

The Adaptive Significance of Male Parental Care
in a Neotropical Frog

by

Daniel S. Townsend

A Dissertation

Submitted to the State University of New York at Albany

in Partial Fulfillment of

the Requirements for the Degree of

Doctor of Philosophy

College of Science and Mathematics

Department of Biological Sciences

1984



CENTER FOR ENERGY AND ENVIRONMENT RESEARCH
UNIVERSITY OF PUERTO RICO -- U.S. DEPARTMENT OF ENERGY

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ABSTRACT

This study describes the reproductive ecology and parental care of the neotropical frog Eleutherodactylus coqui, measures the benefits and costs of parental care to individual reproductive success, and integrates benefits and costs into a single view of the adaptive significance of parental care for this species.

Eleutherodactylus coqui males call from elevated perches at night. Eggs are laid in non-aquatic nest sites and undergo direct development, hatching as tiny frogs in 17-26 days. Clutch size (mean = 28 eggs) is correlated with female body size. Parental care is performed exclusively by males, who attend eggs and hatchlings. Males attend their clutches 97.4% of the time by day and 75.8% of the time at night. Most of this time is spent brooding eggs. Males also defend eggs against cannibalistic intruders. Care is provided throughout development; neither nest fidelity nor brooding frequency changes from oviposition to hatching.

Parental care significantly increases hatching success. In a field experiment, clutches from which parental males were removed failed 3.4 times as often as control clutches (males not removed). Experimental clutches suffered greater mortality from desiccation and cannibalism than controls. Plotting average hatching success of experimental clutches against the developmental stage at which males were removed yielded a convex second order function. Hence, the benefits of parental care accelerate throughout development.

Parental males do less feeding than non-parental males and suffer small but significant losses in body mass during parental care.

Parental males also call less than non-parental males and sacrifice approximately one additional mating during a 20-day period of parental care.

A marginal value model of parental care is proposed which integrates the time-dependent benefits of attending eggs and the consequent costs of reduced reproductive activity. Using empirically determined values for E. coqui, the model indicates that by providing care throughout pre-hatching development, males maximize their lifetime reproductive success. The model also indicates that, starting with no care, either sex would have been favored to evolve care in E. coqui. Male coquíes may have evolved parental care because of their initial association with eggs at defended retreat sites.

Dedication

To my children, Christopher and Carissa,
that theirs will be a better world.

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INTRODUCTION

Parental care has long been of interest to ethologists, ecologists and evolutionary biologists. The potential for males or females to allocate post-fertilization time and energy to offspring is a critical factor in sexual selection (Trivers, 1972; Williams, 1975; Maynard Smith, 1978), the evolution of mating systems (Orians, 1969; Emlen, 1973; Emlen and Oring, 1977; Kleiman, 1977; Walters, 1982) and the evolution of social behavior in general (Alexander, 1974; Wiley, 1974; Brown, 1975; Wilson, 1975; Wittenberger, 1981).

Parental care is an integral part of the reproductive strategies of mammals and birds (Orians, 1969; Kleiman, 1977). In these two vertebrate groups, evolutionary questions revolve around how the allocation of parental time and energy should relate to mating systems, with the central issue being whether one or both parents should be favored to care for the young (Orians, 1969). Parental care by at least one parent is essential because of the large input of energy and thermoregulatory buffering required to raise an endothermic offspring. These stringent physiological requirements are often not the case for lower vertebrates, both because of their ectothermic physiology and because immature and adult animals often occupy markedly different habitats. This is the case with most temperate and many tropical amphibians, in which the typical life history involves an aquatic larval stage that is completely different in its physiology, behavior and trophic niche from the adult. Not surprisingly, parental care is unusual in frogs, known to occur in only about 10% of all species (McDiarmid, 1978). Its rarity raises questions, on one level, about

how, when and why it arose and on a second level provides the ideal situation for investigating the evolution of reproductive strategies in animals within related taxa. Also, because the sex which provides care may differ among congeners, there exists the opportunity to investigate the relationship between environmental conditions, social systems, allocation of parental effort and sex-specific reproductive strategies.

Parental care has been studied mainly in birds and mammals. More recently, the evolution of parental care in lower vertebrates has received attention, especially in fishes and amphibians (McDiarmid, 1978; Perrone and Zaret, 1979; Blumer, 1979; Barlow, 1981; Baylis, 1981; Gross and Shine, 1981; Wells, 1981; Simon, 1982, 1983). In anurans, it should rarely be the case that a second parent can provide much additional effective care (effective in further increasing the survival of the offspring), and since it seems that cooperative behavior is rare in frogs, the dichotomy between whether one parent or both parents should care (as in endothermic vertebrates) is a moot question. The principal issues are whether uniparental care or no parental care should be favored and which sex should perform the care. These two questions have been addressed theoretically (Trivers, 1972; Maynard Smith, 1977; Grafen and Sibly, 1978).

To understand the evolution or maintenance of parental care in any animal, it is necessary to know how reproductive success varies as a function of parental care. More specifically, we need to measure how an increase in offspring survival due to care trades off with the consequences of reduced allocation of time and energy to future offspring. Specification of benefits and costs for each sex is crucial to interpretation of sex-specific parental strategies (Trivers, 1972).

Interspecific differences in cost-benefit relationships, resulting from different breeding systems (Maynard Smith, 1977) or differing degrees of paternal certainty (Werren et al., 1980), may alter predictions about the evolution of parental care.

Parental care has been documented in most frog families (McDiarmid, 1978). There is tremendous diversity in the ways that amphibians provide post-fertilization parental care to their offspring (Salthe and Mecham, 1974; Lamotte and Lescure, 1977; McDiarmid, 1978). Wells (1981) recognized four major categories of anuran parental care: egg attendance, tadpole attendance, egg transport and tadpole transport. In addition, viviparity occurs in a few species (Lamotte and Lescure, 1977; Wake, 1978, 1980), and recent evidence indicates that, in a few species, parents may provide nutrition to larvae (Graeff and Schulte, 1980; Weygoldt, 1980; Zimmermann and Zimmermann, 1981). In anurans, parental care is practiced almost exclusively by species that lay eggs either in terrestrial or in specialized aquatic microhabitats. Many cases of anuran parental care have been suggested to be an adaptive response by parents to the problem of maintaining a vulnerable amphibian egg in a terrestrial environment (Myers, 1969; McDiarmid, 1978; Wells, 1981), either to prevent desiccation of eggs or to protect them from predators and pathogens. There are only a few studies which quantitatively assess the relative importance of anuran parental care in fulfilling these functions (McDiarmid, 1978; Simon, 1982, 1983; Taigen et al., 1984; Townsend et al., 1984).

In addition to the diversity of forms of parental care exhibited by anurans, there is interspecific variation in which sex provides parental care in several anuran groups (Wells, 1981). One of these is

the genus Eleutherodactylus, in which there occur species with paternal care, others with maternal care and some with no parental care at all.

In this study, I have investigated the parental care system of the Puerto Rican frog Eleutherodactylus coqui in which care is performed exclusively by males. My goals in the study were:

- (1) to describe the reproductive biology and ecology of the species;
- (2) to describe, in detail, its parental care behavior;
- (3) to document how provision of parental care affects offspring survival; i.e., to demonstrate the benefits of parental care;
- (4) to estimate how parental care provision affects future fecundity of caring males; i.e, to estimate the costs of parental care;
- (5) to integrate benefits and costs into a single statement about the relationship between parental care behavior and fitness in E. coqui.

Each of these goals is treated as a chapter in the following thesis.

GENERAL METHODS

In this section, I describe some of the general methods used in this study. Methods used only in particular parts of the study will be described in the appropriate chapters.

The Species.

Eleutherodactylus coqui Thomas (Anura: Leptodactylidae) is a terrestrial breeding frog endemic to Puerto Rico. It is the most widespread and generally the most abundant species of sixteen native Eleutherodactylus (Drewry, 1970a, b; Rivero, 1978). The coquí was first described by Bello y Espinosa (1871), who noted that the parent frogs were found with the terrestrial eggs and that development was direct, miniature frogs hatching from the eggs without any intervening free-living larval stage. Until 1966, E. coqui was nominally subsumed under E. portoricensis, but Thomas (1966) recognized the existence of two species and named the second one E. coqui.

There has been an explosion of work on E. coqui since the mid 1960s. Drewry (1970a, b; Drewry and Rand, 1983) worked on the population biology, life history, and vocal behavior of several Puerto Rican Eleutherodactylus. A great deal of descriptive and detailed experimental work on the vocalization, neurobiology, and calling behavior of E. coqui has been done by Narins and his associates (Narins and Capranica, 1976, 1977, 1978, 1980; Narins, 1982a, b, 1983a, b; Narins and Hurley, 1982; Zelick and Narins, 1982, 1983). Aspects of the physiological ecology of E. coqui have been studied by Heatwole et al. (1969), Pough et al. (1983) and Taigen et al. (1984).

The coquí is nocturnal, achieves high densities in the Luquillo Mountains of northeastern Puerto Rico (Stewart and Pough, 1983) and, as with its congeners, has direct development (Townsend and Stewart, in press).

Study Site and Field Work.

The field work was conducted in the Luquillo Experimental Forest near the El Verde Field Station of the Center for Energy and Environment Research in the Luquillo Mountains of northeastern Puerto Rico. The E. coqui population that I studied inhabits second-growth tabonuco (Dacryodes excelsa) forest at about 350 m elevation. Mottillo, Sloanea berteriana, and ausubo, Manilkara bidentata, are also prominent species. Three other trees are important to the population ecology of E. coqui: Cecropia peltata, Didymopanax morototoni, and Prestoea montana, the sierra palm. Dead leaves of Cecropia and fallen fronds of Prestoea are used extensively as diurnal retreat sites and nest sites by coquíes, and the axils of sierra palms are often used as calling sites by males (see below). Cecropia and Didymopanax are both early successional species which take advantage of temporary openings in the forest canopy, while Prestoea is a sub-canopy palm which occurs regularly throughout the forest (Ewel and Whitmore, 1973). The forest has moderately seasonal climate. A cooler, dry season extends from December to March, when mean monthly rainfall is about one third less than in the warmer wet season of April through October. Dry season mean monthly temperatures are about 21 to 22 C, while wet season monthly temperatures average about 23 to 24 C (Odum et al., 1970).

Field work was done during June-August 1979, January-July 1980,

and January, March, June-July, and October 1982. Most of the results discussed in this thesis were from the 1980 and 1982 field work.

Measuring, Sexing and Marking Frogs.

The snout-vent lengths (SVL) of frogs were measured in the field by placing them inside a plastic bag, pressing them against a flat surface and measuring to the nearest 0.5 mm the distance from the tip of the snout to the distal tip of the pubis.

In areas where frogs were marked, males could be sexed when recaptured while calling or in amplexus. The following morphological criteria were used to determine sex as well. Adult males in the El Verde population range from 29.5 mm to 38.0 mm SVL (see Chapter 2) and have a vocal sac of thin flexible skin beneath the throat. Females range from 35.5 mm to 52.0 mm SVL (see Chapter 2), lack the flexible skin of the vocal sac, and often have ovarian eggs visible through the abdominal wall.

Frogs were marked in several areas during the study. This was done by clipping a unique combination of toes; never more than one toe per foot or four toes per frog. Also, the first digit on the hind foot was never clipped. Toes were always clipped proximal to the first joint. This prevented any substantial regrowth of clipped toes, and marked frogs were easily recognized for up to two years following initial marking.

Sampling Methods

Frogs and nests were located in the forest via systematic diurnal surveys of the habitat. Surveys were of two types: complete and

selective. Complete surveys involved thorough searching of the leaf litter, turning logs and rocks, and inspection of all crevices or cavities up to 250 cm off the ground (as high as I could reach). The leaf litter was turned by hand and most curled leaves were opened and inspected. Leaves above the ground were handled similarly. Crevices and cavities, including palm axils, rock crevices, the interstices of prop roots, tree trunk holes and cracks, branch axils, etc. were inspected, if necessary with the aid of a penlight. The objective of a complete survey was to inspect all accessible cavities, crevices and holes in an area that could be potential retreat or nest sites.

Selective surveys represent sampling of only certain types of cavities and crevices in an area. Often, this involved sampling only the sierra palm fronds, Cecropia leaves, large cavities such as tree holes and crevices in rocks, and palm axils. Data from complete surveys indicated that frogs overused these types of cavities relative to their general abundance in the habitat. Hence, selective surveys were used to maximize the efficiency of locating frogs or nests rather than to estimate population parameters.

Bamboo frog houses were used in several parts of this study. These consisted of 15-25 cm lengths of bamboo with an opening near the bottom and a removable plastic top. Bamboo houses were either hung on trees (up to 200 cm above the ground), or placed horizontally on the ground. Frogs readily adopted them as retreat and nest sites. Their use has been of major importance in various work on E. coqui (Townsend et al., 1981, 1984; Stewart and Pough, 1983).

When a nest was found, the attending parent was removed briefly while the number, diameter and developmental stage of the eggs were

recorded. The number of eggs in the clutch and their arrangement (number of layers, number of eggs per layer) were recorded; number of eggs is hereafter referred to as the clutch size. Egg diameter was measured to the nearest 0.5 mm with a small clear plastic mm ruler. Generally, the outside diameter, including the egg capsule, of 5-10 eggs in the clutch was measured. The developmental stage of eggs was determined according to a staging table which I constructed for E. coqui. A complete description of the staging table is given in Appendix I. For many purposes in what follows, I have divided the 15 pre-hatching stages of my table into three developmental intervals, I-III, each corresponding to about one-third of development. Developmental interval IV includes all post-hatching stages. In addition to clutch parameters, I recorded the nature and location of the nest site, height off the ground of the clutch, and several metric characters of the nest, including its largest linear dimension, the longest dimension of the actual nest cavity and the perpendicular to length (e.g., the length and diameter of a palm frond roll), and the angle from horizontal of the substrate on which the clutch rested.

Calling male surveys were conducted on a regular basis in several areas during the study. Calling surveys were designed to record the number of males calling, and several characteristics of their call sites, in prescribed areas of the habitat. A calling male survey consisted of passing slowly through an area while listening for calls. The identity of calling males (where frogs were marked) was determined by visual inspection of exposed toes. In the elevated calling posture, it is possible to do so without capturing the frogs. The characteristics and height above the ground of the calling site were

also recorded. All surveys were done between 2000 hours and 2300 hours, and generally lasted for 1-2 hours. For complete surveys, an area was completely traversed at least twice to ensure that all calling males had been found. Some partial surveys were performed in which an area was only partially covered, or traversed only once.

While sampling was done in many areas of the forest at El Verde, one large plot was sampled continuously and intensively throughout the study. Dicks House Plot was a 500 square meter quadrangular area in which 30 bamboo frog houses had been hung. All major topographic features, palms and trees were plotted within Dicks House Plot. Twelve reference stakes placed in the plot allowed pinpointing the horizontal location of any frog, nest or other feature in the plot. Beginning in 1979, all frogs of 24 mm SVL or greater were marked upon initial capture. Marking continued throughout 1979 and 1980 field work. A complete diurnal survey and a complete calling male survey were done during alternate weeks (each done biweekly) throughout 1979 and 1980. In 1982, a series of 5-7 complete calling surveys were done on consecutive nights prior to each complete diurnal survey.

Statistics.

All means are given \pm 1 SE unless otherwise indicated. Statistical tests used are the Student's t-test, Pearson product-moment correlation and One-Way ANOVA when variables are normally distributed and homoscedastic (Sokal and Rohlf, 1969). When those assumptions are violated or for small sample size, non-parametric statistics such as the Mann-Whitney U-test, the Chi-square test, and the Kruskal-Wallis One-Way ANOVA are used (Siegel, 1956). Alpha is set at 0.05 as the

level of rejection in all hypothesis testing. Data were analyzed and some statistical procedures performed using the computer statistics package SPSS (Nie et al., 1975).

CHAPTER 2

THE REPRODUCTIVE ECOLOGY OF E. COQUI

Males

Body size. Complete calling surveys of Dicks House Plot were conducted on 21 nights in 1980 (Table 1) and 29 nights in 1982 (Table 2). Calling male SVL was recorded on a regular basis only during 1980 (Table 1). Individual males ranged from 29.5 mm to 37.0 mm SVL and nightly means ranged from 32.0 ± 0.57 mm (N=9) to 33.9 ± 0.28 mm (N = 20), with an overall mean of 33.4 ± 0.40 mm (N = 207). There was significant variation among monthly means of calling male SVL (Kruskal-Wallis One-Way ANOVA; $h = 12.49$, $df = 4$, $p < 0.02$). Average body size decreased from dry to wet season, the difference stemming primarily from the increased presence of smaller males in the chorus during April through June (note extension of size range to lower values during those months).

During 1980, 134 clutches were deposited in Dicks House Plot. The SVLs of the parental males were measured in 132 of them (Table 3). The overall mean body size of parental males was 33.4 ± 0.12 mm (N = 132). Although there was a decrease in mean SVL from January to June (as with calling males), there was not significant variation in body size among months (Kruskal-Wallis One-Way ANOVA; $H = 4.93$, $df = 5$, NS; July not included in analysis). The calling male and parental male body size distributions (Tables 1 and 3) in Dicks House Plot during 1980 were not significantly different (Mann-Whitney U with z-transformation: $z = 1.114$, $p = 0.1335$).

Characteristics of calling sites and nest sites. Male coquíes use

Table 1. The numbers and mean body sizes of calling males in Dicks House Plot during 21 complete surveys in 1980. Note that the April 30 sample is combined with May for monthly analyses.

Night	Number of calling males	Snout-Vent Length		
		Mean	SE	Range
Jan. 7	7	33.4	0.34	32.0-34.5
18	0	-	-	-
20	6	33.4	0.29	32.5-34.0
22	4	33.5	0.20	33.0-34.0
Monthly Mean (SE)	4.3 (1.6)	33.4	0.17	
Feb. 2	0	-	-	-
19	2	33.0	-	-
27	0	-	-	-
March 4	16	33.9	0.28	32.5-36.0
11	20	33.9	0.27	32.0-36.5
17	14	33.5	0.43	32.0-37.0
25	9	33.5	0.43	32.0-35.0
Monthly Mean (SE)	14.8(2.3)	33.7	0.19	
April 2	8	33.3	0.46	32.0-35.0
9	22	33.6	0.30	31.5-37.0
16	23	33.8	0.27	32.0-37.0
Monthly Mean (SE)	17.7(4.8)	33.6	0.20	
April 30	12	33.2	0.43	31.0-36.5
May 7	4	33.3	0.80	31.0-34.5
21	24	32.8	0.39	30.0-37.0
Monthly Mean (SE)	13.3(5.8)	32.9	0.28	
June 4	11	32.0	0.57	29.5-34.5
7	20	32.7	0.31	29.5-34.5
21	19	32.8	0.37	29.5-37.0
25	24	33.2	0.27	31.0-36.5
Monthly Mean (SE)	18.5(2.7)	32.8	0.19	
Grand Mean (SE)	11.7(1.9)	33.4	0.40	29.5-37.0

Table 2. The numbers of calling males in Dicks House Plot during 29 complete surveys in 1982.

Night	Number of calling males	Night	Number of calling males
Jan. 7	4	June 10	16
8	3	11	15
9	9	12	8
10	7	13	11
11	9	14	17
12	11	15	17
13	9	Monthly Mean(SE)	14.0(1.5)
14	9	July 8	25
Monthly Mean(SE)	7.6(1.0)	9	28
March 8	16	10	20
9	14	11	29
10	16	12	20
11	18	Monthly Mean(SE)	24.4(1.9)
12	21	Oct. 12	16
13	19	13	20
Monthly Mean(SE)	17.3(1.0)	14	14
		15	8
		Monthly Mean(SE)	14.5(2.5)

Table 3. Mean body sizes of parental males in Dicks House Plot from January to July 1980. Small July sample results from sampling only during the first four days of the month.

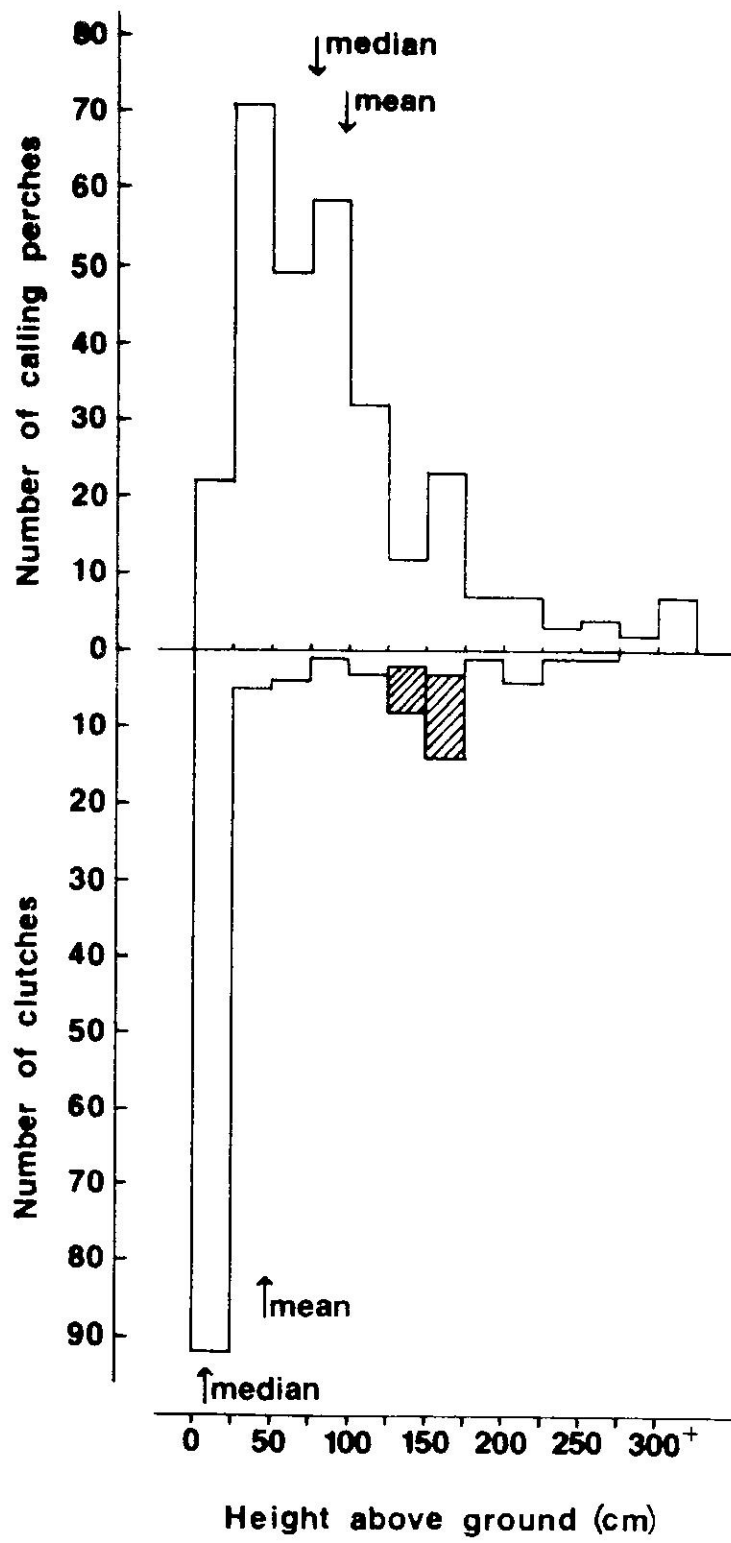
Month	N	Snout-Vent Length		
		Mean	SE	Range
January	5	34.0	0.42	33.0-35.0
February	14	33.8	0.37	32.0-36.0
March	23	33.6	0.28	31.0-36.0
April	23	33.5	0.27	31.0-36.0
May	30	33.4	0.27	31.0-36.5
June	30	33.0	0.28	30.0-37.0
July	7	33.3	0.53	31.5-35.0
Summary	132	33.4	0.12	30.0-37.0

markedly different microhabitats for calling and nesting. Two variables were measured for both types of sites: height above the ground and relative cover. Height above the ground is the vertical distance in cm to a male's calling position or to a clutch. Relative cover was ranked using a qualitative scale devised by Narins and Hurley (1982; p. 289) to categorize the amount of cover around male calling sites, as follows: "1 - open with no surrounding cover; e.g., on an open surface, mudbank, rock, leaf, or bare tree trunk; 2 - generally open, but with sparse surrounding vegetation partially covering the frog; e.g., on a tree trunk amid small leaves or vines; 3 - closely covered by vegetation or substrate but with at least one side and the anterior uncovered; e.g., between bamboo shoots or in a palm axil; 4 - covered closely on all sides by vegetation and substrate but open anteriorly; e.g., in a palm axil with leaves near the dorsal surface of the frog; 5 - completely surrounded by vegetation; e.g., inside a tightly curled leaf." Although Narins and Hurley (1982) used the categories only for ranking calling sites, the scale is entirely appropriate for ranking the relative cover of other microhabitats used by the frogs.

Twenty-one calling male surveys in Dicks House Plot during 1980 (3 partial and 18 complete surveys) yielded a total of 297 calling site observations. Figure 1 illustrates the distribution of perch heights. Most males (92.6%) called from sites that were 25 cm or higher, while only 5 males (1.7%) used sites that were less than 10 cm high. Only a few males (2.4%) used perches higher than 3 m. The average perch height was 93.5 ± 4.6 cm (N=297).

Males employed a wide range of substrates for calling, but the majority of them (252 of 297 = 84.9%) were relatively exposed

Figure 1. The frequency distributions of height above the ground for calling perches (top) and clutch locations (bottom) in Dicks House Plot during January to June 1980. Means and medians were computed using raw data. N of calling perches = 297; N of clutches = 134. Hatched areas of lower figure represent clutches in bamboo houses.



(categories 1-3, Table 4). The majority of these more exposed sites were palm axils (94 of 252 = 37.3%), the surfaces and crevices of tree trunks and branches (65 of 252 = 25.8%) and the open surfaces of leaves (33 of 252 = 13.1%). Thirty-three males (11.1%) used type 4 sites, consisting mainly of the doorways of bamboo frog houses (8 of 33 = 24.2%), the open ends of fallen sierra palm fronds (6 of 33 = 18.2%) and hanging clusters of dead leaves or palm leaflets (7 of 33 = 21.2%). Only 12 sites (4.0%) were of category 5, including calling from inside the curls of Cecropia leaves (6 of 12 = 50.0%) or from inside bamboo frog houses (5 of 12 = 41.7%). Palm axils, as a single category, were the most frequently used call sites, comprising 33.0% (98 of 297) of all sites.

The height distribution of 134 clutches deposited in Dicks House Plot during 1980 is shown in Figure 1. Note that the peak in clutch height frequency between 125 and 200 cm is due entirely to bamboo houses which were concentrated in that height range. The majority of oviposition sites (92 of 134 = 68.7%) were less than 25 cm high (79.3% of 116 natural nests). The average nest site height was 47.8 cm \pm 6.0 cm for all nests; 30.5 \pm 5.4 cm for natural nests (N = 116). The height distributions for calling sites and nest sites are significantly different (Mann-Whitney U with z-transformation: $z = 8.293$, $p < 0.0001$).

The relative cover distribution of Dicks House Plot nest sites is given in Table 4 and is significantly different from the calling site distribution (Chi-square = 309.97, $df = 4$, $p < 0.0001$). Eighty-three percent of all clutches (111 of 134) were located in category 5 nest sites, in which the clutch and attendant male were entirely enclosed by

Table 4. Frequencies of cover categories for calling sites and nest sites in Dicks House Plot during 1980. Categories after Narins and Hurley (1982); see text for definitions.

	Cover Categories				
	1	2	3	4	5
Calling Sites	85	48	119	33	12
Nest Sites	0	0	3	20	111

vegetation or substrate.

