

LARVAL AND POST-METAMORPHIC ECOLOGY OF THE DUSKY GOPHER FROG
(*RANA SEVOSA*)

A thesis presented to the faculty of the Graduate School of Western Carolina University in partial fulfillment of the requirements for the degree of Master of Science in Biology

By

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ABSTRACT

LARVAL AND POST-METAMORPHIC ECOLOGY OF THE DUSKY GOPHER FROG (*RANA SEVOSA*)

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Amphibians are declining worldwide, and research on their habitats and ecology are critical to their continued conservation. I conducted two experiments with the federally endangered dusky gopher frog, *Rana sevosa*. The dusky gopher frog breeds exclusively in isolated, open canopy wetlands, usually with extensive herbaceous growth. It is known that larvae of the dusky gopher frog, and of many other anurans, have higher growth and survival under open canopy than under closed canopy conditions. The mechanisms whereby this occurs are poorly understood, however. In the first study, I conducted a complete factorial experiment in mesocosms to compare the relative influence of factors differing between open and closed canopy ponds. A heat/light treatment consisted of one third of tanks being exposed to full sunlight, one third of tanks being shaded with 70% shade cloth, and the final third being shaded with 70% shade cloth but heated to mimic the temperature of the full sun tanks. In addition, tanks received either a closed canopy tree leaf litter mixture or an open canopy herbaceous vegetation mixture, and vertical vegetation-like structure made of polypropylene rope or not. Cool shaded tanks and heated shaded tanks had identical survival to metamorphosis at 74.7% and produced frogs with an average mass of 2.21 g and 2.09 g respectively, while tanks in full sunlight achieved

significantly higher 93.4% survival to metamorphosis and 2.64 g mass. The open canopy vegetation mixture yielded an average tank survival of 88.3% and mass of 2.94 g, compared to closed canopy vegetation tanks with a significantly lower survival of 73.5% and mass of 1.61 g. Structure had no effect on survival or mass. These results indicate that sunlight (not heat alone) and herbaceous plants are important in increasing dusky gopher frog survival and mass in open canopy conditions, with implications for captive-rearing and habitat restoration. Dusky gopher frog tadpoles are raised in captivity to increase survival and released back into the wild at metamorphosis to augment wild populations (headstarting) and start new populations (translocation). In the second study, I used radiotelemetry to compare post-metamorphic migratory movements and behavior between tank-reared juveniles and juveniles leaving the pond naturally. Frogs were tracked daily, until the signal was lost, the frog shed the transmitter, or the frog was predated, with a range of 1 to 73 days of tracking. Two of twenty frogs successfully found a permanent refugium, at an average distance of 153 meters from the center of the pond basin. No differences in movements or fates were found between groups; however, natural individuals did utilize grass as cover during migration more often than tank-reared frogs. This suggests that current headstarting protocols produce juveniles that differ only minorly from natural juveniles in movement and ecology, and that headstarting is likely to effectively replicate natural recruitment.

CHAPTER ONE: ASSESSING FACTORS THAT INCREASE DUSKY GOPHER FROG
(*RANA SEVOSA*) LARVAL PERFORMANCE IN OPEN-CANOPY WETLANDS

Introduction

Amphibians are one of the most imperiled groups of organisms, as they have undergone precipitous declines in the last half century (Stuart et al. 2004, Grant et al. 2020, Green et al. 2020). Although in many cases the reasons for species' declines and extinctions are unclear, habitat destruction and fragmentation are likely to be significant factors for many amphibian populations (Stuart et al. 2004, Gallant et al. 2007, Green et al. 2020). In the United States, the Southeastern Coastal Plain has an especially speciose amphibian assemblage, with some areas containing more than 40 sympatric amphibian species (Weir et al. 2014). Historically, the Southeastern Coastal Plain was dominated by longleaf pine (*Pinus palustris*) savannahs. The longleaf ecosystem supports an incredible diversity of animal and plant species, including many amphibians that are endemic to longleaf systems (Means 2007). Unfortunately, many of these specialists have drastically reduced ranges due to the loss of the longleaf pine ecosystem; Frost (2007) estimated that less than 3% of the original savannah remains intact. The longleaf pine savannah is a fire-adapted ecosystem, which depends on frequent, low-intensity fire to maintain the open understory and release longleaf pine seedlings from competition with other, less fire-resistant tree species (e.g. loblolly pine, *Pinus taeda*, and hardwoods such as *Quercus spp.*). Thus, not only has this habitat been lost through logging and forestry, clearing for agriculture, and urban/suburban development, but even otherwise undisturbed tracts of longleaf savannah have become significantly degraded simply through human exclusion of fire (Gilliam & Platt 1999, Frost 2007).

All anurans native to the Southeastern Coastal Plain, as well as many salamanders, have a biphasic life cycle including a larval aquatic stage and a terrestrial juvenile and adult stage. As such, they require appropriate breeding wetlands in addition to suitable terrestrial habitat. For habitat specialist species such as the dusky gopher frog, *Rana sevosa*, this dual habitat requirement becomes a double-edged sword in the context of massive ecosystem alteration and loss of native longleaf environments, including the ephemeral wetlands they historically contained. The dusky gopher frog is listed as endangered under the United States Endangered Species Act of 1973 (USFWS 2001). A medium to large sized ranid frog, the dusky gopher frog spends the majority of its adult life in upland habitats, returning to wetlands for only a short period of time to breed. It requires both longleaf savannah with appropriate underground refugia, as well as fishless ephemeral wetlands for breeding and larval development (USFWS 2001, Thurgate 2006). As with many endangered species, habitat loss is a primary cause of the decline of the dusky gopher frog (USFWS 2015), although other threats, such as disease, are also of concern (Sutton et al. 2014, Atkinson 2016). Historically, the dusky gopher frog ranged from southeastern Louisiana, through southern Mississippi, to southwest Alabama west of the Mobile basin (Goin & Netting 1940). It is now known from only a handful of breeding ponds in southern Mississippi, including one natural breeding population and several translocation sites (USFWS 2015).

The wetlands that dusky gopher frogs typically use for breeding share several characteristics, including the absence of fish (through appropriate depth and size resulting in periodic drying, and isolation from permanent lakes and rivers), and an open canopy with abundant herbaceous vegetation (Thurgate 2006, Gregoire & Gunzburger 2008, Holbrook & Dorn 2016). Analogously to the open understory in longleaf uplands, historical fire regimes

maintained the open and herbaceous character of ephemeral wetlands (Kirkman et al. 2000). Fire exclusion or management burning in the dormant season (when these wetlands typically hold the most water) allows hardwood species to invade wetlands and create a dense canopy that changes the character of the pond (Kirkman et al. 2000, Van Lear et al. 2005). The closure of canopies over historical gopher frog wetlands may have been a significant contributor to the frog's decline.

Several species of anurans have been shown to breed preferentially in open canopy wetlands (Skelly et al. 1999), and experimentally, the larvae perform better under open canopies (Werner & Glennemeier 1999, Skelly et al. 2002, Thurgate & Pechmann 2007). Several factors could explain the differences in larval performance between open and closed canopy wetlands. Physiological processes in ectotherms, such as tadpoles, scale with temperature. Ponds with higher levels of direct sunlight can be expected to have higher average temperatures than shaded ponds, which could allow for faster growth and development (Bradford 1990). Open canopy ponds have a higher dissolved oxygen concentration than closed canopy ponds due to increased photosynthesis, which could affect tadpoles physiologically and behaviorally (Skelly et al. 2002). The two pond types can be expected to differ in the composition of benthic substrates due to their different plant communities and different inputs of plant litter, which can affect organisms living and growing in the pond (Stoler & Relyea 2020). Ponds with higher amounts of woody vegetation within and near the pond basin should experience a shorter hydroperiod through increased evapotranspiration. If the hydroperiod is shorter than the minimum larval development time, complete cohort failure can occur (e.g. Richter et al. 2003). Direct sunlight should support higher levels of algal growth and primary production in the open canopy ponds; in addition, the herbaceous vegetation which dominates them would provide additional surface area for algal

growth. Algae (perhaps along with other autotrophic and heterotrophic components of periphyton and plankton) are thought to be the primary food sources for most of the stereotypical, generalist tadpole species (Wassersug 1975, Skelly & Golon 2003, Altig et al. 2007). Although it is likely that each of these aspects has some effect on anuran species distributions and performance, the relative importance of each factor is poorly understood. In order to better elucidate the mechanisms underlying performance increases under an open canopy in the dusky gopher frog and other amphibian species, I performed an experiment with tadpoles of the dusky gopher frog to compare the effects of temperature, sunlight, vegetation type, and three-dimensional structure on their survival and growth.

