HEAVY METAL TISSUE DISTRIBUTIONS

IN

SOUTHWESTERN ALASKAN WATERFOWL;

TOTAL MERCURY ASSAYS FROM MUSCLE, BRAIN, AND BONE

By

Roger F. N. Rothschild

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A

THESIS

Presented to the Faculty

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By

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Abstract

Food containing mercury has been identified as a possible health risk. Total mercury (THg), which is inorganic (Hg^{2+}), and methylmercury (MeHg) species, has been found in the arctic food web. In Alaska, birds are an important seasonal component of the diet, but have not been studied extensively and characterized for the presence of mercury. Birds are good subjects for examination because they feed at different trophic levels, can be long-lived, and are both abundant and widely distributed. Not only can birds monitor local Alaskan food webs, but, if they are migratory, can be used to compare exposure in different regions.

Mercury levels in muscle, brain, and bone tissue of 140 birds taken by subsistence hunters across southwestern Alaska were determined. I tested the null hypothesis of no interspecific differences in total mercury levels in the 18 species of Alaska birds surveyed. There were interspecific differences with the Lesser Scaup (*Aythyra marila mariloides*), and the Black Scoter (*Melanita nigra Americana*), having the highest levels of mercury. In general, mercury levels were higher in muscle than in brain or bone. The mean values for mercury in the species studied were lower than the levels known to cause adverse reproductive or behavioral effects.

Keywords: Mercury, Methylmercury, Subsistence, Birds, Alaska

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Chapter 1

Introduction

Naturally occurring mercury (Hg) is ubiquitous to the environment due to mineral deposits and degassing from the earths crust. Mercury occurs in three oxidations states: Hg^0, Hg^+, and Hg^{2+}. The Hg^0 vapor can reside in the atmosphere for about a year. Mercury can form bonds with carbon (organic mercury), and can also volatilize as MeHgCl or MeHg. This can be redeposited as inorganic Hg^{2+}, after conversion by UV radiation in the atmosphere, under rainwater (wet), or snow or dust (dry), conditions (Watras *et al*, 1994). Hg^{2+} is methylated by sedimentary bacteria such as sulfate reducing bacteria or non-biologically by humic acid/chemical processes in boreal ponds and lakes. It is the methylated form that is of primary interest because of biomagnification up the food chain to increase human health risks (Tsiros and Ambrose, 1999).

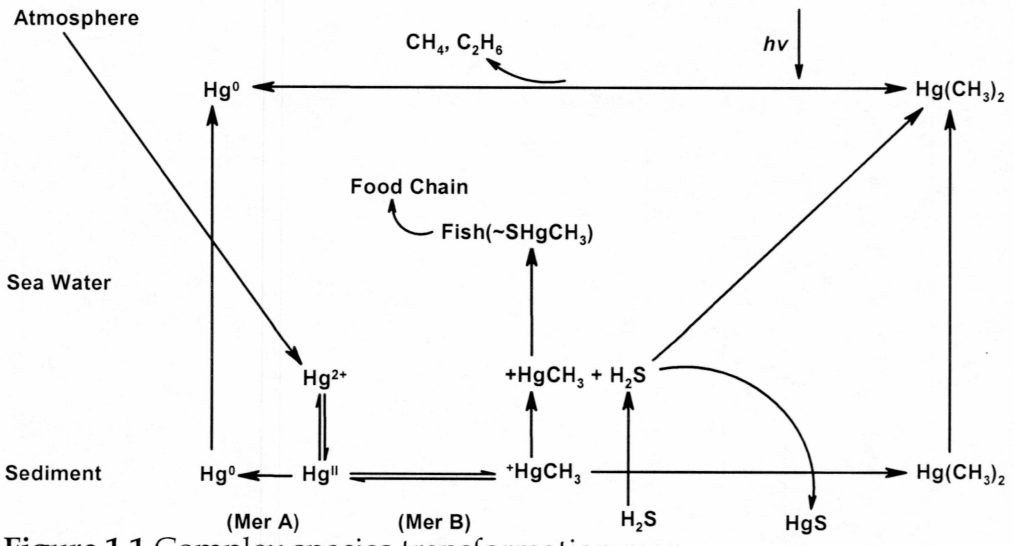

Figure 1.1 Complex species transformation map

All mercury species exist in fresh water, but Hg^{2+} is more soluble in water than Hg^0. In the Arctic, freshwater lakes derive their Hg burdens from snow pack runoff (atmospheric precipitation) and mountain stream outflow (mineral deposits). Clear lakes and outflow by rivers retain these burdens. Demethylation of methylated Hg species has been found to occur in these waters, whereas methylation occurs in "colored water" of wetlands and estuaries (Fjeld *et al* 1992, Louis *et al* 1993, Fitzgerald *et al* 1998, Porvari and Verta 2003).

Increased Hg burdens have been found in the Northern Tier Scandinavian countries, and the Great Lakes of the U. S., which are heavily industrialized. In Alaska, the long-range emission and transport leads to deposition which is retained in the vegetation and soil, increasing the environmental burden to Alaskan wildlife (Porvari and Verta, 2003). This increase of mercury level is not limited to the Northern European countries; remote arctic regions are also seeing an increase. Due to the global transport of mercury, it is now a global pollutant. The primary causes for this global background increase are: coal-fired generators and waste incinerators. The increase is not a recent phenomenon; it began with the industrial revolution (Fitzgerald *et al*, 1998).

Environmental factors such as absorption onto carbonaceous matter, rate of deposition/elimination, pH, anions/cations, hydrated metal oxides and redox

conditions all play a part in the water Hg concentration (Fjeld *et al*, 1992). Several studies suggest Hg methylation is due to sulfate reducing bacteria (biological methyl and sulfide fixing), in the aerobic/anaerobic boundaries, and in the sulfate limiting conditions found in many lake waters and lake sediments particularly peatlands (Porvari and Verta, 2003). The environmental concern of great significance is: does this increase in background mercury make its way into the aquatic food chain where $MeHg^{+}$ biomagnifies up the foodchain beginning with the smallest of aquatic organisms (phytoplankton), and finally, enter human consumption (MacDonald *et al*, 2002)?

Mercury released from industrial spills or mining operations can be carried hundreds of miles from the originating point even though Hg generally settles quickly into the sedimentary beds. A release from the Oak Ridge complexes responsible for producing thermonuclear fission products during the 1950's into the 1960's released an estimated 150 tons of Hg into Polar Creek, near Oak Ridge, Tennessee (Campbell *et al*, 1998). The study, accomplished in 1997 of the local riverine system, found that very high concentrations decrease with distance from the originating point. There was a great deal of variability though, due to fluctuations in water flow during periods of flooding and backflow from a major nearby river (Campbell *et al*, 1998). This is similar to the deposition patterns found in Alaska. Major deposits of cinnabar lie within the Yukon-

Figure 1.2 This figure shows the collection area. Taken from ref. 27.

Kuskokwim drainage. Wintertime low water levels allow mercury-laden particles (mostly cinnabar), to settle out. During the spring breakup and immediately following this, the increased outflow from the melting snows remobilize this accumulation, and begin carrying it farther downstream where the water can eventually slow, and redeposit its burden to the riverbed. The surface water of the rivers do not contain high levels of mercury, it is concentrated in the sediments (Campbell *et al*, 1998, Bloom and Lasorsa, 1999).

Accumulation of mercury in estuarine food chains has been previously reported. The uptake of the inorganic Hg^{2+} and $MeHg^{+}$ occurs through passive diffusion in phytoplankton; the very bottom of the food chain ladder (Lawson and Mason, 1998).

Biomagnification is a geochemical/physiological process of concentration increase in tissues. Mercury exists in the environment; small organism's intake an amount more than they can excrete; a larger organism eats many small organisms, and that amount within the small organisms is now incorporated into the larger organism's tissues. This continues until an animal is eaten by a human. The degree to which biomagnification occurs is related to the number of biomagnificational steps that are between the animal and the last trophic level it inhabits (Ben-David *et al*, 1998).

An example (Ben-David *et al*, 2001):

Algae → duck → man	2 steps
Algae → small fish → large fish → river otter	3 steps
Plankton → small fish → large fish → seal → man	4 steps
Plankton → small fish → large fish → seal → polar bear → man	5 steps

Figure 1.3 Biomagnification steps

There is an increasing trend in mercury tissue concentrations as the trophic level increases. Plankton are low in mercury, while whales that eat plankton are a little higher. But polar bears, that eat seal, that eat fish, tend to be very high in mercury tissue concentration. Man is at the top of the food chain. People who eat large amounts of marine foods are at risk from mercury contamination (Rothschild and Duffy, 2002).

Figure 1.4 easily illustrates the result of biomagnification. In my research, I analyzed samples of halibut tissue from Cold Bay, Alaska. The samples were sent to me marked with the weight of the fish they were taken from. There is a clear increase in the THg concentration as the fish gets larger or older. The one sample marked 10 pounds contained 20.28 ng/g, while the two marked 20 pounds contained 32.82 and 33.32 respectively. Not quite twice the concentration as the 10 pound halibut, but the 23 pound halibut was almost exactly twice the

concentration at 40.73 ng/g. The 90-pounder, an old fish, was virtually nine times the 10-pounders' concentration at 164.35 ng/g.

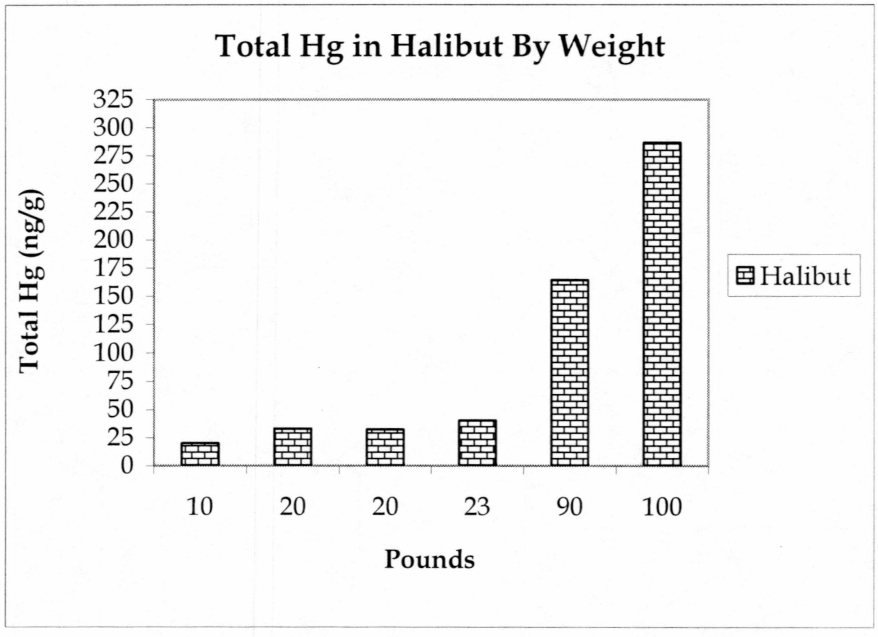

Figure 1.4 Halibut THg by weight.

Birds are efficacious as a monitoring tool for the bioindication of mercury in the environment: many species have long lives; birds are widely distributed; are top-level piscivores down to plankton eaters; and are abundant. Previous research effort has been focused on organ levels of THg, particularly the liver and kidneys, including research on mercury assays in feathers and eggs (Burger, Burger and Gochfeld, 1985, 1992, 1993, 1994, 1997, 2000, 2002, Lewis and Furness 1991, Monteiro and Furness 1995, and Braune *et al*, 2002). Burger and Gochfeld (1997), Thompson and Furness (1989), and Thompson *et al* (1991), reported all mercury in feathers and muscle is in the form of $MeHg^{+}$. If this is correct, $MeHg^{+}$

levels in various tissues of birds in higher trophic levels will be elevated when compared to birds of lower trophic levels. Also, performing THg analysis will be cost efficient as the THg analysis by default, includes $MeHg^{+}$, and is by far analytically more straightforward.

Appendices

Waterfowl Species Surveyed: Species, *N*, and Specific Characteristics

			Habitat		Nesting		
			While	Migrating	Placement	Ave. Distance	
Name	*n* = ?	What	Breeding	and Wintering	Preference	to Water (yds.)	Food
Anas acuta acuta	43	Duck	FW	SW	OG	40	CS
Anas americana	5	Duck	FW	SW	OGC	32	FP
Anas clypeata	6	Duck	FW	SW	OGC	138	PLANK
Anas crecca carolinensis	10	Duck	FW	SW	OGC	31	CS/FP/A
Anas platyrhynchos platyrhyncos	3	Duck	FW	SW	OGC	100	CS/FP/A
Aythyra affinis	1	Duck	FW	SW	OGC	3	SA
Aythyra marila mariloides	17	Duck	FW	SW	OMGC	3	SA/FP
Bucephala albeola	2	Duck	FW	SW	ET	200	SA
Bucephala islandica or clangula	1	Duck	FW	SW	ET	30	FA
Clangula hyemalis	5	Duck	FW	SW	OMGC	3	SA
Melanita fusca deglandi	6	Duck	FW	SW	OGC	unknown	SA
Melanita nigra americana	12	Duck	FW	SW	OGC	35	SA
Anser albifrons frontalis	6	Goose	FW/SW	SW/FW	OGC	3	CS
Branta bernicla nigricans	1	Goose	SW	SW	OG	3	SP
Branta canadensis taverneri	3	Goose	FW	FW	All areas	3	CS/FP
Branta canadensis minima	12	Goose	FW/SW	FW	All areas	3	CS/FP
Grus canadensis	1	Crane	FW	SW/FW	OMGC	3	FA/SA/CS
Limosa lapponica	5	Godwit	FW	SW	OG	unknown	SA
Cygnus columbianus	1	Swan	FW	SW/FW	OMGC	20	FP
	140						

Notes: 1. Distances less than 10 yards were noted as "on floating vegetation", directly adjacent" to water, "very close", or "on small islets in lakes", ect.

2. Where a sequence of Key acronyms are noted, the first is the primary.

Acronym Key

Habitat

Lakes, pools, marshlands, brackish estuaries, and sloughs e.g. mostly freshwater environment	FW
Salt water and brackish coastal waters and estuaries including marshy areas, coastal bays, and/or mudflats. e.g. mostly saltwater environment	SW

Nesting Preference

On ground with sparse cover	OG
On ground with cover	OGC
On marshy ground, sparse cover	OMG
On marshy ground in marshy areas tall grass or other cov	OMGC
On ground on islets, peninsulas, or muskeg	OGIPM
Elevated in trees	ET

Food

Pondweeds and seeds of pondweeds, leafy parts and stems. e.g. Mostly freshwater plants and parts.	FP
Eelgrass, rockgrass, sea lettuce or other saltwater grasses. E.g. Mostly saltwater plants and parts.	SP
Cereal grains such as; wheat, barley, oats, millet sorghum, rice, and other plant seeds. E.g. Mostly seeds.	CS
Cereal grain, freshwater plants and parts, but no animal consumption. E.g. Virtually a pure vegetarian bird.	CS/FP
Cereal grain, freshwater plants and parts with some animal consumption. E.g. <25-30% animal intake.	CS/FP/A
Insects, small crustaceans and mollusks. E.g. Mostly freshwater animals with some plants, seed and parts. E.g. <25-30% vegetarian intake.	FA
Insects, mollusks, crustaceans and other small animal life. E.g. Mostly saltwater animal consumption.	SA
Zooplankton and other phytoplankton. E.g. Freshwater plankton with some small animals and seeds.	PLANK

Chapter 2

Materials and Methods

2.1 Sample Collection

Bird head samples were collected from Native subsistence hunters in villages across the Yukon-Kuskokwim Delta of southwestern Alaska. No effort was made to collect any particular species. The collection period was all of 2002 through spring and summer of 2003. All bird heads were taken from the freezers of the families who agreed to participate in this study.

One "sample" is defined as a bird head with approximately 1-2 inches of neck attached. The samples were placed in Zip-Lock bags and kept frozen for later analysis.

One "tissue sample" is either muscle, brain or bone tissue taken from one head and placed into a vial for analysis for THg or $MeHg^{+}$. Correspondingly, each head had six total tissue samples removed: one (1) each muscle, brain and bone for THg, and one (1) each muscle, brain and bone for $MeHg^{+}$.

Muscle tissue was taken from the cervical area immediately inferior to the occipital region of the skull, due to denser muscle structure in this area. Brain tissue was taken after carefully removing the (or parts of), the parietal, occipital, temporal and frontal bones. No effort was made to acquire any particular part of the brain. Bone tissue was the aforementioned structures, but for some small birds, bone from the mandible was used. No fatty tissues were collected.

2.2 Analytical Methods

2.2.1 Total Mercury (THg) In Tissues

An Overview of the Method

The usual method is to first homogenize the sample to be digested with a stainless steel, high-speed blender (Bloom and Fitzgerald, 1998, and Bloom, 1992). This operation was not performed to prevent any possibility of cross-contamination of samples, and to enhance the robustness of the digestion process.

The sample is a solid piece, or small pieces of tissue, in a 40ml vial, into which, 7mL of 70:30 HNO_3:H_2SO_4 solution is introduced (acid concentrations 69-70% and 98% respectively). After heating and cooling, the "soup" is diluted with 0.2M BrCl. This step is critical as the acid digestion is not robust enough by itself

to completely break down the CH_3Hg^+ to free Hg^{2+}. A known volume aliquot of the oxidized, diluted sample is then introduced into the sparging vessel, and $SnCl_2$ added to reduce the mercuric ions (Hg^{2+}), to elemental mercury ($Hg^{0)}$. Ultra high purity N_2 gas is "bubbled" through the reduced digestate mixed with Milli-Q water in the sparging vessel. The vapor passes through an acid-fume trap composed of Teflon tubing containing soda-lime trapped between tufts of glass wool. The now scrubbed vapor passes into a trap, in which the mercury adsorbs to the surface of gold coated sand particles. This trap is placed into a stream of ultra-high purity Ar gas, and heated rapidly to 400^0 C. The Hg desorbs into the gas stream, immediately adsorbing to the surface of a second "analytical", gold-coated sand trap downstream from the first. This trap concentrates the mercury and allows for interference removal.

(Note: interferences at this point are water vapor and air introduced into the traps and detection train). The analytical trap is then heated desorbing the mercury into the Ar stream which carries them into the cold vapor atomic fluorescence spectrophotometer cell. Here, the Hg atoms are bathed in UV radiation which excites them to a higher energy-state, and they fluoresce as they fall back to ground state: the fluorescence occurs at 253.7nm. A photomultiplier tube detects the released photons creating an electrical output that varies as a function of Hg concentration. The signal is read as a graph on a Kipp & Zonen Model BD11/12, 200mm flatbed recorder.

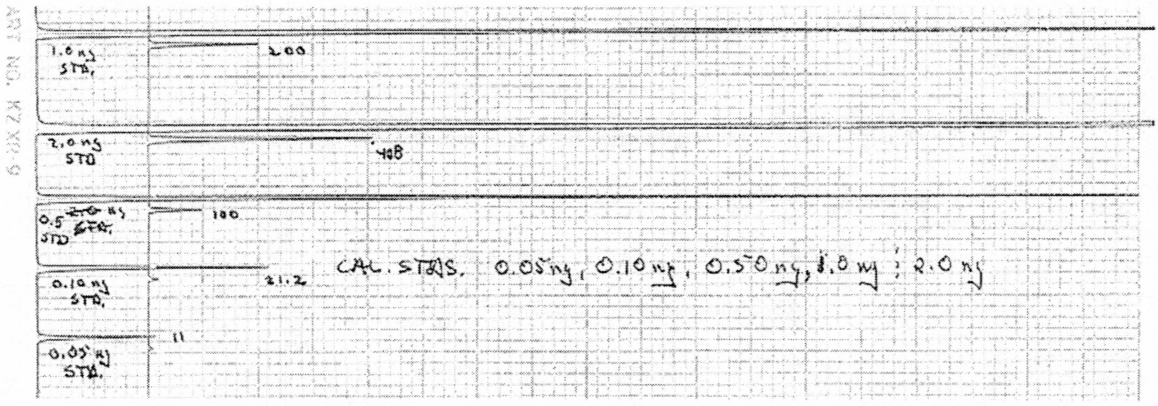

Figure 2.1 Scan of calibration curve showing graphical output of the Kipp & Zonen flatbed recorder

The varying voltage displays are seen as peaks: the higher the concentration of Hg in the gas stream, the more photons released upon UV excitation, the more photons detected, the higher the voltage, and a peak displays from baseline.

Concentration of THg in a sample is rendered from the peak via calibration curve and the aliquot volume. Detection limits were generally <1ng/g, in the range of 0.030ng/g to >400ng/g. THg detected by this method means all HNO_3/H_2SO_4 and BrCl generated species and forms. These include the following: covalently bound organomercurials such as $(CH_3)_2Hg$ and CH_3HgCl, HgS, Hg^0, Hg^{2+}, organocomplexed Hg^{2+} compounds, and adsorbed particulate Hg.

Apparatus and Reagents

Apparatus:

Ultra-high purity purging gases: UHP N_2 and Ar gas cylinders (Air Liquide), with VWR Scientific and Victor Equipment Co., pressure and flow regulators.

