

# Diet composition and trophic niche overlap between two sympatric species of *Physalaemus* (Anura, Leptodactylidae, Leiuperinae) in a subtemperate forest of southern Brazil

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**Abstract.** Comparative studies of the diet of ecologically and phylogenetically close-related species assist in understanding the evolutionary processes underlying feeding specialization. The frogs *Physalaemus lisei* (Braun's Dwarf Frog) and *P. gracilis* (Graceful Dwarf Frog) are good models for comparative diet studies because they occur in sympatry in several forest environments of Brazil. In this study we evaluated the gastrointestinal content of 83 individuals of these two species. We registered 12 prey categories in the diet of *P. gracilis*, and 19 in the diet of *P. lisei*. Formicidae was the most important prey category in the diet of both species, followed by Coleoptera and Araneae. Despite the high importance of ants in the diet of both species, as assessed by the Index of Relative Importance, Coleoptera presented the highest volumetric contribution. Both species presented a similar trophic niche breadth and a high diet overlap. Our results indicate similarities in the diet composition and foraging behavior of *P. lisei* and *P. gracilis*.

**Key words.** Araucaria forest, sympatry, predation, Amphibia.

## Introduction

The ecological niche of a given species is characterized based on its distinct life history components (Putman, 1994), with those concerning the use of food resources being among the most well studied by ecologists (Pianka, 1973; Sih and Christensen, 2001). In fact, the processes related to food obtainment, such as selection of foraging habitats, prey selection and capture, are critical for establishing the trophic niche of a species (Sih and Christensen, 2001). A simple description of some of these components, such as the diet, is relevant because it assists in mapping trophic relationships and establishing models of interspecific interactions. Abundant species represent good models for studying trophic relationships in an ecosystem, as they have a high potential contribution in the transfer of matter and energy between different trophic levels. Anurans, for example, are some of the most abundant terrestrial

vertebrates in Neotropical wetlands, having a key contribution in the matter and energy transfer along the trophic web (Araújo et al., 2007; Huckembeck et al., 2014).

Brazil has the world's greatest richness of amphibians (Segalla et al., 2016), which puts the country in a major position for the advance of the knowledge about the trophic ecology of amphibians. Fortunately, the number of studies on the feeding ecology of Neotropical frogs has increased exponentially in the last years (see Siqueira et al., 2006; Dietl et al., 2009; Rodrigues and Santos-Costa, 2014). However, even though we currently know the diet composition of a significant number of these species, few studies used a comparative approach. To compare the diet of species living under similar habitat conditions (e.g., sympatric species) is an important exercise for formulating hypotheses about the role of competition and phylogenetic or behavioral factors in prey selection (Menin et al., 2005; Sabagh and Carvalho-E-Silva, 2008; Oliveira et al., 2015). This debate is enhanced when we investigate not only sympatric species, but also species sharing morphological, ecological or behavioral traits (e.g., microhabitat selection and activity patterns) (Duré and Kehr, 2001, 2004). Based on this framework, it is expected that congener species sharing habitat requirements would present similarities

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in their diets (Sabagh *et al.*, 2010). This pattern was observed in amphibians (Guidali *et al.*, 2000; Sabagh and Rocha, 2012; Méndez-Narváez *et al.*, 2014) and in other vertebrates as well (Monda and Ratti, 1988; Vieira and Port, 2007; Mata-Silva *et al.*, 2013), suggesting an ecological pattern consistent in various vertebrate taxa. However, the differential use of food resources between similar species could reduce competition and would therefore allow their coexistence (Pianka, 1973). These somewhat contrasting results reinforce the importance of trophic comparative studies.

