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Monitoring Population Trends of *Eleutherodactylus* Frogs

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ABSTRACT.—**Like many Neotropical frogs, a number in the genus *Eleutherodactylus* have declined or gone extinct in the past two decades. However, the extent of *Eleutherodactylus* population declines is unknown. Our objective was to identify a good method for monitoring the density of *Eleutherodactylus* populations to assess the extent of declines. We did this in two ways. First, we compared two methods of directly estimating density, closed population capture-recapture analysis and distance sampling, and one method of indirectly estimating density, visual encounter surveys, for multiple *Eleutherodactylus* species at three sites in Ecuador. We then conducted a power analysis to estimate the power of our current sampling design to detect declines. Distance sampling estimates of density were biased low compared to capture-recapture estimates. When we corrected this bias, distance sampling estimates became imprecise. Estimates of density from visual encounter surveys were also imprecise. In contrast, capture-recapture estimates were fairly precise and most likely unbiased. Moreover, capture-recapture analysis had the most power to detect declines, although even with capture-recapture analysis, power was low with only five years of sampling. We conclude that capture-recapture analysis is a good method for monitoring *Eleutherodactylus* density over time, but the sampling area and/or the number of sampling occasions should be increased from the area and number of occasions used here in order to increase sample sizes and therefore power.**

RESUMEN.—**Como muchas especies de ranas y sapos neotropicales, varias ranas en el género *Eleutherodactylus* han disminuído en número o se han extinguido en las últimas dos décadas, pero no se sabe en que magnitud han disminuido. Nuestro objetivo fue identificar un buen método para monitorear la densidad de poblaciones de *Eleutherodactylus* y de esa forma evaluar la magnitud de sus disminuciones. Esto lo hicimos de dos maneras. Primero, comparamos dos métodos para estimar la densidad directamente, captura-recaptura para poblaciones cerradas y el muestreo de distancia, y un método para estimar densidad indirectamente, registro de encuentros visuales en transectos, en varias especies de *Eleutherodactylus* en tres sitios en Ecuador. Luego hicimos un análisis de poder para estimar el poder estadístico de nuestro diseño de muestreo actualizado para percibir disminuciones. Los cálculos del muestreo de distancia tenían un sesgo a la baja comparados a los cálculos de captura-recaptura. Cuando corregimos este sesgo, los cálculos de muestreo de distancia se volvieron imprecisos. También, los cálculos de registro de encuentros visuales en transectos fueron imprecisos. Los cálculos de captura-recaptura fueron medianamente precisos y probablemente no tuvieron sesgos a la baja o a la alta. Además, el análisis de captura-recaptura tenía el poder estadístico más alto para percibir disminuciones, aunque el poder fue bajo después de cinco años de muestreo. Concluimos que el análisis de captura-recaptura es un buen método para monitorear la densidad de *Eleutherodactylus* a través del tiempo, pero el área de muestreo y/o el número de ocasiones de muestreo deben ser incrementados en relación al área y el número de ocasiones que usamos para aumentar tamaños de muestras y poder.**

Perhaps the greatest obstacle to preventing and reversing amphibian declines is that there are few long-term data on population trends for most amphibians (Blaustein, 1994; Pechmann and Wilbur, 1994). As a result, most amphibian declines are not detected until populations have declined precipitously or gone extinct, by which time it may be too late to infer causes of declines, prevent future declines, or restore populations. Before it is possible to determine the causes of declines and develop management strategies to prevent and reverse declines, researchers and managers first need to know: (1) which species are declining; (2) where they are declining; and (3) the rate at which they are declining. Moreover, it is critical that this information is gathered quickly.

The only reliable way to gather this information is through well-designed amphibian population monitoring programs. Population mon-

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itoring involves estimating population parameters of interest over time and then using regression analysis to test for significant declines or increases in the parameters (Thompson et al., 1998). The parameters of interest in population monitoring programs are usually abundance (the absolute number of animals) or density (the number of animals per unit area) although parameters of interest may also include population growth rates or vital rates (birth and death rates). Monitoring programs should be designed so that biologically significant changes in the parameter of interest can be detected with a desired level of statistical power, the probability of detecting an actual decline or increase in the parameter of interest (Gerrodette, 1987). In turn, it is important to choose a good method for estimating the parameter of interest because the method used will directly affect the power to detect changes.

For most species of amphibians, little is known about which methods are best for estimating abundance or density. The best estimates are those that are both precise and unbiased. Precision is the degree of spread in estimates generated from repeated samples. Bias is the difference between the expected value of a parameter estimate and the true value of the parameter (Thompson et al., 1998). A lack of precision, manifest as high sampling variance, standard error, and coefficients of variation, reduces power. An estimate that is consistently biased high or low will not reduce power but will simply be an overestimate or underestimate of the true parameter, respectively.

There are two classes of methods for estimating abundance and density: direct estimators and indices. Direct estimators are designed to estimate true abundance or density by first estimating the number or proportion of individuals not encountered. In contrast, indices are count statistics that are assumed to be correlated with abundance or density by some functional relationship (Thompson et al., 1998) but do not directly estimate these parameters. Examples of direct estimators are closed population capture-recapture analysis, distance sampling, and removal sampling (White et al., 1982; Seber, 1982; Buckland et al., 1993). Indices that have been applied to amphibian populations include visual encounter surveys, audio strip transects, and breeding site surveys (Heyer et al., 1994).

