I. INTRODUCTION

**A. Title**

Ecological effects of amphibian population declines: consequences of Mountain yellow-legged frog extinctions on Sierra Nevada alpine lake communities

**B. Date of proposal**

5/1/2009

**C. Investigators**

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**E. Abstract**

Worldwide declines in amphibian populations have drawn the attention of researchers across biological disciplines. While a growing field of literature explores the causes of these declines, few studies address their consequences at the community or ecosystem level. Amphibians greatly affect food web dynamics because of their dual aquatic and terrestrial lives and roles as primary and secondary consumers, and their disappearance will affect the structure, function, and stability of aquatic ecosystems. In the Sierra Nevada, the Mountain yellow-legged frogs (*Rana muscosa* and *R. sierrae*) have suffered extensive declines, most recently due to the emergence of infectious disease. The Mountain yellow-legged frogs have been extirpated from >90% of their original range, and remaining populations are unlikely to persist in great numbers beyond the next decade. My objective in this study is to quantify the role of Mountain yellow-legged frogs in the alpine lake communities of the Sierra Nevada, and to describe the ecological consequences of amphibian declines and extinctions. This study addresses the changes in alpine lake communities due to the extinction of major species, and the immediate effects of these changes on aquatic resources in one of California’s major watersheds.

**Keywords**: *amphibian decline, aquatic community, extinction, food web, Sierra Nevada yellow-legged frogs*

**II. OVERVIEW**

**A. Statement of issue**

**II. OVERVIEW**

**A. Statement of issue**

*Amphibian declines*

Amphibian populations are declining on 6 continents, disappearing from broad ranges and high density populations (Stuart et al. 2004). For an incredibly successful group that has been around for 250 million years (Pough 2001), the fact that these dramatic declines have occurred mostly in the last half century (Houlahan et al. 2000) is alarming. Declines are caused by overexploitation, habitat degradation, and a series of enigmatic causes which include pollution, climate change, and emerging infectious disease (Stuart et al. 2004). Declines are not constrained to degraded habitats, but occur in pristine and protected areas (Wake 1991). Declines have been linked to extinctions of entire amphibian species (Skerratt et al. 2007). The literature abounds with studies of the causes and extent of amphibian declines, but the recently emerged lethal fungal pathogen, *Batrachochytrium dendrobatidis* (the cause of the disease chytridiomycosis, hereafter Bd)*,* is cited as one of the proximate causes of enigmatic declines in protected habitats (Collins and Crump 2008). Numerous studies document the effects of Bd on individual frogs and frog populations, but only a handful of studies focus on the broad ecological effects of amphibian decline and extinction (Ranvestel et al. 2004, Connelly et al. 2008, Colón-Gaud et al. 2009).

In the Sierra Nevada, the Mountain yellow-legged frogs (*Rana muscosa and Rana sierrae*) are rapidly declining towards extinction (Vredenburg et al. 2007, Vredenburg et al. 2010). Already extirpated from 90% of their native range by the introduction of non-native fish, the remaining populations face habitat fragmentation, stress from contaminants from upwind pesticide use, and emerging infectious disease (Davidson and Knapp 2007, Vredenburg et al. 2007). *Batrachochytrium dendrobatidis* is dramatically impacting frog populations, causing local extinctions and leaving entire lake basins devoid of Mountain yellow-legged frogs. However, a small number of populations have persisted at low densities despite infection of individuals with Bd. If persisting populations are at such low densities that their ecological interactions are insignificant, then those populations may be considered functionally extinct. In either case, the effects of the loss of frogs from the lake community may be similar.

*Amphibians in food webs*

The role of *R. muscosa* and *R. sierrae* in the high elevation lakes of the Sierra Nevada is partially understood – both species provide links between aquatic and terrestrial food-webs as tadpoles and adults consume algae and invertebrates (Finlay and Vredenburg 2007), and are prey for vertebrates like garter snakes (Jennings et al. 1992), coyotes (Zweifel 1955) and birds such as ravens, Clark’s Nutcrackers, and migratory grebes and gulls (TCS, personal observation). In other aquatic and terrestrial ecosystems amphibians have been shown to have crucial roles as prey, consumers, and cyclers of nutrients (Carpenter et al. 1985a, Beard et al. 2002). Tadpole grazers can exert strong control over algal and macro-invertebrate communities through facilitation and competition (Kupferberg 1997). Frogs, as prey, can also shape predator communities, and *Thamnophis* garter snake populations in the Sierra decline following Mountain yellow-legged frog declines (Jennings et al. 1992). Mountain yellow-legged frogs also provide a link for the flow of energy and nutrients from aquatic to terrestrial habitats (Finlay and Vredenburg 2007).

The loss of frogs from aquatic communities is likely to have far-reaching effects on the community and the associated food webs. It can be predicted that removal of consumers from any community will grant producers a release from grazing pressure, and they will subsequently increase in biomass, which may provide additional resources for other consumers. Removal of a dominant consumer may also allow a formerly excluded consumer the opportunity to invade. Amphibians are consumers at the middle levels of the food webs to which they belong. However, significant life history differences between larval (aquatic and generally herbivorous) and adult amphibians (partially terrestrial and carnivorous) mean they occupy both primary (grazing) and secondary (predatory) consumer positions, so the removal (via extinction) of one amphibian species is equivalent to the removal of two distinct consumers. Ripples of change in trophic levels above or below the level experiencing a perturbation are known as trophic cascades; the influence of upper trophic levels on lower levels and producers is known as top-down control of the food web. Freshwater systems, with generally more linear and less complex food webs, are more apt to experience these phenomena than terrestrial systems (Polis and Strong 1996a, Loreau et al. 2002). Oligotrophic lakes, such as those found in the Sierra (Melack et al. 1985), are especially prone because of their low productivity and nutrient limitation (Polis and Strong 1996a). The relatively low biodiversity (Stoddard 1987) in the extreme environment of Sierra alpine lakes suggests further susceptibility to trophic cascades and community instability.

