The California State University
COUNCIL ON OCEAN AFFAIRS, SCIENCE & TECHNOLOGY (COAST)

Graduate Student Research Award Program
AY 2019-2020 Application Form

Application Deadline: Thursday, January 30, 2020, 5:00 p.m. PST

Save as both a Word document and a PDF file named as follows:
***LastName_FirstName_App.docx* and *LastName_FirstName_App.pdf*.**
Submit both files as email attachments to graduate@share.calstate.edu.

Student Applicant Information

First Name:	Lauren	Last Name:	Cooley
CSU Campus:	Moss Landing Marine Laboratories / SJSU	Student ID#:	
Email:		Phone:	
Degree Program:		Degree Sought (e.g., MS, PhD):	MS
Matriculation Date (mm/yy):		Anticipated graduation date (mm/yy):	
GPA in Major Courses:		Thesis-based? (Y/N):	Y

Advisor Information

First Name:	Birgitte	Last Name:	McDonald
CSU Campus:	MLML / SJSU	Department:	Marine Science
Email:		Phone:	

Research Project Title: The physiological effects of scientific handling on northern elephant seals: an integrated approach

Project Keywords (5-7 keywords related to your project): Stress, marine mammals, northern elephant seals, biologging, cortisol, scientific handling

Budget Summary (must add up to $3,000)

Award amount directly to awardee:	$3,000
Award amount to Department:	$0

Please refer to the Award Announcement for detailed instructions on the information required for each of the following sections.

Project Description (60 points total)-1,500 word maximum; any text over this limit will be redacted

Background

Capture and handling are essential elements of many physiological and ecological studies of wild animals, but physiological stress artifacts may confound such research[1–3]. Captured animals are manually restrained, anesthetized, or confined for minutes to days to sample tissues, collect morphological measurements, and attach dataloggers[4]. These research techniques are paramount in studies of cryptic species like marine mammals where instrumentation provides the only means of collecting crucial behavioral and physiological data from at-sea animals[5]. While these studies have furthered both marine mammal science and conservation, capture and handling are potentially stressful events that alter behavior and physiology, and may cause injury or death[6]. Since physiological stress artifacts likely influence the parameters that researchers are measuring, both science and animal welfare benefit from disentangling the effects of scientific handling on marine mammals.

Stress in marine mammals is typically measured with one of two indicators: hormone levels (e.g. corticosterone, aldosterone)[7,8] or vital signs (e.g. heart rate, respiration rate)[2,9]. Stress hormones are measured in blood, blubber, and fur, allowing for evaluation of stress across different timescales. Vital signs are used less frequently to measure stress, but are advantageous in that they allow for real-time monitoring of animal stress during procedures[10]. While these indicators have been measured separately in pinnipeds and other wildlife taxa, few studies have attempted to monitor both hormone and vital levels simultaneously to assess research effects on stress. Even fewer studies have examined changes in blood parameters that reflect overall physiological status and processes such as metabolism, inflammation, and oxygenation that are likely to be influenced by research handling[11].

An understanding of the magnitude and duration of the acute stress response, beyond just routinely measured cortisol levels, induced by specific capture and handling techniques is critical to ensure research results are not biased by physiological stress artifacts. Moreover, the success of management and conservation initiatives could be improved if any negative fitness consequences from research activities were minimized[7]. Marine mammals, which are already subject to numerous natural and anthropogenic stressors including climate change, fisheries interactions, and ocean noise would especially benefit from reduction in additional research-induced stress[10]. To provide a holistic understanding of the effects of scientific handling on wild marine mammals, I will integrate endocrine, cardiovascular, and blood chemistry data into a single comprehensive study of the acute stress response.

The northern elephant seal (*Mirounga angustirostris*) is a model species to apply an integrated approach to investigate the physiological response to scientific handling. These large pinnipeds are readily accessible to researchers, tolerate instrumentation well[12], have documented cortisol responses[11], and are intensively studied. Routine research handling of elephant seals at Año Nuevo State Park includes chemical immobilization, physical restraint, morphological measurement, and instrumentation with biologgers. More complex studies can involve translocation via truck or boat, surgical procedures conducted under gas anesthesia, and overnight holding[13]. Given that over 500 seals are handled at Año Nuevo annually[14], it is imperative that scientists understand the effects of their research methods on study animals.

