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Source: South American Journal of Herpetology, 35(1): 23-31

Published By: Brazilian Society of Herpetology

URL: https://doi.org/10.2994/SAJH-D-24-00010.1

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New insights on the natural history of *Odontophrynus* asper (Phillippi, 1902) (Anura, Odontophrynidae): relating diet, sexual dimorphism, and longevity

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Abstract. Investigations into aspects of the life history of species such as sexual dimorphism, diet, and longevity are important for understanding the natural history of species. Here, we evaluate sexual size dimorphism and sexual and ontogenetic variation in the diet of the *Odontophrynus asper* in southern Brazil. We found that males are larger than females. Although there is no intersexual difference in diet, there is ontogenetic variation, with juveniles exhibiting a broader niche. Furthermore, males are longer-lived, living up to 8 years, while females live up to 6 years. Overall, eye—snout distance and femur and tibia-fibula length reflected age more accurately than body size and should be included in future studies.

Keywords. Anuran; Generalist; Morphometric characters; Ontogenetic; Skeletochronology.

INTRODUTION

Studies involving life history, such as sexual dimorphism, longevity, and diet, are crucial for understanding the role of a species in an ecosystem (Zug et al., 2001; Stark and Meiri, 2018; Ceron et al., 2022). In anurans, sexual dimorphism is exhibited by most species and is commonly associated with characters such as body size. Females are larger than males in approximately 90% of known anuran species, probably because of reproductive investment (Shine, 1979; Halliday and Verrell, 1988; Zhang and Lu, 2013). In species in which males are larger, dimorphism is often associated with physical combat behavior (Shine, 1979; Bell and Zamudio, 2012) or greater male longevity (Brum et al., 2020). In addition to size, intersexual differences in coloration and ornamentation are also common (e.g., presence of nuptial spines in males, coloration of the vocal sac; Cochran, 1955; Bell and Zamudio, 2012), as well as morphoanatomical characteristics such as mouth width and head size (Katsikaros and Shine, 1997; Arantes et al., 2015; Quiroga et al., 2015; Caldart et al., 2019).

The study of age in amphibians provides information on demographic parameters and life history traits such as growth rate, age at sexual maturity, and longevity (Duellman and Trueb, 1994). To estimate age, skeletochronology has been widely used in anurans. This technique is based on counting lines of arrested growth (LAGs) in long bones, which result from annual growth patterns (Halliday

and Verrell, 1988; Socha and Ogleiska, 2010). Although highly effective (Sinsch, 2015; Peng et al., 2022), studies using this technique are scarce, with only ca. 4% of anuran species having age data descriptions (Peng et al., 2022). Age in anurans is also correlated with diet composition, since feeding can differ between juveniles and adults (Maneyro et al., 2004). Furthermore, selection of food item size is also related to morphological differences between the sexes. Males and females have different energy needs (Slatkin, 1984; Wu et al., 2005) and can present different foraging strategies (Brasileiro et al., 2010).

Odontophrynus asper (Philippi, 1902) is a widely distributed anuran species found in Brazil, Paraguay, Uruguay, and Argentina (Aquino et al., 2010; Rosset et al., 2022). Unlike other species of the genus (O. occidentalis [Berg, 1896]; O. maisuma Rosset, 2008; O. monachus Caramaschi and Napoli, 2012), no sexual size dimorphism was found in this species (Grenat et al., 2012; Brum et al., 2022a). This is likely because there is no difference in the age at sexual maturity between males and females (Brum et al., 2020). The diet of O. asper mainly comprises Isopoda, Coleoptera, and Lepidoptera (Machado et al., 2019). However, no study has evaluated sexual and ontogenetic differences in diet related to variation in morphoanatomical characteristics. Therefore, our objectives were to (i) investigate ontogenetic variation in the diet and morphoanatomical characteristics of O. asper, and (ii) analyze sexual size dimorphism and its correlation with diet and age of sexual maturity.

