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Phenotypic plasticity in the retina of Chiso catfish (*Trichomycterus* aff. *banneaui*: Siluriformes) in response to intermittency in the Cube River

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Plasticidad fenotípica en la retina del pez Chiso *(Trichomycterus* aff. *banneaui*: Siluriformes) en respuesta a la intermitencia en el Río Cube

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RESUMEN

La ecología de la visión de peces es un excelente sistema por el cual explorar la plasticidad fenotípica. Los ríos intermitentes presentan cambios ecológicos que podrían alentar un ajuste a la luz disponible en los peces. Esto se examinó en el bagre *Trichomycterus* aff *banneaui* del Río Cube, en el Chocó ecuatoriano, que debe su intermitencia a la estación seca-lluviosa. Con el objetivo de analizar la plasticidad fenotípica en la retina de *T.* aff. *banneaui* en respuesta a esta propiedad del río, se realizó un análisis de expresión de genes retinales mediante FPKM, para aquellos que codifican para opsinas visuales, y el gen *cyp27c1*, implicados en el funcionamiento de pigmentos visuales, y DESeq2 para las demás secuencias obtenidas de individuos capturados en ambas estaciones. Se encontró que *T.* aff. *banneaui* posee una rodopsina, RH1 (visión nocturna), y dos opsinas (visión diurna a color) sensibles a longitudes de onda medias, RH2 y largas, LWS, que son sobreexpresadas durante la estación lluviosa, y componen el principal mecanismo de ajuste espectral en la especie. Además, se hallaron otros 35 genes diferencialmente expresados, donde resaltaron varios vinculados a inmunología y cáncer durante la estación seca, y se reporta posibles genes implicados en la visión. Este estudio contribuye a entender la relevancia de la visión para bagres, y su operación entre los demás mecanismos de ajuste en la retina al complejo impacto ecológico y evolutivo de la estacionalidad e intermitencia de este tipo de ríos.

Palabras clave: Plasticidad fenotípica, río intermitente, bagre, visión, opsinas, genes diferencialmente expresados, Chocó

ABSTRACT

Fish visual ecology is an excellent system to explore phenotypic plasticity. Intermittent rivers exhibit ecological changes that might motivate adjustment to available light in fish. This was examined in the catfish *Trichomycterus* aff. *banneaui,* from the Cube River in the Ecuadorian Chocó, that owes its intermittency to seasonality (dry-wet). With the objective to analyze retinal phenotypic plasticity in *T.* aff. *banneaui* in response to this river's property, we performed a gene expression analysis using FKPM for visual opsin and *cyp27c1* genes, involved in visual pigment function, and DESeq2 for the accompanying sequences obtained from wild-caught fish during both seasons. We found that *T.* aff. *banneaui* possess one rhodopsin, RH1 (nocturnal vision), and two opsins (diurnal color vision) sensible to medium, RH2, and long, LWS, wavelengths. These two are upregulated during the wet season and are the main spectral tuning mechanism in the species. We also found 35 differentially expressed genes, from which the high prevalence of immune and cancer related genes during the dry season stood out, and some possible vision-related genes are reported. This study contributed to the comprehension of vision relevance for catfishes, and its function among other retinal adjustment mechanisms to the complex ecological and evolutionary impact of seasonality and intermittency in this type of rivers.

Keywords: Phenotypic plasticity, intermittent river, catfish, vision, opsins, differentially expressed genes, Chocó

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INTRODUCTION

Phenotypic plasticity is the capacity of some traits to be manifested as different phenotypes, in response to environmental variation (Pigliucci et al., 2006), and has also shown to be a valuable component for studying species evolutionary trajectories (Fusco & Minelli, 2010). Vision is a critical sensory mechanism that rapidly adapts to environmental changes, making it a suitable system for studying phenotypic plasticity (Hauser & Chang, 2017). Particularly, the study of vision in teleost fish offers the opportunity to better understand phenotypic plasticity because: 1) This large group possess multiple tuning mechanisms to adjust spectral sensitivity to the available light wavelengths; and 2) their aquatic environment exerts a dynamic and broad spectrum of optic conditions (Carleton et al., 2020).

The light absorbing mechanisms in the vertebrate retina have received much attention because it contains visual pigments that are responsible for light reception and processing. These are made of two proteins (Fig. 1). First, the opsin protein, which is classified among five vertebrate opsin classes according to the specific wavelength range to which it is sensible; and second, a chromophore that isomerizes when the correspondent photons are received, inducing a following neurological signaling cascade (Hauser & Chang, 2017). Visual pigments are found in rod and cone photoreceptor cells. While rod cells contain only a single dim light vision opsin, rhodopsin (RH1, ~500 nm), cone cells display four opsin classes related to color vision in day light (Hauser & Chang, 2017). These include short wavelength sensitive (SWS1, 355-455 nm; and SWS2, 400-470 nm), a medium wavelength sensitive (RH2, 480-530 nm) and a long wavelength sensitive opsin (LWS, 500-570 nm) (Hauser & Chang, 2017). At the opsin protein level, fish vision can be shaped by molecular evolution mechanisms, such as mutations in the opsin amino-acid sequence and duplication or loss of opsin genes (Carleton et al., 2020), which ultimately shift the spectral sensitivities of fish visual systems. Notably, fish are the only

vertebrate group which has increased their quantity of opsin genes through these mechanisms (Musilova et al., 2021).

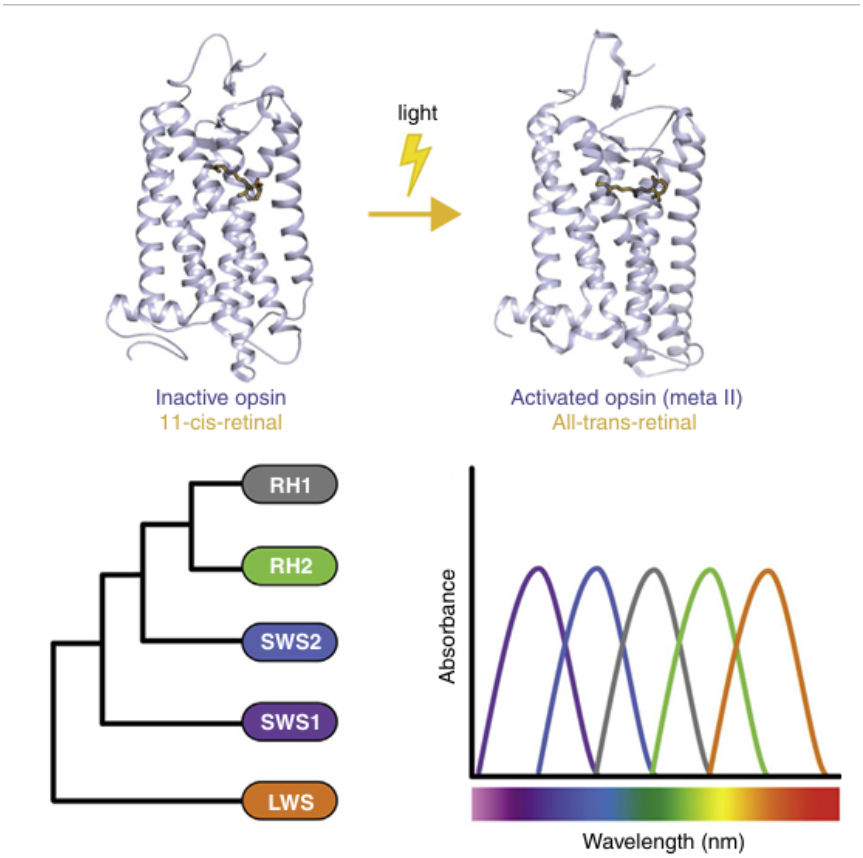


Figure 1. Visual pigment components (Hauser & Chang, 2017)

Above, both opsin, the multiple helix structure, and chromophore, central molecule, are shown. In response to light sensitive to the opsin, the chromophore isomerizes. Below, the five vertebrate opsin classes by their phylogenetic relationship, as well as their sensibility profiles.

Furthermore, fish visual sensitivity can also be tuned within the fish lifespan - 'in-vivo' in order to adjust to variable light environments (Musilova et al., 2021). For example, differential opsin gene expression of the various opsin proteins results in different visual sensitivities throughout the lifecycle of the fish (Carleton et al., 2020). However, there are several mechanisms that don't directly affect opsins, but shape individuals' optic traits in other ways. These include wavelength filtering in other eye tissues (Douglas et al., 1998; Hofmann et al., 2010), retinal photoreceptor density, photoreceptor spatial arrangement, cone-to-rod

ratio (Collin & Shand, 2003); or type of chromophore within the visual pigment. This latter mechanism is common in freshwater fish (Toyama et al., 2008), and is controlled by the *cyp27c1* gene, which codes an enzyme that converts A_1 chromophore type to the red-shifting A_2 type (i.e., increasing spectral sensitivity of the visual pigment by several nm) (Corbo, 2021).

Although there's a great body of literature regarding opsin expression adjustment to ambient light, its implications aren't always clear (Carleton et al., 2020). There are various known processes that also affect vision, making differential expression of accompanying retinal genes a promising effort to understand adjustment to available light. One such study investigated genes in cichlid species that inhabit different depth niches, and thus, different light conditions, in Cameron's Lake Barombi Mbo (Musilova et al., 2019). Change in expression of several retinal genes was determined by depth; most of the identified genes were related to vision, to some degree. For example, some were associated with chromophore synthesis, eye development, or phototransduction pathways, while others were related to circadian clock regulation, as well as eye-pressure and hypoxia regulation, showing that broader phenotypical responses to the ecological context are also crucial to maintain eye functions. Although broad in scale, expression analysis of non-opsin genes in the retina allow to integrally understand spectral tuning and its related pathways.

