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HABITAT USE BY *AGALYCHNIS ANNAE* (ANURA: HYLIDAE) AT AN URBAN GREEN SPACE IN COSTA RICA

USO DEL HÁBITAT POR *AGALYCHNIS ANNAE* (ANURA: HYLIDAE) EN UN ESPACIO VERDE URBANO EN COSTA RICA

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Resumen.– La información sobre el uso del hábitat de algunas especies de hílidos, como *Agalychnis annae*, es limitada. Esta especie se reproduce en estanques y requiere la presencia de plantas cerca de pequeños cuerpos de agua para tener una reproducción exitosa. El objetivo de esta investigación fue examinar los aspectos básicos del uso del hábitat de una población de *A. annae*. Estudiamos una población de esta especie en la laguna natural del Zoológico Nacional Simón Bolívar, San José, Costa Rica. Encontramos que los individuos prefieren diferentes tipos de perchas según la categoría de sexo/edad, observándose un mayor número de hembras adultas y juveniles en las hojas y más machos adultos en los tallos. La ubicación en el sustrato vegetal, así como la altura a la que se posaron los individuos de cada categoría de sexo/edad, fue similar. Encontramos una mayor proporción de individuos tanto juveniles como adultos en dos especies de gramíneas (*Cyperus involucratus* y *Coix lacryma-jobi*). Encontramos 65 masas de huevos en siete especies de plantas, pero la mayoría ubicadas en la gramínea *Coix lacryma-jobi* y el bambú *Rhipidocladum racemiflorum*. Es posible que *A. annae* elija la vegetación para la oviposición en función de su estructura en vez de su abundancia. De manera similar, el hallazgo de que las hembras prefieren las hojas a los tallos para ovopositar la puesta de huevos puede explicarse por el área de superficie y el soporte que ofrece una hoja en comparación con un tallo. Estos hallazgos proporcionan información valiosa sobre las preferencias de hábitat y la etología reproductiva de *A. annae*, por lo tanto, esta aportación puede contribuir a futuras estrategias de manejo y conservación para esta especie.

Palabras clave.– perchas, rana de cafetal, sustrato, Valle Central, Zoológico Simón Bolívar

Abstract.– Information regarding the habitat use of some hylid species, such as *Agalychnis annae*, is limited. This species breeds in ponds and requires the presence of plants near small bodies of water for successful reproduction. The aim of this research was to examine the basic aspects of habitat use in a population of *A. annae*. We studied a population of this species in the natural lagoon at the Simón Bolívar National Zoo, San José, Costa Rica. We found that individuals preferred different types of perches depending on their sex/age categories, with a higher number of adult and juvenile females observed on leaves and more adult males on stems. The location on the vegetative substrate and the height at which individuals perched for each sex/age category were similar. We found a higher proportion of both juvenile and adult individuals on two grass species (*Cyperus involucratus* and *Coix lacryma-jobi*). We found 65 egg masses on seven plant species, with the majority located on the grass *Coix lacryma-jobi* and the bamboo *Rhipidocladum*

racemiflorum. It is possible that *A. annae* selects vegetation for oviposition based on its structure rather than its abundance. Similarly, the finding that females prefer leaves over stems for egg deposition can be explained by the surface area and support offered by a leaf compared to a stem. These findings provide valuable information on habitat preferences and reproductive ethology of *A. annae*, and therefore contribute to future management and conservation strategies for this species.

Key Words. – Blue-sided Treefrog, Central Valley, perches, Simón Bolivar National Zoo, substrate

INTRODUCTION

Understanding the factors that influence population patterns and the parameters defining aspects of behavior, ecology, and genetics of animal populations is critically important in various areas of theoretical and applied biology (Arguedas et al., 2022). For example, habitat use is one of the fundamental parameters in the ecology and conservation of species. Descriptive data on habitat use by anurans have been generated for some tropical communities, including some recent analyses (e.g., Barroso de Andrade, 2019; Zumbado-Ulate et al., 2021; Komanduri et al., 2023). However, for many other communities, information on habitat use is scarce (Prado et al., 2005; Arguedas et al., 2022). Information on habitat use for several hylids, such as the Blue-sided Treefrog (*Agalychnis annae*), is limited, although significant progress has been made in understanding its distribution and reproductive aspects (Hidalgo-Mora et al., 2021; Arguedas et al., 2022).

