Journal of Herpetology, Vol. 36, No. 4, pp. 627–634, 2002

Tadpoles Balance Foraging and Predator Avoidance: Effects of Predation, Pond Drying, and Hunger

CHRISTINE M. BRIDGES[1]

Division of Biological Sciences, University of Missouri-Columbia, Columbia, Missouri 65211-7400, USA

ABSTRACT.—**Organisms are predicted to make trade-offs when foraging and predator avoidance behaviors present conflicting demands. Balancing conflicting demands is important to larval amphibians because adult fitness can be strongly influenced by size at metamorphosis and duration of the larval period. Larvae in temporary ponds must maximize growth within a short time period to achieve metamorphosis before ponds dry, while simultaneously avoiding predators. To determine whether tadpoles trade off between conflicting demands, I examined tadpole (*Pseudacris triseriata*) activity and microhabitat use in the presence of red-spotted newts (*Notopthalmus viridescens*) under varying conditions of pond drying and hunger. Tadpoles significantly decreased activity and increased refuge use when predators were present. The proportion of active time tadpoles spent feeding was significantly greater in predator treatments, suggesting tadpoles adaptively balance the conflicting demands of foraging and predator avoidance without making apparent trade-offs. Tadpoles responded to simulated drying conditions by accelerating development. Pond drying did not modify microhabitat use or activity in the presence of predators, suggesting tadpoles perceived predation and hunger as greater immediate threats than desiccation, and did not take more risks.**

Organisms must often balance growth and predation risk to maximize fitness by making behavioral trade-offs; that is, they incur both a cost and a benefit by adopting a particular strategy. In many taxa feeding is greatly reduced when the risk of predation is perceived to be high (Holomuzki, 1986; Lima and Dill, 1990; Holmes, 1991; Lima, 1991; Angradi, 1992), thus allowing individuals to reduce predation risk; however, long-term reduction in feeding may lead to decreased growth and development and ultimately, reduced fitness (Semlitsch, 1987). There is ample evidence several factors influence prey decisions make, including resource density (Werner, 1991, 1992a), prey speed (Werner and Anholt, 1993), and ontogenetic shifts in vulnerability (Werner and Gilliam, 1984).

Because many factors can affect behavior, it is important to examine how these factors influence trade-offs and contribute to their evolutionary consequences (Werner and Anholt, 1993). In organisms with complex life cycles, a reduction in foraging (resulting from either increases in refuge use or decreases in activity) can reduce size at transformation or lengthen the larval period (Skelly and Werner, 1990; Skelly, 1992; Scrimgeour and Culp, 1994; Juliano and Stoffregen, 1995). These, in turn, can have strong consequences for adult fitness. In amphibians, large size at metamorphosis is correlated with size at first reproduction, increased fecundity (Smith, 1987; Semlitsch et al., 1988) and increased survival to maturity (Berven and Gill, 1983). Although it is beneficial for amphibians to be large at metamorphosis, Wilbur and Collins (1973) concluded amphibian larvae can alter the duration of their larval period in a deteriorating environment by making trade-offs between growth and development.

Pond hydroperiod often determines persistence of amphibian species within a particular habitat (Semlitsch et al., 1996) and can alter larval life-history characteristics (e.g., accelerate development; Semlitsch and Wilbur, 1988; Crump, 1989) and interspecific interactions (Wilbur, 1987). Pond drying decreases survival of amphibians breeding in ephemeral habitats (Semlitsch and Wilbur, 1988) and contributes to reproductive failure in some years (Semlitsch, 1983). Although the repercussions of pond drying are well documented, its effects (alone or in conjunction with other factors) on larval amphibian behavior are less understood. For example, reduced foraging attributable to predator avoidance can be exacerbated in a drying environment where the duration of the larval period is often abbreviated (Semlitsch and Wilbur, 1988), resulting in increased risk of death caused by desiccation. Consequently, larvae exposed to the dual risks of predation and desiccation face conflicting demands; reduced foraging lowers risk of death by predation but increases risk of death by desiccation. Alternatively, if the presence of a predator favors developing quickly to escape a risky environment (Werner, 1986), threats posed by predation

[1] Present address: USGS, Columbia Environmental Research Center, 4200 New Haven Road, Columbia, Missouri 65201, USA; E-mail: christine_bridges@usgs.gov

and desiccation may be equal, and both favor increased feeding rates.

Competition (i.e., the amount of food resources available) can also hasten development in larval amphibians. Wilbur (1977) demonstrated that, when food resources were low (i.e., when larval density was high), metamorphs were small. Because fitness can be correlated with size at metamorphosis (Smith, 1987; Semlitsch et al., 1988), garnering food resources is of paramount importance in tadpoles. Thus, tadpoles may be expected to take greater risks to feed when growth rates are perceived to be low (Ludwig and Rowe, 1990) or perhaps when hunger levels are high. Although Horat and Semlitsch (1994) found hungry tadpoles took no more risks than sated tadpoles, Eklov and Halvarsson (2000) noted tadpoles adopted riskier foraging behavior when food resources were less dense and more difficult to attain. Therefore, under certain conditions, tadpole responses to predation risk may be mediated by hunger.

In this experiment, I reared tadpoles of the western chorus frog (*Pseudacris triseriata*) under three drying regimes and two hunger levels, and then examined their activity and microhabitat use in the presence and absence of predators. Because *P. triseriata* typically breeds in temporary ponds (Skelly, 1995, 1996) in early spring, habitat drying represents a significant source of mortality to its tadpoles (Skelly, 1996, 1997). Although these temporary environments typically lack large fish predators, predatory salamanders and beetle larvae may present predation risks. *Pseudacris triseriata*, therefore, provide an excellent opportunity to test the prediction that tadpoles in drying environments take greater risks in the presence of predators and that this risk-taking behavior is mediated by hunger level.

Materials and Methods

Tadpole Collection and Rearing.—I collected seven egg masses of the western chorus frog (*P. triseriata*) at a farm pond in Englewood, Missouri. This pond fills each spring, serves as a breeding site for several amphibian species (e.g., *Acris crepitans, Ambystoma maculatum, Notopthalmus viridescens, Pseudacris crucifer, Rana palustris, Rana sphenocephala*), and typically dries in August to September. Egg masses were combined in the laboratory and placed in a stainless steel tub filled with 30 liters of well water maintained at 22°C.

Forty-eight 3.78-liter glass jars were arranged in a water bath and maintained at 22°C to provide a constant water temperature regardless of water depth. Jars were randomly assigned to drying regimes (high-constant, low-constant, or drying) and to food levels (high or low). After rearing under these treatments, behavior was examined under two predation levels (newts present or absent). Each of these 12 treatments was replicated four times. Jars were filled with well-water according to the assigned drying regime. The high-constant control had a constant water level of 2 liters, the low-constant control 500 ml, and to simulate drying in ponds, the drying treatment consisted of a decreasing water level. In the drying treatment, the water volume began at 2 liters and was lowered by 200 ml every other day for 10 days and then decreased 100 ml every day for eight days bringing the final volume to 200 ml. Tadpoles were exposed to their respective drying regimes for 19 days before microhabitat use and activity were measured. Although increased dissolved solutes are not thought responsible for inducing metamorphic responses (Denver, 1997), water was changed in all treatments every other day to maintain a constant solute level.

Seven days after hatching, three tadpoles were randomly introduced into each jar (N = 144 total tadpoles) where they were exposed to one of the three drying regimes. All tadpoles were fed a standard diet consisting of a 3:1 mixture of ground rabbit chow and TetraMin fish flakes. Hunger treatments were initiated only six days before the beginning of behavioral observations to avoid creating differences in size between hungry and fed tadpoles. Each tadpole was fed 5 mg of the mixture during the first six days of the drying exposure period (i.e., 15 mg per jar) and received 10 mg each for the next six days. For the final six days, tadpoles in the fed treatment were each given 25 mg. Tadpoles in the hungry treatment continued to receive 10 mg each for four days and were not fed during the two days prior to exposure to predators.

Nonlethal Predator Effects on Tadpole Behavior.—Red spotted newts (*Notopthalmus viridescens*, 2.11 ± 0.15 g SD) were collected at the same farm pond as the chorus frog egg masses, seven days before the end of the drying portion of the experiment. Newts were maintained in stainless steel tubs in the laboratory and were fed chorus frog tadpoles ad libitum.

Tadpole responses to newts were examined in eight 18-liter plastic testing chambers (45 × 25 × 15 cm) filled with 10 liters of well water. Water levels were equal among all testing chambers regardless of drying treatment, to control for concentration of predatory cues (and other confounding factors that may have been present if varying water levels were used). I secured a small plastic aquarium plant (*Bacopa* sp.) to the bottom center 15 cm from one end. A 0.5-liter plastic container (15 × 15 × 5 cm) with mesh sides was placed at the other end, permitting exposure to only the chemical and visual cues

from the predator (Stauffer and Semlitsch, 1993). Containers in the predator treatment each held a single newt, whereas containers in the control treatment were empty. Predators were placed in containers within the testing chamber and fed a single *P. triseriata* tadpole 18 h before beginning the test. Because there were only eight testing chambers and 48 treatments to test, each testing chamber was used six times. Testing chambers used in no predator treatments never held a predator to prevent the chamber from becoming contaminated with predator odor. Order effects were not significant in initial statistical analyses ($P > 0.05$) and were removed from subsequent analyses.

Tadpoles were placed into each testing chamber by removing them from the treatment jar with a net and gently placing them into the end of the chamber farthest from the predator. After a 15-min acclimation period, the three tadpoles were observed 5 s/min for 20 min. At each time interval, I recorded whether each tadpole spent the greatest portion of the observation period feeding, swimming, or resting. Because tadpoles were reared on food requiring grazing, feeding individuals were defined as those exhibiting small tail movements while visibly grazing with the mouth. Swimming tadpoles moved greater distances than those feeding and were not observed grazing. Resting individuals remained stationary. Relative feeding time was calculated as (feeding/feeding + swimming); this response determined the proportion of total active time tadpoles spent feeding (Horat and Semlitsch, 1994). I also recorded whether tadpoles were in refugia, near the edge, or in the open water. ''In refugia,'' was defined as having at least half of the body hidden within the plant, whereas observations near the edge were defined as individuals within 2 cm of the edge of the testing chamber. The remaining tadpoles were characterized as being in open water. At the end of the experiment, I determined developmental stage (Gosner, 1960) and wet mass (nearest 0.1 mg) of each tadpole.

Data Analyses.—Some tadpoles reached metamorphosis before the end of the experiment. Although there were always three individuals per testing chamber, when metamorphs were present only observations of nontransformed tadpoles were included in analyses. Because there were three tadpoles present in each testing chamber, individual observations were not independent. Additionally, resting behavior and time spent in open water were not included in the analyses as these behaviors were uniquely defined by their counterparts (e.g., tadpoles not feeding or swimming must be resting). Therefore, data from the three tadpoles in each testing chamber were pooled and the mean proportion of time spent swimming, feeding, and spent near the edge or in refugia were angularly transformed (Zar, 1984). After transformation, a Shapiro-Wilk test showed that the distributions of all variables, except for the position in refugia, were not significantly different from normal. In refugia data contained a number of zeroes which were difficult to normalize using any transformation; however, the data were more normal after angular transformation. I used a $3 \times 2 \times 2$ analysis of variance (ANOVA) to examine the effects of drying regime, hunger level, predation risk, and the interaction of these three variables on both activity levels and refuge use (SAS Institute, Inc., rele. 6.03 ed., Cary, NC, 1988, unpubl.). Tadpole mass was initially used as a covariate but never affected any response variable significantly and was removed from each model. When necessary, pairwise differences were examined using Bonferroni adjusted multiple *t*-tests.

Tadpole mass was calculated by averaging the wet mass of the three tadpoles in each jar. Mean masses were log-transformed, and, after a Shapiro-Wilk test showed the data were normally distributed, a one-way ANOVA was used to determine the effects of drying regime and hunger level on tadpole mass. Developmental stage was averaged similarly; these data did not meet the assumptions of ANOVA, so nonparametric statistical tests were used. The effect of drying on developmental stage was analyzed using a Kruskal-Wallace tied-ranks test. Significant differences among drying regimes were then determined using a nonparametric multiple comparison test. The effect of hunger level on developmental stage was examined with a Mann-Whitney test performed on mean developmental stage for each treatment. Average mass and developmental stage for treatments containing transformed individuals included only the values for nontransformed individuals.

RESULTS

Tadpole Activity.—The amount of time tadpoles spent swimming was reduced by predator presence and increased by tadpole hunger. Predator presence reduced time spent swimming by 50% (Fig. 1), and hungry tadpoles spent 35% more time swimming than fed tadpoles (Table 1). Drying regime significantly affected time tadpoles spent feeding (Table 1). Tadpoles in the drying treatment spent nearly half as much time feeding as individuals in the high-constant water control (12% vs. 21%, respectively). The proportion of active time spent feeding was significantly higher in the presence of a predator than when it was absent (Table 1, Fig. 1).

Tadpole Microhabitat Use.—Predators caused

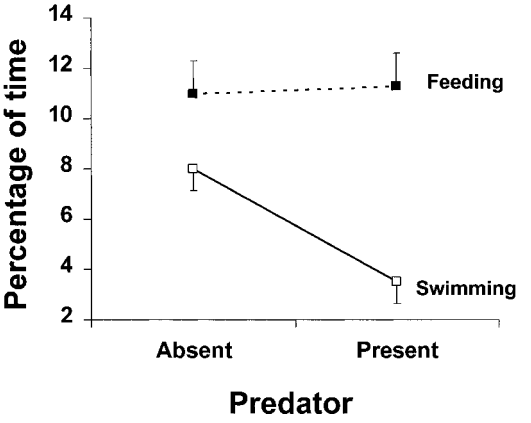

FIG. 1. Percentage of time tadpoles of *Pseudacris triseriata* spent swimming and feeding in the absence and presence of predatory newts (*Notophthalmus viridescens*). Bars represent means ± 1 SE.

tadpoles to spend a significantly greater proportion of time in refugia (50% increase; Table 2). The interaction of predation and hunger significantly affected the amount of time tadpoles spent near the edge of the testing chamber (Table 2). When predators were absent, hungry and fed tadpoles spent approximately the same amount of time near the edge. Conversely, when predators were present, hungry tadpoles spent significantly less time near the edge than did fed tadpoles (Fig. 2).

Tadpole Mass and Stage.—Tadpole mass differed significantly between food treatments as a result of reduced food in the hungry treatment during the last six days of the experiment ($F_{1,42} = 28.16$; $P < 0.0001$; hunger treatment mean ± 1 SE = 0.20 ± 0.08 g; fed treatment mean ± 1 SE = 0.30 ± 0.10 g). However, the developmental stage of tadpoles in hungry and fed treatments did not differ significantly from one another ($U = 196.5$; $P = 0.20$). Although drying did not influence tadpole mass ($F_{2,42} = 0.18$; $P = 0.8332$), it induced faster development compared to tadpoles in high-constant and low-constant controls ($H = 111.9$; $P < 0.0001$). Individuals in the drying treatment were one developmental stage ahead of those in the high-water control, and two ahead of tadpoles in the low-water control (treatment means ± 1 SE in Gosner [1960] stages; drying, 38.7 ± 1.7; high-water control, 37.5 ± 2.0; low-water control, 36.7 ± 2.4). Additionally, although only three and five tadpoles reached metamorphosis in high- and low-water controls, respectively, 11 tadpoles in the drying treatment metamorphosed.

DISCUSSION

The chief finding of this study is that tadpoles reduced swimming time while maintaining the

TABLE 1. Univariate analyses of variance on the effects of drying, predation, and hunger on activity response variables by *Pseudacris triseriata*. Type III sums of squares are reported.

Response variable:	Source	df	SS	*F*	*P*
Time spent swimming	Drying	2	0.0261	1.01	0.3721
	Predation	1	0.1043	8.09	0.0071
	Hunger	1	0.0573	4.44	0.0417
	Drying × Predation	2	0.0473	1.83	0.1737
	Predation × Hunger	1	0.0008	0.06	0.8087
	Drying × Hunger	2	0.0326	1.26	0.2945
	Error	38	0.4905		
	Total	47	0.7591		
Time spent feeding	Drying	2	0.0882	3.46	0.0417
	Predation	1	0.0005	0.04	0.8405
	Hunger	1	0.0381	2.99	0.0920
	Drying × Predation	2	0.0004	0.02	0.9843
	Predation × Hunger	1	0.0241	1.89	0.1770
	Drying × Hunger	2	0.0109	0.43	0.6548
	Error	38	0.4846		
	Total	47	0.6469		
Proportion of active time spent feeding	Drying	2	0.0531	0.48	0.6230
	Predation	1	0.3787	6.83	0.0128
	Hunger	1	0.0482	0.87	0.3569
	Drying × Predation	2	0.0857	0.77	0.4685
	Predation × Hunger	1	0.0760	1.37	0.2488
	Drying × Hunger	2	0.0397	0.36	0.7009
	Error	38	2.1061		
	Total	47	2.7877		

TABLE 2. Univariate analysis of variance of the effects of drying, predation, and hunger on microhabitat use by *Pseudacris triseriata*. Type III sums of squares are reported.

