2 equal portions; one portion was run for total mercury analysis on the DMA-80 Direct Mercury Analyzer, and the other lipid-extracted following Bligh and Dyer (1959). Within a 20ml glass vial, each LE flesh sample was diluted with a 2:1 Chloroform/Methanol mixture. The vial was capped and shaken by hand for approximately 30 seconds. After allowing the mixture to settle for 30 minutes, the solvent layer was extracted with a 5ml disposable polyethylene pipette and discarded into a waste container. The remaining solid portion was diluted to the top of the glass vial with the Chloroform/Methanol mixture once again, the mixture shaken for 30 seconds, left to settle again for 30 minutes and the solvent layer was again extracted. This procedure was repeated until the solvent layer became clear. Upon reaching full extraction, the remaining solid portions were placed into the Virtis Benchtop freeze-dryer (Virtis, Gardiner, New York, US) to dry for 2 days after which

they were weighed out in 0.01-0.05g portions into weigh boats for total mercury analysis on the DMA-80 Direct Mercury Analyser (Milestone Srl, Sorisole, Italy).

2.2.4 Statistics

To reduce non-normality and heteroscadasticity among groups, all total mercury concentrations were $\log_{10}$-transformed before statistical analysis. All analyses were conducted using an NCSS statistical software package (NCSS, Kaysville, Utah, US). Due to non-normal size distribution (size was not a continuous variable within entire species classes), one-way analysis of variance was used to test for differences in total Mercury concentration between size categories of both wild and farmed fish. Where applicable, analysis of co-variance was used to examine the relationship between mercury and body size (fork-length) for both farmed and wild flesh within size categories of species classes. All error is expressed as standard error of the mean (SEM). Paired T-tests and linear regression were used in the comparison of LE and NLE flesh total mercury concentrations across fork lengths.

2.3 Results

2.3.1 Farmed vs. Wild

Within our farmed Atlantic salmon samples, fork-length was of continuous distribution, from smolt measuring ~20 cm to market-sized fish of ~80 cm. Our wild

Atlantic salmon samples were non-normally distributed, with the majority of smolt falling within a range of 14-18 cm in fork-length and adult fish 50-100 cm. Therefore, in order to compare types (farmed and wild) to one another, we categorized size, with "large" Atlantic salmon falling into the 50 to 100 cm category and "small" fish comprising the 14–49 cm fork-length category.

Total mercury concentrations in the flesh of large (50-81 cm fork-length) farmed Atlantic salmon were significantly lower than concentrations found in the flesh of large wild Atlantic salmon of similar fork-length (Table 2.3.1).

Table 2.3.1 Data table for total mercury concentrations in farmed and wild fish flesh and liver along with p values for comparison (ng/g, dry weight).

B.			Flesh				Liver			
Species	Type	Size	N	Mean (mg/kg)	S.E.M.	P	N	Mean (mg/kg)	S.E.M.	P
Atlantic salmon	Farmed	Large	33	0.075	±0.014	p<0.001	9	0.101	±0.011	p<0.001
Atlantic salmon	Wild	Large	47	0.235	±0.011		28	0.237	±0.021	
Atlantic salmon	Farmed	Small	31	0.072	±0.011	p<0.05	-	-	-	-
Atlantic salmon	Wild	Small	38	0.363	±0.021		-	-	-	-
Atlantic cod	Farmed	-	29	0.167	±0.018	p>0.05	6	0.094	±0.009	p>0.05
Atlantic cod	Wild	-	15	0.190	±0.050		15	0.113	±0.016	

Total mercury concentrations in the flesh of small (14-49 cm fork-length) farmed Atlantic salmon were significantly lower than concentrations found in the flesh of small wild Atlantic salmon (Table 2.3.1).

Liver was collected only from the large Atlantic salmon group. Farmed Atlantic salmon liver total mercury concentrations were significantly lower than those of wild Atlantic salmon of similar fork-length (Table 2.3.1). When categorized into size classes, neither farmed nor wild of the large Atlantic salmon group showed change in total mercury concentration with increasing fork length in both flesh and liver concentrations ($p<0.05$).

When examining farmed fish total mercury concentrations in relation to fork length for sites from which samples were taken over the longest period (exemplifying the largest difference in size over time), we found that mercury concentrations tended to decrease over increase in fork length (Figure 2.3.1).

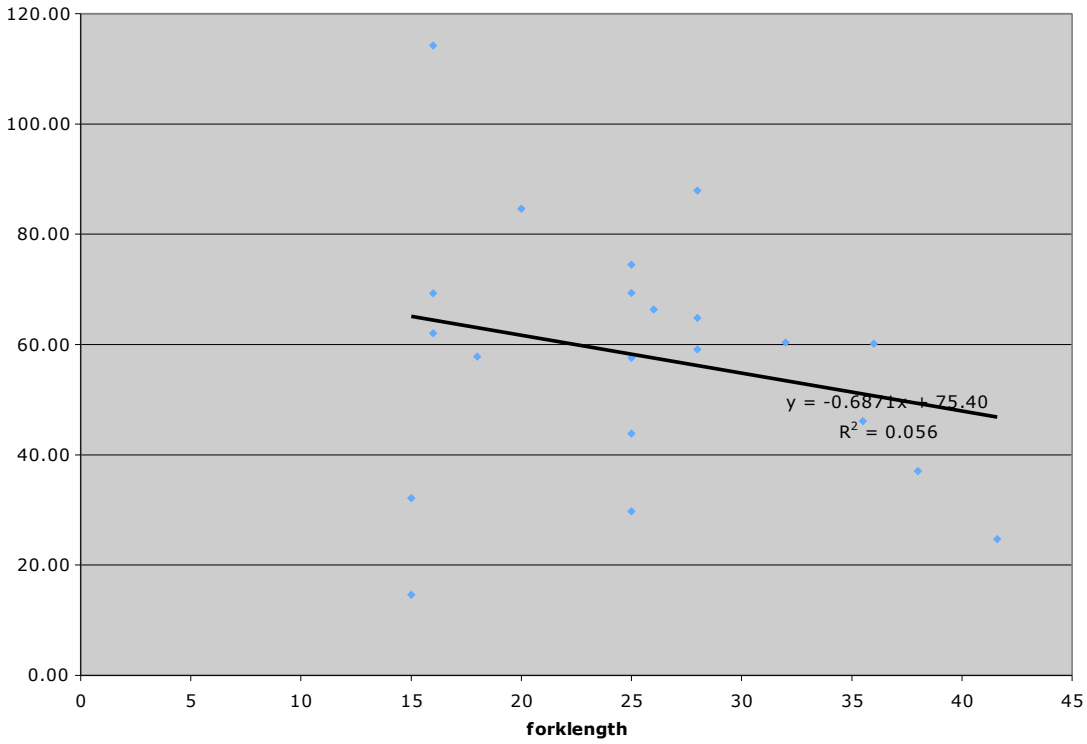

Figure 2.3.1 Comparison of farmed Atlantic salmon total mercury flesh concentrations (µg/kg) in relation to forklength (cm).

Within all Atlantic cod samples (both farmed and wild), fork-length was of continuous distribution, measuring ~16 cm to market-sized fish of ~54 cm. Therefore, in order to compare types (farmed and wild) to one another, all fish were compared within the same size class.

Total mercury concentrations in the flesh of farmed Atlantic cod were not significantly different from concentrations found in the flesh of wild Atlantic cod of similar fork-length (Table 2.3.1).

Farmed Atlantic cod liver total mercury concentrations were not significantly different from concentrations found in the liver of wild Atlantic cod of similar fork-length (Table 2.3.1). We found that there was no change in total mercury concentrations across fork length in either flesh or liver of both farmed and wild Atlantic cod ($p<0.05$).

Between species of farmed fish, Atlantic cod were significantly higher in total mercury concentration of flesh than both undersized and market-sized farmed Atlantic salmon ($p<0.05$) (Table 2.3.1).

Total mercury concentration comparisons between returning wild adult Atlantic salmon and wild Atlantic salmon smolt leaving the Miramichi River system revealed significantly lower total mercury concentrations in returning adult fish ($p<0.05$) (Table 2.3.2).

Table 2.3.2 Total mercury concentration comparison between wild smolt and MSW Atlantic salmon from the Miramichi River system.

	Mean [mercury] ± SEM
smolt (14-18 cm)	363±21.06 mg/kg
MSW adult (50-100 cm)	260±15.03 mg/kg

Health Canada has established consumption guidelines of 0.5 ppm (mg/kg) wet weight for mercury concentrations in commercial fish. The United States Environmental Protection Agency has established guidelines at 0.1 ppm (mg/kg) wet weight. Mean total

mercury concentrations of both farmed and wild Atlantic cod and salmon, when adjusted to wet weight concentrations, do not approach these established guidelines (Table 2.3.3).

Table 2.3.3 Health Canada and USEPA advisory guidelines for fish consumption in comparison with total Mercury concentrations in wild and farmed fish from our study (mg/kg, wet weight)

	Mean [mercury] ± SEM	
	Farmed	Wild
market sized Atlantic salmon	0.015±0.003 mg/kg	0.047±0.002 mg/kg
market sized Atlantic cod	0.031±0.004 mg/kg	0.029±0.005 mg/kg
Health Canada advisory guidelines	0.500 mg/kg	0.500 mg/kg
USEPA advisory guidelines	0.100 mg/kg	0.100 mg/kg

- Our experimental values have been adjusted to reflect wet weight (advisory guidelines are given in wet weight).

2.3.2 Lipid Extraction Trials

Lipid extraction trials were run on Atlantic salmon in order to determine whether farmed and wild tissue would show similar total mercury concentrations upon lipid extraction. These trails were run under the assumption that farmed Atlantic salmon would carry higher lipid-loads than wild Atlantic salmon based on farmed Atlantic salmon's enhanced diet formulation and increased feeding.

Total mercury concentrations were higher in LE tissue compared with NLE (wild; $p<0.05(|t|>2.1009)$, farmed; $p<0.05(|t|>2.1448)$. (Figures 2.3.2 and 2.3.3).

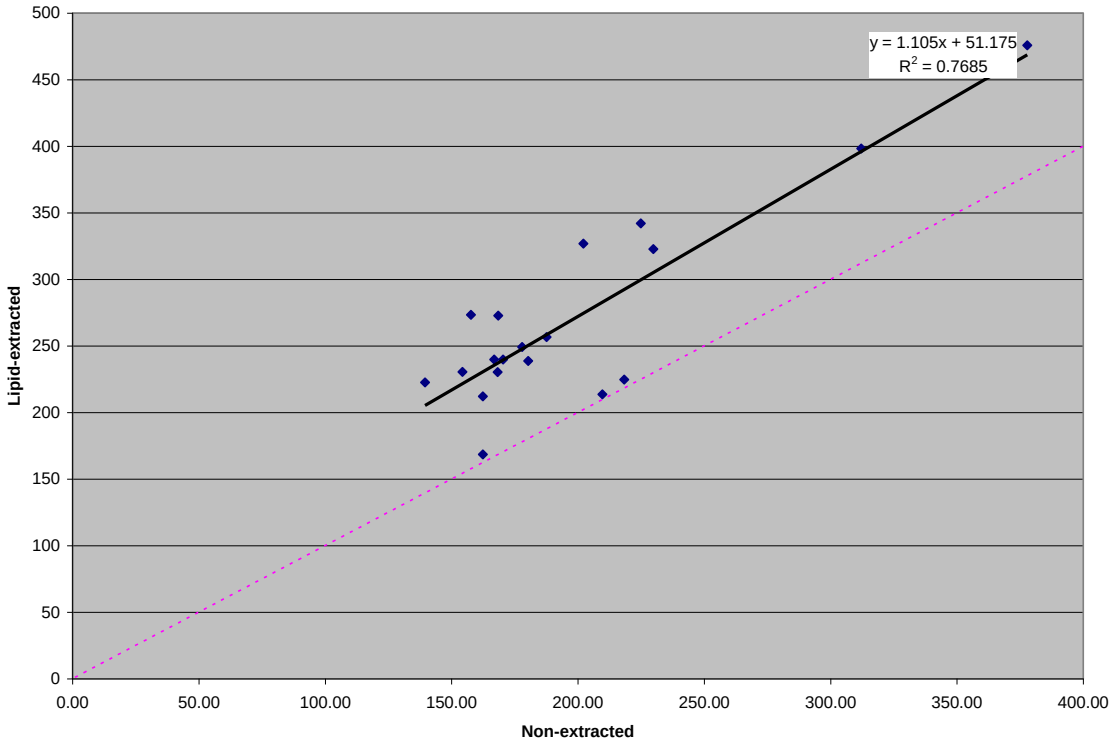

Figure 2.3.2 Comparison of total mercury flesh concentrations (µg/kg) from lipid-extracted (LE) and non lipid-extracted (NLE) flesh of wild Atlantic salmon from the Miramichi River system.

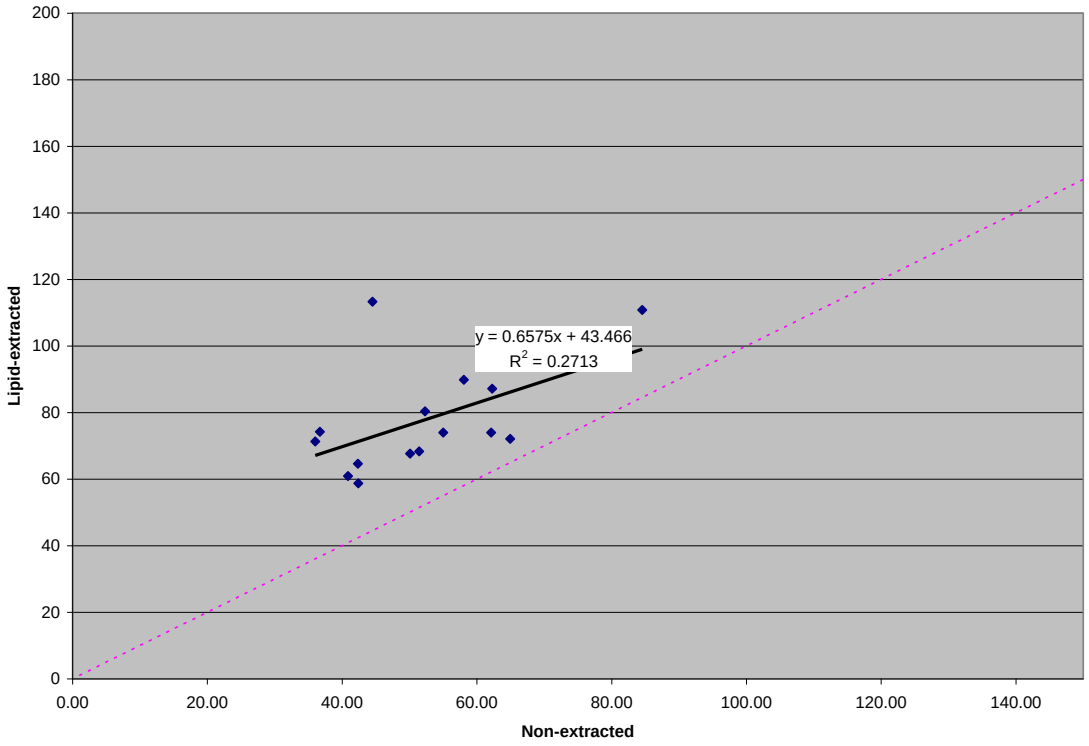

Figure 2.3.3 Comparison of total mercury flesh concentrations (µg/kg) from lipid-extracted (LE) and non lipid-extracted (NLE) flesh (µg/kg) of farmed Atlantic salmon of the Saint John River strain, cultured in the lower Bay of Fundy.

