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Dietary observations of adult *Pleurodema nebulosum* (Anura: Leptodactylidae) from Argentina

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Abstract: This study is the first to examine the diet composition of adult *Pleurodema nebulosum* specimens based on an analysis of the gastrointestinal contents of 78 individuals. Analyzed herein were 40 specimens from a site disturbed due to anthropogenic action and 38 from a protected area in Argentina. Differences were found in the trophic consumption at both sites. In the protected area, *P. nebulosum* presented an active foraging mode, with a diet specialized in prey items from the Formicidae family. At the disturbed site, this amphibian showed a sit-and-wait foraging mode, with a generalist diet where it consumes Hemiptera and Coleoptera. In conclusion, the results support the idea that *P. nebulosum* adapts to local conditions of a disturbed site or protected area. In this way, it modifies its foraging mode according to habitat conditions.

Key words: Amphibian, diet, Monte region, parque presidente sarmiento

Obtaining information on trophic aspects in wild animal populations is essential for conservation decision making. Therefore, the three main areas for wildlife management are conservation, sustained, and conserved performance (Sinclair et al., 2006). Consequently, it is essential to acquire knowledge about the trophic resource (Sinclair et al., 2006). Trophic analyses help evaluate the impact of anthropogenic disturbances on habitats, as they provide information about the types of prey consumed (Castillo et al., 2017; 2019). They also furnish details regarding their specialization and/or opportunistic behavior, along with their active foraging type (widespread foragers), passive behavior (sit and wait), or a combination of both (Huey and Pianka, 1981, Castillo et al., 2017, 2019). This information is crucial for establishing management guidelines and assessing their conservation status (Castillo et al., 2019).

The amphibian *Pleurodema nebulosum* (Burmeister, 1861) has an approximate size of 45 mm (snout-vent length) (Gallardo, 1987), with a wide distribution in various provinces of Argentina. Its distribution range overlaps in saline environments and semi-desert areas from the center of Argentina, with a broad altitudinal distribution (Ferraro and Casagranda, 2009; Vaira et al., 2012). Despite its extensive distribution, biological information is scarce, with contributions related to reproduction, thermal data, diet in juvenile stages, and parasitological aspects (Sanabria et al., 2006; 2007; 2013; Piñeiro-Gómez et al., 2017; Castillo et al., 2023). On the International Union for Conservation of Nature (IUCN) Red List of Threatened Species in 2017, *P. nebulosum* was listed as Least Concern.[1]

The objective of the following work was to compare the trophic consumption pattern of *P. nebulosum* between a protected area and a disturbed site.

In order to generate information on aspects of bioecology influenced by different types of habitats previously unknown, the following questions were raised: 1) What are the main prey items consumed by *P. nebulosum*? 2) What dietary category (insectivorous, omnivorous, or herbivorous) and level of specialization (specialist/opportunist) do they exhibit? 3) What foraging strategy does it have (active foraging/sit and wait)?

In the following research work, a hypothesis was proposed that different types of sites (disturbed site and

[1]IUCN. 2024. The IUCN Red List of Threatened Species. Version 2023-1. Available at: www.iucnredlist.org. [Accessed: 29 May 2024].

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protected area) generate differential variations in the trophic consumption of *P. nebulosum* in Argentina.

The Sarmiento Provincial Park, designated as a conserved site, is situated at the heart of San Juan city (31°55′S, 68°70′W). This park holds the distinction of being the second-largest natural wetland in San Juan Province. This site harbors 15% of the herpetofauna of San Juan (Sanabria and Quiroga, 2010). It is considered a conserved site due to its characteristics as a protected area and for being an environment with stable herpetofaunal assemblages (Sanabria and Quiroga, 2010).

The Matagusanos locality (disturbed site) (31°14′S, 68°38′W; 600 m asl) is located 55 km east of San Juan, Ullúm Department. This site is characterized by deforestation, affecting the diversity of flora and fauna species. The main activity is the extraction of raw materials for the manufacturing of bricks. It is characterized by the presence of a human population, settlements close to the road, and various access points. There is a 70% loss of vegetation due to anthropogenic pressure (Castillo, 2022), and there is also the presence and activity of domestic and farm animals (for example, goats, dogs, and horses). There is also the presence of tractors and trucks that constantly enter the area, carrying out deforestation and soil disturbances (Castillo, 2022).

Twenty samplings were conducted in the summer of 2017 in two types of habitats: Ten samplings were carried out at the disturbed site, and 10 samplings in the protected area.

