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Health implications of mercury, selenium, and a red pelage in Pacific harbor seals (Phoca vitulina richardii) off central California

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HEALTH IMPLICATIONS OF MERCURY, SELENIUM, AND A RED PELAGE IN PACIFIC HARBOR SEALS (*PHOCA VITULINA RICHARDII*) OFF CENTRAL CALIFORNIA

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by

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ABSTRACT

HEALTH IMPLICATIONS OF MERCURY, SELENIUM, AND A RED PELAGE IN PACIFIC HARBOR SEALS (*PHOCA VITULINA RICHARDII*) OFF CENTRAL CALIFORNIA

by Elizabeth A. McHuron

San Francisco Bay (SFB) is the largest estuary on the west coast of the United States. It provides habitat for many species, although is heavily urbanized with a history of selenium (Se) and mercury (Hg) contamination. Harbor seals (*Phoca vitulina*) are good indicators of the health of SFB because they are long-lived, upper-level trophic consumers, and present in the estuary year-round. The objective of this study was to examine the role of Se and Hg contamination on the health of harbor seals in this region, and the role of Se in the development of a red pelage. Between 2009 and 2011, free-ranging seals (n = 146) were sampled at three sites off central California. Harbor seals from SFB and Tomales Bay had greater total Hg (THg) and lesser Se concentrations in hair than seals from Elkhorn Slough. Differences in THg concentrations with location were likely the result of historic gold and Hg mining. Lesser Se concentrations in seals from SFB and Tomales Bay may indicate that these seals have a greater physiologic requirement for Se due to increased Hg exposure. Concentrations of THg measured in this study may be great enough to negatively impact the health of harbor seals; however, seals in SFB did not appear to suffer from chronic Se toxicosis. The development of a red pelage may be the result of external iron deposition because of increased dependence on benthic prey and does not appear to have any short-term health implications.

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INTRODUCTION

San Francisco Bay (SFB) is one of the largest estuaries on the west coast of the United States and is located at the mouth of the Sacramento-San Joaquin River system. Sources of freshwater inputs include the Sacramento and San Joaquin Rivers, the Santa Clara County Water Treatment Plant, and seasonal input from small creeks. San Francisco Bay has been extensively modified by human activity (Nichols *et al.* 1986) and is home to more invasive species than any other estuarine system in the United States (Cohen and Carlton 1998). Inputs of pollutants from municipal and industrial activities and runoff from urban and agricultural lands have flowed into the estuary since the beginning of the Gold Rush in 1848. Toxicants present in SFB included polychlorinated biphenyls (Davis *et al*. 2007), organochlorine pesticides, and polycyclic aromatic hydrocarbons (Anderson *et al.* 2007), and widespread sediment toxicity has been documented since the mid-1980s (Chapman *et al.* 1987, Long *et al.* 1990). Despite the urbanized nature of the estuary, SFB continues to provide habitat for a myriad of species, including invertebrates, fish, shorebirds, ducks, and marine mammals.

The Pacific harbor seal (*Phoca vitulina richardii*) is widely distributed along the mainland coast, islands, and bays of California. San Francisco Bay provides critical habitat for harbor seals for resting ashore (hauling-out), pupping, social interaction, and feeding. Harbor seals are the most abundant marine mammal in SFB and are present year-round (Fancher 1979, Torok 1994, Grigg *et al.* 2002). Because of their trophic

position (Brookens *et al.* 2007), ability to accumulate pollutants, and year-round presence in SFB, harbor seals are a good indicator and sentinel species of the health of SFB.

Harbor seals in SFB have been slow to recover despite the passage of the Marine Mammal Protection Act in 1972 (Harvey *et al.* 1990, Jeffries *et al.* 2003, Brown *et al.* 2005). Between 1970 and 2002, the number of harbor seals in SFB did not increase or increased only slightly at specific haul-out sites (Grigg *et al.* 2004). In contrast, harbor seal populations in California, Oregon, and Washington have increased and/or reached carrying capacity since the 1970s (Harvey *et al.* 1990, Jeffries *et al.* 2003, Brown *et al.* 2005, Lowry *et al.* 2008). The number of harbor seals in coastal estuaries in Washington increased from 1,694 to 7,117 between 1975 and 1999 (Jeffries *et al*. 2003). Between 1975 and 1983, numbers of harbor seals within bays in Oregon also increased (Harvey *et al.* 1990). An increase in the number of seals in California and the recovery of harbor seals in estuaries in other states indicate that factors unique to SFB may affect the reproductive rate or health of harbor seals within the SFB estuary. Possible factors include harassment, a reduction or change in prey resources, and environmental contamination (Kopec and Harvey 1995).

The overarching objective of this study was to examine the role of environmental contamination of mercury (Hg) and selenium (Se) on the health of harbor seals. The objectives of Chapter I were to: 1) determine whether total mercury (THg) and selenium (Se) concentrations in hair differed by location on an individual (*i.e.*, neck *vs.* mid-dorsal), 2) compare THg and Se concentrations and the molar ratio of Se:Hg in hair of

harbor seals at three locations off central California, 3) determine if THg and Se concentrations and the molar ratio of Se:Hg differed with sex, and 4) examine the relationship between measures of chemical feeding ecology (carbon, nitrogen, and sulfur stable isotopes) and THg and Se concentrations. The objectives of Chapter II were to: 1) compile historic and current data on the prevalence of red-coated harbor seals in SFB, 2) describe and document changes to keratinized tissues of red-coated harbor seals, 3) evaluate hematology and serum chemistries of red-coated and normal-pelaged seals from SFB, 4) definitively determine whether a red pelage is the direct result of external iron deposition on the hair shaft, and 5) evaluate the hypothesis that chronic Se toxicosis predisposes seals in SFB to developing a red pelage.

Chapter I

Mercury and selenium concentrations in hair and relationship with stable isotopes for Pacific harbor seals (*Phoca vitulina richardii*) off central California

ABSTRACT

San Francisco Bay (SFB) is an urbanized estuary with a history of environmental contamination, including selenium and mercury. Pacific harbor seals (*Phoca vitulina richardii*) are year-round residents of SFB, which provides habitat for feeding, resting, and pupping. A proportion of harbor seals in SFB may suffer from chronic selenium toxicosis, and the study objective was to evaluate this hypothesis. Total mercury (THg), selenium (Se), the Se:Hg molar ratio, and stable isotopes ($\delta^{15}N$, $\delta^{13}C$, $\delta^{34}S$) were measured in hair of harbor seals at three locations off central California. Total Hg concentrations were 2.96 to 144 μg/g, and differed with location ($P < 0.01$; SFB = Tomales Bay > Elkhorn Slough) and sex ($P < 0.01$; males > females). Seals had greater THg concentrations than previously reported in central California; however, there was no relationship between THg concentrations and $\delta^{15}N$ values (*i.e.*, trophic level). Selenium concentrations in hair ranged from below the detectable limit to 4.93 μg/g, and differed among sites ($P < 0.01$; Elkhorn Slough > SFB = Tomales Bay). Difference in THg concentrations among sites were likely due to contamination from historic mining in SFB and Tomales Bay. Greater THg concentrations in males might be explained by females offloading Hg to their developing fetuses, or by foraging differences between males and females. Lesser concentrations of Se and the Se:Hg ratio from seals in SFB and Tomales Bay is an important nutritional consideration in the context of Hg. Results support previous interpretations that hair may serve as an important excretory route for some toxicants; however, results do not support the chronic Se toxicosis hypothesis.

INTRODUCTION

Essential trace elements occur at relatively low levels and are needed in small quantities for proper growth, development, and physiology of an organism. Those deemed to have no known biological function are known as non-essential elements. For the essential elements, organisms deficient or containing amounts in excess of what is homeostatically required may experience symptoms of deficiency or toxicosis. Trace elements have been well-studied in humans and some other animals (*e.g.*, livestock, domesticated animals; Underwood 1977, Roussel *et al.* 1999); however, less is known about baseline values, symptoms of element imbalance, and interactions of trace elements in pinnipeds, a diverse group of marine mammals comprised of sea lions, fur seals, and true seals. Some pinnipeds are long-lived, and many are upper-level trophic consumers (Pauly *et al.* 1998), which makes them particularly sensitive to accumulation of essential and non-essential elements.

Non-essential trace elements, such as mercury (Hg), have been studied because of their potential toxic effects on pinnipeds and other vertebrates, including humans that depend on pinnipeds for subsistence. Because they are long-lived, have extensive fat stores in the form of blubber, and are upper-level trophic consumers, many species of pinnipeds are especially susceptible to accumulating concentrations of trace elements in excess of concentrations considered toxic to humans. Nearshore species (*e.g.*, harbor seals) often live in close proximity to dense human populations and forage at a similar trophic level as humans, thereby making them good indicators of ecosystem health.

Increased concentrations of certain trace elements in pinniped tissues could potentially be detrimental, or beneficial, to humans that depend on them for subsistence (Moses *et al.* 2009).

Mercury is a heavy metal that is widely present in the environment. Sources of Hg pollution in aquatic systems include atmospheric deposition, erosion, urban discharge, agricultural materials, mining, and combustion and industrial discharge (Wang *et al.* 2004). Dissolved Hg is present as elemental mercury (Hg^0), inorganic mercury (*e.g.*, mercuric sulfide, chloride, and oxide), and organic mercury (*e.g.*, dimethyl, ethyl, and methylmercury). The majority of Hg present in aquatic organisms, such as fish, exists as monomethyl Hg (MeHg; Harris *et al.* 2003), a toxic form of Hg that biomagnifies in aquatic food webs (Dietz *et al.* 2000), and can negatively impact neurological, immunological, and reproductive systems (Zahir *et al.* 2005).

Selenium (Se) is a naturally occurring essential element possibly providing a protective effect against Hg toxicosis **(**Cuvin-Aralar and Furness 1991, Yang *et al.* 2008**)**, therefore, researchers rarely examine the effects of Hg without also considering Se concentrations. Dissolved selenium is present in several oxidation states including selenate (SeVI), selenite (SeIV), elemental selenium (O), and selenide (-II). Particulate selenium can exist as insoluble elemental selenium, selenate, and selenite. Selenium is an important component of numerous proteins, including the antioxidant enzyme, glutathione peroxidase (GSH-Px), which protects cellular membranes and lipid–containing organelles from peroxidative damage (Nakane *et al.* 1998, Behne and

Kriakopoulos 2001). Selenium also competes for binding sites with sulfur, and is incorporated into the sulfur-containing amino acids, cystine and methionine (Behne and Kyriakopoulos 2001).

The protective effects of Se against Hg toxicosis have been relatively well-documented (Cuvin-Aralar and Furness 1991, Yang *et al.* 2008), and this relationship has received particular attention in pinnipeds and other marine mammals because they accumulate increased concentrations of Hg with no apparent negative effects. Mercury and Se primarily are acquired through diet, and although the majority of Hg present in prey species is MeHg, only a small proportion of MeHg is present in certain marine mammal tissues (Reijnders 1980, Ikemoto *et al.* 2004*a*). Selenium plays a role in the conversion of MeHg to inorganic Hg (Iwata *et al*. 1982) and also in the formation of a Se-Hg complex in specific tissues (Yoneda and Suzuki 1997, Wang *et al.* 2001, Ikemoto *et al.* 2004*a*). Additional mechanisms for the detoxification of Hg by Se include the redistribution of Hg to less sensitive organs/tissues and competition for binding sites (Cuvin-Aralar and Furness 1991, Wang *et al*. 2001). Researchers reported that Hg and Se accumulated in the livers of marine mammals in a 1:1 ratio (Koeman *et al.* 1973, Smith and Armstrong 1978), although more recently published ratios deviated from this, especially in young animals (Woshner *et al.* 2001*a, b*; Brookens *et al.* 2007). Because Se can counteract the toxic effects of Hg and reduce the amount of bioavailable Hg, it has been suggested that the Se:Hg molar ratio may be a more appropriate measure than total concentrations of Hg when assessing potential toxicity (Ralston *et al.* 2008).

Mercury and Se concentrations can be measured in a variety of tissues, including liver, kidney, muscle, hair, and blood. Hair is increasingly being used to determine trace element concentrations in pinnipeds as it is relatively non-invasive, easy to collect, and allows for a large sample size and mass. Hair concentrations reflect element levels in circulating blood during the time of hair growth and from external deposition onto the hair shaft. In marine mammals that undergo an annual molt (*e.g.*, pinnipeds), hair may serve as an excretory route for trace elements (Wenzel *et al.* 1993, Saeki *et al.* 1999, Ikemoto *et al.* 2004*b*). The majority of Hg in hair is present in its most toxic form (MeHg; Dolbec *et al.* 2001), therefore, excretion of Hg in hair may be especially important for species that live in urbanized areas with increased toxicant inputs.

San Francisco Bay (SFB) is the largest estuary in California and has been extensively modified by human activity (Nichols *et al.* 1986). San Francisco Bay has a history of Hg and Se contamination, and portions of the bay are listed as impaired for either one or both elements under the Clean Water Act 303(d). The main source of Hg in SFB is from historical mining activities, which allowed Hg to enter the bay from runoff or by re-mobilization of contaminated sediments (MacLeod *et al.* 2005, Conaway *et al.* 2008). Primary sources of Se in SFB have been attributed to discharge from oil refineries and wastewater treatment plants (Cutter 1989), riverine inputs (Cutter and Cutter 2004), resuspension of estuarine sediments, and phytoplankton production (Doblin *et al.* 2006).

Increased concentrations of Hg and Se in sediments and the water column in SFB have led to concerns about potential effects on wildlife inhabiting the bay. Despite being

heavily urbanized, SFB provides habitat for a range of species, including invertebrates, shorebirds, ducks, fish, and marine mammals. Mercury concentrations in excess of concentrations of concern for human health (0.23 μg/g wet wt) were detected in all samples of leopard shark (*Triakis semifasciata*) and striped bass (*Morone saxatilis*) collected from SFB, and in a lesser proportion of samples from California halibut (*Paralichthys californicus*), white sturgeon (*Acipenser transmontanus*), white croaker (*Genyonemus lineatus*), and jacksmelt (*Atherinopsis californiensis*; Davis *et al.* 2002). Schwarzback *et al.* (2006) suggested that Hg contamination was adversely affecting California Clapper Rail (*Rallus longirostris*) reproductive success, and Ackerman *et al*. (2007) found that 17% of Black-necked Stilts (*Himantopus mexicanus*) had blood Hg concentrations in excess of concentrations that could impair reproduction (> 3.0 μg/g wet wt). Selenium concentrations in excess of concentrations of concern to public health were detected in sportfish from SFB (Greenfield *et al.* 2005), and increased Se concentrations were detected in livers of surfscoters (*Melanitta perspicillata*) from south SFB (Ohlendorf *et al.* 1986). Selenium concentrations in the benthic food web increased threefold beginning in the early 1990s, which was attributed to the invasion of a non-native bivalve (*Potamocorbula amurensis*; Linville *et al.* 2002).

Pacific harbor seals (*Phoca vitulina richardii*) are an important top-level predator in the SFB ecosystem, and because they are relatively long-lived and reside in the bay throughout the year, may be exposed to increased concentrations of Hg and/or Se. Brookens *et al.* (2007) found that maximum concentrations of total Hg (THg) in hair of

harbor seals were greater than previously reported, but found no differences in hair concentrations of THg among sites in northern and central California. Selenium concentrations in harbor seals from SFB have not been studied extensively; however, Se concentrations in blood of seals from SFB were greater than other locations (Kopec and Harvey 1995). Increased Se concentrations in blood, coupled with hair loss and shortened vibrissae (symptoms of Se toxicosis; Raisbeck *et al.* 1993; O'Toole and Raisbeck 1995, 1997), led Kopec and Harvey (1995) to conclude that a proportion of harbor seals in SFB may suffer from chronic Se toxicosis.

The objectives of this study were to: 1) determine whether THg and Se concentrations differed with hair location on an individual (*i.e.*, neck *vs.* mid-dorsal), 2) compare THg and Se concentrations and the molar ratio of Se:Hg in hair of harbor seals at three locations off central California, 3) determine if THg and Se concentrations and the molar ratio of Se:Hg differed with sex, and 4) examine the relationship between measures of chemical feeding ecology (nitrogen, carbon, and sulfur stable isotopes) and THg and Se concentrations. I hypothesized that THg and Se concentrations would not differ between the neck and mid-dorsal samples, and that seals captured in SFB would have increased concentrations of THg and Se in hair, and a greater Se:Hg molar ratio than seals from other locations. I also hypothesized that adult males would have greater concentrations of THg and Se than adult females, and that THg concentrations would be greater for seals feeding at an increased trophic level. Selenium can bioaccumulate (Dietz *et al.* 2000), but because Se is an essential element and homeostatically regulated,

I expected that Se concentrations would not differ with tropic level. A relationship between carbon and/or sulfur stable isotopes and THg and Se concentrations may be detected if seals consistently used habitats with different inputs of primary productivity.

METHODS

Sample Collection

Harbor seals (n = 146) were captured in SFB, Tomales Bay, and Elkhorn Slough between August 2009 and February 2011 (Fig. 1). Samples were not collected during the pupping season (March to May) to avoid unnecessary disturbance and/or separation of mother-pup pairs. All samples were collected under permits to either Dr. James T. Harvey, Dr. Sarah G. Allen, or Elizabeth A. McHuron (National Marine Fisheries Services, NMFS, No. 555-1870, No. 373-1868; US Fish and Wildlife Service, 2009-041, 2011-002; SJSU IACUC #933). Seals were captured using tangle nets, salmon nets, or a modified beach seine (Jeffries *et al.* 1993). Seals were manually restrained for sample collection, and when available, were sedated with an IV injection of diazepam (0.2 mg/kg).

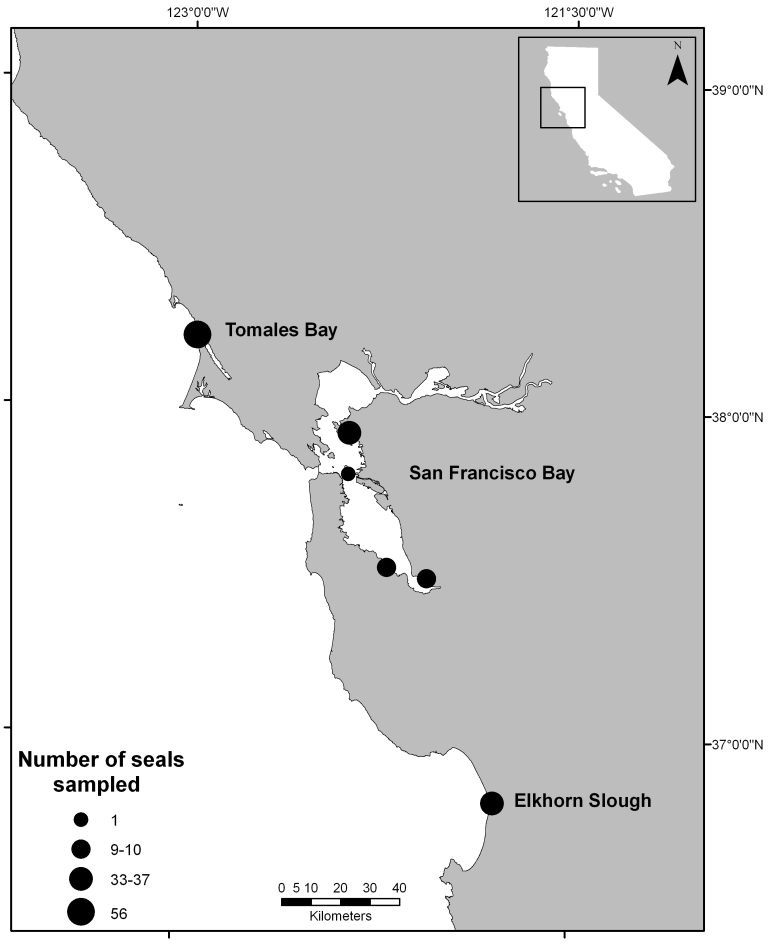

Figure 1. Location and number of harbor seals sampled (denoted by black dots sized relative to sample size) in central California between August 2009 and February 2011.

