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RESEARCH ARTICLE

Bioinformatics studies and examining the tissue distribution of glutathione reductase and glucose-6-phosphate dehydrogenase genes to investigate gender differences in differences in stress tolerance in zebrafish (*Danio rerio*)

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ABSTRACT

The study aimed to investigate the bioinformatics of zebrafish glutathione reductase (*gsr*) and glucose-6-phosphate dehydrogenase (*g6pd*) genes, as well as their tissue-specific distribution. To achieve this, samples of various tissues were taken from female and male zebrafish and total RNA was extracted to obtain cDNA. qPCR was performed to determine the transcripts of *gsr* and *g6pd* genes. The structure of the genes, conserved gene maps, and phylogenetic tree were also designed. The results showed that the liver was the most dominant tissue for both *gsr* and *g6pd* genes in both female and male zebrafish. The expression of *gsr* gene was significantly higher in male zebrafish's liver, intestine, heart, eye, gills, and gonad tissues compared to female fish, while *g6pd* gene transcription was found to be significantly higher in the male liver, intestine, muscle, brain, eye, gill, kidney, stomach, and gonad tissues. Overall, this study provides valuable insights into the bioinformatics of *gsr* and *g6pd* genes in zebrafish and their tissue-specific distribution, which could help in understanding their roles in various physiological and pathological processes in zebrafish and other related species.

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Introduction

Bioinformatics studies deal with topics such as protein structures and functions, enzyme activities and pathways, and are used to analyze genomic data (Kumar et al., 2008). Genetic data, protein structures and functions, enzyme activities and pathways related to glutathione reductase (*gsr*) and glucose-6-phosphate dehydrogenase (*g6pd*) enzyme genes can be investigated with bioinformatics analysis. The *gsr* and *g6pd* are important enzymes involved in maintaining cellular redox balance and energy metabolism, respectively (Li et al., 2020). Dysregulation of these enzymes can lead to various pathological conditions, including cancer, neurodegenerative diseases, and diabetes (Traverso et al., 2013). In fish, both *gsr* and *g6pd* have been studied extensively. For example, a study on *gsr* gene in rainbow trout showed that its expression was induced by oxidative stress caused by exposure to pollutants (García-Meilán et al., 2022). Another study on zebrafish found that *gsr* gene expression was higher in liver tissues of fish exposed to high levels of heavy metals (Xu et al., 2020). As for *g6pd* gene, a study on Nile tilapia (*Oreochromis niloticus*) showed that its expression was significantly higher in liver and muscle tissues of fish exposed to hypoxia (Osman, 2012). Since mitochondria are the primary source of reactive oxygen species (ROS) production in cells, the first release of ROS after exposure to stress in organisms can lead to longer and more extensive ROS release, which can cause long-term negative effects in the organism (Zorov et al., 2014). Therefore, mitochondrial function appears to be a key mechanism that emphasizes phenotypic variation between sexes, and mitonuclear interactions may be critical determinants of the life histories of organisms (Wolff et al., 2014). Consequently, organisms have developed a series of antioxidant defense systems that play a critical role in maintaining cellular function and eliminating the toxic effects of ROS in the face of a threat caused by oxidative stress (Dowling & Simmons, 2009; Hill, 2014; Espinosa-Diez et al., 2015).

Zebrafish (*Danio rerio*) has emerged as an important model organism in biomedical research due to its ease of maintenance, rapid development, and similarity to humans in terms of genetic and physiological characteristics (Kari et al., 2007). Zebrafish has become a popular model organism due to its numerous advantages, such as transparent embryos, rapid development, and genetic similarities with humans (Veldman & Lin, 2008). In recent years, there have been the studies on the bioinformatics and tissue-specific expression of genes in zebrafish (Parmar et al., 2012). These studies have provided valuable insights into the roles of these genes in zebrafish and their potential involvement in various physiological and pathological processes.

