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Sexual Selection and Larval Performance of Two Species of Red-Eyed Treefrogs, *Agalychnis callidryas* and *A. moreletii* of the Chiquibul Forest Reserve, Belize.

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UNIVERSITY OF MIAMI

SEXUAL SELECTION AND LARVAL PERFORMANCE OF TWO SPECIES OF
NEOTROPICAL RED-EYED TREEFROGS, *AGALYCHNIS CALLIDRYAS* AND *A.*
MORELETHI OF THE CHIQUIBUL FOREST RESERVE, BELIZE.

By

Venetia S. Briggs

A DISSERTATION

Submitted to the Faculty
of the University of Miami
in partial fulfillment of the requirements for
the degree of Doctor of Philosophy

Coral Gables, Florida

December 2007

UNIVERSITY OF MIAMI

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Sexual selection and larval performance of two species of neotropical red-eyed treefrogs, *Agalychnis callidryas* and *A. moreletii* of the Chiquibul Forest Reserve, Belize

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Abstract of a dissertation at the University of Miami.

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In species where males do not provide immediate benefits to females in the form of resources or parental care to offspring, adaptive mate choice may be maintained by selection because preferred males sire offspring that are genetically superior. I test the hypothesis that female mating preferences result in enhanced performance of their larvae, owing to genetic variation among sires in two species of red-eyed treefrogs. This variation in males may be manifested as body size or in advertisement call parameters. I examined natural pairing patterns and found that both species display a size-based mating pattern with substantial seasonal effects. In lean years, females mate with males that are larger on average, yet females also seem to favor a constant size ratio of the male relative to her body size. I documented significant call trait variation both within and among males and have evidence to support size-related call traits as indicators of mating success for some properties. Finally, I provide evidence for a pronounced effect of sire size on offspring traits, most notably upon hatching that may have significant post-metamorphic fitness benefits. My study provides data on the basis of female choice and may provide a link between female preference for male traits and enhanced offspring performance by larvae sired by preferred males in the evolution of anuran mating systems.

*To my nucleus: my parents, John Shaw and Aloma Lashley,
and my brother, Farren Basil*

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CHAPTER 1

INTRODUCTION

Sexual selection, female choice, and larval performance

Darwin (1871) argued that many secondary sexually dimorphic traits evolved as a consequence of sexual selection, and he envisioned two components of sexual selection: an inter-sexual component involving the exercise of choice by one sex for members of the opposite sex, and an intra-sexual component involving competition among members of one sex for the opposite sex. Mate choice has associated costs that include the time and energy spent in travel and assessment, increased risk of predation during the search, injury from potential mates, the loss of potential mates to competitors, as well as the fitness cost of choosing a poor mate (Parker 1982; Reynolds and Gross 1990).

Females are often the choosier sex because of the asymmetry in reproductive investment, in which females typically invest substantially more than do males (Maynard Smith 1982). The benefits that accrue to females that exercise such choice and the basis of such a choice have been a major focus of study (Andersson 1994; reviewed by Sullivan et al. 1995; Shuster and Wade 2003), but are still not completely understood.

The benefits afforded females that exercise choice may be direct or indirect. Direct benefits include resources (food, territory, nuptial gifts, and paternal care; reviewed by Andersson 1994) provided to a female by her mate to ensure her current fitness or the survival of her offspring, whereas indirect benefits are in the form of genes transmitted to her offspring (Maynard Smith 1978; Kodric-Brown and Brown 1984).

Choosy females would then produce offspring with a higher fitness relative to other offspring.

There are two main models that address indirect benefits in a non-resource based system: Fisherian “runaway selection” and the “good genes” model of sexual selection. Under the Fisherian process (1930), female choice is directed toward heritable male traits (Fisher 1958; Trivers 1972; Thornhill and Alcock 1983) that increase the mating success of her sons. The trait eventually is chosen because it is linked with the female preference for that trait (Kirkpatrick 1982). Under the “good genes” model, or “viability indicator” model of sexual selection, females prefer males with particular fitness characteristics that signify high genetic quality that will improve offspring survival (Zahavi 1975; Hamilton and Zuk 1982; Heisler 1984; Kodric-Brown and Brown 1984; Andersson 1986; and Sullivan et al. 1995).

While indirect benefits are less conspicuous than direct benefits, there is an increasing number of studies that document mate choice for such indirect benefits (Woodward 1987; Reynolds and Gross 1992; Moore 1994; Sheldon et al. 1997; Alatalo et al. 1998; Welch et al. 1998), suggesting that there must be an inherent mechanism to detect quality and ensure the heritability of fitness traits.

In frogs, a male’s genetic quality may be encoded in body size and/or in vocalizations (which may covary with body size). In species with indeterminate growth, a larger male body size may signify faster growth rate, better competitive ability, or longer life expectancy or some combination of these (Wilbur et al. 1978), all of which may reflect genetic quality.

Indeed, the most common deviation from random mating in frogs involves a large-male mating advantage (reviewed by Woodward 1982) and in some species females are known to respond preferentially to vocalizations that correspond to those of large males (reviewed by Sullivan and Kwiatowski 2007).

Although studies exist on the direct benefits to females (reviewed by Andersson 1994), few investigate the consequences that female choice has on offspring performance (Howard 1978; Woodward 1986, 1987, Woodward et al. 1988; Mitchell 1990; Howard et al. 1994). In systems where there is no direct benefit to females, males of superior genetic quality, manifested in either large body size or in call parameters, may influence offspring and lead to enhanced offspring growth performance. Offspring performance in frogs is typically measured as hatching success, growth and developmental rates, survivorship, and size at metamorphosis. Offspring with enhanced performance traits as larvae may possess survival advantages that carry over to the post-metamorphic stage (Altwegg and Reyer 2003), and which may account in part for variation evident in body size and size-related acoustic variation in the calls of adult frogs.

The red-eyed treefrogs, Agalychnis callidryas and Agalychnis moreletii

Neotropical hylids, such as *Agalychnis callidryas* and *Agalychnis moreletii* offer excellent systems to test hypotheses based upon the “good genes” otherwise known as “viability indicator” model of sexual selection because males do not hold territories, they provide no parental care, they contribute only sperm to reproduction, and fertilization is external.

Male advertisement calls function to attract mates and serve to resolve intrasexual conflicts and a distinct larval stage allows for discrete time points at which larval performance can be measured.

The morphology and vocalizations of both treefrog species are well-described (Duellman 1970, 2001; Lee 1996; Ryan 2001; Savage 2003); and much work has been done on tadpole biology and hatchling plasticity in *A. callidryas* (Warkentin 1995, 1999, 2002, 2005; Warkentin et al. 2007). Additionally, the phylogenetics of Phyllomedusine frogs that include both *A. callidryas* and *A. moreletii*, has only recently been completed (Gomez-Mestre et al. in review). Considering these areas of research, there are few data beyond anecdotal reports documenting the breeding behavior and mating patterns of either species (Pyburn 1970; Scott 1983; Donnelly and Guyer 1994; D'Orgeix and Turner 1995).

In this study, I test the hypothesis that female mating preferences result in enhanced performance of their larvae, owing to genetic variation among sires in both species of red-eyed treefrogs. This variation in males may be manifested as body size or in advertisement call parameters. I investigate size-related traits and test whether variation in larval performance can be explained by paternal variation in the preferred male traits.

To investigate sexual selection and the role of female choice in these systems, I examine natural pairing patterns of both species of *Agalychnis* in Chapter 2. I evaluate the potential roles of female mate preference and male-male competition by testing for the presence of a large-male mating advantage or size-assortative mating in these two species.

In Chapter 3, I compare call traits between males and investigate the presence of a size-related effect in both *A. callidryas* and *A. moreletii*. I describe and quantify call trait variation within and among males and examine the relationship between mating success, size and call properties.

Finally, in Chapter 4, I test hypotheses based on the “good genes” model of a female preference for large males by using a maternal half-sib design. Differences in larval performance between sires can be attributable to genetic differences in paternity under this controlled experimental design. I assess the consequences of female choice for larval performance traits by examining whether sire body size results in enhanced offspring viability that may have post-metamorphic fitness benefits.

CHAPTER 2

MATING PATTERNS OF RED-EYED TREEFROGS, *AGALYCHNIS CALLIDRYAS* AND *A. MORELETHI* IN THE CHIQUIBUL FOREST RESERVE, BELIZE

INTRODUCTION

Male frogs may mate several times throughout a breeding season; however the energetic and time constraints of producing mature eggs severely limit breeding opportunities for a female (Berven 1981; Andersson 1994). Male competition for females is expected because the operational sex ratio is male-biased (Emlen 1976; Kvarnemo and Ahnesjö 1996). However, anisogamy dictates that females are usually the choosier sex (Maynard Smith 1982) with significant associated fitness costs (Parker 1982; Reynolds and Gross 1990). This differential parental investment is thought to directly affect mating patterns (Trivers 1972; Andersson 1994).

Among anurans, considerable variation exists in mating patterns and some of this variation can be explained by the length of breeding season (reviewed by Sullivan et al. 1995). In explosively breeding species that congregate en masse for short periods the opportunity for intense male-male competition is greater and there is typically little occasion for females to exercise choice. Mating instead may involve scramble competition among males for females (Emlen and Oring 1977). In this case, deviations from random mating may be expressed as a large-male mating advantage. However, in species with protracted breeding periods the opportunity for females to exercise choice is greater and may play a greater role in producing deviations from random mating, expressed as a large-male advantage, or size-assortative mating.

A common deviation from random mating in frogs relates to body size, and there are two hypotheses that propose a size-based female preference (reviewed by Woodward 1982; Halliday 1983; Halliday and Tejedo 1995). The first is a female preference for larger males which predicts a large-male mating pattern where males that are successful in fertilizing the eggs of females are larger, on average than unsuccessful males (Wilbur et al. 1978). In species that have indeterminate growth (like frogs), larger individuals may be older and more experienced. In addition, larger individuals may have higher survival ability, faster growth rates and better competitive ability, all of which may be reflections of genetic quality (Wilbur et al. 1978; Halliday and Tejedo 1995).

Under the “good genes” model of sexual selection females are expected to choose males based on behaviors, physiologies, or morphologies that signify high genetic quality (Fisher 1930; Zahavi 1975; Hamilton and Zuk 1982; Heisler 1984; Kodric-Brown and Brown 1984; Andersson 1986; and Sullivan et al. 1995), and this is especially so in species in which males contribute only sperm to reproduction. Because large body size may signify high genetic quality, females that mate with large males could produce more offspring or superior offspring relative to those that mate with males of smaller size (Maynard Smith 1978; Kodric-Brown and Brown 1984). Evidence for a large-male mating advantage in frogs comes from species in which larger males are more often found in amplexus than smaller males or females respond preferentially to vocalizations of large males (Lee 1986; reviewed by Sullivan et al. 1995; Howard and Young 1998).

The second female preference is for males of a given body size relative to their own. The prediction of this hypothesis is that the preference will result in a pattern of size-assortative mating (Licht 1976). The biomechanics of the axillary amplexus process

require that the vents of males and females be in close apposition to ensure effective fertilization (see Figure 2.1). Females are thus expected to choose males of a size relative to their own body size that maximizes fertilization success. This type of positive size-assortative mating occurs in decidedly fewer anuran species (Davies and Halliday 1977; Lee and Crump 1981; Tárano and Herrera 2003; Gutiérrez and Lüddecke 2004).

As a first step toward understanding the breeding behavior and the basis of possible female mate choice, I examined pairing patterns in two Neotropical hyliid frogs, *Agalychnis callidryas* and *A. moreletii*, closely-related phyllomedusine species that share similar life histories (Duellman 2001; Weins et al. 2006; Gomez-Mestre et al. in review). I evaluate the potential roles of female mating preferences and male-male competition by testing for the presence of a large-male mating advantage or size-assortative mating in the breeding behavior of these two species during three consecutive breeding seasons in Belize, Central America. I also investigate potential differences among more subtle morphometric characters that may also serve as indicators of male quality (Lee and Crump 1981; Lee 2001). Finally, I compare the fertilization success of amplexant pairs with various body size ratios (female to male) to determine whether there is selection for size-assortative mating in *A. callidryas* and *A. moreletii*.

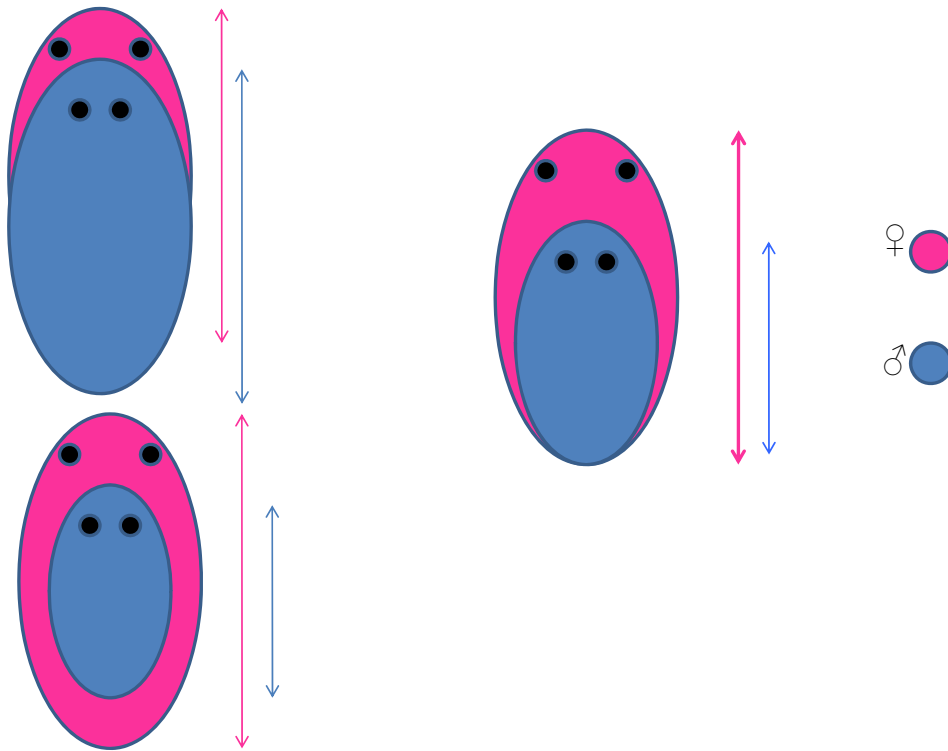


Figure 2.1. Schematic representation of the axillary amplexus of red-eyed treefrogs. Females are depicted in pink, males in blue. If a male is too large relative to the female's body size, or if a male is too small relative to the female, fertilization success is reduced. When cloacal apposition is closer as in the diagram to the right, fertilization success is increased. In either case on the left, successful fertilization is substantially reduced.

METHODS

Study species

Red-eyed treefrogs, *Agalychnis callidryas* (Figure 2.2) and *Agalychnis moreletii* (Figure 2.3) are moderately large Neotropical hylids (Subfamily Phyllomedusinae). Males average 45-55 mm and 55-65 mm snout-vent-length (SVL) respectively (Lee 1996). Females are larger than males in both species ranging from 55-75 mm in *A. callidryas* and 65-85 mm in *A. moreletii* (Lee 1996; Duellman 2001; Savage 2003). The more common *A. callidryas* ranges from central Veracruz, Mexico to western Ecuador (Lee 1996; Duellman 2001; Savage 2003; Santos-Barrera et al. 2004). *Agalychnis moreletii*, recently assigned to a critically threatened status (2004), is restricted to small areas of south-central Veracruz, Mexico, western Belize (Maya Mountains), north-western Honduras, central Guatemala and El Salvador (Santos-Barrera et al. 2004).

Both species are nocturnal and arboreal and inhabit humid lowland and lower montane forests. Males become active with the onset of the rainy season and congregate in choruses, calling from vegetation surrounding permanent pools and temporary ponds (Lee 1996). Females appear at ponds later in the season after heavy rains. Gelatinous egg masses are deposited on a range of oviposition substrates, but typically on the underside of leaves overhanging water. Upon hatching, larvae drop into the water where they complete development (Lee 1996). Clutch sizes in Belize have been reported with a range of 20-50 in *A. callidryas* and 50-75 in *A. moreletii* (Lee 1996); however, these are representative of sub-clutches of a single female (see Figure 2.1, and Chapter 4) that typically produces 200-250 eggs in *A. callidryas* (pers. obs.; pers. comm. I. Gomez-Mestre) and 300-350 in *A. moreletii* (pers. obs.).



Figure 2.2. Male of *Agalychnis callidryas* displaying the red iris and the lateral yellow and blue banding flank pattern.



Figure 2.3. Male of *Agalychnis moreletii* with the darkened iris that is typically blue-rimmed and whose flank pattern shows no banding but is of a solid orange color.

The morphology and vocalizations of both species are well-described (Duellman 1970, 2001; Ryan 2001; Savage 2003).

Study site

I conducted all research at Las Cuevas Research Station (16° 43' N, 88° 59' W) Cayo District, Belize. The station is located in the Chiquibul Forest Reserve, which lies within a much larger area of protected forest in the Maya Mountains of Belize. The rainy season in the western Cayo district of Belize begins toward the end of May or early June (Belize Weather Bureau). The area averages approximately 3,000 mm of rainfall per year (Walker 1973) and has an elevation range from 560-860 m. The vegetation is a mosaic of deciduous semi-evergreen and deciduous seasonal tropical forest punctuated by stands of pine (Penn et al. 2004). There are several seasonally-filled ponds (bajos), and a few permanent water sources (aguadas) that serve as breeding sites for several anuran species (Fitzherbert et al. 2001). I measured the area of each pond that was used as a collection site. Elegans and Warree ponds were within 1 km of the station and Coral and Aguada were within 7 km of the station. At each pond, I counted the number of males to females to determine the operational sex ratio on a given night throughout the breeding season.

Collection and maintenance

To study the mating patterns of *A. callidryas* and *A. moreletii*, I collected amplexant pairs and calling non-amplexant males at breeding aggregations during the hours of 2200-0300 on 7 nights between May 24 to August 3, 2003; on 7 nights between

May 25 to August 3, 2004 and on 26 nights between June 8 and August 25, 2005. Males began calling at dusk and females arrived at the pond between 2100-2200 h. I measured the diameter of each pond using a tape measure to calculate pond area and estimated population density and operational sex ratio by counting the mean maximum number of individuals per pond and the mean number of males to females on a nightly basis throughout the breeding season. I conservatively characterized non-amplectant males as unsuccessful though these males may have been successful in securing a mate prior to or after capture. However, I collected non-amplectant males after amplectant pairs had been located, thus any differences between amplectant and non-amplectant males are likely to reflect true differences in the sample population (Lee and Crump 1981; Howard et al. 1994; Lee 2001).

For each individual, I measured the snout-vent-length (SVL) using a plastic ruler and/or dial calipers, read to the nearest 0.1 mm, and mass using a fine scale Pesola spring balance read to the nearest 0.1 g (2003, 2004) or a fine scale Ohaus Scout Pro balance read to 0.001g in 2005. Females were allowed to extrude the full complement of eggs before being measured on the morning following capture. All measurements are reported as means followed by one standard error. I measured adult individuals on the morning following collection and returned them to the site of capture within two days.

I placed amplectant pairs in individual five-gallon plastic buckets with approximately 3 cm of standing pond-water, a pair of twigs as perch sites and one leaflet of *Chaemadora elegans* palm as an egg deposition site. Non-amplectant males were placed in similar plastic buckets and housed in groups of 10-15 individuals. Palms leaves with deposited egg clutches were clipped to the lip of a separate plastic bucket so that

eggs were suspended above pond-water. I carefully transferred egg clutches to palm leaflets if they were deposited on the walls of the container or the lid of the bucket and similarly suspended these above water. I counted the number of eggs in each clutch both by eye and then placed the leaflet with eggs under the dissecting microscope and also counted the number of eggs to reduce observer error. I misted each clutch daily with pond-water to prevent desiccation. I scored fertilization success as the proportion of healthy eggs to total oviposited eggs 12 h after deposition occurred. Healthy eggs can be differentiated from unfertilized eggs because unfertilized eggs become pale yellow with dark areas after 12 h, have a dimpled yolk surface, and collapsed egg capsules (Duellman and Trueb 1986; pers. obs.).

