Habitat Use and Movement Patterns of the Southern Crawfish Frog (*Rana areolata*)

Final Report to Texas Parks and Wildlife Department

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ABSTRACT

The crawfish frog, *Rana (Lithobates) areolata*, is a species of conservation concern due to declining populations and habitat loss. We tracked 24 crawfish frogs at the Attwater Prairie Chicken National Wildlife Refuge in southeast Texas from March 2015-May 2016. Crawfish frogs utilize crayfish burrows for shelter most of the year. We tracked 11 frogs to burrows. Distance traveled ranged between 63-624 meters, for an average 315 meters. We also placed game cameras on five burrows to monitor activity patterns of the resident frogs. Frogs spent 75% of their time aboveground (69% of the night, 81% of the day). The remaining 25% of their time (31% at night, 19% during the day) was spent underground inside the burrow. The Fordtran soil type contained significantly more crayfish burrows than the Edna, Katy or Crowley soil types. Chi-squared analyses suggest that occupancy of particular soil types is correlated with available soil type area.
INTRODUCTION

Amphibian populations have been declining in every part of the globe for decades (Blaustein & Wake 1990, Sherman & Morton 1993, Houlahan et al. 2000, Stuart et al. 2004). For many declining species, more than one factor may be contributing to their decline (Grant et al. 2016). Sometimes many stressors are present, subsequently or simultaneously, and they can interact with each other in ways that have multiplicative rather than simply additive effects (Vinebrooke et al. 2004, Folt et al. 1999). Stress factors include pollutants, increased UV-B radiation, invasive species, pathogens, parasites, trafficking, road mortality, climate change and habitat loss (e.g., Kerr & McElroy 1993, Alford & Richards 1999, Beebee & Griffiths 2005)

Habitat Destruction and Fragmentation

Habitat destruction and habitat fragmentation - defined here as a reduction in patch size and an increase in patch separation distance (Fahrig 2003) - are two more major threats affecting amphibian populations. Habitat fragmentation has been shown to affect distribution and/or reproductive success of many taxa, including plants (Vellend et al. 2006, Aguilar et al. 2006), insects (Brown & Albrecht 2001, Didham et al. 1996), birds (Herbert 1994, Robinson et al. 1995, Askins et al. 1987), mammals (Diffendorfer et al. 1995, Crooks 2002), and herpetofauna (Beebee 1977, Johansson et al. 2007, Templeton et al. 2001) and to have a negative impact on biodiversity when fragmentation is associated with habitat loss (see review in Fahrig 2003). Amphibians are especially vulnerable to habitat loss and fragmentation because of three factors: relatively low vagility, high mortality when crossing roads and inhospitable terrain, and narrow habitat tolerances (see review in Cushman 2006).

Prairies are among the most rapidly disappearing landscape types in North America. Between 1830 and 1994, the extant area of tallgrass prairie declined by 82.6-99.9% in the majority of its historic range (Samson & Knopf 1994). This includes a decline of 90% in Texas, where much of the lost acreage was coastal prairie (Samson & Knopf 1994). Of course, amphibians are far from the only species affected by prairie destruction. One iconic example would be the prairie chickens, three species in the genus *Tympanuchus* which have all declined by varying degrees in the years since European colonization and westward expansion (Taylor & Guthery 1980, Connelly et al. 1998, Svedarsky et al. 2000). In all three
cases, this decline is primarily attributed to habitat loss and fragmentation as prairies were converted to
enabling the decline is inbreeding depression caused by greatly reduced numbers and fragmentation of
habitats supporting the remaining populations (Westemeier et al. 1998, Bellinger et al. 2003, Bouzat et al.
1998).

**Study Species**

Another species tied to prairie habitats is the crawfish frog, of which there are two subspecies. The
northern subspecies, *Rana (Lithobates) areolata circulosa*, occurs from southwestern Indiana, west
through Illinois and Missouri into Nebraska and northeastern Oklahoma. Populations also occur south of
Indiana in western Tennessee, Kentucky, and Alabama. The southern subspecies, and the focus of this
project, is the southern crawfish frog, *R. a. areolata*, which is found primarily in Louisiana, Arkansas,
central to east Texas, and southeast Oklahoma (Parris & Redmer 2005). Crawfish frogs’ preferred prairie
habitat is greatly reduced from historic levels and still on the decline. It is likely that crawfish frog
populations are also declining. The IUCN Redlist classifies *R. areolata* as a “near threatened” species, as
of an assessment in 2004, primarily due to habitat loss (Hammerson & Parris 2004). The species is
currently listed as “endangered”, “threatened” or “of conservation concern” in seven of the twelve states
from which records of the species exist (Hammerson & Parris 2004) and is believed by some to be
extirpated in Iowa, where the most recent record of the species dates from the 1940’s (Christiansen &
Bailey 1991). In Texas, the 2012 Texas Conservation Action Plan classified crawfish frogs as
“vulnerable.” Additionally, there is evidence of pronounced range contraction within the state of Texas.
Crawfish frogs were once found all across the eastern half of the state, but all records of the species in
the last twenty years have come from one of two known remaining populations close to the coast (Saenz
and Hibbitts, unpublished data). For this reason, it is important to understand the habitat requirements of
the species in the areas where populations remain, especially if those requirements differ from what is
known for populations in the northern range of the species.

The northern subspecies almost exclusively utilizes abandoned crayfish burrows for living space during
the non-breeding season (Heemeyer et al. 2012), which, in Indiana and Kansas, typically lasts from mid-
April to February of the following year (Busby & Brecheisen 1997, Williams et al. 2013). It has been speculated that crayfish burrows are particularly suitable because they go down to the water table and so allow the frog to reside below frozen top layers of soil (Heemeyer et al. 2012). They do not need this protection from freezing on the Texas coastal plain, although they may benefit from any moisture collecting at the base of the burrow. There is typically very little movement of northern crawfish frogs during the nonbreeding season, but during the breeding season they migrate variable distances to the ephemeral wetlands where they call and breed. Upon reaching the breeding ponds, northern crawfish frogs remain for the duration of the breeding period, calling daily. One radio telemetry study found an average migration distance of 0.5km (Heemeyer & Lannoo 2012), and several studies have shown that some northern crawfish frogs travel as far as a kilometer from their burrows to reach breeding ponds (Heemeyer & Lannoo 2012, Heemeyer et al. 2012) before returning to those same burrows (Heemeyer & Lannoo 2012).

