Ecosystem Dynamics of California Red-Legged Frog Habitat (*Rana draytonii*) Throughout a Breeding Season

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INTRODUCTION

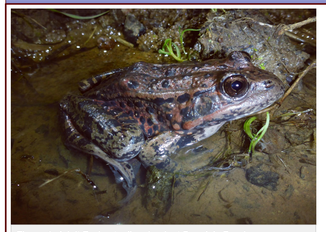

Figure 1. Adult R. draytonii on bank at Bonnie's Pond. Photo: Keith Wellstone

The California Red-Legged Frog (*Rana aurora draytonii*) was federally listed as a threatened species in 1996 and has been extirpated from 70% of its former range[7]. Non-native species have major effects on frog survivability and reproductive success. However, few researchers have focused on how native predators affect the California Red-Legged Frog[4].

A unique situation occurs at Sonoma Mountain Ranch Preserve (SMRP) where California Red-Legged frogs use three ponds, of variable size, depth and cover. Observations at the site revealed that they only utilize one pond for breeding (Bonnie's Pond). SMRP has no introduced species, has breeding and non-breeding *R. draytonii,* and is a great site to study the effects of predaceous aquatic invertebrates and larval amphibians on breeding site selection of Red-Legged Frogs.

Water quality, inter- and intraspecific competition, and abiotic factors of potential breeding sites, can significantly affect successful laying, hatching and development of larval frogs[3] [6]. In terrestrial systems, amphibians prey on insects, but the reverse is observed in aquatic systems. Predaceous macroinvertebrates are found to prey extensively on larval amphibians[2] [5].

Data collection was conducted between the months of November and April through a critical 1-2 week period in which *R.draytonii* migrate from a non-breeding habitat to a breeding habitat.

This study assessed and compared water quality, population dynamics of the Red-legged frog and invertebrate biodiversity between the three ponds at SMRP. Surveying *R. draytonii* populations may elucidate how habitat fragmentation affects population viability. This research will provide important insight to assist mitigation and restoration efforts of *R. draytonii* habitat.

STUDY SITES

Figure 3. Rainfall dispersing the duckweed on Turtle Pond (Jan. 2016)

Figure 6. Elizabeth Grewal sampling water quality at Turtle Pond at FOP (Apr. 2016)

Figure 4. Leaky Lake (Nov. 2015)

Figure 7. Keith Wellstone and Jeff Wilcox sampling newts at Bonnie's Pond (Nov. 2015)

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SOURCES

[1]Alvarez, Jeff A. "Comparative Microhabitat Characteristics at Oviposition Sites of the California Red-Legged Frog (Rana Draytonii)." *Herpetological Conservation and Biology* (2013): 539-51.
[2]Bishop, M. et al.. Food web linkages demonstrate importance of terrestrial prey foR the threatened California Red-Legged frog. *Journal of Herpetology* (2014) 48: 137-143.
[3]Cummins, C.P. Interaction between the effects of pH and density on growth and development in *Rana temporaria l.* tadpoles. *Functional Ecology* (1989) 3: 45-52.
[4]Hayes, M., and M. Jennings. Decline of rancid frog species in Western North America: Are bullfrogs (*Rana catebeiana*) responsible? *Journal of Herpetology* (1986) 20: 490-509.
[5]Ohba, Shinya.. Density dependent effects of amphibian prey on the growth and survival of the endangered Giant Water bug, *Insecta* (2011) 2: 435-446.
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MATERIALS & METHODS

Insect Sampling:

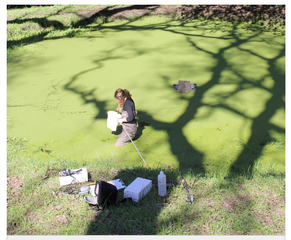

Figure 5. Lizzie sampling insects at Turtle Pond

- Aquatic insects were sampled at Turtle Pond, Bonnie's Pond and Leaky Lake for 8, 12 and 15 minutes respectfully, with a maximum depth of 5 feet. A 12'' diameter aquatic insect net and chest waders were used to collect insects for each sampling period. At the end of each sampling period, any non-insects (ie. rocks, amphipods, tadpoles etc.) sampled were removed and returned to the water.
- Insects were preserved in 70% ethanol. Samples were sorted, quantified and keyed to genus using Aquatic Insects of California (1979) and Aquatic Insects of North America (3rd ed.1996).
- Genus richness was calculated for each site over the entire sampling period and represented graphically (Fig.11).

