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Microhabitat use by *Phalloceros harpagos* Lucinda (Cyprinodontiformes: Poeciliidae) from a coastal stream from Southeast Brazil

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The size-related microhabitat use of *Phalloceros harpagos* Lucinda, 2008 from Mato Grosso stream (Saquarema - RJ) was examined. We analyzed, during 8.3 h of underwater observation, a 200 m long reach in the upper Mato Grosso stream, quantifying the following microhabitat descriptors: (i) distance from the stream bank, (ii) water column depth, (iii) occurrence on riffle, pool or run mesohabitat, (iv) water velocity, (v) substratum, and (vi) subaquatic vegetal cover. Microhabitat selectivity was analyzed by comparing the microhabitat used by fish and the microhabitat available in the study site. Complementary analyses, based in the Ivlev Electivity Index were applied in order to test selectivity for the microhabitat use. We did not found differences in the microhabitat used by juvenile and adult individuals. *Phalloceros harpagos* was selective for five among the six analyzed microhabitat descriptors. The occurrence of *Phalloceros* in the studied stream was limited to shallow pools, close to the river bank, with low water velocities and mud substratum. Vegetal cover was not an important factor in the occurrence of the studied species.

As diferenças de uso do micro-habitat, relacionadas ao tamanho, foram estudadas para *Phalloceros harpagos* do rio Mato Grosso (Saquarema - RJ). Para tal analisamos, durante 8,3 h de observação subaquática, um trecho de 200 m do alto rio Mato Grosso, considerando-se os descritores ambientais de: (i) distância da margem do riacho, (ii) altura da coluna d´água, (iii) ocorrência dos meso-habitats de corredeira, poças ou rápidos, (iv) velocidade da água, (v) substrato e (vi) cobertura vegetal subaquática. A existência de seletividade foi analisada pela comparação dos micro-habitats usados e disponíveis na área de estudo. Análises complementares a partir do Índice de Eletividade de Ivlev foram usadas para testar a seletividade no uso do micro-habitat. Não registramos diferenças para o uso do micro-habitat de jovens e adultos. *Phalloceros harpagos* foi seletivo para cinco dos seis parâmetros de micro-habitat analisados. A ocorrência de *Phalloceros* na área de estudo se deu preferencialmente em poças de pequena profundidade, próximas a margem, baixa velocidade da água e fundo de substrato areno/lodoso. A presença de cobertura vegetal aquática não foi um fator de influência na ocorrência da espécie.

Key words: Focal position, Mata Atlântica stream, Subaquatic observations.

Introduction

Assemblages of stream-dwelling fishes can vary greatly in composition over small spatial scale and an understanding of the mechanisms underlying this spatial variation has been an important point of ecological studies in freshwater systems (Bremset & Berg, 1999; Silva, 2007). Although this is not the only fundamental aspect of fish ecology, it could be considered the basis for developing management tools such as habitat suitability indexes (Pouilly & Souchon, 1994; Martínez-Capel & García de Jalón, 1999; Casatti *et al*., 2009) necessary for environmental conservation. These habitat studies can also provide information to prioritise habitat improvement measures, and manage flow regimes to conserve native fish populations, especially from Mata Atlântica streams, which are being displaced by deforestation and other human sewage outputs (*e.g*., Cunico *et al*., 2006; Pinto *et al*., 2006).

Enormous deleterious human actions over lotic systems had modified and promoted important losses of stream-dwelling fishes from Mata Atlântica (Mazzoni & Lobón-Cerviá, 2000) enhancing the needs of studies that relate habitat use in the lowest scale of resolution (microhabitat associations). Following this, an important point to be held in consideration during microhabitat studies is the investigation of available resources in association to the

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used ones (Rincón, 1999), as microhabitat studies are normally viewed as a multiple variable approach reflecting the complex relationship between environmental descriptors and specific life-history aspects.