Intermittent rivers are the predominant type of river network whose ecological impact has been underestimated in policy and conservation interests (Datry et al., 2023). These are characterized by having reaches that cease to flow or completely dry during periods of their hydrological cycle creating variable conditions that have an impact on biodiversity and ecological processes (Datry et al., 2023). Some intermittent systems, as the Cube River Basin (CRB) in northwestern Ecuador, exhibit their intermittence product of seasonality (i.e., dry and wet seasons) (Datry et al., 2023; Escobar-Camacho et al., 2024). The CRB is part of the Esmeraldas River basin, as the CRB later joins the Viche River downstream, which eventually

joins the Esmeraldas River (Escobar-Camacho et al., 2024), The Esmeraldas River basin is part of the diverse Chocó Darien Global Ecoregion in northwestern Ecuador, an isolated lowland mountainous area critical for fish conservation (Aguirre et al., 2021). Up to 43% of its species are endemic, yet most are poorly understood (Escobar-Camacho et al., 2024). Additionally, the region is heavily impacted by anthropogenic disturbances, which have already affected fish communities and threaten the basins it supports (Aguirre et al., 2021; Leberg et al., 2021).

Due to striking environmental variability, intermittent rivers might act as plasticity-stimulating scenarios for fish visual systems because of their different light conditions between seasons. For example, water tends to be clear during the dry seasons, while turbid and murky during the rainy seasons (Torres-Arias, 2024). Studies in neotropical cichlids and characins in Panama (Escobar-Camacho et al., 2019, 2020) have showed that long wavelength-sensitive opsin palettes tend to be favored in more turbid waters, in comparison to individuals sampled from clearer habitats; additionally, both cases also presented higher usage of A_2 chromophore. Aside from aquatic optical properties, other environmental variables can induce a change in gene expression. This has been observed in the case of neotropical cichlids, as detected by differential gene expression analysis; for instance, expression of genes *HBB, HBAA, HBAB* and *PDFGFRL,* involved in blood circulation, were conversely affected by dissolved oxygen concentration due to turbidity (Escobar-Camacho et al., 2019). The intermittence of CRB as a possible determiner of ecological and evolutionary traits involved in phenotypic plasticity contributes to the significance of drying river networks for conservation efforts considering that this systems are most probably on the rise due to climate change (Datry et al., 2021).

In this study, the visual system of the ‘Chiso’ catfish, *Trichomycterus* aff. *banneaui* (Escobar-Camacho et al., 2024) was examined, whose distribution is confined to the headwaters of the CRB. This investigation will be a valuable addition to the scarce knowledge about vision in catfishes (Lemopoulos & Montoya-Burgos, 2022; Zheng et al., 2021), one of

the most ecologically and taxonomically representative fish groups, and will be useful in characterizing the ecology of these area within the watershed.

This study addresses the following question: ¿How is the expression of visual opsins and other retinal genes in *Trichomycterus* aff. *banneaui* affected by seasonal intermittency in the CRB? To solve it, we aimed to 1) Identify the visual opsin repertoire in this species; 2) Analyze if visual opsins and *cyp27c1* gene are differently expressed within seasons, and 3) Examine the possible set of accompanying differentially expressed genes from the retinal samples. For this, gene isolation on the targeted opsin and *cyp27c1* genes in combination with differential gene expression (DGE) analysis was performed on retinal transcriptomes of wild caught Chiso fish collected in dry and rainy seasons in the CRB. Based on results from neotropical cichlids and characiforms, we hypothesized that the retina of *Trichomycterus* aff. *banneaui* shows phenotypic plasticity by exhibiting different gene expression between seasons.

MATERIALS AND METHODS

Study area and sampling design

The Cube River Basin (CRB) is a seasonal intermittent fluvial system located in the Choco ecoregion, in northwestern Ecuador. The Cube River drains into the Viche River to its north (Fig. 2). Its headwaters, to its south, fall within the borders of private protected reserves and a large portion of the remaining watershed overlaps with the Mache-Chindul Ecological Reserve (Escobar-Camacho et al., 2024). However, the region is covered by a patchy matrix of forests, with native vegetation characteristic of wet tropical rainforest, and transformed farmland (Durães et al., 2013). It is a mountainous area (Escobar-Camacho et al., 2024) with a maximum elevation of 800 m. a. s. l (Leberg et al., 2021), mean annual precipitation between 2000-3000 mm, and seasonal variability: Wet season goes from January to June, while the dry season lasts from July to December (Durães et al., 2013).

To analyze phenotypic plasticity in response to the seasonal environmental variation, we sampled throughout sites with different levels of intermittence and fish communities between seasons during two sampling campaigns. Among the collected species, *Trichomycterus* aff. *banneaui* was only found at a single sampling site (0.37817, -79.66441; 485 m. a. s. l) located in upstream intermittent headwaters within the area owned by the private reserve Fundación para la Conservación de los Andes Tropicales (FCAT) (Fig. 2). Sampling was carried out during the wet and dry season throughout a one-year span. During the dry season (November 29th, 2022), the sampling site consisted mostly of isolated ponds, while in the wet season (April 3rd, 2023) it consisted of shallow flowing streams with some partially connected pools.

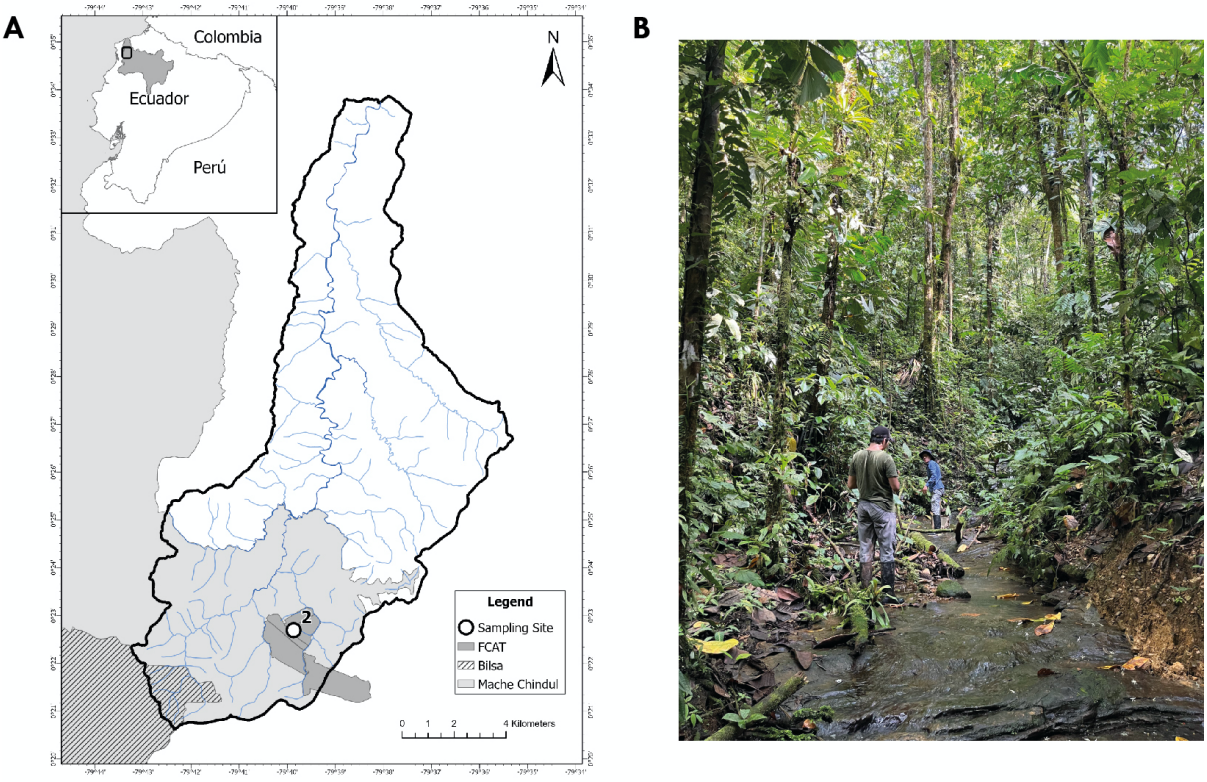


Figure 2. Study area and sampling site within the CRB

A) Spatial drainage area of CRB (black outlined area) and related protected zones (scale of gray). The grey area on the top insert shows the Esmeraldas River Basin. Courtesy of José Daza **B)** Sampling site during the wet season. Image from Karla Barragán

Study Species

Although literature recognizes the existence of different *Trichomycterus* species in the CRB (Jiménez-Prado, 2015; Leberg et al., 2021), recent studies show that the basin's clade record high diversity, and is possibly inhabited by two yet undescribed lineages, including *Trichomycterus* aff. *banneaui* (Escobar-Camacho et al., 2024). This species' visual capabilities can seldomly be inferred. Apparently, the group is confined to the very first waterhead streams and ponds, which are often isolated in the dry season (Escobar-Camacho et al., 2024). They feed on small invertebrates and are presumably nocturnal, staying close to the sediment while inactive (Jiménez-Prado, 2015).

Environmental variables

To examine environmental variation, we used a YSI multi-parameter water quality sonde to measure several physicochemical variables at each sampling site, on both sampling periods (Appendix A). The variables measured included water temperature (°F), turbidity (FNU), dissolved oxygen (mg/L and % saturation), conductivity (µS/cm), pH, oxidation-reduction potential (ORP). We also used a Secchi disk to compare light penetration between seasons. We made two measurements per season, at random ponds, at a depth of 5 and 28 cm for each season. River water level measurements at a close station were also included (Appendix B).

