Reintroduction of a Declining Amphibian: Determining an Ecologically Feasible Approach for the Foothill Yellow-legged Frog (*Rana boylii*) Through Analysis of Decline Factors, Genetic Structure, and Habitat Associations

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ABSTRACT

Species reintroductions provide a model for integrating practical and theoretical aspects of conservation biology. However, we currently lack a clear understanding of the factors that determine the outcome of reintroduction programs for many taxa. Combining pre-reintroduction research on ecology, genetics, and causative factors of declines with experimental reintroductions and rigorous monitoring, offers an approach that should increase reintroduction success rates. Amphibians present challenges as reintroduction subjects due to characteristics such as biphasic life cycles, low mobility, and patchy distributions. This study focused on a stream-dwelling, declining amphibian in California and Oregon, the foothill yellow-legged frog (*Rana boylii*), and included three components: (1) determining primary causes of decline, (2) describing range-wide genetic variation, and (3) quantifying habitat associations. For decline factors, the analysis approach was to spatially relate the current status of *R. boylii* (present or absent) at historic localities to: geographic characteristics, land uses, wind-borne toxins, climatic variables, and proximity and size of dams. Climatic variables showed strong influence in multivariate models. There was also evidence for interactions, especially that negative effects of dams appeared to be exacerbated in areas with low precipitation. For genetic analyses, 1525 total base pairs from sequences of two mtDNA fragments (Cytochrome B and ND2) for 77 individuals from 34 localities were used. Phylogenetic analyses recovered several well-supported, geographically congruent clades within *R. boylii*. Genetic variation was low among populations in the largest, most inclusive clade, but individuals from several localities showed substantial divergence. Hydrologic regions, which represent likely dispersal corridors for *R. boylii*, show promise in explaining patterns of genetic variation. The habitat associations component focused on microhabitat scale oviposition site selection coupled with larger scale evaluations of occurrence and relative abundance at breeding areas. Oviposition microhabitat characteristics such as water depth, water velocity, and stream substrate showed narrow ranges among study localities. These results suggest that habitat selection results in population stability for *R. boylii* even within the substantial temporal and spatial variability of stream environments. I discuss the application of these results to potential reintroductions of *R. boylii* and propose a conceptual model for integrating this and other information into reintroduction programs.

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CHAPTER 1. INTRODUCTION

Background

Conservation biology by its nature is an applied science focusing on the immediate and practical issues of biodiversity loss, declining species, reserve design, and ecological restoration. Strategies that are developed to solve various conservation problems are often based on current ecological and genetic theories and related empirical research. Even though the extent to which these theories can be applied is a matter of controversy, conservation biologists have, for example, gained insights from island biogeography theory and metapopulation dynamics in the development of reserve design strategies (Simberloff 1997). In fact, each conservation problem draws on specific areas of ecological and evolutionary theory and requires a different set of practical tools (e.g. population viability analysis, geographic information systems).

Species reintroductions and translocations provide an interesting model for integrating practical and theoretical issues and developing appropriate sets of analytical tools. Historically, species reintroductions have been used as efforts of last resort in the recovery of endangered species. However, because of the accelerating rate of population decline of many species, reintroductions have become an increasingly important component of conservation strategies (Table 1.1). Reintroduction is defined here as: “an attempt to establish a species in an area which was once part of its historical range, but from which it has been extirpated or become extinct.” A translocation is: “the deliberate and mediated movement of wild individuals or populations from one part of their range to another” (IUCN 1995). For most vertebrate taxa, reintroduction successes have been outstripped by failures, calling into question the approaches, assumptions, and methods used. Recent reviews of reintroduction programs have suggested that both a better grounding in ecological theory and extensive monitoring are needed (May 1991, Sarrazin and Barbault 1996). Specifically, several areas of research are needed to validate the appropriateness of reintroductions and to increase their likelihood of success: (1) an understanding of the original cause of the species decline, (2) knowledge of genetic relationships of remnant and source populations, (3) an understanding of social relationships for translocated animals and the community to which they will be added, and (4) knowledge of habitat suitability (Griffith et al. 1989, Dodd and Siegel 1991, Reinert 1991, May 1991, Sarazin and Barbault 1996, Wolf et al. 1996, Dodd, *In Press*). In addition, robust monitoring of the whole process is essential to gaining a better understanding of why some programs fail and some succeed (Dodd and Siegel 1991, Sarazin and Barbault 1996, Dodd, *In Press*). Along with these rather broad research needs, successful reintroductions depend on local habitat suitability and careful consideration of demographic characteristics and processes (Sarrazin and Legendre 2000). So, a successful reintroduction program should address all of these concerns and develop an objective evaluation process prior to its implementation.

Amphibians present distinctive challenges as reintroduction subjects due to several unique ecological, genetic, and demographic characteristics and the interplay among them. Some of these characteristics include: (1) the presence of "cryptic species" (e.g., Green et al. 1997), (2) patchy distributions on the landscape due to associations with isolated aquatic environments (e.g., ponds and wetlands), (3) high site fidelity and low dispersal rates among habitat patches, (4) local adaptations that strongly influence genetic structure (because of characteristics 1-3), (5) biphasic life cycles and different habitat requirements for eggs, larvae or juveniles, and adults, and (6) variable fecundity and "boom and bust" population cycles (Alford and Richards 1999, Houlahan et al. 2000), and (7) documented environmental sensitivities (Vitt 1990, Welsh and Ollivier 1998). Some of these characteristics have clear consequences for attempted reintroductions while others may manifest differently in different species. For example, the biphasic life cycle means that suitable habitat must be available for all life stages and these habitat types may be quite different from each other. Also, while egg and larval environments (ponds and streams) are fairly well-studied, knowledge of the habitat requirements of the terrestrial life stages (sub-adults and adults) of many species is more limited. Depending on the context of the reintroduction, the often patchy distribution, high site fidelity, and low dispersal rates could be either advantageous or problematic. If the reintroduction program involves a single, isolated site, then site fidelity could prove important for sustaining the new population. However, if the reintroduction program is planning for newly established populations to move into unoccupied areas, then high site fidelity and low dispersal rates could be problematic and multiple populations may need to be established. Additional factors to consider with regard to amphibians are that there has been minimal empirical research on potential genetic and demographic bottlenecks and the mechanisms in recent declines of many species are poorly understood (Collins and Storfer 2003).

Nevertheless, reintroductions are being attempted for declining species around the world. I recently compiled list of amphibian reintroductions in North and Central

American and found that in the last 20 years, at least 46 reintroductions have been attempted for 26 species (Table 1.1). Detailed information on these programs is available at the Declining Amphibians Populations Task Force web site (http://www.open.ac.uk/daptf/about/abou7.htm). It is worth noting that in the western U.S., all reintroduction programs include special status (State or Federally listed) and all focus on ranid frogs and bufonid toads (Table 1.1). While some reintroductions in Europe have been successful (e.g. Denton et. al. 1997), programs in the western hemisphere have either failed, have unknown outcomes, or it is too early to tell (Dodd, *In Press*). These failures as well as other ecological and genetic concerns have led to cautionary articles regarding amphibian reintroductions (Dodd and Siegel 1991, Reinert 1991, Seigel and Dodd 2002, Dodd, *In Press*).

To resolve some of these issues, a number of questions should be addressed prior to implementing a reintroduction program. I used a focal amphibian species and conducted empirical research on several of these questions. My research focused on a declining amphibian species in California and Oregon, the foothill yellow-legged frog (*Rana boylii*), and included three primary objectives: (1) to determine the primary causes of decline of this species through analysis of its distribution and corresponding spatial information on known and potential stressors or threats, (2) to describe the genetic variation and structure of *R. boylii* throughout its range and relative to hydrologic basins, and (3) to quantify habitat associations especially for reproduction and rearing. Finally, with the information garnered from literature review and these three components of my research I have developed a qualitative decision tree for amphibian reintroduction planning. I have also attempted to make some preliminary recommendations on how to apply this information to future reintroductions of *R. boylii*.

Study Species

Rana boylii historically occurred in foothill and mountain streams from northern Baja California to southern Oregon west of the Sierra-Cascade crest, to 1830m (6000 ft) in elevation. This species has experienced significant population declines especially in the southern part of its range (southern Sierra Nevada and south coastal California) and is currently listed as a California State Species of Special Concern and USDA Forest Service California Region Sensitive Species (Jennings and Hayes 1994, California Department of Fish and Game 2004). In the Sierra Nevada, it apparently has disappeared from at least 66% of its historic range (Jennings 1996). There are at least two remnant populations of *R. boylii* in the southern Sierra Nevada, one near the historic southern extreme of its geographic range in that region and a second that falls about midway between that population and more abundant populations to the north on the Stanislus National Forest (pers. obs.). *Rana boylii*'s historical geographic range and the current, reduced one, encompass significant latitudinal, elevational, and geologic variation, which create environmental gradients of temperature, vegetation, and stream hydrologies (e.g. rain only, snow only, and combinations).

The basic ecology of *R. boylii* is known from only a few descriptive studies scattered throughout its range (Zweifel 1955, Hayes and Jennings 1988, Fuller and Lind 1992, Kupferberg 1996a&b). Information on the basic ecology of the species is summarized in Table 1.2. Briefly, *R. boylii* is one of a handful of western North

American anurans that are almost exclusively associated with running waters (lotic environments). Breeding and oviposition occur in spring (March through June) and females deposit a single egg mass which consists of several hundred to over 1000 eggs. Tadpoles (larvae) develop in and near oviposition areas and metamorphose in late summer through early autumn (July through September) (Nussbaum et al. 1983).

Preliminary information on breeding ecology and habitat associations indicate that the main threats and possible causes of declines are human activities that alter hydrologic regimes of streams and rivers. These effects have been most pronounced for the egg and larval stages of this frog that occur in very specific conditions of water temperature, velocity, and depth (Fuller and Lind 1992, Kupferberg 1996a&b). Human activities such as dams and diversions, mining, and livestock grazing, can have significant effects on hydrologic regimes (Lind et al. 1996). In addition, while research on environmental toxin effects on *R. boylii* has not yet been conducted, closely related species in other regions have shown sensitivity to numerous pesticides, herbicides, and fertilizers (Berrill et al. 1997, Davidson et al. 2002).

Chapter Overview

Here I provide a brief overview of each of the chapters that follow. The research in Chapter 2 (Lind et al., *unpublished ms-a*) was modeled after work done by Davidson and colleagues (Davidson et al. 2001, 2002) in which the current status of historic *Rana boylii* localities is geo-spatially and statistically related to a set of potential decline factors (habitat alteration, climatic conditions, etc.) My work here is distinctive (in relation to Davidson et al. 2001, 2002) in that it includes very accurate mapping of each locality of the focal species, covers the entire range of the species

(California and Oregon), and adds a new decline factor (presence and characteristics of dams) to the analysis of the decline of this species. This work is especially relevant to a potential reintroduction of this species since understanding (and reducing or eliminating) decline factors would be essential for choosing target reintroduction locations.

Chapter 3 (Lind et al., *unpublished ms-b*) describes the phylogeography of *Rana boylii* with a focus on conservation implications (reintroductions, land management, potential units for listing, etc.). Two fragments of mitochondrial DNA (Cytochrome B and partial ND2) were sequenced for seventy-seven individual *R. boylii* from 34 localities across the geographic range of the species. The resulting phylogenetic trees and analyses of molecular variance are the first for this species and provide information on patterns of genetic diversity relative to biogeographic history and past and current hydrologic basin affinities. Genetic data are critical for species reintroductions because they can guide selection of source populations for particular target reintroduction sites. Depending on the goals of the reintroduction program, genetic data can provide information that will lead to conservation of populations on similar evolutionary trajectories as well as allow for future adaptations.

Chapter 4 (Lind and Welsh, *unpublished ms*) focuses on the breeding habitat associations of *the R. boylii* through an analysis of characteristics of oviposition sites, associated random sites, and by chronicling the multi-year use of particular oviposition areas within several streams in two geographic areas of the species' range. Oviposition microhabitat was evaluated at 11 localities – seven in the northwestern part of California and four in the central and northern Sierra Nevada. Microhabitat use and availability for oviposition sites and oviposition site fidelity are examined in detail at two of the seven localities in northwestern California. This information is relevant to potential reintroduction programs both in terms of selection of target reintroduction locations with appropriate habitat and in understanding the importance and spatial distribution of particular sites (site fidelity) to the overall success.

Chapter 5 is a synthesis of results from the previous chapters and provides an example of how the information gained at each step (Chapters 2-4) could be used in reintroduction planning for *R. boylii*. I also present a qualitative decision tree for amphibian reintroductions in general.

A final note on format; Chapters 2-4 will ultimately be submitted as separate publications. They have co-authors who have already provided some guidance on the chapters and plural pronouns (i.e. we, instead of I) are used in the text. Also because they were written to stand alone and be submitted at manuscripts, the tables, figures, and appendices have been placed at the end of the appropriate chapter, rather than imbedded within it.

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Table 1.1. Summary[1] of recent (1980-present) and planned reintroduction/ translocation programs for amphibians in North and Central America (compiled via email and telephone inquiries, February – March 2003).

Region	**Number of Active Programs**	**% of Programs with Species State or Federally Listed**	**% of Programs With Captive Breeding or Rearing**	**Species**
Eastern U.S.[2]	24	8.3	5.9	*Bufo fowlerli, B. houstonensis, Hyla versicolor, Pseudacris crucifer, Rana clamitans, R. sylvatica, Scaphiopus holbrooki, Ambystoma annulatum, A. maculatum, Notophthalmus, viridescens, Plethodon cinereus*
Western U.S.	17	100.0	23.5	*Bufo baxteri, B. boreas, Rana boylii,* *R. chiricahuensis,* *R. draytonii, R. lutriventris,* *R. muscosa, R. onca,* *R. pipiens, R. pretiosa,* *R. subaquavocalis,* *R. tarahumara*
Eastern Canada	0			
Western Canada	2	100.0	100.0	*R. pipiens*
Mexico	0			
Caribbean	2	100.0	100.0	*Bufo lemur, Leptodactylus fallax*
Central America	1	100.0	100.0	*Atelopus zeteki*

[1] Details on each program are available at the Declining Amphibians Populations Task Force web site – http://www.open.ac.uk/daptf/about/abou7.htm.

[2] Eastern U.S. is east of the Rocky Mountains. The high number of programs in this region are largely a result of a multi-year effort to translocate several relatively common species into unoccupied areas of the Gateway National Recreation Area in New York, U.S.A. (Cook 2002).

Table 1.2. Ecological relationships of the foothill yellow-legged frog (*Rana boylii*).

Ecological Component	**Documented Requirements/Interactions**	**Relevant Literature**
a. EGGS/LARVAE (Eggs: March-June, depending on location; Larvae: March-August, depending on location)		
Food	algae (preference for *Cladophora* sp. with epiphytic diatoms).	Kupferberg 1996a
Cover		
Water Depth	10-13 cm 7-22 cm 4-43 cm 6-28 cm 6-40 cm	Fitch 1936 Fuller and Lind 1992 Kupferberg 1996a&b VanWagner 1996 Lind and Welsh (*unpublished ms*)
Water Velocity	0.0-0.06 m/s 0.01-0.14 m/s 0.0-0.03 m/s 0.00-0.21 m/s	Fuller and Lind 1992 Kupferberg 1996a&b VanWagner 1996 Lind and Welsh (*unpublished ms*)
Substrate	most common – cobbles and boulders “clean” (no algae/sediment) surface for egg attachment	Kupferberg 1996a&b, VanWagner 1996, Lind and Welsh (*unpublished ms*) Wheeler et al. 2003
Water Quality		
Dissolved Oxygen	unknown, but likely important especially during larval rearing in warmer summer months	see Duellman and Trueb 1986 for info on related species
Thermal Regime	Critical thermal maximum (embryos) - <26^{o}C	Zweifel 1955

Table 1.2, continued		
Predators	garter snakes (larvae) Pacific coast aquatic garter snake Sacramento pike minnow (eggs) rough-skinned newt (eggs) American dipper (larvae) Centrarchid fish crayfish (eggs, small larvae) trout ? bullfrog? raccoons and other mammals?	Fitch 1941, Fox 1952 Lind and Welsh 1994 Ashton and Nakamoto, pers. comm., Corum 2004 Evenden 1948 Lind, pers. obs. Werschkul and Christensen 1977 Wiseman 2004
Competitors	bullfrog	Kupferberg 1997

b. JUVENILES/ADULTS		
Food	aquatic and terrestrial invertebrates and arachnids	Zwiefel 1955, VanWagner 1996
Cover		
Aquatic Habitat Type	most common along pool, glide, riffle	VanWagner 1966, Lind and Welsh (*unpublished ms*)
Near-Stream Cover	shoreline rocks (especially cobble), leaf litter, overhanging vegetation (e.g. *Carex* sp.)	Hayes and Jennings 1988, VanWagner 1996
Riparian Habitat	overhanging vegetation (e.g. *Carex* sp.) moderate shade [ground cover needs not known]	VanWagner 1996 Zweiful 1955, Moyle 1973, Hayes and Jennings 1988
Upland Habitat	[unknown]	
Terrestrial Microclimate		
Humidity	unknown, but likely important for some life functions (e.g cutaneous respiration)	see Duellman and Trueb 1986 for info on related species
Light	unknown, but day-length likely affects breeding readiness	see Duellman and Trueb 1986 for info on related species
Dispersal Habitat	unknown, but likely along streams and in adjacent riparian young-of-the-year frogs may move upstream at greater rates than downstream	 Twitty, et al. 1967

Table 1.2, continued		
Mates	calling behavior – male	MacTague and Northen 1993, Ziesmer 1997
	sex ratios 1:1	VanWagner 1996
	females “clean” substrate prior to oviposition	Wheeler et al. 2003
	male territoriality and female selection of oviposition sites	Wheeler, pers. comm..
	bullfrogs may interfere with successful mating of *Rana boylii*	Lind et al. 2003
Predators	Sacramento pike minnow	Ashton and Nakamoto, pers. comm., Corum 2004
	garter snakes	Fitch 1941, Lind and Welsh, pers. obs.
	bullfrog	
	raccoons and other mammals	Bourque, pers. comm.
Competitors	other Ranids ?	

CHAPTER 2. A RANGE-WIDE ECOGEOGRAPHIC ANALYSIS OF THE DECLINE OF THE FOOTHILL YELLOW-LEGGED FROG (*RANA BOYLII*)

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ABSTRACT

The phenomenon of amphibian decline has been examined at a variety of spatial scales from intensive, experimental studies to coarse scale, primarily correlative research. Six categories of hypotheses for site-level causes of these declines have been generated including: land use change, alien species, over-exploitation, pesticides and other toxins, infectious disease, and global change, which includes both increased ultraviolet (UV) radiation and climatic change. We focused our research on a declining stream-associated frog (*Rana boylii*) and conducted the study over the entire geographic range of this species (Oregon and California, USA). Covering this extensive spatial scale, we used statistical modeling to test three of the six categories of hypothesized causes of decline: land use change, pesticides and other toxins, and global change. Through univariate and multivariate analyses we evaluated up to fourteen environmental and two interaction variables representative of the three hypotheses. In addition, to better understand regional patterns, we conducted separate multivariate analyses for localities within California and for several bioregions. Because previous work had indicated that dams (and resulting downstream hydrologic changes) have negative impacts on *R. boylii* and that precipitation levels could partly explain presence/absence patterns, we explored these two factors in more detail. We analyzed proximity and size of dams and reservoirs and evaluated measures of mean precipitation, variability in precipitation, and indices of dryness. We found evidence for all three of the six proposed amphibian decline hypotheses. There was strong evidence for negative effects of land use change especially the degree to which an area surrounding a frog locality had been converted to agricultural or urban uses. Climatic

affects were the strongest we saw for any individual variable as well as showing strong influence in multivariate models. Specifically, mean annual precipitation was positively related to *R. boylii* presence. Variability of precipitation and percent of dry years were negatively related to *R. boylii* presence. When considered along with the strong positive univariate effect of latitude, it appears that *R. boylii* is responding to recent climate changes and may respond to future changes as well. We found a somewhat weaker signal from pesticides and other toxins with agricultural pesticide drift showing equivocal results in univariate and multivariate analyses and urban pollutant drift showing a consistently negative influence on *R. boylii* presence. We also discovered evidence of interactions and/or synergisms, especially that negative effects of dams appear to be exacerbated by low precipitation. The relative importance of the factors differed among bioregions. We propose that the results of our work can be used immediately in conservation planning, especially in the operation of some dams within the range of *R. boylii* and in selection of protected areas relative to adjacent land use. In addition, our work brings to light recent and potential future responses of *R. boylii* to variation in precipitation that will aid conservation planning efforts in the face of near and long-term climate change.

INTRODUCTION

Understanding factors in the decline of a species is key to developing conservation and recovery plans. Examining potential factors at extensive geographic scales can reveal spatial patterns of decline that could be missed using locality-to-locality assessments. Certain patterns may be indicative of pervasive factors in species declines (e.g. Davidson et al. 2001, 2002). Global amphibian declines, first widely documented in the late 1980s and early 1990s (Blaustein and Wake 1990), have become a major focus in conservation research. A recent review of amphibian declines identified six main hypotheses: land use change, alien species, over-exploitation, pesticides and other toxins, infectious disease, and global change, which includes both increased ultraviolet (UV) radiation and climatic change (Collins and Storfer 2003). Some of these have been tested with correlative or experimental studies or both, yet definitive results remain elusive for most species and geographic areas (Linder et al. 2003, Stuart et al. 2004). Because declines have often been documented in relatively remote and ostensibly pristine ecosystems, windborne pesticides/toxins and global change have been touted as likely factors (Carey and Alexander 2003, Sparling and Cowman 2003).

The foothill yellow-legged frog, *Rana boylii*, is a member of a group of ranid frogs from the western United States that has shown substantial declines. Historically, *R. boylii* occurred in foothill and mountain streams (to 1830 m) from southern California (with one isolated and unverified locality record from northern Baja California, Mexico) to southern Oregon west of the Sierra-Cascade crest. *Rana boylii* females breed and oviposit each spring, attaching one globular egg mass to rocky substrates in all manner of lotic waters, from small creeks to large rivers. Larvae (tadpoles) graze on algae throughout the summer and metamorphose by late summer. Adults are found in and near streams and primarily consume aquatic and terrestrial insects (Nussbaum et al. 1983).

Rana boylii has experienced significant population declines, especially in the southern part of its range (southern Sierra Nevada and southern coast range of California) and is currently listed as a California State Species of Special Concern (Jennings and Hayes 1994, Jennings 1996, California Department of Fish and Game 2004). Recent reviews of *R. boylii* status indicate that a primary hypothesis for this stream-dwelling species' decline involves alteration of hydrologic regimes (Jennings 1996). Dams (power generating and water storage) and water diversions are the primary stressors (influences) on hydrologic regimes in California, especially in the Sierra Nevada (Mount 1995, Kattelmann 1996). In addition, because *R. boylii* occurs primarily at low to mid-elevations in California, they may be affected by urbanization, local and nearby (e.g. via windborne pesticides) agricultural activities, and climate change (especially warming and its affects on precipitation). In an analysis of the California portion of *R. boylii*'s geographic range, Davidson et al. (2002) found that frog presence at a locality was positively associated with latitude and average precipitation and negatively associated with amount and proximity of agricultural land. Though *R. boylii* co-occurs with native fish throughout much of its range, exotic species introductions (both fish and bullfrogs) may also be playing a role in its decline (Ashton and Nakamoto, pers. comm., Kupferberg 1997, Lind et al. 2003).

Due to the somewhat mysterious nature of many amphibian declines it is often the case that long lists of potential threats or decline factors are generated during the conservation planning process. As a result of our work here and other related studies (Davidson et al. 2001, 2002), we propose that analysis of multiple factors and their interactions may best be accomplished initially with coarse scale modeling approaches. By examining the decline hypotheses at an extensive spatial scale, we hope to identify likely causes of decline of *R. boylii* and to understand how they interact and vary across the range of the species. Ideally such approaches would be coupled with more intensive and even experimental studies on a short list of factors at local scales. Here we evaluate three (land use change, global change, and toxins) of the six hypotheses identified above (i.e. Collins and Storfer 2003) as represented by a number of environmental variables (hereafter called decline factors). We analzye range-wide species distribution (locality) data and corresponding spatial information on these factors. Effects of land use change were represented by the degree of agriculture and urban development and presence and characteristics of dams. Global change effects were represented by latitude, elevation, and amount and variability of precipitation. UV-B effects were not expected for this mainly lower elevation-dwelling species. We also evaluated the potential for airborne drift of toxins using wind direction scenarios combined with agricultural and urban use information. Specially, we asked: (1) whether the current status (present or absent) of historical *R. boylii* localities is statistically related to variables representative of known decline factors (e.g. agricultural land use, dams, precipitation, etc.), (2) whether the spatial pattern of the decline and its relationship to the environmental variables implicates one or more of the proposed hypotheses, and (3) whether the resulting patterns are constant throughout the range of the species or vary among bioregions.

Earlier work on spatial patterns of decline of *Rana boylii* is similar in scope (Davidson et al. 2002). However, the approach and methods used in our paper differ from those of Davidson et al. in the following ways: (1) *R. boylii* records were individually mapped based on narrative locality records rather than digitized as a group from less accurate maps; (2) locality records and associated environmental variables cover the entire range of the species in California and Oregon, not just California; (3) a detailed analysis of the presence, proximity, and potential influence of dams is included; and (4) the influence of precipitation is examined in greater detail with the inclusion of a variety of measures of means, variability, and indices of dryness.

Finally, though the primary approach of our study is retrospective, we believe that findings of particular influential variables may provide insights for the future, particularly when it comes to the geographic and climatic variables. As predictions of climate change become more reliable, our results may allow us to predict where and to what extent *R. boylii* is likely to experience the most significant declines or range shifts in the future.

METHODS AND ANALYSIS

The study area for this work encompassed the entire geographic range of *R. boylii* (Fig. 2.1). Our overall approach for examining potential factors in the decline of the *R. boylii* is based on a method developed earlier (Davidson et al. 2001, 2002).

We mapped historic locality records for the species of interest, determined the current status of those localities, and then conducted statistical modeling to identify relationships between current status environmental variables. The environmental variables used in this case were those that are representative of proposed hypotheses for general amphibian decline (e.g. Collins and Storfer 2003) as well as specific variables which have been identified as having effects on the focal species (e.g. dams/diversions - Lind et al. 1996, precipitation and pesticide drift – Davidson et al. 2002).

Localities and Status

For California, we started with a previously compiled database of 3356 narrative descriptions (e.g. "3 miles upstream of Smith Road crossing of Jones Creek") of localities for *R. boylii* from museum collections and to a lesser extent literature and field notes sources (Jennings and Hayes 1994, Davidson et al. 2001, 2002). This database represented the potential set of historic locality records. Of these, 1049 were unique localities and contained information sufficient for detailed mapping using a geographical information system (GIS). We randomly selected a subset of 10 records from each California county in which *R. boylii* occurred; if a county contained less than 10 records, all of them were selected. We mapped each of the resulting 372 localities using 1:100,000-scale U.S. Geological Survey (USGS) digital topographic quadrangles and ArcView version 3.2 GIS software (Environmental Systems Research Institute [ESRI] 1999). For Oregon, a search of museum and other locality records resulted in 105 unique localities, of which 90 contained adequate information for mapping. All of these were mapped. This brought the total number of historic localities to 462. An Albers map projection (datum NAD27) was used for locality mapping as well as for data processing of environmental variables (see below) to achieve the highest accuracy possible given the geographic scope of the data (ESRI 1992).

We determined the current status (presence or absence) of each *Rana boylii* locality using several methods. For California, we reviewed published accounts of field visits to historic localities conducted in the late 1980s and early 1990s (Jennings and Hayes 1994, Jennings 1996), queried species experts and local biologists, and conducted our own field locality visits from 2000-2002. For Oregon, current status was determined by field site visits to all localities in 1997 and 1998. Of the 372 California locality records, we were able to validate current status for all but 44 localities. We were able to determine current status based on field surveys for all 90 Oregon localities. Thus the combined California/Oregon data set included a total of 418 localities with known status throughout the range of the species. Finally, because global amphibian declines are widely believed to have begun in the 1970s, we chose 1975 as a cut-off and omitted localities at which the initial visit was after that date (n=24) from further analyses, resulting in a final sample size of 394 localities.

Decline Factors

We developed four categories of environmental variables that characterized potential direct and indirect effects on *Rana boylii* status and that represented one or more of the hypothesized causes of world-wide amphibian population declines. The rest of this section is organized by these four categories: (1) geographic context, (2) land use, (3) precipitation, and (4) dam presence, proximity, and size. We also targeted environmental conditions that had been identified in previous work as being likely factors in the decline of *R. boylii*. For example, work by Lind et al. (1996) had shown negative impacts from dams in northern California and work by Davidson et al. (2002) identified negative effects of agricultural land use and positive effects of precipitation and geographic location (i.e., more northern latitudes). The set of variables we are using collectively address the land use change, global change (UV and global warming), and toxins hypotheses. We were limited to readily available data because of the coarse scale nature of this work and thus could not address hypotheses on alien species or emerging infectious diseases. Over-exploitation is not likely to be an issue for this species, because it is not captured for food or the pet trade.

Geographic Context

Three variables, elevation, latitude, and longitude, represent the geographic context for each *Rana boylii* locality (Appendix 2.A). Elevation and latitude are included as possible explanatory variables relative to hypotheses of amphibian decline, and latitude along with longitude, provide information on possible spatial autocorrelation effects. Spatial autocorrelation is defined as the likelihood that localities near to each other will have similar status. These variables were included for their potential to indicate historic global change effects as well as to provide insights into where future effects may be most severe. Elevation was derived using ArcGIS version 8.3 (ESRI 2002) and 1:250,000-scale USGS digital elevation models (DEMs). The DEMs were defined by 60 m cells, giving a resolution of 0.36 ha. Latitude and longitude were determined by converting Albers-projected point localities to decimal degrees and outputting the coordinates.

Precipitation

Previous work evaluating decline patterns demonstrated that precipitation levels might explain current *Rana boylii* occurrence (Davidson et al. 2002). This work used one variable, an average value spanning the period 1900-1960, to represent the relationship between current status and precipitation. Because this species is highly aquatic and both natural and human-caused changes in hydrology and stream-flow regimes have been shown to have detrimental impacts (e.g. Kupferberg 1996, Lind et al. 1996), we wanted to explore precipitation in more detail, examining average values as well as measures of variability and indices of low precipitation. To that end, we used precipitation data developed using the PRISM (Parameter-elevation Regressions on Independent Slopes) model (Daly et al. 1994). This model uses real weather station data combined with three-dimensional topographic maps to predict climatic conditions at selected resolutions. We used PRISM-modeled data available from NOAA with a resolution of 4 ha (2 km grid cells), spanning 103 years (1885-1997) (Daly et al. 2002). This data set was available in ASCII format and was converted to GRID format using ArcGIS 8.3 (ESRI 2002) to facilitate spatial linking of precipitation data with *R. boylii* localities. We used ArcView 3.2 (ESRI 1999) with a publicly available custom extension (Davies 2000) to identify total annual precipitation values for each *R. boylii* locality. This resulted in a data matrix of 394 *R. boylli* localities (rows) by 103 years (columns) of precipitation values. These data were then summarized and analyzed to create variables representing average conditions and measures of variability. We used the complete 103-year data set to create two variables for each locality: an overall mean and a coefficient of variation (i.e. standard deviation expressed as a percent of the mean). We used a subset of the precipitation data from 1960 to 1997, covering the likely period when declines began, to create four other variables for each locality: overall mean and coefficient of variation, percent of years below the mean, and percent of years below the mean-1 s.d. (Appendix 2.A). This latter variable was created to represent dry conditions.

