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Short communication

Shelter selection in the Amazonian zebra pleco, *Hypancistrus zebra* Isbrücker & Nijissen, 1991 (Siluriformes: Loricariidae): requirements in rearing conditions

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Introduction

Loricariidae, popularly known as plecos, are attractive and colourful armoured catfishes with significant value in the ornamental fish market. Many species, however, are currently overexploited in the Amazon basin (Camargo and Ghilardi, 2009), provoking social and environmental issues.

The great diversity of microhabitat in the Amazon basin, specifically in the Xingu River, has favoured the rise of many endemic species (Camargo and Ghilardi, 2009) such as the zebra pleco *Hypancistrus zebra*. This species is endemic in the Xingu River and considered as critically threatened in the national Red List (IN 05/2004 of the Ministério do Meio Ambiente). The imminent risk of extinction is mainly owed to overfishing as well as the Belo Monte Hydroelectric Power plant under construction, and which will modify the river flow to a lentic regime.

The culture of loricariids has been stimulated to satisfy market demands and to reduce the pressure on natural populations (Camargo et al., 2004). Torres (2006) reviewed the limited literature on loricariid culture and summarised the available rearing technologies, which comprise many gaps in knowledge. Thus, the development of adequate culture protocols is of great urgency and which could promote an alternative income for riverine families and lower the fishing pressure on the species (Torres et al., 2008).

The behaviour of seeking shelter in burrows, logs or under rocks is a prevalent characteristic in loricariids, and has been associated with the advantages of protection against predators (Power, 1984; Castro, 1999), food availability (Power, 1984; Buck and Sazima, 1995; Casatti and Castro, 1998; Sabino, 2000) and spawning (Evers and Seidel, 2005). Animals in captivity do not achieve their best growth performance or reproductive output under stress conditions (Galhardo and Oliveira, 2006). Stressors in aquaculture are unavoidable, and reducing stress and its harmful effects is fundament to successful production as well as fish welfare (Ashley, 2007). According to Volpato et al. (2007), fish welfare is best evaluated based on the fish-preference approach. Accepting this approach is reasonable, based on the assumption that a sentient creature does not freely choose a condition of discomfort when presented with better conditions (Volpato et al., 2007). Therefore, in considering adequate culture conditions to reduce captivity stress, the aim of this study was to evaluate the preferential use amongst three different artificial shelters in the cultivation of the zebra pleco, *Hypancistrus zebra*.

Materials and methods

Permit restrictions to collect endangered species limited the capture of wild zebra plecos *Hypancistrus zebra* to 20 individuals (Collection License nº 17760-01, IBAMA). Specimens were acclimated in 200-L aquaria with aeration and a recirculation filter (water flow rate 100L h^{-1}). Feed were dried 'bloodworms' (*Tubifex tubifex*) provided *ad libitum* twice a day (early morning and evening). Residues were removed by siphoning the bottom of the tank at the end of the day.

This study evaluated the feasibility of using materials found commonly in the market for alternative shelters for the zebra pleco. The experimental setup consisted of three types of shelter: (i) natural rock from the Xingu River (rectangular shaped shelter), (ii) clay (rectangular bricks from construction work), and (iii) polyvinyl chloride (PVC) pipes (4 cm ø), all having similar internal volumes (ca. 80 cm^2) and which were distributed in the centre area of each aquarium. To minimise the effect of competitive interactions in the fish selection preference, experiments were carried out using a single specimen in each trial. To avoid disturbances, five fish were randomly selected and each individual distributed into a 60-L aquarium provided with aeration and a recirculation filter (flow rate 100 L h^{-1}). Mean total lengths and weights (±SD) of experimental specimens were 6.49 ± 0.52 cm and 3.28 ± 0.72 g, respectively.

The order of the shelters in the tank was randomly permuted daily, and the sequence of the previous day was not repeated. Three sides of the aquarium were covered to minimise disturbance during visual observation, which took place only from the uncovered side. Observation time began at 8 h and finished at 19 h, and the time each fish spent in the shelters was recorded in minutes by three different observers taking hourly turns. The observers stood in front of the three aquaria at 3 m distance so as to not interfere with the fish behaviour and with a view covering all aquaria.

At the end of the observation the fish were fed with dried bloodworms and the shelters removed until the next trial the following morning. This procedure was intended to minimise habituation by the fish prior to the next trial. The removal of excess residues during the 12-day experiment was made

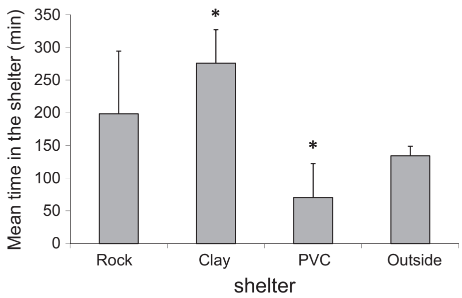

Fig. 1. Mean time (±SD) in minutes spent in each shelter during day-time observations (n = 5 fish). *treatments with significant differences (P < 0.05)

Table 1
Kendall's coefficient of concordance *W* for replicated focal observations per fish, Friedman corrected Chi-square χ^2_r, rank-r and P-value (Zar, 1996)

Fish	*W*	rank-r	χ^2_r	P-value
1	0.2851	0.2151	10.098	<0.05
2	0.0981	0.0161	3.5309	ns
3	0.1989	0.1261	7.1621	ns
4	0.3041	0.2408	10.9459	<0.05
5	0.2603	0.1931	9.3717	<0.05

solely by the recirculation filter in order not to increase fish stress.

