Letters to Editor

What tunes aggression in fish during fighting in environments that vary in enrichment?

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In a recent study, we tested the effects of environmental enrichment (EE) with objects (pebbles and kelp models) compared to those of an environment without these objects (control-non-enriched environment) on the aggressive behavior of pairs of pearl cichlid *Geophagus brasiliensis* (Kadri & Barreto, 2010). This study was published in *Neotropical Ichthyology* (volume 8, issue 2, pages 329-332, Apr-Jun 2010). We used the intruder-resident animal relationship as the experimental paradigm. The resident animals typically win a confrontation and become the dominant in the pair (Beaugrand & Zayan, 1985). We showed that EE decreases aggression and/or leads to co-habitation of individuals without concomitant confrontation in pairs of pearl cichlids. Moreover, we also concluded that, regardless of the condition of enrichment (pebbles and kelp or no enrichment), there is a greater probability that the resident pearl cichlid will win a fight. In volume 9, issue 3, Jul-Sep 2011 of *Neotropical Ichthyology*, however, our study was criticized by Nijman & Heuts (2011). Here, I offer five aspects of counter-arguments that maintain the validity of our study. Furthermore, as the research coordinator of the Kadry & Barreto (2010) publication and as the former supervisor of the undergraduate student V. O. Kadry, I assume any liability in relation thereto.

First, Heuts & Nijman (2011) argue that our data do not show a significant effect of prior residence. We tested 9 pairs in a non-enriched environment (control) in the context of the intruder-resident animal relationship. Of these 9 pairs, 7 residents and 2 intruders became dominant. These authors performed a new statistical analysis (binomial test) and found that the frequencies of resident and intruder fish as dominant were statistically indistinguishable ($P = 0.18$); however, this analysis by Heuts & Nijman (2011) used an incorrect sample size. While the correct sample size is 9 pairs, they used 10 pairs and compared 7 resident winners *vs.* 3 intruder winners. Moreover, we used the Goodman test (Goodman, 1965) and the correct sampling size (9 pairs) and showed different results. Within the same multinomial (resident *vs.* intruder), assuming a critical level of significance $\alpha = 0.05$ and $A = 3.84$, we calculated a lower and an upper limit of 0.0124 and 1.0987, respectively. Because we reject the H0 when this interval does not include zero, the Goodman test indicates that these frequencies are statistically different from each other ($P < 0.05$). In relation to the enriched environment, we could not show any effect of prior residence, as in the 8 pairs, only 4 pairs were involved in fighting, and only 3 resident fish became the dominant animal in these 4 pairs. The most important is the analysis with the total number of pairs. We had 13 pairs of fish that fought, without regard for the condition of environmental enrichment (pooled data) for an analysis of the effect of prior residence. In 10 of these 13 pairs, the resident animal became dominant. By using the binomial test, Heuts & Nijman (2011) found that the proportions of dominant residents and intruder animals are statistically equivalent ($P = 0.092$) and concluded that we were wrong to suggest that there was effect of prior residence regardless of the condition of enrichment. But when we analyzed the same data with Goodman's test, we found that the proportion of dominant resident fish is statistically higher ($P < 0.05$) than the proportion of dominant intruder fish. Within the same multinomial (resident *vs.* intruder) with $\alpha = 0.05$ and $A = 3.84$, we calculated the lower and upper limits as 0.0805 and 0.9964, respectively. Thus, we maintain the conclusion that, regardless of the condition of the enrichment, the effect of prior residence is significant.

Second, Heuts & Nijman (2011) stated that Kadry & Barreto 2010 "...found no prior-residency effect for pearl cichlids but there was clear difference in the amount of aggression displayed by both residents and intruders in the non-enriched condition..." and asked "How to best explain these differences?". In the paragraphs following this question, these authors gave no explanation of the differences in the frequency of aggressive acts and only discussed how we measured the index of dominance. The dominance index is widely used and represents the number of aggressive acts directed toward and received by an individual in the pair (Winberg *et al.*, 1991; Gómez-LaPlaza & Morgan, 1993, Oliveira *et al.*, 1996, Oliveira & Almada, 1996; Lehner, 1996; McCarthy *et al.*, 1999, Bailey *et al.*, 2000; Gonçalves-de-Freitas *et al.*, 2008; Miyai *et al.*, 2011). An attack of a pearl cichlid is followed by a submissive reaction by the other fish, and this act results in escape or retreat in response to the opponent's attacks or redirects the attack to a less vulnerable area of the body, such as the tail. This is well established in the literature to define the winner of an attack (Oliveira *et al.*, 1996, Oliveira & Almada 1996, McCarthy et al., 1999). Therefore, the use of this index in our study cannot be criticized, and this index was only used to set the resolution of the confrontation (identification of the dominant fish). I apologize to the readers of *Neotropical Ichthyology* for not having been explicit about this point in the original paper; however, at that time, we regarded this distinction as obvious to researchers in the field.

