Comparison of Habitat Use and Movement Patterns of Native and Invasive Frogs in a Grassland and Oak Savannah Habitat

By

Lisa L. Surber

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Biology Committee Members:

Dr. Derek Girman

Dr. Daniel Crocker

Mr. Jeff Wilcox

Date:

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Abstract

Research on animal movement patterns and factors that influence these patterns is vital to conservation of endangered species. The California Red-legged frog *(Rana draytonii*) is a threatened species native to California. Their rapid decline has been largely attributed to habitat loss and introduction of invasive species, including the American Bullfrog (*Lithobates catesbeianus*). The aim of this research was to compare the nocturnal habitat use and seasonal movement patterns of *R. draytonii* and *L. catesbeianus*. I conducted a radio telemetry study in Sonoma County, California and mapped the locations of 13 *L. catesbeianus* and 51 *R. draytonii* from May 2017 to June 2018. Using a mixed model, I evaluated the effects of species, sex, size and rainfall on frog movement rate and compared habitat use relative to a water source. Within this model species, size and sex were found to have significant effects on movement rate. Rainfall was not found to have a significant effect on movement rate for either species. When comparing nocturnal habitat movement, I found that in the summer months species occupy different places relative to water sources in a creek environment; *R. draytonii* position themselves higher and further away from the nearest open water than *L. catesbeianus*. My work suggests that there are significant differences between the seasonal movement patterns and nocturnal habitat use of native *R. draytonii* and invasive *L. catesbeianus.* These differences may be helpful to conservation practices facilitating the survival of threatened *R. draytonii.*

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Introduction

Amphibian populations are in decline worldwide (Blaustein and Wake 1990). The factors causing these declines vary, but research indicates habitat loss (Lehtinen et al. 1999), introduced diseases (Carey 1993, Retallick et al. 2004), and introduced invasive species to be leading drivers of declines (Bronmark and Edenhamn 1994, Hayes and Jennings 1996, Moyle 1973). Most amphibian species have a biphasic life cycle, where larvae develop in water and then transition to air-breathing terrestrial adults (Wilbur 1980). In the aquatic stage, larval amphibians are threatened by water pollution (deWijer 2003, Rouse et al.1999), fluctuating hydrological cycles (Salice 2012), and human-altered lotic flow regimes (ie., Kupferberg 2012). Additionally, eggs and aquatic larvae are prone to predation by introduced invasive species (Kats and Ferrer 2003, Cook and Jennings 2001). Vulnerabilities of terrestrial adult amphibians include habitat loss and fragmentation (Cushman 2009), road mortality (Forman and Alexander 1998, Mazerolle 2004), and competition with invasive species (D'Amore 2009). Though some threats are present in all life stages, like invasive species, this complex life cycle can make conservation efforts challenging, as there are both aquatic and terrestrial habitats to consider (Baldwin et al. 2006). It is critical to understand how movement patterns and habitat use of native species may be impacted by invasive species.

Frog dispersal, the movement of organisms between suitable habitats, is a life history trait that may have significant impact on population survival and persistence, drive speciation, local adaptations, and evolution by determining gene flow within and among populations (Andreassen et al. 2002). Ecologists examine dispersal in two ways: behavioral (cues that initiate dispersal) and ecological (mechanisms, route determinants, spacing, navigating landscapes, reproductive and sexual differences, etc.). The dispersal of young influences ecological phenomena such as distribution, species abundance and community structure (Dieckmann et al. 1999). Long distance dispersal may be infrequent, yet important to species distribution within suitable habitats and for genetic exchange. While emigration (e.g., Sinsch 1990) may be critical to frog populations for the reasons discussed above, most temperate frog species are considered to be poor dispersers with high site fidelity (Duellman and Trueb 1986, Sinsch 1990, Blaustein et al. 1994, B). Thus, within populations some frogs emigrate while others remain in suitable habitat within seasonal migration distance of their natal aquatic habitats, where they return to reproduce.

Even frogs exhibiting high site fidelity are compelled to move within their occupied home ranges for reasons such as better access to foraging, reduced competition, predator avoidance, reproductive migrations, and seasonal habitat changes. Movement cues are often specific to species and environment (Sinsch 1990, Bulger et al. 2003; Fellers and Kleeman 2007) but, the terrestrial movement of frogs is constrained by demands of water balance and thermoregulation (Sinch 1990), and there are risks of predation exposure, desiccation and starvation during overland travel between foraging, basking, and breeding habitats (Semlitsch and Bodie 2003).

In contrast to long distance travel, the daily movements of frogs are driven more by maintaining physiological requirements such as food and shelter needs, as well as avoiding predators and attaining growth (Woolbright 1985, Freed 1980, Hodgkison and Hero 2001, Lillywhite 1970). Frog daily activities revolve around gaining size because greater size reduces the risk of predation, increases the range of prey that may be consumed, and confers energetic advantages and earlier sexual maturity (Werner and Gilliam 1984; Sebens 1987). For frogs, the selection of microhabitat, a localized area that may differ from the surrounding environment, is critical because their permeable skin is vulnerable to desiccation (Hodgkison and Hero 2001, Woolbright 1985) and, as ectotherms, their body temperatures are dependent on environmental conditions (Lillywhite 2010). To regulate their body temperatures, semiaquatic frogs display “basking” behavior: they sit in the shallows or outside banks of nearby water sources (Freed 1980, Lillywhite 1970). It has been demonstrated that increased body temperature increases the rate of food digestion, which increases growth rate (Freed 1980). Basking is often observed in warm summer months, whereas in colder months frogs are seen near the middle of water sources rather than the edge, as it is warmer in the water than in the outside air, as the air temperatures are colder than that of the water (Lillywhite 1970). The advantages of basking behavior are offset by the risk of predation and desiccation. Understanding the nature of microhabitat use by basking frogs is critical, as physical structure of the streams and ponds may be impacted by habitat degradation or modification because some kinds of habitat modification have been observed to have negative impacts on native anurans in California (Kupferberg et al. 2012, Lind et al. 1996).

The California red-legged frog (*Rana draytonii*) is a federally threatened species native to California (Storer 1925, Stebbins 1952). In 1996 it was listed as “threatened” under the Endangered Species Act largely due an approximate 70% decline in range since 1900 (Jennings 1994). The current range extends from Mendocino County, California, into northern Baja California, Mexico (US Fish and Wildlife Service 2002, Peralta-Garcia et al. 2016, Storer 1925, Stebbins 1952). This rapid decline has been attributed to habitat loss (Jennings and Hayes 1994), disease (Padgett-Flohr et al. 2009), illegal market hunting, commercial harvesting (Jennings 1994, Jennings and Hayes 1985), and competition and predation by introduced invasive species (Fellers and Kleeman, 2007, Lawler et al. 1999, US Fish and Wildlife Service, 2002). *Rana draytonii* are explosive breeders that migrate to marshes, slow streams, or ponds, to breed (Jennings and Hayes 1985; Wells 2007). Breeding migrations occur in fall to early winter in connection with heavy rain events (Bulger et al. 2003; Fellers and Kleeman 2007); precipitation may facilitate anuran travel across previously dry terrain (e.g. Allaback et al. 2010). Males arrive at breeding ponds before females, as early arrival maximizes breeding opportunities by extending their breeding period. When females arrive, mating occurs opportunistically and thousands of eggs are laid in each clutch.

The American bullfrog (*Lithobates catesbeianus*), a species native to the east coast of the United States, was introduced to the west coast of the United States in the late 1800s to supply frog legs for the restaurant trade (Hayes and Jennings 1986*). Lithobates catesbeianus* are lek breeders, meaning once a year, males establish and defend breeding territories. They use a loud, lowing (like a rutting male cow, hence the name) call to attract potential female mates. In their breeding season, that can occur from March to November (Willis et al. 1956), males migrate to breeding ponds or slow-moving streams before females (Emlen 1968, Emlen 1973, Raney 1940). After females arrive at the breeding pond, they select a male and then oviposit an egg mass in the male's territory (Ryan 1980). Female selection of a male is based on favorable phenotypic traits (like large size) that indicate good genetic quality, as well as available resources in the male's territory that are critical for offspring survival (Howard 1977a, 1997b).