Land Use

Two types of land use variables were developed for each *Rana boylii* locality: (1) percent of each of two land use types (agricultural and urban) in 2 km radius circle surrounding the locality, and (2) percent of each land use type in a 100 km long triangle, spanning 22.5^o, and extending into the predominant wind direction (upwind). Land use data were obtained from the U.S. Geological Survey and Environmental Projection Agency web sites (U.S.Geological Survey 2004). More details on derivation of each of the resulting four variables are in Appendix 2.A. To create upwind triangles, predominate wind direction was first determined for each *R. boylii* locality using streamline wind maps in combination with wind direction data from nearby weather stations. Then an isosceles triangle was constructed with the apex located at the R. *boyli*i locality and the base oriented toward the predominant wind direction. For California localities, further details on development of both the “upwind triangle” and the 2 km radius land use variables were provided in Davidson et al. (2001, 2002). This same approach was used to derive data for Oregon, with the exception that predominant wind direction maps for July were obtained from a map in Jackson and Kimmerling (1993).

Dam Presence, Proximity, and Size

Changes to hydrologic regimes and conditions (e.g. timing of high flows, temperatures, water depths and velocities) downstream of dams have been detrimental to *Rana boylii* (Lind et al. 1996) and have been suggested as causes of their decline (Jennings and Hayes 1994). By evaluating a set of variables that represent the presence, proximity, and size of dams within watersheds inhabited by *R. boylii* we hoped to identify the relative influence that dams and the associated hydrologic changes may have on the status of *R. boylii*. Due to differing availability of data for California and Oregon our methods and level of analysis differ for each state. For California, we had access to a database describing both the location and the characteristics (height, reservoir area, etc.) of 1391 dams in the state, through the U.C. Berkeley Digital Library Project (BDL 2000). This database was created by BDL directly from a California Department of Water Resources (CDWR) document (CDWR 1993). For Oregon, only data on presence of dams within *R. boylii* watersheds was readily available. Thus our range-wide analysis included only one variable relating to dams – presence of at least one dam in the watershed upstream of each *R. boylii* locality (Appendix 2.A).

With the large data set available for California, we were able to develop several variables to address previously identified impacts of dams on *Rana boylii*. Earlier work had shown clear negative effects downstream of dams, mainly resulting from changes to stream flow regimes (volume and timing of water releases) and to the physical environment (water depths, velocities, substrates) (Lind et al. 1996). We thus created a set of variables that would represent these sorts of impacts at an extensive spatial scale. We used the following approach to develop relationships between dam localities and *Rana boylii* localities. First the latitude/longitude values in the BDL dams database were used to create a GIS data layer. A second GIS data layer of dams obtained from Bureau of Reclamation (BOR) was used to check the BDL data set. This data layer contained locations for 1379 dams but lacked details on characteristics of dams. Also, any BOR dams not found in the BDL data set (n=19) were added to the BDL data set. This resulted in a total of 1410 dams for the state of California. The next step was to delineate the specific watershed upstream of each *R. boylii* locality. Using a statewide DEM at 0.36 ha resolution (60 m cells) (U.S. Forest Service 2003), each *R. boylii* point locality was relocated to the lowest elevation point within 300 m. This was necessary because even small errors during mapping of localities resulted in some records being outside of stream courses. Such an approach helped to avoid delineating very small, hydrologically meaningless watersheds. Once the new locality point was established, watersheds were delineated upstream of each locality based on the previously mentioned DEM, using algorithms available in ArcGIS 8.3 (ESRI 2002). Then, using overlay methods in ArcGIS, the dams within each watershed were identified. In addition, we calculated a distance between each dam in the watershed and the associated downstream *Rana boylii* locality. Following this data processing, a subset of watersheds, *R. boylii* localities, and associated dams were visually checked in ArcView 3.2 to confirm the accuracy of the resulting data.

Finally, we derived several variables based on the information on characteristics of dams available in the BDL database. We categorized each dam as to whether it was "large" based on criteria provided by the International Commission on Large Dams (ICOLD 1997). We also developed two variables that represented the potential influence of dams on a watershed level. One was simply a count of dams within each *Rana boylii* watershed and the second was a ratio of total reservoir area to watershed area (Appendix 2.A). While this doesn't address the variety of operating procedures and flow regimes resulting from different types of dams (e.g. water storage vs. hydroelectric), we felt it was a useful way to characterize the relative influence of dams among watersheds.

Bioregions

To aid in our understanding of range-wide presence and absence patterns of *Rana boylii* and to assess whether regional variation was playing a role in these patterns, we categorized each *R. boylii* locality as belonging to a bioregion or ecoregion using overlay techniques in ArcView 3.2. California bioregion definitions were from Welsh (1994) with an associated GIS coverage from the California Department of Fish and Game (M. Parisi, pers. comm.). Oregon ecoregion definitions and a GIS data layer were developed by the Oregon Natural Heritage Program and Oregon Department of Fish and Wildlife (Oregon Geospatial Enterprise Office 2004, Thorson et al. 2004) and originally based on Franklin and Dyrness (1988). Oregon ecoregions were visually assessed to determine their geographic continuity with California bioregions and descriptions of each were interpreted in order to arrive at a set of 12 bioregions appropriate for both states. These were further collapsed for analyses into 6 categories based on geographic proximity and similarity (Table 2.1).

Statistical Analyses

Analysis of Current Status

We examined current status of a locality (present or absent) using both univariate (two sample t-tests, correlations, chi-square contingency tables) and multivariate (logistic regression) approaches. For t-tests, we assumed unequal variances between the sets of present and absent *R. boylii* localities and did separate tests for each environmental variable – the decline factors. We also derived a correlation matrix to better understand the relationships between the status of *R. boylii* and the decline factors. To examine differences in frequencies of presence and absence among bioregions, we used chi-square contingency analyses. Significance levels were set to *alpha*=0.05 for these tests.

All possible subsets logistic regression was used to model the current status (present or absent) of *R. boylii* relative to the set of environmental variables in the multivariate context. We conducted analyses with three sets/subsets of our locality points: (1) range-wide (all localities), (2) California only, and (3) within the four bioregions where sample sizes were large enough (North Coast/Klamath [NCK], Bay-Delta/Valleys [BAV], Sierra Nevada [SNV], and Central Coast/Transverse Ranges [CCT]). We did not have data on two variables (dam count and the ratio of total reservoir area to total watershed area) for Oregon localities, so these variables were only included the California analyses and in the bioregions completely contained within California (Table 2.1). For range-wide and NCK bioregion analyses we started with 14 possible variables and for California, BAV, CCT, and SNV bioregion analyses we started with 16 possible variables (Appendix 2.A).

Because some of the precipitation variables were derived from subsets of the same data set (e.g. the variables including 1960-1997 data only were a subset of the complete 103 year data set), we conducted correlation analyses among them and eliminated one of each pair of highly correlated ($r>0.80$) variables; we kept the variable that had the highest correlation with the dependent variable (current status). We also examined correlations among the other independent variables and found that both latitude and longitude were highly correlated with some of the precipitation variables. Finally, we built a linear regression model with all of our potential explanatory variables because the statistical package we were using provides better multicollinearity diagnostics for linear regression than for logistic regression. This model was used only for diagnostics and confirmed the correlation results showing that latitude, longitude, and coefficient of variation of precipitation were moderately collinear. However, we did not eliminate any of these variables, because latitude and longitude were required for dealing with spatial autocorrelation concerns.

We derived a set of “best” models based on the Akaike Information Criterion (AIC). This information-theoretic criterion provides a balance between model fit (based on likelihood theory) and precision (enforced by a penalty for the number of parameters) such that best models are those with the lowest AIC value (Burnham and Anderson 2002). For each set/subset of locality points we evaluated all models within 2 points of the lowest AIC value. Models within 1 point of the lowest AIC value are considered to be equally valid combinations of explanatory variables. For the range-wide and for California only sets, we present all models as well as information on the best single variable (after latitude and/or longitude). To simplify the results for the bioregional subsets, we present only the best single variable and the best model (lowest AIC) for which a maximum likelihood estimate could be made; i.e. for CCT and SNV convergence criteria were not met for some models due to small sample sizes. Finally, we evaluated the predicted classification success of each model using a cut point value of 0.5 which assumes equal consequences for omission and commission errors.

One concern with data derived from museum or other historic records is that the original data collection may have been done in a non-random way, potentially resulting in geographically close and/or clumped data points. Even with random sampling, it is not uncommon for localities that are close to each other to be more similar than those that are farther apart. This phenomenon of spatial autocorrelation is not often dealt with explicitly in distributional studies (Knapp et al. 2003). We addressed these potential concerns in two ways. First, as described above, our set of historic California localities were randomly selected from each county in the historic geographic range of *R. boylii*. This random approach to locality selection was meant to spread the data points geographically. For Oregon, nearly all historic localities were sampled and these occurred throughout the range of the species in Oregon (Fig. 2.1). Secondly, we assessed the influence of latitude and longitude in our initial logistic regression modeling by examining variable sets with and without these two variables. In all cases, we found that models including latitude and longitude had substantially lower AIC values. Thus, we included them in all modeling from that point forward. With spatial autocorrelation accounted for, we were able to examine the relative influence of the other environmental variables.

Detailed Dam Effects Analyses

Dams and their accompanying altered flow regimes have been hypothesized to be one of the major influences on *R. boylii* reproductive success and survival (Kupferberg 1996, Lind et al. 1996). To explore these potential influences in greater detail for California, we used the dam status (DAMSCR) and dam count (DAMCOUNT) variables to subset our dam data and represent several different scenarios (e.g. presence and number of dams over a particular size or distance or distance from an *R. boylii* locality) and included these variables in our logistic regression modeling along with all the other variables (Appendix 2.A). Our approach was to find the dam inclusion criteria that resulted in the lowest AIC values in logistic regression modeling. We examined 4 scenarios - presence and number of: (1) large dams (definition A – higher than 15 m), (2) large dams (definition B - higher than 15 m or higher than 10 m and with more than 500 m crest length, or more than 1 million m^3 storage capacity [ICOLD 1997]), (3) large (definition B) and (<10 km) dams, and (4) close (<5 km) dams of any size. The second scenario resulted in the lowest AIC values and we present these results along with the results for all dams, in the California analysis results below. Since we also suspected that dam effects might be exacerbated in areas or years with low precipitation, we developed two interaction variables for the range-wide analyses: (1) a dam by precipitation level variable using the dam score variable and the mean annual precipitation from 1960 to 1997, and (2) a dam by precipitation interaction, using the dam score variable and the percent of very low precipitation years variable (Appendix 2.A).

For California, we also examined the influence of the size and proximity of dams in greater detail. We created an additional dam size variable ("very large dams" were those greater than 30 m high) and conducted univariate analyses on this and the previously described dam related variables. Finally, we created a subset of the data that included only those *R. boylii* localities whose watersheds contained a dam of a particular size, and used t-tests to compare distances and heights of dams between the currently present and absent localities for frogs. For these exploratory analyses we set *alpha* at 0.10.

All variables were examined for univariate normality and intercorrelations (among explanatory variables) prior to univariate analyses (t-tests, correlations, chi-square contingency tests) and logistic regression modeling status (currently present versus absent). Non-normal variables were transformed as needed to reduce skewness and kurtosis and to linearize them based on visual inspection of normal probability plots. Missing values for some of the dam-related variables resulted in reduced sample sizes (Table 2.2). All data analyses in this study were conducted using SAS/STAT software, Version 8 of the SAS System for Windows (SAS Institute 1999).

RESULTS

The overall status of *Rana boylii* confirms that it is a species in decline. Our study along with other recent surveys (G. Fellers, pers. comm.) show that large areas of the species' historic range are no longer occupied – especially areas in the southern and extreme northern portions of the range. Two hundred and one of our 394 historic localities (over 50%) no longer have extant populations (Fig. 2.1). Our results provide the first quantitative evidence for the causes of those declines.

Univariate Analyses

For the univariate comparisons of geographic variables we found that the latitudes and longitudes of *Rana boylii* localities showed that present localities were farther north and west than absent localities (Table 2.2). However, examining a graph of proportion of extant localities relative to degrees of latitude indicates a non-linear relationship such that the areas furthest north in the range have a relatively lower likelihood of *R. boylii* presence (Fig. 2.2.). All of the precipitation variables were significantly different between *R. boylii* present and absent localities. Present localities had higher mean precipitation values with lower inter-annual variability and lower percentages of years with low precipitation conditions than did absent localities (Fig. 2.3). Three of the four land use variables demonstrated a negative influence on presence of *R. boylii*. Localities where frogs were present had significantly less agriculture and urban development nearby and were downwind of areas with less urban development than absent localities. Upwind agriculture while negatively associated with *R. boylii* status, was not statistically significant (Table 2.2).

Range-wide analyses did not demonstrate a relationship between dam presence and *R. boylii* status, even though almost a quarter of all *R. boylii* localities (n=98) had at least one dam in the upstream watershed and there are over 800 dams in these 98 watersheds. For all California localities, trends indicated that both the presence and number of dams in the upstream watershed had a negative influence on *R. boylii* status, but only very large dams showed a marginally statistically significant effect (Table 2.3). For localities with at least one dam, the apparent trend was for the closest dam to be farther upstream at localities where *R. boylii* was present than for those where it was absent. Large dams had a stronger effect than small dams, especially when comparing the maximum height of dams for present and absent localities. The height of dams at localities where *R. boylii* was present were significantly lower than dams where it was absent (Table 2.3).

Multivariate Models

Range-wide and California-only logistic regression models consisted of similar sets of variables. For the range-wide set, the best single variable model (after latitude or longitude) was mean annual precipitation for the period 1960-1997; explaining 15% of the variation in *R. boylii* status. Sixteen range-wide multivariate models met the criteria of being within 2 points of the lowest AIC value. Of these, four were within 1 point of the lowest AIC value (Appendix 2.B-1) and are considered to be equally likely models. These four models contain from 8-11 variables and explain 40-41 % of the variation in *R. boylii* status (Appendix 2.B-1). All four models contained latitude and longitude indicating the existence of spatial autocorrelation in the data (Table 2.4). In ecological terms, this means that localities where *R. boylii* are extant are more likely to be near other extant localities than to absent localities. All models also contained elevation; the positive sign of its coefficient was consistent with the univariate correlation (Tables 2.2 and 2.4) indicating a higher likelihood of *R. boylii* presence at higher elevation localities.

Land use variables were prominent in the four models, with agricultural land in the upwind triangle (UPAGTR_P) and urban land in the surrounding 2 kilometers (UR2K_L) occurring in all four and urban land in the upwind triangle (UPURTR_L) and agricultural land in the surrounding 2 km (AG2K_L) occurring in three models and one model respectively (Table 2.4, Appendix 2.B-1). The signs on the coefficients for the land use variables were consistent with those seen in the bivariate correlations (negative) for all but upwind agricultural variable which consistently showed the opposite sign (positive) in the multivariate models.

As with the univariate results, the precipitation variables emerged as significant variables. Mean annual precipitation (MNAN_P60) occurred in all four models (Table 2.4) and was positively associated with *R. boylii* presence. The percent of years with below average precipitation (PMMPC_BA) and the percent of dry years (PMMPB_DR) also occur in all four models, but the below average precipitation variable has a positive sign in the multivariate analyses and a negative sign in the bivariate correlations (Appendix 2.B-1, Table 2.2). Finally, the dam status (DAMSCR) variable did not appear in the top four models but one of the models contained two interaction terms (dam status by precipitation and dam status by dry years – Table 2.4, Appendix 2.B-1, model 2). The occurrence of these interaction terms without the main effect of dam status (DAMSCR) in this model indicates that there is an effect of precipitation or percent of dry years only when dams are present. Thus with dams present, dry years have a more negative effect and precipitation has more positive effect.

Logistic regression models for California localities that included large dams (see definition in Appendix 2.B-2 footnote) only in the number of dams (DAMCOUNT), and dam status (DAMSCR) variables achieved substantially lower AIC values. Results for both sets (all dams and large dams only) are provided in Table 2.4 and Appendix 2.B-2. The best single variable, after latitude and longitude, was the coefficient of variation of precipitation from 1960-1997 (CVAN_P60). On its own, this variable explained 25% of the variation in *R. boylii* status. Of the seven multivariate models with AIC values within 2 points of the lowest AIC value, four fell within one point and were considered to be equally valid models for this dataset. These models contained from 12-15 variables and explained 53% of the variation in *R. boylii* status.

As with the range-wide models, the California-only models all contained latitude and longitude indicating spatial autocorrelation effects (Table 2.4). Elevation and land use variables also occurred in these models and with exception of upwind agricultural land, the signs on the coefficients were consistent with the univariate results such that there was a higher probability of *R. boylii* presence at higher elevations, and in areas with less immediate (UR2K_L) and nearby (UPURBTR_L) urban development and less nearby agricultural land (AG2K_L) (Appendix 2.B-2). Upwind agricultural land use again demonstrated inconsistencies in the signs between multivariate and univariate analyses.

Mean annual precipitation (MNAN_P60) and percent of dry years (PMMPC_DR) occurred in all four models and had coefficient signs that were consistent with univariate analyses such that localities with higher mean annual precipitation values and fewer dry years were more likely to have *R. boylii* present. In addition the coefficient of variation of precipitation (CVAN_P60) entered all four models and played the same role in the multivariate models as it did as the best single variable (Table 2.4, Appendix 2.B-2). The dam related variables also figured prominently in the four top models. The ratio of reservoir area to watershed area (RES_WS_P) occurred in all four models and was negatively related to *R. boylii* presence. The dam status and number of dam variables occurred in one and two of the four models, respectively and the dam and precipitation/dry years interaction variables occurred in all four models (Table 2.4). Together these variables provide evidence that the presence and number of large dams negatively influenced *R. boylii* presence. The occurrence of the interaction variables in the models demonstrate that dam effects on *R. boylii* can be exacerbated in areas where precipitation is low.

Bioregional Differences

The bioregional context was examined both by looking at simple frequencies and by examining multivariate models for some bioregions. In frequency analyses (chi-square contingency) we found a significant association between the current status of *Rana boylii* and particular bioregions (chi-sq=39.60, p=<0.001). Examining the six simplified bioregions revealed that two contributed substantially to the overall chi-square value: central coast/transverse ranges (CCT) and the north coast/Klamath (NCK). In the CCT, *R. boylii* was present less often than expected and absent more often than expected, and in the NCK, they were present more often than expected and absent less often than expected (Fig. 2.4).

Logistic regression modeling within each of the four bioregions with large enough sample sizes indicated that the factors most strongly affecting *R. boylii* status differ by geographic location. The elevation variable occurred in all but the Sierra Nevada bioregion model and indicated that higher elevation areas are more likely to have *R. boylii* present (Appendix 2.B-3). In the North Coast/Klamath bioregion (NCK), latitude and levels of urbanization had the strongest negative influence on *R. boylii* presence; both the best single variable and two variables in the multivariate model represented an urbanization effect. Interestingly here, the negative sign on the latitude coefficient (Appendix 2.B-3) is consistent with the bivariate correlation (not shown).

In the Central Coast/Transverse Range bioregion (CCT), inter-annual variability in precipitation (CVAN_P60) was the best single variable, explaining over 30% of the variation in *R. boylii* status; higher variability meant lower likelihood of *R. boylii* being present. This variable acted similarly in the multivariate model for the CCT which also included a negative effect of urbanization (UPURTR_L) and explained over 50% of the variation in *R. boylii* status (Appendix 2.B-3). The best single variable in the Bay Area / Valleys bioregion (BAV), was mean annual precipitation. This variable was not present in the best multivariate model. That model contained three land use variables and the number of dams. Two of the land use variables (AG2K_L, AND UR2K_L) showed negative effects on *R. boylii* status as did number of dams (DAMCOUNT). Upwind agriculture exhibited a positive relationship with *R. boylii* status (Appendix 2.B-3) which was consistent with the univariate correlation (not shown).

In the Sierra Nevada bioregion (SNV), the best single variable represented inter-annual variation in precipitation (CVAN_P60), which had a negative influence on

R. boylii presence. This variable was also present with the same effect in the multivariate model along with a negative effect of urbanization (UR2K_L). The other two variables in the model represented dam effects (RES_WS_P and DAMCOUNT) but the signs of their coefficients were inconsistent with each other and with bivariate correlations (not shown). Together these variables explained over 60% of the variation in *R. boylii* status (Appendix 2.B-3).

DISCUSSION

Emerging Factors in the Decline of *Rana boylii*

We found evidence for all of the three proposed amphibian decline hypotheses that we studied, in relation to the spatial distribution of *Rana boylii*. Strong evidence was found for effects of land used change and for global climate change, with a somewhat weaker signal from pesticides and other toxins (Tables 2.2 and 2.3, Fig. 2.3). We also discovered evidence of interactions and/or synergisms among these factors and found that the prominent factors differed among bioregions (Appendix 2.B-3). The key explanatory variables in our univariate and multivariate analyses were consistent with findings of previous quantitative (Davidson et al. 2002) and descriptive (Jennings and Hayes 1994, Jennings 1996) work on *Rana boylii*, with few exceptions. Our analysis provides further evidence for the negative effects of dams, which appear to be exacerbated by low precipitation.

Habitat alteration, represented in our study by the degree of agricultural and urban land use and the presence, number, and proximity of dams was also found to be a key factor in a California-wide study of *Rana boylii* (Davidson et al. 2002) and has been suggested by previous status reviews (Jennings and Hayes 1994, Jennings 1996). Davidson, et al. (2002) also found strong evidence for a potential global change effect. In that study, *R. boylii* was found less often at localities in southern latitudes and/or those with lower precipitation, which is consistent with the expectation of warming climate scenarios. In our study, latitude and precipitation were also strongly positively related to *R. boylii* status and elevation was moderately so. These three variables together indicate a climate change impact, though not a UV-B effect. In addition, our finding of a negative relationship with variability in precipitation and with number of years of below average or dry conditions (Table 2.2, Fig. 2.3) further implicates climate change as a likely factor. One key difference between our results and Davidson et al.'s (2002) is our equivocal findings with regard to upwind agricultural effects. We did find upwind effects of urban areas, which were not included in Davidson et al.'s (2002) study. We believe that the combination of systematic-random selection of frog localities (in California) with more detailed mapping of each point and the somewhat different set of variables evaluated have contributed to these different results.

Evidence that dams on their own have played a major role in the decline of *Rana boylii* was weak. Hydrologic and related geomorphic changes in riverine environments resulting from dams have been shown to have strong negative effects on *Rana boylii*, (Lind et al. 1996) but translating those sorts of effects into appropriate variables for the spatial scale we assessed was challenging. Dam operation (e.g. amount, daily and seasonal timing, and duration of water releases), which is a key factor in downstream survival of *R. boylii,* is highly variable and could not be represented directly in our analyses; we used dam presence, number, proximity, and size, and reservoir size as surrogates. Nevertheless, the negative effect of number of very large dams and significantly larger maximum height of dams in watersheds without *R. boylii* (Table 2.3) coupled with evidence of and interaction between precipitation and dams (California results, Appendix 2.B-2), does identify a potentially extensive effects of dams. More work is needed to evaluate the details of historic and current operations of at least some of the 800+ dams within the *R. boylii* watersheds identified in this study. We also recognize the weakness of using only upstream dams in our analyses and encourage future work that would assess the fragmentation and alien species effects of both upstream and downstream dams and reservoirs.

California and Bioregional Trends

California decline factors were similar to range-wide results except that the variables representing effect of dams were more prominent in California results (Table 2.4). Other than the presence of precipitation variables in most models, there was little consistency among the bioregions in terms of the important variables in our multivariate analyses (Appendix 2.B-3). Variables representing levels of urbanization emerged for the North Coast/Klamath (NCK) and Central Coast/Transverse Ranges (CCT) (Appendix 2.B-3). Urban/suburban development and the associated habitat alteration and effects of riverine ecosystems are substantially greater throughout the CCT and in the southern portions of the NCK where *R. boylii* no longer occurs. Also of interest is the (real) negative effect of latitude in the NCK bioregion, which is apparently due to the fact that many localities in the extreme northern regions of *R. boyli*i's range (in Oregon) are not currently occupied. In the Bay Area/Valleys bioregion, the urban and agricultural land use also showed negative effects. The strong negative relationship with numbers of dams for this bioregion is likely due to the fact that the most dams/per watershed occur in this bioregion. In the Sierra Nevada bioregion (SNV), the longitudinal relationship is really representative a latitudinal effect because of the orientation of the mountains; i.e. the more westerly localities are also the more northerly ones. Finally the conflicting relationship between number of dams and reservoir area is likely due to their relatively high intercorrelation (r=0.695) and potential collinearity in this multivariate analysis. This may be due to the fact that there are more dams in the northern portion of the SNV bioregion where there area also more extant populations of *R. boylii*.

Analytical Challenges

There are several analytical challenges for data such as ours – i.e., species presence/absence over a large spatial extent. Ranking statistical models using AIC is one way to examine the influence of several variables individually and together. However, as we found, problems of multicolinearity can come into play. We attempted to mitigate this issue by examining bivariate correlations and eliminating some variables and by using multivariate linear regression diagnostics to identify particularly problematic variables. However, since we were interested in particular hypotheses and were incorporating spatial autocorrelation influences we were not able to eliminate all highly intercorrelated variables. Thus signs (positive/negative) sometimes differed between univariate and multivariate analyses. This primarily occurred for variables that were only marginally (or not significantly) correlated with presence/absence of *R. boylii* (e.g. upwind agriculture - UPAGTR_P). One exception was latitude, which showed strong correlations but had opposite signs in multivariate (negative) and univariate (positive) analyses. This was due to the fact that latitude and longitude, two variables that were included to account for spatial autocorrelation, were highly correlated to each other simply as a result of the geographic distribution of the species (i.e. the more northerly localities are also the more coastal or westerly localities). This complicated our interpretation of latitude and longitude in the multivariate models. The univariate results which indicated higher occupancy rate of *R. boylii* at more northerly localities were strong, though graphical examination indicated that the trend was probably non-linear (Table 2.2, Fig. 2.2). This relationship will be examined in more detail in future analyses of spatial variables and range contraction hypotheses.

Recent papers have raised concerns over the reliability of single resampling studies of historic localities. For example, Skelly et al. (2003) found that studies with 1 or only a few revisits to a localities were more likely to find evidence of a decline of the focal species. We appreciate the message of their analysis but also feel confident in our results. Our goal was primarily to determine the likely factors contributing to the decline of *R. boylii* rather than describing the degree of its decline. We were essentially treating the localities as fixed effects (Shaffer et al. 1998). The scale of our work differs from most other multi-year studies in that our study covers the entire geographic range of the focal species while other studies of decline have been done more intensively at fewer, geographically proximate localities. In addition, we used systematic random sampling to effectively spread out our study localities and eliminate duplicate or highly clumped localities. Finally, the major geographic patterns of decline of *R. boylii* observed in our work (e.g., absence throughout much of the central California coast and southern Sierra Nevada) have been confirmed by recent surveys and local expert contacts (Jennings and Hayes 1994, G. Fellers, pers. comm.).

Spatial Pattern of Decline and Predictions for the Future

The significant geographic (latitutude, longitude, elevation) and precipitation variables provide insights into *Rana boylii* decline in several ways. First, latitude and longitude together are indicative of spatial autocorrelation in our data set. These sorts of relationships are at least partly due to the large number of localities we assessed and the clumped nature of localities derived from museum collections. However, at an extensive geographic scale, this autocorrelation is indicative of a strong spatial pattern in the decline of *R. boylii* while at more local geographic scales it likely represents an ecological phenomenon that is well-documented in amphibians – metapopulation structure. For example, Knapp et al. (2003) recognized the value of explicitly addressing spatial autocorrelation in his analyses of mountain yellow-legged frogs (*Rana muscosa*) patch occupancy. They proposed that the high degree of spatial autocorrelation they saw was indicative of the fact that the frogs were occurring in metapopulation structure; i.e., nearby source populations had a strong influence on individual pond population dynamics.

Secondly, as described above, the latitude, elevation, and precipitation variables together are potentially representative of the susceptibility of this species to global climate change. Recent data shows that the temperatures have increased, especially over the last 30 years, and future climate modeling shows continued warming (Washington et al. 2000, Houghton et al. 2001). In the western U.S., recent research has shown that the timing of spring snowmelt run-off events has shifted to earlier in the spring (Cayan et al. 2001). Models for future precipitation and streamflow show continued shifts earlier though overall amounts of precipitation are expected to remain relatively constant in the near term (Stewart et al. 2004, Dettinger et al. 2004). Shifts in the spring phenologies of taxa including invertebrates, amphibians, birds, and trees over the past 50 years are well documented (Root et al. 2003) but the implications of these shifts for species interactions and long-term survival are unknown.

Temperature, precipitation, and streamflow changes could have a multitude of effects on *R. boylii* since these are key interacting variables that influence the timing and success of oviposition (Kupferberg 1996, Lind et al. 1996, Lind and Welsh 1997). Understanding the relationships among temperatures, precipitation, and streamflows and connecting it to the pattern of decline seen here for *R. boylii* should aid in the prediction of where future declines are likely to occur. Specifically, we propose that *R. boylii* is likely to experience declines to a greater degree in areas where precipitation is low and/or variable. Future climate models for California indicate that temperatures are likely to increase to a greater degree in central and northern regions than in the southern regions of the state. However, precipitation will decrease the most in the southerly areas and snow accumulation will decrease the most in more central and northerly areas of the state (Snyder et al. *In Press*). Given these complex relationships, it appears that populations in the central and southerly areas of *R. boylii's* geographic range are likely to experience continuing declines. In more northerly areas, reduced snow accumulation in and its affect on timing of run-off may also impact the currently more stable *R. boylii* populations. In addition, dams may exacerbate these precipitation effects, especially if the ecological effects of temperature changes and concomitant snowmelt timing changes are not considered in dam operations.

