CONCENTRATIONS OF MERCURY AND SELENIUM IN ALASKAN STELLER SEA LION POPULATIONS

An Undergraduate Research Scholars Thesis

by

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RESEARCH COMPLIANCE CERTIFICATION

Research activities involving the use of human subjects, vertebrate animals, and/or biohazards must be reviewed and approved by the appropriate Texas A&M University regulatory research committee (i.e., IRB, IACUC, IBC) before the activity can commence. This requirement applies to activities conducted at Texas A&M and to activities conducted at non-Texas A&M facilities or institutions. In both cases, students are responsible for working with the relevant Texas A&M research compliance program to ensure and document that all Texas A&M compliance obligations are met before the study begins.

I, Kailey Nicole Wilkin, certify that all research compliance requirements related to this Undergraduate Research Scholars thesis have been addressed with my Research Faculty Advisors prior to the collection of any data used in this final thesis submission.

This project did not require approval from the Texas A&M University Research Compliance & Biosafety office.

TABLE OF CONTENTS

ABSTRACT

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Mercury (Hg) is a neurotoxin that can cause health issues or even death. In Alaskan Steller sea lion (SSL) populations, Hg concentrations were observed to vary by region and time. These observations are of interest because SSLs, particularly the western population segments, are classified as an endangered species. This study intends to investigate what may be causing regional Hg differences and if there are other elemental correlations. Blood and hair samples were obtained from SSL pups. The hair samples were formed while the pup was in utero, so concentrations are reflective of the mother's diet. Hair samples were not received in time to be included for consideration in this document, but the blood samples were digested and then analyzed by inductively coupled plasma mass spectroscopy (ICP-MS) and inductively coupled plasma optical emission spectroscopy (ICP-OES), methods that are used to determine concentrations of elements in solution. These analytical methods were chosen because they may

be used with different sample types, and they are capable of detecting a variety of elements. These techniques allow other elemental trends, in addition to the previously observed behavior of mercury and selenium, to be observed.

DEDICATION

First, I would like to thank my faculty advisors for supporting my laboratory experience and teaching me research skills despite hardships due to the COVID-19 pandemic. Second, I recognize each of my female STEM professors for encouraging my pursuit of higher learning and showing me that becoming a successful woman in science is an attainable goal.

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Guidance concerning the research background and additional sources was provided by Dr. O'Hara. The archived blood samples used for *Concentrations of Mercury and Selenium in Alaskan Steller Sea Lion Populations* were provided by The University of Alaska - Fairbanks. The analytical processing of these samples was conducted by Dr. Taylor in the Texas A&M Trace Elements Research Lab (TERL). Dr. Taylor and Dr. Berrios aided my understanding of interpreting the ICP-MS and ICP-OES data, and Dr. Berrios provided data he obtained about locations of the sample collections.

All other work conducted for the thesis was completed by the student independently.

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NOMENCLATURE

SSL	Steller sea lions, *Eumetopias jubatus*
wDPS	Western distinct population segment
eDPS	Eastern distinct population segment
WAI	Western Aleutian Islands
CAI	Central Aleutian Islands
[THg]	Total mercury concentration
Hg	Mercury
Se	Selenium
[Se]	Concentration of selenium, selenium concentrations
Fe	Iron
Ca	Calcium

1. INTRODUCTION

Steller sea lions (*Eumetopias jubatus*; SSL), which are found off the northwestern coast of the United States and Canada, have historically been hunted as a source of meat, hides, and oil products. These animals, the only living members of their genus, are divided into two different populations – the eastern distinct population segment (eDPS) and the western distinct population segment (wDPS), with 144°W longitude as the dividing line between the two populations. The western DPS is considered endangered on the International Union for Conservation of Nature Red List of Threatened Species, while the eastern DPS has recovered after previously having been listed as threatened. Differing rates of recovery for the eastern and western DPSs are thought to be due to differing threats experienced by each population. Threats that the sea lions may face have both natural and anthropogenic sources. These may include, but are not limited to, diseases or parasitic organisms, climate change and associated phenomena (e.g., temperature changes, sea level rise, etc.), environmental contaminants, predation, and commercial fishing.

