Spatial and Ecological Patterns of Mercury and Arsenic Concentrations in Pacific Ocean Perch (*Sebastes alutus)* from British Columbia

by

Alison Edwards
BSc, The Open University, Milton Keynes, 2001

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Supervisory Committee

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Dr Marc Trudel, Department of Biology, Fisheries and Oceans Canada
Departmental Member

Dr John Dower, Department of Biology
Departmental Member

Dr John Volpe, School of Environmental Studies
Outside Member

Abstract

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Pacific ocean perch (*Sebastes alutus*) is a slow growing species of demersal rockfish, which inhabits the continental slope of the west coast of North America. This species represents a highly commercial fishery and an important part of the groundfish fishery of British Columbia. A variety of biological factors are known to influence the concentrations of trace metals in fish, including, age, body size and diet; all of which vary in this species. Little is known of the concentrations of total mercury and total arsenic in *Sebastes alutus* from British Columbia, a Canadian province where marine groundfish have previously been found with elevated concentrations of mercury. The spatial variability of trace elements across different fishing regions within British Columbia is also unknown.

Substantial variability in concentrations of both mercury and arsenic has been reported in marine fish from around the globe. In the case of arsenic, associations between concentrations in muscle tissue and a variety of biological variables are not frequently reported. This contrasts with mercury, which is known to be intrinsically linked to the biology of fish. Consequently, biological variability in fish has the potential to confound studies of mercury and possibly arsenic in fish.

This thesis examines the influence of biological variables and fishing region on total mercury and total arsenic in *Sebastes alutus* from British Columbia. It also assesses the concentrations found in muscle tissue from a food safety perspective. Samples were obtained from a commercial fish processing plant on Vancouver Island and had been caught by a commercial fishing vessel in Hecate Strait, Queen Charlotte Sound and the west coast of Vancouver Island.

A multivariate outlier determination method was used to quantify the natural background variability across all three fishing regions. Significant differences in concentrations between regions were identified. This spatial variability of total arsenic did not appear to be related to age, body or body size. However, the spatial variability of mercury concentrations appear to be associated with the size and age of the fish sampled. The outlier determination procedure also identified the presence of elevated concentrations of both mercury and arsenic in specimens that were determined to be outliers. Outliers originated predominantly from the west coast of Vancouver Island and exceeded regulatory limits for both mercury and arsenic. In addition to the outliers, estimated quantities of MeHg frequently exceeded Provisional Tolerable Intakes for children and pregnant women.

Generalized Additive Models were produced to examine the effect of age, body size (length and mass) and diet ($\delta^{13}C$ and $\delta^{15}N$) on concentrations of total mercury and total arsenic in *Sebastes alutus* within each fishing region. These identified significant nonlinear, qualitative patterns between mercury and body size in two out of three fishing regions. A significant nonlinear effect of age on arsenic concentrations was found. Model results also suggest an effect on arsenic concentrations of organic carbon sources;

i.e., fish with stronger links to continental slope or inshore food webs (with relatively enriched $\delta^{13}C$ signatures) had greater arsenic concentrations.

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Dedication

I dedicate this thesis to Morgan Thomas Edwards, with love and a wish for cleaner oceans and sustainable marine fisheries in his life time.

Chapter 1: General Introduction

The main objective of this thesis is to investigate the variability of THg and TAs in Pacific Ocean perch, within the context of the biological variables of this fish, in and across fishing regions of British Columbia.

Regardless of species, fish provide a source of high quality protein, fatty acids, vitamins and minerals when consumed (Sheeshka and Murkin 2002). In addition to their nutritional benefits, human fish consumption is associated with protection from several serious diseases including childhood asthma, plaque psoriasis, colon cancer and coronary heart disease in men (Daviglus *et al.* 2002).

Fish may also contain substances that are less beneficial for human health, such as mercury and arsenic. These are both elements which are ubiquitous in the environment (Hansen and Danscher 1997; Davidson *et al.* 2004; Fulladosa *et al.* 2007) and can occur naturally or as the result of anthropogenic activities (Fergusen and Gavis 1972; Santa Maria *et al.* 1986; Liao and Ling 2003). They are present in many marine organisms, including fish (Edmonds and Francesconi 1993), in which they occur predominantly in their organic (methylated) forms; that is, monomethyl mercury (MeHg) (see Table 1.1 for percentages and citations) and arsenobetaine (Asb) (Table 1.2). Both MeHg and Asb are produced by complex processes involving micro-organisms, which usually occur in the environment (Neff 1997; Boening 2000).

Table 1.1 Review of concentrations of methyl mercury (MeHg) as a percentage of total mercury (THg) reported in muscle tissue of marine fishes

% MeHg in THg	**Fish Species and location**	**Citation**
100	2 species from a Brazilian lagoon	Niencheski *et al.* 2001
>99	15 species of aquatic organisms from N. America	Bloom 1992
92.5 to 97.8	15 species of N. Sea fish	Baeyens *et al.*2003
70 to 100	a variety of edible fish species from the Azores	Anderson and Depledge 1997
>85	sardine and pilchard	Joiris *et al*. 1999
Mean = 83	cod	Lansens 1991

Methylmercury (CH_3Hg^+) is formed by methylation, a biological process that transfers a methyl group (CH_3) from one atom to another (Morel 1998; Thayer 2002). This occurs primarily but not exclusively inside sulfur-reducing bacteria in anoxic sediments (Compeau and Bartha 1985). It then biomagnifies through food webs to organisms such as fish, at upper trophic levels (Hammerschmidt and Fitzgerald 2006).

The scientific understanding of the marine arsenic cycle is incomplete (Pengprecha *et al*. 2005). Two hypotheses have been proposed to explain the origin of Asb in marine organisms. The first states that significant amounts of Asb are present in some species of algae which subsequently provide a source for marine food chains (Grotti *et al.*2008). A second hypothesis involves the decomposition of arsenosugars. Decomposition of arsenosugars by anaerobic bacteria in sediments forms dimethyarsinylethanol or arsenocholine (Edmonds and Francesconi 1987; Neff 1997). This may subsequently be bioaccumulated by benthic and demersal marine animals and converted to Asb, which is then transferred through the marine food. This hypothesis is supported by studies which have identified both arsenocholine and Asb in coastal sediment cores (Takeuchi *et al*. 2005).

Table 1.2 Review of arsenobetaine (Asb) as a percentage of total arsenic (TAs) reported in muscle tissue of marine fishes

Asb as a % of TAs	**Species analysed and location**	**Citation**
>98 in all species	needle fish, tuna, flatfish, hairtail, hake and sardine from Chinese seas	Li *et al*. 2003
Mean = 95	plaice, haddock, catfish, coalfish, cod and herring, from the N. Atlantic Ocean.	Ballin *et al.* 1994
62 – 98 (min – max)	mullet from Lake Macquarie, Australia.	Kirby and Maher 1992
59 – 100 (min – max)	luderick from Lake Macquarie, Australia.	Kirby and Maher 1992
86 – 94 (min – max)	tailor from Lake Macquarie, Australia	Kirby and Maher 1992

In the case of the second hypothesis, activity of anaerobic bacteria in marine sediment appears to be a common feature of the marine biogeochemical cycles of both Hg and As. Specifically, the activity of anaerobic bacteria in marine sediments is associated with production of the chemical forms of both Hg and As which predominate in fish.

Chemical Forms

It is important to understand the chemical forms of Hg and As, which are predominantly found in fish, because this affects the way that they interact with biological organisms. The pathways of assimilation and accumulation in biota differ between the chemical forms, as do the toxicological implications. The way that these elements are assimilated and accumulate, contributes to the patterns of their concentrations in fish.

In general, the scientific literature describing the differences between the chemical forms of As and their interactions and effects in fish is relatively scarce in comparison to that of Hg. This may be due to the complex procedures that are required for its determination (Santa Maria *et al*. 1986). Arsenic also occurs in several different organic and inorganic forms, which makes understanding and predicting its bioaccumulation and toxicity difficult and complicates its geochemistry (Meador *et al.*2003). Thus, the factors that control its bioavailability are less well known (Meador *et al.* 2003)

Assimilation

Assimilation of trace elements from food can be thought to result from the passage of the trace element across the gut lining during digestion. Therefore, assimilation efficiency represents the fraction of ingested element that remains in the tissues of an animal after it has emptied its gut of undigested material (Reinfelder *et al.*

1998). This is a physiological process, which can be quantitatively compared among trace elements and animal species under diverse environmental conditions.

Mercury

Each chemical form of Hg is assimilated with different efficiencies. Several experimental studies have shown that MeHg has both a trophic transfer factor (TTF) and assimilation efficiency (*AE*) higher than inorganic Hg in fish (Moore and Sutherland 1981; Boudou and Ribeyre 1985; Riisgard and Hansen 1990; Wang and Wong 2003). This is likely a consequence of the high specificity of the intestine wall toward MeHg absorption and a very low uptake rate of inorganic mercury (Boudou and Ribeyre 1985).

Arsenic

It is possible that arsenic behaves in a specific manner similar to mercury, where marine organisms accumulate arsenobetaine selectively over other forms of arsenic present in marine sediments and waters (Francesconi *et al*. 1989). Observations of fish that consume arsenocholine, yet still retain the majority of their arsenic as arsenobetaine (Francesconi *et al*. 1989) suggest that arsenocholine is converted to arsenobetaine readily in fish, possibly in the gut tract.

Another mechanism that has been proposed for the specific assimilation of arsenobetaine by invertebrates involves glycine betaine, a molecule involved in the osmoregulation of marine invertebrates (Gailer *et al*. 1995). Structurally, glycine betaine is similar to that of arsenobetaine, and it varies in concentration depending on the salinity of the ambient waters (Larson and Francesconi 2003). Efficient assimilation from seawater of Asb in the blue mussel (*Mytilus edulis)* has been observed to decrease in the presence of glycine betaine (Gailer *et al*. 1995). This observation suggests that Asb and glycine betaine compete for the same transporter system in marine invertebrates (Gailer *et al*. 1995). Fish accumulate As predominantly via their diet and hence reflect the concentrations of their prey; therefore, concentrations of As in marine fish also reflect the salinity of their environment via the salinity of their prey (Francesconi and Edmonds 1993; De Gieter 2002). This hypothesis is supported by the finding of a positive linear relationship between arsenic concentration and salinity in cod, flounder and herring in the Baltic and North Seas (Larson and Francesconi 2003).

Accumulation

An organism's ability to accumulate a contaminant is linked to its ability to excrete or store it (Bryan 1979). Bioaccumulation occurs when the rate of uptake exceeds that of elimination (National Academy of Sciences 1978; Trudel and Rasmussen 1997).

Mercury

Most of the methylmercury in fish tissue is covalently bound to sulfhydryl groups of proteins (Global Mercury Assessment Working Group 2003) and elimination is very slow relative to the rate of uptake (Hoffman *et al*. 2002) which leads to accumulation. Methylmercury is excreted via the bile through the liver in mammals (Naganuma and Imura 1984). Methylmercury in the bile is predominantly bound to glutathione and approximately 90% is reabsorbed in the intestine (Naganuma and Imura 1979; Clarkson 1993). This eliminates inorganic mercury, whilst MeHg is re-circulated and absorbed. This is known as enterohepatic recirculation (Riisgard and Hansen 1990; Bjerregaard *et* *al.*1998) which results in accumulation of mercury (primarily methylmercury) within cells of fish (Storelli *et al*. 2002).

Arsenic

Arsenobetaine is present in most major organs of fish (Maher *et al*. 1999; Kirby and Maher 2002; Amlund *et al*. 2006), with the highest retention being in muscle tissue (Francesconi *et al*. 1989; Amlund and Berntssen 2004). This high concentration in muscle tissue relative to other body sites is indicative of a high capacity of muscle to absorb and store arsenobetaine; thus contributing to its accumulation in fish (Amlund *et al*. 2006). In contrast to methylmercury, auto radiographs indicate that arsenobetaine does not covalently bind to macromolecules in the muscle (Amlund *et al*. 2006).

Toxicity

Mercury

Both MeHg and inorganic Hg^{2+} interfere with calcium channels, disrupting their function. This leads to apoptosis and nephro- and hepatotoxicity (Broniatowski and Dynarowicz-Łatka 2009). MeHg is far more toxic than inorganic forms of Hg, due to its additional ability to cross biological barriers. The brain of vertebrates is protected by a biological barrier. This barrier, known as the Blood Brain Barrier (BBB), prevents passive diffusion of solutes from blood into the extracellular space of the central nervous system (CNS) (Janzer and Raff 1987). The BBB is not impenetrable, and substances that are lipophyllic are able to enter the CNS (Zheng *et al.* 2003). However, free MeHg is rarely present in biological fluids because it has a high affinity for sulfhydryl groups. This ensures that MeHg in blood is almost exclusively bound to sulfhydryl-containing compounds, including proteins or amino acids (for example cysteine) (Zheng *et al.* 2003).

The BBB allows highly selective entry to large neutral amino acids via an endothelial carrier known as the L-system amino acid carrier (Reichel *et al*. 2000). When MeHg binds to cysteine it has a structure that is very similar to that of methionine, which is a large neutral amino acid (Clarkson 1993; Zheng *et al.* 2003). This similarity enables the MeHg–cysteine complex to be transported across the BBB by the L system amino acid carrier (Zheng *et al*. 2003).

Once inside the CNS, MeHg is neurotoxic (Environment Agency, Japan 1975; WHO 1989, 1990). Effects of this toxicity include mental disturbances, abnormal sensations, reduced visual fields, hearing loss, disturbances of gait, tremors, and weakness (Clarkson 1993; Rice and Gilbert 1995). Transport of the MeHg–cysteine complex via L-type neutral amino acid carriers also enables MeHg to cross the placenta and enter fetal blood plasma. This causes a wide range of embryo-toxic and feto-toxic effects, from severe cerebral palsy to subtle developmental delays (Clarkson 1993; Kajiwara *et al*. 1996). The developing nervous system of a fetus is the most sensitive toxicological endpoint of Hg in humans.

Arsenic

Inorganic As and Asb have very different toxicities (summarized in Table 1.3). Inorganic As is both a carcinogen and a toxin (Shi *et al*. 2004) and is considered to be increasingly important as an environmental concern (Goyer *et al*. 1995; Han *et al*. 2003). Asb is rapidly metabolized and excreted in urine without any toxic effects in humans or other organisms (Borak and Hosgood 2007).

Table 1.3 Table showing the relative toxicity of the various chemical forms of arsenic

High Toxicity	• arsines (trivalent, inorganic or organic);
	• arsenite (inorganic);
	• arsenoxides (trivalent with two bonds joined to one oxygen, e.g. R-As = O where R is an alkyl group);
	• arsenate (inorganic);
	• pentavalent arsenicals such as arsenic acids;
	• arsonium compounds (four organic groups with a positive charge on the arsenic - akin to arsenobetaine $CH_3{}^+As(CH_3)^{2-}$ CH_2-$CO^-{}_2$)
Low toxicity	• metallic arsenic.

Regulatory Limits and Tolerable Intake

The risks associated with sub-clinical concentrations of MeHg exposure remain uncertain. Consumption of fish is believed to be the major source of human exposure (Larsen *et al*. 2002; Mahaffey 2009). To protect public health, international organizations and the governments of many countries have set limits on the amount of mercury that is acceptable in fish destined for human consumption. In Canada the guideline for THg in commercially-sold fish is 0.5 with the exception of three piscivorous fish, namely, shark, swordfish, and fresh/frozen tuna. These fish regularly exceed 0.5 ppm and are subject to a consumer advisory. The advisory (last re-issued in 2002) recommends that the general adult population limit consumption of these fish to one meal per week. Additionally Pregnant women, women of child-bearing age and young children are recommended to limit consumption to no more than one meal per month.

Public health regulations do not always distinguish between the different chemical forms of arsenic found in fish and other marine organisms, despite their different toxicities (Neff 1997). The Canadian Food and Drugs Act, for example, states in section B.15.001., "Fish are adulterated if arsenic is present therein in an amount exceeding 3.5 μgg^{-1}". Several countries have regulations for the maximum permissible concentration (MPC) of arsenic in seafood consumed by humans (Neff 1997), ranging from 0.1 μgg^{-1} (Venezuela) to 10 μgg^{-1} (Hong Kong). In some countries, the MPC for As is for TAs; in others, it is for inorganic As only (Edmonds and Francesconi 1993; Neff 1997). The rating of marine foods according to their TAs content, which includes the non-toxic Asb, may involve an overcritical assessment (Ballin *et al*. 1994). The WHO and the FAO of the United Nations (World Health Organisation and Food and Agriculture Organization) work together as the Joint Expert Committee on Food Additives (JECFA) to provide a Provisional Tolerable Weekly Intake (PTWI) for Hg and inorganic arsenic in food.

Provisional Tolerable Weekly Intake (PTWI)

The PTWIs produced by the JECFA, are widely used exposure limits for food contaminants considered to be tolerably safe (such as heavy metals). This provides a permissible weekly intake level for many contaminants. The term tolerable is used because it signifies permissibility, rather than acceptability, for the intake of a contaminant that is difficult to avoid because it is associated with certain foods. The assessment of the Joint Expert Committee takes into account possible daily variations, so that consumption of food containing above average levels of the contaminant, on any particular day, may exceed its proportionate share of the weekly tolerable intake (Herrman and Younes 1999). The Committee also agrees that it is impossible to make generalizations concerning the length of time during which consumption of heavy metals such as Hg in excess of the PTWI would be harmful. The approach for establishing acceptable or tolerable intake of substances exhibiting thresholds of toxicity are described by Herrman and Younes. There are many examples of the use of PTWIs for THg and TAs as useful toxicological tools. When combined with consumption quantity, concentration of contaminant and body size of the consumer, they can be used to estimate if levels of exposure are safe (Tressou *et al.* 2004). Recent examples of studies that have applied the PTWI to seafood include (Wilhem *et al*. 2003; Ikem and Egieber 2005; Storelli *et al.* 2005; Endo *et al.* 2007; Sahuquillo *et al*. 2007; Szefer *et al*. 2008).

Concentrations of THg and TAs in ocean fishes, from around the globe

Wide ranging concentrations of mercury and arsenic in marine fishes are a natural phenomenon. The scientific literature includes many examples from diverse parts of the world's oceans, with concentrations ranging from low, to those that greatly exceed regulatory limits. This includes fish with THg concentrations from 3.6 to 18.7 μgg^{-1} (Table 1.4) and TAs concentrations from 0.2 up to 64.0 μgg^{-1} (Table 1.5). Species with reported concentrations that greatly exceed the THg guidelines (Table 1.4) are all examples of large, predatory, long lived ocean fish. This illustrates the current knowledge and understanding of the patterns of Hg in fish. Biological characteristics such as trophic position, size and age are intrinsically linked to concentrations of MeHg (Storelli 2002; Adams 2004).

Some of the intrinsic biological characteristics that influence the THg concentrations of fish include body size, age, sex, growth rate, feeding habits and food web structure. Therefore, the biological variability associated with the species themselves can in part result in variation of THg concentrations in fish muscle (Licata *et al.* 2005). However, despite the wide variation in TAs concentrations that is also expected in accordance with physiological, environmental or dietary changes, the effects of biological variation on TAs concentrations have been less well documented in marine fishes and are still under discussion (De Gieter 2002).

Table 1.4 Summary of examples of THg concentrations (μgg^{-1}) in fish from the worlds oceans, reported in the scientific literature

Species	Ocean	THg w/w Min – Max	THg w/w Mean (s.d)	Citation
Blue marlin, *Makaira nigricans*	N. Atlantic	4.95 – 18.72	10.52 (5.03)	Cai *et al*. (2007)
Ghostshark, *Chimaera monstrosa*	Mediterranean	1.3 – 5.16	3.1 (1.4)	Storelli *et al.*(2002)
Night shark, *Carcharhinus signatus*	S. Atlantic		1.8	Pinho *et al*. (2002)
Lesser spotted dogfish, *Scyliorhinus canicula*	N Sea		0.6 (0.2)	Baeyens *et al.*(2003)
Albacore tuna, *Thunnus alalunga*	N. Atlantic	0.22 - 1.13	0.4	Anderson and) Depledge (1997)
Rockfish, *Helicolenus dactylopterus*	N. Atlantic	0.04 – 1.10	0.3 (0.0)	Monteiro *et al*. (1991)
Rockfish, *Helicolenus dactylopterus*	N. Atlantic	0.12 – 0.70	0.3	Anderson and Depledge (1997)
Sardine, *Sardina aurita*	Mediterranean	0.19 – 0.40	0.3	Joiris *et al.*(1999)
var. commercial species	N. Adriatic	0.02 – 1.05	0.2 (0.1)	Ghidini *et al.*(2003)
Dover Sole, *Solea solea*	N.E. Pacific		0.1 (0.1)	Teeny *et al.*(1984)
Dolphin fish, *Coryphaena hippurus*	N. Atlantic	0.01 – 0.49	0.1 (0.09)	Cai *et al.*(2007)
Plaice, *Pleuronectes platessa*	N Sea		0.045 0.023	Baeyens *et al.*2003
Greater lizardfish, *Saurida tumbil*	Caspian Sea	0.012 - .086	0.043 (.032)	Agah *et al.*2006
Blue jack mackerel, *Trachurus picturatus*	N. Atlantic	0.020 - 0.144	0.043	Anderson and Depledge 1997
Greasy grouper, *Epinephulus. tauvina*	Arabian Gulf	0.029 (0.003)	0.025 – 0.032	Al-Saleh and Shinwari 2002
Atka mackerel, *P. monopterygius*	N.E. Pacific		0.014(0.004)	Teeny *et al.*1984

Biological variation associated with THg and TAs concentrations in marine fishes

Body Size

Mercury:

Results from studies of wild marine fish, indicate that THg concentrations in muscle tissue have a positive relationship with body size (measured interchangeably by either length or weight) (Baeyens *et al.* 2003). This is well documented and represents a typical process of mercury accumulation (Storelli *et al.*2007). Several authors have observed positive relationships (usually linear or exponential) suggesting that the rate of Hg accumulation may be closely tied to growth; i.e., as fish grow they accumulate Hg (Baeyens *et al*. 2003; Cai *et al*. 2007). The relationship for growth is similar to the relationship observed between age and THg (Storelli *et al.*2002; Adams 2004).

Table 1.5 Summary of examples of TAs concentrations in marine fish from the scientific literature

species	Location	TAs w/w Range (ppm)	TAs w/w (ppm) Mean (s.d)	Citation
Dogfish, *Scyliorhinus canicula*	North Sea and Channel (N Atlantic)	21.3 – 64.0		De Gieter (2002)
Plaice, *Pleuronectes platessa*	E. Shetland (N. Atlantic)	16.6 – 55.7	32.0 (14.4)	Ballin *et al.* (1994)
Black- spotted rubber lip, *Pletrohynchus gaterinus*	Arabian Gulf	14.4	9.95 (7.5)	Attar *et al*. (1992)
School whiting, *Sillago bassensis*	Indian Ocean (Western Australia)	3.2 - 14.5	7.7 (2.97)	Edmonds and Francesconi (1981)
A variety of commercial fish	N. Adriatic Sea (Mediterranean)	0.2 – 33.1	5.4 (4.6)	Ghidini *et al.*(2003)
trumpeter whiting, *Sillago maculata*	Indian Ocean (Western Australia)	1.2 – 4.9	2.4 (0.9)	Edmonds and Francesconi (1981)
hake	East Sea, China		0.8 (0.03)	Li *et al. (*2003)
red fish	Yellow Sea, China		0.3 (0.01)	Li *et al.* (2003)

Arsenic:

The relationship between body size and muscle TAs concentrations in marine fish is less clear. Some studies have found positive correlations between TAs and weight in some species; such as, tunas from the Arabian Sea, *Thunnus thynnus* and *Thunnus toggel,* (Ashraf and Jaffar 1988) and school whiting, *Sillago bassensis*, from Western Australia (Edmonds and Francesconi 1981). Yet this relationship was not detected in other species, such as trumpeter whiting, *Sillago maculata* (Edmonds and Francesconi 1981). Another study has shown a significant negative correlation between mass and arsenic concentration in sea mullet, *Mugil cephalus*, from New South Wales, Australia (Maher *et al*. 1999). The available literature indicates that the association between size and TAs concentrations varies and that body size appears to explain little variation in TAs concentrations in fish (Maher *et al*. 1999).