Frog sampling:

- Abundances were determined by conducting weekly nocturnal visual surveys from November 2015 through March 2016. During each survey, two individuals scanned each pond for 12-20 minutes using a headlamp and binoculars simultaneously.
- The primary materials used included a Fenix hp26 180-lumen spotlight and 8x42 Vortex Diamondback binoculars. We used a combination of visual and vocal recognition to determine the species present at each pond.
- We recorded the species present, number of individuals observed, air temperature, and any relevant weather notes that might influence *R. draytonii* presence.
- Each survey was conducted no earlier than 30 minutes after sunset to ensure the frogs would be visible and active. In addition, surveys were not conducted if visibility was obscured due to rain or heavy fog.

Water Sampling and Egg Mass Counts:

- Sampling coincided with the breeding season and was conducted once a week during the daytime from January 1st, 2016 until March 16th, 2016.
- A LabQuest 2 computer system with nitrate, dissolved oxygen, and temperature sensors was used to collect data.
- De-ionized water was used to rinse sensors between sampling in each pond to ensure correct readings.
- Sample sites were selected by their potential for egg mass deposition based on studies by Jeff Alvarez[1].
- Due to concerns of egg mass disturbance, sampling for egg mass abundance was conducted by scanning the water and emergent vegetation from the banks of each pond.

SITE DESCRIPTIONS

- **Turtle Pond** (221 m^2) - Duckweed present, shaded by oaks
  - Species observed: Rough-skinned newts, Wood Ducks, Cooper's Hawk, Bobcat
- **Bonnie's Pond** (704 m^2) - No duckweed present, mostly open grassland bordered by oak woodland
  - Species observed: Western Pond Turtles, Western Toad, Pacific Treefrog, Rough-skinned Newt, California Newt, Canada Geese, Mallard Duck, Gartersnake spp.
- **Leaky Lake** (3,711 m^2) - No duckweed present, lowest elevation, surrounding hills of grassland slope down to water
  - Species observed: Pacific Treefrog, Western Toad, Mallard Ducks, Bufflehead Ducks, Canada Geese, Great Blue Heron, River Otters
- **Fairfield Osborn Preserve** - 2-3 inch layer of duckweed present at FOP Turtle Pond,
  - Species observed: Canada Geese, Pacific Treefrog, American bullfrog

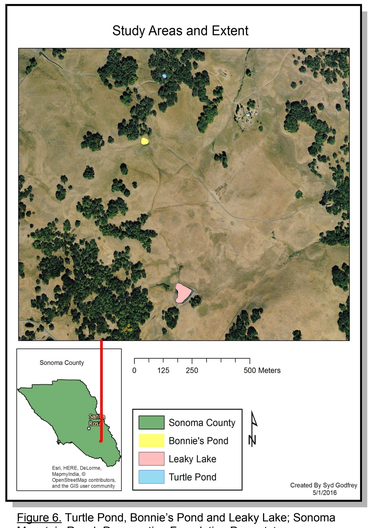

Figure 6. Turtle Pond, Bonnie's Pond and Leaky Lake; Sonoma Mountain Ranch Preservation Foundation Property[1]