To test the hypothesis that large males can supplant small males in amplexus, but rarely the reverse, as has been demonstrated for some temperate species of explosively breeding frogs and toads (Lamb 1984; Howard and Kluge 1985), I conducted 15 male-displacement trials in 2003 and 2004, using *A. callidryas*. Amplectant pairs were collected from the pond and each male's SVL was measured using a plastic ruler; I made more accurate measurements the next day. A recently collected non-amplectant male was measured and placed in the container housing the amplectant pair. Each non-amplectant male differed in the estimated SVL of the amplectant male by a minimum of 0.5 cm. Individual males were identified by the spot coloration on their dorsum. Each group was checked using red-light every hour and at the end of a six hour period, I noted the positions of individuals. A successful displacement is scored if the previously non-amplectant male was found in amplexus and data would be consistent with a "take-over".

Data analyses

I calculated pond area for each pond and counted the total number of frogs, as well as number of male to female frogs observed at each pond on a single night over the course of the breeding season to calculate population density per pond (number of frogs/pond area) and operational sex ratio. I investigated potential inter-annual effects on the SVL and mass of males by conducting a two-tailed multivariate analysis of variance (MANOVA) and I conducted post-hoc comparisons between breeding years to locate differences. I also investigated the potential for an interaction effect between breeding year and mating status of males (amplectant or non-amplectant) and also conducted post-hoc comparisons to determine differences. When data did not meet parametric assumptions I conducted the natural log transformation of each response variable (SVL and mass).

To test for large-male mating advantage within each breeding year of 2003, 2004, and 2005, I compared mean SVL and body mass for amplectant and non-amplectant males using a one-tailed one-way analysis of variance for each year and each species. Wilbur et al. (1978) proposed that the percentage of eggs fertilized in a clutch covaries positively with male body size because larger males presumably produce more sperm (Travis 1980; Emerson 1997) and females should prefer larger males resulting in a large-male mating advantage; therefore I used a Pearson's product moment correlation analyses between male SVL and the number of fertilized eggs.

To test for size-assortative mating, I calculated the Pearson's product moment correlation between the body sizes (SVL) of males and females found in amplexus. I conducted 1000 random permutations of all possible size ratios ($\text{♀ SVL}/\text{♂ SVL}$) to

investigate whether actual size ratios within amplexant pairs differed from random size ratios. As the deviation from the optimum size ratio increases (in either direction) one would predict a non-random pairing pattern that lends support to Licht's (1976) hypothesis that there is an optimum size ratio of amplexant pairs that maximizes fertilization success. The greater the deviation from the optimum (in either direction) the lower the proportion of eggs fertilized. All data analyses were done using SPSS ver. 15.0 and random permutations were done using Microsoft Excel 2003.

RESULTS

Large-male Mating Advantage

Agalychnis callidryas

I captured 153 males of *A. callidryas* on 40 nights for a total of 250 search nights during the three year study period. I counted a mean total of 35 frogs at Elegans pond which measured 15 x 15 m² for a calculated mean population density of 0.16 frogs/m²; Warree pond measured 10 x 12 m² and I counted a mean total of 25 frogs for a mean density of 0.21 frogs/m²; Coral pond at 20 x 25 m² yielded 30 frogs and a density of 0.06 frogs/m² and Aguada pond at 20 x 20 m² yielded 20 frogs for 0.05 frogs/m². The overall operational sex ratio was 3.4 males to one female when females were present.

To determine the effect of seasonality on male size, I conducted a MANOVA. There were significant inter-annual effects on male SVL ($F = 57.195$, $df = 252$, $P < 0.001$; Figure 2.4) and Tukey's HSD post-hoc comparisons reveal that SVL significantly differed between 2004 and 2003 ($P < 0.001$), between 2004 and 2005 ($P < 0.001$) but not between 2003 and 2005 ($P = 0.798$). There were also significant inter-annual effects on

male mass ($F = 5.660$, $df = 147$, $P = 0.004$) and post-hoc comparisons show that mass of males differed between 2003 and 2004 ($P = 0.004$), 2003 and 2005 ($P = 0.014$), but not between 2004 and 2005 ($P = 0.902$). There was also a significant interaction effect of year x mating status of males on SVL ($F = 19.005$, $df = 3$, $P < 0.001$) but not on mass ($F = 0.784$, $df = 3$, $P = 0.505$) illustrating a possible seasonal effect.

There was no difference in mean body size of amplexant males versus non-amplexant males in 2003 and 2005 (Table 2.1). On average, males in 2004 were larger than in either of the other two breeding seasons (Figure 2.4) and there was evidence of a large-male mating advantage because mean SVL of amplexant males were significantly larger than that of non-amplexant males (Table 2.1). There was no difference in mean body mass between amplexant and non-amplexant males for any of the three breeding seasons (Table 2.1).

In none of the 15 trials of the male-displacement experiment, did I observe any attempts by non-amplexant males to supplant amplexant males. At each hourly check, the position of the non-amplexant male was always found in a resting position some distance away from the amplexant pair. In 12 of the 15 trials, original pairs were still found in amplexus six hours later and in three cases the amplexant male was no longer in amplexus and the female was unpaired at that point.

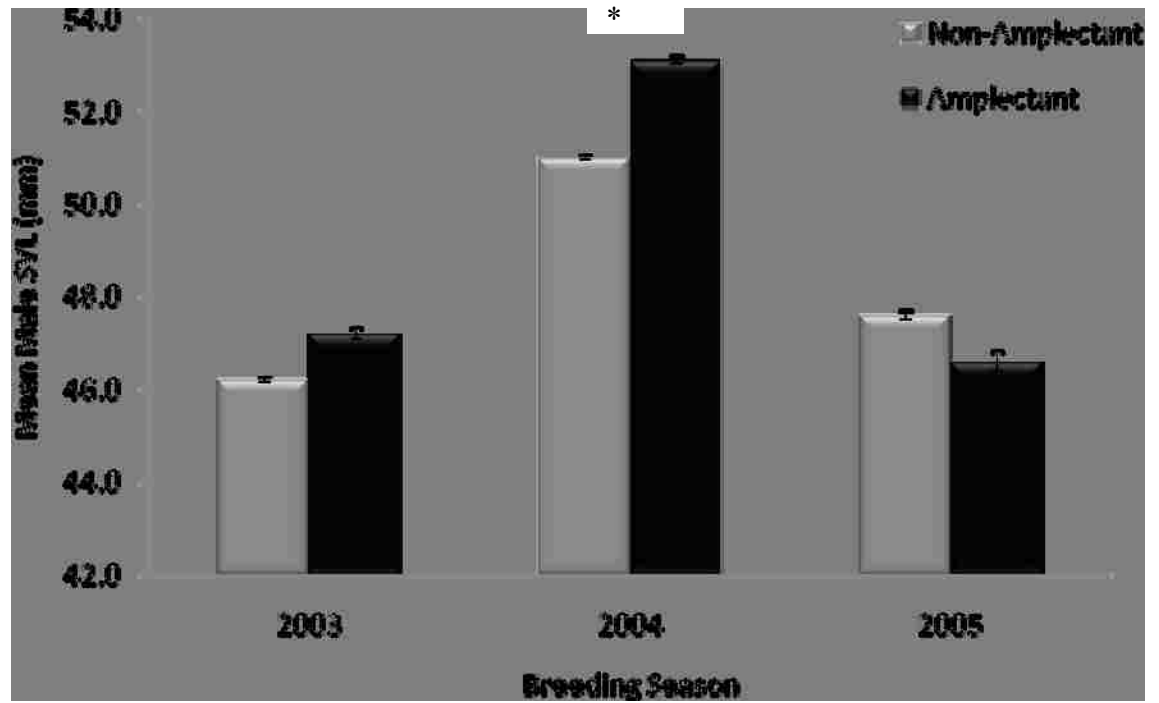


Figure 2.4. Inter-annual effects on mean male SVL for *Agalychnis callidryas* for the three breeding seasons. The mean SVL \pm 1 SE are shown for amplectant males (black bars) and non-amplectant males (gray bars). Amplectant males were significantly larger than non-amplectant males in 2004 ($F = 4.818$, $df = 1$, $P = 0.032$) only.

Table 2.1. Results of one-way analysis of variance of body size traits of *Agalychnis callidryas* between non-amplectant and amplectant males for each breeding season. Values represent mean \pm 1SE for each measure, SVL (mm) and mass (g); * $P < 0.05$.

Size Trait	Non-amplectant (<i>N</i>)	Amplectant (<i>N</i>)	<i>F</i>	<i>P</i>
2003				
SVL	46.2 \pm 0.04 (24)	47.2 \pm 0.10 (10)	1.237	0.274
MASS	3.9 \pm 0.12 (22)	3.9 \pm 0.10 (5)	0.050	0.825
2004				
SVL	51.0 \pm 0.05 (50)	53.1 \pm 0.08 (16)	4.818	0.032*
MASS	4.4 \pm 0.09 (50)	4.5 \pm 0.22 (16)	0.080	0.779
2005				
SVL	47.6 \pm 0.10 (13)	46.6 \pm 0.20 (40)	1.785	0.080
MASS	4.6 \pm 0.10 (14)	4.3 \pm 0.11 (41)	1.221	0.227

Agalychnis moreletii

I captured 119 males of *A. moreletii* on 26 nights for a total of 250 nights of the three year study period. Population density for Elegans pond was 0.20 frogs/m²; Warree pond was 0.21 frogs/m²; Coral pond was 0.10 frogs/m² and Aguada pond had a density of 0.07 frogs/m². On nights when females were present, the operational sex ratio was 4.5 males to each female.

There were significant inter-annual effects on male SVL ($F = 13.391$, $df = 2$, $P < 0.001$; Figure 2.5) and Tukey's HSD post-hoc comparisons reveal that SVL significantly differed between 2004 and 2005 ($P < 0.001$), but sample size for 2003 was too small to allow pairwise comparisons. There was a significant inter-annual effect on male mass ($F = 6.686$, $df = 2$, $P = 0.002$) between the 2004 and 2005 breeding season ($P = 0.024$). There was a significant interaction effect of year x mating status of males on SVL ($F = 5.811$, $df = 1$, $P = 0.017$) but not on mass ($F = 2.028$, $df = 1$, $P = 0.157$).

In 2003, I collected only two pairs of this species and I was therefore unable to make comparisons. During the 2004 breeding season, male frogs were also larger than other breeding seasons and I found evidence of a large-male mating advantage. Amplectant males were significantly larger than non-amplectant males (Table 2.2; Figure 2.5). In 2005, I found no size difference between amplectant and non-amplectant males (Table 2.2; Figure 2.5). I detected no difference in mean body mass between amplectant and non-amplectant males in 2003 and 2004. However in 2005, amplectant males were significantly heavier than non-amplectant males (Table 2.2; Figure 2.5).

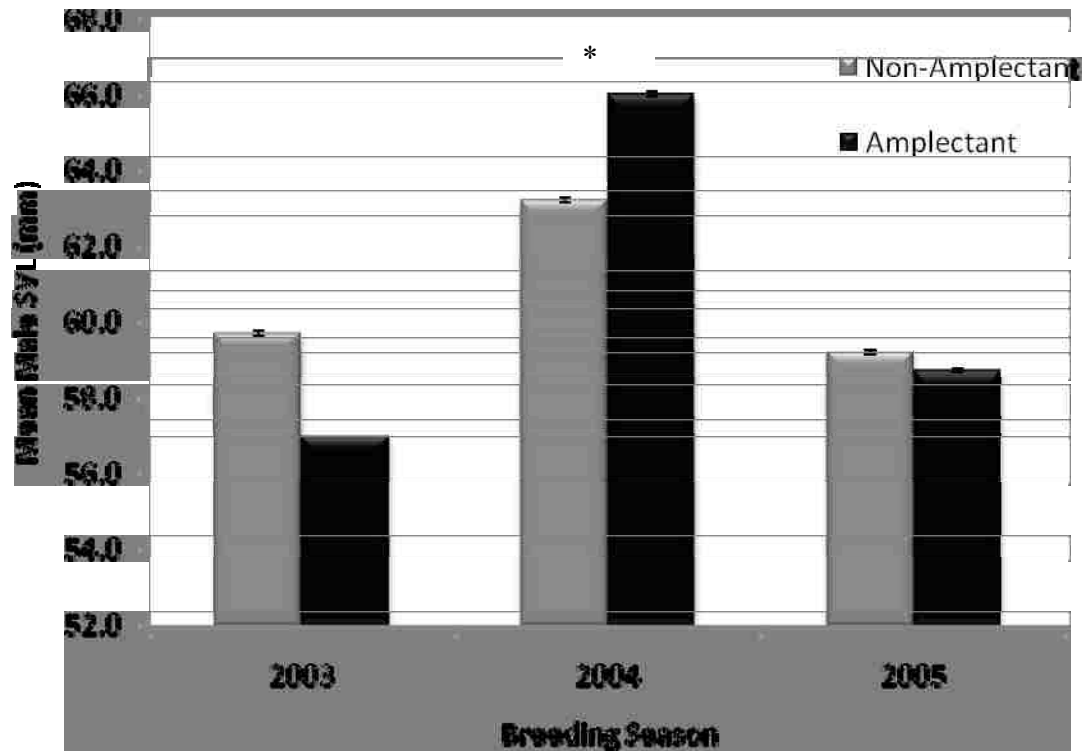


Figure 2.5. Inter-annual effects on mean male SVL for *Agalychnis moreletii* for the three breeding seasons. The mean SVL \pm 1 SE are shown for amplectant males (black bars) and non-amplectant males (gray bars). Amplectant males were larger than non-amplectant males in 2004 ($F = 5.337$, $df = 1$, $P = 0.027$) only.

Table 2.2. Results of one-way analysis of variance of body size traits of *Agalychnis moreletii* between non-amplectant and amplectant males for each breeding season. Values represent mean \pm 1SE for each measure, SVL (mm) and mass (g); * $P < 0.05$; NA = not available.

Size trait	Non-amplectant (<i>N</i>)	Amplectant (<i>N</i>)	<i>F</i>	<i>P</i>
2003				
SVL	59.7 \pm 0.06 (37)	57.0 \pm 0.00 (2)	1.250	0.280
MASS	7.5 \pm 0.20 (37)	NA (0)	NA	NA
2004				
SVL	63.2 \pm 0.07 (24)	66.0 \pm 0.07 (10)	5.337	0.027*
MASS	8.5 \pm 0.30 (24)	8.3 \pm 0.31 (10)	0.237	0.630
2005				
SVL	59.2 \pm 0.06 (25)	58.7 \pm 0.04 (43)	0.604	0.440
MASS	7.7 \pm 0.16 (25)	8.1 \pm 0.13 (43)	4.533	0.037*

Size-assortative Mating

Agalychnis callidryas

Across the three breeding seasons, the body size (SVL) of males and females were positively correlated within amplexant pairs (Pearson's product moment $r = 0.704$, $N = 64$, $P < 0.001$; Table 2.3). The mean SVL ratio between amplexant pairs for all three years was 1.30 ± 0.01 ($N = 64$; see Figure 2.6) and permutation results show that the size ratios within pairs were significantly different from random when the three breeding seasons were combined ($P = 0.001$). I investigated individual breeding seasons to determine annual responses. In 2003, there was a strong positive association between female and male body size within amplexant pairs ($r = 0.868$, $N = 10$, $P = 0.001$; Table 2.3). Larger females tended to be paired with larger males. The mean female: male SVL ratio was 1.34 ± 0.02 SE ($N = 10$). Thus, I found evidence of positive assortative mating with respect to size in *A. callidryas* for 2003. In 2004, the mean SVL ratio was 1.30 ± 0.02 ($N = 15$) and I found no evidence of a correlation in SVL between members of amplexant pairs (Table 2.3). Similarly, in 2005, I found no correlation between SVL within members of amplexant pairs (Table 2.3) and found the mean SVL ratio to be 1.29 ± 0.01 ($N = 39$).



Figure 2.6. Amplectant pair of *Agalychnis callidryas*. Females are always larger than males and in this study there was a constant size ratio of 1.3 of female body size to male body size throughout the three breeding seasons.

A female's body mass was also positively correlated with the body mass of males with whom they were found in amplexus across the breeding seasons ($r = 0.280$, $N = 59$, $P = 0.049$; Table 2.3). Permutation results show that the body weight ratios between pairs were significantly different and smaller than random pairings across all years ($P = 0.001$). I did not find evidence for annual body weight correlations between amplexant pairs in either of the separate breeding seasons (Table 2.3). Permutation results show that the size ratios within pairs were significantly different from random in 2003 ($P = 0.007$) but not in 2004 or 2005 ($P = 0.401$ and $P = 0.423$, respectively).

Data pooled across the three seasons show that male SVL was not positively correlated with the proportion of eggs fertilized ($r = -0.019$, $N = 105$, $P = 0.787$). There was no significant correlation between the size ratio of amplexant pairs and the proportion of eggs fertilized across breeding seasons ($r = -0.202$, $N = 56$, $P = 0.147$). Fertilization success was less than 100% in only 2 of the 56 clutches.

Table 2.3. Results of Pearson's product moment correlations of mean body size traits within males and females found as amplexant pairs of *Agalychnis callidryas*. Values represent mean \pm 1SE for each measure, SVL (mm) and mass (g); * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Size Trait	Female	Male	<i>N</i>	<i>r</i>	<i>P</i>
2003					
SVL	63.3 \pm 0.20	47.2 \pm 0.10	10	0.868	0.001**
MASS	10.1 \pm 1.32	3.9 \pm 0.10	5	0.297	0.628
2004					
SVL	67.9 \pm 0.06	52.5 \pm 0.06	15	0.084	0.766
MASS	10.4 \pm 0.17	4.3 \pm 0.16	15	0.121	0.669
2005					
SVL	60.2 \pm 0.03	46.4 \pm 0.04	39	0.114	0.489
MASS	9.7 \pm 0.15	4.3 \pm 0.11	39	0.021	0.899
TOTAL					
SVL	62.4 \pm 0.06	48.0 \pm 0.04	64	0.704	<0.001***
MASS	9.9 \pm 0.15	4.3 \pm 0.08	59	0.280	0.049*

Agalychnis moreletii

Female SVL was positively correlated with the SVL of the males with which they were found in amplexus when data for all three breeding seasons were combined ($r = 0.417$, $N = 54$, $P = 0.016$; Table 2.4). Mating was significantly size-assortative by SVL, but not for mass when data for all three breeding seasons were combined (Table 2.4). The mean SVL ratio within pairs for the three combined breeding seasons was 1.26 ± 0.03 ($N = 54$). Permutation tests showed that these size ratios differed significantly from random pairing ($P = 0.001$). I conducted separate analyses to determine within year effects on mating patterns. In 2004, I found no relationship within amplexant pairs for SVL or mass (Table 2.4). The mean female: male SVL ratio was 1.30 ± 0.02 ($N = 9$). However, there was a significant positive relationship between the SVL of amplexant males and the females with which they were paired in 2005 ($r = 0.329$, $N = 43$, $P = 0.031$; Table 4), indicating size-assortative mating for SVL but not for mass. The mean SVL ratio within pairs of 2005 was 1.30 ± 0.02 , $N = 43$. Permutation results show that size ratios within pairs differed significantly from random pairings in 2004 ($P = 0.05$) and 2005 ($P = 0.012$).

I found no significant correlation between male SVL and the proportion of eggs fertilized when data were pooled across breeding seasons ($r = -0.190$, $N = 46$, $P = 0.206$). There was no significant correlation between the size ratio of amplexant pairs and the proportion of eggs fertilized across breeding seasons ($r = 0.173$, $N = 46$, $P = 0.234$). Fertilization success was less than 100% in only 1 of the 46 clutches.

Table 2.4. Results of Pearson's product moment correlations of mean morphometric traits within males and females found as amplexant pairs of *Agalychnis moreletii*. Values represent mean \pm 1SE for each measure, SVL (mm) and mass (g); * $P < 0.05$, NA = not available.