Proposed Research

My M.S. research under Dr. Birgitte McDonald at Moss Landing Marine Laboratories is part of a larger study deploying a novel physiological logger on free-ranging seals. The extensive animal capture, handling, and transport involved in this project provide an ideal system in which to examine the effects of such research procedures on pinnipeds. <u>I will use a combination of heart rate data collected by EKG biologgers, cortisol levels measured by radioimmunoassays, and blood parameters to develop a comprehensive picture of the physiological effects of scientific handling on elephant seals.</u>

Objectives

1. Quantify hormonal stress responses using periodic serum cortisol measurements.
2. Measure cardiac stress responses using continuous EKG heart rate data.
3. Examine whole-body physiological changes induced by stress using blood parameters such as erythrocyte sedimentation rate (ESR), lactate, blood glucose, and hematocrit.
4. Determine the impacts of the hormonal stress response on physiology by linking cortisol to heart rate and blood parameters.

Hypotheses

1. Serum cortisol measurements will allow for identification of the procedural stages that elicit the strongest stress response over a longer timescale.
2. Elevated heart rate (i.e. tachycardia) will enable precise identification of specific stressors on a shorter timescale.
3. Stress will alter physiological processes by increasing inflammation (ESR), metabolism (lactate and blood glucose), and blood oxygenation (hematocrit).
4. Cortisol will be positively correlated with mean heart rate, inflammation, metabolic rate, and blood oxygenation.

Methods

Field Work: This project employs a well-established translocation protocol[13] in which juvenile elephant seals are captured at the Año Nuevo colony (San Mateo County, CA) during the April molt season. Following capture, seals are transported by truck to Long Marine Laboratory (UC Santa Cruz) for instrumentation under isoflourane-O_2 gas anesthesia, held overnight, and then transported by truck to Hopkins Marine Station on the other side of Monterey Bay for release. The seals swim back to Año Nuevo over the next two to seven days facilitating the recovery of their biologgers. Throughout the translocation process detailed procedural records and video recordings are maintained to provide a time stamp for each event in relation to the measured parameters. We aim to translocate ~24 seals over two field seasons (2019, 2020). All research work is performed under NMFS permit #1656282 with approval from SJSU and UCSC IACUCs.

Sample Collection & Analysis: I have identified seven distinct stages of the translocation procedure that represent different types of research handling: 1) capture at Año Nuevo; 2) early instrumentation procedure (immediately following gas anesthesia); 3) mid-procedure (50 minutes into gas anesthesia); 4) late procedure (150 minutes into gas anesthesia); 5) early recovery (three hours post-gas anesthesia); 6) late recovery (next morning pre-disturbance); and 7) re-capture (back at Año Nuevo several days later) (Fig 1). Blood samples are collected intravenously from the extradural vein in 10 mL BD Vacutainer tubes during each of these stages. An i-STAT handheld blood analyzer (Abbott Laboratories) is used to rapidly measure blood chemistry parameters including blood glucose, blood urea nitrogen, lactate, electrolytes, and various blood gas indicators. ESR and hematocrit are determined following standard laboratory procedures[15,16]. Cortisol concentrations are measured using commercially available radioimmunoassay kits (Siemens cortisol coat-a-count kit TKCO2) that have been previously validated for this species[17].

