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Habitat Selection and Site Fidelity of Cope's Gray Treefrog (*Hyla chrysoscelis*) at the Aquatic-Terrestrial Ecotone

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Abstract.—**Wetlands are essential breeding sites for many amphibians. The importance of terrestrial habitat for many aquatic-breeding amphibian species is well known, although often understudied and understated. This study examined the recapture rates, habitat use, and site fidelity of Cope's Gray Treefrogs (*Hyla chrysoscelis*) within and surrounding a wetland for 15 months. Using visible implant elastomer and visible implant alpha tags, we tracked individuals as they used a grid of 110 PVC pipes as refugia. PVC pipe refugia allow treefrogs to be sampled when not actively calling or breeding. We captured 82 individual frogs a total of 141 times (59 recaptures). Treefrogs occupied pipes every month except during winter (December, January, and February). Recapture rates decreased during the breeding season (May, June, and July). Preferred pipes were in terrestrial habitat or close to trees instead of in aquatic habitat devoid of trees. Treefrogs displayed high site fidelity; only three frogs were recaptured in pipes different from those in which they were originally captured. Our results suggest that *H. chrysoscelis* select terrestrial habitat adjacent to wetlands and have high site fidelity, which could have important implications for the conservation of treefrogs and other wetland-breeding amphibians.**

Wetlands provide critical habitat for a wide variety of amphibian species. However, many amphibian species also require terrestrial habitat adjacent to wetlands to complete their life cycle (Semlitsch, 1998). Understanding basic ecological requirements is integral for developing effective management and conservation strategies for amphibians and the ecosystems in which they reside (Pechmann et al., 1991). Although research focusing on rare or threatened species is important, common species play important roles in ecosystems, and their demise could have serious consequences to overall ecosystem integrity (Price et al., 2006). Additionally, studies performed on common species are important because they can provide new insight into the ecology of that species and rare species that share similar ecological traits.

Cope's Gray Treefrog (*Hyla chrysoscelis*) is an abundant hylid frog found throughout much of the eastern United States and all of North Carolina (Wright and Wright, 1949; Cline, 2005). This species breeds in a variety of habitats and is often found calling along the edges of ephemeral wetlands, ponds, and roadside ditches (Ritke et al., 1990). As a result of its abundance, broad distribution, and easily detectable call, numerous studies have documented the natural history and dispersal mechanisms of this amphibian (Zweifel, 1970; Ritke et al., 1990, 1992). Ritke et al. (1992) and Johnson and Semlitsch (2003) found *H. chrysoscelis* and the closely related *Hyla versicolor* prefer a single breeding site and are unlikely to disperse to other breeding ponds within or between breeding seasons. Both studies stressed the need to preserve upland habitat surrounding a wetland to ensure that juveniles and adults have sufficient habitat. However, little data exist on upland habitat use by either *H. chrysoscelis* or *H. versicolor.*

Treefrogs are highly reclusive and well camouflaged, making them difficult to study outside of the breeding season and within the terrestrial habitat (Wright and Wright, 1949; Cline, 2005). Previous studies have employed various methods of studying these animals, such as opportunistic searching during periods of high reproductive activity (Ritke et al., 1992), creating artificial pools near breeding sites to encourage colonization (Resetarits and Wilbur, 1991), or performing frog-calling surveys (Zweifel, 1970). These methods are unlikely to allow observations of treefrogs during nonbreeding seasons. One relatively novel method for sampling treefrogs is the use of polyvinyl chloride (PVC) pipe refugia (Moulton et al., 1996; Boughton et al., 2000). This is a passive sampling technique that facilitates the location of treefrogs during the daytime or at times of year when they are not actively breeding (Wright and Wright, 1949; Cline, 2005), thus providing a method to reliably and systematically sample these frogs in aquatic and terrestrial habitats (Johnson, 2005).