Crawfish frogs are generally known to occupy burrows in two different ways, based on the work which has been conducted on the northern subspecies in the Midwest. “Primary” burrows are occupied for the majority of the non-breeding season, while “secondary” burrows are utilized temporarily on the way to or from breeding sites or on feeding forays from the primary burrow (Heemeyer & Lannoo 2012). Frogs use the same primary burrows from year to year, and follow similar routes when migrating from those burrows to the breeding ponds, sometimes stopping at the same secondary burrows on the way (Heemeyer & Lannoo 2012). Movements between secondary burrows are not always directly in line to and from the ultimate destinations – that is to say, they do not always move in straight lines, sometimes backtracking or moving to the side off of the most direct route (Heemeyer & Lannoo 2012, Williams et al 2012).

Heemeyer et al. (2012) found that crawfish frogs in Indiana utilized 1-11 (x̅=3.5) secondary burrows en route to their primary burrows, while another study in the same area (Williams et al. 2012) found that frogs used 0-11 secondary burrows (x̅=4). In the latter study, three frogs used only a primary burrow, while ten frogs used secondary burrows for average occupancy times of 11 days in the post-breeding migration (Williams et al. 2012). Both of these investigations utilized internal transmitters, and thus avoided any problems with transmitter detachment, though they experienced other difficulties (see Heemeyer et al.
In particular, transmitter implantation prior to breeding activity often causes visceral herniation (Heemeyer et al. 2010), necessitating post-breeding capture and implantation. However, capturing frogs when they are leaving the wetland following a breeding event would be difficult. Because of the way that the prairie at APCNWR fills in response to rain, "ponds" are not clearly delineated at the time of breeding activity and frequently cover several acres – too large an area to surround with drift fences to capture departing frogs. It may be necessary to track frogs with external transmitters to relocate them after the breeding event is over, then implant internal transmitters to follow their movement over the next few months.

For purposes of comparison between northern and southern populations of crawfish frogs, it is important to note that preliminary calling data indicates the breeding behavior of the southern subspecies is strikingly different with regards to timing (Saenz & Hibbitts, unpublished data) compared to northern populations (Williams et al. 2013), so it is reasonable to expect that other aspects of breeding behavior deviate from currently published results as well.

Crawfish frog activity, as with other amphibians, is tightly linked to weather patterns which drive reproductive events. Many amphibians require specific rainfall and/or temperature conditions to trigger breeding activities (Oseen & Wassersug 2002, Saenz et al. 2006). For example, rain events exceeding one inch during the appropriate times of year triggered explosive breeding events in *Bufo valliceps* (Blair 1960). Salvador & Carrascal (1990) found that breeding season initiation in two species of frogs was positively correlated with water temperature and amount of rainfall on the preceding day and with air temperature. Similarly, *Hyla chrysoscelis* initiated breeding activity only when presented with a combination of rainfall and warm temperatures (Ritke et al. 1992). In all these cases, as in northern crawfish frogs (Busby & Brecheisen 1997), climatic conditions are important (though not exclusive) factors in determining the timing of breeding season. One study in Kansas found that northern crawfish frogs started calling at a minimum temperature of 13°C and in response to enough rain "to saturate the soil and partially fill breeding ponds" (Busby & Brecheisen 1997). Another study found a positive linear relationship between calling probability and both temperature and the amount of rain 24 hours prior to surveying (Williams et al. 2012). Yet another study found that detection probability was highest above 9°C and when
there was no precipitation in the 24 hours prior to surveying (Williams et al. 2013), though they noted that rain is required to trigger crawfish frog breeding migrations. Preliminary data indicates that southern crawfish frogs may also be affected by rainfall and temperatures, though the specific ways in which they respond to these cues are undoubtedly different.

Crawfish frogs utilize two types of habitat where breeding populations occur. Burrow sites, where the frogs spend most of the year, are usually located in upland habitat, typically native tallgrass prairie or other types of grassland (Bragg 1953, Busby & Brecheisen 1997, Heemeyer et al. 2012, Parris & Redmer 2005). Furthermore, crawfish frogs are unique in that they almost exclusively reside in crayfish burrows for most of the year (Heemeyer et al. 2012, Heemeyer & Lannoo 2012, Hoffman et al. 2010), so they are limited to areas where crayfish burrows are available. While located at their burrows, one study found that northern crawfish frogs spend an average of 60% of the day in a 24 hour period aboveground on small patches of bare ground called “feeding platforms” where they wait to ambush prey (Hoffman et al. 2010). These frogs spent an additional 24% of their time visible at the entrance of the burrow (Hoffman et al. 2010) so they were detectable on game cameras 84% of the time when they were located at the burrow. The area comprised of the burrow and the feeding platform is typically 0.05m², while the interior of the burrow is estimated to be 0.01m³, making for a tiny home range during the non-breeding season, which makes up most of the year (Heemeyer et al. 2012). Breeding sites, which the frogs migrate to, are typically ephemeral to semi-permanent wetlands (Bragg 1953, Busby & Brecheisen 1997, Heemeyer et al. 2012, Parris & Redmer 2005). In both upland and breeding habitats, hard clay soils are favored (Smith 1961, Busby & Brecheisen 1997).

Crawfish frogs are historically among the least studied Ranids in North America (Busby & Brecheisen 1997). Additionally, most of the crawfish frog studies in the past 50 years have been conducted on the northern subspecies (Rana areolata circulosa) which experiences different environmental conditions than the southern subspecies. Since amphibians are heavily influenced by environmental variables, there may be behavioral differences between northern and southern subspecies. Texas crawfish frogs are breeding over a prolonged period which starts earlier and ends later than typical northern breeding periods (Saenz & Hibbitts, unpublished data). It is possible that during this period some frogs make multiple migrations,
rather than the one per year made by the northern subspecies. This shift in breeding pattern may be caused by milder and warmer climatic conditions on Texas coastal tallgrass prairies than on comparable tallgrass prairies at northern sites, potentially allowing for an extended breeding season.

In addition to the variable breeding time frame, there was no data on how far the southern subspecies travels from burrows to breeding ponds or even on what types of habitat crawfish frogs prefer to utilize in Texas. Collecting data on burrow site distance and available soil types could indicate which areas are suitable for sustaining a successful breeding population. These data could also be useful for locating probable areas for any existing unknown populations of crawfish frogs in Texas, especially since detection of the frogs themselves is only feasible during a narrow window of activity.

