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Reproductive behaviour of the glass frog *Hyalinobatrachium cappellei* (Anura: Centrolenidae) in the Southern Amazon

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ABSTRACT

Frog species of the Centrolenidae family exhibit a high variation in reproductive behaviours. Herein we describe the reproductive, ecological, acoustic and behavioural features of *Hyalinobatrachium cappellei* in Southern Amazon. Two hypotheses were also evaluated: (1) are ecological characteristics of the reproductive site related to male size and quantity of eggs and clutches? And (2) do males with one or more existing clutches at reproductive sites accumulate more new clutches than those males initially without them? This study was conducted at two streams, with sampling at 1 km each in Southern Amazon. Species reproduction occurred during the wet season and was considered prolonged breeding. Although male vocalization occurred on both the upper and underside of leaves, clutches were most often deposited on the underside of leaves. Males emitted a courtship call upon female approach. The process of female approach until oviposition lasted approximately eight hours. After oviposition, females left the breeding site while males remained near the clutch, covering it with the belly in ventral brooding behaviour. Thus, egg attendance is reported for the first time for this species. No intraspecific or interspecific aggressive behaviour was recorded. There was no relationship between habitat characteristics of the reproductive sites and male size, number of eggs and clutches. However, after 10 days of monitoring, we found evidences that parental males had a higher number of clutches than those males initially without clutches. Similar to other glass frog species, the behavioural characteristics of *H. cappellei* make this species an excellent model to use in evaluating the cost-effectiveness of parental care and the importance of egg attendance for offspring survival. Therefore, these data contribute towards a better understanding of the complex phylogenetic and biogeographic relationships between centrolenid lineages.

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Introduction

Anurans have the greatest reproductive diversity among all tetrapod vertebrates (Haddad and Prado 2005; Crump 2015). However, detailed information on the reproductive biology of many species is unknown, especially those occurring in the Neotropics, which contains the highest species richness (Rodrigues et al. 2007; Wells 2007). Information about a species' reproductive and ecological aspects contributes to solutions in taxonomic problems (Guayasamin et al. 2009), assessment of conservation status and development of strategies in biodiversity conservation following IUCN established criteria (Hoffmann et al. 2008).

Biological and reproductive strategies are the combination of physiological, morphological and behavioural attributes that each species adopts in response to environmental conditions in aiming to produce the optimal number of offspring (Duellman and Trueb 1994). Understanding the complex interactions between biotic and abiotic factors, and any effects these have on the reproductive strategies of anurans, has been the subject of many studies (e.g. Landeiro et al. 2014; Schalk and Saenz 2016). Some species of centrolenids have peculiar reproductive attributes such as territoriality and parental care (Cisneros-Heredia and Mcdiarmid 2007; Vargas-Salinas et al. 2014), making them important ecological models in the study of environmental factors which contribute to increases in the reproductive success of frogs with arboreal reproduction (Vockenhuber et al. 2009; Valencia-Aguilar et al. 2012; Lehtinen et al. 2014; Mangold et al. 2015; Valencia and Delia 2016; Delia et al. 2017).

Centrolenids are nocturnal and arboreal species which generally deposit their clutches on plants or rocks overhanging water bodies. Tadpoles leave the clutch (stage 25) and 'drip' to the body of water, where they complete their development (Guayasamin et al. 2006; Menin et al. 2009). This behaviour prevents and/or reduces egg predation by aquatic predators (Magnusson and Hero 1991; Touchon and Worley 2015), although it simultaneously increases the possibility of clutch desiccation and predation by animals foraging in arboreal substrate, e.g. grasshoppers, crickets, ants and wasps (Vockenhuber et al. 2008, 2009). To increase offspring fitness and minimize the effects of desiccation and predation, some centrolenid species, mainly those belonging to the genera *Centrolene* and *Hyalinobatrachium*, have developed parental care (Vargas-Salinas et al. 2014). Traditionally, parental care is characterized when the parents increase the survival and growth of their offspring, often at a cost to their own survival and reproduction (Smiseth et al. 2012).

Many studies on centrolenid species have analysed male breeding site selection and the effectiveness of egg attendance in the reproductive success of the group (Vockenhuber et al. 2008, 2009; Valencia-Aguilar et al. 2012; Lehtinen et al. 2014; Valencia and Delia 2016). However, little is known about the factors involved in female selection of breeding sites guarded by territorial males. In some species of fish and harvestmen, females show preference for males already in possession of clutches, as this provides benefits such as dilution of predation, reduction in the costs of male assessment and the certification of parenting skills (Stiver and Alonzo 2011; Requena and Machado 2015). To understand the factors that lead to greater reproductive success, the selection criteria used by females in partner selection is equally as important as the selection of breeding sites by males.

Hyalinobatrachium cappellei was recently the subject of a taxonomic review, resulting in no distinction between lineages of *Hyalinobatrachium crurifasciatum, H. eccentricum, H. ignioculus*, and *H. cappellei*, therefore all were synonymized as *Hyalinobatrachium cappellei* (Castroviejo-Fisher et al. 2011). No detailed information about the reproductive biology of the species has been recorded, apart from that males vocalize in trees and shrubs near water bodies with some individuals found close to one or more clutches during the day and night (Castroviejo-Fisher et al. 2011; Noronha et al. 2012). Knowledge of the ecological and reproductive aspects of centrolenids is important as it contributes to further understanding of the complex phylogenetic and biogeographic relationships between lineages (Guayasamin et al. 2009). Therefore, the objectives of this study were to describe the reproductive, ecological, acoustic and behavioural features of *H. cappellei* in the Southern Amazon. Two hypotheses were also evaluated: (1) are ecological characteristics of the reproductive site (height, distance to water, water depth, leaf area, canopy opening, stream velocity, pH and dissolved oxygen) related to male size and quantity of eggs and clutches? And (2) do males with one or more existing clutches at reproductive sites accumulate more new clutches than those males initially without clutches, as shown in other groups such as harvestmen and fish?

