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Use of PVC Pipe Refugia as a Sampling Technique for Hylid Treefrogs

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ABSTRACT.—We used retreats made from white polyvinyl chloride (PVC) pipes to capture hylids and determined how pipe design and placement influenced the frequency with which hylids used pipes as retreats. Pipes were hung vertically in trees on three sites in north-central Florida. Pipes were checked twice weekly for 10 mo during which 788 individuals of four species (*Hyla squirella, H. cinerea, H. femoralis* and *H. gratiosa*) were captured, with 2658 recaptures. Retreats on hardwoods were used significantly more than retreats on pines, and retreats hung at 2 m and 4 m aboveground were used significantly more than retreats at 0 m. Long and T shaped retreats (both 60 cm long) were used significantly more than short (30 cm) retreats. Retreats capped on the bottom with water inside and 3.81 cm in diameter were used more frequently than retreats of the same diameter that were either capped on the top or not capped and retreats 1.75 cm in diameter with no cap.

INTRODUCTION

In the United States field studies of amphibians that are dependent on wetlands for breeding have been undertaken almost exclusively at the breeding sites (Duellman and Trueb, 1985). Consequently, the life history of most amphibians away from breeding ponds is not well known (McComb and Noble, 1981; Murphy, 1993; Dodd, 1996). Reports of North American hylids in the nonbreeding season are anecdotal accounts of a single or a few individuals. These reports include discovery of hylids at various depths in the soil (Blanchard, 1933; Carr, 1940; Neill, 1952; Ritke and Babb, 1991), under tree bark and logs (Holbrook, 1842; Dickerson, 1906; Neill, 1952; Einem and Ober, 1956), in the axils of bromeliads (*Tillandsia* species) and cabbage palmetto (*Sabal palmetto*) (Einem and Ober, 1956; Lee, 1969) and in knothole cavities of trees (McComb and Noble, 1981; Ritke and Babb, 1991; Meshaka, 1996). In the southeast U.S., treefrogs are also found in and around human habitation (Neill, 1951; Goin, 1958; Meshaka, 1996), in nest boxes (Dickerson, 1906; Van Hyning, 1933; Dawson, 1967; Muncy and Burbank, 1975; McComb and Noble, 1981) and inside pipes driven into the ground for survey or plot markers (pers. obs.).

The ability of treefrogs to evade capture both by climbing out of pitfall traps and moving over drift fences (Gibbons and Bennett, 1974; Jones, 1986; Dodd, 1991) and to evade detection in densely vegetated habitat has led to the development of new capture techniques (Murphy, 1993; Greenberg *et al.*, 1994; Moulton *et al.*, 1996). Successful new techniques include a modified drift fence made from clear plastic sheeting (Murphy, 1993) and artificial refugia such as polyvinyl chloride (PVC) pipe, bamboo, tin cans and wood nest boxes (Goin, 1958; McComb and Noble, 1981; Stewart and Pough, 1983; Buchanan, 1988; Phelps, 1993; O'Neill, 1995; Meshaka, 1996; Moulton *et al.*, 1996). PVC pipe retreats have been

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shown to be highly effective in the study of treefrogs (Buchanan, 1988; Phelps, 1993; O'Neill, 1995; Moulton *et al.*, 1996).

Although artificial refugia are effective for sampling treefrogs, in previous studies capture success has varied both among age classes (Goin, 1958) and among species (Phelps, 1993; O'Neill, 1995). Differential capture success in studies using multiple pipe designs or locations (McComb and Noble, 1981; Lohoefener and Wolfe, 1984; Buchanan, 1988; Townsend, 1989; Phelps, 1993) indicates that treefrogs are selective in their use of refugia.

In this study we sought to design pipe-retreats which would attract all treefrog species and age classes present on each site. We chose pipe designs and locations based on factors which would likely influence treefrog use of retreats, including prevention of desiccation, prevention of predation and the likelihood of encountering a natural retreat on a particular tree. We also assessed the influence of meteorological and site conditions on capture success.

STUDY SITE AND METHODS

This study was conducted on the Katharine Ordway Preserve-Carl Swisher Memorial Sanctuary, Putnam County, Florida (latitude 29°41′N, longitude 82°00′W). The Ordway Preserve consists of approximately 3750 ha of rolling sandhills, wetlands, lakes and ponds. Historically this area was managed as a private fishing reserve, although some areas were farmed for livestock, oranges and other crops.