2.4 Discussion

From our analyses, it appears that flesh and liver from farmed Atlantic salmon are generally lower in total mercury concentration than wild Atlantic salmon that originate from a nearby river system. There appears to be no difference between farmed and wild cod total mercury flesh concentrations while both farmed and wild Atlantic cod tend to be higher in total mercury concentration than farmed Atlantic salmon. Neither farmed nor wild Atlantic salmon or cod total mercury concentrations trigger consumption advisories according to Health Canada or the USEPA (0.5mg/kg wet weight and 0.1mg/kg wet weight respectively) (Table 2.3.3).

Sustained and rapid growth in an aquaculture operation is the goal of farmers, who strive to use the least amount of feed to produce the largest amount of biomass accumulation (with minimal adverse effects). For this reason, using Atlantic salmon as an example, when Atlantic salmon parr are moved from hatchery to marine cages during their smolting phase, they are immediately switched to a refined diet of low fat and high protein, which is gradually modified to higher fat and moderate protein. Diet is derived from fish oil, fish meal, plant meal and poultry by-product in addition to vitamin and mineral complexes which maximize metabolic function to produce enhanced growth throughout the grow out phase to market-sized product (Shearer *et al*., 1994). Under this feeding regime, it takes Atlantic salmon producers in New Brunswick roughly three years to grow a stock of Atlantic salmon eggs through to market-sized fish (roughly 80cm) (Saunders, 1995). In comparison, wild Atlantic salmon in Nova Scotia (NS) and New Brunswick (NB) take on average 2.8 years to reach a smolt length of roughly 13cm when they are ready to leave their native freshwater habitat for migration to the continental shelf (Hutchings & Jones, 1998). Roughly 0.4% of salmon leaving NS and NB rivers will return to freshwater after one year as undersized grilse (ranging in size from 53-56cm); the remainder will migrate between the continental shelf and the western coast of Greenland where they will spend multiple sea-winters feeding (herein referred to as MSW salmon) (Hutchings & Jones, 1998). Typically, MSW salmon will return to their native stream after 1-2 years at sea, during which they may experience 40-45cm of growth each year (Hutchings & Jones, 1998).

Therefore, it may take upwards of 4-5 years for a wild adult MSW salmon to reach a "market size" of roughly 80cm with growth disproportionately distributed throughout its lifespan. Jenson's growth dilution theory (1982) proposed that fast-growing fish assimilate lower concentrations of persistent pollutants than do slow-growing, due to dilution of pollutants by growth within tissue, therefore we would expect returning adults to contain lower mercury concentrations than freshwater smolt. Within our study, in examining the differences between the time that it takes wild Atlantic salmon to reach a similar size to that of farmed, the theory of growth dilution due to their artificially high and sustained growth rates, may hold true since we see that farmed Atlantic salmon appear to have lower mercury concentrations as both juvenile smolts and adults in southwestern New Brunswick. We also see that slow-growing wild Atlantic salmon smolt tend to be lower in mercury concentration then their adult counterparts (MSW or grilse).

Because we did not see the same trend in cod as we did in salmon, it appears that the principal of growth dilution does not adequately explain the discrepancy we see between uptake and excretion of mercury in fish in relation to growth in our study. Examination of growth rates in farmed Atlantic cod taken from industry reports as compared with wild reveals that growth rates are comparable. Although farmed Atlantic cod are only now available commercially, models for Atlantic cod production estimate the egg to market timeframe at between two and three years in order to achieve a market size of 1.1Kg (SIM Corp., 2003). Hutchings modeled roughly the same growth rate of wild Atlantic cod based on data collected from native stocks based on Atlantic cod

growth data collected by Lilly between 1978 and 1996 (Hutchings, 1999). According to the theory of growth dilution, we would expect mercury concentrations to be similar between farmed and wild fish that possess similar growth rates, and this we saw with Atlantic cod.

Hites (2004) claimed that organochlorine accumulation in farmed Atlantic salmon compared to wild Pacific salmon (Chinook (*Onchorhynchus tshawytscha*), Coho (*Onchorhynchus kisutch*), and Chum (*Onchorhynchus keta*)) accumulated more quickly in the faster-growing farmed species. Although species differences are not accounted for within this study, Hites's claims (2004) contradict the theory of bio-dilution by stating that lipid-bound contaminant concentrations accumulate more quickly in faster-growing farmed fish as compared to slower-growing wild. They attribute the contradiction to the increased lipid soluble contaminant loadings in farmed fish diet. We believe that further study between farmed and wild fish of the same species should take place to examine this effect. We propose that lipid storage location is a factor that affects growth dilution, which in turn influences the uptake and assimilation of mercury in farmed fish compared to wild.

During sample preparation we noticed differences in lipid composition between farmed and wild Atlantic salmon samples. This prompted us to examine mercury concentration differences between LE and NLE samples. For Atlantic salmon, mercury concentrations in extracted samples were consistently higher than concentrations in samples that were not lipid-extracted, in both wild and farmed fish. We believe this to

indicate that, along with growth dilution, the assimilation of mercury may be lipid diluted within the flesh of fish with high lipid-fillet content, like Atlantic salmon. That is, due to the low solubility of mercury in lipid, the high presence of lipid in fillet is a deterrent to assimilation within fillet. This proposition is further strengthened by data that show that mercury concentrations are not different between wild and farmed Atlantic cod. This is expected because neither farmed nor wild Atlantic cod store lipid loads within the fillet (Schwalmn & Chouinard, 1999).

Lipid-extraction did not fully account for the differences in mercury concentrations between farmed and wild Atlantic salmon; upon lipid-extraction there were still significant differences between wild and farmed concentrations. This suggests to us that the increased lipid content in the fillet of Atlantic salmon, enhanced in farmed Atlantic salmon, acts as an inhibition to mercury uptake into muscle tissue in addition to the inhibitory effects of growth dilution. Therefore, farmed Atlantic salmon have the advantage of high lipid content and fast growth rate to counteract a potentially increased mercury load consumed in formulated diet (Choi & Cech, 1998). An assessment of lipid content of both LE and NLE samples through Carbon/Nitrogen ratio analysis is an obvious next step to verify our findings. The comparison of LE and NLE flesh samples of both farmed and wild Atlantic cod would also be beneficial.

It is important to establish that for the purposes of our study, unlike previous studies looking at contaminant loadings between wild and farmed fish (Easton *et al*., 2002; Hites *et al*., 2004) our comparisons were made between fish of the same species

and, to the best of our abilities, the same river system. Wild Atlantic salmon are practically unavailable for purchase and consumption by most of the population in Canada due to their current status in Canada and the United States. But, under current regulations, wild Atlantic salmon are still legally being caught and consumed by aboriginal communities within Canada. From a human health perspective comparing simply "wild" and "farmed" mercury concentrations, is a legitimate comparison when those are the only options available at the supermarket. However, the comparison of wild and farmed fish of more than one species, i.e. Atlantic salmon and Pacific salmon (i.e. Chinook, Coho, or Chum) negates species-specific traits such as habitat requirements, trophic level position and diet preferences, growth rates and longevity. These traits vary between species and from previous studies, it is known that they are important factors in contaminant uptake, assimilation and excretion (Hammar *et al.*, 1993; Cabana & Rasmussen, 1994; Storelli *et al.*, 2002). To make the study as relevant to public health as possible, in addition to examining mercury concentrations between farmed and wild Atlantic salmon, we have added comparisons in farmed and wild mercury concentrations of the newest aquaculture finfish species being produced commercially in southwestern New Brunswick, Atlantic cod. We recommend further investigation into Atlantic halibut wild and farmed mercury concentrations.

Although we are comparing farmed and wild Atlantic salmon, (i.e. fish of the same species) our wild and farmed samples are not from the same river system as our sampled wild breeding stock and we make the assumption that the wild mortalities are similar to healthy wild fish in mercury concentration. This may make location, origin of

our samples, age of fish and health of fish potentially confounding variables. Farmed Atlantic salmon in the lower Bay of Fundy are under the provincial Aquaculture Act, of Saint John River breeding stock. Wild fish mortalities sampled for the purposes of our study were taken from the Miramichi River system. Although these fish are of the same species, specific breeds native to different river systems may show genetically based traits that dictate an increased or decreased ability to uptake and excrete contaminants, such as mercury. In addition, there is mounting evidence that the methods employed to select and culture Atlantic salmon have caused, or are causing, a genetic divergence of the cultured and wild species of *Salmo salar*, with farmed exploiting an entirely different niche in the ecosystem (Gross, 1998). Because farmed Atlantic salmon are selected from a broodstock constructed to maximize and minimize specific traits (i.e. growth and maturation, respectively) the ecology, life history, and distribution of the cultured species may be different from that of wild Atlantic salmon. In order to control for health of fish in relation to mercury concentrations, we compared mean mercury concentrations of wild Atlantic salmon within three control lakes in Newfoundland used in French *et al.*'s 1998 reservoir study, with our own results and found them to be similar in mean concentration ($p>0.05$) (Table 2.4.1). We attempted to control for the remaining variables by comparing mercury concentrations across fork-length of all fish, normalizing the population as has been done in similar studies (Storelli *et al*., 2002).

Table 2.4.1 French *et al.*'s mean baseline mercury concentrations (dry wt) for landlocked Atlantic salmon from control sites Middle Gull Pond, Eclipse Point, and Rocky Pond (French *et al.*, 1998) compared to mean concentrations (dry wt) in wild Atlantic salmon mortalities from the Miramichi River system (2005-2006).

	Mean [mercury] ± SEM
Middle Gull Pond (NL)	0.375±0.038 mg/kg
Eclipse Point (NL)	0.270±0.033 mg/kg
Rocky Pond (NL)	0.338±0.053 mg/kg
Miramichi River (NB)- Large	0.235±0.011 mg/kg
Miramichi River (NB)- Small	0.363± 0.021 mg/kg

Therefore, gleaning information from previous studies in addition to this one, we can conclude that although farmed Atlantic salmon contain lower mercury concentrations in relation to their wild counterparts, they may contain higher concentrations of other contaminants (Easton *et al*., 2002; Hites *et al.*, 2004; Foran *et al.*, 2005), none of which yet invoke precautionary warnings from Health Canada or the USFDA. To state that either wild fish or farmed fish are safer to consume based on these data is not accurate if the species considered are not the same. This is especially evident with total Mercury comparisons between farmed Atlantic salmon and farmed Atlantic cod; farmed salmon flesh is lower in Mercury than its wild counterpart, but farmed cod is not different from its wild counterpart. Both of these farmed species are at present available for purchase in the marketplace. Furthermore, understanding why contaminant concentrations differ between wild and farmed species of fish will be paramount in making advisories and potentially targeting species which may pose more of a risk for consumption. We believe, in light of existing contaminant studies and our current work, that farmed

Atlantic salmon fillets may be more likely to contain higher lipid-soluble contaminant burdens than those of wild Atlantic salmon, both of which will be higher than both wild and farmed Atlantic cod based on their increased lipid loads. These claims should be tested further with experiments that have the proper ability to test for differences between wild and farmed fish of the same species. For contaminants more strongly associated with protein binding (i.e. mercury), we expect to see higher fillet concentrations carried in wild Atlantic salmon, as compared with their more lipid-rich farmed counterparts. However, between wild and farmed Atlantic cod we expect mercury concentrations in flesh to be similar based on their physiology, with the majority of fat going directly to the liver to be metabolized rather than assimilated in flesh (Schwalmn & Chouinard, 1999). We also expect that mercury concentrations will be higher in species that have lower lipid content in their flesh, like Atlantic cod, which should also have correspondingly low organochlorine (lipid-soluble POP) concentrations. Therefore, between species of farmed fish there is potential for the production of fish containing lower contaminant loadings based on the physiology of the species.

Based upon results from our study, growth rate and lipid load appear to be playing a role in determining contaminant concentrations in farmed and wild fish. The ability to reduce contaminant loading in farmed fish will be strengthened by further investigation into the possibly interacting effects of lipid content of fillet and mercury assimilation in relation to the principal of growth dilution with the goal of producing a safe, reliable protein source for consumers.

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Chapter 3.0-Total mercury concentrations show dose and time-dependent relationship to uptake and accumulation in farmed Atlantic haddock (*Melanogrammus aeglefinus)* tissue

3.1 Introduction

Reports of methylmercury poisoning in both humans and wildlife such as fish and birds have become common-place in scientific literature since the discovery of the effects of mercury poisoning of human populations in Minamata Bay, Japan during the 1950's. At that time, an industrial plant using mercury to catalyze the chemical process of converting acetylene to acetylaldehyde was releasing unknown quantities of mercury directly into Minamata Bay (Kudo *et al.*,1998). The principal of the bioaccumulation of mercury up the foodchain was borne out of this tragedy in that rather than through the uptake of mercury through water consumption, mercury poisoning evident in the population was derived directly from the consumption of fish from contaminated Minamata Bay (Kudo *et al.*,1977). As the principal of mercury bioaccumulation has been further studied, it is now understood that mercury is deposited into aquatic ecosystems in its inorganic form predominantly through atmospheric deposition rather than through point sources (Gilmour & Henry, 1991). Through relatively poorly understood biological processes within sediment of both freshwater and marine systems, mercury in its inorganic form is methylated to the toxic form of methylmercury and consumed by primary feeders (Gilmour *et al.*, 1992). The study and use of stable nitrogen isotopes has shown that methylmercury accumulation in fish is positively correlated with the trophic

level that it represents within an aquatic ecosystem as determined by the ratio of heavy to light Nitrogen ($\delta^{15}N = ([^{15}N/^{14}N_{sample}/^{15}N/^{14}N_{standard}]-1) \times 1{,}000$) (Cabana & Rasmussen, 1994). This indicates that organisms feeding higher up in the food web tend to accumulate more contaminants than organisms lower in the food web. With the use of improving technology, such as stable isotopes, bioaccumulation of contaminants within foodwebs is becoming more easily monitored and better understood. Along with these improvements, the mechanics of mercury uptake and excretion within species of fish are also becoming better understood. Trudel and Rasmussen (2001) developed a simple Mercury Mass Balance Model (MMBM) which predicts mercury concentrations in fish. This model incorporates the work of previous researchers that had used both the laboratory setting and field studies to predict mercury uptake, assimilation and elimination of mercury in wild fish over time based on food consumption rates, energy expenditure, age and size of fish (Trudel & Rasmussen, 2001). The MMBM has become widely used in ecological and ecotoxicological applications (Essington & Houser, 2003; Debruyn *et al*., 2006) and forms the basis for the MMBM for mercury in farmed fish that will be explored herein.

