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ORIGINAL ARTICLE

The diet of *Stereocyclops incrassatus* Cope, 1870 (Anura: Microhylidae) tadpoles in Atlantic Forest of southern Bahia, Brazil

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Abstract
Tadpole diet studies based in gut content analysis provide information on the diversity of prey consumed, foraging behavior and ecological function of tadpoles. Although trophic ecology studies have increased, tadpole diet is still understudied compared to the high diversity of anurans species, especially in some families such as Microhylidae, which present great morphological and ecological diversity. In this study we describe the diet of the tadpole of *Stereocyclops incrassatus*, from a population in southern Bahia, Brazil. Gut contents of 15 tadpoles were analyzed under light microscope (microscopic items) and stereoscopic microscope (macroscopical items). We found 14 categories of food items in the diet of the *S. incrassatus*, with detritus, composed primarily of degraded plant material, representing the most important food source (69%), followed by microalgae of the phylum Bacillariophyta (18%). The other items of the diet of *S. incrassatus* were composed mainly of Euglenophyta, Placidozoa, Testacea, Fungi, Nematoda, animal and protozoan fragments. Thus, tadpoles of *S. incrassatus* are classified as detritivorous, feeding especially on organic matter. The results of the present study show that suspension-feeder tadpoles besides being primary consumers are also detritivorous, and can play an important role in nutrient cycles in freshwater trophic webs.

Keywords Anuran larvae · Feeding ecology · Gut content analysis · Detritus · Microalgae

Introduction

Studies on feeding ecology help to understand the ecological function, resource partitioning, and trophic levels of consumers in a community (Paine 1980), and are essential to understand trophic interactions and the dynamics of food webs (Whiles and Altig 2010). Among the great diversity of species in continental aquatic environments, amphibian larvae (tadpoles) are among the least studied groups with respect to trophic relationships (Petranka and Kennedy 1999). Due to their high density, tadpoles exert great influence on the function and structure of aquatic ecosystems, shaping the density of algal communities and modifying primary production patterns and organic matter dynamics (e.g. Kupferberg 1997; Flecker et al. 1999). In addition, tadpoles serve as important food items for other consumers, participating directly in the transfer of energy and nutrients between aquatic and terrestrial ecosystems (Capps et al. 2015; Whiles et al. 2006).

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Although studies on the feeding ecology of tadpoles have increased in recent years (e.g. Asrafuzzaman et al. 2018; Mira-Mendes et al. 2020; Protázio et al. 2020), tadpoles diet is still understudied compared to the high diversity of anurans species (Altig et al. 2007; Frost 2021). A recent review on tadpole diets showed that most studies have been conducted on species from the families Hylidae, Bufonidae and Ranidae from Neotropic and Nearctic regions, while species in tropical regions lack trophic ecological data (Montaña et al. 2019). The Microhylidae family has a wide geographic distribution, and has one of the highest species richness among the anurans (> 740 spp.), standing out for its great morphological and ecological diversity (Frost 2023). However, studies of the tadpole trophic ecology of only eight species of microhylids are documented (Montaña et al. 2019). This gap may be related to the fossorial habit and the explosive breeding pattern in adults, observed in most species of microhylids (Duellman and Trueb 1994; Wells 2007), which cause tadpoles of this family to remain in aquatic environments for short periods.

Currently, Brazil is home to 59 species of microhylids, which represents 8% of the global diversity (Segalla et al. 2021). *Stereocyclops incrassatus* Cope, 1870 is distributed in the coastal forests of eastern Brazil from Northeast (Pernambuco Alagoas and Bahia) to Southeast Regions (Minas Gerais and Espírito Santo) (Frost 2023). Adults of this species can be found in the leaf-litter on the floor of primary and secondary forests, as well as in rubber and cocoa agroforestry systems, especially after periods of heavy rains (Dias et al. 2014; Mira-Mendes et al. 2018; Peixoto et al. 2010). It is an explosive breeder using temporary waters within or at the edge of forests, where the tadpoles develop. In this study we describe the diet composition of *S. incrassatus* tadpoles from a population in southern Bahia.