Numerous studies have explored the relationship between fish distribution and the quality or quantity of their habitats in temperate (*e.g.*, Schlosser, 1987; Valladolid & Przybylski, 1996; Lamouroux *et al*., 1999) and Mediterranean (*e.g*., Grossman *et al*., 1987b; Rincón *et al*., 1992; Santos *et al*., 2004; Martínez-Capel *et al*., 2009) rivers, but there are few studies for tropical fishes (Reichad, 2008). With one known exception (Lima *et al*., 2008), these studies in tropical areas, did not provide habitat indexes, but were mostly based on qualitative observation and descriptive data and the term microhabitat was used to refer to general locations in the river bed (Mazzoni & Iglesias-Rios, 2002; Casatti & Castro, 2006; Ferreira, 2007 and references therein). Nonetheless, in studies addressing microhabitat use by fish it should be considered the position of the fish in the water column (focal height and focal velocity) as well as quantitative data of used and available resources, which are essential to understand fish behaviour and to apply advanced river modelling techniques (Martínez-Capel *et al*., 2004). Following this, specific microhabitat requirements are important features to be considered during environmental enhancement projects as they drive community structure and species maintenance in a given environment (Boavida *et al*., 2010).

Poeciliidae species live in a broad array of habitats, occupying from temperate to tropical zones, deserts, rivers, lakes springs, fresh and brackish marshes, seacoasts, and saline mangrove swamps (Nelson, 1994). Many species are ecologically very tolerant, inhabiting in quiet water streams, flood-water ponds, lagoons, lakes and dams, in water clear to turbid or very muddy (Gómez-Márquez *et al*., 2008). Such plastic behavior associated to the high fecundity and a viviparous or ovoviviparous strategy (*sensu* Rosen & Bailey, 1963; Reznick & Endler, 1982) is efficient to maintain viable and abundant populations and could explain their worldwide distribution.

Phalloceros Eigenmann, 1907 is a Poeciliidae genus (Lucinda, 2003) comprising small fishes broadly distributed throughout southern and southeastern river basins of South America. Its intrageneric diversity and relationships remained unknown until 2008, when Lucinda (2008) revised the genus and described *P. harpagos*, among twenty-one new species, some of them previously classified as *P. caudimaculatus* Hensel, 1868. In the present study, we aimed to quantify the used and available habitat resources of juvenile and adult individuals of *Phalloceros harpagos* Lucinda, 2008 from a coastal Mata Atlântica stream, in order to address whether the pattern of microhabitat use (spatial occupation) is selective or stochastic and to provide information to adequately improve management propositions.

Material and Methods

Field work was developed at Mato Grosso fluvial system (Fig. 1) that composes a small coastal drainage located in the northeast of Rio de Janeiro State (22º53'S 42º39'W). Mato Grosso is a 3rd order stream flowing for about 12 km which sources are located in Serra do Mato Grosso, at approximately 500 m asl, and discharges at Saquarema Lagoon. It flows through meadows deforested for agricultural practices and cattle ranching, though pristine Mata Atlântica forest is still common on the slopes and at the top of the surrounding rocky hills. The study site was located in the upper reach of Mato Grosso stream, in an area where riparian vegetation is preserved and no human disturbance is present. Twenty-seven species compose the ichthyofauna of Mato Grosso stream but thirteen species co-occur in the study site. *Phalloceros harpagos* is one of the most abundant species in the study site and was regularly registered during inventory studies through the year.

Two criteria defined the study site chosen. Firstly, the fish density at the site had to be sufficiently high to yield numerous observations of *P. harpagos* and to minimize the probability of repeated observations of the same individuals. Secondly, the site had to have heterogeneous physical properties such as water depth, water velocity and composition of the river bed, to obtain a wide range of available microhabitats with different characteristics allowing the recognition of stochastic or selective use of habitat. Data records were done in two steps: (i) quantification of resources used by fish and (ii) quantification of resources available in the study site. In order to quantify microhabitat resources used by fish, daylight snorkelling observations (diving sessions - Fig. 2) were made in a 200 m long (122.8 ± 2.1 m^2 - Table 2) reach of Mato Grosso stream in two sampling days (15th January and 21st March of 2007) during the wet season and two sampling days (29th June and 1st August of 2007) during the dry season. Five diving sessions, based on a focal individual approach (*sensu* Altman, 1974), were performed at each sampling day and lasted for, at least, 25 min,