Study System

The dusky gopher frog, *Rana sevosa*, is a medium sized ranid frog with dark, patterned, and warty skin. It is a specialist of the longleaf pine ecosystem, living in holes in the ground, such as burrows dug by gopher tortoises or small mammals, or root holes left by tree stumps that have burned out or rotted away (Richter et al. 2001, Roznik and Reichling 2020). Adults migrate to breeding ponds, sometimes over distances of a few hundred meters or more (Richter et al. 2001, Humphries & Sisson 2012), and males call to attract females. Gopher frogs typically breed in the winter and early spring, although they may breed during heavy rainfall any time from late August to April, such as during tropical storms and hurricanes in the fall (Richter et al. 2003, Pechmann et al. 2017). A female deposits her eggs each season as a single mass, almost always attached to a stem of vegetation. Temperature influences developmental rate; the eggs generally hatch 1-2 weeks after deposition, and the larval period usually extends to early summer, 81-179+ days after egg deposition (Richter et al. 2003). After metamorphosis, frogs leave the pond and take refuge in the uplands, returning to breed once they reach sexual maturity after 1-5 years

(Pechmann et al. 2017). The only remaining natural dusky gopher frog population is at Glen's Pond, located on the DeSoto National Forest in Harrison County, Mississippi, USA, though other populations have been established through habitat improvement and reintroductions. Since 2002, a portion of nearly every egg mass laid in Glen's Pond has been taken and reared to metamorphosis in a "headstarting" project using nearby cattle watering tanks (USFWS 2015). Eggs from other breeding sites are also headstarted depending on facility space and egg mass numbers, which can vary substantially from year to year. A portion of these headstarting tanks were used for the experiment described in the next section.

Methods

Experimental Design

Eggs were collected from two translocation sites, TNC1 Pond and Ashe South Pond, on 17 and 18 February 2021, respectively. I obtained eggs from four egg masses from Ashe South Pond and six from TNC1 pond. Roughly one third of each egg mass was collected and transported in pond water to the lab, where each portion was kept in a ~1 liter container until hatching. Water changes of 50% were performed every other day using filtered water. Tadpoles were retained indoors until they had finished hatching and had begun to actively swim. I then stocked the outdoor experimental tanks at a density of 20 tadpoles, two from each egg mass, on 5 March 2021.

I conducted the experiment using plastic cattle watering tanks, approximately 1.8 meters in diameter and 0.6 meters tall, at the Harrison Experimental Forest. Three factor levels were chosen and applied in a split-plot design. The whole-plot factor, which was applied to blocks of four tanks and replicated four times (for a total of 48 tanks), was a heat/light treatment with three levels: tanks in full sunlight, tanks covered with 70% shade cloth, and tanks covered with 70%

shadecloth but heated to the temperature of full sunlight tanks (henceforth sun, shaded, and heated tanks, respectively). These last tanks were heated using an Eheim Jager 300W aquarium heater (Eheim, Deizisau, Germany) attached with suction cups to the bottom of the tank and controlled via a timer to correspond to diel temperature fluctuations in the unshaded tanks (Rudolf and Roman 2018). As needed based on the weather, heaters were manually checked and controlled to match the temperature of the full-sun tanks. Non-heated tanks received sham heaters constructed out of PVC. 70% shadecloth was chosen because it approximated the light conditions under a closed canopy pond near Glen's Pond that may represent a typical fire-suppressed depressional wetland (Thurgate 2006). All tanks were tightly covered with fine mesh window screen to exclude predators and competitors.

The split-plot factor applied to individual tanks within each whole-plot block of four tanks was a two by two factorial of the remaining two factors. The first was a vegetation treatment with two levels. One half of the tanks received an open canopy vegetation mix consisting of *Juncus repens* (lesser creeping rush), *Panicum hemitomon* (maidencane), and *Scirpus cyperinus* (woolgrass), and the other half received a closed canopy leaf litter vegetation mix of *Pinus palustris* (longleaf pine) needles, *Nyssa sylvatica* (black gum) leaves, and *Liquidambar styraciflua* (sweetgum) leaves. The vegetation was air dried completely before being weighed, and each tank received 1.5 kg of vegetation. The closed canopy mixture was evenly divided among the three species, but the open canopy mixture consisted of 350 g *Juncus*, 500 g *Scirpus*, and 650 g *Panicum* per tank due to limited availability at the collection sites. Vegetation was added 3 March 2021 and allowed to sink to the bottom before tadpoles were introduced to the tanks. The final factor was a vertical structure treatment with two levels, designed to imitate the emergent vegetation found in open canopy breeding ponds that may

provide a more natural or efficient feeding environment for tadpoles. Two sections of polypropylene rope, one meter in length, were frayed by hand. I tied one rope to each of two bricks placed in half of the tanks. The other tanks received only two bricks to control for any effect that they would have on the mesocosm community. The polypropylene floated upwards in the water to provide more surface area for algal growth and tadpole feeding. Each treatment combination (three levels of heat/light, two levels of vegetation, and two levels of structure) was replicated four times for a total of 48 tanks in the experiment.

I filled tanks to 15 cm depth on 1 March 2021 and added tadpoles on 3 March. I allowed the tanks to gradually fill to the final depth of 45 cm and an approximate water volume of 1000 liters, controlled with a PVC standpipe inside of the tank, over the next three weeks. This ensured that the tadpoles could easily reach the surface of the water to prevent death from potential anoxia until their swimming abilities improved. Beginning 14 March, I measured temperature, specific conductance, pH, and dissolved oxygen were taken approximately weekly at sunrise and sunset. I measured temperature, specific conductance, and dissolved oxygen with a YSI 600QS (YSI Incorporated, Yellow Springs, OH, USA), and pH with a handheld meter (PH20, Apera Instruments, Columbus, OH, USA), at a depth of 15 cm below the water's surface. In addition, HOBO temperature loggers (MX2201; Onset Computer Corporation, Bourne, MA, USA) were placed within a subset of tanks (the tank with open canopy vegetation mix and structure treatment in each block, 12 total tanks) to record the temperature on an hourly schedule, for fine-scale temperature monitoring and adjustment of the temperature in the heated tanks. Four of these HOBO temperature loggers were placed in natural wetlands, two with closed canopies and two with open canopies, for the duration of the season to collect comparison data. I collected plankton samples with a plankton net approximately monthly starting on 4 March, from

Ashe South (open canopy) and Ashe North (closed canopy) ponds. Samples from both ponds were well mixed, and I added 800 ml aliquots to each tank to inoculate and then maintain plankton communities. As experimental treatments mixed characteristics of open and closed canopy ponds, plankton samples from both ponds were added to all tanks to allow the specific tank environment to select for successful components of the plankton inoculation communities. To measure periphyton/biofilm growth in each tank, I suspended a frosted tip microscope slide on the West, East, and North side of each tank at 15 cm depth. Slides were left in the tanks for three weeks, then the biofilm was dried, scraped off of the slide (an area of 2.5 cm x 5.8 cm), and weighed (Stevenson & Bahls 1999, Skelly et al. 2002, Thurgate 2006). I repeated this process four times with samples retrieved from tanks on 20 April, 19 May, 18 June, and 16 July 2021.