Material and methods

This study was conducted at two streams located at Fazenda São Nicolau (−9.818611°S, −58.260556°W), municipality of Cotriguaçu, in the north-west portion of Mato Grosso, Brazil. Monthly visits were carried out from January 2014 to May 2016 to evaluate the temporal distribution of the species. Sampling was intensified during the wet seasons between the months of October 2014 to May 2015 and October 2015 to May 2016 due to the stability of rainfall and an increase in the abundance of individuals. The region's vegetation is characterized as open and dense Ombrophyle forest (Veloso et al. 1991), 200–300 m above sea level. The region's climate is tropical, hot and humid, with an average temperature of 24°C and relative humidity around 80% (Vourlitis et al. 2002). Average annual rainfall is 2034 mm, with a dry season from April to September and a wet season from October to March (Noronha et al. 2015).

Transects of 1 km in length were established at each stream and monitored for seven nights per month between 17.00 h and midnight. Sporadic diurnal visits were also performed to verify male permanence during the day. Five oviposition events were monitored until complete deposition of eggs, which occurred at approximately 04.00 h. Vocalizing males and clutches were georeferenced, numbered and the breeding site marked with flagging tape for subsequent monitoring. Behavioural data were also recorded using a headlamp with red light. Males were identified using photo-identification through stains and spots on the dorsum of individuals. Behavioural data (photos and videos) was recorded with a Nikon D90 camera (Nikon Corporation, Manaus, Amazonas, Brazil).

Advertisement and courtship calls were recorded using a Marantz PMD 660 recorder adapted with a Sennheiser ME 66 unidirectional microphone. Digital recordings were sampled at 44.1 kHz with 16-bit resolution and saved as wave files. Calls were analysed and digitalized with Raven Pro v1. 3 software (Bioacoustics Research Program 2008). Comparative analyses were performed to detect possible differences between the advertisement call, previously described by Myers and Donnelly (1997), and the courtship call, firstly described in this study. We used the Bray–Curtis distance measure to obtain dissimilarity values between the advertisement and courtship call using non-metric multidimensional scaling (NMDS). The NMDS scores were used as dependent variables in multivariate analysis of variance models (MANOVA) to analyse differences between the two types of calls, relating to the temporal and spectral parameters (call duration and range, number and length of notes, intervals between notes, minimum, maximum and dominant frequency). All statistical analyses were performed with the R software (R Development Core Team 2017), using the *vegan* and *mass* packages.

Physical and chemical parameters were measured to characterize reproductive sites, with measurements performed at the time during which the male or the clutch were located. Clutch/male height and distance to water were measured using a tape measure. Leaf width and length and stream depth were measured with a millimetric ruler (0.1 mm). Canopy openness was measured with a concave spherical densiometer, model C (Lemmon 1957). Stream velocity was measured using the time spent by a float switch to travel 1 m, therefore stream velocity was obtained in $m\ s^{-1}$. Dissolved oxygen (%) and pH within the stream were measured with an Oakton PCD-650 multiparameter (Oakton Instruments, Vernon Hills, Illinois, US). A total of 42 males were measured with a digital calliper (0.01 mm), and 33 males were not measured as they were not near to the clutch during sampling and/or were located in very high places. Of the plants used as reproductive sites that were collected; 82% were identified to genus level and 68% identified to species level. Leaf area was determined using the ellipse area formula (Vockenhuber et al. 2008), i.e. leaf area = (leaf length/2) × (leaf width/2) × π. Multiple regressions were carried out to analyse whether characteristics of the reproductive site or size of the male influenced the amount of clutches and eggs. We transformed the variables using Box–Cox transformation, whenever the criteria for normality and homoscedasticity were not achieved (Box and Cox 1964).

To suggest the hypothesis that males already in possession of one or more clutches at the reproductive site ultimately obtain more clutches than those males initially without clutches, we observed 10 males for 10 days during the rainy season (five males with one or more clutches and five males without clutches). Subsequently, we conducted a *t*-test to evaluate whether there was a difference in the total number of clutches obtained between the two groups at the end of the studied period. We included in the analyses only clutches obtained after observations begun.

Results

Reproductive aspects and site selection

The reproductive period of *H. cappellei* corresponds with the region's wet season ($r^2 = 0.61$, $F = 22.38$, $p = 0.003$) and lasted over six months. Males began calling activity in the month of November and ceased in April. Seventy-five reproductive sites were recorded, all of which were located on leaves. Sixty-six sites were with clutches (single or multiple) and nine without clutches. The average distance between sites was 19.28 m, with a minimum distance of 0.3 m (males sharing the same leaf) and a maximum of 190 m, resulting in 37 reproductive sites per km of sampled stream.