Standard length (± 1 cm; SL), girth (± 1 cm), mass (± 1 kg), age class, and sex were determined for all seals sampled. Age class was determined based upon length and weight (Bigg 1969), body condition, and date of capture. Age classes included pup (young of the year), yearling (1 to 2 years), subadult (2 years to adult), and adult (females, 3+ years; males, 5+ years). Individuals were tagged (flipper and PIT tags) to avoid resampling the same seal, and photographed for future use if necessary.

An approximate 15 x 15 cm patch of hair was shaved from the right side of the neck using an *Oster*® Pro battery-operated shaver with a size 40 cryotech stainless steel blade that had been cleaned in acetone. Hair samples also were collected from the mid-dorsal region and left pelvic area in case additional hair was needed for analyses. Hair samples were stored on ice until transported to a -80°C freezer at Moss Landing Marine Laboratories (MLML).

Sample Analysis

Hair samples were analyzed for THg and Se concentrations in the Wildlife Toxicology Lab at the University of Alaska Fairbanks. Before analysis, external dirt, debris, and oil were removed by soaking hair in a 1% solution of Triton® X-100 for 15 minutes, followed by multiple rinses with ultrapure water (NANOpure Model D4751, Barnstead International, Dubuque, Iowa). Hair samples were then frozen and freeze-dried for a minimum of 24 hours to remove water before weighing. Data for THg and Se concentrations are presented on a dry-weight basis.

Total Hg concentrations were measured in hair (0.003 to 0.01 g) on a DMA-80 Direct Mercury Analyzer (Milestone Inc, Shelton, Connecticut). Samples were run in duplicates or triplicates, depending on the amount of hair available, and the mean THg concentration was calculated for each individual. Quality controls (blanks, liquid standards, standard references) were run in triplicates at the beginning of each batch of samples. The liquid standard contained 1.04 mg THg/kg, and the standard reference was

human hair IAEA-085 (23.2 ± 0.08 mg THg/kg, International Atomic Energy Agency, Vienna, Austria). Total Hg concentrations were determined using a 16-point calibration curve (30.1 to 452.9 ng/g). Mean recoveries for quality controls were 103% (liquid standard) and 99.8% (standard reference), and the coefficient of variation for all but one replicate was less than 14%. Total Hg concentrations reported are representative of the MeHg concentration because the majority (> 80%) of Hg present in hair is in the methylated form.

Samples for Se analysis were prepared using a two-step digestion in a PerkinElmer Multiwave 3000 microwave oven. Hair (0.027 to 0.19 g) was placed in a 3:1 nitric acid (NO_3): hydrogen peroxide (H_2O_2; v/v) solution and heated to 170°C for 15 minutes. Digested samples were transferred into a 50 mL pre-weighed polyethylene vial and diluted to 20 mL with ultrapure water. A sub-sample (2 mL) of the diluted digest underwent a second digestion (heated to 95°C for 60 minutes) with excess hydrochloric acid (HCl; 1:1, v/v) to reduce Se (VI) to Se (IV). Quality control samples (blanks, spikes, duplicates, matrix spikes, and standard reference materials) were included in each digestion batch. Standard reference materials included human hair IAEA-086 (1.00 ± 0.20 mg Se/kg, International Atomic Energy Agency, Vienna, Austria), and fish protein DORM-3 (3.3 mg Se/kg, National Research Council Canada, Institute for National Measurement Standards, Ottawa, Canada).

Selenium concentrations were determined using a mercury/hydride system-flame ionization atomic spectrometry (MHS-FIAS) on a PerkinElmer AAnalyst 800 atomic

absorption spectrometer (AAS) and a six point calibration curve (0.0968 to 4.64 ng/g). Sodium borohydride (0.2% $NaBH_4$ in 0.05% NaOH) was used as the reductant in a 10% HCl carrier solution. Detection limits (0.002 to 0.089 ng/g) varied as new calibration curves were created every 32 samples. The mean percent recoveries were 83% (spikes), 86% (spiked samples), 90% (duplicates), 71% (DORM-3), and 75% (SRM-086). Decreased recoveries for standard reference materials may indicate that measured Se concentrations were less than actual concentrations; however, this should not have affected overall conclusions as samples were randomized among runs and recoveries were relatively consistent among runs.

Hair samples collected from SFB were analyzed for nitrogen, carbon, and sulfur stable isotopes at the USGS Stable Isotope Laboratory in Denver, Colorado. Hair was washed, cleaned, and freeze-dried as described above, and approximately 0.1 mg of hair was placed in an aluminum foil square. Vanadium oxide (V_2O_5; 0.1 to 0.2 mg) was included with hair samples for sulfur analysis. Prepared samples were analyzed by continuous-flow isotope ratio mass spectrometry using an elemental analyzer coupled to a mass spectrometer (Fry *et al.* 1992). Analytical sequences included laboratory standards, and reproducibility of results was generally better than 0.1‰ for nitrogen and carbon, and 0.3‰ for sulfur based on repeated analyses of standards and samples.

The ratio of stable isotopes is expressed in delta (δ) notation and calculated as:

$$\delta X = [(R_{sample}/R_{standard}) - 1]*1000$$

where $X = {}^{15}N$, ^{13}C, or ^{34}S, and $R = {}^{15}N/{}^{14}N$, $^{13}C/^{12}C$, or $^{34}S/^{32}S$ in the sample and standard. Standards used to normalize isotope data were: USGS 40 ($\delta^{13}C = -26.24‰$) and USGS 41 ($\delta^{13}C = -37.76‰$; relative to Vienna-Pee Dee Belemnite), $\delta^{15}N = -4.52$ and 47.57‰ (relative to air), and NBS127 ($\delta^{34}S = 21.1$) and IAEA-SO-6 ($\delta^{34}S = -34.05‰$; relative to Vienna-Canyon Diablo troilite).

Data Analysis

All statistical analyses were conducted using PASWStatistics (version 18.0, IBM, 2010). Assumptions of parametric tests were met before analysis unless otherwise stated and all results are presented using untransformed data. Concentrations of THg and Se did not differ between years or location within SFB (north *vs.* south SFB), therefore, samples from multiple years and sites within SFB were pooled. Mean concentrations of THg and Se were calculated for seals that were captured in successive years to avoid problems with pseudoreplication. Three seals captured in SFB had Se concentrations below the detectable limit (*bdl*), therefore, were excluded from all Se analyses and any analysis where Se was used as a covariate.

A paired samples *t*-test was used to determine whether THg and Se concentrations differed with hair location. Concentrations of THg and Se were determined in a subset of individuals (THg, $n = 17$; Se, $n = 13$). Samples collected from the mid-dorsal region and neck were used in the analysis.

Analysis of Covariance (ANCOVA) was used to determine if THg and Se concentrations and the Se:Hg molar ratio varied with the fixed factors of location and sex. Covariates included SL, THg, and Se. Standard length was included as a covariate to remove any variation in THg and/or Se concentrations associated with age, and THg and Se were considered as covariates in the analysis of the other element because of their antagonistic interaction (Cuvin-Aralar and Furness 1991). Total Hg concentrations and the Se:Hg molar ratio were log transformed to meet the assumption of equal variances. Post-hoc comparisons were made using a Ryan's Q test with a Kramer modification to correct for unequal sample sizes (Day and Quinn 1989).

Stable isotope data were analyzed to determine whether differences in sex, location (north *vs.* south SFB), and age existed, and whether these data could explain any of the variability in THg and Se concentrations. Pups (n = 3) were excluded from the analysis as the stable isotope signatures of pups were representative of their mother's diet during lactation. Eight seals were excluded from sulfur analyses because of poor yield of $\delta^{34}S$. Analysis of Covariance was used to determine whether $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ values differed with the fixed factors of location and sex, and SL was included as a covariate of interest. The significance value of the location*sex interaction was 0.05. Because this interaction was only mildly significant, and was most likely affected by females from south SFB, the main effects were still interpreted for this analysis. Linear regression was used to examine the relationship between $\delta^{15}N$ and SL, $\delta^{13}C$, and $\delta^{34}S$. Stepwise linear regression was used to determine whether any variability in THg and Se concentrations

could be explained by $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ values. Additional variables included in the analysis were %N, %C, %S, SL, THg (Se analysis), and Se (THg analysis). Predictor variables were removed from the model if $P > 0.05$. Problems with multicollinearity were assessed if multiple variables were present in the final model.

RESULTS

Concentrations of THg and Se differed between the neck and the mid-dorsal region, therefore, only hair samples collected from the neck region were used in further analyses. Hair samples from the mid-dorsal region had greater concentrations of THg ($t = 2.458$, $P = 0.024$) and Se ($t = 1.899$, $P = 0.082$) than samples from the neck, but the magnitude of this difference was relatively small (THg = 1.68, Se = 0.55 μg/g dry wt).

Mean THg concentrations differed with location ($F = 10.939$, $P < 0.001$) and sex ($F = 22.355$, $P < 0.001$; Fig. 2). Selenium and SL were included as covariates, although these variables only explained a relatively small proportion of the total variability, especially in seals from SFB and Tomales Bay (Fig. 3, 4, and 5). Total Hg concentrations in SFB (22.20 ± 2.79; $\bar{x}$ μg/g dry wt ± SE) and Tomales Bay (20.83 ± 1.17) were greater than Elkhorn Slough (13.03 ± 1.31), and males had greater concentrations of THg than females at all locations (Table 1). Seals from SFB also had greater variability in THg concentrations (CV = 0.92) than seals from Elkhorn Slough (0.57) and Tomales Bay (0.42). A proportion of seals had THg concentrations that exceeded hair concentrations associated with sub-clinal effects of Hg toxicosis in polar bears (5.4 μg/g dry wt; Basu *et*

al. 2009), maternal ranges of concern for human fetal neurodevelopment (10 to 20 µg/g dry wt; WHO 1990), and the lower (20 µg/g dry wt; Thompson *et al.* 1996) and upper neurological effects limits for fish-eating wildlife (30 µg/g dry wt; Evers *et al.* 2007; Table 2). Mean THg concentrations of seals from Elkhorn Slough exceeded the 5.4 and 10 threshold limits, whereas mean THg concentrations in seals from SFB/Tomales Bay exceeded the lower neurological effect limit for fish-eating wildlife (Fig. 6).

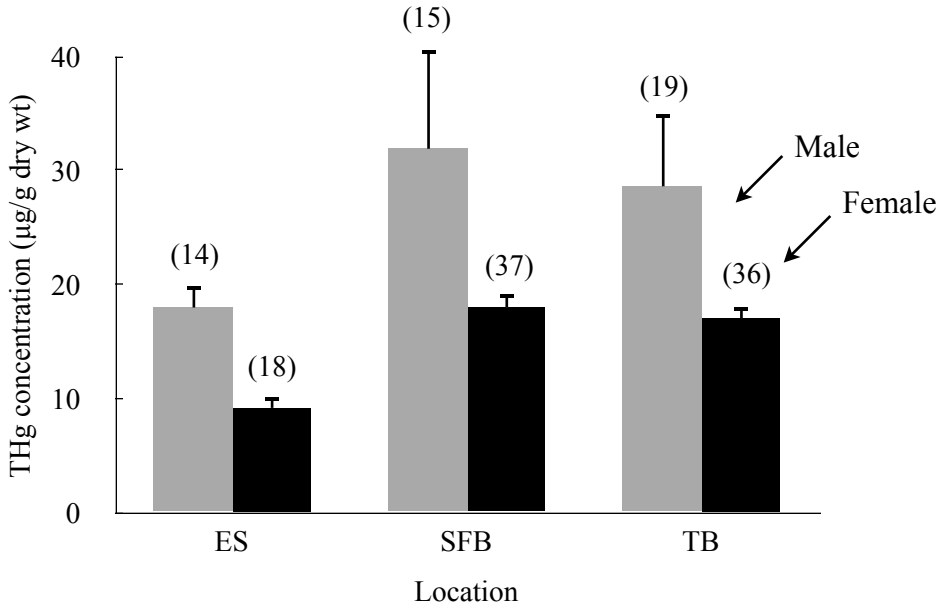

Figure 2. Mean total mercury concentrations (THg; µg/g dry wt) in hair of harbor seals from Elkhorn Slough (ES), San Francisco Bay (SFB), and Tomales Bay (TB). Males are represented by gray bars and females by black bars. Sample sizes are presented in parentheses above standard error lines.

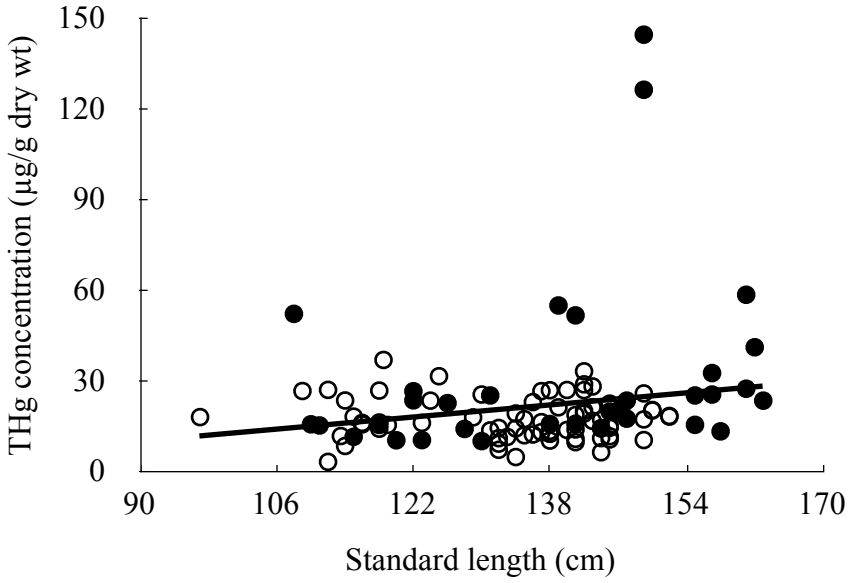

Figure 3. Relationship between total mercury concentration (THg; µg/g dry wt) in hair and standard length (cm) in harbor seals from San Francisco Bay and Tomales Bay ($r^2 = 0.006$, $P = 0.420$). Males are represented by closed and females by open circles.

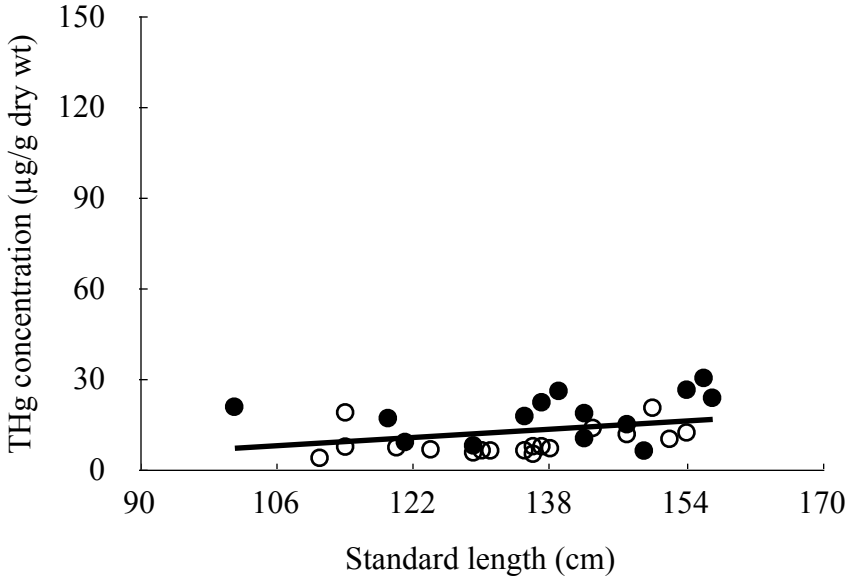

Figure 4. Relationship between total mercury concentration (THg; µg/g dry wt) in hair and standard length (cm) in harbor seals from Elkhorn Slough ($r^2 = 0.116$, $P = 0.056$). Males are represented by closed and females by open circles.

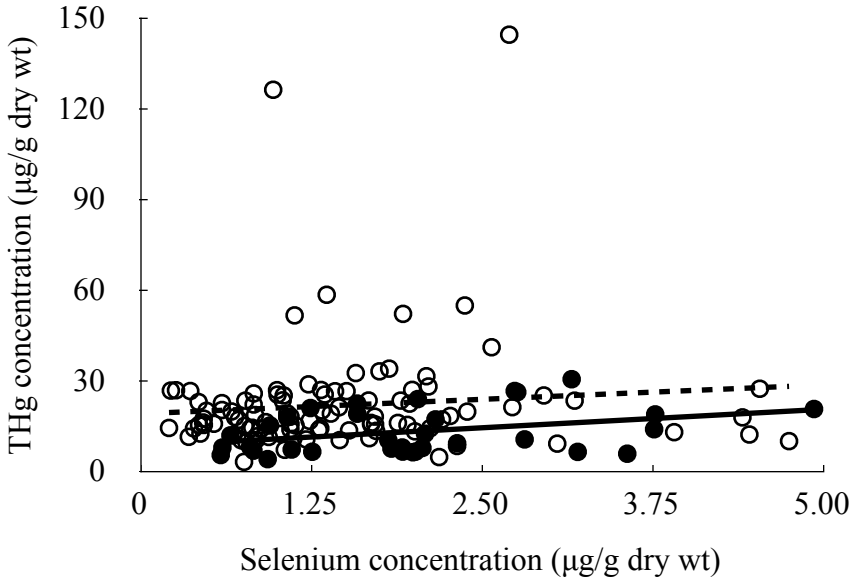

Figure 5. Relationship between total mercury (THg) and selenium concentrations (µg/g dry wt) in hair of harbor seals from San Francisco Bay and Tomales Bay (open circles, dotted line; $r^2 = 0.022$, $P = 0.130$), and Elkhorn Slough (closed circles, solid line; $r^2 = 0.122$, $P = 0.05$).

Table 1. Mean total mercury concentrations (µg/g dry wt ± SE), ranges, and samples sizes in hair of male and female harbor seals from Elkhorn Slough, San Francisco Bay, and Tomales Bay, 2009 to 2011.