Response variable:	Source	df	SS	*F*	*P*
Time near edge	Drying	2	0.0057	0.13	0.8824
	Predation	1	0.0057	0.25	0.6211
	Hunger	1	0.0562	2.46	0.1254
	Drying × Predation	2	0.0223	0.49	0.6176
	Predation × Hunger	1	0.1059	4.63	0.0378
	Drying × Hunger	2	0.0121	0.26	0.7688
	Error	38	0.8689		
	Total	47	1.0770		
Time in refugia	Drying	2	0.0004	0.02	0.9848
	Predation	1	0.0610	4.43	0.0421
	Hunger	1	0.0294	2.13	0.1524
	Drying × Predation	2	0.0002	0.01	0.9942
	Predation × Hunger	1	0.0197	1.43	0.2392
	Drying × Hunger	2	0.0738	2.68	0.0816
	Error	38	0.5238		
	Total	47	0.7084		

same feeding time in the presence of predators (i.e., tadpoles spent more active time feeding when predators were present). In my experiment, resources were not present in the testing chamber. Therefore, tadpoles in the predator-free chambers spent more time swimming, perhaps because they were actively searching for food, whereas when predators were present, tadpoles curtailed searching to avoid predation. I hypothesize that this behavioral response allowed tadpoles to minimize decreases in growth and developmental rates without increasing conspicuousness to predators. In this manner, tadpoles gauged predation risk and altered time spent foraging without making apparent trade-offs. However, tadpoles that reduce time spent swimming in a natural environment may incur costs not evident in the laboratory. For example, reduced swimming activity may confine tadpoles to low quality food patches or to a thermally inferior environment. Therefore, trade-offs that seem adaptive in the laboratory may represent poor decisions in a natural setting. Alternatively, tadpoles may have a decision-making hierarchy that allows them to fine-tune their behaviors depending on environmental cues. In this case, time tadpoles spent feeding in my experiment did not decrease because feeding represented an important behavior within the laboratory setting. In contrast, circumstances in the field (e.g., the need to thermoregulate or find a new food patch) may motivate tadpoles to spend more time swimming and consequently reduce feeding.

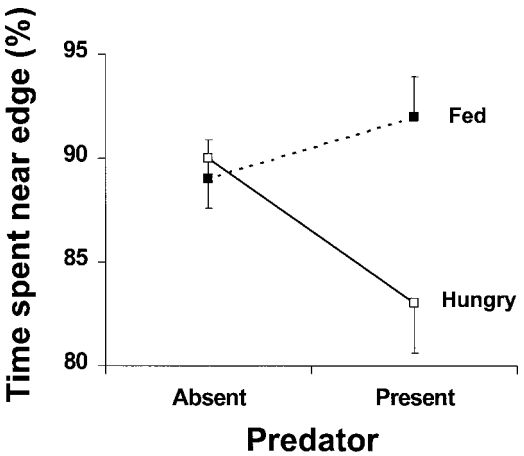

FIG. 2. Percentage of time fed and hungry tadpoles of *Pseudacris triseriata* spend near the edge of the testing chamber in the presence and absence of predatory newts (*Notophthalmus viridescens*). Bars represent means ± 1 SE.

Several studies demonstrate the long-term fitness consequences of short-term foraging decisions. Individuals that forage less in the presence of predators have reduced survival, reach smaller sizes at metamorphosis and have reduced fecundity and egg size (Skelly, 1992; Scrimgeour and Culp, 1994; Juliano and Stoffregen, 1995). Reduced feeding of hungry tadpoles during the last six days of my experiment resulted in significantly smaller tadpoles. Because tadpoles must reach a minimum size before metamorphosis can take place (Wilbur and Collins, 1973), small tadpoles should take greater risks by feeding more in a predator-rich environment to attain this threshold size for metamorphosis. Small tadpoles that feed less remain susceptible to aquatic predators for longer periods of time and risk habitat desiccation before metamorphosis. Consequently, the increased

foraging I observed may reflect strong selection for smaller tadpoles to grow and metamorphose successfully, despite the predation risk. Alternatively, the response of hungry tadpoles may be independent of size. In this instance, hungry tadpoles may have taken more risks than fed tadpoles because the hunger level of these tadpoles was great enough to cause tadpoles to adopt more risky behaviors rather than starve. Godin and Sproul (1988) present similar evidence that sticklebacks with higher energetic demands are willing to take greater risks by increasing their time foraging.

My results are consistent with those of Horat and Semlitsch (1994), who showed a reduction in the time tadpoles spent swimming in the presence of predators. Skelly (1995) also found that tadpoles of *P. triseriata* reduced activity significantly in the nonlethal presence of a predator. Reduced activity can lessen predation rates by visually oriented predators (e.g., *N. viridescens*, unpubl. data) but negatively affects growth and development rates (Skelly and Werner, 1990), which can lead to a smaller size at metamorphosis and reduced adult fitness (Smith, 1987; Semlitsch et al., 1988). Moreover, reduced activity can render an organism competitively inferior (Werner, 1992b), which is important in temporary environments where resources are ephemeral and larval density increases with decreasing water volume.

Tadpoles responded to simulated drying conditions by significantly reducing the time spent feeding compared to the two other treatments. By the end of the experiment, jars in this treatment held only 200 ml of water with a depth of approximately 2 cm. Food in this treatment, therefore, was much more concentrated than in either control, regardless of whether tadpoles were in hungry or fed treatments. Toward the end of the experiment, increased food concentrations in the drying treatment may have influenced tadpoles to decrease foraging time compared to control conditions, a response predicted by Werner and Anholt (1993). Werner (1992b) and Eklov and Halvarsson (2000) both demonstrated tadpole foraging effort decreased with increasing resource density, and Anholt and Werner (1995) found tadpoles reduced overall activity under comparable conditions. Similar reductions in feeding have also been documented in other taxa (e.g., centipedes: Formanowicz and Bradley, 1987; turtles: Formanowicz et al., 1989). Alternatively, it is possible that reduced feeding in the drying treatment could simply represent an ontogenetic shift in feeding needs, because tadpoles in drying treatments were at more advanced developmental stages than control tadpoles. Rowe and Ludwig (1991) predicted individuals with high fitness will take fewer risks for an incremental gain in mass, possibly explaining the reduction of feeding in more developed tadpoles in my experiment.

Fed tadpoles spent similar amounts of time near the edge of the testing chamber in the presence and absence of a predator, whereas hungry tadpoles spent less time near the edge when a predator was present. Because hungry tadpoles were smaller than fed tadpoles, hungry individuals may have adopted a more risky behavior to hasten growth. However, interpreting this interaction as a trade-off between foraging and predator avoidance requires the amount of time spent near the edge of the chamber be considered a predator avoidance mechanism. Other studies demonstrated that tadpoles increased time spent near the edge of an experimental chamber in the presence of predators (Stauffer and Semlitsch, 1993; Laurila et al., 1997). Whether this behavior represents avoidance and increases survival in the presence of a predator remains untested. In my experiment, tadpoles did exhibit increased refugium use in the presence of predators. This is clearly a predator avoidance response that, in a natural setting, would decrease predation rates.

Although all animals must constantly maximize the benefits of foraging while minimizing exposure to predators, amphibian larvae in ephemeral aquatic habitats experience the additional demand of pond drying. Pond drying decreases amphibian survival in temporary environments and can be a major source of reproductive failure in many years because of high larval mortality (Semlitsch et al., 1996). Tadpoles in my experiment responded to habitat drying by developing more rapidly than tadpoles in either control treatment, a response now repeatedly demonstrated for larval anurans (Crump, 1989; Newman, 1988; Tejedo and Reques, 1994). However, pond drying failed to modify tadpole behavioral responses to predators. It is possible that tadpoles perceived starvation and the presence of a predator as more immediate risks than pond drying and, thus, did not adopt more risky behaviors as water levels dropped. Consequently, tadpoles in drying environments did not accelerate growth and development to escape desiccation when predators were present. This in turn could affect the growth and development of predators, which often are organisms with complex life cycles (e.g., salamander and insect larvae), by making predator cues more concentrated and tadpoles less likely to emerge from refugia. Research examining the joint effects of predators and pond drying on the development and behavior of tadpoles is necessary to shed light on these relationships.

Acknowledgments.—This manuscript was im-

proved through the critical comments and suggestions of S. L. Ball, J. E. Fauth, P. S. Hopkins, D. B. Noltie, M. J. Parris, D. Reznick, R. D. Semlitsch, D. K. Skelly, E. E. Werner, and an anonymous reviewer. Thanks to J. A. Bridges for assistance in the laboratory, to the U.S.G.S. Columbia Environmental Research Center, Columbia, MO, for providing facilities, and to F. J. Dwyer for the use of his laboratory space. This research was partially funded through a Sigma Xi Grant-in-Aid of Research.

Literature Cited

Angradi, T. R. 1992. Effects of predation risk on foraging behavior of juvenile rainbow trout (*Oncorhynchus mykiss*). Canadian Journal of Zoology 70: 355–360.

Anholt, B. R., and E. E. Werner. 1995. Interaction between food availability and predation mortality mediated by adaptive behavior. Ecology 76:2230–2234.

Berven, K. E., and D. E. Gill. 1983. Interpreting geographic variation in life-history traits. American Zoologist 23:85–93.

Crump, M. L. 1989. Effect of habitat drying on developmental time and size at metamorphosis in *Hyla pseudopuma*. Copeia 1989:794–797.

Denver, R. J. 1997. Proximate mechanisms of phenotypic plasticity in amphibian metamorphosis. American Zoologist 37:172–184.

Eklov, P., and C. Halvarsson. 2000. The trade-off between foraging activity and predation risk for *Rana temporaria* in different food environments. Canadian Journal of Zoology 78:734–739.

Formanowicz Jr., D. R., and P. J. Bradley. 1987. Fluctuations in prey density: effects on the foraging tactics of scolopendrid centipedes. Animal Behaviour 35:453–461.

Formanowicz Jr., D. R., and E. D. Brodie, and S. C. Wise. 1989. Foraging behavior of matamata turtles: the effects of prey density and the presence of a conspecific. Herpetologica 45:61–67.

Godin, J.-G., and C. D. Sproul. 1988. Risk taking in parasitized sticklebacks under threat of predation: effects of energetic need and food availability. Canadian Journal of Zoology 66:2360–2412.

Gosner, K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16:183–190.

Holmes, W. G. 1991. Predator risk affects foraging behavior of pikas: observational and experimental evidence. Animal Behaviour 42:111–119.

Holomuzki, J. R. 1986. Predator avoidance and diel patterns of microhabitat use by larval tiger salamanders. Ecology 67:737–748.

Horat, P., and R. D. Semlitsch. 1994. Effects of predation risk and hunger on the behaviour of two species of tadpoles. Behavioral Ecology and Sociobiology 34:393–401.

Juliano, S. A., and T. L. Stoffregen. 1995. Effects of habitat drying on size at and time to metamorphosis in the tree hole mosquito *Aedes triseriatus*. Oecologia 97:369–376.

Laurila, A., J. Kujasalo, and E. Ranta. 1997. Different antipredator behaviour in two anuran tadpoles: effects of predator diet. Behavioral Ecology and Sociobiology 40:329–336.

Lima, S. L. 1991. Energy, predators, and the behavior of feeding hummingbirds. Evolutionary Ecology 5: 220–230.

Lima, S. L., and L. M. Dill. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Canadian Journal of Zoology 68: 619–640.

Ludwig, D., and L. Rowe. 1990. Life-history strategies for energy gain and predators avoidance under time constraints. American Naturalist 135:686–707.

Newman, R. A. 1988. Adaptive plasticity in development of *Scaphiopus couchii* tadpoles in desert ponds. Evolution 42:774–783.

Rowe, L., and D. Ludwig. 1991. Size and timing of metamorphosis in complex life cycles: time constraints and variation. Ecology 72:413–427.

Scrimgeour, G. J., and J. M. Culp. 1994. Feeding while evading predators by a lotic mayfly: linking short-term foraging behaviors to long-term fitness consequences. Oecologia 100:128–134.

Semlitsch, R. D. 1983. Structure and dynamics of two breeding populations of the eastern tiger salamander, *Ambystoma tigrinum* Copeia 1983:608–616.

———. 1987. Interactions between fish and salamander larvae: costs of predator avoidance or competition? Oecologia 72:481–486.

Semlitsch, R. D., and H. M. Wilbur. 1988. Effects of pond drying time on metamorphosis and survival in the salamander *Ambystoma talpoideum*. Copeia 1988:978–983.

Semlitsch, R. D., D. E. Scott, and J. H. K. Pechmann. 1988. Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. Ecology 69:184–192.

Semlitsch, R. D., D. E. Scott, J. H. K. Pechmann, and J. W. Gibbons. 1996. Structure and dynamics of an amphibian community. *In* M. L. Cody and J. A. Smallwood (eds.), Long-Term Studies of Vertebrate Communities, pp. 217–248. Academic Press, San Diego, CA.

Skelly, D. K. 1992. Field evidence for a cost of behavioral antipredator response in larval amphibian. Ecology 73:704–708

———. 1995. A behavioral trade-off and its consequences for the distribution of *Pseudacris* treefrog larvae. Ecology 76:150–164.

———. 1996. Pond drying, predators, and the distribution of *Pseudacris* tadpoles. Copeia 1996:599–605

———. 1997. Tadpole communities. American Scientist 85:36–45

Skelly, D. K., and E. E. Werner. 1990. Behavioral and life-historical responses of larval American toads to an odonate predator. Ecology 71:2313–2322.

Smith, D. C. 1987. Adult recruitment in chorus frogs: effects of size and date at metamorphosis. Ecology 68:344–350.

Stauffer, H. P., and R. D. Semlitsch. 1993. Effects of visual, chemical and tactile cues of fish on the behavioural responses of tadpoles. Animal Behaviour 46:355–364.

Tejedo, M., and R. Reques. 1994. Plasticity in meta-

morphic traits of natterjack tadpoles: the interactive effects of density and pond duration. Oikos 71: 295–304.

WERNER, E. E. 1986. Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. American Naturalist 128:319–341.

———. 1991. Nonlethal effects of a predator on competitive interactions between two anuran larvae. Ecology 72:1709–1720.

———. 1992a. Individual behavior and higher-order species interactions. American Naturalist 140:S5–S32

———. 1992b. Competitive interactions between wood frog and northern leopard frog larvae: the influence of size and activity. Copeia 1992:26–35.

WERNER, E. E., AND B. R. ANHOLT. 1993. Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. American Naturalist 142:242–272.

WERNER, E. E., AND J. F. GILLIAM. 1984. The ontogenetic niche and species interactions in size-structured populations. Annual Review of Ecology and Systematics 15:393–425.

WILBUR, H. M. 1977. Density-dependent aspects of growth and metamorphosis in *Bufo americanus*. Ecology 58:196–200.

———. 1987. Regulation of structure in complex systems: experimental temporary pond communities. Ecology 68:1437–1452.

WILBUR, H. M., AND J. P. COLLINS. 1973. Ecological aspects of amphibian metamorphosis. Science 182: 1305–1314.

ZAR, J. H. 1984. Biostatistical Analysis. Prentice Hall, Englewood Cliffs, NJ.

Accepted: 7 March 2002.

Journal of Herpetology, Vol. 36, No. 4, pp. 634–640, 2002

A New Species of *Hyla* (Anura: Hylidae) from the Highlands of Venezuelan Guayana

J. CELSA SEÑARIS[1] AND JOSÉ AYARZAGÜENA[2]

Museo de Historia Natural La Salle, Apartado 1930, Caracas 1010-A, Venezuela

ABSTRACT.—A new species of *Hyla* is described from Cerro Jaua, 1600 m, Bolívar State, Venezuela. The external appearance of the new species closely resembles *Hyla benitezi* and *Hyla lemai* but differs from these species in having cream-colored digits and webbing (in live specimens), a golden iris, less foot webbing, no quadratojugal, and a characteristic advertisement call that consists of a rhythmically repeated note with a dominant frequency between 3260 and 3450 Hz.

During the last few years, the number of genera and species of anurans known from Venezuela has rapidly increased, especially from the highlands of the Venezuelan Guayana (Ayarzagüena, 1992; Ayarzagüena and Señaris, 1994; Ayarzagüena et al., 1992a,b; Donnelly and Myers, 1991; Gorzula and Señaris, 1999; La Marca, 1996; Myers and Donnelly, 1996, 1997, 2001; Señaris et al., 1994, 1996). The Pantepui Region, comprising all high-tepui ecosystems between 1500 and 3000 m elevation (Huber, 1987, 1995), has been shown to be especially notable because of its high levels of specific and generic endemism (Duellman, 1999; Gorzula and Señaris, 1999).

A brief expedition to Cerro Jaua in Bolívar State resulted in an interesting collection of reptiles and amphibians (Señaris and Ayarzagüena, 1996) including species new to science. Some of these species have already been described (Señaris et al., 1996; La Marca, 1996), but among the undescribed specimens is a series of treefrogs that, after a study of their morphology, osteology, and vocalizations, were determined to be a new species of *Hyla* described herein.

MATERIALS AND METHODS

Measurements were made to the nearest 0.1 mm with dial calipers in the manner described by Duellman (1970). Webbing formula follow the methodology of Savage and Heyer (1967) as modified by Myers and Duellman (1982). Cleared-and-stained specimens were prepared in the manner described by Dingerkus and Uhler (1977). Osteological terminology follows Trueb (1993).