Adequate resources are necessary to tadpoles, as no parental care is given to larvae and larvae must compete for resources to survive. *Lithobates catesbeianus* normally defend habitats with perennial water supplies that facilitate the overwintering of larvae. The overwintering behavior of *L. catesbeianus* larvae confers larger size advanced development when native anuran larvae such as *R. draytonii*, hatch in the spring. Larger size and accelerated ontogeny facilitate predation of newly hatched native anuran larvae and a competitive advantage for limited resources (Kiesecker and Blaustein 1997).

Lithobates catesbeianus have had a negative effect on larval and adult life stages of *R. draytonii* as a competitor for food (Cook and Jennings 2007) and as a predator (Cook and Jennings 2001, Wilcox 2011). In addition, *L. catesbeianus* has been observed to alter behavior, growth, and survivorship in larval *R. draytonii* (Kiesecker et al. 2001, Kiesecker and Blaustein 1997, Kiesecker et al. 2001, Lawler et al. 1999). Adult *L. catesbeianus and R. draytonii* compete for food, such as aquatic insects (J. Wilcox unpubl. data). In contrast, Cook and Jennings (2007) found that adult populations of *L. catesbeianus* and *R. draytonii* overlap in their use of microhabitat in a marsh setting, but timing of breeding activity and hibernation appeared to create a niche separation between sympatric populations in this specific habitat. This niche partitioning, a result of independent adaptations, may facilitate the coexistence of these species in this habitat. A comparative study on seasonal and daily movement patterns would highlight other potential key differences between the species such as movement timing and/or land utilization.

The goal of this study was to determine whether native *R. draytonii* and invasive *L. catesbeianus* use a grassland and oak savannah habitat differently for daily and seasonal movements between connecting ponds and streams. I conducted a telemetry study in Sonoma County, California, from May 2017 to June 2018 to map movement patterns of *L. catesbeianus* and *R. draytonii*. I also hypothesized that nightly summer basking habitat would differ, as preliminary night surveys *R. draytonii* were observed further out from water sources than *L. catesbeianus* (J. Wilcox pers. comm). Finally, I hypothesized that seasonal movement rates and timing of long-distance travel would differ by species, as differing breeding seasons would require different migration times. If my hypotheses are accurate, there may be niche divisions between the species that may have implications for conservation of native amphibian populations.

Methods & Materials

Study Area

My study was conducted on the Mitsui Ranch, a 632-acre property owned and operated by the Sonoma Mountain Ranch Preservation Foundation (SMRPF). This working cattle ranch is located on Sonoma Mountain, 8 km east of Petaluma in Sonoma County, California. Elevations on the ranch property range from 612 meters, at the lowest point where the main drainage exits the property, to 732 meters near the mountain summit. Cattle grazing has been the major human activity on the property for the past 150 years and is the also the only continuous natural disturbance on the landscape. Springs and seeps are well-distributed on the ranch landscape, and the existing ponds were constructed around springs and seeps to impound water, year-round for livestock. Vegetation is best characterized as mixed oak (*Quercus spp*.) and grassland savannah that is maintained with cattle grazing. The ranch property encompasses the headwaters for Copeland Creek, the largest of 6 drainages that originate on the ranch. Copeland Creek is a tributary of Laguna de Santa Rosa in the Russian River watershed. Through much of the Mitsui Ranch, it is a first order, intermittent stream with very little vegetative cover aside from dense western rush (*Juncus occidentalis*) and patches of Himalayan blackberry (*Rubus armeniacus*). Just before it picks up the first tributary, it begins a higher gradient fall through riparian forest of California bay-laurel *(Umbellularia californica*), coast live oak (*Quercus agrifiolia*), Oregon white oak (*Quercus garryana*), black oak (*Quercus kelloggii*), and willow (*Salix spp.*). With few exceptions, the riparian forest is closed canopy and the creek has scoured a series of runs, riffles, and pools throughout. Each season, the riffles are normally dry by the end of June, while runs and pools often hold water until September.

Within the property are four small, perennial reservoirs (hereafter referred to as ponds) constructed to water cattle (Fig. 1). The two smallest ponds: Turtle Pond and Poplar Spring are >0.025 ha in surface area, spring fed, shaded, and approximately 2 m deep (Figure 1). The two larger ponds are located within grasslands and largely unshaded. Leaky Lake (not pictured) has a maximum surface area of approximately 0.5 ha and is relatively shallow (2.5–3.0 m). Bonnie's Pond has a surface area of 0.15 ha and the maximum depth is approximately 5 m (Wilcox et al.

2017). Both larger ponds are ringed with littoral vegetation patches comprised of spike rush (Eleochorus *palustris*), bullrush (*Scheoenoplectus californicus*), and tule (*Typha spp.*). *R. draytonii* are known to use both larger ponds for reproduction (Wilcox et al. 2017), but no reproductive activities have been documented at the smaller, shaded ponds. Thus far, *L. catesbeianus* has only used Leak Lake for reproduction. It is not known how and when *L. catesbeianus* arrived on the Mitsui Ranch, but according to the ranch manager they have been present for at least 27 years. For the past 7 years, the *L. catesbeianus* population has been controlled by a regular program of shooting adults (using air rifles) during the warm months of the year.

Rana draytonii are native to the area but their numbers were reduced to the point that repeated searches of the property by consulting biologists produced no results in the early 1990s (J. Wilcox pers. comm.). *R. draytonii* was present when searches were resumed in 2010, and their numbers have increased each year since then (J. Wilcox pers. comm). Three other native Anurans host breeding populations on the Mitsui Ranch, including foothill yellow-legged frogs (*Rana boylii*), Pacific chorus frogs (*Hyliola regilla*), and western toads (*Anaxyrus boreas*).

Frog Capture

Searches were conducted primarily at night, approximately once per week from May 2017 to June 2018. Incidental diurnal searches were occasionally conducted, but most searches were nocturnal as night is when *R. draytonii* are most visible (Fellers and Kleeman 2006). We captured frogs by hand and with dip nets. Species was determined through physical characteristics, such as the presence or absence of dorsolateral folds; Present dorsolateral folds indicated the frog to be *R. draytonii*. Sex was determined differently for each species. *Rana draytonii* sex was determined by the presence/absence of nuptial excrescences on thumbs and confirmed secondarily by webbing extent on the 4th phalanx of the hind foot, or by bilateral expansion of the vocal sacs (Storer 1925, Stebbins 1952). L. *catesbeianus* sex was determined mainly through tympanum size, but also through presence/absence of nuptial excrescences on thumbs. Mass and snout-urostyle length (SUL) were measured for each individual. Mass was measured using a Pesola scale to the nearest gram, and SUL was measured with a ruler to the nearest millimeter. Frogs were captured, handled, and marked under guidelines described in a United States Fish and Wildlife Service permit (TE-068745-5) and a California Department of Fish and Game Scientific Collectors Permit (SC-005654) issued to J. Wilcox.

Tracking Methods

Capture-recapture methods, including radiotelemetry and Passive Integrated Transponder (PIT) tagging, were used to track frog movements. Radio transmitters (Holohil,Inc. Carp, Ontario, Canada), fitted to adult *R. draytonii* and *R. catesbeianus* dorsally via aluminum beaded belts (Rathbun and Murphey 1996), were employed to locate previously captured frogs. Each transmitter (Holohil BD-2), either 1.8g (20-week battery) or 1.4g (11-week battery), emits a frequency (150–151 Mh) that is detected using a directional three-element Yagi antenna channeled through a receiver (R-1000; Communication Specialists). Aluminum belts were made to fit individual frogs by sliding the belt (with slight compression) over extended hind legs to fit loosely around the waist, making them tight enough to be held in place by the bulk of the hind legs but loose enough to avoid causing damage to the skin around the waist (Fellers and Kleeman 2007). When frog leg size increased or decreased, new belts were adjusted or replaced as needed to keep the radio-transmitters on the frog. Combined transmitter and belt masses were always less than ten percent of the animal's body mass (Richards et al. 1994). Both triangulation and direct tracking methods were used to locate frogs. A total of 72 frogs were fitted with radio-transmitters: 21 *L. catesbeianus* and 51 *R. draytonii*. Only 13 L. catesbeianus moved significantly. In total, 54 *L. catesbeianus* and 248 *R. draytonii* GPS points indicating frog location were recorded. 165 visual observations of frogs were recorded: 45 *L. catesbeianus* and 120 R. draytonii.

