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Trace element concentrations in the Pacific harbor seal, Phoca vitulina richardii, in central and northern California : a thesis ...

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California State University, Monterey Bay

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TRACE ELEMENT CONCENTRATIONS IN THE PACIFIC HARBOR SEAL,
PHOCA VITULINA RICHARDII,
IN CENTRAL AND NORTHERN CALIFORNIA

A Thesis Presented to the Faculty of

Moss Landing Marine Laboratories

and

California State University Monterey Bay

In partial Fulfillment

Of the Requirements for the Degree

Master of Science in Marine Science

By

Tiffini Jo Brookens

January 2006

APPROVED FOR MOSS LANDING MARINE LABORATORIES

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Dr. James T. Harvey, Professor, Committee Chair

_____

Dr. Kenneth H. Coale, Professor

_____

Dr. Todd M. O'Hara – University of Alaska at Fairbanks
Institute of Arctic Biology

APPROVED FOR THE UNIVERSITY GRADUATE COMMITTEE

_____

Abstract

To determine trace element concentrations in the Pacific harbor seal (*Phoca vitulina richardii*), live (n=189) and dead seals (n=53) were sampled throughout central and northern California from March 2003 to January 2005. Total mercury (THg) concentrations were determined in blood and hair of live harbor seals and hair and liver of dead harbor seals. Concentrations of monomethyl mercury (MeHg), selenium (Se), and lead (Pb) additionally were determined in liver of dead harbor seals. To assess trophic level, carbon and nitrogen stable isotopes were examined in liver. Age class assignments of live harbor seals were based on morphometrics, and ages of dead harbor seals were determined by cementum annuli in teeth. There were significant differences in THg concentrations in blood and hair based on age (P<0.001). Adult male harbor seals had greater THg concentrations in their hair than adult female harbor seals (P<0.003). THg concentrations in liver increased linearly with age and δ^{15}N (P<0.001); whereas, MeHg concentrations in liver increased with age exponentially with an asymptote at approximately 1.3 ppm wet weight. MeHg expressed as a percentage of THg (% MeHg) was best described by a decay function (r^2=0.7961, P<0.001). As harbor seals aged, % MeHg decreased to a minimum and remained constant. Se in the liver also increased with age and was in equilmolar ratios with THg in adults; whereas, molar ratio of Se:THg in pups did deviate from a 1:1 ratio. Significant differences among locations in THg concentrations in blood and hair were not detected. Assessing the possible affect of location of sampling on mercury concentrations, however, is confounded and limited by lack of equal sample sizes for basic age and sex cohorts.

Acknowledgements

  I want to thank my thesis committee, Dr. James Harvey, Dr. Todd O'Hara, and Dr. Kenneth Coale, for their encouragement and guidance throughout the study. A special thanks to Jim for keeping the study grounded and assuring the study was completed properly and to Todd for his encouragement and expertise. I would like to graciously thank Tam Voss, Mike Gordon, and Amy Byington for analyzing the samples for various trace elements at Moss Landing Marine Laboratories. This study would not have been completed without the generosity of Tam Voss, both analytically and through her thoughtful insights along the way. I also would like to thank Katrina Knott, Sara Moses, and Ben Meyer at University of Alaska at Fairbanks for assisting in stable isotope analyses and age estimations.

  A special thanks to The Marine Mammal Center (Denise Greig and Frances Gulland) and Long Marine Laboratories-UCSC (Elizabeth Wheeler) for their unparalleled commitment to necropsing and sampling a myriad of animals for this study. This study could not have been completed without the help of various students, interns, and staff members from MLML, TMMC, and LML for their assistance in collecting live and dead harbor seal samples: L. Bodensteiner, C. Gibble, T. Goldstein, D. Greig, S. Hansen, S. Hayes, M. Lander, B. Long, H. Nevins, T. Norris, B. Phillips, M. Rutishauser, T. Sigler, S. Simmons, J. Sweeney, K. Thomas, B. Watts, L. Wertz, E. Wheeler, and E. Woolery. A special thanks to Sarah Allen at the National Park Service for aiding in the original brainstorming aspects of the study and facilitating portions of the study at Pt.

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Introduction

Increasing concern about environmental pollution has led to many studies regarding heavy metal burdens in marine mammals. The Pacific harbor seal, *Phoca vitulina richardii*, generally inhabits nearshore areas (Boulva and McLaren 1979) associated with productive waters that provide an adequate source of prey (Roffe and Mate 1984; Payne and Selzer 1989; Harvey et al. 1995; Sydeman and Allen 1999). Harbor seals, situated near the top of the marine food web, consume a considerable amount of fish, thereby accumulating significant amounts of mercury, Hg (Smith and Armstrong 1973 Tohyama et al. 1986; Himeno et al. 1989). Because they primarily eat fish and squid (Smith and Armstrong 1973; Anas 1974; Cappon and Smith 1982), mercury levels in some marine mammals often are orders of magnitude greater than levels found in terrestrial carnivorous mammals (Skaare et al. 1994) and nonfish-eating marine mammals (Woshner et al. 2001a; Dehn et al. in press). Harbor seals, therefore, are useful mammalian biomonitors for Hg because they are endemic to the nearshore coastal environment and are high-level trophic consumers (Miles et al. 1992).

Because harbor seals inhabit marine and estuarine habitats near urban or industrialized regions, they are more vulnerable to contaminated runoff (Miles et al. 1992) than other migratory pinniped species. Since the industrial revolution, anthropogenic Hg emissions have increased atmospheric Hg levels by three- to five-fold (Mason et al. 1994) and have caused corresponding increases in Hg levels in aquatic ecosystems (Wiener et al. 2003). Approximately 70-80% of current Hg emissions to the

atmosphere are of anthropogenic origin, and 50% of these emissions of Hg are deposited locally (Mason et al. 1994). Anthropogenic sources have contributed considerable amounts of Hg to coastal environments via industrial use in combustion of fossil fuels (Nriagu and Pacyna 1988), chlor-alkali plants, pulp and paper mills (Berlin 1979; Goyer 1991), and gold mining (Magos 1997). Mercury has been mined along the Coastal Range of California from the mid 1800s and has been used extensively to recover gold. Most of the Hg used in gold recovery in California was obtained from the mercury belt along the Coastal Range on the western side of California's Central Valley (Bradley 1918). Total Hg production between 1850 and 1981 was more than 1.0×10^8 kg (Churchill 1999), of which the annual loss of Hg to the environment was approximately 30% (Averill 1946). Much of the Hg discarded in mine tailings has been found in nearby rivers that flow from the Central Valley into San Francisco Bay estuary (Choe et al. 2003). In addition, a Hg mine was situated on Walker Creek, which runs south into Tomales Bay, California, (Hornberger et al. 1999). Mercury has been leaching from this abandoned mine since the early 1980s when a strong El Niño flooded the region, and Hg contamination began to flow into the bay. Mercury mines also were present in northern California, however, more Hg contamination is found in the San Francisco Bay estuary because it receives runoff from 40% of California waters, some of which flow through Hg contaminated watersheds (Domagalski et al. 2002).

Once Hg is transported into nearby bays, it is incorporated into aquatic flora and fauna as it is cycled throughout the ecosystem. Nearly all Hg bioaccumulated in fish is

monomethylmercury (MeHg; Bloom 1992), a highly neurotoxic form that biomagnifies to high concentrations in aquatic food webs (Wiener et al. 2003). Production of MeHg via methylation of inorganic mercury, Hg^{+2} (IHg), from microbial sulfate reduction in the environment (Berlin 1979; Compeau and Bartha 1985; Gilmour et al. 1992) is a key process affecting Hg concentration in fish and other aquatic biota. Consumption of fish is the primary route of MeHg exposure to harbor seals (Koeman et al. 1975; Reijnders 1980). It is efficiently absorbed across the gut, crosses intercellular membranes, distributes via blood stream to all internal organs and tissues, and accumulates in exposed organisms (Clarkson 1994).

Mammals, including pinnipeds, can demethylate MeHg into IHg via intestinal flora (Norseth and Clarkson 1971; Ewers and Schlipköter 1991), tissue macrophages, and fetal liver (National Research Council 2000). MeHg and IHg may be retained by the liver, recirculated throughout the body, or excreted. As harbor seals age, they may be unable to demethylate MeHg as efficiently (Dietz et al. 1990). Their overall health may be compromised by disease, which may alter the underlying physiological detoxification mechanisms or their ability to demethylate MeHg (Dehn et al. 2005). They also may be ingesting more organic Hg than can be biotransformed or eliminated. Some organs and tissues tend to accumulate greater amounts of MeHg before it can be excreted (Roberts et al. 1976; Chang and Reuhl 1983; Himeno et al. 1989; Miles et al. 1992). For instance, MeHg concentrations in the liver of ringed seals increased, but % MeHg remained

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somewhat stable because THg and IHg continued to increase in relation to MeHg (Woshner et al. 2001b).

In mammals, Hg can be excreted slowly in the feces and urine after demethylation; whereas, MeHg can be sequestered directly into keratinized structures and eliminated by hair/feather loss or epidermal sloughing (Scheuhammer 1991; Clarkson 1994; Gaggi et al. 1996; National Research Council 2000). The vast majority of THg in hair, therefore, is considered MeHg (National Research Council 2000). Once MeHg is incorporated into the hair, it does not change and becomes an accurate, continuous record of this trace element's concentration found in the body for many months and is based on dietary intake (Jenkins 1979; Subramanian 1991). MeHg also can be transferred to seal pups via the placenta and to a lesser degree via the milk of the mother (Jones et al. 1976; Reijnders 1980; Wagemann et al. 1988). Some researchers have documented that MeHg crosses the placenta (Jernelov 1986) and accumulates in the developing fetus; therefore, the fetus acts as a mercury "trap" or "sink" by sequestering the toxicant from the maternal system (Chang and Reuhl 1983). Adverse health effects of MeHg occur in the reproductive, immune (Koller 1979; Chowdhury and Chandra 1991), and central nervous systems (Scheuhammer 1991; Wiener and Krabbenhoft, 1999; Clarkson 1994).

Some harmful effects of Hg can be counteracted by the presences of selenium, Se, an essential element. Se may provide protective effects for Hg toxicity (Parizek and Ostadalova 1967; Ganther et al. 1972; Stillings et al. 1974; Martoja and Berry 1980). It potentially binds to Hg in equimolar ratios in the liver of marine mammals (Koeman et al.

1973; Koeman et al. 1975; Smith and Armstrong 1978; Braune et al. 1991; Dietz et al.

1995); however, some researchers have determined molar ratios deviating from 1

(Wagemann et al. 1988; Himeno et al. 1989; Woshner et al. 2001b; Dehn et al. 2005).

The 1:1 molar relationship, thus equimolar binding of Hg and Se, may serve as a

protective mechanism against toxic effects of Hg by biotransformation of ingested MeHg

into a less toxic chemical form. Deviations from the 1:1 molar ratio, however, may

indicate that the mechanism for Hg detoxification is overwhelmed or is not completely

Se-dependent (Woshner et al. 2001b).

Accumulation of trace elements (Hg and Se) in marine mammal tissue may be

dependent on age and trophic level (Anas 1974; Roberts et al. 1976; Drescher et al. 1977;

Smith and Armstrong 1978; Reijnders 1980; Himeno et al. 1989; Frank et al. 1992; Miles

et al. 1992; Skaare et al. 1994; Dietz et al. 2000; Woshner et al. 2001b; Watanabe et al.

2002; Bustamante et al. 2004; Dehn et al. 2005). Gender based differences in Hg also

may occur in various tissues of phocids, especially adults (Reijnders 1980; Wagemann et

al. 1988; Skaare et al. 1994). Each tissue type incorporates Hg during various

timeframes. THg concentrations in blood represent Hg exposure during numerous days

and are dependent on types and amounts of prey consumed (National Research Council

2000). THg concentrations in hair represent Hg that was available to the growing pile via

blood during their annual moult (Berlin 1979), which lasts 6-8 weeks (Montagna and

Harrison 1957). The liver functions as a filtering organ, and trace metals tend to

accumulate in this organ because Hg binds to certain components of the cytoplasm of

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hepatocytes and stellate macrophages and is retained (Woshner et al. 2002). THg

concentrations in liver may represent more closely a lifetime exposure because the half-

life of MeHg in phocids is relatively long, 500 days (Tillander et al. 1972), and the half-

life of THg in phocids is longer, 10 years (Wagemann et al. 2000). Increased

concentrations of Hg in one tissue type should be correlated with increased

concentrations in another tissue type. These relationships, though, may be confounded

by age, demethylation efficiency, health of the animal, and form of Hg ingested (Dietz et

al. 1989; Himeno et al. 1989; Miles et al. 1992; National Research Council 2000; Wiener

et al. 2003).

The objectives of this study were (*i*) to determine concentrations of various trace

elements in blood, hair, and liver in harbor seal throughout central and northern

California, (*ii*) to determine if these trace elements differ with age, sex, location, tissue

type, and trophic level, and (*iii*) to evaluate various elemental interactions.

I hypothesized that THg concentrations in the liver of harbor seals should be the

greatest; whereas, THg concentrations in blood of harbor seals should be the least. THg,

MeHg, and Se concentrations in harbor seals should increase with age in all of the tissue

types. Male adult harbor seals are expected to have greater THg concentrations in their

hair than females. Harbor seals sampled in San Francisco Bay should have the greatest

concentrations of THg in their tissues, with intermediate concentrations from seals

sampled in Pt. Reyes and Humboldt, and least concentrations of THg from seals in

Monterey Bay. THg concentrations in blood should be positively related to THg

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concentrations in hair, and THg concentrations in hair should be positively related to THg and MeHg concentrations in liver. Harbor seals feeding at higher trophic levels should have greater concentrations of THg, MeHg, and Se. Molar ratio of THg to Se should deviate from a 1:1 relationship because not all of the bioavailable Se will be bound to THg.

Methods

Sampling of Dead Harbor Seals

Dead, beachcast harbor seals (n=53) were sampled along the central California coast from March 2003 to September 2004 (Fig. 1). Harbor seals were measured, sex determined, and examined externally. Full post-mortem examinations followed when appropriate samples were taken for histology, pathology, parasitology, and cytology. An approximate 15 cm x 15 cm patch of hair, weighing 3 g, was shaved from the dorsal midline region, just anterior of the tail. A battery operated shaver, Oster[®] PowerPro, with a 1mm stainless steel blade was used to remove the hair. Hair was placed in a separate polyethylene bag. Livers (n=40) were removed with stainless steel instruments and placed either whole or subsampled in a separate polyethylene bag. Equal proportions of each lobe of the liver were grossly determined and subsampled. Two upper canines were extracted and placed in polyethylene bags for age estimations. All tissue samples were stored in a -20°C freezer at Moss Landing Marine Laboratories (MLML). Hair was cleaned, digested, and analyzed for THg concentration; whereas, livers were

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homogenized, digested, and analyzed for THg, MeHg, Se, and Pb concentrations (Freeman and Horne 1973; Roberts et al. 1976; Reijnders 1980; Frank et al. 1992; Wenzel et al. 1993; Lake et al. 1995) using standard laboratory procedures. Animals were sampled under permit to Dr. James T. Harvey, MLML (CDF&G#801135-05 and LOA from NMFS) and San Jose State University's Internal Animal Care and Use Committee (IACUC) #835.

Aging of Dead Harbor Seals

One canine tooth per animal was sectioned and prepared by Matson's Laboratories, Milltown, Montana. Teeth were decalcified, thin sectioned across the midline in 14μm sections using a rotary microtome, stained with Giesma® histological stain, and mounted. The mounted sections were examined under transmitted light through a compound microscope at high magnification (100X).

Growth layer groups, GLGs, represent one translucent and one opaque layer. One GLG is deposited each year in the cementum of harbor seal canines (Dietz et al. 1989). Age was estimated at least three times each by two independent, blind readers. Reader 1 (R1) was Tiffini Brookens, MLML, and Reader 2 (R2) was Sara Moses, University of Alaska Fairbanks (UAF). The precision of age determinations was measured for each reader using Average Percent Error index (Beamish and Fournier 1981), Coefficient of Variation (Chang 1982), and Index of Precision (Chang 1982):

Average Percent Error (APE): $\left[\dfrac{1}{R} \left(\left| \dfrac{X_{ij} - \overline{X}_j}{\overline{\overline{X}}_j} \right| \right) \right]$

where: R = number of time each animal is aged
$\underline{X}_{ij}$ = ith age determination for the jth animal
X_j = average age calculated for the jth animal

Coefficient of Variation (CV): $\dfrac{SD_j}{\overline{X}_j}$

where: $\underline{S}D_j$ = standard deviation of ages for jth animal
X_j = average age calculated for the jth animal

Index of Precision (D): $\dfrac{CV}{\sqrt{R}}$

where: CV = coefficient of variation (standard dev/mean)
R = number of times each animal is aged

To determine age precision of the two independent readers, a frequency histogram was used to compare number of times estimated ages differed. Concensus age from R1 was subtracted from concensus age of R2. To further determine precision of estimated ages, an age-bias graph comparing R1 and R2 was used (Campana et al. 1995). A 1:1 equivalence line represented agreement on age; any deviation indicated variation in each reader's aging biases.

