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## A PRELIMINARY INVESTIGATION INTO HAYNE ESTIMATES OF POISON DART FROG (ANURA: DENDROBATIDAE) DENSITIES IN RECOVERING TROPICAL FOREST HABITATS, SOUTHWESTERN COSTA RICA

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**Abstract.**—The global decline of amphibian populations has created a high demand for effective tools to measure how species respond to environmental change. We investigated the effectiveness of the Hayne Estimator for evaluating the population densities of *Dendrobates granuliferus* and *Dendrobates auratus*. We observed frogs in three Costa Rican lowland forest habitats with varying degrees of deforestation recovery. Population densities were highest in selectively-logged riparian forest, substantially lower in non-native bamboo plantation forest, and lowest in pasture-regrowth secondary forest. This trend corresponds to previous research on species recolonization after deforestation and subsequent regrowth. Individuals of both species tended to aggregate near water, but our study design masked this observation. Sighting frequency correlated with rainfall for *D. granuliferus* but not for *D. auratus*. Air temperature did not influence sighting frequency. Time of day, however, influenced the sighting frequencies of both species, with most frogs observed in the early morning and late afternoon. Our results appear to support the robustness of the Hayne Estimator for Poison Dart Frog monitoring and we recommend this technique as a valuable tool for dendrobatid research.

**Key Words.**—Costa Rica; deforestation; Hayne Estimator; Poison Dart Frog; population density

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### INTRODUCTION

In the light of growing international concern over catastrophic declines in amphibian populations worldwide (Stuart et al. 2004; Mendelson et al. 2006; McCallum 2007), benchmark monitoring programs and evaluation tools are increasingly important for determining the responses of at-risk species to environmental change (Young et al. 2001; Biek et al. 2002). Effective monitoring of sensitive species is an essential step in restoration planning for recovering habitats subject to divergent management strategies. In Costa Rica, one amphibian species has gone extinct and 64 species are considered threatened (International Union for Nature and Natural Resources. 2006. 2006 International Union for Nature and Natural Resources Red List of Threatened Species. Available from <http://www.iucnredlist.com> [Accessed 29 November 2006]). Pressures on these declining species include habitat loss, chemical contamination, climate change and the introduction of exotic species and disease (Young et al. 2001).

Dendrobatids may be useful sentinels of anuran habitat condition because their thin, permeable skin and prey preferences make them sensitive to changes in the environment (Gibbs and Stanton 2001; Blaustein and Kiesecker 2002; Rohr and Madison 2003; Darst et al. 2005). Dendrobatids frequent water in the surrounding

rainforest rather than at ponds or streams (Savage 1968; Vences et al. 2000; Jowers and Downie 2005). This may make dendrobatid populations more susceptible to deforestation because they are distributed throughout the larger forest rather than clustered near water. Moreover, deforestation strips the land of the available moisture on which they depend (McGuffie et al. 1994; Zhang et al. 1996).

We tested the utility of the Hayne Estimator as an assessment tool for monitoring population densities of the Green and Black Poison Dart Frog, *Dendrobates auratus*, and the Granular Poison Dart Frog, *D. granuliferus*. We conducted our study in: (1) selectively-logged riparian; (2) post-pasture secondary; and (3) non-native bamboo plantation forests located within the Firestone Reserve (Fig. 1). We followed the Hayne Estimator technique to collect field data (Hayne 1949). Because this method assumes that the investigator will flush and immediately observe animals on approach, the Hayne Estimator is poorly suited for use with cryptic, nocturnal amphibians (Coulson and Raines 1985; Pelletier and Krebs 1997). The Hayne Estimator may be suited for use with dendrobatid frogs because they are easily seen when flushed.

We hypothesized that the Hayne Estimator would be useful for monitoring Poison Dart Frogs if the Hayne Estimator produced significant density differences between riparian and secondary forest habitats (Wenny



**FIGURE 1.** The three habitats of study on the Firestone Reserve, Costa Rica: selectively-logged riparian forest (left), post-pasture secondary forest (center) and non-native bamboo plantation (right). Photographed by Jennifer R. B. Miller.

et al. 1993; Alcalá et al. 2004), and if the average sighting angle was  $\leq 32.7^\circ$  (to avoid bias in the final density estimation; Robinette et al. 1974; Burnham et al. 1980). Additionally, we anticipated lower frog densities in secondary forests because of earlier deforestation (McGuffie et al. 1994; Zhang et al. 1996; Gibbs and Stanton 2001).