Blue-sided Treefrog breeds in ponds, requiring the presence of plants near small water bodies for successful reproduction (Hoffmann, 2005). Plants are needed because eggs are oviposited on vegetation overhanging the water (IUCN SSC Amphibian Specialist Group & NatureServe, 2020). Although it was considered a Costa Rican endemic (Duellman, 1970; Savage, 2002), it was also recorded in the Cerro Colorado region in Panamá (Hertz et al., 2011).

In Costa Rica, the historical habitat of Blue-sided Treefrog covered the Central Valley and adjacent areas, particularly across the premontane region of the Central Mountain range and the premontane forest on the Pacific slope of the Talamanca Mountain range (IUCN SSC Amphibian Specialist Group & NatureServe, 2020; Arguedas, 2018). During the 1980s and 1990s, *A. annae* experienced a decline throughout its entire range, encompassing protected areas and other well-conserved lands (Kubicki, 2004). Like other highland frog species in Costa Rica, this one has lost significant portions of its natural habitat (Pounds & Puschendorf, 2004; Hoffmann, 2006). However, *A. annae* seems to have undergone a recovery in the majority of its historical range and has been observed in previously unknown regions, notably in urban areas (IUCN SSC Amphibian Specialist Group & NatureServe, 2020; Hidalgo-Mora et al., 2021; Zumbado-Ulate et al., 2021; Arguedas et al., 2022).

The potential factors contributing to the declines in a high number of anurans have been documented (Blaustein & Wake, 1995; Lips, 1998, 1999; Kiesecker et al., 2001; Pounds et al., 2006; Carrasco et al., 2021; Valdez et al., 2021). However, the understanding of the possible causes or factors behind these declines is not sufficient, and more information is needed on basic aspects, such as habitat use by anurans like *A. annae*, to determine the causes that have led to their decline.

The objective of this research was to examine the basic aspects of habitat use by *A. annae*, analyzing factors such as substrate preference, perch location, and types of perches used for reproductive activities. This information aims to generate key ecological insights about *A. annae*, which are understudied or unknown at this time and may contribute to future management plans and conservation strategies for the species.

MATERIALS AND METHODS

The study consisted of 53 samplings carried out at intervals of one to two weeks, between August 2007 and December 2008, from 18:00 h to 23:00 h. The study was conducted on a population of *Agalychnis annae* located at a small but natural lagoon of the Simón Bolívar National Zoo (SBNZ), in Barrio Amón, San José, Costa Rica (9° 56' 18.58" N, 84° 04' 23.06" W; 1,147 m a.s.l.; Fig.1). This area has a climate regime with a dry season from December to March and a rainy season from May to October; April and November are transitional months (Herrera, 2016). The average annual temperature for the area during the sampling period was 21.2°C, while the annual precipitation was 1730 mm.

The SBNZ lagoon has an area of approximately 1300 m^2 and a maximum depth of 2.5 m. The vegetation around the lagoon is principally composed of herbaceous plants such as *Cyperus involucratus* (Cyperaceae), *Coix lacryma-jobi*