Fish sampling

Fish were collected with seines and hand nets, leaning against the rock or mud at the ponds' bottom. The sample transect was about 200 m long, along the stream. 12 specimens were selected manually thereafter and placed temporarily on water buckets with an oxygen blower. We kept adult individuals only (TL >4.5 cm), whose size allowed for dissection and collection. Fish were transported to FCAT research station, where they were photographed and humanely euthanized with eugenol oil. Directly after euthanization, dissection was held (following section), and morphological measurements of individuals collected were taken. Only eight specimens (three for dry season, five for wet season) were kept for analysis in this study (Appendix C).

Tissue preparation

Eyes were dissected with microdissection scissors and tweezers. Retinas, including the pigment epithelium, were extracted and preserved in RNAlater solution (ThermoFisher Scientific). Muscle tissue samples from the fish back were extracted and stored in 96% ethanol tubes for additional DNA extraction. Finally, whole specimens were fixed in 10%

formaldehyde bath and consequently preserved in 70% ethanol as vouchers, following museum collection protocols.

This study was carried out accomplishing animal welfare laws, guidelines, and policies approved by the Ministry of Environment, Water and Ecological Transition (Ministerio de Ambiente, Agua y Transición Ecológica del Ecuador—MAATE). Scientific/Collection research permit (MAATE-ARSFC-2022-2286), Mobilization permit (01038; 01159), Export permit (071-2023-EXP-CM-FAU-DBI/MAATE), Permit to access genetic resources (MAATE-DBI-CM-2023-0289).

RNA extraction and sequencing

Retinal tissue samples were transported for RNA extraction to Fabio Cortesi's Laboratory at Marine Sensory Ecology Group in the School of the Environment and Queensland Brain Institute (© The University of Queensland). To isolate total RNA, Monarch Total RNA Miniprep Kit (NEB) was utilized, and samples were subsequently DNA-decontaminated using RNase-free DNase (NEB). RNA quality and yield were evaluated with eukaryotic total RNA 6000 Nano assay in a Bioanalyzer 2100 instrument (Agilent Technologies) (Escobar-Camacho et al., 2017).

Creation of transcript libraries from raw RNA samples was done with NEBNext Ultra RNA library preparation kit for Illumina (NEB) was used, following manufacturer's instructions. Subsequently, library concentrations were assessed with Qubit dsDNA BR Assay kit (ThermoFisher Scientific). Library barcoding was performed thereafter. These were pooled in equimolar ratios and subjected to 150 bp paired-end sequencing in the NovaSeq X Plus (Illumina), applying SBS v4 sequencing chemistry (Fogg et al., 2023). Sequencing was performed at Novo gene, Singapore.

Transcriptome assembly with Galaxy

To perform analysis of differential gene expression, raw sequences were assembled into transcriptomes for each individual using Galaxy (https://usegalaxy.org) web-based platform. An initial quality assessment was conducted on forward and reverse files, using FASTQC tool (Galaxy Version 0.74+galaxy0). Reads were filtered, and adapters were removed using Trimmomatic tool (Galaxy version 0.36.6) (Bolger et al., 2014). To evaluate quality for the new files (Fogg et al., 2023), the resulting FASTQ files were submitted for the second quality assessment with FASTQC tool. After validation, FASTQ sequences were transformed to FASTA format using FASTQ to FASTA converter tool (Galaxy Version 1.1.5) (Blankenberg et al., 2010; Fogg et al., 2023). Finally, de novo transcriptome assembly was performed with forward and reverse FASTA files for each individual using Trinity assembler (Galaxy Version 2.15.1+galaxy1) (Grabherr et al., 2011) with default parameters .

Opsin class identification

To identify possible opsin sequences within the assembled transcripts obtained in the previous section, we performed BLAST hit searches. We used the assembled transcripts as a database, and NCBI opsin (SWS1, SWS2, RH1, RH2, LWS) sequences from the closest species found (*Danio rerio, Sternopygus macrurus, Ictalurus punctatus, Eretmobrycon emperador, Eigenmannia virescens*) and Nile tilapia (*Oreochromis niloticus*) as queries. Additionally, the process was repeated to search for chromophore-type relevant gene *cyp27c1*, using a query sequence obtained from *D. rerio* in NCBI. This analysis was held in ® Geneious Prime (Version 2024.05) (https://www.geneious.com).

To classify opsin classes, we performed an opsin phylogenetic tree using our candidate sequences. Blast hit sequences (candidates) >1000bp from *Trichomycterus* aff. *banneaui* transcripts were first aligned using MAFFT Alignment plugin (Version 1.5.0) (Katoh & Standley, 2013) against the mentioned reference sequences from other teleosts. We later

performed a maximum likelihood phylogenetic tree using IQ-TREE web platform (Version 1.6.12), which also select the best fit model of molecular evolution (http://www.iqtree.org). The resulting tree file was further edited in FigTree (Version 1.4.4).

Opsin and *cyp27c1* differential gene expression

To obtain expression counts for the identified opsins in *Trichomycterus* aff. *banneaui*, we mapped the identified opsin sequences to transcripts of paired read files in Geneious. The latter was created using 'Set as Paired Reads' tool with both forward and reverse transcript FASTA files obtained in Galaxy earlier. We used 'Map to Reference' tool with the following changes to standard parameters: 'Fine tuning = None'; 'Allow gaps'; 'Minimum Overlap = 50'; 'Word Length = 24'; Ignore words repeated more than = 8 times'; 'Maximum Mismatches per read = 2%'; 'Accurately map reads with errors to repeat regions = Deactivated'; 'Only map paired reads which = both map'; 'Maximum gap size = 3'; 'Index Word Length = 14'. These modifications were intended to recognize correspondent opsin reads only. We mapped each opsin sequence with every specimen's paired-read sequencing transcripts.

Expression quantification was obtained using Fragments Per Kilobase Million Mapped Fragments (FPKM) counts. This is a suitable mode of quantification for expression analysis within a single sample, as normalization and calculation are made with reference to particular sequence length and raw read counts (Blankenberg et al., 2010; Zhao et al., 2021). Coherently, FPKM values were calculated with Equation 1, for each opsin of every specimen. Finally, expression levels recorded among samples from different seasons were compared with a Welch t-test in RStudio (Rstudio, Boston, MA, USA; RStudio Version 4. 4. 1) with base functions. The process was also replicated to compare *cyp27c1* expression levels.

$$FPKM = \frac{FragmentCounts * 10^9}{TotalMappedFragments * TranscriptLength}$$

Equation 1. FPKM calculation (Zhao et al., 2021)

Whole retinal transcript differential gene expression

To analyze differentially expressed genes in the retina of fishes we compared expression levels for all genes in each sample. For this we built a hybrid transcriptome to use as a reference. The hybrid transcriptome was elaborated with Trinity assembler (Galaxy Version 2.15.1+galaxy1) in Galaxy platform with default parameters, using a forward FASTA file from a dry season-captured specimen, and a reverse FASTA file from a wet season-captured specimen. By doing this, we strived to include a representative sample of transcripts as a reference for gene mapping. To map genes from each specimen transcript to the hybrid transcriptome, and estimate its expression levels, we used Salmon quant tool (Version 1.10.1+galaxy2) (Patro et al., 2017) followed by HTSeq (Version 2.0.5+galaxy0) (Anders et al., 2015) in Galaxy.

To carry out the DGE analysis, we performed DESeq2 Analysis in RStudio using Bioconductor DESeq package (Version 1.44.0) (Love et al., 2014). This method allows for identification of genes whose expression is most conversely affected by the treatment specified (Love et al., 2014), and whose own normalization methods allow for comparison between different samples (Zhao et al., 2021). First, we built a count matrix with data from HTSeq output, and a design matrix containing sample treatment, as specified by developer. Genes with less than 10 counts, in at least 3 samples (smallest sample group), were filtered out, as recommended by developer. Subsequently, a list of differentially expressed genes under 0.05 p-value adjusted threshold was obtained (Appendix D). An MA-plot for such genes, with normal long fold change shrinkage (Love et al., 2014), taking dry season condition as reference, was drawn in RStudio. To broadly characterize them, their sequence was used as a query in BLAST tool, from NCBI web platform, to identify their possible gene identity, and further research was held using NCBI, UniProt (https://www.uniprot.org), as well as ZFIN

(https://zfin.org) to elaborate a table with known gene ID, related functions and an assigned category in accordance with our review (Appendix D).

Finally, with the aim to have a general view of the effect of seasonality in retinal gene expression, a PCA plot was drown with each individual's expression profile in RStudio, considering the top 500 differentially expressed genes only (Love et al., 2014). The chosen parameters to identify the best PCA model included processing of expression data following regularized logarithm transformation (rlog), as well as non-blind dispersion, as recommended by developer (Love et al., 2014).

