The importance of substrate size and interstitial space in the microhabitat selection by the stream-dwelling catfish Hatcheria macraei (*Actinopterygii, Trichomycteridae*)

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PRIMARY RESEARCH PAPER

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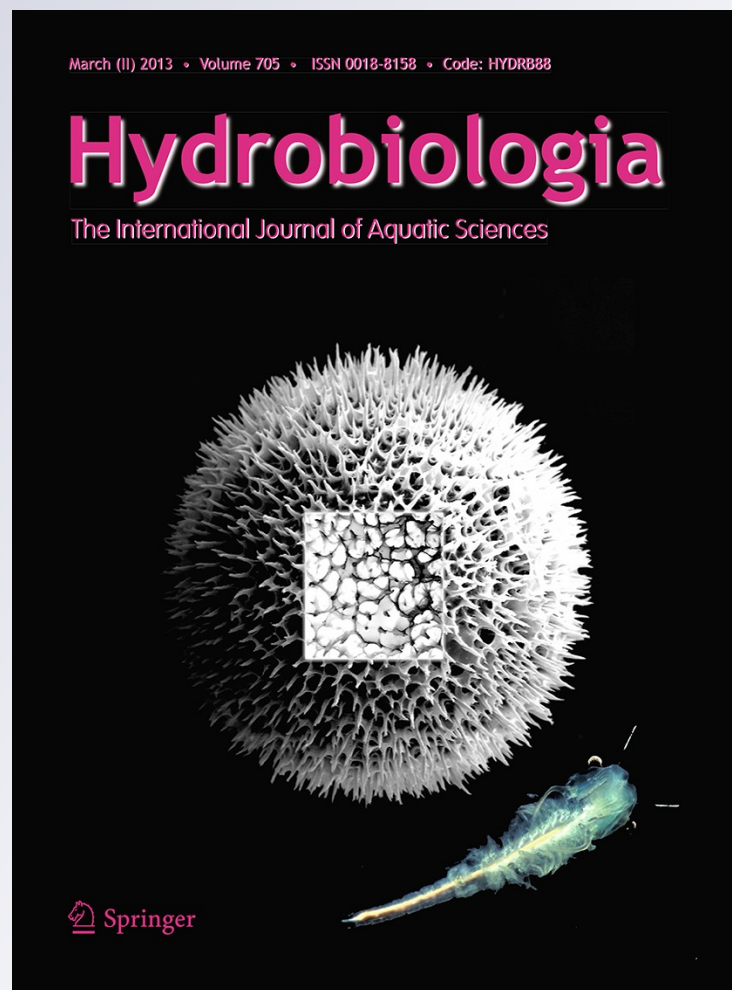
J. P. Barriga · N. A. Espinós · J. M. Chiarello-Sosa · M. A. Battini

Abstract The study tests whether diurnal microhabitat use by *Hatcheria macraei* depends upon specific environmental parameters and/or the abundance of other fish. We carried out a 1-year field study in a low-order river of northern Patagonia, Pichileufu River, and used experimental trials to determine substrate preferences. Fishes were captured during daylight and physicochemical environmental variables were recorded. Headwater zones were dominated by rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*), while native fishes, *H. macraei* and the creole perch (*Percichthys trucha*) were more abundant downstream. *H. macraei* inhabited mostly shallow microhabitats with fast water velocity and substrates having significant interstitial spaces, independently of the abundance of other fishes. Experimental trials pointed out that *H. macraei* preferred mostly coarser substrates (>6 cm), avoiding fine ones. This study highlights the importance of erosional zones with high water velocity, large substrates, and suitable interstitial space in the microhabitat selection of *H. macraei*. The knowledge of microhabitat use by native fish

populations is critical for management and conservation strategies and should be taken into account before any river modification.

Keywords Trichomycteridae · Habitat selection · Fish assemblage variation · Stream substrate preference · Fish density

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Introduction

Fish distribution within a river has been related to microhabitat preferences (Onoda et al., 2009), and it is strongly influenced by predation risk (Clavero et al., 2005), food resources (Tyler & Clapp, 1995), and intra- (Petty & Grossman, 2007) or inter-specific interactions (McIntosh et al., 1992; Hesthagen & Heggenes, 2003; Clavero et al., 2009). The main abiotic microhabitat variables are related to hydraulic factors, such as depth, water velocity, and substrate (e.g., Van Liefferinge et al., 2005). However, the importance of each variable depends upon the fish ecomorphological adaptation to the environment (Wootton, 1998). Pelagic fishes are more affected by water velocity, while bottom dwelling fishes are more likely to be influenced by substrate composition (Hlohowskyj & Wissing, 1986; Onoda et al., 2009). For example, a high degree of heterogeneity and patchiness in rivers due to variation in the composition and arrangement of available substrates are the major causes of the distribution of benthic fish, either by

substrate selectivity (e.g., Dixon & Vokoun, 2009) or indirectly by selecting patches with higher prey densities (e.g., Petty & Grossman, 1996).

The benthic catfish, *Hatcheria macraei* (Girard 1855), belongs to the family Trichomycteridae and is widely distributed in Argentina and Chile. It has a fairly continuous distribution from the northern Colorado River system (Grande River, La Rioja Province, 28°33′S, 67°9′W) south to the Baker River system (Blanco River, Santa Cruz Province, 47°34′S, 71°38′W), being found in both Atlantic and Pacific basins (Menni, 2004; Unmack et al., 2009, 2012). Despite this broad distribution, the biology of this species remains poorly known (Pascual et al., 2007). This is primarily because the majority of studies dealing with the Patagonian ichthyofauna have been carried out in lakes and limited efforts have been applied to the study of rivers. In addition, *H. macraei* are not frequently captured in traditional net surveys because it buries itself in the substrate (Unmack et al., 2012).

Hatcheria macraei has a generalized diet based on benthic invertebrates (Di Prinzio & Casaux, 2012). It is a rheophilic and negatively phototactic species (Menni, 2004), characterized by living in cold and well-oxygenated waters with sandy and rocky substrates (Ringuelet et al., 1967). Arratia & Menu-Marque (1981) mentioned a size-related habitat preference, mostly associated to the type of substrate and the water depth. Individuals up to 20 mm total length (TL) prefer shallow quiet pools with a substrate of sand or small pebbles near shore (<5 cm depth). Larger individuals, from 20 to 60 mm TL are found among plants and stones (<20 cm depth); and the largest individuals choose stony (medium- to large-sized stones) and sandy substrates (<50 cm depth). In addition, Barriga & Battini (2009) determined the indirect ontogeny (sensu Balon, 1990) of this species and related its morphological constraints to habitat and feeding preferences. A complete development of fins allow juvenile colonize deeper and faster water habitats while a bigger mouth gape permitted them to prey on new items and on a larger size prey range. Despite these observations, no specific work has been done to evaluate quantitatively the microhabitat preferences of this species. In the present study, some aspects of the diurnal microhabitat use by *H. macraei* and accompanying species were analyzed. In addition, *H. macraei* substrate preferences were evaluated using experiments. The main objectives of this study were (i) identify the main microhabitat variables that influence the presence and density of *H. macraei*, (ii) characterize diurnal microhabitat and density data of the accompanying species, both native and exotic fishes, and (iii) evaluate the substrate preference of *H. macraei* using trials performed in experimental channels.