Cascading responses to a change in consumers show opposite dynamics at consecutive levels; ie., a decline in a predator has a positive effect on secondary consumers, a negative effect on primary consumers, and a positive effect on the producers in the community. Despite a great deal of debate over the primacy of top-down (upper level consumer dynamics determine community dynamics) vs. bottom up (where producer dynamics determine community dynamics) control of food webs (Power 1992a), decades of study have shown that both are important in shaping community structure and productivity. For example, removal of piscine predators in Sierra lakes had a cascading effect on zooplankton and phytoplankton (Sarnelle and Knapp 2005), illustrating a top-down control in that system. On the other hand, data I collected in 2009 indicates that high epiphyton production may not be limited by the density of Mountain yellow-legged frog or mayfly consumers, suggesting that consumers are less important for control of productivity than are nutrient resources, and thus changes in consumer density will have small impacts on lake production compared to changes in nutrient concentration, such as those predicted to occur in Sierra lakes with changing regional climate (Field et al. 1999). Thus, it appears that even within the Sierra, there will be variability in the dominance of top-down and bottom-up influences in shaping lake communities.

*Stability and species interactions*

The stability of populations and communities are important properties of ecosystems that result from the interactions of organisms and environment (Hooper et al. 2005). Stability can be defined both mathematically, as the ability of a system at equilibrium to resist or recover from a perturbation (Murdoch et al. 2003), and is thus frequently referred to as resistance and resilience. For example, previous work by Knapp and others documents that Sierra alpine lake communities showed low resistance to introductions of non-native fish, but high resilience when fish were removed from lakes (R. A. Knapp et al. 2005, 2001). Other definitions of ecological stability are diverse, but for this study, I choose to define stability as the temporal variability of aggregate community properties such as biomass or diversity (Connell and Sousa 1983, Tilman 1996). Many properties of species and food webs influence the stability of a community, such as food web topology and species interaction strengths (Ives and Carpenter 2007). One of the best ways to quantify the temporal variability of a population or community is through long term censuses, which provide data for the calculation of a coefficient of variation for a metric of choice (e.g. mayfly biomass) which can be compared to the independent variable (e.g. diversity, abundance of an organism of interest, degree of disturbance) (Connell and Sousa 1983, Zar 1984). A well supported method for calculating interaction strengths of species is through the ratio of consumer:resource body sizes (Brose et al. 2005, Emmerson and Raffaelli 2004, Yodzis and Innes 1992).

Species deletions, or extinctions, are intuitively a big deal, and have potentially large effects on system stability (Pimm 2002) because they break links, reduce connectance, and alter food web topology. The importance of biodiversity in the proper functioning and integrity of ecosystems is well documented (Allen-Diaz 2000, Loreau et al. 2002), and diversity can protect ecosystems against radical changes because many species react weakly to strong resource or consumer variability, thus dampening large fluctuations (McCann et al. 1998). However, when extinctions do occur, the risk of additional extinctions in the system depends on the functional position of the species removed, the strength and distribution of the trophic links, (Borrvall et al. 2000, Pimm 2002). Much work remains to describe the basic, pristine alpine lake ecology and to make system-specific predictions about effects of species declines and deletions.

In 2009, I conducted a study in high elevation lakes in Sequoia/King’s Canyon National Park which quantified the strength of the interaction between *R. muscosa* and *R. sierrae*, mayfly nymphs (the most abundant benthic macroinvertebrate grazer, genera *Ameletus* and *Callibaetis*), and epiphytic producers. My results give no indication that tadpoles exert strong effects on their competitors or resources, but that mayflies can reduce epiphyton production in lakes in which production is limited. However, both types of consumers exhibited intraspecific competition effects, with numerical density reducing average individual biomass. This observation indicates that consumers respond to limited resources but may not limit producers through grazing.

*Role of disease*

One of the underlying questions in this study is how diseases can indirectly affect the resources and consumers of their hosts. Many of the causes of amphibian declines are non-specific environmental stressors and directly affect multiple taxa on multiple trophic levels at once (e.g. habitat destruction), but diseases are generally specific to single taxa and therefore create fewer confounding effects when they reduce the density of their hosts. Disease may be one of the best natural manipulations of diversity which affects single species.

*Conclusion*

The fate of the Mountain yellow-legged frogs seems certain, but the fate of the rest of the aquatic community is unresolved. My objective in this study is to elucidate the relationship between the frogs and their resources, consumers, and environment, and to examine the dynamics of all three in relation to the disappearance of the abundant amphibians of Sierra Nevada lakes. Southern populations of Mountain yellow-legged frogs are listed as endangered, and the northern populations have been declared worthy of listing . There are remaining, but very few, large populations of both *R. muscosa* and *R. sierrae* in Sequoia/Kings Canyon National Park and Yosemite National Park. Quantifying the role of these amphibians and the effects of their declines must necessarily take place where frogs are still abundant and in places where frogs have recently declined. The emergence of chytridiomycosis in Sierra Nevada frog populations, and the subsequent decline of the frogs, creates natural manipulations of frog density and presence, but has implications for the longevity of these species. Thus, this study occurs at a time when nature has created the perfect opportunity to address critical questions in community ecology, but the ability to answer those questions is increasingly constrained by dwindling numbers of pristine communities.

