

Population genetics of a sentinel stream-breeding frog (*Rana
boylei*)

By

RYAN A PEEK

B.S. (University of California, Davis) 2002

M.S. (University of San Francisco) 2010

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Ecology

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

Approved:

(Michael R. Miller), Chair

(Peter B. Moyle)

(Mark W. Schwartz)

Committee in Charge

2018

The Dissertation of Ryan A Peek is approved.

Peter B. Moyle

Mark W. Schwartz

Michael R. Miller, Committee Chair

September 2018

“One thing to remember is to talk to the animals. If you do, they will talk back to you. But if you don’t talk to them, they won’t talk back to you, then you won’t understand. And when you don’t understand, you will fear, and when you fear, you will destroy the animals, and if you destroy the animals, you will destroy yourself”

(Chief Dan George, Tseil-Waututh Nation, North Vancouver)

Acknowledgements

For all the curious people who have come before and hopefully after, I want to acknowledge you, and I hope we can do better to inspire and support those voices that may not have had the opportunities or privilege I have had. I am lucky to have had all I have had, and finishing a dissertation requires a community, and this dissertation would not have happened if it wasn't for the amazing community of family, friends, and colleagues who helped me every step of the way. In particular, thank you to my partner, wife and best-friend Leslie—you are my sun and gravity—you held me together, anchored our family, and made it possible to run this crazy academic ultra-marathon. To my dearest little tadpoles, Connor and Genevieve, you inspire me, you make me laugh every day, and you remind me the world still has hope as long as we nourish joy and curiosity. Thank you for being you, and I hope one day you forgive me for the amount of time I've spent staring at a computer. Thanks to my mom for all the support, love, and baked goods. Sibling, thank you for consistently inspiring me, listening to me, and being the best sibling one could ask for. And for all my close friends, bandmates, and officemates (you know who you are), you keep me sane, you motivate me, and you remind me every day that I really love this crazy journey. John, your shed and couch have probably single-handedly kept me anchored in ways I can't even express...also your friendship. Thank you for your time, humor, and general levity. Thanks to my Dad, who has cajoled, pestered, and annoyed me for far too long to "get a PhD". . . thanks for believing it was possible even when I didn't. Also, please never suggest anything like this again. And to my committee and my colleagues at the Center for Watershed Sciences, you have all been an amazing resource in providing feedback, guidance, and support throughout my graduate student career. Finally, to my cohort and fellow students in the GGE, this has been a great place to grow and mature as a scientist and researcher. Thank you all.

Abstract

Rana boylei is an imperiled frog species native to CA and OR, and it is currently designated as a species of special concern (CDFW) in the state of CA. It has been petitioned as candidate for federal (USFWS) and state (CDFW) listing. As a lotic breeding amphibian, *R. boylei* is tied closely to local flow regimes in the watersheds it inhabits and is therefore particularly sensitive to alterations to the natural flow regime. Effective conservation management of this species should consider and prioritize maintenance of genetic diversity as part of any listing decision because it is closely related to the evolutionary capacity for adaptation to environmental changes. Conservation of genetic diversity in this species will require several components, including refining potential conservation units (i.e., distinct population segments) and quantifying of genetic diversity and genetic diversity trajectories across the species range. To assess these components, fine-scale and landscape-scale analyses were conducted using genomic data from over 600 samples from 89 localities across the range of the species. Six genomically-distinct groups were identified, as well as population subdivisions at local watershed scales. One major impact on *R. boylei* populations has been river regulation. River regulation has been implicated as a cause of fundamental changes to downstream aquatic ecosystems. Regulation changes the natural flow regime which may restrict population connectivity and decrease genetic diversity in some species. Since population connectivity and the maintenance of genetic diversity are fundamental drivers of long-term persistence, understanding the extent that river regulation impacts these critical attributes of genetic health is an important goal. However, the extent to which *R. boylei* populations in regulated rivers have maintained

