Marine Mammal Society 2023

Small Grants in Aid of Research

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Project Information

Title Indicators of oxidative stress in tissues of California sea lions in select colonies of the Southern Gulf of California (Mexico) in relation to mercury concentrations

Location La Paz, Baja California Sur, Mexico

Applicant Information

Full Name Taryn Elizabeth Symon

Affiliation Centro de Investigaciones Biológicas del Noroeste S.C. (CIBNOR)

Email tsymon@pg.cibnor.mx

Address Instituto Politécnico Nacional 195, Playa Palo de Santa Rita Sur, La Paz, Baja California Sur. Código postal 23096

☒ **Student** ☐ **Early Career** ☐ **Experienced Investigator (> 5 y post degree or equivalent)**

Biography

Introduce yourself. Provide a short bio starting with "I am…". 200 words max.

I am currently a Ph.D candidate within the Laboratory of Oxidative Stress (Dr. Tania Zenteno-Savín) at Centro de Investigaciones Biológicas del Noroeste (CIBNOR), S.C. in beautiful La Paz, Baja California Sur, Mexico. I have approximately nine years of experience working with bottlenose dolphins (*Tursiops truncatus*) in various capacities. During my M.Sc., I worked on a project analyzing the indicators of oxidative stress of leukocytes isolated from *T. truncatus* and *Homo sapiens* in response to the exposure to lipopolysaccharides *in vitro*. For the past two years, I have been working with an international team of experts on my Ph.D. project in California sea lions (*Zalophus californianus*) within the Gulf of California. Following the termination of my Ph.D, my goal is to continue my research within the fascinating field of marine mammals.

Overview

What do you want to do? Explain the background, rationale, and purpose of the study. Summarize current knowledge of the topic, identify knowledge gaps, and state the specific research question or hypothesis you will address. You may include essential numbered references [1]. 700 words max.

Mercury (Hg) is a naturally occurring toxic element of global concern. Inorganic Hg is released to the environment by natural and anthropogenic means, and can be converted to highly bioavailable organic forms such as monomethylmercury ($MeHg^{+}$) [1]. Due to their longevity, high trophic level, close

proximity to contamination sources, and One Health implications, California sea lions (CSL) are effective sentinels of environmental health [1; 2]. In Mexico, the CSL is the most abundant pinniped species [3], although, in recent years, the majority of the 13 CSL reproductive colonies within the Gulf of California (GC) have experienced population decline [4]. However, the potential cause(s) remain unknown.

In marine mammals, including CSL, exposure to $MeHg^+$ primarily occurs by trophic interactions (dietary intake) [5: 6]. Stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) can be used to evaluate the flow of Hg through aquatic systems. $\delta^{13}C$ and $\delta^{15}N$ indicate where (coastal vs. pelagic) individuals are feeding and the trophic level they occupy, respectively [1]. Furthermore, Hg exposure also occurs during *in utero* development [5]. In CSL, lanugo fur develops *in utero*, and, therefore, reflects Hg exposure of the fetus (via dam diet) over time [5]. However, Hg can also accumulate in internal organs and can cross the blood-brain barrier, causing numerous adverse health effects [5]. Conditions of neurotoxicity and conditions of oxidative stress have been well documented in pinnipeds as a result of Hg accumulation [6-10]).

Oxidative stress is the loss of equilibrium between oxidant production and antioxidant defenses. Selenium (Se) is a non-enzymatic antioxidant and cofactor in various enzymatic antioxidants, including glutathione peroxidase [6]. Interactions of Se with $MeHg^+$ may reduce toxicity through various pathways, including formation of mercuric selenide (HgSe), a biologically inert compound [6] that can be stored or excreted via urine. However, formation of this compound may result in decreased Se bioavailability [6], increasing the likelihood of oxidative tissue damage.

