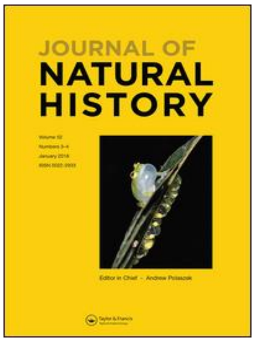

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Ecological aspects of the endemic tree frog *Ololygon kautskyi* (Anura: Hylidae) in an Atlantic Forest area of Southeastern Brazil

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ABSTRACT

We studied several ecological aspects of the endemic *Ololygon kautskyi* in the Reserva Biológica Duas Bocas (RBDB), Espírito Santo, Brazil, including activity, detectability and use of microhabitats. Fieldwork was carried out from February 2018 to March 2019, between 8:00 and 23:00, in 22 transects of 50 m along streams. For each individual frog found, we recorded the hour, the microhabitat in which it was detected, the height above ground, snout-vent length (in mm), and mass (in g). We measured the air temperature (°C), relative humidity (%), stream water temperature (°C), pH, and conductivity. We tested for differences in body size among sexes and estimated the detectability of the species in relation to the environmental variables. We recorded a total of 93 individuals from *O. kautskyi*, with the majority of individuals recorded between 18:00 and 22:00. Females were significantly larger in body size than males. The detection probability of the species was affected by the period of the day, air temperature and relative air humidity. *Ololygon kautskyi* used five different types of microhabitats, and vegetation was the most used microhabitat by the species. We conclude that *O. kautskyi* is nocturnal, mainly uses vegetation on stream banks, is more likely to be detected during cold and dry days, and is dimorphic in body size with females being larger than males. There is currently insufficient information to assess the conservation status of *O. kautskyi*. However, considering that the species is endemic to a relatively small portion of the Atlantic Forest biome in the state of Espírito Santo and that its ecology is strongly associated with riparian zones, we believe that the species would be eligible to be categorised in some threat category.

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Amphibians; conservation; data deficient species; detectability; occupancy models

Introduction

Ololygon kautskyi Carvalho-e-Silva and Peixoto, 1991, is an anuran species belonging to the *Ololygon catharinae* group. This species is endemic to the Espírito Santo State, Southeastern region, Brazil, inside the Atlantic Forest biome (AF), with known records in

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Supplemental data for this article can be accessed here.

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the municipalities of Domingos Martins, Santa Teresa, Aracruz and Cariacica (Almeida et al. 2011; Silva et al. 2018). This species is found in the vegetation bordering streams, where it breeds (Carvalho-e-Silva and Peixoto 1991). Its known range of occurrence is estimated to be 1,793 km^2 (Haddad et al. 2016), but this area may be considerably overestimated because the effective occupancy area of amphibians associated with riparian environments is, generally, considerably smaller (see Almeida-Gomes et al. 2014).

Although the population trend of this frog species was considered as stable by the IUCN (Peixoto and Pimenta 2004), population studies on *O. kautskyi* are non-existent, thus there is no effective data to support such an assumption, with knowledge on the species being scarce and usually being restricted to the simple mention of species occurrence in amphibian community composition studies (e.g. Tonini et al. 2010; Almeida et al. 2011; Silva et al. 2018; Ferreira et al. 2019), or general features in the studies that described the species (Carvalho-e-Silva and Peixoto 1991) and tadpole (Carvalho-e-Silva et al. 1995). Due to the lack of information on species ecology, structure and population trends, distribution, and possible threats, the conservation status of this species has been categorised as *data deficient* at the global level in the IUCN List (Peixoto and Pimenta 2004), at the national level in the List of Endangered Brazilian Fauna (MMA 2014), and also at the regional level in the list of endangered species of Espírito Santo state (Government decree No. 1499-R from 13 de june 2005; Gasparini et al. 2007).