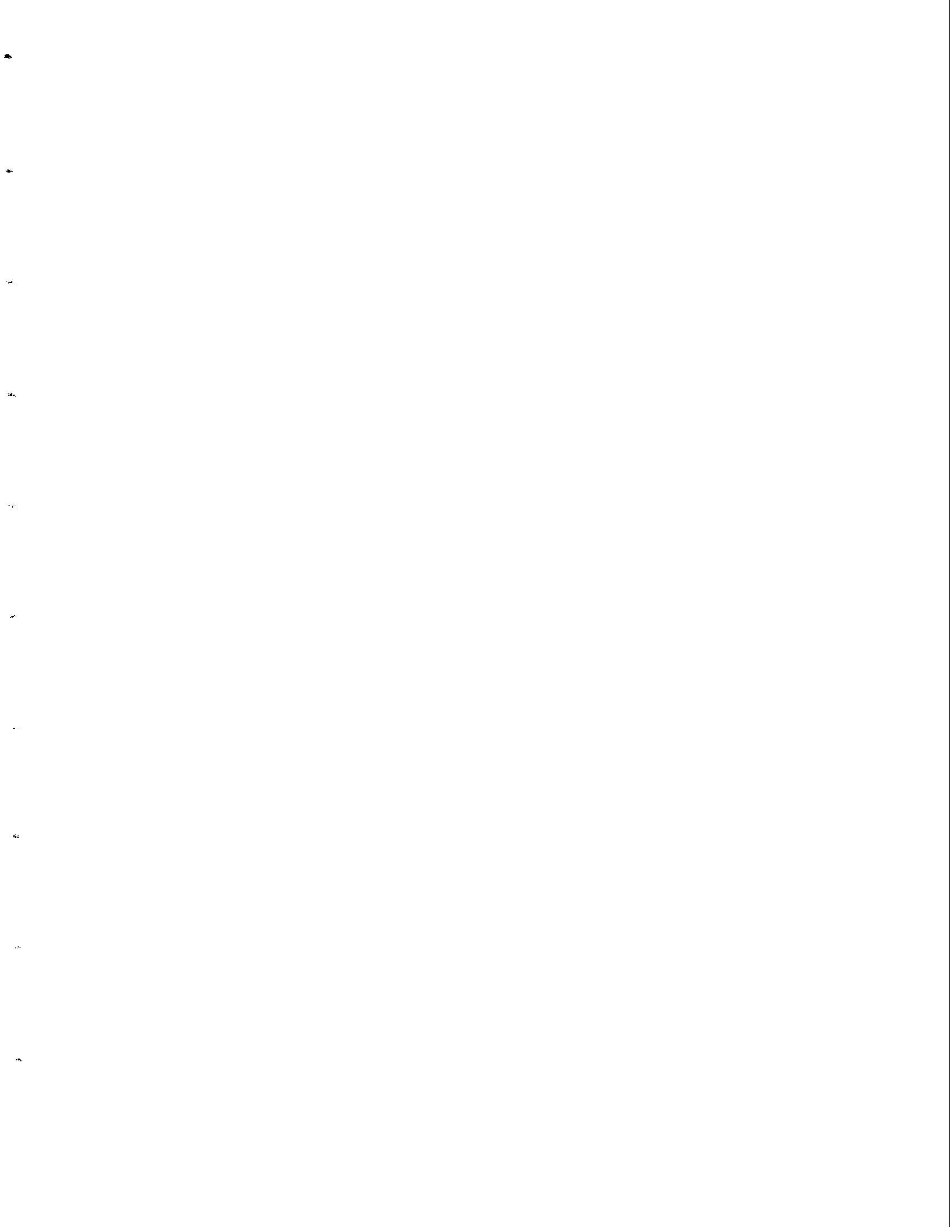
Males are using elevated, relatively open perches as call sites, typified by palm axils, tree trunks and branches, rock surfaces, and open leaf surfaces. Oviposition sites, on the other hand, are in closed cavities which are usually on or near the ground (less than 26 cm high).

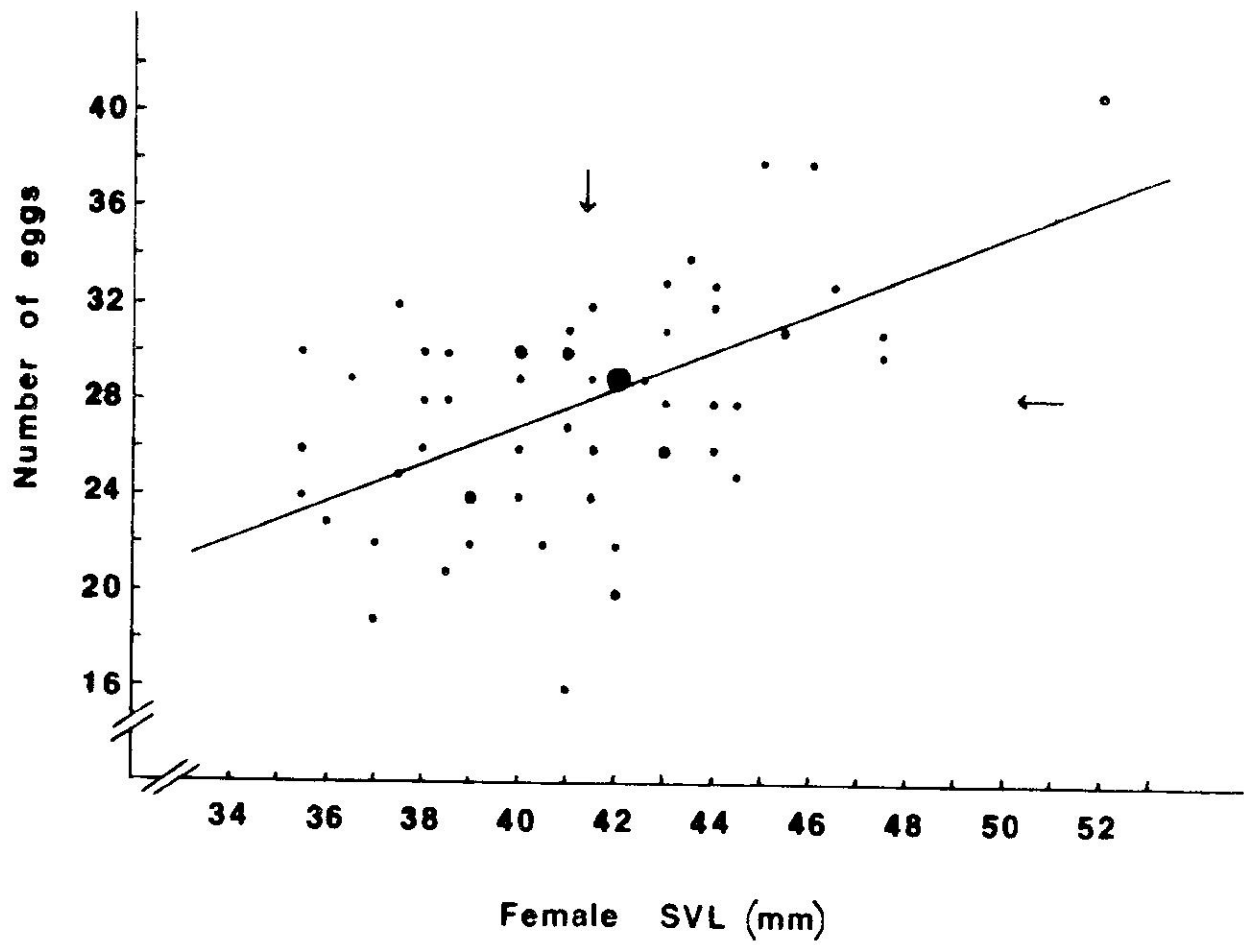
Females

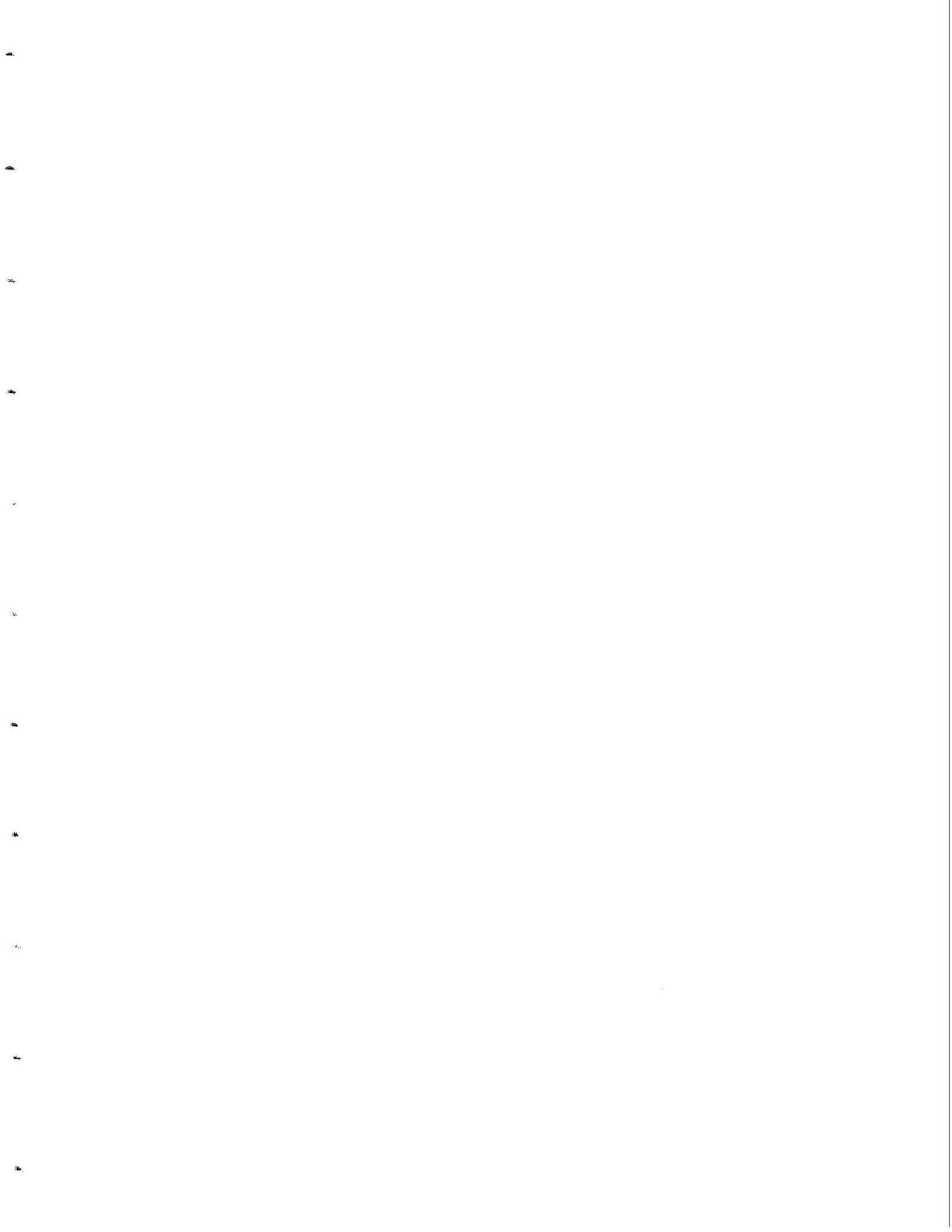
Body size and fecundity. From January to July 1980, 69 females were captured in nest sites immediately following oviposition. Their average SVL was 41.4 ± 0.4 mm (Range = 35.5-52.0 mm) and average clutch size was 28.1 ± 0.6 eggs (N = 58, Range = 16-41). There was a significant correlation between female body size and clutch size ($r = 0.5672$; $p < 0.01$, Figure 2). In 65 cases, the SVL of the amplexant male was also recorded. The regression of male SVL on female SVL for amplexed pairs was not significant (Figure 3; $F < 1.0$). Hence, there does not appear to be positive assortative mating by body size, at least within the range of male body sizes that were sampled (30.0-35.5 mm SVL).

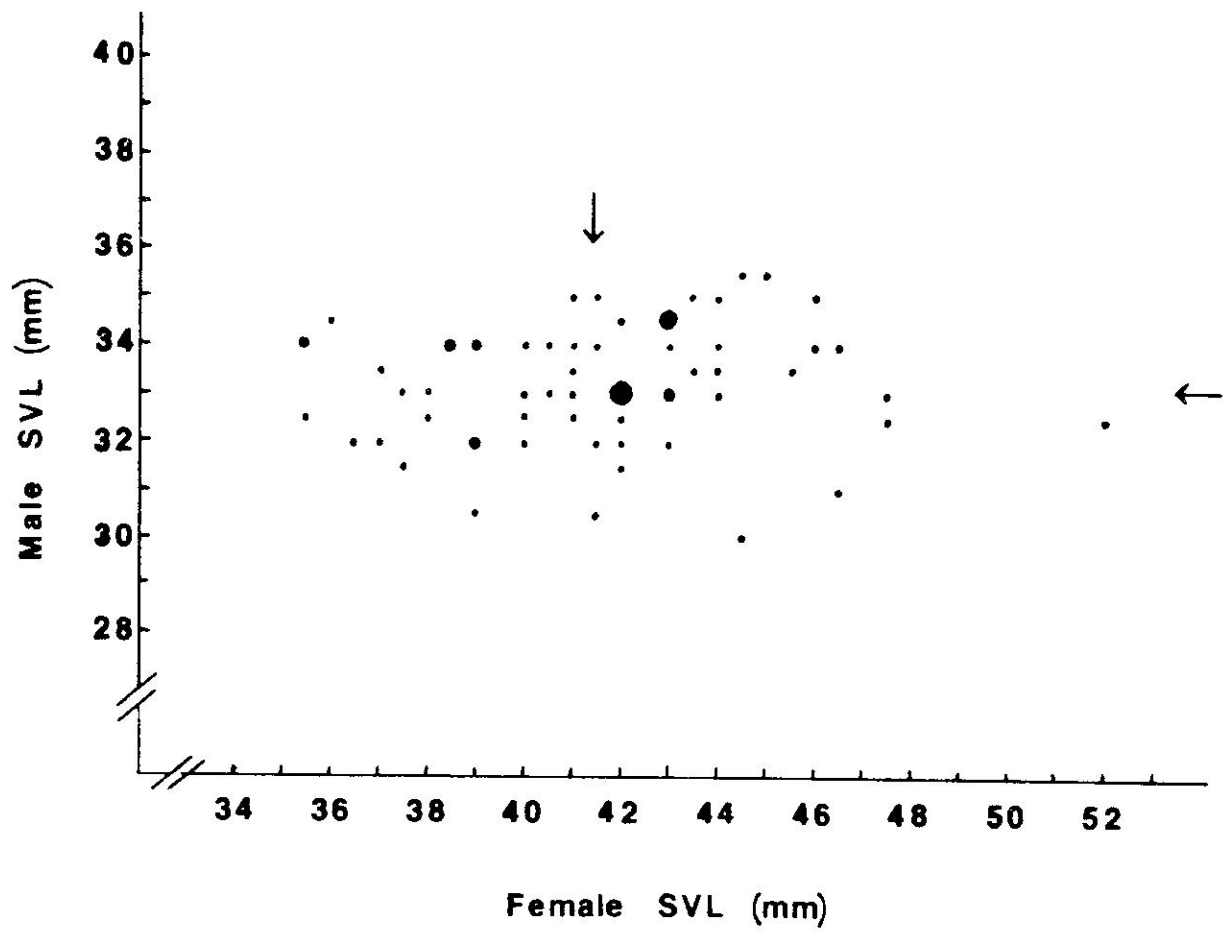
A total of 357 clutches was found in all habitat surveys during 1980 (Table 4), including the 69 mentioned above. Average size of all clutches was 26.7 ± 0.3 eggs (N=357, Range = 3-45 eggs per clutch). Since many of these were found at various stages post-oviposition, small clutches may have suffered partial predation prior to discovery, yielding a lower estimate of clutch size overall.

Two females were recaptured depositing second clutches in 1980. One female laid 33 eggs on 20 April and 34 eggs on 14 June, with an









interclutch interval of 55 days (8 weeks). A second female laid a clutch on 5 May (the eggs were not counted) and a second clutch of 24 eggs on 3 July, yielding an interclutch interval of 59 days (8.5 weeks). A third female was captured laying a clutch (uncounted) on 11 March and had laid a second clutch by 21 May, yielding a maximum interclutch interval of 71 days (10 weeks).

Seasonality of Reproduction

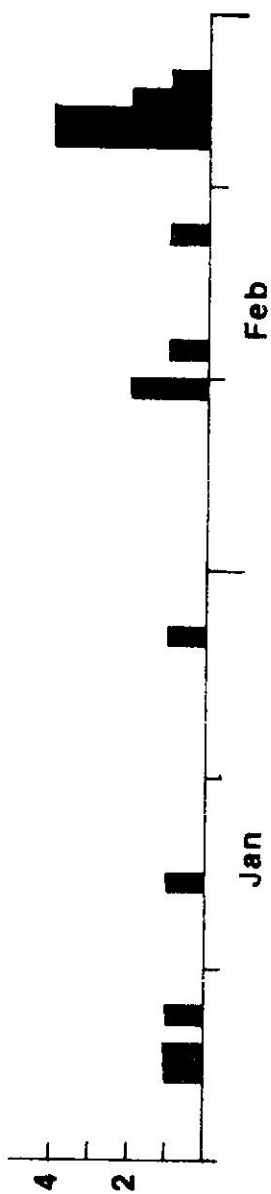
Calling activity was recorded during every month of the study (Tables 1 and 2). Nightly densities in Dicks House Plot ranged from 0 to 29 calling males and varied significantly among months in both years (Kruskal-Wallis One-Way ANOVA; 1980: $H = 13.2$, $df = 5$, $p < 0.025$; 1982: $H = 19.9$, $df = 4$, $p < 0.001$). The principal source of the variation was a difference between dry season (Jan-March) and wet season (April-July) densities in the plot.

Clutches were found during every month of the study (Table 5). There was significant variation among months in the number of clutches deposited in Dicks House Plot when compared to an expected distribution of equal monthly clutch frequency (Chi-square = 22.99, $df = 5$, $p < 0.001$), with the main source of variation being the lower frequency of clutch deposition during the dry season. Figure 4 illustrates the temporal distribution of deposition of 134 clutches in Dicks House Plot during 1980. No more than four females ever deposited eggs in the plot on a single day. At least one female oviposited on 81 of 186 days (43.6%) with an average of 1.65 clutches per deposition day (134/81). Calling activity (Table 1) and egg deposition (Table 3; Figure 4) were low during January and most of February. Following a pulse of

Table 5. The numbers and sizes (number of eggs) of clutches found in Dicks House Plot and in all habitat surveys (including DHP) during 1980.

Month	Total N	Clutch Size		
		Mean	SE	Sample N
Dicks House Plot:				
January	5	19.9	2.89	3
February	15	22.3	2.11	11
March	24	26.4	1.51	19
April	23	27.0	1.21	23
May	31	27.8	1.59	28
June	30	30.7	0.98	29
July	6	27.6	1.29	5
All Surveys:				
January	12	21.1	1.63	9
February	31	23.5	1.27	21
March	67	25.8	0.78	52
April	69	26.3	0.67	63
May	86	27.7	0.78	78
June	77	28.1	0.64	72
July	15	27.6	0.89	13
Summary	357	26.7	0.34	308

Figure 4. The temporal distribution of clutch deposition in Dicks House Plot from January 1 to July 4, 1980. Each bar width equals one day.



Number of Clutches

reproductive activity in late February (Figure 4), reproduction increased into March and continued at that level through July 4, the date of the last complete diurnal survey at Dicks House Plot. From March 1 to July 4, 1980, females deposited eggs on 69 of 126 days (54.8%).

Both calling activity and oviposition were associated with rainfall (Tables 6 and 7). Calling male densities in Dicks House Plot were significantly greater on nights following 24 hour periods in which there was some rainfall than on nights which followed 24 hours without rain (Table 6). Females deposited clutches significantly more often following 24 hour or 48 hour periods in which there was some rainfall than following similar periods with no rainfall (Table 7).

Courtship and Mating Behavior

Eleutherodactylus coqui is a nocturnal frog. Frogs generally leave their diurnal retreat sites and become active in the forest within the first hour after dark. Calling activity begins before dark, the chorus is generally at full strength within a half-hour of full darkness, and calling is heaviest before midnight, declining in the early morning hours until dawn (Drewry, 1970a; Drewry and Rand, 1983; Woolbright and Townsend, Ms.).

I have observed 18 partial or complete courtship sequences in E. coqui. Courtship was always initiated by a female approaching a calling male and making contact. The initial contact by the female often resulted in a sudden pause in the male's calling followed by a switch in the vocalization to a lower amplitude, softer call. Within seconds of the initial contact by the female, the male began to move away from

Table 6. Chorus sizes at Dicks House Plot on 31 complete survey nights during March-June 1980 and March, June-July 1982, as a function of whether or not rainfall occurred during the previous 24 hour period. A. Complete table of chorus sizes. B. Reduced table for statistical analysis.

A.

	Number of Calling Males				
	0-5	6-10	11-15	16-20	21-30
No rainfall	1	3	4	3	0
Rainfall	0	0	3	9	8

B.

	Number of Calling Males	
	0-15	16-30
No rainfall	8	3
Rainfall	3	17

Fisher Exact Test; $p < 0.005$.

Table 7. Frequencies of days when 0, 1, or 2⁺ clutches were deposited in Dicks House Plot from March to July 1980, as a function of immediate past precipitation. A. When rainfall did or did not occur during the previous 24 hours. B. During the previous 48 hours.

A.

	Number of Clutches per Day		
	0	1	2 ⁺
No rainfall	33	12	10
Rainfall	24	26	21

$$\chi^2 = 8.59, df = 2, p < 0.02.$$

B.

	Number of Clutches per Day		
	0	1	2 ⁺
No rainfall	28	6	6
Rainfall	29	32	25

$$\chi^2 = 14.61, df = 2, p < 0.001.$$

the call site, always continuing to call. Typically, a male would move from 10 to 30 cm and stop. The female had to follow the male and either get very close to him or actually make contact in order for the male to move again. Typical courtship involved a long series of these leading-and-stopping moves by the male with following moves by the female. Males always continued to call while leading, although the calls were usually much softer than the advertisement calls that had been given prior to initial contact by the female. No male went more than 50-60 cm in one move before waiting for the female to catch up. On several occasions, when females took too long to follow the male, he turned around and went back to the area of his last stop. Female behavior during the leading phase was variable. Some females did little more than follow the male directly. In two other cases, females spent time investigating various curled leaves and cavities along the courtship route. One female did this to such an extent that the male backtracked three times to find the female and initiate leading again. Without exception, however, the female eventually entered the nest cavity that the male first entered and remained. Once the female had followed the male into the cavity, the female normally initiated amplexus by backing under the male within 15 minutes. The male's calling became progressively softer, with an increase in the number of soft, multiple-note calls (repeated co notes), and eventually stopped. All courtships were observed before 2300 hours, and most were initiated within the first 1.5 hours of dark. Leading courtship lasted from 15 to 60 minutes, the time varying as a function of the distance between the call site and nest site and of the propensity of the female to follow the male directly.

Once amplexus was initiated, the pair remained in amplexus for the rest of the night and into the following day, when eggs were finally laid. Early in amplexus, the male was atop the female in approximately an axillary position, but males were never seen to clasp the female. Prior to oviposition, and often close to dawn (after 5-8 hours of amplexus), the pair assume a secondary amplexic position, in which the female's hind legs come to rest on top of the male's hind legs. As a result, the male's posterior end is firmly pinned against that of the female and against the substrate. This position is maintained throughout the rest of amplexus and oviposition.

At some point between the initiation of amplexus and oviposition the female ovulates her full ovarian complement and the male inseminates her, although not necessarily in that order. Two females that were captured two hours after the initiation of amplexus were found to have all mature ova still in their ovaries, so ovulation apparently does not occur prior to amplexus. Also, females were observed to undergo very heavy abdominal pulsations for up to two hours prior to oviposition; these may correspond to ovulation. Fertilization in E. coqui is internal (Townsend et al., 1981) and while I do not know at what point during amplexus the male inseminates the female, I presume that it occurs after the onset of the secondary amplexic position.

Oviposition begins 7-10 hours after the initiation of amplexus, and requires 3-5 hours to complete. Oviposition begins without any visible interruption of the abdominal pulsations that I have interpreted as ovulation. As eggs are laid, the female moves out from under the male, and eventually comes to sit directly in front of him when

egg-laying is done. The male is then directly over the new clutch in a typical brooding posture (see Chapter 3). The male and female remain in these positions for the rest of the daylight hours. As dusk comes, both parents become more active and, virtually without exception, the new male parent begins giving aggressive calls toward the female and may even make several biting attacks in chasing her out of the nest (see Chapter 3). Females usually return to their regular retreat sites within one or two days of mating.

Females that entered nest sites and initiated amplexus did not always remain and mate with the male. In five courtships witnessed during 1980 and 1982, a female left the nest site after the initiation of amplexus without mating with the male. I did not capture any of these females mating at other sites, so I do not know their subsequent mating history. In two of the five pairs, disturbance by the observer was a probable cause of the aborted mating. However, in the other three pairs, there was negligible interference by the observer, and it seems probable that females were rejecting either the male or the nest site. In none of the five instances had the pair entered the secondary amplexic position; the latest time that a female was observed to leave the nest was 0200 hours.

Developmental Biology

As with all other known Eleutherodactylus, E. coqui has direct development. At oviposition, eggs are large (3.6 ± 0.1 mm in diameter, $N = 21$) and unpigmented. Development occurs entirely within the egg and at hatching a fully pigmented miniature frog emerges bearing a keratinized egg tooth, a tail remnant, and a yolk reserve (Townsend and

Stewart, in press). A staging table describing the development of E. coqui is presented in Appendix 1. Hatchlings average 6.1 ± 0.1 mm SVL. The egg tooth disappears within 36 hours of hatching, the tail is resorbed within 3 days, and the yolk reserve is used up within 6 days, by which time the hatchlings have dispersed from the nest site.

Developmental time (oviposition to hatching) ranges from 26.3 days in January and February to 16.8 days in June, and is strongly and inversely correlated with ambient temperature (Appendix 1).

Discussion

Reproduction is continuous in E. coqui, with some calling activity and egg deposition occurring during every month of the year (this study; Stewart and Pough, 1983). Although continuous it is seasonal in intensity. Male calling activity (Tables 1 and 2) during the dry season (e.g., January-February) is about 25% of that in the wet season (e.g., June-July), although erratic rainfall patterns within season can give rise to some variability. For example, January 1982 was unusually wet, and calling male densities were inflated relative to 1980, while a dry June in 1982 resulted in depressed calling male densities (Tables 1 and 2). Rainfall has a significant effect on calling activity, with chorus densities being greater on wet nights than on dry ones (Table 6).

Likewise, egg deposition varies significantly with season. There was a significant increase in the number of clutches deposited in Dicks House Plot from January to June 1980. Within the wet season, the schedule of egg deposition varies significantly with habitat moisture; females ovipositing more frequently when the habitat is moist than when it is dry (Table 7).

There does not appear to be any non-random mating in the population with respect to male body size. The distributions of calling male SVL and parental male SVL were not different, and mean values were identical for the two groups; 33.4 mm (Tables 1 and 2). Large males are not obtaining mates in higher proportion relative to their occurrence in the chorus than are smaller males. This finding contrasts with a number of studies of other anurans in which male body size is strongly related to male mating success (see Arak, 1983 for a review). Furthermore, there was no significant relationship between the male SVL and female SVL in mated pairs (Figure 3). There is no evidence of positive assortative mating in the population.

Male coquíes are using microhabitats for calling that are markedly different from nest sites. The vast majority of nest sites (79.3%) are within 25 cm of the ground while males almost invariably call from perches above 25 cm (92.6%). In terms of cover, 83% of all nest sites were entirely enclosed, with no opening. Calling sites, on the other hand, are virtually always open at least in front (96.0%), and often (85%) are more exposed than that. The majority of calling sites are comprised by the axils of sierra palm fronds, the open surfaces of shrub and vine leaves, and the surfaces of tree trunks and branches.