Materials and methods

Study site

Field work was conducted in the Pichileufu River, Río Negro Province, Argentina. This river begins in the Carrera Mountains and flows north for around 150 km, through the Patagonian steppe before draining into Piedra del Águila Reservoir on the Limay River, a major tributary of the Negro River (Fig. 1). Sampling was performed at three localities, from October 2008 to December 2009. The three localities from upstream to downstream were: Pilila (41°17′50″S, 71°02′30″W, 1088 m a.s.l.); Pilcaniyeu (41°05′24″S, 70°49′42″W, 926 m a.s.l.); and Corralito (40°43′59″S, 70°41′14″W, 658 m a.s.l.). The river distance from Pilila to Pilcaniyeu is ∼40 km, from Pilcaniyeu to Corralito is ∼75 km, and from Corralito to Piedra del Águila reservoir is ∼20 km (Fig. 1). Sampling dates
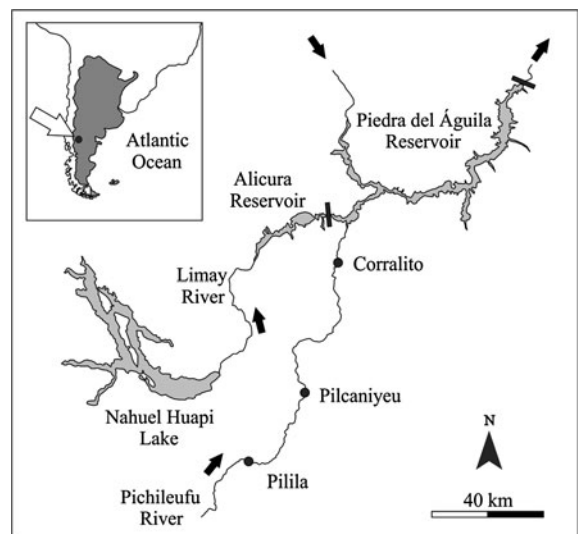

Fig. 1 Study site in Patagonia (*white arrow*) with an *inset box* showing its location in Argentina (*gray area*). *Dots* Sampling locations on Pichileufu River and *black arrows* water flow direction. *Bars* Dams on Limay River

by localities are indicated in Table 1. Pilcaniyeu was also sampled in October 6 and November 17, 2008, and February 9, May 4, June 8, and August 10, 2009.

Fish collection and habitat characterization

Fishes were captured using a 24 V DC backpack electrofishing unit, model 12-B (Smith-Root, Inc., Vancouver, WA, USA) fitted with a diamond shaped anode (diagonals of 36.8 and 19.7 cm). Sampling units consisted of 1 m^2 areas. Once the area was chosen, two dip nets (50 cm wide) were carefully juxtaposed by two people on the bottom to block the area while the electrofisher operated the unit along 1 m upstream of the nets. Fish affected by the electricity, but not in the sampling area, were ignored. Stunned fishes were captured using hand nets (10×10 cm^2) and deposited into the dip nets or released downstream according to their location, inside or outside the sampling area, respectively. Next, substrate was disturbed within the area to find stunned fishes among the interstitial space. Distance between units was at least 7 m to avoid electrical perturbation among sampling areas. A total of 295 units were sampled during daytime (from 10:00 to 15:00 h) throughout the sampling period, always starting downstream and working in an upstream direction at each sampling location. We tried to sample all different available mesohabitats [i.e., riffle, glide, run, backwater, edge-water, and pool (see Maddock, 1999 for a physical description)]. As mesohabitats had different areas, the number of sampling units was not equal in each type, and the number of units per locality varied from 14 to 22 by date. *H. macraei* individuals captured were transported to the laboratory for further analysis, while other fish species were counted, measured, and then released. At the laboratory, catfish were euthanized using an overdose of benzocaine and then weighed (to the nearest 0.01 g), measured (standard length, SL, to the nearest 1 mm), and sexed through examination of their gonads.

Habitat variables were recorded in each of these units. Water velocity of the water column (mean and maximum) and mean water velocity at the bottom were measured with a flowmeter, model FP101 (Global Water, Gold River, California, USA) with an accuracy of 0.03 m s^{-1}. To measure mean and maximum water velocity of the water column the probe was moved slowly and smoothly throughout the

flow, between the surface and bottom, over at least 1 min always inside the quadrant. Because the flow-meter takes one reading per second, both values were obtained from at least 60 readings. A similar procedure was carried out to record the mean water velocity at the bottom, with the difference that the probe was moved slowly perpendicularly to the flow direction above the bottom of the sampled area. Temperature, oxygen concentration, and pH were registered with a Water Quality Meter, model 850081 (Sper Scientific Ltd., Scottsdale, Arizona, USA) with an accuracy of 0.8°C, 0.4 mg l^{-1}, and 0.2, respectively. Mean depth of each square was calculated as the average between the minimum and maximum depths registered with a meter stick, within the sampling area. Substrate size composition was visually estimated as percentage composition by at least two observers, classifying in five diameter size categories as: boulders (>180 mm), cobbles (>64 and <180 mm), gravel (>4 and <64 mm), sand (>1 and <4 mm), and silt (<1 mm). All cutoffs correspond to Wentworth scale with the exception of 180 mm. The mean classification error of each substrate category was less than 5%. This error was calculated comparing 30 sampling units visually ranked with the same units measured with an image analyzer (Digimizer version 4.0, Mariakerke, Belgium) on digital images. This misclassification error was considered negligible due to the codification of data performed previously to the statistic analysis (detailed below).

The interstitial space (or the inverse of embeddedness) as the degree of big substrate particles (>64 mm) surrounded or covered by fine sediment (<4 mm) was coded as: 1, when no interstitial space was detected among particles (they were completely surrounded or covered by the sediment); 2, when more than 50% of the area was embedded by fine sediments; and 3, when less than 50% of the area was embedded by fine sediments and there was substantial interstitial space (many interparticle voids). We also measured the presence or absence (1 or 0, respectively) of submerged macrophytes, filamentous algae, and riparian vegetation. Individual *H. macraei* were separated into two groups, juveniles and adults, according to their SL, sensu Barriga & Battini (2009). Individuals larger than 61.4 and 64.4 mm SL were considered adults, for males and females, respectively. Fish between these values and 25.9 mm SL were classified as juveniles.

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Table 1 Density (fish m^{-2}, mean ± SD) and occupancy in percentage of sampling units where the species was present (into brackets) of native and exotic fishes by sampling location

