

Microhabitat segregation and fine ecomorphological dissimilarity between two closely phylogenetically related grazer fishes in an Atlantic Forest stream, Brazil

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Abstract Habitat segregation is considered the most prevalent resource-partitioning mechanism for stream fishes and the species morphology can be a strong predictor of their spatial distribution. However, most studies addressing morphology-habitat relationships have defined the space in physiognomically homogeneous units (i.e., mesohabitat), probably not detecting segregation among several closely related species. Here we investigated the ecomorphology and the use of habitat in a fine spatial scale (i.e., microhabitat) by two closely phylogenetically related grazer fishes (the loricariids *Parotocinclus maculicauda* and *Hisonotus notatus*), syntopic in an Atlantic Forest stream. We conducted standardized underwater observations in

two 50 m long stream sections differing in canopy condition, totaling 273 individual microhabitat records. We clearly detected microhabitat segregation between the species. In both sample sites, *H. notatus* remained near the stream banks and closer to shelters, while *P. maculicauda* predominantly occurred in more hydrodynamic microhabitats, facing higher focal current velocities and water turbulence. Differences in focal elevation and water depth (i.e., vertical segregation) were exclusively detected in the deforested site. The spatial segregation was congruent with slight interspecific morphological differences, being in accordance with hypotheses about form-function relationships previously reported for fishes. Given that the diel activity and diet of these grazer species were strongly overlapping, we believe that the observed microhabitat segregation favors resource partitioning between *P. maculicauda* and *H. notatus*, facilitating their coexistence in high abundances in the studied system. This study illustrates how the assessment of fine-tuned ecological processes can provide subsidy to management strategies aiming the conservation of tropical stream biodiversity.

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Introduction

One of the main goals of ecologists is to understand how similar species can be to one another and still coexist.

This question emerged after the proposal of competitive exclusion (Hardin 1960) and limiting similarity principles (MacArthur and Levins 1967), which postulate that the magnitude of competition between species is directly proportional to their phylogenetic or ecological closeness. Thus, species with very similar requirements could only coexist by partitioning resources. Among the several dimensions comprising the species ecological niche (sensu Hutchinson 1957), authors have recognized that assessing three of these dimensions (e.g., space, food, and time) is often sufficient to detect partial or non-niche overlap between syntopic species (Iglesias-Rios 2004). Particularly for stream fishes, habitat segregation is considered the most prevalent resource-partitioning mechanism (Grossman et al. 1998; Jackson et al. 2001).

In aquatic systems the use of resources is expected to be strongly correlated with the organism's morphology (Winston 1995). This form-function relationship makes ecomorphological analysis a reliable methodology to infer many aspects of fish's niche, such as ecological interactions and habitat preferences (Gatz 1979a; Winemiller 1991; Langerhans et al. 2003; Casatti and Castro 2006; Leal et al. 2010). However, most studies relating morphology and spatial distribution of fishes in streams and rivers have defined the space in physiognomically homogeneous units (i.e., mesohabitat scale, such as riffles, runs, and pools; Rezende et al. 2010; Kano et al. 2013). Consequently, they probably have not detected niche segregation among several closely related species or morphological types, which in fact tend to occupy similar mesohabitats (e.g., riffles predominately occupied by depressed-body species, whereas pools inhabited by deep-body ones). An assessment based on a finer spatial scale (i.e., microhabitat, where we refer to a set of parameters measured for independent focal individuals, such as water-column depth, vertical position, focal-point velocity, substrate, and cover; Rincón 1999) would potentially lead to different conclusions, given that even slight morphological differences might influence micro-spatial segregation, maximizing resource partitioning among species.

Considering the multiple threats faced by freshwater fishes, it has become urgent to clearly define the species functional roles within communities and their habitat requirements (Rosenfeld 2003; Mitchell 2005). For instance, precisely detecting how fish species occupy the space and share resources in streams may be of great value to build predictive models of winner vs. loser species after discharge reductions, streambed

sedimentation, and several other structural alterations that typically follow riverscape changes (e.g., Roth et al. 1996; Allan et al. 1997; Wilcove et al. 1998; Bojsen and Barriga 2002; Sutherland et al. 2002). Therefore, beyond all the theoretical implications, an approach drawing on the use of microhabitat may bring important insights to suggest priorities for the improved management of stream systems and their biodiversity. Such approach has been scarcely applied to tropical stream ichthyofauna (e.g., Romero and Casatti 2012).