The relationship of female body size to clutch size follows the trend noted in many other anurans, as well as ectothermic vertebrates generally. The significant positive correlation of egg number with female SVL has been described for urodeles (Salthe, 1969) and for anurans (Salthe and Duellman, 1973; Crump, 1974; Wake, 1978). However, one of the obstacles to interpreting life history adaptations concerning fecundity has been the lack of data on how often females

deposit eggs within a breeding season. Lower clutch size to body size regressions in some species may be offset by more frequent clutch deposition. Only recently has it been found that some temperate anuran females can deposit two clutches in one season (Wells, 1976; Howard, 1978; Perrill and Daniel, 1983). For many tropical species, females can almost certainly produce two or more clutches per year (Chibon, 1960; Wells, 1980b; Kluge, 1981; Wells and Schwartz, personal communication). The evidence presented in this study for E. coqui suggests that females can deposit a new clutch about once every two months in the field. In laboratory populations on ad libitum food, female coquíes can produce eggs every six weeks. These data suggest that E. coqui females could produce at least four clutches per year, if egg production is limited primarily to the wet season (March through October), and may be able to lay as many as six or seven clutches if food is abundant and egg production continues, even at a reduced level, during the dry season. Female coquíes maintain a positive energy budget throughout the year and appear to have excess available energy for growth at all body sizes (L.L. Woolbright, personal communication) so that energy limitations to egg production do not appear to be present in this population.

CHAPTER 3

THE MALE PARENTAL BEHAVIOR OF E. COQUI

Introduction

In order to evaluate the net adaptive value of parental care, it is important initially to know the basic care behavior repertoire and to have some idea of the time budget of care provision for a species. This information has been gathered for a number of birds and mammals in which parental care has been studied (Kleiman, 1977; Martindale, 1982; Walters, 1982), but there exist virtually no data of this kind for amphibians (exceptions include Kluge, 1981; Simon, 1982, 1983). Knowing the temporal pattern of care provision is especially crucial for amphibians which exhibit egg attendance (which comprise the vast majority of species with parental care) because without having the eggs physically attached, the parent is free to pursue activities that are alternative to care behavior. Alternate activities may include maintenance functions (feeding, rehydrating) and behaviors that influence mating success (calling, territorial maintenance). The trade-off between caring for one brood and either survival or additional reproduction will be strongly influenced by the allocations of time to alternative activities. Furthermore, it is important to know the details of care behavior because mere presence at an oviposition site may not constitute functional parental care but only coincidental presence in the same microhabitat as eggs (Woodruff, 1977). Testing predictions about the evolution of parental care will require gathering such data, particularly when interspecific comparisons are used to make inferences about evolutionary trends.

In this chapter, I describe the major behavioral characteristics and temporal patterns of parental care in E. coqui.

Methods

The sampling method by which clutches were located was discussed under General Methods. Most clutches were left undisturbed and subsequently checked at various times of day and night. During each nest check, the position and behavior of the parent and the developmental stage and condition of the eggs were recorded. Nest checks did not appear to disturb the nest or parents greatly (but see Methods of Chapter 4).

Aggressive calls (see below) were recorded using a portable cassette recorder (Channel Master Model 6395) and analyzed on a Model 4500 Unigon Uniscan real-time spectrum analyzer and a Tektronix Model 5103N Storage Oscilloscope. Sonagrams were made on a Kay Model 6061B Sona-Graph with the band selector set on wide.

In all analyses, 'day', 'daytime' and 'diurnal' represents the period 0601-1759 hours and 'night' and 'nocturnal' the period 1800-0600 hours.

Results

Sex of the Attending Parent

On the basis of the behavioral and morphological criteria used to determine the sex of an attending parent (General Methods), I have no evidence that females ever attend eggs in this species.

Frequency of Unattended Clutches and Multiple Clutches

I found only 11 clutches (out of 616) which never had an attending parent. Males do not attend eggs in more than one nest simultaneously. Any pattern of complementary presences and absences at two neighboring nest sites would have been suspicious. I never found such a pattern. There were cases of multiple simultaneous clutches in the same nest site (29 double clutches and two triple clutches). However, multiple clutches were usually contiguous inside the nest; only seven of the double clutches had more than one body length separating them.

Description of Parental Care

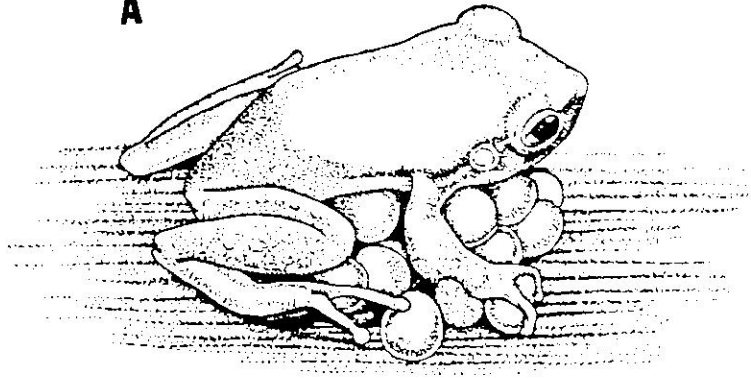
Parental care in E. coqui is characterized by egg attendance beginning at oviposition and lasting throughout development, a period of 17-26 days. Males often continue to attend hatchlings for 1-6 days after hatching. Males perform two major parental activities: brooding of eggs and defense of the nest and eggs.

Brooding. A parent was considered brooding at any time that a part of its venter was in contact with its eggs (Figure 5A). Although males varied with respect to how well they were centered over their eggs, they typically covered most of the clutch. Males that brooded after hatching adopted an elevated posture near or over the egg capsules. Because hatchlings typically grouped together on or near the capsules, they were often clustered beneath the male.

Nest defense. Parental males were observed defending nests against conspecific intruders on 15 occasions during the study. Six contests were observed in sufficient detail and length to allow quantitative analysis. These six ranged from 10-75 min (mean = 30 ± 10.2 min) in duration and took place before midnight.

Figure 5. Parental behaviors in E. coqui. A. Egg brooding.
B. Parental male blocking a nest intruder.
C. Parental male delivering a sustained bite to
a nest intruder.

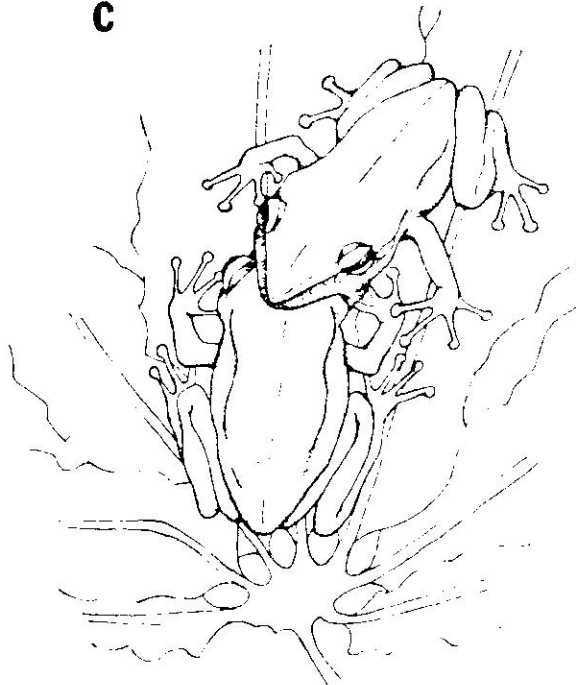
A



B



C

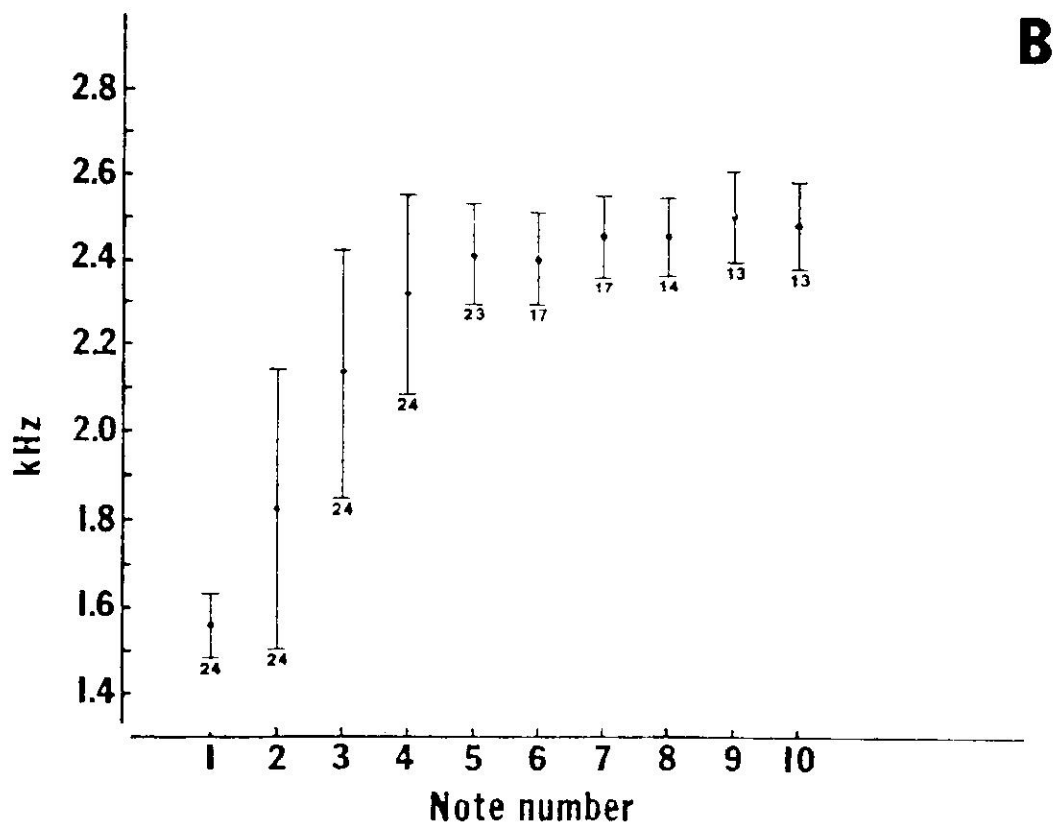
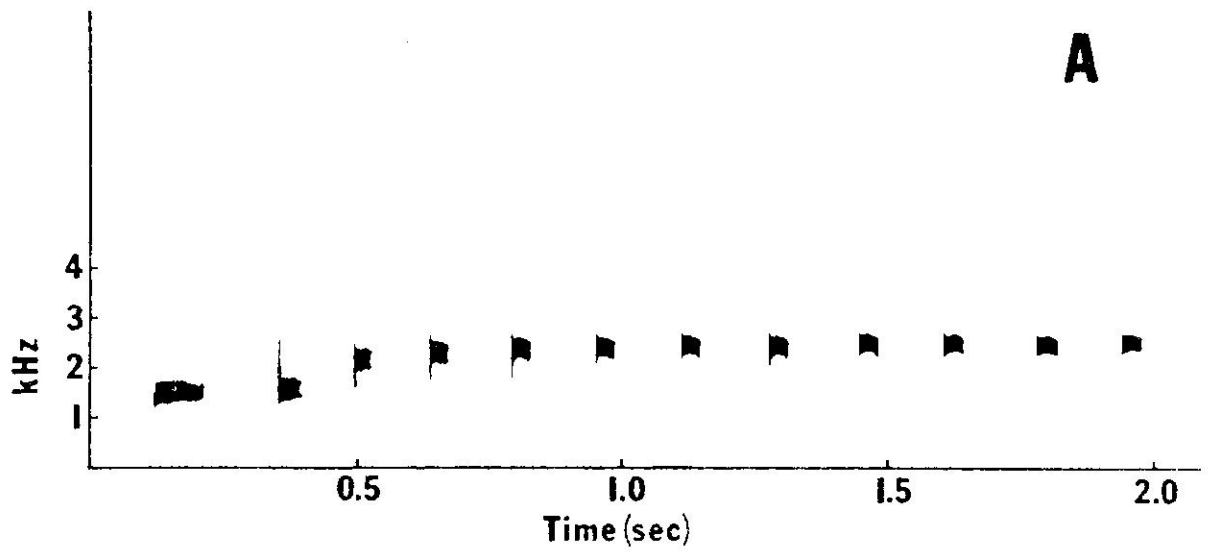


Aggressive behaviors by parental males observed during nest defense included aggressive calling, biting, sustained biting, blocking and wrestling. Contests usually consisted of long periods (minutes) of inactivity punctuated by short bursts (seconds) of activity. Parents spent 5% of total contest time in active behaviors (calling, biting, wrestling and chasing) and the remainder in static positions, whether separated from intruders or in contact with them (blocking or sustained biting). Prolonged contact (lasting more than 5 s) between parent and intruder occurred in five of the six contests. An average of 51% of contest time was spent in contact although the range in the six individual contests was 0% to 98%. There were two types of prolonged contact: blocking and sustained biting. In the typical blocking posture (Figure 5B), a parent was on top of the intruder, its front leg and forebody blocking the intruder's head and forebody, and its hind legs partly extended in a bracing position. In sustained biting (Figure 5C), a parent held some part of an intruder's body, usually the head, in its mouth.

Aggressive calling by parental males was heard in 14 of 15 contests. Although the regular advertisement call of E. coqui has been extensively studied (Drewry, 1970a, b; Narins and Capranica, 1976, 1978, 1980), the aggressive call has never been quantitatively characterized. Therefore, I will describe it briefly. All variance estimates in the following section on aggressive calls are 1 SD.

Aggressive calls were composed of 4-37 (mean = 11 ± 5 notes per call, N = 88) brief, rapidly repeated notes with dominant frequencies of 1.4-2.7 kHz (Figure 6). The calls always began with a note of 120 ± 15 ms duration (range = 100-160 ms), followed by a sequence of notes,

Figure 6. The aggressive call of E. coqui. A. A sonogram of a 12-note call recorded during a nest-defense contest. B. Mean dominant frequencies (bar equals ± 1 SD; sample size below SD bars) of the first 10 notes of aggressive calls by five different males during nest-defense contests. Sample sizes decrease because some calls had fewer than 10 notes.



each of 60 ± 5 ms (range = 40-70 ms) in duration. The period between the first and second notes was 250 ± 20 ms (range = 220-300 ms) and that between all subsequent pairs was 160 ± 15 ms (range = 120-200 ms). Individual aggressive calls ranged from 0.6 s (four-note call) to 5.7 s (37-note call) in length. The average dominant frequencies of notes increased from 1.6 ± 0.1 kHz for the first note to an asymptote at 2.5 ± 0.1 kHz by the tenth note (Figure 6).

Aggressive calls were common in the six contests (mean = 14 ± 9.1 calls per contest, range = 7-31), occurring in bouts of 2-5 calls. The average overall rate of aggressive calls was 0.8 calls/min (range of average call rate for individual contests was 0.4-1.6 calls/min).

Biting by the defending parent was observed in all six contests (mean = 4.5 ± 3.3 bites per contest, range = 2-10). The frequency of biting ranged from 0.2-0.4 bites/min in individual contests, with an overall frequency of 0.4 bites/min. Individual bites were rapid lunges, usually at an intruder's head. On five occasions, in four different contests, the parent bit and held on to an intruder's head or body (sustained bite - Figure 5C) for a period of 0.25-22.0 min (mean = 6.5 ± 8.8 min).

Wrestling occurred during periods of blocking contact. Typically, the parental male grabbed or levered the intruder's head or forebody and pushed the intruder away from the eggs. Wrestling bouts were brief, lasting from 5 to 20 s. Nine wrestling bouts were observed during the three contests in which blocking contact occurred.

Intruder behavior and contest outcome. Intruders did not engage in aggressive behavior (although, during one contest, an intruder gave several aggressive calls). Movement by intruders seemed entirely

directed at getting around the parent and to the eggs.

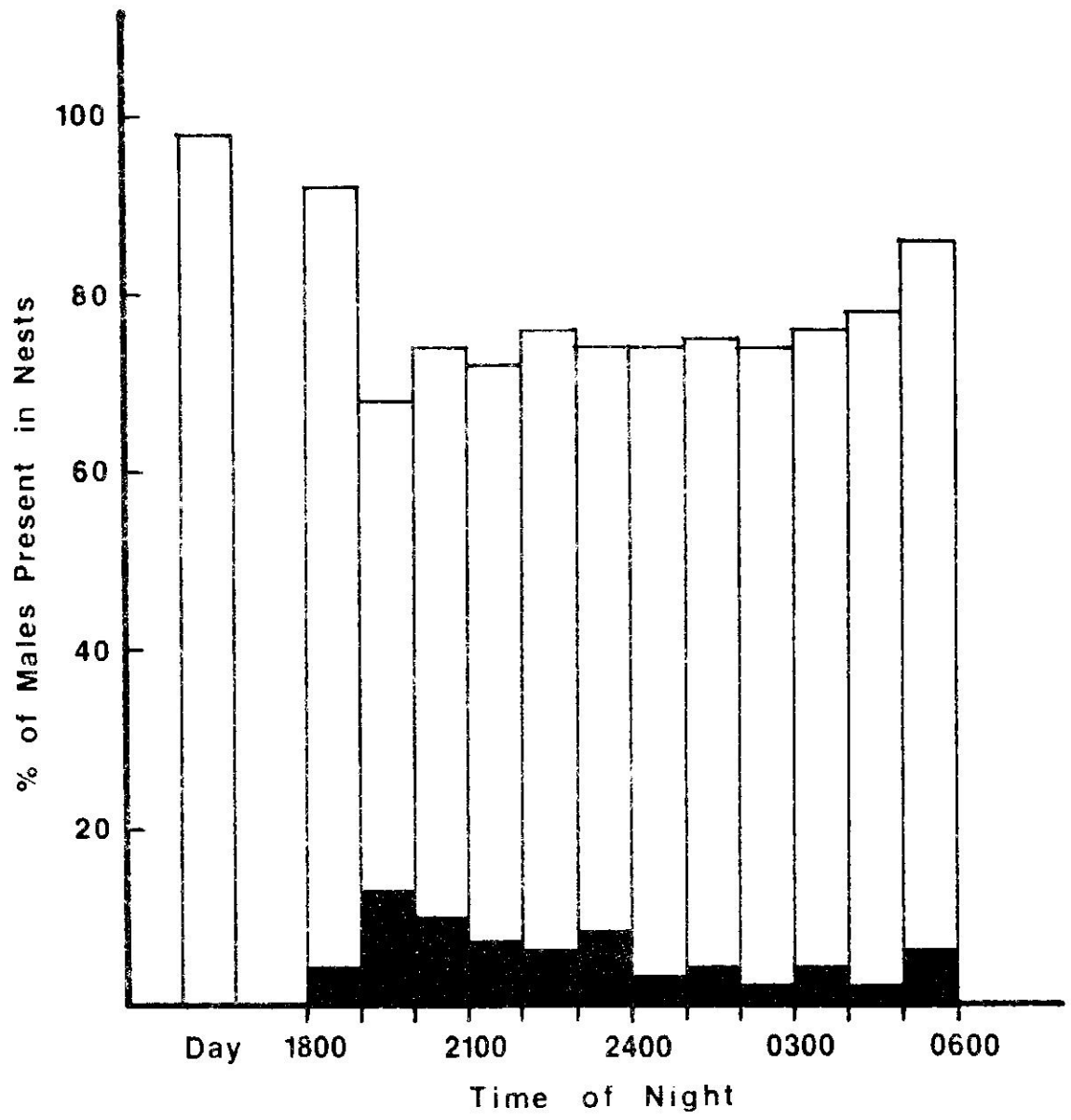
In four of the 15 occasions when nest defense was observed, the outcome was inconclusive because the frogs were disturbed. In six of the other 11 cases, intruders ate most or all of the clutch. Intruders were chased out of the nest and consumed few or no eggs in five contests.

Temporal Patterns of Parental Care

Analysis of temporal aspects of parental care in E. coqui was based on 4879 nest checks made on 510 clutches during 1980 and 1982. Nest checks were divided between day (44.9%) and night (55.1%), and among the four developmental intervals (I: 22.4%, II: 27.2%, III: 39.5%, IV: 10.9%). I arbitrarily established four categories of male position with respect to eggs: brooding, in the nest not brooding, at the nest entrance, and absent from the nest. On about 67% of the occasions that males were absent from their nests, they could not be located.

Diel patterns of nest attendance and brooding behavior. The percentage of nest checks in which males were present in the nest or at the nest entrance was 97.4% during the day and 75.8% at night for pre-hatching clutches, and 90.3% by day and 44.9% at night after hatching. The hourly pattern of nocturnal nest presence of males with pre-hatching clutches is shown in Figure 7. The presence of parental males dropped to 92.2% between 1800 and 1900 hours, as frogs began to emerge at dusk; it was lowest (68.8%) between 1900 and 2000 hours, the first full hour of darkness, and fairly constant at about 74% (range = 72.4-76.4%) for the rest of the night, increasing after 0400 hours as

Figure 7. Percentage of nest checks in which parental males were found in their nests (brooding eggs, in nest not brooding, and at nest entrance categories are combined) during the day and at hourly intervals through the night. The darkened lower area of each bar indicates the percentage of checks in which males were at the nest entrance. Only pre-hatching developmental stages were used.



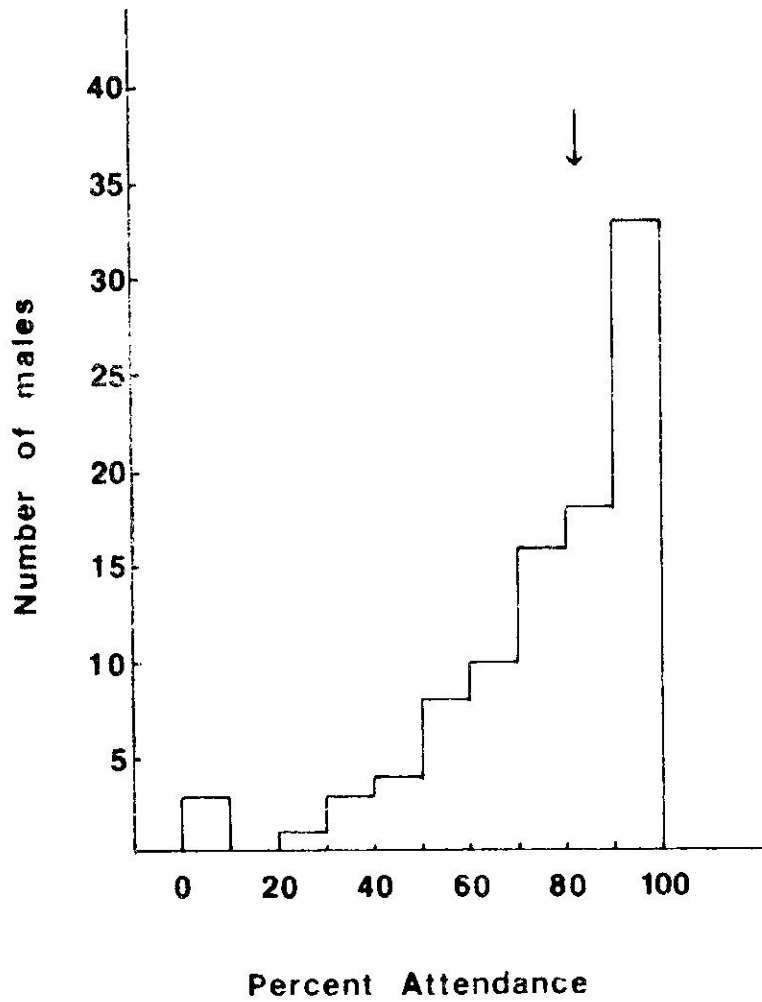
all males returned to their nests towards dawn. There was a concomitant peak in males observed in their nest entrances (12.4%) during 1900-2000 hours (dark area of each bar in Figure 7).

There was individual variation in the population in levels of nocturnal nest attendance. During the two years, there were 96 nests for which I had at least 4 nocturnal nest checks during pre-hatching development. The distribution of percent nest attendance is plotted for these nests in Figure 8. The distribution is quite skewed towards lower levels of nocturnal nest attendance and has a Median of 84% presence. Clearly the majority of parental males exhibited high levels of attendance; 67 of 96 (69.7%) males were in their nests at least 70% of the time at night.

Coquíes are nocturnal and normally spend the day in retreat sites which are similar to nest sites. Evidence that males were actually providing care to eggs, and not merely occupying retreat sites that happened to contain clutches comes from a consideration of parental male position in the nest. When in the nest, a male could sit either on the eggs or somewhere else inside the nest. The circular area whose radius is defined by a clutch plus one male body length is the area where a male could sit and be considered brooding. That area was usually much less than 25% of the interior surface area of a nest. Hence, on a random basis, males should have been found brooding eggs about 25% of the time that they were present in their nests. In fact, when found in their nests, parental males were brooding eggs in 93.1% of all daytime checks and 81.9% of all nocturnal checks.

To test the hypothesis that the high level of nest presence at night (75.8%) represents a departure from the normal nocturnal activity

Figure 8. The frequency histogram of nocturnal nest attendance levels for parental males at 96 nests during 1980 and 1982. Attendance level is expressed as the percentage of nest checks in which a male was present in the nest or at its entrance. Only nests with at least four nocturnal checks were included. Arrow indicates the median = 84%.



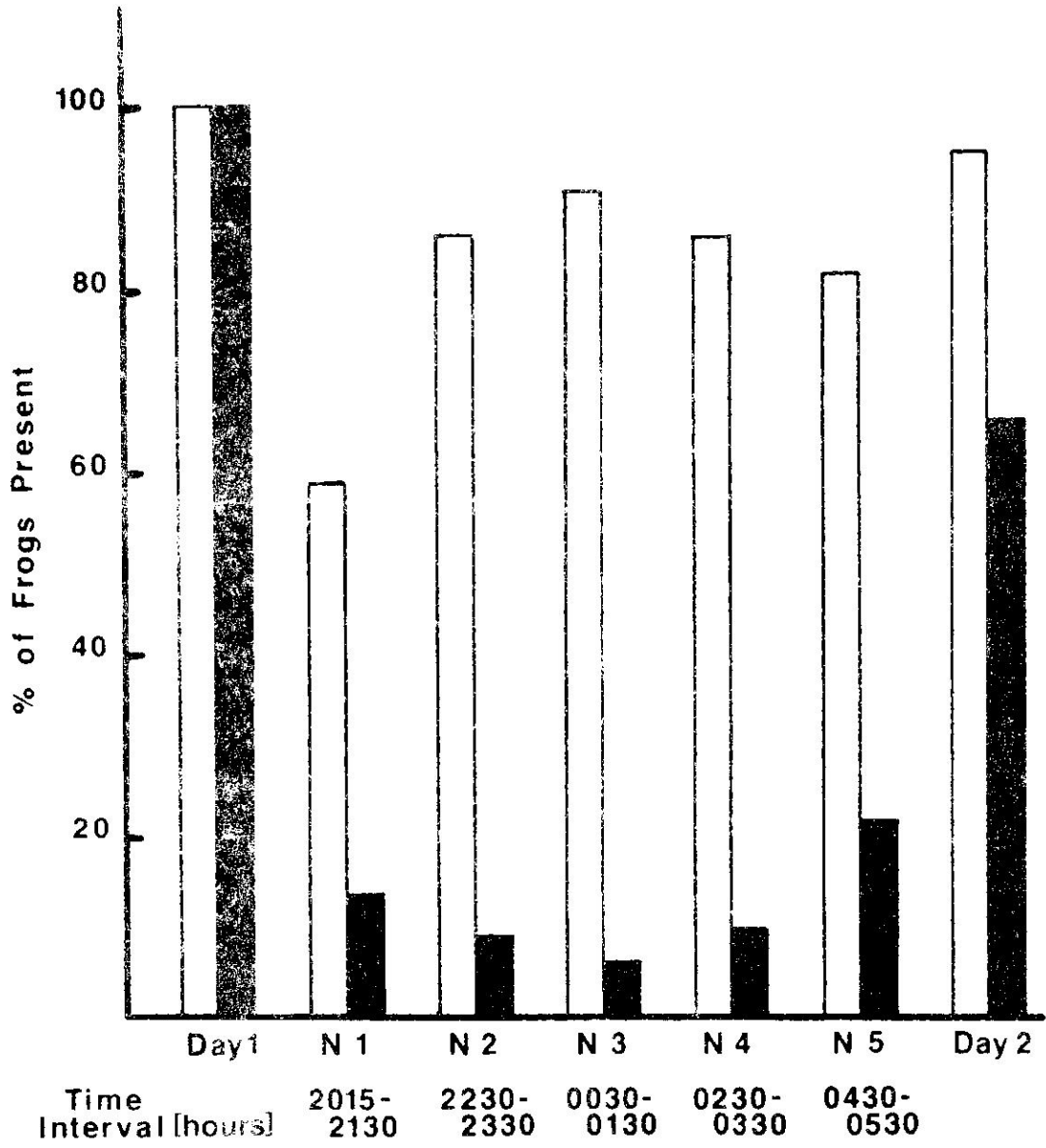
patterns of adult coquíes, two sets of observations were made during 1982 (June 27-28 and July 7-8). During the afternoon of the first day, the bamboo frog houses in each of two plots were surveyed and the numbers of parental males and non-parental adults were recorded. During the following night all houses were re-sampled at 2-h intervals, starting at least 1 h after sunset and continuing until 1 h before dawn (see Figure 9 for nocturnal survey time intervals). All houses were checked a final time during the afternoon of the second day.