In this context, in order to provide additional ecological data to provide support during the evaluation of the conservation status of *O. kautskyi*, we studied several ecological aspects of the species in an AF area in Espírito Santo State, Brazil, such as abundance, body size, activity period and use of microhabitat. We specifically addressed the following questions: i) Are there significant differences in the body size of the species between the sexes? ii) What is the period of activity of the species, and how does activity vary throughout the day? iii) How does species detectability vary throughout the day? iv) Which environmental variables (i.e. air temperature, relative air humidity, water temperature, pH and water electrical conductivity) affect frog detectability? and v) Which microhabitats are preferred by the species?

Material and methods

Study site

The Reserva Biológica Duas Bocas (RBDB) is located in the municipality of Cariacica (20º14′04″ and 20º18′30″ S; 40º28′01″ and 40º32′07″ W), in the state of Espírito Santo, Southeastern Brazil (Figure 1). The RBDB plays an important role in the conservation of biodiversity for the state of Espírito Santo, integrating one of the primary ecological corridors for state conservation, as well as being of fundamental importance to the water supply for the population of Cariacica (Boni et al. 2009; IEMA 2018).

The RBDB is part of the Duas Bocas River Basin which has an area of 92.27 km^2 (9226.88 ha), with about 40% located in the RBDB (Bastos et al. 2015). The main springs of this river basin are the Sertão Velho, Panelas, Naiá-Assú and Pau Amarelo streams.

The RBDB has a total area of 2910 ha, with elevation range between 300 and 738 m. Approximately 80% of the RBDB is covered by native forest, and the remaining 20% is distributed among secondary forests, dam, streams and riparian zones (Tonini et al. 2010).

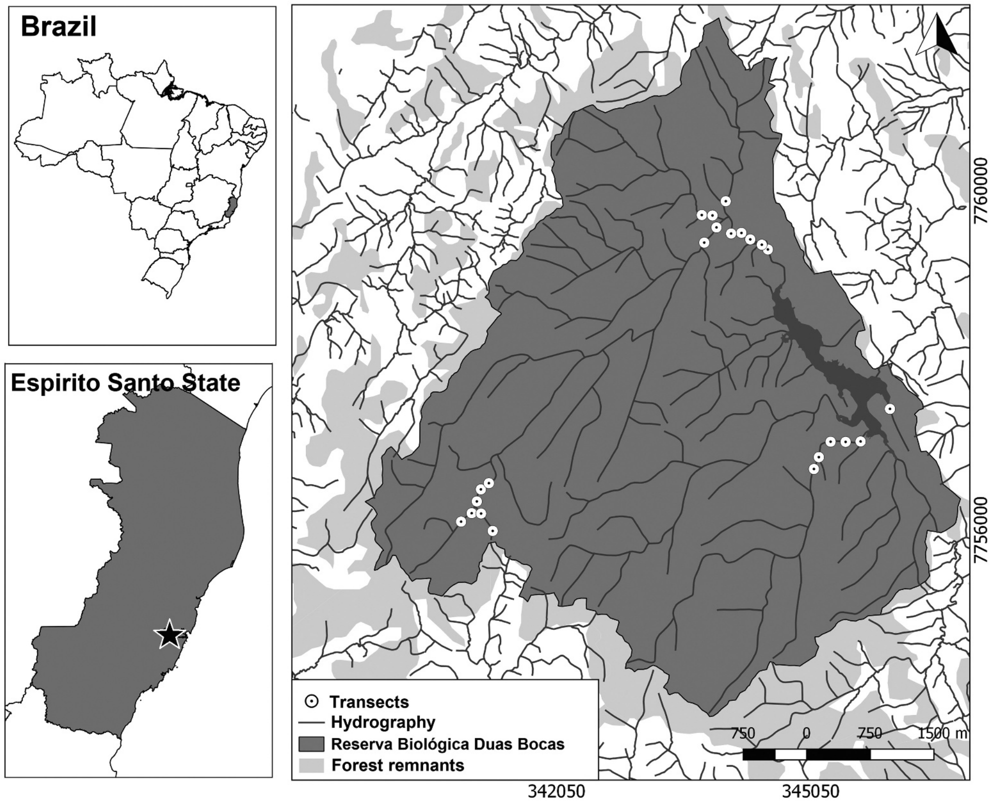

Figure 1. Location of the Reserva Biológica Duas Bocas, Cariacica, Espírito Santo, Brazil.