Age and the 'life span effect'

Mercury

Large fish with elevated concentrations of THg are often species with long life spans and fish with long life spans have a tendency to exhibit high MeHg levels (Kelly *et al*. 2008). Comparisons of THg concentrations in large predatory fish with shorter life spans (typically 4 – 10 years contrasted with > 20 years for the longer-lived species); suggests that a long life span is necessary for fish to accumulate very high concentrations of THg (Cai *et al*. 2007). This is commonly referred to as the "life span effect" (Cai *et al*. 2007). This is similar to the concept that fish with lengths close to the maximum size for their species are older and have accumulated Hg for a longer time than shorter and therefore younger fish (Storelli *et al*. 2002). Age influences mercury concentrations (Braune 1987; Baeyens *et al.* 2003). Older fish tend to have higher concentrations of THg within a species (Riisgard and Hansen 1990; Monterio *et al*. 1991; Joiris *et al*. 1999; West *et al.* 2001). This relationship occurs because uptake exceeds elimination, so that over time, THg accumulates in the tissues of fish (Riisgard & Hansen 1990). Again, models that describe the balance between uptake, elimination, and growth show that as fish age, Hg concentrations may increase. Similarly, because elimination of mercury occurs slowly, bioaccumulation will extend for long periods of time before steady-state is attained, thus the phenomena is accentuated in the longest lived organisms.

Arsenic

There is limited data to illustrate the relationship between arsenic and age in marine fish. One of the few studies to report these results found that age does not influence arsenic concentration in sea mullet from NSW, Australia (Maher *et al*. 1999)

Prey

Mercury

Most of the THg in fish muscle is transferred to fish through their food (Niencheski *et al.* 2001) and the importance of dietary sources of mercury to the concentrations of THg in fish tissue is widely recognized (Phillips *et al.* 1980; Braune 1987; Storelli *et al.*2002, 2007). Benthic fishes or fish which feed mainly on benthic organisms, generally are long lived in the marine environment (Cailliet *et al*, 2001) and tend to accumulate high concentrations of THg (Storelli *et al.* 2002; Ghidini *et al.* 2003).

Arsenic

The accumulation of As in fish is considered to be predominantly through their diet and to be directly related to their food source (De Gieter *et al*. 2002). Benthic marine organisms may contain higher concentrations of arsenic than their pelagic counterparts

(Neff 1997) and a study of arsenic in North Sea fish found that, in general, species that feed on benthic organisms and small fish contain more arsenic than fish that feed on larger fish (De Gieter *et al*. 2002). It is possible that in this habitat, some ingestion of sediments occurs.

Food web structure

Mercury

Biomagnification is the transfer of a xenobiotic chemical (a substance that is foreign to the body) from food to an organism, which results in generally higher concentrations within the predator than the prey (Connell 1989; Connell 1990; Rand *et al*. 1995; Gray 2002). Mercury, unlike most metals is not regulated or excreted. This enables concentrations to increase in predators and is why mercury is the only metal known to biomagnify (Gray 2002). This explains why carnivorous fish are associated with higher THg concentrations than herbivorous species (Storelli *et al*. 2002).

Arsenic

In contrast to mercury, arsenic does not biomagnify (Edmonds and Francesconi 1981; De Gieter *et al*. 2002) and has been reported to biodiminish. That is, animals relatively higher in food chains are predicted to carry lower burdens than animals at the bottom (e.g. predators < prey; piscivores < planktivores) (Wagemann *et al.* 1978).

Stable Isotope Analysis

The effect of food web structure on concentrations of contaminants can be investigated with stable isotope analysis. In aquatic ecology, stable isotopes are useful tools used to quantify feeding relationships in aquatic food webs (Lajtha and Michener 1994). Trophic transfer is the transfer of material from predator to prey (Swartz and Lee 1980). During this process, the ratios of stable isotopes of carbon and nitrogen are altered. This is known as trophic-step fractionation (Vander Zanden and Rasmussen 2001; Sweeting *et al*. 2007). Knowledge of the way in which these isotopes are transferred through food webs, enables their use as a tool to explore feeding relationships in aquatic systems (Vander Zanden and Rasmussen 2001; Sweeting *et al.* 2007).

Trophic-step fractionation of naturally occurring stable isotopes of nitrogen (^{15}N and ^{14}N) takes place during the production of metabolites. The nitrogen contained within proteins is assimilated as food by a variety of digestive processes. These processes include amino acid deamination and transamination, which produce metabolites. The production of these metabolites utilizes the lighter nitrogen isotope (^{14}N) (Mill *et al*. 2007) which is removed during nitrogenous excretion via urine (Ponsard and Averbuch 1999). Therefore, the remaining tissue that is assimilated contains nitrogen that is enriched in ^{15}N relative to ^{14}N. The nitrogen derived from animal tissue is dominated by proteins. This type of fractionation, referred to as 'metabolic fractionation' (Gannes *et al*. 1997) is considered to be the dominant fractionation process in carnivores (Mill *et al.* 2007). Alternatively, 'assimilative fractionation', which is fractionation resulting from isotopic discrimination during nitrogen assimilation also occurs. For herbivores, fractionation of nitrogen isotopes are likely to be affected by both metabolic and assimilative processes (Vander Zanden and Rasmussen 2001).

At each trophic transfer, the predators' tissues are enriched in ^{15}N relative to ^{14}N. The ratio between the two isotopes is highest in organisms feeding at higher trophic positions. Laboratory experiments have shown that the enrichment in ^{15}N occurring at each trophic transfer is predictable (DeNiro and Epstein 1981). Comparing the ratio of stable isotopes to a standard in the following equation produces stable isotope ratios.

$$\delta X = \frac{R_{sample} - R_{standard}}{R_{standard}} \text{x} 1000$$

Equation 1

Where R = $^{15}N/^{14}N$ or $^{13}C/^{12}C$

This ratio (or signature) is expressed using delta (δ) notation, with units of parts per mille (‰).

The increase in $\delta^{15}N$ that occurs with each trophic transfer is referred to as the trophic shift ($\Delta\,\delta^{15}N$). In aquatic food webs, the average $\Delta\,\delta^{15}N$ is an enrichment of 3 – 4 ‰ for each step of trophic transfer (Vander Zanden and Rasmussen 2001). The predictability of $\Delta\,\delta^{15}N$, enables $\delta^{15}N$ signatures of organisms to be used to investigate the relationship between contaminants and vertical food web structure in both freshwater and marine food webs (Spies *et al*. 1989; Broman *et al.* 1992; Kidd *et al.* 1995, Atwell *et al*., 1998; Bowles *et al*. 2001; Power *et al*. 2002). The $\delta^{15}N$ values of consumers are generally subtracted from the $\delta^{15}N$ of primary producers (baseline), in order to estimate trophic position.

Caution should be exercised when comparing $\delta^{15}N$ values between food webs, since baseline $\delta^{15}N$ values may vary from system to system (Cabana and Rasmussen 1996). For this reason, the use of $\delta^{15}N$ values of consumers without a baseline is not sufficient to infer trophic position (Post 2002; Vander Zanden and Rasmussen 2001). Despite this, $\delta^{15}N$ data without a base line has value in food web studies of contaminants, if the organisms are part of the same food web (Atwell *et al*. 1998). In this case, comparisons of consumer $\delta^{15}N$ signatures provide a measure of relative trophic position. This relationship can be utilized to look for correlations between concentrations of contaminants and relative trophic position in the food web in question. Stable isotopes of carbon (^{12}C and ^{13}C) are also useful tools for examinations of trophic pathways in aquatic food webs. The stable isotopes of carbon are fractionated by primary producers during the carbon fixation step of photosynthesis (Emerson and Hedges 2008). This step leads to a ratio of ^{12}C to ^{13}C that is variable. The stable isotope ratios can be compared to a standard in Equation 1.

The variability of $\delta^{13}C$ values of primary producers is the result of physical and chemical variables that alter phytoplankton growth rates (Schell 1998) including salinity, photoperiod (Leboulanger *et al*. 1995), nutrient supply, aqueous CO_2 concentration and temperature (Rau *et al*. 1997). Unlike isotopes of nitrogen, the effect of trophic transfer on the ratio of carbon isotopes is negligible (Chisholm *et al.* 1982; Rau *et al*. 2001). Consequently, the $\delta^{13}C$ values of consumers reflect the isotopic signatures of the primary producers at the base of their food chains. They can, therefore, be used to investigate the influence of horizontal food web structure on contaminant concentrations in fish; i.e., the effect on contaminant concentrations of any dietary shift between prey items at a similar trophic level, linked to food chains with different primary producers (Lindqvist *et al.* 1991; Power *et al.*2002). Off the west coast of North America, the $\delta^{13}C$ of primary producers varies (Schell *et al.* 1998; Kline 1997; Perry *et al.* 1999). These variations are linked to conditions such as upwelling of nutrient rich water (Rau *et al*. 2001), continental shelf versus slope/deep ocean environment (Perry *et al*. 1999), or the difference between a terrestrial or marine derived carbon source (Chisholm *et al.* 1982).

Spatial Variability

In addition to biological variability, spatial variation also appears to be an important determinant of concentrations of Hg and As in marine fishes. Although these elements are reportedly ubiquitous in the environment, they are not evenly distributed around the globe. The scientific literature includes reports of Hg and As concentrations in marine fishes throughout the worlds oceans (Tables 1.4 and 1.5) which illustrate variability both between ocean basins and within different regions of basins. High Hg concentrations are often reported in fishes from the Mediterranean Sea, which is a region with large cinnabar deposits (a natural ore of mercury) beneath the ocean crust (Ferrara *et al.*1999). Similarly, some of the highest concentrations of arsenic ever reported in fish have been from the Middle East, particularly the Gulf of Aden (Attar *et al*. 1992; Fowler *et al*. 1993; Madany *et al*. 1996). This is a region with large petrochemical deposits.

The west coast of British Columbia supports many commercial fisheries, yet little is known about the variation in mercury and arsenic concentrations in fish from different commercial fishing regions. Pacific ocean perch is an important part of the rockfish fishery in British Columbia, in terms of total volume. In 2002, the landed value of this species was $30.6 million, with $1.2 million generated in exports (DFO 2002). Elevated concentrations of heavy metal contaminants could pose a risk to this commercially important resource.

This study produced data on mercury and arsenic concentrations in *Sebastes alutus* from three coastal fishing regions of British Columbia; Hecate Strait, Queen Charlotte Sound and the west coast of Vancouver Island (Figure 2.0). Chapter one is a general thesis introduction which aims to provide substantial background knowledge of both Hg and As in marine fish, by a comparative review of the scientific literature relating to these elements and their concentrations in marine fish. Chapter Two investigates variability of mercury and arsenic concentrations in Pacific ocean perch from different fishing regions. Spatial variability is examined as patterns of concentrations from different fishing regions and these are compared to spatial patterns of several biological variables. Exposure scenarios of spatial patterns are also included, which investigate potential for human health risk from different regions.

Chapter Two also examines several hypotheses. The null hypothesis, that there is no effect of location on the mercury and arsenic concentrations of Pacific ocean perch, and the alternative hypothesis that location does influence these concentrations. The hypothesis that spatial variability reflects the biology of the fish is also considered. In addition, spatial variability of THg and TAs is examined in the context of internationally recommended intake levels and potential health concerns, which are also compared between fishing regions.

Chapter Three considers the variation in THg and TAs concentrations within each fishing regions through quantitative inference, using generalized additive models (GAMs) (Hastie and Tibshirani 1990). This approach uses patterns of concentrations to examine the relationship between concentrations of these elements in fish muscle and a range of biological parameters of fish. These include age, size and stable isotope ratios of Carbon and Nitrogen.

Chapter 4 discusses the contribution that the findings of this thesis make to the knowledge and understanding of mercury and arsenic in fish. Additional avenues for further research relating to the biotic component of the cycles of Hg and As in British Columbia are indicated.

Chapter 2: Spatial variability of mercury and arsenic concentrations in *Sebastes alutus* (Pacific ocean perch) from British Columbia

2.1 Abstract

Biological variables such as age, body size and food web structure are known to influence concentrations of trace elements in fish. Pacific ocean perch are an important part of the commercial groundfish fishery in British Columbia. This species has a varied diet, long life span and a wide variety of potential body sizes. However, the variability of concentrations of mercury and arsenic in fish from different fishing regions is unknown. This study uses a multivariate outlier identification procedure to assess the natural background variability across three fishing regions, Hecate Strait, Queen Charlotte Sound and the west coast of Vancouver Island. These concentrations were compared between fishing regions to identify significant differences in concentrations, biological variables and potential food safety. Elevated concentrations of both mercury and arsenic in specimens were identified as outliers. These specimens originated predominantly from the west coast of Vancouver Island and exceed regulatory limits for both mercury and arsenic.

Comparisons of samples identified as inliers; i.e., within the natural background variability, indicated that the Hecate Strait fishing region had samples with the highest mean mercury concentrations and lowest arsenic concentrations. Samples from this region were relatively older and longer. Estimated MeHg concentrations for a single serving from this region frequently exceeded Provisional Tolerable Intakes for children and pregnant women. Several fish were found to have concentrations of total arsenic

which exceeded regulatory limits. However, exposure scenarios based on estimated inorganic arsenic intakes, indicate that consumption of arsenic from Pacific ocean perch alone is well below tolerable weekly intakes. The spatial variability of arsenic concentrations did not appear to be influenced by the biology of the fish sampled.

2.2 Introduction

This study compares variability in biological variables, with an emphasis on the spatial variability of the concentrations of these elements in Pacific ocean perch within British Columbia. Mercury and arsenic are elements present in marine environments, both as a result of natural and anthropogenic processes. Both elements have multiple chemical forms, at least one of which is toxic. Seafood represents the primary route of human exposure to these metals (Storelli and Marcotrigiano 2004) and the presence of potentially harmful elements in edible tissues of commercial fish has important implications for food safety. In turn, this has repercussions for the market value of specific species (Forrester *et al.*1972;Debruyn *et al*. 2007), some of which (including Pacific ocean perch) are an economically and culturally important resource in British Columbia (DFO, 1999).

Several previous studies have shown a relationship between mercury concentrations and area of catch along the west coast of North America (Hall *et al*. 1976a, 1976b; Cutshall *et al*. 1978). However, these studies investigated large scale spatial variability in terms of latitudinal trends, in fish ranging from the Gulf of Alaska to Southern California. They did not examine spatial variability of Hg concentrations at a smaller spatial scale (such as coastal regions within British Columbia) which is currently unknown. The spatial variability of As concentrations in coastal marine fish from British Columbia is also poorly understood. The objective of this chapter is to investigate variability of THg and TAs in Pacific ocean perch, from different fishing regions, within British Columbia. For this purpose, I examine a variety of hypotheses relating to spatial variability.

Firstly, by examining the extent to which the concentrations of these elements vary spatially, I will be testing the hypothesis that the concentrations of THg and TAs in Pacific ocean perch differ between fishing regions. As discussed in chapter one, earlier investigations of mercury in marine fish indicate that biological factors influence their concentrations. The influence of biology creates expected patterns of concentrations, which may be useful for prediction and monitoring purposes. The biological factors associated with mercury concentrations include body size (Baeyens *et al.* 2003; Storelli *et al.* 2007), age (Braune 1987; Riisgard & Hansen 1990; Monterio *et al*. 1991; Baeyens *et al.* 2003) and trophic position (Jarman *et al.* 1996; Atwell 1998; Storelli *et al*. 2002). These patterns have been reported for a wide range of both freshwater and marine fishes from around the world. There is a pattern that long lived, predatory fish are associated with relatively high concentrations of mercury (Table 1.4). Therefore a second hypothesis, that spatial patterns of mercury concentrations in Pacific ocean perch reflect differences in age and body size, is also considered.

Similar associations between arsenic and the biology of fish are less studied and the results are inconsistent between species (Edmonds and Francesconi 1981; Ashraf and Jaffar 1988; Fattorini *et al.*2006). For example, tunas from coastal Pakistan (Ashraf and Jaffar 1988) and school whiting from Western Australia (Edmonds and Francesconi

1981) showed strong positive associations between weight and arsenic concentration. However, trumpeter whiting from Western Australia and orange spotted grouper, *Epinephelus coioides*, from the Gulf of Oman did not (Edmonds and Francesconi 1981; de Mora *et al.* 1994). Significant interspecific variability in the concentrations of arsenic in fish has been reported, and species-specific characteristics appear to have a greater influence on arsenic bioaccumulation, than geographic location (Gibbs and Maskiewicz 1995; Fattorini *et al.* 2006). Evidence for this comes from reports of highly significant differences in arsenic concentrations between fish species from both the Caribbean and the Mediterranean (Fattorini *et al.* 2006) and also observations of high arsenic concentrations in elasmobranch species compared to other fish worldwide (Gibbs and Maskiewicz 1995).

The evidence of associations between biological variables and arsenic concentrations in fish is limited. This suggests that patterns of spatial variability in arsenic concentrations will not be clearly associated with age and body size. Hence they may be dissimilar to the spatial patterns of mercury. This chapter also considers the hypothesis that patterns of concentrations of TAs from different fishing regions do not reflect the age and body size of the fish sampled.

Pacific Ocean Perch

Coastal British Columbia supports many commercial fisheries, including Pacific ocean perch. This is a culturally and economically important species in British Columbia (Schnute *et al.* 2001) and represents an important part of the total volume of the rockfish fishery (DFO 2002). Pacific ocean perch has a varied diet, a long life span and does not undertake large coast wide migrations, making it is a good choice of species for a study incorporating spatial and biological variability and contaminant concentrations. It is possible that the measurement of $\delta^{13}C$ in Pacific ocean perch may be a useful tracer to investigate a potential relationship between THg and TAs concentrations from different sources of organic carbon.

The $\delta^{13}C$ value of plants is primarily determined by isotope-kinetic effects in the photosynthetic fixation of carbon dioxide (Thayer *et al*. 1983). In animals this is primarily a reflection of their diet. When the diet of animals is derived from different sources, different $\delta^{13}C$ signatures can be used as a tracer of organic carbon source (DeNiro and Epstein 1978; Thayer *et al.*1983).

Differences in the $\delta^{13}C$ of marine organisms from water masses on adjacent continental slope and continental shelf regions of British Columbia have been detected (Perry *et al.* 1999). Their findings indicate that $\delta^{13}C$ in the food web of the continental slope region is depleted, relative to the shelf. That is, the ratio of $^{12}C/^{13}C$ ($\delta^{13}C$) was more negative in the slope region than the shelf region. In this study $\delta^{13}C$ values are used to reflect reliance on different organic carbon sources. Age and body size are also used as biological variables with which spatial variability of THg and TAs concentrations between regions may vary.

Due to the toxicity of some forms of Hg and As, guidelines limit the concentrations recommended in edible tissues for human consumption. In Canada, the guideline for total mercury is 0.5 μgg^{-1}(Health Canada 2007). Arsenic has a legal limit of 3.5 μgg^{-1} in fish (Food and Drugs Act, R.S.C. 1985, c F-27, s. B.15.003)

If elevated or low concentrations are associated with any particular region, then spatial variability in these concentrations may have consequences for food safety. This chapter will explore the hypothesis that spatial variability in the concentrations of THg or TAs in Pacific ocean perch has food safety implications.

The general purpose of this chapter is to explore spatial variability of THg and TAs concentrations between *Sebastes alutus* from different regions of coastal British Columbia. The regions are Hecate Strait (HS), Queen Charlotte Sound (QCS) and the west coast of Vancouver Island (WCVI). The inclusion of quantitative biological data ensures this chapter yields conclusions that can be applied to other geographical regions or periods of time.

Outliers

In the field of geochemistry outliers can be identified and defined as data which differs greatly from the central location (Filzmoser *et al.* 2005). This is generally considered to be the result of an exotic process. Conversely, inliers are defined by location and spread and represent background variability which is uninfluenced by exotic processes. By applying these concepts to contaminant concentrations in fish, the natural background variation within three regions of coastal British Columbia can be characterized. This leads to the hypothesis that the presence of outliers indicates a region where a secondary process has occurred. This results in concentrations which deviate from the dominant data structure, as identified by this multivariate outlier determination method. This method proposed by Filzmoser *et al.* 2005, utilizes all observation in the study. Outliers are present in all three regions, but predominate in the WCVI region. Although not exactly predictable, the region with the greatest proportion of outliers can be hypothesized to contain a location related to the unknown secondary process that has occurred. This may occur due to a stationary feature of the landscape. In this context outliers are identified as a group which deviates from the dominant data structure, based on location and spread of all of the observations.

This method utilizes the robust Mahalanobis distance, which is resistant to the influence of outlying observations. Multivariate outliers can now simply be defined as observations having a large (squared) Mahalanobis distance.

2.3 Materials and Methods.

2.3.1 Sample Collection

Over 230 fish samples were collected over a 12 month period from June 2004 and June 2005. They were collected from a fish processing plant on Southern Vancouver Island and were caught by commercial fishing boats operating in Hecate Strait, Queen Charlotte Sound, or the West Coast of Vancouver Island (Fig 2.1). Of the 233 fish samples analyzed, 30 came from HS, 156 from QCS and 47 from the WCVI region. All of the samples from HS and the WCVI were landed from a single fishing vessel. The Hecate Strait samples were collected in the summer of 2004, the WCVI samples in the summer of 2005 and the QCS samples in between those periods.

All of the samples were in the form of "fish frames", i.e., the filets had been removed. The length of the frames were recorded before being stored at minus 20^{o}C. After defrosting the frames, muscle tissue and otoliths were collected.

2.3.2 Metals analysis

Five muscle tissue from the anterior dorsal region of each fish was removed, homogenized and frozen at minus 20 ^{o}C before analysis for mercury and arsenic. Analysis of both total mercury and total arsenic was undertaken by Maxxam Analytics, Burnaby. In addition to satisfactory QA reports, blind replicates were included in every batch produced identical results. Inductively coupled plasma mass spectrometry *(*ICP-MS*)* was used in accordance with 'Sample preparation procedure for spectrochemical determination of total recoverable elements' (EPA SW846 200.2 rev 2.5 May 1994), followed by US EPA method 200.8, revision 5.4,1994. The minimum detection limits for THg and TAs were 0.01 μgg^{-1} and 0.1 μgg^{-1}, respectively.

2.3.3 Stable Isotope Analysis

A sub-sample of anterior dorsal muscle tissue was removed from each fish, freeze dried and ground to a fine powder with liquid nitrogen in an agate pestle, or a wig L bug. 0.5 – 0.7 mg’s were packed into tin capsules for isotope ratio determination of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ using a Delta Isotope Ratio Mass Spectrometer.

2.3.4 δ^{13}C Signature

Lipid synthesis favours the lighter isotope of carbon (DeNiro and Epstein 1977), so that lipids are often δ^{13}C depleted relative to other tissues. To prevent a bias in the carbon isotope ratio (δ^{13}C) caused by variation in lipid content between individual fish, a mathematical correction model is used. Here the C:N ratio is used to account for the effect of lipids on δ^{13}C values (Power *et al*. 2002; Kiljunen *et al*. 2006; Logan *et al*.

2008). Lipids were extracted from sub-samples of Pacific ocean perch (freeze dried and homogenized dorsal muscle tissue) using a protocol based on Bligh and Dyers 1959.