Sampling of Live Harbor Seals

Harbor seals (n=189) were captured near their haulout sites in Monterey Bay, San Francisco Bay, Pt. Reyes, and Humboldt, CA, from March 2003 to January 2005 (Fig. 2), using methods described by Jeffries et al. (1993). Two-outboard powered boats were used to set a net, approximately 120 m in length and 8 m in depth, in waters adjacent to

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their haulout sites. Each end of the net was pulled ashore, encircling, and capturing

harbor seals. Seals then were removed from the net, placed headfirst into hoop nets, and

physically restrained. Once secured, standard length (± 1 cm), girth (± 1 cm), mass (± 1

kg), sex, and age class were determined. Age classes were defined by criteria from Bigg

(1969) and based on size and pelage of individuals.

Blood from each live harbor seal (n=174) was drawn from the extradural

intervertebral sinus with a 7.5-cm long 18-gauge needle, luer adapter, and Vacutainer®

holder. Approximately 10 mL of blood was collected in royal blue top Vacutainer® with

sodium heparin (Sherlock et al. 1984; Hansen 1991). Blood was stored in 20mL or 40mL

trace metal clean I-Chem™ Teflon™-coated vials in coolers until transported to MLML

and placed in a -20°C freezer.

A patch of hair, approximately 15 cm x 15 cm and weighing 3 g, was shaved from

the dorsal region of each live harbor seal (n=187) using previous stated methods. For live

animals, a 1/10mm cryotech blade was used to shave the hair. After the hair was shaved,

it was placed in a polyethylene bag, stored in a small cooler, and transported back to a

-20°C freezer at MLML. All animals were sampled under permit to Dr. James T. Harvey,

MLML (NMFS permit No. 555-1565-01) and San Jose State University IACUC #835.

Analytical Procedure for THg Concentrations in Blood and Hair

Hair, 0.5 to 2.0 g, from beachcast and live harbor seals was washed in dionized

(D.I.) water with a serial racking method and dried at 65°C in a VWR® 1040E drying

oven for 48 hrs before analysis. Six cleaning blanks and six crossover contamination

blanks also were analyzed to ensure minimal or undetectable Hg contamination occurred

during the washing process. Both types of blanks were below the method detection limit

(MDL) of 0.0107 μg/g wet weight (ww). Hair samples were considered to have 0%

moisture because the moisture attached to the hair was not inherent in the hair but an

artifact of environmental conditions. The dry weight and wet weight for hair, therefore,

were considered equal.

Blood samples were aliquoted directly from the vials. Percentage moisture was

determined for only blood samples. Approximately 3 g of blood were placed in

preweighed drying dishes and dried at 72°C in a VWR$^{®}$ 1040E drying oven for 48 hrs.

Final weights were determined and used to calculate percent moisture:

Percent moisture= |(initial mass – final mass) / initial mass| x 100

Certified Reference Material, CRM (dogfish liver, DOLT-3 NRCC), was used as

the certified standard. Digestion of tissues and THg analyses were based on the

procedure established by Hatch and Ott (1968) with slight modifications. Approximately

1.0 g of blood, 0.5 g of hair, and 0.25 g of CRM were digested in 40mL I-Chem™ vials

with 10 mL of 70:30 v/v HNO_3/H_2SO_4 solution. Vials then were placed on a hot plate

with a reflux cap and heated to 125°C for 2 hours, after onset of refluxing. Samples were

allowed to cool and diluted to 40 mL with a 5% v/v solution of 0.2N BrCl in MilliQ$^{®}$

water.

Quality control (QC) consisted of three method blanks, one CRM, one matrix duplicate (MD), one analytical spike (AS), and one analytical spike duplicate (ASD) per batch. No more than 20 unknowns were analyzed per batch. Quality control criteria from CALFED Quality Assurance Program Plan (QAPP; Puckett and van Buuren 2000) included the following: CRM 75-125% recovery limit, MD 25% relative percent difference (RPD) limit, AS/ASD 75-125% recovery limit, and 25% RPD limit.

Marine Pollutions Studies Laboratories in cooperation with California Department of Fish and Game (CDFG) at MLML analyzed THg concentrations in blood and hair samples. THg samples were analyzed by Atomic Absorption Spectroscopy (AAS) using a Perkin Elmer® Flow Injection Mercury System (FIMS-100) with the software application AA WinLab. A peristaltic pump, in conjunction with an auto-sampler (Perkin Elmer® AS-90), drew 4 mL aliquot of the sample solution into the mixing block. The reducing agent (1.1% Tin (II) chloride in 3% HCl v/v) was pumped simultaneously into the mixing block where a spontaneous reaction took place, reducing ionic mercury to elemental mercury. Argon, the carrier gas, then carried the mercury vapor to the gas/liquid separator, and the gas continued on to the FIMS-cell.

Concentrations of unknowns greater than the highest standard were diluted and reanalyzed until their placement was within the bounds of the standard curve. The MDL for these tissues using the above procedure was 0.0107 μg/g wet weight tissue. Final concentrations of THg were reported in μg/g or ppm dry weight and then converted to

wet weight based on their percent moisture. Additionally, all QC fell within criteria

limits and all method blanks were less than MDL.

Analytical Procedure for THg, Se, and Pb in Livers

Livers from dead beachcast harbor seals were subsampled, approximately the

same proportional volume grossly extracted from each lobe, and then homogenized using

a trace metal clean Brinkman[®] Polytron PT-10/35 homogenizer. Dogfish liver (DOLT-3

NRCC) was used as the CRM, with an additional beluga liver reference material (RM,

QC97LH2-NIST). Approximately 0.5 g liver and 0.25 g of CRM and RM were digested

in Teflon™ vessels with 6 mL of concentrated double distilled HNO_3 in a CEM[®]

MARS5 pressurized microwave. They were digested with a 15-min ramp to 195°C and

250 psi; samples were held at this temperature and pressure for 20 min. A 20-min cool-

down cycle then followed. Samples were removed from the microwave and brought to

room temperature. Once cooled, 7 mL of MilliQ[®] were added, and vessels were gently

shaken. Digestates then were transferred into 30mL precleaned, preweighed

polyethylene bottles and brought up to a mass of 20 g by adding MilliQ[®] (method 3052;

US Environmental Protection Agency 1996).

QC included two method blanks, one CRM/RM, one MD, one AS, and one ASD

per batch of no more than 20 samples using the same criteria as specified previously by

CALFED QAPP (Puckett and van Buuren 2000). Marine Pollution Studies Laboratories

in cooperation with CDFG performed THg analyses on livers using AAS/FIMS as

specified previously. The MDL for these tissues using the above procedure was 0.0107

µg/g wet weight. Percent moisture was calculated using previously described methods.

Final concentrations of THg were reported in µg/g or ppm dry weight and converted to

wet weight based on percent moisture.   If unknowns were greater than the highest

standard, they were diluted and reanalyzed until their placement was within the bounds of

the standard curve. All QC fell within criteria limits, and all method blanks were less than

MDL.

High Resolution Inductive Coupled Plasma-Mass Spectroscopy, HR-ICPMS, was

used to determine Se and lead (Pb) concentrations in liver tissue. ICPMS method

followed criteria of US Environmental Protection Agency method 200.8 (US

Environmental Protection Agency 1994) and was performed at MLML, CA, using a

ThermoFinnigan[®] Element2 HR-ICPMS. The MDL for liver using the above procedure

was 0.012 µg/g wet weight for Se and 0.0072 µg/g wet weight for Pb. Final

concentrations of Se and Pb were reported in µg/g or ppm dry weight and then converted

to wet weight based on percent moisture. Unknowns greater than the highest standard

were diluted and reanalyzed until their placement was within the bounds of the standard

curve. All QC fell within criteria limits, and all method blanks were less than MDL

except two Se blanks and one Pb blank. The absolute values of each of these three blanks

(0.013, 0.014, and 0.0098 µg/g) were well within two times the MDL (0.024 and 0.0144

µg/g); therefore, samples were not blank subtracted (i.e. blank concentrations greater than

MDL are subtracted from concentrations of each sample from the batch).

<u>Analytical Procedure for MeHg Concentrations in Livers</u>

Livers also were analyzed for MeHg. Approximately 0.5 g of liver homogenate and 0.25 g of CRM/RM were transferred to a 40mL I-Chem™ vial. Ten mL of 25% KOH/methanol reagent were added to each sample. The samples were capped, shaken, and placed on a hot plate at 90ºC for 2-4 hours or until all soft tissue was visibly solubilized. Samples then were cooled to room temperature and diluted with methanol to 25.6 mL volume.

Fifty mL of D.I. water and 500 μL of acetate buffer were added to a reaction vessel (solution buffered at pH of 5.0). Then, 5-100 μL aliquot of the digestate were added to the bubbler as follows: A new trace metal-free tip was placed on the pipettor, and the tip was inserted < 1 mm below the surface of the digest. The sample was pipetted, without rinsing the tip in the solution. The tip then was placed > 1 mm deep into the buffered water in the bubbler, and the tip was rinsed three times into the water. A new tip was used for each KOH/methanol aliquot that was pipetted. Addition of 35 μL of 1% sodium tetraethylborate in 2% KOH activated the aqueous phase ethylation. MeHg was determined by isothermal gas chromatography separation of ethyl analogs and cold vapor atomic fluorescence spectrometer (Tekran®Model-2500 CVAFS mercury detector; Bloom 1989).

A peak at approximately 2 minutes corresponded to methyl ethylmercury, the ethylation product of MeHg. To calculate the concentration of MeHg in a sample the

following formula was used:

$$[MeHg] \ (\mu g/g \ or \ ppm) = V(S-B_R)/(A)(v)(M)(1000);$$

where V is the final dilution volume of the digestate in mL, S is the gross area under the

curve of the sample in question, B_R is the mean method blank, A is the slope of the

calibration curve, v is the aliquot size in mL, and M is the mass (in g) of the digested

aliquot.

QC consisted of three method blanks, one CRM/RM, one MD, one AS, and one

ASD per batch. Quality control criteria from CALFED QAPP (Puckett and van Buuren

2000) consisted of the following: CRM/RM 70-130% recovery limit, MD 25% RPD

limit, AS/ASD 70-130% recovery limit, and 25% RPD limit. Marine Pollution Studies

Laboratories in cooperation with CDFG performed MeHg analyses on livers. Percent

moisture was calculated using previous methods, and MeHg concentrations were reported

in $\mu g/g$ or ppm dry weight and then converted to wet weight based on percent moisture.

The MDL for MeHg in liver using the above procedure was 0.002 $\mu g/g$ or ppm wet

weight. All QC fell within criteria limits, and all method blanks were below MDL.

$\delta^{15}N$ and $\delta^{13}C$ Stable Isotope Analyses for Liver

Liver samples (n=40) were analyzed for $\delta^{15}N$ and $\delta^{13}C$ stable isotopes using

Elemental Analysis-Isotope Ratio Mass Spectrometry (EA-IRMS). Lipid content has no

effect on C or N signatures in marine biota tissue (Hoekstra et al. 2002); therefore, lipids

were not extracted from the samples. Liver samples (n=40) were freeze dried for 48

hours. Approximately 0.30 mg were aliquoted into tin capsules and placed in a Costech®

EA (ESC 4010) autosampler then combusted. The N_2 and CO_2 combustion gases were

separated chromatographically and transferred to Finnigan® MAT Conflo III interface

with a Delta+XP® Mass Spectrometer, where the isotopes were measured (Dehn et al. in

press, 2005). International isotope standards used were atmospheric N_2 for nitrogen and

Pee Dee Belemnite limestone for carbon. QC included analyzing tin capsule blanks,

laboratory working standards, and isotope standards. Analyses were performed at the

Alaska Stable Isotope Facility at UAF. The results are expressed as:

$$\delta^{15}N \text{ or } \delta^{13}C = [(R_{sample}/R_{standard}) - 1] \times 1000$$

R$_{sample}$ and R$_{standard}$ are the corresponding ratios of $^{15}N/^{14}N$ or $^{13}C/^{12}C$ for the

sample and the standard. All QC fell within criteria limits.

Statistical Analyses

Assumptions of an Analysis of Variance (ANOVA) include random sampling,

independence, equal variances, and normal distributions. All samples were considered

random and independent of one another. To determine homogeneity among variances,

Cochran's test of equal variances was used. If variances were unequal, data were log

transformed to ensure homogeneity. Normal distributions then were evaluated using

Kolmogorov-Smirnov test. Once all assumptions were met, ANOVAs were used.

To test for differences in THg blood concentrations, a Model I, two-way ANOVA

with Tukey's post-hoc test was used with age class (pups, juveniles, and adults) and site

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(Monterey Bay, San Francisco Bay, Pt. Reyes, and Humboldt) as fixed factors. Age classes were defined as pups (SL $\leq$ 100 cm), juveniles, and adults (males weighing $\leq$ 50 kg, females weighing $\leq$ 45 kg) from criteria by Bigg (1969) and determined in field.

To test for differences in THg concentrations in hair of beachcast and live harbor seals, a Model I, two-way ANOVA was used with dead or live and age class as factors. Once dead or live indicated no effect on the samples, all of the THg concentrations were pooled. To test for differences in THg concentrations in all hair samples, a Model I, three-way ANOVA with Tukey's post-hoc test was used with sex (males and females), age class (pups and nonpups), and site as fixed factors. Sites were Monterey Bay, San Francisco Bay, Pt. Reyes, and Humboldt. To meet homogeneity of variances assumption of the ANOVA, THg hair concentrations were log transformed. If data from dead animals did not fit into one of the site categories, the data were not used in the ANOVA.

To further distinguish gender, age, and location differences, the ANOVA testing differences in mean concentrations of THg in blood and hair were partitioned accordingly. There were seemingly equal sample sizes for gender and age classes in the Pt. Reyes dataset, so a Model I, two-way ANOVA with Tukey's post-hoc test was used only for the THg blood data from Pt. Reyes with sex and age class as fixed factors. Age classes again were defined as pup, juvenile, and adult. Because there were few males in the sample for Monterey Bay, a Model I, two-way ANOVA with Tukey's post-hoc test was used to test differences in mean THg in hair of females with age class and location as fixed factors. In this instance, age classes were defined as pup, juvenile, and adult;

whereas, locations were San Francisco Bay, Pt. Reyes, and Humboldt. Because no

location differences were found and gender differences were likely to be found in hair of

adults, a one-way ANOVA was used with location data pooled for all adults with sex as

the fixed factor. To meet the equal variance assumption of the ANOVA, THg hair

concentrations were log transformed.

To test for differences in mean THg, MeHg, Se, Pb, and % MeHg concentrations

in livers, a Model I, two-way ANOVA was used with sex and age (pups and nonpups) as

fixed factors. The ratio of MeHg to THg is referred to as % MeHg. THg and Se

concentrations in the liver were log transformed to meet assumptions of equal variances.

One Pb outlier was removed from the ANOVA because this animal died of acute Pb

toxicity and that datum would have skewed the results. A Model I, two-way ANOVA

also was used to test for differences in $\delta^{15}N$ and $\delta^{13}C$ signatures with age class (pups and

nonpups) and sex as fixed factors.

To further determine if differences in mean concentrations of THg, Se, and MeHg

in livers were based on age class, sex, and trophic position, a Model III General Linear

Model (GLM) was used. Age class (pups and nonpups) and sex (male and female) were

fixed factors; whereas, $\delta^{15}N$ was a random factor. THg and Se liver concentrations were

log transformed to reduce skewness and kurtosis and to meet GLM assumptions of

normal distribution and homogeneity of variances. All interaction terms were

insignificant according to type III sums of squares, and each model was reduced to:

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log THg, log MeHg, or log Se liver concentrations

$$= \mu \ + \ \text{age class} \ + \ \text{sex} \ + \ \delta^{15}N \ + \text{age class} * \text{sex} + \varepsilon$$

where μ is a constant and ε is the random error component for each model.

To determine if harbor seals with hepatic lesions had greater trace element concentrations in their livers, Fisher's exact test was used. Independence of THg liver concentrations and % MeHg concentrations based on documented lesions in the liver were determined. THg liver concentrations and % MeHg concentrations were partitioned into $\geq$ median and < median. Histological data from post-mortems were used to score livers as with (+) or without (-) lesions. Livers were scored + lesion if there was moderate to severe histological findings described by certified veterinary pathologists at University of California Davis.

To assess relationships between THg concentrations (blood and hair) and length, girth, and mass, simple linear regressions were used. Simple linear regressions also were used to determine relationships between the following: THg hair and THg blood concentrations, THg liver concentrations and age, and Se liver concentrations and age, molar THg and Se liver concentrations, and % MeHg and $\delta^{15}N$ liver concentrations. To determine if the linear regression slope for molar THg liver concentrations based on molar Se liver concentrations is equal to one, a one sample t-test was used. The ratio of molar Se to molar THg also was calculated and compared with one using a one sample t-test. Nonlinear regressions were used to determine the relationships between the following: THg hair and THg liver concentrations, THg hair and MeHg liver

concentrations, MeHg liver concentrations and age, % MeHg and age, MeHg and THg

liver concentrations, THg and δ^{15}N liver concentrations, Se and δ^{15}N liver

concentrations, and MeHg and δ^{15}N liver concentrations. For graphical purposes, some

nonlinear plots were fitted with log-based axes to better distinguish trends.