The climate in the area is a humid tropical climate, with monthly average temperatures ranging from 19°C in the winter to 25.5°C in the summer, and an average annual rainfall of approximately 1500 mm and mean relative air humidity above 70% (Prado and Pombal 2005; Tonini et al. 2010). Dominant vegetation in the region is dense ombrophilous forest type (Novelli 2010).

Data collection

We sampled from February 2018 to March 2019, with monthly campaigns from 2 to 4 days, using an active search method, with visual and auditory sampling (Crump and Scott 1994). We sampled during the day (08:00 hours to 17:00 hours) and night periods (18:00 hours to 23:00 hours) to increase the chances of encountering individuals of the species, since activity for the species has not been previously documented (Haddad et al. 2013). We distributed the transects across different streams in the reserve, totalling 22 standardised transects, each 50 m long, with a distance of at least 50 m apart from each other (Figure 1).

Throughout the study period (i.e. 14 months), we sampled 16 transects four times, twice in the daytime and twice in the night, and six transects were sampled three times, twice in the daytime and once in the night, due to the logistical difficulty of accessing certain sites nightly. In each transect, sampling was performed for 30 to 50 minutes, depending on the number of individuals of the species found in the transect. Before performing each transect, we measured the air temperature (°C) and relative humidity (%) using a thermohygrometer. In addition, we measured stream water temperature (°C), pH, and water conductivity (µS/cm), using a Multi-Parameter (®Simokit pH/EC-983). We searched for individuals with a minimum of two fixed observers looking for individuals using the following habitat types: ground, under and within rock crevices, under vegetation, under leaf litter, near tree roots, in the water, on tree trunks, inland from the steam and on the streams' banks. Along transects, we considered all individuals found (visually or by their calling activity) within up to 2 m wide on either side of the stream bank. For each individual detected, we recorded the period of day and the time, the microhabitat in which it was originally occupying, the height above ground, frog snout-vent length (SVL in mm), and mass (g). Whenever possible to distinguish precisely, the sex was also recorded. We measured the SVL of the individuals using a Vernier Caliper (to the nearest 0.1 mm) and weighed them using a Pesola dynamometer (to the nearest 0.1 g).

Data analysis

We used a Student's t-test to test for differences in mean body size between the sexes. To estimate the detectability of *O. kautskyi* in the RBDB, based on the approach proposed by MacKenzie et al. (2006), we developed single-season occupancy models using the species detection and non-detection matrix in each transect on multiple sampling occasions (total of 4 occasions). To perform this estimate, we selected six covariates *a priori*: air temperature (air_temp); relative humidity (air_humid); water temperature (water_temp); pH (pH); conductivity (conductivity) and sampling period – day or night.

We constructed our single-season occupancy models using the Unmarked package (Fiske and Chandler 2011) in the Program R (R Core Team 2019). Top occupancy models were selected using Akaike information criterion adjusted for small sample size (AICc), and all models with a ΔAICc value lower than two were equivalent (Akaike 1973; Burnham and Anderson 2002). We also used the model weight (AICw), which corresponds to the amount of evidence in favour of a given model, to choose our 'best-fit' model, which we subsequently used to test our specific hypotheses (Akaike 1973; Burnham and Anderson 2002). We assessed the adjustment fit (P) and the over-dispersion parameter ($\hat{c}$) using 2000 bootstraps.

Results

We recorded a total of 93 individuals of *O. kautskyi* in the transects performed (Table S1). Males ranged in body size (SVL) from 21.3 up to 27.1 mm (mean = 24.8 mm, N = 11) and in mass from 0.7 g up to 1.7 g (mean = 1.15 g; N = 11). Females ranged in body size from 24.1 mm up to 38.8 (mean = 30.46 mm, N = 14) and in mass from 0.8 g up to 2.0 g (mean = 1.18 g; N = 14). Females were significantly larger in body size than males (T test; t = 3.372, df = 23, P = 0.002; Figure 2) but not in body mass (T test; t = 0.173, df = 23, P = 0.864; Figure 2).

