



Influence of *Eichhornia crassipes* and *Lemna* sp. on the natural diet of *Astyanax altiparanae* Garutti & Britski, 2000 in the initial stages

Eliana Maria Galdioli^{1*}, Carmino Hayashi², Claudemir Martins Soares¹, Telma Soares³ and Liu Wolff⁴

¹Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura, Universidade Estadual de Maringá, Av. Colombo, 5790, Bloco G-80, 87020-900, Maringá, Paraná, Brazil. ²Departamento de Biologia, Universidade Estadual de Maringá, Maringá, Paraná, Brazil. ³Secretaria Estadual de Educação do Estado do Paraná, Pinhais, Paraná, Brazil. ⁴Escola de Educação Básica Alcuino Gonçalves Vieira, Camboriú, Santa Catarina, Brazil. *Author for correspondence. Email: emgaldioli@uem.br

ABSTRACT. Aiming to study the changes in the diet of *Astyanax altiparanae* in the early developmental stages, in the presence of *Eichhornia crassipes* (EC) and *Lemna* sp. (LM), larvae of *Astyanax altiparanae* were stocked in eight tanks of 900 L (500 ind. tank⁻¹), four with EC and four with LM covering 50% surface. Tanks were inoculated with plankton and received 3.0 g inorganic fertilizer (NPK-7:14:8) and 1.5 g every week. At every three days, three larvae were taken from each tank, fixed in 4% formaldehyde and grouped into four age classes (I: 6 to 12 days, II: 15 to 21 days, III: 24 to 30 days; IV: 33 to 39 days). The analysis of dominance and frequency of occurrence (FO) of food items indicated that, at this stage, *A. altiparanae* is generalist. In class I, there was a greater dominance and FO of *Lecane bulla* and *Alona* sp. in digestive tracts in the presence of both EC and LM, respectively; and larvae started to consume larger organisms, such as Chironomidae, in other age classes with EC. In the presence of LM, *Alona* sp. was dominant in class II; Chironomidae, in class III and *Centropyxis* sp., in class IV. Diatoms have greater FO both in EC and in LM. In class III, with EC, Chironomidae had higher FO, as well as *Arcella* sp. and *Scenedesmus* spp. with LM. The highest FO in class IV was found for *L. bulla* and *Scenedesmus* spp., with EC and LM, respectively. It can be concluded that EC and LM influenced the diet of *Astyanax altiparanae*, with alterations according to the availability of organisms and increase in age.

Keywords: fish larvae, floating macrophyte, plankton, larval development, diet.

Influência de *Eichhornia crassipes* e *Lemna* sp. na alimentação natural de *Astyanax altiparanae* Garutti & Britski, 2000 na fase inicial

RESUMO. Objetivou-se estudar mudanças da dieta nos estágios iniciais de desenvolvimento de *Astyanax altiparanae*, na presença de *Eichhornia crassipes* (EC) e *Lemna* sp. (LM). Larvas de *Astyanax altiparanae* foram estocadas em oito tanques de 900 L (500 ind. tanque⁻¹), sendo quatro com EC e quatro com LM cobrindo 50% da superfície destes. Os tanques foram inoculados com plâncton e receberam 3,0 g de fertilizante inorgânico (NPK-7:14:8) e 1,5 g semanalmente. A cada três dias foram coletadas três larvas de cada tanque, fixadas em formol a 4% e agrupadas em quatro classes de idade (I: 6 a 12 dias, II: 15 a 21 dias, III: 24 a 30 dias e IV: 33 a 39 dias). Relacionando a dominância e frequência de ocorrência (FO) dos itens alimentares verificou-se que, nesta fase, o *A. altiparanae* é generalista. Na classe I, houve maior dominância e FO de *Lecane bulla* e de *Alona* sp. nos tratos digestórios na presença de EC e LM, respectivamente, passando a consumir organismos maiores como Chironomidae nas demais classes com EC. Na presença de LM, *Alona* sp. foi dominante na classe II, Chironomidae na III e *Centropyxis* sp. na IV. As diatomáceas tiveram maior FO tanto em EC quanto em LM. Na classe III, com EC Chironomidae teve maior FO, assim como *Arcella* sp. e *Scenedesmus* spp. com LM. A maior FO na classe IV foi de *L. bulla* e *Scenedesmus* spp. com EC e LM, respectivamente. Concluiu-se que EC e LM influenciaram a dieta de *A. altiparanae*, alterando-a de acordo com a disponibilidade dos organismos e com o aumento da idade.