Size trait	Female	Male	<i>N</i>	<i>r</i>	<i>P</i>
2003					
SVL	77.0 \pm 0.50	57.0 \pm 0.00	2	NA	NA
MASS	NA	NA	-	NA	NA
2004					
SVL	83.4 \pm 0.13	66.4 \pm 0.06	9	0.515	0.156
MASS	18.3 \pm 0.69	8.4 \pm 0.32	9	0.112	0.774
2005					
SVL	75.8 \pm 0.07	59.0 \pm 0.04	43	0.329	0.031*
MASS	19.8 \pm 0.39	8.1 \pm 0.13	43	0.132	0.394
TOTAL					
SVL	75.5 \pm 0.17	60.3 \pm 0.05	54	0.417	0.016*
MASS	19.0 \pm 0.33	8.1 \pm 0.13	52	-0.152	0.298

DISCUSSION

Anuran mating patterns are often non-random with respect to body size (reviewed by Halliday and Tejedo 1995) but deviations from random mating can be consistent with a hypothesis of male-male competition, a hypothesis of female mate choice, or a combination of the two. Two competing size-based patterns related to reproductive success have been found in anurans, one in which larger males have a mating advantage (Wilbur et al. 1978) and size-assortative mating such that there is a relatively constant male to female size ratio (Licht 1978). In this study, *Agalychnis callidryas* and *A. moreletii* displayed non-random mating patterns with respect to body size. However, for each species, there was considerable among-year variation in the pairing pattern. In 2004, frogs of both species were larger in comparison to 2003 and 2005, and amplexant males were significantly larger than non-amplexant males for both species, consistent with there being a large-male mating advantage in that year. In the 2003 and 2005 breeding seasons, however, amplexant and non-amplexant males did not differ in body size. In contrast to 2004, SVLs of amplexant pairs of *A. callidryas* were positively correlated in 2003, and the same was true for *A. moreletii* in 2005, evidence of assortative mating by body size.

In animals that exhibit indeterminate growth, including anurans, large body size may signify older age, better resource-accruing capabilities, superior fitness and the ability to survive fluctuating climatic conditions (Sullivan et al. 1995). Larger males may also have more sperm for fertilization (Smith-Gill and Berven 1980). Thus, females that mate with large males may maximize the proportion of their eggs that are fertilized (Wilbur et al. 1978; Berven 1981). However, effective fertilization may require that

cloacae be properly juxtaposed during amplexus; if a male is too large or too small relative to the female, the cloacal apposition is not efficient with the result that the proportion of eggs fertilized may be reduced (Licht 1976; Figure 2.1). This line of reasoning predicts a positive size-association within pairs. The size of successful males should depend upon the size of females and the optimal size ratio between individuals or pairs (Licht 1976). In the present study, both *A. callidryas* and *A. moreletii* maintained the same average size ratio of 1.3 between females and males in amplexus across sampling years however fertilization success was independent of body size ratios. These results do not support Licht's (1976) optimal size ratio hypothesis.

These results suggest that females mate with males whose SVL insures an optimal size ratio within pairs. There was nearly 100% fertilization success in both *A. callidryas* and *A. moreletii*, suggesting that males may not be sperm limited and male size is not correlated with the ability to fertilize an entire egg complement as has been suggested in some other species (Howard 1978; Kruse 1981; Howard and Kluge 1985; Krupa 1988). My results indicate that the body size ratio of amplexant pairs does not necessarily influence fertilization success as has been found by others (Davies and Halliday 1977; Ryan 1985; Gibbons and McCarthy 1986; Bourne 1993). These contrasting sets of observations may illustrate an alternative and combined hypothesis that postulates a large-male mating advantage, provided the male's size does not exceed an optimal size ratio within pairs. While this is a plausible explanation for the current findings, it does not exclude other contributing factors.

The large-male mating advantage and the size-assortative mating detected in these two species are consistent with the exercise of choice by females, but in this study female choice cannot be separated from male-male competition for access to females (Shuster and Wade 2003); both may be acting in concert (Wilbur et al. 1978). Over the course of 250 nights of observation spanning three field seasons, I found little evidence of male-male competition in *A. callidryas*. Males were found calling within a few meters of amplexant pairs with no attempt to displace the successful male (pers. obs., 2003-2005).

The results of my male-displacement trials in 2003 and 2004 showed that non-amplexant males did not attempt to supplant amplexant males from females as has been observed in other temperate species (Lamb 1984; Howard and Kluge 1985). However, at the considerably higher population densities found at Gamboa, Panama, males often engage in male-male combat and male-takeovers are commonplace (pers. obs., pers. comm. M.S. Caldwell). The intensity of male-male competition may be density-dependent in *A. callidryas* and female choice may be obscured at higher densities such as at Ocelot pond, Gamboa (pers. obs.; pers. comm. K.M. Warkentin), than in small populations, such as at Las Cuevas, where female mate choice may play a greater role in determining pairing patterns.

In *A. moreletii* at Las Cuevas, however, there may be more opportunity for male-male competition because of a higher operational sex ratio and higher population densities of this species. The strong male-biased operational sex ratio produces more intense male-male competition (Emlen 1976; Kvarnemo and Ahnesjö 1996). Individuals of *A. moreletii* seem to arrive at the ponds in much larger numbers than *A. callidryas* and tend to have a more protracted breeding season as they are often the only species found

later in the rainy season at Las Cuevas (August 25; pers. obs.). In marked contrast to *A. callidryas* I observed intense male-male combat in this species in the field where nine males of *A. moreletii* were congregated on a single wooden stick located in the center of a small pond. Males appeared to be grappling for the highest position on the stick. Given the evidence of male-male agonism detected in *A. moreletii*, the large-male mating advantage apparent in 2004 may well be a consequence of male-male competition, although a role for female choice cannot be excluded. In this case, larger males presumably have a higher probability of winning agonistic encounters, thereby increasing their access to females. This may obscure evidence for female choice. More data are needed to quantify the importance of male-male competition during the breeding season for *A. moreletii*.

Although *A. callidryas* and *A. moreletii* are morphologically similar and occupy similar habitats, they appear to exhibit different behavioral strategies with respect to their reproductive biology. In *A. callidryas*, which occurs at relatively low population densities in Belize, female choice may be relatively more important, whereas in *A. moreletii* male-male competition may be most important, particularly at the high population densities found in this study. This begs the question why the pattern is not consistent from year to year within a species.

The relative importance of female mate choice and male-male competition may fluctuate with changing climatic conditions as is obvious from the significant inter-annual effects on both mating patterns and male sizes. In 2003 the rains came on June 1st and in 2005, the first rains came on June 8th. In both years, aguadas and bajos at the study site filled with water. In contrast, 2004 was one of the driest “rainy” seasons on record in the

greater Maya Mountains (Belize Weather Bureau). The onset of the rainy season was delayed for over a month, and aguadas that usually contained water were dry until the first rains arrived on July 10th. In 2004 both species occurred at lower densities, and calling rates were well below those of the other two years and amplexant males were larger than calling, non-amplexant males in both species. I found small aggregations of three to five individuals per pond that usually house at least 15-20 calling males; these males generally called intermittently from branches higher up in the canopy (> 10m). I collected four amplexant pairs on a total of five nights throughout the season until the first night of heavy rain. Egg clutches that were produced throughout the breeding season (but prior to the later heavy rains) were often found desiccated, preyed upon, or if developing, there was no standing water beneath them for hatchlings to fall into.

The reproductive patterns, such as timing and length of the breeding season of frogs are largely influenced by rainfall and temperature (Crump 1974) and reflect life cycle adaptations (Duellman and Trueb 1986). Typically, reproductive activities are seasonal with most species breeding during the rainy months (Aichinger 1987; Bertoluci 1998; Bertoluci and Rodrigues 2002; Kopp and Eterovick 2006). In this study, the lengthy dry period of 2004 may have exerted stress on *A. callidryas* and *A. moreletii* and may have influenced behavioral patterns, reducing the numbers of calling males at ponds.

Vocalizations are energetically costly to males and in gray treefrogs, *Hyla versicolor*, long calls are more costly to produce than short calls and are linked to climatic conditions (Taigen and Wells 1985; Wells and Taigen 1986; Grafe 1997; Welch 2003). Males that produce long calls spend less time calling per night (Wells and Taigen 1986) and attend fewer choruses per season (Sullivan and Hinshaw 1992). Individuals of

Eleutherodactylus coqui reduce calling and minimize activity in response to evaporative water loss during periods of drought (Pough et al. 1983; Beuchat et al. 1984). Several tropical frog species, including hylid species significantly alter behavior during dry periods such that male vocalization, and female response to that vocalization, is depressed (Pough et al. 1983; Prado et al. 2005; Kopp and Eterovick 2006). Under such conditions males of lower quality may be more vulnerable to stress, which may in turn constrain their ability to compete for mates. The magnitude of sexual selection may be altered by harsh environmental conditions and may help to explain why amplexant males were larger than non-amplexant males during the 2004 breeding season.

During harsh conditions, male frogs that are of poor quality are likely to incur greater fitness decrements than males of higher quality (Duellman and Trueb 1994). For example, calling activity may be reduced due to small energy reserves, (Taigen and Wells 1985; Wells and Taigen 1986) or males of lower quality may have slower growth rates and thus be of smaller size. As a result, smaller body size may reduce mating success (Kopp and Eterovick 2006). Male vocalizations often carry information about body size, with smaller males emitting calls with higher fundamental frequencies, whereas larger males have lower fundamental frequencies (Ryan 1980; Gerhardt 1988; Tárano and Herrera 2003). Several studies have shown that females preferentially orient to, and move toward, calls of larger males (Ryan 1980; Lopez and Narins 1991; Howard and Young 1998; Murphy and Gerhardt 2000) as a signal of male quality (Sullivan et al. 1995).

The breeding seasons of 2003 and 2005 were considerably wetter than that of 2004. Desiccation-related stress on red-eyed treefrogs was likely less in those years. Under such relatively benign conditions, lower quality males would not differ notably in body size or condition in comparison with males of higher quality. In the breeding seasons of 2003 and 2004, females were paired with males that were not statistically larger than calling, non-amplexant males, but whose SVLs were positively correlated with that of the female, and maintained a constant body size ratio between males and females within pairs. Substantial among-year variation and population density may greatly affect mating patterns of *A. callidryas* and *A. moreletii*. In lean years, females mate with males that are larger on average, yet females also seem to favor a constant size ratio of the male relative to her body size. Both of these Neotropical hylids display a size-based mating pattern, and findings indicate that the magnitude of environmental stress may influence the nature and intensity of sexual selection.

CHAPTER 3

CALL TRAIT VARIATION IN TWO SPECIES OF RED-EYED TREEFROGS, *AGALYCHNIS CALLIDRYAS* AND *A. MORELETII* (ANURA: HYLIDAE, PHYLLOMEDUSINAE) OF BELIZE

INTRODUCTION

Darwin (1871) observed that there was considerable variation in the structure and development of vocal organs in anurans, all of which he proposed was attributable to sexual selection. An advertisement call of a male frog serves both to attract females and to repel competing males (Wells 1988; Ryan and Rand 1993; Halliday and Tejedo 1995). Calls require an immense energetic investment (Taigen and Wells 1985; Wells and Taigen 1986) and are presumably the single most expensive activity that a male frog engages in during his lifetime (Pough et al. 1992; Grafe 1996).

Call intensity and structure can depend on a male's body condition and the information encoded therein may be a signal of genetic quality to females. Females that discriminate between different calls and choose mates based on call properties can exert sexual selection on the males (reviewed Arak 1983; Sullivan et al. 1995). Females may prefer energy-consuming call traits that transmit mate-quality information (Klump and Call characteristics can show substantial variation within a single population (Sullivan and Wagner 1988). Call properties such as call duration, pulse rate and call intensity can vary among males even when contributing factors such as body size and

temperature are held constant (Ryan 2001). Gerhardt (1991) defined call properties as either static (low within-male variation) or dynamic (high within-male variation) as measured by coefficients of variation. Within-male coefficients of variation were based on means and standard deviations calculated from the calls produced during a single recording session for each individual male. He showed that within-male variation for call properties such as pulse rate, call duration, and call rate typically exceeded 20% (dynamic properties) for several populations of 41 different anuran species (Gerhardt 1991). These dynamic properties are much more variable among males than static properties and as a consequence may be more important in mate choice (Gerhardt 1991, 1994).

There is also a range of variation for call properties within an individual that can differ according to physiological state, ecological conditions and social contexts. As a consequence, these selective pressures may affect individual reproductive success and influence call production in other individuals (Castellan and Giacoma 1998).

Within-male variation in call traits is also influenced by individual constraints, most notably in response to body size. One call property that is constrained by body size is fundamental frequency which is considered a reliable size-assessment signal (Ryan 2001; Bee 2002) because the shape and mass of the laryngeal apparatus which determine the fundamental frequency scale with overall body size (Martin 1972).

In frogs, larger body size is negatively correlated with this fundamental frequency which is a better predictor of male size than other call properties (Robertson 1986; Wagner 1989; Bee et al. 1999; Bee et al. 2001).

In addition to body mass, there are several other factors that influence variation in call parameters. Some call properties can be environmentally-constrained, such as pulse rate, which has a strong positive relationship with temperature (reviewed Ryan 2001). Lower temperatures constrain the laryngeal muscle and decrease the number of contractions during call production (Martin 1972; Wells and Taigen 1986). Some call properties can be influenced by both environmental and physiological constraints, in particular call and pulse duration. Generally lower body temperature corresponds to shorter call duration; however, because calling is energetically demanding, call duration is expected to be under strong energetic constraints. Findings reveal a positive correlation between body condition, as an estimate of energy availability, and call duration. Thus, energetic constraints influence variation in pulse and call duration much more than morphological and environmental constraints (reviewed Ryan 2001).

Research continues to determine the functions of acoustic properties of anuran advertisement calls and their relevance to effective communication (Gerhardt 1991; Wagner and Sullivan 1995; Bee 2002; Tárano and Herrera 2003). More recently a multivariate approach has been adopted to investigate the variability of male traits used in the mate selection process by females (reviewed Sullivan and Kwiatkowski 2007).

The results of sexual selection produce a disproportionate mating pattern for males that produce advertisement calls that are more complex and have longer durations, faster rates, and higher intensities relative to the calls of other males (reviewed Ryan and Keddy-Hector 1992). Therefore, amplexant males are expected to have advertisement call traits that are more attractive to females relative to males not found in amplexus.

My study organisms, *Agalychnis callidryas* and *Agalychnis moreletii*, closely related species (Weins et al. 2006; Gomez-Mestre et al. in review), are excellent model species to compare call trait variation both between and among males because they exhibit stereotyped calling behavior used in intra- and inter-sexual interactions. Males of both species descend from the forest canopy and congregate in choruses shortly after sunset and call from vegetation surrounding bodies of water. The advertisement “chack” calls of *A. callidryas* and the “zwoorp” calls of *A. moreletii* function to attract females and both species emit aggressive “chuckling” calls during intra-sexual encounters (Duellman 1970; Pyburn 1970; Lee 1996; M. S. Caldwell pers. comm.). To date, call trait descriptions have been made for both species (Pyburn 1970; Scott 1983; Gray and Rand 1997; Ryan 2001; Savage 2003), but there has been no quantitative assessment of call properties in relation to male mating success. Previous work has documented seasonal fluctuations of a size-related mating advantage for both of these species but with species differences (Briggs Chapter 2). These data provide the opportunity to investigate size-related call traits in two similar Phyllomedusine species (Duellman 1970).

The goals of the present study were to compare call traits between males, to investigate the potential for within-individual variation of call properties and to investigate the presence of size-related call traits in *Agalychnis callidryas* and *Agalychnis moreletii*. Specifically, I sought to quantify variation in call traits among males and to examine the relationship between mating success, and size and call properties across males.

MATERIALS AND METHODS

Study Species and Field Site

This study was conducted at the Las Cuevas Research Station (16° 43' N, 88° 59' W) in the Cayo District located in the Maya Mountains of Belize. The rainy season in this area begins toward the end of May or early June (Belize Weather Bureau) and the area averages approximately 3,000 mm of rainfall per year (Walker 1973). There are several seasonally-filled ponds (bajos), and a few permanent water sources (aguadas) that serve as breeding sites for several anuran species (Fitzherbert et al. 2001). At this field site *A. callidryas* and *A. moreletii* breed during the rainy season from June to August. Females appear at ponds later in the season (mid-June to July, Briggs Chapter 2) after heavy rains. Males of *A. callidryas* engage in aggressive encounters (M. S. Caldwell pers. comm.) and there is anecdotal evidence of aggression in *A. moreletii* (Briggs Chapter 2).

Collection and maintenance

I collected amplexant pairs and calling non-amplexant males of *A. callidryas* and *A. moreletii* at breeding aggregations during the hours of 2200-0300 h on 26 nights between June 8 and August 25, 2005. Males began calling at dusk and females arrived at the pond between 2100-2200 h. Non-amplexant males were collected after amplexant pairs had been located, and thus any differences between amplexant and non-amplexant males are likely to reflect true differences between successful and unsuccessful males in the sample population (Lee and Crump 1981; Howard et al. 1994; Lee 2001).

All individuals were housed in an open-air laboratory in plastic buckets (from which recordings were taken) with 1 liter of standing water at room temperature (23°C). Buckets contained perch sites and leaflets of *Chaemadora elegans* palm, and were covered with hardware cloth. In pilot recording trials, a small sample of amplexant males ($n = 4$) were removed from their mates and calls were recorded. These males were then paired with gravid females and calls were recorded the following night. Calls from individual males were compared between the two nights and they did not differ in call structure or intensity. These results support previous findings suggesting that amplexus allows for energy conservation and does not affect call structure (Dyson et al. 1998). Thus, amplexant males were recorded the night following initial capture after mating was complete and non-amplexant males were recorded upon capture.

Males called from perch sites placed in the bucket and usually called near the top of the bucket. I began recording at 2000 h and recorded the vocalizations of each male for a 10 min. period using a SONY TC-D5M tape-recorder equipped with a Sennheiser K3 directional microphone. To improve recording quality and facilitate acoustic analyses, the microphone was placed on the top of each housing chamber immediately above the target male. The recording level was adjusted and kept constant throughout each recording session. I used a calibrated Radioshack digital sound pressure level meter to measure call intensity (dB) that was kept within a range of 0.3 to 0.5 m. I recorded air and water temperatures within the laboratory on a nightly basis. On the morning following capture, I measured each individual for snout-vent-length (SVL) using dial calipers, read to the nearest 0.5 mm, and mass using an Ohaus Scout Pro fine scale balance read to 0.001 g.

Call analyses

Each sound file was digitized using Audacity ver. 1.2.6 (Mazzonni 2006) and call analyses were conducted using SoundRuler ver. 0.9.6.0 (Gridi-Papp 2007). A call consists of a series of pulses and call duration is measured as the time from the beginning to the end of one discrete call (Cocroft and Ryan 1995). A pulse refers to any change (rise or fall) in amplitude in a call by 1% (Gridi-Papp 2007) and is generally only visible in an oscillogram. Call attributes that were measured included dominant frequency, fundamental frequency, pulse rate and duration, and call rate and duration. Dominant frequency (Hz) is defined as the frequency containing the greatest energy and fundamental frequency is the first harmonic with the lowest frequency (Cocroft and Ryan 1995). Since, the microphone was kept at a constant distance from the calling male during recordings; I assumed that the relative amplitudes of fundamental and dominant frequencies were not affected by frequency-dependent attenuation with distance (Gerhardt 1976). Calling activity traits include call and pulse rates and call and pulse durations (Pröhl 2003). Pulse rate is the number of pulses per minute and pulse duration is the length of a single pulse from onset to cessation. Call rate is the number of calls per minute (Howard and Young 1998; Murphy and Gerhardt 2000). Each male produced several calls within the 10 minute recording time and each call was analyzed for the list of call traits and the mean value of each call trait was used in comparison among males.

Findings in other frog species indicate a female preference for longer duration and faster call rates (Ryan et al. 1992; Sullivan et al. 1995; Gerhardt et al. 1996; Gerhardt et al. 2000; Tárano and Herrera 2003). I measured the SVL and mass of males and compared the vocalizations of large to small males based on the mean values for each

trait to investigate size-related call traits which may help to explain potential variation in call components. I expected larger males to produce calls with lower fundamental frequencies, as has been demonstrated for a number of species of frogs and toads (Ryan 1980; Doty and Welch 2001; Sullivan and Kwiatkowski 2007). I used a multivariate analysis of variance (MANOVA) to investigate the potential relationship among call traits between amplexant and non-amplexant males and used a follow-up non-parametric univariate analysis to investigate individual call traits among males (Sokal and Rohlf 1995). I conducted Spearman's rank correlation analyses to determine if there was a relationship between male SVL, mass, mating status and various call properties. I did a follow-up comparison by grouping males into larger than average and smaller than average sized males, based on the mean SVL and mass measurements for all recorded males and used the mean of all call traits of interest per individual. All statistical comparisons were done with SPSS ver. 15.0.

