Effects of the North American Bullfrog on Native California Amphibians: A study at Blue Oak Ranch Reserve and Joseph D. Grant County Park

Jacob A. Finkle

ABSTRACT

The North American Bullfrog, *Rana catesbeiana*, is an invasive species in California. However, the effects of its presence on populations of native amphibians in the central Diablo Range (located east of San Jose, California) are not known. I collected data from 18 ponds in two protected areas in the Diablo Range to see how *R. catesbeiana* affected local amphibian populations over a one-year period. I identified and counted larvae in each pond, recorded pond area, depth, turbidity, and vegetation cover, and made subsequent observations for the rest of the year of the interactions between adults. I found that *R. catesbeiana* larvae are confined to perennial ponds and these ponds had fewer individuals of other species, but that not every perennial pond contained *R. catesbeiana* larvae. Pacific tree frog larval populations were negatively related to *R. catesbeiana* populations. All other larval species populations were either significantly related to other measured factors or not related to any measured factors. As adults, *R. catesbeiana* migrated between ponds and likely displaced native species to occupy the best territory surrounding the pond: the pond's edge. Species such as the Pacific tree frog and California toad coexisted as adults. The California red-legged frog occupied the same area as an adult as *R. catesbeiana*, but the two species were not found in the same pond. As larvae, California toads were significantly affected by area and turbidity, California tiger salamanders were observed almost exclusively in ephemeral ponds, and California newts were not affected by any measured factors.

KEYWORDS

Rana catesbeiana, *Pseudacris regilla*, Diablo Range, invasive species, ephemeral pond

INTRODUCTION

The recent human population explosion has been associated with nonnative species invasions throughout the world. Humans have been directly and indirectly responsible for introducing nonnative and invasive species to a wide variety of ecosystems. Invasive species can alter these ecosystems by destabilizing food webs, altering habitats, and changing the relationships between native species in an area. Invasive species are defined as nonnative organisms that survive, reproduce, and cause environmental or economic harm to an area (Boersma et al. 2006). These species compete with native species for food and territory and they reproduce to build self-sustaining populations. Additionally, invasive species alter ecosystems because the ecosystem does not have time to respond to their presence and integrate them into food webs (Sakai et al. 2001). A species that slowly integrates itself into a new ecosystem is not invasive, but a species that enters or is brought into an ecosystem and dominates territory and resources is certainly an invasive species. Examples include *Centaurea solstitialis*, commonly known as yellow star thistle, which has invaded many Western grasslands after being introduced at various times and locations over the past 175 years (Roché et al. 1997).

Invasive amphibian species have been linked to native amphibian population declines (Kats and Ferrer 2003). In California, precipitous declines of native species have been observed at local sites (Diamond 1996) and throughout the state (Davidson et al. 2002). Invasive species have negatively affected amphibian populations through competition for food and territory, predation, and facilitating the spread of disease (Kats and Ferrer 2003). The North American Bullfrog, *Rana catesbeiana*, is one of California's most prolific invaders, and it is a species that is well suited to outcompete local amphibian populations (Snow and Witmer 2010).

The spread of nonnative *R. catesbeiana* has occurred as some native California amphibians have experienced steep population declines during the time which *R. catesbeiana* has established large populations in many areas throughout the state. Under certain conditions, the presence of *R. catesbeiana* has been directly linked to declines in California red-legged frog populations (Kiesecker et al. 2001). The California red-legged frog, *Rana draytonii*, has experienced such a significant decline in its population that it is federally listed as a threatened species. The population declines are not limited to frogs: the California tiger salamander (*Ambystoma californiense*) has also experienced a sharp population decline due to many possible factors and is also a federally-listed threatened species (Barry and Shaffer 1994). *R. catesbeiana* is very aggressive and is a voracious generalist predator, potentially impacting the populations of all animals living in its vicinity (Stebbins 2003, Wright and Wright 1995). In addition to its predatory behavior, *R. catesbeiana* may also accelerate and increase the spread of disease to other amphibian populations. Due to its highly migratory behavior, *R. catesbeiana* is a known carrier of chytridiomycosis, which has devastated many California amphibian populations (Daszak et al. 2004). The invasive characteristics of *R. catesbeiana* coupled with the declines of California native amphibian populations highlight the importance of understanding the interactions between *R. catesbeiana* and California native amphibians.

This purpose of this observational study is to understand the interactions between *R. catesbeiana* and California native amphibian species that live in the Diablo Mountain Range of Northern California. The study sites are located in Joseph D. Grant County Park and Blue Oak Ranch Reserve, both of which are located in Santa Clara County in the Diablo Range. I hypothesize native amphibians that occupy the same ecological niche as *R. catesbeiana* will be negatively affected by its presence. Although all native species will compete with the bullfrogs for food and territory (and must avoid predation as well), species such as *R. draytonii* that occupy the same ecological niche as the bullfrog will be in direct competition with it and will probably be disproportionately affected. Similarly, I predict that species that have different behavior and lifestyle characteristics will not be as affected by *R. catesbeiana*. This will be apparent if there is no correlation between *R. catesbeiana* numbers and dissimilar species' numbers in a pond, although it is essential to realize the presence and effects of other factors acting on these species counts.

METHODS

Study Species

The North American bullfrog (*Rana catesbeiana*) is a highly adaptive amphibian that can live in a variety of different habitats. Its natural range covers much of eastern North America, from the eastern Great Plains to the Atlantic coastline, and into Canada and Mexico (Wright and Wright 1995). *R. catesbeiana* is a durable species, constrained mainly by the availability of perennial freshwater ponds that are necessary for its survival during its multi-year larval stage (Gahl et al. 2009). Larvae are primarily herbivorous and insectivorous, while adults are almost completely carnivorous (Pryor 2003). Adults are excellent predators and can overtake ponds where they have no predators due to their high fecundity rates. Although *R. catesbeiana* larvae are confined to perennial ponds, adults can migrate between ponds (Gahl et al. 2009). The bullfrog can therefore severely threaten other species if its growth is left unchecked. Humans began importing bullfrogs to California in the late 1800s to satisfy the market for frog meat (Jennings and Hayes 1985), and encouraged their spread with the construction of perennial ponds for cattle grazing (Boone et al. 2008, Doubledee et al. 2003).