The main objective of this study is to evaluate the concentrations of total mercury ([THg]), total selenium ([TSe]), the stable isotopes of $\delta^{13}C$ and $\delta^{15}N$, and the indicators of oxidative stress in the tissues of CSL in select colonies of the Southern GC. To achieve this general objective, three specific objectives will be implemented including: 1) quantification of the [THg], [TSe] and the stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ in relation to sex, maturity stage, and year in the hair and internal tissues of CSL in select colonies of the Southern GC; 2) evaluation of the indicators of oxidative stress including the production of superoxide radical, antioxidant defenses, and oxidative damage in the internal tissues of CSL in relation to [THg], [TSe], the stable isotopes of $\delta^{13}C$ and $\delta^{15}N$, sex, and maturity stage; and 3) evaluation of the effects of feeding ecology (inferred by $\delta^{13}C$ and $\delta^{15}N$), sex, maturity stage, the indicators of oxidative stress, and year on [THg] and [TSe].

The hypothesis of this study is that if the accumulation of Hg in the tissues of CSL is influenced by trophic ecology, and sex, increases with maturity stage, decreases the bioavailability of [Se], and results in conditions of oxidative stress, then the tissues of males and older individuals will present greater [THg], higher production of reactive oxygen species, lower Se-dependent antioxidant activity, and greater oxidative damage in comparison to the tissues of females and younger individuals.

Approach

How will you do it? Briefly explain the methods you will use to collect, analyze, and share data. You may include essential numbered references [1]. 700 words max.

Sample collection:

In 2021 and 2022, hair samples were collected from live and deceased CSL neonates between 0-3 months of age captured at Los Islotes, La Paz, Baja California Sur, Mexico. Similarly, samples of internal organs (muscle, brain, kidney, liver, and heart) were collected from recently deceased (< 6 hours) individuals found within the same location. The sex, maturity stage, weight, and total length were recorded for each individual. All samples were collected with permission from the Comisión Nacional de

Áreas Naturales Protegidas (CONANP). Subsequent sample collection will also occur in 2023 and 2024 following annual permit renewal.

Sample preparation:

Hair and internal organ samples were transported on ice to the Oxidative Stress Laboratory (CIBNOR). To prepare samples for the analysis of [THg], [TSe], and $\delta^{13}C$ and $\delta^{15}N$, hair samples were washed with a 1% solution of Triton X and ultrapure water, frozen at -80°C and freeze-dried (Labcono, FreeZone, Kansas City, MI, USA) for 24 hours. Internal organs were weighed (wet weight), frozen at -80°C, freeze-dried for 48 hours, weighed (dry weight), and macerated with an agate mortar and pestle. The percent moisture content of the internal tissues was determined.

To prepare samples for the analysis of the indicators of oxidative stress, a sub-sample of each internal tissue was placed into individually labelled cryovials (Corning Incorporated, Corning, NY, USA) and frozen at -80°C. Prior to analysis, samples will be thawed on ice and each sample will be homogenized in phosphate buffer solution (PBS; Gibco, Paisley, United Kingdom).

Sample analysis:

The [THg] was determined in the hair and internal tissues of CSL using a mercury analyzer (MA-3000; NIC; AG Scientific, Bryan, TX, USA), while the [TSe] was analyzed in the same tissues using in inductively coupled plasma-mass spectrophotometry (ICPMS; NexION 2000, PerkinElmer, Waltham, MA, USA) following acid digestion.

The indicators of oxidative stress, including the production of superoxide radical, (a reactive oxygen species), the activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), thioredoxin reductase (TrxR), the concentration of glutathione (GSH), and the concentration of protein carbonyls (as an indicator of oxidative damage) will be analyzed using spectrophotometry in internal tissues.

Statistical analysis:

Statistical analysis will be conducted using generalized linear models (GLMs) to analyze possible correlations between [THg], [TSe], trophic ecology ($\delta^{13}C$ and $\delta^{15}N$), the indicators of oxidative stress, tissue type, sex, maturity stage, and year.

Significance

Why is it important? Explain the anticipated outcomes of your proposed study and their significance to our understanding of the biology and/or conservation of marine mammals. You may include essential numbered references [1]. 400 words max.

