

preocular are not fused. The specimens in our series have snout-vent length from 16.5 mm (hatchling) to 43.6 mm (largest adult). The eggs, collected from under leaf litter in the plots (see Table 1), are creamy-white to yellow, oval, and leathery and average 8.2 mm long by 4.8 mm wide (after preservation in 10% formalin). Most have a small, purplish-black spot visible through the eggshell. A fully developed skink is visible through the eggshell of two eggs (FMNH 258918). A baby skink hatched from one egg (FMNH 258917) immediately upon immersion of the egg in formalin in the field, making identification unambiguous. Animals with regenerating tails accounted 5.5% of the specimens captured.

The plot results for *S. tridigitus* are summarized in Table 1. All individuals and eggs from the census plots were found under leaf litter. Six of the eight plots (75%) contained this species and it was clear that abundance was related to elevation. Intensive searching over 16 days (10–25 September 1999) at these sites by the authors and two camp assistants (and others sporadically) in the conventional way yielded only two individuals of this species, one under leaf litter when clearing a campsite and the other inside a rotten log. Thus, by the standard methods of expeditionary field surveys, this species would have been considered rare at the study site. In fact, it was the most abundant reptile in the area. Without the plot method the small numbers of individuals otherwise obtained would not have revealed this. No animals were found at low elevations but they were relatively abundant at 1000 m. Population density was lower again at 1200 m. Hence, this species is most abundant at mid-elevations.

At 1000 m on average there was one egg for every 3.5 adults, whereas at 1200 this value dropped to one egg for every 8.75 adults suggesting either that reproductive rate was much lower at the higher elevation, or that the reproductive season differed between the two sites.

This species, rather than being an insignificant rarity, is abundant at higher elevations on the Bolaven Plateau, where it accounted for 86% of the total individuals of the forest-floor lizards and frogs (6 species; snakes inadequately sampled) at 1000 m and 57% of the individuals of forest-floor lizards and frogs (8 species) at 1200 m. At a lower elevation on the plateau where *S. tridigitatus* was not present, it was replaced by a similarly small skink in the *Scincella reevesi* complex (mean density: 0.05/m²; 64% of total individuals of the forest-floor frogs and lizards; four species). These two skinks probably play an important role in the dynamics of the forest floor community as significant predators upon small invertebrates and as food for various snakes.

Rodda et al. (2001a,b), using a censusing technique similar to the present one, also found unexpectedly high densities of some small reptiles and it is likely that many small forest-floor lizards are far more abundant than they appear to be. Estimates of density are used in studies of population biology and structure of assemblages and often play an important role in decisions about conservation. Much of the previous literature, even that based on unfenced plots, probably contains serious underestimates and needs to be reassessed by research using more refined, fenced-plot techniques.

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TECHNIQUES

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Bromeliad Patch Sampling Technique for Canopy Herpetofauna in Neotropical Forests

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The canopy strata of tropical forests are one of the remaining unexplored biotic frontiers. Canopy research is a relatively new discipline facilitated by recent methodological advances in canopy access techniques (Basset et al. 2003b). Forest canopies are among the most species-rich terrestrial habitats on earth, supporting approximately 40% of known extant species and estimated to hold up to 50% of the earth's biodiversity (Basset et al. 2003b; Mitchell et al. 2002). The ecological role of amphibians and reptiles in forest canopies is mostly unknown. Thus far the research focus has been on arthropods, birds, mammals, plants and ecological processes; investigations of canopy herpetofauna have only recently been documented (De Vries et al. 1997; Guayasamin et al. 2006; Schiesari et al. 2003). Kays and Allison (2001) reviewed published ecology and study methods for arboreal tropical forest vertebrates