[1] Corresponding Author. E-mail: celsas@mixmail.com

[2] Present address: Asociación Amigos de Doñana, % Panamá 6, 41012 Sevilla, España; E-mail: ayarza@ccs.internet.ve

Calls were recorded using a Sony WM D6C with a Sennheiser directional microphone model Me-80. The recording was digitized at a sampling rate of 22050 Hz and size of 16 bits and analyzed using Canary 1.2 software (Cornell Univ., Ithaca, NY, 1995, unpubl.) on a Macintosh computer. Advertisement call terminology follows that of Heyer et al. (1990). Museum abbreviations are as follows: MHNLS = Museo de Historia Natural La Salle, Caracas and EBRG = Estación Biológica de Rancho Grande, Ministerio del Ambiente y de los Recursos Naturales, Maracay.

RESULTS

Hyla rhythmicus, sp. nov.

Holotype.—MHNLS 12957, an adult male, from Cerro Jaua, Parque Nacional Jaua-Sarisariñama, Bolívar state, Venezuela (04°49′55″N, 64°25′54″W), 1600 m, collected by J. Celsa Señaris and José Ayarzagüena on 11 June 1994.

Paratypes.—MHNLS 12958, MHNLS 12946 and EBRG 3437, all with the same data as holotype.

Diagnosis.—*Hyla rhythmicus* differs from the other species of the genus by the following combination of characters: (1) snout–vent length 33.4 mm (32.9–34.1, $N = 3$) in males, 33.3 mm in one female; (2) in life dorsum reddish brown or burgandy colored with a fine black reticulation and/or black transverse lines, occasionally with dorsolateral white stripes; forearms and limbs with variable dark banding; (3) venter and hidden surfaces cream; anterior part of the throat with brown flecks; (4) fingers, toes and webbing cream with variable brown suffusion; (5) iris golden color; (6) ulnar and tarsal tubercles or keels absent; (7) axillary membrane absent; (8) vocal sac large, single and subgular; (9) vomerine odontophores prominent, arched, each with 9–11 teeth, narrowly separated medially; (10) dorsal skin smooth, ventral surfaces of chest and gular area finely shagreened and on belly and thighs coarsely granular; (11) tympanum small, concealed under skin but visible in outline; (12) hand with basal webbing between outer fingers; (13) webbing formula for foot **I**$1^{1}/_{2}$–2^{+}**II**1^{+} − $2\frac{1}{2}$**III**$1\frac{1}{2}$–$2\frac{1}{2}$**IV**2–1**V**; (14) quadratojugal absent; (15) prepollex enlarged, with protruding prepollical spine; and (16) advertisement call consists of a train of rhythmically reapeted notes of 111–162 msec in duration, repeated at a rate of 1.4–2 notes per sec, with a dominant frequency at 3260–3450 Hz.

The external appearance of *H. rhythmicus* closely resembles that of *Hyla benitezi* and *Hyla lemai*. From these, *H. rhythmicus* differs by having the fingers, toes and webbing cream (orange in *H. benitezi* and orange or yellow in *H. lemai*), a golden iris (light bronzy brown or tan in *H. benitezi* and silvery or greenish gray in *H. lemai*), less foot webbing, quadratojugal absent (vs. present in *H. benitezi* and *H. lemai*), as well as a different advertisement call that consists of a prolonged repetition of a single note with a dominant frequency at 3260–3450 Hz (vs. 4–6 notes with dominant frequencies at less than 3000 Hz in *H. benitezi* and *H. lemai*).

FIG. 1. Holotype of *Hyla rhythmicus* (MHNLS 12957).

Description of Holotype.—Body moderately robust (Fig.1); head narrower than body, slightly wider than long; head width 34% of snout–vent length (SVL); head length 34% of SVL; snout short, rounded in dorsal view, bluntly rounded in profile; distance from eye to nostril about 73% of the horizontal eye diameter; canthus rostralis rounded and barely evident; loreal region slightly concave, gradually sloping to lip; lips not flared; interorbital distance 152% of width of eyelid; tympanum small, circular, concealed under skin but visible in outline, separated from eye by distance about equal to its length; diameter of tympanum 32% of eye length; supratympanic fold low and ill-defined.

Choanae moderately small, elliptical shaped, length about 40% of interchoanal distance; vomerine odontophores prominent, arched, each with 9 teeth, narrowly separated medially, located between the choanae. Tongue circular, posterior margin free.

Axillary membrane absent; forelimbs moderately robust; ulnar tubercles absent; fingers bearing moderately expanded (about 1.5 times wider than adjacent phalange) round terminal discs; diameter of third finger disc equal to tympanum diameter; relative length of fingers

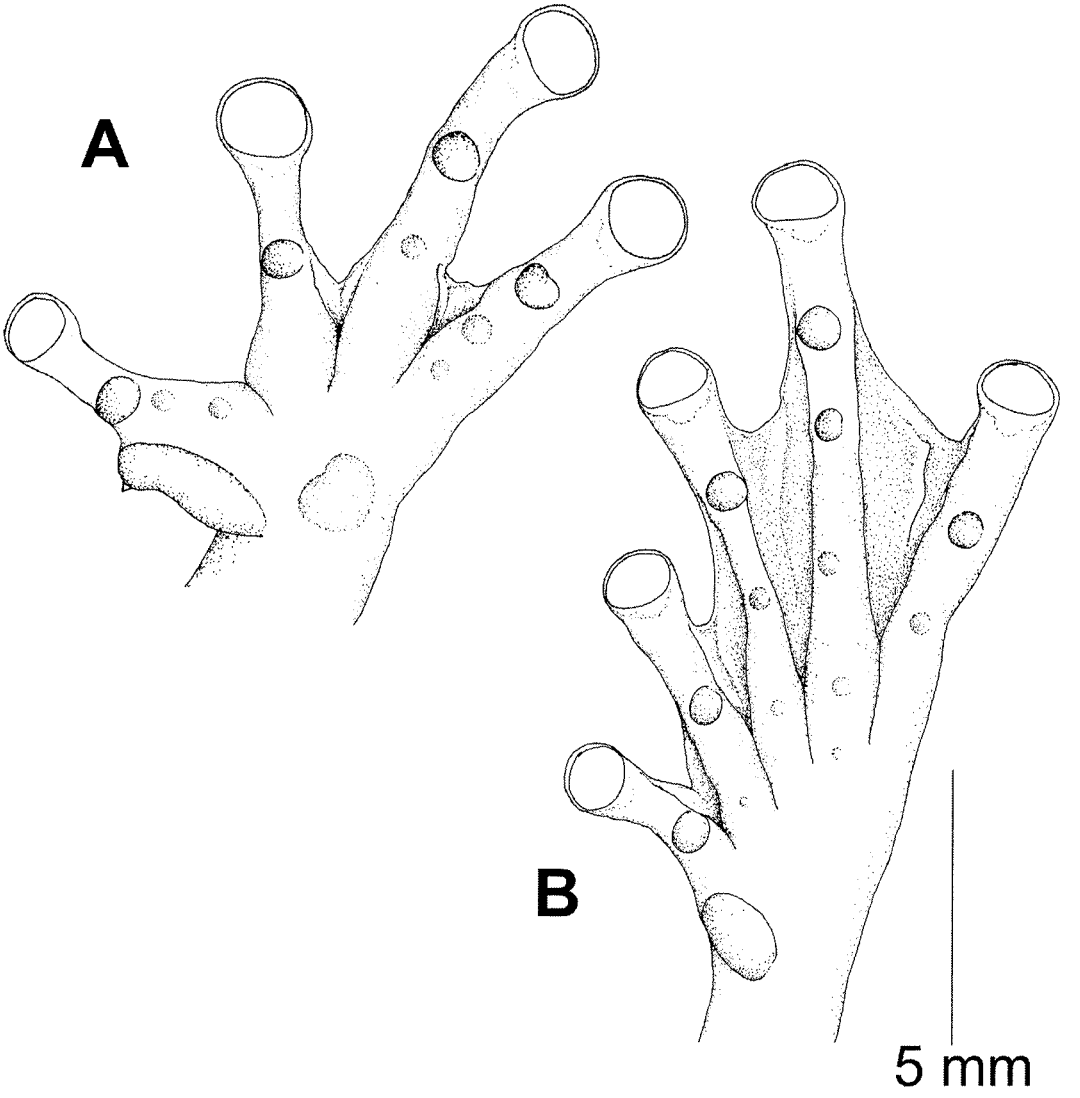

FIG. 2. Ventral view of the (A) left hand and (B) left foot of the holotype of *Hyla rhythmicus* (MHNLS 12957).

1<2<4<3; no webbing between fingers I and II, and basal webbing between fingers II and III; webbing formula for outer digits **III**2½–2**IV**; subarticular tubercles large, round, distinct; supernumerary tubercles on proximal portions of digits low, round, and inconspicuous; palmar tubercle small, round, and ill-defined; thenar tubercle large, elliptical; prepollex enlarged, spine protruding (Fig. 2A). Hind limbs moderately long, slender, tibia length 53% of SVL; tarsal tubercles and inner tarsal fold absent; outer metatarsal tubercle absent; inner metatarsal tubercle oval, flat, distinct; toes moderately long, bearing round terminal discs, slightly smaller than discs on fingers; relative length of toes 1<2<3=5<4; webbing present between toes; webbing formula **I**1½–2^{+}**II**1^{+} – 2½**III**1½–2½**IV**2–1**V**; subarticular tubercles moderately large, round; some small and low supernumerary tubercles on proximal portions of toes (Fig. 2B).

Skin on dorsum (body, head and limbs) and flanks smooth; skin on chest and gular area finely shagreened; skin on belly and ventral thighs coarsely granular, others surfaces smooth. Opening of vent directed posteriorly at upper level of thighs, concealed by a small dorsal flap; small white tubercles below vent.

Coloration in Life.—Dorsum reddish brown with black transverse lines or bands and longitudinal bands along the flanks. Limbs with black reticulations that form bands; forearms slightly paler than dorsum but with similar

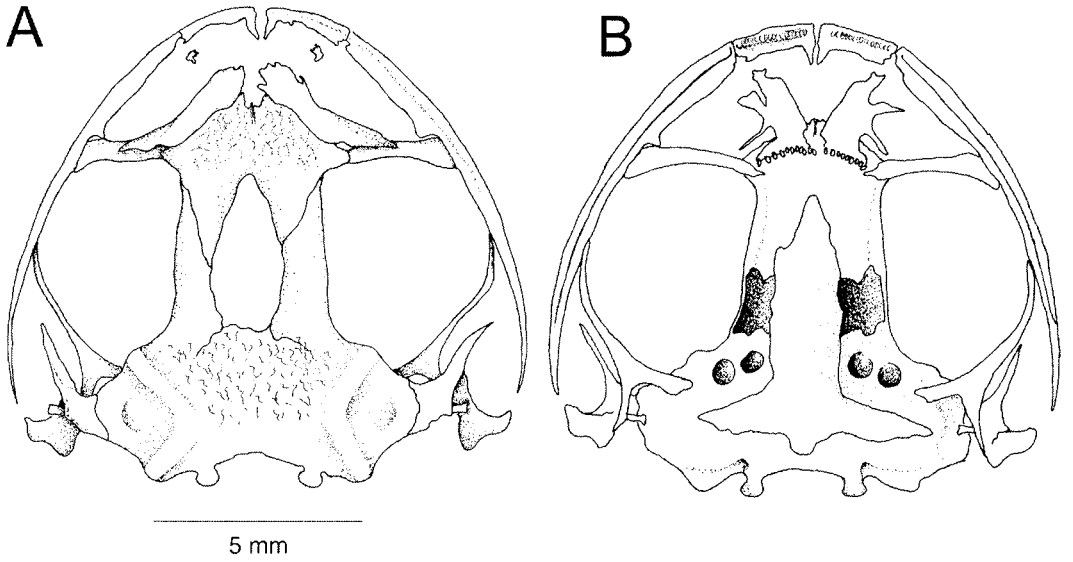

FIG. 3. Skull of *Hyla rhythmicus* (MHNLS 12958). (A) Dorsal; (B) ventral.

dark reticulations; digits and webbing cream with brown flecks; iris brilliant golden with fine dark venation; venter and hidden surfaces of limbs cream with a pink tint; anterior part of the throat and angles of jaw with brown flecks; vocal sac clear white.

Coloration in Preservative.—Dorsum reddish brown with continuous and broken dark brown bands; eyelids dark brown with a dark interorbital bar; forearms slightly paler than dorsum with dark spots resembling bands; canthus rostralis and areas below eye and tympanum dark brown; flanks cream-colored with dense, brown spots becoming diffuse ventrally; posterior thigh surface cream with brown suffusion; ventral surfaces cream; anterior part of the throat and border of lower lip with brown flecks.

Osteology.—Skull wider than long, snout region short and blunt; nasals ossified, widest anteriorly and separated medially by a distance about equal to one half of their greatest width; sphenethmoid ossified and slightly ornamented, broader than long in dorsal view, underlying the nasals and the anterior ends of frontoparietals, forming anterior margin of the frontoparietal fontanelle; frontoparietals slender, not in median contact, partially fused to the prootics; frontoparietal fontanelle oval. Squamosal moderately robust, zygomatic ramus narrow anteriorly, horizontal except anterior tip which is inclined. Alary processes of the premaxilla slender and vertical; maxilla with a moderately high facial process and a slender preorbital process. Quadratojugal absent. Vomers broad, narrowly separated medially, bearing a massive, curved dentigerous process anterior to neopalatines. Neopalatines well separated medially, only slightly overlapping sphenethmoid, articulating with maxillae. Parasphenoid slender anteriorly, the tip not extending to the level of the neopalatines; alary processes narrow, directed posterolaterally; posteromedial process short, not extending to margin of the foramen magnum. Two auditory foramina. Medial ramus of the pterygoid relatively robust, articulating with anterolateral surface of otic capsule; anterior ramus of the pterygoid long, slender, in contact with the maxilla at about midlength of orbit (Fig. 3A–B). The prepollex is enlarged and curved with a sharp distal point.

The postcranial axial skeleton and the appendicular skeleton is typical of hylid frogs (Duellman and Trueb, 1986).

Measurements of the Holotype.—In millimeters. SVL 32.9, head length 11.1, head width 11.3, interorbital distance 3.5, eye–nostril distance 3.2, width of eyelid 2.3, length of eye 4.4, diameter of tympanum 1.4, thigh length 16.7, tibia length 17.3, foot length 10.8.

Variation.—Size and proportions are summarized in Table 1. In life, MHNLS 12958 had a dark red dorsal ground color, with black reticulations and a white canthal line extending continuously over the eye and dorsolaterally to midbody. The other two specimens were dark red with narrow to broad transverse black lines.

Vocalization.—Advertisement calls of the holotype and one of the paratypes of *H. rhythmicus* were recorded between 2030 and 2035 h, at an air temperature of 18°C. The call consists of a train of rhythmically repeated notes given at a rate of 1.4–2 notes per sec; during more than 2.5

TABLE 1. Size and proportions of the holotype and paratypes of *Hyla rhythmicus* (values are means ± SD with range in parentheses).

Character	Males (N = 3)	Female (N = 1)
Snout–vent length (SVL) in mm	33.4 ± 0.7 (32.9–34.2)	33.3
Head width/SVL	0.34 ± 0.003 (0.343–0.344)	0.35
Head length/SVL	0.32 ± 0.02 (0.29–0.34)	0.34
Tibia length/SVL	0.52 ± 0.01 (0.51–0.53)	0.54
Tympanum/eye	0.35 ± 0.04 (0.32–0.39)	0.35
Eye-naris/eye	0.79 ± 0.08 (0.73–0.88)	0.86
Interorbital length/upper eyelid width	1.46 ± 0.11 (1.33–1.52)	1.48

min of continuous recording, each frog produced 84–96 notes per min. The notes are 111–162 msec in duration ($\bar{x}$ ± SD = 132.9 ± 12.19, N = 45) with intervals of 331–798 msec (495.1 ± 126.07, N = 44). The notes are modulated and weakly pulsed (Fig. 4). The notes have a brief initial rise in frequency from 2516 Hz to 4118 Hz. The dominant frequency rangs from 3260 Hz to 3450 Hz (3367.4 ± 53.6, N = 45).

Distribution and Ecology.—This species is known only from the type locality: Cerro Jaua at 1600 m in Bolívar State in the Venezuelan Guayana.

Males were calling from the undersides of leaves (about 1–1.5 m above the water) on a small tree growing on a rock in the middle of a torrential river. Individuals of *H. benitezi* were calling from the upper surfaces of leaves of the same tree. The calls of *H. rhythmicus* and *H. benitezi* also were heard from other bushes growing on rocks in a steep section of the river near a waterfall.

Other anurans found at the same locality included *Colostethus ayarzaguenai, Hyalinobatrachium crurifasciatum, Hyla sibleszi, Stefania percristata*, and *Stefania oculosa*. We also recorded calls of a microhylid (*Otophryne* sp). We collected *Hyla minuta* in a patch of *Brocchinia* sp. adjacent to the gallery forest.

Etymology.—The specific epithet, *rhythmicus*, is a Latin noun in apposition, alluding to the distinctive vocalization which consists of a single, rhythmically repeated note.

