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Application of the physical habitat simulation for fish species to assess environmental flows in an Atlantic Forest Stream in South-eastern Brazil

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The physical habitat simulation sub-routine of the Instream Flow Incremental Methodology (IFIM) uses hydraulic modeling and suitability indices of target fish species to predict how differences in-stream flows affect the microhabitat occupation by fish species. This habitat modelling approach was adopted to assess the ecological effects of running flows on three neotropical fish species of different orders (*Bryconamericus ornaticeps*, *Ancistrus multispinis* and *Geophagus brasiliensis*).The study encompassed two reaches of an Atlantic Forest stream in Southeastern Brazil where topographic and hydraulic (depth, velocity and type of substrate) characteristics were measured to implement one-dimensional hydraulic simulation. Sub aquatic observation of fish was performed to collect data on microhabitat use and these data were used to develop habitat suitability curves that were used in the habitat simulation to obtain the habitat suitability index (HSI) and weighted usable area (WUA) *versus* flow curves. Upon these curves minimum and optimum environmental flows for the target fish species were proposed. *Bryconamericus ornaticeps* and *A. multispinis* selected microhabitats around 0.6 m depth, whereas *G. brasiliensis* showed a wider suitable range (0.35-0.9 m). All the three species were mainly observed in microhabitat with low flow velocity (0.1 m/s). *Bryconamericus ornaticeps* selected more frequently coarse substrate (*e.g.* boulders) but it appeared also over sandy substrate, whereas *A. multispinis* and *G. brasiliensis* selected preferably boulders. The range of 0.65-0.85 m^3/s was found as the optimum to meet the needs of the three fish species. Our results agree with the necessary objective information to perform grounded management actions in the frame of a management program aiming at ecosystem conservation. Thereby it can be considered a successful pilot study in environmental flow assessment in an Atlantic Forest stream of Brazil.

A sub-rotina simulação de habitat físico da metodologia incremental - IFIM usa variáveis hidráulicas e os índices de aptidão das espécies alvo para predizer como diferentes vazões afetam a ocupação de microhabitat por espécies de peixes. Esta abordagem de simulação de hábitat foi adotada para avaliar os efeitos ecológicos sobre três espécies de peixes tropicais de diferentes ordens (*Bryconamericus ornaticeps*, *Ancistrus multispinis* e *Geophagus brasiliensis*) em dois trechos de um riacho de Mata Atlântica do sudeste do Brasil, durante a estação chuvosa de 2013 e a estação seca de 2014. Características topográficas e hidráulicas (profundidade, velocidade e tipo de substrato) foram mensuradas para implementação da simulação de habitat em uma dimensão; observações subaquáticas dos peixes foram realizadas para avaliação do uso do microhabitat. As curvas de aptidão de habitat de cada variável hidráulica foram utilizadas na simulação de habitat para obtenção das curvas de aptidão composta (HSI) e a área útil ponderada (WUA) *versus* fluxo, e propor vazões ambientais mínimas e ideais para as espécies de peixes-alvo. A amplitude de vazão de 0,65-0,85 m^3/s foi registrada como o ótimo para satisfazer as necessidades das três espécies de peixes. Esta é uma informação objetiva para os gestores de recursos hídricos e que pode ser aplicada no planejamento de programas de gestão visando a conservação dos ecossistemas. Desse modo, pode ser considerado um estudo piloto bem sucedido na avaliação do fluxo ambiental em um riacho da Mata Atlântica do Brasil.

Keywords: Habitat modeling, Habitat suitability curves, Neotropical fish.

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Introduction

Flow regimes and physical habitat structure are two major components of fluvial ecosystems that determine the presence, abundance and distribution of aquatic organisms (Karr *et al.,* 1986; Bunn & Arthington, 2002). The models integrating these two components and simulating fluvial and habitat dynamics use geomorphological, hydraulics and biological information directly measured in the aquatic ecosystem. Based on such models for a range of stream flow, the habitat availability and the stream flow rate that potentiates the optimum use by species can be assessed and several indicators can be inferred for instance, the Weighted Usable Area - WUA (Bovee *et al.,* 1998). These indicators allow the inference of the optimum flow rate or the minimum environmental flow (e-flow) as the basic references to assess the e-flow regime in a river reach.

Models assessing e-flow can be performed following a physical habitat simulation (abbreviated as PHABSIM) which was created as part of the Instream Flow Incremental Methodology (IFIM; Bovee, 1982; Bovee *et al.,* 1998) to solve conflicts in projects involving water allocation and impacts on aquatic ecosystems. Among the methods to assess e-flows at a global scale, IFIM is the methodological framework with the highest number of applications in the last decades worldwide (Tharme, 2003; Krstolic *et al.,* 2006; Martínez-Capel *et al*., 2009). Moreover, IFIM has been considered by some practitioners as the most scientifically and legally defensible methodology available for e-flow assessment (Gore & Nestler, 1988; Dunbar *et al*., 1998). Specifically, the phase of the IFIM that includes the habitat simulation, based on hydraulic simulation and models of habitat suitability for aquatic species (univariate curves or multivariate models) at the microhabitat scale (*sensu* Bovee *et al*., 1998). The suitability models for a given species can be used for: (i) predicting the species occurrence according to habitat variables and (ii) improving the understanding of species-habitat relationship (Ahmadi-Nedushan *et al*., 2006).

Fish assemblages that inhabit streams have species-specific patterns of habitat use (*e.g.* for feeding and reproducing) at small spatial scale, with a limited number of species occupying randomly a given space (Roff, 1992). This suggests that patterns of habitat selection are specific; this is one assumption in the development of predictive models of species distribution along environmental gradients (Ahmadi-Nedushan *et al*., 2006).

Stream habitats may be defined at different scales; in e-flow assessment, the microhabitat scale has been referred to as a portion of the river of approximately few meters with specific physical characteristics (*i.e.,* depth, mean flow velocity and type of substrate and cover) which is used by a given species during some part of its life cycle (Bovee *et al*., 1998). The habitat suitability models at microhabitat scale range from simple habitat suitability index (HSI) and bivariate polynomial functions (Lambert & Hanson, 1989) to more complex fuzzy rule base models (Jorde *et al*., 2001; Mouton *et al*., 2008), such as multilayer perceptron ensembles (Muñoz-Mas *et al*., 2014a) or probabilistic neural networks (Muñoz-Mas *et al*., 2014b). The mesohabitat scale areas usually classified as riffles, runs or pools (Angermeier & Schlosser, 1989) that exhibit important differences among them related to water velocity, channel morphology and type of substrate.

