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

Graduate Student Research Awards
AY 2013-2014 Application Form

Application Deadline: Thursday October 24 2013, 5:00 p.m. PDT
Save this file as *LastName_FirstName.docx* and email it as an attachment to:
graduate@share.calstate.edu

Student Applicant Information

First Name:	Garrett	Department or Degree Program:	Biology
Last Name:	Lemons	GPA in Major Courses:	
Student ID#:		Anticipated graduation date (mm/yy):	Summer 14
CSU Campus:	San Diego	Degree Sought (e.g., MS, PhD):	MS
Email:	Lemonsgarrett@gmail.com	Thesis-based? (Y/N):	Y
Phone			

Have you previously received a COAST Research Award? (Y/N)	N
If yes, please provide year of award:	

Faculty Advisor Information

First Name:	Rebecca	Position/Title:	
Last Name:	Lewison	Email:	rlewison@mail.sdsu.edu
CSU Campus:	SDSU	Phone:	
Department:	Biology		

Research Project Title:	**DEVELOPING OF A NOVEL TOOL TO INVESTIGATE SEA TURTLE SPATIAL and TROPHIC ECOLOGY**
Project Keywords (5-7 keywords related to your project):	Isotopes, amino acids, fractionation, ectotherm, enrichment, Chelonia, nitrogen

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

Project Description (50 points)-1500 word maximum

Background

Bulk stable isotope analysis (BSIA) has, for a number of years, been used to explore the foraging ecology of many marine megafauna [1,2,3,4,5,6,]. The value of this technique stems from the fact that isotopic composition of consumer body tissues is derived from its prey and its environment [7,8,9]. However, trophic level determination by way of BSIA can be biased by the influence of nutrient composition and cycling at the primary production level. Furthermore, individual specific metabolic processes can influence isotope values and prevent the correct interpretation of isotopic data and trophic relationships. To address these confounding aspects of BSIA, compound specific isotope analyses (CSIA) has been proposed as an innovative method to more accurately determine trophic position while also characterizing nutrient cycling dynamics at the base of the food web.

CSIA utilizes the isotopic composition of individual amino acids to make inferences about the association between an organism and their environment [10,11]. Using amino acids as the source of isotopic signatures, CSIA eliminates the confounding influence of enrichment and primary production inherent in BSIA [12,11,13]. During metabolism some amino acids are fractionated (cleavage of nitrogen bonds) and enriched as they move up trophic levels ("trophic" amino acids) while some amino acids are minimally enriched or not at all ("source" amino acids) [10]. Using these source and trophic amino acids, CSIA circumvents the uncertainty of BSIA utilizing non-enriched source amino acids to retain isotopic signatures that are indicative of nutrient dynamics at the primary production level, while trophic amino acids can give unbiased trophic position. For example, ^{15}N analysis of trophic amino acids when coupled with source amino acids, can provide exact information on the trophic position of an organism calculated by the following equation:

$$\mathbf{TL_{x/y} = (\delta^{15}N_x - \delta^{15}N_y - \beta_{x/y})/(\Delta_x - \Delta_y) + 1}$$

where $\beta_{x/y}$ represents the isotope difference between amino acids x and y in the primary producers (trophic level = 1.0), and Δx and Δy represent the ^{15}N-enrichment factors with each trophic level for amino acids x and y, respectively. The sub-scripts x and y indicate "trophic" and "source" amino acids that show a large ^{15}N enrichment and little change in $\delta^{15}N$ values with each trophic level, respectively [11].

The successful application of CSIA relies on the determination of enrichment factors between source and trophic amino acids ($\Delta_x - \Delta_y$). Although Chikaraishi et al. [11] has determined this enrichment factor for algae, zooplankton and fish, enrichment factor determination is needed to apply this powerful technique to other taxa.