DISCUSSION

Few species of the genus *Hyla* have been reported from elevations above 1500 m in the Pantepui. Ayarzagüena and Señaris (1994) described *Hyla aromatica* and *Hyla inparquesi* from

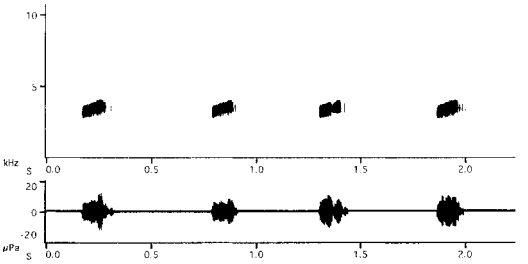

FIG. 4. Audiospectrogram (top) and oscillogram (bottom) of the advertisement call of *Hyla rhythmicus* (air temperature 18°C).

the summits of Cerro Huachamacari (1700 m) and Cerro Marahuaca (2600 m) respectively; *H. sibleszi* is known from some highland localities (Auyán-tepui, Cerro Guanay and Chimantá Massiff, up to 1850 m) in the Venezuelan Guayana (Gorzula and Señaris, 1999). Myers (1997) and Gorzula and Señaris (1999) mentioned specimens of the genus *Hyla* from the Auyántepui summit and Sierra de Maigualida, but these species have not been described. In this paper, in addition to *H. rhythmicus*, we report *H. benitezi* and *H. minuta* from Cerro Jaua at 1600 m (Appendix 1).

Of these species, *H. rhythmicus* can only be confused externally with the members of the *H. aromatica* group (Ayarzagüena and Señaris, 1994) and *H. benitezi. Hyla rhythmicus* differs from *H. aromatica* and *H. inparquesi* by being smaller (32.9–34.2mm vs. 43.6–50.4 mm), dorsum reddish brown with continuous and broken dark brown bands (vs. dorsum brown or greenish brown without dark bands), quadratojugal absent (vs. present), cartilaginous process in the eyelid absent (vs. present) and an advertisement call consisting of a train of modulated notes with a dominant frequency at 3260–3450 Hz (vs. a more clearly pulsed call with frequencies between 1600 and 2400 Hz). Futhermore, living individuals of the *H. aromatica* group have a strong odor (similar to curry), a characteristic that is unknown in other hylids from the Pantepui Region.

After a comparison of the different populations in the Venezuelan Guayana, Myers and Donnelly (1997) concluded that at least two species may be included in *H. benitezi*. Despite significant differences in body size and variation in the presence of the ulnar tubercles and call characteristics among populations, *H. rhythmicus* is easily identified by the absence of orange coloration of the digits and webbing, its characteristic advertisement call and the absence of a quadratojugal. The same characteristics also separate *H. rhythmicus* from *H. lemai*; the latter is known from elevations of 850 and 1200 m in

the Escalera Región (Sierra de Lema) and the northern part of the Gran Sabana, Bolívar State (Duellman, 1997).

Rivero (1961) described *Hyla loveridgei* from the uplands (1000 m) of the Cerro Marahuaca in Amazonas State and mentioned that this species has a reddish brown dorsum marbled with black, a general coloration similar to that of *H. rhythmicus*. Nevertheless, *H. loveridgei* has numerous small whitish spots on its flanks, less webbing and a brown nuptial excrescence, all of these characteristics easily separate it from *H. rhythmicus*.

Acknowledgments.—The Ministerio del Ambiente y de los Recursos Naturales (MARN) and Instituto Nacional de Parques (INPARQUES) provided permits for collection of specimens. Fieldwork was made possible by the Fundación La Salle de Ciencias Naturales (FLASA) and Electrificación del Caroní C. A. (EDELCA). Conrad Vispo kindly translated the preliminary version of the manuscript. We are indebted to W. R. Heyer, W. E. Duellman, C. W. Myers, and M. A. Donnelly for their criticism and comments on the manuscript.

Literature Cited

Ayarzagüena, J. 1992. Los centrolénidos de la Guayana Venezolana. Publicaciones de la Asociación Amigos de Doñana 1:1–48.

Ayarzagüena, J., and J. C. Señaris. 1994. Dos nuevas especies de *Hyla* (Anura; Hylidae) para las cumbres tepuyanas del Estado Amazonas, Venezuela. Memoria de la Sociedad de Ciencias Naturales La Salle 139:127–146.

Ayarzagüena, J., J. C. Señaris, and S. Gorzula. 1992a. El grupo *Osteocephalus rodriguezi* de las tierras altas de la Guayana venezolana: descripción de cinco nuevas especies. Memoria de la Sociedad de Ciencias Naturales La Salle 52:113–142.

———. 1992b. Un nuevo género para las especies del grupo *Osteocephalus rodriguezi* (Anura: Hylidae). Memoria de la Sociedad de Ciencias Naturales La Salle 52:213–221.

Dingerkus, G., and L. D. Uhler. 1977. Enzyme clearing of alcian blue stained whole small vertebrates for demostration of cartilage. Stain Technology 52:229–232.

Donnelly, M. A., and C. W. Myers. 1991. Herpetological results of the 1990 Venezuelan Expedition to the summit of Cerro Guaiquinima, with new tepui reptiles. American Museum Novitates 3017: 1–54.

Duellman, W. E. 1970. The hylid frogs of Middle America. Monograph of the Museum of Natural History, University of Kansas, Lawrence.

———. 1997. Amphibians of the Escalera Region, southeastern Venezuela: taxonomy, ecology and biogeography. Scientific Papers Natural History Museum, University of Kansas 2:1–52.

———. 1999. Distribution patterns of amphibians in South America. *In* W. E. Duellman (ed.), Patterns of Distribution of Amphibians. A Global Perspective, pp. 255–328. Johns Hopkins University Press, Baltimore, MD.

Duellman, W. E., and L. Trueb. 1986. Biology of Amphibians. McGraw-Hill Book Co., New York.

Gorzula, S., and J. C. Señaris. 1999 [''1998'']. Contribution to the herpetofauna of the Venezuelan Guayana I. A data base. Scientia Guaianae 8:1–270.

Heyer, W. R., A. S. Rand, C. A. G. da Cruz, O. L. Peixoto, and C. E. Nelson. 1990. Frogs of Boracéia. Arquivos de Zoologia, Sao Paulo 31:231–410.

Huber, O. 1987. Consideraciones sobre el concepto de Pantepui. Pantepui 2:2–10.

———. 1995. Geographical and physical features. *In* J. Steyermark, P. E. Berry and B. K. Holst (eds.), Flora of the Venezuelan Guayana. Vol. 1. Introduction, pp. 1–61. Timber Press, Inc., Portland, OR.

La Marca, E. 1996. Ranas del género *Colostethus* (Amphibia: Anura: Dendrobatidae) de la Guayana venezolana, con la descripción de siete nuevas especies. Publicaciones de la Asociación Amigos Doñana 8:1–64.

Myers, C. W. 1997. Preliminary remarks on the summit herpetofauna of Auyantepui, eastern Venezuela. Acta Terramaris 10:1–5.

Myers, C. W., and M. A. Donnelly. 1996. A new herpetofauna from Cerro Yaví, Venezuela: first results of the Robert G. Goelet American Museum-Terramar Expedition to the northwestern tepuis. American Museum Novitates 3171:1–56.

———. 1997. A tepui herpetofauna on a granitic mountain (Tamacuri) in the borderland between Venezuela and Brazil: report from the Phipps Tapirapecó Expedition. American Museum Novitates 3213:1–71.

———. 2001. Herpetofauna of the Yutajé-Corocoro Massif, Venezuela: secnd report from the Robert G. Goelet American Museum-Terramar Expedition to the northwestern tepuis. Bulletin of the American Museum of Natural History 261:1–85.

———. 1982. A new species of *Hyla* from Cerro Colorado, and other tree frog records and geographical notes from western Panama. American Museum Novitates 2752:1–32.

Rivero, J. A. 1961. Salientia of Venezuela. Bulletin of the Museum of Comparative Zoology 126:1–207.

Savage, J. M., and W. R. Heyer. 1967. Variation and distribution in the treefrog genus *Phyllomedusa* in Costa Rica, Central America. Beiträge zur Neotropischen Fauna 5:111–131.

Señaris, J. C., and J. Ayarzagüena. 1996. Fauna: anfibios y reptiles. *In* J. Rosales and O. Huber (eds.), Ecología de la Cuenca del Río Caura, Venezuela I. Caracterización general. Scientia Guaianae 6:80–82.

Señaris, J. C., J. Ayarzagüena, and S. Gorzula. 1994. Los sapos de la familia Bufonidae (Amphibia: Anura) de las tierras altas de la Guayana venezolana: descripción de un nuevo género y tres especies. Publicaciones de la Asociación Amigos Doñana 3:1–37.

———. 1996. Revisión taxonómica del género *Stefania* (Anura: Hylidae) en Venezuela, con la descripción de cinco nuevas especies. Publicaciones de la Asociación Amigos Doñana 7:1–57.

Trueb, L. 1993. Patterns of cranial diversity among the Lissamphibia. *In* J. Hanken and B. K. Hall

(eds.), The skull. Vol. 2. Patterns of Structural and Systematic Diversity, pp. 255–343. University of Chicago Press, Chicago.

Accepted: 7 March 2002.

APPENDIX 1

Specimens Examined

Hyla aromatica.—Venezuela: *Amazonas*: Cumbre del tepuy Huachamacari (03°50′N, 65°45′W), 1700 m, MHNLS 12808 (cleared and stained), 12510–12.

Hyla benitezi.—Venezuela: *Amazonas*: Río Negro, Monte Duida (03°29′N, 65°35′W), 1180 m, MHNLS 13548, 13549 (cleared and stained); Cuenca Alta del Río Ventuari (04°14′N, 64°58′W), 1230 m, MHNLS 12052–12053; *Bolívar*: Selva El Abismo, Cantarrana, MHNLS 9496–99; Cerro Jaua, Parque Nacional Jaua-Sarisariñama (04°49′55″N, 64°25′54″W), 1600 m, MHNLS 12947–48, 12964–71, 12972 (cleared and stained).

Hyla inparquesi.—Venezuela: *Amazonas*: Cumbre del tepuy Marahuaca-Sur (03°40′N, 65°27′W), 2600 m, MHNLS 12338.

Hyla lemai.—Venezuela: *Bolívar*: Quebrada a orillas de la carretera El Dorado-Santa Elena de Guairén, km 117, Parque Nacional Canaima (05°58′N, 61°22′W), 1025 m, MHNLS 13430–13432, 13436–13439, 13442 (cleared and stained). Quebrada de Jaspe, San Ignacio de Yuruaní, 1000 m, MHNLS 9437 (cleared and stained).

Journal of Herpetology, Vol. 36, No. 4, pp. 640–648, 2002

Nesting Behavior of a Gladiator Frog *Hyla boans* in Peru

JOANNA BURGER,[1,2] WILFREDO ARIZABAL,[3] AND MICHAEL GOCHFELD[4]

[1]*Division of Life Sciences, Rutgers University, 604 Allison Road, Piscataway, New Jersey 08854-8082, USA; E-mail: burger@biology.rutgers.edu*

[3]*Museo de Historia Natura de la Universidad Nacional de San Antonio, Abad del Cusco, MHNUNSAAC, Urb. Los Angeles E-1, Cusco, Peru*

[4]*Environmental and Community Medicine, UMDNJ-Robert Wood Johnson Medical School, Piscataway, New Jersey 08854, USA*

ABSTRACT.—The temporal pattern, basin construction and egg-laying of the Neotropical gladiator frog *Hyla boans* were studied along a small stream that enters the Manu River in Peru in August 2000. Initially we located 60 basins, which were all destroyed by flood waters; subsequently 89 basins were constructed in the same area. In basins where eggs were deposited, oviposition usually occurred on the night the basin was constructed (60%) or during the following night (30%). Of the breached basins that were initially unused the first night, but were subsequently used, 91% were repaired before oviposition. Significantly more basins were located on the top of sand islands than in the center or along the other edges. Basins with eggs had significantly higher rims than those without eggs, and new basins and those with eggs had deeper water than basins with tadpoles or old basins that were disintegrating. Of the basins constructed following the flood, 55% ultimately had eggs, 89% of the eggs hatched in these basins, and 91% of the tadpoles reached maturity or left the basin when the rims were breached. Of the eggs that hatched, 89% of the clutches hatched on the second day after egg-laying, and 11% hatched on the third day. Of the total 146 basins we located, tadpoles reached maturity in five of the original 60 and in 38 of the 86 basins constructed after the flood, for an overall success rate of 29%. However, considering only those basins with eggs, 43 of 81 clutches were successful (53%).

Frogs deposit eggs in a wide range of habitats, including underground breeding chambers (Kaminsky et al., 1999), foam nests (Haddad et al., 1990), and on tree roots or creek banks (Richards and Alford, 1992), as well as more traditional aquatic habitats. Duellman and Trueb (1986) recognized nearly 30 reproductive modes within the Anura and noted that anurans in tropical regions exhibit the greatest variation. Several species of frogs deposit eggs in basins that males construct, including species in the *Litoria lesueuri* (Richards and Alford, 1992) and *Hyla boans* groups (or complex, Caldwell, 1992; Hobel, 1999; Martins, 1993; Magnusson et al., 1999).

Frogs in the *H. boans* group, known as gladiator frogs, are distributed from Central America (Costa Rica, Panama) into northern and southeastern South America (north of Argenti-

[2] Corresponding Author.

na, south and southeastern Brazil; Kluge, 1979; Frost, 1985; Rodriguez et al., 1993). Of 49 northeastern Ecuadorian frogs for which Crump (1974) had temporal breeding data, only two (including *H. boans*) were considered sporadic dry season breeders. In some species (e.g., *Hyla rosenbergi*), eggs may be laid in puddles or depressions left by cattle footprints on wet ground, as well as in male-constructed basins (Hobel, 1999). The nest is constructed by males, although it may be repaired by females prior to oviposition (Kluge, 1981). Nests are constructed on mud, sand, or other suitable substrates at the edge of streams (Caldwell, 1992). Nests of *H. boans* have previously been described from Brazil (Caldwell, 1992; Magnusson et al., 1999). Magnusson et al. (1999) found that 74% of the nests had connections to streams, and Caldwell (1992) reported that most nests were circular.

This habit of nest building has been interpreted as a method to protect the early developmental stages against the high diversity of aquatic predators (including cannibalism by conspecific tadpoles) in adjacent streams (Kluge, 1981; Martins, 1993; Caldwell, 1992) and as a method to raise water temperature to enhance growth and development (Lamotte and Lescure, 1977). Despite the occurrence of nest building in several species, there are relatively few descriptions (but see Martins, 1993; Martins and Moreira, 1991; Magnusson et al., 1999) and those that have been made involve few nests (e.g., Caldwell, 1992; Magnusson et al., 1999).

In this paper, we examine the temporal and spatial pattern of site selection, construction, and use of basins in *H. boans* breeding in a small stream (quebrada) that flows into the Manu River, in Manu, Peru. Our observations were made in August, near the end of the dry season when the Neotropical gladiator frog, *H. boans*, breeds, unlike others in this group (Magnusson et al., 1999). Our objectives were to compare basin site characteristics with those of matched points, to compare basins ultimately used for egg-laying with unused basins, and to examine the temporal pattern of basin construction and use. We provide information on 146 basins, 59 of which contained eggs or tadpoles.

Initially we located 60 basins and were monitoring them when a two-day rainstorm raised water levels sufficiently to wash out all nests. When the water levels receded, new basins were constructed, allowing us to examine the temporal pattern of basin construction and basin use. Hereafter we refer to the ''nests'' as basins, because the frogs constructed a circular basin with a rim; eggs were sometimes laid in these basins (making them a nest), but this was not always so. In the literature, it is not always clear whether nests contained eggs. It would be helpful if this distinction were clear in future papers because a basin that never has eggs may reflect either a lack of interest on the part of female's (which may in turn reflect a suboptimal location) or that it was constructed by a male that is in some way not optimal (as judged by the female).

Materials and Methods

Observations were made within Manu National Park in Peru between 7 and 20 August 2000 along a narrow stream that empties into the Manu River. The stream entered the river just south of the Machiguenga Ccollpa Biological Station (11°50.292′S; 71°25.546′W), at an elevation of about 350 m, and the site is about 12 h (depending on water depth) upriver by boat from the junction of the Manu and the Alto Madre de Dios Rivers, near the town of Boca Manu. Ecological features are extensively described in the book *Manu* by Wilson and Sandoval (1996). Our study area was selected because it is above the tourist zone, reducing the likelihood of human disturbance. At the stream mouth, there is a dense stand of *Tessaria* trees and giant cane (*Gynerium*), behind which is low-lying *varzea* forest dominated by *Cecropia* trees with a rich flora of palms (Palmaceae); this area floods during the rainy season. This is similar to the vegetation studied in greater detail at Cocha Cashu Biological Station (18 km downriver, Terborgh, 1985).

