Colonizations and Chytridiomycosis: A Case Study of American Bullfrogs in Tilden Regional Park, Orinda, CA

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ABSTRACT

American bullfrogs have been introduced outside their native range in Europe, South America, and Asia, and North America. Not only are they aggressive predators, they are a carrier species for *Batrachochytrium dendrobatidis* (Bd). Bd is a fungal pathogen responsible for the large population declines of amphibian species worldwide. Although Bd has been present in California for nearly 50 years, few specific case studies on its transmission and localized impacts in the San Francisco Bay Area have been conducted. During my study, I counted a total of 9 bullfrogs over the course of my study in the Botanical Garden Pond, Junior Ranger Pond, and Environmental Education Center Ponds. Six Bullfrogs colonized the Environmental Education Center Ponds, 2 bullfrogs colonized the Junior Ranger Pond, and no bullfrogs colonized the Botanical Garden Pond. With the support of the East Bay Parks District staff, I conducted 4 separate collection events at each of the three sink populations along with Lake Anza and Wildcat Creek to sample for Bd presence. We took skin swabs of 11 bullfrogs, 14 Pacific Chorus Frogs, 5 Slender Salamanders, and 37 California Newts. Bd presence was highest among California Newts. The small, seasonal Junior Ranger Pond had the highest Bd prevalence of any source or sink location. The high concentration of Bd positive California Newts in a seasonal body of water should necessitate further study on their potential as a Bd transmission vector. Management efforts should aim to reduce the population of bullfrogs outside of their native range and to prevent existing invasive populations from spreading further.

KEYWORDS

Lithobates catesbeianus, Batrachochytrium dendrobatidis, Invasive Species, Disease Ecology

INTRODUCTION

Invasive species are becoming more and more common in a globalized world, but the extent of their impact is just beginning to be understood. About 42% of the species on the Threatened or Endangered species lists are at risk primarily because of alien-invasive species, and that number is likely going to grow as climate change expands the ranges of more species (Pimentel et al. 2005). One species, the American bullfrog *(Lithobates catesbeianus)*, has a large range of tolerable environmental conditions, which has allowed the species to become a deadly global invader. One study found, using species distribution models, that only 57% of the bullfrogs invasion success was accounted for, meaning they have the potential to spread further and further into more uncolonized, habitable ecosystems (Ficetola et al. 2007). The San Francisco Bay Area is one region where bullfrogs were introduced. In 1896, 4 ponds in El Cerrito were stocked with 36 bullfrogs because the frogging industry had overharvested red-legged frogs and was in need of a new source of frogs similar in size and meat content (Jennings and Hayes 1985). The California Department of Fish and Wildlife is currently considering a significant reduction or total ban on the live amphibian trade (Titus and Stinson 2014), and appropriate management efforts are being determined in invaded regions all over the world.

Bullfrogs have been introduced in Europe, South America, and Asia, and almost the entirety of North America outside their native range. In most cases, bullfrog introduction is due to a demand for recreational hunting or for food, but in some cases they are introduced just for the aesthetic value of having frogs in a pond (Sepulveda et al. 2015, Luja and Rodriguez-Estrella 2010). Once introduced, bullfrogs can establish breeding populations and disperse at an alarming rate. A 2014 study of bullfrogs in the Yellowstone River revealed that the number of breeding sites (presence of bullfrog eggs or larvae) increased from 12 sites in 2010 to 45 sites in 2013 (Sepulveda et al. 2015). Another study of invasive bullfrogs in oases in the Baja California desert showed a 50% increase in bullfrog-invaded oases in just 8 years (Luja and Rodriguez-Estrella 2010). This is concerning, because bullfrogs have demonstrated the ability to colonize a variety of habitats, including a harsh desert environment. Also, a bullfrog's diet consists of a diverse range of fauna, including insects, amphibians, reptiles, arthropods, larvae, fish, and even small birds. Their voracious appetite allows them to prey on nearly any species that can fit in their mouths. One study in Korea demonstrated that bullfrog-invaded sites had lower native frog density and species richness and higher submerged vegetation cover than non-invaded sites (Li et al. 2011). Not only are bullfrogs voracious predators and fierce competitors, they are also a carrier for a deadly fungal pathogen, *Batrachochytrium dendrobatidis* (Bd).

Bd is a deadly fungal infection that has been shown to cause reduced growth rates and exacerbated rates of skin sloughing in amphibian species (Davidson et al. 2007). A recent global study revealed that Bd is directly responsible for the population declines of 501 amphibian species worldwide, 90 of which are presumed extinct in the wild. An additional 124 are experiencing an over 90% decline in population size (Scheele et al. 2019). Bd's large global impact can be attributed to its ability to infect resistant carrier species. Examples of Bd carrier species include the coqui frog in Hawai'i and the American bullfrog in Korea (Beard and O'Neill 2005, Borzee et al. 2017). Bd has also been identified as the main cause of the sharp decline in common midwife toads in central Spain (Bosch et al. 2001). Researchers at the Museum of Vertebrate Zoology have linked the spread of Bd in the Western United States to the bullfrog (Yap et al. 2018), which means that many native amphibians could face population declines as the bullfrogs expand their range. Even more concerning, Bd persists in ecosystems for long periods of time without a host. Bd can persist in water for months at a time, and a study of Bd dissemination in Australia found that Bd can persist up to 9 months in moist soil (Johnson and Speare 2005), putting native species at even greater risk of infection. While a number of environmental and anthropogenic variables must be considered on a species to species basis with regards to population declines (Blaustein and Kiesecker 2002), it is clear that Bd is a significant threat to amphibian populations worldwide and that more research needs to be conducted on its impact and the dispersal of its carriers.

