J Anim Behav Biometeorol
v.2, n.2, p.47-53 (2014)

ISSN 2318-1265

ARTIGO ORIGINAL
Original Article

Daily behavioral activities of bullfrog *Lithobates catesbeianus* (Shaw 1802)

Atividades comportamentais diárias de rãs-touro Lithobates catesbeianus (Shaw 1802)

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Received: 15 April 2014 ▪ Accepted: 28 April 2014

Abstract Studies on the daily behavioral activities of bullfrogs in laboratories may indicate avenues for appropriate technical management for this specie culture. Twenty-four post-metamorphic phase *Lithobates catesbeianus* (Shaw 1802) bullfrogs had their daily activities described. The animals were housed in six experimental stalls and feed was offered once a day at random times over 10 days of filming. Behavioral activity in each stall was recorded for the first 15 minutes of every hour in a 24-hour period. As results, we observed that ingestion and movement on dry ground were more common at dawn and rare in dark; inactivity on dry ground occurred more in the late night and at dawn. Consequently, there was preference for sheltering less at dawn and in the end of dark phases. The inactivity behavior in the water was more frequent during the early dark phase, showing an inverse relationship with respect to preferred times for feed ingestion. As a result of observed behaviors, we recommended that feed offerings for bullfrogs in captivity are during the light phase of day and that it is available until the following morning.

Keywords amphibian, animal behavior, applied ethology, frog culture

Resumo Pesquisas relativas às atividades diárias de rãs touro em laboratório podem fornecer subsídios para definir técnicas de manejo apropriadas para o cultivo desta espécie. Vinte e quarto rãs da espécie *Lithobates catesbeianus* (Shaw 1802) na fase pós-metamórfica tiveram suas atividades diárias descritas. Os animais foram alojados em seis baias experimentais, seis em cada, com o alimento ofertado uma vez ao dia em horários aleatórios ao longo de 10 dias de filmagem. As atividades comportamentais foram registradas em cada baia nos primeiros 15 minutos de cada hora, por um período de 24 horas. Como resultados, nós observamos que a ingestão do alimento e movimentos na área seca foram mais comuns ao amanhecer e raros na fase escura do dia; a inatividade na área seca ocorreu mais frequentemente tarde da noite e de madrugada. Consequentemente, a utilização do abrigo foi menor no amanhecer e no final da fase escura. O comportamento de inatividade na água foi mais frequente no início da fase escura, mostrando uma relação inversa com relação com o horário preferido para ingestão do alimento. Com base nos resultados obtidos, nós recomendamos que o alimento artificial seja ofertado, para rãs em cativeiro, durante a fase clara do dia e permaneça disponível até a manhã seguinte.

Palavras-chave anfíbio, comportamento animal, etologia aplicada, ranicultura

DOI http://dx.doi.org/10.14269/2318-1265.v02n02a03

ISSN 2318-1265

Introduction

Brazil possesses its own technology for breeding frogs in captivity (frog farming). The activity began in the country in the 1930s and enjoyed periods of growth and technical development in the 1980s and 1990s. According to IBAMA (2009), Brazil produced 603 t of frog meat in 2007, generating an estimated current US$ 4.049.758,74.

The bullfrog, *Lithobates catesbeianus* (Shaw 1802), is an exotic species in Brazil, originally from North America (Coppo 2003), being well adapted to the Brazilian climate. Due to its prolificacy, adaptation to captivity, rapid growth and tolerance to low temperatures, it is the most widely used species in the country (Vieira 1980; Lima and Agostinho 1992).

Despite the use of artificial feeding in farms, little is known about the feeding habits of frogs. In commercial ponds, feed is usually offered once a day, usually in the morning. However, no research has been done to determine whether this is appropriate for the species. Studying animal feeding behavior may therefore indicate adequate management techniques in captivity, favoring the animal welfare and productivity growth (Huningford et al 2012). This would allow the costly feed currently used to be fully exploited and avoid waste that may pollute the environment.