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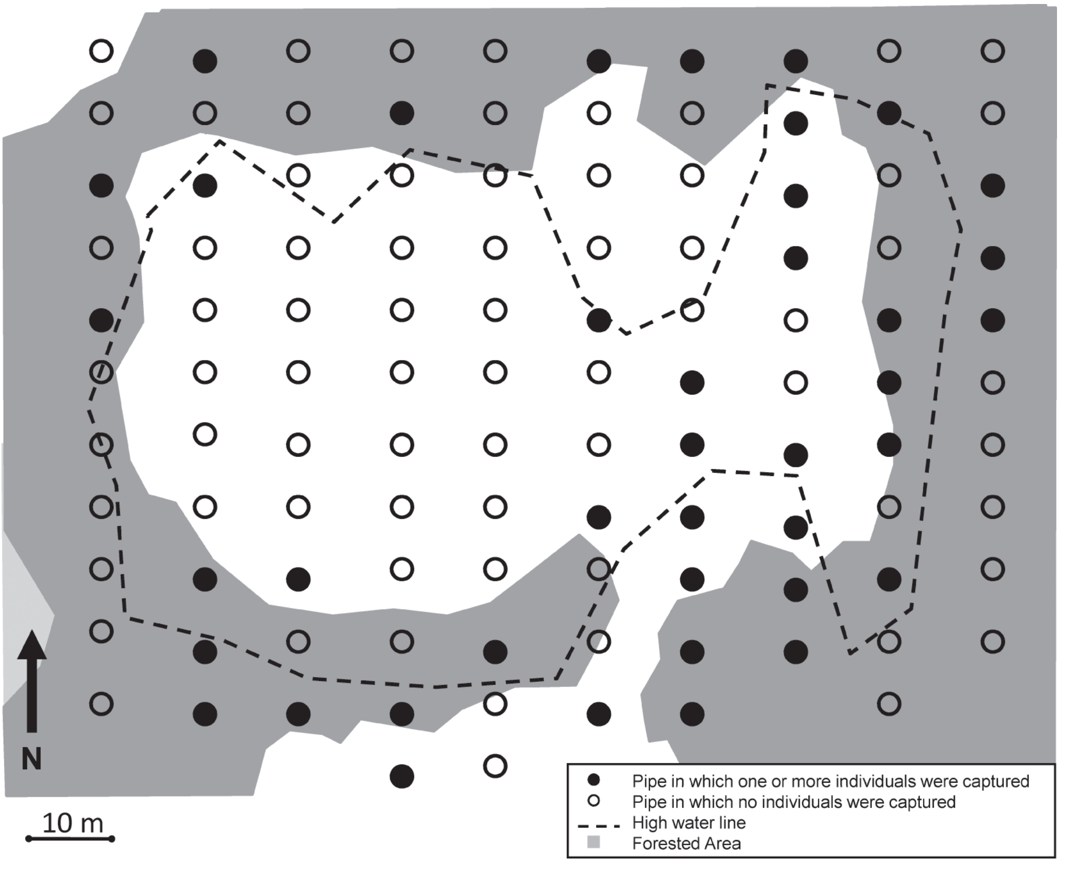

FIG. 1. Diagram of wetland showing pipe array and indicating pipes occupied by treefrogs; 110 pipes were spaced at 90° angles, 10 m apart. Note that occupied pipes were primarily in terrestrial habitat, close to the trees.

In this study, we used PVC pipe refugia to study the ecology of *H. chrysoscelis* within and around a wetland for 15 months (September 2005 to November 2006). Specifically, we examined (1) capture and recapture rates throughout the study, (2) habitat selection of *H. chrysoscelis* throughout a 15-month period, (3) movement and site fidelity of individuals, and (4) pipe occupancy during the breeding and nonbreeding seasons.

MATERIALS AND METHODS

We conducted this study within and around an ephemeral wetland at Cowan's Ford Wildlife Refuge in Huntersville, North Carolina. Cowan's Ford Wildlife Refuge is a 270-ha area surrounded on three sides by the Catawba River. Our study was confined to a 0.93-ha wetland and adjacent terrestrial habitat within the refuge. Vegetation surrounding the wetland consisted mostly of deciduous and pine forest, with some areas dominated by grasses and shrubs. We placed 110 PVC pipes in a 100 × 110 m grid over the wetland and surrounding woodland habitat; pipes were arranged with 10 m spacing between them at 90° angles (Fig. 1). Pipes had an inside diameter of 3.8 cm, were approximately 1.5 m tall, and were driven approximately 15 cm into the ground.