**B. Literature summary**

A vast amount of literature exists regarding the causes of recent amphibian declines, the roles of amphibians in aquatic ecosystems, and the aquatic ecosystems of the Sierra Nevada. Much has been published on food webs, trophic cascades, and the effects of species extinction on the structure and function of ecosystems. The following is a brief summary of notable relevant publications. In 2004, the first worldwide assessment of the status of amphibian populations was published (Stuart et al. 2004). This synthesis highlighted the number of rapidly declining amphibian species according to IUCN red-list criteria, and associated each with one or more reasons for decline. The authors defined three categories for causes of declines: over-exploitation, reduced habitat, and so-called enigmatic declines which include climate change and emerging infectious disease. A number of papers have reviewed the phenomenon: in addition to Stuart (2004), Wake (1991), Blaustein, Wake, and Sousa (1994), Blaustein and Kiesecker (2002), Collins and Storfer (Collins and Storfer 2003), McCallum (2007), Collins and Crump (2008) all provide reviews of the scale and scope of the declines as well as the causes and potential results of amphibian declines.

In recent decades, amphibian declines have received a lot of attention – a Google Scholar search for articles containing the phrase “amphibian declines” between 2008 and 2010 yields over 1000 citations, 2000 to 2010 over 3000 citations; inclusion of the previous decade yields an additional 300 citations. Prior to that only a few dozen appear. Inclusion of the search term “disease” finds approximately 1900 citations over the past two decades (Google Scholar, April 2010). Infectious disease as a proximate cause of amphibian mortality and population decline is well studied throughout the world. The disease chytridiomycosis and its causative agent – the fungus Batrachochytrium dendrobatidis (Bd) – are arguably the most studied and most culpable of any emerging infectious disease. In a 2007 review, Skerratt et al., wrote that Bd has caused worldwide amphibian declines and extinctions, highlighting evidence for chytridiomycosis as an emerging disease. This echoes nearly a decade of work saying the same. Berger et al., (1998) documented Bd in dying amphibians in Australia, as did Lips et al., (2006) in Central America, and Rachowicz et al. (2006) and Vredenburg et al. (2010) in the Sierra Nevada of California, USA.

In the Sierra Nevada, the decline of the Mountain yellow-legged frogs (*Rana muscosa* and *R. sierrae*, (Vredenburg et al. 2007)) has been heavily studied for decades. Even as early as the 1920’s Grinnell and Storer commented on the possibility that fish stocking excluded *Rana boyleii sierrae* (later to become *R. muscosa*) from otherwise suitable habitat (Grinnell and Storer 1924). This relationship has a strong body of evidence supporting it: conspicuous taxa (including tadpoles and macroinvertebrates) were negatively correlated to the presence of non-native trout (Bradford et al. 1993, Knapp 2005, Knapp and Matthews 2000), and removal of these trout facilitated re-colonization of lakes by frogs (Knapp et al. 2007, Vredenburg 2004).

The optimism of the latter studies is tempered by the emergence of the disease chytridiomycosis in *Rana muscosa* and *R. sierrae*. *Batrachochytrium dendrobatidis*, the causative agent of chytridiomycosis was first detected in the MYLF in 2001 (Fellers et al. 2001), but analyses of museum specimens suggest its presence in MYLFs in the 1970s (Green and Sherman 2001, Ouellet et al. 2005). Other studies show that MYLF mass mortality events were occurring throughout the 1990s (Bradford 1991, Sherman and Morton 1993, Drost and Fellers 1996). Disease induced mortality continues to drive MYLF declines, as described in Briggs et al. (2005) and Rachowicz et al. (2006). Vredenburg et al. (2010) demonstrates a wave-like spread of Bd through amphibian populations in individual lake basins and subsequent local extinctions of Mountain *R. muscosa* and *R. sierrae*.

The loss of amphibians in aquatic ecosystems of the Sierra Nevada and worldwide is likely to have far reaching consequences. The role of aquatic anuran larvae is well described (Dickman 1968, Seale 1980, Kupferberg et al. 1994, Kupferberg 1997, Alford 1999, Kiffney and Richardson 2001, Loman 2001). Given the magnitude of amphibian losses worldwide, changes in aquatic communities can be anticipated, but few studies have examined this. Ranvestal et al. (2004) ,Whiles et al. (2006), Connelly et al. (Connelly et al. 2008), and Colon-Gaud (2009) show ecosystem level effects of amphibian extinctions in Central America. The ecological roles of MYLFs have been explored by Grinnell and Storer (1924), Mullaly and Cunningham (1956), Jennings, Bradford, and Johnson, (1992), and Finlay and Vredenburg (2007).

The effects of species extinctions on ecosystems and trophic cascades both have vast bodies of literature supporting them, as does basic food web ecology. For a review of literature describing the interaction of biodiversity and ecosystem function see Loreau et al., (2002). Carpenter, Kitchell, and Hodgson (1985b) contribute a review of trophic cascades in freshwater systems, and Carpenter and Kitchell published a text on the topic (Carpenter and Kitchell 1993). Power (1992b) and Polis and Strong (1996b) offer summaries of food-web issues, and Pimm (2002) and Murdoch, Briggs, and Nisbet (2003) provide a foundation for investigations into food-web and consumer-resource dynamics.

Statistical methods such as coefficient of variation have been used in many studies of community stability, see and O’Gorman and Emmerson (2009), Caldeira et al., (2005), Ives and Hughes (2002), and Tilman (1996), and most graduate level statistics texts, e.g. Zar, (1984).

**C. Scope of study**

At the local level, this entire study includes and affects Sequoia/Kings Canyon and Yosemite National Parks and areas in the John Muir Wilderness. The information in this proposal is pertinent to all lacustrine habitats in Sequoia/Kings Canyon National Park. It is my hope that my findings can be applied to aquatic ecosystems throughout the Sierra Nevada, as well as aquatic ecosystems throughout the world which are affected by the disappearance of endemic amphibians.