Palavras-chave: larva de peixe, macrófita flutuante, plâncton, desenvolvimento larval, alimentação.

Introduction

Fish hatchery, which sustains the large scale production of quality fingerlings, has faced many problems with frequent failures, and the food factor

is the most important (Cestarolli, Portella, & Rojas, 1997; Gerking, 1994).

The early stage of most fish is a period of rapid morpho-anatomical changes and during this life

cycle period they feed on plankton. Natural food is of paramount importance as it contributes with essential nutrients for growth, development and survival (Nikolsky, 1969; Mills, Widzowski, & Jones, 1987).

The shift from endogenous to exogenous feeding is a major difficulty faced in farming juveniles of rheophilic species (Gerking, 1994), as well as cannibalism that occurs in the first days of larval life (Feiden, Hayashi, & Boscolo, 2006). Therefore, studies on the diet during the early stages of fish development are of great importance to understand its role in the community, an opportunity to know one of the most critical steps for fish hatchery, which can help in the development of fish farming (Makrakis et al., 2005; Qu et al., 2012) and in determining priority areas for the maintenance of natural stocks.

Different fish species may have a preference for a certain type of food-organism, and feeding and sensory organs become adapted to it. This adaptation may be the result of morphological changes that occur in the course of development in fish (Nikolsky, 1969). In this way, the consumption of food-organisms can vary according to their size and the systematic group (Sipaúba-Tavares, Bachion, & Rocha, 1994).

Thus, it is required the availability of food of good nutritional quality, such as planktonic organisms in aquatic environment (Furuya, Hayashi, Furuya, Soares, & Galdioli, 1999; Gerking, 1994), for presenting all the essential dietary components. Protein, high content of free amino acids, fatty acids, enzymes and water are important for initial exogenous feeding of fish larvae, being necessary to ensure growth and high survival of juveniles during the initial stages (Herbert, 1978; Gerking, 1994).

Planas (1999) reports that the stomach content analysis is an important tool for understanding the aspects involved in the feeding of fish larvae. In order to understand the factors that lead to mortality of larvae in natural environment and its implications for the recruitment of populations, it is important to know their feeding habits (Goshorn & Epifanio, 1991; Sánchez-Velasco, 1998).

Because fish larvae are small and poorly developed, they have different feeding compared to the adult stage of the same species. Therefore, they are essentially treated as 'separate species' from their adult forms. This inequality between larvae and adult forms, in both morphology and physiology, leads to different feeding habits (Gerking, 1994; Osse, Van Den Boogaart, & Van Snik, 1997). In this way, they have different ecological requirements,

mainly regarding habitat, feeding and behavior. Most larvae of different fish species are planktivorous, primarily zooplanktivorous, even those herbivorous when adult. Therefore, these studies are essential to understanding the self-ecology and population dynamics (Gerking, 1994; Nakatani et al., 2001).

Fish assemblage is strongly influenced by the presence of aquatic macrophytes, in which they find suitable conditions for maintenance and, because of its structural complexity, there may be a greater species richness within the stands than outside of them. These plants can also serve as a means of transportation, promoting the dispersion of fish species. The fish fauna that lives among macrophyte stands is characterized by being small or juveniles of larger species, which generally need shelter from predators, places for feeding or breeding (Machado-Allison, 1990; Delariva, Agostinho, Nakatani, & Baumgartner, 1994; Scremin-Dias, Pott, Hora, & Souza, 1999). Macrophytes favor the growth of a great number of fish species, and represent important substrate for a wide array of planktonic organisms (Thomaz & Bini, 2003).

Floating aquatic macrophytes, as well as other types of macrophytes, due to their structural complexity, play an important role in the trophic structure of fish assemblages as they increase the availability of shelter for forage fish species and juveniles of large species, reducing mortality and influencing interspecific interactions (Mittlebach, 1981; Savino & Stein, 1982). Also, macrophytes provide substrate for the development of organisms used as food by most freshwater fish species, at least during the early stages of development, as well as serving as spawning sites (Iversen, Thorup, Hansen, Lodal, & Olsen, 1985; Dibble, Killgore, & Harrel, 1996).