RESULTS

Opsin repertoire of *Trichomycterus* aff. *banneaui*

BLAST results using opsin queries show that *Trichomycterus* aff. *banneaui* possess two cone opsin pigments sensitive to green (RH2) and red (LWS) wavelengths, and a dim light (RH1) rhodopsin pigment in rods (Musilova et al., 2021). Chiso opsin sequences clustered coherently with the additional opsin sequences from other teleost species for each opsin class, supporting this finding (Fig. 3)

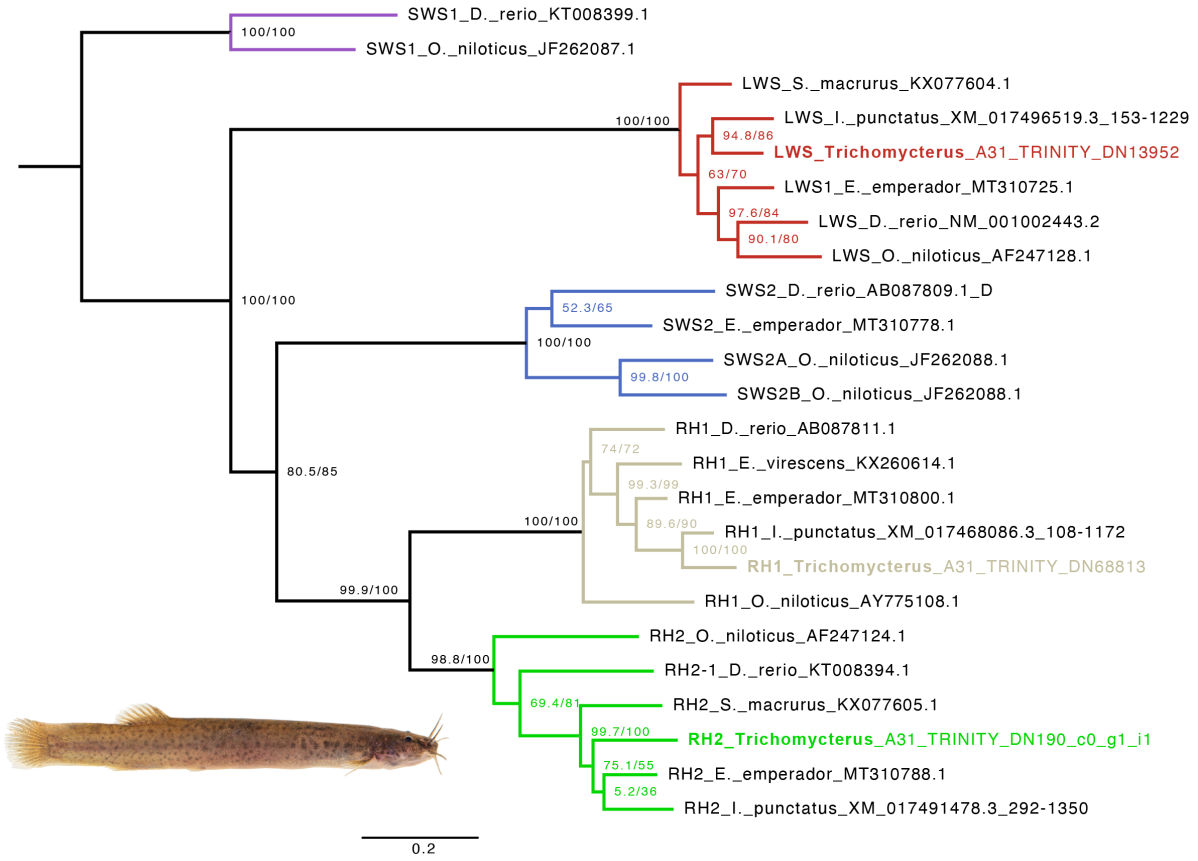


Figure 3. Opsin repertoire of *Trichomycterus sp*.

Opsin maximum likelihood phylogenetic tree of *Trichomycterus* aff *banneaui, D. rerio, S. macrurus, I. punctatus, E. emperador, E. virescens* and *O. niloticus*. Colored samples indicate opsin sequences obtained from *Trichomycterus* aff. *banneaui* specimens. Node values are read as SH-aLRT support (%) / ultrafast bootstrap support (%).

Visual pigment differential gene expression in *Trichomycterus* aff. *banneaui*

T-test results recorded significant differences for cone opsin expression (FPKM) among seasons. LWS showed the strongest difference (df = 5.58, t = -2.69, p = 0.038), followed by RH2 (df = 4.94, t = -2.69, p = 0.044), which was the least expressed opsin (Table 1). Both opsins were upregulated during the wet season (Fig. 4). Non-significant differences were found for RH1 (df = 5.76, t = -0.86, p = 0.426), whose expression greatly exceeded that of color opsins. *cyp27c1* gene was minimally expressed, and no significant differences were found either (df = 5.80, t = -1.08, p = 0.324).

Table 1. Average opsin & *cyp27c1* expression (FPKM) between seasons

	Dry Season	**Wet Season**
LWS	592.70 ± 388.71	1929.81 ± 989.32
RH2	186.29 ± 137.10	811.68 ± 489.49
RH1	8568.55 ± 4949.26	12301.14 ± 7348.05
cyp27c1	25.25 ±19.81	44.28 ± 30.08

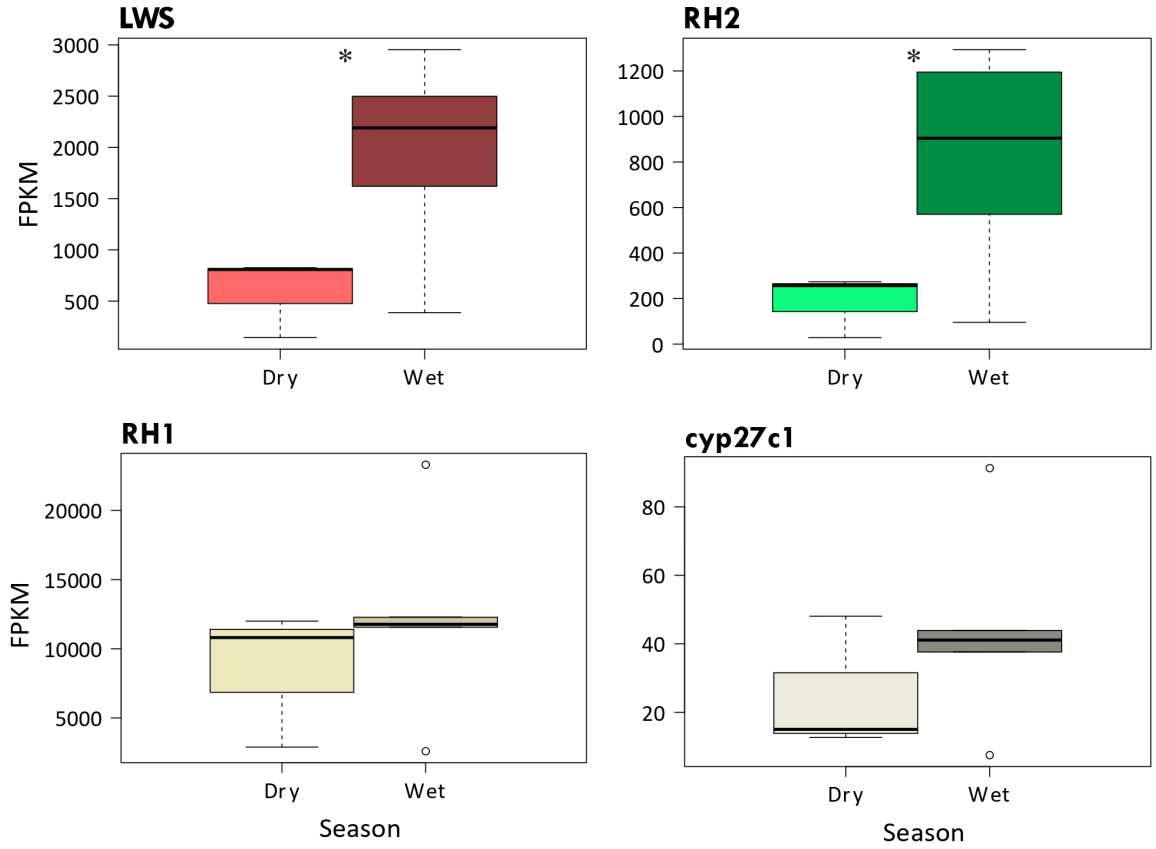


Figure 4. *Trichomycterus* aff. *banneaui* opsin & cyp27c1 expression (FPKM) within seasons Significant differences are shown with (*) next to gene name

Whole transcriptome differential gene expression in *Trichomycterus* aff. *banneaui*

DESeq2 analysis with whole retinal transcripts proposed a total of 35 significant differentially expressed genes between seasons (Appendix D), of which 20 were upregulated during the dry season, while the remaining 15 were upregulated during the wet season (Fig. 5). A total of 32 genes were characterized using external databases. Based on assigned functional categories, 'Immunology' (13 genes), 'Development' (12) and 'Expression' (11) were the most represented gene classes during the dry season (18 characterized genes). Upregulated characterized genes during the wet season (14) were more widespread in their categories; the most represented were 'Immunology' (6), 'Metabolism' (5) and 'Expression' (3). Interestingly, nine of the total upregulated genes for the dry season were related to cancer as one of the gene's associated highlight functions -in comparison to only two, for the wet season-. The complete

list of these genes, their key functions and assigned categories can be found in the appendix section.

