

Effects of structural heterogeneity provided by the floating macrophyte *Eichhornia azurea* on the predation efficiency and habitat use of the small Neotropical fish *Moenkhausia sanctaefilomenae*

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Received: 27 February 2008 / Revised: 8 December 2008 / Accepted: 14 December 2008 / Published online: 4 January 2009
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Abstract Macrophytes have a fundamental structuring role in aquatic environments. Several authors have suggested that trophic interactions are particularly mediated by aquatic plants. In the current article, we evaluated the effects of the structural heterogeneity provided by *Eichhornia azurea* (Kunth) roots on predation and habitat use by the small fish *Moenkhausia sanctaefilomenae* (Steindachner). We tested the hypotheses that (i) high structural heterogeneity protects macroinvertebrates against predation by *M. sanctaefilomenae*; (ii) distinct prey types are differently protected by the refuge provided by roots; and (iii) the behavior of *M. sanctaefilomenae* is affected by the structural heterogeneity provided by macrophyte roots. To test these hypotheses, we performed an experiment in 20 l aquaria in which macroinvertebrates (*Cypricercus* sp. and *Chironomus* sp.) were exposed to *M. sanctaefilomenae* predation for

4 h under three structural heterogeneities, represented by different root densities. High structural heterogeneity protected macroinvertebrates against predation. Additionally, *E. azurea* roots similarly protected different prey species. The macrophyte spatial structure substantially changed the habitat use of *M. sanctaefilomenae*. In general, our results corroborated the hypothesis that the structural heterogeneity provided by *E. azurea* roots significantly affects predation and habitat use by *M. sanctaefilomenae*.

Keywords Structural heterogeneity · *Eichhornia azurea* · *Moenkhausia sanctaefilomenae* · Macroinvertebrates · Trophic interactions

Handling editor: S. Declerck

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Introduction

Macrophytes play an important structuring role in aquatic ecosystems, not least in the (sub)tropics (Jeppesen et al., 1998; Agostinho et al., 2007; Meerhoff et al., 2007a, b). Aquatic habitats with high structural heterogeneity provide refuge against predators and suitable spawning and foraging substrates, thus supporting more individuals and species of invertebrates and fish (Declerck et al., 2005; Agostinho et al., 2007; Vieira et al., 2007; Thomaz et al., 2008). Moreover, several authors have suggested that trophic interactions are particularly mediated by

aquatic macrophytes (see Savino & Stein, 1982; Diehl, 1988; Pelicice & Agostinho, 2006; Jeppesen et al., 2007).

A myriad of factors should be considered when attempting to elucidate trophic interactions in vegetated habitats. Particularly important are the ability of predators to forage in structured habitats (Jacobsen & Perrow, 1998; Priyadarshana et al., 2001), the spatial scale (Dibble et al., 2006), the macrophyte density (Savino & Stein, 1982; Snickars et al., 2004), the macrophyte morphological type (Warfe & Barmuta, 2004), and the structural heterogeneity provided by aquatic plants (Meerhoff et al., 2006; Thomaz et al., 2008). Despite the importance of determining spatial scale and structural heterogeneity, few investigations have controlled for these factors (Dibble et al., 2006).

According to the optimal foraging theory, the choice of a prey by a predator depends on the trade-off between prey availability and energetic contribution to the predator (Wootton, 1999). In other words, the preference comes down to choosing the prey with the correct balance of capture, ingestion, digestion, and absorption costs, and energetic and nutritional benefits (Wootton, 1999). According to this theory, because of the difficulty of finding prey in an environment with high structural heterogeneity, small fishes will choose a prey with a higher availability. On the other hand, in environments with low structural heterogeneity, in which prey capture is less costly, the choice of a particular prey with a higher energetic value is more favorable. Therefore, the feeding preference of predators can be mediated by structural heterogeneity.

The majority of studies aimed at understanding the trophic interactions between fishes and invertebrates in vegetated habitats has considered only the role of submerged macrophytes (Jeppesen et al., 1998). However, Meerhoff et al. (2003) showed that *Eichhornia crassipes* (Mart.) Solms, a Neotropical free-floating macrophyte, has a fundamental structuring role in subtropical lagoons. In spite of that, some researchers (e.g., Brendonck et al., 2003; Meerhoff et al., 2006, 2007b; Iglesias et al., 2008) have suggested that free-floating macrophytes do not play an important role as refuges for large zooplankton (e.g., cladocerans); however, they may be particularly important for macroinvertebrates (Meerhoff et al., 2007a). Therefore, the role of the structural complexity of macrophytes on predator–prey interactions

should be clarified in Neotropical ecosystems. The influence of macrophyte structure on predator–prey interactions is also reflected in predator behavior in vegetated habitats (Savino & Stein, 1982). Low swimming activity and high exploration of the interstices among plant roots are common behavior patterns observed in fishes that explore habitats with macrophytes (Priyadarshana et al., 2001).

Most of the conclusions about the abundance of invertebrates in littoral vegetated areas come from observational data (e.g., Lansac-Tôha et al., 2003; Takeda et al., 2003). Few experimental studies have formally tested the hypothesis that macrophytes provide refuge against predation for associated animals (but see Burks et al., 2001; Meerhoff et al. 2006, 2007a, b), mainly with fish–invertebrate interactions. However, some researchers cast doubt on the structuring role of macrophytes for invertebrates (mainly large zooplankton, see Meerhoff et al. 2006, 2007b; Iglesias et al., 2008).