PIT tags (Biomark MiniHPT8, Boise, ID) are unique markers for frogs that allow identification upon recapture. PIT tags were used in addition to telemetry transmitters, to ensure that frogs could still be identified if transmitters fell off. PIT tags were inserted into frogs via a small upper dorsal incision made using surgical scissors (Miltex 5-SC-304) and softly coaxed below the sacral hump to reduce risk of the PIT tag working itself back out of the incision. An alpha-numeric code was scanned and read using a hand-held tag reader (Biomark GPR Plus, Boise, ID).

Frog locations were recorded (WGS-1984) with a hand-held GPS unit (Garmin 60csx). The location point data were uploaded to a computer (DNR Garmin, Minnesota Dept. of Nat Res.) and transferred to ArcGIS Pro (ArcGIS, ESRI, Redlands, CA) for analysis.

Nocturnal Habitat Use Measurements

While conducting night searches from June 29th to September 14th, 2017, pin flags were placed to mark locations of frogs basking outside of ponds and the creek. A five-meter tape measure (to the nearest cm) was used to measure the linear distance from the base of the flag to the nearest water edge. To measure the height from the closest water surface, a laser level (Bosch GL55) was placed on a tripod over the pin flag and aimed at the five-meter tape measure, held vertically with the zero end of the tape on the water surface. The height between the laser and the ground under the tripod (at the pin flag) was subtracted from the total measurement to calculate the frog's elevation on land relative to the water surface. Frogs basking at the water's edge, or in water, were recorded as zero height from the water surface and zero distance from the water edge.

Mapping

I used the "XY table to point" tool in ArcGis Pro to overlay each point on the map to visualize frog positions (Fig. 2). To calculate straight-line movement distances for each frog I used the "points-to-line" tool and sorted by individual (PIT tag) and Julian date. The results of this analysis were input into the "split line at vertices" tool to determine the length of distances travelled between each observed point (Fig. 3). The "add geometry attribute" tool was used to convert the output distance length to meters. The calculated distance was divided by the number of days between observations to find the rate of movement. This controlled for the inconsistencies and variability of days between frog observations, as some frogs were located more often than others.

Rainfall measurements

Rainfall data were obtained through the Western Weather Group at the Fairfield Osborne Preserve (Sonoma State University) weather station (DataLynx Instruments, Tipping Bucket Model 260-300), located approximately 3 kilometers northwest of the study site. Rainfall, originally recorded in inches, was converted to centimeters in Excel (Microsoft Corp).

Data Analysis

All statistics were analyzed using JMP (Version 14, SAS Institute 2018). I used a linear regression analysis to test whether SUL determined variation in mass, as to avoid duplicating a variable in further analyses. To compare the size of *L. catesbeianus* and *R. draytonii*, I used a general linear model with species as a model effect and SUL as the response variable. To analyze movement rate, I ran a linear mixed model that included species, sex, SUL and rainfall as model effects, as well as cross effects like species-sex and species-mass and set movement rate as the response variable. PIT tag number was included in the model as a random effect to control for pseudo-replication. I also used a general linear model to compare the estimated arrival dates of female and male *R. draytonii* traveling overland to their breeding sites, setting arrival date in Julian day as the response variable and sex as the model effect. Estimated date of arrival is defined as the date an individual was first observed at the breeding pond.

Rainfall was calculated by adding the rainfall across the three-day period before an individual frog was located. Rainfall was calculated this way because observations of frogs were not always consistent, but a three-day period was focused enough to see a potential effect on movement from the rainfall event while broad enough to account for the spacing of movement observations. To compare the microhabitat use of the species in relation to the water source in a creek setting, I ran two general linear models with species as the model effect; one model tested distance from water edge as the response variable and the other model tested height from the water surface as the response variable. To compare the habitat use of *R. draytonii* in a creek setting and a pond setting, I ran two general linear models with water source type (creek or pond) as the model effect; the first model tested distance from water edge as the response variable, the other with height from water surface as the response variable. Statistical significance was accepted at $\alpha=0.05$ level.

Results

Size

Mass and Snout Urostyle Length (SUL) are strongly correlated across both species ($r^2 = 0.52$, $F_{1,40}=904.9492$, $p<0.0001$) (Fig. 4) thus, only SUL was included in this model. SUL was chosen over mass because individual SUL is less likely to fluctuate. Mean Snout Urostyle Length (SUL) of *L. catesbeianus* (110.6mm) was found to be greater than *R. draytonii* (95.99mm) ($F_{1,296}=62.8772$, $p<0.0001$) (Fig. 5). Within *R. draytonii*, females were found to be significantly larger than males ($F_{1, 243}$17.54, $p<0.001$). There was no significant difference in size between male and female *L. catesbeianus* ($F_{1, 53}=1.94$, $p=0.1695$). The cross of SUL and species was found to have a significant effect on movement rate ($F_{1, 52.99}=9.7391$, $p=0.0029$), but SUL alone did not ($F_{1, 55.45}=2.034$, $p=0.1594$). When the species were run separately, SUL of *L. catesbeianus* was found to have a significant effect on movement rate ($F_{1, 40}=5.8351$, $p=0.0203$) (Fig. 6A), with smaller frogs moving faster. Snout-Urostyle Length of *R. draytonii* was found to have a significant effect on rate of movement ($F_{1,193}=17.5491$, $p<0.0001$), with larger frogs moving at faster rates (Fig. 6B).

Seasonal Movement

Male *R. draytonii* arrived at the breeding pond earlier than female R. draytonii ($F_{1,9}=3.852$, $p=0.0813$) (Fig 7), but the significance was marginal. The earliest male arrival to the breeding pond was recorded in October 2017 and the earliest female arrival was observed in January 2018 (*R. draytonii* egg masses were first observed in February 2018). Within the mixed model analyzing breeding migration, the variable with the most significant effect on movement rate was the cross effect of sex and species (Table 1) ($F_{1, 56.98}=14.19$, $p=0.0005$), meaning movement is different between sexes for each frog species (Fig. 8). Overall, male *L. catesbeianus* were the fastest moving within the study. Male *L. catesbeianus* moved at a faster rate over land than females. Conversely, female *R. draytonii* moved at faster rates than males. Sex was also a significant variable determining movement rate ($F_{1, 56.07}$= 4.9995, p=0.0294). Frog species was found to have a significant effect on movement rate ($F_{1, 62.64}$=8.124, p=0.0059). Rainfall was not found to have a significant effect on movement rate overall ($F_{1, 21.3}$=0.0218, p=0.8828), nor when crossed with species ($F_{1, 218.5}$=0.1198, p=0.7296). Individual (PIT) made up 5.728% of total effect within the model.

Nocturnal Habitat Use

No radioed *L. catesbeianus* were observed in any ponds during this time, thus we could only compare *L. catesbeianus* and *R. draytonii* in a creek setting. *R. draytonii height location* was observed to be significantly higher on average from the nearest water surface (103.7cm) than *L. catesbeianus* (3.2cm) ($F_{1,112}$=77.8153, p<0.0001) (Fig. 9A). The mean distance from nearest water edge for *R. draytonii* was significantly greater (95.5cm) than *L. catesbeianus* (4.2cm) ($F_{1, 112}$=54.3884, p<0.0001) (Fig. 9B). When comparing *R. draytonii* habitat use within creek and pond environments, we found that they prefer to locate further from the water edge in pond settings than in creeks (creek=95.5cm, pond=205.6cm; $F_{1, 118}$=21.8827, p<0.0001), and higher from the water surface in creek settings than in ponds (creek=103.7cm, pond=58.0cm; $F_{1, 118}$=14.48, p<0.0001) (Figure 10).

Discussion

My research results indicate key differences in how a sympatric population of *L. catesbeianus* and *R. draytonii* move seasonally between breeding and non-breeding habitats. In addition, my research points to a clear difference in nocturnal niche occupation between the two species where they co-occur in a seasonal stream during the summer months in a grassland and oak savannah habitat.