Location	Male	Female
Elkhorn Slough	**17.98 ± 2.00**	**9.18 ± 1.09**
	6.27 - 30.31	3.87 - 20.45
	n = 14	*n* = 18
San Francisco Bay	**31.91 ± 8.83**	**18.00 ± 1.34**
	9.83 - 144.31	2.96 - 36.70
	n = 15	*n* = 37
Tomales Bay	**28.61 ± 6.35**	**17.05 ± 0.99**
	11.25 - 126.09	9.00 - 33.81
	n = 19	*n* = 36

Table 2. The percentage of harbor seals with total mercury concentrations in hair exceeding hair concentrations associated with sub-clinical effects of Hg toxicosis in polar bears (5.4; Basu *et al.* 2009), maternal ranges of concern for human fetal neurodevelopment (10 to 20; WHO 1990), and the lower (20; Thompson 1996) and upper (30; Evers *et al.* 2007) neurological threshold limits for fish-eating wildlife.

		Percentage exceeding hair effects level for other species (µg/g dry wt)			
Location	*n*	5.4	10	20	30
Elkhorn Slough	32	94	53	22	3
San Francisco Bay	52	96	87	44	12
Tomales Bay	55	100	96	33	9

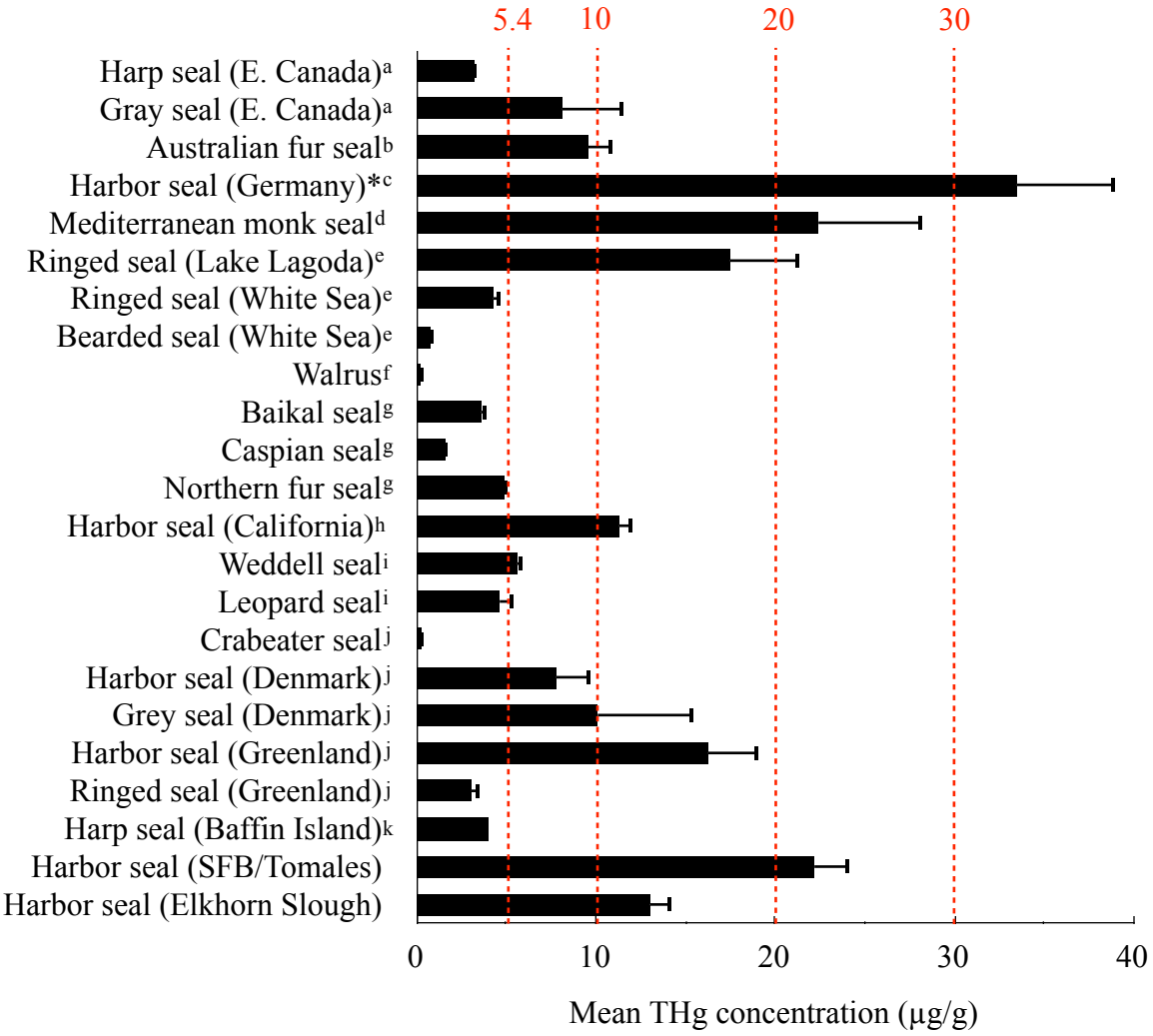

Figure 6. Mean total mercury concentrations (THg; µg/g dry wt ± SE) in hair of pinnipeds. Dotted lines correspond to hair concentrations (µg/g dry wt) associated with sub-clinical effects of Hg toxicosis in polar bears (5.4; Basu *et al.* 2009), maternal ranges of concern for human fetal neurodevelopment (10 to 20; WHO 1990), and the lower (20; Thompson *et al.* 1996) and upper (30; Evers *et al.* 2007) neurological threshold limits for fish-eating wildlife. * corresponds to concentrations presented as wet wt. [a] Freeman and Horne 1973, [b] Bacher 1985, [c] Wenzel *et al.* 1993, [d] Yediler *et al.* 1993, [e] Medvedev *et al.* 1997, [f] Wiig *et al.* 1999, [g] Ikemoto *et al.* 2004*b,* [h] Brookens *et al.* 2007, [i] Gray *et al.* 2008, [j] Aubail *et al.* 2011, [k] Agusa *et al.* 2011.

Mean Se concentrations differed with location ($F = 5.036$, $P = 0.008$). Selenium concentrations were greater in seals from Elkhorn Slough (2.00 ± 0.19; $\bar{x}$ μg/g dry wt ± SE) than SFB (1.50 ± 0.12) or Tomales Bay (1.44 ± 0.14; Fig. 7). A proportion of seals had Se concentrations below concentrations in hair associated with deficiency in livestock and humans; however, some individuals also had Se concentrations in hair greater than those associated with toxicosis in cattle and pigs (Table 3). Mean Se concentrations in seals were greater than mean hair concentrations in humans from regions in China with known Se deficiency, concentrations associated with deficiency in livestock, and concentrations associated with toxicosis in cattle (Fig. 8). There also was a locational difference in the Se:Hg molar ratio ($F = 15.411$; $P < 0.001$), with seals from Elkhorn Slough having a greater Se:Hg molar ratio (0.48 ± 0.058; $\bar{x}$ ± SE) than seals from SFB (0.26 ± 0.034) and Tomales Bay (0.21 ± 0.020). Females also had a greater Se:Hg molar ratio than males at all locations ($F = 5.221$, $P = 0.024$; Fig. 9).

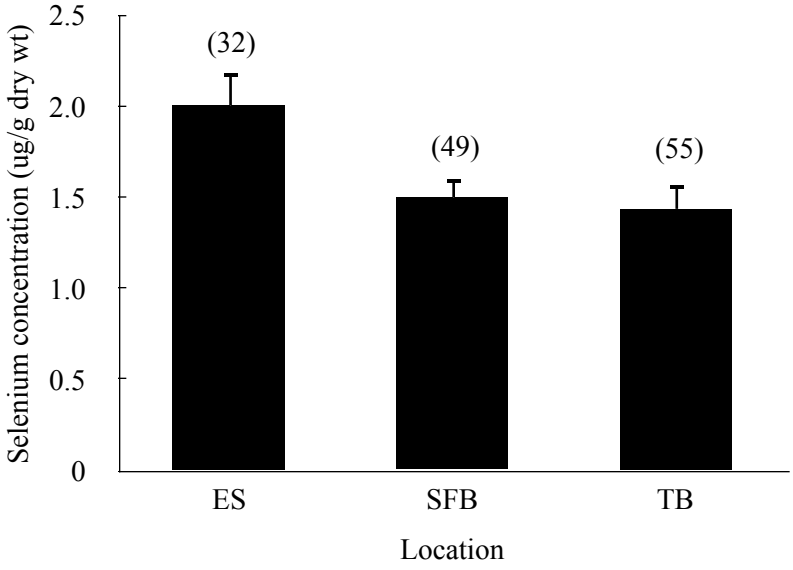

Figure 7. Mean selenium concentrations in hair (µg/g dry wt) of harbor seals from Elkhorn Slough (ES), San Francisco Bay (SFB), and Tomales Bay (TB). Sample sizes are presented in parentheses above standard error lines.

Table 3. The percentage of harbor seals with selenium (Se) concentrations in hair less than (deficiency) or in excess of (toxicosis) hair effects levels for cattle (0.23, 1.4), pigs (4.0), and horses (0.50, 7.0; Puls 1994). The effects level of 0.304 corresponds to mean hair concentrations in humans from Se-deficient regions in China (Fordyce *et al.* 2000).

		Percentage below (deficiency) or exceeding (toxicosis) hair effects level for other species (µg/g dry wt)					
		Deficiency			Toxicosis		
Location	*n*	0.23	0.304	0.50	1.4	4.0	7.0
Elkhorn Slough	32	0	0	0	63	3	0
San Francisco Bay	52	6	8	15	50	2	0
Tomales Bay	55	4	4	16	35	5	0

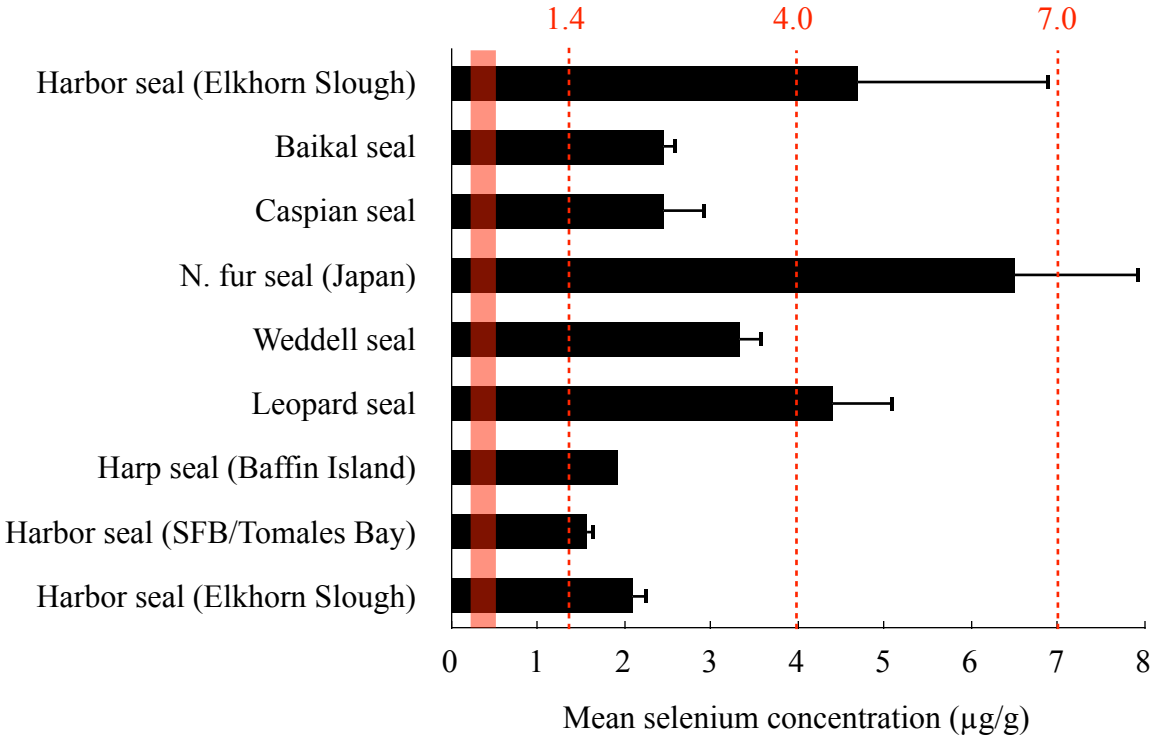

Figure 8. Mean selenium concentrations in hair (µg/g dry wt ± SE) of pinnipeds. Rectangle corresponds to a range of hair concentrations that resulted in deficiency in livestock, and humans in China from known Se-deficient areas (0.18 to 0.5; Puls 1994, Fordyce *et al.* 2000). Dotted lines correspond to mean hair concentrations associated with toxicosis in cattle (1.4), pigs (4.0), and horses (7.0; Puls 1994).

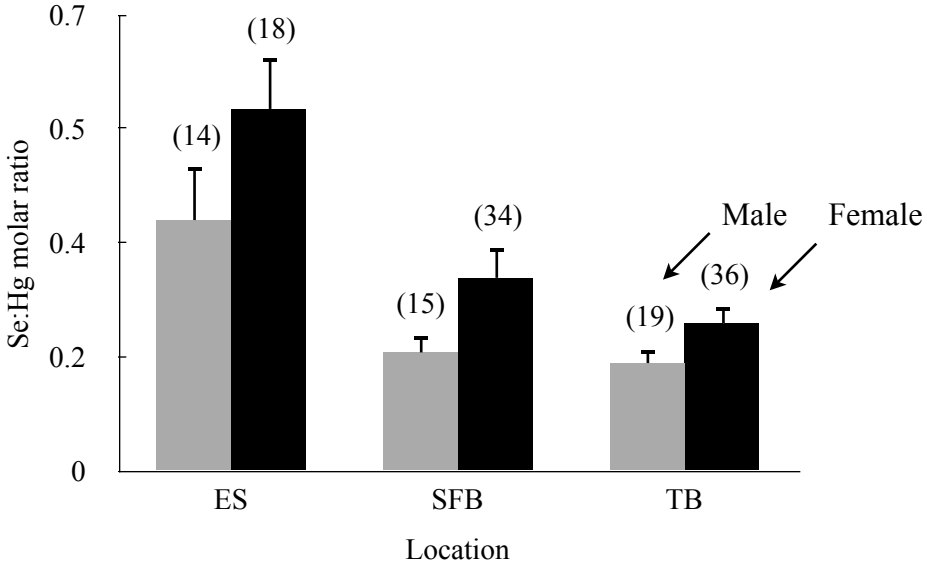

Figure 9. Mean Se:Hg molar ratio in hair of harbor seals from Elkhorn Slough (ES), San Francisco Bay (SFB), and Tomales Bay (TB). Males are represented by gray bars and females by black bars. Sample sizes are presented in parentheses above standard error lines.

Stable isotopes were measured in 51 unique adults and juveniles (subadults and yearlings) from SFB (Table 4). No differences in sex or location (north *vs.* south SFB) were detected for any of the stable isotopes; however, the significance value for the location factor for $\delta^{15}N$ was 0.066. Standard length was included as a covariate in $\delta^{15}N$ and $\delta^{34}S$ analyses, with $\delta^{15}N$ values increasing with SL, and $\delta^{34}S$ values decreasing with SL. Therefore, adults had greater $\delta^{15}N$ values (+1.7‰), and lesser $\delta^{34}S$ values than juveniles (-1.3‰; Table 4). Greater than 50% of the variability in $\delta^{15}N$ values was explained by $\delta^{34}S$ and $\delta^{13}C$ values ($F = 29.586$, $P < 0.001$); however, this relationship primarily was driven by $\delta^{34}S$ values (Fig. 10). Stepwise linear regression revealed that a model containing $\delta^{34}S$ was the best predictor of THg concentrations ($F = 6.349$, $P =$

0.017; Fig. 10). A relatively small amount of variability was explained by $\delta^{34}S$ (r^2 = 0.161), although this relationship improved when extreme values (n = 2) were removed from the dataset (r^2 = 0.330). None of the predictor variables explained a significant amount of variability in Se concentrations.

Table 4. Mean values (‰ ± SE) of stable isotopes of nitrogen, carbon, and sulfur for seals captured in San Francisco Bay separated by sex and age. Juvenile age class represents subadults and yearlings.

	$\delta^{15}N$		$\delta^{13}C$		$\delta^{34}S$	
Age class	Male	Female	Male	Female	Male	Female
Adult	19.50 (0.77)	19.18 (0.54)	-14.26 (0.38)	-14.49 (0.25)	16.06 (0.79)	16.75 (0.42)
Juvenile	17.57 (0.60)	17.66 (0.30)	-14.35 (0.33)	-14.51 (0.25)	17.45 (0.33)	18.04 (0.20)

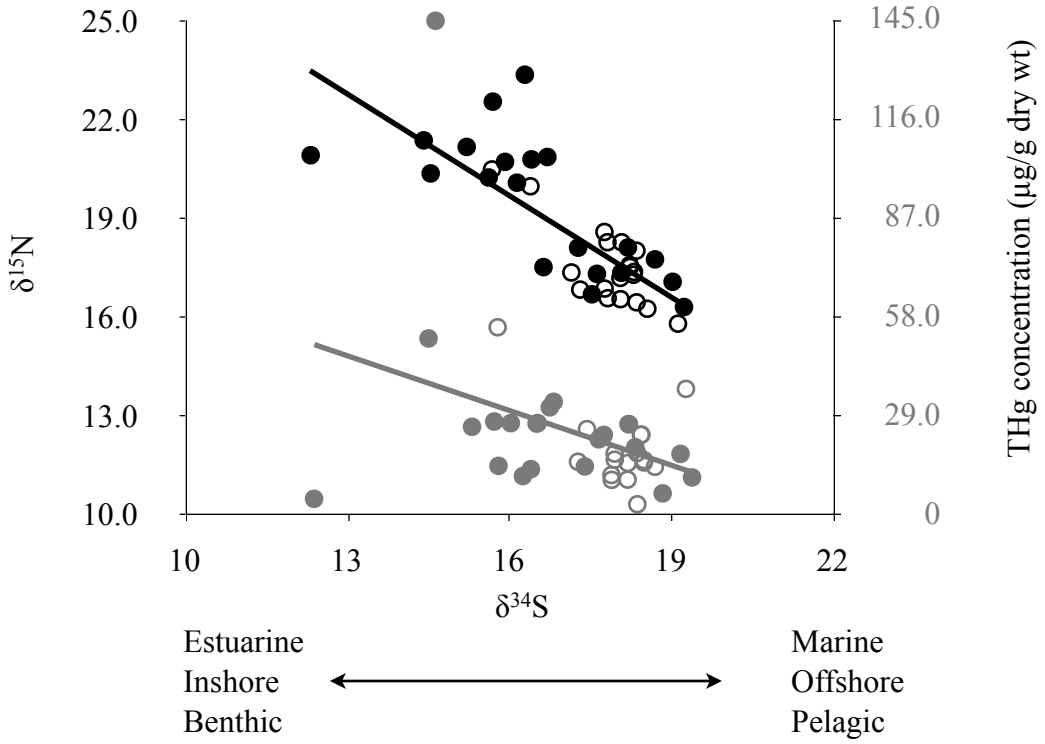

Figure 10. Relationship between $\delta^{15}N$ and $\delta^{34}S$ (black; $r^2 = 0.598$, $P < 0.001$), and total mercury concentrations (THg; µg/g dry wt) and $\delta^{34}S$ (gray; $r^2 = 0.161$, $P = 0.017$) in hair of harbor seals from San Francisco Bay. Adult values are represented with closed and subadults and yearlings with open circles.