connectivity and genetic diversity is unknown. The impacts of river regulation on *R. boylei* were investigated with genomic data to explore the potential for long-term persistence of *R. boylei* under continued regulation. *R. boylei* in regulated rivers showed striking patterns of isolation and trajectories of genetic diversity loss relative to unregulated rivers. For example, river regulation explained the greatest amount of variance in population genetic differentiation compared with other covariates including geographic distance. Importantly, patterns of connectivity and genetic diversity loss were observed regardless of regulation level but were most prominent in locations with the greatest regulation intensity. Using the same genomic data, fine-scale analyses of *R. boylei* and *R. sierrae* in a single region of the Sierra Nevada of California was conducted to evaluate the potential for hybridization between species. Hybridization between species may combine parental genotypes in ways that yield reproductively sterile or isolated lineages, and hybridization events may be short-lived and difficult to detect. Limited hybridization between the species was detected in the Feather basin, though it appears these are terminal events based on PCA, admixture, and tests of heterozygosity using species diagnostic SNPs. Finally, rangewide quantification and comparison of genomic variation across populations indicates the southern coast, southern Sierra Nevada, and Northern Sierra/Feather basin in California should have high prioritization in conservation efforts due to low genomic diversity and trajectories of diversity loss. More broadly, these results demonstrate both the critical need for regional conservation in a sentinel river species, and the utility and power of genetic methods for assessing and monitoring sensitive species across many scales.

Contents

Abstract	v
1 UW thesis fields	1
2 Flow regulation associated with decreased genetic health of a river-breeding frog species	2
Introduction	3
Methods	3
Results	3
Discussion	3
3 This chunk ensures that the huskydown package is	4
Introduction	5
Materials and Methods	5
Results	5
4 Refining conservation unit boundaries of a sentinel stream-breeding frog (<i>Rana boylei</i>) using population genomics	6
INTRODUCTION	6
A The First Appendix	10
B The Second Appendix, for Fun	11
Colophon	12

Chapter 1

UW thesis fields

Placeholder

Chapter 2

Flow regulation associated with
decreased genetic health of a
river-breeding frog species

INTRODUCTION

Introduction

Methods

Sample collection

De novo assembly

Rapture sequencing

Principal component analysis

Genetic differentiation and diversity estimates

Boosted regression tree modeling of variance in F_{ST}

Results

Discussion

Chapter 3

This chunk ensures that the
huskydown package is

INTRODUCTION

Introduction

Materials and Methods

Sampling and DNA Extraction

Rapture Sequencing

PCA & Admixture

F1 vs F2 Test with Species Diagnostic SNPs

Demographic Modeling with fastsimcoal2

Results

Rapture produced high quality genomic data for both *R. sierrae* and *R. boylii*

Chapter 4

Refining conservation unit boundaries of a sentinel stream-breeding frog (*Rana boylei*) using population genomics

```
#include_graphics(path = "figure/uw.png")
```

```
# Here is a reference to the UW logo: Figure \@ref(fig:uwlogo). Note the use of th
```

INTRODUCTION

The use of modern genomic sequencing technology has greatly advanced the ability for higher resolution analyses of both geographic and ecological patterns in populations (Nunziata et al. 2017, Hendricks et al. 2018, Barbosa et al. 2018). Reduced repre-

INTRODUCTION

sentation sequencing methods such as restriction site-associated DNA sequencing (RAD-Seq) (Miller et al. 2007, Baird et al. 2008, Ali et al. 2016) provides a powerful tool to address ecological genomics questions at scales that were previously impossible using traditional field methods. Furthermore, new methods such as RAD Capture (Rapture) (Ali et al. 2016) adapt RADSeq to target desired loci and allow highly efficient genotyping of thousands of individuals at once. As historical and future landscape use can influence species demography and migration patterns (Burkey 1989, Anderson and Beer 2009, Barbosa et al. 2018), these genomic tools will be invaluable for assessing critical factors for long-term persistence in sensitive populations or species. The ecological integrity of freshwater systems and their constituent biota are rapidly declining globally (Ricciardi and Rasmussen 1999), and conservation efforts will require assessment of the adaptive capacity of populations to rapid environmental change. Given limited capacity to conserve, it is important to define and establish clear geographic boundaries for conservation units such as distinct population segments across a species' range. Delineation of distinct population segments can be used for prioritizing objectives in conservation management. Furthermore, quantification and comparison of relative genetic diversity within and among populations can provide additional information as a benchmark for future assessment responses to conservation actions. Thus, quantifying and linking landscape change with genetic diversity metrics may provide an important baseline to track how sensitive populations respond to future environmental change (through reduced adaptive potential) as well as evaluating whether restoration efforts are effective (i.e., increasing genetic connectivity, diversity, effective breeder/population size).