There are two species closely related to crawfish frogs: *Rana (Lithobates) capito* and *R. sevosa*, the latter of which was considered a subspecies of the former (Goin & Netting 1940) until recently (Young & Crother 2001). Both *R. capito* and *R. sevosa* are similar to crawfish frogs in that they split their time between breeding wetlands and upland burrows, stopping at temporary shelters along the way (Jensen & Richter 2005, Richter & Jensen 2005). Adult *R. capito* move between an average of 180.3 meters (Roznik et al. 2009) and an average of 1300 meters (Humphries & Sisson 2012) in a migration, though they sometimes travel as far as 2000 meters in the course of their migrations (Franz et al. 1988). *Rana sevosa* migrations are less well documented, but in one study adult frogs moved an average of 173.0 meters to breeding wetlands (Richter et al. 2001).

Crawfish frogs and their relatives in the *Rana capito-sevosa* complex are somewhat unique among anurans in their movement patterns. Compared to other frog species they utilize a tiny area during the non-breeding season. Some individuals exhibit ranging behaviors under certain conditions, moving away from their primary burrows for short periods of time only to return within several days (Heemeyer 2011), but for the most part the only movement that occurs is the breeding migration(s) from burrow to wetlands and back (Franz et al. 1988, Heemeyer 2011, Heemeyer et al. 2012, Humphries & Sisson 2012, Jensen & Richter 2005, Richter & Jensen 2005, Richter et al. 2001, Williams et al. 2012).
In a review of studies on anuran movement, Lemckert (2004) found that behavior varied widely by species. In 17 studies on 15 species, the anurans studied did not have separate breeding and non-breeding habitat, instead remaining at the breeding site year round regardless of whether mating was occurring (Lemckert 2004). In an additional six studies on five species, the anurans studied migrated from the breeding wetland to a non-breeding wetland (Lemckert 2004). In an additional 27 studies on 13 species, the anurans studied moved from the breeding site to a terrestrial habitat some distance away (Lemckert 2004). Crawfish frogs, of course, fit into this last category. There was a great deal of variation in both maximum (55-2800m) and average (30-900m) distance traveled between species (Lemckert 2004), to a degree that emphasizes the importance of individual studies on species of conservation concern. Mean travel distance for Ranids was 334 meters (Lemckert 2004), similar to the 376.9 meters (Heemeyer 2011) reported for the northern subspecies of crawfish frogs.

Most of the current research on *Rana areolata* has been conducted in the northern part of their range (primarily Indiana) on the northern subspecies. Crawfish frogs in the South exhibit different behaviors than their northern conspecifics (Saenz & Hibbitts, unpublished data), probably due to the milder winter climate at the lower latitudes and the different temperature and precipitation patterns year round. The objectives of this project were: 1) determine how far southern crawfish frogs travel between burrows and breeding sites on the coastal prairies of Texas, to learn how much space they are utilizing on the landscape; 2) learn how southern crawfish frogs spend their time when residing in their home burrows; and 3) identify whether there were differences in southern crawfish frog occurrence among different soil types, since soils with different characteristics vary in their ability to retain water and support burrow structures (see comments in Methods below on why soil data was collected rather than vegetation data).

**MATERIALS AND METHODS**

**Study Site**

The Attwater Prairie Chicken National Wildlife Refuge is located in Southeast Texas. Established and managed primarily to protect the Attwater’s Prairie Chicken, it covers 4,260 hectares of the roughly 40,000-50,000 remaining hectares of coastal prairie in Texas. Because of this, the refuge represents one of the largest contiguous sections of coastal prairie habitat remaining in Texas, and is home to possibly...
the largest population of crawfish frogs in the state (Saenz & Hibbitts, unpublished data). Between 800 to 1,200 hectares of the property are burned annually, with each area being burned approximately every four years, and most of it is grazed on rotation by cattle. Annual precipitation averages between 0.75 – 1.1 meters, with rain events forming temporary wetlands. Additional, artificial wetlands are provided by windmill-created ponds used for watering stock. Crawfish frogs utilize both types of ponds for mating choruses (Saenz & Hibbitts, unpublished data). The three ponds used for this project were all ephemeral wetlands. The boundaries of these temporary ponds vary dramatically with just a few inches of precipitation (from a few square meters, to several hectares), so GPS coordinates for each pond were set at the center of the area which vegetation indicated to be the “core” area most often filled.

**Preliminary Efforts**

Initially, we sought to capture frogs to tag with transmitters in the fall of 2014. We located several ponds where big choruses of crawfish frogs have occurred in previous years. We set up six three-foot high, 100-foot long silt fences in the ponds to guide the frogs into 20 minnow traps, which we placed in the water overnight. We did this for ten nights of trapping, and on five of those nights at least one crawfish frog was actually calling in the trapped pond. This effort yielded no crawfish frogs, though it did capture dozens of similarly sized leopard frogs (so we think it is unlikely to have been an issue of trap suitability).

When trapping ponds proved ineffective, we sought to increase sample size in the spring of 2015 by locating frogs at their burrows when they had returned from their breeding migration. Only recently burned areas could be surveyed due to the thick, interwoven vegetation present after just one or two growing seasons. Walking surveys during both day and night yielded no crawfish frog activity, so we planned a camera survey effort. We used a website (http://www.geomidpoint.com/random) to generate 100 random points within the bounds of the refuge, then used a random number generator to select ten of these which did not occur on roads or refuge boundaries or in standing water. We traveled to each of these points and marked out a 25x25 meter plot centered on the GPS point. Within this plot, we counted all of the crayfish holes present, placing numbered irrigation flags on each hole which was greater than or equal to three centimeters in diameter and which was not visibly blocked or constricted to a sliver below the opening. We then used a random number generator to select twelve of these holes to place Bushnell game
cameras on to monitor overnight to see if a frog was in residence. Ten nights of this, plus haphazard
sampling of any burrows encountered on walking surveys which had the right "look" (large enough
diameter, appeared to have a feeding platform) yielded only one occupied burrow, which was abandoned
by the time we returned with a camera three days later. Hence, we abandoned these effort.

**Tagging Frogs**

We were able to reliably capture crawfish frogs with dip nets during large mating choruses. We examined
captured frogs and determined mass and snout-vent length, as well as sex when possible by visual
inspection (i.e. via calling behavior). All but one of the tracked frogs was male.

