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Martins Soares, Claudemir; Hayashi, Carmino; Esper Faria-Soares, Anna Christina; Galdioli, Eliana Maria; Soares, Telma
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Diet of fish (Characiformes: Characidae) during early developmental stages in the Upper Paraná river basin, Brazil

Claudemir Martins Soares[1*], Carmino Hayashi[2], Anna Christina Esper Faria-Soares[3], Eliana Maria Galdioli[1] and Telma Soares[1]

*[1]Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura, Universidade Estadual de Maringá, Av. Colombo, 5790, bloco, G-80, sala 12, 87020-900, Maringá, Paraná, Brazil. [2]Departamento de Biologia, Universidade Estadual de Maringá, Maringá, Paraná, Brazil; [3]Programa de Pós-graduação em Análise Ambiental, Centro Universitário de Maringá, Maringá, Paraná, Brazil. *Author for correspondence. E-mail: cmsoares@uem.br*

ABSTRACT. This study analyzed the diet during early developmental stages of *Astyanax lacustris* (AA), *Piaractus mesopotamicus* (PM), *Megaleporinus obtusidens* (MO) and *Prochilodus lineatus* (PL), under experimental conditions. Fish larvae, 350 of each species, were stocked separately in 16 fiber-cement tanks (500 L), from which, three larvae of each species were collected every three days, for 36 days. Tanks were fertilized (1.5 g NPK-7: 14: 8) and had 50% of the surface with macrophytes. Larvae were grouped in age classes = I: 5 to 11, II: 14 to 20, III: 23 to 29 and IV: 32 to 38 days and, dissected for analysis of the digestive tract. Methodologies of dominance, frequency of occurrence and points of food items were used. For AA and PM larvae, measurements were taken for the number and size of organisms to determine the percent participation in biovolume. Changes in diets of larvae in different age classes were found as well as between different species, with the same age. Rotifers were dominant in the content of digestive tracts of all species at Class I, and the larvae diets became more distinct with increasing age, in which AA consumed mainly rotifers, PM, larger organisms (mainly cladocerans), PL, algae (diatoms as the main item) and PP with a more diversified diet (rotifers, ostracodes and algae). In conclusion, early stages of these species presented distinct diets, undergoing remarkable changes in the first 38 days of life.

Keywords: fish larval, stomach content, *Astyanax lacustris, Megaleporinus obtusidens*, *Piaractus mesopotamicus*, *Prochilodus lineatus*.

Alimentação de peixes (Characiformes: Charicidae) nas fases iniciais de desenvolvimento da bacia do Alto rio Paraná, Brasil

RESUMO. Estudou-se a alimentação dos estágios iniciais de desenvolvimento de *Astyanax lacustris* (AA), *Piaractus mesopotamicus* (PM), *Megaleporinus obtusidens* (MO) e *Prochilodus lineatus* (PL) em condições experimentais. As larvas, 350 cada espécie, foram estocadas separadamente, em 16 tanques de fibrocimento (500 L) dos quais foram coletados três indivíduos de cada um, a cada três dias, por 36 dias. Os tanques foram fertilizados (1,5 g de NPK-7:14:8) e tinham 50% da superfície com macrófitas. As larvas foram agrupadas em classes de idade = I: 5 a 11, II: 14 a 20, III: 23 a 29 e IV: 32 a 38 dias e, dissecadas para a abertura do trato digestório. Foram empregadas as metodologias de dominância, frequência de ocorrência e pontos dos itens alimentares. Para as larvas de AA e PM, foram contados e tomadas medidas das dimensões dos organismos para determinar a participação percentual do biovolume. Observaram-se mudanças nas dietas das larvas em diferentes classes de idade e também entre larvas de espécies diferentes, com mesma idade. Houve predomínio de rotíferos nos conteúdos dos tratos digestórios de todas as espécies na classe I, as dietas das larvas passaram a ser mais distintas com o incremento da idade, com AA consumindo principalmente rotíferos, PM organismos maiores (principalmente cladóceros), PL tendo algas (diatomáceas como item principal) e PP com uma dieta mais variada (rotíferos, ostracodes e algas). Conclui-se que as formas jovens destas espécies apresentaram dietas distintas, passando por nítidas alterações nos primeiros 38 dias de vida.

Palavras-chave: larva de peixe conteúdo estomacal, *Astyanax lacustris*, *Megaleporinus obtusidens*, *Piaractus mesopotamicus*, *Prochilodus lineatus*.