How to cite this article: Miolo L.B., Brum A.J.C., Avila F.R., Tozetti A.M., Cechin S.Z.2025. New insights on the natural history of *Odontophrynus asper* (Phillippi, 1902) (Anura, Odontophrynidae): relating diet, sexual dimorphism, and longevity. *South American Journal of Herpetology* 35: 23–31. https://doi.org/10.2994/SAJH-D-24-00010.1

Submitted: 26 July 2024 Accepted: 24 February 2025 Available Online: 22 August 2025 Handling Editor: Rafael Omar de Sá DOI: https://doi.org/10.2994/SAJH-D-24-00010.1

MATERIALS AND METHODS

Data collection

A total of 100 specimens of *Odontophrynus asper* (26 males, 38 females, and 36 juveniles) were analyzed. All specimens were collected in the state of Rio Grande do Sul, Brazil (Fig. 1), including 54 deposited in the Herpetological Collection of the Federal University of Santa Maria (UFSM) and 46 in the collection of the University of Vale do Rio



Figure 1. Sites where *Odontophrynus asper* specimens were collected in the state of Rio Grande do Sul, Brazil.

dos Sinos (UNISINOS). Specimens examined are listed in the Appendix. Due to the small number of specimens, we treated all individuals as being from the same population. Information on the diet of 20 individuals from a previously published study by Machado et al. (2019) was used for comparison. Individuals from the UFSM herpetological collection were identified as adults when gametes were available in their reproductive tracts (analysis previously carried out by Brum et al., 2020). Individuals from the UNISINOS collection were identified as adults based on the presence of secondary sexual characteristics.

Sexual dimorphism

Sex and body mass (measured using a precision scale of 0.0001 g; Shimadzu UniBloc AUY-220) were recorded for all individuals. Ten measurements were taken (Freitas et al., 2008; Fig. 2A; Table 1): snout–vent length (SVL), mouth width (MW), head length (HL), head height (HH), distance between the eye and nostril (EN), distance between the eye and snout (ESD), radius and ulna length (RUL), humerus length (HUL), femur length (FL), and tibia and fibula length (TBL).

Age

Ontogenetic stage was determined through skele-tochronological analysis of the penultimate phalanx of the third and fourth toes of the right hindlimb (Fig. 2B). Histological processing following Caputo et al. (2010), namley decalcification in 10% EDTA, cross sectioning at

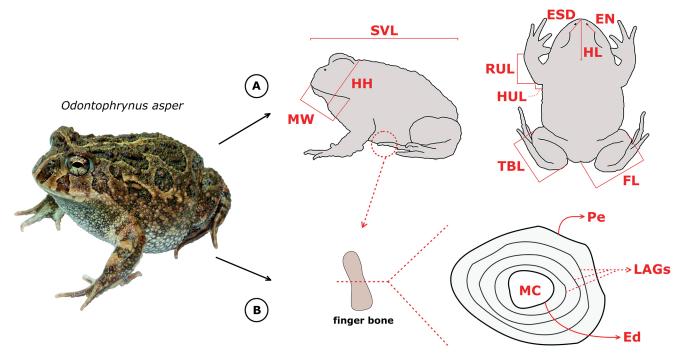


Figure 2. (A) Morphoanatomical measurements taken for each specimen of *Odontophrynus asper*. (B) Representation of the histological sectioning of the *O. asper* phalanx. SVL = snout—vent length; MW = mouth width; HL = head length; HH = head height; EN = eye—nostril distance; ESD = eye—snout distance; RUL = radius-ulna length; HUL = humerus length; FL = femur length; TBL = tibia-fibula length; MC = medullary canal; Pe = periosteum; Ed = endosteum; LAGs = lines of arrested growth.

Table 1. Morphometric measurements of male, female, and juvenile *Odontophrynus asper*. Linear measurements are given in millimeters and body mass in grams. Data are expressed by $\bar{x} \pm SD$. n = sample size; BM = body mass; SVL = snout vent-length; MW = mouth width; HL = head length; HH = head height; EN = eye—nostril distance; ESD = eye—snout distance; RUL = radius-ulna length; HUL = humerus length; FL = femur length; TBL = tibia-fibula length.