Amphibians are particularly sensitive to changes in the ecosystem due to their physiology and ontogeny (Davidson et al. 2002, Beebee and Griffiths 2005), thus the ability to utilize environmental variables as life history cues can be especially important. In highly dynamic riverine environments, organisms must constantly adapt to temporal and spa-

tial changes. One such sentinel stream-breeding species is the Foothill yellow-legged frog (*Rana boylei*), a native to California and Oregon which historically occurred in lower elevation (0-1500m) streams and rivers from Southern Oregon to northern Baja California west of the Sierra-Cascade crest (Stebbins 2003). As a lotic breeding amphibian, *R. boylei* is tied closely to the local hydrology in watersheds it inhabits, and therefore it is particularly sensitive to alterations to flow regimes (Kupferberg 1996, Lind et al. 1996, Kupferberg et al. 2012). As with many amphibians in California (Davidson 2004, Vredenburg and Wake 2007, Peek 2010, Kupferberg et al. 2012, Thomson et al. 2016), there have been significant population declines across the former range of this species, particularly in southern California and the Sierra Nevada where it has been extirpated from approximately 50 percent of its historical range (Jennings and Hayes 1994, Davidson et al. 2002). *Rana boylei*, currently designated as a species of special concern (CDFW) in the state of CA, has been petitioned as candidate for listing under the federal (USFWS) Endangered Species Act (USFWS 2014) as well as the state (CDFW) Endangered Species Act. Effective conservation management of this species will need to consider and prioritize maintenance of genetic diversity as part of any listing decision because it is closely related to the evolutionary capacity for adaptation to environmental changes (Lande and Shannon 1996). Thus, utilizing genetic data provides a potentially informative process for identifying the impacts of anthropogenic and environmental change on the process of adaptation. Establishing high-resolution genetic boundaries for populations across the species range as well as delineation of distinct population segments that can be more effective in conservation management when coupled with quantification of relative genomic diversity metrics (i.e., genomic diversity, population connectivity). A recent study by McCartney-Melstad et al. (2018) identified five major clades in *R. boylei* with strong geographically structured genetic subdivision across its range in California and Oregon. Here we provide an additional population genomic analysis across the range

INTRODUCTION

of this declining sentinel stream species that is currently a candidate for listing. We provide additional geographic and genetic resolution to McCartney-Melstad et al. (2018), as well as quantify genetic diversity metrics across subpopulations and clades as both a reference and assessment of the potential for long-term persistence across this species' range.

Appendix A

The First Appendix

This first appendix includes all of the R chunks of code that were hidden throughout the document (using the `include = FALSE` chunk tag) to help with readability and/or setup.

In the main Rmd file

In Chapter ??:

Appendix B

The Second Appendix, for Fun

Colophon

This document is set in **EB Garamond**, **Source Code Pro** and **Lato**. The body text is set at 11pt with *lmr*.

It was written in R Markdown and *LaTeX*, and rendered into PDF using **huskydown** and **bookdown**.

This document was typeset using the XeTeX typesetting system, and the **University of Washington Thesis class** class created by Jim Fox. Under the hood, the **University of Washington Thesis LaTeX template** is used to ensure that documents conform precisely to submission standards. Other elements of the document formatting source code have been taken from the **Latex, Knitr, and RMarkdown templates for UC Berkeley's graduate thesis**, and **Dissertate: a LaTeX dissertation template to support the production and typesetting of a PhD dissertation at Harvard, Princeton, and NYU**

The source files for this thesis, along with all the data files, have been organised into an R package, `xxx`, which is available at <https://github.com/xxx/xxx>. A hard copy of the thesis can be found in the University of Washington library.

This version of the thesis was generated on 2018-09-10 15:59:58. The repository is currently at this commit:

The computational environment that was used to generate this version is as follows:

Session info -----

```
setting  value
version  R version 3.5.1 (2018-07-02)
system   x86_64, darwin15.6.0
ui        X11
language (EN)
collate   en_US.UTF-8
tz        America/Los_Angeles
date      2018-09-10
```

Packages -----

package	* version	date	source
assertthat	0.2.0	2017-04-11	CRAN (R 3.5.0)
backports	1.1.2	2017-12-13	CRAN (R 3.5.0)
base	* 3.5.1	2018-07-05	local
bindr	0.1.1	2018-03-13	CRAN (R 3.5.0)
bindrcpp	0.2.2	2018-03-29	CRAN (R 3.5.0)
bookdown	0.7	2018-02-18	CRAN (R 3.5.0)
colorspace	1.3-2	2016-12-14	CRAN (R 3.5.0)
compiler	3.5.1	2018-07-05	local
crayon	1.3.4	2017-09-16	CRAN (R 3.5.0)
datasets	* 3.5.1	2018-07-05	local
devtools	* 1.13.6	2018-06-27	CRAN (R 3.5.0)
digest	0.6.16	2018-08-22	CRAN (R 3.5.1)
dplyr	* 0.7.6	2018-06-29	CRAN (R 3.5.0)

