RESEARCH ARTICLE

Thermal biology of *Amphisbaena munoai* (Squamata: Amphisbaenidae)

Nathalia Rocha Matias[1], **Laura Verrastro**[1]

[1]*Programa de Pós-Graduação em Biologia Animal, Laboratório de Herpetologia, Departamento de Zoologia, Universidade Federal do Rio Grande do Sul. Avenida Bento Gonçalves 9500, prédio 43435, sala 105, 91501-970 Porto Alegre, RS, Brazil.*
Corresponding author: Nathalia Rocha Matias (nathimatias@hotmail.com)

http://zoobank.org/4941B13F-7CCE-4C10-98AD-AC13522F6593

ABSTRACT. Studies on the thermal biology of fossorial reptiles that examine the relationship between the body temperature and thermal environment are needed to determine the extent of their thermoregulation abilities. This study assessed the thermal biology of *Amphisbaena munoai* Klappenbach, 1969 in the rocky fields of the Rio Grande do Sul and in the laboratory. The body temperature of most individuals was between 24 and 30 °C, both in the field (n = 81) and laboratory (n = 19). More individuals were caught in winter (n = 55) and spring (n = 60) than in summer (n = 25) and fall (n = 45), and in spring, individuals showed similar nocturnal and diurnal activities. In the laboratory, we found individuals with body temperatures up to 5 °C higher than the ambient temperature (n = 4), suggesting that some physiological mechanisms participate in the thermoregulation of these animals. *Amphisbaena munoai* is a thigmothermic species that is capable of actively regulating its temperature by selecting microhabitats such that its various activities occur within an ideal temperature range. This study is the first to evaluate the effect of seasonality and diurnal and nocturnal variations on the thermoregulation of an amphisbaenid.

KEY WORDS. Amphisbaenians, microhabitat, seasonal, thermoregulation.

INTRODUCTION

All organisms employ strategies that allow them to cope with the heterogeneity in the thermal environment. These strategies include various combinations of thermosensitivity and thermoregulation (Vitt and Caldwell 2014). Thus, organisms may vary between thermal specialists and thermal generalists and between perfect thermoregulators and perfect thermoconformists (Angilletta 2009).

When active, Squamata maintain their body temperatures within a certain range, which varies among species (Avery 1982, Adolph and Porter 1993). The idea that different species of lizards maintain a certain "optimal" temperature was predominant until the mid-20th century; however, it has since then been shown that there is no single optimal temperature but rather several optima depending on the activity (Rocha et al. 2009). The extent of thermoregulation is also known to vary intraspecifically due to various factors, such as altitude (Gvoždík 2002), seasonality (Huey and Pianka 1977), habitat structure (Sears and Angilletta 2015), sex (Ortega et al. 2016), or even competition with other species (Žagar et al. 2015). Moreover, an individual may be

a thermal specialist at specific times but a thermal generalist overall throughout the year (Angilletta 2009).

Fossorial reptiles have a lower thermal preference than most basking reptiles (Brattstrom 1965, Templeton 1970, Rocha et al. 2009). Consequently, amphisbaenians exhibit lower body temperatures than heliotherm diurnal lizards during their periods of activity, and their body temperatures are related to variations in the surrounding substrate (López et al. 2002).

To determine whether fossorial reptiles have the ability to thermoregulate or are conformists, it is necessary to study the thermal biology of species with regard to their body temperature and the thermal environment (López et al. 2002). Furthermore, preferred body temperatures should be measured under artificial conditions that allow an individual to select between equally accessible microclimates, whereby the average of the selected temperatures is considered the preferred temperature and the range of selected temperatures is considered the preferred range (Angilletta 2009).

To date, nine studies have been conducted on the thermal ecology of amphisbaenians, encompassing three of the six extant families (Uetz 2012). These have shown that *Amphisbaena*

mertensii Strauch, 1881 (Abe, 1984), *Blanus cinereus* (Vandelli, 1797) (Martín et al. 1990, 1991, Gil et al. 1993, López et al. 1998), *Diplometopon zarudny* Nikolsky, 1907 (Al-Johany 1999), and *Trogonophis wiegmanni* Kaup, 1830 (Gatten and McClung 1981, López et al. 2002, Civantos et al. 2003) thermoregulate and select particular microhabitats. However, only one study has investigated the thermal biology of the family Amphisbaenidae Gray, 1825, which was conducted by Abe (1984) and determined the preferred temperature of *A. mertensii* Strauch, 1881 in Brazil under field and laboratory conditions.

Previous studies have shown that lizards modify their body temperatures and the selection of microhabitats according to the season (Díaz et al. 2006, Basson and Clusella-Trullas 2015, Ortega and Pérez-Mellado 2016), highlighting the importance of considering this effect in studies of thermal ecology. However, the importance of seasonality with respect to the thermal biology of amphisbaenids has never been verified.

Balestrin and Cappellari (2011) reported a high abundance of amphisbaenids in the rocky fields of the Rio Grande do Sul, providing an excellent opportunity for studying the ecology of *Amphisbaena munoai* Klappenbach, 1969. This species occurs in the south of Brazil and in Uruguay (Gans 2005). Two studies have investigated the diet and reproduction of this species in the Shield of Rio Grande do Sul (Bernardo-Silva et al. 2006, Balestrin and Cappellari 2011). Both studies showed that the diet is diverse but mainly consists of termites, whereas Balestrin and Cappellari (2011) found that this species has a seasonal reproductive cycle between September and November.