Initially, the stream was shallow, with numerous small sand islands, and sand bars or flats along the stream banks since it had not rained for several weeks (F. Fernandez, pers. comm.). It rained heavily from 7–10 August, swelling the creek by 1 m and flooding all the existing basins. Thereafter the stream level dropped, and daily censusing established the timing of basin construction and egg deposition by *H. boans*. All basins were examined for their location, and nests initiated after the flood were examined for temporal patterns and success. Without marked males, it was impossible to determine whether the same frog displayed from the basin on subsequent nights.

A 2-km section of stream was censused each morning, during which the contents of all basins were recorded, and new basins were marked by affixing a numbered tape to a nearby tree, fallen log, or post. For each basin, we recorded the type of basin (newly constructed, with eggs, with tadpoles, old), the number of eggs or tadpoles, rim (or rampart) height, and the distance to the nearest basin. An old nest was one that did not have well-constructed walls and appeared to be washed over with water.

For analysis of basin site selection, the char-

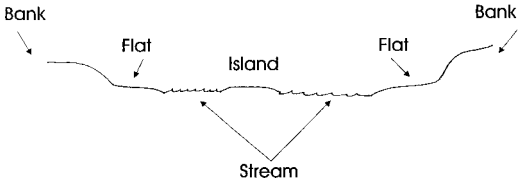

FIG. 1. Schemmatic of study area showing major habitat examined.

acteristics of basins were compared to a set of matched points located 2 m from the basin in a randomly selected direction from that basin. This method was used rather than points chosen completely at random because the stream meandered, the frogs built basins in and adjacent to the stream rather than on dry land, and there were long sections of the stream without any nests. Further, the frogs could easily have picked a different site within 2 m of the one they chose but may not have constructed one several hundred meters away (as would occur with random points). The following characteristics were recorded: location (on low sandy island, in shallow water at edge of stream or island, on flat or sand bar at edge of stream, or adjacent to bank, Fig. 1), length of the island or sand bar, water depth, and the distance to stream water, the closest current, and the bank. For basins, water depth was recorded in the middle of the basin, and for the matched points, it was recorded at the point. For those basins on islands, we recorded basin location on the island (at apex [leading edge], on any other edge, or in the center more than 30 cm from the edge), and the location on the island relative to the stream flow (top 25% of the island, second 25%, third 25%, or bottom 25%). A basin could be on the leading apex of the island, facing into the current, or at the bottom point of the island. A nest was considered successful if the eggs hatched and tadpoles grew to a size where they maneuvered out of the nest into the main stream on their own. The number of eggs in basins was determined by counting of eggs from nine basins in which eggs were laid the night the basin was constructed. We counted the number of eggs using a grid superimposed on photographs.

We used Kruskal-Wallis ANOVA (Wilcoxon matched pairs option) on the data with a Fisher Exact test to examine for differences between basins and matched points (Statistical Analysis Institute, Inc. Cary, NC, 1995, unpubl.). To compare the characteristics among basin types, we used a Kruskal-Wallis ANOVA with three degrees of freedom. In comparing the temporal patterns, we used a contingency chi-square test. The level for statistical significance was designated as < 0.05, but values between this level and 0.1 are presented.

RESULTS

Temporal Patterns and Basin Use.—Hyla boans males began to call at sunset from the surrounding trees and started descending to the creek level about one-half hour after sunset. After this time, we found males on logs, on sand, swimming in the water, constructing basins, and amplexing with females until about 2100 h; thereafter males were scarce although they continued to call from low shrubs and trees until about 2200. Males did not call from the water, logs in the water, or nest basins.

Most basins constructed by *H. boans* were nearly circular (mean of 34 cm long), had intact rims, and were filled with water (Fig. 2). Only one egg mass of *H. boans* was located outside of a constructed basin, and it was in a depression caused by intersecting branches and twigs forming walls. The 60 basins initially located were all destroyed by flooding; the waters rose slowly, however, and some of the tadpoles that were washed out of basins found their way to the edge of the stream and clung to logs and branches. We estimated that at least five of the original basins with tadpoles were successful, based on finding *H. boans* tadpoles close to the original basin, and clinging to branches or debris. However, we found no intact eggs after the water rose. The rains stopped on 10 August, and the water level began dropping. Most of the first basins that were constructed after the water level fell did not have eggs (Fig. 3). By nine days after the cessation of the rains, almost no new basins were being constructed.

Of the 86 basins constructed in the week following the cessation of rains, 55% ($N = 47$) ultimately had eggs; mean eggs per nest = 1980 (range of 1160–3987). The mean was somewhat lower than Crump (1974) obtained by counting ovarian eggs (mean of 3154). The eggs in 89% of the basins hatched, and the tadpoles reached maturity and left the basins when the rims were breached by slow-moving currents in 91% of the nests (Table 1). Of the nests in which eggs hatched, 89% of the clutches were partially or completely hatched on the second day after egg-laying, and 11% hatched on the third day. Of the entire 146 basins we located, tadpoles reached maturity in five of the original 60 basins, and 38 of the 86 basins constructed after the flood, for an overall success rate of 29%. However, considering only those basins with eggs, 43 of 81 clutches were successful (53%, Table 1).

Most new basins were initially intact, and were not breached (76%). Significantly more of the new basins in which eggs were laid the

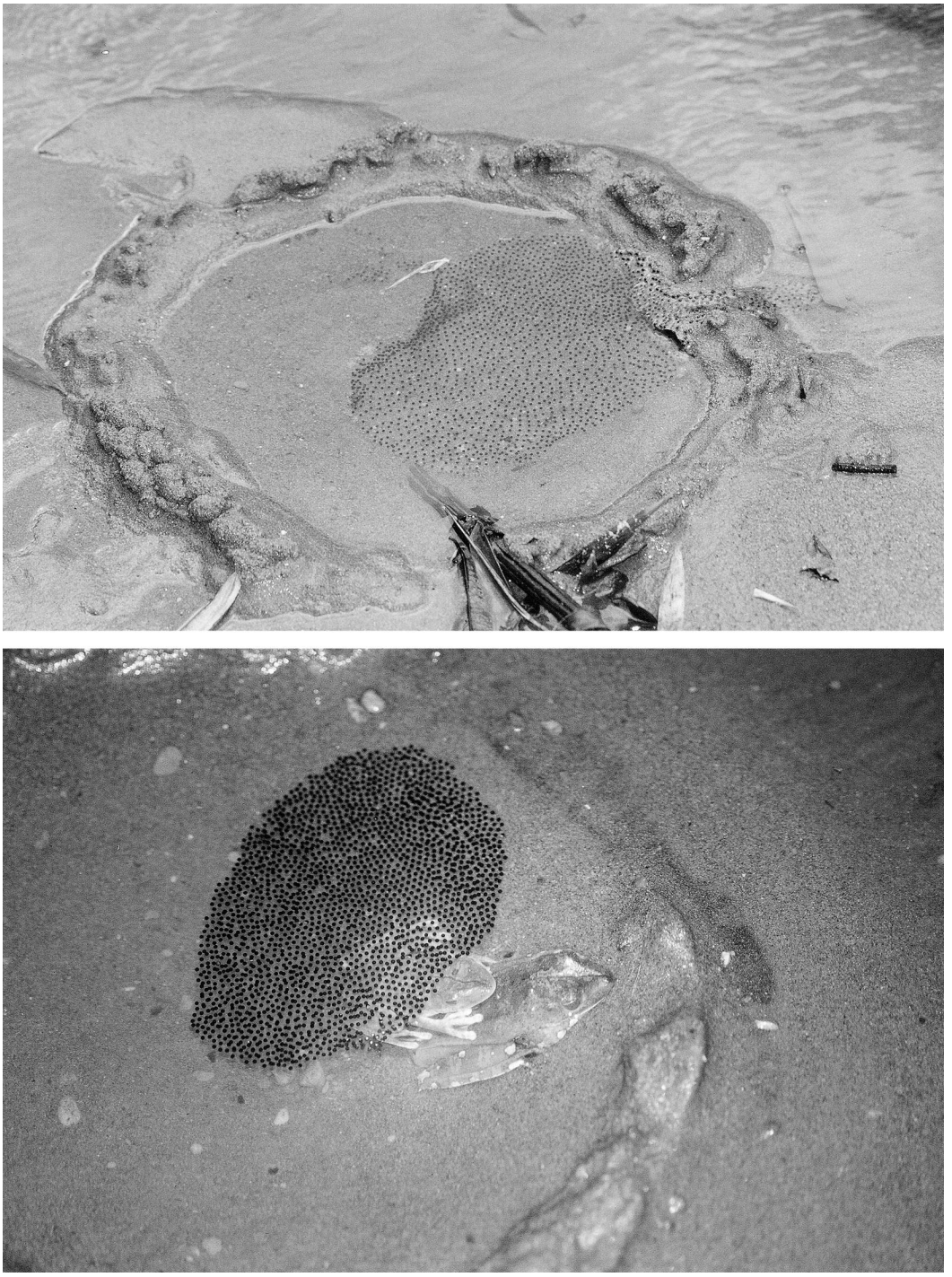

FIG. 2. Photograph of recently constructed basin and amplexing frogs with eggs.

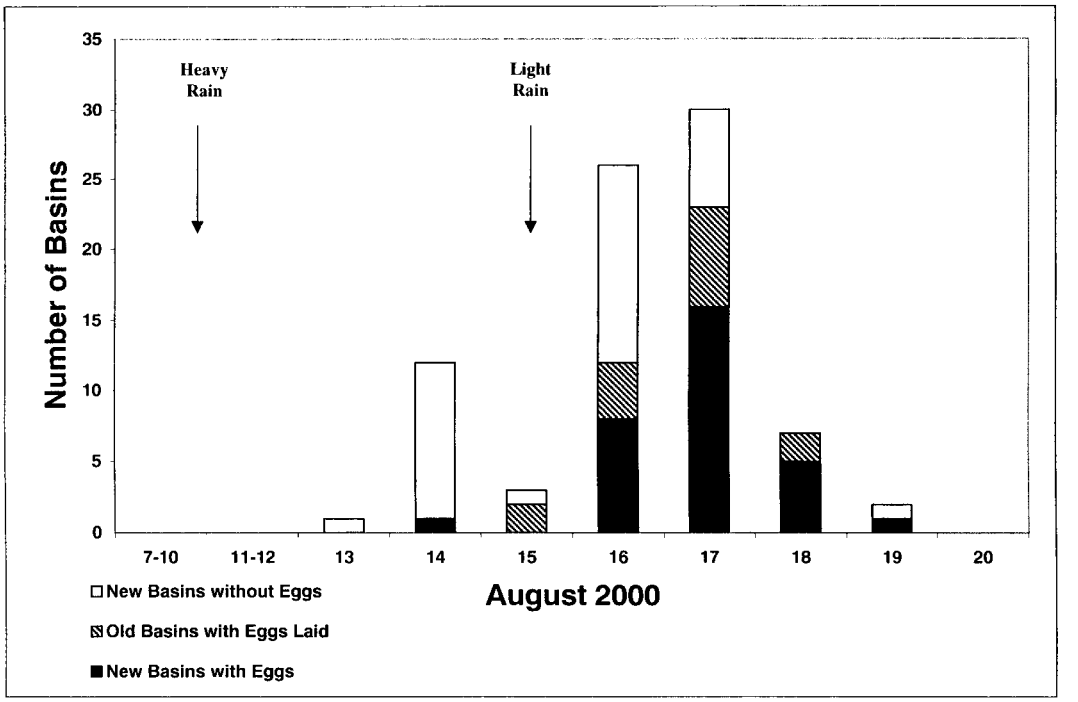

FIG. 3. Number of basins of *Hyla boans* initiated in August after the heavy rains of 7–10 August 2000 (Manu National Park, Peru).

night they were constructed were unbreached (85%), compared to those without eggs (67%, Fisher Exact Test, $P = 0.015$). However, after the first day, some of these basins were breached by adjacent currents, and in two, some of the eggs floated out of the basins.

In most basins, eggs were laid the night the basin was constructed or during the following night (Fig. 4). Of the breached basins that were initially unused the first night, but were subsequently used, 91% were repaired the night eggs were laid in them. Thus, basins that were not used initially were more often breached than those that were used, and basins that were used on subsequent nights were repaired before eggs were laid in them. Basins in which no eggs are laid eventually are worn away by currents; all such basins disappeared within six days of construction (Fig. 4).

TABLE 1. Reproductive success in basins of *Hyla boans* in a tributary of Manu River Machiquenga Ccollpa Biological Station in Peru.

	Preflood basins	Postflood basins
Initial basins	60	86
Contained eggs	12	47/86 (55%)
Contained tadpoles	22	—
Basin flooded out	29/34 (85%)	0
Hatched	—	42/47 (89%)
Basin from which hatched tadpoles reached stream	5/22 (23%)[a]	38/42 (91%)
Basin with eggs from which hatched tadpoles reached stream successfully	5/34 (15%)	38/47 (81%)

[a] During flooding.

Basin Site Selection.—Although there were some solitary basins, basins were generally clumped, with up to six on islands 3–6 m long. Distances between solitary or groups of clumped basins were often greater than 25 m. Basins constructed after the rain were in the same areas of the stream as the washed-out basins, although their exact location varied because of the creation of new sandbars and islands.

There were significant differences between the matched points and the nest basins (regardless of nest contents, Fig. 5). Basins could be constructed on sandy islands, in the water, on sandy flats on the sides of streams, adjacent to banks, or on banks, but most basins were located on islands, with others on flats or adjacent to banks, which differed significantly from the

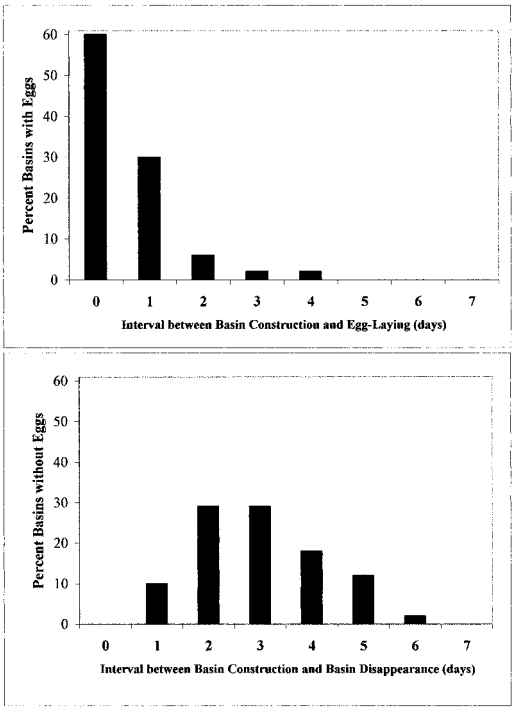

FIG. 4. Interval between basin construction and egg-laying of *Hyla boans* of 47 nests with known fate that were initiated after heavy rains washed out all previous nests (top); interval between basin construction and basin disappearance (never having eggs) for 39 nests initiated in August 2000 (bottom).

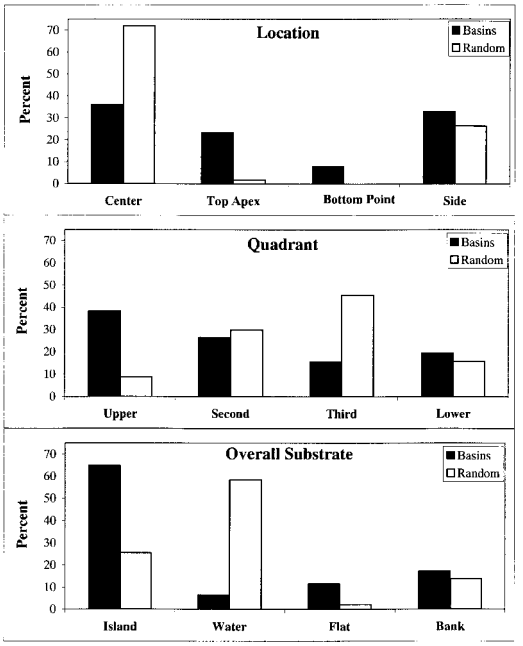

FIG. 5. Comparison of basin sites of *Hyla boans* with random points on sandy islands (top and middle), and overall substrate of all basins (bottom). Shown are whether nests were in the center of the island, along the top or bottom point, or along the sides of islands (top), or in the upper, second, third, or lower quarter of the island relative to stream flow (middle). All basins differed significantly ($P < 0.0001$) from matched points.

matched points (Fig. 5). Significantly more basins were located on the apex of sand islands, and at the upstream end, than in the center or along the other edges than were the matched points. Further, significantly more basins were located on the upstream quarter of islands (Fig. 5), perhaps to ensure that tadpoles would eventually make it to the stream. Basins were closer to the stream than were matched points, and the water was shallower in the basins than in the matched points (Table 2).

We compared characteristics of basins that initially had eggs, tadpoles (of the first 60 located before floods), were new, or were old and

TABLE 2. Comparison of all *Hyla boans* basins with matched points (Wilcoxon matched-pairs test). All distances and dimensions in centimeters. NS = Not Significant ($P > 0.09$).