Lifestyle characteristics of native amphibians may make certain species more susceptible to the negative effects of *R. catesbeiana* intrusion. The California tiger salamander (*Ambystoma californiense*) uses ponds to breed but is otherwise terrestrial. It would interact with the bullfrog in the larval stage or during the time that it spends in its burrow, since bullfrogs can hibernate in the same burrows (Stebbins 2003). The California newt (*Taricha torosa*) is extremely poisonous as an adult and is only eaten by the common gartersnake (*Thamnophis sirtalis*). Its larvae mainly experience predation from introduced fishes (Stebbins 2003), but they could also be prey for bullfrogs in the late spring and early summer. The California red-legged frog has many similar physical characteristics to the North American bullfrog, and the two prefer similar breeding habitats (*R. draytonii* prefers to use stock ponds for breeding sites in some areas), which places the two species in direct competition with each other for much of their life cycle (Fellers and Kleeman 2007). *Pseudacris regilla*, the Pacific tree frog, is one of the most prolific amphibian species in California and is a very resilient species (Stebbins 2003). The California toad (*Anaxyrus boreas halophilus*/*Bufo boreas halophilus*) is known for its schools of tadpoles, which could be consumed by adult bullfrogs. It is largely terrestrial in its adult stage (Stebbins 2003). Although it shares habitat with the bullfrog for much of its life, adult California toads tend to stay farther from the water's edge than bullfrogs, which could result in the bullfrogs being less of a threat to the toad's survival. Taken together, the differences in lifestyle traits can result in different outcomes for native species living in the presence of *R. catesbeiana*.

Study Location

I sampled from a total of 18 ponds in Joseph D. Grant County Park and Blue Oak Ranch Reserve. Eight of the ponds were perennial ponds that contained a sufficient amount of water to provide habitat for *R. catesbeiana*. Ten of the ponds were ephemeral (they dry out completely at some point during the year and therefore are not suitable habitat for *R. catesbeiana* larvae). All the ponds were located in an oak woodland ecosystem in the areas around and northeast of Hall's Valley and Poverty Ridge, and all areas were on preserve property. The total distance between the most distant ponds was 10.4 miles. All larval samplings took place in early June 2011 before metamorphosing amphibians left the ponds (late June-early August). The ponds' characteristics covered a range of surface areas and depths, although in general perennial ponds were larger than ephemeral ponds.

Sampling Design

For the first stage of my study, I sampled larval populations of amphibians in my study area. I also gathered the following data for each pond: location and area (using a GPS with accuracy to the nearest yard), maximum depth, maximum potential depth, turbidity, vegetation cover, and any other observations. To calculate the pond's location and area, I walked around the perimeter of the pond with a GPS mapping device, staying as close as possible to its shore. The device calculated the pond area. I calculated the maximum depth by either dropping a marked measurement stick to the bottom of the pond at the assumed deepest point (usually near the dam of a manmade pond or near the center of a natural ephemeral pond), or dropping a rock with a string attached to the bottom and measuring the length of string that was submerged. Vegetation cover is the estimated percent coverage of the emergent vegetation within a pond, which I estimated by eye. I used a turbidometer to calculate each pond's turbidity, measured in Nephelometric Turbidity Units (NTU).

To sample each of the ponds, I used seine nets to count their larval populations. Each individual seine sample covered an area of 300 square feet: the seine net is 10 feet long and the people carrying the net through the water walk a distance of 30 feet in the pond. Samples were not uniform in volume due to the fact that I sampled at different depths in the ponds. Seining techniques involved starting the seine in deeper areas of the pond and walking toward the shore to capture all amphibian larvae in the 300 square foot area. We dragged the seine nets along the pond bottom or as close to the bottom as possible to capture ground-dwelling amphibians. After each seine, all amphibians were identified to the species level, counted, and recorded.

The second stage of the study focused on the adult stages of the amphibians' lives. I visited the pond sites beginning in mid-July and continued through October (before the start of the rainy season) to see how the population makeup at the ponds changed throughout this time. I visited each pond multiple times during this period, and I recorded the following: changes in water level, vegetation cover, any noticeable changes in water quality, and the relative amounts of frogs or other species based on visual or auditory recognition. I then examined the areas away from the edge of the shoreline for other amphibian species that may be found farther from the immediate vicinity of the pond. It is important to note that this portion of the study was entirely qualitative, no quantities were recorded, only relative amounts of species.

Data Analysis

I used several data analysis and visualization techniques to determine if *R. catesbeiana* is responsible for population differences between ponds. I created box plots and XY comparison plots using R that show the spread of larval populations according to pond type, and native amphibian populations versus *Rana catesbeiana* populations, respectively. From my counts, I calculated species richness, species evenness, and species diversity for each pond. Finally, I used multivariate methods, including a generalized linear mixed model (glmm) for counts and a principal components analysis (PCA) to look for relationships between distinct community assemblages. For my qualitative data, I summarized my observations and I was able to understand how population structures of the ponds change as the amphibians matured throughout the season. I paid particular attention to bullfrog migrations to ponds, especially at the ponds where I did not encounter bullfrog larvae during my larval sampling. The analysis of the larval and adult stages of the amphibian life cycles showed the changing populations as the amphibians matured.

RESULTS

Analysis of Larval Data

I visited a total of 18 ponds located in Blue Oak Ranch Reserve and Joseph D. Grant County Park. Ten of the ponds from which I collected data were ephemeral ponds, and 8 of the ponds were perennial. In these ponds, I found members of every expected amphibian species: the California tiger salamander (*Ambystoma californiense*), California toad (*Anaxyrus boreas*), Pacific tree frog (*Pseudacris regilla*), North American bullfrog (*Rana catesbeiana*), California red-legged frog (*Rana draytonii*), and California newt (*Taricha torosa*).

In general, ephemeral ponds were smaller, shallower, more turbid, and had a smaller vegetation cover (Table 1).

Table 1: Average and Standard Deviation for Measured Variables.

Variable	Ephemeral	Perennial
n	21	47
Area (ft^2)	4108.24 (±3000.57)	42646.87 (±36198.64)
Depth (ft)	4.03 (±1.61)	5.15 (±0.36)
Max. Potential Depth	4.94 (±2.45)	8.87 (±3.57)
Turbidity (NTU)	115.88 (±249.59)	4.65 (±2.58)
Vegetation Cover (%)	14.95 (±23.47)	15.61(±8.25)

Certain species were more confined to ephemeral ponds (Figure 1), other species were confined to perennial ponds (Figure 4), and some species did not appear to be affected by the seasonality of the ponds (Figure 3). *A. californiense* is almost completely confined to ephemeral ponds (Figure 1). I plotted distributions of *A. boreas* in perennial and ephemeral ponds, and I found that *A. boreas* was more prevalent in perennial ponds than in ephemeral ponds (Figure 2). I collected *P. regilla* in both perennial and ephemeral ponds, and I collected approximately even amounts of specimens (Figure 3). I only found *R. catesbeiana* in perennial ponds (Figure 4), but I did not find members of the species in all perennial ponds included in my study. I collected *R. draytonii* in only one of the ponds from which I sampled (Figure 5), and the pond was perennial. I did not collect any specimens of *R. catesbeiana* from the pond where I collected *R. draytonii*. I found *T.*

torosa in both ephemeral and perennial ponds, although numbers of species appeared to fluctuate between the different pond types.

