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COLLEGE of
CHARLESTON

UNDERGRADUATE RESEARCH
AND CREATIVE ACTIVITIES

Application Cover Page

Application for Summer Undergraduate Research with Faculty (SURF) Grant

**Applicants should refer to the SURF Program Description and Guidelines and the SURF Application Check List to ensure a complete application.
Incomplete applications will not be considered for funding.**

PROPOSAL TITLE: Salinity effects on early life stages in squirrel treefrogs

PRIMARY MENTOR APPLICANT*:

NAME: _____

CofC Email: _____

Department: Biology

Faculty Status:

- Tenured/Tenure-track
 Instructor
 Visiting
 Adjunct
 Other (please specify _____)

UNDERGRADUATE APPLICANT**:

NAME: _____

CofC Email: _____

CofC ID Number: _____

Primary Major: Biology

Current Enrollment Status:

- Full-time (12 hours or more)
 Part-time (less than 12 hours)

*Please note in the guidelines the eligibility criteria for faculty mentors and limits on number of grants per faculty mentor.

**If more than one student will work on a project, separate applications must be submitted for each student.

SECONDARY MENTOR APPLICANT:

NAME: _____

Institutional Affiliation: _____

Department: _____

Title: _____

Email: _____



Project Information Page

REQUESTED DATES OF PROJECT SUPPORT (mm/dd/yy): From 05/18/15 To 07/24/15

TOTAL AMOUNT REQUESTED FROM URCA: \$ 6500

1. Does the proposal involve research on human subjects? Yes No
If yes, status of the IRB request (no funds can be awarded without IRB approval):
 Submitted Approved
2. Does the proposal involve research with live vertebrate animal subjects? Yes No
If yes, status of the IACUC request (no funds can be awarded without IACUC approval):
 Submitted Approved
3. Have student or faculty applicants received URCA support for this or any other project since September 2013 or do they currently hold funding through the URCA program?
 Yes No
If yes, which type? SURF MAYS RPG AYRA
If the applicant holds funding in the current cycle, specify name of applicant and award amount:
2013 RPG - Jonathan Brown & Allison Welch (AW) - \$500; 2013 RPG - Sarah Turner & AW - \$500; 2014 SURF - Emily Beam & AW - \$3900; 2014 RPG - Luke Rein & AW - \$500
4. Does the student have another proposal under consideration by URCA during the current cycle? Yes No
If yes, what type of grant proposal is it? SURF MAYS RPG AYRA
5. Does the faculty mentor have another proposal under consideration by URCA during the current cycle? Yes No
If yes, what type of grant proposal is it? SURF MAYS RPG AYRA
6. Is there another internal proposal current or pending for this research/creative work?
 Yes No
If yes, please list the source(s) as well as amount of request and dates of award:
7. Is there an external proposal current or pending for this research/creative work?
 Yes No
If yes, please list the source(s) as well as amount of request and dates of award:
8. Does the project involve biohazards or other safety issues? Yes No
9. Does the project have potential for copyright or invention? Yes No

FERPA WAIVER

The Family Educational Rights and Privacy Act (FERPA) of 1974 establishes the rights of students with regard to educational records. The act makes provision for inspection, review and amendment of educational records by the students and requires, in most instances, prior consent from the student or their parent/guardian if under the age of 18 for disclosure of such records to third parties. The consent must be in writing, signed and dated by the student and must specify records to be released, reason for release, and the names of the parties whom such records shall be released. The act applies to all persons formerly and currently enrolled at an educational institution. Access to educational records does not give permission to make changes to the student's record. For more information visit:

<http://www2.ed.gov/policy/gen/guid/fpco/ferpa/index.html>

I hereby give permission for the College of Charleston Undergraduate Research and Creative Activities personnel and committee members to obtain

- information concerning my academic transcript
- information concerning my academic advising notes
- information concerning my in-class performance and grades

This waiver will be in effect as long as I am a student at the College of Charleston, or seeking the services of faculty and staff on the College of Charleston campus.

Signatures (Required for All participants): Please read the SURF Guidelines prior to signing this page. Signatures below indicate awareness of and intention to follow appropriate Program, FERPA Waiver, Departmental, School, College and State rules and regulation for conducting projects, travel, and expenditure of funds.