PVC pipes were set out in the wetland in July 2005. All pipes were checked for the presence of frogs by looking into the pipe from the top once every two weeks from September 2005 through November 2006—a total of 15 months. Sampling was sporadic during July and August 2005; therefore, we only report data from September 2005 until November 2006.

To coerce frogs out of a pipe, we removed the pipe from the ground and gently shook the pipe, allowing the frog to fall into our hands or a plastic bag (Boughton et al., 2000). The date of last rainfall was determined for each sampling period, and we recorded the sampling periods during which the wetland was dry. Rainfall was recorded hourly at McGuire Nuclear Research Station (Duke Power) approximately 14.5 km from the study site. Temperature to the nearest

1°C was recorded during each sampling period. Daily minimum temperature (recorded hourly) was obtained from the Davidson College Biology Department's weather station in Mecklenburg County, approximately 27.4 km northeast of the study site.

We individually marked adult and subadult frogs and gave all recently metamorphosed frogs a cohort mark. Individual marks were created with visible alphanumeric tags (VI alpha; Visible Implant [VI] Tags Alphanumeric Format, Northwest Marine Technology, Shaw Island, WA; Buckmeier and Irwin, 2000). These tags were inserted under the skin of each frog in the tibial region of the left hind leg. Cohort marks were created by injecting visible implant elastomer (VIE; Visible Implant Elastomer Tags, Northwest Marine Technology, Shaw Island, WA; Marold, 2001) under the skin within the tibial region of the right hind leg. Additionally, we photographed the back patterns of each frog to aid in identification (Kurashina et al., 2003). We determined the sex and approximate age of each frog. Age was determined by SVL and presence of discernible back pattern; frogs were categorized as follows: adults, SVLs of 30 mm or more; subadults, SVLs of more than 20 mm but less than 30 mm; and recently metamorphosed frogs, SVLs of 20 mm or less (Wright and Wright, 1949; Harding, 1997). Subadult frogs were those frogs with discernible back patterns and smaller SVLs than the lower cutoff for adult frogs as determined by previous studies (Wright and Wright, 1949; Harding, 1997). Frogs with SVLs less than 20 mm did not have discernible back patterns and, therefore, were considered recent metamorphs. Sex was determined by examining the throat coloration of each animal (males had darker throats than females, even during the nonbreeding season). Frogs were processed on site and returned to the pipe in which they had been found within 60 min.

We considered the peak breeding season to be May, June, and July (Dorcas et al., 2007). The shortest distance of each pipe from the high-water line of the wetland was measured to the nearest meter (Fig. 1). The distance of each pipe to the nearest tree trunk was measured to the nearest centimeter (Fig. 1). Trees were considered to be any vegetation with a trunk diameter greater than 4.8 cm at breast height (15-cm circumference).

To evaluate habitat selection, we compared the habitat surrounding pipes in which frogs were found to the distribution of pipes in and around the wetland. We separated pipes into groups based on distance to the high water line of the wetland so that there were similar numbers of pipes in each group. We used a Chi-squared analysis to compare number of pipes at each distance range with number of frogs captured at each distance range to determine whether frogs were more likely to be captured at certain distances. We also compared the distribution of frogs in pipes between the breeding and nonbreeding seasons. We separated pipes into groups based on distance to the nearest tree so that there were similar numbers of pipes in each group. We used a Chi-squared analysis to compare number of pipes at each distance range with number of frogs captured at each distance range to determine whether frogs were more likely in pipes closer to trees. We used a regression analysis to determine whether the number of frogs captured was affected by either monthly rainfall or rainfall three days prior to the sampling date. We also used a Chi-squared analysis to determine whether capture rates of males were different than females. We used an alpha of 0.05 to determine significance for all tests.

Results

We captured 82 individual frogs, a total of 141 times (59 recaptures). Of the frogs captured, 61 were adults, 16 were subadults, and five were classified as metamorphs. Of the 61 adults captured, 54 were male and seven were female. Males were captured more often than females ($X^2_1 = 36.20$, $P < 0.05$). The most often recaptured individual was a subadult male that was captured nine times from 15 October 2005 to 28 October 2006. Most frogs ($N = 56$) were captured only once.