To date, only two studies have analyzed the [THg] and [TSe] within the tissues of CSL in the GC [2; 3]. Furthermore, samples for these studies were collected in 1997 and between 1978-1994, respectively, indicating that the [THg] and [TSe] have been unexplored for the past two decades. Additionally, only one [2] of these studies analyzed how feeding ecology (as determined by $\delta^{13}C$ and $\delta^{15}N$), may be reflective of Hg accumulation, while neither of these studies compared [THg], [TSe] or oxidative stress indicators between sexes, maturity stages, or tissue types. Therefore, our study aims to fill in numerous knowledge gaps in relation to Hg and Se accumulation in the tissues of CSL in the GC. Within this study, various tissue samples will be collected. Furthermore, this study will allow us to better understand how

Hg and Se bioaccumulation in the tissues of CSL is influenced by factors including trophic ecology, sex, maturity stage, tissue type, and year. The results from this study may allow the formation of plans to improve the conservation status of the CSL in reproductive colonies experiencing population decline, as well as other geographical locations. Furthermore, the results from this study may be indicative of the local human population due One Health implications, including shared dietary resources (fish and crustaceans) and habitat.

Timeline

What is your timeline? Give a brief timeline of expected research milestones. Funds should be used within 12 months of the award. A short summary report will be requested at the end of the year. An extension may be requested and granted at that time if needed. 200 words max.

Year one (September 2021-September 2022): Bibliography/thesis redaction, divulgation products, sample collection/analysis, international laboratory exchange, data analysis, and scientific manuscript formation.

In 2021, the following biological samples were collected: hair (N = 36; n = 34 live pups; n = 2 deceased pups), muscle (N = 2 deceased pups), kidney (N = 2 deceased pups), brain (N = 1 deceased pup), heart (N = 1 deceased pup), and liver (N = 2 deceased pups).

As of June 2022, the following biological samples were collected: hair (N = 31; n = 30 live pups; n = 1 deceased pup), muscle (N = 1 deceased pup), kidney (N = 1 deceased pup), brain (N = 1 deceased pup), heart (N = 1 deceased pup), and liver (N = 1 deceased pup). Further fieldwork is programmed for 2022.

Year two (October 2022- September 2023): Bibliography/thesis redaction, divulgation products, sample collection/analysis, data analysis, and predoctoral exam.

Year three (October 2023- September 2024): Bibliography/thesis redaction, divulgation products, sample/data analysis, laboratory exchange, participation in national/international conferences, and scientific manuscript formation

Year four (October 2024- September 2025): Bibliography/thesis redaction, divulgation products, data analysis, participation in national/international conferences, and final doctoral exam.

Expenses

What support is needed and how will it be used? Provide a brief project budget and justify the expenses (breakdown by category and explain requested costs within each category). A maximum of 25% of the budget can be requested as a stipend. As these awards are modest, please describe other available funding or explain how the project can be completed with available resources. Grants are awarded to individuals, therefore no grant fees or overhead should be included. Budget not to exceed US $2000.

Funding for sample analysis has been obtained from CIBNOR and TAMU as part of an international collaboration between these facilities. The applicant for this grant is receiving a four-year student scholarship from the Consejo Nacional de Ciencia y Tecnología (CONACYT). Proposals will be submitted for financial assistance to attend national/international conferences, to publish scientific articles, and to attend external courses critical for academic and professional development.

The current proposal being submitted to SMM is sought as a means to fund an international laboratory exchange from CIBNOR to TAMU for one person and to fund fieldwork during the 2023 field season. A Round trip laboratory exchange visit between CIBNOR and TAMU for one person is proposed for August-September 2023 and would be beneficial as a means to analyze the [THg] and [TSe] in tissues obtained during the 2022 and 2023 field seasons. Similarly, funding for fieldwork would allow us to increase our sampling efforts (number of samples obtained and geographical regions analyzed) during the 2023 field season which will allow us to set up a long-term data base of [THg] and [TSe] in the tissues of CSL in the GC in the past two decades.

We plan to allocate the proposed budget ($2000 USD) to the following areas:

a) Round-trip flight: A laboratory exchange visit for one person is proposed for August-September 2023 using American Airlines. Please note that flight schedules for these dates have not yet been released, and as such, the following breakdown is subject to change:
• Departure: San José del Cabo (Mexico) to College Station (USA)
• Return: College Station (USA) to San José del Cabo (Mexico)
• Round trip cost: 653 USD

b) Baggage fees: Using American Airlines, each checked bag has a cost of 30 USD, although by the time of travel, costs are subject to change:
• Departure price: 30 USD
• Return price: 30 USD
• Round trip cost: 60 USD

c) Personal stipend: This stipend will be put towards additional travel-related expenses including food, transport, and any unexpected travel-related fees during the laboratory exchange. We propose this personal stipend be 25% of the proposed grant total:
• 2000 USD x 25% = 500 USD personal stipend
However, if the entirety of the personal stipend is not used during the 2023 laboratory exchange, we propose to allocate the left-over budget to fieldwork expenses.