RESULTS

Single Regression Analysis	Factors	R-squared	p-value
Bonnie's Pond	Water temperature (°C) x abundance of egg masses	0	0.84398
	Dissolved Oxygen (mg/L) x abundance of egg masses	0.41562	0.118
	Nitrates (mg/L) x abundance of egg masses	0.42702	0.06645
	Air temperature (°C) x abundance of adult *R.draytonii*	0.07653	0.22293
Turtle Pond	Air temperature (°C) x abundance of adult *R.draytonii*	0	0.71083
Leaky Lake	Air temperature (°C) x abundance of adult *R.draytonii*	0	0.51196

Figure 9. Single Regression Analyses; regressions were used to assess correlations between individual abiotic factors and egg mass abundance

Multiple Regression Analysis	Factors	R-squared	p-value
Bonnie's Pond	Water Temperature (°C), Dissolved Oxygen (mg/L), Nitrates (mg/L) x abundance of egg masses	0.61651	0.13404

Figure 10. Multiple Regression Analysis; a multiple variable regression was used to assess a correlation between water quality and egg mass abundance

Figure 13. R. draytonii egg mass attached to submerged substrate in Bonnie's Pond

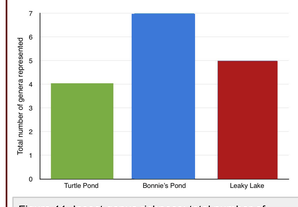

Figure 11. Insect genus richness; total number of genera identified at each site

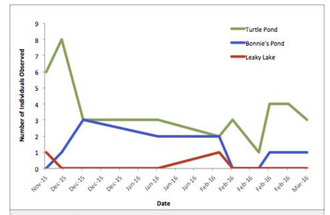

Figure 12. Observed change of R. draytonii presence in each site from November 2015 through March 2016.

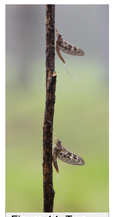

Figure 14. Two adult mayflies at Bonnie's Pond (Mar. 2016)

- Genus richness was highest in Bonnie's Pond (Fig. 11).
- Larger proportions of amphipods and glassworm larvae (G: Chaoborus) were found in Turtle Pond compared to Bonnie's Pond and Leaky Lake.
- Mayflies (G: Parameletus) were collected in Bonnie's Pond, both as naiads and breeding adults (Fig. 14).
- Predaceous insects, such as damselfly naiads and backswimmers, were found prevalently in both Bonnie's Pond and Leaky Lake. Dragonfly naiads and water boatmen were found exclusively in Bonnie's Pond.

DISCUSSION

- Though data was not collected for the abundance of Western Toads or Pacific Chorus Frogs, both species were observed in amplexus during CRLF surveys in Bonnie's Pond and Leaky Lake. In addition, egg masses for all three species were present in these two ponds indicating a correlation between habitat characteristics and oviposition sites. No egg masses were observed in Turtle pond for any of these species, though additional study would be optimal due to the higher presence of duckweed on the surface that obstructed field-of-view.
- Sampling methods for egg mass abundance were impacted by water turbidity and inclement weather on many of the survey days, resulting in incomplete or flawed data sets.
- The results of the multiple regression analysis of abiotic factors of water quality indicate that a significant trend could be observed if we increased our sample size by extending the data collection period and adding more study sites.
- Although we were able to run statistical analysis, we can not be confident that these models reflect the presence or absence of trends between water quality and egg mass deposition due to the low levels of significance.
- Bonnie's Pond, the observed breeding site of *R.draytonii,* supported the largest biodiversity of predaceous insect genera. Originally, we hypothesized that *R.draytonii* would choose a breeding site with fewer predaceous insects to limit the effect of predation on larval amphibians. The higher genera richness found at this site could be indicative of a complex food web and good water quality, supported by an abundance of producers. Bonnie's Pond has several species of aquatic plants that can act as a substrate for egg masses (Fig. 12) and might also provide shelter for tadpoles. We hypothesize these characteristics might make Bonnie's pond a better choice for tadpoles.
- Further study should be conducted in order to inform mitigation and restoration efforts regarding the abiotic and biotic factors that define the California Red-Legged frogs breeding and non-breeding habitat.