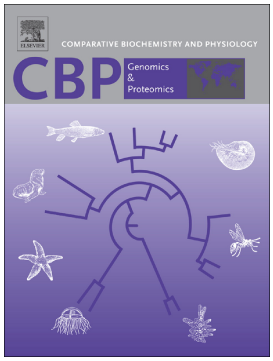
Journal Pre-proof



Different transcriptomic architecture of the gill epithelia in Nile and Mozambique tilapia after salinity challenge

Aurora Campo, Tali Nitzan, Tatiana Slosman, Adi Doron-Faigenboim, Helena D'Cotta, Jean-François Baroiller, Avner Cnaani

PII: S1744-117X(21)00139-8

DOI: https://doi.org/10.1016/j.cbd.2021.100927

Reference: CBD 100927

To appear in: *Comparative Biochemistry and Physiology - Part D: Genomics and Proteomics*

Received date: 23 August 2021

Revised date: 5 October 2021

Accepted date: 20 October 2021

Please cite this article as: A. Campo, T. Nitzan, T. Slosman, et al., Different transcriptomic architecture of the gill epithelia in Nile and Mozambique tilapia after salinity challenge, *Comparative Biochemistry and Physiology - Part D: Genomics and Proteomics* (2021), https://doi.org/10.1016/j.cbd.2021.100927

Different transcriptomic architecture of the gill epithelia in Nile and Mozambique tilapia after salinity challenge

Aurora Campo[a*], Tali Nitzan[a], Tatiana Slosman[a], Adi Doron-Faigenboim[a], Helena D'Cotta[b,c], Jean-François Baroiller[b,c], Avner Cnaani[a*]

[a] Institute of Animal Science, Agricultural Research Organization, Rishon LeZion, Israel.

[b] ISEM, Université de Montpellier, CNRS, IRD, EPHE, Montpellier, France

[c] UMR116 ISEM, CIRAD, Montpellier, France

*Corresponding authors: ayla.bcn@gmail.com (A. Campo) and avnerc@agri.gov.il (A. Cnaani)

Abstract

Tilapiine fishes of the genus *Oreochromis* vary in their euryhaline capabilities, therefore inhabiting aquatic environments of different salinities across the African continent. We analyzed the differential gene expression in the gills before and after 6 weeks salinity challenge between the highly tolerant Mozambique tilapia (*Oreochromis mossambicus*) and the less tolerant Nile tilapia (*O. niloticus*). The pathways triggered by salinity in both tilapia species reveal immune and cell stress responses as well as turnover of ionocytes. Nevertheless, the actual differential expressed genes vary between these two species, pointing at differential transcriptomic architecture, which likely contribute to the species osmoregulation capabilities in elevated salinities.

Keywords: Epitellium turnover, immune response, RNAseq, cichlids, osmotic regulation, differentially expressed genes, pseudogenes

Introduction

The distribution of aquatic species is determined by various environmental and physical factors, one of them is water salinity. The gradients between freshwater and sea water environments increase the alternatives for the propagation of species, and different salinities select for the most efficient metabolic pathway to cope with the osmotic challenge. Freshwater species may have diversified from euryhaline

ancestors through processes such as landlocking (Schultz and McCormick, 2012). Actinopterygian fishes in particular have adapted to freshwater by developing ion uptake through the gills, absorption of ions in the gastrointestinal tract (Chourasia et al., 2018) and ion reabsorption in the kidney (Edwards and Marshall, 2012). This is also the case of the euryhaline tilapias, of the family Cichlidae (Cnaani and Hulata, 2011). Nile tilapia (*Oreochromis niloticus*) is a freshwater species of major economic importance as one of the most aquacultured species worldwide. This species has shown very low survivability in seawater (Kamal and Mair, 2005; Villegas, 1990) and furthermore, its least growth rate is inhibited by small increases in salinity (Likongwe et al., 1996). In contrast, its sister species the Mozambique tilapia (*O. mossambicus*) has low growth rates but it can be found in salt lakes that exhibit salinities up to 99ppt, highlighting its strong ability to withstand saline waters (Morgan et al., 2004). Popper and Licatowich (1975) conducted a study in which Mozambique tilapia fish were still able to grow well at a salinity of 40ppt. It is still not clear if the salinity tolerance of Mozambique tilapia is a conservation of an ancestral seawater/euryhaline genetic background, or a *de novo* feature in this species convergent with other saline tolerants.

Maintenance of homeostasis in seawater is energetically expensive, for teleosts and fish in general, as they lose water to their surroundings through passive diffusion (Loretz, 1995; McCormick, 2001). In order to compensate for this loss of hydration, actinopterygians are obligated to continuously drink seawater. Therefore, they must exude the excessive ions acquired from seawater consumption in order to restore the normal osmolarity. Hypo-osmoregulation involves reflexive drinking, intestinal absorption of salts and water, ion secretion by the gills against a concentration gradient and renal ion excretion (Edwards and Marshall, 2012; Evans et al., 2005; McCormick, 2001). In tilapias, the findings of Uchida et al. (2000) suggest that the impressive salinity tolerance of *O. mossambicus* is likely due to an increased differentiation of ionocytes during seawater acclimation. Ionocytes (previously known as mitochondria-rich cells or chloride cells) are specialized cells found in the gills, containing ion pumps and transporters,

that are responsible for the expulsion of excess ions to the surrounding environment (Zadunaisky, 1996). An epithelium turnover after salinity challenge has been described in Mozambique tilapia (Hiroi et al., 2005) and four types of ionocytes have been identified in its embryos (Hiroi and McCormick, 2012): two in charge of ion uptake, one involved in salt secretion and one of unknown function.

Studies in European sprat (*Sprattus sprattus*) concluded that salinity could be an important environmental driver for selection (Quintela et al., 2021). The gill's role in osmoregulation has been well characterized by differential gene expression in euryhaline killifish species *Lucania parva* and *L. goodei* (Kozak et al. 2014). They found gene pathways critical for osmoregulation in ion transport and cell volume regulation that are differentially expressed after salinity challenge. Another killifish species, *Fundulus heteroclitus*, presented a transcriptomic response for cell volume regulation, nucleosome maintenance, ion transport, energetics, mitochondrion function, transcriptional regulation and apoptosis (Brennan et al., 2015; Whitehead et al., 2012, 2011). The cytokine and kinase signaling pathways seem to coordinate the response to the osmotic challenge through the transcription factor HNF-4α. A comparison between *F. heteroclitus* and the sea water adapted sister species *F. majalis* found differences in the transcriptome of both species but not in the gene sequence, indicating that the evolutionary process involved gene expression divergence (Whitehead et al., 2013).

As a consequence of climate change and global warming there is a decrease of freshwater environments, which are occasionally salinized, imposing an osmotic challenge to native freshwater species. Therefore, the study of osmotic regulation in euryhaline and freshwater species, such as tilapias, can illustrate key differences in metabolic pathways and speciation mechanisms driven by climate changes. In order to study the differences between high and low salinity tolerant species, we compared Mozambique and Nile tilapias, analyzing the gene expressions in the gills after 6 weeks exposure to freshwater and salty water.

Materials and methods

Experimental animals, salinity challenge and sampling

The fish used and the salinity challenge procedure are described by Chourasia et al. (2018). Briefly, the *O. mossambicus* stock originated in Natal, South Africa and the *O. niloticus* was from the Thai Chitralada strain. Two consecutive experiments were conducted, one with *O. mossambicus* (24 ± 0.6 g) and the other with *O. niloticus* (62 ± 0.9 g). In each experiment, thirty-six male fish were individually kept in an aquarium of 40 liters. Only males were used, to reduce any sex effects in our study. Fish were maintained in freshwater, and subsequently in 18 aquaria the salinity was increased gradually (5 ppt/day) using marine salt (Red Sea salt). Watanabe et al., (1990) and Lemarié et al., (2004), have previously recommended this daily rate of salinity increase as optimal for the acclimation of various tilapias species including *O. niloticus*. For *O. mossambicus* salinity was increased to 30 ppt and for *O. niloticus* to 20 ppt. After six weeks, blood samples were taken from the caudal vein and plasmas were separated by centrifugation for 15 min at 4°C. Fish were sacrificed and all the gills' arches were sampled, partly kept in RNAlater© buffer (Ambion®) at −20 °C until used, and the rest frozen immediately.

RNA extraction

Total RNA was extracted from the tissue samples using the TRIzol® reagent (Thermo Fisher Scientific, Waltham, MA, United States), and then purified to remove DNA contamination using the TURBO DNA-free™ kit (Invitrogen, Carlsbad, CA, United States). RNA concentration and quality were determined using an Epoch Microplate Spectrophotometer (BioTek, Winooski, VT).

RNA sequencing and mapping

Twelve samples were used for RNA sequencing (3 from each species in each salinity), as described by Nitzan et al. (2019). RNA samples (2 µg) were sent on dry-ice to the Technion Genome Center (Haifa, Israel) for sequencing on two lanes on an Illumina Hi-Seq 2500 device. The retrieved fastq files were mapped against the Tilapia genome (NMBU 2018, accession GCF_001858045.2) with STAR (v2.7.1a, Dobin et al., 2013) and further processed with Picard (v2.22.4) for addition of read groups and deletion of read duplicates. The annotation used for identifying the genes was the same version as the genome.