Seasonal Movement

Seasonal, long-distance movement timing was compared between species and sex, and by correlating movement rate of species, size and sex, and rainfall. The long-distance movements observed in this study were attributed to breeding migrations based on the timing and patterns of the movements aligning with previous research on breeding seasons and mating strategies (e.g., Wells 2007).

The difference in arrival times between male and female *R. draytonii*, with males arriving first, followed the expected pattern for *R. draytonii* as explosive breeders (Wells 2007). *Rana draytonii* likely evolved an explosive breeding system as a result of living in vernal pools and ephemeral ponds, where breeding season length was determined by limited water availability. In contrast, lek breeders like *L. catesbeianus,* evolved from having long breeding seasons made possible by long-lasting sources of water to breed in (Wells 2007). Since only two male *L. catesbeianus* were seen at the breeding pond during the entire study period (Both male L. catesbeianus arrived in June 2017, corresponding with the summer breeding season of the species), a statistical comparison of arrival times to breeding ponds could not be made. Although 9 of the 21 bullfrogs radioed were sexually mature females, no females were observed to arrive at breeding ponds so a comparison of breeding pond arrival time of male and female *L. catesbeianus* also could not be made.

The results of the mixed model analyzing movement rate indicated that *L. catesbeianus* move at a faster rate over land than *R. draytonii.* Within a species, larger amphibians are better equipped to travel across dry habitats; their large surface to volume ratio confers the ability to retain water better than smaller individuals (Thorson 1955). Male *L. catesbeianus* were found to move faster than females; however, this result may be because of an outlier in a small sample size. It may also be attributable to a difference in movement types: males moved to breeding ponds whereas female movements did not. Therefore, the differences in rate of movement between male and female *L. catesbeianus* in my study may not be comparable due to a difference in motivational impetus. In *R. draytonii,* female frogs were found to move significantly faster than male frogs. This result may be explained by the ability of differing sizes of the sexes, as female *R. draytonii* were found to be larger than males in my study.

In a statistical model that only included *R. draytonii* as an effect, SUL alone was found to have a significant effect on movement where, as Thorson (1955) predicted, that within a species, larger frogs moved faster than smaller frogs. Contrastingly, in my study *L. catesbeianus* SUL was found to have the opposite effect, although only marginally significant, with smaller frogs moving slightly faster than larger frogs. This may be due to a small *L. catesbeianus* outlier, in a small sample, that moved very quickly to a breeding pond in June 2017. A larger sample size may have balanced out this result and changed the significance of the effect. In addition, other behavioral variables aside from capabilities conferred by size may influence the travel rate of smaller frogs such as the need to avoid detection or seeking a more direct route to avoid desiccation. Finally, a more frequent interval of detection may have refined travel rate calculations and determined a greater significance of frog size related to movement rate.

In my statistical movement model, rainfall was not found to have a significant effect overall, nor when crossed for species. This result was surprising since rainfall has been correlated with *R. draytonii* movement events in previous studies (Bulger et al. 2003; Fellers and Kleeman 2007). My observational intervals may have been insufficient in detecting movement timing precisely enough to establish a correlation between rainfall and frog movements. To find a connection between precipitation and movement rate may require more frequent location monitoring, and more localized data on rainfall.

Nocturnal Habitat Use

Comparison of nocturnal basking behavior in *L. catesbeianus* and *R. draytonii* could only be made in a creek setting since *L. catesbeianus* was not observed in ponds during this study. In Copeland Creek, *L. catesbeianus* was only observed out of the water on two occasions, but never out of the thalweg portion of the stream. *R. draytonii* sat significantly further and higher from the water surface than *L. catesbeianus,* and very often located themselves out of the thalweg and even outside of the stream channel. If this “basking” behavior is thermoregulatory, as suggested by Freed (1980) and Lillywhite (1970), it may be an indication that *R. draytonii* and *L. catesbeianus* do not have the same thermoregulatory requirements. This might be explained by a difference in environmental adaptation since *R. draytonii* evolved in a drought-prone environment where summer relative humidity is often low, while *L. catesbeianus* evolved in the southeastern United States where summers feature frequent rain and high humidity. If the nocturnal niche partitioning of the stream habitats by the two species is a result of competition between them, either via interference or exploitation, *R. draytonii* would be expected to behave differently in the absence of *L. catesbeianus*.

Comparing frog position of *R. draytonii* in creek versus pond environments revealed their similar use of different aquatic landscape features in the absence of *L*. *catesbeianus*. *Rana draytonii* were found to sit further from the water surface in pond settings than creek settings, yet they sat higher from the water surface in creek settings than pond settings. In both aquatic settings, *R. draytonii* remained close enough to the water to escape to refuge (water source) just a couple of jumps away, yet still exhibited the characteristic of basking far from water whether L. catesbeianus was present or absent. At Turtle Pond, much of the edge of the pond is fairly level, with manna grass (*Glyceria leptostachys*) and a few clumps of western rush (*Juncus occidentalis*) plants surrounding much of the perimeter. Within the creek setting, the steep bank provides more space away from the water vertically than horizontally. *Rana draytonii* observed in creeks were surrounded by steep leaf-littered slopes, tangled roots of trees, and short grasses. In both pond and creek settings, individuals were found to exhibit strong site fidelity by returning to the same spot across multiple nights; these positions may be optimal for a fast escape into the water.

Rana draytonii utilizes available aquatic landscape features similarly with and without *L. catesbeianus* present (Alvarez et al., Ecological Restoration, in review). The habitual upland habitat use in pond and streams illustrates that the banks surrounding water sources are regularly used by *R. draytonii* for basking opportunities. It may be inferred that *R. draytonii* are leaving the water at night, not as a response to the presence of the invasive *L. catesbeianus*, but strictly for thermoregulatory purposes. Protecting these areas of upland landscape may be vital to *R. draytonii* life history and behavior, especially in the summer months. My results suggest that habitat buffer zones around creeks and ponds may be necessary to protect *R. draytonii* in similar environments. To investigate whether the behavior observed is strictly a thermoregulatory behavior, future studies should compare internal temperature measurements of *R. draytonii* to those of ambient air and water (Freed 1980, Lillywhite 1970).

Conclusions

I found differences between how invasive *L. catesbeianus* and native *R. draytonii* use their shared habitats within the creeks and their uplands, and in their varied rates of movement across a grassland and oak savannah. Differences in timing of large-scale movements of each species follow published differences in breeding patterns. Use of the near-shore terrestrial landscape also differed between the species. Cook and Jennings (2007) suggested that independent adaptations create divisions in habitat use, but whether those divisions facilitate coexistence (niche partitioning) between the species is unknown. There may be differences within shared environments, like those I found in movement patterns, that make *L. catesbeianus* better able to survive human-induced habitat alteration. For example, *R. draytonii* may be more vulnerable to changes in creek structure, as they utilize banks for thermoregulation. *Lithobates catesbeianus* may be better able to take advantage of altered habitats (Hayes and Jennings 1986). Further study may be required to determine whether coexistence of these two species is due to variations in environment. The results of this study may be used to inform conservation efforts for the threatened *R. draytonii* in stream and pond habitats.