On June 27-28, 90 houses were checked. Five parental males and 16 non-parental adults were found during the first afternoon survey. On July 7-8, 187 houses were checked; the initial count was 17 parental males and 54 non-parental adults. The results of the two nights were combined and the initial counts of parental and non-parental frogs during the first afternoon survey were designated as 100% (Figure 9). During the first nocturnal check, parental male presence dropped to 59%, while that of non-parental adults dropped to 14%. During the second check, parental presence rose to 86% and subsequently remained at that level. The presence of non-parental adults dropped to about 10% and remained there until the last check, when it rose to 22% as frogs returned to their retreats prior to dawn. The average presence for all nocturnal checks was 82% for parental males and 12% for non-parental adults. The difference was significant (Chi-square = 193.5, $p < 0.001$).

On the following day one of the 22 parental males (4.5%) failed to return to his nest, whereas 23 of the 70 non-parental adults (32.8%) failed to re-enter their retreat sites of the preceding day (Figure 9).

Nest attendance as a function of developmental state of the

Figure 9. The percentage presence of parental males (clear bars) and non-parental adults (dark bars) in their nest sites and retreat sites, respectively, during an initial afternoon survey (Day 1), five nocturnal surveys (N1-5) and the following afternoon survey (Day 2) of two house plots on June 27-28 and July 7-8, 1982. The number of frogs in each category on Day 1 (parental males: N = 22; non-parental adults: N = 70) was set as 100%.



clutch. To investigate whether parental care behavior varied with the developmental state of eggs, all nocturnal nest checks were partitioned among the four developmental intervals. For this analysis, the male position categories 'in nest not brooding' and 'at nest entrance' were combined into one 'not brooding' category. There was a significant difference in parental attendance across developmental intervals (Table 8) only when post-hatching nest checks were included. There was no significant difference in parental behavior of attendant males across the pre-hatching developmental intervals.

Discussion

Male parental care in E. coqui is characterized by attendance of eggs at a terrestrial oviposition site. Males exhibit very high levels of nest fidelity. They are found in their nests 97.4% of the time during the day and 75.8% of the time at night during pre-hatching development. Night-time attendance, and hence presence in the nest site, is significantly higher than night-time presence of non-parental adults in their retreat sites (Figure 9). Hence, parental care represents a significant shift in the nocturnal activity budget of parental males. Furthermore, the distribution of attendance levels is very skewed towards lower levels, with the majority of parental males (69.7%) spending at least 70% of their time at night in the nest (Figure 8).

Within the context of egg attendance, parental males exhibit two types of care-giving behavior: egg brooding and nest defense. Egg brooding is the major activity of parents. Males maintained contact with the eggs 89.1% of the time that they are in the nest at night and

Table 8. Brooding and nest attendance patterns of parental males during each developmental interval for nocturnal nest checks (Percentage of column total in parentheses).

Male position	Developmental Interval			
	I	II	III	IV
Brooding	376 (62.5)	493 (61.5)	671 (62.6)	59 (27.6)
In nest, not brooding	85 (14.1)	107 (13.3)	144 (13.4)	37 (17.3)
Absent from nest	141 (24.4)	202 (25.2)	257 (24.0)	118 (55.1)

Whole table: $\chi^2 = 112.9$, $df = 6$, $p < 0.01$.

Table without IV: $\chi^2 = 0.78$, $df = 4$, $p > 0.05$.

93.1% of the time during the day. Overall, then, parental males spent 67.5% (89.1% of 75.8%) of the night and 90.0% (93.1% of 97.4%) of the day brooding their clutches.

Comparable data for temporal patterns of parental behavior and details of care behavior are virtually unavailable for anurans. Simon (1983) found that Cophixalus parkeri parents remained close to their clutches throughout an 85-100 day developmental period, and were invariably found brooding the eggs. However, he did not differentiate between daytime and nighttime attendance nor did he provide sample sizes for evaluating attendance data. Stomach contents of brooding parents indicate markedly lower foraging rates, suggesting that attendance levels are high enough to preclude normal foraging activities at night. Kluge (1981) found that male Hyla rosenbergi exhibited high nest attendance during pre-hatching development but that once eggs hatched, attendance ceased (although in a second year pre-hatching attendance levels were much lower). In this species, eggs are aquatic and pre-hatching development lasts only 1.5-3 days, so the temporal and behavioral constraints on parental care provision are quite different from those operating in terrestrial species with long pre-hatching periods.

Brooding is probably the most common type of parental behavior in amphibians (Salthe and Mecham, 1974; McDiarmid, 1978; Wells, 1981). This is not terribly surprising. Brooding is generally a simple behavior which would require relatively little innovation in motor patterns or neural pathways in order to evolve. Also, since eggs are often deposited in the regular retreat site of the parent, there may be no need for a microhabitat shift in order to provide care. There

should be no added energetic cost of brooding, at least initially. Finally, brooding may have a relatively large effect in increasing offspring survival. The benefits and costs of brooding behavior in E. coqui will be addressed in the next two chapters.

The evolution of nest defense behavior probably represents the redirection of territorial behavior to a parental care function. In E. coqui, both males and females will defend retreat sites from conspecifics (personal observation; M.M. Stewart, personal communication). Nest defense by parental males may be more intense and prolonged than retreat site defense, but now serves a function more directly related to fitness. That nest defense is more intense than retreat site defense and constitutes a somewhat separate category as a parental care behavior is supported by several observations. First, intruder males were never observed to engage in aggressive behavior, but rather appeared intent on approaching and ingesting the eggs. Second, parental males never pursued intruders beyond the entrance of the nest site. Third, nest defense contests lasted longer and generally involved higher levels of wrestling, biting and sustained biting than did retreat site defense.

CHAPTER 4

THE BENEFITS OF MALE PARENTAL CARE

Methods

During April-June 1980 and June-July 1982 attendant males were removed from their clutches in some nests and the fate of these experimental clutches was compared to that of control clutches which had attendant males throughout development. Parental males were removed over the whole range of pre-hatching developmental stages, from immediately post-oviposition to Stage 13. Experimental and control clutches were monitored daily until they hatched or failed. There were two major causes of clutch failure: desiccation and predation. I observed two types of egg predators: invertebrates and coquíes. In cases where the predator was not evident, I used criteria based on field and laboratory observations of predation to identify the predator. All invertebrate predators left a residue from their feeding activity, whether empty egg capsules or partially chewed eggs. Coquíes always ate eggs whole. Hence when eggs had been chewed or otherwise partly consumed, invertebrate predation was inferred; when entire eggs disappeared, cannibalism was presumed to have taken place. I have no evidence that any other vertebrates consume the eggs of E. coqui. In the course of almost 4900 individual nest checks (see below) I never had an instance of actual or suspected predation on coquí eggs by vertebrates other than coquíes.

I have observed both filial cannibalism, the parent eats its own eggs, and heterocannibalism, a conspecific eats the eggs (Rohwer, 1978), in E. coqui. Filial cannibalism was associated with major

disturbance of the nest, parent or eggs. There were two sources of such disturbance. One was the investigator: when a nest was first discovered, the parent was captured and various parameters of nest and eggs were measured. Following such initial disturbance, a parent was observed to eat its eggs on several occasions, but only when eggs were in early developmental stages (i.e., during developmental interval I). The second type of disturbance occurred when a conspecific entered a nest and ate most of the eggs in the clutch. The parent sometimes consumed the remaining eggs but, again, only when they were in early stages of development. There were instances when heterocannibals ate part of a clutch but the parent continued to care for the rest of the eggs. On the basis of this evidence, I used the following criteria to assign cannibalized clutches: (1) if, at the first nest check following initial disturbance, a clutch had disappeared, I considered it filial cannibalism; (2) if the parent was found attending the eggs during checks subsequent to the initial disturbance and the clutch later disappeared, I considered it heterocannibalism. Using these conservative criteria I may overestimate the incidence of heterocannibalism on control clutches. However, any bias will act against the hypothesis that removal of parents causes increased clutch failure from heterocannibalism.

Results

Male Removal Experiments

Parental males were removed from 31 clutches in 1980 and 73 clutches in 1982. The fate of these experimental clutches was compared with that of 98 control clutches in 1980 and 108 controls in 1982.

Average initial clutch sizes for experimental and control clutches were 26.6 ± 0.9 and 28.2 ± 0.5 eggs/clutch respectively in 1980, and 24.5 ± 0.5 and 24.6 ± 0.5 eggs/clutch in 1982 (the control and experimental clutch sizes were not significantly different in either year).

Most clutches either succeeded or failed completely. Of 206 control clutches and 104 experimental clutches, 280 (90.3%) had either 0-10% or 90-100% hatching, where % hatching = (number of eggs that hatched/initial egg count) X 100. Hence clutches were scored as successes (50% or greater hatching) or failures (less than 50% hatched). There were very few borderline cases: 10 clutches (3.2%) had between 40-60% hatching. In a few clutches, more than one agent of egg mortality was involved in causing failure. In those cases, the agent responsible for most of the of egg mortality was cited as the cause of clutch failure.

Fungal infection and developmental abnormalities, major causes of egg mortality in other anuran species (Salthe and Mecham, 1974; Simon, 1982, 1983), were of minor impact on the eggs of E. coqui. Fungi infected only dead eggs, usually those that had failed to develop. A low incidence (1-3 eggs/clutch) of abnormal or non-developing eggs was found in many clutches. There were no differences in the incidence of fungal infection or abnormal development between experimental and control clutches.

In the 2 years, 37 experimental clutches (11 in 1980 and 26 in 1982) failed from predation. On the basis of direct evidence I could assign one instance to invertebrate predation (IP) and 21 to heterocannibalism (HC). Using the criteria outlined in the Methods for the remaining clutches, I assigned 3 to IP and 12 to HC. Sixty-nine of

the control clutches failed from predation during the 2 years (40 in 1980 and 29 in 1982). I could assign 26 on the basis of direct evidence: 8 to IP, 2 to HC and 16 to filial cannibalism (FC). On the basis of the Methods criteria, I assigned the rest as follows: 5 to IP, 23 to HC and 15 to FC. Because experimental clutches were not subject to failure from filial cannibalism, the 31 control clutches that failed for this reason are excluded from further analysis.

The success rate of experimental clutches was significantly different from that of control clutches in both years (Table 9). Combining the results from both years, 76.9% (80 of 104) of all experimental clutches failed, as against 22.5% (40 of 175) of all control clutches. The increased frequency of failure in experimental clutches was caused by significantly more frequent desiccation (Chi-square = 77.94, df = 1, $p < 0.001$; both years) and cannibalism (Chi-square = 12.06, df = 1, $p < 0.001$; both years). Predation by invertebrates was not significantly different between experimental and control clutches (Chi-square = 1.46, df = 1, $p < 0.05$; both years).

When clutches were assigned to the three developmental intervals according to the stage of the eggs at discovery or male removal, there was a significant decrease in clutch failure from developmental interval I to III for both experimental and control clutch groups (Table 10). However, when the fate of clutches at each developmental interval were compared between treatments, there were significant differences in hatching success for each developmental interval. Thus, although there was a significant increase in hatching success of experimental clutches from early to late development, hatching success was still significantly lower than for control clutches at every

Table 9. The fates of experimental and control clutches in male-removal experiments during 1980 and 1982 (proportion of row totals in parentheses).

Year and treatment	N	No. of clutches that hatched	No. of clutches that failed from:		
			Desiccation	Heterocannibalism	Invertebrate predation
1980					
Experimental	31	5 (0.161)	15 (0.484)	9 (0.290)	2 (0.065)
Control	78	56 (0.718)	2 (0.025)	13 (0.167)	7 (0.090)
1982					
Experimental	73	19 (0.260)	28 (0.384)	24 (0.329)	2 (0.027)
Control	97	79 (0.814)	0 (0.000)	12 (0.124)	6 (0.062)

1980: $\chi^2 = 44.00$, $df = 3$, $p < 0.001$.

1982: $\chi^2 = 68.72$, $df = 3$, $p < 0.001$.

Table 10. The fates of experimental clutches divided according to initial developmental interval for 1980 and 1982 combined (proportion of row totals for each treatment in parentheses).

Developmental interval	Experimental clutches		Control clutches		χ^2	df
	Hatched	Failed	Hatched	Failed		
I	4 (0.064)	59 (0.936)	52 (0.627)	31 (0.373)	48.02*	1
II	8 (0.333)	16 (0.667)	36 (0.837)	7 (0.163)	17.35*	1
III	12 (0.706)	5 (0.294)	47 (0.959)	2 (0.041)	8.54*	1
					20.74*	
						2
						2

* $p < 0.01$.

developmental interval.

Evidence from Non-Experimental Nests

Corroborative evidence that parental care results in significantly increased hatching success comes from combining the observations of nocturnal nest attendance (Figure 8, Chapter 3) with records of hatching success for those nests. Of 96 nests for which I have at least four nocturnal nest check records (Figure 8), I also know the hatching success in 64 of them. All 64 were clutches in natural nest sites (did not include any bamboo houses); 45 were on the ground (less than 25 cm off the ground) and 19 were elevated. Hatching success was extremely bimodal, as with experimental clutches, and was scored similarly; clutches with 50% or greater hatching were considered successful, those with less than 50% as failures. Also, because of the small number of males that exhibited low levels of nest attendance, I combined percent attendance into two categories, 0-75% and 76-100%. The relationship between nocturnal attendance level and hatching success (Table 11) was significant (Chi-Square = 5.14, $p < 0.025$), with significantly more clutches hatching (76%) when nocturnal attendance was high than when it was low (48%).

Discussion

In the first part of the discussion, I will examine the proximate functional benefits of male parental care in E. coqui in the context of what we know about the functional significance of parental care in amphibians. In the second part, I will consider how the enhancement of hatching success that results from parental care affects reproductive success and hence fitness of caring males.

Table 11. The frequencies of two levels of hatching success as a function of the level of nocturnal attendance, computed as the percent of nest checks when the parental male was in the nest.

Percent attendance	Percent hatching	
	0-49	50-100
0-75	14	13
76-100	9	28

$$\chi^2 = 5.14, p < 0.025.$$

Proximate Benefits of Parental Care

At least four functional roles resulting in increased offspring survival have been ascribed to amphibian parental care (McDiarmid, 1978; Wells, 1981): (1) provision of an appropriate hydric regime, (2) reduction of predation, (3) reduction of fungal infection and (4) reduction of developmental abnormalities.

Provision of an appropriate hydric regime. A major function of parental care in E. coqui is to prevent desiccation of eggs. When male parents were removed from their eggs, 41% of all clutches (54% of those clutches that failed) died from desiccation (both years combined). Provision of parental care (control clutches) reduced desiccation to negligible levels (1.1% of all clutches; 5.0% of clutches that failed). The physiological basis of egg hydroregulation by coquí males has been documented by Taigen et al. (1984), who proposed a mechanism for the transfer of water from parent to egg across the male's ventral integument and suggested that brooding also functions to reduce evaporative water loss. Further, they showed that increased egg water content had a significant positive effect on hatchling mass. Thus, extensive brooding by male coquíes may serve both to insure hatching and to maximize hatchling size. Both consequences are potentially important components of fitness. Although the provision of an appropriate hydric regime may be an important function of parental care in other anurans (Jameson, 1950; Myers, 1969; Wells, 1980a; Weygoldt, 1980), our study is the first to demonstrate its importance in the field. In some anurans parental care apparently does not serve this function (Kluge, 1981; Blommers-Schlösser, 1975; Scheel, 1970;

McDiarmid, 1978; Simon, 1982, 1983).

Reduction of predation. Conspecifics are the principal egg predators in E. coqui. Nest defense by parental males is a significant counter to this threat. In male-removal experiments, 31.7% of the experimental clutches were consumed by conspecifics, as against 14.3% of the control clutches, a two-fold difference in the incidence of heterocannibalism. Parental defense of eggs against conspecifics appears to be important in at least two other anurans, Hyla rosenbergi (Kluge, 1981) and Cophixalus parkeri (Simon, 1982, 1983). Filial cannibalism occurred in E. coqui only when disturbance of the eggs by the investigator or an intruding conspecific occurred during early development. Filial cannibalism probably is not an adaptive response in this species, either as a source of nutrients for an energy-stressed parent (Rohwer, 1978), or to remove eggs infected with fungi, as has been found in some salamanders (Highton and Savage, 1961; Tilley, 1972; Forester, 1979a). Once a male coquí began to ingest his own eggs, he ate most or all of them. There was no evidence of discriminatory ingestion of only dead eggs.

Protecting eggs against invertebrate predators may be an important function of parental care in other amphibians (McDiarmid, 1978; Simon, 1982, 1983). In E. coqui, egg predation by invertebrates was infrequent and the presence of parental males did not change its relative incidence. The results of the male removal experiments suggested that males are either indifferent to invertebrate egg predators or unable to defend clutches against them. Observations of egg predators in the field and laboratory corroborate this conclusion. The main invertebrate egg predators were an ant (Paratrichina sp.) and

the larvae of a small phorid fly (Megaselia scalaris)(Villa and Townsend, 1983). The ants occasionally invaded coquí nests by the thousands and decimated any clutch therein. Megaselia scalaris is a very small fly that could oviposit on coquí eggs without being noticed by male frogs or while males were temporarily out of the nest. The larvae develop very quickly and completely destroy a clutch of coquí eggs, reducing it to a slimy mass, within 48 hours. The incidence of predation by either of these predators was less than 2%. There were several other invertebrate egg predators of more minor effect (taking only 1-3 eggs at a time), including a cricket (Amphiacusta portoricensis), two land snails, and a spider.

Reduction of fungal infection. It has been suggested for some frogs (Blommers-Schlösser, 1975; Simon, 1982, 1983) and some salamanders (Highton and Savage, 1961; Tilley, 1972; Forester, 1979a) that an attending parent may reduce fungal infection of eggs either by secreting an antibiotic substance or by eating infected eggs to prevent infection of other eggs. However, neither Forester (1979a) nor Simon (1983) were able to isolate a substance or to demonstrate that skin swabs had a fungicidal effect. In E. coqui, fungi grew only on eggs that were already dead and never appeared to spread to normal living eggs. Fungi were observed to infect dead eggs in clutches with and without attending males.

Reduction of developmental abnormalities. Attending parents may reduce the incidence of developmental abnormalities by jostling or turning eggs during early development (Salthe and Mecham, 1974; Forester, 1979a; Simon, 1982, 1983). In E. coqui, eggs are adhesive and are often deposited on vertical surfaces. Hence, there is little

chance for the male to manipulate the eggs. There was no indication in the male-removal experiments that eggs deviated from normal development.

From this brief review, it is clear that there is marked variability among species in the incidence and relative importance of the four functions proposed for amphibian parental care. From these limited data, no clear relationship emerges between form and function of parental care in anurans. For example, Cophixalus parkeri (Simon, 1982, 1983), Centrolenella valerioi (McDiarmid, 1978) and Eleutherodactylus coqui (this study) seem to differ substantially in the functional basis of parental care, yet all three exhibit prolonged attendance of eggs.

Fitness Benefits of Male Parental Care

Male parental care in E. coqui involves high levels of nest attendance, extensive brooding and elaborate nest defense against conspecific cannibals (Chapter 3). The results of the male-removal experiments (Tables 9 and 10) demonstrate that this parental care significantly increases egg survival and hatching success. Only 23.1% of the clutches from which attendant males were removed hatched as compared to 77.5% of the clutches receiving care.

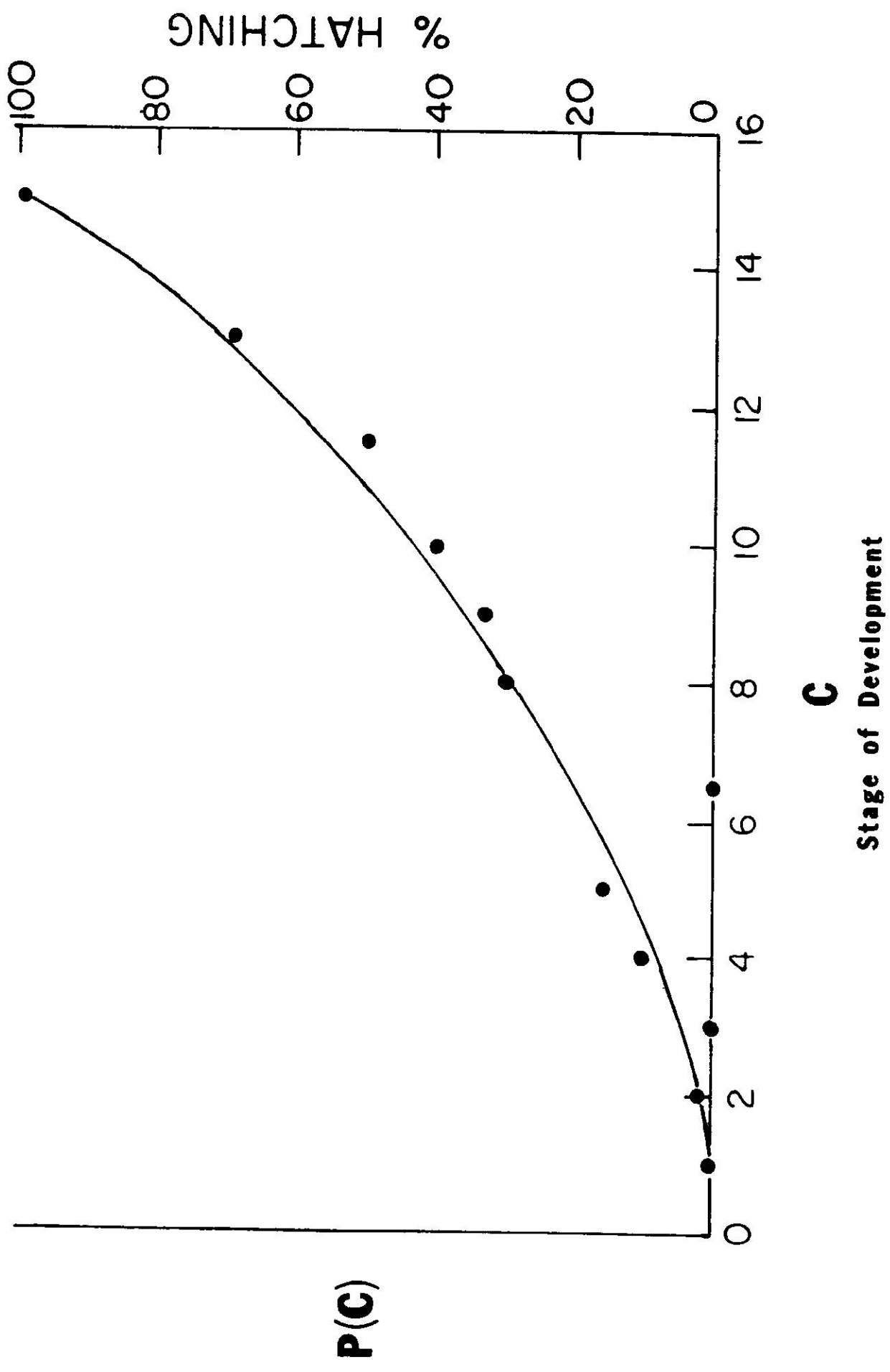
Another way of examining the results is to see how the expectation of hatching changes as a function of the length of the care period, i.e., how hatching success changes as a function of removing males at progressively later stages of development. I did this by plotting the mean percent hatching of clutches against the stage of development during which the attendant male was removed. The mean percent hatching

(+ 1SE) was computed for each stage for which $N \geq 5$; when sample size was smaller, the results for adjacent stages were combined, and the value plotted against the average value of the two stages. The results are shown in Figure 10. No eggs hatched when males were removed immediately after oviposition or during Stage 1. After Stage 1, hatching success increased slowly through mid-development and then accelerated more quickly towards Stage 15 and hatching. In order to approximate the data and obtain a benefit function, I used a multiple linear regression model (Nie et al., 1975), in which $P(C)$, percent hatching as a function of male-removal at Stage C , was the dependent variable and C and C^2 were entered as the independent variables. A quadratic polynomial gave a significantly better fit than a linear model, yielding the function:

$$P(C) = 0.50 - 0.26C + 0.42C^2$$

The benefit function, $P(C)$, drawn in Figure 10 describes the expected hatching success of a clutch after Stage X (when the male was removed). However, the probability of a clutch surviving to Stage X is not incorporated in $P(C)$. Incorporation of this pre-removal survivorship may be important in examining the net fitness of parental care strategies in E. coqui, especially since clutches do not have 100% hatching success even with complete parental care (Table 10). The devaluation of expected hatching success by stage-specific survival will obviously change the benefit function of Figure 10 to a new function. Since a consideration of how benefits and costs interact to determine net fitness will require specification of the benefit function. I will suggest here the circumstances under which one or the other type of benefit function would be more appropriate, and derive

Figure 10. The percent hatching success of clutches as a function of the developmental stage at which the parental male was removed. Each point represents the mean hatching success of at least five clutches which had the parental male removed at that stage of development. The line represents the best fit curve from a multiple regression analysis in which $P(C)$, the percent hatching, was entered as the dependent variable and C and C^2 , the non-transformed and squared forms of the stage of male removal, were entered as the independent variables.



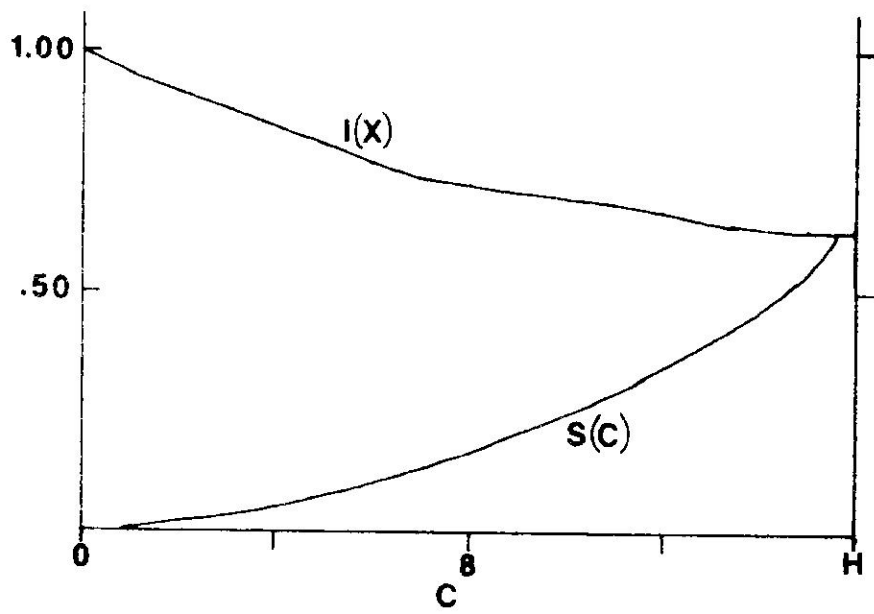
the function which incorporates survivorship up to the stage when the male was removed.

If we posit that parental males are capable of assessing the developmental stage of their clutches, and that selection can operate (via differential hatching success) on short-term decision making by parental males, then males might decide how long to care on a day-by-day basis. If that is so, males would be favored to 'decide' whether to stay and care or to abandon solely on the basis of future expectation of hatching, without regard to the clutch's past survivorship. Simply put, a male can only decide to stay if he still has a clutch, and if he has one then past survivorship is 1.00. Hence, past survivorship is not important in making a decision. In this case, the first benefit curve (Figure 10), in which hatching success is plotted only as a function of what happens after male removal, is the appropriate one.