There are two problems with indices (Thompson et al., 1998). First, use of an index assumes that there is a functional relationship between the index and the parameter of interest, but often this relationship is unknown. Moreover, even if the function relating the index and parameter is known in a particular case, it is likely not constant over time, space, species, or observers. Second, indices often have high sampling variance. Because of these problems, direct estimators are expected to give better estimates than indices, both in terms of precision and bias, as long as their assumptions are met. However, direct estimators generally require more effort at a greater cost. Because indices are relatively easy and cheap, they are much more commonly used for studies of amphibian populations than are direct estimators.

The objective of the present study was to identify a good method for monitoring population density of *Eleutherodactylus* frogs and, in particular, to identify a method that has a high probability of quickly detecting rapid declines because many tropical amphibian declines have occurred rapidly (Lips, 1999; Young et al., 2001). *Eleutherodactylus* are direct developing frogs found throughout the Neotropics, some of which have experienced declines and that are in immediate need of population monitoring. The genus is represented by over 600 described species and dozens of undescribed species, making it the most speciose vertebrate genus in the world (Lynch, 1999). At least three *Eleutherodactylus* species have declined or gone extinct in Costa Rica and Panama (Lips, 1999), nine species in Puerto Rico (Hedges, 1993; Joglar and Burrowes, 1996), and several others from other Latin American countries (Hedges, 1993; Young et al., 2001). Because of the extreme species richness of the genus, continued *Eleutherodactylus* population declines could result in a major loss of Neotropical and global amphibian diversity.

We used two direct estimators and one index to estimate the density of multiple *Eleutherodactylus* species from Ecuador and evaluated the relative performance of each method in terms of the precision and bias of its estimates. We then estimated the power to detect *Eleutherodactylus* population declines using these three methods. The two direct estimators we tested were closed population capture-recapture analysis and distance sampling. The index we tested was visual encounter surveys. Capture-recapture analysis uses capture histories of individually marked animals to estimate capture probabilities and from these probabilities, the number of individuals not found. Distance sampling uses the distribution of distances of animals from transect centerlines to estimate a detection function, which is then used to estimate the proportion of animals not encountered. Finally, visual encounter surveys involve systematically searching an area and estimating the number of animals found per person-hour of searching. Capture-recapture analysis is the most labor-intensive of these three methods and was therefore expected to provide the most power to detect declines. However, this is the first study to

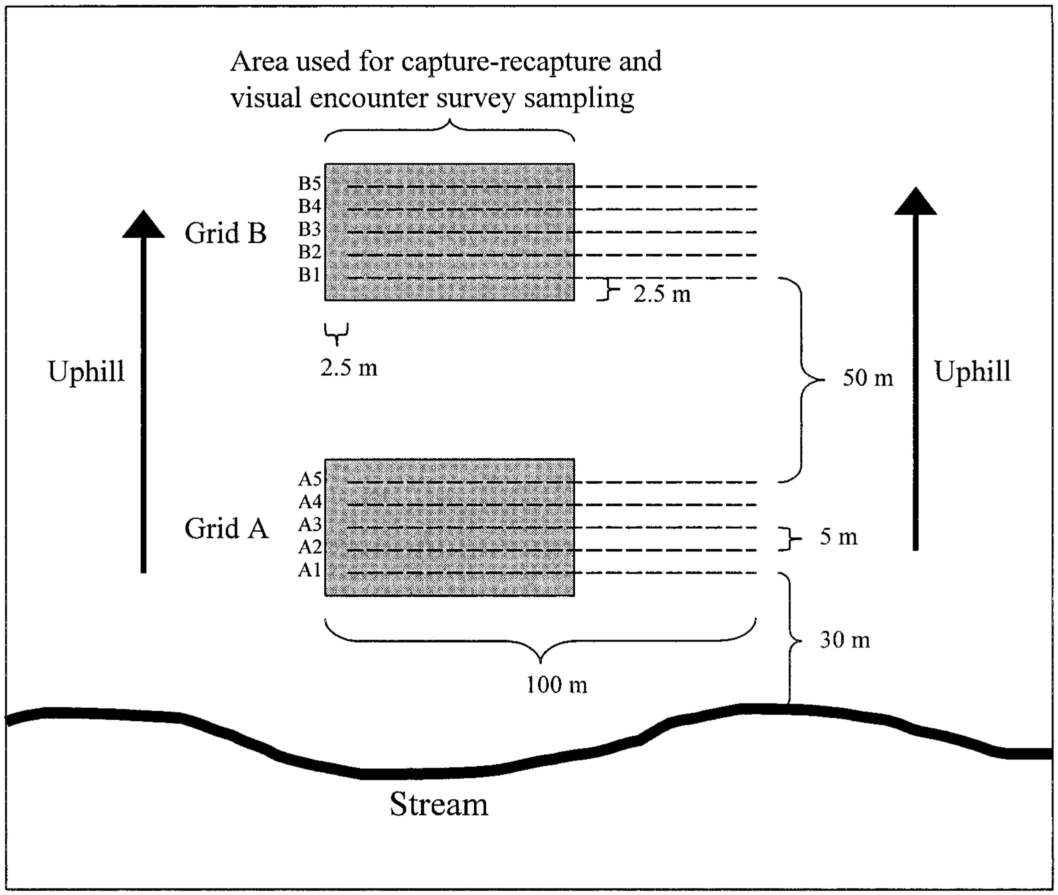

FIG. 1. Grid (A and B) and transect (A1-5 and B1-5) layout used for estimating the density of *Eleutherodactylus* in cloud forest (Cashca Totoras and Yanayacu) and lowland rain forest (Sacha Lodge) using capture-recapture analysis, distance sampling, and visual encounter surveys. The entire length of transects (100 m) was used for distance sampling, but only the area encompassing the first half of transects (0–50 m) was used for capture-recapture and visual encounter survey sampling. Five transects were used in each grid at Cashca Totoras (total transect length (L) = 1000 m), but only four were used in each grid at Yanayacu and Sacha Lodge (L = 800 m). The area used for capture-recapture and visual encounter survey sampling was 55 m × 25 m = 1375 m^2 of each grid at Cashca Totoras and 55 m × 20 m = 1100 m^2 at Yanayacu and Sacha Lodge.

quantify the power of these methods for tropical frogs to assess whether capture-recapture analysis is sufficiently more powerful than the other two methods to warrant its higher cost.