Undergraduate Applicant: _____
Signature _____ Date 1/30/2015

Faculty/Mentor Applicant: _____
Signature _____ Date 1/30/2015

Faculty/Mentor Applicant: _____
Signature _____ Date _____

Chair: I acknowledge that the above student and faculty mentor(s) are applying for URCA Funding and that the funds for successful proposals will be transferred into the departmental R & D account for dispersal based on the budget included in this proposal.

Chair: _____
Signature _____ Date 2/2/15

Proposed Budget Table

	I	II	III	IV	V
	SURF Funding Requested*		Dept/School Other Internal Support for Project	External Support for Project	Total Cost of SURF Project (Add Columns I-IV for each row)
	For Use Prior to June 30	For Use After July 1			
A. Student Salary (taxable amount)	\$1750	\$1750			3500
B. Faculty Salary (taxable amount)	\$1137	\$1363			2500
C. Student Travel**	\$176	\$98			274
D. Faculty Travel**	\$78	\$39			117
E. Supplies & Materials	\$109				109
F. Other					0
G. Total Costs Per Column (Add values from cells A-F for each column)	3250	3250	0	0	6500
TOTAL SURF REQUEST: (Add values from cells G-I + G-II)	6500				

COLLEGE of
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UNDERGRADUATE RESEARCH
AND CREATIVE ACTIVITIES

Summer Undergraduate Research with Faculty (SURF) Grant Application

PART II

**To be submitted electronically as a PDF to urca@cofc.edu by 5pm,
February 2, 2015**

**Part I and Part II of the application must be submitted in order to be considered for
funding. Part I of the application can be accessed at www.urca.cofc.edu.**

**PROPOSAL TITLE: Salinity effects on early life stages in squirrel treefrogs: from
fertilization through embryonic and larval development**

PRIMARY MENTOR APPLICANT: [REDACTED]

UNDERGRADUATE APPLICANT: [REDACTED]

1. STUDENT STATEMENT OF INTENT

For as long as I can remember, I have been fascinated by the wide variety of animals that lived in the same world as me. During my childhood, I could always be found wandering through my backyard searching for interesting creatures to study. I knew I wanted to study and interact with the natural world. Although I had considered becoming a veterinarian, I realized that it wouldn't be enough for me. I knew that in addition to working with animals, I wanted to educate people about environmental issues. Instead I have decided I would like to pursue a career in conservation biology. After completing my undergraduate degree, I am not entirely sure whether I want to attend graduate school or go straight into the work force. This previous summer, I worked as a recreator at a state park where I designed educational kids programs. This experience really sparked my interest in the educational side of environmental conservation, and as a result I am now considering a career as an educational facilitator in a state or national park. I am also considering attending graduate school to gain more research experience and eventually teaching at the college level. This SURF grant will allow me the opportunity to gain research experience and help me decide on what career path I would like to take, whether I will continue my schooling or begin a career that educates the public on environmental issues. Through education, I will strive to spark others' interests in the conservation effort.

For the past two semesters, I have been working in [REDACTED] lab on animal care. When the opportunity presented itself to conduct my own research, I was thrilled. I have always been fascinated with frogs, and I am intrigued that so little is known about the effects of salinity on the different life stages, so this research is a perfect fit. I am excited to play a part in observing these unstudied effects and to contribute to new and exciting discoveries in herpetology conservation. I feel that through this research project, I have the opportunity to contribute interesting and relevant new information on the effects of salinity on amphibians. I intend to continue this research with another independent study in the fall semester to finish up data analysis, work on preparing a presentation for a national conference, and work on a manuscript for publication.

I will not receive academic credit for research this semester, I will not be receiving any academic credit for the summer research project. My interest in pursuing this research is purely for my own personal desire to increase my understanding of the effects of environmental stressors on amphibians. To fully focus all my attention on my research, I will have no other commitments and I will be able to spend 40 hours a week in the lab and out in the field completing the project.

This previous semester I undertook a rigorous course academic course load which included organic chemistry 231. In addition to organic chemistry, I was enrolled in environmental psychology, plant taxonomy, and environmental ethics. All of these classes are pertinent to my major and I put a lot of effort into each one. As you can see from all my other grades from last semester, only my organic chemistry grade suffered. Unfortunately, despite a tremendous amount of studying, I received a C overall in the class. As challenging as the course was, I applied my best effort while also staying on top of the rest of my course load rather well. It is very unusual for me to have that much difficulty in a course, as can be seen from my previous semesters here at the College. Although organic chemistry is a requirement to earn my bachelor's of science in biology, I do not feel that it will impact my performance during the summer research project. My research will involve laboratory and research techniques that I will refine over the course of the current semester that are largely unrelated to the course material from organic chemistry.