Introduction

Several fish species produce a large number of larvae in the breeding season, but survival is usually very low during this critical period. As a consequence, small changes in mortality rates can significantly affect recruitment of populations and this effect will be reflected in the subsequent years

(Gerking, 1994, Osse, van den Boogaard, van Snik, & van der Sluys, 1997; Sánchez-Velasco, 1998; Rabe & Brown, 2000; Bunnell, González & Stein, 2003). For many fish species, the breeding season and site occur simultaneously with high densities of zooplankton, which is the main food resource for their young forms (James, Pitchford, & Brindle., 2003; Bremigan & Stein, 1999; Sammons, Bettoli, & Greear, 2001). The survival of these young forms depends on environmental conditions, availability of adequate food, and other biotic interactions (Miller, Crowder, Rice, & Marschall, 1988; Goshorn & Epifanio, 1991; Meng & Orsi, 1991; Rabe & Brown, 2000). Rapid growth of larvae is an important parameter to reduce loss by predation. For this, it is important to have an adequate diet that contributes with the necessary energy (Hartig & Werner, 1987; Miller, Crowder, & Binkowski, 1990; Pedersen, 1997).

In terms of feeding, fish larvae are essentially a 'species apart' when compared to their adults. In fish, larvae and adult are often completely different from each other, and may even be considered ecospecies, presenting peculiarities as to the type of habitat, feeding and behavior (Nakatani, Baumgartner, & Cavichioli, 1997, Osse et al., 1997). Newly hatched larvae are so small and undeveloped that their feeding ecology is greatly differentiated from the feeding ecology of the same species for the rest of the life cycle (Gerking, 1994; Osse et al., 1997). The micro-habitat used by the early stages of fish and their response to environmental variations is generally different from juveniles and adults of the same species (Childs, Clarkson, & Robinson, 1998).

Although the larvae of most fish species are very small compared to their adults, the larvae of different species may present different ecological responses to changes in abiotic and biotic variables. Some, because of size at hatch, swimming ability and capacity to utilize available resources, are more vulnerable to hunger, while others are more affected by predation and other biological factors (Miller et al., 1988).

Changes in the preferential food items of young fish stages depending on the species and also between individuals of different sizes in the initial phase, as well as diet plasticity of larger individuals, are reported for *Colossoma macropomum* and hybrid of *C. macropomum* ♀ x *Piaractus mesopotamicus* ♂ by Sipaúba-Tavares (1993), for the *Rhinelepis aspera* by Soares, Hayashi, Furuya, and Maranhão (1997) and for species of the genus *Bryconamericus* by Borges, Assakawa, Cunha, Bialetzki, and Nakatani (2006), Suiberto, Galuch, Bialetzki, and Nakatani (2009).

Unlike marine fish species, freshwater fish larvae depend more on small cladocerans than copepods, and rotifers are also common as the first food (Gerking, 1994; Soares et al., 1997). Larvae of tropical freshwater fish generally use rotifers as the first food, consuming cladocerans, nauplii, copepods and insect larvae, and present greater dietary plasticity as they develop (Miller et al., 1990; Soares et al., 1997, Suiberto et al., 2009).

This study aimed to characterize and compare the natural diet of the Characidae fishes *Astyanax lacustris*, *Piaractus mesopotamicus*, *Megaleporinus obtusidens* and *Prochilodus lineatus* in different stages of early development under experimental conditions.

Material and methods

Larvae of *A. lacustris*, *P. mesopotamicus*, *M. obtusidens* and *P. lineatus* witch 48 to 72 hours after hatching, were stocked at a density of 350 individuals per fiber-cement tank with 500 L capacity; 16 tanks were used, four for each species, for 36 days, during January and February 2001. Larvae were obtained by induced breeding in commercial fish farms with matrices and reproducers originating from Paraná River basin.

Tanks, coated with non-toxic water-based paint, were previously washed and dried, exposed to the sun, for two days and received water from artesian wells and treated, using 15 mL 12.6% sodium thiosulphate to inactivate chlorine. Fertilization with 1.5 g of inorganic fertilizer (NPK-7: 14: 8) was performed with weekly applications of 0.75 g NPK to each of the tanks. Tanks were inoculated with water from lakes of the Paraná River basin, in the region of Porto Rico, State of Paraná, seven days before larval storage to allow the proliferation of planktonic organisms.