I hung a minnow trap at the water line in each tank on 13 May 2021 to capture metamorphosed individuals attempting to leave the water. I checked traps daily, and I weighed and measured captured metamorphs. Depending on release site, some metamorphs were tagged with a Visual Implant Elastomer Alphanumeric Tag (VI-Alpha) for ongoing mark-recapture demographic studies. All frogs were released at Glen's Pond or a translocation site within five days of metamorphosis. When almost all surviving tadpoles had metamorphosed, the water in the tanks was gradually drawn down to mimic natural drying of the pond. The tanks were lowered in 5 cm increments every 3 days starting on 26 July. On 16 August, when the tanks were lowered to 5 cm depth, I meticulously searched the litter to remove any remaining tadpoles or frogs for recording and release.

Statistical Analysis

I used (generalized) linear mixed models (GLMM) to examine the relationship between tank treatments and frog mass, snout to vent length (SVL), days to metamorphosis, and tank

survival, with block and tank as random effects. Days to metamorphosis, mass, and length were log transformed to improve model fit and meet model assumptions. Survival to metamorphosis within each tank was analyzed as a binomial family GLMM, using only block as a random effect. P-values reported for main effects and interactions in LMMs are the result of F tests using Satterthwaite approximations for denominator degrees of freedom, and an alpha level of 0.05 was set *a priori*. I tested the significance of effects and their interactions in GLMMs using likelihood ratio tests. Chemical data and periphyton mass were analyzed analogously, as LMMs with time as a fixed effect. Treatment contrasts were produced using Tukey's HSD test. Analyses and graphs were produced in R (version 4.1.2, R Core Team 2020), using the tidyverse (Wickham et al. 2019), lme4 (Bates et al. 2015), lmerTest (Kuznetsova et al. 2017), car (Fox & Weisberg 2019), multcomp (Hothorn et al. 2008), and DHARMA (Hartig 2021) packages.

Results

The average temperature recorded in full sun, heated, and shaded tanks was 24.8 °C, 24.7 °C, and 23.0 °C, respectively. Tukey's post hoc comparison revealed that the temperature of sun and heated tanks was significantly different ($z = 2.873$, $p = 0.01$); however, the difference was likely too small ($\sim 0.1^\circ\text{C}$) to result in biologically meaningful differences in comparison to the much larger difference between sun and heated tanks and the cooler shaded tanks ($\sim 1.75^\circ\text{C}$ cooler, both $|z| > 14$, $p < 0.001$). Cold temperatures in April lowered water temperatures, which subsequently rose and remained high through the summer. One HOBO logger in an open canopy natural pond was lost before any data could be retrieved. The averages for the remaining open canopy pond and the two closed canopy ponds (combined) were 27.9 °C and 24.3 °C, respectively. There appear to be differences in temperature variance due to canopy closure and experimental heat. The natural closed canopy ponds had an average standard deviation of

3.76 °C, and the shaded, unheated mesocosms had an average standard deviation of 3.83 °C. On the other hand, the natural open canopy pond, full sunlight tanks, and heated tanks had standard deviations of 4.03 °C, 4.37 °C, and 4.17 °C, respectively. The ranges of the temperatures were similar across these categories, with the minimum and maximum temperatures shifted several degrees higher in natural ponds, as indicated by the averages reported above.

Specific conductance was significantly higher ($F_{1, 1557} = 66.5$, $p < 0.001$) in tanks with open canopy vegetation than with closed canopy vegetation. Specific conductance was also observed to decline throughout the season, although effects were complicated (all interactions of experimental effects and time significant, $p \leq 0.01$). Dissolved oxygen (DO) rose through the spring and reached a peak near early May, and then maintained average levels of around 5.0 mg/L throughout June and July (Figure 1). Full sun tanks exceeded heated and shaded tanks, which did not statistically differ from one another, by over 1 mg/L (Tukey's HSD: $z > 8.5$, $p < 0.0001$). In the shaded and heated tanks, closed canopy vegetation tanks had higher DO levels by approximately 0.3 mg/L, but this effect disappeared in the full sunlight treatment (significant interaction, $F_{2, 1557} = 7.04$, $p < 0.001$). There was also a significant interaction ($F_{2, 1557} = 7.08$, $p < 0.001$) between the heat/light treatment and structure treatment. The trend was for structure to have a positive effect on DO in shaded tanks, a negative effect in heated tanks, and no effect in full sunlight tanks, although the size of these trends was small (approximately 0.2 mg/L) in comparison to the main effects and the two-way interaction between heat/light and vegetation. No experimental treatments or interactions had a significant effect on tank pH. The average pH was 6.26, although there was a significant downward trend during the season, from an average of 7.47 in March to 5.52 in July ($F_{34, 1564} = 210.9$, $p < 0.001$). Periphyton mass was almost twice as great in open canopy vegetation tanks compared to closed canopy vegetation tanks (0.041

mg/cm² vs 0.0225 mg/cm²; $F_{1, 179} = 18.5$, $p < 0.001$). Mass was highest for the June sampling, lowest for the July sampling, and intermediate for the April and May samplings. None of the other experimental treatments or interactions had a significant effect on periphyton mass, although there was a nonsignificant trend towards higher mass in full sunlight tanks.

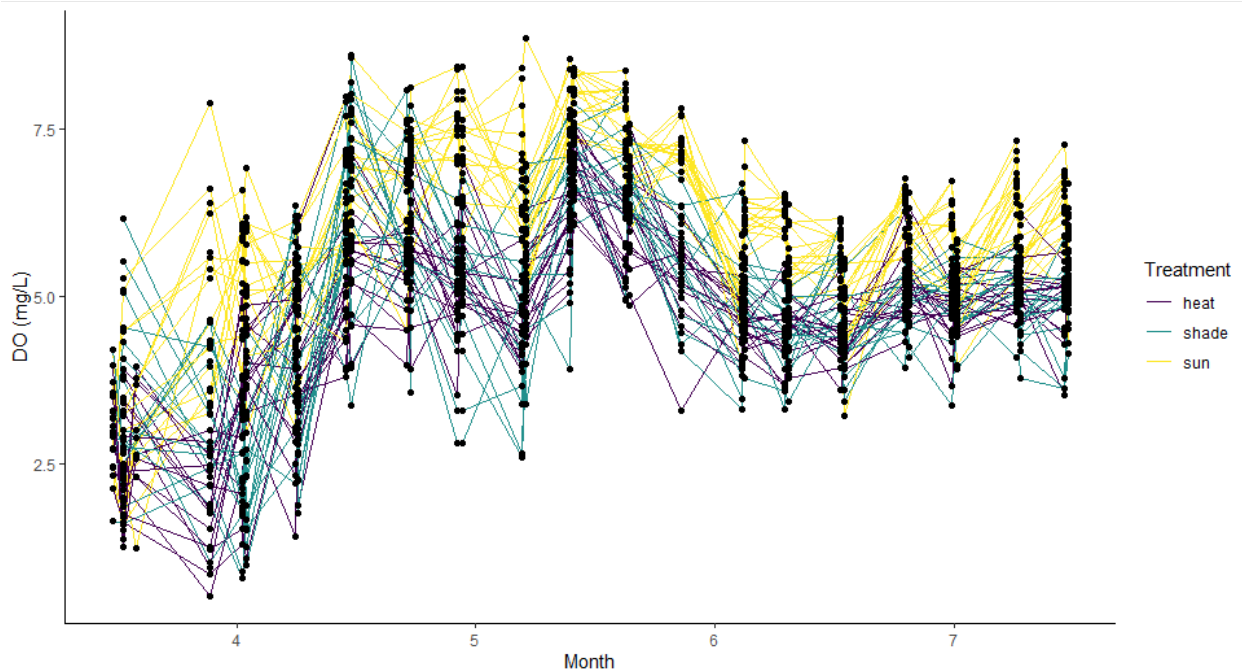


Figure 1: Dissolved oxygen levels over time. Lines connect measurement values for individual tanks, and colors represent the main plot heat/light treatment factor.