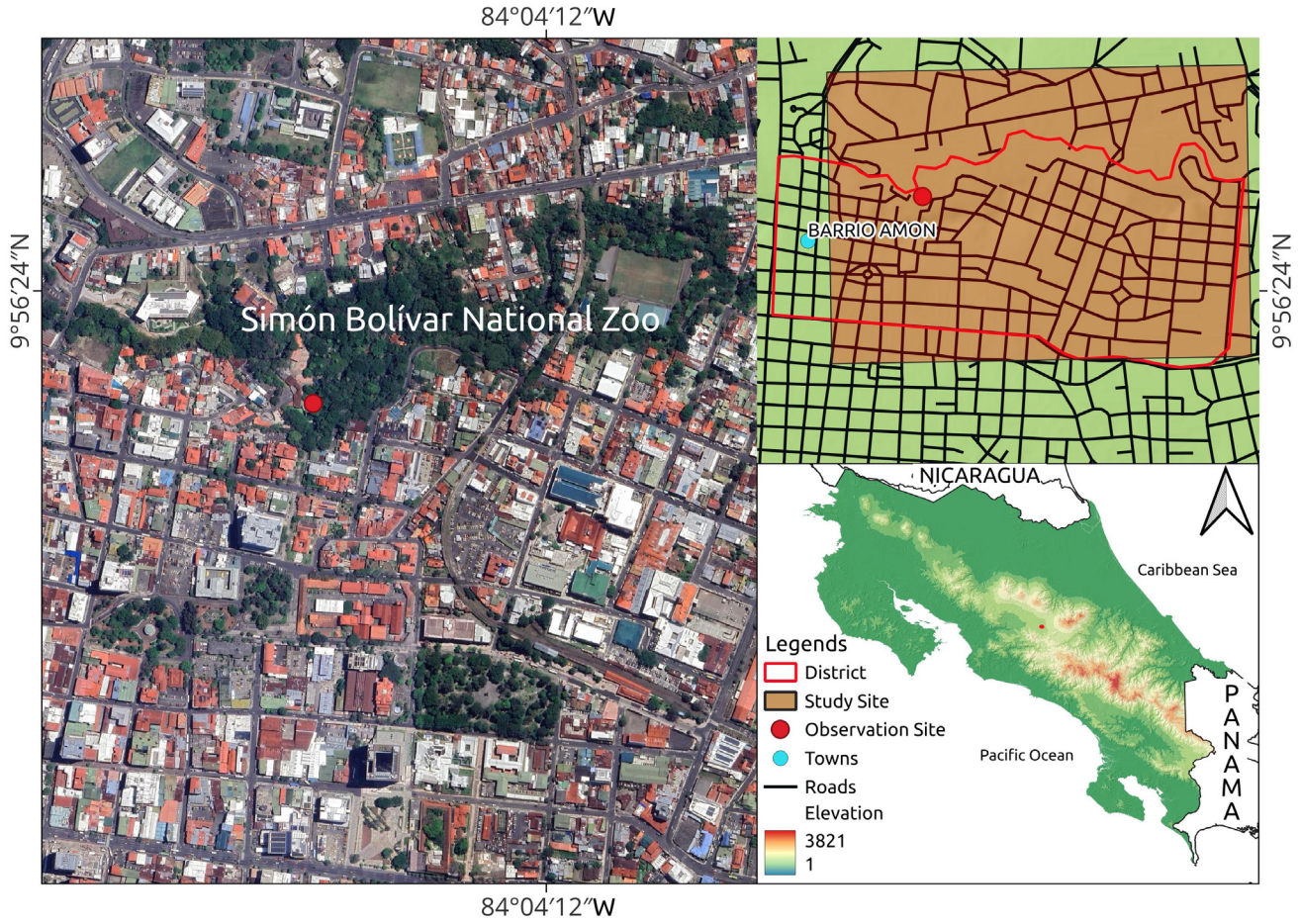

Figura 1. Ubicación de la laguna del Zoológico Nacional Simón Bolívar, San José, Costa Rica. Mapa por G. Chaves.

Figure 1. Location of the lagoon of the Simón Bolívar National Zoo, San José, Costa Rica. Map by G. Chaves.