The ample array of approaches for habitat suitability modeling have a wide application in management of natural river systems and water resources since they allow the assessment of the degree of preference of a given species for specific mesohabitat or microhabitat types (Bovee, 1982, 1986). According to Wootton (1998) heterogeneous systems supply a large variety of microhabitats with sites where fish species can shelter against predators and find a wide variety of feeding resources and places for reproduction. Recently, some studies using habitat simulation models have reinforced that physical habitat variables and cover availability determine patterns of habitat use in rivers and streams (Lee & Suen, 2012; Costa *et al*., 2012; Teresa & Casatti, 2013; Costa *et al*., 2013). However, the capability of prediction of these models depends on the understanding of how the species respond to environmental gradient conditions. Such information is usually obtained by means of statistical analysis developing Habitat Suitability Curves (HSCs), or multivariate models.

The efforts to develop models that present the real habitat use (*i.e.,* at the mesohabitat or the microhabitat scales) by multiple fish species have increased due to degradation of hydric resources and increased biodiversity impoverishment (*e.g.* Lamouroux *et al*., 1999; Vadas & Orth, 2001; Strakosh *et al*., 2003). In the United States, the IFIM methodology was created at the end of the seventies (*e.g.* Bovee & Cochnauer, 1977) and rapidly spread out in various environmental institutions as a tool to support river management and water resources planning. In many cases its spread was favored by its inclusion in specific legislative frameworks (García de Jalón, 2003) such as the European Water Framework Directive (Directive 2000/60/Ec, 2000) and the corresponding transposition to national bills.

Studies developing HSCs for Neotropical fish species are rare in Brazil (Pelissari & Sarmento, 2003; Leal *et al*., 2011; Costa *et al*., 2013; Teresa & Casatti, 2013) and their results are incipient in relation to the use of such curves for physical habitat simulation to determine the minimum flow for a river or stream. Moreover, some concerns should be raised because changes in natural flow regimes are occurring in a disorderly manner, and hydrological alteration is among the main anthropogenic threats to conservation of Neotropical fish species in lotic systems (Barletta *et al.,* 2010). Conversely, in other countries such as United States of America, United Kingdom or Spain where habitat simulation have been applied to different configuration in habitat analysis (García de Jalón, 2003; Acreman & Dunbar, 2004; Krstolic *et al*., 2006), a considerable number of microhabitat studies have been carried out in the last decades (Bowen *et al*., 2001; Armstrong *et al*., 2001; Martínez- Capel *et al*., 2009, 2011; Muñoz-Mas *et al*., 2014a).

Atlantic Forest streams, such as the riacho São Pedro, represents a good opportunity to study e-flows, mainly because part of its waters have been diverted to public supply and because of its location within an environmental protected area – the Tinguá Biological Reserve, with well-preserved conditions in the upper mountain reaches. The main objectives of this study were: (i) to develop habitat suitability curves of microhabitat use for three fish species representative of the fish community in riacho São Pedro; (ii) to apply the physical habitat simulation in this stream; and (iii) to assess the relationship between flow rate and habitat availability for the three species and the consequences for water and habitat management.

Materials and Methods

Study area. Was carried out in the riacho São Pedro (22º 35’ S 43º 33’ W), a second order tributary of the rio Guandu basin, located in the metropolitan area of Rio de Janeiro, RJ, Brazil. The riacho São Pedro basin drains an area of 98 km^2 and has a length of 28 km, flowing into the upper reaches of the rio Guandu (Fig. 1). Its natural flow is, approximately, 1.8 m^3/s and ca. 0.58 m^3/s (measured “*in situ*”) is diverted by a small substation of CEDAE (Water and Sewer Treatment Works of Rio de Janeiro State) in the upper reaches (above the study area) for supplying some municipalities nearby. This abstraction is always lower that the mean monthly stream flow, therefore the stream flow pattern is similar to the natural flow regime throughout the year. The climate is mild, subtropical with dry winters and wet hot summers in a forested region, according to Köppen-Geiger classification. Annual average rainfall ranges from 1,000 to 2,300 mm, with the mountain areas having comparatively higher rainfall. The highest rainfall occurs from December to March and the driest period from June to August (Rio de Janeiro, 2001). Two well-preserved stream reaches in the mountain area within the Tinguá Biological Reserve were studied.

Field work for fish habitat suitability/ Microhabitat suitability for fish/ Microhabitat selection by fish. The method for determining the microhabitat use by the three fish species was designed with an approach of equal effort (Bovee *et al.,* 1998), in order to produce HSCs without the over-correction of the preference curves (Payne & Allen, 2009). In our study, the design was done to obtain habitat use curves (category II ½). All the habitats were visually classified into three classes; deep-slow, deep-fast and shallow-fast. The class shallow-slow was not recorded in the study area. We selected similar areas of each of the habitat classes (with error estimated of ± 10 %), which were surveyed by snorkeling to observe fish locations.

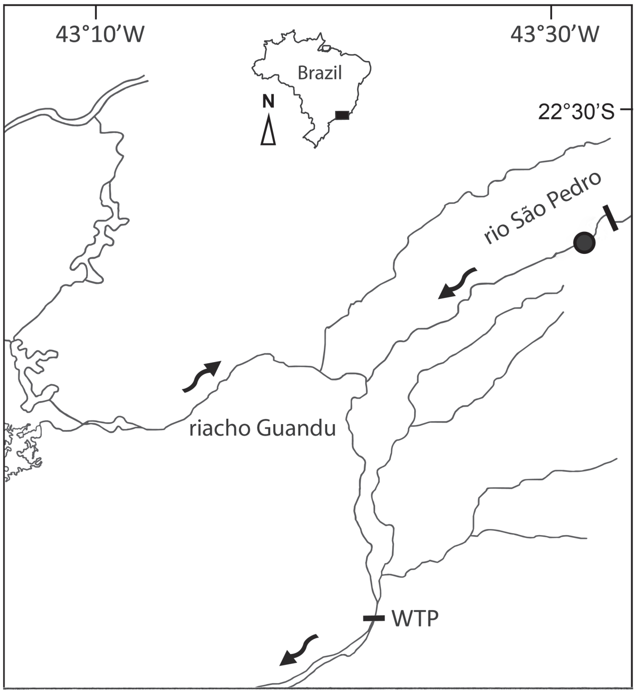

Fig. 1. Map of the rio Guandu basin, with indication of the studied reach in riacho São Pedro. Solid dash = small substation of CEDAE (Water and Sewer Treatment Works of Rio de Janeiro State). WTP: Water Treatment Plant.

