APPLICATION CHECKLIST & BUDGET FORM
Research, Scholarship, and Creativity Grant

Deadline February 10th

Please complete this checklist and attach it as the cover page of your grant application, whether you submit electronically or via hard copy.

Faculty Information

Name:____Joel L. Carlin_____

Dept:_____Biology (and Environmental Studies program) _____

Email: _____jcarlin@gustavus.edu_____

Rank:____Assistant Professor_____

Checklist

☑ **Description of previous projects (and outcomes) funded by RSC grants**

☑ **Complete project description, including separate statements of:**

1. **Purpose.** What are the intellectual, conceptual, or artistic issues? How does your work fit into other endeavors being done in this field?

2. **Feasibility.** What qualifications do you bring to this project? What have you done/will you do to prepare for this project? What is the time period, i.e. summer, summer and academic year, academic year only? Is the work's scope commensurate with the time period of the project?

3. **Project Design.** This should include a specific description of the project design and activities, including location, staff, schedules or itineraries, and desired outcomes.

☑ **RSC Budget Proposal Form**

☑ **If successful, my proposal can be used as an example to assist future faculty applications. This decision will not in any way influence the evaluation of my application. Check box to give permission.**

Submission instructions

Electronic — Submit a single document containing the entire application to rsc-proposals@gustavus.edu.

Paper — Submit one (1) copy of completed application to the John S. Kendall Center for Engaged Learning (SSC 119).

Research, Scholarship, and Creativity Grant Application

GENETIC VARIATION IN FISHES EXPOSED TO THE *DEEPWATER HORIZON* OIL SPILL (GULF OF MEXICO, USA)

submitted by
Dr. Joel Carlin, Assistant Professor of Biology, 10 February 2012

Previous Projects funded by RSC

I have not previously been funded by the RSC. This is my first application.

Complete project description

1. Purpose.

The 2010 oil spill from the blown out platform *Deepwater Horizon* released 83,000 barrels of oil per day for three months (Robertson and Krauss 2010), a cumulative volume capable of filling 184 gymnasiums of 100'x60'x30' size. Only a fraction of the slick floated the surface, and bottom-dwelling fishes are potentially some of the first vertebrates to be affected by a bottom sludge, as has been seen in other oil spill disasters (Peterson et al. 2003). Such habitats in the Gulf of Mexico already have been altered by fishing practices (trawling) and the presence of functional oil drilling platforms (O'Bannon 2001, MMS 2002).

I plan on examining genetic diversity in several species of demurral fishes to: 1) verify species identity, 2) quantify the amount of inbreeding in each species, and 3) detect possible genetic effects of the oil spill and oil drilling platforms upon these populations. Typically, publications in the fields of marine population genetics and phylogeography (e.g., Gold et al. 2009, Jue 2006, Mobley et al. 2010, Pumitinsee et al. 2009) utilize approximately 100 or more individuals per species. At a minimum DNA sequencing cost of $12.86 per gene per fish, I obviously require a competitive research proposal (e.g., NSF-Division of Environmental Biology, Environmental Protection Agency, Marine Fisheries Initiative) to financially support my efforts. I am requesting RSC funding to allow the collection of data for such a proposal.

2. Feasibility.

My Qualifications.. I have >10 years of experience obtaining and comparing DNA sequences from wild animal populations, in both natural and actively harvested populations of marine fishes (Carlin *in press*, Bowen et al. 2006, Carlin et al. 2003, Ball et al. 2003).

Sample Collection. US government research vessels annually sample bottom fishes as part of monitoring both the Gulf ecosystem and the shrimp fishing industry. I currently have 893 tissue samples from 22 species of fishes obtained by the NOAA Pascagoula Marine Laboratory during these sampling cruises. Three of these species are sampled commonly enough to warrant study, with the most common species being the inshore lizardfish *Synodus foetens*. These tissue samples are held at Gustavus' -80C freezer. I believe that these samples are a reservoir of genetic information about the animals that lived in 2009 and

2010 in areas both adjacent and distant from the oil spill. I also expect a shipment of approximately 500 additional frozen fishes at the end of 2012. It is important to note that my students and I follow the ethical guidelines outlined in AFS (2004).

Molecular Genetics. A difficult part of a molecular biology study is tailoring the experimental conditions published in previous studies to meet the needs of the samples and genes of interest (Altshuler 2006). However my students and I have conducted a pilot study of the feasibility of this study. The work performed in my lab in Fall 2011 and January 2012 resulted in the first gene sequences from three species at two genes each. These successful lab procedures were developed by GAC students Kimberley Sukhum ('11) and Grant Walters ('15), in which they spent all of their funds from student Grants-in-Aid supplied by the Gustavus chapter of Sigma Xi. Now that protocols are apparently working, it is vital that they be applied to as many of the samples as can be funded, strengthening my proposition that original research can be conducted in the liberal arts setting.

3. Project Design.

I am conducting new research on the genetic effects of human activity under the sea. I have chosen to examine genes that react to toxins and genes that do not, and both are viewed in a set of animals not purposely harvested by humans. It is unlikely that I or anyone can definitively prove that any one activity causes any one genetic effect. However, the geographic diversity of samples should allow comparison of areas relatively free of human interference with areas exposed to an oil spill, oil dispersant toxins, oil drilling, anoxia and accidental catch in fishing gear.