The principle advantages CSIA affords over the bulk method are (i) exact trophic level can be determined without the influence of base level nutrients and (ii) a single tissue sample reflects an integrated value for the isotope signatures of the primary producers in the studied food webs [11]. The

first advantage is important because unlike BSIA, CSIA does not require separate determination of isotope values of primary producers to infer trophic level. Exact trophic level determined by CSIA overcomes the spatial and temporal variability of nutrients at the base of environments allowing for insights into mediation of nutrients through systems. Hannides et al. [14] demonstrated this by showing temporal variation in bulk $\delta^{15}N$ values of zooplankton, but a steady trophic level was measured via CSIA for over 10 years. The second advantage is that it allows for the characterization of nutrient composition at the base of the food web. This advantage is especially promising for investigations into systems facing nutrient loading [15], understanding spatial differences in nutrient composition [16] and response to criticisms about the limitations of isotope analysis in determining trophodynamics [17].

Project Description

This project, divided into experimental and applied parts, takes the first step toward an ultimate goal of characterizing the trophic and spatial ecology of Eastern Pacific green turtles (EPGTs) as they transition from oceanic to neritic habitats. The goal of the experimental phase of the project is to validate two critical assumptions for EPGTs, (1) the trophic discrimination factor (TDF) and (2)tissue turnover rates. Using a controlled feeding study, this first part of the experiment will determining the trophic discrimination factor for EPGTs. The second phase will determine the isotopic turnover rates for different tissues. The applied portion will apply newly validated CSIA to understand wild EPGT populations. The experimental part of this project is centered on three hypotheses:

1.) The ^{15}N isotopic enrichment factor for EPGTs is greater than the established 7.8‰ [11].
2.) A single green turtle tissue sample analyzed by CSIA will reveal both trophic position and the primary production isotope values for ^{15}N.
3.) Turnover rates will vary between skin and plasma.

Materials and Methods

The experiment uses 4 captive green turtles located at the Living Coast Discovery Center, a coastal wildlife and ecosystem education center in Chula Vista, CA. Due to nature of conducting research on threatened species that are difficult to access, the small samples size is not ideal but can still provide valuable data with sufficient replication during analysis. Data collection and analysis for the first phase of the experimental portion of the project, TDF determination, is complete. The second phase, determining the isotopic turnover rates for skin and plasma will be initiated in November this year. To determine isotopic turnover rates in tissues a diet shift will be initiated by feeding the turtles a diet of isotopically enriched food and sampling tissues at appropriate intervals.

Tissues in a living organism are constantly in flux, renewing and degrading as the body grows and expends energy. Moreover amino acids constituents of tissues that degrade enter the blood stream and mix with diet derived amino acids (18). Therefore, the turnover rate and isotope half-life of a given piece of green turtle tissue is a mix of fractional turnover rates and isotope half lives that vary based on the different pools that are drawn from to supply amino acids to the different parts of a tissue (19,20). Essentially, the overall incorporation rate and half-life of a tissue can potentially be a product of multiple isotopic pools resulting in the isotope incorporation into a specific tissue not being adequately modeled using the exponential fit model (Eq. 4). In the context of ^{15}N analysis of amino acids, it is especially important to account for multiple turnover pools as previous studies (10,11) show differential ^{15}N enrichment and depletion among amino acids of the same tissue. Neglecting multiple turnover pools can result in the true half-life of an isotope in a certain tissue being inaccurately estimated which in turn can confound interpretation of applied SIA (21). The design of this experiment allows for determination of isotope turnover pools in tissues by sampling at specified intervals and using the **reaction progress variable** (**RPV**; 22)

Preliminary Results and Anticipated Data Analysis

Trophic discrimination factor preliminary results:

Preliminary data from the first part of the experiment has yielded trophic and source amino acids and the TDFs for EPGT skin and plasma (Table 1). These data represent the first determination of trophic and source amino acids for the EPGT and highly mobile marine megafauna and the first validation of CSIA TDFs for EPGTs (Table 1).

Table 1. Characterization of trophic and source amino acids and TDFs for EPGT skin and plasma.