Suppose, instead, that a male's care strategy is more or less set prior to breeding. This could be a consequence of either genetic or ontogenetic factors. Imagine that a male's strategy is to 'care for X days, then abandon'. In that case, selection should act on the strategy as a whole, adjusting X to maximize reproductive success. The expectation of hatching must take into account the entire history of the clutch, including its survivorship to X (with parental care) and its survivorship from X onwards (without parental care). I will designate this second function as $S(C)$. To calculate $S(C)$, I first describe the $l(X)$ curve for clutches which received parental care throughout development. The $l(X)$ curve represents the probability of a clutch surviving from oviposition to Stage X, given normal parental

care to Stage X. Only clutches that were found within one day of oviposition and followed to hatching or failure were used. There were 60 such clutches during April-June 1980 and June-July 1982. I designate the total number of eggs at oviposition as $N(0)$ and the number still viable at Stage X as $N(X)$. The ratio, $N(X)/N(0)$, then constitutes the stage-specific survivorship of clutches receiving parental care and is plotted against stage of development to generate $l(X)$ (Figure 11). To obtain $S(C)$, the total expectation of hatching of a clutch as a function of care to X and no care after X, I compute the product, $l(X)$ times $P(X)$, for each X. $S(C)$ is plotted as the lower curve in Figure 11. Note that the incorporation of $l(X)$ does not change the shape of the benefit curve; it only lowers the asymptote.

Figure 11. A plot of the stage-specific survivorship, $l(X)$, of 60 clutches followed from oviposition to hatching or failure. $S(C)$ is the conjugate curve derived from plotting the product of $P(C)$ and $l(X)$ for each developmental stage at which parental males were removed. See text for details of curve computation and plotting.



CHAPTER 5
THE COSTS OF MALE PARENTAL CARE

Introduction

Male coquíes invest a great deal of time in caring for developing eggs (Chapter 3). While this time investment results in highly significant benefits in hatching success (Chapter 4), any analysis of how natural selection acts to maintain parental behavior in the population must consider the costs of providing care in terms of male reproductive success.

Costs of parental care are postulated to be of two types; (1) increased risk of mortality and (2) decreased future fecundity. A variety of causes may give rise to these costs. Performing parental care might involve a temporal or spatial shift in activity or microhabitat of the care-giver into a state in which exposure to predation was greater (Forester, 1979b). Alternatively, behavioral or physiological processes specific to care-giving may use energy that results in a decrement in the care-givers physiological condition and hence increases the risk of mortality. While an energetic cost may result from greater metabolic expenditure in giving care, such a cost may also originate from reduced energy intake if foraging activity is constrained by the temporal demands of care-giving (Fitzpatrick, 1976; Rohwer, 1978; Krzysik, 1980; Forester, 1981; Simon, 1983; for an exception, see Kaplan and Crump, 1978). While the consequences of a negative energy budget caused by providing care may not be severe enough to increase mortality risk, they may decrease the energy that can be devoted to future reproductive effort (Walters, 1982). In

females, this may correspond to reduced production of mature ova. For female frogs, which have indeterminate growth and in which fecundity is highly correlated with body size (see Chapter 2), energetic demands of care could result in slower growth rates with consequences for fecundity throughout the rest of a female's lifetime. In males, an energy cost probably translates into a reduction in reproductive activity following the period of care while they make up the energy deficit; hence it increases the time until males can begin to advertise for mates and translates into a lower rate of success in fertilizing eggs. For males, the time spent caring may itself constitute a cost since it may preclude reproductive activity, leading to the same outcome.

In this section, I (1) consider what the costs of parental care are, (2) present estimates of the proximate magnitude of those costs, and (3) suggest how they might affect male reproductive success. A discussion of how benefits and costs interact to determine net fitness (reproductive success) is postponed until the next chapter.

I examined two types of costs of parental care for E. coqui males resulting from the prolonged period of nocturnal egg attendance: reduced feeding opportunities and reduced calling activity.

Methods

Estimating energetic costs.

Attendance of eggs in an enclosed nest site may reduce the opportunities for male parents to feed. The availability of food items in nest sites such as Cecropia leaves, rolled sierra palm fronds,

bamboo houses and other enclosed cavities is assumed to be lower than levels of food available in the open habitat, where most non-parental adults are found at night. Hence, remaining inside a nest site with a clutch may reduce nocturnal food intake of parental males relative to that of non-parental adults that are active in the habitat.

To measure the feeding levels of parental males, I sampled the stomachs of brooding males just after dawn on two mornings in July 1982. A syringe stomach pump (Legler and Sullivan, 1979) was used to flush the stomach contents of parental males taken from their nests on the mornings of 4 July (0515-0830 hours) and 14 July (0630-1030 hours). This technique is highly effective for recovering total stomach contents and, since gut passage time for E. coqui was estimated to be 14-18 hours at temperatures similar to those in this study, stomach contents are assumed to represent those items eaten during the previous night (L.L. Woolbright, personal communication). To be sure that all contents were regurgitated, each frog was pumped three times. Stomach contents were immediately preserved in 70% ethyl alcohol and later examined under a dissecting microscope (12X). Each item was identified to order and measured for average width and length to the nearest 0.01 mm. By treating items as cylinders, volume was calculated using width as the diameter.

No male was sampled on more than one day (four males that were resampled on July 14 were not included in the analyses), and males that were attending hatchlings were not used. Comparisons of the stomach contents of these brooding males are made with those of non-brooding adults which were sampled at about 0600 hours on five mornings in 1982 between July 4-19 by L.L. Woolbright (personal communication). The

non-brooding adult sample was taken by capturing and preserving frogs, later dissecting out their stomachs and analyzing the contents in the same manner.

Another way of estimating an energetic cost of parental care is to monitor weight changes of parental frogs over the course of the care period. During May-June 1980, I did this by weighing brooding frogs early and late in development. Males were removed from their nests, placed in a plastic bag and measured for SVL to the nearest 0.5 mm with a mm ruler. The male was then removed from the bag, gentle pressure applied to the groin to empty the bladder (bladder contents were usually voluntarily expelled while the frog was in the first bag), patted dry with a paper towel and placed into a second bag. The bag and frog were weighed to the nearest 0.05 g using a 10 g Pesola balance. The frog was then removed, the bag reweighed, and the frog's weight determined by subtraction. Both weighing procedures were performed until three consecutive weights were obtained that varied by less than 0.1 g and the average of those three was used.

In eight cases the same male was weighed early and late in development. Additional independent samples of 22 males early in development and 12 males late in development were also taken. All weights and measures were made between 1100 hours and 1700 hours (average times of measurement are given in results).

Estimating reproductive costs.

Calling activity was monitored from January through July 1980 in Dicks House Plot at biweekly intervals. At each survey, the entire plot was searched for calling males. For each male, its position in the

plot, the characteristics of the call site (type, height off the ground, orientation), and the male's identity (frogs were marked by toe-clips) were recorded. Because Dicks House Plot was surveyed biweekly (complete surveys) for frogs and nests, I also knew the location of every nest and the identity of each parental male on any given night. Thus, I knew which of the calling males on any night were parental males and which were not. I then estimated the percentages of parental males that were calling and of non-parental males that were calling as the ratios of calling males to total males in each category. The total number of non-parental males in a plot on any night was estimated from the mark-recapture records. Because surveying and marking in Dicks House Plot were begun in January, well before the dates used here for estimating these two ratios, I used the number of marked males present at any time as the total mature male complement. A male was counted as present in Dicks House Plot on any date between its initial capture (or after it attained an SVL = 29.0 mm) and its final capture. Males that were captured only once were not counted unless the capture date coincided with a particular survey date (and then only for that date).

To estimate how successful parental males were at obtaining new clutches versus non-parental males, I compared the proportion of non-parental males that obtained clutches during 1980 in Dicks House Plot with the proportion of parental males that obtained second clutches during that time.

Results

Energetic costs.

Five (33.3%) of the 15 brooding males had food in their stomachs on 4 July, while 15 (50%) of 30 males pumped on 14 July had food. The remaining males on both dates had empty stomachs. Only two of the 21 non-parental, calling males (9.5%) sampled during July had empty stomachs. The difference in frequency of empty stomachs between parental and non-parental calling males was highly significant (Table 12A). Of the 20 parental males that had food in their stomachs, the mean number of items per stomach was 6.0 ± 1.8 (Range=1-31, N=19; one male had a large item that could not be extracted and is not included in volume statistics). Non-parental calling males had 2.1 ± 0.4 items per stomach (Range=1-8, N=19). Average volume of food (Table 12B) was not significantly different between the two groups when only males with food in their stomachs were compared. However, when groups were compared using all males (empty and with food), they were significantly different (Table 12B). The large variance within groups in food volume is a problem in making very strong inference from the volume results.

In order to assess whether parental males change their foraging habits (and hence their food intake) as development of their clutches proceeds, I compared the frequency of empty stomachs among the three developmental intervals (Table 13). Although there was a trend towards a decreasing frequency of empty stomachs from early to late development, it was not significant.

I next consider the results from weighing parental males. Every one of the eight males that were weighed twice lost weight between the first and second measurements (Table 14). Mean weight loss was 0.36 ± 0.07 g over 10 to 13 day intervals. When weight loss was corrected for the number of days between measurements, the mean weight loss per day

Table 12. The stomach contents of brooding males and non-parental calling males sampled at dawn on **two** mornings and five mornings, respectively, during July 1982. A. The frequencies of stomachs that were either empty or contained food. B. Mean \pm SE (N) volume of stomach contents in the two groups, in mm^3 .

A.

	Empty stomachs	Stomachs with food
Brooding males	25	20
Calling males	2	19

$$\chi^2 = 12.55, p < 0.001.$$

B.

	Volume of stomach contents		U*	p
	Brooding males	Calling males		
All stomachs	135.4 \pm 56.9 (N = 19)	225.3 \pm 74.0 (N = 19)	174	.326
Only stomachs with food	58.5 \pm 29.5 (N = 44)	184.5 \pm 64.2 (N = 21)	243	<.001

*Mann-Whitney U-test.

Table 13. The frequencies of empty stomachs and stomachs with food of parental males at dawn as a function of the developmental interval of their clutches at the time of sampling. The proportion of row totals are in parentheses.

Developmental interval	Empty stomachs	Stomachs with food
I	6 (.67)	3 (.33)
II	9 (.56)	7 (.44)
III	10 (.50)	10 (.50)

$$\chi^2 = 0.70, \text{ NS.}$$

Table 14. Body weights of parental males measured early (before Stage 4) and late (after Stage 12) in the developmental periods of their clutches. Means \pm SE.

	Early development	Late development	Student's t
Same males measured early and late (N=8)			
Mean time of measurement	1440 h.	1550 h.	
SVL	33.1 \pm 0.57	-	
Mass (g)	2.86 \pm 0.12	2.49 \pm 0.11	5.86***
All males (includ- ing eight above)			
Mean time of measurement	1420 h.	1410 h.	
SVL	33.1 \pm 0.26	33.3 \pm 0.36	NS
Mass (g)	2.81 \pm 0.07	2.58 \pm 0.08	2.10*
N	30	20	

* $p < .05$.

*** $p < .001$.

NS - not significantly different.

was 0.031 ± 0.005 g per day. If I assume that per diem weight loss is constant throughout the parental care period, a total weight loss of 0.527 g (SD $\pm .255$) is obtained over a 17 day period (the average developmental period in May-June). This means that the average male would lose 18.2% ($0.53/2.86$) of his initial body weight by caring from oviposition to hatching. Average initial weights for these eight males were 0.086 ± 0.008 g/mm (2.86 ± 0.34 g per frog) with final average weights of 0.076 ± 0.006 g/mm (2.49 ± 0.30 g per frog).

Reproductive costs.

A potential cost to parental males in missed mating opportunities would exist if, (1) by providing care, males reduced the time spent calling or the effectiveness of calling (if nests are inferior call sites) and (2) gravid females are available. Nest sites and calling sites for E. coqui are almost mutually exclusive microhabitats (Chapter 2). Nest sites, as closed cavities that are near the ground, make very poor calling sites and are never used as such by parental males. Hence, by spending a lot of time in their nests, parental males might reduce the time they spent calling and pay a cost in reduced probability of attracting gravid females in the area.

To estimate and compare the frequencies of parental males that called versus those of non-parental males, I used seven of the complete calling surveys of Dicks House Plot made between March 1 and July 4, 1980 (Table 15). The average number of marked males present in Dicks House Plot on those dates was 81 (67 non-parental males and 14 parental males). The proportion of non-parental males that were calling (Table 15) ranged from 23.4% to 31.9% on individual nights, while 0-10% of

Table 15. The proportions of non-parental and parental males that were calling in Dicks House Plot on seven nights from March to June 1980. A. Records for individual nights. B. The frequencies of calling versus non-calling states for non-parental and parental males; cell totals represent summed frequencies for all seven nights.

A.

Calling survey dates	Non-parental males			Parental males		
	N	Number Calling	Percent Calling	N	Number Calling	Percent Calling
March 4	64	15	23.4	15	1	6.7
March 11	64	20	31.3	16	0	0.0
March 17	61	14	23.3	19	0	0.0
April 9	71	22	31.0	10	0	0.0
April 16	72	23	31.9	15	1	6.7
May 21	72	23	31.9	12	1	8.3
June 7	67	19	28.4	10	1	10.0
Column Means	67.3	19.4	28.8	13.9	0.6	4.3

B.

	Calling	Non-calling
Non-parental males	136	335
Parental males	4	93

$$\chi^2 = 26.53, p < 0.001.$$

parental males called on any of those nights. Combining the results for all seven nights, parental males called significantly less frequently than did non-parental males (Chi-square = 26.53, df = 1, $p < 0.001$).

During the 126 day period from 1 March to 4 July 1980, 114 clutches were deposited in Dicks House Plot on 69 days (1.65 clutches per deposition day)(Chapter 2). At least one clutch was laid in Dicks House Plot on 54.8% of the days during that period. There is no question therefore that gravid females were available on a regular basis during March-July 1980 and that parental males that did not call were missing potential opportunities to mate.

In order to obtain an estimate of a mating cost from parental care, I calculated a nightly probability of mating for calling males in Dicks House Plot on each night during April-June 1980 and June-July 1982 when a complete calling survey had been performed (Table 16). The nightly probability of mating was computed as the number of clutches that were deposited in Dicks House Plot on a particular day divided by the number of males that were calling on the previous night. Clutch deposition for 1980 is derived from Figure 4, I have similar information for the clutch deposition chronology in 1982. Calling male densities are obtained from Tables 1 and 2. The resultant nightly mating probabilities are presented in Table 16.

Nightly mating probabilities ranged from 0.0 to 0.235 females per calling male-night, with a median value of 0.050 ($N = 21$). I have included nights when no females mated (probability of 0.0) because by calling on those nights, some males will have expended energy that may preclude their calling on some other night when gravid females are available.

Table 16. The nightly probability of mating for males in Dicks House Plot calculated for 21 nights during April through June 1980 and June and July 1982. The Nightly Probability of Mating (NPM) was computed as the ratio of the number of females depositing clutches on a particular day (the second date given below) to the number of calling males on the previous night (the first date of the pairs below).

Dates	Number of calling males	Number of mating females	NPM
1980			
April 2-3	8	0	0.000
April 9-10	22	2	0.091
April 16-17	23	1	0.044
April 30-May 1	12	0	0.000
May 7-8	4	0	0.000
May 21-22	24	2	0.083
June 4-5	11	1	0.091
June 7-8	20	0	0.000
June 21-22	19	2	0.105
June 24-25	24	0	0.000
1982			
June 10-11	16	1	0.063
June 11-12	15	1	0.067
June 12-13	8	1	0.125
June 13-14	11	1	0.091
June 14-15	17	4	0.235
June 15-16	17	0	0.000
July 8-9	25	0	0.000
July 9-10	28	1	0.036
July 10-11	20	1	0.050
July 11-12	29	1	0.035
July 12-13	20	1	0.050
Median			0.050

During the period 1 January to 4 July 1980, 129 clutches were deposited in Dicks House Plot. Of those, only 2 (1.6%) were laid in the nest sites of males that were attending a clutch at the time. The other 127 (98.4%) clutches were laid with non-parental males.

Discussion

The results suggest that there are costs incurred as a result of parental care by male coquíes. Energetic costs appear to stem from a reduction in the number of nights that parental males spend foraging compared to calling non-parental males, since parental males had empty stomachs significantly more often than non-parental calling males, but there was no difference between the two groups in the stomach volume of those males with stomachs containing food. It seems that when parental males do forage, they are obtaining about as much food as non-parental calling males.

The reduced energy intake as a function of nest attendance in E. coqui may not result overall in a depressed energy budget for parental males relative to non-parental calling males. Because nest attendance is generally passive, consisting largely of brooding (Chapter 3), metabolic rate is probably little different from resting rates, and certainly lower than for a frog that is active or calling. Taigen et al. (1982) estimated that oxygen consumption rates for active frogs were about seven times higher than for resting frogs. Woolbright (personal communication) measured oxygen consumption rates of inactive males and inflated males in the field at night (when they were exposed to the chorus) and found they were significantly different, with inflated males having rates about 3.5 times higher than inactive males.

Either of these estimates would suggest that the passive nature of egg brooding requires much lower energy expenditure than that of activities such as calling.

The weight loss data suggest that brooding males are at an energetic disadvantage during the period of parental attendance. The eight parental males that were weighed twice lost an average of 18.2% (Range = 7.3% - 31.7%) of their initial body weight over a 17-day developmental period.

Evidence of energetic costs from parental care in amphibians has been found in several species. Tilley (1972) and Krzysik (1980) found that brooding females of Desmognathus ochrophaeus had significantly reduced food intake relative to non-brooding females in the population. Fitzpatrick (1973) reported that the brooding period in the same salamander resulted in a depletion of fat body and carcass lipid content in females. In contrast, Kaplan and Crump (1978) found that females of Ambystoma opacum brooding clutches in early developmental stages did not differ in dry weight, total calories, or weight-specific caloric content from females brooding clutches in late developmental stages and concluded that there was no energetic cost to parental brooding in this salamander. The only attempt to measure energetic costs of parental care in anurans is Simon's (1982, 1983) work with Cophyxalus parkeri, a New Guinea microhylid frog in which both sexes may care for separate clutches. Simon (1983) found that attending parents, of both sexes, had significantly less food and more plant material in their guts than non-brooding adults. Further, fat body weights of brooding males was negatively correlated with developmental stage. Because the developmental period of C. parkeri is 85-100 days,

4-5 times that of E. coqui, energetic costs are likely to be more important for the former.

It appears that the reduced calling and consequent loss of additional mating opportunities may be a more substantial cost of parental care in E. coqui. Parental males call significantly less frequently than non-parental males, and obtain significantly fewer clutches. The incidence of double clutches in Dicks House Plot is very small suggesting that parental males are sacrificing opportunities to mate. How many opportunities to remate does a male miss during a period of parental care?

One way of calculating such an estimate of mating opportunities missed would be to transform a nightly probability of mating into a probability of mating for an entire period of parental care. In the above Results, I derived an estimate of a nightly mating probability by dividing the number of receptive gravid females in an area (Dicks House Plot) by the number of males calling in the area for each night (Table 16). This is essentially a random-mating estimate, since I am assuming that any calling male has an equal chance of getting a mate if females are available. The median nightly probability of mating in Dicks House Plot for 21 nights during 1980 and 1982 was 0.050.

Another estimate is generated by taking the most successful males in Dicks House Plot during March-July 1980 and calculating an a posteriori estimate of their mating probability. Two males in Dicks House Plot each obtained 5 clutches during March-July 1980. Assuming that such a male called on 55% of the nights during that period (the average long-term calling rate from Woolbright and Townsend, Ms.) and that his calling schedule was independent of female availability

(females mated on 55% of the nights during that period, Figure 4), such a male would have called on 30.3% ($.55 \times .55$) of the nights when females mated in Dicks House Plot. This is equivalent to 38 nights (0.303×126 nights). To obtain 5 clutches by calling on 38 nights, the nightly probability of mating for such a male was $5/38 = 0.13$. This is a reasonable estimate of the maximum nightly mating probability for an individual male in Dicks House Plot.

I now have two estimates of male mating probability; a random mating estimate and a maximum mating estimate. If X is the nightly probability that a male can get a female, then $1/X$ will be an estimate of the number of days required for a male to get one female. Hence, on a random mating basis, a male should be able to mate once every 20 nights ($1/.050$) while a male with the maximum probability of mating could get a female every 7.7 nights ($1/.13$). These require that the male call every night of the period. Another way of envisioning a cost of parental care would be to calculate how many females a male would pass up by not calling during a period of parental care. Using a 20-day care period (the average developmental period for March-July), the male in a random mating population would miss one female $(.05)(20)$, while a maximum male would miss 2.6 females $(.13)(20)$. Thus, a male with a mating probability somewhere between that of a random male and a maximum male would sacrifice 1.0-2.6 potential additional clutches if he did not call while caring for a clutch. This is a substantial cost. The next chapter addresses the question of what sort of benefit level is necessary to offset such a cost and account for the high fidelity, complex care behavior observed in male coquíes.

CHAPTER 6

WHY SHOULD MALE COQUIES CARE? - MODEL AND SUMMARY

I have shown that parental care by male coquíes yields significant benefits in offspring survival but that there are costs from providing that care in terms of reduced future reproduction. In this chapter, I present a model which integrates the benefits and costs of parental care into a single statement about the relationship of parental behavior to fitness. I then use the model to analyze the net adaptive value of male parental care in E. coqui and to examine situations in which the model would make different predictions about the evolution of parental care in anurans.

A Graphical Model of Anuran Parental Care Evolution

The model is an adaptation of the marginal value model of Charnov (1976). The basic form of the model as it applies to parental investment and the evolution of mating systems was anticipated by Trivers (1972), and has been applied to assessment of male mating strategies in dung flies (Parker and Stuart, 1976; Parker, 1978). Maynard Smith (1977) and Grafen and Sibly (1978) have applied it to examinations of parental investment. As with the foraging applications for which it was first developed, I am interested in examining how an animal can maximize its rate of return when it can choose between two alternate types of behavior each of which can contribute to the return. Choices could be either of a proximate or evolutionary type. In the foraging model, it is the problem of how long to stay and forage in a patch, and the rate of return measured is energy per unit time. In my

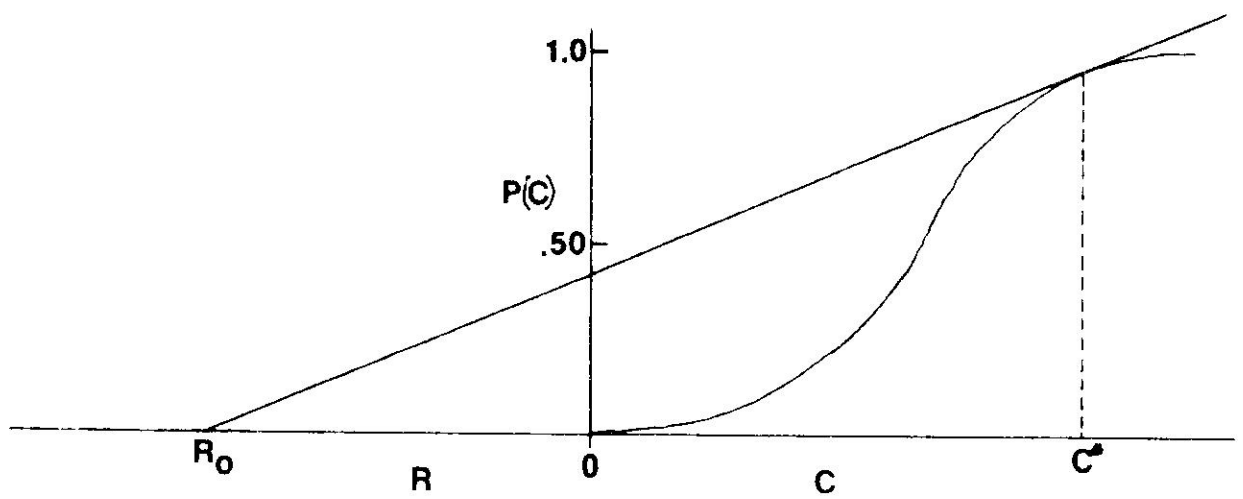
application (after Maynard Smith, 1977) the dichotomy is between continuing to provide care to a current clutch versus attempting to mate again. In males, an attempt to mate would involve engaging in the appropriate reproductive behavior to attract a female, such as calling. For females, the attempt to remate would involve foraging at an appropriate level to mature a new complement of eggs. The currency in this case is the rate of offspring production. The marginal value theorem indicates that when the rate of return from caring declines to a level that is equal to the mean rate of return from both activities, then the animal should switch to the alternate activity. The switching value is the marginal rate of return, below which an animal loses fitness as it delays switching.

The model is presented in Figure 12. It assumes a continuous breeding season, such that males and females breed more than once per season and, in particular, that females are relatively asynchronous in their oviposition schedules (Emlen and Oring, 1977; Maynard Smith, 1977). The ordinate represents the probability of hatching for a clutch $P(C)$, as a function of the amount of time that a parent provides care, C . There is some amount of time, R , that an animal must spend before it can mate again. For a male, this might be the number of nights he must call to get another female. For a female, it is the time required to produce another clutch. The remating time, R , is incorporated into the model by extending the abscissa beyond the origin to the left. If we know the form of $P(C)$, the care benefit function, and specify R then we can calculate the rate of offspring production for any given period of care, C^* , by computing the ratio $P(C^*)/(C^*+R)$. That rate of offspring production is equivalent to the slope of a line

Figure 12. A marginal value model of parental care evolution.

C is time spent caring for a clutch; $P(C)$ is the probability of hatching as a function of the amount of care, C ; R is the time before an animal can remate. The maximal rate of offspring production, when $R = R_0$, is predicted by the slope of the tangent line drawn in the figure. See text for details.

$$\text{Slope of tangent} = \frac{P(C^*)}{C^* + R_0}$$



drawn from R through the point $[C^*, P(C^*)]$. According to the marginal value theorem, the maximal rate of offspring production will be given by the tangent to $P(C)$, drawn from R, that has the highest slope. In Figure 12, that tangent has been drawn and yields a value of C, C^* , which is associated with the maximum rate of offspring production, and corresponds to the Stage at which the parent should abandon the current clutch and attempt to remate.