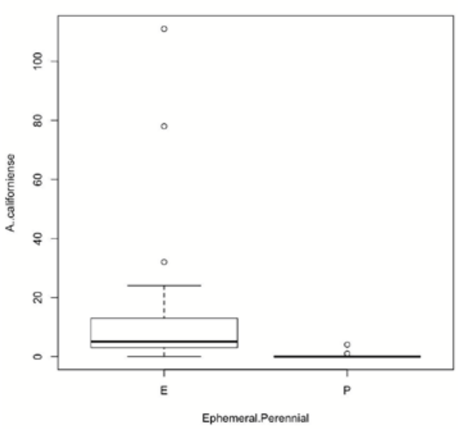


Fig. 1: A. californiense distribution.

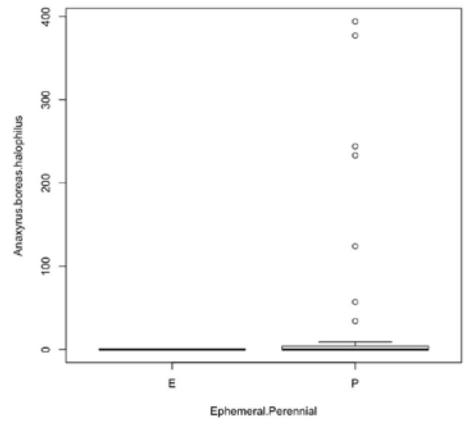
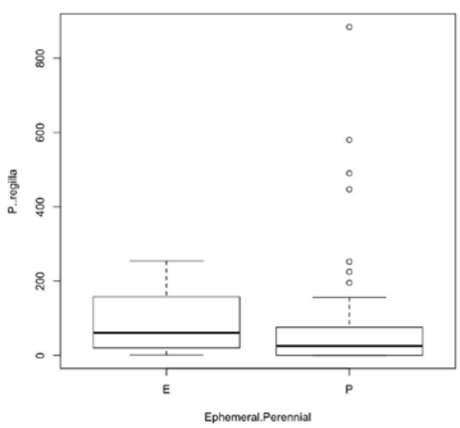


Fig. 2: A. boreas distribution.



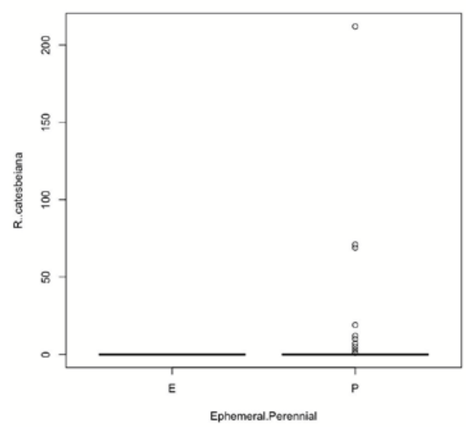
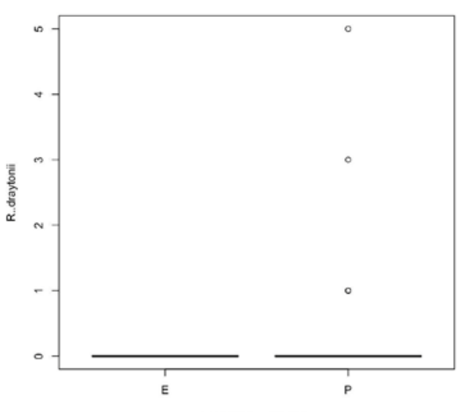


Fig. 4: R. catesbeiana Distribution.



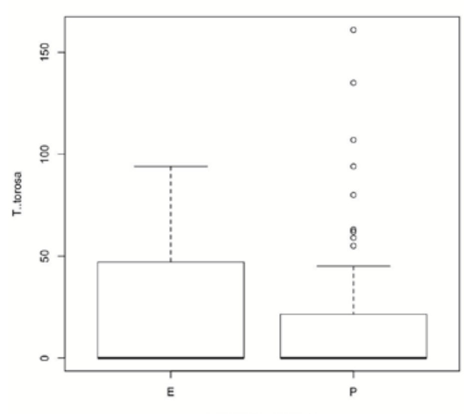


Fig. 5: R. draytonii Distribution.

Fig. 6: T. torosa Distribution.

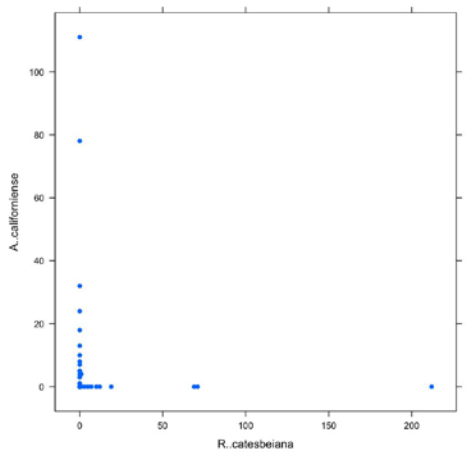


Fig. 7: A. californiense vs. R. catesbeiana.

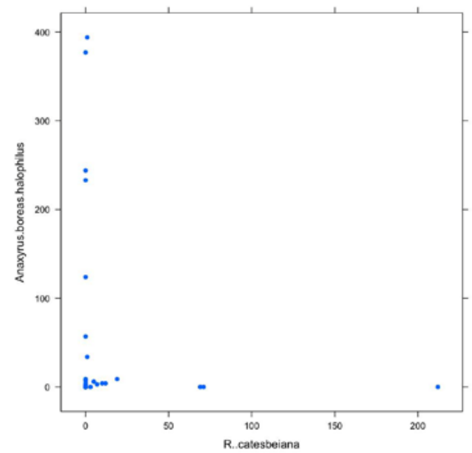


Fig. 8: A. boreas vs. R. catesbeiana

I found that certain native California amphibian species varied inversely with populations of *Rana catesbeiana*, while other species counts did not appear to have a relationship. Based on the data, populations of *A. californiense* have a negative relationship with populations of *R. catesbeiana* (Figure 7). In the case of *A. boreas* (Figure 8), there is a more positive relationship between species than the case of *A. californiense*. Differences for some species counts were significant between ephemeral and perennial ponds.

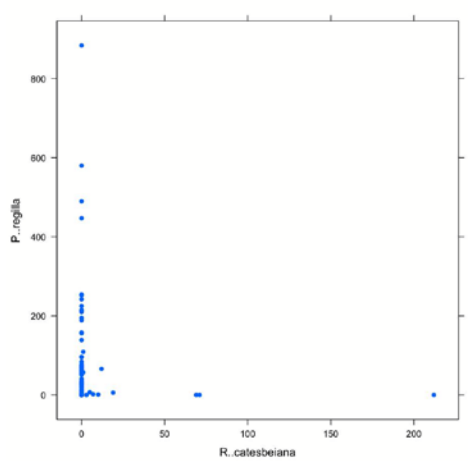


Fig. 9: P. regilla vs. R. catesbeiana.