In the first field season, 2015, we used Holohil BD-2 1.6 g and 1.8 g transmitters, attached via a beaded
aluminum chain at the frogs’ waists (Rathbun & Murphey 1996, Heemeyer et al. 2012, Heemeyer &
Lannoo 2012, Matthews & Pope 1999). We used the largest transmitters which, when combined with
attachment methods, would still weigh in at under 10% of any tagged frogs’ body mass (Heyer et. al.,
1994). We chose to use external transmitters because of problems reported in previous studies with
intraperitoneal transmitters resulting in visceral herniation in calling male crawfish frogs (Heemeyer et al.
2010). We selected aluminum bead chains because elastic-threaded glass beads have caused irritation
in similar trials (Heemeyer & Lannoo 2012).

Tagged frogs were then released at the pond where they were captured. When placed back in the water
immediately after being tagged, frogs often floated in a way that concerned us. They continued this
behavior for thirty minutes or more, sometimes began clawing at the belts with their rear legs and in some
cases appeared to have a foot temporarily stuck inside the belt. After retrieving any frogs which appeared
to be struggling, we were able to greatly reduce this behavior by keeping the frogs overnight before
release, with the transmitter belts already attached. Following this change in procedure, many frogs
immediately jumped into the water upon release and swam away, while a few floated nearby for 5-15
minutes before regaining their bearings and departing.

Some difficulties were encountered with this attachment methods following successful release of the
frogs. The transmitters did not reliably remain on the frog. There were two separate, common means of
failure. In the first and most common situation, the belt was too loose and slipped off the frogs’ legs, sometimes within a meter of the location where the frog was released. This was difficult to avoid because the best way to attach the belt was sliding it over the thick part of the frogs’ legs to reach the narrower waist, requiring the belt to be just barely loose enough to be forced around that width (and thus also just barely loose enough to be forced off if the frog maneuvered in just the right way). Five of the thirteen tagged frogs lost their transmitters in this manner before we were able to locate a burrow, and an additional three lost them in this manner once we had already located at least one burrow occupied by that frog. In the second type of situation, the transmitter snapped when the connection between the epoxy and the metal of the belt or the connection between the epoxy and the plastic coating on the transmitter failed. This occurred for one of the thirteen frogs before we could record any tracking information, and also for two of the thirteen tagged frogs after they had already been tracked to burrows. One transmitter was recovered in the remains of a frog which had been partially digested by a predator, and the final transmitter was removed from a previously tracked frog.

In the second field season, 2016, we used Holohil BD-2 1.6 g transmitters, threaded onto dissolvable veterinary suture thread. We threaded five to eight brown 5mm glass beads onto either side of the transmitters, then tied the suture thread around the frogs “waists” (Jonathan Swan, personal communication, similar to Muths 2003) as shown in Figure 1.
Though skin irritation was initially a concern with this method, no abrasions were observed on tagged frogs which were relocated approximately two weeks later, after which time no transmitter belts remained on frogs. This method allowed for the transmitters to be fitted more securely to the frogs, and we did not lose any due to overly loose fits. However, the frogs remained in the grass at the breeding sites for one to two weeks longer in the 2016 season (perhaps due to different precipitation patterns), and of the ten we initially tagged, at least three were depredated before making any movement toward their burrows. An additional four of the ten initial frogs lost their transmitters when the veterinary suture thread used to attach transmitters snapped. The final four frogs made it to burrows before the transmitter detached, apparently while they were underground traveling through the burrows (the frogs did not remain at these burrows, and the entrances were sealed by crayfish or the trampling of cattle within several days of the frogs’ arrival when the water receded).
**Radio-tracking**

We returned to the field every 1-3 days and located previously tagged frogs with a Communications Specialists R1000 telemetry receiver. Each time we located a frog, we logged its location with a GPS unit (accuracy 3m). Each time a frog was located in a burrow, we also placed an irrigation line marking flag within 30cm of the burrow entrance for easier relocation. Radio tracking occurred March - April in both 2015 and 2016. We then conducted a correlation analysis to determine whether relationships existed between the mass, SVL, and “body condition” (measured as a ratio of mass/SVL) of frogs and the distance they traveled. Given that body size and condition variables may be correlated, we also used stepwise multiple regression to investigate which body variable provides predictive information for distance moved from the pond. Forward stepwise regression was performed in JMP 10.0.0 (2012 SAS Institute Inc.). A P-value threshold of 0.1 was used to allow independent variables to enter the model.

**Burrow monitoring**

When the identified occupied burrow was not underwater, we placed a Bushnell Trophy Camera mounted on a wooden frame in a position to monitor the mouth of the burrow. We covered up the motion sensors and set the cameras to take a single picture every five minutes. This allowed us to see what times of day the frogs were active, and to confirm that they were alive and present at a burrow site between times we located them with radio-tracking equipment. The cameras automatically took photographs in infrared when light conditions were low enough, so we considered photos taken in infrared to be “night” activity and photos taken in the normal mode to be “day” activity for the purposes of analysis. Photo monitoring occurred in March and April of 2015, and ceased only when the frogs appeared to leave the burrows (no sightings were observed for a week or more). Unfortunately the radio transmitters had fallen off by that point, but the fact that crayfish began using two of the burrows shortly after the frogs disappeared supports their departure.

**Vegetation Analyses at Burrow**

We initially sought to sample the vegetation directly around each burrow site. However, the regular prescribed burns conducted at the refuge precluded data collection on vegetation. These burns resulted in rapid successional changes where a burrow may have been associated with bare ground, several sets
of various small herbaceous plants of varying families and heights, and thick waist high grass on each area that was burned. Sometimes the area was flooded with up to a meter of water, sometimes it was dry. In the end, conditions changed too rapidly to make meaningful comparisons. Accordingly, we switched efforts of habitat analyses from vegetation to soil (see below).

Radio-tracking

Following the field data collection we calculated the distance each frog traveled from breeding pond to burrow using GPS coordinates taken while tracking the frogs. Since crawfish frog behavior typically does not include movement except this migration between the burrow and the breeding pond, this distance is essentially the home range size. In this project, we considered home range to be “…that area traversed by the individual in its normal activities of food gathering, mating, and caring for young” (Burt 1943) in the course of one year as an adult. While this definition includes “caring for young”, which is not relevant for crawfish frogs, it also includes movement to breeding ponds. Although frogs spend relatively little time there, compared to their burrows, we included the ponds in home range calculations.