Studies on frog behavior in nature are common, like bullfrog reproduction (Moorea and Jessop 2003), vocal communication (Bee 2004) and studies of their stomach contents (Moreira and Barreto 1996; Daza and Castro 1999; Coppo 2003; Wu et al 2005). In addition, these studies do not apply to commercial frog farming, where animals are subject to injury and stress (Dias et al 2010), high population densities, and artificial feed (pellets).

Applied ethology studies for frogs in captivity are scarce. No research was found in the specialized literature describing the feeding behavior of bullfrogs in captivity. The present study aims to describing the distribution of their daily behavioral activities of bullfrogs, *L. catesbeianus*, under laboratory conditions. This may serve as well as an important tool to improve the animal welfare and positively influence commercial pond breeding.

Materials and Methods

Twenty-four bullfrogs *Lithobates catesbeianus* from the same spawning, inseminated by semen from four different individuals males (Agostinho et al 2000), were studied approximately 60 days after metamorphosis at an initial weight of 70.5 ± 25.6 g. Behavior was recorded in a laboratory using six small stalls, each housing 4 animals (25 frogs m^{-2}). Each stall (54 × 33 × 31 cm) was composed of a wet area with shelter and a pool, and a dry area with ramps and a trough. The mean maximum and minimum temperatures in the environment were 28.7 ºC and 23.9 ºC, respectively. Median maximum and minimum humidity was 85.9% and 75.4%, respectively. Luminosity during the light phase (5 to 17 h) was 61 ± 19 lx and 0 ± 0 lx in the dark phase (18 to 4 h).

During the 10 days of adaptation to the environment and the following 10 days of filmed observations, fresh feed was offered once a day at random times to avoid anticipatory feeding behavior. Each stall was cleaned and had pool water changed at the time new feed was offered. A standard commercial extruded frog feed was available all the time. The pellets were 3-4 mm long and contained the following levels guaranteed by the manufacturer: 45% crude Protein, 4% Ether Extract, 6% Crude Fiber, 14% Mineral Matter and 87.5% Dry Matter. *Musca domestica* (Linnaeus) larvae were used as feed attractants (15% of feed supplied) by causing feed mobility.

Each of the six stalls was continuously filmed 24 hours during 10 days, using a camera equipped with infrared beams. The method used was scan sampling, with instantaneous recording in every minute during the first 15 minutes of each hour, resulting in 86.400 filming episodes. For the following analyses we used the 10 days frequencies, by stall and time periods.

In order to summarize our results, the day period was grouped in eight clusters of three hours each: (1) Dawn: hour 4 to 6; (2) Light 1: hour 7 to 9; (3) Light 2: hour 10 to 12; (4) Light 3: hour 13 to 15; (5) Twilight: hour 16 to 18; (6) Dark 1: hour 19 to 21; (7) Dark 2: hour 22 to 24; and (8) Dark 3: hour 1 to 3.

Behavioral activities recorded were previously catalogued in a previous pilot study, as follows: (a) Inactivity in the water: being static or moving a limb without displacement, such as floating or fully submerged underwater; (b) Movement in water: animals swimming or executing small jumps towards the border; (c) Time inside shelter: the animal entered the shelter and could not be seen; (d) Inactivity on dry ground: static animals or those just moving the limbs, without lateral displacement, both on the ramps, edges or bottom of the trough; (e) Movement on dry ground: animals walking or performing small jumps on the ramps towards the trough, as well as its edges or bottom; and (f) Ingestion: animal ingesting larvae or feed in the trough or on the ramp.

Given the non-adherence of the data to normal distribution and the heterogeneity of factor variances verified by Shapiro-Wilks and Levene tests, respectively, Kruskal-Wallis non-parametric variance analysis was used. When significant differences were found, the post hoc Tamhane's T2 was applied, except for the activity "ingestion", where the Chi-Square test was used. We compared frequencies of each behavior (dependent variables) among time periods

DOI http://dx.doi.org/10.14269/2318-1265.v02n02a03

ISSN 2318-1265

(independent variables). Wilcoxon test was used in order to compare the frequencies in light and dark phases observed during the experiment (Zar 1999). The significance level of 5% was set to assess the results, which are graphically represented by their median and interquartile ranges (75-25%).