The stress responses of vertebrates involve different interactions between physiological pathways that can be characterized in both acute and chronic conditions (Bayır et al., 2020). Genetic similarities among species that are found in all organisms mean that studies conducted on one organism can serve as a source of data for other species (Collins et al., 1998; Bayır & Arslan, 2021). Therefore, completing the bioinformatics studies of the *gsr* and *g6pd* genes in the zebrafish, a model aquatic organism, will provide pioneering data for molecular studies in other species. The current study employed molecular biology techniques, such as qPCR and RT-qPCR, to determine the transcripts and transcript amounts of *gsr* and *g6pd* genes in various tissues of male and female zebrafish. This study aims to investigate the differences in expression levels of antioxidant enzyme genes between male and female zebrafish, the differences in stress tolerance of fish in males and females, and the causes of these differences. The study also included *in silico* analyses, such as conserved gene maps and phylogenetic tree construction, to determine the evolutionary relationship and similarity of these genes with other related fish species. The findings of this study could have significant implications in understanding the molecular mechanisms underlying various physiological processes in zebrafish and other related species.

Material and Method

Fish Sampling and Experimental Designs

A total of 6 zebrafish (*Danio rerio*), weighting approximately 2.49±0.1 g were obtained from the Trout Production and Research Center of Atatürk University and transferred to the Laboratory of Agricultural Biotechnology. Three females and three males were used to determine the distribution of tissue-specific gene expression. Fish were anaesthetized with clove oil before dissection. Atatürk University Local Ethical Committee for Animal Studies Experimental protocols were followed. The liver, gill, testis, ovary, intestine, kidney, stomach, eye, heart, muscle, spleen, and brain tissues from fish which were kept at 28°C taken for determine the mRNA expression. To ensure the integrity of RNA in the samples, strict measures were taken to avoid RNA degradation. Prior to the dissection, all instruments and the working bench were thoroughly sterilized and cleaned using a cleaning agent designed to remove RNases, which are enzymes that can degrade RNA. After the dissection, the samples were

Marine Science and Technology Bulletin

immediately transferred into nuclease-free tubes containing RNAlater, a solution that stabilizes RNA and prevents degradation. The tubes were kept at 4°C overnight to allow the RNAlater to penetrate the tissues and stabilize the RNA. The samples were then stored at -80°C until RNA isolation, to further prevent RNA degradation. These measures ensure the quality of the RNA extracted from the samples, which is crucial for downstream applications such as qPCR and RT-qPCR.

RNA Isolation and cDNA Synthesis and Real-Time PCR (qPCR) Analysis

Tissue samples were firstly removed from the RNAlater and transferred to nuclease-free tubes containing 1 ml of trizol reagent (Life Technologies) and homogenized. Trizol protocol was applied for RNA isolation. RNA concentrations were measured by Nanodrop 8000 spectrophotometer and quality of total RNA determined by agarose gel-electrophoresis. Before the cDNA synthesis all the RNAs were treated with DNase (DNase I, Amplification Grade, Life Technologies) for to avoid genomic contamination and the High-Capacity cDNA Reverse Transcription Kit (Life Technologies) was used for the cDNA synthesis.

Quantitative PCR A Rotor-Gene 6000 thermal cycler system (Qiagen GmbH, Düsseldorf, Germany) and QuantiTect SYBR Green PCR kit (Qiagen) were used for determination of tissue-specific distribution ((copy number/µL) of zebrafish target (*gsr* and *g6pd)* and reference (*rpl7* and *eef1a1)* genes. 20µl (10 µL SYBR Green, 4 µL forward and reverse primer, 5 µL nuclease free water and 1 µL cDNA) for each tissue and a negatif control which includes nuclease free water instead of cDNA were used for each Quantitative PCR reaction. RT-qPCR steps were 1) initial denaturation (95.0°C for 15 min), 2) 40 cycles-denaturation (95.0°C for 20 s), 3) primer annealing [optimum temperature for each primer (Table 1) for 30 s] and elongation (72.0°C for 30 s). mRNA transcript level of *gsr* and *g6pd* genes in zebrafish tissues were normalized to *rpl7* and e*ef1a1* for evaluate tissue-specific distribution after the qPCR reaction. The level of the gene expression was given consistent to the mean value of the control groups (Torstensen et al., 2009).

Primer Optimization

The forward and reverse primers were designed using NCBI Primer-BLAST for real time qPCR amplification of zebrafish target (*gsr* and *g6pd*) and reference genes (*eef1α1*and *rpl7*) (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. 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.