Variable	Male	Female	Juvenile	
n	26	38	36	
BM	10.73 ± 4.12	9.86 ± 4.60	1.76 ± 0.51	
SVL	41.62 ± 5.58	40.42 ± 5.89	22.22 ± 2.89	
MW	18.03 ± 2.67	17.59 ± 2.33	9.64 ± 1.58	
HL	5.38 ± 0.72	5.09 ± 1.36	2.76 ± 0.35	
НН	11.27 ± 2.14	10.51 ± 2.00	5.56 ± 0.88	
EN	5.88 ± 0.81	5.82 ± 1.04	3.03 ± 0.68	
ESD	7.54 ± 1.01	7.54 ± 1.22	3.91 ± 0.69	
RUL	9.11 ± 1.53	9.47 ± 1.57	5.31 ± 0.75	
HUL	12.33 ± 1.81	12.52 ± 1.99	6.93 ± 1.11	
FL	14.91 ± 2.45	15.33 ± 2.59	7.89 ± 1.388	
TBL	14.82 ± 1.84	14.45 ± 2.18	7.75 ± 1.05	

 $4~\mu m$ using a rotary microtome (Leica RM2245), and staining with toluidine blue. Four sections from the diaphyse-al region were obtained to count LAGs and estimate age (Castanet et al., 1993; Castanet et al., 2003). Bone resorption was determined based on the absence of remaining cartilaginous tissue from the larval cartilage, situated between the bone tissue and the endosteum (Rozenblut and Ogielska, 2005).

Diet

Specimens were dissected to remove the gastrointestinal contents (stomach and intestines), which were preserved in 70% ethanol for analysis. With the assistance of a stereomicroscope, we performed taxonomic identification of the contents to order. For each recognized prey category, the numerical quantity (N), volume (P), absolute frequency (% F), were determined. N is defined as the total number of individuals found of each prey category, while %F is defined as the relative frequency of prey occurrence. To calculate P, each prey category was mashed and spread in a Petri dish, maintained at a consistent height of 1 mm. This value was then multiplied by the area (in mm²) occupied by each prey category (Oliveira et al., 2015).

Statistical analysis

To assess the presence of sexual dimorphism in morphological characters, we first estimated the sexual dimorphism index (SDI), following Lovich and Gibbons (1992), calculated as follows: SDI = (mean length of the larger sex / mean length of the smaller sex) \pm 1; \pm 1 if males are larger or \pm 1 if females are larger. According to this index, sexual size dimorphism (SSD) = 0 if the sexes are equal in size, \pm 0 if males are larger, and \pm 0 if females are larger. To investigate sex differences in male and female size and morphology, we performed principal component analysis

(PCA) using the correlation matrix for a pooled dataset. The first PC calculated from a set of morphometric measurements is generally interpreted as an axis of body size variation when all traits load strongly and in the same direction (Reyment et al., 1984; Bookstein et al., 1985). The remaining variance describes the relative shape differences expressed in subsequent components (Schäuble, 2004). Next, we conducted a univariate analysis of covariance (ANCOVA), with sex as a factor and the PC1 score as the covariate (Guillaumet et al., 2005; Romano et al., 2009), for each morphological variable independently. To investigate the relationship between age and morphoanatomical characters, first we excluded the effect of body size on the morphometric variables, and then we used linear regressions for each sex and measure separately. To investigate the relative importance of each prey category for the species as a whole and for each sex, the index of relative importance (IRI) was calculated using the formula IRI = (% N + % V) % F, where % N = relative abundance of each prey in the diet; % V = percentage of volume of each prey in the diet; % F = relative frequency of prey occurrence. The higher the IRI value, the greater the importance of the prey in the diet. The level of dietary specialization was calculated using Levin's index of trophic niche width, defined as B = 1 / Σ pi^2, where p = proportion of individuals of a specific resource i (taxon) found in the diet. To investigate if there is an intersexual difference in the total volume of prey consumed, we used ANOVA. Linear regression analysis was employed to investigate the potential relationship between SVL and total prey volume, as well as the relationships between age and total prey volume. All analyses were conducted using the R programming language (R Core Team, 2022), with a significance level of P < 0.05. All variables were tested for normality using the Shapiro-Wilk test and log-transformed when necessary.