DISCUSSION

The use of hair to determine trace element concentrations in marine mammals has been used since the 1980s (Bacher 1985, Wenzel *et al.* 1993, Yediler *et al.* 1993), but its frequency of use appears to be increasing (Brookens *et al.* 2007, Elorriaga-Verplanken and Aurioles-Gamboa 2008, Gray *et al.* 2008, Aubail *et al.* 2011). Hair concentrations represent element levels circulating in blood at the time of hair growth, which spans one to two months in harbor seals (Ashwell-Erickson *et al.* 1986, Daniel *et al.* 2003). Molt

progression in harbor seals typically begins around the face, neck, flippers and body openings followed by the ventrum, dorsum, and lastly the dorsal-lateral sides (Ashwell-Erickson *et al.* 1986, Daniel *et al.* 2003). Researchers have addressed whether element concentrations differ with type of hair (molt *vs.* new; Wenzel *et al.* 1993, Gray *et al.* 2008), but to my knowledge no studies have addressed whether concentrations differ with hair location. Total Hg and Se concentrations in this study were greater in samples collected from the mid-dorsal region than neck, although this trend was not observed in all seals. Because harbor seals do not molt all at once, differences in THg and Se concentrations between samples collected from different locations were likely the result of differences in the timing of molt. Individual variability in molt progression likely contributed to the lack of, or the opposite trend in differences in THg and Se concentrations between the neck and mid-dorsal for some seals. Differences in THg and Se concentrations with hair location may be species-specific, as the length of molt varies among pinnipeds. These differences have implications for sample design, comparisons within and among studies, and relationships between tissue types (*e.g.*, hair *vs.* blood). This may be especially important to address for studies that rely on hair collected from the substrate of haul-out sites (*e.g.*, Yediler *et al.* 1993, Gray *et al.* 2008), or for studies where the trace element/s measured may be present in relatively low concentrations.

The overall mean concentration of THg in hair (19.60 ± 1.44; μg/g dry wt ± SE) and locational means were greater than previously reported for central California (11.3 ± 0.797; Brookens *et al.* 2007), and greater than most previously published values for

pinnipeds (*e.g.*, Watanabe *et al.* 1996, Medvedev *et al.* 1997, Wiig *et al.* 1999, Ikemoto *et al.* 2004*b*, Gray *et al.* 2008). The maximum THg concentrations in hair from SFB and Tomales Bay seals also were greater than published concentrations for harbor seals and other pinniped species (*e.g.*, Watanabe *et al.* 1996, Medvedev *et al.* 1997, Wiig *et al.* 1999, Ikemoto *et al.* 2004*b*, Gray *et al.* 2008). Differences in mean THg concentrations in hair between seals in this study and Brookens *et al.* (2007) could be due to a number of factors, including an increase in Hg input into the system, differences in the foraging behavior of seals or their prey, and differences in data collection and/or analysis.

Variability in Hg input into an ecosystem can occur over a variety of time scales, including long-term (years), annually, or seasonally. No long-term increases in THg concentrations in sediments nor fish were detected in SFB (Greenfield *et al.* 2005, Conaway *et al.* 2007, Greenfield *et al.* 2011). Significant decreases in THg sediment concentrations between 1993 and 2001 were detected at eight stations in SFB, which were attributed to the transport of relatively cleaner sediment into the bay (Conaway *et al.* 2007). Corresponding decreases in THg concentrations of forage fish did not occur (Greenfield *et al.* 2005, 2011), although sediment was considered a primary source of Hg to the nearshore food web (Gehrke *et al.* 2011). Although interannual variation in THg concentrations were observed in striped bass (Greenfield *et al.* 2005) and Mississippi silverside (*Menidia audens*; Greenfield *et al.* 2011), seasonal variation and site location explained the majority of variability in THg concentrations. Although long-term increases in THg concentrations in sediment and forage fish have not been detected, the

decoupling between sediment and forage fish indicate that other factors influence how Hg is transferred through the food web (Conaway *et al.* 2007). Therefore, it is possible that the observed differences in THg in harbor seal hair between 2005 to 2007 (Brookens *et al.* 2007) and 2009 to 2011 (present study) could be the result of long-term increases in Hg concentrations in harbor seals in SFB. Interannual variation in Hg concentrations in prey could have contributed to the increase, but this hypothesis could not be tested because studies were not conducted during the molt period and because THg concentrations for common prey items of harbor seals were not collected during the time of these studies (2004 to 2010). Because hair is molted at approximately the same time every year, seasonal variation is an unlikely cause of the increase.

Total Hg concentrations in this region vary not only with time, but also with location and prey species (Conaway *et al.* 2007, Heim *et al.* 2007, Greenfield and Jahn 2010, Ridolfi *et al.* 2010). Spatial variations of sediment THg concentrations and species differences in THg concentrations occurred in SFB and Tomales Bay (Conaway *et al.* 2007, Heim *et al.* 2007, Ridolfi *et al.* 2010); however, Hg has received more attention, therefore, has been well-researched in SFB. Concentrations of THg in sediments in SFB were greatest at the southern tributaries (including the Guadalupe River), followed by the south bay and northern estuary (San Pablo and Suisan Bays), the central bay, and the San Joaquin and Sacramento Rivers (Conaway *et al.* 2007). These spatial variations were reflected in THg concentrations in forage fish, with the greatest concentrations detected in fish at sites closest to the Guadalupe River (Greenfield and Jahn 2010). Additionally,

increased THg concentrations were found in wetland, mudflat, and tidal species compared with offshore species (Greenfield and Jahn 2010). These spatial gradients were consistent despite differences in THg concentrations among years and fish species, and were likely to be less pronounced in species with large home ranges, variable movement patterns, and/or seasonal migration such as striped bass and salmon (Greenfield *et al.* 2005). Total Hg concentrations also varied among prey species and were less in species such as the bay goby (16 ± 8; $\bar{x}$ ng/g wet wt ± SD), Pacific herring (*Clupea pallasii*; 17 ± 1), and yellowfin goby (33 ± 7), and greater in northern anchovy (*Engraulis mordax*; 45 ± 21) and staghorn sculpin (*Leptocottus armatus*; 50 ± 9; Greenfield and Jahn 2010).

Because of this variability, differences in foraging patterns or habitat use by prey, or in the size, location, or type of prey eaten by harbor seals may have resulted in increased THg concentrations in harbor seals from this study. A greater dependence of seals on prey with greater THg concentrations (*e.g.*, striped bass, northern anchovy, staghorn sculpin) than prey with lesser THg concentrations (*e.g.*, bay goby, Pacific herring) would likely result in the observed differences. Gobies are an important prey source for harbor seals (Torok 1994, Gibble 2011), and increased abundance of recent invasive species, such as the shimofuri goby (*Tridentiger bifasciatus*), that have greater concentrations of THg than other species of gobies also could result in the increased THg in harbor seals from this study (Greenfield and Jahn 2010). Diet data are not available for either time period (2005 to 2007, 2009 to 2010), therefore, it is unknown whether the diet of harbor seals differed between 2005/2007 and 2009/2010. Differences in diet

between these periods is feasible given that harbor seals are generalist predators and that the diet of seals in SFB changed between the early 1990s and late 2000s, with an increased dependence on invasive species in later years (Torok 1994, Gibble 2011). Increased THg concentrations in seals from this study also could be the result of interannual variation in prey THg concentrations, particularly if prey had greater THg concentrations during the years of this study than the previous study (Brookens *et al.* 2007). A greater dependence by harbor seals on prey with increased THg concentrations, or a combination of these factors also could be responsible for the observed differences.

Collection and analysis techniques differed slightly between this study and Brookens *et al.* (2007), which could have contributed to the observed differences. Total Hg concentrations in hair samples differed with location in this study (*e.g.*, neck *vs.* mid-dorsal), therefore, it is not easy to compare samples collected in this study (neck) with those from the previous study (pelvic region). Samples from northern California were included in Brookens *et al.* (2007), whereas samples from northern California were not collected in this study. Whereas different methods (DMA-80 vs. AAS) and labs were used to determine THg concentrations, it is unlikely that this had a significant effect as the concentration of Hg measured was relatively high, differences in concentrations measured among labs and between methods were relatively low (Butala *et al.* 2006), and quality assurance/control criteria were met for both studies. Discrepancies in data analysis methodology may have contributed to the difference in THg concentrations between studies, as samples from Brookens *et al.* (2007) were pooled for all locations in

northern and central California, whereas samples were separated by location for this study. Because no samples were collected from northern California sites in this study, the addition of these data in the previous study may have resulted in decreased mean THg concentrations if THg concentrations in northern California were less than concentrations in other areas. Despite this, it is unlikely that the inclusion of data from northern California significantly affected the mean THg concentration because no significant differences among locations were detected during the previous study.

Multiple factors may have contributed to the increased mean THg concentrations in seals from this study compared with Brookens *et al.* (2007), and given the available data, it is not possible to definitively determine whether this increase was real, an artifact of differences in sampling design and analysis, or a combination of both. Despite this, the maximum concentration of THg measured in this study was 52 μg/g greater than concentrations from the previous study (Brookens *et al.* 2007), indicating that it is unlikely that differences in sample design and analysis were solely responsible for the increase. This issue deserves further attention because increases in THg concentrations, whether interannual or long-term, places seals in this region at greater risk for Hg toxicosis, and also has implications for water quality standards and the choice of target species used to determine and evaluate the effectiveness of these standards.

Fine-scale locational differences in trace element concentrations often are difficult to detect and previously have not been found for harbor seals off central and northern California (Brookens *et al.* 2007). Differences among sites were detected in this study,

with increased mean THg concentrations in seals captured in SFB and Tomales Bay compared with Elkhorn Slough. These differences were likely the result of increased Hg input due to historical gold and mercury mining activities in the Sierra Nevada and Coast Range. Although atmospheric inputs, storm-water runoff, and geologic weathering also contribute to Hg input, the fluxes into SFB are largely dominated by contamination from historic mining (Conaway *et al.* 2003, Alpers *et al.* 2005). Data regarding Hg concentrations are lacking for the Elkhorn Slough/Monterey Bay region; however, Hg concentrations in sediment and fish tissues were less in Monterey Bay compared with samples from SFB (Meador *et al.* 1998, 2005).

The lack of difference in THg concentrations in harbor seal hair between SFB and Tomales Bay may be a combination of similar levels of environmental contamination and movement of seals between these regions. Whereas harbor seals exhibit strong site fidelity to several resting (haul-out) sites and make predominantly short-distance movements, long-distance movements among sites does occur (Harvey 1987, Yochem *et al.* 1987, Torok 1994). Torok (1994) found that seals tagged in SFB made frequent trips to the outer coast, traveling south to Pillar Point and north to Point Reyes. Eleven seals tagged in Tomales Bay and five seals tagged in SFB used haul-out sites in other locations; however, the majority of individuals moved among local haul-out sites (< 10 km; Harvey and Goley 2011). Nickel (2003) found that 9 out of 10 seals tagged in SFB used areas within 10 km of a known haul-out site, and foraged one to five km from this site. Seals in Elkhorn Slough mainly moved and foraged within Monterey Bay, although one female

seal tagged in Elkhorn Slough moved north to SFB, presumably to pup (Oxman 1995, Trumble 1995, Eguchi 1998, Greig 2002). Whereas it was possible for harbor seals sampled in this study to travel among all site locations, previous telemetry work indicated that overlap of foraging habitat was more likely for seals using SFB and Tomales Bay.

Differences in THg concentrations between sexes also were found in this study, with males at all locations having greater concentrations than females. Differences in THg concentrations with sex in hair of pinnipeds varies (*e.g.*, Gray *et al.* 2008, Aubail *et al.* 2011), and may be species-specific given that life history characteristics differ among pinnipeds. Brookens *et al.* (2007) also detected increased THg concentrations in hair of adult males compared with adult females, which was attributed to a female's ability to offload Hg to her pup during fetal development and lactation. Although Wenzel *et al.* (1993) found greater mean concentrations of THg in hair of female harbor seals found dead along the northern coast of Germany, differences between sexes were not significant because of increased variability in THg concentrations. Because adult females were able to transfer Hg to their developing fetuses (Wagemann *et al.* 1988), this likely contributed to the observed difference between males and females in central California; however, it also is possible that other factors may have contributed to this difference.

Additional factors that may have resulted in the observed differences in THg concentrations between male and females include physiological differences that affect Hg toxicodistribution and differences in foraging behavior. There was no consistent pattern with sex in the relationship between THg and SL (*e.g.*, THg concentrations of males >

females at all size classes). Therefore, it is more likely that foraging differences, and not physiology, may have contributed to the observed differences in THg concentrations between males and females. The foraging behavior of pinnipeds often differs with sex and tends to be more pronounced in species with extreme sexual dimorphism (Le Boeuf *et al.* 1993, Beck *et al.* 2003). Foraging behavior may vary with time, location, depth, prey type, and prey size, but determining differences between sexes is difficult, though not impossible, using traditional diet methods such as scat collection.

Stable isotope analysis has proved useful in detecting differences in diet among age classes and between sexes as certain elements have multiple isotopes that vary predictably as they cycle through an ecosystem (Peterson and Fry 1987, Newsome *et al*. 2010). Nitrogen is typically representative of trophic level, with increasing $\delta^{15}N$ values indicative of increasing trophic levels. Additionally, $\delta^{15}N$ values also change with latitude and nutritional status (Newsome *et al.* 2010). Carbon often is used to determine the source of primary production input, and nearshore areas tend to have increased (less negative) $\delta^{13}C$ values compared with offshore areas, and temperate systems are more enriched than high latitude systems. The use of sulfur stable isotopes to distinguish the contributions of different producers to food webs primarily has been confined to estuarine systems, and sulfur is not often measured in marine mammals (Connelly *et al.* 2004). As a general rule, marine sources of primary productivity tend to be more enriched in ^{34}S (greater $\delta^{34}S$ values) than estuarine and marsh plants, and pelagic, offshore sources tend

to be more enriched than benthic, inshore sources (Peterson *et al.* 1985, Peterson and Howarth 1987, Barros *et al.* 2010).

Differences in stable isotope values with age were detected in seals from SFB. Adults had greater $\delta^{15}N$ values than juvenile animals, potentially indicating adults foraged on species occupying greater trophic levels than juveniles. Oates (2005) found that newly-weaned harbor seals off Monterey primarily fed on shrimp, small schooling fishes, and small cephalopods before switching to larger schooling fishes, cephalopods, and benthic fishes as their foraging and diving skills developed. Newly-weaned pups were not included in my study; however, several diet studies from SFB indicated that seals of unknown age classes and sexes fed on a variety of prey, including several species of gobies, northern anchovy, striped bass, staghorn sculpin, and crangonid shrimp (Torok 1994, Gibble 2011). Germain *et al.* (2011) found that seals between age one and two had lesser $\delta^{15}N$ values in blood than seals greater than two years, which indicated that younger animals were feeding at a trophic level slightly less than older animals.

Enrichment of ^{15}N also is influenced by nutritional status, and $\delta^{15}N$ values tend to increase in fasting animals (Hobson *et al.* 1993, Cherel *et al.* 2005). Nutritional stress may have contributed to increased $\delta^{15}N$ values in this study because adults are at their poorest body condition following breeding when new hair is forming (Coltman *et al.* 1996, Greig 2002). Several seals in this study had $\delta^{15}N$ values equivalent to or greater than values found in hair of polar bears, a predator of seals (Cardona-Marek *et al.* 2009). These $\delta^{15}N$ values were more than 3‰ greater than prey species found in SFB, further

supporting the conclusion that during molt adult seals may be catabolizing their own tissues (Hobson *et al.* 1996, Stewart *et al.* 2004).

Adult seals also had lesser $\delta^{34}S$ values than juveniles, which could be the result of differences in foraging locations or diet during the time period when seals were molting. During molt, adult seals may forage in locations within the bay, whereas juveniles may depend more on foraging areas outside of the bay. Locational foraging differences with age have not been detected in seals tagged in SFB; however, juvenile harbor seals in Prince William Sound had larger home ranges than adults (Lowry *et al.* 2001), and all long distance movements made by subadult harbor seals in Monterey Bay occurred before or during the breeding season (Oates 2005). Juveniles may choose to forage in areas outside of SFB during breeding to reduce competition with adults and also to avoid confrontations with adult males patrolling aquatic territories (Hayes *et al.* 2004, Boness *et al.* 2006). Sulfur stable isotopes also are affected by the protein content of the diet, with a greater trophic shift between prey and consumer with greater protein quality of the prey (McCutchan *et al.* 2003). Despite this, it is unlikely that differences in $\delta^{34}S$ values were the result of greater juvenile dependence on prey with a greater protein content than prey of adult seals because juveniles from other areas had a greater dependence on crustaceans and smaller forage fish than adults (Oates 2005).

More recently, stable isotopes have been used to examine trophic relationships and trace metal transfer (Dehn *et al.* 2006, Cardona-Marek *et al.* 2009), which was the primary purpose of this study. No relationship between THg and $\delta^{15}N$ was detected,

indicating that biomagnification of Hg was not the primary reason for the variation in THg concentrations in harbor seals. Previous studies on the relationship between THg and trophic level produced mixed results. Aubail *et al.* (2011) found a positive correlation between THg and $\delta^{15}N$ in hair of seven species of phocids, although this relationship only was assessed among and not within a species. This relationship may have been driven primarily by the fact that species were from vastly different locations (Antarctic and Arctic) and foraged at a greater range of trophic levels than harbor seals in this study, as the relationship within a species appeared much less clear or even absent. Cardona-Marek *et al.* (2009) found no direct relationship between THg concentrations in hair of polar bears and $\delta^{15}N$ values, although a model including $\delta^{15}N$ and $\delta^{13}C$ was a good predictor of THg concentrations among bears. Mercury concentrations in harbor seal prey species in SFB do not always increase with trophic level, and similar species (*e.g.*, gobies) may have a wide range of Hg concentrations. Spatial variations in Hg concentrations within a species also exist in SFB, which likely contributed to the lack of a relationship between THg concentrations and $\delta^{15}N$ values. There was a weak relationship between THg concentrations and $\delta^{34}S$, which could be the result of locational foraging differences, especially if juveniles were feeding on prey species outside of SFB where Hg concentrations may be less.

Stable isotopes only explained approximately 20% of the variability in THg concentrations in seal hair, and because no differences in nitrogen, carbon, nor sulfur were detected between sexes, other factors likely contributed to the observed differences

between males and females. Total Hg concentrations in hair of harbor seals represents blood concentrations during molt, which follows reproduction and typically spans June and July in SFB. Whereas data on the age- and sex-specific timing of molt for seals in SFB were not available, studies in other regions indicated that juveniles molted first, followed by adult females, and lastly adult males (Thompson and Rothery 1987, Daniel *et al.* 2003, Reder *et al.* 2003). Differences in the timing of molt may ultimately be the result of differences in energetic costs associated with reproduction. Males and females tend to lose mass during reproduction (Coltman *et al.* 1996, Greig 2002); however, the energetic costs associated with lactation may be greater than costs incurred by males while patrolling aquatic territories. Thompson *et al.* (1989) found that female harbor seals in Scotland spent more time at sea during the period before molt, and suggested that the need to feed intensively after weaning a pup outweighed the benefits associated with a slower molt. Harbor seals in the high Arctic displayed a similar pattern, with adult males hauling-out regularly for extended periods of time at the beginning of molt, whereas females spent a greater time at sea following lactation and did not spend extended periods of time hauled-out during molt (Reder *et al.* 2003).