MATERIALS AND METHODS

Study Sites and Sampling Design.—We estimated the population density of multiple *Eleutherodactylus* species in different forest types at three sites in Ecuador to test the relative performance of closed population capture-recapture analysis, distance sampling, and visual encounter surveys. The three sites we used were the Bosque Protector Cashca Totoras, Yanayacu Biological Station, and Sacha Lodge Biological Station. The Bosque Protector Cashca Totoras is located at approximately 3200 m on the west side of the Cordillera Occidental of the Andes in Provincia Bolívar at 01°43′S, 78°58′W. The reserve consists of a mixture of pasture and secondary and primary montane cloud forest with a 15–20 m canopy. Yanayacu Biological Station is located at approximately 2100 m on the east side of the Cordillera Oriental of the Andes in Provincia Napo at 00°35′S, 77°53′W. Yanayacu is surrounded by pasture and primary cloud forest with a 20–25 m high canopy. Sacha Lodge Biological Station is located at 250 m in lowland Amazonia in Provincia Sucumbíos at 00°26′S, 76°27′W. The forest at Sacha Lodge is a mixture of secondary and primary lowland rain forest and has a 25–30 m canopy.

At each site, we set up two grids in forest (Fig. 1). Grids were at least 100 m from forest edge at Yanayacu and Sacha Lodge and 50 m from edge at Cashca Totoras. Each grid consisted of 100 m parallel transects spaced 5 m apart. At Cashca Totoras, each grid had five transects and at Yanayacu and Sacha Lodge each grid had four. We set up the first grid (designated grid A) approximately 30 m uphill from a stream and the second grid (designated grid B) 50 m further uphill of grid A to allow us to test for differences in density at different distances from streams. We used distance sampling along the entire length of transects. For capture-recapture and visual encounter surveys, we only sampled the area encompassing the first 50 m of transects because capture-recapture sampling took more time than distance sampling. The area sampled for capture-recapture analysis and visual encounter surveys was 55 × 25 m = 1375 m^2 of each grid at Cashca Totoras and 55 × 20 m = 1100 m^2 of each grid at Yanayacu and Sacha Lodge.

At each site, we sampled grids using all three methods for six to seven consecutive nights. Grids were searched at night because most *Eleutherodactylus* are nocturnal. We first sampled grids using capture-recapture and visual encounter surveys for five nights at Cashca Totoras and six nights at Yanayacu and Sacha Lodge. Each night was considered one sampling occasion. After capture-recapture and visual encounter surveys, we sampled grids for one night using distance sampling. We started sampling at nightfall (1900–2000 h) and continued until we finished which took approximately 2–7 h depending on the number of frogs that were found and processed. After estimating the density of *Eleutherodactylus* species using these three methods, we compared each method in terms of bias and precision. We considered a method to be ''good'' if it gave unbiased, precise estimates of density and ''poor'' if it gave biased and/or imprecise estimates.

A potential problem with using capture-recapture analysis, distance sampling, and visual encounter surveys to estimate frog density on the same grid is that frogs may hop away, hide, and/or become more difficult to catch over time because of the added handling time required for capture-recapture sampling, thereby biasing estimates low. To assess whether this was a problem in our study, we tested whether the number of *Eleutherodactylus* caught per person-hour decreased over time within nights and/or across nights. In the first analysis, we divided each night into two equal time periods, calculated the mean number of *Eleutherodactylus* caught during the first half of the night and the second half of the night for the entire sampling period at each site, and tested whether there was a significant decrease in the mean number of *Eleutherodactylus* caught in the second half of nights at each site. In the second analysis, we tested whether there was a significant decrease in the number of *Eleutherodactylus* caught per person-hour over nights at each site. In neither analysis did we find a reduction in the number of frogs caught per person-hour over time at any of our three sites. Therefore, the added time of handling frogs during capture-recapture sampling does not appear to bias estimates low.

Voucher specimens of each species sampled were stored at the Museo de Zoología of the Pontificia Universidad Católica del Ecuador in Quito, Ecuador.

Closed Population Capture-Recapture Analysis.— Grids were searched by walking along transects and searching the entire area within 2.5 m of transect centerlines, moving off of transects when this area was not visible from centerlines. Because transects were separated by 5 m, this method assured that the entire area within grids was searched. When frogs were found, we captured them by hand, recorded their locations, and marked animals larger than or equal to 10 mm snout–vent length. Frogs were marked by clipping 3–5 toes in unique combinations similar to those used by Waichman (1992) except that we did not clip thumbs (Finger I) from the forefeet or the longest digits (Toe IV) from the hind feet. We sterilized cut toes with Bactine® and released frogs where they were found. If a frog had already been marked, we recorded its code and location and released it where it was caught.