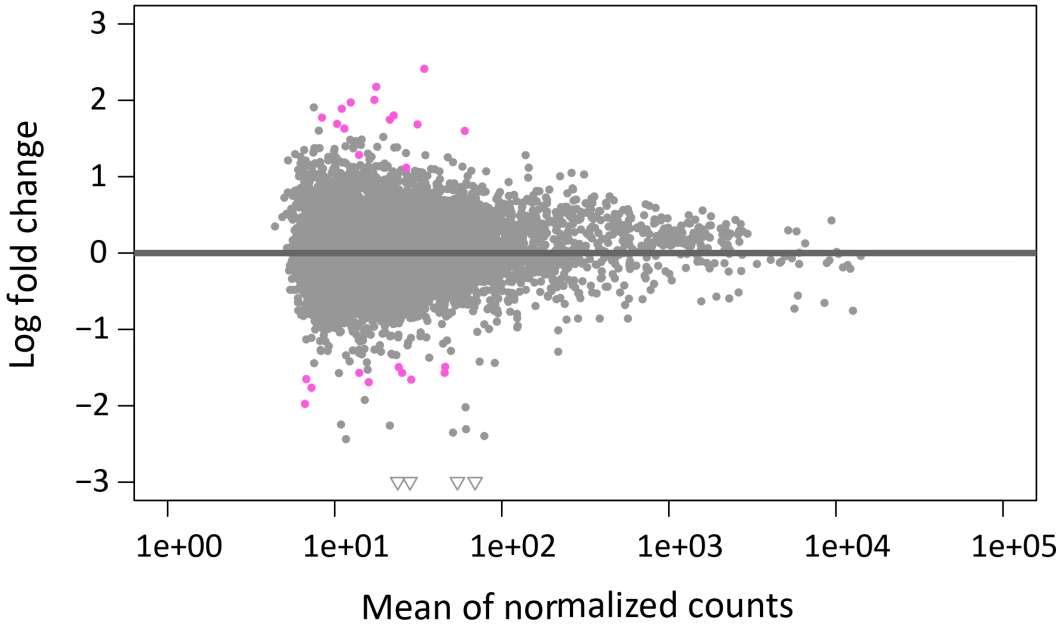


Figure 5. MA Plot with retinal gene expression levels

Graph shows up and down regulated genes (log fold change) in reference to dry season. Genes below 0 in y axis are downregulated in the dry season, but upregulated in the wet season, while genes above 0 are upregulated in the dry season but downregulated in the wet season. Significant differentially expressed genes are shown in pink. Downregulated outliers are printed as triangles.

Finally, expression profiles based on the top 500 differentially expressed genes from each individual analyzed show a loose, yet recognizable effect of seasonality on retinal genes expression in the Chiso catfish (Fig. 6).

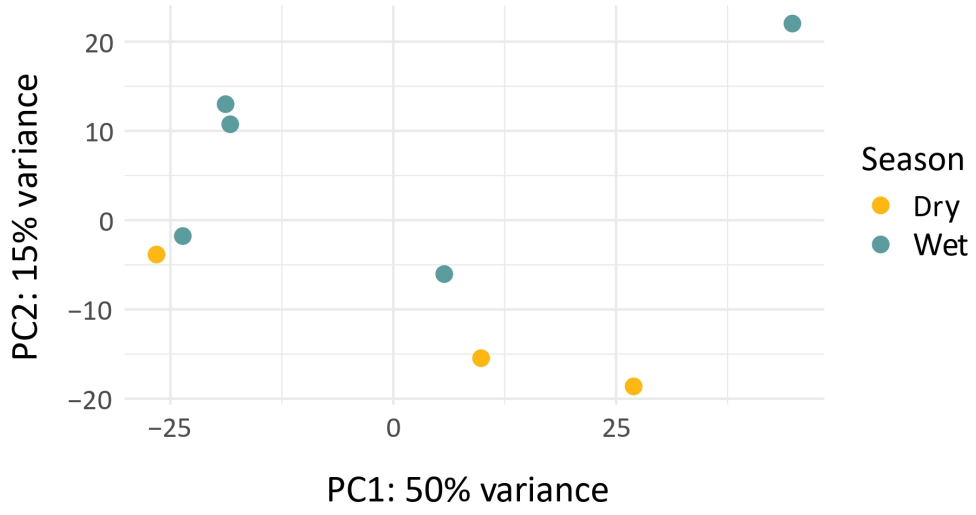


Figure 6. PCA of Chiso individuals' expression profiles

DISCUSSION

Opsin repertoire of *Trichomycterus* aff. *banneaui*

A long-wavelength sensitive opsin (LWS), a medium-wavelength sensitive opsin (RH2), a rhodopsin (RH1) and chromophore type-controlling gene, *cyp27c1*, were found in *Trichomycterus* aff. *banneaui* retinal transcriptomes. This aligns with opsin repertoires found for other nocturnal silurid species, including members from both Siluroidei and the neotropical Loricarioidei groups -the greatest and most diverse catfish suborders-, which possess the visual opsin repertoire reported here (Lemopoulos & Montoya-Burgos, 2022; Zheng et al., 2021); one exception being the European freshwater catfish, *Silurus glanis*, which possess SWS2 in addition (Musilova et al., 2021). Yet, this convergence supports the idea that this reduced opsin repertoire, in comparison to other teleost fish (Musilova et al., 2021), was present in the benthic ancestor to these groups' divergence (Lemopoulos & Montoya-Burgos, 2022) and might be expected for most catfish species. Indeed, opsin repertoires tend to be reduced in fish groups that live in turbid and dim environments, and that possess other sensing mechanisms (Policarpo et al., 2024), as is the case of barbels in catfishes. This last pattern might probably be a consequence of redundancy between sensorial functions (Policarpo et al., 2024). Accordingly, catfish species generally present a less complex retinal tissue; it is usually small and thin, with low photoreceptor cell density and cones present in the simplest disposition category for fish (Ali & Anctil, 1976). For instance, the catfish *Silurus meridionalis,* actively and genetically promotes the simplification of its visual capacities (Zheng et al., 2021).

No short wavelength sensing opsins -SWS1, nor SWS2- were found in *T.* aff. *banneaui*. As mentioned, this likely responds to loss of these genes in the benthic-dueling ancestor of catfishes (Lemopoulos & Montoya-Burgos, 2022; Zheng et al., 2021). Yet, the absence of these opsin genes in *T.* aff. *banneaui* shall not be mistaken for the absence of the correspondent wavelengths in our sampling site, since the stream and ponds were shallow and clear, especially

during the dry season (pers. obs). In fact, UV-sensing non visual opsins have been found in the benthic nocturnal neotropical loriicarid *Ancistrus triradiatus,* which also inhabits similar habitats (Lemopoulos & Montoya-Burgos, 2022).

Visual pigment differential gene expression in *Trichomycterus* aff. *banneaui*

Supporting our hypothesis, phenotypic plasticity in the Chiso retina was documented for cone opsin genes only. LWS and RH2 were upregulated during the wet season, while RH1 and *cyp27c1* expression remained unaffected by seasonality. These results are novel for siluriforms; Although long-wavelength shifting tends to occur in turbid environments, as expected for the wet season in the CRB, this pattern is usually accompanied by downregulation of short wavelength sensitive opsins in other fish groups (Carleton et al., 2020), which are absent in the catfish. We hypothesize that, although wavelength filtering by turbidity is most efficient for violet and blue wavelengths (Lemopoulos & Montoya-Burgos, 2022), other portions of the spectrum are still affected. Then, having more opsins might compensate for a comparable amount of visual information. Additionally, it has also been reported that red wavelengths are the most crucial range of light for cichlid fish to maintain their position when visual cues are lost, by swimming against the current (Smith et al., 2012), which is also expected to increase during wet periods in the CRB. These findings suggest that turbidity and water current are possibly key visual constraints of cone opsin expression for *T.* aff. *banneaui*, exerted by CRB's seasonal regime.

Regarding expression numbers in these genes, LWS had a higher expression than RH2 during both seasons, while RH1 recorded the highest, and *cyp27c1*, the lowest overall. This pattern for cone opsin expression has also been reported for neotropical cichlids and characins (Escobar-Camacho et al., 2017, 2020), implying that the relative relevance of the longest wavelengths sensitivity is not only convergent in the region, but in the case of *T.* aff. *banneaui*, is constant despite CRBs seasonal variation. The minimal expression seen for *cyp27c1*

indicates that chromophore conversion isn't relevant for spectral tuning, which was somehow unexpected given that this mechanism tends to prevail in fish species that live in turbid environments (Escobar-Camacho et al., 2019). Meanwhile, although higher expression for RH1 is expected among vertebrates (Hauser & Chang, 2017), its elevated expression in this study may also reflect the nocturnal and benthic niche occupied by the Chiso catfish, as has been shown for other nocturnal catfishes. For instance, *S. meririonalis* exhibits a high rod to cone ratio (Zheng et al., 2021) that leads to greater expression of rod visual pigments. In combination with differential expression results, this pattern may expose RH1 conserved status in *T.* aff. *banneaui*, regardless of seasonality.

A parallel study made in the cichlid *Andinoacara rivulatus* opsin expression, captured downstream in the CRB, revealed a larger set of visual opsins which didn't present expression differences in response to seasonality (Torres-Arias, 2024). These findings suggest three implications: 1) Different responses to seasonal intermittency are expected between different teleost lineages, possibly due to different light tuning relevancies determined by evolutionary history, niche, and basin locations inhabited; 2) Opsin repertoire size, nor opsin expression, alone, are indicative of daylight vision adaptation. Despite having a simpler opsin set, *T.* aff. *banneaui* seems to be well adapted for color vision, as has been reported for other nocturnal catfish species (Kawamura et al., 2017; Lemopoulos & Montoya-Burgos, 2022), and; 3) Although the visual pigment related genes are the candidate system to exhibit phenotypic plasticity in the fish retina, analysis of other retinal genes expression might complete our look into plasticity in these systems, as was seen in our results.

Whole transcriptome differential gene expression in *Trichomycterus* aff. *banneaui*

Phenotypic plasticity was also detected in 35 differentially expressed retinal genes in the Chiso catfish. The dry season strongly favored immunology, development and expression related genes, while the wet had a more diverse effect on gene category. Although our analysis

doesn't allow for deep examination, there was a prevalence of immune related genes for both seasons, as is the case of the Chiso catfish niche. For example, the dry season-upregulated lysozyme g-like coding gene (Appendix D, 1) is part of fish innate response to bacterial infection (Li et al., 2021). This finding might reflect the expansion of immunity associated gene families in neotropical catfishes, probably in response to occupying pathogen-rich benthic habitats and often warm waters that favor pathogen proliferation (Lemopoulos & Montoya-Burgos, 2022), as is the case of the Chiso catfish niche.