2. NON-TECHNICAL PROJECT ABSTRACT

Salinity levels are increasing in some freshwater environments due to human activities including road salt runoff, land use changes from agriculture, and rising sea levels. Amphibians are particularly vulnerable to habitat salinization because they need freshwater to complete their life cycle. Increased salinity can harm amphibian larvae by decreasing survival, reducing growth, and increasing the time to reach metamorphosis. Salinization of breeding habitats may also affect other stages of the frog life cycle, including fertilization and embryo development. Exposure to salinity during fertilization could affect sperm function, decreasing the number of eggs fertilized. Increased salinity can also slow or halt embryonic development. Testing the salinity tolerance of each of these life stages will help us identify which life stages are most sensitive to habitat salinization. In addition, we will test how salinity exposure during fertilization and embryonic development affects tadpole success. One possibility is that early exposure will allow acclimation to salinity, increasing the ability of tadpoles to survive and thrive at elevated salinity. On the other hand, early salinity exposure could have a cumulative effect and decrease a tadpole's ability to tolerate additional stress from salinity exposure, ultimately leading to poorer performance or even death. Studying how salinity exposure during early development affects the fitness of tadpoles will improve our understanding of the effects of habitat salinization during different life stages. Overall, our work will help conservation efforts address the impact of elevated salinity on the most susceptible amphibian life stages.

3. PROJECT DESCRIPTION

(a) Technical Project Abstract: Freshwater salinization is an emerging threat for amphibians, which typically rely on freshwater environments for reproduction and larval development. Tadpoles are poor osmoregulators, and even the most salinity-tolerant species cannot complete larval development at salinities above ~10 ppt (~25% seawater). Embryonic development is also impaired at elevated salinities, but the effects of elevated salinity on naturally-occurring external fertilization have not been tested, and the relative tolerances of these different life stages have seldom been compared. We will compare the effects of elevated salinity on fertilization, embryos and tadpoles of squirrel treefrogs (*Hyla squirella*) by experimentally exposing each stage to a range of salinities. Further, because environmental challenges experienced early in development often alter the expression of later phenotypes, we will test the effects of elevated salinity during fertilization and embryonic development on fitness during the tadpole stage. Understanding how elevated salinity affects these early stages of the amphibian life cycle will help us identify the most vulnerable life stages and better evaluate the threats posed habitat salinization.

(b) Project Objectives and Expected Outcomes: This research will investigate the effects of elevated salinity on early amphibian life stages, from fertilization through metamorphosis. Salinity tolerance has been studied in various species of amphibian larvae, but the relative tolerance of different stages of development is not well understood. Further, the effects of environmental salinity on frogs' external fertilization have rarely been studied. Two specific research objectives will be addressed using locally-abundant squirrel treefrogs: (1) to compare salinity tolerance across three early life stages – fertilization, embryonic and tadpole development, and (2) to test effects of salinity exposure during early developmental stages on fitness during later stages. We predict that all three stages will be impaired at moderate salinities and will totally cease at higher salinities, but that the tadpole stage will be more sensitive than fertilization or embryonic development, given its longer duration. Further, we predict that elevated salinity experienced during fertilization and embryonic development will have long-lasting effects on the resulting tadpoles, leading to impaired growth, development and survival.

Short-term objectives to be completed from 5/18-7/24/2015: By the end of the funded period we will have completed two studies examining (1) salinity tolerance of fertilization, embryos and tadpoles, and (2) effects of salinity exposure across these stages. All experiments, most digital image analysis, and preliminary data analyses will be completed.

Long-term objectives: During the upcoming academic semester, we will complete remaining image analysis, refine data analyses and prepare a manuscript for publication.