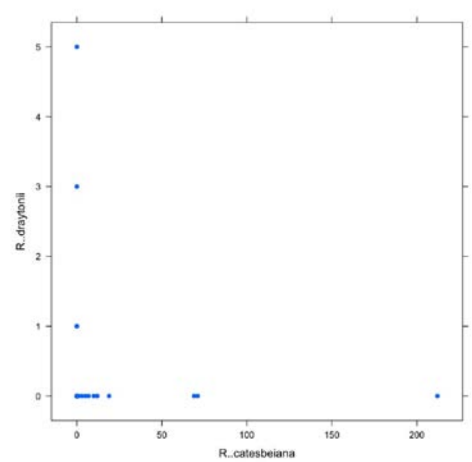


Fig. 10: R. draytonii vs. R. catesbeiana.

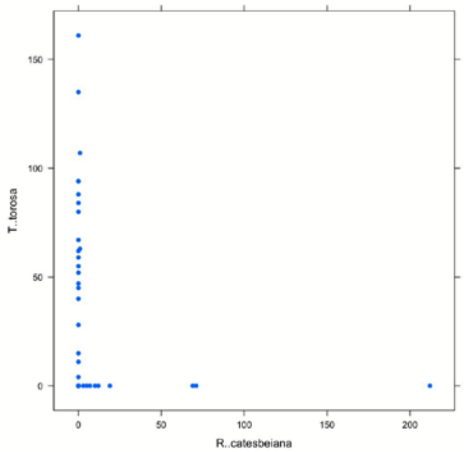


Fig. 11: T. torosa vs. R. catesbeiana.

Table 2: Average Counts by Species. Total average counts of species from all ponds.

Pond Type	*A. californiense*	*A. boreas*	*P. regilla*	*R. catesbeiana*	*R. draytonii*	*T. torosa*
Ephemeral	5.6	15.9	84.3	0	0	21.3
Perennial	32	0.1	89.5	10.9	0.2	20.1

I fit a generalized linear mixed model to amphibian counts with random effects for each pond. Populations of *A. boreas* were significantly affected by pond surface area and turbidity, but not vegetation cover. *A. boreas* populations were not significantly related to *R. catesbeiana* presence. *A. californiense* populations were not significantly affected by *R. catesbeiana* presence, pond surface area, turbidity, or vegetation cover. Populations were nearly significantly related to the seasonality of the ponds, however the p-value was slightly greater than 0.05. *P. regilla* populations were significantly affected by *R. catesbeiana* presence, but they were not affected by the seasonality of the pond, the surface area of the pond, turbidity, or vegetation cover. I was not able to determine *R. draytonii* populations' relatedness with my measured factors because the sample size was too small and restricted to only one pond. *T. torosa* populations were not significantly related to any of the measured variables: *R. catesbeiana* presence, pond area, seasonality of the pond, turbidity, or vegetation cover. Species richness was significantly related to pond area, but it was not significantly related to *R. catesbeiana* presence, turbidity, or vegetation cover. Neither species evenness nor species diversity was significantly related to any of the measured factors, including *R. catesbeiana* presence.

I used the results from the principal components analysis (Figure 12) to visualize the possible relationships between the species themselves as well as between species and the biotic and abiotic factors that I measured. The PCA plot of the ponds (shown by their number on the plot) places them according to how strongly they exhibited the included factors. Ponds 2 and 7, for example, had the highest counts of *R. catesbeiana* and also had large surface areas. Also, the "R..catesbeiana" arrow and "P..regilla" arrow point in different directions, which was confirmed by the fact that the two were related negatively (as the generalized linear mixed model showed).

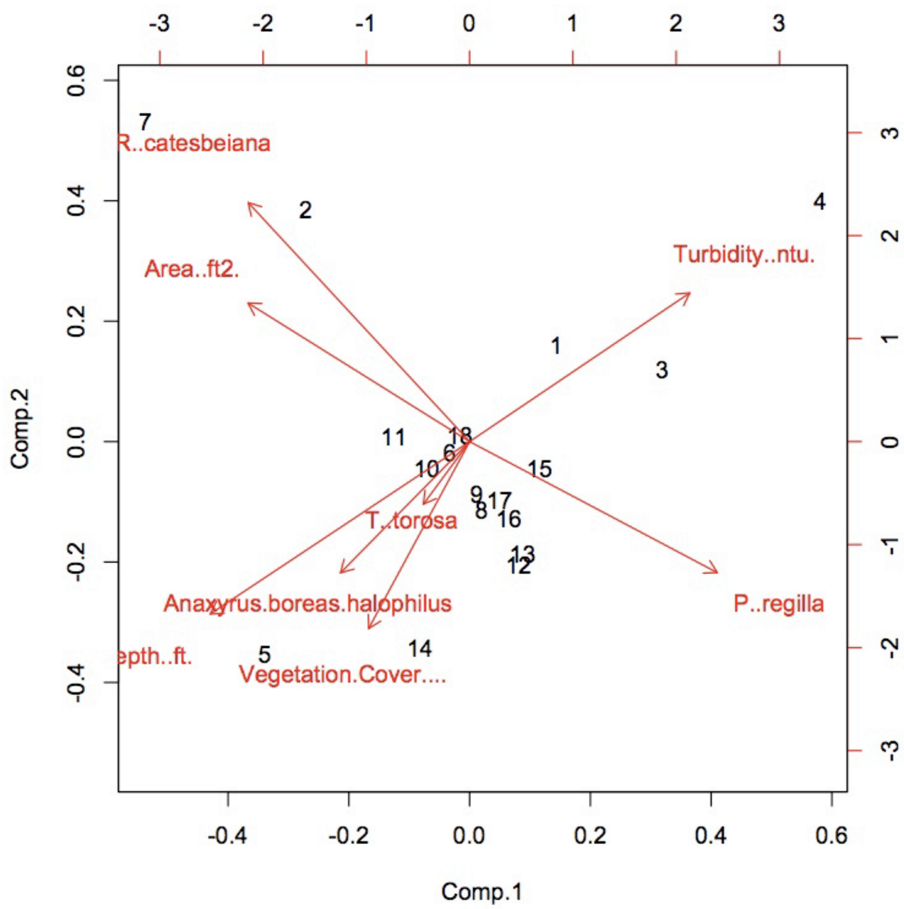


Fig. 12: PCA plot. The numbers, 1-18, in the plot correspond to each individual pond. The plot shows relationships between measured biotic and abiotic factors, and between species.

