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The diurnal aggregation behavior in *Phasmahyla cochranae* tadpoles (Anura: Phyllomedusidae)

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We investigated schooling behavior of *Phasmahyla cochranae* including its periodicity based on periodic regression models. The school structure and differences between day and night were discussed. We found that tadpoles formed aggregative schools, which were significantly more frequent during the day than at night. During the day, from 06:30 to 18:00 h, tadpoles formed one or two polarized schools at the water surface. Based on these results and on observations of specific behaviors, we suggest that daylight may be a significant environmental factor related to schooling behavior in *P. cochranae*, although this hypothesis needs to be further investigated.

Key words: amphibians; cyclic data; diel preference; gregarious behavior; schools.

Many amphibian species show gregarious behavior during the larval stage in aquatic environments (McDiarmid & Altig, 1999). In fact, there are at least nine families of anurans, including Phyllomedusidae, in which tadpoles form aggregations (Wells, 2007). This aggregation behavior can exhibit irregular distribution of individuals in constant rotation, or large compact polarized groups usually called "schools" (Wassersug *et al.*, 1981). Schooling behavior may be the result of individual responses to the presence of other tadpoles and / or of other external stimuli (Wassersug & Hessler, 1971; Beiswenger, 1975), such as cannibalism or predation (Brattstrom, 1962; Beiswenger, 1977), variations in water temperature (Brattstrom, 1962) and daylight (Brattstrom, 1962; Katz *et al.*, 1981; Wassersug *et al.*, 1981; Branch, 1983).

Daylight has been shown to trigger aggregative behavior (Beiswenger, 1977; Branch, 1983; Eterovick & Sazima, 1999; Surova, 2006) and to influence orientation of tadpoles in ponds (Katz *et al.*, 1981; Wassersug *et al.*, 1981). Low water visibility in ponds caused by diminishing of daylight or by increased water turbidity negatively affects tadpole aggregations, and may even cause dispersal of tadpoles (Beiswenger, 1977; Branch, 1983). Increased visibility under high light conditions may favor visually-oriented aquatic

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predators (MORIN, 1983; WILBUR *et al.*, 1983; LIMA & DILL, 1990). With reduced sunlight at dusk, predation risks probably decrease because of low prey detectability, and therefore sunlight could have a direct and indirect influence on tadpole behavior.

Aggregation behavior in tadpoles has been well studied in numerous species, including bufonids (*Anaxyrus americanus, Bufo bufo, B. woodhousei*), ranids (*Rana cascadae, R. temporaria*), or pipids (*Xenopus laevis*) (BLAUSTEIN & O'HARA, 1987; SUROVA *et al.*, 2009). Within the family Phyllomedusidae, this behavior has been described in some species from the subfamily Phyllomedusinae (BRANCH, 1983; BRANDÃO *et al.*, 2009), and more specifically in the genus *Phasmahyla* (LUTZ & LUTZ, 1939; BOKERMANN & SAZIMA, 1978), although no report existed so far about the presence of this behavior in *Phasmahyla cochranae* (Bokermann, 1966). In the present study, we tested the aggregation behavior of *P. cochranae* tadpoles and the diel preference for displaying such behavior.

Phasmahyla cochranae is a small-sized species (adult snout-vent length: 29.0-45.5 mm, tadpole maximum total length: 55.0 mm; BOKERMANN, 1966). Tadpoles (Fig. 1a) inhabit small ponds formed in lotic environments, such as creeks and streams. It occurs in the states of São Paulo, Rio de Janeiro and Minas Gerais in southeastern Brazil, generally in coastal mountain ranges such as Serra da Bocaina, Serra do Mar and Serra da Mantiqueira (CRUZ & CARVALHO-E-SILVA, 2004; ROSSA-FERES *et al.*, 2011). Our study was conducted on February 23rd and 24th, 1995 in the Parque Municipal do Itapetinga (23°14′23″ S, 46°30′18″ W), Atibaia municipality, São Paulo. This is a reserve with a total area of approximately 10 000 ha, and occupies part of the municipalities of Mairiporã, Nazaré Paulista, Atibaia and Bom Jesus dos Perdões, in a mountainous region with elevations ranging between 900 and 1400 m and annual rainfall from 1400 to 1700 mm. The vegetation type is high elevation, semi-deciduous forest (NETO *et al.*, 1989; GROMBONE *et al.*, 1990). Natural water bodies include ponds, lakes and forest streams. Focal observations were conducted in a rock pond with approximately 4 m of diameter formed within a stream (Fig. 1b). The specimens were identified by authors in accordance with species description (Fig.1a; BOKERMANN, 1966).