(c) Project Significance: Habitat modification due to human activities is an important threat to global biodiversity (Vitousek et al. 1997). One increasingly common form of habitat degradation is increased salinity of freshwater habitats. Salinization of freshwater can result from road de-icing salts, irrigation, and land conversion for agriculture (Kaushal et al. 2005; Williams 2001). In coastal regions, climate change is also contributing to freshwater salinization from rising sea levels, increased storm surge, and saltwater intrusion into groundwater (e.g., Gornitz 1991).

Amphibians are at particular risk from habitat salinization. Amphibians are the most imperiled major group of vertebrates, with almost one-third of all amphibian species considered threatened or endangered and nearly one-half having experienced population declines (Stuart et al. 2004). Typically, amphibians rely on freshwater environments for reproduction and larval development, as most amphibian larvae are poorly equipped to handle osmotic stress (Gomez-

Mestre et al. 2004). In frogs, fertilization also occurs externally in freshwater. Tadpoles of several species have been shown to suffer reduced survival and growth when exposed to elevated salinity, although sensitivity varies among species (Alexander et al. 2012).

Salinity within freshwater habitats can fluctuate on short time scales, increasing with storm surge or evaporation and decreasing with rainfall (Brown and Walls 2013). However, although salinity exposure can vary during the aquatic stages of an amphibian's life, little is known about how salinity tolerance compares across life stages. Amphibian embryos are harmed by elevated salinity, but the relative tolerance of the embryos and tadpoles is poorly understood because the two stages have rarely been studied in concert (Alexander et al. 2012). Further, although laboratory studies show that elevated salinity can impair frog sperm function (Wilder and Welch 2014) and can inhibit in-vitro fertilization (Edwards et al. 2004), the effects of increased salinity on natural fertilization have not, to our knowledge, been tested. Thus, to fully understand the consequences of habitat salinization for amphibians we need better information about effects of salinity at all aquatic life stages, from fertilization through metamorphosis.

Because salinity can vary during development, we also need to understand how salinity exposure at one developmental stage affects the organism's fitness at later stages. For example, compensatory growth has been observed after tadpoles were removed from salinity stress, allowing them to make up for earlier growth impairment (Squires et al. 2010). Other studies, however, found the opposite: that early salinity exposure led to poorer growth later in development (Wu et al. 2012). For some contaminants, exposure early in development can promote acclimation and thereby increase tolerance later in development (e.g., Herkovitz and Pérez-Coll 2007). Acclimation to salinity has been suggested by reports of tadpoles naturally occurring in salinities beyond those tolerated by naïve tadpoles during laboratory tests (e.g., Gordon and Tucker 1965). However in experiments, salinity exposure early in tadpole development made later salinity exposure more, rather than less, harmful (Wu et al. 2012; Hua and Pierce 2013), suggesting that rather than promoting acclimation, the stress of early salinity exposure has cumulative effects throughout development. But these tests did not investigate effects of salinity exposure during fertilization or embryonic development, which could have dramatic effects on later development.

By investigating both the immediate and delayed effects of elevated salinity experienced during the earliest amphibian life stages, the proposed research seeks an improved understanding of how increased salinity in freshwater environments may impact amphibian populations. We predict that transient exposure to elevated salinity will be less detrimental than exposure throughout aquatic development, but that even brief exposures during critical stages of development may have important effects. In such a case, even transient increases in salinity could impact population persistence. On the other hand, if early exposure provides protective effects via acclimation, populations may have a previously unappreciated ability to withstand modest levels of salinization. Either way, our results will help to refine predictions about the effects of habitat salinization on amphibian populations.

(d) Methods of Work: We will investigate the effects of salinity on early life stages of the squirrel treefrog, *Hyla squirella*. Squirrel treefrogs are locally abundant throughout the southeastern coastal plain, with large clutch sizes and a typical amphibian life cycle, which will increase the applicability of our results to other amphibian species. In South Carolina, squirrel treefrogs breed after summer storms from April through August (Conant and Collins 1998). Breeding pairs of treefrogs will be collected from the College of Charleston's Dixie Plantation

field site and transported back to the lab for experiments. Frogs, embryos and tadpoles that are no longer needed for experiments will be returned promptly to the collection site. All collections will be done with proper permits from the South Carolina Department of Natural Resources. This work is approved under IACUC protocol 2012-012.