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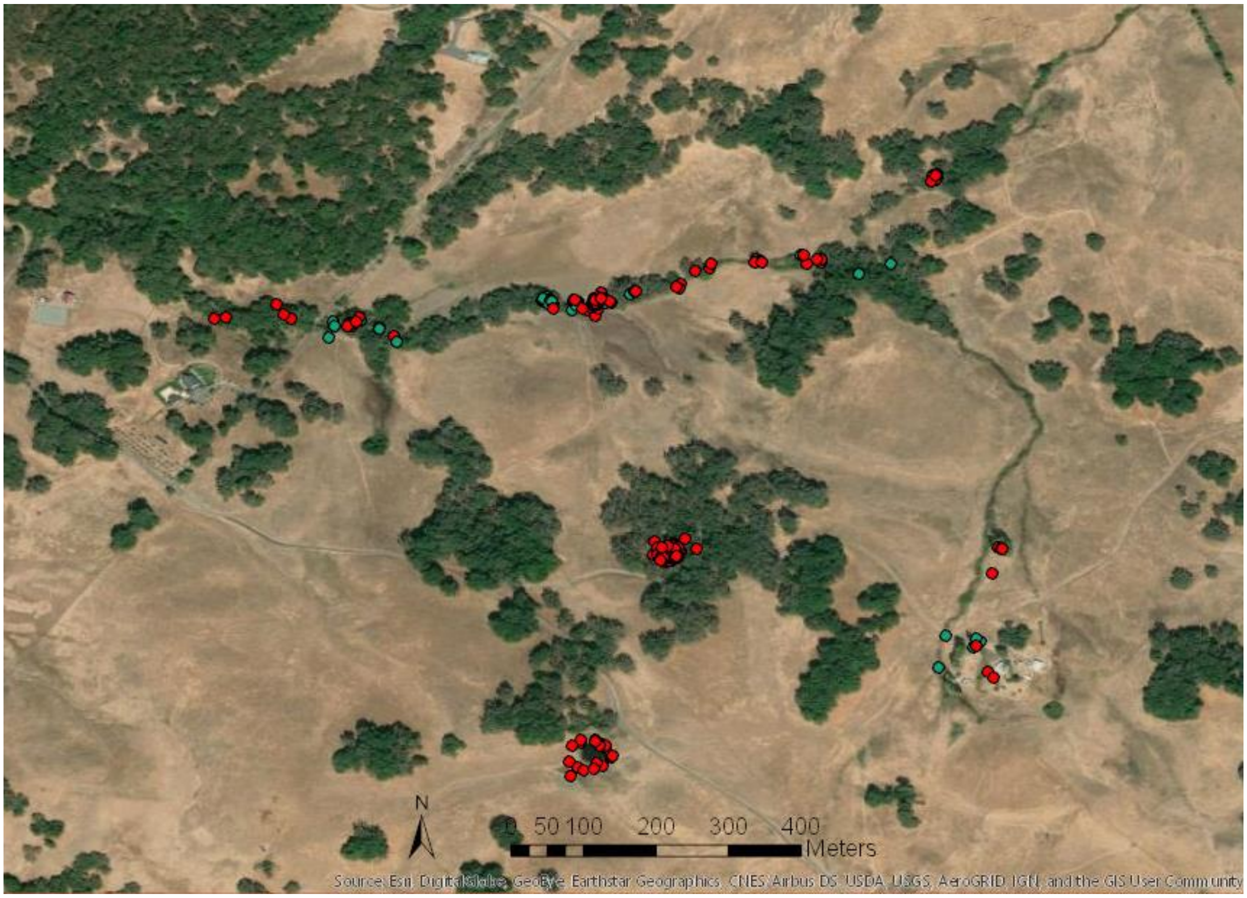
Tables & Figures

Table 1: Results from movement rate model.

Variable	Degrees of Freedom	F Ratio	P value
Species*Sex	1, 56.98	F = 13.8007	P=0.0005
SUL*Species	1, 52.99	F= 9.7391	P=0.0029
Species	1, 62.64	F = 8.124	P=0.0059
Sex	1, 56.07	F = 4.9995	P=0.0294
SUL	1, 55.45	F = 2.0340	p = 0.1594
Rainfall	1, 218.3	F = 0.0218	p = 0.8828
Rainfall*Species	1, 218.5	F = 0.1198	P = 0.7296

Figure 1. Map of the Mitsui Ranch. *R. draytonii* non-breeding areas (white) include French Drain, Poplar Spring, Turtle Pond and Copeland Creek. Bonnie's Pond (yellow) is a successful breeding pond for *R. draytonii.*



Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

Figure 2. Map of Sonoma Mountain Ranch Preservation Foundation with GPS locations of *L. catesbeianus* (green) and *R. draytonii* (red).

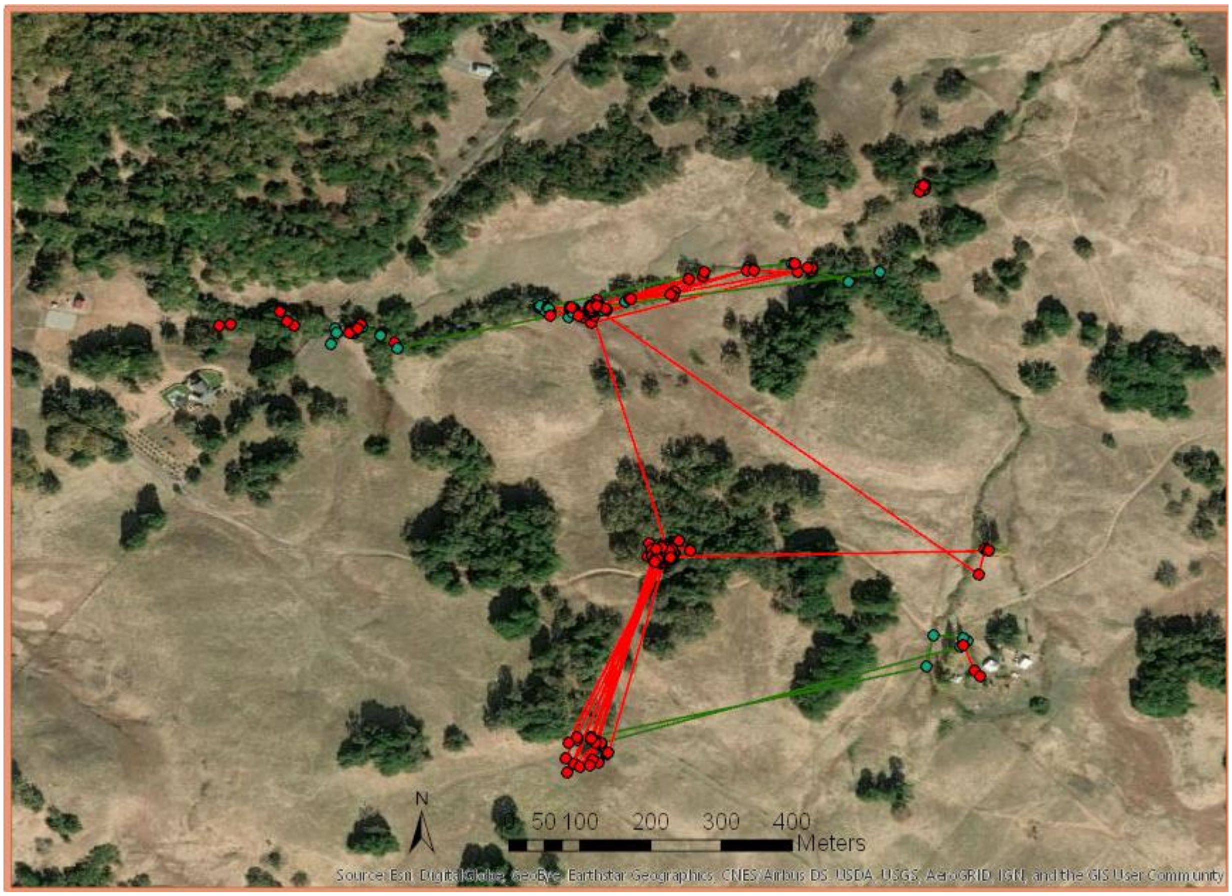


Figure 3. Map of movement patterns of *L. catesbeianus (green)* and *R. draytonii* (red*).*