My study will address issues across ecological disciplines, as I examine the interactions between the distribution and abundance of amphibians and their ecological neighbors, and an emerging infectious disease. Within the scope of community ecology, I will be studying food webs and trophic cascades, and the relationship between community structure, function, and stability, and basic consumer-resource dynamics. Aquatic ecology will be studied as I monitor the trophic interactions of freshwater communities, and the interactions these plants and animals have with their biotic and abiotic environments. The field of disease ecology will be addressed with regards to the effect of diseases and pathogens on a community level.

**D. Intended use of results**

The products of the study will be used to build academic knowledge about the effects of pathogens and species extinctions on ecosystems and about the ecological dynamics of Sierra Nevada aquatic ecosystems. That information may have conservation relevance and is likely to be used by wildlife managers. Papers and data generated will be compiled into a dissertation in fulfillment of requirements for a PhD degree from the University of California, Santa Barbara, Department of Ecology, Evolution, and Marine Biology. Many of these papers will also be submitted for publication in scientific journals.

**III. OBJECTIVES/HYPOTHESES TO BE TESTED**

*Objective:* The objective of the study is to quantify the role of the Mountain yellow-legged frogs in the high-elevation lake communities of the Sierra Nevada, and to examine the effect of declines and local extirpations of this species on Sierra Nevada aquatic ecosystems. Across a gradient of frog density, I will measure diversity and abundance of limiting nutrient resources, producers, and other consumers. Variation in these components will be related to variation in density of corresponding frog populations.

*Hypotheses:* Mountain yellow-legged frog declines will reduce the stability of Sierra Nevada alpine lake communities. Mountain yellow legged frogs have strong interspecific interactions which link dynamics in their populations to the abundances of their resources, competitors, and predators. The density of frogs in a lake is related to the diversity and abundance of algal flora, invertebrates, and vertebrates in the community. Lower density and local extinctions of Mountain yellow-legged frogs will increase the temporal variability of aggregate community properties such as community biomass, diversity, and primary and secondary production.

**IV. METHODS**

**A. Description of study area**

All remaining *R. sierrae* populations are of interest for this study, so I will work in lakes throughout Sequoia/Kings Canyon National Park, especially near the Sierra crest. All lakes are found in wilderness areas, and while most are remote, some are in high use areas, e.g. near the Pacific Crest Trail/John Muir Trail. A summary list of sites is included below, chosen according to methods outlined in section IV-B.

At each site, work will occur on the shorelines of selected lakes (typically the northern, south-facing shore), in water of ca. 1m depth and on and along the terrestrial shoreline.

**B. Procedures**

Throughout this section, I refer to *frogs*. This is a brief reference in shorthand only to Sierra Nevada yellow-legged frogs (*Rana sierrae*) and /or Southern mountain yellow-legged frogs (*Rana muscosa*) of all life stages, but excludes Pacific tree frogs (*Pseudacris regilla*) or Yosemite toads (*Bufo canorus*).

*Large-Scale Survey*

To quantify the effect of frog density on lake community, and thus the effect of declines/local extinctions of frogs, I will conduct a large scale survey comparing abundance and diversity of lake community members across a gradient of frog density and presence. This study will include approximately 30 lakes in the high Sierra; each will be surveyed 2-4 times each season for three seasons. The majority of these lakes are located in Sequoia/Kings Canyon National Park and the John Muir Wilderness, but a small set of persisting and recently manipulated frog populations remains in Yosemite.

*Selection of Study Lakes*

Lakes used in the survey will be selected as follows, with assistance from and credit to Dr. Roland Knapp and his long-term data on Sierra Nevada lakes:

1. *Fish history*. This is not a study of the effect of introduced fish on lake communities, (but see Knapp, 2005 [28]), therefore the absence of fish, duration absent, and stocking history will all be considered to minimize the confounding effects that would result from a recent or continued presence of non-native fish.
2. *Lake characteristics*. I will minimize effects from morphometric features and limnologic processes by selecting lakes above minimum depth (2m) and surface area (approximately 1ha).
3. *Environmental characteristics*. Lakes will be above 3000m/9000ft to put them all in a similar elevational class, which will minimize differences in temperature regimes and surrounding vegetation/land cover (and thus inputs into the lakes).
4. *Frog population history*. Since *Bad*-caused die-offs often last a few years from onset to local extinction, populations can simultaneously be found along a gradient of decline, from naïve, to initially declining, to drastically reduced, to extinct. One or two lakes from each category will be chosen. Additionally, any lakes involved in whole-lake frog population experiments (e.g. experimental anti-fungal treatments) by Drs. Cheryl Briggs and Roland Knapp (University of California, Santa Barbara) will also be surveyed. If experimental manipulation changes population dynamics, I will be able to measure the response of the lake community to complement measurement of community response to amphibian decline.
5. *Ease of access*.After all other factors are considered, priority will be given to lakes which are close to one another and to major trailheads.

Though frog and tadpole abundances are continuous variables throughout Sierra lakes, many lakes fall into one of the following four categories. I have included lake IDs here as examples of lakes in each category; greater detail about proposed lakes can be found in Table 1.

Lakes with high frog abundances

*Repeat surveys of these populations over the past eight years have documented relatively stable numbers of frogs of all stages through time.*

10090, 10196, 10201, 10263

Lakes with declining frog populations

*This category includes populations, in which more recent surveys have found significantly fewer adult and subadult frogs than previous surveys, following a documented arrival of the fungal pathogen Bd. Population sizes range from large to very small, and are in flux. Presumably some of these lakes will move into the ‘no frog’ category during the course of the study.*

10037, 10055, 10206, 10220, 10222, 10223, 10225, 10227, 10228, 10478

Lakes with low abundances or no frogs

*Frogs are absent from the lake, and have been for many years. Cause of decline or extirpation varies.*

10036, 10103, 10323

Lakes in which frog populations may be increasing or have recently reached high abundance.