Conclusions and Recommendations

Analyses of the type we conducted cannot reveal details on the actual mechanisms of the decline factors, but some discussion of mechanisms is warranted, if only to generate testable hypotheses and encourage future studies. Experimental and intensive local scale studies would provide an important compliment to our range-wide analyses. For habitat alteration effects, potential mechanisms are fairly straightforward. Physical and biotic systems that are changed to a significant degree do not provide the conditions of cover and microclimate necessary for survival and/or reproduction and eventually populations dwindle and become extinct (Bishop et al. 2003, Boone et al. 2003). In our study, negative associations between *R. boylii* status and levels of surrounding agricultural and urban land use indicate direct effects of habitat alteration. Effects of dams are more complex because both direct (off-seasonal water releases that wash out eggs/larvae) and indirect (physical habitat changes resulting from modification of the natural hydrology) effects are possible (Lind et al. 1996). Temperature changes downstream of dams may have sublethal effects on *R. boylii* or may affect their prey/food base. In addition, because the reservoirs associated with dams harbor (and are purposefully stocked with) alien fish predators that can move both up and downstream, the potential interactions of these factors should be considered in future studies.

Because of their ectothermic nature and often narrow temperature and moisture requirements, the potential impact of global climate change on amphibians is clear (Boone et. al 2003, Carey and Alexander 2003). However, understanding the actual mechanisms will require more research. For *R. boylii*, further investigation of precipitation effects is warranted; both through assessment of how predicted changes in precipitation patterns will play out in different areas of this species' range, and through more detailed studies of impacts at individual localities. For instance, localities with low levels of precipitation likely have smaller and/or more variable population sizes and are thus more susceptible to other stressors. It is also possible that precipitation levels could be so low as to directly affect reproduction, survival, dispersal, and/or metapopulation processes in and among stream drainages and watersheds. In a recent global assessment of amphibian declines, Neotropical montane stream-associated species were found to be the most at risk group of amphibians (Stuart et al. 2004), further high-lighting the need to understand potential mechanisms in lotic environments.

While questions and research needs remain, there are obvious steps that could be taken toward the conservation of *R. boylii*. All of the factors we found to be related to *R. boylii* decline present challenges for land managers. Extensive land use changes and dams (especially large dams) are not easily restored. Thus efforts should be made to identify and provide protection of areas where these impacts are already minimal or can be mitigated (e.g. with changes in dam operations for example). Climate change effects on their own will obviously be more difficult to overcome in future management or conservation plans for *R. boylii*. But, we do have some predictive ability that should help with selection of protected areas across suitable latitudinal and elevational gradients. Potential interactions between dams and precipitation may be mitigated via dam operations in areas where there is flexibility in the timing and amount of water releases. Finally, even though more detailed work on the mechanisms of these decline factors is sorely needed, land managers and planners who consider these factors in combination with basic ecological knowledge will have more options for *R. boylii* conservation in the future.

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Analysis and Visualization (IDAV; formerly known as Center for Image Processing and Integrated Computing [CIPIC]) provided computer time and trouble-shooting for analyses requiring a campus computer bank. U.C. Berkeley Digital Library provided an electronic version of California Department of Water Resources Bulletin 17 which included information on dams in California. The U.S. Bureau of Reclamation also provided a draft GIS coverage of dams for California. A GIS coverage of bioregions of California (as proposed by Welsh 1994) was provided by the California Department of Fish and Game and an ecoregion coverage of Oregon was obtained from

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Table 2.1. Bioregions of California and Ecoregions of Oregon in which *Rana boylii* occurs. California bioregions are from Welsh (1994) and Oregon ecoregions are from Thorson et al. (2004).

California	**Oregon**	**Simplified Categories (CODE)**	**Description**	**Num. of Pres. Locs.**	**Num. of Abs. Locs.**
North Coast	Coast Range	North Coast / Klamath (NCK)	Coastal near coastal mountain ranges extending from the San Fransico Bay Area in California north to central coastal Oregon	94	49
Klamath	Klamath				
Cascade	Western and Eastern Cascades	Cascade (CAS)	Volcanic origin mountains in north central California and central interior Oregon.	6	9
Sacramento Valley	NA	Bay/Delta and Valleys (BAV)	The great central valley of California and the areas surrounding its outlet to the Pacific ocean.	60	73
San Joaquin Valley	NA				
San Francisco Bay/Delta	NA				
Northern Sierra Nevada	NA	Sierra Nevada (SNV)	Synonymous with the mountain range of the same name in eastern California.	23	24
Southern Sierra Nevada	NA				
Central Coast	NA	Central Coast and Transverse Ranges (CCT)	Coastal mountains and east-west running mountains in central California.	10	42
Transverse Ranges	NA				
NA	Willamette Valley	Willamette Valley (WIV)	Lowland areas surrounding the river of the same name.	0	4

Table 2.2. Characteristics of explanatory variables relative to *Rana boylii* presence or absence at all localities (n=193 present; 201 absent), with tests for differences between means. Variables with *'s were natural log-transformed prior to t-tests and correlations, though untransformed means are shown in the table.

Variable (units)	***R. boylii* present**	***R. boylii* absent**		
	mean (s.e.)	mean (s.e.)	t-test - *t*, *P*	corr - *r*, *P*
Geographic Context				
Elevation (m)	442.93 (25.04)	440.52 (27.10)	ns	0.003, ns
Latitude (dd)[1]	39.80 (0.14)	38.43 (0.21)	5.46, <0.0001	0.264, <0.0001
Longitude (dd)	122.55 (0.08)	121.24 (0.12)	8.94, <0.0001	0.409, <0.0001
Precipitation				
1895-1997 mean annual precipitation (mm)	1200.89 (40.43)	791.32 (30.58)	8.08 <0.0001	0.379 <0.0001
1895-1997 annual precipitation CV	28.83 (0.38)	31.99 (0.58)	4.53 <0.0001	-0.221 <0.0001
1960-1997 mean annual precipitation (mm)	1247.85 (41.47)	818.55 (31.36)	8.26 <0.0001	0.387 <0.0001
1960-1997 annual precipitation CV	30.23 (0.41)	33.66 (0.63)	4.53 <0.0001	-0.221 <0.0001
1960-1997 years below mean (%)	54.38 (0.29)	56.22 (0.36)	4.00 <0.0001	-0.197 <0.0001
1960-1997 dry years (%)	13.45 (0.21)	14.24 (0.23)	2.53 0.0118	-0.126 0.012
Land Use				
Agriculture in upwind triangle (%)*	17.94 (1.91)	18.88 (1.91)	ns	-0.026 ns
Agriculture in a 2km radius (%)*	2.16 (0.51)	7.57 (1.27)	3.53 0.0005	-0.174 0.0005
Urban areas in upwind triangle (%)*	4.43 (0.56)	6.72 (0.70)	3.31 0.0010	-0.165 0.001
Urban areas in a 2km radius (%)*	1.72 (0.36)	8.23 (1.16)	5.33 <0.0001	-0.258 <0.0001
Dam Presence, Proximity and Size	freq., percent	freq., percent	Chi-sq, *P*	
Dam status - frequency and percent of localities with at least one dam in upstream watershed (any size and distance)	47, 24.35 %	51, 25.37 %	ns	----

[1] dd=decimal degrees

Table 2.3. Characteristics of dam variables relative to *Rana boylii* presence or absence at California localities, with tests for differences between means. For all California localities, n=157 present localities; 153 absent localities. For localities with dams only, sample sizes are provided in the table.

Variable (units)	***R. boylii* present**	***R. boylii* absent**		
All Localities	freq., percent	freq., percent	Chi-sq, *P*	
Dam status - frequency and percent of localities with at least one large[1] dam in upstream watershed	35, 22.29 %	37, 24.18 %	ns	-----
	mean (s.e.)	mean (s.e.)	t-test - *t*, *P*	corr - *r*, *P*
Number of all dams in upstream watershed	1.83 (0.44)	3.44 (1.38)	ns	-0.064, ns
Number of large[1] dams in upstream watershed	0.85 (0.19)	1.36 (0.38)	ns	-0.068, ns
Number of very large[2] dams in the upstream watershed	0.24 (0.06)	0.50 (0.14)	1.72, 0.09	-0.098, ns
Reservoir / Watershed Area Ratio (%) (all reservoirs)	0.34 (0.09)	0.32 (0.08)	ns	0.010, ns
Localities With Upstream Dams Only	n, mean (s.e.)	n, mean (s.e.)	t-test - *t*, *P*	corr - *r*, *P*
Distance to closest dam (any size) (km)	46, 11.23 (1.58)	39, 9.11 (1.67)	ns	0.101, ns
Distance to closest large[1] dam (km)	35, 12.65 (2.12)	37, 9.83 (1.78)	ns	0.121, ns
Distance to closest very large[2] dam (km)	19, 19.64 (5.52)	26, 15.27 (3.20)	ns	0.110, ns
Max height of any dam in upstream watershed (m)	46, 40.78 (5.51)	39, 62.54 (7.35)	2.37, 0.021	-0.256, 0.018

[1] large = dams higher than 15 m or higher than 10 m and with more than 500 m crest length or more than 1 million m^3 storage capacity (ICOLD 1997).

[2] very large = dams higher than 30 m.

Table 2.4. Summary of results from *Rana boylii* range-wide logistic regression analysis. An "_L" at the end of a variable name means natural log-transformed prior to analysis. For each set/subset, the number of models within one point of the lowest AIC value and the number of times each variable entered those models is presented. See Appendices 2.B-1 and 2.B-2 for the details of each model.

	Data Set/Subset		
	All Localities	**Calif. – All Dams**	**Calif. - Large Dams Only**
Number of Low AIC Models	4	5	4
Variables			
Elevation	4	5	4
Latitude	4	5	4
Longitude	4	5	4
1960-1997 mean annual precipitation	4	5	4
1960-1997 annual precipitation CV	0	5	4
1960-1997 years below mean	4	1	0
1960-1997 dry years	4	5	4
Agriculture in upwind triangle	4	5	4
Agriculture in a 2km radius	1	5	3
Urban areas in upwind triangle	3	5	4
Urban areas in a 2km radius	4	5	4
Dam status (present/absent)	0	3	1
Dam count	na	5	2
Reservoir / watershed ratio	na	5	4
Dam presence X precipitation interaction	1	4	4
Dam presence X dry years interaction	1	4	4

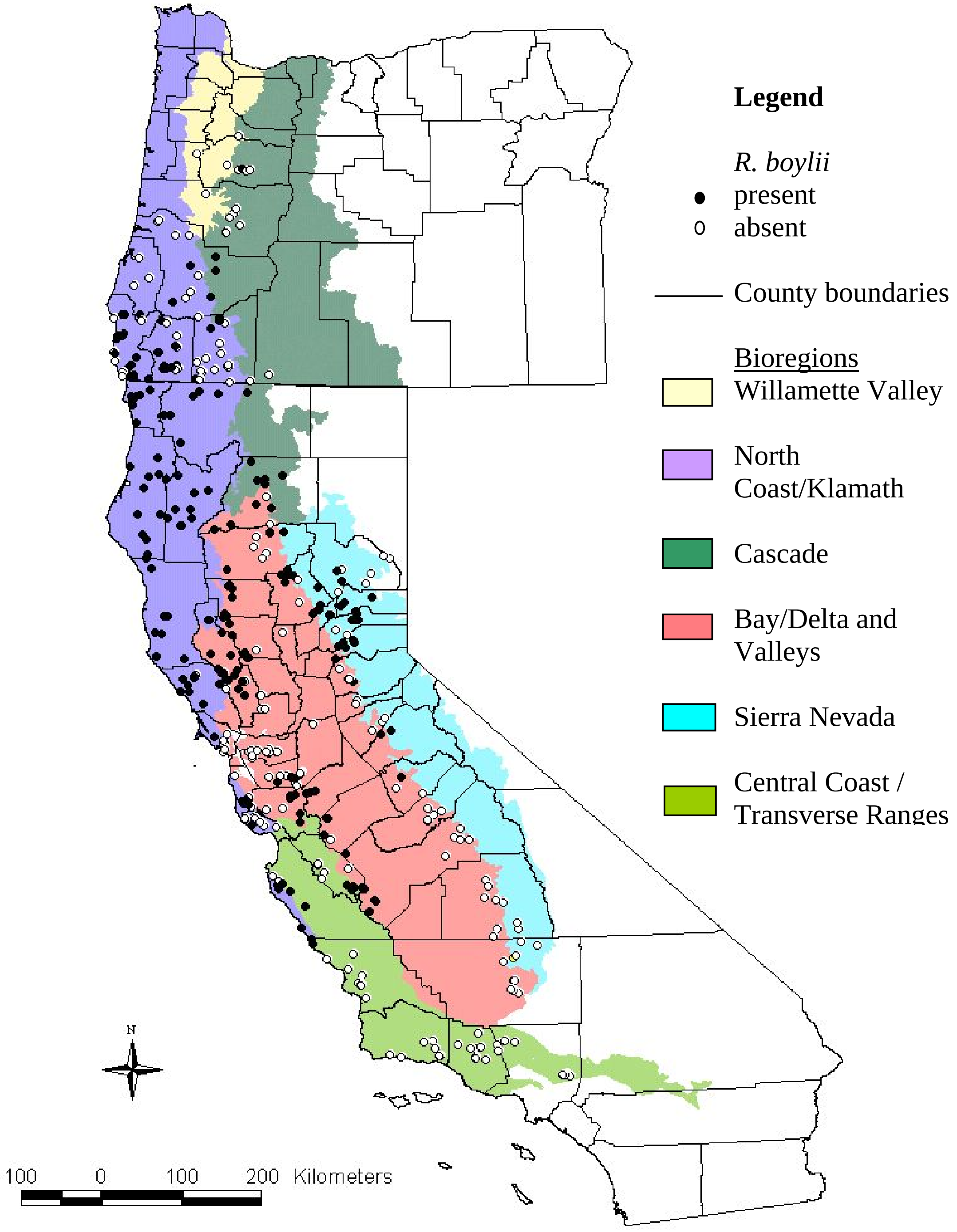

Figure 2.1. Current status (filled circles=present, open circles=absent) of *Rana boylii* throughout its geographic range (California and Oregon, USA). Recent surveys in the southeastern portion of the geographic range (southern Sierra Nevada) have resulted in the discovery of only two “new” populations (H. Eddinger, pers. comm., G. Fellers, pers. comm.). These populations were not included in the analysis because their historic status is unknown.

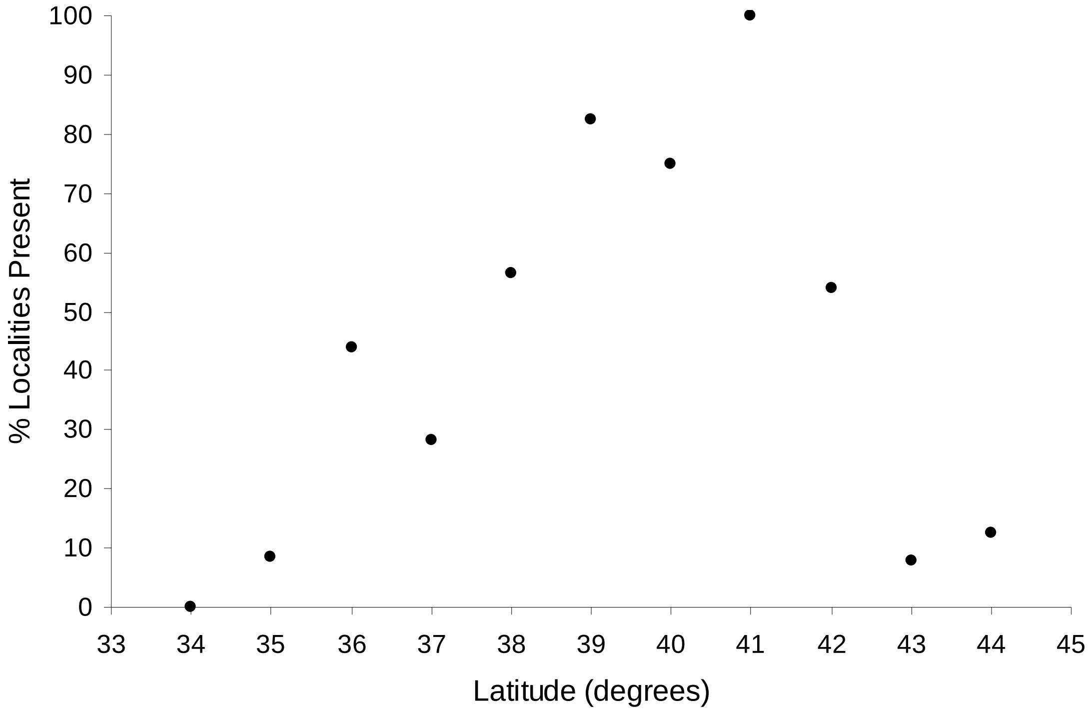

Figure 2.2. Percent of localities with *R. boylii* present for each degree of latitude across its geographic range in California and Oregon.

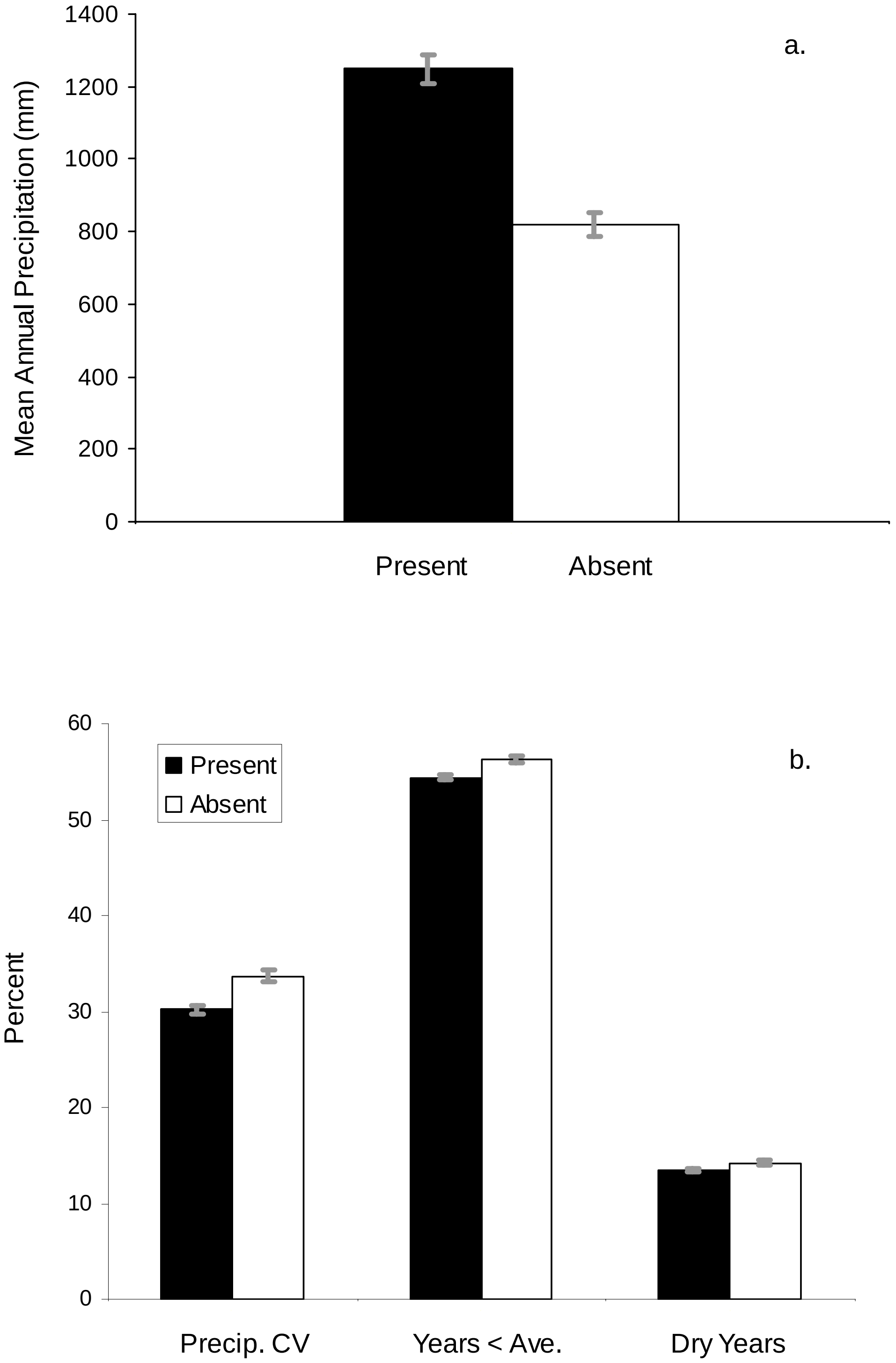

Figure 2.3. Precipitation values for 1960-1997 by *Rana boylii* locality status (present, n=193; absent n=201): (a) mean annual precipitation with standard error bars, (b) coefficient of variation of precipitation, percent of years below average, and percent of dry years (below mean – 1 s.d.); standard error bars less visible due to small values.

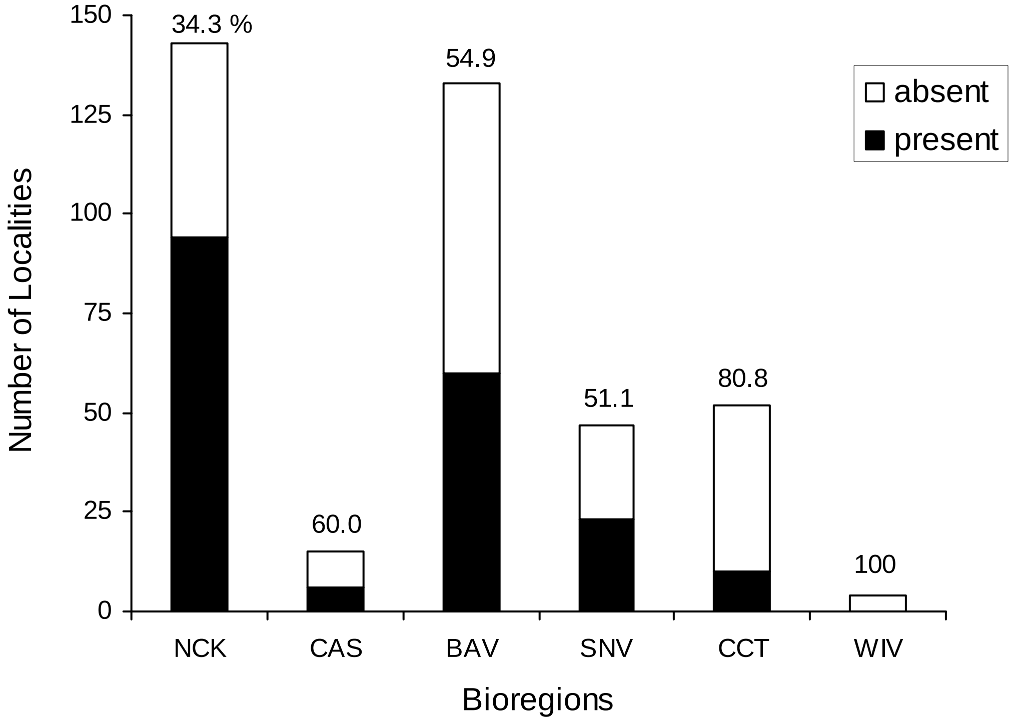

Figure 2.4. Presence and absence of *Rana boylii* at localities for each bioregion (see Table 2.1 for definitions). The number above each bar is the percent of absent localities within the bioregion.

Appendix 2.A. Variables used in analyses of decline of the foothill yellow-legged frog (*Rana boylii*). CA=California, OR=Oregon. RW=range-wide, CA=California only, BR=bioregion analyses. An* indicates that a variable was available to be chosen in all possible subsets logistic regression. A "c" indicates variables used as criteria for inclusion of observations (e.g. dams of a certain height were included or excluded in some analyses). An "s" indicates the variable was used in some of the bioregional analyses – i.e. for the bioregions completely contained within California (BAV, SNV, CCT).

			Used in Logistic Regr. Modeling		
Variable and Abbreviation	Description	Sources and Methods	RW	CA	BR
***Rana boylii* Status** STATUS	Current (1990-present) status of *R. boylii* at a locality, identified as present, absent, or unknown.	Correspondence with species experts, local biologists, and field checking.	*	*	*
Geographic Context					
Elevation (m) ELEV_M	Elevation of each *R. boylii* locality.	Derived using GIS from digital elevation models (dem) for each *R. boylii* locality.	*	*	*
Latitude (decimal degrees - dd) LAT	Latitude of each *R. boylii* locality.	Derived using GIS from dem's for each *R. boylii* locality.	*	*	*
Longitude (dd) LONG	Longitude of each *R. boylii* locality.	Derived using GIS from dem's for each *R. boylii* locality.	*	*	*
Precipitation					
1895-1997 mean annual precipitation (mm) MNAN_PMM	Mean of 103 years of precipitation for each *R. boy*lii locality.	Derived from PRISM modeled precipitation data (4km resolution) available at NOAA web site (Daly et al. 2002).			
1895-1997 precipitation coefficient of variation CVAN_PMM	Coefficient of variation of 103 years of precipitation for each *R. boylii* locality.	Derived from PRISM modeled precipitation data (4km resolution) available at NOAA web site (Daly et al. 2002).			
1960-1997 mean annual precipitation (mm) MNAN_P60	Mean of 38 years of precipitation for each *R. boy*lii locality.	Derived from PRISM modeled precipitation data (4km resolution)available at NOAA web site (Daly et al. 2002).	*	*	*
1960-1997 precipitation coefficient of variation CVAN_P60	Coefficient of variation of 38 years of precipitation for each *R. boylii* locality.	Derived from PRISM modeled precipitation data (4km resolution) available at NOAA web site (Daly et al. 2002).	*	*	*

Appendix 2.A., ***Precipitation*** *continued.*					
1960-1997 years below mean PMMPC_BA	Percent of years which fell below the mean annual precipitation value for each *R. boylii* locality.	Derived from PRISM modeled precipitation data (4km resolution)available at NOAA web site (Daly et al. 2002).	*	*	*
1960-1997 dry years PMMPC_DR	Percent of years which fell below the mean-1 s.d. for each *R. boylii* locality.	Derived from PRISM modeled precipitation data (4km resolution) available at NOAA web site (Daly et al. 2002).	*	*	*
Land Use					
Agriculture in an upwind triangle UPAGTR_P	Percent of a 100 km long triangle, spanning 33.75° extending into the predominant wind direction (upwind) that is defined as agricultural land use for each *R. boylii* locality.	From USGS and EPA land use / land cover data at 1:250,000 (U.S. Geological Survey 2004); see Davidson, et al. (2001) for details of methods.	*	*	*
Agriculture in a 2km radius AG2K_P	Percent of a 2km radius circle around each *R. boylii* locality that is defined as agricultural land use.	From USGS and EPA land use / land cover data at 1:250,000 (U.S. Geological Survey 2004); see Davidson, et al. (2001) for details of methods.	*	*	*
Urban areas in an upwind triangle UPURTR_P	Percent of a 100 km long triangle, spanning 33.75° extending into the predominant wind direction (upwind) that is defined as urban land use for each *R. boylii* locality.	From USGS and EPA land use / land cover data at 1:250,000 (U.S. Geological Survey 2004); see Davidson, et al. (2001) for details of methods.	*	*	*
Urban areas in a 2km radius UR2K_P	Percent of a 2km radius circle around each *R. boylii* locality that is defined as urban land use.	From USGS and EPA land use / land cover data at 1:250,000 (U.S. Geological Survey 2004); see Davidson, et al. (2001) for details of methods.	*	*	*
Dam Presence, Proximity, and Size					
Dam Status DAMSCR	Occurance of dams in the watershed upstream of each *R. boylii* locality: 1=at least one, 0=none.	CA: Latitude/longitude values in U.C. Berkeley Digitial Library database of CA Department of Water Resources Bulletin 17 were used to create a GIS coverage which was then compared to a coverage from the Bureau of Reclamation.	*	*	*

Appendix 2.A., ***Dam Presence, Proximity, and Size***, *Dam Status, continued.*					
		OR: Documented from current USGS topographic maps and verified during field visits.			
Dam count (CA only) DAMCOUNT	Count of dams in the watershed upstream of each *R. boylii* locality.	Derived as described above in “Dam Status”.		*	s
Reservoir / Watershed Ratio (%) (CA only) RES_WS_P	Ratio of sum of all reservoir surface areas in a watershed to the total area of the watershed.	Derived from data provided in in U.C. Berkeley Digitial Library database of CA Department of Water Resources Bulletin 17 for each dam and upstream watershed areas derived from GIS for each *R. boylii* locality.		*	s
Dam Distance (km) (CA only) DAMDIST	Distance of each dam within a watershed to the downstream *R. boylii* locality defining the watershed.	Derived when each dams was linked to *R. boylii* watersheds using GIS		c	
Dam Size (Large) (CA only) LGDAM	From criteria developed by the International Commission on Large Dams (ICOLD 1997). Dams over 15m high or over 10m high with a reservoir volume ≥ 2000 cubic m. or crest length of ≥ 500 m. We also examined very large dams – defined by us to be >30m high.	Derived from information provided in in U.C. Berkeley Digitial Library database of CA Department of Water Resources Bulletin 17 for each dam (e.g. height, reservoir area, etc.)		c	
Max dam height (CA only) MAX_HTM	Maximum dam height in a watershed, for localities with upstream dams.	Derived from information provided in in U.C. Berkeley Digitial Library database of CA Department of Water Resources Bulletin 17 for each dam (e.g. height, reservoir area, etc.)			
Dam presence by precipitation interact. DAMPRC	DAMSCR X MNAN_P60	Created prior to logistic regression analyses with a simple mathematical function.	*	*	*
Dam presence by dry years interact. DAMDRT	DAMSCR X PMMPC_DR	Created prior to logistic regression analyses with a simple mathematical function.	*	*	*

Appendix 2.B-1. Results from *Rana boylii* range-wide logistic regression analysis (n=394 localities). An "_L" at the end of a variable name means it was natural log-transformed prior to analysis. For each set/subset, all models within two points of the lowest AIC value are presented and ordered from lowest to highest AIC. R-square values and total classification success are given as additional model diagnostics. Model numbers/AIC's in bold are considered equally valid models for each set/subset.