Most individuals of *O. kautskyi* were found active between 18:00 hours and 22:00 hours with peak activity between 19:00 hours and 20:00 hours (Figure 3(a)).

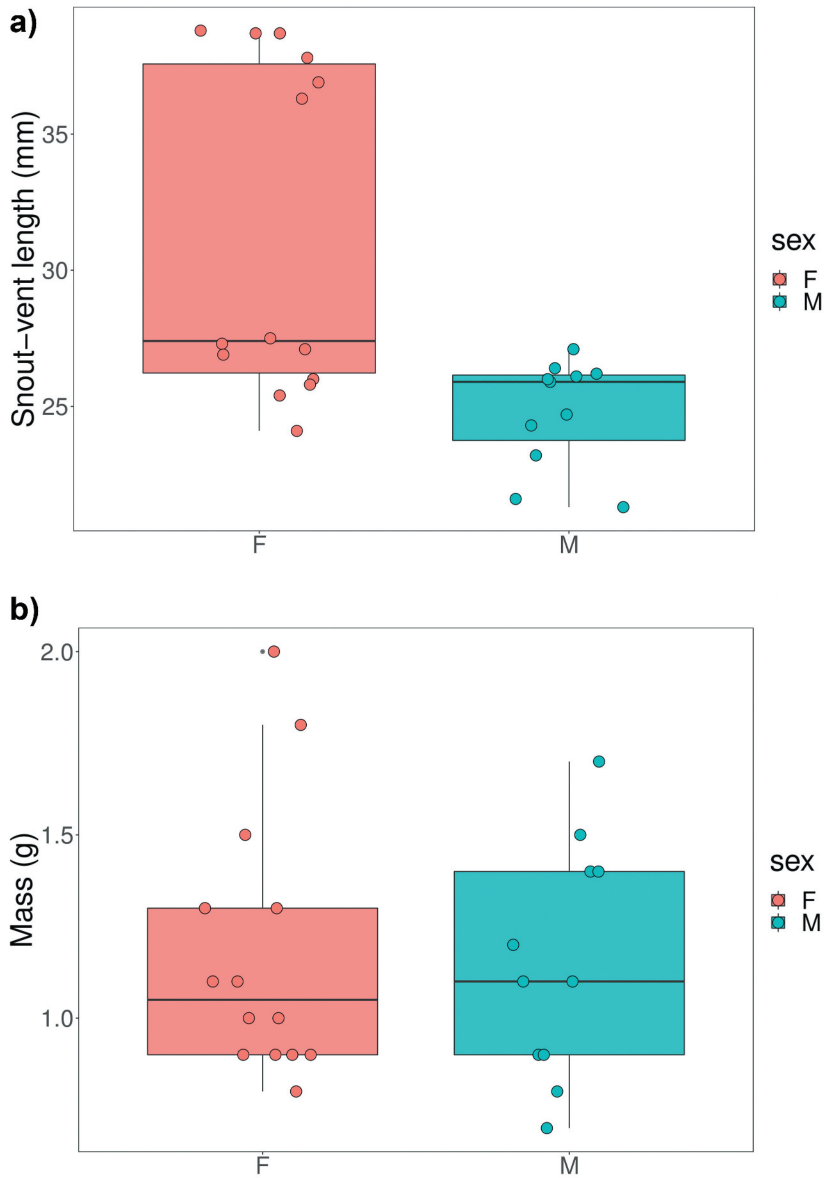

Figure 2. Differences in body size (a) and mass (b) between males and females of *Ololygon kautskyi* in the Reserva Biológica Duas Bocas, municipality of Cariacica, Espírito Santo state, Southeastern Brazil. F = females, M = males.