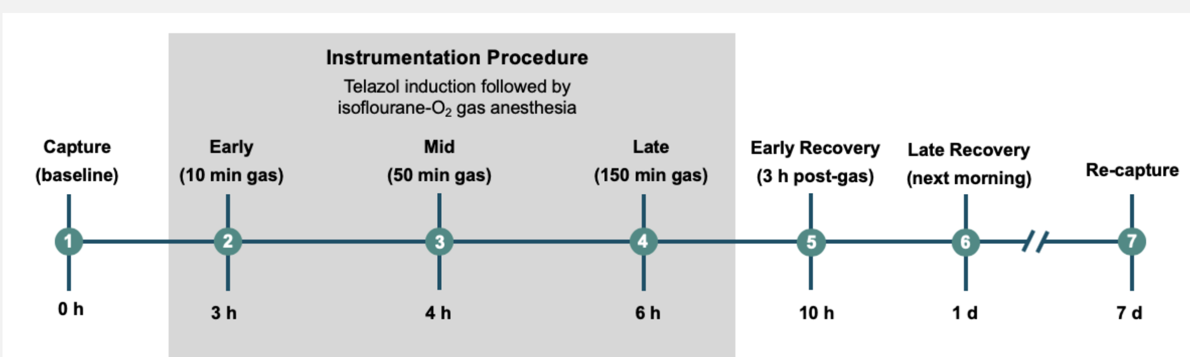

Fig 1. Blood sample collection timeline. Spacing between time points not to scale.

Heart Rate Data Collection & Processing: Each seal will be instrumented with an OxyLog custom physiological logger (UFI Instruments) that records the electrocardiogram (EKG) at 50-100 Hz. A sterile subcutaneous EKG electrode is inserted percutaneously into the extradural vein through a peel-away catheter, protected with a neoprene patch, and then connected to the datalogger with a cable. These instrumentation techniques follow standard protocol and have proven to not interfere with seal locomotion[13,18]. Using custom processing scripts in MATLAB and R, I will convert EKG data into instantaneous heart rate (beats min^{-1}).

Data Analysis: I will calculate mean heart rate over 10-second intervals and then categorize each mean in two ways: broadly according to stage and then more narrowly according to specific stressor (e.g. extubation, cage moved in lab, transport on truck, etc.) using information from the procedural records and video recordings. I will then analyze the heart rate, cortisol, and blood parameter data using separate repeated measures ANOVAs to test for differences between the seven procedural stages and/or stressors, as applicable. If significant differences between groups are found, I will use Fisher's least significant difference post hoc tests to compare specific stages and/or stressors. Finally, I will determine the relationship between cortisol and the other stress metrics (mean heart rate and each blood parameter) using separate mixed model linear regressions. All statistical analyses will be performed in R.

Significance

The effects of scientific handling on the acute stress response of wildlife are rarely analyzed as a complex physiological interaction. I propose a conceptual step forward in characterizing the stressful effects of research activities on marine mammals by integrating diverse physiological parameters traditionally analyzed separately into a single comprehensive model. Cortisol and heart rate are well validated stress indicators, but the relationship between them remains ill-defined[19]. Beyond evaluating just endocrine and cardiac stress markers, this study will advance our understanding of foundational stress physiology by using blood parameters to investigate the effects of stress on metabolism, inflammation, and oxygen transport.

This integrated approach will identify the most stressful handling activities and further clarify the complex physiological changes induced by the acute stress response in northern elephant seals. Only when such activities have been identified will we be able to re-assess and modify handling protocols to reduce stress loads. Beyond improving animal welfare, such reductions limit the potential confounding effects of physiological stress artifacts on measured parameters, thus benefitting scientists and elephant seals alike. The holistic approach developed here in a model species can then be applied to other more vulnerable marine mammal species to further both physiological and ecological research goals and conservation initiatives[10].

References (0 points)-no limit

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5. Hooker, S. K., Biuw, M., McConnell, B. J., Miller, P. J. O. & Sparling, C. E. Bio-logging science: Logging and relaying physical and biological data using animal-attached tags. *Deep. Res. Part II Top. Stud. Oceanogr.* 54, 177–182 (2007).
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11. Champagne, C. D., Houser, D. S., Costa, D. P. & Crocker, D. E. The effects of handling and anesthetic agents on the stress response and carbohydrate metabolism in Northern elephant seals. *PLoS One* 7, e38442 (2012).
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13. Meir, J. U., Champagne, C. D., Costa, D. P., Williams, C. L. & Ponganis, P. J. Extreme hypoxemic tolerance and blood oxygen depletion in diving elephant seals. *Am. J. Physiol. - Regul. Integr. Comp. Physiol.* 297, 927–939 (2009).
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Timeline (10 points total)-250 word maximum