Sites	Date	Hm (%)	Pt (%)	Natives (%)	Om (%)	St (%)	Salmonids (%)
Corralito	18 Dec 2008	2.22 ± 3.30 (47)	0.41 ± 1.14 (12)	2.65 ± 3.22 (59)	0.06 ± 0.24 (6)	0.12 ± 0.47 (6)	0.18 ± 0.51 (12)
	16 Mar 2009	2.43 ± 4.25 (36)	0.14 ± 0.35 (14)	2.57 ± 4.19 (50)	0.14 ± 0.52 (7)	0 ± 0 (0)	0.14 ± 0.52 (7)
	8 Jul 2009	0.20 ± 0.54 (13)	0.27 ± 0.44 (27)	0.47 ± 0.72 (33)	0 ± 0 (0)	0.13 ± 0.34 (13)	0.13 ± 0.34 (13)
	16 Sep 2009	1.33 ± 1.92 (47)	0 ± 0 (0)	1.33 ± 1.92 (47)	0.07 ± 0.25 (7)	0 ± 0 (0)	0.07 ± 0.25 (7)
	Total (n = 61)	1.56 ± 3.01 (36)	0.21 ± 0.69 (13)	1.77 ± 2.99 (48)	0.07 ± 0.31 (5)	0.07 ± 0.31 (5)	0.13 ± 0.43 (10)
Pilcaniyeu	19 Dec 2008	1.95 ± 2.77 (41)	0.82 ± 2.04 (23)	2.77 ± 2.94 (64)	0.36 ± 0.77 (23)	0.41 ± 0.78 (27)	0.77 ± 1.13 (41)
	01 Apr 2009	4.67 ± 7.44 (47)	0.13 ± 0.34 (13)	4.80 ± 7.41 (53)	0 ± 0 (0)	0.07 ± 0.25 (7)	0.07 ± 0.25 (7)
	13 Jul 2009	1.13 ± 1.36 (60)	0.07 ± 0.25 (7)	1.20 ± 1.38 (60)	0.20 ± 0.54 (13)	0.07 ± 0.25 (7)	0.27 ± 0.57 (20)
	9 Sept 2009	3.53 ± 4.18 (67)	0 ± 0 (0)	3.53 ± 4.18 (67)	0.07 ± 0.25 (7)	0 ± 0 (0)	0.07 ± 0.25 (7)
	Total (n = 67)	2.73 ± 4.61 (52)	0.31 ± 1.25 (12)	3.04 ± 4.62 (61)	0.18 ± 0.55 (12)	0.16 ± 0.51 (12)	0.34 ± 0.79 (21)
Pilila	11 Dec 2008	0.13 ± 0.34 (18)	0 ± 0 (0)	0.13 ± 0.34 (18)	0.33 ± 0.60 (24)	0.07 ± 0.25 (6)	0.40 ± 0.71 (24)
	18 Mar 2009	0.60 ± 0.86 (40)	0 ± 0 (0)	0.60 ± 0.86 (40)	0.55 ± 0.97 (30)	0.35 ± 0.79 (20)	0.90 ± 1.48 (40)
	29 Jun 2009	0.13 ± 0.34 (13)	0 ± 0 (0)	0.13 ± 0.34 (13)	0.07 ± 0.25 (7)	0.13 ± 0.50 (7)	0.20 ± 0.54 (13)
	20 Sept 2009	0 ± 0 (0)	0 ± 0 (0)	0 ± 0 (0)	0.07 ± 0.25 (7)	0.13 ± 0.34 (13)	0.20 ± 0.54 (13)
	Total (n = 67)	0.25 ± 0.59 (19)	0 ± 0 (0)	0.25 ± 0.59 (19)	0.27 ± 0.66 (18)	0.18 ± 0.55 (12)	0.45 ± 1.00 (24)

Hm, Hatcheria macraei; Pt, Percichthys trucha; Om, Oncorhynchus mykiss; St, Salmo trutta

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Experimental design of trials

To isolate the effect of great heterogeneity in substrate sizes and food availability in rivers, experiment trails were performed. In this way, the hypothesis that microhabitat selection by *H. macraei* depends on the size of the substrate particles, which was tested. Fish captured at the middle location (Pilcaniyeu) were transported to facilities of the salmonid hatchery belonging to the Centro de Ecología Aplicada de Neuquén, Argentina (CEAN) to let them adapt to captive conditions for at least 1 week. Substrate selection experiments were conducted in four flow-regulated channels (5 m long × 0.30 m wide × 0.40 m high). Each channel was subdivided in four equal size sections and contained four different size substrate categories as those defined for the river (boulders, cobbles, gravel, and sand). The relative position of the substrates varied from channel to channel in a way that each substrate was placed in the four possible positions with respect to the water entrance (Fig. 2). These channels were placed outside and exposed to natural photoperiod. Four fish were introduced in each channel and left to acclimatize for 2 days before the experiment. Catfish density in each channel was 3.1 fish m^{-2}, based on preliminary sampling data in Pichileufu River considering only those sampling units, where *H. macraei* was present. Both acclimation and the experiment were carried out in the absence of prey organisms to avoid the effect of non-random distribution of prey. The location of each fish was recorded eight times during the experiment, four times per day (i.e., dawn, midday, dusk, and midnight) during two consecutive days. As no

statistical differences were detected between both days in substrate selection (see "Results"), data generated from the same group of experimental fish ($n = 4$) were pooled by time of the day. Thus, we investigated whether the utilization of different substrates was related to the time of the day during 24 h cycle. These experiments were performed four times, using a total of 64 individuals ranging from 4.2 to 10.5 cm SL (mean ± SD, 7.64 ± 1.44 cm), and from 0.36 to 6.09 g total mass (mean ± SD, 2.37 ± 1.20 g).

Trials were performed during 2009. The first series of experiments were run from March 1 to 5, the second from April 12 to 16, the third from June 2 to 6, and the last from August 1 to 5. After 2 days acclimatization, each trial began with the dusk observation and finished with the mid-day observation, approximately 40 h after the experiment began (depending on the change in natural photoperiod). The experimental water supply came from the Chimehuín River (Neuquén Province) which has similar temperatures, mean water velocities, and mean depths to the Pichileufu River (Table 2). Water discharge of each channel during acclimatization and experiment was maintained constant at 0.2 l s^{-1}.

Experimental channels were laterally covered with an opaque nylon of 2 m height to avoid fish disturbance. Before counting, each substrate was isolated using a mobile sluice that prevented fish moving from one section to another as a result of the presence of the observer. This sluice was removed after each observation. Substrate preference was evaluated as the percentage of *H. macraei* present in each substrate category.
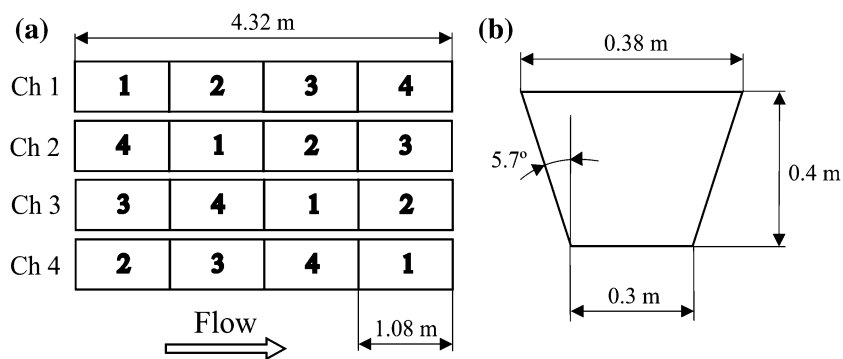

Fig. 2 Experimental design used in substrate preference trials of *H. macraei*. **a** Dimensions of channels (Ch 1–4), substrate size and relative position of each substrate (1–4) to water flow (*arrow*) are indicated. *1* boulders (>180 mm), *2* cobbles (>64 and <180 mm), *3* gravel (>4 and <64 mm), and *4* sand (>1 and <4 mm). **b** Transversal channel section

Table 2 Comparison of parameters (mean ± SD) between experimental trials and the closest sampling dates in Pichileufu River taking those sites where *H. macraei* was captured

Date (2009)	Site	Temperature (°C)	Water velocity (m s^{-1})	Depth (cm)
March 16, 18	Pichileufu	16.4 ± 2.6	0.21 ± 0.19	13.9 ± 8.5
March 1–5	Experiment	14.8 ± 2.0	0.004	22.5 ± 2.5
April 1	Pichileufu	11.4 ± 0.3	0.08 ± 0.11	10.0 ± 4.9
April 12–16	Experiment	11.7 ± 1.6	0.004	22.5 ± 2.5
June 8, 29	Pichileufu	4.3 ± 1.3	0.22 ± 0.22	13.3 ± 3.6
June 2–6	Experiment	6.7 ± 1.2	0.004	22.5 ± 2.5
August 10	Pichileufu	7.9 ± 0.2	0.37 ± 0.34	13.0 ± 4.8
August 1–5	Experiment	4.8 ± 0.8	0.004	22.5 ± 2.5

Data analyses

The hypothesis that fish density vary along the river was analyzed using a non-parametric ANOVA, Kruskal–Wallis analysis, and Mann–Whitney test as the data were not normally distributed. A multiple comparison procedure (Dunn's method) was used to detect differences between each pairwise comparison. For this analysis, we compared the seasonal samplings from Pilila and Corralito sites, and similar sampling dates from Pilcaniyeu (Table 1).