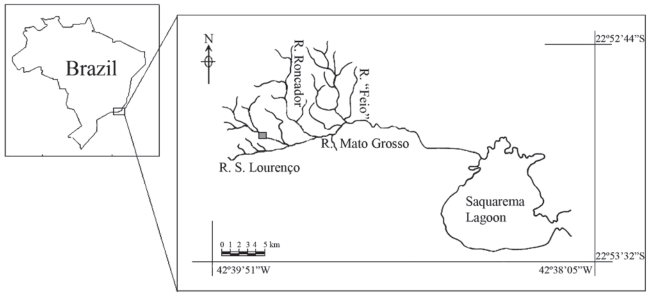

Fig. 1. Mato Grosso fluvial system with indication of the sample site (grey square).

Fig. 2. Microhabitat underwater observation. In detail substratum measurements (Photo: Rosana Mazzoni).

totalling 8.3 h of underwater observation during the whole study. Thus, we performed 4.16 h of diving observations at each season (10 diving sessions * 25 min of observations at dry and wet seasons).

At the beginning of each diving session the diver lay calm for 10 min downstream of the site, to let the fish become accustomed to the observer. Since few fishes reacted when the diver approached, we recorded only fishes that appeared to be undisturbed. During observation, the diver moved from the lower to the upper section of the study site and, for each fish sighting, the descriptors presented in Table 1 were registered for the fish focal position. Fish size of each observed individual was also registered. Microhabitat availability was quantified along the study site where fish observations were made. Forty-eight transversal transects were established, 5 m apart from each other, through the 200 m long study site. At each transect the six microhabitat measurements presented in Table 1 were recorded in one or two 0.5x0.5 m (0.25 m^2) quadrats, according to the total area (see details of total area and quadrats of each sampling day on Table 2). In order to avoid taking measurements at a constant distance from the shore, the position of each quadrat was sorted among four points of the transect: (1) position in the right shore, (2) position in the first third of the channel, (3) position in the second third of the channel and (4) position in the left shore. Thus, at each sampling day we sampled a number of quadrats representing 10% of the total studied stream area.

Voucher specimens of *P. harpargos*, obtained in previous studies developed in Mato Grosso stream were placed in the collection of the Museu Nacional do Rio de Janeiro and Universidade Federal do Tocantins (MNRJ and UFT).

The significance of the differences in frequency of each category within each variable between used and available resources was tested with contingency chi-square tests and Yates correction when necessary, assuming as significant values of $P<0.05$ (Zar, 1984). Electivity Index (E_i) (Krebs, 1989): $E_i = (Ui - Ai)/(Ui+Ai)$, where, Ui = fish proportion using resource i and Ai = available proportion of resource i, was applied to test preference/avoidance of each microhabitat resource. Ei ranges from -1 to +1, where positive values indicate preference and negative values indicate avoidance of a giving resource. STATISTICA 7.0 (StatSoft, 2000) and Pcord v. 4.0 (McCune & Mefford, 1997) were used for all statistical analysis.

Table 1. Microhabitat descriptors, and their respective measurements for fish use and environmental availability measurements, applied for microhabitat study of *Phalloceros harpagos* from Mato Grosso stream.