The detection probability of the species was affected by three of the variables studied, represented in two best-fitted models with a summed AICw of 0.72 (the models are shown in Table 1): (1) period of the day, when the detectability was higher during the night (Figure 3(b)); (2) air temperature, which had a negative relationship, with higher detectability of the species under comparatively colder temperatures (Figure 4(a)), and (3) relative air humidity, which had a negative relationship, showing that the higher the humidity, the less likely to detect the species (Figure 4(b)).

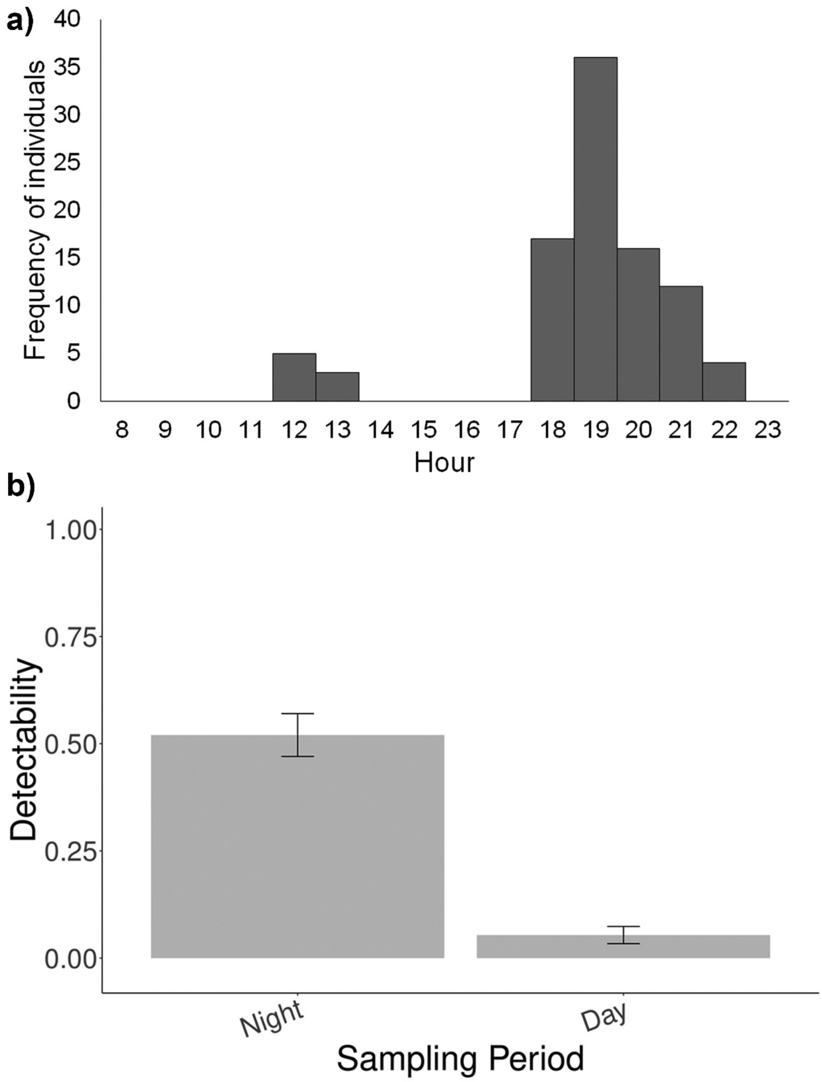

Figure 3. *Ololygon kautskyi* activity period at Reserva Biológica Duas Bocas, Espírito Santo, Brazil: (a) Number of individuals recorded between 08:00 hours and 23:00 hours in transects. (b) *O. kautskyi* detectability during day and night.

Ololygon kautskyi used, in general, five different types of microhabitats (n = 93) (Figure 5), with perching on vegetation being the most used microhabitat (70%; n = 65). Individuals were found on the ground and perched on vegetation above ground, with a median perch-height of 71.8 cm (first quartile = 40 cm, third quartile = 100 cm, n = 93).