Identification and Structural Determination of Zebrafish gsr and g6pd Genes

The Ensembl database was used for bioinformatic identification of glutathione reductase *gsr* and *g6pd* genes. To confirm the accuracy of the obtained cDNA from this database, a BLAST search was performed in the NCBI database (https://www.ncbi.nlm.nih.gov/). It was found that both *gsr* and *g6pd* genes, which were the target genes used in this study, have a single isoform each. The Ensembl gene IDs, NCBI accession numbers, amino acid numbers, and chromosomal locations of these genes and their respective regions on the chromosome are provided in the Table 2.

The BLOSUM62 matrix algorithm (Gromiha, 2010) was used to determine the similarity and identity rates of zebrafish *gsr* and *g6pd* genes with those of other teleost fish and vertebrates. Similarity-identity rates were calculated using the protein sequences synthesized by *gsr* and *g6pd* genes of medaka (*Oryzias latipes*), stickleback (*Gasterosteus aculeatus*), goldfish (*Carassius auratus*), fugu (*Fugu rubripes*), platyfish (*Xiphophorus maculatus*), and vertebrates such as human (*Homo sapiens*) and mouse (*Mus musculus*) through the BioEdit program (Table 3).

Table 1. Primer sequences of *gsr*, *g6pd, eef1a1* ve *rpl7* genes in zebrafish

Zebrafish	Forward Primer (5′ → 3′)	Reverse Primer (5′ → 3′)	Tm (°C)
gsr	CCTGCGTCAATGTTGGATGT	CAACTGTGGGTTCAGGGTCA	62
g6pd	ATGACAAGATGAGCCTTCCG	GAGCGAGCAAAACCCACAAA	61.5
eef1α1	TGCAGAGATGGGAAAAGGCT	ACACCAGCCGCTACAATCAA	61
rpl7	ATGAGCAGGATGGCTCGTAA	GTAGCCCCAGGCAATGTAGG	62

Table 2. The Ensembl gene IDs, NCBI accession numbers, amino acid numbers, and chromosomal locations of *gsr* and *g6pd* genes in zebrafish

Gen	Ensembl gen ID	NCBI ID	Amino Acid Numbers	Chromosome Regions
Zebrafish *gsr*	ENSDART00000047050.8	NM_001020554.1	425	Chromosome 14: 38,852,969
Zebrafish *g6pd*	ENSDARG00000071065	LR812085.1	523	Chromosome 23: 13,844,920

Table 3. The nucleotide sequences of zebrafish *gsr* and *g6pd* genes

ENSDART00000047050.8 (*gsr*)

5’aaaatctgcggaattctgcgtgcgcaggtcccttgttggcctaattatgaggcatatt
ttaaaacgtatttcaatatttttaagtgtttttagtgcaatttattgtttactccctat
aaatatacatttgtacatatttatcatcatttttaaacttgctaacctttatcagccct
gaatatggccattttagtcatcaaaaaaaaaataat**TATA**ttccctcctgtgccgctgct
gactccgcgcatgcgcagtccagctcagctccacacggttccgttccgaaacattccgtt
+1
ATAAACAAAACCGAAGATGGGCACCATAGCTAACCCTAAAGAGCTCATATCGTAACACCT
CTAAATAATGATACTCCATTTACTTCAAGGATGTTAATAATATAAGTTACAGCAAACGGC
TTTCGGCTACAGGTTTAAGCTGCACTCCACCGgtaag’N356’ctcagTCTCGGACGCTC
GCTGTCATCTTCCTGCAAACTCCTTGGTCGCAGCATGGCTTCTGGATCCGTCTCGCGCTT
TGATTTTCTGGTGGTC**ATGGCTTCTGGATCCGTCTCGCGCTTTGATTTTCTGGTGGTCGG**
-M--A--S--G--S--V--S--R--F--D--F--L--V--V--G
CGGAGGATCCGGTGGGCTGGCCGGTGCGAGGAGAGCGGCTGAACTCGGTGCCACCACTGC
--G--G--S--G--G--L--A--G--A--R--R--A--A--E--L--G--A--T--T--A
CGTGATCGAAAGTCACAGACTCGGAGGTACgtgag’N1680’ctcag**CTGCGTCAATGTT**
--V--I--E--S--H--R--L--G--G--T --C--V--N--V-
GGATGTGTTCCTAAAAAGgtaat’N878’tccag**GTTATGTGGAACACATCCACTCATGC**