The purpose of the present study was to describe the thermal biology of *A. munoai* in the Shield of Rio Grande do Sul, identifying this species' behavior regarding heterogeneity of the thermal environment. This is the first study of this kind to be conducted in the south of Brazil, a region that is subjected to severe, low-temperature winters.

Building on current knowledge on this subject, this study addressed the following questions: 1) Is *A. munoai* a thermoregulator or thermoconformist species? 2) What is the set point range of temperatures for *A. munoai*? 3) Is this set point range similar to that of other amphisbaenas? 4) Is this species able to select microhabitats for thermoregulation? 5) What source of environmental heat is most related to the body temperature of *A. munoai*? 6) Do the answers to these questions differ between seasons?

MATERIAL AND METHODS

The study area is known as the Shield of Rio Grande do Sul or the southeastern mountain range. This area has granite intrusions that form the Dom Feliciano Belt (Rambo 2005), also known as the rocky fields of the Pampa biome. It is located at the São Jerônimo municipality in Rio Grande do Sul, Brazil (30°21′55″S, 51°54′35″W) at an altitude of approximately 500–600 m. The area is covered with typical steppe vegetation (gallery forest) and semi-deciduous seasonal forest (Semi-decid-

uous Tropical Forest). The Pampa biome is under the influence of the temperate mesothermal super-humid climate without droughts, with an average temperature of 10–15 °C (IBGE 2010).

We studied the thermal ecology of *A. munoai* daily throughout the annual study period. The field study took place between March 2011 and October 2012, during which time monthly data were collected from 8:00 am to 6:00 pm for two consecutive days. Nocturnal field studies were also conducted between January and October 2012, during which time observations were made from 6:00 pm to 8:00 am for four consecutive days in each season. Thus, the behavior of *A. munoai* in the field was observed over a full 24 hours period in every season of the year.

A method of active searching was used, whereby the same transect was covered each month. This transect was 870 m long and covered an area of 60 ha, calculated on the basis of a polygon. This area encompassed the largest possible number of habitats (rocks, tree trunks, termite mounds, and anthills) for amphisbaenians. The following environmental parameters were measured every hour: air temperature (Ta); substrate temperature (Ts); substrate temperature 1 cm above the ground at the same location as Ts [Ts (1 cm)]; substrate temperature 5 cm below ground (Tsub); temperature under a rock in contact with the substrate (Tur); and relative humidity of the air. Each time an individual was found and captured, its body temperature (Tb) was taken by inserting a thermometer into the cloaca and recorded (up to 15 s after first contact with the individual), its snout-vent length (SVL) was measured in centimeters with a measuring tape, and all of the abovementioned microhabitat temperatures were recorded at the capture site. All temperatures were measured with a K-Type Minipa® MT-450 thermometer (0.1% + 1 °C) over an approximate time of 5 s or until the thermometer stabilized (up to 15 s). Body temperature was not measured in individuals an SVL of < 7 cm to prevent injuries. All measurements were made away from direct sunlight, ensuring that the collector did not interfere with the animal's temperature.

The height and circumference of the rock under which the animal was found were also measured in centimeters with a measuring tape. Relative humidity was measured with a portable weather station (Portable Digital Hygro-Thermo-Anemometer-Light Meter, model THAL-300; Instrutherm).

To determine the preferred temperature and behavior of *A. munoai* over a 24 hours period under laboratory conditions, 14 individuals were captured from the study site in January and March 2013. Individuals with an SVL of < 9.5 cm were deemed immature according to the study by Balestrin and Cappellari (2011), indicating that this sample contained seven juveniles and seven adults.

These individuals were taken to the Herpetology Laboratory of the Federal University of Rio Grande do Sul, Porto Alegre, Brazil, where they were immediately placed in a glass terrarium (89.5 × 50.0 × 45.0 cm high) containing a 5-cm layer of soil from the collection site, a 100-W "Sun Glo" lamp to simulate sunlight, a 15-W "Night Glo" lamp to simulate moonlight, three natural

rocks of different sizes collected in the field (length × height: Rock 1, 60.0 × 17.0 cm; Rock 2, 29.0 × 6.0 cm; and Rock 3, 37.5 × 5.5 cm), an artificial thermal rock (length × height: Rock 4, 36.0 × 3.0 cm), and a thermal mat (length × width: 18 × 10 cm). This terrarium simulated the natural environment by containing various equally accessible microclimates and microhabitats.

Two experiments were conducted: one in January 2013 with four amphisbaenas and another in March 2013 with 10 amphisbaenas. In both cases, these individuals were captured two days before the observations. The "Sun Glo" lamp was turned on at 7:00 am and turned off at 5:30 pm, whereas the "Night Glo" lamp was turned on at 7:00 pm and turned off at 6:00 am, maintaining the accessible temperatures to the amphisbaenas between 23 and 41.3 °C.

The behavior of the amphisbaenas was monitored over 12 hours for two consecutive days, giving a total of 24 hours for each observation. The microhabitats were assessed every two hours, at which time the available rocks were lifted and the underground layer was readily seen because the terrarium was made of transparent glass. During each inspection, all temperatures in the artificial environment were measured, including all rocks, the substrate temperature 3 cm below the ground (thermal mat), and the air temperature inside the terrarium (Ta). Whenever an individual was found, it was captured by hand and its cloacal temperature was measured over a duration of 5–15 s, avoiding any interference with the animal's temperature by the collector. All temperatures were measured using the same K-Type Minipa® thermometer used in the field. After the experiments, all animals were returned to the capture site in the field.