Soil Type and Burrow density

We sampled four main soil types on the refuge (Edna, Fordtran, Katy and Crowley) to see if there were differences in crayfish burrow density among soil types. It is important to note that these four soil types do not account for all of the area of the refuge. They do, however, include most of the areas where crawfish frogs have been found. One additional soil type which is present in these areas and in the results, the Gessner formation, was excluded from this sampling because it was uncommon and almost exclusively present in some “pond” areas which were often filled with water and too small to source more than one sample from. We used a soil map of the refuge to identify locations for samples, utilizing ArcMap to generate ten to twelve random points within the bounds of each of the four surveyed soil types. We navigated to the GPS coordinates of each selected point and marked out a 25 by 25 meter plot. We took a voucher soil sample from the center point of the plot for later analysis to confirm the accuracy of the broad scale soil map used to select points by checking the soil sample identification against what the map claims is present. We then counted the number of crayfish burrows present within the plot. The broad scale soil map used to select points was not entirely accurate in the field, so sample sizes were reduced
from the intended 10-15 samples of each type once the voucher specimens were examined in a laboratory environment. We then used a one-way ANOVA to determine whether there were statistical differences between the mean number of burrows on the four soil types, followed by a Duncan’s Multiple Comparison Test to identify which types were different.

To estimate the area of each soil type surrounding the ponds, we used the distance from each pond to the farthest known burrow (occupied by a crawfish frog which was captured from that pond) away from it to form the radius of a circle around the pond. Within that circle we calculated the percentages of each soil type and area covered by each soil type in square meters. We tabulated the number of known occupied crayfish burrows in each soil type. We also counted the number of times a tagged frog was tracked to a location in each soil type. We performed a chi-squared analysis to test for an association between occupancy or times frogs were logged in a soil type and the area of that soil type which was present at that site.

**RESULTS**

**Radio-telemetry**

Between the two years of this study, we collected data on eleven frogs that moved back to crayfish burrows within the course of the radio-tracking period from three different ponds (Table 1, Figures 2-4). Seven of the burrows were located in 2015, with the distance ranging from 72 to 624 meters from each of the two source ponds, and an average of 326.71 meters. The remaining four burrows were documented in 2016, with the distance ranging from 63 to 430 meters and an average distance of 295 meters. The combined average for all eleven burrows was 315 meters. It is important to reiterate that these “distance from the pond” measurements are actually the distance from the center point of the pond that fills up first when it rains, not the actual capture locations. The ponds themselves change drastically in extent in response to rain, ranging from completely dry impressions up to broad pools across several acres. Because of this, some of the burrow locations measured as being less than 100 meters away from the pond were actually underwater when a frog was tracked to them.
Of the eleven burrows identified, only two could potentially be classified as “primary” burrows in the vein of previously published research - that is, they were occupied consistently over a period of several weeks. But since the frogs left after those few weeks, they were probably not primary burrows. All the other burrows we located were potentially “secondary” burrows inhabited for a period of only 1-7 days before the frog moved on, sometimes tracked to another potential secondary burrow, and sometimes lost while moving to an unknown location. Frogs at APCNWR used only 1-3 burrows each before losing their transmitters. These results do not provide comprehensive enough evidence to conclude that a system of primary and secondary burrows is actually utilized at the APCNWR.

**Table 1.** Distance traveled from pond to burrow(s) ranged from 63-624 meters away from the center of the pond where the frog was collected between the two seasons (combined mean: 315 meters).

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<th>Pond #</th>
<th>Distance (m)</th>
<th>Transmitter</th>
<th>Pond #</th>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>603</td>
<td>5</td>
<td>297</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>619</td>
<td>5</td>
<td>303</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>327</td>
<td>Mean</td>
<td></td>
<td>295</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>207</td>
<td>SD</td>
<td></td>
<td>160</td>
</tr>
</tbody>
</table>

All eleven frogs tracked back to burrows were males. Mass of tracked frogs ranged from 30.3 grams to 66.5 grams, with an average of 49.7 grams (Table 2). Snout-vent-length of tracked frogs ranged from 65.8 to 85.4 mm, with an average of 74.7 mm (Table 2). Correlation analyses revealed a correlation coefficient of 0.6477 between mass of frogs and the distance they traveled to burrows, a correlation coefficient of 0.3091 between snout-vent-length and distance traveled, and a correlation coefficient of
0.6682 between “condition” (mass/SVL) and distance traveled. Step-wise multiple regression analysis on mass, SVL and “condition” versus distance traveled yielded a probability below 0.1 for the mass only and an R-Square value of 0.4195 for mass (Table 3).

Table 2. Morphological data on all frogs fitted with radio transmitters.

