INFLUENCE OF LIFE HISTORY TRAITS ON ACCUMULATION OF POLYBROMINATED DIPHENYL ETHERS AND POLYCHLORINATED BIPHENYLS IN THREE LAKE TROUT POPULATIONS FROM LAKE SUPERIOR

By

Laura Christine Claus

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ABSTRACT

INFLUENCE OF LIFE HISTORY TRAITS ON ACCUMULATION OF POLYBROMINATED DIPHENYL ETHERS AND POLYCHLORINATED BIPHENYLS IN THREE LAKE TROUT POPULATIONS FROM LAKE SUPERIOR

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Bioaccumulation of lipophilic contaminants can be influenced by many factors, including life history. Life history traits, such as age at maturity and reproductive schedules evolve to optimize the allocation of available energy to reproduction and growth, which may have implications for the distribution and effects of lipophilic contaminants. The goal of this study was to understand how life history traits can influence the distribution of lipophilic contaminants in three distinct lake trout (*Salvelinus namaycush*) populations from Lake Superior: siscowet and lean morphotypes from Marquette and Michipicoten Island leans. The siscowet morphotype is characterized by higher body fat content, slower growth and lower fecundity than the lean. The Michipicoten Island lean is a slow growing lean population with high reproductive investment at older ages. The concentrations of PCBs and PBDEs in the muscle, ovary and liver were measured and compared to population relevant metrics such as fecundity, muscle fat, length and age. As predicted, based on higher body fat, siscowets had higher concentration of contaminants in the muscle tissue when adjusted for exposure. The Michipicoten leans had relatively higher concentrations of contaminants in the ovary tissue compared to siscowets of similar ages, which is consistent with their higher fecundities and gonad weights. The patterns of contaminant bioaccumulation between the three populations suggest that it is necessary to consider life history traits when monitoring for lipophilic contaminants because such patterns allow for the inference of the effects of contaminants on the general health of the fish population.

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KEY TO SYMBOLS OR ABBREVIATIONS

AIC	Akaike information criterion
Concen.	concentration
CI	confidence interval
GC/MS	Gas chromatography / mass spectrometry
GSI	Gonadosomatic index
g	gram
µL	microliter
mL	milliliter
ng/g	nanogram / gram
K_{ow}	octanol-water partition coefficient
OMOE	Ontario Ministry of the Environment
PBDE	polybrominated diphenyl ether
PCB	polychlorinated biphenyl
Pop.	Population
β	slope of the regression line
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
v/v	volume / volume
w/w	weight / weight
wt/w	wet weight

Introduction

Lipophilic contaminants, such as polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs), are usually transferred throughout the body of a fish by binding to lipoproteins and triglycerides. The movement and distribution of triglycerides and lipoproteins as a result of metabolism, growth and reproduction, constantly reposition the lipophilic contaminants, often leading to uneven accumulation patterns in different tissues (Dulfer et al., 1998). Growth and reproductive processes are influenced heavily by life history (Roff, 2002) and in this study, I will explore how the life history traits of three different populations of lake trout (*Salvelinus namaycush*) can potentially influence PBDE and PCB accumulation in different tissues. The potential link between life history traits and lipophilic contaminant accumulation patterns could have implications for management and monitoring of lake trout in the Great Lakes.

Environmental challenges faced by populations will dictate birth and death rates and therefore will influence population structure including size at age (growth), age structure, age at maturity, fecundity and survival rates (Roff, 2002). Life history traits are heritable characteristics that are theoretically a result of evolution and optimize survival and reproduction and include traits such as age and size at maturity, growth rate and reproductive effort with age. These traits directly influence population structure. Populations that face different environmental challenges could select for traits that allow for different tradeoffs between survival and reproduction, and such tradeoffs could lead to different population growth trajectories and reproductive schedules. For example, a population that experiences high adult mortality may reach sexual maturity sooner, exhibit increased growth rates and show high reproductive investment at younger ages when compared to a longer-lived population (Roff,

2002). These differences in growth rates and reproductive outputs between populations could also lead to different patterns of lipophilic contaminant bioaccumulation.

Lipids are an energy source that can either be stored or mobilized for use in growth or reproductive processes. In lake trout, the dominant storage lipids are triacylglyercols (DiGiulio & Hinton, 2008). Stored triacylglycerols provide a moderate degree of independence from a constant forage food sources (DiGiulio & Hinton, 2008). Additionally, lipid storage in some morphotypes compensates for vertical lift and drag by reducing an individual's specific gravity (Henderson & Anderson, 2002). Lipids can be used for egg production and are transferred into the developing egg mass or incorporated into vitellogenin, the yolk-precursor protein (Kleinow et al., 2008). Environmental pressures on populations will dictate whether lipids are stored, used for growth or reproduction at particular ages. Lake trout that live at a greater depth and store more lipids as muscle fat expend less energy to migrate through the water column because energy is no longer required to use a swim bladder (Henderson & Anderson, 2002). Inversely, lake trout that experience higher rates of adult mortality are likely to allocate lipids towards growth and reproduction earlier in life. The trade-offs between energy allocations to growth or reproduction is meant to optimize survival of the population, but has implications for the distribution of lipophilic contaminants that bioaccumulate in the body of a fish.

Lipophilic contaminants are moved through the body by binding with lipoproteins, such as vitellogenin (Ungerer & Thomas, 1996). The movement of lipophilic contaminants within the body begins at the time of exposure. When PCBs and PBDEs enter the body, they are metabolized by the cytochrome P450 (CYP) enzyme system once they enter the liver (Schlenk et al., 2008). The cytochrome P450 enzyme system is the most dominant system in the phase I biotransformation oxidation processes (Schlenk et al., 2008). The CYP enzymes convert

lipophilic pollutants into water-soluble products that can be excreted (Schlenk et al., 2008). The particular CYP isozyme involved depends on the congener. PCBs with coplanar structures interact with the Ah receptor and induce CYP1A (James, 2001). CYP2B isozymes are induced by ortho-substituted non-planar PCBs (Arnold & Feeley, 2002). Oxygen is introduced into the PCB via the cytochrome P450 enzyme system and the hydroxylated metabolite may be conjugated with a glucuronide or sulfate with UDP-glucuronosyltransferase or PAPS-sulfotransferase as catalysts (James, 2001). The metabolite is then excreted through the bile; however, when organisms are exposed to lipophilic pollutants at a higher rate than the contaminants are metabolized, the contaminants begin to bioaccumulate in the organism.

Lipophilic contaminants can bioaccumulate disproportionately throughout the body in a pattern related to the lipid concentration of the organs. Additionally, the accumulation efficiencies of tissues vary (Karjalainen et al., 2006). For example, when brook trout (*Salvelinus fontinalis*) were exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the total assimilation through dietary exposure for females was 84 % (Tietge et al., 1998). The ovaries only retained 6% of the total body burden of TCDD and 43% of muscle concentrations(Tietge et al., 1998; Walker et al., 1994). In previous studies, it was found that patterns of lipophilic contaminant accumulation follows lipid concentrations throughout the body, including the blood, liver, ovary, muscle and eggs (Tietge et al., 1998).

The ease at which lipophilic contaminants bind to lipoproteins and triglycerides is a process that is determined by the contaminant's octanol-water partition coefficient (K_{ow}) (Streit et al., 1991). K_{ow} is the ratio of the concentration of a chemical in octanol and water at equilibrium (United Geological Survey, 2010). The octanol is considered a surrogate for lipid matter, and examining the K_{ow} allows for an understanding of the fate of chemicals in the

environment. A chemical with a high K_{ow}, ranging between 2 and 7, will bioaccumulate at greater rates than a chemical with a lower K_{ow} (Streets et al., 2006).

The lipophilic contaminants of interest in this study include two industrial persistent contaminants with high K_{ow}, PCBs and PBDEs. PCBs, in the past, were used in a variety of commercial and industrial applications including fluorescent light ballasts, electrical transformers, plasticizers in plastic and rubber, and cable insulation. The manufacture and use of PCBs was prohibited in 1976, however the release of PCBs into the environment continues due to existing equipment and processes (United Nations Environment Programme, 2002). PCBs are highly lipid soluble; the logarithms of K_{ow} values for tetra, penta, hexa and octa PCBs range from 5.46 to 7.67 (Hawker & Connell, 1988). PBDEs are a class of brominated flame retardants used in items such as furniture foam, plastics and draperies. PBDEs are also highly lipid soluble; the logarithmic K_{ow} values for pentaBDE-99 range from 6.5 to 8.5 (US EPA, 2008b). Production of these compounds are not banned in the United States, however the manufacture of pentaBDE and octaBDE ceased starting in 2005 (US EPA, 2011). Both PCBs and PBDEs can be transported atmospherically from heavily polluted areas and deposited in more remote areas (Datta et al., 1999 and Schmide et al., 2007). The atmospheric deposition of these contaminants complicates management of these contaminants in the Great Lakes ecosystem.

PCBs and PBDEs are ubiquitous in the Great Lakes and in fish because of their widespread usage and chemical composition and may contribute to adverse health effects within aquatic ecosystems. As top predators in the Great Lakes ecosystem, lake trout have historically been known to bioaccumulate lipophilic contaminants. PCB levels in whole lake trout from the Great Lakes have declined since the 1970s, although recently the levels have stabilized and

remain above levels requiring consumption warnings in some locations (US EPA & Environment Canada, 2009). Concentrations of PBDEs in lake trout eggs from the Great Lakes have increased in the last 20 years (Valters et al., 2005). The PBDE levels in whole lake trout from all five of the Great Lakes was increasing through the early 2000s, but more recently we are beginning to see a decline (US EPA & Environment Canada, 2009). Concentrations of these contaminants in particular tissues have health implications for humans that consume lake trout (Devault et al., 1996) and the health of the fish populations (Maule et al., 2005; Olsson et al., 1999).

PBDEs and PCBs induce sublethal toxic effects, which can lead to stress on human and fish populations. PCBs have been found to affect the immune system in fish and can be embyotoxic (Maule et al., 2005; Olsson et al., 1999). PCBs are endocrine disruptors that reduce the uptake of vitellogenin by oocytes and reduce fecundity (Halden et al., 2010; Rose et al., 2003). Additionally, the planar PCB congeners produce dioxin-like effects (Tillitt et al., 2008).

PBDEs, as endocrine disruptors, show many structural similarities to dioxins, which suggest that they may have toxic properties that are similar to dioxins (US EPA, 2010b). The structural similarities between PBDEs and thyroid hormones suggest disruption of the immune system (Luthe et al., 2008; Birchmeier et al., 2005).

The siscowet and lean morphotypes from Lake Superior along with the Michipicoten Island lean population were chosen for this study because of the unique life history traits exhibited for each population. Populations of lake trout from Lake Superior can be grouped into two ecologically distinct morphotypes (Figure 1). Lean and siscowet lake trout morphotypes are estimated to have diverged from each other around the time of the Wisconsin Glacial Episode, although debate exists as to whether the morphotypes diverged before or after recolonization

(Page et al., 2004; Eshenroder, 2008). The bathymetric distribution of siscowets and leans result in key life history differences, most noticeable is the relative lipid content (Sitar et al., 2008). Populations of siscowets are found in large abundances at depths greater than 100 meters and are robust with higher body fat content, whereas lean lake trout populations are most abundant in depths less than 50 meters and are slender with low body fat content (Eschmeyer & Phillips, 1965; Hansen et al., 1995). The higher lipid content in siscowets is hypothesized to overcome drag and reduce energy expenditures during vertical migration (Henderson & Anderson, 2002). Whether these morphological differences result from phenotypic plasticity or genetic differences is still under investigation, but studies indicate a genetic basis for differences in lipid levels between leans and siscowets (Goetz et al., 2010, Eschmeyer and Phillips, 1965).

In addition to differences in lipid accumulation and distribution patterns, siscowets and leans also show differences in growth and fecundity, which may have implications for contaminant distribution. Lean lake trout grow faster than siscowets and have a higher relative fecundity (Hansen et al., 1995). The Michipicoten Island lake trout is believed to be a separate genetic strain of lean lake trout (Meredith, 2008). Michipicoten leans grow slower than the Marquette leans and live in north-eastern Lake Superior where they are best able to persist because fishing pressure and sea lamprey (*Petromyzon marinus*) parasitism rates are low (Bronte et al., 2007; Ebener, 1998). The growth rate of the individual is important to the accumulation of lipophilic contaminants because of the effect of biodilution: a faster growing individual will have lower concentrations of lipophilic contaminants at a specific length compared to a slower growing individual (Simoneau et al., 2005). However, this theory of biodilution assumes individuals with different growth rates experienced similar contaminant exposure rates.

Figure1. The phenotypic differences between lean and siscowet lake trout (courtesy Shawn Sitar).

The interaction of life history traits of the populations and lipophilic contaminants like PBDEs and PCBs causes unique concerns for human health and lake trout population management. Accumulation of lipophilic contaminants into the muscle tissue of lake trout that are being consumed by humans creates human health concerns. Siscowets are being considered for use as high-grade fish oil dietary supplements because of their high population numbers and lipid content. Human consumption of fish oils has been associated with many human health benefits including prevention of cardiovascular diseases (Marik & Varon, 2009) and siscowet lake trout have been found to contain very high levels of omega-3 fatty acids (Wang et al., 1990). Fish oil extracted from siscowets could be used as nutritional supplements and used to produce high-value fish meal. However, if siscowets store large amounts of lipids, and thus lipophilic contaminants, the benefits of consumption of omega-3 fatty acids could be compromised by increased exposure to halogenated contaminants. Life history traits can vary within species and I suspect that life history traits should be taken into consideration when using lake trout data for public health recommendations and when considering the response of a population to a stressor such as PBDEs and PCBs.

Additionally, mobilization of lipophilic contaminants through the body allows for the maternal transfer of contaminants into the ovaries. Accumulation of endocrine disrupting contaminants in the ovaries, and thus the eggs, presents potential health concerns for the developing embryo (Miller et al., 1993). The additional stress of lipophilic contaminants adds to the multitude of stressors faced by lake trout in the Great Lakes and contributes to the decline of naturally reproducing lean lake trout populations.

My study investigates the role of life history traits on patterns of lipophilic contaminant accumulation in three lake trout populations. The energy trade-offs between growth and reproduction for leans and siscowets have been reported in previous studies (Eschmeyer and Philips, 1965; Hansen et al., 1995, Miller et al., 2000). My hypothesis is that the distribution of PCBs and PBDEs will be unique to each population and will reflect life history trade-offs. I predict that lean populations with a greater reproductive investment will have higher concentrations of PCBs and PBDEs in the ovary tissue than siscowets. The siscowets, due to larger stores of muscle fat are predicted to have higher concentrations of PCBs and PBDEs in the muscle tissue. My research investigated this hypothesis by sampling these three lake trout populations from Lake Superior and collecting life history data, such as age, fecundity, muscle lipid concentration and weight. Analyses of muscle, ovary and liver tissue samples for selected PCB and PBDE congener concentrations were performed using gas chromatography/mass spectrometry methods. The life history and contaminant data were then analyzed in tandem to understand the interaction between life history of a population and contaminant concentrations. My study will improve our understanding of the relationship between life history and lipophilic contaminants; a relationship that can have implications for the management and consumption of lake trout.

Methods

Field Sampling

Sexually mature female lake trout were collected during September and October of 2008 and 2009 off the coast of Marquette, USA and Michipicoten Island, Canada; both in Lake Superior (Figure 2A). Lean and siscowet lake trout were collected offshore of Marquette as part of the Michigan Department of Natural Resources project sampling. The Marquette sampling used bottom gill nets with individual nylon, mutifilament panels (91.4 m x 1.8 m) and mesh sizes including 10.2, 11.4, 12.7 and 14.0 cm. The nets were set at two sites for twenty-four hours before lifting. The first site, that targeted siscowets, was between 375 meters and 385 meters deep (latitude 46°37.10' and longitude 87°16.20'). The second site was selected to capture leans, and was between 109 and 145 meters (latitude 46°33.17' and longitude 87°18.90') (Figure 2B). The mesh sizes were selected in order to target mature lake trout (greater than 550 mm). The minimum length requirement was based on current knowledge of average mature lake trout lengths (Goetz et al., in review). The Marquette sampling resulted in the capture of thirty-four female lake trout, but only nine leans and twelve siscowets were mature females. The lake trout sampled from Michipicoten Island were part of the Ontario Ministry of Natural Resource's effort to stock their hatchery programs (Felt, unpublished). Lake trout were caught by gill nets with 50 and 100 foot panels of 2 and 2.5 inch monofilament. The nets were set for between 3 and 70 hours before lifting at seven main sites (Figure 2C). The sampling trip occurred over the course of about 20 days in both 2008 and 2009. A total of 73 lake trout were caught in 2008, but only twenty mature females were caught near Michipicoten Island between 2008 and 2009. Ten

lake trout from the Michipicoten population were included in this study.

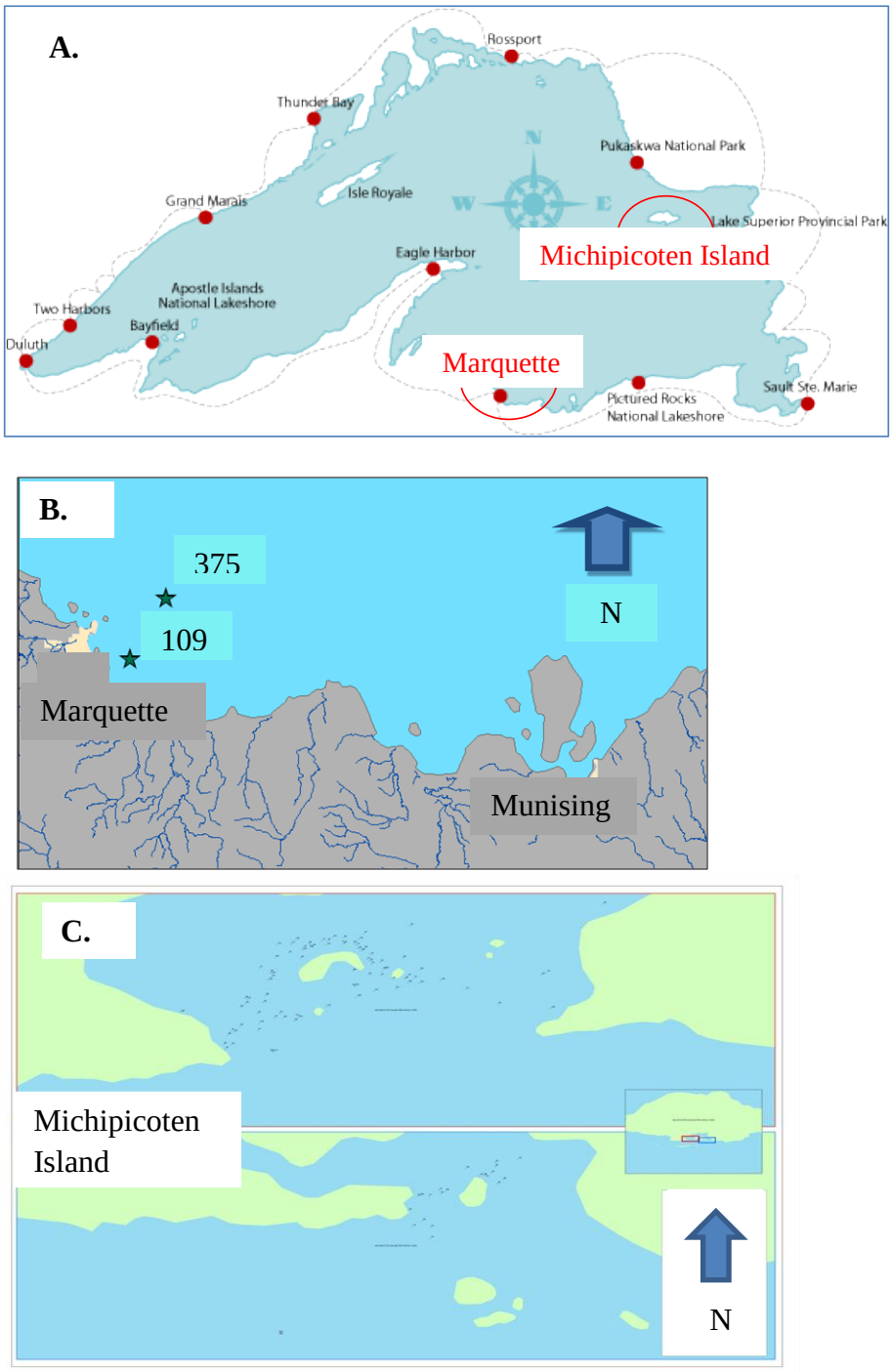

Figure 2. A) Map of Lake Superior. Large circles indicate sampling locations (map courtesy of mspmag.com). B) Location of two sites of gill net sets off the coast of Marquette. The numbers on the map indicate the depth in meters at each site (map courtesy of Ralph Tingley III). C) Locations of gill nets set for lake trout sampling near Michipicoten Island (map courtesy of the Ontario Ministry of Natural Resources).