Historically, relatively few controlled exposure laboratory studies looking at methylmercury uptake in fish through diet have been undertaken with proportionately more studies having been focused on methylmercury uptake through gills by controlled dosages administered through the aqueous environment (Houck & Cech, 2004; Berntssen *et al*., 2004). However, it is accepted that dietary intake (food) has been shown to be the primary pathway for methylmercury uptake by fish, not the aqueous environment (Hall *et*

al., 1996). In laboratory studies looking at dietary uptake to date, many have focused on a continuous administration of methylmercury spiked diet with sampling taking place at the end of an experiment in order to quantify accumulation in organ tissue over the period. Berntssen et al (2004) determined that fish fed continuously over a four month period with moderate levels of methylmercury (5 µg/g) showed accumulation of methylmercury primarily in the blood, gills, muscle, brain, liver, kidney and intestine. However, fish fed continuously with higher levels (20 µg/g) showed higher accumulation of methylmercury in the flesh (up to 92%) (Berntssen *et al*., 2004). The study also determined that intestinal cell proliferation and liver metallothionein are quantifiable early indicators of toxic mercury exposure (Berntssen *et al*., 2004). Earlier controlled exposure diet studies have shown similar toxic effects by way of the induction of stress hormones and reduced growth (Friedmann *et al*., 1996) and impaired reproductive capacity (Drevnick & Sandheinrick, 2003). Ruohtula and Miettinen (1975) were able to quantify methylmercury retention in Rainbow trout (*Oncorhynchus mykiss*) through the use of radioactively labelled mercury by various means of methylmercury uptake including uptake by gills, injection into muscle tissue and direct uptake into the stomach via intubation. Houck and Cech (2004) also undertook a study on the effects of methylmercury exposure on juvenile Sacremento blackfish (*Orthodon microlepidotus*) bioenergetics, treating four experimental treatment groups to a continuous diet of methylmercury laden feed at 0.00 mg/kg (control), 0.45 mg/kg (low), 20 mg/kg (medium), and 50 mg/kg (high). Control and low dietary levels were chosen to simulate mercury levels encountered in the native environment (Houck & Cech, 2004). A portion of the experimental group of fish were sacrificed at regular intervals and analysed for

mercury concentrations in body tissues. All fish within all treatment groups were weighed and measured at these intervals. The addition of the growth parameter and metabolic rates in relation to bioaccumulation was useful in concluding that depressed growth and feed conversion inefficiencies at high dose treatments resulted due to decreased digestive and absorptive capacities.

Studies have shown that depending on feeding regime, more aggressive (dominant) fish are more likely to consume more feed then less aggressive (subordinate) fish and therefore in a controlled dosage study, actual dosage consumed is difficult to control (Jobling, 1994; Sloman & Armstrong, 2002). In addition, unequal distribution of contaminant in the feed may also lead to differences in dosage consumption. This problem was approached methodologically by Houck & Cech (2004) where feed was administered by a vertical tube into the tank in order to prevent feed from escaping down the standpipe of the tank which was fitted with a fine-mesh floating collar to prevent loss. They then worked under the assumption that all feed was consumed at some rate by all fish present in the tank. Our study attempts methodologically to get around these problems and assumptions by directly administering a known quantity of methylmercury enclosed within a gelatine capsule directly into the stomach of the experimental fish by the use of a plastic catheter. This method being a variation of the Ruohtula and Miettinen 1975 study.

In this study, we examine and model the assimilation of methylmercury as a single pulse of a discrete quantity, administered through diet over a long-term

consecutive sampling study. Tissues measured include blood, flesh, liver and gut. Our goal was to understand physiological processes that might affect mercury concentrations in various tissues of farmed fish with the idea of improving information for both fish farmers and health authorities regarding consumption guidelines.

3.2 Methods

3.2.1 Animal husbandry

Approximately six hundred fifty 15g hatchery-reared Atlantic haddock (*Melanogrammus aeglefinus*) juveniles were acquired from the Marine Centre in Shippagan, New Brunswick (NB) and transported to the Huntsman Marine Science Centre (HMSC, St. Andrews, NB, Canada) in a holding tank in June of 2005. Fish were randomly distributed into nine 100 cm diameter x 33 cm depth circular fibreglass aquaria at a density of ~100 fish/tank and reared under 24h continuous lighting (100 Lux) replicating hatchery conditions in an ambient temperature flow-through (3 l/min) system. Water flow into each aquarium was dispersed into half inch spraybars which acted to aid in offgassing as well as increase dissolved oxygen (D.O.) levels within each experimental unit. Nitrogen levels were verified by the use of a YSI Multiparameter Instrument (YSI Incorporated, Yellowstone, OH, USA) upon initial entry of fish into individual aquaria. D.O. and temperature data in each tank were collected daily by use of an Oakton oxygen/°C meter, DO 100 series (OAKTON Instruments, Vernon Hills, IL, USA).

Quarter inch mesh netting was used to cover individual aquaria to ensure that experimental subjects remained in the appropriate tank. Fish were hand fed on a daily basis to satiation as Atlantic haddock are capable of reaching satiation under a normal daylight period feeding regime (Trippel & Neil, 2003). Juvenile haddock were reared on Skretting nutra-fry (Skretting, St Andrews, NB, Canada) extruded dry feed and were subjected to eight weeks of acclimation at HMSC in order to reach an experimental start weight of ~60g.

3.2.2 Capsule Preparation

Commercially produced Nutra Fry NP (Moore-Clark / Skretting, crude protein 50%, crude fat 20%, crude fiber 1.5%, crude calcium 1.6%, crude phosphorus 1.3%, crude sodium 0.5%, vitamin A 5000 IU/kg, vitamin D 2400 IU/kg, vitamin E 200 IU/kg) diet was ground into a homogenous powder. The powder was then mixed into a homogeneous solution of deionized water and methylmercuric chloride **(**Fisher Scientific Canada**,** Ottawa, ON, Canada**)** dissolved in 100% ethanol at appropriate concentrations for the desired dosages, 0ppm (control dosage), 5ppm (moderate dosage) and 10ppm (high dosage). Spiked feed was then freeze-dried for 5 days in a Virtis Benchtop freeze-dryer (Virtis, Gardiner, New York, USA). To ensure that available moisture was extracted, feed was repeatedly weighed until weight did not change. 80% was the average moisture content for feed samples. Dried feed was then ground into a uniform powder and 10ml portions were measured into gelatine capsules (10ml volume). The capsules were placed in cold storage (-20°) until further use. Randomly selected capsules

from each dosage group were further analysed for total mercury concentration by atomic absorption spectrometry (Milestone DMA-80 Direct Mercury Analyzer (Milestone Srl, Sorisole, Italy)) and found to reflect corresponding dosages of methyl mercury (0.00, 5.42, 11.24 ppm for control, moderate and high dosages, respectively). Earlier studies have confirmed that mercury loadings in commercial fishfeed diet are attributed to residual traces in all ingredients (Choi & Cech, 1998).

3.2.3 Experimental Design

The controlled dosage spiking trial was run over a period of 63 days from initial spiking to final sacrificial sampling. Nine aquaria were randomly designated as treatment tanks with triplicate replication of control (0ppm), moderate (5ppm) and high (10ppm) dosage treatments of methylmercury. Experimental fish were kept on their respective diets for 140 days (acclimation through final sacrificial sampling) and mortalities were routinely sampled for weight, length and total mercury analysis to determine baseline total mercury concentrations prior to spiking. Upon reaching an average weight of 60g, the remaining ~60 fish/aquaria were designated as experimental animals. The nine experimental units representing triplicate aquaria of the three meHg exposure groups (control, moderate and high dosage were chosen to allow comparison with similar studies (Choi & Cech, 1998; Berntssen *et al*., 2004). All 60 fish/aquaria were anesthetised, weighed, forklength measured and intubated. A syringe filled with saline solution isotonic with cell cytoplasm (0.9 ppt) and fitted with a flexible Teflon capsule holding

tube to retain the 10ml gelatin capsules containing spiked fish feed was used for intubation. The capsules were inserted directly into the stomach cavity, with the flexible tubing minimizing irritation as the instrument passed the esophagus. Intubated fish were placed in a recovery unit and monitored for capsule regurgitation. If intubation was successful, the fish was placed back into its respective tank. If capsules were regurgitated, the third dorsal fin of the affected subject was clipped and the fish was then released into its respective tank. Experimental endpoints were assimilation rates and bioaccumulation rates in flesh, blood, gut and liver tissue following meHg spiked dosage administration.

3.2.4 Sampling

Juvenile haddock sampling was achieved by randomly sampling five fish per tank (in all treatment groups) according to a pre-determined sampling schedule based on predicted outcome. Fish were starved 24 hours prior to sampling. Upon each sampling, five fish were killed by a sharp blow to the head, weighed, measured for forklength and given a label used for identification purposes upon sampling. In addition, the first out of every five fish from all treatment tanks at each sampling was bled from the caudal vein into a labelled vacutainer tube (Benton Dickinson VACUTAINER systems USA, Rutherford, NJ, USA). Liver, flesh and intestinal samples were taken from these fish and cold-stored in labelled 5ml centrifuge tubes at -80°C. Upon each sacrifice, sampled fish from each treatment tank were individually wrapped in labelled plastic bags and stored along with the first fish from the group in a ziplock® bag. At each sampling, the

remaining fish were fed immediately following the collection of water samples from each tank. Fish feed samples were taken from both bags of fish feed consumed throughout the sampling period and levels were found to be similar to baseline concentrations found in capsule preparation (17-49 ppb). Water samples from each experimental tank were taken after each sacrificial sampling prior to feeding. These samples were frozen at -20°C and later analyzed for total mercury by CVAFS.

3.2.5 Growth

Atlantic haddock growth was determined by the change in weight between the groups of fish (n=5) sampled from each tank and the initial mean weight of the entire tank, prior to intubation. Sampled fish were removed from each aquarium, killed by a sharp blow to the skull, blotted with a kim wipe® to remove excess moisture and weighed individually on a tared balance. Due to unaccounted weight variability between tanks due to tank effects (Figure 3.2.1), Specific Growth Rates (SGR, the % body weight gained per day) have been calculated in order to more accurately portray growth relative to each tank over time. SGR was calculated using the formula:

$$SGR = 100 (\ln m_2 - \ln m_1) (t)^{-1}$$

Where t = time period (in days) having passed from intubation day until specific sampling (1,4,7,10,13,17,24,31,38,45,52 or 59 days) and m_1 and m_2 = wet fish mass (g) initially and at time of sampling, respectively. All fish within treatments were found to

be uniform in weight prior to intubation ($p<0.05$), however there were tank effects (Figure 3.2.1).

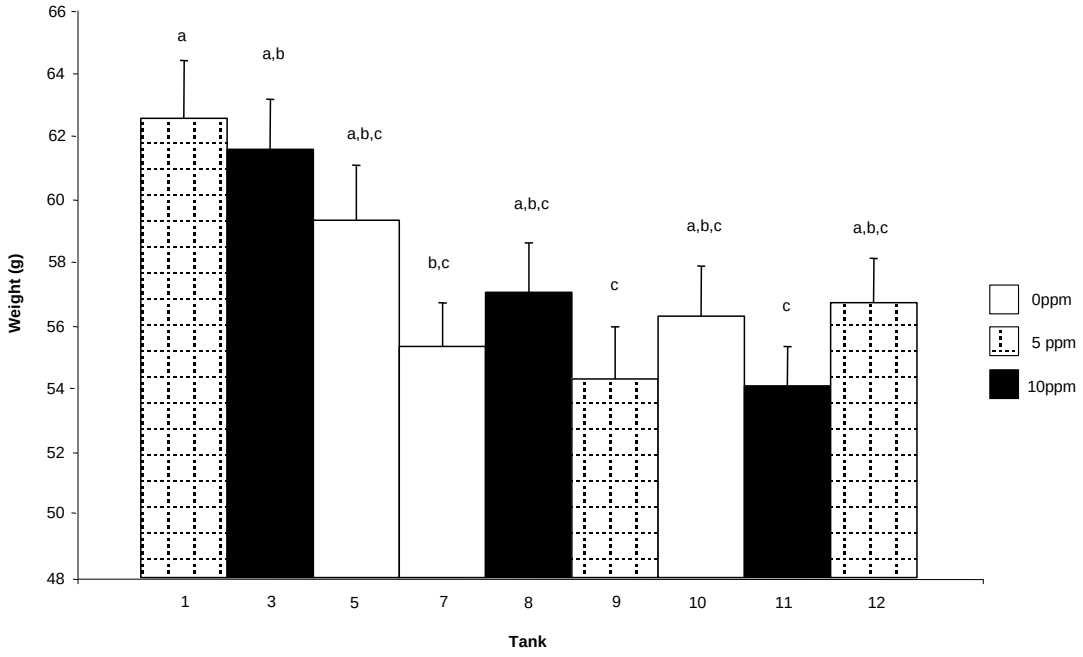

Figure 3.2.1 Mean treatment group weights of Atlantic haddock (g) at intubation. Tank 1 and 2 fish are significantly larger than tanks 7, 9, and 11 ($p<0.05$). Due to this unforeseen discrepancy, growth was considered on a tank-specific basis.

Condition factor (K) was determined by the calculation in Busaker *et al*. (1990).

$$K = \text{weight}/\text{length}^3$$

3.2.6 Total Mercury Analysis of Fish Tissue

Whole fish, organ samples and water were transported on ice from HMSC cold-storage to cold-storage at the UNB mercury lab until processed for total mercury analysis. Each labelled fish was identified and matched with corresponding weight and lengths from sampling data collected.

3.2.6.1 Muscle Tissue

Prior to total mercury analysis, verification was made to see if the third dorsal fin was clipped from the subject in order to determine whether it had regurgitated the capsule upon administration. An aliquot of muscle tissue was removed with a scalpel from the dorsal region posterior to the operculum, anterior to the first dorsal fin, on the left side of the fish. This tissue was placed into a 20ml glass vial and freeze dried for 2 days. Following freeze-drying, 0.01 to 0.05g samples were analysed for total mercury on a DMA-80. In a previous study with Atlantic salmon, Sweeney *et al.* (2006) compared samples of tissue from the dorsal, tail, and stomach region for total mercury to verify that concentrations were consistent throughout the entire fish. All regions were found to be similar ($p>0.05$). We are therefore confident that each muscle sample, taken from a consistent location on the haddock in the current study, will give a representative mercury concentration for flesh.