Environmental Descriptors	Description	Use Measurement	Availability Measurement
Distance from the nearest bank (cm)	Distance from the stream bank to a given point in the stream channel, measured through a measuring tape	Distance from to the stream bank to the fish focal position	Distance from the stream bank to a place where the quadrats were placed
Water Column Depth (cm)	Distance from the water surface to the stream bottom	Distance from the water surface to the fish focal position	Distance from the water surface to a place where the quadrats were placed
Mesohabitat	Occurrence of the three mesohabitats (pools, runs and riffles) present in the study site	Kind of mesohabitats predominating in the fish focal position	Mesohabitat types predominating in a place where the quadrats where placed
Water Velocity (m/seg)	Water velocity measured through a fluxometer probe	Water velocity measured at the fish focal position	Water velocity measured where the quadrats were placed
Substratum	Substratum types occurring in the study site: (i) mud – particles < 0.2 cm and subject to suspension; (ii) sand - particles 0.2 cm ≥ 2.5cm; (iii) gravel - particles 2.5 cm > 3.0 cm; (iv) boulder - particles 3.5 cm > 30 cm and (v) bedrock - particles > 30 cm	Percentual of each substratum type measured just below the fish focal position	Percentual of each substratum type measured just below the quadrats
Vegetal Cover	Quantity of instream vegetation serving as underwater refuge	Percentual of instream vegetation measured just above at the fish focal position	Percentual of instream vegetation measured just above the quadrats

Table 2. Environmental characterization of the study site at Mato Grosso stream considering (1) maximum (Mx), minimum (Mn) and mean (Me) depth; (2) percentual values of mesohabitat cathegories: riffles (Ri), runs (Ru) and pools (Po); (3) percentual values of substratum type: mud (Md), sand (Sd), gravel (Gr), boulder (Bd), and bedrock (Br); (4) maximum (Mx), minimum (Mn), and mean (Me) channel width ; total sampled area (TSA); total quadrat area (TQA); total of analysed quadrats (nQ) and percentual of aquatic vegetation (Veg).

		Depth (cm)			Mesohabitat (%)			Substratum (%)					C. Width (cm)			TSA	TQA	nQ	Veg (%)
		Mx	Mn	Me	Ri	Ru	Po	Md	Sd	Gr	Bd	Br	Mx	Mn	Me	(m^2)	(m^2)		
Wet	January 15th 2007	52.0	15.1	17.1	22	67	11	25	12	18	22	23	5.5	0.9	2.2	125	12.5	50	23
Season	March 21st 2007	52.0	15.3	19.4	21	68	11	25	13	17	22	23	5.5	1.3	2.2	120	12.0	48	25
Dry	June 29th 2007	50.0	14.2	18.7	22	68	10	24	13	17	23	23	4.8	0.8	2.0	123	12.3	49	31
Season	August 1st 2007	51.0	13.9	15.9	20	67	13	25	11	19	21	24	5.0	0.9	2.4	123	12.3	49	29
	Mean	51.3	14.6	17.8	21.3	67.5	11.3	24.8	12.3	17.8	22.0	23.3	5.2	1.0	2.2	122.8	12.3		
	SD	0.96	0.68	1.58	0.96	0.58	1.26	0.50	0.96	0.96	0.82	0.50	0.36	0.22	0.16	2.06	0.21		

Results

The environmental parameters characterizing possible seasonal variation (Table 2) did not vary between dry and wet seasons. Available resources were quantified in 196 quadrats totalling 49 m^2, established according to the total sampling area (Table 2). Quantification of microhabitat use was done for 184 specimens (117 juveniles and 67 adults). Microhabitat availability analysis revealed that the studied site was quite heterogeneous being represented by all substrata and encompassed run, riffle and pool habitats (Table 2).