Discussion

Our data shows that the sexes in *O. kautskyi* differ in body size, with females being larger than males. A larger body size in females occurs in many frog species and is adaptive. This

Table 1. Single-season detectability models for the *Ololygon kautskyi* in the Reserva Biológica Duas Bocas, Brazil, estimated by four occasions. Covariates: air temperature (air_temp); relative humidity (air_humid); water temperature (water_temp); pH (pH); electrical conductivity (conductivity) and sampling period – day or night (period).C, occupancy; p, detectability; AIC, corrected Akaike information criterion; ΔAIC, the difference between AIC and the lowest of all the AIC values; AICw, Akaike weight.

Model	AIC	ΔAIC	AICw	nº parameters
Ψ(.) p(period; air_temp; air_humid)	69.58	0	0.53	5
Ψ(.) p(period; air_temp)	70.23	0.65	0.19	4
Ψ(.) p(period)	72.27	2.69	0.11	3
Ψ(.) p(.)	76.82	7.24	0.06	2
Ψ(.) p(period; air_humid)	76.97	7.39	0.03	4
Ψ(.) p(air_temp)	77.82	8.24	0.03	3
Ψ(.) p(air_temp; air_humid)	78.14	8.56	0.01	4
Ψ(.) p(period; water_temp)	80.21	10.63	0.01	4
Ψ(.) p(air_temp; conductivity)	80.59	11.01	0.01	4
Ψ(.) p(period; conductivity)	82.27	12.69	0.01	4
Ψ(.) p(air_humid; water_temp)	82.33	12.75	< 0.01	4
Ψ(.) p(period; air_temp; air_humid; conductivity)	82.81	13.23	< 0.01	6
Ψ(.) p(period; air_temp; pH)	84.17	14.59	< 0.01	5
Ψ(.) p(period; air_temp; conductivity)	84.61	15.03	< 0.01	5
Ψ(.) p(air_temp; air_humid; conductivity)	84.69	15.11	< 0.01	5
Model fit = 0.31, and c-hat = 1.32 obtained with 2,000 bootstraps				

usually results from intrasexual selection acting on females to favour an increase in body size compared to males in order to allow for the production of larger clutches (Shine 1979; Woolbright 1983). The differences in body size of *O. kautskyi* follows a trend found in most species of *Ololygon* from the *Ololygon catharinae* group (see Lourenço et al. 2010), for example, *O. albicans* (Bokermann, 1967) (SVL; males = 26.6 mm–32.0 mm, females = 39.0 mm–43.8 mm; Lourenço et al. 2009), *O. skaios* (Pombal, Carvalho, Canelas, and Bastos, 2010) (SVL; males = 23.2 mm–29.6 mm, females = 30.7 mm–36.1 mm; Pombal et al. 2010) and *O. pombali* (Lourenço, Carvalho, Baêta, Pezzuti, and Leite, 2013) (SVL; males = 20.2 mm–25.1 mm, females = 28.6 mm–34.9 mm; Lourenço et al. 2013).

Our data indicates that *O. kautskyi* is a predominantly nocturnal species, although some individuals may remain active during the day, with the detection probability of *O. kautskyi* increasing by almost 50% at night when compared to daytime. In general, some anuran species living associated with streams and riparian zones exhibit a pattern of daytime activity (e.g. Almeida-Gomes et al. 2007; Machado et al. 2016). However, although *O. kautskyi* lives associated with riparian zones (Carvalho-e-Silva et al. 1995), the activity of the species follows the predominantly nocturnal trend of most AF amphibian species (Rocha et al. 2015). A study conducted in 10 areas of the AF of Rio de Janeiro State, Brazil analysing frog´s species period of activity showed that 86% of recorded amphibian species had a twilight-nocturnal activity pattern (Rocha et al. 2015). Similarly, another study in the AF showed that 88% of the recorded species exhibited nocturnal activity, with detection probabilities being almost 80% higher at night (Pereira-Ribeiro et al. 2019).