RESULTS

The SDI revealed that males were larger in size than females (SDI = 2.04). PC1 explained the majority of the overall data variation (Fig. 3; Table 2), with all variables pointing to the same direction on this component (negatively). Thus, individual PC1 scores were used to estimate differences in overall body size. No significant intersexual differences were found in any morphometric characters (Table 3).

The age of *Odontophrynus asper* was 0–8 years, with females of 1–6 years, males of 1–8 years, and juveniles from 0–1 years (Figs. 4 and 5). For males, a significant positive relationship was found between age and ESD (P=0.03, $R_{aj}=0.15$; Fig. 6A), FL (P=0.03, $R_{aj}=0.16$; Fig. 6B), and TBL (P=0.01, $R_{aj}=0.20$; Fig. 6C). No relationship was found between age and total prey volume (P=0.28, F = 1.244). Nonetheless, individuals with larger SVL (P=0.0005, F = 3.57) and body mass (P=0.0006, F = 3.57) consumed prey with a larger volume.

Gastrointestinal content was found in 76.76% of the analyzed specimens. A total of 238 prey items belonging

Table 2. First three principal component (PC) scores for the size-adjusted morphological variables, proportion of variance, and cumulative proportion of variance of male and female of *Odontophrynus asper*. MW = mouth width; HL = head length; HH = head height; EN = eye—nostril distance; ESD = eye—snout distance; RUL = radius-ulna length; HUL = humerus length; FL = femur length; TBL = tibia-fibula length.

Variables	Principal Components					
variables	PC1	PC2	PC3			
MW	-0.8695179	-0.8695179 -0.05752697				
HL	-0.6316814	0.47789281	-0.45206055			
нн	-0.7435344	0.37939929	-0.26398249			
EN	-0.7439720	0.27589175	0.55459308			
ESD	-0.8057277	0.32480863	0.37254889			
RUL	-0.7309137	-0.49966884	-0.02731859			
HUL	-0.8373478	-0.33940403	0.07780032			
FL	-0.8680015	-0.20988905	-0.23328165			
TBL	-0.9358491	-0.18092303	-0.00922354			
Proportion of Variance						
	64.1% 11.0% 8.7%					
Cumulative Proportion of variance						
	64.1% 75.2% 84%					

Table 3. Results of analysis of covariance (ANCOVA) with first principal component (PC1) scores as covariate tests for differences in morphological variables. None of the variables was statistically significant at P < 0.05. MW = mouth width; HL = head length; HH = head height; EN = eye-nostril distance; ESD = eye—snout distance; RUL = radius-ulna length; HUL = humerus length; FL = femur length; TBL = tibia-fibula length.

Variables	F	Р
MW	2.97	0.09
HL	2.66	0.10
НН	0.58	0.44
EN	0.003	0.96
ESD	0.07	0.78
RUL	0.04	0.83
HUL	0.81	0.37
FL	1.15	0.28
TBL	0.36	0.54

to 16 distinct categories were identified: Gastropoda, Araneae, Coleoptera, Hymenoptera, Orthoptera, Lepidoptera, Isopoda, Chilopoda, Diplopoda, Annelida, Blattodea, Diptera, Acari, Dermaptera, Neuroptera, and Odonata (Table 4).