Differential Gene Expression and Statistical Analysis

A count matrix with the reads of each genes by sample was produced with htseq package (v. 0.13.5,

(Anders et al., 2010)) in R v.3.6. The resulting bam files were submitted to DESeq2 package DESeq package (Anders et al., 2010) in R (v 3.6.3, Development Core Team, 2013). The experimental design consisted of testing the differential gene expression between salinity treatment (fresh and salty water) and species (Nile and Mozambique tilapias). The effect of the salinity was evaluated in four different contrast tests, as follows: Test A - comparing Nile tilapia in freshwater vs. salty water; Test B - comparing Nile tilapia vs. Mozambique tilapia in freshwater; Test C - comparing Mozambique tilapia in freshwater vs. salty water; Test D - comparing Nile tilapia vs. Mozambique tilapia in salty water (Table 1). The p-value was adjusted with the Bonferroni method and the significance was established below 0.05. Volcano and micro array MA plots with and without shrinkage of effect size on LFC (log fold-change) were used for data quality assessment. A Venn graph was plotted to compare the up- and down-regulated differentially expressed genes (DEGs) in each species. The most relevant genes were identified with the annotation of the reference genome and those uncharacterized were analysed by its orthologous in close species found with the TBLASTN alignment tool (NCBI, ENSEMBL).

Functional and Enrichment analysis

The significant DEGs were annotated for functional analysis by using a database created from the annotation of the genome and the GO and KEGG terms provided by gprofiler (Raudevere et al., 2019). The data was mined with the dplyr R package (v. 0.7.8) and plotted for graphical evaluation. The toolbox of www.kegg.jp (Kanehisa, 2019) was used to retrieve the KEGG metabolic pathways involved in the differential expression between Nile and Mozambique tilapias after 6 weeks in salinity (Table 1, tests A and C).

Results

Quality of the data

The dispersion of the data for each contrast test was plotted on a Volcano graph (Figure 1) and a MA plot (Supplemental 1). The graphs indicate that there are more DEGs in Mozambique tilapia than in the

Nile tilapia in response to the 6 weeks salinity challenge (tests C and A respectively). In addition, there are more DEGs when the two species of tilapia were compared (tests B and D). The tests for salinity treatment in each species, tests A and C, offered a narrower range of dispersion for the log Fold Change than the tests for species in each salinity, tests B and C. These results are consistent with Volcano and MA plots (Figure 1, Supplemental 1).

Differentially expressed genes

Out of 25,885 genes with read counts, 63 to 2,235 genes were found differentially expressed under salinity treatment between the two tilapia species, depending on the interaction test (table 1). The differences between salinity tests offered 63 differentially expressed genes in Nile tilapia (test A) and 267 genes in Mozambique tilapia (test C), and a wider number of genes comparing the basal expression of the two species in freshwater (1358 genes, test B) or in salty water (2235, test D).

The significant differentially expressed genes of each test were identified and analyzed for gene ontology (GO) enrichment and KEGG pathway (Supplemental 2-5 and 8).

Up to 32 chains of the kappa immunoglobulins (κ-Igs) and few lambda immunoglobulins (λ-Igs) were found among the differentially expressed genes after the salinity challenge in Mozambique tilapia (Figure 2, Table 2, test C). Differential expression was also found between the species in freshwater and in salt water (table 2, tests B and D).

In total, 9 ncRNA were identified in the salinity challenge for Nile tilapia (test A) and 47 ncRNA for the Mozambique tilapia in test C (Table 3). The species comparison in fresh and sea water showed a total of 647 ncRNA (Test B and D respectively, Supplemental 7). The possible orthologous were found by TBLASTN analysis (Supplemental 6).

Pseudogenes appeared differentially expressed in this analysis. In some cases, the pseudogenes correspond to a wrong annotation and the ensemble gene reference is provided. In other cases, it is reported as pseudogenes also in ensemble or not reported (Table 4).

The Venn graph compares the up- and down-regulated genes in Nile and Mozambique tilapia in response to the 6 weeks salinity treatment (Figure 3). Two genes transcripts, 100711639 and 109199608, were downregulated in both species after salinity treatment. The gene 100711639 corresponds to centrosomal protein 55-like (*cep55*) and the gene 109199608 is uncharacterized in NCBI. The 100705715 gene stands for the *cytochrome P450 26B1* gene, which is down-regulated in Nile tilapia and up-regulated in Mozambique tilapia in response to the 6 weeks salinity challenge. Finally, the Venn graph shows a gene commonly up-regulated, 100702028 encoding *probable polypeptide N-acetylgalactosaminyltransferase 8* (*galnt8*).

GO Enrichment analysis also revealed differences in the pathways triggered by differential expression of the genes (Figure 4). According to the KEGG analysis (Figure 5, Supplemental 8 and 9), the salinity challenge triggered differential expression in 45 metabolic pathways in Nile tilapia and 51 pathways in Mozambique tilapia (tests A and C).

Discussion

We found that the responses were clearly district between the two tilapia species. Euryhaline fish need between 1 and 3 days to fully remodel gill epithelium, ionocytes and ion transporters in order to compensate for the increase in tissues' ion concentration by active transport (reviewd in T. G. Evans & Kültz, 2020; Foskett et al., 1981). Therefore, the study reported here indicates differences in the hypo-osmoregulatory physiology, as reflected by gene expressions, after long term exposure to high salinity conditions of the highly tolerant Mozambique tilapia and the less tolerant Nile tilapia.

Previous studies comparing differential expression of genes in gills of Nile and Mozambique tilapias in response to salinity challenge were performed with more acute exposures, from 2h (Breves et al., 2010) to 1 week (Velan et al., 2011). In contrast, our main objective in this analysis was to identify what gene expression differences occurred in the gills between these two species, in response to a long-term salinity challenge. Therefore, our analysis provides an overview of the osmoregulation in a chronic

challenge for both species.

Differences between Nile and Mozambique tilapias facing 6 weeks salinity challenge

The differential regulating gene network between the two species includes a number of genes and pathways such as membrane transporters, cytoskeletal fibers, cell cycle proteins and immune related peptides, among others. Mozambique tilapia changed the expression pattern of 267 genes, around 4 times more genes than Nile tilapia (Figure 3). The ncRNA genes are also different in number with only one transcript shared between both species (Table 3). Interestingly, in Mozambique tilapia the GO enrichment analysis showed that the expressed genes are highly related to ATP binding, sequence-specific DNA binding as well as nucleic acid binding according to their molecular function. Some genes are also enriched for the regulation of transcription DNA-template (Figure 4). Therefore, the transcriptomic activity is widely triggered in the Mozambique tilapia when compared with the Nile tilapia.

The two studied tilapias regulate different transporters for sodium and potassium secretion as well as water transport by aquaporins (Table 5, Supplemental 2, and Supplemental 4). Different regulation of the transporters after short-term salinity challenge have been previously reported in the gills for these two species (Breves et al., 2010; Velan et al., 2011). This has also been observed in the intestine (Ronkin et al., 2015), particularly for the aquaporin 8 gene (Velan et al. 2011). Our long-term study reports additional differences in potassium regulation. Therefore, both species regulate differentially the concentration of ions, and therefore, also the cell stress of during the osmotic challenge.

In addition, we found that the regulation of the cell adhesion molecules is also different between the two tilapia species. As shown in the KEGG analysis (Figure 5), Nile tilapia upregulates a single gene, *cadherin-1-like*, affecting tight junctions in epithelial cells. Studies based on transepithelial electrical resistance and diffusion of fluorescent sphingomyelin showed that E-CADHERIN is essential for the sealing of heterotypic tight junctions, which in turn include CLAUDINS-1 and 2 among other proteins

(Contreras et al., 2002). Therefore, the permeability of the tight junctions in the gills of Nile tilapia may be reduced after the salinity challenge by upregulating *cadherin-1-like* gene, thus increasing the sealing of the junctions in the epithelial cells of the gills. In contrast, Mozambique tilapia shows a downregulation of *cadherin-1* and *blastomere cadherin*, and upregulation of *claudin-10* gene. CLAUDIN-10 is qualified as pore-forming protein with increase of permeability in anions by CLAUDIN-10b and cations by CLAUDIN-10a (Günzel and Yu, 2013; Van Itallie et al., 2006). The upregulation of this gene may increase the tolerance to ions of Mozambique tilapia by the tight junctions of the gills. Studies on primary cultured gill epithelia proved that the mRNA of *claudin-10c*, *-10d* and *-33b* were detected only in preparations that included ionocytes (Kolosov et al., 2014, 2018). All these differences appear to indicate ionocytes changes occurring in both the Mozambique and Nile tilapias when confronted to a long-term salinity challenge, but they are of a distinct nature in each species.

Another major interspecific difference observed in our study regards the immune response. The Mozambique tilapia seem to experience an upregulation of many κ-Igs chains (Figure 2, Table 2), the modulation of the communication with lymphocytes and the regulation of other molecules such as toll-like receptors (Figure 5, Table 6, Supplemental 4). Other cell adhesion molecules upregulated in Mozambique tilapia of the immune system consist in the T-cell receptor-signaling pathway (Figure 5). *T-cell surface CD2* (CD2) and the genes *b7h1* (gene 106097210, aka PD-L1) and *b7h3* (gene 106097212) are down-regulated (Figure 5, Supplemental 4 and 10). The gene *b7h1* codifies for programmed death-ligand 1 that promotes T-cell apoptosis (Dong et al., 2002) and the gene *b7h3* encodes the Butyrophilin-like protein 2, which in turn may inhibit T-cell activation (Zang et al., 2003). In Nile tilapia on the other hand, the immune response regarding lymphocytes was reduced with the down-regulation of the *chemokines 8* and *17* genes that would attract T-cells. The complement cascade in Nile tilapia is up-regulated by *complement C3* (*c3*) and *c1qtnf3/ctrp3* thus, upregulating the innate immune system (Janeway Jr et al., 2001). These results point to a different immune strategy in the Nile tilapia with lower

response than what is observed in the Mozambique tilapia.

