Mar. Sci. Tech. Bull. (2023) 12(2): 212-224
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1266381

Marine Science and Technology Bulletin

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https://prensip.gen.tr/

RESEARCH ARTICLE

In silico analysis and tissue-specific transcription of platyfish (*Xiphophorus maculatus*) catalase gene

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ARTICLE INFO

Article History:
Received: 16.03.2023
Received in revised form: 03.05.2023
Accepted: 12.06.2023
Available online: 20.06.2023

Keywords:
Bioinformatics
Catalase
Gene expression
Platyfish

ABSTRACT

The present study focused on conducting in silico analysis and investigating the tissue-specific distribution and expression of the catalase gene in platyfish (*Xiphophorus maculatus*), which can be used as a model organism for studying stress responses in fish. Assay of the steady-state levels of *cat* gene transcripts by real time PCR revealed. The steady-state level of platyfish *cat* transcript was abundant liver (2162.21) compared with the level of *cat* transcript in intestine (1270.94), heart (1241.25), muscle (419.157), brain (46.205), eye (47.57), swimming bladder (28.99), gills (81.18), spleen (95.45), kidney (20.25) ovary (91.16) and testis (113.22). The results suggest that the liver is the major site of *cat* expression in platyfish, with significantly higher expression levels compared to other tissues. In addition, the research involved using bioinformatics tools to analyze the genetic sequence of the catalase gene and predict its structure and function. The results of the study indicated that the *cat* in Platyfish shares a high sequence identity and similarity with its orthologs in other teleost species, including medaka, fugu, and zebrafish. This observation suggests that the *cat* gene is conserved among these fish species, and the gene's function and regulatory mechanisms are likely to be similar. The high conservation of the *cat* gene among teleost fish species highlights the importance of this gene in the antioxidant defense system and its potential role in responding to environmental stressors. Platyfish *cat* gene exhibits a conserved gene structure, as evidenced by its conserved gene synteny with the orthologous *cat/CAT* genes in other teleost fish and humans. Overall, the study provides evidence for the highly conserved gene structure of the *cat* gene in platyfish, which contributes to its functional stability and the maintenance of its critical role in antioxidant defense and stress response mechanisms.

Please cite this paper as follows:

Çapan, E. C., Arslan, G., & Bayır, M. (2023). In silico analysis and tissue-specific transcription of platyfish (*Xiphophorus maculatus*) catalase gene. *Marine Science and Technology Bulletin*, *12*(2), 212-224. https://doi.org/10.33714/masteb.1266381

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Introduction

The catalase gene is an essential component of the antioxidant defense system, which helps to protect cells against oxidative stress caused by various environmental stressors (Sies, 2017). Catalase is an enzyme that plays a crucial role in protecting cells from oxidative damage by breaking down hydrogen peroxide into water and oxygen (Chance & Maehly, 1955). Many studies have investigated the expression of catalase genes in fish, which can provide insights into the antioxidant defense mechanisms of fish and how they respond to environmental stressors. Zhao et al. (2019) examined the expression of catalase genes in the liver, muscle, and gill tissues of grass carp (*Ctenopharyngodon idella*) under different environmental stressors, including hypoxia, ammonia, and nitrite exposure. The results showed that catalase gene expression was upregulated in all three tissues under hypoxia, while it was downregulated in the liver and gill tissues under ammonia and nitrite exposure. Bopp et al. (2008) investigated the expression of catalase genes in the liver and kidney tissues of rainbow trout (*Oncorhynchus mykiss*) exposed to copper. The researchers found that catalase gene expression was significantly increased in both tissues after exposure to copper, indicating that the fish were using this antioxidant defense mechanism to combat the oxidative stress caused by the copper exposure. Miryaghefi et al. (2016) found that catalase gene expression was upregulated in both tissues when fish were fed a diet with higher levels of vitamin C, suggesting that vitamin C may enhance the antioxidant defense mechanisms of fish. Fish are frequently exposed to such stressors, making them an excellent model organism for studying stress responses in animals (Balasch & Tort, 2019). Platyfish (*Xiphophorus maculatus*) has been extensively used to study stress responses in fish (Heston, 1982) is an omnivorous freshwater fish live from Northern Mexico to Central and South America (Zaret, 1984) and prefers warm waters, canals and slow-flowing watery trenches, alluvial bases and grassy shores (Arthington, 1989). As known, zebrafish and medaka are the most widely used fish species as a model organism (Iwamatsu, 2004; Howe et al., 2013). Platyfish also has an important place in genetic studies such as these two aquatic organisms (Schartl, 2014). Platyfish has been used as a research model in various fields ranging from genomics and genetics, evolution, ecology, to systematic, since the early 1930s (Kang et al., 2013; Schartl et al., 2013). Platyfish is also in an important point for logistical view of all the other evolutionary models. Its genome sequences are made (Schartl et al., 2013) and possible to reach the sequences in the ENSEMBL genome database (https://www.ensembl.org/Xiphophorus_maculatus/Info/Index).

In recent years, there has been a growing interest in investigating the tissue-specific distribution and expression of the antioxidant enzyme genes in fish to better understand its role in stress responses. In this context, a recent study has conducted in silico analysis and examined the tissue-specific distribution of the *cat* gene in platyfish. The study also employed bioinformatics tools to analyze the genetic sequence of the cat gene in platyfish and predict its structure and function. The study's findings highlight the importance of the cat gene in the antioxidant defense system and its potential role in responding to environmental stressors. Overall, the study provides important insights into the tissue-specific distribution and expression of the *cat* gene in platyfish and its highly conserved gene structure. These findings contribute to a better understanding of the cat gene's role in antioxidant defense and stress response mechanisms in fish and could have implications for developing novel approaches for mitigating the impact of environmental stressors on aquatic organisms. Overall, these studies highlight the importance of catalase gene expression in fish and how it can be influenced by environmental stressors and dietary factors. Further research on catalase gene expression in fish may provide valuable insights into the antioxidant defense mechanisms of fish and how they respond to different environmental conditions.

Material and Methods

Husbandry and Dissection of Platyfish (Xiphophorus maculatus)

The study used a total of 6 platyfish (*X. maculatus*) that were obtained from a commercial supplier in Erzurum, Turkey. The fish, weighing approximately 3.2±0.3 g, were transferred to the Laboratory of Agricultural Biotechnology and fed with commercial fish feed. The water temperature of the aquarium system was maintained at 26±0.4°C. To determine the distribution of tissue-specific expression of the catalase gene, six fish (3 females and 3 males) were used. Liver, gill, testis, ovary, intestine, kidney, stomach, eye, heart, muscle, spleen, and brain tissues were collected from fish. The study followed experimental protocols approved by the Atatürk University Local Ethical Committee for Animal Studies. Before dissection, the fish were anaesthetized with clove oil, and all dissecting instruments and the working bench were sterilized and cleaned

with a cleaning agent for RNase (RNase ZAP, Invitrogen™). The samples were transferred to nuclease-free tubes containing 1 ml RNAlater (RNAlater™ Stabilization Solution, Invitrogen) and kept at 4°C overnight. The samples were then stored at -80°C until RNA isolation.