3.2.6.2 Liver Tissue

Liver tissue was removed from frozen storage and placed into a 20ml glass vial. Due to abnormally high amounts of lipid associated with liver tissue, we lipid extracted the tissue before freeze-drying by modifying the method of Bligh and Dyer (1959). Liver in the 20ml vial was diluted with a Chloroform/Methanol mixture at a 2:1 ratio. The vial was capped and shaken by hand for approximately 30 seconds. After allowing the mixture to settle for 30 minutes, the solvent layer was extracted with a 5ml plastic pipette and discarded into a waste container. The remaining solid portion was diluted to the top of the glass vial with the Chloroform/Methanol mixture once again, the mixture shaken for 30 seconds, left to settle again for 30 minutes and the solvent layer was then extracted. Upon reaching complete extraction, the remaining solid portions were freeze-dried for 2 days and analysed for total mercury as before.

3.2.6.3 Blood Tissue

BD Vacutainers containing blood samples were uncapped and weight of sample was determined upon taking the samples from cold storage. Due to the very small amounts of extracted blood for each sample, accurate sample weights were obtained by adding 500µg of 0.01% KOH to each sample and allowed to mix overnight on a shaker unit. The day following, 0.1g of the mixture was analysed for total mercury as before. Resulting values were adjusted for wet-weight and a dilution factor.

3.2.6.4 Gut Tissue

Intestinal tissue was removed from frozen storage, placed into a 20ml glass vial and freeze dried for 2 days. Due to lipid associated with this tissue, we modified the suggested analytical preparation method by digesting the gut tissue in 200µl of 10% Potassium Hydroxide. Upon digestion, the remaining material was run for total mercury analysis. The resulting readings were adjusted for a dilution factor.

Differences between sample preparation for blood, flesh, liver and gut tissue (lipid extraction, addition of 10%KOH) were necessary within the confines of the technological requirements of equipment within the mercury laboratory at UNB Fredericton. Trends seen within tissues over the course of our experiment are more valuable as information for this study than simple differences between tissues.

3.2.6.5 Data Analysis

Differences in survival, growth and condition of fish, and mercury concentrations in water before and after intubation were analysed using single factor ANOVA ($p<0.05$) and appropriate post-hoc comparison methods (Day & Quinn, 1989) were run on NCSS™ software. Modelmaker™ software was used to plot raw data and compare treatment groups and effects over time. All data are presented as means ±S.E.M. Due to interactions between tissue and time among treatments, no formal statistical hypothesis testing was conducted for mercury concentrations.

In special cases, P-values for moderate and control treatment groups were set at <0.01 in order to decrease our probability of Type 1 error due to the increase in treatment groups and decrease in sample size because of tank effects.

3.2.7 Mercury Mass Balance Model for Mercury in Farmed Fish

Previous studies have shown that although mercury can be taken up across gills, the majority of mercury that is accumulated within fish tissue is typically accumulated through diet (>99.9%) (Hall *et al*., 1996). We have based our MMBM on the work of Trudel & Rasmussen (2001), however, where mercury ingested by fish was previously an unknown, we know exactly what concentration of mercury was intubated directly into the stomach of fish. Therefore, we can estimate assimilation into tissues based on tissue sampling results. We assume that daily loss of mercury to gonadal development is zero.

Therefore, our model is based on the following parameters

Inputs:
[mercury] in feed = known
[mercury] in fish = known

We know that feeding rate contributes to both fish weight and mercury uptake due to higher feed volumes being fed to larger fish and the dilution of mercury through growth (Jensen *et al*., 1982).

Outputs:

[mercury] in blood = calibrated with known

[mercury] in liver = calibrated with known

[mercury] in muscle = calibrated with known

[mercury] in gut = calibrated with known

[mercury] in waste = unknown

Our experimental outputs allow us to estimate elimination rates from tissues and therefore the assimilation of mercury within subjects of our experiments can be determined by the following equation:

Total mercury uptake (μ) = gut[Hg]+blood[Hg]+liver[Hg]+flesh[Hg]

3.3 Results

The mean values for length, weight, liver weight and hepatosomatic index (HSI) are presented in Appendix 2. Mean values for total mercury concentrations within all tissues are presented in Appendix 1.

3.3.1 Survival, Condition and SGR (Growth)

Experimental fish mortality rates were <0.005%/day during the 60 day experimental period with no significant differences between treatment groups ($p<0.05$). Over the entire duration of the experiment (including acclimation) there were no

significant changes in mortality rates within treatment groups nor between treatment groups ($p>0.05$) on a weekly basis. There were no significant differences between condition factor (K) of the entire experimental group from acclimation throughout all sampling days ($p<0.05$) and all fish in each tank appeared similar in body color throughout the experiment. There was no significant change ($p>0.05$) in Atlantic haddock SGR in the high dose tanks compared with moderate and control dose tanks over the entire experimental period, however, fish from all treatments showed a drop in SGR immediately following intubation, likely associated with handling stress (Figure 3.3.1).

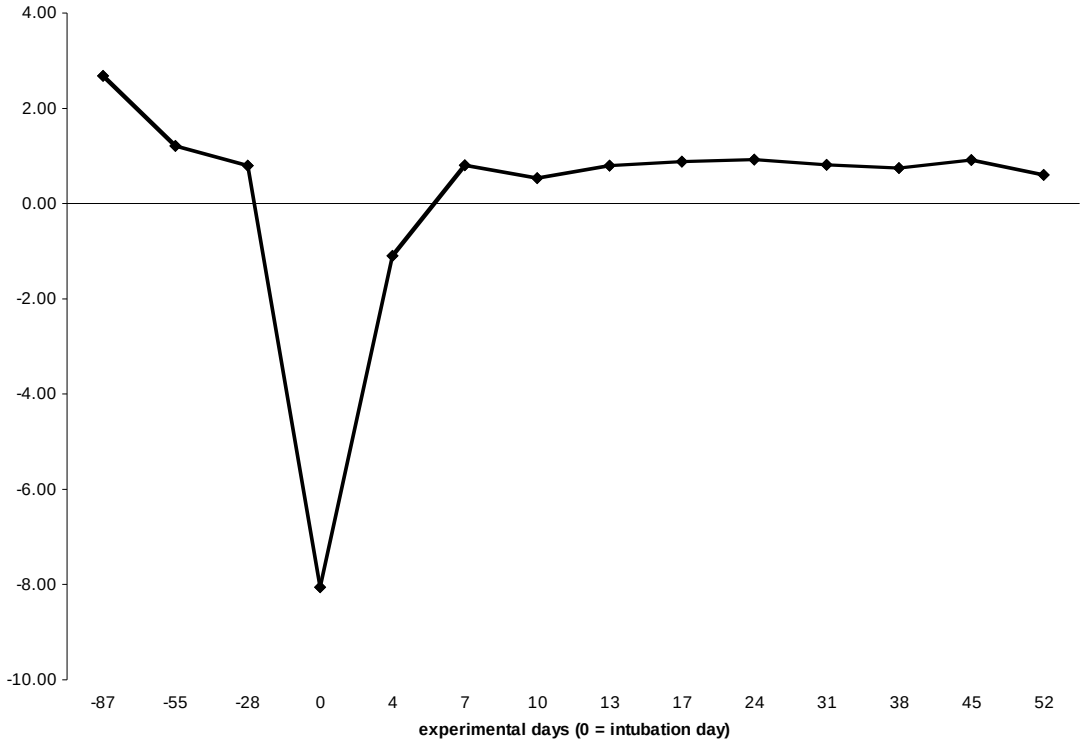

Figure 3.3.1 Specific Growth Rate of experimental Atlantic haddock throughout the duration of the acclimation and experimental period of the controlled dosage trials.

To ensure that mercury uptake in Atlantic haddock took place primarily through direct contact with diet, water samples from both prior and immediately following intubation were taken. In order to be assured that there were no influxes of mercury through the flow-through system over the 60 day sampling period (residual mercury within system or introduced by diet), upon each sacrificial sampling (with the exception of sampling days 13 and 17) representative water samples were also taken. Results from comparisons of total mercury levels in water prior to re-stocking tanks with intubated fish with water samples taken immediately following re-stocking show that there is no significant difference between the two periods of sampling in high, moderate and control dosage tanks ($p>0.05$) and concentrations never exceeding 3 ng/l. Results from repeated sampling over the course of the entire experiment show no significant changes in total mercury levels within treatment groups nor between treatment groups ($p>0.05$ and $p>0.05$, respectively) and ranged from 0.31 to 5.88ng/l.

3.3.2 Assimilation and Bioaccumulation of Mercury

Assimilation of dietary MeHg showed a dose-dependent trend in flesh, liver, gut and blood.

3.3.3 Flesh

Fish spiked at the highest dosage showed total mercury concentrations in flesh which ranged from 64ppb (day 10) to 455ppb (day 17) with a mean of 144ppb and showed no statistically significant difference in concentrations over the entire sampling period. Flesh concentrations of the highest dosed fish were significantly higher than moderate dose (5ppm) and control (0ppm, placebo) dosed fish over the entire 60 day sampling period ($p<0.05$) (Figure 3.3.2). Due to statistical interaction caused by tank effects in the moderate dosage group, the triplicate tanks for the moderate dose treatment were analysed separately. In two of the three tanks there was no statistically significant difference in total mercury flesh concentrations over the entire 60 day sampling period and concentrations ranged from 67 ppb (day 45) to 200ppb (day 45) with a mean of 101ppb. In the third tank we saw a statistically significant increase in total mercury flesh concentrations on days 13 and 17 ($p<0.01$) with a range and mean of 37ppb to 336ppb and 120ppb, respectively. In the control dosage group we saw that although one of the three triplicate treatment tanks had total mercury flesh concentrations which were significantly lower than the other two, that there was no statistically significant difference in flesh concentrations across the entire sampling period ($p>0.01$).

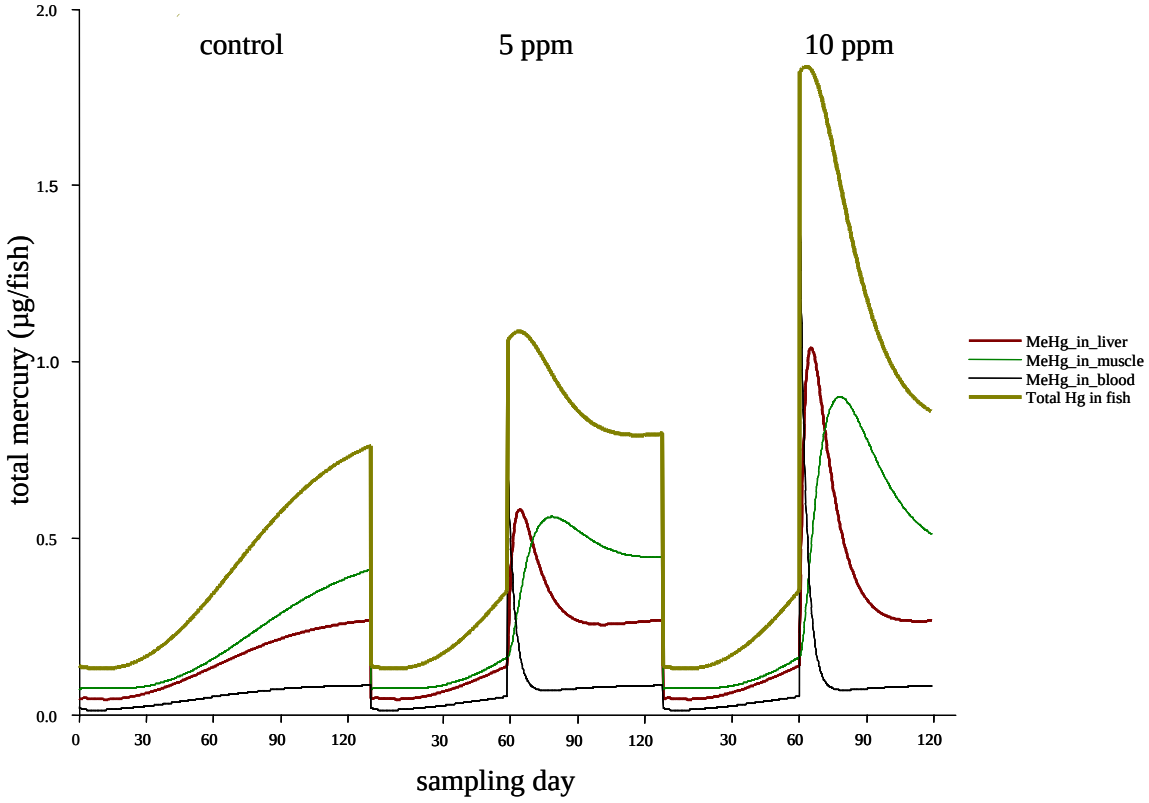

Figure 3.3.2 Total mercury (µ/fish) model output for the controlled dosage trial with sampling day represented in three 125 day sections on the x axis (control group, 5ppm group, and 10ppm group) is represented by the thick green line. The components of the total mercury count are represented by the thin red (liver), green (muscle) and blue (blood) lines. Gut is not represented as it was the site of administration and not necessarily reflective of uptake.

3.3.4 Liver

Liver from fish spiked with the highest dosage of methylmercury showed total mercury concentrations which ranged from 59 ppb (day 45) to 615 ppb (day 4) with a mean of 234 ppb over the entire experimental duration. We saw a significant effect of sampling day on liver concentrations for the highest dosage treatment group ($p<0.0001$). Liver concentrations in the moderate dosage groups range from 84 ppb (day 52) to 444 ppb (day 4) with a mean concentration across all sampling days of 204ppb. There was no

statistically significant effect of sampling day on liver concentration in the moderately spiked experimental group. In the control group, we saw a range of liver total mercury concentrations of 61 ppb to 196 ppb with a mean of 132ppb across the entire sampling period. There was no significant effect of sampling day on liver total mercury concentrations in the control group. Due to effects of interaction between dosage concentration and sampling day, predictable patterns of high and moderate dosage concentrations in liver are unclear. There appears to be no statistically significant separation of the three dosage groups over the entire sampling period ($p<0.05$)

3.3.5 Gut

Gut content from fish spiked with the highest dosage of methylmercury showed total mercury concentrations which ranged from 1757 ppb (day 1) to 34 ppb (day 31) with a mean of 365±136 ppb over the entire experimental duration. We saw a significant effect of sampling day on gut concentrations for the highest dosage treatment group ($p<0.001$). Gut concentrations in the moderate dosage groups range from 489 ppb (day 1) to 38 ppb (day 24) with a mean concentration across all sampling days of 145±27.2 ppb. There was no statistically significant effect of sampling day on gut concentration in the moderately spiked experimental group ($p>0.05$). In the control group, we saw a range of gut total mercury concentrations of 143 ppb to 39 ppb with a mean of 83±6.4 ppb across the entire sampling period. There was no significant effect of sampling day on gut total mercury concentrations in the control group ($p>0.05$). We also saw effects of interaction between dosage concentration and sampling day within gut treatment groups.