<table>
<thead>
<tr>
<th>Tag</th>
<th>Mass (g)</th>
<th>SVL (mm)</th>
<th>Condition (mass/SVL)</th>
<th>Distance traveled (m)</th>
<th>Number of burrows</th>
</tr>
</thead>
<tbody>
<tr>
<td>603</td>
<td>38.6</td>
<td>65.8</td>
<td>0.6</td>
<td>297.0</td>
<td>1</td>
</tr>
<tr>
<td>619</td>
<td>45.8</td>
<td>68.7</td>
<td>0.7</td>
<td>302.6</td>
<td>1</td>
</tr>
<tr>
<td>604</td>
<td>30.3</td>
<td>66.8</td>
<td>0.5</td>
<td>72.0</td>
<td>1</td>
</tr>
<tr>
<td>612</td>
<td>50.3</td>
<td>82.8</td>
<td>0.6</td>
<td>584.0</td>
<td>1</td>
</tr>
<tr>
<td>613</td>
<td>66.5</td>
<td>79.2</td>
<td>0.8</td>
<td>623.7</td>
<td>1</td>
</tr>
<tr>
<td>611</td>
<td>38.5</td>
<td>72.3</td>
<td>0.5</td>
<td>150.8</td>
<td>1</td>
</tr>
<tr>
<td>617</td>
<td>65.1</td>
<td>85.4</td>
<td>0.8</td>
<td>255.9</td>
<td>3</td>
</tr>
<tr>
<td>538</td>
<td>60.3</td>
<td>72.8</td>
<td>0.8</td>
<td>430.5</td>
<td>1</td>
</tr>
<tr>
<td>536</td>
<td>51.2</td>
<td>76.2</td>
<td>0.7</td>
<td>339.5</td>
<td>1</td>
</tr>
<tr>
<td>528</td>
<td>46.0</td>
<td>80.4</td>
<td>0.6</td>
<td>62.7</td>
<td>1</td>
</tr>
<tr>
<td>540</td>
<td>53.7</td>
<td>71.0</td>
<td>0.8</td>
<td>347.8</td>
<td>1</td>
</tr>
<tr>
<td>Mean</td>
<td>49.7</td>
<td>74.7</td>
<td>0.7</td>
<td>315.1</td>
<td>1.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tag</th>
<th>Mass (g)</th>
<th>SVL (mm)</th>
<th>Condition (mass/SVL)</th>
<th>Distance traveled (m)</th>
<th>Number of burrows</th>
</tr>
</thead>
<tbody>
<tr>
<td>529</td>
<td>45.4</td>
<td>61.6</td>
<td>0.7</td>
<td>n/a</td>
<td>0</td>
</tr>
<tr>
<td>530</td>
<td>52.4</td>
<td>74.9</td>
<td>0.7</td>
<td>n/a</td>
<td>0</td>
</tr>
<tr>
<td>539</td>
<td>77.4</td>
<td>80.0</td>
<td>1.0</td>
<td>n/a</td>
<td>0</td>
</tr>
<tr>
<td>537</td>
<td>56.0</td>
<td>76.2</td>
<td>0.7</td>
<td>n/a</td>
<td>0</td>
</tr>
<tr>
<td>535</td>
<td>44.0</td>
<td>75.6</td>
<td>0.6</td>
<td>n/a</td>
<td>0</td>
</tr>
<tr>
<td>527</td>
<td>70.8</td>
<td>85.4</td>
<td>0.8</td>
<td>n/a</td>
<td>0</td>
</tr>
<tr>
<td>614</td>
<td>60.6</td>
<td>79.1</td>
<td>0.8</td>
<td>n/a</td>
<td>0</td>
</tr>
<tr>
<td>608</td>
<td>67.1</td>
<td>77.3</td>
<td>0.9</td>
<td>n/a</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 2. Satellite imagery showing Pond 2 burrow locations.
Figure 3. Satellite imagery showing Pond 5 burrow locations.
Figure 4. Satellite imagery showing Pond 7 burrow locations.

Table 3. Stepwise Multiple Regression values

<table>
<thead>
<tr>
<th></th>
<th>F Ratio</th>
<th>Prob&gt;F</th>
<th>R Squared value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass</td>
<td>7.226</td>
<td>0.02277</td>
<td>0.4195</td>
</tr>
<tr>
<td>SVL</td>
<td>0.375</td>
<td>0.55559</td>
<td>n/a</td>
</tr>
<tr>
<td>Mass/SVL</td>
<td>0.051</td>
<td>0.82585</td>
<td>n/a</td>
</tr>
</tbody>
</table>
One frog was tracked to multiple burrow locations within a small area before its transmitter fell off (Figure 5). These movements show it did not travel in a straight line from the breeding pond, but in fact moved back and forth horizontally in relation to it after reaching some distance away.

**Figure 5.** All burrows occupied by Frog 617.

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**Dial Activity**

We collected 5033 images of three burrows occupied by crawfish frogs between 17 March and 31 March 2015 from game cameras. We were able to collect 945 images of the first burrow, 3453 images of the second burrow, and 635 images of the third burrow (Table 4). All three burrows were in areas which had been burned within the last six months, so they had similar vegetative structure at any given time.

Analysis of activity based on the photo indicated that frogs spent 70% of their time aboveground (60% of their time at night, 80% of their time during the day), either paused in the burrow entrance with their head facing out, or else sitting on the ground within half a meter of the entrance, presumably feeding (Table 5, Figure 6). The remaining 30% of the time (40% of the night, 20% of the day) was spent underground inside the burrow (Table 5, Figure 6).
Table 4. Breakdown of the number of diurnal/nocturnal activity photographs by burrow.

<table>
<thead>
<tr>
<th>Camera 1 (Crowley)</th>
<th>24 hours</th>
<th>Day only</th>
<th>Night only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not visible</td>
<td>272</td>
<td>20</td>
<td>252</td>
</tr>
<tr>
<td>Visible</td>
<td>673</td>
<td>439</td>
<td>234</td>
</tr>
<tr>
<td>Sum total</td>
<td>945</td>
<td>459</td>
<td>486</td>
</tr>
<tr>
<td>Active Time Percentage</td>
<td>71.2%</td>
<td>95.6%</td>
<td>48.1%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Camera 2 (Fordtran)</th>
<th>24 hours</th>
<th>Day only</th>
<th>Night only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not visible</td>
<td>706</td>
<td>332</td>
<td>374</td>
</tr>
<tr>
<td>Visible</td>
<td>2747</td>
<td>1389</td>
<td>1358</td>
</tr>
<tr>
<td>Sum total</td>
<td>3453</td>
<td>1721</td>
<td>1732</td>
</tr>
<tr>
<td>Active Time Percentage</td>
<td>79.6%</td>
<td>80.7%</td>
<td>78.4%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Camera 3 (Crowley-Gessner Complex)</th>
<th>24 hours</th>
<th>Day only</th>
<th>Night only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not visible</td>
<td>274</td>
<td>105</td>
<td>169</td>
</tr>
<tr>
<td>Visible</td>
<td>361</td>
<td>177</td>
<td>184</td>
</tr>
<tr>
<td>Sum total</td>
<td>635</td>
<td>282</td>
<td>353</td>
</tr>
<tr>
<td>Active Time Percentage</td>
<td>56.9%</td>
<td>62.8%</td>
<td>52.1%</td>
</tr>
</tbody>
</table>

Table 5. Overall measures of the percentages of activity for the three frogs.

<table>
<thead>
<tr>
<th></th>
<th>24 hours</th>
<th>Day only</th>
<th>Night only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average of the three frogs</td>
<td>69.2</td>
<td>79.7</td>
<td>59.5</td>
</tr>
<tr>
<td>Standard error of the mean</td>
<td>6.6</td>
<td>9.5</td>
<td>9.5</td>
</tr>
</tbody>
</table>
**Figure 6.** Pie chart showing frog activity broken down by time of day. Proportions shown are percentages of the 5033 total images.