RNA Isolation, Reverse Transcriptase (RT) and Real-time PCR (qPCR) Analysis

Samples were removed from RNA later before homogenization with Trizol reagent (Life Technologies) was used for total RNA extraction. Nanodrop 8000 spectrophotometer was used for determine the RNA concentrations and RNA agarose gel-electrophoresis was used for determine the quality of total RNA. RNA was converted to mRNA into cDNA by employing the High-Capacity cDNA Reverse Transcription Kit (Life Technologies) after DNase treatment (DNase I, Amplification Grade, Life Technologies). A Rotor-Gene 6000 thermal cycler system (Qiagen GmbH, Düsseldorf, Germany) and a QuantiTect SYBR Green PCR kit (Qiagen) were used for RT-qPCR analyses to determining transcription of target (*cat*) and reference (ß-actin (*actb*) and elongation factor 1 alpha (*ef1α*)) (number of copies/µL) in different tissues of platyfish. Quantitative PCR was run in the final volume of 20µl by using 10 µL SYBR Green, 5 µL DNAse/RNAse free 2 µL forward primer, 2 µL reverse primer and 1 µL cDNA template. A tube which doesn't include cDNA was used as a negative control for each qPCR reaction. RT-qPCR conditions were initial denaturation (95.0°C for 15 min), 40 cycles-denaturation (95.0°C for 20 s), primer annealing [optimum temperature for primers for 30 s] and elongation (72.0°C for 30 s). To calculate normalized steady-state levels of platyfish *cat* mRNA transcripts in each tissue, copy number of *cat* mRNA transcripts divided to copy number of two reference genes (*actb* and *ef1α*) and constitutively expressed at approximately the same steady-state levels in all tissues and the mean of both values were taken.

Primer Optimization

The forward and reverse primers were designed using NCBI Primer-BLAST for real time qPCR amplification of platyfish *cat* and reference genes (*actb* and *ef1α*) (Table 1). Exon-exon junction model was used for primers designing for avoid PCR amplification of a product from any contaminating hnRNA or genomic DNA (Keşan et al., 2022). Ordered lyophilized primers diluted in TE buffer (10mM Tris, 1mM EDTA and pH 8.0) in such a way that the stock concentration for each primer was100 pmol/µl.

Quantitative Polymerase Chain Reaction

PCR was performed in the final volume of 25µl using 0.4 µM forward and reverse primer as well as 100 ng template cDNA. Platinum PCR Supermix (Invitrogen, Carlsbad, California, USA) was utilized to run a PCR. The annealing temperature was optimized by taking six different temperatures for the target and reference genes. Agarose gel (1%) electrophoresis was performed to confirm the anticipated size of PCR products and the optimum annealing temperature was determined for each primer set by observing thermal gradients.

Identification and Structural Determination of Platyfish Catalase Gene

Sequence identity and similarity among platyfish, medaka, fugu zebrafish, mouse, rat and human *cat* gene was designed using BIOEDIT Program. For this reason, it was collected the amino acid sequences of *cat* in these organisms from NCBI database. Teleost fish exhibits strict evolutionary conservation for gene structure in same gene family. Platyfish *cat* gene sequence was acquired by performing BLAST (http://www.ensembl.org/Multi/Tools/Blast) searches with identical orthologous zebrafish (*Danio rerio*). Ensembl IDs were determined as ENSXMAG00000025437 and ENSDARG00000104702 for catalase gene in platyfish and zebrafish and NCBI cDNA IDs of platyfish and zebrafish were XM_005815279 and NM_130912, respectively.

Phylogenetic Analysis

CLUSTALW (Thompson et al., 1994) at BioEdit software (http://www.mbio.ncsu.edu/bioedit/page2.html) used for sequence alignment of *cat* gene in platyfish (*X. maculatus*). The protein sequence of platyfish Cat was aligned with *Cat/CAT* protein sequences from platyfish, amazon molly, tilapia, fugu, tetraodon, zebrafish, common carp, goldfish, Atlantic salmon, brown trout, chicken, mouse and human. Human LCP2 was used as an external group for phylogenetic tree (Figure 1).

To determine the phylogenetic relationship of the platyfish and its *cat* gene sequence with other fish species, as well as mouse and chicken, a pairwise alignment was performed using the BLOSUM62 matrix to calculate sequence identity and similarity. A maximum-likelihood tree was constructed using the Poisson correction distance model based on amino acid

Table 1. Sequences of primers used as target and reference genes

Platyfish	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)	Tm (°C)
cat1	TCTGTGGCTGGGGAGTCTG	GCTGAACAGGAACGACACCT	58.3
ß-actin	ACCCAGATCATGTTTGAGACC	ATGTCACGCACGATTTCCCT	55.8
Elongation factor1	ACGTCAAGATGGAGAGAACTCG	GTGAAGTGAACCCAGAGCGA	56.8

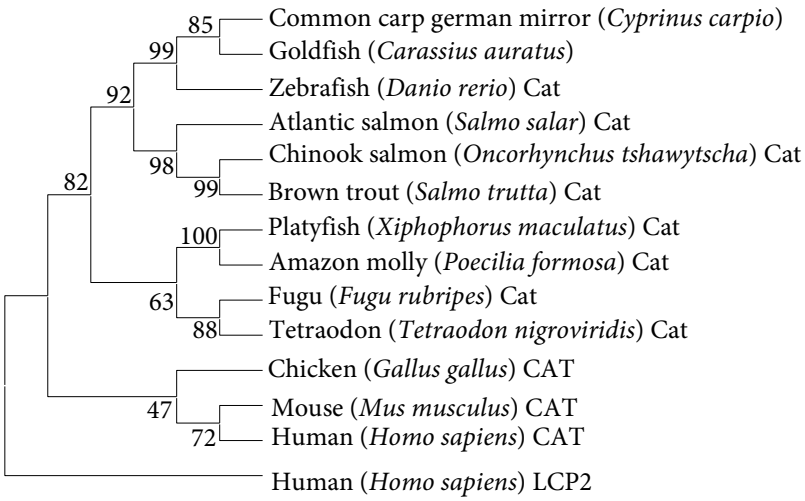

Figure 1. Phylogenetic tree of catalase gene in platyfish

substitution per site, using MEGA11 software. The tree was used to detect the evolutionary relationship of the platyfish with other fish species, mouse, and chicken. To confirm the phylogeny of the cat gene, a bootstrapped neighbor-joining tree was constructed prior to building the maximum-likelihood tree. In addition, the protein sequence of the human lymphocyte cytosolic protein (LCP2) was used as an external group, as done in a previous study (Kell et al., 2018).