	Basins	Random	Kruskal-Wallis χ^2
All basins ($N = 139$)			
Island length	1,010 ± 71.3	1,570 ± 151	6.93 (0.009)
Water depth	5.37 ± 0.11	7.64 ± 2.97	35.2 (<.0001)
Distance to bank	250 ± 17.7	245 ± 18.1	0.04 (NS)
Distance to stream	16.7 ± 3.20	35.6 ± 6.55	2.93 (0.09)
Distance to current	103 ± 8.10	112 ± 11.7	2.33 (NS)
Occupied basins only ($N = 59$)			
Island length	928 ± 119	1,630 ± 286	5.47 (0.02)
Water depth	5.39 ± 0.14	5.40 ± 1.06	12.8 (0.0003)
Distance to bank	244 ± 28.0	247 ± 29.3	0.01 (NS)
Distance to stream	13.9 ± 3.32	26.8 ± 7.30	2.82 (0.09)
Distance to current	106 ± 14.48	104 ± 18.9	2.82 (0.09)

TABLE 3. Characteristics of *Hyla boans* basins in a stream near the Manu River, Machiquenga Ccollpa Biological Station. Different letters differ significantly, using the Duncan Posthoc Test. NS = Not Significant ($P > 0.09$).

	New	Eggs	Tadpoles	Old	ANOVA F (P)
	61	43	22	19	
Island length (cm)	998 ± 82.4 AB	795 ± 101 B	1,110 ± 243 AB	1,300 ± 239 A	3.64 (NS)
Nearest neighbor (cm)	772 ± 132 A	238 ± 41 B	490 ± 116 AB	367 ±134 AB	9.73 (0.02)
Basin dimension: (cm)					
Length	34.3 ± 0.80 A	34.2 ± 0.9 A	34.8 ± 0.97 A	31.7 ± 1.03 A	4.45 (NS)
Width	29.3 ± 0.56 A	28.9 ± 0.7 A	30.7 ± 0.81 A	29.0 ± 1.44 A	2.87 (NS)
Rim height	3.87 ± 0.16 A	4.44 ± 0.2 A	3.83 ± 0.30 A	2.83 ± 0.27 B	19.7 (0.0002)
Water depth	5.76 ± 0.14 A	5.6 ± 0.2 AB	5.03 ± 0.24 B	4.07 ± 0.37 C	19.9 (0.0002)
Distance to: (cm)					
Stream	13.1 ± 4.51 B	12.2 ± 2.8 B	16.8 ± 7.65 B	36.7 ± 14.8 A	4.85 (NS)
Bank	206 ± 23.2 B	203 ± 33.5 B	316 ± 46.6 A	406 ± 48.5 A	16.5 (0.0009)
Current	101 ± 10.4 A	113 ± 20.8 A	95.2 ± 17.3 A	101 ± 20.2 A	0.63 (NS)

disintegrating. Basin site selection was examined when basins were first located; thus sample sizes reflect conditions at that time, and some new basins without eggs subsequently had eggs. Basins with eggs had significantly higher basin rims than others, and new basins and those with eggs had deeper water than nests with tadpoles or old basins that were disintegrating (Table 3). Distances to nearest neighbor basins were greater for new basins compared to those with eggs or tadpoles (Table 3). New basins and those with eggs were closer to stream banks than were other types of basins (Table 3).

DISCUSSION

The *H. boans* group shows diversity in basin or nest sites; nests can be on leaf litter or roots (Magnusson et al., 1999; Martins and Moreira, 1991), on pond margins (Martins, 1993), or on margins of swamps or on wet ground up to 30 m away from a swamp (Hobel, 1999). Other species in the *H. boans* group construct basins nearly identical to those of *H. boans*, including *Hyla faber* (Lutz, 1960; Martins and Haddad, 1988). In *H. rosenbergi* the male-constructed nests (only 29%) were depressions in mud in vegetation and did not have rims or ramparts (Hobel, 1999). The nests of *Hyla wavrini*, located in roots and depressions, do not have distinct rims or ramparts (Martins and Moreira, 1991). Members of the *Litoria lesueuri* group in Australian rain forests construct basins that resemble those of *H. boans* (Richards and Alford, 1992). These basins are also circular, and some nests are located at the edge of stream channels or on stream banks (Richards and Alford, 1992). Nests on wet mud or in vegetation away from swamps or streams may be less vulnerable to heavy rains and floods because the rain can seep into the ground, whereas in the stream we studied the rains resulted in rising water levels sufficient to flood out and obliterate all *H. boans* basins. Although the basins constructed by *H. boans* in this study had solid rims, they were not sufficient to prevent flooding.

August is generally the dry season in Peru, but in 2000, there were heavy rains that raised the water levels in streams and rivers. The 146 basins of *H. boans* fell into two categories: those that experienced a heavy flood and were destroyed; and those that were not exposed to heavy flooding. Both groups of basins were constructed following rains, but the second group was followed by a period without rain. These two groups clearly illustrate the effects of rain and flooding on nests that are located in streams.

None of the 146 basins of *H. boans* in this study were on sandy banks, and only one egg mass was placed in a natural basin created by twigs. Magnusson et al. (1999) reported that 10 of 16 nests were in natural pools with no evidence of modification by the frogs, in sharp con-

trast to our study. Further, Magnusson et al. (1999) reported that four of six nests were modifications of preexisting pools formed by root mats. Partly this difference may relate to an absence of pools of sufficient depth for egg-laying and development and to the presence of wet malleable mud in our study area. There were few roots in the stream that could have provided natural basins.

The basins of *H. boans* in Manu averaged 34 cm in diameter, which is comparable with the 36 cm reported by Caldwell (1992) but smaller than the 44 cm reported by Magnusson et al. (1999). Magnusson et al. (1999) reported mean nest depths of 8.5 cm, which is equivalent to what we found (adding water depth and rim height). Caldwell (1992) reported that water depth of newly formed basins at the center averaged 5.2 cm, which is similar to the 5.8 cm we found. Thus, the basins of *H. boans* constructed in the Manu stream are similar to those reported elsewhere. In Manu, all the *H. boans* basins located had only one clutch of eggs, in contrast to *H. faber*, where up to six clutches were deposited in one basin (Martins, 1993). Similarly, we only observed one male in any basin, whereas Martins (1993) reported up to four.

The evolutionary significance of male-constructed basins for deposition of eggs in the *H. boans* group has been the subject of debate (Caldwell, 1992). The function of the nest may be to prevent desiccation, increase temperature or reduce predation (Kluge, 1981; Caldwell, 1992; Martins, 1993; Magnusson et al., 1999). Predators can have an effect when basins are breached and connected to streams (Martins et al., 1993). Kluge (1981) observed other species of tadpoles preying on eggs and embryos of *H. rosenbergi* and that cannibalism was highly efficient. Caldwell (1992) reported that more mortality occurred in clutches in nests as opposed to those not in nests, but both predation and drying were sources of mortality.

In the Magnusson et al. (1999) study, 75% of the nests were connected to streams by channels deep enough for fish and macroinvertebrate predators to enter, and these basins frequently contained fish. Martins (1993) reported that aquatic insects accounted for egg and larvae loss in *H. faber*. Both vertebrate and invertebrate predators were observed feeding on *H. faber* eggs or tadpoles (Martins et al., 1993). Once the tadpoles left the basins, they were preyed upon by a range of insects, and metamorphosing froglets were preyed upon by snakes and adult frogs (Martins et al., 1993).

In contrast, only 24% of new basins constructed in our study were breached, only 15% of those with eggs were breached, and there were no fish, invertebrates, or tadpoles of other species in these nests. The *H. boans* in the Manu study site constructed nests with strong intact rims providing a barrier for fish and macroinvertebrates that could have been predators. This resulted in a relatively high hatching rate of eggs (89%).

In the basins of *H. boans* observed in Manu, breached basins were repaired before eggs were laid, implying that having an intact nest was important. Further, in basins not exposed to flooding, success was relatively high, perhaps because of a lack of aquatic predators because the basins were intact. With time, however, nests began to disintegrate, creating connections with the nearby stream. Because most basins in this study were very close to stream waters, the sand was wet, and with time, the rims began to sink. Eventually, water seeped through, but rarely before the tadpoles were two or three days old. We did not observe any desiccation in the basins. Frogs did not lay eggs in all basins; most basins that were used for eggs had them deposited there within two nights of construction. Basins never used for eggs disintegrated more quickly than those used for eggs, suggesting that their location was suboptimal and eggs may not have survived until the tadpoles were old enough to enter the main stream.

Acknowledgments.—The authors thank F. Fernandez, A. Flores, and M. Palacio for logistical help while in Peru; K. Herring and the U.S. and Peruvian staffs of Inkanatura for logistical arrangements and provision of a well-trained field staff; and Tiko for financial support. We thank the Peruvian government and the Manu National Park for permits for work in the restricted area of the park (019-00).

Literature Cited

Caldwell, J. P. 1992. Diversity of reproductive modes in Anurans: facultative nest construction in gladiator frogs. *In* W. C. Hamlett (ed.), Reproductive Biology of South American Vertebrates, pp 85–97. Springer-Verlag, New York.

Crump, M. L. 1974. Reproductive strategies in a tropical anuran community. University of Kansas Museum of Natural History Miscellaneous Publication 61:1–68.

Duellman, W. E., and L. Trueb. 1986. Biology of Amphibians. McGraw-Hill Book Co., New York.

Frost, D. R. (ed.). 1985. Amphibian Species of the World. Allen Press, Lawrence, KS.

Hadad, C. F. B., J. P. Pombal Jr., and M. Gordo. 1990. Foam nesting in a Hylid frog (Amphibia, Anura). Journal of Herpetology 24:225–226.

Hobel, G. 1999. Facultative nest construction in the gladiator frog *Hyla rosenbergi* (Anura: Hylidae). Copeia 1999:797–801.

Kamindky, S. K., K. E. Linsemair, and T. U. Grafe. 1999. Reproductive timing, nest construction and tadpole guidance in the African pig-nosed frog,

Hemisus marmoratus. Journal of Herpetology 33: 119–123.

KLUGE, A. G. 1979. The gladiator frogs of Middle America and Colombia—a reevaluation of their systematics (Anura: Hylidae). Occassional Papers of the Museum of Zoology, University of Michigan 688:1–24.

———. 1981. The life history, social organization, and parental behaviour of *Hyla rosenbergi* Boulenger, a nest-building gladiator frog. Miscellaneious Publications of the Museum of Zoology, University of Michigan 160:1–170.

LAMOTTE, M., AND J. LESCURE. 1977. Tendances adaptatives a l'affranchissement du milieu aquatique chez les amphibiens anoures. Terre Vie 31:225–311.

LUTZ, B. 1960. Fighting and an incipient notion of territory in male tree frogs. Copeia 1960:61–63.

MAGNUSSON, W. E., A. P. LIMA, J. HERO, AND M. C. DE ARAUJO. 1999. The rise and fall of a population of *Hyla boans*: reproduction in a Neotropical gladiator frog. Journal of Herpetology 33:647–656.

MARTINS, M. 1993. Observations on nest dynamics and embryonic and larval development in the nest building gladiator frog, *Hyla faber*. Amphibia-Reptilia 14:411–421.

MARTINS, M., AND C. F. B. HADDAD. 1988. Vocalizations and reproductive behaviour in the Smith frog, *Hyla faber* Wied (Amphibia: Hylidae). Amphibia-Reptilia 9:49–60.

MARTINS, M., AND G. MOREIRA. 1991. The nest and the tadpole of *Hyla wavrini*, Parker (Amphibia, Anura). Mem Institute Butantan 53:197–204.

MARTINS, M., I. SAZIMA, AND S. G. EGLER. 1993. Predators of the nest building gladiator frog, *Hyla faber,* in southeastern Brazil. Amphibia-Reptilia 14:307–309.

RICHARDS, S. J., AND R. A. ALFORD. 1992. Nest construction by an Australian rainforest frog of the *Litoria lesueuri* complex (Anura: Hylidae). Copeia 1992:1120–1123.

RODRIGUEZ, L. O., J. H. CORDOVA, AND I. JAVIER. 1993. Lista preliminar de los anfibios del Peru. Museo de Historia Natural, Universidad Nacional Mayor de San Marcos. Serie A Zoologia 45:1–22.

TERBOUGH, J. 1985. Habitat selection in Amazonian birds. *In* M. L. Cody (ed.), Habitat Selection in Birds, pp. 311–338. Academic Press, New York.

WILSON, D. E, AND A. SANDOVAL. 1996. Manu: The Biodiversity of Southeastern Peru. Smithsonian Institution, Washington DC

Accepted: 7 March 2002.

Journal of Herpetology, Vol. 36, No. 4, pp. 648–655, 2002

Mitochondrial Variation in Sharp-Tailed Snakes (*Contia tenuis*): Evidence of a Cryptic Species

CHRIS R. FELDMAN[1] AND GREG S. SPICER

Department of Biology, San Francisco State University, San Francisco, California 94132, USA

ABSTRACT.—We examined genetic variation and structure in mitochondrial DNA sequences of sharp-tailed snakes (*Contia tenuis*) from California and southern Oregon. Maximum parsimony and maximum likelihood analyses distinguish two mitochondrial lineages: a north coast clade restricted to cool evergreen forest along the Pacific Coast; and an interior/south clade widespread throughout California. The southern limit of the north coast clade is congruent with that of several other vertebrate taxa, a historical pattern consistent with a long-term marine embayment. We interpret additional phylogeographic pattern as resulting from either gene flow or incomplete lineage sorting. Genetic, distributional, ecological, and morphological data suggest that north coast and interior/south mitochondrial lineages of *C. tenuis* are distinct at the species level.

Knowledge of genetic variation is critical to our understanding of population genetics, speciation, and historical biogeography. Examination of variation within species has led to the discovery of genetic diversity, geographic pattern, and cryptic species (e.g., Tilley et al., 1978; Yanev, 1980; Good, 1989; Highton, 1989; Omland et al., 2000; Jockusch et al., 2001). One common approach to the study of genetic variation involves analysis of mitochondrial DNA collected at the population level and interpreted in a phylogenetic context. Resultant intraspecific phylogenies, or phylogeographies (Avise et al., 1987; Avise, 1989), allow inferences about historical patterns of vicariance, dispersal, gene flow, and population subdivision (Templeton et al., 1995; Walker and Avise, 1998). When unre-

[1] Corresponding Author. Present address: Department of Biology, Utah State University, 5305 Old Main Hill, Logan, Utah 84322, USA; E-mail: elgaria@biology.usu.edu

lated taxa with similar distributions and ecologies are examined via the phylogeographic method, congruent phylogeographic patterns often suggest shared geologic and evolutionary history (Avise, 2000).

The sharp-tailed snake (*Contia tenuis*) is one of North America's least studied ophidians; there is little natural history information available (Leonard and Ovaska, 1998), and its phylogenetic placement among xenodontine colubrids is uncertain (Cadle, 1984). One of the smallest western snakes (rarely attaining 40 cm), it is secretive, ground dwelling, and seasonally active (Cook, 1960; Leonard and Ovaska, 1998). *Contia* ranges from northern Oregon south into California with disjunct populations in British Columbia and Washington (Leonard and Ovaska, 1998; Fig. 1).

We characterized patterns of genetic variation and structure in this poorly studied snake to assess whether these patterns were consistent with geography and with patterns described in other taxa. Information on *Contia tenuis* mtDNA will be useful for testing the influence of common historical events on the evolution of other vertebrates sharing its habitat and distribution (Feldman, 2000).

Materials and Methods

Population Sampling.—We collected mitochondrial DNA sequence data from 22 individuals representing 19 localities in the southern two-thirds of the geographic distribution of *C. tenuis* (Fig. 1; Table 1). We deposited voucher specimens in the Museum of Vertebrate Zoology (MVZ) and California Academy of Sciences (CAS; Table 1).

Laboratory Protocols.—We isolated genomic DNA from liver tissue, scales and/or tail tips by standard proteinase K digestion and phenol/chloroform purification (Maniatis et al., 1982). To amplify a 900 base pair region of the ND4 gene and flanking $tRNA^{his}$, $tRNA^{ser}$, and $tRNA^{leu}$, we conducted PCR using primers ND4 (5′-CAC CTA TGA CTA CCA AAA GCT CAT GTA GAA GC-3′) and Leu (5′-ACC ACG TTT AGG TTC ATT TTC ATT AC-3′; Arevalo et al., 1994) with the following thermal cycle parameters: 35 cycles: 1 min 94°C; 1 min 52°C; 2 min 72°C. The 5′ end of primers ND4 and Leu match nucleotide positions 11,671 and 12,594, respectively, of the heavy strand of the mitochondrial genome of the snake *Dinodon semicarinatus* (Kumazawa et al., 1998). We purified PCR products with the Wizard Prep Mini Column Purification Kit (Promega, Inc.) and used purified template in 10μl dideoxy chain-termination sequencing reactions (Sanger et al., 1977) using ABI Big Dye (Perkin-Elmer Applied Biosystems, Inc.) and primers ND4 and Leu. Following an isopropanol/ethanol precipitation, we ran cycle-sequenced products on an ABI 377 automated sequencer (Perkin-Elmer Applied Biosystems, Inc.).