We used our capture-recapture data to estimate the abundance and density of the four *Eleutherodactylus* species with the largest sample sizes and numbers of recaptures. One species was from Cashca Totoras, one was from Yanayacu, and two were from Sacha Lodge. Initially, we analyzed our capture-recapture data using program MARK (White and Burnham, 1999) rather than the Lincoln-Petersen estimator (Lincoln, 1930) or program CAPTURE (White et al., 1982) for a number of reasons. First, the Lincoln-Petersen estimator requires an assumption which program MARK and program CAPTURE do not. Although all three methods assume that populations are closed (no births, deaths, immigration, or emigration) during capture sessions and that marks are not lost, the Lincoln-Petersen estimator also assumes that all animals have the same probability of being caught during sampling occasions (Thompson et al., 1998). Second, MARK allows the development of more user-defined models than program CAPTURE (White and Burnham, 1999), including models with group covariates, which permits testing alternative hypotheses for differences among groups such as sex or habitat type. Finally, program MARK has more advanced model selection features than CAPTURE. Specifically, program MARK uses Akaike's Information Criterion values adjusted for sample size (AICc; Akaike, 1973) to identify the best models in terms of parsimony and fit to the data.

However, for all species analyzed, the best models selected by program MARK gave estimates of abundance and standard error that were very different from each other despite the fact that the models had similar AICc values and therefore similar levels of support. This suggested that some of the abundance estimates and standard error estimates were poor in that the abundance estimates were biased low or high, and the standard error estimates were either unreasonably large or unrealistically small. The likely reason for the poor estimates is that program MARK has little power to select the best model(s) with small sample sizes, such as we had, as has been demonstrated for program CAPTURE (Menkens and Anderson, 1988).

As a result of the poor estimates obtained using program MARK, we decided to use Chapman's unbiased version of the Lincoln-Petersen estimator to estimate abundance and its associated variance (Seber, 1982). Chapman's estimator has been shown to perform well with small sample sizes except when there is extreme individual heterogeneity in capture probabilities and/or extreme behavioral responses in capture probabilities (Menkens and Anderson, 1988). Potential sources of individual heterogeneity in capture probabilities for frogs are heterogeneity among males and females and/or among adults and juveniles. Males may have higher capture probabilities than females because they advertise their locations with calls. Likewise, adults may have higher capture probabilities than juveniles because adults are larger and potentially easier to see. Moreover, there could be a behavioral response in frogs if frogs become more wary and more difficult to catch over time (termed ''trap shy'' in small mammal trap studies) or if researchers become better at locating and/or capturing animals through time (termed ''trap happy'' in trap studies).

As recommended by Menkens and Anderson (1988), we tested for evidence of individual heterogeneity and/or behavioral responses in capture probabilities using chi-square tests in program CAPTURE and found no evidence for heterogeneity or behavioral responses for any of the *Eleutherodactylus* species we analyzed. Lack of evidence for heterogeneity and/or behavioral responses may be caused by low power of the chi-square tests, but it does suggest that any existing heterogeneity or behavioral responses or both were not extreme. Therefore, we proceeded to estimate abundance with Chapman's estimator. At each site, the first half of the capture-recapture sampling period was designated as the capture and marking period (three days at all sites) and the second half was designated as the recapture period (two days at Cashca Totoras and three days at Yanayacu and Sacha Lodge).

To convert our estimates of abundance into estimates of density, we calculated the effective capture area for each species analyzed using the mean maximum distance moved procedure as described by Wilson and Anderson (1985). We also used the procedures they described for estimating the variance associated with density estimates.

Distance Sampling.—Prior to sampling, we laid out nylon string along the centerlines of transects to facilitate accurate measurement of distances of frogs from centerlines. During sampling, we walked along transect centerlines and searched on both sides of transects. In contrast to capture-recapture and visual encounter surveys, we remained on centerlines while searching for frogs during distance sampling. Because we rarely observed frogs at a distance of greater than 2 m from centerlines, the probability of observing the same frog twice from different transects was minimal. When a frog was observed, we caught the frog to identify it and then used a metal tape measure to measure the distance of the frog from the centerline to the nearest centimeter. Measurements were likely accurate because frogs did not move away from their original positions when approached. Only frogs equal to or larger than 10 mm snout–vent length were included in the distance sampling analysis.

We used program DISTANCE version 3.5 (Buckland et al., 1993) to analyze our distance data for the two *Eleutherodactylus* species that had total sample sizes of at least 30 (*Eleutherodactylus simonbolivari* from Cascha Totoras and *Eleutherodactylus* sp. 3 from Yanayacu). Program DISTANCE fits distance data to various detection functions and evaluates the detection functions using Akaike's Information Criterion (AIC). The detection function with the lowest AIC value is considered the best function based on the criteria of parsimony and fit to the data (Akaike, 1973). This function is then used to estimate density. We fit our data to all nine of the detection functions available in program DISTANCE, each of which is defined by a key function (uniform, half-normal, or hazard-rate) and series expansion (cosine, simple polynomial, or hermite polynomial). Prior to analyzing our data, we examined them using histograms to make sure that there was no heaping of observations at zero (defined as the disproportionate accumulation of observations near zero distance from the centerline) and to identify outlying observations because both heaping and outliers can result in poor density estimates (Buckland et al., 1993). We did not find heaping for either of the species we analyzed. We removed observations identified as outliers (Buckland et al., 1993) by truncating the distance data at 1.6 m (four of 54 observations) for *E. simonbolivari* and 1.8 m (four of 35 observations) for *Eleutherodactylus* sp. 3.

Distance sampling requires three main assumptions (Buckland et al., 1993). The first assumption is that objects on the transect are detected with certainty so that the probability of detection on the centerline ($g[0]$) is one. When $g(0)$ is less than one, density estimates ($\hat{D}$) are biased low by the factor $g(0)$. The second assumption is that objects are detected at their initial location. The third assumption is that measurements are exact. We were confident that the last two assumptions were met for our analysis, but were skeptical that the first assumption was met because some frogs may not be active every night, and others may simply be overlooked. We therefore estimated $g(0)$ for *E. simonbolivari* from the ratio of $\hat{D}$ obtained from distance sampling to $\hat{D}$ obtained from capture-recapture analysis under the assumption that $\hat{D}$ obtained from capture-recapture analysis was an unbiased estimate. We only used data from the first 50 m of distance sampling transects for estimating $g(0)$ because this is the portion of grids that were sampled using capture-recapture (Fig. 1). We used the method described by Mood et al. (1974) to estimate the variance associated with this ratio, assuming that covariance among the two estimates was zero.

