## Effects of divergent waters colours on Amazon fish evolution

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# 1. Introduction

Amazon has the highest freshwater fish fauna in the world with more than 3000 species formally described. This biodiversity inhabits black-, clear- and white- waters. Many hypotheses considering different factors as evolutionary drivers of the Amazon fish biodiversity have been developed. They mainly focused on speciation through allopatric processes. However, the Amazon aquatic environment highly varies in physical and chemical parameters, affecting the sensory systems of the aquatic biota. For example, black waters are rich in dissolved organic carbon, which reduces the pH and biases the underwater light toward red colours. Clear waters show a nearly neutral pH and show no apparent colour biases. These water features have a high potential to drive the sensory systems of the individuals exposed to these divergent waters apart. As a consequence of such divergent evolution of the sensory systems, local adaptations are expected to lead populations to be reproductively isolated. Such effect can be observed since sexual communication can be hampered by the divergent adaptations to the different water types.

I used the sailfin tetra *Crenuchus spilurus*, an Amazon small fish species, as a model to investigate how divergent underwater lighting condition drives population apart through behavioural and genetic experiments. The sailfin tetra is a sexually dimorphic fish species in which males possess dorsal and anal fins conspicuously ornaments in red and yellow colours. These ornaments are used during an elaborated courtship behaviour. Males perform exclusive parental care that lasts for around eight days. Therefore, it is expected a high sexual selection pressure in this species. The sailfin tetra is composed of two phylogenetic lineages, one inhabiting small forest streams of black and the second one inhabiting clear waters. These lineages are reproductively isolated. I evaluated the effects of black and clear water lighting condition on males' ornaments, female mating choice, their red colour preference and the light sensitivity

## 2 Material and Methods

## 2.1 Effects of water colour on males ornaments

I created a black water light colour filter, simulating the black water lighting condition. I took photos of the males from black and clear waters under the black water filter and full-spectrum light, simulating both black and clear water lighting conditions, respectively. I analyzed the intensity of the red colour on males dorsal and anal fins ornaments.

### 2.2 Effects of water colour on female mating choice

I then set up couples, composed for male and female from either black or clear waters, under the full spectrum and black water light condition and analysed their male mating female mating acceptance.

## 2.3 Female red colour preference

I also investigated female colour preference through environmental colour choice. For this experiment, a female was inserted into an experimental tank that was half illuminated by the red and half illuminated by the full spectrum light. Females from black and clear water lineages were tested.

## 2.4 Evaluation of the colour sensitivity

RNA samples from the fish eyes were extracted and sequenced. I aligned the RNAseq data to a reference genome to identify the opsin genes in the fishes eyes. Once they were found, I checked for the genetic diversity, the expected light peak sensitivity and expression level of each opsin gene.

## 3. Results

# 3.1 Effects of water colour on males ornaments

Black water light increased the perception of the males ornaments red colours. However, it also decreased the among-individual variation of the ornament red colouration for all males, irrespective of their lineage.

#### 3.2 Effects of water colour on males ornaments

Females from clear and black waters were more likely to accept larger ornamented mates. Clear water lineage female mating acceptance was higher under black water lighting condition than under the full spectrum light. On the other hand, black water lineage females' mating acceptance was not affected by the different lighting conditions.

#### 3.3 Female red colour preference

Females from black waters and clear waters preferred red-lightened environments. However, this red colour preference was stronger in the females from clear than in those from black waters.

## 3.4 Colour vision

I found five copies of the LWS genes and a copy of the SWS2 gene in all samples. All these genes were genetically similar and the expected  $\lambda$  max did not differ among the populations. The expression levels of most of the LWS genes were similar for all populations; however, it can be expected since individuals were kept under similar captivity conditions before the acquisition of the genetic samples.

## 4. Discussion

The red bias of the black water is expected to increase the intensity of the red colours in the males ornaments. However, it also decreased the among-individual variation on the males ornaments colours. Therefore, it may be more difficult to females to correctly access male quality based on a trait that show little variation due to the environmental effects on male colouration. Thus, I suggest that the lower among-individual variation in male ornaments colouration due to the environmental lighting condition under black water represents a potential fitness cost to females living in such waters. Therefore, females living in black waters may have shifted their mating choice criteria, from ornaments colouration to ornaments size.

Even though the importance of the red colour differs among lineages for the mate choice, females from black and clear waters preferred red colours over full-spectrum light environments. This preference can be related to several ecological processes, such as finding food resources and predator avoidance. More importantly, such stronger preference for red colour in females living in clear waters may relate to their colour preference in mating behaviour.

Finally, the opsin genes, and especially the LWS genes, were conserved among populations inhabiting black and clear waters. Because of the high environmental variation in the forest stream waters in Amazon, I propose that the visual adaptations may occur in the gene expression profile rather than the direct correlation between the LWS gene and environmental conditions. Thus, individuals may express different genes according to the transitory environmental colour.

Here, I suggest that the Amazon water types (and colours) affect the evolution of morphologic, genetic and behavioural traits in an Amazon fish species. I also encourage future investigations into Amazon forest stream biota for a better understanding of how the biota evolves and the drives of such huge biodiversity. Ultimately, understanding the evolutionary mechanisms in Amazon fish fauna can help us manage the actual biodiversity and propose optimal conservation efforts.