Eichhornia azurea, structurally similar to *E. crassipes* but with long floating stems and secondary submerged roots (coming from stem nodes), is one of the most abundant macrophytes in the upper Paraná River floodplain. This macrophyte is characterized by the great morphological plasticity of its secondary submerged roots, which change morphologically depending on the water nutrient concentrations (particularly phosphorus) (Camargo & Esteves, 1996). *E. azurea* provides a high level of structural heterogeneity due to its submerged roots, which may supply a great diversity of underwater shelters for macroinvertebrates (Higuti et al., 2007) and fishes (Agostinho et al., 2007). Root length varies greatly. The length is usually shorter than 5 cm in the younger portions of the stems but can reach up to 1 m in the older portions. Thus, the free space for aquatic fauna may be reduced below dense stands colonizing littoral regions in floodplain lakes, which are generally shallow (in the Paraná floodplain, for example, depths are usually lower than 1.5 m). Therefore, *E. azurea* provides different levels of structural heterogeneity in aquatic environments, which can affect the efficiency of predation and the behavior of small fishes.

Moenkhausia sanctaefilomenae (Perciformes, Characidae), popularly known as “maconheirinho,” is a fish native to South America and is found in the Parnaíba, São Francisco, and de la Plata river basins (Graça & Pavanelli, 2007). According to Agostinho et al. (2007),

this is the second most abundant species colonizing *E. azurea* roots in the upper Paraná lakes, reaching densities of 4–49 individuals m^{-2} . This species is zooplankti-benthivorous, feeding mainly on microcrustaceans and insect larvae such as ostracodes and chironomids (Loureiro-Crippa, unpublished data). Together with its high abundance, the feeding habits of this fish species make it suitable to study predator–prey interactions inside vegetated habitats.

The main purpose of our study was to evaluate the effect of the structural heterogeneity provided by the secondary submerged roots of *E. azurea* on predator–prey interactions involving the small fish *M. sanctaefilomenae* as the predator and two different invertebrate species, the ostracod *Cypricercus* sp. (Podocopa; Crustacea) and the chironomid *Chironomus* sp. (Diptera; Insecta), as the prey. We chose these prey species because they are highly abundant in the littoral regions in the upper Paraná River floodplain lakes and have different behaviors (the former is more active and swims within the macrophytes' interstices, whereas the latter remains attached to macrophytes' roots and leaves; Takeda et al., 2003; Mormul et al., 2006; Higuti et al., 2007). Specifically, we evaluated the influence of the structural heterogeneity on the predator–prey interactions. Additionally, we recorded the behavior of the fish to determine how this species explores the vegetated environment. We tested the following hypotheses: (i) high structural heterogeneity protects macroinvertebrates from *M. sanctaefilomenae*, reducing the efficiency of predation and (ii) the pattern of habitat use by *M. sanctaefilomenae* is significantly affected by the structural heterogeneity provided by *E. azurea* roots.

Materials and methods

Experimental design

To test our hypotheses, we used 50 individuals of the genus *Cypricercus* (Ostracoda, approximately 1 mm length) and 20 individuals of the genus *Chironomus* (Diptera, approximately 6.5 mm length) as prey in 20 l aquaria, each with a stem of *E. azurea* containing secondary roots with different structural heterogeneities. We noticed that once they were put in the aquaria, the macroinvertebrates immediately searched for *E. azurea* roots. Each aquarium received three

M. sanctaefilomenae individuals as predators. Fish size ranged from 3.2 to 4.5 cm (mean 3.9 cm). We designed the experiment with three conditions, corresponding to two levels of structural heterogeneity provided by *E. azurea* roots and one without plant roots, and five replicates of each condition. Experiments ran for 4 h, always between 10:00 am and 2:00 pm, over 5 days in April 2006.

In order to estimate the invertebrate mortality caused by predation alone, it was necessary to quantify the background (non-predation) level of invertebrate mortality. For this purpose, each experimental aquarium had a control with the same conditions but lacking fish. Therefore, each day represented one independent experimental replicate with six aquaria (three aquaria with fish and three corresponding controls; total of 30 independent observations). We always used different fish and macroinvertebrates in the aquaria on different days. Hence, each individual was only used once during the course of the experiments. In order to reduce the stress caused by the external environment, which could affect the results, we randomly placed the aquaria on a table, separating them from each other by an opaque cover in a silent and closed room with artificial illumination. The three aquaria containing fish were laterally arranged and in front of three corresponding controls.