Visual Encounter Surveys.—We conducted visual encounter surveys concurrently with capture-recapture sampling. However, the only data we collected for visual encounter surveys was the number of each *Eleutherodactylus* species equal to or larger than 10 mm snout–vent length found during sampling occasions and the time spent searching. These data were then used to calculate an index: the number of frogs seen per person-hour for each sampling occasion (each night). The main assumptions of visual encounter surveys are that (1) all individuals of all species have the same probability of being observed; (2) the probability of being observed is constant over time and space; and (3) there are no differences in the ability of observers to detect animals (Heyer et al., 1994). If all of these assumptions hold, then the functional relationship between the index and density will be constant, and the index can theoretically be used as a surrogate for direct estimates of density.

We used linear regression analysis in program SPSS version 7.0 to test whether there was a significant positive linear relationship between $\hat{D}$ obtained from capture-recapture analysis and the mean number of frogs caught per person-hour per night ($\hat{I}$) averaged over five consecutive nights of sampling. We then used the linear regression model relating these two variables to predict $\hat{D}$ and its associated variance from $\hat{I}$ after one, three, or five nights of sampling.

Power Analysis.—We conducted a power analysis using program TRENDS (Gerrodette, 1993) to estimate the power to detect declines in density using capture-recapture, distance sampling, and visual encounter survey estimates of *Eleutherodactylus* density. Power is defined as the probability of detecting an actual decline ($1 - \beta$) where β is the probability of concluding no decline when a decline actually exists (a Type II error). We estimated the power to detect a major decline (20% exponential decline per year) and a less severe, but still substantial, decline (10% exponential decline per year) after five or 10 years of annual sampling given the coefficients of variation obtained from capture-recapture analysis, distance sampling, and visual encounter surveys. We were particularly interested in estimating the power to detect major declines over a short time interval given that many declines of tropical amphibian populations have occurred rapidly (Lips, 1999; Young et al., 2001). We set $\alpha = 0.05$ and used a directional test with 20% of alpha allocated for detecting a positive trend (Rice and Gaines, 1994). Estimates of power obtained from program TRENDS are maximum estimates because program TRENDS does not consider temporal or spatial process variation, which will decrease power to detect population trends (Thompson et al., 1998).

RESULTS

Closed Population Capture-Recapture Analysis.—Sample sizes and numbers of frogs recaptured at least twice were small for all of the *Eleutherodactylus* species encountered during capture-recapture sessions (Table 1). We only analyzed capture-recapture data for *E. simonbolivari* from Cashca Totoras, *Eleutherodactylus eriphus* from Yanayacu, and *Eleutherodactylus lanthanites* and *Eleutherodactylus martiae* from Sacha Lodge because numbers of recaptures and/or sample sizes were very small for the other species.

Capture-recapture estimates of density varied substantially among the four species analyzed, but coefficients of variation were not as variable (Fig. 2). *Eleutherodactylus simonbolivari* had the highest density with $\hat{D} \pm \hat{SE}(\hat{D}) = 564 \pm 112$ frogs/ha. The other three species, *E. eriphus, E. lanthanites*, and *E. martiae* had much lower densities of $\hat{D} = 154 \pm 42$ frogs/ha, $\hat{D} = 129 \pm 27$ frogs/ha, and $\hat{D} = 99 \pm 30$ frogs/ha, respec-

TABLE 1. *Eleutherodactylus* species sampled during capture-recapture sessions at three sites in Ecuador. A = total area (m^2) sampled at each site, M_{t+1} = number of individuals caught, recaptures = number of individuals caught on at least two different nights.

Site	A	Species	Grid	M_{t+}	Recaptures
Cashca Totoras	2750	*E. phoxocephalus*	A	2	0
			B	0	0
			Total	2	0
		E. simonbolivari	A	53	13
			B	39	7
			Total	92	20
		E. truebae	A	3	1
			B	0	0
			Total	3	1
Yanayacu	2200	*E. eriphus*	A	8	1
			B	14	7
			Total	22	8
		Eleutherodactylus sp. 2	A	16	1
			B	11	1
			Total	27	2
		Eleutherodactylus sp. 3	A	29	1
			B	8	0
			Total	37	1
Sacha Lodge	2200	*E. altamazonicus*	A	5	1
			B	0	0
			Total	5	1
		E. lanthanites	A	12	2
			B	15	8
			Total	27	10
		E. martiae	A	12	5
			B	13	2
			Total	25	7
		E. ockendeni	A	4	4
			B	3	1
			Total	7	5
		E. variabilis	A	2	1
			B	2	0
			Total	4	1

tively. The corresponding coefficients of variation for *E. simonbolivari, E. eriphus, E. lanthanites*, and *E. martiae* were $CV(\hat{D})$ = 0.20, 0.27, 0.21, and 0.31, respectively. There were no differences in density among grids A and B for any species that could not be attributed to sampling error.