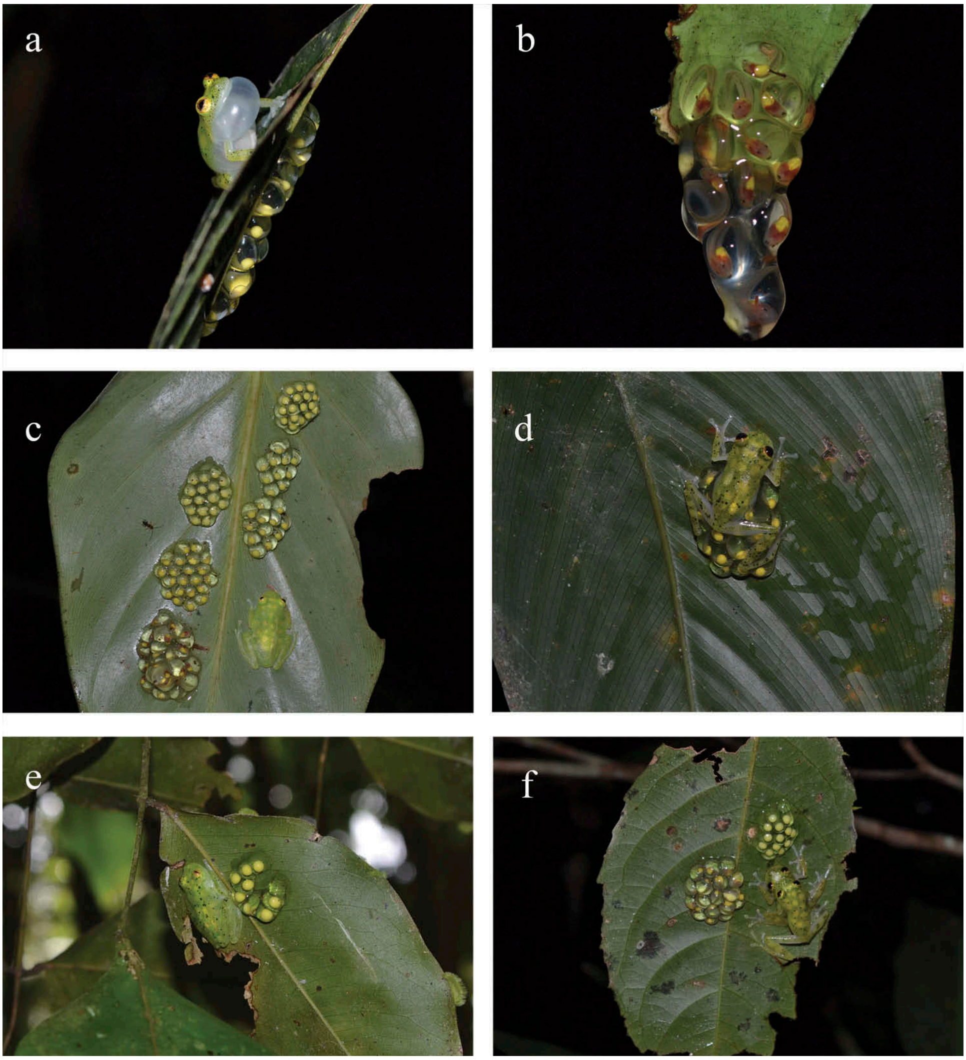

Figure 1. Reproductive behaviours of *Hyalinobatrachium cappellei* at Cotriguaçu, Mato Grosso, Brazil. (a) Vocalizing male on the upper face of leaf with a clutch on the lower face; (b) clutch with hatching tadpoles; (c) multiple disposition of clutches on the same leaf; (d) male ventral brooding behaviour after remaining for a few minutes in the rain; (e) male near clutch during the day; (f) couple in amplexus.

Calling males (Figure 1(a)) and clutches (Figure 1(b)) were found on trees and shrubs overhanging the water. The males alternated vocalization cycles on upper and lower surface of a leaf during the night; however, 95.45% of clutches were found on the lower face (Figure 1(c)). The plants most used for reproductive sites were *Anaxagorea dolichocarpa* Sprague and Sandwith (Annonaceae) and *Phenakospermum guyannense* (Rich.) Endl. (Strelitziaceae), 18.6% and 5.3% respectively. The average number of clutches per leaf was 1.70 with an average of 17.20 eggs per clutch (Table 1). Males began calling shortly before sunset, at approximately 17.40 h, ending at around 05.00 h. A peak in calling activity was recorded

Table 1. Snout–vent length of male/female and biotic and abiotic data related to the reproductive sites of *Hyalinobatrachium cappellei* in Cotriguaçu, Mato Grosso, Brazil.

	Average	Maximum	Minimum	Total number of observed events (*N*)
Male size (SVL) (mm)	22.24 ± 0.74	23.7	20.63	42
Female size (SVL) (mm)	22.47 ± 1.33	23.90	20.27	5
Number of clutches per leaf	1.70 ± 1.24	6	1	128
Number of eggs per clutch	17.20 ± 6.92	32	4	2133
Height (cm)	188 ± 64.57	500	89	75
Water horizontal distance (cm)	119.40 ± 126.67	700	0	75
Leaf area (cm^2)	405,76 ± 931.26	4647.2	3.29	75
Canopy opening (%)	16.60 ± 4.99	33.28	9.36	75
Water depth (cm)	15.89 ± 14.64	58	0	75
Stream velocity (m s^{-1})	0.09 ± 0.10	0.33	0	75
pH	4.81 ± 2.03	6.71	0	75
Dissolved oxygen (%)	18.79 ± 15.19	67.9	0	75

Table 2. Relationship between male size, number of clutches and number of eggs in respect to characteristics of the reproductive sites for *Hyalinobatrachium cappellei* in Cotriguaçu, Mato Grosso, Brazil.

Biotic variables	Male size	Number of clutches	Number of eggs
Abiotic variables	$r^2 = 0.12; F_{8,32} = 0.58$	$r^2 = 0.16; F_{8,32} = 0.78$	$r^2 = 0.12; F_{8,32} = 0.58$
Height (cm)	$p = 0.79$	$p = 0.38$	$p = 0.79$
Water horizontal distance (cm)	$p = 0.60$	$p = 0.80$	$p = 0.60$
Leaf area (cm^2)	$p = 0.93$	$p = 0.67$	$p = 0.93$
Canopy opening (%)	$p = 0.37$	$p = 0.43$	$p = 0.37$
Water depth (cm)	$p = 0.92$	$p = 0.35$	$p = 0.92$
Stream velocity (m s^{-1})	$p = 0.35$	$p = 0.10$	$p = 0.33$
pH	$p = 0.91$	$p = 0.36$	$p = 0.91$
Dissolved oxygen (%)	$p = 0.93$	$p = 0.61$	$p = 0.93$

between 20.00 h to 01.00 h. Several males were observed with their ventral surface in contact with the clutches during the nocturnal period (Figure 1(d), ventral brooding *sensu* Vockenhuber et al. 2009), and remaining near to the clutch during the day (Figure 1(e)). The multiple regression analysis performed to evaluate the relationship between the habitat characteristics of the reproductive site (height, distance to water, water depth, leaf area, canopy opening, stream velocity, pH and dissolved oxygen) and male size, number of eggs and clutches (Table 1) did not show any significant relationship (Table 2).