The main question I seek to answer with my research is how and to what extent are bullfrogs colonizing the manmade and seasonal ponds in Tilden Regional Park. More specifically, this question will be answered by two sub-questions: how do bullfrog population sizes in the population sinks, the Environmental Education Center ponds, Junior Ranger pond, and Botanical Garden pond, vary with season, and how quickly each are colonized following eradication or, in the case of the Environmental Education Center ponds and Junior Ranger pond, filling with rainwater. I predict that Jewel Lake, Lake Anza, and Wildcat Creek will act as source populations that will allow colonization of these manmade or seasonal ponds through immigration. I also hypothesize that Wildcat Creek acts as a wildlife corridor to facilitate bullfrog dispersal and that population size will steadily increase through the winter as bullfrogs move upland. Population size will likely boom in early spring when breeding begins.

My research also aims to find out if bullfrogs in Tilden Regional Park are carrying Bd and infecting native amphibians. Bd has caused huge amphibian population declines globally, and no studies have of its dispersal or transmission have been conducted in my study site. I hypothesize that bullfrogs are infecting native amphibians and that native amphibian species that are in closest contact with bullfrogs will have higher Bd infection rates.

METHODS

Study site

I conducted my study in Tilden Regional Park, a large park in the Berkeley Hills of the San Francisco Bay Area. The park lies between Kensington and Richmond with vast tracts of pine and deciduous forest. The park lies is in a Mediterranean climate with hot, dry summers and cool, wet winters. The park was created in 1936 and has a vast network of aquatic habitats. Lake Anza and Jewel Lake are man-made reservoir lakes created after construction of the Tilden Park Dam finished in 1938. This project was part of the Public Works Administration, created during the New Deal. Wildcat Creek is a natural creek that flows through Wildcat Canyon from the Berkeley Hills and empties out into San Pablo Bay, covering 13.4 miles.

Smaller water bodies in the park vary in size and use. The Botanical Garden pond is a 30' x 15' x 3' deep, man-made pond in the Tilden Botanical Garden that is surrounded by a small wall and filled with aquatic vegetation. This vegetation provides ample habitat for amphibians and is home to a large population of California Newts. The Botanical Garden Pond is 32.43 meters from Wildcat Creek, the closest source population. The Environmental Education Center ponds are man-made, seasonal ponds that were built with the hope of recreating a natural aquatic ecosystem that could be used for educational purposes and lie just North of the Environmental Education Center. The larger pond is 130' x 80' x 7' deep and the smaller pond is 80' x 60' x 7' deep, and they are 217.87 meters and 179.69 meters from Jewel Lake, respectively. The Junior Ranger pond is a 7' x 15' x 4' deep seasonal pond just East of the Environmental Education Center and is 136.92 meters from Wildcat Creek.

Tilden is home to multiple established populations of invasive bullfrogs, who have colonized multiple bodies of water and have the potential to spread to more. Currently, Lake Anza, Jewel Lake, and Wildcat Creek have established populations of bullfrogs, and the seasonal and man-made ponds nearby are perfect ecosystems for bullfrog invasions. These three water bodies act as source populations, and the Botanical Garden pond, EEC ponds, and Junior Ranger pond are all potential population sinks (Figure 1B).

Study species

Lithobates catesbeianus

The bullfrog, *Lithobates catesbeianus,* is a large olive-green frog native to the East Coast and Midwest of the United States and Canada. It is considered a "true frog" as a member of the Ranidae family and is considered invasive in the Western United States. Bullfrogs tend to inhabit large, permanent bodies of water and usually live in the shallows around the banks. Bullfrogs breed through external fertilization in the spring and early summer months, and females can lay up to 20,000 eggs (Ryan 1980). Full metamorphosis from egg to adult can take a few months to nearly three years, and their lifespan in the wild is roughly 8-10 years (Ryan 1980). Bullfrogs are the most commonly farmed frog, which has resulted in its intentional introductions around the world. Bullfrogs were introduced to California in 1896 and have been harmful to native species diversity and health. Their competition with other amphibians and spread of Bd, which causes chytridiomycosis in amphibians, have had significant impacts on freshwater ecosystems in California.