Three common fish species of the Atlantic Forest streams were studied. In the selection of target species we followed three criteria adapted from Bovee (1986): (i) ecological importance – abundant in the study area; (ii) river adaptation – inhabit the river reach during most of life cycle; and (iii) availability of information in the literature. *Bryconamericus ornaticeps* Bizerril & Perez-Neto, 1995 is a small-bodied Characiformes of the family Characidae (10 about cm total length) that is part of a group of 15 species described for the Atlantic streams from Southern and Southeastern Brazil (Silva, 1997). Species of the genus *Bryconamericus* (*e.g. Bryconamericus stramineus*) are able to swim faster than individuals of other species of similar length (Castro *et al.,* 2010). Sampaio *et al.* (2012) comparing swimming performance of five characin species found that *B. stramineus* ranked among the highest speed values. In general, characin species are omnivores, visually dependent to feed, showing high feeding plasticity, capturing prey both at the water column as well as in the substratum (Ferreira *et al.,* 2007). *Ancistrus multispinis* (Regan, 1912) is a medium-sized catfish of the Loricariidae family that reaches up to 14.2 cm of total length (Fisch-Muller, 2003). This species has a specialized buccal apparatus that helps to adhere to hard substratum (rocky, stones and logs) (Geerinckx *et al*., 2008), dwelling small rivers and streams (Oyakawa *et al.,* 2006) and having herbivore habits (Assis *et al*., 2009). *Geophagus brasiliensis* (Quoy & Gaimard, 1824) is a medium-sized Perciformes of the Family Cichlidae reaching up to 22.5 cm of total length (Costa *et al*., 2015). This species shows territorial defensive behavior (Paraguassú *et al*., 2005), with omnivore-opportunist habits, occupying pools with diurnal activity and visual orientation (Sabino & Castro, 1990).

Direct underwater observation by snorkeling was applied to identify fish presence/absence at the microhabitat scale. The two reaches (upper and lower) were studied in two seasons to characterize the real fish behavior and the position in the water column (Heggenes *et al*., 1990; Martínez-Capel & García de Jalón Lastra, 1999; Martínez-Capel *et al*., 2009). The observations were made in cross-sections or transects in the stream channel; at the beginning of each observation, the observer waited for 10 minutes downstream of each reach to allow the fish to get used to the presence of the observer. Then, the diver moved slowly zigzagging in a systematic way (covering a virtual transect related to wet perimeter). After the fish or school was observed for some time (≅ 2 minutes) showing undisturbed behavior, the observer recorded the presence of the species/school in a subaquatic slate, the number of individuals and behavior (feeding or holding position). After the observation procedure, the position of each fish species or school was marked with a small weight with a flag. All observations were recorded between 08:00 and 16:00 hours totaling 60 hours of snorkeling, and individual observation lasting between 60 and 90 minutes per transect, depending on the number of fish recorded. After completing the observations in a group of transects, the marks were found again and the hydraulic variables were recorded at each of the marks' locations, *i.e.* depth, mean flow velocity (average of three measurements at sub-surface, middle depth, and near to the bottom) and type of substrate (within a 0.5 x 0.5 m square centered in the marker).

Survey of the stream channel and hydraulics. The surveys were conducted in two seasons, a wet season in 2013, and a dry season in 2014, covering two reaches (upper and lower) in the headwaters segment of the riacho São Pedro. The two reaches had ca. 100 m long and were 50 m far apart. In each reach, cross-sections were established to survey the topographic profile and the stream flow. Water levels were also measured for each section in order to calibrate the hydraulic component in the physical habitat simulation. The topographic survey was performed using a Leyca© total station and data were depurated using the software GNSS Solution and Data GeoOffice.

In all the cross-sections (nine in the upper reach and ten in the lower reach) the wet perimeter was virtually divided in 0.5 x 0.5m cells for measuring the hydraulic variables (depth, velocity and type of substrate) and to perform the subaquatic observation of the target species. The types of mesohabitat units in each of the stream reaches were determined by visual observation and also based on the hydraulic characteristics at the cross-sections. Depth was measured with a wading rod (at the nearest cm). Mean velocity measurements were performed concomitant with depth measurements in all cells using a flow meter (model Flowatch FL-K2) whereas the type of dominant substrate in each cell was determined visually by adapting the Krstolic *et al.* (2006) protocol. The substrate was classified as: large boulder, 1000 - 450 mm; boulder, 450 - 180 mm; large cobble, 180 - 25 mm; gravel, 25 - 12 mm; fine gravel, 12 - 2 mm; sand, < 2 mm and vegetation debris. The complete velocity profile in each transect was performed in one survey only ("1-vel" simulation method) whereas the water level was measured twice.

Development of Habitat Suitability Curves - HSCs. The database for the development of Habitat Suitability Curves (HSCs) consisted of the microhabitat data at the locations where fish were present, for each fish species separately. First, a frequency analysis by intervals or classes for each microhabitat variable, *i.e.*, depth, mean velocity, and type of substrate was performed. Depth was classified at intervals of 5 cm and mean flow velocity of 5 cm/s whereas the substrate types were analyzed following the aforementioned classification. The frequencies of fish observed at each interval of a variable were plotted in the form of histograms. For each microhabitat variable, such frequencies were tested with the chi-squared test to assess eventual differences between the upper and the lower stream reach. The histogram for each variable was normalized from zero (minimum suitability or intolerable for the fish) to one (maximum suitability) and converted into a continuous curve in the cases of continuous variables corresponding to category II ½ HSCs (Bovee *et al*., 1998; Payne & Allen, 2009). According to the knowledge about the target species, such curves were smoothed to fill gaps due to missing values and, finally, the tail of each curve (right end) was also corrected by applying and expert-knowledge-based modification out of the range of the survey. Then a minimum constant suitability index for mean flow velocity and depth was assigned (0.2 and 0.4, respectively). Likewise a minimum suitability of 0.2 was assigned to the substrate types with suitability lower than this value (Muñoz-Mas *et al*., 2012).

One-dimensional physical habitat simulation. The flow measurements of each survey were made in the same stream reaches where the microhabitat measurements were carried out, aiming at performing the physical habitat simulation for each reach independently. The stream flow rate obtained from the measurements at the cross-sections, and the water level, allowed us the calibration of the model and calculation of the rating curves at each cross-section, in the software RHYHABSIM (Jowett, 1989) for physical habitat simulation. For a set of simulation flows, the model provides with the depth, mean flow velocity and channel attributes (substrate only, in this case) for each cell of the model at each flow rate. Based on the aforementioned HSCs, the model assesses the suitability for each fish species and variable at each cell. Finally, the software combines (by product in this case) the suitability indices of the three variables to determine the composed suitability index (HSI_c). This adimensional index (ranging from 0 to 1) is expressed as follows:

$$HSIc_{q,i} = (\mathrm{hsi}_{q,i,v})_s \cdot (hsi_{q,i,d})s \cdot (hsi_{q,i,d})_s \cdot (hsi_{q,i,ts})_s$$

where:
$HSIc_{q,i}$ = index of suitability composed of the cell (i), flow (q) and species (s)
$hsi_{q,i,v}$ = index of suitability of mean flow velocity for the cell (i), flow (q) and species (s)
$hsi_{q,i,d}$= index of suitability of depth for the cell (i), flow (q) and species (s)
$hsi_{q,i,ts}$ = index of suitability of the type of substrate in the cell (i), flow (q) and species (s)

When the HSI_c of all the cells in the model are averaged (for the whole stream reach) the indicator HSI for each flow rate is obtained, and can be presented in the form of curves as a function of the flow rate whereas when the HSI_c of a cell is multiplied by the area representative of each cell (a_i), corresponds to the weighted suitability. The sum of the weighted suitability for the whole stream reach gives and indicator of the overall microhabitat suitability, for a stream flow rate and a fish species. This value is named Weighted Usable Area (WUA), expressed by the equation:

$$\mathrm{WUA}_{q,s} = \sum(\mathrm{HSIc}_{q,i}) * (a_{q,i})$$

where:
$\mathrm{WUA}_{q,s}$ = index of weighted suitability for the representative stream reach in flow (q) and species (s).