Adult Stage

I visited each pond at least twice between July 15, 2011 and October 2, 2011. I heard *R. catesbeiana* calls and saw both juveniles and adults in every remaining pond by August 21, 2011, except for two ponds. By August 21, 2011, the following ephemeral ponds did not contain any water: Pond #3, Pond #4, Pond #10, North Pond (#12), Windmill Pond (#13), Lower Turtle Pond (#15), and South Pond (#18). Barn Pond (#14) contained no more than four square feet of water, with a depth of 0.5 feet. I also witnessed adult *A. boreas*, *P. regilla* and *R. catesbeiana* around the edges of every pond (that had not desiccated after August 21, 2011) at each visit, aside from Barn Pond (no amphibians) and Cabin Pond (no *P. regilla* and no *R. catesbeiana*). *P. regilla* could generally be found within 20-30 feet of the pond's edge, especially if there was thick, low vegetation. Juvenile *A. boreas* could generally be found 20-50 feet from the pond's edge. Both *R. catesbeiana* and *R. draytonii* could either be found at the pond's edge or sitting in the pond. Both species occupied a band around the pond that extended about 5-10 feet inland from the shore. In the ponds where *R. catesbeiana* was present, I did not see any other amphibian species within this 5-10 foot band, but I saw overlapping habitats of *A. boreas* and *R. draytonii* and *A. boreas* and *P. regilla*. I did not see any specimens of *A. californiense* or *T. torosa* during my visits to the ponds.

I witnessed more than 10 adult and juvenile *R. draytonii* at Cabin Pond on October 1, 2011.

DISCUSSION

Although the results I obtained from my data did not allow me to make strong conclusions concerning the effects of *R. catesbeiana* on every individual native amphibian species, analysis of my data revealed that *R. catesbeiana* negatively influences populations of native amphibians. *R. catesbeiana* that lived in perennial ponds seemed to affect populations of native species negatively overall, according to my results. Different habitat preferences between some native species and *R. catesbeiana* rendered their relationships untestable, but it is possible that these species could not coexist with *R. catesbeiana*. If this is the case, *R. catesbeiana* is capable of excluding native species of amphibians from their habitat when the two species occupy the same niche, which confirms its classification as an invasive species.

Specifically, the adult frog species *A. boreas* and *P. regilla* were able survive and maintain sizable populations in the presence of *R. catesbeiana*, however it seemed that adult *R. catesbeiana* dominated their preferred habitat and did not live in the same zone with other species of frogs. Below I discuss the implications of my findings for each native amphibian species.

***Rana catesbeiana* (North American Bullfrog)**

I only found *R. catesbeiana* larvae in perennial ponds, an observation that is consistent with past literature (Adams 2000). Since these ponds were usually deeper and covered a larger surface area, my tests showed that *R. catesbeiana* populations were positively related to pond surface area and depth. In the majority of cases where *R. catesbeiana* were present, there were few other species and low species abundances. Past literature suggests that invasive *R. catesbeiana* tends to exclude native species from its territory because of its physical and behavioral characteristics (Kupferberg 1997).

As adults, *R. catesbeiana* remained at the pond's edge while two other observed species, *P. regilla* and *A. boreas*, occupied a niche farther away from the shore of the pond. Adult *R. catesbeiana* are aggressive predators and can consume a variety of organisms, which may explain the *R. catesbeiana*-dominated area surrounding the pond's edge; possibly *R. catesbeiana* consumed species that intruded into its habitat in and around the pond's shore (Stebbins 2003, Wright and Wright 1995). *R. catesbeiana* also exhibited migratory behavior; I observed in more than 10 individuals in several ponds where it was formerly absent or in very small numbers during larval sampling (Stebbins 2003). The fact that *R. catesbeiana* migrates means that it is capable of disturbing native species-dominated ponds, and that eradication efforts could be hindered by returning bullfrogs each season.

My calculations of species richness, species evenness, and species diversity revealed that species richness was directly related to pond area, a finding consistent with past literature as well as with my expectations (Werner et al. 2007). However, species diversity was not significantly related to any of the measured variables, even though the competitive and invasive behavior of

R. catesbeiana can decrease the populations of certain species in their habitat, as my data and past studies show (Doubledee et al. 2003).

***Pseudacris regilla* (Pacific Treefrog)**

The comparatively larger sample sizes of larval *P. regilla* throughout the study enabled me to draw stronger conclusions from my data. *P. regilla* was the most ubiquitous amphibian species, occurring in all but two ponds in my study. This echoes previous claims that the frogs are found in a variety of California's aquatic ecosystems (Adams 2000). Fluctuations in numbers of *P. regilla* between ponds were related to the presence or absence of *R. catesbeiana*, showing that the two species are antagonistic towards each other in the larval stage. The glmm confirmed that there was a significant difference in *P. regilla* populations depending on the presence of *R. catesbeiana*. This was surprising since, aside from the fact that *R. catesbeiana* is known to be an aggressive competitor as a tadpole, I could not find any literature that shows that the two species have an especially antagonistic relationship, only that *P. regilla* is among the hardiest of California amphibians and can survive in a variety of habitats (Fisher and Shaffer 1996). Although *P. regilla* is negatively affected by *R. catesbeiana*, its resilience as a species may allow it to survive despite *R. catesbeiana* intrusion. Also, *P. regilla*'s lack of any preference for all other measured factors meant that, of the factors I measured, *R. catesbeiana* presence was the only significant variable in determining population sizes of *P. regilla*.

My subsequent qualitative observations of the interactions between species as adults showed that the *P. regilla* was able to coexist with *R. catesbeiana* when each species occupied different habitat zones. In the same niche, *R. catesbeiana* likely displaced *P. regilla* from their habitat in this case. Although the frogs are capable of limited movement away from the pond for breeding purposes, they normally do not venture far from the pond (Schaub 1978). The fact that there were far more adult *P. regilla* outside of the band of habitat occupied by *R. catesbeiana* strengthens the case for *R. catesbeiana* exclusion or predation of *P. regilla*.

***Ambystoma californiense* (California Tiger Salamander)**

I observed few interactions between *A. californiense* and *R. catesbeiana* because of *A. californiense*'s preference for ephemeral ponds and *R. catesbeiana*'s requirement for perennial ponds. This observation is supported by past literature documenting the species' preferred habitats (Loredo and Van Vuren 1996, Jennings and Hayes 1986). Even in the one pond that contained both *A. californiense* and *R. catesbeiana*, the existence of *A. californiense* only in the shallow, more vegetated regions of this pond as opposed to *R. catesbeiana*'s habitat in the more open waters meant the two species probably did not interact very much. *A. californiense* is known to breed and develop in ephemeral ponds (Barry and Schaffer 1994), which also meant that they preferred more turbid, shallower, and smaller ponds, and they were not strongly correlated with any other measured variables, as the glmm showed. As a result, the seasonality, depth, size and turbidity of the pond appear to be more significant factors in determining *A. californiense* locations than the presence or absence of *R. catesbeiana*; *A. californiense* prefers ephemeral ponds.