The detectability of *Ololygon kautskyi* was negatively influenced by air temperature and relative humidity, and this species was more likely to be detected under colder and

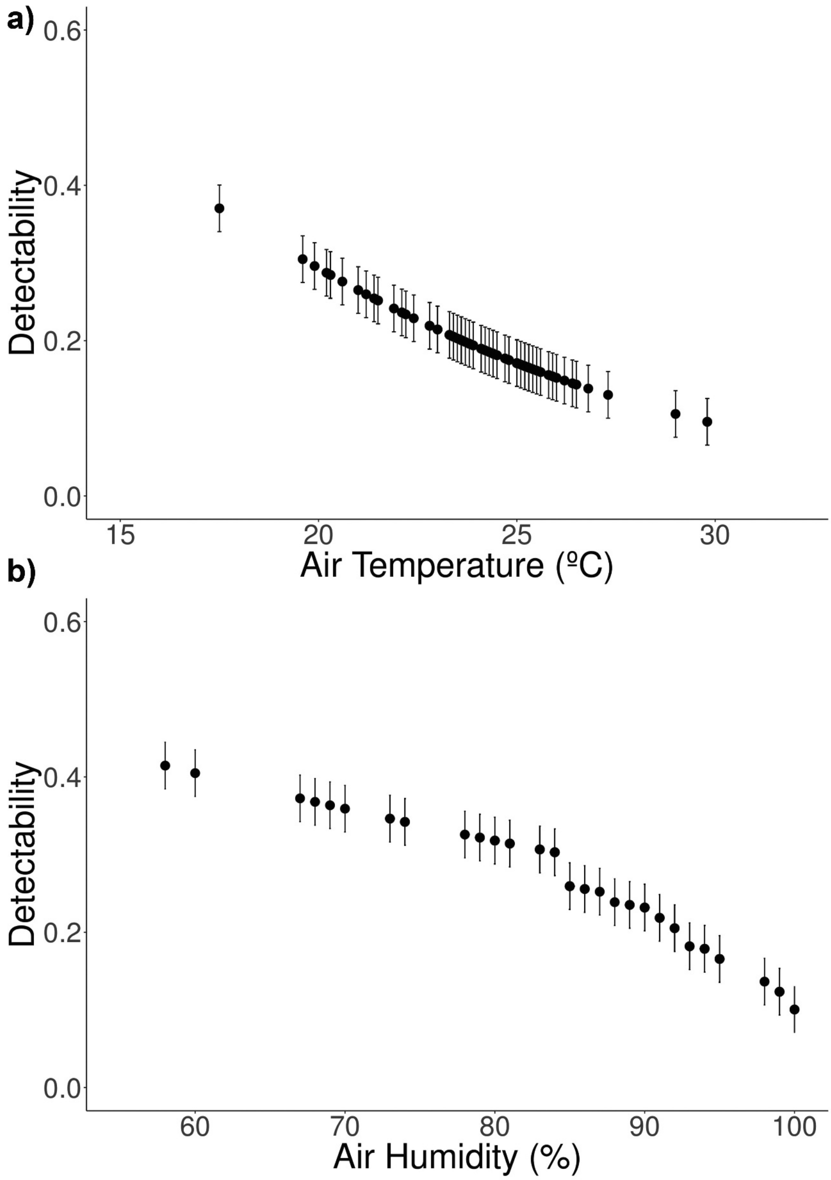

Figure 4. Relationship between detectability of *Ololygon kautskyi* and (a) air temperature (ºC) and (b) relative humidity (%) in Reserva Biológica Duas Bocas, Espírito Santo, southeastern Brazil, from February 2018 to March 2019.

drier weather conditions. We expected the probability of detection of *O. kautskyi* to be higher on warmer nights and under comparatively higher air humidity, as this is the most frequently observed pattern in tropical anurans as these conditions favour increased reproductive activity (Duellman and Trueb 1986). However, certain studies (e.g. Nascimento 2003; Lourenço et al. 2010) show that some species belonging to the same species group as *O. kautskyi* (the *Ololygon catharinae* group) tend to be more abundant during the cold season and under lower air humidity. For example, the study describing