-G--C--V--P--K--K- -V--M--W--N--T--S--T--H--A
AGAGTATCTCCATGATCATGAAGACTATGGATTTGAGGGAGCAAAAGCACATTTCAGCTG
--E--Y--L--H--D--H--E--D--Y--G--F--E--G--A--K--A--H--F--S--W
GCAgtaag’N1207’ttcag**AATCATAAAACACAAAAGGGATGCTTACGTGAGTCGCCTG**
--Q --I--I--K--H--K--R--D--A--Y--V--S--R--L-
AATCAGATTTACAGGAGCAACCTTGAAAAGgtaag’N2151’cacag**GGCAAAATTGAGT**
-N--Q--I--Y--R--S--N--L--E--K- -G--K--I--E--
TTATTCATGGCTATGCAAGGTTCACAGATGACCCTGAACCCACAGTTGAAGTCAATGGGA
F--I--H--G--Y--A--R--F--T--D--D--P--E--P--T--V--E--V--N--G--
AGAAATACACAGCAACCCATATCTTAATCTCCACTGGCGGCCATCCATCCACAGTCAGTG
K--K--Y--T--A--T--H--I--L--I--S--T--G--G--H--P--S--T--V--S--
AGGATGATGTGCCAGgtggg’N124’ttcag**GATCCAGTTTAGGCATCACCAGTGATGGG**
E--D--D--V--P-- G--S--S--L--G--I--T--S--D--G-
TTCTTTGAACTTGAGTCTTGCCCTAAgtaag’N62’cctag**ACGTAGTGTTATAGTTGGA**
-F--F--E--L--E--S--C--P--K --R--S--V--I--V--G-
GCAGGCTATATTGCTGTGGAAATGGCTGGTATTCTTTCCACTCTTGGGTCTAAAACGTCC
-A--G--Y--I--A--V--E--M--A--G--I--L--S--T--L--G--S--K--T--S-
ATCATCATACGACAAGGAGGGgtaag’N77’ccaag**GTGCTGAGGAACTTCGATGCCTTG**
-I--I--I--R--Q--G--G- -V--L--R--N--F--D--A--L-
ATAAGCTCCAATTGCACCAAAGAATTGCAAAATAATGGTATTGACTTACGGAAAAATACT
-I--S--S--N--C--T--K--E--L--Q--N--N--G--I--D--L--R--K--N--T-
CAGgtaat’N2879’cacag**GTGAAGTCAGTGAAGAAGAATGGCAAAGGCCTCTCTATAA**
-Q- -V--K--S--V--K--K--N--G--K--G--L--S--I--
CACTGGTTACAAAAGACCCTGATGACAAGGATTCACAGGAGAAGTTTGACACTATTAATG
T--L--V--T--K--D--P--D--D--K--D--S--Q--E--K--F--D--T--I--N--
ATGTAGACTGTCTGCTGTGGGCCATTGGCAGAGAACCCAACACCGCCGGCCTCAACCTCA
D--V--D--C--L--L--W--A--I--G--R--E--P--N--T--A--G--L--N--L--
GTCAAATAgtagg’N1766’ttcag**GGTGTGAAACTTGATGAACGGGGTCATATCGTGGT**