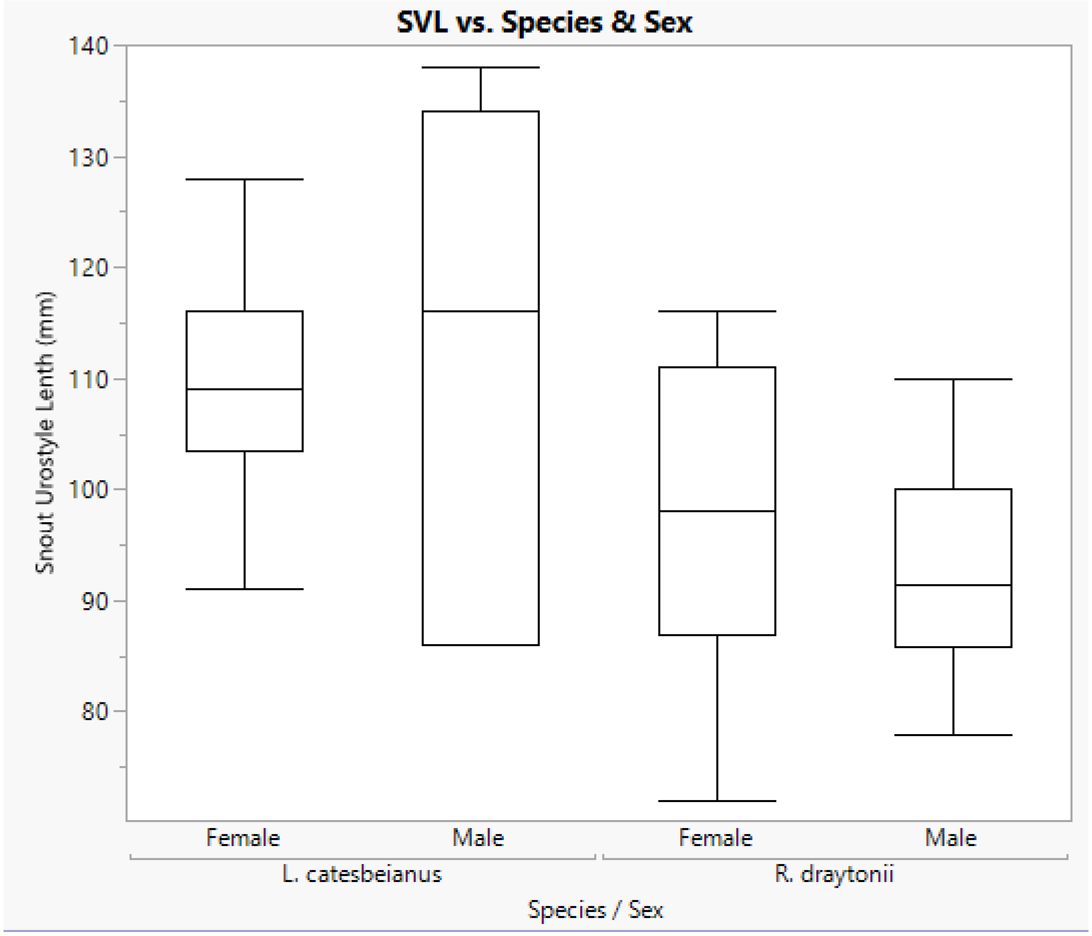


Figure 4: Comparison Snout Urostyle Length (SUL) between male and female *L. catesbeianus* and *R. draytonii.* Average *L. catesbeianus* SUL is significantly higher than *R. draytonii* ($F_{1,1}$=62.8772, $p<0.0001$). Within *R. draytonii,* females were found to be nearly significantly larger than males ($F_{1,1}$=17.54, $p<0.0001$). There was no significant difference in size between male and female *L. catesbeianus* ($F_{1,1}$=1.94, $p=0.1695$). Boxes represent interquartile ranges and middle line in each box is the median value.

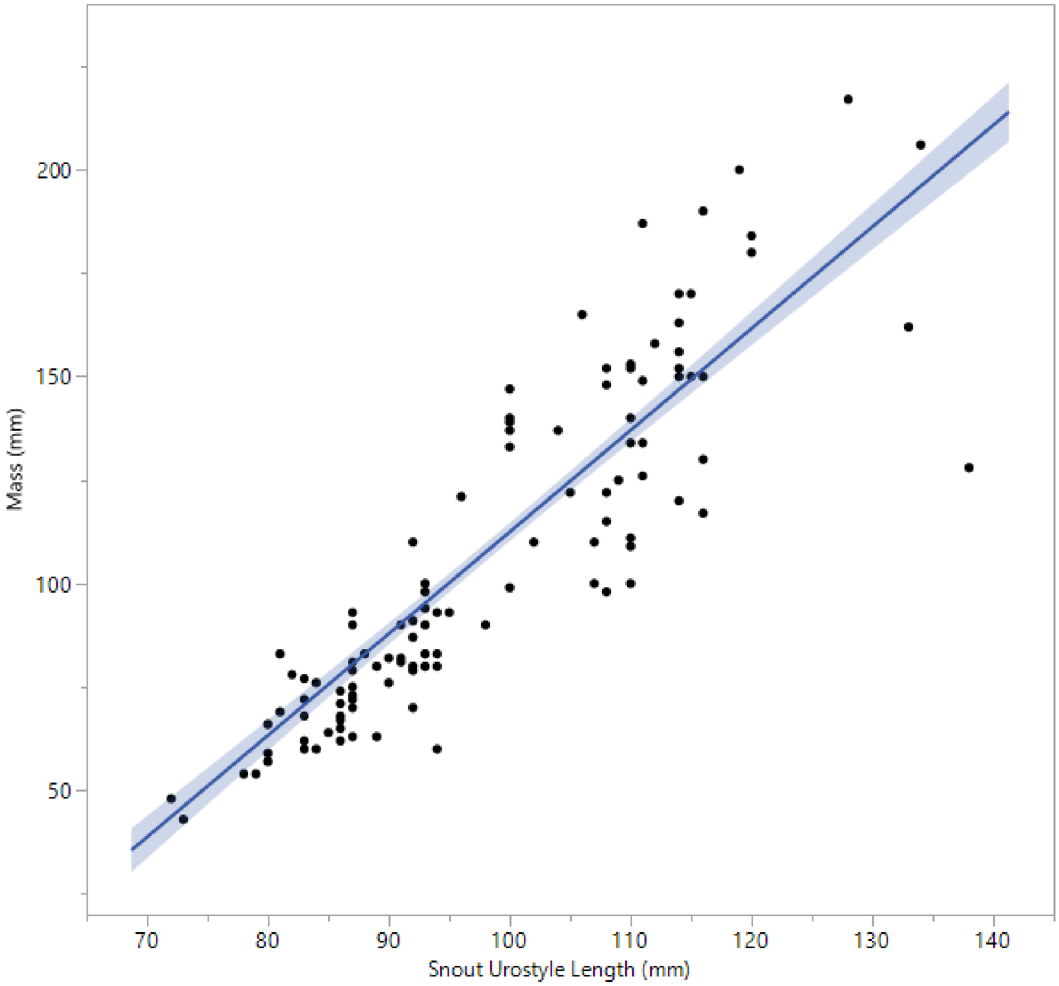


Figure 5. Regression analysis of Snout Urostyle Length (mm) and Mass (g) for *L. catesbeianus* and *R. draytonii* (r^2 = 0.523, F=904.9 p<0.0001).