The first metamorphosed frog was captured on 13 May 2021. Metamorphosis peaked in early June, then declined until the experiment was ended on 16 August. A total of 777 frogs achieved metamorphosis, out of a total of 960 stocked, before the end of the experiment. The heat/light and vegetation treatments had a significant effect on tank survival to metamorphosis (respectively, $\chi^2 = 20.4$, $df = 2$, $p < 0.001$; $\chi^2 = 14.8$, $df = 1$, $p < 0.001$; Figure 2). Neither the

main effect of structure nor any of the treatment interactions were significant. Tukey’s post hoc comparisons revealed that full sun tanks had significantly higher survival rates ($z \approx 4$, $p < 0.001$), with an average of 93.4% metamorphosis, than the shaded tanks and the shaded and heated tanks, which did not differ from one another ($z = -0.1$, $p = 0.994$), with both treatments having an average of 74.7% survival to metamorphosis. The open canopy vegetation mixture also raised tank survival to an average of 88.3% compared to closed canopy vegetation tanks, with an average survival of 73.5%.

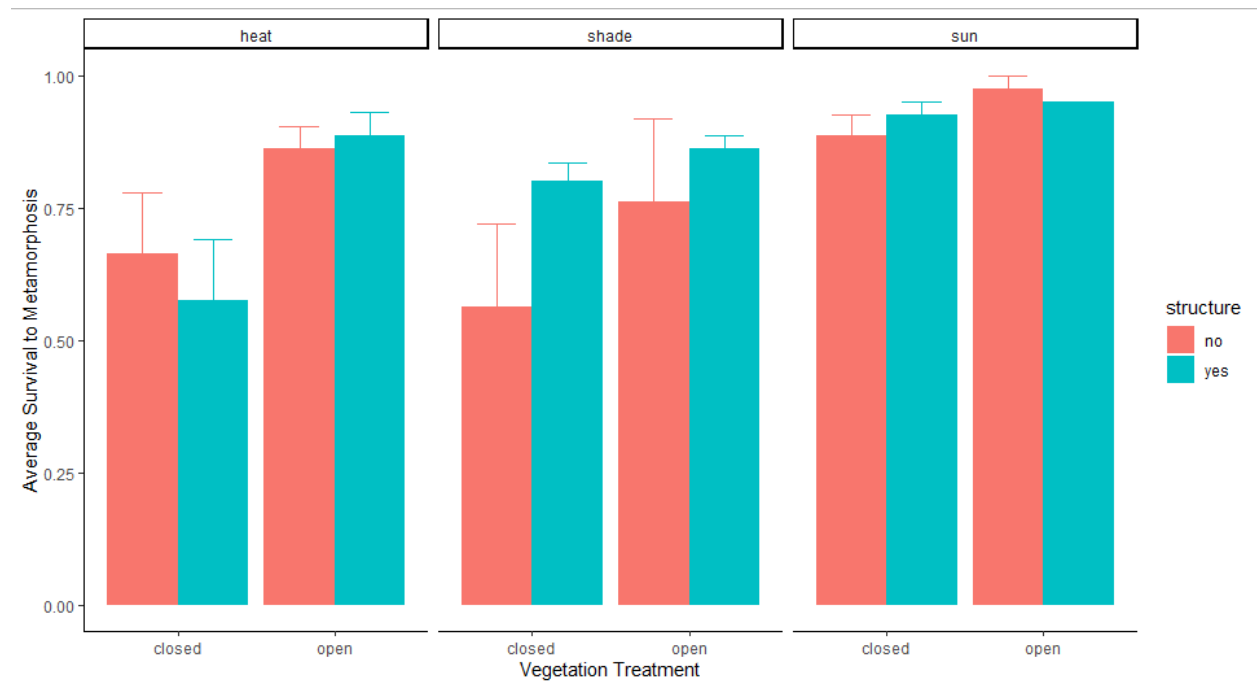


Figure 2: Average tank survival by treatment. Error bars represent standard error.

A significant three-way interaction ($F_{2, 24} = 6.4$, $p = 0.006$) among the three treatments was found in a model of the length of the larval period (measured in days to metamorphosis; Figure 3). This appears to be driven by a two-way interaction between structure and vegetation

that reverses in the heated treatment compared to the shade and sun treatments. In the shade and sun treatments, structure has a negative effect on number of days to metamorphosis in the open canopy vegetation tanks but not in the closed canopy vegetation tanks. For heated tanks, this pattern reverses with a negative effect of structure in the closed canopy vegetation but not the open canopy vegetation tanks. Additionally, the signal of a main effect of both heat/light and vegetation, consistent across treatment combinations, remains detectable statistically and graphically (Figure 3). Frogs achieved metamorphosis in an average of 94.6 days in the sun tanks, 105 in the heat tanks, and 116 in the shade tanks. Grouping by vegetation treatment, frogs achieved metamorphosis in 94.7 days with open canopy vegetation and 116 days with closed canopy vegetation.

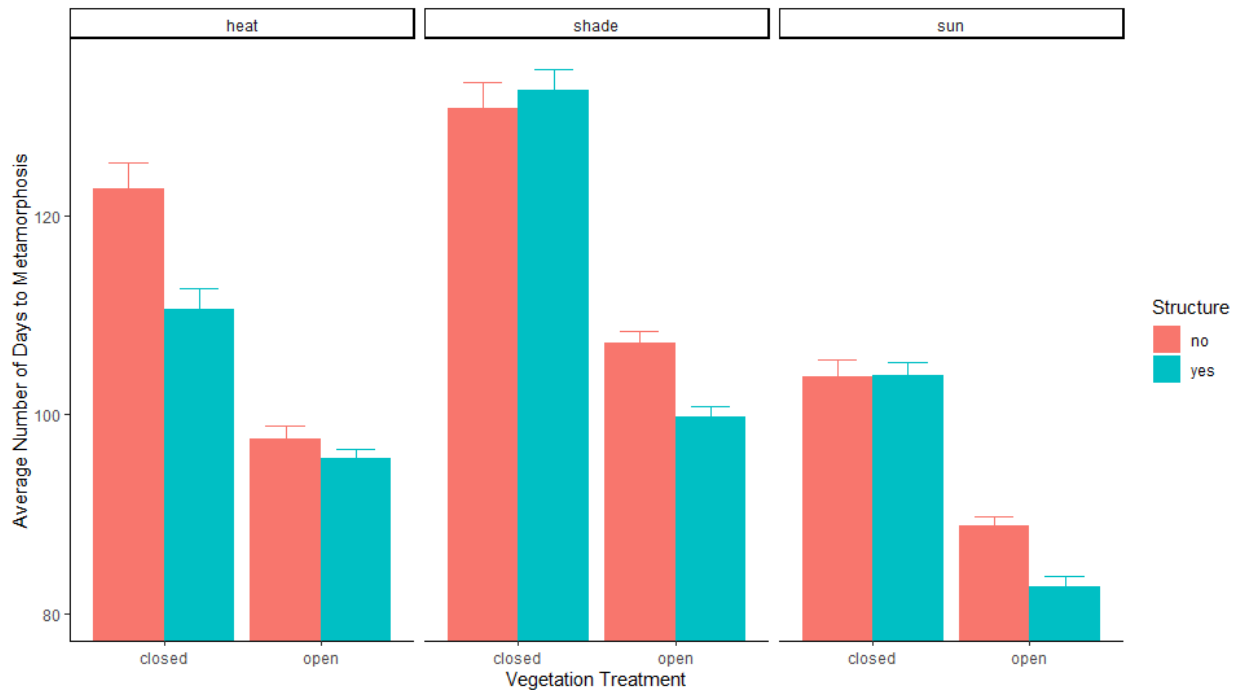


Figure 3: Average number of days to metamorphosis by treatment. Error bars represent standard error. Note the scale of the Y axis does not begin at 0.