	Trophic amino acid	Source amino acid	TDF (Trophic – source)
SKIN	Serine	Lysine	9.33‰
PLASMA	Serine	Methionine	8.68‰

Tissue turnover and isotopic incorporation rates:

The RPV (21, 22) can determine if more than one isotope turnover pool is present and can aid in determining the relative fractional contribution of the isotope pool to a specific tissue. The RPV is characterized by the equation below:

$$[\delta_{t} - \delta_{ss} / \delta_{i} - \delta_{ss}] = (1-F)$$

Where δ_t is the isotopic value at time t during the experiment δ_i is the data derived initial isotopic value and δ_{ss} is the data derived isotope final steady-state value. **F** is defined as **0** at the beginning of the experiment and **1** at the final steady state. Following mathematical transformation identification and modeling of each linearized turnover pool can be characterized as a function of either carapace length or weight of the captive turtles. The best representation will be chosen after the model is fit to both measurements of growth following procedures outlined in [26].

Significance

The intellectual merit of this project and its contribution to the scientific and conservation community stems from the fact that it innovates stable isotope methodology. This project provides a new analytical methodology to study sea turtle ecology. This new methodology will elucidate dynamics of green turtle ecology in cryptic life stages and throughout their entire life cycle. More broadly, this methodology holds promise to determine trophic dynamics of oceanic food webs, nutrient pathways, primary productivity and the ecology of other taxa. In the face of increasing anthropogenic threats, development of more accurate methodology for ecological studies is compulsory to the development and implementation of effective conservation measures in oceanic systems. In the case of EPGTs, understanding the dynamics of their spatial and trophic ecology is a crucial part to their conservation and future persistence.

Relation to COAST goals (20 points)-250 word maximum

This project promotes the advancement of coastal and marine related research by developing a novel research tool for the investigation of East Pacific green turtles, animals which inhabit California waterways, coastal environments and oceanic waters off the coast of California. Not only will this project advance research of the trophic ecology of oceanic and neritic foraging green turtles, areas of research impeded by the cryptic nature of these life stages, the proposed methodology is also relevant to the use of other marine mega fauna to understand nutrient flow through food webs and the nutrient composition at the base of marine food webs. By developing a novel research technique, this project will provide a new methodology for CSU researchers to utilize in education and research. This project will also provide meaningful learning-through-research opportunities for undergraduate research assistants, enhancing their science education experiences through exposure to applied ecological science in the field and the lab. As a collaborative effort between SDSU and the Learning Coast Discover Center (LCDC), this project has access to the general public in a unique way. The Living Coast Discover Center's mission is to inspire care and exploration of the living earth by connecting people with coastal animals, plants and habitats. Because this project is being conducted at the LCDC, local residents, students and tourists will learn of the research and the current status of endangered sea turtles, the ecology of oceanic systems and gain awareness to emerging ecological science, investigative techniques and local conservation issues.

Need for Support (10 points)-250 word maximum plus timeline including graduation date

A majority of this project has been funded but support is still needed for CSIA for the turnover part of the project. Funding from COAST will help cover remaining costs for CSIA of skin and plasma. A breakdown of support needed is in the following table:

Tissue type	No. of samples	Analysis cost total	Amount Funded	Needed
Skin	60	$3840.00	$2340.00	**$1500.00**
Plasma	80	$5120.00	$3620.00	**$1500.00**
			Total Support Needed	**$3000.00**

Support from COAST would be crucial to the completion of this project. Collecting and analyzing enough samples is crucial to experimentally validating this new isotope methodology. Not only will this support help move this project forward to completion but additional funding from COAST with allow for the most robust and proper sample analysis in order to make this methodology reliable for use in future investigations. A timeline for the remainder of the project and degree completion is shown below:

	Spring 2013	Summer 2013	Spring 2014
Steady state determination (BSIA)	X	X	
TDF and Turnover (CSIA)		X	X
Graduation			X

References (10 points)-no limit

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