We obtained the three structural heterogeneities by clipping the *E. azurea* secondary submerged roots. The plant fragments used (one fragment of approximately 30 cm per aquarium) always contained exactly four nodes of root ramification. In this plant, each node contains several root ramifications. In the condition considered more heterogeneous (high structural heterogeneity, HH), all roots were left intact (Fig. 1). In the condition with low structural heterogeneity (LH), we clipped the roots and kept just two root ramifications per node (Fig. 1). Finally, in the null structural heterogeneity condition (NH), we clipped all roots, leaving only nude stems floating in the experimental aquaria (Fig. 1). We distinguished these structural heterogeneities using the complexity index proposed by Dibble et al. (1996) (Eq. 1):

$$\text{Complexity index} = \frac{f_v}{\mu_v} + \frac{f_h}{\mu_h} \quad (1)$$

in which f_v is frequency of vertical interstices, μ_v is mean size of vertical interstices, f_h is frequency of

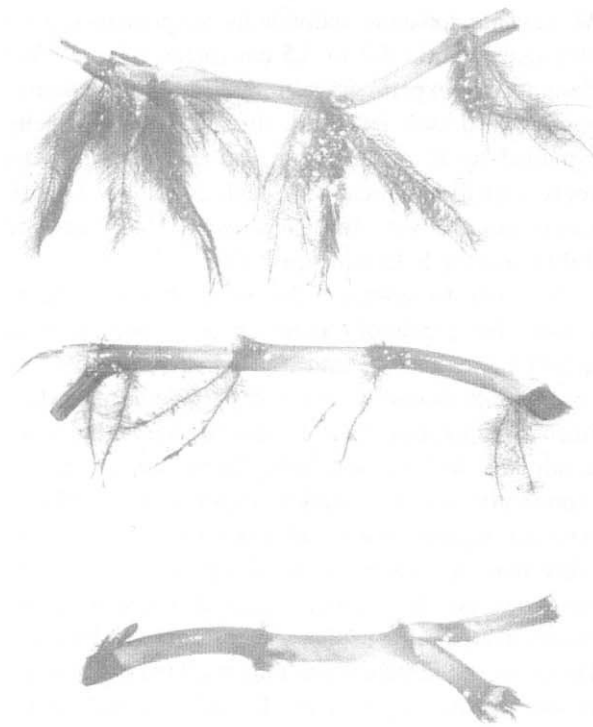


Fig. 1 Representative photographs of the structural heterogeneities. The fragments (one fragment per aquarium) are approximately 30 cm in length; HH (high structural heterogeneity; *upper photograph*), LH (low structural heterogeneity; *middle photograph*) and NH (null structural heterogeneity, *lower photograph*) conditions. The mean interstice size for the HH and LH conditions ranged from 0.13 to 0.20 cm and from 0.29 to 0.35 cm, respectively. In the NH condition all roots were clipped

horizontal interstices, and μ_h is mean size of horizontal interstices.

This dimensionless index considers the frequency and size of vertical and horizontal interstices (free holes among plant structures that animals can inhabit or explore) of a plant in a determined spatial resolution. The mean interstice size in the HH and LH conditions ranged from 0.13 to 0.20 cm and from 0.29 to 0.35 cm, respectively. We measured interstices using digital photographs of the stem fragments suspended in water in a tray before the beginning of the experiment. In each photograph (corresponding to each replicate), we took three measurements at a spatial resolution of 10 cm \times 10 cm. We chose this scale because it is within the spatial scale perceived by small fishes (Dibble et al., 2006).

We collected fish, *Chironomus* sp., and plants from a lake in the upper Paraná River floodplain. We

starved the fish for 3 days in 1,000 l tanks and transferred them to the aquaria 1 h before the beginning of the experiment. We cultivated *Cypricercus* sp. individuals, also collected from the same lake, in tanks from the Aquiculture Laboratory of the Maringá State University. Thus, we could easily achieve the desired density of individuals. We chose the densities for all groups according to a pilot experiment. Further, we used three fish per aquaria with the goal of eliminating individual feeding differences.

Prior to the experiment, we washed the plant roots and placed them in ice water for approximately 30 min to eliminate any undesired attached organisms. To minimize problems with fish acclimatizing, the water used in the aquaria was the same in which the fish were acclimatized. We used filtered (0.02 mm mesh) water to eliminate undesired feeding resources for the fish. Also, several authors have argued that prey search for protected habitat when predators' chemical signals are present in the water (e.g., Lehtiniemi, 2005; Richmond & Lasenby, 2006). Thus, this enhanced the similarity of the experiment to natural conditions.

Assessment of predation rates

We evaluated predation by calculating the difference between the number of *Cypricercus* sp. and *Chironomus* sp. before and after exposure to the fish. The results were corrected for non-predation losses using the results from the control aquaria (i.e., aquaria with the same structural heterogeneity and without predators).

After the experiment, we carefully washed the roots and cautiously inspected them to recover any attached macroinvertebrates. In addition, we filtered (0.02 mm mesh) the water used in this wash together with the water used in the aquaria, after removing the fish. We also carefully inspected the aquaria glass to count any remaining macroinvertebrates. The number of lost macroinvertebrates in the controls (aquaria in the same conditions but without fish) was relatively low (<5%). Nonetheless, we considered these losses to enhance the accuracy of the experiment. In this case, we subtracted the number of lost macroinvertebrates in each control replicate from the number of invertebrates in the corresponding aquarium containing fish.

Fish behavior analysis

We evaluated the behavior of the fish through videography. We placed one analog video-camera in front of the three aquaria containing fish and recorded the videos during the entire experimental period (4 h) for all replicates. We measured the swimming activity of the fish since this variable is frequently related to foraging (Abrahams & Sutterlin, 1999). It is usually shown that swimming activity of small fish inhabiting macrophytes is negatively related to foraging in littoral regions (Savino & Stein, 1982). We also quantified the time spent by the fish in the upper region of the aquarium, since roots were concentrated in this region. More time spent near the roots suggested more exploration of the interstices and, consequently, more searching for prey. To measure the time spent in the upper region, we divided the video representation of each aquarium into two equal parts (upper and lower).