Application of the Model to Parental Care in E. coqui

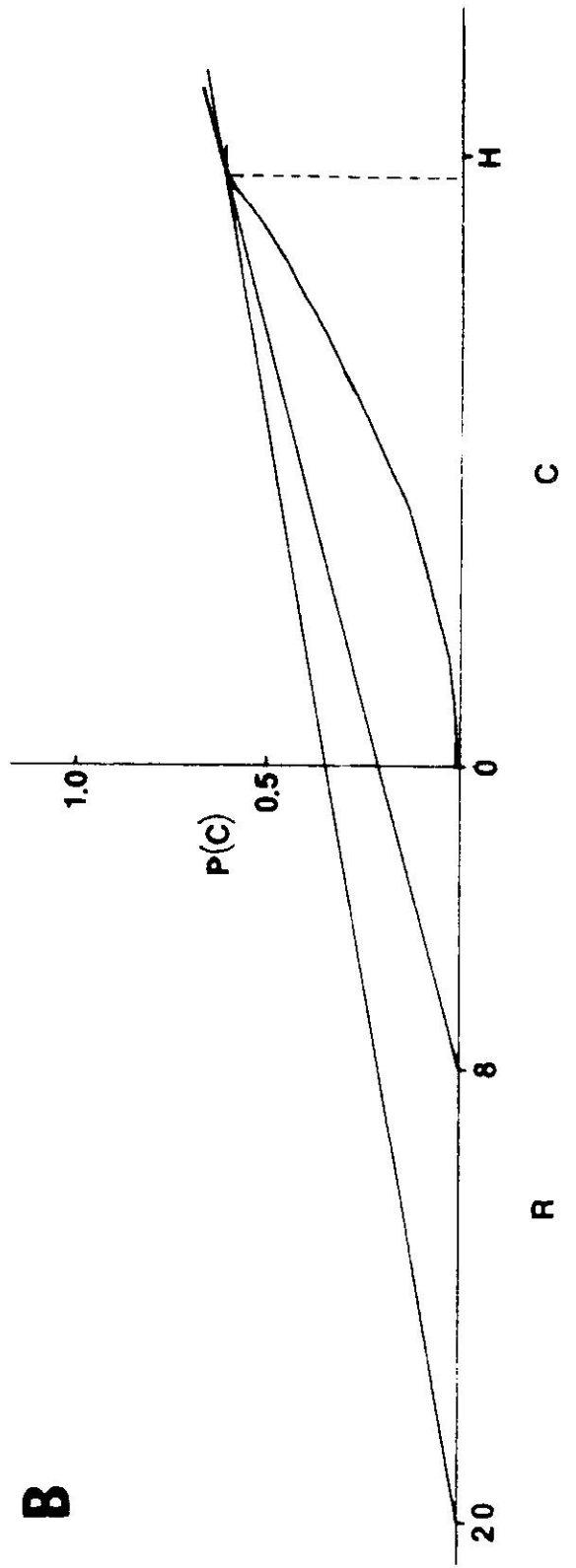
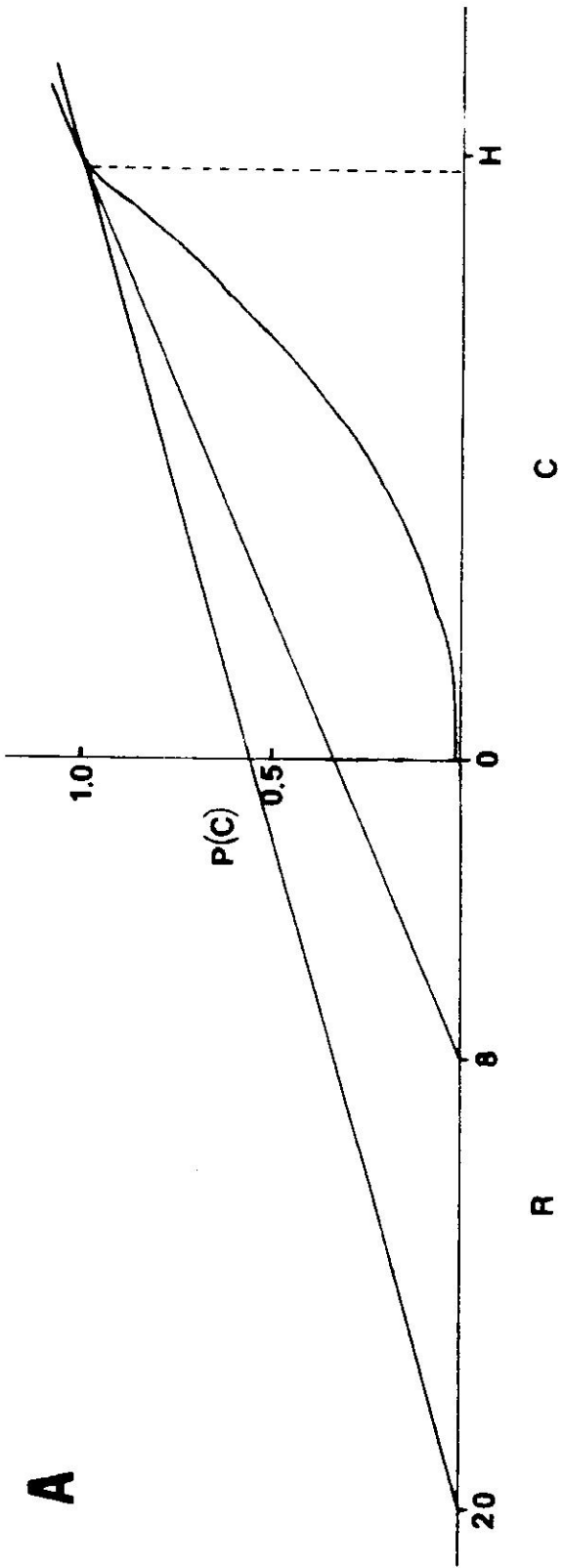
In order to evaluate the net adaptive value of parental care in E. coqui we need to simultaneously analyze those consequences that contribute to fitness and those that decrease fitness. Put another way, we need to express and analyze, using a common currency, the benefits and costs of parental care. The model presented above gives us the means to do so. Reproductive success is expressed as a rate that incorporates both benefits [the probability of hatching per clutch per unit of parental care time, $P(C)$] and costs (the number of clutches per unit of calling time) to yield an estimate of net reproductive success per unit of total time (care time plus calling time).

For E. coqui, $P(C)$ is the benefit function that was derived in Chapter 4 (Figure 10) using the results from the male-removal experiments. A remating time for males (R) was estimated using the nightly probabilities of mating estimates in Chapter 5 (Table 16). The median value of nightly mating probability in Dicks House Plot was 0.05 females per calling male-night. By taking the inverse of this value, I obtained 20 as the number of nights that a male would have to call to obtain one female. I also calculated a remating time for the most

successful male in the plot as 8 days.

We now have the two necessary parts of the model to integrate costs and benefits and examine the net adaptive value of male parental care in E. coqui. In Figure 13A I have plotted $P(C)$ and drawn the tangent lines for the two remating times, $R = 8$ and $R = 20$. For either value of R , the tangent to $P(C)$ with the highest slope yields a value of parental attendance, C^* , of Stage 15. Thus, the model indicates that, in order to maximize reproductive success, a male parent should provide care right up to hatching, then leave the clutch (or hatchlings) and begin calling to attract another female. This result is robust over a wide range of remating times because of the y-intercept and shape of the benefit curve. First, $P(C)$ goes through the origin, which means that without provision of some parental care no eggs will hatch. Males will never be favored to abandon eggs completely, since abandoning the clutch yields zero reproductive success, regardless of how fast they can get another clutch. Second, the benefit of providing care accelerates throughout pre-hatching development; i.e., the second derivative of $P(C)$ is positive. The benefit from parental care accelerates most rapidly toward the end of development, and then becomes asymptotic after Stage 15. Once parental care starts, parental males get progressively greater increments of hatching success for each additional unit of time spent providing care. As long as the costs of care are fairly constant over the period of parental care, males will realize the highest reproductive success by staying with their eggs through Stage 15. Because of the sharp asymptote, large changes in remating time cause negligible shifts in predictions of how long males should care. In Chapter 4, I showed that there was a distinction

Figure 13. Application of the model to male parental care in E. coqui. Tangent lines are drawn for two values of remating time, $R = 6$ days and $R = 20$ days. A. Using the benefit function $P(C)$, after Figure 10. B. Using the benefit function $S(C)$, after Figure 11.



between $P(C)$, a function describing hatching success from the stage of male-removal onwards (Figure 10), and $S(C)$, which describes expectation of hatching over the entire developmental period (Figure 11). In Figure 13B, $S(C)$ is used as the benefit function, and generates the same predictions about length of the parental care period, for the same reasons discussed for $P(C)$.

The results of the model are in close agreement with the observed parental behavior exhibited by E. coqui males (Chapter 3). Parental males showed high fidelity to their clutches throughout development, spending 97.4% of the time during the day and 75.8% of the time at night in their nests (Table 8, Figures 7-9). Nest fidelity did not change from early to late developmental intervals (Table 8). Males were in their nests at night as often during late development (76.0%) as during early development (76.6%). However, after hatching nest fidelity dropped significantly (Table 8), such that males changed from being in the nest 76.0% of the time at night during developmental interval III to 44.9% of the time at night during interval IV (post-hatching).

The application of the model to parental care in E. coqui is somewhat trivial. Knowing that hatching success is zero in the absence of parental care, it is obvious that males should never be selected to abandon eggs altogether. On the other hand, only by knowing the shape of the benefit curve is it evident why males should continue to care throughout pre-hatching development. In the next section of the chapter, I consider some less trivial cases in which the model may yield predictions that are more useful in hypothesizing about the evolution or maintenance of parental care in anurans.

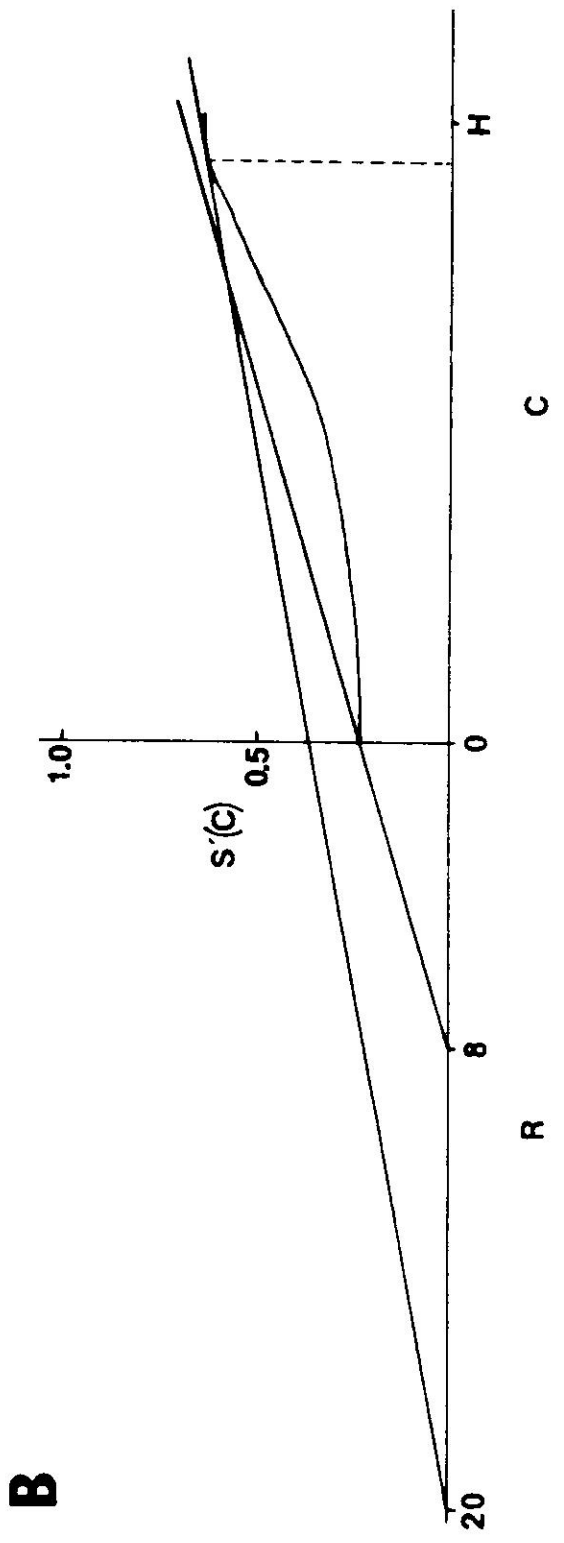
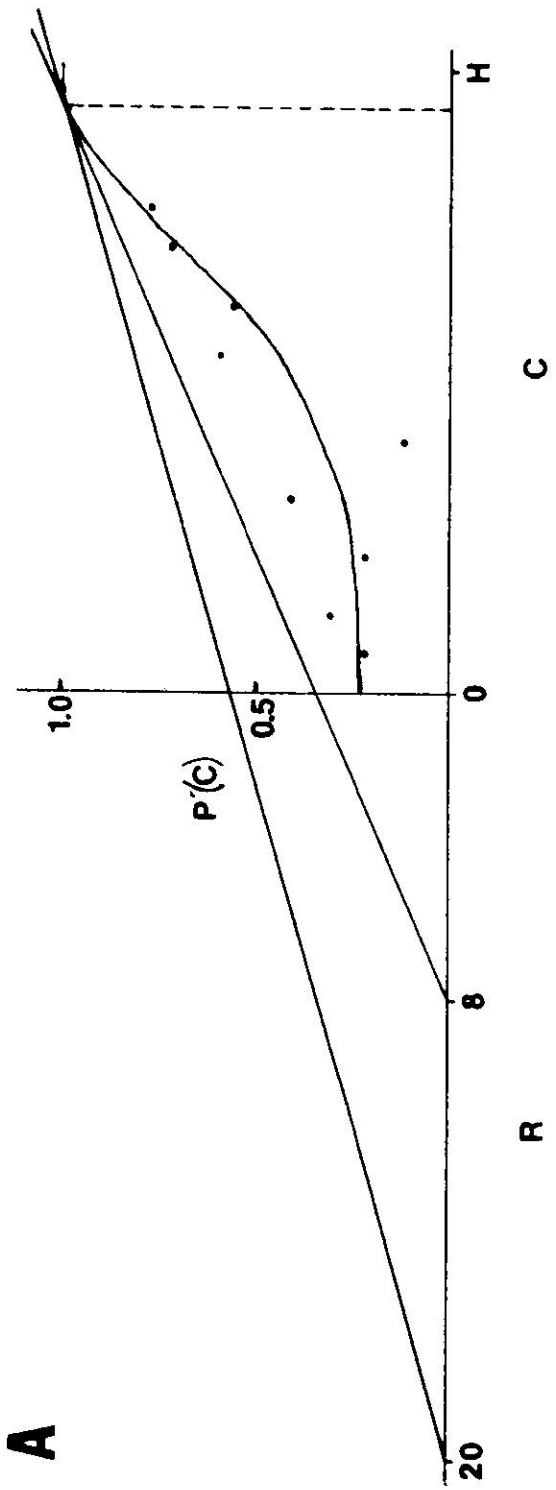
More General Applications of the Model to Anuran Parental Care

In the remainder of the chapter, I will consider first a more realistic evaluation of temporal patterns of parental care in E. coqui, as a means of examining how short-term variations in parental attendance can be incorporated into the model and how they affect the consequent predictions. Also, I will expand the view from parental care in a single species to a more general consideration of the evolution (or maintenance) of parental care in anuran amphibians.

In the initial exploration of the model's application to parental care in E. coqui, I used one benefit curve, $P(C)$, that was based on hatching success after male removal and a second curve, $S(C)$, that combined post-removal hatching success with pre-removal survivorship. For both curves I am plotting the results of male removals and, in effect, simulating the consequences of a male completely abandoning its clutch at some developmental stage. In fact, it seems unreasonable to expect that males would shift from a high level of parental care to complete abandonment of their eggs. It seems more reasonable to expect that males might decrease their level of care by some intermediate amount. Because nest sites are often the same as their normal retreat sites (both prior to and after parental duties), it is more realistic to presume that parental males would, at a minimum, spend the daylight hours in the nest site with their eggs. In that case, egg brooding during the day could contribute to maintenance of an appropriate hydric state and contribute to reproductive success, albeit at a potentially lower level. The experimental removal of males probably misrepresents the real situation of how males might abandon their eggs. Since I did

not do any field experiments in which I removed males and then provided the equivalent of some parental care (rather than none) after removal, I will adopt the following scenario. Suppose that a male cares continuously for his eggs (both day and night) up to Stage X, then adopts a behavioral pattern of attendance during the day and absence at night. Using the data from the male removal experiments I will assume that, if a parental male were to adopt this strategy, the rate of clutch failure from desiccation would be one-half of that observed in the actual experimental removals, for any developmental stage. The incidence of cannibalism and predation would not be altered, since they occur, virtually without exception, at night. The results of this analysis are presented in Figure 14, with Figure 14A showing the new benefit function $P'(C)$, and Figure 14B illustrating the new $S'(C)$ function. Note that the primary effect of incorporating these new results is to raise the y-intercept of the curves by a considerable amount. In the case of $P'(C)$, the change in the intercept is not sufficient to change the prediction for either level of remating time. Alternatively, in the case of $S'(C)$ the tangent with the highest slope for a male with a remating time of 8 days intersects at the ordinate; such a male would have a higher lifetime reproductive success if he adopted a strategy of daytime egg brooding only, abandoning the eggs and calling at night. A male with $R = 20$ should still be favored to give complete care through pre-hatching development. In fact, one can calculate the point of trade-off between the two endpoint strategies (where lifetime reproductive success is equivalent) for either $P'(C)$ or $S'(C)$. To reiterate, the two strategies are (1) give care continuously both day and night from oviposition to hatching, and (2) stay with the

Figure 14. Application of the model to E. cogui using new benefit functions which incorporate partial hatching when there is no parental care ($C = 0$). A. Using the new benefit function $P'(C)$. B. Using the new benefit function $S'(C)$. See the text for more details.



eggs during the day but leave at night (i.e., maintain a normal diel activity pattern but brood the eggs when in the nest site). The trade-off point is represented by the x-intercept of the straight line that passes through the y-intercept and is maximally tangent to the function. For $P'(C)$ this occurs at $R = 4.5$ days, for $S'(C)$, at $R = 9$ days.

In the above analysis, it appears that a male with the maximum nightly probability of mating (yielding an $R = 8$ days) should not provide care at night if, by adopting such a strategy, his clutch suffers only half the level of desiccation of a clutch that received complete parental care. There are two reasons why such a parental strategy is unlikely in this population of E. coqui. First, a remating time of 8 days probably represents an unattainably low remating time for any male in the population. Second, even with a strategy of care only during the day, the rate of desiccation of eggs would be sufficiently great during early development that few clutches would survive if parental males did not care continuously for at least the first few days of development. Hence, my assumption that only half of the clutches that perished from desiccation in the male removal experiments would have done so under a daytime only brooding schedule is probably unrealistically low for the first few days of development. Consequently, the y-intercept for either $P'(C)$ or $S'(C)$ would be lower than depicted in Figure 14. Taigen et al. (1984) showed that coquí eggs lost water rapidly, regardless of their developmental stage. However, eggs that had had a longer period of parental brooding gain in water content as a result, and would be expected to better withstand desiccation from a change in parental behavior than would younger eggs

(Taigen et al., 1984).

The crucial point of the above exposition is that if survival of eggs without parental care is high enough there is the possibility of variability in remating time resulting in markedly different predictions about optimal care strategies. It is important to reiterate the point that the only reason for the existence of a sharp trade-off point between no parental care and complete parental care is the shape of the benefit function. As long as the function is convex (bowed toward the abscissa), only the two endpoint strategies can yield maximal reproductive success.

What are the implications of incorporating this new benefit curve, in which there is some level of hatching success without any parental care, into the model? The principal result is that remating time becomes a critical factor in making predictions about parental strategies. If there is individual variation in the remating times in the population, there could arise the case in which males that had relatively great success in attracting females (e.g., were larger, had better territories, etc.) might be expected not to care for eggs, since their remating times would be short enough to yield a maximum tangent to the benefit curve with an intersection at the ordinate (such as the male with $R = 8$ days in Figure 14B). Males that were less successful (i.e., had longer remating times) would have a maximum tangent to the benefit curve that intersected it near the asymptote (such as the male with $R = 20$ in Figure 14B). Such males would be predicted to exhibit complete care. If individual variation in remating times was sufficiently great so that some males fell on either side of the trade-off point, we might predict a behavioral polymorphism in the

population with respect to parental care. Some individuals would exhibit complete parental care while others would provide no care. There is no intermediate strategy which would be optimal in terms of lifetime reproductive success.

A similar argument can be made for the evolution of facultative parental care (Wells, 1981). If adverse environmental conditions which caused total clutch failure in the absence of parental care during one part of the year (such as Figure 13B) were less harsh during another season, survivorship of abandoned clutches might be increased (as in Figure 14B). Under such seasonal variation, it would be advantageous for males to care for clutches during one part of the year (e.g., $R = 8$ days in Figure 13B) but to abandon clutches during times of more favorable conditions (e.g., $R = 8$ days in Figure 14B).

Up to this point, I have considered only the question, "If the male is the care-giver, how should he provide care in order to maximize his lifetime reproductive success?". A fundamentally more interesting question is, "When parental care evolves, which sex should provide it?" In the next section, I will consider how the model described herein may be useful in making predictions about the evolution of sex-specific parental strategies in anurans.

In the above discussion, I made the point that a benefit curves, such as $P'(C)$ or $S'(C)$, which intersected the ordinate sufficiently above the origin could generate circumstances wherein individual variation in the remating times of males could lead to markedly different predictions about the presence or absence of parental care. Consider the following situation. Imagine a species like E. coqui, but without parental care. Given a mutation which causes its bearer to

begin attending eggs, which sex (if either) should evolve parental care? I will suppose, as is likely for anurans, that one parent is better than none in improving hatching success, but two parents are little better than one (Maynard Smith, 1977). Hence, if one sex evolves parental care, the other sex will not, since it can contribute little more to hatching success. I also assume that there is no difference in the quality of parental care that either sex could provide. This is reasonable for egg attendance in anurans. There exists a benefit curve of the form $P'(C)$ in Figure 14A, and we can estimate the relative remating times of males and females. Remating time for females would correspond to the time it took to produce a new clutch.

There are two possible alternative situations. In the first (similar to Figure 14B), the two sexes have different remating times. If the y-intercept of $P'(C)$ is sufficiently high (relative to its asymptote) and the remating time of one sex is sufficiently short, we would predict that the sex with the longer remating time should evolve care, the other sex should not. In *E. coqui*, the male has the shorter remating time. A male in a randomly mated population had a remating time of about 20 days. Females, on the other hand, require 8 to 10 weeks to produce a new clutch in the field, a remating time that is about three times longer than males. If the intercept of $P'(C)$ or $S'(C)$ was higher (or developmental time was longer such that the slope of the tangent line through the intercept was lower), the trade-off point might fall between R for females and R for males, in which case we would predict that females should care, but males should not. Again, the issue here is not to focus specifically on *E. coqui* but to investigate a general prediction of the model. If the benefit function

from providing parental care intersects the ordinate at a sufficiently high value (as in Figure 14B) relative to the height of the asymptote, and remating times differ between the sexes, there exists the possibility that selection would act against the evolution of parental care by the sex having the shorter remating time and favor its evolution in the sex having the longer remating time.

In the alternative situation (similar to Figure 14A), it is irrelevant whether or not the sexes differ in their remating times since both have remating times that are greater than the trade-off point on the abscissa. The maximum tangent lines drawn from R of either sex will intersect the benefit function at the asymptote. In this case, either sex would have higher fitness by evolving parental care. Which sex does so would depend on initial conditions in the population, a point that Maynard Smith (1977) made in examining the applications of game theory to parental care evolution. When one parent is much better than none, and two are little better than one, there are two possible ESS's, one in which the female deserts, one in which the male deserts. "Given that two ESS's often exist, it is hard to predict which will actually evolve, since this depends on initial conditions" (p.4).

The situation in E. coqui is akin to that of Figure 14A rather than Figure 14B, as can be seen by imagining the tangent line for females with $R = 60-70$ days on Figure 14A or 14B and comparing it to the tangent line of the random males ($R = 20$ days). Clearly, if these remating times for male and female coquíes are representative, then from an initial condition of no parental care, either sex would be favored to evolve it. Why males, rather than females, became the care-giving sex is a question that we can address only by a consideration of what

factors, other than fitness benefits and costs, may have predisposed one sex to care over the other. We need to examine the possible initial conditions (Maynard Smith, 1977) which could bias the possibility of one sex versus the other providing parental care.

Wells (1981) provided a thorough review of the initial conditions which may influence the evolution of sex-specific parental strategies in anurans. The following analysis owes much to his paper. It has long been realized that there is a strong qualitative association between mode of fertilization and sex-specific parental care patterns. Species with internal fertilization more often have at least female parental care. Many internal fertilizers, such as birds, have biparental care but few have exclusively male parental care. Species with external fertilization more often have male parental care, although there appear to be a fair number of species with external fertilization (presumed in the case of many frogs) and female parental care (Gross and Shine, 1981).

Several hypotheses for this association have been suggested. The first and perhaps most compelling is that in species with internal fertilization, males should be less certain of their genetic relatedness to their brood (females should never be uncertain about genetic relatedness) and hence less apt to invest heavily in post-fertilization care if they are raising someone else's offspring. By this argument, male parental care should be rare in species with internal fertilization. Since, with external fertilization, both parents on average should have a high certainty of relatedness to offspring, this argument does not predict which sex should be favored to provide care under the latter mode of fertilization. However, in a

recent review of parental care patterns in fishes and amphibians (the two groups in which both external and internal fertilization are found) Gross and Shine (1981) tested predictions from a paternal certainty model against the parental patterns of the two groups and found no correspondence. Also, it has been pointed out by Maynard Smith (1978), and demonstrated by Werren et al. (1980) that when paternity is uncertain in a population (i.e., cuckoldry occurs), then all males will suffer potential for cuckoldry, and relative reproductive success will depend only on hatching success of the clutch. Only under special conditions will level of paternity play an important role in whether males evolve care. Put another way, low paternity may be a cost of mating in a population with internal fertilization, but it will not represent any special cost in the provision of parental care by males.

A second hypothesis that has been advanced to explain the association between external fertilization and male parental care is that males must be present when eggs are laid, and thereby have the opportunity to evolve care, if it is advantageous to do so. In internal fertilizers, of course, females either lay eggs after the male has left or gestate the young and consequently males have a low probability of being present to provide care during development or after hatching. As Wells (1981) pointed out, this hypothesis may be sufficient to explain how male parental care is maintained once it arises, but not to predict which path evolution would take from a starting point of no parental care.

Another hypothesis to explain the evolution of male parental care postulates the occurrence of a special preadaptive association between a male and his brood. In particular, if males defend territories which

include oviposition sites, then by having clutches laid within their territories, males may have the opportunity to associate with eggs, and increase hatching success, while continuing to attract new females (Williams, 1975; Ridley, 1978; Perrone and Zaret, 1979; Wells, 1981; Gross and Shine, 1981). This is an attractive and parsimonious argument for the evolution of male parental care, and seems to agree with the few data which are available for anurans (Wells, 1981).

Eleutherodactylus coqui is a good species with which to examine some of these hypotheses. In the first place it has internal fertilization. On the basis of the paternity hypothesis, we should not expect male parental care. Even if the initial conditions under which parental care evolved in this species were such that the level of paternity was an important factor in whether males or females were selected to care (Werren et al., 1980), and low certainty of paternity selected against male parental care, there does not appear to be a potential for cuckoldry. There are three lines of evidence against cuckoldry in E. coqui. First, my observations of courtship, amplexus, and oviposition suggest that male insemination and female ovulation occur very close together in time, such that there is only a small difference in the time at which males and females commit themselves to a mating. Presumably, if males committed themselves very early in amplexus, there might be time for a female to leave the nest and find another male with which to mate, thereby cuckolding the second mate. Second, the secondary amplexic position is assumed very close to dawn, and is presumably followed by insemination, such that a female leaving at that point would have to wait at least another day to mate. Third, females seem to have no control over whether or not to lay eggs once

they have ovulated. In situations where I separated amplexed pairs, handled females and placed them in plastic bags in the laboratory (conditions under which females might be expected to reverse the decision to oviposit, and retain their ovulated eggs if they could), all females laid their eggs within the time frame of a normal mating. This suggests that, once ovulated, the process is irreversible and non-interruptable. It may be that an irreversible commitment to lay eggs is made even earlier, before ovulation occurs, via a physiological sequence that is triggered by some other amplexic event, such as the secondary amplexic position. In summary, even if low paternal certainty could be a factor in E. coqui, there does not seem to be a potential for cuckoldry in the species and hence low paternal certainty has not been an obstacle to the evolution of male parental care in the species.

The hypothesis that males are less likely to be present when the eggs are laid with internal fertilization does not hold for E. coqui, since males are present throughout oviposition, and both the male and female remain in the nest following oviposition for the remainder of the daylight hours.

The territoriality hypothesis appears to be a strong candidate for the evolution of male parental care in E. coqui, as was suggested by Drewry (1970b) and Wells (1981). Nest sites are usually within 2 m of the usual calling site of parental males, and are certainly contained within the male's usual acoustically defended area. Females travel to males to mate and are lead by the male to a nest site that is usually close to his call site. Often, a nest site has been a former retreat site of the male, not of a female. Females have been found to travel up

to 20 m from their normal diurnal retreat sites to mate. Both males and females will defend diurnal retreat sites, so male defense of a oviposition site itself is likely to have been the case. The required predisposition of males to evolve care is certainly present. Once a male obtained a clutch in his usual diurnal retreat site, any tendency to maintain contact with it might increase its probability of hatching. Strictly diurnal attendance would probably increase a male's reproductive success, and involve relatively little cost in reduced future matings, as suggested by Figure 14. However, given the relationship between benefits and costs from more prolonged care, again as in Figure 14, we should expect any initial association to evolve to the situation of prolonged, high fidelity care that we find in E. coqui today. One interesting bit of behavioral evidence for the territorial association of a male with a clutch is that, when dusk comes on the day of oviposition, the parental male begins to give aggressive calls, of the same type as those in nest and retreat defense encounters. These are undoubtedly directed at the female that is still in the nest site, and result in her imminent departure from the nest. In three cases, I have even seen the male deliver several rapid bites at the female's posterior as she left.