On site, various measurements were made including morphology metrics, length and fork length, weight and fatmeter readings. Lean and siscowet lake trout were identified based on their morphometry that included fin size, eye location, eye size and visceral fat (Bronte & Moore, 2007; Eschmeyer and Philips, 1965). Length and fork length were measured onsite to the nearest milimeter. Weight was measured to the nearest gram and ovary and liver mass was taken to 0.1 gram. Muscle fat measurements were taken along the outer body cavity with a fish fatmeter (Model FM-692, Distell Inc.,West Lothian, Scotland). Fish were then filleted with the skin on and the muscle tissue, liver and ovaries were removed. The tissue samples were stored separately in aluminum foil and frozen at -80°C until analysis.

The fatmeter readings were taken at consistent locations along the right side of the outer body (Figure 3). The fatmeter is a noninvasive microwave sensor that emits a low frequency microwave beam (2 GHz ±20 MHz) that efficiently heats water dielectrically and provides a reading of water content of the tissue. Fatmeter readings were converted to lipid percentages based on a relationship of lipid to water content in fish, calibrated using lipid extraction data (see Lipid Extraction section).

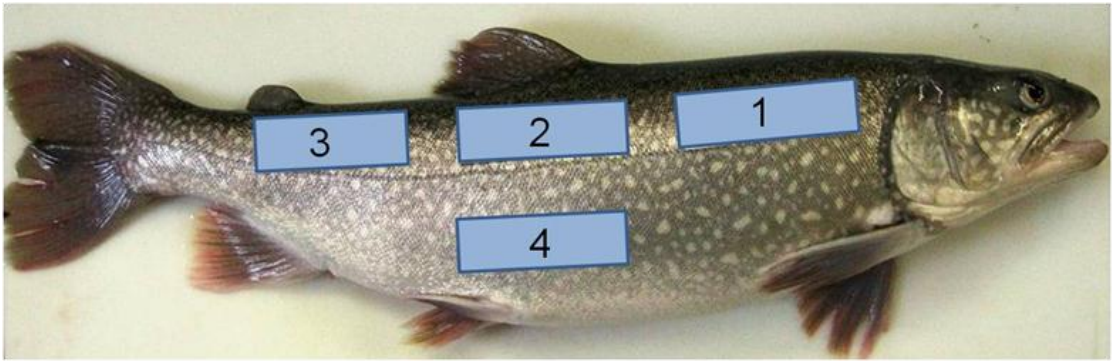

Figure 3. Location of the four fatmeter readings taken along right lateral body. Readings were then calibrated with lipid extraction data to generate muscle fat percentages for lake trout. (Photograph courtesy of Sara Smith)

Additional information was collected offsite after collections. Ages were determined by counting the annuli on the otoliths. Fecundity was later determined by extracting four fresh ovary samples approximately 15 grams, which were weighed and all eggs were counted in each sample. The number of eggs per gram of each sample was averaged and multiplied by gonad mass to determine fecundity. Photos of approximately 25 eggs from each fish were taken on a Leica MZ6 dissecting scope. The pictures were then imported into Axiovision Rel. 4.7 where the numbers of pixels along the diameter of the egg were measured. The pixel number of a known length was used to convert the pixel measurements into egg diameter measurements.

Contaminant Analysis

PBDEs and PCBs were quantified using a contaminant extraction procedure and quantified using gas chromatography/mass spectrometry (GC/MS) methods. This section details the column construction, contaminant extraction, quality control and contaminant quantification.

Column Construction

Two chromatography columns were constructed using mixtures of packing materials and used for contaminant extraction (see Contaminant Extraction). Before the chromatography columns were constructed, packing materials were prepared. First, anhydrous sodium sulfate and silica gel with a mesh of 70/230 were activated by placing in an oven at 215°C for at least 70 hours before use. Once the silica gel was activated, it was mixed with a variety of chemicals in order to create packing materials used in the column construction. The 44% (weight/weight) sulfuric acid/silica packing material was made by mixing 56 grams of activated silica with 25 mL of concentrated sulfuric acid until the packing was free flowing. The 10% (w/w) silver nitrate/silica packing material was made by dissolving 6 grams of silver nitrate in 23 mL of HPLC grade water. The silver nitrate/water mixture was then combined with 53 grams of activated silica and shaken until the packing was free flowing. The 33% (w/w) 1 M sodium hydroxide/silica packing material was prepared by weighing 1.3 grams of sodium hydroxide and dissolving it into 33 mL of HPLC grade water. The water/sodium hydroxide mixture was combined with 67 grams of activated silica and shaken until the packing was free flowing. All packing materials were stored in desiccators until use.

The extraction column was prepared by plugging a 250 mL cylindrical funnel first with glass wool and then with 25 g of 44% sulfuric acid/silica and finally with 8 g of anhydrous

sodium sulfate. The amount of 44% sulfuric-acid/silica in the extraction column was increased for more samples with higher concentrations of lipids, which was indicated by the color change of the silica gel mixtures.

The acid-base column was prepared by plugging a chromatography column (14 x 3.5 cm i.d.) with glass wool and adding, in order from bottom to top: 1.5 g 10% silver nitrate/silica packing, 1.0 g activated silica packing, 2.0 g 33% sodium hydroxide/silica packing, 1.0 g activated silica packing, 4.0 g 44% sulfuric acid/silica packing, 2.0 g activated silica packing and 2.0 g anhydrous sodium sulfate.

All glassware was rinsed three times with acetone, three times with toluene and twice with dichloromethane between each sample.

Contaminant Extraction

All digestion, extraction and GC/MS procedures were modified from the Ontario Ministry of the Environment's method (OMOE, 2004).

Approximately 5 grams of muscle, ovary and liver tissue was homogenized before contaminant extraction. Muscle tissue was homogenized with dry ice in a commercial grade blender. Ovary tissue and liver tissue was thawed and manually homogenized using a sharp blade. Each sample was digested overnight in 50 mL of concentrated hydrochloric acid. The hydrochloric acid was pre-extracted before contact with the sample using a 3:1:1 ratio of hydrochloric acid, dichloromethane and hexane to prevent contamination of the sample. The first column used during the extraction process was the extraction column (see Column Construction). The extraction column was pre-rinsed with 50 mL of hexane and the eluate was discarded. The digested tissue solution (also referred to as the acid-layer) was extracted with 40

mL of hexane by mixing in a separatory funnel for 2 minutes. The hexane and acid layers were then allowed to separate and then the hexane extract was run through the extraction column to remove water, excess lipids and other polar compounds. The acid-layer was returned to the original flask and the extraction was repeated twice and added the hexane from each extraction to the extraction column and at the end, discarded the remaining acid-layer. To ensure maximization of extraction, the original flask and the separatory funnel were rinsed twice with 20 mL of hexane, which was added to the extraction column. Eluate from extractions was collected in a round bottom flask and placed on a rotary-evaporator at 45°C until only about 2 mL of the eluate remained. In between individual sample extractions, the rotary evaporator was rinsed with 150-200 mL of hexane.

Each sample was subjected to chromatographic cleanup with the acid-base column, which was made with silica gel mixtures (see Column Construction). The acid-base column was pre-rinsed with 50 mL of hexane and the eluate was discarded. Then each sample extract was added to the acid-base column. The original round-bottom flask containing the sample was rinsed three times with 2 mL hexane and added to the acid-base column. Then, 100 mL of hexane was added to the acid-base column followed by 80 mL of 50:50 dichloromethane and hexane mixture. The acid-base column was allowed to drain to bed level between each addition. Once the column was well drained, the round bottom flask containing the eluate was placed on the rotary evaporator until approximately 1 mL was remaining in the flask. The concentrated sample extract was then transferred to a clean glass vial and the round bottom flask rinsed twice with hexane and then twice with dichloromethane with each rinse was added to the glass vial. The glass vial was stored at -80°C until evaporated using a nitrogen evaporator. The sample was

not completely evaporated and the remaining sample was transferred into an amber glass autosampler vial with a 100 µL insert.

Quality Control

The native standards were purchased from Wellington Laboratories (Guelph, Ontario) and were spiked in the precision and recovery (PAR) sample between every 20 tissue samples in order to understand recovery of native PBDEs. The native standard includes BDE 28, 47, 66, 99, 100, 153 and 154 (Table 1).

The internal standard [$^{13}C_{12}$]- 2,2',4,4'-TeBDE (47L) was also purchased from Wellington Laboratories (Guelph, Ontario). A spiked procedure blank (SPB) was analyzed between every 10 samples and included a known concentration of internal standard and about 50mL of pre-extracted hydrochloric acid (extracted as described below). The internal standard was added to ever$_{12}$y sample at a known concentration in order to determine recovery rates, but concentrations were not corrected based on recovery.

Calibration standards were purchased from Wellington Laboratories (Guelph, Ontario) and included [$^{13}C_{12}$]- 2,2',4,4'-TeBDE (47L), 2,4,4'-TrBDE (28), 2,2',4,4'-TeBDE (47), 2,3',4,4'-TeBDE (66), 2,2',4,4',5-PeBDE (99), 2,2',4,4',6-PeBDE (100), 2,2',4,4',5,5'-HxBDE (153) and 2,2',4,4',5,6'-HxBDE (154) (Table 1). This set of calibration standards generated a calibration curve, which included data from 4 different concentrations. The R^2 values of the calibration curves were all greater than 0.90 suggesting a strong relationship between concentration and area under the curve of the peak generated by the GC/MS.

The PCB calibration standards were purchased from Cambridge Laboratories (Andover, MA). The calibration standards generated a calibration curve with data at 6 different concentrations and included 2',3,4,4',5-PeCB (123), 2,3,3',4,4',5-HxCB (156), and 2,3',4,4',5,5'-HxCB (167) (Table 1). The internal standard BDE 47L was added to each calibration standard at a known concentration. The R^2 values of the calibration curves were all above 0.88.

Table 1. Standards and congeners used for analysis

Type of Standard	Source purchased	Congeners included in Standard	
Precision and Recovery (PAR)	Wellington Laboratories (Guelph, Ontario)		
		BDE 28	2,4,4'-TrBDE
		BDE 47	2,2',4,4'-TeBDE
		BDE 66	2,3',4,4'-TeBDE
		BDE 99	2,3',4,4'-TeBDE
		BDE 100	2,2',4,4',6-PeBDE
		BDE 153	2,2',4,4',5,5'-HxBDE
		BDE 154	2,2',4,4',5,6'-HxBDE
Internal Standard	Wellington Laboratories (Guelph, Ontario)	BDE 47L	[$^{13}C_{12}$]- 2,2',4,4'-TeBDE
PBDE Calibration Standard	Wellington Laboratories (Guelph, Ontario)		
		BDE 47L	[$^{13}C_{12}$]- 2,2',4,4'-TeBDE
		BDE 28	2,4,4'-TrBDE
		BDE 47	2,2',4,4'-TeBDE
		BDE 66	2,3',4,4'-TeBDE
PBDE Calibration Standard (continued)	Wellington Laboratories (Guelph, Ontario)		
		BDE 99	2,3',4,4'-TeBDE
		BDE 100	2,2',4,4',6-PeBDE
		BDE 153	2,2',4,4',5,5'-HxBDE
		BDE 154	2,2',4,4',5,6'-HxBDE
PCB Calibration Standard	Cambridge Laboratories (Andover, MA)		
		PCB 123	2',3,4,4',5-PeCB
		PCB 156	2,3,3',4,4',5-HxCB
		PCB 167	2,3',4,4',5,5'-HxCB
		BDE 47L	[$^{13}C_{12}$]- 2,2',4,4'-TeBDE

All solvents were either analytical reagent grade or HPLC grade. All samples were analyzed in duplicate.

The average percent recoveries for the PAR samples were reported along with the amount of each contaminant found in the blanks (Table 2).

Table 2. Amount of contaminants found in blanks and the average percent recoveries for the PAR samples

Congener number	Greatest amount found in blank (ng)	Average % recovery for the Precision and Recovery Samples
BDE 28	0.18	62.82
BDE 47	2.44	95.9
BDE 66	0.78	101.42
BDE 99	0.52	111.3
BDE 100	49.15	91.22
BDE 153	2.56	268.23
BDE 154	2.15	249.97
PCB tetra unknown	44.56	PCB standards were not included in PAR
PCB 123	31.02	
PCB penta unknown #1	163.2	
PCB penta unknown #2	25.44	
PCB 156	1.45	
PCB 167	5.81	
PCB hexa unknown #1	58.24	
PCB hexa unknown #2	49.04	

Contaminant Quantification

The samples were analyzed using an Agilent 6890N gas chromatograph coupled to an Agilent 5973 mass spectrometer with helium as the carrier gas (Michigan State University Mass Spectrometry Facility). The samples were injected as 1 µL in splitless mode with a 2 minute purge time and the injection port was held at 270°C. The GC column used was a 30 m x 0.25 mm (i.d.) capillary tube coated with DB-5MS and 0.25 µm film thickness (J&W Scientific, Folsom CA). The GC temperature program was as follows: 110°C for 1 min., 45°C/min to

200°C, 6°C/min to 240°C, 4°C/min to 325°C and held at 325°C for 5 min. The MS transfer line was held at 280°C. Selected ion monitoring was used to detect two masses for the tri-, penta- and hexa-BDEs and the tri-, penta- and hexa-PCBs. The selected ion monitoring reported only the two ion masses determined to correspond to the molecular ion of the selected congeners of PBDEs and PCBs. Samples with low signal-to-noise ratios were reinjected with 2 µL of extract. A signal to noise ratio less than 10 was considered not quantifiable and less than 3 was considered not detectable. The detection limits and limits of quantification were used to replace contaminant readings with low signal to noise ratios with a random number (Table 3). A sample with a signal to noise ratio less than 3 for a particular congener was assigned a random number between the zero and the detection limit. A sample with a signal to noise ratio less than 10 for a particular congener was assigned a random number between the detection limit and the limit of quantification. The use of random numbers was based on my assumption that the overall concentrations for a given tissue against the frequency of fish with that concentration would form a bell-curve. However, the limits detection of the GC/MS machine would truncate this curve at the detection limit and limit of quantification. Therefore, samples with low signal-to-noise ratios were assigned random numbers in place of zeros in order to provide a more normal distribution of PBDE and PCB concentrations.

Table 3. Detection Limits and Limits of Quantification

Congener number	Detection limit (ng)	Limit of Quantification (ng)
BDE 28	0.34	1.22
BDE 47	0.42	n/a
BDE 66	0.17	1.05
BDE 99	0.75	5.76
BDE 100	0.36	3.16
BDE 153	0.71	3.73
BDE 154	0.24	1.67
PCB tetra unknown	64.5	125
PCB 123	86.2	96.1
PCB penta unknown #1	534	958
PCB penta unknown #2	120	979
PCB 156	0.01	34.3
PCB 167	5.35	13.6
PCB hexa unknown #1	0.01	90.7
PCB hexa unknown #2	n/a	n/a

Calibration curves for both PBDEs and PCBs were generated in order to relate the output of the GC/MS data to output from known concentrations. A calibration curve for PBDEs was generated before and after every set of samples run through the GC/MS. The PCB calibration curve was generated once for all samples injected at 1 µL and once for samples injected at 2 µL. The isotopic ratio between primary and secondary ion masses was within ±10% of theoretical values. The detection of a second ion mass allowed me to correctly determine that the compound was a PCB or PBDE; additionally the use of the secondary ion mass allowed for the inclusion of several PCB congeners that were not identified in the standard. One unknown tetra-PCB, two unknown penta-PCBs and two unknown hexa-PCBs were detected with enough frequency throughout the samples to add to the analysis. These unknown PCB congeners were identified based the known molecular mass of a primary and secondary ion and an isotopic ratio within ±10% of theoretical values.

The concentrations were determined using MassLynx V4.1 and QuanLynx software (Waters Corp.), after conversion of the ChemStation data files to MassLynx file format.

Lipid Extraction

Fatmeter readings were calibrated by extracting lipids from muscle tissue scanned by the fat meter. Muscle samples (approximately 5 cm^2) were collected from areas on the fish where the fatmeter readings were taken (Figure 3) and stored in -20°C freezer. At the time of lipid extraction, the tissue was thawed and placed in a drying oven at 75°C until fully dehydrated (i.e. the mass of the sample did not change). The percent water in the muscle tissue was calculated as the dried tissue mass divided by the wet tissue mass; percent water was later used to estimate a wet mass of the dried sample used for the lipid extraction.

The dehydrated tissue was homogenized and lipid content was estimated using a procedure described by Bligh and Dyer (1959). Approximately 1.5 g of dried tissue was weighed and the sample was hydrated by adding 12 mL of distilled water and allowed to settle. After 2 minutes, 45 mL of a 1:2 (v/v) mixture of chloroform and methanol was added to the hydrated sample. A small amount of Whatman ashless powder was added to the sample and the sample was homogenized for an additional 3 minutes. An additional 15 mL of chloroform was added and the mixture was homogenized with the sample for 30 seconds. Then 15 mL of distilled water was added and the mixture was homogenized for 30 more seconds. The sample was then filtered through a Buchner funnel using Whatman #1 filter paper. The filter paper was transferred back into the homogenizer and homogenized for 3 minutes with 50 mL of chloroform. The sample was then filtered with a new filter paper through the Buchner funnel. To ensure the entire sample was captured, the homogenizer was rinsed with about 25 mL of chloroform and the rinsate was filtered. Filtrate was transferred to a graduated cylinder and allowed to sit for 5 hours to allow the methanol/water and chloroform/lipid layers to separate.

The upper layer of methanol/water was removed and the remaining chloroform/lipid layer was allowed to stand for 12 hours in a fume hood to evaporate the chloroform. The remaining lipid layer was then dried in a drying oven for 30 minutes at 100°C. The percent lipid was calculated using wet weight as the amount of lipid, adjusted for the volume removed with the methanol/water layer, and reported as a percentage. Each tissue was analyzed as duplicate subsamples. The log-transformed percentages of lipid obtained for each sample were associated with their respective log-transformed fatmeter reading using a linear equation of best fit.

The data included in this equation (lipid concentration from muscle tissue) was collected over several years and included fatmeter readings from three separate fatmeters along with muscle samples and fatmeter readings from lake trout from several additional stocks beyond the three stocks examined in this study.

Statistical Analysis

ANCOVA

Life history traits that include fork length, body weight, fecundity, gonad weight, gonadosomatic indices and lipid concentration were analyzed using an ANCOVA with age as a covariate. Fecundity was further adjusted for body weight (Qunice et al., 2008) and an ANCOVA was conducted with age as a covariate. Gonadosomatic indices (GSIs) were determined as the gonad weight divided by the body weight, multiplied by 100 and were further analyzed using an ANCOVA with age as a covariate. All variables were tested for normality and all life history traits except for adjusted fecundity, GSI and lipid concentration, which were originally normally distributed, were log-transformed to achieve normality. The ANCOVAs were tested for homogeneity of slopes. If the slopes of the regression lines within each population were significantly different, then an interaction term between population type and age was included in the model and reported. Additionally, if the slopes were significantly different, the populations' marginal means were compared at three ages in order to obtain an adequate understanding of the population differences.

There was a lack of fecundity and gonad weight data from the Michipicoten population because of the remote location of the island and the delay in arrival of fresh ovaries to the laboratory. Most of the Michipicoten lake trout tissue that were included in the contaminant analyses did not have associated fecundity and gonad weight data. However, there were twenty additional lake trout collected from Michipicoten Island. Life history data from these additional twenty lake trout were included in the analyses to help describe the population. The addition of these data allowed for a stronger comparison of the fecundity and gonad weight of Michipicoten population against the lean and siscowet populations.

