Warren J. Baker Endowment
for Excellence in Project-Based Learning
Robert D. Koob Endowment *for Student Success*

PROPOSAL NARRATIVE

I. Project Title

Does the acorn barnacle *Balanus glandula* exhibit predictable gradients in metabolic performance across the intertidal zone?

II. Abstract

Intertidal organisms live in one of the most environmentally stressful habitats on the planet, and daily fluctuations they experience in abiotic factors (e.g., temperature, oxygen, salinity, pH) are predicted to intensify as our global climate continues to change. Elevated temperature and oxygen limitation are two dominant stressors associated with periodic air emersion in the littoral zone; both of which have substantial direct effects on metabolism in ectotherms. In the proposed study, we aim to profile the *'metabolic phenotype'* of acorn barnacles (*Balanus glandula*) from different intertidal zones (low, mid, high). We define metabolic phenotype as the individual's baseline metabolic performance, and will characterize this parameter with a comprehensive suite of biochemical (e.g., citrate synthase and lactate dehydrogenase activity, [lactate]), physiological (VO_2, aerobic scope) and behavioral (e.g., feeding rate) indices of metabolism. We hypothesize that there will be predictable gradients in metabolic performance across the tidal zone directly resulting from environmental variation. With few exceptions (e.g., Stillman and Somero, 1996), data of this kind is nearly absent from the current literature. The proposed project has inherent value in its potential to identify physiological responses to emersion and climate-driven environmental variation, as well as document tidal position-dependent patterns in metabolic phenotype and capacity for plasticity that can be quantitatively integrated into predictive models of population persistence during climate change.

III. Introduction

Intertidal organisms experience a wide range of stressors that can vary considerably over daily, seasonal, and annual timescales. Such extreme variation includes abiotic factors such as temperature, oxygen, salinity, and pH, along with biotic factors like food availability and predation. The ability to cope with extreme environmental changes is the product of their adept capacity for phenotypic plasticity in anatomical structure, physiology and behavior, which ensures adequate fitness in the face of a constantly shifting environment (Matzelle et al., 2015; Seebacher et al., 2015). Studies have documented such remarkable plasticity in other shoreline organisms following variation in air exposure, predator density, and temperature (Finke, 2007; Trussel, 1996). From these studies we find that there are often predictable gradients in the resulting phenotypes across the intertidal zone owing to predictable variation in the environmental conditions that define this habitat.

Daily fluctuations in temperature and oxygen are associated with periodic air exposure (emersion) in the intertidal zone and are considered to be two of the primary abiotic drivers of physiology in resident species. Temperatures here can change drastically over a 24h period depending on tidal height and time of day, whereas sea surface temperatures off the California coast remain relatively stable. In a mussel bed, for example, there can be an 8°C change in temperature between low and high tide (Zippay et al., 2012). And though oxygen levels stay relatively constant in the air, intertidal species vary substantially in abilities to uptake oxygen during emersion. In ectothermic marine invertebrates, increased temperatures elevate metabolic rates and hypoxia typically induces a metabolic depression (Storey, 1990; Guppy, 2004). Air emersion is therefore especially challenging since these events often occur simultaneously. These issues become increasingly interesting in the context of global climate change, given that the intertidal zone is predicted to experience even greater extremes in these abiotic conditions (Harley et al., 2006). We feel it is important to try and unravel the physiological responses of keystone intertidal organisms to variation in these stressors, as well as to explore the *extent* to which inhabitant species can acclimate (and ultimately adapt) to changing conditions.

In this project we aim to describe the baseline 'metabolic phenotype' of an intertidal organism - the locally ubiquitous acorn barnacle *Balanus glandula* - from different locations across the intertidal zone (e.g., low, mid and high). We hypothesize that predictable gradients in metabolic performance will be found across this zone owing to the established variation in environmental stressors respectively experienced in each tidal site (e.g., differences in daily temperature fluctuations, hemolymph pO2, food availability, etc.). To this end, we will collect *B. glandula* from low- mid- and high- intertidal regions from San Luis Bay, CA and use an integrative suite of biochemical (lactate dehydrogenase and citrate synthase activity), physiological (O_2 consumption rates), and behavioral (cirri beat frequency, operculum open/closed) measurements to collectively define a baseline for metabolic performance in each tidal site for comparison. This kind of data is relatively absent in current research (Stillman and Somero, 1996) and so we feel it will be a valuable contribution to the fields of comparative intertidal physiology and climate change research. In the future, we intend for this research to serve as a baseline for studies of the *capacity* for phenotypic plasticity in barnacles (and other species) across a vertical zonation.

IV. Objectives

PRIMARY OBJECTIVE: Determine if there are gradients in the metabolic phenotype of *B. glandula* across the intertidal zone.

- *Objective 1*: Characterize the daily and seasonal variation in abiotic stressors in the field and in the animals from our collection site in San Luis Bay, CA.
- *Objective 2*: Characterize the metabolic capacity (via biochemical analysis) of *B. glandula* from the low-, mid- and high-intertidal zone.
- *Objective 3:* Compare the standard metabolic rate (SMR) between *B. glandula* from the low-, mid- and high- intertidal zone.
- *Objective 4*: Compare activity levels (of feeding and respiratory behaviors) between *B. glandula* from the low-, mid- and high-intertidal zone.