Courtship, oviposition and egg attendance behaviour

Five female approach, pre-amplexus and oviposition events were recorded. Courtship behaviour displayed between couples was similar, thus the description follows the behaviour of the couple in Figure 1(f) (a video recording of this couple is provided as supplementary material). At approximately 20.30 h, we observed the female at 2 m height approaching the male (Figure 2), jumping from branches adjacent to the perched calling male (1.44 m). This approach process lasted on average 10 min (SD ± 1.92 min). Once the female arrived on the male's leaf, the male remained stationary and changed his vocalization to a courtship call. The female approached the male and touched his forelimb and snout, while the male remained motionless and calling. After making

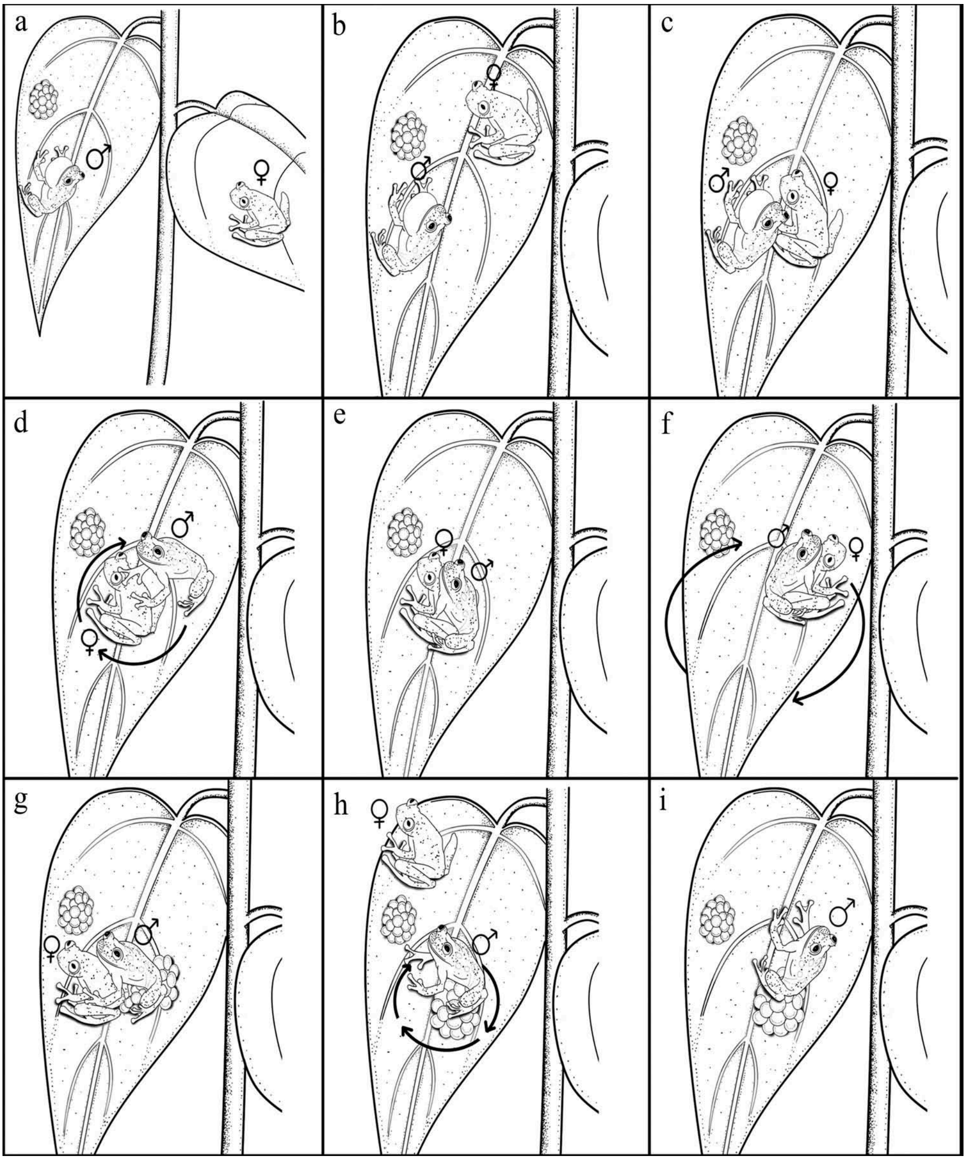

Figure 2. Sequence of courtship and oviposition behaviour in *Hyalinobatrachium cappellei* (a, b) Female approaching the calling male which initiates the vocalization of courtship call; (c) female touching the male; (d) male 'turns' its body around the female; (e) amplexus formation; (f) couple traverses both the upper and lower face of the leaf; (g) oviposition, (h) female leaves the top of clutch while male remains, rotating the body; (i) male remains on clutch while the female abandons the reproductive site. Illustration by Milton Cordova.

contact with the male, the female positioned itself in parallel to the male and amplexus occurred. On three occasions before the completion of mating, the male performed a 360° rotation over the female's body.

During amplexus, the couple moved slowly and repeatedly between the lower and upper faces of the leaf. This process lasted approximately seven hours until