Given the above, the present study aimed to analyze the natural diet of early developmental stages of *Astyanax altiparanae*, as well as possible dietary changes throughout the development, in the presence of *Eichhornia crassipes* and *Lemna* sp.

Material and methods

The experiment was conducted at the *Laboratório de Aquicultura, Departamento de Biologia, Universidade Estadual de Maringá*, state of Paraná, from January to March 2001, for a period of 40 days.

Aquatic macrophytes were obtained from stocks of the *Laboratório de Aquicultura*. This experiment used four tanks with *Eichhornia crassipes* (EC) and four with *Lemna* sp. (LM). These plants covered 50% of the surface of each fiber cement tank, delimited by a wooden structure with a length,

height and width of 110 x 6 x 2 cm, respectively, and attached at edges of the tanks.

Tanks, with individual working volume of 900 L water, were washed, dried and exposed to sunlight for three days. Subsequently, they were filled with water from an artesian well and SANEPAR - Sanitation Company of the state of Paraná, which was treated by adding 15 mL sodium thiosulfate to neutralize chlorine. The renewal of water was performed at 10% of the total volume per day with continuous circulation. The inlet and outlet of water were individual by means of taps and siphon at the bottom of the tank, respectively, on opposite sides.

For fertilization of tanks, it was initially applied 3.0 g inorganic fertilizer (NPK-7:14:8), and 1.5 g every seven days. For inoculation, we used 5 L wild plankton from eutrophic lakes and tanks. The initial inoculation was performed together with the first fertilization, seven days before larval introduction.

Along with the macrophytes, we stocked 500 larvae of *Astyanax altiparanae* per tank (density of 0.56 larvae L⁻¹). Larvae with 48-72 hours post-hatching were obtained by induced breeding in a Fish Farming Center, initially weighing, on average, 0.25 mg and with initial average length of 3.72 ± 0.23 mm.

An analytical scale accurate to 0.0001 g and a stereomicroscope with ocular micrometer were used to obtain the initial weight and length, respectively, in a sample of 79 fish larvae.

Every three days, three *A. altiparanae* individuals, in the early stage of development, were taken from each tank. These were fixed in 4% formaldehyde solution, buffered with calcium carbonate for analysis of the contents of digestive tracts. Larvae were grouped into four age classes (I: 6 to 12 days, II: 15 to 21 days, III: 24 to 30 days and IV: 33 to 39 days).

The removal of digestive tracts was performed under a stereomicroscope using a stylus. The content of the digestive tracts was analyzed and identified under an optical microscope. However, for fish at post-flexion stage (Ahlstrom & Ball, 1954), only the stomach content was examined. For analysis of digestive tract and stomach contents, we used the methodologies of dominance and frequency of occurrence, according to Hynes (1950), in order to determine the contribution of food items.

A food item (phytoplankton and zooplankton organisms) was considered dominant when presented visually larger volume in the stomach content of each fish. The frequency of occurrence of food items was determined by the number of stomachs containing a particular item in relation to the total number of stomachs.

The feeding strategy of *A. altiparanae* was evaluated using the Graphic Method of Amundsen,

Gabler, and Staldvik (1996) modified from Costello (1990), which measures the tendency of a species to a generalist or specialist diet, at the individual and population level, using the relationship between Dominance (Bennemann, Casatti, & Oliveira, 2006) (expressed as the percentage of the number of times the item occupies most of the content of each stomach according to the total number of analyzed individuals) and Frequency of Occurrence. Food items were determined from the identification of the most dominant and frequent items; for this, dominance (y-axis) of each item was plotted as a function of frequency of occurrence (x-axis).

The identification of phytoplankton and zooplankton was supported by the studies of Bicudo & Bicudo (1969, 1970), Hino and Tundisi (1977), Koste (1978), Pontin (1978), Lewis Jr. (1979), Needham and Needham (1982), Sendacz and Kubo (1982), Matsumura-Tundisi and Rocha (1983), Picelli-Vicentim (1987), Xavier (1993), Huszar, Werneck, and Esteves (1994), Oliveira, Train, and Rodrigues (1994) and Segers (1995).