In this thesis, I have addressed the issue of parental care in a neotropical frog from an evolutionary point of view, considering how costs and benefits to lifetime reproductive success contribute to an animal's fitness under different patterns of parental care. An equally interesting area of investigation is that of the physiological control of parental behavior. Some of the observations made in this study suggest several intriguing questions about how parental behavior may be

turned on or off and what mediates the transition from high levels of calling and call site defense to high levels of nest site attendance and nest site defense at night. Perhaps the proximate control of parental behavior is by changes in the levels of reproductive hormones. The development and maintenance of the brooding pouch in some marsupial hylid frogs is mediated by progesterone (del Pino, 1983). There are very few data on the hormonal control of reproductive behavior in amphibians (but see Moore, 1983). A partial explanation for the dichotomy of sex-specific parental strategies in anurans that has perplexed Maynard Smith (1977) and others could lie in sex-specific hormonal attributes that have served as preadaptations for parental care. We need detailed studies of parental care (and its absence) in many more anurans before we can understand whether the evolution of parental care has a single explanation or results from some combination of demographic, behavioral and physiological factors.

Summary

A marginal value model of parental care is presented that incorporates the time dependent benefits of attending eggs and the consequent costs of reduced calling activity, and relates the two parameters via a common currency of rate of offspring production. The model indicates that male coquíes maximize their lifetime reproductive success by providing high fidelity care from oviposition to hatching. Additional analysis of the model indicates that three critical parameters in analyzing the evolution of parental care strategies in anurans are the shape of the benefit function (hatching success as a function of the care provided), the level of hatching success realized

when no parental care is provided, and the relative lengths of time before individuals of either sex can mate again. Under some circumstances (with certain values of these three parameters), the model makes specific predictions of which sex should evolve care. Under other circumstances, either sex would be favored to evolve care from an initial condition of no parental care, and initial conditions such as mode of fertilization and territoriality must be examined to account for the evolution of sex-specific parental strategies. In E. coqui, the model indicates that either sex would have been favored to evolve parental care initially, since either sex would have realized a significant increase in reproductive success over no parental care by doing so. It appears likely that males evolved care in E. coqui because of their initial association with eggs stemming from territorial defense of oviposition sites. Regardless of whether the model can predict which sex will evolve care, it predicts the extent to which parental care should be provided. It also may serve to detect situations in which we should expect to see facultative parental care.

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APPENDIX I

DIRECT DEVELOPMENT IN ELEUTHERODACTYLUS COQUI: A STAGING TABLE
AND THE EFFECTS OF TEMPERATURE

MATERIAL AND METHODS

Descriptions of morphological features are based on laboratory observation of living and preserved material. During July and August 1979, eggs were collected in the field at various stages of development and observed in the laboratory through hatching. Eggs were examined twice daily by eye and under a stereo microscope at 7-30X. Laboratory temperatures ranged from 21 to 24.5 C. Additional observations were made on material collected and preserved in 10% buffered formalin during April-July 1980 and June-July 1982. Preserved embryos were measured using an ocular micrometer on a Bausch and Lomb stereo microscope at 10X (to the nearest 0.05 mm) or 25X (to the nearest 0.01 mm). Drawings were made using a Wild stereo microscope with a camera lucida attached. Egg membranes were removed for measurements and drawings, except for early stages of development (through neurulation).

During 1980 and 1982, the staging table presented here was used in the field to record development in over 500 clutches of E. coqui. In many clutches the diameter of 5-10 eggs was periodically measured to the nearest 0.5 mm with a plastic mm ruler as development proceeded. Also, records of daily minimum and maximum temperatures were available at the El Verde Station.

RESULTS

Staging Table for Eleutherodactylus coqui

Pre-hatching development was arbitrarily divided into fifteen stages, based on readily discernible changes in major aspects of external morphology (e.g., eyes, limbs, gills, etc.). Each stage represents at least one day of development during the warm wet season of April-July. The major features of each stage are summarized in Table 17, with more detail provided in the following section.

Eyes.- The large eyes of E. coqui embryos are distinctive throughout development. Their position is evident as large anterior bulges in the cephalic region by Stage 4 (Figures 15 A-B). The iris first shows pigment in Stage 6 (Figures 15 G-H) and darkens progressively until black in Stage 10 (Figures 16 E-F). The pupil remains clear throughout this period. During Stage 10 the pupil begins to darken and the iris to lighten. By Stage 13, the pupil is dark and the iris attains the adult color characteristics with the upper half golden and the lower half bronze-brown.

Gills and gill circulation.- The embryos of E. coqui bear a single pair of small gills for less than one third of the developmental period. The gills first appear as buds from the gill arches during Stage 5 (Figures 15 C-D), elaborate into short stalks bearing 2-4 terminal convolutions by Stage 7 (Figures 15 I-J, 16 B), and then regress rapidly, disappearing from external view by Stage 9 (Figures 15 M, 16 D). Pale yellow to amber blood can be seen circulating during Stage 5. At full development in Stages 6-7, the blood is orange. As the gills develop, progressively higher densities of red blood cells are observed moving through them, accounting for the change in hue.

Table 17. A SYNOPSIS OF THE MAJOR DIAGNOSTIC FEATURES USED TO STAGE THE EGGS OF E. COCUL.

See the text for definitions of special terms.

Stage	Color	Diagnostic Features
1	egg white-cream	<ul style="list-style-type: none"> - stage begins at oviposition, lasts until formation of blastopore - egg featureless, reticulate pattern of cleavage furrows may be visible
2	egg white-cream	<ul style="list-style-type: none"> - from blastopore through first evidence of neural folds outlining the neural plate
3	egg white-cream	<ul style="list-style-type: none"> - neural groove forms and closes to form neural tube - no limb buds - rapid rotation of the embryo
4	egg white-cream	<ul style="list-style-type: none"> - neural groove closed - limb buds first evident as round swellings lateral to and separated by a groove from the trunk - eye bulges distinct but unpigmented - gill arches apparent as lateral bulges caudal to the eyes, antero-medial to the forelimb buds; no gills evident - rotation moderate

Table 17 (continued).

Stage	Color	Diagnostic Features
5	egg white-cream	<ul style="list-style-type: none"> - limb buds elongate, "attach" to trunk; forelimbs round to ovoid, hindlimbs round to ovoid-elongate - eyes prominent but unpigmented - gill buds first appear from gill arches, one gill on each side as a short stalk with 1-2 distal convolutions by end of stage, pale yellow to amber blood circulates through gills
6	embryo light pink; yolk white-cream	<ul style="list-style-type: none"> - tail bud (first apparent microscopically in stage 4) elongates enough to bend at membrane; small thin fin develops - rotation slow to absent; may be weak lateral flexing at mid-body - limbs elongate; elbow and knee joints evident as slight constrictions - iris with light gray pigment; eye becomes distinct from rest of head; pupil clear and distinct from iris - gills are stalks with several convolutions; amber-orange blood circulates - tail over one-half of its final length; small translucent fin - endolymphatic calcium deposits (ECD) first evident as symmetric white points - widely scattered melanophores on trunk - no rotation; much mid-body flexion; weak tail thrashing

Table 17 (continued).

Stage	Color	Diagnostic Features
7	<ul style="list-style-type: none"> embryo light pink with pale gray overtones; yolk white-cream 	<ul style="list-style-type: none"> - limbs with obvious elbow and knee joints; foot paddles first evident - iris gray; pupil clear - gills fully developed; single stalks bearing several distal convolutions; orange blood circulates - tail two-thirds of its final length, with well-vascularized fin - ECD small, somewhat quadrangular patches from dorsal view - small disc (future pigmented body wall) first appears flanking trunk;
8	<ul style="list-style-type: none"> embryo pink with gray overtones; exposed yolk white-cream 	<ul style="list-style-type: none"> anterior edge covers base of forelimbs; area and border of disc marked by scattered melanophores - tail thrashing moves embryo - limbs elongate; nubs of digits first evident on feet - iris slate gray; pupil clear - gills regressing in size; still have orange blood - tail about two-thirds full length; fin almost full size; blood vessels obvious - ECD quadrangular patches; beginnings of forward extensions from antero-lateral corners of ECD towards eyes - disc expanded, border even with line from tips of front to hind limbs

Table 17 (continued).

Stage	Color	Diagnostic Features
9	embryo mostly pink, trunk gray; exposed yolk white-cream	<ul style="list-style-type: none"> - limbs elongate; digits discernible on all feet - iris dark gray; pupil clear - gills gone (at least from external view) - tail two-thirds to full length with full size fin - ECD are triangular patches with forward extensions partially or completely to rear medial area of eyes - disc expanded laterally, encloses one-third to one-half of yolk - moderate concentration of melanophores on trunk and disc; fewer on head; none on distal halves of limbs or on tail - tail thrashing moves embryo
10	embryo light gray with pink undertones; exposed yolk white-cream	<ul style="list-style-type: none"> - toes to one-third of their eventual length at hatching - iris dark gray to black; pupil clear and distinct but darkens late in stage - tail full length with full fin - ECD with full forward extensions to eyes; posterior areas expand medially - disc encloses one-half to two-thirds of yolk - pigment heavy on trunk and disc; less so on head - strong tail thrashing; legs twitch and flex for first time

Table 17 (continued).

Stage	Color	Diagnostic Features
11	embryo gray; exposed yolk white-cream	<ul style="list-style-type: none"> - toes to one-half their length at hatching; heels held together - iris black; pupil darkening but still distinct - tail full length with full fin - posterior portions of ECD expand medially and longitudinally; two sides separated by thin gap at midline of embryo - disc encloses two-thirds to all-but-a-sliver of the yolk - tail thrashing strong, legs flexing
12	embryo gray- brown	<ul style="list-style-type: none"> - toes two-thirds of length at hatching; heels usually together - iris and pupil about equally dark early in stage; later, iris lightens - tail full - ECD joined at midline of embryo; reach fullest development; begin to be masked by increasingly heavy pigmentation on head - disc encloses all-but-a-sliver to all of yolk - movement mostly with tail; leg movements strong

Table 17 (continued).

Stage	Color	Diagnostic Features
13	embryo gray- brown	<ul style="list-style-type: none"> - toes full length, toepads first evident - iris lighter, approaching adult coloration; eyelid first evident - tail regressing in size - ECD more vague, increasingly masked by pigmentation on head - yolk completely enclosed in body wall; yolk reserve still large - pigmentation heavy over whole body; heaviest on dorsum and sides, lighter ventrally, approximating adult color - movement with legs, tail almost useless
14	embryo gray- brown; banding on hind legs	<ul style="list-style-type: none"> - toes full length with toepads - iris golden above, bronze-brown below (adult coloration); pupil dark; eyelid distinct - tail regressing, useless - ECD masked by heavy pigment - yolk reserve still large and prominent - pigmentation almost full; banding patterns on legs evident - all movement with legs

Table 17 (continued).

Stage	Color	Diagnostic Features
15	embryos fully pigmented	<ul style="list-style-type: none"> - ECD no longer visible - dorsal morph pattern obvious - much movement by embryo; may poke at egg capsule with snout - hatching occurs at any time during stage 15
At hatching		<ul style="list-style-type: none"> - yolk reserve still prominent at hatching (varies with hatching time); requires up to five days to disappear - floppy tail remnant one-half or less of full length

Figure 15. Camera lucida drawings of E. coqui embryos. A) Stage 4, dorsal view. B) Stage 4, caudal view. C) early Stage 5, dorsal view. D) early Stage 5, lateral view. E) late Stage 5, dorsal view. F) late Stage 5, lateral view. G) Stage 6, dorsal view. H) Stage 6, lateral view. I) Stage 7, dorsal view. J) Stage 7, lateral view. K) Stage 8, dorsal view. L) Stage 8, lateral view. M) Stage 9, lateral view. N) Stage 11, lateral view. O) Stage 12, dorsal view. P) Stage 12, ventrolateral view. ecd = endolymphatic calcium deposit, flb = front limb bud, g = gill, gb = gill bud, hlb = hind limb bud. Scale = 1.0 mm.

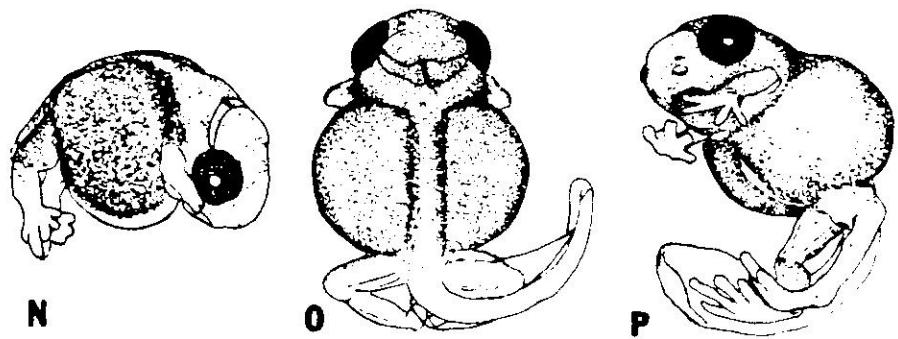
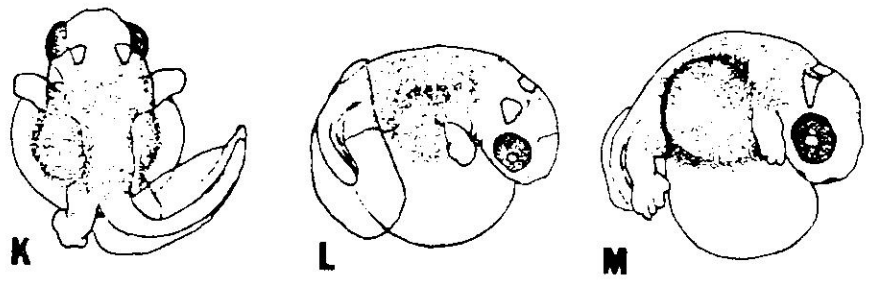
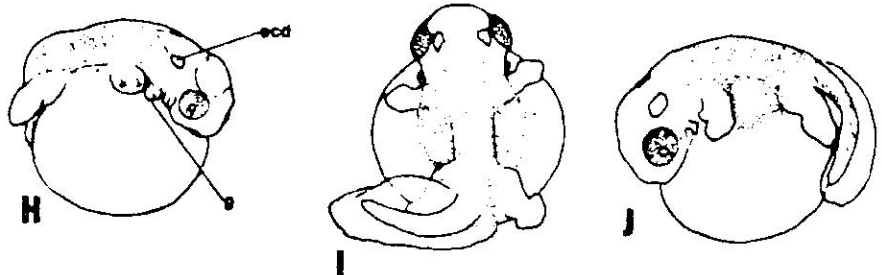
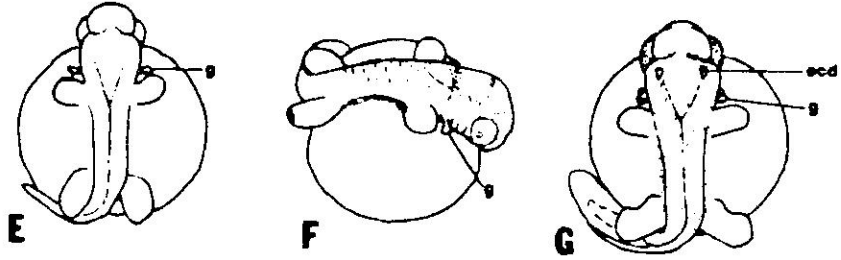
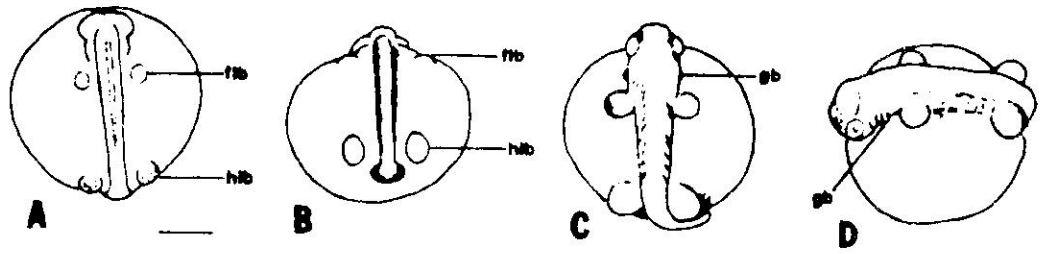
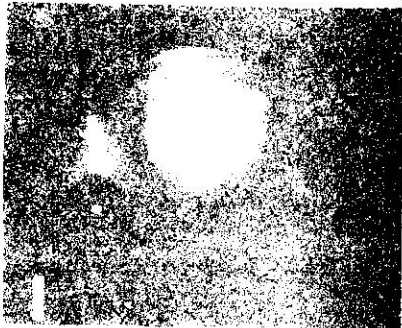
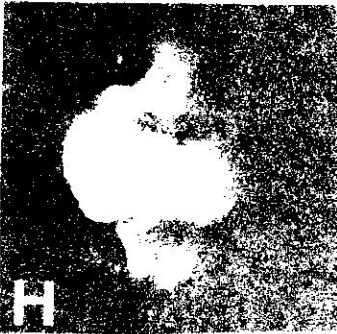
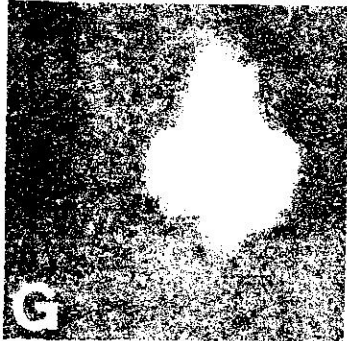
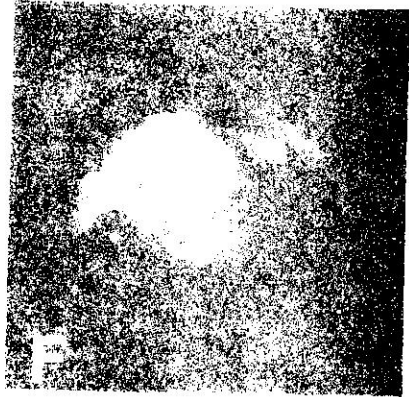
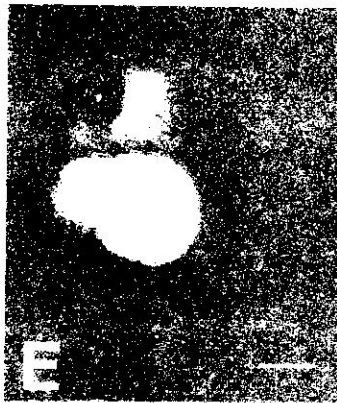
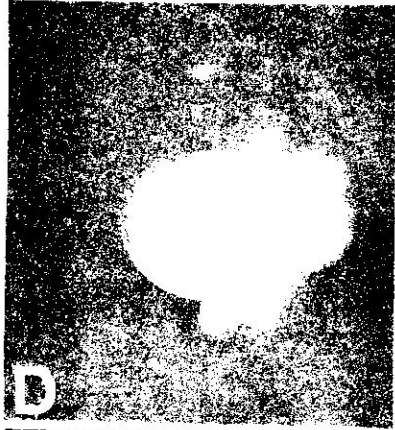
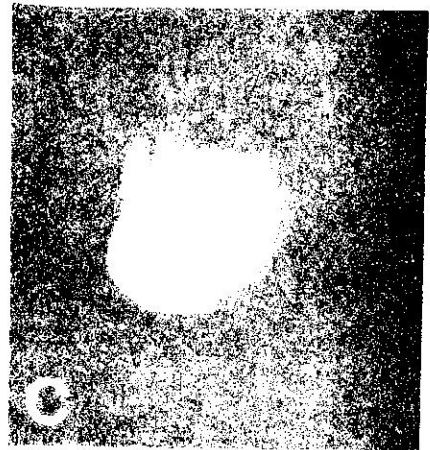
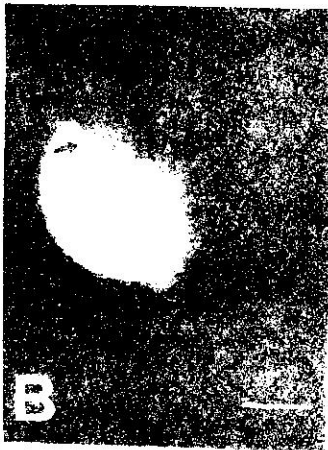
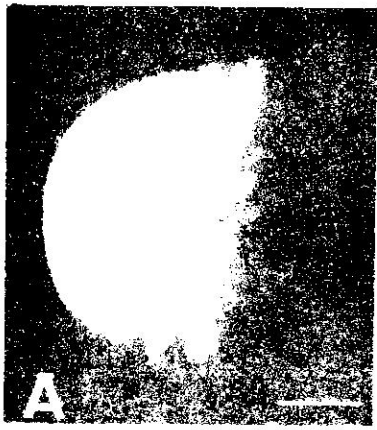


Figure 16. Photomicrographs of E. coqui embryos. A) early Stage 5, dorsal view. B) Stage 7, dorsolateral view (arrow indicates gill bud). C) Stage 7, lateral view. D) Stage 9, dorso-lateral view. E) Stage 10, ventral view. F) Stage 10, lateral view. G) Stage 10, dorsal view. H) Stage 12, ventral view. I) Stage 12, lateral view. J) Stage 14, ventrolateral view. K) Egg tooth of a Stage 14 embryo. Scale = 1.0 mm; scale in B applies to B-D, scale in E applies to E-J.



Endolymphatic Calcium Deposits.- During Stage 6, there first appears a pair of symmetric points of pure white material immediately posterior to the mesencephalon and anterolateral to the rhombencephalon (Figures 15 G-H). These initial points enlarge into small quadrangular patches by Stage 8 (Figures 15 I-L, 16 B-C), then develop forward extensions from their anterior lateral corners toward the rear medial region of the eye. From external view, these extensions reach the eye by the end of Stage 9 (Figures 15 M, 16 D). During Stages 10-12, the forward extensions widen dorsoventrally (as seen in lateral view), and the original posterior areas widen cephalo-caudally and medially. By late Stage 12, the white areas on the two sides are joined at the midline (Figures 15 O, 16 I) and, viewed dorsally, the ECD resembles a shallow horseshoe with a thickened base and slightly spread arms. After Stage 12, it begins to fade and is no longer visible by Stage 15. This is due to two processes. There is regression of the calcium deposit, such that by hatching, it is about one-third of its size at full development (late Stage 12). Concurrent increasing pigmentation masks what remains of the structures from Stage 14 on.

Preliminary analysis of these structures dissected from a Stage 11 embryo (Figure 15 N) indicates that they are 28% calcium by weight. Their position at Stage 6 (Figures 15 G-H) involves quite clearly the endolymphatic sac (Lynn, 1942) with subsequent elaboration and forward extension during Stages 7 through 12 (Figures 15 I-O, 16 B-D, F-G, I). There is little doubt that they are homologous with the calcium carbonate deposits which have been described in the endolymphatic sacs of many aquatic anuran larvae (Dempster, 1930; Carlstrom, 1963; Guardabassi, 1960; Pilkington and Simkiss, 1966; Etkin, 1964). I will

refer to them as endolymphatic calcium deposits (ECD).

Dissection of the ECD at full development, from Stage 12 embryos, reveals a structure with a posterior portion that is more or less dorsoventrally depressed. The forward extensions from the anterior lateral corners begin as narrow terete stalks becoming progressively wider and laterally compressed towards the eye. Deterioration of the ECD, beginning in late Stage 12, involves progressive reduction of all parts. By Stage 14 (Figure 16 J) the narrow terete base of the forward extensions completely disappears, leaving separate anterior and posterior sections. By hatching, the entire structure is reduced to about one-third of its fullest extent at Stage 12. Their size continues to decrease after hatching.

Egg tooth.- An egg tooth first appears at the premaxillary symphysis during the latter part of Stage 12 (Figure 15 P), and develops into a prominent, black, bicuspid structure by Stage 14 (Figures 16 J-K). The tooth projects perpendicularly from tip of the upper jaw, and is 0.12 ± 0.01 mm (mean \pm SD) wide and 0.07 ± 0.01 mm (mean \pm SD) long at full size. It sloughs off within 1.5 days of hatching.

Body wall.- The pigmented body wall appears in Stage 7 as a small disc flanking the trunk between the front and hind limbs (Figures 15 I-J, 16 B). This disc and its leading edge, which covers the base of the forelimbs, are well-defined by melanophores which are not evident elsewhere. No pigmentation appears on the yolk surface beyond the borders of the body wall. The disc expands from Stage 8 through Stage 12 (Figures 15 I-N, P, 16 D-F, H), progressively surrounding the large yolk reserve. The body wall completely encloses the yolk by the end of

Stage 12 (Figures 15 P, 16 H).

Limbs.- Limb buds first appear in Stage 4 as rounded swellings lateral to, and slightly separated from, the neural tube (Figure 15 A-B). The buds increase in size and join the trunk in Stage 5 (Figures 15 C-D, 16 A). The hind limb buds are slightly larger than the fore limbs throughout development. Both front and hind limb buds are roundish in appearance early in Stage 5, becoming more oblong as the stage progresses (Figures 15 E-F). Elbow and knee joints appear as constrictions during Stage 6 (Figure 15 G) and are quite evident in Stage 7 (Figures 15 I-J, 16 B-C). Foot paddles are also evident in Stage 7. Nubs of at least three digits on front and hind feet are apparent in Stage 8 (Figure 15 K). Limbs and toes elongate from Stage 9 through 13 (Figures 15 M-N, P, 16 E-J), at which point they reach full length, relative to their length at hatching. Toepads appear in Stage 13 (Figure 16 J).

Tail.- A tail bud covers the cloaca in Stage 4 embryos (Figure 15 B). The tail stem curves to one side in Stage 5 (Figures 15 C, 16 A) and has a small membranous fin in Stage 6 (Figure 15 G). Elongation of the tail to full length with a large membranous fin is accomplished by Stage 10 (Table 18, Figures 16 E-G) and remains so through Stage 12 (Figures 15 O-P, 16 H-I). At full length, it bends at the vitelline membrane and extends laterally about two-thirds of the way to the snout. It begins to regress after Stage 12 and at hatching is about one-half of its former length (Table 18). It is resorbed completely within 2 days of hatching. The tail is translucent, heavily vascularized and unpigmented throughout development.

Table 13. MEASUREMENTS OF EMBRYONIC FEATURES (Mean \pm 1 SE) FOR EACH DEVELOPMENTAL STAGE AND

POST-HATCHING. All measurements in mm. Membranes removed for all eggs except those in stages 1-3. Sample size (N) is the number of embryos measured at each stage.

Stage	N	Total length	Snout-vent length	Yolk width	Tail length
1-3	21	3.6 \pm 0.05	-	-	-
4	11	3.7 \pm 0.05	3.2 \pm 0.05	3.3 \pm 0.03	0.7 \pm 0.12
5	20	4.0 \pm 0.02	3.5 \pm 0.05	3.6 \pm 0.03	1.7 \pm 0.04
6	14	4.1 \pm 0.05	3.5 \pm 0.03	3.5 \pm 0.03	3.1 \pm 0.05
7	4	4.3 \pm 0.05	3.7 \pm 0.05	3.4 \pm 0.05	2.3 \pm 0.10
8	6	4.5 \pm 0.07	3.8 \pm 0.07	3.3 \pm 0.04	2.7 \pm 0.07
9	7	4.9 \pm 0.05	4.2 \pm 0.05	3.5 \pm 0.05	3.1 \pm 0.06
10	10	5.0 \pm 0.05	4.3 \pm 0.05	3.7 \pm 0.05	3.4 \pm 0.03
11	10	5.2 \pm 0.06	4.4 \pm 0.03	3.9 \pm 0.05	3.2 \pm 0.06
12	23	5.6 \pm 0.04	4.7 \pm 0.02	3.9 \pm 0.04	3.4 \pm 0.06
13	15	5.6 \pm 0.08	4.9 \pm 0.03	3.4 \pm 0.05	3.1 \pm 0.10
14-15	14	-	5.5 \pm 0.11	3.3 \pm 0.05	2.7 \pm 0.08
Hatching	16	-	6.1 \pm 0.10	2.8 \pm 0.05	1.7 \pm 0.08
Two days post-hatching	12	-	6.3 \pm 0.06	-	0.2 \pm 0.09

Behavior. - There is a progression of behavioral events which are readily visible in living embryos. Rapid rotation of embryos begins in Stage 3. Rotation ceases in late Stage 5 or early Stage 6, when the first evidence of mid-body flexion is observed. The tail moves for the first time during Stage 6, but tail thrashing strong enough to move the embryo does not occur until Stage 7. Tail thrashing is the main source of locomotion through Stage 12. Twitching and flexing of the limbs is first apparent during Stage 10, and by Stage 13 the embryo moves inside the egg solely with its limbs. Hatchlings are fully mobile.