Conserved Gene Synteny

To identify co-localized genes and create a conserved gene synteny map for the platyfish *cat* gene and its counterparts in zebrafish and medaka, we manually arranged the gene synteny using the Ensembl database. Specifically, we used the region conceptus selection to select the relevant regions of the genomes and identified the co-localized genes within those regions. By doing so, they were able to generate a conserved gene synteny map that showed the relationship between the *cat* genes of the three species.

Statistical Analysis

In this study, the researchers used SPSS Statistics 17.0 software to perform a one-way analysis of variance (ANOVA) and Duncan's multiple comparison tests. We used these tests to compare the levels of *cat* gene expression platyfish tissues. The experiments were conducted three times, each time in triplicate. The researchers considered a p-value of less than 0.05 to indicate a significant difference between the groups being compared.

RESULTS AND DISCUSSION

Bioinformatics and Computational Analysis of Platyfish Cat Gene

In the bioinformatics section of this study, statistical knowledge was gathered using biological data from Ensembl genomic database, NCBI database, BioEdit software, pairwise alignment of BLOSUM62 matrix program, and MEGA11 program (Tamura et al., 2021). The researchers used the Ensembl genomic database to obtain the longest cDNA sequence, which was used to determine the exon-intron structure of the platyfish *cat* gene. The researchers discovered that these genes have 13 exons and 12 introns, which follow the gt-ag rule. Additionally, the researchers identified putative TATA and CAAT boxes and polyadenylation signal for *cat* gene. Finally, a computational algorithm was tested and evaluated for showing the gene structure (Table 2).

Table 2. Nucleotide Sequence of platyfish (*Xiphophorus maculatus*) catalase gene

ENSXMAG00000025437

```
5'gctataacgttatatatatatatatatatatatatatatatatatatatatatatatata     -300
tatatatatatatatatatatatatatatataccaaggtgtaggttgtgcaaggaggccc     -240
ctgagacagtccagcacataacagcagggtgcaagatactggcagggaaagcgtacatgg     -180
aacgacataaccaagttgcaggcatagtgtacagaaacatctgtgcagAATATggactgg     -121
aaaccccgagatcaaagtgggaaacacccccaaaggtggcggagaacgccagagctaata      -61
+1
TCCTGTGGGCCTTCCAGATCCAGACAGACAAAATGGTAATGGCGAACCAACCAGACATTG       60
TCGTAGTGGATAAACAACAGAGGAAAGCCGTTGTGATAGATGTAGCAATACCAAGCGACT      120
GCAACATCAGGAAAAAGGAGCACGAGAAACTAGAGAAATACCAGGGCCTCAGGGAGGAAC      180
TGGAGAGGGCCTGGAAGGTGAAGACCACAGTGGTGCCTGTGGTCATCGGGGCCCTCGGGG      240
CAGTCACCCCCAAACTGGACCAATGGCTACAACAGATCCCAGGAACAACATCAGACATCT      300
CAGTCCAGAAATGTGCAGTCCTTGGCACAGCCAAGATACTGCGCAGAACCCTCAAGCTCC      360
CAGGCCTCTGGTAGAGGACCCGAGCTCAGAGGATAAGAACCACCCGCGGTGGGTGAGAAG      420
GGAATTTTTTTTTTTTTTTATATATATATATATATATTTTTTTTATTTTTTTTTTTTTTT      480
GTTTCCTCTTCGTCTTTTGATAAATTTACTCGAAGTAGCTAAAACGTTATACAAGGTCAG      540
CTGCAGCGCTTTTTTTTTTCTTTTAAAGACAAATAGGTCGCGTTAGTCCTGTCAGCCAAT      600
CAGAAATGAGGGGCGAAGTAGAGGCGGAGTCACGTGGAACAAAGTCCAGGAAGACTTTCT      660
CTGGCATTGAGGTAAACAGATAACGCTTTTCTCCAAGACACCTACCGTGCAGTTAGTGGT      720
CCCGACGCGCTGTTTTGTCGAGATGGCGGAGACCAGAGATAAAACTACCGACCAGATGAA      780
                      -M--A--E--T--R--D--K--T--T--D--Q--M--K
GATATGGAAGGAGAACCGAGGCTCCCAGgtttgtacaaatgctgcatttttatcaagggt      840
--I--W--K--E--N--R--G--S--Q-
tttgtttgctccgtgtccgacttgcaggaattgggtgacaggtcgtttggggtaacaaga      900
gtcgaagcccgtcggttgttcagtctttgctcgttattaacgtgagctaatctcaaacat      960
gctaacacatgttgtttaaatcagcactccggttttgtagtcgttttgttgcagtaatga     1020
gagaggttgctaatgttttgctagctgaaatttaaacaacgccatcaagcgagggctttc     1080
tcctccaagcattttggtcgcgacattaagaagggagctcctaaaatgtctcccgtgggc     1140
taaaattaagttgaaagacgtgaaagtgggtcgtttttattgcacatggtggaacttgag     1200
cctttagttgcataacaagaccacagttcagtttacattagtgcgctaaactgactgaag     1260
tctacctgatcctgcagctgaatgtgagggtttaacacaattcagctctgaaaggattct     1320
tcttattcaatttgccttattgacgcacttttatttatcacaatatataaactattcatg     1380
tttatgcaaacaaacacatttcatcgggtaaagtcagtgttattacacttaaaatgggtt     1440
tagttaaaccacatttttttcaaaactatcataatatgtgaacaacttagtaagttaatgt     1500
aaaataaagatacatttataaaaaaaacaatgcatttcaaagcacatgatttcttgtgaa     1560
aagtaagaacagagctattgacatgtgtatatatattatgtttttataaactgacatttg     1620
cagggtgttaagaaccagtcaggtcaatttatttccttcgatttacattgtaggttgtga     1680
aaagttttgaccgaaattcatgatatttgatggatgttgtaacttttcaatggttaaaat     1740
aaaggcctgttagttgacatgtgttagtcctttgttttactcaaccacaagatcatattc     1800
gttttcagtataattcaacccttaattttaaaaaaaataacacatatttcagttcaggtg     1860
ttttttctattatcttcacatgcatcaaatcaatgttcatgtcacaaacctacacagttt     1920
ttttgtaaaaaaaaaaaaaagtataaatatgcttcctatatcttgacgtctgatccagta     1980
tgttatgagtgtagaaagctaactagctgtgctcatcttgaagacggggctacaccgtat     2040
tcttaaccaaatattagcctaatatttatgttcacactcgcctcctatcatgccggtttt     2100
attggcccattactcttctttttatttcgtgaaggaagtcggacgacgacaaagctgaaa     2160
ctgtcctttcagacaatttttggacctttctgtgaggtgtgaataaaaatgtcaggaata     2200
tagttgtttttttttgccctccatagacgggtaaataaacatgctgccttttctgttttc     2280
cttcttgcccaatctaggtctgtgggtgatcaacagactctcgttggattgattaataca     2340
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agtccacttcttttatgctttttttttttatagttttacctgctctgctttttaagttcat     2460
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taaaatgaaagactgtctgaaattaattggaaaatgtacaagttggccttagacacgtta     2580
atcacaggtcttaaactttgttctggcaggattattccatgtagttcattgttctcccgg     2640
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taccgctaactagctgctaataaacactagctagctagctttttttgtgttcatcattgat     2760
aatggcaaatttatcggcagttggctggatgaccttcatcattgccactggctcacttct     2820
gctaccaattcgtacgatgcaatgtgcgttctgttcaaaagaatgaaataaaaatctgga     2880
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agtcttaaatttgagttggtgaaaccggcagagatcgtgtttacttctgttgtctttgtt     3000
cagaggaatccaaatcctctcttctcatccaaagaaggttttgacattaaactgaattta     3060
aatgctattcatattgttgtgttaataaacatgtaaagttaggggtgtagttgttagcca     3120
```