A group of mammalian innate-like lymphocytes (mILL) with properties between adaptive and innate immune functions have been reviewed recently by their connection with fish lymphocytes (Scapigliati et al., 2018). These lymphocytes include γδT-Cells (Wang et al., 2017; Wu et al., 2009) and B1-B cells (Tung et al., 2006) and could represent a "lower layer" of extant, evolutionary-related, analogs of fish lymohcytes (Scapigliati et al., 2018). Thus, pointing to a common origin of lymphocytes back to the origin of all vertebrates (Flajnik, 2018). These innate-lymphocytes would be highly triggered in Mozambique tilapia when facing osmotic challenge, but it would not be of much importance in the Nile tilapia.

In other pathways of cell adhesion molecules, the *B-cell receptor CD22* (CD22) was upregulated only in Mozambique tilapia (Figure 5). This gene is in charge of B-cell adhesions and binds to α2-6—linked sialic acid via an amino-terminal Ig-like domain and this interaction has a turnover by endocytosis (Zhang and Varki, 2004). This last gene is leading the formation of Sialyl-Tn antigens in Mucin type O-glycan biosynthesis pathways (Brockhausen, 1999, Varki et al. 1999). Interestingly, the *alpha-N-acetylgalactosaminide alpha-2,6-sialyltransferase 1-like* (*st6galnac1-like*) is also upregulated (Table 6, Supplemental 4 and Supplemental 12b), thus suggesting that the B-cells of gills in Mozambique tilapia may be secreting Igs, in agreement with previous studies on non-mammalian B-cell (Danilova et al., 2005; Hansen et al., 2005; Isu et al., 2006; Picchietti et al., 2017). However, the high increase in the κ-light chain and not so important upregulation in λ-light chain of immunoglobulins noticed in the Mozambique tilapia may change the ratio of the chains and also the type of Ig that is produced.

Our results suggest that Mozambique tilapia are activating the T-cell and B-cell like lymphocytes as part of the innate immune response to face the long-term salinity challenge (Scapigliati et al., 2018). In contrast, Nile tilapia do not reach a similar magnitude of this response.

Similarities between Nile and Mozambique tilapias

The GO enrichment analysis reports a similar approach for cell components in both species (Figure 4).

The membrane and integral components of the membrane are consistent with the differential expression of the transporters, aquaporins and the innate immune proteins described previously.

Proteolysis is the most notable biological process responding to salinity in both tilapias, with a high number of genes related to proteolysis and catabolism found in our analysis (Supplemental 10). Proteolysis is the breakdown of proteins into smaller chains for its degradation. Recent analysis of quantification of protein content in Nile tilapia after short term salinity challenge proved some non-linear effects between differentially expressed genes and proteins (Roy et al., 2021). However, more research is needed to find out the exact role of proteolysis in the epithelium turnover after the salinity challenge interfering with quality of translation and protein folding.

Both tilapias upregulate genes encoding for the tight junctions proteins involved in transepithelial migration of leukocytes through adhesive interactions (*cxadr* and *cxadrh*, Table 6) for the activation of gamma-delta T-cells residing in epithelia (Witherden et al., 2010). In addition, the inflammation was modulated in both species by different genes. The negative regulator of inflammatory response, the *c1qtnf3* gene, (Table 5, Supplemental 2) was down-regulated in Nile tilapia while *tnfaip2*, *nlrp12, pycard*, *granulins and S100a6*, were modulated (up- or down-regulated according to their function) to induce inflammation also in the Mozambique tilapia. Therefore, both species modulate an innate immune response including innate lymphocytes and inflammation to face the long-term salinity challenge. Furthermore, the mucin type O-glycan biosynthesis was up regulated by *galnt8* (Table 6), thus suggesting certain parallelism between the two species in the barrier defense.

Cytoskeletal changes, cell repair, and apoptosis as indicators of epithelium turnover

In the two studied tilapias we found differentialy expressed genes related to changes in cytoskeleton rearrangement, cell geometry, tissue remodeling and turnover and processes related to different metabolic pathways that revealed major structural changes (Supplemental 8). In Mozambique tilapia these processess are modulated by a set of genes (*hmt, khc, pycard, tlr2a, tlr2btnrfrsf14*; Table 5 and

Table 6) that are not expressed in the Nile tilapia, which in turn, is expressing apoptotic genes instead (*cep55*, *casp1*, *casp1-a*, *ifi27a*, *ifi27b*, *ctrp3*, *gzmb*; Table 5).

Additionally, endocytosis was down-regulated by *kinesin* in Mozambique tilapia and the gene *cep55* was generally down-regulated in both species (Table 5, Supplemental 8b). This gene is associated to mitosis and cytokinesis (Van Der Horst et al., 2009), promoting cell proliferation and inhibiting apoptosis (Li et al., 2018) thus indicating the cytoskeleton. Nile tilapia seems to trigger the intracellular rearrangement by the up-regulation of *unconventional myosin-1c* (*myo1C*) *isoform 1* (Table 5, Supplemental 2) which regulates intracellular movements (Münnich et al., 2014).

Finally, COLLAGEN is an important extra cellular matrix protein related to tensile strength that affects the structural constitution of the tissue. Mozambique tilapia down-regulated the gene encoding for the *collagen alpha-4 (IV)* chain while Nile tilapia down-regulated the *la-related protein 6* (*larp6*) gene (Table 5, Supplemental 12b), in charge of COLLAGEN translation (Cai et al., 2010; Challa and Stefanovic, 2011) and stabilization (Manojlovic and Stefanovic, 2012). Our recent study on Nile tilapia indicates that the concentration of COLLAGEN IV protein was significantly different after short-term salinity challenge (Root et al., 2021). The transcription and translation of collagen was triggered in both species by the long-term salinity challenge, but each species follows a different approach through the transcription of different genes. Studies in smoltification of Atlantic salmon found that collagen is up-regulated in the gills during salinity acclimation and is related to tissue growth and reorganization (Seear et al., 2010). Therefore, the regulation of collagen may be related to the changes in the gill epithelium under adaptation to salinity in some teleost species.

Inokuchi and Kaneko (2012) revealed that ionocytes adapted to freshwater enter in an apoptotic process and seawater-type ionocytes are recruited in the gills of the Mozambique tilapia after a salinity challenge. The various ionocytes' types in Mozambique tilapia were classified by the different transporters that they express (Hiroi et al., 2005). This was in agreement to our long-term salinity

treatment with the differential expression of transporters found in both tilapias as well as the presence of genes involved in apoptosis modulation and the rearrangement of cell geometry and tensile strength. Therefore our present results suggest that in the Nile tilapia, ionocytes are also reorganized and there is an epithelium turnover occurring but with differences observed to those previously reported in the Mozambique tilapia.

Regulation of transcription

The Up- and down-regulated genes after the six-week salinity challenge show a transcriptional divergence between the two species, as only 4 genes were commonly regulated (Figure 3, tests A and C). These differences include DEGs in the Mozambique tilapia that had undergone a pseudogenization process in Nile tilapia (Table 4) and a wide differential expression of lncRNA and ncRNA, with 9 DEGs in Nile tilapia and 47 DEGs in Mozambique tilapia (Table 3). The GO analysis also reveals an increased activity of sequence-specific DNA binding and nucleic acid binding within the DEGs in Mozambique tilapia than DNA binding activity in Nile tilapia (GO accessions 0043565, 0003676 and 0003677 respectively, Figure 4).

Salinity tolerance and speciation in euryhaline tilapia species

Adaptation to freshwater as a speciation phenomena has been studied between euryhaline killifish species, finding transcriptomic divergences in similar metabolic pathways to those observed in our analysis: cell volume regulation, nucleosome maintenance, ion transport, energetics, mitochondrion function, transcriptional regulation and apoptosis (Whitehead et al., 2011). This convergence of metabolic pathways is consistent with our analysis and may be required to cope with cell stress after a salinity challenge, as reviewed recently (Evans and Kültz, 2020).

The teleost whole genome duplication (TWGD) had an important role in evolutionary diversification (Campo et al., 2018; Fleming et al., 2019; Henkel et al., 2012; Kratochwil et al., 2017; Musilova et al., 2018; Van De Peer et al., 2010; Venkatesh, 2003). In addition, species of the family Cichlidae went

through extensive local duplication events, thus increasing their molecular diversity (Fruciano et al., 2019; Kautt et al., 2020; Xiong et al., 2020). Interestingly, genes reported as pseudogenes in the Nile tilapia were found in this study to be differentially expressed in Mozambique tilapia (Table 4). The role of pseudogenes have been recently reviewed, recommending their reassessment in order to identify elements of genome function and evolution (Cheetham et al., 2020). These Nile tilapia pseudogenes that were found to be differentially transcribed in the Mozambique tilapia agree with the perspective of pseudogenization as one possible speciation method (Cheetham et al., 2020). However, this observation may result also from wrong annotations, as one pseudogene was found differentially expressed in Nile tilapia (gene 109195272, Table 4). The current lack of an annotated reference genome from the Mozambique tilapia delays further study in this species.

Conclusions

Here we identified inter-specific changes between Nile and Mozambique tilapias in the gills transcriptome in response to a six-week salinity challenge. Our results suggest that both species undergo an epithelium turnover in the gills after this salinity challenge with inter-species differences. Cytoskeletal rearrangements that could modify cell geometry, differences in transporters both in cell membrane and mitochondrion, and the extracellular matrix, together with a different up-regulation of the immune system are the most remarkable differences found. Our comparative analysis reveals a different architecture in the genes expressed in freshwater *vs* salty water. Our data suggests the regulation of the transcription as one of the key factors for a successful osmoregulation. We showed species differences are not only in response to salinity challenge, but in the high number of differentially expressed genes. Some of these are related to osmoregulation, which were already observed in freshwater between both species.