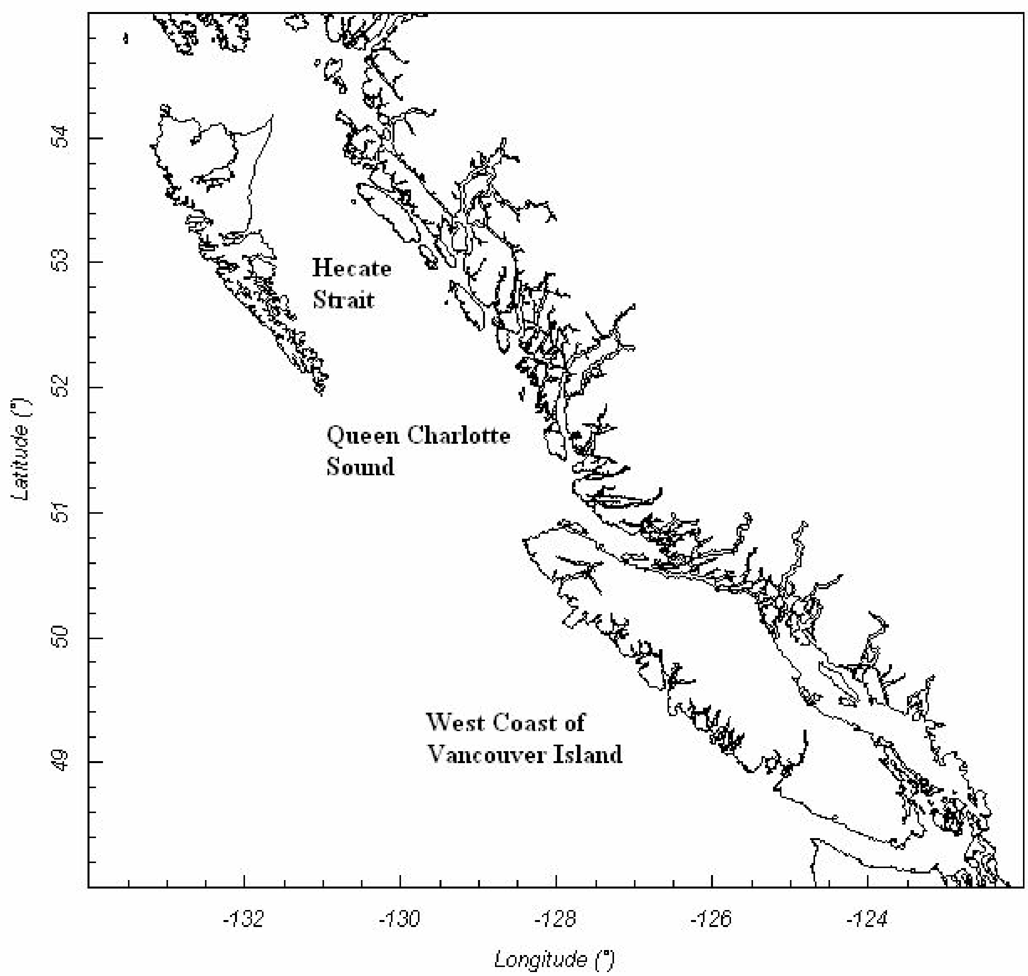


Figure 2.1 Map of British Columbia indicating the regions that *S. alutus* were harvested by commercial fishing vessels

The relationship between C:N ratio and lipid content is strong in aquatic biota (Dempson *et al*. 2009). Figure 2.2 and Equation 3 show how $\delta^{13}C$ varies with C:N in Pacific ocean perch from this study.

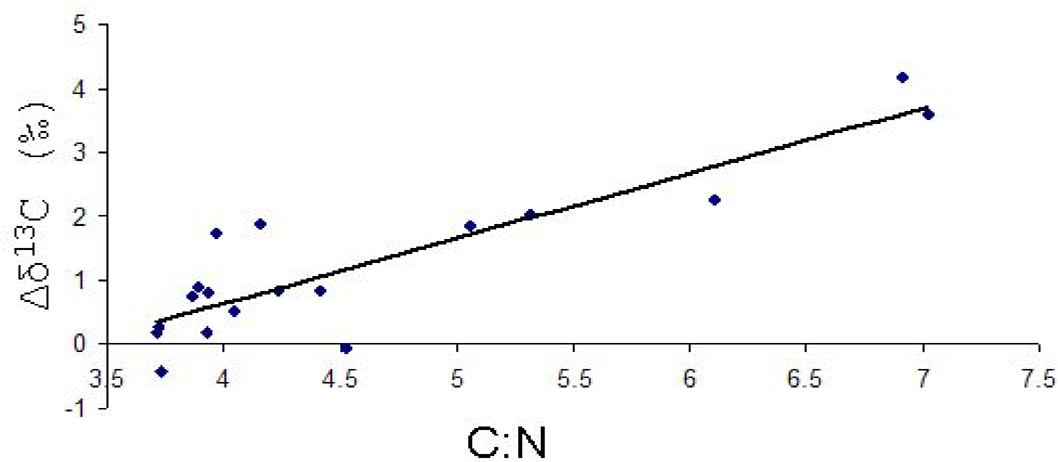


Figure 2.2 Scatter plot with regression line showing the relationship between $\Delta\ \delta^{13}C$ and C:N ratio in Pacific ocean perch. The $\delta^{13}C$ of individual fish samples were determined both before and after lipid extraction in order to obtain the effect due to lipid content of the tissue ($\Delta\ \delta^{13}C$).

$$\Delta\ \delta^{13}C = \delta^{13}C_{(WT)} - \delta^{13}C_{(LE)}$$ **Equation 2**

Where $\delta^{13}C_{(WT)}$ is the $\delta^{13}C$ of whole tissue, $\delta^{13}C_{(LE)}$ is the $\delta^{13}C$ of tissue after lipid extraction and the change in $\delta^{13}C$ due to lipid extraction is = $\Delta\ \delta^{13}C$. The relationship between $\Delta\ \delta^{13}C$ and C:N ratio (Figure 2.2) was described by a straight line (Equation 4).

$$\Delta\ \delta^{13}C = 1.146(C{:}N) - 3.415$$ **Equation 3**

Where carbon to nitrogen ratio (C:N) is used as a proxy for lipid content in fish. The r-squared value for this relationship is 0.78.

Equation 4 was used to estimate the $\delta^{13}C$ for each sample, based on the individual C:N; which was then used to adjust the $\delta^{13}C$ to standardise the $\delta^{13}C_{(WT)}$ to a lipid extracted $\delta^{13}C$ (expressed as $\delta^{13}C'$) for each fish specimen. Lipid normalization methods including both chemical lipid extraction and arithmetic lipid correction have been used in food web studies over the last 30 years (McConnaughey and McRoy 1979; Sweeting *et al*. 2005; Sweeting *et al.* 2007).

2.3.5 Statistical Methods

Outlier Identification

A robust, two dimensional, multivariate outlier identification procedure was used to identify observations as outliers (Filzmoser *et al*. 2005). This used a multivariate outlier plot to identify points that depart from the dominant data structure (Figure 2.2). The method was implemented using the mvoutlier package in R. For more detailed information see Filzmoser *et al*. (2005). The Mahalanobis distance is a well-known distance measure which takes into account the covariance matrix. This method of outlier detection calculated the ordered squared robust Mahalanobis distances from the THg and TAs concentrations of all 233 fish samples. The robust Mahalanobis distance (MD_i) is defined as Equation 5 (Filzmoser *et al*. 2005).

$$MD_i := ((x_i - t)^T C^{-1}(x_i - t))^{1/2} \text{ for I} = 1,\dots\dots\dots, n,$$

Equation 4

Where t is the estimated multivariate location and C is the estimated covariance matrix.

The 0.975 quantile of the chi-square distribution of the ordered squared robust Mahalanobis distance was used as the critical value for outlier detection (Filzmoser *et al*. 2005), (Figure 2.3).

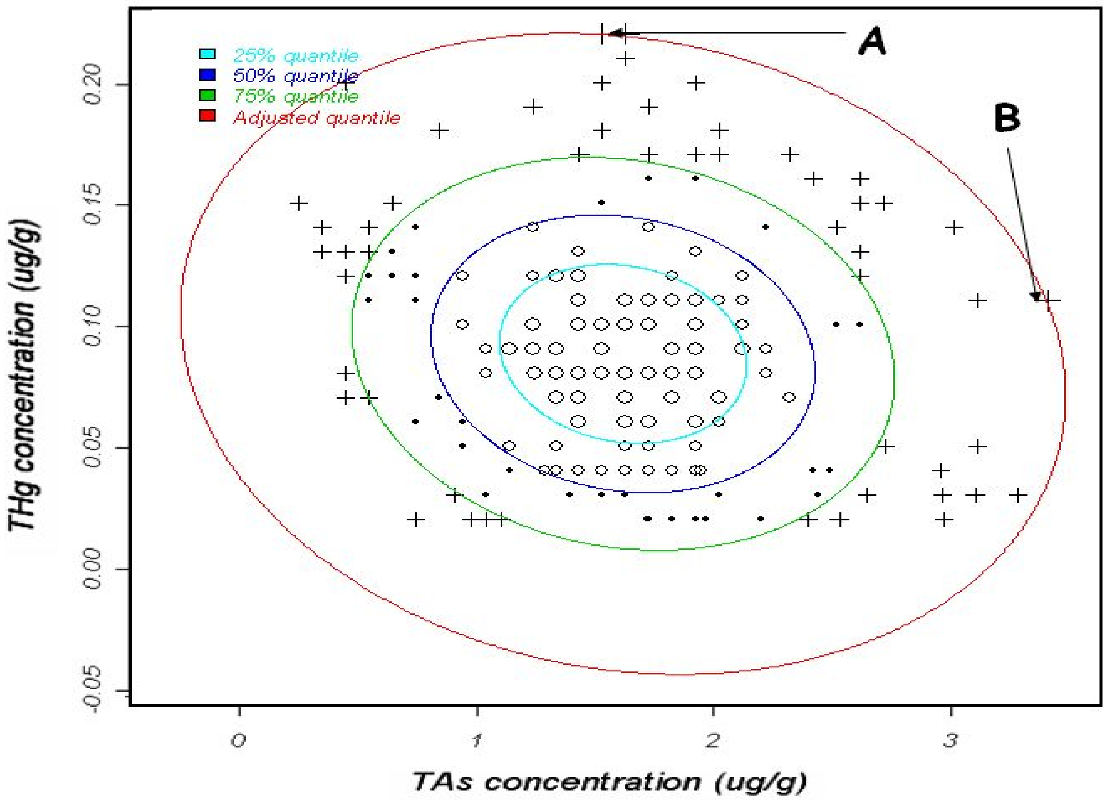


Figure 2.3 Plot of the Mahalanobis distances of THg and TAs concentrations. Different symbols represent Mahalanobis distances. Ellipsoids represent constant Mahalanobis distances, which correspond to the 25%, 50%, 75% and the 97.5% quantiles of the chi-square distribution. The 97.5% quantile was used as the critical value for outlier detection. This corresponds to the THg concentration at Point A = 0.23 μgg^{-1}, TAs concentration at Point B = 3.6μgg^{-1}. Thirty one samples with concentrations of either THg that exceeded Point A, or TAs that exceeded Point B were identified as outliers. These points are not shown on this figure.

Statistical Comparisons

Due to non-normality of the data, the Kruskal-Wallis Rank Sum Test was used to compare samples from the three regions. Unless otherwise indicated, p-values <0.05 were considered to be significant. The Kruskal-Wallis Rank Sum Test, tests the hypothesis that all samples are drawn from different populations with the same distribution. When significant differences were found, post hoc comparisons were done to identify which region was different. Nonparametic, two sided Steel type (unbalanced) multiple comparisons, were used for this purpose. All statistics were produced with the free statistical software package R: a language and environment for statistical computing (Version 2.4.0, see http://cran.r-project.org). The following contributed packages were used; Rcmdr, PBSmapping, mvoutlier and npmc.

2.3.6 Food Safety Scenarios

Consumption calculations were based on 60 kg for an adult body size and 26 kg for children (Health Canada 2007). Although potentially an underestimate, 60 kg is the standard body weight frequently used for PTWI calculations in the scientific literature. It is used in this study for ease of comparison. Serving sizes for a non-aboriginal adult was 145g and 116g for a child (Health Canada 2007). Based on a diet survey carried out in an aboriginal community in coastal British Columbia, a serving size for aboriginal adults was determined to be 278 g (Frosst, G. personal communication, University of Victoria 2006). When estimating health risks some studies assume that all of the THg in fish is methylated, such as Hoover *et al*. (1997). Applying the assumption that all of the mercury in fish is present as Methylmercury, results in a more cautious assessment (Health Canada 2007).

The range of the proportions of methylated mercury reported in marine fishes (Table 1.1) show that the range of methylmercury in marine fish varies from 70 – 100 %. Therefore, to provide as balanced an approach as possible in this study, I have estimated the MeHg quantities of both 85% and 100% of THg (Appendix B). Despite this source of uncertainty, the spatial patterns shown by 85% and 100% MeHg are very similar and the conclusions are not affected. For ease of comparison with other studies, I have used the 100% proportion, as does Health Canada (Health Canada 2007).

Tolerable intakes of arsenic are based on inorganic arsenic. The proportion of TAs that is present as inorganic arsenic is estimated as 0.1%, which is the proportion reported in quillback rockfish (*Sebastes maliger)* from eastern Juan de Fuca Strait (Washington State Department of Ecology 2002).

Tolerable intakes were based on the following PTWIs

- THg for adults: 0.005 mg/kg bw/week (JECFA 1978). (That is equivalent to 5 μg/kg bw/week).
- MeHg for adults: 1.6 μg/kg bw/week (JECFA 2006).
- MeHg for children and pregnant women 0.20 μg/kg bw/day (Health Canada 1997).
- $As_{inorganic}$: 0.015 mg/kg bw/week (JECFA 1988).

The tolerable intake of MeHg for Children and pregnant women is estimated per day, which corresponds to its use by Health Canada (Health Canada 2001) and reflects the increased vulnerability of these groups, as the most sensitive toxicological endpoints for MeHg (JECFA, 2006).

2.3.7 Age Determination

Otoliths were stored in a glycerin-thymol solution (C.A.R.E. 2000) prior to age determination. Age determination was by the "break and burn" method described by MacLellan (1997). Age readers were trained by staff at the Pacific Biological Station (PBS), Nanaimo. Multiple axes of each otolith were aged by two age readers at the University of Victoria. The determined age was by agreement with two age readers. All ages below 40 are accurate to ± 1 year. Otoliths with ages exceeding 40 year are considered to be accurate to ± 5 years. For quality control purposes, a subsample of otoliths aged at the University of Victoria were brought to PBS and the ages were confirmed.

2.4 Results

2.4.1 Inliers and Outliers

Initial examination of the Hg and As concentrations in muscle tissue of Pacific ocean perch from all three regions indicates the presence of observations that are very different from the rest of the samples (Figure 2.5 (data at top right)). This suggests the presence of potentially confounding outliers. Therefore, for the purpose of this thesis, each sample is identified as either an outlier or an inlier (see Methods section and Figure 2.3 for details).

The number of outliers identified and the THg and TAs concentrations of both inliers and outliers from each region are described in Table 2.1. With respect to the data from this study, samples are identified as outliers if either of the following conditions are met.

THg concentration>0.23 (μgg^{-1}).
TAs concentration > 3.6 (μgg^{-1}).

A total of 31 outliers were identified from 233 samples, thus, 86.7% of the fish sampled were determined to be inliers and 13.3% to be outliers. The number and pproportion of fish identified as outliers differ among regions (χ^2 = 8.9, df=2, p=0.01). There were 5 (16%) in HS, 14 (8%) in QCS and 12 (25%) in fish from the WCVI region. With the exception of one sample, all of the specimens identified as outliers on the basis of their mercury concentrations also had TAs concentrations that identified them as outliers. The THg and TAs concentrations of the outliers (n=31) are strongly correlated to each other (Pearson correlation coefficient = 0.8).

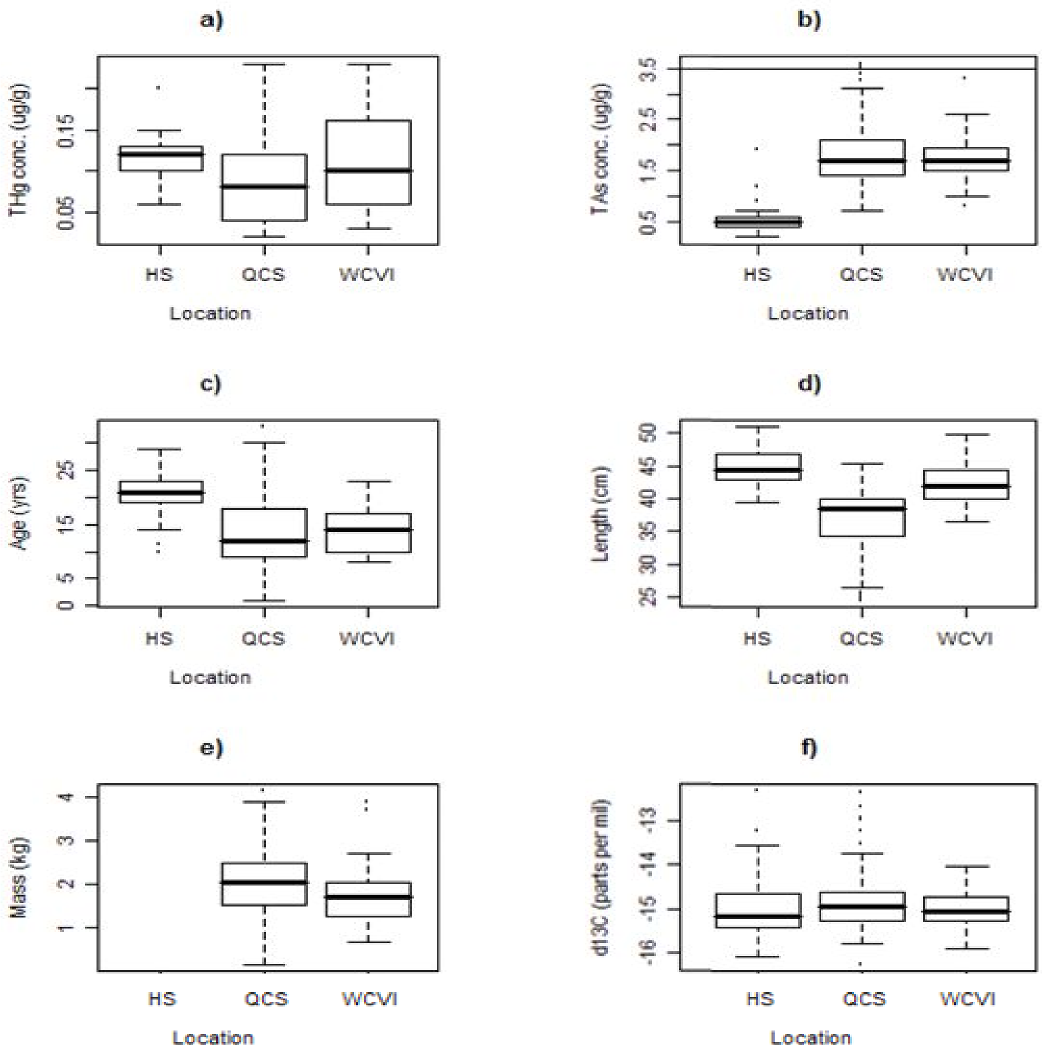


Figure 2.4 Box plots of a) THg concentrations, b) TAs concentrations, c) age, d) length, e) mass and f) lipid normalized δ13C signature of Pacific ocean perch (INLIERS). Fish were caught in Hecate Strait (HS), Queen Charlotte Sound (QCS) or the west coast of Vancouver Island (WCVI) regions of British Columbia. Horizontal line in b) represents the legal limit for arsenic in food, 3.5 (Food and Drug Regulations, C.R.C., c. 870, s. B .15.003).

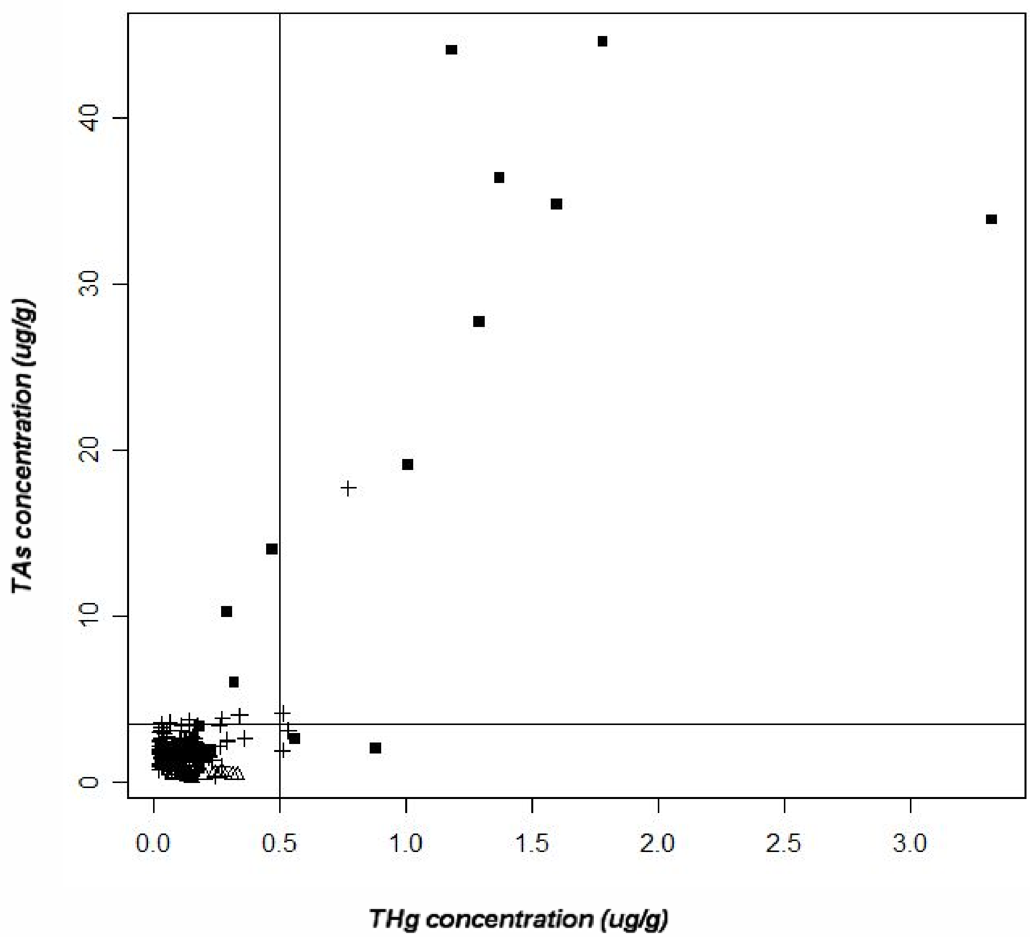


Figure 2.5 Scatter plot of the concentrations of THg and TAs in Pacific ocean perch from three regions of British Columbia (n=233). Symbols represent fish from different regions; Hecate Strait (Δ), Queen Charlotte Sound (+) and the west coast of Vancouver Island (■). The horizontal line represents the legal limit for As in food (3.5 $\mu g g^{-1}$) and the vertical line represents the Health Canada guideline for Hg in food (0.5 $\mu g g^{-1}$) (Health Canada 2007).

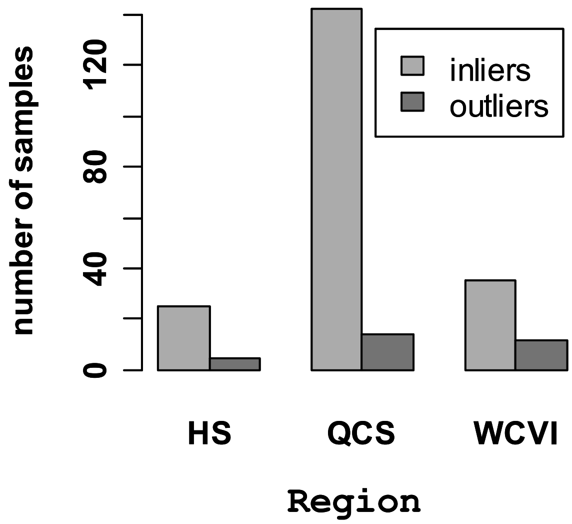


Figure 2.6 Bar Graph of the number of samples identified as inliers or outliers in each region.

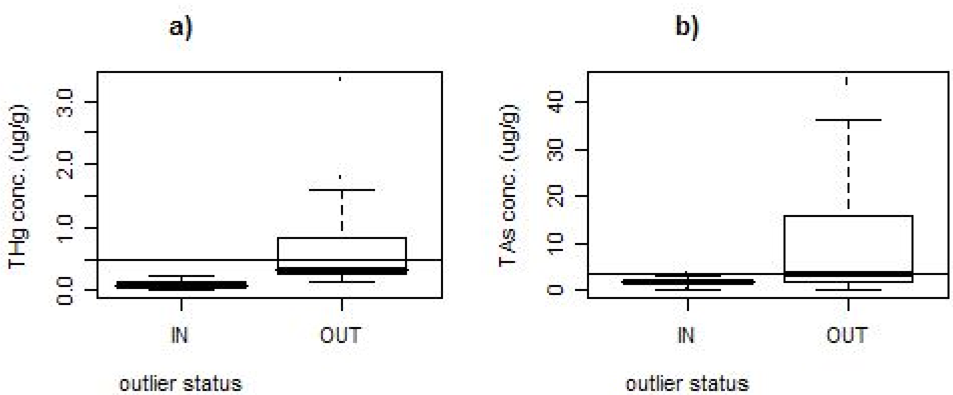


Figure 2.7 Box plots of a) THg concentrations, b) TAs concentrations in muscle tissue of Pacific ocean perch from British Columbia. Fish were identified as either inliers (IN) or outliers (OUT).