Tanks were covered with 50% shading screen to prevent predator action. Aquatic macrophytes were placed, water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes*) in a proportion of 1: 1 in the tanks covering half of the surface, using wires fixed to the edges to delimit this space.

Every three days, three larvae were collected from each tank. These were fixed in 4% neutral formalin solution. The collected larvae were grouped into four classes, according to age as follows: I: 5 to 11 days, II: 14 to 20 days, III: 23 to 29 days and IV: 32 to 38 days. Subsequently, larvae were measured for total length under a stereomicroscope with a millimeter eyepiece.

For the analysis of the consumed organisms, the digestive tracts of the young stages were removed under a stereoscopic microscope, at magnifications of 10 and 40 X, with the aid of stilettos. Subsequently, with the appropriately separated content, under optical microscopy, the material was analyzed for number, frequency of occurrence, points and dominance, according to Hynes (1950). Analyses of the total content of the digestive tract were performed, except for larvae at the post-flexion stage, from which only the contents of the half or the initial 2/3 of the digestive tract were analyzed because of the lower degree of digestion of the organisms in these portions. For de *A. lacustris* and *P. mesopotamicus* larvae, we counted the organisms present to evaluate the percent participation of food items.

In the point methodology, a scoring scale was established according to the visual volumetric participation of each food item. In this way, the following procedure was adopted: points 1, 2, 3 and 4 for organisms that had a reduced participation (less than 5%), low (5 to 10%), moderate (10 to 30%) and important (above 30%) participation in the total volume analyzed for each fish, respectively. As in the case of the young stages of *A. lacustris* and *P. mesopotamicus*, a total count of the organisms present in the analyzed portions can be performed, measurements were taken total length, height and width (excluding spines) of at least 30 individuals of each food-organism for the determination of participation in biovolume of these in the digestive tubes of these fish. For the calculation of biovolume of food items, we used the methodology proposed by Ruttner-Kolisko (1977).

The identification of food-organisms was performed according to Bicudo and Bicudo (1970) Hyno and Tundisi (1977), Pontin (1978), Lewis (1979), Needham and Needham (1982), Sendacz and Kubo (1982) and Picelli-Vicentim (1987).

Results

There were differences in diets of larvae of different species with the same age and changes in diets of larvae of the same species in different age classes (Figure 1). In the first age class (5 to 11 days), there was predominance of rotifers in the diets of all species, with dominance in most of the gastrointestinal tract of these species. There was a predominance of *Brachionus* sp. both in dominance (Figure 1) and percentage of estimated biomass (Figure 2), in the contents of larvae of *A. lacustris* and *P. mesopotamicus* at this stage. However, for larvae of *M. obtusidens* and *P. lineatus*, there was a greater dominance of smaller organisms, such as small rotifers (*Lecane bulla* and Bdelloidea) and algae like *Scenedesmus* sp. (Figure 1). Regarding the score of food items (Figure 3), *Brachionus* sp. had the highest frequency (score 4) for larvae of *A. lacustris* and *P. mesopotamicus*, followed by *L. bulla* and cladoceran *Macrothrix* sp., respectively. For *M. obtusidens* and *P. lineatus,* higher frequencies of higher scores were found for Bdelloidea and *L. bulla*, respectively. It is noteworthy that there have already been differences in the diet of these fish in their first days of life. In the second age class (14 to 20 days), a more significant participation of cladocerans (*Macrothrix* sp. and *Simocephalus* sp.) was verified for all larvae, and *Macrothrix* exhibited the highest percentage of dominance in the content of the digestive tract (Figure 1). Cladocerans received the highest frequency of highest scores (score 4) for *A. lacustris, P. mesopotamicus* and *P. lineatus* (Figure 3), whereas for *M. obtusidens*, *L. bulla* was the item with the highest frequency of high scores. In the contents of *A. lacustris*, there was a greater participation of Chironomidae larvae and ostracods in terms of both dominance and biomass (Figure 2). The reduced dominance of rotifers and higher frequencies of lower scores for these were more evidenced for *P. mesopotamicus* followed by *A. lacustris*.

In the third age class (27 to 30 days), there were higher percentages of dominance and biomass in the contents (Figures 1 and 2) and higher scores in a greater percentage of gastrointestinal contents (Figure 3) of rotifers (*Brachionus* and *L. bulla*) and cladocerans (*Macrothrix* sp. and *Simocephalus* sp.) for *A. lacustris* and *P. mesopotamicus*, respectively. For *M. obtusidens*, the highest percentages of dominance and score 4 were observed for *Brachionus* sp., ostracods and periphyton protozoa, while for *P. lineatus,* the predominance was observed, based on the dominance and high scores of diatoms (unicellular and filamentous) typical of the periphyton.