Here we investigate the use of microhabitat by two grazer fishes, the loricariids *Parotocinclus maculicauda* (Steindachner 1877) and *Hisonotus notatus* Eigenmann and Eigenmann 1889, syntopic in an Atlantic Forest stream from southeastern Brazil. During 1 year of surveys, they constituted dominant species in the stream fish community (Leitão et al. unpubl. data). Considering their close phylogenetic relationship (Gauger and Buckup 2005; F. Martins, pers. comm.), and supposedly similar requirements, we hypothesized that *P. maculicauda* and *H. notatus* occupy distinct microhabitats, maintaining their high densities by spatial segregation. By performing an ecomorphological analysis, we examine key morphological traits that potentially favor this segregation. Complementarily, we assessed the diel activity and diet of the species to estimate the degree of interspecific overlapping in these two niche dimensions.

Material and methods

Study area

This study was conducted in Ouro stream (22°17'S 42°00'W), a fourth-order tributary of the Macaé River basin, northern Rio de Janeiro state. Two 50 m long stream stretches were sampled, selected for the presence and absence of riparian vegetation, termed Forested and Deforested (Fig. 1) site. The Forested site runs into an Atlantic Forest fragment (about 30 km perimeter) contiguous to a Conservation Unit (Parque Municipal do Atalaia). This site is characterized by a shaded stretch (>75 % mean canopy cover) with clear water and presence of rocks of different sizes, wood debris, and decaying leaves distributed over sandy bottom. The Deforested site, located 2 km downstream from Forested, runs through a pasture area with total absence of arboreal vegetation (0 % canopy cover). In this



Fig. 1 Forested (FOR) and Deforested (DEF) stretches sampled for the use of microhabitat by grazer fishes in Ouro stream, state of Rio de Janeiro, Brazil

section, the stream banks are completely occupied by grassy vegetation, and the substratum is mainly composed by sand and clay, with few hard structures in mid-channel restricted to scattered fragments of logs and branches. Both sites were less than 1 m deep and c. 7 m wide. Current velocity, discharge and limnological conditions, systematically measured within each sampling campaign, were similar between sites. Thus, besides the canopy openness the differences were restricted to the percentage of substrate categories (see Online Resource Table 1).

Microhabitat assessment

We made direct daytime observations of microhabitat use by the loricariids *Hisonotus notatus* and *Parotocinclus maculicauda* (Fig. 2) while snorkeling in an upstream direction. The entire area of each site, including shoreline and mid-channel, was sampled by

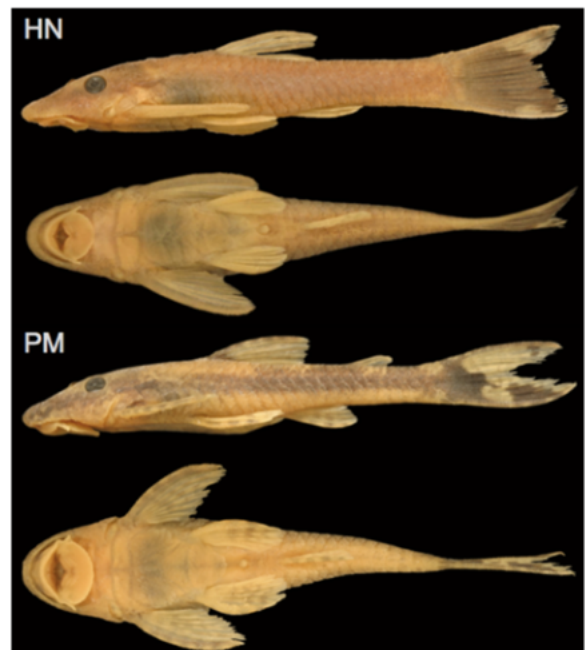


Fig. 2 Lateral and ventral view of the loricariids *Hisonotus notatus* (HN; 37.6 mm standard length) and *Parotocinclus maculicauda* (PM; 35.7 mm standard length) from Ouro stream. Image: M. Brito

visual scanning. To avoid sampler bias, only one and always the same investigator conducted the observations. Each monthly session lasted about 260 min, and were conducted in January, February, March, and July 2005, totaling four sessions and 1040 min of sampling at each site.

After an undisturbed fish was located, the observer remained motionless for two minutes before collecting the data, which we assumed as a conservative method to avoid observer-induced changes in the fish behavior. The fish' standard length was then visually estimated, and the following microhabitat measurements were taken for each focal individual: distance from nearest bank; water column depth; focal elevation (distance from the streambed); average current velocity (taken at 60 % of the water depth); focal-point velocity (taken at the tip of the fish's snout); ventral substrate; distance from current shelter (any upstream structure capable of concealing at least 50 % of the fish's body); surface-water turbulence; submerged cover (any submerged structure located above focal fish); and above-water cover (any structure located up to 50 cm from the water surface above focal fish).