To test the variance in body temperatures of the amphisbaenas, we first tested the homoscedasticity of the seasonal and diurnal/nocturnal data with D'Agostino's K-squared test (Ayres et al. 2007). Data from all seasons except summer (p < 0.01) were normally distributed, as were the nocturnal data. However, the diurnal and summer data had a non-normal distribution (p < 0.01). Consequently, we performed the Kruskal–Wallis test to analyze the variances in body temperatures.

Multiple linear regression analysis was used to define the relationship between the body temperatures of amphisbaenas and ambient temperatures. Forward stepwise regression analysis was used to determine the relative importance of each thermal source because this allows selection of the predictive variables that are most strongly related to the dependent variable (Ayres et al. 2007). Because the independent variables (ambient temperatures) could be highly correlated (multicollinearity; Graham 2003), we tested the pairwise correlations between these variables with Pearson's correlation and analyzed the main components using principal component analysis (PCA; Hair et al. 1984).

To define the thermal heterogeneity of *A. munoai*, we used two equations. Hertz et al. (1993) developed a way to quantify thermoregulation behavior that combines preferred body temperatures in an artificial thermal gradient with operative temperatures in a natural environment: E = 1 – db/de.

This index is called "Effectiveness of thermoregulation" and is calculated from two indexes: the first is known as "Accuracy of thermoregulation" (db), which equals the absolute average deviation of the specimen's body temperature in an artificial gradient, and the second is known as "Thermal quality of the environment" (de), which equals the absolute average deviation of operative temperatures. The value of "E" is the relative intensity of thermoregulation: when E = 0, random use of thermal microhabitats; when E = 1, thermoregulation is perfect; and when E = 0.5, it can be concluded that there is thermoregulation with moderate effectiveness (Hertz et al. 1993, Angilletta 2009). This index can only be used in studies with animals weighing up to 1 kg (Hertz et al. 1993, Seebacher and Shine 2004), which applies to *A. munoai* because it only grows up to 172 mm (Bernardo-Silva et al. 2006); individuals in the present study weighed a maximum of 1.3 g.

Mean absolute deviance of the body temperature of 14 specimens kept in laboratory was used to define (db), and mean absolute deviance of the ambient temperature Ts (1 cm) of the whole field study was used to define (de). In a burrow, where an organism receives no solar radiation, the operative temperature equals the ambient temperature (Chappell and Whitman 1990).

To define the degree of thermal heterogeneity in areas where *A. munoai* can be found (ΔT), it was necessary to calculate the differences between the recorded body temperatures and the temperatures of the microhabitats according to the following equation: ΔT = ΔTc – ΔTs, where ΔTc represents the variation in body temperatures of amphisbaenas and ΔTs represents the variation in environmental temperatures [Tur, Ts, Ts (1 cm), Tsub, and Ta]. The higher the absolute value of that difference, the higher the degree of thermal regulation (active thermoregulation), as stated by Vrcibradic and Rocha (1998).

Canonical correlation analysis (CCA) was used to test microhabitat selection through rock size and to determine whether the humidity affected the rate at which amphisbaenas were found. This multivariate analysis allows the relationship between two groups of variables to be assessed (Ayres et al. 2007).

RESULTS

Field study

The 15-month-long field study included a total of 1104 collector hours of capture effort, during which time 192 *A. munoai* individuals were found. Between March 2011 and March 2012, 877 collector hours of diurnal capture effort were recorded, during which time 168 *A. munoai* individuals were found. During the warmer months (November and December 2011), the mean rate of amphisbaenas findings decreased. To determine whether this decrease was related to the time of year, nocturnal field studies were also conducted. Hence, 227 collector hours of active nocturnal searching were recorded in January (summer), May (fall), July (winter), and October (Spring) 2012, during which

time 24 *A. munoai* individuals were found. All individuals were found under rocks.

We found more individuals in winter and spring than in other seasons (capture rate = 0.24 individuals/collector hour vs. 0.15 individuals/collector hour in fall and 0.08 individuals/collector hour in summer). When the diurnal and nocturnal studies were considered separately, we found that the values were higher for both studies in spring, with a rate of 0.3 individuals/collector hour for the diurnal study and 0.21 individuals/collector hour for the nocturnal study. In contrast, only the diurnal capture rate was higher in winter, with 0.28 individuals/collector hour compared with 0.07 individuals/collector hour at night (n = 3 individuals).

Summer and fall had lower capture rates in the diurnal study and across the entire study. In contrast, all seasons except spring showed similar capture rates in the nocturnal study. During the breeding season (September–November), activity was similar between the day (0.3 individuals/collector hour) and night (0.21 individuals/collector hour), whereas it remained below 0.07 individuals/collector hour in all other seasons in the nocturnal study.

No correlation (CCA) was found between humidity and the rate of finding amphisbaenas across the entire study (Rc = 0, χ^2 = 3.5, gl = 1, p > 0.06, n = 351), or in spring (Rc = 0.1, χ^2 = 1.8, gl = 1, p > 0.1, n = 77), summer (Rc = 0, χ^2 = 0, gl = 1, p > 0.9, n = 91), or fall (Rc = 0.1, χ^2 = 1.7, gl = 1, p > 0.2, n = 98). However, there was a positive correlation in winter, the wettest season (Rc = 0.2, χ^2 = 5.5, gl = 1, p < 0.05, n = 85) (Table 1).