In the southern Brazilian plateau, some species of *Physalaemus* (Leptodactylidae) are usually recorded in high numerical abundance, occurring in sympatry with congeners in many localities. The genus *Physalaemus* is widely distributed in the Neotropical region and currently comprises 47 species (Frost, 2016). Species of *Physalaemus* usually forage in the leaf litter and feed primarily on ants, beetles and spiders (López *et al.*, 2003; Becker *et al.*, 2007; Santana and Juncá, 2007; Rodrigues and Santo-Costa, 2014; Olivera *et al.*, 2015). The morphological, behavioral and phylogenetic similarities make this an adequate genus for comparative studies on the diet of similar, sympatric pairs of species. The sympatric distribution of *Physalaemus lisei* Braun and Braun 1977 and *Physalaemus gracilis* Boulenger 1883 is well studied in the subtropical forests of the southern region of Brazil (see IUCN, 2015). While *Physalaemus lisei* is endemic to southern Brazil, *Physalaemus gracilis* has a wider geographical distribution, though restricted to southern Brazil and Uruguay (Frost, 2016). In this study we compared the diet of *P. lisei* and *P. gracilis*, testing the hypothesis that due to morphological, ecological (“*latu sensu*”) and phylogenetic similarities between the two species, there should exist a high overlap in the composition of their diets.

## Materials and Methods

**Study site:** The study was conducted in a subtropical Araucaria forest, which is a phytogeography of the Atlantic Forest biome of southern Brazil. The study area is located in the municipality of São Francisco de Paula (29°23'S 50°22'W), state of Rio Grande do Sul, Brazil. The region has a subtropical climate, with an annual rainfall of 2162 mm and a mean annual air temperature of 14.5 °C (Backes, 1999; Maluf, 2000). In the winter, minimum air temperature often reaches zero Celsius degree (Maluf, 2000).

**Data collection:** Samples were collected using the active search method (Crump and Scott Jr, 1994) in

October and November 2014, with samplings performed between 0900 and 1400 h.

The specimens captured were placed in plastic bags, identified, and kept in a hermetic cooled cage in order to reduce their physiological activities, thereby reducing the digestion process (Oliveira, 2014). Samples were collected under the collecting permit provided by the competent Federal Agency (SISBIO license # 45861-1). Later on the same day of capture, the frogs were euthanized with Xylocaine, fixed with 10% formaldehyde, and preserved in 70% alcohol in the Universidade do Vale do Rio dos Sinos Herpetological Collection (see Appendix). Afterwards, specimens were dissected to remove the gastrointestinal contents, which were maintained in alcohol 70% and sorted using a stereomicroscope. The gastrointestinal content of each specimen was considered as a sample. For each identified prey category we calculated the numeric and volumetric contribution, as well as the absolute and relative frequency of occurrence. Prey items were identified to the taxonomic levels of order or family, as for the case of ants. The food items had their volume (V) estimated as follow: each individual prey was macerated and evenly spread on a Petri dish, which was then disposed over a millimeter paper. The macerated prey was maintained at a regular height of 1 mm, and this value was multiplied by the area (in mm<sup>2</sup>) occupied by the prey in the millimeter paper (Oliveira *et al.*, 2015).

**Data analysis:** The assessment of the importance of each prey category in the diet was calculated by the Index of Relative Importance (IRI), using the following equation:  $IRI = (\%N + \%V) \%FO$ , where %N is the relative abundance of each prey category in the diet, %V is the relative volumetric contribution of the prey in the diet, and %FO is its relative frequency of occurrence in the diet (Pinkas *et al.*, 1971; Krebs, 1999). The higher the IRI value, the greater is the importance of a given prey category in the diet.

To analyze the dimension of the trophic niches we calculated the Levin's Standardized Niche Breadth Index (B<sub>st</sub>) (Krebs, 1999), which allows comparing the degree of feeding specialization between species. The index ranges from 0 to 1, and is calculated according to the following equation:  $B_{st} = (B-1) / (n-1)$ , where n is the number of resources registered in the diet (prey categories), and  $B = 1 / \sum p_i^2$ , p represents the proportion of individuals of a given prey category (i) found in the diet. Values near 0 indicate a specialist diet (narrow niche breadth), while values near 1 indicate a generalist diet (wide niche breadth).