To test the hypothesis that microhabitat selection by *H. macraei* depends upon specific environmental parameters, which was performed using principal component analysis (PCA). The PCA was carried out to summarize the main habitat gradients in the Pichileufu River. Environmental variables were transformed for statistical analysis. Cover proportion variables were coded in a 0–5 semi-quantitative scale, where: 0, 0%; 1, 1–10%; 2, 11–25%; 3, 26–50%; 4, 51–75%, and 5, 76–100% (Clavero et al., 2009). Continuous variables were $\log_{10}(X + 1)$ transformed. Components that had eigenvalues >1 were retained (Grossman & Freeman, 1987). First, microhabitat characteristics of sites where *H. macraei* was present were compared with sites where it was absent. A one way ANOVA was conducted using the factor scores of the PCA. The three sampling sites on the Pichileufu River were introduced as co-variables in the analysis, as well as months, in order to analyze microhabitat variation in the whole river taking into account variation due to seasonal conditions (e.g., physicochemical variables).

To assess the dependence of *H. macraei* density, we evaluated different models using the information-theoretic approach (Burnham & Anderson, 2002). For this analysis only those areas with *H. macraei* presences

($n = 127$) were used. Density of each fishes species [i.e., *H. macraei*, creole perch *Percichthys trucha* (Valenciennes 1833), rainbow trout *Oncorhynchus mykiss* (Walbaum 1792), and brown trout *Salmo trutta* L. 1758] was previously transformed as $\log_{10}$ (density + 1). We constructed linear regression models by incorporating all possible combinations of the following independent variables: *P. trucha*, *O. mykiss*, and *S. trutta* densities; and the three main environmental gradients, principal component (PC) 1, PC2, and PC3 (see "Results"). We constructed individual global regression models with all variables. Models were fit to the data using linear regression and then the Akaike's Information Criterion adjusted for small sample size (AICc; Burnham & Anderson, 2002; Johnson & Omland, 2004) was applied to evaluate the models. First, we calculated the difference between model with the lowest AICc and the other models as: $\Delta AICc_i = AICc_i - \min AICc$, where $\Delta AICc_i$ is the difference between the AICc of the best-fitting model and that of model i. AICc$_i$ is AICc for model i, and min AICc is the minimum AICc value among all models. Then, we normalized the relative likelihood values as: $w_i = [\exp(-0.5 \Delta AICc_i)] [\sum \exp(-0.5 \Delta AICc_n)]^{-1}$, where w_i is known as Akaike weight for model i and the denominator is simply the sum of the relative likelihoods for all candidate models. Values of w_i range from 0 (complete information loss) to 1 (no information loss). The w_i can be interpreted as the probability that i is the best model, given the data and set of candidate models (Burnham & Anderson, 2002). We incorporated model selection uncertainty in analyses of the predictor variables of interpretable models (i.e., those with w_i 10% of the best model) by calculating model-averaged estimates based on the unconditional variance of an estimate (Burnham & Anderson, 2002). We estimated the relative importance of predictor variables by

summing their w_i over all interpretable models. For interpretation, the precision of model-averaged parameter estimates were reported with the aid of 95% confidence intervals (CI) using a t statistic with $n - 1$ degrees of freedom.

Finally, a partial canonical correspondence analysis (CCA) was performed to identify the habitat preferences of each fish species present in Pichileufu River. In this direct gradient analysis (ter Braak & Šmilauer, 1998), the density of each fish species can be directly related to environmental data by performing both ordination and multiple regression techniques. Thus, CCA is used to explore species data in relation to the environmental data, and also the relationship among species. The species matrix considered in the CCA consisted of 165 sites (only sites with fish presence) × the $\log_{10}$ (density + 1) of 5 groups (*H. macraei* juveniles, *H. macraei* adults, *P. trucha*, *O. mykiss*, and *S. trutta*). Variables highly correlated (i.e., correlation level greater than 0.75), with high variance of inflation factor (VIF > 10) were removed from this analysis, such as mean water velocity at bottom and maximum water velocity in the water column. Environmental variables were selected employing manual forward selection, where significance of each variable was tested by Monte Carlo permutations ($n = 499$), and only those variables with $P < 0.05$ were retained (ter Braak & Šmilauer, 1998). The three sampling locations on Pichileufu River were incorporated as co-variables as well as the month of sampling. As advised

by ter Braak & Šmilauer (1998), a biplot scaling and a downweighing of rare species were used in this analysis. Statistical analyses were performed using SPSS 15.0 and CANOCO 4.5.

Differences in *H. macraei* substrate preference during experiments were assessed using Mann–Whitney and Kruskal–Wallis tests as normality and homoscedasticity assumptions of the data failed. A multiple comparison procedure (Tukey test) was used to detect differences between each pairwise comparison.

Results

Fish assemblage variation along the river

Four fish species were captured during the sampling period, two native, *H. macraei* and *P. trucha*; and two introduced salmonids, *O. mykiss* and *S. trutta*. Fishes, other than *H. macraei*, were all juveniles within a size range from ∼20 to ∼100 mm SL. Fish assemblage varied along the longitudinal axis of the river (Fig. 3). Native species abundances varied among sampling sites (Kruskal–Wallis, $H = 31.88$, df = 2, $P < 0.001$), but not the abundance of salmonid species (Kruskal–Wallis, $H = 4.91$, df = 2, $P > 0.05$). Native species were less abundant at Pilila with 0.25 ± 0.59 fish m^{-2}, and differed from Pilcaniyeu and Corralito (Dunn, $P < 0.05$, Table 1). Natives species were more abundant than salmonid species in Corralito (Mann–Whitney,

Fig. 3 Fish assemblage variation by sampling site and date in Pichileufu River. Pie charts represent the total number of fish captured (in percentage) grouped by species. *H. macraei* (*stripped*), *P. trucha* (*white*), *O. mykiss* (*black*), *S. trutta* (*gray*)
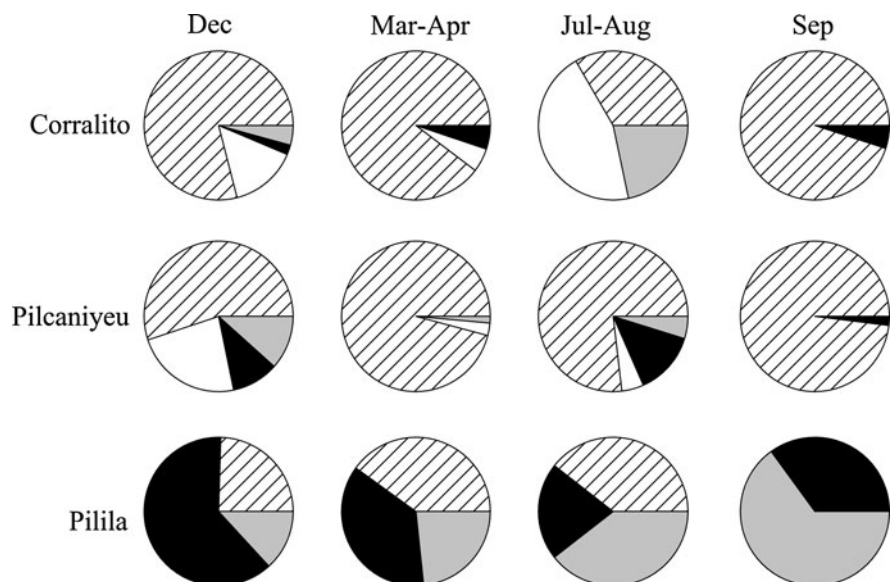
$U = 1,116.00$, $P < 0.001$) and Pilcaniyeu (Mann–Whitney, $U = 1,185.00$, $P < 0.001$); however, in Pilila no difference was found between both groups (Mann–Whitney $U = 4,388.50$, $P > 0.05$, Table 1).