The young stages with 32 to 38 days of age (fourth phase) presented distinct differences in the diets with predominance of rotifers (mainly *L. bulla*), cladocerans and diatoms in terms of percentages obtained for dominance and high scores for the young forms of *A. lacustris*, *P. mesopotamicus* and *P. lineatus*, respectively. On the other hand, *M. obtusidens* presented a differentiated diet with greater dominance of rotifers, especially *Brachionus* sp., however, with relevant participation for ostracods, unicellular and filamentous diatoms. These differences in the food items found in the diets of young forms were also evident with respect to the score obtained by these items in the gastrointestinal contents (Figure 3).

In relation to the frequency of occurrence of food items (Figure 4) in the gastrointestinal contents, changes in fish diets are also found both for the age of the same species and for groups of different species with the same age.

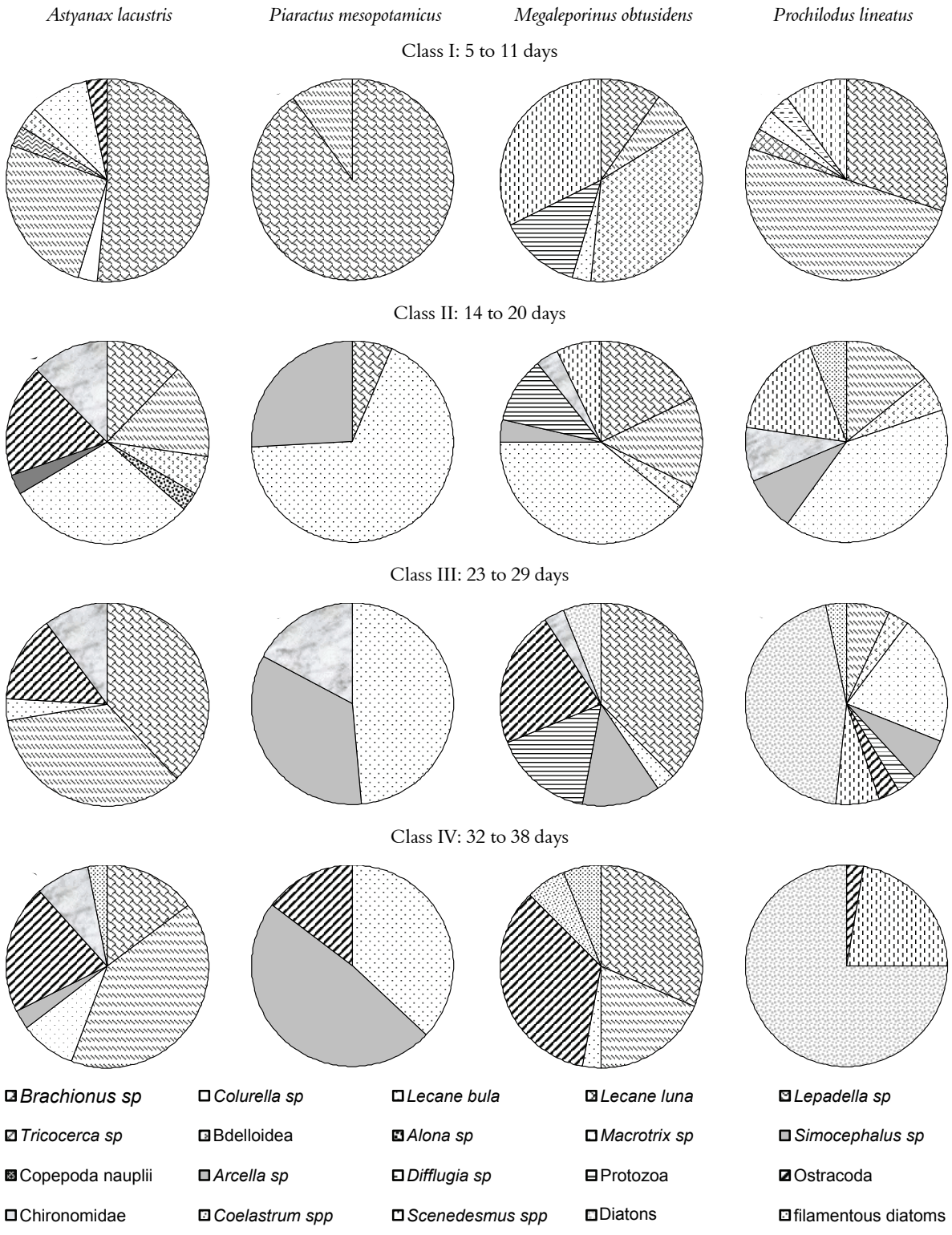

Figure 1. Percentages of dominant items in the gastrointestinal contents of young stages of *Astyanax lacustris*, *Piaractus mesopotamicus* *Megaleporinus obtusidens* and *Prochilodus lineatus* with different ages.

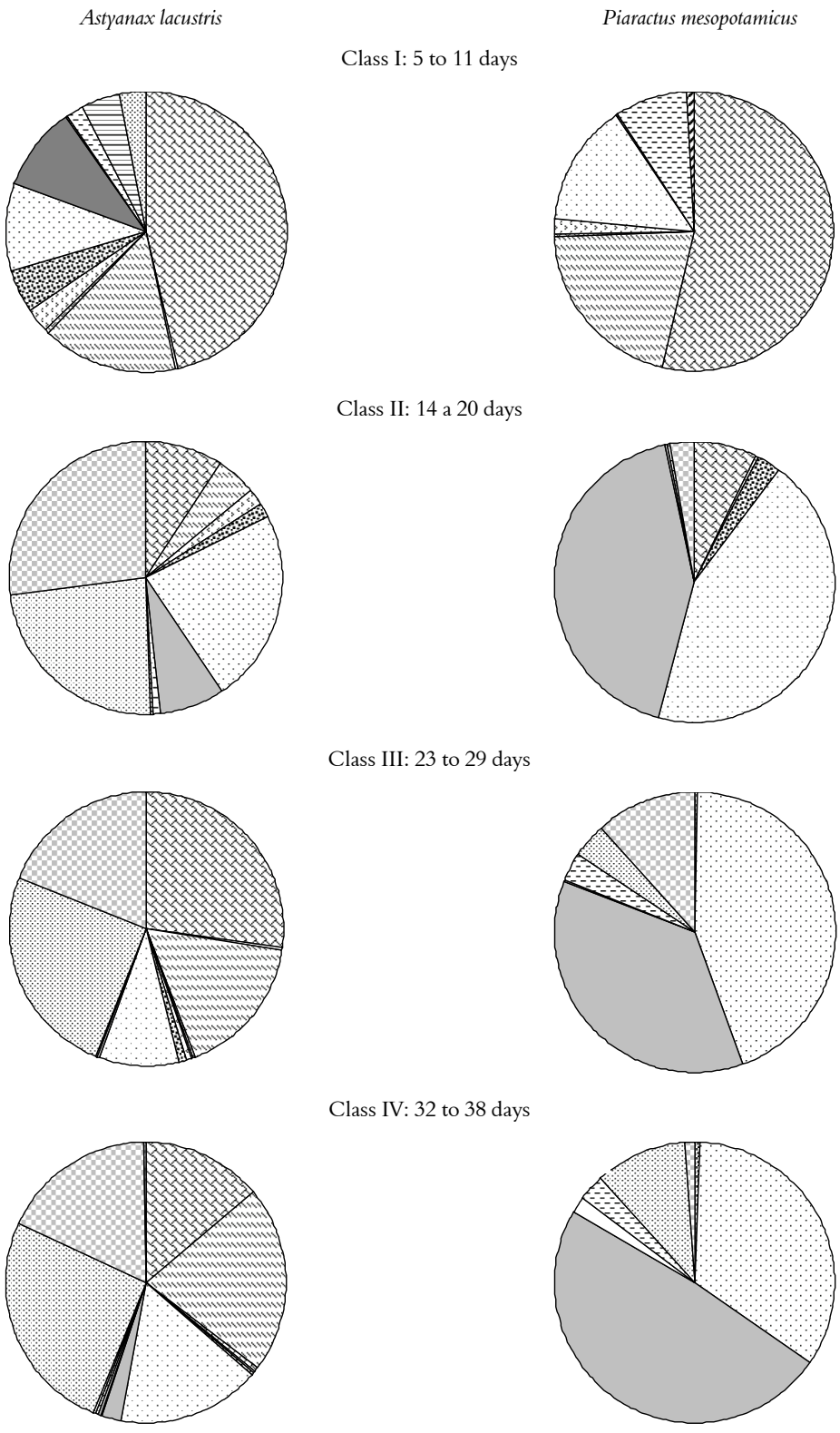

Figure 2. Percentages of estimated biovolume of items in the gastrointestinal contents of young stages of *Astyanax lacustris* and *Piaractus mesopotamicus* with different ages.