Juveniles differed regarding the most important prey categories that were Coleoptera (IRI = 2,526.5), Isopoda (IRI = 551.18), and Gastropoda (IRI = 401.32). Furthermore, Diptera and Acari were only preyed on by juveniles (Tables S1–S3). According to the Levins' index of trophic niche width, the dietary specialization level of juveniles is more generalist (1.13) than males (0.61) and females (0.43). A statistically insignificant index was only found for males (P = 0.07). Differences in the total volume of prey consumed between sexes were evident (P = 0.001, F = 7.504), with females consuming a greater volume of prey.

DISCUSSION

Our results indicate that there is sexual dimorphism in *Odontophrynus asper*, with males being larger in body

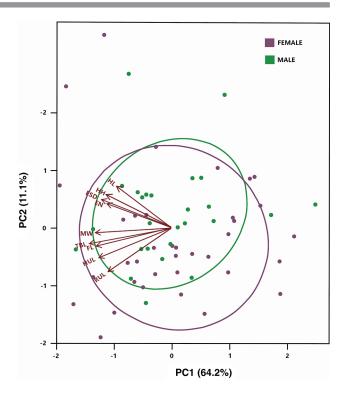


Figure 3. Principal component (PC) analysis calculated for all tested morphoanatomical measurements. MW = mouth width; HL = head length; HH = head height; EN = eye-nostril distance; ESD = eye-snout distance; RUL = radius-ulna length; HUL = humerus length; FL = femur length; TBL = tibia-fibula length.

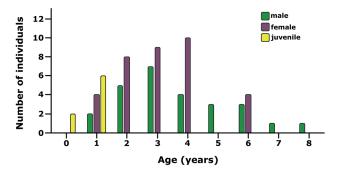


Figure 4. Age distribution frequency for males (green), females (purple), and juveniles (yellow) of *Odontophrynus asper*.

size than females. Although most anuran species exhibit sexual size dimorphism, which can be attributed to differences in age, growth rate, longevity, and reproductive investment of the species (Shine, 1979; Sinsch et al., 2010; Otero et al., 2017), previous studies did not demonstrate differences in body size between sexes for *O. asper* (Grenat et al., 2012; Brum et al., 2020). It is possible that this distinction was not observed previously because we analyzed a different population, thus highlighting possible interpopulational differences in the body size.

Male-biased sexual dimorphism is often associated with physical combat behavior and territoriality (Schäuble, 2004; Wells, 2007). However, there is no record of combat between males in *Odontophrynus asper*. Thus, we believe that this intersexual size distinction may be associated with greater agility in males during reproductive events and favored choice by females (Shine, 1979; Nali et al., 2014). In fact, the reproductive temporal pattern can directly

Table 4. Prey categories consumed by *Odontophrynus asper.* $n = \text{number of individuals; } V = \text{total volume of prey (mm}^3); F = \text{frequency of occurrence of each prey category; } IRI = \text{Index of relative importance; } (%) = \text{percentage values over total; } NI = \text{undetermined prey. } \text{Results presented in descending order of relative importance index.}$

Prey categories	N	%N	V	%V	F	%F	IRI
Coleoptera	85	35.714	5,050	19,444	36	45	2,481.93
Orthoptera	21	8.82	5,348	20,591	13	16.25	477.92
Isopoda	30	12.605	784	3,018	10	12.5	195.28
Lepidoptera	13	5.462	2,010	7,739	10	12.5	165.01
Hymenoptera	17	7.142	226	0,87	12	15	120.18
Gastropoda	15	6.302	1,159	5,871	4	5	60.86
Araneae	7	2.941	1,152	4,435	7	8.75	64.54
Annelida	12	5.042	1,159	4,462	4	5	47.51
Blattodea	12	5.042	277	1,066	6	7.5	45.81
Acari	9	3.781	28	0,106	5	6.25	24.28
Diptera	4	1.68	30	0,114	4	5	8.97
Dermaptera	4	1.68	230	0,885	2	2.5	6.41
Chilopoda	5	2.1	82	0,315	4	5	3.67
Diplopoda	2	0.84	114	0,438	2	2.5	3.19
Neuroptera	1	0.42	100	0,385	1	1.25	1.00
Odonata	1	0.42	25	0,09	1	1.25	0.63
NI			7,832	30,155	49	61.25	

influence the occurrence and intensity of body size selection (Wells, 1977). In species with explosive reproduction, such as *O. asper* (Achaval and Olmos, 2007), where there is a high density of individuals, larger males would have an advantage in actively searching for females and in displacing other males in amplexus, even when there is no territory defense or guarding of individual females (Wells, 1977; Herrel et al., 2012; Brum et al., 2020).