The average body temperature of *A. munoai* in this study was 23.7 °C (SD = 5.2, range = 12.8–33.8 °C, n = 185), with most individuals exhibiting body temperatures between 24 °C and 30 °C (Fig. 1). Furthermore, most individuals that were found between 8:00 am and 6:00 pm had body temperatures between 28 and 30 °C (Fig. 2), whereas those that were found between 6:00 pm and 8:00 am had body temperatures between 16 and 18 °C (Fig. 3). In the diurnal study, the average body temperature of amphisbaenas was 24.1 °C (SD = 5.2, range = 13.2–33.8 °C, n = 163), which was significantly higher than that in the nocturnal study (mean = 20.5 °C, SD = 4.7, range = 12.8–28 °C, n = 22; Kruskall-Wallis: H = 9.4, gl = 1, p < 0.01, Fig. 4). The body temperatures of *A. munoai* (Tb) also significantly differed between seasons (Kruskall-Wallis: H = 59.6, gl = 3, p < 0.0001), with the exception of the comparison between spring and fall (p > 0.05) (Fig. 5 and Table 2).

According to the index "Effectiveness of thermoregulation," the thermoregulation behavior of *A. munoai* was E = 0.6 in spring and fall and E = 0.5 in winter, indicating thermoregulation with moderate effectiveness. The index was E = 0.4 in summer, indicating random use of microhabitats.

The lowest difference between body temperature and microhabitat temperature (ΔT) for all seasons of the study was ΔTur = 2.1. In the nocturnal study, where ambient temperatures were generally lower than those during the day, all ΔT values were higher, with the exception of ΔTsub. Only in spring, nocturnal ΔT values were lower than diurnal ΔT values (Table 3).

Table 1. Seasonal variation in the relative humidity of the air at São Jerônimo between March 2011 and October 2012.

	Average humidity (%)	SD	Minimum	Maximum	n
Winter	75.5	9.7	53.8	94.1	85
Spring	52.6	18.4	24.6	87.1	77
Summer	60.6	16.7	26.7	94.7	91
Fall	65.7	13.7	32.6	89.4	98
Total	63.9	16.8	24.6	94.7	351
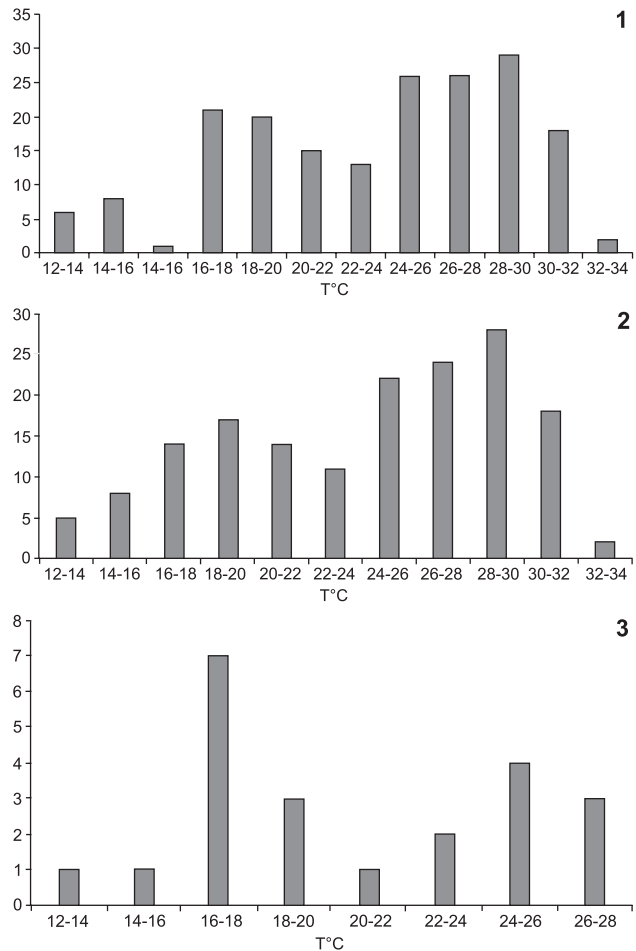
Figures 1–3. Frequency distribution of body temperatures of *Amphisbaena munoai* found at São Jerônimo between March 2011 and October 2012 (n = 185). Body temperatures are shown for: (1) the entire study; (2) the diurnal study; (3) the nocturnal study.

Multiple linear regression showed that there was a significant relationship between body temperature and environmental sources of heat (F = 93.7, p < 0.0001, n = 185). However, when we verified the relationship between Tb and each of the environmental variables included in this regression, we only
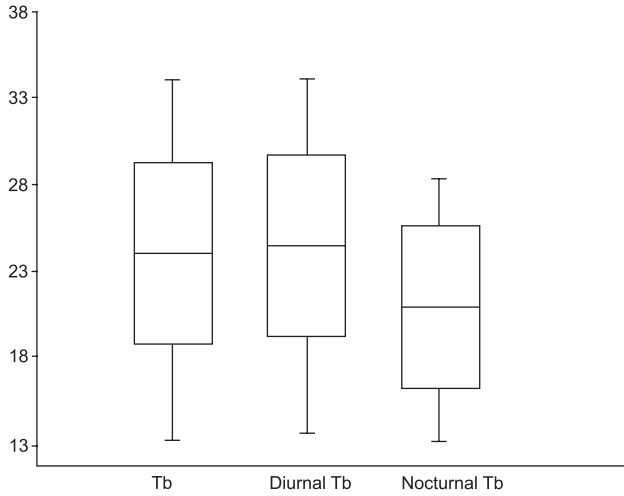
Figure 4. Descriptive statistics (mean and standard deviation) for the body temperatures of *Amphisbaena munoai* found at São Jerônimo between March 2011 and October 2012. Tb: body temperatures for the entire study (n = 183); diurnal Tb: body temperatures for the diurnal study (n = 163); total nocturnal Tb: body temperatures for the nocturnal study (n = 22).