Both the heat/light and the vegetation treatments had a significant ($F_{2, 8.2} = 20.6, p < 0.001$; $F_{1, 32.7} = 384.6, p < 0.001$, respectively; Figure 4) effect on frog mass at metamorphosis, as well as snout-vent length at metamorphosis (for heat/light: $F_{2, 8.4} = 15.3, p = 0.0016$; vegetation: $F_{1, 32.1} = 325.1, p < 0.001$; Figure 5). Frogs raised with an open-canopy vegetation mixture weighed almost twice as much as frogs raised in the closed-canopy vegetation (2.94 g vs 1.61 g) and were also significantly longer (32.8 mm vs 27.5 mm SVL). Frogs in the full sun tanks were significantly larger (mean 2.64 g and 31.4 mm SVL) than those in the heated (mean 2.09 g and 29.5 mm; $z \geq 3.9, p < 0.001$) or shaded (mean 2.21 g and 30 mm; $z \geq 3.17, p < 0.004$) tanks, which did not significantly differ from one another for either variable ($z < 2.3, p > 0.056$). The main effect of structure was not significant for either variable ($F_{1, 32.8} = 1.23, p = 0.276$ for mass and $F_{1, 32.3} = 0.7, p = 0.408$ for SVL). No interaction effects were significant for mass or SVL.

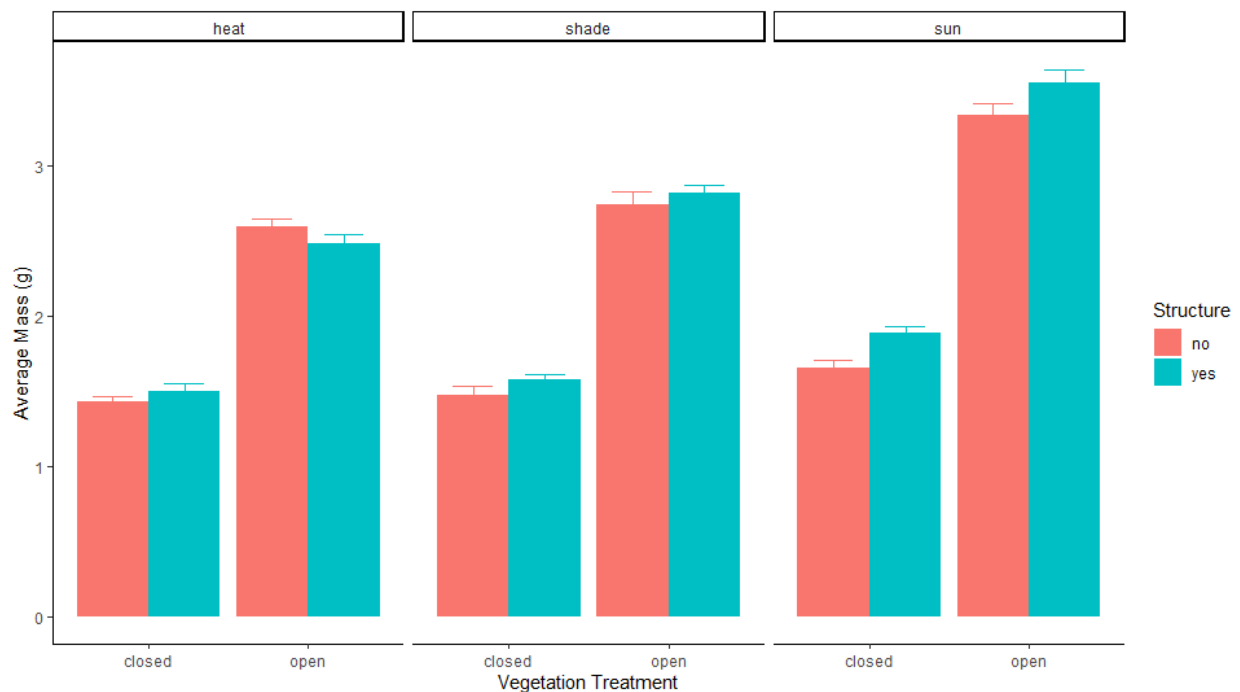


Figure 4: Average mass at metamorphosis, in grams, by treatment. Error bars represent standard error.

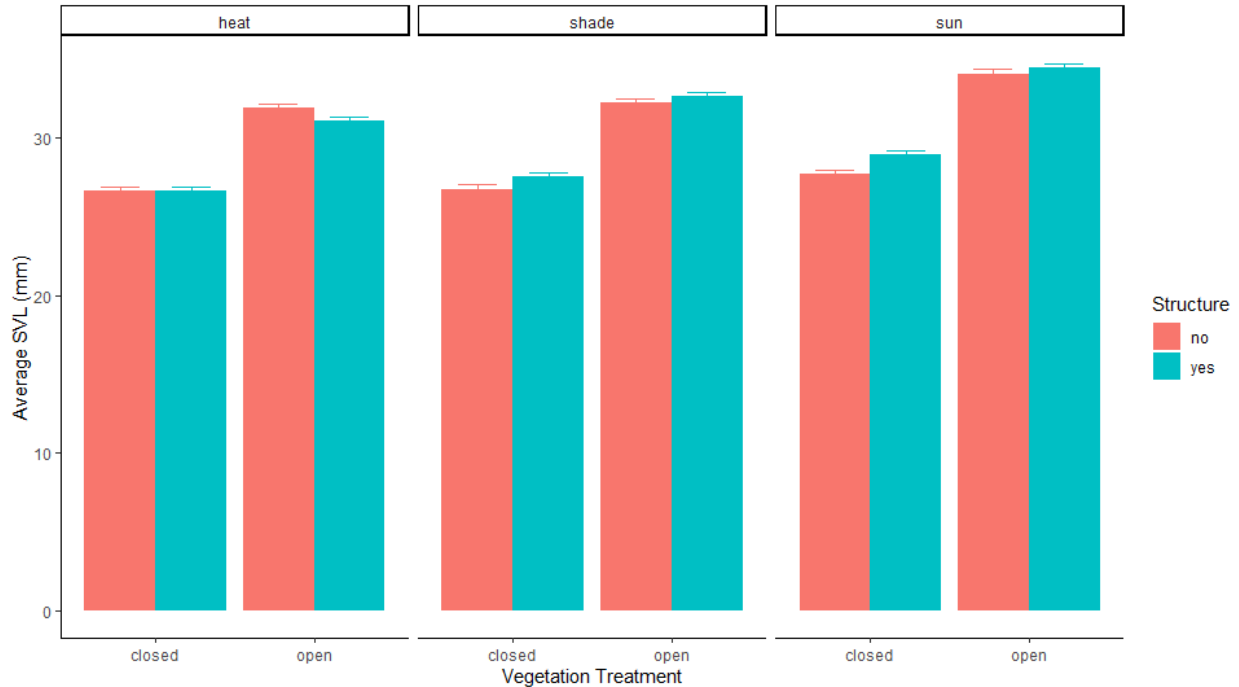


Figure 5: Average length (snout to vent) at metamorphosis, in millimeters, by treatment. Error bars represent standard error.

Discussion

The results of this experiment support previous findings that open canopy conditions maximize the larval performance of dusky gopher frogs (Thurgate 2006, Thurgate & Pechmann 2007) and many other amphibians (Werner & Glennemeier 1999, Skelly et al. 2002). In addition, they provide insight into the relative influences of several different factors. Recently published work (Burrow & Maerz 2021) also indicated that shading and leaf litter from closed canopy ponds reduced larval performance and survival in the Carolina gopher frog, *Rana capito*. This experiment further shows that it is primarily the sunlight, rather than temperature, and the

chemical composition of vegetation, rather than changes in physical structure, that increases tadpole survival, growth, and rate of development in open canopy wetlands.

