Abstract

Amphisbaenians are fossorial reptiles that have a cylindrical and elongated body covered with scales arranged in rings, and are all apodal, except for the three species of the genus *Bipes*. The amphisbaenian diet consists of a variety of invertebrates and small vertebrates. As these animals live underground, many aspects of their natural history are difficult to study. Most feeding studies of amphisbaenians have focused on the composition of the diet and feeding ecology, and the data available on feeding behavior are based on precursory observations. The present study describes the food capture behavior of *Leposternon microcephalum* Wagler, 1824 in captivity. In this experiment we used non-live bait (moist cat food), which was placed near a burrow opening, on the surface of the substrate. Three animals were monitored visually and filmed using cellphone cameras deployed at fixed points, to capture images from the dorsal and lateral perspectives of the study subjects. Two principal types of behavior were observed: the capture of food and defense mechanisms. The strategies used to capture the food were similar to those observed in other fossorial species. Although the backward movement has already been observed and described, we were able to record this movement being used as an escape strategy. These findings enrich our knowledge on different aspects of the natural history of the amphisbaenians.

Keywords: feeding, worm lizard, behavior, escape.

Resumo

Anfisbênas são répteis fossoriais caracterizadas por apresentarem corpo cilíndrico e alongado coberto por escamas dispostas exclusivamente em anéis e todas são ápodas, com exceção das três espécies do gênero *Bipes*. Sua dieta consiste em uma variedade de invertebrados e pequenos vertebrados. Por viverem no subsolo, muitos aspectos de sua história natural são difíceis de observar. A maioria dos estudos sobre alimentação em anfisbênas se concentra na dieta e na ecologia alimentar, enquanto as informações sobre o comportamento alimentar se baseiam em observações preliminares. O objetivo deste artigo foi descrever o comportamento de captura de alimentos exibido por *Leposternon microcephalum* Wagler, 1824, fora da galeria, em cativeiro. Para o experimento foi utilizada uma isca não viva, ração úmida de gato, que foi colocada próxima a uma das aberturas da galeria, na superfície do solo. Um total de três animais foi analisado utilizando imagens visuais e registros de câmeras de telefones celulares posicionadas em um ponto fixo, captando imagens de suas vistas dorsal e lateral. Foram detectados dois tipos principais de comportamento: captura de recursos alimentares e mecanismo de defesa. As estratégias utilizadas para capturar o recurso alimentar foram semelhantes às observadas em outras espécies fossoriais. Embora o movimento de “marcha-à-ré” tenha sido observado e descrito, o registramos sendo usado como uma estratégia de fuga. Esses resultados contribuem para enriquecer o conhecimento sobre diferentes aspectos da história natural dos Amphisbaenia.

Palavras-chave: alimentação, cobra-de-duas-cabeças, comportamento, fuga.
1. Introduction

The worm lizards are reptiles of the order Squamata, which form a monophyletic group, the suborder Amphisbaenia (Gans, 1969; Kearney, 2003; Kearney and Stuart, 2004). The name of the suborder is derived from the Greek amphis = double and baena = move, due to the unique ability of these animals to reverse their movement in their tunnels (Gans, 1977).

Amphisbaenians have a cylindrical and elongated body covered with scales arranged in rings (Gans, 1969) and all forms are apodal, except for the three species of the genus Bipes [B. biporus (Cope, 1894), B. canaliculatus Latreille, 1801; and B. tridactylus (Dugès, 1894)] (Uetz and Hoşek, 2020). Currently, the Amphisbaenia has approximately 205 species (Uetz and Hoşek, 2020), distributed in six families, the Amphisbaenidae, Bipedidae, Rhineuridae, Trogonophidae (Gans, 2005), Blanidae (Kearney and Stuart, 2004), and Cadeidae (Vidal et al., 2008). These reptiles are found in southern Europe and northern sub-Saharan Africa, Asia Minor, some regions of the Middle East, and North, South, and Central America (Al-Sadoon, 1988; Gans, 2005; Hembree, 2006; Measey and Tolley, 2013). The Amphisbaenidae is the only taxon found in Brazil, where it is represented by three genera, Amphisbaena Linnaeus, 1758, Leposternon Wagler, 1824, and Mesobaena Mertens, 1925, which is known to be primarily found in Brazil, where it is represented by three genera, Amphisbaena Linnaeus, 1758, Leposternon Wagler, 1824, and Mesobaena Mertens, 1925, Hoogoord, Pinto, Rocha, Pereira, 2009 (Tavares, 2015; Uetz and Hoşek, 2020).