Native Amphibians

To assess the bullfrogs' impact on Bd spread, I conducted 4 collections of bullfrog specimens in these ponds along with bullfrogs in Lake Anza, Jewel Lake, and Wildcat Creek while also collecting specimens of native amphibians. Slender Salamanders, *Batrachoseps attenuatus,* are small, terrestrial salamanders; Pacific Chorus Frogs, *Pseudacris regilla,* are small tree frogs that spend much of their time in bodies of water; and California Newts, *Taricha torosa*, a mildly toxic brown newt with a bright orange underside. The collections occurred every month to six weeks from the end of November to the beginning of April. Pacific Chorus Frogs and California Newts both share aquatic habitats with bullfrogs and are often in close contact with each other, making them a prime target for Bd infection. Slender Salamanders are terrestrial amphibians, but tend to live under rocks, logs, etc. near bodies of water. Studying their infection rates will give greater insight to the magnitude of Bd spread.

Bullfrog population surveys

To determine bullfrog population sizes and distributions in each of the potential sink populations, I conducted daytime population surveys each week from September to April. These surveys took place in Tilden's Botanical Garden pond, which had undergone a bullfrog eradication event just prior to my data collection, and the Environmental Education Center ponds and Junior Ranger pond, which were initially unoccupied because they are seasonal ponds and had only filled with rainwater in January. I used ten-minute point counts at each pond; point counts were at least five minutes apart from each other to give the bullfrogs the chance to move or other bullfrogs to surface. The specific number of point counts was based off the pond characteristics, which include visibility, presence of vegetation, and size. I conducted monthly night time counts to reinforce daytime population count results, which required laser pointers, binoculars, and flashlights to locate bullfrog eye shine, which produced a pale blue light. I documented weather conditions and pond characteristics in my field notes journal before each point count. Lastly, I measured the distance to the nearest bullfrog source population and pond circumference in meters using tape measures.

Bullfrog colonization modeling

To model colonization rate, I used my weekly point counts to measure arrival rate; this gave me an idea of how many frogs were migrating to the sink ponds from the source populations in Lake Anza, Jewel Lake, and Wildcat Creek. I used these weekly data points to make a graph showing the changes in total population over time. I assumed that bullfrog colonization rate was independent of total population in the sink ponds, as monthly collection events removed some bullfrogs from each sink pond where they were present.

Batrachochytrium dendrobatidis **sampling**

Swab collection

To determine if bullfrogs are spreading Bd to native amphibian populations, I collected bullfrog skin swabs in the Environmental Education Center ponds, Junior Ranger pond, Botanical Garden pond, Lake Anza, Jewel Lake, and Wildcat Creek every 4 to 6 weeks. I also collected skin swabs of slender salamanders, *Batrachoseps attenuatus*; Pacific chorus frogs, *Pseudacris regilla*; and California newts, *Taricha torosa*, all of which are native Californian amphibians. Pacific Chorus Frogs and California Newts both share aquatic habitats with bullfrogs and are often in close contact with each other, making them a prime target for Bd infection. Slender Salamanders are terrestrial amphibians and tend to live under rocks, logs, and other small spaces near bodies of water. Each collected sample was swabbed under the collection permits held by East Bay Regional Parks District Resource Analyst Tammy Lim. Additionally, I took water samples of the source and sink populations every 10 weeks over the course of my study. At each location, I took 2 liters of water from each side. The water collections were used to give us a better understanding of how abundant Bd is in the park and if locations with more bullfrogs had higher concentrations of Bd.

Bd assay

I placed each swab in a separate capped vial, labeled with species and location. I recorded the location of capture along with the weather conditions and time. I swabbed each specimen 5 times on dorsal, ventral, right and left sides, and feet with a sterile swab, and the swab was placed in a sterile 1.5ml vial and transferred to a -20*C freezer. I filtered each water sample through a vacuum flask and cut each vacuum filter in half and stored each one individually in a -20*C freezer. For all test tubes containing filters or swabs, I followed the recommended manufacturer's protocol for the Qiagen DNEasy Blood and Tissue DNA extraction kit. I performed the final lowTE wash twice, giving me 100 microLiters of DNA in lowTE solution for each sample.

Once the DNA was extracted from the swabs and filters, they were then tested for Bd DNA via qPCR (Boyle et al. 2004). In a 96 well well-plate, we added 20 microliters of a master mix, including primers (ITS and 5.8S), BSA, water, and a fluorescent probe. 24 samples were placed in triplicate in the well-plate with 7 standards and one negative control, also in triplicate. For filters, we included an additional step to remove PCR inhibitors before testing (Zymo Research One-step PCR inhibitor removal kit). Also, we filtered distilled water and used the same extraction protocol used on the other samples to get a negative control. Each sample was tested in triplicate, and a sample with at least 2 of 3 wells coming back positive was considered positive. Samples with only 1 of 3 coming back positive were considered unknown. We used qPCR analysis to determine the Bd concentration of lowTE solution. I then created graphs of infection rates of each species over time at different locations using these results and compared rates of infection among species and location.