and found amphibians and reptiles to be grossly understudied compared to mammals, primarily due to their cryptic habits and sampling difficulties. Of 752 articles on tropical forest arboreal vertebrates published between 1988 and 1998 only 4% focused on reptiles and amphibians, with the majority of those covering reptiles (Kays and Allison 2001). While many studies report arboreal occupancy by an extensive number of amphibian species, few have documented ecological characteristics besides presence/absence data based on calling males and new species descriptions (Duellman and Trueb 1986; Guayasamin et al. 2006; Schiesari et al. 2003). Most data for arboreal amphibians were obtained through collection and observation during reproduction of those species that descend from the canopy to breed in water bodies at the forest floor level (Duellman 1978; Duellman 2005; Ron and Pramuk 1999). Standard survey techniques for amphibians, such as those at breeding sites, only encompass a small stratum (~2 m vertical height) of forest diversity (McCracken et al. 2007). Amphibians that specialize within the upper canopy remain mostly unaccounted for as a result of this limited vertical sampling bias (Guayasamin et al. 2006). More practical methods for studying canopy amphibians and reptiles is a high priority to facilitate the need for more survey and natural history work (Kays and Allison 2001).

A component of neotropical rainforest canopies that provide rich fauna microhabitats are the phytotelmata, defined as plants or parts of plants which hold rainwater (e.g. bromeliads, fruits, inflorescences, palm fronds and tree holes). In some tropical locations the availability of this habitat for aquatic organisms is up to 50,000 liters per hectare, literally a “wetland in the sky” (Kitching 2000; McCracken and Forstner 2006). In particular, epiphytic tank bromeliads are capable of holding relatively large amounts of water and play a principal role as a “keystone resource” and microhabitat for invertebrates, vertebrates and other plants (Nadkarni 1994). Canopy bromeliad arthropod surveys have reported them as reservoirs of incredibly high biodiversity (Basset et al. 2003a; Kitching 2000). Typically, tank bromeliads occur in the upper canopy and overstory trees of lowland rainforest at vertical heights between 5–45 m. Bromeliads normally range in number of individuals from ~5 to >150 on a single tree. Herein, we describe a technique for canopy bromeliad patch sampling of herpetofauna in lowland neotropical forests which is similar to those used in other canopy research disciplines but has not been documented for herpetofaunal investigations.

Methods.—Bromeliad patch sampling was conducted during 2004 and 2006 at the Tiputini Biodiversity Station (TBS)—Universidad San Francisco de Quito (USFQ), Orellana Province, Ecuador (00.63847°S, 076.14908°W, 217 m elev.). The vegetation type of the site has been defined as Amazonian Evergreen Lowland Forest (Palacios et al. 1999). Sampling units consisted of five bromeliads from each of 16 trees for a total of 80 bromeliads sampled. A tree was not sampled if less than 15 bromeliads of any species to be sampled were present to ensure continued persistence of the bromeliad community. Host trees were measured for diameter at 1.5 m above ground, height using a clinometer, and canopy cover using hemispherical photography with the Gap Light Analyzer (GLA) software. A leader line was positioned in the tree using a large slingshot (Sherrill™ Big Shot) which enables setting lines at 30+ m. The canopy was accessed using single-rope technique, which should only be performed by trained and

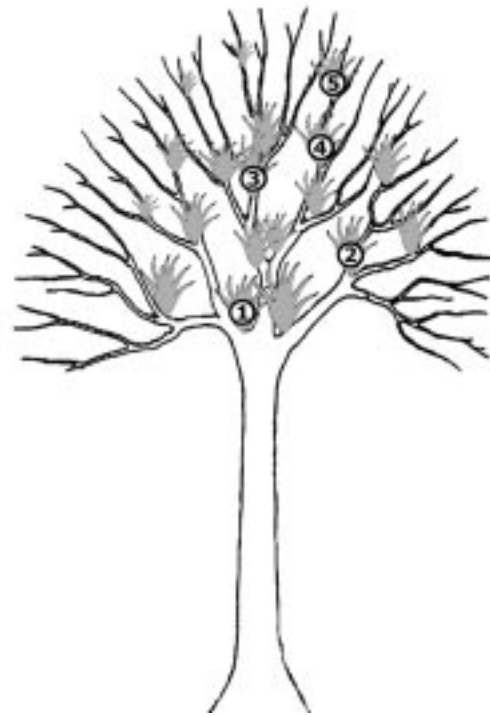


Fig. 1. Schematic of tree with bromeliads illustrating distribution strategy for sampling units (bromeliads), numbers denote bromeliads sampled.