Marine Science and Technology Bulletin

Table 3 (continued)

```
S--Q--I-                    -G--V--K--L--D--E--R--G--H--I--V--V
GGATGAGTTCCAGAACACCTCTCGTCCAGGCGTCTATGCAGTCGGGGATGTTTGCGGACG
--D--E--F--Q--N--T--S--R--P--G--V--Y--A--V--G--D--V--C--G--R
AGCCCTTCTGACACCTGgtacg'N6775'tgtagATGAAGCAGTTAAGACGTATGGAAAA
--A--L--L--T--P--                  D--E--A--V--K--T--Y--G--K-
GACAAGGTGAAGGTTTACACCACTTCTTTCACCCCCATGTATTACGCCATTACCACTCGA
-D--K--V--K--V--Y--T--T--S--F--T--P--M--Y--Y--A--I--T--T--R-
AAGAGTCAGTGCATCATGAAGTTGGTGTGCGCTGGTGAAAATGAAAAGgtgag'N96'at
-K--S--Q--C--I--M--K--L--V--C--A--G--E--N--E--K-
tagGTGGTCGGTCTCCACATGCAGGGTTTTGGCTGTGATGAGATGCTTCAGGGTTTTGCC
   -V--V--G--L--H--M--Q--G--F--G--C--D--E--M--L--Q--G--F--A-
GTAGCCGTTAACATGGGGGCGACTAAAGCAGACTTTGACAGAACCATTGCCATCCACCCA
-V--A--V--N--M--G--A--T--K--A--D--F--D--R--T--I--A--I--H--P-
ACGTCCTCAGAGGAGCTAGTAACACTGCGCTAAtcagtgccttttcattacatctccact
-T--S--S--E--E--L--V--T--L--R--*-
gcaatccaaagagtgtaaatgtaaacaaatgtaattccctggactattgttccatctaca
gaactaacagtgtaacaccacaagcatatgtttgatattggttgtgtagaagttgcacac
agtacaaccatttatcaggctgcgtctcttgtacagtacctcagatttttcacaggtatt
attgtctgcttggaacggagtcaggtaaaggcttgattatgttatagggtaAAATTAAAt
ctgtttaaagtcaaacactgttgcttttctcttatatttccagaatattatattcag 3'
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ENSDART00000138696.2 (*g6pd*)

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5'atatgtatacaaatacatctacataaaaataagcgccgtgttttaatgtgatccagtt
atgttgaaattttacaactgtgtttctgtgactgttggtgttggttgttatatagtttaa
aagcctgacaaataaagtaactaggctaatttctgattaaattaaataaaacattagcca
atatttagggcgttttttgcgggttttggacagcttttgggctggaaaccgtcagcTATA
tctggcgaccctgacatttacacacaaataaataaatggactaaatgatggcctaaaaat
+1
GTGCTACTTTCTGCGTGCACAGATTCCGTGTGGGCCCAGTCACCAATGTTTGACTACTTT
TGACTACAAAAGgtaaa'N3037'tctagCGTCGTCGCGTCGCTGACGTCAGTTTACTCC
AGCCTTCTGAAATGATGGGCAGTCGAGCGAGCGCTGgtgag'N2140'ggcagACAAGAT
           -M--M--G--S--R--A--S--A--                  D--K--M
GAGCCTTCCGCTCTCACGTTCAGAAGTGTTTGGGCAGTTGAGGAAAGAACTGCATGATGA
--S--L--P--L--S--R--S--E--V--F--G--Q--L--R--K--E--L--H--D--D
TACCGCATTTCACCAGTCAGATGTGCATATTTTCATCATCATGGGCGCTTCGgtaag'N8923'
--T--A--F--H--Q--S--D--V--H--I--F--I--I--M--G--A--S-
tgtagGGAGATCTGGCCAAAAAGAAAATCTATCCAACTTTGTGgtgag'N1932'tgtag
```

```
-G--D--L--A--K--K--K--I--Y--P--T--L--W
GTGGTTATTCAGAGATGGTCTCCTCCCTGAACAGACATATTTTGTGGGTTTTGCTCGCTC
--W--L--F--R--D--G--L--L--P--E--Q--T--Y--F--V--G--F--A--R--S
AGATCTGACTGTGGATGCCATACGCATAGCCTGCATGCCCTACATGAAGgtcac'N866'
--D--L--T--V--D--A--I--R--I--A--C--M--P--Y--M--K-
ttcagGTAGTAGACAATGAGGCAGAGCGTCTTGCTGCTTTCTTCAGCCGAAACTCTTACA
     -V--V--D--N--E--A--E--R--L--A--A--F--F--S--R--N--S--Y--
TCAGTGGGAAGTATGTGGAAGAATCCTCTTTCTCTGACCTGAACACACACCTACTGTCTC
I--S--G--K--Y--V--E--E--S--S--F--S--D--L--N--T--H--L--L--S--
TGCCTGGAGGTGCTGAGGCCAACCGGCTCTTCTACCTGGCCCTGCCACCCAGCGTCTACC
L--P--G--G--A--E--A--N--R--L--F--Y--L--A--L--P--P--S--V--Y--
ATGATGTCACCAAAAATATCAAACATCAGTGCATGAGCACCAAAgtgag'N2812'cgca
H--D--V--T--K--N--I--K--H--Q--C--M--S--T--K-
gGGCTGGAACAGGGTGATTGTGGAGAAGCCGTTTGGCCGTGATCTGCAGAGCTCAGAGGA
 -G--W--N--R--V--I--V--E--K--P--F--G--R--D--L--Q--S--S--E--E
GTTATCCAGTCATCTATCCTCTCTCTTCACTGAGGAGCAAATCTACCGTATAGACCATTA
--L--S--S--H--L--S--S--L--F--T--E--E--Q--I--Y--R--I--D--H--Y
CCTGGGCAAAGAGATGGTGCAGAACCTGATGGTCCTCAGgtgtg'N156'taaagGTTTG
--L--G--K--E--M--V--Q--N--L--M--V--L--R                --F—
```