Model variables (with standardized coefficients for each variable)	**Num. Vars.**	*AIC*	**Rsq**	**Total Class. success**
Best single variable (after LAT, LONG) - MNAN_P60 (0.505)	1	486.117	0.15	65.7
1. LAT (-0.971), LONG (1.942), ELEV_M (0.553), UPAGTR_L (0.5159), UPURTR_L (-0.139), UR2K_L (-0.422), MNAN_P60 (0.415), PMMPC_BA (0.257), PMMPC_DR (-0.154)	**9**	**359.150**	0.41	77.9
2. LAT (-0.971), LONG (1.990), ELEV_M (0.544), UPAGTR_L (0.510), UPURTR_L (-0.1439), UR2K_L (-0.438), MNAN_P60 (0.320), PMMPC_BA (0.098), PMMPC_DR (-0.1174), DAMPRC (0.359), DAMDRT (-0.345)	**11**	**359.347**	0.41	76.6
3. LAT (-0.9554), LONG (1.948), ELEV_M (0.555), UPAGTR_L (0.473), UR2K_L (-0.405), MNAN_P60 (0.449), PMMPC_BA (0.277), PMMPC_DR (-0.1318)	**8**	**359.812**	0.40	78.9
4. LAT (-0.952), LONG (1.960), ELEV_M (0.544), UPAGTR_L (0.534), UPURTR_L (-0.141), AG2K_L (-0.097), UR2K_L (-0.394), MNAN_P60 (0.379), PMMPC_BA (0.246), PMMPC_DR (-0.155)	**10**	**359.864**	0.41	77.7
5. LAT (-0.958), LONG (2.001), ELEV_M (0.547), UPAGTR_L (0.469), UR2K_L (-0.420), MNAN_P60 (0.360), PMMPC_BA (0.2737), PMMPC_DR (-0.093), DAMPRC (0.345), DAMDRT (-0.337)	10	360.168	0.41	76.9
6. LAT (-0.934), LONG (1.993), ELEV_M (0.561), UPAGTR_L (0.517), UR2K_L (-0.411), MNAN_P60 (0.440), PMMPC_BA (0.306)	7	360.418	0.40	78.9
7. LAT (-0.953), LONG (2.008), ELEV_M (0.538), UPAGTR_L (0.522), UPURTR_L (-0.146), AG2K_L (-0.080), UR2K_L (-0.414), MNAN_P60 (0.295), PMMPC_BA (0.243), PMMPC_DR (-0.121), DAMPRC (0.344), DAMDRT (-0.328)	12	360.500	0.42	77.7
8. LAT (-0.942), LONG (1.998), ELEV_M (0.559), UPAGTR_L (0.558), UPURTR_L (-0.115), UR2K_L (-0.426), MNAN_P60 (0.411), PMMPC_BA (0.292)	8	360.548	0.40	77.9
9. LAT (-0.962), LONG (1.943), ELEV_M (0.554), UPAGTR_L (0.502), UPURTR_L (-0.143), UR2K_L (-0.426), MNAN_P60 (0.393), PMMPC_BA (0.257), PMMPC_DR (-0.151), DAMPRC (0.055)	10	360.619	0.41	78.2

Appendix 2.B-1, continued.

10. LAT (-0.935), LONG (1.962), ELEV_M (0.546), UPAGTR_L (0.488), AG2K_L (-0.094), UR2K_L (-0.377), MNAN_P60 (0.415), PMMPC_BA (0.261), PMMPC_DR (-0.132)	9	360.621	0.41	77.9
11. LAT (-1.067), LONG (1.963), ELEV_M (0.564), UPAGTR_L (0.526), UPURTR_L (-0.138), UR2K_L (-0.423), MNAN_P60 (0.416), CVAN_P60 (-0.103), PMMPC_BA (0.266), PMMPC_DR (-0.152)	10	360.921	0.41	77.9
12. LAT (-1.095), LONG (2.019), ELEV_M (0.559), UPAGTR_L (0.523), UPURTR_L (-0.144), UR2K_L (-0.440), MNAN_P60 (0.318), CVAN_P60 (-0.132), PMMPC_BA (0.264), PMMPC_DR (-0.115), DAMPRC (0.367), DAMDRT (-0.350)	12	360.993	0.41	76.6
13. LAT (-0.973), LONG (1.998), ELEV_M (0.552), UPAGTR_L (0.506), UPURTR_L (-0.143), UR2K_L (-0.443), MNAN_P60 (0.330), PMMPC_BA (0.252), PMMPC_DR (-0.094), DAMSCR (0.226), DAMPRC (0.317), DAMDRT (-0.532)	12	361.007	0.41	77.7
14. LAT (-0.968), LONG (1.966), ELEV_M (0.541), UPAGTR_L (0.511), UPURTR_L (-0.145), UR2K_L (-0.428), MNAN_P60 (0.340), PMMPC_BA (0.255), PMMPC_DR (-0.157), DAMSCR (-0.244), DAMPRC (0.276)	11	361.056	0.41	77.9
15. LAT (-0.969), LONG (1.941), ELEV_M (0.554), UPAGTR_L (0.513), UPURTR_L (-0.139), UR2K_L (-0.422), MNAN_P60 (0.413), PMMPC_BA (0.257), PMMPC_DR (-0.153), DAMSCR (0.012)	10	361.124	0.41	77.7
16. LAT (-0.972), LONG (1.943), ELEV_M (0.552), UPAGTR_L (0.518), UPURTR_L (-0.138), UR2K_L (-0.421), MNAN_P60 (0.416), PMMPC_BA (0.257), PMMPC_DR (-0.153), DAMDRT (-0.009)	10	361.135	0.41	77.4

Appendix 2.B-2. Results from *Rana boylii* logistic regression analysis for California localities (n=310). An "_L" at the end of a variable name means it was natural log-transformed prior to analysis. For each set/subset, all models within two points of the lowest AIC value are presented and ordered from lowest to highest AIC. R-square values and total classification success are given as additional model diagnostics. Model numbers/AIC's in bold are considered equally valid models for each set/subset.

Model variables (with standardized coefficients for each variable)	**Num. Vars.**	*AIC*	**Rsq**	**Total Class. success**
All Dams				
Best single variable (after LAT, LONG) - CVAN_P60 (-0.893)	1	342.613	0.25	64.8
1. LAT (-0.654), LONG (1.937), ELEV_M (0.518), UPAGTR_L (0.559), UPURTR_L (-0.251), AG2K_L (-0.243), UR2K_L (-0.483), MNAN_P60 (0.143), CVAN_P60 (-0.750), PMMPC_DR (-0.191), RES_WS_P (-0.175), DAMCOUNT (-0.176), DAMPRC (0.224)	**13**	**232.581**	0.52	82.3
2. LAT (-0.701), LONG (2.006), ELEV_M (0.519), UPAGTR_L (0.574), UPURTR_L (-0.256), AG2K_L (-0.225), UR2K_L (-0.501), MNAN_P60 (0.096), CVAN_P60 (-0.772), PMMPC_DR (-0.166), RES_WS_P (-0.189), DAMCOUNT (-0.172), DAMPRC (0.552), DAMDRT (-0.362)	**14**	**232.692**	0.52	81.6
3. LAT (-0.682), LONG (2.010), ELEV_M (0.522), UPAGTR_L (0.556), UPURTR_L (-0.254), AG2K_L (-0.225), UR2K_L (-0.527), MNAN_P60 (0.135), CVAN_P60 (-0.747), PMMPC_DR (-0.099), RES_WS_P (-0.232), DAMSCR (0.710), DAMCOUNT (-0.219), DAMPRC (0.454), DAMDRT (-0.944)	**15**	**232.694**	0.52	82.6
4. LAT (-0.633), LONG (1.923), ELEV_M (0.519), UPAGTR_L (0.535), UPURTR_L (-0.237), AG2K_L (-0.231), UR2K_L (-0.510), MNAN_P60 (0.234), CVAN_P60 (-0.728), PMMPC_DR (-0.093), RES_WS_P (-0.196), DAMSCR (0.909), DAMCOUNT (-0.213), DAMDRT (-0.738)	**14**	**233.345**	0.52	82.9
5. LAT (-0.784), LONG (2.194), ELEV_M (0.597), UPAGTR_L (0.596), UPURTR_L (-0.228), AG2K_L (-0.215), UR2K_L (-0.559), MNAN_P60 (0.153), CVAN_P60 (-0.784), PMMPC_BA (0.177), PMMPC_DR (-0.069), RES_WS_P (-0.225), DAMSCR (0.769), DAMCOUNT (-0.233), DAMPRC (0.472), DAMDRT (-1.020)	**16**	**233.380**	0.52	82.9
6. LAT (-0.794), LONG (2.162), ELEV_M (0.582), UPAGTR_L (0.611), UPURTR_L (-0.237), AG2K_L (-0.218), UR2K_L (-0.527), MNAN_P60 (0.109), CVAN_P60 (-0.807), PMMPC_BA (0.153), PMMPC_DR (-0.152), RES_WS_P (-0.179), DAMCOUNT (-0.182), DAMPRC (0.568), DAMDRT (-0.379)	15	233.712	0.52	81.3

Appendix 2.B-2, continued.				
7. LAT (-0.731), LONG (2.069), ELEV_M (0.574), UPAGTR_L (0.589), UPURTR_L (-0.235), AG2K_L (-0.238), UR2K_L (-0.505), MNAN_P60 (0.158), CVAN_P60 (-0.776), PMMPC_BA (0.136), PMMPC_DR (-0.180), RES_WS_P (-0.164), DAMCOUNT (-0.187), DAMPRC (0.225)	14	233.800	0.52	81.9
8. LAT (-0.636), LONG (1.891), ELEV_M (0.515), UPAGTR_L (0.548), UPURTR_L (-0.244), AG2K_L (-0.243), UR2K_L (-0.476), MNAN_P60 (0.206), CVAN_P60 (-0.745), PMMPC_DR (-0.183), RES_WS_P (-0.155), DAMSCR (0.172), DAMCOUNT (-0.174)	13	233.928	0.51	82.9
9. LAT (-0.714), LONG (2.076), ELEV_M (0.582), UPAGTR_L (0.567), UPURTR_L (-0.213), AG2K_L (-0.223), UR2K_L (-0.536), MNAN_P60 (0.255), CVAN_P60 (-0.756), PMMPC_BA (0.156), PMMPC_DR (-0.065), RES_WS_P (-0.186), DAMSCR (0.960), DAMCOUNT (-0.226), DAMDRT (-0.791)	15	234.305	0.52	82.9
10. LAT (-0.669), LONG (1.953), ELEV_M (0.518), UPAGTR_L (0.566), UPURTR_L (-0.252), AG2K_L (-0.238), UR2K_L (-0.483), MNAN_P60 (0.125), CVAN_P60 (-0.760), PMMPC_DR (-0.195), RES_WS_P (-0.171), DAMSCR (-0.106), DAMCOUNT (-0.169), DAMPRC (0.317)	14	234.416	0.52	81.3
Large^1 dams only				
Best single variable (after LAT, LONG) - CVAN_P60 (-0.893)	1	342.613	0.25	64.8
1. LAT (-0.858), LONG (2.176), ELEV_M (0.527), UPAGTR_L (0.613), UPURTR_L (-0.308), AG2K_L (-0.185), UR2K_L (-0.533), MNAN_P60 (0.154), CVAN_P60 (-0.785), PMMPC_DR (-0.137), RES_WS_P (-0.227), DAMPRC (1.062), DAMDRT (-1.018)	**13**	**224.067**	0.53	80.6
2. LAT (-0.918), LONG (2,064), ELEV_M (0.525), UPAGTR_L (0.548), UPURTR_L (-0.307), UR2K_L (-0.556), MNAN_P60 (0.205), CVAN_P60 (-0.855), PMMPC_DR (-0.143), RES_WS_P (-0.209),DAMPRC (1.059), DAMDRT (-1.024)	**12**	**224.798**	0.53	80.6
3. LAT (-0.784), LONG (2.179), ELEV_M (0.509), UPAGTR_L (0.595), UPURTR_L (-0.312), AG2K_L (-0.215), UR2K_L (-0.586), MNAN_P60 (0.153), CVAN_P60 (-0.767), PMMPC_DR (-0.108), RES_WS_P (-0.158), DAMSCR (0.987), DAMCOUNT (-0.245), DAMPRC (0.893), DAMDRT (-1.761)	**15**	**224.855**	0.53	80.0

Appendix 2.B-2, continued.				
4. LAT (-0.797), LONG (2.136), ELEV_M (0.499), UPAGTR_L (0.610), UPURTR_L (-0.307), AG2K_L (-0.205), UR2K_L (-0.538), MNAN_P60 (0.125), CVAN_P60 (-0.790), PMMPC_DR (-0.151), RES_WS_P (-0.199), DAMCOUNT (-0.120), DAMPRC (1.069), DAMDRT (-0.980)	**14**	**224.900**	0.53	80.6
5. LAT (-0.893), LONG (2.339), ELEV_M (0.581), UPAGTR_L (0.612), UPURTR_L (-0.291), AG2K_L (-0.191), UR2K_L (-0.621), MNAN_P60 (0.166), CVAN_P60 (-0.871), PMMPC_BA (0.181), PMMPC_DR (-0.078), DAMSCR (1.198), DAMCOUNT (-0.329), DAMPRC (0.786), DAMDRT (-1.951)	15	225.456	0.53	80.0
6. LAT (-0.901), LONG (2.368), ELEV_M (0.585), UPAGTR_L (0.636), UPURTR_L (-0.287), AG2K_L (-0.200), UR2K_L (-0.619), MNAN_P60 (0.179), CVAN_P60 (-0.808), PMMPC_BA (0.187), PMMPC_DR (-0.080), RES_WS_P (-0.162), DAMSCR (1.035), DAMCOUNT (-0.253), DAMPRC (0.903), DAMDRT (-1.816)	16	225.500	0.54	81.3
7. LAT (-0.906), LONG (2.296), ELEV_M (0.565), UPAGTR_L (0.649), UPURTR_L (-0.287), AG2K_L (-0.194), UR2K_L (-0.565), MNAN_P60 (0.146), CVAN_P60 (-0.827), PMMPC_BA (0.164), PMMPC_DR (-0.136), RES_WS_P (-0.206), DAMCOUNT (-0.128), DAMPRC (1.081), DAMDRT (-0.985)	15	225.856	0.53	80.6

[1] large = dams higher than 15 m or higher than 10 m and with more than 500 m crest length or more than 1 million m^3 storage capacity (ICOLD 1997).

Appendix 2.B-3. Results from logistic regression analysis for each bioregion (sample sizes are give in table). An "_L" at the end of a variable name means it was natural log-transformed prior to analysis. For each bioregion, the best single variable and the best model are presented. R-square values and total classification success are given as additional model diagnostics.

Model variables (with standardized coefficients for each variable)	**Num. Vars.**	*AIC*	**Rsq**	**Total Class. success**
North Coast / Klamath (NCK) (n=143)				
Best single variable (after LAT, LONG) – UR2K_L (-0.385)	1	172.307	0.10	69.9
Best model - LAT (-0.963), LONG (0.988), ELEV_M (0.615), UPURTR_L (-0.271), UR2K_L (-0.331)	5	143.730	0.31	75.5
Central Coast / Transverse Ranges (CCT) (n=52)				
Best single variable (after LAT, LONG) - CVAN_P60 (-1.411)	1	35.293	0.31	78.8
Best model - ELEV_M (1.819), UPURTR_L (-1.800), CVAN_P60 (-2.506)	3	21.464	0.51	96.2
Bay Area / Valleys (BAV) (n=133)				
Best single variable (after LAT, LONG) - MNAN_P60 (0.493)	1	167.543	0.14	66.9
Best model – LONG (1.579), ELEV_M (0.476), UPAGTR_L (0.915), AG2K_L (-0.429), UR2K_L (-0.463), DAMCOUNT (-1.051)	6	105.846	0.50	77.4
Sierra Nevada (SNV) (n=47)				
Best single variable (after LAT, LONG) - CVAN_P60 (-1.368)	1	44.697	0.41	76.6
Best model - LONG (2.654), UR2K_L (-0.776), CVAN_P60 (-1.497), RES_WS_P (-0.716), DAMCOUNT (2.073),	5	32.105	0.62	89.4

CHAPTER 3. PHYLOGEOGRAPHY OF *RANA BOYLII*:

IMPLICATIONS FOR THE CONSERVATION OF FROGS AND RIVERS

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ABSTRACT

Genetic data are increasingly being used in conservation and restoration efforts of declining species. In this paper, we combine basic ecological associations with a phylogeographic and population genetic analyses to describe patterns of genetic variation for the foothill yellow-legged frog, *Rana boylii*, a declining amphibian species. We used 1525 total bp of Cytochrome B and ND2 mtDNA fragements for 77 individuals from 34 localities and conducted phylogenetic and population genetic analyses. We constructed both neighbor-joining and Bayesian trees to identify well-supported monophyletic clades. We also examined population genetic relationships through analysis of haplotype networks and partitioning of molecular variance (via AMOVA). Specifically, we evaluated hydrologic regions, river basins, and mountain provinces in terms of within and among levels of molecular variance and tested these groupings using F_{st} statistics. Our results demonstrated that several well-supported geographically congruent clades exist within *R. boylii*. While genetic variation was low among populations in the largest, most inclusive clade, samples from several localities demonstrated substantial genetic divergence. Hydrologic regions and river basins, which represent likely dispersal corridors for *R. boylii*, show more promise in terms of explaining historic patterns of genetic variation than do mountain ranges. The combined results from our phylogeographic and population genetic analyses provide useful information for conservation planning.

INTRODUCTION

Successful conservation and restoration of declining species is dependent on numerous factors, from the availability of information on basic ecology to human social/cultural forces. Species status designations (endangered, red list, etc.) are increasingly based on genetic information, especially for definitions of appropriate taxonomic units to conserve. Designations such as Evolutionary Significant Units (ESU) and Distinct Population Segments (DPS), and Management Units have been developed to define intraspecific groupings of animals that share an evolutionary history (Moritz 2002, Avise 2004). Because it incorporates information on both genetic and geographic patterns of taxa, phylogeographic analysis has been used to describe historical patterns of gene flow and potential evolutionary trajectories (Avise 2000, Moritz 2002). However, the spatial scale of genetic data collection and analysis is key to the interpretation of phylogeographic patterns and to the development of resulting conservation designations. We investigate these ideas by using information on basic ecological associations and spatial distribution to guide genetic sampling for a declining frog species and then discuss the conservation implications of the resulting phylogeographic analyses.

The foothill yellow-legged frog, *Rana boylii*, was first described by Baird in 1854, but a half-century of taxonomic uncertainty followed, including several name changes (Zweifel 1968). Since 1955, *R. boylii* has been recognized as a distinct species in the family Ranidae (Zweifel 1955). Zweifel (1955) grouped five other western North American Ranids, including the mountain yellow-legged frog (*R. muscosa*) and four Mexican species, into what he termed the *Rana boylii* (*boylei*) species group. However, more recent genetic work has defined a somewhat different set of five western North American species as the *Rana boylii* group: *R. aurora, R. boylii, R. cascadae, R. muscosa, and R. pretiosa* (Case 1978a, Macey et al. 2001). While early morpological and geographic work suggested that *R. boylii* and *R. muscosa* were each other's closest relatives, most recent genetic work indicates they are not (Case 1978b, Hillis and Davis 1986, Macey et al. 2001, but see Green 1986). Instead, *R. boylii* appears to be more closely allied with *R. lutriventris* (*pretiosa*), the Columbia spotted frog (Macey et al. 2001). The intraspecific genetic relationships of *R. boylii* have not been thoroughly examined. One study of six populations indicated high levels of variability in allozymes both within and among populations. However, no conclusions were made about evolutionary relationships (Case 1978b).

Rana boylii, historically occurred in foothill and mountain streams from northern Baja California to southern Oregon west of the Sierra-Cascade crest, from sea level to 1830m (6000 ft) elevation (Fig. 3.1). This species has experienced significant populations declines, especially in the southern part of its range (southern Sierra Nevada Mountains and south coastal California), and is currently listed as a California State Species of Special Concern (Jennings and Hayes 1994, Jennings 1996, California Department of Fish and Game 2004). *R. boylii* is almost exclusively a species of lotic (running water) environments. As such, when studying the genetic structure of this species throughout its range, we were interested in whether there would be congruencies between patterns of genetic variation and boundaries of river basins and smaller watersheds or whether large geologic events and processes representing longer time scales would overshadow these sorts of patterns. For example, recent analyses of other species (both plants and animals) in California indicated that congruent genetic breaks seen in many species correspond with major mountain-building events in the region (Calsbeek et al. 2003). If reliable phylogeographic scenarios (or sets of scenarios) emerged from our analyses, our goal was to propose approaches for future conservation for *R. boylii*. Specifically, our objectives were to describe the intraspecific genetic variation and structure of *R. boylii* throughout its geographic range and determine whether the observed patterns were indicative of: (1) gene flow barriers due to river basin divides/boundaries, (2) more extensive temporal and spatial geologic events, such as glaciations and mountain-building, or (3) some combination of processes acting at different spatial and temporal scales.

METHODS AND ANALYSIS

Genetic Sampling, Mapping, and Marker Selection

Previous allozyme work (Case 1978a) and research on genetic relationships above the species level for western North American *Ranid* frogs (Green 1986, Macey et al 2001) provided both context and potential outgroups for phylogenetic analysis. The relatively large geographic range of *R. boylii* precluded sampling every population, so our goal for tissue collection was to cover the extent of the historic range. Because this species is primarily associated with streams, and we were interested in whether or not genetic differentiation could be detected among river basins and larger hydrologic units, our sampling was structured based on major river drainages and hydrologic regions. The California Department of Water Resources has defined 10 hydrologic regions in the state which integrate current and past geology, tectonics, and climate (Mount 1995). *R. boylii* historically occurred in seven of these regions and currently occurs in six. These six regions include over 30 major river drainages. Thus tissue sampling was conducted in a nested design, with the first priority being to collect from several populations in each of the six hydrologic regions, and secondarily to collect from as many of the major river drainages as possible within each of the regions. Populations of *R. boylii* are not known from the floor of the Central Valley of California, though there is one historic location in the Sutter Buttes (a small mountain range completely within the northern Central Valley). There are an additional six major river drainages in southwestern Oregon within the geographic range of the *R. boylii*. Collections were made in three of these rivers covering the north-south extent of the range in Oregon. From 1 to 30 individuals (whole larvae or sub-adults, adult toe tips) were collected at each locality. Efforts were made to collect larvae from multiple parental groups by spreading sample collecting out at each stream location.

We mapped all sample localities using ArcView 3.2 Geographical Information System (GIS) software (Environmental Systems Research Institute [ESRI] 1999). The CALWATER database/GIS coverage (CALWATER 2.2. – California Interagency Watershed Mapping Committee 1997) was used to determine in which river basins and hydrologic regions each sampling locality occurred. Other widely available GIS coverages such as topographic shaded relief, counties, and streams/rivers were used to determine mountain range associations of sampling localities and to visually assess phylogenetic tree relationships in the context of geographic features.

Mitochondrial DNA typically evolves more quickly than single copy nuclear DNA but more slowly than nuclear microsatellite regions and can provide information on genetic differentiation over relatively long time periods and large geographic areas (Avise 2004). MtDNA markers have been successfully used to examine genetic differentiation over both local and larger geographic scales in amphibians (Shaffer and McKnight 1996, Shaffer et al. 2000). Within the mitochondrial genome, we focused on two fragments – nearly complete Cytochrome *b* (1026 bp) and a portion of ND2 (499 bp). Both of these fragments have been used for inter and intra-specific analyses in western Ranid frogs (e.g. Bos and Sites 2001, Macey et al. 2001, Shaffer et al. 2004), so using them will ultimately allow for comparisons with data on related species.

Laboratory Methods

We extracted total genomic DNA from 77 individual *R. boylii* from 34 localities (1 to 4 per locality, Appendix 3.A.) using a standard salt extraction (Sambook and Russell 2001). Several primer sets were used to amplify the focal mtDNA fragments using polymerase chain reactions (PCR). For Cytochrome *b*, we used the following primer set: 5’ end, *Glu_F* (5’to3’ gaaaagctatcgctgttattcaac; T. Engstrom, pers. comm.) and 3’ end, *CytbAR-H_R* (5’ to 3’ tawaagggtcttctactggttg; Goebel 1999). For ND2, we developed a primer set for amplification based available *R. boylii* mtDNA sequence (Macey et al. 2001): 5’end, *ND2_2_F* (5’to 3’ tattggccaaacccagcttc) and 3‘ end, *tRNAtrp_R* (5’ to 3’ ttaaagggcctgagttgcatt). For Cytochrome *b*, we sequenced the resulting amplified product using the original PCR primers and two internal primers, *CB755_R* (5’to 3’ ctggtgtaaaattgtctgggtctc; T.

Engstrom, pers. comm.) and *Cytb18rL_F* (5' to 3' ccacgcctaytaytcytacaaagac; Goebel 1999). To sequence ND2 we used the 5'end PCR primer and developed an internal 3' end primer *tRNAtrp2_R* (5'to 3' ctttgaaggcctttggtctgtatta).

We used different temperature profiles for PCR reactions of the two mtDNA fragments. For Cytochrome *b*, we ran 30 cycles of denaturation at 94^{o}C, annealing at 50^{o}C and extension at 72^{o}C. For ND2, we ran 40 cycles of denaturation at 94^{o}C, annealing at 60^{o}C and extension at 72^{o}C. Sequencing of all amplified fragments was done by the University of California Division Biological Sciences DNA Sequencing Facility (http://dnaseq.ucdavis.ed) using either an ABI 3100 or ABI 3730 automated DNA sequencer. Alignments are available from AJL and all sequences will be deposited in GenBank.

Preliminary analyses of a subsample of our data showed low mtDNA sequence variation across the geographic range of *R. boylii*. To confirm that the low levels of variation were "real" and not the result of a selective sweep, we sequenced 10 individuals from 10 localities spanning the geographic range (Appendix 3.A, Localities 1, 6, 9, 20, 21, 25, 28, 29, 32, and 34) for the nuclear gene Tropomyosin. We used the following primer set from Friesen et al. (1999) for amplification and sequencing: 5'end *TROPex5* (5' to 3' gagttggatcgkgctcaggagcg) and 3'end *TROPex6* (5' to 3' cggtcagcmtcttcagcaatgtgctt). We obtained 517 bp of the nuclear gene Tropomyosin for each of the 10 individuals. Only 2 of the 517 positions were variable and only in 3 individuals. Uncorrected *p* distances ranged from 0.002 to 0.004 for pairwise comparisons of these 3 individuals with the other 7. Because variation was substantially lower than for mtDNA, we did not explore any other individuals or localities for the nuclear gene.

Phylogenetic and Population Genetic Analyses

Two primary methods were used for analysis of mtDNA sequence data. To examine range-wide genetic and biogeographic history and structure, we used phylogenetic tree-building. To examine population genetic relationships among hydrologic regions, river basins, and mountain ranges, we used Analysis of Molecular Variance (AMOVA). Prior to these analyses, sequences were confirmed by aligning and viewing forward and reverse primed fragments in the program SeqEdit v. 1.0.3 (G. Olsen, Applied Biosystems) and checked for reading frame using vertebrate mitochondrial genetic code in Gene Jockey (Taylor 1990). Multiple alignments were done using Clustal X v. 1.8 (Thompson et al. 1997) and subsequently checked by eye. We constructed a haplotype network using TCS 1.1.3 (Clement et al. 2000) to provide visualization of spatial relationships of haplotypes within and among sampling localities.

Neighbor-joining (NJ) phylogenetic trees (Saitou and Nei 1987) were constructed using PAUP* v.4.0.b10 (Swofford 2000) and Bayesian trees (Huelsenbeck and Ronquist 2001) were constructed using MrBayes v.3.04b (Huelsenbeck 2000). For these analyses, all nucleotide positions were considered equally weighted characters and gaps (outgroups only) were treated as missing data. We concatenated Cytochrome *b* and ND2 for phylogenetic analyses and used sequence data from two individudal *Rana muscosa* as an outgroup in phylogenetic analyses. For the NJ tree, uncorrected *p* distances were used for construction. For Bayesian analysis, we used 4 chains (3 heated and 1 cold) for one million generations, sampling the chains every 100th generation (= approximately 10,000 trees per run). Six partitions of the data were created based on two fragments (Cytochrome *b* and ND2) and 3 possible codon positions for base pairs within each fragment; all parameters were unlinked based on these partitions. Once a run was complete, we determined the point of stationarity through visual examination of scatterplots of the resulting likelihood scores versus generation number. Trees produced prior to stationarity were considered “burn in” and discarded. We conducted three separate *MrBayes* runs under these conditions. After eliminating “burn in” trees, we appended the 3 tree files and used PAUP* to generate a 95% majority-rule consensus tree. Posterior probabilities for a clade were determined as the percent of trees recovering that particular clade.

We used AMOVA for sequence data (Excoffier et al. 1992) to explore genetic variation relative to current and historic geologic features and clarify the phylogeographic history of *R. boylii*. For this analysis, we set up our data to address several possible explanations for genetic affinities. First, we were interested in how genetic variation was partitioned within and among 7 hydrologic regions: North Coast, San Francisco Bay, Central Coast, Sacramento, San Joaquin, Tulare Lake (California Department of Water Resources [Mount 1995]) and South Santium River, Willamette Valley, Oregon. Oregon boundaries were drawn based on a U.S. Geological Survey map (USGS 2004). We were also interested in whether or not river basins within these hydrologic regions showed clear patterns of within and among genetic variation. The hydrologic regions representing coastal areas (North Coast and Central Coast) were somewhat artificial and not truly inter-connected for this freshwater frog. So, we conducted the river basin analysis using a subset of populations from drainages that flow into the Great Central Valley of California. These drainages are currently hydrologically connected, via the Sacramento and San Joaquin Rivers, and share a common geologic history. Thus we excluded populations in Oregon, populations in the coastal regions, and populations in the Tulare Lake hydrologic region. Even though the Tulare Lake region is geographically at the southern end of the Central Valley, the drainages within it are not hydrologically connected to the San Joaquin River. Finally, we were interested in how genetic variation was partitioned within and among 4 mountain provinces (as defined by Oakeshott [1978]): Coast Ranges, Klamath, Cascade-Modoc, and Sierra Nevada. Each mountain province was defined by features of geology, relief, precipitation, and landforms resulting from a common geologic and climatic history (Oakeshott 1978). River basins and hydrologic regions represented dispersal corridors and areas within which substantial gene flow was expected. For the mountain provinces, we assumed that the common geologic history within a province, especially the timing of mountain formation, would explain some spatial patterns of genetic variation in *R. boylii*.

Arlequin v. 2.0 software (Schneider et al. 2000) was used to conduct three AMOVA tests; one on sets of populations defined by their occurrence within one of 7 hydrologic regions, one on sets of populations defined by their occurrence in one of 5 river basins flowing into the Sacramento or San Joaquin Rivers of California, and one on sets of populations defined by their occurrence in one of 4 mountain provinces. All tests were followed by calculation of pairwise F_{st}'s among hydrologic regions, river basins, or mountain provinces.

RESULTS

Sequence Variation

We derived mtDNA sequences for 77 individual *R. boylii* and 2 individual *R. muscosa* as the outgroup (Fig. 3.1, Appendix 3.A.) The concatenated Cytochrome B and ND2 mtDNA fragments provided a total of 1525 bp for analyses. For the *R. boylii* ingroup, only 81 of the 1525 positions were variable and we found no insertions or deletions. Three deletions were present in the ND2 fragment of the *R. muscosa* outgroup. In the heart of the geographic range of *R. boylii*, genetic variation was relatively low within and among localities (uncorrected *p* range: 0.00 – 0.009). However, populations at the northern and southern extremes of the range (i.e. Appendix 3.A. localities 1, 32, 33, and 34) were substantially different from the core (uncorrected *p* range: 0.006 – 0.022). By comparison, all *R. boylii* sequences differed from *R. muscosa* outgroup taxa by more than 14% (uncorrected *p* range: 0.142 – 0.148).

