POPULATION STRUCTURE AND REPRODUCTIVE PATTERN OF PRISTIMANTIS AFF. FENESTRATUS (ANURA: STRABOMANTIDAE) IN TWO NON-FLOODED FORESTS OF CENTRAL AMAZONIA, BRAZIL

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Abstract. Pristimantis aff. fenestratus is a common species in non-flooded forests of central Amazonia, Brazil. Herein, we show the reproductive pattern and data on population structure of this species from two sites, using standardized sample methods (visual and auditory surveys), during three rainy seasons. The higher number of males in calling activity was detected in the beginning of the rainy season. However, sporadic male calls were also heard in the dry season. Females were significantly larger than males, and males captured at the end of the rainy season were larger than those captured in other periods. Females with eggs visible through the abdominal wall were found in all samplings at one site, but a larger number of juveniles were found at the end of the rainy season. The size of juveniles was variable and the smallest individuals were found at the end of the rainy season. According to nocturnal visual surveys males were significantly more abundant than females (1.9:1 adult sex ratio). According to data collected on gravid female occurrence, juvenile number variation, and presence of calling males, we suggest that P. aff. fenestratus reproduces during the rainy season showing marked seasonality.

Keywords. Abundance, gravid females, juveniles, size, temporal variation.

Introduction

Neotropical anurans exhibit different patterns of activity related to rainfall distribution throughout the year. In some areas with seasonal climate, calling males and gravid females are generally found throughout the rainy season (Aichinger, 1987), due to the presence of water bodies for reproduction. For species with terrestrial clutches and direct development, such as the genus Eleutherodactylus (see Hedges et al., 2008 for new taxonomic arrangement), reproduction is expected to be continuous due to their independence from water bodies for reproduction. However, studies of “Eleutherodactylus” in the Neotropical region, indicate that population recruitment and the occurrence of juveniles is variable, occurring either during the rainy season (e.g., Ovaska, 1991), the dry season (e.g., Fogarty and Vilella, 2002; Watling and Donnelly, 2002) or throughout the whole year (e.g., Crump, 1974; Towsend and Stuart, 1994; Bourne, 1997; Donnelly, 1999; Ortega et al., 2005; Arroyo et al., 2008; Fong et al., 2010). Some species (e.g., Craugastor transfordinii) vary from seasonal to continuous reproduction (Crump, 1974). A juvenile biased population structure was reported for C. transfordinii in Costa Rica (Donnelly, 1999).

The genus Pristimantis (Strabomantidae, Strabomantinae) has about 439 named species, distributed mainly in Central and South America (Frost, 2011) but most of the species of the genus are only known taxonomically. Members of this genus and others from related families (see Hedges et al., 2008) are terrestrial frogs with direct development (Lynch and Duellman, 1997) or viviparity (Wake, 1978). Although these species are common components of most Neotropical anuran communities (for example they account for 32% of the anuran fauna at La Selva Biological Station in Costa Rica) (Donnelly, 1999), aspects of distribution, population structure and reproduction are unknown for this high number of species.

Pristimantis aff. fenestratus (Steindachner, 1864) (= Eleutherodactylus fenestratus in Lima et al., 2006) is a common species in central Amazonia, Brazil (Zimmerman and Rodrigues, 1990; Lima et al., 2006; Menin et al., 2007, 2008; Rojas-Ahumada and Menin, 2010), occurring in sympatry with Pristimantis ockendeni and Pristimantis zimmermanae (Lima et al., 2006; Menin et al., 2007, 2008). Based on nocturnal samples in the non-flooded forest of central Amazonia, the distribution of this species is related to topographic and edaphic factors (Menin et al., 2007).

Males of this species call above the ground from the leaf litter, branches or shrubs at low height (Zimmerman and Rodrigues, 1990; Lima et al., 2006). The calls can be heard at dusk and dawn throughout the forest and individuals call sporadically both during the day and at night (Zimmerman and Bogart, 1984). According to Lima et al. (2006) reproduction occurs...
from November to May, a period when clutches are deposited in the soil under fallen leaves, suggesting that this species is a seasonal breeder in central Amazonia. However, data on population structure and reproductive pattern are not available for this species. Herein, we present data on abundance, calling activity, size of adults and juveniles, sex ration and presence of gravid females of *P. aff. fenestratus* in two areas of terra firme forest in central Amazonia, based on different rainy seasons using standardized sampling methods.