Size during development. - Measurements of embryonic dimensions are presented in Table 18. Through the end of Stage 3, the developing embryo is shorter than the diameter of the egg. In Stage 4, the embryo first exceeds the egg diameter and begins to curl around the large yolk reserve (Figure 15 A-B). From that point on, embryo length is the longest intraoval dimension. Total length is the longest dimension of the embryo in dorsal view, and is measured to the most posterior edge of the curled tail stem. Snout-vent length (SVL) is measured to the angle which the hind limbs make with the base of the tail. Because embryos are curled around a large yolk reserve during most of development, SVL is not strictly representative of their true length. From Stage 14 on, the yolk is reduced sufficiently in size to allow flattening the embryos or hatchlings and measuring a true SVL. Hence there is a large change in SVL from Stage 13 to hatching. Tail length is a measurement of the central tail stem. Yolk width was measured as the greatest transverse width of the embryo in dorsal view. It is relatively constant through Stage 8, as the embryo lengthens. In Stages

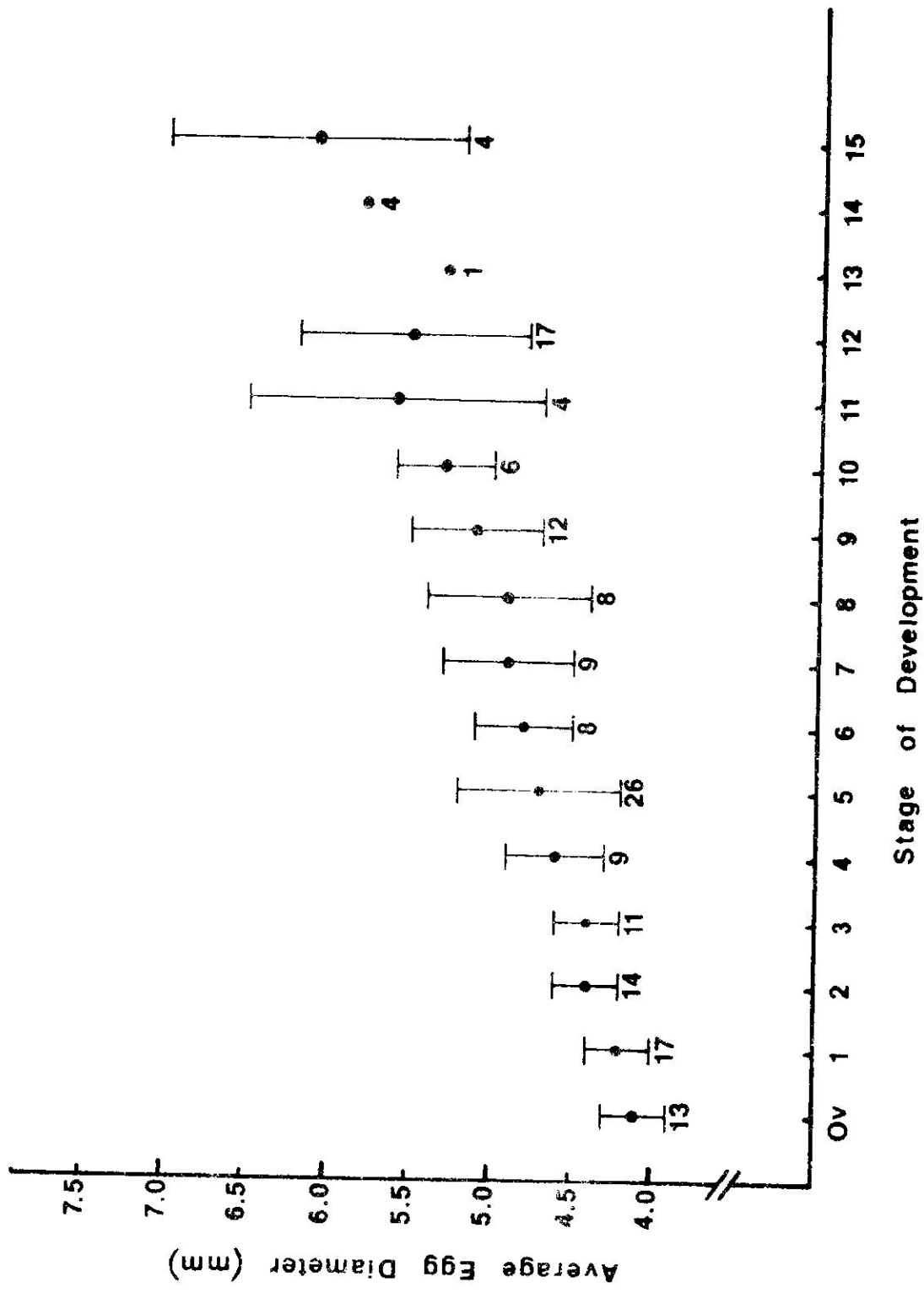
9-12, the elongating embryo progressively pinches the yolk antero-posteriorly while the expanding disc envelopes it laterally. This apparently causes the yolk to bulge laterally (Table 18). From Stage 13 on, yolk width decreases.

Measurements of egg diameter in the field, although subject to greater error due to variation in the hydric state of eggs, closely parallel laboratory measurements of total length (Table 18, Figure 17). Egg diameter was significantly correlated with developmental stage ($r = 0.78$, $p < 0.01$).

Comparisons with Other Puerto Rican Eleutherodactylus

I used this staging table to record development in two other Eleutherodactylus: E. portoricensis and E. antillensis. Microscopic examination of developmental series of these species reveals morphological detail and developmental chronology which are virtually identical to E. coqui. Embryos are almost indistinguishable from those of E. coqui through Stage 11. At that stage, the sharper angle of the canthus rostralis and narrower snout, diagnostic of adults (Rivero, 1978), are evident in the embryos of both species and pigmentation differences begin to appear. Laterally and ventrally, the embryos of E. antillensis develop an almost black ground color which is flecked with white, a pattern that is quite distinct at hatching. In E. portoricensis color differences are not so marked although hatchlings are a light orange-brown rather than dark brown as in E. coqui. Development of the ECD, gills, limbs and tail, and expansion of the pigmented body wall are equivalent, in external view, to that of E. coqui. Hatchlings of both E. portoricensis and E. antillensis have

Figure 17. Average egg diameter (measured in the field) as a function of developmental stage. For each stage, mean \pm 1 SD and sample size (below brackets) are given. Total sample = 163 clutches. Ov = Oviposition. Regression line:
 $Y = 0.12X + 4.1$; correlation coefficient: $r = 0.78$,
 $p < 0.01$.



pigmented egg teeth at hatching.

Temperature and Development

It was not possible to raise eggs under constant temperature conditions at El Verde. Hence, I examined the relationship of temperature and development by regressing the developmental period (number of days from oviposition to hatching) of clutches in the field against ambient temperature as measured by daily minima and maxima recorded at the El Verde Field Station. For each clutch, I calculated the average of the minimum and maximum for each day of development, then took the mean of the daily averages (Figure 18). Developmental period and mean daily temperature were significantly and negatively correlated ($r = -0.96$, $p < 0.01$). This relationship also holds for regressions of developmental period on mean daily minima ($r = -0.94$, $p < 0.01$) and mean daily maxima ($r = -0.94$, $p < 0.01$). The slope of the regression line (Figure 18) illustrates the strong effect of temperature on development. A drop of 1 C in mean daily temperature results in 2.5 day prolongation of development.

The strong negative correlation of temperature and developmental rate in E. coqui was reflected in a strong seasonal effect on developmental rate. In Table 19 I present the mean daily temperature for each month (computed as above) and the average developmental period for clutches which had the major portion of development during a month (for any month, this included clutches laid between the 21st of the previous month and the 20th of the current month). From Jan-Feb to June average developmental rate dropped by 9.5 days, corresponding to an increase in average daily temperature of 4.0 C (Table 19).

Figure 18. Scattergram of developmental period against mean daily temperature for 27 clutches of E. coqui. Regression line: $Y = -2.47X + 78.2$; correlation coefficient: $r = -0.96$, $p < 0.01$.

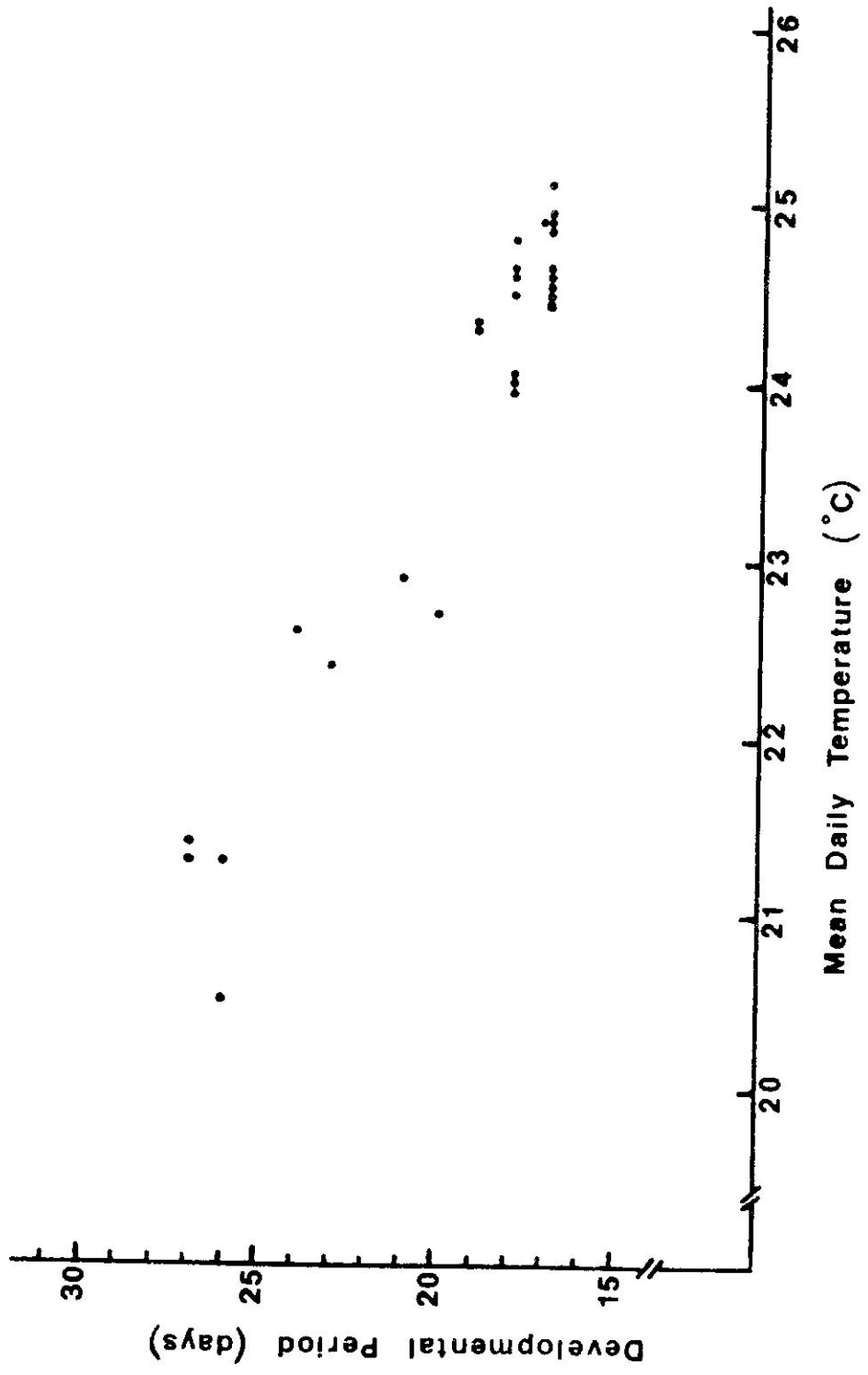


Table 19. MEAN DEVELOPMENTAL PERIODS FOR CLUTCHES IN THE FIELD AND MEAN DAILY TEMPERATURES FOR EACH MONTH OF 1980 (Δ is the difference in means between successive months, N is the number of clutches).

Month(s)	N	Mean Developmental Period (days)		Mean Daily Temperature ($^{\circ}$ C)	
		Mean \pm SD	Δ	Mean \pm SD	Δ
Jan-Feb	12	26.3 \pm 0.9	-	21.1 \pm 1.4	-
March	35	24.6 \pm 1.5	-1.7	21.7 \pm 1.0	+0.6
April	36	20.6 \pm 1.7	-4.0	22.9 \pm 1.3	+1.2
May	25	18.1 \pm 0.7	-2.5	24.5 \pm 1.1	+1.6
June	27	16.8 \pm 1.0	-1.3	25.1 \pm 1.2	+0.6
July	16	17.1 \pm 0.4	+0.3	24.6 \pm 0.6	-0.5

DISCUSSION

I have described a continuum of external morphological changes in living eggs of E. coqui which form the basis of a developmental staging table for this species. The table has been used repeatedly in the field to stage the eggs of two other Eleutherodactylus as well. In order to examine its potential as a general scheme for staging the eggs of Eleutherodactylus, I present (Table 20) equivalent stages and figures from other developmental studies of Eleutherodactylus and discuss similarities and differences.

The reduced nature of external gills has been noted in other Eleutherodactylus embryos. Gitlin (1944) noted the brief tenure of reduced gills in E. antillensis. He found gill buds in embryos of about 3.5 days (his Figures 2, 3, 16, 17), corresponding to early Stage 5 of my scheme. Gills were reduced at seven days (presumably reaching peak development prior to that time), almost gone at 8.75 days, and completely gone by 9.75 days. Because reduced gills at seven days were contained within a dermal pocket, they would not have been visible externally after that time in Gitlin's specimens. Since Gitlin's seven day embryos correspond to my Stage 9, disappearance of the gills at that stage in my scheme may represent a similar phenomenon. I have observed the small gills in embryos of E. antillensis as well. Chibon (1960) also reported the presence of reduced gills in E. martinicensis, which were visible at three days (his Figure 5), corresponding to my Stages 7-8, but had disappeared by 4.5 days (his Figure 6), corresponding to Stage 8 or early Stage 9 of my scheme. Adamson et al. (1960) described transitory external gills arising in Stage III (my

Table 20. THE EQUIVALENT STAGES AND FIGURES FROM A GENERAL STAGING TABLE (Gosner, 1960) AND FROM OTHER PAPERS ON DEVELOPMENT IN ELEUTHERODACTYLUS (d = days, h = hours, F = Figure).

Other staging tables	Stages for <u>Eleutherodactylus coqui</u>			
	1	2	3	4
Gosner (1960)	1-10	11-13	14-16	17-18
<u>Eleutherodactylus</u>				
<u>E. nubicola</u> ^a	-	F 5	I (F 6)	II-III
<u>E. nubicola</u> ^b	-26d	-25d	-24d (F 41+43)	-23d
<u>E. antillensis</u> ^c	-	-	1d 14h (F 9)	2d 16-22h (F 10-13)
<u>E. guentheri</u> ^d	-	-	I (F 1)	-
<u>E. planirostris</u> ^e	-	-	36h	60h
<u>E. johnstonei</u> ^f	-	I	-	II
<u>E. martinicensis</u> ^g	12h (F 1)	18h (F 2)	24h (F 3)	-
<u>E. nasutus</u> ^h	-	-	-	-27 + -26d (F 1)

a. Sampson, 1904; b. Lynn, 1942; c. Gitlin, 1944; d. Lynn and Lutz, 1946; e. Goin, 1947;

f. Adamson et al., 1960; g. Chibon, 1960; h. Lynn and Lutz, 1947.

Table 20 (continued).

Stages for <u>Eleutherodactylus coqui</u>									
5	6	7	8	9	10				
-	-	-	-	-	-				
IV (F 8-9)	V	VI (F 10)	-	VII	VIII				
-22 + -21d (F 43)	-20 + -19d	-18d (F 45)	-17d	-15 + -15d (F 46)	-14d				
3d 10-18h (F 14-17)	-	-	-	-	-				
II	F 2	-	F 3	III (F 5)	-				
84h	-	5.5d	-	-	-				
III	-	-	IV	-	-				
2.5d (F 4)	-	3d (F 5)	-	4.5d (F 6)	-				
-26 + -25d (F 2)	-	F 3	-	-	F 4				

Table 20 (continued).

Stages for <u>Eleutherodactylus coqui</u>					
	11	12	13	14	15
	-	-	-	-	-
IX		X + XI	XII (F 12)	XIII (F 13)	XIV (F 14)
-13d		-12 + -11d	-10 + -9d	-	-
-		-	-	-	-
-		IV (F 6)	F 7	-	F 8
-		-	-	-	-
-		VII	-	-	-
-		7d (F 7)	-	-	11d (F 8)
F 5		F 6	F 7-8	-	-

Stage 5) and disappearing in Stage IV (my Stage 8) in E. johnstonei, and Hughes (1959) found blunt vascular papillae on the surface of the third pharyngeal arches (third fill arch?) in E. planirostris. Noble (1925, 1927) described two pairs of gills in E. inoptatus of Hispaniola, the anterior pair larger than the posterior pair.

A complete absence of gills has been reported for other Eleutherodactylus. Jameson (1950) and Vallett and Jameson (1961) reported that gills were absent in Hylactophryne (Eleutherodactylus) augusti. However, they examined embryos which, according to other features in my scheme, were already past the point at which external gills disappear. Earlier developmental stages of this species would have to be examined to determine whether or not external gills occur. Reported absence of gills in several other species is more credible since larger ranges of developmental stages were examined. External gills apparently are absent in the embryos of E. nubicola (Lynn, 1942), one or two other species of Jamaican Eleutherodactylus (Sampson, 1904), E. abbotti and E. flavescens of Hispaniola (Noble, 1925), and Brazilian E. guentheri (Lynn and Lutz, 1946).

The reduction or absence of gills in Eleutherodactylus is coincident with possession of a very large, membranous, vascularized tail. The hypothesis that respiratory function is carried out primarily by the tail has been proposed repeatedly since Peters' (1876) early observations and is probably true. My observations suggest that the tail is also a motile organ during part of development. Tail beating is an important source of movement for at least one-third of development in E. coqui.

The early appearance of limb buds in Eleutherodactylus embryos was

one of the first aspects noted in early reports of direct development in this genus. Peters (1876) stated that Gundlach found all four extremities present as short stumps at an early stage in E. coqui. Sampson (1904) found that the limbs appeared early and simultaneously in the Jamaican species she examined. Gitlin (1944) noted that the hind limbs actually appeared about six hours before the front limbs in E. antillensis (portoricensis).

An egg tooth has been seen in embryos of every Eleutherodactylus reported thus far (Lynn, 1942; Lynn and Lutz, 1946; Lutz, 1944; Bayley, 1950; Adamson et al., 1960; Bogert, 1969; Wake, 1978; this study), although in some species it may be quite small (Noble, 1926). Typically, it is described as a bicuspid structure (single-spined in a few; Noble, 1926; Goin, 1947), partly or completely black, and situated at the premaxillary symphysis approximately perpendicular to the upper jaw. In E. coqui and E. antillensis, the egg tooth is black over its distal half. Several authors have also observed the hatching of Eleutherodactylus and have described the scraping action of embryos prior to hatching. Stage 15 embryos in this study have been observed on many occasions to poke at the egg capsule with their snouts just prior to hatching, apparently rupturing the membranes with the egg tooth.

The pigmented disc that appears during Stage 7 in my table and expands to form the body wall is analogous to the "dermal fold" of Sampson (1904). She described the dermal fold as a sandwich layer of mesoderm surrounded by ectoderm whose free anterior edge covered the base of the forelimbs early in development, but later disappeared. In embryos of E. nasutus (Lynn and Lutz, 1947, p. 4), the "dermal fold does not only envelop the base of the forelimbs but continues backward

to the insertion of the hind limbs." In E. coqui and E. antillensis, the disc formed in Stage 7 covers the base of the forelimbs and extends back to the base of the hind limbs. The anterior edge of the disc, which covers the forelimb base, disappears as the limbs grow and the disc expands. The dermal fold has been described in E. nubicola (Lynn, 1942), E. guentheri (Lynn and Lutz, 1946) and E. planirostris (Goin, 1947).

Endolymphatic calcium desposits (ECD) have been reported in the embryos of several Eleutherodactylus. Lynn and Lutz (1946), in observations of living E. guentheri, found small symmetrical centers of ossification on the occiput in two specimens that roughly correspond to Stages 7 and 8 of my table, during which ECD are prominent. Lynn and Lutz (1947, p. 4) described "a pair of arches of incipient ossification on the occiput" in living embryos of E. nasutus that were at a stage equivalent to my stage 7. Later in the paper (p. 5) they state that these "ossification arches...(are) more contiguous and are sending prolongations down the middorsal region" in embryos equivalent to my stage 11. Chibon (1960) noted the appearance of "otocysts" at three days (his Figure 5) in E. martinicensis and stated that they are well-developed at 4.5 days (his Figure 6). From his description and illustrations, these structures are clearly equivalent to the ECD in E. coqui. Chibon's (1960) Figures 5 and 6 correspond to Stages 7-8 and Stage 9, respectively, in my scheme. In E. johnstonei from Jamaica, Hughes (1962) observed that otoliths first appeared at 5 days and were large by day 7. In his Text-Figure 1, a 6 1/2 day embryo is illustrated which bears a shaded area posterior and dorsal to the eye which corresponds exactly to the position and shape of the ECD in a Stage 10

embryo of E. coqui. I have also observed ECD in the embryos of Puerto Rican E. antillensis and E. portoricensis.

The apparent absence of ECD in the embryos of other Eleutherodactylus embryos may be coincidental artifact of the heavy concentrations of calcium in the ECD and fixation of material in acidic media [i.e., picric acid, Sampson, 1904; Bouin's fluid and Zenker's fluid, Lynn, 1942; Lavdowsky's mixture (formalin, alcohol and acetic acid), Goin, 1947; Bouin's fluid, Hughes, 1959; Heidenhain's Susa reagent, Adamson et al., 1960]. Indeed, in my study, ECD were always obvious in live embryos but when I fixed two initial series of eggs in Bouin's Solution and in 10% unbuffered formalin all traces of the ECD were lost. All later series were fixed in 10% buffered formalin, which preserved the ECD completely. If the ECD are to serve as a diagnostic feature for staging Eleutherodactylus embryos, it is imperative to fix material in buffered or alkaline media.

The endolymphatic sac, an insignificant dorsal outpocketing of the endolymphatic duct in most vertebrates, is excessively enlarged in amphibians (Dempster, 1930). In anurans, right and left sacs are fused and the structure extends over the hindbrain and vertebral column with anterior extensions around the midbrain and protrusions between the vertebrae of adults. Deposits of calcium carbonate are found in most parts of the endolymphatic sacs of larval and adult anurans, with crystals often filling the entire structure during the latter part of metamorphosis. The presence of ECD in Eleutherodactylus therefore appears to be the homologous situation in these terrestrial frogs. In aquatic anuran larvae, the deposits can only be seen in situ in cleared specimens because of the heavy pigmentation early in development. In

Eleutherodactylus however, the much later appearance of pigment renders the progressive formation of ECD quite visible, providing an important diagnostic feature for staging embryos. The early appearance of ECD in E. coqui is similar to early concentration of calcium carbonate in post-hatching aquatic larvae. Whiteside (1922) noted that calcareous deposits exist almost from the earliest stages of formation of the endolymphatic sac in Rana temporaria larvae.

The functional significance of ECD in anuran larvae was long the subject of speculation. Early suggestions (e.g., Gaupp, 1897 as cited in Dempster, 1930) that calcium stored in the endolymphatic sacs of pre-metamorphic larvae might be mobilized for bone formation during metamorphic climax awaited definitive proof by Guardabassi (1960), Kreiner (1954) and Pilkington and Simkiss (1966). The latter study showed conclusively that endolymphatic calcium carbonate is resorbed during metamorphic climax to provide calcium for skeletal ossification. Pilkington and Simkiss suggested that the process of early storage and later mobilization was an adaptation to allow continued skeletal ossification during the extensive morphological changes of metamorphic climax, when no feeding occurs. My observations of the early formation and later dissolution of ECD in E. coqui suggest a similar calcium storage and mobilization scenario for these terrestrial embryos. The ECD first appear early in development (Stage 6 in my scheme), long before ossification begins in any Eleutherodactylus (Lynn, 1942; Lynn and Lutz, 1946; Vallett and Jameson, 1961). They enlarge during the next one-third of development, and begin to disintegrate coincident with the beginning of skeletal ossification. In E. nubicola, ossification begins in the limbs at -12 days, in the vertebral column

at -11 days and in the head at -10 days (Lynn, 1942). These times are equivalent to late Stage 12 and Stage 13 in E. coqui, when the ECD are starting to disintegrate. In E. johnstonei (Adanson et al., 1960), ossification of the limbs begins in Stage VII (equivalent to my Stage 12).

While the calcium storage function of the endolymphatic organ is probably similar in aquatic anuran larvae and direct-developing embryos, the source of the calcium is probably different. Calcium stored by aquatic larvae almost certainly is derived from larval food. In eggs with direct development, the calcium must be invested in the egg by the female prior to oviposition.

Temperature and Development

Temperature has a strong effect on the developmental rate in embryos and larvae of aquatic-breeding amphibians (Moore, 1939, 1942; Pettus and Angleton, 1967; Licht, 1971; Howard, 1978; Smith-Gill and Berven, 1979). I have found a significant inverse relationship between ambient temperature and developmental rate for the terrestrial eggs of E. coqui. Over the range of temperatures encountered in the field (approximately 15-28 C), a 1 C drop in mean daily temperature caused a change in mean developmental period of 2.5 days. This strong relationship between temperature and developmental rate results in an average winter developmental period (Jan-Feb: 26.3 d) which is 1.57 times the summer developmental period (June: 16.8 d)(Table 19).

Two principal effects of growing amphibian larvae at lower temperatures are longer larval periods and larger size at metamorphosis (Uhlenuth, 1919; Stewart, 1956; Etkin, 1964; Kollros, 1961; Smith-Gill

and Berven, 1979). Because the longer developmental period of E. coqui which results from lower temperatures is spent wholly within the egg, there is no reason to expect that hatchlings should be larger at lower temperatures. Unlike free-living aquatic larvae, E. coqui embryos cannot feed for some more extended period.

A more important result of slower development in E. coqui is the longer period during which eggs are vulnerable to mortality. Desiccation and cannibalism are major causes of egg mortality in E. coqui (Townsend et al., 1984). Although male parental care significantly reduces egg mortality, a 1.57X increase in developmental period could result in much lower hatching success despite full-term parental care. The vulnerability of eggs due to longer developmental periods may be further compounded by the drier conditions during the winter months.

Although the lower temperatures and drier conditions of the winter months may have a direct effect on the physiology and reproductive behavior of adult E. coqui, the increased vulnerability and hence lower hatching success of eggs laid during that season may be a selection pressure that reinforces (or underlies) the decreased level of reproductive activity which is observed during that season. There is marked seasonality in the breeding intensity of E. coqui, with less than one-third the number of clutches being laid in Jan-Feb as are deposited in May-June (Chapter 2). It is reasonable to expect that females that delayed vitellogenesis or ovulation during the winter and put energy into growth may have much higher reproductive success.