Differences in PBDE and PCB concentrations of the muscle, ovary and liver tissues between each population were examined within each tissue type using an ANCOVA. The PBDE and PCB concentrations were all log-transformed to achieve normality. Age was included as the covariate and the homogeneity of the slopes was tested using an interaction term between age and population type. If the slopes of each population's regression line were significantly different, the interaction term was reported. A box-plot analysis was used to ensure there were no outliers and consequently two outliers were removed from the PCB muscle analysis and the ANCOVA was rerun.

The different lake trout populations exhibited extremely different age structures such that Marquette lean and siscowet age ranges did not overlap; thereby making comparisons between populations problematic. Therefore, in certain situations, lake trout were compared within a certain age group to reduce the influence of age. Ovary and liver concentrations of PCB and PBDES were compared between siscowets and Michipicotens age 20 years or older using an ANCOVA. Although the ANCOVA included age as a covariate, truncating the ages in this way allowed for a stronger comparison between fish of the same age. Liver concentrations were compared with an ANCOVA between Michipicoten and lean lake trout age 14 year and younger in order to examine the homogeneity of the regression slopes of lake trout of similar ages.

Multiple Regression

A multiple regression was used to determine which variables best predicted PCB and PBDE concentrations in the muscle tissue. The regression analysis used the Akaike information criterion (AIC) in order to understand how contaminant concentrations for a specific tissue can be predicted by demographic variables and contaminant concentrations from other tissues.

Because concentrations of PCBs were an order of magnitude higher than PBDEs, in order to simultaneously compare the contaminants, the muscle concentrations of contaminants were standardized by dividing by the mean contaminant concentration. PBDE and PCB concentrations of the liver were standardized in a similar way using the mean of the concentrations. The variables included in the AIC analysis for the muscle tissue included age, population type, liver concentration, lipid concentration and fork length. All PBDE and PCB concentrations used in the AIC analyses were log-transformed to achieve normality. All variables were tested for correlation and removed if correlation was high between variables.

The AIC revealed that the best regression models that predicted muscle PBDE and PCB concentrations included the liver concentration of the contaminant. Liver concentrations could be representing the current exposure rate. Therefore, to correct for potential differences in exposure rate and thus isolate life history differences, the muscle concentrations of PBDEs and PCBs were divided by corresponding liver concentrations.

Lake trout are mainly exposed to PCBs and PBDEs through ingestion of prey and the liver, because its main function is the metabolism of lipophilic compounds (Hinton et al., 2008), is one of the first organs to be exposed to lipophilic contaminants. PCBs taken in by organisms are removed from the liver within 14 days (Karjalainen et al., 2006); whereas muscle tissues accumulate lipophilic contaminants at a slower rate than other organs based on the internal transfer of the contaminants and has been found to continue accumulating even after exposure (Antunes et al., 2008). Therefore the accumulation of PCBs and PBDEs in the muscle tissue is representative of a longer accumulation period, whereas the liver concentrations of PCBs and PBDEs would be more indicative of the present exposure rate. Based on the residence times of contaminants in both the liver and the muscle, I assume that liver concentrations of PCBs and

PBDEs can provide an indication of current exposure rates and I attempted to control for the exposure variable in order to understand the role of life history variations without the confounding effect of difference in exposure. These adjusted PBDE and PCB muscle concentrations were then used in an ANCOVA analysis with age as a covariate to determine if muscle concentrations are different between populations after correcting for potentially different exposure rates. Since ovary concentrations were not predicted by liver concentrations in the AIC, ovary concentrations were not adjusted for liver concentrations.

A multiple regression, using AIC, was used to determine which variables best predicted PCB and PBDE concentrations in the ovary tissue and liver tissue. All contaminant concentrations were log-transformed. Two outliers were identified, using a Cook's distance test, and removed in the regression analyses for the PCB ovary data. Two outliers were identified and removed for the PBDE ovary analysis based on the residual and Cook's distance test. One outlier was removed in the PBDE liver analysis due to the Cook's distance test. The variables included to predict ovary concentrations were age, population type, liver and muscle concentrations, lipid concentration, fork length, fecundity, egg diameter and gonad weight. The variables included to predict liver concentrations were age, population type, muscle concentration, lipid concentration and fork length. All statistical analyses were conducted using SAS version 9.1 (Table 4).

Table 4. List of statistical tests used

Statistical analysis	***Additional information- transformations and manipulations***
ANCOVA (with age as covariate)	
Fork length	log-transformed
Body weight	log-transformed
Fecundity	log-transformed, included additional Michipicoten data
Gonad weight	log-transformed, included additional Michipicoten data
Adjusted fecundity	divided by body weight, included additional Michipicoten data
Gonadosomatic Index	Gonad weight divided by body weight and multiplied by 100, included additional Michipicoten data
Muscle lipid concentrations	
PBDE muscle concentration	log-transformed
PCB muscle concentration	log-transformed, two outliers removed
PBDE ovary concentration	log-transformed, additional test with only lake trout ≥ 20 years
PCB ovary concentration	log-transformed, additional test with only lake trout ≥ 20 years
PBDE liver concentration	log-transformed, additional test with only lake trout ≥ 20 years, and third test with lake trout ≤ 14 years
PCB liver concentration	log-transformed, additional test with only lake trout ≥ 20 years and third test with lake trout ≤ 14 years
Adjusted PBDE muscle concentration	divided by liver concentrations for each individual
Adjusted PCB muscle concentration	divided by liver concentrations for each individual
Multiple regression using Akaike information criterion (AIC) to predict best model	
PBDE muscle concentrations	Concentrations were standardized by dividing by the mean and log-transformed
PCB muscle concentration	Concentrations were standardized by dividing by the mean and log-transformed
PBDE ovary concentration	log-transformed, two outliers removed
PCB ovary concentration	log-transformed, two outliers removed
PBDE liver concentration	log-transformed, one outlier removed
PCB liver concentration	log-transformed

Results

Life History Results

On average, siscowets were older than Marquette leans, while Michipicoten leans had the largest age range. Mature female siscowets' ages ranged from 15 to 26 years (n=12), while mature female Marquette leans only ranged from 6 to 14 years (n=9). Twenty mature females were caught between 2008 and 2009 near Michipicoten Island, ON and ten Michipicotens were included in the analysis. The Michipicoten Island leans' ages spanned both the Marquette leans and siscowets with an age range from 6 to 27 years (Figure 4).

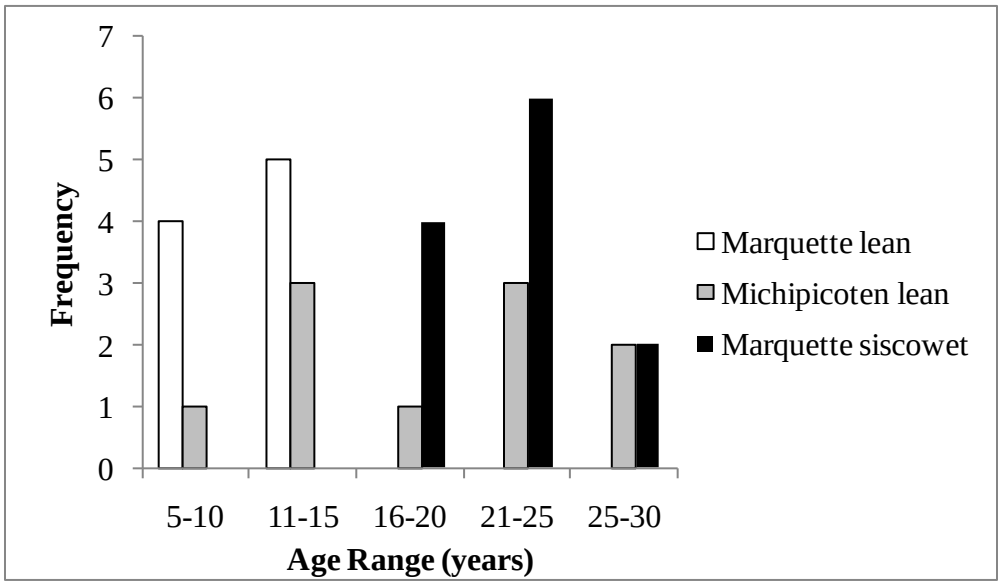

Figure 4. Histogram of the age ranges (years) of each population. The vertical axis contains the frequency counts for each age range. Marquette leans (n=9), Marquette siscowets (n=12) and Michipicoten Island leans (n=10).

The growth rates of both Michipicoten and Marquette leans were much higher than siscowets. The fork lengths (FL) -at-age of the siscowets were significantly lower than both the Marquette leans and Michipicoten populations (ANCOVA, Table 5; Figure 5A). Michipicoten leans had the highest population marginal mean fork length (means adjusted for confounding variable of age), but were not significantly different than the Marquette leans. The interaction

between age and population was not significant in the FL-at-age model (similar slopes) and therefore not included in the final ANCOVA model, however a t-test indicated that Michipicotens had a significantly greater slope between age and FL than siscowets ($t=-2.53$, $p=0.0185$; Figure 5B) indicating a higher growth rate. The Marquette leans did not appear to have a significantly different growth rate than either the Michipicoten leans or siscowets.

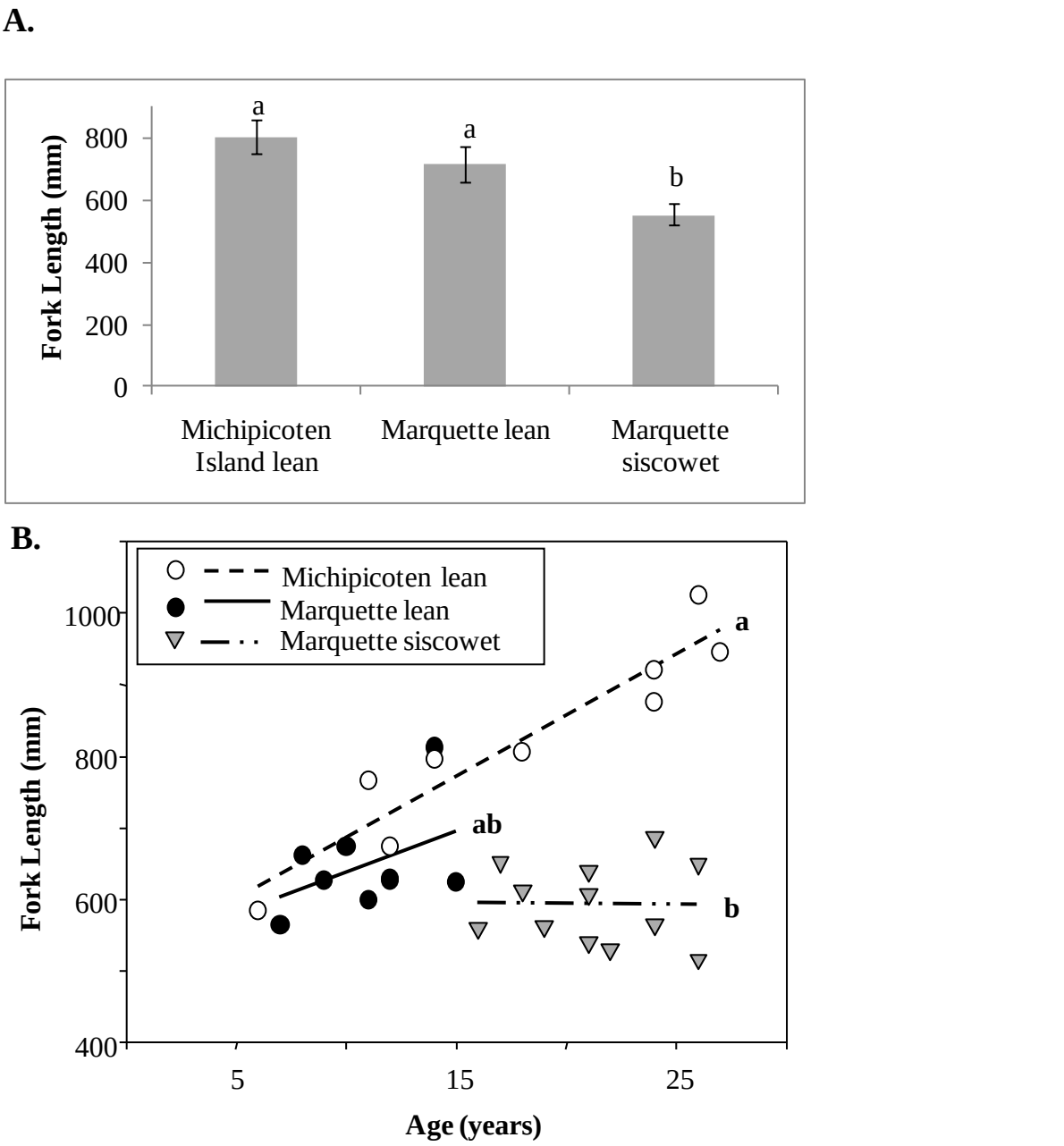

Figure 5. The marginal mean fork length (FL) and FL-at-age relationships were compared between the Marquette leans (n=9), siscowets (n=12) and Michipicoten leans (n=10). A) Comparison of the marginal mean fork lengths (mm) between lake trout populations. Error bars indicate the 95% confidence interval of the population marginal means. Letters indicate populations that are significantly different based on the results of an ANCOVA. B) Relationship between fork length (mm) and age (years) of the three lake trout populations. Regression lines are included for each population (Michipicoten leans: β=0.02, R^2=0.88; Marquette leans: β=0.02, R^2=0.21and siscowets: β=0, R^2=0). Letters indicate populations that have significantly different slopes of the regression line based on the results of an ANCOVA.

Table 5. Differences in life history characteristics based on ANCOVA

Dependent Variable	Independent Variables	Degrees of freedom	F-value	*P*-value
Fork Length (mm)				
	Age[+]	1	19.75	0.0001**
	Population	2	35.42	<0.0001**
Total Model		3	25.72	<0.0001**
Body Weights (g)				
	Age[+]	1	15.15	0.0006**
	Population	2	27.00	<0.0001**
Total Model		3	20.76	<0.0001**
Fecundity (total eggs)				
	Age[+]	1	6.25	0.0181*
	Population	2	18.96	<0.0001**
Total Model		3	13.88	<0.0001**
Gonad weight (g)				
	Age[+]	1	9.62	0.0042*
	Population	2	20.46	<0.0001**
Total Model		3	15.36	<0.0001**
Fecundity adjusted for body weight (total eggs / g)				
	Age[+]	1	1.44	0.2408
	Population	2	1.12	0.3401
Total Model		3	1.43	0.2562
Gonadosomatic index				
	Age[+]	1	4.15	0.0511
	Population	2	4.30	0.0236*
Total Model		3	3.68	0.0237*
Muscle Fat (% lipid)				
	Age[+]	1	0.28	0.6025
	Population	2	4.77	0.0168*
Total Model		3	3.77	0.0220*

[+]Age was covariate for all ANCOVA models; *significant to <0.05; ** significant to <0.001

Similar to fork lengths, lake trout body weights were also significantly different among the lake trout populations with siscowets smaller than the other populations (ANCOVA with age as the covariate; Table 5). Siscowets had significantly lower body weights than both the lean and Michipicoten populations (Figure 6A); however there were no significant difference between the slopes of the regression lines between the populations (Figure 6B). Despite the lack of differences in the slopes between each population, there appears to be different patterns in growth (Figure 6B). Siscowets have a lower mean body weight despite being older than the Marquette leans and the Michipicoten leans are also heavier on average than the siscowets.

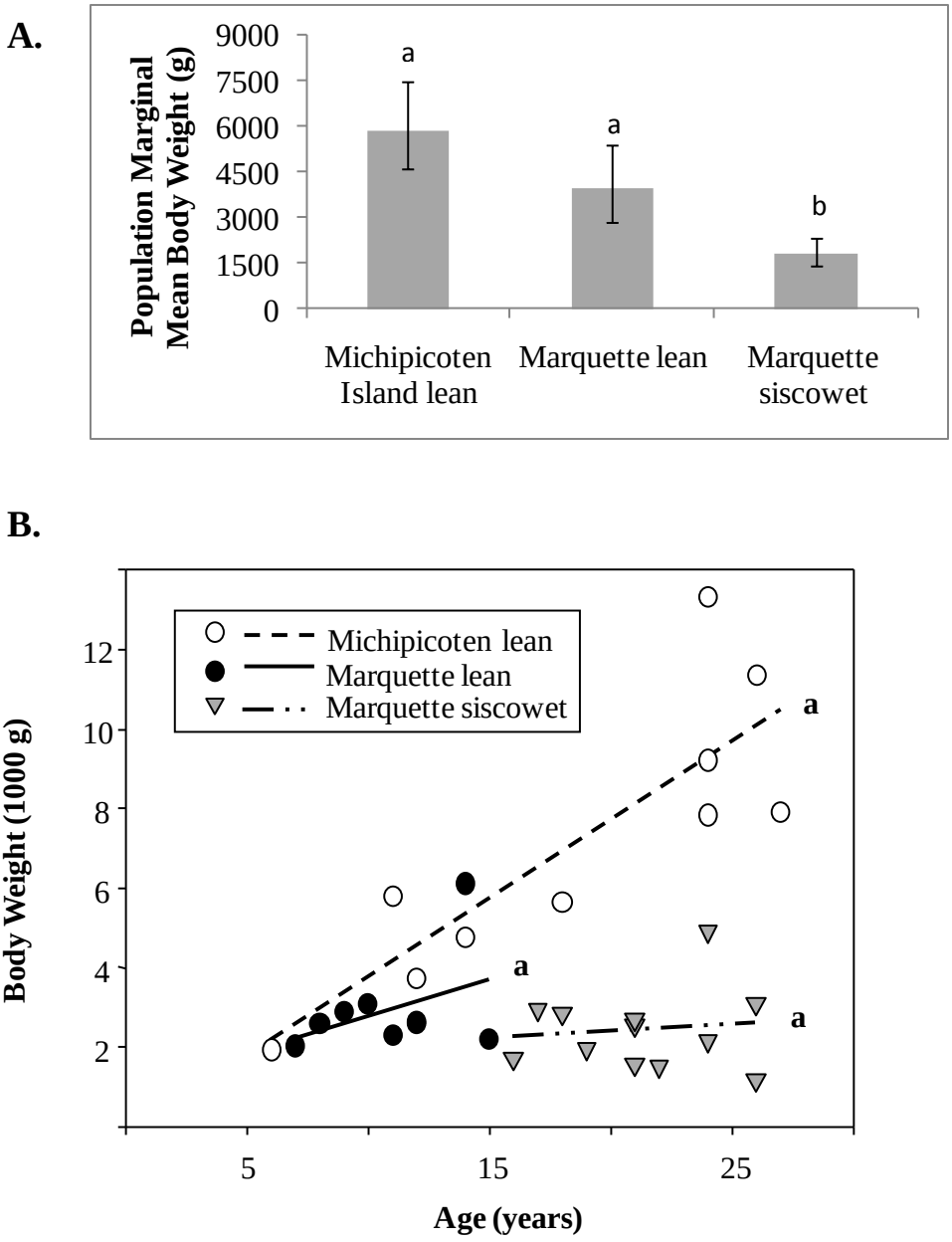

Figure 6. The marginal mean body weights and the relationships between weight and age were compared between the populations: Marquette leans (n=9), siscowets (n=12) and Michipicoten leans (n=10). A) Comparison of population marginal mean body weights (g) between lake trout populations. Error bars indicate the 95% confidence interval of the population marginal means. Letters indicate populations that are significantly different based on the results of an ANCOVA. B) Relationship between body weight (1000 g) and age (years) between the Marquette lean, siscowet and Michipicoten lean lake trout populations. Regression lines are included for each population (Michipicoten leans: β=0.07, R^2=0.80; Marquette leans: β= 0.04, R^2=0.14 and siscowets: β=0, R^2=0). Letters indicate populations that have significantly different slopes of the regression line based on an ANCOVA. There were no significant differences between slopes.