RESULTS

Agalychnis callidryas

I recorded vocalizations from 11 males of *A. callidryas*, of which 7 were collected in amplexus and 4 were non-amplexant males. Recorded males ranged in SVL from 45.4 to 49.0 mm and from 3.7 to 5.6 g in body mass. Call frequency ranged between 1741 Hz and 2012 Hz (mean = 1882 Hz) and had a pulsed structure (Figure 3.1A). Call intensity ranged from 52 – 68 dB (Table 3.1). Considerable variation existed among males for several call traits, including total number of calls, call duration and pulse rate (Table 3.1). Variation in these call properties was higher for call and pulse rates than for

dominant frequency and fundamental frequency (Table 3.1). Each male produced multiple vocalizations ranging from 7 to 49 calls within a recording period and each call was typically comprised of several pulses ranging from 1 to 14 pulses per call (see Figure 3.1A).

Amplectant males did not differ from non-amplectant males in SVL (Mann-Whitney $U = -0.945$, $n = 11$, $P = 0.345$; Table 3.2) nor in body mass in this sample ($U = -0.378$, $n = 11$, $P = 0.705$, Table 3.2). A multivariate analysis of variance showed that none of the call properties differed between amplectant and non-amplectant males (MANOVA $F = 20.323$, $df = 4$, $P = 0.175$; Figures 3.2A-3.2C). Follow-up univariate comparisons of each call trait produced similar results (all $P > 0.25$; Table 3.2). Spearman's rank correlation analysis showed that there was a relationship between SVL and mass (Spearman's $r = 0.609$, $n = 11$, $P = 0.047$). Neither SVL nor mass of males were correlated with call traits (all $P > 0.05$, Figures 3.3A and 3.4A).

Table 3.1. Variation of call traits between males of *Agalychnis callidryas* and *Agalychnis moreletii*. *N* = total number of recorded males followed by total number of calls analyzed, CV = coefficient of variation, total calls = mean number of calls per individual, rate = mean total number of calls per minute, total pulses = mean number of pulses per call, DF = mean call dominant frequency, FF = mean call fundamental frequency, and call intensity as MinAmp = mean minimum call amplitude, and MaxAmp = mean maximum call amplitude.

Property	<i>N</i>	Mean \pm SD	Range	CV (%)
<i>Agalychnis callidryas</i>				
<i>Call Characteristics</i>				
Total calls	11 (271)	24.6 \pm 13.3	7.0 - 49.0	54.1
Rate (#/min)	11 (271)	2.3 \pm 1.4	0.6 - 5.4	60.9
Duration (ms)	11 (271)	55.0 \pm 15.7	29.3 - 71.9	28.7
MinAmp (dB)	11 (271)	56.4 \pm 3.5	52 - 63	6.2
MaxAmp (dB)	11 (271)	62.4 \pm 3.2	58 - 68	5.1
<i>Pulse Characteristics</i>				
Total pulses	11 (1091)	99.2 \pm 85.8	0 - 283	86.5
Rate (#/min)	11 (1091)	9.9 \pm 7.8	2.2 - 24.9	78.8
Duration (ms)	11 (1091)	1.2 \pm 0.3	0.8 - 1.7	25.0
DF (Hz)	11 (1091)	1882 \pm 102	1741 - 2012	5.4
FF (Hz)	11 (1091)	941 \pm 51	871 - 1006	5.4
<i>Agalychnis moreletii</i>				
<i>Call Characteristics</i>				
Total calls	28 (575)	19.2 \pm 14.8	4 - 68	77.1
Rate (#/min)	28 (575)	1.8 \pm 1.3	0.3 - 6.1	72.2
Duration (ms)	28 (575)	38.8 \pm 18.4	21.6 - 87.8	47.4
MinAmp (db)	28 (575)	54.2 \pm 3.4	50 - 62	6.3
MaxAmp (dB)	28 (575)	60.6 \pm 5.0	53 - 68	8.3
<i>Pulse Characteristics</i>				
Total pulses	28 (2362)	76.1 \pm 97.9	4 - 436	128.6
Rate (#/min)	28 (2362)	7.0 \pm 9.0	0.5 - 39.7	128.6
Duration (ms)	28 (2362)	0.5 \pm 0.5	-1.0 - 1.5	100.0
DF (Hz)	28 (2362)	1234 \pm 91	1046 - 1396	7.4
FF (Hz)	28 (2362)	617 \pm 45	523 - 698	7.3

Figure 3.1. Advertisement call of (A): *Agalychnis callidryas* and (B) *Agalychnis moreletii* as (a) an oscillogram, and (b) a sonogram, both of which show the pulsed structure of the call and (c) a power spectrum shows the energy distribution across the call.

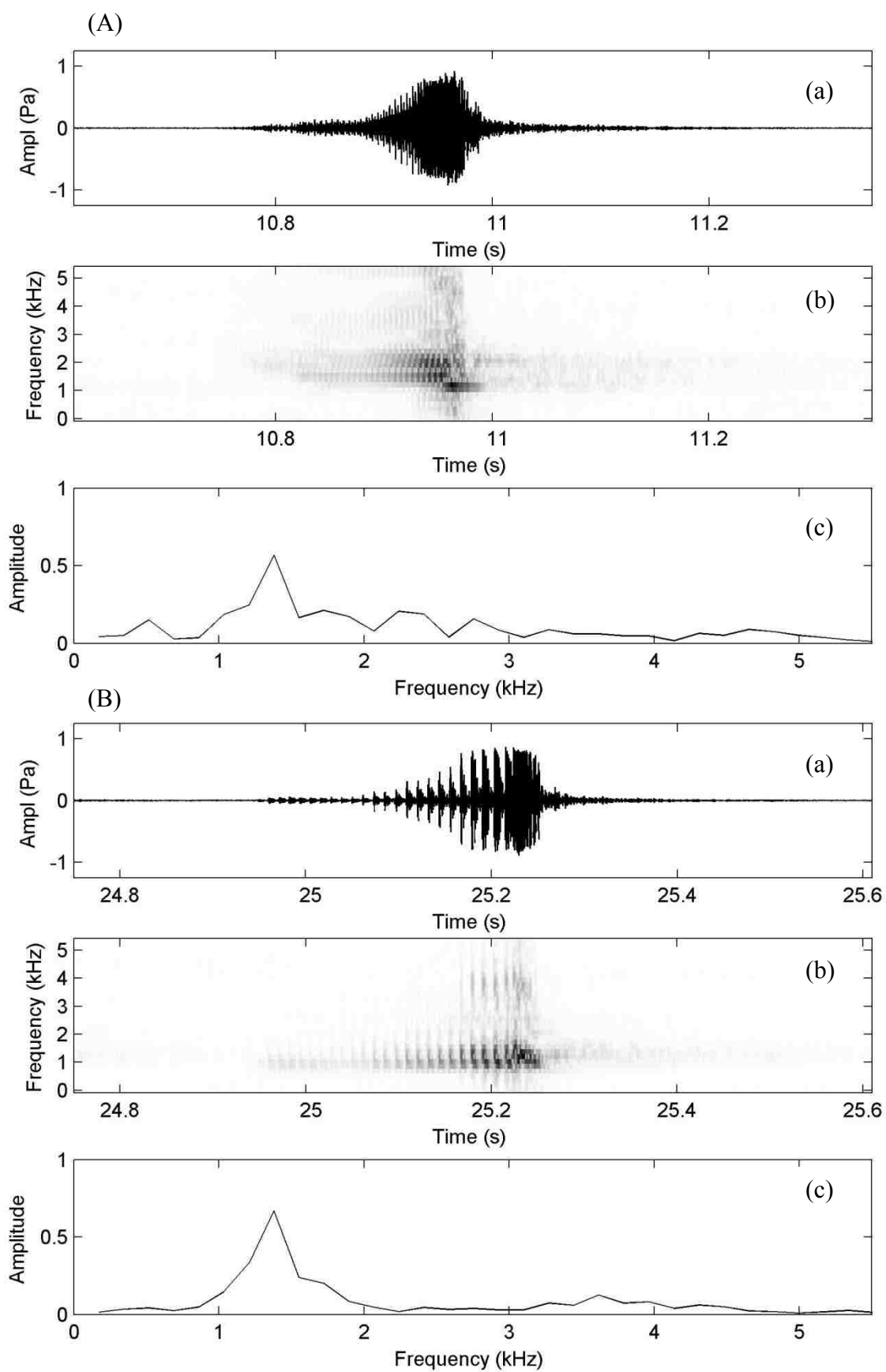
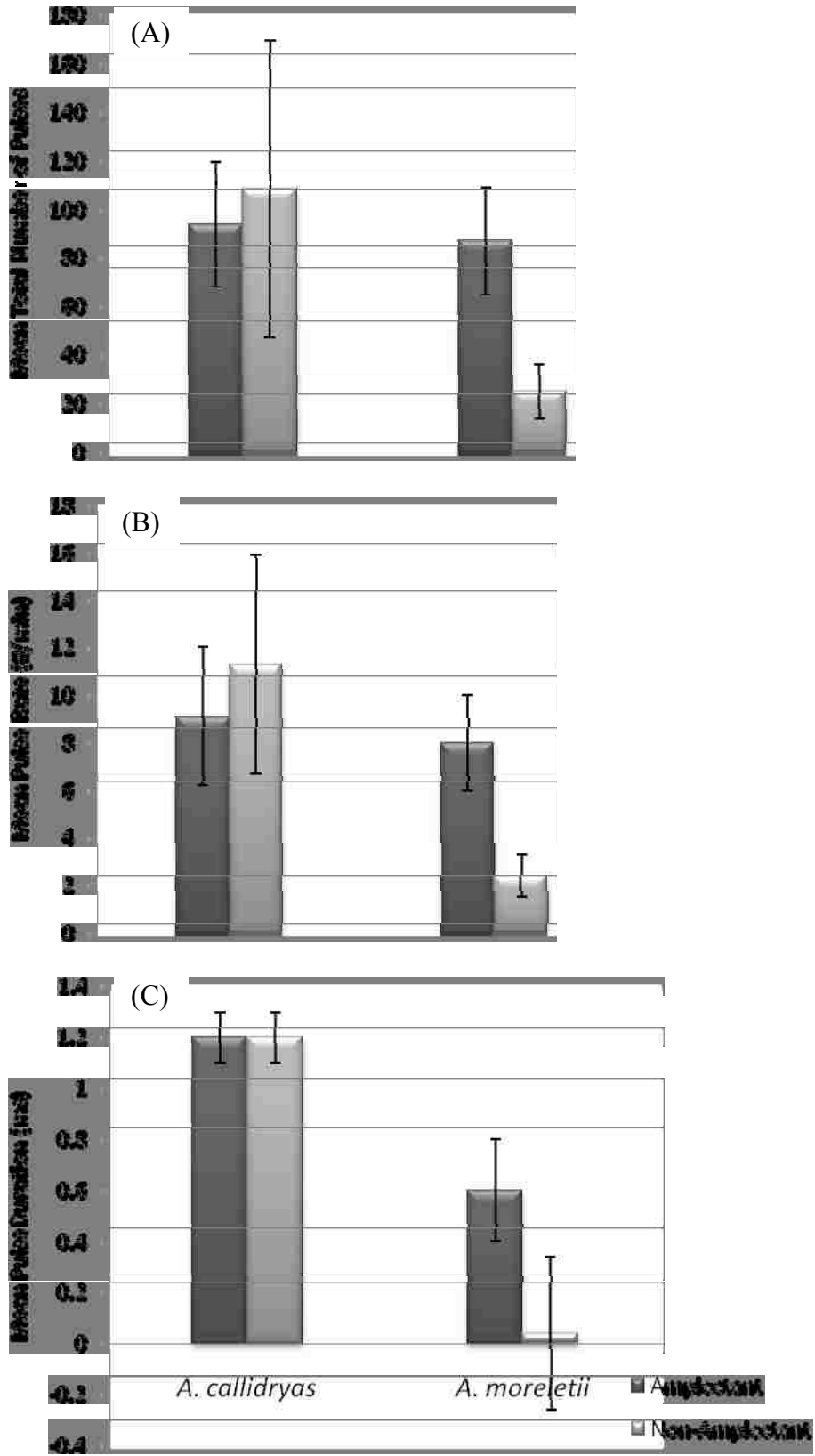


Table 3.2. Mann-Whitney U results on call properties of amplexant and non-amplexant males of *Agalychnis callidryas* and *Agalychnis moreletii*. SVL = snout-vent-length, total calls = mean number of calls per individual, call rate = mean total number of calls per minute, total pulses = mean number of pulses per call, pulse rate = mean total number of pulses per minute, DF = mean call dominant frequency, FF = mean call fundamental frequency, and call intensity as MinAmp = mean minimum call amplitude, and MaxAmp = mean maximum call amplitude.

Property	Amplexant male	Non-amplexant male	<i>U</i>	<i>P</i>
<i>Agalychnis callidryas</i> (n = 7)		(n = 4)		
SVL (mm)	47.6 ± 0.4	47.1 ± 0.7	-0.945	0.345
Mass (g)	4.6 ± 0.2	4.8 ± 0.3	-0.378	0.705
Total calls	24.0 ± 5.8	25.8 ± 5.3	-0.475	0.635
Call rate (#/min)	2.3 ± 0.6	2.3 ± 0.4	-0.567	0.571
Call duration (ms)	47.7 ± 10.0	59.2 ± 4.8	-1.134	0.257
Total pulses	93.9 ± 25.8	108.5 ± 61.3	-0.189	0.850
Pulse rate (#/min)	9.1 ± 2.9	11.3 ± 4.6	-0.378	0.705
Pulse duration (ms)	1.2 ± 0.1	1.2 ± 0.2	-0.567	0.571
DF (Hz)	1874 ± 41	1897 ± 51	-0.567	0.571
FF (Hz)	937 ± 21	948 ± 26	-0.567	0.571
MinAmp (dB)	55.9 ± 0.8	57.3 ± 2.8	-0.285	0.775
MaxAmp (dB)	62.4 ± 1.0	62.3 ± 2.2	-0.286	0.775
<i>Agalychnis moreletii</i> (n= 23)		(n = 5)		
SVL (mm)	58.7 ± 1.0	58.7 ± 0.4	-0.090	0.928
Mass (g)	8.0 ± 0.1	8.0 ± 0.5	-0.187	0.851
Total calls	21.0 ± 3.2	11.2 ± 3.2	-1.532	0.126
Call rate (#/min)	1.9 ± 0.3	1.1 ± 0.3	-1.230	0.219
Call duration (ms)	40.8 ± 4.0	29.9 ± 4.4	-1.350	0.177
Total pulses	87.2 ± 22.0	25.0 ± 11.1	-1.950	0.051*
Pulse rate (#/min)	8.0 ± 2.0	2.4 ± 0.9	-1.950	0.051*
Pulse duration (ms)	0.6 ± 0.1	0.04 ± 0.3	-2.381	0.017*
DF (Hz)	1241 ± 19	1199 ± 42	-0.605	0.545
FF (Hz)	620 ± 9	600 ± 21	-0.605	0.545
MinAmp (dB)	53.5 ± 0.7	59.5 ± 2.5	-1.918	0.055*
MaxAmp (dB)	60.2 ± 1.3	64.0 ± 3.0	-0.847	0.397

Figure 3.2. Histograms of a subset of call traits between amplexant males (dark bars) and non-amplexant males (light bars) of *Agalychnis callidryas* (depicted on the left) and *A. moreletii* (depicted on the right). Each histogram illustrates the comparison of a different call trait: (A) mean total number of pulses produced per call; (B) mean pulse rate as the mean total number of pulses produced within one minute; (C) mean pulse duration within a call. For each of the three call traits, represented, amplexant males of *A. moreletii* produced a greater total number of pulses, had a faster call rate and had pulse rates that were of a longer duration relative to those produced by non-amplexant males.



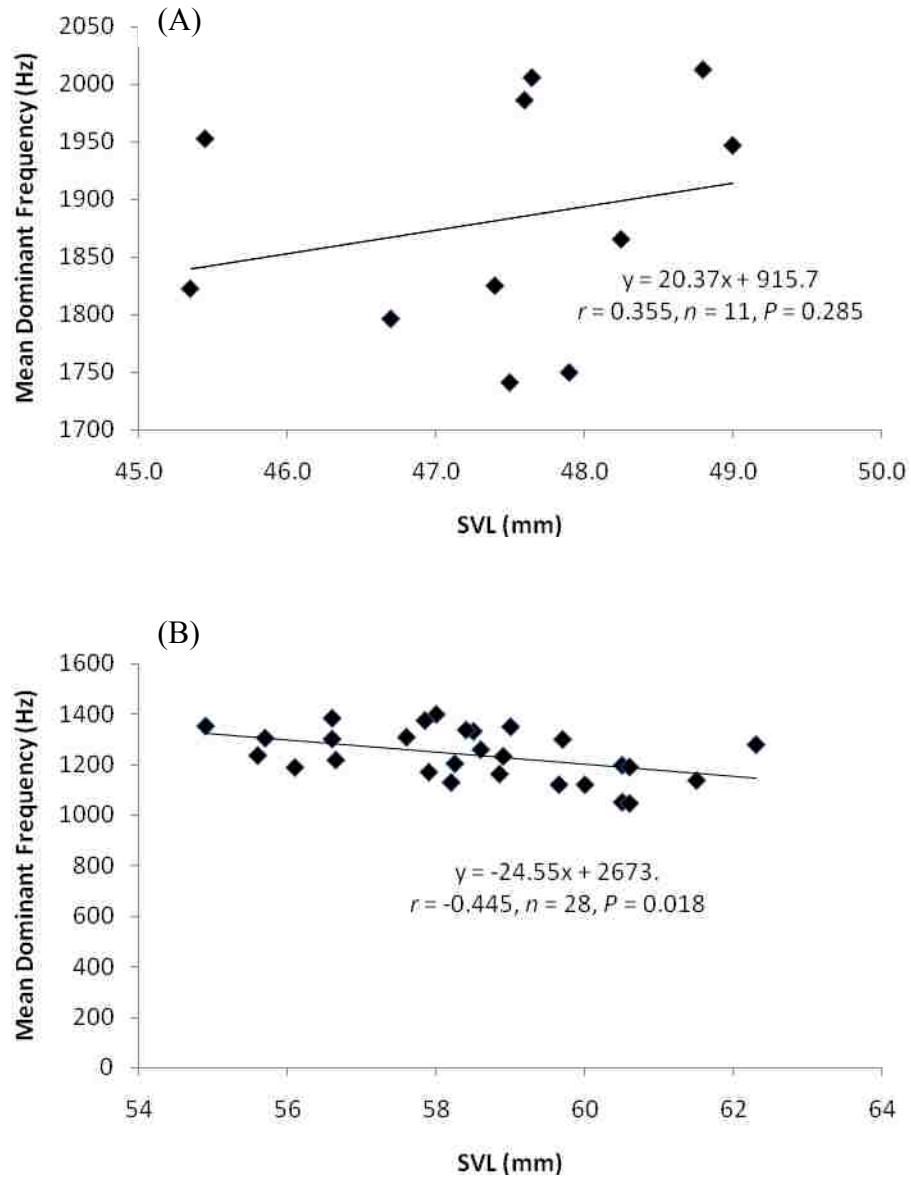


Figure 3.3. Call dominant frequency in relation to male SVL in *Agalychnis callidryas* (A) and in *Agalychnis moreletii* (B) with regression coefficients and a best fit line.