When applicable, outliers were eliminated from various datasets to meet statistical

assumptions. All statistical tests were calculated using Systat® version 10.0 (SPSS Inc.

2000) with α=0.05, and all statistical assumptions were met.

Results

Harbor seals ranged in age from a fetus to 21 years old. The average percent error

of harbor seal age estimates was 5.6%, the coefficient of variation was 14.6%, and

precision was 1.8% for R1. R2's age estimates resulted in an average percent error of

5.2%, a coefficient of variation of 16.6%, and a precision of 0.7%. Relative differences

between readers appeared normally distributed with no differences between estimates

with some estimated ages differing by +3 or -2 (Fig. 3). The age-bias regression also

indicates a normal variation around the 1:1 equivalence line indicating variation in age

estimates were not skewed toward one reader (Fig. 4).

Mean concentrations of trace elements (THg, MeHg, Se, and Pb) and stable

isotope signatures (δ^{15}N and δ^{15}C) in blood, hair, and liver of harbor seals from central

and northern California were partitioned based on age (Table 1). Approximately 5% (9

values out of 175) of THg blood concentrations were less than the lowest standard of the

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instrument but greater than the MDL; whereas, 25% (10 values out of 40) of Pb liver

concentrations were less than the MDL. It should be noted there is greater uncertainty

with these values because they were slightly outside the QA/QC range, but they also were

so close to "zero" that inclusion or exclusion of these data do not significantly alter the

associated statistics.

THg concentrations in blood differed significantly among age class (P<0.001) but

not location (P=0.307, Table 2). Mean (± SE) blood THg concentrations in pups (0.054 ±

0.008) was significantly less than juveniles (0.259 ± 0.068, P<0.001) and adults (0.258 ±

0.037, P<0.001). To further distinguish gender differences, the ANOVA was reanalyzed

using only the Pt. Reyes dataset partitioned into gender and age class. There was no

significant difference in THg blood concentrations based on gender (P=0.322) for the Pt.

Reyes dataset, but there was a significant difference among age classes (P<0.002).

Significant linear relationships were determined for THg concentrations in blood

based on length (Fig.5a), girth (Fig. 5b), and mass (Fig. 5c); however, the relationships

were not strong. All of the linear relationships had r^2 around 0.08, which implied 8% of

the variability of THg blood concentrations was explained by the three independent

variables. All linear regression slopes were significantly greater than 0 (P<0.001).

These three morphometric measurements increase as the animals' age increases and are

the closest approximation for age that can be assessed in live harbor seals without

collecting a tooth.

There were no significant differences in mean concentrations of THg in hair between dead and live animals (P=0.907, Table 3). To achieve 80% statistical power, 147 samples were needed for each treatment but only 53 dead and 186 live harbor seal samples were collected; therefore, there were not enough samples from dead animals to test for effects. There was an effect of age (P=0.010), Mean (± SE) THg concentrations in hair of adults (15.11 ± 1.17) was significantly greater than pups (8.20 ± 0.61, P<0.001) and juveniles (9.87 ± 0.73, P=0.002).

The assumptions of the Model I, three-way ANOVA were met after concentrations of THg in hair were log transformed. There were no significant differences in mean THg concentrations in hair based on location (P=0.619) or sex (P=0.993, Table 4). Mean (± SE) THg concentrations in hair for pups (7.30 ± 0.64) was significantly less than non pups (12.27 ±0.55, P<0.001). For the hair ANOVA partitioned into age class and locations (except Monterey Bay), all assumptions were met without log transforming data. With dead and live animals pooled, there were no significant differences in concentrations of THg in hair amongst seals in San Francisco Bay, Pt. Reyes, and Humboldt (Table 5, P=0.092), but there was a significant difference among age classes (P<0.001). Mean (± SE) concentrations of THg in hair of adults (12.49 ± 0.85) was significantly greater than pups (7.94 ± 0.67, P=0.003) and juveniles (8.79 ± 0.68, P=0.02). Gender differences were assessed by analyzing log transformed THg concentrations in hair of adults with pooled data of live and dead individuals and

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location. Males (18.03 ± 2.15, mean ± SE) had significantly greater concentrations of THg in hair than female harbor seals (13.13 ± 1.28, P<0.003).

After removal of two outliers, linear regressions were conducted to determine relationships between THg concentrations in hair and morphometrics. THg concentrations in hair were significantly related to length (Fig. 6a), girth (Fig. 6b), and mass (Fig. 6c); the relationships, however, were not strong. The greatest r^2 was 0.158; therefore, only 15.8% of the variability in THg concentrations in hair could be explained by the independent variables. All slopes of the linear regressions were significantly different from 0 (P<0.001). THg concentrations in hair had the strongest relationship with girth; whereas, the relationship between THg concentrations in hair and mass was weakest. There was a significant relationship between concentrations of THg in hair and in blood (Fig. 7, P<0.001), which was stronger (r^2=0.324) than the prior blood and hair regressions that were based on morphometrics.

Assumptions of Model I, two-way ANOVAs were met after THg and Se liver data were log transformed. THg (Table 6), MeHg (Table 7), Se (Table 8), % MeHg (Table 9) concentrations in the liver differed significantly based on age class (P<0.001, P=0.004, P<0.001, and P<0.001) but not sex (P=0.140, P=0.097, P=0.097, and P=0.511). Nonpups had significantly greater THg, MeHg, and Se concentrations in their livers than pups (Table 1); whereas, pups had significantly greater % MeHg than nonpups. In all of these instances the majority of nonpups were mature adults. No significant differences were determined for Pb concentrations, δ^{13}C, or δ^{15}N in liver based on age or sex

(P>0.05). Pb statistical analyses were calculated without a 62.06 ppm ww outlier, a harbor seal that died of acute Pb toxicity.

Liver concentrations of THg were dependent on trophic position as determined by δ^{15}N (GLM, P<0.007) and age (P<0.001). Increased δ^{15}N was related to increased concentrations of THg in liver. MeHg and Se concentrations in the liver were dependent only on age (P=0.011 and P<0.001), not trophic position (P=0.335 and P=0.117). Once again, sex was not a significant factor for differences in THg, MeHg, or Se concentrations in liver. To determine if moderate to severe histological findings in liver affected THg concentrations or % MeHg concentrations, a Fisher exact test was used. Neither THg concentrations nor % MeHg concentrations were related to the presence or absence of lesions in the liver (P=0.167 and P=0.283), but a lack of significance probably was an artifact of minimal sample size and low power.

There were significant linear relationships between THg (r^2=0.7047, P<0.001) and Se (r^2=0.7129, P<0.001) concentrations in liver and age (Figs. 8 and 9); whereas, a significant exponential relationship existed between MeHg (r^2=0.3335, P<0.001) concentrations in the liver and age (Fig. 10). Concentrations of MeHg in liver increased exponentially reaching an asymptote around 1.3 ppm ww.   The relationship between % MeHg and age (r^2=0.7961, P<0.001) was best modeled as a decay function (Fig. 11). As harbor seals aged, % MeHg in the liver reached a minimum after approximately 4 years of age.

There were significant relationships between THg, MeHg, and Se concentrations in the liver. MeHg concentrations in the liver (r^2=0.6725, P<0.001) increased exponentially with THg concentrations reaching an asymptote around 2 ppm ww (Fig. 12). There was a significant linear relationship between molar THg and Se concentrations in liver (r^2=0.9917, P<0.001; Fig. 13), with a slope approaching one (β=1.07) but not equal to one (t-test, P<0.001). The molar ratio of Se to THg of 1.88 was significantly different from one (t-test, P<0.001).

There was a weak relationship between Hg concentrations in hair and liver. Concentrations of THg in hair increased exponentially with concentrations of THg in liver (r^2=0.267, P<0.001; Fig. 14) and MeHg in liver (r^2=0.4812, P<0.001; Fig. 15). A stronger relationship existed between concentrations of THg in hair and MeHg in livers.

Most harbor seals sampled had δ^{15}N and δ^{13}C values near mean values of each isotope; however, two outliers exist (Fig. 16). One harbor seal (HS 1) was feeding one trophic level less than the mean; whereas, the other harbor seal (HS 2) was feeding one trophic level greater than the mean. THg (r^2=0.2952, P<0.001) and Se (r^2=0.2162, P<0.001) concentrations in liver increased significantly with δ^{15}N in pups (Figs. 17a and 17b); whereas, minimal data were available to determine these relationships in adults. MeHg (r^2=0.1745, P<0.001) concentrations in the liver increased significantly with δ^{15}N using pooled age classes (Figs.18). Additionally, δ^{15}N decreases significantly (r^2=0.1453, P<0.001) with increasing % MeHg (Fig. 19).

Discussion

Trace Element Concentrations in Various Tissue Types

THg concentrations in blood are not routinely measured in wild marine mammals. Mean concentrations of THg in blood of harbor seals in this study were greater than values previously published (Kopec and Harvey 1995; Moser 1996). These prior studies also were completed in the eastern Pacific, with sampling sites in the San Francisco and Monterey Bay areas. THg concentrations in sportfish in San Francisco Bay have been determined (Fairey et al. 1997; Thompson et al. 2000; Davis et al. 2002) but not compared through time. Trace metal concentrations in benthic and pelagic schooling fish that comprise the majority of harbor seal's diet have yet to be investigated. Because valuable data on Hg concentrations in harbor seal prey species from this area is lacking, it would be impossible to determine if greater concentrations of THg in blood from the present study represents an increase in MeHg in the prey. These apparent differences also may be caused by differences in sampling design and overall sample size. More samples were collected during a shorter timeframe in a more confined area in the present study than in two previous studies.

Mean THg concentrations in hair were less than previously published values (Wenzel et al. 1993; Moser 1996). The maximum concentration of THg in hair, however, was greater than that reported by others (Sergeant and Armstrong 1973; Freeman and Horne 1974; Wenzel et al. 1993; Moser 1996), which may be an artifact of sample size or

that some individuals recently were exposed to greater amounts of Hg than reported previously.

Hg concentrations in the liver of harbor seals have been measured extensively. Mean THg concentrations in liver of harbor seals from central California are within the mid range of previously published values (Gaskin et al. 1973; Heppleston and French 1973; Koeman et al. 1973; Sergeant and Armstrong 1973; Anas 1974; Holden et al. 1975; Koeman et al. 1975; Roberts et al. 1976; Drescher et al. 1977; van de Ven et al. 1979; Reijnders 1980; Calambokidis et al. 1984; Himeno et al. 1989; Skaare et al. 1990; Law et al. 1991; Frank et al. 1992; Law et al. 1992; Miles et al. 1992; Skaare et al. 1993; Olsson et al. 1994; Lake et al. 1995; Moser 1996; Table 10). Mean Se concentrations in liver of harbor seals from central California, however, were greater than most previous studies (Himeno et al. 1989; Skaare et al. 1990; Frank et al. 1992; Miles et al. 1992; Skaare et al. 1993; Olsson et al. 1994; Moser 1996) except for harbor seals in Wadden Sea (Koeman et al. 1973; Koeman et al. 1975; Reijnders 1980; Table 10). Mean THg and Se concentrations in livers of harbor seals in this study also were greater than concentrations associated with toxicity in terrestrial animals (>50 ppm ww for THg and >7 ppm ww for Se; Puls 1994). It is interesting to note, that mean concentrations of THg (62.9 ± 15.1, mean ± SE) and Se (23.9 ± 5.4) in livers of harbor seals in this study were greater than a prior study completed in the same sampling locales (43.7 ± 17.7 and 3.9 ± 2.1; Moser 1996). Fewer researchers have investigated MeHg concentrations in harbor seals. Mean MeHg concentrations in the present study were generally greater than those published for

harbor seals on the east coast of North America (Gaskin et al. 1973) and less than those published for harbor seals in the Wadden Sea (Reijnders 1980).

Harbor seals in the present study had lesser baseline Pb concentrations than previously reported (Holden et al. 1975; Roberts et al. 1976; Drescher et al. 1977; Duinker et al. 1979; Calambokidis et al. 1984; Law et al. 1991; Frank et al. 1992; Law et al. 1992; Moser 1996). One harbor seal, however, was removed from all statistical analyses because it had the greatest Pb liver concentration reported and subsequently died of acute Pb toxicity (Tzabka et al. in press).

Influence of Age

Age-related differences in concentrations of THg in blood and hair corroborated past work that indicated older harbor seals had greater concentrations of THg in their liver and kidneys than younger harbor seals (Heppleston and French 1973; Anas 1974; Koeman et al. 1975; Roberts et al 1976; Reijnders 1980; Himeno et al. 1989; Skaare et al. 1990; Frank et al.; 1992; Miles et al. 1992; Skaare et al. 1994). Newly weaned pups are not as efficient at foraging as adults, and pups tend to consume more crustaceans than older, mature harbor seals (Oates 2005). THg concentrations in crustaceans should be less than in fish because fish are at a higher trophic level than crustaceans (Cappon and Smith 1982; Bargagli et al. 1998; Muir et al. 1999; Horn and Ferry-Graham 2006). It follows that adult harbor seals should have greater concentrations of THg in blood and hair than pups. Juveniles, on the other hand, had similar concentrations of THg in blood

as adults and similar concentrations of THg in hair as pups. Juveniles, consisting of

yearlings and subadults, probably forage on similar prey species as adults and feed on

more prey types than newly weaned pups (Oates 2005), which would explain similar

concentrations of THg in blood of juveniles and adults. Greater THg concentrations in

hair, however, would be expected in adults compared with pups or juveniles. Because

THg bioaccumulates through time and MeHg is slowly excreted from the body (Berglund

and Berlin 1969; Goyer 1991), pups and juveniles have a lesser overall burden of THg

because they have been exposed to MeHg for a shorter timeframe than mature adults.

Pups and juveniles, therefore, should have lesser concentrations of THg incorporated into

their hair than adults.

Numerous researchers have determined a positive relationship between

concentrations of THg, MeHg, or Se in liver and age in harbor seals (Heppleston and

French 1973; Anas 1974; Koeman et al. 1975; Roberts et al 1976; Reijnders 1980;

Himeno et al. 1989; Skaare et al. 1990; Frank et al. 1992; Miles et al. 1992; Skaare et al.

1994). The continuous uptake of THg (i.e. MeHg) via prey, slow elimination, storage,

and relatively long half-life of THg (Wagemann et al. 2000) explain this positive

relationship. Although the half-life of whole body MeHg is approximately 500 days in

ringed seal (Tillander et al. 1972), MeHg concentrations become asymptotic in older

individuals with age, which indicates animals reach an equilibrium when enhanced

detoxification capabilities balance new pollutant intake (Aguilar et al. 1999). Because

MeHg can be demethylated, % MeHg in the liver is greater in pups and decreases to a

minimum of less than 10% at 4 years of age and then remains constant with increasing age. In the livers of younger animals, the greater % MeHg suggests that the demethylation process was not yet well developed (Caurant et al. 1996); whereas, various researchers of pinnipeds have determined comparable % MeHg levels between 5 and 15% in adults (Freeman and Horne 1973; Koeman et al. 1973; Koeman et al. 1975; Gaskin et al. 1973; Roberts et al. 1976; Reijnders 1980; Dietz et al. 1990; Woshner et al. 2001b; Dehn et al. 2005). These lesser values of % MeHg in adults indicate dietary uptake of MeHg in harbor seals remaining in equilibrium with physiological detoxification processes (Dehn et al. 2005).

Mammalian fetuses, however, are exposed to MeHg through direct placental transfer from the mother (Berglund and Berlin 1969; Suzuki et al. 1967; Jones et al. 1976; Chang and Reuhl 1983; Itano et al. 1984; Suzuki et al. 1984; Jernelov 1986; Wagemann et al. 1988; Hansen 1991; Aguilar et al. 1999; Watanabe et al. 1999; Ask et al. 2002; Watanabe et al. 2002). Two fetuses (4-month and full-term) were opportunistically examined in this study. A 4-month old fetus had minimal amounts of THg in its liver, but % MeHg was approximately 100%. The THg concentrations in the amniotic sac and total body (minus liver) of the 4-month old fetus were an order of magnitude greater than the fetal liver, and % MeHg also was approximately 100% for both the amniotic sac and total body. In the liver of the full-term fetus, THg concentration was 60 times that of the liver of the 4-month old fetus, but % MeHg (65.3%) was less than in the liver of the 4-month old fetus. Fetuses in early gestational stages do not have

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fully matured and functioning organ systems, including the liver (Beath 2003, Teitelbaum 2003). Detoxifying and demethylating mechanisms within the fetus, therefore, probably were not operating at early gestational stages, but were beginning in the full-term fetus. IHg accumulates in the placenta but is not necessarily transferred in large concentrations into the umbilical blood; whereas, MeHg readily crosses the placental barrier and accumulates in the developing fetus (Ask et al. 2002). These embryonic stages of development are crucial to the survival of the fetus. MeHg suppresses immune responses in the embryo and neonate of mice during their critical periods of development (Koller, 1979), which may have detrimental long-term effects on the animal. Lastly, the mother of the 4-month old fetus had a greater % MeHg (7.48%) than the mother of the full-term fetus (2.28%). The mother in the latter gestational stage should be expending more energy and providing more nutrition (Trivers 1974; Thompson and Nicoll 1986; Crespi and Semeniuk 2004), thus, more MeHg to a full-term fetus than the mother in an early gestational stage, which could account for the difference in % MeHg in the mothers.

