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**Proposal Cover Sheet**

**Term: Fall\_\_**X**\_\_\_ Spring \_\_\_\_\_ Year** 2011

**Instructor \_\_\_\_\_\_**Nora Demers**\_\_\_\_\_\_\_\_**

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Present Year in Education (e.g., freshman, sophomore, etc.): \_Senior\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

E-mail Address: reditter@eagle.fgcu.edu\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Major \_Marine Science\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Have you identified a research mentor for a senior thesis (if applicable)?

\_\_\_X\_\_ Yes \_\_\_\_\_ No.

If yes, please identify.

Name: \_\_Dr. Robert Erdman\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Title of Proposal:**

Gene Flow Among Populations of Caridean Shrimp (*Barbouria cubensis* and *Parhippolyte sterreri*) in Anchialine Lakes on San Salvador Island, Bahamas.\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Keywords

\_Gene Flow, Caridean, ITS, Anchialine, Karst\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Checklist:**

All required portions of the first submission are included \_X\_\_\_\_ Yes \_\_\_\_\_ No

I had an external reviewer read the proposal \_\_\_X\_\_ Yes \_\_\_\_\_ No

If Yes, who \_\_Robert Erdman and Matt Palmtag\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ When \_10/16/2011\_\_

I authorize the use of this proposal as an example in future courses \_\_X\_\_\_ Yes \_\_\_\_\_ No

**Gene Flow Among Populations of Caridean Shrimp (*Barbouria cubensis* and *Parhippolyte sterreri*) in Anchialine Lakes on San Salvador Island, Bahamas.**

By: Robert Ditter

**Abstract**

San Salvador is a small tropical Island in the Bahamas. Of the little land mass the island has, nearly half is covered by two types of saline lakes, that display either hypersaline or marine conditions. The lakes with marine conditions have conduits large enough for subsurface tidal flow to occur. Although the locations of the conduits within the lakes have been established, no study has successfully traced these conduits or established if any connection between them exists. It is the goal of this study to find if there is any connectivity among the conduits by measuring gene flow among populations of Caridean cave shrimp that reside within them.

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**Introduction**

San Salvador is a small karst island in the Bahamas, with no surface freshwater supply, and only a small underground freshwater lens (Davis and Johnson 1989). Nearly half of its land mass is covered by two types of anchialine lakes (hypersaline and marine) (Mylroie et al. 1995). These lakes have been surficially isolated for roughly 140,000 years and act as ecological “islands” within an island (Hearty and Kindler 1993, and Edwards 1996). Yet, subsurface tidal flows permit marine conditions to exist in many of the lakes via conduits created from the dissolution of the carbonate platform (Mylorie and Carew 2003). This topography is rather unique to tropical islands, and no study has successfully traced these conduits, either to the outside ocean, or to each other (Whitelaw 2001).

On San Salvador most anchialine lakes have few common species (primarily Mollusca), including two species of Caridean cave shrimp (*Barbouria cubensis* and *Parhippolyte sterreri*) that inhabit the conduits (Iliffe 1986, and Lanterman et al. 2007). In studies by Santos (2006) in Hawaii, and Zaksek et al. (2009) in the Balkan Peninsula, gene flow between populations of cave shrimp were used to examine connections of similar karst lake systems.

Gene flow between populations is difficult to measure. Comparison of variations in microsatellites located on the ribosomal DNA (rDNA) is the most widely accepted method (Edsman et al. 2002), but this requires a minimum of 30 samples from individuals from each population. Identifying variations in the ribosomal RNA (rRNA) may also be used to establish similarities in populations’ genetics and any genetic connectivity (Santos 2006). Due to the rarity of anchialine lake habitats with Caridean shrimp populations, using variations among the first and second Internal Transcribed Spacers (ITS1 and ITS2) and 16S and 28S regions of rDNA has been used in some studies (Santos 2006, Wang et al. 2007, and Zaksek et al. 2009). This method only requires one sample from each population to establish the presence of gene flow and phylogenetic differences, and many microsatellite loci are found within these regions (Harris and Crandall 2000).

ITS1, ITS2, 16S and 28S of rRNA act as molecular markers for phylogenetics and population analysis (Chu et al. 2001). Regions of rRNA can be isolated using primers, composed of 18 nucleotides, and replicated via polymerase chain reaction (PCR) for comparison (Zaksek et al. 2007, and Harris and Crandall 2000). These regions of rRNA, and the primers used to isolate them for Carideans has been identified (Feidler et al. 2010, White et al. 1990). If genetic similarities are present a connection between the conduits exists, and if no gene flow is present speciation maybe be occurring within the population of *B. cubensis* and *P. sterreri*.