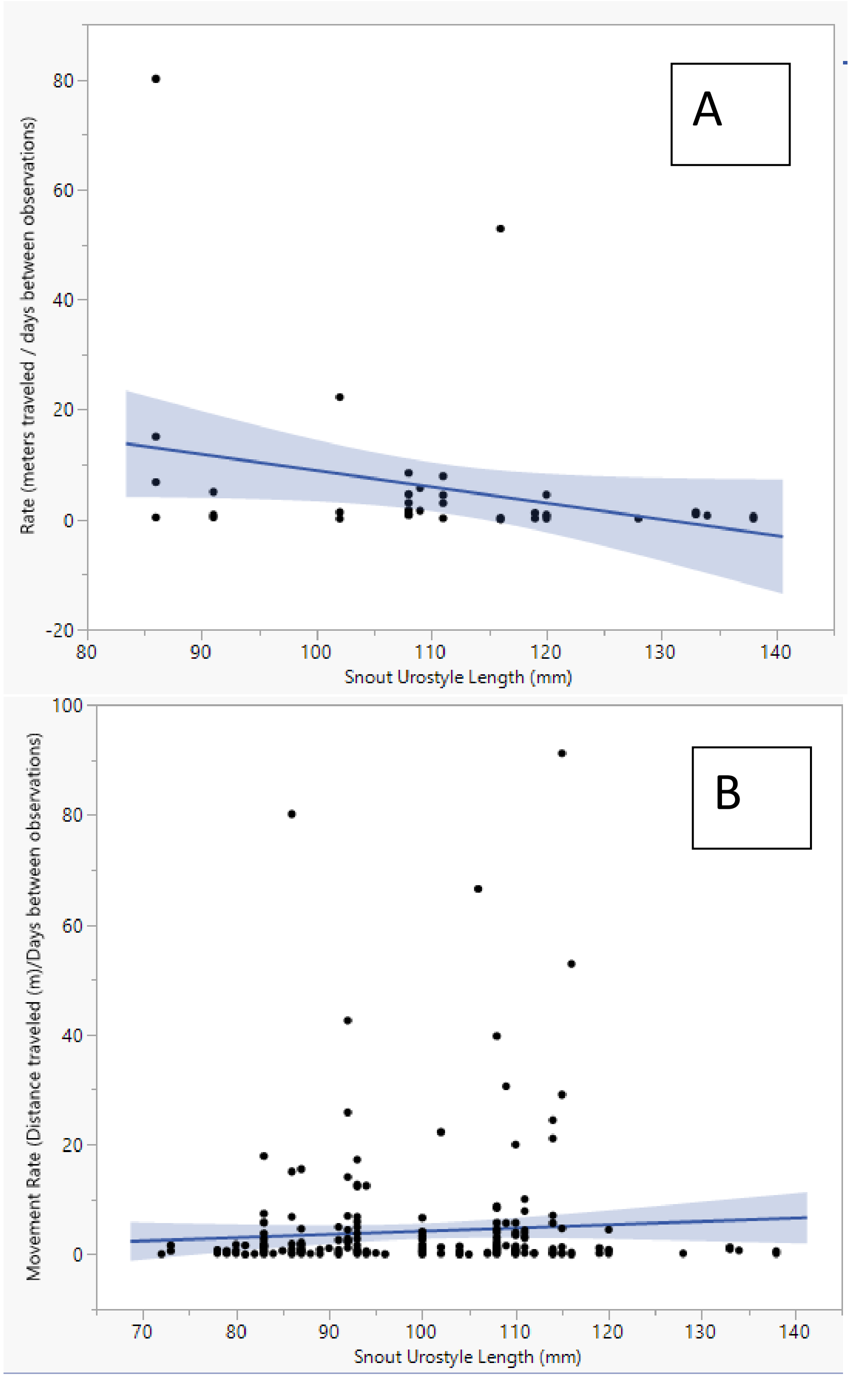


Figure 6. *L. catesbeianus* (A) with smaller SUL were found to move marginally faster than those with larger SUL ($F_{1, 1}$=5.8351, p=0.0203). SUL of *R. draytonii* (B) was found to have a significant effect on rate of movement ($F_{1, 1}$=17.5491, p<0.0001), with larger frogs moving at faster rates.

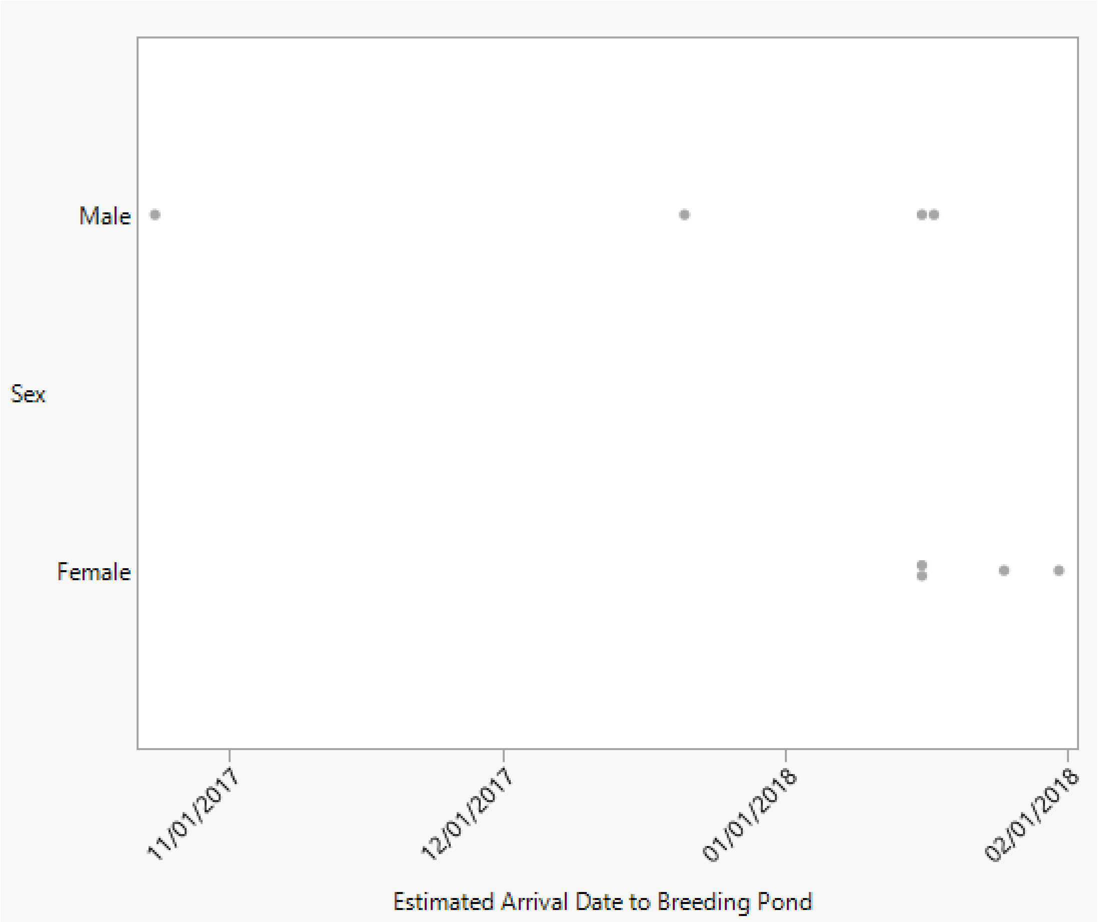


Figure 7. Male *R. draytonii* (n=4) arrived at the breeding pond marginally earlier than female *R. draytonii* (n=4) ($F_{1,1}$=3.85, p=0.0813)