Influence of Sex

No significant differences were determined between sexes in THg concentrations of blood. Harbor seals lack a marked sexual dimorphism and do not fast during lactation (Bigg 1969; Riedman 1990; Reeves et al. 1992); therefore, animals at any specific age of either gender should be ingesting the same relative amounts of prey, i.e. MeHg. Significant differences based on gender in THg concentrations of hair for mature, adult

harbor seals were expected and determined when locations were pooled. Adult male harbor seals were expected to have greater concentrations of THg (i.e. MeHg) in their hair than adult females, because females transfer MeHg to their pups via gestation and minimally via lactation (Jones et al. 1976; Reijnders 1980; Wagemann et al. 1988). Both sexes slowly eliminate Hg in urine and bile, but Hg in bile can be reabsorbed in the intestines or eliminated via feces (Klaassen and Rozman 1991). A significant source of removal of THg for males then would be sequestering it in growing hair follicles; similar gender differences have been postulated for humans (Bencko 1991). Because there were overall more females (n=133) than males (n=85) sampled in this study testing gender differences was more difficult especially when only a portion were mature adults. Given a more equal sample size of adult females and males, gender differences would likely be determined for the entire dataset.

THg, MeHg, and Se concentrations in the liver did not differ based on gender. Several studies have indicated differences in Hg concentrations between sexes because mature, females excreted THg, mainly in the form of MeHg, across the placenta into the developing fetus (Berglund and Berlin 1969; Suzuki et al. 1967; Chang and Reuhl 1983; Suzuki et al. 1994; Wagemann et al. 1988; Hansen 1991; Aguilar et al. 1999; Watanabe et al. 1999; Ask et al. 2002; Watanabe et al. 2002). There also were no differences in % MeHg based on gender in this study. There were no differences in trace element concentrations in liver based on gender because sample size was minimal for mature adults (n=12), especially adult males (n=2). Recovering carcasses of fresh dead, adult

male harbor seals was problematic in central California. Neonates and weaners more commonly stranded than mature animals (Aguilar et al. 1999). Fewer mature males were recovered because adult animals were not washing up in areas where the public frequents or they were in poor condition once they stranded. Rarely were adult male harbor seals brought into rehabilitation facilities, live or dead.

Influence of Location

Significant site-based differences in THg concentrations of blood and hair were not found. This is surprising because THg concentrations in sediment and other faunal species differ among these four sites (Downing et al.. 1998; Hunt et al. 1998; Jacobi et al. 1998). Concentrations of THg in blood and hair of harbor seals at a fixed trophic level, therefore, were expected to differ among sites. Tomales and San Francisco Bays, CA, have a considerable amount of Hg cycling in their ecosystems due to runoff from rivers in close proximity to abandoned cinnabar mines; specifically, the presence of the New Almaden mine drains into South San Francisco Bay (Bradley 1918; Johnson 1963). Sections of South San Francisco Bay also are considered a semi-enclosed estuary that is not tidally flushed and has a great hydraulic residence time (Flegal et al. 1991). Hg concentrations in sediments and unfiltered water of San Francisco Bay are greater downstream of Hg mining activities, especially in areas with greatest wetland land cover (Domagalski 2001). Enrichment of Hg in a benthic species commonly has been

accompanied by enrichment throughout the benthic community (Luoma and Phillips 1988).

Prey from these areas should contribute to increased concentrations of MeHg and should constitute the majority of the seal's THg burden. Fish, oysters, and birds had increased concentrations of Hg in San Francisco and Tomales Bays compared with other bays and estuaries (Ohlendorf et al. 1988; Martin et al. 1993; Davis et al. 2002). In San Francisco Bay, shiner surfperch (*Cymatogaster aggregata*) and white croaker (*Genyonemus lineatus*) had levels of THg in their muscle/skin, ranging from 0.146 to 0.420 µg/g wet weight (Fairey et al. 1997). Jack smelt (*Atherinopsis californiensis*) also had levels of THg ranging from 0.20 to 0.27 µg/g wet weight (Thompson et al. 2000). All THg concentrations were greater than the EPA screening value of 0.23 µg/g wet weight. Benthic fishes in Tomales Bay also were expected to have increased levels of Hg, however, they may be slightly less than those found in San Francisco Bay. Tomales Bay has only one major source of Hg contamination from Walker Creek (Hornberger et al. 1999); whereas, San Francisco Bay has multiple sources of Hg contamination from many tributaries and rivers that drain from the Sierra Nevadas through the Central Valley. Fluxes of trace elements originating from the delta and smaller creeks indicate that these inputs have increased background concentrations in the estuary upon which the effects of local anthropogenic inputs were superimposed. This should cause increased Hg concentrations in fish and shellfish (Luoma and Phillips 1988) and, therefore, increased THg concentrations in harbor seals.

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The Humboldt area, conversely, has or had pulp mills, petroleum plants, fossil fuel and nuclear power plants, and coal and oil gasification plants that add pollutants to the ecosystem. Although Hg emissions may be lesser and from different sources than in central California, Humboldt Bay has a narrow opening to the Pacific Ocean causing circulation and flushing to be severely restricted, resulting in high potential for pollutant deposition (Jacobi et al. 1998). Although flushing is severely restricted, San Francisco Bay should have greater Hg levels than Humboldt because more Hg contamination drains and is retained in the San Francisco Bay estuary.

Lesser concentrations of THg were expected in harbor seals in Monterey Bay, because there is only one known Hg mine at New Idria (Bradley 1918) possibly contaminating the Pajaro and Salinas Rivers that flow into this bay. Monterey Bay has the largest opening to the Pacific Ocean of the four sites so with increased flushing it could feasibly sustain more industrial pollution with lesser effects. Previous research also has determined that only one site, Santa Cruz Yacht Harbor, had increased levels of Hg in the sediment (Downing et al. 1998) compared with NOAA guidelines. The other sampling locations had multiple sites with increased levels of Hg in the sediment; whereas, Monterey Bay had one site with increased levels of Hg in the sediment. With an increase in sample size per location (blood n=102 and hair n=145) and equal sampling of age and sexes (blood n=154 and hair n=92), power of statistical tests would have increased to 80% and significant site differences may have been determined. To determine location differences in THg concentrations in blood, at least 102 blood samples

will be needed at each location partitioned equally amongst age classes without taking into account gender because animals of the same age should be ingesting the same type and amount of prey no matter their sex. To determine location differences in THg concentrations in hair, however, at least 145 blood samples will be needed for each gender partitioned equally amongst age classes at each location.

Some of the southerly sites were within 50 km of one another. The distance between the two extreme sampling sites, Monterey Bay and Humboldt, also were relatively short, approximately 560 km. There was a possibility of movement of harbor seal among all areas sampled. Radio-tag data from the same sampling timeframe has indicated that harbor seals from Monterey Bay and Humboldt remained in their prospective areas; whereas, some harbor seals from San Francisco Bay and Pt. Reyes traveled greater distances (Harvey and Goley 2005). A few harbor seals (n=7) tagged in Tomales Bay traveled south to the Marin Headlands and Año Nuevo Island; whereas, a few seals (n=4) tagged in San Francisco Bay traveled north to Pt. Reyes and south to Half Moon Bay (Harvey and Goley 2005). Harbor seal prey also have the ability to migrate great distances. Pelagic schooling fish, which are at a lower trophic level than benthic fish (Horn and Ferry-Graham 2006) may have constituted more of the seal's overall diet during this sampling period than more sedentary benthic fish. Because benthic fish are sedentary, they reflect Hg contamination of a distinct locale; whereas, pelagic schooling fish are more mobile and would have a Hg contamination based on various

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locales. Location differences, therefore, may not be easily determined because either the seals or prey are moving among sampling locations.

Tissue Comparisons

There was a significant positive relationship between THg concentrations in blood and hair. It is important to note, however, THg concentrations in these hair samples were indicative of the bioavailable Hg during their moult, summer of 2003, not 2004 when most animals were sampled. The amount of THg sequestered in the hair is dependent on amount of Hg cycling in the ecosystem, bioaccumulated in prey species, consumed as prey, retained in blood, and sequestered into the hair of harbor seals through the growing follicle during moult. Hair may function as an excretory tissue for harbor seals because accumulation of metals in this keratinized structure indicates considerable amounts of toxic substances can be removed by the blood and retained in the hair (Wenzel et al. 1993; Wiig et al. 1999).

There were significant positive relationship between concentrations of THg in hair and concentrations of THg and MeHg in liver. THg in hair was comprised primarily of MeHg and was more related to MeHg concentrations in liver than THg concentrations in liver. It had been proposed, though, that THg concentrations in seal fur could be used as a general indicator of the extent of Hg contamination in the animal (Freeman and Horne 1973). The indicator function for metal pollution in hair alone, however, could be determined by comparing seal hair between two moults (Wenzel et al. 1993), which did

not occur in this study because lack of sample size for a moult other than summer of

2003. A more significant relationship between THg concentrations in fur and blood or

other internal tissues likely could be found if sampled shortly after the old fur has

moulted, and the new fur has formed (Watanabe et al. 1996).

Influence of Trophic Level

^{15}N enriches with increasing trophic levels and thus reflects trophic position;

whereas ^{13}C indicates sources of primary productivity (DeNiro and Epstein 1981;

Minagawa and Wada 1984; Peterson and Fry 1987; Hobson and Welch 1992; Hobson et

al. 1996). The majority of harbor seals from this study had comparable δ^{15}N and δ^{13}C

and subsequently were feeding at the same trophic level, only two outliers were found.

One animal was probably feeding a trophic level greater than the mean, and gross

necropsy and histological results indicated that this animal died of adenosquamous

genital carcinoma. Ninety-five percent of the liver was infiltrated with cancer, and

Kupffer cell and hepatocellular hemosiderosis were present. Pathways by which proteins

are taken up and digested in lysosomes are non-selective and inducible, being active

primarily under circumstances of nutrient deprivation or stress (Dice and Chiang 1989).

During periods of net protein loss and wasting, protein turnover equates with rates of

protein synthesis (Hawkins 1991); and during periods of partial or complete starvation,

protein turnover is decreased in mammals and humans (McNurlan and Garlick 1989).

Because the major portion of the liver was neoplastic, the harbor seal was likely

catabolizing its own liver as opposed to feeding one true trophic level higher. A comparable stepwise increase in $\delta^{15}N$ of 3.7 $^o/_{oo}$ was found in Arctic char that were practicing cannibalism (Hobson and Welch 1995). Another harbor seal had lesser values of $\delta^{15}N$ and $\delta^{13}C$ and was feeding a trophic level less than the mean. This animal was brought into rehabilitation as a neonate and was fed formula for an entire month before it was euthanized due to complications from pylonephritis and possible aspiration pneumonia. The formula is primarily a zoologic milk matrix, which is probably either corn- or soy-based, and supplemented with salmon oil (TMMC pers. comm.). Because trophic positioning of organisms by stable nitrogen analyses is based on assimilated food of many meals (Tieszen et al. 1983) and liver has a great protein turnover rate on the order of days to weeks (Welle 1999), this animal's $\delta^{13}C$ and $\delta^{15}N$ are probably an artifact of rehabilitation not of typical prey or lactation.

THg concentrations in liver increased with $\delta^{15}N$. The plot was biphasic indicating differences also existed based on age class of individual harbor seals. Differences in concentrations of MeHg and Se in liver also were expected based on $\delta^{15}N$ but were not significant although positive relationships existed. Biomagnification of mercury with trophic level was reported by some researchers (Riisgard and Hansen 1990; Futter 1994; Jarman et al. 1986; Atwell et al. 1998) but not by other researchers (Williams and Weiss 1973; Wagemann and Muir 1984; Atwell et al. 1998; Dehn et al. 2005). These discrepancies seem to be dependent on the type of prey the animal consumes and type of tissue sampled. Some researchers have sampled muscle for stable isotope and Hg

determinations. THg concentrations in the liver, however, were approximately two orders of magnitude greater than muscle (Sergeant and Armstrong 1973) and are dependent on the demethylating efficiency and health status of the animal (Himeno et al. 1989; Dietz et al. 1990; Aguilar et al. 1999; National Research Council 2000; Wiener et al. 2003).

Approximately a third of the livers that were examined histologically had hepatic lesions, but presence of lesions was not significantly related to greater concentrations of trace elements. Previous researchers have found seals with hepatic lesions had a greater relative occurrence of MeHg in liver and elevated % MeHg (Dehn et al. 2005). MeHg can inhibit primary, secondary, and memory immune responses in mammals (Koller 1979). % MeHg decreased linearly with increasing $\delta^{15}N$ in the presence and absence of hepatic lesions. It has been postulated that animals with greater % MeHg were unable to efficiently demethylate MeHg or that the underlying physiological detoxification mechanism was altered (Dietz et al. 1990, Dehn et al. 2005). Many diseases affect metabolism, thus, the capacity of diseased animals to metabolize or excrete pollutants may be less efficient than healthy animals (Aguilar et al. 1999). Detoxification mechanisms may be altered in animals with suppressed immune systems or those with infectious diseases. Few researchers have found that seals affected by epizootics did not have greater concentrations of environmental contaminants or more lesions than those unaffected by epizootics (Frank et al. 1992; Olsson et al. 1994). Biases associated with beachcast animals also may be insignificant in the case of Hg given the wide range of

results obtained (Law et al. 1991). Determining status of trace elements in stranded or

compromised animals may or may not represent normal healthy populations (Simmonds

and Johnson 1989; Joiris et al. 1991; Law et al. 1991; Frank et al. 1992; Olsson et al.

1994; Aguilar et al. 1999; Anan et al. 2002; Dehn et al. 2005). Relationship between

susceptibility to disease and high pollutant levels is highly contested and may be

explained by depressed immunocompetence caused by pollutants, mobilization of

pollutants stored in reserve tissues in individuals thinned by disease (Joiris et al. 1991), or

alterations in physiological functions leading to increased concentrations (Aguilar et al.

1999).

Interaction Amongst Trace Elements

Concentrations of MeHg in liver increased with respect to THg concentrations to

a maximum around 2 ppm. MeHg concentrations are asymptotic, with % MeHg

remaining constant with increasing THg concentrations.   Hepatic lesions had no

statistically significant effect on Hg concentrations, although statistical power was

extremely low. For statistical power of 80%, 121 liver samples were needed to determine

differences in THg concentrations based on hepatic lesions; whereas, at least 1091

samples were needed to determine differences in % MeHg based on hepatic lesions.

Therefore, harbor seals in this study were not compromised in their ability to demethylate

MeHg.   Thereby, an equilibrium has occurred between dietary intake of MeHg from prey

and detoxification processes of MeHg.

Se is an essential element that is involved in enzymatic activity of glutathione

peroxidase (Rotruck et al. 1972), namely in the formation and utilization of glutathione

(Fishbein 1986). Glutathione and cysteine are thiol compounds that act as antioxidants

and radical scavengers (Fishbein 1986). In liver cells, MeHg has a high affinity for

sulfhydryl groups and forms soluble complexes of glutathione, which are secreted in bile

and reabsorbed from the gastrointestinal tract. MeHg also undergoes biotransformation

to divalent mercury compounds (i.e. IHg) in tissues by cleavage of the carbon mercury

bond (Goyer and Clarkson 2001). Because the majority of Hg in liver is IHg (Wagemann

et al. 1988; Dietz et al. 1990), it has been suggested that the liver is a demethylation site

(Caurant et al. 1996). The demethylation mechanism may have evolved in response to

biomagnification of MeHg either in species at high trophic levels or in individuals of the

same species with greatest levels of Hg (Bryan 1984).

After MeHg has been demethylated, IHg forms stable complexes with Se

(Martoja and Berry 1980) associated with glutathione (Goyer and Clarkson 2001),

inhibiting this enzyme by lessening available Se that binds to antioxidants in the cells

(Fishbein 1987). Se, however, may provide marine mammals with protection against

toxicity caused by Hg (Martoja and Berry 1980; Wagemann and Muir 1984), because

tiemmanite (HgSe complex) can be stored indefinitely in the liver of marine mammals

(Nigro and Leonzio 1996). Se was first reported to counteract acute mercuric chloride

toxicity by Parizek and Ostadalova (1967). Numerous researchers have discussed the

protective effect of Se on Hg toxicosis in marine mammals and subsequent association of

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the two trace elements, namely in a molar ratio of 1:1 for THg:Se (Koeman et al. 1973; Koeman et al. 1975; Smith and Armstrong 1978; Itano et al. 1984; Frank et al. 1992; Caurant et al. 1994; Dietz et al. 1995; Dietz et al. 2000; Bustamante et al. 2004). Other researchers, however, have determined a molar ratio deviating from unity (Wagemann and Stewart 1994; Woshner et al. 2001b; Anan et al. 2002; Dehn et al. 2005). Mean molar Se:THg ratio did deviate from one in the present study, but if molar ratios were partitioned age-wise, adults (0.99:1) had a more unified molar Se:THg ratio than pups (2.3:1). It has been proposed that the typical 1:1 molar ratio is only found in adults with greater concentrations of Hg (Dietz et al. 2000; Wagemann et al 2000; Dehn et al. 2005) with pups having a greater molar ratio of Se:THg (Wagemann et al. 1988; Frank et al. 1992).