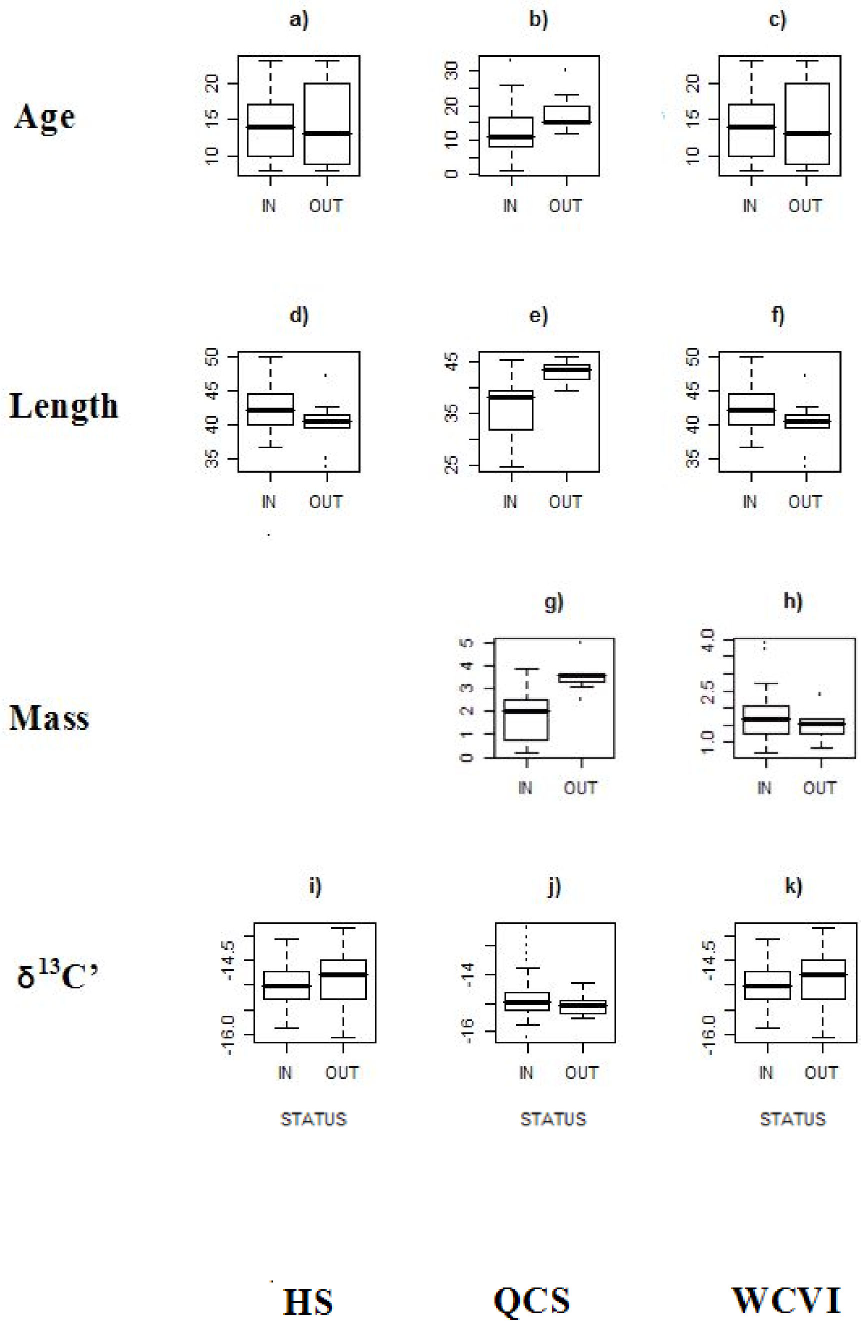


Figure 2.8 Box plots comparing age in years a) b) and c), length in cm d) e) and f), mass in kg g) and h), $\delta^{13}C$ (%) i) j) and k) and $\delta^{15}N$ (%) l) m) n) between Pacific ocean perch identified as either inliers (IN) or outliers (OUT). Columns indicate the region of British Columbia where the fish were caught: Hecate Strait (HS), Queen Charlotte Sound (QCS) or the west coast of Vancouver Island (WCVI).

Table 2.1 Summary statistics for muscle tissue of Pacific ocean perch caught in Hecate Strait, Queen Charlotte Sound or the west coast of Vancouver Island between June 2004 and June 2005. Samples are identified as either inliers or outliers.

[1]Mass data was not available for specimens from the Hecate Strait region.

All Data	**Hecate Strait**	**Queen Charlotte Sound**	**West Coast of Vancouver Island**
(n=233)	(n=30)	(n=156)	(n=47)
THg (mean ± SEM) ($\mu g g^{-1}$)	0.14 ± 0.01	0.11 ± 0.01	0.38 ± 0.10
TAs (mean ± SEM) ($\mu g g^{-1}$)	0.57 ± 0.1	1.9 ± 0.1	7.2 ± 1.8
Age (mean ± SEM) (years)	22.7 ± 1.4	13.8 ± 0.5	14.1 ± 0.7
Length (mean ± SEM) (cm)	45.2 ± 0.5	37.2 ± 0.4	41.9 ± 0.6
Mass (mean ± SEM) (Kg)	NA[1]	2.0 ± 0.1	1.7 ± 0.1
$\delta^{13}C$’ (mean ± SEM) (‰)	-14.9 ± 0.2	-14.9 ± 0.1	-14.9 ± 0.1
$\delta^{14}N$ (mean ± SEM) (‰)	14.8 ± 0.2	14.9 ± 0.1	15.1 ± 0.1
Inliers only (n=202)	(n=25)	(n=142)	(n=35)
THg (mean ± SEM) ($\mu g g^{-1}$)	0.114 ± 0.01	0.09 ± 0.004	0.108 ± 0.01
TAs (mean ± SEM) ($\mu g g^{-1}$)	0.6 ± 0.17	1.8 ± 0.05	1.7 ± 0.1
Age (mean ± SEM) (years)	20.6 ± 0.9	13.2 ± 0.5	14.0 ± 0.8
Length (mean ± SEM) (cm)	45.1 ± 0.1	36.8 ± 0.4	42.5 ± 0.6
Mass (mean ± SEM) (Kg)	NA[1]	1.9 ± 0.1	1.8 ± 0.1
$\delta^{13}C$ (mean ± SEM) (‰)	-14.8 ± 0.2	-14.8 ± 0.1	-15.0 ± 0.1
$\delta^{15}N$ (mean ± SEM) (‰)	14.8, ± 0.2	14.8 ± 0.1	15.1 ± 0.1
Outliers only (n=31)	(n=5)	(n=14)	(n=12)
THg (mean ± SEM) ($\mu g g^{-1}$)	0.28 ± 0.02	0.36 ± 0.04	1.17 ± 0.24
TAs (mean ± SEM) ($\mu g g^{-1}$)	0.5 ± 0.04	3.8 ± 1.1	23.0 ± 4.6
Age (mean ± SEM) (years)	33.1 ± 4.6	20.0 ± 2.5	14.3 ± 1.7
Length (mean ± SEM) (cm)	46.1 ± 1.0	41.5 ± 0.8	40.1 ± 1.1
Mass (mean ± SEM) (Kg)	NA[1]	3.1 ± 0.2	1.5 ± 0.1
$\delta^{13}C$ (mean ± SEM) (‰)	-14.9 ± 0.3	-15.1 ± 0.1	-14.9 ± 0.2
$\delta^{14}N$ (mean ± SEM) (‰)	15.0 ± 0.3	14.9 ± 0.2	15.1 ± 0.3

2.4.2 Spatial variability of THg and TAs concentrations in Pacific ocean perch.

A wide range of concentrations of THg and TAs were found in the muscle tissue of *Sebastes alutus* from the three commercial fishing regions (Table 2.0, Figures 2.5 and 2.9). Some of this variability can be attributed to the presence of outliers with extremely elevated concentrations. However, when only the inliers are examined, spatial variability between regions is still evident. In the inliers (Figure 2.9) the rank order of mean TAs concentrations is QCS>WCVI>HS. For mean THg concentrations, the rank order was HS>WCVI>QCS (Figure 2.9, panel a) and b), Table 2.0). Kruskal Wallace Rank Sum Test confirms that both THg and TAs concentrations were significantly different ($p<0.05$) between regions.

2.4.3 Spatial variability of biological variables in Pacific ocean perch

Mean and standard error of the mean (SEM) of age, length, mass, and $\delta^{13}C'$ of samples from each region are shown in Table 2.0. Box plots illustrate the age, length, mass and $\delta^{13}C'$ values of the inliers (Figure 2.2, panels c, d, e and f). These biological variables are all statistically different between regions (Kruskal Wallis Rank Sum Test, p-value<0.05), with the exception of $\delta^{13}C'$ (Kruskal Wallis Rank Sum Test, p-value>0.3). The significant differences in the biological variables between regions are found in the outlier, the inliers and the complete (inliers and outliers combined) data sets. The pattern of the rank order of age and length in the inliers (Figure 2.9, panel a and b, Table 2.0, Figure 2.4) matches the pattern of the rank order of THg in these fish, i.e., HS>WCVI>QCS. In general, this study found that fish from HS (both inliers and outliers) have average age and lengths greater than fish from QCS and the WCVI. Fish from the WCVI have a mean mass lower than HS and QCS than fish from QCS (both inliers and outliers).

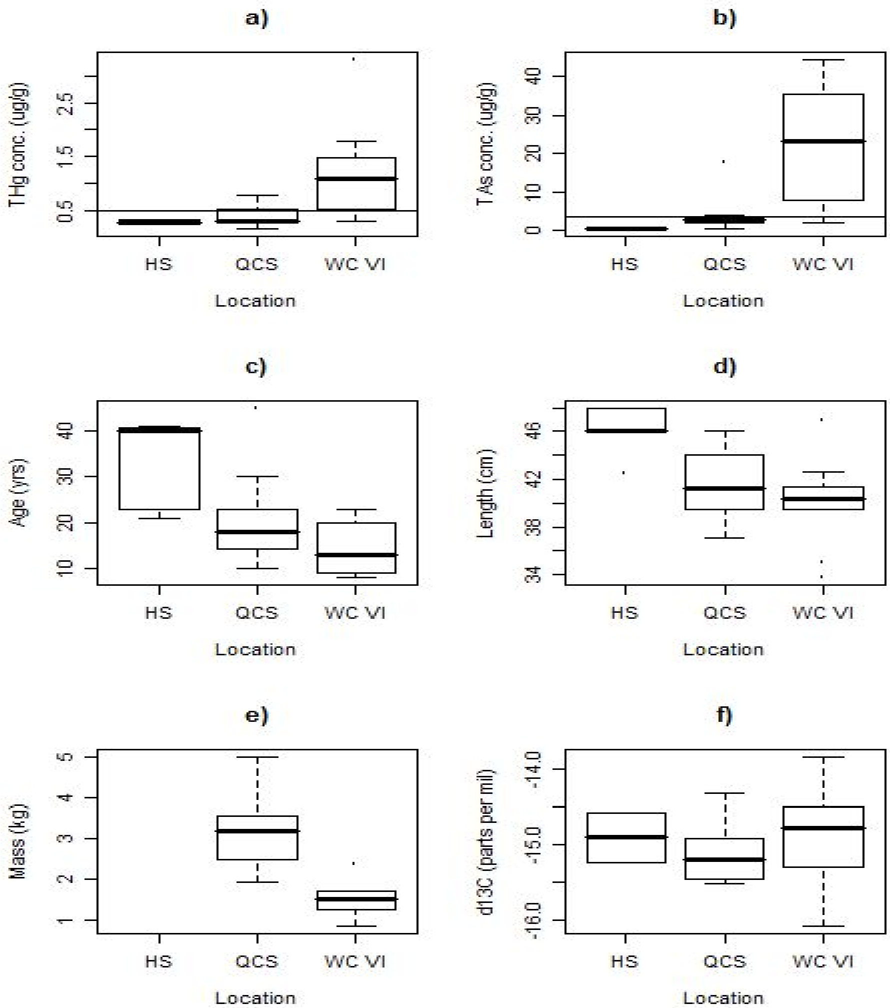


Figure 2.9 Box plots of a) THg concentrations, b) TAs concentrations, c) age, d) length, e) mass and f) lipid extracted $\delta^{13}C$ signature of Pacific ocean perch (OUTLIERS ONLY). Fish were caught in Hecate Strait (HS), Queen Charlotte Sound (QCS) or the west coast of Vancouver Island (WCVI) regions of British Columbia. Horizontal lines in a) and b) represent the guidelines and legal limits for each element.

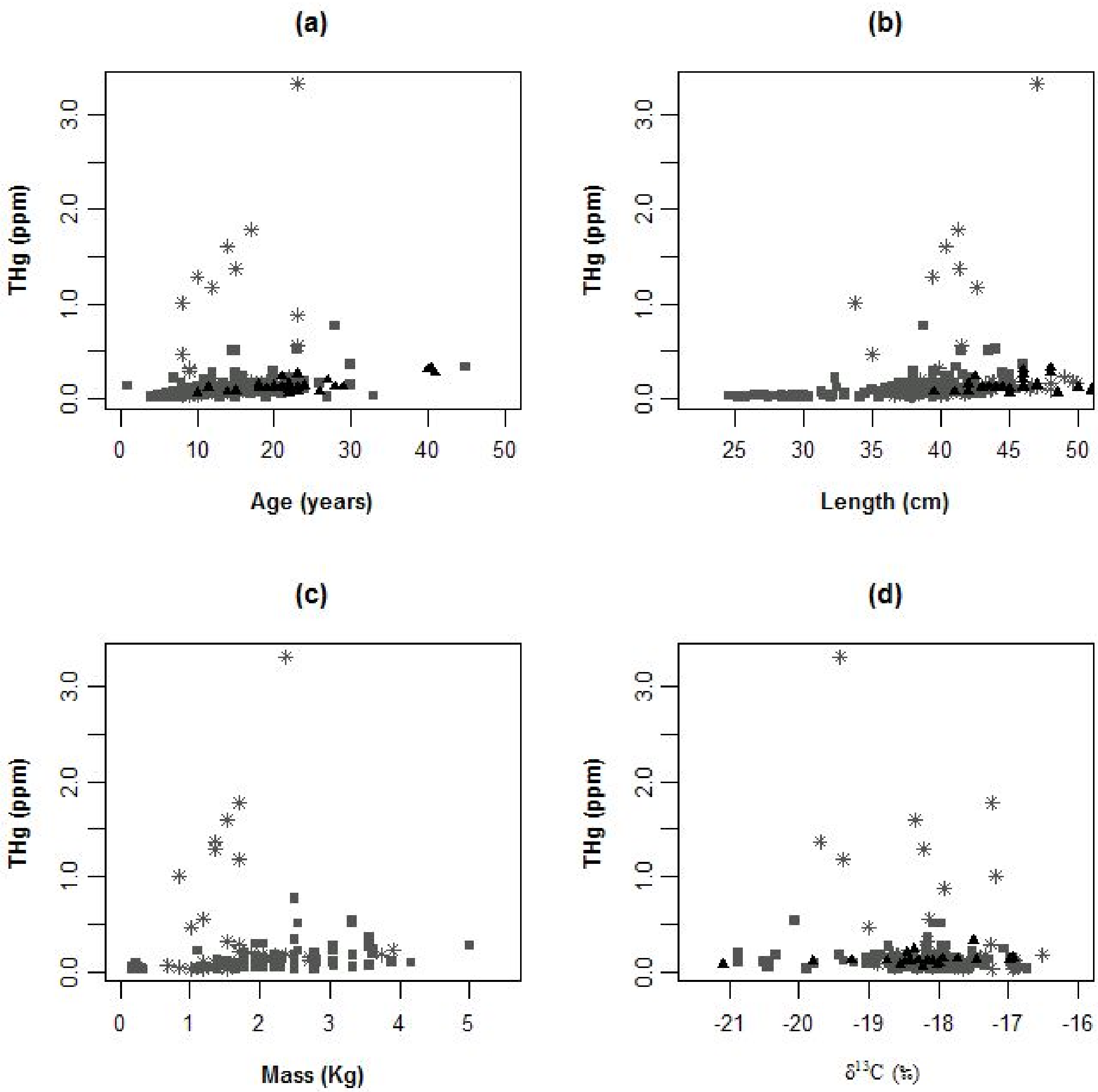


Figure 2.10 Scatter plots of THg concentrations (μgg^{-1}) against a) age, b) length, c) mass, d) $\delta^{13}C$ in all data (inliers and outliers) from all three regions, ▲ = Hecate Strait, ■ = Queen Charlotte Sound and *= West Coast of Vancouver Island.

2.4.4 Food Safety

Exposure scenarios, using estimates of MeHg, suggest that tolerable intakes for children and pregnant women are frequently (40 – 100%), exceeded from a single serving in all three regions (Figure 2.12 and Appendix A). The tolerable intakes for THg in both aboriginal and nonaboriginal adults is similar to the pattern of concentration of THg in the outliers (i.e. HS>WCVI>QCS, Section 2.4.2).

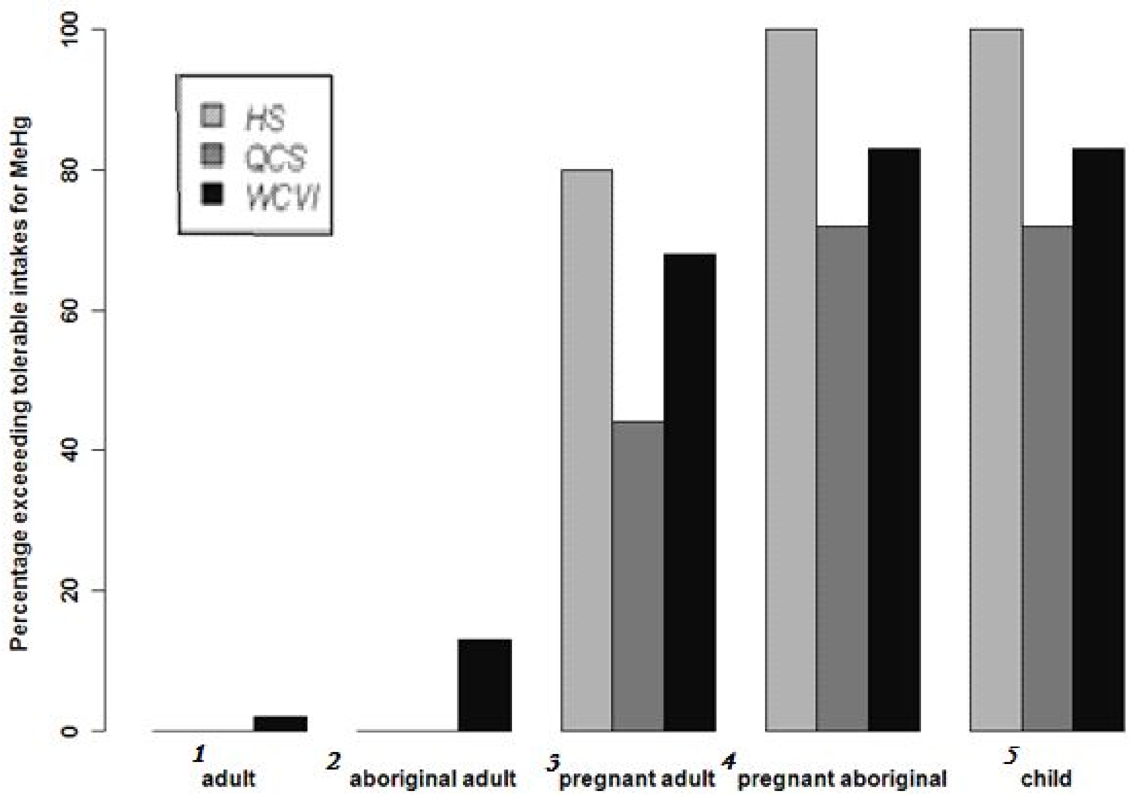


Figure 2.11 Exposure Scenario bar graph: proportion (as a percentage) of fish from each region that exceed a variety of tolerable intakes for MeHg of different subpopulations, from a single serving of Pacific ocean perch (assuming 100% of THg is MeHg). For details of calculation of tolerable intakes for each population sub group see Methods 2.3.5.3.

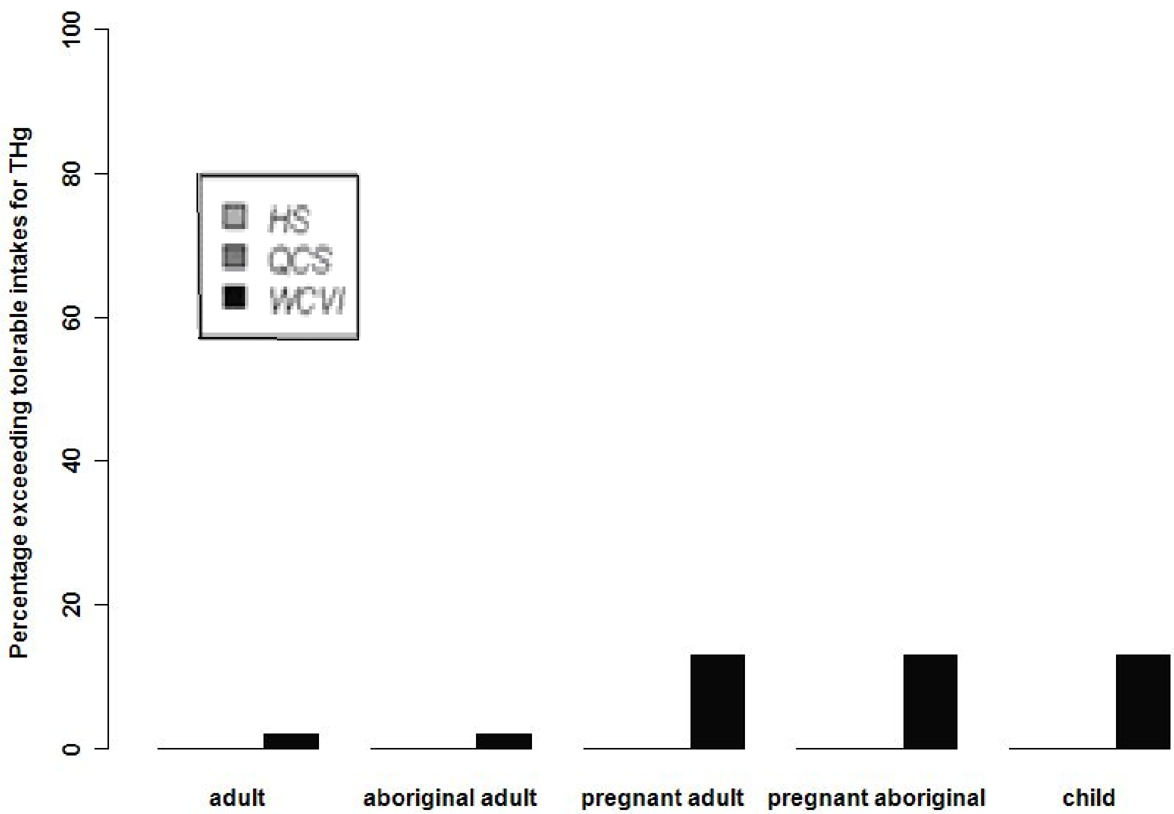


Figure 2.12 Exposure Scenario bar graph: proportion (as a percentage) of fish from each region that exceed a variety of tolerable intakes for THg of different subpopulations, from a single serving of Pacific ocean perch. For details of calculation of tolerable intakes for each population subgroup see Methods 2.3.5.3.

Single servings of fish that exceeded T.I.s for THg originated from the WCVI region only. None of the estimated intakes for $As_{(inorganic)}$ exceeded tolerable weekly consumption (rows 13 - 16 of Appendix A). As such, the variability in the percentage of samples from each region that exceed exposure scenarios for arsenic intake did not vary between regions. Therefore based on current knowledge, it does not appear that concentrations of arsenic in Pacific ocean perch have any public health implications.