Linear distance measurements were taken with rulers, and velocities with a digital flow meter (Global

Water FP101). Ventral substrate was classified into five types: boulder (rocks >15 cm in diameter); rubble-gravel (rocks <15 cm in diameter); wood debris; decaying leaves; and submerged grassy vegetation. The proportion of each type was calculated using a square (25 × 25 cm) positioned around the focal specimen. Sand, clay and fine detritus were not included in the analysis because both species are exclusively associated to hard substrates. Distance from current shelter was categorized as: <5 cm, between 6–20 cm, and >20 cm in distance. Surface-water turbulence was categorized as: low (water surface completely smooth), medium (wavy surface), and high turbulence (forming bubbles). The cover densities were visually estimated using a square (25 × 25 cm; Online Resource Fig. 1) and categorized as: absent (0 %), low (1–25 %), medium (26–75 %), and high (>75 %).

Interspecific differences in the use of microhabitat between species were firstly tested independently for each variable. Linear measurements were compared with Student's *t*-test; when necessary, it was replaced by the non-parametric analogue Wilcoxon Rank Sum test (*W*). The distributions of frequencies among classes of substrate, distance from shelter, turbulence, and cover were compared using two-sample Kolmogorov-Smirnov test (D_{KS} ; Siegel 1975). To identify linear combinations of variables that maximized microhabitat segregation, we performed a Linear Discriminant Analysis (LDA).

To avoid ontogenetic bias on the interpretation of microhabitat use by the fishes we restricted the analysis to adult individuals (>30 mm standard length, according to Brito 2007). To confirm the diurnal habits of the species (reported for the genera; Buck and Sazima 1995; Schaefer 2003) and consequently their temporal co-occurrence in the stream stretches, we carried out two complementary nocturnal ad libitum dive sessions, totaling 240 min of observation.

Ecomorphological analysis

We performed an ecomorphological analysis on 30 adult individuals of each species previously collected in the same stream sites. Specimens were euthanized by immersion in an anesthetic solution of Eugenol and were later preserved in 10 % formalin solution. The following morphometric attributes were selected for their potential importance

to locomotion and attachment to substrate: body-flatness index; body depth; trunk-shape index; ventral-flatness index; peduncle length; peduncle-flatness index; oral-disc area; pectoral-fin length; pectoral-fin area; pectoral-ray thickness; pelvic-fin length; pelvic-fin area; pelvic-ray thickness; relative depth of caudal-fin fork. These measurements and supposed functions followed previous ecomorphological studies (Gatz 1979b; Watson and Balon 1984; Winemiller 1991; Casatti et al. 2005), except for ray thickness and for relative depth of caudal-fin fork. Ray thickness refers to the width of the anteriormost unbranched ray taken at its longitudinal middle-point; we propose that higher values favor more efficient holding to the substrate by benthonic fishes. Relative depth of caudal-fin fork (CdF) was calculated as:

$$\text{CdF} = (\text{CdUR} - \text{CdMR})/\text{CdUR}$$

where: CdUR is the length of the longest caudal-fin unbranched ray, and CdMR is the length of the smallest middle caudal-fin ray. CdF is a metric of caudal-fin shape and relates to propulsion efficiency and drag reduction; higher values indicating better station holding and faster-swimming fishes (adapted from Webb and Smith 1980).

Linear distance measurements were taken with digital caliper to the nearest 0.01 mm, under binocular microscope. Measurements of areas were taken on digital pictures with the image processing software ImageJ. In order to reduce body-size effects, we used the residuals from regressions of each measurement of linear distances and areas against fish standard length and body area, respectively (Reist 1985). A Principal Component Analysis (PCA) on the ecomorphological data was then conducted using a correlation matrix, and axes with eigenvalues greater than 1.0 were retained. Differences of PCA sample scores between species were tested by Student's *t*-test.

Sexual dimorphism related to pelvic-fin length was recorded for some loricariids (Garavello and Britski 2003). To assess possible sexual dimorphism in *P. maculicauda* and *H. notatus* that could bias our interpretations, we distinguished males and females by examination of urogenital papilla.

Diet analysis

As a complementary approach, to identify the level of diet similarity between *P. maculicauda* and *H. notatus*, we analyzed the diet of 10 adult individuals from each species per site. The contents of the upper third of the intestine were diluted, identified, and quantified in a Sedgwick-Rafter cell under an optical microscope (adapted from Aranha 1993). Food items were identified to the lowest appropriate taxonomic category and the asymptote was attained in the accumulation curve with fewer than 10 samples. The importance of each food category was assessed by the Feeding Importance Index – FII (Granado-Lorencio and Garcia-Novo 1981), which combines frequency of occurrence and a semi-quantitative scale of volume. Both Schoener's (S) and Pianka's overlap indexes were applied to assess the diet similarity between the two species (Krebs 1999). The former index was expressed as a percentage of dietary overlap (biologically significant when $S > 60\%$, according to Wallace 1981), whereas the second varies from 0 (no overlap) to 1 (total overlap).