The later occurrence of molt in male harbor seals, coupled with changes in foraging behavior between males and females may result in differences in THg intake. Males could be foraging on different prey than females, or prey concentrations of THg may be increased during the time when adult males are molting. Greenfield *et al.* (2011) found that THg concentrations in the arrow goby (*Clevelandia ios*) in SFB were greatest

in late July and into August, but the timing of seasonal peaks was strongly dependent on location. Kopec and Harvey (1995) found that male harbor seals captured in SFB between May 1989 and September 1992 had greater Hg concentrations in blood than females, indicating that differences in foraging behavior may not be confined to molt. Thompson *et al.* (1998) found that male harbor seals made significantly longer trips than females and that mean foraging ranges were greater for males than females, indicating that foraging differences can exist in the absence of extreme sexual dimorphism. Additional data on sex- and age-specific diet in harbor seals from central California, coupled with fine-scale movement patterns and behavior, THg concentrations, and stable isotope values of prey are needed to elucidate whether foraging differences contributed to the differences in THg concentrations between male and female seals.

In contrast to Hg, Se has not been as extensively studied in marine mammals and often is not measured in studies examining hair concentrations of trace elements in pinnipeds. Selenium concentrations measured in this study ranged from *bdl* to 4.53 μg/g, and mean concentrations in seals at all locations were less than those reported for Weddell seals (*Leptonychotes weddellii*), Baikal seals (*Pusa sibirica*), Caspian seals (*Pusa caspica*), northern fur seals (*Callorhinus ursinus*), and harbor seals from Elkhorn Slough (Moser 1996, Ikemoto *et al.* 2004*b*, Gray *et al.* 2008). The decreased concentrations of Se in seals at all locations and greater concentrations in seals from Elkhorn Slough compared with elsewhere were unexpected, especially because elevated

Se concentrations have been detected in surfscoters and sportfish from SFB (Ohlendorf *et al.* 1986, Greenfield *et al.* 2005).

The molar ratio of Se:Hg may be an important indicator of element status, as Se ameliorates the toxic effects of Hg, therefore, Se:Hg may approach 1:1 in certain tissues of adult animals (Koeman *et al.* 1973, Brookens *et al.* 2007). Deviations from this 1:1 ratio have been observed, and were attributed to several factors including: 1) Hg and Se only may occur in a consistent proportion when a physiologic threshold is surpassed, thereby stimulating Se uptake and binding, 2) adherence to a 1:1 ratio is not necessary to protect against Hg toxicosis, 3) alternative detoxification mechanisms exist, and 4) the Se:Hg ratio may be species- or age-specific (Woshner *et al.* 2001*a, b*; Brookens *et al.* 2007). Molar ratios in my study deviated from this relationship; however, because the majority of Hg present in hair is MeHg and hair is not a likely target tissue for accumulation of a Se-Hg complex, adherence to a 1:1 ratio in hair is not expected. The Se:Hg molar ratios were less in seals from SFB and Tomales compared with Elkhorn Slough, which was expected given increased THg and decreased Se concentrations in seals from SFB and Tomales Bay. The physiologic need for Se in animals from sites with increased Hg may be greater because Se is needed to bind to Hg and maintain normal selenoenzyme activities, thereby resulting in lesser Se concentrations and a lesser Se:Hg ratio in hair.

Marine mammals often accumulate concentrations of trace elements in excess of what is considered safe for humans and domestic animals without any apparent negative

effects. Seals captured in this study appeared in relatively good health with no noticeable signs of illness, indicating that Hg concentrations were not great enough to induce health problems, that Se concentrations were sufficient to mitigate effects of Hg, or that symptoms were not severe enough to be detected *via* external examination. Several threshold limits were used to assess whether seals in this study were at risk for Hg toxicosis, including the mean concentration in hair of polar bears with sub-clinical effects associated with Hg toxicosis (5.4 µg/g dry wt; Basu *et al.* 2009), and the lower (20 µg/g dry wt; Thompson *et al.* 1996) and upper neurological thresholds for fish-eating wildlife (30 µg/g dry wt; Evers *et al.* 2007). Use of these threshold limits were not meant to imply that harbor seals were suffering from Hg toxicosis, but simply to place values in context in the absence of threshold limits for phocids. Because mean THg concentrations in seals from all locations exceeded 5.4 µg/g, seals in central California may potentially be at risk for Hg toxicosis. Seals in SFB are likely the most at risk given that 44% of seals had THg concentrations in hair that exceeded 20 µg/g and 12% had concentrations that exceeded 30 µg/g. Although harbor seals in this study appeared in good health, the amount of Hg they were exposed to may be great enough to induce sub-clinical, and in some instances neurological effects. Pups may be at an increased risk during gestation and lactation *via* milk, as they may represent a sink for Hg, and appear unable to demethylate it as efficiently as adults (van de Ven *et al.* 1979, Wagemann *et al.* 1988, Brookens *et al.* 2008). An assessment of these risks requires further attention and should be evaluated in future studies.

Results from this study do not support the chronic Se toxicosis hypothesis previously suggested by Kopec and Harvey (1995), and decreased concentrations of Se and a Se:Hg molar ratio indicate that seals from SFB and Tomales Bay may potentially be at risk for Se deficiency. Examining Se concentrations in hair is an important first step in determining exposure and whether concentrations are of concern; however, future research should focus on measuring the activity of selenium-dependent proteins, such as GSH-Px, to determine the Se status of harbor seals. This may be especially important as large-scale tidal restoration of salt ponds in south SFB may result in increased Hg input to the estuary and increased MeHg production at these sites (Davis *et al.* 2003). Increased Hg input, coupled with increased MeHg production, could result in increased Hg uptake by seals foraging in south SFB, thereby increasing their risk for Hg toxicosis. As top-level predators and a critical component of the SFB ecosystem, it is important to continue to monitor and understand how future changes in Hg and Se concentrations will affect harbor seals both in the context of Hg toxicosis and/or Se deficiency.

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Chapter II

Red-coated harbor seals (*Phoca vitulina richardii*) in San Francisco Bay: prevalence, potential causes, and health implications

ABSTRACT

A proportion of harbor seals (*Phoca vitulina*) in San Francisco Bay (SFB) accumulate iron on their hair, thereby resulting in a rusty coloration to their pelage. Previous research indicated that selenium (Se) toxicosis may play a role in the development of a red pelage, therefore, the main study objective was to evaluate this hypothesis. Additional objectives were to examine trends with time, whether foraging differences existed between red- and normal-pelaged seals, and whether there were health implications associated with the development of a red pelage. Data were compiled from aerial and land counts to evaluate trends in the proportion of red-coated harbor seals with time. Samples were collected from seals captured in SFB between 2009 and 2011 (n = 57). The proportion of red-coated harbor seals increased between the early 1970s and 1980s, but was relatively stable from 1984 to 2010. Red-coated seals had hair loss (n = 6), shortened vibrissae (n = 5), and nose lesions (n = 6), but were of similar body condition and had similar concentrations of Se in hair as normal-pelaged seals. Differences in blood variables (cholesterol, phosphorous, MCV, and SDH), mercury concentrations (females), and chemical measures of feeding ecology (nitrogen and sulfur stable isotopes) were detected between red- and normal-pelaged seals. Results do not support previous suggestions that chronic Se toxicosis predisposed seals to developing a red pelage and instead support the conclusion that exposure to iron in sediments due to increased dependence on benthic prey may be the cause. There do not appear to be any serious short-term health implications associated with a red pelage.

INTRODUCTION

Population censuses of harbor seals in San Francisco Bay (SFB) began in the late 1960s (Fancher 1979, Risebrough *et al.* 1980), and in 1968 the first known observation of "rust-colored" seals was documented (Paulbitski 1971 as cited in Kopec and Harvey 1995). Allen *et al.* (1993) found that between 1979 and 1985, red-coated harbor seals accounted for 4 to 32% of the total number of seals counted, and suggested that the prevalence of red-coated harbor seals in SFB was one of the greatest world-wide. Between 1989 and 1992, greater than 20% of seals at the seven primary haul-out sites in SFB had a red pelage (Kopec and Harvey 1995). Red pelage was observed equally among all sex and age classes, with the exception of pups (Allen *et al.* 1993, Kopec and Harvey 1995). Allen *et al.* (1993) reported that the percentage of red-coated harbor seals was greatest at haul-out sites in north SFB; however, Kopec and Harvey (1995) observed a greater proportion of red-coated seals at haul-out sites in south SFB. The percentage of red-coated harbor seals increased until the time of molt (late June to early August), with some harbor seals completely red by June (Kopec and Harvey 1995).

Red-coated harbor seals have been observed in other locations and red pelage has been documented in several other phocids. A lesser number of red-coated harbor seals have been observed at haul-out sites in nearby Tomales Bay (pers. observ.) and also at a haul-out site in Humboldt County (Neumann and Schmahl 1999). Lydersen *et al.* (2001) found that bearded seals (*Erignathus barbatus*), and a lesser number of ringed seals (*Phoca hispida*), in Norway had a rusty coloration on their faces and foreflippers.

Red pelage is not believed to be a normal color morph in harbor seals because the red coloration disappeared following molt, the number of red-coated seals increased until the time of molt, and the spatial area of the body covered by red pelage progressed posteriorly until the time of molt (Allen *et al.* 1993, Kopec and Harvey 1995). Normal pelage in harbor seals ranges from a dark or light background with various dark or light spotting patterns. Allen *et al.* (1993) suggested that the red coloration was not the result of diet or algal growth, and scanning-electron-microscopy (SEM) micrographs of the hair shaft revealed layered deposits that were not present on hair of normal-pelaged seals (Allen *et al.* 1993, Moser 1996). Elemental analyses revealed that the red was the result of iron oxide precipitates on the hair shaft (Allen *et al.* 1993); however, another study in this region found similar amounts of iron on hair shafts of red-coated and normal-pelaged seals (Moser 1996). Lydersen *et al.* (2001) found increased concentrations of iron, vanadium, and manganese on hair of red-coated bearded and ringed seals. Whereas all the above studies had small sample sizes ($n \leq 4$), it was generally agreed that one or more elements were responsible for the red coloration.

The mechanism of element deposition may differ among species and/or areas and is not entirely understood. Allen *et al.* (1993) suggested that conditions unique to SFB allowed for the resuspension of sediments that were then deposited on the pelage of seals by either flocculation or precipitation. This suggestion was criticized by Neumann and Schmahl (1999) as unlikely because this would require supersaturation of the water by iron, which would likely result in widespread precipitation of iron oxides on haul-out

sites and beaches. They suggested that the red coloration on harbor seals hauled-out at the Mad River in Humboldt County was the result of direct contact with sediment on the haul-out site that contained increased levels of iron oxide and hydroxide particles. The deposition of elements on the pelage of bearded and ringed seals in Norway was believed to be from direct contact while feeding in soft-bottom sediments (Lydersen *et al.* 2001). Lydersen *et al.* (2001) suggested that while feeding, the face and flippers of seals came into contact with rich deposits of iron monosulfide that oxidized when exposed to the air. There were a greater number of red-coated bearded than ringed seals, which was explained by the greater dependence of bearded seals on benthic prey (Lydersen *et al.* 2001).

Few researchers have investigated why some seals develop a red pelage and others do not. Kopec and Harvey (1995) suggested that chronic selenium (Se) toxicosis may play a role in the development of a red pelage by causing degradation of the hair shaft, thereby providing a greater surface area for element deposition. Although selenium toxicosis has not been documented in free-ranging marine mammals, symptoms of chronic Se toxicosis were similar among other species and included emaciation and changes to keratinized tissues (Raisbeck *et al.* 1993; O'Toole and Raisbeck 1995, 1997). Selenium toxicosis was implicated as the causative agent in the deaths of three captive California sea lions (*Zalophus californianus*); however, this diagnosis was based on comparisons with concentrations associated with toxicosis in livestock (cattle, sheep, and horses; Edwards and Whitenack 1989), which may not be valid. Concentrations of Se in

the liver and kidney of free-ranging pinnipeds were similar or greater than concentrations reported by Edwards and Whitenack (1989), therefore, it is unlikely that Se toxicosis was the causative agent in the the deaths of these captive sea lions.

Selenium is an essential element that is an important component of many proteins (Behne and Kriakopoulos 2001) and may provide a protective effect against mercury (Hg) toxicosis (Cuvin-Aralar and Furness 1991, Yang *et al.* 2008). One of the primary mechanisms that Se mitigates the toxic effects of Hg (and *vice versa*) is through the formation of a non-toxic Se-Hg complex (Yoneda and Suzuki 1997, Wang *et al.* 2001, Ikemoto *et al.* 2004). Previous researchers have suggested that the Se:Hg molar ratio may be a more appropriate measure than total concentrations of Hg when assessing potential toxicity (Ralston *et al.* 2008). The use of this ratio only may be appropriate for certain tissue types given that this complex does not appear to accumulate in all tissues (*e.g.*, Dietz *et al.* 2000).

Assessing the elemental status of free-ranging pinnipeds is difficult because the range encompassing deficiency and toxicity for Se and Hg is unknown. Therefore, researchers often make comparisons among regions to determine the potential risk for Se and/or Hg toxicosis. Increased Se concentrations in whole blood were detected in harbor seals from SFB compared with seals from Monterey County, Puget Sound, and San Nicolas Island (Kopec and Harvey 1995). Moser (1996) found no significant difference between Se concentrations in liver and hair of red- and normal-pelaged seals, but sample size thus power were minimal. Some red-coated harbor seals also had shortened

vibrissae and noticeable hair loss around the eyes, face, throat, and body (Kopec and Harvey 1995). Shortened vibrissae and hair loss were not observed in seals with normal pelage (Kopec and Harvey 1995), nor have they been reported in red-coated seals from other locations. Despite the fact that Se toxicosis has not been documented in free-ranging marine mammals, the increased Se concentrations and changes to keratinized tissues warrant investigation of the hypothesis that chronic Se toxicosis may predispose seals in SFB to developing a red pelage.

Whereas several studies have addressed the phenomenon of red-coated harbor seals in SFB, it remains unclear as to why some seals develop a red pelage, what the exact method of iron accumulation is, and whether there are significant health implications associated with the development of a red pelage. The objectives of this study were to: 1) compile historic and current data on the prevalence of red-coated harbor seals in SFB, 2) describe and document changes to keratinized tissues of red-coated harbor seals, 3) evaluate hematology and serum chemistries of red-coated and normal-pelaged seals from SFB, 4) definitively determine whether iron is the direct cause of the red pelage, and 5) evaluate the hypothesis that chronic Se toxicosis is a contributing factor in the development of a red pelage. Specific hypotheses included: 1) red-coated harbor seals would have changes to keratinized tissues, including hair loss, shortened and brittle vibrissae, and greater damage to hair shafts than normal-pelaged seals, 2) differences in hematology between red-coated and normal-pelaged seals would exist, with red-coated seals showing signs of decreased immunity or immune stress, 3) red-

coated seals would have increased concentrations of iron on their hair shafts compared with normal-pelaged seals, and 4) red-coated seals would have increased Se concentrations and/or an increased Se:Hg molar ratio in hair compared with normal-pelaged seals, and that histologic lesions typical of selenium toxicosis (Green and Albers 1997) would be present in areas of hair loss in red-coated seals.

METHODS

Prevalence

Data on the prevalence of red-coated seals at the three main haul-out sites in SFB (Castro Rocks, Yerba Buena Island (YBI), and Mowry Slough; Fig. 1) were compiled from previous publications and reports (Fancher 1979, Risebrough 1980, Allen *et al.* 1993, Kopec and Harvey 1995, Green *et al*. 2006) and from recent, unpublished counts conducted by The Marine Mammal Center and the National Parks Service (2007 to 2010; Castro Rocks and YBI), and myself (2009 to 2010; Mowry Slough). The resulting dataset included counts conducted between 1972 and 2010. There were occasional reports of red-coated seals from other haul-out sites within SFB, but these observations only spanned one to several years, therefore, were not included in the dataset. Counts conducted for the purposes of this project were in accordance with permits (IACUC #2009-E, US Fish and Wildlife Service, 2009-041) and authorizations (CalTrans, US Coast Guard, Cargill Salt Company).

Data on the number of seals hauled-out were primarily from land counts, with the exception of counts conducted *via* aerial surveys between 1979 and 1984. During land counts, the total number of seals hauled-out and the number of seals with red pelage were determined using binoculars or a spotting scope from nearby vantage points. Counts were conducted at Castro Rocks and YBI from an observation area above the haul-out site, whereas counts at Mowry Slough were conducted from levees bordering the slough. These levees had to be accessed through the Cargill Salt Company or the TriCities Sanitary Waste and Recycling Facility and could not be accessed during or several days after periods of moderate to heavy rain. Surveys at Castro Rocks and YBI primarily were conducted at low tide, whereas surveys at Mowry Slough were conducted at varying tide levels depending upon the study and time of year. Counts at Castro Rocks were conducted aperiodically (1979 to 1980, 1984, 1989 to 1992), monthly (1974 to 1975), bimonthly (2007 to 2010), and several times per week (1999 to 2005), whereas counts at YBI were conducted aperiodically (1989 to 1992), several times per week (1999 to 2005), and bimonthly (2007 to 2010). Because of the logistical difficulty in accessing Mowry Slough, counts were conducted aperiodically (1972 to 1975, 1979, 1980, 1984, 1989 to 1992), several times per month (1999 to 2005), and bimonthly (2009 to 2010).

Data are presented as the proportion of red-coated seals to the total number of seals hauled-out. Pups were not included in the total count for data collected between 2007 and 2010. When multiple surveys were conducted per year, the mean proportion and standard error (SE) were calculated. Counts conducted at Mowry Slough represent a

minimum number of red-coated seals as the observer distance from the haul-out site is greater than the other two sites and seals are frequently covered in mud, which obscures the red coloration. Because harbor seals molt in late June through early August, counts for any given year were from August to July of the following year. For example, data presented for 2009 represent counts conducted in August 2009 through July 2010. Raw count data from 1989 to 1992 (Kopec and Harvey 1995) and 1999 to 2005 (Green *et al.* 2006) were not available, therefore, the mean for each year range was used. Counts conducted between 1999 and 2005 classified Newark Slough and Mowry Slough as one haul-out site (termed Mowry Slough), despite the fact that these two areas typically are considered separate haul-out sites. This may have resulted in an overestimation of the proportion of red-coated seals present as Kopec and Harvey (1995) found that a greater proportion of harbor seals hauled-out out at Newark Slough were red; however, it was not possible to separate counts from the two sites. Counts only were included in the dataset if greater than five animals were present at the haul-out site, except for data collected between 1989 and 1992 because that information was not available (Kopec and Harvey 1995).

Sample Collection

Harbor seals were captured in SFB as described in Chapter I. Samples in addition to those described in Chapter I (hair, morphometrics) were collected, including vibrissae and vibrissae measurements, blood samples, and skin biopsies. Individuals with a red

pelage were photographed, and the severity of hair loss and amount of red coloration was determined as percentage cover for each individual. Location of hair loss and other lesions also were documented with photographs.