Therefore, predictable patterns of high, moderate and control dosage concentrations in gut are unclear. We also determined for gut treatment groups that there appears to be no statistically significant separation of the three dosage groups over the entire sampling period ($p>0.05$).

3.3.6 Blood

Blood from fish spiked with the highest dosage of methylmercury showed total mercury concentrations which ranged from 339 ppb (day 1) to 23 ppb (day 3) with a mean of 119±20.9 ppb over the entire experimental duration. We saw no significant effect of sampling day on blood concentrations for the highest dosage treatment group ($p>0.05$). Blood concentrations in the moderate dosage groups range from 269 ppb (day 17) to 13 ppb (day 31) with a mean concentration across all sampling days of 109±18.5 ppb. There was no statistically significant effect of sampling day on blood concentration in the moderately spiked experimental group ($p>0.05$). In the control group, we saw a range of blood total mercury concentrations of 243 ppb (day 1) to 13 ppb (both day 1 and day 45) with a mean of 95±14.2 ppb across the entire sampling period. There was no significant effect of sampling day on blood total mercury concentrations in the control group ($p>0.05$), as expected. Within the entire experiment we were unable to detect an effect of dosage, day and interaction on blood mercury concentrations ($p>0.05$).

3.3.7 Mercury Mass Balance Model for Mercury in Farmed Fish

Output results from our model show that total mercury (μ) increased over the duration of our study within our control group. Mercury administered in the form of a controlled dose of 5ppm showed a peak in total mercury immediately following administration, and dropping to control group levels within 30 days (Figure 3.3.2) The highest dosage group showed a similar trend, an immediate peak following dose administration and drop back to control group levels following a 60 day duration.

3.4 Discussion

3.4.1 Assimilation and Bioaccumulation of Mercury

According to our data, within 24 hours mercury was detected in highest concentrations within the gut, while liver mercury concentrations peaked at 5 days post sampling. Flesh concentrations peaked at roughly one week post-sampling. Blood data was highly variable but appears to reflect similar concentrations as flesh. Mercury appeared to be quickly sequestered into liver tissue upon dosage administration and resides for the longest duration within flesh tissue (Figures 3.3.2 and 3.4.1).

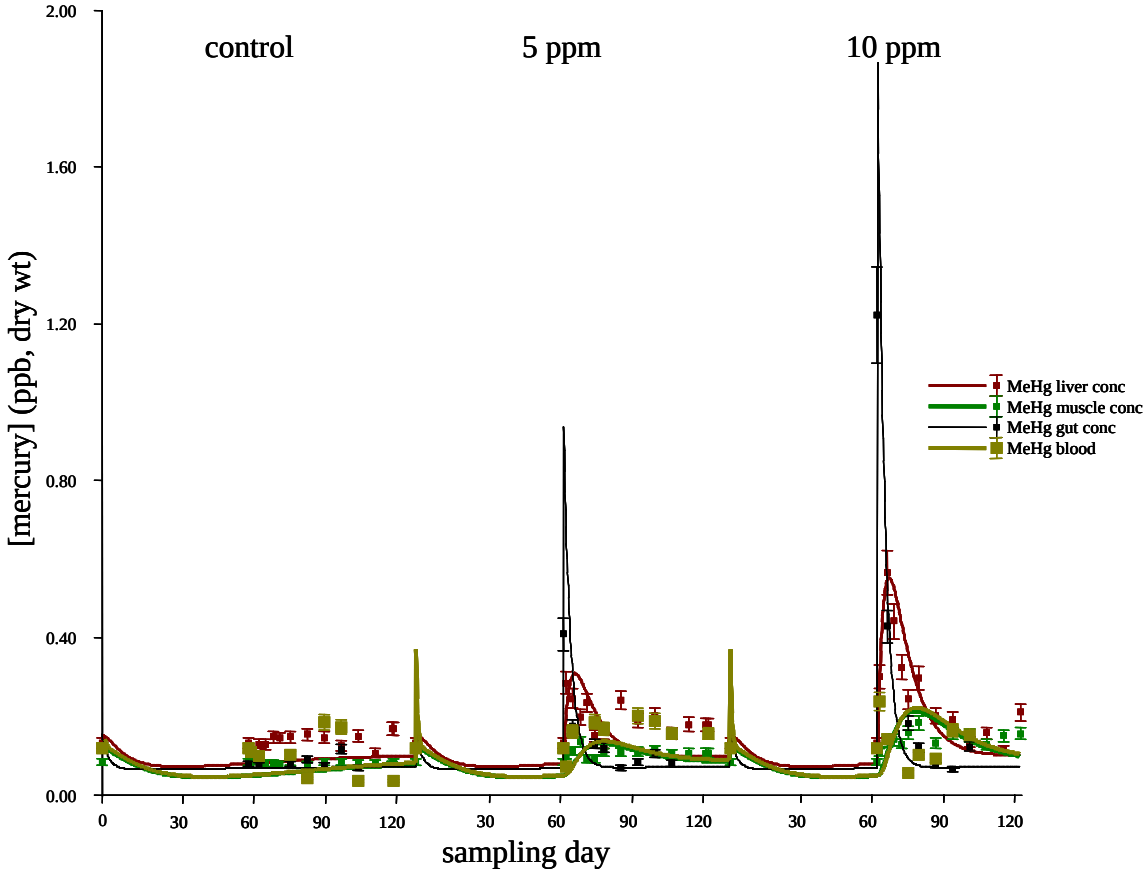

Figure 3.4.1 Mercury concentration (ng/kg) model output for controlled dosage trial. 125 sampling days for each dosage treatment group are represented on the x axis. Mercury concentrations in liver, muscle, gut, and blood are represented by red, green, blue and thick green lines respectively.

Our results fall within the range of data from previous studies. In three generations of brook trout (*Salvelinus fontinalis*) which were exposed to methylmercuric chloride through water, uptake of mercury into body tissues showed similar trends. Uptake into blood and gill tissue of first generation fish showed their highest rates of increase within the first two weeks of administration of methylmercuric chloride through the water system, initially reaching higher levels than those of liver and muscle tissue (gut tissue was not analysed) (McKim *et al.*, 1976). Because this was a continuous dosage study, we see that mercury concentrations within different tissues do not decrease

over time and therefore results are not comparable to the rest of our study. Through the use of radioisotopes, Ruohtula & Miettinen (1975) showed that through various means of ingestion (gill, intramuscular and directly deposited into the stomach cavity via intubation of protein bound mercury) the contaminant was taken up immediately into whole body tissue and showed a half-life which varied from 202 to 516 days. This suggests that the metabolism of mercury in fish is quite slow although this particular study was not able to determine which tissues retained mercury the longest. Fish which were given the highest dosage of mercury (5ppm) through intubation, retained a mean of 3ppm of mercury (Ruohtula & Miettinen, 1975). Our results in comparison show a much shorter half-life of roughly 20-30 days for a similar dosage, this may be a reflection of increased metabolism within our fish due to handling stress.

We have considered blood circulation pathways throughout the organs of fish in order to determine how, where and when ingested mercury is assimilated. We also have considered where experimental blood was extracted from, in terms of what that blood has picked up along its circulatory pathway and where it will be redistributed.

Under normal circumstances, according to Smith and Bell (1975), once blood is taken up across the gills and oxygenated, it may follow one of three or a combination of these circuits throughout the body of fish. One blood distribution pathway is directly into capillary beds located in both skeletal muscle and visceral organs where blood nourishes tissue and is then shunted through either the renal or hepatic portal system which would then involve either the excretion or redistribution of the remaining nutrients, un-

distributed toxins and waste bi-product from organs. The second blood distribution pathway is from the dorsal aorta and typically into segmental veins (ventral, dorsal) located anterior to the caudal vein which are redistributed through capillary beds back to the heart, or nearby, without passing through a portal system bringing oxygenated blood to the heart. The final distribution route is directly from the gills into the efferent branchial arches and into the head region where oxygenated blood is again distributed. Based on this information, we can see that results may be obscured by location from which blood is drawn in relation to mercury circulation.

In an experiment where mercury was taken up across the gills, the first place that an increased mercury concentration would be seen immediately after uptake is within the blood where it would then be distributed throughout the pathways described. Blood samples taken from the caudal vein, as in the current study would reflect this increase in mercury due to the distribution of blood from the dorsal aorta to the caudal artery which eventually drains into the caudal vein via the trunk muscles (Smith & Bell, 1975). However, since the uptake of mercury in our study was not across the gills, but directly from the gut, the first place that we should have seen increased mercury levels appear would be the liver and the route to the liver from the gut, the hepatic portal vein. Having only sampled blood from the caudal vein which is not closely linked with the hepatic pathway, we may have missed the initial spike in blood due to a less appropriate sampling location for blood.

3.4.2 Survival, Condition and SGR (Growth)

Mortality rates within treatment groups were negligible throughout the duration of the experiment and were at no time significantly higher than during any other time period. This helps us conclude that there was no mortality directly associated with the administration of mercury into the experimental fish. Although Specific Growth Rates throughout the duration of the experiment were not significantly different between groups over time, there is evidence that growth rates were negatively impacted by either the administration of mercury, or by a toxic implication of the levels given. Since all treatment groups showed the same trends in SGR over a similar time period, it is unlikely that the initial negative trend is a response to toxic methylmercury as much as it is a physiological response to the stress of intubation and repeated handling of sacrificial fish from tank populations over the first 10 days of sampling. Handling stress is defined as netting, grading, marking and transport of fish according to Pennell and Barton (1996). Excessive handling is understood to contribute directly to the environmental stress in experimental fish by eliciting a compensatory physiological response of varying degrees depending on the nature of the stress (Jobling, 1994). In teleost fishes, like many organisms (Wedemeyer *et al*., 1990), the primary response to both acute and chronic environmental stressors is evidenced by an increase in catecholamine (epinephrine) plasma levels along with cortisol plasma levels which can remain elevated for long periods of time depending on length and frequency of exposure (Jobling, 1994; Pennell & Barton, 1996). Secondary stress responses result from prolonged periods of primary response to stress and although organ responses vary, they are easily characterized by the

mobilization of glycogen reserves within the liver which increase plasma glucose levels (Jobling, 1994). Tertiary responses are indicative of longer-term or highly frequent environmental stressors including excessive and frequent handling and may manifest in depressed growth rates, impaired immunity and poor reproductive success (Jobling, 1994; Pennell & Barton, 1996). Techniques for experimentally monitoring primary and secondary stress response in order to quantify the degree of chronic stress upon a population are common in literature (Acerete *et al*., 2004). A weakness of our experimental design is the fact that despite the projected amount of handling which would be undertaken to attain the goal of our study, we did not set in place means of quantifying both chronic and acute stressors. Hoskonen and Pirhonen (2006) have recently shown that repeated handling of Rainbow trout (*Onchorhynchus mykiss*) without anaesthetic resulted in a significantly reduced feed intake and in turn, reduced growth, and further confirmed that certain types of anaesthetic do have properties which reduce the effect of stress response due to adverse conditions. Although our experimental fish were anaesthetised for the gelatine capsule exposure, there were no means for us to gauge the impact of stress that netting of the fish the day following intubation and every three days out of the next two weeks following, had upon the experimental fish. In Bonga's (1997) review of the stress response in fish it is shown that along with the consequences of a reduced appetite, the metabolic rate as influenced by both acute and chronic stress is commonly increased. We have considered the tradeoff that exists between the confounding variable that increased handing imposes upon fish within our study and the benefits of having continuous data.

On average, over the period of intubation to final sacrifice, the group of experimental haddock grew from 55g to roughly 70g whereas over the acclimation period of the same duration leading up to intubation, the fish grew from an average weight of 10g to 55g. Because we witnessed a sharp decrease in SGR in all groups that is most marked within the period immediately following intubation, we assume that handling intensity of the experiment may have resulted in decreased growth rates compared to typical experimental haddock trials (Trippel & Neil, 2003).

Wet weight gain may be indicative of a gain in lipid content which is not always accepted as growth (Busaker, 1990). Considering the high hepatosomatic indices found in our fish as a result of fatty liver (consistent with Treasurer *et al.* (2006)), increased weight is more likely a reflection of the increased lipid intake and uptake from diet. Furthermore, lipid storage in gadoid fishes heavily favours liver tissue, while lipid uptake in muscle tissue is quite low (Schwalmn & Chouinard, 1999; Treasurer *et al*., 2006). This may have consequences on our results as we have also suggested in a related study that lipid concentration is negatively correlated with mercury concentration in flesh of fish (Sweeney *et al*., 2006). Therefore, the trends that we see in Atlantic haddock, that store lipids primarily within liver tissue, will differ from a species that stores lipid in flesh tissue.

3.4.3 Future Consideration

This study gives a clear and accurate picture of how mercury is sequestered within fish, especially in light of similar studies which have used different methods to achieve the same goal. It gives us the enhanced ability to monitor and predict how mercury taken up through diet will be reflected in blood, liver, and flesh from the gut. From a human health perspective, this model enables health authorities to make relevant fish consumption guidelines based on residence time and tissue under consideration. From an aquaculturalist point of view, the model allows for knowing mercury concentrations within diet fed and residence time of mercury in muscle tissue, and can become an important biomonitoring tool. It can help to ensure that farmed fish are a safe, low mercury source of protein which also allows a market advantage for farmers.

Future consideration should be focused on establishing model parameters for other species of farmed fish under laboratory conditions. Sweeney *et al*., 2006 have shown differences in mercury bioaccumulation between farmed species of Atlantic salmon and Atlantic cod and have attributed these differences to lipid content in desired tissue.

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Chapter 4.0-Mercury cycling through finfish aquaculture in the lower Bay of Fundy: a conceptual mercury mass-balance model

4.1 Introduction

It is commonly understood that both industrial and agricultural development during the past century has been responsible for increased emissions of the toxic heavy metal mercury, both atmospherically and directly into aquatic systems. This has led to contamination of many freshwater and marine systems (Downs *et al*., 1998). Through various biological processes, contamination has led to increased levels of methyl mercury in long-lived wildlife at higher trophic levels which inhabit these systems and in turn, has been responsible for mercury poisoning of human consumers of fish and wildlife inhabiting these waters (Walcek *et al*., 2003). The human health effects seen from its poisoning are most notably expressed in neurological impacts in young children and developing fetuses (French *et al*., 1998). Fish consumption is the primary means of mercury poisoning in humans. This fact was brought to light with the fatal poisoning of at least 100 people that lived on a diet composed primarily of fish contaminated with methyl mercury of industrial origin, in Minimata Bay, Japan. Thousands more were negatively affected within a relatively short time period (Walcek *et al*., 2003). The event has brought mercury contamination to the attention of the general public and scientific community as a global food safety concern.