Voucher specimens of *P. maculicauda* and *H. notatus* from Ouro stream were deposited in the Fish Collection of the Museu Nacional do Rio de Janeiro (MNRJ 30882, MNRJ 30883, MNRJ 30884). All statistical analyses were carried out in R 3.0.2 (R Core Team 2013).

Results

Microhabitat

A total of 273 microhabitat observations were conducted during the study. In the Forested site, we recorded 46 and 143 individuals of *Hisonotus notatus* and *Parotocinclus maculicauda*, respectively; whereas in the Deforested site we observed 34 *H. notatus* and 50 *P. maculicauda*. In both stretches, *P. maculicauda* occupied areas farther from the bank (Forested: $W=1505.5$; $p < 0.0001$; Deforested: $t=4.38$; $p < 0.0001$; Fig. 3), with swifter average current velocity (Forested: $W=2009.5$; $p < 0.0001$; Deforested: $W=557.5$; $p=0.0077$; Fig. 3) and focal-point velocity (Forested: $W=1314.5$; $p < 0.0001$; Deforested: $t=3.99$; $p=0.0001$; Fig. 3). Particularly in the Forested stretch, the mean difference of focal-point velocity between the species reached *c.* 20 cm/s (Fig. 3). *H. notatus* was

more frequently observed closer to current shelters (Forested: $D_{KS}=0.33$; $p < 0.05$; Deforested: $D_{KS}=0.49$; $p < 0.05$), and in areas of lower surface-water turbulence (Forested: $D_{KS}=0.48$; $p < 0.05$; Deforested: $D_{KS}=0.35$; $p < 0.05$). Only in the Deforested stretch the species showed differences in relation to water depth (Forested: $W=3330.0$; $p=0.90$; Deforested: $W=525.5$; $p=0.0031$; Fig. 3) and focal elevation (Forested: $W=3584.5$; $p=0.35$; Deforested: $W=268$; $p < 0.0001$; Fig. 3). At this site, individuals of *H. notatus* were more frequently observed in shallower areas and nearer the bottom (mean difference over 10 cm; Fig. 3). In the Forested site, the species were similar on the use of substrate ($D_{KS}=0.22$; $p > 0.05$), and the most important types were wood debris and boulder (Fig. 4). In the Deforested site, the frequency of individuals of each species were different among substrate types ($D_{KS}=0.32$; $p < 0.05$); 75 % of individuals of *P. maculicauda* were observed on wood debris, whereas *H. notatus* was more frequently associated to grassy vegetation (Fig. 4). Cover densities were similar between the species in both sites (submerged cover: Forested: $D_{KS}=0.17$; $p > 0.05$; Deforested: $D_{KS}=0.23$; $p > 0.05$; above-water cover: Forested: $D_{KS}=0.08$; $p > 0.05$; Deforested: $D_{KS}=0.30$; $p=0.05$).

The Linear Discriminant Analysis (LDA) produced one axis that maximized microhabitat segregation (Fig. 5). Posterior probability analysis showed that most individuals were classified into the correct group by the LDA (*H. notatus*, Forested: 74 %, Deforested: 82 %; *P. maculicauda*, Forested: 93 %, Deforested: 88 %). In both sites, *P. maculicauda* showed mainly positive scores, contrasting with the negative scores of *H. notatus* (Fig. 5). The LDA-axis of Forested site was highly positively loaded on focal-point velocity, distance from nearest bank, and surface-water turbulence; and negatively loaded on % decaying leaves (Fig. 5, Online Resource Table 2). The LDA-axis of Deforested site was highly positively loaded on focal elevation, % wood debris, distance from current shelter, and distance from nearest bank; and negatively loaded on % grassy vegetation (Fig. 5, Online Resource Table 2). This analysis corroborated the main microhabitat segregation patterns identified with the univariate tests, evidencing the effects of focal current velocity and turbulence in Forested site, and vertical position and ventral substrate in Deforested site (Fig. 5).