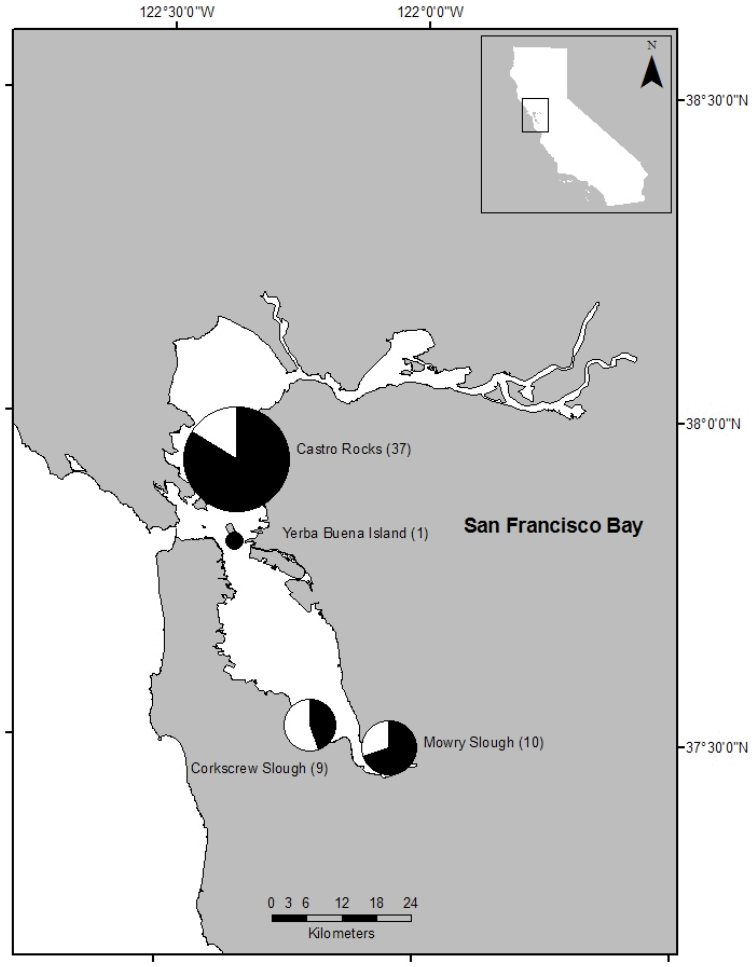

Figure 1. Capture locations and the number of harbor seals captured in San Francisco Bay between 2009 and 2011. Circles are proportional to sample size, and samples sizes are listed in parentheses following the haul-out site. The number of red-coated seals (white) in relation to the number of normal-pelaged seals (black) is presented.

Three vibrissae were measured from the left and the right side of the face from a pre-selected location. Every attempt was made to measure vibrissae from the same location on each individual. This was not always possible because seals were alert and often tried to bite. One vibrissa was collected by cutting as close to the base as possible, and vibrissae were stored in polyethylene bags in a -80°C freezer at Moss Landing Marine Laboratories (MLML).

Blood samples were collected from the extradural invertebral sinus with an 18 gauge sterile needle into one 3.5 ml BD Vacutainer® tube containing a clot activator, and one 3 ml BD Vacutainer® tube containing an anti coagulant (K_2EDTA). Every effort was made to obtain one tube type for each seal captured; however, this was not always possible due to difficulty in locating the invertebral sinus, or because of concern about stress to the individual. Blood samples were stored on ice until transported to The Marine Mammal Center (TMMC) in Sausalito, CA, where they were refrigerated until processed.

One 6 mm sterile biopsy punch of the dermis and epidermis was collected from the left side of the neck and stored in a container with 10% buffered formalin. The neck was chosen as a biopsy site as this was a likely region where red pelage would occur, and was safer to biopsy than an area closer to the face. An additional biopsy punch was taken from individuals with hair loss or other lesions at the interface (margin) of the area of alopecia/lesion when possible. It was not possible to biopsy individuals with hair loss

around their face as this would have caused undue pain to the seal and a danger to the individual collecting samples.

All statistical analyses were conducted using PASWStatistics (version 18.0, IBM, 2010) and assumptions of parametric tests were met before analysis unless otherwise stated. All results are presented using untransformed data unless otherwise stated.

Morphometric Analyses

Differences in body condition with coat color were analyzed with an Analysis of Covariance (ANCOVA). The residuals from the regression of mass (kg) versus standard length (cm; SL) were used as an index of body condition (BCI), as the residuals represent all of the variability in mass not explained by length. Additional individuals captured outside of the study period (n = 17; July 2011) were included in the body condition assessment to increase sample size. Month of capture was considered as a covariate because body condition of adult harbor seals fluctuates throughout the year due to reproductive costs (Coltman *et al.* 1996, Greig 2002). Males and females were analyzed separately because of a significant sex*coat interaction, and the BCI for males was log transformed to meet assumptions of equal variances.

Differences in vibrissae length with coat color were assessed using a Welch's one-tailed independent samples t-test. The mean of the two outermost vibrissae (left and right side) was used for each individual as these were the longest, easiest, and most

consistently measured vibrissae. A Welch's *t*-test was used because of unequal variances that could not be corrected with transformations.

The brittleness of vibrissae were analyzed using a custom-built device at MLML designed to test the force (g) required to break an individual vibrissa. Length (mm), width (mm), and thickness (mm) of each vibrissa were measured using digital calipers, as vibrissae were of varying lengths. The difference in strength of vibrissae with coat color was analyzed using an ANCOVA, with coat color as a fixed factor and height as a covariate. Height of vibrissae was chosen as the only covariate because it explained a significant amount of variation in force, and explained more variability than the other two measurements (all three were strongly correlated).

Blood Analyses

Blood samples were analyzed for hematology and serum chemistries within 24 to 48 hours after collection. The only exception to this was blood from two individuals that was accidentally frozen before analysis, therefore, serum chemistries were conducted on serum that had been frozen for approximately two months. Two seals were recaptured during the course of this study, one within the same year (ID# 1781/1780), and one the following year (ID# 1764/1754). Blood was not collected a second time from 1781/1780, although blood was collected both times from 1764/1765. To avoid pseudoreplication, blood collected in 2010 from 1764/1765 was excluded from the dataset because it was lipemic, which can affect blood parameter values (see below).

Before analyzing differences in coat color, blood variables were assessed to determine if samples that were hemolyzed, lipemic, or clotted affected any of the blood values. Hemolyzed samples have ruptured red blood cells that have leaked into the surrounding fluid. The level of hemolysis varies, and is characterized by pale to cherry red serum. Lipemic samples have an excess of lipids in the blood, characterized by milky white serum. Hematology parameters were analyzed using a three-factor ANOVA with hemolyzed, lipemic, and clots as fixed factors after testing for homogeneity and heterozygosity. If the assumptions were not met, a non-parametric bootstrap analysis using the R programming language (http://www.r-project.org; R Development Core Team, 2011) was used. Ninety-five percent confidence intervals (CI) were created from data of seals whose blood samples were not hemolyzed, lipemic, or clotted. Mean values for each parameter of hemolyzed, lipemic, and clotted samples were calculated, and if they fell within the 95% CI values for that parameter, were retained in the dataset. Serum chemistries were analyzed using the same method, with the exception that lipemic and hemolyzed were the only fixed factors.

Blood variables of subadult and adults were analyzed with ANCOVA and Analysis of Variance (ANOVA). Pups and yearlings were excluded from the analysis to minimize difference in blood variables with age, and because no red-coated pups were captured. Fixed factors included sex and coat color, and SL and BCI were included as covariates as needed. Standard length and body condition were chosen as covariates because age and body condition affect some blood parameters (Greig *et al.* 2010).

Season can affect certain blood variables (Kopec and Harvey 1995, Trumble *et al.* 2006); however, neither season nor date of capture were included in the analysis because the majority of captures in SFB occurred during winter, with only six animals caught in May and June. Neutrophils (band) were not included in the analysis because of a large number of zeros in the dataset. Males and females were analyzed separately for mean cell volume (MCV) and total iron because of a significant sex*coat color interaction.

Blood parameters were grouped into related dependent variables (hematology, protein, energy, minerals, kidney function, liver function, and other) to assess condition within different physiological systems (Hall *et al.* 2007). As described by Hall *et al*. (2007), these groupings were not intended to be diagnostic in veterinary terms, but to allow for the interpretation of trends with respect to coat color.

Skin Biopsies

Skin biopsies were analyzed for all red-coated seals and a randomly selected subset of biopsies from normal-pelaged seals that matched sex/age class combinations of red-coated seals. Biopsy samples were prepared at the California Department of Fish and Game (CDFG), and sent to UC Davis to be embedded in paraffin, sectioned, and stained. Samples were stained for hemotoxylin and eosin, Pearl's (iron), Fontana Masson (melanin), Von Kossa (calcium), and copper. Slides were read by Dr. Melissa Miller, a pathologist at CDFG. Slides were initially read without knowing sex, age, animal ID, or coat color.

Hair Analyses

The structure of the hair shafts of red-coated seals and a randomly selected subset of hair shafts from normal-pelaged seals that matched sex/age class combinations of red-coated seals was examined using a SEM (S-3400N, Hitachi). Hair shafts were rinsed in MilliQ® water to remove any sand or mud present on the sample, dried, and placed on a SEM stub. Because of the possibility that the structure of the hair shaft would be obscured by deposits (*e.g.*, iron), hair shafts were cleaned with approximately 10 mL of 30 mM ascorbic acid, and placed in a sonicator for one hour (Moser 1996). Samples were subsequently rinsed with MilliQ® water, dried, and placed on the same stub as the uncleaned hair. A mark was used to divide the stub so that it was possible to determine which samples had been cleaned. Each stub was labeled with the seals ID number, and contained approximately five to ten hairs of each type (cleaned and uncleaned). Stubs were gold-coated, and individually cleaned hair shafts were examined for signs of damage, including upturned cuticle edges and pitting (Wyatt *et al.* 1972, Weisel *et al.* 2005).

The external composition of elements on the hair shafts was determined using X-ray microanalysis (20 mm^2 X-Max SDD, Oxford Instruments). Hair shafts were treated as described above for the uncleaned hair, and three shafts from each individual were placed on stubs that were subsequently gold-coated. The composition of external elements was determined for an approximate 100 x 100 μm square of hair, and a mean value was calculated for each individual seal. Differences in the amount of sulfur, silica,

and aluminum with coat color were determined using a two-tailed independent samples *t*-test. Differences in the amount of iron with coat color were assessed with a one-tailed one sample *t*-test with zero as the comparison value because iron was not detected on hair samples from normal-pelaged seals. Differences were not analyzed for magnesium, chloride, phosphorous, calcium, bromide, and copper as these elements were measured in only one or two animals. The relationship between the amount of iron on hair and the percentage of the body covered with red pelage was assessed using linear regression.

Hair samples were analyzed for total mercury (THg), selenium (Se), and stable isotopes (nitrogen, carbon, and sulfur) as described in Chapter I. Because there were no differences in hair THg and Se concentrations between seals from SFB and Tomales Bay (Chapter I), samples from these locations were combined. Pups were removed from the dataset as they were underrepresented ($n = 3$) and no red-coated pups were captured. Differences in THg concentrations were analyzed separately for each sex because the assumptions of ANCOVA were not met when sexes were combined. Coat color was considered a fixed factor, with SL and Se included as covariates. An ANCOVA was used to determine whether Se or Se:Hg differed with coat color, with SL and THg (Se analysis only) included as covariates. Differences in stable isotopes with location (north *vs*. south SFB), coat color, and sex were analyzed using ANCOVA.

RESULTS

The greatest number of red-coated seals were observed at Castro Rocks (n = 69; 2007 to 2010), followed by YBI (n = 33; 2007 to 2010) and Mowry Slough (n = 17; 2009 to 2010). The greatest proportion of red-coated seals were observed at Castro Rocks, followed by Mowry Slough, and YBI (Fig. 2). The mean proportion of red-coated seals for all years was 0.19 (SE = 0.034; Castro Rocks), 0.17 (0.03; Mowry Slough), and 0.097 (0.02; YBI). There was an increasing trend in the proportion of red-coated harbor seals at Castro Rocks between the early 1970s and 1980s, with the proportion increasing from approximately 0.01 in 1974 to 0.4 in 1984. The proportion of red-coated seals at Castro Rocks stabilized around 0.2 from the late 1980s onward, although there was variability among years. The proportion of red-coated seals at YBI was relatively constant across years, with the exception of 1999 to 2005 when the proportion increased to 0.2. An increasing trend also was observed at Mowry Slough, although the proportion appeared to stabilize around 0.2 in 1984, with the exception of 1999 to 2005 when the proportion of red-coated seals increased above 0.3 (Fig. 2).

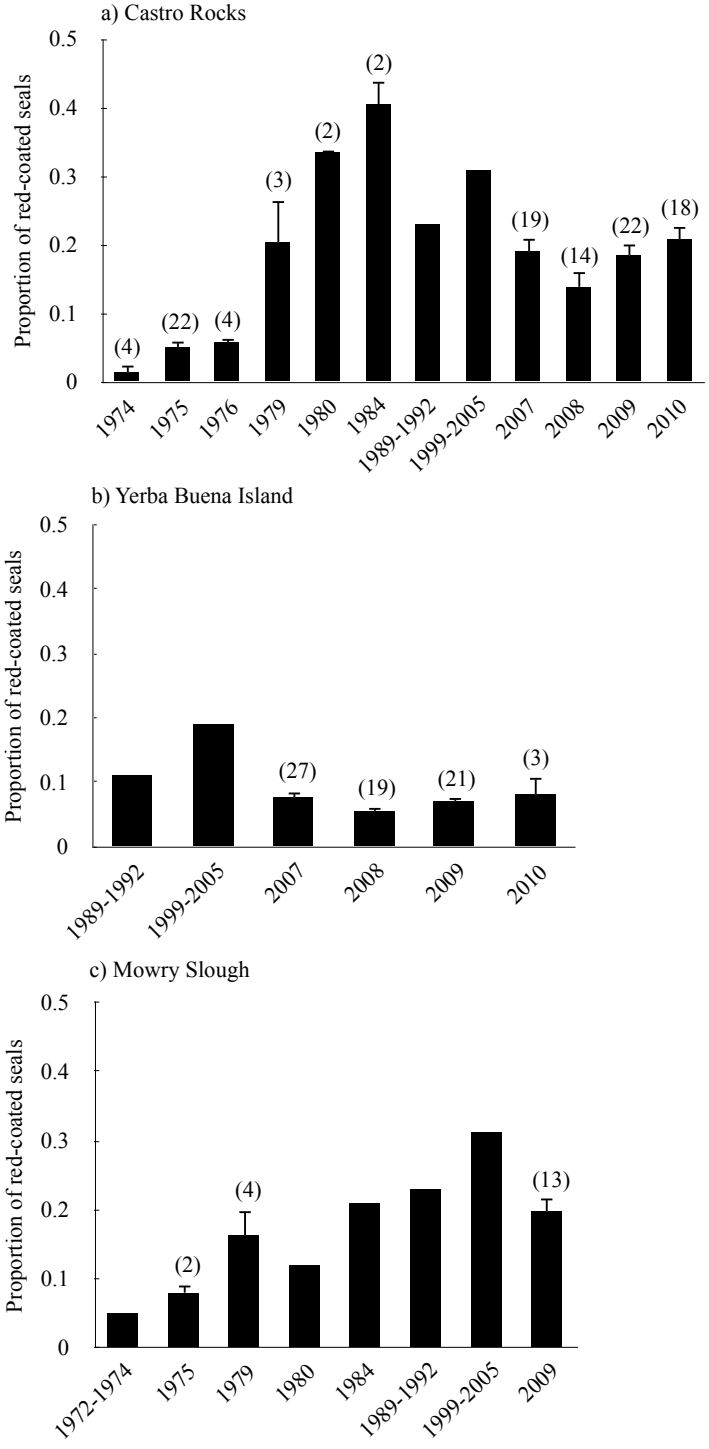

Figure 2. The proportion of red-coated harbor seals to the total number of seals present ashore through time (1972 to 2010) at Castro Rocks (a), Yerba Buena Island (b), and Mowry Slough (c). The mean proportion, sample size, and associated standard error (black line) are presented when available.

Fifty-seven harbor seals were captured in SFB, of which 14 individuals had red pelage (Fig. 1). Additionally, 56 seals were captured in nearby Tomales Bay, of which one individual had red pelage. One seal was captured in successive years and at both times had a red pelage. The greatest proportion of red-coated seals in SFB were captured at Corkscrew Slough (0.55), followed by Mowry Slough (0.30) and Castro Rocks (0.16). The percentage of red pelage varied from 5 to 100%, and appeared to begin around the head, neck, and foreflippers. Some red-coated seals had noticeably shortened vibrissae (n = 6; Fig. 3) and hair loss (n = 5; Fig. 4). Hair loss typically occurred around the face, although one individual had patchy hair loss over much of her body. Red-coated seals (n = 6) also had lesions on their noses of varying severity not believed to be associated with capture (Fig. 4).

Figure 3. Shortened (left) and normal-sized vibrissae (right) in harbor seals from San Francisco Bay.

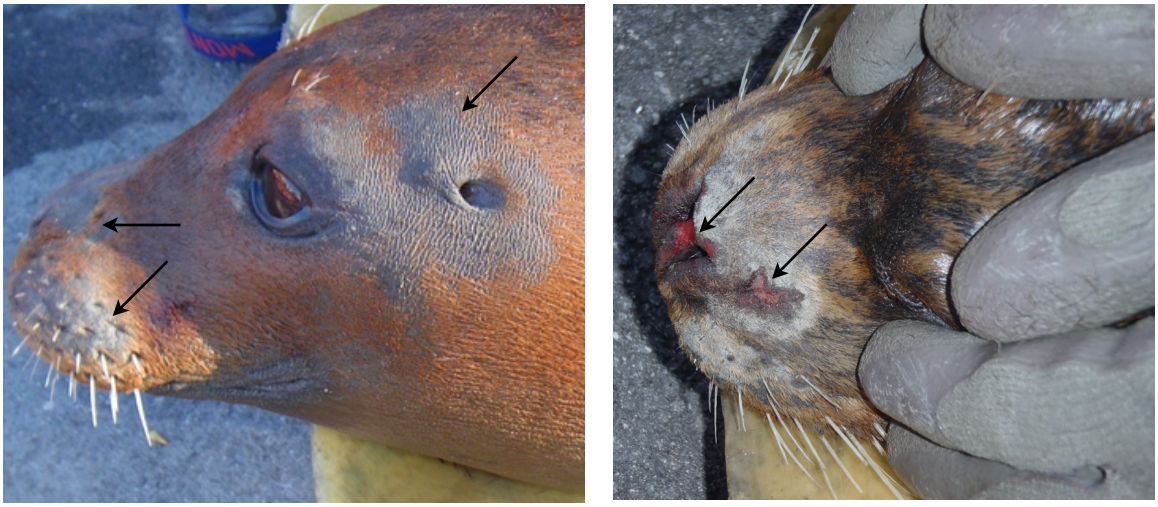

Figure 4. Hair loss (left) and nose lesions (right) from two red-coated seals captured in San Francisco Bay. Arrows point to affected areas.