Objective 1: Comparing life stage sensitivity to increased salinity – To investigate the effects of increases in salinity during reproduction and development, we will conduct separate studies on three distinct life stages: fertilization, embryos, and tadpoles. Each stage will be experimentally exposed to six salinity treatments to determine salinity tolerance. The six treatments will include 2, 4, 6, 8 and 10 parts per thousand (ppt) salinity, with 0.4 ppt used as a control providing an optimal salinity for amphibian gametes and larvae (Armstrong et al. 1989). The six treatment solutions will be prepared with Instant Ocean aquarium salt in carbon-filtered tap water.

For the fertilization study, pairs of male and female squirrel treefrogs will be collected while in amplexus, indicating that the release of gametes for external fertilization is imminent. Amplexed pairs will be transported to our laboratory at the College of Charleston and randomly assigned to salinity treatments. Each pair will be placed in 1.0 L of the appropriate treatment solution within a 1.5 L plastic container covered with a ventilated lid and allowed to oviposit. Approximately 2 hr post-fertilization, eggs will be collected and photographed in a monolayer under a dissecting microscope. These images will be used to determine whether each egg has undergone cleavage and thereby to ascertain fertilization success. Over the course of the summer, we will collect 60 breeding pairs, with 10 pairs assigned to each salinity treatment.

The embryo study will make use of eggs that were successfully fertilized in the control treatment (i.e., 0.4 ppt salinity). From each clutch, 50 embryos will be assigned to each of the six salinity treatments, divided equally between two petri dishes to allow within-clutch replication. Since an average clutch contains approximately 1000 eggs (Martof et al. 1980), we will easily be able to obtain the required 300 embryos per clutch. This design will be replicated across five separate clutches from unique male-female pairs. Each petri dish will contain 100 mL of the appropriate treatment solution, replenished daily. Embryos will be photographed every 24 hours under a dissecting microscope until they have reached the feeding stage (Gosner stage 25). From these images, each embryo will be staged daily and assessed for developmental abnormalities.

The tadpole study will begin at stage 25, with tadpoles resulting from fertilization and embryonic development in the control solution. From each clutch, six tadpoles will be assigned to each of the six salinity treatments, with three tadpoles per container. Tadpoles will be reared in 1.5 L containers with 1.0 L of solution. Every three days, solutions will be replenished and precisely measured rations of finely ground fish food will be added. Tadpoles will be weighed every six days for 18 days in order to determine growth rates at each salinity.

We predict that salinities ≥ 8 ppt will be lethal for developing embryos and tadpoles, while 4 and 6 ppt will lead to impaired growth and development with more pronounced effects in the tadpole stage due to the energetic demands of maintaining osmotic balance for an extended period of time. Although we expect poor fertilization at ≥ 8 ppt, we predict that any reductions in sperm activity at 6 ppt and below will not result in reduced fertilization due to high sperm concentrations at the time of fertilization. Thus we expect salinity tolerance to decrease from fertilization through tadpole development.

Objective 2: Cumulative effects of increased salinity – To investigate the effects of salinity exposure across life stages, we will expose developing young to moderate salinity for varying portions of development from fertilization through the larval stage. Seven different treatments

will consist of exposure to moderate salinity (4.0 ppt) or the control solution (0.4 ppt) for each of three stages – fertilization, embryonic development, and larval development – in various combinations (Figure 1). Sublethal effects have been observed in other tadpole species at 4 ppt (Alexander et al. 2012). This experimental design will allow us to determine how exposure to salinity in one life stage affects growth, development and survival in later stages.

The experiment will begin with six breeding pairs, allowed to oviposit in the lab. Three pairs will be exposed first to the control solution and then switched to the 4 ppt solution after approximately half of the clutch has been released. The other three pairs will be allowed to oviposit first in the 4 ppt solution in order to control for order of presentation. From the resulting clutches, successfully fertilized eggs will be randomly

Stage(s) exposed	Fertiliz.	Embryo	Tadpole
1) control	—	—	—
2) fertilization	X	—	—
3) embryo	—	X	—
4) tadpole	—	—	X
5) fert. + embryo	X	X	—
6) embryo + tadpole	—	X	X
7) fert. + embryo + tad.	X	X	X

Figure 1. Seven treatments will be used to expose tree-frogs to elevated salinity for different portions of early life. Lines represent exposure to the control (0.4 ppt) whereas X represents exposure to moderate salinity (4.0 ppt).

selected and assigned to either the 4 ppt or control solution for embryonic development. At stage 25, tadpoles will be randomly assigned to the two solutions and reared through metamorphosis. Each treatment will be replicated across six breeding pairs, with three groups of tadpoles per treatment per pair. Tadpoles will be weighed after 15 days and again at metamorphosis.