We captured frogs throughout the 15-month sampling period except for: December 2005 and January, February, and November 2006 (Fig. 2). Forty of the 110 pipes were occupied at least once by a treefrog. The greatest number of frogs ($N = 29$) was captured in October 2005 (Fig. 2). The highest relative recapture rates (number of unmarked vs. marked frogs) occurred during November 2005, March 2006, September 2006, and October 2006, directly before and after the winter months (Fig. 2). We observed a decrease in recapture rates in May, June, and July 2006 (Fig. 2).

The lowest daily minimum air temperature at which a frog was found was 0.3°C in March 2006. Daytime air temperature at the time of this capture was 12.7°C. The lowest monthly rainfall occurred during September 2005, in which only 4.1 mm of rain fell (Fig. 2). Mean monthly rainfall between June 2005 and November 2006 was 99.1 mm. Monthly rainfall was not related to the number of captures or recaptures per month ($y = 0.08x + 8.98$, $P = 0.96$, $R^2 =$

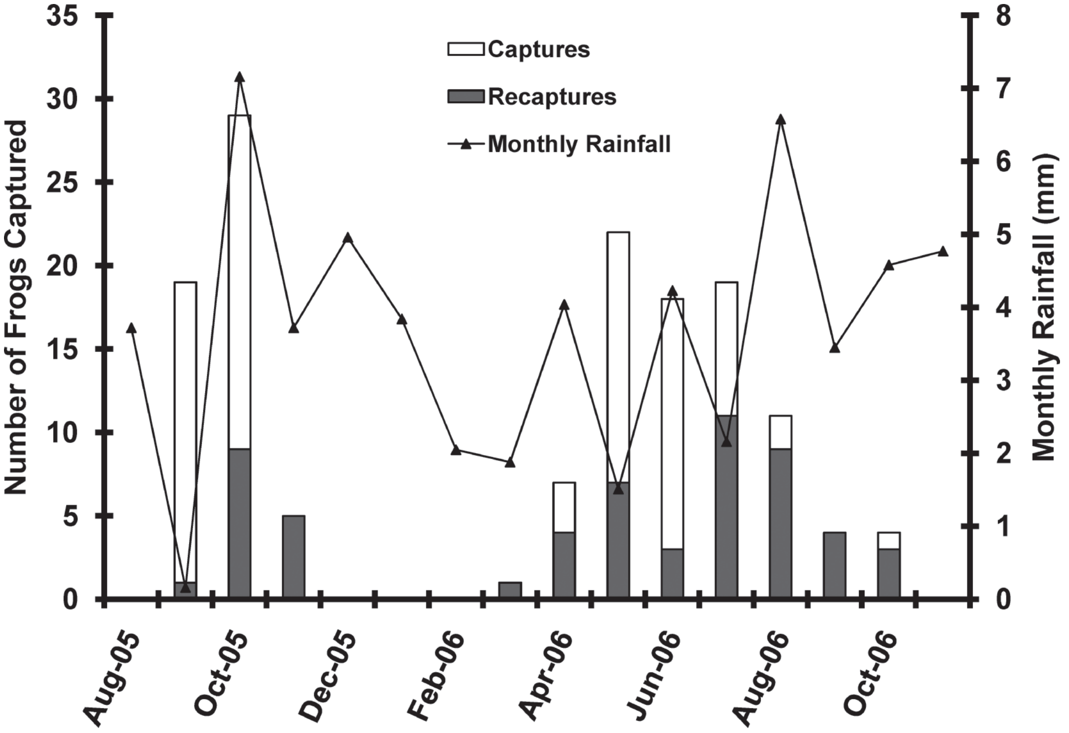

FIG. 2. Number of captures and recaptures by month and year and total monthly rainfall. Note that no frogs were captured during the winter months, and a low relative number of recaptures occurred at the beginning of the breeding season.

0.0002, $N = 15$). We also found no relationship between pipe occupancy and cumulative rainfall three days prior to the sampling date ($y = -1.12x + 5.83$, $P = 0.82$, $R^2 = 0.0022$, $N = 25$). The wetland was completely dry during sampling periods in November 2005, July 2006, and August 2006.