Materials and methods

Specimen collection and preparation

The tadpoles of *S. incrassatus* were collected on August 30, 2019 in a semi-permanent pond located in an agroforestry system, named "cabruca", in which cacao grows under the shade of native tree species [14°47'45.8"S, 39°10'20.9"W, 34 m above sea level], municipality of Ilhéus, state of Bahia, Brazil. The pond measured about 140 square meters, was ~ 1 m deep at the deepest area, and was mostly covered by aquatic vegetation (Fig. 1). Tadpoles were collected using a 20 cm diameter hand net with 2 mm steel mesh. They were immediately euthanized in 5% lidocaine solution and then preserved in 10% formalin. In order to identify the tadpoles, comparisons were made with specimens kept in the herpetological collection of Museu de Zoologia da Universidade Estadual de Santa Cruz, Ilhéus, State of Bahia, Brazil (MZUESC) and we consulted literature describing the tadpole species (Wogel et al. 2000; Dubeux et al. 2020). The tadpoles collected were between development stages 31 and 37 (Gosner 1960).

Fig. 1 A semi-permanent pond inside "cabruca" in the campus of the Universidade Estadual de Santa Cruz (UESC), municipality of Ilhéus, state of Bahia, Brazil

Diet analysis

We dissected digestive tracts of each specimen ($n = 15$) through a ventral incision. Then, we separated the first third of the intestine of each individual and placed the contents in a microtube with 2 mL of Transeau solution. The remaining part of the intestine was placed in another microtube in the same solution. Then, we homogenized the solution with the first third of the intestine, and with a plastic pipette we deposited approximately 0.5 mL of each sample on a glass slide of 75×24 mm covered with a coverslip (50×20 mm) for analysis under a light microscope. The items found in the entire area of glass slide were identified under a ZEISS Axio Scope light microscope. If there was any uncertainty during the identification process of an item, we consulted specific literature (Bicudo and Menezes 2017) and utilized a Canon Powershot G5 digital camera attached to a microscope to take a photograph of it, which was then sent to an expert (one of the authors, KPC). As suggested by Mira-Mendes et al. (2020) we analyzed the remaining intestinal content of each sample under a stereoscopic microscope in order to verify the presence of larger food items such as macroinvertebrates. For this purpose, the remaining intestinal contents were spread with 5 mL of water in a petri dish. The food items were identified at the lowest possible taxonomic level.

We calculated numeric percentage (NP), percentage of the frequency of occurrence (FO) and the importance index "I" proposed by Kawakami and Vazzoler (1980), with numerical percentage replaced by volume as suggested by Huckembeck et al. (2016):

$$I = F_i \times NP_i / \sum (F_i / NP_i) \times 100$$

where F*i* is the frequency of occurrence of item *i* in the sample; and NP*i* is the numeric percentage of the item.

Results

We found 2401 microscopic food items in the diet of the *S. incrassatus* tadpoles, distributed in 14 categories. Detritus, composed primarily of degraded plant material, was present in large amounts in all samples (N% = 64.5; FO% = 100), and represented the most important food source (I = 69.23). The other items were composed mainly of algae, although Placidozoa, Testacea, Fungi, Nematoda, animal and protozoan fragments were also found (Table 1).

After the detritus, microalgae of the phylum Bacillariophyta accounted for the highest numerical percentage (NP = 18.9%) and frequency of occurrence (FO = 93.3%), and also were the second most important food source (I = 18.98) to *S. incrassatus* tadpoles. Bacillariophyta was composed of seven families, and eight genera, of which *Gomphonema* and *Pinnularia* were the most representative, corresponding to 35.2 and 25.9% from the remaining genera of the phylum, respectively.

The importance index of food of Euglenophyta was 4.65, with a predominance of the genera *Trachelomonas* (NP = 52.5%) and *Phacus* (NP = 36%). Cyanobacteria (I = 3.37) was represented mostly by the genus *Oscillatoria* (NP = 77%). The other food items (Streptophyta, Chlorophyta, Ciliophora, Cryptophyta, Placidozoa, Testacea, Protozoa, Fungi, Nematoda and animal fragments), represent together the remaining value of the importance index of food (I = 3.77). We did not identify macroscopic food items in the diet of *S. incrassatus* tadpoles.

Discussion

Tadpoles are generally present in high densities in aquatic environments, usually have a high rate of ingestion of a variety of food items and can be considered the major consumers of freshwater aquatic ecosystems, particularly in temporary ponds (Alford 1999). Tadpoles of *S. incrassatus* are in the suspension-feeder ecomorphological guild (*sensu* Altig and Johnston 1989) by presenting the following characteristics: keratinized mouth parts absent, presence of tail flagellum; inhabit the water column; lateral eyes, body usually strongly depressed and low fins similar. Suspension-feeder tadpoles have been classified as primary consumers, as they occupy lower trophic levels (Altig et al. 2007; Whiles and Altig 2010; Montaña et al. 2019). Our results, based on the analysis of the tadpole gut contents, shows the diet of *S. incrassatus* is composed primarily of detritus (organic matter).