Results

The percentage values of dominance and frequency of occurrence in the contents of the digestive tracts of *Astyanax altiparanae*, when represented by the Graphic Method of Amundsen, et al. (1996) modified from Costello (1990), clearly indicate that this fish, at the early stages of development, in different age classes, in tanks with *Eichhornia crassipes* and *Lemna* sp., shows tendency to the generalist feeding strategy (Figure 1). This is because there were higher values of frequency of occurrence and low values of dominance for various food items. Thus, *A. altiparanae* larvae consumed various food items, many of them at low amounts.

There were changes in the percentage values of dominance and frequency of occurrence of different food items in the diet of *A. altiparanae* with the same age, in tanks with *E. crassipes* compared to those with *Lemna* sp. and also variations in the diet of larvae of different ages, in tanks with the same macrophyte species.

In tanks with *E. crassipes*, the rotifer *Lecane bulla* was the food item with the highest dominance and frequency of occurrence, followed by the rotifer Bdelloidea and unicellular diatom in the contents of digestive tracts of *A. altiparanae* in the first age class. In the presence of *Lemna* sp., the cladoceran *Alona* sp. exhibited a greater dominance, followed by *Mytilina ventralis macracantha* in relation to other items, but *Alona* sp. had a higher frequency of occurrence, followed by filamentous and unicellular diatoms.

