A COMPARISON OF GROUND-BASED AND TREE-BASED POLYVINYL CHLORIDE PIPE REFUGIA FOR CAPTURING *PSEUDACRIS REGILLA* IN NORTHWESTERN CALIFORNIA

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ABSTRACT—Creating artificial refugia through the use of polyvinyl chloride (PVC) pipes to capture hylid treefrogs has been examined in the Eastern United States and Caribbean, but has not been evaluated in the Pacific Northwest. We compared the effectiveness of ground-based and tree-based PVC pipes for capturing the Pacific Treefrog (*Pseudacris regilla*) in northwestern California. We recorded a total of 464 *P. regilla* captures. Probability of use increased asymptotically over our 28-d sampling period and was higher (6–20%) for tree-based than ground-based pipe refugia. Tree-based pipe refugia caught 81% more *P. regilla* than ground-based pipe refugia but latency to first detection did not vary significantly. Probability of use was higher for pipe refugia that had been used in a previous study than for new pipes, but age did not influence capture rate or latency to detection. Our results support the hypothesis that tree-based pipe refugia are more effective than ground-based pipes for capturing *P. regilla,* as well as the hypothesis that *P. regilla* actively discriminate among refugia. Using tree-based PVC pipe refugia to monitor *P. regilla* may be more effective than current techniques for addressing many research questions.

Key words: Pacific Treefrog, *Hyla regilla, Pseudacris regilla,* polyvinyl chloride, pipe refugia, northwestern California

Traditional amphibian sampling techniques, such as the use of pitfall traps, drift fences, and visual encounter surveys are ineffective for addressing many research questions for hylid treefrogs due to their ability to evade pitfall traps and drift fences, and observer biases associated with visual encounter surveys (Dodd 1991; Moulton and others 1996; Lamb and others 1998; Boughton and others 2000). Despite these findings, traditional techniques are still employed to assess trends of the Pacific Treefrog (*Pseudacris regilla,* also referred to as *Hyla regilla*; family *Hylidae*), the most abundant amphibian in western North America (Matthews and others 2001).

Urbanization, habitat destruction, and the introduction of exotic trout have reduced abundances and distributions of *P. regilla* in many parts of its range (Bull 2002; Pearl and others 2003; Knapp 2005; Pearl and others 2005; Reid 2005; Riley and others 2005). In addition, *P. regilla* populations show great natural fluctuation, making it difficult to assess trends of the species (Matthews and others 2001). In order to accurately monitor *P. regilla* populations, specific and efficient capture techniques are needed (Johnson 2005).

Constructing artificial refugia by means of polyvinyl chloride (PVC) pipes has been established as an effective monitoring technique for hylids in the eastern United States and Caribbean (McComb and Noble 1981; Stewart and Pough 1983; Meshaka 1996; Moulton and others 1996; Boughton and others 2000), but to our knowledge there have been no studies investigating PVC pipe refugium design for capturing hylids in the Pacific Northwest or for *P. regilla* in particular. Our objective was to monitor and assess how ground-based and tree-based positioning of PVC pipe refugia influence capture success for *P. regilla.*

Previous studies examining pipe refugia for other hylids have demonstrated that pipe design and positioning can influence capture success (Townsend 1989; Moulton and others 1996; Boughton and others 2000; Zacharow and others 2003; Bartareau 2004). Pacific Treefrogs seek refuge beneath ground cover and within trees (Nussbaum and others 1983), but it is unknown if tree-based PVC pipe refugia are more effective than ground-based pipe refugia for capturing the species. Hylids have shown a preference for elevated retreat sites in other studies examining pipe refugium design (Townsend 1989; Greenberg and others 1996; Boughton and others 2000), and we hypothesized that tree-based PVC pipe refugia would be more effective than ground-based PVC pipe refugia for capturing *P. regilla.* We evaluated this hypothesis by testing the following predictions: relative to ground-based pipe refugia, tree-based pipe refugia should have (1) a higher probability of use, (2) a higher capture rate, and (3) a shorter latency to detection for *P. regilla.*

METHODS

We conducted our study on the Humboldt Bay National Wildlife Refuge, located on the coast of northwestern California, approximately 150 km south of the Oregon border. The refuge has a variety of habitats including marshes, seasonally flooded wetlands, riparian wetlands, streams, and forests (Kwansy 2000), and *P. regilla* are known to inhabit these types of habitats at elevations ranging from sea level to 2440 m above sea level (Corkran and Thoms 1996). Our study site consisted of a forested riparian area with a flowing water source (Salmon Creek). Red Alder (*Alnus rubra*) and willows (*Salix* spp.) dominated the canopy layer while Salmonberry (*Rubus spectabilis*), Twinberry (*Lonicera involucrata*), and California Blackberry (*Rubus ursinus)* comprised the under story vegetation. *Pseudacris regilla* have been observed in the area year-round, as the area supports a large amount of invertebrate prey (Verhey 1992) and wet areas for breeding (Stebbins 2003). The climate throughout the region is relatively cool and moist, with annual rainfall averaging 99 cm and mean annual temperatures ranging from 9.5 to 15° C (NOAA 2006).