(Poaceae), *Rhipidocladum racemiflorum* (Poaceae), *Calathea lutea* (Marantaceae), and *Molineria capitulata* (Amaryllidaceae). Additionally, there are some tree and palm species such as *Eupatorium* sp. (Asteraceae), *Schinus terebinthifolia* (Anacardiaceae), and the palm (Arecaceae) *Chamaedorea costaricana* (Arguedas et al., 2022). The fauna species recorded in the lagoon includes three species of fish (*Poecilia reticulata*, *Poecilia gillii*, and *Xiphophorus hellerii*), three species of turtles (*Kinosternon scorpioides*, *Rhinoclemys pulcherrima*, and *Trachemys grayi*), Spectacled Caiman (*Caiman crocodylus*), Muscovy Duck (*Cairina moschata*), and some arthropods such as spiders, dragonflies, and cockroaches, among others. Among the mentioned fauna species, only the arthropods and the two species of *Poecilia* are native to the lagoon (Arguedas et al., 2022).

Agalychnis annae is the exclusive anuran inhabiting the SBNZ lagoon. It is a nocturnal and arboreal species, distinguished by its uniform blue sides and yellowish-orange eyes. Adult males typically measure 57–74 mm in snout-to-vent length (SVL), while adult females range from 67–84 mm (Duellman, 1963; Savage, 2002). This species is predominantly found in pre-montane regions and rainforests situated at elevations ranging from 600 to 1650 m a.s.l. (IUCN SSC Amphibian Specialist Group & NatureServe, 2020). However, it has also been observed in disturbed areas like coffee plantations, vacant lots, and gardens within urban locations (Hoffmann, 2005; Kubicki, 2004; Hidalgo-Mora et al., 2021; Arguedas et al., 2022).

The breeding behavior of female *A. annae* is not well-known, but their presence at breeding ponds leads to increased male competition for mating (Kubicki, 2004). Male frogs primarily call during the rainy season, although reproductive activity can occur year-round in nonseasonal habitats. During amplexus, males embrace females on vegetation located 3–10 m above the

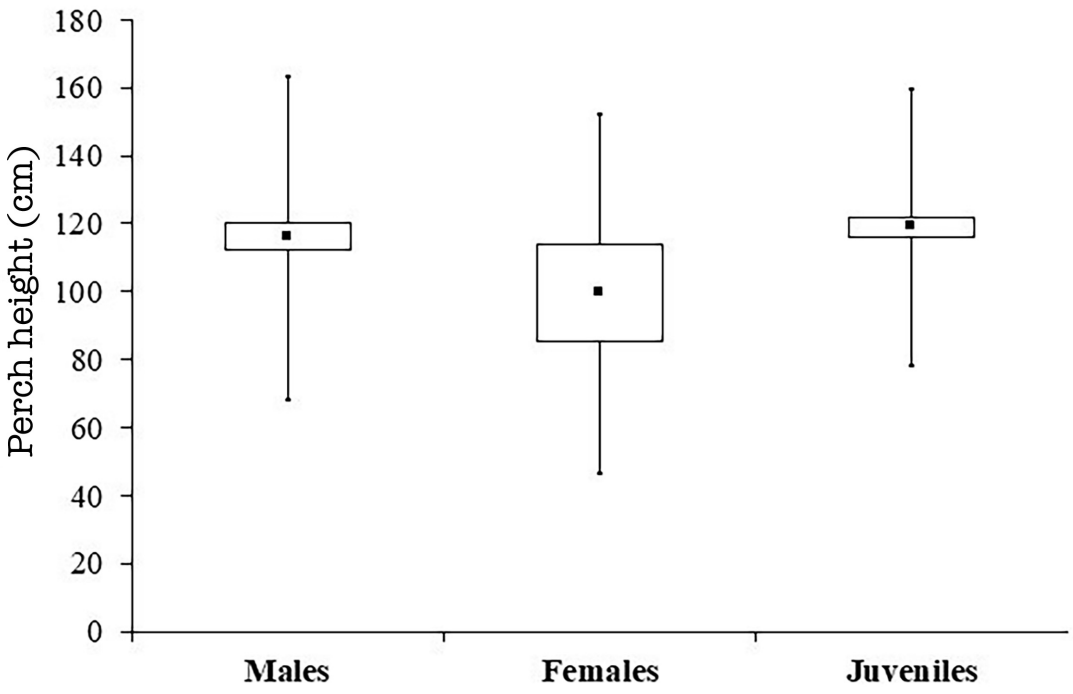

Figura 2. Promedio de altura a la cual perchan los individuos de *Agalychnis annae* según las categorías de sexo/edad en el Zoológico Nacional Simón Bolívar, San José, Costa Rica. Las cajas corresponden a ± Error Estándar, y las líneas a ± Desviación Estándar.