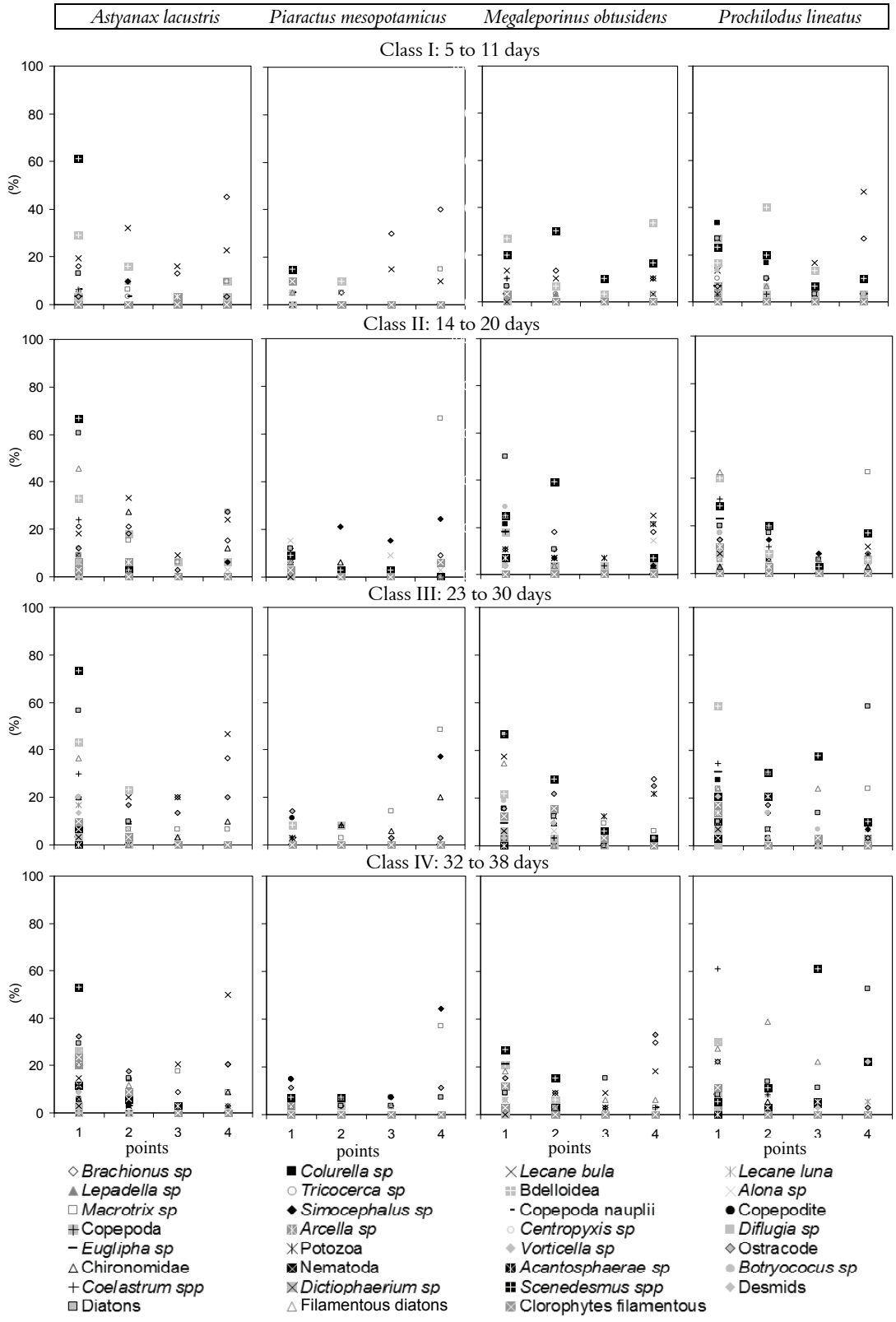

Figure 3. Percentages of scores of food items in the gastrointestinal contents of young stages of *Astyanax lacustris*, *Piaractus mesopotamicus*, *Megaleporinus obtusidens* and *Prochilodus lineatus* with different ages.