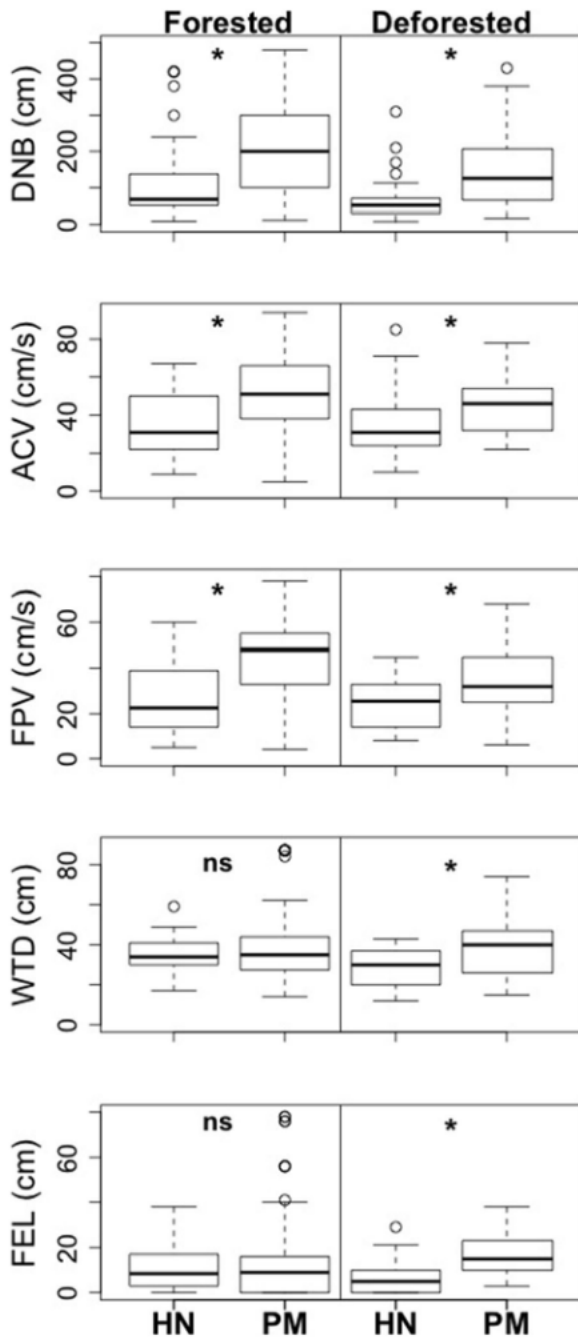


Fig. 3 Mean distance from nearest bank (DNB), average (ACV) and focal-point current velocity (FPV), water depth (WTD), and focal elevation (FEL) used by *Hisonotus notatus* (HN) and *Parotocinclus maculicauda* (PM) in Forested and Deforested sites of Ouro stream. (*) $p < 0.05$, and (ns) $p > 0.05$

During nocturnal diving sessions, both *P. maculicauda* and *H. notatus* were observed in very low abundances compared to the diurnal samples. Foraging activities

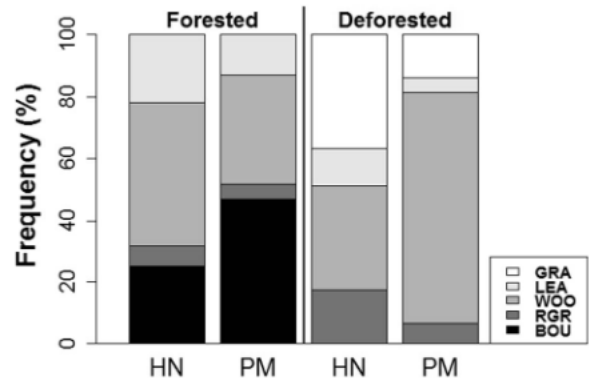


Fig. 4 Frequency of individuals of *Hisonotus notatus* (HN) and *Parotocinclus maculicauda* (PM) observed on each type of substrate in Forested and Deforested sites of Ouro stream. *GRA* submerged grassy vegetation, *LEA* decaying leaves, *WOO* wood debris, *RGR* rubble-gravel (rocks < 15 cm in diameter); *BOU*: boulder (rocks > 15 cm in diameter)

(sensu Buck and Sazima 1995; Leitão et al. 2007) were not recorded at night for both species.

Ecomorphology

The set of ecomorphological-traits values was significantly different between *H. notatus* and *P. maculicauda* along the first PCA dimension ($t=15.0$; $p < 0.0001$; Fig. 6). Compared to *H. notatus*, individuals of *P. maculicauda* have flatter body, deeper forked caudal fin, wider pelvic-fin rays, and greater oral-disc and pelvic-fin areas (Fig. 6, Online Resource Table 3). There were no evidences of sexual dimorphism related to the ecomorphological attributes analyzed for both species (Fig. 6).

Diet

The diet analysis indicated high importance of organic detritus, inorganic sediment, and diatoms in the digestive content of *H. notatus* and *P. maculicauda*. Despite differences on rare food items between the two species, the overlap indexes indicated high similarity on their diets in both stream sites (Table 1).