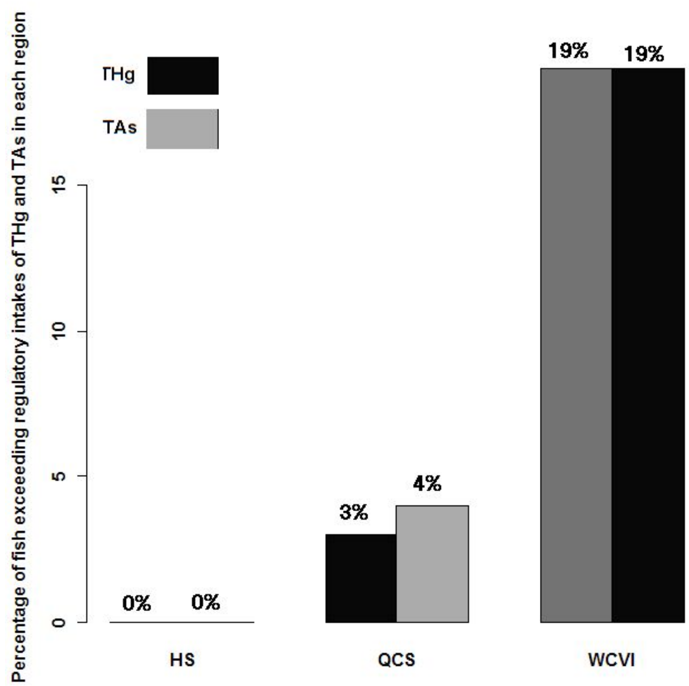


Figure 2.13 Bar graph illustrating the percentage of fish sampled from each region which exceed regulatory limits. 0.5 μgg^{-1} for THg and 3.5 μgg^{-1} for TAs. HS = Hecate Strait, QCS = Queen Charlotte Sound, WCVI = West Coast of Vancouver Island.

2.5 Discussion

2.5.1 Inliers and Outliers

The unexpected finding of fish with extremely elevated concentrations of both THg and TAs, determined statistically to be outliers, has implications for the analysis and interpretation of the data produced for this thesis. The detection of outliers and unusual data structures is often included in the statistical analysis of geochemical data sets (Filzmoser *et al.* 2005). In the context of geochemical samples, the natural variation of the material being studied in a specific area (the background) can be defined by the location and spread of the data. The natural background variation indicates samples that are not influenced by extraneous or exotic processes. These could be associated with rare rock types, processes resulting in mineral deposits, or anthropogenic contamination. In the field of geochemistry, outliers are therefore generally considered to be observations which are the result of secondary processes, rather than extreme values from the background distribution (Filzmoser *et al.* 2005). This chapter transfers this concept, where background is defined by location and spread, to trace element concentrations in marine biota. Therefore, outliers are interpreted as the result of a secondary process, and inliers are interpreted as the natural background variation of the trace element concentration.

Other studies of mercury in marine fish have also reported extreme variability in concentrations. For example, THg concentrations in white seabream landed in Sao Miguel Island in the Azores ranged from 0.132 to 24.6 μgg^{-1} (Anderson and Depledge 1997). These specimens were all of intermediate size, and the increase in concentrations a factor of 186. This is greater than the increase factor in concentrations of THg found in the WCVI region in this study (0.03 to 3.32 μgg^{-1}) which varied by a factor of 111. In turn, this is a greater increase factor than sea bass from the Ria de Aveiro lagoon, Portugal (Abreu *et al.* 2000) where concentrations increased by less than a factor of 57. In this case the fish that were assessed were all juveniles (Abreu *et al.* 2000). Examples of studies where concentrations increase by a much smaller factor (less than ten) include horse mackerel, rockfish and albacore tuna from the N. Atlantic and greater lizardfish from the Caspian Sea (Table 4) (Anderson and Depledge 1997; Agah *et al.* 2006).

Studies of THg in fish frequently show a positive linear relationship between THg concentrations and length (Anderson and Depledge 1997 and 2000). It is worth noting that high variability in THg concentrations can occur in a narrow range of lengths, and be independent of the influence of size. This pattern and mercury concentrations in fish with high increase factors (similar to this thesis) suggest the presence of a hot spot. That is the presence of a local area with raised environmental levels of mercury. Examples of this include white seabream, *Diplodus sargus*, from the Azores (Anderson and Depledge 1997) and sea bass, *Dicentrarchus labrax*, from the Ria de Aveiro Lagoon, Portugal (Abreu *et al.* 2000). In these examples, the Ria de Aveiro Lagoon contains a basin known to be contaminated with the effluent from a chlor-alkali plant. The source in the Azores is unknown, but may be related to submarine volcanism in the area. Another example of this pattern is seen in whitemouth croaker (*Micropogonias fornieri)* from the Patos Lagoon, Brazil. The fish that had higher than expected and widely variable THg concentrations were sampled close to the city of Rio Grande, a potential source of anthropogenic THg (Niencheski *et al.* 2001).

Again this pattern is similar to that found in this study in concentrations of both THg and TAs in Pacific ocean perch (Figures 2.4, 2.5 and 2.10) and where the outliers in Pacific ocean perch originated predominantly in the WCVI region, which have highly correlated concentrations of TAs and THg. This finding suggests that this region contains an area of localized high environmental concentrations of both mercury and arsenic, an interpretation that is supported by studies of other species of marine biota, which have found higher concentrations of mercury in this region compared to others in British Columbia (Elliot and Scheuhammer 1997).

Other studies of mercury in the marine environment have investigated geochemical processes. The potential for bioaccumulation and biomagnification in food webs is thought to be determined by the transformation of inorganic Hg to MeHg. This process is greatly influenced by the biogeochemical condition of sediments (Wagemann *et al.* 1978*)*. Thus, MeHg production and bioaccumulation in coastal and pelagic systems are thought to be strongly influenced by biogeochemical conditions at local scales (Wagemann *et al.* 1978).

In British Columbia, investigations of the marine sediment pore waters from coastal sites demonstrate the importance of redox interconversions of inorganic arsenic compounds (Reimer and Thompson 1998). They showed that *in situ* microbial methylation of inorganic arsenic produces an array of organo-arsenic derivatives. The authors suggest that methyl-arsenic distributions are governed by a balance between methylating and demethylating bacteria and by adsorption phenomena (Holm *et al*. 1980). Sulfate reducing bacteria appear to be involved in the marine biogeochemical cycles of both of these elements (Wagemann *et al.* 1978). The findings of this chapter suggest the possible presence of a 'hot spot' (i.e., an area of raised environmental levels of Hg and As) in the WCVI region. The link between Hg and As suggests anaerobic sediments (as a site for *in situ* microbial methylation). Further research is required, however Fjords with a shallow submarine sill may be sites of sediment anoxia, such as Effingham Inlet in Barkley Sound on the south west coast of Vancouver Island. (Sannigrahi Ingall, 2005). . This has potentially important implications for the monitoring of contaminants in Pacific ocean perch from this region, because the outliers contain concentrations of Hg and As that exceed consumption guidelines (0.5 and 3.5 μgg^{-1}) and tolerable intakes for humans.

2.5.2 Spatial variability of THg and TAs concentrations

The significant statistical differences and the heterogeneity in the ranges of concentrations of THg and TAs in samples from different regions support the hypothesis that the concentrations of these two elements vary spatially in this commercial species of fish in different regions of the marine coastal environment of British Columbia. The difference in mean THg concentrations from different regions of the same ocean basin in this chapter (0.27 μgg^{-1}), is within the range of differences in means between sampling locations reported in other studies. Examples include snapper (*Chrysophrys auratus*) from New South Wales Australia (Chvojka *et al*. 1990), black bellied redfish *(Helicolenus dactylopterus)* from offshore waters in the North Atlantic, and spiny dogfish (*Squalus acanthias*) from two locations on Georges Bank, North Atlantic (Greig *et al.* 1977). The differences between the mean THg concentrations between locations in these studies were 0.21, 0.05 and 0.30 μgg^{-1} respectively.

Similarly, the difference between mean TAs concentrations in fish from different marine regions in this study (6.94 μgg^{-1}) is within the range reported between sampling locations in the scientific literature. These include both Atlantic cod (*Gadus morhua)* and Atlantic herring (*Clupea harengus)* from the North Atlantic (Ballin *et al*. 1994) and orange spotted grouper, *Epinephelus coioides,* from Bahrain (de Mora *et al*. 2004). The differences in mean TAs concentrations from these studies were 2.4, 2.61 and 7.8 μgg^{-1} respectively. Previous studies of mercury in commercial marine fishes sable fish, *Anoplopoma fimbria,* and pacific hake, *Merluccius productus,* on the Pacific coast of North America have indicated a relationship between mercury and area of catch. These studies took place in the 1970s and did not include samples from British Columbia (Hall *et al.* 1976a; Cutshall *et al.* 1978). The findings of this chapter not only fill a gap in the literature, but also update the knowledge of the concentrations of these elements in commercial fish from the west coast of North America.

2.5.3 Spatial variability of biological variables in Pacific ocean perch

In this study, the regional patterns of mean background THg concentrations (inliers) matched the regional patterns of mean age and length. This is in contrast with the results of other studies of the spatial distribution of mercury in marine fish from different locations, which did not show this association (Greig *et al.* 1977; Chvojka *et al.* 1990). However, the importance of both size and location in relation to THg has been shown; for example, a multiple linear regression model of mercury on length and latitude of Pacific hake produced an r^2 value of 0.89, where both length and latitude were highly significant (Cutshall *et al*. 1978). They noted that fish sampled at higher latitudes on the west coast of North America were larger, and mercury concentrations increased with latitude. As in this study, locations with larger (longer) fish also have higher mercury concentrations. Cutshall *et al*. 1978 also suggests that the variation in mercury is more dependent on latitude than length and that length of fish and latitude are not independent.

The relationship between locations with larger fish and higher mercury concentrations is certainly interesting. Without further investigation of other factors, however, including abiotic variables, the hypothesis that spatial variability in Hg concentrations reflects the spatial distribution of the biological characteristics of the fish sampled cannot be supported or disproven.

Differences in $\delta^{13}C$ among various carbon reservoirs are passed through marine food webs (Hobson *et al.* 1994), with an estimated +1.0 ‰ fractionation between consumer muscle tissue and dietary protein (DeNiro and Epstein 1978; Tieszen *et al*. 1983). Evidence of an inshore/offshore or benthic/pelagic gradient comes from $\delta^{13}C$ of seabird prey organisms from Vancouver Island (Hobson *et al.* 1994). Other studies from this region, indicate differences in marine $\delta^{13}C$ between food webs of the continental shelf and the slope (Perry *et al.* 1999). The $\delta^{13}C$ signatures of Pacific ocean perch from all three regions in this study were very alike, with no statistically significant differences (Kruskal Wallis Rank Sum Test $p>0.05$). This finding also applies to the $\delta^{13}C$ signatures of the inliers and the outliers. The implication of this is that the organic carbon in each region originates in habitats or food web compartments which are similar and not isotopically distinct. Because concentrations of THg and TAs in Pacific ocean perch from each of the three regions show spatial variability, which is not present in the $\delta^{13}C$ values, the results of this study suggest that differences between regions in the relative contribution of organic carbon from the continental shelf or slope are minimal and do not influence THg or TAs concentrations in the Pacific ocean perch from this study. It is possible that dietary differences between different habitats could have been isotopically indistinguishable, but this was not addressed in this study.

Pacific ocean perch were found to be slightly enriched in $\delta^{13}C$ (which are lipid normalized), relative to the $\delta^{13}C$ values (lipid extracted or normalized) of the muscle tissue of other marine consumers from the coastal northeast Pacific Ocean. For example, a study of trophic relationships in seabirds from the west coast of North America, reports mean $\delta^{13}C$ values between -15.7 ± 0.1‰ and -18.5 ± 0.5‰ (Hobson *et al*. 1994). These values are depleted (more negative) in comparison to the mean values from Pacific ocean perch in this study (-14.85 ± .01‰, -14.85 ±.065‰ and -14.99 ± .07‰ in HS, QCS and WCVI regions respectively). In seabirds, this enrichment in $\delta^{13}C$ is consistent with inshore foraging behaviours. In particular, pigeon guillemots, off the west coast of Vancouver Island, showed the greatest $\delta^{13}C$ enrichment, which is consistent with their inshore benthic feeding preference where they may have consumed carbon of detrital origin, (Hobson *et al*. 1994).

Another example of marine consumers with $\delta^{13}C$ values that are enriched $\delta^{13}C$ (less negative) comes from a stable isotope study of the food web in the Astoria submarine canyon, Oregon, USA. This study reported $\delta^{13}C$ of five species of rockfish, with values ranging from -16‰ to -18‰ (Bosley *et al.* 2004). The rockfish species from Astoria canyon, most enriched in $\delta^{13}C$ (i.e. with values closest to those of the Pacific ocean perch in British Columbia (this study) was bocaccio (*Sebastes paucispinis).* Coincidentally, Pacific ocean perch remnants were found in the stomach contents of the bocaccio (Bosley *et al*. 2004). The $\delta^{13}C$ values of Pacific ocean perch from this study are within the range reported for marine amphipods (-14.6‰ to -16.4‰), collected from the stomachs of owls, *Aegolius acadicu*, on the Queen Charlotte Islands (Hobson and Sealy 1991). Assuming 1–2‰ enrichment in $\delta^{13}C$ between trophic levels (DeNiro and

Epstein 1978), it is possible that Pacific ocean perch and saw-whet owls from the Queen Charlotte Islands share a common habitat source of organic carbon (Hobson and Sealy 1991).

Larval fish from water masses on adjacent continental slope and continental helf regions of British Columbia sampled in spring 1992, indicated depletion of $\delta^{13}C$ in the food web in the slope region relative to the food web in the shelf region (Perry *et al.* 1999). In comparison to $\delta^{13}C$ data reported in the literature, the $\delta^{13}C$ signatures of Pacific ocean perch (lipid normalized muscle tissue) in this study were relatively enriched (less negative). In marine consumers, this is associated with inshore or shelf sources of organic carbon.

A long-lived rockfish would probably reflect an integration of isotopic signatures over a long period of time (Bosley *et al*. 2004). However, because the $\delta^{13}C$ values in this study are at the limit of the range reported in the literature for this coast, it is unlikely that this represents a mixture of distinct signatures. Despite the main adult habitat of this species being the continental slope, the source of organic carbon in Pacific ocean perch in British Columbia originates predominantly in continental shelf or inshore food webs. This is supported by reports of summer inshore feeding migrations (Love *et al.* 2002)

2.5.4 Spatial Variability of Food Safety

Spatial variability of concentrations which exceed regulatory limits is shown in Figure 2.13. Additionally the results of exposure scenarios for different population subgroups (Figures 2.11 and 2.12) are based on different tolerable intakes. The tolerable intake for pregnant women and children is for MeHg and is calculated on the basis of daily consumption. This differs from that of non-pregnant adults, whose tolerable intakes of mercury are determined on a weekly basis. Figure 2.8 and Appendix B show that estimated MeHg concentrations in a large proportion of fish samples collected from Hecate Strait would exceed the tolerable daily intake for children and pregnant women in a single serving. The proportion of samples that exceed tolerable intake quantities reflects the concentrations of the inliers. The concentrations of THg vary between regions, as do their potential food safety concerns.

On the other hand, concentrations of THg have to be relatively high to exceed tolerable intakes for non pregnant adults in a single serving. In this study only a small proportion of fish sampled exceeded this quantity (Figure 2.13) all of which came from the WCVI region. In these cases, the specimens were outliers.

This is in contrast to the results of a survey of mercury intake, which estimated weekly dietary intakes of adults and children from two Canadian cities (Whitehorse and Ottawa) (Dabeka *et al.* 2003). The authors found that fish contributed to more than half of the ingested mercury, but all estimated daily intakes were well below both the PTWI for THg and the maximum recommended methylmercury intake of 0.20 μg/kg bw/day for children and women of child-bearing age (Dabeka *et al.* 2003). Other studies of marine fish utilizing weekly intakes for THg or MeHg have determined that mercury concentrations in the fish in question do not constitute a health threat. Examples include THg in perch (*Perca fluviatilis*) from the southern Baltic Sea based on weekly intakes (Szefer 2003), MeHg in fish and seafood products frequently consumed by Spanish people with a body weight >60 kg (Sahuquillo *et al.* 2007) and the dietary intake of mercury by children with high fish consumption from a German island in the North Sea (Wilhelm *et al.* 2003). These studies did not reveal a health risk due to Hg dietary intake, based on the PTWI for THg.

Adult exposure scenarios for Pacific ocean perch from HS and QCS in this study produce results very similar to studies of other species. For example, a study of hake (*Merluccius merluccius)* and striped mullet (*Mullus barbatus)* from the Mediterranean Sea reports estimated weekly intakes below the PTWI for THg (Storelli *et al.* 2005), despite consumption of methylmercury which was determined to be higher than the WHO safety limit. However, nine percent of the fish from WCVI would exceed this limit from a single serving. Children and pregnant women have a recommended daily intake due to their toxicological sensitivity. In this case, a single serving of many fish from this study would exceed the recommended daily intake.

The benchmark dose (BMD) is the dose of a substance that is expected to result in a pre-specified level of effect (Setzer and Kimmel 2003). Setting acceptable exposure levels of toxic substances with the use of BMDs is outside the scope of this thesis, but is discussed in Kodell (2009).

PTWIs for MeHg are based on an average BMD of 14mgkg^{-1} (14 µgg^{-1}) of mercury in maternal hair in the studies of neurodevelopmental effects in cohorts of children from the Faroe Islands and the Seychelles. The MeHg concentration in maternal blood equivalent to the MeHg concentration in maternal hair was calculated to be 0.056 mg/l (56 µgg^{-1}), which was subsequently calculated to occur at a daily MeHg intake of 1.5 µgg^{-1}/kg bw. The PTWI was derived by dividing this intake by a total uncertainty factor of 6.4 to give a value of 1.6 $\mu g g^{-1}$/kg bw. There are several studies of the neurodevelopmental effects of prenatal exposure to MeHg in cohorts of children. Most notably, a study from the Faeroe Islands (Grandjean *et al*. 1997) and the Seychelles (Myers *et al*. 2003). These two studies produced dissimilar results, which have been discussed by Lyketsos (2003). It is plausible that the opposing findings may be due to different doses produced by variations in the concentration of MeHg in the seafood consumed. This would not necessarily be apparent from concentrations in maternal hair (Lyketsos 2003). If the hypothesis that a small bolus dose of MeHg can be detoxified by a developing fetus (Myers *et al.* 2003) is true, and that a large bolus dose could be problematic for a developing fetus (Lyketsos 2003), then it would be advisable for pregnant women and young children to avoid consumption of the fish that were identified in this study as outliers. The findings of this chapter are significant, because 25% of the samples from the WCVI region had concentrations of THg that identified them as outliers.

Despite the assumption that 100 % of THg present in fish is meHg, the tolerable intakes for these contaminants differ. This may be because the PTWI for THg is somewhat redundant, as the meHg PTWI has been updated in light of scientific evidence. Therefore the THg PTWI may be more useful to foods that contain limited amounts of sea food. It is included in this thesis for comparison purposes.

Chapter 3: Generalized Additive Models of Mercury and Arsenic Concentrations in *Sebastes alutus* from British Columbia

3.1 Abstract

Potential concern for public health in relation to contaminants in fish justifies scientific interest in patterns of contaminant concentrations and accumulation. This study used Generalized Additive Models to examine linear or nonlinear patterns of total mercury and total arsenic concentrations in a slow growing deep water rockfish from the continental slope of British Columbia.

After outliers were identified and removed, the effects of age, body size (length and mass) and food web structure ($\delta^{13}C$ and $\delta^{15}N$) on the variability of mercury and arsenic concentrations in Pacific ocean perch (*Sebastes alutus*) was examined. These effects were assessed within three fishing regions; Hecate Strait, Queen Charlotte Sound and the west coast of Vancouver Island.

Statistical models explained a larger proportion of the variability of mercury concentrations relative to total arsenic. Model results from two of the three fishing regions (Hecate Strait and Queen Charlotte Sound) suggest a nonlinear plateau effect on mercury concentrations as an effect of body size. The effect of body size on arsenic concentrations was inconsistent between regions. Age had a significant positive effect on mercury concentrations in all three regions. This effect was significant but nonlinear on arsenic concentrations in samples from Hecate strait and the west coast of Vancouver Island fishing regions. The results of this study also suggest an effect of of organic carbon

sources on arsenic concentrations, i.e., fish with stronger links to the continental slope or inshore food webs (relatively enriched $\delta^{13}C$ signatures) had greater arsenic concentrations. Food web structure did not appear to be an important regulator of mercury concentrations.

3.2 Introduction

Potential concern for public health in relation to contaminants in fish justifies scientific interest in patterns of contaminant concentrations and accumulation. Concentrations of THg and TAs in marine fish have been linked to multiple biological variables including fish size, trophic position and age (age has been linked to THg, but not TAs concentrations). These patterns often involve simple linear regression models, which illustrate the effect of one biological variable at a time on THg or TAs in fish.

Generalized additive models (GAMs) are non-parametric modifications of generalized linear models (GLMs), where each predictor term is included in the model as a non-parametric smoothing function (Hastie and Tibshirani 1990). There are two major differences between GAMs and GLMs. The first is that GAMs are not constrained to any parametric form. GAM models are more flexible as they do not include any assumption about the shape of the function being estimated. The second is that the response variable is modeled as the sum of the functions of each predictor, thus the term additive. As THg and TAs accumulate in fish, there is a clear biological rationale for the use of models that are additive. GAMs are used to examine the effect of several individual biological variables. Ontogenetic changes in the biological variables which influence trace metal concentrations may cause the rate of accumulation to fluctuate. The flexibility of these models will allow non linear patterns associated with biological variables to be visible.

In this Chapter I use GAMs to explore the variability in THg and TAs concentrations within three fishing regions, by describing the effect of several biological variables in Pacific ocean perch on concentrations of THg and TAs. There are several expected patterns of THg associated with biological variables in fish. These include positive relationships between Hg and body size, age and trophic position (Gray 2002; Storelli *et al.* 2002; Adams 2004). Arsenic does not biomagnify, it biodiminishes. In contrast to mercury, concentrations of TAs are expected to decrease when trophic position (inferred by $\delta^{15}N$) increases. The relationships between TAs and age and body weight of fish have been inconsistent between studies and species. There are few expectations for these patterns in Pacific ocean perch. The only example consistently reported in the scientific literature is a negative relationship between TAs concentration and trophic position (Wagemann *et al.* 1978).

3.3 Methods

Sample and data collection is described in Chapter 2. The samples identified as outliers in Chapter 2, were not included in these modelling assessments. Age, length, mass, $\delta^{13}C$ and $\delta^{15}N$ data was used to model the THg and TAs concentrations in muscle tissue of Pacific ocean perch from each of the three fishing regions (HS, QCS, WCVI) using Generalized Additive models (GAMs) (Hastie and Tibshirani 1990). The analysis presented here was performed using the "mgcv" package in R, which is available from the Comprehensive R Archive Network, (http://cran.r-project.org). A further description of GAM methods and their applications can be found in the literature (Hastie and Tibshirani 1990; Wood 2006; Zarauz *et al*. 2008).

The fit of each GAM was measured by deviance and Akaike information criterion (AIC). The explanatory terms were age, length, mass, $\delta^{13}C$ and $\delta^{15}N$. A GAM was created for every possible combination of these terms and selection of the 'best' model was primarily based upon the (AIC). If the difference in AIC between models was very small (a fraction) then the % deviance and number of significant terms were also considered. For both THg and TAs in each region, the AIC, percentage of deviance in concentrations explained by each GAM and the significant covariates are shown in Appendix B. (Tables B.1 (HS); B.2 (QCS) and B.3 (WCVI) for THg and Tables B.4 (HS); B.5 (QCS) and B.6 (WCVI) for TAs. The fit of models with and without particular terms were used to assess the contribution of each predictor term, and its inclusion in the best fitting model was evaluated. Each predictor term included in the best fitting model was visualised with a smooth. Each smooth represents the effect of the predictor term on concentrations of THg or TAs. These are illustrated by partial plots of the smooth, with 95% confidence intervals and Pearson residuals.

For the purpose of this thesis, $\delta^{15}N$ values from Pacific ocean perch are only used in Chapter 3. This chapter incorporates the data into Generalized Additive Models (GAMs) for each fishing region separately and I make the assumption that all of the fish from each fishing region are part of the same food web. All of the fish from the WCVI region were collected by a single fishing vessel and were landed on the same day. This is also the case for fish from Hecate Strait. The fish from Queen Charlotte Sound were collected from multiple fishing trips.