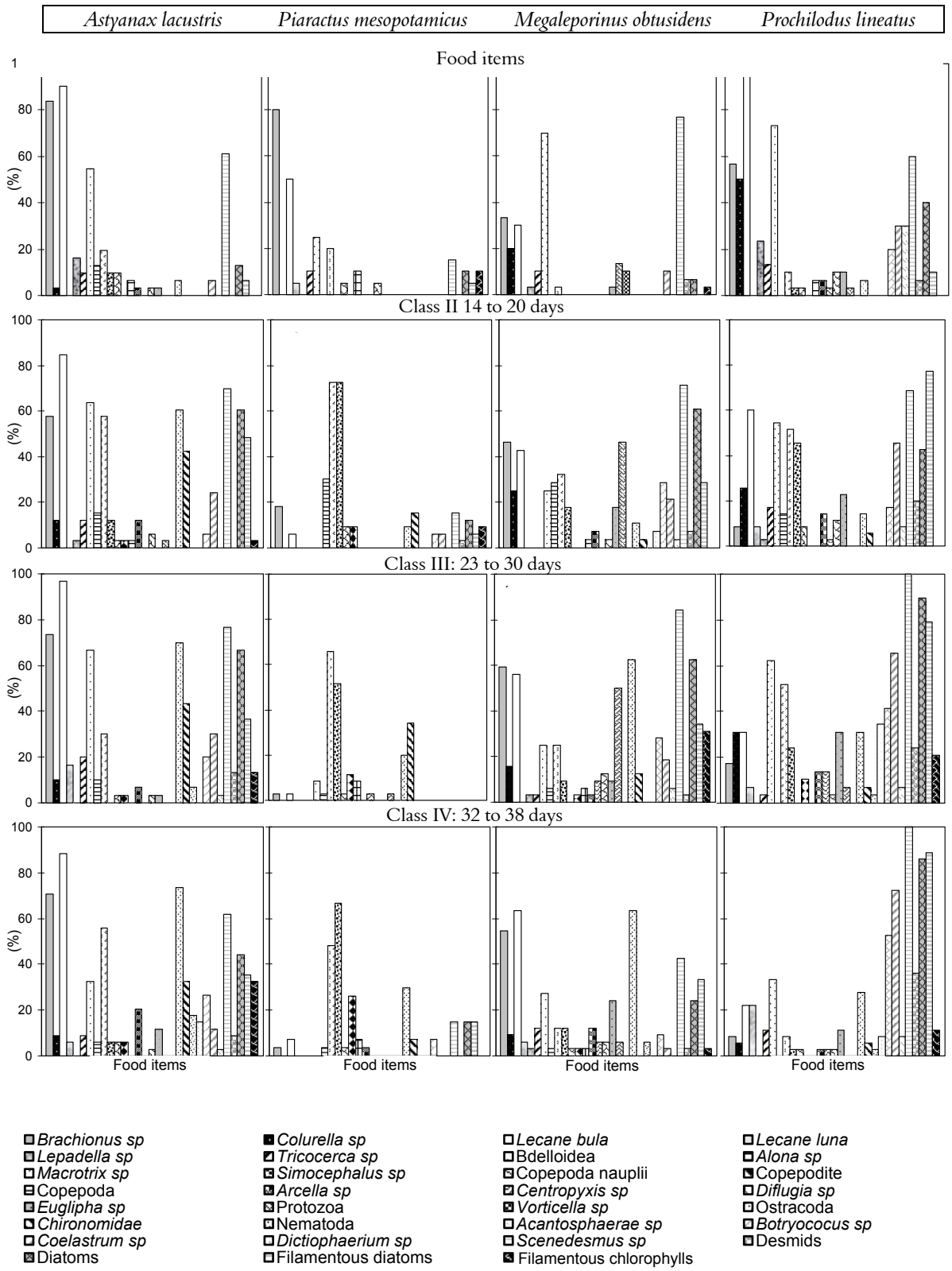

Figure 4. Frequency of occurrence of food items in the gastrointestinal contents of young stages of *Astyanax lacustris*, *Piaractus mesopotamicus*, *Megaleporinus obtusidens* and *Prochilodus lineatus* with different ages.