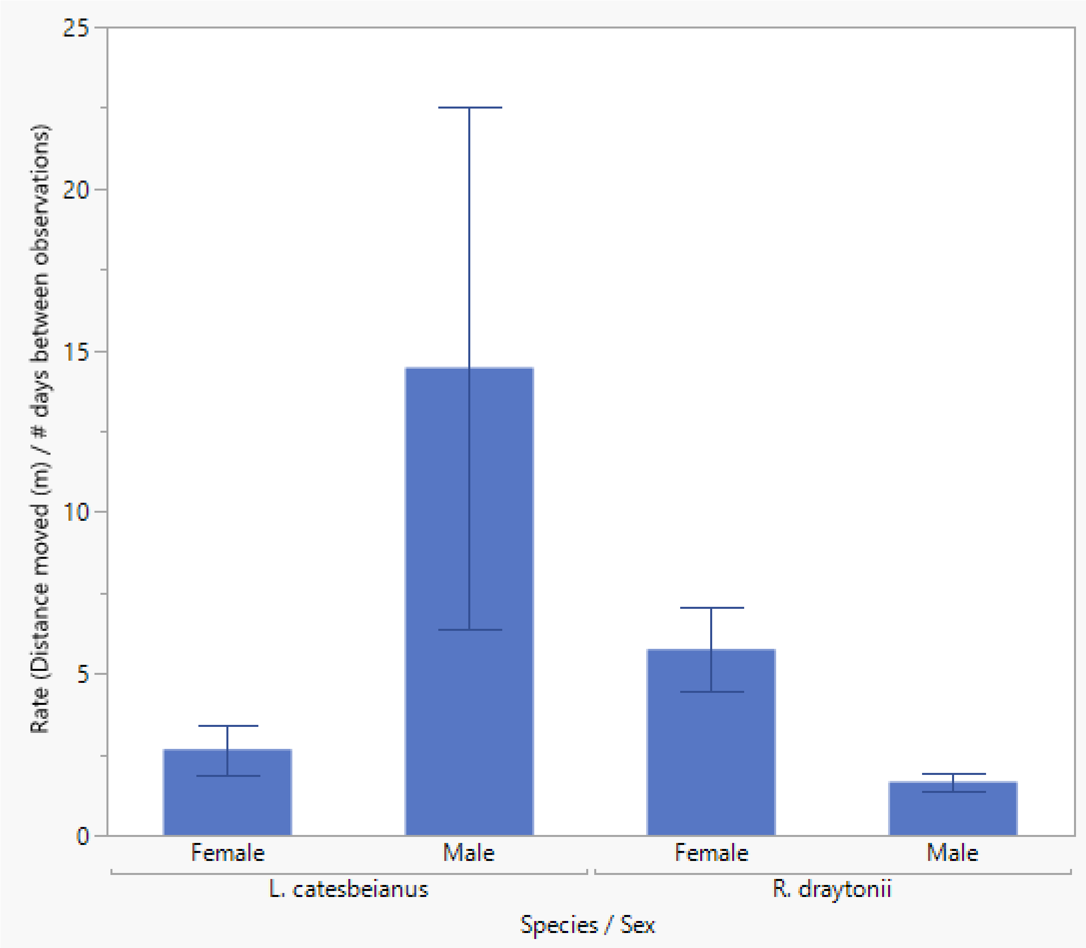


Figure 8. Species was found to have a significant effect on movement rate ($F_{1, 62.64}$=8.1240, p<0.0059). Movement rate differed significantly between sexes for each species ($F_{1. 56.98}$=14.19, p<0.0005)). Male *L. catesbeianus* (n=5) moved at a faster rate than females (n=8). Female *R. draytonii* (n=28) moved at faster rates than males. Overall, male *L. catesbeianus* were the fastest moving within the study.

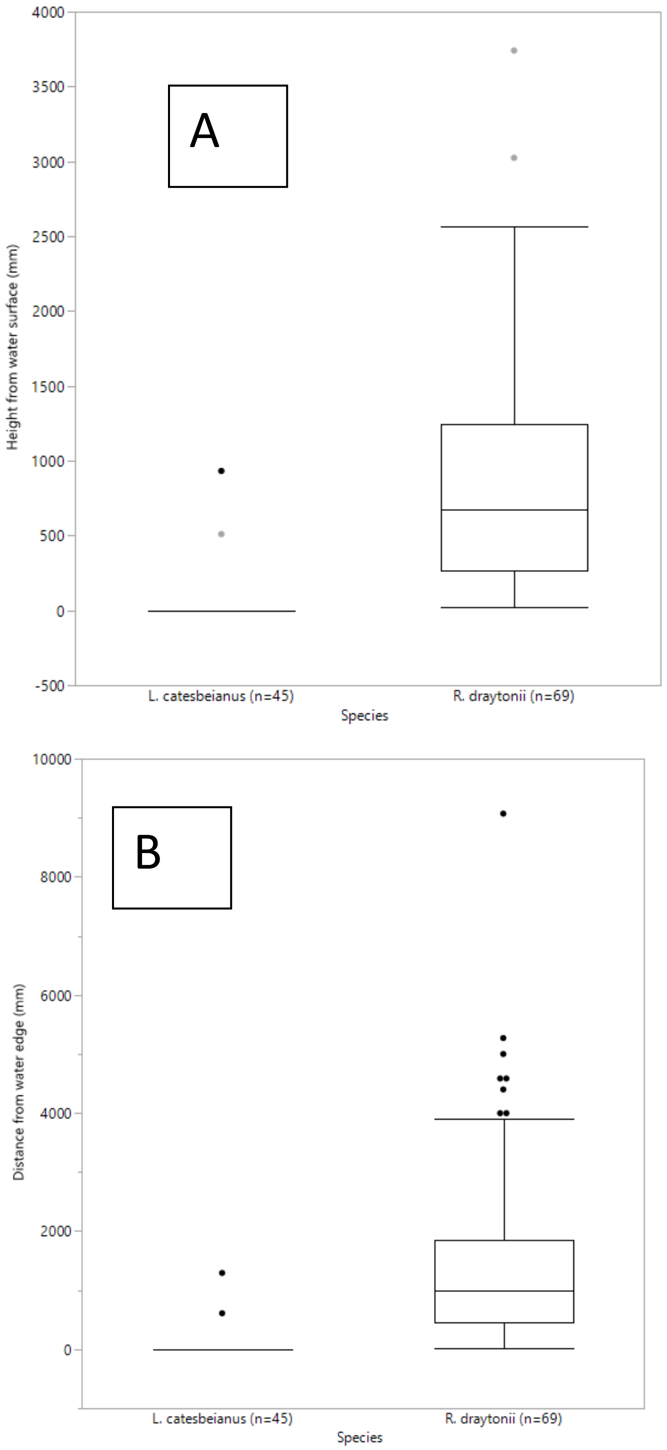


Figure 9. Comparing *L. catesbeianus* and *R. draytonii* basking location in relation to a) height from water surface ($F_{1,1}$=77.8153, p<0.0001) and b) distance from water edge in creek environment ($F_{1,1}$=54.3884, p<0.0001). Boxes represent interquartile ranges and middle line in each box is the median value.

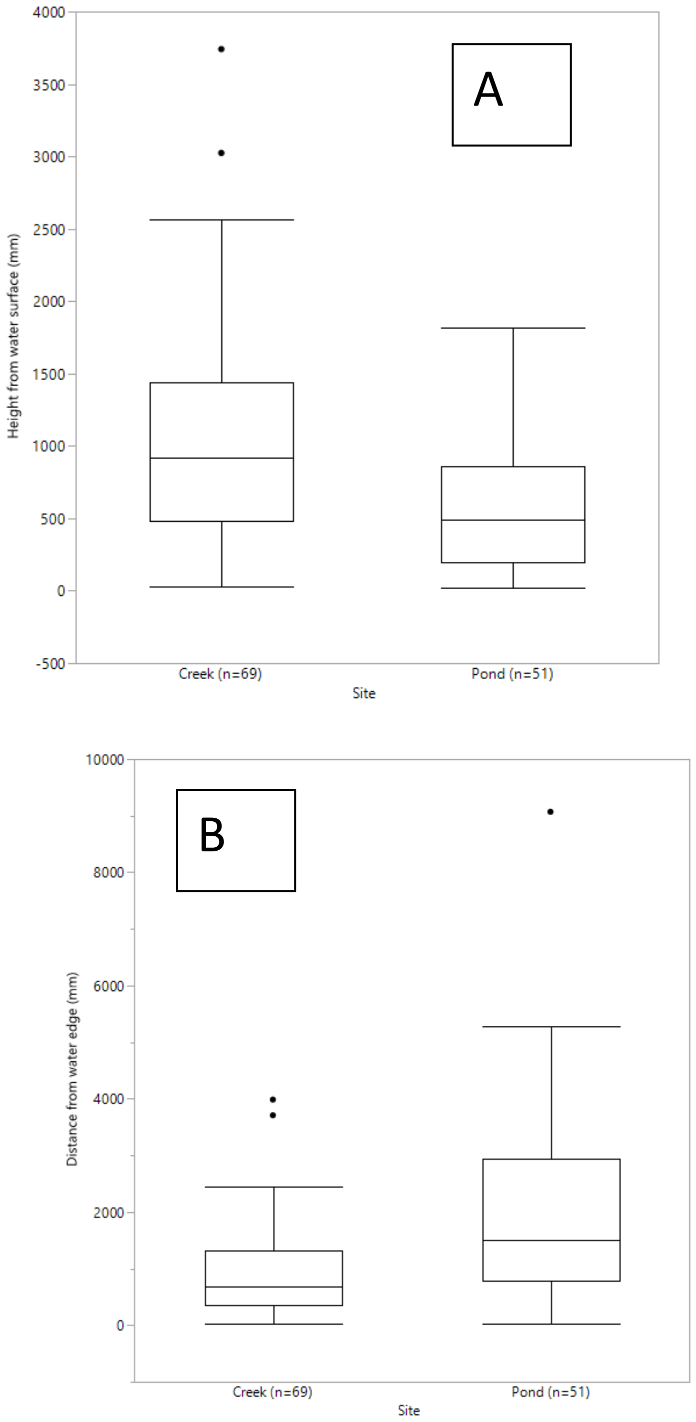


Figure 10. Comparing *R. draytonii* habitat use in creek and pond environments with basking position relative to A) Height from water surface and ($F_{1,1}$=14.48, p<0.0001) and B) Distance from water edge ($F_{1,1}$=21.8827, p<0.0001). Boxes represent interquartile ranges and middle line in each box is the median value.