The trials for each specimen were analysed using Kendall's coefficient of concordance (*W*) to evaluate plasticity in behaviour. The 12 repeated observations for each fish were then averaged and followed the recommendations of Roa (1992) and Lockwood (1998); data of multiple-offer experiments were analysed by Friedman's nonparametric test (Zar, 1996).

Results

During the experimental period no mortalities or major feeding behaviour changes were observed compared to fish maintained in the acclimation aquarium. Fish spent on average 80% of their time in the shelters during the observations. Seidel (1996) reported that zebra plecos have diurnal activity, but in the trials any alterations in the surroundings or movements near the aquarium drove the fish to seek shelter, demonstrating a shy behaviour. When the disturbance ended, the fish frequently returned to search for food.

The ranking order of time spent in shelters was the longest in clay shelters (276 ± 51 min day^{-1}; mean±SD) followed by the rock shelter (198 ± 96 min day^{-1}), outside the shelters (134 ± 15 min day^{-1}), and in the PVC shelter (70 ± 52 min day^{-1}) (Fig. 1). The variability in the results of concordance amongst the replicated trials for each fish demonstrated the inherent plasticity of the shelter selection in this species. Kendall's concordance coefficient for each individual tested demonstrated the variability of the results, which indicates some level of plasticity in the refuge selection. Two individuals in five tests showed no preference in the degree of concordance amongst the repetitions of the trials (Table 1) and even in the three specimens that showed significant concordance ($X_r^2 > 9.371$, P < 0.05) the *W* ranged between 0.245 and 0.323.

Times spent in the refuges were significantly different amongst the shelter types (F_r= 8,76; P < 0.05). Nonetheless, only differences between times spent in the clay and PVC shelters were significantly different (P < 0.05) (Fig. 1), indicating a low preference for the PVC pipes.

Discussion

Understanding fish requirements and behaviour in cultivations are essential for the establishment of good handling practices (Galhardo and Oliveira, 2006) and to achieve fish welfare (*sensu* Volpato et al., 2007). Fish respond to poor and stressful confinement conditions by triggering physiological mechanisms to adapt to the new conditions; when responses exceed a critical threshold, then changes in swimming behaviour, feed reduction, an increase in aggression (Schreck et al., 1997) and reproduction reduction can all be observed (Val et al., 2004).

The present study demonstrated that shelter selection in *Hypancistrus zebra* is not random, however, lack of agreement in observations of the same fish (Table 1) indicate that selection is sufficiently amenable to allow alternative materials for shelter use. Nevertheless, the nature of the material can significantly affect acceptance, and although often described by aquarists as a suitable shelter, PVC pipes were not appropriate because the fish spent as much time inside the pipes as outside of the shelters.

In natural habitats, zebra pleco inhabit rocky substrates in high water flow stretches of the Xingu River at depths between 1-30 m (Gonçalves et al., 2009; Roman, 2011). Our alternative shelters made of bricks (clay shelters) showed occupation rates comparable to natural rocky shelters and indicated that clay shelter could be used with similar acceptance. Such material enables a range of sizes or shapes to be tailor-made, and could prove a valuable asset to induce reproductive behaviour in the species.

In addition, the presence of refuges could minimise the stress-induced effects of disturbances caused by shadows or during tank cleaning. The presence of shelters in holding tanks of the catfish *Rhamdia quelen* significantly lowered their cortisol levels, indicating lower stress levels (Barcellos et al., 2009). In juvenile perch, *Perca fluviatilis,* the energy growth efficiency for disturbed fish was reduced by more than 19% compared to undisturbed fish that caused a weight reduction of 24 – 56% (Strand et al., 2007). Decreasing growth performances of the cobitid fish, *Lefua echigonia,* an endangered benthic dweller (Matsuzaki et al., 2012), and salmonids (Millidine et al., 2006) were also attributed to the absence of shelter. In both species, increased metabolism maintenance and elevated energy expenditure were probably caused by high levels of flight readiness (Hawkins et al., 2004) and increased mental alertness (Finstad et al., 2007).

In more vulnerable developmental stages such as in fry and early juveniles, the provision of shelter improved growth performance in the benthic African catfish *Clarias gariepinus* (Hossain et al., 1998), and the Arctic charr. Alevins in the latter not only grew faster, but mortality was also lower within shelter treatments. They initiated exogenous feeding later, demonstrating a lower consumption of energy reserves with the availability of shelter (Benhaim et al., 2009).

Zebra pleco show a reduced maximum size, which suggests that this species is particularly vulnerable to predation in the Xingu River. Hence, the use of more acceptable refuges will undoubtedly lower stress levels in *Hypancistrus zebra* and potentially allow the assimilated energy to be directed toward somatic growth or reproduction.

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