The relationship between body measurements and longevity in anurans is still uncertain. It is widely accepted that anurans increase in size as they age (Duellman and Trueb, 1994; Halliday and Verrell, 1988). Nonetheless, age is not the only factor that influences growth. In fact, studies using skeletochronology have shown that size can be highly variable (e.g., Baraquet et al., 2021; Brum et al., 2020, 2022). Latitude and environmental conditions also play a role, resulting in differences in size across populations (Khonsue et al., 2001; Wells, 2007) and making body size alone unreliable to assess age. Most studies that assess the relationship between body measurements and age use only SVL and body mass (Li et al., 2010; Otero et al., 2017; Baraquet et al., 2018), while other morphometric measurements remain understudied (Baraquet et al., 2021). Therefore, we recommend incorporating HL, HH, RUL, HUL, FL and TBL in future studies to allow better comparison.

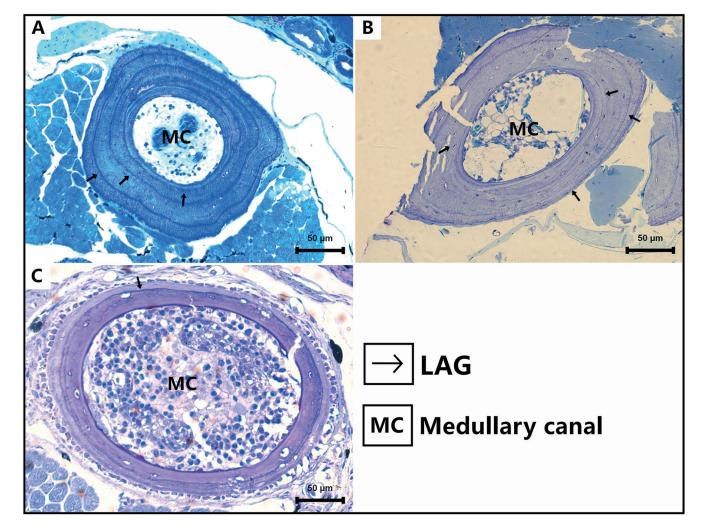
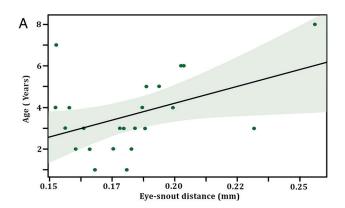
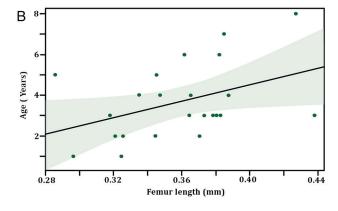


Figure 5. Cross-sections of the phalanges of Odontophrynus asper showing the LAGS (lines of arrested growth) of (A) a female, (B) a male, and (C) a juvenile.