Dietz et al. (2000) proposed that molar ratios near one could indicate compromised health. Harbor seals with equimolar ratios in this study, however, were no more compromised health-wise based on hepatic lesions than those animals with ratios deviating from one. These elements also may occur in consistent proportions only when a physiologic threshold has been reached or when adherence to this ratio is not physiologically necessary (Woshner et al. 2001b). It has been suggested that a threshold concentration of Se is equivalent to what is physiologically necessary, plus an additional reservoir for which Hg has a far greater binding capacity (Hansen et al. 1990; Krone et al., 1999; Woshner et al. 2001a). Once Hg concentrations exceed baseline Se concentrations around 3 ppm ww, Se is likely to increase in parallel with Hg

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concentrations. Coaccumulation may be a result of compensation by the organism for the depletion of the physiologically essential levels of selenium as mercury is accumulated and linked to selenium present, a normal homeostatic regulation (Beijer and Jernelov 1978). Below this baseline Se level, molar Se:THg deviate from one (Krone et al. 1999). This continued uptake and subsequent binding of THg to Se was observed in adult harbor seals, with deviations from the 1:1 molar ratio only in pups, when overall Se levels were suspected below normal physiological concentrations.

Conclusions

Hg, namely due to anthropogenic sources from gold mining, accumulated in harbor seals in central and northern California via various prey species. THg concentrations in blood and hair of harbor seals increased with age but did not differ among locations in central and northern California. Gender differences were only present in hair of adult harbor seals. Males sequestered more THg, hence MeHg, in hair than females. Hair was a key source of Hg excretion in males; whereas, females excreted Hg via hair, placenta, and milk.

THg and MeHg increased in livers with age and increased with greater δ^{15}N. MeHg can be demethylated into IHg; therefore, % MeHg was at minimum levels in older harbor seals although they had greater THg concentrations than younger harbor seals. Se in the liver also increased with age and was in equilmolar ratios with THg in adults. Molar ratio of Se:THg in pups did deviate from a 1:1 ratio because THg did not bind with

all Se found below baseline physiological concentrations. Harbor seals from central and northern California had significantly greater concentrations of Hg and Se than terrestrial mammals, in some instances these concentrations were considered toxic to terrestrial mammals. Their health, in terms of hepatic lesions, however, did not seem to be weakened by presence of these trace elements. This finding may not represent the population as a whole due to insufficient sample size (i.e. low statistical power).

THg concentrations in blood is useful in determining present day contamination associated with a specific locale; whereas, THg concentrations in hair is beneficial in comparing differences during various moults over approximately the same timeframe each year, which could be valuable in monitoring changes in Hg levels through time. THg, MeHg, and Se concentrations in liver represent long-term exposure and retention with additional insight into proportions and potential binding of these trace elements. Trophic level assessments are advantageous in terms of comparisons among individuals. Nevertheless, overall health status, evaluated using basic blood chemistries, liver function tests, and complete postmortems on all individuals, needs to be assessed to determine if harbor seals are expressing toxic effects of trace metal accumulation. Finally, the appropriate sample size is needed to investigate and determine age, sex, and location affects on trace element concentrations.

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Table 1. Mean (± SE) trace element (ppm wet weight) and stable isotope ($^o/_{oo}$) concentration, range, and sample size in tissues of harbor seals in central and northern California based on age class.

	THg Blood	THg Hair	THg Liver	MeHg Liver	Se Liver	Pb Liver	$\delta^{13}C$	$\delta^{15}N$
Pups	0.0928 ± 0.0235	8.200 ± 0.611	1.444 ± 0.255	0.4491 ± 0.0701	0.400 ± 1.303	0.029 ± 0.008	-17.65 ± 0.29	17.01 ± 0.31
	0.0153 - 0.5545	0.41 - 22.30	0.151 - 7.003	0.0678 - 1.845	0.0477 - 0.7473	<0.007 - 0.174	-21.89 - -14.37	12.37 - 20.18
	n=23	n=63	n=28	n=28	n=28	n=28	n=28	n=28
Juveniles	0.2842 ± 0.0263	9.869 ± 0.732						
	0.0612 - 0.9805	1.52 - 30.73						
	n=46	n=70						
Adults	0.3029 ± 0.0228	15.11 ± 1.17	62.91 ± 15.08	1.211 ± 0.179	23.89 ± 5.35	0.027 ± 0.007*	-17.41 ± 0.52	18.20 ± 0.38
	0.0519 - 1.398	2.38 - 92.78	13.57 - 162.8	0.5064 - 2.852	4.68 - 57.97	<0.007 - 62.06	-21.42 - -15.61	16.81 - 21.70
	n=106	n=106	n=12	n=12	n=12	n=12	n=12	n=12

*denotes mean ± SE calculated without 62.06 ppm wet weight outlier

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Table 2. Model I, two-way ANOVA table for blood THg concentrations with age class and location as fixed factors. Locations were defined as Monterey Bay, San Francisco Bay, Pt. Reyes, and Humboldt; whereas, age classes were defined as pups, juveniles, and adults. SS is sums of squares, DF is degrees of freedom, and P is probability value.

Source of variation	SS	DF	F-ratio	P
Location	0.1581	3	1.2115	0.3074
Age Class	0.7218	2	8.2969	0.0004
Location*Age Class	0.1166	6	0.4469	0.8464
Error	7.1336	164		

Table 3. Model I, two-way ANOVA table for hair THg concentrations with dead or live animals and age class as fixed factors. Age classes were pups, juveniles, and adults.

Source of variation	SS	DF	F-ratio	P
Dead/Live	1.127	1	0.014	0.9070
Age class	764.3	2	4.676	0.0100
Dead Live*Age class	92.54	2	0.566	0.5690
Error	19043	233		

Table 4. Model I, three-way ANOVA table for hair THg concentrations with location, sex, and age class as fixed factors. Locations were Monterey Bay, San Francisco Bay, Pt. Reyes, and Humboldt; whereas, age classes were pups and nonpups. Dead and live animals were pooled for this analysis. THg hair concentrations were log transformed to meet statistical assumptions.

Source of variation	SS	DF	F-ratio	P
Location	0.69845	3	0.59571	0.61850
Sex	0.00003	1	0.00009	0.99256
Age class	8.687	1	22.2276	<0.00001
Location*Sex	0.9244	3	0.78843	0.50163
Location*Age class	0.2575	3	0.21962	0.88271
Sex*Age class	1.30804	1	3.34691	0.06881
Location*Age class*Sex	0.94447	3	0.80554	0.49212

Table 5. Model I, two-way ANOVA table for THg hair concentrations of females with location and age class as fixed factors. Locations were San Francisco Bay, Pt. Reyes, and Humboldt; Monterey has been removed from analysis. Age classes were pups, juveniles, and adults.

Source of variation	SS	DF	F-ratio	P
Location	129.57	2	2.44522	0.09178
Age Class	518.48	2	9.78485	0.00013
Location*Age Class	39.946	4	0.37694	0.82464
Error	2702.4	102		

Table 6. Model I, two-way ANOVA table for THg liver concentrations with age class and sex as fixed factors. Age class was defined as pups and nonpups. THg liver concentrations were log transformed to meet statistical assumptions.

Source of variation	SS	DF	F-ratio	P
Age Class	6971.4	1	26.2427	0.00001
Sex	117.16	1	0.44103	0.51085
Age Class*Sex	110.41	1	0.41562	0.52322
Error	9563.4	36		

Table 7. Model I, two-way ANOVA table for MeHg liver concentrations with age class and sex as fixed factors. Age class was defined as pups and nonpups.

Source of variation	SS	DF	F-ratio	P
Age Class	1.947	1	9.500	0.004
Sex	0.594	1	2.900	0.097
Age Class*Sex	0.085	1	0.414	0.524
Error	7.379	36		

Table 8. Model I, two-way ANOVA table for Se liver concentrations with age class and sex as fixed factors. Age class was defined as pups and nonpups. Se concentrations were log transformed to meet statistical assumptions.

Source of variation	SS	DF	F-ratio	P
Age Class	48.5636	1	171.403	<0.00001
Sex	0.82265	1	2.90352	0.09701
Age Class*Sex	0.31541	1	1.11323	0.29841
Error	10.1999	36		

Table 9. Model I, two-way ANOVA table for % MeHg liver concentrations with age class and sex as fixed factors. Age class was defined as pups and nonpups.

Source of variation	SS	DF	F-ratio	P
Age Class	6971.4	1	26.2427	0.00001
Sex	117.16	1	0.44103	0.51085
Age Class*Sex	110.41	1	0.41562	0.52322
Error	9563.4	36		

Table 10. THg and Se concentrations (means or medians) in livers of harbor seals from present and previous studies partitioned by age and location. Sample size included. Text in red denotes results from present study.

Age	THg Concentrations in Liver (ppm ww)	Se Concentrations in Liver (ppm ww)	n	Locale
pup	1.44	0.747	28	central California[1]
pup	2.91		4	eastern Canada[2]
pup	4.3		3	Britian[3]
pup	8.5		49	Germany[4]
pup	15.95		9	Britian[5]
pup	55.5		2	Netherlands[6]
subadult	0.4	0.92	8	Jarfjord, Norway[7]
subadult	0.44	1.02	10	Sweden[8]
subadult	1.3		1	Washington[9]
subadult	2.42	2.07	10	Sweden[8]
subadult	2.45		2	New Brunswick[10]
subadult	2.96		4	Maine[10]
subadult	3.35	2.74	15	Japan[11]
subadult	3.56	2.04	10	Sweden[8]
subadult	6.77	5.07	11	Vesteralen, Norway[7]
subadult	7.71		2	eastern Canada[2]
subadult	8.4	2.8	6	Netherlands[12]
subadult	10.2	2.6	7	Denmark[12]
subadult	20.6		5	Germany[4]
subadult	23.83		3	Oregon[9]
subadult	26.9		8	Britian[5]
subadult	36		2	Washington[9]
subadult	45.83		3	Britian[3]
subadult	60		1	Britian[13]
adult	0.69	2.79	3	Norway[7]
adult	3.4		2	Maine[10]
adult	4.23		3	Pribilof Islands[9]
adult	6.22	2.54	5	Norway[7]
adult	30.1		2	eastern Canada[2]
adult	32		2	New Brunswick[10]

adult	43.7	17	23	western US[14]
adult	62.9	23.9	12	central California[1]
adult	72.6		12	Britian[5]
adult	83.7		6	Britian[3]
adult	96		4	Germany[4]
adult	152.5		2	Netherlands[6]
adult	214	112	5	Denmark[12]
adult	269		4	San Miguel Islands[9]
adult	293	109	8	Netherlands[12]