Flow manifold: Cole-Parmer acrylic plastic, four (4) output, individual flow-adjustable manifold, capable of flowing up to 400 ml/min. gas per output.

Sparging vessels: 125mL Florence flasks with 24/40 necks, fitted with glass stoppers incorporating inflow/outflow tubes, with coarse glass frits on the end of the inflow tubes, which extend to within 0.2 cm of the flask bottom.

Acid-fume scrubber: A Teflon tube, 10 cm x 0.9cm, filled with reagent grade, non-indication, 40 mesh soda-lime ($Ca(OH)_2$ + NaOH), packed between tufts of silica glass-wool. The assembly includes Teflon fittings on the ends for attachment to the sparging vessels.

Gold-coated sand traps/analytical columns: 10 cm x 6.5 mm O.D. x 4 mm I.D. quartz glass tubing. The reagent grade 40/60 mesh quartz gold-coated sand is contained

within the tubing by two (2), quartz glass wool tufts, one of which is 2.0 from one end, restrained by a gentle four-way crimp in the tube.

Analytical column heating/cooling apparatus: Two (2) Staco Energy Products Type 3PN1010 120V 50/60Hz transformers connected to a platinum wire coil heating element. Two (2) 750CFM Active Air shaded pole squirrel cage fans to cool the traps. One (1) GraLab Model 545 main timer controlling two (2) GraLab Model 171 slave timers executing the individual trap heating and cooling cycles of the analytical and sample columns.

Tekran Model 2500 Cold Vapor Atomic Fluorescence Spectrometry detector: The CVAFS contains five (5) major sub-assemblies:

1. Flow meter.
2. Photomultiplier tube.
3. Quartz flow-through fluorescence cell.
4. Low-pressure Hg vapor lamp producing the 253.7 nm UV radiation.
5. Lamp voltage controlling feedback circuit.

Teflon piping and fittings: All piping for gases is Teflon, and all component connections are 6.4mm O.D. Teflon using Teflon friction-fit and/or threaded connectors.

Reagents:

All are trace metals grade reagents, either JT Baker Instra-Analyzed, or ACS Certified.

Nitric/Sulfuric acid digestion solution: Add 300ml concentrated (98%), sulfuric acid to 700 ml concentrated (69-70%), nitric acid.

0.2M Bromine monochloride solution: 27g KBr are added to a 2.5 L jug of 12M (36-38%) hydrochloric acid. The solution is stirred in the hood with a stir bar for one (1) hour, after which, 38g $KBrO_3$ are slowly added to the solution.

Mercury Stock Standard: An NIST certified 10,000 mg/L mercury atomic absorption standard is used to make all lower concentration laboratory stock solutions.

Argon: Grade 5.0 Ultra-High Purity (Air Liquide), argon further scrubbed with a gas-stream gold-coated sand trap, which is checked to be free of Hg before placement in the gas stream.

Nitrogen: Grade 4.5 Ultra-High Purity (Air Liquide), nitrogen additionally purified as aforementioned.

Sample Digestion:

Bird tissue samples analyzed for THg were slightly thawed. Skin was dissected back, but not removed, to prevent any contamination from the polyethylene cutting board. A new stainless steel scalpel was used for every few birds, and washed in 12M hydrochloric acid after each sample tissue (muscle, brain or bone), was taken. In addition, stainless steel scissors, needles, and tweezers were used, and washed after every collection.

The tissues were placed directly into a 40 ml certified, pre-cleaned quartz glass sample vial, while sitting on a Mettler Toledo Model AE166 analytical balance for accurate weighing. The vials were stored at -60^0 C until analysis. For THg, approximately 1 gram of sample was digested by placing 7 ml of 70:30 HNO_3/H_2SO_4 in the vial, and heating on a hot plate to 90^0C for 2 hours or until all soft tissue was dissolved. After cooling, the digests were diluted to a final volume of 40 ml with 10% (v/v) 0.2N BrCl in Milli-Q water.

Reduction:

The sparging vessel is filled with approximately 100 ml Milli-Q water, and 300 µl $SnCl_2$ is added. A soda-lime acid-fume trap is placed in the outflow, and the vessel is purged with UHP N_2 for 20 minutes at 400 ml/min. A gold-coated sand trap is placed on the soda-lime trap, and the vessel purged another 20

minutes to acquire a bubbler blank. A calibration curve is constructed using standards analyzed by adding 0.05 – 2.0 ng aliquots to the vessel, adding 300 µl $SnCl_2$, swirling to mix, and purging as aforementioned. Tissue samples were analyzed by adding 300 µl $SnCl_2$, and 200 µl of digestate to the vessel, gently swirling to mix, and purging 20 minutes into a clean, gold-coated sand trap placed on the soda-lime trap. (Note: at the beginning of the day, all gold-coated sand traps are heated in the detector stream to desorb any Hg from atmospheric sources, and to establish their cleanliness by a lack of a peak on the Kipp & Zonen when run a second time.) A sparging vessel may thus be used for five (5) sequential samples before the water and spent digestate solution needs to be replaced with clean Milli-Q water, 300 µl $SnCl_2$ and additional digestate aliquots.

Detection of Mercury

The gold-coated sand trap that has been on the sparging vessel 20 minutes is removed and placed into the analyzer train apparatus. Here, there are two (2) traps, two (2) transformers (for the heater coils), and two (2) cooling fans controlled by a master controller and slave timers. The trap removed from the sparging vessel is placed inside a Nichrome wire coil, in the incoming UHP Ar gas stream, just ahead of the analytical column. Argon flows through the

columns at approximately 25-35 ml/min. The sequential operations executed by the master controller are thus:

First column

A slave timer is energized, turning on the transformer which supplies 10 VDC at 18A to heat the first gold coated sand trap for 2 minutes 30 seconds, the transformer is turned off, and the 750 CFM squirrel cage fan turns on rapidly cooling the trap for another 3 minutes. Any mercury is desorbed into the Ar stream and flows to the analytical column.

Second column

Once the heat has been turned off the first trap, the second slave timer is energized, and the analytical column undergoes heating for 1 minute 20 seconds. The timer returns to zero, turning off the transformer and turning on the second fan to rapidly cool the analytical column for 2 minutes 15 seconds. This is the last step in the detection process, as any thermally desorbed Hg (as Hg^0), that may have been contained in a sample is taken into the detector by the gas flow. If any Hg is present, an excursion from baseline will be seen within 40-45 seconds of onset of heating the analytical column. The excursion is seen as a sharp peak on the Kipp & Zonen graph paper.

Once the result is recorded, the sample column is replaced on the reloaded sparging vessel to begin another analysis, while the next sample column is

placed into the analytical train. Thus, an analysis can be executed approximately every 6 minutes.

The peaks generated by this technique are sharp and symmetrical, usually base width is no wider than 1mm unless there is a significant presence of Hg in the analyzed sample (at 2mm/min. chart speed). Any asymmetrical peaks, broad peaks or a small pre/post peak are indicative of problems. A pre-peak is water vapor and is seen almost immediately upon placing the sample column in the train. A small post peak is due to migration of gold off the analytical column from overheating and/or degradation by chemical fumes or age. When blanking the traps at the beginning of the day, a trap that shows a peak after the first heating needs to be retired, it has degraded from use/age.

2.2.2 Methylmercury ($MeHg^+$) in Tissues

An Overview of the Method

Samples are digested in 25% KOH/methanol solution, and diluted to 40 ml with methanol. An aliquot of digestate is added to Milli-Q water in a sparging vessel, buffered to pH 5.0, and reacted with sodium tetraethyl borate in an aqueous phase ethylation. This produces a volatile methylethylmercury derivative of $MeHg^+$. Ethyl analogs are separated by isothermal GC and detected

by using a cold vapor atomic fluorescence spectrometer (CVAFS) detector. The typical minimum detection limit is <1 ppb as Hg. $MeHg^+$ as defined by this method means all methylmercury forms and species in the digestate including, but not limited to, CH_3HgS-R, CH_3HgCl, CH_3HgOH, and CH_3Hg^+.

Apparatus and Reagents:

In addition to the apparatus described in section 2.2.1, the following were used:

Carbotrap column: This is a 6.5 mm O.D. x 4 mm I.D. silanized quartz glass tube 10 cm long, with two tufts of silanized glass wool restraining 3.4 cm (30/45 mesh) Carbotrap (activated carbon), one of the tufts being held by a gentle crimp 2.0 cm from the end of the tube.

Isothermal GC Unit: A 1.3 meter long, ¼ inch O.D. x 4 mm I.D. borosilicate glass column tubing. The tube is formed into an 8 cm diameter coil of 1.0 m length with two (2) 15 cm arms extending parallel up from the coil. The column is silanized, and packed in the coil section only, preconditioned 60/80 mesh 15% OV-3 on Chromosorb WAW-DMSC, held in place by silanized glass wool plugs (A custom Supelco, Div. of Sigma-Aldrich, product). The column is held in a small temperature-controlled isothermal oven made from a heating mantle (Glas-Col TM-616), interfaced with a Cole-Parmer Digi-Sense temperature controller.

The column is held at a constant temperature of 110 ± 2^0 C using the temperature controller.

Pyrolytic organomercury breakdown column: A quartz glass column 20 cm long x 7 mm O.D. x 4.5 mm I.D. with the central 10 cm packed with quartz glass wool. This column is heated continuously during analysis to 700^0 C by a wrapped 1.5 m length of 22 ga. Nichrome wire, heated by a Staco Energy Products Type 3PN1010 120V 50/60Hz transformer.

Reagents:

All reagents are either JT Baker Intra-Analyzed trace metals grade or ACS certified.

Acetate buffer: 2 moles sodium acetate (272g), and 2 moles glacial acetic acid (118ml), are dissolved in Milli-Q water to give a final volume of 1.0 L.

25% Potassium Hydroxide/Methanol solution: 250g KOH are slowly dissolved, with cooling, in methanol to make a final solution volume of 1.0 L.

Sodium tetraethyl-borate solution: This reagent is acquired from Strem Chemicals (Newburyport, MA) in 1.0 gram argon-packed, paraffin-sealed bottles. 100 ml of

12% KOH in Milli-Q water is prepared in a Teflon bottle and chilled to approximately 0^0 C. The seal on the $NaBEt_4$ bottle is carefully removed, the cap rapidly removed and the bottle filled with about 5 ml of the cold KOH solution. The cap quickly replaced and the bottle shaken to dissolve the $NaBEt_4$. This solution is poured back into the 100 ml bottle of remaining KOH and shaken to mix thoroughly.

Methylmercury Stock Solution: Methylmercury solutions are prepared by serial dilution of an initial concentrated solution of methylmercuric chloride in Milli-Q water containing 0.5% (v/v), glacial acetic acid and 0.2% (v/v), HCl.

Sample Digestion

Bird tissue samples analyzed for THg were slightly thawed. Skin was dissected back, but not removed, to prevent any contamination from the polyethylene cutting board. A new stainless steel scalpel was used for every few birds, and washed in 12M hydrochloric acid after each sample tissue (muscle, brain or bone), was taken. In addition, stainless steel scissors, needles, and tweezers were used, and washed after every collection.

The tissues were placed directly into a 40 ml certified, pre-cleaned quartz glass sample vial, while sitting on a Mettler Toledo Model AE166 analytical balance for accurate weighing. The vials were stored at -60^0 C until analysis. For

MeHg analysis, 10.0 ml 25% KOH/methanol is added to approximately 1.0 gram of tissue sample in each vial. The sample is capped, shaken and placed on a hot plate and slowly heated to 90^0 C for 2-4 hours or until all soft tissue is visibly dissolved. The samples are then diluted to 40 ml with methanol.

Trapping Procedure

Approximately 100 ml Milli-Q water and 500 µl acetate buffer were placed in a sparging vessel. An aliquot of digestate is added, followed by 35 µl $NaBEt_4$ solution, activating the aqueous phase ethylation. The solution is left to react in the sparging vessel for 17 minutes. A Carbotrap is placed on the outflow of the glass stopper and the solution purged with UHP N_2 for 17 minutes at 400 ml/min. After the time has expired, the trap is removed and connected directly to the UHP N_2 coming from the flow manifold. N_2 is allowed to flow through the trap for seven (7) minutes to dry any water-vapor present (this technique does not use a soda-lime pre-trap).

Mercury Detection

The dried Carbotrap is slipped into a Nichrome wire coil and connected to the argon carrier gas flowing to the input side of the isothermal GC column. The output side of the isothermal GC column is connected to the pyrolytic

breakdown column, connected to the CVAFS by Teflon tubing to form the MeHg detector train. After allowing argon to flow through the fully connected train for one (1) minute to stabilize the system, the Nichrome coil is energized for 30 seconds to approximately 400^0 C, the mercury species transferring to the isothermal GC column. The column separates the species according to molecular weights, and the pyrolytic breakdown column, heated by another Nichrome wire coil to a constant 700^0 C, further separates the species so they elute as follows:

1. A peak at about one (1) minute corresponding to Hg^0, usually a decomposition product of diethyl mercury, as Hg^0 is not retained by Carbotrap. A small Hg^0 peak is always present simply due to Hg being released upon heating the Carbotrap.
2. A peak at about two (2) minutes corresponds to methyl ethyl mercury. This is the peak of interest, the ethylation product of methyl mercury.
3. A peak at about three (3) minutes corresponds to diethyl mercury, which results from the ethylation of Hg (II) in the reagents and sample. This is not quantitative for Hg (II) though as most Hg (II) is excluded by the distillation procedure.

2.3 Quality Control/Quality Assurance

Certified dogfish tissue (DORM-2), from the National Research Council of Canada was used to asses the accuracy of THg and MeHg determinations. This

tissue contains 4640 ± 260ng/g THg. A THg solution is made by digesting approximately 1.0 gram of dogfish tissue in 25 ml 70:30 (v/v), HNO_3:H_2SO_4 and diluting to 1000.0 ml with 0.0001 M BrCl solution (Bloom and Crecelius, 1983).

2.4 Statistical Procedures

No ANOVA statistical procedures were attempted due to the study design in setting up this survey.

Problems are:

1. I was not able to place birds with location.
2. I should have identified female and male differentiation.
3. I should have made an estimate of age.
4. I should have identified spring or fall plumage.

Chapter 3

Results

3.1 THg Distribution in Tissues

Mercury was detected in all species, but not in all tissues analyzed (Table 3.1).

Table 3.1 Distribution of mercury levels in different species in Alaska birds

Name	Species	Tissue	*n*	Mean (ng/g)
American Wigeon	*Anas americana*	M	5	15
		BR	5	8.7
		BN	5	5.7
Bar-Tailed Godwit	*Limosa lapponica*	M	5	48
		BR	5	54.4
		BN	0	0
Black Scoter	*Melanita nigra*	M	12	134.7
		BR	12	105.2
		BN	12	422.9
Brent Goose	*Brant bernicla*	M	7	2.3
		BR	7	1.4
		BN	7	0.7
Bufflehead	*Bucephala albeola*	M	2	78.1
		BR	2	49.4
		BN	2	41
Common Goldeneye	*Bucephala clangula*	M	1	39.3
		BR	0	0
		BN	1	29.9
Canada Goose	*Branta c. taverneri*	M	3	2.3
		BR	3	0.8
		BN	0	0
Cackling Goose	*B. c. minima*	M	12	2.5
		BR	12	2
		BN		2

Table 3.1, continued

Greater Scaup	*Aythya marila*	M	17	69.5
		BR	17	47.2
		BN	17	53.9
Greater White-Fronted Goose	*Anser albifrons*	M	6	2.3
		BR	6	1.4
		BN	6	2.4
Lesser Scaup	*Aythya affinis*	M	1	268.6
		BR	1	197.7
		BN	0	0
Mallard	*Anas p. platyryhncos*	M	3	89.3
		BR	3	78
		BN	3	14.9
Northern Pintail	*Anas acuta acuta*	M	43	38.5
		BR	43	35.5
		BN	43	14.5
Northern Shoveler	*Anas clypeata*	M	6	64.9
		BR	6	66.4
		BN	6	38.9
Oldsquaw	*Clangula hyemalis*	M	5	151.2
		BR	5	105.1
		BN	5	108.6
Ptarmigan	*Lagopus mutus*	M	5	1.4
		BR	0	ND
		BN	0	ND
Sandhill Crane	*Grus canadensis*	M	1	0.8
		BR	1	0.4
		BN	0	ND
Teal	*Anas crecea*	M	10	28.3
		BR	13	27.4
		BN	13	112.1
Tundra Swan	*Cygnus columbianus*	M	1	2.7
		BR	1	1.9
		BN	0	ND
Velvet Scooter	*Melanita fusca*	M	6	87.9
		BR	6	83.8
		BN	6	44.1

The overall mean THg value in muscle was 55 ng/g wet weight (ww), and in brain tissue was 44 ng/g (ww).

THg in bone varied widely, from non-detectable (ND), to a very high 422.9 ng/g for the black scoter, while muscle and brain were 134.7 and 105.2 ng/g respectively. Teal (*Anas crecea*), also returned higher bone (112.1 ng/g), values than muscle and brain. The mean THg for bone in the oldsquaw and greater scaup were also higher than brain tissues.

The most sampled species, the northern pintail (*Anas a. acuta*, $n = 43$), shows brain THg being 92% of muscle THg and bone THg levels 38% of muscle (Table 3.1 and Fig. 3.1).

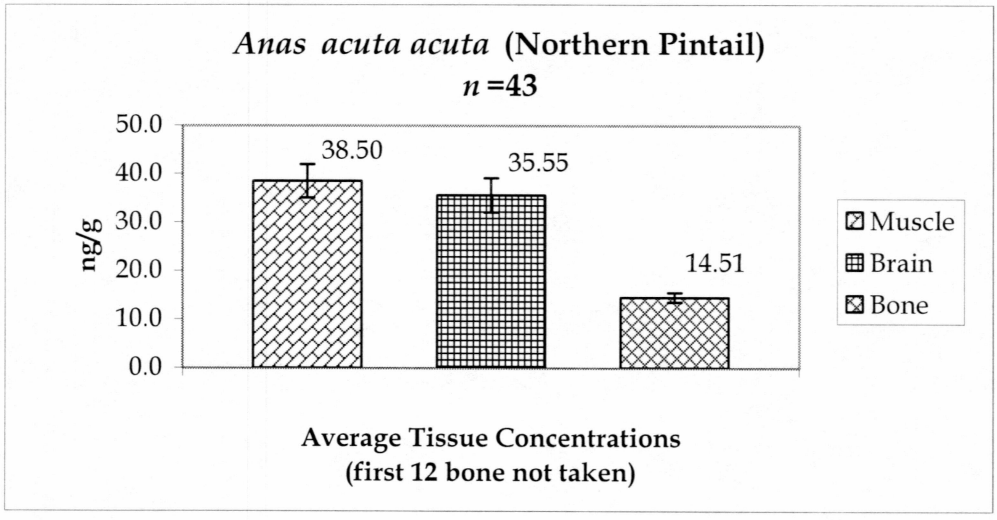

Figure 3.1 *Anas a. acuta* tissue distributions

A better indicator of THg in bone in these birds is the median value rather than the THg mean. Black scoter and teal high levels of THg in their bones (422 ng/g and 112 ng/g respectively), and this is driving the mean bone value out of proportion to the muscle and brain. Figure 3.2 summarizes the mean and median values for all bird tissues (Fig. 3.2).

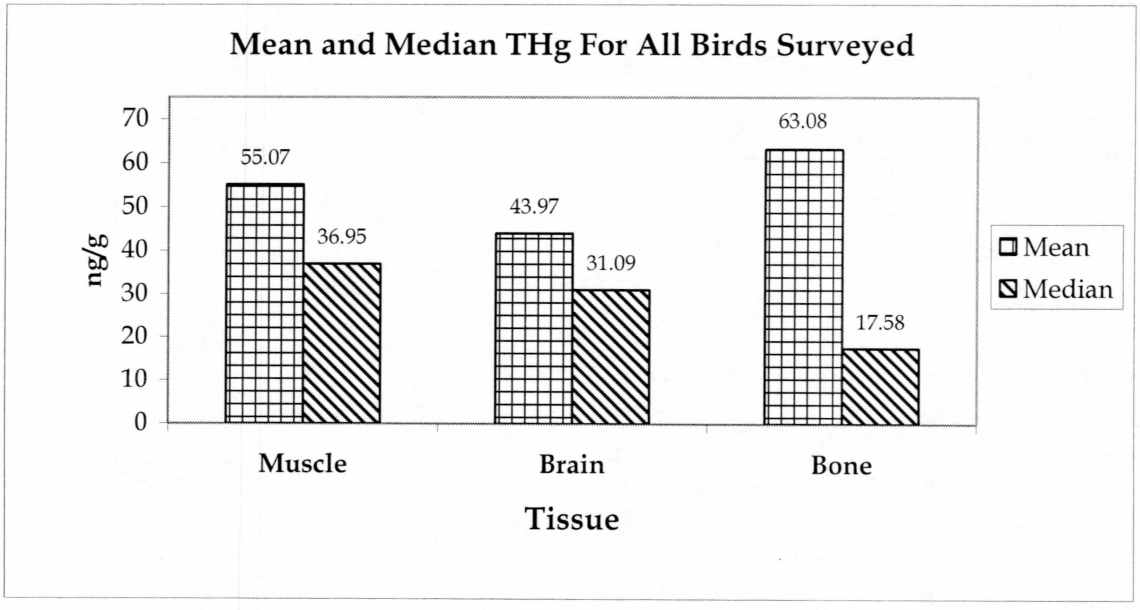

Figure 3.2 Median THg mercury values for all birds sampled

3.2 Mercury Distribution by Trophic Level

Interspecies differences in mercury levels in muscle for the lesser scaup and oldsquaw showed them having high levels while Canada goose, brant and sandhill crane were very low. Mean values varied by an order of magnitude between the swans, geese and cranes, and the oldsquaw, scoter/scaup birds. Highest THg levels were found in birds at the highest trophic levels.