Fecundity and gonad weight were compared between populations to determine differences in reproductive investment. Michipicoten leans had the largest fecundities and gonad weights (ANCOVA, age as the covariate; Table 5; Figure 7 and 8). Fecundities, adjusted for body weight, showed no significant difference between the populations (Figure 9A). The interaction between age and population type for body weight adjusted fecundities was also not significant, however there was a significant difference in the slopes of the regression lines between Michipicoten leans and Marquette leans, with Marquette leans changing production of eggs with age more rapidly than Michipicoten leans ($t=2.25$, $p=0.03$; Figure 9B). The body-weight-adjusted fecundity decreased with age in the Michipicoten leans and increased with age in the Marquette leans. Similarly, Michipicoten leans had a significantly higher marginal mean GSI than the Marquette leans (Figure 10A), however there was no difference in slopes of the regression lines (Figure 10B). Analysis of fecundity, gonad weight and GSI all suggest that Michipicotens produce more eggs with age, but this does not reflect an increase in relative reproductive investment; Michipicotens get much larger with age (Figure 5B) and as a result can produce more eggs.

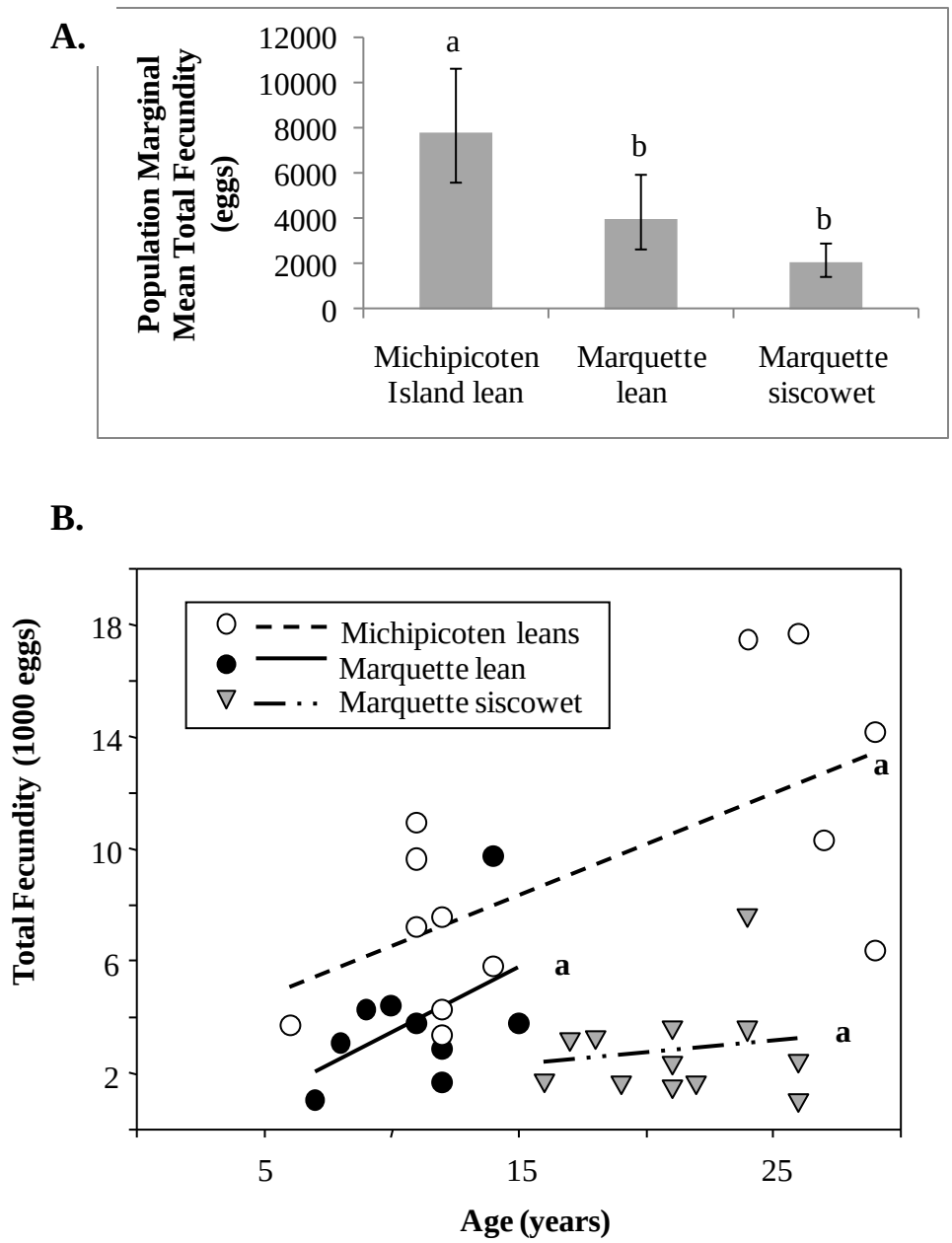

Figure 7. The marginal mean fecundities and the relationships between fecundity and age were compared between the populations: Marquette leans (n=9), siscowets (n=12) and Michipicoten leans (n=11). A) Comparison of population marginal mean fecundities (number of eggs) between lake trout populations including data from Michipicoten lake trout that was not included in this study. Error bars indicate the 95% confidence interval of the population marginal means. Letters indicate populations that are significantly different to an alpha value of 0.05 based on the results of an ANCOVA. B) Relationship between total fecundity (1000 eggs) and age (years) between lean, siscowet and Michipicoten lake trout populations. Regression lines are included for each population (Michipicoten leans: β=0.33, R^2=0.03; Marquette lean: β=0.12, R^2=0.27; siscowet: β=0, R^2=0). There were no significant differences between slopes.

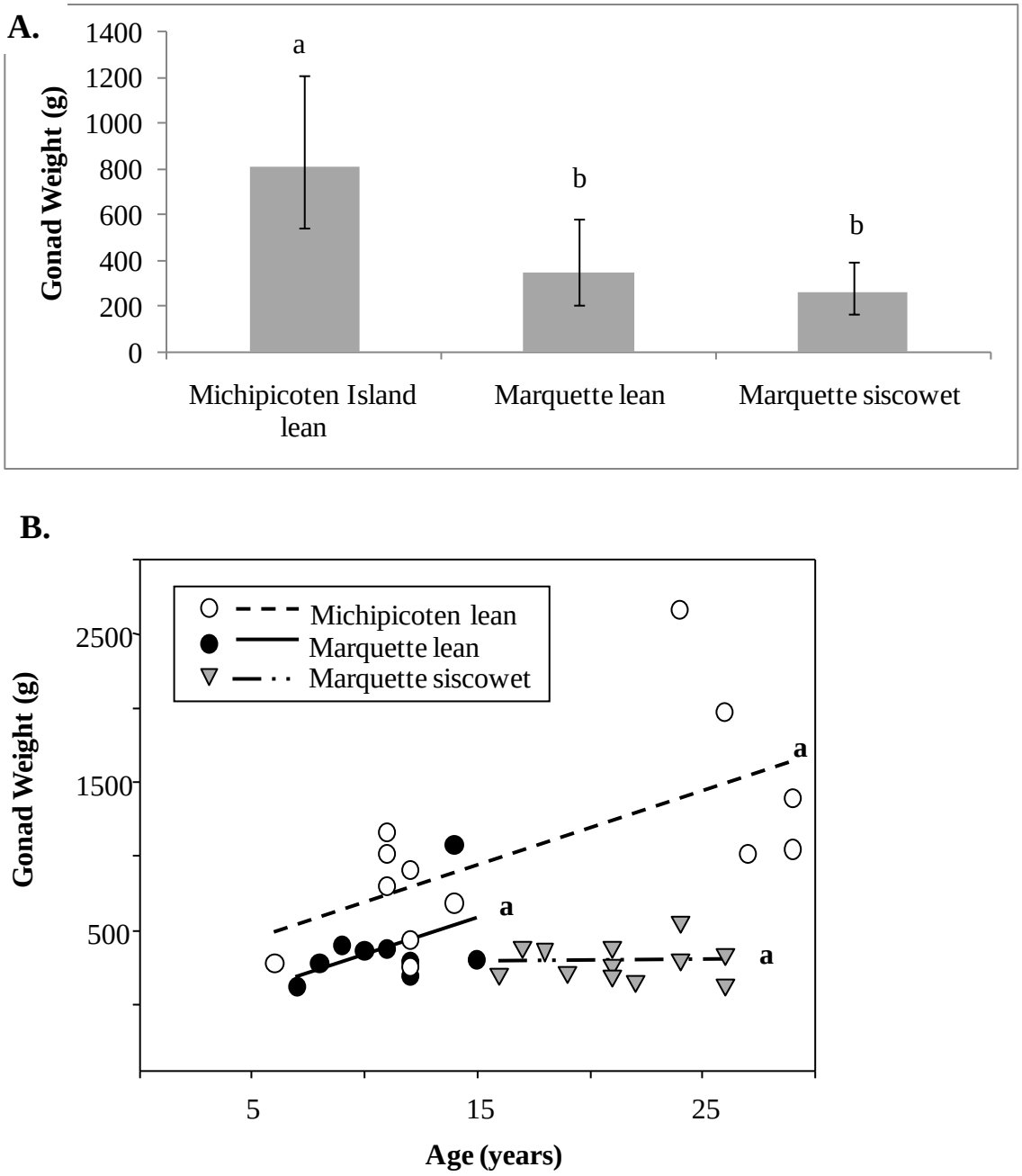

Figure 8. The marginal mean gonad weights and the relationships between gonad weight and age were compared between the populations: Marquette leans (n=9), siscowets (n=12) and Michipicoten leans (n=11). A) Comparison of population marginal mean gonad weights (g) between lake trout populations including data from Michipicoten lake trout that was not included in this study. Error bars indicate the 95% confidence interval of the population marginal means. Letters indicate populations that are significantly different to an alpha value of 0.05 based on the results of an ANCOVA. B) Relationship between gonad weight (g) and age (years) between the lake trout populations. Regression lines are included for each population (Michipicoten leans: β=0.01, R^2=0.01; Marquette leans: β=0.11, R^2=0.27, siscowets: β= -0.01, R^2=0.01). There were no significant differences between slopes.

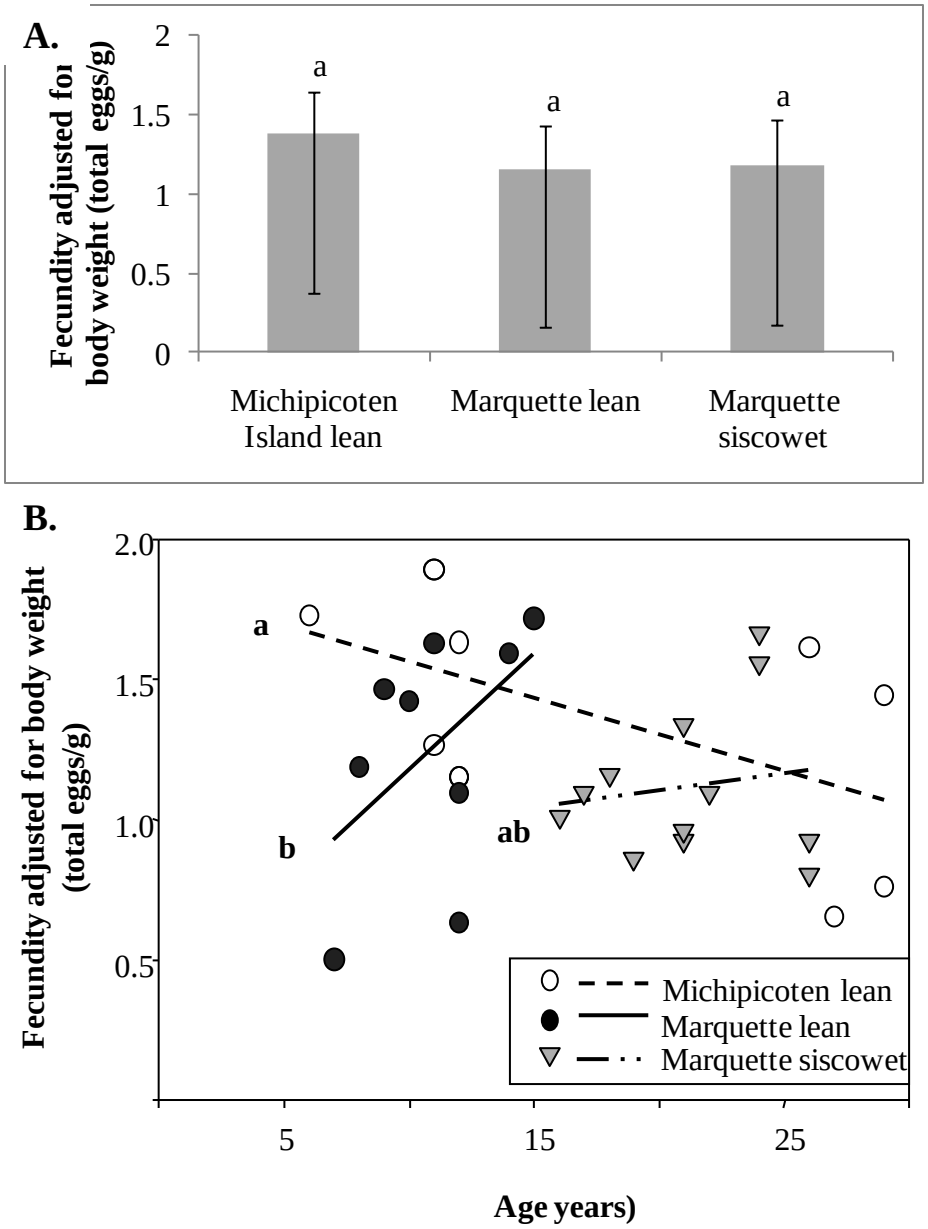

Figure 9. The marginal mean fecundities adjusted for body weight and the relationships between adjusted fecundity and age were compared between the populations: Marquette leans (n=9), siscowets (n=12) and Michipicoten leans (n=11). A) Comparison of population marginal mean fecundity adjusted for body weight (total eggs/g) between lake trout populations including data from Michipicoten lake trout that was not included in this study. Error bars indicate the 95% confidence interval of the population marginal means. Letters indicate populations that are significantly different to an alpha value of 0.05 based on the results of an ANCOVA. B) Relationship between fecundity adjusted for body weight (total eggs/g) and age (years) between the lake trout populations. Regression lines are included for each population (Michipicoten leans: β= -0.03, R^2=0.29; Marquette leans: β=0.08, R^2=0.26; siscowets: β=0.01, R^2=0.02).

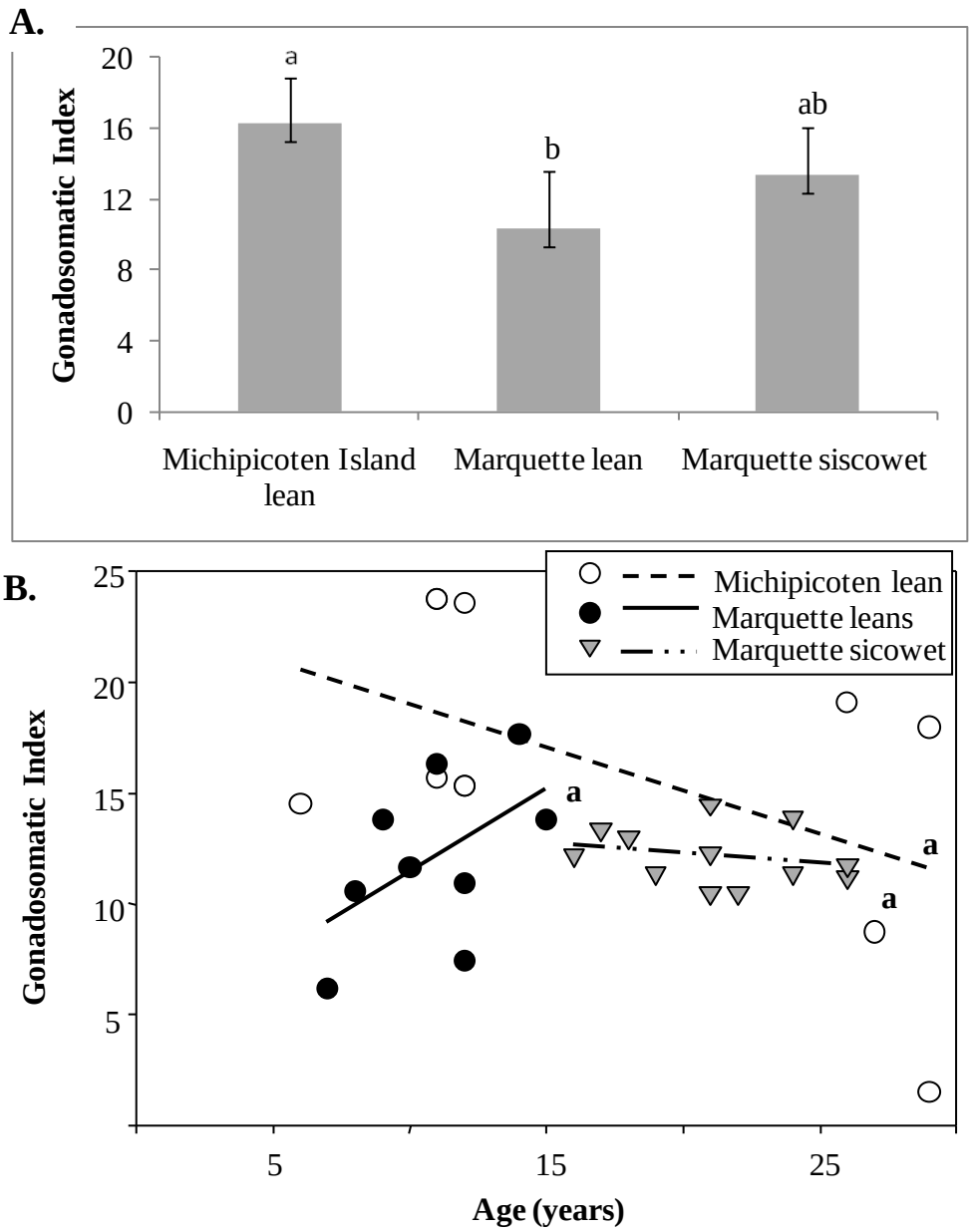

Figure 10. The marginal mean gonadosomatic indices (GSI) and the relationships between GSI and age were compared between the populations: Marquette leans (n=9), siscowets (n=12) and Michipicoten leans (n=11). A) Comparison of population marginal mean GSIs between lake trout populations including data from Michipicoten lake trout that was not included in this study. Error bars indicate the 95% confidence interval of the population marginal means. Letters indicate populations that are significantly different to an alpha value of 0.05 based on the results of an ANCOVA. B) Relationship between GSI and age (years) between the lake trout populations. Regression lines are included for each population (Michipicoten leans: β= -0.39, R^2=0.26, Marquette leans: β=0.75, R^2=0.28, siscowets: β= -0.09, R^2=0.06). There were no significant differences between slopes.

The percentage of lipid in the muscle tissue was significantly different between populations (ANCOVA; Table 5). Fatmeter readings were converted to percent lipid using the following equation ($R^2 = 0.6117$).

$$muscle\ lipid\ concentration = \left(e^{(1.9436 * \ln(fatmeter\ reading)) - 9.5022}\right) * 100\ ;$$

Siscowets had significantly higher levels of muscle fat compared to Michipicoten leans, but were not significantly different than Marquette leans (Figure 11A). There were no changes with age in muscle lipid concentrations for all stocks (Figure 11B).