[1] This study
[2] Sergeant and Armstrong 1973
[3] Law et al. 1992
[4] Drescher et al. 1977
[5] Law et al. 1991
[6] van de Ven et al. 1979
[7] Skaare et al. 1993
[8] Frank et al. 1992
[9] Anas 1974
[10] Gaskin et al. 1973
[11] Himeno et al. 1989
[12] Reijnders 1980
[13] Holden et al. 1975
[14] Moser 1996
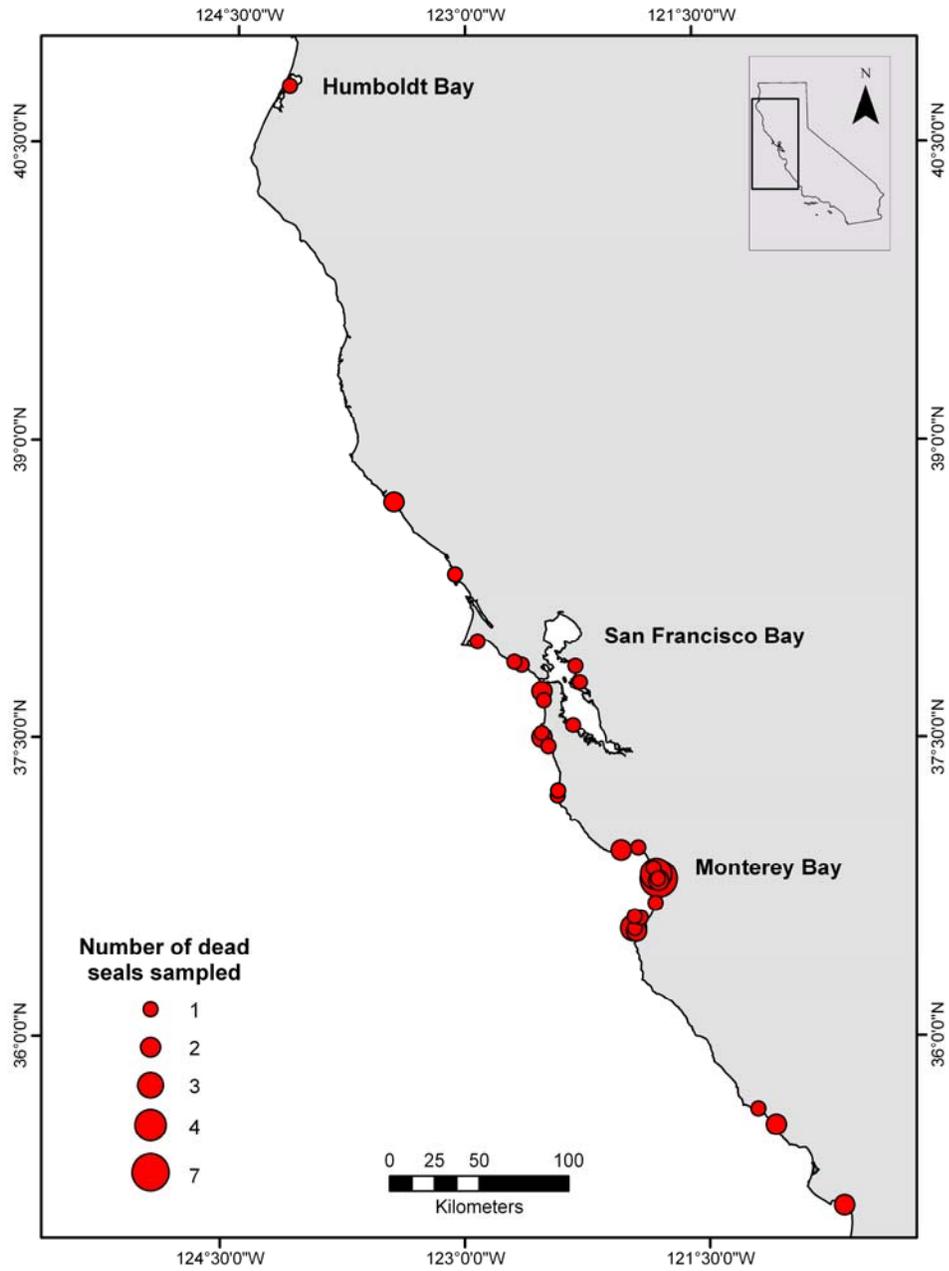
Figure 1. Locations of dead beachcast harbor seals sampled in central and northern California from 2003-2005 for the present study.
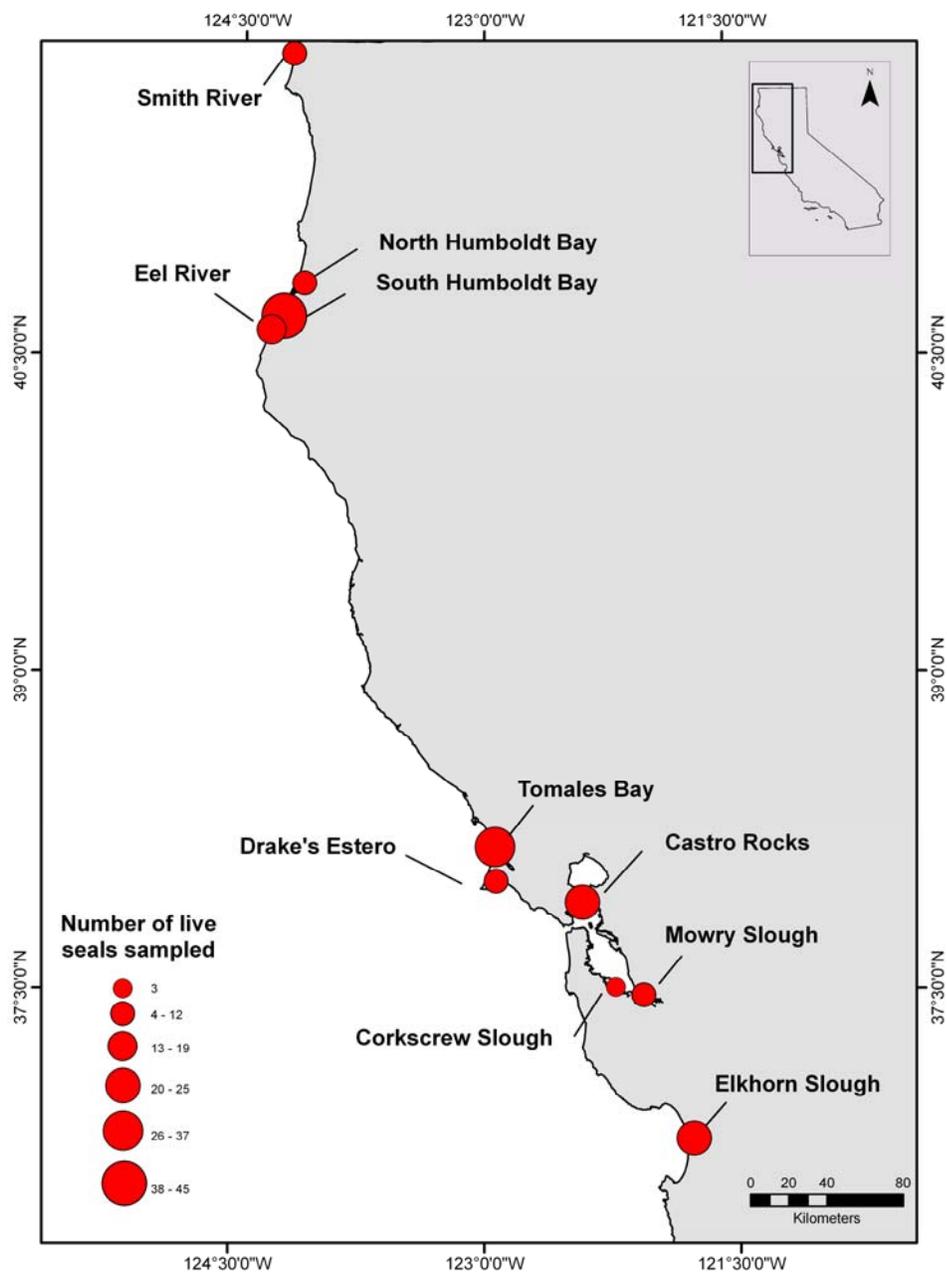
Figure 2. Locations of live harbor seals sampled in central and northern California from 2003-2005 for the present study.
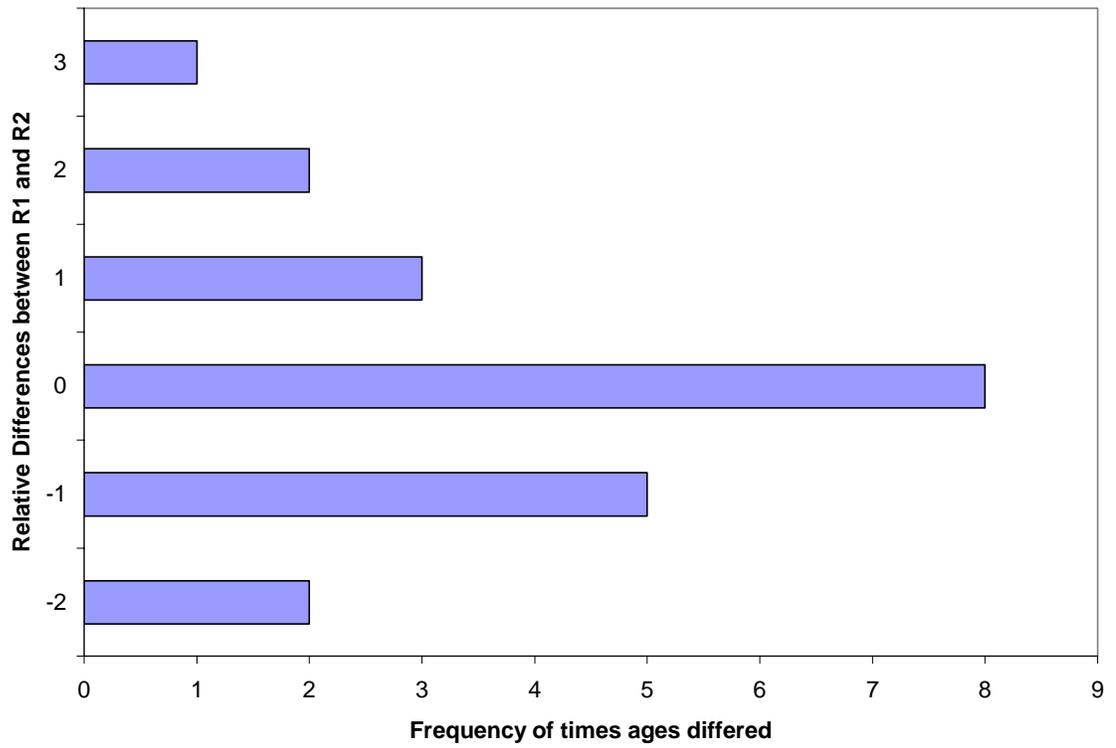
Figure 3. Age precision analysis based on relative difference of estimated ages of harbor seals between reader 1 (R1) and reader 2 (R2) compared with frequency of times that ages differed.
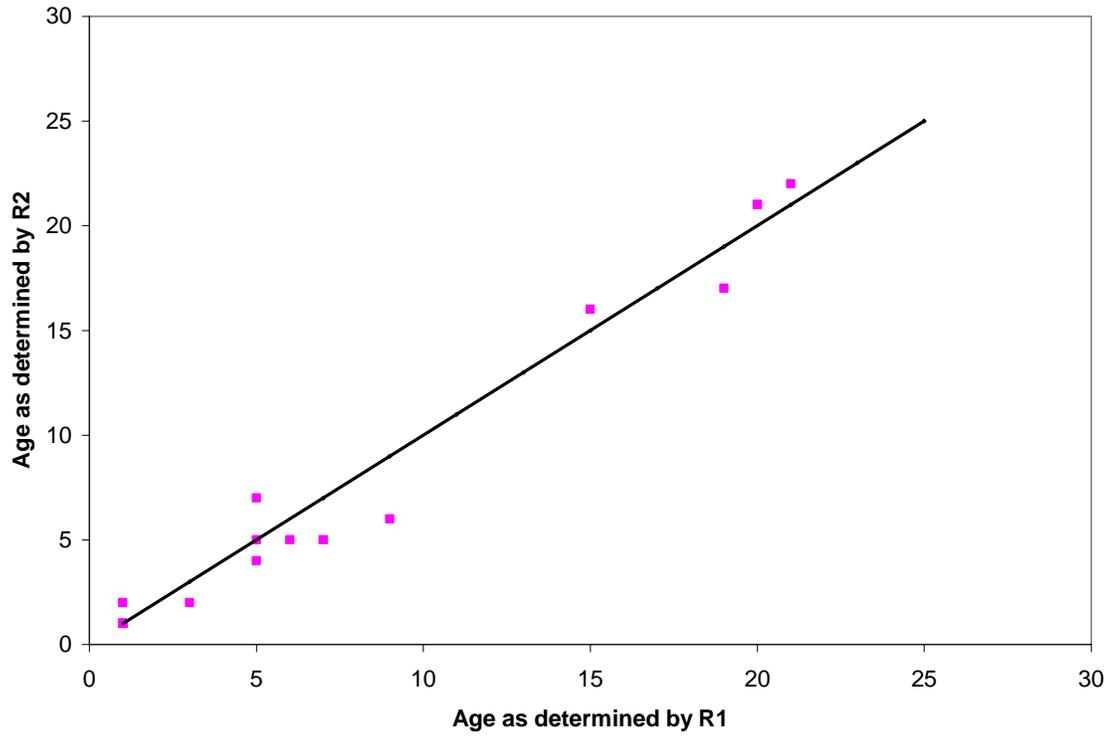
Figure 4. Age-bias curve with 1:1 equivalence line. Points denote estimated age of harbor seals based on both reader 1 (R1) and reader 2 (R2).
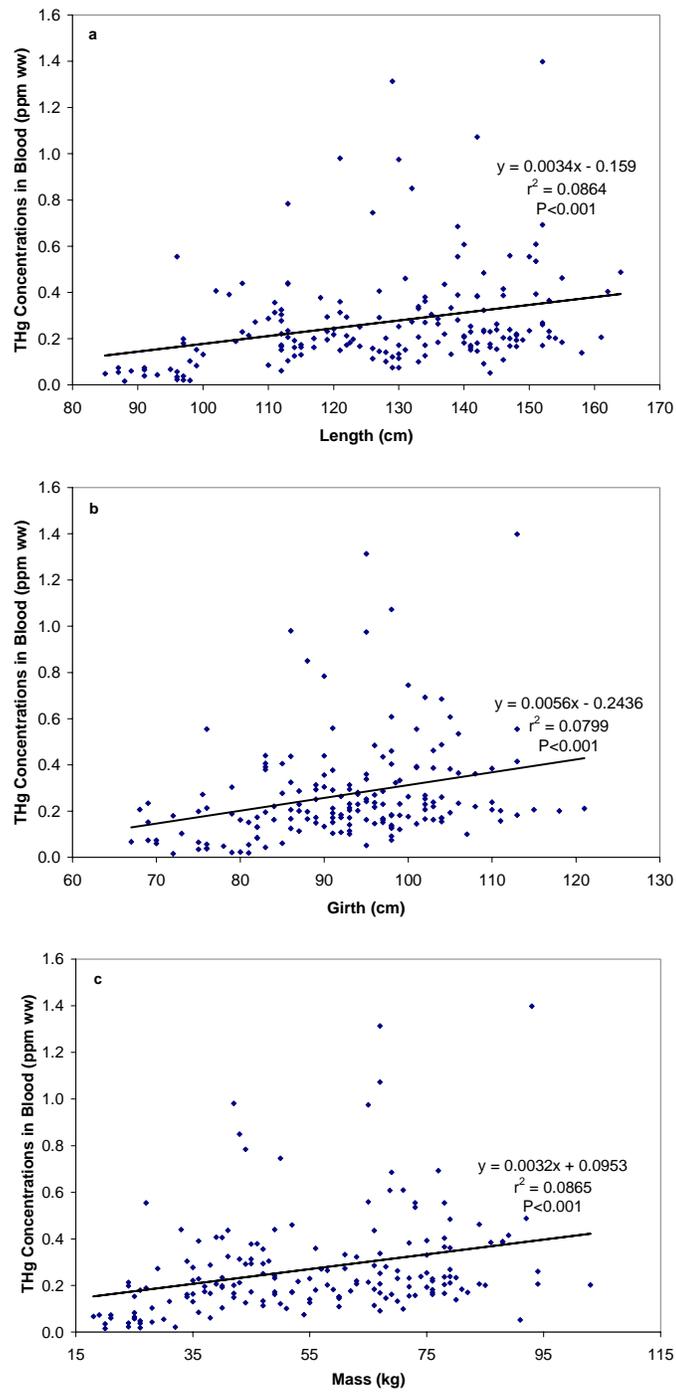
Figure 5. Linear regression of THg concentrations in blood based on length (a), girth (b), and mass (c) of harbor seals from central and northern California.
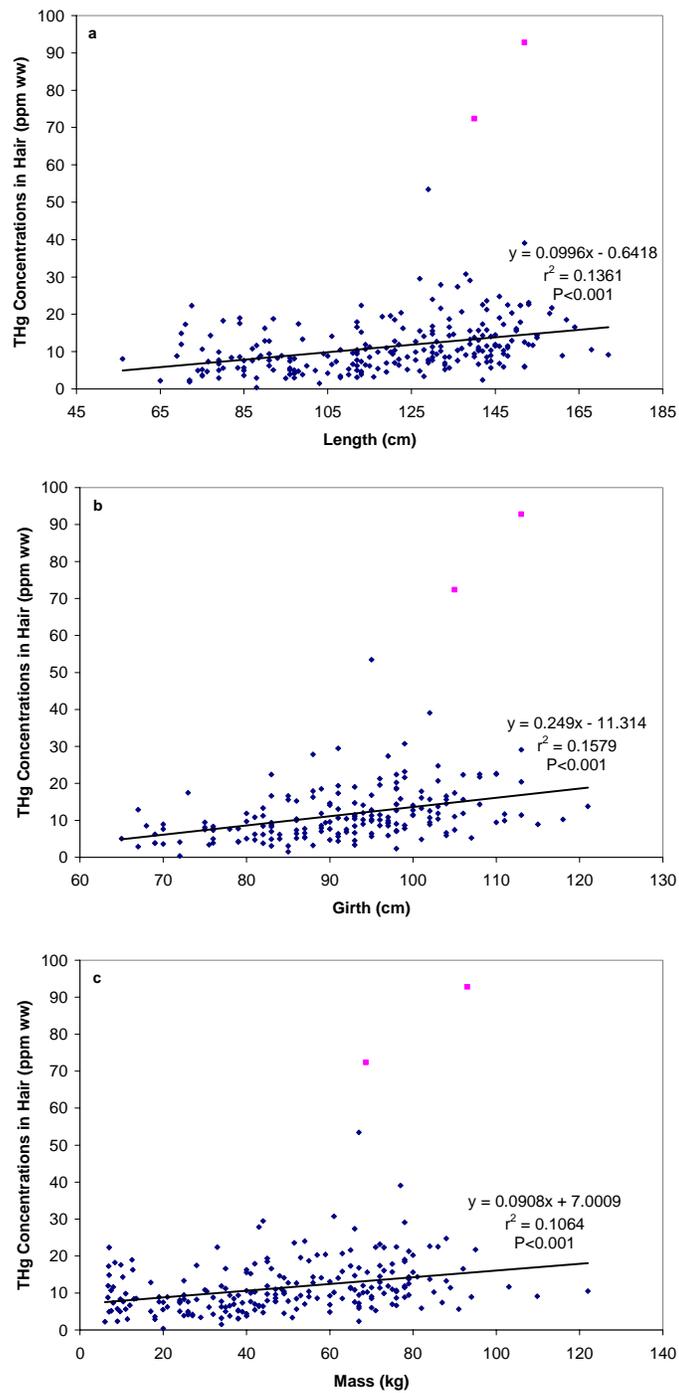
Figure 6. Linear regression of THg concentrations in hair based on length (a),
girth (b), and mass (c) in harbor seals from central and northern California.
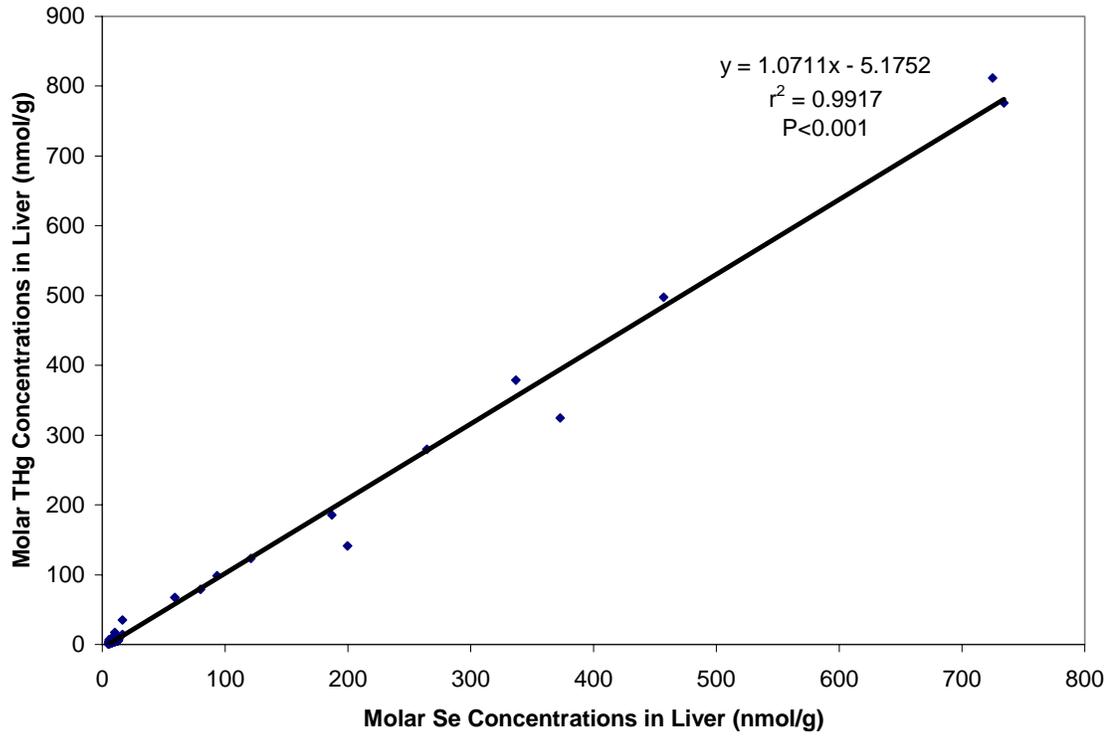Two outliers (red squares) removed from analyses.
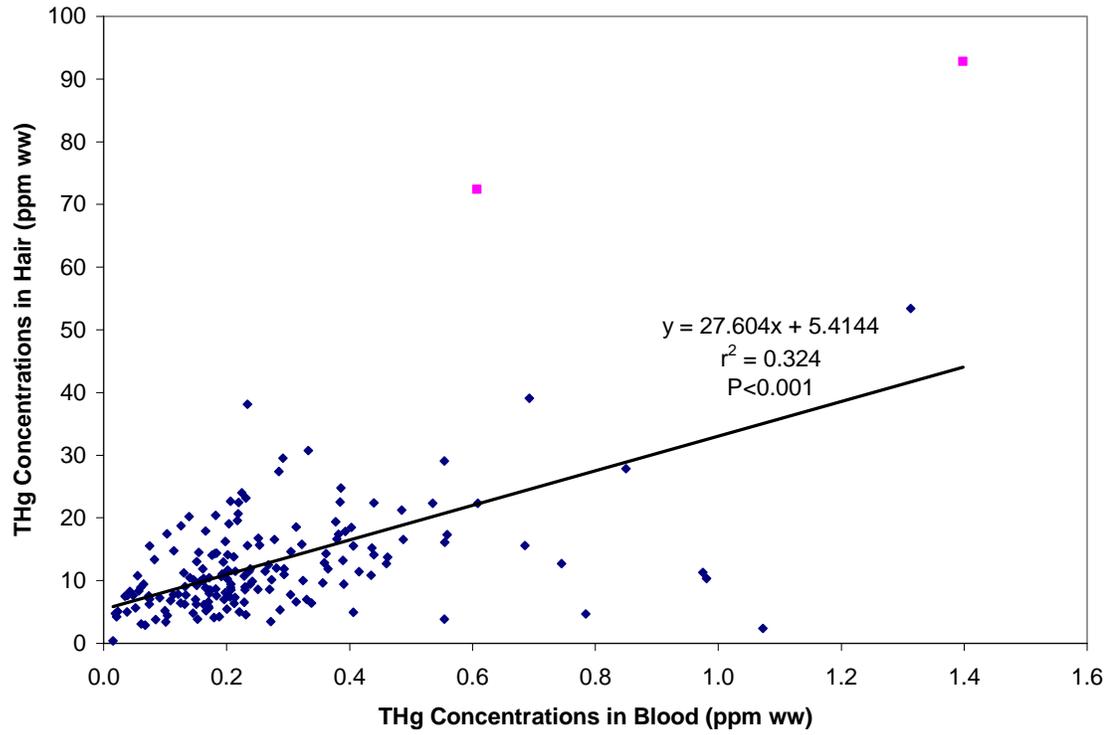
Figure 7. Linear regression of THg concentrations in hair and THg concentrations in blood of harbor seals from central and northern California. Two outliers (red squares) removed from analyses.
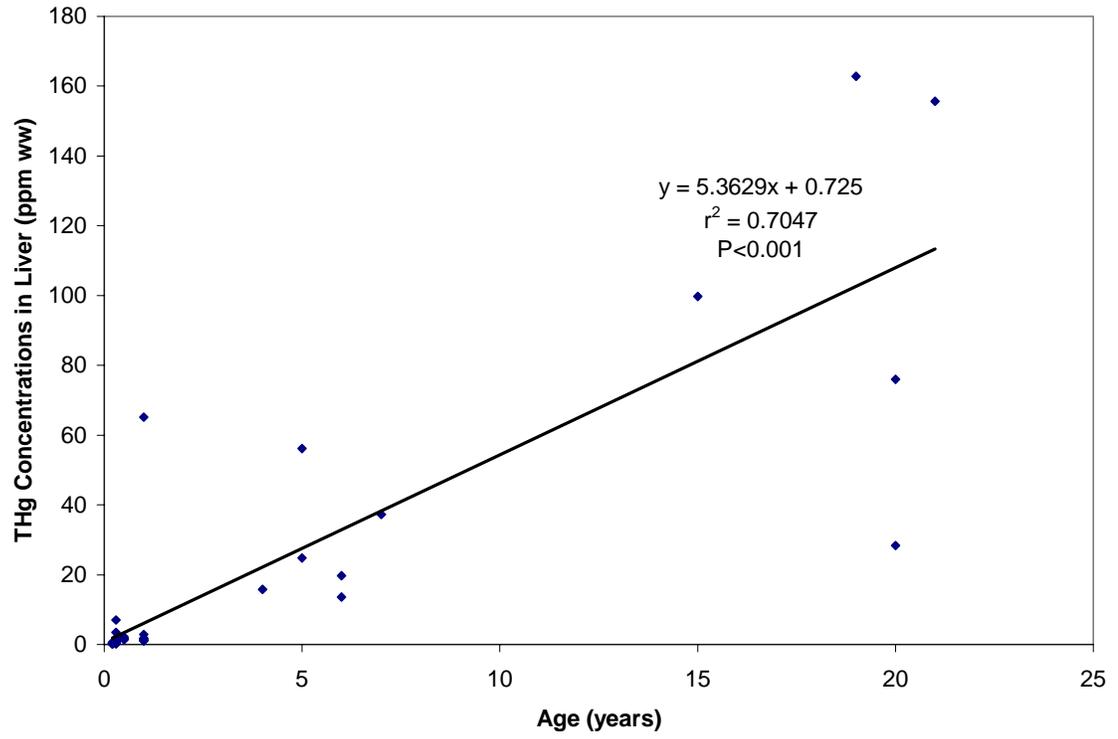
Figure 8. Linear regression of THg concentrations in liver and age of harbor seals from central and northern California.
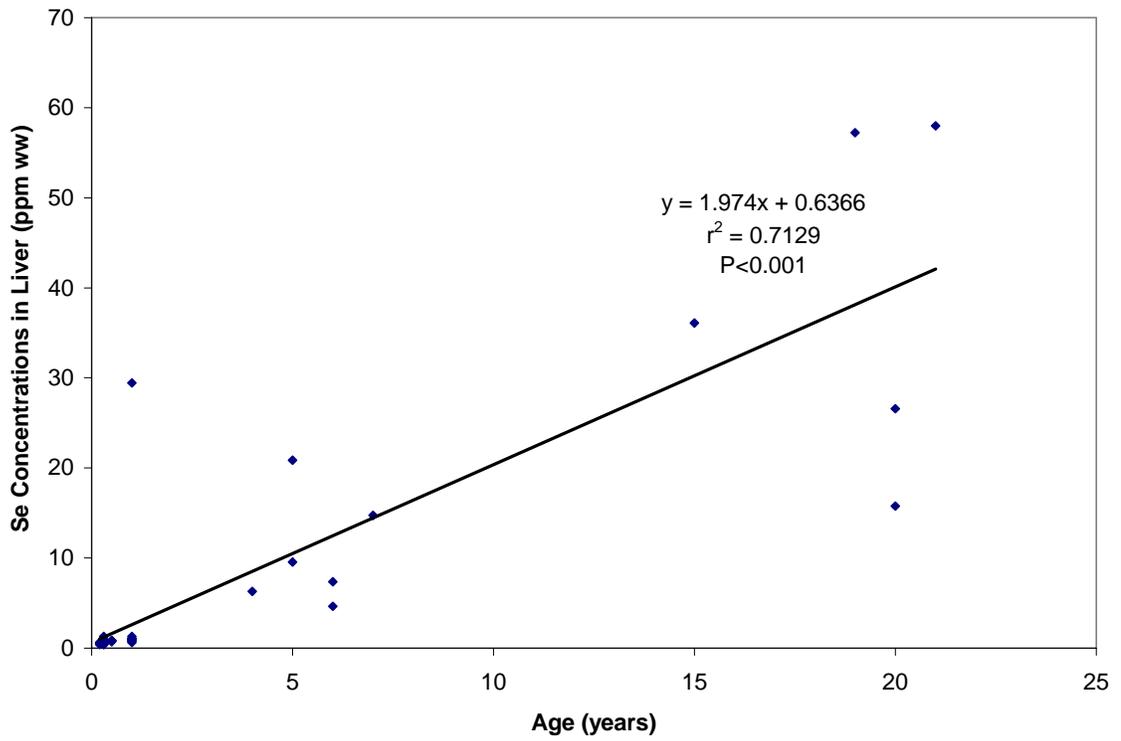
Figure 9. Linear regression of Se concentrations in liver and age of harbor seals from central and northern California.
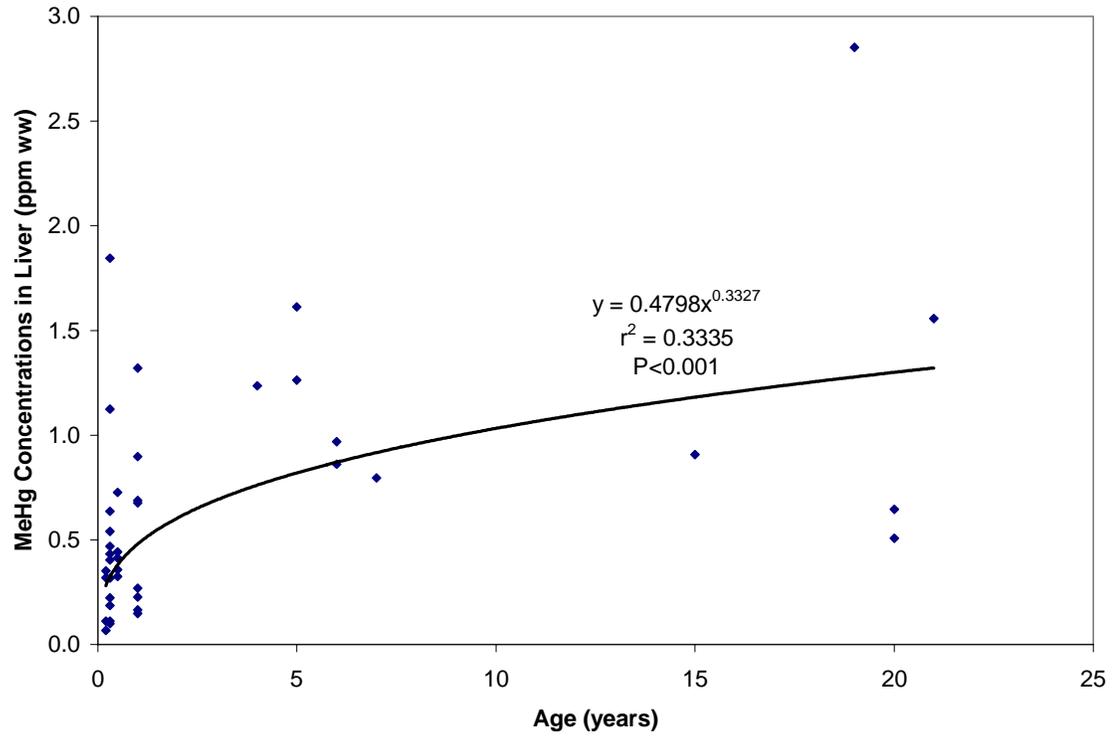
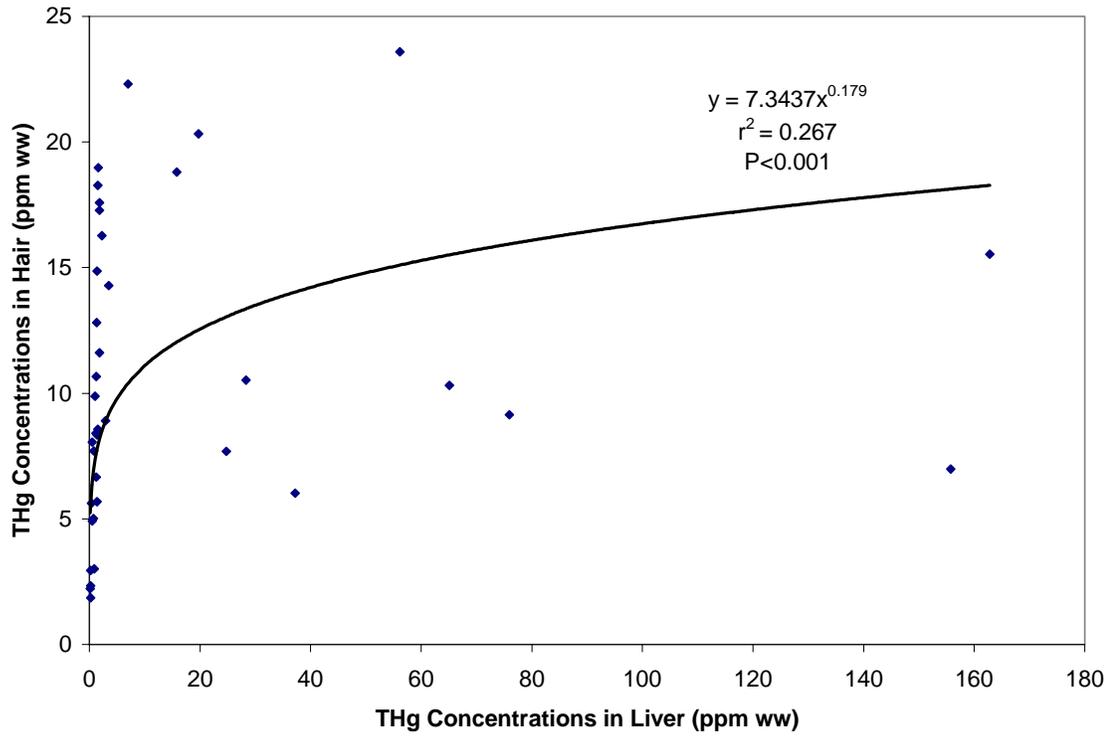Figure 10. Power-based exponential increase of MeHg concentrations in liver and age of harbor seals from central and northern California.
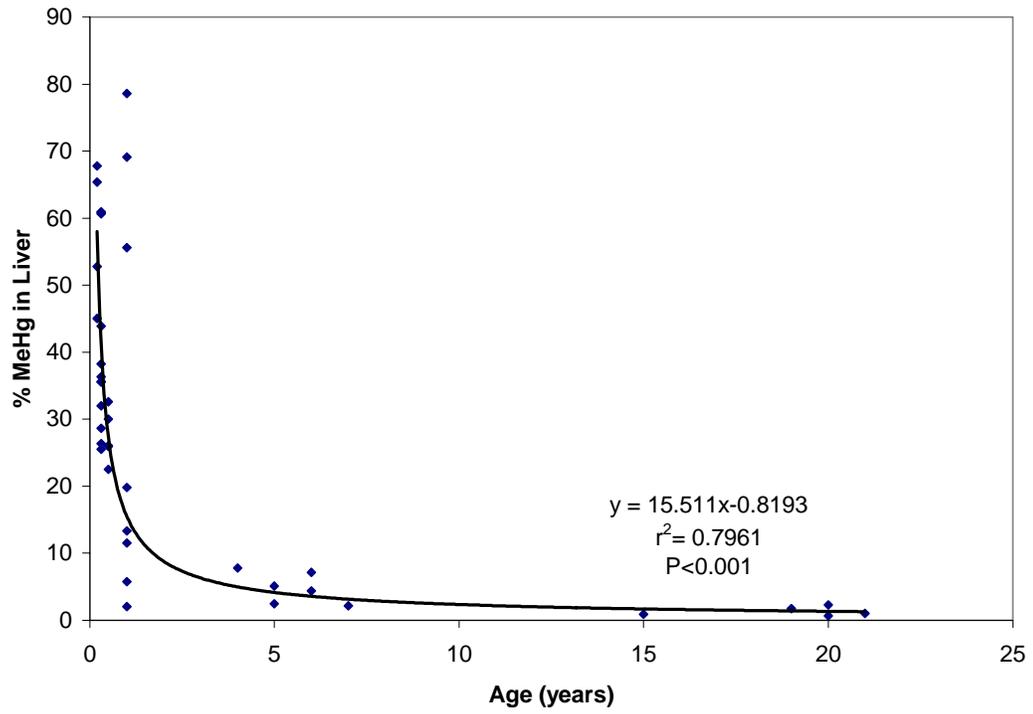
Figure 11. Power-based decay function for % MeHg in the liver and age of harbor seals from central and northern California.