*Restoration of lakes via removal of non-native trout over several years has allowed frog populations to recover.*

10102, 10474, 10475, 10476, 10477, 10490

*SURVEY METHODS*

*The following survey components will be performed two to four times throughout the ice-free season at all lakes in the survey, for at least two consecutive seasons:*

*Survey of algae*

Algae will be surveyed by scouring the surface of natural substrate. Sampling points will be selected randomly. Ten random numbers between 0 and 100 will be generated (e.g. using the milliseconds on a stopwatch (Gotelli and Ellison 2004)); I will walk the perimeter starting at the inlet or outlet, using the random numbers as distances between sampling point. At each location, I will scour substrate or sediment at two points less than 1m depth, so as to represent each in relation to the percent of lake bottom it occupies. All samples will be composited into one container, mixed homogenously, then a 30ml subsample will be taken and preserved in 1% Formalin, and another 30ml subsample will be filtered on a preweighed glass fiber filter for estimation of biomass (Hauer and Lamberti 2007). These methods will reflect the algal community across a heterogeneous benthic surface.

*Benthic Macro-invertebrate Survey*

Benthic invertebrates will be sampled by benthic sweeps with a fine mesh net (mesh size of .5mm). I will perform 15 sweeps along the north shoreline of each lake, as possible. Each sample will then be visually sorted in the field, to remove visible invertebrates from detritus and to preserve them in ethyl alcohol (R. A. Knapp et al. 2001). Identification to the lowest taxonomic level possible (usually genus (Merritt and Cummins 1996)), enumeration, and measurement of body sizes will be performed following the field season.

*Visual Surveys*

Visual shoreline surveys are a reliable way of counting Mountain yellow legged frog adults and tadpoles (Knapp and Matthews, 2000), and do not require capture or contact with the animals. These surveys will be coordinated with Dr. Roland Knapp’s research of dynamics of MYLF populations so that populations are not surveyed multiple times, and to maximize the number of populations surveyed in a season. I will walk the shoreline of lakes, counting and recording the number of amphibians of each species and life-stage that I observe in and out of the water, as well as anything else of note, like conspicuous but rare invertebrates (e.g. adult *Odonata* or large *Dytiscid* beetles). Visual surveys will also qualify presence of other vertebrate consumers along the lake shore, especially predators such as garter snakes, coyote, bear, or avifauna which appear to be consuming amphibians. Reliable anecdotes and sources document birds such as Clark’s nutcrackers, Brewer’s blackbirds, ravens, and migratory birds consuming amphibians in Sierra lakes.

**NOTE:** No amphibians will be captured or handled in this study. All amphibian surveys and information are purely observational.

*Other Measurements:*

*Temperature:*

Water and air temperatures will be taken at each survey. Water temperature will be measured 1m from the shoreline, at depths of 10cm and 1m. Air temperature will be measured at the shoreline at a high of 1m in the shade.

*Lake Nutrients:*

Water samples will be taken to quantify concentrations of phosphorus and nitrogen. In each lake, two water samples will be gathered in a 30ml acid-washed bottle from the point near the outlet where water transitions from still to moving. One sample will be filtered on a glass fiber filter to separate particulate nutrient sources and dissolved nutrients, and the other sample will remain unfiltered for analysis of total nutrients. Upon return to the front country, samples and filters will be frozen and stored at -20C. Analysis of these samples will be contracted to dedicated laboratories at UC Santa Barbara.

*AMPHIBIAN BODY SIZE MEASUREMENTS*

To quantify species interaction strengths using consumer-resource body size ratios, I will collect data on body size distributions of most species. Much of this data is available from scientific literature, collected but unmeasured specimens, or from data from other researchers. However, insufficient data exists on *Rana muscosa* and *R. sierrae* tadpoles. In lakes where tadpoles are abundant, such as in Upper LeConte Canyon (10100, 10102) or Spur Basin (10475), I will capture 30-60 tadpoles. I will measure tadpole length, width, tail length, tail height, and length of developing limbs, as well as wet weight, and Gosner stage. In addition, I will perform mouthpart inspections, which are a reliable and low stress method of estimating the prevalence of the fungal pathogen *Batrachochytrium dendrobatidis* (Knapp and Morgan 2006); that data may be useful to other researchers. Tadpoles will be released into the lake.

*SMALL SCALE NUTRIENT SURVEY*

I will quantify the effect that tadpoles have on lake water nutrient concentration at a very local level. While it is unlikely that tadpoles have an effect on whole lake nutrient concentrations, aggregations of tadpoles could excrete measurable amounts of nutrients such as ammonium, nitrogen, and phosphorus. In mid-summer, I will locate large tadpole aggregations, probably in Barrett Lakes Basin or Spur Basin. At 1m intervals along 5 transects radiating from the center of each tadpole aggregation, I will collect nutrient samples similarly to those collected near the outlets.

Fig. 1 Layout of small-scale nutrient sampling transects in relation to a tadpole aggregation.

*OTHER METHODS*

*Prevention of disease dispersal: Disinfection of field equipment*

To prevent moving *Bd* between frog populations and lakes, I will treat field gear that has come into contact with water (e.g. nets, sandals, containers) in a 0.1% solution of quaternary ammonia. This will be mixed on site daily in a dry-bag, and retained for the course of the day. The solution remains effective for approximately 24 hours, so I will use a new solution each day. Waste will dumped at least 100m from water, on trails or other disturbed areas, or in areas with copious organic soil where it will break down. In areas where neither is possible, the waste will be cast as far from water as is possible and as widely as possible.