Frogs selected pipes in terrestrial habitat or wetland habitat near the high-water line more frequently than wetland habitat far from the high-water line ($X^2_9 = 18.48$, $P = 0.03$; Fig. 3). During the nonbreeding season, we captured the greatest number of frogs in pipes 12–21 m into the terrestrial habitat from the high-water line of the wetland (Fig. 3). During the peak breeding season, the greatest number of frogs was captured 10–11.5 m into the terrestrial habitat from the high-water line (Fig. 3). Frogs were captured at a distance greater than 10 m into the wetland only during the breeding season (Fig. 3). However, the distribution of frogs throughout the wetland during the peak breeding season did not differ significantly from that during the remainder of the year ($X^2_9 = 13.16$, $P = 0.16$; Fig. 3).

Frogs selected pipes that were closer to trees than those further away from trees ($X^2_9 = 24.05$, $P = 0.004$; Fig. 4). Only one frog was captured in the 27 pipes that were farther than 4.5 m away from a tree. Most frogs were captured in pipes 1.6–2.0 m from a tree (18 frogs, Fig. 4). Of the 82 individual frogs captured, 26 were recaptured at least once. Of the 26 recaptured, 11.5% (three males) were found in pipes different from those in which they were originally captured.

DISCUSSION

Hyla chrysoscelis preferred pipes located along the edges of the wetland and close to trees, suggesting that the terrestrial habitat surrounding wetlands is an important component of the ecology of this species. We found that individuals had high site-fidelity and often returned to specific refugia.

We did not find a strong correlation between air temperature or rainfall and the number of frogs captured during each sampling period. Previous studies on anurans have found that environmental variables, such as temperature, relative humidity, and wetness of vegetation, were positively correlated with anuran activity (Bellis, 1962; Cree, 1989). Johnson (2005) found that higher humidity and rainfall decreased number of *H. versicolor* captures in artificial

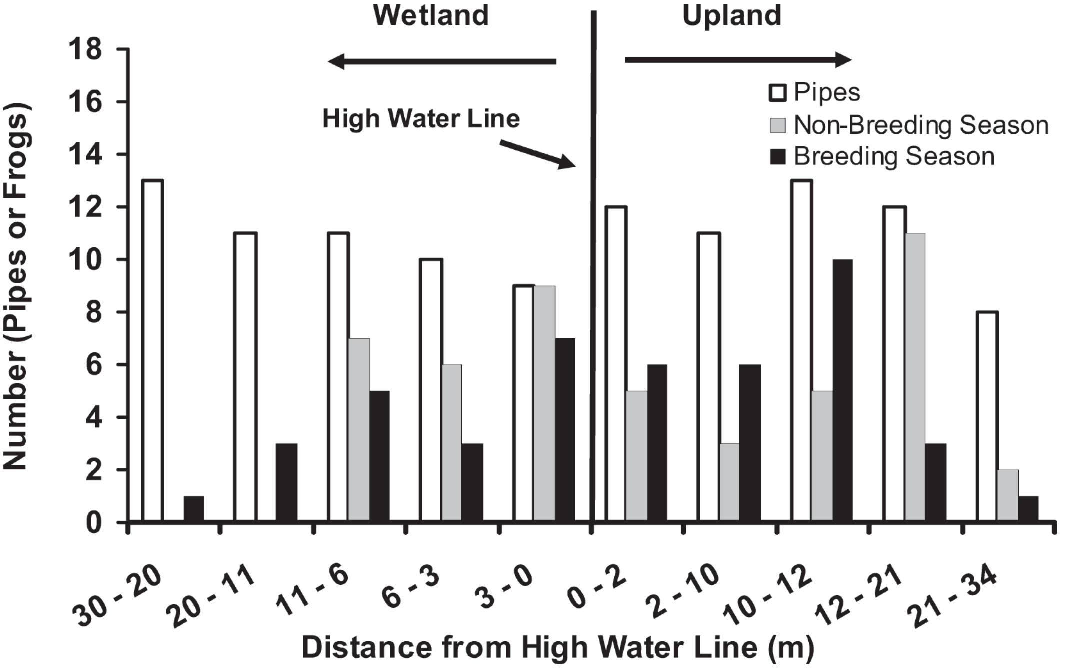

FIG. 3. Habitat selection in the breeding and nonbreeding seasons determined by distance to high-water line. The distribution of pipes (open bars) and the distribution of frogs (gray and black bars) were statistically different ($P = 0.03$). Frogs preferred pipes in upland habitats or the edge of the wetland. The distribution of frogs was not statistically different between the breeding and nonbreeding seasons ($P = 0.16$).