Taken all together, our analysis suggests that Nile tilapia do not have the capacity for responding at a transcriptome level to the high osmotic pressure of salt waters compared to the Mozambique tilapia,

which in turn transcribes a broader range of genes including some annotated as pseudogenes in Nile tilapia.

New genomic approaches are necessary to identify if this differential transcription corresponds to conservation, duplication, pseudogenization and diversification or loss of gene copies produced during species radiation.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was supported by grant 3-11898 from the Israeli-French High Council for Scientific and Technology Cooperation, and by grant 2016611 from the US-Israel Binational Science Foundation (BSF).

RNA sequences and GenBank Accession numbers

All transcriptomic data and metadata have been deposited and are publicly accessible at NCBI under accession number PRJNA (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA756879).

Bibliography

Anders, S., Huber, W., Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., Gingeras, T.R., Gu, Z., Eils, R., Schlesner, M., Huber, W., Carey, V.J., Gentleman, R., Anders, S., Carlson, M., Carvalho, B.S., Bravo, H.C., Davis, S., Gatto, L., Girke, T., Gottardo, R., Hahne, F., Hansen, K.D., Irizarry, R.A., Lawrence, M., Love, M.I., MaCdonald, J., Obenchain, V., Oleš, A.K., Pagès, H., Reyes, A., Shannon, P., Smyth, G.K., Tenenbaum, D., Waldron, L., Morgan, M., Liao, Y., Smyth, G.K., Shi, W., Love, M.I., Huber, W., Anders, S., Narayana, A., Mathew, M., Tam, M., Kannan, R., Madden, K.M., Golfinos, J.G., Parker, E.C., Ott, P.A., Pavlick, A.C., Saeidipour, B., Bakhshi, S., 2010. Differential expression analysis for sequence count data via mixtures of negative binomials. Adv. Environ. Biol. 7, 2803–2809.

Brennan, R.S., Galvez, F., Whitehead, A., 2015. Reciprocal osmotic challenges reveal mechanisms of divergence in phenotypic plasticity in the killifish Fundulus heteroclitus. J. Exp. Biol. 218, 1212–1222. https://doi.org/10.1242/jeb.110445

Breves, J.P., Hasegawa, S., Yoshioka, M., Fox, B.K., Davis, L.K., Lerner, D.T., Takei, Y., Hirano, T., Grau, E.G., 2010. Acute salinity challenges in Mozambique and Nile tilapia: Differential responses of plasma prolactin, growth hormone and branchial expression of ion transporters. Gen. Comp. Endocrinol. 167, 135–142. https://doi.org/10.1016/j.ygcen.2010.01.022

Brockhausen, I., 1999. Pathways of O-glycan biosynthesis in cancer cells. Biochim. Biophys. Acta (BBA)-General Subj. 1473, 67–95.

Cai, L., Fritz, D., Stefanovic, L., Stefanovic, B., 2010. Nonmuscle myosin-dependent synthesis of Type I collagen. J. Mol. Biol. 401, 564–578. https://doi.org/10.1016/j.jmb.2010.06.057

Campo, A., Lafont, A.G., Lefranc, B., Leprince, J., Tostivint, H., Kamech, N., Dufour, S., Rousseau, K., 2018. Tachykinin-3 genes and peptides characterized in a basal teleost, the european eel: Evolutionary perspective and pituitary role. Front. Endocrinol. (Lausanne). 9, 1–14. https://doi.org/10.3389/fendo.2018.00304

Challa, A.A., Stefanovic, B., 2011. A Novel Role of Vimentin Filaments: Binding and Stabilization of Collagen mRNAs. Mol. Cell. Biol. 31, 3773–3789. https://doi.org/10.1128/mcb.05263-11

Cheetham, S.W., Faulkner, G.J., Dinger, M.E., 2020. Overcoming challenges and dogmas to understand the functions of pseudogenes. Nat. Rev. Genet. 21, 191–201. https://doi.org/10.1038/s41576-019-0196-1

Chourasia, T.K., D'Cotta, H., Baroiller, J.F., Slosman, T., Cnaani, A., 2018. Effects of the acclimation to high salinity on intestinal ion and peptide transporters in two tilapia species that differ in their salinity tolerance. Comp. Biochem. Physiol. -Part A Mol. Integr. Physiol. 218, 16–23. https://doi.org/10.1016/j.cbpa.2018.01.004

Cnaani, A., Hulata, G., 2011. Improving salinity tolerance in tilapias: Past experience and future prospects. Isr. J. Aquac. - Bamidgeh 63.

Contreras, R.G., Shoshani, L., Flores-Maldonado, C., Lázaro, A., Monroy, A.O., Roldán, M.L., Fiorentino, R., Cereijido, M., 2002. E-cadherin and tight junctions between epithelial cells of different animal species. Pflugers Arch. Eur. J. Physiol. 444, 467–475. https://doi.org/10.1007/s00424-002-0827-8

Danilova, N., Bussmann, J., Jekosch, K., Steiner, L.A., 2005. The immunoglobulin heavy-chain locus in zebrafish : identification and expression of a previously unknown isotype , immunoglobulin Z 6, 295–302. https://doi.org/10.1038/ni1166

Development Core Team, R., 2013. R: A language and environment for statistical computing. R Foundation for statistical computing. cran.univ-paris1.fr.

Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., Gingeras, T.R., 2013. STAR: Ultrafast universal RNA-seq aligner. Bioinformatics 29, 15–21. https://doi.org/10.1093/bioinformatics/bts635

Dong, H., Strome, S.E., Salomao, D.R., Tamura, H., Hirano, F., Flies, D.B., Roche, P.C., Lu, J., Zhu, G., Tamada, K., Lennon, V.A., Cells, E., Chen, L., 2002. Tumor-associated B7-H1 promotes T-cell apoptosis: A potential mechanism of immune evasion. Nat. Med. 8, 793–800. https://doi.org/10.1038/nm730

Edwards, S.L., Marshall, W.S., 2012. Principles and Patterns of Osmoregulation and Euryhalinity in Fishes, Fish Physiology. Elsevier Inc. https://doi.org/10.1016/B978-0-12-396951-4.00001-3

Evans, D.H., Piermarini, P.M., Choe, K.P., 2005. The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. Physiol. Rev. 85, 97–177. https://doi.org/10.1152/physrev.00050.2003

Evans, T.G., Kültz, D., 2020. The cellular stress response in fish exposed to salinity fluctuations. J. Exp. Zool. Part A Ecol. Integr. Physiol. 333, 421–435. https://doi.org/10.1002/jez.2350

Flajnik, M.F., 2018. A cold- blooded view of adaptive immunity. Nat. Rev. Immunol. 18. https://doi.org/10.1038/s41577-018-0003-9

Fleming, M.S., Maugars, G., Lafont, A., Rancon, J., Fontaine, R., Nourizadeh-lillabadi, R., Weltzien, F.,

Yebra-pimentel, E.S., Dirks, R., Mccormick, S.D., Rousseau, K., Martin, P., Dufour, S., 2019. Functional divergence of thyrotropin beta-subunit paralogs gives new insights into salmon smoltification metamorphosis 1–15. https://doi.org/10.1038/s41598-019-40019-5

Foskett, B.Y.J.K., Logsdon, C.D., Machen, T.E., Bern, H.A., 1981. By j. kevin foskett, craig d. logsdon,* timothy turner, terry e. machen* and howard a. bern.

Fruciano, C., Meyer, A., Franchini, P., 2019. Divergent Allometric Trajectories in Gene Expression and Coexpression Produce Species Differences in Sympatrically Speciating Midas Cichlid Fish. Genome Biol. Evol. 11, 1644–1657. https://doi.org/10.1093/gbe/evz108

Günzel, D., Yu, A.S.L., 2013. Claudins and the modulation of tight junction permeability, Physiological Reviews. https://doi.org/10.1152/physrev.00019.2012

Hansen, J.D., Landis, E.D., Phillips, R.B., 2005. Discovery of a unique Ig heavy-chain isotype (IgT) in rainbow trout : Implications for a distinctive B cell developmental pathway in teleost fish 102.

Henkel, C. V, Burgerhout, E., de Wijze, D.L., Dirks, R.P., Minegishi, Y., Jansen, H.J., Spaink, H.P., Dufour, S., Weltzien, F.-A., Tsukamoto, K., van den Thillart, G.E.E.J.M., 2012. Primitive Duplicate Hox Clusters in the European Eel's Genome. PLoS One 7, e32231. https://doi.org/10.1371/journal.pone.0032231

Hiroi, J., McCormick, S.D., 2012. New insights into gill ionocyte and ion transporter function in euryhaline and diadromous fish. Respir. Physiol. Neurobiol. 184, 257–268.

Hiroi, J., McCormick, S.D., Ohtani-Kaneko, R., Kaneko, T., 2005. Functional classification of mitochondrion-rich cells in euryhaline Mozambique tilapia (Oreochromis mossambicus) embryos, by means of triple immunofluorescence staining for Na+/K+-ATPase, Na +/K+/2Cl- cotransporter and CFTR anion channel. J. Exp. Biol. 208, 2023–2036. https://doi.org/10.1242/jeb.01611

Hsu, E., Criscitiello, M.F., Hsu, E., Criscitiello, M.F., 2006. Diverse Immunoglobulin Light Chain Organizations in Fish Retain Potential to Rearrange B Cell Receptor Specificities. J. Immunol. 177, 2452–2462. https://doi.org/10.4049/jimmunol.177.4.2452

Inokuchi, M., Kaneko, T., 2012. Recruitment and degeneration of mitochondrion-rich cells in the gills of Mozambique tilapia Oreochromis mossambicus during adaptation to a hyperosmotic environment. Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 162, 245–251. https://doi.org/10.1016/j.cbpa.2012.03.018

Janeway Jr, C.A., Travers, P., Walport, M., Shlomchik, M.J., 2001. The complement system and innate immunity, in: Immunobiology. The Immune System in Health and Disease. 5th Edition. Garland Science.