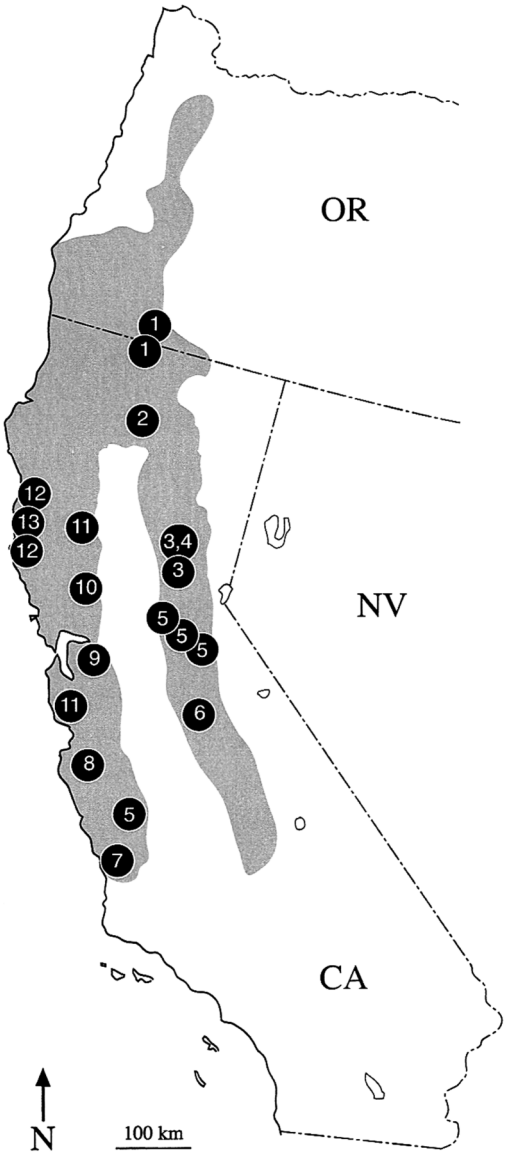

FIG. 1. Geographic range of *Contia tenuis* in California and Southern Oregon (after Stebbins, 1985). Dots indicate sample localities, numbers refer to unique mtDNA haplotypes.

Sequence Analyses.—We aligned DNA sequences with the program Sequencher™ 3.0 (Gene Codes Corp.) and translated protein coding DNA into amino acid sequences using MacClade 3.06 (W. P. Maddison and D. R. Maddison, Sinauer Assoc., Inc., Sunderland, MA, 1992, unpubl.). In addition, we identified tRNA

TABLE 1. Unique *Contia tenuis* and outgroup mtDNA haplotypes. CAS: California Academy of Sciences; MVZ: Museum of Vertebrate Zoology.

Haplotype	Locality	Museum	GenBank
1	N. of Hilt, Jackson Co., OR	no voucher	AF258879
	Hilt, Siskiyou Co., CA	CAS 210367	AF258879
2	Potter Creek, Shasta Co., CA	MVZ 164926	AF258880
3	Pike City, Sierra Co., CA	CAS 207044	AF402656
	Golden Trout, Butte Co., CA	CAS 205639	AF402656
4	Golden Trout, Butte Co., CA	CAS 205652	AF258881
5	S. of Georgetown, El Dorado Co., CA	CAS 208587	AF258882
	Rocklin, Placer Co., CA	CAS 210366	AF258882
	Chumash Circle, Calaveras Co., CA	MVZ 230096	AF258882
	Hwy 198, W. of Fresno Co. line, Monterey Co., CA	MVZ 208157	AF258882
6	Bear Valley, Mariposa Co., CA	CAS 205778	AF258883
7	Vineyard Drive, San Luis Obispo Co., CA	MVZ 208158	AF258884
	Vineyard Drive, San Luis Obispo Co., CA	MVZ 208160	AF258884
8	Hastings U.C. Reserve, Monterey Co., CA	CAS 205788	AF258885
9	Pleasant Hill, Contra Costa Co., CA	MVZ 232671	AF258886
10	Cache Creek, Yolo Co., CA	CAS 214873	AF402657
11	China Grade Rd., Santa Cruz Co., CA	CAS 205802	AF258887
	Brittan Ranch, Glenn Co., CA	CAS 202582	AF258887
12	Hwy 1 and 128 jct., Mendocino Co., CA	no voucher	AF258888
	Angelo Coast U.C. Reserve, Mendocino Co., CA	MVZ 230270	AF258888
13	Jackson State Forest, Mendocino Co., CA	MVZ 232650	AF402658
	Jackson State Forest, Mendocino Co., CA	MVZ 232651	AF402658
D. punctatus	Crystal Springs, San Mateo Co., CA	CAS 204287	AF258889
H. platirhinos	Southern Pines, Moore Co., NC	MVZ 175928	AF402659

genes by drawing their secondary structures following the criteria of Kumazawa and Nishida (1993). We deposited all sequences in GenBank (Table 1).

Using both uncorrected and maximum likelihood estimated distances, we computed pairwise sequence differences between haplotypes in PAUP* 4.0b8 (D. L. Swofford, Sinauer Assoc., Inc., Sunderland, MA, 1998, unpubl.). To test for deviations from neutrality, we calculated Tajima's D (Tajima, 1989) using the program DnaSp 3 (Rozas and Rozas, 1999).

Phylogenetic Analyses.—We used molecular genetic data to determine evolutionary histories of populations by constructing intraspecific phylogenies. To infer haplotype relationships, we used maximum parsimony (MP; Swofford et al., 1996) and maximum likelihood (ML; Felsenstein, 1981) phylogenetic methods. We conducted all phylogenetic analyses in PAUP* and coded tRNA in/dels as fifth character states. Last, we polarized the phylogeny via outgroup comparison (Maddison et al., 1984) using the xenodontine snakes *Diadophis punctatus* and *Heterodon platirhinos*.

We executed MP reconstructions with the branch-and-bound search algorithm (Hendy and Penny, 1982) using equally weighted characters. To assess nodal support, we performed a bootstrap analysis (Felsenstein, 1985) employing 1000 replicates of heuristic searches in PAUP*. Additionally, we calculated branch support (Bremer, 1988; 1994) for all nodes using the program TreeRot 2 (M. D. Sorenson, TreeRot, vers. 2, Boston, MA, 1999, unpubl.).

To estimate branch lengths and search for additional tree topologies, we performed ML analyses. To determine the most appropriate model of DNA substitution for reconstructing haplotype relationships under ML, we executed a hierarchical likelihood ratio test (LRT; J. Felsenstein, PHYLIP, vers, 3.5c, Seattle, WA, 1993, unpubl.; Goldman, 1993; Yang, 1996) via Modeltest 3.0 (Posada and Crandall, 1998). We then used a MP tree as our starting tree and estimated the ML tree successively until we obtained a stable topology (Wilgenbusch and de Queiroz, 2000).

Estimating Divergence Times.—We used gene trees, a molecular clock hypothesis, and geographic information to estimate evolutionary diversification and the degree of congruence between *C. tenuis* phylogeny and geologic events. To determine whether ND4 data are evolving in a clocklike fashion, we compared differences in log-likelihood scores for the same tree built under two different, nested models of molecular evolution (optimal model vs. molecular clock) using a LRT. Finally, we dated cladogenesis using uncorrected pairwise average distances between well-supported clades and a pairwise rate of sequence divergence of 1.3% per million years (reviewed in Macey et al., 1999, 2001).

RESULTS

Genetic Variation.—We did not find gene rearrangements in the sequenced region and ND4 appeared functional (Kumazawa and Nishida, 1995; Kumazawa et al., 1996; Macey and Verma, 1997; Macey et al., 1997). The final sequenced product was 860 bp; 694 bp of ND4 and 166 bp of tRNAs. Including outgroup, 229 nucleotide positions were variable and 101 were parsimony informative. Among *C. tenuis* samples, 73 nucleotide positions were variable, and 66 were parsimony informative. We found 13 unique mtDNA haplotypes in the 22 sharp-tailed snakes surveyed (Table 1). The model of DNA evolution that best fit these sequence data was the Hasegawa, Kishino and Yano model (HKY; Hasegawa et al., 1985) of nucleotide substitution in conjunction with gamma (Γ; Yang, 1994a,b). This model accommodates unequal base composition, estimates transition and transversion substitution ratios, and accounts for heterogeneous rates of nucleotide substitutions across all sites. The ML HKY + Γ estimated pairwise distance comparisons between *C. tenuis* and outgroup taxa revealed sequence divergences ranging from 22.95% to 34.45%, whereas sequence divergence among *C. tenuis* haplotypes ranged from 0.12% (a single transition) to 8.97% (64 nucleotide differences; Table 2). The HKY + Γ model of DNA substitution estimated a ti:tv ratio of 5.38 for the ingroup, characteristic of ND4 sequence data for snakes (e.g., Zamudio and Greene, 1997; Kraus and Brown, 1998; Rodriguez-Robles and Jesus-Escobar, 1999, 2000). A high number of nonsynonymous substitutions have occurred in ND4 but mostly between two main clades of *Contia* (see below). Thus, we cannot reject the hypothesis of neutral evolution for haplotypes in either clade using Tajima's D statistic (D = 1.4427; $P > 0.10$; Tajima, 1989).

Phylogenetic Relationships.—The MP analysis produced nine shortest trees (L = 282; CI = 0.933; RI = 0.957; Fig. 2A). The ML HKY+ Γ search yielded one optimal tree (−LnL = 2398.159; α = 0.391; ti:tv = 2.81) identical to one of the nine most parsimonious trees (Fig. 2B).

Both MP and ML analyses reveal a basal divergence of *C. tenuis* populations in California into two major clades: a north coast clade and an interior/south clade (Fig. 2). *Contia tenuis* populations of the interior/south clade (Sierra Nevada Mtns., Cascade Range, Klamath Mtns., central and interior north coast of California; 100% bootstrap; decay index 24) are identified by haplotypes 1-10, whereas those of the north coast clade (Santa Cruz Mtns. and northern Coast Range; 100% bootstrap value; decay 26) are represented by haplotypes 11–13. The interior/south clade can be divided into subclades

TABLE 2. Pairwise comparisons of mtDNA sequences among all unique *Contia tenuis* and outgroup haplotypes. Uncorrected nucleotide differences (%) above diagonal, ML HKY + Γ corrected sequence divergences below diagonal.

Haplotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	—	0.12	0.23	1.07	0.94	1.17	0.12	1.18	0.23	1.17	7.14	7.14	7.25	14.63	17.45
2	0.12	—	0.35	1.18	1.05	1.29	0.23	1.30	0.35	1.29	7.25	7.25	7.37	14.75	17.57
3	0.24	0.35	—	1.06	0.94	1.17	0.12	1.18	0.23	1.17	7.14	7.14	7.25	14.51	17.34
4	1.09	1.22	1.09	—	0.12	0.35	0.95	0.35	1.06	0.36	7.20	7.20	7.32	14.87	17.24
5	0.96	1.08	0.96	0.12	—	0.23	0.82	0.24	0.94	0.23	7.14	7.14	7.25	14.75	17.45
6	1.20	1.33	1.20	0.36	0.24	—	1.05	0.47	1.17	0.47	7.14	7.14	7.25	14.75	17.45
7	0.12	0.24	0.12	0.97	0.83	1.08	—	1.06	0.12	1.05	7.02	7.02	7.14	14.51	17.34
8	1.21	1.34	1.21	0.36	0.24	0.48	1.09	—	1.18	0.47	7.30	7.30	7.42	14.72	17.45
9	0.24	0.36	0.24	1.10	0.96	1.21	0.12	1.22	—	1.17	7.15	7.15	7.26	14.53	17.35
10	1.21	1.33	1.21	0.36	0.24	0.48	1.09	0.48	1.22	—	7.37	7.37	7.49	14.75	17.45
11	8.43	8.59	8.42	8.53	8.42	8.42	8.26	8.69	8.47	8.81	—	0.12	0.23	14.75	18.03
12	8.39	8.55	8.39	8.50	8.39	8.39	8.23	8.65	8.44	8.78	0.12	—	0.12	14.63	18.03
13	8.59	8.75	8.59	8.70	8.58	8.58	8.43	8.85	8.63	8.97	0.24	0.12	—	14.63	18.03
14 *D. punct*	23.19	23.43	22.95	23.80	23.42	23.43	22.95	23.22	23.09	23.42	24.48	24.12	24.24	—	16.71
15 *H. plat*	32.43	32.72	32.13	31.95	32.41	32.42	32.13	32.20	32.05	32.41	34.45	34.29	34.45	30.19	—

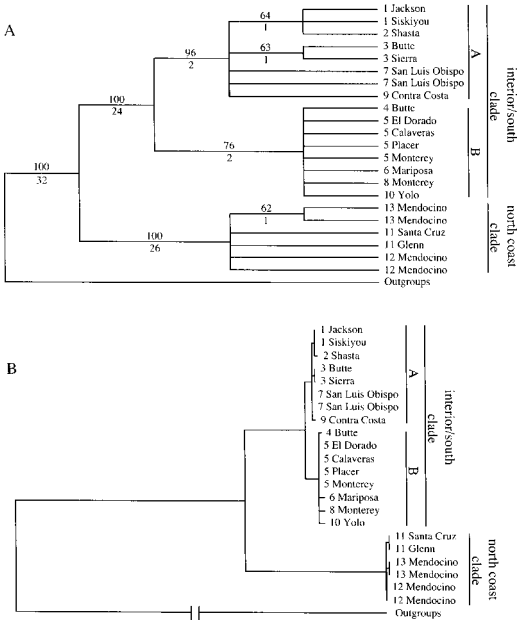

FIG. 2. Phylogenetic relationships of mtDNA lineages. (A) Strict consensus of nine equally parsimonious trees (L = 282; CI = 0.933; RI = 0.957). Numbers above nodes indicate bootstrap support, numbers below denote decay indices. (B) Maximum likelihood tree constructed under the HKY + Γ model (−LnL = 2398.159; $\alpha = 0.391$; ti:tv = 2.81). Branch lengths proportionate to ML estimates of genetic distances.

(A and B) that receive strong support but lack phylogeographic structure. Subclade A (Cascade and Klamath Mtns. and central coast; 96% bootstrap; decay 2) consists of haplotypes 1–3, 7, and 9, whereas subclade B (Sierra Nevada and Coast Ranges; 83% bootstrap; decay 2) contains haplotypes 4–6, 8, and 10.

Divergence Times.—The LRT could not reject a molecular clock hypothesis ($P = 0.09$). The average uncorrected distances are 7.2% between the north coast clade and interior/south clade and 1.03% between the A and B subclades. The estimated mtDNA divergence rate of 1.3% sequence/million years (Macey et al., 1999, 2001) suggests that north coast and interior/south clades split roughly 5.5 million years ago, whereas subclades A and B split about 0.8 million years ago.

DISCUSSION

Phylogeography.—Sharp-tailed snakes belong to two major mitochondrial lineages: a north coast clade; and an interior/south clade (Fig. 2). Both clades, recovered by MP and ML methods, are strongly supported by bootstrap and decay analyses, and each is associated with different habitat types. Snakes of the north coast clade are restricted to wet Douglas fir and redwood forest along the Pacific Coast, whereas interior/south clade *Contia* occupy drier woodland and forest characterized by grey pine, ponderosa pine, and oak. The north coast clade and interior/south clade differ by more than 7% sequence divergence (uncorrected), distance values that suggest a partition between clades over five million years old. The northern limit of each clade is unknown, but our sampling places the southern border of the north coast clade in the Monterey Bay region.

The Monterey Bay area is an important biogeographic region for many vertebrate species; it is the southern distributional limit for *Ambystoma macrodactylum, Aneides flavipunctatus, Batrachoseps attenuatus, Dicamptodon ensatus, Ensatina eschscholtzii xanthopicta*, and *Taricha granulosa*, and the northern distributional limit for *Batrachoseps luciae* and *Ensatina eschscholtzii eschscholtzii* (Yanev, 1980; Jockusch et al., 2001). Taxa distributed around Monterey Bay, such as *Taricha torosa* (Tan and Wake, 1995), *Elgaria multicarinata* (Feldman, 2000), *Thamnophis atratus* (Boundy, 1999), and *Strix occidentalis* (Aves: Strigiformes; Barrowclough et al., 1999), display genetic or morphological discontinuities across this area. Geologic evidence indicates that the Pacific Ocean invaded interior California around 5–24 million years ago through the present day Monterey Bay, and did not recede until about a million years ago (Oakeshott, 1978; Howard, 1979; Dupre, 1990). Uncertainties involved with dating geologic events and calibrating molecular clocks, and confounding issues with plate tectonic data, prohibit our positively establishing the Monterey seaway as the causal factor in *Contia* cladeogenesis. Nevertheless, biogeographic congruence among vertebrates in this region, and the degree of concordance between *Contia tenuis* diversification and the Monterey embayment suggest this long-standing, marine barrier has played an important role in sharp-tailed snake evolution.

The distribution of genetic variation in *Contia* is not congruent with geography at lower levels in the phylogeny. The widespread interior/south clade displays internal haplotype structure inconsistent with geography as evidenced by the strongly supported subclades A and B. Three inner Coast Range populations group with Sierran populations (subclade B) and populations of sharp-tailed snakes from the Cascade, Klamath, and Sierra Nevada Mountains cluster with two coastal populations far to the south (subclade A). Additionally, one Sierran population (Butte Co.) contains members from subclades A and B. These data could indicate that *C. tenuis* disperses well and maintains gene flow throughout California. Sharp-tailed snakes are distributed along riparian corridors flanking

the American and Sacramento Rivers, and these and other drainages flow from the Sierra Nevada to the coast, possibly explaining the sharing of haplotype 5 in Sierran and coastal populations.