The amphisbaenian diet consists of a variety of invertebrates and small vertebrates (Gans, 1969; Navega-Gonçalves and Benites, 2019), and most amphibian species appear to be dietary generalists (Bernardo-Silva et al., 2006; Gomes et al., 2009; Balestrin and Cappellari, 2011; Martin et al., 2013), although some species have a more selective diet, which indicates that they are specialists (Webb et al., 2000; Vega, 2001; Al-Sadoon et al., 2016). These studies showed that the amphisbaenian diet consists primarily of small arthropods found in the soil, including ants, termites, beetles, and spiders. Species of the genus Leposternon, such as Leposternon microcephalum Wagler, 1824, and Leposternon wuchereri (Peters, 1879), feed mainly on earthworms, while Leposternon polystegum (Duméril, 1851) feeds preferentially on termites, but also on ants and beetle larvae (Goëldi, 1902 apud Gomes et al., 2009; Barros-Filho and Valverde, 1996; Amorim et al., 2019). Since wormlizards are limbless and have vestigial eyes, their preys are preferably slow-moving and easy to capture (Al-Sadoon et al., 1999, 2016).

As they live underground, many aspects of the natural history of the amphibians are difficult to study (Gans, 1978; Navas et al., 2004; Gomes et al., 2005, 2009). Most studies of amphibian feeding have focused on the composition of the diet and feeding ecology. The available data on feeding behaviour is based on precursory observations (e.g. Gans, 1974). Few published studies provide any detailed description of amphibian prey capture or feeding behavior. López and Salvador (1992) and López et al. (2013) focused on the predatory behavior of Blanus cinereus Vandelli, 1797 in artificial burrows that simulated the fossorial environment in captivity. This species uses the vomeronasal sense to identify odors of the prey (López and Salvador, 1992) in addition to be able to use different strategies to capture prey of different sizes, thus a diversified diet could allow amphisbaenian to exploit variable underground trophic resources, circumventing the restrictions imposed by fossoriality (López et al., 2013).

The present study describes the food capture behavior of L. microcephalum on the substrate surface in captivity. This species has the shovel-headed morphotype, considered the most specialized for digging (Gans, 1974; Hohl et al., 2017). Gans (1968) suggested that shovel-headed amphisbaenians are less efficient predators than species that share the rounded-headed morphotype. For instance, prey size that an amphisbaenian can capture and ingest may be constrained by morphological specializations for burrowing (Pough et al., 2003; Gomes et al., 2009).

Leposternon microcephalum has an ample distribution, occurring in several regions of Brazil, as well as in Bolivia, Paraguay, Argentina, and Uruguay (Perez and Ribeiro, 2008; Ribeiro et al., 2011). Different aspects of the locomotion of this species have been studied since the 1950s (see Kaiser, 1955; Navas et al., 2004; Barros-Filho et al., 2008; Hohl et al., 2014; Hohl et al., 2017).

2. Material and Methods

The collection of amphisbaenians in the field was authorized by the Chico Mendes Institute for the Conservation of Biodiversity (ICMBio), the environmental agency of the Brazilian government, through a permanent license (number 15337) for the collection of zoological material, emitted on May 28th, 2008. This behavioral study was approved by the Ethics Committee for the Care and Experimental Use of Animals of the Roberto Alcantara Gomes Biological Institute (CEUA/IBRAG/015/2017). Three adult individuals of L. microcephalum from the municipality of Rio de Janeiro were maintained in captivity at the Vertebrate-Tetrapod Zoology Laboratory (LAZOVERTE) of Rio de Janeiro State University (UERJ) in Rio de Janeiro, where they were kept individually in terrariums containing humus-rich soil that was maintained humid. The photoperiod was natural. Each animal was provisioned with earthworms once a week. The amphisbaenians were healthy and active during the experiments, and were subsequently released back into the wild. The experiments were conducted during different periods, according to the availability of subjects.