Figure 12. Power-based exponential increase of MeHg concentrations and THg concentrations in liver of harbor seals from central and northern California.
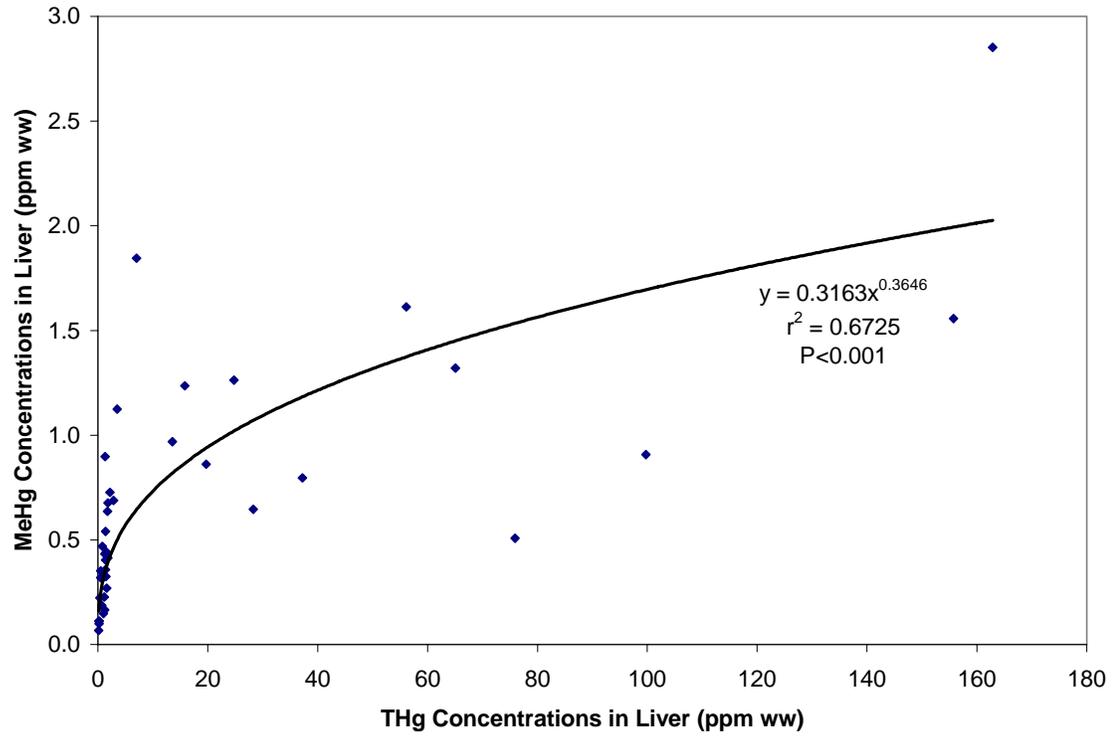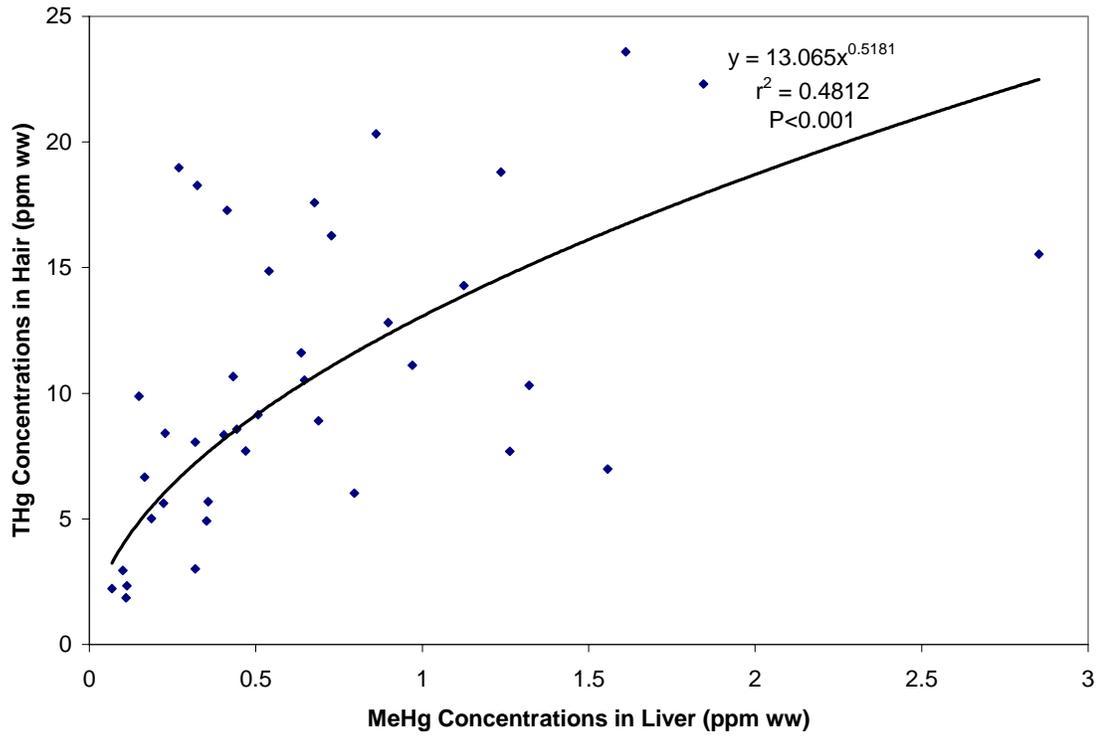
Figure 13. Linear regression of molar THg concentrations and molar Se concentrations in liver of harbor seals from central and northern California.