In the last age class (32 to 38 days), we observed a clear difference between the items with the highest percentages of occurrence in the gastrointestinal contents of *P. mesopotamicus* and *P. lineatus*, with the predominance of cladocerans and algae (*Coelastrum*, *Scenedesmus*, unicellular and filamentous diatoms), respectively. In some cases, especially for *A. lacustris*, in the last age group, although some items presented high frequency of occurrence, they do not stand out in terms of dominance and scores in the contents and this is because, although they were present in many of the contents of digestive tracts, they were recorded in small quantities.

Discussion

The finding of the greatest importance of rotifers and cladocerans in the first 20 days of the larvae of the four fish species of this study is in accordance with the report of Gerking (1994), who considers that zooplankton is the main food for fish larvae and that this initial 'carnivorous' habit also occurs with those species that, when adult, are herbivorous or omnivorous, as is the case of the target species of this study.

To *M. obtusidens* in this work, *L. bulla* was the most dominant item. Sipaúba-Tavares (1993), with tambaqui larvae (*Colossoma macropomum*) and tambacu (*C. macropomum* ♀ x *Piaractus mesopotamicus* ♂), observed a greater dominance of rotifers in the first six days of life. Bremigan and Stein (1999) emphasize the importance of small zooplankton for the recruitment of *Dorossoma cepedianum*, observing a higher number of larvae in the weekly cohorts of larvae during periods with high biomass of small zooplankton, which is evidenced for fish in this study, especially in the first days of life.

In the first class (5 to 11 days), there were higher values of frequency of occurrence of rotifers mainly for *A. lacustris* and *P. mesopotamicus*, although, for *P. lineatus*, algae had already presented high values of occurrence. In the second and third classes (14 to 20 and 23 to 29 days of life), there were changes with increase in the frequency of occurrence of algae, mainly with the young stages of *A. lacustris*, *M. obtusidens* and *P. lineatus*; and for *P. mesopotamicus*, there were higher values for cladocerans. Furuya, Hayashi, Furuya, and Sakaguti (2002), on *Pseudoplatystoma corruscans,* Borges et al. (2006) and Suiberto et al. (2009), on species of the genus *Bryconamericus*, observed an increase in the frequency of occurrence of larger items (copepods, ostracods and insect larvae) and reduction in the occurrence of smaller items (rotifers), according to increasing age of the post-larvae, which was also observed by Galdioli, Hayashi, Soares, and Marques (2017) for *Astyanax lacustris* at early developmental stages.

Changes in the most important items were observed in the diets with increasing age of the young stages of all the species of this study, and the differences regarding the preferential items for each species became more evident in older individuals. Similar results were obtained by Sipaúba-Tavares (1993), with larvae of *C. macropomum* and hybrids of *C. macropomum* x *P. mesopotamicus*, which prefer smaller organisms, mainly rotifers at the beginning of development, starting to consume larger organisms (cladocerans and copepods) as they grow. In studies with *A. lacustris*, Galdioli et al. (2017), there were changes in the diet of these fish as they develop, with preference for *Lecane* sp. during the first days of development.

The importance of algae and rotifers in the diet of young stages of periphyton-feeding fish was reported by Soares et al. (1997), with *Rhinelepis aspera*, during the first 32 days of life, where rotifers and *Scenedesmus* spp were the most important items in the diet of this species. Shifts from more frequent items, as the fish grow, as obtained for the young stages of *A. lacustris* and *P. mesopotamicus* in this study, with reduction in *Brachionus* and *Lecane* percentage, to the increase in consumption of larger organisms such as cladocerans, were registered for larvae of *I. labrosus* and *H. edentatus* by Makrakis et al. (2005).

Differences in the diets of these fries, whether intraspecific or interspecific, should be related to changes in the morphology of both the digestive tract, ie due to differences in the size of the mouth, in the form and function of the stomach and in the length of the intestine, which influence the capacity of capture, digestion and use of the different diets, such as those that confer swimming ability and visual acuity. These factors can lead to species segregation by food and micro habitats.

Conclusion

The early stages of *A. lacustris*, *P. mesopotamicus*, *M. obtusidens* and *P. lineatus* present distinct diets, undergoing clear changes in the first 38 days of life. Rotifers represent the main item for the young stages of all species studied, with predominance of *Brachionus* for *A. lacustris* and *P. mesopotamicus*. As larvae of *P. mesopotamicus* grow, they begin to consume larger organisms, especially cladocerans, while larvae of *A. lacustris* and *M. obtusidens* consume rotifers and ostracods and *P. lineatus* consumes principally periphyton diatoms.

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