observed a significant relationship with Tur (p < 0.05) and Ta (p = 0.05), indicating multicollinearity between these variables. We also verified that there was a high correlation between the environmental variables when pairs were analyzed with Pearson's correlation (Table 4). The variable Tur represented 90.3% of the total variance in PCA.

To analyze the relative importance of each thermal source, we conducted forward stepwise regression analyses. Given the high correlation between Ta and Ts (1 cm) (r = 0.96, p < 0.0001, n = 185), we excluded the independent variable Ta from this analysis. We found that the independent variable Ts (1 cm) was most strongly related to the body temperature of amphisbaenas throughout the year (all seasons, F = 437.8, r = 0.84, r² = 70.41%, gl = 1.2, p < 0.01) and in each individual season (winter, F = 117.2, r = 0.83, r² = 68.9%, gl = 1.5, p < 0.01; spring, F = 11.9, r = 0.40, r² = 15.5%, gl = 1.6, p < 0.01; summer, F = 13.2, r = 0.60, r² = 36.4%, gl = 1.2, p < 0.01; and fall, F = 34.5, r = 0.66, r² = 44.0%, gl = 1.4, p < 0.01). Tur was then selected as the second variable in all seasons except fall. In all cases, Tsub had the weakest relationship with body temperature.

In the analysis of microhabitat selection through rock size, Tb and SVL formed one group of variables, whereas rock circumference, rock height, and the time at which individuals were
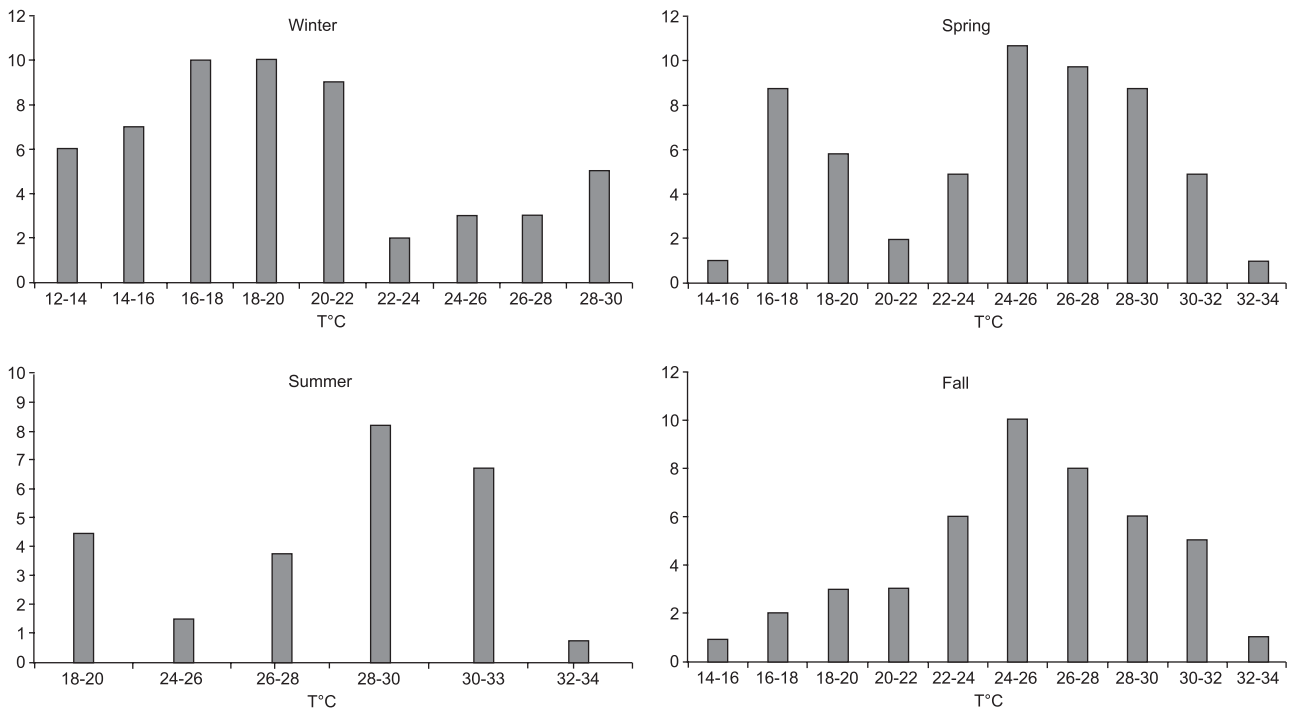

Figure 5. Seasonal frequency distribution of the body temperatures of *Amphisbaena munoai* found at São Jerônimo between March 2011 and October 2012 (n = 185).

Table 2. Seasonal variations in the body temperatures of *Amphisbaena munoai* and the environmental temperatures at São Jerônimo between March 2011 and October 2012.