Following the experimental procedures of López et al. (2013), the animals were acclimatized to laboratory conditions and the presence of observers for at least one month prior to the experiments. During this period, each of them was provisioned once a week, always at the same time and on the same day of the week, with the experimental bait. As we did not know how long it would take for the animals to detect the food, we used non-live bait for the experiment, that is, moist cat food (Whiskas flavored meatsachets). This bait was offered invariably in the morning, between 7:00 h and 9:00 h, when it was placed on a Petri dish near one of the burrow openings, on the surface of the substrate. Behavioral observations typically lasted approximately one hour. During each
experiment, the observer would occasionally snap their fingers close to the animal to check its reactivity to potential predators (“predator test”). The whole experiment lasted four months approximately.

The food capture behavior of the study subjects was recorded by cellphone cameras deployed at fixed points to capture video footage from the dorsal and lateral perspectives of the subject. The video images were analyzed frame by frame to determine the type of movement exhibited by the subject during displacement and foraging. In addition to the other standard behavioral parameters analyzed here, we evaluated some of the variables proposed by López et al. (2013), that is, “latency to first bite”, which was measured to the nearest second, and “handling behavior”. This included whether the bait was consumed in one or multiple actions, interspersed with pauses during which the bait was released, whether the bait was ingested completely, whether the subject needed support from the terrarium structure or substrate to strike or ingest the “prey”, and whether the bait was shaken. No significant differences were detected in the behaviors exhibit by the individuals. Thus, the results were generalized described.

3. Results

Differences in behavior were not found among the three observed amphibiaenians. In a typical event, the subject appears at the opening of the burrow a few minutes after the bait is deployed, and begins to exhibit behaviors that suggest the detection of food, such as moving its head back and forth, and flicking its bifid tongue. The subject subsequently moves toward the bait on the Petri dish, where it encounters the food and captures it using its mouth. When the pieces are small, the subject seizes the bait in its mouth, chews, and then swallows the pieces whole. When the pieces are larger, the subject first removes a part, by taking the food in its mouth and pressing it against the substrate using muscle contractions along its body, with internal concertina movements and torsional movements of the head. During food capture, approximately half of the body of the animal remains above the substrate with the other half still inside the burrow. After feeding, the subject returns completely back into the burrow using the same entrance, using backward movements. The whole process lasts approximately 20 minutes.

During the “predator tests” (finger snapping), the subject stopped foraging immediately, and retracted back into the burrow using backward movements. By raising its head and apparently moving other parts of the body, the animal tapped the roof at the entrance to the burrow, causing it to collapse and close the entrance. After approximately five minutes, the animal exited the burrow through a new entrance excavated in the vicinity of the Petri dish containing the bait. The subject would then return to feed. Subsequently, the observer snapped fingers once again, and, despite a more delayed reaction, the amphibiaenian reproduced the behavior observed previously.

Occasionally, after feeding, the animal would move to a part of the burrow adjacent to the glass of the terrarium, where we would be able to observe it underground. At this moment, the animal was at rest, when it would occasionally open and close its mouth repeatedly, while simultaneously poking out its bifid tongue from time to time.

Two principal types of behavior were detected in the present study: (i) the capture of food, and (ii) defense mechanisms. The food capture behavior was divided in two principal stages: (i) foraging and detection, when the animal emerged from the burrow, moving its head, and flicking its tongue (Figure 1A), and (ii) contact and capture - the seizing and ingestion of the food using the mouth (Figure 1B).

When consuming small pieces of bait, L. microcephalum presented the “single swallow” pattern of behavior, approaching the bait and attacking it rapidly with a single bite, bringing the whole of the bait into its mouth. The animal then ingested the bait in a single movement, shaking violently while the bait was in the mouth, and then swallowing it rapidly (see López et al., 2013 for comparison). For large pieces of bait, L. microcephalum presented “cut and swallow” behavior, in which the food was crushed and divided using bites, and then ingested piece by piece (see López et al., 2013 for comparison).

The defense mechanisms had three principal steps: (i) threat perception – foraging is halted (Figure 2A), (ii) backward movement, when the animal returns completely to the interior of the burrow (Figure 2B-D), and (iii) closure of the burrow opening, when the animal strikes its head against the roof of the burrow, causing it to collapse and obstruct the entrance (Figure 2E).

All three subjects presented the same foraging behavior patterns. The escape behavior was observed in only two of the individuals, however, and only one sealed the entrance of the burrow. This animal repeated the behavior on two occasions.