We designed two separate studies at three locations. The main study was conducted on two sites; Tucker upland, a mixed evergreen-deciduous hammock and Tucker Lake, a bottomland lake margin. Common tree species on these sites included slash pine (*Pinus elliottii*), laurel oak (*Quercus hemisphaerica*) and sand live oak (*Q. geminata*) in the upland, and the same species plus sweet gum (*Liquidambar styraciflua*) at the lake. A second study was conducted at Porter Hole, a hardwood hammock of laurel oak, sand live oak and sweet gum surrounding an upland sinkhole pond.

Retreats were constructed from white PVC pipe. At the Tucker sites, the five retreat design variables used were site, tree type, pipe shape, pipe height and configuration. All pipes at the Tucker sites were hung in trees. At Porter Hole, pipes of four shapes were hung in trees and additional pipes were driven vertically into the ground. Retreats in trees were hung vertically by drilling a small hole near the top end of each pipe and hanging it on a nail. Retreats were hung on hardwoods (*Quercus geminata*, *Q. hemisphaerica* and *Liquidambar styraciflua*) and on pines (*Pinus elliottii*) that were 18 cm or greater in diameter at breast height (dbh). At Tucker Lake all trees were within 25 m of the lake margin. At Porter Hole trees were within 55 m of the pond edge. All retreats were shaded except those installed in the ground around the edge of the pond at Porter Hole.

All pipes were 3.81 cm inside diameter (ID) except where otherwise noted. At the Tucker sites the three retreat shapes used were short pipes (30 cm), long pipes (60 cm) and long pipes (60 cm) with a T fitting on the top. Seventy-two trees were selected at each site, 36 pines and 36 hardwoods. Retreats were hung on all selected trees so that the lower opening was at each of three heights, at the base of the tree (0 m), at 2 m and at 4 m. Retreats were hung on each tree in one of two configurations: with one pipe at each height or with three pipes at each height. In the latter configuration, one long, one short and one T shaped pipe were hung side by side at each height. We hypothesized that treefrogs would not randomly select trees to ascend and consequently, the number of captures per tree also would not be random. Therefore, an individual frog could select both the retreat height and shape on these trees. Each possible combination of retreat variables was represented equally on both sites (lake and upland) and both tree types (pines and hardwoods).

Preliminary results from the Tucker sites (July–September 1995) were used to determine retreat design and placement at Porter Hole. All retreats at Porter Hole were hung vertically at 2 m on hardwood trees. Four 60 cm long pipes were hung on each of 25 trees. One pipe was open on both ends, the second had a PVC cap at the top end and the third had a cap on the bottom end and was filled with 5–15 cm of water. A small drainage hole (0.5 cm) was drilled approximately 15 cm from the bottom of these pipes to control the water level. The fourth retreat type was 1.75 cm (ID) and was open on both ends. Finally, 32 pipes—5 cm (ID) and 90 cm long were driven upright into the ground at random locations throughout the site.

All retreats were checked twice weekly on nonconsecutive days between 0800 and 1800 h from July 1995 through May 1996 at the Tucker sites and from October 1995 through May 1996 at Porter Hole. The Tucker sites and Porter Hole were not necessarily checked on the same days. Each retreat was inspected and cleaned of debris. Frogs were removed with a sponge plunger—a cut kitchen sponge glued to the end of a 60 cm dowel. A plastic bag was wrapped around one end of the retreat and the frog was pushed gently into it with the plunger. Retreats hung at 4 m were inspected from the ground. When a frog was present, the retreat was removed with a hooked steel pole that could be inserted into a small hole drilled in the pipe.

Captured frogs were marked by toe clipping and recaptured frogs were reclipped as necessary. Frogs which escaped before inspection for toe clips were classified as unknown. Snout-urostyle length (SUL) was measured in millimeters with a clear plastic ruler and frogs were classified as juvenile or adult. Individuals were classified as juvenile when snout-vent length was $<$23 mm for *Hyla squirella*, $<$37 mm for *H. cinerea*, $<$50 mm for *H. gratiosa* or $<$24 mm for *H. femoralis* (Wright and Wright, 1949). All treefrogs were replaced inside retreats. Measurements of dbh were taken for all trees and distance from water was measured for trees at Tucker Lake and Porter Hole.