Winter	Tb	Ta	Ts	Ts (1 cm)	Tsub	Tur
CV (%)	22.9	42.6	29.1	34.3	26.0	29.1
Average (°C)	19.7	13.7	15.1	14.6	15.0	15.3
Minimum (°C)	12.8	2.4	4.5	4.5	6.3	4.7
Maximum (°C)	29.3	25	24.7	26.6	24.0	28.5
SD	4.5	5.8	4.4	5.0	4.0	5.3
n	55	85	85	85	85	85
Spring						
CV (%)	20.1	28.6	31.0	29.8	29.8	34.4
Average (°C)	24.2	19.0	21.7	20.4	21.0	23.5
Minimum (°C)	14.8	9.7	8.8	10.3	9.4	9.4
Maximum (°C)	32.5	31.5	40.3	34.8	38.6	47.3
SD	4.9	5.4	6.7	6.1	6.3	8.1
n	60	77	77	77	77	77
Summer						
CV (%)	9.5	15.2	15.0	15.5	14.7	23.2
Average (°C)	28.6	22.0	25.2	23.7	25.2	26.9
Minimum (°C)	19.0	16.0	19.0	16.5	18.3	16.6
Maximum (°C)	32.0	29.5	34.6	32.9	34.4	44.7
SD	2.7	3.4	3.8	3.7	3.7	6.2
n	25	91	91	91	91	90
Fall						
CV (%)	16.8	34.8	28.3	32.9	24.0	43.9
Average (°C)	25.1	16.8	19.0	17.8	19.0	19.5
Minimum (°C)	14.6	6.9	10.2	7.9	10.2	8.7
Maximum (°C)	33.8	30.0	34.5	33.2	32.2	42.2
SD	4.2	5.8	5.4	5.9	4.6	8.5
n	45	98	98	98	98	98

found formed another group of variables. In the overall analysis, a canonical relationship was found between the two groups (Rc = 0.2, χ^2 = 13.4, gl = 6, p < 0.04); however, when the variables were subjected to individual analysis between groups, there was no significant relationship (Table 5). The seasonal analysis of the canonical relationship showed that there was only a correlation between the two groups in spring (spring, Rc = 0.4, χ^2 = 13.5, gl = 6, p < 0.05, n = 59; summer, Rc = 0.4, χ^2 = 7.2, gl = 6, p > 0.2, n = 25; fall, Rc = 0.4, χ^2 = 11.3, gl = 6, p > 0.07, n = 44; and winter, Rc = 0.3, χ^2 = 6.2, gl = 4, p > 0.1, n = 54). The spring and fall analyses also showed a correlation between rock circumference and both spring Tb (p < 0.05) and fall SVL (p < 0.05).

Laboratory experiment

Microhabitats were surveyed 31 times in the laboratory. In 19 of these surveys, one or more individuals were found in the same or different microhabitats at the same time during the 48 hours observation period. Amphisbaenas activity was observed during both the day (n = 13) and night (n = 19). Amphisbaenas

were found at the highest frequency under rocks on the ground (n = 22), followed by in the subterranean environment (n = 9). A displacement was observed above ground near the Petri dish containing water.

Individuals were found under Rock 1, the largest rock in terms of both height and length, at 12 occasions and under artificial Rock 4, which attained the highest temperature, at eight occasions. In addition, individuals were found in the subterranean environment, which was heated by the thermal mat, at nine occasions. In contrast, individuals were only found under Rock 3 at two occasions and were never found under Rock 2, the smallest of the rocks (Table 6).

The average preferred temperature for *A. munoai* in the laboratory was 28 °C (SD = 2.3, range = 24.7 °C–31.8 °C, n = 25), matching the mean activity temperature observed in the natural habitat of this species in summer (28.6 °C). However, unlike the field study, there was no significant difference between day and night in the body temperatures of *A. munoai* in the laboratory (ANOVA: F = 0.1, p > 0.7).

DISCUSSION

Amphisbaenians are a group of reptiles that are specialized for fossorial life, making it difficult to study their biological and ecological characteristics (Martín et al. 2011). In general, the finding of an amphisbaenian under a rock suggests that there is ongoing thermoregulation activity. Although some of the observations made in this study suggest that *A. munoai* are occasionally inactive under rocks, in majority of the cases, activity of this species was confirmed. Therefore, the rate of *A. munoai* findings indicates their diurnal and nocturnal activity during all seasons of the year.

The average body temperature of *A. munoai* found in this study (23.7 °C, SD = 5.2, range = 12.8–33.8 °C, n = 185) is similar to that of other amphisbaenas (15.5–27 °C). The only other species of the Amphisbaenidae that has previously been studied, *A. mertensi*, presented an average body temperature of 21.4 °C (SD = 2.7, range = 15.5–26.0 °C, n = 52) (Abe 1984). Similarly, in studies with *Trogonophis wiegmanni* Kaup, 1830, the range was between 17.6 and 27.6 °C, and thermoregulation and microhabitat selection were confirmed (López et al. 2002, Civantos et al. 2003); in studies with *Blanus cinereus*, the range was 19.6–25.2 °C (Martín et al. 1990), and selection of soil depth and rocks during the day was observed (Martín et al. 1991, López et al. 1998). *Diplometopon zarudny* Nikolsky, 1907, an amphisbaenid with nocturnal habits, has a similar average body temperature (26.4 °C) in Central Saudi Arabia (Al-Johany 1999).