Biological variables are often correlated to each other. Variables associated with bioaccumulation such as body size, trophic position and age are all closely linked. For example, ontogenetic diet shifts have been documented in both predatory (Estrada *et al*. 2006) and herbivorous (Clements and Choat 1993) species of marine fish. There are also correlations between body size and trophic level (Estrada *et al*. 2006) and distinct size-based feeding strategies in several species of marine fishes (Scharf *et al*. 2000). When explanatory variables in GAMs are correlated to each other, concurvity (the nonparametric analogue of multicollinearity) occurs. Concurvity has been problematic in GAMs implemented in S plus (Ramsay *et al*. 2003). If concurvity is present between predictor terms, GAMs produce confidence intervals that are too narrow and P-values that are misleading as a result of downward biased variance estimates. Fortunately, these problems can be avoided by using R. The mgcv package in R produces GAMs that do not use the same approximations which cause the problem in S-Plus (Wood, S. University of Bath, personal communication, 2007; Wood 2008) and offers a reliable way of fitting generalized additive models.

Linear correlations are included for the purpose of comparison with the literature (past present or future) but are considered restrictive in a study which uses models with greater flexibility to investigate a variety of patterns.

GAMs are specialized forms of regression, where smooths are functions that are used to describe the relationship between a predictor variable and a determinant (X and Y). Essentially smooths are used to fit a curve to the relationship between Y an X that is not restricted to a specific model structure (such as linear).

3.4 Results

The best fitting GAMs of concentrations of THg and TAs were identified within each region (Tables 3.1 and 3.3). Smooths of the effect of each covariate, plotted against the value of each explanatory variable are shown (Figures 3.0 to 3.13), i.e., the additive effect of the variable was conditional on the covariates listed in Tables 3.1 and 3.3, being include in the model. A summary of the statistically significant predictor terms in the GAMs from each region with the best fit are shown in Tables 7 and 9.

3.4.1 THg results

In all three regions, GAM results indicate that THg concentrations are best represented by a model which includes the explanatory covariates of age, $\delta^{13}C$, and a measure of body size (length or mass) (Table 3.1).

Correlations between THg concentrations and stable isotope ratios are consistently weaker than those between THg and age, length or mass, across all three regions (Table 3.2).

Table 3.1 GAMs with the best fit to THg concentrations in Pacific ocean perch from each region. HS = Hecate Strait, QCS = Queen Charlotte Sound and WCVI = the west coast of Vancouver Island. Terms are significant if $p<0.05$, terms in parenthesis are included if $0.05< p <0.1$ as weakly significant. A=age, L=length, M=mass, C= $\delta^{13}C$, N= $\delta^{15}N$.

Region	Explanatory variables	Sig terms	Deviance explained (%)	n
HS	~s(A and L and $\delta^{13}C$)	A	37	19
QCS	~s(A and M and $\delta^{13}C$)	M	42.8	102
WCVI	~s(A and L and M and $\delta^{13}C$ and $\delta^{15}N$)	A L M (C) N	80.2	35

Table 3.2 Pearson correlation coefficients (2 decimal places) between explanatory variables and THg concentrations (x) in inliers of Pacific ocean perch from three fishing regions in British Columbia. HS = Hecate Strait, QCS = Queen Charlotte Sound and WCVI = west coast of Vancouver Island.

	HS	**QCS**	**WCVI**	**mean**
Age	0.38	0.44	0.80	0.54
Length	0.19	0.61	0.66	0.486667
Mass	-	0.61	0.77	0.69
$\delta^{13}C$	-0.25	-0.13	-0.007	-0.129
$\delta^{15}N$	0.09	0.04	-0.27	-0.04667
mean	0.10	.314	0.39	

3.4.2 Smooths of biological variables, from Generalized Additive Models of THg concentrations

Age

Smooths are indicating the effect of age on THg concentrations show a positive linear relationship. This is consistent in GAMs from all three fishing regions (Figure 3.01).

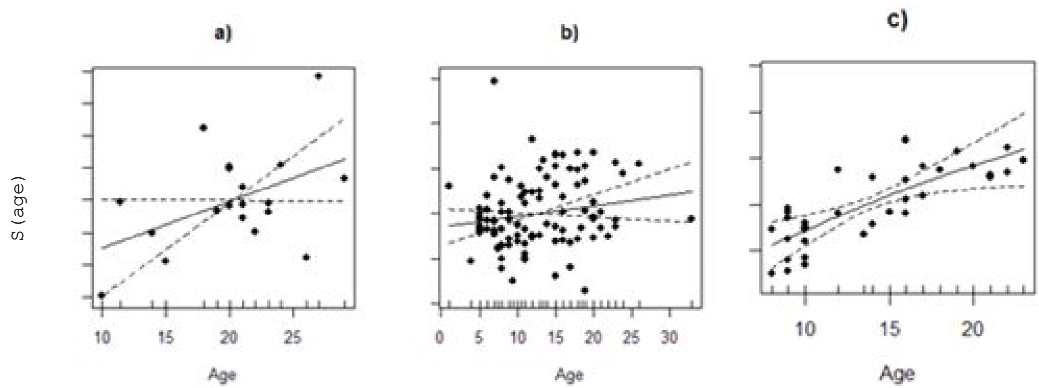


Figure 3.01 Results of Generalized Additive Models showing the smooth of age (years) from the best fitting generalized models (GAMs) of total mercury concentrations in Pacific ocean perch from a) HS, b) QCS and c) WCVI fishing regions. The solid line represents the smooth of age from models of THg concentrations; dashed line indicates 95% confidence intervals and data points are Pearson residuals. Age is a significant explanatory variable ($p<0.05$) in the GAMs from HS and WCVI, it is not significant in the model from QCS.

Body size (length, mass and mass to length ratio)

Smooths that relate to body size in the WCVI region, i.e., length and mass, show contrasting effects on THg concentrations. The smooth of length shows a negative linear relationship (Figure 3.02 b) and the smooth of mass is also linear, but positive (Figures 3.03 b) and 3.04 b). This model indicates that THg concentrations are greatest in short, heavy fish and lowest in long, lightweight fish. At any length, heavier fish have higher m:l, and at any mass, shorter fish have a higher m:l (Figure 3.06). Substituting mass and length for mass to length ratio (m:l) does not compromise the model (Table A3). THg concentrations increase linearly with an increase in m:l in this region (Figure 3.05 a).

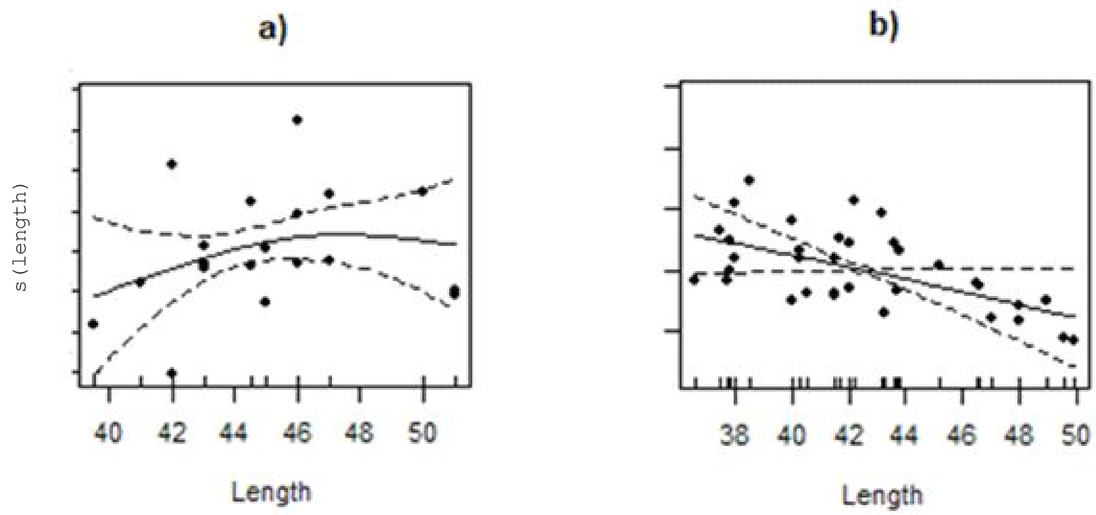


Figure 3.02 Results of Generalized Additive Models (GAMs) showing smooth functions of the effect of length (cm) on THg concentrations from the best fitting GAMs of THg concentrations in Pacific ocean perch from a) HS, b) WCVI fishing regions. The solid line represents the tensor product smooth of length in models of THg concentrations; dashed line indicates Bayesian 95% confidence intervals and data points are Pearson residuals. Length is a significant explanatory variable ($p<0.05$) in the WCVI GAM. Check marks on the x-axis indicate the density of data points for the covariate included in the model.

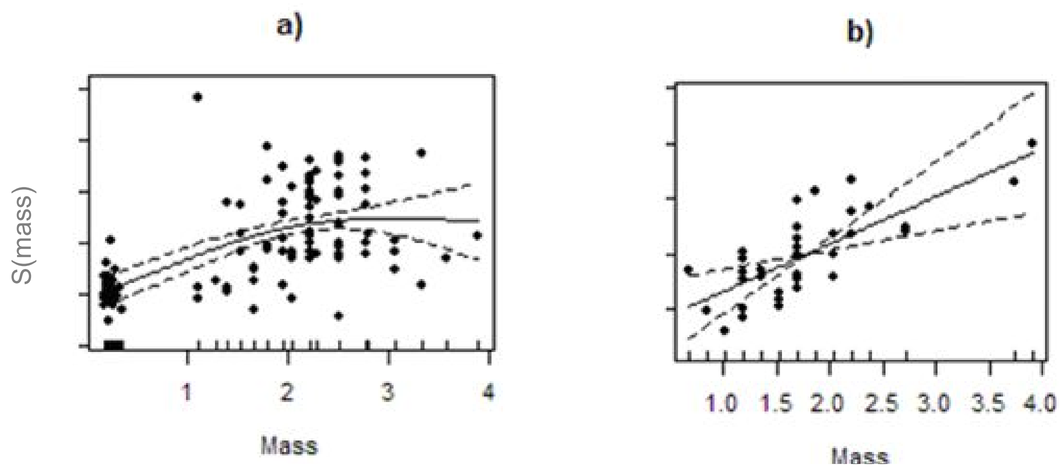


Figure 3.03 Results of Generalized Additive Models (GAMs) showing the smooth of mass (Kg) from the best fitting GAMs of THg concentrations in Pacific ocean perch from a) QCS and b) WCVI fishing regions. The solid line represents the tensor product smooth of length in models of THg concentrations; dashed line indicates Bayesian 95% confidence intervals and data points are Pearson residuals. Mass is significant ($p<0.05$) in the GAMs from QCS and WCVI regions.

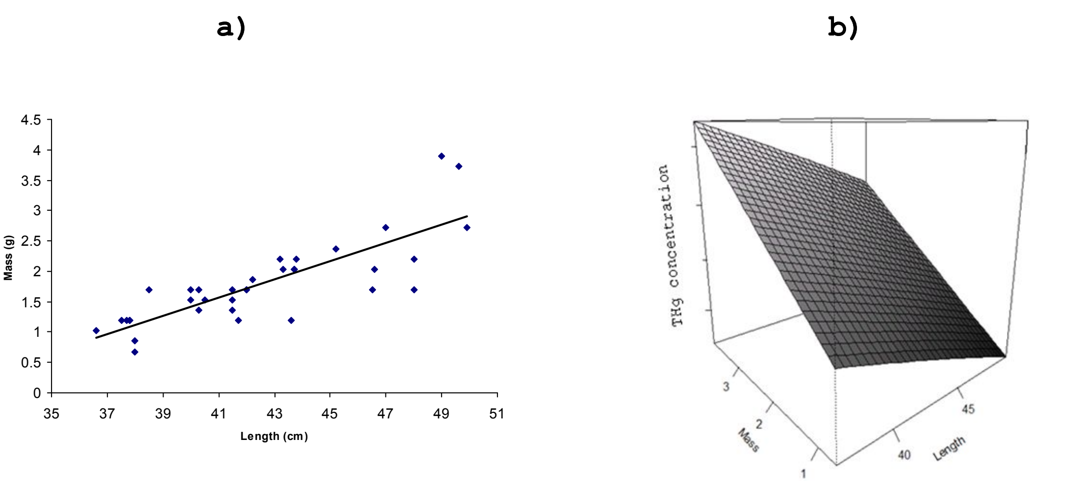


Figure 3.04 a) Plot of length versus mass in inliers of Pacific ocean perch from the WCVI region (correlation coefficient=0.81). b) Three-dimensional visualisation of the smooths of mass and length from the best fitting GAM of THg concentrations in *Sebastes alutus* from the WCVI. Both length and mass are significant ($p<0.05$) covariates.

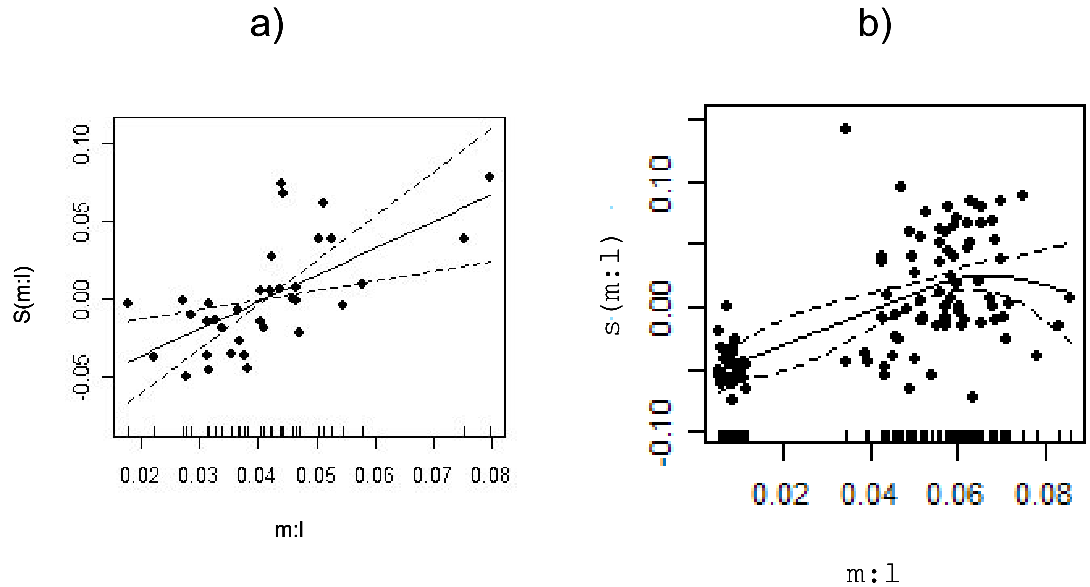


Figure 3.05 Estimated smooth of mass to length ratio (m:l) from generalized additive models of THg concentrations in Pacific ocean perch from a) WCVI and b) QCS fishing regions. Solid lines represent the effect of m:l on THg concentrations; dashed line indicates Bayesian 95% confidence intervals and data points are Pearson residuals. Mass to length ratio is a significant explanatory variable in both models ($p<0.05$). When substituted for mass and length, the fit of the model improves slightly (Table A3).

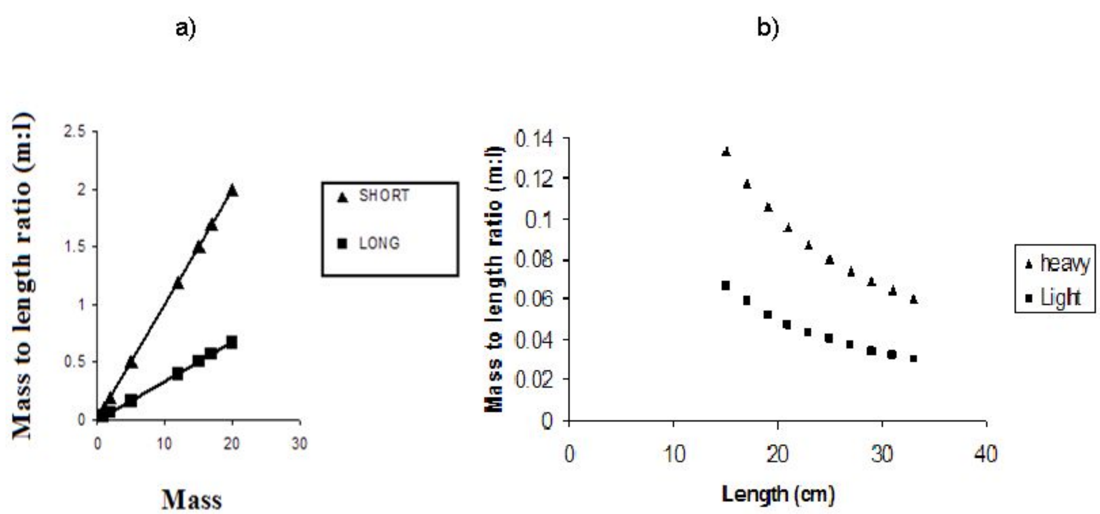


Figure 3.06 Hypothetical plots of m:l in large and small fish at different a) mass and b) length.

All of the smooths of covariates that relate to body size, (i.e., length and mass) in fish from the WCVI region are linear. However, equivalent smooths from QCS (mass and m:l) and HS (length) show similar nonlinear features; that is, relatively low THg concentrations in small fish, which increase in concentration to a maximum in mid sizes and appear to plateau in larger fish (Figures 3.02 a), 3.03 b) and 3.05 b).

Food Web

Smooths of the effect of $\delta^{13}C$ on THg concentrations do not show a consistent pattern between regions (Figure 3.07) and all of the smooths are relatively flat. The partial residuals are not evenly scattered around the smooth, or frequently within the 95% confidence intervals. As an explanatory covariate, $\delta^{15}N$ is only included in the best fitting model from the WCVI region. Residuals are not evenly distributed around the smooth, or well within the confidence intervals. The smooth shows a weakly significant negative linear effect (Figure 3.08).

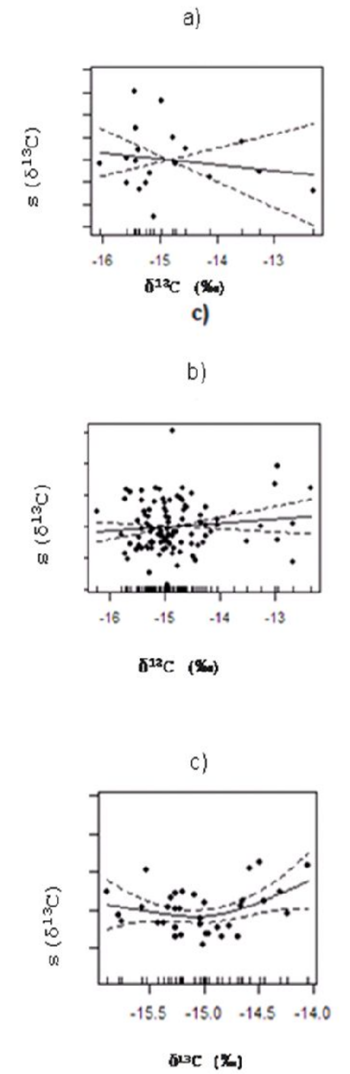


Figure 3.07 Estimated smooths of $\delta^{13}C$ from generalized additive models (GAMs) of total mercury (THg) concentrations in Pacific ocean perch from a) HS, b) QCS and c) WCVI fishing region. Solid line represents the smooth of $\delta^{13}C$ (‰) on THg concentrations; dashed line indicates 95% confidence intervals and data points are Pearson residuals. As an explanatory variable $\delta^{13}C$ is weakly significant ($p<0.1$) in the WCVI region.

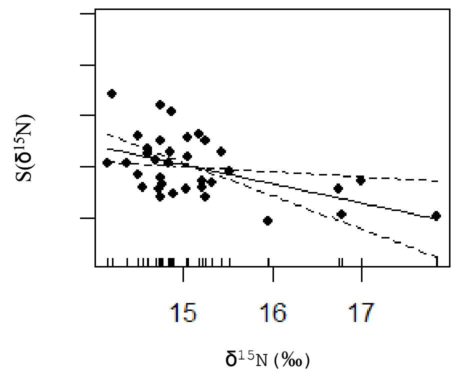


Figure 3.08 Estimated smooth of $\delta^{15}N$ from generalized additive model (GAM) of total mercury (THg) concentrations in Pacific ocean perch from WCVI. The solid line indicates the smooth; dashed line indicates 95% confidence intervals and data points are Pearson residuals. $\delta^{15}N$ is weakly significant ($p<0.1$).

3.4.3 TAs Results

The covariates included in the best fitting GAM of TAs concentration from each region are not consistent in all fishing regions. The proportion of deviance explained by these models varied from 18.9 to 71.2 percent (Table 3.2), and were lower in each region compared to the GAMs based on THg concentrations. Similarly, the correlation coefficients between each of the explanatory variables, with the exception of $\delta^{13}C$, were lower for TAs than THg concentrations (Tables 3.2 and 3.4) and overall the correlation between mercury and biological variables is greater than arsenic to biological variables on average .

Table 3.3 GAMs with the best fit to TAs concentrations in Pacific ocean perch from each region. HS=Hecate Strait, QCS=Queen Charlotte Sound and WCVI=the west coast of Vancouver Island. Terms are significant if $p<0.05$. Terms in parenthesis are included if $0.05< p <0.1$ and $p>0.05$ as weakly significant. A=age, L=length, M=mass, C= $\delta^{13}C$ and N= $\delta^{15}N$ model parameters of all models are shown in the Appendix.

Region	Model Terms	Sig terms	Dev (%)	n
HS	~s(A and L and $\delta^{13}C$)	A	71.2	19
QCS	~s(L and M and $\delta^{15}N$)	(M)	18.9	102
WCVI	~s(A and L and M)	L (M) (A)	37.4	35

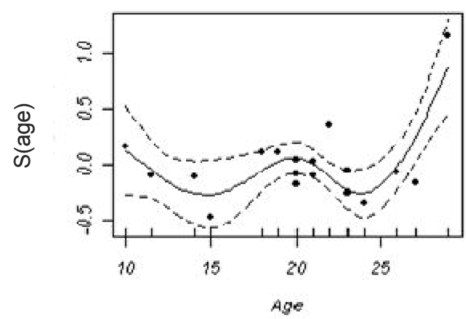
Table 3.4 Pearson correlation coefficients (2 decimal places) between explanatory variables and TAs concentrations (x) in inliers of Pacific ocean perch from three fishing regions in British Columbia. HS = Hecate Strait, QCS = Queen Charlotte Sound and WCVI = west coast of Vancouver Island.

	HS	**QCS**	**WCVI**	mean
Age	0.22	-0.11	0.44	0.18
Length	0.35	-0.28	0.38	0.45
Mass	-	-0.32	0.25	-0.07
$\delta^{13}C$	0.33	0.06	0.15	0.54
$\delta^{15}N$	0.19	-0.2	0.2	0.19
mean	0.2725	-0.17	0.284	

3.4.4 Smooths of biological variables, from Generalized Additive Models of TAs concentrations.

Age

a)



b)

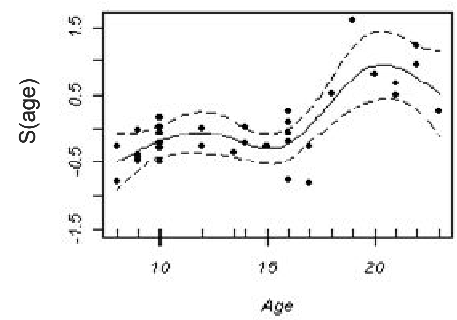


Figure 3.09 Smooths of the effect of age (years) from generalized additive models (GAMs) of total arsenic (TAs) concentrations from a) HS and b) WCVI. The solid line indicates the additive effect of age on THg concentrations; dashed line indicates 95% confidence intervals and data points are Pearson residuals. Age is significant ($p<0.05$) in HS and weakly significant ($p<0.1$) in WCVI.

Model results indicate effects of age on TAs concentrations from HS and WCVI. The smooths show similar, nonlinear characteristics in each of these regions. That is a pattern of peak-trough-peak-trough corresponding to 10,15,20 and 25 year old fish in both regions (Figure 3.09).

Body Size (length and mass)

Length is the only covariate included in the best fitting model of TAs concentrations from all three regions; however, the pattern of the effect of length is dissimilar (Figure 3.10). The residuals are not evenly distributed along the smooth and the residuals are not frequently within the confidence intervals. This is also the case for the smooths of mass from HS and QCS. The residuals are unevenly distributed and show a dissimilar pattern between regions (Figure 3.11).