RESULTS

Population surveys

I counted a total of 9 bullfrogs over the course of my study in the Botanical Garden Pond, Junior Ranger Pond, and Environmental Education Center Ponds. There was 1 bullfrog present in the Botanical Garden Pond by the end of my point counts, 2 in the Junior Ranger Pond, and 6 in the EEC Ponds (Table 1). The Botanical Garden Pond had 16 more survey events than the Junior Ranger and EEC Ponds. This is because the Botanical Garden Pond is a manmade pond that is maintained year-round, while the Junior Ranger Pond and EEC Ponds are seasonal ponds and only filled up with water during December and January.

Bullfrog colonization modelling

Bullfrogs disproportionately colonized the Environmental Education Center Ponds, few bullfrogs arrived in the Junior Ranger Pond, and no bullfrogs colonized the Botanical Garden Pond (Table 2). 1 bullfrog was present in the Botanical Garden pond but was present at the beginning of the study, so it was not counted as an arrival. That bullfrog likely avoided capture when the Botanical Garden pond was eradicated in October. Bullfrogs colonized the sink ponds in much higher numbers during the Spring months than the Fall or Winter (Figure 2). This was anticipated, as bullfrogs are usually much more sessile during the colder months of the year.

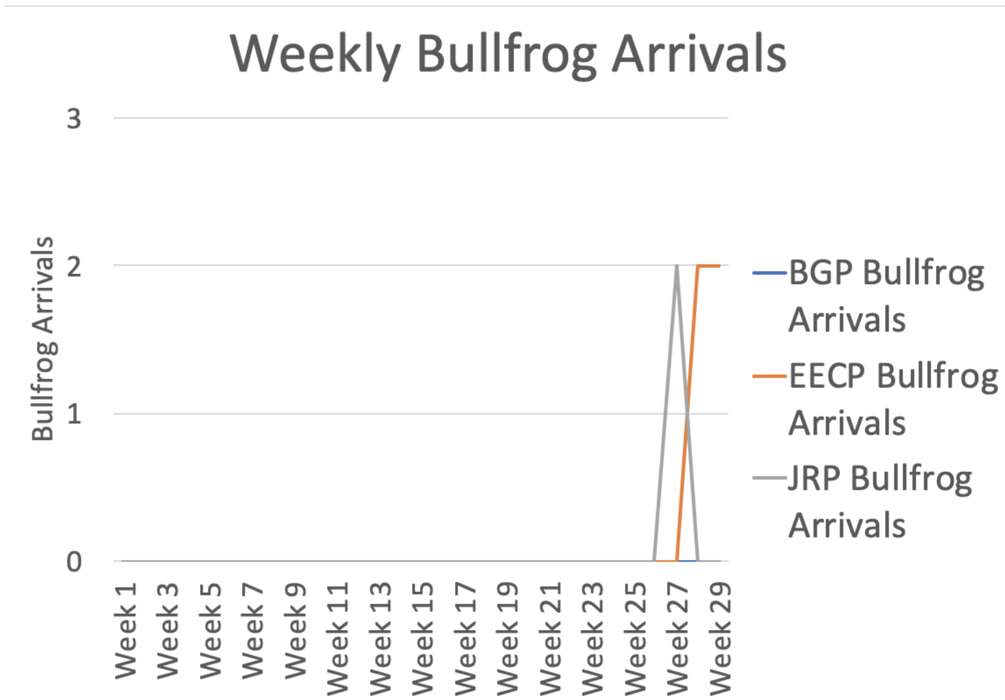

Figure 2. Weekly Bullfrog Arrivals. This graph demonstrates the increase in colonization by bullfrogs of the three sink populations from October 2018 to April 2019.

***Batrachochytrium dendrobatidis* sampling**

Specimen collection

With the help of the East Bay Parks District staff, I conducted 4 separate swab collection events at each of the three source populations along with Lake Anza and Wildcat Creek. In 4 collections, we collected swabs from 11 bullfrogs, 14 Pacific Chorus Frogs, 5 Slender Salamanders, and 37 California Newts (Figure 3). Swab totals for each collection event were 13, 16, 17, and 21 respectively (Table 3), and we collected the most samples, 19, from the Junior Ranger Pond (Table 4).

Bd assay

After using qPCR to determine which specimens were infected, I found that Bd presence was highest among California Newts (Figure 4) and that the Junior Ranger Pond had the highest Bd presence of any of the source or sink populations (Figure 5). No bullfrogs sampled in this study tested positive for Bd. I observed that smaller habitats with high species densities, mostly the sink population locations, generally had higher rates of infection.