Kamal, A.H.M.M., Mair, G.C., 2005. Salinity tolerance in superior genotypes of tilapia, Oreochromis niloticus, Oreochromis mossambicus and their hybrids. Aquaculture 247, 189–201. https://doi.org/10.1016/j.aquaculture.2005.02.008

Kanehisa, M., 2019. Toward understanding the origin and evolution of cellular organisms. Protein Sci. 28, 1947–1951. https://doi.org/10.1002/pro.3715

Kautt, A.F., Kratochwil, C.F., Nater, A., Machado-Schiaffino, G., Olave, M., Henning, F., Torres-Dowdall, J., Härer, A., Hulsey, C.D., Franchini, P., Pippel, M., Myers, E.W., Meyer, A., 2020. Contrasting signatures of genomic divergence during sympatric speciation. Nature 588, 106–111. https://doi.org/10.1038/s41586-020-2845-0

Kolosov, D., Bui, P., Chasiotis, H., Kelly, S.P., 2013. Claudins in teleost fishes. Tissue Barriers 1, e25391. https://doi.org/10.4161/tisb.25391

Kolosov, D., Chasiotis, H., Kelly, S.P., 2014. Tight junction protein gene expression patterns and changes in transcript abundance during development of model fish gill epithelia. J. Exp. Biol. 217, 1667–1681. https://doi.org/10.1242/jeb.098731

Kozak, G.M., Brennan, R.S., Berdan, E.L., Fuller, R.C., Whitehead, A., 2014. Functional and population genomic divergence within and between two species of killifish adapted to different osmotic

niches. Evolution (N. Y). 68, 63–80. https://doi.org/10.1111/evo.12265
Kratochwil, C.F., Sefton, M.M., Liang, Y., Meyer, A., 2017. Tol2 transposon-mediated transgenesis in the Midas cichlid (Amphilophus citrinellus) - Towards understanding gene function and regulatory evolution in an ecological model system for rapid phenotypic diversification. BMC Dev. Biol. 17, 1–11. https://doi.org/10.1186/s12861-017-0157-x
Li, F., Jin, D., Tang, C., Gao, D., 2018. CEP55 promotes cell proliferation and inhibits apoptosis via the pi3k/akt/p21 signaling pathway in human glioma u251 cells. Oncol. Lett. 15, 4789–4796. https://doi.org/10.3892/ol.2018.7934
Likongwe, J.S., Stecko, T.D., Stauffer, J.R., Carline, R.F., 1996. Combined effects of water temperature and salinity on growth and feed utilization of juvenile Nile tilapia Oreochromis niloticus (Linneaus). Aquaculture 146, 37–46. https://doi.org/10.1016/S0044-8486(96)01360-9
Loretz, C.A., 1995. 2 Electrophysiology of ion transport in teleost intestinal cells, in: Fish Physiology. Elsevier, pp. 25–56.
Manojlovic, Z., Stefanovic, B., 2012. A novel role of RNA helicase A in regulation of translation of type I collagen mRNAs. Rna 18, 321–334. https://doi.org/10.1261/rna.030788.111
McCormick, S.D., 2001. Endocrine control of osmoregulation in teleost fish. Am. Zool. 41, 781–794. https://doi.org/10.1093/icb/41.4.781
Morgan, D.L., Gill, H.S., Maddern, M.G., Beatty, S.J., 2004. Distribution and impacts of introduced freshwater fishes in Western Australia. New Zeal. J. Mar. Freshw. Res. 38, 511–523. https://doi.org/10.1080/00288330.2004.9517257
Münnich, S., Taft, M.H., Manstein, D.J., 2014. Crystal structure of human Myosin 1c - The motor in GLUT4 exocytosis: Implications for Ca2 + regulation and 14-3-3 binding. J. Mol. Biol. 426, 2070–2081. https://doi.org/10.1016/j.jmb.2014.03.004
Musilova, Z., Cortesi, F., Matschiner, M., Davies, W.I.L., Stieb, S.M., de Busserolles, F., Malmstrøm, M., Tørresen, O.K., Mountford, J.K., Hanel, R., Jakobsen, K.S., Carleton, K.L., Jentoft, S., Marshall, J., Salzburger, W., 2018. Vision using multiple distinct rod opsins in deep-sea fishes. bioRxiv 592, 588–592. https://doi.org/10.1101/424895
Picchietti, S., Nuñez-Ortiz, N., Stocchi, V., Randelli, E., Buonocore, F., Guerra, L., Scapigliati, G., 2017. Evolution of lymphocytes. Immunoglobulin T of the teleost sea bass (Dicentrarchus labrax): Quantitation of gene expressing and immunoreactive cells. Fish Shellfish Immunol. 63, 40–52. https://doi.org/https://doi.org/10.1016/j.fsi.2017.02.002
Popper, D., Lichatowich, T., 1975. Preliminary success in predator control of Tilapia mossambica. Aquaculture 5, 213–214.
Quintela, M., Richter-Boix, A., Bekkevold, D., Kvamme, C., Berg, F., Jansson, E., Dahle, G., Besnier, F., Nash, R.D.M., Glover, K.A., 2021. Genetic response to human-induced habitat changes in the marine environment: A century of evolution of European sprat in Landvikvannet, Norway. Ecol. Evol. 1691–1718. https://doi.org/10.1002/ece3.7160
Raudevere, U., Kolberg, L., Kusmin, I., Arak, T., Alder, P., Peterson, H., Vilo, J., 2019. g : Profiler : a web server for functional enrichment analysis and conversions of gene lists (2019 update). Nucleic Acids Res. 47, 191–198. https://doi.org/10.1093/nar/gkz369
Ronkin, D., Seroussi, E., Nitzan, T., Doron-Faigenboim, A., Cnaani, A., 2015. Intestinal transcriptome analysis revealed differential salinity adaptation between two tilapiine species. Comp. Biochem. Physiol. - Part D Genomics Proteomics 13, 35–43. https://doi.org/10.1016/j.cbd.2015.01.003
Root, L., Campo, A., Macniven, L., Con, P., Cnaani, A., Kûltz, D., 2021. A data-independent acquisition (DIA) assay library for quantitation of environmental effects on the kidney proteome of Oreochromis niloticus. Molecuar Ecol. Resour. https://doi.org/10.22541/au.160553713.37893872/v1
Scapigliati, G., Fausto, A.M., Picchietti, S., 2018. Fish Lymphocytes : An evolutionary equivalent of

Mammalian innate-Like Lymphocytes ? Front. Immunol. 9, 1–8. https://doi.org/10.3389/fimmu.2018.00971

Schultz, E.T., McCormick, S.D., 2012. Euryhalinity in An Evolutionary Context, Fish Physiology. Elsevier Inc. https://doi.org/10.1016/B978-0-12-396951-4.00010-4

Seear, P.J., Carmichael, S.N., Talbot, R., Taggart, J.B., Bron, J.E., Sweeney, G.E., 2010. Differential gene expression during smoltification of Atlantic salmon (Salmo salar L.): A first large-scale microarray study. Mar. Biotechnol. 12, 126–140. https://doi.org/10.1007/s10126-009-9218-x

Tung, J.W., Mrazek, M.D., Yang, Y., Herzenberg, Leonard A, Herzenberg, Leonore A, Med, K.J.E., 2006. Phenotypically distinct B cell development pathways map to the three B cell lineages in the mouse. Pnas 103, 16–21.

Uchida, K., Kaneko, T., Miyazaki, H., Hasegawa, S., Hirano, T., 2000. Excellent salinity tolerance of mozambique tilapia (Oreochromis mossambicus): Elevated chloride cell activity in the branchial and opercular epithelia of the fish adapted to concentrated seawater. Zoolog. Sci. 17, 149–160. https://doi.org/10.2108/zsj.17.149

Van De Peer, Y., Maere, S., Meyer, A., 2010. 2R or not 2R is not the question anymore. Nat. Rev. Genet. 11, 166. https://doi.org/10.1038/nrg2600-c2

Van Der Horst, A., Simmons, J., Khanna, K.K., 2009. Cep55 stabilization is required for normal execution of cytokinesis. Cell Cycle 8, 3742–3749. https://doi.org/10.4161/cc.8.22.10047

Van Itallie, C.M., Rogan, S., Yu, A., Vidal, L.S., Holmes, J., Anderson, J.M., 2006. Two splice variants of claudin-10 in the kidney create paracellular pores with different ion selectivities. Am. J. Physiol. - Ren. Physiol. 291, 1288–1299. https://doi.org/10.1152/ajprenal.00138.2006

Velan, A., Hulata, G., Ron, M., Cnaani, A., 2011. Comparative time-course study on pituitary and branchial response to salinity challenge in Mozambique tilapia (Oreochromis mossambicus) and Nile tilapia (O. niloticus). Fish Physiol. Biochem. 37, 863–873. https://doi.org/10.1007/s10695-011-9484-1

Venkatesh, B., 2003. Evolution and diversity of fish genomes. Curr. Opin. Genet. Dev. 13, 588–592. https://doi.org/10.1016/j.gde.2003.09.001

Villegas, C.T., 1990. Evaluation of the salinity tolerance of Oreochromis mossambicus, O. niloticus and their F1 hybrids. Aquaculture 85, 281–292.