Capture probabilities during the capture and marking session (the first three nights of sampling at all sites) and recapture session (the last two nights of sampling at Cascha Totoras and last three nights at the other two sites) were fairly low for the species analyzed. Estimated capture probabilities for the capture and marking session ($\hat{p}_1$) were 0.28, 0.29, 0.41, and 0.34 for *E. simonbolivari, E. eriphus, E. lanthanites*, and *E. martiae*, respectively. Estimated capture probabilities for the recapture session ($\hat{p}_2$) were 0.24, 0.40, 0.46, and 0.39 for *E. simonbolivari, E. eriphus, E. lanthanites*, and *E. martiae*, respectively.

Distance Sampling.—Sample sizes from distance sampling were also small (Table 2). We only analyzed distance sampling data for *E. simonbolivari* from Cashca Totoras and *Eleutherodactylus* sp. 3 from Yanayacu because sample sizes were very small for the other species.

Unadjusted ($g[0] = 1$) distance sampling estimates of density for *E. simonbolivari* and *Eleutherodactylus* sp. 3 were substantially different from each other, but their coefficients of variation were the same (Fig. 2). *Eleutherodactylus simonbolivari* had a higher density with $\hat{D}$ = 260 ± 49 frogs/ha compared to $\hat{D}$ = 162 ± 31 frogs/ha for *Eleutherodactylus* sp. 3. The coefficient of variation was $CV(\hat{D})$ = 0.19 for both species.

The density estimate obtained for *E. simonbolivari* from distance sampling was much lower than the estimate obtained from capture-recapture analysis (Fig. 2), suggesting that the assumption that $g(0) = 1$ was violated and that $g(0) < 1$. Based on the ratio of distance sampling and capture-recapture estimates of density for *E. simonbolivari*, $\hat{g} = 0.35 \pm 0.12$ $\{CV\ [\hat{g}\ (0)] = 0.34\}$. When we adjusted density estimates for *E. simonbolivari* and *Eleutherodactylus* sp. 3 using this estimate of *g(0)*, the density estimates and

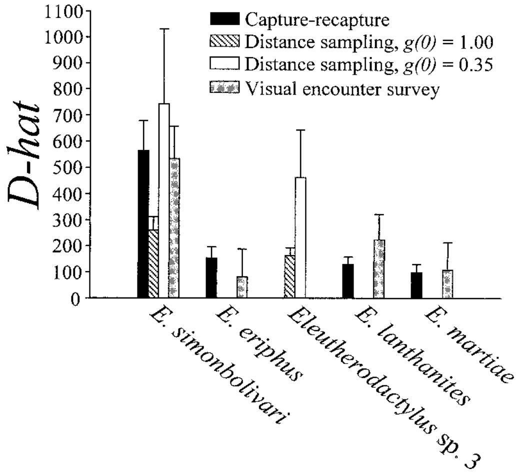

FIG. 2. Density estimates ($\hat{D}$) for *Eleutherodactylus* obtained from capture-recapture analysis, distance sampling, and visual encounter surveys. Density is expressed as individuals/hectare and error bars represent standard error estimates associated with each density estimate ($\widehat{SE}[\hat{D}]$). Distance sampling estimates are shown with the probability of detection on transect centerlines ($\hat{g}[0]$) not adjusted $\{\hat{g}[0] = 1.00; \widehat{SE}[\hat{g}(0)] = 0.00\}$ and adjusted $\{\hat{g}[0] = 0.35; \widehat{SE}[\hat{g}(0)] = 0.12\}$ for incomplete detection. Visual encounter survey estimates were derived from the mean number of frogs caught per person-hour per night ($\hat{I}$) averaged over five consecutive nights of sampling using the regression equation $\hat{D} = 298 \cdot \hat{I} - 125$.

their associated variance increased substantially for both species, as expected (Fig. 2). With the adjusted $g(0)$, $\hat{D} = 742 \pm 289$ frogs/ha ($CV[\hat{D}] = 0.39$) for *E. simonbolivari* and $\hat{D} = 461 \pm 180$ frogs/hectare ($CV[\hat{D}] = 0.39$) for *Eleutherodactylus* sp. 3.

Visual Encounter Surveys.—The linear regression model relating the mean number of frogs caught per person-hour per night ($\hat{I}$) averaged over five nights of sampling to $\hat{D}$ estimated from capture-recapture analysis ($\hat{D} = 298 \cdot \hat{I} - 125$) was marginally significant ($F = 16.841$, $r = 0.945$, $P = 0.055$). However, the regression model was very poor at predicting $\hat{D}$ from $\hat{I}$, giving density estimates that tended to be very imprecise (Fig. 2). The regression model gave particularly poor estimates of density when $\hat{I}$ was low, even predicting negative densities for *E. eriphus* and *E. martiae* after one night of sampling. All of the 95% prediction intervals for $\hat{D}$ included zero except for the prediction interval for *E. simonbolivari* after five nights of sampling, indicating that in most cases, estimates were indistinguishable from zero. Coefficients of variation for density estimates were also extremely large, ranging from 0.23–1.99 and were negative in two cases based on the negative density estimates for *E. eriphus* and *E. martiae* after one night of sampling.

Power Analysis.—The predicted power to detect declines in density for the *Eleutherodactylus* species analyzed depended strongly on the method used to estimate density, the number of years of sampling, the rate of decline, and the coefficient of variation of density estimates (Fig. 3). As expected, power was higher to detect a 20% decline per year (Fig. 3A–B) than a 10% decline (Fig. 3C–D) and was higher after 10 years of annual sampling (Fig. 3B, D) than after five years (Fig. 3A, C). Moreover, power tended to be highest with capture-recapture estimates of density because of the relatively small coefficients of variation associated with these estimates. In particular, capture-recapture analysis had the highest power to detect a 20% decline per year after 5 years of sampling for all species analyzed (Fig. 3A). Under this scenario, power ranged from 0.35–0.61 for capture-recapture analysis and 0.10–0.52 for visual encounter surveys and was 0.27 for both species analyzed using distance sampling.