Sampling Scheme

We utilized a systematic sampling scheme consisting of 2 separate 250-m transects. Each transect consisted of 50 ground-based and 50 tree-based pipe refugia. Pipe refugia were designed following previously successful protocols for other species of treefrogs (Boughton and others 2000; Staiger and others 2002): pipes were white, schedule 40-weight, 60 cm in length, 5.08 cm in diameter, open on the top and capped on the bottom. To ensure a moist, humid environment within the confines of the pipe, we drilled a drain-hole 15 cm from the bottom of the pipe and filled it to the hole with water. To hang pipe refugia on screws, we drilled another hole approximately 10 cm from the top of the pipe. We mounted tree-based pipe refugia vertically on tree trunks at a height of 2.5 m above the ground while ground-based pipe refugia were mounted vertically on wooden stakes (1.27 × 5.08 × 122 cm) so that the bases of the pipes were resting on the ground. All pipe refugia faced north to control for potential differences stemming from orientation. We positioned tree-based pipe refugia on alder trees at approximately 5-m intervals, while ground-based pipe refugia were also positioned at 5-m intervals, 1 m from each tree-based pipe refugium and directly in line with the transect. We obtained 100 PVC pipes from a previous study (summer 2006) and purchased 100 additional pipes for our study (hereafter, ''old'' and ''new'' pipe refugia). We distributed old and new pipe refugia evenly among tree and ground sites. During the course of our study, we noticed that 10 tree-based pipe refugia were placed onto trees used in this previous study. To reduce possible biases associated with these locations, we removed these pipe refugia from our analyses.

Pipe refugia were deployed on 30 September 2006 and we inspected pipe refugia on every 3rd day from 2–29 October 2006, resulting in a total of 10 surveys. To avoid predation and desiccation, hylids seek out retreats during the day (Boughton and others 2000); therefore, we conducted surveys between 09:00–16:00 PST. With the aid of flashlights, survey teams (consisting of 2 field technicians at each transect) tallied the number of frogs in each pipe. Due to budget constraints, we were unable to mark any *P. regilla* in this study. We designed our methods to be minimally intrusive so frogs were not sexed, aged, weighed, or removed from pipe refugia at any time during this study. We removed debris only if it interfered with our ability to accurately observe or count the number of frogs in each pipe refugium. We refilled each pipe to the 15 cm mark with water during each survey when necessary.

Data Analyses

We measured 3 response variables to determine if the positioning (ground vs. tree) and age (old vs. new) of pipe refugia influenced their effectiveness: probability of use, capture rate, and latency to initial detection (LTD; Foresman and Pearson 1998). Our unit of replication was a pipe ($n = 190$). Because capture rates can be difficult to interpret if the independence of captures is unknown, probability of use and LTD are useful in comparing pipe refugium efficacy (Gompper and others 2006).

Probability of Use.—We used the occupancy modeling software program PRESENCE 2.0 (MacKenzie and others 2006) to determine the probability of pipe refugium use. The data were simplified to reflect frog presence or absence in a pipe for each survey, regardless of the number of frogs present. Occupancy modeling of animal ''presence-absence'' data can be conceptualized by considering 3 probabilities: (1) the probability a general site is occupied by an animal; (2) the conditional probability, given the animal occupies a site, that the animal used the precise surveyed location when it was surveyed; and (3) the conditional probability, given that the animal used the surveyed location, that the animal was detected by the researcher (MacKenzie and others 2006). The 1st probability, called occupancy probability (ψ), is often the premier parameter of interest in understanding the spatial distribution of a species. The product of the 2nd and 3rd probabilities is called detection probability (p). PRESENCE allows the simultaneous estimation of both ψ and p via a maximum-likelihood information-theoretic model selection procedure (MacKenzie and others. 2006).