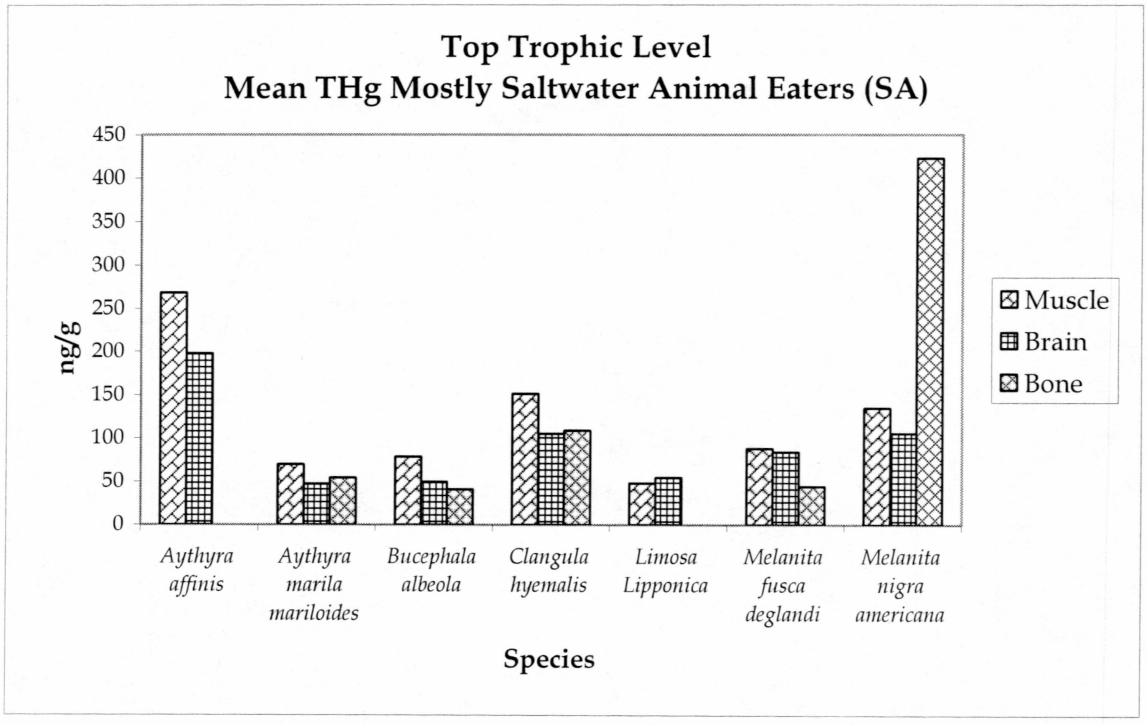

Figure 3.3 Top trophic level mean THg by species

Birds feeding on saltwater animals, insect and other animal life (SA, Table 1.1 and 1.2), had the highest THg concentrations in their tissues (Fig. 3.3). Mean THg values had high levels of variation due to the bone concentrations in Black Scoter (Fig. 3.4 next page).

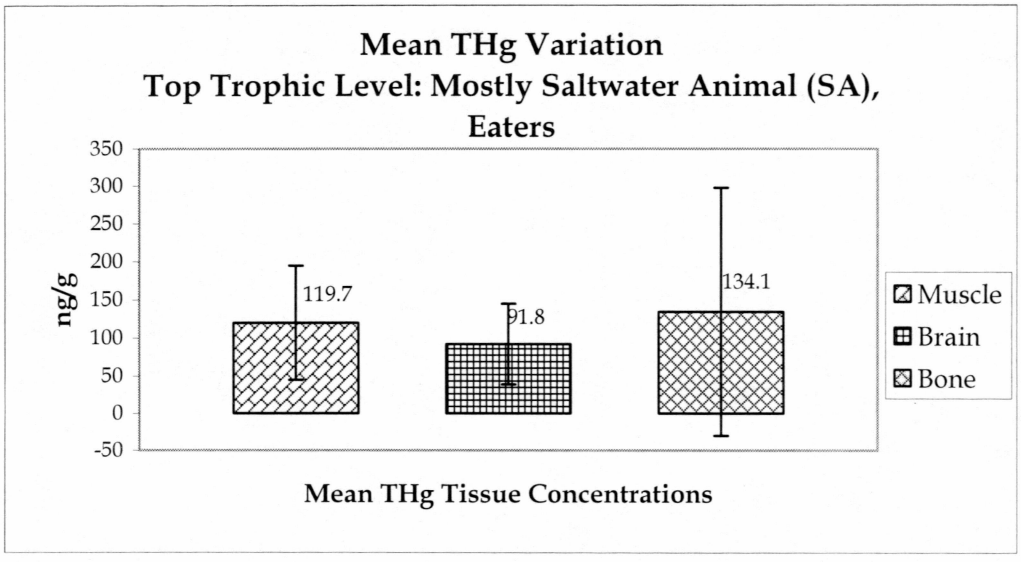

Figure 3.4 Variation in mean THg levels

Those birds feeding on grass, cereal grains and seed had the lowest THg concentrations in their tissues (Fig. 3.5).

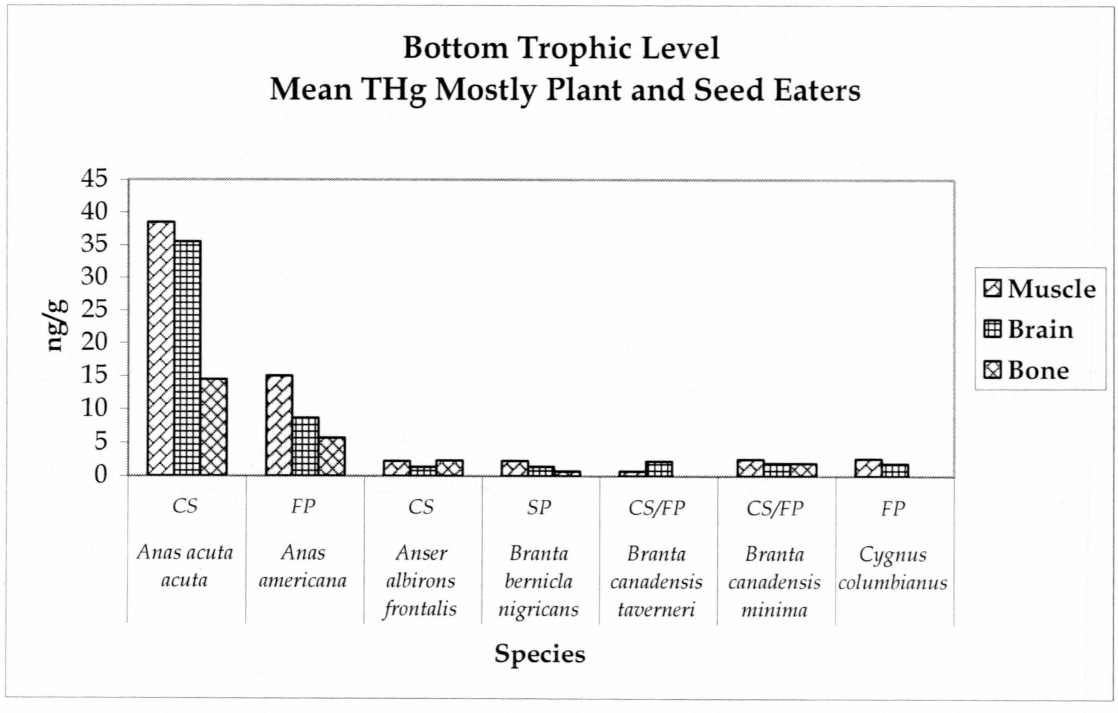

Figure 3.5 Bottom trophic level THg by species (acronym key pgs. 18 and 19)

Variation in the bottom trophic level is much less than in the animal eaters (Fig. 3.6).

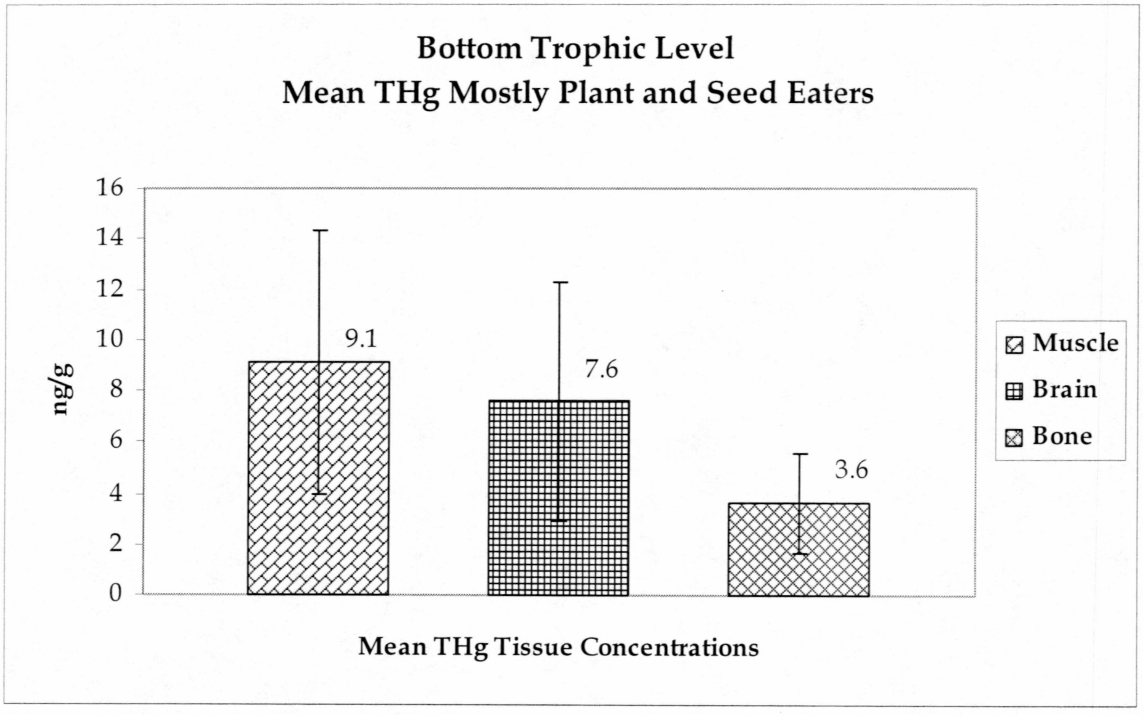

Figure 3.6 Bottom trophic level mean THg

However, if the birds add some animal life to their diets (mallard is higher in THg than Canada goose), the THg mean tissue concentrations increase (Table 3.1).

3.3 Mercury Distribution by Wintering Area

The bird flyway, or migration, maps in Bellroses' book (1976), are comprehensive in their detail of all flyways taken by a specific species, based on population in that flyway. Because of this, the maps can be quite cluttered. I

decided clarity would be best, so I looked at THg in species by the wintering area. Flyways roughly correspond to the wintering area geographically, but while I suspect most people (even internationally), will instantly recognize "California", as a wintering area, they would probably have to look up a map of the Pacific Flyway if I used that term.

There is no correlation between wintering area and THg concentrations in bird tissues. The following figure shows several species that winter in California and along the Baja Peninsula and some down into Southern Mexico (Fig. 3.7).

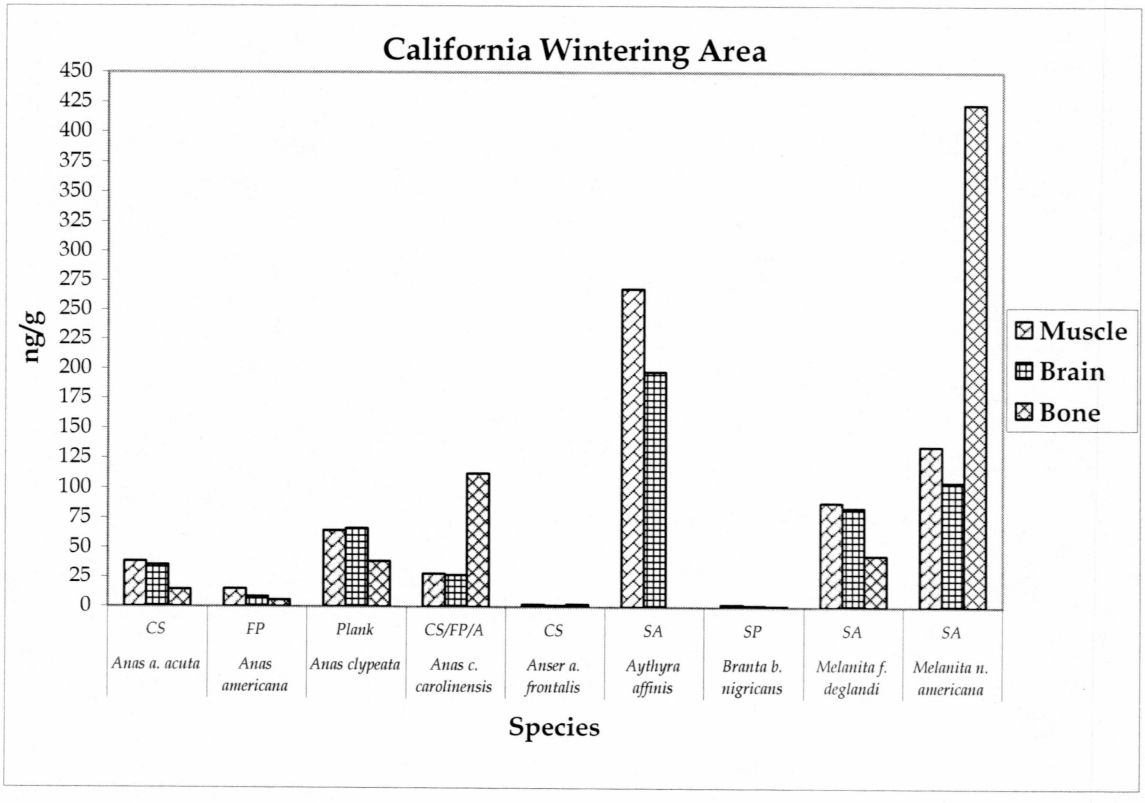

Figure 3.7 California wintering area species THg levels (acronym Key pgs. 18 and 19)

Cereal grain eaters are low trophic level birds, but when even a small portion of their diet is supplemented by animals, their THg levels increase. This increase can be seen in all of the other wintering area species comparisons for the species in this survey (Figs. 3.8, 3.9, 3.10, 3.11)

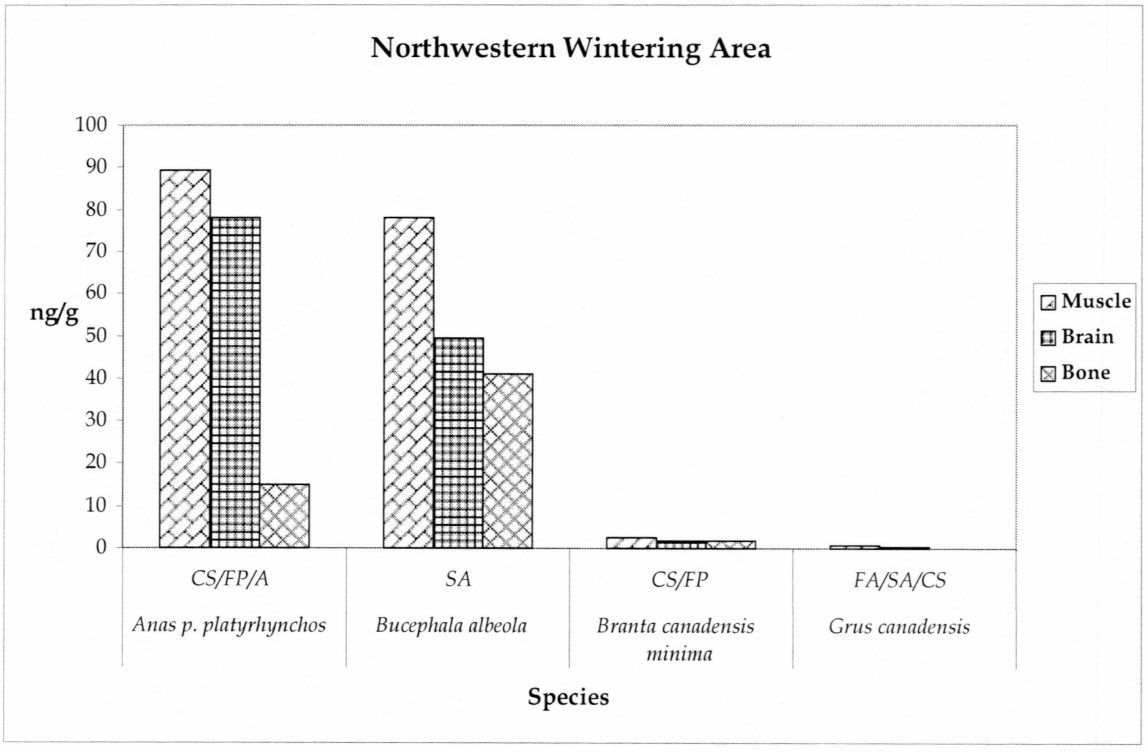

Figure 3.8 Northwestern wintering area species THg levels

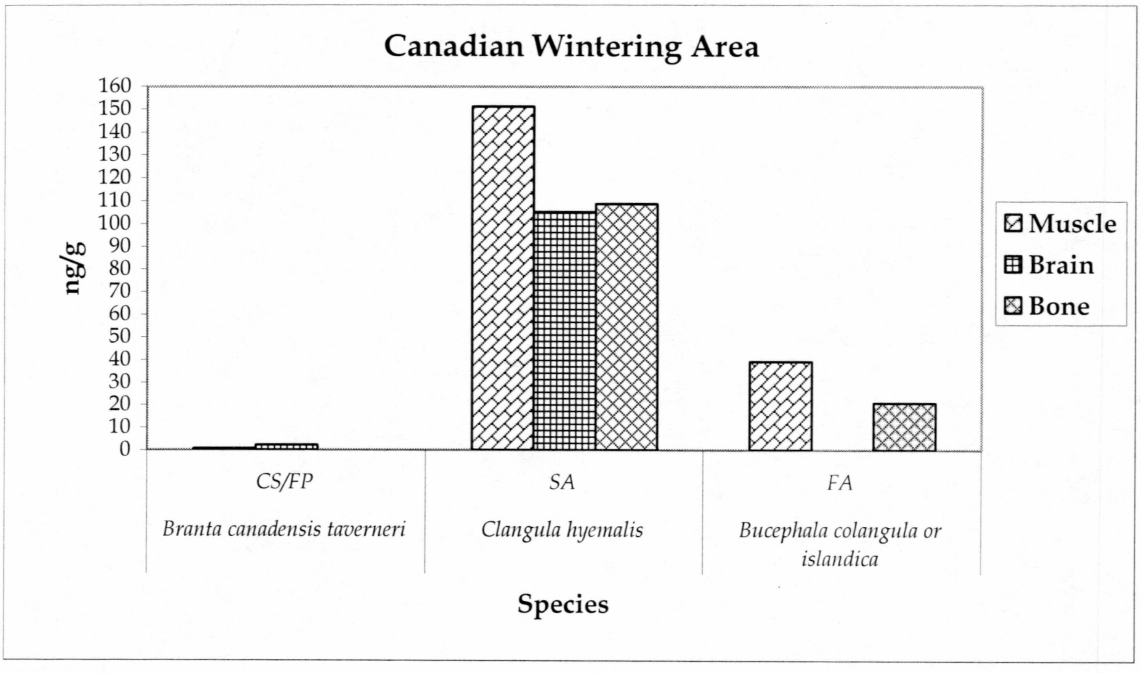

Figure 3.9 Canadian wintering area species THg levels

Figures 3.10 and 3.11 show dramatic differences in THg levels. *Aythyra* feeds primarily on saltwater animals and supplements its' diet with freshwater plants. *Cygnus* eats primarily freshwater plants. *Limosa* eats saltwater animals on mudflats in Alaska, and Australia and New Zealand.