Detritus is frequently observed in the diet of tadpoles (Altig et al. 2007; Souza-Filho et al. 2007) and has been considered a primary food source for aquatic organisms in the trophic webs of several tropical freshwater ecosystems. Some studies have shown that the consumption by detritivorous tadpoles of the most nutrient-rich detritus fractions can increase their growth and survival rates (Kupferberg et al. 1994; Barrett et al. 2017). Furthermore, associated microbes and fungal biomass contribute significantly more to the nutritional value of detritus than the particles themselves (Cummins and Klug 1979; Methvin and Suberkropp 2003). Stable isotope analyses of some centrolenid tadpoles have corroborated this approach, indicating that they are primarily assimilating microbes rather than the detritus they consume (Whiles et al. 2006). Although it is difficult to differentiate between morphic (nutritionally poor) and amorphic detritus (nutritionally rich) in tadpole guts (Montãna et al. 2019), detritus may represent an important

Table 1 Microscopic diet of *Stereocyclops incrassatus* tadpoles

Food items	N	N%	FO	FO%	I
Bacillariophyta	**455**	**18.95**	**14**	**93.33**	**18.98**
Pinnulariaceae					
Pinnularia	118	4.91	8	53.33	
Gomphonemataceae					
Gomphonema	160	6.66	8	53.33	
Naviculaceae					
Navicula	21	0.87	2	13.33	
Eunotiaceae					
Eunotia	23	0.96	3	20.00	
Bacillariaceae					
Nitzschia	48	2.00	3	20.00	
Diadesmidaceae					
Luticola	13	0.54	1	6.67	
Diadesmis	5	0.21	1	6.67	
Sellaphoraceae					
Sellaphora	67	2.79	1	6.67	
Streptophyta	**47**	**1.96**	**8**	**53.33**	**1.12**
Zygnemataceae					
Mougeotia	26	1.08	3	20.00	
Closteriaceae					
Closterium	21	0.87	6	40.00	
Chlorophyta	**36**	**1.50**	**5**	**33.33**	**1.07**
Oedogoniaceae					
Oedogonium	13	0.54	3	20.00	
Chlamydomonadaceae					
Chlamydomonas	11	0.46	3	20.00	
Chlorophytes (NI)	12	0.50	5	33.33	
Ciliophora	**20**	**0.83**	**6**	**40.00**	**0.36**
Vorticellidae					
Vorticela	3	0.12	3	20.00	
Ciliates (NI)	17	0.71	3	20.00	
Cryptophyta	**2**	**0.08**	**1**	**6.67**	**0.01**
Cryptomonadaceae	2	0.08	1	6.67	
Cyanobacteria	**87**	**3.62**	**13**	**86.67**	**3.37**
Borziaceae					
Borzia	1	0.04	1	6.67	
Spirulinaceae					
Spirulina	14	0.58	5	33.33	
Oscillatoriaceae					
Oscillatoria	67	2.79	9	60.00	
Aphanizomenonaceae					
Dolichospermum	3	0.12	1	6.67	
Pseudanabaenaceae					
Pseudoanabaena	2	0.08	2	13.33	
Euglenophyta	**120**	**5.00**	**13**	**86.67**	**4.65**
Euglenaceae					
Trachelomonas	63	2.62	13	86.67	
Euglena	6	0.25	3	20.00	
Phacaceae					
Lepocinclis	15	0.62	5	33.33	
Phacus	36	1.50	12	80.00	
Placidozoa	**3**	**0.12**	**1**	**6.67**	**0.01**
Opalinidae					

Table 1 (continued)

Food items	N	N%	FO	FO%	I
Opalina	3	0.12	1	6.67	
Testacea	**27**	**1.12**	**7**	**46.67**	**0.56**
Arcellidae					
Arcella	7	0.29	3	20.00	
Lesquereusiidae					
Lesquereusia	2	0.08	1	6.67	
Trinematidae					
Trinema	12	0.50	6	40.00	
Euglyphidae					
Euglypha	6	0.25	3	20.00	
Protozoa	**18**	**0.75**	**1**	**6.67**	**0.05**
Fungi	**22**	**0.92**	**6**	**40.00**	**0.39**
Nematoda	**6**	**0.25**	**5**	**33.33**	**0.09**
Animal fragments	**9**	**0.37**	**4**	**26.67**	**0.11**
Detritus (organic matter)	**1549**	**64.51**	**15**	**100.00**	**69.23**

N = total number per item; NP (%) = numerical percentage; F = total frequency per item; FO = frequency of occurrence in percentage; I = importance index

item in the diet of *S. incrassatus* because it was present in large amounts in the gut of all examined tadpoles.