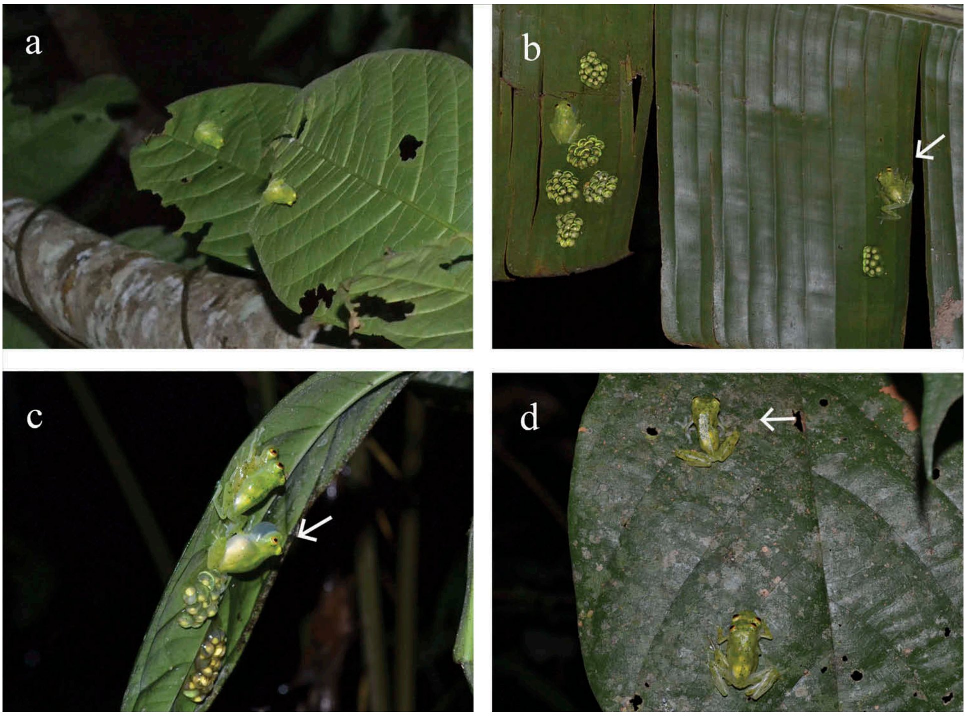

Figure 3. Non-aggressive behaviours of *Hyalinobatrachium cappellei* in Cotriguaçu, Mato Grosso, Brazil. (a) Two males vocalizing on different leaves of the same plant; (b) two males sharing the same leaf, the arrow indicates couple in amplexus; (c) invasive couple in amplexus, with the arrow indicating the male resident; (d) female retreating from a couple in amplexus.

oviposition (mean = 7.08 h, SD ± 1.06 h, n = 5). At approximately 04.00 h the couple positioned themselves on the leaf's lower face and initiated oviposition. As the eggs were deposited, the female moved forward, disengaging from amplexus, while the male remained on the deposited eggs. After ending oviposition, the female remained on the leaf for three minutes, then stepped away from the male and the clutch. The time from oviposition to female retreat lasted approximately 25 min (SD ± 3.70 min).

No aggressive behaviour among conspecific males or between males and predators was observed. Two cases of males calling close to each other (0.3 m) were recorded (Figure 3(a)). In one of these cases, two males were vocalizing on the same leaf (Figure 3(b)). On a single occasion we observed a couple in amplexus invading the reproductive site of another male. Although the male resident showed signs of agitation, no aggressive interactions were observed (Figure 3(c)). In a separate event, we observed two females approaching the same male. One of the females soon approached and made contact with the male, subsequently forming amplexus. Shortly after the second female arrived near the couple (Figure 3(d)) on the same leaf and remained stationary for two minutes before moving to another leaf.

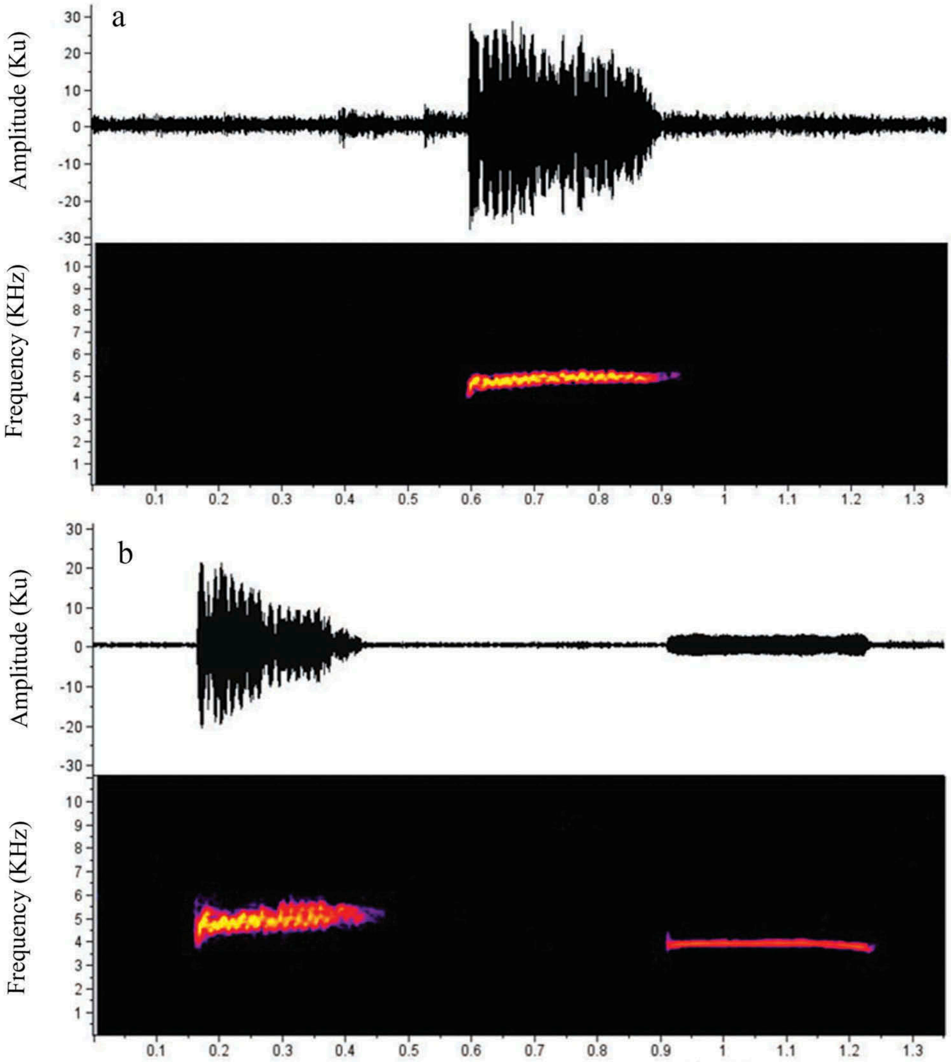

Figure 4. Sonogram and oscillogram of (a) advertisement call and (b) courtship call of *Hyalinobatrachium cappellei* at Cotriguaçu, Mato Grosso, Brazil.

Bioacoustic analysis

The advertisement (Figure 4(a)) and courtship calls (Figure 4(b)) showed a multipulse pattern. However, acoustic parameters measured between the two call types were varied (Table 3). The advertisement call is composed of a single note characterized as a 'peeeep' lasting on average 0.319 seconds (± 0.014), while the courtship call has two notes with an average duration of 1.077 seconds (± 0.06), and is characterized as 'peeeep…fîîî' (the calls are provided as supplementary material, inter-call intervals were reduced). We found significant differences between the advertisement and courtship calls (MANOVA, Pillai trace = 0.65; $F_{2,28} = 26.52$; $p < 0.01$; Figure 5). The first two axes captured 95% of the variation of the original data.