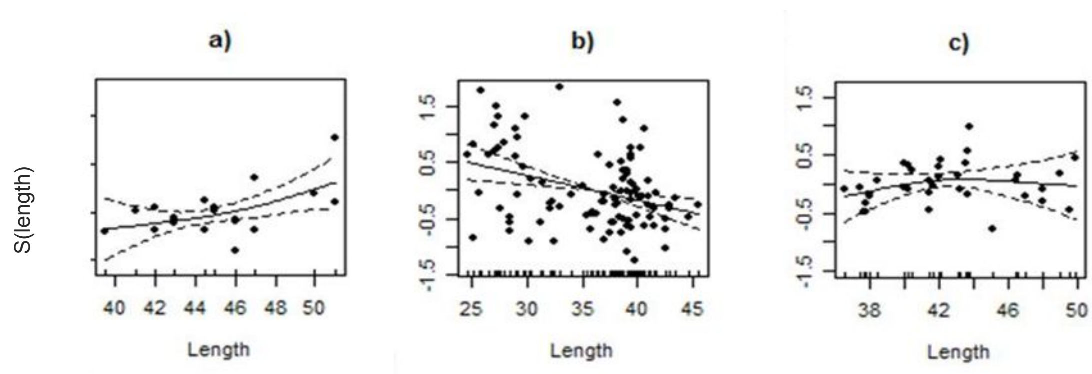


Figure 3.10 Smooths of length (cm) from generalized additive models (GAMs) of total arsenic (TAs) concentrations in Pacific ocean perch from a) HS, b) QCS and c) WCVI. The solid line indicates the smooth of length on THg concentrations; dashed line indicates 95% confidence intervals and data points are Pearson residuals. Length is not significant in any of these GAMs ($p<0.1$).

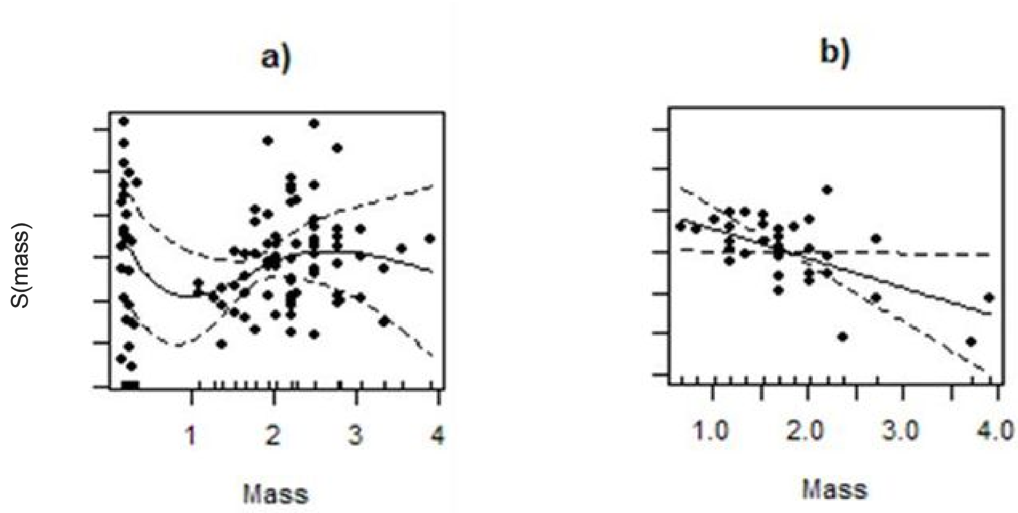


Figure 3.11 Smooths of mass (Kg) from generalized additive models (GAMs) of total arsenic (TAs) concentrations in Pacific ocean perch from a) QCS and b) the WCVI fishing region. The solid line indicates the smooth of length on TAs concentrations; dashed line indicates 95% confidence intervals and data points are Pearson residuals. Mass is weakly significant in both of these GAMs ($p<0.1$).

Food web

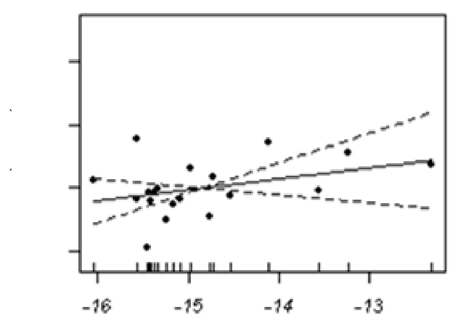


Figure 3.12 Smooth showing the additive effect of $\delta^{13}C$(‰) on total arsenic (TAs) concentrations in Pacific ocean perch from a generalized additive model (GAM) of total arsenic (TAs) concentrations in specimens from the HS fishing region. The solid line indicates the smooth of length on TAs concentrations.

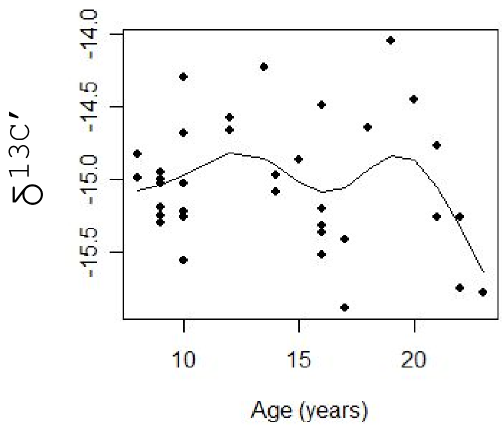


Figure 3.13 Smoothing spline of $\delta^{13}C$' against age in inliers of Pacific ocean perch from the WCVI fishing region.

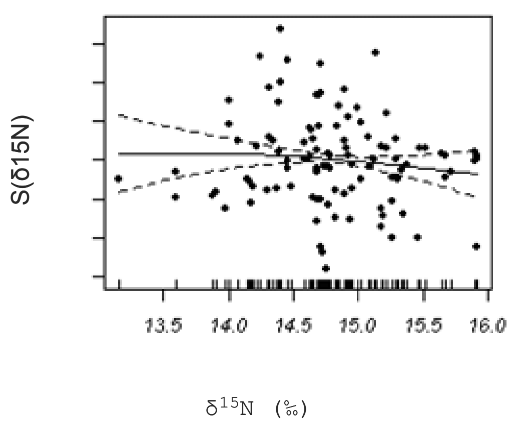


Figure 3.14 Estimated smooth of $\delta^{15}N$ from a generalized additive model of total arsenic concentrations in Pacific ocean perch from QCS. The solid line indicates the smooth; dashed line indicates 95% confidence intervals and data points are Pearson residuals. $\delta^{15}N$ is not significant.

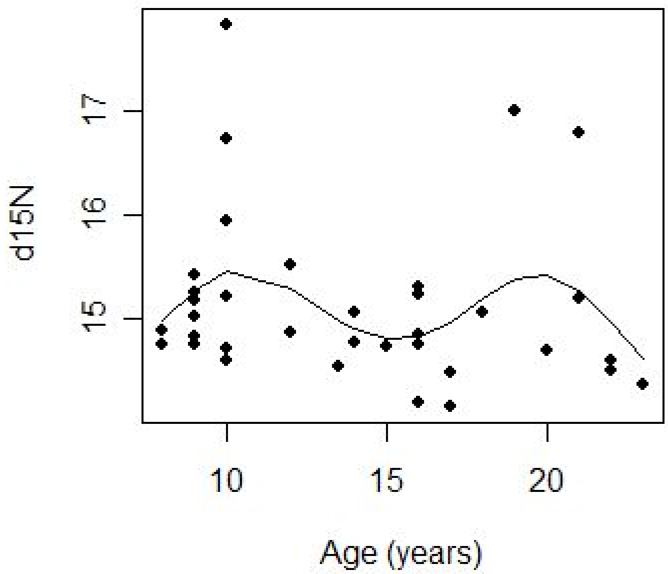


Figure 3.15 Smoothing spline of $\delta^{15}N$ against age in inliers of Pacific ocean perch from the WCVI fishing region.

3.5 Discussion

3.5.1 THg Discussion.

Age

Best fitting GAMs of fish from all three regions include age as an explanatory variable for THg concentrations. They all show a positive linear effect between THg concentrations and GAM plots. This result suggests that age has an important effect on variability in THg concentrations in this species. Age is significant in both the HS and WCVI GAMS, but not the GAM from QCS; suggesting that the effect of age in this region (QCS) should be treated with caution. The proportion of deviance explained by the complete GAM from the WCVI is 80.2%, which is considerably higher than the other two regions. The partial residuals were evenly distributed around the smooth of the effect of age in the WCVI GAM , indicating a well fitting model (Wood 2006). The smooth of the effect of age fits the data well and is a significant component of a model explaining a large proportion of the variation in THg in this region. The effect of age as shown in Figure 3.01c) is a reliable estimate of the effect of age on THg concentrations. Despite variability in the statistical significance of age in the models, the qualitative pattern of the effect of age is consistent across regions.

This positive relationship can be explained by two basic concepts; firstly, by the simple "exposure-over-time hypothesis". This theory suggests that fish which have lived longer have higher THg concentrations because accumulation has occurred over a longer time period. This is supported by reports of large fish with elevated concentrations of THg, which are often species with long life spans (Storelli *et al.* 2002). Long lived fish appear to have a tendency to exhibit high MeHg levels (Kelly *et al*. 2008) and a long life may, in fact, be necessary for fish to accumulate very high concentrations of THg. This is called the "life span effect" (Cai *et al*. 2007).

Secondly, models of bioenergetics processes describe THg in terms of physiological or bioenergetics changes in fish. These models include changes in rates of growth, metabolic activity, ingestion or excretion rates (Braune 1987 and Trudel and Rasmussen 2006) and their general applicability has been demonstrated (Braune 1987). Bioenergetics models show that mercury concentrations increase with age and size, either because older and longer fish feed on more contaminated prey, or because growth efficiency decreases with age or size.

If concentrations of THg in food are known, the bioenergetics of fish can be assessed independently of prey contamination (Trudel and Rasmussen 2006; Rowan *et al*. 1998; Thomann 1981). This enables bioenergetics models to provide an alternative explanation for increasing THg concentrations with age

The positive linear association between age and THg concentration is not an unexpected finding. This association has been documented in other studies of marine fishes; for example, muscle THg concentrations are also positively correlated with age class in Atlantic herring from the Bay of Fundy (Braune 1987) and several other fish species from the continental slope of the North Atlantic (Cronin *et al*. 1998). Similarly, monitoring programmes of contaminants in rockfish from Puget Sound, USA, report increasing mercury concentrations with age (West and O'Neill 2001).

Body Size (length, mass and m:l)

The best fitting models of THg concentrations from all three regions include a measure of body size, either length or mass. However, length and mass smooths from the WCVI region show contradictory effects. This apparent paradox can be explained by mass to length ratio (m:l). For the range of sizes found in this study: at any mass, shorter fish have a higher m:l, as do heavier fish at any length. In fish from the WCVI region, THg concentrations are higher in fish with a higher m:l. Smooths of the effect of both mass and m:l on THg concentrations from the WCVI show positive linear associations with THg concentrations and. This suggests that as fish become heavier, their THg concentrations increase specific growth rate decreases faster than ingestion rate with increasing size (Trudel and Rasmussen 2006.

Positive relationships between fish length and THg concentrations are the pattern most commonly found in the scientific literature, and are generally regarded as the norm. (Gonul and Kucuksezgin 2007). Statistically significant examples of this pattern include, blackfin tuna (*Thunnus atlanticus)*, yellowfin tuna (*Thunnus albacares)*, carcharinid sharks from the Gulf of Mexico (Cai *et al.* 2007), several species of deep sea fishes from the north Atlantic (Cronin *et al*. 1998), pacific hake (*Merluccius productus)* from the north east Pacific (Cutshall 1978), sea bream (*Mullus barbatus*) from the eastern Aegean (Gonul and Kucuksezgin 2007) and Atlantic herring, (*Clupea harengus)* from the Bay of Fundy, Canada (Braune 1987). Similarly, mercuy concentrations increased with the size of *S. alutus* in all the sampled regions of this study. However, the use of GAMs in this study suggests that the positive length – THg association, requires mass to increase relative to length; in other words, a THg increase occurs if the m:l increases with length.

Food Web ($\delta^{13}C$ and $\delta^{15}N$)

The inclusion of $\delta^{13}C$ as an explanatory covariate improves the fit of models of THg concentrations in all three regions (Table 3.1). However, $\delta^{13}C$ was weakly significant at $p<0.1$ in the GAM from the WCVI region (Table 3.1, Figure 3.07). Residuals are not evenly distributed around the smooths of the effect of $\delta^{13}C$, indicating a weakness in this part of each model. The lack of a discernable functional form relating differences in $\delta^{13}C$ to THg, suggests that its usefulness as an explanatory variable in this study is limited. This finding is similar to that of Jarman *et al.* 1996, and Cai *et al.* 2007. Cai *et al*'s study reported $\delta^{13}C$ values which could be used to distinguish between groups of fish with common carbon sources or prey resources. The groups identified, however, did not appear to be linked to Hg bioaccumulation. The range of $\delta^{13}C$ values in this thesis is similar to the range reported by Cai *et al* (2007) and my results support the hypothesis that the $\delta^{13}C$ values of Pacific ocean perch explain little of the variation in THg explained by the covariates. This is conditional on the other variables being included in the model (Table 3.1). The possibility of over-fitting should be considered.

As an explanatory covariate, $\delta^{15}N$ was included in the best fitting GAM from the WCVI region only. Including $\delta^{15}N$ did not improve the fit of GAMs of THg concentrations in Pacific ocean perch from either HS or QCS (Tables A1 and A2). Residuals were not evenly distributed around the smooth, indicating a poor fit. Nevertheless, the smooth of the effect of $\delta^{15}N$ from the WCVI GAM indicates a linear, weakly negative association (Figure 3.08); suggesting that fish with relatively shorter food chains have higher THg concentrations. This is an unusual finding; as has been widely reported, mercury biomagnifies within aquatic food webs (Jarman *et al.* 1996; Atwell *et al.* 1998; Gray 2002; Bank *et al*. 2007; Cai *et al.* 2007). These findings were based on organisms from multiple trophic levels and included a wider range of $\delta^{15}N$ values than this study. However, $\delta^{15}N$ values reported by Bank *et al*. (2007) covered a similar range of values, but were pooled data from two species. The $\delta^{15}N$ data in this study is from a single species. Compared to others, this study shows a relatively short range of $\delta^{15}N$ values associated with large variation in THg concentrations. Therefore the lack of a significant general trend in $\delta^{15}N$ associated with THg concentrations in this species, does not indicate that biomagnification is not occurring. Rather, it is difficult to detect.

3.5.2 TAs Discussion

As previously discussed in relation to mercury, bioenergetics (or kinetic) models provide a unifying conceptual framework for trace metal accumulation in aquatic organisms. Steady state models based on the balance between physiological parameters and trace metal accumulation, include rates of metal influx rates from food and water, and constants of loss and growth (Reinfelder *et al.* 1998; Luoma and Rainbow 2005

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Age

Age, as an explanatory variable of arsenic concentrations, improves the fit of models from HS and the WCVI region. Figure 3.09 show similar patterns of the effect of age in both models. The age specificity of each peak suggests that whatever factor influenced these peaks was during the part of the life cycle when fish of similar ages are relatively isolated from other year classes in the ecosystem. This most likely occurs during the first three years after hatching. During this time, Pacific ocean perch inhabit the continental shelf rather than the continental slope (Love 2002). The peaks in TAs concentration in HS and WCVI specimens are in fish which were approximately 10, 20 and 30 years of age at the time of death, thus, the peaks in TAs concentrations occurred in fish that inhabited the continental shelves of HS and WCVI during approximately 1974 to 1977, 1984 to 1987 and 1994 to 1997. Interestingly, all of these periods included La Nina events. However, there does not appear to be a link between the years that the juvenile Pacific ocean perch with peak TAs concentrations inhabited the continental slope, and atmospheric or ocean climate conditions reported in the north Pacific during this period. These include the North Pacific Index (Francis and Hare 1994) indices for the Pacific Decadal Oscillation (PDO) and El Niño Southern Oscillation (ENSO) (Hare and Mantua 2000).

Studies of trace elements in fish do not always include age determination, and the number of studies of arsenic concentrations in marine fish is relatively low (compared to studies of other elements such as mercury). This increases the difficulty of comparing the association found in this thesis to other studies which found that the relationship between arsenic concentration and age was not statistically significant. There was no statistical significance, for example, between these variables in fish from an Australian estuary (Maher *et al.*1999) and in the North Sea (Falconer *et al*. 1993). It is possible that climatic signals may be seen in increased arsenic concentrations, possibly as a result of increased uptake of arsenate instead of phosphate by phytoplankton. This hypothesis is outside the scope of this thesis. There is a lot of unexplained variation in these models, so that the pattern seen is interesting, but not particularly strong.

Body size

The patterns between the concentrations of TAs and length and TAs and mass in Pacific ocean perch differ in each of the three fishing regions (Figures 3.10 and 3.11). This corresponds well with other studies of TAs in fish, where contrasting patterns of TAs concentrations in different species within a genus were observed across different sampling locations (Edmonds and Francesconi 1981).

Food web

Fish that may obtain a greater proportion of their organic carbon from sources derived from continental shelf or inshore food webs (i.e., $\delta^{13}C$ is relatively enriched) appear to have higher TAs concentrations. This effect of $\delta^{13}C$ is illustrated by the trend of the smooth of $\delta^{13}C$ in the best fitting TAs model from HS . This shows that TAs concentrations increase when $\delta^{13}C$ values are enriched. HS is the only region where $\delta^{13}C$ is included in the best fitting GAM (Table 3.3). However, the hypothesis that $\delta^{13}C$ depletion is associated with increased background concentrations of TAs is also supported by the finding of peak $\delta^{13}C$ values at ages associated with peak TAs in samples from the WCVI region.

Fractionation of stable isotopes of carbon is linked to conditions that occur during photosynthesis at the base of the food chain. This finding suggests a possible link between primary production on continental shelves and TAs concentrations in marine fish, in the context of the current understanding of arsenic accumulation in marine organisms. This is a reasonable suggestion which complements existing knowledge of the biogeochemical cycle of arsenic. Evidence that supports this includes observations of arsenic concentrations that are highest in herbivores (Wagemann *et al.* 1978). However, size dilution may also be involved.

The range of $\delta^{13}C$ values reported in this thesis compared to the scientific literature (Hobson and Sealy 1991; Hobson *et al*. 1994; Perry *et al.*; 1999Bosley *et al*. 2004) confirm that in this study the $\delta^{13}C$ values associated with relatively higher TAs concentrations are linked to inshore/shelf sources of organic carbon. I am not aware of any other studies of commercial marine fish that examine ratios of stable isotopes of carbon and arsenic concentrations, consequently the finding of this association requires further investigation.

δ^{15}N-arsenic relationship

Although not statistically significant, the slight negative association between δ^{15}N and concentrations of TAs, supports other studies which report that arsenic does not biomagnify (Edmonds and Francesconi 1981; De Gieter 2002) and suggest that arsenic biodiminishes. That is, animals at the top of the food chain are predicted to carry lower burdens than animals at the bottom, for example, predators < prey; piscivores < planktivores) (Wagemann *et al.* 1978). The range of δ^{15}N values in fish reported in this thesis is narrow, which make discerning any trends difficult.

Chapter 4: General Conclusions

The overarching theme of this thesis has been to investigate the variability of THg and TAs in Pacific ocean perch, within the context of the biological variables of this fish and across different regions of the Pacific Coast. The scientific outcomes of this work can be divided between two main objectives, which make up Chapters 2 and 3. The first objective was to examine the variability in concentrations between regions (Chapter 2) while the second objective was to examine the variability within regions (Chapter 3). Each chapter included several sub-questions.

Chapter two examined the variability between regions and raised three main research questions;

1. How do concentrations of THg and TAs in pacific ocean perch vary spatially?
2. Does the spatial variation of THg and TAs reflect biological variation?
3. Does the spatial variation of THg and TAs have public health implications?

Chapter three used Generalized Additive Models to examine the patterns between THg and TAs concentrations of Pacific ocean perch within each region. Combinations of age, length, mass, $\delta^{15}N$ and $\delta^{13}C$ were used to qualitatively model the effect of each variable on concentrations of both THg and TAs. By visualizing the patterns between each of these variables and the concentrations of THg and TAs, Chapter 3 examined the following sub-questions:

1. How are concentrations of THg associated with age, body size and food web structure of Pacific ocean perch?

2. How are concentrations of TAs associated with age, body size and food web structure of Pacific ocean perch?

The current chapter (Chapter 4) provides a general conclusion to the thesis. The outcome of each sub question is summarized, compared to previous findings in the field, and the contributions of the thesis to the scientific literature are highlighted.

4.1 Spatial Variability

4.1.1 Spatial variability of THg and TAs concentrations

The question of spatial variability in concentrations of THg and TAs was investigated by comparing concentrations from different regions (Chapter 2). The results showed statistically significant differences in median concentrations between regions. Mean concentrations were also reported. Differences between mean concentrations from each region in this thesis are similar to the differences observed in other studies (Chvojka *et al.* 1990; Davis *et al.* 1997; Teeny *et al,* 1984; Greig *et al*. 1977). Sablefish, *Anoplopoma fimbria*, for example, from different regions off the west coast of North America, show differences in mean THg concentrations of up to 0.56 μgg^{-1} (Hall *et al*. 1976). Lower mean differences between regions (0.002 μgg^{-1}) have also been reported in Pacific whiting from the Northeast Pacific (Teeny *et al.* 1984). Consequently, differences between means in each fishing region in this thesis are comparable to the range reported in the literature for marine fish, despite differences in the geographic scale, locations and migratory behaviours between these studies.

Studies of mercury in sablefish and Pacific halibut (*Hippoglosses stenolepis*) from the west coast of North America found that mean THg concentrations in these fish increased from north to south (Hall *et al*. 1976a; Hall *et al*. 1976b). The geographic range of this study was restricted to British Columbia and is much smaller than the range of the sablefish and halibut studies (Hall *et al*. 1976a, 1976b). If one considers the mercury concentrations of the outliers in this thesis, one could also infer qualitative north to south variations; however, a larger data set would be required to confirm these qualitative observations; i.e., with an increase from north to south.

Findings of the studies of sablefish and halibut contrast with results from a study of Pacific hake (*Merluccius productus*) (Cutshall *et al*. 1978). Cutshall's study found a positive correlation between latitude and THg concentration (i.e., concentrations decreased from north to south) within the same geographic area. These studies took place during the 1970s and did not include multiple sampling locations within British Columbia. This thesis updates the knowledge of the spatial variability of THg concentrations in commercial marine fish from British Columbia which is otherwise absent from the scientific literature.

In addition to comparisons of concentrations between locations, Chapter 2 used a statistical outlier determination procedure. This approach, where outliers are considered to be the result of an exotic process and inliers represent the natural background variation, has previously been used in geochemical studies (Filzmozer *et al*. 2005). The finding of outliers with elevated concentrations of both THg and TAs that occur predominantly in fish from the WCVI region, suggests that this region may contain a hot spot. Similar patterns of THg concentrations in marine fish from areas known to have elevated environmental concentrations support this finding. Further support is provided by reports of unusually high concentrations in other marine biota from the WCVI region (Elliot and Scheuhammer 1997).

The use of outlier detection methods to identify potential hot spots from biological data is a potentially useful contribution to the field of environmental monitoring that may be employed in other geographic locations. This study utilizes a robust method where the data structure is used to characterize background variation and identify specimens that may be influenced by additional processes and/or a hot spot. Additionally, this is the first report (to my knowledge) of a correlation between THg and TAs concentrations in marine fish with elevated concentrations of these elements.

4.1.2 Biological variability between regions.

The patterns of spatial variation in concentrations, considered in relation to biological factors of the fish are complex. In this study (after outliers were excluded), the fishing region with the oldest and longest fish (mean values) had the highest mean THg concentrations. This pattern does suggest that the spatial variability of THg concentrations reflect the age and size of the fish to some degree; however, because these were wild–caught, marine fish, there are many more factors or variables that were not measured. While the observed association supports the hypothesis, it does not prove a causal relationship. Nevertheless, in this study, mean THg concentrations of Pacific ocean perch from regions of British Columbia, are related to fish age and length.

