

**INSTITUTION:** San Francisco State University  
**PROJECT DIRECTOR:** Vance Vredenburg  
**TITLE:** The arboreal and Ensatina salamanders of California  
**SPONSOR:** USFWS Farallones Islands  
**DURATION:** 1 year  
**GRANT ADMINISTRATOR:** Chao Guan

**PERSONNEL**

Vance Vredenburg, Principal Investigator

\$85,092 Academic Year Salary  
 \$7,091 Per Month  
 \$9,455 Intersession Salary Per Month  
 \$436 Intersession Salary Daily

0% RRT  
 62.70% RRT Benefit

0.25 Intersession Month  
 10.70% Fringe Benefits

Graduate Student

\$17.25 Per Hour  
 0 Hours  
 2.70% Fringe Benefits

**Total Salaries**

**Fringe Benefits**

**TOTAL SALARIES AND BENEFITS**

**TRAVEL**

Domestic Travel

International Travel

**Travel Expense Subtotal**

**SUPPLIES & SERVICES**

General Research Supplies

**Supplies Expense Subtotal**

**TOTAL DIRECT COSTS**

**INDIRECT COSTS**

26.0%

**TOTAL COSTS**

**MTDC**

Requested Year 1			Total Project Costs
0			0
0			0
2,435			2,435
260			260
0			0
0			0
2,435			2,435
260			260
<b>2,695</b>			<b>2,695</b>
500			500
0			0
<b>500</b>			<b>500</b>
5,000			5,000
<b>5,000</b>			<b>5,000</b>
<b>8,195</b>			<b>8,195</b>
<b>2,131</b>			<b>2,131</b>
<b>10,326</b>			<b>10,326</b>
8,195			8,195

\*Modified Total Direct Costs (MTDC) consists of all salaries and wages, fringe benefits, materials, supplies, services, travel and subgrants and subcontracts up to the first \$25,000 of each subgrant or subcontract (regardless of the period covered by the subgrant or subcontract). Modified total direct costs shall exclude equipment (hardware which exceeds the unit cost of \$5000) capital expenditures, charges for patient care tuition remission, rental costs of off-site facilities, scholarships, and fellowships as well as the portion of each subgrant and subcontract in excess of \$25,000.

## **Budget Justification**

### **PERSONNEL**

#### **Senior Personnel**

**Principal Investigator Vance Vredenburg, Ph.D.** In order to fulfill the project, PI Dr. Vredenburg requests 0.25 month of summer salary for the project. The proposed level of commitment for this proposal is appropriate to the scope of work and is required in order to fulfill the objectives of this project within the proposed timeframe. Dr. Vredenburg will manage the SFSU component of the project and will coordinate with PI Gerry McChesney, US Fish and Wildlife Service. Project management includes coordination with activities performed by PI Dr. Vredenburg at SFSU including data management, and submission of progress and final reports as well as analyses of data and preparation of manuscripts to be submitted to peer-review journals. The SFSU component includes field-collection of salamanders, pathogen disease assays for each animal, and animal housing during the initial part of the project.

**Real-time PCR assay for the chytrid fungal pathogen: at the SFSU molecular lab**—Vredenburg run samples using standard real time PCR techniques to detect disease. Vredenburg will capture and handle salamanders, collect non-harmful skin swabs from live animals, extract and amplify DNA, and analyze chytrid fungal pathogen PCR assays to test for pathogen presence. Vredenburg will estimate infection intensity (Bsal and Bd) on hosts can help predict the occurrence of a disease epidemic.

**FRINGE BENEFITS:** Fringe benefits for each of the above positions are requested based on the following calculations: PI (10.67% for intersession salaries) and graduate student (2.67%). All amounts are in accordance with current university policies and applicable collective bargaining agreement.

### **TRAVEL**

**Domestic Travel:** Budgeted funds (\$500) are requested to cover costs of local field work

### **OTHER DIRECT COSTS**

**Materials and Supplies:** Funds (\$5,000) have been budgeted for the materials and supplies necessary for carrying out the aspects of the research project. Skin swab costs are approximately \$20 per swab including expendable supplies used for the swabs, vials and for the qPCR assay (e.g. Prepman, Mastermix, etc. from Applied Biosystems, International). The budget will allow for the analysis of approximately 250 swabs for both Bsal and Bd.

### **INDIRECT COSTS**

Indirect costs are budgeted at 26% of Modified Total Direct Costs (MTDC), in accordance with San Francisco State University's federally negotiated agreement dated 04/11/2011.

## **Plethodontids and disease: Determining the fungal infection status of Arboreal and Ensatina Salamanders in California**

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The fungal pathogen, *Batrachochytrium dendrobatidis* (*Bd*) causes the disease chytridiomycosis and has been linked to mass mortality events and population collapse in many species of frogs and salamanders (e.g., Lips et al. 2006, Vredenburg et al. 2010, Cheng et al. 2011). The effect of chytridiomycosis varies among species, and the outcome of amphibian-pathogen interactions depends on amphibian community composition, host species traits, pathogen genotype, and the environment. When exposed to a novel pathogen, naive populations experience mass mortality, population declines and extirpations (e.g., Lips et al. 2006, Vredenburg et al. 2010, Cheng et al. 2011). Yet other populations of the same species potentially invaded decades earlier coexist with low levels of infection and mortality (Fisher et al. 2009, Briggs et al. 2010).