Results

Comparing ingestion frequency in the light and dark phases we observed significant differences (test results in Table 1). Differences were also found when comparing the time periods ($\chi^2(5) = 32.8$, $P < 0.001$). Ingestion levels were highest at dawn and lowest during dark 1, 2 and 3 (Figure1a). Time periods Dark 1 and Dark 3 were not included due showed no ingestion events.

Table 1 Median and Wilcoxon test comparison for light and dark phases
Note: The * represents significant difference between light and dark phases

	Median		Wilcoxon Test		
	Light	Dark	*T*	*z*	*P*
Ingestion	4.0	0.0	0.0	-2.20	0.027*
Movement on dry ground	21.5	3.0	0.0	-2.20	0.027*
Inactivity on dry ground	3086.5	2709.5	10.0	-0.11	0.917
Time inside shelter	3883.0	2127.0	2.0	-1.78	0.075
Movement in water	18.0	31.0	9.0	-0.31	0.753
Inactivity in the water	820.0	1608.5	0.0	-2.20	0.028*

For Movement on dry ground, we found significant difference between light and dark phases (Table 1). Significant differences were found for this activity ($H(7) = 31.07$; $P = 0.001$) among the time periods (Figure1c), but no difference was found in pairwise comparisons ($P > 0.108$).

We found no difference between light and dark phases for Inactivity on dry ground (Table 1). Differences were found ($H(7) = 24.59$ $P = 0.001$) among time periods (Figure1d). This behavior showed similar frequencies during dawn and dark 3 ($P = 0.999$) and animals were more inactive in these time periods than during dark 1 and twilight ($P < 0.029$); dark 1 and twilight also showed similar frequencies ($P=1.000$). The remaining comparisons between time periods found no significant differences ($P > 0.057$).

Comparing the Time inside shelter frequency between light and dark phases, we also found no difference (Table 1). However, differences ($H(7) = 27.97$; $P = 0.001$) were found among time periods (Figure1b). This behavior was less frequent during dark 2, dark 3 and dawn, with no differences between these time periods ($P > 0.371$) but we verified a difference between them and dark 1 ($P < 0.013$). Dark 3 and dawn showed lower frequencies than twilight ($P < 0.022$). The remaining comparisons between time periods found no significant differences ($P > 0.115$).

We observed no difference between light and dark phases (Table 1) or among time periods (Figure 1e), for movement in water frequency ($H(7) = 4.86$; $P = 0.677$).

The comparison between light and dark phases showed a significant difference for the behavioral frequency during the experiment for Inactivity in the water (Table 1). Differences in behavioral frequency were recorded ($H(7) = 30.94$; $P = 0.001$) among the different time periods (Figure1f). This behavior showed similar frequencies during dark 1 and dark 2 ($P = 1.000$) and these time period were both more frequent than light 2 and light 3 ($P < 0.009$); light 2 and light 3 also showed similar frequencies ($P = 0.999$). We also found that dark 3 was different from light 2 and light 3 ($P < 0.045$), and dark 1 and dark 2 were different from light 1 ($P < 0.024$). The remaining comparisons between time periods found no significant differences ($P>0.097$).

Discussion

Ingestions were observed more at dawn, and were rare during the dark phase. Movement on dry ground was rare too during the night, and seems to coincide with ingestion. Inactivity on dry ground increased in frequency in late dark phase and at dawn, when there is a pike of ingestion. Conversely, the frogs sheltered less at dawn and in late dark phases. Inactivity in water was more common in the early dark phase, which is probably linked to absence of ingestions. Like inactivity in water, movement in water was more frequent in the early dark phase.

The bullfrog spawn has 5,000 to 40,000 eggs, so it is normal to find both producers and researchers who used only one spawning for cycle of production or research. In this work, only one spawn inseminated by semen from four different individuals males (Agostinho et al 2000) was used, for it is recommended that future studies should address this issue to examine possible interference of genetic lineages.