The idea of capturing and culturing wild fish in a controlled environment is not a new concept to Canadians. Aboriginal communities are believed to have been

participating in proto-aquaculture activities prior to the confederation of Canada while government, dating as far back as 1850, has been recorded to have participated in the incubation and hatching of different species of shellfish and finfish (OCAD, 2003). The Bay of Fundy is known worldwide for its extreme tidal range, reaching up to 16m at the mouth of the Bay. It is roughly 16,000 km^2 in area, not more than 200m in depth and hosts a wide variety and abundance of aquatic life because of its shallow bays and sheltered inlets. For these reasons, the Bay of Fundy has also proven ideal for the development of finfish aquaculture in Eastern Canada. Since the 1970's, marine finfish aquaculture in the lower Bay of Fundy has expanded from a relatively small number of wooden cage farms to an industry composed of 98 farms which maintains positive growth, is currently commercially producing three species of finfish, occupies over 1,500 hectares of coastline, and has become New Brunswick's largest agrifood producer (NBDAFA, 2004). The numerous sheltered coves along the lower Bay of Fundy coastline provide refuge for marine cages from storm surges and, early in the development of finfish aquaculture in Atlantic Canada, it was thought that strong tidal currents would act as a "flush" for organic buildup produced at marine aquaculture sites, essentially washing the waste out to sea. This theory proposed that organic material produced would be swept out of the vicinity of the aquaculture site and become diluted within the larger ocean upon each tidal cycle. The "flushing" theory has been contested by oceanographic studies which indicate that organic waste from aquaculture netpens may reside within close proximity of cage sites for extended periods (over years in some cases) (Ernst *et al*., 2001, Sather *et al*., 2006). Despite this fact, finfish aquaculture in New Brunswick has continued to develop, bringing economic prosperity to the region in

the form of spin-off businesses and in drawing larger companies to the area while providing jobs to a large number of workers under the age of 40 (Stewart, 2001). The finfish aquaculture industry in New Brunswick is the second largest aquaculture industry in Canada and it has doubled in value over the past decade to annual sales of roughly $283 million with export revenues of $150 million alone. Atlantic salmon (*Salmo salar*) production has been the most successful form of finfish aquaculture in New Brunswick (NBDAFA, 2004).

Studies particular to the Bay of Fundy have revealed that anthropogenically released atmospheric mercury (i.e. fuel combustion) in industrialized regions of central Canada, the eastern United States and eastern Canada have historically and currently influence mercury levels in sediment and wildlife inhabiting the bay (Walcek *et al*., 2003; Sunderland *et al*., 2000). Sunderland *et al*. (2004) have shown that the biological process of methylation of inorganic mercury into toxic methylmercury within Passamaquoddy Bay, an inlet Bay within the lower Bay of Fundy, is enhanced by the physical mixing of sediments, resulting in the Bay of Fundy having a highly impacted area based on the continual conversion of inorganic to organic mercury of historical atmospheric mercury depositions. The geo-chemical climate suitable for methylation requires a) an input of inorganic mercury, b) organic matter as a substrate for methylation and c) presence of sulfate reducing bacteria (SRB) (Hintelmann *et al*., 2000). Sunderland *et al*., (2004) show that the active mixing of sediment layers within Passamaquoddy Bay enhances the availability of all three components and thus the availability of toxic methylmercury to organisms residing within the Bay. Thus, although atmospheric emissions of mercury

have decreased in recent years, inorganic mercury from historical deposition within the Bay of Fundy remains available for methylation for many years following initial entry into the aquatic system, more-so than it would be in depositional (non-mixing) sediments (Sunderland *et al*., 2004).

The general goal of this project is to determine mercury inputs into and relationships within the finfish aquaculture cycle and whether marketable product may be influenced by increased levels of mercury within the local marine ecosystem. Previous studies have shown that mercury most likely enters the aquaculture production cycle by means of fishmeal-based diet and is reflected almost immediately in blood, gill and muscle tissue (Berntssen *et al*., 2004; Choi & Cech 1998). Using feed and growth information collected from growers along with data gleaned from sampling and experimental trials, we develop a conceptual model to quantify biomass and mercury accumulation through two species farmed in southwestern New Brunswick, Atlantic salmon and Atlantic cod (*Gadus morhua*). Using data collected from Passamaquoddy Bay from 2002 through 2006 along with published data from other authors, this model attempts to trace mercury from fish feed to various fish tissues, along with sediment collected from underneath aquaculture sites from participating aquaculture farms in southwestern New Brunswick. The model will help us to address whether there is an obvious link between mercury levels in fish feed, tissues, and sediment. Because the intensive sampling period has taken place over almost a two year period, we have the ability to model mercury accumulation within finfish species over the time period in which they are held in marine cages in relation to growth and growth rates as well.

4.2 Methods

4.2.1 Farmed fish sampling

Six active marine finfish aquaculture sites were selected from 96 sites which are currently operational within the lower Bay of Fundy, New Brunswick, Canada. These sites were chosen based on site location and partnership availability. One of six of the sites commercially produces Atlantic cod, a second site contains both Atlantic salmon and Atlantic halibut, the remaining four sites are solely Atlantic salmon producing. The New Brunswick Department of Agriculture, Fisheries and Aquaculture (NBDAFA) has divided the aquaculture-intensive lower Bay of Fundy area into Bay Management Areas (BMA) that determine the yearclass (even or odd) of Atlantic salmon smolt being entered for each particular zone. Three of our sites are located within even yearclass BMAs while the remaining three sites are located within odd-yearclass BMAs. Yearclass separation was introduced as a tool to prevent the spread of disease from older fish to younger fish or vice versa by separating them according to the year that the fish are entered into marine cages as smolt (Bay of Fundy Marine Aquaculture Site Allocation Policy). Upon commencement of our project, one of our sites contained no fish (all had been harvested prior to the Fall of 2004 and not restocked until the Spring of 2005), two sites contained newly entered smolt, two sites contained fish that had been held in marine cages for over one year and one site contained Atlantic halibut of multiple yearclasses.

This spread of yearclasses provided fish samples from various stages of marine growout and ages while giving a more clear overall picture of mercury concentrations within farmed species in the lower Bay of Fundy. Samples of five fish per site were collected from a single cage every two months if possible from August, 2004 until July, 2005. Samples were labeled, placed on ice at the site and later frozen at -20°C until preparation for total mercury analysis. In earlier studies, Atlantic salmon, samples of tissue from dorsal, tail, and stomach region were compared for total mercury concentration to verify that concentrations were consistent throughout the entire fish and were found to be similar ($p>0.05$).

4.2.2 Farmed fish feed sampling

Farmed fish feed was collected from each site upon initial sampling. Samples were labeled, placed on ice at the site and later frozen at -20°C until preparation for total mercury analysis. Feed samples were collected from four different suppliers local to New Brunswick and seven different types of feed were represented, of which two were "moist" and the remaining five samples were "dry". "Dry" formulated diet is typically composed of crude protein, crude fat, crude fiber, crude calcium, crude phosphorus, crude sodium, vitamin A, vitamin D, and vitamin E at varying concentrations according to species requirements. Moist feed is a non-extruded pellet feed which is usually locally produced and has a higher percentage of wild fish as its base. In southwestern New Brunswick, moist diet is composed mainly of Atlantic herring (*Clupea harengas*) from local stocks supplemented with formulated nutritional additives.

4.2.3 Substrate sampling

We attempted to collect sediment from each industry partner location during the Fall of 2004 and 2005 during the year and a half sampling period. Diver-collected core samples were taken according to New Brunswick Department of Environment and Local Government's Environmental Management Guidelines for the Marine Finfish Cage Aquaculture Industry (EMG). Guidelines dictate the amount of transects per site based on the allowable production limit (APL) of the site and number of fish being held/cage in the Fall of each year. According to the EMG, triplicate core samples are taken from sediment at 50m from cage edge, cage edge and 10m underneath cage edge. Fresh samples were kept on ice until they could be frozen at -20°C until prepared for total mercury analysis. Control sediment samples from areas not directly influenced by deposition from nearby aquaculture operations were taken throughout the duration of the study and were used in comparison with pre-existing data collected from other researchers examining the behaviour of mercury cycling in the lower Bay of Fundy.

4.2.4 Total Mercury Analysis

Preparation for analysis for fish included fork-length measurement (tip of nose to fork of tail) and the excision of a 10 g aliquot of muscle tissue from the dorsal region anterior to the first dorsal fin. Samples of fish feed, sediment and fish tissue were

homogenized and then freeze-dried in a Virtis Benchtop Freeze-dryer (Virtis, Gardiner, New York, USA) until all moisture was removed and weight fluctuations of dried material ceased. All samples were then analyzed for total mercury by Cold Vapour Atomic Fluorescence Spectrometry on a Tekran 2500 (Tekran; Knoxville, Tennassee, USA).

4.2.5 Mercury mass balance in the system

Data records were collected from Site 3 following one lot of Atlantic salmon from smolt entry through to final harvest. Using data averages from all partner sites we calculated total mercury input (g) based on mercury concentrations within the total "available" biomass of smolt entered into Cage 2 of Site 3. "Available" biomass refers to the percentage of biomass which is most likely to take up and retain the majority of mercury; in the case of Atlantic salmon, 80-100% of mercury is stored within muscle tissue (Berntssen *et al*., 2004), which makes up roughly 64% of the body weight of adult fish (Einen *et al*., 1998). Average mercury concentration within feed used was derived from results of field sampling for Site 3 and used to determine total mercury (g) entered into the cage from the total amount of feed entered from May 2004 until November 2005. Total mercury output (g) from Cage 2 was calculated from the total "available" biomass removed from the cage at final harvest.

4.2.6 Statistical analysis

To reduce non-normality and heteroscadasticity among groups within datasets, all total mercury concentrations were log10-transformed before statistical analysis. All analyses were conducted using an NCSS statistical software package (NCSS, Kaysville, Utah, USA). All error is expressed as standard error of the mean (S.E.M.). One-way analysis of variance (ANOVA) was used to compare between and within site differences in sediment, flesh and feed. Due to failure to meet implied ANOVA assumptions of residual, kurtosis and omnibus normality, data was first log-transformed and outliers were removed (1 Atlantic salmon flesh sample from Site 3 (496 ng/g)). Post-hoc multiple comparisons were carried out with the Kruskal-Wallis Multiple Comparison Z-Value Test.

The relationship between mercury in fish feed, flesh and in sediment collected from beneath aquaculture cages for each location was qualitatively assessed due to small sample sizes that limited quantitative analysis.

4.3 Results

4.3.1 Mercury in farmed fish

Farmed Atlantic salmon from different sites are significantly different in total mercury flesh concentrations (Figure 4.3.1). Both Atlantic cod and Atlantic halibut show mercury concentrations higher than mercury concentrations in farmed Atlantic salmon, for fish grown in the lower Bay of Fundy. However, no mean concentrations for any species trigger consumption advisories dictated by Health Canada of 0.100mg/Kg wet weight.

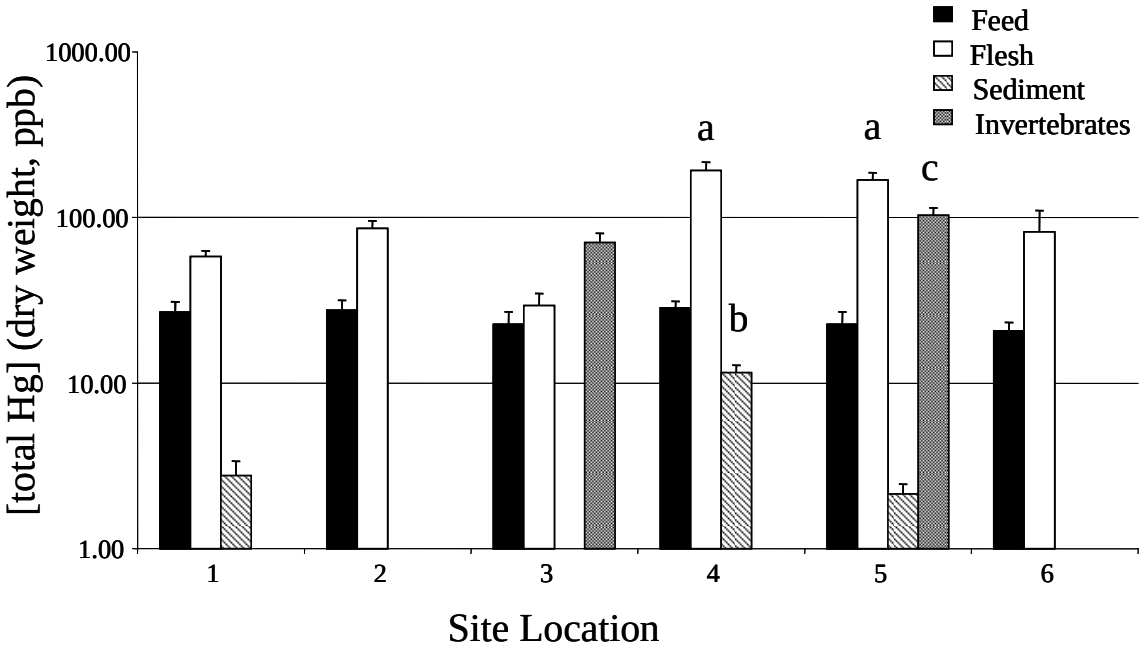

Figure 4.3.1 Mean flesh, sediment and feed mercury concentrations for samples taken from six Marine Aquaculture Sites located within the lower Bay of Fundy from the Fall of 2004 until the Spring of 2006.

4.3.2 Mercury in farmed fish feed

Eight different feed samples were collected throughout the entire field sampling period. There are no significant differences between any of the samples ($p>0.05$) (Figure 4.3.1).