Houlahan et al. (2000) described widespread amphibian declines in North America and Europe in the 1950-60s, followed by a second round of declines in the 1970's and 80s. A similar pattern of slow, ongoing, population declines was reported in the US by Adams et al. (2013) with a 3.7% annual decline in occupancy in protected habitats (southern species and especially salamanders showing the greatest declines). One of the largest and most widespread declines comes from the Appalachian Mountains from terrestrial forest salamanders (Genus *Plethodon*, Family Plethodontidae) throughout the eastern US in the late 1970's to early 1980's (Highton 2005), although die-offs were not noticed and no definitive cause has been identified. Caruso and Lips (2012) resurveyed many of Highton's historic collecting sites and found that occupancy and detection declined for many species of *Plethodon* salamanders in the Great Smoky Mountain N.P. They have since resurveyed more species at Highton's historic sites and found many populations of multiple genera have declined in both occupancy and detection (Caruso et al. unpublished data). This might indicate the presence of novel lineages of chytrid fungi in the Eastern US that are not detected by existing PCR primers.

*Bd* is widespread in North America (Olson and Ronnenberg 2014), and although it generally occurs at low prevalence and low intensity in the eastern US (James et al. 2015), in Illinois (an area without mass-die offs) it is found at both high prevalence and intensity (Talley et al. 2015), suggesting variation in virulence of eastern lineages of *Bd*. A particular lineage of *Bd*, called *Bd*-GPL2, has caused rapid, sudden and widespread declines in Mexican salamanders (Rovito et al. 2009, Cheng et al. 2011) as well as causing mortality of many tropical salamanders as it moved through Central America (Lips et al. 2006, Lips et al. 2008). In California, local extinctions and species declines of frogs are documented with emergence of *Bd*-GPL1 (Vredenburg et al. 2010, Piovato-Scott et al. 2011), but less is known about the role of *Bd* in salamander populations (Sette et al. 2015).

Chytrid fungal pathogens are more diverse than recognized (Fisher et al. 2012; James et al. 2015), isolates vary in genotype and phenotype (Farrer et al. 2011), and variation is associated with differences in virulence (Fisher et al. 2012). *Bd* itself is no longer considered one entity, but is comprised of at least two clades: 1) *Bd*-GPL1 (Global Pandemic Lineage) which mostly infects North American species and which caused epidemics in California, and 2) *Bd*-GPL2 which is highly virulent, has been found globally, and has caused die-offs in Panama, Colombia, Australia and other tropical regions (James et al. 2015). *Bd*-GPL1 has been cultured from many wild amphibians in California and in the eastern US while *Bd*-GPL2 has more often been cultured from Central and South America.

The newly described *Batrachochytrium salamandrivorans* (*Bsal*) is salamander-specific chytrid causing die-offs of European salamanders (Martel et al. 2013, 2014). Field and museum sampling indicates an Asian origin (Martel et al. 2014) and introduction into Europe through the pet trade. Martel et al. 2014 found no evidence of *Bsal* in wild populations of salamanders in Eastern North America and Panama, although no wild-caught salamanders from western US, Mexico, or South America were tested. They found 23 species from 4 families were susceptible to infection by *Bsal* in lab assays. Both eastern and western North American newts (Family Salamandridae) experienced 100% mortality, although other genera were infected but rarely died. North America is a global hotspot for salamanders, with 10 families and 675 species or (~65% of global species; AmphibiaWeb, 2015). Especially concerning are the 4 million live amphibians imported into the US every year (US Fish and Wildlife Service LEMIS data; Yap et al. 2015b) that are likely to be carriers of *Bd*, *Bsal*, and other pathogens. Testing of US species and disease surveillance across the US are critical needs (USFWS recently announced a ban on live trade for 201 amphibian species; Federal Register, 2016).

This project will test for disease prevalence of two fungal pathogens *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans* in two host species of salamanders native to California, the Arboreal salamander (*Aneides lugubris*) and the Ensatina salamander (*Ensatina eschscholzii*). We will collect up to 90 Ensatina salamanders and up to 30 arboreal salamanders. In the field, we will collect individual data on species identity, size (snout / vent length and tail length), and infection status. Animals will be housed in individual containers and transported to the animal care facility at San Francisco State University (SFSU). Collection sites will all be in the San Francisco Bay Area, and will be no further than a 3 hour drive from SFSU campus. In captivity, the salamanders will be cared for according to the Vredenburg SOPs for terrestrial salamanders. Animals will be held at SFSU for 3 weeks, allowing for 3-5 independent analyses of infection status for each animal. After that time, animals will be shipped alive to the research collaborator at Colorado State University, Fort Collins, Colorado, 80523.

#### Salamander collections:

Arboreal salamander (*Aneides lugubris*): up to 30

Ensatina salamander (*Ensatina eschscholzii*): up to 90

Methods: Vredenburg and assistants will collect animals, determine their infection status, house and feed them for up to three weeks before shipping to collaborators at Colorado State University. The animals will be mailed alive to Colorado via Fed EX for further study. Each animal will be captured by hand, placed in a small container with a lid, and will be housed without contact with other animals to ensure that the infection status of each animal will be known before the exposure experiment begins. Each animal will be tested for chytrid pathogens (*Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans*) in the Vredenburg Lab using a standard qPCR assay (Boyle, et al., 2004; M. Blooi et al., 2013). The Vredenburg lab routinely runs these tests on wild caught and captive amphibians to determine infection status.

#### References:

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