Intra-specific differences in density among sites were found for *H. macraei* (Kruskal–Wallis, $H = 21.84$, df $= 2$, $P < 0.001$) and *P. trucha* (Kruskal–Wallis, $H = 9.11$, df $= 2$, $P < 0.05$), but not for *O. mykiss* (Kruskal–Wallis, $H = 5.16$, df $= 2$, $P > 0.05$) and *S. trutta* (Kruskal–Wallis, $H = 2.34$, df $= 2$, $P > 0.05$). Both native species were more abundant in Pilcaniyeu, with a mean density 2.73 ± 4.61 and 0.31 ± 1.25 fish m^{-2} for *H. macraei* and *P. trucha*, respectively. A similar pattern was found in the percentage of occupancy (Table 1).

Microhabitat selection

The 17 environmental variables registered in 295 sampling units along the Pichileufu River showed a high degree of variation due to the great diversity of microhabitats sampled. Non-transformed data grouped by the presence or absence of *H. macraei* per sampling site are indicated in Table 3. The PCA including these environmental variables (transformed) produced six components (i.e., habitat gradients) that explained 73.2% of the total variance. PC1 (27% variance explained) represented a gradient from depositional to erosional habitats. Positive values on PC1 indicated high water velocity, with medium to large substrate and without silt, denoting more interstitial space. Negative values indicated depositional microhabitat with opposite characteristics. PC2 (13% variance explained) described mainly physicochemical water quality, such as pH, temperature, and dissolved oxygen. PC3 (11% variance explained) appeared to represent microhabitats in a depth gradient associated inversely to medium size particle substrates (i.e., gravel). The remaining components (PC4, PC5, and PC6) were not ecologically interpretable and therefore excluded from further analyses (Table 4). There was a significant difference between areas occupied and unoccupied by *H. macraei* along PC1 (ANOVA, $F_{1,158} = 54.01$, $P < 0.001$) and PC2 (ANOVA, $F_{1,158} = 6.76$, $P < 0.01$, Table 5). *H. macraei* utilized those habitats with fast water velocities, with more interstitial space and without submerged macrophytes. The occupied habitats were also characterized by low pH, low temperature, and by less

proportion of cobbles (Table 4). Months incorporated as covariable proved to be significant for PC2 (ANCOVA, $F_{1,156} = 25.56$, $P < 0.001$), and PC3 (ANCOVA, $F_{1,156} = 18.72$, $P < 0.001$); similarly sampling site was significant for PC2 (ANCOVA, $F_{1,156} = 7.38$, $P < 0.05$) and PC3 (ANCOVA, $F_{1,156} = 6.80$, $P < 0.05$).

The information theory approach evidenced that the best significant fitting model to explain *H. macraei* density in Pichileufu River took into account PC1, PC2, and PC3, but not densities of the other fish species (Table 6). The best model had the greatest explanatory power, and was almost 3 times more likely to be true than when incorporating densities of the other fishes (Table 6). The components more explicative for the most plausible models were PC1 and PC2 based on their w_i. Only environmental gradients (i.e., PC1, PC2, and PC3) estimates and model intercept did not include zero into the 95% CI (Table 7). In consequence, despite the fish density of other species was incorporated to plausible models, *H. macraei* density was better explained by environmental gradients, specifically PC1 and PC2 rather than the interaction with other fish species.

Eleven of a total of 17 environmental variables were retained in the final solution of the CCA. The covariables (month and site) included in the analysis explained 4.8% of the variation in the species data. The first CCA axis (eigenvalue $= 0.394$, 64.8% of the species-environment relationship) was primarily related to habitat features modeled by the water velocity. On the left (negative scores of x axis), habitats with fast water velocity were characterized by having different size of rocks, shallow waters, and a significant interstitial space among the substrate. On the right (positive scores), deeper habitats had very low water velocities in depositional zones with high proportion of fine sediment (silt) and submerged macrophytes. The second CCA axis (eigenvalue $= 0.112$, 18.3% of the species–environment relationship) was directly related with the presence of bedrocks, boulders, riparian vegetation, and depth. The availability of different microhabitats was similar among sampling localities (Fig. 4a).

Three groups of fishes were clustered by the CCA: *H. macraei* (juveniles and adults), *P. trucha*, and salmonids (*O. mykiss* and *S. trutta*) (Fig. 4b). *H. macraei* occupied habitats with high water velocity characterized by larger interstitial spaces among median to large size substrate. Differences between

Table 3 Microhabitat non-transformed variables registered in sampling units (1 m^2) surveyed with and without *H. macraei* (*Hm*) presence