**Material and Methods**

**Study area**

Our study took place at Reserva Florestal Adolpho Ducke (RFAD: 02°55’ and 03°01’S, 59°53’ and 59°59’W), and Fazenda Experimental of the Universidade Federal do Amazonas (Fazenda UFAM: 02°37’17” and 02°39’41”S, 60°03’29” and 60°07’57”W), both near the city of Manaus, Amazonas state, Brazil. RFAD is site 1 of the Brazilian Long Term Ecological Research (PELD) program and the two areas are part of the Programa de Pesquisa em Biodiversidade (PPBio) of the Brazilian Ministry of Science and Technology (MCT) (http://ppbio.inpa.gov.br). RFAD covers 10,000 ha of terra firme (non-flooded) rainforest, a well-drained forest not subject to seasonal inundation. Fazenda UFAM has an area of 3,000 ha. A 30-37 m tall closed canopy characterizes the forest in both areas, with emergent trees growing to 40-45 m (Ribeiro *et al.*, 1999). The understory contains abundant sessile palms (*Astrocaryum* spp. and *Attalea* spp., Ribeiro *et al.*, 1999). The climate is characterized by a rainy season from November to May and a dry season during the rest of the year (Marques Filho *et al.*, 1981). Mean annual temperature is approximately 26°C (Marques Filho *et al.*, 1981) and mean annual rainfall was 2,489 mm between 1985 and 2004.

**Data collection and analyses**

We sampled adults and juveniles of *Pristimantis aff. fenestratus* during three diurnal surveys (November-December 2002, February-April 2003 and January-February 2004) and five nocturnal surveys (November-December 2002, March-May 2003, November-December 2003, February-March 2004 and April-May 2004) at RFAD and three nocturnal surveys at Fazenda UFAM (November-December 2008, January-February 2009 and April-May 2009). The sampling periods were determined according to the rainy season in central Amazonia: beginning (November-December), middle (January-March) and end of the rainy season (April-May). Sporadic observations were made throughout the dry season of the same years. Data were collected in 72 plots systematically distributed over a 64-km² grid formed by 8-km long trails at RFAD (Figure 1) and 41 plots distributed over a 24-km² grid formed by four 8-km long trails in the east-west direction and nine 3-km long trails in the north-south direction at Fazenda UFAM (Figure 1). At Fazenda UFAM each plot was at least 500 m distant from any other, but 31 plots were systematically distributed at every 1 km (Rojas-Ahumada and Menin, 2010). Plots were 250 m long and positioned to follow altitudinal contour lines, thus minimizing altitudinal and soil variation within each plot (Magnusson *et al.*, 2005). All plots were at least 1 km away from the edge of the study areas. Surveys occurred only during the rainy season (November to May). At RFAD diurnal surveys required a mean of 46 days to cover all 72 plots, and nocturnal surveys required a mean of 49 days to survey all 72 plots. Each sampling period lasted between 19 and 21 days at Fazenda UFAM.

The three diurnal surveys carried out at RFAD lasted about 2 hours per plot and were conducted between 08:00 and 16:00 h by two people walking along a 250 × 1 m (0.025 ha) plot (total sampled area was 1.8 ha/sampling period). Observers visually scanned and gently turned over the leaf-litter, detecting individuals by visual encounter. We conducted nocturnal surveys at the two areas by simultaneous visual encounter and auditory sampling (Crump and Scott, 1994; Zimmerman, 1994). These methods are complementary and adequate for surveying the distribution and abundance of anurans in long and short-term studies (Doan, 2003). Nocturnal sampling of each plot lasted about one hour between 18:30 and 22:00 h. At every 5 m, the two observers stopped and recorded the number of vocalizing individuals and searched the litter and vegetation for anurans. Only one observer recorded the number of calling males to avoid counting the same individual more than once during the survey. All individuals located within 20 m of the central line of the plot were recorded so that 1 ha was searched per plot (total sampled area was 72 ha by sampling period at RFAD and 41 ha by sampling period at Fazenda UFAM). We considered abundance in this study as the number of detections...
of individuals using each sampling method in each period (diurnal and nocturnal samples).

Each time we captured a frog in the nocturnal visual survey we (1) recorded its snout-vent length (SVL) with a vernier calliper (0.05 mm), (2) classified it as adult or juvenile, and (3) recorded female reproductive condition (presence/absence of eggs visible through the abdominal wall). We considered juveniles individuals with SVL < 22.0 mm (the size of the smallest male found in calling activity). In diurnal samples, observers estimated the SVL and the sex of individuals was not determined.

Figure 1. Geographical location of the study areas, Reserva Florestal Adolpho Ducke (RFAD) and Fazenda Experimental of the Universidade Federal do Amazonas (Fazenda UFAM), north of Manaus city, Amazonas State, Brazil, and the grid system inside the study areas. Circles indicate the plots.
We compared the sizes (snout-vent length, SVL) of both sexes by using the Mann-Whitney U-test (Zar, 1999). We checked the adult sex ratio by using the Chi-square test with Yates’ Correction (Fowler et al., 1998). The differences in size between males and females along sampling periods were tested by Kruskal-Wallis test (Zar, 1999). Voucher specimens were deposited in the Amphibians and Reptiles Collection of the Instituto Nacional de Pesquisas da Amazônia (INPA-H 10872, 10879, 10882, 10885, 26016, 26022) in Manaus, Amazonas, Brazil.