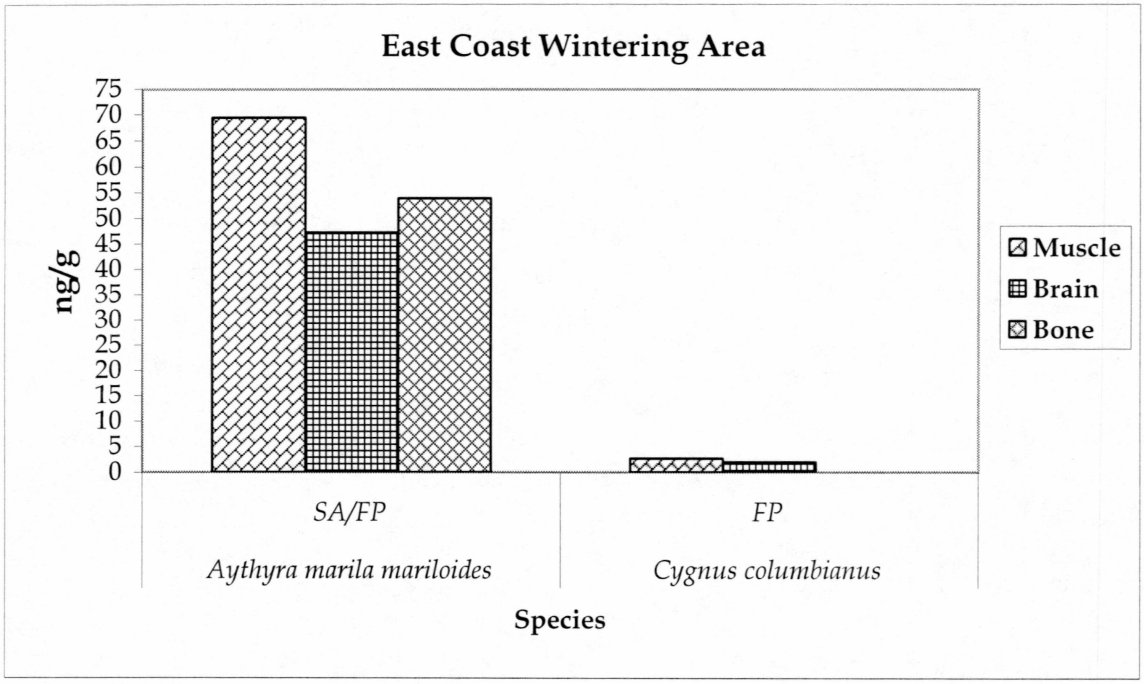

Figure 3.10 East coast wintering areas species THg levels

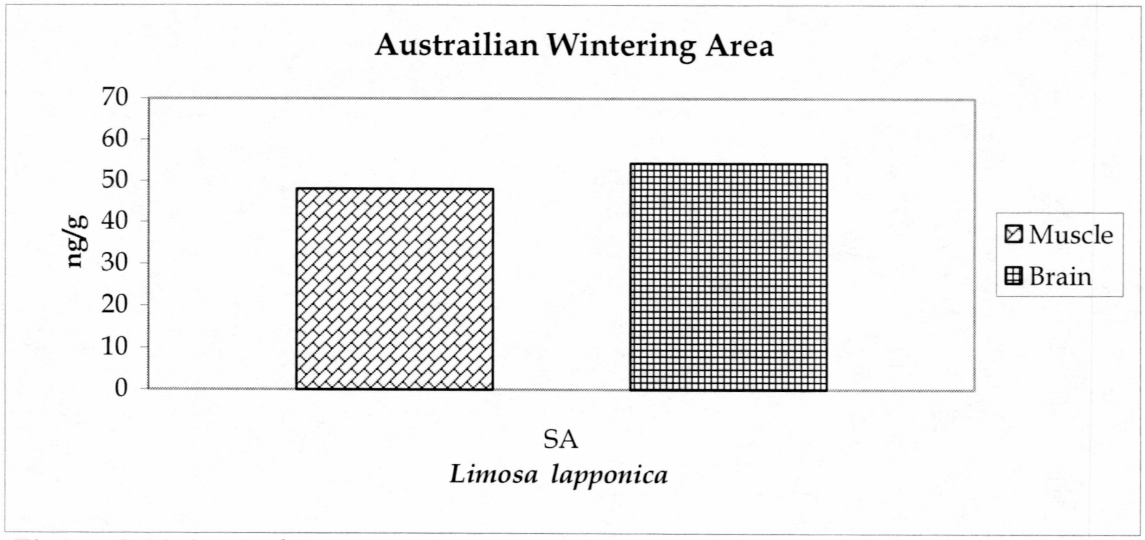

Figure 3.11 Australian wintering area species THg levels

3.4 MeHg Analysis Difficulties

I was unable to accomplish the analysis for $MeHg^+$ that would have provided a comparison ratio of $MeHg^+$ to THg for the tissues. This section is included to show the problems I encountered. It was pointed out to me by a committee member I was forcing the trendline through the origin as per Bloom (Bloom 1992). Due to systematic error the equation for the slope of the line was off. If I did not force the trendline through the origin, I could have used the calibrations and used statistical methods to account for the systematic error. This was a classic case of not seeing the forest for the trees.

Beginning May 2004, I ran a standard to compare all the sparging vessels (bubblers), and Carbo-traps to determine variations between them, the results are shown below (Table 3.2).

Table 3.2 MeHg bubblers with existing Carbo-traps.

	Bubbler 1		Bubbler 2		Bubbler 3		Bubbler 4	
	#	Peak Hgt.	#	Peak Hgt.	#	Peak Hgt.	#	Peak Hgt.
Carbo-trap	12	103	11	67.7	9	95.1	6	130
Carbo-trap	10	130	7	112	5	129	2	120
Carbo-trap	3	118	2	98.7	11	123	12	111
Carbo-trap	2	90.8	10	119	12	145	3	89.1
Carbo-trap	7	91	5	93.8	3	72.3	9	84.5
Carbo-trap	6	120	3	37.4	2	98	7	102
Carbo-trap	11	87.3	12	96.1	7	75.5	10	102
Carbo-trap	5	105	9	83.4	6	103	11	74
Carbo-trap	9	87.5	6	112	10	102	5	101.5
	Ave.	103.62		91.12		104.77		101.57
	Median	103.00		96.10		102.00		102.00
	SD	15.90		25.57		23.98		17.48

There were seven (7) Carbo-traps in the lab that were used to work up the table. All gave widely dispersed values for the 0.40 ng $MeHg^+$ standard that was used. The standard deviations and disparate peak heights were too unreliable to be used for a serious analysis. The following figure shows the data points graphed (Fig. 3.12).

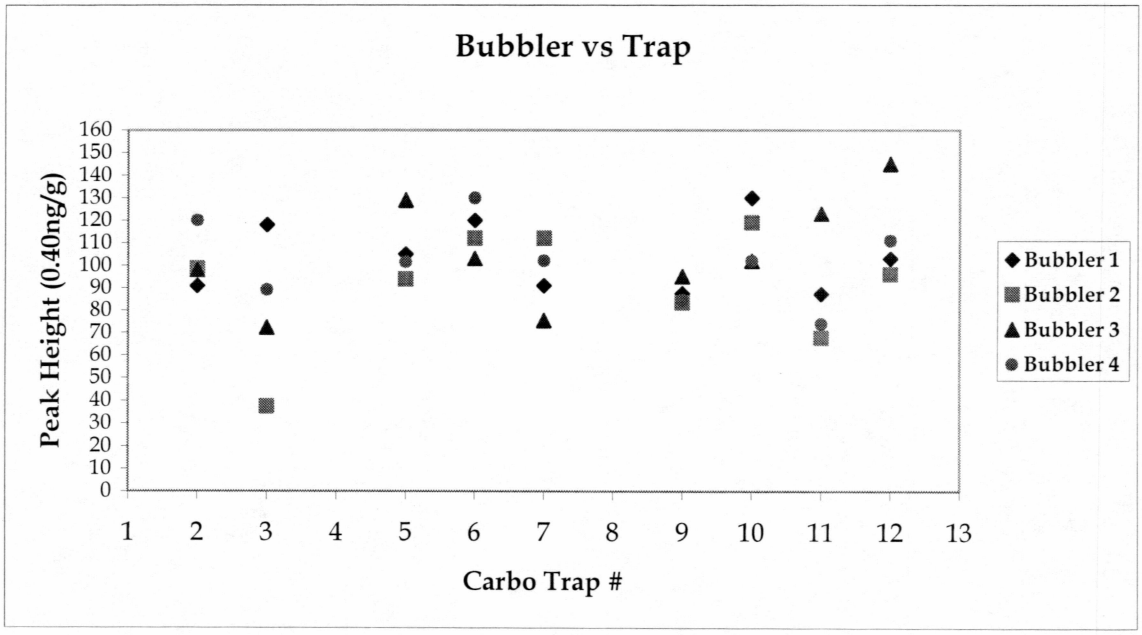

Figure 3.12 Original Carbo-traps and bubblers compared

The decision was made to purchase new Carbo-traps and wash the bubblers and associated connections with *aqua-regia* to assure their cleanliness.

The bubblers and associated glassware (spargers and Teflon connections), were submerged overnight in, and washed thoroughly with, *aqua-regia*. The new Carbo-traps were blanked (placed in the detector train and heated with no

sample being run through them), then placed onto the bubblers and a 0.40 ng $MeHg^+$ standard was run. The following table shows the results (Table 3.3).

Table 3.3 Washed bubbler and new Carbo-traps

	Bubbler #1	Peak Hgt.	Bubbler #2	Peak Hgt.	Bubbler #3	Peak Hgt.	Bubbler #4	Peak Hgt.
Carbo-trap	1	67	2	67	3	77.2	4	77.2
Carbo-trap	4	118	2	93.1	3	97	1	95
Carbo-trap	2	85.4	1	103	4	106	3	100.5
Carbo-trap	3	110	4	106	1	93.1	2	104
Carbo-trap	1	96	3	103	2	101.5	4	96.9
Ave		102.35		101.275		99.4		99.1
Median		103		103		99.25		98.7
SD		14.50		5.63		5.58		3.98

As can be seen in the table, the peak heights are still not consistent across the bubblers. While some Carbo-traps are consistent, such as #3, the results for all of them are disappointing. The following figure graphically shows how the data points are dispersed over a wide range (Fig. 3.13).

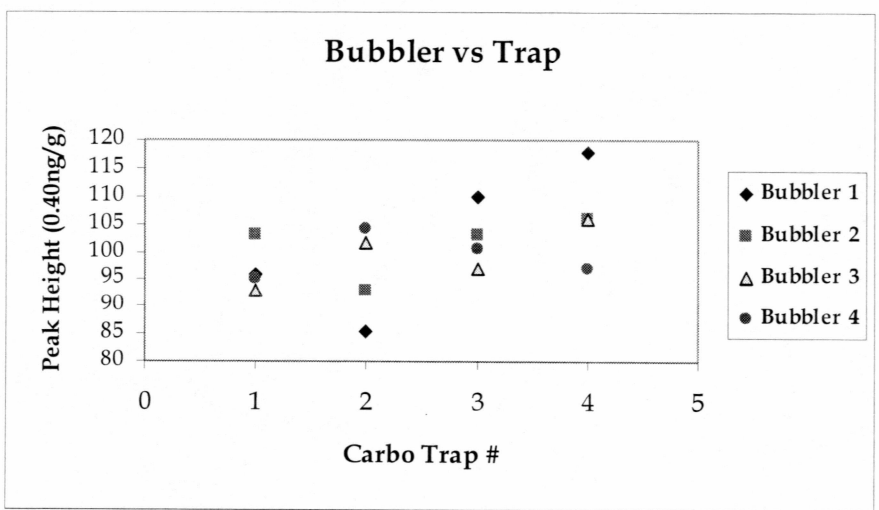

Figure 3.13 Bubblers versus traps: data point dispersion

The standard deviations were much better, so I decided to work on some samples and analyze the DORM (Canadian Dogfish) standards. New DORM had been purchased with the new Carbo-traps, so this would afford the opportunity to check the known, old DORM standard, against the new DORM. Also, a European tuna-fish standard had been purchased (BCR-464), as a triple-check to the DORM. I made two (2) stock solutions (one used twice the first mass), for the new DORM and one (1) stock solution from the BCR-464. I ran a calibration curve and proceeded to run the standards.

As table 3.4 shows, the results are consistent, in that, they show the systematic error as the standards concentrations go down, percent recovered rises, and as standards concentration increases, percent recovery falls (Table 3.4). These figures were calculated with the trendline being forced through the origin.

Table 3.4 Tissue standards and percent recovery

Sample ID	Digested Mass [g]	Analyzed Volume [ml]	DORM(analyzed) /DORM(real)*100	Percent MeHg Recovered
Dorm-2 A	0.0423	0.010	265.06	265.06
Dorm-2 A	0.0423	0.025	172.75	172.75
Dorm-2 A	0.0423	0.025	130.57	130.57
Dorm-2 A	0.0423	0.025	151.26	151.26
Dorm-2 A	0.0423	0.025	179.51	179.51
Dorm-2 A	0.0423	0.025	169.96	169.96
Dorm-2 B	0.0824	0.010	137.23	137.23
Dorm-2 B	0.0824	0.025	125.98	125.98
Dorm-2 B	0.0824	0.025	78.34	78.34
Dorm-2 B	0.0824	0.025	113.71	113.71
Dorm-2 B	0.0824	0.025	119.23	119.23
Dorm-2 B	0.0824	0.025	124.35	124.35
Dorm 2 LCS (old)	1.0000	0.025	125.95	125.95
BCR-464	1.1016	0.010	91.72	91.72
BCR-464	1.1016	0.010	76.74	76.74
BCR-464	1.1016	0.010	98.73	98.73
BCR-464	1.1016	0.010	94.91	94.91
BCR-464	1.1016	0.010	97.45	97.45
BCR-464	1.1016	0.010	98.73	98.73

The above table was constructed from the following calibration curve shown in Figure 3.14 on the next page. The standards used to construct the curve are listed in Table 3.5, which immediately follows Figure 3.14, for reference.

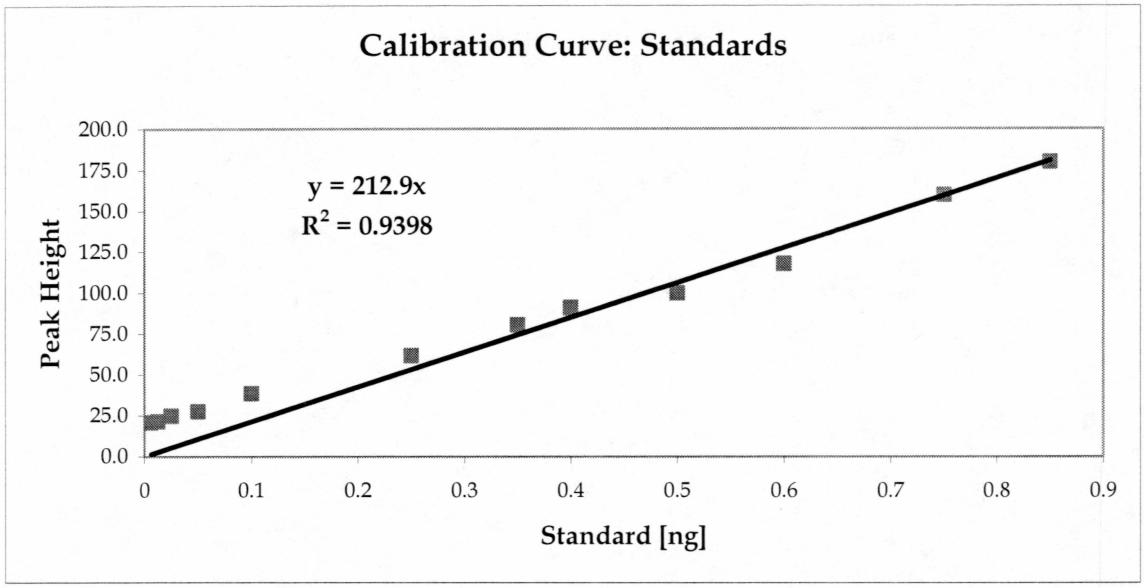

Figure 3.14 Calibration curve used for standards

Standards used to construct the above figure. The falling off at higher concentrations is easily seen (Fig. 3.14, Table 3.5).

Table 3.5 Standards table for calibration curve in Figure 3.14

Standard (ng)	Peak Height
0.00625	23
0.0125	23.6
0.025	27
0.05	29.7
0.1	40.6
0.25	63.8
0.35	82.7
0.4	93.2
0.5	102
0.6	120
0.75	162
0.85	182

The following day I ran more standards and calibration curves to verify previous data. The following tables and graphs again show the inconsistency of low concentrations with high peak heights, and high concentrations with low peak heights (Tables 3.6, 3.7, and Fig.'s 3.15 and 3.16).

Table 3.6 Standards used for calibration curve in Figure 3.15

Standard (ng)	Peak Height
0.05	62.5
0.1	74.2
0.25	108.0
0.50	160.0

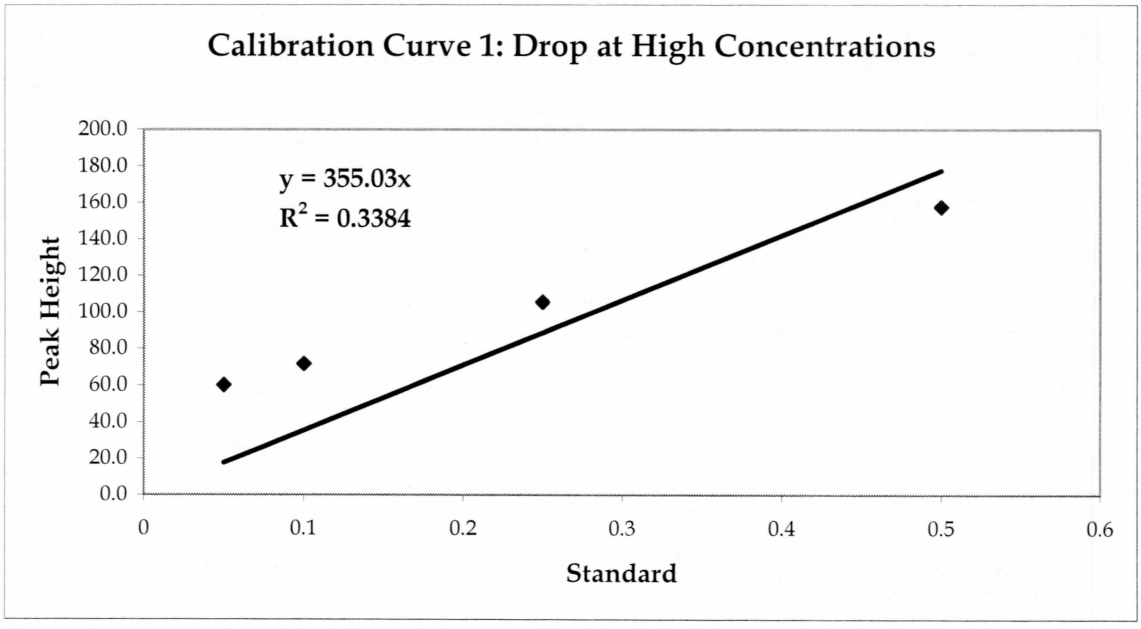

Figure 3.15 Calibration curve 1 showing drop at high concentrations

Table 3.7 Standards used for calibration curve in Figure 3.16

Standard (ng)	Peak Height
.05	32.6
0.10	43.0
0.25	77.8
0.50	130.0

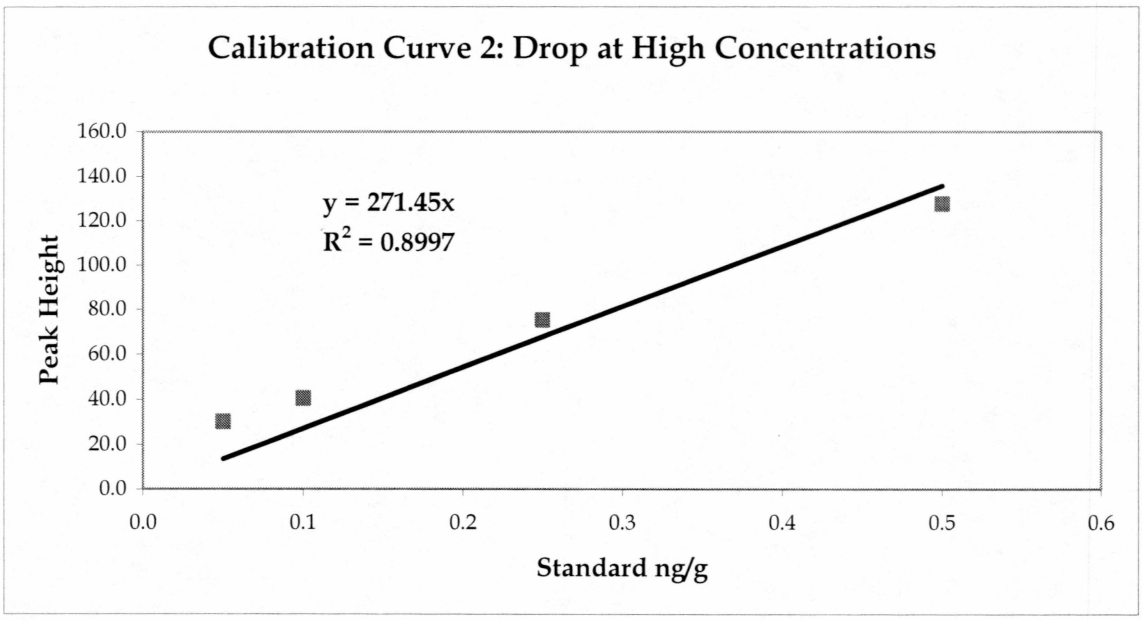

Figure 3.16 Calibration curve 2 showing drop at high concentrations

After three weeks of inability to recognize what I was doing incorrectly, I decided to terminate my efforts. I could not proceed with any analysis until I determined what was happening. A few months later, Dr. Lara Dehn used the lab and she stated she was able to get near perfect curves and near 100% recovery rates on the tissue standards stock solutions I made.