Differences in the use and availability of the various microhabitat descriptors revealed non size-related and non stochastic patterns of microhabitat use by *P. harpargos*. Distance from the nearest bank availability did not vary along the study site but significant differences ($\chi^2_{adults} = 63.8$ and $\chi^2_{juvenile} = 118.6$; df = 4; $p<0.001$) in the use by juvenile (Fig. 3a) and adult (Fig. 4a) were registered with higher use of positions close to the bank. The positive values of Ivlev index to positions between 0 and 24 cm from the river bank and the negative ones to position far from 24 cm denoted, for both adult and juveniles, preference and avoidance for these positions, respectively. Water column depth varied from 2 to 52 cm without availability differences along the study site. Nonetheless, both juveniles (Fig. 3b) and adult (Fig. 4b) showed significant differences ($\chi^2_{adults} = 50.3$ and $\chi^2_{juvenile} = 106.1$; df = 4; $p<0.001$) in the use of this microhabitat. According to Ivlev index both juveniles and adult individuals showed preference for depths between 22 and 32 cm and avoidance for all the other depth classes present in the study area. Among the three mesohabitats available in the study site, run was the one with the highest incidence. Nonetheless, pool habitat was the most used one ($\chi^2_{adults} = 54.1$ and $\chi^2_{juvenile} = 76.6$; df = 4; $p<0.001$) for both adult (Fig. 4c) and juvenile (Fig. 3c) individuals, with low to non use of the other two available mesohabitats. Ivlev index corroborated these findings. Water velocity varied from 0 m/seg to 3.38 m/seg, but only two velocity classes were effectively used, being the [0-0.7] m/seg the one with significantly ($\chi^2_{adults} = 107.2$ and $\chi^2_{juvenile} = 119.4$; df = 4; $p<0.001$) greater use for both adult (Fig. 4d) and juvenile (Fig. 3d) individuals. Positive values of Ivlev index, denoting preference, were registered only for water velocities between 0 and 0.5 m/seg, the negative values of positions between 0.7 and 3.5 m/seg denoted avoidance for these positions. All kinds of substrata occurred in the same proportion along the study site, but juvenile (Fig. 3e) and adult (Fig. 4e) of *P. harpagos* used significant ($\chi^2_{adults} = 87.4$ and $\chi^2_{juvenile} = 120.8$; df = 4; $p<0.001$) higher proportion of mud substratum. Sand and gravel were used by less individuals and the other substrata were not used. Ivlev index values, of both juveniles and adults, denoted preference for mud and sand substratum and avoidance for the others. Instream vegetation was patchlly distributed along the study site. The use of such microhabitat was not selective as all vegetal cover classes were equally used by juvenile (Fig. 3f) and adult (Fig. 4f) individuals ($\chi^2_{adults} = 2.5$ and $\chi^2_{juvenile} = 9.1$; df = 4; $p>0.05$). Ivlev index showed, for both juvenile and adult individuals values close to zero, denoting absence of preference or avoidance.

Ivlev Electivity Index confirmed that microhabitat use was not stochastic with both adult and juvenile individuals of *P. harpagos* showing markedly preference for the low lateral positions, medium water column deep, pool mesohabitat use, low water velocity and mud substrata (Fig. 3 a, b, c, d, and e). The only environmental parameter that did not show preference was the one related to vegetal cover (Fig. 3f).

Discussion

Habitat use by fish is related to morphological and physiological characters including size, form and position of fins as morphological features (Wootton, 1990), trophic (Zavala-Camin, 1996) and reproductive requirements as well as physiological ones (Balon, 1975; Munro, 1990). According to previous studies conducted in the Ubatiba River (*e.g.*, Mazzoni & Lobón-Cerviá, 2000; Mazzoni *et al.*, 2006), the habitat sampling covered in this study was broader enough to be considered as predictive of preferences of *P. harpagos*. Active individuals of *P. harpagos* showed non size-related and non-random patterns of microhabitat use, positively selecting fine-grained substratum, pools (slow waters) and shallow positions with or without vegetal cover. This is a kind of microhabitat frequently registered for Poeciliidae species (*i.e.*, Mazzoni & Iglesias-Rios, 2002; Lima *et al.*, 2008).