The quantification of the behavior variables described above occurred as follows: videotapes were continuously watched, and every 20 s we counted the number of fish in the upper region of the aquarium (3, 2, 1, or 0 fish) and the number of fish in motion (3, 2, 1, or 0 fish). Thus, we recorded approximately 720 observations for each aquarium and each variable during the 4-h duration of the experiments. We used this strategy because the quantification of the total time spent by each fish in movement or in the upper region of aquaria would be difficult to obtain due to the erratic movements of these fish. In this case, we assumed that results from these observations are representative of the time spent by fish near macrophytes and in motion.

We measured the number of observations of fish in the upper region and the number of observations of fish in motion in all replicates, generating five values for the aquaria of each structural heterogeneity. In this case, the response variables were the total number of observations of each behavior, calculated by summing the number of observations of 0 fish multiplied by 0, plus 1 fish multiplied by 1, plus 2 fish multiplied by 2, plus 3 fish multiplied by 3. Given that we did approximately 720 observations, the maximum value would be 2,160, corresponding to 720 observations (i.e., at all time points) of 3 fish in the upper region or in motion. Then, we could determine the total number of observations corresponding to each behavior

(our response variables) for further statistical comparisons among aquaria with different structural heterogeneities.

Data analysis

We tested for differences between the HH and LH conditions using *t* tests for the complexity index. To test the effects of the plant root density (structural heterogeneity) on predation, we used the difference between the initial and the final number of macro-invertebrates, after correcting for the losses recorded in the control aquaria, as our response variable (i.e., prey mortality due to predation). We used two one-way ANOVA tests (for each prey species) to evaluate the effects of the structural heterogeneity on the prey mortality due to predation. Data transformation was not necessary because the ANOVA assumptions were met (i.e., a normal distribution and variance homogeneity). In the case of significant differences, we applied Tukey a posteriori tests to identify which structural heterogeneity was different.

We also used two one-way ANOVA tests to assess differences in the fish behavior. In the first, we tested for the effects of the structural heterogeneity on the number of observations of fish in the upper region of the aquaria. The other test involved the effects of the structural heterogeneity on the number of observations of fish swimming. For this last ANOVA, we used log-transformed data to meet the ANOVA assumptions. For all tests, we considered differences to be significant when the probability of type I error was lower than 0.01. We used STATISTICA software (Statsoft, 2005) for the statistical analyses and graphics.

Results

The HH condition had a significantly higher value for the complexity index (Mean \pm SD: 411.1 \pm 71.1) than the LH condition did (Mean \pm SD: 144.5 \pm 36.8; *t*-test: *t* = 10.53, *df* = 1, *P* < 0.01). The NH condition had no underwater complexity, and we assumed that the complexity index for this condition was 0.

The effects of the structural heterogeneity were significant for predation of both *Cypricercus* sp. and *Chironomus* sp. (Table 1). In addition, by using the

Table 1 One-way ANOVA results showing the effects of the structural heterogeneity on prey mortality due to predation using two prey types

Prey	Sum of squares	Mean squares	Degrees of freedom	F-values	P-values
<i>Cypricerus</i> sp.	7,571.7	3,785.9	2	18.30	<0.01
<i>Chironomus</i> sp.	7,453.3	3,726.7	2	11.65	<0.01

percentage of prey mortality due to predation, it seems that the structure provided by *E. azurea* is similarly protective for both prey types (Fig. 2). The loss of macroinvertebrates was the lowest in the HH condition for both prey types. This result was significant according to a Tukey a posteriori test ($P < 0.01$, see Fig. 2). In addition, this test also showed that the predation rates were significantly similar in the LH and NH conditions considering the two prey types ($P = 0.29$ and 0.22 for *Cypricerus* sp. and *Chironomus* sp., respectively, see Fig. 2).

There was significant effect of the structural heterogeneity on the number of *M. sanctaefilomenae* individuals observed at the upper region of the aquaria (Table 2). Accordingly, the effect of the structural heterogeneity on fish movement was also significant (Table 2). Thus, the time spent at the aquaria's upper region and the time spent in motion

depended on the structural heterogeneity provided by the macrophyte roots. In the HH condition, fish had significantly reduced swimming activity and spent a longer time in the upper region of the aquaria near the macrophytes than fish in the LH and NH conditions (Fig. 3).

Discussion

It is widely accepted, although rarely shown, that littoral habitats dominated by macrophytes provide refuge areas against predators and are foraging habitats for small-sized fishes in Neotropical ecosystems (Agostinho et al., 2003, 2007). However, some studies have suggested that the high structural heterogeneity provided by aquatic plants also protects invertebrates against predation by small fish (Rantala et al., 2004; Meerhoff et al., 2006, 2007a). In general, in the presence of predators, prey search for protected habitats, avoiding suitable predator foraging areas (Jacobsen et al., 1997). Our experimental results obtained with a small Neotropical fish species, *M. sanctaefilomenae*, clearly showed the effects of the structural heterogeneity provided by *E. azurea* secondary roots on the predator–prey interactions (involving fishes and macroinvertebrates). The shelter provided by enhancing the structural heterogeneity for both prey types (*Cypricerus* sp. and *Chironomus* sp.) was clearly evident. This effect has been widely shown, but previous studies were mainly conducted for temperate environment species and under temperate conditions (e.g., Snickars et al., 2004). Additionally, recent research in subtropical ecosystems has shown that free-floating macrophytes can repel large zooplankton (e.g., cladocerans), whereas submersed macrophytes are a better refuge for these invertebrates (e.g., Meerhoff et al., 2006, 2007a). Despite the fact that these investigations have shown the effects of macrophytes on zooplankton, studies validating the shelter effect of macrophyte structure on macrophyte-associated macroinvertebrates (such