To analyze if the trophic niche overlaps between the species, with regards to the degree of similarity between their diets, we used the Trophic Niche Overlap Index of Pianka ( $O_{jk}$ ) (Pianka, 1973), defined by the following equation:

$$O_{jk} = \frac{\sum_{n=1}^o p_{ij} \times p_{ik}}{\sqrt{\sum_{n=1}^o p_{ij}^2 \times \sum_{n=1}^o p_{ik}^2}}$$

where  $O_{jk}$  is the niche overlap index between the species  $j$  and  $k$ ;  $p_{ij}$  is equivalent to the proportion of the resource type  $i$  relative to the total of resources used by the species  $j$ ;  $p_{ik}$  is the proportion of resource  $i$  relative to the total of resources used by the species  $k$ ; and  $n$  is the total number of resource categories used by the species  $j$  and  $k$ . The index ranges from 0 to 1, when there is no overlap or a complete overlap between the species diets, respectively (Krebs, 1999).

## Results

We analyzed the gastrointestinal contents from 19 individuals of *Physalaemus gracilis* and 64 individuals of *Physalaemus lisei*, totaling 83 individuals; from the total, only one individual of *P. lisei* had no gut content. We identified 12 prey categories for *P. gracilis* and 19 for *P. lisei* (Table I). Overall, the IRI showed that for both *P. gracilis* and *P. lisei* the most important prey category was Formicidae (IRI = 6469.9 and IRI = 4522, respectively). Two other important prey categories were Araneae and Coleoptera, though Coleoptera was more important for *P. gracilis* (IRI = 1722.5) than for *P. lisei* (IRI = 720.9). On the other hand, Araneae was more important for *P. lisei* (IRI = 868.7) than for *P. gracilis* (IRI = 623.3) (Table I). Seven prey categories were consumed exclusively by *P. lisei* (Isoptera, Blattodea, Collembola-larvae, Diplopoda, Chilopoda, Diptera and Orthoptera), while only one prey category was exclusively consumed by *P. gracilis* (Escorpionida). Nevertheless, these prey were recorded infrequently, with most of them showing IRI values below 25, with the exception for Orthoptera (IRI = 67.2).

Although Formicidae presented the high value of IRI for both species, this category had low volumetric importance. On the other hand, Coleoptera was the most representative prey category for both species in volumetric terms (*P. lisei* = 20.7%; *P. gracilis* = 14.1%) (Table I). Furthermore, Formicidae had a volumetric contribution similar to that of Araneae, though the IRI of the latter was about 10 times lower than the IRI of Formicidae for *P. gracilis*, and five times lower for *P.*

*lisei* (Table I).

The trophic niche breadth was narrow and similar for both species (*P. gracilis*,  $B_{sta} = 0.15$ ; *P. lisei*,  $B_{sta} = 0.11$ ). Likewise, the Pianka Index of Niche Overlap indicated a high overlap in the diet of both species ( $O_{jk} = 0.98$ ).

## Discussion

*Physalaemus lisei* consumed 19 prey categories, whilst *P. gracilis* consumed 12, a pattern consistent with other studies recently conducted with these species. For example, Becker et al. (2007) recorded 18 prey categories in the diet of *P. lisei*, while Oliveira et al. (2015) found 13 for *P. gracilis*.

The diet of both species had Formicidae as the most important prey category. The importance of ants in the diet of species of *Physalaemus* was also observed in other regions of Brazil for *P. biligonigerus* (Oliveira et al., 2015), *P. cuvieri* (Santos et al., 2004) and *P. ephippifer* (Rodrigues and Santos-Costa, 2014), and in Argentina for *P. albonotatus* (Falico et al., 2012 b) and *P. riograndensis* (López et al., 2003). Ants tend to be unpalatable and of difficult metabolic assimilation to various predators (Hirai and Matsui, 2000), but some species of *Physalaemus* seem to consume ants often. This behavior may guarantee access to an abundant food source which is exploited by few predators (Clarke, 1974). Consumption of Formicidae has been linked to the alkaloid sequestration process in some amphibians (Saporito et al., 2004); however there is no evidence in the literature that such a process occurs in species of *Physalaemus*. We cannot exclude the possibility that the high frequency of Formicidae in the diet of both species may be due to an opportunistic feeding behavior related to the abundance of ants in the environment. The hypothesis of opportunistic predation of an abundant food resource was raised by Becker et al. (2007) when studying the diet of *P. lisei* in a locality near to the present work study site. According to Becker et al. (2007), the high environmental availability of ants caused the high frequency of these insects in the diet of *P. lisei*.