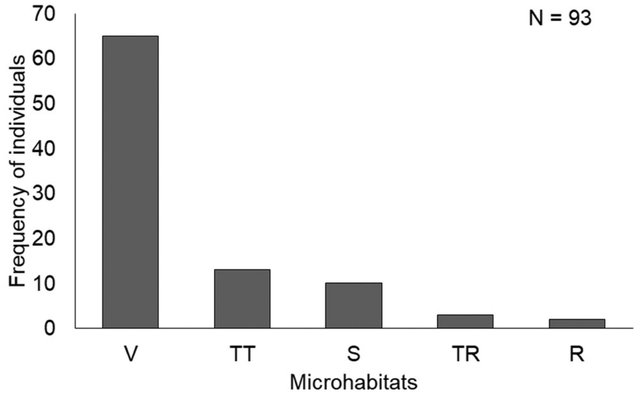

Figure 5. Use of microhabitats by *Ololygon kautskyi* in the Reserva Biológica Duas Bocas, Espírito Santo, Brazil. V = over vegetation, TT = tree trunk, S = sand, TR = tree root, R = over rock.

the species *O. tripui* (Lourenço, Nascimento, and Pires, 2010) showed that individuals were observed mainly during the dry and cold season, synchronic with the period when males were actively calling for females (Lourenço et al. 2010). In another study on *O. albicans* reproductive behaviour, the species reproduced mainly on dry days and had no reproductive activity on most rainy nights or nights which had been preceded by rainy days (Nascimento 2003). In general, anuran species that use streams for breeding usually have reproductive activity at times of low rainfall (and consequently lower humidity), when the water level is lower and the current is slower (Duellman and Trueb 1986).

The use of foliage and tree trunks at the stream's margins by over 80% of the individuals in this study indicates that this riparian vegetation is the main microhabitat of the species, and highlights the importance of riparian forest for this frog species conservation. Riparian zones are known to be responsible for controlling and maintaining different factors, such as water flow, temperature, air humidity and shading (Pusey and Arthington 2003; Decamps et al. 2004; Olson et al. 2007), which in turn may influence the occurrence and abundance of anuran species. In addition, riparian vegetation cover has been shown to be an important factor influencing the abundance of AF anurans, especially species with habitat-specific requirements (Almeida-Gomes et al. 2015). Removal of riparian vegetation can result in strong physical and structural changes in streams and their margins (Gomi et al. 2006), including changes in stream temperature regimes (Johnson and Jones 2000; Wilkerson et al. 2006). Remnants of riparian forests in fragmented areas may not preserve the microclimatic conditions necessary for certain species and may not provide enough habitat to maintain the abundance for some frog species (Marczak et al. 2010). Therefore, considering that *O. kautskyi* mainly uses riparian vegetation and is more frequently detected at lower temperatures, the preservation of RBDB riparian forests is of fundamental importance. Additionally, studies of *O. kautskyi* distribution within and outside of protected areas are needed to investigate the relationship between forest cover and occurrence and abundance of this species.

In this study, we provide the first information on aspects of the ecology of *O. kautskyi*. We conclude that *O. kautskyi* is a species of nocturnal habits, which mainly uses vegetation on stream banks and is more likely to be detected on cold and dry days. In addition, the species is dimorphic in body size with females being larger than males, probably as a result of selection favouring larger body sizes in females to produce larger clutch sizes. There is currently insufficient information to assess the conservation status of *O. kautskyi*. However, considering that the species is endemic to a relatively small portion of the AF of the state of Espírito Santo and that it is strongly associated with riparian zones (i.e. its occupancy is restricted to those zones), we believe that the species is eligible to be categorised in the status 'Near threatened'. Therefore, we believe that additional studies examining population trends and effective areas of occupancy will likely place the species under a threat category soon.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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