Variables	Corralito		Pilcaniyeu		Pilila	
	Hm absence N = 39	*Hm* presence N = 22	*Hm* absence N = 75	*Hm* presence N = 92	*Hm* absence N = 54	*Hm* presence N = 13
Temperature (°C)	12.2 ± 8.3 (23.6–2.2)	14.8 ± 6.7 (27.4–2.2)	11.7 ± 5.7 (21.5–2.3)	10.8 ± 6.1 (22.2–2.3)	7.8 ± 4.3 (18.0–2.8)	12.8 ± 5.4 (20.4–3.5)
pH	8.4 ± 0.4 (9.1–8.0)	8.4 ± 0.4 (9.7–8.1)	8.0 ± 0.3 (8.9–7.4)	8.1 ± 0.3 (8.9–7.0)	7.8 ± 0.4 (9.1–7.1)	8.3 ± 0.6 (9.3–7.3)
Oxygen (mg l^{-1})	10.2 ± 0.9 (11.5–8.6)	9.7 ± 0.7 (10.9–8.1)	10.2 ± 1.4 (13.4–6.4)	10.3 ± 1.4 (13.4–8.5)	10.3 ± 0.5 (11.7–9.6)	10.4 ± 0.6 (11.7–9.6)
Max. depth (cm)	28.9 ± 16.4 (70.0–7.0)	28.9 ± 22.2 (80.0–9.0)	20.8 ± 11.3 (60.0–7.0)	21.3 ± 14.4 (65.0–6.0)	20.1 ± 11.4 (58.0–6.0)	13.8 ± 8.1 (31.0–4.0)
Mean water velocity (m s^{-1})	0.24 ± 0.21 (0.79–0.00)	0.56 ± 0.33 (1.10–0.00)	0.19 ± 0.30 (1.26–0.00)	0.47 ± 0.42 (1.64–0.00)	0.39 ± 0.28 (1.27–0.00)	0.31 ± 0.26 (0.77–0.00)
Max. water velocity (m s^{-1})	0.36 ± 0.29 (1.10–0.00)	0.76 ± 0.39 (1.40–0.00)	0.26 ± 0.42 (1.60–0.00)	0.67 ± 0.57 (2.10–0.00)	0.53 ± 0.32 (1.40–0.00)	0.45 ± 0.39 (1.20–0.00)
Bottom water velocity (m s^{-1})	0.13 ± 0.12 (0.44–0.00)	0.33 ± 0.17 (0.66–0.00)	0.13 ± 0.21 (1.00–0.00)	0.27 ± 0.23 (0.93–0.00)	0.24 ± 0.22 (1.01–0.00)	0.17 ± 0.16 (0.57–0.00)
Interstitial space (1, 2, or 3)	1.49 ± 0.64 (3–1)	2.00 ± 0.98 (3–1)	1.53 ± 0.84 (3–1)	2.64 ± 0.74 (3–1)	2.07 ± 1.01 (3–1)	2.54 ± 0.88 (3–1)
Boulders (%)	17 ± 24 (80–0)	32 ± 19 (60–0)	4 ± 10 (45–0)	19 ± 23 (80–0)	16 ± 24 (80–0)	24 ± 25 (80–0)
Cobbles (%)	21 ± 20 (71–0)	29 ± 11 (50–10)	27 ± 27 (75–0)	49 ± 19 (80–3)	30 ± 28 (95–0)	45 ± 23 (70–0)
Gravel (%)	27 ± 24 (85–0)	34 ± 17 (75–10)	26 ± 31 (100–0)	31 ± 21 (97–0)	23 ± 24 (95–0)	23 ± 23 (83–0)
Sand (%)	7 ± 13 (50–0)	5 ± 15 (70–0)	9 ± 25 (100–0)	1 ± 3 (15–0)	18 ± 38 (100–0)	1 ± 2 (5–0)
Silt (%)	18 ± 39 (100–0)	0 (–)	28 ± 44 (100–0)	1 ± 1 (10–0)	0 (–)	0 (–)
Bedrock (%)	0 (–)	0 (–)	5 ± 20 (100–0)	0 (–)	13 ± 34 (100–0)	8 ± 28 (100–0)
Riparian vegetation (%)	0 (–)	0 (–)	28 ± 45 (100–0)	4 ± 21 (100–0)	100 ± 0 (–)	100 ± 0 (–)
Macrophytes (%)	28 ± 46 (100–0)	0 (–)	20 ± 40 (100–0)	2 ± 15 (100–0)	22 ± 42 (100–0)	8 ± 28 (100–0)
Filamentous algae (%)	0 (–)	0 (–)	21 ± 41 (100–0)	17 ± 38 (100–0)	0 (–)	0 (–)

Mean ± SD, and range (max–min, in brackets) of each variable is indicated by sampling station during a 1-year period in Pichileufu River

Table 4 PCA based on 17 environmental variables recorded in 295 sampling units in the Pichileufu River

Environmental variables	PC1	PC2	PC3	PC4	PC5	PC6
Maximum water velocity	0.85					
Mean water velocity	0.84					
Bottom water velocity	0.82					
Cobbles	0.76					
Interstitial space	0.66					
Submerged macrophytes	−0.65	0.40				
pH		−0.73				
Temperature		−0.68	0.48			
Boulders	0.41	−0.43				
Mean depth			0.67			
Gravel	0.47		−0.50			
Filamentous algae				0.60		
Silt	−0.56			0.58		
Bedrock				−0.45	0.67	
Oxygen concentration		0.40			0.56	
Sand		0.42			−0.54	
Riparian vegetation				−0.53		0.63
Eigenvalues	4.65	2.25	1.84	1.41	1.19	1.12
% Cumulative explained variance	27.3	40.6	51.4	59.7	66.6	73.2

The highest contributions of variables for each PC (absolute value > 0.4) are indicated

juveniles and adults *H. macraei* in their utilized habitat was negligible. A subtle difference in substrate size could be associated with fish size. Juveniles preferred cobbles and gravel while adults were more associated with boulders. With respect to depth preference, juveniles occupied shallower habitats than adults. Juvenile individuals of *P. trucha* were captured in depositional zones, where water velocity was the lowest. These habitats were characterized by submerged macrophytes rooted on a silty substrate or filamentous algae. The third group, exotic salmonids, was associated with bedrock zones where riparian trees were present. Salmonid sites were the deepest and had an intermediate water velocity (Fig. 4b).

Substrate experiments

No differences were found between the first and second day of experiment in *H. macraei* substrate selection, for any series of experiments (Mann–Whitney, $U >$ 100.00, $P > 0.05$). Thus, data from both days were pooled for later analyses. There were differences in substrate selection (Kruskal–Wallis, $H = 126.20$, df = 3, $P < 0.001$, $n = 256$). *H. macraei* showed a marked preference for substrates of large size. Boulder

Table 5 Comparisons of the mean values of the main environmental gradients (PCs) in Pichileufu River, between sites with and without *H. macraei*

Component	*t* test			
	Mean absences ($n = 38$)	Mean presences ($n = 127$)	*t* value	*P*
PC1	−0.57	0.49	−5.91	<0.001
PC2	0.10	−0.33	2.78	0.007
PC3	0.22	−0.01	1.28	0.202

was significantly selected (mean ± SD, 52 ± 26) more than the other substrates followed by cobbles (35 ± 27) (Tukey, $P < 0.05$). Gravel (8 ± 17) and sand (6 ± 10) were scarcely selected and no difference were found between them (Tukey, $P > 0.05$). The same pattern was observed when analyzing the substrate preference within each of the four periods analyzed during the 24-h cycle (Kruskal–Wallis, $462.20 > H > 206.90$, $P < 0.001$, $n = 128$). Both larger substrates were preferred than the two smaller ones (Fig. 5). Although there were some differences (Tukey, $P < 0.05$) in the substrate preference among positions with respect to

Table 6 Model evaluation results for linear regression analyses of environmental gradients (PC1, PC2, and PC3) and other fish densities on the *H. macraei* density (Den *Hm*)

Candidate model	AICc[a]	ΔAICc[b]	w_i^c
Den *Hm* = PC1 + PC2 + PC3	−341.784	0.000	0.305
Den *Hm* = PC1 + PC2 + PC3 + Den *Om*	−344.026	2.060	0.109 (2.8×)
Den *Hm* = PC1 + PC2 + PC3 + Den *St*	−334.718	2.122	0.106 (2.9×)
Den *Hm* = PC1 + PC2 + PC3 + Den *Pt*	−343.939	2.160	0.104 (2.9×)
Den *Hm* = PC1 + PC2	−343.996	2.600	0.083 (3.7×)
Den *Hm* = PC1 + PC2 + PC3 + Den *Om* + Den *St*	−324.758	4.235	0.037 (8.3×)
Den *Hm* = PC1 + PC2 + PC3 + Den *Pt* + Den *Om*	−339.529	4.264	0.036 (8.4×)
Den *Hm* = PC1 + PC2 + PC3 + Den *Pt* + Den *St*	−346.138	4.321	0.035 (8.7×)
Den *Hm* = PC1 + PC2 + Den *Pt*	−335.826	4.479	0.032 (9.4×)

Den *Pt*, *Perchichthys trucha* density; Den *Om*, *Oncorhynchus mykiss* density; Den *St*, *Salmo trutta* density