Table 3. Temporal and spectral parameters of the advertisement (four individuals) and courtship call (five individuals) of *Hyalinobatrachium cappellei* in Cotriguaçu, Mato Grosso, Brazil.

	Advertisement call (n = 19)	Courtship call (n = 12)	
Number of notes	1	2	
Call duration (s)	0.319 ± 0.014 (0.298–0.350)	1.07 ± 0.06 (1.017–1.254)	
Inter-call interval (s)	24.885 ± 10.653 (4.200–41.241)	36.594 ± 14.129 (24.555–57.120)	
Inter-notes interval (s)	-	0.434 ± 0.055 (0.326–0.517)	
		Note 1	Note 2
Minimum Frequency (Hz)	3658.5 ± 173.6 (3381–3947.7)	3498.3 ± 201.2 (3165.9–3839.7)	3396.4 ± 128 (3267–3661.5)
Maximum Frequency (Hz)	5339.6 ± 125.7 (5178.7–5664.2)	5802.4 ± 240.6 (5421.5–6149.7)	4347 ± 385.8 (4086.3–5512.5)
Dominant Frequency (Hz)	4850.6 ± 76.2 (4737.3–4995.7)	4873.6 ± 112.9 (4651.2–4995.7)	3962.1 ± 307.2 (3789.9–4909.6)

Notes: Time is given in seconds (s) and frequency in Hertz (Hz). All recordings were done between 24.8 and 27°C. Values are presented as mean ± standard deviation (minimum–maximum); n = number of notes analysed. Individuals were not collected and were recorded about 0.50 m.

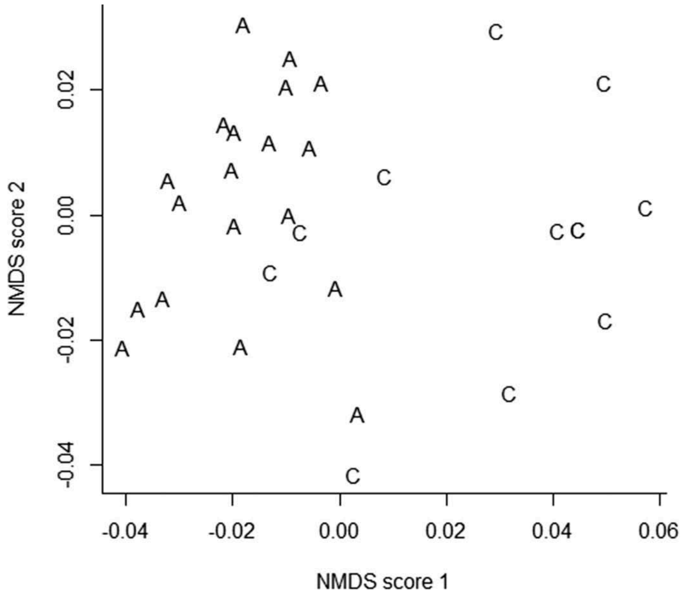

Figure 5. First two axes of nonmetric multidimensional scaling (NMDS), composed by acoustics parameters of advertisement call (A) and courtship call (C) of *Hyalinobatrachium cappellei* in Cotriguaçu, Mato Grosso, Brazil.

Predation

Six predation events were observed, four involving clutches abandoned by males and two involving clutches with parental attendance. Abandoned clutches suffered from predation by different types of insects (cockroaches, moths, bush crickets and ants, Figure 6(a–d)), as well as desiccation and the development of fungi (Figure 6(e), 6(f)). Clutches with parental attendance were partially predated (we do not know if the male