Continuous meteorological data were obtained from a 12 m tower approximately 6.5 km from the study site. Data were collected and recorded by a datalogger (model CR10, Campbell Scientific, Logan, Utah) and meteorological sensors. Air temperature data were collected using a thermocouple. Rainfall data were collected with a tipping bucket rain gage (Texas Electronics). Rainfall data were integrated over 10 min intervals and temperature data were averaged over 10 min intervals for data logging. On each Tucker trap day rainfall since last trap day was measured with a bucket rain gauge (All-Weather, See-Thru Rain Gauge).

Capture data were analyzed for "all species" and for *Hyla squirella* and *H. cinerea* separately. Capture rates were not high enough for *H. gratiosa* ($n = 46$) or *H. femoralis* ($n = 42$) to analyze data for these species separately. Analysis of variance (ANOVA) was used to detect significant differences between number of captures in retreats of each design and location. Captures and recaptures were both included in the analysis because the response of interest was the capacity of each retreat to attract frogs. Recaptures did not alter the capacity of the retreats to attract new frogs, so the response of interest was not adversely affected; that is, each capture was independent of the previous capture. Equal numbers of pipes of each design assured that under the null hypothesis, captures and recaptures were equally likely in all pipes. Fisher's least significant difference test then was used to determine significant differences between the levels of each significant variable. Total number of captures in each retreat were square root transformed in order to normalize large variances.

The effect of meteorological conditions, dbh and lake distance on capture rate were examined using separate regression analyses. In these tests count data were log-linear transformed to normalize the variance. Statistical analyses were performed using the SAS program (SAS Institute, Cary, North Carolina)

RESULTS

Of 3441 total captures, there were 788 individuals of four species, with 2401 recaptures (252 unknown). *Hyla squirella* were captured most frequently (n = 2660 captures) followed by *H. cinerea* (n = 691 captures), *H. gratiosa* (n = 48 captures) and *H. femoralis* (n = 42 captures). Frogs were found in retreats on each site within one week of pipe installation. Peak hylid capture occurred in August (average = 40/d) and October (43.2/d) at the Tucker sites and in November (53/d) at Porter Hole (Fig. 1). Excluding the first (incomplete) month on each site, minimum captures occurred in December at both sites (Tucker = 5.5/d, Porter Hole = 28/d).

At the Tucker sites the number of hylids captured varied with the retreat height, shape and tree type but not configuration (Table 1). Frogs were found in retreats hung at 2 m and 4 m significantly more often than in retreats at 0 m (Table 2). However, frog captures were not significantly different between retreats at 2 m and 4 m. *Hyla squirella* was captured in long retreats and T shaped retreats significantly more than in short retreats (Table 2). *Hyla cinerea* was captured in long retreats significantly more than in T shaped and short retreats (Table 2). Frogs were captured significantly more in retreats on hardwoods than in retreats on pines (Table 2). Only one significant difference in capture results occurred between the upland and lake sites. At the upland site *H. squirella* was captured in retreats on hardwoods significantly more than in retreats on pines (df = 1, t = 6.450, P =0.0001), whereas at the lake site no significant difference was detected between hardwood and pine captures (df = 1, t = 0.994, P =0.3239).

At Porter Hole there were significant differences between the number of hylids captured in retreats of each shape and between the number of hylids captured in tree (78%) vs. ground (22%) retreats (Tables 1, 2). Of captures made in trees, retreats with water were used significantly more than all other retreats (Table 2). Eighty-four percent of *Hyla squirella* captures were in retreats in trees; however, *H. cinerea* was captured equally in tree and ground retreats (Table 2).

Both juvenile and adult frogs were captured in retreats on all sites. At the Tucker sites a small proportion of all captures were juveniles (*Hyla squirella*—2.4%, *H. cinerea*—1.6%, *H. gratiosa*—27.5%, *H. femoralis*—8.3%). Porter Hole captures of juveniles were similarly small for *H. squirella* (0.9%) and *H. cinerea* (3.2%). However, all captures of *H. gratiosa* (n = 6) and none of *H. femoralis* (n = 17) were juveniles at Porter Hole.