Specific ecomorphological attributes should reflect important

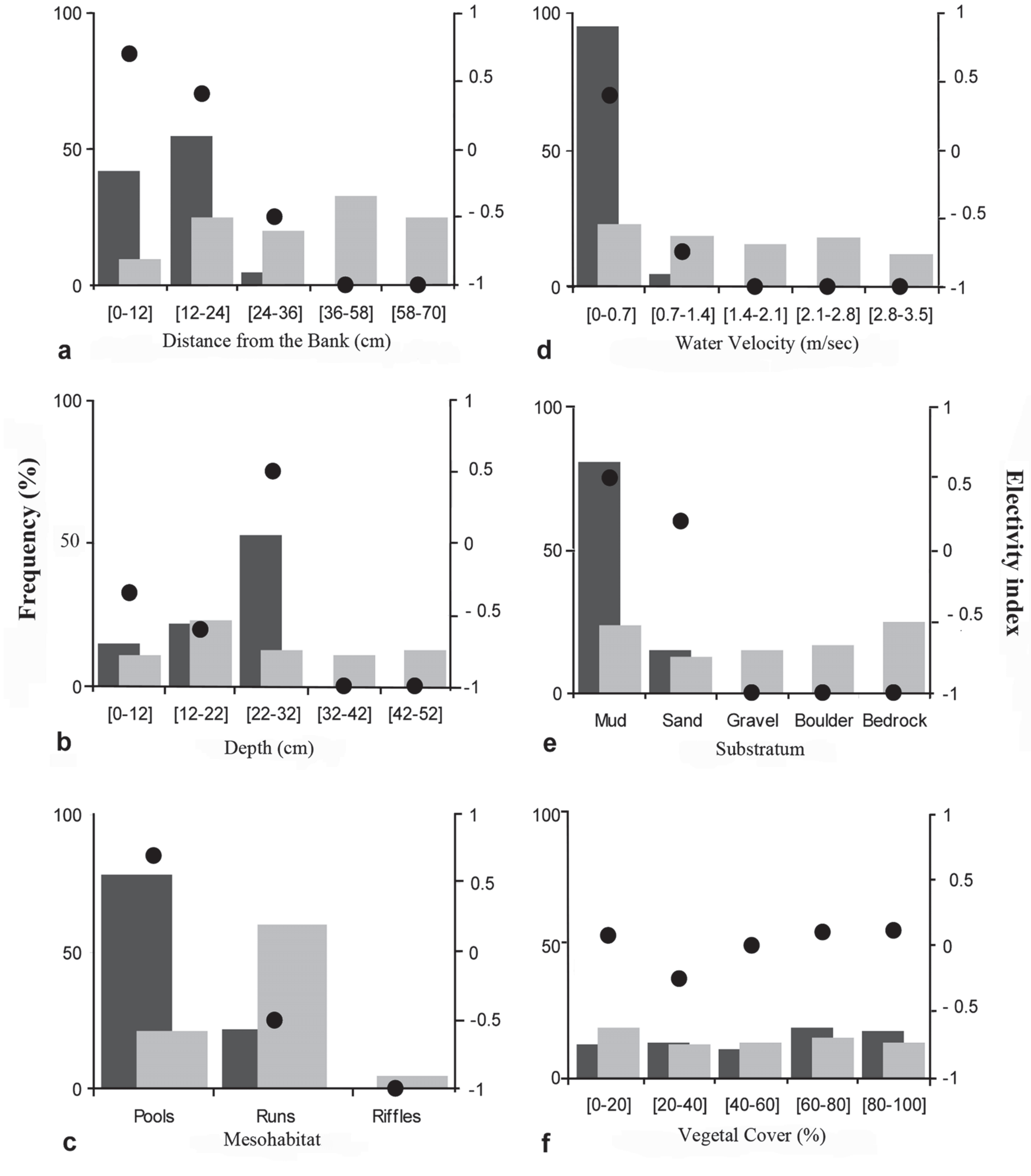

Fig. 3. Availablity (light grey bars), use (dark grey bars) and Ivlev Index (black circles) for the six studied microhabitat descriptors affecting juvenile individuals of *Phalloceros harpagos* populations from Mato Grosso stream: (**a**) distance from the nearest bank; (**b**) total depth; (**c**) mesohabitat; (**d**) focal water velocity; (**e**) substratum, and (**f**) vegetal cover density.