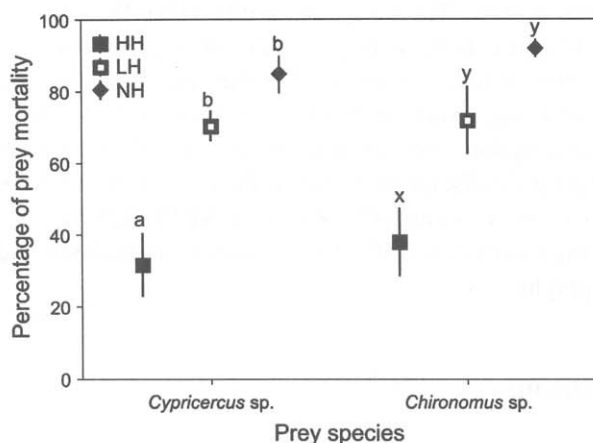


Fig. 2 Percentage of prey mortality due to predation by *M. sanctaefilomenae* in the three structural heterogeneities. The percentages indicate the mean of the five replicates for each structural heterogeneity; the replicates were completed over 5 days in April 2006. Bars indicate the standard error. HH high structural heterogeneity, LH low structural heterogeneity, NH null structural heterogeneity. Values labeled with the same letters are not significantly different according to the values from the Tukey test. These results are prey mortality values (corrected for losses in the control aquarium, see “Materials and methods” section) for each prey species

Table 2 One-way ANOVA results showing the effects of the structural heterogeneity on the number of observations of *M. sanctaefilomenae* individuals in movement and in the upper region of the aquaria

Behavior	Sum of squares	Mean squares	Degrees of freedom	F-values	P-values
Fish in movement	1,745,252	872,626	2	11.76	<0.01
Fish in the upper region	1,949,568	974,784	2	101.85	<0.01

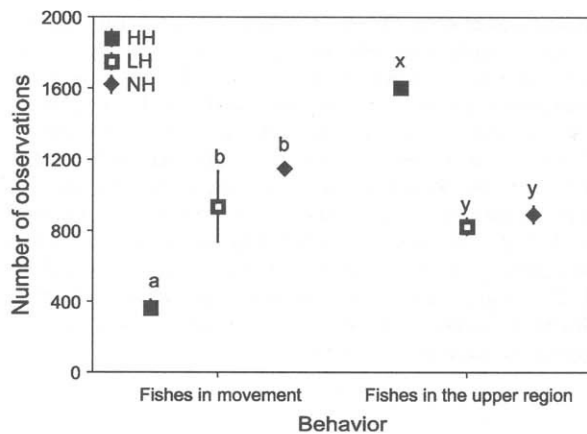


Fig. 3 Number of observations of fish in movement and in the upper region of the aquarium in the structural heterogeneities. The observation counts indicate the mean of the five replicates for each structural heterogeneity; the replicates were completed over 5 days in April 2006. Bars indicate the standard error. *HH* high structural heterogeneity, *LH* low structural heterogeneity, *NH* null structural heterogeneity. Values labeled with the same letters are not significantly different according to the values in the Tukey test considering observation counts for each behavior

as *Cypricercus* sp. and *Chironomus* sp.) in (sub)tropical habitats are rare (but see Takeda et al., 2003).

For small fishes, high structural heterogeneity means low foraging rates. Therefore, it has been suggested that the highest fish abundance is found in environments with intermediate structural heterogeneity, in which prey are sufficiently available for predators (Grenouillet et al., 2002). In areas of high structural heterogeneity, there is a high prey abundance because predation is reduced. However, fish movement (and prey capture) is restricted due to the physical barriers provided by the roots. On the other hand, in areas of low structural heterogeneity, there is little impediment to fish movement, but prey are scarce.

Nevertheless, for a better understanding of the predator–prey interactions in natural conditions, experimental investigations analyzing the effects

of different structural heterogeneities, the feeding preferences and the behavior of predators are also necessary. The structural heterogeneities used in our experiment represented real situations observed in situ and simulated different structural heterogeneities provided by *E. azurea* roots. According to our results, low structural heterogeneity would not be sufficient to protect *Cypricercus* sp. and *Chironomus* sp. against *M. sanctaefilomenae* predation, since similar levels of predation were recorded in the LH and NH conditions for both prey species. This suggests that if the environment has a low habitat structure, the predatory efficiency of this small fish is not affected by structural heterogeneity. This suggestion is supported by field evidence, showing that at *E. azurea* stands in the upper Paraná River floodplain, the highest density and diversity of small fish are found at the edge of the stands where the structural heterogeneity is intermediate, since roots are younger and less developed (Agostinho et al., 2007).