experienced individuals (Fig. 2d). The lowest and highest elevation bromeliads were sampled with the remaining three sampled at estimated even intervals in between (Fig. 1). Before removal of each bromeliad a wide-angle photograph was taken and the following variables collected: elevation, ambient air temperature, relative humidity, barometric pressure, water temperature and pH are measured inside one of the outer leaf bracts, and a 50 ml water sample is collected by siphon. Ambient air temperature, relative humidity and barometric pressure were also collected at 1.5 m elevation. The bromeliad was removed by holding several leaves at the tips in one hand and cutting its base support stem with a pruning saw. The response of most animals is to retreat into the bromeliad bracts and therefore alleviates loss of specimens due to escape. The bromeliad was placed in a 55 gal. plastic bag with minimal disturbance, sealed, and placed in a tarp connected to a rope that is threaded through a carabiner on the climbers harness and the other end held by a ground support person. It was then gently lowered to the forest floor by the ground support person. Another photograph was taken of the site where the bromeliad was removed. After removal of the five bromeliads, a herbarium sample was collected from the tree to confirm identification and deposit in a herbarium. Bromeliads were processed at camp in a screen tent to prevent escape of animals (Fig. 2c). Bromeliad water was strained through a 1 mm mesh screen to separate arthropods, leaf litter, and detritus. Water volume was measured with a graduated cylinder. Bromeliads were measured, number of leaves counted, and photographed including a meter stick for scale reference (Fig. 2a, b). Individual leaves were removed to facilitate collection of herpetofauna, which were temporarily stored in bags for further processing. Herpetofauna species were photographed, measured and weighed. Blood or tissue samples were collected



FIG. 2A. Side view photo of *A. zebrina* bromeliad with meter stick in background. Bar = 20 cm. 2B. Top view of *A. zebrina* bromeliad with meter stick below. Bar = 20 cm. 2C. Senior author in screened tent with sampled bromeliad to prevent escape of herpetofauna. 2D. Senior author ascending into canopy to access bromeliads for sampling using single-rope technique (SRT) to climb.

and stored in blood storage buffer or 95% ethanol, respectively. Animals were euthanized in 10% ethyl alcohol or by ventral application of 20% benzocaine (Orajel®) and preserved using 10% formalin before being transferred to 70% ethyl alcohol for storage.

Results.—In 2004, eight trees were surveyed for a total of 40 bromeliads sampled. Three species of bromeliads were sampled: 20 individuals of *Aechmea zebrina*, 17 of *Aechmea* sp., and three of an unidentified tankless bromeliad. In 2006, eight trees were surveyed for a total of 40 *A. zebrina* bromeliads sampled as part of a current study. Bromeliads were collected at elevations of 5.7–38.0 m (mean 27.0 ± 6.2 m) above ground. *Aechmea zebrina* bromeliads were 58.5–125.0 cm (mean 79.9 ± 13.9 cm, $N = 40$) tall and 54.0–147.5 cm (mean 89.5 ± 22.2 cm, $N = 40$) in diameter, *A* sp. bromeliads were 32.0–58.0 cm (mean 47.2 ± 9.7 cm, $N = 17$) and 54.0–94.0 cm (mean 66.8 ± 12.6 cm, $N = 17$) in diameter, and the unknown tankless bromeliads were 41.0–47.0 cm (mean 43.8 ± 3.1 cm, $N = 3$) and 33.0–43.0 cm (mean 37.3 ± 5.1 cm, $N = 3$) in diameter.