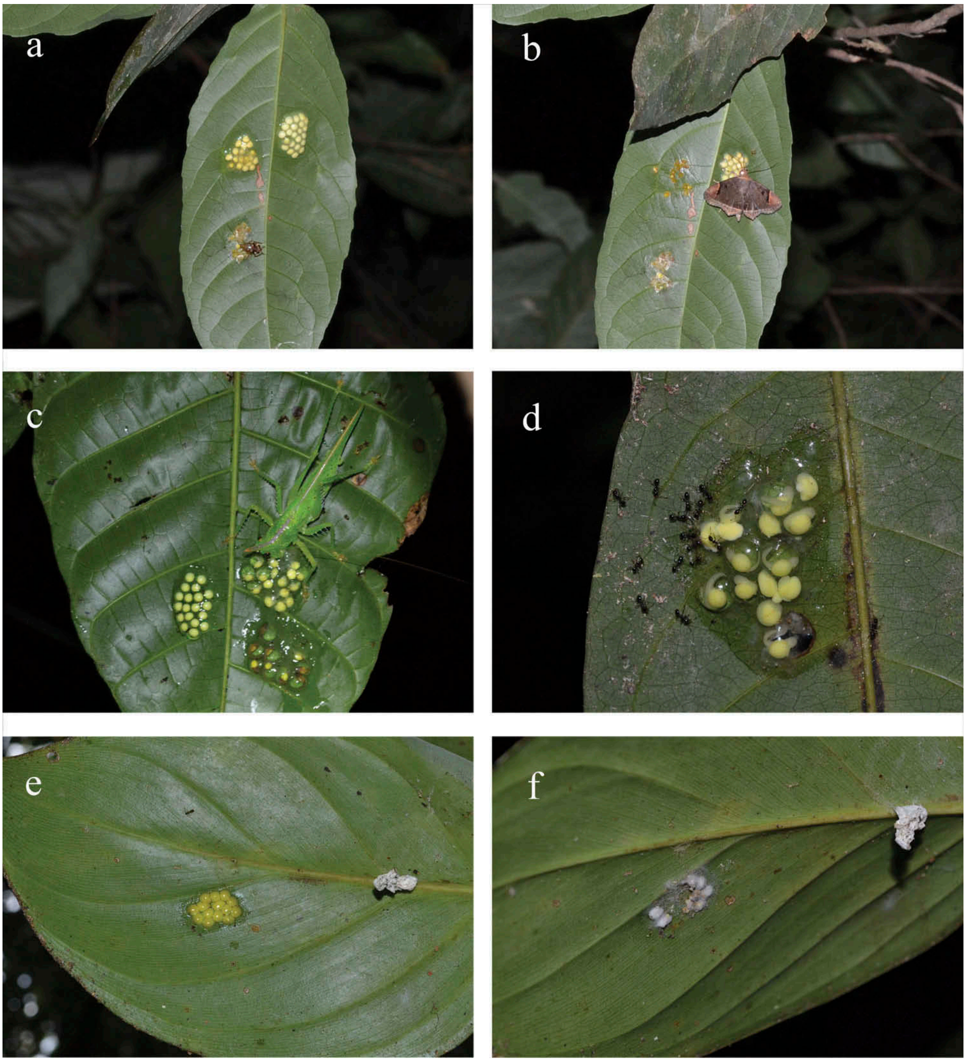

Figure 6. Observed predation and parasitoid events on *Hyalinobatrachium cappellei* clutches in Cotriguaçu, Mato Grosso, Brazil. Clutches without male attendance suffering predation by (a) Blattodea; (b) Noctuidae moth family; (c) Tettigoniidae bush crickets; and (d) ants, *Pheidole* sp. (e, f) Abandoned clutch, process of dehydration and initiated fungi development after four days.

was or was not near to the clutch during predation). However, the males continued to care for the remaining eggs, which reached the hatching stage.

Number of clutches between males with and without pre-existing egg masses

Our data suggested that males with one or more clutches obtained more new clutches than those males without initial clutches during the period studied (t = 2.62, df = 4, p = 0.04; Figure 7). Males with initial clutches gained an average of 3.80 new clutches (maximum = 6; minimum = 2; SD ±1.78, n = 5), and males without initial clutches gained an average of 1.80 new clutches (maximum = 3; minimum = 2; SD ± 0.83, n = 5).

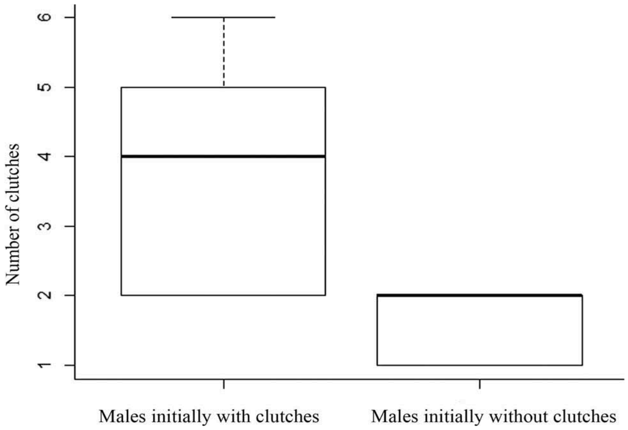

Figure 7. Number of clutches recorded in a 10-day period between *Hyalinobatrachium cappellei* males initially with clutches and males initially without clutches at Cotriguaçu, Mato Grosso, Brazil.

Discussion

Reproductive aspects and site selection

Hyalinobatrachyum cappellei can be classified as prolonged breeder (Wells 1977; Rodrigues et al. 2007) whose reproductive period lasts approximately six months and coincides with the region's wet season. We believe that the prolonged reproductive period facilitates a reduction in intraspecific competition and increases the reproductive success of *H. cappellei*. Further, as the wet season contains higher precipitation and increase of stream water, this creates a suitable environment for tadpole development for species with moisture-dependent arboreal clutches, and enables them to avoid egg and embryo desiccation (Prado et al. 2005; Rodrigues et al. 2007).

Most centrolenid genera typically deposit their clutches on the upper faces of leaves (e.g. *Centrolene, Vitreorana* and *Teratohyla*); however, the majority of species in the *Hyalinobatrachium* genus deposit their clutches on the lower faces of leaves (Guayasamin et al. 2009; Nokhbatolfoghahai et al. 2015). It is possible that this strategy has been adopted to reduce clutch desiccation by providing protection from direct exposure to wind and solar radiation. However, this positioning also precludes them from directly receiving rain and mist, which is probably compensated by the male's hydration process (Delia et al. 2010).

Our results did not find any relationship between characteristics of the reproductive site and male size or quantity of eggs and clutches. Similarly, no significant difference between habitat characteristics used by males attending one or multiple clutches was observed in *H. aureoguttatum* and *Nymphargus ignotus* (Restrepo and Naranjo 1999; Valencia-Aguilar et al. 2012). In *C. savagei*, larger males obtained a greater number of mating events (Vargas-Salinas et al. 2014), and *H. fleischmanni* males located at higher sites had a higher frequency of new clutches (Greer and Wells 1980). Calling from higher sites and also on larger leaves allows a greater propagation of sound (Wells and Schwartz 1982); however, there is no evidence which confirms *H. fleischmanni* males choose a location by its acoustic characteristics. *Anaxagorea dolichocarpa* is a common plant found within the riparian zone of the present study (pers. obs.) and the males may be using the most available resource in the area. But many centrolenid species show preference for depositing clutches on plants with large and smooth leaves (Vockenhuber et al. 2008; Valencia-Aguilar et al. 2012), such as *Anaxagorea dolichocarpa* and *Phenakospermum guyannense*, the plants most used by *H. cappellei*. Cabanzo-Olarte et al. (2013) found through the application of an electivity index that male and female *Espadarana andina* almost strictly selected green leaves of the broad-leaved species *Hedychium coronarium* (Zingiberaceae) for calling and oviposition. We believe that these arboreal plant species are the most used due to their leaf size and smooth surface (absence of trichomes), which facilitates egg adhesion and offers more space for new clutch additions (Vockenhuber et al. 2008; Valencia-Aguilar et al. 2012; Cabanzo-Olarte et al. 2013; Rojas-Morales and Escobar-Lasso 2013). We also believe that the choice of reproductive site involves a complex balance between biotic and abiotic characteristics which should be analysed in the future to better understand the selection criteria used by centrolenid species.

Courtship, oviposition and egg attendance behaviour

The passivity of *H. cappellei* males, which remain motionless waiting for tactile stimulation from the female, was also observed in *H. fleischmanni* (Jacobson 1985). However in *Centrolene savagei*, males approached first, providing visual and tactile stimulation to the females (Vargas-Salinas et al. 2014). There are few descriptions of courtship behaviour in Centrolenidae, though the courtship behaviour observed in *H. cappellei* is similar to that described for *C. savagei* (Vargas-Salinas et al. 2014), *H. fleischmanni* (Greer and Wells 1980; Jacobson 1985) and *H. orientale* (Nokhbatolfoghahai et al. 2015).