This project began in spring 2019 with the successful completion of the first field season. Our research team translocated 11 juvenile northern elephant seals and I collected blood samples, EKG heart rate data, and behavioral records from each animal. Over summer and fall 2019, I processed heart rate data in MATLAB, drafted my thesis proposal, and mentored an undergraduate student through the NSF REU program. During the upcoming field season in spring 2020 we aim to translocate an additional 12-14 seals.

The remaining work for my thesis consists of laboratory analysis, data processing, statistical analysis, and manuscript writing. I will complete cortisol assays on all blood samples in summer 2020 in partnership with the Crocker Lab at Sonoma State University. Throughout summer and fall 2020, I will complete any remaining heart rate data processing, perform all statistical analyses, and begin writing my thesis manuscript. I aim to finish writing my manuscript and defend in spring 2021. Additional data dissemination will occur through open access journal publications and conference presentations (e.g. Society for Marine Mammalogy, Western Society of Naturalists, International Bio-Logging Society).

Relation to COAST Goals (15 points total)-300 word maximum

In accordance with COAST goals, this project aims to advance our knowledge of coastal and marine resources. California is home to a diverse range of over 30 marine mammal species, including pinnipeds, cetaceans, and sea otters. While non-subsistence hunting of marine mammals was banned in 1972, these uniquely vulnerable species now face a new gauntlet of anthropogenic threats including fisheries interactions, pollution, ocean noise, and climate change[10]. Before the deleterious effects of such actions can be effectively mitigated, we must understand their impacts on animal physiology, behavior, and ecology. Stress physiology research is an integral component of this endeavor, but physiological stress artifacts resulting from the capture and handling requisite in many marine mammal research projects may confound results. By investigating northern elephant seal endocrine, cardiac, and blood chemistry stress indicators throughout a translocation procedure, my project will help clarify the largely unresolved effects of research artifacts on stress levels. As elephant seals are an excellent model species in which to investigate stress physiology, the holistic approach developed here will benefit future research into other marine mammal species of concern in California and beyond.

COAST also strives to promote environmental literacy. By collaborating with the educational team at Año Nuevo State Park to help develop new displays that synthesize elephant seal physiology research in an engaging and accessible format, I will help educate the public about the importance of marine research and conservation. Furthermore, I will partner with two distance learning programs, “Skype A Scientist” and “California State Parks Online Resources for Teachers and Students”, to reach students from underserved communities who would otherwise have limited exposure to marine science education or direct interaction with a marine biologist.

Budget and Justification (15 points total)

Item Description	**Unit Price**	**Quantity**	**Amount to Awardee (via Financial Aid)**
Living expenses	-	-	$1920.00
Tuition	$270/unit	4 units	$1080.00
		Grand Total	**$3,000.00**

Justification (250-word maximum):

This COAST Graduate Student Research Award would provide me the financial security to allocate more time to my thesis work over summer 2020. While the biodiversity and unique oceanographic conditions of Monterey Bay make it a hotspot for marine science research, the high cost of living here makes it difficult to afford basic living expenses on a graduate student income. I currently work three jobs to cover my living expenses: (1) library assistant at the MLML/MBARI Research Library; (2) graduate research assistant in my lab; (3) petsitter for local families.

Last summer I worked 25-40 hours/week at the library to save up enough money to pay rent throughout the school year when students are capped at 20 hours/week. The funds from this award would allow me to reduce my average weekly hours at the library this summer to have more time to focus on data processing. I would also have the ability to take 1-2 weeks off from work to travel to Sonoma State University to complete my hormone assays, an essential element of my thesis project. The remainder of the funds would be used to cover my tuition fees for fall 2020.

Thank you for your time and consideration of my application.

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