Notes: My thesis committee members pointed out I forced the trendline through the origin in the calibration curves. I did this per Bloom; to show no systematic error was occurring. I changed the parameters in Excel to allow the trendline to follow the data points, and the following figures illustrate while there was some systematic error, it was not my pipetting technique as I originally thought (fig. 3.17, 3.18, and 3.19). The R^2 values are now near perfect. This is a classic mistake of not seeing the forest for the trees. I could have used any of these curves to complete the analysis of the samples to build the THg:$MeHg^+$ ratio base. Sadly, the lab had been cleaned two weeks previously and my MeHg tissue samples had been destroyed.

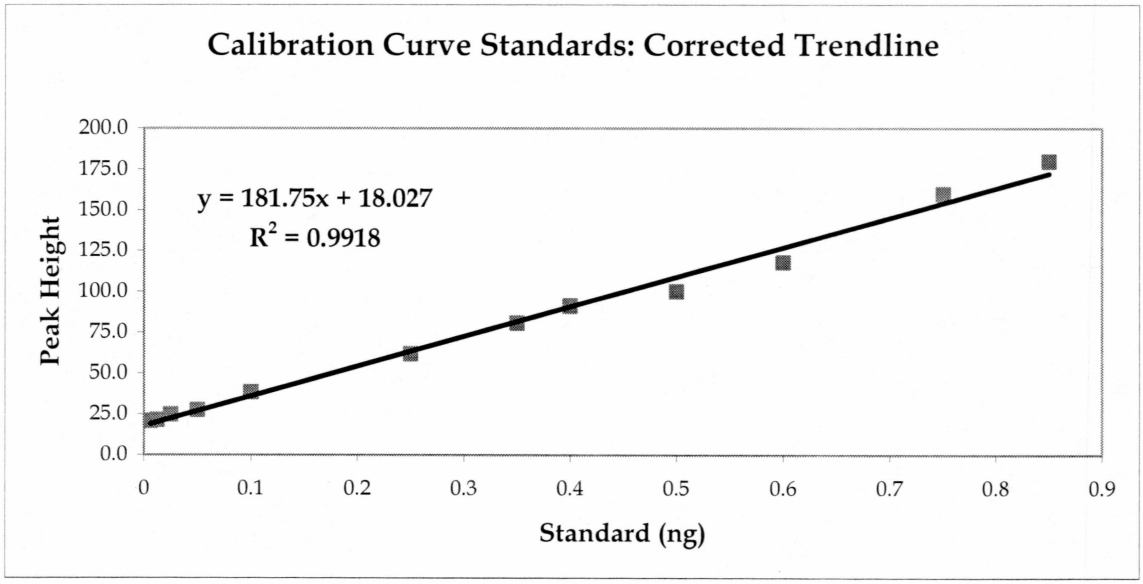

Figure 3.17 Calibration curve: standards (Fig.3.14) corrected trendline.

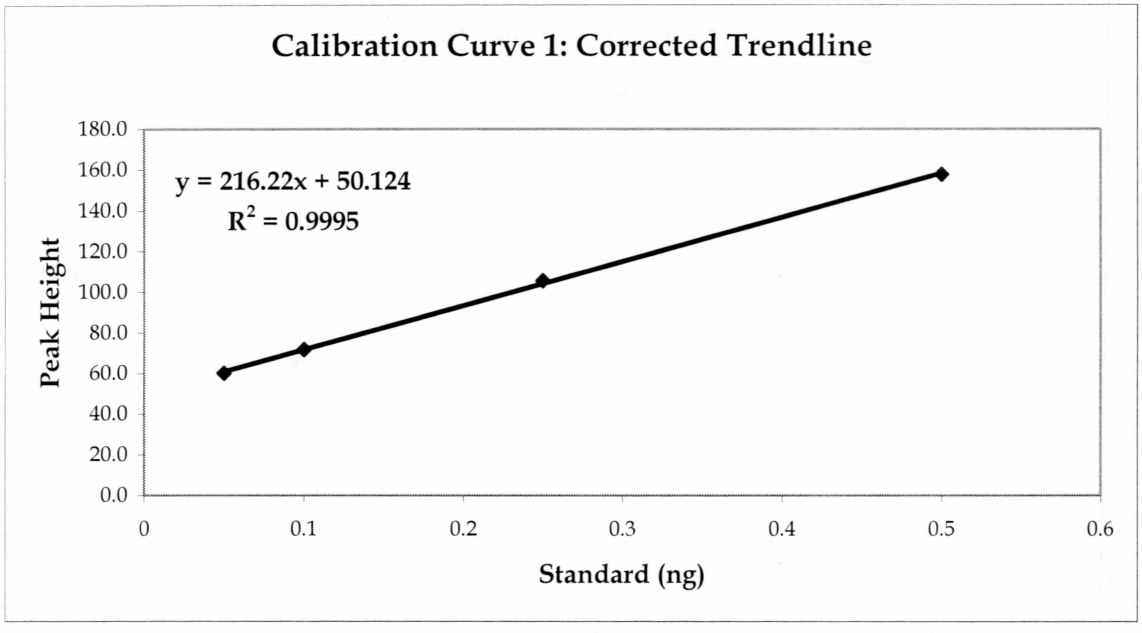

Figure 3.18 Calibration curve (Fig. 3.15) corrected trendline.

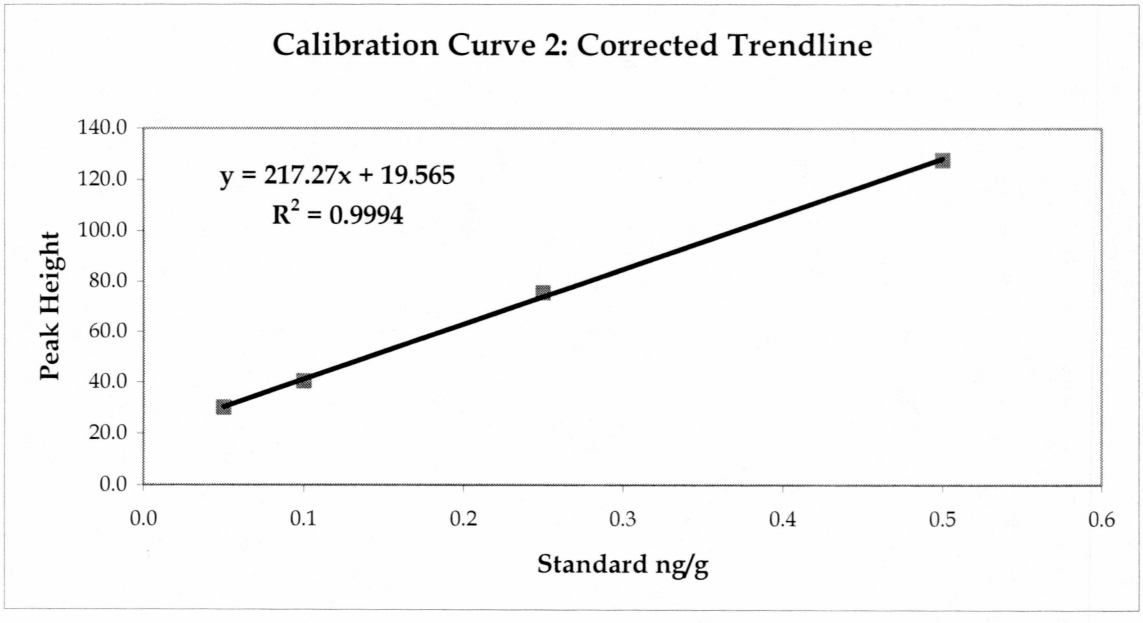

Figure 3.19 Calibration curve (Fig. 3.16) corrected trendline.

Chapter 4

Discussion

For many years there has been a desire by both Alaskans and policy-makers to know the methylmercury ($MeHg^+$), and total mercury (THg), burdens in Alaskan subsistence food sources, as they could relate to any negative health effects from both mercury forms in the Alaskan Native diet (Egeland et al., 1998; Rothschild and Duffy, 2001; Weis, 2000). The subsistence lifestyle provides not only nutrition and healthy living inexpensively, but also the intangible, but real, benefits of social and cultural values (Hild, 1998). $MeHg^+$ accumulates in the edible portions of plants and prey, and is biomagnified up the food chain (Van Oostdam et al., 1999). There is a clear relationship between trophic level of the surveyed bird species and their mean THg. As birds make up roughly 3 to 6% of the subsistence diet in Western Alaska (Arnold and Middaugh, 2004), the THg in birds should be monitored frequently, as well as the other subsistence food sources. Burger (2004), and Rocque and Winker (2004), wrote that birds are good indicators of environmental exposure to mercury, this study supports that concept. Initially this study had ambitiously hoped to show a relationship between a species Hg level and its' flyway and/or wintering area. No relationship was observed even though 18 species were sampled. The variation

in the Hg data and the low sample number may be responsible for this. However, there might not be a relationship. Rocque and Winkler (2004), failed to detect a relationship between contaminant levels and the migration of albatross species.

Variations in THg concentrations in muscle range from less than 1 to greater than 268 ng/g, strongly reflecting the trophic levels inhabited by the birds, but could be affected by local factors as in the previous paragraph. Trophic levels are dominant and have been reported for different taxa (Burger and Gochfeld, 2000). There is a clear relationship between the mercury tissue concentrations in prey predator interactions. Geological location and size of prey are factors that are additional influences on trophic level relationships (Burger and Gochfeld, 2000). The trophic levels in this study strongly suggest overlapping diets of plants and animals can influence tissue concentration of mercury. Wintering and summer feeding areas should be considered as influences, but the data is too coarse to say with any degree of certainty.

The level of mercury in these birds was expected to be low, considering the vast distance of the Yukon-Kuskokwim Delta from heavily populated, urbanized, and industrialized areas in North America. Burger (1993; 1994; et al, 2004), summarized mercury levels in feather and eggs, and the median for many

species was a very high 21,000 ng/g (Burger and Gochfeld, 2000). Decreased egg size, low hatch-rate, and decreased chick survival can be seen with concentrations approaching 5,000 ng/g. This study did not gather feathers to provide a ratio of Hg in muscle, brain, and bone to feathers, the low concentrations should not affect the overall health of the birds for the species studied.

Burger and Gochfeld (1997), and Thompson and Furness (1989), and Thompson *et al* (1991), showed all mercury in feathers and muscle is $MeHg^+$. If this is correct, $MeHg^+$ analysis should be eliminated as the total analysis, by default, includes $MeHg^+$ and is by far analytically, more straightforward and cost-efficient.

Chapter 5

Conclusion and Future Directions

Results of this study show there are differences in mercury tissue concentrations for subsistence-use birds in southwestern Alaska. Levels were higher in high trophic level, animal eating birds as opposed to low-level, plant eaters. There is a strong need to provide reference means and THg ranges in Alaskan birds. With the continued blossoming of the Near-East Asian continent as a major industrial power-house, the continued, periodic sampling is even more critical as a real-world tool to connect trans-national airborne pollutants to existing theoretical global environmental transport models. More detailed research is necessary on accumulation factors and pathways for this region.

Because of the error with the $MeHg^{+}$ calibration curves, more bird heads should be gathered, of the same species previously analyzed for THg, and the $MeHg^{+}$ analysis performed. Doing another THg assay parallel to the $MeHg^{+}$ will reinforce the THg data that already exists.

Chapter 6

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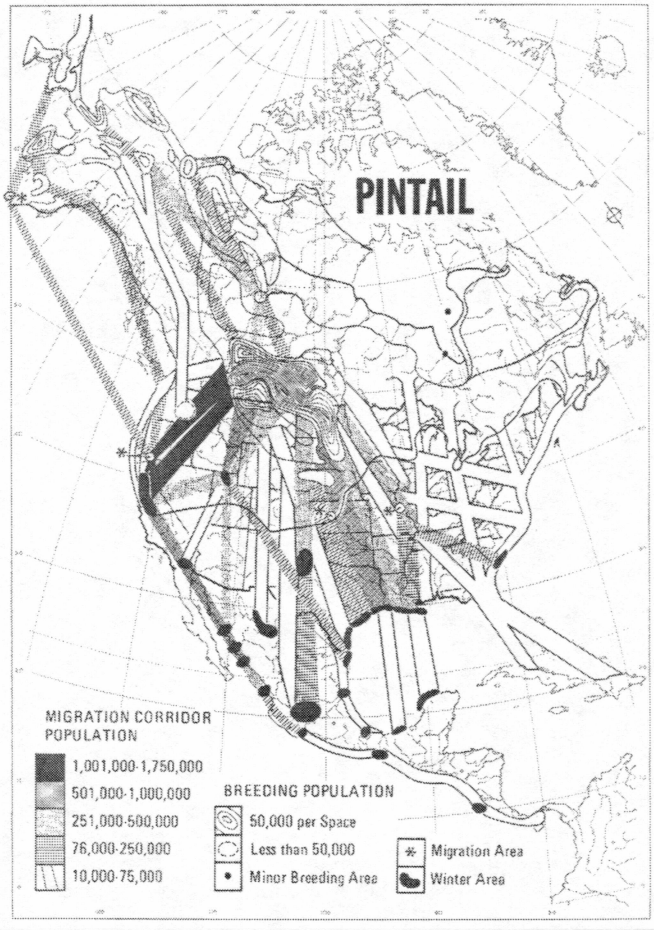
Appendix A
Bird Flyway Maps and Species THg



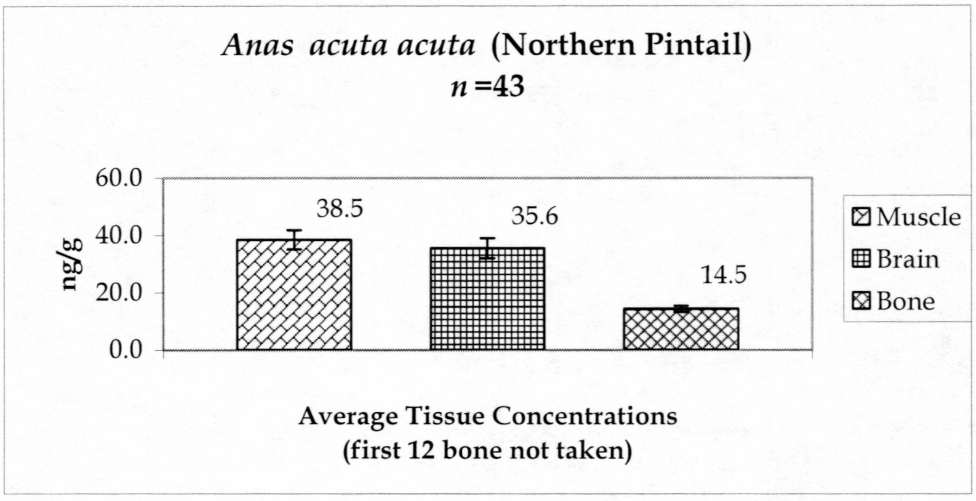
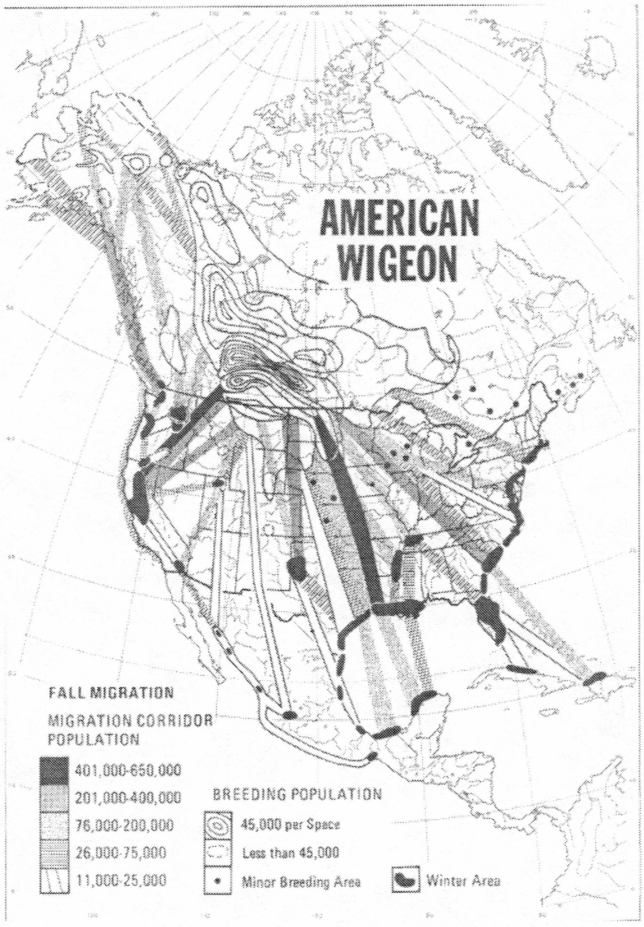