Phylogenenetic Analyses

The recovered neighbor-joining and Bayesian trees were nearly identical with strong bootstrap support for several geographically distributed clades. The neighbor-joining tree is presented as a phylogram (Fig. 3.2) so that relative genetic divergence can be visualized. Clade posterior probabilities (pp) are presented above the branches on the Bayesian tree (Fig. 3.3). The following results are relevant to both trees. We found a large and well-supported (pp=100) clade that included samples from all but four localities: (1) the northernmost in Oregon (south Santium River, Locality 1), (2) the southernmost in the Sierra Nevada (Kern River, Locality 32), (3) the southernmost locality on the coast (Monterey Co., Locality 34), and (4) two of four individuals from an inland draining river (Los Burros Creek, Locality 33) (Clade A, Fig.3.3).

Individuals from these four clades showed substantial genetic divergence from the rest of the *R. boylii* samples (Fig. 3.2). Within the large clade there were three well-supported (pp=99-100) and for the most part geographically congruent clades (B, C, and D, Fig.3.3). Clade B represented *R. boylii* from several streams flowing into the upper Sacramento River, the Umpqua River in southern Oregon, and two coastal streams in Marin and Sonoma counties (localities 3, 7, 8, 9, 11, 23, and 25). Clade C included only individuals from streams draining the Sierra Nevada mountains of California; the Feather, Yuba, Bear, American, Calaveras, and upper San Joaquin Rivers (localities 13-18, 20-22, 24, and 28). Clade D included individuals from the central Coast Range of California, from both coastal and inland draining streams (localities 19, 26, 27, 29, 30, 31, and 33). This clade included the other two individuals from Los Burros Creek (Locality 33). Several localities were included in the large clade (A) but are not in one of the three well-supported clades (B,C,D) (Fig. 3.3). These localities were all in the coastal mountain ranges of northern California and southern Oregon and were from the Chetco, Smith, and Mattole Rivers and Redwood and Thistle Glade Creeks (localities 4, 5, 6, 10, and 12).

Population Genetics

Even though the phylogenetic analysis indicated that the several well-supported clades were geographically consistent, there was mixed evidence for the historic influence of river basins. Some haplotypes were shared among hydrologic regions and rivers basins within these clades, but AMOVA results indicated a significant portion of the variation was among these groups. The haplotype network we constructed showed a combination of a few very divergent haplotypes with several nodes of shared haplotypes among geographically close localities (Fig. 3.4). Node 33-AJL144 represented two individuals from a stream locality that drains into the Salinas River valley of central California. This node could not be connected to the network within the limits of the TCS software (Fig. 3.4, 17 steps, i.e. ”missing haplotypes”). Node 34-37109 represented 4 individuals sampled on the central coast of California at the current southern extreme of the species’ range and is the most steps from the rest of the network. Two nodes are of interest because they include haplotypes shared among several localities. Node 25-30444 represented five samples from two localities that are geographically separated; one in northern coastal California (Marin County) and one in interior northern California in the upper Sacramento River drainage (Fig. 3.4). This node was also separated from individuals at several other localities (e.g. 7-371105 and 23-15138) by only one step (one missing haplotype). Another node (13-KM1) represented 9 individuals from 6 localities in the Feather, Yuba, American, and Bear river drainages of the northern Sierra Nevada, California (Fig. 3.4). All of the other samples from these drainages and from all but one Sierra Nevada stream link to this node. The one Sierra Nevada stream that doesn’t directly link to that node is 32-37100 was found closest to the central coast node (34-37109) described earlier (Fig. 3.4).

Analyses of genetic variation (AMOVA) within and among hydrologic regions indicated that a significant component of the variation was among regions (F_{st} = 0.3996, P=0.000), however a greater overall portion of the variation was within

(60.04%) than among (39.96%) (Table 3.1). Analyses of populations within Sacramento-San Joaquin river basins showed a pronounced pattern of higher and significant variation among (60.56%) than within river basins (39.44%) (Table 3.1). These results are on a subset of the data and so are not directly comparable to those from the hydrologic regions. The mountain provinces we used indicated that more genetic variation was present within (73.34%) than among them (26.6%), though the among portion was still statistically significant (Table 3.1). The somewhat higher level of genetic variation explained by the hydrologic regions and river basins suggests that historical influences of river formation, may be a more likely explanation of historical patterns of gene flow than mountain range affinities.

When examining pairwise F_{st} values among hydrologic region populations, all but two pairs were significantly (*alpha* <0.05) differentiated; the Central Coast and San Francisco Bay regions and the Central Coast and Tulare Lake regions (Table 3.2a). The most differentiation appears to be occurring between the northernmost hydrologic region in Oregon (Santimum/Willamette) and three others (North Coast, San Francisco Bay, and San Joaquin). However, the relatively consistent F_{st} values among other pairs indicates that the overall percent variation (Table 3.1) is not being driven by any one particular locality (Table 3.2a). Levels of haplotype diversity were high for several of the hydrologic regions and nucleotide diversity was highest for the Central Coast region (Table 3.2a). For the Sacramento-San Joaquin river basin populations, three of the populations (Bear-Feather, Yuba, and American) were not significantly different from each other while the other river basins were different from each other and different from those three (Table 3.2b). Haplotype diversity was relatively high for all but one river basin (Bear and Feather) with no standouts in nucleotide diversity (Table 3.2b). For the mountain province populations, all were significantly differentiated with none showing particularly high pairwise F_{st} values. Haplotype and nucleotide diversities were highest in the Coast Ranges (Table 3.2c).

Examining the distribution of populations that were associated with clades in our Bayesian tree relative to the hydrologic regions and mountain provinces reveals that neither approach to grouping populations is particularly congruent with phylogeographic history (Fig. 3.5). However, the Sierra Nevada mountain province is fairly consistent with clade C and the Sacramento hydrologic regions is somewhat consistent with clade B (Fig. 3.5). Another feature of note that is evident from examination of Fig. 3.5 is the fact that the phylogenetic break between Clade B and Clade C, and the mountain province break, between the Sierra Nevada and Cascade-Modoc provinces, appear to be coincident.

DISCUSSION

We present the first extensive analysis of *R. boylii* genetic variation and demonstrate that several well-supported geographically congruent clades exist within this species. While genetic variation was low among populations in the largest, most inclusive clade, several localities demonstrated substantial genetic divergence. Hydrologic regions and river basins, which represent likely dispersal corridors for *R. boylii,* seem to show more promise in terms of explaining historic patterns of genetic variation than do mountain ranges. The combined results from our phylogeographic and population genetic analyses provide insights for potential areas for conservation actions.

Phylogeography of *Rana boylii*

With the exception of one population from the extreme northern area of *R. boylii*'s geographic range, we found more genetic variation in southern California than in northern California and southern Oregon (Fig. 3.2). Samples from three localities (32, 33, 34) in central and southern California appeared to be distinct from the rest. In particular, the southern Sierra Nevada mountain range sample (Locality 32) fell far outside of the clade containing the rest of the samples from the Sierra Nevada mountain range. This break is consistent with mtDNA data for other taxa in the region and implies a shared biogeographic history. For example, *Rana muscosa* (Macey et al. 2001), *Emys marmorata* (Spinks and Shaffer, *unpublished ms*), and *Lampropeltis zonata* (Rodriguez-Robles et al. 1999) all show a split in this area of the Sierra Nevada mountain range, south of the San Joaquin River. However, unlike these three species, there does not appear to be evidence for recent gene flow across the San Joaquin Valley and Tulare Lake hydrologic regions for *R. boylii*. This is demonstrated by the existence of two well-supported monophyletic clades; one on the east side of the San Joaquin Valley (Fig. 3.3, Clade C), and a second on the west side of the valley (Fig. 3.3, Clade D).

Our northern California *R. boylii* clade is congruent with another hypothesized biogeographic break (e.g. Good 1989, Shaffer et al. 2004). The strong support for this clade, which represented a combination of coastal localities and upper Sacramento River localities (Fig. 3.3, Clade B), is consistent with mtDNA results for another ranid with a geographic distribution similar to *R. boylii*. The *R. aurora/draytonii* species complex shows a phylogenetic split in the same area of the coastal northern California that is deep enough to warrant species status for each clade (Shaffer et al. 2004). However, unlike Shaffer et al.'s (2004) results for *R. aurora*, the *R. boylii* populations sampled to the north of that area along the coast do not form a distinct monophyletic clade.

There are undoubtedly geologic events that have shaped these strong congruencies in genetic breaks among taxa (e.g. Calsbeek et al. 2003). However, identifying these events over the appropriate time scale is challenging. In a couple of cases however, the pattern of genetic variation and phylogenetic breaks we found for *R. boylii* is consistent with geologic history. The large genetic divergence of samples from the most southern California coastal localities (Fig. 3.2, localities 33 and 34) relative to the rest of *R. boylii* is indicative of a history of isolation. The apparent lack of gene flow across the San Joaquin Valley and Tulare Lake hydrologic regions (Fig. 3.3) provides further evidence for lack of gene flow between these central coastal California localities and others to the north and east. These patterns are congruent with geologic evidence of marine intrusions in this area and the presence of a large freshwater lake in what is now the San Joaquin Valley/Tulare Lake region (Dupre et al. 1991). Both of these features would have been significant dispersal barriers for this freshwater, stream-associated frog. For the break in the southern Sierra Nevada, Macey et al. (2001) have suggested that Pleistocene climatic changes, especially the extent of glacial activity were key factors for *R. muscosa*. Recent studies have shown that precipitation amount and variability can influence the presence of *R. boylii* (Lind et al., *unpublished ms*; this dissertation, Chapter 2) so it is conceivable that these factors acted in the past as well. Finally, the co-occurrence of a phylogenetic break (between Clade B and Clade C) and the mountain province break between the Sierra Nevada and Cascade-Modoc provinces is indicative of an historic vicariance event (Fig. 3.5).

Population Genetics, Rivers, and Mountains

Our phylogeographic analysis demonstrates that genetic similarity is for the most part consistent with geographic proximity. The results of our analyses of hydrologic regions and mountain provinces provide more details on how genetic variation is partitioned geographically. Even though hydrologic regions and mountain provinces both demonstrated higher genetic variation within than among regions or provinces, the among component was still significant. There are several possible reasons for this result which warrant discussion and beg further research and analysis. For the hydrologic regions, we had low numbers of samples within each river and thus lumped rivers basins into hydrologic regions. Our analysis of a subset of hydrologically connected river basins (Sacramento-San Joaquin) indicates that some river basins may have genetically distinct populations. However, even within this analysis, pairwise results showed that 3 of 5 river basins were not significally different. Mitochondrial DNA may not be the ideal marker for examining variation at the hydrologic region scale and especially at finer scales such as within and among river basins. Research assessing the utility of microsatellite markers to describe gene flow and genetic variation in *R. boylii* is now being conducted in several streams of one northern California river basin (J. Dever, pers. comm..).

The two approaches (rivers and mountains) we used to partition genetic variation are not mutually exclusive. It has been proposed that the dendritic nature of river systems and the inherent terrestrial barriers would result in hierarchical patterns of genetic variation for fish and other aquatic species (Meffe and Vrijenhoek 1988). In practice however, rainbow trout (*Oncorhynchus mykiss*) show evidence of genetic uniqueness in some river basins but not others (Bagley and Gall 1998). In Siberian salmonid fishes, Pleistocene climate fluctuations and recent hydrologic mixing among major river basins have been invoked to explain geographically congruent patterns for some species and the lack there of for others (Froufe et al. 2003). For *R. boylii,* it is likely that the combined historical effects of mountain building events and associated river basin formation/connectivity and climatic shifts have acted in concert and perhaps at different temporal and spatial scales to influence *R. boylii* gene flow to produce the patterns of genetic variation we discovered here. Mountain building events along with climatic changes may have played a major role in early in the history of *R. boylii* gene flow while river connectivity may be the primary organizing factors in more recent times.

Frog Genetics and River Conservation

The hydrologic regions we used to evaluate genetic structuring in *R. boylii* populations had been previously defined to integrate current and past geology and to some degree climate. We expected that for this highly aquatic, stream-associated frog, strong patterns of congruency between genetic structure and hydrologic structure would emerge. We found some support for genetic affinities to hydrologic regions and more evidence in the subset of river basins we examined. Both our population genetic and phylogenetic results provide insights into the genetic variation across the range of *R. boylii* and may be useful in identification of areas that deserve immediate conservation measures. Several other ranid frog taxa exhibit significant intra-specific mtDNA genetic variation, potentially warranting designation as Evolutionary Significant Units (ESU) or full species (Green et al. 1997, Macey et al. 2001, Shaffer, et al. 2004). In the case of *R. boylii*, certainly, the populations in the southern portion of its range (especially localities 32, 33, and 34) are quite divergent from the rest of the species and deserve special conservation focus. The population at the northern extreme of the range in Oregon may also warrant some special management efforts though having data on intervening populations in Oregon would help to clarify the uniqueness of that population. Both our phylogenetic analyses and hydrologic region analyses indicated that genetic variation in *R. boylii* has a strong geographic component. Future conservation efforts and especially genetic management for *R. boylii* should strive to preserve these patterns, as they represent elements of historical lineages that could not be recovered if lost (Moritz 2002). Finally, river conservation may best be accomplished in California and Oregon by using *R. boylii* genetic data in combination with data from other aquatic species (fish, invertebrates, etc.) to prioritize and protect areas of unique and historic biological diversity.

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Table 3.1. Genetic variation of populations of *R. boylii* as determined by AMOVA within and among hydrologic regions, Sacramento-San Joaquin river basins, and mountain provinces.

Source	**d.f.**	**Percent of Variation, *P* for F_{st}**
Among hydrologic regions	6	39.96, *P* =0.000
Within hydrologic regions	70	60.04
Among Sacramento – San Joaquin river basins	4	60.56, *P*=0.000
Within Sacramento – San Joaquin river basins	26	39.44
Among mountain provinces	3	26.66, *P*=0.000
Within mountain provinces	73	73.34

Table 3.2. Pairwise F_{st} values (below diagonal) and associated *P* values (above diagonal) for populations of *Rana boylii* from AMOVA's of (a) hydrologic regions, (b) Sacramento-San Joaquin river basins, and (c) mountain provinces. Nucleotide and haplotype (gene) diversity estimates include an estimate of variance in parentheses.

(a)

Hydrologic Region	**Nucleotide Diversity**	**Haplotype Diversity**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1. Willamette (Santium)	0.000 (0.000)	0.000 (0.000)	----	0.000	0.018	0.000	0.009	0.000	0.000
2. North Coast	0.001 (0.001)	0.914 (0.425)	0.853	----	0.000	0.000	0.000	0.000	0.000
3. San Francisco Bay	0.002 (0.002)	0.700 (0.218)	0.818	0.251	----	0.099	0.000	0.000	0.045
4. Central Coast	0.011 (0.006)	0.901 (0.058)	0.376	0.305	0.163	----	0.000	0.000	0.090
5. Sacramento	0.004 (0.002)	0.857 (0.059)	0.673	0.278	0.282	0.364	----	0.000	0.000
6. San Joaquin	0.003 (0.002)	0.867 (0.129)	0.841	0.681	0.598	0.346	0.322	----	0.000
7. Tulare Lake	0.006 (0.003)	0.679 (0.122)	0.582	0.518	0.279	0.076	0.486	0.570	----

(b)

River Basin	**Nucleotide Diversity**	**Haplotype Diversity**	**1**	**2**	**3**	**4**	**5**
1. Upper Sacramento	0.001 (0.001)	0.821 (0.101)	----	0.000	0.000	0.009	0.000
2. Bear and Feather	0.001 (0.001)	0.476 (0.171)	0.845	----	0.135	0.081	0.000
3. Yuba	0.003 (0.002)	0.933 (0.122)	0.712	-0.002	----	0.081	0.000
4. American	0.003 (0.002)	0.833 (0.222)	0.782	0.272	0.229	----	0.045
5. San Joaquin	0.003 (0.002)	0.867 (0.129)	0.759	0.488	0.409	0.335	----

(c)

Mountain Province	**Nucleotide Diversity**	**Haplotype Diversity**	**1**	**2**	**3**	**4**
1. Coast Ranges	0.007 (0.004)	0.956 (0.015)	----	0.018	0.000	0.000
2. Klamath	0.001 (0.001)	0.762 (0.115)	0.149	----	0.036	0.000
3. Cascade-Modoc	0.005 (0.003)	0.679 (0.122)	0.206	0.336	----	0.000
4. Sierra Nevada	0.005 (0.003)	0.869 (0.051)	0.246	0.373	0.385	----

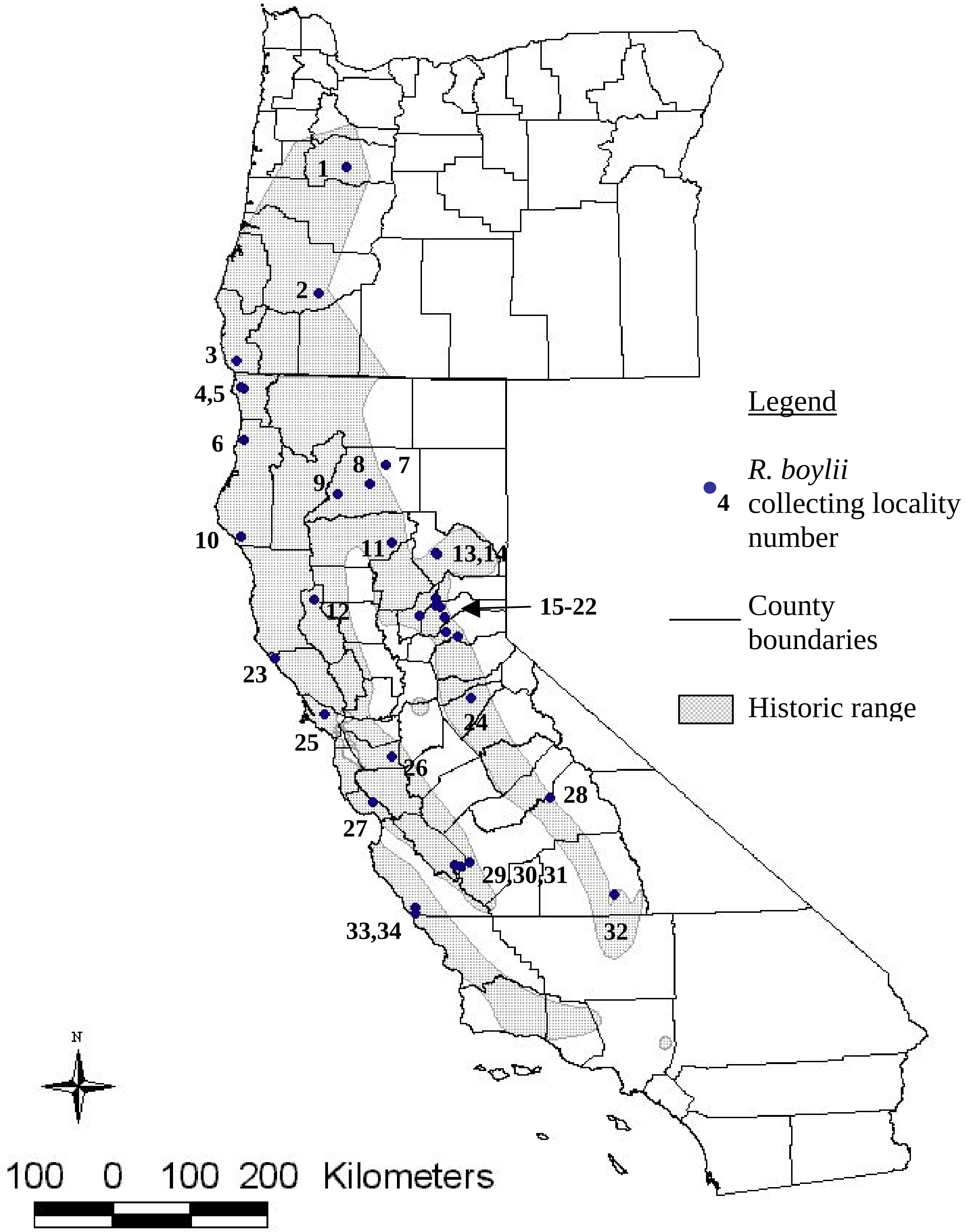

Figure 3.1. *Rana boylii* collecting localities relative to its historic range in California and Oregon.

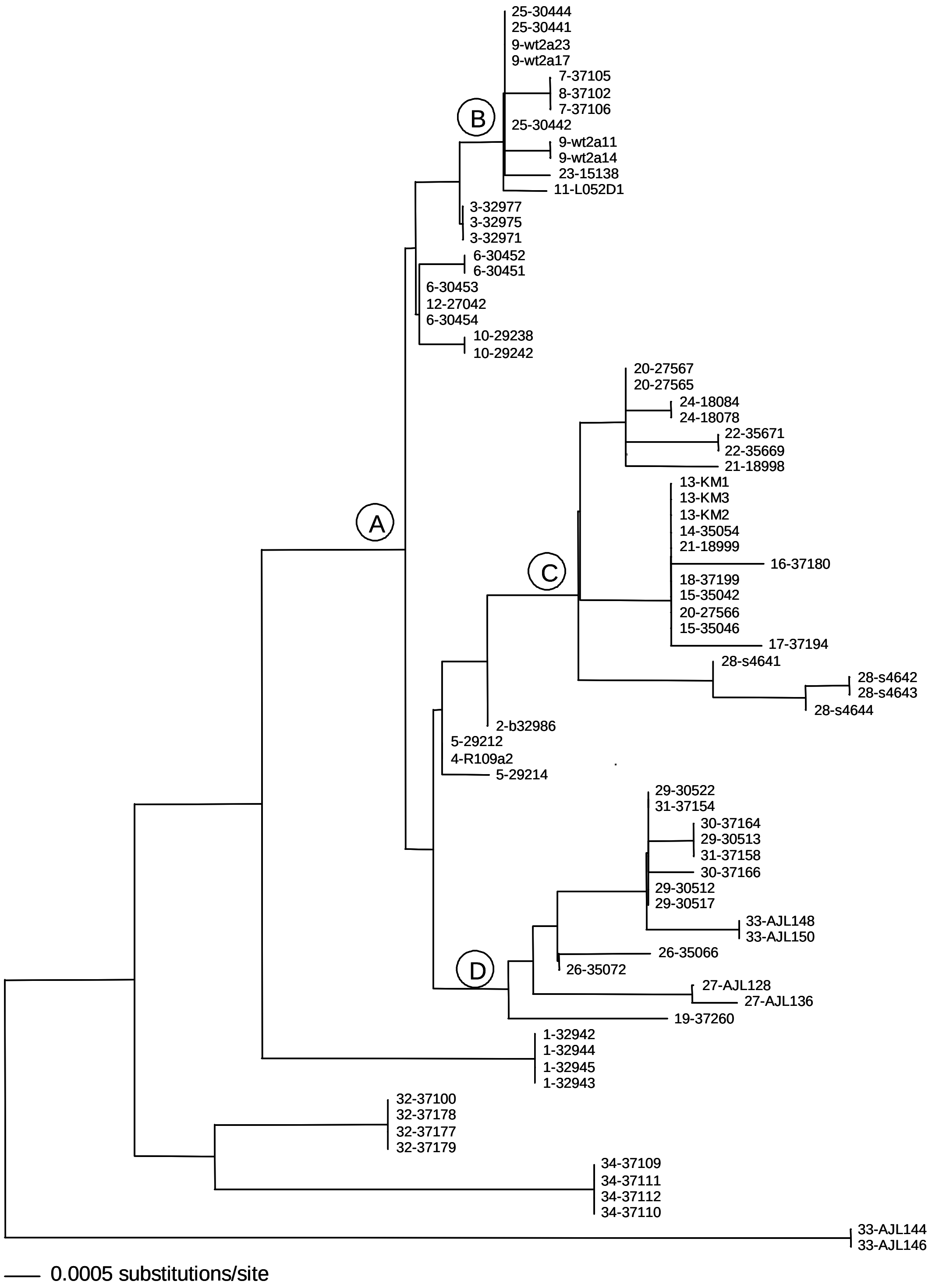

Figure 3.2. Neighbor-joining tree (phylogram)for 77 *Rana boylii* and one *R. muscosa* Cytochrome B/ND2 mtDNA sequences (1525 b) from 34 localities across its geographic range in California and Oregon. Node labels (A-D) correspond to the same nodes in the Bayesian tree (Fig. 3.3) and are discussed in the text.

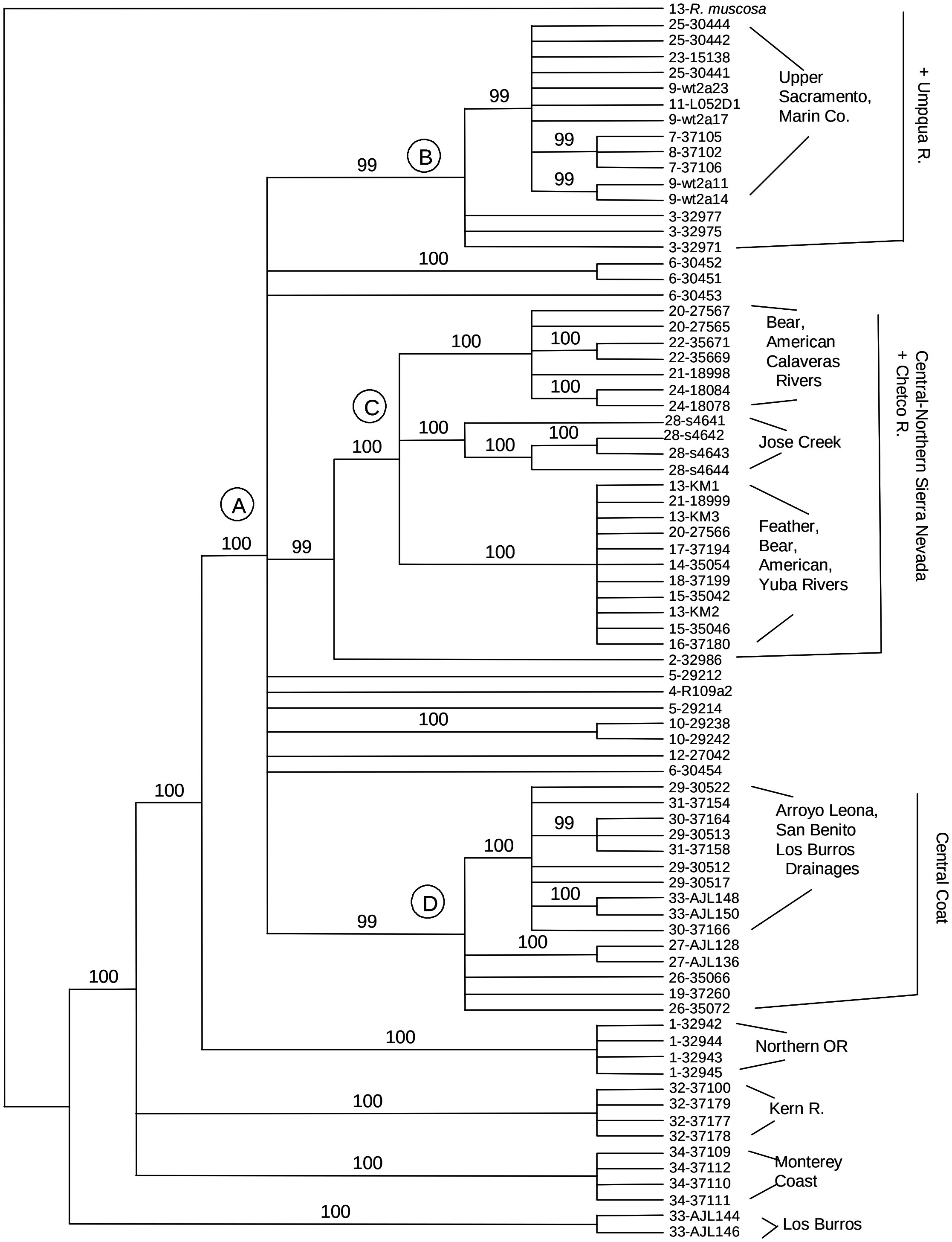

Figure 3.3. Bayesian tree for 77 *Rana boylii* and one *R. muscosa* Cytochrome B/ND2 mtDNA sequences (1525 bp) from 34 localities across its geographic range in California and Oregon. Posterior probabilities (as percents) from 95% majority-rule consensus tree appear above the branches. Node labels (A-D) are discussed in the text.

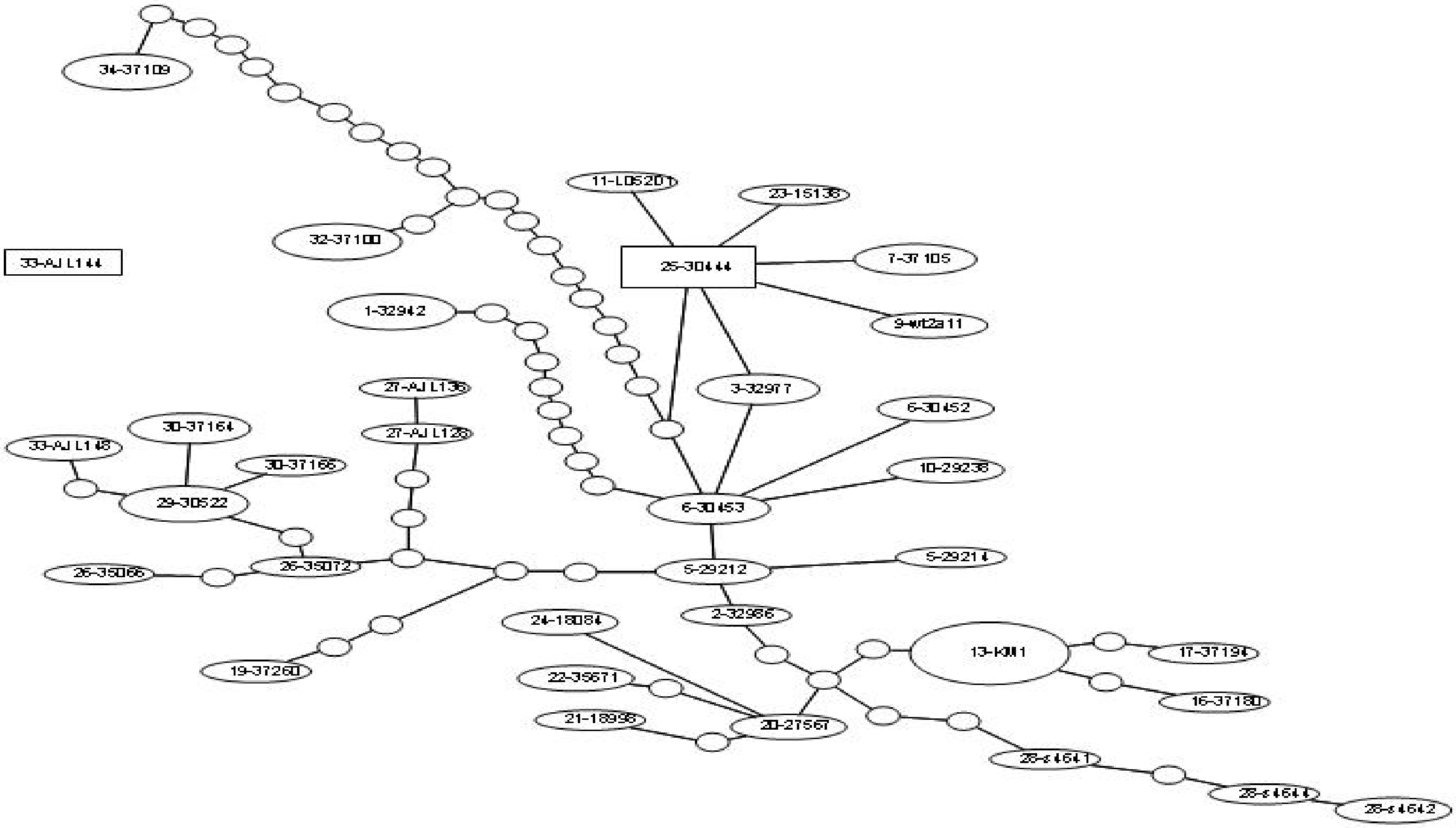

Figure 3.4. A haplotype network for 77 *Rana boylii* Cytochrome B/ND2 mtDNA sequences (1525 bp) from 34 localities across its geographic range in California and Oregon. Labeled nodes represent haplotypes and their size is proportional to the haplotype frequency. Nodes separated by only one line represent haplotypes that are one base pair different (one "missing haplotype"). Small unlabeled nodes represent additional missing haplotypes. Node 33-AJL144 could not be connected to the network by the maximum number of the steps allowed in the TCS software.