This index of microhabitat (WUA) combines area and quality (suitability) of the stream habitats to sustain one aquatic species in a given phase of its life cycle. The WUA was calculated by the RHYHABSIM software for the set of simulated flows, resulting in the relationship between stream flow and WUA.

Brazil lacks of specific legislation on e-flow assessment based on physical habitat simulation therefore the recommendations included in the Spanish norm for hydrologic planning (España, 2008) were used to infer the critical values of the environmental flow regime. This norm establishes that the minimum e-flow should correspond with 50 % to 80 % of the maximum WUA. If no maximum e-flow could be observed in the WUA-Flow curve and it presents a monotonic increment, the inflection point should determine the minimum e-flow.

Results

The profiles for the two stream reaches are shown in Fig. 2. The upper reach consisted of deep-fast habitats (depth > 0.5 m and velocity ≥ 0.1 m/s), its water surface area was 205 m^2 and the average wet perimeter was 10.05 m per transect. The lower reach had two types of mesohabitat units, deep-slow in its upper part (depth > 0.5 m and velocity < 0.1 m/s) with area of 191 m^2 and average wet perimeter of 10 m; and the shallow-fast in the lower part (depth ≤ 0.5 m and velocity ≥ 0.1 m/s) with area of 210 m^2 and average wet perimeter of 18 m (Fig. 2).

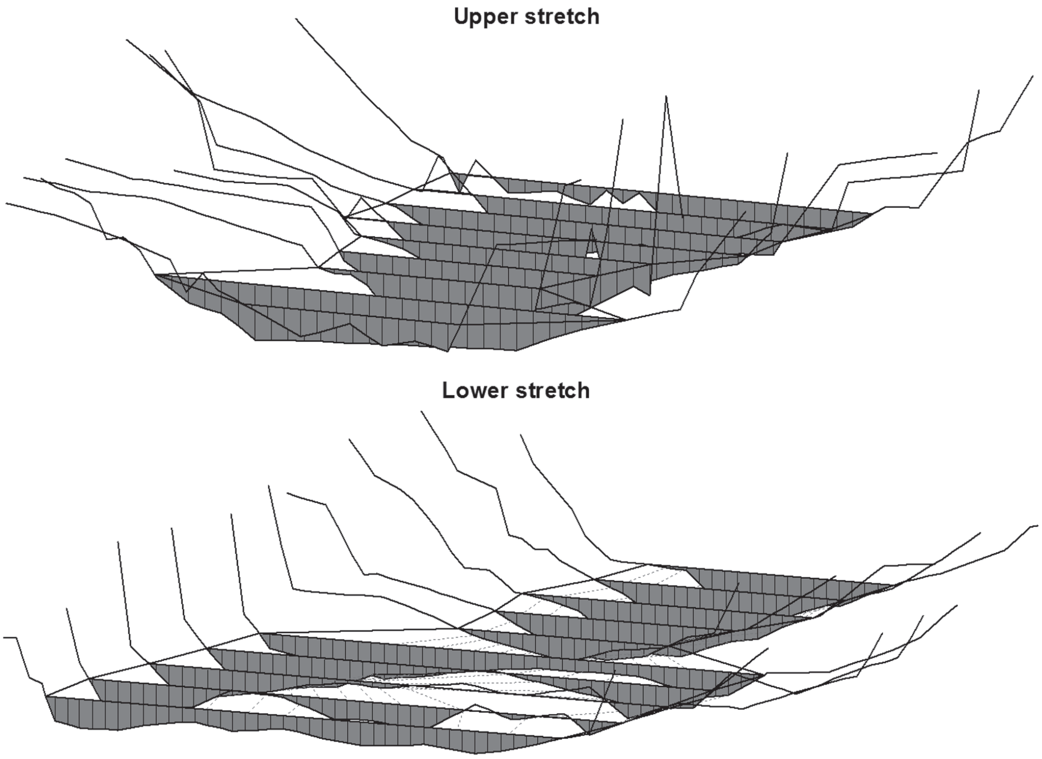

Fig. 2. Isometric view of the cross-sections in the two target reaches of the riacho São Pedro. Grey color indicates water; solid line indicates the contour of the cross-section (in wet areas and banks).

The hydraulic characteristics for both reaches were pooled together for a brief description of the study area (Fig. 3). Depth ranged from 0.25 to 1.25 m, with a single mode of *ca.* 0.65 m. Velocity ranged from 0.05 to 0.65 m s^{-1}, with 70 % of data showing mean velocity of 0.05 and 0.1 m s^{-1}. Type of substrate had two modes, *i.e.* large boulder and sand. Boulder also had a relative relevance with 25% of the total frequency; the remaining types of substrate had frequencies lower than 10 %.

During the two field surveys for assessing fish habitat suitability, a total of 335 fish observations were carried out, as follows: *A. multispinis* (presence data = 123; absence data = 212); *B. ornaticeps* (presence data = 120; absence data = 215); and *G. brasiliensis* (presence data = 92; absence data = 242). No significant differences in the frequencies of the number of individuals, for each habitat variable, were found between the two stream reaches (p >0,05). Therefore, all the data of microhabitat use were pooled together in a single dataset for further analysis.

The Habitat Suitability Curves (Fig. 4) for depth showed that *B. ornaticeps* and *A. multispinis* selected microhabitats with small/medium depth (0.6 m), whereas *G. brasiliensis* showed a more ample plasticity for this variable and was associated to both shallow (0.35 m) and deep (0.9 m) areas. Concerning mean velocity, all the three species were mainly observed in reaches with velocities near to 0.1 m/s, (Fig. 4). The curves for substrate type indicated that *B. ornaticeps* was associated with a wide variety of substrate classes, such as large boulders, boulders, cobble and sand. The curves for *A. multispinis* indicated preference for large boulders and boulders, whereas *G. brasiliensis* for large boulders only (Fig. 4).