In the Aleutian Islands, where the majority of the western DPS is located, SSL total mercury concentrations ([THg]) were observed to vary, with the highest levels reported near the Western/Central Aleutian Islands area (Rea et al., 2020). It is possible that this may be due to the biogeographical differences in the western DPS. High levels of mercury in SSLs may be related to the introduction of contaminants into their environment or to the abundance and composition of prey species. For example, some fish known to be SSL prey were found to have higher [THg] values in the western Aleutian Islands than other areas.

These elemental trends are of great concern due to the endangered status of the western DPS, and because of mercury's ability to bioaccumulate and bio-magnify. Methylmercury (MeHg), which is easily absorbed by the brain and placental tissues, can cause harm to an organism's nervous system and can be lethal at high doses (Castellini et al., 2012). Since MeHg can cross the placenta, SSLs may receive doses of mercury before they are even born. To investigate the transfer of MeHg between mother and fetus, blood and lanugo (a hair coat formed in utero) samples are taken from SSL pups. It should also be noted that the concentrations of mercury and selenium have been observed to oscillate over the course of decades. Although the geo-temporal reasons behind the cycling and differences in elemental concentrations are not clear, some contributing factors may include aging individuals (bioaccumulation of mercury over time), changes in diet composition, or other cyclic affecters.

Selenium (Se), which is an element that binds to mercury, can offset mercury's potential for harm. When it binds to mercury, it may form crystals in which both elements are immobilized, so mercury cannot interact with other tissues (Rea et al, 2020). Lower Se levels were observed to correspond with higher [THg] (Rea et al, 2020). This may indicate a dietary deficiency of selenium in SSLs in the Western and Central Aleutian Islands – if less Se is available to protect against mercury and allow it to be excreted, mercury levels may increase.

Selenium's interaction with mercury is beneficial, in that it provides some level of protection to the organism. In order to explore other possible elemental relationships to Hg in SSL tissues, this study evaluated other micronutrient and trace element levels within SSL pup blood.

2. METHODS

2.1 Equipment Preparation

This project utilized quartz (SiO_2) tubes for digestion of the SSL blood samples. This material was chosen because of its chemical purity, its resistance to strong acids and high temperatures (digestion conditions), and its low cost when compared to fluoropolymers such as Teflon. Quartz tubes and Teflon caps were cleaned in 10% nitric acid (HNO_3) baths, rinsed with deionized (DI) water, and allowed to dry between digestions. Once dry, tubes and caps were covered with polyethylene film to eliminate contamination via airborne dust until used. Before the blood samples were introduced, tare weights were taken of each tube to be used in later calculations of final digestion volumes.

2.2 Sample Preparation

Frozen SSL pup blood samples were received from colleagues at the University of Alaska Fairbanks in polyethylene cryovials on dry ice. Samples were transferred to ultra-low temperature freezers until subsampled for preparation and analysis.

Sample preparation is necessary before analysis by both inductively coupled plasma – mass spectroscopy (ICP-MS) and inductively coupled plasma – optical emission spectroscopy (ICP-OES) can be performed. Preparation began by thawing the samples under contamination-free conditions. Because many elements are preferentially associated with red blood cells in whole blood, samples were homogenized within their cryovials by vigorous agitation with polyethylene transfer pipets. A new polyethylene transfer pipette was used for each sample to limit contamination. Approximately 0.5 mL of each blood sample was then added to tare-weighted quartz digestion tubes, and the sample weight was recorded to the nearest 0.0001 g. In

addition to the SSL blood samples, five laboratory quality control (QC) samples were prepared with each group of project samples in order to evaluate possible laboratory contamination and laboratory precision and accuracy. These included a method blank, a spiked blank, a standard reference material (NIST SRM 2976, Marine Mussel), and duplicate and spiked SSL blood samples.

Samples were digested with a mixture of nitric acid (HNO_3), hydrogen peroxide (H_2O_2), and hydrochloric acid (HCl) in a Milestone UltraWAVE microwave digestion system using the method of Nobrega et al. (2012). This instrument reaches high digestion temperatures (250°C) as the result of microwave heating within a pressurized vessel, resulting in total breakdown of organic material. After digestion was completed, samples were allowed to cool to room temperature and then diluted to volume with 10 mL of deionized water. Final digest volumes were determined gravimetrically, using final weights, tare weights of the quartz digestion tubes, and digest densities. Finally, samples were transferred to 15 mL polypropylene centrifuge tubes for analysis.