Inactivity on dry ground, inactivity in water and time inside shelter were the more common behaviors recorded during the 24 h period. These were characterized as activities also preferred by the animals. Ingestion, movement in water and on dry ground had lower frequencies. According to Duellman and Trueb (1994), most amphibians are “passive predators” (sit-and-wait), however other species may have intermediate strategies between the “active” and “passive” types (Toft 1981). Inactivity on dry ground may therefore be related to the anticipation of feed.

DOI http://dx.doi.org/10.14269/2318-1265.v02n02a03

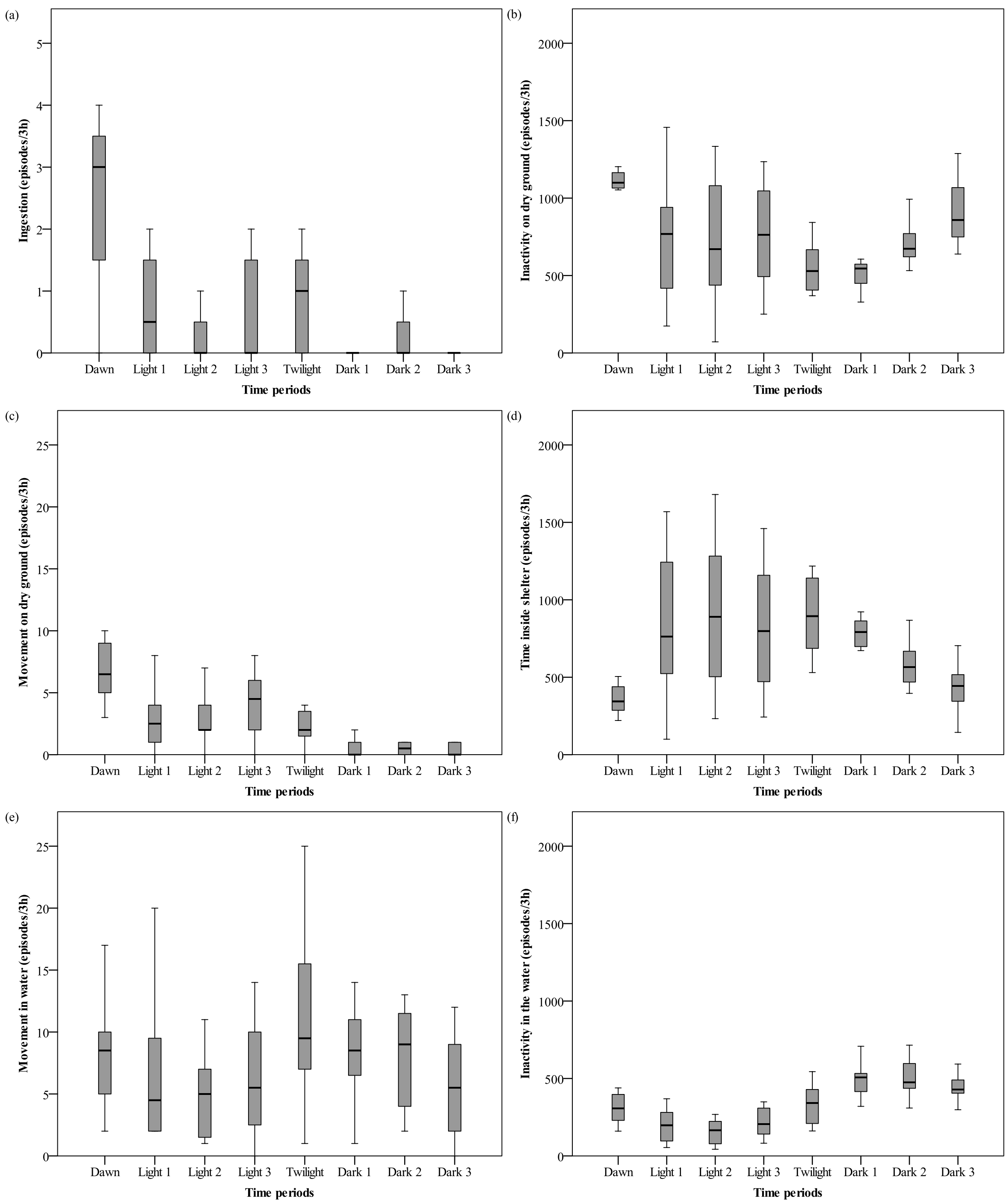

Figure 1 Frequency behaviors of bullfrogs according to time periods

The water has a great importance on metabolic changes after frog feeding (Busk et al 2000). Also, amphibians absorb water through their skin and must therefore submerge their bodies in water after feeding. This may be related to the increased levels of inactivity in water recorded in this study during the early dark phase. Since feeding activity is low in the dark phase, the animals could use this period for adequate water absorption, thereby assisting the metabolic changes.

In accordance with Castro (1998), eight hours after feed ingestion (cornmeal), feed was found in the stomach (61%) and small and large intestine (19% and 5.33% respectively), associated with the appearance of feces. Only after 48 hours, feed considered to have passed through the gastrointestinal tract completely. The water absorption also occurs during the day, so the demand of the water environment at night may also have other reasons not yet understood.

DOI http://dx.doi.org/10.14269/2318-1265.v02n02a03

 ISSN 2318-1265

Anurans generally use their tongues to capture food, with some differences reported between the species. Nishikawa (1999) recorded measurements of 252 ms during food capture movement, with speeds of 250-400 cm s-1 for *Bufo marinus* (Linnaeus, 1758). This type of movement is known as “inertial elongation”, as seen in the bullfrog. Anderson (1993) presented a video of *Rana pipiens* showing the capture of small prey at 308 ms. Food ingestion by the bullfrog is therefore extremely fast, lasting less than a second, and seldom recorded by instantaneous sampling. This characteristic is probably the cause for the low feeding frequency recorded.