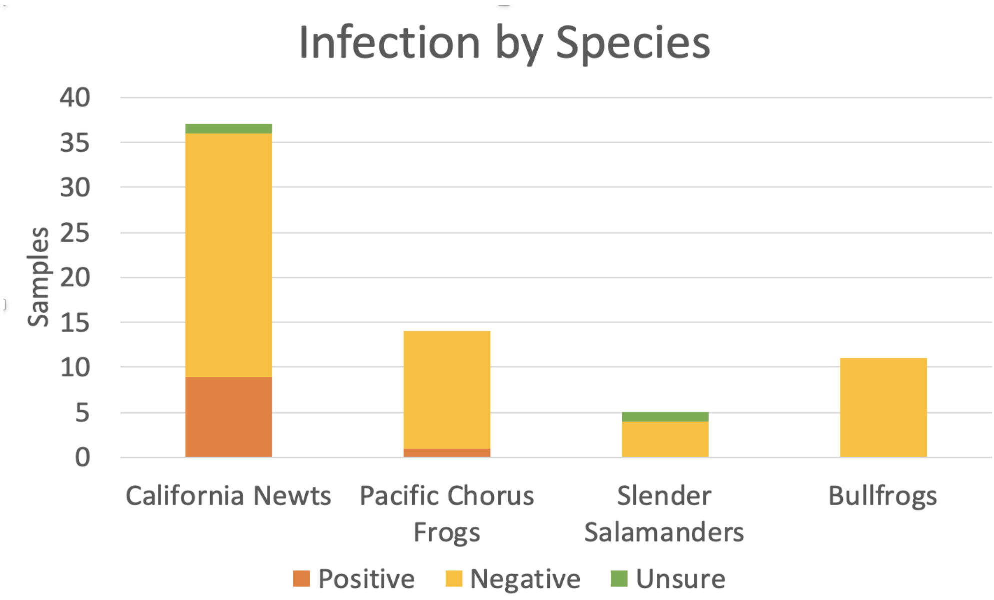

Figure 4. Infection by Species.

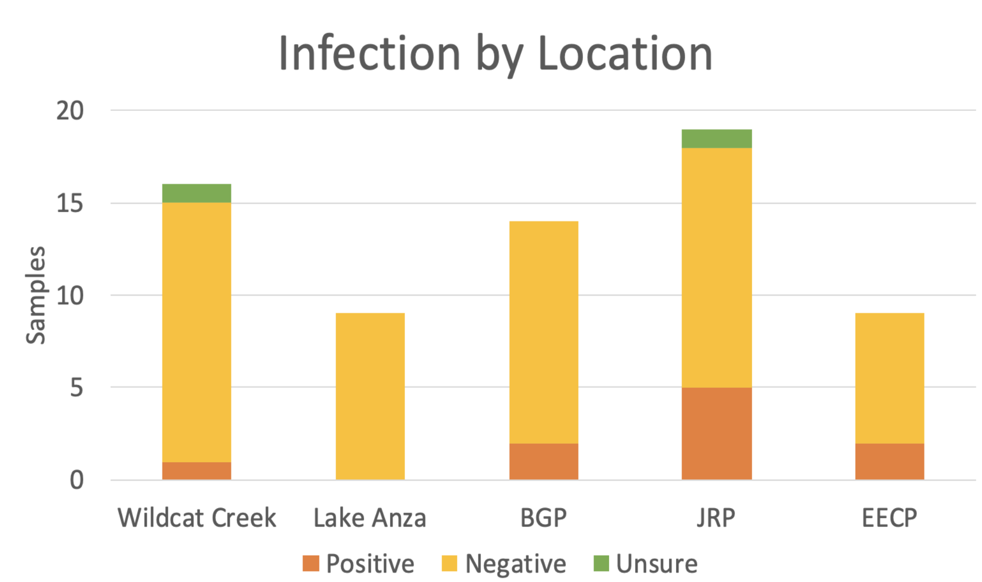

Figure 5. Infection by Location.

DISCUSSION

Introduction

Despite the limitations to my study, the results were very unexpected. With such little movement of bullfrogs during the period of my study, I was unable to substantiate my main hypothesis regarding bullfrog colonization. However, my Bd tests raise concerns of the health of the aquatic ecosystems in Tilden Regional Park and should necessitate new management techniques to curb the spread of Bd in Tilden Regional Park and the San Francisco Bay Area.

Population surveys

Over 30 weeks of conducting point count surveys, I counted 6 bullfrog arrivals in the Environmental Education Center Ponds, 2 bullfrog arrivals in the Junior Ranger Pond, and 0 bullfrog arrivals in the Botanical Garden Pond. These low numbers are likely the result of poor weather conditions, discussed further in the Limitations and Future Directions section, but also natural barriers making the journey from source to sink more difficult. Because we only counted 8 total bullfrog arrivals at all of our sink locations, we did not create generalized linear models to show or predict their movement patterns. The sample sizes were too small, and the short time frame of my study did not include the late Spring and Summer months when movement is much more frequent.

Bullfrog colonizations

The Botanical Garden Pond underwent a bullfrog eradication just prior to the beginning of my study, which removed 46 bullfrogs. The presence of one bullfrog following the eradication is likely a bullfrog that avoided capture. The re-emergence of one bullfrog in my population surveys in March is likely the same bullfrog emerging from a winter hibernation period. Though this population sink was closest to a source population, 32.43m from Wildcat Creek, the source is surrounded by a tall rock wall, making it difficult for any amphibians to travel from source to sink.