Discussion

Our results clearly indicate microhabitat segregation between *Parotocinclus maculicauda* and *Hisonotus notatus*. As a general pattern, *H. notatus* remained near the banks and closer to current shelters, whereas

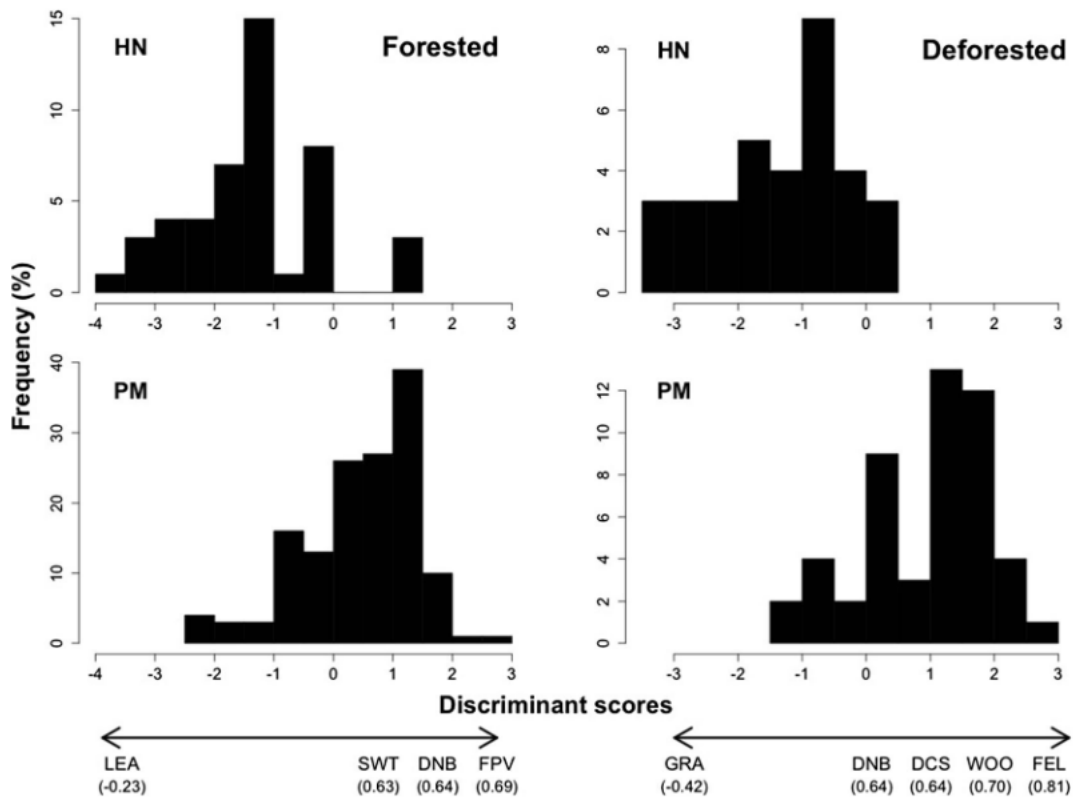


Fig. 5 Frequency distribution of discriminant scores (Linear Discriminant Analysis on microhabitat variables) for *Hisonotus notatus* (HN) and *Parotocinclus maculicauda* (PM) in Forested and Deforested sites of Ouro stream. Highest loaded variables listed below axes (loadings in parentheses). *DNB* distance from

nearest bank, *DCS* distance from current shelter, *FEL* focal elevation, *FPV* focal-point current velocity, *GRA* submerged grassy vegetation, *LEA* decaying leaves, *SWT* surface-water turbulence, *WOO* wood debris

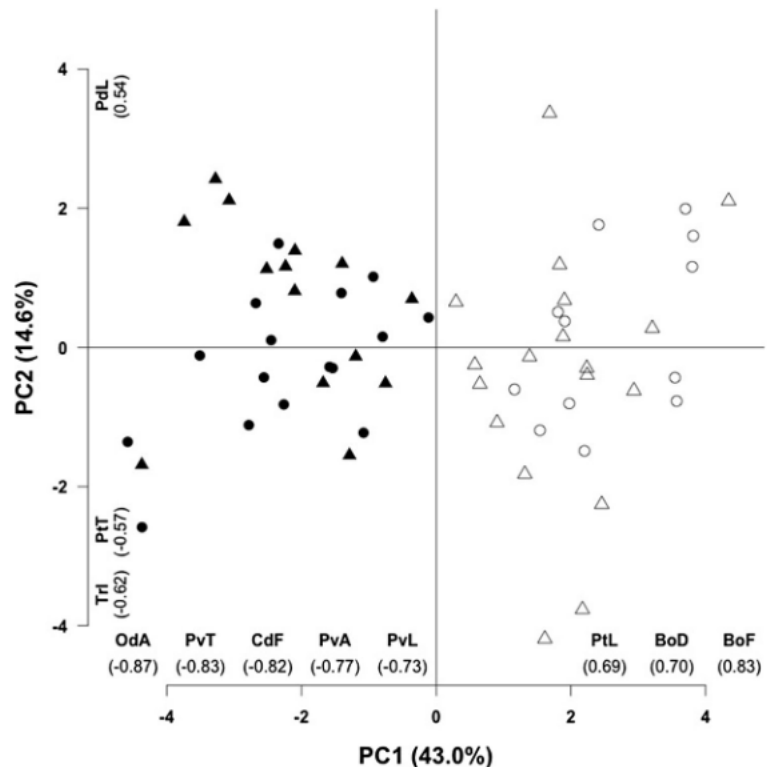
P. maculicauda inhabited the mid-channel, facing higher focal current velocities and water turbulence. We found high overlap on diel activity and diet between *P. maculicauda* and *H. notatus*: both species have strictly diurnal habits and feed mostly on diatoms and organic detritus associated to hard substrates (i.e., periphyton). The strong similarity in these two niche dimensions (time and food) reinforces the importance of spatial segregation to their coexistence in the studied stream.