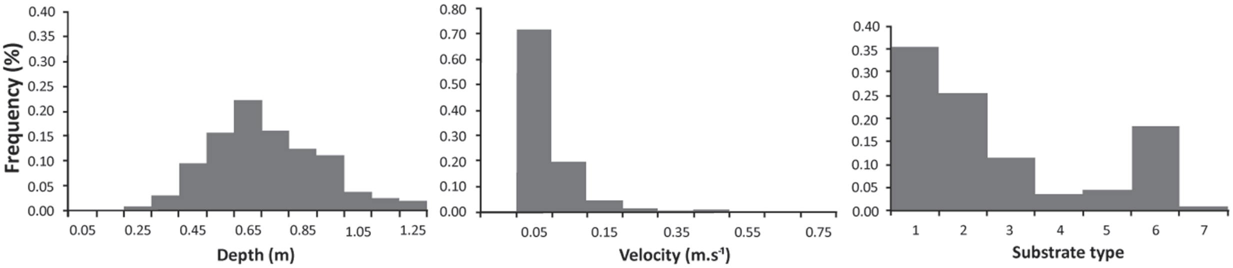

Fig. 3. Frequencies of habitat availability by classes of depth (m), mean velocity (m/s) and type of substrate (1- large boulder; 2- boulder; 3- cobble; 4- gravel; 5- fine gravel; 6- sand and 7- vegetation), obtained by direct survey in the riacho São Pedro.

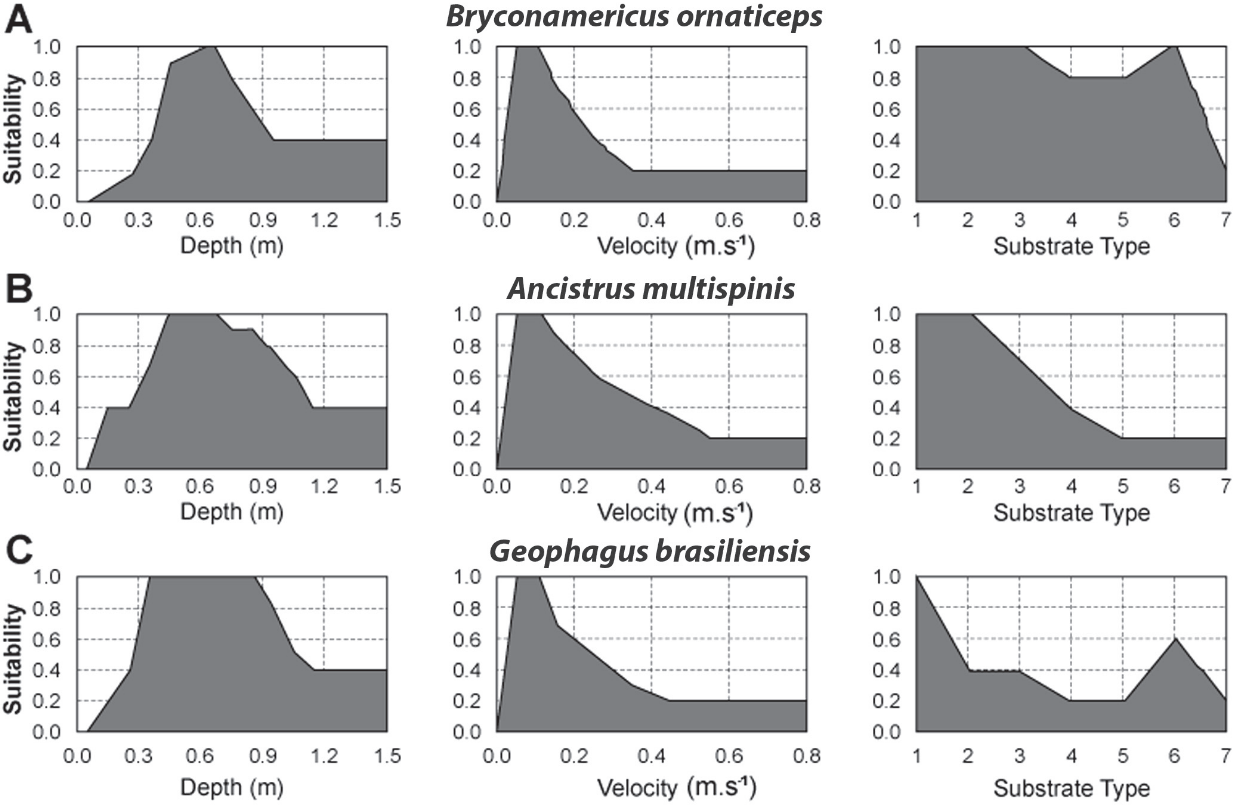

Fig. 4. Habitat suitability curves for depth (m), mean water column velocity (m/s) and type of substrate for the three fish species in the riacho São Pedro. Substrate types are; 1- large boulder, 2- boulder, 3- cobble, 4- gravel, 5- fine gravel, 6- sand and 7- vegetation.

The average flow rate used in both hydraulic and habitat models were: 1) upper reach in the 1st survey (dry season) = 0.124 m^3/s; in 2nd survey (wet season) = 1.102 m^3/s; 2) lower reach: 1st survey = 0.123 m^3/s; in 2nd survey = 0.965 m^3/s. The HSI for the upper reach (Fig. 5) showed maximum values at flow rates higher than 0.75 m^3/s for *B. ornaticeps*, between 0.45 and 0.75 m^3/s for *A. multispinis* and at flows higher than 0.55 m^3/s for *G. brasiliensis*.

Unlike the upper reach, the lower reach did not show a clear asymptote for any fish species. Nevertheless, the highest values of HSI were relatively stable over 0.65 m^3/s for *B. ornaticeps*, over 0.75 m^3/s for *A. multispinis* and over 0.85 m^3/s for *G. brasiliensis*. Considering both reaches, there was an overlap of very high HSI for the three fish species between 0.65 and 0.85 m^3/s.

The relationships between WUA and flow indicated the following optimum or asymptotic values (Fig. 6). In the upper reach, the maximum WUA were ca. 3.19 m^2/m with an asymptotic trend (at $Q \geq 1.15$ m^3/s) for *A. multispinis,* and 3.34 m^2/m ($Q \geq 0.95$ m^3/s) for *G. brasiliensis*; the curve for *B. ornaticeps* did not show an asymptote but a change of gradient at 0.25 m^3/s and a gradual increase up to the maximum flow rate. Likewise, in the lower reach there were changes in gradient as the flow increased, but WUA did not show a clear trend or an asymptote. Such changes in gradient occurred at 0.55 m^3/s in the curves of the three fish species. Due to the monotonous increase of the WUA with flow in the lower reach, the flow rate that maximizes the WUA is the highest flow simulated, which is not consistent with the upper reach. The maximum values of WUA were always lower for *A. multispinis* in comparison with the other two fish species.