As we observed in the diet of *P. gracilis* and *P. lisei*, Coleoptera and Araneae were also considered important prey to several Neotropical species of Hylidae (Miranda et al., 2006; Rosa et al., 2011), Bufonidae (Duré et al., 2009) and other Leptodactylidae (Maneyro et al., 2004; Oliveira et al., 2015). These taxa of prey can be preferentially consumed by these anurans, but alternative hypotheses should also be considered, including the one stating that the numerical dominance of beetles and

**Table 3.** Relation between parasite load and biometric parameters (Number of turtles=53).

Prey categories	<i>P. gracilis</i>							<i>P. lisei</i>						
	N	%N	V	%V	FO	%FO	IRI	N	%N	V	%V	FO	%FO	IRI
Araneae	9	7.8	192	6.9	8	42.1	623.3	42	10.2	404	8.9	29	45.3	868.7
Acarina	1	0.9	1	0.003	1	5.3	4.8	40	9.8	5	0.1	17	26.6	262.1
Opilionida	1	0.9	35	1.3	1	5.3	11.3	1	0.2	40	0.9	1	1.6	1.8
Escorpionida	1	0.9	100	3.6	1	5.3	23.7	0	0	0	0	0	0	0
Hymenoptera	8	6.9	47	1.7	2	10.5	91.2	9	2.2	24	0.5	1	1.6	4.3
Formicidae	65	56.5	225	8.2	19	100	6469.8	225	54.9	427	9.4	45	70.3	4522.1
Coleoptera	18	15.6	570	20.7	9	47.4	1722.5	28	6.8	640	14.1	22	34.4	720.9
Coleoptera-larvae	0	0	0	0	0	0	0	5	1.2	195	4.3	3	4.7	25.9
Hemiptera	4	3.5	457	16.6	4	21.1	422.8	11	2.7	401	8.9	8	12.5	144.3
Dermaptera	1	0.9	60	2.2	1	5.3	16.1	1	0.2	25	0.6	1	1.6	1.2
Lepidoptera	2	1.7	94	3.4	1	5.3	27.1	2	0.5	1	0.02	2	3.1	1.6
Lepidoptera-larvae	2	1.7	55	2	2	10.5	39.3	1	0.2	8	0.2	1	1.6	0.7
Isoptera	0	0	0	0	0	0	0	3	0.7	6	0.1	1	1.6	1.3
Blattodea	0	0	0	0	0	0	0	2	0.5	50	1.1	2	3.1	5
Isopoda	3	2.6	65	2.4	1	5.3	26.2	20	4.9	311	6.9	16	25	293.8
Collembola-larvae	0	0	0	0	0	0	0	1	0.2	1	0.02	1	1.6	0.4
Diplopoda	0	0	0	0	0	0	0	2	0.5	49	1.1	2	3.1	4.9
Chilopoda	0	0	0	0	0	0	0	1	0.2	8	0.2	1	1.6	0.7
Diptera	0	0	0	0	0	0	0	5	1.2	28	0.6	3	4.7	8.6
Orthoptera	0	0	0	0	0	0	0	11	2.7	95	2.1	9	14.1	67.2
Plant material	-	-	26	0.9	-	-	-	-	-	29	0.6	-	-	-
Other	-	-	825	30	-	-	-	-	-	1778	39.3	-	-	-
<b>Total of categories</b>					12							19		

spiders would favor their consumption (Baretta, 2007). Moreover, not only the abundance of these preys would favor their predation; in addition, the high mobility in the soil by beetles and many spiders could also favor their encounter and consumption by frogs (Oliveira, 2014). The importance of spiders has been reported for the diet of other species of *Physalaemus*, including *P. ephippifer* (Rodrigues and Santos-Costa, 2014), *P. riograndensis* (López et al., 2003) and also *P. lisei*, (Becker et al., 2007). However, for *P. albonotatus* (Falico et al., 2012 b), *P. biligonigerus* (Oliveira et al. 2015), *P. cicada* (Santana and Juncá, 2007), and for at least a population of *P. gracilis* from southern Brazil (Oliveira et al., 2015) the spiders did not represent a major prey category in diet. Some studies suggest that species of *Physalaemus* often consume a wide variety of prey, though with most prey categories having little importance in the diet composition. In fact, it is more common that only two or three prey categories show a high importance in the diet of these frogs (López et al., 2005; Falico et al., 2012b). Moreover, the diet composition tends to vary among

populations, generally reflecting the most abundant arthropods in the environment (Falico et al., 2012b), thereby suggesting an opportunistic feeding behavior in some anurans (Falico et al., 2012a).