4.3.3 Mercury in substrate samples

Samples from the same Marine Aquaculture Site which were taken on different years were compared to determine whether temporal variation was a factor influencing mercury sediment concentrations. The single site from which we were able to collect two years worth of data (Fall 2004, Fall 2005) showed a significant difference in mean total mercury concentration between 2004 and 2005 ($p<0.05$). Transect samples taken from the same site were analyzed in order to assess variability of mercury concentrations in sediment between transects within sites. We found that there were significant differences between mercury concentrations between transects taken from the same site at both Site 1 and Site 6 while there were no significant differences in mercury concentrations between transects at Site 2. Samples within each transect were compared using nested ANOVA and we found that there were no significant differences in mercury concentration within any sampled transects ($p>0.05$). Because we know that there are potential differences in mercury concentration within sediment between years, we have compared site concentrations which were taken within the same year. There was no significant difference in mercury concentration detected between Site 1 and Site 6 in the Fall of 2005 ($p>0.05$). In the Fall of 2004, Site 2 sediment mercury concentrations were found to be significantly higher than those of Site 1 ($p<0.05$). However, due to the variability which is unaccounted for within sites, it may not be accurate to compare sediment mercury concentrations between sites as the differences that we see are not necessarily due to the variables that we were testing.

Qualitative assessments of the relationship between fish feed, flesh and sediment mercury concentrations reveal that although all fish have received similar mercury concentrations in their feed, flesh and sediment concentrations do not positively respond.

4.3.4 Mercury mass balance in the system

Based on metadata collected from industry partners, we are able to approximate mercury inputs into a simplified mercury mass balance aquaculture model and compare these numbers to actual outputs from total mercury analysis. Site three contained a group of 10 marine cages, each of which contained on average, approximately 17,000 fish prior to harvest (November 15th, 2005). Production data for Cage 2 from May 2003 through to November 2005 is presented in Appendix 4. Approximately 30,000 Atlantic salmon smolt (representing 5040 Kg of biomass) were entered into the marine cage at roughly 60 ng/g (dry wt.) of mercury. Over the next 17 months, roughly 156,000 kg of fish feed at a mercury concentration of approximately 24±0.87 ng/g was fed to Cage 2. In November 2005, 28,800 market-sized Atlantic salmon (representing 13,250 Kg) at approximate mercury concentrations of 43±2.86 ng/g were harvested from Cage 2. The sum input of total mercury into Cage 2 which is accounted for by mercury in smolt and in feed over 17 months, is roughly 3800g, the output in the form of market fish harvested and mortalities incurred is approximately 3700g. The net gain of mercury into Cage 2's surrounding ecosystem is roughly 100g. If we assume that Cage 2 is representative, there are 10 cages per site and roughly 100 Marine Aquaculture Sites within the entire lower Bay of Fundy.

The net input of total mercury into the system is 100,000g into roughly 3200 Km^3 (31.25 g/ Km^3).

4.4 Discussion

The relationship between fish feed, flesh and sediment mercury concentrations is poorly defined based on results from our study. Whether aquaculture is contributing to mercury cycling within the lower Bay of Fundy is also unexplained with the confines of the methodology of our field study. However, our results have shown at the very least, that an assumed direct relationship between mercury concentration in feed and corresponding concentrations in flesh is most likely incorrect. As we saw in each of our sites, the mercury concentration in feed was similar across all sites whereas the mercury concentrations in flesh varied both within sites and among sites. Therefore we propose four potential factors that may account for the differences observed across sites: 1) mixing of sediments with the lower Bay of Fundy which increases methylation of mercury and its availability to consumers (Sunderland *et al.*, 2004), 2) mercury spikes in feed that were not detected in our sampling (Sweeney *et al*, 2006), 3) differences in lipid levels in fish across sites (Sweeney *et al*., 2006), and 4) differences in growth rates across sites (Jensen *et al*., 1982). Species differences will be discussed in relation to 3) and 4).

Based on results from the Sunderland (2004) study which revealed higher concentrations of toxic organic mercury within Passamaquoddy Bay due to the enhanced mixing of sediment layers, we anticipated seeing a trend towards lower mercury in

sediment and consequently within flesh of fish within Marine Aquaculture Sites the further one traveled away from Passamaquoddy Bay. Due to small sample size and inconsistent sampling methods, this proved difficult to assess. Based on the ecosystem connectivity between Pasamaquoddy Bay and surrounding areas within the lower Bay of Fundy, the expectation of this trend may have been unrealistic because mobile communities most likely transport mercury outside of Passamaquoddy Bay. A comparison of farmed fish feed, flesh and sediment concentrations from marine locations outside of the lower Bay of Fundy would have allowed for such comparisons.

Sweeney *et al*. (2006) undertook a controlled dosage study in which farmed Atlantic haddock were intubated with high (10ppm), moderate (5ppm), or control (0ppm) dosages of methylmercury. Fish were sampled from within spiked populations over a two month period and were found to retain high doses of mercury within flesh tissue for at least two months following dosage administration (Sweeney *et al*., 2006). Based on these results, we assume that if farmed fish received a dose of mercury throughout their marine growout phase (in a contaminated batch of feed, for example), this pulse may reside within flesh for an extended period (> two months). This possibility may also explain variability between sites within flesh mercury concentrations. Within our experiment, we might have been able to monitor for pulses of mercury in the form of feed had we monitored feed more vigorously. Because this was not done, we compared fish feed mercury concentrations to flesh concentrations of fish sampled at the same time as feed was collected.

Preliminary differences in lipid composition during sample preparation of farmed Atlantic salmon samples prompted us to question mercury concentration differences between lipid-extracted and non-lipid-extracted samples. Furthermore, among sites and species we found that the amount of obvious lipid within flesh tissue varied (personal observation). For Atlantic salmon, mercury concentrations in lipid-extracted aliquots were found to be consistently higher than concentrations in samples that were not lipid-extracted. We believe this to be preliminary indication that the assimilation of mercury may be "lipid-diluted" within the flesh of fish with high lipid-fillet content, like Atlantic salmon. That is, due to the low solubility of mercury in lipid, the high presence of lipid in fillet is a deterrent to assimilation of mercury within fillet. This idea is further strengthened by data that show that between Atlantic salmon and Atlantic cod, mercury concentrations in Atlantic cod are significantly lower ($p<0.05$) (Sweeney *et al*., 2006). Given that fillets from Atlantic cod contain <1% of the lipid stored within the fish (Schwalmn & Chouinard, 1999), while Atlantic salmon fillets contain the majority of the lipid load of the fish (Johnston *et al*., 2006), the differences may be partially explained by the deterrence of mercury assimilation within high-lipid fillet. Therefore, we expect that mercury concentrations will be higher in species that have lower lipid content in their flesh, like Atlantic cod, that theoretically have correspondingly low organochlorine (lipid-soluble POP) concentrations. Because we not only saw high variability among sites for lipid concentrations within samples, but also tested low flesh-lipid species, such as Atlantic cod, to high flesh-lipid species such as Atlantic salmon, the variability in flesh mercury concentration among sites despite similar feed mercury concentrations is perhaps not unexpected.

Growth rate of fish at individual sites is most likely also influencing mercury uptake and sequestering within fish flesh. It is commonly understood that fast-growing fish assimilate lower concentrations of persistent pollutants than do slow-growing, due to "growth dilution" of pollutants by growth within tissue (Jensen *et al*., 1982). Therefore, if this factor was looked at independently, we would likely see that farmers that grow their fish at faster rates would tend to produce fish of lower mercury concentrations. Due to the difficulties associated with sample collection in relation to growth data and lipid content, growth rates were not isolated as an experimental variable. However, when we consider that growing fish faster will bring higher economic returns to farmers, we can assume that species which have been farmed longer will have better growth rates based on the research and money which has gone in to achieving enhanced growth. In our study we compared farmed Atlantic salmon which have been cultured in the lower Bay of Fundy for roughly 30 years, and Atlantic halibut and cod, which have been cultured in the Bay of Fundy for less than 10 years. In reality, we see that the marine grow-out phase of Atlantic salmon takes on average, 18 months. Both Atlantic cod and halibut have taken no less than 28 months of grow-out within the Bay of Fundy before market. Therefore, based on species differences in cultured growth rates, we predict that slower growing Atlantic cod and halibut will carry higher mercury concentrations as compared to Atlantic salmon, which we saw in this study. In addition, we have data which shows that over an increase in forklength, farmed Atlantic salmon from all of our sites tend to remain consistently low in mercury concentration (with a tendency to decrease) over forklength (Sweeney *et al*., 2006) (Figure 4.4.1).

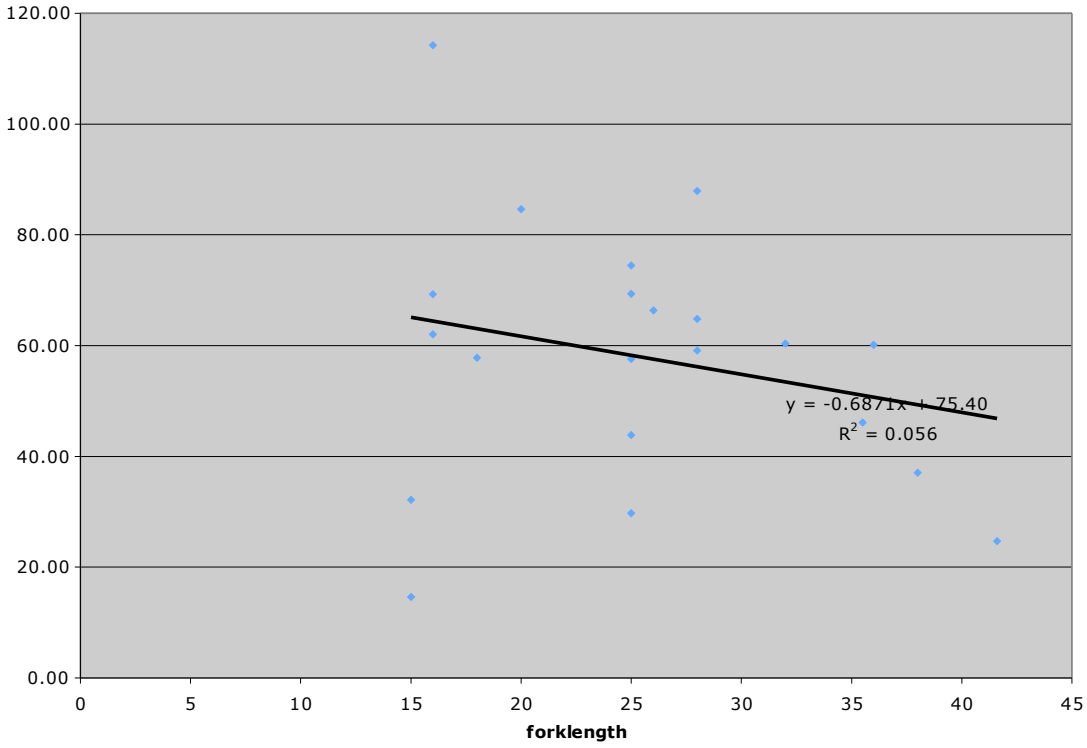

Figure 4.4.1 Comparison of mercury flesh concentrations (ng/g, dry wt) across forklength of farmed Atlantic salmon from the lower Bay of Fundy.

Our simplified mercury mass balance model showed that for the case of Cage 2 on the Marine Aquaculture Site of industry partner 3, mercury input into the system was roughly equivalent to mercury which was taken out, in the form of market fish. When Cage 2 data is extrapolated to an entire site (on average 10 cages), and furthermore, to the entire lower Bay of Fundy (~100 sites), we see that the input of mercury into the entire Bay of Fundy because of the existence of marine finfish aquaculture within the Bay is most likely negligible. This simplified model assumes that mercury comes into the system through two primary routes of smolt and feed and exits in the form of market fish. Therefore, it does not account for feed which is not consumed and assumes a ratio of mercury in feed:mercury in flesh to be 1:1. Patterns from field sampling reveal that sites

which had higher mercury concentrations in flesh showed similar trends in sediment mercury concentrations even though all sites were fed feed of similar mercury concentration. Based on our model and field sampling findings, we predict that sites that consistently overfeed fish will be more likely to show higher concentrations of mercury in sediment because it will not be assimilated within flesh tissue. This would keep mercury within the aquaculture system for extended periods based on current patterns within the site location. Furthermore, in the case of overfeeding, we also predict that those fish which live in conditions of excess feed within sediment below cages, will have an increased opportunity for mercury uptake from the enhanced breakdown of organic material below cages.

This study did not achieve our preliminary goal of quantifying mercury inputs into the finfish aquaculture cycle or in determining whether marketable product may be influenced by increased levels of mercury within the local marine ecosystem. A more thorough study that included larger sample sizes, more consistent sampling and more focused collection and analysis of metadata from growers would have to be conducted in order to accurately quantify this cycle and the influence that the local ecosystem may have on it. Factors such as tidal influence, site characteristics, along with proper control sites would have to be included in order to fully assess whether aquaculture within the lower Bay of Fundy is altering ecosystem mercury cycling patterns within this coastal zone. We saw that although the primary input of mercury into the system was assumed to be through fish feed, similar concentrations of mercury going into fish at different site locations produced large variations within flesh concentrations. We have predicted that

species differences in flesh lipid content and growth rates along with site husbandry (likelihood of overfeeding) and physical site characteristics (current patterns dictating sediment mixing and distribution) are important factors to consider in the assessment of impact that marine finfish aquaculture is having on mercury cycling within the Bay of Fundy.

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Chapter 5.0-Conclusion

5.1 Stated goals

With the rapid development of modern marine finfish aquaculture over the past 30 years, both globally and locally we have seen numerous examples of how the industry has been targeted for its potential impacts on the marine environment and the quality of product produced. In most cases, both government and industry have responded and continue to respond by improving practices and policy. In collaboration with major partners within the finfish aquaculture industry in south western New Brunswick, we have undertaken to determine the potential for mercury uptake in farmed fish and the potential for it to add alternate pathways for mercury into its surrounding ecosystem through fish feed and waste.

5.2 Methods Overview

We undertook three separate studies in order to assess mercury cycling within the lower Bay of Fundy through aquaculture. Our first undertaking was to test for statistically valid comparisons between contaminant levels in wild and farmed fish of the same species, including New Brunswick's newest commercially developed "alternative" species, Atlantic cod. We believe that comparisons between contaminant levels in wild and farmed fish have been erroneous in the past in that species differences have not been accounted for; therefore, we have tested mercury concentrations between farmed and

wild fish of the same species as well as between species of both wild and farmed fish. Food safety and environmental sustainability are major concerns for aquaculture producers in taking responsibility for the safety of the product to be consumed and in ensuring that there will be suitable environments to farm fish in the future.

Aside from inter and intraspecies comparisons, the general goal of this project was to determine and model mercury inputs into and relationships within the finfish aquaculture cycle in southwestern New Brunswick in order to recommend solutions to further minimize mercury inputs and outputs in connection with this food chain. Based on previous studies, we believed that mercury was most likely to enter the finfish aquaculture production cycle by means of fishmeal-based diet (Berntssen *et al*., 2004). Studies had already shown that mercury is also reflected almost immediately in blood, gill and muscle tissue of fish as well (Choi & Cech, 1998). Using feed and growth information collected from growers in addition to controlled laboratory dosage administration, two mass-balance models were developed to help quantify biomass and mercury accumulation and concentrations in finfish aquaculture.