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Table 3 (continued)

```
GAAATCGGATTTTTGGTCCCATATGGAACCGAGACAGCGTGGCGTGTGTGGTTCTGACCT
G--N--R--I--F--G--P--I--W--N--R--D--S--V--A--C--V--V--L--T--
TCAAAGAGCCGTTTGGTACCCAGGGCCGCGGAGGATATTTTGACGATTTTGGTATCATTC
F--K--E--P--F--G--T--Q--G--R--G--G--Y--F--D--D--F--G--I--I--
Ggttag'N1980'tgcagTGATGTGATGCAGAACCACCTGCTTCAGATGCTGAGTCTGGT
R                --D--V--M--Q--N--H--L--L--Q--M--L--S--L--V
GGCGATGGAGAAGCCTGCTTCCACCAGCTCTGATGATGTGCGGGATGAGAAGgtaac'N3500'
--A--M--E--K--P--A--S--T--S--S--D--D--V--R--D--E--K-
tacagGTAAAAGTGCTGAAGTGCATTGAGCCGGTCACTCTCTCAGATGTGGTTCTGGGTC
     -V--K--V--L--K--C--I--E--P--V--T--L--S--D--V--V--L--G--
AGTATGTCGGAGATCCAGATGGAGAAGGGGAGGCAAAACTGGGGTATCTAGATGACAAAA
Q--Y--V--G--D--P--D--G--E--G--E--A--K--L--G--Y--L--D--D--K--
CGGTCCCGAAAGGCTCCACTCAGGCTACATTTGCCACAGCAGTGCTTTATGTAAAGAACG
T--V--P--K--G--S--T--Q--A--T--F--A--T--A--V--L--Y--V--K--N--
AACGCTGGGATGgtgag'N772'tctagGCGTCCCGTTTATTCTCCGGTGTGGAAAGGCT
E--R--W--D--               G--V--P--F--I--L--R--C--G--K--A-
CTGAATGAGAGGAAAGCGGAGGTGCGTCTGCAGTTCACTGATGTTCCTGGAGACATCTTT
-L--N--E--R--K--A--E--V--R--L--Q--F--T--D--V--P--G--D--I--F-
AGCTCTCAGTGCCGGAGAAATGAGCTGGTGGTCCGTGTGCAGCCCAATGAGGCCATTTAC
-S--S--Q--C--R--R--N--E--L--V--V--R--V--Q--P--N--E--A--I--Y-
GCCAAGATGATGAGCAAGAAACCTGGAGTCTATTTTAGCCCTGAGGAGACCGAGCTGGAC
-A--K--M--M--S--K--K--P--G--V--Y--F--S--P--E--E--T--E--L--D-
CTGACCTACCATAGCAGATACAGGgtaaa'N1667'tacagGATGTAAAGCTGCCTGATG
-L--T--Y--H--S--R--Y--R-                  -D--V--K--L--P--D—
CTTATGAACGTCTGATTTTGGACGTCTTTTGTGGCAGTCAGATGCATTTTGTACGCAGgt
A--Y--E--R--L--I--L--D--V--F--C--G--S--Q--M--H--F--V--R--S
aag'N909'cacagTGATGAGTTGAGGGAAGCCTGGAGGATCTTCACTCCTCTCCTTCAT
             --D--E--L--R--E--A--W--R--I--F--T--P--L--L--H-
CAGATAGAGTCTGAGAAAACACCACCCATCAAATACAAATACGGGAGgtaat'N75'taa
-Q--I--E--S--E--K--T--P--P--I--K--Y--K--Y--G--S
agTCGTGGTCCCGCTGAGGCTGATGAGCTGGTGCAGAAAGTGGGCTTCCGCTACGAGGGA
  --R--G--P--A--E--A--D--E--L--V--Q--K--V--G--F--R--Y--E--G-
ACATACAAATGGGTGAATCCACACAAACTGTGAaagcagcagatgcggaagctgaagcct
-T--Y--K--W--V--N--P--H--K--L--*-
gtttaaaaacaaaaaatcagcagtcgagtgcctgaaacgagcactttaaacaaactaaat
attgaaaattcgttctgtttaagcactgcatttacagaaagaccaacgtgattggaaaac
ctttttttttagcatgttatgaatcataaccagctccttgtggtatatgatttagaacag
tgtttgttaaccaagttcctggaggaccaccagctctgcacattttccatgtcgttttaa
ctaaacacacctgattaagatgatcagctcattagcagagactgaaagacctgtaacggt
tgtgacagacaaaggagacatccaaaacatgcagtgttggtggtcctccggcaacgtggt
tgagaaacaatgctttAAAATAtcttattattatttcattttatattttattgtacat 3'
```