2.3 Sample Analysis

Samples were analyzed without further dilution by ICP-OES using a Perkin Elmer Avio 500 instrument equipped with both axial and radial viewing. Depending upon concentration and emission line chosen, this analysis involved from 3 to 6 external calibration standards in addition to the calibration blank. Both yttrium and ytterbium were utilized as internal standards to compensate for instrument drift and performance differences between samples and standards. Continuing calibration verification standards and continuing calibration blanks were analyzed following instrument calibration, after each group of ten samples, and at the end of the analytical sequence in order to ensure that instrument performance was within acceptable limits. Elements

that were too low in concentration to result in reliable SSL blood data by ICP-OES were then analyzed by the more sensitive ICP-MS method using a Perkin Elmer DRC 2 instrument operated in both “standard” mode and “reaction cell” mode. Reaction cell mode involved sequential use of ammonia and oxygen gasses in order to overcome molecular ion interferences. Calibration utilized three standards in addition to the calibration blank. Internal standards included isotopes of gallium, rhodium, indium, and bismuth to compensate for instrument drift and performance differences between samples and standards. Periodic calibration checks were the same as those followed in the ICP-OES method. Sample analysis via ICP-MS was performed without further dilution of the original digest solutions.

3. RESULTS

Nine elements were sufficiently high in SSL blood samples to be determined by ICP-OES: aluminum, boron, calcium, iron, potassium, magnesium, sodium, phosphorus, and sulfur. Thirteen elements were analyzed by ICP-MS: arsenic, barium, beryllium, cadmium, cobalt, copper, lithium, manganese, lead, selenium, strontium, uranium, vanadium, and zinc. Quality control samples showed that laboratory contamination (method blanks), precision (duplicate analyses) and accuracy (standard reference materials and spiked blanks and samples) were acceptable. [THg] data was obtained from the University of Alaska Fairbanks.

Some notable trends within SSL blood levels include regional differences between Se concentrations and [THg] (Tables 3.1 and 3.2; Figure 3.1). Samples from the Central Aleutian Islands (CAI) showed higher Se concentrations and lower [THg]. In the Western Aleutian Islands (WAI), higher [THg] values corresponded with lower Se concentrations. In addition, the average value for THg (ug/g) in whole blood in the WAI was 0.1253 ug/g, almost 3 times higher than the average in the CAI (0.0446 ug/g). Another trend that was observed for both the CAI and WAI regions was an inverse relationship between iron (Fe) and calcium (Ca) (Figure 3.2). This relationship did not seem to have strong regional differences but, due to different prey species for the two SSL populations, bears further evaluation as it may impact health.

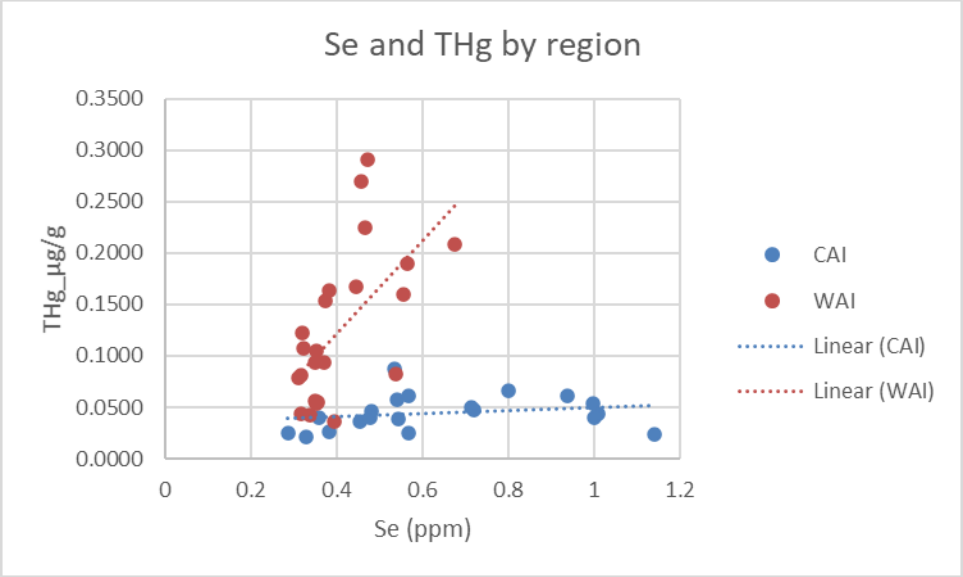

Figure 3.1: Graph depicting correlation between Se and THg concentrations.