Furthermore, most of the immunity related genes in our dataset during the dry season were found to have a reported connection with cancer. This was the case of *junbb* (Appendix D, 3), part of a group of genes that are immediately transcribed after exposure to carcinogens in *D. rerio*, which enables cell proliferation and blocks apoptosis (Chen et al., 2014); or *ADAMTS1* (Appendix D, 26), which has also been related to apoptosis avoidance in renal carcinoma in *D. rerio* (Wen et al., 2024). Although retinal tissues experience increased light exposition and accelerated metabolism (Yourick et al., 2019), documentation of neoplasms in fish retinas are extremely rare (Jurk, 2002). Tumors in fish are usually reported due to pollutants (Baumann, 1992, 1998), which would be rare in our study site given the river headwater's conservancy state. Then, carcinogenic stress could have arisen due to viral or genetical origins (Baumann, 1992) which tend to appear in hybrid, small, unconnected or inbred populations (Pesavento et al., 2018). This might be the case of *T.* aff. *banneaui* populations, which record elevated genus diversity in the region (Escobar-Camacho et al., 2024) coherent with hybridization scenarios, and inhabit headwater habitats which favor isolation of ponds and streams, especially during the dry season. This could imply that seasonality in the CRB affects retinal gene expression through evolutionary consequences of intermittency in habitat structure. The small sample number for this season (three individuals) may also explain this pattern.

Although vision related genes are to be expected in differential gene expression analysis of retinal transcripts (Escobar-Camacho et al., 2019; Musilova et al., 2019), a few upregulated genes possibly associated with vision were only found during the wet season. For example, *SIK1* (Appendix D, 13) expression in rat retinas probably has neural transduction functions (Vural, 2008), meanwhile *sst7* (Appendix D, 18), part of the somatostin family of genes, has been linked with avoidance of photoreceptor cell apoptosis induced by diabetic conditions in mice (Arroba et al., 2016). Absence of vision related genes during the dry season might be explained by the apparent immunological and carcinogenic stress suffered by these individuals, which highlights the complex effects that seasonality might pose over gene expression in Chiso retinas, whose sensing functions are parallel to its complex ecological context.

CONCLUSIONS

In this study, different lines of evidence supported the exhibition of phenotypic plasticity in the Chiso catfish (*T.* aff. *banneaui*) retina, through differential gene expression. These differences were induced by seasonality (dry-wet) in an intermittent Ecuadorian Chocó river. Having identified a rhodopsin, a medium and a long wavelength sensitive opsins in *T.* aff. *banneaui,* we found that cone opsin genes were upregulated during the wet season, most probably due to increased water turbidity and current. This finding suggests that neotropical catfish vision, although relatively simpler due to evolutionary histories, is well equipped for daylight vision and should not be considered as a secondary sensing mechanism. Analysis of accompanying retinal genes highlighted other eco-physiological contrasting effects of seasonality, such as immunology and evolutionary consequences of both pond isolation due to lowered water flow in the dry season, and the catfishes' benthic and passive niches. Identification of genes possibly related to vision supports the complexity of visual sensing, as well as its adjustment relevance for the group. Additional hydrological records, behavioral observations of specimens sensing responses to light and fitness evaluations would strengthen this study's conclusions. Nonetheless, these results contribute to the knowledge of the ecologically and taxonomically representative catfish group visual system, as well as to the ecological implications of season-induced intermittent rivers on sensory systems.

REFERENCES

Aguirre, W. E., Alvarez-Mieles, G., Anaguano-Yancha, F., Burgos Morán, R., Cucalón, R. V., Escobar-Camacho, D., Jácome-Negrete, I., Jiménez Prado, P., Laaz, E., Miranda-Troya, K., Navarrete-Amaya, R., Nugra Salazar, F., Revelo, W., Rivadeneira, J. F., Valdiviezo Rivera, J., & Zárate Hugo, E. (2021). Conservation threats and future prospects for the freshwater fishes of Ecuador: A hotspot of Neotropical fish diversity. *Journal of Fish Biology*, *99*(4), 1158-1189. https://doi.org/10.1111/jfb.14844

Ali, M.-A., & Anctil, M. (1976). *Retinas of Fishes*. Springer. https://doi.org/10.1007/978-3-642-66435-9

Anders, S., Pyl, P. T., & Huber, W. (2015). HTSeq—A Python framework to work with high-throughput sequencing data. *Bioinformatics*, *31*(2), 166-169. https://doi.org/10.1093/bioinformatics/btu638

Arroba, A. I., Mazzeo, A., Cazzoni, D., Beltramo, E., Hernández, C., Porta, M., Simó, R., & Valverde, Á. M. (2016). Somatostatin protects photoreceptor cells against high glucose–induced apoptosis. *Molecular Vision*, *22*, 1522-1531.

Baumann, P. C. (1992). The use of tumors in wild populations of fish to assess ecosystem health. *Journal of Aquatic Ecosystem Health*, *1*(2), 135-146. https://doi.org/10.1007/BF00044045

Baumann, P. C. (1998). Epizootics of cancer in fish associated with genotoxins in sediment and water. *Mutation Research/Reviews in Mutation Research*, *411*(3), 227-233. https://doi.org/10.1016/S1383-5742(98)00015-5

Blankenberg, D., Gordon, A., Von Kuster, G., Coraor, N., Taylor, J., Nekrutenko, A., & the Galaxy Team. (2010). Manipulation of FASTQ data with Galaxy. *Bioinformatics*, *26*(14), 1783-1785. https://doi.org/10.1093/bioinformatics/btq281

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*(15), 2114-2120. https://doi.org/10.1093/bioinformatics/btu170

Carleton, K. L., Escobar-Camacho, D., Stieb, S. M., Cortesi, F., & Marshall, N. J. (2020). Seeing the rainbow: Mechanisms underlying spectral sensitivity in teleost fishes. *Journal of Experimental Biology*, *223*(8), jeb193334. https://doi.org/10.1242/jeb.193334

Chen, Y. Y., Zhu, J. Y., & Chan, K. M. (2014). Effects of cadmium on cell proliferation, apoptosis, and proto-oncogene expression in zebrafish liver cells. *Aquatic Toxicology*, *157*, 196-206. https://doi.org/10.1016/j.aquatox.2014.10.018

Collin, S. P., & Shand, J. (2003). Retinal Sampling and the Visual Field in Fishes. En S. P. Collin & N. J. Marshall (Eds.), *Sensory Processing in Aquatic Environments* (pp. 139-169). Springer. https://doi.org/10.1007/978-0-387-22628-6_8

Corbo, J. C. (2021). Vitamin A1/A2 chromophore exchange: Its role in spectral tuning and visual plasticity. *Developmental Biology*, *475*, 145-155. https://doi.org/10.1016/j.ydbio.2021.03.002

Datry, T., Allen, D., Argelich, R., Barquin, J., Bonada, N., Boulton, A., Branger, F., Cai, Y., Cañedo-Argüelles, M., Cid, N., Csabai, Z., Dallimer, M., Araújo, J. C. de, Declerck, S., Dekker, T., Döll, P., Encalada, A., Forcellini, M., Foulquier, A., … Vinyoles, D. (2021). Securing Biodiversity, Functional Integrity, and Ecosystem Services in Drying River Networks (DRYvER). *Research Ideas and Outcomes*, *7*, e77750. https://doi.org/10.3897/rio.7.e77750

Datry, T., Boulton, A. J., Fritz, K., Stubbington, R., Cid, N., Crabot, J., & Tockner, K. (2023). Non-perennial segments in river networks. *Nature Reviews Earth & Environment*, *4*(12), 815-830. https://doi.org/10.1038/s43017-023-00495-w

Douglas, R. H., Partridge, J. C., & Marshall, N. J. (1998). The eyes of deep-sea fish I: Lens pigmentation, tapeta and visual pigments. *Progress in Retinal and Eye Research*, *17*(4), 597-636. https://doi.org/10.1016/S1350-9462(98)00002-0

Durães, R., Carrasco, L., Smith, T. B., & Karubian, J. (2013). Effects of forest disturbance and habitat loss on avian communities in a Neotropical biodiversity hotspot. *Biological Conservation*, *166*, 203-211. https://doi.org/10.1016/j.biocon.2013.07.007

Escobar-Camacho, D., Barragán, K. S., Guayasamin, J. M., Gavilanes, G., & Encalada, A. C. (2024). New records of native and introduced fish species in a river basin of Western Ecuador, the Chocó-Darien Ecoregion, using DNA barcoding. *PLOS ONE*, *19*(3), e0298970. https://doi.org/10.1371/journal.pone.0298970

Escobar-Camacho, D., Carleton, K. L., Narain, D. W., & Pierotti, M. E. R. (2020). Visual pigment evolution in Characiformes: The dynamic interplay of teleost whole-genome duplication, surviving opsins and spectral tuning. *Molecular Ecology*, *29*(12), 2234-2253. https://doi.org/10.1111/mec.15474

Escobar-Camacho, D., Pierotti, M. E. R., Ferenc, V., Sharpe, D. M. T., Ramos, E., Martins, C., & Carleton, K. L. (2019). Variable vision in variable environments: The visual system of an invasive cichlid (Cichla monoculus) in Lake Gatun, Panama. *Journal of Experimental Biology*, *222*(6), jeb188300. https://doi.org/10.1242/jeb.188300

Escobar-Camacho, D., Ramos, E., Martins, C., & Carleton, K. L. (2017). The opsin genes of amazonian cichlids. *Molecular Ecology*, *26*(5), 1343-1356. https://doi.org/10.1111/mec.13957