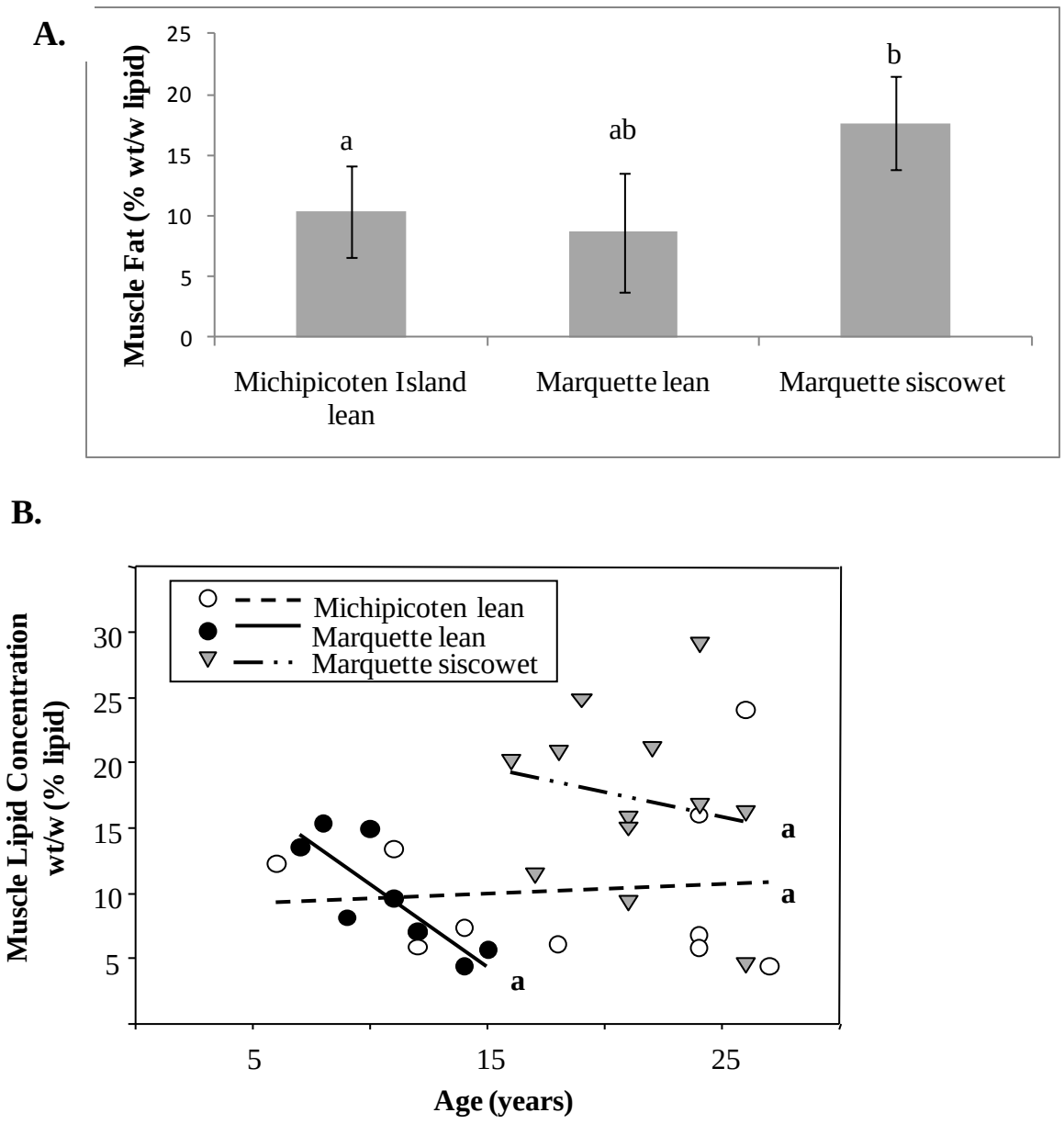

Figure 11. The marginal mean lipid concentrations and the relationships between lipid concentrations and age were compared between the populations: Marquette leans (n=9), siscowets (n=12) and Michipicoten leans (n=10). A) Comparison of population marginal mean lipid concentrations between lake trout populations. The lipid concentrations were taken from muscle tissue and reported as a percentage in wet weight. Error bars indicate the 95% confidence interval of the population marginal means. Letters indicate populations that are significantly different to an alpha value of 0.05 based on the results of an ANCOVA. B) Relationship between lipid concentration (% lipid wt/w) and age (years) between the lake trout populations. Regression lines are included for each population (Michipicoten leans: β=0.07, R^2=0.01; Marquette leans: β= -1.26, R^2=0.67; siscowets: β= -0.39, R^2=0.04). There were no significant differences between slopes.

Contaminant Results

According to the AIC method, empirical support for multiple linear regression models to predict muscle contaminant concentrations based on age, population type, liver concentration, lipid concentration and fork-length was strongest for the model that included population type, liver contaminant concentration (PCB or PBDE) and fork-length (Table 6). The PBDE model also included muscle lipid concentration (Table 6).

Variables important to predicting the contaminant concentrations in the ovary were different between contaminants. Using the AIC method, PBDE concentrations in the ovary were best predicted by age, population type, PBDE muscle concentration, gonad weight and egg diameter (Table 6). The PCB concentrations in the ovary were best predicted by age, population type, muscle PCB concentration, fecundity and egg diameter (Table 6).

PBDE and PCB concentrations in the liver were predicted by nearly the same variables. Multiple regressions using the AIC method found that PBDE liver concentrations were best predicted by the population type, PBDE muscle concentration, muscle lipid concentration and fork length (Table 6). The PCB liver concentrations were best predicted by the population type, PCB muscle concentration and muscle lipid concentration (Table 6).

Table 6. Best models based on AIC regression method

Modeled to predict	Variables	AIC	R-squared
PBDE muscle concentration		-46.85	0.85
	Population		
	PBDE liver concentration		
	Muscle lipid concentration		
	Fork length		
PCB muscle concentration		-52.70	0.90
	Population		
	PCB liver concentration		
	Fork length		
PBDE ovary concentration		-42.81	0.83
	Age		
	Population		
	PBDE muscle concentration		
	Gonad weight		
	Egg diameter		
PCB ovary concentration		-56.40	0.77
	Age		
	Population		
	PCB muscle concentration		
	Egg diameter		
	Fecundity		
PBDE liver concentration		-29.08	0.80
	Population		
	Muscle lipid concentration		
	PBDE muscle concentration		
	Fork length		
PCB liver concentration		-17.94	0.69
	Population		
	PCB muscle concentration		
	Lipid concentration		

Liver PBDE and PCB concentrations showed significant changes in bioaccumulation rates with age between the populations (ANCOVA). For liver contaminant concentrations, results indicated a significant interaction between age and population; the slopes of the populations' regression lines were significantly different (Table 7). There were no significant differences between the marginal means of the populations for both PBDEs and PCBs (Figure 12A and 12B). However, there were significant differences between the slopes of Michipicotens and Marquette leans for both PBDEs and PCBs with the rate of PBDE and PCB accumulation in the livers increasing with age for the Michipicoten leans and show a declining trend with age for the Marquette leans (Figure 12C and 12D).

Liver concentrations can be considered an indicator of exposure levels, and when Michipicotens and Marquette leans younger than 15 were compared for PBDE and PCB concentrations, there were no significant differences in the slopes of the regression lines (Table 7; Figure 13A and B), indicating that the lean morphotypes were similarly exposed to contaminants. In contrast, when Michipicotens and siscowets of the same age (≥age 20) were compared, there was a significant difference in the marginal mean PBDE and PCB liver concentrations with siscowets having much lower concentrations in the liver (Table 7; Figure 13C and D); thereby indicating that the siscowet morphotypes is potentially exposed to lower levels of contaminants.

Table 7. Differences in PBDE and PCB concentrations in the liver tissue based on ANCOVA results

Dependent Variable	Independent Variables	Degrees of freedom	F-value	*P*-value
PBDE liver concentration (ng/g)				
	Age[+]	1	0.42	0.5209
	Population	2	0.98	0.3891
	Interaction term	2	4.07	0.0301*
Total Model		5	4.95	0.0030*
PCB liver concentrations (ng/g)				
	Age[+]	1	2.28	0.1440
	Population	2	1.66	0.2118
	Interaction term	2	4.12	0.0289*
Total Model		5	9.41	<0.0001**
PBDE liver concentrations for lake trout ≤ age 14 (ng/g)				
	Age[+]	1	0.10	0.7579
	Population	1	0.24	0.6389
	Interaction term	1	1.02	0.3379
Total Model		3	0.72	0.5633
PCB liver concentrations for lake trout ≤ age 14 (ng/g)				
	Age[+]	1	0.76	0.4066
	Population	1	1.00	0.3429
	Interaction Term	1	1.84	0.2076
Total Model		3	0.83	0.5079
PBDE liver concentrations for lake trout ≥ age 20 (ng/g)				
	Age[+]	1	1.62	0.2347
	Population	1	10.93	0.0092*
Total Model		2	10.83	0.0040*
PCB liver concentrations for lake trout ≥ age 20 (ng/g)				
	Age[+]	1	1.21	0.3000
	Population	2	7.89	0.0204*
Total Model		2	7.88	0.0105*

[+]Age was covariate for all ANCOVA models

* significant to <0.05

** significant to <0.001

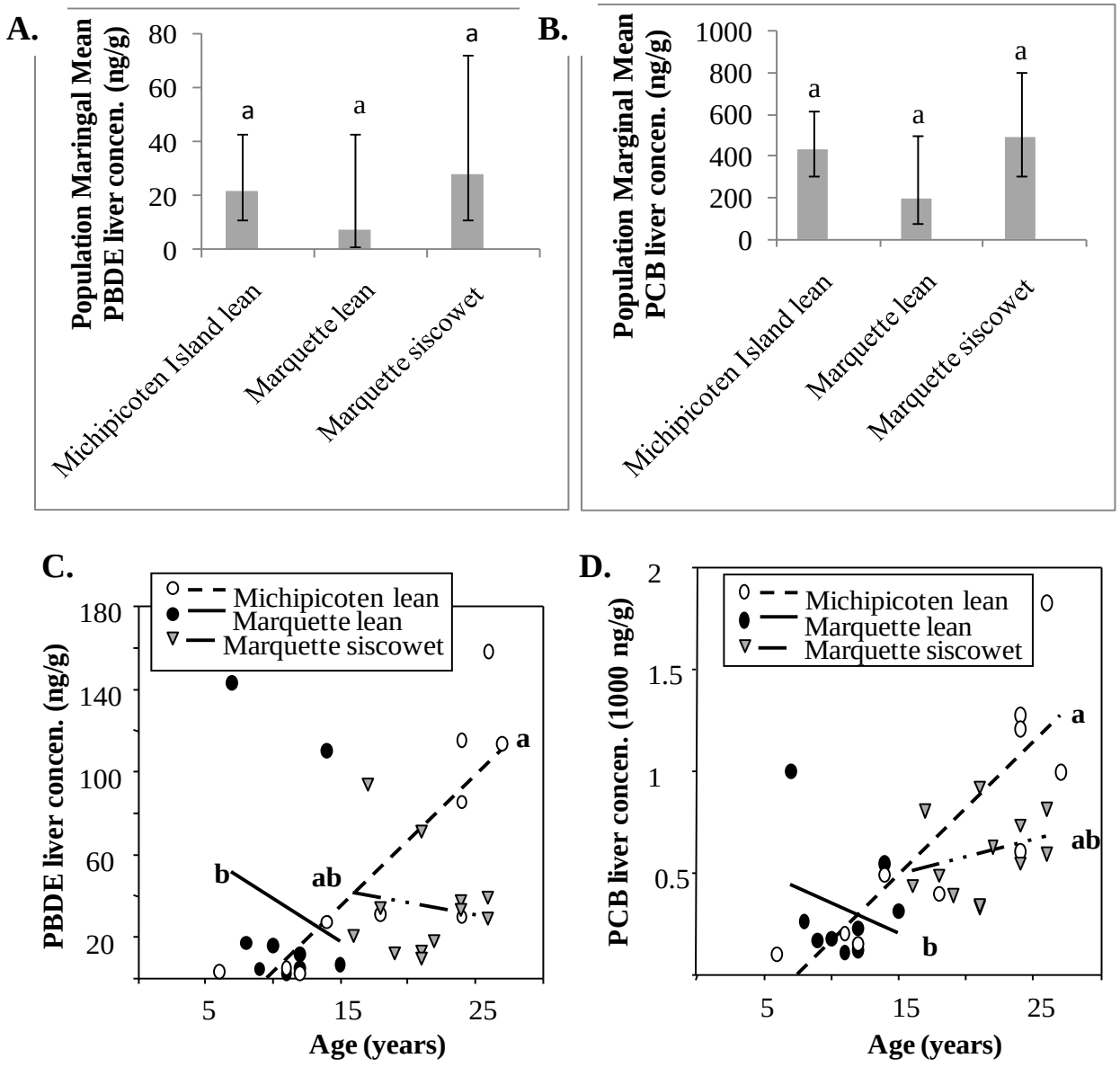

Figure 12. The marginal means of PBDE and PCB concentrations in the liver were compared between the populations along with the relationship between PBDE and PCB concentrations in the liver and age. A) Comparison of population marginal mean PBDE liver concentrations (ng/g) between lake trout populations. B) Comparison of the population marginal mean PCB liver concentrations. Error bars indicate the 95% confidence interval of the population marginal means. C) Relationship between PBDE liver concentrations (ng/g) and age (years) between the lake trout populations (Michipicoten leans: β=0.20, R^2=0.87, n=9; Marquette leans: β=0.11, R^2=0.05, n=9; siscowets: β=0, R^2=0, n=12). D) Relationship between PCB liver concentration (ng/g) and age (years) between the lake trout populations (Michipicoten leans: β=0.13, R^2=0.91, n=10; Marquette leans: β=-0.04, R^2=0.05, n=9; siscowets: β=0, R^2=0, n-12). Letters indicate populations that have significantly different slopes of the regression line or population marginal means based on the results of an ANCOVA.

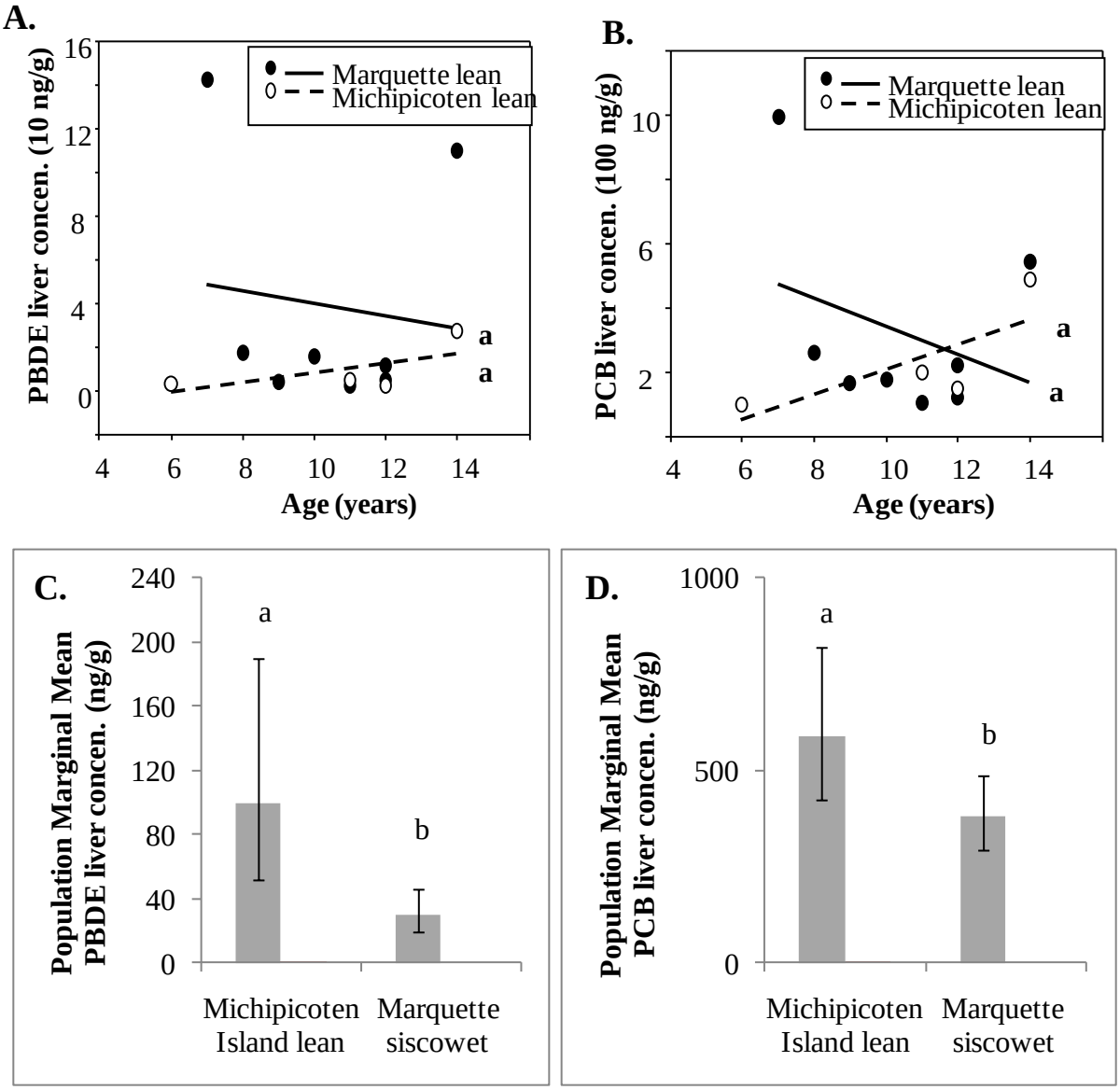

Figure 13. The marginal means of PBDE and PCB concentrations in the liver were compared between the populations along with the relationship between PBDE and PCB concentrations in the liver and age at particular age ranges. A) Relationship between PBDE liver concentration (ng/g) and age (years) for ages less than or equal to 14. Regression lines are included for each population (Michipicoten leans: β=0.20, R^2=0.39, n=4; Marquette leans: β=0.25, n=, R^2=0.17, n=9). B) Relationship between PCB liver concentration (ng/g) and age (years) for ages less than or equal to age 14. Regression lines are included for each population (Michipicoten leans: β=0.17, R^2=0.72, n=9; Marquette leans: β= -0.08, R^2=0.06, n=4). C) Comparison of the marginal mean PBDE liver concentrations for lake trout at or above age 20: siscowets (n=8), Michipicoten leans (n=5). D) Comparison of the marginal mean PCB liver concentrations for lake trout at or above age 20. Error bars indicate the standard errors of the population marginal mean. Letters indicate populations that have significantly different slopes of the regression line or population marginal means based on the results of an ANCOVA.

PBDE concentrations in the muscle tissue accumulated in significantly higher concentrations in siscowets than in leans (ANCOVA, Table 8; Figure 14A; intercepts significantly different ($t=-2.37$, $p=0.03$)). The difference in the rate of accumulation of muscle PBDE concentrations with age across different populations made it difficult to compare muscle PBDE concentrations directly. Therefore, I compared PBDE muscle concentrations at the mean age 17, age 12 and age 22. At age 17 and 22, Michipicoten lake trout had significantly lower concentrations of muscle PBDEs than siscowets; however, there were no significant differences between the populations at age 12 (Figure 14B).

PCB muscle concentrations were significantly higher in siscowets than in Michipicoten and Marquette leans. The statistical analyses were performed with the two outliers removed from the Michipicoten population (see Methods), and when muscle PCB concentration was compared with age across the three stocks, slopes were not significant, but the siscowet intercepts were significantly higher than the other stocks (Table 8, Figure 15A). Marginal means of PCB muscle concentrations for each stock also showed that siscowets had significantly higher concentrations (Figure 15B).

To tease out life history effects on the muscle concentrations of contaminants, I corrected for exposure by dividing the muscle concentration of contaminant by liver concentration of contaminant; this procedure is justified because of the multiple regression (Table 6) indicated that PCB and PBDE muscle concentrations could be predicted by their respective liver concentrations. The PBDE muscle concentrations when adjusted for liver concentrations showed that Michipicotens had significantly lower concentrations compared to siscowets, but siscowets and Marquette leans were not significantly different from each other (Figure 16A and 16B). When muscle PCB concentration was adjusted for liver concentrations, there were also

significant differences between populations (Table 8). Michipicotens and Marquette leans had significantly lower PCB muscle concentrations than siscowets (Figure 16C and 16D).