Table 2. continued

gggtaatatgtattaagaatttttcagtcggctttccattgggttactctttgcttgtca	3180
cctcatgagaagcacttttaataagaataagtatgaatggtttgagcccaaacttttaat	3240
atatatttttttaaattcttgaactgcatttggttccag**AGACCAGATGTGCTAACGAC**	3300
-R--P--D--V--L--T--T	
AGGAGGTGGCCATCCCGTTGGGGACAAGCTCAACCTGCAGACTGCGGGGCCGAGGGGCCC	3360
--G--G--G--H--P--V--G--D--K--L--N--L--Q--T--A--G--P--R--G--P	
TCTGCTCGTGCAGGATGTGGTCTTCACCGATGAGATGGCCCACTTCGACCGGGAGCGAAT	3420
--L--L--V--Q--D--V--V--F--T--D--E--M--A--H--F--D--R--E--R--I	
CCCAGAGAGAGTGGTGCACGCTAAAGGCGCAGgttagctaacggttgtcttacagctcat	3480
--P--E--R--V--V--H--A--K--G--A--	
gtgttttggactcaaggtctcattttgctttgataaacaagtttcacgcaataaattcaa	3540
cccctccagacagcaagagttaagtttttattgtagaaactgcttttcgtaaataataat	3600
ttaacttttaccgtacgtgtgtaatttaatttctgagctctttttgttcaaaggaacgt	3720
tctagaagctttagaacgggacaatttttttccacatccagactttaaacaaacagcaagc	3780
cctcctgaaaagctgctacgttgttctcaatgctgatacgctgaaacattcaacagcatc	3840
gatgtaaaggtgattttgtatttttcttcccatgatgcag**GCGCGTTCGGCTACTTTGAG**	3900
G--A--F--G--Y--F--E-	
GTCACTCACGACATCACCCGCTACTGCAAGGCCAAGGTGTTTGAACATGTTGGGAAAACG	3960
-V--T--H--D--I--T--R--Y--C--K--A--K--V--F--E--H--V--G--K--T-	
ACTCCCATCGCTGTCCGGTTCTCCTCTGTGGgtgagagattcactttcatttccaaggaa	4020
-T--P--I--A--V--R--F--S--S--V--	
cattctcactctttgtcgtcgtaggaatagctgtgccatgtttgtgcgcaataatttaat	4080
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gccgcagtgttgcaatatgtgtatttttttatttaaccatgatgttcacacgcctctact	4260
cctgcagcacccatattctgttctttttatgatggcagagggcgctgttgtgcaactttta	4320
atatgtggctttgttgcttctgttactgctgtagaaaatataaggtctttgtatgatgaa	4380
gctgcacacaacaaactgaatattataaacgacgattaaaatgcttgtgcgctcag**CTGG**	4440
A--G	
GGAGTCTGGATCAGCCGACACTGTCCGAGACCCCCGAGGCTTCGCTGTAAAGTTTTACAC	4500
--E--S--G--S--A--D--T--V--R--D--P--R--G--F--A--V--K--F--Y--T	
CGAGGAGGGGAACTGGGACCTGACGGGCAACAACACCCCCATCTTCTTCATCAGGGACGC	4560
--E--E--G--N--W--D--L--T--G--N--N--T--P--I--F--F--I--R--D--A	
AATGCTTgtaagttggcgtttaaaatttggagttcaaccactttatttgttattttatcg	4620
--M--L-	
gtgctgtttgttttctcttcttccgccctgcag**TTTCCGTCCTTCATTCATTCCCAGAAG**	4680
-F--P--S--F--I--H--S--Q--K-	
CGTAACCCTCAAACCCACATGAAGGATCCTGACATGGTGTGGGACTTCTGGAGCCTGAGG	4740
-R--N--P--Q--T--H--M--K--D--P--D--M--V--W--D--F--W--S--L--R-	
CCTGAGAGTCTGCATCAGgtagccgcggatattcaacctttagcacttccaggaactaaa	4800
-P--E--S--L--H--Q-	
ataaacacatgttctaaaatgatttggtaatggggaacttgcaaaccacagtcgctcccg	4860
ctgatgaacatttttatctgttttatttgtcagtatctactttaacacgctctttttcta	4920
ccttctgtctatgaagacaatctgtttgtcacagattgtctgaaaccctccacacatcca	4980
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gtcagtgatcaaagctaatttttcttactataatcaaatgaagcatctccatttgatttaa	5760
aatccatttgacagcaataacagtgataaaacatatttttttttgtgtaaataaactcggg	5820
taaacctttttttttttttctttttccctctcag**GTGTCGTTCCTGTTCAGCGACCGTGGC**	5880
-V--S--F--L--F--S--D--R--G-	
CTGCCTGACGGCCACCGCCACATGAACGGCTACGGTTCCCACACCTTCAAGCTGGTCAAC	5940