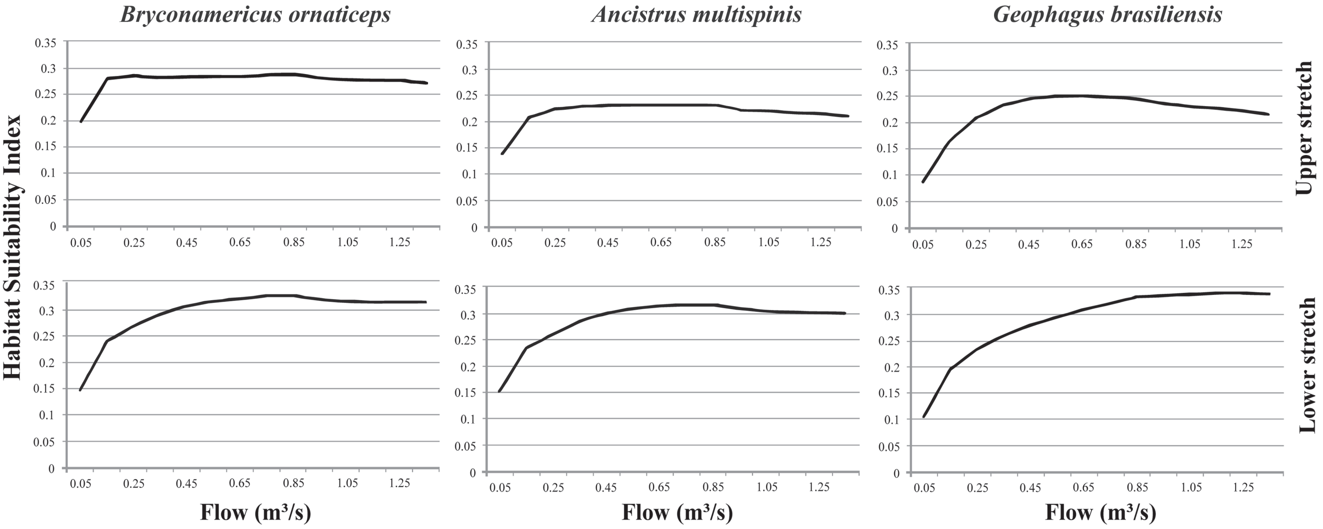

Fig. 5. Average habitat suitability index –HSI– in the upper and lower reach of the riacho São Pedro, for the three fish species.

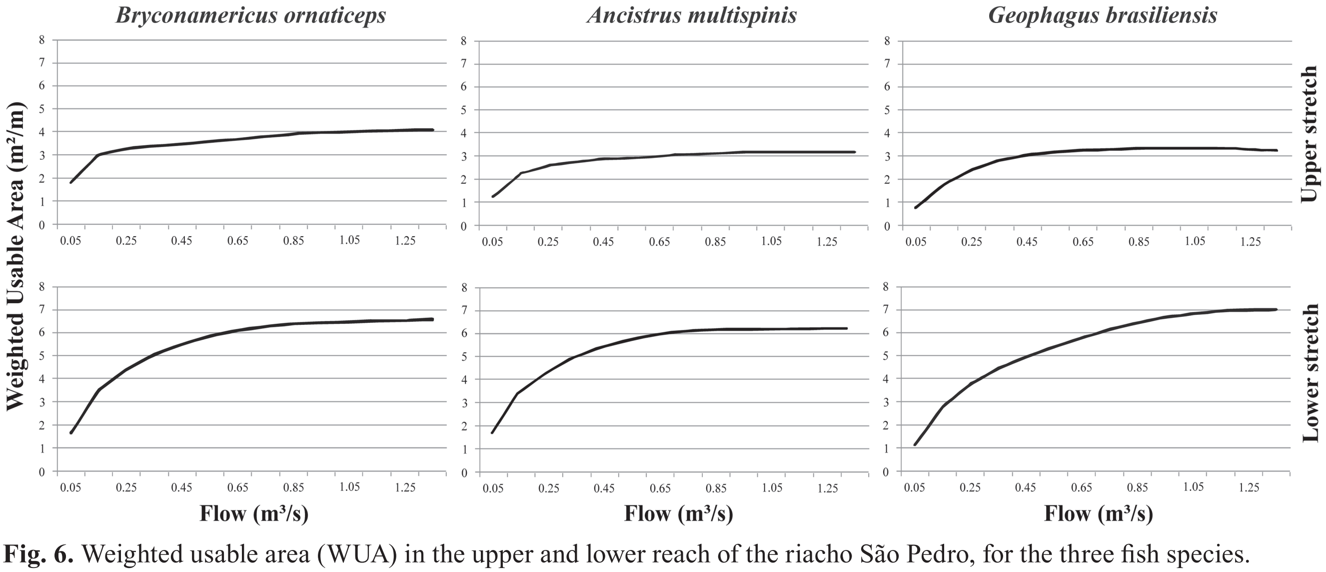

Fig. 6. Weighted usable area (WUA) in the upper and lower reach of the riacho São Pedro, for the three fish species.

Discussion

Habitat use by the three fish species. The patterns of microhabitat use by the three native fish species were analyzed in the riacho São Pedro. Overall, these species appear to select their habitats according to specific microhabitat characteristics related to hydraulics (depth, velocity, type of substrate), highlighting the importance of hydraulic variables as predictors of patterns of species distribution in lotic systems (Vadas & Orth, 2001; Schwartz & Herricks, 2008; Leal *et al*., 2011). We observed that the three species exhibited selection for distinct habitats, but with considerable overlap. As the HSC are fundamental in the physical habitat simulation, the WUA-flow curves also indicated some differences in habitat suitability for the three species as the flow rate increases.

The main type of available habitats and the microhabitat partition by the three fish species in this Atlantic Forest stream were assessed in this study; *Bryconamericus ornaticeps* showed the narrowest range of habitat selection for depth whereas *G. brasiliensis* had the widest range. An opposite pattern was observed for the type of substrate, with *B. ornaticeps* occurring in different substrate types including large boulders, boulders, cobble and sand; whereas *G. brasiliensis* was restricted to a single type of substrate (large boulders). *Bryconamericus ornaticeps* is a water column dweller (Nelson, 2006), therefore, no clear relationship with the type of substrate should be expected, but in most of *in situ* observations this fish was associated with runs and riffles habitats, which were observed at the deep-fast upper reach. On the other hand, *G. brasiliensis* had an ample adaptive plasticity that enables this species to occur in a wide variety of mesohabitat types (Sabino & Castro, 1990; Meschiatti, 1995; Aguiaro & Caramaschi, 1998; Agostinho & Júlio Jr., 1999; Vono & Barbosa, 2001) including large boulders and boulders that ensure high habitat structuration (*i.e.,* deep-slow areas typical of the upper reach and the upper part of the lower reach), as well as at the proximity of sandy banks with organic matter such as twigs and leaf litter (*i.e.,* the shallow-fast areas of the lower part of the lower reach). Furthermore, *A. multispinis* was closely associated to the bottom, favored by its flatted body and ventral funnel-like sucking mouth to adhere to boulders and large boulders (Geerinckx *et al.,* 2008). Such characteristics enable this species to explore a particular niche dimension of both reaches.