Table 8. Differences in PBDE and PCB concentration in the muscle tissue based on ANCOVA results

Dependent Variable	Independent Variables	Degrees of freedom	F-value	*P*-value
PBDE muscle concentration (ng/g)				
	Age[+]	1	2.89	0.1016
	Population	2	5.50	0.0105*
	Interaction term	2	3.22	0.0570
	Total Model	5	11.50	<0.0001**
PCB muscle concentrations (ng/g)				
	Age[+]	1	10.78	0.0030*
	Population	2	6.65	0.0048*
	Total Model	3	24.34	<0.0001**
PBDE muscle concentrations adjusted for liver concentrations				
	Age[+]	1	0.93	0.3440
	Population	2	5.25	0.0122*
	Total Model	3	3.74	0.0232*
PCB muscle concentrations adjusted for liver concentrations				
	Age[+]	1	1.10	0.3035
	Population	2	9.06	0.0010**
	Total Model	3	14.33	<0.0001**

[+]Age was covariate for all ANCOVA models; * significant to <0.05; ** significant to <0.001

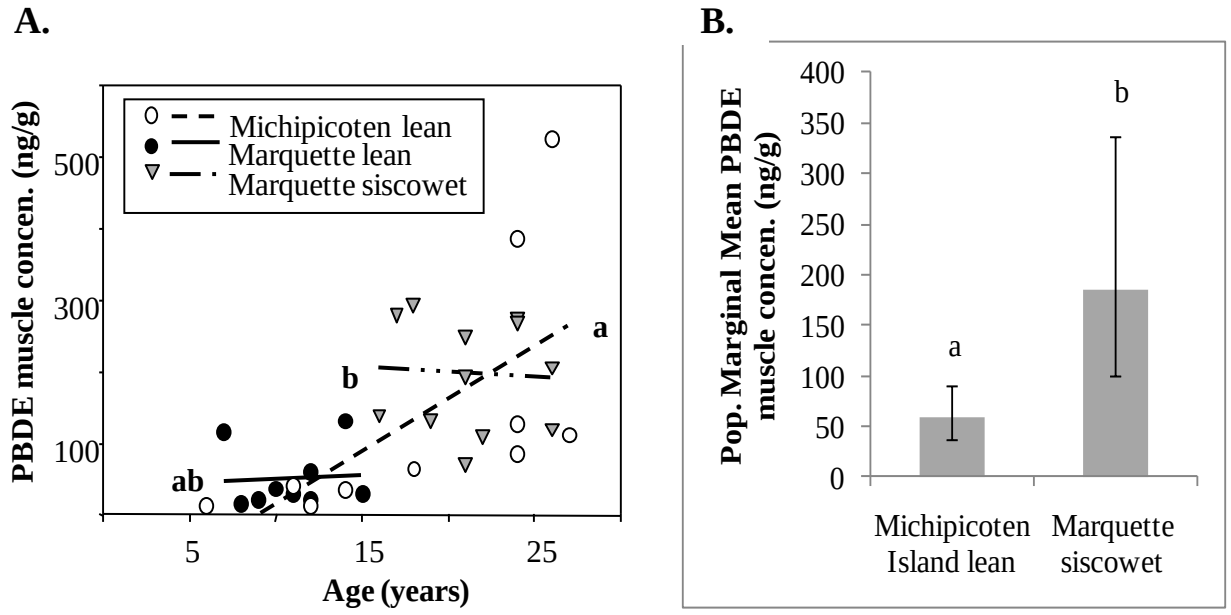

Figure 14. The relationship between PBDE concentrations in the muscle tissue and age were compared between the populations: Marquette leans (n=9), siscowets (n=12) and Michipicoten leans (n=10). Muscle PBDE concentrations were also compared between populations at three ages. Comparing three ages are necessary for a strong comparison because of the differences in the slopes of the regression lines for each population. A) Relationship between PBDE muscle concentration (ng/g) and age (years). Regression lines are included for each population (Michipicoten leans: β=0.15 R^2=0.76; Marquette leans: β=0.04, R^2=0.02; siscowets: β= -0.01, R^2=0). Letters indicate populations that have significantly different slopes of the regression line to an alpha value of 0.05 based on the results of an ANCOVA. B) Comparison of PBDE muscle concentrations (ng/g) at age 17 and 22 years. Error bars indicate the standard errors of the population marginal mean. Comparison of population marginal mean PBDE muscle concentrations (ng/g) between lake trout populations for ages 12 years showed no significant differences between populations. Letters indicate the significant difference between marginal means. Error bars indicate the standard errors of the population marginal mean.

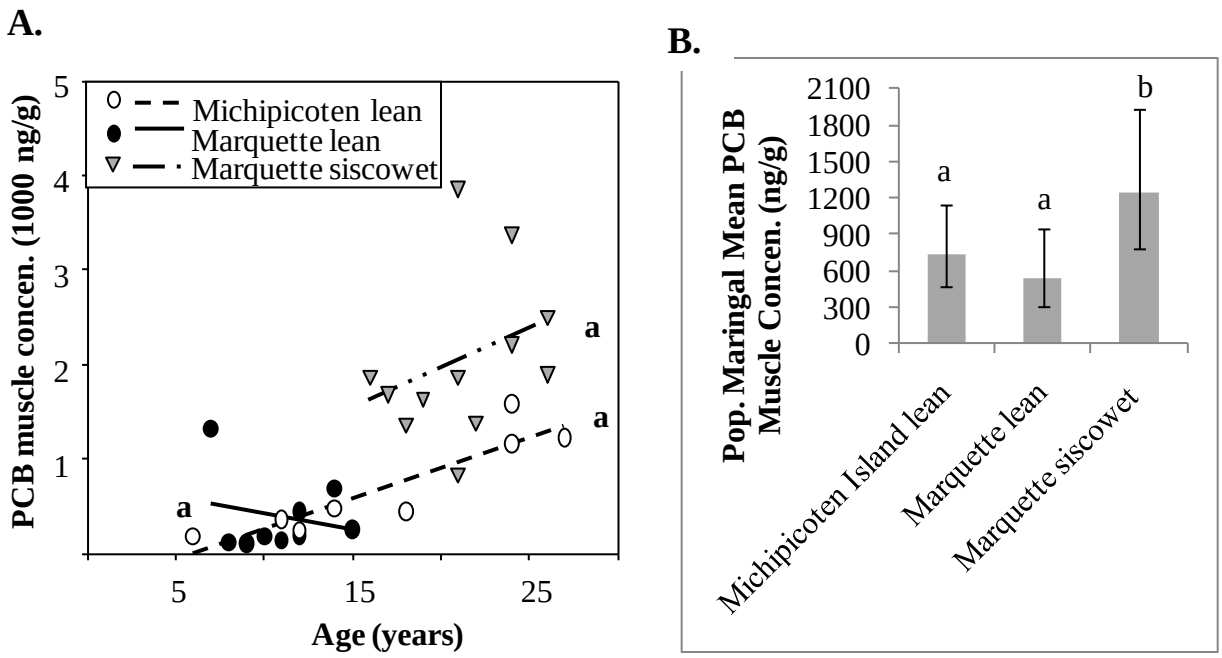

Figure 15. The marginal mean PCB concentrations in the muscle were compared between the populations along with the relationship between PBDE and PCB concentrations in the muscle: Marquette leans (n=9), siscowets (n=12) and Michipicoten leans (n=10). A) Relationship between PCB muscle concentration (ng/g) and age (years). Regression lines are included for each population (Michipicoten leans: β=0.14, R^2=0.76, Marquette leans: β=0.02, R^2=0; siscowets: β=0.04, R^2=0.11). Letters indicate populations that have significantly different slopes of the regression line based on the results of an ANCOVA. B) Comparison of the population marginal means PCB muscle concentrations with two outliers removed (ng/g). Error bars indicate the standard errors of the population marginal mean. Letters indicate population marginal means across that are significantly different to an alpha value of 0.05 based on the results of an ANCOVA.

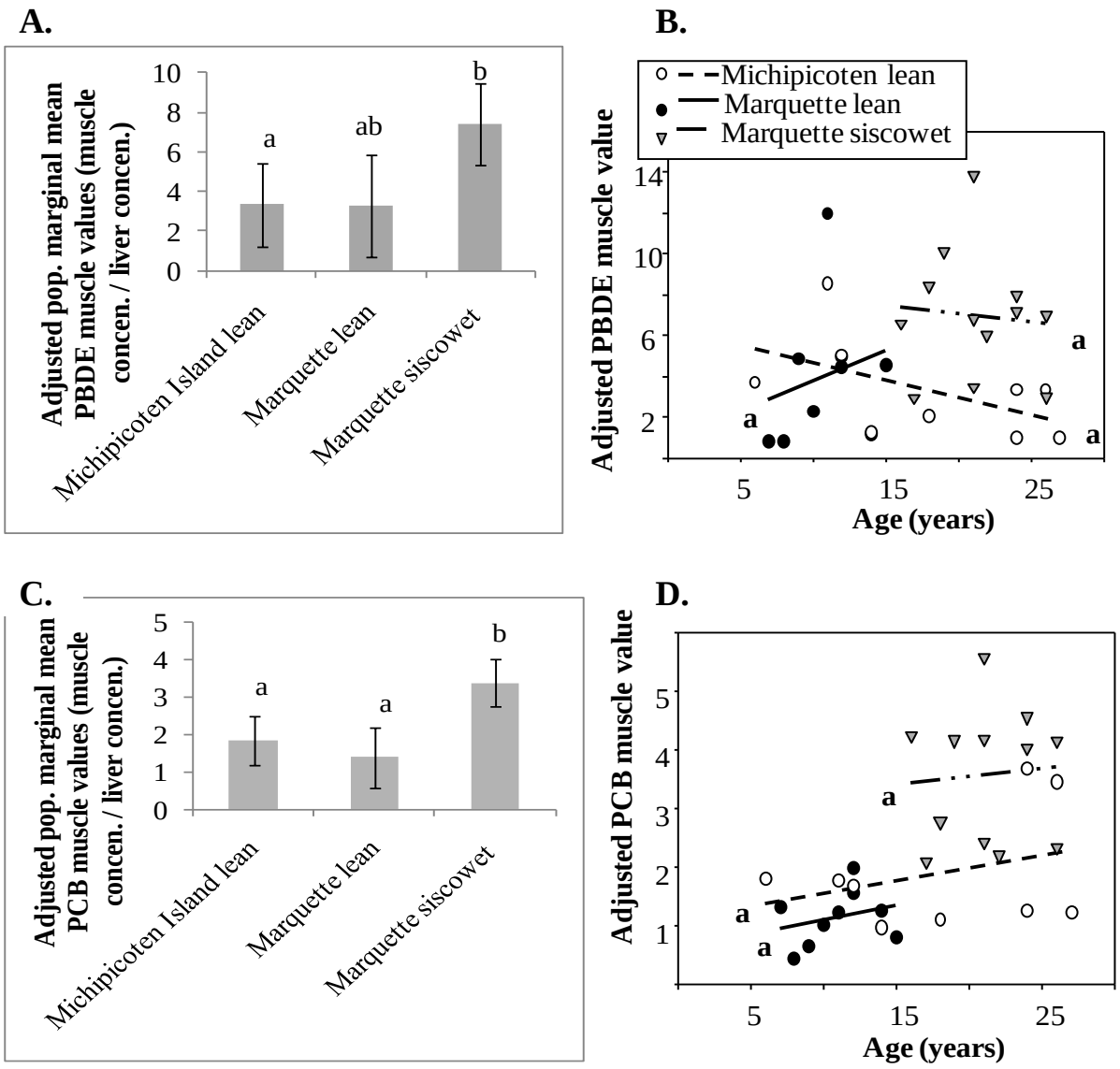

Figure 16. The marginal means of PBDE and PCB concentrations in the muscle adjusted for liver concentrations were compared between the populations along with the relationship between PBDE and PCB adjusted muscle concentrations and age: Marquette leans (n=9), siscowets (n=12) and Michipicoten leans (n=10). A) Comparison of PBDE muscle concentrations (ng/g) that have been adjusted for liver PBDE concentrations (ng/g). Error bars indicate the 95% confidence interval of the population marginal means. B) Relationship between the PBDE muscle concentrations (ng/g) adjusted for PBDE liver concentrations (ng/g) and age (years) between the lake trout populations (Michipicoten leans: β= -0.20, R^2=0.34; Marquette leans: β=0.09, R^2=0; siscowets: β= -0.08, R^2=0.01). C) Comparison of PCB muscle concentrations (ng/g) that have been adjusted for liver PBDE concentrations (ng/g). Error bars indicate the 95% CIs of the population marginal means. D) Relationship between PCB muscle concentrations (ng/g) adjusted for PCB liver concentrations (ng/g) and age (years) between the lake trout populations (Michipicoten leans: β=0.04, R^2=0.11; Marquette leans: β=0.05, R^2=0.07; siscowets: β=0.03, R^2=0.01). Letters indicate populations that have significantly different slopes or population marginal means based on the results of an ANCOVA.

PBDE and PCB ovary concentrations did not differ significantly between populations (Table 9). There were also no significant differences between the marginal mean PBDE or PCB concentrations of the populations (Figure 17A and 17B). Siscowets had a significantly different slope between PBDE concentration in the ovary and age compared to both the Marquette leans and the Michipicoten leans (Figure 17C). The concentration of PBDE in the ovary tissue was decreasing with age in the siscowets, but increasing with age for both lean populations. There were no differences in rates of increase in ovary PCB concentration with age between populations (Figure 17D).

However, despite the lack of significant differences between the marginal means, the difference in slopes of the regression lines suggest that the Marquette and Michipicoten lean populations are accumulating PBDEs into the ovary tissue at different rates based on age (Figure 17C). To further investigate this, the ages were truncated and siscowets were compared to Michipicoten leans age twenty and older. Due to lack of Marquette leans older than age twenty, Marquette leans were not included in this analysis. When the older ages were compared, siscowets and Michipicotens did not have significantly different slopes between age and PBDE or PCB concentrations (Figure 18A and 18B). However, siscowets older than 20 have a lower marginal mean concentration of PBDEs and PCBs in their ovaries than Michipicotens in the same age range (Figure 18C and 18D).

Table 9. Differences in PBDE and PCB concentrations in the ovary tissue based on ANCOVA results

Dependent Variable	Independent Variables	Degrees of freedom	F-value	*P*-value
PBDE ovary concentrations (ng/g)				
	Age[+]	1	10.17	0.0038*
	Population	2	1.08	0.3553
	Interaction term	2	3.73	0.0382*
Total Model		5	7.89	0.0001**
PCB ovary concentrations (ng/g)				
	Age[+]	1	34.64	<0.0001**
	Population	2	0.72	0.4981
Total Model		3	18.79	<0.0001**
PBDE ovary concentrations for lake trout ≥ age 20 (ng/g)				
	Age[+]	1	1.26	0.2884
	Population	1	22.09	0.0008**
Total Model		2	17.81	0.0005**
PCB ovary concentrations for lake trout ≥ age 20 (ng/g)				
	Age[+]	1	1.05	0.3304
	Population	1	5.12	0.0471*
Total Model		2	5.24	0.0277*

[+]Age was covariate for all ANCOVA models

* significant to <0.05

** significant to <0.001

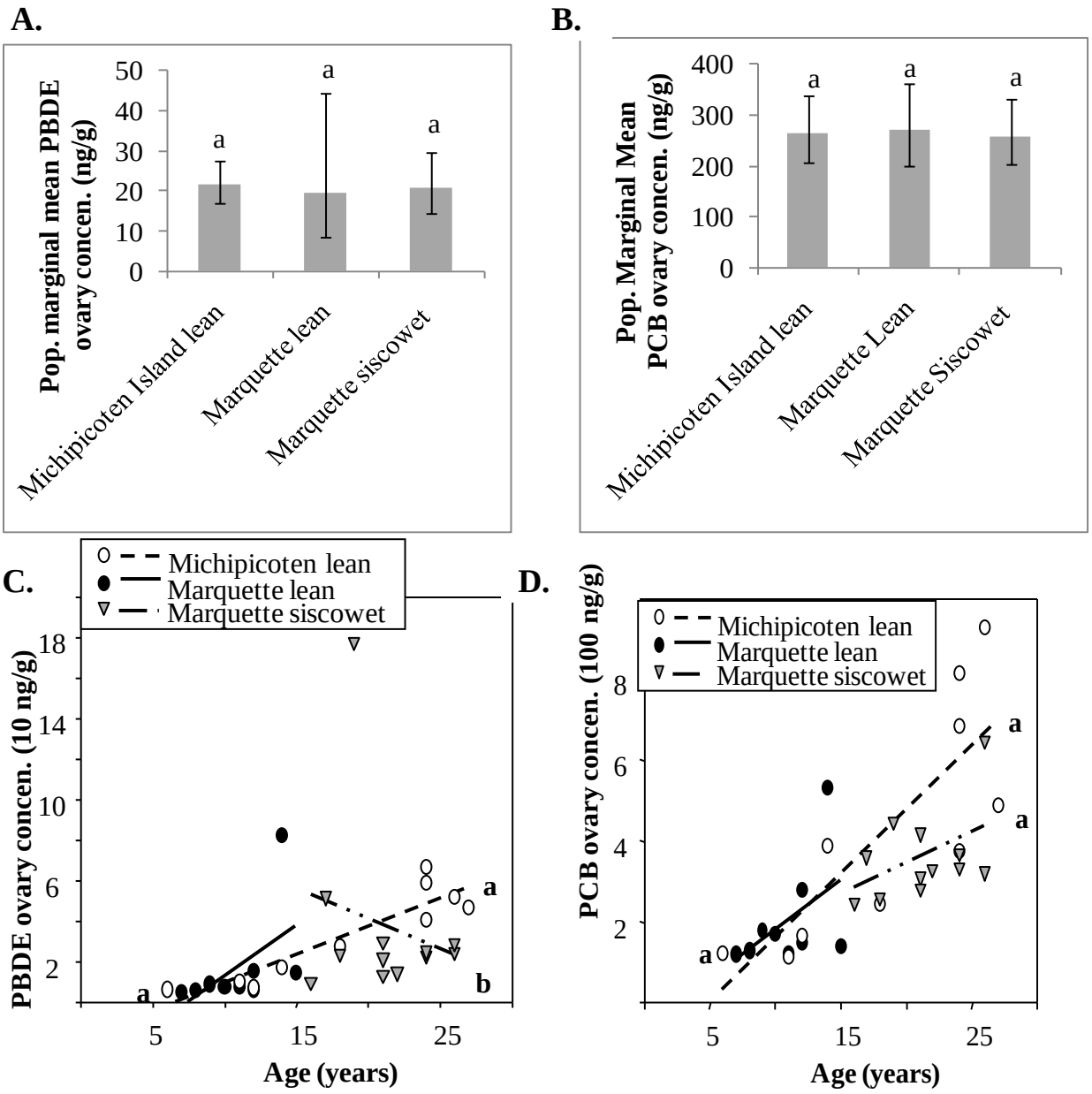

Figure 17. The marginal means of PBDE and PCB concentrations in the ovary were compared between the populations along with the relationship between PBDE and PCB concentrations in the ovary and age. A) Comparison of population marginal mean PBDE ovary concentrations (ng/g). Error bars indicate the 95% CI of the population marginal mean. B) Comparison of population marginal mean PCB ovary concentrations (ng/g). C) Relationship between PBDE ovary concentrations (ng/g) and age (years) between the lake trout populations (Michipicoten leans: β=0.12, R^2=0.91, n=10; Marquette leans: β=0.13, R^2=0.61, n=9; siscowets: β=0.02, R^2=0.02, n=10). D) Relationship between PCB ovary concentration (ng/g) and age (years) between the lake trout populations (Michipicoten leans: β=0.09, R^2=0.78, n=10; Marquette leans: β=0.09, n=9, R^2=0.24; siscowets: β=0.04, R^2=0.23, n=12). Letters indicate populations that have significantly different slopes of the regression line or population marginal means based on the results of an ANCOVA.