Wang, F., Hu, C., Ma, J., Gao, K., Xiang, L., Shao, J., 2017. Characterization of γδ T Cells from Zebrafish Provides Insights into Their Important Role in Adaptive Humoral Immunity. Front. Immunol. 7. https://doi.org/10.3389/fimmu.2016.00675

Whitehead, A., Roach, J.L., Zhang, S., Galvez, F., 2012. Salinity- and population-dependent genome regulatory response during osmotic acclimation in the killifish (Fundulus heteroclitus) gill. J. Exp. Biol. 215, 1293–1305. https://doi.org/10.1242/jeb.062075

Whitehead, A., Roach, J.L., Zhang, S., Galvez, F., 2011. Genomic mechanisms of evolved physiological plasticity in killifish distributed along an environmental salinity gradient. Proc. Natl. Acad. Sci. U. S. A. 108, 6193–6198. https://doi.org/10.1073/pnas.1017542108

Whitehead, A., Zhang, S., Roach, J.L., Galvez, F., 2013. Common functional targets of adaptive micro- and macro-evolutionary divergence in killifish. Mol. Ecol. 22, 3780–3796. https://doi.org/10.1111/mec.12316

Witherden, D.A., Verdino, P., Rieder, S.E., Garijo, O., Mills, R.E., Teyton, L., Fischer, W.H., Wilson, I.A., Havran, W.L., 2010. References and notes. Science (80-.). 329, 55–57. https://doi.org/10.14217/9781848590663-8-en

Wu, Y., Wu, W., Wong, W.M., Ward, E., Thrasher, A.J., Goldblatt, D., Osman, M., Digard, P., Canaday, D.H., Gustafsson, K., 2009. Human γδT Cells : A Lymphoid Lineage Cell Capable of Professional Phagocytosis. J. Immunol. 183, 5622–5629. https://doi.org/10.4049/jimmunol.0901772

Xiong, P., Hulsey, C.D., Fruciano, C., Wong, W.Y., Nater, A., Kautt, A.F., Simakov, O., Pippel, M., Kuraku,

S., Meyer, A., Franchini, P., 2020. The comparative genomic landscape of adaptive radiation in crater lake cichlid fishes. Mol. Ecol. 955–972. https://doi.org/10.1111/mec.15774

Zadunaisky, J.A., 1996. Chloride cells and osmoregulation. Kidney Int. 49, 1563–1567. https://doi.org/10.1038/ki.1996.225

Zang, X., Loke, P., Kim, J., Murphy, K., Waitz, R., Allison, J.P., 2003. B7x: A widely expressed B7 family member that inhibits T cell activation. Proc. Natl. Acad. Sci. U. S. A. 100, 10388–10392. https://doi.org/10.1073/pnas.1434299100

Zhang, M., Varki, A., 2004. Cell surface sialic acids do not affect primary CD22 interactions with CD45 and surface IgM nor the rate of constitutive Cd22 endocytosis. Glycobiology 14, 939–949. https://doi.org/10.1093/glycob/cwh126

Figure 1: Volcano plot indicating the significant differentially expressed genes (DEGs) according to p-adjusted value in the four tests. Blue indicates significance with log FoldChange below 2 and red significance with log-FoldChange above 2.

Figure 2: Regulation kappa and lambda chains of immunoglobulins differentially expressed (p-adj < 0.05). Studied from the normalized counts.

Figure 3: Venn diagram indicating the genes differentially expressed (p-adj < 0.05) for the two salinity tests, namely: Test A, NF NS: Nile tilapia contrast from fresh water to salty water; Test C: MF MS: Mozambique tilapia contrast from fresh water to salty water.

Figure 4: Enrichment analysis indicating frequency of the differentially expressed genes (p-adj < 0.05) in each of the GO categories for tests A (Nile tilapia in fresh to salty water) and test C (Mozambique tilapia in fresh to salty water). Pink color scale indicates Biological Process, orange color scale indicates Cell Component and green color scale indicates Molecular Function. In the Nile tilapia the most important enrichment is transmembrane transport and proteolysis for Biological Process, Integral component of membrane for Cell Component and Channel Activity for Molecular Function. In Mozambique tilapia Proteolysis is the predominant enrichment in Biological Process, Integral component of membrane in Cell Component and ATP-binding and Calcium ion binding in Molecular Function.

Figure 5: KEGG pathway of the Cell Adhesion Molecules indicating Mozambique tilapia is triggered for tight and adherens junctions in epithelial cells by *claudin* (CLDN) and *cadherin* (CDHE); for synapses by *protein tyrosine phosphatase receptor type F* (PTPRF) and *leucine-rich repeat-containing protein 4b* (NGL3); for immune system by *T-cell surface antigen cd2* (CD2), *B-cell receptor cd22* (CD22), *b7 homolog 1* (PD-L1) and *b7 homolog 3* (B7H3). Nile tilapia is triggered for epithelial cells tight and adherens junctions by *cadherin* (CDHE).

Table 1: Genes with significant differential gene expression (DEGs, p-adj < 0.05) in each contrast test performed with DESeq2 in R. The four groups were Nile tilapia in fresh water, Nile tilapia in salty water, Mozambique tilapia in fresh water and Mozambique tilapia in salty water. Control group was Nile tilapia in fresh water for tests A and B, Mozambique tilapia in fresh water for test C and Nile tilapia in salty water for test D.

Test	Description of the test	DEGs	Upregulated	Downregulated
A	Nile fresh water us Nile salty water	63	39	24
B	Nile fresh water us Mozambique fresh water	1359	756	603
C	Mozambique fresh water us M. Salty water	267	114	153
D	Nile salty water us Mozambique salty water	2235	1105	1130

Table 2: Light chain of immunoglobulins differentially expressed in the contrast test for salinity in Mozambique tilapia (Test C, p-adj < 0.05).

Gene ID	Gene name	DEGs
100695241	Ig kappa chain V-III region CBPC 101-like	Test D
100699464	Ig kappa chain C region, B allele-like	Test B
102078942	Ig kappa-b4 chain C region-like	Test D
102079018	Ig kappa-b4 chain C region-like	Test B
102081724	Ig kappa chain V region 3381-like	Test C and D
102083289	Ig kappa-b4 chain C region-like	Test C and D
102083336	Ig lambda-3 chain C region-like	Test D
106097557	Ig kappa chain V region 3381-like	Test D
109195808	Ig kappa chain C region, B allele-like	Test C
109195812	Ig kappa chain C region, B allele-like	Test C
109195905	Ig lambda-1 chain C region-like	Test D
109196386	Ig kappa chain V region 3381-like	Test D
109196593	Ig kappa-b4 chain C region-like	Test C and D
109199541	Ig kappa chain V region 3381-like	Test C and D
109199542	Ig kappa chain V region 120-like	Test C and D
109199546	Ig kappa-b4 chain C region-like	Test C
109199555	Ig kappa chain V region 3381-like	Test C and D
109199556	Ig kappa chain V region 3381-like	Test C and D
109199558	Ig kappa chain V region 3381-like	Test D
109199562	Ig kappa chain V region K-25-like	Test D
109199568	Ig kappa chain V region 3381-like	Test D
109199569	Ig kappa chain V region 3381-like	Test D
109199570	Ig kappa chain V region 3381-like	Test B, C and D
109199571	Ig kappa chain V region 3381-like	Test C and D
109199572	Ig kappa-b4 chain C region-like	Test C
109199573	Ig kappa chain V region 3381-like	Test C and D
109199574	Ig kappa chain V region 120-like	Test C and D
109199577	Ig kappa chain V region 2717-like	Test C
109199581	Ig kappa chain V region 120-like	Test C
109199582	Ig kappa chain V region BS-5-like	Test C
109199583	Ig kappa-b4 chain C region-like	Test B
109199878	Ig kappa-b4 chain C region-like	Test C
109199882	Ig kappa chain V region 4135-like	Test B and C
109199883	Ig kappa chain V region 3381-like	Test C
109199884	Ig kappa-b4 chain C region-like	Test D
109199885	Ig kappa chain V region 3381-like	Test C and D
109199887	Ig kappa chain V region 3381-like	Test C and D
109199890	Ig kappa chain V region 3381-like	Test C and D
109199894	Ig kappa chain V region BS-5-like	Test D
109199896	Ig kappa chain V region 3381-like	Test D
109199897	Ig kappa-b4 chain C region-like	Test C
109199898	Ig kappa-b4 chain C region-like	Test C
112843187	Ig lambda-3 chain C region-like	Test C
112843192	Ig lambda chain C region-like	Test C
112843450	Ig kappa chain V region 3381-like	Test D
112844677	Ig kappa chain V region K-25-like	Test C and D
112844682	Ig kappa chain V region 120-like	Test D
112844716	Ig kappa chain V region K-25-like	Test C
112844717	Ig kappa chain V region K-25-like	Test B and C

112844719	Ig kappa chain V region 4135-like	Test C

Table 3: Accession numbers of the ncRNA found differentially expressed in all the contrast tests (p-adj < 0.05).

Genebank	DEGs
XR_001223425.3	Test B and C
XR_001223464.2	Test B and C
XR_001224495.3	Test C
XR_001224894.3	Test B and C
XR_002056725.2	Test A and D
XR_002056988.2	Test B, C and D
XR_002057277.2	Test C
XR_002057544.2	Test B and C
XR_002057953.2	Test A and D
XR_002058234.2	Test B and C
XR_002058390.2	Test B and C
XR_002059107.2	Test C and D
XR_002059355.1	Test A and D
XR_002059550.2	Test C and D
XR_002059813.2	Test B and C
XR_002059841.2	Test B and C
XR_002059926.2	Test A and C
XR_002060137.2	Test B and C
XR_002060753.2	Test C and D
XR_002060890.2	Test C and D
XR_002060997.2	Test C
XR_002061966.2	Test A and D
XR_002062267.2	Test C and D
XR_002063084.2	Test C
XR_002064106.2	Test C and D
XR_003213240.1	Test C and D
XR_003213263.1	Test B and C
XR_003213631.1	Test C
XR_003213941.1	Test B and C
XR_003214998.1	Test B and C
XR_003215136.1	Test B and C
XR_003215488.1	Test C and D
XR_003215633.1	Test C and D
XR_003216294.1	Test C and D
XR_003216969.1	Test C and D
XR_003217047.1	Test B, C and D
XR_003217376.1	Test B and C
XR_003217412.1	Test A and D
XR_003217646.1	Test C and D
XR_003218937.1	Test C
XR_003219042.1	Test B and C
XR_003219572.1	Test B and C
XR_003220791.1	Test B, C and D
XR_003220938.1	Test C and D
XR_003221098.1	Test C

XR_003221291.1	Test B and C
XR_003221655.1	Test C
XR_003221787.1	Test C
XR_003221798.1	Test B and C
XR_003221798.1	Test C
XR_003222128.1	Test A and D
XR_266276.4	Test A and D
XR_268103.4	Test B and C
XR_268359.3	Test C
XR_269581.3	Test A

Table 4: Annotated pseudogenes found differentially expressed (p-adj < 0.05).