4. Discussion

*Leposternon microcephalum* presented typical foraging behavior, coming out of the burrow to seek and detect the food, moving its head back and forth, and flicking its tongue regularly. Active reptilian foragers tongue-flick regularly during foraging and can detect and recognize prey by chemical cues (López and Salvador, 1992). Amphibiaenians have enhanced auditory and olfactory capabilities (Gans and Wever, 1975; Gans, 1978).

Here, *L. microcephalum* was observed engaging in internal concertina movements even when moving across the surface of the substrate, with part of its body out outside the burrow. This may be a discreet type of displacement, used to approach prey without alerting it to the presence of the predator. Amphibiaenians travel on the surface of the substrate by the lateral undulation of the body, which may be associated with concertina movements when the surface is smooth (Gans, 1974). Rectilinear locomotion is also an option in this situation (Gans, 1978). In order to move within the galleries of the burrow, amphibiaenians may use either rectilinear or concertina locomotion (Gans, 1978) or a variant of the latter known as “internal concertina” (Gans, 1973), which was
Figure 1. Images showing the different phases of the capture of food by *Leposternon microcephalum*: (A) Foraging and Detection – displacement, with head movements and tongue flicking, and (B) Contact and Capture - capture and ingestion of the food using the mouth. In detail, the bifid tongue.

Figure 2. Images showing the different phases of the predator defense behavior in *Leposternon microcephalum*: (A) Threat perception – foraging is halted; (B-D) Backward movement – the animal returns to the inside of the burrow, and (E) Obstruction of the burrow opening - the animal strikes its head on the roof of the burrow, causing it to collapse and close the entrance.
denominated “vermiform locomotion” by Gaymer (1971). López et al. (2013) observed that *B. cinereus* advances through artificial burrows using continuous slow forward concertina movements to approach its prey. Although the internal concertina is used to move inside the burrow (Gans, 1973), *L. microcephalum* relied on the physical support of the substrate to capture and process the food.

In the present study, *L. microcephalum* presented different modes of handling and ingesting the bait according to the size of the pieces encountered. López et al. (2013) concluded that the specific characteristics of the prey determine the adoption of different prey-handling modes by *B. cinereus*. This amphisbaenian follows the swallowing threshold model, which predicts that prey will only be reduced in size when the forager is unable to swallow it whole (Kaspari, 1990; López et al., 2013). The behavior of *L. microcephalum* was similar to that of *B. cinereus*, with the “cut and swallow” mode being used when the bait was large and the “single swallow” being used when the piece of bait was small enough to be ingested whole.

López et al. (2013) highlighted the mechanical adaptations that allow amphisbaenians to pierce and tear prey, such as the arrangement of the teeth and the ability to twist the head. *Leposternon microcephalum* also used torsional movements of the head to help cut off pieces of the bait, in a manner similar to *B. cinereus* (López et al., 2013) and the caecilians *Boulengerula taitana* Loveridge, 1935 and *Schistometopum thomense* (Bocage, 1873) (Measey and Herrel, 2006; Herrel and Measey, 2012).

In the present case, when *L. microcephalum* left the burrow to forage, exposing part of its body, it would have been at a much greater risk of predation. During this behavior, the “predation test” (finger clicking) caused the subject to stop foraging and return to the burrow using backward movements and, in one case, tapping the substrate to collapse the roof of the entrance, isolating the burrow. The capacity to detect potential predators is diminished during the consumption of prey (López et al., 2013). Epigean saurians can be detected easily by terrestrial or aerial predators while feeding (López and Salvador, 1992; López et al., 2013).

Here, the capacity of *L. microcephalum* to move backward when faced with a potential threat was decisive for escaping from a potential predator. Amphisbaenians are the only burrowing reptiles capable of moving backwards in their tunnels (Gans, 1969), a characteristic that determined the name of the suborder, from the Greek *amphis* = double and *baena* = move (Gans, 1977). This backward movement, described in full and analyzed by Hohl et al. (2014) in *L. microcephalum*, is typically used to move backwards within the galleries of the burrow.

Much of the natural history of the amphisbaenians is difficult to study due to their fossorial lifestyle. Given this, captive studies may provide important insights into the behavior and ecology of these reptiles that might otherwise remain unknown. The results of the present study provide important evidence on both foraging behavior and predator defense, which may be typical of the patterns occurring in the wild. The main contribution of this study was to describe in detail the food capture behavior of *L. microcephalum* contributing with information that help to understand the biology of these animals that are still poorly studied.

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