The Junior Ranger Pond is the smallest sink population and sits atop the hill near the Tilden Little Farm. There were only 2 bullfrog colonizations recorded here during my study. Though this may seem small, the change in elevation from source to sink along with the human disturbance associated with its proximity to a popular attraction in the park would understandably prevent more bullfrogs from colonizing the pond. The bullfrogs found here may have even travelled from Jewel Lake, stopped in the Environmental Education Center Ponds, then made the trek to the Junior Ranger Pond, as this path would limit exposure to human disturbance.

The Environmental Education Center Ponds had 6 recorded bullfrog colonizations over my period of study, all of which came in April. These bullfrogs most likely came from Jewel Lake, as there is a cleared, dirt walking path from Jewel Lake to these ponds, offering the easiest travel route from source to sink of any of my other study sites. This site also was larger than the other sink populations and had more resources, including hiding space and food, in the form of native amphibians, to maintain a population of bullfrogs. Unlike the other 2 sink populations, there was no significant change in elevation for bullfrogs travelling from source to sink population.

Bd assay

Water samples

We sampled the water in the source populations and the Botanical Garden Pond 3 times and the Junior Ranger Pond and Environmental Education Center Ponds twice. All samples from the third collection event, which included samples from all sources and sinks, were determined contaminated. Negative controls came back positive in both rounds of qPCR on the samples from the third collection event. However, we found that water samples from Jewel Lake, Lake Anza, and the Botanical Garden Pond, all of which currently have or recently had a large bullfrog population, tested positive for Bd. Samples from South Wildcat Creek, the section closest to the Botanical Garden Pond, and the smaller Environmental Education Center Pond also had 1 positive result when tested in triplicate.

Skin samples

We found 10 positive samples over the course of my sampling period, all of which were either California Newts or Pacific Chorus Frog. We were uncertain of Bd infection for one Slender Salamander and one California Newt that both only had 1 positive DNA sample when tested in triplicate. All bullfrogs sampled in this study came back negative. Because our bullfrog sample size was only 11 and predominantly composed of tadpoles and subadults, we cannot interpret our data as being representative of the entire bullfrog population in Tilden Regional Park.

The high concentration of positive samples in the Junior Ranger Pond was very unexpected. Despite Bd usually spreading less during the winter due to colder temperatures (Weinstein, 2009), there were 5 positive samples. California Newts were the only recorded Bd-positive amphibian species in the Junior Ranger Pond, which, because both bullfrogs in the Junior Ranger Pond were negative for Bd, may mean that Bd has already spread to large populations of California Newts and that they are now spreading Bd amongst themselves. Bd, however, may not be as lethal to California Newts as it is for many other amphibian species. Because Bd has been present in California for at least 50 years, it also may be possible that the California Newts' almost yearly breeding cycles and short period from egg to sexually mature adult (3 years) has produced enough generations for some genetic resistance to Bd to be naturally selected.

Limitations and future direction

I conducted my study from early October of 2018 to late April of 2019, and during this time period, there were major fluctuations in weather patterns. The winter in the San Francisco Bay Area during my period of study was 1°F warmer than average, and the abundance of individual bullfrogs usually is positively related to longer photoperiods and higher temperatures (Medeiros et al, 2016). However, February was particularly cold: 3.6°F below average (National Weather Service). A warm February would have provided more opportunity for bullfrog colonization, especially coupled with a rainy Winter: 1.73" more rainfall than usual (National Weather Service). Not only did weather significantly limit my data, human disturbance was also a factor in decreased bullfrog movement in Tilden Regional Park. The Environmental Education Center Ponds are not only seasonal, like the Junior Ranger Pond, but they were also undergoing a restoration project, which included building paths, planting trees, and increased human activity. This likely limited bullfrog movement from Jewel Lake to the EEC Ponds and Junior Ranger Pond.

Second, I ran into many logistical limitations that limited the scale and scope of my study. Because my study took place in a public park, I needed specific collection permits to conduct my research. These were provided by East Bay Parks District Resource Analyst Tammy Lim but took nearly a month to confirm. Because of this, my first collection was in late November, which was not an optimal time for bullfrog activity. Being able to begin my research earlier would have provided more comprehensive data, as a particularly warm Fall allowed for heightened bullfrog activity. In an ideal situation, my study would have taken place over at least a full year, so I could collect bullfrog population and amphibian skin swab data from all four seasons.

My third water sample collection underwent 2 separate qPCR events, and both came back with unreasonably high concentrations of Bd, including the negative controls. Because of this we were forced to throw out all the samples from that event and were unable to determine the Bd concentrations in the water of the sources and sinks after January. We are still unsure how all of our samples were contaminated but suspect the contamination occurred during the DNA extraction stage. Unfortunately, we were unable to infer any trends in Bd concentration in the environment and bullfrog colonization.

The high concentration of Bd positive California Newts in a seasonal body of water necessitate further study. Specifically, the ability for California Newts to carry Bd and the symptoms associated with being Bd positive should be researched more thoroughly. There were no significant, unexplained die-offs of California Newts, and not a single sample collected during my study showed any obvious signs of Bd infection, which is very unusual for species who have historically not been introduced to Bd. California Newts are one of the most abundant amphibian species in Northern California, and if they have the same ability as bullfrogs to act as a Bd carrier species, or at least act as a vector of transmission, there could be drastic consequences for the rest of Northern California's amphibian population.