With our methods, the probability that a frog was overlooked inside a pipe was essentially zero. Therefore, $\hat{p}$ is an estimate of the probability that a frog used a pipe given that a frog occupied the general site where the pipe occurred (hereafter ''the probability of use''). This variable thus eliminated potentially confounding effects of variation in occupancy in evaluating the effectiveness of a capture technique and represents an improvement over more conventional comparisons of capture success. We developed a set of a priori candidate models for $\hat{p}$ and $\hat{\psi}$ based on our hypotheses, experience, and knowledge of frog natural history. We considered pipe refugium positioning (tree or ground) and pipe refugium age (old or new) as factors potentially influencing $\hat{\psi}$ and $\hat{p}$. In addition, we modeled $\hat{p}$ as a function of time (survey number 1–10) by examining a linear trend in $\hat{p}$ over survey number, an asymptotic increase in $\hat{p}$ over survey number, a unique value for $\hat{p}$ at each survey, or a unique value for $\hat{p}$ for surveys 1–5 and 6–10. We used Akaike's Information Criterion (AIC) for model selection and considered models with ΔAIC values of <3 to be competitive models (Burnham and Anderson 2002). We presented estimates of occupancy and pipe refugium use probabilities as $\bar{x} \pm s_x$ by model-averaging the top models (ΔAIC <3).

Capture Rate and Latency to Detection.—We defined capture rate as the mean number of frogs captured/pipe refugium over the 10 surveys. Latency to initial detection was defined as the amount of time that lapsed until initial detection of a frog in pipe refugia. To calculate LTD, we assigned a numerical value (ranging from 1 to 10) to each pipe corresponding with the number of surveys made until initial detection (assigning no numerical value to pipe refugia where no detection was made). We used 2-way ANOVAs to test for significant differences ($\alpha = 0.05$) in capture rate and LTD between ground-based vs. tree-based pipe refugia and old vs. new pipe refugia. Capture rate was transformed (log + 1) for analysis; raw values are reported for graphical purposes.

RESULTS

We recorded a total of 464 *P. regilla* captures in 80 PVC pipe refugia from 2–29 October 2006, with an average of 37 pipe refugia occupied/survey. Of the 464 *P. regilla* captures, 299 captures (64%) occurred in tree-based pipe refugia. The total number of *P. regilla* counted/survey ranged from 22–113 frogs.

Probability of Use

The best of the candidate models indicated that the probability of use, *p*, varied with pipe refugium positioning and age, and it increased asymptotically over the 10 surveys (Table 1). The top 4 models (ΔAIC <3) contained this parameterization for *p*, and in each case the model coefficients (β) did not overlap zero, indicating strong support for the effect of these parameters on *p*. Model averaging indicated that the

TABLE 1. Model selection results for PRESENCE models of *Pseudacris regilla* occupancy (ψ) and probability of use (p) at the Humbolt Bay National Wildlife Refuge, California, 2–29 October 2006; Akaike's information criterion values (AIC), change in AIC from top model (ΔAIC), number of estimable parameters (K), and AIC Weights (w_i). Subscripts give parameterization for ψ and p: '.' = constant over group and time variables; 'placement' = tree- or ground-based pipe refugia; 'age' = new pipe refugia or pipe refugia used in a previous study; 'time_linear' = p increased linearly over 10 surveys; 'time_asymptote' = p increased asymptotically over the surveys. Subscripts joined by a '+' or an '*' indicate additive and factorial models, respectively. Only the top 6 models are shown, all others had w_i <0.01.

Model parameterization	AIC	ΔAIC	K	w_i
$\psi_{(placement)}, p_{(placement+age+time\_asymptote)}$	1252.6	0.00	6	0.413
$\psi_{(.)}, p_{(placement+age+time\_asymptote)}$	1253.6	0.98	5	0.253
$\psi_{(placement+age)}, p_{(placement+age+time\_asymptote)}$	1254.6	1.94	7	0.157
$\psi_{(age)}, p_{(placement+age+time\_asymptote)}$	1255.6	2.93	6	0.095
$\psi_{(placement*age)}, p_{(placement+age+time\_asymptote)}$	1256.5	3.88	8	0.059
$\psi_{(placement+age)}, p_{(placement+age+time\_linear)}$	1259.8	7.14	7	0.017

probability of use was 6–20% higher for old pipe refugia placed on trees than for the other pipe refugium categories (Fig. 1). Old pipe refugia placed on the ground and new pipe refugia placed on trees had similar probabilities of use, while new pipe refugia placed on the ground had the lowest probability of use (Fig. 1). For all pipe refugium categories, $\hat{p}$ increased asymptotically with increases in $\hat{p}$ as much as 10% between consecutive surveys early in our study, and smaller increases (2–3%) between the later surveys. Model averaging indicates that $\hat{p}$ was peaking by the end of our study, as models containing an asymptotic increase were over 57 times more likely to fit the data than those with a linear increase. The best models