Trophic analysis

In order to determine the diet (insectivorous/omnivorous/herbivorous), the plant material content in the stomachs of the individuals was analyzed based on percentages. This content was quantified following the criteria of Espinoza et al. (2004), Astudillo et al. (2015), Córdoba et al. (2015), and Castillo et al. (2017). For plant quantification (stems, leaves, flowers, fruits, and seeds), a calculation was made of the percentage that the plant material occupied in the stomach in relation to that occupied by arthropod prey. Stomach contents were analyzed to describe the qualitative (type) and quantitative (number) prey composition. All the variables recorded for each prey were systematically determined at the order, family, or genus level, following Brewer and Arguello (1980). The maximum length and width of the prey items were measured, and their volume was calculated using the formula proposed by Dunham (1983):

$V = 4/3\pi\ (½L)\ (½W)^2$

Here, L is prey maximum length and W is prey maximum width. The index of relative importance (IRI) was calculated for each prey category using the following formula (Pinkas et al., 1971):

IRI = % frequency of occurrence FO (% volume V + % numerousness N)

In order to establish the hierarchy ranking of the diet, the highest IRI value was considered as 100% and other values were calculated as relative percentages. If the percentage of prey fell between 100% and 75%, it was considered fundamental, between 75% and 50%, was secondary, between 50% and 25% was accessory, and below 25% was accidental (Aun and Martori, 1998).

The criterion of prey item consumption was taken into account to determine the feeding strategy (active/sit and wait) (Huey and Pianka, 1981). When local prey consumption is numerous, a tendency toward an active search mode is considered. On the contrary, a lower consumption of larger solitary prey is considered as sit and wait (Pianka, 1982; Vidal and Labra, 2008; Vitt and Caldwell, 2009; Castillo et al., 2017; 2019). Considering that the type of diet (specialist/generalist) is related to feeding strategy, specialists are associated with active foraging mode, while generalists (opportunists) are linked to sit and wait (Huey and Pianka, 1981; Castillo et al., 2017; 2019).

To establish whether there are trophic similarities between protected area and disturbed environments, the richness of the prey items was determined, and Jaccard (JI), Sorensen (SI), and Simpson (SD) indices (González et al., 2006) were used for each sampled site. The specimens were deposited in the Herpetological collection, Department of Biology, Faculty of Exact Physical and Natural Sciences, National University of San Juan (*Pleurodema nebulosum*: UNSJ 4053-4096).

A total of 78 individuals of *P. nebulosum* were captured, with 40 specimens collected from a site disturbed due to anthropogenic activities and 38 from a protected area. All the specimens analyzed provided relevant information for the trophic analysis.

Trophic consumption was not differentiated between sexes because the quantity of samples for the females was insufficient, and in this way, the data were prevented from being biased. A total of 358 prey items were recorded in the gastrointestinal content of *P. nebulosum*, with 332 prey items in the protected area and 26 in the disturbed site. These prey items were classified into 10 categories: four Hymenoptera, four Coleoptera, one Hemiptera, and one Araneae. The Table shows the values of percentages of numerosity, volume, occurrence frequency, IRI, and categories in the protected area and disturbed site.

In the protected area, the 332 prey items were arthropods and plant material was not detected. The order Formicidae showed higher relative importance (IRI). Ant genus *Solenopsis* sp. showed a higher percentage of abundance and occurrence frequency, although the genus *Componatus* sp. showed greater volume. *P. nebulosum* in the protected area exhibited an active foraging mode and Formicidae specialist diet, mainly consuming ants of the genus *Solenopsis* sp.

Table. Trophic data of *P. nebulosum*.

		Protected area					Disturbed site				
Prey items		%N	%V	%FO	%IRI	Category	%N	%V	%FO	%IRI	Category
Hymenoptera (Formicidae)	*Solenopsis* sp.	72.2	6	24.3	100	Fundamental	28.5	1.11	16.6	17.4	Accidental
	Componatus sp.	7.2	35.9	9.7	12.5	Accidental	0	0	0	0	Accidental
	Acromirmex sp.	10.5	15.9	9.7	7.7	Accidental	0	0	0	0	Accidental
Hymenoptera (Vespidae)	S/I	0.3	4.2	2.4	0.3	Accidental	0	0	0	0	Accidental
Coleoptera	Scarabidae	0.9	1.1	7.3	0.4	Accidental	10.7	49.8	16.6	35.4	Secondary
	Colliphoridae	1.5	0.4	7.3	0.4	Accidental	0	0	0	0	Accidental
	Tenebrionidae	4.2	23.1	21.9	17.9	Accidental	25	7.8	22.2	25.6	Secondary
	Trogossitidae	0.3	0.1	2.4	0.03	Accidental	0	0	0	0	Accidental
Hemiptera	S/I	1.8	10.1	7.3	2.6	Accidental	32.1	40	38.8	100	Fundamental
Araneae	S/I	0.9	2.6	7.3	0.7	Accidental	3.5	0.2	5.5	0.7	Accidental

S/I: unidentified. %N: numerousness percentage, %V: volume percentage, %FO: frequency of occurrence percentage, and %IRI: index of relative importance percentage.