Although retreats in 93% of the study trees on the Tucker sites and 100% of those at Porter Hole were used at least once by frogs, certain individual trees were used considerably more than others. Some of the variation in total captures on each tree can be attributed to one or a few individual frogs which used certain trees as habitual retreats. Variation may be further explained by the significant relationship between dbh and total captures on each tree at the Tucker sites; total captures increased with an increase in dbh (df = 1, r^2 = 0.027, P = 0.049). Distance to the lake was significant only for *Hyla cinerea* at Tucker Lake; captures decreased with distance from the lake (df = 1, r^2 = 0.067, P = 0.029).

Similar patterns were observed between meteorological conditions and capture rate at the Tucker sites and at Porter Hole. Total daily captures were positively correlated with mean daily temperature at both sites (df = 1, r^2 = 0.245, P = 0.0001 and df = 1, r^2 = 0.338, P = 0.0001, respectively). When analyzed separately by season, temperature was significant in summer and winter at the Tucker sites (df = 1, r^2 = 0.525, P = 0.008 and df = 1, r^2 = 0.526, P = 0.0002, respectively) and in winter only at Porter Hole (df = 1, r^2 =

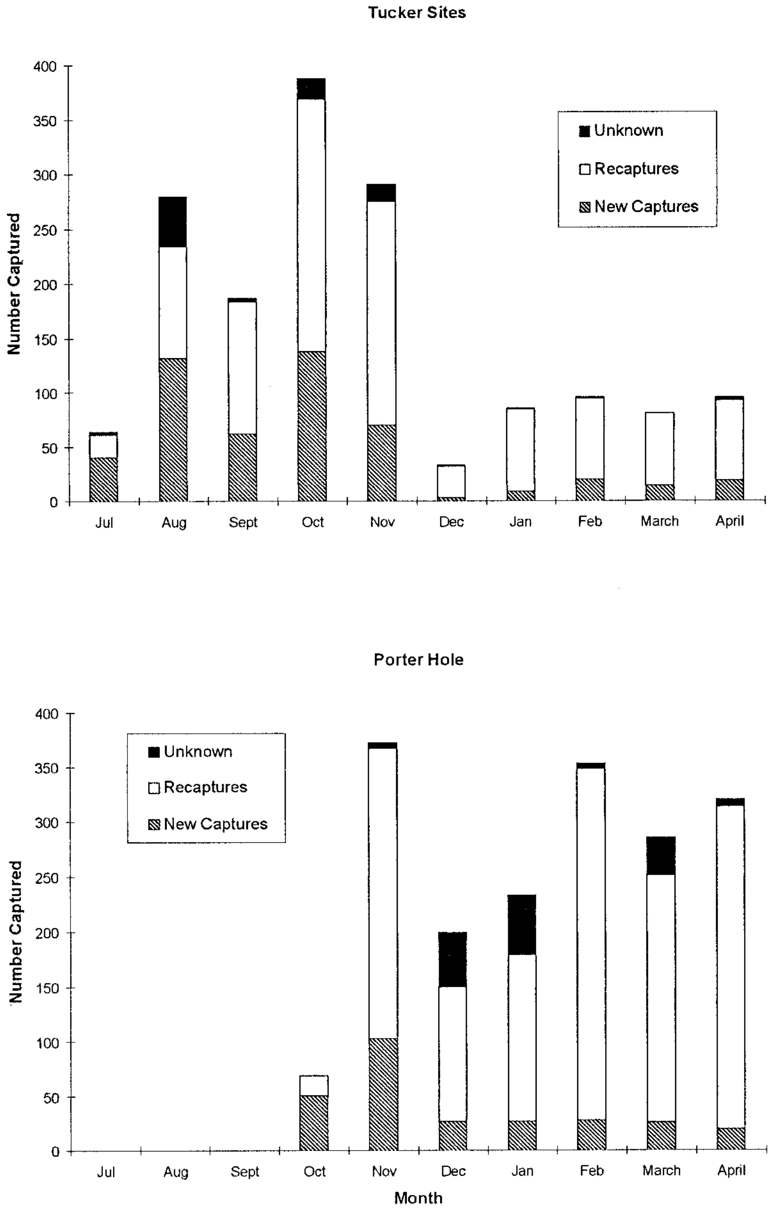

FIG. 1.—Total number of new captures, recaptures and unknowns of all hylid frogs in each month at the Tucker sites (above) and Porter Hole (below)

0.780, $P = 0.0001$). Total daily captures did not correspond well with rainfall in the last 24 h, 48 h, or total rainfall since last trap day at any site. Detailed results of the relationship between capture rates and meteorological conditions are reported elsewhere (Boughton, 1997).