refugia, whereas higher temperatures increased captures, both of which suggest that pipes may serve as protection during hot, dry periods. However, we did not observe this relationship, which may be because our study encompassed only 15 months. Frogs were found in pipes throughout the year except during winter months, suggesting that occupancy of the wetland and surrounding terrestrial habitat may be related to foraging or

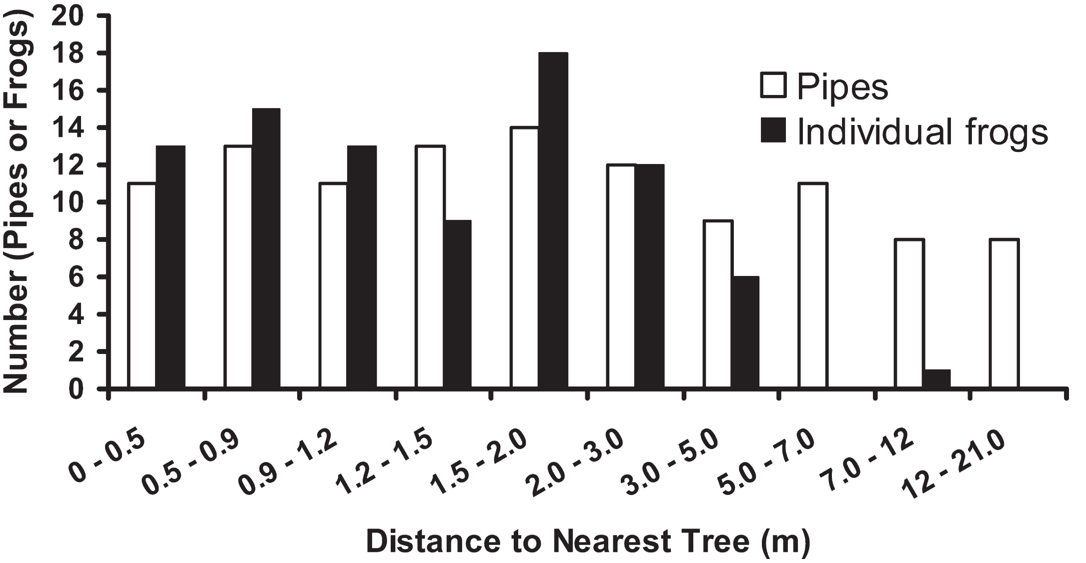

FIG. 4. Habitat selection determined by distance to nearest tree. Note that few frogs were captured in pipes farther than 5 m away from a tree ($P = 0.004$).

habitat preferences and not solely to reproductive activities.

Relative numbers of recaptures varied considerably throughout our study. In May and June 2006, the relative number of recaptures decreased as the breeding season began. The increase in unmarked frogs during May and June of 2006 may have been a result of an influx of frogs moving to the wetland from upland habitat for breeding. A decrease in unmarked frogs in March, April, August, September, and October suggested that frogs captured in pipes at these times resided close to the wetland for longer periods of time and, therefore, were more likely to be recaptured.

We found that relative numbers of recaptures were high and that many frogs were captured in the same pipes immediately before and after winter, suggesting that frogs captured at these times may have overwintered near their pipes. Studies show that *H. chrysoscelis*, like *H. versicolor*, are freeze tolerant (Schmid, 1982; Costanzo et al., 1992). However, previous studies also indicate that *H. chrysoscelis* prefer to overwinter within insulated refugia, such as under logs and leaf litter and inside hollow trees (Johnson, 2005; Trauth et al., 2006), presumably to avoid freezing temperatures. Because winter temperatures in North Carolina are frequently below freezing, frogs may prefer insulated habitat within which to overwinter. It appears unlikely that a PVC pipe provided suitable winter refugia for *H. chrysoscelis*. However, insulation may have been sufficient under leaf litter near or at the bottom of the pipe, and it is possible that frogs in our study overwintered there. Johnson (2005) found that *H. versicolor* overwintered close to their foraging grounds, supporting our conclusion that frogs captured often in pipes likely overwintered near these same pipes.