In all cases, higher body temperatures were recorded than the environmental temperatures (Fig. 6), matching the findings of other amphisbaenid thermoregulation studies (Abe 1984, Martín et al. 1990, 1991, Gil et al. 1993, López et al. 1998, 2002, Al-Johany 1999, Civantos et al. 2003). It is not currently known what causes this pattern, but Tossini et al. (1994) suggested that it could be related to foraging, whereby amphisbeanids are se-

Table 3. Difference between the body temperatures of *Amphisbaena munoai* and the thermal environment (ΔT values) for individuals found in São Jerônimo between March 2011 and October 2012 (n = 185). Tur, temperature under a rock; Ts, substrate temperature; Ts (1 cm), substrate temperature 1 cm above the ground; Tsub, substrate temperature 5 cm below the ground; Ta, air temperature.

ΔT/season	Winter				Spring				Summer				Fall			
ΔTur	n	Mean ± SD	Range	ΔTur	n	Mean ± SD	Range	ΔTur	n	Mean ± SD	Range	ΔTur	n	Mean ± SD	Range	ΔTur
Total	55	17.6 ± 4.3	9.6–29.3	2.1	60	21.2 ± 5.0	9.2–30.9	3	25	25.4 ± 3.4	18.6–32.4	3.2	44	20.9 ± 5.0	12.3–36.9	4.2
Diurnal	52	18.0 ± 4.1	12.2–29.3	2	49	22.0 ± 5.0	9.230.9	3.2	20	26.3 ± 2.9	19.7–32.4	2.8	40	21.5 ± 4.8	13.0–36.9	4
Nocturnal	3	10.8 ± 1.2	9.6–12.0	4.8	11	17.4 ± 2.4	14.9–23.0	1.9	5	21.9 ± 3.1	18.6–25.7	5	4	15.0 ± 3.2	12.3–19.3	5.6
ΔTs	n	Mean ± SD	Range	ΔTs	n	Mean ± SD	Range	ΔTs	n	Mean ± SD	Range	ΔTs	n	Mean ± SD	Range	ΔTs
Total	55	17.1 ± 3.6	8.4–24.9	2.6	60	20.2 ± 4.5	10–29.6	4	25	25.0 ± 2.6	20.1–31.3	3.6	44	19.7 ± 3.5	13.0–30.6	5.4
Diurnal	52	17.4 ± 3.4	11.3–24.9	2.6	49	20.6 ± 4.8	10–29.6	4.6	20	25.4 ± 2.6	20.1–31.3	3.7	40	20.1 ± 3.3	14.5–30.6	5.4
Nocturnal	3	11.0 ± 2.3	8.4–13.0	4.6	11	18.3 ± 2.4	16–24.2	1	5	23.3 ± 2.4	20.5–26.2	3.6	4	15.8 ± 2.6	13.0–18.6	4.8
ΔTs (1 cm)	n	Mean ± SD	Range	ΔTs (1 cm)	n	Mean ± SD	Range	ΔTs (1 cm)	n	Mean ± SD	Range	ΔTs (1 cm)	n	Mean ± SD	Range	ΔTs (1 cm)
Total	55	16.7 ± 3.9	6.6–25.1	3	60	19.1 ± 4.9	11.5–30.3	5.1	25	23.6 ± 3.0	18.3–30.1	5	44	19.3 ± 4.0	11.0–28.9	5.8
Diurnal	52	17.1 ± 3.6	11.2–25.1	2.9	49	19.6 ± 5.0	11.5–30.3	5.6	20	24.6 ± 2.4	18.6–30.1	4.5	40	19.9 ± 3.6	13.5–28.9	5.6
Nocturnal	3	9.5 ± 2.5	6.6–11.3	6.1	11	15.7 ± 1.9	16.4–28.1	3.6	5	19.9 ± 1.8	18.3–22.4	7	4	13.2 ± 2.7	11.0–17.2	7.4
ΔTsub	n	Mean ± SD	Range	ΔTsub	n	Mean ± SD	Range	ΔTsub	n	Mean ± SD	Range	ΔTsub	n	Mean ± SD	Range	ΔTsub
Total	55	16.3 ± 3.5	8.7–22.5	3.4	60	19.6 ± 4.3	10.7–28.1	4.6	25	25.3 ± 2.6	21.2–31.0	3.3	44	19.6 ± 3.0	14.5–27.5	5.5
Diurnal	52	16.5 ± 3.3	11.2–22.5	3.5	49	19.6 ± 4.5	10.7–26.8	5.6	20	25.1 ± 2.5	21.2–31.0	4	40	19.8 ± 3.1	14.5–27.5	5.7
Nocturnal	3	11.5 ± 2.7	8.7–14.0	4.1	11	20.0 ± 3.3	16.4–28.1	–0.7	5	25.8 ± 3.3	22.5–30.5	1.1	4	17.4 ± 1.8	15.4–19.0	3.2
ΔTa	n	Mean ± SD	Range	ΔTa	n	Mean ± SD	Range	ΔTa	n	Mean ± SD	Range	ΔTa	n	Mean ± SD	Range	ΔTa
Total	55	15.8 ± 4.8	2.4–24.6	3.9	60	17.7 ± 4.7	9.5–30.9	6.5	25	22.2 ± 3.0	16.3–28.2	6.4	44	18.8 ± 4.0	10–27.6	6.3
Diurnal	52	16.3 ± 4.3	9.6–24.6	3.7	49	18.6 ± 4.8	9.5–30.9	6.6	20	23.1 ± 2.3	18.1–28.2	6	40	19.5 ± 3.5	13.3–27.6	6
Nocturnal	3	6.7 ± 3.7	2.4–9.3	8.9	11	14.0 ± 1.6	12.2–17.8	5.3	5	18.2 ± 2.1	16.3–21.7	8.7	4	12.3 ± 2.4	10.0–15.7	8.3

Table 4. Pairwise Pearson's correlation values (r) for the environmental temperatures at São Jerônimo between March 2011 and October 2012. Tur, temperature under a rock; Ts, substrate temperature; Ts (1 cm), substrate temperature 1 cm above the ground; Tsub, substrate temperature 5 cm below the ground.