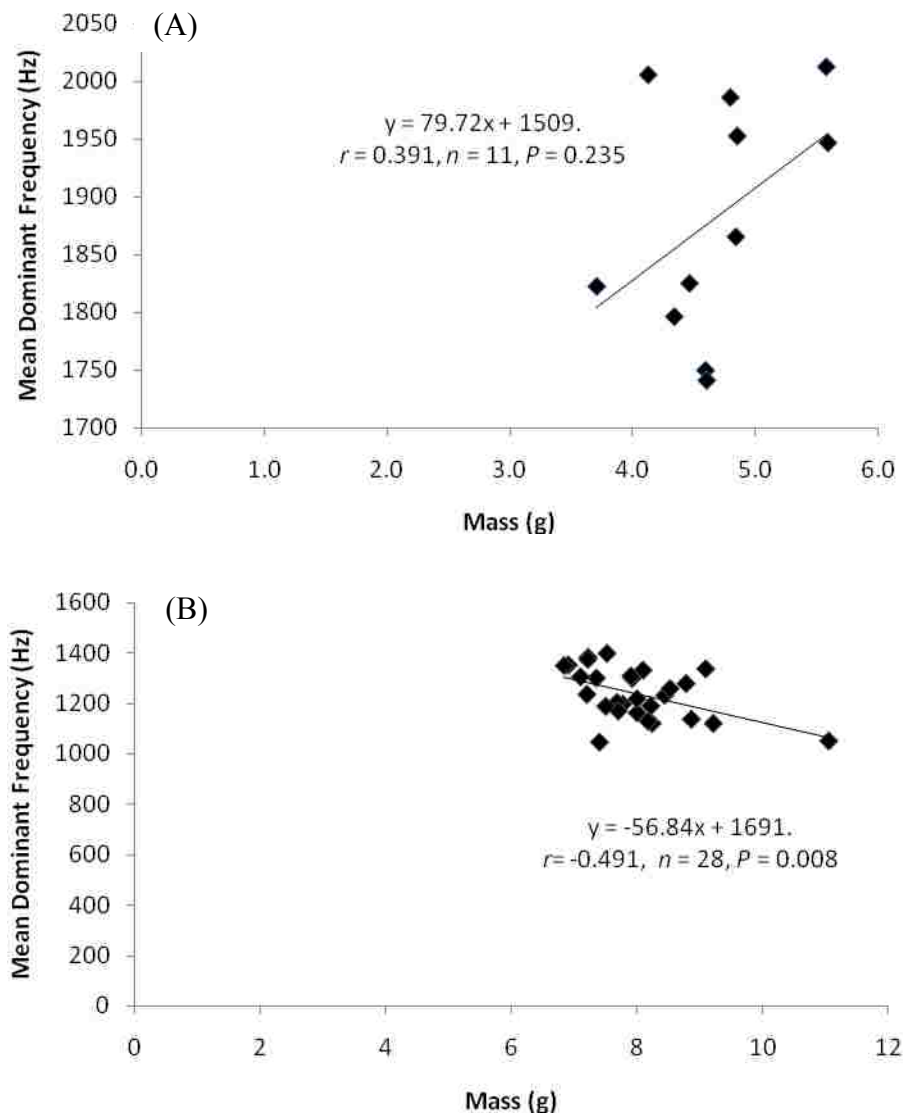


Figure 3.4. Call dominant frequency in relation to male body mass in *Agalychnis callidryas* (A) and *Agalychnis moreletii* (B) with regression coefficients and a best fit line.

A Spearman rank correlation analysis showed that dominant frequency was positively correlated with total number of calls ($r = 0.735$, $P = 0.010$), call rate ($r = 0.709$, $P = 0.015$) and pulse rate ($r = 0.627$, $P = 0.039$). Call duration was also positively correlated with total number of pulses ($r = 0.673$, $P = 0.023$), and pulse duration was correlated with pulse rate ($r = 0.645$, $P = 0.023$).

Agalychnis moreletii

I obtained recordings from 28 males, including 23 amplexant males and 5 non-amplexant males. SVL ranged from 54.9 to 63.6 mm and mass ranged from 6.8 to 9.4 g. Call frequency ranged between 1045 Hz and 1396 Hz (mean = 1882 Hz) comprised of a series of pulses (Figure 3.1B) and call intensity ranged from 50 – 68 dB (Table 3.1). The total numbers of calls and pulses and call and pulse rates were highly variable between males in comparison to call duration. As with *A. callidryas* dominant and fundamental frequencies and call intensity were the least variable call traits (Table 3.1). Males produced multiple vocalizations per recording period ranging from 4 to 68 calls that contained 1 to 22 pulses per call.

Amplesant males were neither larger (Mann-Whitney $U = -0.090$, $P = 0.928$; Table 3.2) nor heavier ($U = -0.187$, $P = 0.851$; Table 3.2) than non-amplexant males. A multivariate analysis of variance showed that there was a trend toward a difference in the call properties amongst males (MANOVA $F = 4.098$, $df = 9$, $P = 0.067$). Follow-up univariate comparisons of each call trait produced showed that amplexant males produced a greater total number of pulses (87.2 ± 21.8) than non-amplexant males (25.2 ± 11.1) that was virtually statistically significant ($U = -1.950$, $P = 0.051$; Table 3.2;

Figure 3.2A). Pulse rate also tended to differ significantly between amplectant and non-amplectant males ($U = -1.950$, $P = 0.051$; Table 3.2; Figure 3.2B). Males found in amplexus (a) also produced pulses with longer duration than non-amplectant males (na) ($a = 0.64 \pm 0.1$ ms, $na = 0.04 \pm 0.3$ ms, $U = -2.381$, $P = 0.017$; Table 3.2; Figure 3.2C). Amplectant males also called at a lower minimum sound intensity in comparison to non-amplectant males (Table 3.2). Other call properties did not differ between amplectant and non-amplectant males (all $P > 0.10$; Table 3.2).

A Spearman rank correlation analysis showed that both SVL and mass were negatively correlated with dominant frequency ($r_{svl} = -0.445$, $n = 28$, $P = 0.018$; Figure 3.3B; $r_{mass} = -0.491$, $n = 28$, $P = 0.008$; Figure 3.4B) and fundamental frequency ($r_{svl} = -0.495$, $n = 28$, $P = 0.007$; $r_{mass} = -0.491$, $n = 28$, $P = 0.008$). A closer comparison of size-related traits showed that larger than average males by both SVL and mass (based on the mean) produced vocalizations with significantly lower dominant frequency (1164 ± 25 Hz) than smaller males (1262 ± 19 Hz; $U = -2.079$, $P = 0.038$). Larger males also produced calls with a lower fundamental frequency ($U = -2.518$, $P = 0.012$). Other call traits were not correlated with male size or mass (all $P > 0.05$). Among call traits, there were significant correlations between call rate and call duration ($r = 0.610$, $P = 0.001$); dominant frequency and call duration ($r = 0.556$, $P = 0.002$); fundamental frequency and call duration ($r = 0.558$, $P = 0.002$); call rate and dominant frequency ($r = 0.431$, $P = 0.022$); and pulse rate and dominant frequency ($r = 0.476$, $P = 0.011$).

DISCUSSION

The results of this study reveal individual variation in male calling behavior for both species. My study shows that (1) there is considerable variation in the call properties within and among males, (2) there are size-related call properties in *Agalychnis moreletii*, i.e. dominant and fundamental frequencies, (3) calling activity traits are the most variable traits among males, (4) dominant and fundamental frequencies and call intensity are the least variable traits among males, and (5) pulse rate and call duration may be important traits that influence mating success, measured by amplectant versus non-amplectant status, in *Agalychnis moreletii* but not *A. callidryas* (Table 3.2).

The subset of recorded males used in this study show that amplectant males of neither *A. callidryas* nor *A. moreletii* differed significantly in body size or mass when compared to non-amplectant males. These findings do not support previous results of mating patterns with these two species of red-eyed treefrogs that show a large-male mating advantage in some breeding seasons (Briggs Chapter 2), and may reflect a small sample size. In *A. moreletii*, however, amplectant males called with increased activity levels as measured by total number of pulses, pulse rate and pulse duration, all presumably energetically costly traits, (Taigen and Wells 1985) in comparison to non-amplectant males. This differential in energy expenditure may be attractive to females and result in males that call with higher energy levels gaining more mating opportunities (reviewed by Halliday and Tejedo 1995; Sullivan and Kwiatkowski 2007) as has been found in several anuran species (Ryan 1983; Sullivan and Hinshaw 1992; Gerhardt 1994; Pröhl 2003; Tárano and Herrera 2003).

In many species of frogs, dominant call frequency is inversely related to male body size, as has been found in several bufonids and hylids (Gerhardt 1991, 1994; Sullivan and Hinshaw 1992; Howard and Young 1998; Searcy and Nowicki 2005). The results of this study show that this relationship holds true for males of *A. moreletii*; larger males produce calls with lower dominant frequencies. Results on *A. callidryas* do not follow this trend, perhaps due to my small sample size. Males of *A. moreletii* called more regularly under the laboratory conditions, in contrast to *A. callidryas*, which tended to assume a non-calling resting posture during recording periods.

Gerhardt (1991, 1994a) described the difference between static and dynamic call properties, of which static properties would be the least variable traits among males and tend to be under stabilizing selection with strong morphological and/or physiological constraints (Castellano and Giacoma 1998). The more variable dynamic properties are typically under directional selection and are mostly under energetic constraints and might therefore serve as an honest indicator of male condition and explain more variation in mating success from one male to another. Static properties are characterized by low coefficients of variation ($CV < 5\%$) and dynamic properties would have CV's in excess of 12%. Results of this study show that dominant and fundamental frequencies and call intensity had the lowest coefficients of variation in both *A. callidryas* and *A. moreletii* and would be characterized as static call properties. This low variation among males for these call properties may reflect body size constraints because dominant and fundamental frequencies depend on the resonance frequency of the vocal cords, and thus on length, mass and tension of the cords (Gerhardt and Huber 2000). Larger males tend to have more massive laryngeal structures and call at lower frequencies relative to smaller males.

Calling intensity is related to the contraction of the trunk muscles. Clearly these call traits are constrained by a male's body size (Martin 1972; Ryan 1975; Robertson 1986; Wagner 1989; Howard and Young 1998; Bee et al. 1999; Zimmitti 1999; Bee et al. 2001) and would indicate an honest signal to females. Similar findings of low coefficients of variation among males for dominant call frequency and call intensity have been found in several other anuran species (Howard and Young 1998; Bosch et al. 2000; Márquez et al. 2001; Pröhl 2003).

By Gerhardt's (1991, 1994) definition, dynamic call properties would include call duration and call rate in these species. Larger males of both species of *Agalychnis* produced call durations similar to those of smaller males, much like the findings for *Bufo americanus* (Howard and Young 1998) and *Hyla versicolor* (Gerhardt et al. 1996). However, although call rate was a highly variable trait within individual males it did not differ among males of either species. Of all call properties measured in this study, males of *A. callidryas* and *A. moreletii* produced a wide range of total number of pulses per call, with the highest coefficients of variation, adding to the findings in several other species (Howard and Young 1998; Bosch et al. 2000; Márquez et al. 2001; Pröhl 2003). This variation in temporal call traits may be influenced by physical conditions as well as the social contexts surrounding calling males but may still transmit mate-quality information (reviewed Howard and Young 1998; Bee et al. 2001; Castellano et al. 2002).

Environmental factors, morphological traits and behavioral contexts can all be contributing factors to the within-individual male variation often observed in call properties. Body temperature typically affects call rate (Wagner 1989) and is often a reflection of water temperature for terrestrial species or air temperature for arboreal

species. In this study, all males perched above water of a standard room temperature that fluctuated between 22.5° and 23° on a nightly basis. Thus, there was no difference in body temperature to influence call rate. Morphological traits such as body size and body mass have the potential to affect call rate and call duration. Larger males with a heavier body mass may have more energy reserves and thus, are in better condition. These males are likely to be able to better “afford” the energetic costs of vocalization and are capable of calling with greater intensity and for longer time periods (Wagner and Sullivan 1995). Though male body size of some species has a positive correlation with call duration, the results of my study with two different species, does not support this because large and small-sized males produced calls of similar call rates and call duration.

Interestingly, a difference in behavioral contexts of *A. moreletii* in this study may help explain the male variation of call properties such as pulse rate and pulse duration among males. Males that were found in amplexus produced a greater number of pulses per call and for a longer duration when compared to non-amplectant males. Increased pulse rates and longer durations may eventually translate into a faster call rate and longer call durations. These may provide females with information regarding motivation, availability, and energy levels, i.e. male quality, particularly since at least one mating had been procured by amplectant males (Dyson et al. 1998).

Calling frequency has been shown to be the most important determinant of male mating success in several species of frogs, which also includes the number of days present and calling at a breeding pond (Arak 1983; Ryan 1983; Sullivan and Hinshaw 1992; Murphy 1994; Pröhl 2003). In several anuran species, females prefer faster calls (Lopez and Narins 1990; Tárano and Herrera 2003), more complex calls (Ryan 1980),

calls of longer duration (Gerhardt et al. 2000; Tárano and Herrera 2003), calls of lower dominant frequency (Ryan 1983; Howard and Young 1998), or a combination of call parameters (e.g. Sullivan 1992; Sullivan & Hinshaw 1992; Welch et al. 1998; Murphy and Gerhardt 2000; Witte et al. 2001).

The results of this study show that in *A. moreletii*, males that were found in amplexus produced call traits of significantly higher energy levels and that dominant and fundamental call frequencies were size-related traits. This is the first study to characterize the variation in call properties of these two species of red-eyed treefrogs and it is the only study to document call properties as potential determinants of mating success. To understand fully the nature of acoustic properties and their role in social interactions, including mating behavior, it is first necessary to investigate individual variation in call production and female preference for that range of variation.

CHAPTER 4

PATERNAL EFFECTS ON LARVAL PERFORMANCE IN RED-EYED TREEFROGS, *AGALYCHNIS CALLIDRYAS* AND *A. MORELETHI* OF BELIZE.

INTRODUCTION

Darwin (1871) identified mate choice as one mechanism by which sexual selection could occur and since then, female mate choice has been documented across numerous taxa (reviewed by Andersson 1994; Sullivan et al. 1995; Shuster and Wade 2003). Sexual selection theory suggests that the variation in male characters is largely responsible for shaping female choice patterns and as a consequence female fitness (Darwin 1871; Trivers 1972; Emlen and Oring 1977; Thornhill and Alcock 1983; Andersson 1994; Searcy and Nowicki 2005).

Two kinds of mating systems exist where there is a female preference for a specific male. In resource-based systems, the evolution of female choice has been clearly demonstrated to be driven by the immediate fitness benefits gained by the female. In these systems, males provide direct benefits to ensure the female's current fitness or the survival of her offspring in the form of food, territory, oviposition sites, nuptial gifts, and paternal care (reviewed Andersson 1994). Females choose males offering superior resources to increase reproductive success.

However, in non-resource based systems, the maintenance of female preference is less clear because males only contribute sperm. In this case, females receive the indirect benefits of genes transmitted to her offspring (Maynard Smith 1978; Kodric-Brown and Brown 1984). Choosy females would then produce offspring with a higher fitness relative to the offspring of less choosy females.

Two models have been proposed to maintain female preference for males in a non-resource based system. The Fisherian process (1930) suggests that female choice is directed toward heritable male traits (Fisher 1958; Trivers 1972; Thornhill and Alcock 1983). The attractive male trait is passed on from the sire possessing the trait to his sons, who in turn become preferred males to “choosy” females in the next generations. Since the choosiest female mates with the most attractive male, the male trait and the preference for that trait become genetically correlated; hence the Fisherian process is also known as “runaway selection” (Kirkpatrick 1982). Under the “good genes” model, or “viability indicator” model of sexual selection, females prefer males with particular fitness characteristics that signify high genetic quality that will improve offspring survival (Zahavi 1975; Hamilton and Zuk 1982; Heisler 1984; Kodric-Brown and Brown 1984; Andersson 1986; and Sullivan et al. 1995). While “good genes” effects are not particularly large, there is a solid body of empirical evidence for female mating preference for indirect benefits of enhanced offspring viability across taxa (Woodward 1987; Reynolds and Gross 1992; Moore 1994; Sheldon et al. 1997; Alatalo et al. 1998; Welch et al. 1998).

Frogs, such as Neotropical hylids offer an excellent system to test hypotheses based upon the “good genes” or “indicator” model because males of most species do not hold territories, provide no parental care, and contribute only sperm to reproduction, and because fertilization is external. Frogs undergo indeterminate growth and a large body size may signify high genetic quality and indicate faster growth rate, better competitive ability, or longer life expectancy (Wilbur et al. 1978). Previous research documents a large-male mating advantage where larger males are more often found in amplexus relative to smaller males (Lee 1986; reviewed by Sullivan et al. 1995; Howard and Young 1998; Briggs Chapter 2) or females respond preferentially to vocalizations of large males (reviewed by Sullivan and Kwiatowski 2007). As a result, females that mate with large males could produce more offspring or superior offspring relative to those sired by males of smaller size (Maynard Smith 1978; Wilbur et al. 1978; Kodric-Brown and Brown 1984).

In anurans, the distinct larval stage provides a discrete time point to measure the effects of paternity on offspring performance typically measured as hatching success, growth and developmental rates, survivorship, and size at metamorphosis. The larval period is a critical stage where mortality can be catastrophic (Wassersug 1973, 1989; Wassersug and Sperry 1977) and offspring with enhanced performance traits as larvae may possess survival advantages that carry over to the post-metamorphic stage. These advantages may account in part for variation evident in body size and size-related acoustic variation in the calls of adult frogs. Female mating preferences, then, may be based on male traits that correlate with larval performance measures.

Larval performance may be an indicator of fitness and provide a link to adult reproductive success. The larval period provides discrete time points and/or tangible anatomical features that can be measured as indicators of growth and development (Altwegg and Reyer 2003).

In this study I test hypotheses based on the “good genes” model of a female preference for large males in two species of Neotropical hylids, *Agalychnis callidryas* and *A. moreletii*. Previous work on these species have documented a large-male mating advantage that is seasonally-dependent (Briggs Chapter 2), so these species provide a model system to investigate female preferences for “good genes”. Females of both of these treefrog species produce moderately large clutch sizes and eggs are large and relatively hardy which facilitates the manipulation of clutches (Warkentin 1995, 1999a, 1999b). I assess the consequences of female choice for larval performance traits by examining whether sire body size results in enhanced offspring viability measured as: 1) fertilization success of egg clutch, 2) hatching success of egg clutch, 3) larval growth, 4) development, 5) survivorship to metamorphosis, and 6) size at metamorphosis. I produced maternal half-sibs by mating a large and a small male to each female. Differences in larval performance between sires can be attributable to genetic differences in paternity because environmental conditions were held constant in the experimental design.

METHODS

Collection and maintenance

Amplectant pairs and calling non-amplectant males of *Agalychnis callidryas* and *Agalychnis moreletii* were collected from breeding aggregations at Las Cuevas Research Station (16° 43' N, 88° 59' W) Cayo District, Belize during the hours of 2200-0100 h on 26 nights between June 8 and August 25, 2005. Males began calling at dusk, with peak calling activity between 2000-0100 and females arrived at the pond between 2100-2200. I collected single males after amplectant pairs had been located; thus any potential differences between amplectant and non-amplectant males are likely to reflect true differences in the sample population (Lee and Crump 1981; Howard et al. 1994; Lee 2001; Briggs Chapter 2). Amplectant pairs were housed in an open-air laboratory in plastic buckets with 3cm of standing water at room temperature (23°C), perch sites, leaflets of *Chaemadora elegans* palm and covered with hardware cloth. The snout-vent-length (SVL) of amplectant males (while in amplexus) and non-amplectant males were measured to the nearest mm using a plastic ruler.

Each amplectant pair was monitored at 30 minute intervals until the first clutch of eggs was deposited (see Figure 4.1) and the pair was separated when the female descended to the water to refill her bladder. The female was rinsed with pond water to remove any remaining sperm, thereby minimizing the chance of subsequent eggs being fertilized by the sperm of the first sire and placed in a different bucket with a second male that was of a different size relative to her first mate. In all cases, females were paired with at least two males to produce maternal half-sibs and the order of the size of males being used was haphazardly assigned.

On the morning following collection, I measured individuals for SVL using dial calipers, read to the nearest 0.5 mm, and mass using an Ohaus Scout Pro balance read to 0.001g. All measurements are mean values followed by one standard error. All individuals were returned to site of capture after measurements were obtained.

Palms leaves with deposited egg clutches were labeled according to parenthood then clipped to the lip of plastic buckets and suspended above 3cm of standing pond water. I counted the number of eggs in each clutch both by eye and then by microscope and misted each clutch daily with pond-water to prevent desiccation.

Each clutch or half-sib was housed separately in plastic buckets and upon hatching (6-7 days) half-sibs were reared under identical laboratory conditions, controlling for larval density and food levels. Tadpoles were housed at a density of 15 tadpoles/liter and fed 0.1g chow and 2g of leaf litter per liter of water during the first month. Food amounts were doubled every month thereafter. Larvae were fed Fluker's powdered tadpole food every two days and each half-sib was provided with a standard mass of leaf litter collected from the natal pond that is presumed to optimize general growth conditions (D. Fenolio pers. comm.). Every third day, 25% of the pond water was replaced in each bucket during the first month and increased to a 50% water change by the second month. Once a week, all housing containers were cleaned and 100% of the water was replaced. Tadpole survivorship of each clutch was simultaneously assessed during this weekly cleaning regime.



Figure 4.1. A typical sub-clutch of eggs of *Agalychnis callidryas* and *A. moreletii*. In a single night while paired with the same male, a female will oviposit a larger complement of eggs that is subdivided into sub-clutches similar to the one above. As a sub-clutch is laid, the amplexant pair descends to the water and the female refills her bladder to lay the next allocation of eggs. A female is usually reproductively active once during the breeding season.