*Analysis:*

For long term community variables, a coefficient of variation will be calculated. These will be compared to MYLF abundance in a lake using regression methods. For small scale nutrient data, nutrient concentration of each nutrient type will be regressed against distance of sample from center of tadpole aggregation.

**C. Collections**

Each algal survey produces up to one 30ml samples (includes algae and formalin preservative), therefore up to 90mL of total algae sample will be taken per lake per season, or a combined total of approximately 1200ml. Three filtered algae samples will be collected from each lake throughout the season. Invertebrate surveys produce an average sample totaling 60mL (includes invertebrates and ethanol preservative), so up to 250mL total sample per lake per season will be collected, or a combined total of 2.5L. These estimates are based on three repeat surveys.

**D. Analysis**

Analysis of survey data will use regression methods, either linear, multiple, or generalized linear models.

**E. Schedule**

2010

* *June 20*: Arrive at University of California field station (SNARL, Mammoth Lakes)
* *Late June-Late September*: surveys in lakes in Sequoia/King’s Canyon National Park, as well as in Yosemite NP and the John Muir Wilderness
* *October – March 2010*: Laboratory processing of samples from 2009 and 2010. Analysis and publication of results of field work.

2011

* Perform consumer exclusion experiments to quantify interaction strengths.
* Resurvey large scale survey lakes. Perform tadpole feeding ecology studies.

2012

* Resurvey large scale survey lakes.
* Wrap-up and unfinished work in field. Analysis and publication of results of field work

**F. Budget**

|  |  |  |
| --- | --- | --- |
| Table 4. Estimated Budget |  |  |
| **Item** | **Description** | **Cost** |
| *Equipment:* |  |  |
| Aquatic Net - Invertebrates |  | $73.00 |
| Lightweight Waders |  | $200.00 |
| Key to Algae |  | $75.00 |
| Insect Sorting Tools | various tweezers, pencils, etc. – ca. $100.00 | $100.00 |
| Weatherproof notebooks |  | $6.50 |
|  |  |  |
| *Consumables:* |  |  |
| Nalgene 60ml bottles | $1.02 ea x 6/lake/survey x 90 surveys | $550.80 |
| Other Nalgene bottles | Various | $200.00 |
| 95% EtOH, gallon | $46.23 x 4/season | $184.91 |
|  |  |  |
| *Services:* |  |  |
| Dissolved Nutrient Analysis @ UCSB MSI Analytical Lab | 1.88/sample/analyte x 2 analytes x approximately 90 samples | $338.40 |
| Total Nutrient Analysis | TBD, est. similar to Dissolved Nutrient Analysis |  |
|  |  |  |
| *Field Station Costs:* |  |  |
| Housing, 1 persons | $275/person/month x 3 months | $875.00 |
|  |  |  |
| *Vehicle Travel Costs:* |  |  |
| Santa Barbara to SNARL | 365mi. x 2 x.585¢/mi. | $427.05 |
| Travel W/in Eastern Sierra | 800mi. x 0.585¢/mi. | $468.00 |
|  |  |  |
| *Subsistence Costs:* | various | $500.00 |
|  |  |  |
| *Personnel Costs:* |  |  |
| Field Assistant | $1600.00/month x 3 months | $2,100.00 |
| Housing at SNARL | $125/month /person x 1 people x 3 months | $375.00 |
|  | **TOTALS** | $9173.66 |

*Funding sources:*

* $3000, Luce Environmental Science to Solutions Fellowship
* Personal savings
* NSF Ecology of Infectious Disease grant to my advisor, Dr. Cheryl Briggs, and NSF REU supplement for stipend for field assistant.

**V. PRODUCTS**

**A. Publications and reports**

Publications coming directly out of this study will consist of summarization of the large scale survey of communities throughout Sierra lakes, including those surveyed in Sequoia/Kings Canyon National Park and the data collected therein. These documents will include summaries which will describe the many interacting components of the lake community. I anticipate that my results will also be applicable to research by colleagues studying disease dynamics of the Mountain yellow-legged frog/*Bd* system, and I will continue to contribute to their work as well.

**B. Collections**

Apart from small reference collections for use in subsequent identification of samples, all algae, invertebrate, and water samples will be destroyed in analysis.

**C. Data and other materials**

I will digitally photograph as much of the study as possible, both the organisms involved and the field methods used. I aim to produce at least one poster and several presentations for public outreach and academic communication. I will retain these materials but make copies of all available to the NPS as necessary.

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**VII. QUALIFICATIONS**

I worked for four years as a technician for Dr. Roland Knapp of the University of California Sierra Nevada Aquatic Research Laboratory in Mammoth Lakes, and for Dr. Cheryl Briggs of UC Santa Barbara as a laboratory technician. During that time, I was primarily responsible for the field and lab-work involved in describing the increasing distribution of the amphibian pathogen *Batrachochytrium dendrobatidis* in the Sierra Nevada and the subsequent decline of its host, the Mountain yellow-legged frog. My field work took me throughout Sequoia/Kings Canyon and Yosemite National Parks and other parts of the high Sierra.

In that time, I collected most of the samples and data which describe recent frog population and disease history and are referred to throughout this document. I have intimate knowledge of many of the park’s amphibian populations. I have a long and continuing working relationship with Roland Knapp, and any information I have mentioned as coming from him, is current and accurate.

I have completed two field seasons for my graduate research, in which I designed and conducted the entire survey and field experiments independently, alone, and self-supported. I have visited hundreds of lakes in the Sierra, and have spent approximately almost 450 work-related days in the Sierra backcountry, almost entirely in the National Parks. In recent seasons, I have cultivated working relationships with park biologists and backcountry rangers stationed near my field sites.

Throughout my work experience, I gained experience counting and handling both invertebrates and vertebrates as large as wood rats, and in conducting independent research. Furthermore, I have held the leadership role in most of my field jobs, and especially in my work in the Sierra. Previously to that work, I was a technician on an avian malaria project, which operated out of Hawaii Volcanoes National Park.