We did not find any relationship between age and morphological characteristics in females, reinforcing that body size (i.e., SVL and other morphological variables) might be not good predictors of the age in Odontophrynus asper. However, for males, we observed that ESD, FL and TBL increases significantly with age; thus, older males have longer ESD and hindlimbs. The continuous growth of hindlimbs throughout could provide advantages to males during amplexus or a locomotor advantage in the search for breeding sites, especially for explosive reproducers like O. asper, in which males must reach breeding sites quickly (Wells, 2007; Herrel et al., 2012; Brum et al., 2020). Furthermore, an increase in the length of any element of the hindlimbs increases jumping capacity (Gomes et al., 2009; Citadini et al., 2018; Li et al., 2021). Jumping is associated with predator evasion (Heyer, 1978), which is more intense for males during the calling season (Woolbright,





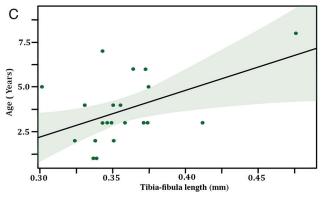


Figure 6. Relationship between male *Odontophrynus asper* age and **(A)** eye—snout distance, **(B)** femur length, and **(C)** tibia-fibula length.

1989; Wells, 2007). Thus, older males would have advantages over younger males in escaping predators.

Although female anurans typically have a longer lifespan than males (e.g., Woolbright, 1989; Guarino et al., 2003; Quiroga et al., 2015; Arantes et al., 2015; Otero et al., 2017), this pattern did not hold true for *Odontophrynus asper*. Our results indicate that males reached a longevity of 8 years and females six years, similar to the findings of Brum et al. (2020). This could be related to the high reproductive investment of females (e.g., size and number of ovarian follicles), which results in reduced adult survival (Pough et al., 1998; Brum et al., 2020). Furthermore, the greater male longevity could be related to a relationship between better escape ability and greater tibia length, resulting in a higher survival rate, as discussed previously.

According to Toft (1980), the dietary composition of anurans can be classified into three types depending on the proportion of different taxa they consume: ant specialists, non-ant specialists, and generalists. Odontophrynus asper can be classified as a generalist, as it consumes a wide diversity of taxa (16 orders). A wide niche breadth is shared by the majority of studied anurans (Duellman and Trueb, 1994; Wells, 2007). Among the consumed prey, we found high consumption of Coleoptera, similar to previous findings (Machado et al., 2019). This appears to be a trend among other odontophrynid species as well (Giaretta et al., 1998; Brito et al., 2012a, b; Almeida-Santos et al., 2017). However, both male and female adults consumed a significant amount of Orthoptera, differing from a previous study (Machado et al., 2019). Prey selection by anurans depends on prey availability and abundance, which can fluctuate throughout the year (Krebs and Davies, 1978; Duellman and Trueb, 1994; Hirai and Matsui, 1999), which could explain the observed divergence. Furthermore, generalist species can alter their diet if certain food items become more abundant than others, as explained by the optimal foraging theory (Pyke, 1984).

Ontogeny influences diet composition and trophic niche breadth of anurans (Hirai, 2002; Maneyro et al., 2004; Quiroga et al., 2009; Luría-Manzano and Bautista, 2019; Luría-Manzano et al., 2022). Although females and males consumed similar food items in our study, juveniles showed a wider niche breadth, including items that adults did not consume (i.e., Diptera and Acari). There was a clear trend of decreasing consumption of smaller prey items (e.g., Acari, Diptera, Isopoda, and Gastropoda) and an increase in the consumption of larger prey items (e.g., Coleoptera and Orthoptera) as individuals grew in size and body mass, an effect widely found in anurans (Peltzer and Lajmanovich, 1999; Hirai, 2002; Luría-Manzano and Bautista, 2019). These variations in diet between juveniles and adults can be explained by several factors. Juveniles could have morphological constraints limiting the size of prey they can consume (Lima and Moreira, 1993; Luría-Manzano and Bautista, 2019). Another possible explanation is differences in foraging method between juveniles and adults (Toft, 1980; Duellman and Trueb, 1994; Lima and Magnusson, 2000), as foraging method is not necessarily related to anuran body mass (Lima and Moreira, 1993). Overall, *Odontophrynus asper* is a generalist species that consumed most taxa across all age classes, albeit in different proportions. Hence, the relationships between morphology and diet seem more likely. A third possibility is that a diet that varies ontogenetically can help avoid intraspecific competition between juveniles and adults, expanding the trophic resource domain exploited by the population (Silva et al., 2021).