Figure 2. Average height at which individuals of *Agalychnis annae* perch according to sex/age categories in the lagoon of Simón Bolivar Zoo, Costa Rica. The boxes correspond to ± Standard Error, and the lines to ± Standard Deviation.

ground, and gelatinous egg masses, each containing 45–126 eggs, are deposited on surrounding vegetation, especially on leaves, near standing water bodies (Duellman, 1963; Kubicki, 2004; Savage, 2002). After 5–7 days, hatchlings emerge, and they undergo metamorphosis over a period of 247 days, reaching a size of 20–23 mm as juveniles (Duellman, 1963).

During each sampling, we either walked or boated along the edges of the lagoon, capturing and placing each observed individual into a bag for tagging, except for juvenile individuals. Each adult frog captured was hand-caught and marked with a visible implant alphanumeric tag of 1.5 × 3.5 mm (Northwest Marine Technology®), injected in the posterior thigh area (Arguedas et al., 2022). The use of this marking method has been on the rise in amphibian research, and it has demonstrated favorable outcomes, as reported by Heard et al. in 2008. The procedures for implantation and sterilization were carried out in accordance with the protocols outlined by Heard et al. (2008). We documented the sex/age categories (adult male, adult female, juvenile) and measured the SVL using a ruler to the nearest 0.5 mm for each individual. Additionally, for the purposes of this research, we recorded the perch data for each individual, including the type of perch (stem, leaf, or branch), location (over water or land), and height, measured with a 2 m tape measure. We also recorded the activity that each individual was engaged in at the time of observation, e.g., calling, amplexus, silent.

During each sampling, we marked and counted egg masses with a topographic tape for later location and identification. We collected the following data for each egg mass: the type of perch it was placed on (stem, leaf, or branch), its position (over water or land), and the height of the oviposition site, measured with a 2 m tape measure. Additionally, we determined the presence of predation in the egg masses through direct observations.

We obtained the climatic variables data from the meteorological station of Barrio Aranjuez, from the National Meteorological Institute, which is located approximately 1 km away from the study area. These variables were: daily precipitation, daily relative humidity, and average daily

temperature.

We compared habitat use (perch type and location) among sex/age categories using Chi-square tests ($\chi 2$). Additionally, we performed an Analysis of Variance (ANOVA) to compare the height at which males, females, and juvenile individuals perched. We used Chi-square tests ($\chi 2$) and G tests to determine the preference of females for perch type and location where their egg masses were located.

Furthermore, we analyzed the normality of the variables used in all parametric tests using Shapiro-Wilk and Jarque-Bera tests. We logarithmically transformed variables that did not follow a normal distribution with base 10. We performed all statistical analyses using the PAST (Hammer et al., 2001) and SYSTAT 11 programs

RESULTS

Individuals were found to prefer different types of perches based on sex/age categories, with a higher number of adult females and juveniles observed on leaves, and more adult males observed on stems ($\chi 2$= 64.54, df= 4, P< 0.001, Table 1). The location on the substrate for individuals of each sex/age categories was similar,

Tabla 1. Tipo de percha y sustrato usado por individuos de *Agalychnis annae*, según las categorías de sexo/edad, en la laguna del Zoológico Nacional Simón Bolívar, San José, Costa Rica.

Table 1. Type of perch and substrate used by individuals of *Agalychnis annae*, according to sex/age categories, in the lagoon of Simón Bolivar Zoo, San José, Costa Rica.