Table 2. continued

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-L--P--D--G--H--R--H--M--N--G--Y--G--S--H--T--F--K--L--V--N-
GCTGACGGCGAATGCATCTACTGCAAGTTCCATTACAAGgtctgtttctgaaactggttt      6000
-A--D--G--E--C--I--Y--C--K--F--H--Y--K-
gttggtttgcagagctctgcgatggattttatttatttatttattttctctaaagACTG      6060
                                                        -T--
ATCAAGGAATAAAAAACCTGTCTGTGGAGGAGGCGGAGCGCCTCGCAGCCACCAACCCAG      6120
D--Q--G--I--K--N--L--S--V--E--E--A--E--R--L--A--A--T--N--P--
ATTACAGCATCGGAGACCTGTTCAACGCCATCGCTAACGGCGACTTTCCGTCCTGGACCT      6180
D--Y--S--I--G--D--L--F--N--A--I--A--N--G--D--F--P--S--W--T--
TTTACATCCAAGTCATGACCTTTGACCAGGCGGAGAGGTTCCAGTTCAACCCCTTTGATG      6240
F--Y--I--Q--V--M--T--F--D--Q--A--E--R--F--Q--F--N--P--F--D--
TCACCAAGgtacggagtcccaacaaagtgaaacggctgactatcccgccggtttgaactt      6300
V--T--K-
ttggtaaaaagctaaactctgggttcttcgatgccagcaaagtgttactctagtagagga      6360
gaacctttaatcaaaccgcctccttcaatatctctcttgaatgcattcgtctcattttc      6420
aatctgcaatatctaaatctggggctacttttccaacgctcccagGTGTGGTCGCATAA      6480
                                                -V--W--S--H--K
GGAGTTCCCTCTGATCCCTGTGGGCAAACTGGTCCTCAACCGGAACCCGGTCAACTACTT      6540
--E--F--P--L--I--P--V--G--K--L--V--L--N--R--N--P--V--N--Y--F
CGCAGAGGTGGAGCAGCTGGCCTTTGACCCCAGCAACATGCCTCCAGGCATCGAGGCCAG      6600
--A--E--V--E--Q--L--A--F--D--P--S--N--M--P--P--G--I--E--A--S
CCCCGACAAGATGCTGCAGgtgacgcagtaaaccgaatcagctgagaaaggagtgttgaa      6660
--P--D--K--M--L--Q-
atttaccagaggttaacctcgtccggctcggcgcagGGTCGGCTCTTCTCCTACCCAGAC      6720
                                    -G--R--L--F--S--Y--P--D-
ACGCATCGCCATCGCCTGGGAGCCAACTACCTGCAGATCCCGGTCAACTGTCCCTTCAGG      6780
-T--H--R--H--R--L--G--A--N--Y--L--Q--I--P--V--N--C--P--F--R-
GCCCGCGTGGCCAACTACCAACGAGACGGCCCCATGTGCATGTCTGATAACCAGGgtgag      6840
-A--R--V--A--N--Y--Q--R--D--G--P--M--C--M--S--D--N--Q--
ttcttcacctgctgctcagccttaagtctttaagtcttaaattcatgtctaaaattaagg      6900
ccttaaaagtaatgtgttaatcgcaggtcttaaattttatcttgacacgtctgtttattc      6960
atagattatatatttatttaatttttttgttgttttttttgctggaaatttctgtccgg      7020
caaaacgttgagggttctgccacccactgctaacataccgtcgctaaataactagctagc      7080
ttttttgcatcctgggtaaatgctcatttatcaccagttggctggacaaagtttgcaag      7140
tttttacggagatataggtattaaatttaattcttaacagtcttattaatacttacattt      7200
gaattggtaaaacatgcagaaataattattaattattaattattattattaatattattg      7260
cacttatgctgtctggttcacgatcagtgaggtcaattaaagctgtaattctacattttg      7320
caaatttgtaacaccttaaaactaaactgtcatcattttataacaaaggtcagtttgctc      7380
ccagaaattgtggcgctaaattttcagggttatgacagagctgtaagaattaaaagtatg      7440
aacaacaagaataagtacaaataaataaagttagtctcttatgtattcacaaatataaaa      7500
aaattatctttggttagtccaaggggaaaaaacaacgtcctactatacatttggtttatg      7560
gacactatgactttaaaatgaccagaattggacatgtttatgtataataaatgtataaat      7620
catatatttacccatgtgattaaaacatgtttgtgttgatgattaatcaactgatgtgaa      7680
tttcttcacaccttcttctgtctttgtcccttctgatgtttatatgctcaatccaatatc      7740
atttttttttaaaaatctgcctcttaaactcaacaagttcttgcttcgtacgacctctagG      7800
                                                            G
TGGCGCTCCAAACTACTACCCCAACAGCTTCAGCGCCCCTCAGAACCAGCCTCGCTTCGT      7860
--G--A--P--N--Y--Y--P--N--S--F--S--A--P--Q--N--Q--P--R--F--V
GGAGACCAGGTTCGGCGTTTCGCCCGACGTCGCCCGTTACAACAGCGAAGACGAAGACAA      7920
--E--T--R--F--G--V--S--P--D--V--A--R--Y--N--S--E--D--E--D--N
CGTGACGCAGgtgacgatgacgccgccgcctctaatagcctctctgtggctgtttgccag      7980
--V--T--Q-
tcgcacacaggaagtcgatgttttcagcctgttgaattcaatgtagaaaatggtctaaat      8040
atatccttcgaaggccacaccagtaaaacaataaacagttacgtggaatcactatcagag      8100
tataacagagttgtcatgcatctaaaaatgttggcacatccgccgtccttgtagcttcat      8160
gtacgtttggtaacttgaaactggagcgcaatggaaacttaacaattagaccaaatcagc      8220
ttcttctcatcaacaacgctcactccatctctcatgacgacacttttaatgcaaagttgc      8280
attaaaagtgtcattatttaccaaatgacactacgtgacatcagtcatgaacattcataa      8340
agactctttcatgttcatgacgtcatttttacctttaaataaaagtgttaccagaataag      8400
tttcaacccctctgtgaaataaacgaccttcccagtaaggttgctgaggtaagtgcagag      8460
aagtgattggttctgtaggactgtgtttaccgtgtctggttctgaccgggtccgtgtgcg      8520
gccggctgcagGTCCGGGCCTTCTACACCCAGGTGCTGAACGAGGACGAGCGCCAGCGGC      8580
```

Table 2. continued