Larval Performance Traits

To investigate maternal investment to each half-sib, I measured ova diameter using a dissecting microscope on the morning following egg deposition. To compare the paternal effects on offspring traits, I measured 1) fertilization success of each sire scored as the proportion of healthy eggs to total oviposited eggs 12 h after deposition occurred. Unfertilized eggs become pale yellow with dark areas after 12 h and have a dimpled yolk surface and collapsed egg capsules (Duellman and Trueb 1986; Briggs Chapter 2). Of the viable eggs, I scored 2) hatching success of each sire when all tadpoles in each half-sib completed the hatching process. I measured a haphazardly selected subset of tadpoles ($N = 10$) from each half-sib for larval performance traits such as 3) hatchling size at one-week post-hatching to obtain an initial growth measurement, and 4) larval growth and 5) development every 15 days until metamorphic climax.

Each tadpole was measured for mass using an Ohaus Scout Pro balance read to 0.001g and staged for development using the Gosner (1960) anuran staging table with the aid of a dissecting microscope. SVL was measured from the tip of the snout to the end of the vent tube and total body length was measured from the tip of the snout to the end of the tail. Both SVL and total body length were measured using an ocular micrometer in the dissecting microscope read to 1.0 micrometer unit and calibrated against a stage ruler. Later when tadpoles ($N = 10$) were large enough, SVL was measured using a pair of dial calipers read to 0.01 mm. All tadpole measurements were done by a blinded observer. Tadpoles undergoing metamorphosis at Gosner stage 40-41 were removed from sibship containers and reared individually in smaller containers placed at an angle to facilitate metamorphic climax and prevent drowning. Tadpoles that completed metamorphosis

into froglets were scored as a proportion of the original clutch for larval performance traits between sires for 6) survivorship to metamorphosis and a subset of these individuals were measured for SVL and mass to determine 7) size at metamorphosis. All froglets and remaining tadpoles were returned to natal ponds at Las Cuevas.

Data analyses

Maternal Investment

Ova diameters were compared across dams for differences in individual maternal investment using a Chi-squared test and then across maternal half-sibs to investigate potential within-individual differences in egg size. A Spearman's rank correlation analysis was used to investigate the potential relationship between the size of dam and ova diameter.

Paternal Effects

I used Spearman's rank correlation analyses to examine whether male body size (SVL) was correlated with hatchling size (SVL, total body length, and mass). I used a one-way analysis of variance to determine whether there was an overall paternal effect of body size on offspring traits. I then compared offspring traits (fertilization success, hatching success, hatchling size [SVL, total length, mass] larval growth, development measured as larval duration, survivorship and size at metamorphosis) between large and small sized sires when mated to the same female. I used a multivariate General Linear Model (GLM): Pillai-Bartlett trace analysis of variance (Olson 1976; Gill 2001) with sire body size (large vs. small) and identity of dam as fixed factor effects and located

potential interaction effects between dam and sire size on offspring traits. I then used a follow-up between subject-effects test to locate the origin of potential differences within maternal half-sibs when controlling for female (Sokal and Rohlf 1995). All data analyses were done using SPSS ver. 15.0.

RESULTS

Maternal Investment

Agalychnis callidryas

Female body mass measures ranged from 8.6 to 9.8 g with a mean of 9.2 ± 0.05 g ($N = 4$) and SVL ranged from 57.8 to 63.1 mm with a mean of 59.8 ± 0.24 mm. Mean ovum size ($N = 80$) amongst the four females was 4.1 ± 0.03 mm ranging from 3.5 to 4.6 mm and there was a significant difference among females for mean ova diameter ($F = 6.202$, $df = 3$, $P = 0.001$). Results of a Spearman's rank correlation show that ova diameter was positively correlated with maternal body size for mass ($r = 0.241$, $N = 4$, $P = 0.031$) and SVL; Figure 4.2).

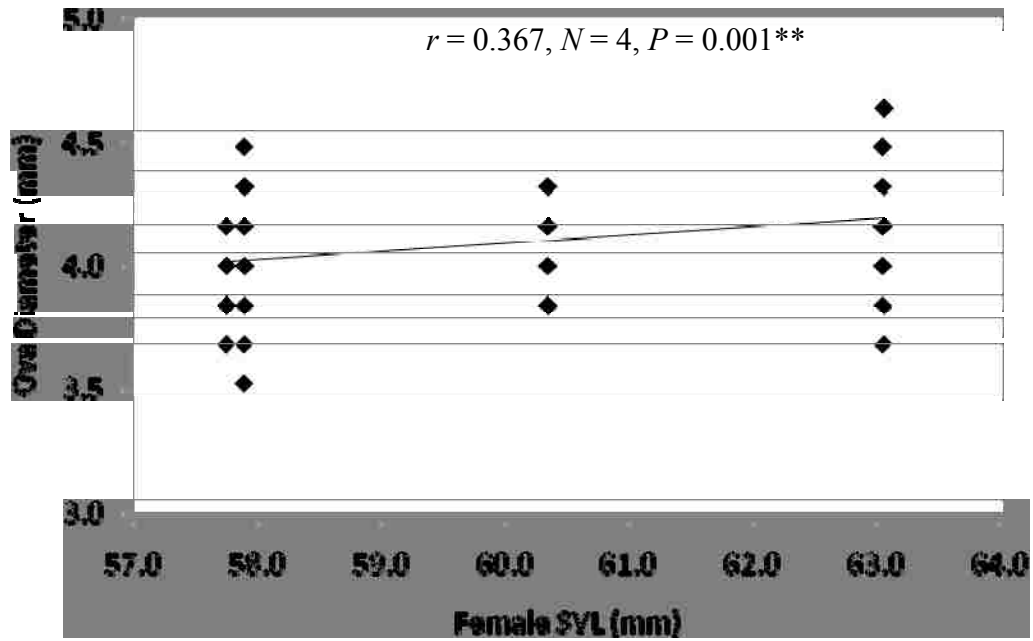


Figure 4.2. Scatterplot depicting the relationship between female body size as SVL ($N = 4$) of *Agalychnis callidryas* and mean ovum diameter ($N = 80$). Female body size is positively correlated with ova diameter; larger females produced eggs with a larger diameter.

Agalychnis moreletii

Mean maternal mass was 19.3 ± 0.40 g ranging from 16.2 to 23.0 g and SVL ranged from 74.2 – 85.0 mm with a mean of 78.1 ± 0.6 mm ($N = 22$). Mean ovum diameter was 4.9 ± 0.03 mm ($N = 560$) with a range of 3.8 to 7.7 mm and there was a significant difference across females for ovum diameter ($F = 69.338$, $df = 21$, $P < 0.001$). A Spearman's rank correlation analysis similarly showed that maternal body size was correlated with mean ova diameter for mass ($r = -0.181$, $N = 560$, $P < 0.001$) and for SVL ($r = 0.202$, $N = 560$, $P < 0.001$; Figure 4.3).

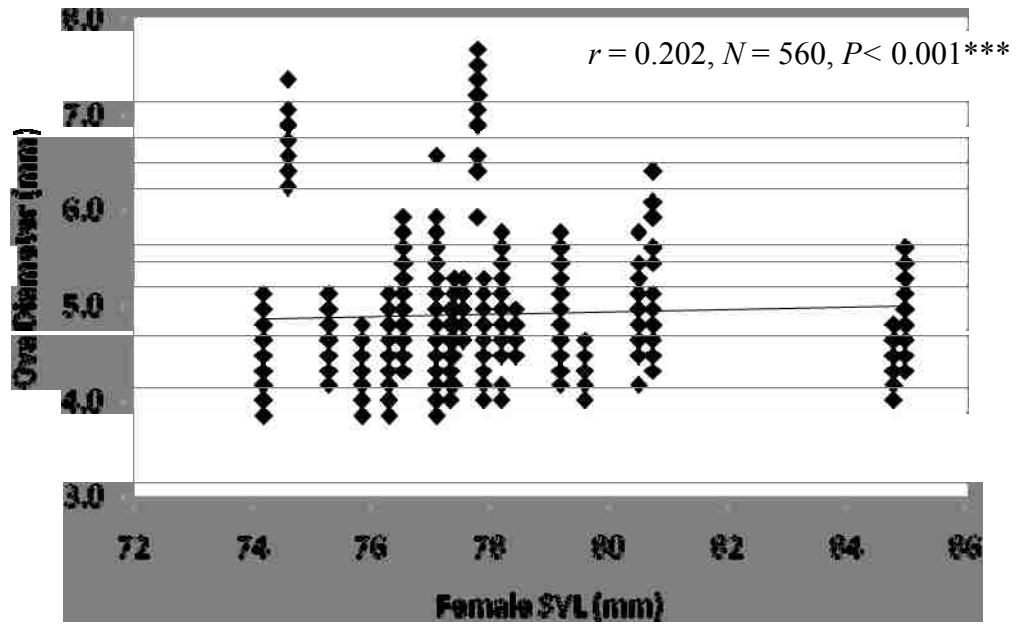


Figure 4.3. Scatterplot depicting the relationship between female body size as SVL ($N = 22$) of *Agalychnis moreletii* and mean ovum diameter ($N = 560$). Female body size is positively correlated with ova diameter; larger females produced eggs with a larger diameter.

Overall Paternal Effects of Body Size

There were significant effects of paternal body size on offspring traits in *Agalychnis callidryas* (Table 4.1). Larger males had greater larval performance measures when compared to smaller males, with the exception of growth rates for SVL where smaller males sired tadpoles that grew more rapidly within the first week and within the first month of development (Table 4.1).

In *A. moreletii*, offspring of larger males had significantly greater performance in most larval traits when compared to offspring sired by smaller males with the exception of hatchling mass, tadpole survivorship and froglet mass for which there were no statistically significant differences between sires (Table 4.2).

Table 4.1. Larval performance characteristics of *Agalychnis callidryas* investigating the overall effect of paternal body size. Offspring of larger than average males were compared to the offspring of smaller than average males. Fertilization success, hatching success and survivorship are calculated as a proportion of the original egg clutch. All other performance measures are the mean \pm SE (n) for total number of tadpoles measured. Hatching Total = total length of hatchling, SVL 14d = size of tadpole at day 14. Growth week 1, month 1 = growth within the first week and 30 days post-hatching * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Character	Mean \pm SE		F	ANOVA	
	Small sire	Large sire			P
Fertilization success (%)	97.5 \pm 1.2 (25)	97.4 \pm 1.1 (30)	0.002		0.961
Hatching success (%)	38.8 \pm 5.38 (52)	48.0 \pm 6.14 (36)	1.250		0.267
Hatchling SVL (mm)	6.7 \pm 0.06 (230)	7.2 \pm 0.09 (130)	17.478		<0.001***
Hatchling Total (mm)	19.0 \pm 0.16 (230)	20.4 \pm 0.22 (130)	26.180		<0.001***
Hatchling mass (g)	0.07 \pm 0.00 (220)	0.09 \pm 0.00 (119)	18.643		<0.001***
SVL 14d (mm)	8.1 \pm 0.06 (226)	8.4 \pm 0.08 (130)	10.353		<0.001***
Mass 14d (g)	0.11 \pm 0.00 (226)	0.13 \pm 0.00 (130)	27.022		<0.001***
SVL Growth week1 (mm)	1.6 \pm 0.10 (226)	1.2 \pm 0.09 (130)	6.367		0.012*
Mass Growth week 1 (g)	0.04 \pm 0.00 (226)	0.05 \pm 0.00 (130)	5.796		0.017*
SVL Growth month 1 (mm)	2.5 \pm 0.13 (178)	2.1 \pm 0.10 (110)	6.897		0.009**
Mass Growth month 1 (g)	0.08 \pm 0.00 (166)	0.09 \pm 0.01 (90)	3.691		0.056*
Larval duration (days)	124.9 \pm 2.01 (179)	132.2 \pm 2.06 (205)	6.363		0.012*
Survivorship (%)	30.2 \pm 4.81 (16)	44.9 \pm 6.42 (10)	3.434		0.076
SVL at metamorphosis (mm)	16.6 \pm 0.12 (158)	18.0 \pm 0.12 (178)	57.603		<0.001***
Mass at metamorphosis (g)	0.43 \pm 0.01 (159)	0.85 \pm 0.21 (178)	3.420		0.065

Table 4.2. Larval performance characteristics of *Agalychnis moreletii* investigating the overall effect of paternal body size. Offspring of larger than average males were compared to the offspring of smaller than average males. Fertilization success, hatching success and survivorship are calculated as a proportion of the original egg clutch. All other performance measures are the mean \pm SE (n) for total number of tadpoles measured. Hatching Total = total length of hatchling, SVL 14d = size of tadpole at day 14. Growth week 1, month 1 = growth within the first week and 30 days post-hatching * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Character	Mean \pm SE		F	ANOVA	
	Small sire	Large sire		P	
Fertilization success (%)	100.0 \pm 0.0 (46)	99.9 \pm 0.1 (25)	1.863		0.177
Hatching success (%)	39.9 \pm 5.8 (35)	36.2 \pm 4.3 (77)	0.241		0.625
Hatchling SVL (mm)	7.2 \pm 0.03 (319)	7.3 \pm 0.05 (200)	3.782		0.052*
Hatchling Total (mm)	18.9 \pm 0.09 (319)	19.5 \pm 0.14 (200)	12.793		0.009**
Hatchling mass (g)	0.08 \pm 0.002 (246)	0.09 \pm 0.002 (136)	23.751		0.160
SVL 14d (mm)	8.1 \pm 0.04 (317)	8.5 \pm 0.06 (200)	31.084		<0.001***
Mass 14d (g)	0.11 \pm 0.01 (207)	0.16 \pm 0.01 (70)	33.100		<0.001***
SVL Growth week1 (mm)	0.91 \pm 0.04 (317)	1.2 \pm 0.05 (200)	15.125		<0.001***
Mass Growth week 1 (g)	0.02 \pm 0.01 (207)	0.07 \pm 0.01 (70)	30.309		<0.001***
SVL Growth month 1 (mm)	1.9 \pm 0.05 (314)	2.4 \pm 0.08 (200)	32.769		<0.001***
Mass Growth month 1 (g)	0.10 \pm 0.00 (314)	0.14 \pm 0.01 (200)	42.590		<0.001***
Larval duration (days)	116.5 \pm 1.76 (339)	129.1 \pm 1.13 (257)	31.633		<0.001***
Survivorship (%)	56.9 \pm 4.68 (30)	51.6 \pm 5.98 (19)	0.485		0.489
SVL at metamorphosis (mm)	18.2 \pm 0.08 (294)	18.5 \pm 0.09 (234)	8.315		0.004**
Mass at metamorphosis (g)	0.52 \pm 0.01 (294)	1.08 \pm 0.38 (234)	2.649		0.104

Paternal Effects within Maternal Half-sibs

Fertilization and hatching success

The first assessment of paternal effects of fertilization success between large and small sized males of both *A. callidryas* (Table 4.1) and *A. moreletii* (Table 4.2) showed that there was no difference in the number of eggs that were successfully fertilized. There were also no differences in fertilization success between maternal half-sibs between large and small sized sires when mated with the same female in either *A. callidryas* (Table 4.3) or *A. moreletii* (Table 4.4). Both species yielded high fertilization success and very few clutches were not completely fertilized. Similarly, hatching success between maternal half-sibs was not affected by sire size in either *A. callidryas* (Table 4.3) or *A. moreletii* (Table 4.4).

Hatchling size within Maternal Half-sibs

Agalychnis callidryas

Average hatchling sizes at one-week post-hatchling varied in SVL from 3.8 to 9.6 mm with a mean of 6.9 ± 0.05 mm ($N = 360$) and in total length from 12.5 to 25.3 mm with a mean of 19.5 ± 0.14 mm. Mass measurements one-week post-hatching ranged from 0.02 to 0.16 g, mean = 0.08 ± 0.002 g ($N = 339$). Male SVL was positively correlated with hatchling SVL and total length (Spearman's $r_{svl} = 0.198$, $P < 0.001$; $r_{total} = 0.287$, $P < 0.001$) and also between male SVL and hatchling mass ($r = 0.188$, $P < 0.001$). Results of the GLM MANOVA show that sire body size had a significant effect on hatchling traits (Pillai Bartlett's $V = 0.081$, $F_{3, 307} = 9.031$, $P < 0.001$) and there was a significant interaction effect between sire size and dam identity ($V = 0.584$, $F_{36, 927} =$

6.222, $P < 0.001$). There were significant differences in hatchling traits (SVL, total length and mass) when maternal half-sibs were compared by sire size (Table 4.3). Larger males sired larger (Figure 4.4) and heavier hatchlings (Figure 4.5).

Agalychnis moreletii

Average hatchling sizes (SVL) ranged from 5.6 to 9.9 mm, a mean of 7.2 ± 0.03 mm, and in total length tadpole sizes ranged from 12.2 to 25.1 mm, and a mean of 19.1 ± 0.08 mm. Mass ranged from 0.03 to 0.18 g with a mean of 0.08 ± 0.00 g. Male SVL was positively correlated with tadpole SVL and total length (Spearman's $r_{svl} = 0.204$, $P < 0.001$; $r_{total} = 0.263$, $P < 0.001$) and also between male SVL and tadpole mass ($r = 0.304$, $P < 0.001$). Sire body size had a significant effect on hatchling traits (GLM Pillai Bartlett's MANOVA $V = 0.046$, $F_{3, 475} = 7.606$, $P < 0.001$) and there was a significant sire size \times dam identity interaction ($V = 0.427$, $F_{48, 1431} = 4.953$, $P < 0.001$). Within maternal half-sibs, there was a non-significant trend for larger males to sire larger hatchlings as measured by SVL (Figure 4.4), but smaller males sired significantly heavier hatchlings (Table 4.4; Figure 4.5).

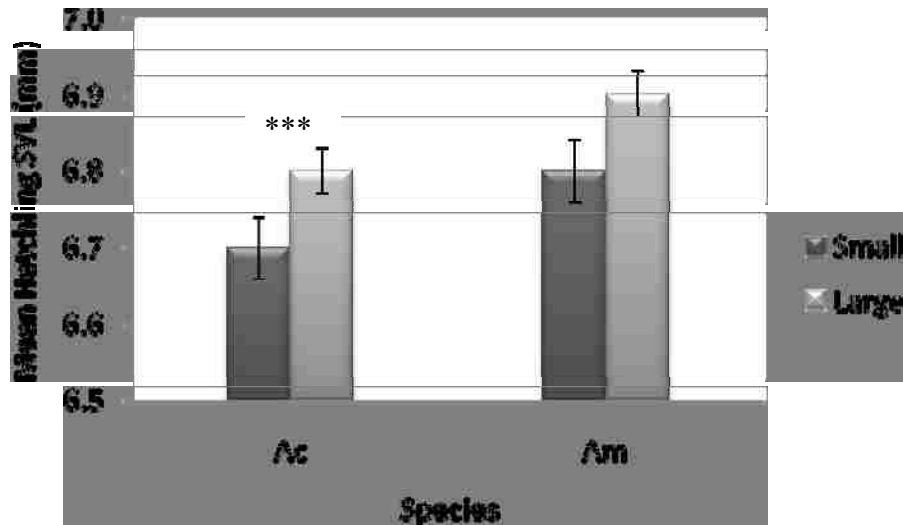


Figure 4.4. Mean body size of hatchlings (SVL) of maternal half-sibs sired by a large male (light gray bars) and small males (dark gray bars). Each bar represents the mean \pm 1 SE for hatchling SVL. Data are presented for *Agalychnis callidryas* (Ac) on the left and *A. moreletii* (Am) on the right. Larger males of *A. callidryas* sired significantly larger hatchlings than smaller males ($F = 23.765$, $df = 1$, $P < 0.001$) and larger males of *A. moreletii* sired tadpoles that were larger, but not statistically significant to those sired by smaller males ($F = 3.359$, $df = 1$, $P = 0.067$).