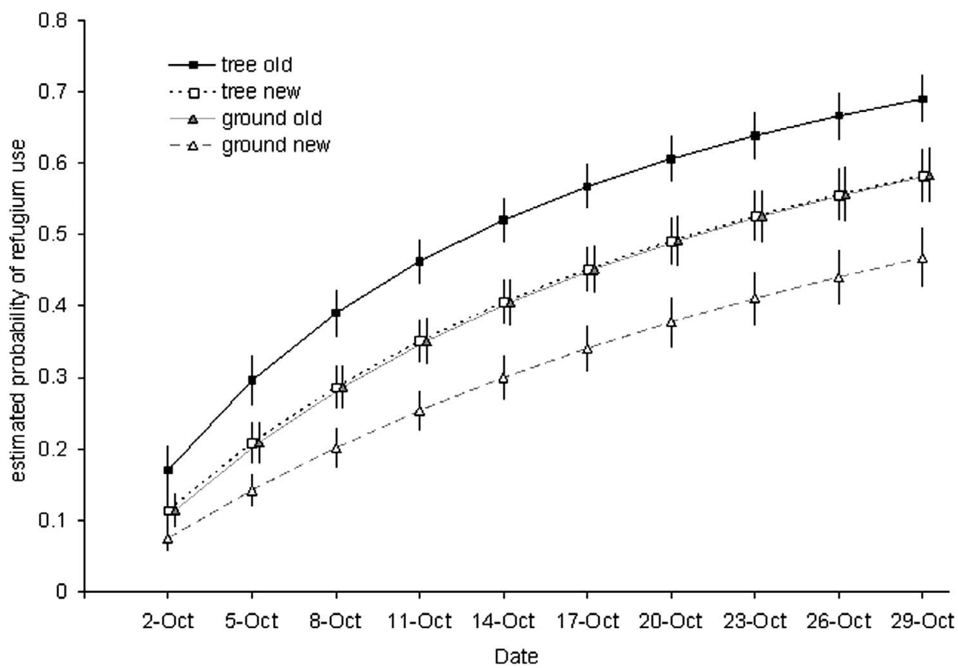

FIGURE 1. Estimated probability of use by *Pseudacris regilla* over 10 survey periods at the Humboldt Bay National Wildlife Refuge, California, 2–29 October 2006. Use probabilities are shown for tree- versus ground-based pipe refugia and for pipe refugia used in a previous study (old) versus new pipe refugia. Values are based on model-averaging (see Table 1) and are offset slightly on the x-axis to show error bars, which are ± 1 $s_{\bar{x}}$.

indicated that occupancy probability (ψ) also varied by location (tree or ground), with 2 of the top 3 models containing this parameter. Pipe refugium age was included in the 3rd and 4th ranked models, but its coefficient overlapped zero, lending little support for its influence on ψ. Model-averaged $\hat{\psi}$ was 0.46, $s_{\bar{x}} = 0.12$ and 0.38, $s_{\bar{x}} = 0.05$ for tree and ground locations, respectively.

Capture Rate and Latency to Detection

Tree-based pipe refugia caught 81% more *P. regilla* than ground-based pipe refugia ($F_{1,186} = 5.95$, $P = 0.016$). Pipe refugium age (old vs. new) did not influence capture rate ($F_{1,186} = 0.57$, $P = 0.45$), nor was there an interaction between positioning and age ($F_{1,186} = 0.10$, $P = 0.75$). Average latency to detection was 5.17 and 4.33 surveys for ground-based and tree-based pipe refugia respectively, although this difference was not statistically significant ($F_{1,75} = 1.98$, $P = 0.16$). Pipe refugium age also did not influence latency to detection ($F_{1,75} = 0.39$, $P = 0.53$), nor was there an interaction between positioning and age ($F_{1,75} = 0.02$, $P = 0.89$).

DISCUSSION

Probability of use and capture rates for *P. regilla* were higher in tree-based than in ground-based PVC pipe refugia, supporting the hypothesis that tree-based pipe refugia are more effective for capturing *P. regilla.* These findings are consistent with studies from other regions conducted on other hylids (Townsend 1989; Greenberg and others 1996; Boughton and others 2000; Bartareau 2004) and support the hypothesis that hylids actively discriminate among artificial refuge sites (McComb and Noble 1981; Boughton and others 2000; Bartareau 2004).

It remains unknown why some hylids prefer elevated retreat sites. Boughton and others (2000) suggested that elevated retreat sites offer favorable microclimates. Although our study was conducted during the non-breeding season, other studies (Greer and Wells 1980; Narins and Hurley 1982; Mitchell 1991) have hypothesized that elevated sites offer better acoustical properties than ground sites for calling male hylids.