Soil Analysis

Laboratory examination of the soil samples collected from the plots surveyed for burrow counts revealed that some samples did not match the profile of the soils the map had suggested were present. Any plots whose samples represented different soil series from those we intended to examine were excluded from the analysis, leaving remaining sample sizes of eight (Edna), eight (Fordtran), eight (Katy), and thirteen (Crowley) (Table 6). Crayfish burrow concentration differed significantly among the soil types \[ F(3,33)=6.95, p=0.0009 \]. A post-hoc Duncan’s Multiple Range Test (\( \alpha=0.05 \)) revealed that the Fordtran soil type had a greater concentration of crayfish burrows than the Katy, Edna, and Crowley soil types, suggesting that areas with Fordtran soils have higher availability of potential habitat for crayfish frogs.

**Table 6.** Numbers of crayfish burrows on 25 meter\(^2\) plots of each soil type.

<table>
<thead>
<tr>
<th>Soil Type</th>
<th>Fordtran(^a)</th>
<th>Katy(^b)</th>
<th>Edna(^c)</th>
<th>Crowley(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Mean</td>
<td>106.00</td>
<td>26.00</td>
<td>20.92</td>
<td>18.75</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>88.50</td>
<td>38.93</td>
<td>15.81</td>
<td>19.17</td>
</tr>
</tbody>
</table>
In terms of habitat usage, we examined the area in a circle around each pond (with the radius being the distance from the pond to the farthest burrow occupied by a crawfish frog from that site) (Figures 7-9, Table 7). We conducted a chi-squared analysis to compare the number of occupied frog burrows in each soil type and the number of times frogs were located in each soil type to what would be expected based on the habitat available (Table 8). All of the chi-squared analyses except for the frog activity analysis at Pond 2 had at least one expected value less than five, so p-values should be interpreted with caution.

**Figure 7.** Soil map of Pond 2 showing the circle created by using the farthest burrow from the pond center as the endpoint of the radius.
Figure 8. Soil map of Pond 5 showing the circle created by using the farthest burrow from the pond center as the endpoint of the radius.
Figure 9. Soil map of Pond 7 showing the circle created by using the farthest burrow from the pond center as the endpoint of the radius.
Table 7. Soil type characteristics and occupancy data for each pond within a circular area where the radius of the area analyzed is the distance from the pond center to the farthest burrow.

<table>
<thead>
<tr>
<th>Pond 2</th>
<th>Soil type</th>
<th>Percentage of soil type</th>
<th>Area in soil type (m²)</th>
<th>Number of frog burrows in soil type</th>
<th>Times frogs logged in soil type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Edna</td>
<td>34.4</td>
<td>929.6</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Katy</td>
<td>65.6</td>
<td>1774.7</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>Pond 5</td>
<td>Soil type</td>
<td>Percentage of soil type</td>
<td>Area in soil type (m²)</td>
<td>Number of frog burrows in soil type</td>
<td>Times frogs logged in soil type</td>
</tr>
<tr>
<td></td>
<td>Gessner</td>
<td>5.5</td>
<td>120.2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Katy</td>
<td>12</td>
<td>262.2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Crowley-Gessner Complex</td>
<td>82.5</td>
<td>1802.7</td>
<td>4</td>
<td>17</td>
</tr>
<tr>
<td>Pond 7</td>
<td>Soil type</td>
<td>Percentage of soil type</td>
<td>Area in soil type (m²)</td>
<td>Number of frog burrows in soil type</td>
<td>Times frogs logged in soil type</td>
</tr>
<tr>
<td></td>
<td>Edna</td>
<td>30</td>
<td>940.3</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Gessner</td>
<td>15</td>
<td>470.2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Fordtran</td>
<td>25</td>
<td>783.6</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Crowley</td>
<td>30</td>
<td>940.3</td>
<td>2</td>
<td>10</td>
</tr>
</tbody>
</table>
Table 8. Chi-squared analyses comparing the observed number of occupied frog burrows in each soil type (“occupied burrows”) and the number of times which a frog was tracked to a location within each soil type (“frog activity”) to the values which would be expected based on the amount of available area of that soil type around the pond. All of the chi-squared analyses except for the frog activity analysis at Pond 2 had at least one expected value less than five, so those p-values may be incorrect.

<table>
<thead>
<tr>
<th></th>
<th>Occupied burrows</th>
<th>Frog activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pond 2</td>
<td>X² value 0</td>
<td>X² value 3.266</td>
</tr>
<tr>
<td></td>
<td>P-value 1.0000</td>
<td>P-value 0.0707</td>
</tr>
<tr>
<td></td>
<td>df 1</td>
<td>df 1</td>
</tr>
<tr>
<td>Pond 5</td>
<td>X² value 0.848</td>
<td>X² value 3.606</td>
</tr>
<tr>
<td></td>
<td>P-value 0.6543</td>
<td>P-value 0.1648</td>
</tr>
<tr>
<td></td>
<td>df 2</td>
<td>df 2</td>
</tr>
<tr>
<td>Pond 7</td>
<td>X² value 3.8</td>
<td>X² value 2.536</td>
</tr>
<tr>
<td></td>
<td>P-value 0.2839</td>
<td>P-value 0.4688</td>
</tr>
<tr>
<td></td>
<td>df 3</td>
<td>df 3</td>
</tr>
</tbody>
</table>

Discussion

Radio-telemetry

In the course of this work, only two burrows were located which might be considered primary burrows in the style of previously published work (Heemeyer et al. 2012) and even those are questionable because the frogs left after several weeks. Therefore, if in fact the crawfish frogs studied at APCNWR do utilize a system of primary and secondary burrows similar to that which was observed in the previously mentioned studies in Indiana, it is probable that the external transmitters fell off before the frogs reached their primary burrows. Burrows at APCNWR flooded several times during radio tracking in 2015, remaining submerged below several inches of water for days at a time, and flooding has been observed to
precipitate crawfish frog departure from burrows in Indiana (Heemeyer 2011). One of the frogs monitored with a game camera followed this pattern.