Moreover, it seems that the effects of structural heterogeneity are independent of the prey type; i.e., the refuge provided by *E. azurea* roots offered similar protection for both *Cypricercus* sp. and *Chironomus* sp (see Fig. 2). This is surprising given the great differences in size and activity of these two species. Additionally, the capture of prey in similar proportions (even with different initial densities) may suggest that *M. sanctaefilomenae* has an opportunist feeding strategy, consuming the prey that is most available at a given moment. The opportunist strategy of this fish was also shown by Hahn et al. (2004), who observed that its broad diet is composed of several insect species.

M. sanctaefilomenae behavior was also highly affected by the structural heterogeneity of *E. azurea* roots. Priyadarshana et al. (2001) argued that the presence of physical barriers provided by macrophytes is the main reason for the low swimming activity in highly structured habitats. This is a possible explanation for the lower swimming activity

in the HH condition. Furthermore, *M. sanctaefilomenae* probably preferred the upper region of the aquaria dominated by macrophytes in order to search for food, although shelter against predation by piscivorous fish and birds is an alternative explanation for fish searching among macrophytes roots (Agostinho et al., 2007). Despite the high exploration of this habitat, the prey mortality was diminished by increased structural heterogeneity. We emphasize that our interpretation is restricted to macroinvertebrates (*Cypricercus* sp. and *Chironomus* sp.) associated with floating macrophyte (*E. azurea*) roots. Submersed macrophytes, on the other hand, seem to be more efficient than the free-floating species as a refuge for large zooplankton in (sub)tropical ecosystems (Meerhoff et al., 2006, 2007a, b). Thus, in habitats that are highly colonized by free-floating macrophytes, the role of small fish in plankton control, and in top-down mechanisms, should still be explored.

Our observations that *Cypricercus* sp. and *Chironomus* sp. predation is reduced by the structural heterogeneity provided by *E. azurea* roots and that *M. sanctaefilomenae* is attracted to regions with high macroinvertebrate abundance have practical consequences. For example, during periods of low prey population densities, when the risk of local extinction is higher, efficient refuges against predation are very important, highlighting the relevance of these refuges in conservation (Piana et al., 2006). In fact, the structural heterogeneity provided by the vegetation has deep implications for community structures (e.g., Vieira et al., 2007), affecting the spatial distribution and survival of prey (Grabowski & Powers, 2004; Warfe & Barmuta, 2006) and predators (Sammons & Maceina, 2006), bottom-up and top-down processes (Jeppesen et al., 2004; Hembre & Megard, 2005), zooplankton horizontal migration (Burks et al., 2001), and even stable states of an environment (Sheffer, 1997). At coarser spatial scales, the biodiversity of aquatic ecosystems also has been related to structural heterogeneity (Grenouillet et al., 2002) and plant cover (Declerck et al., 2005).

In general, our results corroborate the hypothesis that *E. azurea* roots provide protection for *Cypricercus* sp. and *Chironomus* sp. against *M. sanctaefilomenae* predation. The results also suggest that the fish uses an opportunist foraging strategy and that the structure of this macrophyte protects very distinct macroinvertebrates in a similar fashion. In addition, *M. sanctaefilomenae* behavior is highly affected by

structural heterogeneity. These results are relevant because they were obtained experimentally and with common Neotropical organisms. However, for a better understanding of the trophic interactions in Neotropical environments, future investigations should test the effects of the spatial scale and different macrophyte types on fishes and macroinvertebrates.

Acknowledgments We acknowledge Dr. Leonardo Maltchik for his valuable comments during the creation of this article. We are also grateful to Dr. Steven Declerck and one anonymous reviewer for valuable comments during previous drafts of this article. We also acknowledge the Program of Graduation in Ecology of Inland Waters and the NUPELIA (Nucleon of Research in Limnology, Ichthyology and Aquaculture) of Maringá State University for the facilities. A. A. Padiã received a student fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). S. M. Thomaz and A. A. Agostinho are researchers of the Brazilian Council of Research (CNPq) and acknowledge this agency for long-term funding.

References

- Abrahams, M. V. & A. Sutterlin, 1999. The foraging and antipredator behaviour of growth-enhanced transgenic Atlantic salmon. *Animal Behaviour* 58: 933–942.
- Agostinho, A. A., L. C. Gomes & H. F. Julio Jr., 2003. Relações entre macrófitas aquáticas e peixes. In Thomaz, S. M. & L. M. Bini (eds), *Ecologia e manejo de macrófitas aquáticas*. Eduem, Maringá: 261–280.
- Agostinho, A. A., S. M. Thomaz, L. C. Gomes & L. S. M. A. Baltar, 2007. Influence of the macrophyte *Eichhornia azurea* on fish assemblage of the Upper Paraná River Floodplain (Brazil). *Aquatic Ecology* 41: 611–619.
- Brendonck, L., J. Maes, W. Rommens, N. Dekeza, T. Nihwatiwa, M. Barson, V. Callebaut, C. Phiri, K. Moreau, B. Gratwicke, M. Stevens, N. Alyn, E. Holsters, F. Ollevier & B. Marshall, 2003. The impact of water hyacinth (*Eichhornia crassipes*) in a eutrophic subtropical impoundment (Lake Chivero, Zimbabwe). II. Species diversity. *Archiv für Hydrobiologie* 158: 389–405.
- Burks, R. L., E. Jeppesen & D. M. Lodge, 2001. Littoral zone structures as *Daphnia* refugia against fish predators. *Limnology and Oceanography* 46: 230–237.
- Camargo, A. F. M. & F. A. Esteves, 1996. Influence of water level variation on biomass and chemical composition of the aquatic macrophyte *Eichhornia azurea* (KUNTH) in an oxbow lake of the Rio Mogi-Guaçu (São Paulo, Brazil). *Archiv für Hydrobiologie* 135: 423–432.
- Declerck, S., J. Vandekerckhove, L. Johansson, K. Muylaert, J. M. Conde-Porcuna, K. Van der Gucht, C. Pérez-Martínez, T. Lauridsen, K. Schwenk, G. Zwart, W. Rommens, J. López-Ramos, E. Jeppesen, W. Vyverman, L. Brendonck & L. De Meester, 2005. Multi-group biodiversity in shallow lakes along gradients of phosphorus and water plant cover. *Ecology* 86: 1905–1915.