Figure A-1 Northern Pintail average THg concentrations



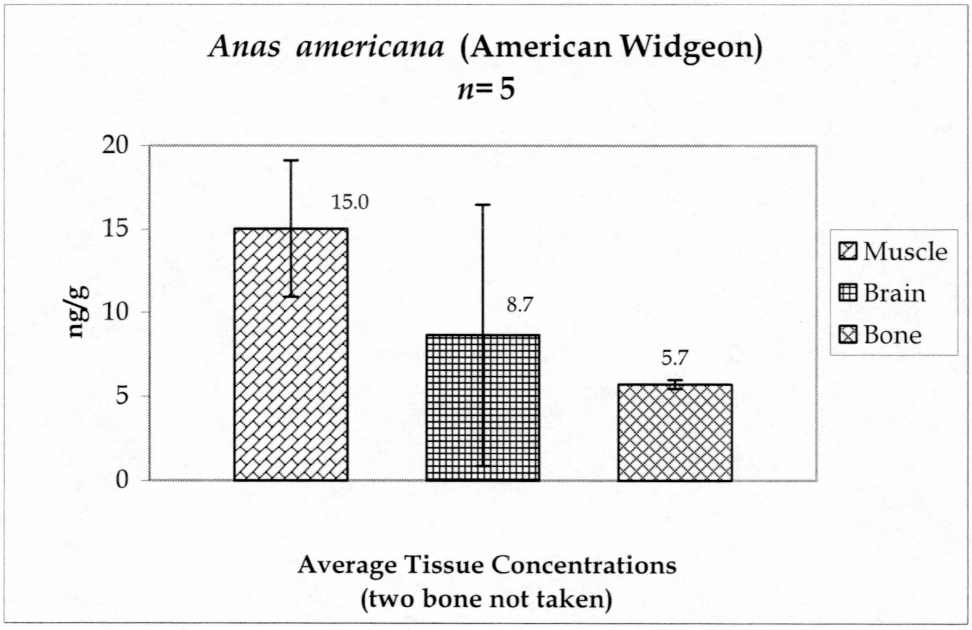


Figure A-2 American Widgeon average THg concentrations

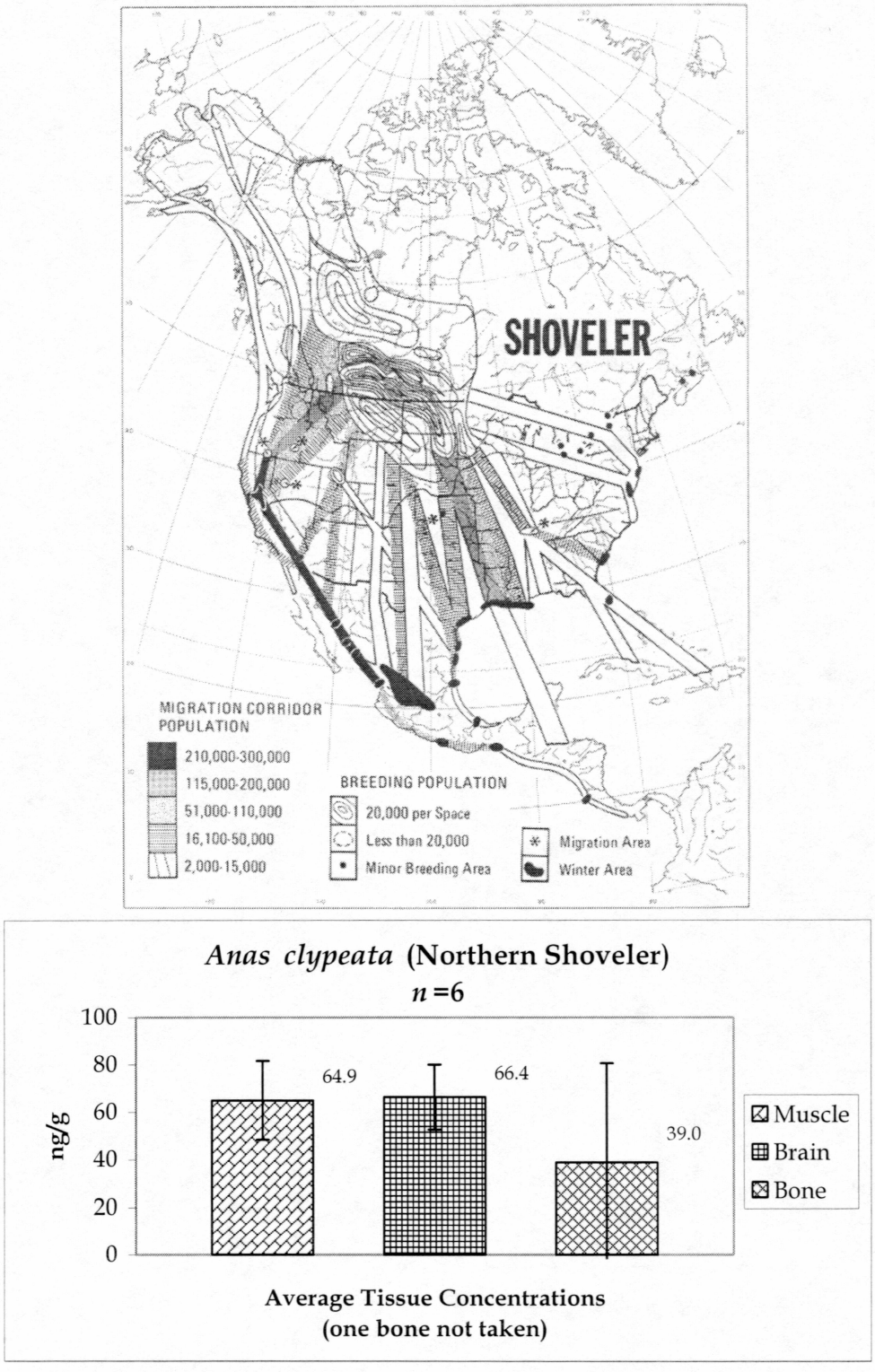
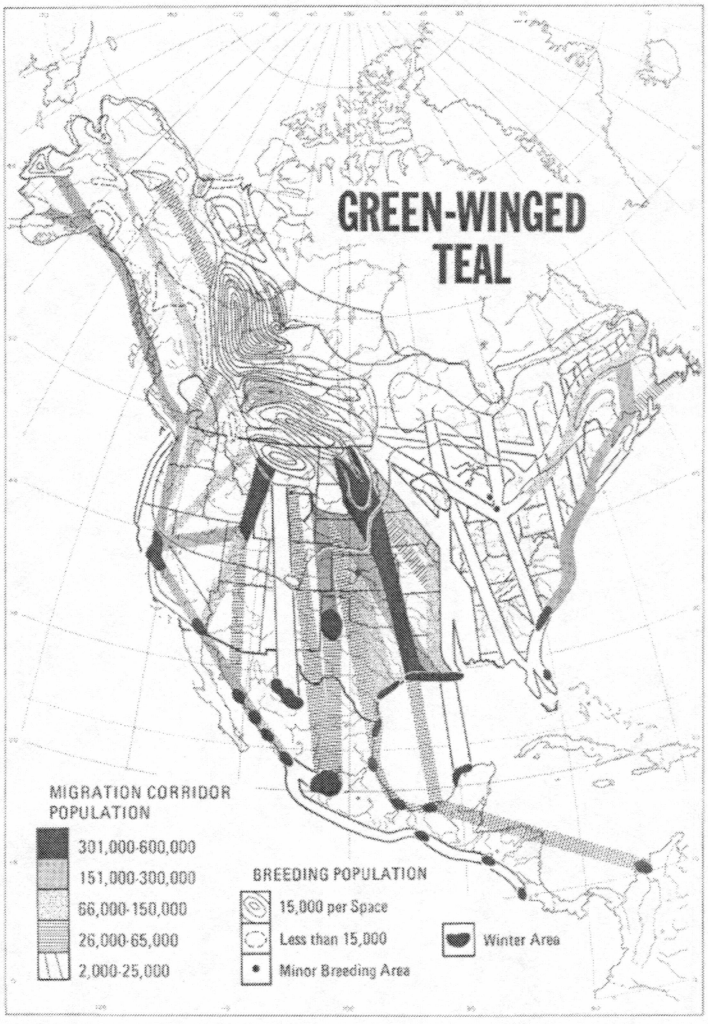
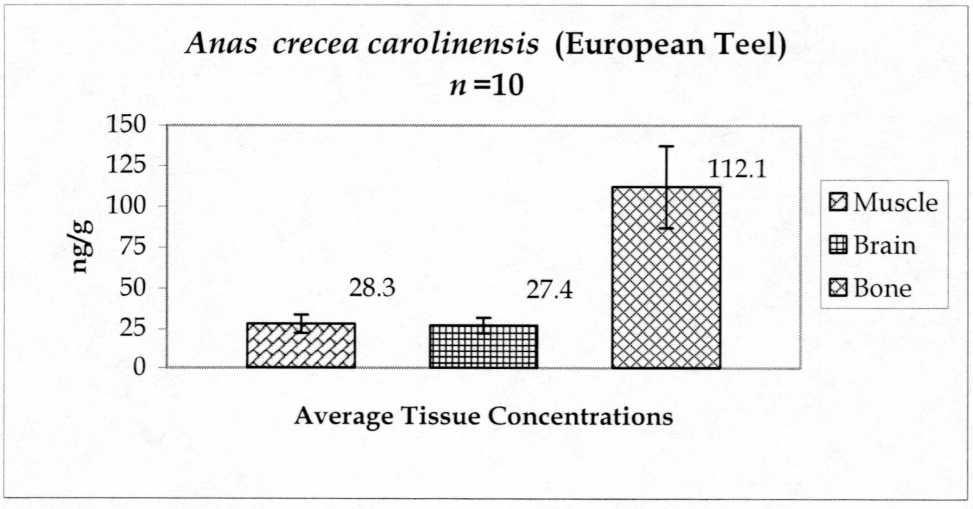


Figure A-3 Northern Shoveler average THg concentrations





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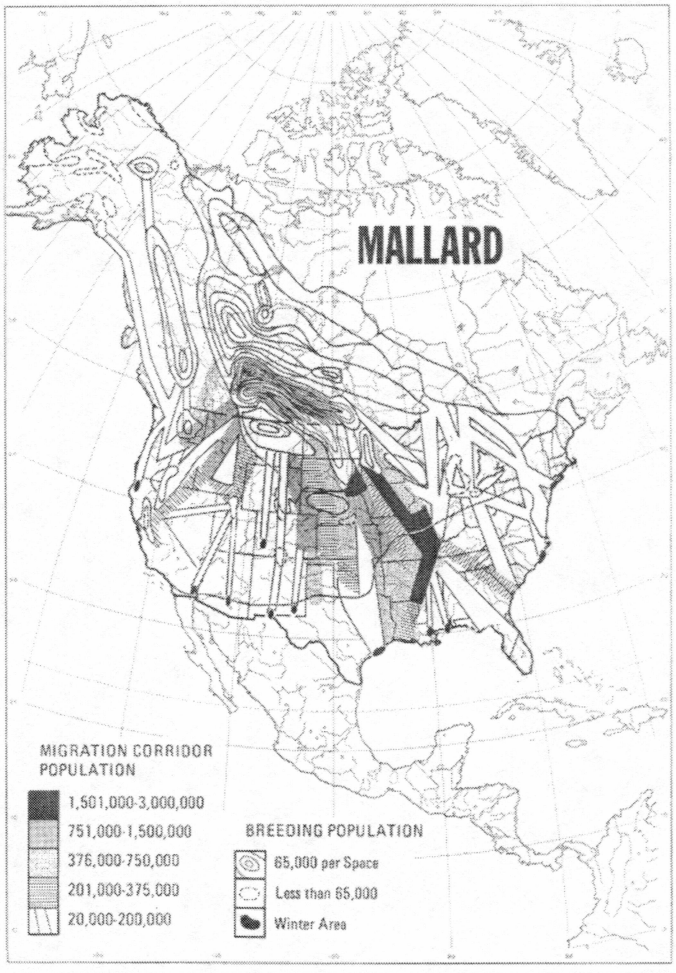

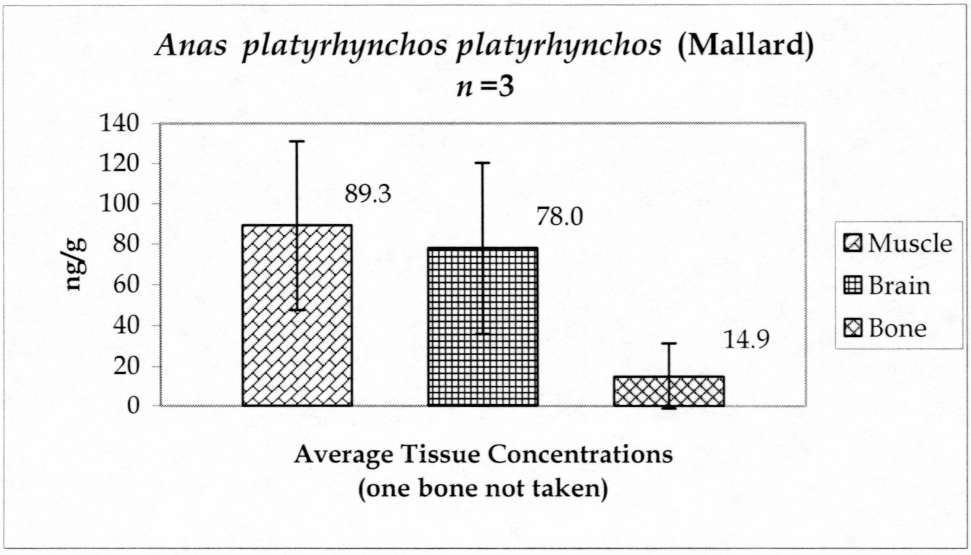

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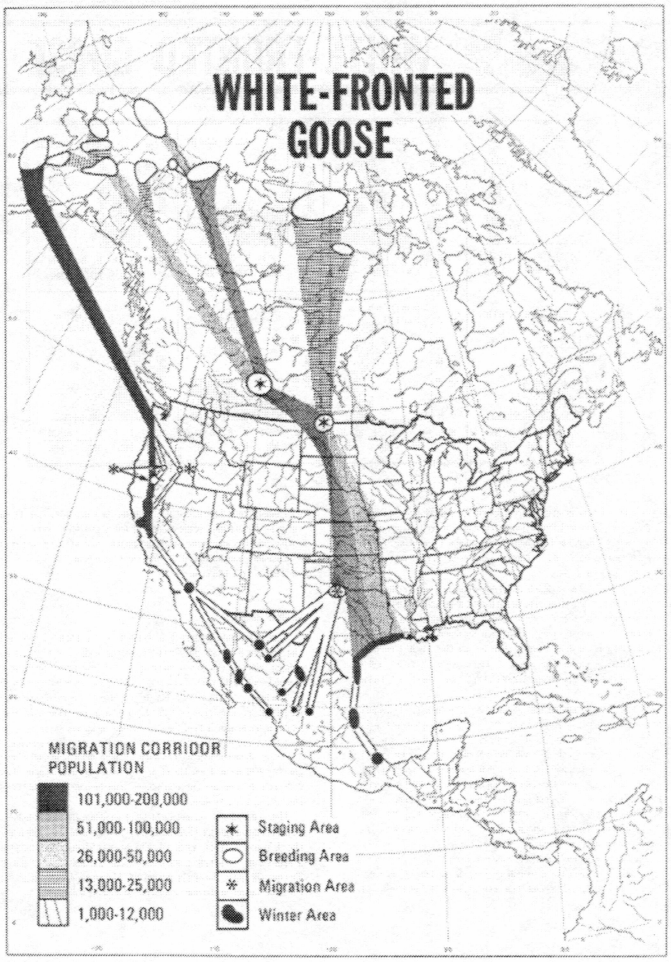


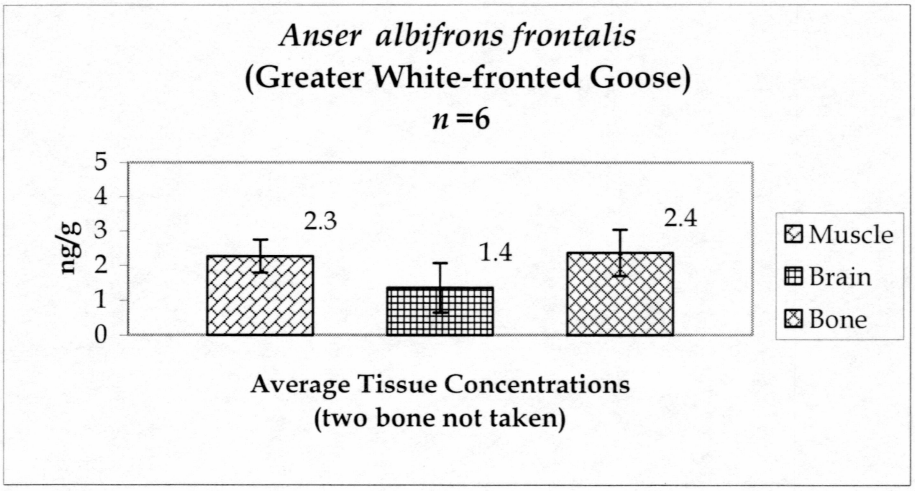
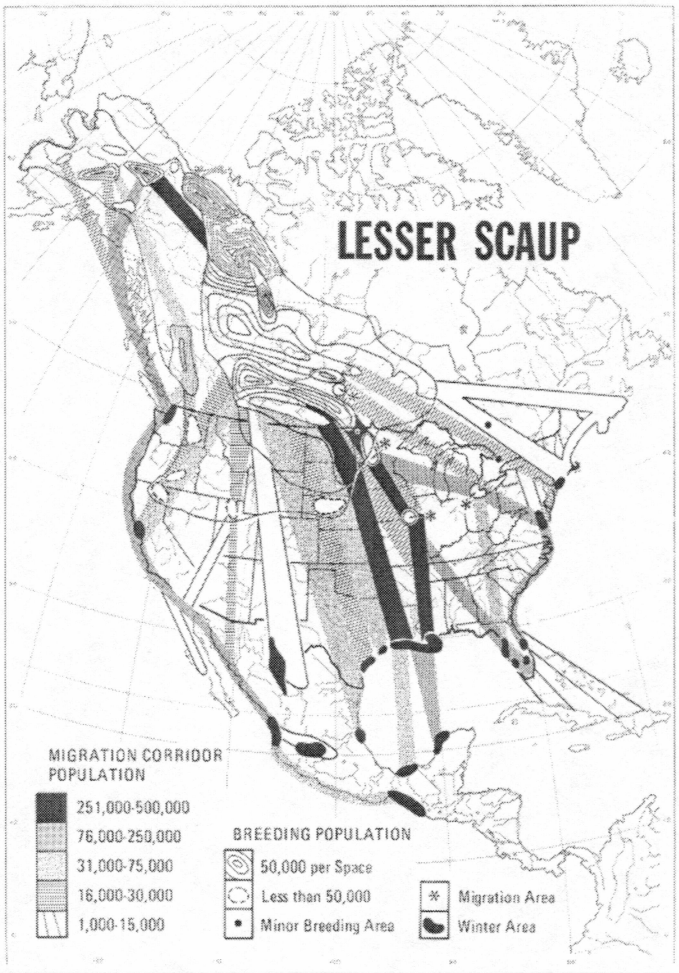
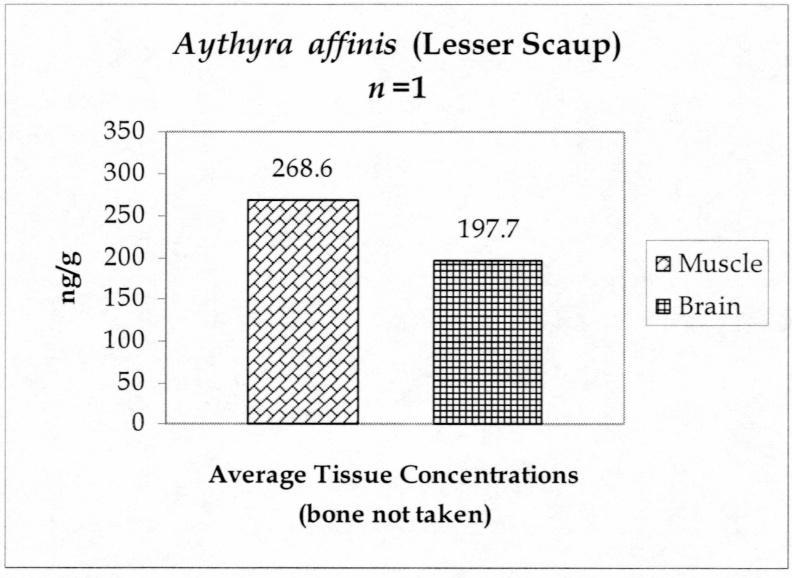


Figure A-6 Greater White-fronted Goose average THg concentrations





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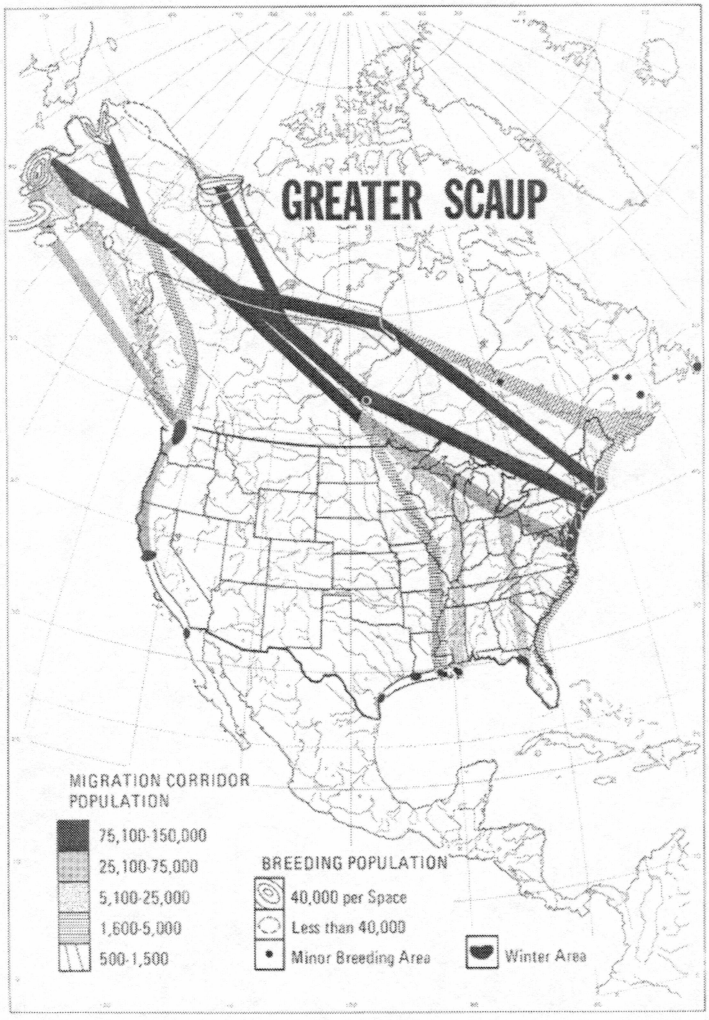