Thirty-four adults, 10 juveniles, 15 tadpoles, and 17 eggs of anurans representing at least four species were collected during the two survey periods. The identified adult and juvenile species included *Dendrobates (Ranitomeya) ventrimaculatus*,

Eleutherodactylus (Pristimantis) aureolineatus, *Eleutherodactylus (Pristimantis) waoranii*, and *Osteocephalus taurinus*. Eight of the tadpole specimens were easily identified as *D. ventrimaculatus* due to their advanced stages of development. The remaining tadpole specimens are to be identified using morphological and/or molecular techniques. One gecko, *Thecadactylus rapicauda*, was collected in an *A. zebrina* in 2006. Only one anuran was observed jumping from a bromeliad during removal and was visually identified when it landed on a nearby bromeliad before retreating into the leaf bracts.

Of the three bromeliad species, no anurans were found in the three tankless bromeliads, nine tadpoles of *D. ventrimaculatus* and three adult *E. waoranii* in five *Aechmea* sp., and the remainder in 26 *A. zebrina* (65% of *A. zebrina* sampled had anurans). All anurans were collected in bromeliads between 20.0–36.0 m (mean 28.3 ± 5.3 m) above ground.

Discussion.—Visual encounter surveys, focal point observations, and inspection of individual bromeliads along a 100 m-long canopy walkway and two ~40 m high observation towers built around emergent trees at TBS–USFQ revealed 13 species of anurans; these surveys were conducted 3–4 times a year from 1998 to 2001 during the morning, afternoon, and night for 4–5 days duration (Cisneros-Heredia 2003; D. F. Cisneros-Heredia, pers. comm.).

During one week in May 2002 canopy searches targeted at calling anurans were conducted using tree-climbing spurs at the Yasuni Scientific Research Station–Universidad Católica del Ecuador and resulted in the discovery of six anuran species occupying canopy habitat (S. Ron, pers. comm.). Canopy bromeliad patch sampling revealed a minimum of four species and the additional species *E. waoranii* (McCracken et al. 2007). Results from our surveys contributed significantly to the new species description for *E. aureolineatus* and a manuscript on the reproductive ecology and behavior; they are wholly responsible for the new species description of *E. waoranii* (Guayasamin et al. 2006; McCracken and Forstner 2006; McCracken et al. 2007). Three other species found during these canopy surveys are newly described since 1999, demonstrating the value of such research techniques (Guayasamin et al. 2006). The potential for the discovery of additional new species and collection of detailed ecological data at other sites is evident in the fact that our surveys and the previous canopy surveys represent a limited sampling effort at two sites within close geographic proximity (~28 km) and similar habitat structure.

The technique provides a labor intensive but successful method for surveying the otherwise inaccessible microhabitats of the upper forest canopy strata herpetofauna. While our bromeliad patch sampling technique recovered less than a third of the number of species collected during the canopy walkway/tower surveys it represents a much less intensive sampling effort. Our sampling focused on the specific microhabitat provided by bromeliads, of which we only investigated three species. Our results indicate that the largest tank bromeliad in our surveys, *A. zebrina*, had the greatest occurrence rate with 65% of those sampled having anurans present. The use of canopy bromeliad patch sampling is also supported by the limited availability of canopy walkways and towers for research in Amazonia, and the financially prohibitive construction costs of such infrastructure for most research projects. Canopy bromeliad patch sampling can be employed anywhere the forest is accessible and facilitates the collection of independent replicate sampling units with associated biotic and abiotic factors for the analysis of ecological correlates of species diversity and abundance in a robust sampling design. Our current study targets a species specific (*A. zebrina*) tank bromeliad microhabitat, but the technique may be applied to other species and microhabitats (e.g. tree holes/cavities) within the canopy. The technique may also be used to survey other forest canopies and their specific microhabitats.