We found considerably more males than females in our study, indicating that (1) there may be fewer females residing in and around this wetland, (2) PVC pipes may be a better sampling method for male frogs, or (3) male treefrogs may remain at the wetland for longer periods of time. Previous studies have indicated that male and female frogs may use different factors to choose a calling or oviposition site (Resetarits and Wilbur, 1989). For example, female *H. chrysoscelis* strongly avoid breeding sites containing *Ambystoma maculatum* larvae, whereas male frogs do not (Resetarits and Wilbur, 1991). Because *A. maculatum* larvae were present at our study site (SEP, unpubl. data), fewer female frogs may have chosen this site to breed. Johnson (2005) found that female *H. versicolor* spent considerably less time at a breeding site than male frogs and, after mating, would return to upland habitat an average of 80 m away from the breeding site.

Habitat Selection.—Distance to the high-water line of the wetland and proximity to trees were important factors in determining occupancy of pipes by treefrogs. Frogs were found more often in forested areas that were rarely inundated than they were in pipes more than 10 m into the wetland from the high-water line, suggesting that forested habitat surrounding wetlands is important for this species (Johnson and Semlitsch, 2003; Johnson, 2005). Because water was rarely at the high-water line, frogs that were captured within the delineated wetland boundary were often captured in pipes not surrounded by water. Even when the wetland was completely dry, frogs preferred pipes close to or beyond the delineated wetland boundary. Frog distributions did not vary between the breeding and nonbreeding seasons, indicating that terrestrial habitat is important habitat for frogs even during the breeding season. Although our study did not find a significant difference in treefrog distributions between the breeding and nonbreeding seasons, Johnson (2005) extended artificial refugia 200 m into upland habitat and found that distributions were shifted toward breeding sites during the breeding season. Pipes in our study did not extend far enough into upland habitat to distinguish this trend. Johnson and Semlitsch (2003) suggest a minimum 60 m of core terrestrial habitat surrounding a wetland for *H. versicolor*; however, they found that the greatest number of oviposition instances occurred in ponds 15 m or closer to the main breeding pond. Our findings also indicate that preservation of terrestrial habitat close to the wetland is critical for *H. chrysoscelis*.

Site Fidelity.—We found a high rate of site fidelity; only three frogs were recaptured in pipes other than those in which they were originally captured. High breeding-site fidelity has been demonstrated in *H. chrysoscelis* both within and between breeding seasons (Ritke et al., 1992). We found that even within a breeding site, treefrogs used the same refugia repeatedly. Often, several months passed between first capture and recapture, but in most cases, treefrogs were captured in the pipe of their previous capture. A study by Freda and Gonzalez (1986) followed the daily movements of *Hyla andersonii* using radioactive tags and found that frogs could move up to 102 m in a day and would often move more than 30 m in a day. Of the three frogs that moved in our study, two were recaptured more than once, and both moved back to, or in the direction of, the pipe of their original capture.

Implications of high site fidelity in treefrogs include possible negative effects of forced emigration. Previous studies have found that short distance translocation of anurans induces stress, disorientation, and, in most cases, a decreased ability to breed and forage (Oldham, 1967; Matthews, 2003). *Rana clamitans* has been found to show strict site fidelity both to a breeding site and to specific locations within a breeding site (Oldham, 1967). Therefore, disruption of habitat via development or other anthropogenic causes may have considerable negative effects on a treefrog's ability to orient itself to needed refugia to survive.

Conclusions.—Similar to other studies of amphibians and turtles (Burke and Gibbons, 1995; Semlitsch, 1998; Joyal et al., 2001), we found that terrestrial habitat adjacent to wetlands is important for *H. chrysoscelis*. Because most treefrogs we captured were male, terrestrial habitat extending even farther into upland habitat from wetlands could be critical for female *H. chrysoscelis* (Johnson, 2005). High site fidelity exhibited by most treefrogs suggests that frogs have acute directional abilities and that forced emigration as a result of habitat destruction may seriously upset the ability of these frogs to breed, forage, or find suitable refugia. Conservation and management strategies for treefrogs should include the conservation of upland, wooded habitat adjacent to breeding sites and habitat alteration close to the wetland should be avoided or minimized.

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