Broader Implications

Bullfrogs remain arguably the largest threat to native amphibians and aquatic environments in California. Their ability to spread Bd along with their voracious appetite and aggressive territoriality make them a huge threat to native wildlife populations. Management efforts should continue to aim to reduce the population of bullfrogs outside of their native range and prevent their populations from spreading to new areas. It is clear now, however, that Bd is already very widespread among California Newts. This suggests the need for management efforts to contain the spread of Bd, and more research should be conducted on the California Newt's potential as a vector for Bd transmission.

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APPENDIX A

Figure 1A. Tilden in the Bay. This map shows where Tilden Regional Park is located in the Bay Area.

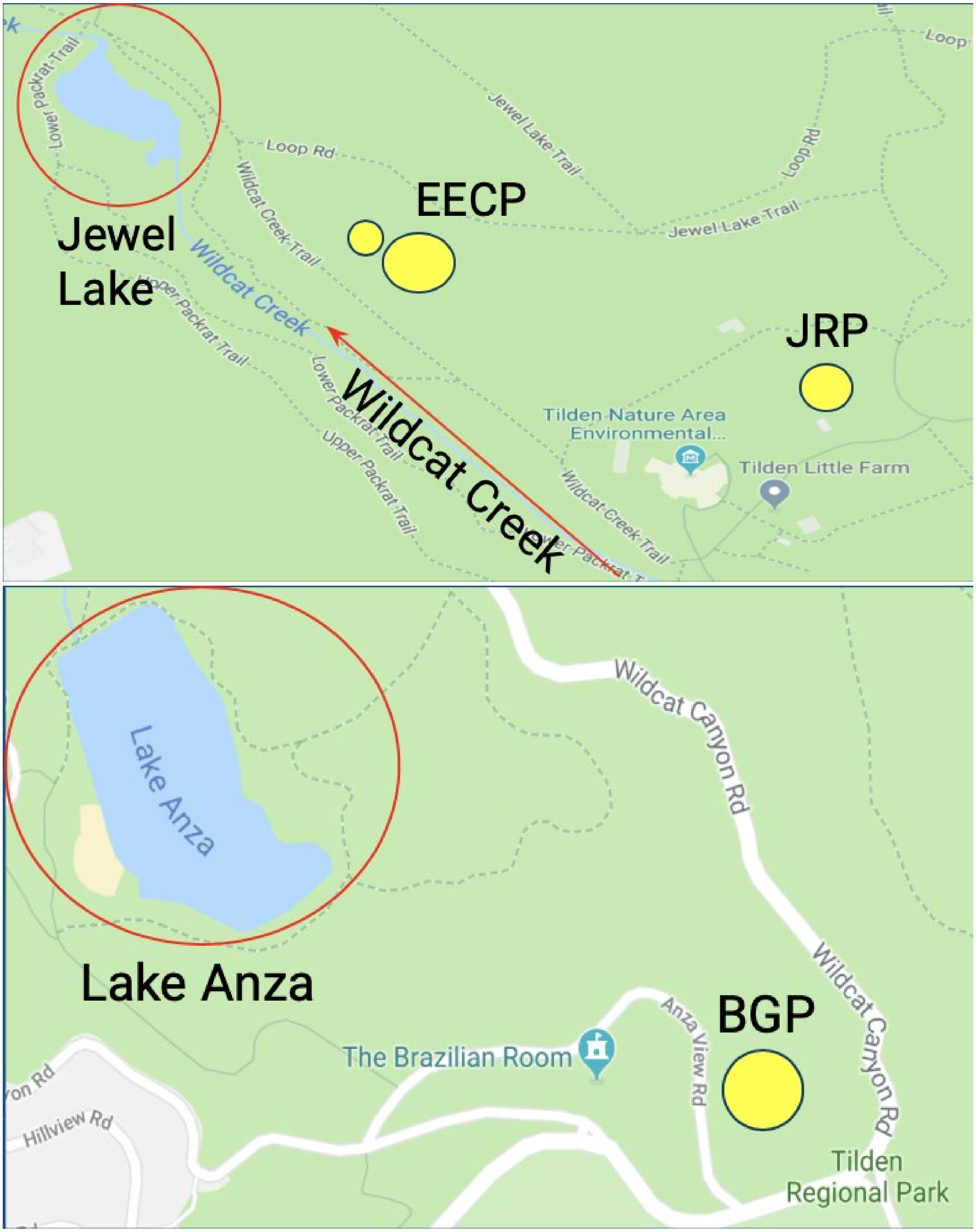

Figure 1B. Sources and Sinks of Tilden Regional Park. These maps show the spatial layout of the source populations (red) and sink populations (yellow) that were surveyed in my study. There is a section of Wildcat Creek, not displayed on the map, that curves above the Botanical Garden Pond and under Wildcat Canyon Road that connects to the Southeast corner of Lake Anza. This section of the creek was considered the closest source population to the Botanical Garden Pond.