	Males	Females	Juveniles	Total
Perch type				
Leaf	48	9	155	**212**
Branch	16	1	2	**19**
Stem	79	4	50	**133**
Others	0	0	2	**2**
Substrate				
Over water	120	13	159	**292**
Over ground	23	1	50	**74**
Total	**143**	**14**	**209**	**366**

Tabla 2. Especies de plantas usadas como percha por individuos de *Agalychnis annae* según las categorías de sexo/edad en la laguna del Zoológico Nacional Simón Bolívar, San José, Costa Rica.

Table 2. Plant species used as perches by individuals of *Agalychnis annae* according to sex/age categories in the lagoon of Simón Bolivar Zoo, San José, Costa Rica.

Species	Cover (%)	Categories (sex / age)			Total
		Adult Females	Adult Males	Juveniles	
Calathea lutea	2.84	2	3	1	**6**
Chamaedorea costaricana	10.29	1	8	5	**14**
Coix lacryma-jobi	13.36	2	26	47	**75**
Cyperus involucratus	26.06	2	33	129	**164**
Eupatorium sp.	5.42	0	10	2	**12**
Molineria capitulata	12.79	3	13	1	**17**
Rhipidocladum racemiflorum	20.74	2	25	5	**32**
Others	8.49	2	25	19	**46**
Total	**100**	**14**	**143**	**209**	**366**

as they were mostly located over water ($\chi2$= 4.78, df= 2, *P*= 0.092, Table 1). The height at which males, females, and juvenile individuals perched was also similar (*F*2,363= 1.34, *P*= 0.262, Fig. 2).

We identified seven plant species as perching sites for the Blue-sided Treefrog (Table 2), with *Cyperus involucratus*, *Rhipidocladum racemiflorum*, and *Coix lacryma-jobi* occupying the highest percentage of coverage. Additionally, we observed some individuals on unidentified perches, such as vines, creepers, dry branches, fallen trunks, and a tree of the Asteraceae family (Table 2). Overall, we found a higher proportion of both juvenile individuals ($\chi2$= 198.25, df= 7, *P*< 0.001) and adult individuals ($\chi2$= 22.50, df= 7, *P*< 0.002) on the sedge *Cyperus involucratus* and the grass *Coix lacryma-jobi* (Table 2).

We found 65 egg masses of the Blue-sided Treefrog on seven plant species, with the majority located on the grass *Coix lacryma-jobi* and the bamboo *Rhipidocladum racemiflorum* (*G*= 52.69, df= 7, *P*< 0.001, Table 3). Additionally, egg masses were almost exclusively found on leaves, with only eight cases found on stems ($\chi2$= 36.94, df= 1, *P*< 0.001). Egg masses found on leaves were mostly located on the upper side of the leaves ($\chi2$= 16.86, df= 1, *P*< 0.001, Table 3). All egg masses were found over water, except for one on land. The average height at which these egg masses were located was 98.35 cm (SE= 4.5) based on 65 egg masses.

DISCUSSION

Regarding *A. annae* males 'preferences for stems, it is possible this type of perch provides more support than leaves, as the latter represent less solid surfaces that do not provide the necessary support for male reproductive activity. Males of this frog species spend several hours in the lagoon, so it is important for them to be located on firm structures that allow them to maintain their calling activity during this period of time.

As for juvenile individuals, being lighter, they can perch on leaves. Also, the fact that all individuals preferred perching over water is in accordance with the behavior observed in members of the genus *Agalychnis* (Savage, 2002). Adult individuals of this genus gather in sites with stagnant water during the reproductive season, so it is expected that the majority of individuals use this type of perches (Savage, 2002; Hoffmann, 2005).

The perch height among sex/age categories was similar (Fig. 2). In other *Agalychnis* species, it has been determined that adult individuals perch higher than juvenile individuals (Donnelly & Guyer, 1994). This can be explained by the fact that adults need to dedicate more time to reproductive activities and thus need to be more exposed, while juvenile individuals spend more time in low vegetation, perhaps for protection from predators. Both adult and juvenile individuals used perches at a similar height, which could be explained in several ways. For example, predation pressure at the study site may not be intense enough to cause juvenile individuals to seek less exposure at low vegetation, or food availability for both adult and juvenile individuals may be

Tabla 3. Especies de plantas usadas como percha para ovopositor por las hembras de *Agalychnis annae* en la laguna del Zoológico Nacional Simón Bolívar, San José, Costa Rica.