Only a few species of Caridean shrimp are unique to anchialine lake conduits (De Grave et al 2009). The typical features that are present in Carideans are a reddish coloration, small size, a short armored rostrum, modified first and second pleopods, the third somite of the pleura overlapping the forth, reduced eyes and specialized chelicera (Borradaile 1907). All Carideans are broadcast spawners with planktonic larval forms (Bohonak 1999, and McConaugha 1992). Because of the isolation of populations of these shrimp, it is believed they display sexual hermaphroditism to increase fitness (Bauer 2000). This allows migrating individuals to mate with any other population, regardless the populations’ gender composition.

*Barbouria cubensis* and the more recently described *Parhippolyte sterreri* have both been found on the Island of San Salvador, and because of the uniqueness of their habitats, both are very rare (Manning and Hart 1984, and Erdman and Ditter; personal observations). The recent discovery of *P. sterreri* on San Salvador is a minor range extension in the Bahamas (Erdman and Ditter; personal observations). Both species prefer shallow inland anchialine lakes with solution conduits (Wicksten 1996). But this is the first time both species have been found inhabiting the same island. There are few morphological differences between these species (Kensley 1988, and Wicksten 1996). The only females recorded between the two species were *B. cubensis*, and were indistinguishable from the male except one, which had a numerous small oocytes Hobbs (1978). Because these shrimp have planktonic larvae, live in caves located directly in or above tidal flows, and the relative species diversities of the lakes; this makes studying the genetic flow among populations the best possible way to establish the existence of any connection between lakes on San Salvador.

**Research Objectives**

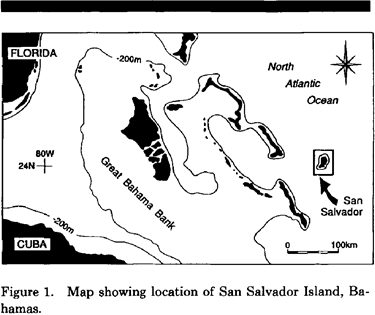
I intend to use genetic connections among populations to establish if any connections exist between the anchialine lakes of San Salvador Island, Bahamas. I will do this by measuring variations in rRNA (ITS1 and ITS2, and 16S and 28S). If no genetic connections are found then it is likely speciation is occurring within the populations of shrimp, which too would be significant findings. This study is an excellent opportunity to further examine the poorly understood geology of the island, and two threatened cave shrimp never before described on the same island.

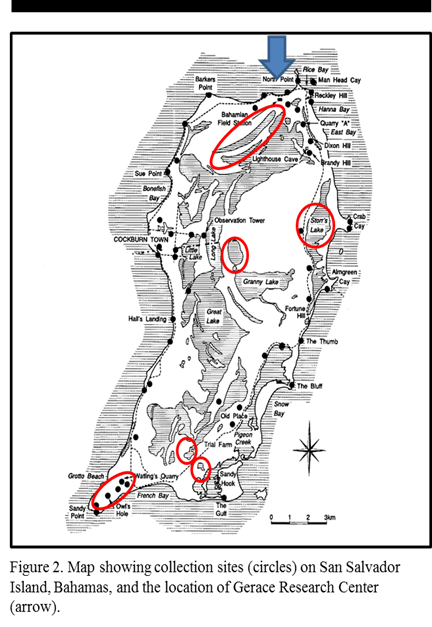
**Methods**

*Study Design*

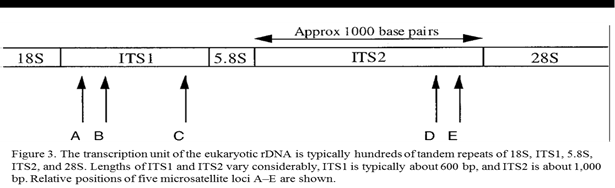
For this study I will obtain the required research permits from the Bahamian Department of Marine Resources Management. I will be collecting specimens of the cave shrimp from conduits in six anchialine lakes (3 lakes containing *Barbouria cubensis* and 3 containing *Parhippolyte sterreri*) on the Island of San Salvador in the Bahamas, during an outgoing tide. No more than three specimens from each population will be collected due to the rarity of these organisms.

*Data Collection*

I will record the exact coordinates of the conduits where specimens are collected, using GPS. This will provide an accurate measure of distance between populations. I will also measure the physical characteristics of the lakes (Button et al. 2007). I will collect specimens by SCUBA diving to the conduit, and use hand nets to catch individuals (Iliffe 1986). I will only collect samples from one lake per day to prevent any accidental transfer of biota between lakes. Once the specimens are transported to Gerace Research Center (GRC), I will record basic physical characteristics of each specimen, identify exactly which species was collected, and the sex of each individual (Hobbs et al. 1977, and Wicksten 1996). Once this is complete, I will then collect tissue samples.

Tissue samples will be collected from each individual via removal of one leg and claw, fixed in 96% ethanol and stored at -20ᵒC until RNA extraction (Zaksek et al. 2007). Live individuals will be maintained separately as a voucher, and for morphology identification at GRC. Once this preliminary work is complete the specimens will be safely returned to their respective lakes. In the case a specimen does not survive this process it will be preserved, and deposited at the American Museum of Natural History. I will then transport the tissue samples to Florida Gulf Coast University for genetic analysis.