We predict that exposure to salinity during fertilization and/or embryonic development will lead to reductions in growth and development during the tadpole stage. Further, individuals exposed during both early in development and during the tadpole stage are predicted to fare more poorly than those exposed only as tadpoles. Such a result would indicate that the deleterious effects of elevated salinity are cumulative during development, likely due to increased energetic demands associated with maintaining osmotic balance. Alternatively, individuals exposed early in development might acclimate to elevated salinities and thereby show improved ability to withstand salinity exposure during the tadpole stage. Regardless, our results will lead to an improved understanding of the ways in which habitat salinization may impact amphibian populations.

Project Timeline:

May	June	July
Begin cumulative exposure experiment	Assess salinity tolerance of fertilization, embryos, tadpoles; Rear tadpoles in cumulative experiment	Continue testing tolerance of fertilization; Complete cumulative experiment; Image analysis; Begin data analysis

(e) Faculty Mentor and Student Participant Roles: During the funded period, the faculty mentor, A. Welch, will work closely with the student, A. Ruby, on all stages of the research. The mentor’s focus during this time will be solely on research, ensuring sufficient time and availability to devote to this project. The mentor anticipates working with one or two other undergraduates on a different project during this time.

The faculty mentor will train the student in all aspects of the work, including collecting amphibians, mixing solutions, setting up and maintaining a controlled experiment, assessing

fertilization status and developmental stages, rearing frog embryos and tadpoles, and weighing and measuring tadpoles and newly-metamorphosed frogs. The mentor will also work collaboratively with the student during the most labor-intensive phases of the project, including collecting animals, assessing fertilization, rearing embryos, setting up the experiment, and weighing tadpoles. During all stages of the research, the mentor will have daily contact with the student to check progress, answer questions, and adjust plans, with more lengthy meetings at least twice weekly. Some of this contact will occur during collaborative activities, while other contact will occur during scheduled and impromptu meetings. During the upcoming academic semester, the faculty mentor will meet with the student at least weekly to supervise additional planned research and to collaborate on a manuscript based on this work.

(f) Current and Pending Support: None

(g) Student Development: SURF funding will allow the student to pursue an in-depth research project exploring the effects of habitat degradation on amphibians. ██████████ has a keen interest in environmental science and conservation biology and intends to pursue a career that builds on these interests, either through graduate study or by working in a conservation field. After working in my lab caring for research animals during the past two semesters, ██████████ is embarking on her own research project this semester. This semester, she will investigate effects of salinity on embryos of various local amphibian species. Her SURF project will complement this research by comparing the effects of elevated salinity on the embryo stage with its effects on fertilization and the later tadpole stage and to investigate how the experience of salinity at early life stages (i.e., fertilization, embryo) affects tolerance in later stages (i.e., tadpoles). The ultimate goal of her research is to understand the consequences of habitat salinization in the context of the complex life cycle of amphibians. For this research, ██████████ participating in every stage of the research process, from conducting literature searches and designing and conducting experiments to analyzing data and, ultimately, to communicating results in written and poster forms. These experiences with research will be critical for preparing ██████████ for competitive graduate programs or for work addressing environmental and conservation issues.

(h) Project Dissemination: The results of this work will be presented by ██████████ to the College of Charleston community as a poster during the SSM Scientific Research Poster Session in spring semester 2016. ██████████ also hopes to present a poster at a national meeting (Society for Integrative and Comparative Biology), in January 2016. We also anticipate a peer-reviewed publication to result from this work, co-authored by ██████████. Both the College of Charleston and the URCA program will be formally acknowledged in all presentations and publications resulting from this work.

(i) Student Involvement in Application Process: ██████████ suggested investigating salinity effects on fertilization in squirrel treefrogs, and ██████████ conducted a literature search on the topic. Based on these readings, we collaboratively decided to investigate embryonic and larval stages in addition to fertilization and together designed the proposed series of experiments. ██████████ wrote the first draft of the non-technical abstract, significance and methods sections, with input from ██████████. ██████████ revised these sections and wrote the remainder of the project description.