Additionally, because *A. californiense* leave the ponds after metamorphosis (Trenham et al. 2000), they have very little contact with adult *R. catesbeiana*; they emerge from burrows during *R. catesbeiana*'s dormant period during January or February to breed (Willis et al. 1956) and therefore probably do not come into contact with the adults. My data supported these claims; I did not see any adult California tiger salamanders while I carried out my study.

***Anaxyrus bufo boreas* (California Toad)**

I expected that *A. boreas*'s preference for perennial ponds and therefore its probable interactions with *R. catesbeiana* caused its populations to be affected by *R. catesbeiana*, most likely negatively. However, the effects of *R. catesbeiana* on *A. boreas* were not significant despite the fact that the highest counts of *A. boreas* I obtained came from ponds with low or absent populations of *R. catesbeiana*. It is possible that *A. boreas* was more affected by surface area and turbidity than by *R. catesbeiana* presence, or that the sample size was simply not large enough to draw a definitive conclusion.

In the adult stage, I observed *A. boreas* individuals in a band outside of ponds inhabited by *R. catesbeiana*- all ponds at that time- and I hypothesized that, as in the case of *P. regilla*, *R. catesbeiana* could be excluding or consuming *A. boreas* juveniles. *P. regilla* and *A. boreas* frequently occupied the same niche, as evidenced by the fact that I frequently observed them overlapping in each others' habitat. Nevertheless, it is not obvious whether these species would be present at the ponds' edges if *R. catesbeiana* was not present, since *A. boreas* frequently venture relatively far away from their ponds (Tracy and Dole 1969).

Rana draytonii **(California Red-legged Frog)**

Although the samples of *R. draytonii* I obtained were too small to draw definitive conclusions through statistical tests based on my data, I compared the habitat of the population I observed to habitat described in the literature. The one pond in which I observed *R. draytonii* larvae was a deep, perennial pond with very low turbidity- habitat preferred by *R. draytonii* (Tatarian 2010). This pond should also be ideal habitat for *R. catesbeiana* (Adams 2010, Stebbins 2003), but the preserve in which the pond is located has prioritized the removal *R. catesbeiana* from its property. The similarities in terms of surface area, depth, turbidity, and vegetation cover between this pond and the *R. catesbeiana*-dominated ponds indicate that *R. draytonii* should be able to exist in those ponds. However, the two species' are essentially unable to coexist in the same areas because of competition for territory and resources (Cook and Jennings 2007).

Each time I returned to Cabin pond I never witnessed an intrusion of *R. catesbeiana*, I always only observed *R. draytonii*. This pond was therefore different from other ponds, which contained adult *R. catesbeiana* by late summer. In this case, *R. draytonii* occupied the same areas (the shoreline areas) of the pond as *R. catesbeiana* did in its invaded ponds, revealing the species' inability to coexist in the same area (Kiesecker et al. 2001).

Taricha torosa **(California Newt)**

T. torosa's populations were unaffected by any of the variables I measured due to the fact that they are not likely to associate with many of the species in the ponds; they are not specific in terms of the ponds they use for breeding, and their larvae exit the ponds during the terrestrial stage of their lives (Stebbins 2003). Relationships between *A. californiense* and *T. torosa*, two species that were often found together in ponds, confirm past studies of their behavior as habitat generalists (Ryan 2005, and Ryan forthcoming).

Limitations

Because species diversity and species evenness depend on the numbers and diversity of species in each pond, the relatively small number of ponds that I sampled may account for the unexpected results I obtained.

The most important limitation to me in carrying out this study was my sample size. A larger sample size is necessary to more accurately observe interactions between native amphibian species (Padgett-Flohr and Hopkins II 2010), and I need to include more perennial ponds in my study. Also, it would be beneficial to include more ponds in which bullfrog eradication is taking place to compare their species compositions. A larger sample size would enable me to generate more reliable statistics from a larger and more accurate dataset. Additionally, other environmental variables can affect amphibian populations, and those I did not measure may have played an important role at my sites (Smith 1999). Finally, in designing my study, I need to adequately address the fact that ephemeral and perennial ponds cannot be compared in this region because *R. catesbeiana* presence only occurs in perennial ponds, therefore *R. catesbeiana* presence or absence is confounded with the seasonality of the pond.

Future Directions

To determine the possible effects of *R. catesbeiana* on native California amphibians, future studies should include more ponds for a larger sample size (especially more perennial ponds) and employ a variety of sampling techniques to ensure data is obtained from every niche of every pond. I also need to sample throughout the larval stage, since I collected during the end of the larval period (towards the time that larvae begin metamorphosing). New types of data to collect include pond temperature, soil type, and data on pond flora. Also, future studies should collect other species in the pond including invertebrates since they may provide insight into the ecology of each pond.

Conclusion

My observational study revealed that the presence of *Rana catesbeiana* does have some effect on the five native California amphibian species I encountered at my study site in terms of effects on individual species, but that I need to investigate further to understand the relationship between the native species and *R. catesbeiana*. It is possible that the implications of understanding the relationships between these species could shed light on many current phenomena in the area, including species declines, prevalence of the fungus *Batrachochytrium dendrobatidis* that is severely affecting amphibian species populations worldwide, and possible ways to improve the situation for native amphibians. This study can also improve our understanding of what species are most affected by *R. catesbeiana*, and how species that are not affected or not as affected are able to cope with exotic species intrusion. Finally, this study could be used to test the effectiveness of *R. catesbeiana* removal and the return of native species to perennial ponds, such as that which is occurring throughout Blue Oak Ranch Reserve.

Acknowledgments

This project would not have been possible without the assistance of the following people: from ES 196, Professor Patina Mendez and Professor Kurt Spreyer, ES 196 Instructors, and Melissa Eitzel, ES 196 GSI who helped me analyze my data and write my report. John Vollmar of Vollmar Natural Lands Consulting inspired me to carry out this project and provided equipment and expertise. Dr. Rudolf von May assisted with data collection and provided knowledge of the most effective sampling techniques. Erick Mahood assisted with data collection. Donnette Lucas and Don Rocha of Santa Clara County Parks allowed me to conduct my study in Joseph D. Grant County Park, and Dr. Michael Hamilton allowed me to conduct my study in Blue Oak Ranch Reserve. Luca Pozzi helped me analyze my data using statistics and R. Dr. Kevin Lunde provided me with ideas for analyzing my data and awareness of how effective projects are carried out. My ES 196 classmates helped me communicate what I learned from my project through peer reviews. This project was also made possible by the Federal Fish and Wildlife permit TE035336-3 and State of California Fish and Game Collection Permit SC-07774.