[a] Akaike's information criterion values adjusted for small samples

[b] Difference between the AICc of the best-fitting model and the other models

[c] Akaike weights for each model. Only models that had up to 10% w_i of the model with the highest w_i were interpreted. Values in parentheses represent the probability that the best-fitting model is true, given the data, in comparison to the model being examined

Table 7 Model-averaged parameter estimates for *H. macraei* density, standard error (SE), 95% CIs, and model-averaged Akaike importance weights for parameters in interpretable models

Regression parameters	Estimates	SE	Upper 95% CI	Lower 95% CI	w_i^a
Intercept	0.476	0.044	0.562	0.389	–
PC1	0.162	0.057	0.272	0.051	0.847
PC2	−0.138	0.032	−0.074	−0.201	0.847
PC3	−0.062	0.029	−0.006	−0.118	0.731
Den *Pt*	−0.068	0.507	0.920	−1.057	0.207
Den *Om*	0.180	0.572	1.296	−0.936	0.182
Den *St*	0.090	0.449	0.966	−0.787	0.177

Den *Pt*, *Perchichthys trucha* density; Den *Om*, *Oncorhynchus mykiss* density; Den *St*, *Salmo trutta* density

[a] Sum of Akaike importance weights for all models that include the parameter

water inflow, preference for larger substrates was maintained (Fig. 6).

Discussion

Density of fish species was variable along the longitudinal axis of the Pichileufu River. Headwater zones were dominated by salmonids while native fishes were more abundant downstream. This pattern has been found in nearby rivers in Chile (Habit et al., 2007) and the central region of Argentina (Bistoni & Hued, 2002). Changes in richness, composition, and diversity of fish assemblages along the longitudinal river axis have been attributed to

gradual changes of environmental variables (Ibanez et al., 2007; Kouamé et al., 2008; McGarvey, 2011). Headwater sections of a river are characterized by cold water temperature, fast water velocity, and substrate of large size. In downstream sections the flow is slow. In terms of food availability, lower values of aquatic invertebrate biomass (and density) have been recorded in the headwater than in downstream sections of Pichileufu River (Navone, 2006). Fish density showed the same pattern as invertebrates. Even in this river, where Pilila is located in headwater zone and both Pilcaniyeu and Corralito in a transition section between headwater and downstream sections, differences in fish assemblages were evident.
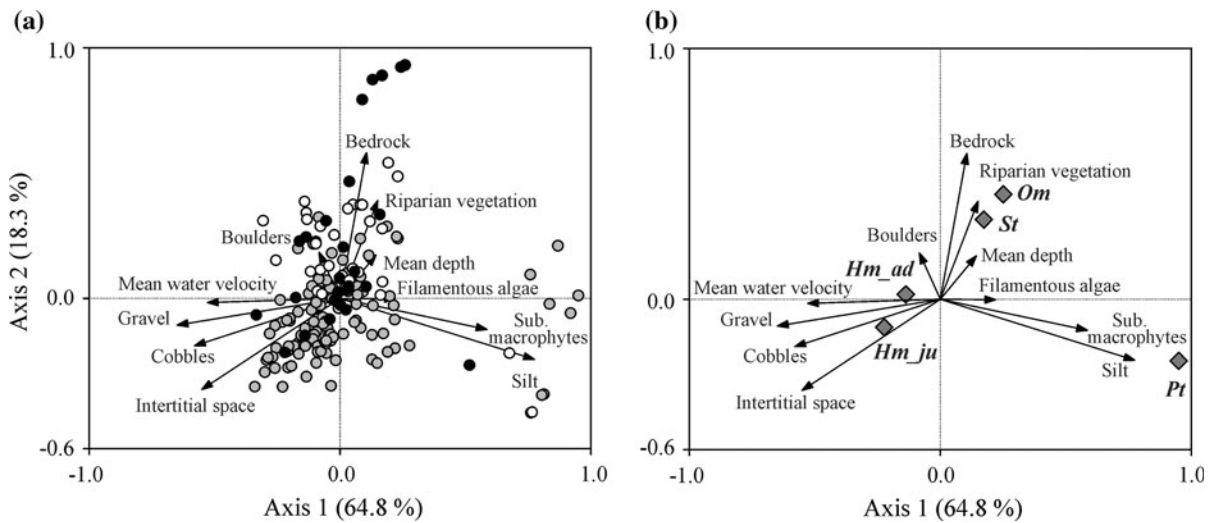
Fig. 4 Partial CCA relating **a** 165 sampling units from Corralito (*white circle*), Pilcaniyeu (*gray circle*), and Pilila (*black circle*); and **b** fish species (*gray diamond*) to environmental variables (*arrows*) in the Pichileufu River. Eleven variables were selected through a forward selection procedure

from 17 original variables (see Table 4). The percentage of the explained variance of the species–environment relationship is indicated in each axis. *Hm_ju* *Hatcheria macraei* juveniles, *Hm_ad* *H. macraei* adults, *Pt* *Percichthys trucha*, *Om* *Oncorhynchus mykiss*, *St* *Salmo trutta*
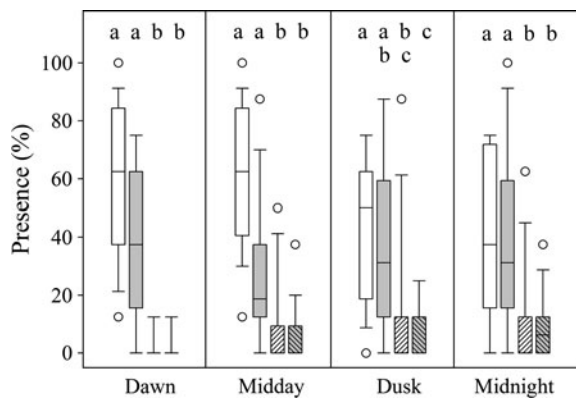
Fig. 5 Substrate selection of *H. macraei* measured as percentage of individuals present in each substrate. Median, quartiles and data outside 10th and 90th percentiles are indicated for boulders (*white*), cobbles (*gray*), gravel (*white stripped*), and sand (*gray stripped*). Significant differences between substrates within each time of the day (dawn, midday, dusk, and midnight) are indicated by *different letters* (Tukey, $P < 0.05$)

Both juvenile and adult *H. macraei* mostly inhabit microhabitats with faster water velocity and larger interstitial space among substrate, such as those present in riffles or runs. This agrees with Ringuelet et al. (1967), who said that *H. macraei* inhabits fast flowing well-oxygenated waters. This species, as do all Trichomycteridae catfishes, has a highly specialized morphological system for anchoring to the

substratum (Adriaens et al., 2010). The presence of spines or odontodes in both opercular and interopercular bones helps *H. macraei* to remain fixed on the substrate in fast flowing waters. Furthermore, this morphological system permits an "elbowing move", allowing this catfish to move between rocks (Adriaens et al., 2010). Also, *H. macraei* is negatively phototactic (Menni, 2004), and they use the interstitial space to rest or hide during daylight. Similar results were found for *Trichomycterus corduvensis* Weyenbergh 1877 by Hued & Bistoni (2006), where the microhabitats preferred were associated with faster water velocities from 0.465 to 0.650 m s^{-1} and substrates between 15 and 30 cm size. At odds, others related species of *Trichomycterus*, such as *T. chapmani* (Eigenmann 1912) and *T. caliensis* (Eigenmann 1912) from Andean streams of Colombia, occurred over sand and mud substrates with slow water flow (Chará et al., 2006).