Rabeni & Jacobson (1993) showed that the distribution and abundance of fish is influenced by the depth, velocity and substrate type, and also responding to specific combinations of these variables. Our results suggest that the depth and substrate type are more determinant of the microhabitat selection for the three species, in comparison with mean velocity. Diverse studies in different types of rivers have supported the idea that depth can be the key variable to understand the microhabitat selection by some fish species, especially cyprinids (Copp & Jurajda, 1993; Grossman & De Sostoa, 1994; Martínez-Capel *et al*., 2009). The substrate composition is partially related with the feeding habits of the species, and this can be related with the position in the water column (Martínez-Capel *et al*., 2009). Another important factor determining substrate type is the stream slope. The longitudinal profiles in riacho São Pedro is formed by interspersion of rapids and pools maintaining the system connection. Accumulation of leafs and logs in pools create diverse microhabitats and substrates for fishes (Crook & Robertson, 1999), whereas in rapids and runs the presence of periphyton and microalgae on the substrate determine the pattern of microhabitat use (Schneider & Winemiller, 2008).

We observed lower flow in both season, which limit all understanding of flow variation. Seasons of high flow, with larger habitat heterogeneity, mean velocity, depth and position in the water column can be fundamental to understand the habitat partitioning in the fish community, as it was demonstrated for three different fish species in Iberian streams with larger habitat variability (Martinez-Capel *et al*., 2009). We consider that habitat partitioning is a relevant aspect of further research on the ecology of Neotropical fish communities, which could be addressed in future studies under a wide range of flow conditions (Leal *et al*., 2011; Costa *et al*., 2013; Teresa & Casatti, 2013). Physical habitat simulation encompasses characteristics of low and high water level, favoring more tolerant species in dry season, and more competitive species in wet season when a wide range of microhabitats are available.

The variability in abundance is also a result of species association with limited habitat diversity. Specialization in habitat use varies among fish species with some species found in almost all sites whereas others have preferences for very specific conditions, such as a particular type of substrate or narrow range of depth or velocity. The fish morphology is frequently interpreted as a series of physic attributes related to habitat use (Wood & Bain, 1995; Blanck *et al*., 2007; Leal *et al*., 2011). Moreover, fish size determines habitat selection (*e.g.* shallow or deep, slow or fast) as part of a strategy to avoid predation (Power, 1984) and/or facilitates obtaining food resources (Rincón *et al*., 2002). In this study we did not consider fish size classes because there is not literature available on the biological characteristics of these species, such as the size at the young of the year, juveniles or adults or size at first maturity. However, the range of sizes of the three species was relatively narrow.

The three targeted species have distinct morphological characteristics with divergent responses in reference to microhabitat use. For example *B. ornaticeps* has fusiform body, forked caudal fin that confer good swimming ability to explore the water column (Castro *et al*., 2010). *Ancistrus multispinis* has a flatted body, a ventrally placed suckermouth and the ability to use the fins to move and adhere to hard substrate, a hydrodynamic advantage that reduces current drag when the fish is positioned against substrate in fast-flowing (Geerinckx *et al*., 2008). *Geophagus brasiliensis* is characterized by a compressed and deep body, pectoral fins in lateral position that confer great maneuver capacity and vertical movements, allowing to explore both lotic and lentic systems (Oyakawa *et al*., 2006).

Habitat suitability curves. The three fish species showed different Habitat Suitability Curves for depth and type of substrate, with a considerable range of selection, whereas the velocity curves were similar for the three species with a narrow range of variation. Since the application of this methodology is uncommon in Brazil, there are not HSCs for these species that could be used to establish comparisons. However, when the velocity curves were compared with other species, we realized that the range of variation is narrow and consequently the assessments of habitat suitability are very sensitive to this limitation (Lamouroux & Capra, 2002; Martínez-Capel *et al*., 2011). Such narrow "optimum" velocity for the three species, the depth for *B. ornaticeps*, the type of substrate for *G. brasiliensis*, and the very steep parts of the curves (*e.g.* for very low velocity) can yield habitat assessments very sensitive to small errors of hydraulic modeling.

In this work, the input of expert-knowledge was limited to minimum, in order to show and discuss the empirical data obtained. Muñoz-Mas *et al.* (2012) pointed the necessity of integrating knowledge of specialist, coupled with empirical data in habitat suitability modelling, specially where data are scarce (*e.g.* high flows) and/or when scientific information is available in different format of limited access. Both techniques, field searches and specialist knowledge, should corroborate for the development of more reliable habitat suitability models (Mouton *et al*., 2011). Therefore, the acquisition of more data around the whole river basin, in different streams and rivers of different order, is a very important aspect that must be considered for future research, as well as for the application of e-flows in the frame of the water resources management in the rio Guandu basin.

From a regional perspective, the curves could be validated in similar river systems containing the same species and similar flow regime (0.12 to 1.10 m^3/s). A process of spatial explicit validation (e.g. Muñoz-Mas *et al*., 2014a) or a transferability test (*e.g.* Thomas & Bovee, 1993) should be followed in that case. However, for an effective application, other issues should be considered, for instance: season (specially the dry season), mean water temperature, hydraulic gradient, dominant types of substrate and species composition. In that case, with a relatively reduced number of observations, the HSCs presented here could be validated and further improved or generalized, thus they could be applied in rivers with similar morphological and ecological characteristics.

The HSIs were developed for periods of low flows (even in the wet season the flow was relatively low), aiming at determining minimum e-flows capable to maintain the fluvial ecosystem functioning, composition and structure similar to natural condition. Moreover, the establishment of e-flow regimes also should consider periods of high flow that adjust to natural variation, in order to remove sediments, shape the river channel, provide migration and spawning cues for some fish, and made available areas for species reproduction and feeding (Richter *et al*., 2006; Schwartz & Herricks, 2008). Furthermore, it will be fundamental to apply holistic approaches to maintain or partially restore important characteristics of the natural flow regime required to maintain the biophysical components and ecological processes of rivers, floodplains, aquifers and estuaries in this river basin (Arthington & Pusey, 2003; King & Brown, 2006; Poff *et al*., 2010). Therefore, the e-flow regime is justified for fauna and flora preservation, as well as for the maintenance of fluvial environment integrity.

Relationships WUA *versus* flow. The average habitat suitability HSI is higher in the lower reach for the three species and its values stabilize at higher flows in comparison with the upper reach. We found that the WUA curves for *A. multispinis* and *G. brasiliensis* tend to stabilize above ca. 0.85 m^3/s in the upper reach, but did not reach an asymptote in the lower reach. This pattern is similar for *B. ornaticeps* in both reaches. The range of flow rate of 0.65 - 0.85 m^3/s was associated to maximum habitat quality for the three species, in terms of HSI; the WUA indicated stable values above 0.85 m^3/s in the upper reach only, and the lower reach showed a change of gradient at 0.55 m^3/s with steeper curves below this threshold. Due to the sensitivity of habitat to the flow reduction, in general the proposal of interim minimum flows should not consider values below this threshold. However, this value is far above the 80% of maximum WUA, corresponding to a flow rate of 0.32 m^3/s; this flow rate would meet or exceed that percentage for the three species in the upper reach. In the lower reach, this percentage corresponded to 0.60 m^3/s.