No single pipe refugium design is appropriate for all hylid species in all locations (Martin and others 2004; Johnson 2005). Staiger and others (2002) found ground-based pipe refugia more effective than tree-based pipe refugia for capturing the Pine Woods Treefrog (*Hyla femoralis*), the Green Treefrog (*H. cinerea*), and the Squirrel Treefrog (*H. squirella*). No single pipe diameter, pipe length, or elevation above ground can be considered the most effective technique and prior to conducting detailed studies, preliminary studies are needed to determine the optimal pipe refugium design for the hylid species under investigation (Martin and others 2004; Johnson 2005). Though we used pipe diameters shown effective elsewhere (5.08 cm), it remains unknown if this is optimal for *P. regilla.*

Although not significantly different, latencies to detection were approximately 5.2 and 4.3 surveys (16 and 13 d) for ground-based and tree-based pipe refugia, respectively. A possible explanation for this pattern is that hylids have to search to find refuge after the initial placement of pipe refugia into a habitat (Staiger and Boughton 1999; Zacharow and others 2003).

Probability of use by *P. regilla* increased asymptotically with each successive survey (Fig. 1). Understanding this pattern is important for managers using PVC pipe refugia to capture *P. regilla,* as they should be aware that pipe refugium use will likely increase as a function of time following initial placement. In our study, new tree-based pipe refugia did not reach a probability of use of 50% until the 8th survey (24 d). For managers to discern how long pipe refugia should be deployed, it is necessary to conduct studies to determine an asymptote for total *P. regilla* captures as a function of time following initial placement. In our system, that time was about a month, but may fluctuate if a study is conducted over a longer period of time, during a different season, or in a different habitat.

Pipe refugium age did not influence capture rate or latency to initial detection, but old pipe refugia had somewhat lower probabilities of use than did pipe refugia constructed from newly purchased PVC pipe. Though all pipe refugia were cleaned before being deployed and the purchased PVC was housed outdoors at the supplier (and thus somewhat weathered), it is possible that the 2 groups differed in odors or off-gassing. Researchers considering PVC pipe refugia may seek to standardize odors across all pipe refugia in a study, either by making all purchases simultaneously, or ''weathering'' new pipe refugia in the field before deployment.

Several confounding factors may have influenced our results. First, we tried to eliminate biases associated with a preliminary experiment in the same area, but *P. regilla* may have grown accustomed to occupying pipe refugia during the preliminary work, and future studies involving naive frogs may not achieve capture rates as high as ours. Second, our study was only conducted over a 4-wk period in midfall, and longer studies have demonstrated a reduction in the effectiveness of tree-based pipe refugia during the hylid breeding season (Staiger and Boughton 1999). During the *P. regilla* breeding season, frogs are known to inhabit permanent and semi-permanent breeding ponds that can be found in open areas (Calhoon and Jameson 1970). We did not measure the effectiveness of pipe refugia under these conditions. Future studies should be designed to place refugia out over the breeding season as well as the non-breeding season. Third, budget constraints prevented us from conducting a mark and recapture analysis to differentiate among individual *P. regilla,* and we may have recaptured many of the same frogs. Boughton and others (2000) demonstrated high levels of retreat fidelity in a mark and recapture analysis among 6 different hylid species. Zacharow and others (2003) recognized that hylids take up residency in the same PVC pipe for various amounts of time. Future studies should investigate retreat site fidelity for *P. regilla* by incorporating alpha numeric tag marking techniques (Buchan and others 2005). In addition, future studies should analyze *P. regilla* demographics such as sex, age, body size, and color with respect to pipe refugium design. It is possible that certain pipe refugium designs may be more effective for targeting a certain demographic group of *P. regilla*

To our knowledge, this is the 1st study to examine the effectiveness of PVC pipe refugium design for capturing *P. regilla* and investigate PVC pipe refugium design for capturing hylids in the Pacific Northwest. Additional studies examining other aspects of pipe refugium design, such as pipe diameter, pipe length, and pipe height above ground for capturing *P. regilla* would be beneficial (Martin and others 2004; Johnson 2005). Nevertheless, our study supports the use of tree-based PVC pipe refugia over ground-based PVC pipe refugia for capturing *P. regilla.* Other studies (Moulton and others 1996; Lamb and others 1998; Boughton and others 2000) have suggested that sampling techniques currently utilized to monitor *P. regilla* are ineffective for addressing many research questions for the species. Tree-based PVC pipe refugia may offer a valuable tool for monitoring *P. regilla* in the Pacific Northwest.

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