Gene ID	Gene name	Ensemble accession	DEGs
100691342	IgGFc-binding protein-like	NOT FOUND	Test B, C
100692118	40S ribosomal protein S28-like	ENSONIG00000029466	Test B, D
100692864	E3 ubiquitin-protein ligase RNF19B-like	ENSONIG00000043202	Test B
100694850	myosin heavy chain, fast skeletal muscle-like	NOT FOUND	Test D
100696151	histone H4-like	NOT FOUND	Test D
100706869	toll-like receptor 13	ENSONIG00000003939	Test B
100707751	L-amino-acid oxidase-like	ENSONIG00000012419	Test B, D
100707825	C-type lectin domain family 4 member G-like	ENSONIG00000006732	Test D
102075750	junctional adhesion molecule C-like	ENSONIG00000024317	Test B, C
102077866	pancreatic secretory granule membrane major glycoprotein GP2-like	NOT FOUND	Test B, D
102081088	mucin-2-like	ENSONIG00000015352 ENSONIG00000033567	Test D
102081111	leukocyte elastase inhibitor-like	NOT FOUND	Test D
102081490	protein NLRC3-like	ENSONIG00000019181	Test B
106096965	protein FAM92A	ENSONIG00000042069	Test D
106097126	alpha-1,3-galactosyltransferase 2-like	ENSONIG00000039724 ENSONIG00000027321 (lncRNA)	Test D
106098296	macrophage-expressed gene 1 protein-like	ENSONIG00000042060	Test B, D
106098607	Uncharacterized	ENSONIG00000033912	Test D
109194404	G2/M phase-specific E3 ubiquitin-protein ligase-like	NOT FOUND	Test B, D
109194512	angiopoietin-related protein 5-like	ENSONIG00000036859	Test D
109194612	uncharacterized similar to Mucin5AC (id 80.34%, e-value 0.00E+00, GeneID 100707479)	NOT FOUND	Test B, D
109195036	NXPE family member 3-like	ENSONIG00000036555	Test B, D
109195165	NXPE family member 3-like	ENSONIG00000040498	Test D
109195272	neuroligin-2-like	ENSONIG00000030850	Test A, B
109195819	tonsoku-like protein DNA repair protein	NOT FOUND	Test D

109196331	GTPase IMAP family member 4	ENSONIG00000000440	Test B
109196379	E3 ubiquitin-protein ligase TRIM21	ENSONIG00000028400	Test B, C
109196472	uncharacterized protein DDB_G0283357-like	ENSONIG00000043168 (lncRNA) ENSONIG00000037075 (lncRNA)	Test B
109196993	OX-2 membrane glycoprotein-like	NOT FOUND	Test D
109199344	nuclear factor 7, brain-like	ENSONIG00000036602 (pseudo)	Test D
109199564	Ig kappa chain V region 3381-like	NOT FOUND	Test C, D
109199778	GTPase IMAP family member 4	NOT FOUND	Test B
109200293	Uncharacterized	NOT FOUND	Test B
109200311	Uncharacterized	ENSONIG00000007068	Test D
109202019	carnitine O-palmitoyltransferase 1	ENSONIG00000039610	Test B
109202856	Uncharacterized	NOT FOUND	Test B, D
109204626	Uncharacterized	NOT FOUND	Test D
109204648	nuclear factor 7	ENSONIG00000009240	Test B, D
112841846	Uncharacterized	NOT FOUND	Test B
112841875	interferon-induced very large GTPase 1-like	NOT FOUND	Test D
112842405	N-acetyllactosaminide beta-1,3-N-acetylglucosaminyltransferase 3	ENSONIG00000040022	Test D
112842610	mucin-2-like	ENSONIG00000041410	Test D
112845090	probable serine/threonine-protein kinase clkA	NOT FOUND	Test B, D
112845663	HTH_Tnp_4 domain-containing protein	ENSONIG00000003544	Test B
112845814	28S ribosomal RNA	NOT FOUND	Test D
112845936	28S ribosomal RNA	NOT FOUND	Test D
112846649	SE-cephalotoxin-like	NOT FOUND	Test D
112847079	85/88 kDa calcium-independent phospholipase A2-like	ENSONIG00000013107	Test D
112847455	putative nuclease HARBI1	ENSONIG00000029067 (pseudo)	Test D

Table 5: Transcriptomic architecture in Nile and Mozambique tilapias indicating the genes triggered for transporters, nucleic acid repair, cytoskeletal proteins and epithelium permeability and cytoskeletal changes, cell repair and apoptosis as indicators of epithelium turnover (p-adj < 0.05).

Pathway	Gene	LFC	Regulation	Common name	Function
Transporters					
Nile	scl12a3	-5,91	down	Na/Cl co-transporter NCCT	Secretion of sodium
	scl6a7	0,79	up	Neurotransmitter/sodium symporter	Reduction of sodium intake
	aqua3	-2,71	down	Aquaporin 3	Water secretion from the cell
	aqua3-like	-5,34	down	Aquaporin 3 -like	Water secretion from the cell
	kcnj1	3,09	up	ATP-sensitive inward rectifier potassium channel 1	Tendency to allow potassium to flow into the cell rather than out of it
Mozambique					
	cmoat1	-1,18	down	Canalicular multispecific organic anion transporter 1	Organic anion transmembrane transporter activity

	scl1a3	-2,08	down	Glutamate aspartate transporter 1	Reduction of sodium intake
	scl1a5	-1,49	down	Neutral aminoacid transporter B	Reduction of sodium intake
	scl2a2	-3,87	down	Glucose transporter	Reduction of sodium intake
Nucleic acid repair					
Nile	scl25a36	1,07	up	Antiport specific for substrate	Transport of pyrimidine in mitochondria, DNA and RNA maintenance
Mozambique	trim39	2,51	up	E3 Ubiquitin-Protein ligase TRIM39	DNA repair
	trim21	1,58	up	E3 Ubiquitin-Protein ligase TRIM21	DNA repair
	slc25a24	-0,82	down	calcium-binding mitochondrial carrier protein SCaMC-1	Transport of adenine in mitochondria, protection against oxydative stress-induced cell death
	DNA-ligase	2,68	up	DNA ligase	DNA repair
	h4c1	-1,26	up	Histone 4	DNA repair
	urcgp	1,33	up	Upregulator of cell-proliferation-like	DNA repair
	msh3	5,65	up	DNA mismatch repair protein	DNA repair
	pif1	3,47	up	ATP-dependent DNA helicase PIF1 like	DNA repair
Cytoskeletal proteins and epithelium permeability					
Nile	cdh1-like	5,69	up	Cadherin-1-like	Increase the sealing of tight junctions in epithelial cells
Mozambique	cdh1	-1,56	down	Cadherin-1	Increase the sealing of tight junctions in epithelial cells
	bcdh	-1,54	down	Blastomere cadherin	Increase the sealing of tight junctions in epithelial cells
	cldn10	2,27	up	Claudin-10 like	Pore forming
Cytoskeletal changes, cell repair, and apoptosis as indicators of epithelium turnover					
Nile	larp6	-1,30	down	la-related protein 6	COLLAGEN I translation and stabilization
	cep55	-0,75	down	Centrosomal protein 55	Mitosis and cytokinesis, promotes cell differentation and inhibits apoptosis
	myo1C	1,35	up	Unconventional myosin-1c isoform 1	Intracellular movements
	casp1	-4,02	down	Caspase 1	Necroptosis and apoptosis
	casp1a	-0,71	down	Caspase 1 A	Necroptosis and apoptosis
	ifi27a	-2,12	down	Interferon alpha-inducible protein 27-like protein 2A	Apoptosis, placed in the mitochondria as part of the signaling pathways that lead to apoptosis (Gytz et al., 2017; Liu et al., 2014; Rosebeck & Leaman, 2008)
	ifi27b	-2,82	down	Interferon alpha-inducible protein 27-like protein 2A	Apoptosis, placed in the mitochondria as part of the signaling pathways that lead to apoptosis (Gytz et al., 2017; Liu et al., 2014; Rosebeck & Leaman, 2008)
	ctrp3	-2,94	down	Complement C1q tumor necrosis factor-related protein 3	Apoptosis
	gzmb	-1,74	down	Granzyme B	Apoptosis
Mozambique	col4a4	-1,86	up	Collagen alpha-4 (IV)	ECM-receptor interaction and focal adhesion pathways
	hmt	-2,78	down	Histone-lysine N-methiltransferase	Cytoskeleton rearrangement for changes in cell geometry, tissue remodeling and turnover of the tissue revealing structural changes
	trim39	2,51	up	E3 ubiquitin-protein ligase TRIM39	Regulates the G1/S transition of the cell cycle and DNA damage-induced G2 arrest by stabilizing CDKN1A/p21 (PubMed:23213251)

	col7a1	1,50	up	collagen alpha-1(VII) chain	May contribute to epithelial basement membrane organization and adherence by interacting with extracellular matrix (ECM) proteins such as type IV collagen
	hebp2	1,65	up	Heme-binding protein 2	Can promote mitochondrial permeability trnasition and facilitate necrotic cell death under different types of stress conditions
	bcor	-1,57	down	BCL6 corepressor	The protein encoded by this gene was identified as an interacting corepressor of BCL6, a POZ/zinc finger transcription repressor that is required for germinal center formation and may influence apoptosis
	khc	-1,57	down	Kinesin heavy chain	Cytoskeleton rearrangement for changes in cell geometry, tissue remodeling and turnover of the tissue revealing structural changes
	pycard	5,51	up	Apoptosis-associated speck-like protein containing a CARD	Functions as key mediator in apoptosis and inflammation.
	kinesin	-2,70	down	Kinesin	Endocytosis
	cep55	-0,78	down	Centrosomal protein 55	Mitosis and cytokinesis, promotes cell differentation and inhibits apoptosis

Table 6: Transcriptomic architecture in Nile and Mozambique tilapias for the differentially expressed genes in the immune system (p-adj < 0.05).