TABLE 1.—ANOVA examining the main effects of PVC retreat design and placement. The first five rows of data refer to design variables at the main study site (Tucker Lake and upland) and the last row refers to the supplementary field site (Porter Hole)

	Degrees of freedom	All species		*H. squirella*		*H. cinerea*	
		F	P	F	P	F	P
Tucker site	1	26.02	0.0001	64.49	0.0001	38.31	0.0001
Tree type	1	34.97	0.0001	24.04	0.0001	31.43	0.0001
Height	2	22.39	0.0001	12.96	0.0001	20.53	0.0001
Configuration	1	0.86	0.3590	0.05	0.8248	1.99	0.1646
Shape	2	34.23	0.0001	40.46	0.0001	13.96	0.0001
Porter shape	4	19.97	0.0001	17.84	0.0001	7.62	0.0001

DISCUSSION

The difficulties encountered in the study of hylids away from breeding ponds have resulted in a paucity of information on the nonbreeding season habits of treefrogs (McComb and Noble, 1981; Dodd, 1996). However, the protocol described here offers a highly effective method for studying hylids in all seasons. In this study 788 individual hylid treefrogs used PVC pipes hung in trees and placed in the ground as diurnal retreats in all months (July to May). Recapture data indicate that individual treefrogs adopted pipes as diurnal refugia for periods from approximately one day to ten months.

Significant differences between the number of captures in retreats of each shape and location in this study indicate strong preferences in these frogs' choice of refugia. In general, frogs selected elevated, long, pipe-retreats, 3.81 cm (ID), hung in hardwood trees and retreats with water. Studies employing multiple retreat designs or placement have shown similar results. In Puerto Rico 66% of *Eleutherodactylus coqui* nests were found in elevated (100–150 cm) bamboo retreats compared with retreats located at the base of trees (Townsend, 1989). In Louisiana, where PVC pipes were wired to palmetto fronds, 83% of *Hyla squirella* captures were in long PVC pipes (8 cm) with 17% in short pipes (4.5 cm) (Buchanan, 1988). In North Carolina, where PVC pipes were driven upright into the ground, 98% of hylid captures were in small diameter pipes (2 cm ID) and only 2% in large pipes (5 cm ID) (Moulton *et al.*, 1996). These studies support the hypothesis that frogs discriminate between retreat sites (McComb and Noble, 1981; Lohoefener and Wolfe, 1984; Buchanan, 1988; Townsend, 1989; Phelps, 1993).

The use of natural or artificial retreats is likely to depend in part on the interaction of a variety of factors including meteorological conditions, site, season and life history of frogs. Provision of retreats which ameliorate the effects of adverse meteorological conditions in different seasons should contribute to higher capture rates. Pipe diameter, length and location, as well as the presence of water in pipes, are characteristics which are likely to afford protection from climatic conditions. For example, in the winter months (December through February), captures at the Tucker sites dropped to low levels whereas at Porter Hole high capture numbers were sustained. This difference may be attributable to the presence of water in pipes at Porter Hole which provided relief from moisture stress. Although only 19% of Porter Hole retreats contained water, 63% of winter captures were in these retreats.

Six species of hylids have been captured in this and other studies using PVC retreats: *Hyla squirella, H. cinerea, H. gratiosa, H. femoralis, H. chrysoscelis* and *Pseudacris ocularis* (Buchanan, 1988; Phelps, 1993; O'Neill, 1995; Moulton *et al.*, 1996). In this study all Hylidae

TABLE 2.—Total number of hylid frog captures in PVC pipes for each variable of retreat design. Significant differences at the P = 0.01 level are indicated by letters. Numbers of frogs caught per trap check are noted in parentheses