	Tur	Ts	Ts (1 cm)	Tsub
Tur	–	–	–	–
Ts	0.94	–	–	–
Ts (1 cm)	0.91	0.89	–	–
Tsub	0.84	0.94	0.79	–
Ta	0.88	0.84	0.96	0.75

Table 5. Canonical relationship between two groups of variables: Tb (body temperature of *Amphisbaena munoai*) and SVL (snout-vent length) × time, rock circumference, and rock height for the total study (n = 182).

	SVL	p	Tb	p
Time	−0.044	>0.05	−0.126	>0.05
Circumference	0.142	>0.05	−0.020	>0.05
Height	−0.030	>0.05	0.130	>0.05

Table 6. Variation in the temperatures of microhabitats observed in the laboratory at the Federal University do Rio Grande do Sul (UFRGS) in January and March 2013.

	Average temperature (°C)	SD	Minimum	Maximum	n
Rock 1	26.5	2.4	23.2	32.9	31
Rock 2	26.0	1.8	23.5	30.3	31
Rock 3	26.2	1.8	23.5	29.4	31
Rock 4 (artificial)	32.6	5.4	23.4	41.3	31
Thermal mat	28.3	3.2	23.0	35.2	31
T(ar)	28.1	2.1	23.0	32.0	31

lecting higher body temperatures after feeding to aid digestion. In addition, Kamel and Gatten (1983) argued that the selection of higher body temperatures by amphisbaenids was due to the high energetic cost of moving underground. However, even in the controlled environment of the laboratory, we detected individuals with body temperatures up to 5 °C higher than the ambient temperature (n = 4). Thus, it is possible that some physiological mechanism is involved in thermoregulation in these animals, which will be the subject of future studies.

In spring, which is the reproductive season, more *A. munoai* individuals were found in both the diurnal and nocturnal studies, and there was greater conformity with the diurnal environmental temperatures. Furthermore, nocturnal ΔT values were always smaller than the diurnal ΔT values, suggesting either that the preferred temperature for reproductive activity is lower than that for other activities or that the conformity of body tempera-
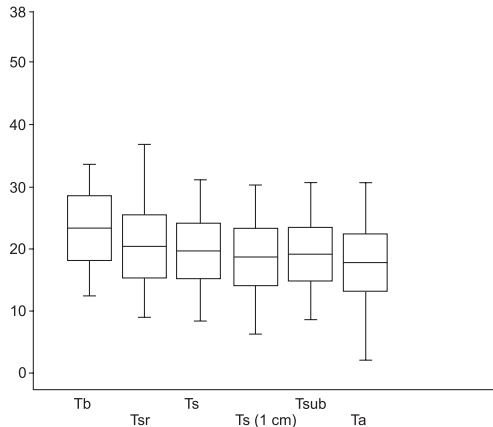
Figure 6. Relationship between ambient temperatures and the body temperatures of *Amphisbaena munoai* found at São Jerônimo between March 2011 and October 2012 (n = 185).

ture to the environment is beneficial for reproductive activity. This was the only season in which low and negative ΔT values occurred, showing conformity with the subterranean environment during the night (Tsub = –0.7). However, CCA showed that in this season, amphisbaenas select their microhabitat based on the size of rocks (Table 5). Therefore, we suggest that *A. munoai* thermoregulate by selecting particular sized rocks to stabilize their body temperatures during the reproductive period.

We found a canonical correlation for total microhabitat selection, but such a correlation could only be found in spring in the seasonal study. However, this result may have been influenced by the lower number of samples when we separated the data by season. In the laboratory, the larger rock (Rock 1) was most used, whereas the smallest rock (Rock 2) was never used, despite there being no significant difference between the temperatures under these rocks (Tukey's test: p < 0.0001) (Table 6). This behavior also indicates that amphisbaenas select different rock sizes as well as different thermal microhabitats. A similar situation was found for the amphisbaenas *B. cinereus* (Martín et al. 1991, López et al. 1998) and *Trogonophis wiegmanni* (López et al. 2002, Civantos et al. 2003), both of which thermoregulate and select microhabitats.

In conclusion, *A. munoai* is a thigmothermal species that is able to actively thermoregulate by selecting microhabitats, allowing it to perform its different activities within an ideal temperature range. This study contributes to our knowledge of the thermal ecology of fossorial reptiles because it is only the second study to describe the thermal biology of a species from the largest family of the clade Amphisbaenia. Furthermore, it is the first study to analyze the thermoregulation of an *Amphisbaena* over the entire 24 hours period in the laboratory and the field and to verify seasonal differences in the thermal biology of an amphisbaenid. More studies on the thermal ecology of these fossorial reptiles are required to allow their behavioral patterns to be compared with other similar species.

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