Although there was a significant difference in temperature among all three heat/light treatments, the magnitude of the difference between the heated and sun tanks was very small in comparison to the difference between either of those two and the shaded tanks. As such, I consider the heated treatment to have successfully mimicked the temperature of the sun tanks. Notably, both the shaded and sun tanks were cooler than the ponds they were meant to mimic, and the average temperature difference between the open canopy and closed canopy ponds was larger for the natural ponds (3.6 °C) than for the tanks. I hypothesize that the cooler temperatures of the tanks results from their sitting on top of the ground, not insulated by the soil, and their smaller volume allowing proportionably more heat transfer to the atmosphere. Both minimum and maximum temperatures were higher in the natural bodies of water, with ranges not greatly differing between ponds and tanks, indicating a consistent shift instead of less cooling at night or heating during the day alone. There were slight differences in variances among treatment and pond categories. Full sun tanks and heated tanks had higher variances, shaded tanks and canopied ponds had lower variances, and the open canopy pond had intermediate variance. This indicates that canopy cover or artificial shading moderated temperature fluctuations. Direct solar irradiation seems to have increased the variance in open canopy water bodies, although less so in the large pond with greater thermal inertia than the smaller experimental tanks (and the heated tanks that mimicked their temperature regime).

Despite the large temperature difference between heated and shaded treatment tanks, average values for survival, mass, and length of *R. sevosia* were identical between the two and significantly smaller than in the sun tanks. This suggests that sunlight is a critical component in

the increased performance of *R. sevosia* larvae under open-canopy conditions. Heat may have been a superfluous factor in this study system; it may be that although the shaded treatment was cooler, it was still more than warm enough in the spring and summer of southern Mississippi to not limit frog growth and development in comparison to other factors. *Rana capito*, the sister taxon of *R. sevosia* and a candidate for federal listing under the Endangered Species Act, ranges as far north as Tennessee and North Carolina. It is possible that in these more northerly localities, shaded ponds or tanks may maintain cooler temperatures, and the heated treatment could have a larger effect and produce results intermediate between the shaded and sun treatments. In this experiment, a slight nonsignificant trend was seen towards higher mass and length in the shaded treatment compared to the heated treatment, along with a nonsignificant trend towards shorter larval period in the heated treatment compared to the shaded treatment. This is consistent with the theory that development and growth respond differentially to environmental factors such as temperature, with tadpoles in warm environments developing faster and reaching metamorphosis at a smaller size (Berven et al. 1979, Smith-Gill & Berven 1979). This effect may have been outweighed by increased algal food sources due to greater photosynthesis in the sunlight treatment of this experiment, such that full sun tanks produced frogs that both developed faster and reached a larger size than those in either of the other two treatments. However, it should be noted that only a nonsignificant positive trend in biofilm growth was found in the sun tanks among heat/light treatments; sunlight may have increased primary production available for tadpole consumption without substantially increasing the standing stock of algal populations.

The vegetation substrate had a marked effect on frog survival, growth, and development, as well as the amount of biofilm accumulating on the microscope slides. These results could potentially be explained by a negative influence of secondary compounds in the tree leaves (Earl

et al. 2011, Burrow & Maerz 2021). Tannins have been shown to directly impair survival in other species of tadpoles (e.g. Earl & Semlitsch 2015), and among plant species with values available in the literature, those used in the closed canopy vegetation treatment have higher phenolics and tannins (Pritchard et al. 1997, Osborne et al. 2007, Jacinthe et al. 2010, McElrone et al. 2010). However, it may also be the case that the vegetation effect on tadpoles was primarily mediated through effects on periphyton and phytoplankton in the mesocosms. Secondary compounds from the closed canopy vegetation may have inhibited primary production (Williams et al. 2008). Also, increased breakdown rates were observed during the experiment in the open canopy vegetation compared to the closed canopy vegetation, consistent with the low lignin content and higher lability of the species utilized (Osborne et al. 2007). This may have allowed a bottom-up increase in primary productivity due to increased nutrients released from decaying plant matter. This would explain both the increased amounts of periphyton as well as the drastically increased size of juveniles from open canopy vegetation tanks, due to increased food resources (Williams et al. 2008).

The physical structure treatment did not increase periphyton levels in the tanks as we measured it and had no effect on tadpole survival or size at metamorphosis. However, the structure may have had an unanticipated benefit, in some cases, on development rate through tadpole behavior. The polypropylene rope did not float to the surface perpendicularly, but at a slight angle. Tadpoles were often observed to rest on the rope near the water's surface, especially on sunny days. This behavior may have been thermoregulatory, enabling tadpoles to easily access the boundary layer of water heated by the sun and speeding their development. If this is true, it remains to be determined why the structure did not influence days to metamorphosis in all treatment combinations. I hypothesize that in shaded and sun tanks, development was food-

limited with closed canopy vegetation, so structure did not decrease time to metamorphosis, but did so in the presence of open canopy vegetation and plenty of food resources. In the heated tanks, the aquarium heater was placed at the bottom of the tank. In that case, the top of the water may no longer be the warmest place in the mesocosm, removing the effect of structure in open canopy vegetation tanks. However, increased heat at the bottom of the tank may have increased release of phenolic compounds from the closed canopy litter, negatively impacting development and increasing time to metamorphosis in those tanks. These hypotheses could be further explored in future studies along with more exact determinations of temperature gradients within mesocosms. Although not tested in this experiment, structure may have an effect in a natural environment containing predators by providing refuge and decreasing predation success on tadpoles (Holomuzki 1986, Chuang et al. 2019).

The results of this experiment provide some evidence for the relative influences of different factors. As expected, sunlight had the greatest impact on survival to metamorphosis, with open canopy vegetation having a smaller effect. Surprisingly, there were no significant differences for any response variables between heated and shaded tanks; it appears that all of the benefits of the sun tanks come from sunlight rather than increased temperature. On the other hand, the herbaceous vegetation treatment, likely moderated through food resources, had a larger positive effect on frog mass and SVL than did the heat/light treatment. Frogs raised with open canopy vegetation were markedly larger, a result that has meaningful fitness consequences in the form of increased fecundity, reduced time to sexual maturity, and potentially higher survival (Smith 1987, Semlitsch et al. 1988, Cabrera-Guzmán et al. 2013, Earl & Whiteman 2015). The effect sizes of heat/light and vegetation were nearly equal for days to metamorphosis, when comparing estimated means for sun and shaded tanks with those for open canopy and closed

canopy vegetation. Finally, physical structure had an effect only on the number of days to metamorphosis under some treatment combinations. Nevertheless, even small reductions in the length of the larval period could be important for this species. The ephemeral ponds that gopher frogs breed in are subject to drying, sometimes resulting in cohort failure (Richter et al. 2003).

Management Implications

This experiment clarifies our understanding of habitat requirements for the larval stage of *Rana sevosa* and suggests specific actions to be taken for habitat restoration. While the most salient feature of open canopy breeding ponds may be the lack of trees, and this is indeed important, it is critical in habitat restoration that the leaf litter be removed and herbaceous plant growth encouraged. Following mechanical removal of trees from a pond basin, prescribed fire can be a tremendously useful tool to restore the pond to conditions suitable for gopher frogs, through burning away leaf litter harmful to tadpole development and favoring the establishment of native wetland grasses (Martin & Kirkman 2009). Dusky gopher frog tadpoles have been headstarted in captivity since 2002 (USFWS 2015), and the same conditions of sunlight and open canopy vegetation should be applied to these rearing practices. The physical structure mimicked by polypropylene rope may provide a slight benefit by reducing time to metamorphosis, while being easy to implement and very unlikely to harm animals. For managers raising frogs in headstarting facilities, reduced time to metamorphosis could reduce financial and labor costs by shortening project length, provided that there are no tradeoffs in size at metamorphosis which might reduce project success. Applying these results with a combined strategy of headstarting and habitat restoration may contribute further to recovery of the dusky gopher frog, while simultaneously providing benefits to many other amphibian species.

