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

By

AMY JO LIND
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Approved:

H. Bradley Shaffer

Peter B. Moyle

Steven E. Greco

Trevor J.C. Beebee

Committee in Charge

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ABSTRACT (entire dissertation)

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.

Chapters 1 and 5 are introductory and conclusory and do not have individual abstracts. Following are title pages and abstracts from Chapters 2-4. Each of these chapters is being submitted separately for publication; co-authors for those submissions are identified on each title page.

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

Amy J. Lind[1]
Carlos D. Davidson[2]
Aaron Borisenko[3]

[1] USDA Forest Service, Pacific Southwest Research Station, Sierra Nevada Research Center, 2121 Second Street, Davis, California 95616 and Department of Evolution and Ecology, University of California, Davis, CA 95616.

[2] Department of Environmental Studies, California State University, Sacramento, 6000 J Street, Sacramento, CA 95819.

[3] Oregon Department of Environmental Quality, Laboratory Division - Northwest Region, Watershed Assessment Section, Portland, OR

CHAPTER 2. 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.

CHAPTER 3. PHYLOGEOGRAPHY OF *RANA BOYLII*:

IMPLICATIONS FOR THE CONSERVATION OF FROGS AND RIVERS

Amy J. Lind[1]
H. Bradley Shaffer
Gary M. Fellers[2]

Department of Evolution and Ecology, One Shields Ave.
University of California, Davis, CA 95616

[1] Current Address: USDA Forest Service, Pacific Southwest Research Station, Sierra Nevada Research Center, 2121 Second Street, Davis, CA 95616
email: alind@fs.fed.us

[2] Western Ecological Research Center, U.S. Geological Survey, Point Reyes National Seashore, Point Reyes, CA 94956.

CHAPTER 3. 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 fragments 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.

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

Amy J. Lind[1] and Hartwell H. Welsh, Jr.

USDA Forest Service, Pacific Southwest Research Station, Redwood Sciences Laboratory,1700 Bayview Drive, Arcata, CA 95521

[1] Current address: USDA Forest Service, Pacific Southwest Research Station, Sierra Nevada Research Center, 2121 Second Street, Davis, CA 95616 and Department of Evolution and Ecology, One Shields Avenue, University of California, Davis, CA 95616.

CHAPTER 4. 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.