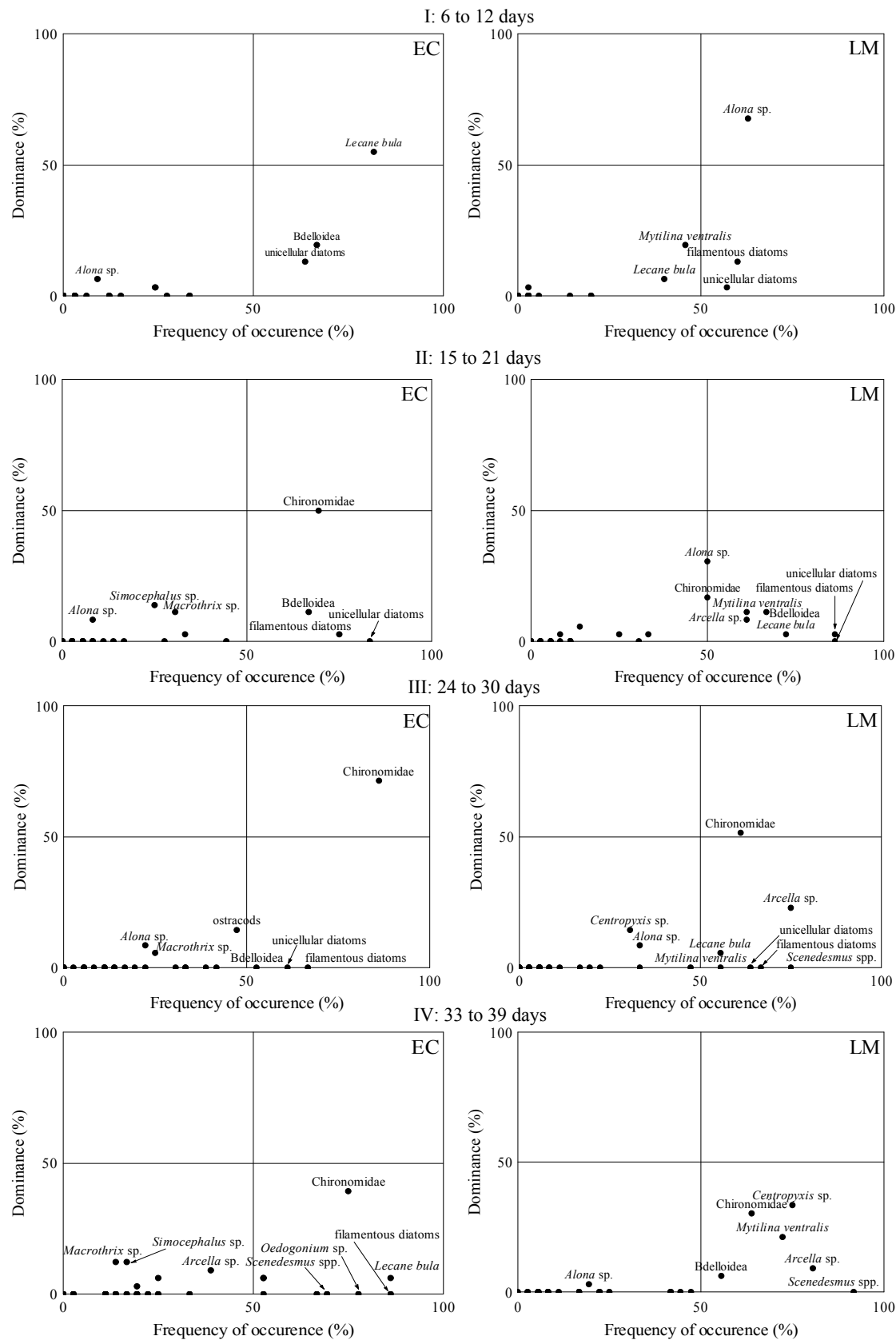


Figure 1. Graphic representation of dominance (axis y) and frequency of occurrence (axis x) of the food items in the digestive tract of *A. altiparanae* at the early developmental stages, in different ages, in tanks with *Eichhornia crassipes* (EC) and *Lemna* sp. (LM).

In the second age class, there was a higher dominance of Chironomidae immature and *Alona* sp. in the digestive tracts of *A. altiparanae* in tanks with *E. crassipes* and *Lemna* sp., respectively. However, in the same age class, the unicellular and filamentous diatoms, showed the highest frequency of occurrence in both treatments. Also, Chironomidae immature and Bdelloidea, in the presence of *E. crassipes*, and *L. bulla*, Bdelloidea, *Arcella* sp. and *M. ventralis macracanthra*, in the presence of *Lemna* sp., had a higher frequency of occurrence compared to other organisms.

Food items with higher dominance in digestive tracts of *A. altiparanae* in the third age class were: Chironomidae immature in both tanks with *E. crassipes* and *Lemna* sp., followed by ostracod, in tanks with *E. crassipes*, and protozoa *Arcella* sp. and *Centropyxis* sp., in tanks with *Lemna* sp.. Regarding the percentage of frequency of occurrence, Chironomidae immature, filamentous and unicellular diatoms and Bdelloidea were more frequent in tanks with *E. crassipes*. In tanks with *Lemna* sp., there were higher values for *Arcella* sp., *Scenedesmus* spp., filamentous and unicellular diatoms and Chironomidae immature.

In the analysis of digestive tracts of *A. altiparanae* in the fourth age class, the dominant food item in tanks with *E. crassipes* were Chironomidae immature, and the items with the highest frequency of occurrence were *L. bulla*, filamentous diatoms, *Oedogonium* sp. and Chironomidae immature. In tanks with *Lemna* sp., there was a greater dominance of *Centropyxis* sp. and Chironomidae immature, followed by the rotifer *M. ventralis macracanthra* and *Arcella* sp. and the greater values of frequency of occurrence were found for *Scenedesmus* spp., *Arcella* sp., *Centropyxis* sp. and *M. ventralis macracanthra* and Chironomidae immature.

Discussion

Our findings indicate that *Astyanax altiparanae* shows a generalist feeding strategy (Figure 1), which corroborates data found in the literature for different environments. Ribeiro & Nuñez (2008) reported that cladocerans and insect larvae were the major food items of *Salminus brasiliensis* post-larvae, being generalist, consuming a varied amount of food items and exhibiting tendency to different feeding specializations according to the developmental stage. Results described by Bennemann, Gealh, Orsi, & Souza (2005) indicate generalist habit for four species of *Astyanax* studied in different rivers of the Tibagi River basin, state of Paraná, including *A. altiparanae*. Other studies agree that species of the

same genus are generalist (Andrian, Silva, & Peretti, 2001; Deus & Petrere-Junior, 2003; Pinto & Uieda, 2007; Corrêa & Silva, 2010; Manna, Rezende, & Mazzoni, 2012).