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CHAPTER TWO: MOVEMENT ECOLOGY OF NATURAL AND CAPTIVE-RAISED
DUSKY GOPHER FROG (*RANA SEVOSA*) JUVENILES

Introduction

The recovery of endangered species relies on a broad range of tactics, in part because many species are threatened in more than one way (Lawler et al. 2002, Leu et al. 2019). An important aspect of a successful recovery strategy is to evaluate its assumptions and assess the efficacy of management actions (Canessa et al. 2019). Headstarting and captive breeding are commonly used tactics to help augment the populations of rare and endangered reptiles and amphibians (e.g. Burke 2015; Harding et al. 2016). These captive-raised animals may be released at some point during their lives into the wild. Wild juveniles may be raised in captivity for a time and released after reaching a certain size or surpassing a high-mortality life stage (i.e. headstarting), or populations may be maintained indefinitely as a captive assurance, with or without release of offspring into the wild. If animals are released into the wild, it is desirable that they behave as naturally as possible in order to avoid any negative impacts to the species and ensure an appropriate return on investment in the form of wild population growth or attenuation of population decline. If differences in behavior are found, then a reassessment of program methods and protocol modifications may be necessary. Potential modifications may include training (e.g. Crane & Mathis 2011), soft releases, where an animal is gradually introduced into the environment using an enclosure (e.g. Tuberville et al. 2005), or alteration of release timing (e.g. Canessa et al. 2019).

Dusky gopher frogs, *Rana sevosa*, are a federally endangered species native to the southeastern United States. Formerly ranging from Louisiana to Alabama, only one natural

population and several populations created through human intervention remain in southern Mississippi. Frogs from the remaining natural population, Glen's Pond, naturally colonized a nearby wetland, Pony Ranch Pond, after management actions improved the habitat at Pony Ranch. A portion of nearly every egg mass laid has been collected from Glen's Pond since 2002 for use in a headstarting project (USFWS 2015). Larvae hatched in captivity are subsequently raised in tanks until the tadpoles metamorphose into juvenile frogs. These animals have been used to establish captive breeding populations at zoos and reintroduced populations in nearby areas of southern Mississippi. Previous work has examined the movements of dusky gopher frogs at Glen's Pond using radiotelemetry, both in adults and juveniles (Richter et al. 2001, Tupy 2012). However, no direct comparison has been made between the movements made by headstarted individuals and completely natural individuals. In this study, I used radiotelemetry to examine the post-release movements and behavior of newly metamorphosed dusky gopher frogs to look for differences between captive-reared and natural individuals. I performed this work at Pony Ranch Pond, where telemetry had not yet been conducted with this species, in order to expand our understanding of dusky gopher frog behavior in diverse habitats.

Methods

Juvenile frogs were fitted with custom ATS T15 radio transmitters (Advanced Telemetry Systems, Isanti, Minnesota, US). Each transmitter weighed 0.15 grams and was attached to the frog with a belt consisting of small glass beads strung on 0.5 mm diameter elastic thread. The thread was chosen to ensure that it would eventually rot and fall off without harming the animal if the individual could not be recovered to remove the transmitter. The belts weighed 0.2 g each, bringing the total transmitter and belt combination to a final weight of 0.35 g. I used frogs with a

minimum mass of 3.50 g at metamorphosis in order to keep the attached transmitter at or under 10% of the animal's body weight (Blomquist & Hunter 2007, Madison et al. 2010).

I decided a priori to track 20 juvenile dusky gopher frogs, with a target sample size of 10 natural and 10 tank-reared juveniles. However, due to supply chain issues related to the ongoing COVID-19 pandemic, I was unable to obtain the equipment until late June 2021. This limited the ability to capture natural juveniles for radiotelemetry due to timing of metamorphosis in the spring and summer of 2021, and as a result the sample size for that group was only three individuals. The remaining 17 transmitters were deployed on tank-reared juveniles. Tank-reared dusky gopher frogs were sourced from ongoing studies and headstart programs. Six tank-reared frogs were taken from the experiment described in Chapter I. Seven frogs were taken from routine headstarting at the Harrison Experimental Forest. These animals were housed in 1000 L cattle watering tanks, in full sunlight, with a vegetation substrate of *Panicum hemitomon* (maidencane) or a mixture of *Panicum hemitomon* and *Scirpus cyperinus* (woolgrass), and fed algae wafers ad libitum. The remaining 4 animals were sourced from a headstarting project at Camp Shelby run by The Nature Conservancy, raised under similar conditions to those just described.

Timing of transmitter attachment and release differed between groups. Natural frogs were captured nocturnally via a drift fence encircling Pony Ranch Pond (Harrison County, MS, US) as part of a long-term demography project. They were marked with visual implant alpha-numeric tags (Northwest Marine Technology, Anacortes, WA, US), measured from snout to vent, weighed, and if of sufficient mass, fitted with a transmitter belt and released immediately on the opposite side of the fence. The distance from the edge of the pond to the encircling fence varied from zero to approximately 10 m depending on location and current water level. Processing took

less than 15 minutes per frog. Tank-reared frogs were marked with visual implant alpha-numeric tags, fitted with a belt beforehand in the lab, and released at the edge of the pond after nightfall, in line with procedures for normal release of headstarted individuals. Release locations were chosen to represent locations around the pond where natural juveniles tended to emerge.

The first individuals were released to be tracked on July 1, 2021. Transmitters were attached to natural individuals when captured, and when natural recruitment ended for the season, the remaining transmitters were deployed on tank-reared juveniles, with the last individual released on August 5, 2021. Individuals were tracked at least once every 24 hours, in the daytime, until the transmitter was recovered or the signal was lost. On days 3 and 6 of tracking every individual, the animal was captured to examine the fit of the belt and look for evidence of skin abrasion. If any abrasions or other harm was identified, the transmitter was removed and the animal immediately released. On each tracking occasion, the frog's location was recorded with a handheld GPS, along with the microhabitat it was utilizing or appeared to be utilizing and any relevant notes. Tracking continued as long as the batteries in the transmitters lasted, or the animal slipped the transmitter or was otherwise lost. The animal was considered to have reached a permanent destination if it remained in the same underground refugium for at least 7 days and remained there until the end of the study.

Data were analyzed using R v. 4.1.2 (R Core Team 2020) and the following packages: tidyverse (Wickham et al. 2020), lme4 (Bates et al. 2015), lmerTest (Kuznetsova et al. 2017), car (Fox & Weisberg 2019), and DHARMA (Hartig 2021); geographical data were mapped and analyzed in ArcMap v. 10.8 (Esri, Redlands, CA, US). The total distance moved by each individual and number of days tracked were compared between groups using Welch's t test. Correlations between movement and mass were created using simple linear regression.

Microhabitat use and average daily movement were compared between groups using (generalized) linear mixed models (GLMMs) with individual as a random effect. Microhabitat was encoded as a dummy variable and analyzed with a binomial error distribution, with p-values calculated by likelihood ratio tests. Average daily movements were log-transformed and analyzed with a linear mixed model (LMM), and p-values reported from the model are the result of F tests with Satterthwaite approximations. All alpha levels were set a priori to 0.05.

Results

Frogs were tracked an average of 22 days (range 1-73 days). The average number of days that headstarted frogs remained in the pond before emigrating was 0.65 (range: 0 to 6). Of the 20 juveniles that we attached transmitters to, 2 found a permanent refuge, 3 were confirmed or suspected to fall to predation, 9 slipped their transmitter belt before reaching a permanent refuge, and 6 had their transmitter die before they reached a permanent refuge, or their fate was otherwise unknown. The identity of one predator was confirmed to be an eastern ribbon snake, *Thamnophis sauritus*; the snake was tracked, visually confirmed, and the transmitter was later found in snake urate and feces. The other predators were never confirmed visually. All of the natural frogs were tracked for at least 20 days (and up to 73) before two of them slipped out of their belts, and the signal was lost before the third found a permanent refuge.