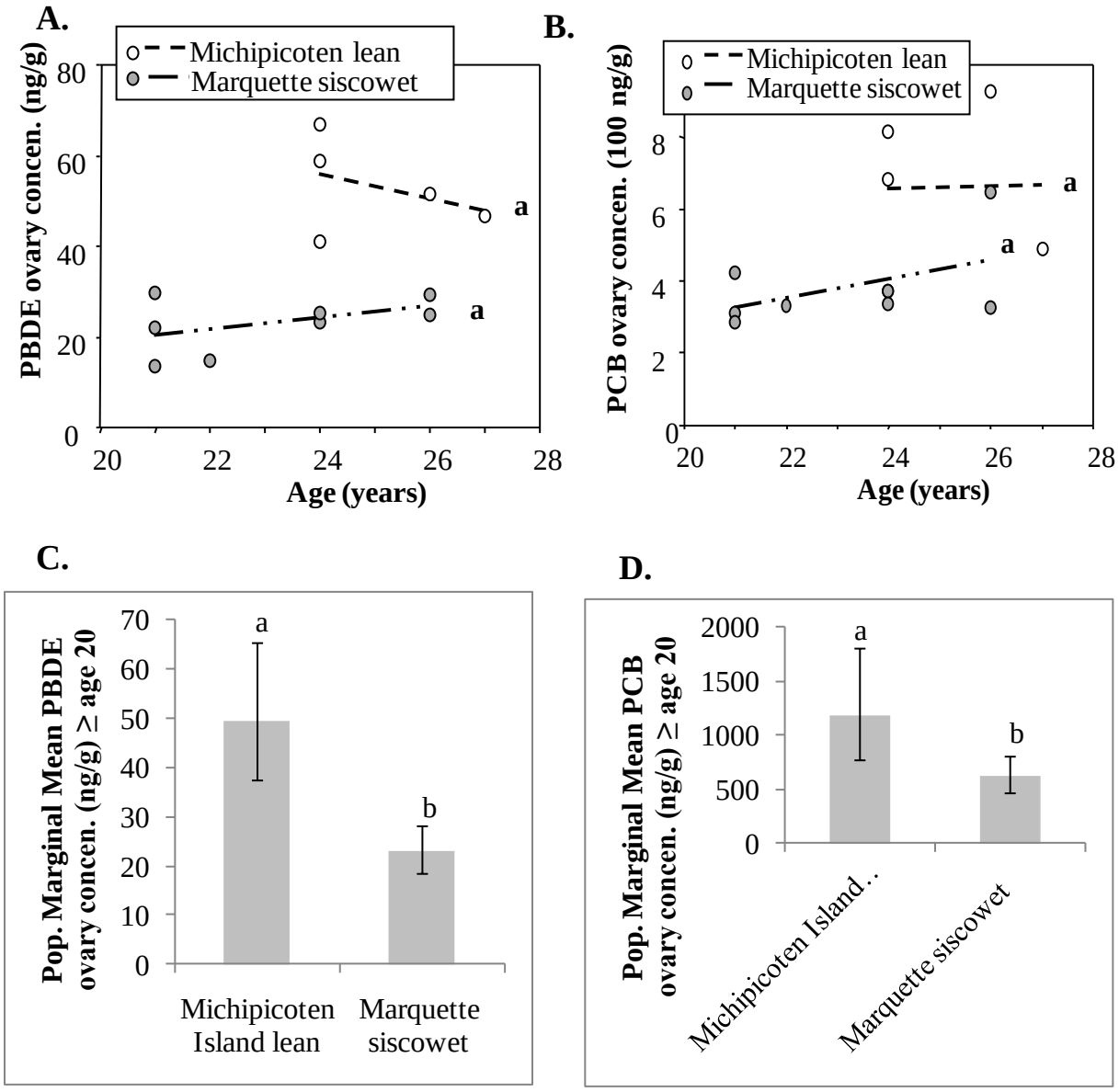

Figure 18. The marginal means of PBDE and PCB concentrations in the ovary were compared between the populations along with the relationship between PBDE and PCB concentrations in the ovary and age: Marquette siscowets (n=8) and Michipicoten leans (n=5). A) Relationship between PBDE ovary concentration (ng/g) and age (years) for ages greater than age 20. Regression lines are included for each population (Michipicoten leans: β= -0.04, R^2=0.11; siscowets: β=0.07, R^2=0.24). B) Relationship between PCB ovary concentration (ng/g) and age (years) for ages greater than 20 (Michipicoten leans: β=0.01, R^2=0; siscowets: β=0.06, R^2=0.24). C) Comparison of the marginal mean PBDE ovary concentration for ages ≥20 years. Error bars indicate the 95% confidence interval of the population marginal mean. D) Comparison of the marginal mean PCB ovary concentrations for ages ≥20 years. Letters indicate populations that have significantly different slopes of the regression line or population marginal means to an alpha value of 0.05 based on the results of an ANCOVA.

Discussion

The results of this study indicate that there may be a potential link between lipophilic contaminants, such as PCBs and PBDEs and the rate of bioaccumulation in different areas of female lake trout's body (muscle, liver and ovary) in a manner that is population specific (Table 6). The most striking differences in bioaccumulation patterns reveled in this study is that Michipicoten lean lake trout older than age 20 have higher concentrations in the ovary tissue compared to siscowets of the similar ages (Figure 18C and 18D). This finding has implications for reproductive success and offspring survival of lean lake trout. Additionally, siscowets accumulate higher concentrations of PCBs and PBDEs in the muscle (Figure 16A and 16C), which is consistent with the higher concentrations of lipids in the muscle tissue and has been supported in other studies (Miller et al., 2000). Aspects of the population that could contribute to the specific bioaccumulation patterns found in this study include life history traits or habitat specific environmental variables. The role of life history traits and habitat on PBDE and PCB bioaccumulation patterns will be discussed along with how bioaccumulation patterns can cause unique issues based on the area of accumulation.

It is not surprising that PBDEs and PCBs are transported and deposited throughout the body of the fish; the chemical composition of PBDEs and PCBs allow them to be transported with triglycerides and lipoproteins (Dulfer et al., 1998). Lipophilic contaminants can be incorporated into bile salt micelles in the liver and then be transported throughout the body by lipoproteins (Dulfer et al., 1998). Due to the high solubility of lipophilic contaminants by lipoproteins, lipoproteins serve as the main transport of contaminants through the plasma (Dulfer et al., 1998). More specifically, lipophilic contaminants can be transported into the ovaries by binding with lipoproteins. Based on in vitro binding assays, it is believed that the interaction

between vitellogenin, a yolk-precursor protein, and a common lipophilic contaminant is a non-specific association between the lipophilic contaminant and the large lipid moiety of the vitellogenin protein (Monteverdi & Giulio, 2000). Lipoproteins such as vitellogenin can then transfer lipophilic contaminant such as PBDEs and PCBs in to the eggs (Monteverdi & Giulio, 2000).

Life History Influence

Population was a consistent predictor of PBDE and PCB tissue concentrations (Table 6). This suggests that life history traits could be influencing PBDE and PCB accumulation patterns in lake trout. The difference in life history traits between lean and siscowet populations has been previously reported (Eschmeyer & Phillips, 1965; Hansen et al., 1995; Bronte et al., 2007; Ebener, 1998) and my study adds additional support to those findings. Below I discuss key life differences before I describe how these life history patterns affect distribution of contaminants in various tissues.

There were several key life history traits differences between the populations. The fork length and body weight data from my study suggest that leans grow much faster than siscowets. Across all age, both the fork lengths and body weights of siscowets were significantly lower than the two populations of leans (Figure 5A and 6A). In addition, the Michipicotens have a faster growth rate compared to siscowets and are maturing at a younger age than siscowets (Figure 5B). This higher growth rate, shown by both lean populations, may lead to biodilution of contaminants as contaminants are dispersed throughout the growing tissues (Lavigne et al., 2010; Hammar et al., 1993) and may explain the higher concentrations of PBDEs and PCBs in the muscle tissue of siscowets. Increased growth rates have been shown to heavily influence

bioaccumulation of PCBs in the body of fish (Ng & Gray, 2009). Although, the theory of biodilution relies on the assumption that populations are exposed to similar levels of contaminants and that populations are metabolizing and excreting contaminants at similar rates. Populations of Altantic salmon (*Salmo salar* L) and lake trout demonstrate different metabolic rates based on life history traits, such as growth rates and lipid storage (Rossignol et al., 2011; Henderson & Anderson, 2002).

Across all the ages of the Michipicoten leans and Marquette leans, the Michipicoten lake trout had higher GSIs although siscowets were not significantly different from either population of leans (Figure 10A). Additional research that compares Michipicoten leans to other genetic strains of lean lake trout has found that Michipicotens have increased reproductive effort with age and they invest more effort into reproduction at older ages than the other genetic strains (Felt, unpublished). This rate of reproductive investment with age was determined in a common garden experiment and suggests that high reproductive investment at older ages is heritable (Felt, unpublished).

The Marquette leans had significantly greater rate of increase in fecundity, adjusted for body weight, with age than the Michipicoten leans (Figure 9B). This suggests that the Marquette leans are producing more eggs as they age. However, the Marquette leans only ranged in age from 6 to 15 and this lack of older leans from Marquette as well as the small sample size makes it difficult to interpret this trend. Additionally, the Michipicoten leans had higher GSIs than Marquette leans, but Marquette leans appear to be increasing fecundity with age. Perhaps since Michipicotens span a range of ages that include older lake trout, the Michipicotens are producing fewer larger eggs, whereas the Marquette leans are producing greater amounts of smaller eggs. However, PCBs and PBDEs are endocrine disruptors and have the ability to disrupt the ability of

lake trout to reproduce (Halden et al., 2010). The exposure to PCBs has been linked to reduction in fecundity (Rose et al., 2003). A difference in exposure rates between Marquette and Michipicoten leans could lead to differences in fecundity due to endocrine disruption by PCBs and PBDEs and exposure to other endocrine-disrupting pollutants.

There were no Marquette leans captured that were older than age 15, suggesting that Marquette leans face higher mortality rates than the other populations; as a result, the Marquette lean population is facing different pressures that result in the production of higher volumes of eggs at younger ages (Figure 9B). However, the use of size-selective gill nets may have contributed to the lack of older lean lake trout collected from Marquette (Sitar, personal communication). Michigan DNR surveys have shown that Marquette lean lake trout live past the age of 15, however the abundance of lean lake trout older than 15 near Marquette is low (Sitar, personal communication).

The Michipicoten leans had significantly higher unadjusted fecundities and gonad weights than the Marquette leans and the siscowets, particularly at older ages (Figure 7A and 8A). The higher fecundities and gonad weight of the Michipicotens indicate a greater investment into reproduction than siscowets over their lifespan, which is consistent with other studies regarding the energy investments of leans and siscowets (Hansen et al., 1995). However, the leans and siscowets from Marquette did not have significantly different fecundities adjusted for body weight, gonad weights or GSIs (Figure 9A, 8A, 10A). The Marquette leans are not producing more eggs per gram of body weight than the Michipicotens, despite the age differences. Additionally, comparing the leans and siscowets from Marquette, it appears that the investment into reproduction is similar based on fecundity and gonad weight despite Marquette leans being younger.

Marquette siscowets had significantly higher concentrations of lipids in the muscle compared to Michipicoten leans (Figure 11A). The amount of muscle fat in lake trout has been determined to be a heritable trait (Goetz et al., 2010; Felt, unpublished). When placed in common environments and exposed to different rations of food, lake trout follow a predictable pattern of lipid accumulation in the muscle that is likely heritable (Felt, unpublished). Work done specifically on lean and siscowet lake trout found a genetic basis for the higher concentrations of lipids in siscowets (Goetz et al., 2010). Siscowets contain higher amounts of a gene in their liver containing an apolipoprotein AII gene, which is associated with increased levels of plasma fatty acids and triglycerides (Goetz et al., 2010). While environmental plasticity may still play a role in the higher lipid concentrations in the muscle tissue of siscowets, there evidence to suggest that this trait is heritable.

Liver Contaminant Patterns

The patterns of PBDE and PCB accumulation in the liver appeared to be population specific with Michipicotens accumulating contaminants at higher rates. The differences in PBDE and PCB concentrations in the livers between the populations could be linked to the energy demands required to maintain particular life history patterns, such as age structure. Alternatively, the difference in accumulation rates of PBDEs and PCBs in the liver with age should be considered within the limited age ranges of the Marquette leans and siscowets. Although there were no differences in PBDE or PCB liver concentrations between the three populations across all ages (Figure 12A and 12B), the Michipicotens and siscowets age 20 and older were different with siscowets having lower liver concentrations of both PBDEs and PCBs (Figure 13C and 13D). The higher concentrations of both PBDEs and PCBs in the liver of older

Michipicotens may be a result of the greater energy requirements for larger fish. Michipicotens had much higher body weights and larger fork-lengths than the siscowets resulting from increased somatic growth and increased energy intake. Basal metabolic rates increase with increased body size (Moyle & Cech, 2004). Additionally, lean lake trout have higher metabolic requirements of the swimbladder due to their higher specific density (Henderson & Anderson, 2002). The higher energy demands due to the larger body size of the Michipicoten leans require more foraging and consumption of more prey items, and consequently more PCBs and PBDEs through ingestion of contaminated prey (Hinton et al., 2008). Once a prey item is consumed, the liver is one of the first organs to be exposed to lipophilic contaminants within the prey (Hinton et al., 2008). While a study has found that the percentage of empty stomach space in leans and siscowets differed only by about ten percent, the size of the stomach would be relative to body size (Conner et al., 1993). Therefore, it is not surprising that fork length is a good predictor of PBDE and PCB liver concentrations; the larger the fish, the more likely to have consumed contaminated prey.

Michipicoten leans and Marquette leans demonstrated potentially different trends in PBDE and PCB accumulation with age. Michipicoten leans appeared to accumulate more contaminants in the liver with increasing age while Marquette leans appear to be stable, although a larger sample size is necessary to confirm these trends (Figure 12C and 12D). The reason for the increase of PBDEs and PCBs concentrations in the Michipicoten leans with age may be due to the differences in growth rates and egg production. The Marquette leans in this study were all under age 14, while Michipicoten leans ranged from 6 to 27 years old. Michipicoten leans could be accumulating more contaminants in their livers because of the slowing of somatic growth and the production of fewer eggs (O'Gorman et al., 1998); Michipicoten leans showed a decreasing

trend of fecundity adjusted for body weight with age whereas Marquette leans had an increasing trend, although the small sample size makes it difficult to confirm these trends (Figure 9B). The Marquette leans, through the higher production of eggs and use of lipids towards growth, could sequester more lipophilic contaminants within muscle tissue and the ovaries. Although, when Michipicoten leans and Marquette leans under age 14 years were compared there was no difference in accumulation patterns with age. This suggests that perhaps the difference in accumulation rates in the liver is due to the difference in exposure rates. The older Michipicoten leans (greater than age 14) are likely consuming more fish and therefore in taking more contaminants. The exposure through food intake would manifest within days and influence the liver concentration because lipophilic contaminants can be eliminated from the liver within weeks of exposure (Karjalainen et al., 2006). Although it is difficult to speculate, if Marquette leans lived as old as Michipicotens, the pattern of PBDE and PCB accumulation in the liver would be expected to increase with age until Marquette leans reached senescence and reduced the elimination of contaminants through egg production.

The concentrations of PBDEs and PCBs in the liver were also predicted by muscle lipid concentrations (Table 6). Muscle lipids could influence the concentration of lipophilic contaminants in the liver if considered within the context of lipid mobilization. The stress of low food availability and consequently low lipid concentrations in the body could mobilize lipids to supplement energy demands (Paterson et al, 2005). When lipids are mobilized, lipophilic contaminants such as PCBs and PBDEs would be moved with the lipids, which would result in higher elimination rates of the contaminants. The elimination of the contaminants could cause higher liver concentrations as the contaminants are mobilized and processed through the liver. This concept has been supported by work on fasted and fed arctic charr (*Salvelinus alpines*)

exposed to PCBs (Vijayan et al., 2006). This study found an increase in PCB accumulation and response of CYP1A enzyme in fasted fish, suggesting increased hepatic activity due to lipophilic contaminants when food availability is low (Vijayan et al., 2006). Populations facing reduced lipid concentrations in the liver may be more susceptible to effects of PCBs and PBDEs.

The relatively rapid elimination rate of PBDEs and PCBs from the liver suggested that the liver could be used as a quantitative indicator of exposure that could be used to adjust for exposure and tease out effects related to life history. To adjust for exposure, I made the assumption that the muscle concentrations could be divided by the liver concentrations based on information generated in my study and guided by information from previous research. In my regression analyses, the liver concentrations were consistent predictors of muscle concentrations and a previous study found that PCBs do not differ between the muscle and liver tissue of rainbow trout (Janz et al., 1992). Also, as previously mentioned, PCBs have been found to be removed from the liver within 14 days (Karjalainen et al., 2006); whereas muscle tissues accumulate lipophilic contaminants at a slower rate than other organs based on the internal transfer of the contaminants (Antunes et al., 2008). Additionally, lake trout in Lake Superior are chronically exposed to PBDEs and PCBs based on historical understanding of accumulation patterns, retention times in Lake Superior and delay in atmospheric deposition (Batterman et al., 2007; Gewurtz et al., 2008). The continuous exposure to these contaminants allows the utilization of the liver as an indicator of the long-term exposure rates. Therefore, muscle PBDE and PCB concentrations were adjusted for exposure rates using liver concentrations (see Muscle Contaminant Patterns below).

Muscle Contaminant Patterns

Muscle tissue also showed a population specific pattern of accumulation. A previous study had found that the concentration of PCBs in the muscle tissue of siscowets increased with the concentration of lipids in the muscle and concentrations were higher in siscowets than leans (Miller et al., 2000). My study also showed that siscowets have greater percentages of body fat and lower growth rates than leans, consistent with previous research (Eschenmeyer and Philips, 1965, Hansen et al., 1995). Also, in my study, siscowets had higher percentages of muscle fat than Michipicoten leans based on fat meter readings from localized areas along the body (Figure 11A). Marquette siscowets had higher concentrations of PCBs than both lean populations and higher concentrations of PBDEs than Michipicoten leans when compared at age 17 and 22 (Figure 14B and 15B). Interestingly, the Marquette siscowets showed a slight trend of increasing accumulation of PBDEs in the muscle with age whereas the Michipicoten leans showed PBDEs a decreasing trend with age (Figure 14A). The limited age range of the siscowets makes it difficult to properly interpret patterns of accumulation with age; however, this difference may be due to depuration of the PBDEs each spawning season and because Michipicoten leans have much higher fecundities, more PBDEs are subsequently eliminated. On the other hand, PCBs did not show a different rate of accumulation with age between the Marquette siscowets and the Michipicoten leans (Figure 15A). The lack of difference in PCB accumulation with age may be caused in a change in exposure to PBDEs in the relative locations of Michipicoten leans and Marquette siscowets. This similar pattern of decreasing PBDE concentrations for Marquette siscowets with age, but increasing patterns of PCBs with age was also shown in the ovary tissue suggesting that lake trout are not exposed to PBDEs and PCBs in similar ratios; suggesting contaminant exposure is site specific (see Ovary Contaminant Patterns below).

The difference in the rate of PBDE muscle accumulation between Michipicoten leans and Marquette siscowets may also indicate a difference in exposure levels. When I adjusted the muscle PBDE and PCB concentrations by liver concentrations and compared between populations, Michipicotens had significantly lower PBDE muscle concentrations than siscowets (Figure 16A). The PCB muscle concentrations of both the lean populations were also lower than the siscowets (Figure 16C). The adjusted muscle data provides similar trends as the unadjusted data; however after adjustment, there were no longer any differences in the rate of PBDE muscle accumulation with age between the Marquette siscowets and the Michipicoten leans. This suggests that the adjustment of muscle concentrations by liver concentrations corrected for potential differences in exposure rates between the populations.

Muscle PBDE and PCB concentrations were predicted by fork-length (Table 6). The influence of fork-length on accumulation of lipophilic contaminants is most likely caused by increased exposure to contaminants because of increased consumption of contaminated prey items to maintain a larger body size.

Ovary Contaminant Patterns

Based on the AIC method, the concentrations of PBDEs and PCBs in the ovary tissue were predicted by egg diameter and fecundity or gonad weight, which are all life history specific traits (Table 6). These results suggest that the amount of energy (in the form of lipoproteins) taken up by the developing eggs (number and size) will directly influence the concentration of these contaminants in the ovaries. The Michipicoten leans had significantly higher fecundities and gonad weights than the siscowets, suggesting that reproductive investment lead to higher accumulation of lipophilic contaminants in the ovaries.

The PBDE concentrations in the ovaries increased with age for Michipicoten leans, but decreased with age for Marquette siscowets (Figure 17C). However, there was no difference in accumulation rates for PCBs between Marquette siscowets and Michipicoten leans (Figure 17D). This may be related to changes in exposure rates in the locations of the populations as similar patterns were shown in the muscle tissue.

Additionally, age was a consistent predictor of PBDE and PCB ovary concentrations (Table 6). When the ovary concentrations of PBDEs and PCBs in lake trout older than age 20 were compared, the Michipicoten leans had higher concentrations compared to siscowets (18A and 18C).

Habitat Specific Influences

My research suggests that life history traits can influence the accumulation patterns of PCBs and PBDEs; however there are also other factors indirectly related to life history traits that could influence the intake of contaminants. Siscowets and leans have unique bathymetric distributions that contribute to the life history differences. However, the bathymetric distribution may also influence PBDE and PCB concentrations through diet and water temperature. Also, exposure to PBDEs and PCBs could be attributed to location and the proximity to a pollution source.