Third, we wish to explain again the difference in the aggressiveness between the pearl cichlid in the enriched and non-enriched tanks. In relation to aggression, the main finding of our study was that EE decreases aggressive activities in pearl cichlids. This is clear in our study: although only 4 of the 8 pairs fought in the enriched environment (50%), all pairs

(9 of 9, 100%), fought in the non-enriched environment (control). These proportions were statistically different (Goodman test, between multinomials; $G_{observed} = 2.83 > G_{expected} = 1.96$, $P < 0.05$ and two-tailed Fisher's exact test, $P = 0.029$). Furthermore, as shown in our article (Kadry & Barreto, 2010), the frequency of aggressive interactions was significantly lower in the enriched environment compared to the non-enriched environment for both resident ($Z = 3.27$, $p < 0.01$) and intruder fish ($Z = 2.17$, $p < 0.05$). Moreover, in the non-enriched environment, the frequency of attacks directed by the resident animal was statistically higher than for the frequency of attacks directed by the intruder animals ($Z = 2.07$, $p < 0.05$). Reinforcing our conclusion that EE reduces aggression in pearl cichlids, in this condition, there was no difference between the frequency of attacks displayed by the intruder and that by the resident fish ($Z = 1.10$, $p = 0.27$). Here, I also provide a new analysis that considers the frequency of aggressive interactions in pairs of pearl cichlids in the different EE conditions (regardless of the condition of residence or social status). At the pair level, the frequency of aggressive interactions was statistically higher for fish in the non-enriched environment compared with those in the enriched environment ($Z = 3.27$, $p < 0.005$; Fig. 1). This new analysis reinforces our initial observation that EE reduces aggression in pearl cichlids. Thus, our conclusion is maintained.

The fourth point concerns a false dichotomy made by Nijman & Heuts (2011). They said that as the resident animals tend to value their territory, they tend to defend that territory more forcefully, increasing the likelihood of winning a fight against an intruder animal. In the case of an enriched environment (an area with more resources), this necessarily must occur due to either the effect of prior residence or to the prior residence effect with increased aggressiveness (the false dichotomy). No other possibilities exist. I partially agree with this prediction. In fact, some colleagues and I reported findings linked to this predicted effect in the cichlid Nile tilapia (*Oreochromis niloticus*), where EE increased aggressiveness, but in a neutral arena (Barreto et al., 2011). Other possibilities could also be suggested. In the case of our study (Kadry & Barreto, 2010), how can we explain our data differently if EE reduced aggression or led to cohabitation without confrontation in pearl cichlids? We assume that the data should be above expectations, irrespective of how nice expectations should be. Moreover, the new analysis presented herein in this comment (Fig. 1) reinforces the existence of other possibilities. This new analysis considers the frequency of aggressive activities in the pair without regard to the effects of prior residence and social status (dominant or submissive). Even then, our conclusion regarding the EE and aggression remains. Moreover, during our manuscript preparation and publication of our article (published in *Neotropical Ichthyology* Apr-Jun/2010 but online since Feb/2010), other reports were published that showed that EE reduces aggression or the territory size defended aggressively (leading to increased population density - cohabitation) in salmonids (Imre et al. 2002; Höjesjö et al., 2004), and these reports were included in our article (Kadry &
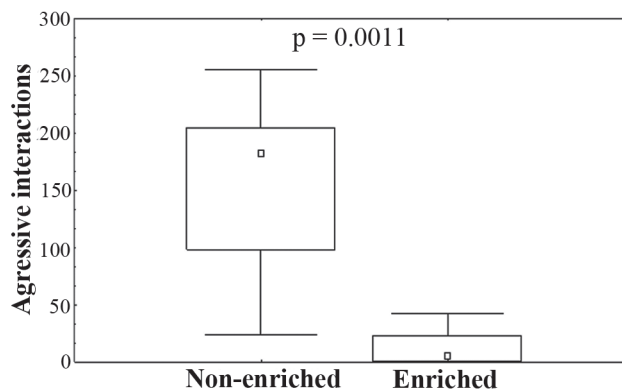
Fig. 1. The frequency of aggressive interactions in pairs of pearl cichlids (*Geophagus brasiliensis*) in different environmental enrichment conditions (pebbles and kelp or no enrichment). At the pair level, the frequency of aggressive interactions was statistically higher for fish in the non-enriched environment compared with those in the enriched environment (Mann-Whitney U test; $Z = 3.27$, $P = 0.0011$). Boxes represent the interquartile range containing 50% of the values. The whiskers are lines that extend from the box to the highest and lowest values. The small squares inside the boxes indicate the median.

Baker, 2010). Heuts & Nijman (2011), however, arbitrarily restricted their analysis to cichlids and ignored those articles about salmonids and the article about convict cichlids (*Archocentrus nigrofasciatus*) by Barley & Coleman (2010). At the same time as the online publication of our article and in the February 2010 issue of *Current Zoology*, Barley & Coleman (2010) reported that "...the number of bites directed at the subordinate fish increased when there was a low amount of structure..." in convict cichlids and also that "These results indicate that increased habitat structural complexity decreases aggressive behavior in convict cichlids." This type of response has also been reported for interspecific confrontations between cichlids (Danley, 2011).

A final comment is that Nijman & Heuts (2011) suggest that the sample size is sufficient to discredit our study. Although larger samples are always well received, statistical tests consider these variations by adjusting the critical assumptions accordingly: a calculated value from a statistical test, that is compared with a critical value in order to reject the null hypothesis in favour of the alternative hypothesis and vice versa, depends on the degree of freedom (Zar, 2009). Confirming the status of quantitative science, we see that others, not included in the work of Nijaman & Heuts (2011), have similar conclusions to ours as shown above [for instance, Barley & Coleman (2010)].

Based on the above arguments, we maintain the conclusion that EE reduces aggressiveness in pearl cichlids and that, regardless of the condition of enrichment, the pearl cichlid residents are more likely to be the dominant individuals when confronted with intruders of the same species. I believe

that science is a great debate and such initiatives are positive. Hence, I fully agree with Nijman & Heuts (2011) who say that "...there is much to learn about the interrelationships between dominance and aggression and what influence the environment plays in this…" Thus, I propose the following question: What tunes aggression during fighting in fish in environments that vary in complexity?

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