The studied pond was limited by a rock -strewn formation that restricted the water flow, forming an ovoid pool. A grid built with PVC frames and nylon twine delimiting 252 squares (10 x 10 cm) identified with letters and numbers was installed above this pool (Fig. 1b). We observed the swimming behavior and location of individuals in the grid from a stationary observation point (adjacent, 4 m above the pool), with assistance of binoculars and artificial light. We started sampling at 16:30 h on February 23rd and finished at 16:00 h the following day, with a total of 48 records made at regular intervals of 30 minutes. For each observation, we recorded the number of tadpoles in each square of the grid, and whether individuals were polarized (i.e. oriented in the same direction) or not.

To verify if tadpoles exhibited gregarious behavior, and if this behavior was variable during the 24-h sampling period, we defined an index of aggregation as the ratio between the number of tadpoles and the approximate area that they occupied. This calculation was achieved by dividing the number of observed individuals (minimum = 4, maximum = 8) by the sum of squares occupied by at least one individual and the number of empty squares located between two occupied squares.

In order to evaluate the aggregation behavior and the periodicity of the data, we employed a periodic regression model (BATSCHELET, 1981). This analytical tool fit observed data to a period model, assessing its possible periodicity. The model generates a cosine function given by the equation

$$y = M + A \cos \omega (t - \theta)$$

where t represents the independent variable time, M represents the mean values of the dependent variable (aggregation index), A is the amplitude, or the range be-

Figure 1: (a) Tadpole of *Phasmahyla cochranae* (Gosner stage 35, total length 41.12 mm). Author: Mario Sacramento. (b) Grid built with PVC frame and nylon twine delimiting numbered squares of 10 x 10 cm in the studied pond at Parque Estadual de Itapetinga, Atibaia municipality, São Paulo state, Brazil. Author: Ricardo J. Sawaya.

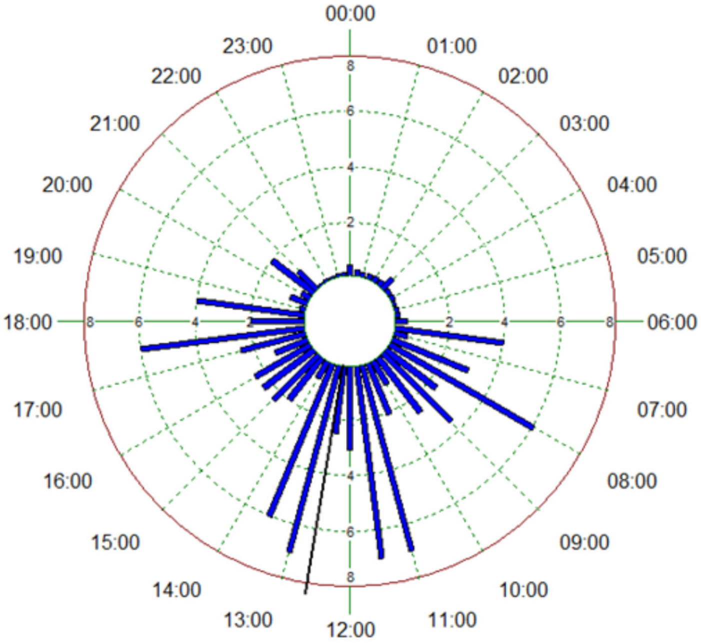

Figure 2: Circular-linear plot of the aggregation index of *Phasmahyla cochranae* during 24 hours of observation. The blue bars represent the aggregation index values and the black line represents the mean time of the day for the observed aggregations.

tween minimum and maximum values of the dependent variable, ω represents the angular frequency, and θ represents the acrophase or time of maximum peak of the dependent variable. With the aim of testing if the periodicity variable (time) was significant in the model, we used an analysis of the variance (ANOVA) to compare the model with time and the model without time as independent variable.