Several morphometric measurements differed with coat color. Whereas the body condition of females did not differ with coat color ($F = 0.200$, $P = 0.656$), body condition was greater in red-coated than normal-pelaged males ($F = 8.425$, $P = 0.012$). Vibrissae of red-coated seals were shorter ($\bar{x} = 6.4$ cm ± 1.3 (SE), $t = 2.331$, $P = 0.0165$) than normal pelaged seals (9.6 cm ± 0.4), but did not require less force to break ($F = 0.02$, $P = 0.969$).

Hematology ($n = 43$) and serum chemistry variables ($n = 46$) were analyzed for adult and subadult seals from SFB, and one red-coated seal from Tomales Bay. Blood variables that were affected by hemolysis included: creatine kinase, chloride, blood urea nitrogen (BUN), triglycerides (TRIG), red cell distribution width, mean cell hemoglobin concentration (MCHC), and glucose (GLU). Blood variables that were affected by lipemia included: BUN, total protein, TRIG, MCHC, and globulin. Total iron values

were affected by samples that were hemolyzed and lipemic. Platelets were the only variable affected by clotted blood.

There were four blood variables that differed with coat color (Table 1). Mean values of total cholesterol, phosphorous, and sorbitol dehydrogenase (SDH) were less in red-coated than normal-pelaged seals. Female red-coated seals had greater MCV values than normal-pelaged females; however, the reverse relationship was true for males.

Table 1. Blood variables for adult and subadult seals from San Francisco Bay that differed with coat color. Estimated marginal means ± standard error (SE), and sample size (*n*) are presented. Values for females (F) and males (M) are presented when appropriate. Only variables with $P < 0.1$ are shown.

Blood variable	$\bar{x}$ ± SE		*P* value
	Normal *n* = 17 (F), *n* = 9 (M)	Red *n* = 8 (F), *n* = 2 (M)	
Hematology			
MCV (fl)	118.16 ± 0.69 (F) 119.60 ± 0.98 (M)	121.27 ± 1.12 (F) 115.52 ± 1.70 (M)	0.027 (F) 0.068 (M)
Energy			
Total cholesterol (mg/dl)	211 ± 6.17	180 ± 9.3	0.009
Kidney function			
Phosphorous (mg/dl)	5.05 ± 0.36	3.76 ± 0.56	0.060
Liver function			
SDH (U/l)	41.83 ± 4.71	24.74 ± 7.44	0.061

External damage to the hair shaft was observed in all hair shafts, regardless of coat color. The type of damage included cuticular scaling, erosion and splitting (longitudinal erosion), and breakage of the hair shaft (Fig. 5). There were no apparent trends in damage with respect to coat color.

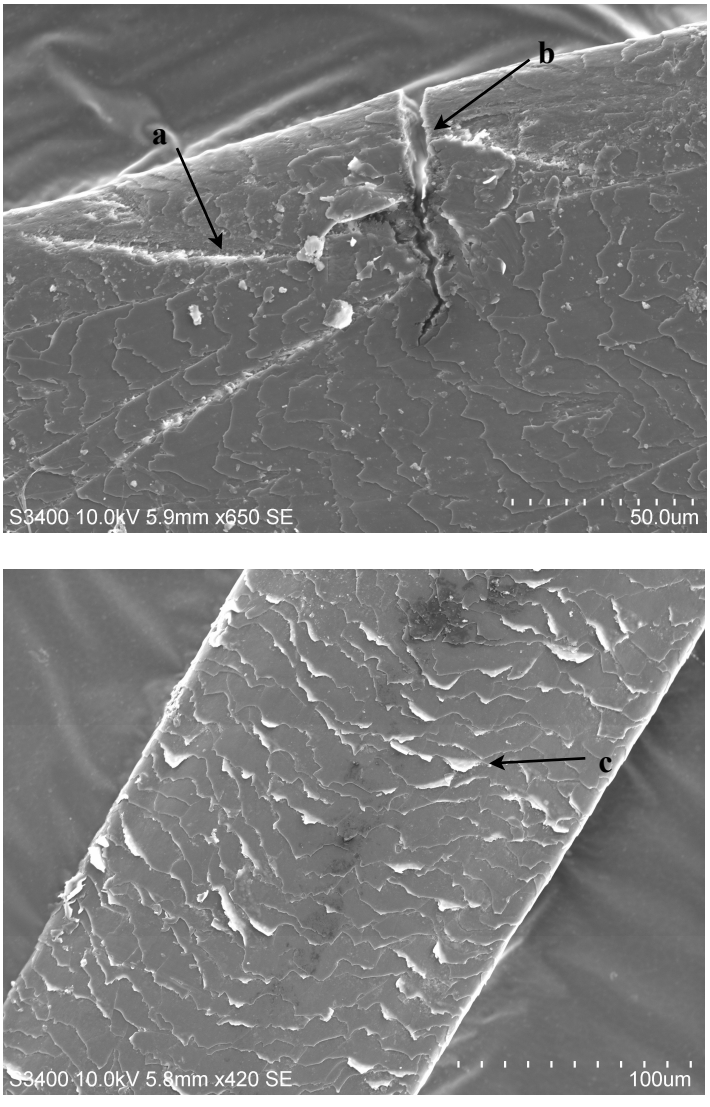

Figure 5. Examples of damage observed in harbor seals hairs including erosion (a), breakage (b), and cuticular flaking (c).

Twelve elements were detected on harbor seal hair shafts, with carbon and oxygen comprising the greatest percentage weight of all detected elements. In general, more elements were detected on hair shafts of red-coated seals than normal-pelaged seals (Fig. 6). No differences with coat color were detected for sulfur ($t = 0.631$, $P = 0.538$), silica ($t = -1.635$, $P = 0.124$), or aluminum ($t = -1.235$, $P = 0.237$). The amount of iron on hair shafts of red-coated seals was greater than zero ($t = 2.661$, $P = 0.0149$; Fig. 6), and there was a significant relationship between the amount of iron on hair shafts and the percentage of the body covered by a red pelage ($P = 0.045$, $r^2 = 0.460$).

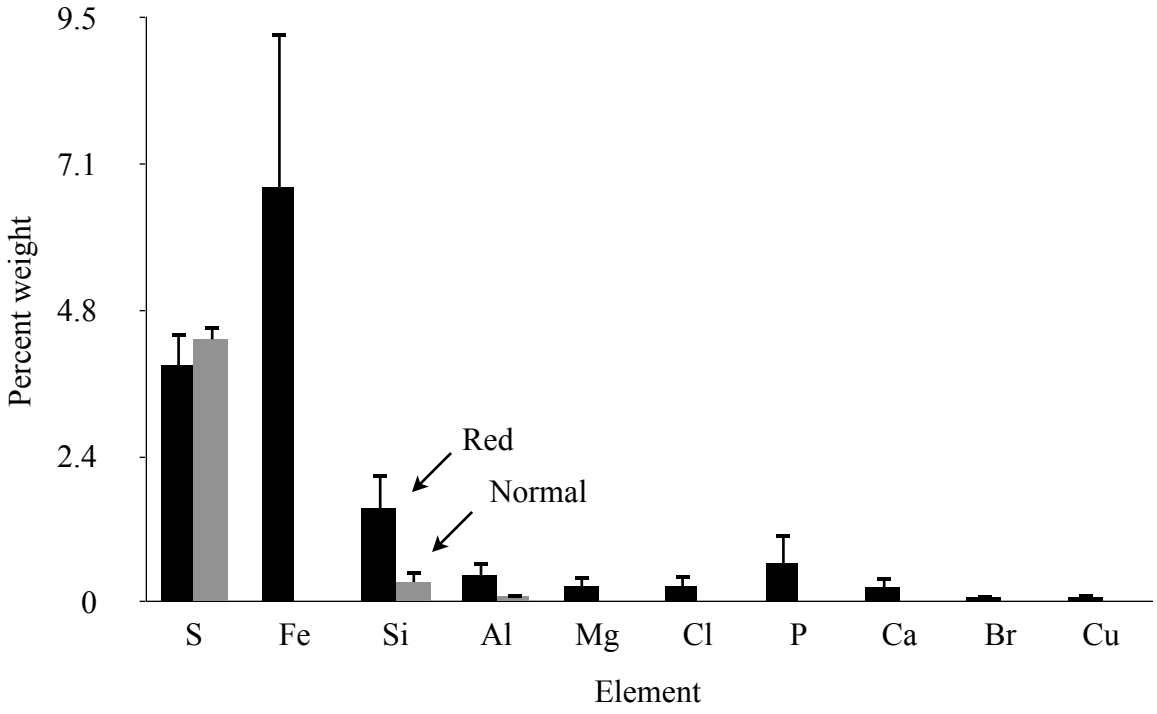

Figure 6. Percentage weight of elements (± SE) other than carbon and oxygen detected on red-coated (black; $n = 8$) and normal-pelaged seals (gray; $n = 8$).

Histology samples were analyzed for 28 seals and included four biopsies from areas of hair loss, one of which was a biopsy from a normal-pelaged seal. A yellow-brown refractile coating was found on the hair shafts of 11/14 biopsies from red-coated seals, and the severity of the coating ranged from mild to marked (Fig. 7). This outer coating was not present on hair from red-coated seals that did not have a red pelage at the biopsy site. All slides showed non-specific staining with respect to the Fontana Masson, Von Kossa, and copper stains. The coating observed on the hair shafts of some red-coated seals stained positive for iron (Fig. 7). Hairs just emerging did not stain as strongly for iron (Fig. 7), and hairs beneath the surface of the skin also did not stain positive for iron.

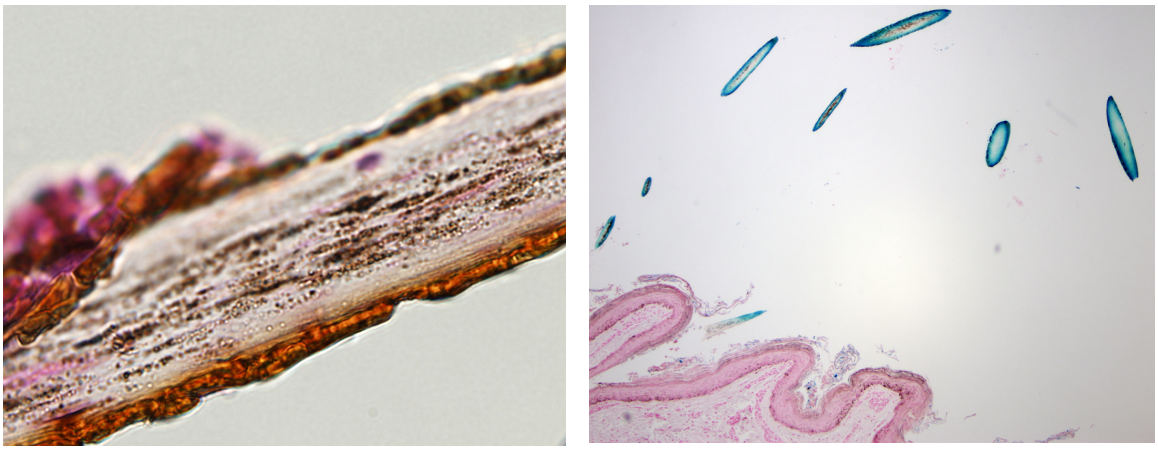

Figure 7. Yellow-brown refractile coating found on the hair shaft of red-coated harbor seals (left), and positive Pearl's iron staining indicated in blue on the hair of a biopsy from a red-pelaged seal (right).

There were no differences in Se concentrations ($F = 0.149$, $P = 0.709$) nor the molar ratio of Se:Hg ($F = 2.198$, $P = 0.141$) with coat color; however, differences between red-coated and normal-pelaged seals were detected for THg concentrations and stable isotopes. Female red-coated seals had lesser THg concentrations (11.63 ± 2.0; $\bar{x}$ µg/g dry wt ± SE) than normal-pelaged females (18.27 ± 0.86; $F = 4.236$, $P = 0.043$), although no difference with coat color was detected for male seals ($F = 0.001$, $P = 0.972$). Red-coated seals had greater $\delta^{15}N$ values than normal-pelaged seals ($F = 9.006$, $P = 0.005$), and seals in south SFB had greater $\delta^{15}N$ values than seals in north SFB ($F = 5.930$, $P = 0.019$; Fig. 8). No differences in $\delta^{13}C$ values with coat color were detected ($F = 1.563$, $P = 0.217$; Fig. 8). Mean $\delta^{34}S$ values were less in red-coated seals ($F = 10.935$, $P = 0.002$) compared with normal-pelaged seals (Fig. 9).

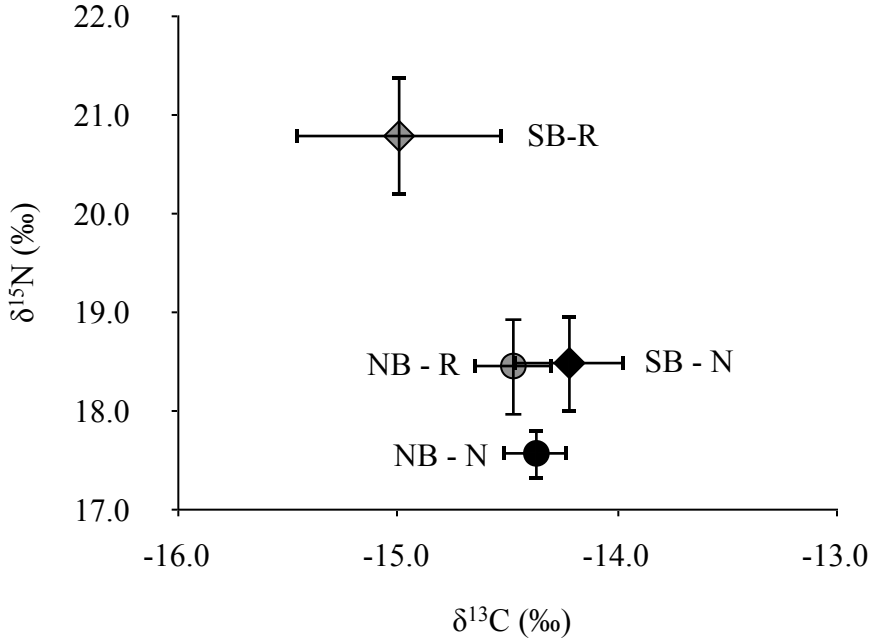

Figure 8. Mean $\delta^{15}N$ and $\delta^{13}C$ values (‰ ± SE) for red-coated (R; gray) and normal-pelaged seals (N; black) in the north (NB; circles) and south bay (SB; diamonds).

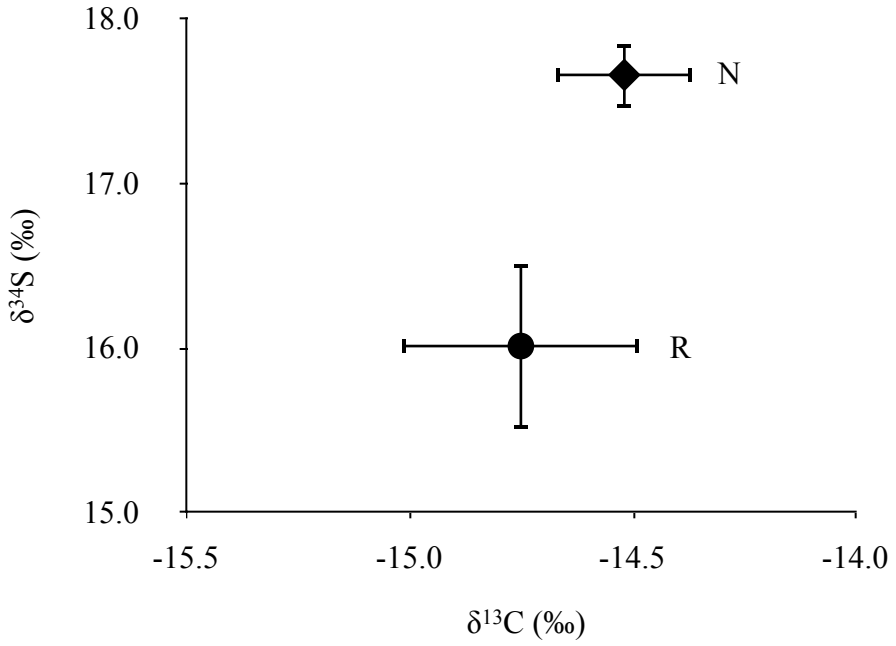

Figure 9. Mean $\delta^{34}S$ and $\delta^{13}C$ values (‰ ± SE) for red-coated (R; circle) and normal-pelaged seals (N; diamond) from San Francisco Bay.

DISCUSSION

Red discoloration of the fur or feathers has been observed in a number of species, including several species of birds and pinnipeds (Allen *et al.* 1993, Lydersen *et al.* 2001, Delhey *et al.* 2007). Red-coated seals first were observed in SFB in 1968, but given that this coincided with increased survey effort of harbor seals, it is possible that red-coated seals were present in the area before this time. Although observations before the late 1960s did not mention red-coated seals, these were not comprehensive surveys of SFB and were of one harbor seal haul-out site or all pinnipeds along the entire California coast (Bonnot 1928, Bartholomew 1949). The number and proportion of red-coated seals in

SFB increased dramatically between 1972 and 1984, therefore, if red-coated seals were present before 1968, it likely was in decreased numbers. There were annual fluctuations in the proportion of red-coated seals between 1984 and 2010; however, these were probably the result of differences in survey method (aerial *vs.* ground), the number of surveys per year, and differences in how seals were classified as red-coated. This was apparent in counts conducted between 1999 and 2005 because the proportion of red-coated seals at all haul-out sites increased during this time period. Between 1999 and 2005, seals were classified as red-coated if they had a slight tinge of red to their pelage, compared with recent counts where only individuals with obvious red pelage were classified as red-coated (D. Greig, pers. comm). Despite annual fluctuations in the number of red-coated seals, SFB continues to have some of the greatest numbers and proportions of red-coated seals world-wide (Allen *et al*. 1993).

Differences in the proportion of red-coated seals among haul out-sites have previously been detected (Allen *et al.* 1993, Kopec and Harvey 1995). Allen *et al.* (1993) found a greater proportion of red-coated seals at haul-out sites in north SFB, but a later study found a greater proportion in south SFB (Kopec and Harvey 1995). Recent land-based surveys indicated that the proportion at Castro Rocks was similar to the proportion at Mowry Slough; however, estimates of red-coated seals in south SFB likely were underestimated as seals often were covered in mud and the observer distance was much greater than for other haul-out sites. There may be a greater proportion of red-coated seals at less-monitored haul-out sites in south SFB given that greater than 50% of

seals at Corkscrew Slough were red-coated (Kopec and Harvey 1995). The greatest proportion of red-coated seals in this study were captured in south SFB, supporting the conclusion that a greater proportion of red-coated seals occurred at haul-out sites in south SFB. The lesser proportion of red-coated seals at YBI compared with other haul-out sites may be an important indicator of how a red pelage is acquired, as this site is closer to the mouth of the bay and outer coastline.