DISCUSSION

Density Estimation.—Distance sampling and visual encounter survey estimates of *Eleutherodactylus* density were imprecise and biased relative to capture-recapture estimates, pointing to capture-recapture analysis as the best method for estimating *Eleutherodactylus* density. The main problem with distance sampling estimates was that they were biased very low, most likely caused by violation of the assumption of complete detection on transect centerlines. When we corrected this bias using an estimate of the probability of detection on transect centerlines, the bias was removed, but variance was greatly increased (Fig. 2) giving coefficients of variation of approximately 0.39.

Visual encounter survey estimates of density were also imprecise (Fig. 2). In some cases, imprecision reached astronomical levels, yielding coefficients of variation greater than one. The imprecision of visual encounter survey estimates stem from problems associated with the regression model used to predict density ($\hat{D}$) from the index ($\hat{I}$). These problems include small sample size (only four datapoints), among species variation in the functional relationship between $\hat{I}$ and $\hat{D}$, sampling error of $\hat{I}$ and $\hat{D}$, and a nonzero y-intercept. It may be possible to partially improve the regression model by increasing sample size, developing different regression models for each species and obtaining more precise estimates of the index and density. However, this would likely require at least as much work as directly estimating density using cap-

TABLE 2. *Eleutherodactylus* species sampled during distance sampling sessions at three sites in Ecuador. L = total length (m) of transects, N = sample size.

Site	L	Species	Grid	N
Cashca Totoras	1000	*E. simonbolivari*	A	26
			B	28
			Total	54
		E. truebae	A	0
			B	1
			Total	1
Yanayacu	800	*E. eriphus*	A	2
			B	10
			Total	12
		Eleutherodactylus sp. 1	A	1
			B	0
			Total	1
		Eleutherodactylus sp. 2	A	2
			B	4
			Total	6
		Eleutherodactylus sp. 3	A	23
			B	12
			Total	35
Sacha Lodge	800	*E. lanthanites*	A	4
			B	8
			Total	12
		E. martiae	A	10
			B	1
			Total	100
		E. ockendeni	A	2
			B	3
			Total	5
		E. variabilis	A	1
			B	2
			Total	3

ture-recapture analysis, defeating the one advantage of indices: lower effort and cost.

The conclusion that capture-recapture analysis gives better estimates of density than distance sampling or visual encounter surveys rests upon the assumption that our capture-recapture estimates were not biased. We believe that this is a good assumption given that the capture-recapture estimator we used, Chapman's estimator, has been shown to give unbiased estimates, even with small sample sizes, as long as there is not extreme heterogeneity or extreme behavioral responses or both in capture probabilities (Menkens and Anderson, 1988). We did not find evidence of heterogeneity or behavioral responses, so we concluded that our estimates should not be biased.

We recommend designing sampling grids so that sample sizes are large enough to allow analysis of capture-recapture data with programs MARK and CAPTURE. These programs are more flexible than Chapman's estimator because they provide estimators of abundance when capture probabilities vary among individuals within sampling occasions. The minimum sample size necessary for model selection procedures in both programs to function properly depends on capture probabilities. With low capture probabilities, as observed in this study, White et al. (1982) recommend sample sizes of 200 or more animals.

One way of increasing sample sizes is to increase the size of the area sampled. For example, we found 92 *E. simonbolivari* on both of our grids, which together encompassed an area of 2750 m^2 (Table 1). To increase the sample size to 200 individuals, the area sampled would need to be increased 2.17-fold (= 200 individuals desired/92 individuals observed) to approximately 6000 m^2 ($= 2.17 \times 2750$ $m^2 = 5968$ m^2). Alternatively, sample size could be increased by increasing the number of sampling occasions or a combination of increasing sampling area and sampling occasions. However, the number of sampling occasions should not be increased beyond 1–2 weeks, because after this time frame, frogs may begin to emigrate out of grids or immigrate into grids, which will violate the assumption of closure necessary for closed population capture-recapture analysis.

Power Analysis.—The power to detect declines in *Eleutherodactylus* density was generally highest with capture-recapture estimates because these estimates had the smallest coefficients of variation (Fig. 3). In particular, capture-recapture analysis had much more power to detect a rapid decline of 20% per year after five years of annual sampling than did distance sampling or visual encounter surveys (Fig. 3A). An exponential decline of 20% per year translates into a 67% decline after five years {calculated from 100% × [1 − $(1 - 0.20)^5$]} and is similar in magnitude to the rapid declines observed in many tropical frogs (Lips, 1999; Young et al., 2001). A decline of this magnitude is certainly of conservation concern and monitoring programs should be designed so that they have high power to detect such declines. Because distance sampling and visual encounter surveys will generally not have the power to detect these declines, capture-recapture analysis will usually be the most appropriate method for monitoring *Eleutherodactylus* density.

Nonetheless, even with capture-recapture sampling, power was low to detect an annual decline of 20% in *Eleutherodactylus* density after five years of sampling with the current sampling design (Fig. 3A). Moreover, our estimates of power are maximum estimates because we did not account for temporal or spatial process variation in density. When process variation is included, CVs will increase (Thompson et al., 1998), thereby reducing power, so our power estimates are optimistic. Therefore, we emphasize once again that it is important to increase sample sizes by increasing grid size, the number of nights of sampling, or both as previously described to increase the precision of density estimates and the power to detect declines.