Although gene flow may link Sierran and coastal populations of *Contia*, long-term ecological and demographic processes may also explain intraclade patterns. Gene flow seems an unlikely explanation for the sharing of haplotype 11 between north coast clade populations on opposite sides of the San Francisco Bay and Sacramento-San Joaquin Delta. The Sacramento-San Joaquin Delta formed during the mid-Pleistocene (Dupre et al., 1991), influencing genetic and morphological evolution in *Thomomys bottae* (Mammalia: Rodentia; Patton and Smith, 1990), *Lampropeltis zonata* (Rodriguez-Robles et al., 1999), *Neotoma fuscipes* (Mammalia: Rodentia; Matocq, 2002) and *Sorex ornatus* (Mammalia: Insectivora; Maldonado et al., 2001). Taxa such as *Ensatina eschscholtzii* (Wake, 1997), *Diadophis punctatus, Elgaria multicarinata* (Feldman, 2000) and *Charina bottae* (Rodriguez-Robles et al., 2001) do not exhibit genetic subdivisions, however, indicating dispersal across the delta or maintenance of large effective population sizes in which reciprocal monophyly has not yet evolved. The San Francisco Bay and Sacramento-San Joaquin Delta effectively divide suitable habitat for the north coast clade; thus the sharing of haplotype 11 in *C. tenuis* across this region is likely caused by retained ancestral polymorphism. Sharp-tailed snakes probably maintain large effective population sizes, possibly because of their tendency to aggregate (Cook, 1960). Unfortunately, patterns of historical and contemporary gene flow and incomplete lineage sorting are difficult to distinguish (Matocq et al., 2000).

Taxonomic Implications.—Mitochondrial DNA evidence indicates that the north coast clade and interior/south clade of *C. tenuis* are exclusive lineages with separate and ancient evolutionary histories (i.e., phylogenetic species; Cracraft, 1983). Making species decisions based solely upon exclusivity is tenuous (de Queiroz, 1998), especially when those decisions are based entirely on a single molecular marker (Moritz et al., 1992; Wake and Schneider, 1998); multiple lines of evidence should be used to diagnose distinctness and permanence of independent units (de Queiroz, 1998). We recognize groups of populations as species if they are monophyletic and possess additional, independent characters suggestive of long-term evolutionary independence. Under these criteria, two species of *Contia* could be recognized.

Sequence divergence between north coast and interior/south clades of *C. tenuis* (Table 2) is equivalent or greater than genetic distances in the same mitochondrial region between *Lampropeltis zonata* and *L. pyromelana* (Rodriguez-Robles and Jesus-Escobar, 1999), and various species of *Pituophis* (Rodriguez-Robles and Jesus-Escobar, 2000), *Lachesis* (Zamudio and Greene, 1997) and *Agkistrodon* (Parkinson et al., 2000). Members of the north coast clade occur in wet Douglas fir and redwood evergreen forest while populations of the interior/south clade occupy drier grey pine, ponderosa pine and oak habitat. North coast and interior/south clades are diagnosable by caudal scale counts, ventral scale counts and color differences (Hoyer, 2001). The two mtDNA clades appear parapatric along the inner Coast Range north of the Monterey Bay area, but more samples are needed to determine the integrity of these clades where their ranges meet.

Although we are confident that additional morphological, ecological and behavioral characters will further elucidate the two clades of *Contia*, we refrain from a taxonomic decision until a molecular or morphological survey throughout the entire range of *C. tenuis* delimits the geographical distribution of each clade. *Contia tenuis*, considered a morphologically uniform colubrid, now joins a growing list of Californian herpetofauna whose patterns of genetic variation and structure reveal more complex histories.

Acknowledgments.—We thank T. Papenfuss, D. Wake, C. Cicero (Museum of Vertebrate Zoology); J. Vindum, R. Drewes (California Academy of Sciences); D. Mulcahy, J. Parham, R. Hoyer, and J. Buskirk for tissue samples; T. Reeder, S. Busack, J. Rodriguez, M. Matocq, E. Routman, T. Papenfuss, K. Omland, G. Pauly, R. Hoyer, and an anonymous reviewer for useful comments and suggestions; M. Blouin and B. Leonard for sharing unpublished data, and M. Koo for mapping assistance. Field assistance from D. Mulcahy, J. Parham and M. Matocq, and permits from the California Department of Fish and Game are gratefully acknowledged. This work was supported by National Science Foundation grant DEB-9629546 to GSS.

Literature Cited

Arevalo, E., S. K. Davis, and J. W. Sites. 1994. Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynostomatidae) in Central Mexico. Systematic Biology 43:387–418.

Avise, J. C. 1989. Gene trees and organismal histories: a phylogenetic approach to population biology. Evolution 43:1192–1208.

———. 2000. Phylogeography: The History and Formation of Species. Harvard University Press, Cambridge, MA.

Avise, J. C., J. Arnold, R. M. Ball, E. Bermingham, T. Lamb, J. E. Neigel, C. A. Reeb, and N. C. Saunders. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population ge-

netics and systematics. Annual Review of Ecology and Systematics 18:489–522.

BARROWCLOUGH, G., R. J. GUTIERREZ, AND J. G. GROTH. 1999. Phylogeography of the spotted owl (*Strix occidentalis*) populations based on mitochondrial DNA sequences: gene flow, genetic structure and a novel biogeographic pattern. Evolution 53:919–931.

BOUNDY, J. 1999. Systematics of the garter snake *Thamnophis atratus* at the southern end of its range. Proceedings of the California Academy of Sciences 51:311–336.

BREMER, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution 42:795–803.

———. 1994. Branch support and tree stability. Cladistics 10:295–304.

CADLE, J. E. 1984. Molecular systematics of Neotropical xenodontine snakes III. Overview of xenodontine phylogeny and the history of new world snakes. Copeia 1984:641–652.

COOK JR., S. F. 1960. On the occurrence and life history of *Contia tenuis*. Herpetologica 16:163–173.

CRACRAFT, J. 1983. Species concepts and speciation analysis. *In* R. Johnston (ed.), Current Ornithology, pp. 159–187. Plenum Press, New York.

DE QUEIROZ, K. 1998. The general lineage concept of species, species criteria, and the process of speciation: a conceptual unification and terminological recommendations. *In* D. J. Howard and S. H. Berlocher (eds.), Endless Forms: Species and Speciation, pp. 57–75. Oxford University Press, New York.

DUPRE, W. R. 1990. Quaternary geology of the Monterey Bay region, California. *In* R. E. Garrison (ed.), Geology and Tectonics of the Central California Coast Region, San Francisco to Monterey, pp. 185–191. Society of Economic Paleontology and Mineralogy, Bakersfield, CA.

DUPRE, W. R., R. B. MORRISON, H. E. CLIFTON, K. R. LAJOIE, D. J. PONTI, C. L. POWELL III, S. A. MATHIESON, A. M. SARNA-WOJCICKI, E. L. LEITHOLD, W. R. LETTIS, P. F. MCDOWELL, T. K. ROCKWELL, J. R. UNRUH, AND R. S. YEATS. 1991. Quaternary geology of the Pacific margin. *In* R. B. Morrison (ed), The Geology of North America. Quaternary Nonglacial Geology: Conterminous U.S. Vol. K-2, pp. 141–214. Geology Society of America, Boulder, CO.

FELDMAN, C. R. 2000. Comparative phylogeography of three Californian reptiles: *Contia tenuis, Diadophis punctatus, Elgaria multicarinata*. Unpubl. master's thesis. San Francisco State University, San Francisco, CA.

FELSENSTEIN, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. Journal of Molecular Evolution 17:368–376.

———. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791.

GOLDMAN, N. 1993. Statistical tests of models of DNA substitution. Journal of Molecular Evolution 63: 182–198.

GOOD, D. A. 1989. Hybridization and cryptic species in *Dicamptodon* (Caudata: Dicamptodontidae). Evolution 43:728–744.

HASEGAWA, M., H. KISHINO, AND T. YANO. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 21:160–174.

HENDY, M. D., AND D. PENNY. 1982. Branch and bound algorithms to determine minimal evolutionary trees. Mathematical Biosciences 59:277–290.

HIGHTON, R. 1989. Biochemical evolution in the slimy salamanders of the *Plethodon glutinosus* complex in the Eastern United States. I. Geographic protein variation. Illinois Biological Monographs 57:1–78.

HOWARD, A. D. 1979. Geologic History of Middle California. University of California Press, Berkeley.

HOYER, R. F. 2001. Discovery of a probable new species in the genus *Contia*. Northwestern Naturalist 82: 116–122.

JOCKUSCH, E. L., K. P. YANEV, AND D. B. WAKE. 2001. Molecular phylogenetic analysis of slender salamanders genus *Batrachosep*s (Amphibia: Plethodontidae), from central coastal California with descriptions of four new species. Herpetological Monographs 15:54–99.

KRAUS, F., AND W. M. BROWN. 1998. Phylogentic relationships of colubriod snakes based on mitochondrial DNA sequences. Zoological Journal of the Linnean Society London 122:455–487.

KUMAZAWA, Y., AND M. NISHIDA. 1993. Sequence evolution of mitochondrial tRNA genes and deep-branch animal phylogenetics. Journal of Molecular Evolution 37:380–398.

———. 1995. Variations in mitochondrial tRNA gene organization of reptiles as a phylogenetic marker. Molecular Biology and Evolution 12:759–772.

KUMAZAWA, Y., H. OTA, M. NISHIDA, AND T. OZAWA. 1996. Gene rearrangements in snake mitochondrial genomes: highly concerted evolution of control–region-like sequences duplicated and inserted into a tRNA gene cluster. Molecular Biology and Evolution 13:1242–1254.

———. 1998. The complete nucleotide sequence of a snake (*Dinodon semicarinatus*) mitochondrial genome with two identical control regions. Genetics 150:313–329.

LEONARD, W. P., AND K. OVASKA. 1998. *Contia* (Girard). Catalogue of American Amphibians and Reptiles 677.1–677.7.

MACEY, J. R., AND A. VERMA. 1997. Homology in phylogenetic analysis: alignment of transfer RNA genes and the phylogenetic position of snakes. Molecular Phylogenetics and Evolution 7:272–279.

MACEY, J. R., A. LARSON, N. B. ANANJEVA, Z. FANG, AND T. J. PAPENFUSS. 1997. Two novel gene orders and the role of light-strand replication in rearrangement of the vertebrate mitochondrial genome. Molecular Biology and Evolution 14:91–104.

MACEY, J. R., Y. WANG, N. B. ANANJEVA, A. LARSON, AND T. J. PAPENFUSS. 1999. Vicariant patterns of fragmentation among gekkonid lizards of the genus *Teratoscincus* produced by the Indian collision: a molecular phylogenetic perspective and an area cladogram for Central Asia. Molecular Phylogenetics and Evolution 12:250–272.

MACEY, J. R., J. L. STRASBURG, J. A. BRISSON, V. T. VREDENBURG, M. JENNINGS, AND A. LARSON. 2001. Molecular phylogenetics of western North American frogs of the *Rana boylii* species group. Molecular Phylogenetics and Evolution 19:131–143.

MADDISON, W. P., M. J. DONOGHUE, AND D. R. MAD-

DISON. 1984. Outgroup analysis using parsimony. Systematic Zoology 33:83–103.

MALDONADO, J. E., C. VILA, AND R. K. WAYNE. 2001. Tripartite genetic subdivisions in the ornate shrew (*Sorex ornatus*). Molecular Ecology 10:127–147.

MANIATIS, T., E. F. FRISTCH, AND J. SAMBROOK. 1982. Molecular Cloning; A Laboratory Manual. Cold Spring Harbor Publication, Cold Spring Harbor, NY.

MATOCQ, M. D. 2002. Phylogeographic structure and regional history of the dusky-footed woodrat (*Neotoma fuscipes*). Molecular Ecology 11:229–242.

MATOCQ, M. D., M. N. F. DA SILVA, AND J. L. PATTON. 2000. Population genetic structure of two ecologically distinct spiny rats: separating history and current ecology. Evolution 54:1423–1432.

MORITZ, C., C. J. SCHNEIDER, AND D. B. WAKE. 1992. Evolutionary relationships within the *Ensatina eschscholtzii* complex confirm the ring species interpretation. Systematic Biology 41:273–291.

OAKESHOTT, G. B. 1978. California's Changing Landscapes: A Guide to the Geology of the State. 2nd ed. McGraw-Hill Co., Inc., New York.

OMLAND, K. E., C. L. TARR, W. I. BOARMAN, J. M. MARZLUFF, AND R. C. FLEISCHER. 2000. Cryptic genetic variation and paraphyly in ravens. Proceedings of the Royal Society of London B Biological Sciences 267:2475–2482.

PARKINSON, C. L., K. R. ZAMUDIO, AND H. W. GREENE. 2000. Phylogeography of the pit viper clade *Agkistrodon*: historical ecology, species status, and conservation of cantils. Molecular Ecology 9:411–420.

PATTON, J. L., AND M. F. SMITH. 1990. The evolutionary dynamics of the pocket gopher *Thomomys bottae*, with emphasis on California populations. University of California Publications in Zoology 123: 1–161.

POSADA, D., AND K. A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818.

RODRIGUEZ-ROBLES, J. A., AND J. M. DE JESUS-ESCOBAR. 1999. Molecular systematics of new world lampropeltinine snakes: implications for biogeography and evolution of food habits. Biological Journal of the Linnean Society 68:355–385.

———. 2000. Molecular systematics of new world gopher, bull, and pine snakes (*Pituophis*: Colubridae), a transcontinental species complex. Molecular Phylogenetics and Evolution 14:35–50.

RODRIGUEZ-ROBLES, J. A., D. F. DENARDO, AND R. E. STAUB. 1999. Phylogeography of the California mountain kingsnake, *Lampropeltis zonata* (Colubridae). Molecular Ecology 8:1923–1934.

RODRIGUEZ-ROBLES, J. A., G. R. STEWART, AND T. J. PAPENFUSS. 2001. Mitochondrial DNA-based phylogeography of North American rubber boas, *Charina bottae*. Molecular Phylogenetics and Evolution 18:227–237.

ROZAS, J., AND R. ROZAS. 1999. DnaSp version 3: integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics 15:174–175.

SANGER, F., S. NICKLEN, AND A. R. COULSON. 1977. DNA sequencing with chain-terminating inhibitors. Proceedings of the National Academy of Sciences 74:5463–5467.

STEBBINS, R. C. 1985. A Field Guide to the Western Reptiles and Amphibians. Houghton Mifflin Co., Boston, MA.

SWOFFORD, D. L., G. J. OLSEN, P. J. WADDELL, AND D. M. HILLIS. 1996. Phylogenetic inference. *In* D. M. Hillis, C. Moritz, and B. K. Mable (eds.), Molecular Systematics. 2nd ed., pp. 407–543. Sinauer Associates, Inc., Sunderland, MA.

TAN, A. M., AND D. B. WAKE. 1995. MtDNA phylogeography of the California newt, *Taricha torosa* (Caudata, Salimandridae). Molecular Phylogenetics and Evolution 4:383–394.

TAJIMA, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595.

TEMPLETON, A. R., E. ROUTMAN, AND C. A. PHILLIPS. 1995. Separating population structure from population history: a cladistic analysis of the geographical distribution of mitochondrial DNA haplotypes in the tiger salamander, *Ambystoma tigrinum*. Genetics 140:767–782.

TILLEY, S. G., R. B. MERRITT, B. WU, AND R. HIGHTON. 1978. Genetic differentiation in salamanders of the *Desmognathus ochrophaeus* complex (Plethodontidae). Evolution 32:93–115.

WAKE, D. B. 1997. Incipient species formation in salamanders of the *Ensatina* complex. Proceedings of the National Academy of Sciences 94:7761–7767.

WAKE, D. B., AND C. J. SCHNEIDER. 1998. Taxonomy of the plethodontid salamander genus *Ensatina*. Herpetologica 54:279–298.

WALKER, D., AND J. C. AVISE. 1998. Principles of phylogeography as illustrated by freshwater and terrestrial turtles in the southeastern United States. Annual Review of Ecology and Systematics 29:23–58.

WILGENBUSCH, J., AND K. DE QUEIROZ. 2000. Phylogenetic relationships among the phrynosomatid sand lizards inferred from mitochondrial DNA sequences generated by heterogeneous evolutionary processes. Systematic Biology 99:592–612.

YANEV, K. P. 1980. Biogeography and distribution of three parapatric salamander species in coastal and borderland California. *In* D. M. Power (ed.), The California Islands: Proceedings of a Multidisciplinary Symposium, pp. 531–550. Santa Barbara Museum of Natural History, Santa Barbara, CA.

YANG, Z. 1994a. Estimating patterns of nucleotide substitution. Journal of Molecular Evolution 39: 105–111.

———. 1994b. Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. Journal of Molecular Evolution 39:306–314.

———. 1996. Maximum likelihood models for combined analyses of multiple sequence data. Journal of Molecular Evolution 42:587–596.

ZAMUDIO, K. R., AND H. W. GREENE. 1997. Phylogeography of the bushmaster (*Lachesis muta*: Viperidae): implications for Neotropical biogeography, systematics, and conservation. Biological Journal of the Linnean Society 62:421–442.

Accepted: 19 March 2002.