Although red-coated seals have been observed in other areas, the pattern of coloration and changes to keratinized tissues in red-coated seals from SFB are unique (Kopec and Harvey 1995). Whereas the initial pattern of red coloration (face and foreflippers) was consistent with bearded and ringed seals from Svalbard, some seals in this study had red pelage covering greater than 50% of their body. Red-coated harbor seals at the Mad River in northern California had red pelage over their entire body; however, the red was more prominent on the ventral surface and sides of the seals than on their back, head, and neck (Neumann and Schmahl 1999). The presence of hair loss and shortened vibrissae have previously been noted in harbor seals from SFB, but have not been mentioned in red-coated seals from other areas (Kopec and Harvey 1995). In this study, hair loss was concentrated around the face, and shortened vibrissae were observed in some, but not all red-coated seals. These observations were consistent with those made by Kopec and Harvey (1995), although some red-coated seals in this study also had nose lesions of varying severity likely not associated with capture. Hair loss, shortened vibrissae, and nose lesions appeared to be more common in seals with a greater

percentage of red pelage, and were not observed in animals that had less than approximately 30% of their body covered by red pelage.

Hair loss in pinnipeds can be caused by a wide range of conditions, and by itself is not diagnostic of any one causative agent (Dierauf and Gulland 2001). Additionally, pinnipeds either continuously or annually shed and regrow hair (molt). Although harbor seals undergo an annual molt where hair is shed and replaced by new hair in a period of one to two months (Thompson and Rothery 1987), it is unlikely that molt was the cause of hair loss in red-coated seals. Molt in SFB primarily occurs during late June through early August, and new hair is typically exposed when the old hair is shed (Ashwell-Erickson *et al.* 1986). Histology revealed the presence of a previously undescribed nematode worm (in harbor seals) in the hair follicles of the red-coated seal from Tomales Bay that had patchy hair loss over her entire body. This nematode was not found in any of the other biopsies, and histology revealed no noticeable differences between areas of hair loss and normal growth nor between red-coated and normal-pelaged seals. The lack of any histopathological changes could be because biopsies were not collected from areas where hair loss was most prominent (*e.g.*, face), although in most cases biopsies were collected from areas close to the face (*e.g.*, neck). Results from histopathology were inconclusive in that they did not reveal any clear health condition responsible for the hair loss, and the cause may be related to the observed vibrissal changes.

Shortened vibrissae have previously been observed in red-coated harbor seals from SFB. Researchers suggested that vibrissae were more brittle, therefore, were more

susceptible to breaking than vibrissae of normal-pelaged seals (Kopec and Harvey 1995). Vibrissal breakage was observed in at least one red-coated seal in this study; however, the amount of force required to break an individual vibrissa did not differ with coat color. The force required to break each vibrissa may not have been an accurate indication of brittleness because breakage likely occurred towards the end of the vibrissae, and not at the base where force was measured in this study. The majority of short vibrissae did not appear to be broken and came to a taper at the end, indicating that either vibrissae were breaking and then being worn, or that some other mechanism contributed to shortness, such as slower growth or increased loss of vibrissae. Harbor seal vibrissae are shed annually and undergo periods of rapid growth followed by inactivity (Hirons *et al.* 2001). Because red-coated and normal-pelaged seals were captured at similar times throughout the year, it is unlikely that annual shedding of vibrissae was the cause of the shortened vibrissae. Despite that data from this study did not support the hypothesis that vibrissae of red-coated seals were more brittle than normal-pelaged seals, atypical breakage remains the most plausible explanation for shortened vibrissae. It may be that vibrissae were breaking, sometimes to the point where the entire vibrissa fell out, and subsequently regrew, which would account for the tapered appearance of shortened vibrissae.

Vibrissae play an important role in foraging (Denhardt *et al.* 2001), and harbor seals with shortened vibrissae may have decreased foraging efficiency. Harbor seals were able to detect and follow hydrodynamic fish trails by protracting vibrissae forward and performing lateral head movements (Denhardt *et al.* 2001). Harbor seals with

experimentally shortened vibrissae spent more time pursuing prey in turbid water conditions (Renouf 1980), indicating that vibrissae may be especially important in SFB where the water is relatively shallow and cloudy from sediment resuspension. Despite this, red-coated seals in SFB were of similar or better body condition than normal-pelaged seals, indicating that the lack of vibrissae did not impact foraging success. Red-coated males were likely in better body condition than normal-pelaged males because the largest male was red-coated (49 kg > than the next largest male), which influenced the estimation of the mean given the small sample size (n = 3). Trumble *et al.* (2003) found that captive harbor seals maintained their supply of energy and nutrients when fed diets of differing prey quality. Therefore, similar BCIs between red-coated and normal-pelaged seals are not necessarily indicative that seals ate the same type and quality of prey.

Hematology and serum chemistries typically are used to assess the health of free-ranging and captive pinnipeds; however, blood variables also can reflect differences in diet, such as nutritional intake and diving behaviors related to prey capture (Thompson *et al.* 1997, Melish *et al.* 2006, Trumble *et al.* 2006). Trumble *et al.* (2006) found that alanine aminotransferase, aspartate aminotransferase, γ-glutamyltransferase, creatine, and BUN:creatine differed among harbor seals fed a diet of pollock, herring, or a mix of the two prey types. Another study found that cholesterol, total protein, and globulins increased between intake and release in captive Steller sea lions (*Eumetopias jubatus*) that participated in feeding studies. Thompson *et al.* (1997) found significant differences

in leukocyte and erythrocyte parameters, including MCV, in free-ranging seals in relation to diet composition. Mean cell volume increased during periods when seals preyed on more benthic gadoids (*e.g.*, whiting, sandeels) compared with periods when the diet was dominated by clupeids (*e.g.*, herring, sprat), indicating that MCV values were affected by diving behavior specific to certain prey types or nutritional quality of different prey (Thompson *et al.* 1997).

The majority of blood variables in this study did not differ between red-coated and normal-pelaged seals, and almost all variables fell within the 90% CI of values reported for subadult and adult harbor seals from SFB and Tomales Bay (Greig *et al.* 2010). Exceptions to this included magnesium for both red-coated and normal-pelaged seals, and total protein and globulin for red-coated seals. The lack of differences in blood variables with coat color that have been associated with clinically sick pinnipeds do not support the hypothesis that an underlying health condition was responsible for the development of a red pelage (Roletto 1993). The differences in MCV and cholesterol between red-coated and normal-pelaged seals, coupled with the fact that total protein and globulin for red-coated seals were outside the range for free-ranging seals from the same area, indicated that there may be differences in the nutritional quality of prey consumed between red-coated and normal-pelaged seals.

Although it has been suggested that an underlying health condition may predispose seals in SFB to developing a red pelage, the actual presence of a red pelage is not believed to be detrimental to an individuals health. Several researchers found that the

red coloration was from deposition of iron oxide precipitates (Allen *et al.* 1993, Lydersen *et al.* 2001), although an alternative study found no difference in the amount of iron on the hair shaft between red-coated and normal-pelaged seals (Moser 1996). Results from this study support the conclusion that external deposition of iron followed by oxidation was the cause of the red pelage. Iron was not detected on hair shafts of normal-pelaged seals, nor on red-coated seals that were not red where the hair or biopsy sample was collected. The fact that hairs below the surface did not stain positive for iron, and hairs just emerging stained mildly positive, supports the conclusion that deposition occurred externally and not internally. The significant relationship between the amount of iron on the hair and the percentage of the body covered by red pelage indicate that the pattern of coloration may be related to the frequency of exposure to iron.

External exposure to iron could occur through a number of mechanisms, including suspended sediments in the water column (Allen *et al.* 1993), contact with sediments on the haul-out site (Neumann and Schmahl 1999), or through contact with sediments during benthic foraging (Lydersen *et al.* 2001). Neumann and Schmahl (1999) suggested that it was unlikely that iron from the water precipitated directly onto hair shafts as this would require supersaturation of the water with respect to iron, and would result in widespread precipitation of iron oxides on haul-out sites. Although iron oxides were deposited on one haul-out site in Humboldt County, they were visible and believed to be from weathering of rocks from a man-made reinforcement of the river bank (Neumann and Schmahl 1999). These reinforcements are not present near haul-out sites

in SFB, nor have iron oxide deposits on haul-out sites been observed, making contact with sediments on the benthos the most likely exposure mechanism. The amount of iron in sediments has not been measured specifically at sites where harbor seals foraged; however, previous studies indicated that iron concentrations in sediment were similar among sites in SFB and Tomales Bay (Chapman *et al.* 1987, Long *et al.* 1990, Hornberger *et al.* 1999). Therefore, it is unlikely that red-coated seals are more abundant in SFB simply because of greater iron concentrations in sediment, indicating that other conditions unique to SFB may be the driving factor for the development of a red pelage.

Research on red-coated seals primarily has focused on the cause of the red coloration and not why some seals develop a red pelage and others do not. Kopec and Harvey (1995) hypothesized that chronic Se toxicosis predisposed seals in SFB to developing a red pelage because seals in this region had greater Se concentrations in whole blood than seals from other regions, and changes to keratinized tissues. Chronic Se toxicosis affects keratinized tissues, such as hair and vibrissae (Raisbeck *et al.* 1993; O'Toole and Raisbeck 1995, 1997), which may result in external damage to hair shafts, thereby creating a greater surface area for iron accumulation (Kopec and Harvey 1995). In this study, seals from SFB had similar Se concentrations and Se:Hg molar ratios in hair as seals from nearby Tomales Bay, and lesser concentrations than seals from Elkhorn Slough (Chapter I). Additionally, no differences in Se concentrations and Se:Hg molar ratio were detected with coat color, and SEM micrographs revealed that all seals had damage to their hair shafts, regardless of coat color.

The locational differences in Se and Se:Hg molar ratio found in this study (Chapter I) were not in agreement with results from Kopec and Harvey (1995), which may be due to a number of factors, including differences in tissue type (hair *vs.* blood), and time between studies. Elemental concentrations in blood represent dietary intake over a period of a week or two, whereas hair concentrations are representative of blood concentrations during the time of hair growth (one to two months). Because of this, hair collected throughout the year always represents concentrations during the months of molt, whereas blood concentrations of certain elements may vary seasonally. Kopec and Harvey (1995) found that Se concentrations were greater in the winter than in the summer; however, samples only were collected from seals in other regions in May (Puget Sound), June (San Nicolas Island), and September (Monterey). In contrast, samples were collected from seals in SFB in August, September, December, and February, which may have contributed to the increased Se concentrations in whole blood in seals from SFB compared with these other regions. Additionally, these studies were conducted almost 20 years apart, and action has been taken in the last decade by the San Francisco Bay Regional Water Quality Board to reduce agricultural and refinery discharges of Se to the bay. Therefore, even though Kopec and Harvey (1995) found increased Se concentration in whole blood of seals from SFB, results from this study indicate that the development of a red pelage is not a bioindicator of chronic Se toxicosis because red-coated harbor seals are still present in SFB even in the absence of increased Se concentrations.

The development of a red pelage in bearded and ringed seals from Norway has been attributed to benthic foraging activity (Lydersen *et al.* 2001); however, deposition of iron from sediment during foraging has not previously been suggested as a reason for red-coated harbor seals in SFB. This may be a viable explanation given that harbor seals primarily forage on benthic and schooling fishes in areas close to haul-out sites (10 to 20 km; Tollit *et al.* 1998, Nickel 2003, Orr *et al.* 2004). Harbor seals typically are considered generalist, opportunistic predators, although individuals may display specificity for certain locations and/or prey types. Although harbor seals in Scotland and Norway foraged on a variety of prey, individuals consistently returned to the same foraging areas (Bjørge *et al.* 1995, Tollit *et al.* 1998). Movements of seals in SFB were variable; however, seals also consistently returned to one or several foraging areas (Nickel 2003). The use of different foraging areas may result in differences in diet among seals, such as differences in prey species or prey type (*i.e.*, schooling fishes *vs.* benthic prey).

Seals in SFB primarily preyed on juvenile benthic and schooling fish, and diet differed with location (north *vs.* south SFB) and season (March to May *vs.* June to March; Gibble 2011). During the non-pupping season (June to March), when a red pelage primarily is acquired, the diet of seals in south SFB was dominated by yellowfin goby (*Acanthogobius flavimanus*), with several other species of gobies constituting a lesser proportion of the diet. In contrast, the diet of seals in north SFB primarily consisted of northern anchovy (*Engraulis mordax*), followed by plainfin midshipman (*Porichthys*

melanostictus), and yellowfin goby (Gibble 2011). Harbor seals generally preyed on the most abundant species, although several species that were abundant in trawls were noticeably absent or found in reduced numbers in the diet of harbor seals (Torok 1994, Gibble 2011). This could be the result of biases in trawl data, minimal overlap between fish habitat and harbor seal foraging areas, or selectivity by harbor seals for certain species. Whereas the first two explanations are more likely, the fidelity of individual harbor seals to specific foraging and haul-out sites coupled with differences in movement patterns among individuals are indicative of individual preference for certain locations and potentially certain prey types. Because it has been suggested that contact with iron-rich sediments can result in a red pelage, harbor seals that have a greater dependence on benthic species may be more likely to develop a red pelage.

Stable isotope data support this hypothesis that differences in diet exist between red-coated and normal-pelaged seals. Red-coated seals had significantly greater $\delta^{15}N$ and lesser $\delta^{34}S$ values than normal-pelaged seals; however, because both isotopes were likely influenced by a number of factors (Chapter I), it is impossible to determine the specific cause of these changes without additional data. Excluding red-coated seals from south SFB, all seal groups had mean $\delta^{15}N$ values approximately 2 to 3‰ greater than mean $\delta^{15}N$ values of known prey items in SFB (Stewart *et al.* 2004), which is consistent with captive studies that demonstrated a $\delta^{15}N$ diet to tissue fractionation of 3‰ in harbor seal hair (Hobson *et al.* 1996). The locational differences in $\delta^{15}N$ values found in this study were likely a result of dietary differences because differences in harbor seal diet between

north and south SFB previously have been detected (Gibble 2011); therefore, differences in $\delta^{15}N$ values with coat color also could be the result of diet. Differences in $\delta^{34}S$ between red-coated and normal-pelaged seals were relatively small (~1.6‰) compared with differences between bottlenose dolphins (*Tursiops truncatus*) that depended on estuarine *vs.* offshore food webs (~9.4‰; Barros *et al.* 2010). This indicates that differences in $\delta^{34}S$ values in this study may be statistically, but not biologically significant. Habitat use of harbor seals in this study overlapped, whereas bottlenose dolphins in the Barros *et al.* (2010) study exhibited a distinct inshore to offshore gradient; therefore, the difference in $\delta^{34}S$ values between red-coated and normal-pelaged seals likely were relatively small because of the potential for overlap in foraging habitat.

Comprehensive data on the stable isotope composition of harbor seal prey items are lacking, although $\delta^{15}N$ and $\delta^{13}C$ values have been measured in northern anchovy and yellowfin goby, which were the most important prey items for seals in north (anchovy) and south SFB (yellowfin goby) from June to March (Gibble 2011). The mean $\delta^{15}N$ value in muscle of northern anchovy collected at the Farallon Islands was 13.9 ± 0.8 (SD; Sydeman *et al.* 1997), whereas the approximate mean $\delta^{15}N$ value in muscle of yellowfin goby in SFB was 16 ± 1 (Stewart *et al.* 2004). Because seals in south SFB depended more on yellowfin goby and had increased $\delta^{15}N$ values compared with seals in north SFB, increased $\delta^{15}N$ values may be indicative of a diet rich in yellowfin goby.

The yellowfin goby is native to estuarine waters of Asia and is an invasive species in SFB. The first two specimens were detected in SFB in 1963 (Brittan *et al.* 1963), and

by 1966 the species had spread throughout the bay (Brittan *et al.* 1970). The number of yellowfin goby in SFB increased greatly after 1966 when they became widely established throughout the bay (Brittan *et al.* 1970). They are the largest goby in SFB and are primarily benthic omnivores that feed on a variety of prey, including polychaetes and crustaceans (Kanou *et al.* 2005, Cohen and Bollens 2008).

The occurrence and subsequent increase of yellowfin goby in SFB in the late 1960s, followed closely by an increase in the number and proportion of red-coated seals in the 1970s, may indicate that development of a red pelage is a direct result of increased benthic foraging, primarily on yellowfin goby. Differences in the proportion of red-coated seals among haul-out sites, coupled with differences in blood variables, THg concentrations, and stable isotope values between red-coated and normal-pelaged seals all support the conclusion that foraging differences exist between these two groups. The greater proportion of red-coated seals in south SFB may be because benthic prey, such as yellowfin goby, are a more important component of the diet of seals in south SFB compared with seals in north SFB. It also may be because of differences in the type of foraging habitat exploited by seals using north and south SFB. It remains unclear as to why some red-coated seals had hair loss, shortened vibrissae, and nose lesions, and histopathology of skin biopsies from areas of hair loss did not provide any insight. Given that blood, THg, and stable isotope data indicate that foraging differences may exist between red-coated and normal-pelaged seals, these symptoms may be the result of differences in nutritional quality of prey, such as protein content. The concentration of

symptoms to the face and neck may indicate that external forces, such as contact with the benthos, possibly contribute to the development of hair loss, shortened vibrissae, and nose lesions.

The development of a red pelage does not appear to affect short-term survival because red-coated seals were in good body condition and the majority of blood variables fell within ranges reported for seals from this region; however, it is possible that long-term survival may be affected. Data on the movements and foraging behavior (diet, foraging sites, and foraging tactics) of red-coated harbor seals in SFB are needed to determine whether the development of a red pelage is truly the result of benthic foraging, and whether their diet is of differing nutritional quality than normal-pelaged seals. The long-term survival and reproductive success of red-coated seals also should be assessed to determine if the development of a red pelage affects the long-term survival or fitness of these individuals.

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CONCLUSIONS

Harbor seals play an important role in the nearshore ecosystems, both as predator and prey for fish and larger marine mammals. Therefore, understanding concentrations and effects of toxicants (*e.g.*, Hg, Se) and other factors potentially influencing health and survival of harbor seals (*e.g.*, development of a red pelage) is important in understanding the health of the nearshore ecosystem. This may be especially critical in areas, such as SFB, where seals are in close proximity to dense human populations, and the number of seals has failed to increase since the passage of the Marine Mammal Protection Act in 1972.

Results from this study indicated that seals from SFB and Tomales Bay had increased THg and decreased Se concentrations in hair compared with seals from Elkhorn Slough. It is unknown whether these concentrations were great enough to cause Hg toxicosis; however, concentrations in some seals exceeded concentrations or effects levels associated with Hg toxicosis in other species. In contrast, mean Se concentrations in seals from SFB and Tomales Bay were less than concentrations measured in hair of other pinnipeds, and may indicate that the physiologic need for Se in animals from sites with increased Hg may be greater because Se is needed to bind to Hg as well as maintain normal selenoenzyme activities. Future researchers should continue to investigate concentrations of THg and Se in seals from SFB and Tomales Bay to understand how future changes in Hg and Se concentrations will affect harbor seals both in the context of Hg toxicosis and/or Se deficiency.

San Francisco Bay continues to have one of the greatest prevalences of red-coated seals world-wide, and because upwards of 20% of seals at haul-out sites in SFB had a red pelage, understanding this phenomenon and its effect on harbor seal health deserves attention. The development of a red pelage appeared to be the result of iron accumulation, possibly as a result of contact with sediment during benthic foraging. This did not appear to affect the short-term survival of seals, but it remains unknown if the development of a red pelage affects long-term survival or fitness. Because some red-coated seals had shortened vibrissae, understanding what predisposes a seal to developing a red pelage and the foraging implications this may have (*e.g.*, diet diversity, nutritional quality of prey), is an important component in evaluating the health of harbor seals inhabiting SFB.

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