Most of the remaining items present in the diet of *S. incrassatus* tadpoles are periphytic taxa loosely attached to substrate or associated with the matrix of their own or other algae mucilage (e.g. *Pinnularia* spp., Bacillariophyta; *Mougeotia* spp., Streptophyta; *Oscillatoria* spp., Cyanophyta) (Burliga and Schwarzbold 2013). Although these organisms are not essentially planktonic, this means that depending on the hydrodynamics of the environment (e.g. shallower or deeper ponds), these taxa detach from the substrate and become available in the water column and can then be consumed by suspension-feeder species. Such hydrodynamic fluctuations often occur in shallow environments, as is the case in the semi-permanent pond where *S. incrassatus* tadpoles were collected. Furthermore, about 18% of the food items are represented by genera of flagellates or ciliates (e.g. *Chlamydomonas* spp., Chlorophyta; *Vorticela* spp., Ciliophora; *Trachelomonas* spp., Euglenophyta), which have structures that allow their movement in the water column for consumption by suspension-feeder species.

After detritus, Bacillariophyta, Cyanophyta and Euglenophyta also represented important items in the diet of *S. incrassatus* tadpoles. Algae are considered one of the most common food items present in the diet of tadpoles of different trophic guilds (e.g., benthic as *Macrogenioglottus alipioi* Carvalho, 1946, nektonic as *Scinax similis* (Cochran, 1952), suspension-rasper as *Pithecopus nordestinus* (Caramaschi, 2006)) because they represent a very abundant group in aquatic environments (Rossa-Feres et al. 2004; Mira-Mendes et al. 2020; Protázio et al. 2020). Some studies have demonstrated that the consumption of algae with diatoms epiphytes can accelerate the development and growth of tadpoles (e.g. Kupferberg et al. 1994; Kupferberg 1997). This is very important for microhylid tadpoles, which exhibit an explosive breeding behaviour and need to leave the ephemeral/temporary ponds in a short period of time. Studies describing the diet of microhylids tadpoles, such as *Elachistocleis bicolor* (Guérin-Méneville, 1838), *Dermatonotus muelleri* (Boettger, 1885), *Kaloula pulchra* Gray, 1831 and *Microhyla ornata* (Duméril and Bibron, 1841) (Rossa-Feres et al. 2004; Candioti 2007; Dey and Goswami 2015; Lalremsanga et al. 2017), showed that diatoms and filamentous algae also accounted for a significant proportion of consumed items.

Zooplankton items, composed of Placidozoa, Testacea, Protozoa (NI), Nematoda and Animal fragments parts, represented only 2.7 of the importance index and are of lesser importance in the diet of *S. incrassatus* tadpoles. The percentage of zooplankton items in the diet of other microhylid species is also low (e.g. Rossa-Feres et al. 2004; Echeverría et al. 2007; Asrafuzzaman et al. 2018). According to Altig et al. (2007) the presence of animal items in the diet of tadpoles can lead to an accelerated growth rate, even if in small proportions (Alford 1999).

Our results show that the *S. incrassatus* tadpoles can be classified as detritivores, feeding especially on organic matter. The results provided in the present study show that suspension-feeder tadpoles besides being primary consumers (Altig et al. 2007; Whiles and Altig 2010; Montaña et al. 2019) are also detritivores, and can play an important role in nutrient cycles in freshwater trophic webs. Gut content analysis studies are important because they provide information on the diversity of prey consumed, foraging behavior and ecological function of tadpoles. However, complementary techniques such as stable isotope analysis are essential to validate the results of gut contents studies and to provide a better understanding of the resources assimilated by tadpoles.

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Author Contribution Conceptualization: CVMM; Methodology: CVMM, EMSN, JC; Formal analysis and investigation: CVMM, EMSN, JBC, KPC, SMMSR, IRD; Writing - original draft preparation: CVMM; Supervision: CVMM, YLP. All authors approved the final version of the manuscript.

Declarations

Conflict of interest There are no conflicts of interest related to this work.

Ethics approval No approval of research ethics committees was required to accomplish the goals of this study because no experimental work was conducted. Tadpoles were collected under the permanent permit n 13708-1 issued to Mirco Solé by Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA) and Instituto Chico Mendes de Conservacão da Biodiversidade (ICMBio).

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