Table 3. Plant species used as perches for nesting by females of *Agalychnis annae* in the lagoon of Simón Bolivar Zoo, San Jose, Costa Rica.

Species	Cover (%)	Perch			Total
		Leaf surface		Stem	
		Abaxial	Adaxial		
Calathea lutea	2.84	1	1	0	**2**
Chamaedorea costaricana	10.29	0	1	0	**1**
Coix lacryma-jobi	13.36	5	21	0	**26**
Cyperus involucratus	26.06	0	5	0	**5**
Eupatorium sp.	5.42	2	2	0	**4**
Molineria capitulata	12.79	2	0	0	**2**
Rhipidocladum racemiflorum	20.74	2	11	8	**21**
Ohers	8.49	1	3	0	**4**
Total	**100**	**13**	**44**	**8**	**65**

found at a certain height. Although it is true that the number of natural predators in the SBNZ is low due to its urban location, there are domestic cats in the area that may pose a threat to this natural frog population (López de Buen, 1995), something that has not been studied.

Both adult and juvenile individuals of *A. annae* were mainly found on the sedge *Cyperus involucratus* (Table 2), perhaps because it is the species with the highest coverage area in the lagoon. However, it is interesting that the second plant species, the grass *Coix lacryma-jobi*, showed a higher number of adult and juvenile individuals despite having a lower coverage percentage compared to other plant species in the lagoon, where the number of individuals of both age categories was lower, especially in the case of juvenile individuals (Table 2). This observation suggests a potential structural or location preference by *A. annae* individuals for the grass *Coix lacryma-jobi*.

Regarding egg masses, most were located on leaves of the grass *Coix lacryma-jobi* and the bamboo *Rhipidocladum racemiflorum* (Table 3). This could be because the area of the leaves of *C. lacryma-jobi* is relatively large, providing a larger surface area for placement of egg masses. Although bamboo leaves are small, they are closely grouped, so like *C. lacryma-jobi*, they also provide a large surface area.

The results obtained indicate that *A. annae* may choose vegetation for oviposition based on its structure rather than its abundance. For instance, even though the species with the highest coverage percentage (*C. involucratus*) barely had 7% of egg masses on it, *C. lacryma-jobi*, whose coverage percentage is lower, had 40% of egg masses located on it (Table 3). Similarly, the finding that females prefer leaves over stems for placing eggs can be explained by the surface area and support that a leaf offers compared to a stem. Similar to the findings of Kubicki (2004) also on *A. annae*, we found that this species showed a preference for placing its eggs on the upper side of leaves at the SBNZ (Table 3). Perhaps this choice is due to the fact that in this part of the leaf, eggs are more exposed to rain, which could facilitate the larvae falling more easily into the water during the hatching process.

Finally, we found that *A. annae* placed its eggs between 8 and 214 cm in height above the water surface. When comparing these heights with those mentioned by other authors (e.g., Savage, 2002; Hoffmann, 2005) for the same species, which range between 35 and 350 cm, it was determined that both the minimum and maximum values are lower in our study. Gómez-Mestre et al. (2008) determined that at very low heights, the risk of an egg mass being covered by natural fluctuations in water level increases, promoting premature hatching. Therefore, the range of heights where egg masses were found could generate plasticity in hatching times.

In the case of this population and the one studied by Hoffmann (2005), the absence of egg predators may be prolonging the hatching time. It is worth noting that although the study lagoon is natural, it shares certain similarities with the study site sampled by Hoffmann (2005). Both cases involve lagoons or ponds surrounded by urban environments. On the other hand, the data from Savage (2002) and Kubicki (2004) may come from observations made in more natural and pristine environments, where egg masses could potentially be more exposed to native predators, leading to accelerated hatching time.

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