APPENDIX B. THE SECOND APPENDIX, FOR FUN

evaluate	0.11	2018-07-17 CRAN (R 3.5.0)
ggplot2	* 3.0.0.9000	2018-09-04 Github (tidyverse/ggplot2@6e545dc)
git2r	0.23.0	2018-07-17 CRAN (R 3.5.0)
glue	1.3.0	2018-07-17 CRAN (R 3.5.0)
graphics	* 3.5.1	2018-07-05 local
grDevices	* 3.5.1	2018-07-05 local
grid	3.5.1	2018-07-05 local
gtable	0.2.0	2016-02-26 CRAN (R 3.5.0)
hms	0.4.2	2018-03-10 CRAN (R 3.5.0)
htmltools	0.3.6	2017-04-28 CRAN (R 3.5.0)
httr	1.3.1	2017-08-20 CRAN (R 3.5.0)
huskydown	0.0.5	2018-09-04 Github (benmarwick/huskydown@3ef00c9)
kableExtra	* 0.9.0	2018-05-21 CRAN (R 3.5.0)
knitr	* 1.20	2018-02-20 CRAN (R 3.5.0)
lazyeval	0.2.1	2017-10-29 CRAN (R 3.5.0)
magrittr	1.5	2014-11-22 CRAN (R 3.5.0)
memoise	1.1.0	2017-04-21 CRAN (R 3.5.0)
methods	* 3.5.1	2018-07-05 local
munsell	0.5.0	2018-06-12 CRAN (R 3.5.0)
pillar	1.3.0	2018-07-14 CRAN (R 3.5.0)
pkgconfig	2.0.2	2018-08-16 CRAN (R 3.5.0)
plyr	1.8.4	2016-06-08 CRAN (R 3.5.0)
purrr	0.2.5	2018-05-29 CRAN (R 3.5.0)
R6	2.2.2	2017-06-17 CRAN (R 3.5.0)
Rcpp	0.12.18	2018-07-23 CRAN (R 3.5.1)
readr	* 1.1.1	2017-05-16 CRAN (R 3.5.0)

rlang	0.2.2	2018-08-16 CRAN (R 3.5.0)
rmarkdown	1.10	2018-06-11 cran (@1.10)
rprojroot	1.3-2	2018-01-03 CRAN (R 3.5.0)
rstudioapi	0.7	2017-09-07 CRAN (R 3.5.0)
rvest	0.3.2	2016-06-17 CRAN (R 3.5.0)
scales	1.0.0.9000	2018-08-29 Github (hadley/scales@0f7a186)
stats	* 3.5.1	2018-07-05 local
stringi	1.2.4	2018-07-20 CRAN (R 3.5.0)
stringr	1.3.1	2018-05-10 CRAN (R 3.5.0)
tibble	1.4.2	2018-01-22 CRAN (R 3.5.0)
tidyselect	0.2.4	2018-02-26 CRAN (R 3.5.0)
tools	3.5.1	2018-07-05 local
utils	* 3.5.1	2018-07-05 local
viridisLite	0.3.0	2018-02-01 CRAN (R 3.5.0)
withr	2.1.2	2018-08-29 Github (jimhester/withr@8b9cee2)
xfun	0.3	2018-07-06 CRAN (R 3.5.0)
xml2	1.2.0	2018-01-24 CRAN (R 3.5.0)
yaml	2.2.0	2018-07-25 CRAN (R 3.5.0)

References

Placeholder

Barbosa, S., F. Mestre, T. A. White, J. Paupério, P. C. Alves, and J. B. Searle. 2018. Integrative approaches to guide conservation decisions: Using genomics to define conservation units and functional corridors. *Molecular ecology*.

Hendricks, S., E. C. Anderson, T. Antao, L. Bernatchez, B. R. Forester, B. Garner, B. K. Hand, P. A. Hohenlohe, M. Kardos, B. Koop, A. Sethuraman, R. S. Waples, and G. Luikart. 2018. Recent advances in conservation and population genomics data analysis. *Evolutionary applications*.

Nunziata, S. O., S. L. Lance, D. E. Scott, E. M. Lemmon, and D. W. Weisrock. 2017. Genomic data detect corresponding signatures of population size change on an ecological time scale in two salamander species. *Molecular ecology* 26:1060–1074.