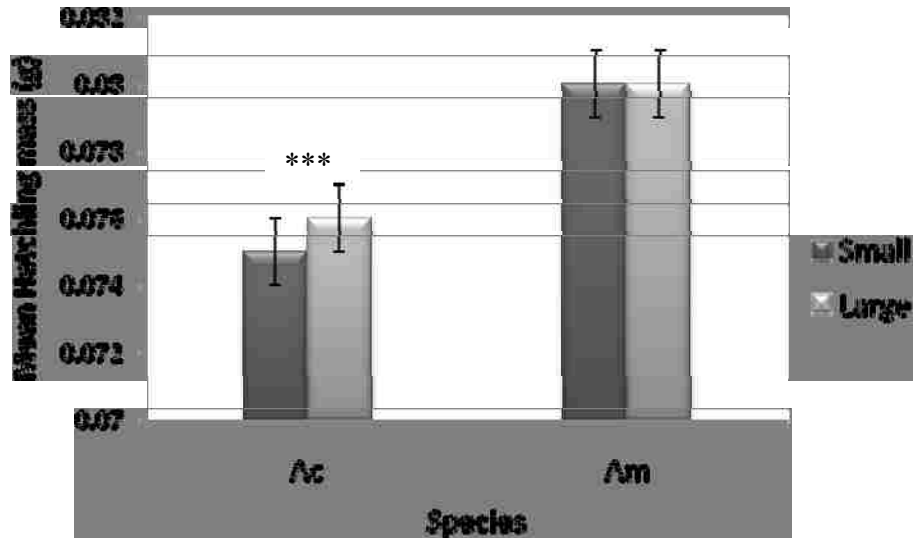


Figure 4.5. Mean body mass of hatchlings of maternal half-sibs sired by a large male (light gray bars) and small males (dark gray bars). Each bar represents the mean \pm 1 SE for hatchling SVL. Data are presented for *Agalychnis moreletii* (Ac) on the left and *A. moreletii* (Am) on the right. Larger males of *A. callidryas* sired significantly heavier hatchlings than smaller males ($F = 14.211$, $df = 1$, $P < 0.001$), but there was no difference in mass between hatchlings that were sired by a large and a small male in *A. moreletii* ($F = 1.100$, $df = 1$, $P = 0.295$).

Table 4.3. Larval performance characteristics of maternal half-sibs of *Agalychnis callidryas* investigating the effect of paternal body size. Fertilization success, hatching success and survivorship are calculated as a proportion of the original egg clutch. All other performance measures are the mean \pm SE (n) for total number of tadpoles measured. Hatching Total = total length of hatching, SVL 14d = size of tadpole at day 14. Growth week 1, month 1 = growth within the first week and 30 days post-hatching * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Character	Mean \pm SE		Test of Between-Subject Effects		
	Small sire	Large sire	MS	F	P
Fertilization success (%)	98.3 \pm 1.2 (29)	96.5 \pm 1.3 (26)	34.166	0.520	0.911
Hatching success (%)	40.6 \pm 5.52 (47)	40.9 \pm 5.74 (44)	10.455	0.008	0.931
Hatching SVL (mm)	6.7 \pm 0.04 (157)	6.8 \pm 0.04 (182)	5.774	23.675	<0.001***
Hatching Total (mm)	18.9 \pm 0.13 (157)	19.3 \pm 0.12 (182)	48.740	21.019	<0.001***
Hatching mass (g)	0.075 \pm 0.001 (157)	0.076 \pm 0.001 (182)	0.004	14.211	<0.001***
SVL 14d (mm)	8.1 \pm 0.06 (164)	8.1 \pm 0.05 (192)	0.010	0.028	0.868
Mass 14d (g)	0.11 \pm 0.002 (164)	0.11 \pm 0.002 (192)	0.000	0.212	0.646
SVL Growth week1 (mm)	1.8 \pm 0.07 (164)	1.2 \pm 0.05 (192)	27.055	50.226	<0.001***
Mass Growth week 1 (g)	0.04 \pm 0.003 (164)	0.04 \pm 0.002 (192)	0.001	1.109	0.293
SVL Growth month 1 (mm)	2.4 \pm 0.09 (126)	2.0 \pm 0.07 (130)	6.139	8.968	0.003**
Mass Growth month 1 (g)	0.08 \pm 0.004 (126)	0.09 \pm 0.003 (130)	0.010	6.505	0.011*
Larval duration (days)	130.2 \pm 3.69 (147)	142.2 \pm 2.66 (189)	4789.837	7.477	0.007**
Survivorship (%)	30.2 \pm 5.33 (14)	42.5 \pm 5.79 (12)	716.919	1.978	0.295
SVL at metamorphosis (mm)	17.0 \pm 0.22 (147)	17.5 \pm 0.16 (189)	8.984	4.101	0.044*
Mass at metamorphosis (g)	0.44 \pm 0.31 (147)	0.68 \pm 0.22 (189)	1.415	0.321	0.572

Table 4.4. Larval performance characteristics of maternal half-sibs of *Agalychnis moreletii* investigating the effect of paternal body size. Fertilization success, hatching success and survivorship are calculated as a proportion of the original egg clutch. All other performance measures are the mean \pm SE (n) for total number of tadpoles measured. Hatching Total = total length of hatching, SVL 14d = size of tadpole at day 14. Growth week 1, month 1 = growth within the first week and 30 days post-hatching * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Character	Mean \pm SE		Tests of Between Subject Effects			
	Small sire	Large sire	MS	F	P	
Fertilization success (%)	100.0 \pm 0.08 (35)	100.0 \pm 0.08 (32)	0.028	0.140	0.715	
Hatching success (%)	32.1 \pm 3.79 (55)	36.9 \pm 3.75 (56)	793.881	1.129	0.296	
Hatching SVL (mm)	6.8 \pm 0.03 (309)	6.9 \pm 0.03 (209)	21.342	3.359	0.067	
Hatching Total (mm)	18.2 \pm 0.08 (309)	18.1 \pm 0.07 (209)	447.672	10.502	0.001**	
Hatching mass (g)	0.08 \pm 0.001 (309)	0.08 \pm 0.001 (209)	0.000	1.100	0.295	
SVL 14d (mm)	8.5 \pm 0.05 (141)	8.4 \pm 0.05 (100)	0.381	1.294	0.256	
Mass 14d (g)	0.13 \pm 0.01 (141)	0.13 \pm 0.01 (100)	0.000	0.032	0.858	
SVL Growth week1 (mm)	1.07 \pm 0.05 (167)	1.11 \pm 0.06 (110)	1.267	3.199	0.075	
Mass Growth week 1 (g)	0.02 \pm 0.01 (167)	0.04 \pm 0.01 (110)	0.001	0.253	0.615	
SVL Growth month 1 (mm)	2.16 \pm 0.05 (264)	2.25 \pm 0.05 (250)	0.974	1.430	0.232	
Mass Growth month 1 (g)	0.12 \pm 0.004 (264)	0.12 \pm 0.004 (250)	0.001	0.457	0.499	
Larval duration (days)	120.1 \pm 2.27 (277)	121.3 \pm 2.47 (250)	87.168	0.128	0.720	
Survivorship (%)	53.7 \pm 4.13 (27)	54.6 \pm 4.49 (22)	118.265	0.279	0.614	
SVL at metamorphosis (mm)	18.4 \pm 0.11 (277)	18.3 \pm 0.12 (250)	0.183	0.112	0.738	
Mass at metamorphosis (g)	0.52 \pm 0.34 (277)	1.00 \pm 0.37 (250)	13.809	0.898	0.344	

Larval Growth, Development, and Metamorphic Climax

Agalychnis callidryas

At one week, post-hatching, there were no paternal size effects on tadpole size (GLM Pillai's Bartlett's trace MANOVA $V = 0.001$, $F_{2, 322} = 0.156$, $P = 0.856$) but there was a significant sire size \times dam interaction effect ($V = 0.279$, $F_{30, 646} = 3.498$, $P < 0.001$). When tadpoles were compared between maternal half-sibs, there was also not a sire effect of size with nearly identical tadpole measurements (Table 4.3). Growth rates within the first week of development were affected by sire size ($V = 0.203$, $F_{2, 322} = 41.039$, $P < 0.001$) and there was also sire \times dam interaction effect ($V = 0.908$, $F_{30, 644} = 17.923$, $P < 0.001$). Tadpoles that were sired by small males grew more rapidly in SVL, but not mass, than their half-sibs sired by large males (Table 4.3).

Growth within the first month was also significantly affected by sire size (GLM $V = 0.227$, $F_{2, 231} = 33.926$, $P < 0.001$) and there was an interaction effect between sire size and dam ($V = 0.976$, $F_{20, 464} = 22.123$, $P < 0.001$). Results illustrate that tadpole growth rates of smaller males were greater for SVL within half-sibs, but curiously, larger males sired tadpoles that grew more rapidly in mass within the first month (Table 4.3).

There were significant paternal effects on offspring traits in newly metamorphosed froglets ($V = 0.028$, $F_{3, 310} = 2.944$, $P = 0.033$) but there was not a statistically significant interaction between sire size and dam in the GLM ($V = 0.132$, $F_{30, 936} = 1.434$, $P = 0.062$). Larger males sired tadpoles that had a significantly longer larval period than tadpoles sired by smaller males (Figure 4.6) and emerging metamorphosed froglets were larger in SVL (Figure 4.7) but not in mass (Figure 4.8) than those sired by smaller males (Table 4.3). Larval duration was positively correlated with froglet SVL (r

= 0.300, $N = 336$, $P < 0.001$) and froglet mass ($r = 0.325$, $N = 337$, $P < 0.001$). There were also significant correlations between male SVL and froglet SVL (Spearman's $r = 0.275$, $N = 336$, $P < 0.001$), froglet mass ($r = 0.366$, $N = 337$, $P < 0.001$) and larval period ($r = 0.187$, $N = 384$, $P < 0.001$). Within maternal half-sibs, larger males sired a greater number of survivors to reach metamorphosis, but this difference was not statistically significant (Table 4.3).

Agalychnis moreletii

Sire body size did not affect tadpole growth within the first week of development from the GLM ($V = 0.006$, $F_{2,220} = 0.654$, $P = 0.521$) and there was not a sire size \times dam interaction effect ($V = 0.113$, $F_{18,442} = 1.466$, $P = 0.098$). Thus, there were no paternal effects within maternal half-sibs for tadpole measures within the first week (Table 4.4).

Growth rates within the first week of development were also not affected by sire size ($V = 0.012$, $F_{2,252} = 1.594$, $P = 0.205$) nor was there an interaction between sire size and dam ($V = 0.069$, $F_{20,506} = 0.908$, $P = 0.577$). However, tadpoles that were sired by large males grew slightly more rapidly in SVL than their half-sibs sired by small males (Table 4.4).

Growth within the first month, however, was significantly affected by sire size ($V = 0.014$, $F_{2,469} = 3.333$, $P = 0.037$) and there was an interaction effect between sire size and dam ($V = 0.397$, $F_{42,940} = 5.551$, $P < 0.001$), but these effects were not apparent within maternal half-sibs (Table 4.3).

At the end of metamorphosis, there were no significant paternal effects on offspring traits ($V = 0.003$, $F_{3, 482} = 0.420$, $P = 0.739$) nor was there a sire size \times dam interaction ($V = 0.133$, $F_{60, 1452} = 1.123$, $P = 0.245$). As a result, there were no differences in offspring traits between large and small sires within the same female (Table 4.4). The length of the larval period did not differ according to sire size (Figure 4.6) and emerging froglets were not different according to SVL (Figure 4.7) or mass (Figure 4.8). However, there was a positive relationship between larval duration and froglet SVL ($r = 0.440$, $N = 527$, $P < 0.001$) and froglet mass ($r = 0.517$, $N = 528$, $P < 0.001$). Male SVL was correlated with froglet SVL (Spearman's $r = 0.148$, $N = 528$, $P = 0.001$), froglet mass ($r = 0.160$, $N = 529$, $P < 0.001$) and larval period ($r = 0.279$, $N = 596$, $P < 0.001$). Survivorship to metamorphosis did not differ between large and small sires (Table 4.4).

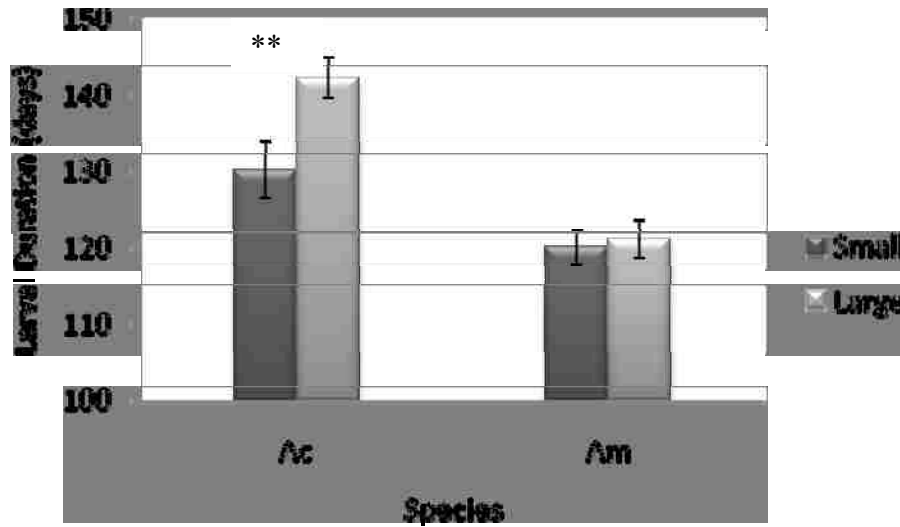


Figure 4.6. The length of the larval period between maternal half-sibs sired by large males (light gray bars) and small males (dark gray bars). Each bar represents the mean larval duration (days) \pm 1 SE for tadpoles of *Agalychnis callidryas* (Ac) on the left and *A. moreletii* (Am) on the right. Tadpoles sired by larger males of *A. callidryas* emerged from metamorphosis later than half-sibs that were sired by smaller males ($F = 7.477$, $df = 1$, $P = 0.007$). There was no difference in the length of the larval period between tadpoles that were sired by a large and a small male in *A. moreletii* ($F = 0.128$, $df = 1$, $P = 0.720$).

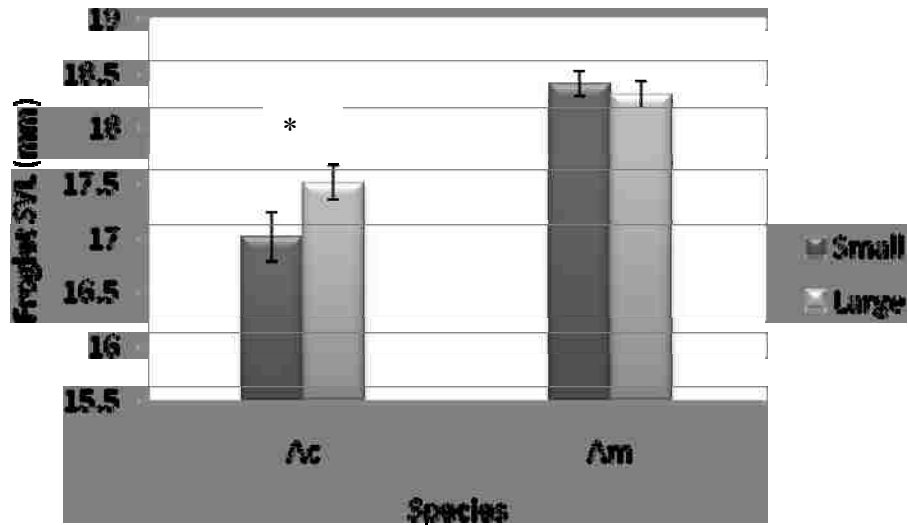


Figure 4.7. The mean froglet (newly metamorphosed tadpole) body size measured as SVL between maternal half-sibs sired by large males (light gray bars) and small males (dark gray bars). Each bar represents the mean froglet SVL (mm) \pm 1 SE for froglets of *Agalychnis callidryas* (Ac) on the left and *A. moreletii* (Am) on the right. Large males of *A. callidryas* sired froglets that emerged with a greater SVL than those sired by small males ($F = 4.101$, $df = 1$, $P = 0.044$). There was no difference in froglet SVL between large and small sires of *A. moreletii* ($F = 0.112$, $df = 1$, $P = 0.738$).

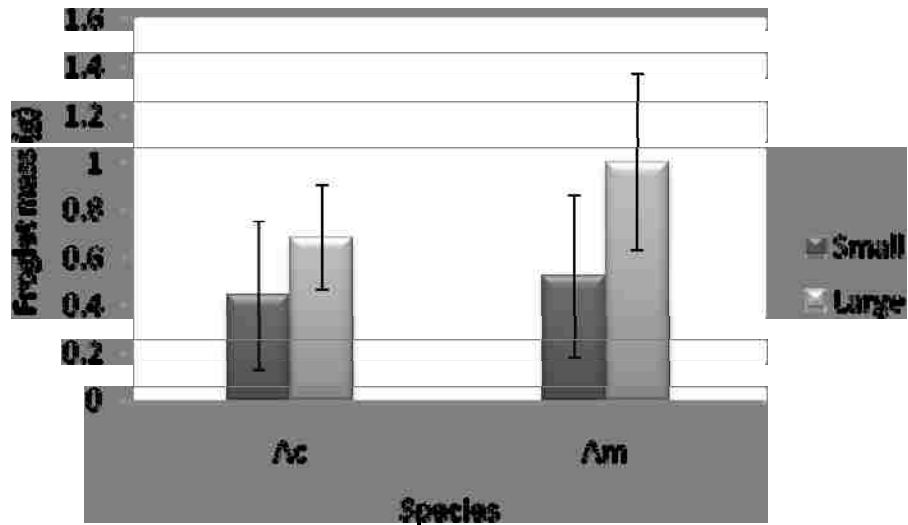


Figure 4.8. The mean froglet (newly metamorphosed tadpole) body size measured as mass between maternal half-sibs sired by large males (light gray bars) and small males (dark gray bars). Each bar represents the mean froglet mass (mm) \pm 1 SE for froglets of *Agalychnis callidryas* (Ac) on the left and *A. moreletii* (Am) on the right. There was no difference in the mass of froglets that were sired by a large or a small male in either *A. callidryas* ($F = 0.321$, $df = 1$, $P = 0.572$) nor *A. moreletii* ($F = 0.898$, $df = 1$, $P = 0.344$).

DISCUSSION

Across taxa, it is widely recognized that maternal contribution greatly affects offspring traits, but until recently it was assumed that paternal effects had little or no influence on the outcome of offspring phenotype, particularly in a non-resource based system where males provide no parental care or immediate benefits to the female (Bonduriansky and Head 2007). However, sexual selection may directly affect the transfer of parental effects of both dam and sire to offspring. Parental effects may act on different phenotypic traits and may occur at different time points in an offspring's lifetime (Bonduriansky and Head 2007).

In my study, there were significant maternal effects in the form of maternal investment to ova diameter. Species with an intermediate mode of reproduction that lay eggs terrestrially but have an aquatic larval stage, such as these two species of *Agalychnis*, tend to have smaller clutch sizes but with larger eggs (see Figure 4.1). Presumably, as the larval development becomes less aquatic, there is a greater initial investment that is tied to the body size of the dam (Salthe and Duellman 1973; Crump and Kaplan 1979). The physical constraints of carrying a larger number of eggs is less for a larger female and a greater body mass allows for a greater resource investment toward the development of larger eggs (Kaplan 1989). Traditionally, maternal investment as yolk allocation has been shown to influence size and growth rates during early stages of development (Travis et al. 1987; Newman 1988b; Mousseau and Fox 1998), but these effects have also been shown to diminish during the larval period and have no effect later in offspring growth or in frogs at metamorphic climax (Newman 1988a; Mousseau and Fox 1998).

Certainly maternal investment can influence the size of offspring; however, when an individual female's clutch is sired by two different males, paternal effects can be observed under similar environmental conditions. Contrary to findings on maternal investment, the few studies that document paternal influence have found that paternal factors to have a greater effect on offspring size which may be directly related to adult reproductive success (Mitchell 1990; Bonduriansky and Head 2007). In such systems, male phenotype may honestly indicate good genes and could drive indirect selection for female preferences (Kodric-Brown and Brown 1984; Andersson 1994; Searcy and Nowicki 2005).

In many frog species, male body size has been documented as a suitable indicator of male quality and females have been shown to choose males that are larger on average (Wilbur et al. 1978; reviewed by Woodward 1982; Halliday 1983; Halliday and Tejedo 1995). Females have also been shown to choose males relative to their own body in *Bufo americanus* (Licht 1976), *Bufo bufo* (Davies and Halliday 1977), *Tripurion petasatus* (Lee and Crump 1981), and *Hyla labialis* (Gutiérrez and Lüddecke 2004) to name a few. In several cases females do not have the opportunity to exercise choice because of strong male-male competition (Davies and Halliday 1979; Wells 1979; Berven 1981; Arak 1983; reviewed Sullivan et al. 1995). However, when females choose larger males, these larger males may be more able to fertilize a greater number of eggs (Emerson 1997) as a function of their resource-accruing capability or enhance offspring survival by passing on their "superior genes" to offspring as evidenced by their large-body size.