```
          -V--R--A--F--Y--T--Q--V--L--N--E--D--E--R--Q--R--
TGTGCCAGAACCTTGCCGGGTTCCTGAAGGGAGCCCAGCTCTTCATCCAGAAACGAATGg      8640
L--C--Q--N--L--A--G--F--L--K--G--A--Q--L--F--I--Q--K--R--M-
tgagttcccgtcagactccatgcggagccaaagatgctttctgtcgtgaagatgagtcac      8700
tgcggagggcatttaaaagaaggaaaaacattgttcctctctttaatccacttcaaccac      8760
agggtgctgctgcatgaaactaaacatgcacaccggcctgctttgtggtaatgtggttgc      8820
tacggaaacaaaacatttttaaacaaggaagaatgatggtgaacagagcccaacgtttaa      8880
agcaacacaatctgtatttttttttttttttttaagttttctgctttcaagttctgtaagg      8940
ctttgactaggacatttaaaatgttattttagtaattcaaccacattgtgcaaaagtgaa      9000
tccaaatctttgacattttaagattcaagctattattccttgtaaatttagataattatg      9060
aagtacagataatgaaatcctgacattcagtgtcaaaaataaatgagattatttacataa      9120
caaccattaaaaaccagcatttttaatgtgtgaatgttgtgtaatgcaccatgtcatggc      9180
ccgagttgaacaccagaacggatctatgaggaagatgaggaacgtcaacaacggacactt      9240
tcaaaacaacttgtgtttcagtttcctctcggctgacagcctccgtgccacgccgcatcg      9300
acgcagttattcatgctaaacaagcctcaaccaatcactgagtgcactgaaataaattct      9360
cacatttctgtttaaaatatccttttttttgtaatatatactatatgttctaattgtctg      9420
aaatacagaattgtggcttactgtgctccaatatttggtttattttaagtcatgattgtg      9480
aaccttcagatgttgcgcagtttagaagaactccctctgaaagtgggaaccttaaatgac      9540
atttatttttcttctcagttgttttcctgagcgtctttttctcgctttgtctccaccgg      9600
tgtgactcgccaccgcaaagttaatttttaaacatttgaatgagaaataaaaggtgatgt      9660
catttatcgatgtcacagcagccacatataccgctctggtttcctgttttatggaggaaa      9720
aaaaaacaaattcctgtttgcacgtgttgtaaatctgctgccagagttgtaatttgcgca      9780
acctttgacttttgctagtctcgcatttttgtgtgcatcgctttaaaaacaggaaac      9840
ctgaatatagatgcatttattcaatgttcttcctgcagGTGGAAAACCTGAAGGCCGTCC      9900
                                      -V--E--N--L--K--A--V--
ATCCAGACTACGGGAACCGGGTCCAGACCTTACTCAACAAGTACAACGCCGAAGCCAAGA      9960
H--P--D--Y--G--N--R--V--Q--T--L--L--N--K--Y--N--A--E--A--K--
AGgtaaccaaacagttggtttgttatcgcggcgtaaaaaagttcagttaacggaaacctg      10020
K-
ctcgtttcctgatgcgctcatttcccgtctgcgtcccagAACACGAACGTCCGCGTCTAC      10080
                                        -N--T--N--V--R--V--Y-
GGCCGTCCAGGAGCCGCCGCCATCGCCGCGTCCTCCAAGATGTGAtcagcggatcggctc      10140
-G--R--P--G--A--A--A--I--A--A--S--S--K--M--*-
acatgaccaccgactggttccaccagcagctggaccagcctcagcggcctaattactgtc      10200
gccgtagtttgctcgagcttggttttggttcattgcaaacagtcaagccttacttttta      10260
attaattatcaaaaattaattttattgcattgaatgctgtaaggtaacactgttgagatt      10320
aggctgcagttttattaaattacctttaatacatcgatgccttattactgtttcagttaa      10380
attcagttaaccaacaaaaactgatgaacctttttctctaatgttgtaatactggaagatg      10440
tttgtagcgactcaagcaaagttaattttgaaagaacatctgtttgaaacgatgctgatc      10500
tttgtgcctggctcgtaccgcaacgcaatcgatcgcttggtaaagcttctggttttgctg      10560
aggacattattaacgtctgggttgtttaactcggacttgactgaatcacttactgttgaa      10620
tgttaatatagaaaagtattatcctgttttcaaatAATAAAAtcattcaaaatgtt 3'      10680
```

Note: ** Platyfish (*Xiphophorus maculatus*) catalase (*cat*) gene. The exons of the catalase gene are shown in capital letters and the nucleotide positions are numbered at the end of each line. The starting site of transcription is +1, 5 'upstream sequence, 3' downstream sequence and introns are shown in lower case. The TATA box and the poly adenylation signal (AATAAAA) are shown in upper case letters and painted in yellow. Stop codon (TGA) is specified asterisk. The used forward primer is shown in yellow and the reverse primer is shown in red.

Sequence identity and similarity among platyfish, medaka, fugu, zebrafish, mouse, rat, and human *cat/CAT* gene was given in Table 3. The highest identity and similarity rates for platyfish *cat* gene was determined with its orthologs which are medaka (89 and 96%), fugu (87 and 95%) and zebrafish (85 and 93%). However, the study revealed that platyfish has the same identity and similarity rates with mouse, rat, and human (79% and 87%, respectively). The high sequence identity and similarity rates of platyfish *cat* gene with its orthologs in medaka, fugu, and zebrafish suggest a conservation of the *cat* gene among these teleost species. This finding is consistent with previous studies that have reported a high degree of conservation of the catalase gene in vertebrates (Gotoh, 2012; Pan et al., 2022). The high conservation of catalase gene sequence among different species may be due to the importance of this gene in protecting cells from oxidative stress. It is interesting to note that despite the high sequence identity and similarity rates between platyfish and mammalian *cat/CAT* genes, the response of these genes to

Table 3. The rate of sequence identity and similarity between platyfish (Pf) *cat* gene and the other vertebrates such as medaka (Me), fugu (Fu), zebrafish (Zf), mouse (Mo), rat (Ra) and human (Hu) *CAT* gene

```
Pf Cat   1   MAETRDKTTDQMKIWKENRGSQRPDVLTTGGGHPVGDKLNLQTAGPRGPLLVQDVVFTDE
Me Cat   1   ...N.........T.......RK.......A...I.........................
Fu Cat   1   ..DK...A.....L...S..Y....I........I...........K............
Zf Cat   1   ..DD.E.S.....L...G............A.V.I.....AM..................
Mo CAT   1   .SDS..PAS....Q...Q.A............N.I.....IM...S..............
Ra CAT   1   ..DS..PAS....Q...Q.AP.K.........N.I.....IM..................
Hu CAT   1   ..DS..PAS...QH...Q.AA.KA......A.N.......VI.V...............

Pf Cat  61   MAHFDRERIPERVVHAKGAGAFGYFEVTHDITRYCKAKVFEHVGKTTPIAVRFSSVAGES
Me Cat  61   ..........................................I...........T.....
Fu Cat  61   .......................................L..............T.G...
Zf Cat  61   ................................S.......I...........T....A
Mo CAT  61   ................................S.......I..R........T.T...
Ra CAT  61   ................................S.......I..R........T.....
Hu CAT  61   ................................K.S.......I..K........T.....

Pf Cat 121 GSADTVRDPRGFAVKFYTEEGNWDLTGNNTPIFFIRDAMLFPSFIHSQKRNPQTHMKDPD
Me Cat 121 ..................D............V......L.....V.T.............
Fu Cat 121 ..................................L.......T.............
Zf Cat 121 ..S..............D..................TL................L....
Mo CAT 121 ..................D.....V............I................L....
Ra CAT 121 ..................D.....V.............................L....
Hu CAT 121 ..................D.....V............PI................L....

Pf Cat 181 MVWDFWSLRPESLHQVSFLFSDRGLPDGHRHMNGYGSHTFKLVNADGECIYCKFHYKTDQ
Me Cat 181 ..........C................Y..................DRV..........
Fu Cat 181 ..................M.........Y................K...V.....F....
Zf Cat 181 .......................I...Y................Q.QPV........N.
Mo CAT 181 .......................I.......................AV..........
Ra CAT 181 ........C.......T.......I...................N..AV..........
Hu CAT 181 .......................I...................N..AV..........