features of fish ecology and, therefore, be indicative of its habits and adaptations to different habitats (Gatz, 1979; Mahon, 1984; Balon *et al.*, 1986, Casatti & Castro, 2006). According to Heggenes & Traaen (1988), Hill & Grossman (1993) and Lamouroux *et al.* (1999), among others, larger fishes tend to select deeper habitats than shallow ones, probably as a strategy to avoid predation (Power, 1984) or because of trophic requirements, as such deeper habitats have greater variety of food resources (Rincón *et al.*, 2002). Size-related differences in the microhabitat use of *P. harpagos* were not registered. Adult and juvenile individuals used the same space, suggesting that trophic requirements and avoidance to predation should be developed in the same way between both adult and juvenile. Thus, in the case of *P. harpagos*, the non-random microhabitat use could be related to its small size and reduced swimming capability. In fact, *P. harpagos*, as all Poeciliidae species, is highly specialized, presenting superior

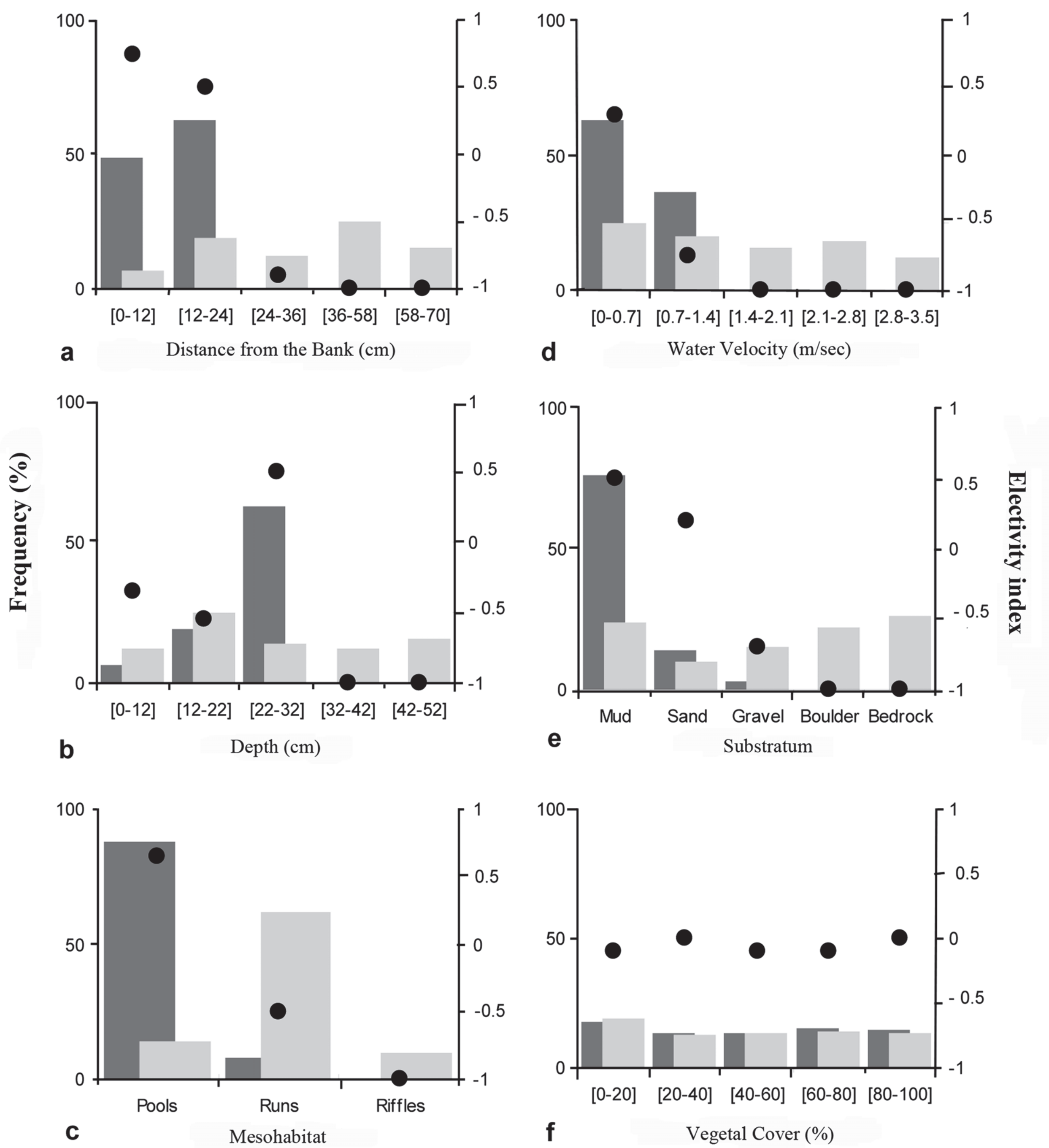

Fig. 4. Availablity (light grey bars), use (dark grey bars) and Ivlev Index (black circles) for the six studied microhabitat descriptors affecting adult individuals of *Phalloceros harpagos* populations from Mato Grosso stream: (**a**) distance from the nearest bank; (**b**) total depth; (**c**) mesohabitat; (**d**) focal water velocity; (**e**) substratum, and (**f**) vegetal cover density.

mouth and small fins, being this last one a morphological trait related to life spent in low flowing waters. Although, we don't have information about feeding ecology of the studied species, many studies have shown that *Phalloceros* fed mainly on detritus and periphyton from shallow waters (Costa, 1987; Teixeira, 1989; Aranha & Caramaschi, 1999; Casatti, 2002; Mazzoni *et al*., 2010), reinforcing the results presented therein.

Hoplias malabaricus is the top predator living in Mato Grosso system. It uses shallow and vegetated sites to feed on small sized Poeciliids (Caramaschi, 1978; Uieda, 1984; Castro & Casatti, 1997; Mazzoni & Iglesias-Rios, 2002) but, in the present study, it was not registered in co-occurrence with *P. harpagos* (Miranda, 2009). Thus the lack of selection in relation to protected areas with vegetal cover (a