- Dibble, E. D., K. J. Killgore & G. O. Dick, 1996. Measurement of plant architecture in seven aquatic plants. *Journal of Freshwater Ecology* 11: 311–318.
- Dibble, E. D., S. M. Thomaz & A. A. Padial, 2006. Spatial-complexity measured at a multi-scale among three aquatic plant species. *Journal of Freshwater Ecology* 21: 239–247.
- Diehl, S., 1988. Foraging efficiency of three freshwater fishes: effects of structural complexity and light. *Oikos* 53: 207–214.
- Grabowski, J. H. & S. P. Powers, 2004. Habitat complexity mitigates trophic transfer on oyster reefs. *Marine Ecology Progress Series* 277: 291–295.
- Graça, W. J. & C. S. Pavanelli, 2007. Peixes da planície de inundação do alto rio Paraná e áreas adjacentes. EDUEM, Maringá.
- Grenouillet, G., D. Pont & K. L. Seip, 2002. Abundance and species richness as a function of food resources and vegetation structure: juvenile fish assemblages in rivers. *Ecography* 25: 641–650.
- Hahn, N. S., R. Fugi & I. F. Andrian, 2004. Trophic ecology of fish assemblages. In Thomaz, S. M., A. A. Agostinho & N. S. Hahn (eds), *The upper Paraná River and its floodplain: physical aspects, ecology and conservation*. Backhuys Publishers, Leiden: 247–270.
- Hembre, L. K. & R. O. Megard, 2005. Timing of predation by rainbow trout controls *Daphnia* demography and the trophic status of a Minnesota lake. *Freshwater Biology* 50: 1064–1080.
- Higuti, J., L. F. M. Velho, F. A. Lansac-Tôha & K. Martens, 2007. Pleuston communities are buffered from regional flood pulses: the example of ostracods in the Paraná River floodplain, Brazil. *Freshwater Biology* 52: 1930–1943.
- Iglesias, C., N. Mazzeo, G. Goyenola, C. Fosalba, F. T. Mello, S. García & E. Jeppesen, 2008. Field and experimental evidence of the effect of *Jenynsia multidentata*, a small omnivorous–planktivorous fish, on the size distribution of zooplankton in subtropical lakes. *Freshwater Biology*. doi: 10.1111/j.1365-2427.2008.02007.x.
- Jacobsen, L. & M. R. Perrow, 1998. Predation risk from piscivorous fish influencing the diel use of macrophytes by planktivorous fish in experimental ponds. *Ecology of Freshwater Fish* 7: 78–86.
- Jacobsen, L., M. R. Perrow, F. Landkildehus, M. Hjørne, T. L. Lauridsen & S. Berg, 1997. Interactions between piscivores, zooplanktivores and zooplankton in submerged macrophytes: preliminary observations from enclosure and pond experiments. *Hydrobiologia* 342/343: 197–205.
- Jeppesen, E., M. Søndergaard, M. Søndergaard & K. Cristoffersen, 1998. The structuring role of submerged macrophytes in lakes. Springer, New York.
- Jeppesen, E., J. P. Jensen, M. Søndergaard, M. Fenger-Grøn, M. E. Bramm, K. Sandby, P. H. Møller & H. U. Rasmussen, 2004. Impact of fish predation on cladoceran body weight distribution and zooplankton grazing in lakes during winter. *Freshwater Biology* 49: 432–447.
- Jeppesen, E., M. Meerhoff, B. A. Jacobsen, R. S. Hansen, M. Søndergaard, J. P. Jensen, T. L. Lauridsen, N. Mazzeo & C. W. C. Branco, 2007. Restoration of shallow lakes by nutrient control and biomanipulation—the successful strategy varies with lake size and climate. *Hydrobiologia* 581: 269–285.
- Lansac-Tôha, F. A., L. F. M. Velho & C. C. Bonecker, 2003. Influência de macrófitas aquáticas sobre a estrutura da comunidade zooplanctônica. In Thomaz, S. M. & L. M. Bini (eds), *Ecologia e manejo de macrófitas aquáticas*. Eduem, Maringá: 231–242.
- Lehtiniemi, M., 2005. Swim or hide: predator cues cause species specific reactions in young fish larvae. *Journal of Fish Biology* 66: 1285–1299.
- Loureiro-Crippa, V. E., 2006. Dieta, hábitos alimentares e morfologia trófica de peixes de pequeno porte, em lagoas da planície do alto rio Paraná, Brasil. Ph.D. thesis, Maringá State University, Maringá, Paraná, Brazil.
- Meerhoff, M., N. Mazzeo, B. Moss & L. Rodriguez-Gallego, 2003. The structuring role of free-floating versus submerged plants in a subtropical shallow lake. *Aquatic Ecology* 37: 377–391.
- Meerhoff, M., C. Fosalba, C. Bruzzone, N. Mazzeo, W. Norrdoven & E. Jeppesen, 2006. An experimental study of habitat choice by *Daphnia*: plants signal danger more than refuge in subtropical lakes. *Freshwater Biology* 51: 1320–1330.
- Meerhoff, M., J. M. Clemente, F. T. Mello, C. Iglesias, A. R. Pedersen & E. Jeppesen, 2007a. Can warm climate-related structure of littoral predator assemblages weaken the clear water state in shallow lakes? *Global Change Biology* 13: 1888–1897.
- Meerhoff, M., C. Iglesias, F. T. Mello, J. M. Clemente, E. Jensen, T. L. Lauridsen & E. Jeppesen, 2007b. Effects of habitat complexity on community structure and predator avoidance behaviour of littoral zooplankton in temperate versus subtropical shallow lakes. *Freshwater Biology* 52: 1009–1021.
- Mormul, R. P., L. A. Vieira, S. P. Júnior, A. Monkolski & A. M. dos Santos, 2006. Sucessão de invertebrados durante o processo de decomposição de duas plantas aquáticas (*Eichhornia azurea* e *Polygonum ferrugineum*). *Acta Scientiarum Biological Sciences* 28: 109–115.
- Pellicice, F. M. & A. A. Agostinho, 2006. Feeding ecology of fishes associated with *Egeria* spp. patches in a tropical reservoir, Brazil. *Ecology of Freshwater Fish* 15: 10–19.
- Piana, P. A., L. C. Gomes & A. A. Agostinho, 2006. Comparison of predator-prey interaction models for fish assemblages from the neotropical region. *Ecological Modelling* 192: 259–270.
- Priyadarshana, T., T. Asaeda & J. Manatunge, 2001. Foraging behaviour of planktivorous fish in artificial vegetation: the effects on swimming and feeding. *Hydrobiologia* 442: 231–239.
- Rantala, M. J., J. Ilmonen, J. Koskimäki, J. Suhonen & K. Tynkkynen, 2004. The macrophyte, *Stratiotes aloides*, protects larvae of dragonfly *Aeshna viridis* against fish predation. *Aquatic Ecology* 38: 77–82.
- Richmond, S. & D. C. Lasenby, 2006. The behavioural response of mayfly nymphs (*Stenonema* sp.) to chemical cues from crayfish (*Orconectes rusticus*). *Hydrobiologia* 560: 335–343.
- Sammons, S. M. & M. J. Maceina, 2006. Changes in diet and food consumption of largemouth bass following large-scale hydrilla reduction in Lake Seminole, Georgia. *Hydrobiologia* 560: 109–120.