REFERENCES

Adams M. J. 2000. Pond Permanence and the Effects of Exotic Vertebrates on Anurans. Ecological Applications 10: 559-568.

Adams M. J., C. A. Pearl. 2007. Problems and opportunities managing invasive Bullfrogs: is there any hope? Pages 679-693 *In* F. Gherardi, editor. Invading Nature - Springer Series in Invasion Ecology, Springer, PO Box 17, 3300 AA Dordrecht, Netherlands.

Barry S. J., H. B. Shaffer. 1994. The Status of the California Tiger Salamander (Ambystoma californiense) at Lagunita: A 50-Year Update. Journal of Herpetology 28: 159-164.

Boersma, P. D., S. H. Reichard, and A. N. Van Buren. 2006. Invasive Species in the Pacific Northwest. University of Washington Press. Seattle, Washington, United States.

Boone M. D., R. D. Semlitsch, and C. Mosby. 2008. Suitability of Golf Course Ponds for Amphibian Metamorphosis When Bullfrogs Are Removed. Conservation Biology 22: 172-179.

Broström, G., and H. Holmberg. 2011. glmmML: Generalized linear models with clustering. R package. http://CRAN.R-project.org/package=glmmML

Cook D. G., M. R. Jennings. 2007. Microhabitat use of the California red-legged frog and introduced bullfrog in a seasonal marsh. Herpetologica 63: 430-440.

Daszak, P., A. Strieby, A. A. Cunningham, J. E. Longcore, C. C. Brown, and D. Porter. 2004. Experimental evidence that the bullfrog (Rana catesbeiana) is a potential carrier of chytridiomycosis, an emerging fungal disease of amphibians. Herpetological Journal 14: 201-207.

Davidson, C., H. B. Shaffer, M. R. Jennings. 2002. Spatial Tests of the Pesticide Drift, Habitat Destruction, UV-B, and Climate Change Hypotheses for California Amphibian Declines. Conservation Biology 16: 1588-1601.

Diamond, J.M. 1996. A-bombs against amphibians. Nature 383: 386-387.

Doubledee R. A., E. B. Muller, and R. M. Nisbet. 2003. Bullfrogs, Disturbance Regimes, and the Persistence of California Red-Legged Frogs. The Journal of Wildlife Management 67: 424-438.

Fellers G., P. Kleeman. 2007. California Red-legged Frog (Rana Draytonii) Movement and Habitat Use: Implications for Conservation. Journal of Herpetology 41: 276-286.

Fisher R. N., H. B. Shaffer. 1996. The Decline of Amphibians in California's Great Central Valley. Conservation Biology 10: 1387-1397.

Gahl M. K., A. J. K. Calhoun, and R. Graves. 2009. Facultative use of Seasonal Pools by American Bullfrogs (Rana Catesbeiana). Wetlands 29: 697-703.

Hayes M. P., M. R. Jennings. 1986. Decline of Ranid Frog Species in Western North America: Are Bullfrogs (Rana catesbeiana) Responsible? Journal of Herpetology 20: 490-509.

Jennings M. R., M. P. Hayes. 1985. Pre-1900 Overharvest of California Red-Legged Frogs (Rana aurora draytonii): The Inducement for Bullfrog (Rana catesbeiana) Introduction. Herpetologica 41: 94-103.

Kats L. B., and R. P. Ferrer. 2003. Alien Predators and Amphibian Declines: Review of Two Decades of Science and the Transition to Conservation. Diversity and Distributions 9: 99-110.

Kiesecker J. M., A. R. Blaustein, and C. L. Miller. 2001. Potential Mechanisms Underlying the Displacement of Native Red-Legged Frogs by Introduced Bullfrogs. Ecology 82: 1964-1970.

Kupferberg S. J. 1997. Bullfrog (Rana Catesbeiana) Invasion of a California River: The Role of Larval Competition. Ecology 78: 1736-1751.

Loredo I., D. v. Vuren. 1996. Reproductive Ecology of a Population of the California Tiger Salamander. Copeia 1996: 895-901.

Padgett-Flohr G. E., R. L. Hopkins II. 2010. Landscape epidemiology of Batrachochytrium dendrobatidis in central California. Ecography 33: 688-697.

Pryor G. S. 2003. Growth rates and digestive abilities of bullfrog tadpoles (Rana catesbeiana) fed algal diets. Journal of Herpetology 37: 560-566.

R Development Core Team. 2012. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing http://www.R-project.org/

Ryan M. E., J. R. Johnson, B. M. Fitzpatrick, and J. H. Brown. 2009. Invasive Hybrid Tiger Salamander Genotypes Impact Native Amphibians. Proceedings of the National Academy of Sciences of the United States of America 106: 11166-11171.

Roché, C. T., D. C. Thill, and B. Shafii. 1997. Reproductive Phenology in Yellow Starthistle (*Centaurea solstitialis*). Weed Science 45: 763-770.

Sakai A. K., F. W. Allendorf, J. S. Holt, D. M. Lodge, J. Molofsky, K. A. With, S. Baughman, R. J. Cabin, J. E. Cohen, N. C. Ellstrand, D. E. McCauley, P. O'Neil, I. M. Parker, J. N. Thompson, and S. G. Weller. 2001. The Population Biology of Invasive Specie. Annual Review of Ecology and Systematics 32: 305-332.

Schaub, D. L. and J. H. Larsen, Jr. 1978. The Reproductive Ecology of the Pacific Treefrog (Hylla regilla). Herpetologica 34: 409-416.

Smith, G. R. 1999. Microhabitat Preferences of Bullfrog Tadpoles (*Rana catesbeiana*) of Different Ages. Transactions of the Nebraska Academy of Sciences 25: 73-76.

Snow, N. P., and G. Witmer. 2010. American Bullfrogs as Invasive Species: A Review of the Introduction, Subsequent Problems, Management Options, and Future Directions. 24th Vertebrate Pest Conference, USDA APHIS Wildlife Services, National Wildlife Research Center, Fort Collins, CO. University of California, Davis.

Stebbins, R. C. 2003. Bullfrog (American Bullfrog) *Rana catesbeiana*. Pages 240-242 *In* Western Reptiles and Amphibians, Third Edition. Houghton Mifflin Company, New York, New York.

Tatarian, P. J. 2008. Movement Patterns of California Red-Legged Frogs (*Rana draytonii*) in an Inland California Environment. Herpetological Conservation and Biology 3: 155-169.

Tracy, C. R., J. W. Dole. 1969. Orientation of Displaced California Toads, Bufo boreas, to Their Breeding Sites. Copeia 1969: 693-700.