Pf Cat  241 GIKNLSVEEAERLAATNPDYSIGDLFNAIANGDFPSWTFYIQVMTFDQAERFQFNPFDVT
Me Cat  241 ..........H..S.....A...........NY............E...K.......L.
Fu Cat  241 ..........G...SA....A...........NY............E...K.H.....L.
Zf Cat  241 ....IP....D.....D.....R..Y......N............E...NWKW....L.
Mo CAT  241 .....P.G..G...QED...GLR.........NY............KE..T.P.....L.
Ra CAT  241 .....P....G...QED...GLR.......S.NY............KE..T.P.....L.
Hu CAT  241 ........D.A..SQED...G.R.......T.KY............N...T.P.....L.

Pf Cat  301 KVWSHKEFPLIPVGKLVLNRNPVNYFAEVEQLAFDPSNMPPGIEASPDKMLQGRLFSYPD
Me Cat  301 ..............RM.............................P...............
Fu Cat  301 .......Y.......M...........Q...M.H..........P...............
Zf Cat  301 ..............RF.............................P...............
Mo CAT  301 ...P..DY...........K...........M............P...........A...
Ra CAT  301 ...P..DY.............A........M............P...........A...
Hu CAT  301 ...P..DY.......................I.............................A...

Pf Cat  361 THRHRLGANYLQIPVNCPFRARVANYQRDGPMCMSDNQGGAPNYYPNSFSAPQNQPRFVE
Me Cat  361 ...................T............F................ET..Q...
Fu Cat  361 .................Y..............................EI..QC..
Zf Cat  361 ............L.....Y.T............H................DV....L.
Mo CAT  361 .......P.........Y.............H................EQ.RSAL.
Ra CAT  361 .......P.........Y.............H................EQ.GSAL.
Hu CAT  361 .......P...H.....Y.............Q............G..EQ..SAL.
```

Table 3. continued

```
Pf Cat  421 TRFGVSPDVARYNSEDEDNVTQVRAFYTQVLNEDERQRLCQNLAGFLKGAQLFIQKRMVE
Me Cat  421 SK.Q.........A........CT.F.K............M..S..E...........
Fu Cat  421 SK.K.Y........S.........T...E...DE......E.F..S.............
Zf Cat  421 SKCK..........A.D.......T.F......A..E.....M..H............Q
Mo CAT  421 HSVQCAV..K.F..AN........T...K....E..K...E.I..H..D.......KA.K
Ra CAT  421 HHSQC.A..K.F..AN........T...K....E..K...E.I.NH..D......RKA.K
Hu CAT  421 HSIQY.GE.R.F.TAND..........VN....EQ.K...E.I..H..D..I....KA.K