### MATERIALS AND METHODS

We surveyed frogs on the Firestone Reserve during the wet season between 9 and 14 October 2006 in riparian forest, secondary forest, and bamboo plantation. The Firestone Reserve is a 60 ha protected preserve of lowland (15-303 m) Pacific Moist Forest in southwestern Costa Rica near Dominical (16.684 N, 51.643 W). Prior to 1993, the characteristic habitat of this area was deforested rangeland. Afterwards, managers removed the livestock and planted half the property with bamboo (*Guadua aculeata*, *G. angustifolia*, *Dendrocalamus asper*, *D. latiflorus*) and other non-native agricultural species. Since 2005, habitat throughout the property regrew without active restoration activities.

We surveyed for both *Dendrobates granuliferus* and *D. auratus*. *Dendrobates granuliferus* is vulnerable to extinction (IUCN 2006) due to human harvesting, habitat loss and degradation, and its restricted range (about 5,579 km<sup>2</sup>; Global Amphibian Assessment 2006. <http://www.globalamphibians.org> [Accessed December 2006]). *Dendrobates auratus* is of lesser concern, largely because of its greater range of 11,944 km<sup>2</sup> (Global Amphibian Assessment 2006, *ibid*).

We chose maintained trails of the reserve as study transects because they permitted an accessible and repeatable loop through the forest and traversed all three habitats of interest. We divided these trails (Total length = 72.6 km) into six segments (range = 101-960 m), and randomized their observation order to avoid multicollinearity with time. We typically conducted two sessions of observations each day. The first session lasted from ~ 0700-1100 h and the second was from ~ 330-1630 h.

For each sighting we recorded: (1) the distance to the frog's original location; (2) the magnetic bearings of each transect and frog; (3) the time; and (4) the location along each transect. We trigonometrically corrected sighting distances from incline distances (i.e., from the height of the hand-held instrument) to true plan distances. We ensured this study met the nine assumptions of the Hayne Estimator (Hayne 1949): (1) animals occur randomly and independently throughout the study area; (2) investigators observe animals on the transect line with a probability of one; (3) each sighting is an independent event; (4) animals remain motionless until flushed by the observer; (5) each animal has a specific circle of detection in which the animal will flush when stimulated by the observer's presence; (6) the mean sighting angle is  $32.7^\circ$ ; (7) each animal is counted only once; (8) distance measurements are error free; and (9) sighting conditions remain consistent during the study.

We calculated the population densities of *D. granuliferus* and *D. auratus* in all habitats for each of the 11 observation sessions using the Hayne Estimator

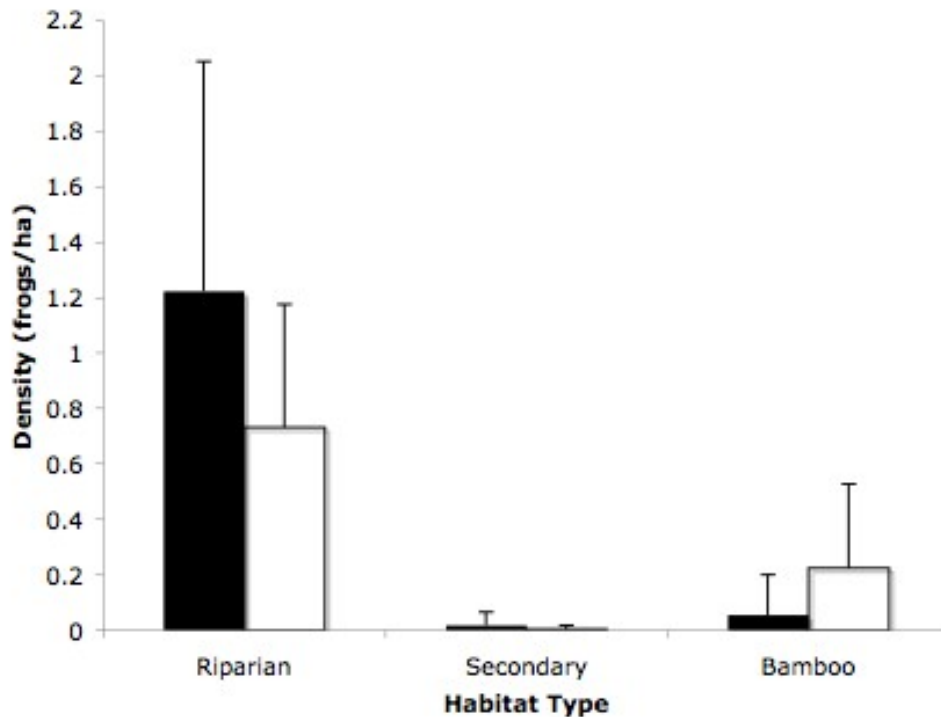


FIGURE 2. Average population densities of *Dendrobates granuliferus* (solid) and *D. auratus* (open) in the three habitat types on the Firestone Reserve, Costa Rica. Vertical lines are 1 standard deviation intervals.