Trenham, P. C., H. B. Shaffer, W. D. Koenig, M. R. Stromberg, and S. T. Ross. 2000. Life History and Demographic Variation in the California Tiger Salamander (*Ambystoma californiense*). Copeia 2000: 365-377.

Werner, E. E., D. K. Skelly, R. A. Relyea, and K. L. Yurewicz. 2007. Amphibian species richness across environmental gradients. Oikos 116: 1697-1712.

Wright, A. H., and A. A. Wright. 1995. Bullfrog, Bloody Nouns, Bully, Jug-o'-Rum, North American Bullfrog, American Bullfrog. Pages 444-449 *In* Handbook or Frogs and Toads of the United States and Canada. Cornell University Press, Ithaca, New York.

Willis, Y. L., Moyle, D. L., and Baskett, T. S. 1956. Emergence, Breeding, Hibernation, Movements and Transformation of the Bullfrog, *Rana catesbeiana*, in Missouri. Copeia 1: 30-41.

APPENDIX A: SPECIES IDENTIFICATION

Larval California tiger salamander (*Ambystoma californiense*) looks like a salamander with external gills attached behind its head. Larvae can be a variety of colors depending on the turbidity of the water, but they usually range from light grey to dark grey or dark green. Below is a picture of *A. californiense* larva (Figure 13). Note its gill structure, which looks like three protrusions originating at the base of its head.

Fig. 13: *A. californiense* larva.

Larval California newt (*Taricha torosa*) is very small in its earliest larval stage and often appears colorless and transparent. More mature *T. torosa* larvae are dark brown in color and can be differentiated from *A. californiense* by the fact that they lack the external gill structure and their eyes do not sit on top of their head, but are instead placed at either side. Figure 14 shows a very young *T. torosa* larva, and Figure 15 left is a more mature specimen.

Fig. 14: Very young *T. torosa*.

Fig. 15: More mature *T. torosa* larva.

California toad (*Anaxyrus bufo boreas*) tadpoles are small and black and they often can be found in large groups at the waters' edge. Figure 16 below is an example of a school of *A. boreas* tadpoles:

Fig. 16: School of *A. boreas* larvae.

Pacific tree frog (*Pseudacris regilla*) tadpoles are identifiable by their small size, generally dark body color, and eyes placed at the side of their head (as opposed to placement on top of the head). North American bullfrog (*Rana catesbeiana*) tadpoles are very large, dark in color, and have a bulb-shaped body, to which a long tail attaches. Their eyes are placed at either side of their head, and they often have visible black spots covering their body (Figure 17).

Fig. 17: *R. catesbeiana* larva.

California red-legged frog (*Rana draytonii*) tadpoles are also very large and are similar in appearance to the bullfrog tadpoles. I distinguished *R. draytonii* tadpoles from *R. catesbeiana* tadpoles by a line of circular pores extending from the eye to the tail of *R. draytonii* tadpoles.

This line of pores can be observed in Figure 18; the line of white pores begins below the eye and extends almost to the end of the tail.

Fig. 18: *R. draytonii* larva.

The identification process needed to be completed quickly to prevent injury or death to larvae due to the lack of water during counting. To prevent harm to amphibians, I identified and counted larvae at the pond's edge immediately after seining and I placed amphibians back in the pond as soon as I finished identifying and counting them. Oftentimes I would place individual specimens back in the pond after I counted them to allow the least exposure.

The second stage of the study focuses on the adult amphibian populations of the same 18 ponds that were used in the first part of the study. It is a qualitative survey of the adult amphibian population. Beginning in late July 2011, I visited each of the pond sites and observed the populations of amphibians at each pond. I based my observations on the physical traits of the adult amphibians, although I also scanned the pond perimeter to see if any larvae were present. *A. boreas* is a medium-sized toad that has a combination of colors that give it an overall dark yellow to light brown appearance. It also has a very bumpy skin, and has a large poison gland behind each eye (Figure 19).

Fig. 19: Adult *A. boreas*.

Pseudacris regilla remain small into adulthood; they generally do not exceed 5 cm snout to tail length (Stebbins 2003). They can be recognized by a dark band extending from their snout that runs across the eye and down to their underside (Figure 19). They can be green or brown and have a distinct "ribbit" call.

Fig. 20: Adult *P. regilla*.

Adult *Rana catesbeiana* are large, green frogs. They have large circular eardrums located behind their eyes, and have faint black stripes along their hind legs. Figure 21 shows a juvenile bullfrog.

Fig. 21: Juvenile *R. catesbeiana*.

R. catesbeiana can be distinguished by the loud chirps it makes when jumping in the water in response to movement and by their very deep mating call. *Rana draytonii* is also a large frog and from afar can be confused with the bullfrog. However, *R. draytonii* has very obviously colored red to orange legs, and mature adults assume a reddish or orange tint over their entire body (Figure 22).

I visited each pond several times between July and November 2011, and I also recorded observations of pond size, vegetation cover change, and any other phenomena that I observed. I visited the ponds at dusk or later in the evening because the frogs sit outside of the ponds and can easily be observed. This is in contrast to mid-day, where many frogs remain out of sight due to the heat, sunlight, and lack of insects or other organisms to eat. This is based on personal observation. All images are my own.

Fig. 22: Adult *R. draytonii*.

APPENDIX B: POND NAMES AND LOCATIONS

Table 3: Pond names and locations.

Pond Number	Pond Name	Location (Geographic Coordinates)
1	No name	37°19’41.152”N 121°40’55.691”W
2	No name	37°19’12.798”N 121°40’22.508”W
3	No name	37°19’7.558”N 121°40’14.932”W
4	No name	37°19’3.449”N 121°39’55.053”W
5	No name	37°18’42.748”N 121°39’26.93”W
6	No name	37°18’22.279”N 121°40’28.184”W
7	No name	37°18’29.804”N 121°40’41.231”W
8	No name	37°20'48.078"N 121°41'15.55"W
9	Bass Lake	37°19'58.924"N 121°42'5.776"W
10	No name	37°19.54.41"N 121°42'9.464"W
11	West Pond	37°22'46.311"N 121°44'48.065"W
12	North Pond	37°23'15.229"N 121°44'54.924"W
13	Windmill Pond	37°23'17.44"N 121°44'49.492"W
14	Barn Pond	37°23'0.188"N 121°44'17.655"W
15	Lower Turtle Pond	37°23'20.141"N 121°44'6.735"W

Table 3: Pond names and locations.

Pond Number	Pond Name	Location (Geographic Coordinates)
16	Upper Turtle Pond	37°23'23.078"N 121°43'58.303"W
17	Cabin Pond	37°22'46.288"N 121°43'54.548"W
18	South Pond	37°22'7.499"N 121°43'41.369"W