V. Methodology

Objective 1: We will measure changes in temperature, pO2, pH, and salinity across the intertidal zone (subtidal to high intertidal) over multiple 24-hour survey periods during both the coolest (~January) and warmest (~September) times of the year. Measures of temperature and salinity (YSI 85), oxygen (YSI ProODO dissolved oxygen meter) and pH (Loligo WTW pH 3310) will be collected from the water directly. To determine thermal profiles of the resident barnacles we will use 1) iButton temperature loggers anchored (with silicone glue) inside of empty barnacle shells and glued in position on rocks in all intertidal zones across our site, and 2) digital thermocouples to measure temperature from inside of a subset of living, anchored barnacles. Internal pO2 (hemolymph and/or mantle cavity) will be measured in the lab across a simulated tidal cycle from barnacles collected in the field (low, mid high) using a PreSens Microx oxygen meter fitted with a needle-type oxygen microsensor. Wave action data, as well as temperature, pH, salinity and oxygen will be utilized from an anchored CTD device that has been continuously monitoring San Luis Bay for years. This component of my work will involve collaborations with oceanographers in the physics (Dr. Ryan Walter), chemistry (Dr. Emily Bockman) and biology (Dr. Alexis Pasulka, Ian Robbins) departments. Collectively, these data will help us understand exactly what conditions our study organisms experience throughout the year.

Objective 2: Anaerobic and aerobic metabolic capacity will be approximated from measures of lactate dehydrogenase (LDH) and citrate synthase (CS) activity. *B. glandula* (~n=30/tidal region) will be collected from low- mid- and high- intertidal zones, and whole-animal tissues will be excised immediately after collection, flash frozen and stored in -80 C^{o} for future analysis. Biochemical analysis for LDH and CS will involve spectrophotometric assays commonly performed in our lab. Additionally, we will assess the amount of anaerobic metabolism carried out by each group of barnacles during air emersion (20°C), by measuring [lactate] from extracts of whole-animal homogenates. Lactate concentrations will be determined using standard spectrophotometric assays based on D-lactate kits.

Objective 3: SMR (15°C; oxygen consumption rates; VO_2) of barnacles from each tidal zone will be determined using intermittent respirometry in aquatic conditions (Loligo Systems respirometry).

Objective 4: Behavioral measurements (percentage of barnacles feeding and cirri beat frequencies) will be analyzed from video recordings of barnacles collected in each intertidal zone (n=20/zone). Behavior data will be collected from immersed barnacles held under common temperature conditions (15°C) over a 2h time period.

Data Analysis: Data collected in these experiments will be analyzed using JMP software (version 13). We will use one-way ANOVA models to look for a main effect of intertidal position (low, mid or high) on enzyme activity, SMR, and behavioral indices. Where significant main effects are found we will use *post hoc* Tukey HSD tests to compare between means.

VI. Timeline

TIME LINE	Fall 2017	Winter 2018	Spring 2018	Summer 2018	Fall 2018	Winer 2019	Spring 2019
Data Collection: Abiotic	X	X	X	X	X		
Data Collection: Respirometry & Behavior		X	X	X	X		
Experiment: Biochemical			X	X	X	X	
Data Analysis				X	X	X	
Manuscript Writing						X	X
Present						X	
Graduate							X

VII. Final Products and Dissemination

The final results of this project will be written into a manuscript and submitted for publication in the *Journal of Experimental Biology* or *Marine Biology*. Along the way, I will disseminate my results via poster and oral presentations at the Society for Integrative and Comparative Biology (December, 2019, Tampa, FL) and Western Society of Naturalist (November, 2018) Conferences.

VIII. Budget Justification

Though our lab is already in possession of all the research equipment required to complete the proposed study, many of the necessary reagents and consumables are extremely expensive and get used up quickly [e.g., D-lactate colorimetric kits ($376.00 each; we will need >6) and acetyl CoA sodium salt ($164.50/5mg); iButtons ($75.00 each; we could use 10 more]. This endowment will allow me to purchase these supplies and, thus, support the completion of my objectives. Further, funds will be used to support conference travel, during which time I will present the results of this study. Departmental travel funds have decreased in the last several years, and by securing this award I will be guaranteed the opportunity to attend both of these meetings. As a graduate student I have little funds saved up due to loans from my undergraduate degree. Out side of graduate courses, research, and teaching as a teaching associate, there is no time to work another job to pay for basic life needs, tuition and travel to conferences.

IX. Appendix: References

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CAL POLY

PROPOSAL BUDGET

Student Applicant(s):	
Faculty Advisor:	Kristin Hardy
Project Title:	**Requested Endowment Funding**
Travel *subtotal*	**$ 910**
Travel: In-state	**$ 200**
Travel: Out-of-state	**$ 710**
Travel: International	**$**
Operating Expenses *subtotal*	**$ 1,590**
Non-computer Supplies & Materials	**$ 1,290**
Computer Supplies & Materials	**$**
Software/Software Licenses	**$**
Printing/Duplication	**$**
Postage/Shipping	**$**
Registration	**$ 300**
Membership Dues & Subscriptions	**$**
Multimedia Services	**$**
Advertising	**$**
Journal Publication Costs	**$**
Contractual Services *subtotal*	**$**
Contracted Services	**$**
Equipment Rental/Lease Agreements	**$**
Service/Maintenance Agreements	**$**
TOTAL	**$2,500**