I will obtain the genomic information for both species from GenBank. Using the tissue samples I will isolate genomic RNA using GenElute Mammalian Genomic DNA min prep kit from Sigma-Aldrich (Zaksek et al. 2007). I will then amplify and sequence the 16S and 28S rRNA using primers and polymerase chain reaction (PCR) based on the procedure described by Zaksek et al. (2007).



For ribosomal internal transcribed spacer (ITS) I will use the primers 5’-TTGATCATCGACACTTCGAACGCAC-3’ described by White et al. (1990) and for ITS1: GTAAAAGTCGTAACAAGG and TCCTCCGCTWAWTGATATGC; ITS2: TGYGAACTGCAGGACACA and TGTGTCCTGCAGTTCRCA (5’–3’) as per Harris and Crandall (2000). Standard PCR reactions will be performed on a Perkin-Elmer 9700 machine via the procedure described by Imai et al. (2004). Each fragment will be sequenced in both directions using PCR amplification primers by Macrogen, and generated on an ABI 377XL automated sequencer using ABI Big-Dye Ready-Reaction kit (Harris and Crandall 2000). Overlapping segments will be assembled and edited using ChromasPro, and then be aligned using Clustal\_X (Edgar et al. 2004).

*Data Analysis*

I will carry out phylogenetic analyses on each data set (ITS1, ITS2, 16s, 28s and total) using maximum-likelihood (ML), maximum parsimony (MP) and Bayesian methods as described by Zaksek et al. (2009). To reduce computing time I will only use unique haplotypes. I will conduct MP analyses using heuristic searches of random sequence addition with 100 replicates and tree-bisection-reconnection (TBR) branch swapping using PAUP\* (Swofford 2002). I will perform ML bootstrap analyses (Felsenstein 1981) using 1,000 bootstrap replicates to test between monophyly and nonmonophyly using the CONSEL program as proposed by Shimordaira and Hasegawa (2001). I will then calculate likelihood scores for minimal parsimony using a chi-square test with degrees of freedom equal to the difference in free parameters between the models (Nikulin 1973). If heterogeneity is found, with significance, among the populations than it will suggest a connection between the lakes, through which gene flow is occurring. But if the results show homogeneity then it is likely that the populations are isolated and speciation is occurring.

**Broader Implications**

If the conduits are connected, either to each other or the ocean, the organisms in them can freely travel between the lakes, and since these species of shrimp are so similar they may be competing for resources. If the conduits are not connected, then speciation may be occurring in each population, given that the lakes have been isolated for roughly 140,000 years. Hopefully this study will aid in future work in taxonomy and phylogenetics using RNA, studies of speciation, and that these methods may be applied to establishing connectivity in other anchialine systems.

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**Biographical Sketch**

Robert E. Ditter (Oct 6, 2011)

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**Education**

2010-Present: I am currently pursuing a BS in Marine Science at Florida Gulf Coast University, Fort Myers, Florida

* Senior Thesis: “Gene flow presence among populations of Caridean cave shrimp (*Barbouria cubensis* and *Parhippolyte sterreri*) in the anchianline lakes on San Salvador Island, Bahamas.

2002-2009: Graduated from University of Missouri, Columbia with a BS in Business Administration

**Supplementary**

2011: Attended Tropical Island Biology, Gerace Research Center, San Salvador Island, Bahamas

2010: Received PADI Divemaster SCUBA certification through Deans Dive Center, Fort Myers, Florida

2001: Attended MarineLab Aquanaut & Research Experience, Key Largo, Florida

Received special training in:

* + - Marine Species Identification
    - Surface Supplies Breathing
    - Underwater Habitation
    - Marine Research Methods
    - Underwater Archeology

**Work Experience**

2008-2010: Sales Associate at Noah’s Pets and More Columbia, Missouri

* Aquaria expert: assisting store and costumer with information
* Maintained over 100 aquariums
* Initiated aquaculturing within store

1999-2010: General Employee for Dsport in Columbia, Missouri

My father owns this business and working for him has instilled a strong work ethic in me.

**Professional Societies**

2011: Founding member of Florida Gulf Coast University Rugby club

2011: Founding member, Treasurer and Recruitment Chair of FGCU Aquarium and Aquaculture Club

2011: Initiated into Golden Key Honor Society

2002: Initiated into Phi Kappa Psi Fraternity at University of Missouri, Columbia

2001: Joined REEF/Project AWARE

**Awards**:

* Dean’s List, Florida Gulf Coast University 2010 – present.
* Eagle Scout, Boy Scouts of America 2001

**Scientific Skills**

* Microsoft Excel and Project Manager
* ARC GIS
* Maintaining live specimens
* Preserving specimens
* Microscopy
* Specimen collecting
* Professional SCUBA certification
* Skilled artist
* Literature Review
* Marine Organism Identification
* Heavy lifting