In the scientific literature, a similar pattern was not found in snapper (*Chrysophrys auratus: Sparidae*) in Australian waters (Chvojka *et al.* 1990) nor was it found in a study of black bellied redfish, (*Helicolenus dactylopterus)* from Atlantic

Ocean waters off the north-eastern coast of the United States (Greig *et al.* 1977). However, other species of wild marine fish do show a pattern where lower mean THg concentrations in fish were found in locations where fish size was smaller. Examples include tusk (*Brosme brosrae*) and spiny dogfish (*Squalus acanthias*), from locations in the north west Atlantic Ocean (Greig *et al.* 1972). A study of THg in Pacific hake from the north east Pacific Ocean, found that at any latitude, larger fish had higher THg (Cutshall *et al*. 1978). However, as discussed by Cutshall *et. al.*, size is also related to location. Because size and location may not be independent, conclusions based solely on location of wild caught fish are extremely difficult to make.

Unlike this study, others did not determine the age of fish, so that this was not compared between regions. The scope of the reported biological data included in this thesis ensures that the conclusions yielded are applicable to more than the particular site or time when the data were taken. Pacific ocean perch are slow growing, demersal and inhabit the continental slope of the west coast of North America (DFO 1999; Munk 2001). This study is the first to document THg and TAs concentrations of a demersal slope species from multiple fishing regions of British Columbia.

4.1.3 Variability in Public Health implications between regions

The Provisional Tolerable Weekly Intake (PTWI) is used in studies of mercury concentrations in seafood as a method of risk assessment. This process characterizes the degree and nature of risk which can subsequently be used to determine the need (if any) for risk management. In this thesis none of the exposure scenarios for fish from HS exceed the PTWI for either THg or MeHg, when consumed once per week by non pregnant adults' (≤ 60 kg). This is similar to results reported for a variety of fish consumed in both Spain (Sahuquillo *et al.* 2007) and Poland (Szefer *et al.* 2003; Usydus *et al.* 2009).

Compared to studies which utilize the PTWI for MeHg, Pacific ocean perch sampled from HS compare favourably in terms of food safety for adults. This differs from the exposure scenarios for children and pregnant women in this thesis. These groups represent the most sensitive toxicological endpoints for MeHg and in recognition of this, the tolerable intakes for these groups are lower and expressed in terms of daily consumption (instead of weekly). Exposure scenarios for these sensitive groups indicate that recommended daily intakes of THg and MeHg could be exceeded from a single serving of the majority of fish from each of the three fishing regions in this study. This finding contrasts with the findings of a diet study of children living on a North Sea Island (Amrum), where less than 25% of the children involved in the study had intakes in excess of the PTDI for MeHg on days that fish were consumed and none exceeded the PTDI for THg (Wilhelm *et al.* 2003). Overall, enhanced monitoring and assessment would be wise in British Columbia to ensure children and pregnant women are not adversely affected by excess Hg in their diets. WCVI is the only region in this thesis where fish sampled exceeded the PTWI for THg. Other studies which have interpreted THg concentrations with PTWIs do not report scenarios that exceed this level of intake (Ikem and Egiebor 2005;Storelli *et al*. 2005; Herreros *et al*. 2008). It is more common to interpret THg in the context of a 0.5 $\mu g g^{-1}$ guideline limit. Some large predatory fishes frequently exceed this limit for a variety of ecological and physiological reasons (Storelli *et al*. 2005). Examples include louvar (*Luvarus imperialis*) and swordfish (*Xiphias gladius*), from the

Mediterranean Sea and sold in Madrid, which exceeded the PTWI for $\mu g g^{-1}$ in 88.2 and 35.1 percent of fish sampled (Herreros *et al*. 2008). Similarly, 29% of canned tuna samples in a study in the southern United States exceeded this guideline (Ikem and Egiebor 2004). An investigation in 2007 by the Canadian Broadcasting Corporation (CBC) revealed that canned albacore tuna sold in Canada exceeded the guidelines in 13% of samples. This is similar to findings from Quebec and Costa Rica (Lean, D., University of Ottawa, personal communication 2010). This thesis shows that 19.15 % of fish sampled from the WCVI region exceeded this guideline.

The third outcome of Chapter two is that there is spatial variability in the degree and nature of food safety, illustrated by the PTWI. This indicates the need for further prevention and control options to reduce potential risk to pregnant women and children under twelve. This thesis highlights the importance of fishing region (spatial variability) in monitoring mercury concentrations for health risk purposes.

4.2 Nonlinear Patterns within fishing regions

Chapter three used Generalized Additive Models to examine the variation of THg and TAs concentrations in Pacific ocean perch within groups (each fishing region). This is the first study to examine patterns of THg and TAs concentrations in a slow growing deep water rockfish from the continental slope of British Columbia using nonlinear models. The first outcome of chapter 3 is that patterns are inconsistent between regions. Other outcomes relate to the patterns found between concentrations of THg or TAs and the biological variables of age, length, mass, $\delta^{13}C$ and $\delta^{15}N$. These relationships are compared to other studies and their contribution to the literature is discussed below.

4.2.1 Age- THg

Generalized Additive Models indicate that age has an important effect on the variability in THg concentrations in the Pacific ocean perch assessed in this study, and the effect is consistently a positive linear relationship. This is similar to the findings of Braune (1987) and Cronin *et al*. (1998). Interestingly, the correlation coefficients between THg concentrations and age from HS and QCS regions (Table 3.1) are substantially lower than the correlation coefficients in both the WCVI region and other studies (Cronin *et al*. 1998). This suggests that although this relationship is consistently reported in fish, the strength of the relationship is variable, even across different fishing regions within the same species. This study highlights this relationship in a commercial groundfish species from the marine continental slope of British Columbia.

4.2.2 Mass – THg

The associations between THg and mass in each region are also positive; however, unlike age and THg, the patterns between mass and THg are not exclusively linear. Previous studies of marine fish have reported positive linear relationships between THg concentrations and both age and length. These include a study of several species from the continental slope of the North Atlantic Ocean with ages ranging from 3 to >70 years (Cronin *et al*. 1998) and Atlantic herring (Braune 1987). Both of these studies show plots of THg against age, from which one can infer plateau patterns. This is the pattern observed in the WCVI region in Chapter 3. However, non-linear models were not used in the Cronin and Braune studies. The patterns found between THg and length in the HS region and THg and mass in the QCS region were also plateaux. These are similar to the findings of other studies in that they show a partially positive pattern, albeit convex, but differ from previous studies because they were not linear. Linear relationships have been shown between THg and length in marine fish from the Azores (Anderson and Depledge 1997), several species of North Sea fish (Baeyens *et al.* 2003) and cartilaginous fish from the Mediterranean Sea (Storelli *et al*. 2002). Linear relationships have also been shown between THg and mass in a variety of predatory pelagic fishes from the central North Pacific Ocean (Choy *et al.* 2009). This thesis contributes to the scientific literature model results that show significant nonlinear, positive relationships (plateaux) between THg and age, mass and length in a marine fish species.

4.2.3 Length – THg

Chapter 3 of this thesis shows that the length – THg relationship is not a simple linear pattern and may be influenced by the mass to length ratio of the fish sampled. Positive linear relationships between fish length and THg concentrations are the pattern most commonly found in the scientific literature, and are generally regarded as the norm (Gonul and Kucuksezgin 2007). Statistically significant examples of this pattern include blackfin tuna (*Thunnus atlanticus)*, yellowfin tuna (*Thunnus albacares)* and carcharinid sharks from the Gulf of Mexico (Cai *et al.* 2007), several species of deep sea fishes from the North Atlantic (Cronin *et al*. 1998), Pacific hake from the Northeast Pacific (Cutshall *et al.*1978), sea bream from the eastern Aegean (Gonul and Kucuksezgin 2007) and Atlantic herring from the Bay of Fundy, Canada. This chapter adds to the variety of patterns found between mass and length and THg documented in the scientific literature on mercury in marine fish.

4.2.4 δ^{13}C – THg

The δ^{13}C values in this thesis do not show any clear patterns with the THg or TAs concentrations. They most closely resemble values in the literature from marine biota associated with habitat sources of carbon derived on the continental shelf ; suggesting that the THg and TAs in Pacific ocean perch predominantly originate on the continental shelf. The lack of a functional form relating differences in δ^{13}C to THg highlights its limitations as a useful explanatory variable in this study.

4.2.5 δ^{15}N – THg

I found no significant trend in Pacific ocean perch in δ^{15}N associated with THg concentrations. Possibly due to the narrow range of δ^{15}N values. Other studies that investigated δ^{15}N and THg concentrations in marine organisms from multiple trophic levels have found evidence of biomagnification (Jarman *et al.* 1996; Atwell *et al*. 1998; Gray 2002; Bank *et al.* 2007; Cai *et al.* 2007. The range of δ^{15}N within a single species is narrow and makes it difficult to detect biomagnification.

4.2.6 Age – Tas

This study found an interesting nonlinear pattern between age and arsenic concentrations that consists of peaks and troughs in TAs concentration that correspond to the fish of similar ages (i.e., 10, 20, 30 years old) from both samples from the WCVI and HS regions. Studies of arsenic concentrations in marine fish are not abundant and those that exist do not frequently include age data or use nonlinear statistical analyses, making it difficult to compare this pattern with the literature. Other studies have found that the relationship between arsenic concentration and age was not statistically significant; e.g., fish from an Australian estuary (Maher *et al.* 1999) and the North Sea (Falconer *et al*. 1993). If I had applied a purely linear statistical approach, a similar result would have occurred. This study documents a statistically significant nonlinear association between age and TAs concentrations, which appears to be synchronous in distinct regions within the same ocean basin.

4.2.7 Body Size (length and mass) – TAs

The patterns between the natural background variability in concentrations of TAs and both length and mass in Pacific ocean perch in this study differ in each of the three fishing regions. This is similar to the contrasting patterns of TAs concentrations found in different species within a genus from varied locations (Edmonds and Francesconi 1981). This study documents the patterns between length and mass and natural background concentrations in a commercial rockfish that inhabit different regions of the continental slope of British Columbia.

4.2.8 $\delta^{13}C$ – TAs

$\delta^{13}C$ was included in the best fitting GAM of TAs in samples from HS because it improved the fit of the model. However, the poor fit of residuals within 95% confidence intervals (Figure 3.12) and the lack of significance indicate this result should be viewed with caution. The smooth suggests that within the natural background variability of TAs concentrations, fish samples from HS with relatively enriched (less negative) $\delta^{13}C$ are associated with relatively higher concentrations of TAs. Comparisons to marine $\delta^{13}C$ values reported in the literature (Hobson and Sealy 1991; Hobson *et al*. 1994; Perry *et al.* 1999; Bosley *et al*. 2004) suggest that, within the inliers from this region, the fish with stronger links to continental slope or inshore food webs organic carbon sources (relatively enriched) may have higher TAs concentrations. This could potentially be due to more extensive inshore summer feeding migrations (Love *et al.* 2002). I am not aware of any other studies of marine fish from British Columbia that examine ratios of stable isotopes of carbon and arsenic concentrations. Due to the amount of unexplained variation in TAs concentrations in the model and the nonsignificance of the HS smooth, this finding is not conclusive and requires further investigation.

4.2.9 $\delta^{15}N$ – Tas

The slight negative association between $\delta^{15}N$ and concentrations of TAs shown in Figure 3.08, although not statistically significant, suggests that arsenic may biodiminish; i.e., animals at the top of the food chain are predicted to carry lower TAs burdens than animals at the bottom (e.g. predators < prey; piscivores < planktivores) (Wagemann *et al.* 1978). This is supported by other studies which report that arsenic does not biomagnify (Edmonds and Francesconi 1981; De Gieter 2002). On the other hand, reports of arsenic biomagnification in marine biota, such as cormorants from the North Pacific (Rocque and Winker 2004) and pelagic organisms and fish from the Sulu Sea, Philippines which showed significant positive correlations ($p < 0.05$) with $\delta^{15}N$, suggest that different chemical forms of arsenic may accumulate by different processes, either biomagnification or biodilution (Hayase *et al.* 2010). The range of $\delta^{15}N$ values in fish reported in this thesis is narrow, which makes it difficult to elucidate any patterns. Scientific studies that report both arsenic concentrations and $\delta^{15}N$ signatures in marine fish are scarce. This thesis is the first to document this data in a slow growing, demersal species that inhabits the continental slope of British Columbia.

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Appendix A

Table A1 The number of Pacific ocean perch samples from each region that exceeded THg, MeHg and $As_{(inorganic)}$ intake limits from one serving of fish. Percentages of n are shown in (), n=sample size.

PTWI ref #'s refer to
1) 0.005 mg/kg bw/week (JECFA 1978). (That is equivalent to 5 µg/kg bw/week)
2) 1.6 µg/kg bw/week (JECFA 2006)
3) 0.20 µg/kg bw/day (Health Canada 1997)
4) 0.015 mg/kg bw/week (JECFA 1988)
5) 0.3 to 0.7 µg/kg bw/day (Dickman and Leung 1998)
6) 3.3 µg/kg bw/week (JECFA 1988 and 2003)
For details of how exposure scenarios were estimated see Methods section 2.3.6 Food Safety Scenarios

Row #	**chemical[1]**	**Population**	**Tolerable intake Ref #**	**Body size (Kg)**	**HS n=30**	**QCS n=156**	**WCVI n=47**
1	THg	adult	1	60	0	0	1 (2)
2	THg	adult male	5	60	2 to 15 (7 to 50)	8 to 45 (5 to 39)	12 to 23 (26 to 49)
5	THg	aboriginal adult	1	60	0	0	6 (13)
6	THg	aboriginal adult male	5	60	6 to 28 (20 to 93)	32 to 103 (20 to 66)	22 to 37 (47 to 79)
7	[1]MeHg	adult	6	60	0	0	4(9)
8	[1]MeHg	Pregnant adult	3	60	24 (80)	80 (51)	33 (70)
9	[1]MeHg	child	3	[4]26	30 (100)	120(77)	42 (89)
10	[1]MeHg	aboriginal adult	6	60	0	1 (1)	8 (17)
11	[1]MeHg	Pregnant aboriginal adult	3	60	30 (100)	120(77)	42 (89)
12	[2]MeHg	adult	6	60	0	0	2 (4)
13	[2]MeHg	Pregnant adult	3	60	24 (80)	69 (44)	32 (68)
14	[2]MeHg	child	3	[4]26	30 (100)	113(72)	39 (83)
15	[2]MeHg	aboriginal adult	6	60	0	0	8 (17)
16	[2]MeHg	pregnant aboriginal adult	3		30 (100)	113(72)	39 (83)
17	[3]$As_{(in)}$	adult	4	60	0	0	0
18	[3]$As_{(in)}$	aboriginal adult	4	60	0	0	0
19	[3]$As_{(in)}$	child	4	[4]26	0	0	0

Appendix B

Table B1 Model parameters for GAMs of THg in Pacific ocean perch from Hecate Strait. Explanatory covariates are A=age, L=length, M=mass, N=$\delta^{15}N$ and C=$\delta^{13}C$. Significant terms have p-values < 0.05, unless in parenthesis, where p<1. Models are presented in order of goodness of fit, assessed by AIC value, top of table = best fit, bottom of table = worst fit.

Model Ref #	GAM terms	Sig Terms[1]	AIC	Dev (%)
HSHg5	~s(A and L and δ^{13}C)	A	-73.504	37
HSHg4	~s(A and L and δ^{15}N)	(A)	-73.2457	36.8
HSHg3	~s(A and δ^{13}C and δ^{15}N)	(A)	-71.5808	26.9
HSHg1	~s(A and L and δ^{13}C and δ^{15}N)	(A)	-71.509	36.5
HSHg2	~s(L and δ^{13}C and δ^{15}N)		-70.0468	26

Table B2 Model parameters for GAMs of THg in Pacific ocean perch from Queen Charlotte Sound. Explanatory covariates are A=age, L=length, M=mass, N=$\delta^{15}N$ and C=$\delta^{13}C$. Significant terms have p-values < 0.05, unless in parenthesis, where p<1. Models are presented in order of goodness of fit, assessed by AIC value, Top of table = best fit, bottom of table = worst fit.

Model Ref #	model terms	Sig terms	AIC	Dev (%)
QCSHg13	~s(A and M and δ^{13}C)	M	-365.472	42.8
QCSHg16	~s(A and L and M)	M	-365.395	42.7
QCSHg15	~s(A and L and δ^{13}C)	L	-365.037	42.6
QCSHg17	~s(A and m:l and δ^{13}C)	m:l	-364.6727	43.1
QCSHg4	~s(A and L and δ^{13}C and δ^{15}N)	M	-364.562	43.3
QCSHg6	~s(A and L and M and δ^{13}C)	M	-364.562	43.3
QCSHg12	~s(A and M and δ^{15}N)	M	-364.538	42.3
QCSHg10	~s(L and M and δ^{13}C)	M	-364.331	42.2
QCSHg7	~s(M and δ^{13}C and δ^{15}N)	M	-364.109	42.1
QCSHg14	~s(A and L and δ^{15}N)	L (N)	-364.024	42
QCSHg9	~s(L and M and δ^{15}N)	(M)	-363.958	42
QCSHg3	~s(A and M and δ^{13}C and δ^{15}N)	M	-363.753	43
QCSHg5	~s(A and L and M and δ^{15}N)	M	-363.526	42.8
QCSHg1	~s(A and L and M and δ^{13}C and δ^{15}N)	M	-362.757	43.4
QCSHg2	~s(L and M and δ^{13}C and δ^{15}N)	M	-362.744	42.4
QCSHg8	~s(L and δ^{13}C and δ^{15}N)	L N	-362.316	41.4
QCSHg11	~s(A and δ^{13}C and δ^{15}N)	N	-350.089	39

Table B3 Model parameters for GAMs of THg in Pacific ocean perch from the west Coast of Vancouver Island. Explanatory covariates are A=age, L=length, M=mass, N=δ^{15}N, C=δ^{13}C and m:l=mass to length ratio. Significant terms have p-values < 0.05, unless in parenthesis, where p<1. Models are presented in order of goodness of fit, assessed by AIC value, Top of table = best fit, bottom of table = worst fit.

Model Ref #	model terms	Sig terms	AIC	Dev (%)
WCVIHg1	~s(A and L and M and δ^{13}C and δ^{15}N)	A L M (C) N	-142.71	80.6
WCVI17	~s(A and m:l and δ^{13}C and δ^{15}N)	A m:l N	-142.62	79
WCVIHg5	~s(A and L and M and δ^{15}N)	A L M N	-135.695	76.1
WCVIHg3	~s(A and M and δ^{13}C and δ^{15}N)	A M N (C)	-135.689	76.6
WCVIHg16	~s(A and L and M)	AM	-132.029	72
WCVIHg4	~s(A and L and δ^{13}C and δ^{15}N)	A M	-131.874	74.3
WCVIHg6	~s(A and L and M and δ^{13}C)	A M	-131.874	74.3
WCVIHg12	~s(A and M and δ^{15}N)	A M	-131.8	71.1
WCVIHg13	~s(A and M and δ^{13}C)	A M	-131.357	72.1
WCVIHg7	~s(M and δ^{13}C and δ^{15}N)	M C N	-131.188	72.2
WCVIHg11	~s(A and δ^{13}C and δ^{15}N)	AN	-130.486	70.9
WCVIHg2	~s(L and M and δ^{13}C and δ^{15}N)	M C N	-129.289	72.2
WCVIHg10	~s(L and M and δ^{13}C)	M	-128.199	73.2
WCVIHg9	~s(L and M and δ^{15}N)	M	-127.496	71.5
WCVIHg14	~s(A and L and δ^{15}N)	A (N)	-126.59	66
WCVIHg15	~s(A and L and δ^{13}C)	A	-124.009	64.1
WCVIHg8	~s(L and δ^{13}C and δ^{15}N)	L C	-116.137	56.4

Table B4 Model parameters for GAMs of TAs in Pacific ocean perch from Hecate Strait. Explanatory covariates are A=age, L=length, M=mass, N=δ^{15}N and C=δ^{13}C. Significant terms have p-values < 0.05, unless in parenthesis, where p<1. Models are presented in order of goodness of fit, assessed by AIC value, top of table = best fit, bottom of table = worst fit.

Model Ref #	model terms	Sig terms	AIC	Dev (%)
HSAs5	~s(A and L and δ^{13}C)	A	7.3647	71.2
HSAs3	~s(A and δ^{13}C and δ^{15}N)	A	14.9489	53.8
HSAs4	~s(A and L and δ^{15}N)	(A)	18.4598	33.2
HSAs2	~s(L and δ^{13}C and δ^{15}N)	(L)	19.0532	26.1
HSAs1	~s(A and L and δ^{13}C and δ^{15}N)	(L)	19.55	31.7

Table B5 Model parameters for GAMs of TAs in Pacific ocean perch from Queen Charlotte Sound. Explanatory covariates are A=age, L=length, M=mass, N=$\delta^{15}N$ and C=$\delta^{13}C$. Significant terms have p-values < 0.05, unless in parenthesis, where p<1. Models are presented in order of goodness of fit, assessed by AIC value, top of table = best fit, bottom of table = worst fit.

Model Ref #	model terms	Sig terms	AIC	Dev (%)
QCSAs9	~s(L and M and $\delta^{15}N$)	(M)	193.2343	18.9
QCSAs12	~s(A and M and $\delta^{15}N$)	M	193.2969	18.9
QCSAs7	~s(M and $\delta^{13}C$ and , $\delta^{15}N$)	M	193.8734	18.4
QCSAs10	~s(L and M and $\delta^{13}C$)	M	194.0248	18.2
QCSAs13	~s(A and M and $\delta^{13}C$)	M	194.2733	17.9
QCSAs3	~s(A and M and $\delta^{13}C$ and , $\delta^{15}N$)	M	194.5454	20.4
QCSAs5	~s(A and L and M and $\delta^{15}N$)	M	194.7204	19.3
QCSAs2	~s(L and M and $\delta^{13}C$ and , $\delta^{15}N$)	M	195.0465	19
QCSAs14	~s(A and L and $\delta^{15}N$)	L (N)	195.0494	16.3
QCSAs4	~s(A and L and $\delta^{13}C$ and , $\delta^{15}N$)	M	195.627	18.4
QCSAs6	~s(A and L and M and $\delta^{13}C$)	M	195.627	18.4
QCSAs8	~s(L and $\delta^{13}C$ and , $\delta^{15}N$)	L N	195.7818	16.1
QCSAs1	~s(A and L and M and $\delta^{13}C$ and , $\delta^{15}N$)	M	196.6284	19.4
QCSAs11	~s(A and $\delta^{13}C$ and , $\delta^{15}N$)	N	198.7548	15.2
QCSAs15	~s(A and L and $\delta^{13}C$)	L	200.412	9.71
QCSAs16	~s(A and L and M)	M	256.9217	16.6

Table B6 Model parameters for GAMs of TAs in pacific ocean perch from the West Coast of Vancouver Island region. Explanatory covariates are A=age, L=length, M=mass, N=δ^{15}N and C=δ^{13}C. Significant terms have p-values < 0.05, unless in parenthesis, where p<1. Models are presented in order of goodness of fit, assessed by AIC value, Top of table = best fit, bottom of table = worst fit.

Model Ref #	model terms	Sig terms	AIC	Dev (%)
WCVIAs16	~s(A and L and M)	L (M) (A)	36.23	37.4
WCVIAs13	~s(A and M and δ^{13}C)	A(C)	40.44365	33.6
WCVIAs6	~s(A and L and M and δ^{13}C)	C (A) (M)	40.6487	37.4
WCVIAs1	~s(A and L and M and δ^{13}C and δ^{15}N)	.	41.48623	38.8
WCVIAs11	~s(A and δ^{13}C and δ^{15}N)	A	41.487	29.6
WCVIAs3	~s(A and M and δ^{13}C and δ^{15}N)	A	41.9133	33.7
WCVIAs12	~s(A and M and δ^{15}N)	A	42.06	28.4
WCVIAs5	~s(A and L and M and δ^{15}N)	(N)	42.41587	31.8
WCVIAs14	~s(A and L and δ^{15}N)	(N)	42.4932	27.5
WCVIAs15	~s(A and L and δ^{13}C)	(C)	42.76	26.9
WCVIAs8	~s(L and δ^{13}C and δ^{15}N)	L	42.98155	26.5
WCVIAs4	~s(A and L and δ^{13}C and δ^{15}N)	.	43.23	30.2
WCVIAs9	~s(L and M and δ^{15}N)	L N	43.30944	25.8
WCVIAs2	~s(L and M and δ^{13}C and δ^{15}N)	L	43.9228	28.7
WCVIAs10	~s(L and M and δ^{13}C)	L (C)	44.4769	23.2
WCVIAs7	~s(M and δ^{13}C and δ^{15}N)	.	48.1059	15.9