Table 1. Surveys and Sightings.

Location	Number of Surveys	Number of Bullfrogs Counted	Period of Survey
Botanical Garden Pond	30	1	October to April
Junior Ranger Pond	14	2	January to April
Environmental Education Center Ponds	14	6	January to April

Table 2. Bullfrog Arrivals by Month.

	Bot. Garden Pond	Junior Ranger Pond	EEC Ponds
October	0	No Data	No Data
November	0	No Data	No Data
December	0	No Data	No Data
January	0	No Data	No Data
February	0	0	0
March	0	0	0
April	0	2	6

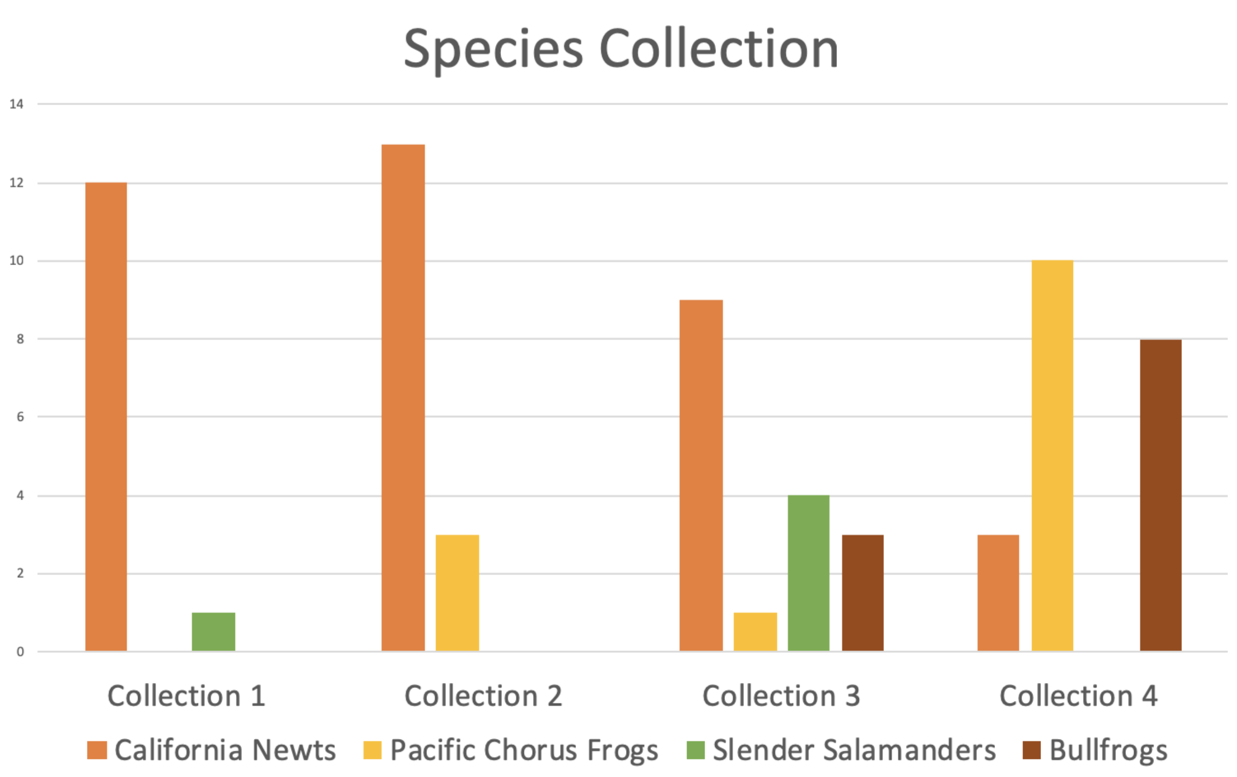

Figure 3. Species Collection. This chart shows the raw counts of each collection event by species.

Table 3. Collection Data. This table contains the number of specimens collected at each collection event by location (Source or Sink).

Population Sinks	Collection 1 (11/28/18)	Collection 2 (2/4/19)	Collection 3 (3/1/19)	Collection 4 (4/4/19)
Junior Ranger Pond	N/A	7	8	4
Botanical Garden Pond	N/A	5	3	6
Environmental Education Center Ponds	N/A	4	N/A	5
Total	N/A	16	11	15

Population Sources	Collection 1 (11/28/18)	Collection 2 (2/4/19)	Collection 3 (3/1/19)	Collection 4 (4/4/19)
Lake Anza	N/A	N/A	3	6
Wildcat Creek	13	N/A	3	N/A
Total	13	N/A	6	6

Table 4. Species Totals by Site.

Species	EEC Pond	Junior Ranger Pond	Botanical Garden Pond	Lake Anza	Wildcat Creek
Bullfrog	0	2	0	9	0
Pacific Chorus Frog	7	2	5	0	0
Slender Salamander	0	4	0	0	1
California Newt	2	11	9	0	15
Total	9	19	14	9	16