formula (Hayne 1949):

$$D_h = \frac{n}{2L} \left( \frac{1}{n} \sum_{i=1}^n \frac{1}{r_i} \right)$$

where  $D_h$  is the Hayne density estimate,  $n$  is the number of animals observed,  $L$  is the transect length and  $r_i$  is the sighting distance to the  $i$ th animal. We calculated the standard deviation by taking the square root of the variance:

$$\text{Variance}(D_H) \approx D_H^2 \left[ \frac{\text{var}(n)}{n^2} + \frac{\sum_{i=1}^n \left( \frac{1}{r_i} - R \right)^2}{R^2 n(n-1)} \right]$$

where  $R$  is the mean of the reciprocal of the sighting distances calculated as:

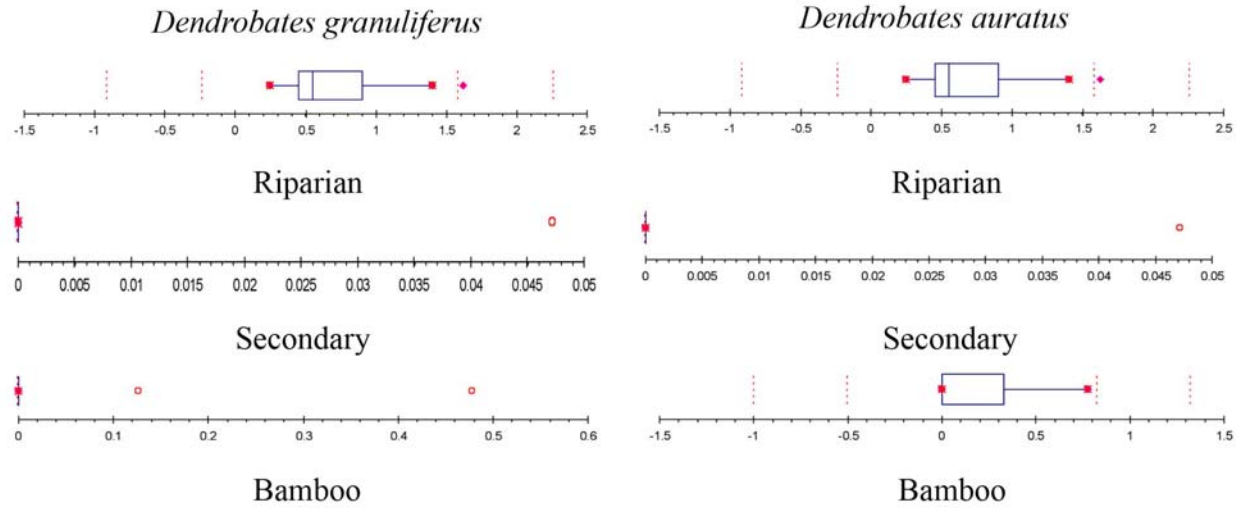
$$R = \frac{1}{n} \sum_{i=1}^n \frac{1}{r_i}$$

We determined the proximity of each frog sighting to water with COMPASS (Version 5.05; Fish, L. 2005. COMPASS v. 5.05. <http://fountainware.com/compass/>. Accessed November 2006.) by calculating a linear regression with VassarStats (Lowry, R. 2007. VassarStats: Web Site for Statistical Computation. Accessed from <http://faculty.vassar.edu/lowry/VassarStats.html> [Accessed February 2007]) on the number of frogs sighted at a location versus the straight-line distance between the sighting location and the closest water source. We then analyzed whether the transect distribution proportionally represented the amount of water in each habitat by comparing the following ratios:

$$\frac{\text{area of habitat within 50m of water}}{\text{total area of habitat}}$$

$$\frac{\text{transect length in habitat within 50m of water}}{\text{total length of transect in habitat}}$$

We used One-way ANOVA to test for differences in densities of frogs among habitats and Tukey Post-Hoc tests for pair-wise comparisons if overall differences were found. To determine if there was a correlation between the number of sightings of each species and yearly rainfall amounts, we used Pearson's chi-square 2 x 2 contingency table test. We used linear regression to determine if there was a correlation between the number of frog sightings and temperature. For all tests,  $\alpha = 0.05$ .



**Figure 3.** Box plots of density summary data used for ANOVA tests ( $n = 11$  for each habitat) for *Dendrobates granuliferus* (left) and *D. auratus* (right) sighted at the Firestone Reserve, Costa Rica.