**RESULTS**

We detected 6,702 individuals of *Pristimantis* aff. *fenestratus* in nocturnal samplings at RFAD, both in visual and auditory surveys. Of these, 334 were found in visual surveys: 69 males, 35 females, and 162 juveniles (SVL < 22.0 mm). We could not determine the sex of 68 individuals (22.7 mm < SVL < 33.8 mm) due to the absence of sexual dimorphism to distinguish between them (51 individuals were captured and released and 17 individuals escaped). Among individuals sampled, males were significantly more abundant than females (sex ratio 1.9:1; Chi-square = 10.47, df = 1, p < 0.005; Figure 2). Females with eggs visible through the abdominal wall were found in all sampling periods (Figure 2). The higher number of juveniles was found at the end of the second rainy season (Table 1 and Figure 2). Juveniles were found resting on leaves or small shrubs during the night. Based on audio surveys, the highest number of males in calling activity was detected at the beginning of the second rainy season (November-December 2003, auditory survey at Table 1) and the smallest number of males was found at the end of the two rainy seasons (second and fifth samplings, Table 1). Sporadic calls of males were heard in the dry season (June, July, September and October).

In diurnal samplings at RFAD, we detected 460 individuals (35 in November-December 2002; 127 in February-April 2003; 298 in January-February 2004), all on the ground in the leaf litter. The number of individuals increased from the first to the second sampling. Comparisons with data of the third sampling were ignored because the size of the frogs was not determined. The highest number of individuals was detected in the first size class (5 to 15 mm), representing juveniles (Figure 3).

At Fazenda UFAM, we detected 667 individuals, 20 of which were found by visual surveys (7 males, 6 juveniles, 7 unidentified) (Table 1). The higher number of calling males was detected at the beginning of the rainy season and the smaller number was found at the end of the rainy season.

Based on nocturnal visual surveys (at RFAD and Fazenda UFAM), adult males averaged 29.77 mm SVL (SD = 3.10; range 22.0-36.2; N = 72); those captured at the end of the second rainy season were larger than those captured in other periods (Kruskal-Wallis = 10.459; df = 4; p = 0.033; Figure 4A). Calling males were found perched on tree trunks, on shrubs, or on fallen tree trunks. Adult females averaged 43.52 mm SVL (SD = 2.79; range 36.7-47.8; N = 35); those captured at the end of the second rainy season were smaller than those captured in other periods, but differences were not significant (Kruskal-Wallis = 4.193; df = 4; p = 0.380; Figure 4B). The minimum size of gravid females was 39.6 mm SVL. Females were found in the leaf-litter or on fallen tree leaves.

![Figure 2](image1.png)

**Figure 2.** Number of males, females (total and only gravid), and juveniles of *Pristimantis* aff. *fenestratus* found in each nocturnal sampling by visual survey at Reserva Florestal Adolpho Ducke, Manaus, Brazil.

![Figure 3](image2.png)

**Figure 3.** Number of individuals of *Pristimantis* aff. *fenestratus* recorded in diurnal samplings in different size categories of SVL (snout to vent length), Reserva Florestal Adolpho Ducke, Manaus, Brazil. Black bars: November-December 2002; white bars: February-April 2003.
trunks. Females were 47% larger than males in SVL (Mann-Whitney U = 2520.00; df = 1; p < 0.001). Juveniles were found in great number only in the middle and end of the second rainy season. Juvenile sizes varied and smaller individuals were found at the end of the two rainy seasons (Figure 4C). The smallest juvenile was 7.3 mm SVL.

**DISCUSSION**

Adult males and females (gravid and non-gravid) of *Pristimantis* aff. *fenestratus* were captured in all samplings of the study period, which were conducted at different times during the rainy season. Juveniles were found mainly in the middle (February-March) and at the end (April-May) of the rainy season, and were more abundant than adults at the end of the rainy season. A similar pattern was also observed for *Craugastor bransfordii* (= *Eleutherodactylus bransfordii*) in Costa Rica (Donnelly, 1999) and other anuran species in central Amazonia (Menin et al., 2008; Rojas-Ahumada and Menin, 2010). In some studies the peak of reproductive activity and growth of juveniles occurs in the rainy season for species with terrestrial reproduction (Moreira and Lima, 1991; Ovaska, 1991).