The average total distance traveled by the frogs was 306 m, and average daily distance was 14.1 m. The total distance estimates reflect the sum of daily movements, i.e. the distances that frogs traveled, not their final distances from the pond. These values are likely overestimates due to errors in signal triangulation and possibly movements representing gopher frog predators carrying transmitters with them. For the two frogs that reached a permanent refugium, straight line distances between permanent burrow sites and the center of the breeding pond basin were

approximately 185 m and 120 m (Figure 6). Natural and tank-reared juveniles did not statistically differ in the total distance traveled ($t_{3,9} = 0.65$, $p = 0.553$) or average distance moved each day ($F_{1, 11.3} = 0.72$, $p = 0.414$). There was no significant relationship between mass at metamorphosis and total distance traveled ($F_{1, 14} = 4.29$, $p = 0.0573$, $R^2 = 0.18$). Comparing the two most commonly used microhabitats during migration, grass tufts and tree litter, comprising 80% of observations, it was found that natural juveniles used grass as cover significantly more than tank-reared juveniles ($\chi^2 = 5.09$, $df = 1$, $p = 0.024$; Table 1). There was no significant relationship between mass at metamorphosis and the number of days that frogs were successfully tracked ($F_{1, 14} = 3.03$, $p = 0.103$). Origin (tanks vs natural) did not have a significant effect on the number of days tracked ($F_{1, 18} = 3.04$, $p = 0.098$; mean \pm standard error: 19.1 ± 3.96 days for tank-reared animals, 39 ± 17 days for natural animals).

Permanent Refuge Locations



Figure 6: Permanent refuge locations of the two juveniles who survived and were tracked to an underground refugium. Pony Ranch Pond is visible in the center.

Table 1: Observational counts of microhabitat use by natural and tank-reared dusky gopher frog juveniles. Generalized linear mixed model results indicate a significant difference between natural and tank-reared individuals ($\chi^2 = 5.09$, $df = 1$, $p = 0.024$).

	GRASS	TREE LITTER
NATURAL	75	7
TANK-REARED	131	82

Discussion

The results of this study indicate that there are few differences in the movement patterns of headstarted dusky gopher frogs compared to natural dusky gopher frogs. Unfortunately, our sample size was limited due to logistical problems related to the COVID-19 pandemic. It would be advantageous to capitalize on future natural recruitment to follow-up on these results and increase the sample size of the study. Nevertheless, this evidence supports the hypothesis that headstarted dusky gopher frogs are just as vagile and move just as far as wild juveniles.

Additionally, although the release protocols differed between natural and headstarted juveniles, the emigration of headstarted juveniles was only slightly delayed. Naturally emigrating juveniles were intercepted during migration with a drift fence and immediately released on the other side. Headstarted juveniles were released at the water's edge just after dark, and typically left the pond the same or the following evening. This provides evidence that the method of releasing headstarted animals has minimal impact on the timing of emigration, and as noted, natural and tank-reared frogs moved in similar ways once they left the pond. The distances moved in this

study are intermediate among those reported for gopher frogs previously (Richter et al. 2001, Roznik & Johnson 2009, Humphries & Sisson 2012, Tupy 2012, Roznik & Reichling 2021).

Although differences in movements were not found, a surprising dependence between origin and microhabitat was found. Natural frogs emigrating from the pond almost exclusively used tufts of grass as refuge during migration. In contrast, headstarted individuals used fallen tree litter, primarily pine needles, as cover in almost 40% of observations. It is possible that this represents a form of habitat imprinting in gopher frogs which may depend on the environment during the larval stage or immediately after metamorphosis. Newly metamorphosed frogs may alter their behavior during migration due to olfactory imprinting on their natal pond during development (Ogurtsov 2004), and tadpoles of some ranids have been shown to alter behavior after exposure to olfactory cues in the embryonic stage (Hepper & Waldman 1992). Habitat selection in other taxa is also known to sometimes be affected by the natal environment (reviewed in Davis & Stamps 2004). Natural frogs may have been able to shelter under grass clumps in the shallows of the pond before, during, and immediately after metamorphosis, producing a preference for that type of cover. Headstarted frogs, on the other hand, were kept under moist paper towels in the lab prior to release; all types of natural cover may have been novel to them. Future work may address whether this association results in survival differences or differences in the characteristics of selected final refugia. If meaningful differences are found, potential mitigation strategies could be implemented, such as giving headstarted juveniles holding enclosures with natural substrates in order to acclimate them to appropriate cover types before release.

Mass at metamorphosis was not found to be related to distances traveled, contrary to expectations, but in accordance with prior studies in gopher frogs and related species (Richter et

al. 2001, Blomquist & Hunter 2007). The number of days tracked, a crude proxy for survivorship over the course of the study, did not have a significant relationship with mass at metamorphosis either. The frogs used in this study were the largest available; only 10% of juvenile frogs in the study described in Chapter 1 met the minimum mass requirements for radiotelemetry. It is possible that if smaller frogs were able to be tracked without encumbering their movement and behavior, an effect of mass would be found. While this study was not able to address any effects of the transmitter belt on frog behavior directly, it is likely that movement and behavior was minimally affected (Blomquist & Hunter 2007, Rowley & Alford 2007). In fact, the current study reports a lower predation rate than has been reported previously in juvenile gopher frogs (Roznik & Johnson 2009, Roznik & Reichling 2021), although the transmitter belt exceeding 10% of frog mass in some previously published work (Roznik & Reichling 2021) may have hindered the individual's escape capabilities compared to the current study.

The most common outcome in this study was the transmitter slipping off the frog's waist, a commonly reported outcome with transmitter attachment of this type (Muths 2003, Madison et al. 2010). There is a tradeoff between attaching waist belts loose enough that slippage is a possibility and attaching them tight enough to increase the likelihood of abrasion or other detrimental effects on the animal (Madison et al. 2010). I opted for the first option in order to avoid accidental deaths or injuries. All instances wherein a transmitter belt was found alone with no other evidence were classed as a slippage; however, it is impossible to rule out the possibility that these frogs succumbed to predation or another fate, leaving only the belt behind. In one case, a transmitter signal was triangulated to a small area with many foraging invasive red fire ants, *Solenopsis invicta*, in the immediate vicinity, although no visual on either the frog or belt was obtained. The following day, no ants were present, and the belt was found with no trace of frog

remains. It is possible that this represented predation or scavenging by ants (as observed in Roznik & Reichling 2021), but it was classed as a slippage due to lack of direct evidence. Three cases were classified as suspected or confirmed predation. One of these involved a direct visual of the predator and the later finding of the transmitter in feces. The other two involved real-time movements during tracking that were impossible to account for by the movement of a juvenile gopher frog, usually ending in the signal moving up to 100 m into a burned-out stump hole. In both cases, this was repeated on multiple days, ending in different stump holes, and verified by multiple observers. Based on our observations, I hypothesize that both of these cases represent predation by the racer, *Coluber constrictor*, a snake that readily predated juvenile gopher frogs (Roznik & Johnson 2009, W.J. Humphries, personal communication).

Taken together, the results of this study provide preliminary evidence that the behaviors of headstarted and naturally raised dusky gopher frogs are similar, and that headstarting is not deleteriously affecting the survival or movements of emigrating juveniles after their release at the pond. A connection between microhabitat use during migration and origin of the individual was found, and if replicated and strengthened through further data collection, future studies should also address the underlying reasons for and effects of this association. Managers should continue to headstart this endangered amphibian as a viable method of augmenting current populations and establishing new populations.

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