d) Fieldwork: Additional fieldwork will take place in 2023. In order to reach the CSL reproductive colony of Los Islotes, a boat and the transport of at least 6 people are required in order to safely capture and restrain pups for the collection of lanugo samples.
• Per day base cost of boat transportation for 6 people: $196.7 USD
o Four one-day trips from June to August: $787 USD fieldwork expenses

e) Grand total of all proposed expenses: 2000 USD

Preparation

My preparation for this project consists of training in areas including sample collection, restraint techniques, physiology and immunology of marine mammals, as well as One Health, contaminants, and oxidative stress. However, as I am currently completing my Ph.D., my preparation is ongoing. Within this project, I will be working with a team of experts that comprise my research committee. These experts include individuals from different research institutes, including Dr. Tania Zenteno-Savín (CIBNOR), Dr. Daniela A. Murillo-Cisneros (CIBNOR), Dr. Claudia J. Hernández-Camacho (Centro Interdisciplinario de Ciencias Marinas, CICIMAR), Dr. José Pablo Vázquez-Medina (University of California, Berkeley), and Dr. Todd M. O´Hara (Texas A&M University). This group of experts will contribute valuable information on marine mammals, contaminants, stable isotopes, oxidative stress, ecology, physiology, and One Health to this project and my academic and professional formation.

Animal Welfare Considerations

What are the legislative requirements for this study in this region and have the necessary permits been applied for or received? Legal requirements for this study include holding valid permits to collect biological samples. All sample collection permits emitted by the Comisión Nacional de Áreas Naturales Protegidas (CONANP) are currently up-to-date. Permits are renewed on an annual basis in compliance with the aforementioned organization.

Are there additional animal welfare requirements and does this study have the necessary approvals applied for or received? This study complies with the Guidelines of Treatment of Marine Mammals in Field Research.

If applicable, does this study comply with the Guidelines of Treatment of Marine Mammals in Field Research? (**yes**/no)

Societal and Research Ethics Considerations

The Society for Marine Mammalogy recognizes the importance of advancing diversity, equity, and inclusion (DEI), and its essential role in generating novel research and conservation practices that truly reflect local needs.

Does this study comply with the Ethical Standards of the Society for Marine Mammalogy? Yes.

Please affirm that you have reviewed these important standards and best practices by stating so here.

I have reviewed and understand the standards and best practices outlined by the Ethical Standards of the Society for Marine Mammalogy.

Please provide a short statement that highlights how throughout your project you will be contributing to the Society's DEI goals. We recognize that this section might not be applicable to everyone. However, we would appreciate it if you could envision ways in which you can advance diversity, equity, and inclusion (DEI) in the country where your research will take place.

Throughout this project, our team is dedicated to following the professional ethics involved in the scientific method, and the timely dissemination of divulgation and scientific products, including providing accurate information free of plagiarism, misrepresentation, etc. We are also dedicated to treating all animals (including humans) we directly and indirectly interact with, as well as their environment, with respect and dignity.

Citations

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[7]. Lalancette, A., Y. Morin., L. Measures., M. Fournier. 2003. Developmental and Comparative Immunology. 27(8): 735–747.

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[9]. Van Hoomissen, S., FMD. Gulland., DJ. Greig., MJ. Castellini., TM. O'Hara. 2015. EcoHealth, 12(3): 490–500.

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[i] The Society's International Relations Committee and Committee of Scientific Advisors have defined all countries except the following as eligible countries: Applicants cannot be from Australia, Austria, Belgium, Canada, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Japan, Korea, Luxembourg, Netherlands, New Zealand, Norway, Poland, Portugal, Singapore, Slovenia, Spain, Sweden, Switzerland, Taiwan, United Arab Emirates, United Kingdom, and United States. See https://marinemammalscience.org/awards-funding/awards-and-scholarships/grants-in-aid-of-research-information/