As for the distances traveled, frogs in Indiana took an average of 13.2 days (as few as 1 to as many as 39 days) to travel an average of 376.9 meters (as few as 28.1 to as many as 1,187.8 meters) in a direct line from the breeding ponds to their primary burrows during the post-breeding migration (Heemeyer 2011). If the two consistently occupied burrows at APCNWR were, in fact, primary burrows, then those frogs traveled 302.60 and 623.69 meters, taking 6 days and somewhere between 3-5 days, respectively. If they were secondary burrows, comparisons should be treated with caution. Additionally, distance traveled was positively correlated with the mass of the frog in both a simple correlation analysis (Table 2) and in a multiple stepwise regression analysis (Table 3), suggesting that frogs with greater mass travel farther to burrows. Since larger frogs may have greater fitness (Berven 1981 & 1990, Wilbur et al. 1978), this suggests that larger areas around the ponds may be important for local crawfish frog populations.

**Dial Activity**

Depending on the method of calculation (see Tables 3 and 4), crawfish frogs at APCNWR were visible at or outside the burrow entrance 69.2% of the time when they were present at a burrow. At night, they were visible 59.5% of the time. During the day, this rose to 79.7%. This stands in contrast to studies conducted in Indiana on the northern subspecies of crawfish frogs. One study (with a sample size five times greater than mine, and which covered a longer period of time) comparing frog activity between recently burned and vegetated areas found an activity level of 20.8% during the night and 69.7% during the day in vegetated areas, while in burned areas, they found that frogs were visible 18.3% of the time during the night and 59.9% during the day (Engbrecht & Lannoo 2012). Another Indiana study, this one with a similar sample size to mine, found crawfish frogs to be active 39% of the day and 45% of the night (Hoffman et al. 2010). The Hoffman et al. (2010) study had a 1 hour interval between sample photos, while this study and the study by Engbrecht & Lannoo (2012) utilized 5-minute photo intervals. From this, it appears that crawfish frogs in Indiana are spending less time outside their burrows, whether in the entrance or on the feeding platform. It is possible that the difference is attributable to the fact that these are secondary burrows, rather than the primary burrows observed in Indiana. Furthermore, the crawfish
frogs we observed were often experiencing especially wet conditions. In particular, compared to Engbrecht & Lannoo (2012), crawfish frogs at APCNWR are much more active at night than their northern counterparts. Interestingly, they are still just as active during the day. Data has shown that frogs which were previously active aboveground will not emerge from their burrows as frequently when temperatures drop (Engbrecht & Lannoo 2012, Hoffman et al. 2010).

**Soil Analysis**

The crayfish burrows which crawfish frogs seem to favor are dug by various species of the invertebrate family Cambaridae, which utilize them for habitation. Burrowing crayfish tend to favor soils with a high clay composition (Correia & Ferreira 1995, Grow & Merchant 1980, Hobbs & Whiteman 1991) and soils which are fine-grained (Barbaresi et al. 2004, Grow 1982). These soil types are easier to form into shapes and hold their shapes better. Additionally, crayfish burrow densities are higher in areas without soil compaction (March & Robson 2006). Many vertebrate and invertebrate species utilize crayfish burrows commensally (Heemeyer 2011, Loughman 2010, Welch et al. 2008) though most crawfish frogs are found in burrows which are not otherwise occupied the majority of the time (Heemeyer 2011). In many fossorial species, the number of burrows present in an area correlates positively to the number of individuals occupying burrows in that area, whether those individuals are members of the species which create the burrows (Nomani et al. 2008, Welch et al. 2008, Wilson et al. 2010) or members of other species which occupy the burrows commensally or following abandonment (Tipton et al. 2008).

In light of all this information, results from the burrow count soil surveys are interesting. The greatest concentration of crayfish burrows occurred in the Fordtran soil type. While Fordtran is classified as both “clayey” and “smectitic”, the top 71 centimeters of Fordtran layers tend to be loamy fine sand with lots of roots (Soil Survey Staff). As previously discussed, fine sand, while more suitable for burrowing than coarse sand, is not a soil type typically favored by crayfish. However, the next lowest several layers of Fordtran, from 71-122 centimeters, are composed primarily of very suitable clay (Soil Survey Staff).

Crayfish burrows can be anywhere from 15 centimeters to five meters deep (Grow & Merchant 1980) depending on species, location, and soil types, so it is certainly possible that crayfish burrows are more common on the Fordtran soil at APCNWR because of these lower clay layers. Interestingly, burrow
counts for the Edna soil type, which is also mostly composed of clay (Soil Survey Staff), were not significantly different from the counts for the other two, primarily loamy soil features. The small sample sizes may have obscured a pattern, or there may be other factors at work which make the Edna soil type less suitable than Fordtran despite the similarities. The Edna series does tend toward a coarser structure (Soil Survey Staff).

There was no difference between soil type available and soil type occupied at Ponds 5 and 7 (Table 7), suggesting that the soils crawfish frogs occupy may simply be determined by what is available. For Pond 2, however, the analysis of the three occupied burrows showed that observed values differed from expected values (Table 7), indicating that there may be factors other than soil type influencing burrow location, or perhaps just that sample sizes are low. All of the chi-squared analyses except for the frog activity analysis at Pond 2 had at least one expected value less than five, so results should be interpreted in light of these low sample sizes.

CONCLUSION

Overview

The most interesting results from this project are the differences in daily activity and burrow distances, compared to currently published metrics. Overall, these data suggest there are differences in behavior between the northern subspecies and the southern subspecies. It is important to make management decisions based on accurate information, and this research highlights the importance of basing those decisions on information gathered on ecologically similar populations of the species when behavior could be altered by environmental variables.

Conservation Implications

As is common in anurans which have separate breeding and non-breeding sites (Lemckert 2004), the breeding site is a concentrated area, with non-breeding habitat radiating out through most of the area around it in all directions. Therefore, it is important to protect the area around the ponds in all directions where suitable habitat (with crayfish burrows) occurs. A buffer radius of 625 meters would cover 100% of the burrows located in this study, though we cannot be sure whether those are, in fact, primary burrows or
just transitional stopovers. Frogs in these burrows are an average of 5.4°C warmer than the air temperature (Heemeyer 2011).

**Further Investigations**

We recommend additional research to distinguish between primary and secondary burrow usage. Internal transmitters are probably advisable, because from our experience it seems that external transmitters, however well attached, are likely to fall off within the first few weeks of travel. Internal transmitters have issues of their own, but they might be necessary in order to illuminate more clearly the differences between northern and southern subspecies which have been touched on in this project.
ACKNOWLEDGEMENTS

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