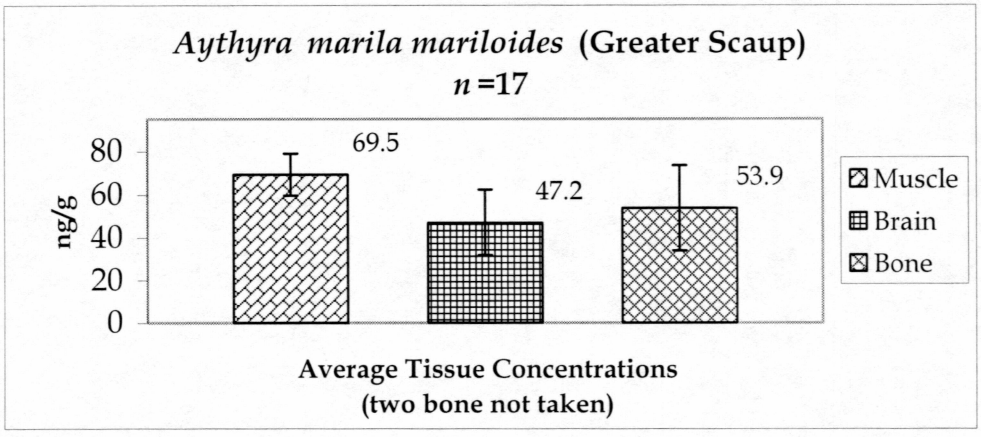

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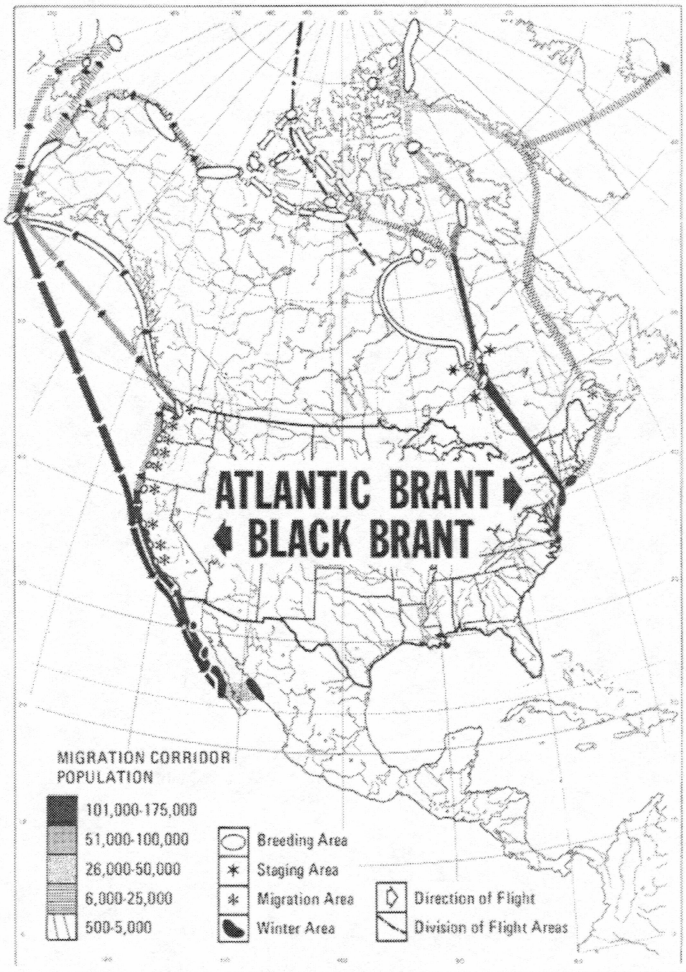


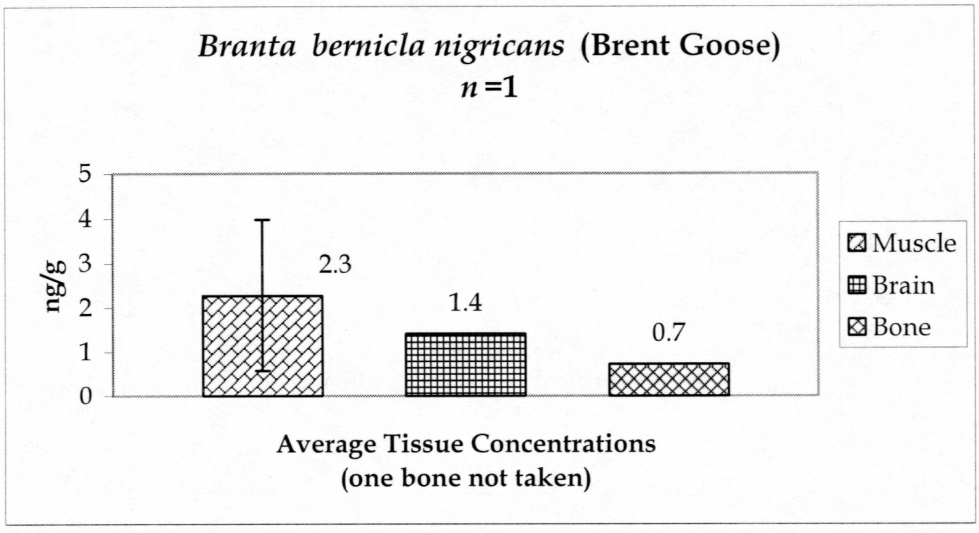
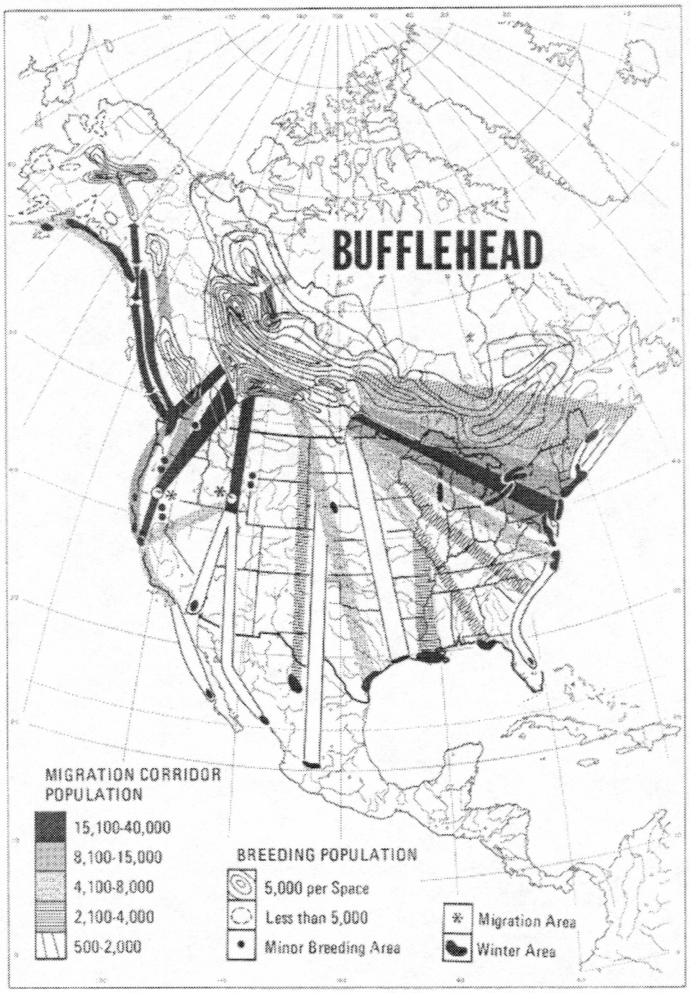


Figure A-9 Brent Goose average THg concentrations



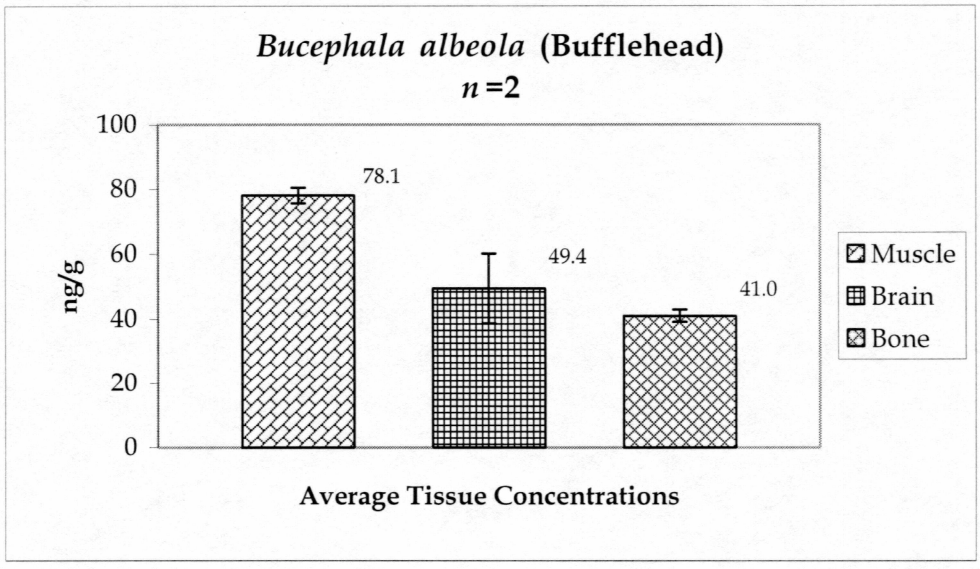
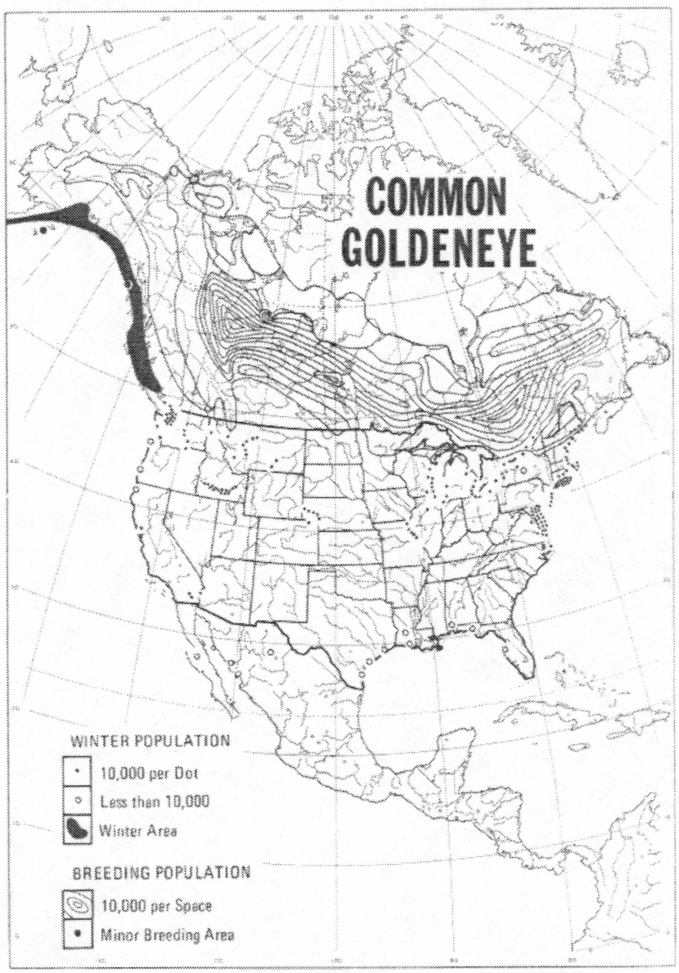


Figure A-10 Bufflehead average THg concentrations



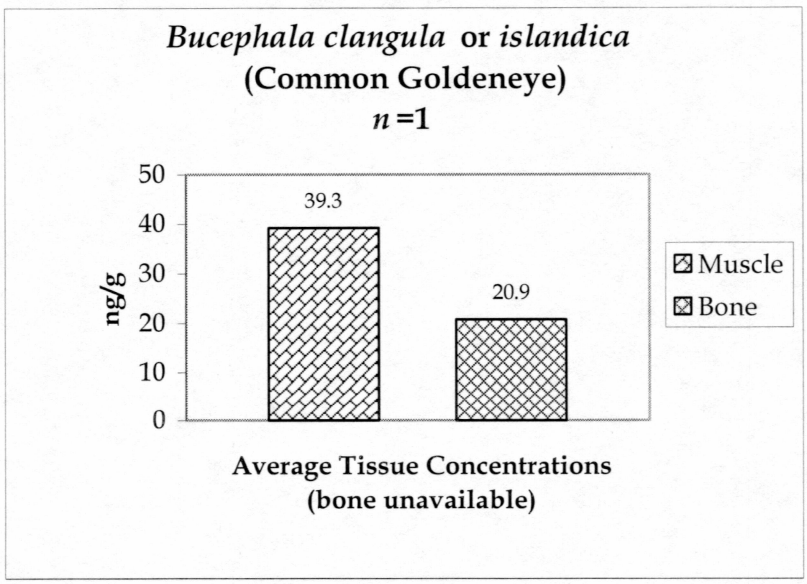
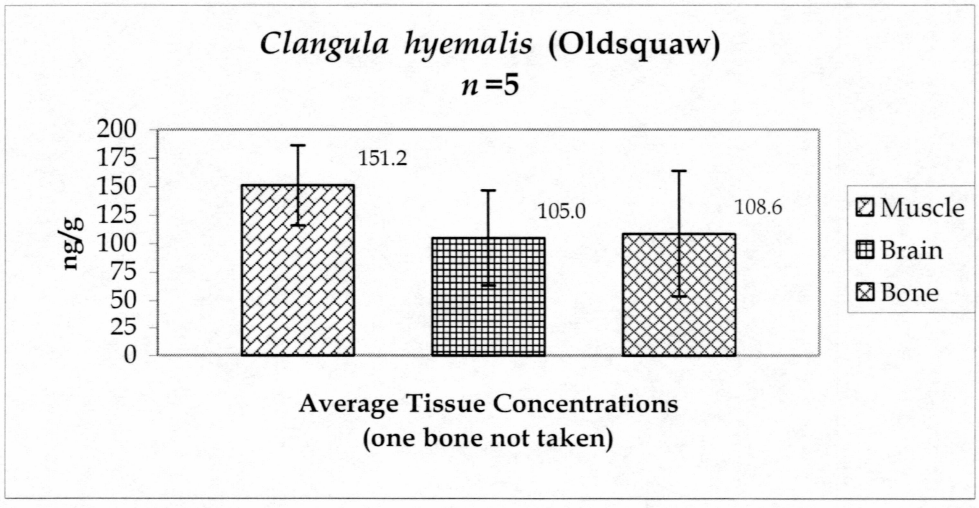


Figure A-11 Common Goldeneye average THg concentrations



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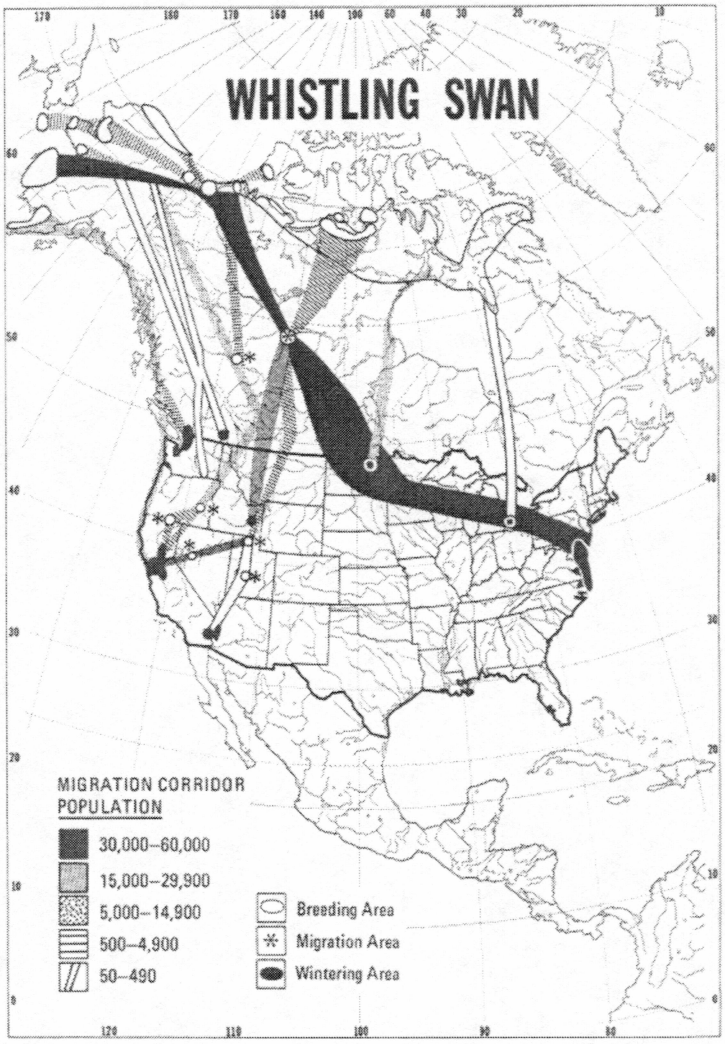


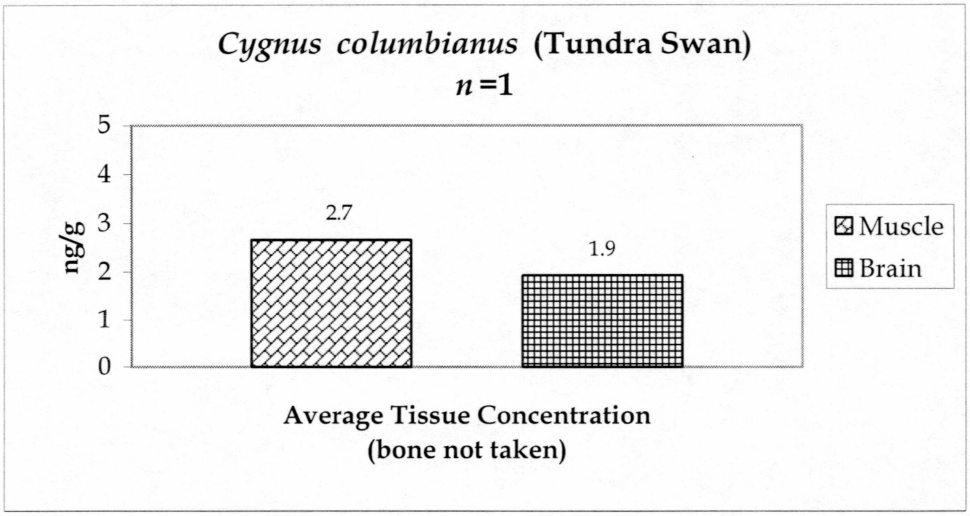


Figure A-13 Tundra Swan average THg concentrations

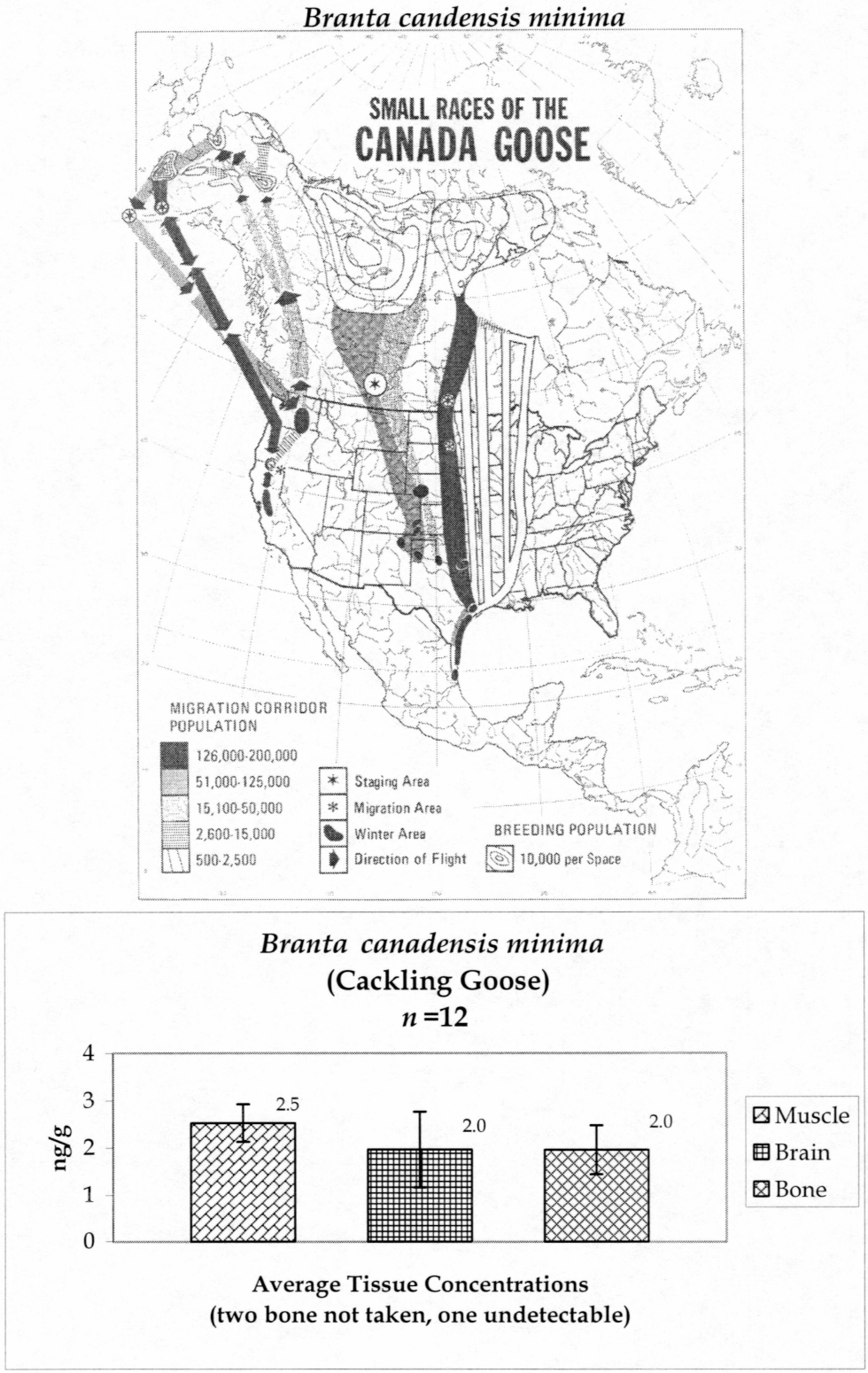
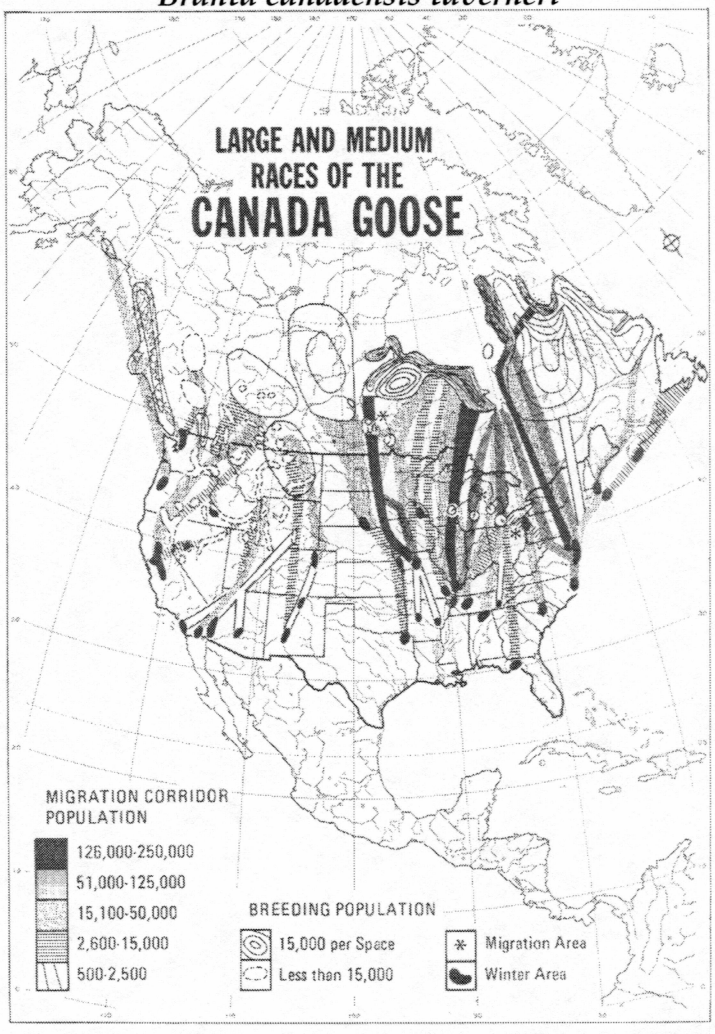
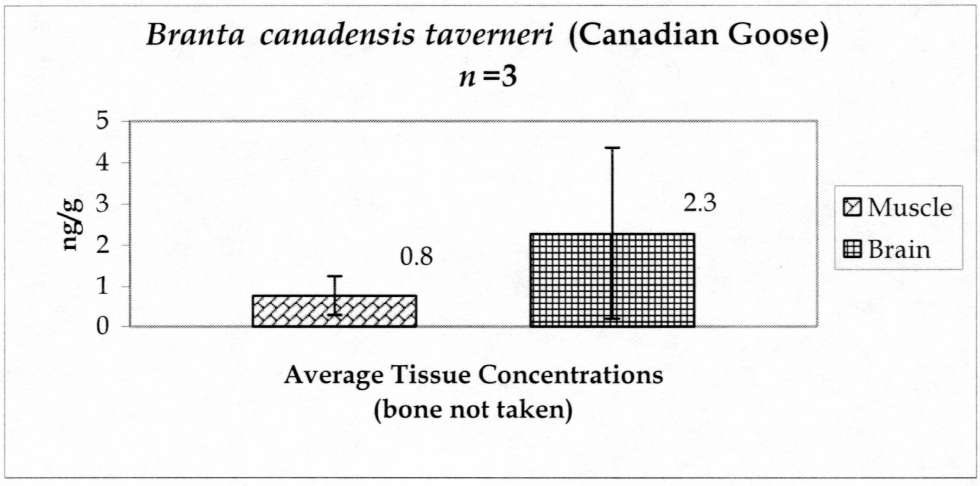