At the disturbed site, the 26 prey items were arthropods. No presence of plant matter was observed. The order Hemiptera showed higher occurrence frequency, abundance, and relative importance (IRI). The order Coleoptera, families Scarabidae and Tenebrionidae were secondary categories in trophic consumption. *P. nebulosum* at the disturbed site exhibited a sit and wait feeding mode and opportunistic diet, where it consumed orders Hemiptera and Coleoptera.

The Jaccard index showed trophic differences of JI = 0.5, as did the Sorensen index SI = 0.6, indicating a consumption of different prey items at both sites. The Simpson diversity index showed SD = 0.2 for the disturbed site and SD = 0.5 for the protected area, indicating a difference in trophic consumption between the two sites. The richness of the protected area was 10 prey items, and that for the disturbed site was five.

This work represents the first study on the diet of *P. nebulosum* in Argentina using the digestive contents of adult specimens in a protected area. Previously, Sanabria et al. (2007a) analyzed the feeding habits of *P. nebulosum* in western Argentina, although the specimens were in the infantile stages. In contrast to the work of Sanabria et al. (2007), in which they studied one population of infants in a specific area of western Argentina, two populations were studied herein, and comparisons were made between a protected area and a disturbed site.

When comparing the two study sites, differences in the trophic consumption were observed. In the protected area, the most representative items were ants, mainly of the genus *Solenopsis* sp. (Westwood, 1840). At the disturbed site, the main item of trophic consumption was bedbugs of order Hemiptera. Thus far, it is known that juveniles of *P. nebulosum* prefer prey items of the order Hemiptera, followed by ants of the order Hymenoptera (Sanabria et al., 2007a). These results were not very different than those observed in the current work, where, depending on the site, the *P. nebulosum* populations consumed Hemiptera and Hymenoptera (Formicidae) as the main items.

The present results showed no presence of plant material in the gastrointestinal contents. The prey items of *P. nebulosum* were similar to those recorded in *Pleurodema bufoninum* Bell, 1843, *Pleurodema thaul* Lesson, 1827, *Pleurodema diplolistris* (Peters 1870) and *Pleurodema somuncurense* (Cei 1969), where the diet was composed of arthropods and in which no plant material was recorded (Díaz-Páez and Ortiz, 2003; Alves et al., 2003; Bello et al., 2005; Velasco et al., 2019). Herein, it was found that the adult *P. nebulosum* varied their foraging mode depending on the site (disturbed or protected area). *P. nebulosum* in the protected area showed an active foraging mode and specialist diet of Formicidae. However, at the disturbed site, it exhibited a stalking foraging mode and opportunistic diet, consuming the orders Hemiptera and Coleoptera. For the genus *Pleurodema*, there is a variation in diet, such as the case that juvenile *P. nebulosum* show certain specialization (Sanabria et al., 2007a), probably related to active foraging mode. The population of *P. bufoninum* is opportunistic, adapting to local conditions (Bello et al., 2005) and is considered a generalist. With *P. thaul*, although the type of diet is not very clear, apparently it would be generalist (Díaz-Páez and Ortiz, 2003) and probably more associated with a sit-and-wait foraging mode.

The richness of prey item types at the disturbed site was five. It was reported that the low diversity found could reflect scarce prey availability at that time (Sanabria et al., 2007a). However, 16 years after this last study, at the same site, the number of recorded prey items was low. Therefore, it was presumed that the Matagusanos site actually had a low diversity of arthropods available for *P. nebulosum*. In the protected area, 10 types of prey items were recorded, this is probably due to the characteristics of site. Being a protected area, it is conserved, with a greater availability of arthropods available for consumption by *P. nebulosum*. The results for the Jaccard index (JI = 0.5) and Sorensen index (SI = 0.6) indicated different consumptions of some prey items. The Simpson diversity index (SD) showed differences in the trophic consumption between the disturbed site (SD = 0.2) and protected area (SD = 0.5).

As a final conclusion, the results support the notion that *P. nebulosum* adapts to local conditions. It modifies foraging patterns depending on habitat conditions. It was assumed that adult *P. nebulosum* mainly consume arthropods based on the obtained data, with no plant material present. The species presents an active search mode or adopts a sit-and-wait strategy, exhibiting specialization or generalization depending on the site of the population. This study provides the first trophic data on adult *P. nebulosum* in Argentina.

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Conflict of interest
The authors declare no conflicts of interest.

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