Significant differences in sex ratios were probably the result of greater male conspicuousness throughout the study period, as observed by Ortega et al. (2005) for a population of *Eleutherodactylus johnstonei* from Colombia and *E. coqui* from Puerto Rico (Fogarty and Vilella, 2002). A male-biased sample was also observed in another anuran species and can be related to behavioral differences between sexes (Giaretta and Menin, 2004) or trapping/sampling methods (Fogarty and Vilella, 2002). Sexual size dimorphism was observed in 90% of anuran species and can be related to female fecundity (Shine, 1979), including “*Eleutherodactylus*” (Lynch and Duellman, 1997).

Gravid females were found throughout the rainy season, in contrast to that observed for *Haddadus binotatus* (= *Eleutherodactylus binotatus*) from the Atlantic Forest (Canedo and Rickli, 2006), in which the reproduction and call activity occur in the beginning of the rainy season. Seasonal reproduction was also observed in *Eleutherodactylus cooki* from Puerto Rico (Rogowitz et al., 2001).

**Table 1. Number of individuals of *Pristimantis* aff. *fenestratus* recorded in nocturnal samplings based on two sample methods (visual and auditory surveys) conducted in 72 plots at Reserva Florestal Adolpho Ducke (RFAD) and 41 plots at Fazenda Experimental da Universidade Federal do Amazonas (Fazenda UFAM), Manaus, Brazil. Adults: snout-vent length (SVL) > 22.0 mm.**

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<td>138</td>
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**Table 1. Number of individuals of *Pristimantis* aff. *fenestratus* recorded in nocturnal samplings based on two sample methods (visual and auditory surveys) conducted in 72 plots at Reserva Florestal Adolpho Ducke (RFAD) and 41 plots at Fazenda Experimental da Universidade Federal do Amazonas (Fazenda UFAM), Manaus, Brazil. Adults: snout-vent length (SVL) > 22.0 mm.**
Our data show that *P. aff. fenestratus*, which is a terrestrial breeder, calls continuously, but shows a strong peak at the beginning of the rainy season. The data agrees partially with that from Gottsberger and Gruber (2004) for French Guiana on another species with direct development, where a slight peak in the calling activity was observed in the beginning of the rainy season, and from Menin *et al.* (2008) and Rojas-Ahumada and Menin (2010) for *Pristimantis zimmermanae* and *P. ockendeni* from central Amazonia. Other species in the Neotropical region have a continuous reproductive activity, showing seasonal variation only in the frequency of the reproductive activity (for example *C. bransfordii*, *E. cuneatus*, *E. johnstonei* and *E. coqui*; Bourne, 1997; Towsend and Stuart, 1994; Donnelly, 1999; Fong *et al.*, 2010). Continuous reproduction was also observed in *Pristimantis croceoinguinis* (= *Eleutherodactylus croceoinguinis*), *P. lanthanites* (= *E. lanthanites*), *Pristimantis variabilis* (= *E. variabilis*) and *E. coqui* (Crump, 1974; Towsend and Stuart, 1994). The pattern observed in central Amazonia could be related to differences in rainfall volume throughout the year, with months with lower rainfall than other tropical forest areas studied. Terrestrial eggs deposited by this species group are susceptible to dehydration, depending on substrate moisture available for embryo development (Duellman and Trueb, 1994). Areas with a low number of dry months favor the occurrence of continuous reproduction, mainly in Central America, in which the dry season is shorter and wetter than other areas of Amazonia (Watling and Donnelly, 2002).

Based on the occurrence and variation of gravid females, the presence of a higher number of juveniles at the end of the rainy season and the variation in the number of calling males, our data suggest that *P. aff. fenestratus* reproduces during the rainy season, showing marked seasonality.

**Resumo**

*Pristimantis aff. fenestratus* é uma espécie comum e abundante em florestas de terra firme na Amazônia Central. Neste estudo, apresentamos dados de reprodução e estrutura populacional dessa espécie em duas áreas, durante três estações chuvosas, utilizando métodos de amostragem padronizados. Um maior número de machos em atividade de vocalização foi registrado no início das estações chuvosas. No entanto, cantos esporádicos de machos foram detectados na
estação seca. Machos capturados no final da estação chuvosa foram maiores que aqueles registrados em outros períodos. Fêmeas apresentaram óvulos visíveis através da parede do abdômen e foram detectadas em todas as amostragens de uma das áreas estudadas. Houve dimorfismo sexual no tamanho, sendo as fêmeas maiores que os machos. O maior número de jovens foi encontrado no final das estações chuvosas. O tamanho dos jovens foi variável e os menores indivíduos foram registrados no final das estações chuvosas. Com base nas amostragens noturnas, a razão sexual dos adultos foi 1,9:1 (machos:fêmeas). De acordo com a presença de fêmeas maduras, variação no número de jovens e variação na atividade de vocalização dos machos, acreditamos que essa espécie se reproduz durante a estação chuvosa e apresenta marcação sazonalidade.

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