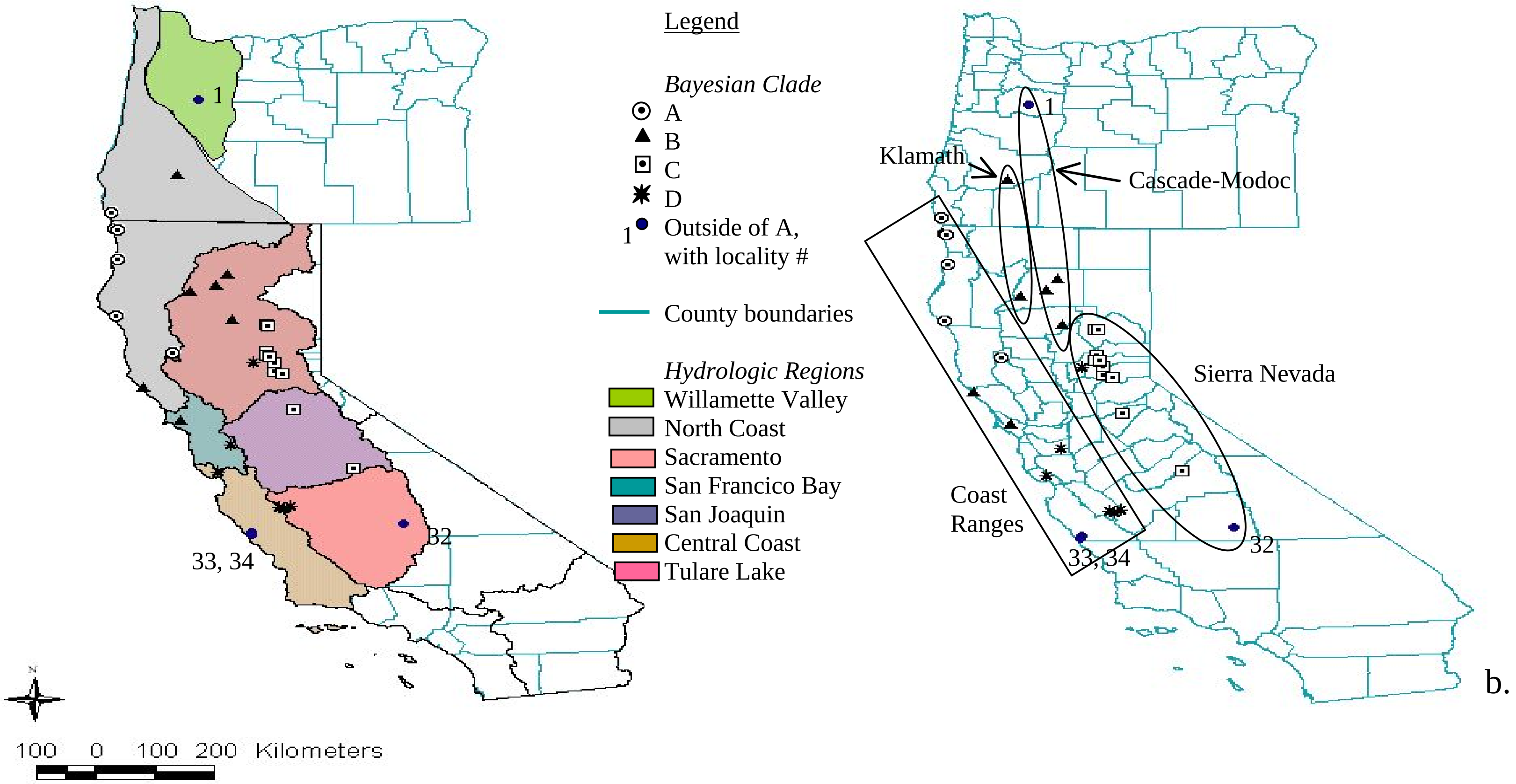

Figure 3.5. *Rana boylii* Bayesian tree clades (A-D) relative to (a) hydrologic regions and (b)mountain provinces. In this depiction, localities associated with clade A are those that were within that inclusive clade but outside of clades B, C, or D (Fig. 3.3). For points designated as "outside of A" locality numbers to indicate their unique status (Fig. 3.3).

Appendix 3.A. Locality information for *Rana boylii* and outgroup samples. Localities are arranged approximately north to south and numbered sequentially. Latitudes and longitudes when presented are in decimal degrees.

Rana boylii

1. HBS 32942, 32943, 32944, 32945 South Santium River, approx. 6km upstream of Foster Reservoir, above Sweet Home, Linn Co., OR. 44.405 N, 122.565 W.

2. HBS 32986 Chetco River, ~1.5 km upriver from Loeb State Park off of N. Bank Chetco Rd., Curry Co., OR. 42.125 N, 124.187 W.

3. HBS 32971, 32975, 32977 South Umpqua River, ~2 km upriver (along S. Umpqua Rd.) from Tiller Trail Highway, Douglas, Co., OR. 42.935 N, 122.939 W.

4. GMF R109a-2 Smith River near mouth of Cedar Creek, Del Norte Co., CA.

5. HBS 29121, 29214 Smith River near junction of Hwy 199 and South Fork Rd, Del Norte Co., CA.

6. HBS 30451, 30452, 30453, 30454 Redwood Creek, near Tall Trees via Tom McDonald Creek, 11.4 km from M-Line intersection, Del Norte Co., CA.

7. HBS 37105, 37106 Deep Creek near confluence with Pit River, Shasta Co., CA 40.97 N, 121.84 W.

8. HBS 37102 Little Cow Creek, along Hwy 299, 3.5 km (along road) southwest of turn-off to Oak Run Rd. 40.74 N, 122.07 W.

9. GMF WT002a-11,14,17,23 Brandy Creek at picnic area, Whiskeytown Recreation Area, Shasta Co., CA.

10. HBS 29238, 29242 Mattole River, near mouth of Big Finley Creek, Humboldt Co., CA.

11. GMF L052d-1 Deer Creek from Little Pine Creek upstream to bridge crossing of USFS Road 28N29, Ishi Wiilderness, Lassen National Forest, Tehama Co., CA.

12. HBS 27042 Thistle Glade Creek at Rice Creek Rd. / M3 Crossing, Lake Co., CA.

13. KM-1,2,3 Bean Creek, near crossing of USFS road 25N17, Plumas National Forest, Plumas Co., CA. 39.98 N, 121.09 W.

14. HBS 35054 Spanish Creek (trib to East Branch of NF Feather River), ~ 0.8 km downstream (along Bucks Lake Rd) from turn-off to Snake Lake, Plumas Co., CA. 39.95 N, 121.03 W.

15. HBS 35042, 35046 Oregon Creek, 100-150m upstream of Log Cabin Reservoir, Middle Fork Yuba River drainage, off Hwy 49, Yuba Co., CA. 39.44 N, 121.06 W.

16. HBS 37180 Shady Creek, ~ 0.4 km above Purdon Rd. crossing., Nevada Co., CA. 39.35 N, 121.06 W.

17. HBS 37194 Spring Creek, ~ 250m upstream of mouth (South Fork Yuba River), Nevada Co., CA. 39.33 N, 120.99 W.

18. HBS 37199 South Fork Yuba River, upstream and near mouth of Spring Creek, Nevada Co., CA. 39.33, 120.98.

19. HBS 37260 "Shubert Creek" in Shubert Watershed, ~0.4 km upstream of mouth (Yuba River), at University of California Foothills Research and Extension Center, Yuba Co., CA 39.24 N, 121.29 W.

20. HBS 27565, 27566, 27567 Missouri Canyon, tributary to Bear River, Nevada Co., CA. 39.22 N, 120.92 W.

21. HBS 18998, 18999 Small Stream in Shirt Tail Canyon, along Shirt Tail Canyon Rd. 1.1 km west of intersection with Yankee Jims Rd., Placer Co., CA

22. HBS 35669, 35671 Middle Fork American River, upstream of junction with USFS road 23, Tahoe National Forest, Placer Co., CA. 39.01 N, 120.73 W.

23. HBS 15138 South Fork Gualala River, 1 km east of Sea Ranch Airport., Sonoma Co., CA.

24. HBS 18078, 18084 Esperanza Creek, Calaveras Co., CA.

25. HBS 30441, 30442, 30444 Halleck Creek, Marin Co., CA.

26. HBS 35066, 35072 Arroyo Mocho Creek, ~4.7 km southeast along Mines Rd. from junction of Mines Rd. and Del Valle Rd., Alameda Co., CA. 37.60 N, 121.67 W.

27. AJL-128, 136 East Branch of Soquel Creek, near bridge along Hihnvilles Rd., ~ 200m downstream from mouth of Amaya Creek, Santa Cruz Co., CA.

28. GMF S464-1, 2, 3, 4 Jose Creek, tributary to San Joaquin River off Italian Bar Rd. off Jose Basin Rd., Fresno Co., CA.

29. HBS 30512, 30513, 30517, 30522 Arroyo Leona Creek, Interstate-5, Derrick-Halan exit, 2.1 km south, 17.5 km on dirt road, Fresno Co., CA.

30. HBS 37164, 37166 Clear Creek at "ORV staging area 2", Road R001, 5.5 km east of Coalinga Rd., San Benito Co., CA 36.37 N, 120.74 W.

31. HBS 37154, 37158 San Benito River at Sawmill Creek confluence, San Benito Co., CA. 36.34 N, 120.66 W.

32. HBS 37100, 37177, 37178, 37179 3rd unnamed tributary to Kern River north of Rincon Trailhead at Sherman Pass Rd., Rincon Roadless Area, Sequoia National Forest, Tulare Co., CA 36.02 N, 118.46 W.

33. AJL-144, 146, 148, 150 Los Burros Creek, near downstream road crossing of unnamed road, Fort Hunter Liggett, Monterey Co., CA.

34. HBS 37109, 37110, 37111, 37112 Dutra Creek, ~ 0.5 km above confluence with San Carpoforo Creek., Monterey Co., CA. 35.80 N, 121.29 W.

Rana muscosa

13. KM-7,8 Bean Creek, near crossing of USFS road 25N17, Plumas National Forest, Plumas Co., CA. 39.98 N, 121.09 W.

CHAPTER 4. MULTI-SCALE OVIPOSTION SITE CHOICE BY THE FOOTHILL YELLOW-LEGGED FROG (*RANA BOYLII*) IN CALIFORNIA: RESPONSES TO A STOCHASTIC ENVIRONMENT

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ABSTRACT

Studies of habitat selection are critical to understanding basic ecological and evolutionary attributes of species as well as for developing sound conservation practices. In this paper, we present an analysis of foothill yellow-legged frog (*Rana boylii*) relative abundance, habitat use, and selection, using data gathered from 11 localities across two geographic regions in California (Northern Coast Range mountains and Sierra Nevada mountains) from 1991-2002. This species is one of only a handful of anuran species associated with stream environments in the western United States. Our work focused on microhabitat scale oviposition site use and selection coupled with larger scale evaluations of occurrence and relative abundance at breeding areas. Oviposition microhabitat characteristics such as water depth, water velocity, and stream substrate varied little among large and small streams and between the disjunct geographic regions. Descriptive statistics showed very narrow ranges for these characteristics among study localities. At two localities where habitat availability data were recorded, variances were significantly lower for oviposition microsites than for randomly chosen points within breeding areas, indicating that habitat selection was occurring. Multi-year studies at two localities showed consistently high densities of egg masses and juvenile and adult frogs. At one locality where breeding area characteristics were quantified in detail, egg mass density showed negative relationships with mean water depths and variances of water depth and water velocity and a positive relationship with a variable representing the width (distance from the shoreline) of the potential breeding area. In total, these relationships indicate that breeding areas with large numbers of egg masses are generally in wide shallow

areas of streams with low water velocity. Our assessment of repeated use of breeding areas at two localities showed high repeated use rates at one locality, with 63% of breeding areas used consecutively for three years. At the other locality multi-year use was less consistent but several areas (“hot spots”) were used in 11 or more of the 16 years of the study. Our analyses of oviposition habitats at 11 localities spanning a substantial portion of *R. boylii*’s geographic range are consistent with earlier qualitative descriptions of breeding habitats and one more recent quantitative study conducted at a single locality. Together these results suggest that oviposition habitat selection and microhabitat specificity result in population stability for *R. boylii* even within the substantial temporal and spatial variability of stream environments. By selecting habitats at both meso and microscales that provide optimum conditions for egg development and rearing of larvae, frogs are optimizing reproductive success in these stochastic environments. Management approaches for stream environments and conservation plans for *R. boylii* should include strategies to preserve the hydrologic processes that produce these habitats as well as identifying and protecting high use breeding areas.

INTRODUCTION

The physical environment is the matrix within which organisms hone their ecological strategies and test their evolutionary mettle (Southwood 1977, 1988). A detailed knowledge of a species' habitat relationships then is key to understanding that organism's fundamental strategies for persistence (Holt 1987). Plasticity in a life history trait like habitat choice can be particularly vital in stochastic or seasonally changing environments (Fretwell 1972). Because amphibians have multiple life stages (i.e. complex life cycles, Wilbur 1980), it can be particularly challenging to unravel stage-specific habitat relationships and overall implications for species persistence. Information on habitat associations, and especially habitat selection, can contribute to an understanding of species' population dynamics and lead to more appropriate conservation and management approaches (Morris 2003). Land use change is one of the primary and best-supported hypotheses for explaining amphibian declines world wide (Collins and Storfer 2003). Consequently, the ability to quantity characteristics of environments that promote high fecundity and survival rates provides an important first step in improving management and restoring altered habitats for imperiled species. In fact, the surrounding environment (e.g. vegetation structure, hydrology, temperature) is often one of the only aspects of a species' ecology that can be immediately managed. This may be especially true for declining species, where opportunities for direct population intervention are limited by reduced ranges and low abundances. With these concepts in mind, we set out to quantitatively describe the oviposition habitat of a declining frog species in California, the foothill yellow-legged frog (*Rana boylii*).

Rana boylii historically occurred in foothill and mountain streams (to 1830m) from northern Baja California, Mexico to southern Oregon west of the Sierra-Cascade crest. This species has experienced significant populations declines, especially in the southern part of its range (southern Sierra Nevada and southern coast range of California) and is currently listed as a California State Species of Special Concern (Jennings and Hayes 1994, Jennings 1996, California Department of Fish and Game 2004). *R. boylii* is one of a handful of western North American anurans that are almost exclusively associated with running waters (lotic environments); most other frogs occur in lentic environments such as ponds and wetlands. Breeding and oviposition occur in spring and females deposit a single egg mass which consists of from several hundred to over 1000 eggs. Tadpoles (larvae) rear in and near oviposition areas and metamorphose in early autumn (Nussbaum et al. 1983).

Early natural historians depicted *R. boylii* breeding habitat as relatively open river bars in streams with rocky substrates. Egg masses were described as being laid in spring and early summer, on or near the bottom of shallow, low water velocity areas, attached to pebbles or cobbles (Storer 1925, Fitch 1936). Recent research confirms these historical observations (Kupferberg 1996, Lind et al. 1996) and indicates that egg attachment substrates are sometimes “cleaned” (of algae or fine sediment) by adults prior to oviposition (Wheeler et al. 2003), presumably to increase adhesion. Apart from these primarily qualitative accounts, little is known about the basic natural history characteristics and habitat associations of *R. boylii*. The seminal paper on the ecology and systematic relationships of this species in relation to other ranid frogs, is now 50 years old (Zweifel 1955). Research since then has been piecemeal, focusing on aspects of habitat associations (Hayes and Jennings 1988, Fuller and Lind 1992, Kupferberg 1996), vocalizations (MacTague and Northen 1993, Ziesmer 1997), and environmental stressors and population declines (Jennings 1988, Jennings and Hayes 1994, Drost and Fellers 1996, Lind et. al. 1996, Davidson et al. 2002). Recent Master's theses have provided new information on basic ecology, physical habitat, and movements for populations in the northern Sierra Nevada mountains of California (Van Wagner 1996, Yarnell 2000) and Oregon (Borisenko 2000). Our goal in this study was to examine breeding habitat associations over a relatively large portion of this species' geographic range and develop quantitative models.

Habitat associations can be described at a variety of spatial scales from microenvironment to landscape. In stream (lotic) environments, spatial and temporal heterogeneity are the rule (Frissell, et al. 1986, Ward 1989). Understanding how lotic-associated species have adapted to the variable conditions of their environment at multiple scales is essential for management and conservation, especially in regulated (dammed or diverted) streams. This paper addresses habitat associations of the *R. boylii* at multiple spatial scales in an attempt to understand both the distribution and use of breeding areas and the characteristics of oviposition microsites. We focused on oviposition habitats because previous work had suggested that survival rates of egg and larval life stages were strongly influenced by variability in stream flows and channel morphology (Kupferberg 1996, Lind et al.1996). Our primary objectives were to: (1) quantify oviposition microhabitat associations of *R. boylii* in two geographic regions of California (Northern Coast Range and Sierra Nevada), (2) examine relative abundance of this species over multiple breeding seasons and relate it to breeding area characteristics, and (3) document breeding area use and spatial distribution over time (years). This work was conducted at the stream reach (segment), breeding area, and the microenvironment scale. Stream reaches (on the order of kilometers in length) were studied to determine relative abundance, distribution of breeding areas, and breeding area use over time. Characteristics of breeding areas (on the order of tens of square meters) were related to reproductive effort. Within breeding areas, the microhabitat characteristics of individual egg masses were described in terms of use and selection (use relative to available environments).

STUDY AREAS

Rana boylii habitat associations data were collected at 11 localities – 7 in the Northern Coast Range region of California and 4 in the central and northern Sierra Nevada (Table 4.1, Fig. 4.1). The Northern Coast Range region included areas north of the San Francisco Bay and west of the Sacramento River drainage in California. The Sierra Nevada region was synonymous with the mountain range of the same name in eastern California. Localities were categorized as rivers or creeks, based on both their name and on their designation in the CALWATER database (CALWATER 2.2. – California Interagency Watershed Mapping Committee 1997). All rivers in this study were CALWATER “hydrologic units or areas” and their corresponding watersheds ranged in size from 63,000 to 174,000 hectares. All creeks were CALWATER “planning watersheds” or smaller and their corresponding watersheds ranged in size from 1200 to 4000 hectares. The term stream is used throughout the paper to designate both creeks and rivers. Oviposition microhabitat use was evaluated at all 11 localities. Microhabitat use relative to availability, relative abundance of one or more life stages, and breeding area characteristics and use were examined in detail at 3 of the 7 localities in the Northern Coast Range of California (Hurdygurdy Creek, mainstem Trinity River, and South Fork Trinity River) (Table 4.1).

METHODS AND ANALYSIS

Microhabitat Use

We gathered on data oviposition microhabitat of *R. boylii* over the course of 12 years (1991-2002). One measurement of each of 10 continuous and categorical variables was recorded immediately adjacent to each egg mass (= microhabitat variables): stream habitat type, water velocity, water depth, water temperature, distance from shore, substrate size, attachment substrate, orientation of egg mass, direction of water flow, and gosner stage. Appendix 4.A lists describes how each variable was measured and treated in subsequent analyses. We first conducted descriptive analyses on all variables to examine mean values among stream localities, stream size (river or creek), and geographic regions (Northern Coast Range and Sierra Nevada). We also used graphical interpretation of principal components analysis of the five continuous microhabitat variables (water velocity, water depth, distance from shore, water temperature, and substrate surrounding the egg mass) to evaluate variability relative to stream size and region.

To examine variation in oviposition microhabitat among sampled localities and address habitat associations in a more quantitative way, the five continuous oviposition microhabitat variables were analyzed using analysis of variance (ANOVA). We developed one “mixed” model (Littell et al. 1996) for each microhabitat variable such that the stream locality factor and the egg masses within stream locality were defined as random effects and the stream size (creek or river) and geographic region (Northern Coast Range or Sierra Nevada) factors were considered fixed effects. An interaction term of stream size and geographic region was also included as a fixed effect. We used contrasts to compare microhabitat differences among streams within stream size or geographic region if we determined that stream localities differed within one of these factors. The unit of analysis for these models were individual egg masses (n= 285). Missing values for some variables meant that not all streams could be evaluated for each variable and actual sample sizes ranged from 223-276. For the substrate variable, only stream size could be evaluated because no substrate data were recorded for the Sierra Nevada localities. All of these analyses were done with an appropriate covariance structure in place, allowing for differences in variance between the two stream sizes and/or among geographic regions. For all tests, *alpha* was set at 0.05 and adjusted as necessary for multiple tests using a Bonferroni correction.

Habitat Selection

The descriptive data that we recorded where egg masses were found (i.e. use information) provided a context for understanding habitat associations and the distribution of *R. boylii* egg masses within breeding areas, but was not a definitive metric for habitat choice. Recording analogous data at a set of randomly chosen, unused points provided an assessment of available habitat which allowed us to make conclusions about habitat selection. Our expectation was that used sites would exhibit less variability than the surrounding available environment but would necessarily be a subset of that environment. Thus differences between both means and variances of used and available site variables were evaluated. We examined microhabitat characteristics of egg mass positions and randomly selected points (within the breeding area) at two localities – the mainstem and South Fork of the Trinity River. We conducted surveys of potential breeding areas at both localities in the spring of 1992. Based on literature accounts and experience, we defined potential breeding habitat as the stream environments along sparsely-vegetated, rocky river bars. A bar is a geomorphologic feature created by deposition of stream-mobilized sediments (all sizes) on the inside of stream/river bends (Trush et al. 2000). Used microsites were defined by the presence of an egg mass. Availability of habitat attributes was assessed by collecting data at randomly selected points along line transects placed in the vicinity of egg masses. Both continuous and categorical data were recorded at egg masses and for randomly selected points (see Appendix 4.B for details).

For both the mainstem and South Fork Trinity River data, we used a multivariate paired t-test (Hotelling's T-Square) to evaluate use vs. availability for four variables simultaneously: water depth, distance from shore, water temperature, and water velocity. This analysis was followed by univariate paired t-tests of each variable to provide better resolution of the overall difference. We also compared the variances of our paired samples using a modified t statistic (Snedecor and Cochran 1989: 196-197). This was done separately for each of the four variables because we were not aware of a multivariate equivalent. For all tests, significance was evaluated at *alpha* = 0.05.

Relative Abundance and Breeding Area Use

We conducted studies of relative abundance of egg masses and breeding area use over time at three stream localities: Hurdygurdy Creek, mainstem Trinity River, and South Fork Trinity River. Reproductive activities were observed at Hurdygurdy Creek from 1987-2002, but detailed data were not recorded for all years. Surveys at this locality involved two to three observers (at least one on each stream bank) moving upstream searching the near shore environment of a 4.7 km stream reach, in the spring and/or early summer of each year. Egg masses were counted and located based on a stream habitat units (e.g. riffle, run, pool) map developed to study an ophidian frog predator at the same locality (Welsh and Lind, *In Prep*.; see Fig. 4.7 inset for an example). Presence of *R. boylii* larvae at mapped habitat units was also recorded both during the breeding season (late April-June) and in mid-summer.

Multi-year surveys of breeding areas were also conducted at the mainstem Trinity River from 1991-1994 and at the South Fork Trinity River from 1992-1995. Results from the mainstem Trinity surveys were presented elsewhere (Lind et al. 1996) and are included only in the discussion of this paper. At the South Fork Trinity River locality, surveys were conducted on a 15.6 km section of the river over several days each spring. Observers traveled downriver by boat and stopped to survey all river bars greater than 5 m in length. Each river bar was given a unique number and mapped on an aerial photograph to allow identification in subsequent years. One observer searched each river bar and near shore waters by walking along the shoreline, first upstream then downstream, scanning for all life stages of *R. boylii*. Egg masses, juvenile frogs (less than ~50 mm snout to vent length), and adult frogs were counted individually and larval numbers were estimated or sub-sampled. All river bars in the 15.6 km reach (n=80) were searched in 1992-1994, and a subset (n=29) on a 5.7 km section of river in the middle of the study reach, were re-visited in 1995. Data were analyzed for both the 80 bars surveyed for three years and the 29 bars surveyed in all 4 years.

For both localities (Hurdygurdy Creek and South Fork Trinity River), abundance data were summarized and presented descriptively relative to the total length of each study area. For the South Fork Trinity River, we also calculated a relative density based on the actual length of each river bar surveyed. Having habitat availability data for the South Fork Trinity from 1992 (described above) allowed us to relate egg mass densities to characteristics of river bars using regression analysis. Here, the river bar was the unit of analysis. All points along line transects (rather than one random point per transect, see Appendix 4.B) were used to derive means and variances for four continuous variables (distance from shore, water depth, water velocity, and water temperature) for each river bar. These values were then related to egg mass densities using All Possible Subsets regression. The "best" models were selected based on the highest adjusted R^2 values. Egg mass densities exhibited substantial departure from normality and were natural log-transformed prior to analyses.

Repeated use of breeding areas (river bars for the South Fork Trinity River localities and habitat units for Hurdygurdy Creek) over time and occupancy rates (percent of river bars used each year) were analyzed using descriptive statistics and maps. Use was defined as the presence of either egg masses or larvae during the breeding season or larvae later in the summer. Because of the observational and opportunistic nature of the data collection at Hurdygurdy Creek, we limited our data analysis as follows. A subset of five years (1991, 1992, 1998, 1999, 2000) in which standardized surveys were done, was used to develop estimates of the relative abundance and density for the entire 4.7 km study reach. Oviposition “hot spots” were identified as those stream habitat units that were observed to contain egg masses or larvae in at least one third of the total years of this study (six or more of 16 years). All breeding areas were mapped using ArcView 3.2 G.I.S. (Environmental Systems Research Institute [ESRI]1999) but descriptive analyses of nearest neighbor distances between breeding areas, and distances from breeding areas to tributary streams, were calculated only for hot spots. These distances were computed using a measuring tool available in the G.I.S. software.

All data analyses in this study were conducted using SAS/STAT software, Version 8 of the SAS System for Windows (SAS Institute 1999). Prior to statistical analyses, the normality of all variables was examined and transformations were used where necessary.

RESULTS

Microhabitat Use

The five continuous variables describing oviposition microhabitats (water velocity, water depth, distance from shore, water temperature, and surrounding substrate size) fell in fairly narrow ranges both within and across localities (Table 4.2). Microhabitat characteristics were remarkably similar among stream sizes and geographic regions, and within these factors among stream localities. In fact, we found only one significant result in our ANOVAs – water depth of egg masses was significantly greater for river localities than for creek localities (Table 4.2). Egg mass positions as described by categorical variables indicated that the majority of egg masses among all localities were attached to cobble and boulder substrates in glide and edgewater pool stream habitats. Egg masses were typically attached on the downstream side of substrates in such a way that water would flow along the side of the mass (Fig. 4.2, Appendix 4.C).

Based on a graphical examination of principal components analysis in the Northern Coast Range region, oviposition sites in creek localities grouped more tightly than did sites at river localities on two axes of variation (Fig. 4.3a). These first two components accounted for a total of 57% of the total variation in the data set. Water depth, water temperature, and substrate size were associated with the first component and water velocity and distance from shore were associated with the second. Though overlap between the two sizes of stream (creek and river) on these axes of variation was complete, egg masses at creek localities tended to be closer to shore in shallower, cooler, lower velocity water, with larger substrates (Fig. 4.3a). Comparing the Northern Coast Range and Sierra Nevada regions there was complete overlap between the regions on the first two principal components (Fig. 4.3b). However, the Sierra Nevada localities grouped more tightly in both cases. The first two components accounted for 78% of the total variation. Water depth and water temperature were associated with the first component and distance from shore with the second. Sierra Nevada oviposition sites were typically closer to shore in relatively deeper and warmer water (Fig. 4.3b).

Habitat Selection

For the mainstem Trinity River, we found differences between used and available microhabitat both in the multivariate means and in the univariate variances of point characteristics. Two variables, water velocity and water temperature, were responsible for the overall mean differences, whereas water depth and water velocity showed differences in their variances (Table 4.3). In terms of the direction of differences, the mean and variance of all variables were lower for oviposition site microhabitat (used) than for the available microhabitat (random points) (Table 4.3).

For the South Fork Trinity River, we found no differences in the overall mean between used and available for any of the microhabitat attributes (distance from shore, water depth, water velocity, and water temperature) with the multivariate approach. Univariate paired t-tests confirmed this result, with water temperature showing only a slight tendency toward a difference in the mean conditions (p=0.0995) (Table 4.3). However, the variances of available and used microenvironment attributes were significantly different (as determined by paired t-tests of variance) for three of the four variables (water depth, water velocity, and water temperature). In all cases, the variances of oviposition point locations (used) were less than those of available point locations indicating that used areas represent a specific subset of conditions within the available environment (Table 4.3).