Figure A-14 Cackling Goose average THg concentrations

Branta canadensis taverneri





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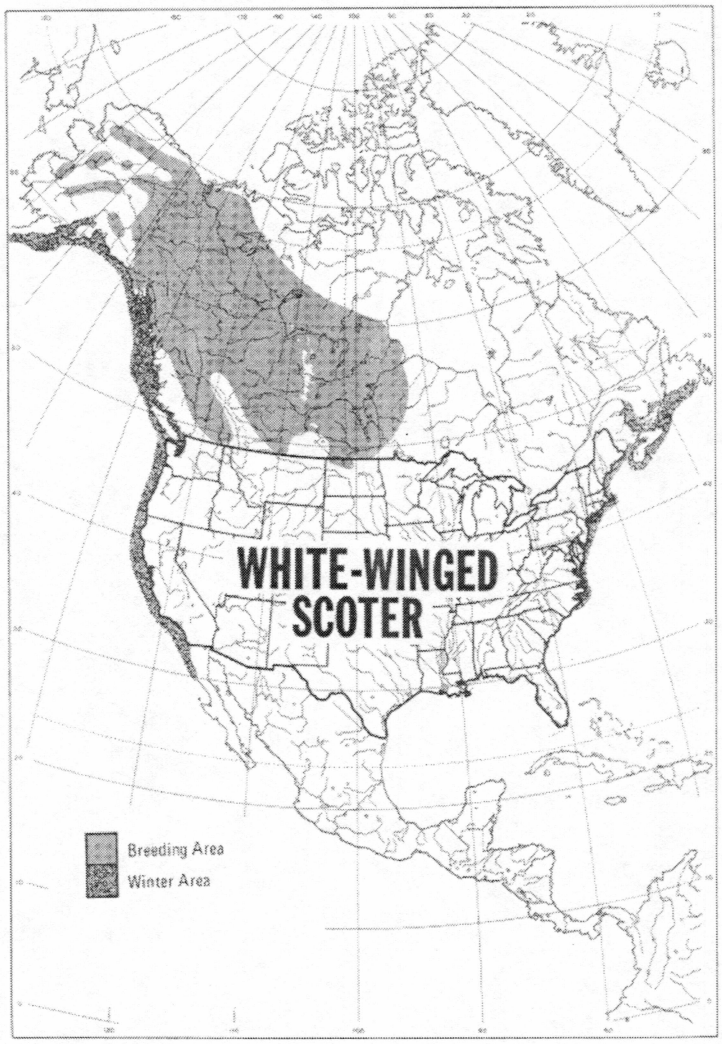


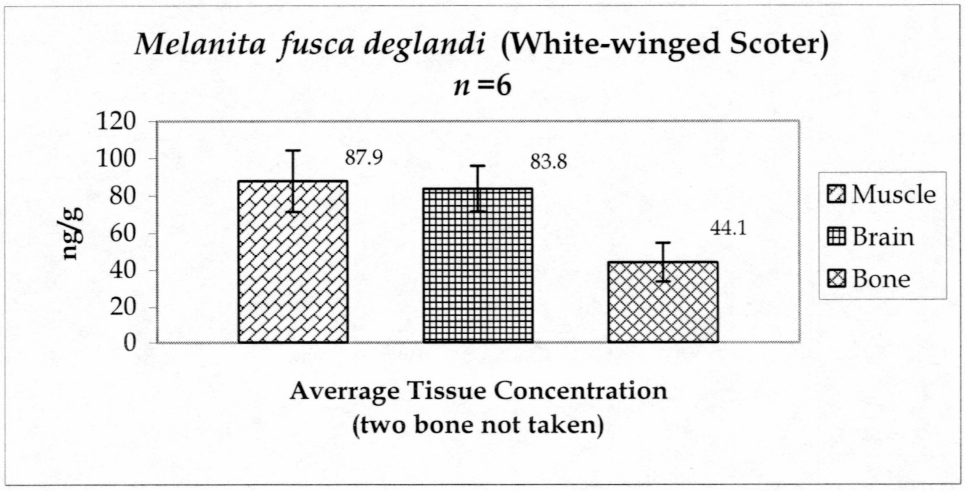


Figure A-16 White-winged Scoter average THg concentrations

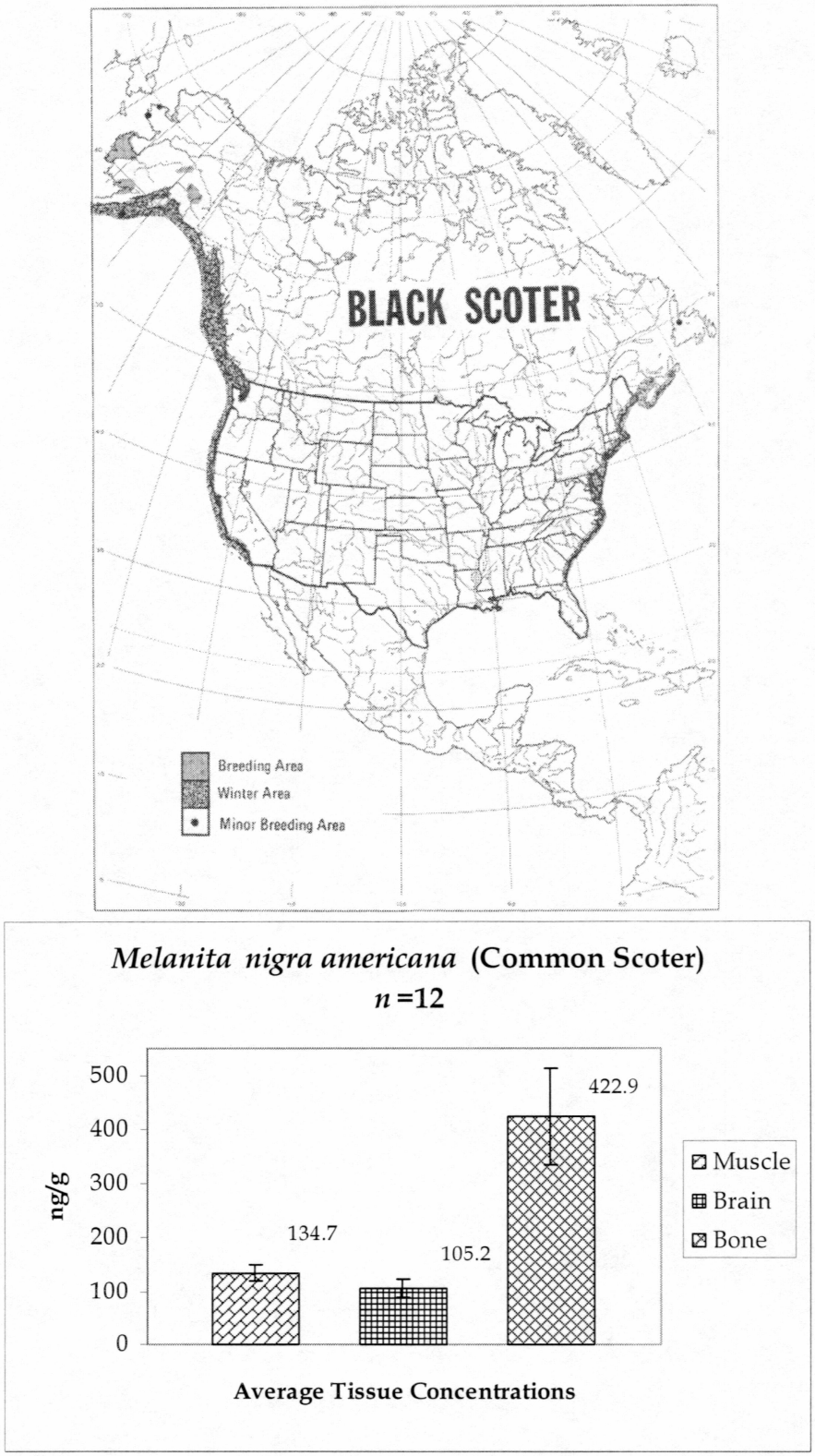
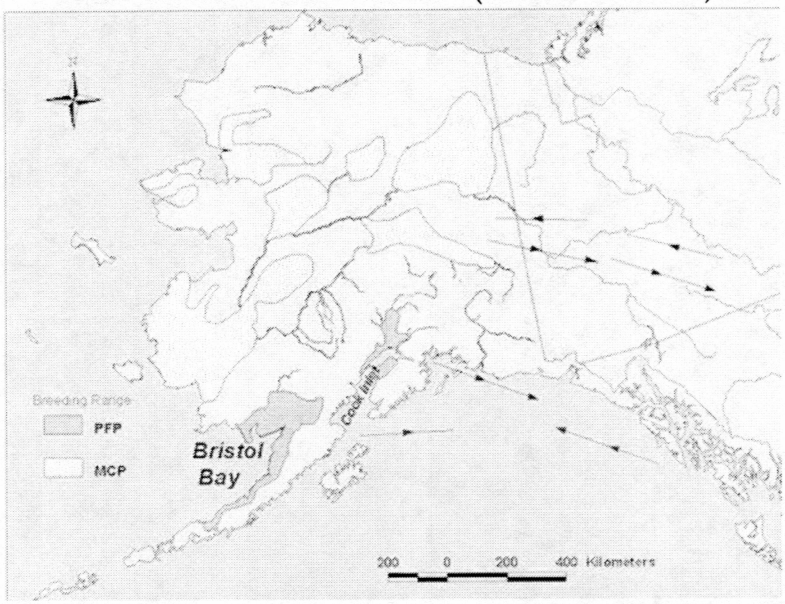


Figure A-17 Common Scoter average THg concentrations

***Grus canadensis canadensis* (Sandhill Crane)**



Pacific flyway, MCP: Mid-continent population, PFP: Pacific flyway population
Courtesy State of Alaska Department of Fish Game

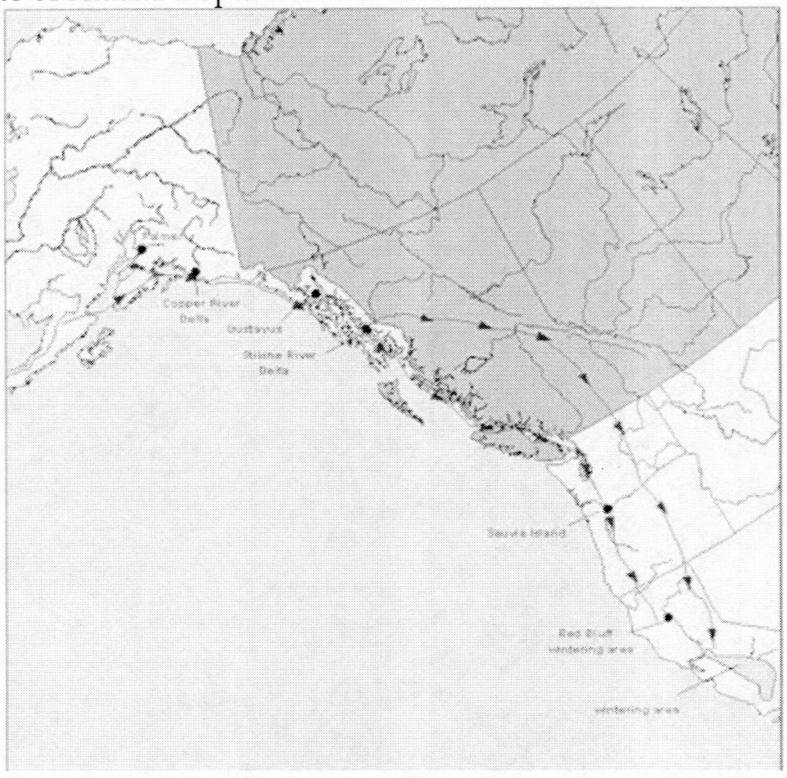


Figure A-18 Sandhill Crane Flyways

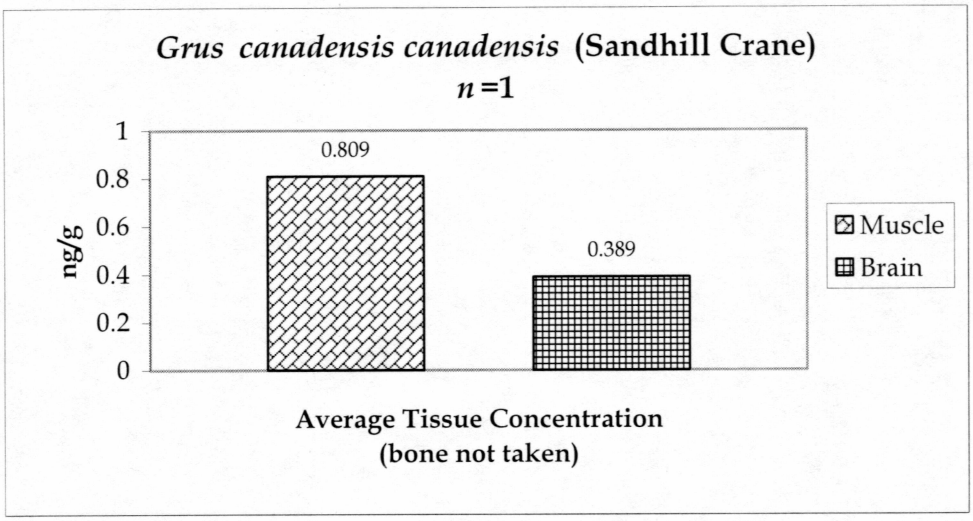
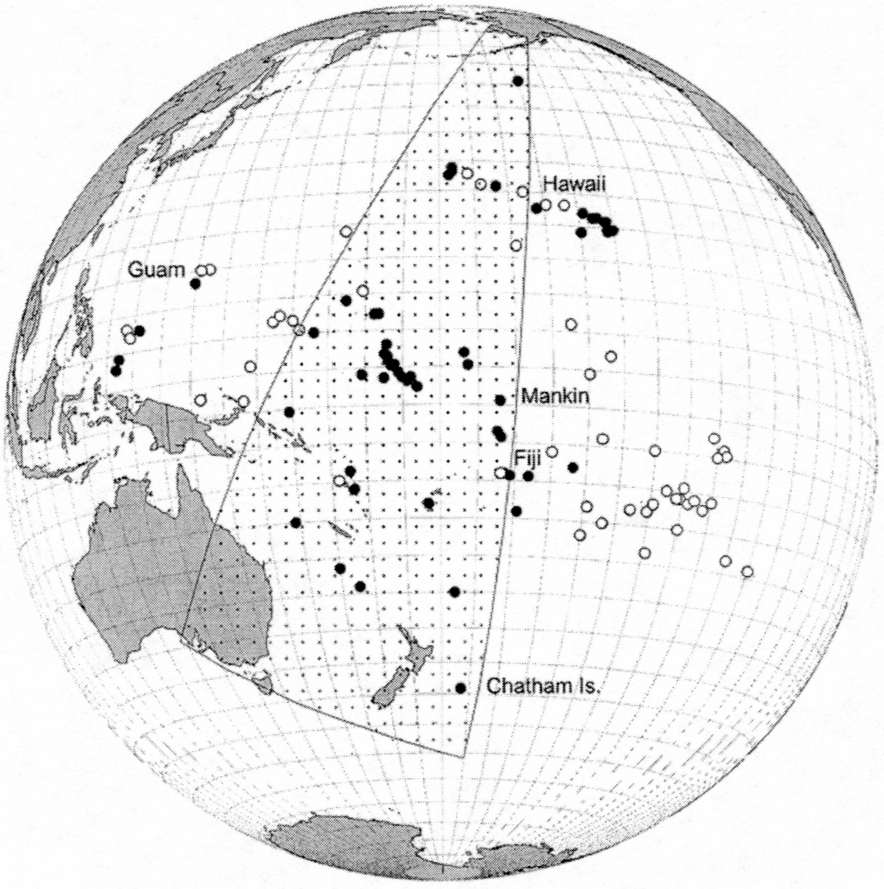


Figure A-19 Sandhill Crane average THg concentrations



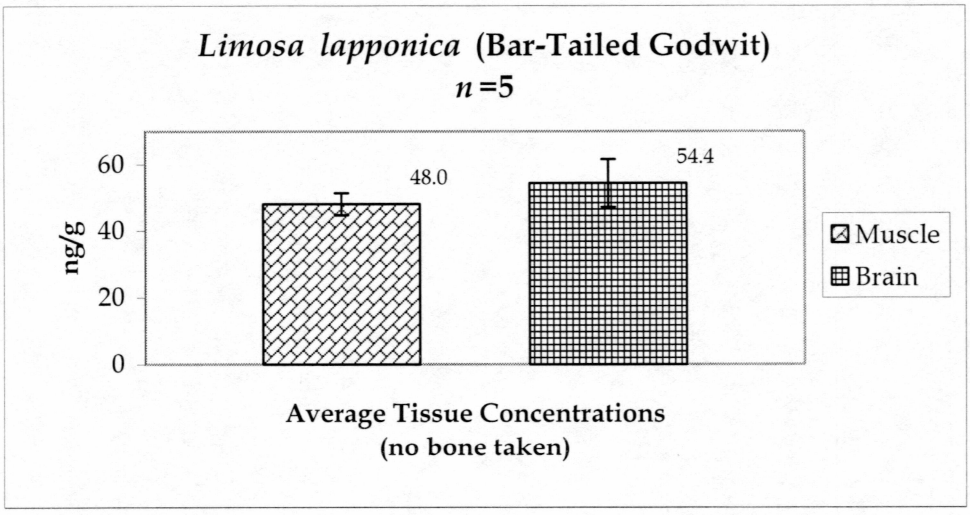


Figure A-20 Bar-tailed Gotwit average THg concentrations