In both *Agalychnis callidryas* and *Agalychnis moreletii*, larger males were found disproportionately mated during lean breeding seasons which may have emphasized body size differences between males in those years (Briggs Chapter 2). Despite this large-male mating advantage, fertilization success documented in this study was not affected and both small and large-sized males had high fertilization success (> 97%). In this case, females may be choosing males based on a different set of criteria that does not reflect fertilization success.

Results of this study show that there was a pronounced paternal effect on initial size of hatchlings. Larger males of both *A. callidryas* and *A. moreletii* produced hatchlings that began the larval period at a larger size than half-sibs sired by small males. Larger hatchlings have an immediate growth advantage because they have the ability to acquire more resources (Ficetola and de Bernardi 2006), potentially avoid gape-limited predators at an earlier age (reviewed Werner 1986; Semlitsch and Gibbons 1988), and may maintain the head start in growth and emerge at a larger size (Altwegg and Reyer 2003).

Tadpoles of both species increased their SVL by a minimum of 1 mm within the first week of development. In *A. callidryas*, tadpoles between sires were identical in size but curiously, tadpoles of smaller males grew significantly more than those sired by large males despite having started the larval period at a smaller hatchling size. This noticeable growth spurt may be in response to competitive stress that selects for increased growth rate to maintain similar body sizes of siblings (Relyea and Auld 2005) in an effort to maintain metamorphic synchrony (Breden and Kelly 1982).

Conversely, in *A. moreletii*, growth rate of tadpoles sired by larger males were slightly greater after one week, in comparison to their half-sibs though they both had similar size measures at one-week post-hatching.

An early growth rate, such as within the first week of hatching may be advantageous for avoiding death from drying ephemeral ponds and associated aquatic predators and greatly enhance survival (Woodward 1986; Banks and Beebee, 1988; Newman, 1988a; Ficetola and de Bernardi 2006). A fast larval growth and development may also increase future growth rate and the chance of survival after metamorphosis (Pechenik et al., 1998; Altwegg and Reyer, 2003). Interestingly, after a month of growth, tadpoles among maternal half-sibs were different in body size and mass in *A. callidryas*. Tadpoles sired by smaller males had a greater growth rate measured as SVL but tadpoles sired by larger males were heavier than their half-sibs within that first month of growth. However, there was no difference in growth rate after a month among maternal half-sibs in *A. moreletii*.

At each 15 day interval when growth was measured tadpoles were substantially larger and/or heavier than the previous time point, but this was not clearly related to the paternal effects of sire body size. Potentially, competition that may have acted on tadpole size within the first week of growth also had an effect into the first month but there may have been more opportunity for compensation to reduce competitive advantages (Relyea and Auld 2005). Compensatory growth effects may be due in part to the physical factors of the aquatic environment during the rearing period. Tadpoles of both *A. callidryas* and *A. moreletii* are mid-water suspension feeders that hang head-up in the water column and may have a preferred depth for optimal feeding opportunities (Satel and Wassersug 1981;

Warkentin 1999b; Savage 2003). In my study, though I controlled for water volume per container, the shape of the container did not change and as a consequence, tadpoles that were at lower densities may have experienced a shallower water depth. A shallower water depth may have affected growth rates across all tadpoles, independent of paternal effects. Additionally, the opportunity for air-water oxygen exchange may have been greater in containers that had a lower water depth. This would provide tadpoles living in these containers with a higher oxygen content which may have also affected growth (Warkentin 2002), but I did not measure oxygen levels during this study and have no evidence in support of this hypothesis.

On the other hand, the larval period was longer for tadpoles that were sired by larger males in *A. callidryas* but not for *A. moreletii*. There was a positive correlation between larval duration and size at metamorphosis in both species. This positive correlation has been found in a few anuran species (Berven 1987; Woodward et al. 1988); however, there are more examples of a negative relationship between larval duration and metamorphic size in *Bufo woodhousei* (Mitchell 1990), *Hyla crucifer* (Travis et al. 1987) and *Scaphiopus couchii* (Newman 1988b); and more recently in *Rana lessonae* and *R. esculenta* (Altwegg and Reyer 2003). In most species, there is a tradeoff between length of larval period and size at metamorphosis. Usually, faster larval growth and developmental rates increase post-metamorphic growth and survival (Pechenik et al. 1998; Girish and Saidapur 2002; Altwegg and Reyer 2003). However, larger metamorphosed froglets have been shown to have an increased chance of survival than those metamorphosing at smaller sizes (Altwegg and Reyer 2003).

The larval period is under intense selection with developmental constraints and carry over effects (Fischer et al. 2004) but the fitness benefits may not be detectable until a later post-metamorphic stage (Ficetola and de Bernardi 2006). There are disadvantages to a prolonged larval period that include an increased competition for local resources, an increased risk of aquatic predation and an increased chance of desiccation particularly when in an ephemeral pond (Travis et al. 1985; Newman 1988a, 1988b; Ficetola and de Bernardi 2006). In addition, tadpoles that emerge from metamorphosis earlier get a head start on the adult stage and may have higher fitness benefits as a consequence of locating mates earlier (Altwegg and Reyer 2003). However, there are obvious advantages to remaining in the larval period in a favorable environment, particularly for increasing body size and there is evidence that growth rate is highly dependent upon local conditions and may not be completely geared toward producing the fastest growing tadpoles (Werner 1986; Arendt 1997; Fischer et al., 2004; Stoks et al., 2005).

Similarly, the benefits of a prolonged larval period in a favorable environment include increased feeding opportunities that will enhance growth, avoidance of gape-limited predators, and larger size upon emergence from metamorphosis (reviewed Werner 1986; Travis et al. 1987; Semlitsch and Gibbons 1988; Ficetola and de Bernardi 2006). This early size difference may translate into advantages into adulthood and have far-reaching fitness consequences (Altwegg and Reyer 2003). Larger froglets have a greater probability of surviving until the next year as juveniles (Travis 1981); larger females will have larger clutches (Kaplan 1989); and larger males may obtain more matings (Wilbur et al. 1978; Altwegg and Reyer 2003; Bonduriansky and Head 2007).

Though there is typically selection against a prolonged larval period under stressful conditions with predators (Travis et al. 1987; Relyea 2007) and intense competition (Semlitsch and Caldwell 1982), in this study, aquatic predators were not a threat to tadpole survival and food was not a limited resource. Thus, a tadpole could only stand to gain fitness benefits by prolonging the larval period to emerge with a size advantage in this system. If a slower growing individual in nature, as in the laboratory, emerges from metamorphosis at a larger size (having survived the larval period), then these individuals may have higher fitness benefits than the tadpoles that grew faster in the larval period. An individual's fitness, then, may not be tightly linked with larval growth (Wilbur and Collins 1973) but with size at metamorphosis (Travis 1980).

Larger males produced froglets that were larger but not heavier in *A. callidryas* only, and froglets of *A. moreletii* were not different between maternal half-sibs. However, in both species, tadpole survivorship was not affected by paternal body size. There are few studies that investigate paternal effects on offspring performance and have focused on temperate species and have produced results that are not completely conclusive. Travis (1980, 1981) demonstrated in *Hyla gratiosa* and *Pseudacris triseriata* that tadpoles of different parentage differed in their growth rate and survivorship. These differences were attributed to both genetic (paternal effects) and non-genetic (maternal investment) factors. Howard (1978) showed that larger males defended better oviposition sites which reduced early embryo mortality in *Rana catesbiana*. Woodward (1986) found that larger males of *Scaphiopus multiplicatus* sired larger offspring relative to those sired by smaller males. In *S. couchii*, Woodward (1987) did not find a difference in offspring performance traits relative to sire body size, but sire size did have an effect on

tadpole survivorship. This finding was confounded by unequal rearing densities.

Woodward et al. (1988) tested the same hypothesis on *Hyla crucifer* and found that larger males sired offspring with a 6% higher growth rate, but other performance measures could not be detected according to size.

Mitchell (1990) questioned the validity of laboratory studies when the paternal effects of *Bufo woodhousei* were more pronounced in the field than in the laboratory, despite the degree of environmental variation in the field. In the lab, neither sire body size nor mating status affected larval performance traits, but in the field larger males produced offspring that were 10% heavier at metamorphosis than offspring sired by small males. Howard et al. (1994) tested the “good genes” hypothesis in *Bufo americanus* and found that males differed in genetic quality because age and mass of metamorphs showed significant heritability within maternal half-sibs and were genetically correlated with each other. However, metamorphs sired by larger males did not differ from those of small sires. Semlitsch (1994) found that sire body size does not affect offspring traits in *Bufo bufo*. Collectively these studies reveal that some variation in larval performance is related to variation in paternal size, but this is not consistent across all offspring performance traits or among all species.

More recent studies have focused on female preference for call parameters of males (which may covary with body size) and the effects on offspring performance. Welch et al. (1998) showed that offspring of male gray treefrogs that produced long calls showed better performance during larval and juvenile stages than did offspring of males that produced short calls.

Similar results were found with *Hyla versicolor* (Doty and Welch 2001; Welch 2003), however, there was a significant genotype-by-environment interaction suggesting plastic response by females to environmental conditions.

However, of the few studies that have found paternal effects on offspring traits, size at metamorphosis has been a reliable indicator trait of post-metamorphic success (Woodward et al. 1978; Mitchell 1990). Arguably, a larger size at metamorphosis ought to be better because it can translate into a larger body size later and reduce the risk of predation of size-limited predators beyond the larval stage (reviewed Travis et al. 1987) or enhance growth and survival in competitive systems (Woodward 1987).

Overall in this study, larger males sired larger hatchlings that experienced a longer larval period and whose growth rates leading up to metamorphosis were outperformed by tadpoles of smaller males. Tadpoles of larger males, however, still emerged at a greater size. This may be a compensatory effect where growth is enhanced in some tadpoles or where growth effects of a sib-ship is masked by “super tadpoles” that release a growth inhibiting hormone in an attempt to reduce competition by producing asynchronous growth rates (Rot-Nikcevic and Wassersug 2004; Etkin and Gona 2005; Rot-Nikcevic et al. 2005; Denver and Crespi 2006).

In several cases in my study, there were at least one or two “super tadpoles” per group that grew much more rapidly than the rest of the cohort, but did not necessarily emerge from metamorphosis earlier. Potential chemical affects ought to have been reduced with the regular water changes, but dissolved oxygen levels were not measured and this may have had an effect on growth by providing tadpoles at lower densities with a greater air-water interface for oxygen exchange (Warkentin 2002). Additionally, when a

tadpole neared metamorphosis and was removed from the container to complete the metamorphic process, the remaining water density was standardized per number of remaining tadpoles, however, water depth changed. This change may have kept tadpoles in the larval period longer by merely not providing sufficient room to swim and/or feed in an upright posture (Satel and Wassersug 1981; Warkentin 1999b) and thus, have prolonged the larval duration (Relyea 2007).

In this study, I present data illustrating that the mating system affects some offspring fitness measures, but the effects on other traits may be masked by the interactions of contributing factors (Bonduriansky and Head 2007). Here, there are paternal effects for the initial hatchling size of tadpoles that may be sufficient in some cases to provide a growth advantage into adulthood (Altwegg and Reyer 2003). However, in nature maternal investment and uncontrolled environmental conditions may obscure and/or enhance the potential paternal effects throughout the larval period. Despite the diffused paternal effect on larval performance traits, it is noteworthy that larger males sired tadpoles that emerged as metamorphosed froglets after a longer larval period.

Laboratory conditions that approximate a natural environment may not completely simulate what occurs under field conditions. Rearing tadpoles in the laboratory may serve to increase the range of genetic expression beyond what is found in nature (Travis 1980) and substantially reduces the role of selective mortality in response, for example, to natural predators and competitive stress. Future studies may need to include more measures of tadpole performance and include both laboratory and field components to determine consistent and predictable paternal effects.

My study provided an opportunity to compare paternal effects between two closely-related, yet obviously different hyloid species, which occupy similar niches (Lee 1996) and have similar mating patterns (Briggs Chapter 2). Hatchlings of both species cannot be told apart but within a week of development, tadpoles of *A. moreletii* (Figure 4.9) are darker in color and obviously more globose with a greater body and tail depth, whereas tadpoles of *A. callidryas* (Figure 4.10) are nearly translucent and are more slender in body form (Lee 1996). This difference in body form may affect escape capability from aquatic predators with *A. moreletii* having an advantage versus gape-limited predators and *A. callidryas* being more stream-lined for escape from pursuit of predators.

Much work has shown that predators can induce phenotypic plasticity in a tadpole's morphology and that the traits that confer predator-avoidance advantages are subject to selective pressures (Blair and Wassersug 2000; Schalk et al. 2002; Relyea 2007). Furthermore there may be a trade-off between predator-avoidance mechanisms and competitive advantages used against conspecifics (Relyea and Auld 2005). In such cases, traits under selection by predators, afford a predator-avoidance/resistance advantage but typically reduce growth levels whereas, traits that enhance growth against competition are usually less advantageous for predator-avoidance/resistance (Travis 1980; Relyea and Auld 2005). The phenotypic and behavioral differences of the two species may be as a result of responses to different selective pressures typically experienced in nature. Tadpoles of *A. moreletii* may thus be more suited for intra- and inter-specific competition against other tadpoles, whereas, tadpoles of *A. callidryas* may be more suited for predator avoidance.



Figure 4.9. Tadpoles of *Agalychnis moreletii* are dark and globose and spend more time feeding and swimming.



Figure 4.10. Tadpoles of *Agalychnis callidryas* are translucent and slender and spend more time suspended in the water column.

Clutch sizes of *A. moreletii* are much larger (> 100 tadpoles) than in *A. callidryas* and tadpoles of both species are often found in schools in nature (Savage 2003) and aggregates in my experimental buckets. However, throughout the experiment tadpoles of *A. moreletii* were more active than *A. callidryas* and spent a large amount of time swimming and feeding, whereas tadpoles of *A. callidryas* were less active and spent more time suspended in the water column (see Figures 4.9 and 4.10). This increased activity level in *A. moreletii* may allow for a greater amount of particulate matter to be stirred up in the water column and provide more feeding opportunities and enhance the competitive advantage (Breden and Kelly 1982; Foster and McDiarmid 1982; Eterovick 2000). Here, increased activity levels may account for the increased growth of *A. moreletii* and allow for a larger size at metamorphic climax despite potential paternal effects in this study.

My study shows that, offspring of *A. moreletii* grow more in size and at a faster rate and emerge from metamorphosis earlier than offspring of *A. callidryas*. This difference may help to explain the emergence pattern of adults during the breeding season at Las Cuevas. Adults of *A. callidryas* emerge en masse after the first heavy rains and are found in smaller numbers throughout the breeding season. They also appear to complete breeding before the end of the rainy season. Adults of *A. moreletii*, though they also emerge after the first rains, are initially fewer in numbers, but are consistently found throughout the breeding season. Toward the end of the rainy season *A. moreletii* are often the only species found at a pond (Briggs, Chapter 2). Tadpoles of *A. moreletii* may accelerate growth and development during the larval period to emerge from metamorphosis and thus reach adulthood earlier, but are able to withstand a prolonged breeding period to avoid being outcompeted by *A. callidryas* at sites where they co-occur.

The fact that there are such differing effects of parental traits on larval performance between the species suggests that sire and dam effects may not be the only contributing factors to offspring growth. In this study, the effects of density was presumably reduced as water volume and food were adjusted across half-sibs, however, there may have been some effect on larval growth and development that obscured the potentially greater effects of genetic factors. Tadpoles of *A. moreletii* may be more resilient and less affected by crowding and thus, are better able to maintain high growth and development rates. This would produce half-sibs that do not have a pronounced difference in offspring traits between sires. Interestingly, in virtually every performance measure, paternal size had an overall effect. It is also noteworthy that though dam effects potentially disappear during development in some systems, there is a noticeable maternal contribution to offspring traits in this study. As is suggested in several other studies (Wassersug and Sperry 1977; Howard 1978; Woodward 1986, 1987; Altwegg and Reyer 2003; Bonduriansky and Head 2007), the larval period has far-reaching post-metamorphic consequences and fitness advantages gained during this period may greatly affect adult fitness.

Female choice may still be acting in this system and because a large-male mating advantage has been documented in both species of *Agalychnis*, females may be choosing males based on “good genes” for body size. Hatchling size and larval duration may be adaptive tadpole traits and female choice may result in offspring that hatch larger and have the opportunity to emerge from metamorphosis at a larger size, thus increasing further reproductive success.

CHAPTER 5

GENERAL CONCLUSIONS

Sexual selection

By investigating the natural pairing patterns in both *Agalychnis callidryas* and *Agalychnis moreletii* across three breeding seasons, I demonstrate that both species subscribe to a size-related non-random mating pattern, and is one of the most common deviations from random mating in anurans (reviewed by Halliday and Tejedo 1995). I document evidence for a large-male mating advantage (Wilbur et al. 1978) in 2003 and 2005 and also provide evidence for size-assortative mating (Licht 1976) in 2004.

The substantial seasonal differences across the three breeding periods may indicate that under harsh conditions larger males, of presumably higher quality, have a mating advantage because they are better able to withstand climatic fluctuations. As a consequence, larger males were found in amplexus more so than smaller males in 2004. In 2003 and 2005, conditions were much more benign and there was not a significant difference between amplexant and non-amplexant males for body size, however, a constant female to male body size ratio was maintained across years.

These findings may suggest a revised hypothesis that favors a large-male mating advantage, provided the male's size does not exceed an optimal size ratio within pairs. Results indicate considerable inter-annual effects and plastic behavioral response of reproductive biology to environmental stress in these two species of red-eyed treefrogs.

I also provide evidence for little or no male-male competition in *A. callidryas*, contrary to findings at higher population densities (M.S. Caldwell pers. comm.), but am unable to separate the effects of male-male competition from female choice in *A. moreletii*.

Call trait variation

By examining the advertisement calls of *A. callidryas* and *A. moreletii*, I documented significant call trait variation both within and among males. I categorized dominant and fundamental frequencies as static call properties with the lowest coefficients of variation among males. This low level of variation of call traits in both species of *Agalychnis* suggests body-size constraints (Martin 1972; Ryan 1985; Robertson 1986; Wagner 1989; Howard and Young 1998; Bee et al. 1999; Zimmitti 1999; Bee et al. 2001) much like the findings on other species (Howard and Young 1998; Bosch et al. 2000; Márquez et al. 2001; Pröhl 2003). However, there was a pronounced size-related effect on dominant and fundamental frequencies in *A. moreletii* owing to a difference in body size among males. Selected males of *A. callidryas* did not demonstrate this pattern however this may be due to a small sample size.

Among males of both species call and pulse rates were the most variable traits. The dynamic call properties of call rate and call duration are energetically costly and may be indicators of male quality (reviewed Howard and Young 1998; Bee et al. 2001; Castellano et al. 2002).

A female preference for these call traits may be present because amplexant males produced a greater number of pulses per call and for a longer duration which may translate into a faster call rate and longer call duration. Here, I characterize call trait variation and document call properties as indicators of mating success.

Larval performance

I provide evidence for a pronounced effect of sire size on offspring traits, most notably upon hatching. Larger males of both *A. callidryas* and *A. moreletii* produced hatchlings that began the larval period at a larger size than half-sibs sired by small males. Larger hatchlings may have immediate growth and survival advantages that propel them through the larval period and suggests the potential for post-metamorphic advantages (reviewed Werner 1986; Altwegg and Reyer 2003).

A compensatory effect was apparent in the growth rates of tadpoles sired by smaller males within the first week of development and grew more rapidly in comparison to their half-sibs sired by larger males. This effect diminished toward the end of metamorphosis and newly metamorphosed froglets of *A. callidryas* were larger when sired by larger males relative to those sired by smaller males.

I also demonstrate that larger males of *A. callidryas* sired tadpoles that remained in the larval period for a longer duration which may be due in part to an increased growth benefit under favorable conditions. These results add to the few studies that document a positive correlation between larval duration and size at metamorphosis (Berven 1987; Woodward et al. 1988).

Here, I present data that support a maternal contribution as well as the effect of uncontrolled environmental conditions that may diffuse potentially stronger sire size effects upon offspring traits. There is also evidence for considerable inter-specific differences of paternal effects between *A. callidryas* and *A. moreletii* which may further help to explain the variation in breeding behavior and tadpole biology within these two species.

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