Relative Abundance and Breeding Area Use

At the South Fork Trinity River locality, egg mass densities were high (range 35-47 / km) and consistent among years for both the three year (Fig. 4.4a) and the four year (Table 4.4) data sets. Over 63% of the 80 river bars were used for breeding in all 3 years (1992-1994) (Fig. 4.4b) and over 72% of 29 river bars used for breeding in all four years (1992-1995) (not shown). The average (over four years) occupancy rate of the 29 river bars was 83.2% (± 4.5 se) (Table 4.4). Detection rates of juvenile and adult frogs were more variable throughout the study, possibly due to the timing of sampling; sampling occurred in May in 1992 and 1993 but not until late June/early July in 1994 (Fig. 4.4a). Examining density of egg masses relative to characteristics of river bars at the South Fork Trinity River in 1992, indicated that habitat means and variances both demonstrated explanatory power. The best multi-variable model for mean characteristics (chosen by adjusted R^2) included both distance from shore (+) and water depth (-) and explained 24% of the variation in egg mass density. The best multi-variable model for variance characteristics (chosen by adjusted R^2) included distance from shore (+), water depth (-), and water velocity (-) and explained over 38% of the variation in egg mass density (Table 4.5). The positive relationship with distance from shore combined with the negative relationship with water depth is indicative of wide shallow areas as optimum breeding sites (Fig. 4.5).

At Hurdygurdy Creek, egg mass densities were consistently high for the five years with systematic surveys (range 19-30 / km; Fig. 4.6). Breeding area occupancy rates exhibited a more complex pattern than at the South Fork Trinity locality in that a few habitat units had high levels of repeated use, but most did not. Specifically, of the 51 habitat units found to have breeding over 16 years, five were used as oviposition sites in 10 or more years, and 10 were used in six to nine years. Thirty-six stream habitat units were used for oviposition in five or fewer years (Fig. 4.7). For the 15 habitat units used as oviposition sites in six or more years (hot spots), most averaged moderate numbers of egg masses over the 16 years (2-8 per habitat unit, per year) but one habitat unit averaged 26 egg masses per year, with evidence of reproduction (as either eggs or larvae) in all years. Results from the G.I.S. analysis indicated the average stream distance between all nearest neighbor pairs of breeding hot spots was about 300 m and the average distance to the closest up or downstream tributary was almost 800 m (Table 4.6, Fig. 4.7). All hot spots were associated with braided or multi-channel areas of the stream; 11 of 15 were immediately upstream of these areas and 4 were in secondary channels within such areas.

DISCUSSION

To date, the majority of research on *R. boylii* has been descriptive and qualitative and many aspects of this frog's ecology are poorly known. Nevertheless, the results from our work are concordant with both previous qualitative and quantitative accounts of *R. boylii* habitat associations. The overall picture that emerges from our analyses is that *R. boylii* prefers shallow, low water velocity areas, in protected locations, with cobble-sized substrates for oviposition and the characteristics of these sites are less variable than the surrounding environment.

The most intriguing results of our work on habitat associations and breeding area use of *R. boylii* are the parallels across widely separated geographic regions. These similarities provide strong support for breeding habitat selection by this species and have significant repercussions for its survival in both regulated and non-regulated river systems. All of our analyses indicated that a narrow range of environmental conditions were used for oviposition sites in both small and large streams and from the Northern Coast Range to the Sierra Nevada. These conditions were also clearly selected for as demonstrated by results from the Trinity River basin. The geographic scope of our work provides evidence that these affinities may be pervasive in *R. boylii*.

Microhabitat Use and Habitat Selection

The narrow ranges of environmental conditions used by breeding *R. boylii* are consistent with both earlier qualitative descriptions (Storer 1925, Fitch 1936, Zweifel 1955) and more recent quantitative work (Kupferberg 1996). The fact that creek localities grouped more tightly than river localities, and Sierran localities grouped more tightly than Northern Coast Range localities, on principal component axes (Fig. 4.3), may indicate differences in availability of conditions across this range of basin sizes and geographic regions. These results highlight the need for collection of site-specific ambient environmental conditions to better understand habitat use and selection. However, even with this inherent variability, *R. boylii* oviposition sites at localities that were widely separated geographically and with substantial variability in stream size, demonstrated surprisingly similar characteristics for all but one variable (Table 4.2). The differences we observed in water depth at oviposition sites between creek and river localities can be attributed to differences in the geomorphologic features of these stream types. Available habitat in large rivers is likely to include more deep water than shallow water areas due to the higher volume of water passing though these systems.

Beyond these descriptive results and univariate analyses, results from comparisons with the available environment in breeding areas make an even stronger case for habitat selection. While logic dictates that used environments should be a subset of available conditions, this has not often be demonstrated quantitatively, especially for amphibians (but see Welsh and Lind 2002). Our results from two localities confirm that for three variables describing oviposition sites (water depth, water velocity, and water temperature), the mean and/or variance differed between used sites and a set of random points representing the available environment. In all cases, the values at the used sites were lower than those of the available points (Table 4.3). However, use of point measurements (as opposed to continuously recorded data) of water temperatures can be problematic. In our study, data were collected at nearly the same time for the egg mass points and the representative available points, though data on egg mass points was usually collected first. The potential bias from this approach is that water temperatures rise throughout the day. For the mainstem Trinity River locality, we did find significantly higher mean temperatures for the available point data than for the used oviposition points. However, at the South Fork Trinity locality, we found only the variance of water temperature to differ significantly, indicating that perhaps the data are not biased in one direction or another. Evidence for selection of breeding areas at both the microenvironmental and stream reach spatial scales has also been documented at the South Fork Eel River (Kupferberg

1996). In that work, water velocity was also a key variable, and it was closely linked to selection of breeding areas that offered refuge from high flow events. Our results along with Kupferberg's, provide strong evidence for habitat selection of particular environmental variables.

Relative Abundance and Breeding Area Use

The models that best explained egg mass density at the South Fork Trinity River incorporated distance from shore and water depth as key variables. Together these variables were indicative of wide, shallow areas as prime breeding sites for many frogs or at least areas that can accommodate large numbers of egg masses and ultimately larvae. In addition the negative association with variance of water velocity demonstrated the importance of the availability of uniform low velocity areas for oviposition (Table 4.5, Fig. 4.5). Water depths in such areas would also fluctuate less dramatically as water pulsed through these stream systems during spring rain and snow melt events.

At both localities where detailed multi-year work was done (South Fork Trinity River and Hurdygurdy Creek), *R. boylii* showed consistently high densities, high occupancy rates of habitat and high repeated use of breeding areas. However there were some apparent differences among these two localities and the mainstem Trinity River. The South Fork Trinity River had similar egg density and rates of repeated breeding area use to those at Hurdygurdy Creek. Both the South Fork locality and Hurdygurdy Creek had substantially higher egg mass densities (ranging from 19-47 / km) than did the mainstem Trinity (range <1 – 1.7 / km; see Table 1 in Lind et al. 1996). The mainstem Trinity River locality was downstream of a dam and the amount of potentially suitable breeding habitat was very low compared to the other two localities as a result of altered environmental conditions in this highly regulated river (Lind et al. 1996). At the South Fork Trinity River locality, breeding habitats were limited to shallow water adjacent to river bars which occurred in a patchy pattern along the river. At Hurdygurdy Creek, potential breeding habitats were more continuous because shallow edgewater habitats were available along much of this smaller stream, though wide, shallow water areas that could accommodate large numbers of egg masses had a more limited distribution. Nevertheless, there were several breeding hot spots at Hurdygurdy Creek where repeated use over many years was similar to the South Fork Trinity River – i.e. four breeding areas were used in 10 or more years and one breeding area was used in all 16 years of the study.

Hot spots at Hurdygurdy Creek appeared to be associated with some geomorphic features such as braided channel areas, but not others (i.e. tributary streams). The wide, low gradient, open canopy characteristics of braided reaches offer more suitable oviposition sites because they have warmer water and foster greater algae growth, allowing more larvae to survive and develop quickly. Conditions such as these were more common at the South Fork Trinity locality, where high water flows scour riparian vegetation from river bars annually, than at Hurdygurdy Creek. Depositional areas similar to those used by *R. boylii* in the Northern Coast Range have been identified as biodiversity hot spots in bedrock type Sierra Nevada streams (McBain and Trush 2004). Kupferberg (1996) found that at the stream reach scale, breeding areas were closer to tributary streams than would be expected by chance. This pattern did not hold at Hurdygurdy Creek where breeding hot spots were distributed throughout the study reach with no apparent patterns relative to tributary location (Table 4.6, Fig. 4.7). We suspect that this may be due to the relative sizes of these drainages. In the larger South Fork Eel River locality studied by Kupferberg (1996), perennial tributary streams may have played a more important role as refugia from both summer heat and winter/spring high flows along the river. At Hurdygurdy Creek shaded areas are found in close proximity to breeding areas along the main channel and few of the tributary streams are perennial.

Though much of the work on amphibian site fidelity has been done on species that use lentic environments (e.g. Reading et al. 1991, Sinsch 1992, Hels 2002), amphibians clearly differentiate between suitable and unsuitable breeding areas even in more continuous lotic environments. Repeated use of the same river bar or habitat unit over many years (at Hurdygurdy, many years beyond the suspected life expectancy) indicates that adults likely return to the same areas to breed each year and that their offspring may also use these areas. The details of these behaviors are under investigation by researchers currently working at Hurdygurdy Creek (e.g. Wheeler et al. 2003), but some explanations for the advantages of use of these areas by multiple frogs over multiple years can be generated based on our work. For instance, in one breeding hot spot at Hurdygurdy Creek, an average of 26 egg masses were found per year over the 16 years this area was surveyed. Breeding aggregations such as these may increase the likelihood that more individuals will successfully find mates (Wells 1977). Survival rates of larvae are likely enhanced in these areas due to optimum conditions of temperature and food availability, even with increased competition from conspecifics (Kupferberg 1997a&b). Individual predator risk would be reduced with faster developmental rates and as a consequence of being in a large aggregation (Wilbur 1980). Use of shallow water environments could reduce predation by some (salmonid fish and Pacific giant salamanders, *Dicamptodon tenebrosus*) but not all (garter snakes and wading birds) predators.

The Role of Environmental Stochasticity in Habitat Selection and Breeding Area Use

Our results indicate that habitat selection by *R. boylii* may lead to large population sizes which can both resist and recover from disturbances at multiple spatial and temporal scales (e.g. Holt 1987, Townsend and Hildrew 1994). Streams are extremely dynamic systems with disturbance regimes that span a large range of temporal and spatial scales (Townsend and Hildrew 1994, Allan 2000). Stream fauna experience annual disturbance events that change their environment through transport and deposition of small substrates, changes in pool depths, and minor shifts in channel positions (Trush et al 2000). These changes may alter conditions (e.g. water depths and velocities) for *R. boylii* at the scale of oviposition sites and require local (on the order of meters) shifts in habitat use. Perhaps of more significance are large scale flood events (10-20+ year recurrence) that alter stream channels extensively, changing the position and order of habitat units (e.g. the pool-riffle sequence) along the stream as well as shifting water pathways into previously dry channels (Frissel et al. 1986, Trush et al. 2000). This scale of change could result in relocation of *R. boylii* breeding areas to newly created stream habitat units, bars, or channels. By examining breeding area use and habitat selection at small (oviposition sites) and medium (breeding areas – river bars or habitat units within a stream reach) spatial scales and over relatively long time frames, an understanding of the adaptations of *R. boylii* to these heterogeneous habitat templates (e.g. Southwood 1988, Townsend and Hildrew 1994) begins to emerge.

Temporal and spatial dimensions are two well-defined and studied aspects of stream environments (Townsend and Hildrew 1994). *Rana boylii* adjusts to variable conditions in these dimensions by shifting their breeding habitat use in both space and time. When environments are altered by large flood events, frogs may shift breeding areas up or downstream to find appropriate conditions (Fig. 4.7). Selection for breeding areas and specific characteristics within these areas then ensures higher survival rates. In terms of temporal shifts, *R. boylii* has both an extended breeding season (on the order of weeks) and is known to respond to variability in temperature and flow regimes by changing the timing of initiation of breeding (Kupferberg 1996, Lind and Welsh 1997 and unpublished data). Overall then, these characteristics are indicative of adaptation to disturbances at several spatial and temporal scales. Holt (1987) theorized that recovery from disturbance would be improved for small population sizes if habitat selection was present. Thus *R. boylii* presumably has developed affinities to particular breeding habitat conditions to increase survivorship following both small, annual disturbance events (winter/spring high flows) as well as larger events (e.g.10-20+ year floods). However, extreme events have been blamed for at least one regional extinction of *R. boylii*. Sweet (1983) proposed that a 500-year flood event may have been responsible for the regional extinction of *R. boylii* in the Southern Coast Ranges of California in 1969. Recovery from events of this magnitude would depend not just on local conditions but on the status of the species in surrounding regions, the hospitability of intervening habitat, and dispersal abilities (Blaustein et al. 1994).

Implications for Conservation and Management

Rana boylii is declining in some areas of its geographic range with suspected factors being habitat alteration (especially hydrologic changes resulting from dams), and alien predators/competitors (Jennings and Hayes 1994, Kupferberg 1996 and 1997a, Lind et al. 1996 and 2003, Davidson et al. 2002). Our abundance data from relatively robust populations in the Northern Coast Range (Tables 4.4 and 4.6, Figs. 4.4a and 4.6), provide baseline information and target recovery population sizes for areas where *R. boylii* is declining. The repeated use of the same river bar or habitat units seen in this work is an indication that habitat selection is occurring not just at the microscale (as shown by use and availability analyses) but at larger spatial scales. Knowledge of repeated use of breeding areas should lead to conservation approaches that protect reaches of stream that contain high-use areas and allow for these areas to shift over time in response to disturbance events. Townsend and Hildrew (1994) predicted that populations in areas with high spatial environmental heterogeneity are less like to be affected by temporal variations. Similarly, because populations at equilibrium use both high and low quality (sink) environments, it may be of equal relevance to managers to understand the role of these lower quality areas in the overall dynamics of populations and metapopulations (Morris 2003). Thus dynamic protection of hot spots and attention to the processes that produce such areas is recommended.

Because *R. boylii* is clearly adapted to the natural disturbance regimes in streams, restoration of natural flow regimes should be a priority in the conservation and recovery of this species. Environments downstream of dams exhibit lower habitat quality for *R. boylii* and have flow regimes that mask or alter ambient environmental conditions such as rainfall events and water temperature fluctuations (Lind et al. 1996). The synthesis of habitat association information provided here along with the specific results of this study (especially Tables 4.2 and 4.3 and Figs. 4.2 and 4.3) could be used to develop both qualitative and quantitative oviposition habitat suitability models for this species. With the addition of recent work on habitat affinities of other life stages (VanWagner 1996, Yarnell 2000) and forthcoming work (e.g Wheeler et al. 2003), our understanding of the ecology of *R. boylii* is improving dramatically. It is essential for the conservation of *R. boylii* that the results of these studies be applied to land and water management activities across the range of this species.

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Table 4.1. Localities (stream number, name, river basin and county), water management status (regulated or unregulated), years studied, and numbers of egg masses for *Rana boylii* ovipostion habitat research in Calfornia. Regulated means the presence of a dam in the proximity of the study area.

Stream Locality	**Regulated (reg) or non-regulated (non) System**	**Years**	**Study Reach Length (km)**
Northern Coast Range			
1. Hurdygurdy Creek, Smith River, Del Norte Co.	non	1987-2002	4.70
2. Red Cap Creek, Klamath River, Humboldt Co.	non	1994	0.10
3. Mainstem Trinity River, Trinity Co.	reg	1991-1994	22.50
4. South Fork Trinity River, Trinity Co.	non	1992-1995	15.60 (5.7, 1995)
5. Mattole River, Humboldt Co.	non	1994-1995	0.10
6. South Fork Eel River, Mendocino Co.	non	1994	0.10
7. Trib.Mark West Creek, Russian River, Sonoma Co.	non	1995	0.10
Sierra Nevada			
8. North Fork Feather River, Plumas Co.	reg	2002	1.00
9. Clear Creek, Yuba River, Nevada Co.	non	1993-1995	0.81
10. Rose Creek, Stanislaus River, Tuolumne Co.	non	2001	0.65
11. North Fork Tuolumne River, Tuolumne Co.	non	2001	0.30

Table 4.2. Summary statistics for four continuous variables describing oviposition microhabitat of foothill yellow-legged frogs (*Rana boylii*) from eleven localities/rivers in the Northern Coast Range and Sierra Nevada regions of California. Analyses of variance (ANOVA), presented at the bottom of the table, included fixed main effects of stream size (creek or river) and geographic region (Northern Coast Range and Sierra Nevada), an interaction term for these two factors, random effects of stream locality overall and pairwise contrasts within each level of each fixed factor. However, only simple main effects results are presented because no interactions or contrasts were found to be significant. For all tests, alpha was set at 0.05 and adjusted as necessary for multiple tests using a Bonferroni correction. Least square means (lsmeans) are presented for significant results.

Stream Locality	**Water Velocity (m/sec)**			**Water Depth (cm)**			**Distance from Shore (m)**			**Water Temperature (^{o}C)**			**Substrate Size (weighted mean, in mm)**		
	n	**$\bar{x}$**	**sd**	**n**	**$\bar{x}$**	**sd**	**n**	**$\bar{x}$**	**sd**	**n**	**$\bar{x}$**	**sd**	**n**	**$\bar{x}$**	**sd**
Northern Coast Range															
Hurdygurdy Creek	28	0.034	0.027	28	13.54	3.64	28	1.50	0.89	28	11.6	1.82	28	112.8	43.6
Red Cap Creek	5	0.010	0.000	5	9.10	1.81	5	0.44	0.15	5	14.0	0.55	5	58.5	1.8
Main Trinity River	77	0.044	0.040	77	21.34	7.17	77	2.68	2.27	69	16.4	2.75	78	80.9	23.2
SF Trinity River	52	0.036	0.035	52	21.33	6.43	52	2.13	1.56	51	19.3	1.69	52	82.7	43.1
Mattole River	23	0.049	0.031	26	12.20	5.48	26	3.79	3.60	26	12.7	1.09	26	113.4	23.3
SF Eel River	16	0.044	0.032	16	14.99	3.61	16	1.81	1.99	16	12.9	0.93	16	147.3	25.4
Trib. to Mark West Creek	-	-	-	6	13.25	5.38	8	0.55	0.51	8	15.0	1.39	8	66.6	58.6
Sierra Nevada															
NF Feather River	4	0.038	0.043	4	32.25	5.56	4	1.68	0.46	4	15.9	2.09	-	-	-
Clear Creek	18	0.009	0.009	35	11.83	3.85	43	0.50	0.33	-	-	-	-	-	-
Rose Creek	-	-	-	10	21.20	7.93	14	1.11	0.35	15	19.0	1.27	-	-	-
NF Tuolumne River	-	-	-	3	21.33	9.02	3	0.70	0.44	3	19.0	0.00	-	-	-

Table 4.2, continued.

ANOVA Results	*F*	*P*		*F*	*P*	lsmean (+SE)	*F*	*P*		*F*	*P*		*F*	*P*	
Stream size	3.21	0.21		6.51	0.03	creek: 14.17 (2.03), river: 22.28 (2.44)	3.62	0.08		0.01	0.95		1.07	0.35	
Geographic region	0.71	0.49		4.53	0.07		2.75	0.12		5.22	0.06		NA		

Table 4.3. Analysis of foothill yellow-legged frog (*Rana boylii*) oviposition site use and available microhabitat for the mainstem and South Fork Trinity Rivers in Northern Coast Range California, 1992. Results are for paired t-tests of both means and variances for each variable. * = $p<0.05$, ** = $p <0.01$, *** = $p <0.001$, ns = not significantly different. Direction of difference is indicated as used (u) > available (a) or u < a.

		Distance from Shore		Water Depth		Water Velocity		Water Temperature	
Stream Locality	n	mean (SD)	var	mean (SD)	variance	mean (SD)	variance	mean (SD)	variance
Mainstem	31	ns	ns	ns	*** u < a 54.31 238.07	** u < a 0.053 0.095 (0.049) (0.089)	*** u < a 0.002 0.008	*** u < a 17.1 17.6 (2.23) (2.36)	ns
South Fork	48	ns	ns	ns	*** u < a 36.66 126.34	ns	* u < a 0.001 0.002	ns	* u < a 1.56 1.98

Table 4.4. Occupancy rates of river bars and relative densities of *Rana boylii* egg masses, juveniles (<50mm svl), and adults on the South Fork Trinity River. Results are for actual km of surveyed habitat and for total km of river covered (as measured along the center line of the river channel). Data presented are mean (± standard error) for four years of surveys (n=4) of 29 river bars.

Life Stage	% occupied river bars	number / km of actual surveyed habitat (range 4.6km – 15.6 km)	number/ km for total river covered (5.7-15.6 km)
Egg Masses	83.2 (± 4.5)	54.8 (± 9.4)	47.4 (± 7.1)
Juveniles	53.7 (±10.5)	10.5 (± 4.3)	9.2 (± 3.6)
Adults	42.9 (± 8.9)	5.3 (± 1.9)	4.6 (± 1.5)

Table 4.5. Relationship between densities of egg masses at the South Fork Trinity River and habitat quality of river bars. Models were selected using the best adjusted R^2 value in an All Possible Subsets regression.

Model	Standarized Coefficients	R^2 (adj.)	*F*	*P*
Distance From Shore Mean Water Depth Mean	0.432 -0.230	0.240	5.58	0.0094
Distance From Shore Variance Water Depth Variance Water Velocity Variance	0.588 -0.260 -0.159	0.381	6.95	0.0014

Table 4.6. Descriptive statistics for fifteen hot spots[1] at Hurdygurdy Creek. Distance to closest tributary if for either up or downstream tributaries. Hot spots were stream habitat units that were used for breeding in at least one third of the years of the study (or six or more of 16 years).

Variable	mean	s.e.	range
Egg masses per hot spot / per year	7.41	2.21	2.33 – 26.38
Distance between hot spots (pairs of nearest neighbors)	0.33 km	0.08	0.03 – 1.07
Distance to closest tributary stream (up or downstream)	0.79 km	0.16	0.13 – 2.26

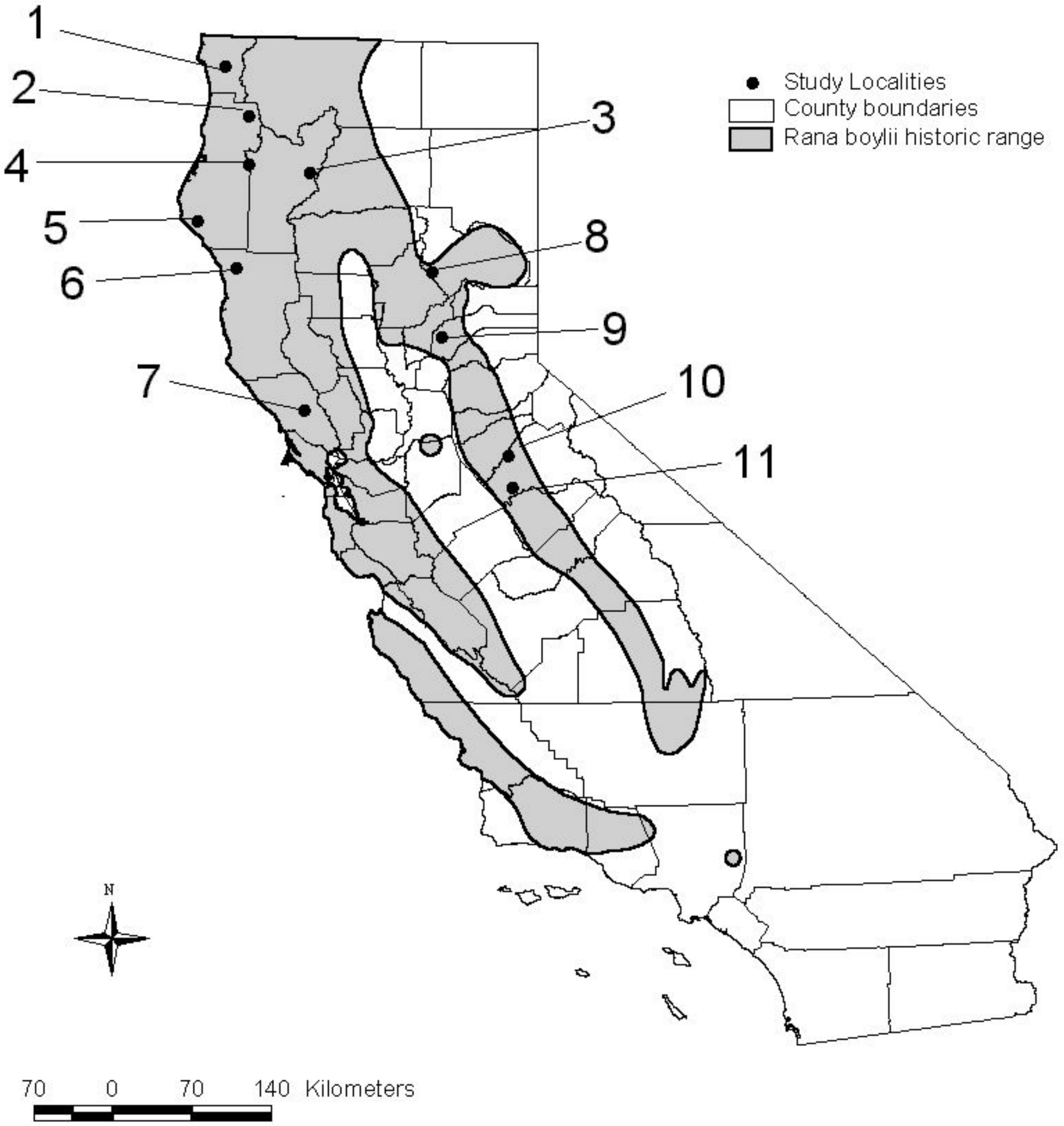

Figure 4.1. *Rana boylii* study localities and historic distribution in California. Study locality numbers correspond to stream localities in Table 4.1.

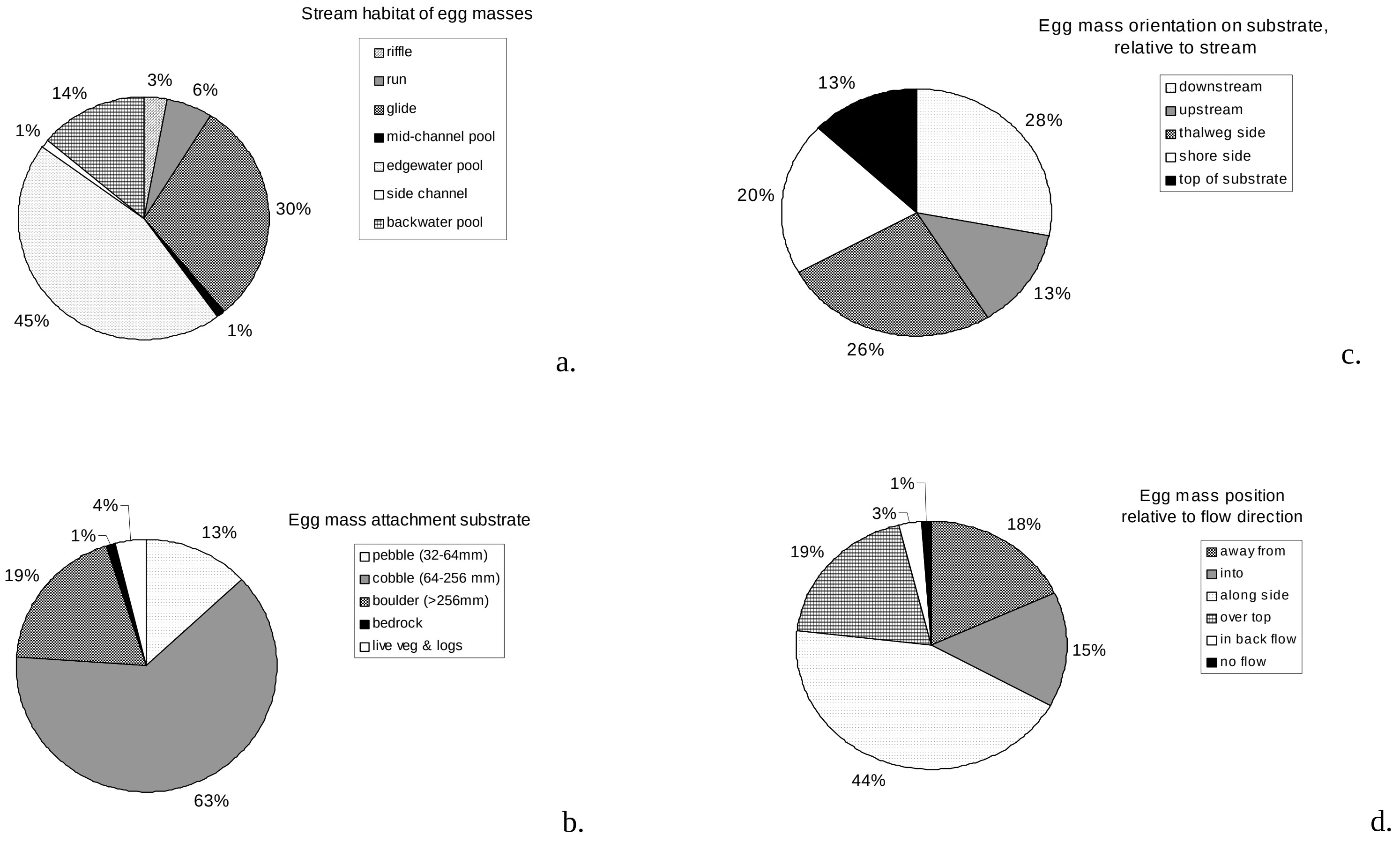

Figure 4.2. Egg deposition site characteristics by (a) stream habitat type (Hawkins et al.1993) and (b) egg mass attachment substrate and orientation of egg masses relative to (c) attachment substrate, and (d) direction of water flow for *Rana boylii* at Northern Coast Range localities from 1991-1995.