5.3 Results Overview

Mercury concentrations in the flesh and liver of farmed Atlantic salmon were significantly lower than concentrations found in the flesh and liver of wild Atlantic salmon of similar fork-length ($p<0.001$). Mercury concentrations in the flesh and liver of farmed Atlantic cod were not significantly different from concentrations found in the

flesh and liver of wild Atlantic cod of similar fork-length ($p>0.05$). Between species of farmed fish, Atlantic cod were found to be significantly higher in mercury concentration than farmed Atlantic salmon ($p<0.05$). As a side-study, we compared mercury concentrations of lipid-rich tissue to lipid-extracted tissue and found that mercury levels were consistently higher in lipid-extracted tissue. We therefore predict that lipids within lipid-rich tissue act to dilute mercury within that tissue. However lipid-extraction did not account fully for differences in mercury concentrations between farmed and wild salmon (farmed Atlantic salmon fillets are consistently higher in lipid content), suggesting that the rapid growth rate of the former may be resulting in the growth dilution of mercury within the species.

Neither farmed nor wild Atlantic salmon mercury concentrations are at concentrations that trigger consumption advisories according to Health Canada and the USEPA (0.5mg/kg wet weight and 0.1mg/kg wet weight respectively).

We determined that when a dose of mercury is administered directly into the stomach cavity of Atlantic haddock, after being detected immediately within the gut and blood at its highest concentration, mercury is first assimilated within liver tissue within 24 hours in the highest dosage concentration. The next detectable mercury peak is within blood within the next 24 hours. Mercury detected in both liver and blood show a single short-term peak and quick drop to control concentrations. Mercury peaks within fillet within the first four days upon administration and gradually drops over time, however, fillet concentrations take roughly two months to drop back to control levels.

Our overall field sampling revealed that feed mercury concentrations (ng/g) were not significantly different across all six partner sites ($p>0.05$). However, significant differences were seen between flesh mercury concentrations (ng/g) across sites ($p<0.05$). Sediment mercury concentrations were significantly different across sites and time (year to year) ($p<0.05$). By qualitative assessment only, it appeared that on sites in which flesh mercury concentrations were high, sediment below cages tends to be higher in mercury concentration as well.

When empirically tested with Atlantic salmon metadata obtained from growers, our mass balance model predicted that mercury inputs into the Bay of Fundy from aquaculture (in the form of smolt entered and feed fed throughout the 18 month grow out period) was roughly equivalent to mercury output (in the form of harvested fish and mortalities obtained over the 18 month grow out period).

5.4 Limitations Overview

As our first study component, we collected samples of wild and farmed Atlantic salmon and Atlantic cod of various sizes to assess changes in mercury with size and differences between wild and farmed fish. Farmed Atlantic salmon samples of all ages were taken from our six industry participant sites (located throughout the Bay of Fundy) and wild samples were adult and juvenile mortalities taken from the Miramichi River system. This method presents a number of potentially confounding variables in that

farmed Atlantic salmon are under the provincial Aquaculture Act, of the Saint John River strain, while our wild samples are of Miramichi River stock origin and that we were comparing healthy farmed fish to wild mortalities. Our method of controlling for these variables was to look at mercury concentrations across fork lengths of all sizes of fish. We compared the mercury concentrations of the wild mortalities to wild Atlantic salmon mercury concentrations from a population in Newfoundland and found them to be similar; therefore we assumed that the wild mortalities did not die as a result of increased mercury loadings. Farmed and wild Atlantic cod samples were of the same stock origin.

Results from our controlled dosage study revealed quick uptake of mercury first seen within the gut/blood and then within liver and flesh. A limitation which we have found with our own methods was that blood was drawn from the caudal vein of our fish samples. In retrospect, having drawn blood from the hepatic portal vein might have shown a more accurate portrayal of immediate mercury concentrations within the blood. Excess handling of fish also may have played a role in mercury uptake within this study. Stress in the form of handling may have serious implications on growth within laboratory trials and this factor was not controlled for within our study. Upon intubation, we saw a large drop in SGR which is indicative of halted growth (decreased food intake, increased metabolic function) as a result of an environmental stress. This appeared to be corrected within the first four days of sampling and our remaining SGR's were comparable to similar studies (Trippel & Neil, 2003).

Variability associated with our field sampling dataset can be attributed to the inconsistency in data collection and small sample sizes. Thus, statistically significant comparisons were not able to be made within the majority of data due to effects of interaction. Our mass balance model created from industry metadata was taken from one cage that was a part of one Marine Aquaculture Site of the ~100 which are in the lower Bay of Fundy now. Therefore we do not know whether this data is representative of all sites within the industry.

5.5 Recommendations

We conclude from our first study that farmed fish product can be a safe alternative to wild product of the same species in terms of mercury concentration, according to USEPA and Health Canada guidelines. An examination of other contaminants in wild and farmed fish of the same species will be an important future consideration. An assessment of lipid content of both LE and NLE samples through Carbon/Nitrogen ratio analysis is also an important next step to verify how important lipid concentration of fillet may be in mercury uptake (and the subsequent uptake of other contaminants). The comparison of LE and NLE flesh samples of both farmed and wild Atlantic cod would also be beneficial. If our predictions are correct and lipid content of fillet is important in the uptake of mercury, then choosing species to culture in relation to contaminant uptake may become a tool that fish farmers can use to their benefit.

With information gleaned from our controlled dosage study, we have a better idea of how, when and where mercury resides upon consumption within farmed Atlantic haddock. As a result of these studies, we know that mercury that may be consumed may not be cleared completely from muscle tissue for greater than a two-month period. Future research into the metabolism of mercury within other farmed species would be beneficial, potentially strengthening our lipid-dilution predictions.

The field sampling which forms the basis for our conceptual model showed that despite the fact that feed sampled on all sites was found to be similar in mercury concentration, there were differences within flesh and sediment concentrations of fish across the same sites. Based on these findings, we predict that proper husbandry will play an important role in mercury cycling within aquaculture in the Bay of Fundy. Marine Aquaculture Sites which are feeding in such a way that all feed is consumed by fish (and no extra feed enters the cage), we predict have the lowest mercury concentrations of mercury within fish and sediment based on two principals. The first being that fish that are fed well have improved growth rates, this thereby dilutes mercury uptake by gain in body mass. Secondly, when extra feed is not consumed by fish, it is left available to organisms within the vicinity of marine cages to assimilate or to remain available to the farmed fish through mixing of sediments.

We predict that species differences within lipid content of fish, achieved growth rates along with physical site characteristics and feed management are all important factors affecting mercury cycling within the Bay of Fundy.

5.6 References

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Appendix 1

All experimental data from treatment groups for the controlled dosage trial (M=muscle, L=liver, B=blood, G=gut).

		1			2			3			4			5			6		
		19-Aug			22-Aug			25-Aug			28-Aug			31-Aug			07-Sep		
		0	5	10	0	5	10	0	5	10	0	5	10	0	5	10	0	5	10
M	n	14	12	13	14	13	15	15	14	13	14	13	15	15	13	14	14	14	15
	x	88.15	99.02	108.22	88.39	110.53	131.21	79.37	133.03	142.8	80.65	90.15	129.29	77.14	92.18	157.24	84.29	108.04	182.95
	sd	7.99	17.11	23.35	9.44	23.73	22.54	9.36	60.73	39.15	12.8	12.54	35.34	9.53	25.65	65.86	11.68	23.39	89.71
	se	2.14	4.94	6.48	2.52	6.58	5.82	2.42	16.23	10.86	3.42	3.48	9.12	2.46	7.11	17.61	3.12	6.25	23.16
L	n	8	4	6	3	7	2	2	8	3	3	3	3	3	5	6	3	3	3
	x	109.23	283.85	300.58	129.16	245.36	566.38	128.17	198.66	441.61	145.99	232.62	324.81	143.6	150.25	242.99	146.68	128.78	298.41
	sd	22.8	131.91	95.71	27.43	116.9	68.77	33.43	58.92	106.07	25.86	84.95	174.05	37.35	35.63	76.36	36.48	25.95	78.27
	se	8.06	65.96	39.07	15.84	44.18	48.63	23.64	20.83	61.24	14.93	49.05	100.49	21.56	15.93	31.17	21.06	14.99	45.19
B	n	9	3	5	3	3	3	0	0	0	0	0	0	0	2	1	3	3	3
	x	115.83	72.43	235.98	98.65	159.85	141								185.33	56	99.82	167.29	102.32
	sd	96.5	18.45	143.05	62.62	56.71	97.59								115.16		46.98	164.1	50.41
	se	32.17	10.65	63.97	36.15	32.74	56.35								81.43		27.12	94.75	29.1
G	n	6	3	3	3		1					3			1	1	3	3	3
	x	78	294.2	1222.1	75.1		428.6					172.8			129.1	182.1	77.66	119.4	119.7
	sd	35.8	213.7	469	23							46.4					41.4	38	12.1
	se	14.6	123.4	271	13.3							26.8					23.9	22	7

		7			8			9			10			11			12		
		14-Sep			21-Sep			28-Sep			05-Oct			12-Oct			19-Oct		
		0	5	10	0	5	10	0	5	10	0	5	10	0	5	10	0	5	10
M	n	15	11	13	15	10	12	15	13	13	16	11	12	10	14	11	4	6	12
	x	76.76	107.72	130.7	84.08	109.28	156.33	82.11	112.71	151.47	80.15	104.72	126.98	82.91	106.24	150	77.65	98.2	155.07
	sd	9.2	33.6	33.42	13.49	16.46	38.77	7.77	15.76	30.94	11.18	34.68	24.31	9.51	28.18	35.19	4.41	12.76	71.5
	se	2.38	10.13	9.27	3.48	5.21	11.19	2	4.37	8.58	2.79	10.46	7.02	3.01	7.53	10.61	2.21	5.21	20.64
L	n	3	3	7	3	3	5	3	3	4	3	3	5	3	6	7	1	1	6
	x	153.06	240.52	202.07	144.92	187.99	189.85	124.34	200.95	134.61	148.57	155.57	156.2	106.16	178.65	113.69	166.76	176.58	209.9
	sd	38.04	20.32	66.16	9.85	76.72	67.2	17.93	70.73	48.36	23.71	10.87	58.91	66.35	99.58	42.29			111.12
	se	21.96	11.73	25.01	5.69	44.29	30.05	10.35	40.84	24.18	13.69	6.27	26.34	38.31	40.65	15.98			45.36
B	n	1	0	1	3	1	2	3	2	2	1	1	0	0	0	0	0	0	0
	x	41.34		91.84	183.8	19	164.44	172.37	354.05	152.01	34.93	155.1							
	sd				68.25		69.2	61.95	235.56	80.72									
	se				39.4		48.93	35.77	166.57	57.08									
G	n	3	2	3	4	2	2	3	1	1		2							
	x	89.4	68.52	73.8	75.1	82.5	63.5	115.3	106.2	122.6		80.6							
	sd	38.3	43.5	8.7	26.2	15	42	17.3				14.6							
	se	22.1	30.8	5	13.1	10.64	29.7	10				10.34							

Appendix 2

Growth data for all experimental tanks for the controlled dosage trial.

		Sampling											
		1	2	3	4	5	6	7	8	9	10	11	12
Weight (g fish $^{-1}$)	N	39	45	45	45	45	43	44	44	45	43	41	22
	$\bar{x}$	47 ± 2	55 ± 2	61 ± 2	61 ± 2	64 ± 2	67 ± 2	72 ± 3	74 ± 2	76 ± 3	87 ± 4	78 ± 2	81 ± 3
Length (cm fish $^{-1}$)	N	18	45	45	45	45	43	44	44	45	43	41	22
	$\bar{x}$	17 ± 0.2	17 ± 0.2	17 ± 0.2	17 ± 0.2	17 ± 0.2	18 ± 0.2	18 ± 0.2	19 ± 0.2	19 ± 0.2	19 ± 0.2	19 ± 0.2	19 ± 0.6
Liver Weight (g fish $^{-1}$)	N	17	9	9	9	9	9	9	9	9	9		22
	$\bar{x}$	8 ± 0.4	8 ± 0.5	9 ± 0.3	10 ± 1	10 ± 0.7	11 ± 1	10 ± 1	11 ± 0.6	10 ± 1	13 ± 0.9		11 ± 0.5
HIS (% liver:body fish $^{-1}$)	N	17	9	9	9	9	9	9	9	9	9		22
	$\bar{x}$	14 ± 0.3	14 ± 0.4	13 ± 0.4	14 ± 0.4	14 ± 0.5	14 ± 0.4	13 ± 0.5	14 ± 0.4	14 ± 0.7	14 ± 0.5		13 ± 0.4

Appendix 3

Complete database of all total mercury results for six sampling Marine Aquaculture Sites located within the lower Bay of Fundy. Sub sectioned into letter categories: A: fishfeed, B: finfish flesh, C: site effect, D: finfish tissue.

A. site	n/group	species	type	mean	S.E.M.	range
2	7	Halibut	dry	29.7	±4.01	19.4-43.9
2	7	Atlantic salmon	dry	27.1	±3.81	13.6-40.8
1	7	Atlantic salmon	dry	26.7	±4.21	14.8-41.6
4	7	Atlantic salmon	dry	22.6	±4.28	13.1-40.1
6	7	Atlantic cod	moist	36.1	±5.71	17.1-47.2
3	4	Atlantic salmon	dry	20.6	±2.64	14.8-27.5
5	4	Atlantic salmon	moist	27.5	±4.16	17.2-37.6

B. Flesh				
species	n	mean (ng/g)	S.E.M.	range (ng/g)
Atlantic salmon	64	73	±13	14.6-293.1
Atlantic cod	29	167	±11	46.8-514.3
Atlantic halibut	12	236	±160	22.6-1507.1

C. site	location	species	n	mean	S.E.M.
1	Andy's Ledge	Atlantic salmon	12	55	±5.35
2	Dark Harbour	Atlantic salmon	10	97	±13.19
3	Harbour Deloutre	Atlantic halibut	12	330	±118.76
		Atlantic salmon	7	158	±27.52
4	Seal Cove	Atlantic salmon	17	68	±26.8
5	Kelly Cove	Atlantic cod	8	160	±14.23
6	Davidson's Head	Atlantic salmon	5	29	±5.45

D. species	tissue	n	mean (ng/g)	S.E.M.	range (ng/g)
Atlantic salmon	anterior fillet	30	82.7	±6.52	39-173
Atlantic salmon	dorsal fillet	30	83.1	±6.49	52-168
Atlantic salmon	ventral fillet	30	58.4	±7.82	28-200