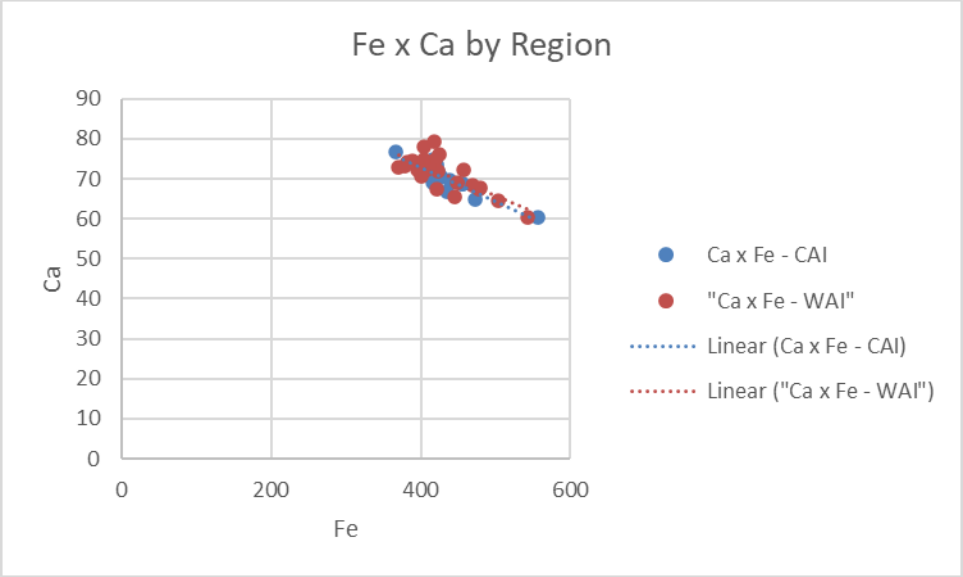

Figure 3.2: Graph depicting inverse relationship between Fe and Ca.

Table 3.1: [THg] values of CAI samples tested in TERL

SampleID	***Capture Site***	***Metapopulation***	***THg_µg/g***
SSL2019>160AL	*Ulak Island*	*CAI*	*0.0658*
SSL2019>163AL	*Ulak Island*	*CAI*	*0.0241*
SSL2019>166AL	*Ulak Island*	*CAI*	*0.0246*
SSL2019>168AL	*Ulak Island*	*CAI*	*0.0879*
SSL2019>169AL	*Ulak Island*	*CAI*	*0.0460*
SSL2019>171AL	*Ulak Island*	*CAI*	*0.0387*
SSL2019>172AL	*Ulak Island*	*CAI*	*0.0471*
SSL2019>174AL	*Ulak Island*	*CAI*	*0.0441*
SSL2019>177AL	*Ulak Island*	*CAI*	*0.0401*
SSL2019>179AL	*Ulak Island*	*CAI*	*0.0501*
SSL2019>183AL	*Ulak Island*	*CAI*	*0.0575*
SSL2019>186AL	*Ulak Island*	*CAI*	*0.0262*
SSL2019>188AL	*Ulak Island*	*CAI*	*0.0538*
SSL2019>190AL	*Ulak Island*	*CAI*	*0.0618*
SSL2019>194AL	*Ulak Island*	*CAI*	*0.0401*
SSL2019>198AL	*Ulak Island*	*CAI*	*0.0217*
SSL2019>199AL	*Ulak Island*	*CAI*	*0.0248*
SSL2019>201AL	*Ulak Island*	*CAI*	*0.0366*
SSL2019>203AL	*Ulak Island*	*CAI*	*0.0398*
SSL2019>204AL	*Ulak Island*	*CAI*	*0.0609*