Larval growth and survival depend on appropriate densities of zooplankton prey, and the availability of types of prey for a predator may be the result of biological and behavioral processes presented by both (Sipaúba-Tavares, 1993). Interactions between predator and prey can be an important factor in structuring the freshwater zooplankton community (Confer & Blades, 1975; Zaret, 1980).

Trophic plasticity is a strategy that allows the exploitation of different food resources temporarily available in the environment (Abelha, Agostinho, & Goulart, 2001; Luz-Agostinho, Bini, Fugl, Agostinho, & Julio-Junior, 2006; Hahn & Fugl, 2007). Studies revealed that Neotropical fish have high trophic plasticity, which reflects the predominance of opportunistic and/or generalist species, especially in tropical ecosystems, where there is a high diversity of food resources (Abelha et al., 2001). The genus *Astyanax* has omnivorous feeding habits (Menezes et al., 2007), considered a generalist species with high trophic plasticity in response to environmental changes and resource availability and has a greater chance of surviving extreme alterations in the habitat (Lobón Cerviá & Bennemann, 2000; Menezes et al., 2007).

The consumption rates of larvae of *A. altiparanae* with 6 to 39 days on protozoa, rotifers, cladocerans, ostracods, Chironomidae immature and algae (unicellular and filamentous diatoms) in tanks with *Eichhornia crassipes* or *Lemna* sp. confirm that the zooplankton is the main food for fish, especially in the first days of life, as reported to larvae of different species of fish by Fregadolli (1993), Sipaúba-Tavares (1993), Gerking (1994), Soares, Hayashi, Furuya, & Maranhão (1997), Furuya, Hayashi, Furuya, & Sakaguti (2002), Makrakis et al., 2005, Feiden et al. (2006), Qu et al. (2012).

The rotifer *Lecane bulla* was prevalent in the diet of *A. altiparanae* in tanks with *E. crassipes*, in the first age class (6-12 days), while in tanks with *Lemna* sp., the predominant item was the cladoceran *Alona* sp., in the first and second age classes (6 to 21 days), which is possibly related to the structural complexity of roots of the macrophytes investigated (Iversen et al., 1985), which can interfere with both the development of planktonic organisms and the predation by fish (Magalhães, Yamamoto, Anjos, Loebens, & Soares, 2015; Casatti, Mendes, & Ferreira, 2003). Plants like *E. crassipes* (Cyr & Downing, 1988) are used as a refuge by a wide

variety of planktonic organisms, against the action of predatory fish, especially in early stages of development (Rozas & Odum, 1988; Thomaz & Bini, 2003; Casatti et al., 2003). Rhizosphere of aquatic macrophytes can determine the distribution of prey organisms (Jurkevitch, Minz, Ramati, & Barel, 2000). Rhizosphere of *E. crassipes* is larger than that of *Lemna* sp. and provides suitable conditions for refuge of *Alona* sp., making difficult the observation and capture by *A. altiparanae* larvae. Thus, *L. bulla* is consumed by these larvae. However, in tanks containing *Lemna* sp., *Alona* sp. becomes more susceptible to attack by larvae due to the smaller size of roots, with a smaller refuge area. Therefore, the impact on the population of smaller planktonic organisms decreases in the presence of *Lemna* sp..

The result observed herein corroborates Savino & Stein (1982), who claimed that an intermediate structural complexity within a habitat results in optimal conditions for predator growth because it ensures food supply. Conversely, many fish species show a decline in predation efficiency on zooplankton in structured habitats, leading to a reduced predation pressure on zooplankton (Winfield, 1986).

Rotifers represented an important resource for *A. altiparanae* larvae up to 12 days of age, especially in the presence of *E. crassipes*. The results are similar to that reported by Fregadolli (1993) and Gerking (1994). Rotifers were considered important by Soares et al. (1997) in feeding larvae of *Rhinelepis aspera* in rearing tanks in the first 32 days of age of the fish.