                                                                Identity Similarity
                                                                    (%)      (%)
Pf Cat 481 NLKAVHPDYGNRVQTLLNKYNAEAKKNTNVRVYGRPGAAAIAASSKM-    100      100
Me Cat 481 ..................D....Q...SAS.H..N....S........-     89       96
Fu Cat 481 ....I....AS...IF.D...E..E..AH....T....S.V......-     87       95
Zf Cat 481 ..M...S.......A..D.H...G....-.H..S.G..S.V..A...-     85       93
Mo CAT 481 .FTD......A.I.A..D.....KP..A-IHT.TQA.SHMA.KGKANL     79       87
Ra CAT 481 .FTD......A...A..DQ..SQKP..A-IHT.VQA.SHIA.KGKANL     79       87
Hu CAT 481 .FTE......SHI.A..D.....KP..A-IHTFVQS.SHLA.REKANL     79       87
```

cold stress may be different. This could be due to differences in the regulatory mechanisms controlling the expression of the catalase gene in different species, as well as differences in the physiological responses of fish and mammals to cold stress. Further studies are needed to investigate the functional implications of the observed sequence conservation of the catalase gene in different species. Overall, the results of this study suggest that platyfish can be a useful model organism for studying the effects of cold stress on the expression and activity of the catalase gene. The high sequence identity and similarity rates of the platyfish catalase gene with its orthologs in other teleost species and mammals provide a basis for comparative studies aimed at understanding the evolutionary and functional aspects of this important gene.

Phylogenetic Analysis

It was used identity and similarity data to construct a phylogenetic tree (Figure 1). The analysis revealed a strong clustering of the *cat* gene in platyfish and its orthologs, indicating a close evolutionary relationship. The tree also showed the phylogenetic relationships between the *cat* sequence in platyfish and *cat* sequences from other vertebrates. We used the Maximum Likelihood method (Felsenstein, 1981) to generate the tree. Accession numbers of the sequences used for phylogenetic tree are the following: Platyfish (Cat: XP_005815336, Amazon molly Cat XP_007546846, Fugu Cat ENSTRUT00000041456, Tetraodon Cat ENSTNIT00000008553, Zebrafish Cat: NP_570987, Atlantic salmon Cat:106564824, Chinook salmon Cat: 112218377, Brown trout Cat: 115197208, Common carp German mirror Cat: 30068, Goldfish Cat: 113066695, Chicken CAT: NP_001026386, Mouse CAT: NP_033934, Human CAT: NP_001743.1, Human LCP2: NP_005556.

The phylogenetic analysis conducted in this study revealed a close evolutionary relationship between the *cat* gene in platyfish and its orthologs in other teleost species, particularly in the amazon molly. The high degree of sequence identity and similarity among the *cat/CAT* genes in different species suggests that these genes have been conserved throughout evolution, indicating their importance in cellular metabolism and protection against oxidative stress. The results of the phylogenetic analysis are also in agreement with previous studies that have suggested a close relationship between platyfish and other teleost species such as medaka, fugu, and zebrafish based on their genome sequences (Schartl et al., 2013). The close phylogenetic relationship between these species suggests that they may share similar physiological and molecular responses to environmental stressors such as cold temperature. Overall, the findings of this study provide new insights into the evolution and function of the *cat* gene in platyfish and other teleost species. Further research is needed to better understand the role of this gene in cellular metabolism and stress responses in fish.

Molecular Studies

Catalase *(cat)* transcription levels of the different tissues of platyfish were determined by RT-qPCR. The highest level of *cat* gene transcription in platyfish was found in liver (2162.21). Transcription of the intestine (1270.94) and heart (1241.25) tissues were found significantly lower than liver although transcription was not significantly different from each other. However intestine (1270.94) and heart (1241.25) tissues

transcriptions were found higher than the all other tissues (muscle (419.157), brain (46.205), eye (47.57), swim bladder (28.99), gill (81.18), spleen (95.45), kidney (20.25), ovarium (91.16), testis (113.22). Although there was no difference in the levels of *cat* gene transcripts between male and female platyfish tissues, it was observed that *cat* gene was highly expressed in liver, intestine and heart tissues (Figure 2).

Conserved Gene Synteny

We observed that the *cat* gene of platyfish showed conserved gene synteny with the orthologous *cat/CAT* genes of other teleost fishes and humans (Figure 3). Specifically, we found that the syntenic genes of the platyfish *cat* gene, located on chromosome 2, had conserved gene synteny with the *cat* genes of zebrafish (located on chromosome 25) and medaka (located on chromosome 6). This suggests that the *cat* gene in platyfish has a highly conserved gene structure. It was also investigated whether the *cat* gene was duplicated in other teleost fish, but did not detect any duplicate copies in the Ensembl database, except for pike (*Esox lucius*) and some cichlid fish (*Zebra mbuna*, *Maylandia zebra, Neolamprologus brichardi, Astatotilapia calliptera*). These findings suggest that

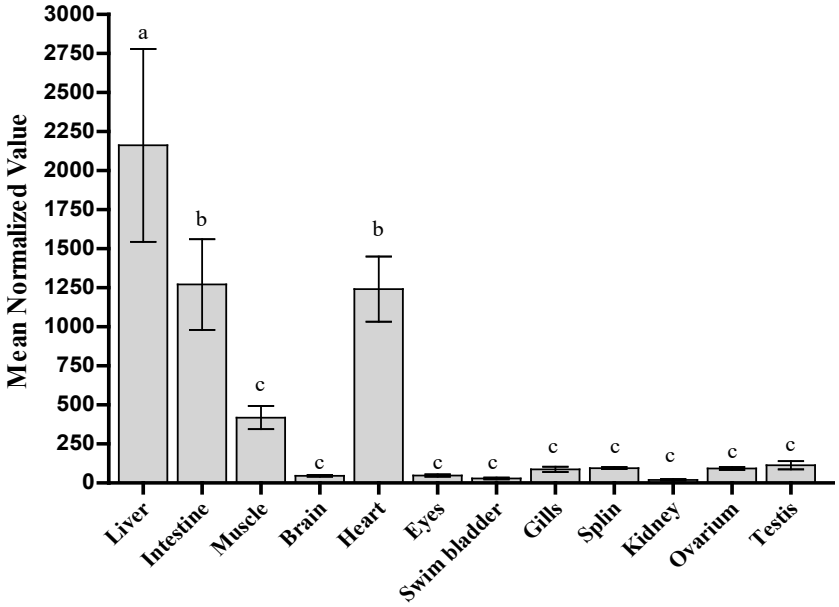

Figure 2. Tissue-specific distribution of catalase transcripts in platyfish

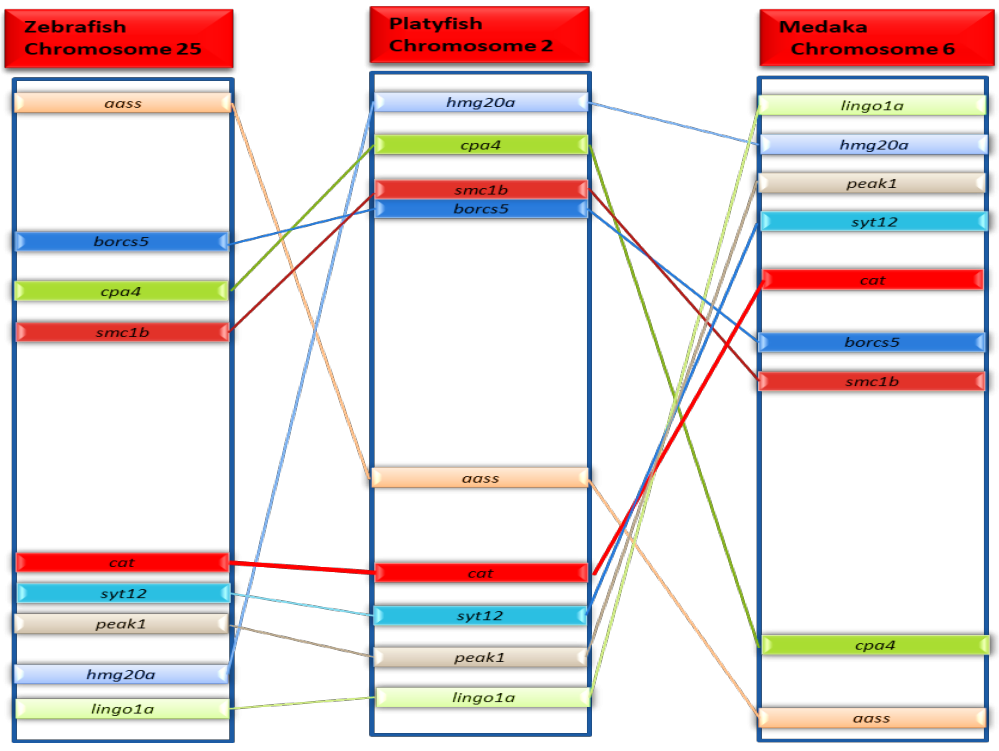

Figure 3. Conserved gene synteny of catalase gene in platyfish (*Xiphophorus maculatus*)

the loss of a copy of the duplicated *cat* gene is a common occurrence in teleost fish following the teleost-specific whole genome duplication. Overall, these results demonstrate the highly conserved gene structure of the *cat* gene in platyfish. The fact that no duplicate copies of the *cat* gene were detected in the Ensembl database for most teleost fish species, except for a few exceptional cases, suggests that the loss of one copy of the duplicated gene is a common occurrence following the teleost-specific whole genome duplication. This may be due to the fact that duplicate genes can sometimes be non-functional or even detrimental to the organism, leading to their loss over time. There are numerous studies that have investigated the evolution and function of gene duplication events in various organisms, including teleost fish. For example, a study explored the evolution of gene families in teleost fish following the whole-genome duplication event, and found that gene loss and subfunctionalization were common outcomes of this process (Volff, 2005). Another study investigated the evolution of the cytochrome P450 gene family in teleost fish, and found evidence for multiple gene duplication events followed by gene loss and functional diversification (Gesto et al., 2018). These and other studies highlight the importance of understanding the evolutionary dynamics of gene duplication events and their impact on gene function and diversity. Overall, the researchers' findings have important implications for our understanding of the evolution and function of the *cat* gene and other genes in teleost fish. They also underscore the importance of considering gene synteny and duplication events when studying the evolution and function of genes and genomes. By considering gene synteny and other genomic features, researchers can gain insights into the mechanisms and outcomes of gene duplication events in various organisms.

Conclusion

The identification and characterization of stress genes, such as the *cat* in platyfish, can provide important genetic markers for improving stress tolerance in aquaculture and serve as a model for studying stress response in other vertebrates, including humans. In summary, the study found that the cat gene was highly expressed in the liver, intestine, and heart tissues of platyfish, with the liver showing the highest level of expression.

Acknowledgements

This manuscript is produced from Esra Can Çapan's master thesis.

Compliance With Ethical Standards

Authors' Contributions

This manuscript is produced from Esra Can Çapan's master thesis.

ECÇ: Literature review, Drafting, Writing, Laboratory experiments

GA: Data analysis and management

MB: Conceptualization, Drafting, Writing, Review, Editing, Supervision

All authors have reviewed and approved the final version of the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

This study was conducted in 2019, did not receive support from any project and did not require ethics committee approval.

Data Availability Statements

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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