Bullfrogs display a preference for feeding during the light phase. The dark phase in this study had zero lux (0 lx) luminosity, meaning a total lack of light. Sit-and-wait predators generally develop good eyesight to locate food (Nishikawa 2000; Duellman and Trueb 1994), behaving differently in accordance with the type of prey (Anderson 1993; Anderson and Nishikawa 1993; Anderson and Nishikawa 1996; Deban 1997; Valdez and Nishikawa 1997).

Fly larvae were used to provide movement cues to the feed and attract the animals as recommended by Lima and Agostinho (1992). Since fly larvae are photophobic, they migrate below the pellets. The animals therefore see movement and are stimulated to ingest the pellets. The importance of visualizing the insect was tested by Miles et al (2004) in *Rana temporaria*. They established that mechanical vibration was less efficient than the movement caused by fly larvae. In the same study, the author isolated larvae beneath the pellets using a transparent membrane. It was concluded that the sight of the larvae was more important than its detection by smell and that animals only ate with the visual stimulus of larvae. Difficulty to see movement during the dark phase, probably lead animals to feed during the light phase.

Anticipatory feeding behavior may provide important information for the adjustment of feed management in captivity as it might indicate a preferred feeding time for the animals. According to Boujard and Leatherland (1992), fish display feeding anticipation behavior when fed at a consistent time. A prior search of the specific location designated for feeding was also described for frogs. Bergeijk (1967) showed that the bullfrog is capable of learning feeding times in small adaptation periods. Indicators in the present study show that animals migrate out of the shelter for the dry area of the stall until the end of the dark phase. High frequencies of inactivity on dry ground were recorded during this period, and may represent the time waiting for feed prior to peak ingestion, which occurs at dawn (with the arrival of light).