Fogg, L. G., Cortesi, F., Gache, C., Lecchini, D., Marshall, N. J., & de Busserolles, F. (2023). Developing and adult reef fish show rapid light-induced plasticity in their visual system. *Molecular Ecology*, *32*(1), 167-181. https://doi.org/10.1111/mec.16744

Fusco, G., & Minelli, A. (2010). Phenotypic plasticity in development and evolution: Facts and concepts. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1540), 547-556. https://doi.org/10.1098/rstb.2009.0267

Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. A., Amit, I., Adiconis, X., Fan, L., Raychowdhury, R., Zeng, Q., Chen, Z., Mauceli, E., Hacohen, N., Gnirke, A., Rhind, N., di Palma, F., Birren, B. W., Nusbaum, C., Lindblad-Toh, K., … Regev, A. (2011). Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology*, *29*(7), 644-652. https://doi.org/10.1038/nbt.1883

Hauser, F. E., & Chang, B. S. (2017). Insights into visual pigment adaptation and diversity from model ecological and evolutionary systems. *Current Opinion in Genetics & Development*, *47*, 110-120. https://doi.org/10.1016/j.gde.2017.09.005

Hofmann, C. M., O'Quin, K. E., Justin Marshall, N., & Carleton, K. L. (2010). The relationship between lens transmission and opsin gene expression in cichlids from Lake Malawi. *Vision Research*, *50*(3), 357-363. https://doi.org/10.1016/j.visres.2009.12.004

Jiménez Prado, P. (2015). *Guía de peces para aguas continentales en la vertiente occidental del Ecuador* [Text]. Biblioteca Hernán Malo González de la Universidad del Azuay; Biblioteca Hernán Malo González. https://biblioteca.uazuay.edu.ec/buscar/item/79255

Jurk, I. (2002). Ophthalmic disease of fish. *Veterinary Clinics: Exotic Animal Practice*, *5*(2), 243-260. https://doi.org/10.1016/S1094-9194(02)00006-3

Katoh, K., & Standley, D. M. (2013). MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Molecular Biology and Evolution*, *30*(4), 772-780. https://doi.org/10.1093/molbev/mst010

Kawamura, G., Bagarinao, T., Hoo, P. K., Justin, J., & Lim, L. S. (2017). Colour discrimination in dim light by the larvae of the African catfish Clarias gariepinus. *Ichthyological Research*, *64*(2), 204-211. https://doi.org/10.1007/s10228-016-0557-0

Leberg, S. S., Barriga, R., Bart, H., Olivo, A., Narasimhan, K., & Karubian, J. (2021). Richness and abundance of stream fish communities in a fragmented neotropical landscape. *Environmental Biology of Fishes*, *104*(3), 239-251. https://doi.org/10.1007/s10641-021-01066-y

Lemopoulos, A., & Montoya-Burgos, J. I. (2022). Whole genome assembly of the armored loricariid catfish Ancistrus triradiatus highlights herbivory signatures. *Molecular Genetics and Genomics*, *297*(6), 1627-1642. https://doi.org/10.1007/s00438-022-01947-6

Li, L., Cardoso, J. C. R., Félix, R. C., Mateus, A. P., Canário, A. V. M., & Power, D. M. (2021). Fish lysozyme gene family evolution and divergent function in early development. *Developmental & Comparative Immunology*, *114*, 103772. https://doi.org/10.1016/j.dci.2020.103772

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*(12), 550. https://doi.org/10.1186/s13059-014-0550-8

Musilova, Z., Indermaur, A., Bitja-Nyom, A. R., Omelchenko, D., Kłodawska, M., Albergati, L., Remišová, K., & Salzburger, W. (2019). Evolution of the visual sensory system in cichlid fishes from crater lake Barombi Mbo in Cameroon. *Molecular Ecology*, *28*(23), 5010-5031. https://doi.org/10.1111/mec.15217

Musilova, Z., Salzburger, W., & Cortesi, F. (2021). The Visual Opsin Gene Repertoires of Teleost Fishes: Evolution, Ecology, and Function. *Annual Review of Cell and*

Developmental Biology, *37*(Volume 37, 2021), 441-468. https://doi.org/10.1146/annurev-cellbio-120219-024915

Patro, R., Duggal, G., Love, M. I., Irizarry, R. A., & Kingsford, C. (2017). Salmon provides fast and bias-aware quantification of transcript expression. *Nature Methods*, *14*(4), 417-419. https://doi.org/10.1038/nmeth.4197

Pesavento, P. A., Agnew, D., Keel, M. K., & Woolard, K. D. (2018). Cancer in wildlife: Patterns of emergence. *Nature Reviews Cancer*, *18*(10), 646-661. https://doi.org/10.1038/s41568-018-0045-0

Pigliucci, M., Murren, C. J., & Schlichting, C. D. (2006). Phenotypic plasticity and evolution by genetic assimilation. *Journal of Experimental Biology*, *209*(12), 2362-2367. https://doi.org/10.1242/jeb.02070

Policarpo, M., Fogg, L. G., Cortesi, F., & Salzburger, W. (2024). *Evolution of the non-visual and visual opsin gene repertoire in ray-finned fishes* (p. 2024.10.03.616438). bioRxiv. https://doi.org/10.1101/2024.10.03.616438

Smith, A. R., Ma, K., Soares, D., & Carleton, K. L. (2012). Relative LWS cone opsin expression determines optomotor thresholds in Malawi cichlid fish. *Genes, Brain and Behavior*, *11*(2), 185-192. https://doi.org/10.1111/j.1601-183X.2011.00739.x

Torres-Arias, A. (2024). *Conserved opsin gene expression in a cichlid fish (Andinoacara rivulatus) across seasons in northwestern Ecuador* [Undergraduate dissertation, Universidad San Francisco de Quito]. http://repositorio.usfq.edu.ec/handle/23000/13940

Toyama, M., Hironaka, M., Yamahama, Y., Horiguchi, H., Tsukada, O., Uto, N., Ueno, Y., Tokunaga, F., Seno, K., & Hariyama, T. (2008). Presence of Rhodopsin and Porphyropsin in the Eyes of 164 Fishes, Representing Marine, Diadromous, Coastal

and Freshwater Species—A Qualitative and Comparative Study. *Photochemistry and Photobiology*, *84*(4), 996-1002. https://doi.org/10.1111/j.1751-1097.2008.00344.x

Vural, M. E. (2008). *Expression of SIK1 and SIK3 in postnatal rat retina*. http://digitalarchive.boun.edu.tr/handle/123456789/15410

Wen, Y.-C., Lin, Y.-W., Ho, K.-H., Yang, Y.-C., Lai, F.-R., Chu, C.-Y., Chen, J.-Q., Lee, W.-J., & Chien, M.-H. (2024). The oncogenic ADAMTS1–VCAN–EGFR cyclic axis drives anoikis resistance and invasion in renal cell carcinoma. *Cellular & Molecular Biology Letters*, *29*(1), 126. https://doi.org/10.1186/s11658-024-00643-0

Yourick, M. R., Sandkam, B. A., Gammerdinger, W. J., Escobar-Camacho, D., Nandamuri, S. P., Clark, F. E., Joyce, B., Conte, M. A., Kocher, T. D., & Carleton, K. L. (2019). Diurnal variation in opsin expression and common housekeeping genes necessitates comprehensive normalization methods for quantitative real-time PCR analyses. *Molecular Ecology Resources*, *19*(6), 1447-1460. https://doi.org/10.1111/1755-0998.13062

Zhao, Y., Li, M.-C., Konaté, M. M., Chen, L., Das, B., Karlovich, C., Williams, P. M., Evrard, Y. A., Doroshow, J. H., & McShane, L. M. (2021). TPM, FPKM, or Normalized Counts? A Comparative Study of Quantification Measures for the Analysis of RNA-seq Data from the NCI Patient-Derived Models Repository. *Journal of Translational Medicine*, *19*(1), 269. https://doi.org/10.1186/s12967-021-02936-w

Zheng, S., Shao, F., Tao, W., Liu, Z., Long, J., Wang, X., Zhang, S., Zhao, Q., Carleton, K. L., Kocher, T. D., Jin, L., Wang, Z., Peng, Z., Wang, D., & Zhang, Y. (2021). Chromosome-level assembly of southern catfish (silurus meridionalis) provides insights into visual adaptation to nocturnal and benthic lifestyles. *Molecular Ecology Resources*, *21*(5), 1575-1592. https://doi.org/10.1111/1755-0998.13338