The trophic niche breadth did not differ between the two species (*P. gracilis*, Bsta = 0.15; *P. lisei*, Bsta = 0.11). These values are similar to the value found for *P. ephippifer* (Bsta = 0.19) (Rodrigues and Santos-Costa, 2014), but higher than that reported for *P. biligonigerus* (Bsta = 0.04) (Oliveira et al., 2015). Although relatively low, we believe that these values do not indicate that *P. gracilis* or *P. lisei* are specialized in any prey category; instead, we argue that their narrow trophic niche is the result of preferential predation of a small prey group that is highly available in the habitat (Oliveira et al., 2015). According to the optimal foraging theory, generalist species may specialize their diet if some food items become more abundant than others (Pyke, 1984). The opportunistic behavior is reinforced because the trophic niche breadth of *P. gracilis* found in this study was lower than that registered for wetland populations

( $B_{sta} = 0.23$ ; Oliveira et al., 2015). Such an intraspecific variation in the diet amplitude has been observed for populations of *Dendropsophus nanus*, *Hypsiboas pulchellus*, *H. punctatus* and *Lysapsus limellum* (López et al., 2015), which suggests that these species have plastic feeding habits, i.e., they are capable to adjust their diet composition as a response to fluctuations in prey availability.

As expected, we observed a high dietary overlap between the studied species that likely exists due to their ecological similarities and phylogenetic proximity. However, Oliveira et al., (2015) reported a low overlap between the diet of sympatric populations of *P. gracilis* and *P. biligonigerus* ( $O_{jk} = 0.28$ ) in the wetlands of southern Brazil. Although they are congeners, the closer phylogenetic relationship between *P. lisei* and *P. gracilis*, in comparison to that between *P. gracilis* and *P. biligonigerus* (Lourenço et al., 2015), may explain the higher overlap between the diet of *P. lisei* and *P. gracilis*. However, the phylogenetic-based argument may be not as relevant if we consider the differences between the habitats in which these studies were conducted. While the present study occurred in a relatively stable forested habitat, Oliveira et al., (2015) evaluated the diet of frog species in wetlands, a type of habitat that undergoes seasonal changes depending on the flooding cycles. The dynamism of wetlands generates many different microhabitats that would be used differently by each species. Based on the relationship between the diet composition and the prey availability in the environment (Falico et al., 2012 b), we could expect that more dynamic habitats would favor more different diets, even between similar species. The high overlap between the diet of *P. lisei* and *P. gracilis* may be explained by similar patterns of microhabitat use (Becker et al., 2010; Kwet et al., 2010), which would be more pronounced in relatively stable habitats as the subtemperate forest where this study was conducted.

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### Appendix

The specimens used in this study are preserved at UNISINOS University (Universidade do Vale do Rio dos Sinos), in the Herpetological Collection of the Terrestrial Vertebrates Ecology Lab (CHLEVT). Here we provide the voucher number of each specimen.

*Physalaemus lisei*: 1017, 1026 to 1028, 1040, 1043, 1044, 1046 to 1051, 1053 to 1056, 1058 to 1063, 1065, 1067 to 1069, 1072, 1076, 1077, 1085, 1086, 1089 to 1092, 1098 to 1101, 1102 to 1105, 1110, 1112, 1113, 1116 to 1118, 1120 to 1133 and 1145

*Physalaemus gracilis*: 1023, 1025, 1033 to 1035, 1037, 1038, 1057, 1070, 1073, 1075, 1080 to 1083, 1087, 1088, 1095 and 1096.