The amphibians exhibit a wide range of behaviors in accordance with the environment, physiological condition and reproductive status (Cardoso et al 1984; Dias and Cruz 1993; Coddington and Moore 2003; Rossa-Feres et al 2004). These behavioral responses may vary among individuals in different strategies, according to the social and environmental context the animal is exposed to (Moorea and Jessop 2003). Since young animals (± 70 g) were used in this study, outside the reproductive state, interaction between them was not observed. Feeding was the primary activity recorded in this age group.

According to Hodgkison and Hero (2001), the behavior of many amphibians is alike due to their similarities. This includes skin permeability, which makes them susceptible to dehydration since they are nocturnal animals that hide during the day and are active at night (Duellman and Trueb 1994). Animals were found to be more active during the day. This may be due to the captive environment that offers protection against the sun.

Figueredo et al (2001) verified a relationship of animal movement between dry and wet areas (stall pool) with the thermoregulation needs of bullfrogs. High temperatures modified animal preference for the pool. As per Duellman and Trueb (1994), amphibian activity depends on the characteristics of their habitat. Øverli et al (2004) reported that adaptation to the environment provokes neuroendocrine and metabolic behavioral responses. This is a common biological phenomenon involving stress and reactions to the level of hypothalamic-pituitary-adrenal axis, as well as the sympathetic nervous system.

Fish, which are also poikilotherms like amphibians, have been the focus of many studies on breeding in captivity. Carnivorous fish have similar ecology and physiology to frogs, which can be considered carnivores in their post-metamorphic phase (Reeder 1964; Nishikawa 2000). Fish can therefore be used for comparison in the absence of specific data on amphibians. In a study on carnivorous fish (Salmon), Valdimarsson et al (1997) drew a parallel between light and temperature. They suggested that poikilothermic animals are less active when temperatures fall; however, owing to luminosity, their behavioral rhythm remains the same. According to the authors, temperature change would be a less reliable time indicator than a variation in light intensity.

In the present study, temperature and luminosity conditions were homogenous for all the animals. Luminosity was constant during the light phase and temperature variations were small. The latter were considered very close to the mean of 25-27ºC recommended for frog farming (Figueiredo et al 1999; Braga and Lima 2001) and probably not sufficient to alter animal behavior. Feed was available 24 h a day and there were no predators in the environment. Conditions were therefore considered adequate for animals to express behavior without interference. It is important to note that commercial frog farms maintain higher population

DOI http://dx.doi.org/10.14269/2318-1265.v02n02a03

 ISSN 2318-1265

densities, larger stalls and less protection from excess light, which should be investigated in future studies.

As in all aquaculture, in frog farming artificial feed is responsible for approximately 40% to 60% of production costs. Lima and Agostinho (1992) estimated this cost at 54.8 to 63.0%. Offering feed at times of more intense searching by the animals is an appropriate feed management strategy to improve growth in captive frogs.

Since ingestion was preferred in the light phase with greater interest in pellets and larvae at dawn (4 to 6 h), the supply of fresh feed is suggested immediately before dawn (3 to 4 h) for the species to express a time preference without human interference. However, this practice requires further study as it would result in higher costs for animal management and illumination (early hours of the morning) and consequently alter the natural photoperiod. Such changes may modify animal behavior. According to Alanärä (1996), the photoperiod supplies visual feeding indicators, as well as information on the time of day and season.

When studying a diurnal anuran, Hantano et al (2002) reported that standard activities of the circadian cycle are finely regulated by the photoperiod and that the daily activities of the *Hyllodes phyllodes* (Fitzinger 1826) species were influenced by light as well as other factors. The same phenomenon was described by Duellman and Trueb (1994) and Cree (1989) for nocturnal species.

In sum, inactivity on dry ground, inactivity in water and time inside shelter are the more common behaviors of bullfrogs. The feeding is the behavior that most influenced the others studied, so the feed supply is recommended during the light phase, which must remain with attractiveness until dawn the next day.

Acknowledgements

To National Counsel of Technological and Scientific Development (CNPq), the Psychobiology Postgraduate Program at UFRN and the Laboratory of Frog Culture and Aquiculture Products of Department of Administration and Agro-industrial Technology at CCHSA-UFPB.

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