The high dominance of Chironomidae immature with increasing age of *A. altiparanae*, in the presence of *E. crassipes*, may be related to the development of this food-organism associated with macrophytes with large rhizosphere, such as *E. crassipes*, leading to an increased density of these organisms compared to tanks with *Lemna* sp., which has a smaller rhizosphere. Our findings differ from those verified by Soares, Hayashi, Faria-Soares and Galdioli (2016), in tanks with macrophytes (*E. crassipes* and *Pistia stratiotes*), in which were found reduction of cladocerans and copepods and increase rotifers in plankton of tanks with *A. altiparanae*, *Piaractus mesopotamicus*, *P. lineatus* and *L. obtusidens*.

In tanks with *Lemna* sp., in the last two age classes, Chironomidae immature and testate amoebae (*Centropyxis* sp. and *Arcella* sp.) showed increased representativeness in the diet of *A. altiparanae*. This indicates that the number of *Alona* sp. may have decreased due to increased predation by *A. altiparanae* larvae, or also because this

cladoceran has a short life cycle. Thus, larvae began to feed on organisms with higher availability for these age classes, such as Chironomidae immature, which, although *Lemna* sp. provides a smaller substrate area for their development due to the small size of roots, also have the walls of the tanks as substrate and can be found in high number. These results differ from those obtained by Soares et al. (2016), who related that testate amoebae increase in *A. altiparanae* presence compared with tanks without fish fry.

In tanks with *Lemna* sp., the cladoceran *Alona* sp. predominated in the diet of *A. altiparanae* larvae in the first two age classes. Meantime, these larvae started to consume larger organisms, such as Chironomidae immature, in the last two age classes in this treatment, as also observed in tanks with *E. crassipes*, from 15 to 39 days of age. These results confirm those presented by Marques, Hayashi, Galdioli, & Fernandes (2007), where the cladoceran *Macrothrix* sp. was the dominant item in the diet of *P. mesopotamicus* in the early developmental stages from 6 to 33 days of age and *P. lineatus*, from 6 to 15 days, in tanks with or without *P. stratiotes*.

The digestive tracts of individuals in tanks with *E. crassipes* showed higher values of frequency of occurrence of rotifers and algae in the first (6 to 12 days), second (15 to 21 days) and fourth (33 to 39 days) age classes and Chironomidae, in the third age class (24 to 30 days). In tanks containing *Lemna* sp., rotifers and algae were the most frequent in the second, third and fourth age classes, and cladocerans showed a higher frequency in the first class. Similar results were registered by Marques et al. (2007) reported that smaller organisms, such as *Diffugia* sp., *Lecane* sp. and algae, had higher frequency of occurrence in the diet of *P. lineatus* in tanks with macrophytes (*P. stratiotes*) with increasing age, as obtained in this study for *A. altiparanae* at early developmental stage in tanks with *Lemna* sp. and *E. crassipes*, except for the third age class.

In some studies, such as Furuya et al. (2002) on *Pseudoplatystoma corruscans*, it has been found that smaller organisms like rotifers becomes less frequent with increasing age of the post-larvae, with an increase in the frequency of occurrence of larger organisms, such as cladocerans, copepods, ostracods and insect larvae, as also verified by Makrakis et al. (2005) for larvae of *Iheringichthys labrosus* and *Hypophthalmus edentatus*, for *P. mesopotamicus* and *P. lineatus* by Marques et al. (2007) and for *Plectropomus leopardus* by Qu et al. (2012). Changes that take place with increasing age of fish larvae, in which some species prefer smaller organisms and other species, larger ones, depend on feeding habits of each species

(Gerking, 1994; Makrakis et al., 2005; Qu et al., 2012).

It is worth noting that, although algae and rotifers have higher frequency of occurrence in the diet of *A. altiparanae* with similar or different age classes, in tanks with *E. crassipes* and *Lemna* sp., and are important in the diet of larvae, these food items were not dominant in the content of digestive tracts. This indicates that, despite at greater number, they did not contribute so significantly in relation to the volume occupied in the digestive tracts when compared to larger organisms (cladocerans, ostracods and Chironomidae immature).

Conclusion

It can be concluded that *E. crassipes* and *Lemna* sp. provide an environment with different composition, in terms of zooplankton organisms, due to their structural complexity, thus directly influencing the diet of *A. altiparanae*, and altering it according to the availability and consumption of organisms. Besides that, there are dietary changes during the early developmental stages of this fish species.

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