The target animals for study should not already have a history of human exploitation, for our selection of the largest or most marketable fish may have changed the natural variety of fish genes. Large generalist predators, such as tunas and sharks, are easily capable of outswimming oil spills and anoxic events. Therefore I have chosen small fishes (adult size 10-50cm) with no commercial value. An example target species is therefore the inshore lizardfish *Synodus foetens.* The lizardfish is an eel-like species that populates coastal waters (to 200m deep) of the western Atlantic including the Gulf of Mexico (Robins and Ray 1986). There is almost no scientific research upon the species, except for its role as a prey item to larger organisms (Cruz-Escalona et al. 2005). Other target species, such as cusk-eels (*Lepophidium* spp.) and pike-congers (*Hoplunnis* spp.), share the same general eel-like shape and lack of fishery interest but are otherwise very distantly related to lizardfish. I hope that an examination of unrelated creatures with a similar lifestyle creates a logical platform for experimentation: any genetic pattern found in 2-3 unrelated species must be due to a shared environment.

I am interested in the genetic variation at three different genes, creating a range of expectations and testing. One gene, cytochrome oxidase, should not vary much at all and will be used to verify my samples against known DNA sequences online (GenBank). In fact this gene is used as a species identification marker (e.g., Ward et al. 2005). A second gene, cytochrome *b*, is a very common target of investigations that try to document population-level genetic differences – 'neutral' evolution that should reflect local varieties (i.e., distinguishing "western" from "eastern" lizardfish). Finally, to detect the presence of selection against some genotypes, I will sequence cytochrome P450, a gene that helps destroy substances harmful to the metabolism.

After isolating DNA from the fish tissues, I will use the polymerase chain reaction (PCR; Saiki et al. 1985) to generate copies at each of the three target genes. I will ship the PCR products off campus for DNA sequencing (e.g., Nevada Genomics Inc.), who will make the resulting data available for download. I will check each sequence by eye for potential errors, then compare the sequenced genetic codes of each fish, looking at how often each allele occurs and where the sample is from. Uncertain results will be re-sequenced. The DNA sequences at each target gene (cytochrome oxidase, cytochrome *b* and cytochrome P450) will be aligned and unique sequences (alleles) noted. Using an Analysis of Molecular Variance, I will calculate the relative abundances of each allele and look for patterns of distribution. Basically, my question is simple: does a random collection of samples contain the same alleles, in the same proportion, of a sample taken from a particular area or timeframe? A mathematical means of answering this question is the statistic Φ_{ST}. If the Φ_{ST} is very close to zero then the population is genetically homogeneous. If Φ_{ST} is a large number then the samples are most likely from reproductively separated units (Excoffier et al. 2005). Differences could be found spatially (samples near the oil spill versus samples distant from the oil spill) or temporally (2009 versus 2010 versus 2012).

The outcomes expected from RSC support are: a scholarly presentation of preliminary data at a scientific conference (e.g., Society of Integrative and Comparative Biologists or Society for the Study of Evolution), preparation of a full research proposal to a US federal agency, and an eventual open sharing of data with international DNA database (GenBank).

Literature Cited.

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Research, Scholarship, and Creativity Grant: BUDGET INFORMATION

Directions:
1. Enter your **Name**
2. Enter the **Stipend Costs**
3. Enter the Project Costs (both individual costs and **Total Project Cost**)
4. Enter **Total Amount Requested** (Total Project Cost + Stipend)

NAME __Joel L. Carlin_____

STIPEND (Please check one box to indicate your distribution preference)
*Note: The RSC grant will fund up to 1,500 towards **Project Costs.** If your project costs will exceed this amount, you may opt to apply a portion (or all) of your stipend to cover these additional costs. If this option is your preference, please select "Partial Amount".*

☑ **Full Amount** ($700- assistant professor; $600-associate professor; $500-full professor)

☐ **Partial Amount** (apply a portion of the full amount to project costs)

Partial Amount: Please indicate the amount that you would like to apply towards project costs ($ _____) and the remaining stipend after this deduction ($ _____)

PROJECT COSTS: List each item individually with its cost. Attach additional sheets if necessary.	Project Costs Amount
I. Equipment (e.g. transcription machine, camera, digital recorder—but not computer hardware)	***I. Equipment***
1.	1.
2.	2.
3.	3.
II. Materials (e.g. books, printing, software, lab supplies)	***II. Materials***
1. DNA sequencing (30 fish x 2 primers x 3 rxns x $6.43/rxn)	1. 1,157
2. *Tfi* DNA polymerase (enzyme for PCR)	2. 143
3. Shipping of frozen PCR reactions to DNA sequencing facility	3. 200
III. Personnel (e.g. typist, transcriptionist, student assistant)	***III. Personnel***
1.	1.
2.	2.
3._____	3.
IV. Travel (cannot include conference travel, see http://gustavus.edu/finance/travel.php for allowable expenses)	***IV. Travel***
1.	1.
2.	2.
3.	3.

TOTAL PROJECT COSTS	**$__1,500_____**
TOTAL AMOUNT REQUESTED (Total Project Costs + Stipend)	**$__2,200_____**

(Note: The RSC grant will fund up to an amount equal to your Full Stipend + 1,500 for Project Costs)