Recommendations for Monitoring Eleutherodactylus Frogs.—For the *Eleutherodactylus* species analyzed in this study, we recommend monitoring density using capture-recapture analysis with larger sample sizes. Capture-recapture estimates of density were more precise than estimates generated from distance sampling or visual encounter surveys (Fig. 2), which allowed greater power to detect declines using capture-recapture (Fig. 3). In particular, capture-recapture analysis had the most power to quickly detect rapid declines. Because many declines of tropical amphibians have been rapid, we feel that all amphibian monitoring programs in the tropics should be designed so that they have a high probability of detecting these declines. For

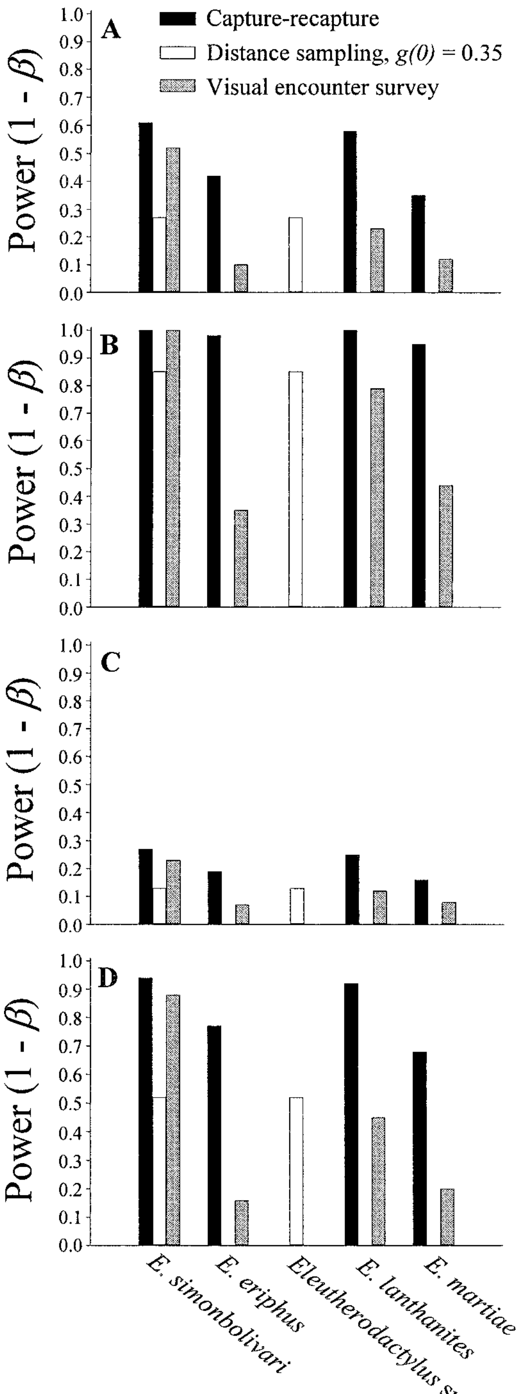

FIG. 3. Predicted power (1 − β) to detect exponential declines in the density of *Eleutherodactylus* using capture-recapture analysis, distance sampling corrected for incomplete detection on transect centerlines {$\hat{g}(0) = 0.35$; $\hat{SE}[\hat{g}(0)] = 0.12$}, or visual encounter surveys. (A) 20% decline per year, five years of annual sampling; (B) 20% decline per year, 10 years of annual sampling; (C) 10% decline per year, five years of annual sampling; (D) 10% decline per year, 10 years of annual sampling.

←

TABLE 3. Advantages and disadvantages of capture-recapture, distance sampling, and visual encounter surveys for monitoring the density of *Eleutherodactylus* species.

Method	Advantages	Disadvantages
Capture-recapture	1. More precise and less biased estimates 2. Higher power to detect declines, particularly to quickly detect rapid declines 3. Allows estimation of other parameters such as survival probability if study correctly designed	1. More labor intensive, which may require reducing the number of species and/or sites monitored
Distance sampling	1. Less labor intensive	1. Biased low 2. Correcting bias causes estimates to become imprecise 3. Lower power so that may not detect rapid declines
Visual encounter surveys	1. Less labor intensive	1. Imprecise 2. Lower power so that may not detect rapid declines

all of the species that we analyzed except for *Eleutherodactylus* sp. 3 from Yanayacu, capture-recapture analysis is the best method for detecting rapid declines. In the case of *Eleutherodactylus* sp. 3, there were not enough recaptures to permit capture-recapture analysis (Table 1). Therefore, either distance sampling or visual encounter surveys need to be used to monitor the density of *Eleutherodactylus* sp. 3.

We would also generally recommend using capture-recapture analysis for monitoring the density of other species of *Eleutherodactylus* not analyzed here because of the much higher precision and greater power of this method (Table 3). In addition, capture-recapture analysis can be used to estimate other parameters such as survival probability when studies are designed appropriately (Cormack, 1964; Jolly, 1965; Seber, 1965; Pollock et al., 1990; W. C. Funk and L. S. Mills, unpubl. data). Survival estimates and other vital rate (birth and death rate) estimates are useful because they can be used to conduct ecological sensitivity analyses to help identify likely causes of declines and develop management strategies for preventing and reversing declines (Caswell, 2001; Biek et al., 2002). Although capture-recapture studies are more labor intensive than distance sampling or visual encounter surveys, which may limit the number of species or sites included in a monitoring program (Table 3), we believe that it is much more valuable to have high power to detect declines of one or a few species at fewer sites than it is to have low power to detect declines of many species at many sites. However, for some species such as *Eleutherodactylus* sp. 3 from Yanayacu, it may not be possible to use capture-recapture to estimate density. Therefore, we also strongly recommend conducting pilot studies prior to implementing long-term monitoring programs to determine which method or combination of methods yield the highest power to detect declines for each species.

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