The spatial niche segregation related to fish length, and probably with ontogeny, could be a frequent pattern found within Trichomycteridae. Species mentioned previously (i.e., *T. corduvensis*, *T. chapmani*, and *T. caliensis*), and also *T. chiltoni* (Eigenmann 1928) (Arratia, 1983), *T. areolatus* Valenciennes 1846 (Arratia, 1983; Manriquez et al., 1988), and *T. maracaya* Bockmann & Sazima 2004 prefer mostly
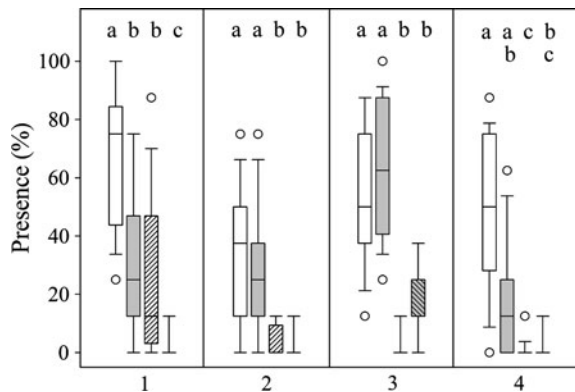
Fig. 6 Substrate selection of *H. macraei* measured as percentage of individuals present in each substrate. Median, quartiles and data outside 10th and 90th percentiles are indicated for boulders (*white*), cobbles (*gray*), gravel (*white stripped*), and sand (*gray stripped*). Significant differences between substrates within each channel position with respect to water inflow (direction from 1 to 4) are indicated by *different letters* (Tukey, $P < 0.05$)

shallow areas as juveniles, while adults choose deeper habitats. However, in *H. macraei*, differences between juveniles and adults microhabitat use were not strikingly different. However, small individuals did occupy gravel and cobbles while bigger catfish utilized boulders and deeper zones (Fig. 4). The main difference in *H. macraei* may primarily be between larvae and both juveniles and adults, with larvae inhabiting shallow marginal pools (<10 cm depth, Barriga & Battini, 2009). Larvae have not developed their swimming and feeding structures, thus restricting both their microhabitat and food use during early life of *H. macraei*.

By contrast, *P. trucha* was associated to low water velocity habitats, depositional zones with aquatic vegetation on silty substrates, i.e., macrophytes or filamentous algae. Juveniles of *P. trucha* were caught among vegetation probably because they used it as shelter. Similarly, juveniles of this species have been found mostly associated with macrophytes in the littoral zone of lakes (Lattuca et al., 2008). Other ecologically similar species, such as *Perca flavescens* (Mitchil 1814) (Fullerton & Lamberti, 2006) or *Percafluviatilis* Linnaeus 1758 (Byström et al., 2003; Lewin et al., 2004) in the northern hemisphere, also use vegetated areas as an anti-predatory strategy during their early life stages (Snickars et al., 2004).

Exotic salmonids (*O. mykiss* and *S. trutta*) were mainly associated with bedrock zones and the presence

of riparian trees. This pattern of trout occurrence was directly related to sampling locations, as the riparian vegetation increased from downstream (Corralito = 0%) to the headwaters (Pilila = 100%). Salmonid habitats were also the deepest, characterized by having an intermediate water velocity. It is widely known that both *O. mykiss* and *S. trutta* are drift feeders, preying on aquatic and terrestrial invertebrates (Bridcut, 2000; Baxter et al., 2004). Results would indicate that both salmonid species in Pichileufu River primarily utilized those areas where the terrestrial subsidies of the allochthonous prey would be higher, i.e., sites near to the tree canopy. Similarly, Kawaguchi et al. (2003) have found a direct relationship between riparian vegetation and salmonid abundance.

The AIC analysis always included PC1 and PC2 parameters in the more explicative models of *H. macraei* density, and with the highest explanatory power (Table 7). This, in agreement with the ANOVA results, points out that *H. macraei* utilized erosional habitats with low pH and temperature. Model of diurnal *H. macraei* density at microhabitat level was better explained by these environmental gradients rather than incorporating density of the other fishes. However, Penaluna et al. (2009) using a before–after control-impact manipulation in rivers of Chile, found a change of mesohabitat in the closely related catfish species *T. areolatus* due to the presence of salmonids. After both *O. mykiss* and *S. trutta* were excluded from experimental segments of the river, *T. areolatus* showed more preference for runs than riffles.

The experimental results showed that *H. macraei* preferred coarser substrates, larger than 6 cm. The selection was independent of the substrate position in the channel and the time of the day. This substrate selection pattern was not modified with low light intensities despite of the high swimming activity performed by *H. macraei* above the substrate during dusk and night (N. Espinós, unpublished data). Thus, the present results confirm the importance of substrate size and suggest that it was actively selected by *H. macraei* rather than a chance association. Other benthic stream fishes have also been associated with coarse substrates, such as *Cottus* spp., *Etheostoma rufilineatum* (Cope 1870), *Percina* sp. (Greenberg & Stiles 1993), and *Catostomus santaanae* (Snyder 1908) (Thompson et al., 2010). We should note that *H. macraei* utilize erosional sites (without fine sediment) with substantial interstitial space, places

associated with fast waters. However, as this catfish lives among the substrate during daylight, water velocity is much lower than the mean water column velocity, even zero. Similarly Chun et al. (2011) found for *Mylopharodon conocephalus* (Baird & Girard 1854), *O. mykiss*, and *Catostomus occidentalis* Ayres 1854 that fish used the substrate to occupy locations with lower velocities than the average. Further experimental trials to test the importance of water velocity in the microhabitat selection are needed.

Substrate selection was not directly associated with food availability because the experiments were performed without aquatic invertebrates. However, taking into account the positive relationship between substrate size and invertebrate abundance found in nature (Velásquez & Miserendino, 2003; Brooks et al., 2005; Pan et al., 2011) we cannot rule out that the selectivity of larger substrate particles by *H. macraei* may be used as a cue for finding prey. Prey abundance has been frequently related to microhabitat selection by small benthic stream fishes [e.g., *Cottus* sp. (Grossman et al., 2006), *Rhinichthys cataractae* (Valenciennes 1842) (Thompson et al. 2001), *Etheostoma olmstedi* Storer 1842 (Henry & Grossman, 2007) or *Trichomycterus* sp. (Chará et al., 2006)]. Furthermore, due to highly heterogeneous conditions within streams prey availability is patchily distributed. In this context, territorial behavior adds complexity to models of fish distribution because the patch utilization is strongly influenced by the intra-specific individual hierarchy (Petty & Grossman, 2007). Nevertheless, *H. macraei* has not been characterized as a territorial fish (Arratia & Menu-Marque, 1981). In this sense, within the dichotomy of the ideal free or ideal pre-emptive distributions (see Petty & Grossman, 2010) the habitat use of this catfish should be better predicted by the former model. Future works must be focused on the effect of food distribution on microhabitat choice of this stream benthic catfish.

Conclusion

This study provides the first quantifiable baseline information of microhabitat use of *H. macraei*. Our results highlight the importance of erosional zones with high water velocity, large substrates, and suitable interstitial space in the microhabitat selection of this species. The microhabitat use during daytime in the Pichileufu River is strongly influenced by the innate hiding behavior of this species in suitable shelters. This response could be an adaptive behavior not only for diminishing predation risk but also to increase the chance of encountering prey. The association with coarse substrate in lotic systems (i.e., runs and riffles) suggests that *H. macraei* may be vulnerable in situations of loss of this habitat type, such as river damming. Furthering the knowledge of microhabitat preferences will increase the ability to protect habitat for *H. macraei* populations and will allow the development of management strategies to improve the conservation status of the species.

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