We found that tadpoles of *P. cochranae* exhibited a periodic gregarious behavior ($F = 21.42$, $P < 0.001$; Fig. 2), in which up to seven tadpoles occupied the same 100 cm^2 square. The amplitude value was $A = 1.20$ and acrophase was $\theta = -3.00$ in radians or 12:30 in hours, indicating that the highest values of the aggregation index happened approximately in the middle of the day.

According to our results and field observations, during the day (from 06:30 to 18:00 h), tadpoles often formed cohesive and generally polarized schools at the water surface, with stationary positions and heads directed towards the water flow. Individuals were distributed leaving small distances among them (usually < 20 mm), but not in physical contact, and the aggregate shape was either two small groups or one large group of eight individuals. During the night (18:30-06:00 h), individuals dispersed to the bottom of the pond, with greater distances among them (usually > 30 mm) and no evidence of aggregation (Fig. 2).

Additionally, we observed four specific types of behaviors in tadpoles during the monitoring: 1) all tadpoles swimming at the surface in a polarized form, with mouth parts on the water surface, supposedly for foraging, and generally motionless except for tail tip movements; 2) floating at mid-water, motionless and polarized, with the head facing upward at an angle of about 45° and tail tip moving; 3) swimming alongside each other, but stopping and / or changing their position in the grid; and 4) individuals swimming and making series of short displacements (a few centimeters) with intervals of short stops and direction changes, presumably not polarized but always staying close to each other. Although schooling behavior was not directly tested, these behaviors often shown by tadpoles of *P. cochranae* are related to the description of schooling behavior made by CALDWELL (1989).

Our results demonstrate that *P. cochranae* tadpoles may show aggregation behavior, particularly during the day, and contrast with the description by Bokermann (1966), who describe this species tadpoles as with nocturnal activity and surface foragers, while during the day they hid under stones and debris. Thus, although our results point out to a diurnal activity preference, this should be confirmed by means of further research on spatial and temporal replicates. During the day, *P. cochranae* tadpoles were often recorded in diurnal schools, polarized, moving slowly through the water surface, and swimming near and alongside each other in the same direction. Such type of aggregation behavior, although in different display forms, has been described in different anuran families such as Bufonidae, Ranidae, Pipidae and Phyllomedusidae (e.g. WALDMAN, 1981, 1982; FOSTER & MCDIARMID, 1982), including the subfamily Phyllomedusinae (BRANCH, 1983), which indicates that this is a relative widespread behavior in anuran tadpoles.

Experimental observations suggest that temperature and light levels in ponds may influence tadpole behavior (BRANCH, 1983; WOLLMUTH *et al.*, 1987). Water transparency, for instance, explained most of the variability in aggregation size of tadpoles of *Phrynomantis microps* (SPIELER, 2003). Tadpoles of *Phyllomedusa vaillanti* (BRANCH, 1983) and *A. americanus* (BEISWENGER, 1977) formed exclusively diurnal schools. Experiments including the effects of light in tadpoles of *P. vaillanti* showed that behavior could shift when exposed to light, rather than because of other stimuli (SPIELER, 2003). We also observed a strong association between daytime and aggregation behavior in this study. Our results seem to support the patterns observed in other studies, suggesting that daylight could be a factor related to aggregation behavior. However, other anuran species such as *Scaphiopus hurterii* aggregate at night (BRAGG, 1948).

This study represents the first record of aggregation behavior and the diurnal preference of such behavior in *P. cochranae*. Although this first record is still not enough to generalize for all populations of the species, our observations agree with other studies conducted on other species of Phyllomedusinae, such as *P. vaillanti*, and even on other species of the genus *Phasmahyla* like *P. guttata* and *P. jandaia* (LUTZ & LUTZ, 1939; BRANCH, 1983; ETEROVICK & SAZIMA, 1999; SUROVA, 2006; COSTA & CARVALHO-E-SILVA, 2008), supporting the existence of schooling behavior in *P. cochranae.*

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