Pathway	Gene	LFC	Regulation	Common name	Function
Immune system					
Nile	cxadr	1,03	up	Coxsackievirus and adenovirus receptor homolog	Tight junctions and transepithelial migration of leukocytes through adesiveinteractions
	gimap4	-1,03	down	GTPase IMAP family member 4	The encoded protein of this gene may be negatively regulated by T-cell acute lymphocytic leukemia 1 (TAL1)
	ccl8	-2,02	down	C-C motif chemokine 8	Chemotactic factor that attracts monocytes, lymphocytes, basophils and eosinophils
	ccl17	-2,08	down	C-C motif chemokine 17	Chemotactic factor for T-lymphocytes but not monocytes or granulocytes. May play a role in T-cell development in thymus and in trafficking and activation of mature T-cells
	c1qtnf3 / ctrp3	-2,94	down	Complement C1q tumor necrosis factor-related protein 3	Diseases associated with C1QTNF3 include Bleeding Disorder, Platelet-Type, 14 and Chromosome 5P13 Duplication Syndrome.
	c3	3,06	up	Complement C3	Innate inmmune system
Mozambique	ifit1	1,32	up	Interferon-induced protein with tetratricopeptide repeats 1	Antiviral RNA-binding protein
	mx1	1,67	up	Interferon induced GTP-binding protein Mx-like	Antiviral activity against a wide range of RNA viruses and some DNA viruses
	itgam	1,01	up	Integrin alpha M	Expressed on the surface of many leukocytes involved in the innate immune system
	mrc1	2,41	up	Macrophage mannose receptor 1	Mediates the endocytosis of glycoproteins by macrophages. Acts as phagocytic receptor for bacteria, fungi and other pathogens.

cd2	1,31	up	T-cell surface CD2	CD2 mediates adhesion between T-cells and other cell types. Is implicated in trigger T-cells
grna	3,95	up	Granulin a	Inflammation and wound healing, cell proliferation, lysosomal function, development
grnb	2,89	up	Granulin b	Inflammation and wound healing, cell proliferation, lysosomal function, development
s100a6	1,05	up	S100 calcium binding protein A6	Regulator of inflammation, intracellular and extracellular functions through regulating calcium balance, cell apoptosis, migration, proliferation, differentiation, energy metabolism, and inflammation.
ly6g	3,49	up	Lymphocyte antigen 6G-like	Marker to identify neutrofils, monocytes and granulocytes
trl2a	1,29	up	Toll-like receptor number 2	Plays fundamental role in pathogen recognition and activation of innate immunity
trl2b	2,39	up	Toll-like receptor number 2	Plays fundamental role in pathogen recognition and activation of innate immunity
tnfrsf14	2,84	up	Tumor necrosis factor receptor superfamily member 14	Herpes simplex virus 1 infection and cytokine-cytokine receptor interaction paths
cd22a	1,54	up	B-cell receptor CD22a	Cell adhesion molecules
cd22b	1,44	up	B-cell receptor CD22b	Cell adhesion molecules
cd2	1,31	up	T-cell surface CD2	Cell adhesion molecules
b7h1 (106097210)	-2,65	down	Programmed death-ligand 1	T-cell apoptosis (Dong et al., 2002)
b7h3 (106097212)	-1,67	down	Butyrophilin-like protein 2	Inhibit T-cell activation (Zang et al., 2003)
clec10a	-4,75	down	C-type lectin domain family 10 member A	May participate in the interaction between tumoricidal macrophages and tumor cells. Plays a role in the recruitment of inflammatory monocytes to adipose tissue in diet-induced obesity.
cxadr	-1,35	down	Coxsackievirus and adenovirus receptor 2C transcript variant X3	Tight junctions and transepithelial migration of leukocytes through adesiveinteractions
ifit5	1,32	up	Interferon-induced protein with tetratricopeptide repeats 5	Interferon-induced RNA-binding protein involved in the human innate immune response
btn3a2	-0,93	down	Butyrophilin subfamily 3 member A2-like	Plays a role in T-cell responses in the adaptive immune response. Inhibits the release of IFNG from activated T-cells.
dmbt1	-2,74	down	Deleted in malignant brain tumors 1 protein-like	May play roles in mucosal defense system, cellular immune defense and epithelial differentiation. May play a role as an opsonin receptor for SFTPD and SPAR in macrophage tissues throughout the body, including epithelial cells lining the gastrointestinal tract.
nlrp1b	-1,84	down	NACHT%2C LRR and PYD domains-containing protein 1b allele 2-like	As the sensor component of the NLRP1 inflammasome, plays a crucial role in innate immunity and inflammation

grnc	4,75	up	Progranulin	Progranulin levels are elevated when tissue is inflamed. After wounding, keratinocytes, macrophages and neutrophils increase production of progranulin.
samhd1	4,28	up	Deoxynucleoside triphosphate triphosphohydrolase SAMHD1	Protein that acts both as a host restriction factor involved in defense response to virus and as a regulator of DNA end resection at stalled replication forks
samhd1-like	3,13	up	Deoxynucleoside triphosphate triphosphohydrolase SAMHD1-like	Protein that acts both as a host restriction factor involved in defense response to virus and as a regulator of DNA end resection at stalled replication forks
tnfaip2	1,15	up	Tumor necrosis factor alpha-induced protein 2	May play a role as a mediator of inflammation and angiogenesis
cd209e	-3,84	down	CD209 antigen-like protein E	Putative pathogen-recognition receptor. May mediate the endocytosis of pathogens which are subsequently degraded in lysosomal compartments
p2ry12	1,43	up	P2Y purinoceptor 12	Required for normal platelet aggregation and blood coagulation
ian12	-2,24	down	Immune-associated nucleotide-binding protein 12	Not defined
siglec10	3,18	up	Sialic acid-binding Ig-like lectin 10	Involved in negative regulation of B-cell antigen receptor signaling. Preferentially binds to alpha-2,3- or alpha-2,6-linked sialic acid (by similarity)
cd83	0,86	up	CD83 molecule	The transmembrane domain of membrane-bound CD83 stabilizes MHC II, costimulatory molecules and CD28 in the membrane by antagonizing MARCH-family E3 ubiquitin ligases
nlrp12	-0,66	down	NACHT, LRR and PYD domains-containing protein 12	Plays an essential role as an potent mitigator of inflammation (PubMed:30559449). Primarily expressed in dendritic cells and macrophages, inhibits both canonical and non-canonical NF-kappa-B and ERK activation pathway
vwa7	3,01	up	Von Willebrand factor A domain-containing protein 7-like	Found in the major histocompatibility complex class III region
mx1	1,67	up	Interferon-induced GTP-binding protein Mx-like	Interferon-induced dynamin-like GTPase with antiviral activity against a wide range of RNA viruses and some DNA viruses. Its target viruses include negative-stranded RNA viruses and HBV through binding and inactivation of their ribonucleocapsid.
eotaxin	-1,53	down	Eotaxin	Chemokine subfamily of eosinophil chemotactil proteins
nlrc3	3,12	up	NLR family CARD domain-containing protein 3 - protein NLRC3	Negative regulator of the innate immune response. Attenuates signalling pathways activated by Toll-llike receptors and the DNA sensor STING/TMEM173 in response to pathogen-associated molecular patterns or in response to a DNA virus infection

st6galnac1-like	-3,48	down	Alpha-N-acetylgalactosaminide alpha-2,6-sialyltransferase 1-like	Sialyl-Tn antigens in Mucin type O-glycan biosynthesis pathways
pycard	5,51	up	Apoptosis-associated speck-like protein containing a CARD	Functions as key mediator in apoptosis and inflammation.
lgals3bpa	5,77	up	Galectin-3-binding protein A a	Promotes integrin-mediated cell adhesion. May stimulate host defense against viruses and tumor cells.
lgalsbp3b	5,78	up	Galectin-3-binding protein A b	Promotes integrin-mediated cell adhesion. May stimulate host defense against viruses and tumor cells.
ian12	-2,24	down	Immune-associated nucleotide-binding protein 12	Functionally uncharacterized GTP-binding proteins expressed in vertebrate immune cells and in plant cells during antibacterial responses

Graphical abstract

Highlights

- Different transcriptomic architecture after salinity challenge between tolerant and sensitive tilapiine species
- RNAseq technique reveals expression of pseudogenomes in close species under osmotic stress
- Immune strategy varies in gills of two tilapia species after salinity exposure
- Same physiological pathways triggered by different genes between two species for coping with cell stress induced by salinity