Fauna of forest canopy habitats are at risk due to high rates of deforestation and habitat fragmentation, which are primary reasons for the rapid decline in amphibian populations worldwide with nearly one-third of all amphibians being threatened and at least 43% declining in population size (IUCN et al. 2006). The rapid exploitation of natural resources is having a profound effect on the rainforests and its inhabitants of the Ecuadorian Amazon. Yet, little is known about the effects of canopy biota loss. Epiphytes are considered hypersensitive to climatic conditions, requiring the very conditions they promote for existence (Benzing 1998, 2000; Hietz 1998). This hypersensitivity makes them particularly susceptible to forest microclimate changes as a result of anthropogenic disturbance, making epiphytes suitable as a bioindicator of diversity and forest ecosystem functions (Benzing 1998; Brighigna et al. 2002; Hietz 1998). Loss of epiphyte diver-

sity will degrade all biodiversity within inclusive ecosystems by causing shifts in faunal resource availability, nutrient budgets and cycling, system energetics, and hydrology (Benzing 1998). Amphibians may be considered a vertebrate counterpart to epiphytes as bioindicator species and their utilization of epiphytic tank bromeliad habitat provides the researcher with a unique system for monitoring anthropogenic disturbance in forest canopies. Bromeliad patch sampling surveys are essential to documentation of the faunal diversity in neotropical forest canopies and promoting the conservation of these important “wetlands in the sky” (McCracken and Forstner 2006).

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Efficacy of PIT Tags for Tracking the Terrestrial Anurans *Rana pipiens* and *Rana sylvatica*

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The terrestrial ecology of many amphibians is poorly known compared with the aquatic stages (e.g., Regosin et al. 2003). Although advances have employed radiotelemetry on terrestrial adults (e.g., Hodgkison and Hero 2001; Watson et al. 2003), the size and battery life of transmitters are limitations on the use of radiotelemetry for smaller amphibian species and life stages. Other approaches for following small amphibians have included powder tracking, radioactive tags, and harmonic radar diodes, but each of these techniques has significant limitations (Heyer et al. 1994; Langkilde and Alford 2002).

Passive integrated transponders (PIT tags) overcome many limitations of these other techniques. PIT tags are small, glass-encased electromagnetic coils with a microchip containing a 10-space unique alphanumeric code that is emitted at a radio frequency (typically 134.2 kHz) when the coil is activated. PIT tags are easily applied and relatively benign to the tagged animal, provide a unique and essentially permanent mark, and can be cost-effective (Arntzen et al. 2004; Gibbons and Andrews 2004; Ott and Scott 1999). As a result, PIT tags have been increasingly used for marking fish, amphibians, reptiles, and other animals for demographic and behavioral studies (e.g., Camper and Dixon 1988; Kurth et al. 2007; Reaser 2000; Rowe and Kelly 2005; Sinsch 1992). Usually, PIT tag detection relies on the physical recapture of the tagged organism because the tag needs to be within range (usually ~ 0.3 m) of an antenna to transmit the alphanumeric identification code to the transceiver (see review by Gibbons and Andrews 2004). Portable antenna and transceiver systems (PIT-packs) are a new approach to locating and identifying a tagged organism without physical recapture, thereby minimizing associated disturbances (Hill et al. 2006; Kurth et al. 2007; Zydlewski et al. 2001).

We evaluated a PIT-pack as a tool to locate and identify confined individuals of two pond-breeding amphibian species, recently metamorphosed *Rana pipiens* (Northern Leopard Frogs) and adult *R. sylvatica* (Wood Frogs). We evaluated the detection range of the PIT-pack using PIT tags alone and the detection probability of frogs implanted with PIT tags and held in terrestrial enclosures. We used the PIT-pack to identify breeding pairs in a small vernal pool and collect information on the breeding ecology of *R. sylvatica*. In addition, we evaluated three surgical implant locations and PIT-tag retention in recently metamorphosed *R. pipiens*.