random habitat use) could be due to the absence of a top predator fish occurring in the study site as many studies showed that in the presence of aquatic predators Poeciliidae species present a non-random pattern of habitat use and avoid habitats with vegetal coverage (Caramaschi, 1978; Mazzoni & Iglesias-Rios, 2002). In fact, predation risk seems to be an important factor affecting fish distribution in streams and could explain the pattern of non selective use of vegetal covered sites.

Although being a place used by top predator acting on Poeciliidae specimens (Mazzoni & Iglesias-Rios, 2002), vegetal cover can also be an effective protection from terrestrial predators (Power, 1984). The responses of prey fishes in relation to these two types of predation should be different and normally are related to swimming movements down and up in the water column (Skyfield & Grossman, 2008). Since Poeciliids are potential prey fishes with limited swimming capability their evasion to predation is dependent of the ability to shift between covered and opened sites and selecting between fish and bird predation seems to be a trade-off dependent of a given circumstance. Among the studied species there was a stronger selection for low depths and marginal positions than the one observed for refuge (vegetal covered sites), reinforcing that complementary analysis of microhabitat use by *P. harpagos*, in sites with controlled presence of aquatic and terrestrial predators, is needed for conclusive results.

Regarding the variables involved, depth, distance from the river bank, substratum and water velocity were the most relevant factors in microhabitat selection of *P. harpagos*. General observations of the species suggests no age class differences in microhabitat use; however, complementary studies, involving the other fish species co-existing with *P. harpagos*, are needed for a complete knowledge about microhabitat selection and assemblage organization at Mato Grosso stream. The information regarding fish microhabitat use also allows the application of habitat models and can be useful to managers involved in the application of conservation measures for critical habitats to maintain and enhance the native stream-dwelling fish from Mata Atlântica.

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