Figure 14. Power-based exponential increase of THg concentrations in hair and THg concentrations in liver of harbor seals from central and northern California.
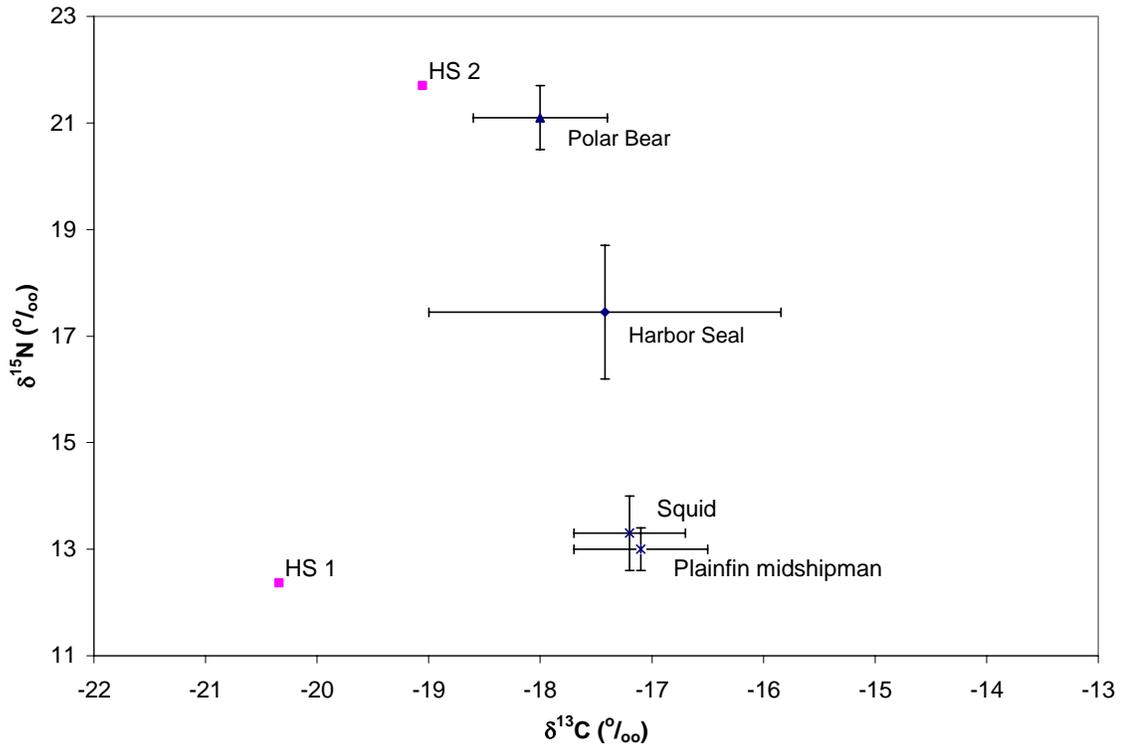
Figure 15. Power-based exponential increase in THg concentrations in hair and MeHg concentrations in liver of harbor seals from central and northern California.

Figure 16. Mean (± SD) δ^{15}N and δ^{13}C values for liver tissue of harbor seals from central and northern California. Two outliers (red squares) were not included in mean calculation. One harbor seal (HS 1), having δ^{15}N and δ^{13}C values less than expected, was a female pup consuming formula during rehabilitation; whereas, the other harbor seal (HS 2), having δ^{15}N and δ^{13}C values greater than expected, was a female adult with a cancerous liver. Polar bear (Hobson and Welch 1992) and prey values (Toperoff 2002) included for graphical comparison.
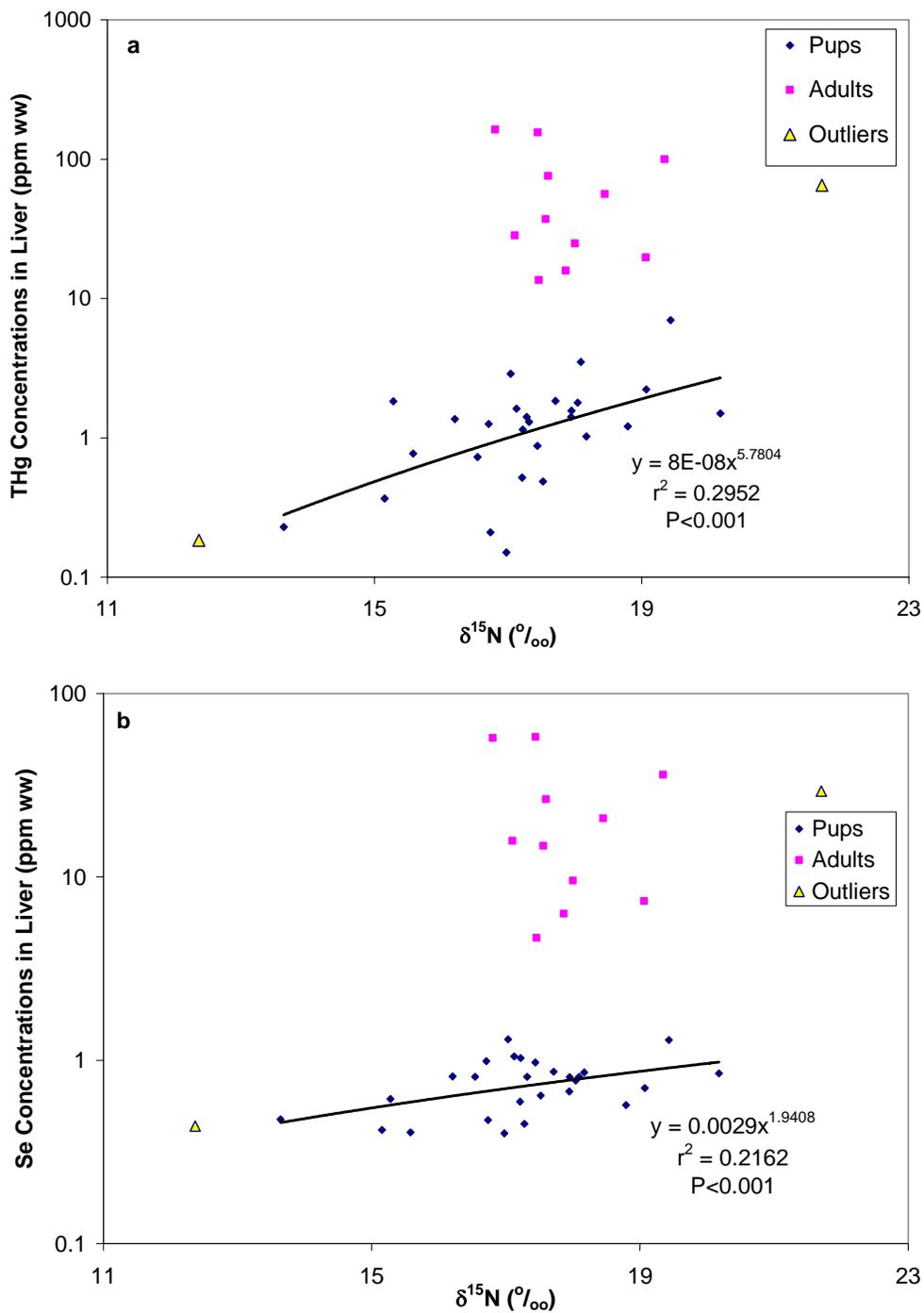
Figure 17. Linear increase of THg (a) and Se (b) concentrations in liver relative to $\delta^{15}N$ in harbor seals from central and northern California based on age class using power-based exponential regressions and log-scaled axes. Two outliers (yellow triangles) removed from analyses.
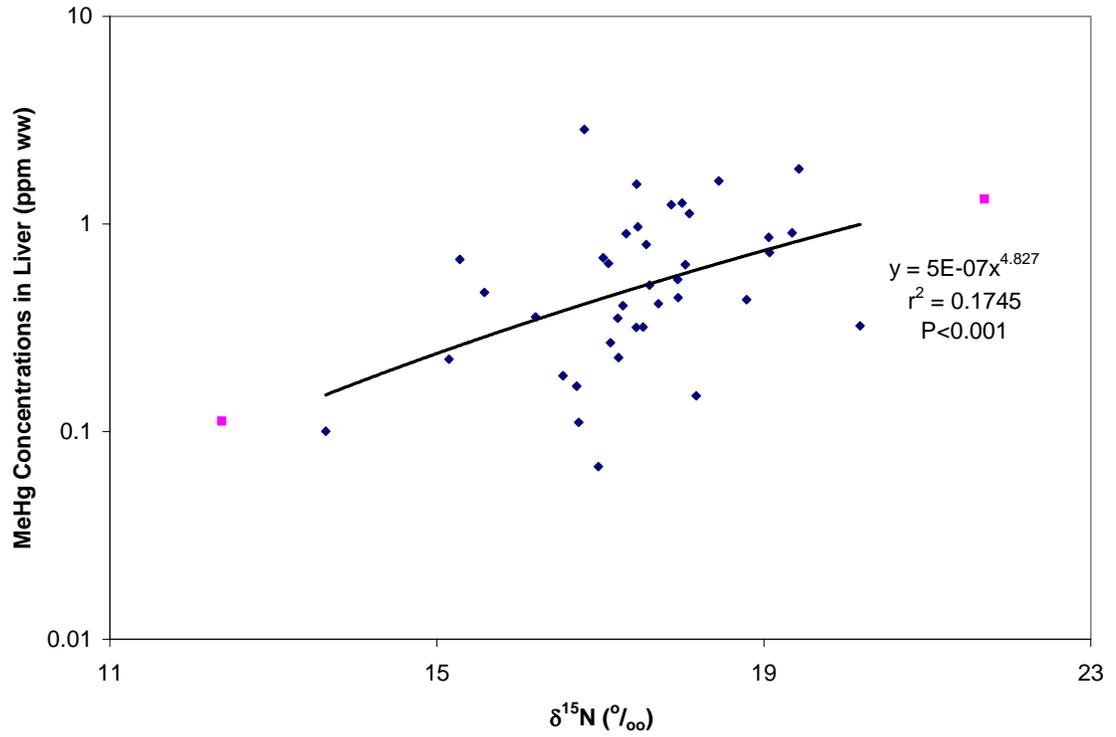
Figure 18. Linear increase of MeHg concentrations in liver relative to $\delta^{15}N$ in harbor seals from central and northern California using power-based exponential regression and log-scaled axes. Two outliers (red squares) removed from analyses.
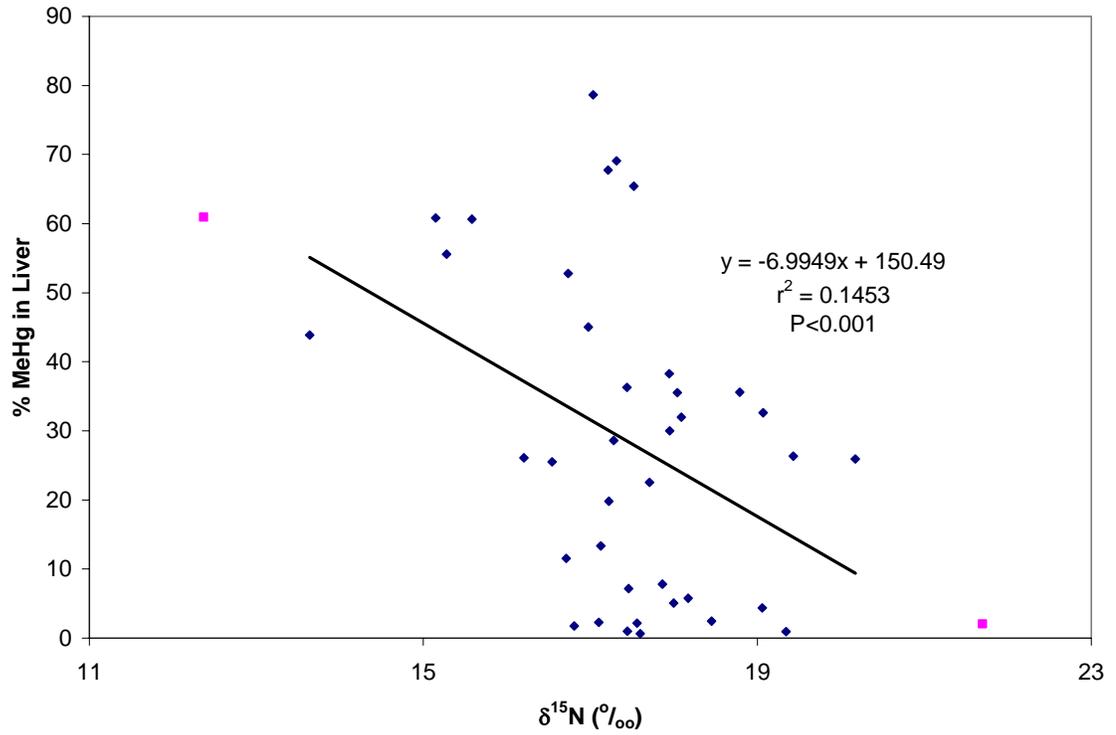
Figure 19. Linear decrease of % MeHg in liver relative to δ^{15}N in harbor seals from central and northern California using power-based exponential regression and log-scaled axes. Two outliers (red squares) removed from analyses.