While we did not find sexual dimorphism in prey selection, we found that females feed on larger volumes of prey. This could be associated with the high reproductive investment in gonadal production by females, leading to higher energy demand and a preference for larger prey (Halliday and Verrell, 1988; Shine, 1979; Zhang and Lu, 2013).

Our study provided new information on the natural history of *Odontophrynus asper*, relating important aspects of the life history of the species, such as sexual dimorphism, diet and longevity. We report for the first time male-based sexual size dimorphism for the species, not found in previous studies, indicating that intersexual size varies among populations. In addition to being larger than females, our results also showed that males are older, and, as they age, they tend to increase the length of their hindlimbs. Furthermore, here we report ontogenetic variation dietary composition in O. asper, whereby young individuals are more generalist and feed on different prey than adults. In conclusion, the relationship between sexual size dimorphism and its relationship to age raises questions about the ecology and behavior of the species. Furthermore, studying the effects of ontogeny on diet and patterns in measurements of anurans will bring connections about the life history of the species, aiding in future phylogenetic studies and conservation measures.

ACKNOWLEDGMENTS

We thank Andressa Paladini for her assistance in the taxonomic identification of prey items, and our colleagues at the Herpetology Laboratory (UFSM) for all their help and suggestions on how to improve this work. We also thank the SAJH editor and reviewers who took the time to read our work and made excellent contributions, which certainly made the manuscript better. LBM is grateful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the research fellowship. SZC thanks the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), for a research fellowship (307135/2020-9).

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ONLINE SUPPORTING INFORMATION

The following Supporting Information is available for this article online:

- **Table S1.** Prey categories consumed by female *Odontophrynus asper* in southern Brazil.
- **Table S2.** Prey categories consumed by male *Odontophrynus asper* in southern Brazil.
- **Table S3.** Prey categories consumed by juvenile *Odontophrynus asper* in southern Brazil.

APPENDIX

Odontophrynus asper (n = 107): BRAZIL: **Rio Grande do Sul**: Agudo: ZUFSM 2283, 2491, 2493, 2495, 2496, 2497, 2591; Alegrete: ZUFSM 8505; Cachoeira do Sul: ZUFSM 6002; Candiota: ZUFSM 4867; Derrubadas: ZUFSM 4520, 4522, 4523, 4524; Dom Feliciano: ZUFSM 3039, 3040, 3055, 3056; Herval: ZUFSM 6054; Ibarama: ZUFSM 2258, 2301, 2304; Itaqui: ZUFSM 2412, 6049; Maçambará: ZUFSM 5534, 6314, 6315, 6325; Nova Palma: ZUFSM 2499; Pelotas: ZUFSM 3843; Porto Alegre: U 2151, 2162, 2163, 2168, 2184, 2193, 2197, 2198, 2199, 2201, 2204, 2206, 2209, 2212, 2215, 2217, 2219, 2220, 2221, 2224, 2225, 2227, 2231, 2232, 2233, 2235, 2254, 2255, 2257, 2258, 2262, 2265, 2267, 2268, 2270, 2271, 2272, 2273, 2275, 2276, 2277, 2278, 2283, 2284, 2285, 2309; Quaraí: ZUFSM 10389; Rolador: ZUFSM 4017; Roque Gonzales: ZUFSM 4025, 4026; Rosário do Sul: ZUFSM 8503; Santa Margarida do Sul: ZUFSM 8292; Santa Maria: ZUFSM 130, 1451, 1534, 1623, 1632, 1925; Santana do Livramento: ZUFSM 8447, 8487; Santo Antônio da Patrulha: ZUFSM 6030; São Francisco de Assis: ZUFSM 4291, 4462, 4463, 4464; São Gabriel: ZUFSM 4726, 4727, 4733, 4801, 9152, 9351, 9720; São Sepé: ZUFSM 9965; São Vicente do Sul: ZUFSM 11203, 11211, 11242, 11559.