### RESULTS

The average population densities of *D. granuliferus* were  $1.22 \pm 0.83$  frogs/ha in riparian forest,  $1.8 \times 10^{-2} \pm 4.8 \times 10^{-2}$  frogs/ha in secondary forest and  $5.4 \times 10^{-2} \pm 0.15$  frogs/ha in bamboo habitat (Fig. 2). Average population densities for *D. auratus* were  $0.73 \pm 0.44$  frogs/ha in riparian forest,  $4.7 \times 10^{-3} \pm 1.4 \times 10^{-2}$  frogs/ha in secondary forest and  $0.22 \pm 0.30$  frogs/ha in bamboo habitat. There were significant differences in densities among habitats for both *D. granuliferus* and *D. auratus* ( $F = 21.88$ ,  $df = 2$ ,  $P < 0.001$ ;  $F = 15.82$ ,  $df = 2$ ,  $P < 0.001$ , respectively; Fig. 3). Densities of both species were significantly higher in riparian habitat than either bamboo or secondary habitats, but densities were not significantly different between secondary and bamboo habitats.

The transect-habitat analysis for proportional water source representation yielded a ratio of 1.00 for riparian habitat, 0.70 for secondary habitat, and 0.88 for bamboo habitat (Table 1). There was no significant correlation between rainfall and the number of *D. auratus* sightings ( $X^2 = \dots$ ,  $P = 0.24$ ), but the number of sightings of *D. granuliferus* were significantly correlated to rainfall amounts ( $X^2 = \dots$ ,  $P = 0.02$ ). Similarly, we found no significant correlation between temperature and frog sightings for *D. auratus* ( $F = 1.45$ ,  $df = 1$ ,  $P = 0.13$ ,  $r^2 = 0.13$ ), but there was a significant relationship for *D. granuliferus* ( $F = 3.76$ ,  $df = 1$ ,  $P = 0.06$ ,  $r^2 = 0.09$ ). Temperature varied only 9°C during the time of observation and ranged from 23°C and 32°C.

Data separated by species fluctuated less over time. Sighting frequency of *D. granuliferus* remained constant except for an increase in the early morning

(0700 to 0800 h) and a decrease in the mid afternoon (1400 to 1500 h). The sighting frequency of *D. auratus* increased in the early morning (0700 to 0800 h) and decreased in the later morning (1000 to 1100 h) and mid afternoon (1400 to 1500 h).

### DISCUSSION

Higher population densities of both *D. granuliferus* and *D. auratus* occurred in riparian habitat than in secondary or bamboo habitats. This suggests that the Hayne Estimator accurately reflected the general density differences between the deforested and pristine areas. The densities in the secondary and bamboo habitats were too low for the Tukey Post-Hoc test to detect a difference. While no formal conclusions can be reached, the similarity of results between secondary and bamboo habitats suggests that they may be unsuitable habitats for dendrobatids. This hypothesis requires further study of frog densities in secondary and bamboo habitats to more rigorously test the suitability of these habitats.

The stark difference between frog densities in riparian and secondary forests is consistent with the results of past studies (Wenny et al. 1993; Alcalá et al. 2004) and supports that Hayne Estimates accurately represented population density trends in these habitats. Although overestimates are possible when the sighting angle exceeds 32.7° (Gates 1969; Burnham and Anderson 1976; Hayes and Buckland 1983), our mean sighting angle ( $29.2 \pm 4.1^\circ$ ) was not significantly different from the theoretical value of 32.7° ( $z = 0.854$ ,  $P = 0.197$ ). Our results provide preliminary evidence that the Hayne Estimator may be a valuable tool for evaluating population trends in Poison Dart Frogs.

The lack or a correlation between rainfall and sighting frequency for *D. auratus* was consistent with previous findings but the negative correlation between *D. granuliferus* sighting frequency and rainfall is inconsistent (Graves 1999). Activity of *D. pumilio*, whose biology is similar to *D. granuliferus*, positively correlated with rainfall. These disparities may arise from inconsistent rainfall during our study. Differences in sighting frequency over the day echo previous studies on Poison Dart Frog activity. The activity levels of both *D. granuliferus* and *D. auratus* were higher in the early morning, which parallels the prime activity level in *D. pumilio* (Graves 1999).

The Hayne Estimator should be further explored for identifying the relationships between deforestation and amphibian population size. Despite 13 years of unrestricted regrowth, secondary forests in our study showed much lower population densities for both species. Future studies are needed to evaluate similar parameters in other Poison Dart Frog species to expand our understanding of this group's sensitivity to habitat destruction.

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