- Savino, J. & R. A. Stein, 1982. Predator-prey interaction between largemouth bass and bluegills as influenced by simulated, submerged vegetation. *Transactions of the American Fisheries Society* 111: 255–266.
- Sheffer, M., 1997. On the implications of predator avoidance. *Aquatic Ecology* 31: 99–107.
- Snickars, M., A. Sandström & J. Mattila, 2004. Antipredator behaviour of 0+ year *Perca fluviatilis*: effect of vegetation density and turbidity. *Journal of Fish Biology* 65: 1604–1613.
- Statsoft, Inc., 2005. STATISTICA (data analysis software system) version 7.1 for Windows: statistics. STATSOFT, Inc., Tulsa.
- Takeda, A. M., G. M. Souza-Franco, S. M. Melo & A. Monkolski, 2003. Invertebrados associados às macrófitas aquáticas da planície de inundação do alto rio Paraná. In Thomaz, S. M. & L. M. Bini (eds), *Ecologia e manejo de macrófitas aquáticas*. Eduem, Maringá: 243–260.
- Thomaz, S. M., E. D. Dibble, L. R. Evangelista, J. Higuti & L. M. Bini, 2008. Influence of aquatic macrophyte habitat complexity on invertebrate abundance and richness in tropical lagoons. *Freshwater Biology* 53: 358–367.
- Vieira, L. C. G., L. M. Bini, L. F. M. Velho & G. R. Mazão, 2007. Influence of spatial complexity on the density and diversity of periphytic rotifers, microcrustaceans and testate amoebae. *Fundamental and Applied Limnology* 170: 77–85.
- Warfe, D. M. & L. A. Barmuta, 2004. Habitat structural complexity mediates the foraging success of multiple predator species. *Oecologia* 141: 171–178.
- Warfe, D. M. & L. A. Barmuta, 2006. Habitat structural complexity mediates food web dynamics in a freshwater macrophyte community. *Oecologia* 150: 141–154.
- Wootton, R. J., 1999. *Ecology of Teleost Fishes*, 2nd ed. Kluwer Academic Publishers, Dordrecht.