Interspecific differences in the use of habitat by stream fishes were observed at different scales and parameters, and studies generally agree that spatial segregation facilitates the coexistence of closely related organisms (Grossman and Freeman 1987; Grossman et al. 1987; Sabino and Zuanon 1998; Erős et al. 2003; Santos et al. 2004; Casatti et al. 2005; Leal et al. 2010). Several authors assigned distinct microhabitat preferences to trade-off-based mechanisms driven by current or past competition (Gray and Stauffer 1999; Taniguchi and Nakano 2000; Herder and Freyhof 2006). In a larger

spatial scale (i.e., regional assessment), Winston (1995) demonstrated by randomization tests that the degree of co-occurrence between stream-fish species is inversely related to their morphological closeness and habitat similarity, strengthening the limiting similarity/competitive hypothesis. On the opposite view, the disparities in the use of space and the observed patterns of species co-occurrence were attributed to environmental unpredictability, anti-predatory mechanisms and independently evolved adaptations, rather than competitive interactions for resources (Douglas and Matthews 1992; Brooks and McLennan 1993; Peres-Neto 2004). Therefore, the causes of differential occupation of space are still a subject of debate, mainly because testing competitive hypotheses is operationally difficult and nonconsensual (Jackson et al. 2001).

Particularly for loricariids, habitat displacement associated to agonistic interactions during grazing has been recorded (Power 1984a; Buck and Sazima 1995), but we did not observe such aggressive behavior

Fig. 6 Principal component analysis on ecomorphological traits measured on *Hisonotus notatus* (white symbols) and *Parotocinclus maculicauda* (black symbols). Males (circle) and females (triangles). Highest loaded attributes listed along axes (loadings in parentheses). *BoD* body depth, *BoF* body-flatness index, *CdF* relative depth of caudal-fin fork, *OdA* oral-disc area, *PdL* peduncle length, *PtL* pectoral-fin length, *PtT* pectoral-ray thickness, *PvA* pelvic-fin area, *PvL* pelvic-fin length, *PvT* pelvic-ray thickness, *TrI* trunk-shape index



between *P. maculicauda* and *H. notatus*. Experiments controlling microhabitat occupancy in the absence of the opposing species could also be an elucidative way to compare fundamental and realized niches and to contrast with our field data. This approach may be a promising way to infer and understand the effects of

competition resulting in spatial segregation among species. The possibility of past-competition effect on spatial occupancy pattern can't be discarded, but it is out of scope of this study. Whatever the origin, we believe that the present microhabitat segregation between *P. maculicauda* and *H. notatus* favors resource

Table 1 Values of Feeding Importance Index (FII) and diet-overlap indexes (Schoener and Pianka) between *Hisonotus notatus* and *Parotocinclus maculicauda* in Forested and Deforested sites of Ouro stream

Food item	Forested		Deforested	
	<i>H.notatus</i>	<i>P.maculicauda</i>	<i>H.notatus</i>	<i>P.maculicauda</i>
Animal debris	0.30	0.00	0.30	0.00
Chironomidae	0.00	0.20	0.10	0.00
Closteriaceae	0.40	0.20	1.20	0.60
Desmidiaceae	0.20	0.10	0.70	0.20
Diatomaceae	15.40	11.90	21.70	15.10
Filamentous algae	0.50	0.20	0.20	0.30
Invertebrate eggs	0.10	0.30	1.30	0.70
Organic detritus	58.20	57.60	65.20	49.60
Sediment	56.30	44.00	58.90	42.20
Vegetal debris	4.10	8.80	2.00	1.50
Schoener's index	91.75		97.82	
Pianka's index	0.99		0.99	

partitioning, and can facilitate their coexistence in high abundances in the studied stream.