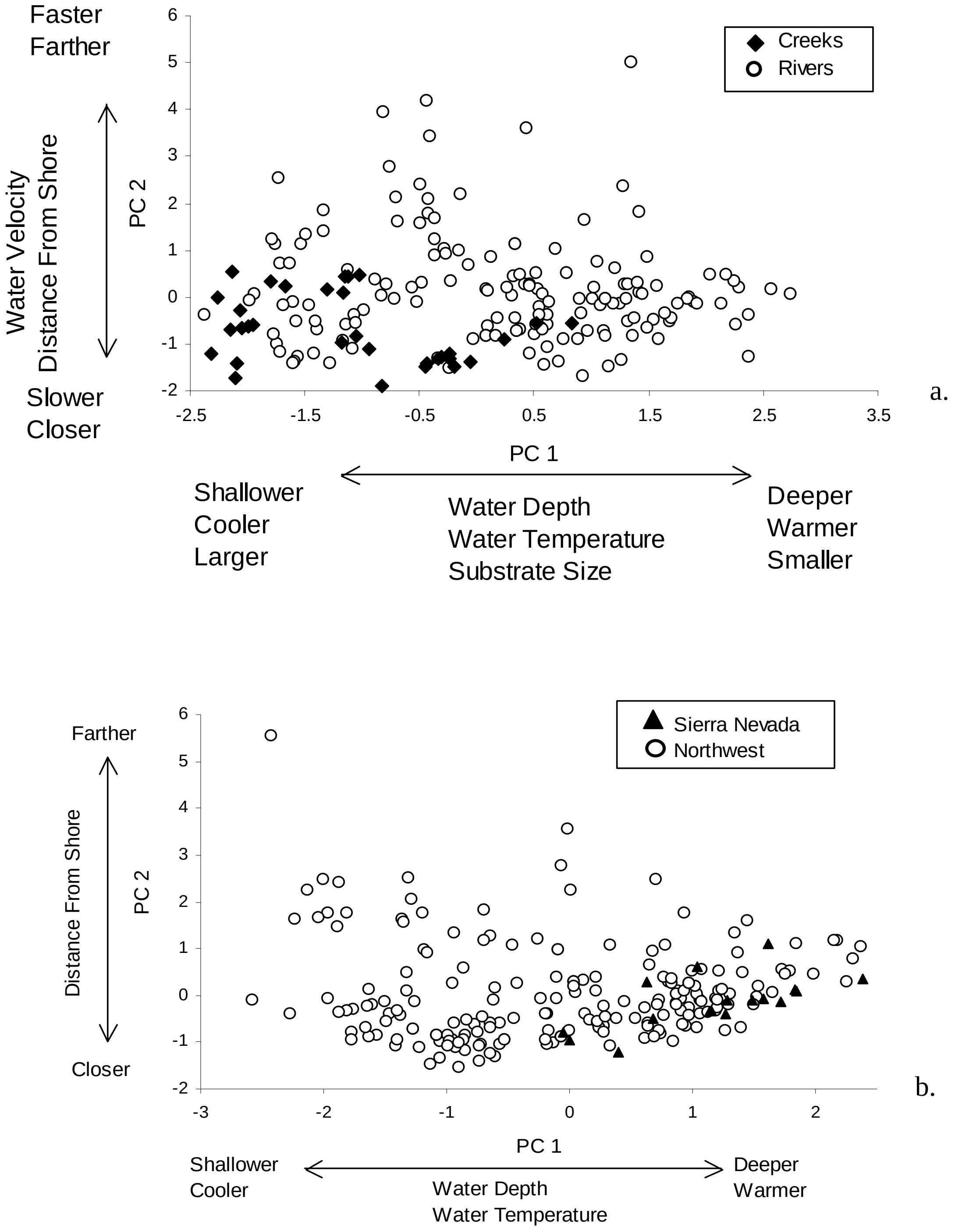

Figure 4.3. The first two principal components relative to (a) stream size (creek or river) and (b) geographic region for *Rana boylii* oviposition site characteristics.

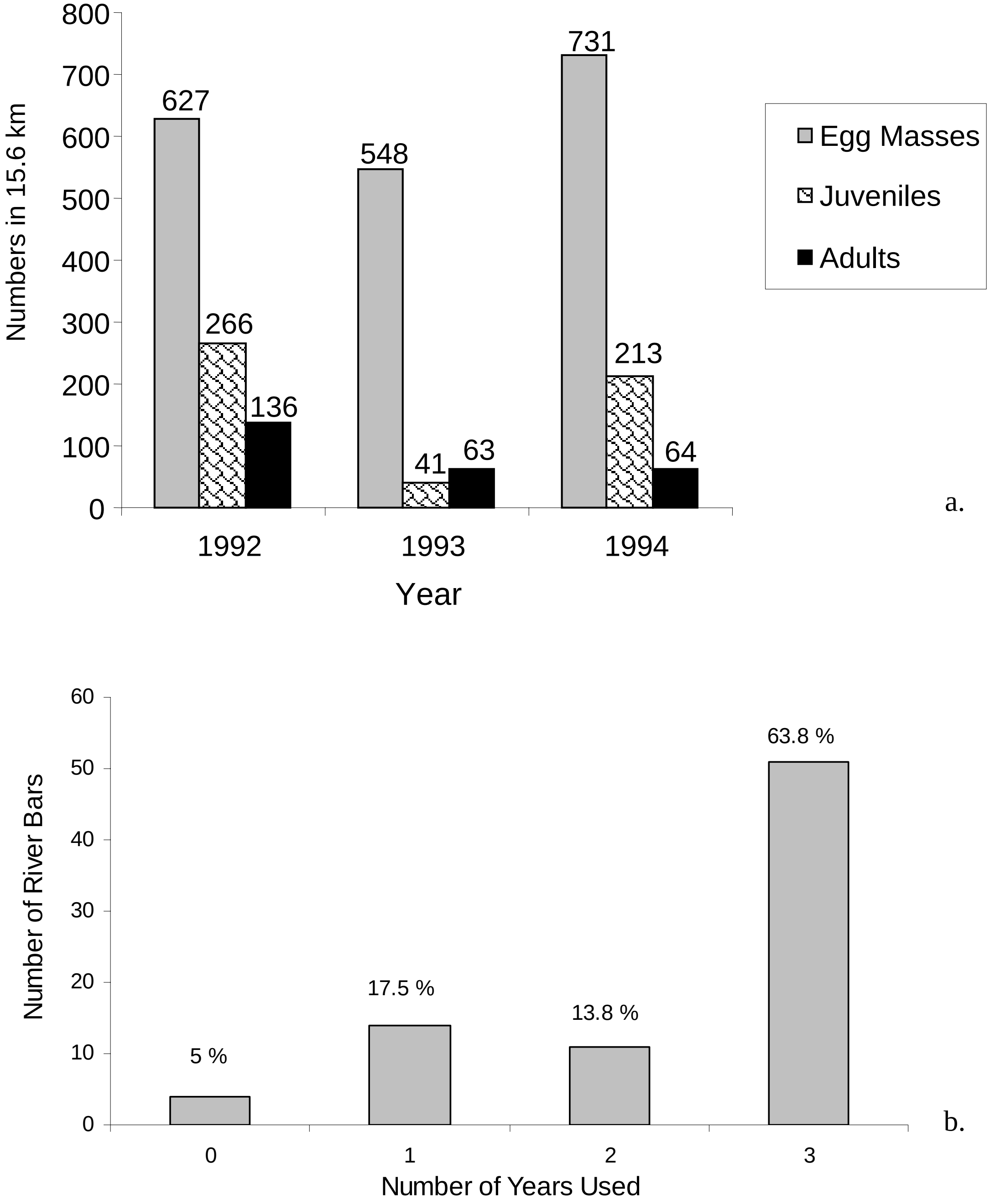

Figure 4.4. South Fork Trinity River *Rana boylii* (a) relative abundance of all life stages of along a 15.6 km study reach from 1992-1994 and (b) frequency of use of river bars for 80 river bars surveyed from 1992-1994.

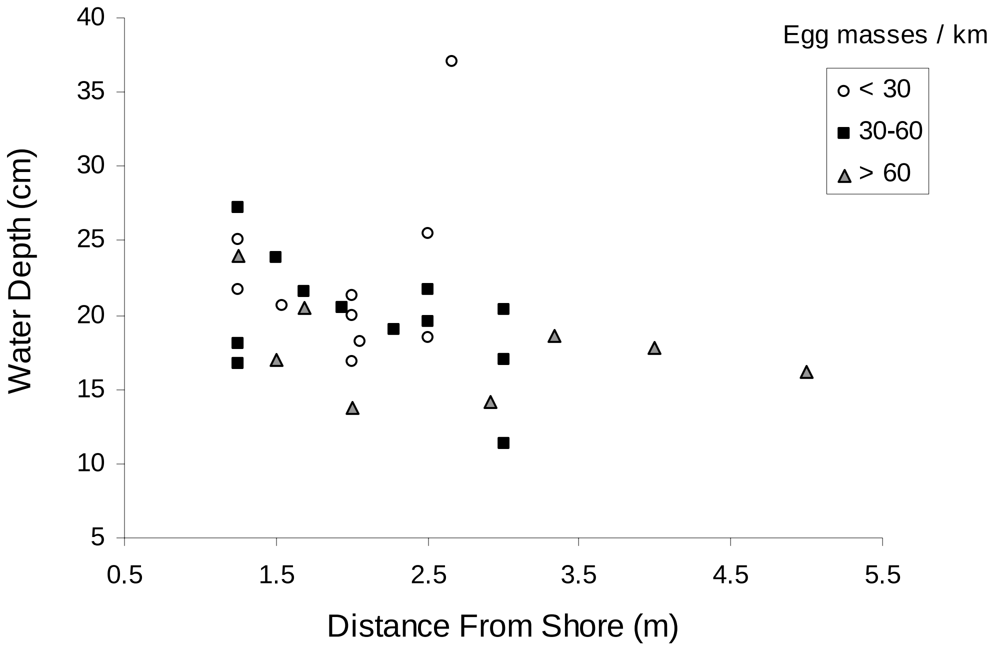

Figure 4.5. Egg mass density relative to mean distance from shore and water depth for the South Fork Trinity River in 1992. Egg mass densities are presented as number/km and placed in three categories shown here with different symbols.

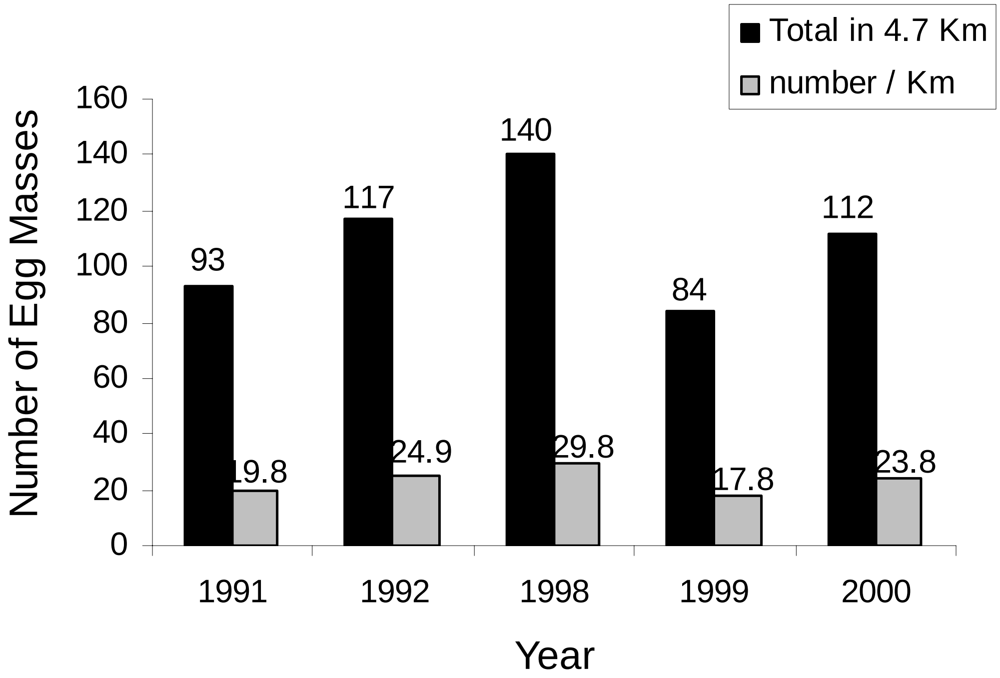

Figure 4.6. Relative abundance and density of *Rana boylii* egg masses at Hurdygurdy Creek (Northern Coast Range) for years with systematic surveys.

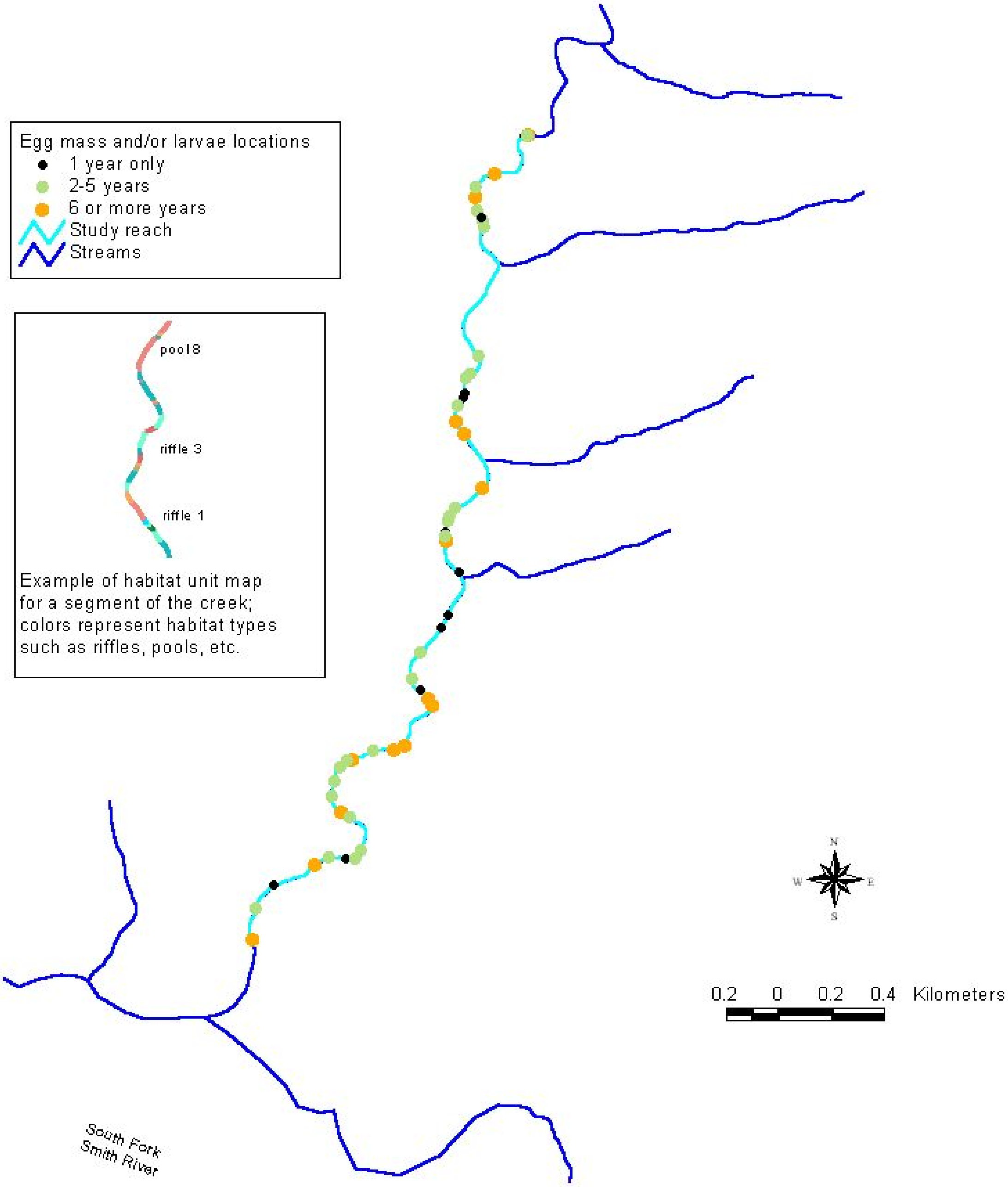

Figure 4.7. Spatial distribution of breeding activity (eggs and/or larvae) at Hurdygurdy Creek from 1987-2002. Orange circles represent locations used in 6 or more years, green circle represent locations used in 2-5 years, and black circle represent locations used in only one year.

Appendix 4.A. Field measurement methods and analysis approaches for oviposition microhabitat variables. Data on categorical variables were gathered in the Northern Coast Range geographic region only.

Variable	**Type**	**Measurement Method**	**Analysis**
Stream habitat type	categorical	Visual assessment – 8 categories (see Appendix 4.C for details).	descriptive
Water Velocity	continuous	Current meter, in m/s.	descriptive, PCA, ANOVA, use and availability
Water Depth	continuous	Depth of water adjacent at egg mass, in cm.	descriptive, PCA, ANOVA, use and availability
Water Temperature	continuous	Mercury thermometer next to egg mass, in ^{o}C.	descriptive, PCA, ANOVA, use and availability
Distance from shore	continuous	Distance from focal egg mass to closest dry shore line, in m.	descriptive, PCA, ANOVA, use and availability
Substrate Size (Weighted Mean)	continuous	Visually estimated percents by size classes (i.e. sand, gravel, pebble, cobble, boulder) in 1m radius circle around focal egg mass.	descriptive, PCA, ANOVA, use and availability
Attachment substrate	categorical	Visual assessment, 6 categories (see Appendix 4.C for details)	descriptive
Orientation of mass	categorical	Visual assessment – 5 categories (see Appendix 4.C for details).	descriptive
Direction of water flow	categorical	Visual assessment – 6 categories (see Appendix 4.C for details).	descriptive
Gosner stage	ordinal	Examined several eggs with hand lens and selected using staging tables (Gosner 1960).	use and availability

Appendix 4.B. Detailed Field and Analysis Methods for Microhabitat Selection

Oviposition microhabitat selection was studied in 1992 along river bars at two localities - the mainstem and South Forks of the Trinity River. At the mainstem Trinity River, two surveys were conducted to evaluate egg microsites - one in late April and early May and the second in late May and early June. Egg mass descriptors and microhabitat associations data was taken on all egg masses during each pass: egg mass diameter, average single egg diameter, gosner stage of eggs (Gosner 1960), attachment substrate, % silt on eggs, orientation, relative to substrate, placement relative to stream topography (in depression?), flow direction, main stream (thalweg) and immediate river habitat types (e.g. riffle, pool, etc.), distance from shore (m), water temperature (^{o}C), water depth (cm), water velocity (m/sec), % surface substrate distribution in a 1m circle around the egg mass (detritus, silt, sand, gravel, pebble, cobble, boulder, bedrock, woody material, live vegetation, stream bank).

Availability of habitat attributes was assessed by collecting data from points along line transects placed in the vicinity of egg masses. Each egg mass site was categorized as one of four types - backwater pool, island, main river margin, or side channel. A minimum of two transects were laid out at each site. If the site was longer than 25 m or had two distinct areas, 3 transects were done. Transects were systematically spaced along the long axis of the shore (e.g. if the area of site was 30 m long, transects were placed every 10 m). Line transects were placed perpendicular to the shore (or direction of main river flow) into the water. At island sites, four transects were set up; two on each side of the island. Transects started at the edge of the grass or other vegetation that indicated the usual summer water line. At main river and island sites, transects extended 3m out into the water. At backwater and side channel sites, transects extended across all the potential habitat. Data was recorded at either 0.5m, 1m, or 2m point intervals depending on the total length of each transect so that at least three points would be recorded per transect. Longer or shorter transects (i.e. those at backwater or island type sites) required closer or farther point measurements. The following data was recorded at each point: distance from shore (m), water depth (cm), and water velocity (m/sec). Water temperatures (^{o}C) were recorded at every other meter point. The first measurement was taken starting at either 0.5 or 1 m from the main shoreline depending on the total transect length.

Points were later randomly drawn from line transects to derive a data set for analyses that represented the available egg deposition habitat. For each egg mass, a point from the adjacent line transect was randomly selected using a random number table. The point location with the egg mass and the randomly selected point from the transect were treated as paired data. If no water temperature data was recorded for the point, the average of the two flanking points was used for the temperature. At one egg mass site on the mainstem Trinity River, 11 egg masses occurred very close together. To avoid undue influence of this particular location, we randomly selected 3 egg masses to represent this site, which was more in keeping with the numbers at other sites. At the mainstem Trinity River, this resulted in a total of 31 paired points representing 11 bar sites.

Methods of data collection for the South Fork Trinity River were the same as those described for the mainstem Trinity, except for the following. We conducted only one survey of this study area, in late May. Because of the large of number of egg deposition sites (n=80) and the large number of egg masses at each site, we sub-sampled to gather egg microhabitat use and availability data. We randomly chose 30 sites with egg masses and collected data as follows. Because bars were substantially longer at the South Fork than at the mainstem, we divided each bar (= site) into three sections – top, middle, and bottom – based on the topography of the bar and areas of low and high velocity water flows. These sections were used to spread sampling of available habitat along the bar rather than placing transects systematically. For each location (top, middle, bottom) at the site that had egg masses, we collected data on only the one most recently laid egg mass. This resulted in a total of 48 paired points for the South Fork study area. At most locations this mass was also the farthest out from shore. If it was not the farthest out, we also recorded the distance of the farthest egg mass from shore. Line transects to determine habitat availability were placed perpendicular to the shore (or direction of main river flow) and out into the water habitat to 1m beyond the distance of the furthest mass. One transect was set up at each location on the site (i.e. top, middle, bottom) that had egg masses. Transects were placed in the center of the conglomeration egg masses. If there was more than one conglomeration of masses at a location, we chose the group that contained the most recently laid egg mass and located the transect near that group.

Appendix 4.C. Summary statistics for categorical variables (percent of egg masses at a locality) describing oviposition habitat of foothill yellow-legged frogs (*Rana boylii*) from seven stream localities in the Northern Coast Range region of California.

		Stream Habitat[1]								Attachment Substrate[2]						Orientation of Egg Mass On Substrate					Flow Direction Relative to Egg Mass					
Stream Locality	n	Low gradient riffle	High gradient riffle	Run	Glide	Mid-channel pool	Edgewater pool	Side Channel	Backwater Pool	Pebble (33-64mm)	Cobble (65-256mm)	Boulder (>256mm)	Bedrock	Woody Material	Live Vegetation	Upstream side	Downstream side	Shre side	Thalwdg side	Mass on top of substr.	Into flow	Away from flow	Flow along side	In backflow from shore	Flow over the top	No flow
Hurdygurdy Creek	28	7	0	0	29	0	64	0	0	25	54	21	0	0	0	21	14	29	32	4	18	4	64	0	14	0
Red Cap Creek	5	0	0	0	0	0	0	0	100	0	100	0	0	0	0	60	0	20	20	0	60	0	40	0	0	0
Main Trinity River	78	0	3	12	28	0	31	1	25	5	82	7	0	6	0	12	23	22	34	9	22	15	54	3	6	0
SF Trinity River	52	0	0	2	12	0	66	2	18	31	40	25	0	0	4	12	25	19	13	31	10	13	25	6	42	4
Mattole River	26	4	0	8	76	0	12	0	0	0	73	23	0	0	4	4	54	13	25	4	4	46	42	0	8	0
SF Eel River	16	0	0	0	50	0	50	0	0	0	44	56	0	0	0	19	37	19	25	0	0	19	44	0	37	0
Trib. Mark West Crk	8	12	0	0	0	13	75	0	0	12	50	25	13	0	0	0	62	13	0	25	0	50	25	12	13	0

[1] – Stream habitats were adapted from Hawkins et al. 1993.

[2] – Substrate diameter size categories for rocky substrates from Platts et al. 1983. Note that egg masses were not found attached to smaller than pebble-sized substrates.

CHAPTER 5. CONCLUSION – APPLICATIONS TO AMPHIBIAN REINTRODUCTION PROGRAMS

Successful reintroductions depend on the appropriate application of both theoretical and empirical ecological information for the focal species (May 1991). Based on a review of the literature (Chapter 1) and the results of the research in this disseration, I have developed a qualitative decision tree for amphibian reintroduction planning (Fig. 5.1). This decision tree provides guidance on when a species reintroduction is warranted and identifies points where either ecological research is critical or experimental approaches could be used. For example, the very first decision point involves knowledge of the original cause of decline of the species (Fig. 5.1). Without that information and methods to mitigate those causes, the likelihood of a successful reintroduction is low (Dodd, *In Press*). The Global Amphibian Assessment (a new assessment and database) may be a useful starting point for identifying causes of decline (Stuart et al. 2004); however risk categories are general and information is primarily qualitative. Progressing through the decision tree, after the basic needs of the species are understood and met (i.e. threats reduced, genetic context understood, suitable habitat), other aspects of the reintroduction could be treated in an experimental fashion (Sarrazin and Barbault 1996). For example, if there is limited knowledge of demographic characteristics of a typical population, releases of various combinations of different life stages (i.e. eggs, larvae, sub-adults, adults) could be attempted and the results rigorously documented (Fig. 5.1). In any case, gathering enough data throughout the project to understand why the reintroduction program succeeded or failed is critical to the success of future efforts. A comprehensive analysis of amphibian reintroduction programs is planned using a database that is currently under development (Table 1.1 and Amphibian Reintroduction Database details at: http://www.open.ac.uk/daptf/about/abou7.htm). In the long run, the ability to relate successes and failures of reintroduction programs to predictions from both empirical and theoretical data will further the fields of both theoretical and applied ecology (Griffith et. al. 1989).

By accomplishing the research described in the previous chapters I have made a substantial contribution to knowledge of the basic ecology and genetics of *Rana boylii*. The information I have gathered on decline factors, genetic structure, and habitat associations can be used for both assessing the ecological feasibility (i.e. the straightforwardness and likelihood of success from the standpoint of the biology of the species) of doing a reintroduction and for predicting appropriate source populations for target reintroduction locations. The details on how this information could be applied will depend on the particulars of the reintroduction program, but some general recommendations are possible.

In terms of the causes of decline of *R. boylii*, my research strongly points to habitat alteration and climatic effects including synergisms with the downstream hydrologic changes caused by dams. Because climatic factors and large dams cannot be easily mitigated, target reintroduction sites should be in places where these effects are already minimized. In addition, conservation and reintroduction planning should include consideration of predicted future climatic changes at both local and range-wide geographic scales.

Analysis of the mitochondrial genome of *R. boylii*, indicate that there is overall low genetic variability throughout the range of the species. However, phylogenetic tree-based analyses consistently identified geographically distinct clades and there are several populations containing relatively divergent haplotypes. Selection of source populations for target reintroduction sites should be within rather than between geographically identified monophyletic groups for this species. Populations in southern Sierra Nevada and central coast of California, and in north/central Oregon are geographically isolated and show genetic uniqueness. It would be risky to supplement the populations in these regions with individuals from other geographic areas. Thus, *in situ* conservation efforts, like head-starting (e.g. Demlong 1997), that enhance survival of local populations may be the best approach.

Habitat associations analyses provide information useful for both captive breeding/rearing and for selection of target reintroduction sites. My results show that very specific conditions of water depth, velocity, and substrate are used at oviposition and during larval rearing stages of *R. boylii*. These conditions along with others identified from previous research (Table 1.2) need to be available at any target reintroduction site. In addition, mimicking these conditions for *in situ* or *ex situ* captive breeding or rearing programs should increase the success rates of these programs.

Apart from the data I have collected here, other information, such as an assessment of the potential for captive breeding and rearing of *R. boylii* and a thorough understanding of demographic dynamics, are needed to fully implement a reintroduction program. As described above, these additional research needs could be considered in an experimental framework. In addition, the survival rates of captive-bred individuals could be compared with translocated, naturally-derived individuals. My research lays the groundwork for such experiments and could lead to successful conservation programs, including reintroductions, for *R. boylii*.

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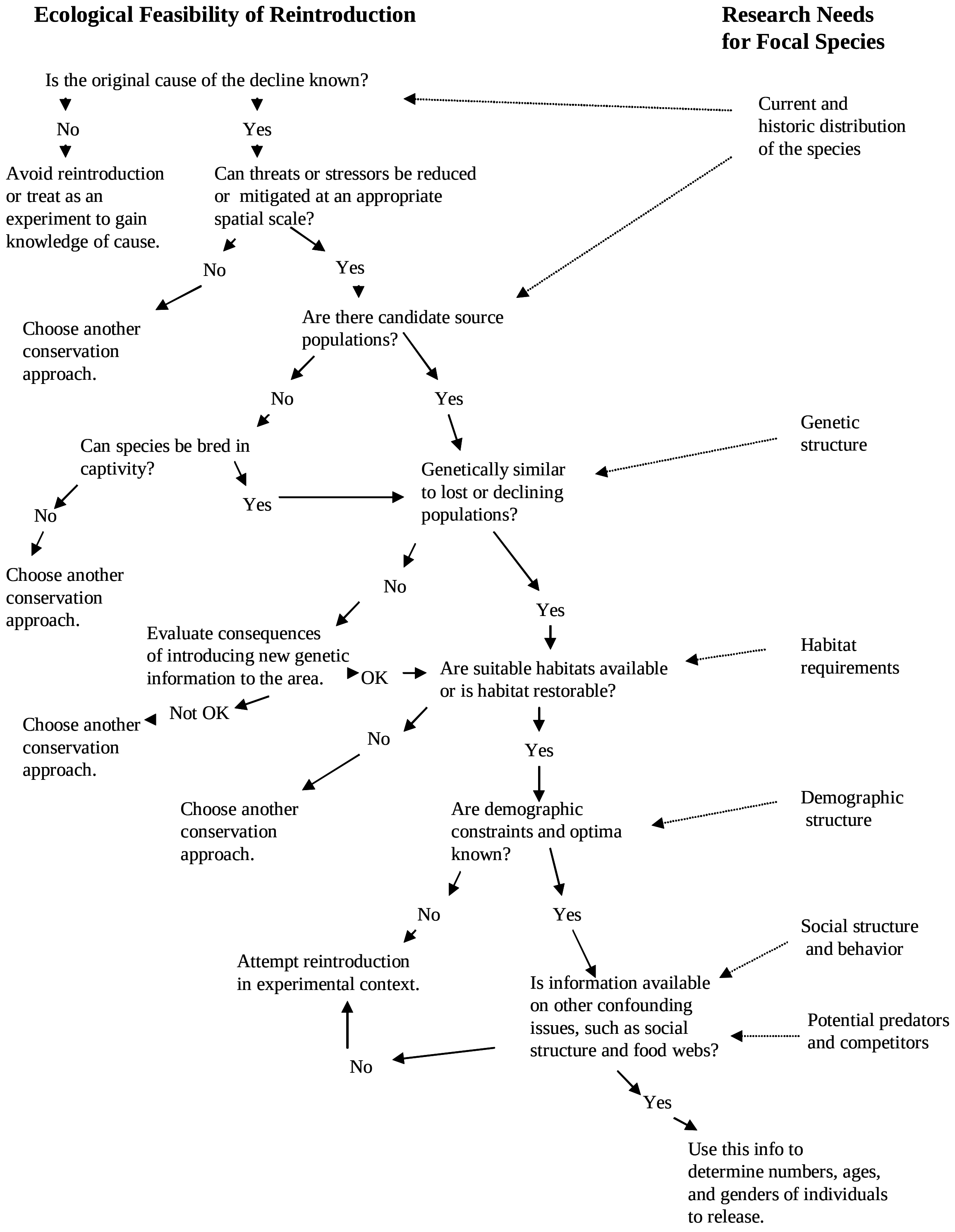

Figure 5.1. A conceptual model for determining the ecological feasibility of a species reintroduction.