The differences in bathymetric distribution of leans and siscowets result in different diets, which could influence accumulation and exposure of PBDEs and PCBs. Because PBDEs and PCBs are highly lipid soluble, the common route of exposure involves trophic transfer (Wong et al., 2004) and leans and siscowets have shown limited dietary overlap (Harvey & Kitchell, 2000; Harvey et al., 2003). Rainbow smelt (*Osmerus mordax*) are the primary prey item for inshore

lake trout whereas siscowets consume deepwater and slimy sculpins (*Myoxocephalus thompsonii* and *Cottus congatus*) (Conner et al., 1993). There is also a marked difference between inshore and offshore lake trout diets. Offshore lake trout consume more coregonines (*Coregonus* sp.), opossum shrimp (*Mysidacida* sp.) and sculpins (Conner et al., 1993). These dietary differences need to be further explored to fully understand the effect on PCB and PBDE concentrations or accumulation. Future studies could compare contaminant concentrations in prey fish and such data would fill data gaps identified by my research.

In addition to diets, the bathymetric distribution may also result in different water temperatures in the habitats occupied by each population, which could influence the concentrations of PBDEs and PCBs. The half-life of PCB congeners decreased in rainbow trout held in warmer water (Buckman et al., 2007). Additionally, biotransformation of PCBs also increased with increasing water temperature (Buckman et al., 2007). Buckman's study showed decreases in biotransformation within a 4°C water temperature decrease. The surface temperature of Lake Superior ranges between 2 and 4°C (NOAA, 2011) and the greater depth occupied by the siscowet morphotype would lead to differences in the water temperature occupied by leans and siscowets, which could influence biotransformation rates of contaminants, including PCBs and PBDEs.

The location of each population and its relationship to industrialized settings could create differences in exposure to PBDEs and PCBs. The Michipicoten leans inhabit waters near a less industrialized area than the Marquette leans and siscowets. Michipicoten Island is uninhabited by humans and the entire island is controlled by the Canadian government as a provincial park. Whereas Marquette, Michigan is home to several industries, including a coal-fired power plant located on the shores of Lake Superior (We-Energies, 2010). This could influence the

concentration of contaminants based on the localized pollution rates. However, PCBs and PBDEs travel atmospherically and the more northern island of Michipicoten could result in higher deposition rates of PCBs and PBDEs. The evaporation of contaminants in warmer areas and subsequent condensation in colder climates near the poles has been termed 'global distillation' and results in atmospheric deposition of persistent organic pollutants in remote areas (Schmid et al., 2007; Datta et al, 199). Atmospheric deposition would be consistent with our findings of higher liver concentrations of PBDEs and PCBs in Michipicotens older than age 20 compared to Marquette siscowets. The exposure rates of each population could have been included as part of this study by including contaminant analysis of sediment and water samples from each fish sampling site. Inclusion of exposure data would have strengthened the evidence for differences in exposure rates between populations.

The concentrations of PBDEs and PCBs within each tissue did not always follow similar patterns. PCB concentrations were higher than PBDE concentrations in all tissues. This can be contributed to the longer period of exposure to PCBs, which began in the early 20th century (Batterman et al., 2007; Gewurtz et al., 2008; USEPA, 2008a). PBDEs and PCBs also did not accumulate in similar patterns within tissues. Siscowets and Michipicoten leans had different rates of accumulation of PBDEs in the muscle and ovary tissue, while accumulation rates of PCBs in these tissues were not significantly different. This seems to suggest that siscowets are exposed to different concentrations of PBDEs than Michipicoten leans. The difference between PCB and PBDE concentrations in this study suggests that populations are experiencing unique exposure rates to both PBDEs and PCBs.

Health Effects of Contaminants

The elevated concentrations of PCBs and PBDEs in the ovary are of concern because of the potential for adverse effects to the offspring. A previous study found that juvenile lake trout, exposed to concentrations of PBDEs as low as 6 ng/g dry weight, had lower thyroxine levels (T4). This thyroid disruption persisted for over 100 days after exposure (Tomy et al., 2004). In my study, PBDE concentrations in the ovary tissue were found to range from 20 ng/g to 42 ng/g reported in wet weight. This suggests that offspring of these lake trout may have decreased levels of T4, which may disrupt the development of the immune system. PCBs have also been found to directly affect the immune system in fish and cause a decrease in survival when exposed to a bacterial pathogen (Maule et al., 2005). Furthermore, epidemiological studies suggest that lake trout eggs show a negative correlation between egg hatchability and total PCB concentrations (Mac et al., 1992). Strengthening the epidemiological study, a laboratory study also found PCBs to be estrogenic and embryotoxic, causing caused delayed egg hatchability in zebrafish (*Danio rerio*) (Olsson et al., 1999). When zebrafish hatched from mothers exposed to PCBs were raised to adulthood, they had craniofacial malformations, severe curvature of the tail and no development of the caudal fin (Olsson et al., 1999). PCB exposure can slow swimming speeds and slow predator avoidance response, which could impact the survival rates of hatched offspring (Rose et al., 2003).

The higher concentrations of PBDEs and PCBs found in the muscle tissues of lake trout are of concern for the health of the adult individual and the population as a whole. A study has found that exposure to a mixture of PCBs suppressed cortisol dynamics in arctic charr experiencing handling stress (Jorgensen et al., 2002). Usually, the primary stress response in fish is to increase cortisol secretion in efforts to maintain homeostasis (Mommsen et al. 1999). The

lake trout in Jorgensen's study had muscle wet weight concentrations of 8.07 µg/g of PCBs, whereas lake trout in my study had an average of 44.48 µg/g of the sum of the PCB congeners measured. This suggests that lake trout in Lake Superior are exposed to concentrations of PCBs high enough to potentially disturb cortisol excretion and therefore disrupt appropriate stress responses. Additionally, PCBs can interact with the immune system. Lower levels of PCBs can induce thymocyte apoptosis and higher concentrations can results in thymocyte necrosis (Sweet et al., 1998). If thymocytes are reduced or damaged, the body may become more susceptible to disease (Birchmeier et al., 2005). PBDEs have also been found to increase necrosis of thymocytes and decrease viability (Birchmeier et al., 2005). Chinook salmon (*Oncorhynchus tshawytscha*) exposed to an infectious pathogen, *Listonella anguillarum,* had significantly higher mortality rates if the salmon had been exposed to PBDEs (Arkoosh et al., 2010). The salmon in Arkoosh's study had concentrations of PBDEs in their stomachs averaging 190 ng/g dry weight (Arkoosh et al., 2010), whereas my study found an average muscle PBDE concentration of 135 ng/g wet weight. However, Arkoosh's study used juvenile subyearling salmon and another study has shown that juveniles are more sensitive to the effects of PCBs (Duffy et al., 2003). While my study examined mature adult lake trout, it is important to also consider the effects to juvenile lake trout exposed to PBDEs and PCBs.

The effects of PBDEs and PCBs have the potential to be detrimental to lake trout populations in the Great Lakes. The ability for individuals of a population to not only produce offspring, but to produce successful offspring that survive past the juvenile stage and continue reproducing is required for the stability of a population. Exposure of adult lake trout to PBDEs and PCBs can result in disruption of the immune system and difficulty maintaining homeostasis due to stress. These effects may lead to the decreased production of offspring or reduced quality

of offspring. Additionally, the effect of PBDEs and PCBs on the success of the offspring due to the maternal transfer of contaminants can result in failure of the offspring to reach maturity. Although not reported, the observed tissue levels suggest that the combination of the effect of PBDEs and PCBs on lake trout at all life stages has the potential to cause a population decline.

Assumptions, Limitations and Future directions

The population age structure differed greatly between populations making it difficult to tease out the effects of life history on contaminant bioaccumulation because age plays an important role in both life history traits and contaminant accumulation. The mature female leans and siscowets from Marquette showed no overlap in ages (Figure 5). While this apparent age difference suggests that each morphotype is experiencing different life history pressures, it created complications for between population comparisons. Mature siscowets were not collected at the spawning grounds younger than age 15, suggesting the siscowets are a slower growing population that matures at an older age. Mature leans were not collected in this sample past the age of 14, suggesting a higher mortality rate than the other populations and thus they reach maturity at a younger age. Lean lake trout from Michipicoten had an age range that overlapped both the leans and siscowets from Marquette. The Michipicoten lean lake trout mature as young as the Marquette lean lake trout and live longer. The Michipicoten lean lake trout are not under the same fishing pressure or predation from sea lamprey as the Marquette leans (Ebener, 1998) and their life history is influenced by the lower adult mortality compared to the Marquette leans. I addressed the issue of age by using an ANCOVA with age as a covariate for the statistical analyses. In some cases, I truncated the ages at a particular year to compare between populations of the same age for a stronger comparison. However, future studies should include more varied

populations of lake trout and sample lake trout from a variety of locations in order to avoid comparing populations with no age overlap. Additionally, a lower minimum length used for sampling may result in wider age ranges for each population as my study targeted mature lake trout based on a minimum length requirement (Goetz et al., in review). The number of lake trout analyzed was relatively low and the age range was limited for this study, so further studies should include larger sample sizes for each population.

In addition to the difficulties encountered by the age structures of the populations, it is difficult to separate the effect of life history traits and exposure due to habitat variations. Populations examined in this study were exposed to different concentrations of contaminants due to their habitats and prey items. I adjusted for exposure by dividing muscle concentrations by liver concentrations. However, future studies should consider the influence of exposure and include data collection and necessary controls for exposure that could be due to both prey items consumed and habitat. Future sampling events should consider stomach contents, soil samples and water samples, and compare these samples for differences in contaminant concentrations. Such a sampling regime would allow for a better understanding of the influence of life history traits on lipophilic contaminant bioaccumulation patterns.

Implications for Monitoring

While further research is needed to better understand the differences in life history and their effects on lipophilic contaminants, fish monitoring programs that focus on lean lake trout should consider life history when making decisions about consumption advisories. Lean and siscowet lake trout can produce half-breeds that have an intermediate lipid concentration (Eschmeyer and Phillips, 1965; Burnham-Curtis & Smith, 1994). There is currently no literature

that supports these half-breeds are truly genetic hybrids of leans and siscowets, however the potential for inter-breeding makes it difficult to distinguish morphotypes without determining lipid concentrations, which can complicate monitoring programs' aims. The consumption of lean and siscowet half-breeds may have health consequences for the public who are basing their consumption rates on the consumption advisories for lean lake trout. Therefore, a better understanding of lipid concentrations and lake trout morphotypes is necessary to properly guide lake trout consumption warnings so that morphotypes with potentially higher contaminant concentrations in their muscle are avoided. Fish consumption advisories would have to be based on contaminant concentrations observed in the fattiest morphotypes so that human consumers who would not be able to easily distinguish between a lean, a siscowet or a half-breed, are protected.

Due to the presence of distinct morphotypes and half-breeds, and based on my data suggesting a connection between PBDE and PCB concentrations and life history, fish advisory programs need a comprehensive way to incorporate life history into fish advisories. The inclusion of life history information in fish advisors is necessary to minimize human consumption of halogenated pollutants. Federal fish contaminant monitoring in the United States waters of the Great Lakes consists of a two part program, the first part is the targeted to monitor contaminant trends and assess the effects of toxics on fish (US EPA, 2010a). This program analyses lake trout from the Great Lakes by targeting a similar size fish (US EPA, 2010a) and PCBs and PBDEs are included in the list of contaminants analyzed. However, these data are not used to issue fish consumption advisories. The U.S.'s federal program to monitor sport fish using fillets was disbanded in order to create a program that monitors for emerging contaminants. Each Great Lake state and tribe in the U.S. are responsible for issuing fish

consumption advice. The states of Wisconsin, Minnesota and Michigan issue a separate PCB advisory for siscowet and lake trout, however no information was provided on the states' website distinguishing lean and siscowet lake trout (WDNR, 2010; MDH, 2010 and MDCH, 2010). The Canadian government issues fish advisories through the Ontario Ministry of the Environment (OMOE). A fisheries advisory guide is provided and includes advisories for PCBs, however only one advisory is given for both morphotypes of lake trout (OMOE, 2011). The OMOE had the most comprehensive guide because it divided Lake Superior into distinct sections and gave individual advisories for each region and fish identification information; however there was no mention of life history differences between morphotypes of lake trout (OMOE, 2011). Fisheries managers releasing fish advisories to the public should present advisories unique to the lean and siscowet morphotypes and should provide a description of each morphotypes and the way to distinguish each morphotype.

In addition to directly consuming siscowets or siscowet/lean half-breeds, due to the abundance and high lipid content of siscowets, they are being considered for use as high-grade fish oil (Sitar et al., 2008). Siscowet lake trout contain high levels of omega-3 fatty acids (Wang et al., 1990). This fish oil would be used as nutritional supplements and to produce high-value fish meal. Fish oils have many human health benefits including prevention of cardiovascular diseases (Marik & Varon, 2009). However, if siscowet store large amounts of lipophilic contaminants in their muscle tissue, consumption of siscowet oils may pose an unacceptable risk of human exposure to these contaminants.

Fisheries managers should also consider the impact of life history on the response of a population to a stressor such as PBDEs and PCBs. A study modeled the effect of chronic toxicity through immune suppression, reproductive toxicity and growth suppression on three

species of salmonids (*Salmonidae* sp.) (Spromberg & Meador, 2006). These salmonids shared a reproductive strategy, but had different life spans, age of maturity and reproductive contributions, similar to the lake trout populations used in my study. The work by Spromberg and Meador demonstrated that contaminant exposure has population-level effects and that populations can be uniquely susceptible to chronic contaminant exposure; therefore demographic and life history traits should be included in risk assessments of contaminant exposure (Spromberg & Meador, 2006).

Implications for Management: Multiple Stressors

Fisheries managers could use the knowledge of the influence of lipophilic contaminants in tandem with other stressors to understand the lack of naturally reproducing lean lake trout populations in the Great Lakes. The decline of lean lake trout populations resulted in the establishment of stocking programs throughout the Great Lakes. However, stocking programs have yet to see the successful restoration envisioned at the onset of the programs because there has been no evidence of natural reproduction in Lake Michigan or Lake Erie (Madenjian et al., 2008; Bronte et al., 2007; Cornelius et al., 1995). The cause for the decline of naturally reproducing populations as well as the mediocre success of stocking programs could be contributed to a variety of stressors faced at different life stages. During the adult life stage, lean lake trout have been under increased fishing effort as a result of increased fishing efficiency post-World War II (Wilberg et al., 2004). Lean lake trout have greater expression of immune-related genes than siscowets, which may have been caused by greater exposure to pathogens (Goetz et al., 2010). The parasitic sea lamprey (*Petromyzon marinus*), another invasive species, attack and prey on adult lake trout. Studies conducted on lake trout who have suffered from sea

lamprey attacks, found mortality rates between 40% and 69% depending on the genetic strain of the lake trout (Swink, 2003). The siscowet populations experience sea lamprey wounding that is comparable to lean wounding rates (Sitar et al., 2008); however siscowets occupy greater depths and lower host mortality from sea lamprey attacks has been associated with lower water temperatures (Swink, 2003). Sea lamprey attacks also have sublethal effects. Changes in blood serum levels were associated with stress from sea lamprey attacks (Edsall & Swink, 2001). More research needs to be conducted to better understand the sublethal effects of sea lamprey attacks and how it might interact with additional stressors as these stressors may exacerbate the effect induced by lipophilic contaminants. As shown in my study, older lean lake trout have higher concentrations of contaminants, which may also affect survivability and contribute to the problem of multiples stressors on Great Lakes lake trout populations, thereby preventing successful recovery.

Multiple stressors are also observed at the egg and larval stages. I observed significant differences in age structures between lean and siscowet populations in my study. My study did not find mature siscowets younger than age 16 years; however the mature leans were as young as 6 years (Figure 5). Other studies suggest that older fish produce higher quality offspring (Green, 2008; Venturelli et al., 2010). While there are additional physiological factors that can also influence offspring viability, the age of the lake trout may influence the quality of the offspring. Lean lake trout that are reproducing at a younger age may not have as high of a concentration of PBDEs or PCBs in the eggs as older lake trout, but the lower quality of eggs may result in a decreased ability of the offspring to overcome the effect of the contaminants. Additionally, hatchery lake trout have a higher incidence of deformed fry and egg mortality (McDermid et al., 2009). Other effects on larvae include thiamine deficiency resulting in early mortality syndrome

in fry (Fitzsimons et al., 1999). Swim-up syndrome is characterized by anorexia, loss of equilibrium and death in lake trout fry and leads to slow growth, reduced foraging ability and reduced predator avoidance (Fitzsimons, 1995; Fitzsimons et al., 2009). Physical disturbances, such as wind fetch, can cause mortality to lake trout eggs (Fitzsimons et al., 2007). Finally, as my study demonstrates, older lean lake trout may be delivering higher than average levels of contaminants to their offspring.

Although studies have found that concentrations of certain contaminants were not high enough to single-handedly cause mortality of lake trout fry, it has been suggested that exposure to contaminants could interact with other factors to cause egg mortality (Fitzsimons, 1995). A previous study determined that exposure levels to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in Lake Ontario and Lake Superior were below the no observable adverse effect level for lake trout sac fry mortality (Guiney et al., 1996). TCDD can lead to symptoms resembling blue-sac disease (Guiney et al., 1996) and certain congeners of PCBs can produce dioxin-like effects (Tillitt et al., 2008). Lake trout sac fry are the most sensitive to the lethal effects of TCDD than any other freshwater fish species investigated (Walker et al., 1991).

My study only quantified PCBs and PBDEs, however, there are other contaminants that are ubiquitous that should be considered as stressors to Great Lakes lake trout populations. My study only documented PCBs and PBDEs, but it seems that other lipophilic contaminants, such as mercury, hexachlorodibenzene, polychlorinated dibenzo-*p*-dioxins and dichlorodiphenyltrichloroethane (DDT) are likely to follow a similar pattern of accumulation in lake trout and may be found if they had been measured as part of my study. A recent study found trends towards synergistic effects of mercury and PCBs on brain function (Andersen et al., 2009). Lake Superior fish, including lake trout, have the highest concentrations of mercury than

fish from the other Great Lakes (Bhavsar et al., 2010). Few studies that examine the mixture effect of multiple contaminants, however, the potentially additive and synergistic effects of contaminants should be considered in understanding the stressors faced by lake trout populations.

Contaminants are only one of many stressors faced by lean lake trout of the Great Lakes; however the additional stress of contaminants could contribute to the lack of natural recruitment occurring in lean lake trout populations. Despite the low abundances of lean lake trout populations, siscowet lake trout abundances have increased in the previous four decades (Sitar et al., 2008). The increasing abundances have been contributed to lower fishing pressures on siscowet populations, although the increase in abundances could perhaps be understood in part by the distribution of lipophilic contaminants within the body of the lake trout; siscowet do not show as high levels of contaminants in their ovaries as similarly aged leans.

Conclusions

My study suggests that there is a potential link between life history traits and lipophilic contaminant accumulation patterns in lake trout populations. Life history traits are a population's response to environmental constraints and can result in different morphotypes. Lake trout have evolved into the lean and siscowet morphotypes and within each morphotypes, different populations have adopted different life history traits for different environments. When the lean and siscowet morphotypes were compared, there were several major life history differences, the most dominant difference that the siscowets allocate more energy into storage as muscle fat, while the leans proportion more energy into reproductive efforts. These life history differences between leans and siscowets have been well documented in other studies and my

results provide more evidence to this body of literature (Miller et al., 2000, Sitar et al., 2008, Eschmeyer and Phillips, 1965; Hansen et al., 1995, Goetz et al., 2010). These life history differences appear to impact the accumulation of lipophilic contaminants within the body, with siscowets accumulating more contaminants in the muscle tissue and older leans transferring more contaminants to their ovaries. However, these patterns of accumulation of lipophilic contaminants can also be strongly linked to habitat-specific exposure. It is difficult to separate direct exposure from life history because of how life history dictates potential for exposure through habitat selection and energetic requirements due to age schedules and reproductive efforts. Nevertheless, in addition to the apparent differences in life history traits, the Marquette siscowet, Marquette lean and Michipicoten Island lean populations in my study show marked differences in accumulation patterns of PBDEs and PCBs in the muscle and ovary tissues, which may have implications for the health and survival of lake trout populations in the Great Lakes and for human consumption.

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