In my second year as a graduate student, I have taken multiple courses in mathematical modeling, and been teaching assistant for herpetology and disease ecology/parasitology courses. By the beginning of the 2009 portion of the study, I will have taken multiple statistics courses, and been teaching assistant for an invertebrate/insect zoology course. My undergraduate education focused heavily on general and theoretical ecology, ecosystem management, herpetology, and parasitology.

As an avid lifelong outdoorsman and adventurer, I explored the mountains of Vermont year-round from childhood, and continue to pursue outdoor challenges such as backpacking, climbing, mountain biking, and ocean sports. Seven days a week, I spend 1-5 hours per day exercising outdoors. I have taken and taught several formal courses on technical wilderness skills, and have been continually certified as a Wilderness First Responder since 2001.

*Curriculum Vitae for Thomas C. Smith:*

**EDUCATION**

University of California, Santa Barbara; Department of Ecology, Evolution, and Marine Biology. Advisor: Dr. Cheryl Briggs; Committee Members: Drs. Scott Cooper, Sally Holbrook. September 2007 – present. Degree Anticipated: Ph.D., Spring 2013.

B.S. University of Vermont; Environmental Sciences, Concentration in Biology. 2002.

**HONORS and AWARDS**

Honorable Mention, National Science Foundation Graduate Research Fellowship Program, 2009

Dean's List, University of Vermont, 1999-2002

Vermont Scholar’s Award, merit based full tuition scholarship, University of Vermont, 1998-2002

**GRANTS, MONETARY AWARDS, FELLOWSHIPS**

The Henry Luce FoundationEnvironmental Science to Solutions Fellowship 2010-2012, $6,000

UCSB EEMB Graduate Student Fee Fellowship 2010, $418.00

Valentine Eastern Sierra Reserve Graduate Student Research Grant 2009, $300.00

UCSB EEMB Graduate Student Fee Fellowship Fall 2008, $214.43

UCSB EEMB Graduate Student Research Grant Spring 2008, $509.19

UC Natural Reserve System Mildred E. Mathias Graduate Student Research Grant 2008, $2500

UVM HELiX grant for undergraduate research, University of Vermont, 2001-2002, $3000

UVM SUGR/FAME grant for undergraduate research, University of Vermont, 200,1$3500

**PROFESSIONAL EXPERIENCE**

Teaching Assistant, UC Santa Barbara Dept of Ecology, Evolution, Marine Biology, 2008 – present.

Courses: *Invertebrate Zoology* (x2)*, Parasitology* (x2)*, Herpetology, Ecology of Disease*

Research Assistant, UC Santa Barbara Dept of Ecology, Evolution, Marine Biology, 2007 – present.

Staff Research Assistant, UC Santa Barbara, Marine Science Institute, 2004 – 2007.

Staff Research Assistant, UC Berkeley, Department of Integrative Biology, 2005 – 2007.

Field Crew Member, Oregon State University/United States Geological Survey, 2005.

Entomological Assistant III, Research Corporation of the University of Hawaii/USGS, 2002 – 2004.

Laboratory/Field Assistant, UVM, Department of Biology, 1998 – 2002.

Field Crew Member, National Wildlife Federation/Vermont Public Interest Research Group, 1999.

Field Crew Member, The Conservation Agency, 1997.

**PUBLICATIONS**

1. Schall, J.J., T.C. Smith. 2006. Detection of a Malaria Parasite (*Plasmodium mexicanum*) in ectoparasites (Mites and Ticks), and Possible Significance for Transmission. J. Parasitol., 92(2), 2006, pp. 413–415
2. Woodworth, B.L., C.T. Atkinson, D.A. LaPointe, P.J. Hart, C.S. Spiegel, E.J. Tweed, C. Henneman, J. LeBrun, T. Denette, R. DeMots, K.L. Kozar, D. Triglia, D. Lease, A. Gregor, T. Smith, and D. Duffy. 2005. Host population persistence in the face of introduced vector-borne diseases: Hawaii amakihi and avian malaria. PNAS 102:1531-1536.

**PRESENTATIONS**

1. Smith, T.C., A. Gregor, A.D. Lease, R. DeMots, D. LaPointe (2003). Diversity, abundance, seasonality, and infection of mosquitoes on windward Mauna Loa (July 11). Presentation at Hawaii Conservation Conference, Honolulu, HI.
2. Smith, T.C. (2009) Amphibian Declines, Species Interactions, and Ecological Stability (November 14). Presentation at 2009 IRCEB Amphibian Disease Meeting, Tempe, AZ.
3. Smith, T.C. (2010) Interactions between tadpoles, mayflies, and epiphyton in alpine  
   lakes of the Sierra Nevada (February 20).Presentation at UC Santa Barbara Dept of Ecology, Evolution, and Marine Biology Graduate Student Symposium, Santa Barbara, CA.
4. Smith, T.C. (2010) Interactions between tadpoles, mayflies, and epiphyton in alpine  
   lakes of the Sierra Nevada (February 27).Presentation at UC Natural Reserve System Mildred E. Mathias Graduate Student Research Symposium, Bodega Bay, CA.