Table 3.2: [THg] values of WAI samples tested in TERL

SampleID	***Capture Site***	***Metapopulation***	***THg_µg/g***
SSL2019~207AL	*Agattu Island*	*WAI*	*0.0806*
SSL2019~208AL	*Agattu Island*	*WAI*	*0.0783*
SSL2019~209AL	*Agattu Island*	*WAI*	*0.1069*
SSL2019~211AL	*Agattu Island*	*WAI*	*0.2694*
SSL2019~213AL	*Agattu Island*	*WAI*	*0.0545*
SSL2019~214AL	*Agattu Island*	*WAI*	*0.1677*
SSL2019~217AL	*Agattu Island*	*WAI*	*0.2914*
SSL2019~219AL	*Agattu Island*	*WAI*	*0.1904*
SSL2019~221AL	*Agattu Island*	*WAI*	*0.0542*
SSL2019~223AL	*Agattu Island*	*WAI*	*0.1539*
SSL2019~227AL	*Agattu Island*	*WAI*	*0.1636*
SSL2019~230AL	*Agattu Island*	*WAI*	*0.2250*
SSL2019~232AL	*Agattu Island*	*WAI*	*0.1223*
SSL2019~234AL	*Agattu Island*	*WAI*	*0.0940*
SSL2019~236AL	*Agattu Island*	*WAI*	*0.1603*
SSL2019~237AL	*Agattu Island*	*WAI*	*0.2087*
SSL2019~242AL	*Agattu Island*	*WAI*	*0.0440*
SSL2019~245AL	*Agattu Island*	*WAI*	*0.0365*
SSL2019~248AL	*Agattu Island*	*WAI*	*0.0560*
SSL2019~250AL	*Agattu Island*	*WAI*	*0.0430*
SSL2019~252AL	*Agattu Island*	*WAI*	*0.0931*

Table 3.2: Continued

SSL2019~255AL	*Agattu Island*	*WAI*	*0.0825*
SSL2019~257AL	*Agattu Island*	*WAI*	*0.1047*

4. CONCLUSION

This project was originally designed to analyze both SSL blood samples and lanugo samples since both sample types are able to capture different pools of elements of interest. However, lanugo samples were not received in time to be included for consideration in this project writing.

Because Se has a known antioxidant function, it is of interest to researchers for its potential to outweigh the effects of $MeHg^+$. If Se concentrations exceed those of $MeHg^+$, the available $MeHg^+$ targets Se until there is no biological effects of $MeHg^+$ (Lian et al.). If there is not enough Se available, $MeHg^+$ will impact Se biochemistry and may potentially be damaging.

The quality control samples that were included in digestion and analysis (blanks, laboratory control samples, standard reference materials, duplicates, and spiked samples) did not raise any cause for concern. Blanks were under the detection limits, less than 2x the detection limits, and/or relatively low in proportion to the measured sample value. These conditions are acceptable results for blanks, so there is limited concern for sample contamination. Most duplicates had low relative percent differences (RPD), where

RPD = |range of duplicate| / average of duplicate

This measurement is used to indicate precision. Duplicates with high RPDs tended to have results that were very close to the detection limits. After reviewing the data, no duplicates were flagged for concern. Lab control samples (LCS) and spiked samples returned the expected elemental concentrations that corresponded to the added spike solution. This solution contained known concentrations of certain elements. Several elements (Li, S, U) were not present in the spike solution and so their recoveries could not be calculated. Finally, the standard reference

material (SRM) was used to compared certified element concentrations of a Marine Mussel (#2976) with those observed during analysis. All SRM recoveries were within acceptable limits. The inverse relationship between Se and Hg described in previous relationships (Rea, et al., 2020), (Correa, et al., 2014) was replicated in this project. There are still elevated levels of Hg in the WAI, the driving factor of which is still unknown. Further research should investigate potential sources of mercury in the area. It is suspected that heightened Hg levels are hindering the ability of the wDPS to recover from their endangered status.

It is not clear what could cause the observed relationship between Ca and Fe, although it has long been recognized that increased Ca intake can reduce Fe absorption (Lynch, 2000). Additional trace element studies may be necessary to replicate these results and deepen an understanding of this phenomenon.

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