This observed pattern of micro-spatial segregation is congruent with slight differences in the ecomorphological characteristics of the species, being in accordance with hypotheses about form-function relationships previously reported for fishes. Species with more depressed body tend to inhabit swifter and more turbulent areas due to the lessening of water resistance over the organism (Gatz 1979b; Watson and Balon 1984). Paired fins of benthic fishes are also described as crucial structures for station-holding in fast-flowing waters, since they act as hydrofoils to deflect the current upwards and thereby maintain the fish firmly attached to the substrate (Keenleyside 1979; Matthews 1985). Specifically for armored catfishes, the suctorial oral disc is an important structure for attachment to the substrate (Buck and Sazima 1995); larger areas are then hypothesized to help the fish to hold position in faster currents (Casatti et al. 2005). The shape of caudal fin is recognized as indicator of swimming and propulsion ability (Webb and Smith 1980); in this case, the deeply forked caudal fin may help minimize the turbulence generated by the current at the tip of the fin and so contributing for station holding. Despite the phylogenetic closeness and the consequently general morphological similarity between *P. maculicauda* and *H. notatus*, almost all of these ecomorphological traits are subtly more developed in *P. maculicauda*, which occupies stronger currents and more hydrodynamic microhabitats. This may indicate that even slight morphological dissimilarities can favor fine-scale spatial segregation among stream fishes. It is important to be said, however, that we are not advocating that these morphological differences are straightforwardly determining the observed microhabitat segregation. Moreover, our study was not designed to test adaptive hypotheses of morphological divergence induced by competitive processes. In fact, we are describing patterns of form-function relationships that corroborate previous studies on ecomorphology and functional morphology, but that have been scarcely investigated in the field and in such a fine spatial scale.

Some interesting outcomes emerge when comparing the two sample sites. For instance, interspecific differences in turbulence and current velocity occupation were much more noteworthy in the Forested stretch, whereas ventral substrate and vertical position were significant factors for spatial segregation only in the Deforested site. These results are likely explained by between-sites differences on the availability and

distribution of hard substrates along the stream. In the Forested stretch, rocks and wood debris were abundant and widely distributed in both mid-channel and margins. Thus, the observed microhabitat segregation is mainly related to hydrodynamic factors, independent of substrate type. On the other hand, the proportion of those consolidated structures was almost five times lower in the Deforested site (see Online Resource Table 1), being the mid-channel predominantly composed by sand and clay, with few scattered pieces of hanging logs. At this site, the major amount of hard substrate (i.e., grassy vegetation) occurs exclusively along the stream banks, resulting in substantial increase in *P. maculicauda* records closer (<1 m) to the margins (15 % of the individuals in Forested vs. 42 % in Deforested site), and consequent increased overlapping in the use of these areas with *H. notatus*. The vertical segregation (i.e., differences in focal elevation) exclusively observed therein can thus represent a modified strategy of resource partitioning between the species in order to offset their horizontal overlap.

Parotocinclus maculicauda was almost three times more abundant in Forested stretch than in Deforested in all field campaigns. This contrasts with previous studies that showed increased density of grazer fishes in open-canopy areas as a result of higher algal productivity (Power 1984b; Burcham 1988; Bojsen and Barriga 2002). In fact, previous experiments in Ouro stream found higher amount of periphyton biomass/area in the Deforested site (Leitão et al., unpubl. data). However, despite its greater photosynthetic capacity this stretch has much less hard substrate available to periphyton colonization than the Forested site, especially at the fast flowing mid-channel where *P. maculicauda* is dominant. Unlike the great difference observed for *P. maculicauda*, *H. notatus* (inhabitant of the stream banks, where hard substrates are widely available in both sites) had very similar densities between Forested and Deforested sites. Romero and Casatti (2012) predicted a drastic simplification of stream ichthyofauna (more than 50 % of species loss) with the elimination of hard benthic structures, with rheophilic loricariids (such as *P. maculicauda*) among the most affected groups.

We recognize that the lack of a broader sampling, including stream sites under wide gradients of canopy cover and across other river basins, prevents us to attribute between-sites results to deforestation. However, we suppose that our findings offer interesting insights concerning the potential effects of instream habitat

changes on the patterns of micro-spatial occupation and segregation by fish species. We also believe that this study is a starting point, and its limitations suggest fruitful opportunities for future investigations; for example, linking differential responses to landscape changes with differences in fine-scale habitat requirements between species. Particularly for tropical streams, which support enormous biodiversity and where precise natural history data (e.g., microhabitat use) lacks for most species (Carvalho et al. 2009), implementing more accessible tools (e.g., ecomorphological analysis) to indirectly estimate how fish species use and share the resources within streams is a reasonable remedy, even if provisional, to reach this goal. Finally, this study illustrates how fine-tuned ecological processes (e.g., micro-spatial segregation) potentially affect patterns of population dynamics and community structure in stream systems. Consequently, it can provide subsidy to effective management strategies aiming the conservation of their biodiversity.

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