The courtship and oviposition behaviour of *H. orientale* lasts approximately seven hours (Nokhbatolfoghahai et al. 2015) and is quite similar to that observed in *H. cappellei* (eight hours). During a large proportion of this time, for both species, the couple move around both sides of the leaf's surface, where oviposition will occur. This prolonged period in amplexus before oviposition is probably used by the female to analyse the quality of the males and the reproductive site. This type of prolonged amplexus was observed by Greer and Wells (1980) in *H. fleischmanni*; these authors suggested this inspection by the female, but before amplexus formation. Anuran selection of oviposition sites includes the recognition and discrimination of the physical and chemical structure of the water body, as well as avoiding risk factors such as desiccation, competition, parasitism, cannibalism and predation (Silva and Giaretta 2008; Touchon and Worley 2015). Thus, the species can select the most beneficial oviposition site for their offspring to maximize fitness (Resetarits and Wilbur 1989; Pintar and Resetarits 2017). In species with terrestrial oviposition and aquatic tadpoles, the mechanisms of choice require differentiated criteria in the selection process, assuming conflicting risks, such as arboreal egg desiccation and aquatic egg predation (Touchon and Worley 2015).

The diurnal and nocturnal male egg attendance observed in this study has been reported for other centrolenid species (Vockenhuber et al. 2008). However, this is the first record of egg attendance for *H. cappellei*. Parental care in centrolenids has always been described as being exclusively performed by males, but recently, Valencia and Delia (2016) recorded for the first time prolonged maternal care in centrolenids in the monotypic *Ikakogi tayrona*, and a brief period of maternal care (first night behaviours) for many centrolenid species (Delia et al. 2017). In *H. cappellei*, males remained close to the clutches during a large part of the day, and at night was often seen on clutches, with the belly covering the eggs (ventral brooding behaviour). This behaviour is considered a process which aims to hydrate the clutch, resulting in the transfer of water from the male's abdomen to the clutch (Taigen et al. 1984; Vockenhuber et al. 2008), or through the emptying of the parent's bladder onto the eggs (Delia et al. 2017). In addition, recent studies have demonstrated that the direct contact between adult and clutch causes a vertical transmission (when symbionts are transferred from parents to offspring) of innate defences by changing the microbial community and reducing the infestation of eggs by fungi (Walke et al. 2011; Valencia and Delia 2016). Egg attendance in amphibians is typically associated with species possessing terrestrial or arboreal oviposition, with the main function being to reduce mortality of embryos that may be vulnerable to predators, pathogens and desiccation (Lehtinen et al. 2014).

Bioacoustic analysis

The advertisement call, the most common anuran vocalization, is emitted by males to attract conspecific females for reproduction and to segregate calling males. In contrast, the courtship call is a close-range communication between male and female, prior to amplexus, for stimulation and orientation of the females (Toledo et al. 2014). Change between the advertisement and courtship calls typically occurs in other species of anurans, including other centrolenid species such as *C. savagei* and *H. fleischmanni* (Jacobson 1985; Vargas-Salinas et al. 2014), and apparently in *H. orientale* (Nokhbatolfoghahai et al. 2015). There is no description of acoustic parameters of the courtship call in *H. orientale*, only *C. savagei* and *H. fleischmanni*, which share similar frequencies to *H. cappellei*. The difference in the courtship call of *H. cappellei* is the emission of two notes, while *C. savagei* and *H. fleischmanni* emit only one note (Greer and Wells 1980; Vargas-Salinas et al. 2014). The courtship call is seen as a strategy adopted by the chosen male in order to appear more conspicuous in a choir of males. Courtship calling during the female's approach is probably common between different anuran species, but it has not been reported for many species due to the lack of detailed observations during courtship behaviour (Wells 2007). More detailed behavioural studies on centrolenids are necessary to detect, record and analyse courtship calls to help explain the factors that determine acoustic changes during courting between species.

Predation

Predation occurred on abandoned clutches, and also on clutches in the presence of a monitoring male. However, in the clutches with a male present, some viable eggs (tadpoles hatched) always remained after predation, unlike abandoned clutches, where all eggs were completely lost to predation, desiccation and fungal growth. These observations empirically corroborate several studies that experimentally verified the importance of egg attendance in centrolenids, demonstrating their efficacy against predation and desiccation (Vockenhuber et al. 2009; Valencia-Aguilar et al. 2012; Lehtinen et al. 2014; Valencia and Delia 2016). Therefore, we suggest that future studies evaluate the effectiveness of male presence against predation and development of pathogens in *H. cappellei*.

Number of clutches between males with and without pre-existing egg masses

In species where parental care is exclusively provided by the male, females might judge a male's quality according to his parenting skills. Some female species of harvestmen and fish prefer to mate with males that already have eggs (Stiver and Alonzo 2011; Requena and Machado 2015). Thus, females can benefit their offspring by reducing the probability of predation and increase the chances of survival through parental care given by the male (Requena and Machado 2015). Female sexual preference based on male parenting skills is suggested as the main selective pressure that favours the evolution and maintenance of parental care exclusively in males (Klug et al. 2012). However, parental care involves a cost-benefit to the male because of the greater risk of predation, reduced foraging time, and loss of opportunities for new mates (Cheng et al. 2013). Our data suggest the females prefer males already in possession of clutches; however, we believe that experimental manipulations are necessary to corroborate this hypothesis. However, it is important evidence for use in further research.

In summary, *Hyalinobatrachium cappellei* showed similar behavioural, acoustic, and reproductive characteristics to other species of the genus *Hyalinobatrachium*. However, we register behaviours previously unknown for this species, such as egg attendance and courtship calls. More research is needed to relate the number of clutches under male care with the frequency of call emission, frequency of new clutches, and the number of hatched tadpoles, to understand male parental skills and female mating preferences in this species. To this extent, the behavioural characteristics of *H. cappellei* make this species an excellent model to evaluate the cost-effectiveness of parental care and the importance of egg attendance for reproductive success.

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