**COMMITTEES and LEADERSHIP POSITIONS**

UCSB Dept. of Ecology, Evolution, Marine Biology Graduate Student Advisory Committee, September 2009 to present

UCSB Natural Reserve Advisory Committee, Graduate Student Representative, 2008 to present

UCSB Dept. of Ecology, Evolution, Marine Biology Graduate Student Research Symposium planning committee, food and social events planner, May 2008-March 2009

**CERTIFICATIONS**

Wilderness First Responder, 2001-present

VIII. **SUPPORTING DOCUMENTATION AND SPECIAL CONCERNS**

**A. Safety**

In Sequoia/Kings Canyon National Park, all field sites will be located in wilderness, and accessed via foot travel, hence extensive wilderness travel will be required, often with extremely heavy loads. Most sites will be accessed via little traveled cross-country routes. I have extensive experience in this type of field, so I am aware of the risks it poses and the prevention required. I am a certified Wilderness First Responder. I will provide accurate itineraries to my advisor at UCSB, and to the staff at the Sierra Nevada Aquatic Research Laboratory field station where I will reside when not in the field.

Invertebrate, algae, and water sample collection requires extended periods of wading in lakes and exposure to cold water. I will use waterproof waders, neoprene socks, insulative clothing, and gloves to minimize the exposure.

**B. Access to study sites**

Sites will be accessed by foot travel. Trips will be up to 10 days long, or several back to back day trips or 2-3 day trips, with trips into other parts of the Sierra in between. So I will be entering the SEKI regularly throughout the season. In 2009 I will have a field assistant, but will continue to conduct much of the work alone; I hope to have volunteers (other graduate students) assist with the setup of the experiment. I have no need to enter restricted areas.

**C. Use of mechanized and other equipment**

i. No mechanized equipment will be used, and all field survey equipment can be carried by foot into and out of the backcountry. However, to expedite the work and improve quality of data, I am requesting that experimental cages be transported into the backcountry on already scheduled helicopter flights (see section VIII-G-NPS Assistance).

ii. *Temporary structures:*

* I will temporarily place paired three inch by four inch ceramic tile arrays into survey lakes, to provide a standardized surface for algal growth and survey (see photo, Fig. 1, in sect IV-Methods). Tiles will be stationary on the benthic surface of the lake for several weeks. Tiles will be removed at the end of the summer, to be replaced again the following spring.
* I will temporarily place cages into several lakes for experimental manipulation of alpine lake consumer density. See photo, Fig. 2, in section IV-Methods. Cages will be removed in late September or early October.

**D. Chemical use**

*Quaternary Ammonia,* (hereafter Quat), is used to disinfect field gear which comes into contact with lake water and amphibians, in order to prevent the spread of the *Bd* pathogen. A total of 120ml are likely to be used throughout each field season. The concentrated Quat is transported in a 30ml plastic bottle. A 0.1% solution of Quat is mixed in a dry bag by adding 30 drops of Quat to 2 liters of lake water. Field gear is then soaked in this solution in the dry bag for 5 minutes. After disinfecting gear, it is rinsed with water from the next survey site prior to using it. Items such as boots, clothing, and your legs are not practical to treat, so these are dried completely before moving to another survey site. Because Quat will retain its effectiveness for 24 hours, the most efficient disinfection method is to mix solution in the morning use this solution for all disinfections for the remainder of the day. Quat breaks down when it comes into contact with organic material. Therefore, the best disposal sites are those containing disturbed organic soil, >100 m from water. Accidental release will be remediated by application of organic material, followed by copious amounts of water.

*95% Ethanol* will be used to preserve invertebrate specimens. A total of several gallons will be used each season. It will be transported in small plastic bottles. Ethanol evaporates quickly in an open space, so no steps will be taken in the event of a spill in the field.

*Formalin* will be used for preservation of algae and tadpole samples. We will use gloves when handling. Great care will be taken to prevent spillage, and to work with formalin away from water sources. Only very small amounts will be handled at a time. If spillage occurs, what can be soaked up in sand will be placed in a closed bottle. The rest will remain in the environment, as formalin oxidizes with exposure to oxygen with no hazardous by products stated.

*MSDS Sheets for all Chemicals are attached at end of document.*

**E. Ground disturbance**

N/A

**F. Animal welfare**

All animal use is approved by UCSB IACUC Protocol #762.

**G. NPS assistance**

I am capable of transporting all materials into/out of the backcountry under my own power with the assistance of volunteers.

**H. Wilderness “minimum requirement” protocols**

*Excerpt from the Wilderness Act:*

*“Except as otherwise provided in this chapter, each agency administering any area designated as wilderness shall be responsible for preserving the wilderness character of the area and shall so administer such area for such other purposes for which it may have been established as also to preserve its wilderness character. Except as otherwise provided in this Act, wilderness areas shall be devoted to the public purposes of recreational, scenic, scientific, educational, conservation, and historical use.*

*Except as specifically provided for in this chapter, and subject to existing private rights, there shall be no commercial enterprise and no permanent road within any wilderness area designated by this Act and, except as necessary to meet minimum requirements for the administration of the area for the purpose of this Act (including measures required in emergencies involving the health and safety of persons within the area), there shall be no temporary road, no use of motor vehicles, motorized equipment or motorboats, no landing of aircraft, no other form of mechanical transport, and no structure or installation within any such area.*

*Within wilderness areas designated by this chapter the use of aircraft or motorboats, where these uses have already become established, may be permitted to continue subject to such restrictions as the Secretary of Agriculture deems desirable. In addition, such measures may be taken as may be necessary in the control of fire, insects, and diseases, subject to such conditions as the Secretary deems desirable.”*

This study requires a significant presence in Wilderness areas. The Wilderness Act states that wilderness will be partially devoted to scientific purposes. This study asks scientific questions addressing the consequences of an emerging infectious disease and species extinction on the aquatic resources of the Sierra Nevada, and the appropriate techniques require some serious time spent at the sites where ecological changes are occurring, within Wilderness areas. Lakes off the beaten path will be used as much as possible, but scientific interest dictates use of some lakes near trails, but therein research activities will occur well away from trails or campsites.

The proposed activities have virtually zero impact on the benthic surface of lakes, nor on lake occupants or the animals captured and released.