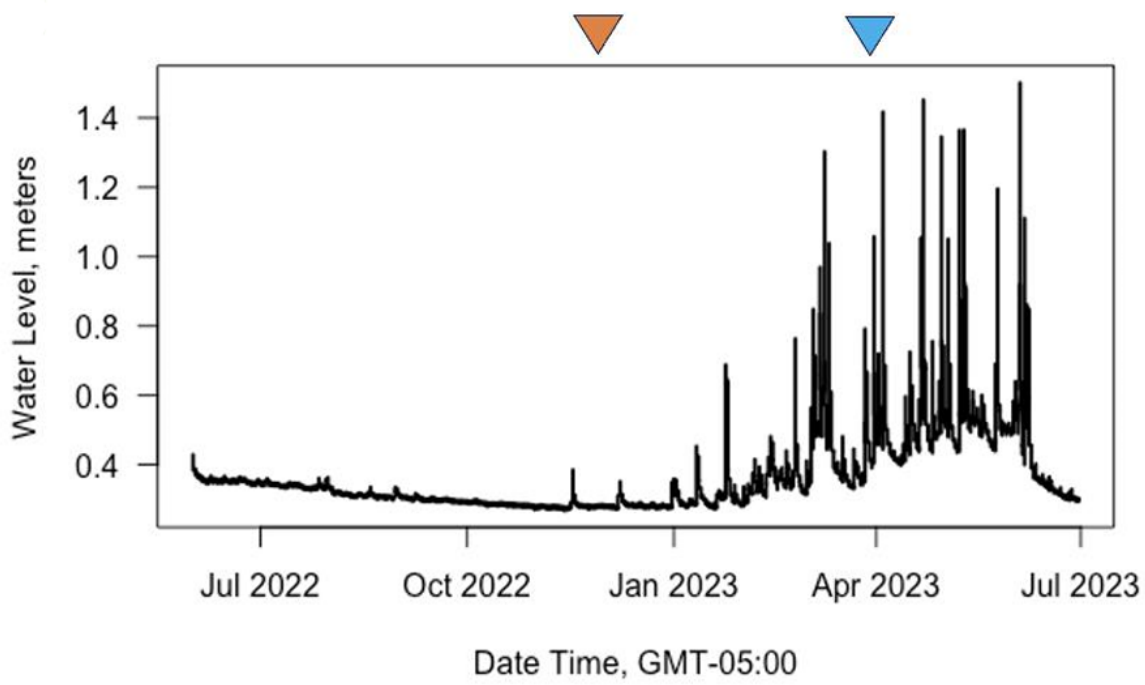
APPENDIX

A. Environmental variables at sampling site

SEASON	TIME	TEMP	DO		SPC	COND	pH	ORP	TURB	ALT	Is Secchi visible?	
		[°C]	[%]	[mg/L]	[us/cm]	[us/cm]			[NTU]	[msnm]	5 cm	28 cm
Dry	8:47 a. m.	20.00	36.50	3.30	193.20	174.90	7.19	-9.20	107.80	485.00	Yes	Yes
Wet	3:30:04 p. m.	22.91	85.81	7.37	52.08	50.00	7.90	165.04	19.50*	485.00	Yes	Yes

*An erroneous measurement, most probably

B. Water level in Cube River (2022-2023).



Data took from close station to sampling site. Triangles in the top part show the specific dates in which fish were samples during the dry (orange) and wet seasons (blue). From (Torres-Arias, 2024)

C. Morphological measurements of analyzed *Trichomycterus* specimens

Individual	FL (mm)	TL (mm)	W (g)	Season
A31	5.21	5.62	1.27	Dry
A32	5.03	5.45	1.01	Dry
A33*	-	-	-	Dry
A143	4.75	5.19	0.8	Wet
A144	5.89	6.39	1.5	Wet
A145	4.94	5.5	0.5	Wet
A146	4.34	4.84	0.7	Wet
A147	5.6	6.06	1.5	Wet

*Measurements for specimen A33 weren't collected on field.

D. Complete list of significantly differentially expressed genes in *Trichomycterus* aff. *banneaui* retina

N°	Gene ID	Category	Encoded protein function	Size (bp)	padj
1	Lysozyme G-like	Immunology	Lysozyme activity. Defense against gram-positive bacterium. Killing of alien cells. In extracellulalr region. Macrophages.	1517	1.49E-11
2	v-fos FBJ murine osteosarcoma viral oncogene homolog Ab	Development. Expression. Immunology	DNA binding activity. Heart tissue regeneration. Cell differentiation. Response to stress. In nucleous.	1353	5.99E-11
3	JunB proto-oncogene, AP-1 transcription factor subunit b (junbb)	Expression. Immunology	DNA binding activity. Allows transcription by RNA polymerase II. In nucleoplasm. Part of transcription factor AP-1 complex. Head and neck squamous cell carcinoma and melanoma. Gastrointestinal and lung cancer. Lymphoma.	1346	2.35E-10
4	Early gowth response 1 (EGR1)	Development. Immunology. Expression	C2H2-type zinc-finger proteins.In nucleus. Transcriptional regulator. Activates genes for differentitation and mitogenesis. Cancer suppressor gene	3087	4.48E-10
5	IER2 immediate early response 2	Development. Expression. Immunology	DNA binding activity. Cell motility. Allows transcription by RNA polymerase II. In cytoplasm and nucleoplasm. Colorectal cancer, colorectal adenocarcinoma and hepatocellular carcinoma. Myeloid leukemia; colorectal adenocarcinoma, Melanoma.	1213	1.48E-06
6	Celullar Communication Network Factor 1	Development. Immunology. Mobility	Growth factor-inducible. Adhesion of endothelial cells. Cell proliferation, differentiation, angiogenesis, apoptosis, mobility, senescence and extracellular matrix formation. Inflammation and tissue repair. Related to chronic inflammation diseases	1952	1.91E-06
7	F-box protein 32 (fbxo32)	Metabolism. Development	Muscle atrophy. Phosphorylation dependent ubiquitination	257	1.57E-05

8	ADAMTS1	Immunology. Development	ADAMTS protein family. Inflammatory processes. Cancer cachexia. Growth, organ morphology. Fertility.	586	2.25E-05
9	JunB proto-oncogene, AP-1 transcription factor subunit b (junbb)	Development. Expression. Immunology	DNA binding activity. Allows transcription by RNA polymerase II. In nucleoplasm. Part of transcription factor AP-1 complex. Head and neck squamous cell carcinoma and melanoma. Gastrointestinal and lung cancer. Lymphoma.	686	4.23E-05
10	Nuclear receptor subfamily 4, group A, member 1 (nr4a1)	Expression. Hormonal. Immunology	Steroid-thyroid hormone-retinoid receptor. Transcription factor. Cell cycle mediation. Inflammation and apoptosis when in mitochondria. In macrophages. Survival and death of cells.	736	7.86E-05
11	FIP1 like 1a (S. cerevisiae) (fip111a)	Expression	mRNA processing. In nucleus. mRNA cleavage and polyadenylation specificity factor complex.	236	0.0001
12	hairy-related 6 (her6)	Expression. Development	DNA-binding transcription factor activity, RNA polymerase II regulation. Notch signaling pathway; regionalization, thalamus development. In nucleus.	1314	0.0005
13	SIK1	Metabolism. Development. Immunology	Cell cycle regulation. Sugar and lipid regulation. Muscle growth and differentaition. Tumor supression	421	0.0008
14	Uncharacterized			515	0.0014
15	H2-Q9	Inmunology	Presentation of foreign antigens to immune system. Peptide antigen binding activity. Defense response to symbiont. T cell mediated cytotoxicity. Type II interferon production. In plasma membrane. Part of MHC class Ib protein complex.	274	0.0038
16	cAMP responsive element modulator a (crema)	Development. Expression. Signaling	bZIP transcription factor. Interacts with viral and cellular promoters. cAMP-mediated signal transduction. Regulation of transcription.	742	0.0040

17	YME1-like 1a (yme1l1a),	Metabolism. Mitochondria	In mitochondria. Protein metabolism in mitochondria. Mitochondria maphotologies.	210	0.0040
18	somatostatin family member 7 (sst7)	Signaling. Hormonal. Mobility	In extracellular space. G protein-coupled receptor binding. Neuropeptide hormone activity. Adenylate cyclase-inhibiting G protein-coupled receptor signaling. Cell migration	562	0.0058
19	ZG57	Metabolism	Metal ion binding activity	218	0.0058
20	Pdlim1	Mobility. Metabolism	Cytoskeleton protein. Brings proteins to cytoskeleton. Motion of stress fibers in fibroblasts. Cell migration. Polarity of fibroblasts	302	0.0071
21	Complexin 3b (cplx3b)	Neurological	SNARE binding activity. Neurotransmitter secretion. Synaptic vescicle exocytosis. In females	629	0.0090
22	cysteine-serine-rich nuclear protein 1b (csrnp1b)	Neurological. Expression	Active in nucleus. Brain development. Transcription factor. DNA binding activity.	474	0.0096
23	insulin-like growth factor binding protein 1a (igfbp1a)	Metabolism. Mobility. Development	Binds to insulin like growth factors. Alters interaction of insulin growth factors. Cell migration. Metabolism. Low levels related to impaired glucose tolerance, vascular disease, hypertension.	1074	0.0130
24	No match			215	0.0185
25	Nt5c3b	Expression	Hydrolizes methylguanosines. Allows incorporation of methylguanosines to nucleic acids.	376	0.0197
26	ADAMTS1	Immunology. Development	ADAMTS protein family. Inflammatory processes. Cancer cachexia. Growth, organ morphology. Fertility.	3168	0.0197
27	adrenomedullin a (adma)	Hormonal. Immunology	Extracellular region. Hormone activity. Brain infarction. Various types of cancers.	1224	0.0197
28	Uncharacterized			1906	0.0197
29	interferon alpha-inducible protein 27-like protein 2A	Metabolism. Immunology. Mitochondria	In nuclear envelope and mitochondrial membrane. Identical protein binding activity. Response to virus.	275	0.0310

30	Jun dimerization protein 2b (jdp2b)	Expression	In nucleus. DNA binding activity. Transcription.	250	0.0388
31	HLA-A	Immonology	TAP complex binding. Microglobulin binding. Signaling receptor binding. Antigen processing and presentation. T cell activation.	296	0.0394
32	DUSP1	Metabolism. Immunology	Phosphatase. Aminoacid phosphatase. Inhibits celullar proliferation. Response to celular stress. Related to cancer	1869	0.0397
33	KH domain containing, RNA binding, signal transduction associated 1b (khdrbs1b)	Immunology. Expression	Alternative splicing. Cell cycle regulation. Regulation of Immunodefitiency. Tumorigenesis.	284	0.0422
34	fibrinogen-like protein 1 (si:ch211-203k16.3)	Immunology	Immune supressive molecule	1096	0.0430
35	CRH	Neurological	Stress response. Related to alzheimer or depression.	786	0.0442

Upregulated genes in the dry season appear yellow, while upregulated genes in the wet season appear blue