	Tucker sites								Porter hole				
	Tree type		Height			Shape			Shape				
	Hdwd	Pine	0 m	2 m	4 m	Short	Long	T	Water	Topcap	Narrow	Open	Grnd
H. squirella	855[A]	315	170[B,C]	496	504	69[D,E]	531	570	969[G]	134	67	77	243[I]
	(0.04)	(0.01)	(0.01)	(0.03)	(0.03)	(0.004)	(0.03)	(0.04)	(0.86)	(0.12)	(0.06)	(0.07)	(0.34)
H. cinerea	279[A]	96	51[B,C]	169	154	78[D]	203[F]	94	142[H]	2	4	20	148
	(0.01)	(0.004)	(0.003)	(0.01)	(0.01)	(0.005)	(0.01)	(0.006)	(0.13)	(0.002)	(0.004)	(0.02)	(0.21)
All species	1164[A]	448	232[B,C]	690	687	168[D,E]	760	682	1122[G]	136	72	99	400[I]
	(0.05)	(0.02)	(0.01)	(0.04)	(0.04)	(0.01)	(0.05)	(0.04)	(1.00)	(0.12)	(0.06)	(0.09)	(0.55)

[A] Hardwood captures significantly higher than pine captures
[B] 0 m captures significantly lower than 2 m captures
[C] 0 m captures significantly lower than 4 m captures
[D] Short captures significantly lower than long captures
[E] Short captures significantly lower than ‘‘T’’ captures
[F] Long captures significantly higher than ‘‘T’’ captures
[G] Water captures significantly higher than all other pipe shape captures
[H] Water captures significantly higher than all other pipe shape captures except ground captures
[I] Ground captures significantly higher than all other pipe shape captures except water captures

that were known to breed near the study sites (Franz, 1995) were captured in retreats except *P. crucifer.* Although juveniles represented a small proportion of captures, juveniles of each species were found in pipes. Although *P. crucifer* was absent from retreats, it was heard calling from treetops in the vicinity of the retreats on many occasions. *Pseudacris crucifer* is rarely encountered at the Preserve outside the breeding season (Richard Franz, pers. obs.) and its habits in the non-breeding season remain a puzzle.

Differences in the habits of treefrogs of different species, size classes and sexes are an important determinant of retreat use. At the Tucker sites frogs which did not ascend or descend tree boles could not encounter retreats. The absence of *Pseudacris crucifer* and juvenile *Hyla cinerea* in retreats may indicate this bias. At Porter Hole species differences in the use of tree vs. ground retreats were evident. Inclusion of retreats in shrubs and at heights above 4 m may have improved representation of absent species and groups or even of the species captured in the study. Consequently, the application of this technique to assessments of abundance or sex ratio of populations should be tested on a species by species basis.

High recapture rates in retreats indicate high levels of retreat fidelity which may have affected dispersal from breeding sites and may also indicate that retreat use increased survivorship. On average, individuals were recaptured 5 times at Porter Hole and 2.2 times at the Tucker sites. However, individuals were recaptured up to 44 times. Frogs dispersing from breeding ponds which encounter retreats may curtail further dispersal because retreats provide a quality refuge. Stewart and Pough (1983) found that introducing bamboo frog houses onto plots in Puerto Rico increased the density of *Eleutherodactylus coqui* on those plots. Similarly, Buchanan (1988) found treefrog density was higher in plots containing PVC pipe-retreats than in control plots. Nonetheless, these capture biases may be minimized in some circumstances by installing retreats after the normal dispersal period from breeding ponds.

Removing frogs from retreats, toe-clipping and measuring affected frog behavior once they were returned to retreats in trees. Frogs frequently attempted escape when they were returned to retreats. Goin (1958) found that frogs tended not to return to the same retreats following disturbance by researchers. However, other researchers observed no such effect (Buchanan, 1988; Townsend, 1989; O'Neill, 1995). In this study it is likely that retreat use by frogs would have been higher if frogs had not been disturbed.

This method of sampling treefrogs is very efficient in comparison with traditional herpetofaunal trapping techniques for capturing treefrogs. PVC pipe is inexpensive, installation time is short and trap mortality is eliminated. PVC pipes have been successfully used for mark-recapture population analysis (Moulton *et al.*, 1996), to improve representation of hylids in amphibian surveys (Phelps, 1993; O'Neill, 1995) and to assess the effects of experimental treatments (Moulton, 1996). PVC or bamboo retreats placed in elevated situations have been used to study site tenacity, territoriality and the effects of limitation of retreat sites (Stewart and Pough, 1983; Buchanan, 1988). Potential applications of this technique include studying dispersal distances from breeding ponds, habitat requirements in the non-breeding seasons, homing behavior to non-breeding season retreats and home range size in the non-breeding season. The use of PVC pipe retreats may also be an important addition to regional amphibian monitoring programs.

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