The flow rate of 0.32 m^3/s is approximately three times the flow rate observed during the hydraulic survey; therefore, so far in this study, a critical flow rate of 0.32 m^3/s could be considered as an interim minimum flow, or *minimum-minimorum* during the dry season, until further studies of fish habitat and stream hydrology could indicate more specific values of suitable flows to conserve the ichthyofauna and other aquatic organisms. For a second order stream like riacho São Pedro, with an average flow of 1.8 m^3/s and several water abstractions, this value might be reasonable for the maintenance of the system integrity during period of reduced flow. The critical value of 0.55 m^3/s could be considered as the minimum flow in wet years during the low flow period (dry season).

These values cannot be considered as a permanent proposal for e-flows, since there is lack of robust and historical hydrological data to infer the natural flow regime, and consequently, the expected average flows during the dry season with minimum flows. The natural flow regime, with characteristic intra and inter-annual flow variability is a primary driver of the structure and function of riverine ecosystems and many of the adaptations of its biota (Poff *et al*., 1997; Lytle & Poff, 2004; Naiman *et al*., 2008). Due to the lack of suitable gauged flow data, the calibration of a rainfall-runoff model for the riacho São Pedro basin will be fundamental, which is actually considered a critical step of further research.

Therefore, these interim recommendations do not mean to neglect the need of validation in the near future. Further studies should validate these values, considering at least the following criteria; meaning of the minimum flows in the context of the natural flow regime; capability to maintain habitat connectivity for the fish species migrating during the dry season (if any); and provision of suitable habitat for the fish spawning in the dry season.

An objective of the physical habitat simulation (according to the IFIM methodology) is the development of a functional relationship between physical habitat parameters at different scales and flow regime. This functional relationship between flow and weighted usable area (WUA) enables the integration of the microhabitat with the macrohabitat as well as the assessment of the potential effect of flow regime using time series of stream flow (Payne, 2007). The three studied species showed different variations in WUA dependent on the flow. Regarding site-specific effects, flow-habitat curves (Q-WUA) showed a higher gradient of the WUA in the lower reach in comparison with the upper one for *A. multispinis* and *B. ornaticeps*, but such a difference was less relevant for *G. brasiliensis*. Further studies on influences of flow on WUA are needed to best clarify the causes of these findings.

Although the relationships are a fundamental part of the study, it is necessary to establish further research steps. In this regard, the hydrological simulation of the stream basin will make possible to evaluate time series of WUA in a reference (natural) condition in comparison with different scenarios of regulation, water abstraction or climatic changes. These scenarios based on curves of total habitat, habitat duration curves and other indicators illustrated in specific documentation (Milhous *et al.,* 1990; Bovee *et al.,* 1998) have been incorporated in tools of e-flows (Payne & Jowett, 2012) and recently in decision support systems for water management (Paredes-Arquiola *et al*., 2013, 2014). The hydrological assessment will also allow the consideration of very important components of the flow regime, such as the flow pulses, the small floods and large floods, with specific importance for the conservation of the aquatic biodiversity. Such considerations are especially included in the implementation of holistic approaches of e-flows, such as the Building Block Methodology - BBM (King *et al.,* 2008) and ELOHA (Poff *et al*., 2010).

On the other hand, such criteria of optimization used to favor biodiversity require adjustment and consultation to specialist in distinct studied areas to assure that all requirements of maintenance of biotic communities are addressed, because the method is targeted to habitat assessment within the limits of each hydrographic basin.

In Rio de Janeiro State, $Q_{7,10}$ has been referred as the percentage (50%) of minimum average flow, along seven consecutive years and 10 year of return interval. In other words, the procedure adopted in this State as flow acceptable to remain in the hydrological system is approximately the half of the average flow observed during the highest dry yearly period with recurrence of 10 years. If one applies the 50% of $Q_{7,10}$ in the studied area to assess the maintenance of fish species in the system, we would adopt a flow of ca. 0.05 m^3/s, based on the minimum observed in the present study during the field work (0.1 m^3/s). The e-flow calculated to ensure high habitat values (at least 80% of maximum WUA) for the three species was approximately 0.32 m^3/s in the upper reach, that is, a flow of six times higher than the 50% of $Q_{7,10}$. In our opinion, with such a low flow of 0.05 m^3/s the species are likely to be unable to complete their life cycles. Thus ending up in a strong anthropogenic pressure that hardly would allow viable populations of native fish.

Consequently, the minimum and the optimum values for the maintenance of the stream habitat for the three fish species could be the fundamental blocks to produce an interim e-flow regime in the riacho São Pedro. Besides, more studies on this subject should be performed, aiming at the extensive determination of the habitat suitability models (univariate or multivariate) for stream species in different sub-basins, the consequent updating of Q-WUA curves, and the validation and correction of e-flows considering at least the different foregoing criteria. The regular monitoring of the aquatic and riparian communities is a fundamental step in the implementation of e-flows, since no method is able to predict a perfect and permanent flow regime under conditions of relevant flow regulation. The implementation of the e-flows presented here, together with regular improvements in the scientific and technical knowledge, should help the decision makers to manage the water resources. This approach also represents an initial effort to integrate this methodology to the present legislation and the multiple uses of the water, re-enforcing the scientific support for revising the actual water resources legislation.

In conclusion, the two main findings of this study could be stated as: (1) the three species showed distinct habitat selection in terms of depth and type of substrate. Such patterns of habitat selection at the microhabitat scale were also meaningful for habitat selection at the larger scale of mesohabitat units; *B. ornaticeps* was associated to the upper reach; *A. multispinis* occurred in both reaches always associated to boulders and large boulders; and *G. brasiliensis* was associated to deeper areas in both reaches and to shallow banks with organic matter in the lower organic matter (shallow-fast); (2) independently of the experimental nature of this study, the methodology of the physical habitat simulation succeeded to provide with a minimum flow (0.32 m^3/s) and optimum flow rate (0.85 m^3/s) which can constitute key values for setting e-flow regimes for the maintenance of three fish species in this Atlantic Forest Stream.

Further studies are necessary to develop habitat suitability curves and multivariate models for distinct species, based on experimental data and expert knowledge. The study of the natural flow regime in this river basin is also a fundamental step to improve the assessment of e-flows and to apply fundamental analysis complementing the physical habitat simulation in a long-term context. Further studies would allow the validation and correction of e-flows based on this study, considering different criteria such as habitat connectivity, fish migration and habitat for spawning.

The relevant stream flows obtained in the present study, during the dry season, can be used as building blocks for the construction of interim e-flow regimes in riacho São Pedro. Although more in-deep ecological studies are necessary to address different aspects of the flow regime and the aquatic communities, these flows can support the first proposal of an e-flow regime in the frame of an adaptive management process of the water resources. In such a process, the e-flow regime should be revisited and improved over time, based on new scientific knowledge, technical advances and a carefully-designed monitoring program of the status of the aquatic and riparian communities. The adoption of an adaptive management approach, including a consistent and holistic monitoring, should help the water managers to establish stream flow protection and support healthy rivers with the diverse social benefits they provide.

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