Note: * The exons of the *gsr* and *g6pd* genes of zebrafish are shown in capital letters. The starting points of transcription are indicated as +1, while the 5' upstream and 3' downstream sequences are shown in lowercase letters. The TATA boxes and polyadenylation signals are shown in capital letters and highlighted in yellow.

Phylogenetic Analysis

CLUSTALW (Thompson et al., 1994) at BioEdit software (http://www.mbio.ncsu.edu/bioedit/page2.html) used for sequence alignment of *gsr* and *g6pd* genes in zebrafish. The protein sequence of zebrafish Gsr was aligned with Gsr/GSR and protein sequences from zebrafish ENSDART00000047050.8, goldfish (*Carassius auratus*) ENSCART00000094324.1, Makobe Island cichlid (*Pundamilia nyererei*) ENSPNYT00000010747.1, platyfish (*Xiphophorus maculatus*) ENSXMAT00000016312.2, coho salmon (*Oncorhynchus kisutch*) ENSOKIT00005024463.1, golden line barbel (*Sinocyclocheilus grahami*) ENSSGRT00000113615.1, guppy (*Poecilia reticulata*) ENSPRET00000020656.1, amazon molly (*Poecilia formosa*) ENSPFOT00000030758.1, brown trout (*Salmo trutta*) ENSSTUT00000009463.1, chinook salmon (*Oncorhynchus tshawytscha*) ENSOTST00005067468.1, northern pike (*Esox lucius*) ENSELUT00000029343.2, channel

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bull blenny (*Cottoperca gobio*) ENSCGOT00000009735.1, Three-spined stickleback (*Gasterosteus aculeatus*) ENSGACT00000023248.1, pike perch (*Sander lucioperca*) ENSSLUT00000058070.1, *common carp* (*Cyprinus carpio*) ENSCCRT00000052099.2, spotted gar (*Lepisosteus oculatus*) ENSLOCG00000012126, human (*Homo sapiens*) ENST00000221130.11, mouse (*Mus musculus*) ENSMUST00000033992.9 and protein sequence of zebrafish G6pd was aligned with G6pd/G6PD protein sequences from zebrafish (*Danio rerio*) ENSDART00000138696.2, *common carp* (*Cyprinus carpio*) ENSCCRT00000130811.1, goldfish (*Carassius auratus*), Nile tilapia *(Oreochromis niloticus)* ENSONIT00000083037.1, Three-spined stickleback (*Gasterosteus aculeatus*) ENSGACT00000004028.1, Mexican tetra (*Astyanax mexicanus*) ENSAMXT00000018040.2, gilthead seabream (*Sparus aurata*) ENSSAUT00010033966.1, princess cichlid (*Neolamprologus brichardi*) ENSNBRT00000031013.1, electric ell (*Electrophorus electricus*) ENSEEET00000052901.1, human (*Homo sapiens*) ENST00000393562.10, mouse *(Mus musculus)* ENSMUSG00000089992. The protein sequence of human (*Homo sapiens*) catalase (ENST00000241052.5) was used as an external reference in this study (Figure 1).

In order to determine the evolutionary relationship of zebrafish and its *gsr* and *g6pd* genes with other fish species, as well as mouse and human, a pairwise alignment was conducted using the BLOSUM62 matrix to calculate sequence identity and similarity. Then, a maximum-likelihood tree was created using MEGA11 software, which was based on the Poisson correction distance model and the number of amino acid substitutions per site. The constructed tree was used to identify the evolutionary relationships of zebrafish with other fish species, mouse, and human.

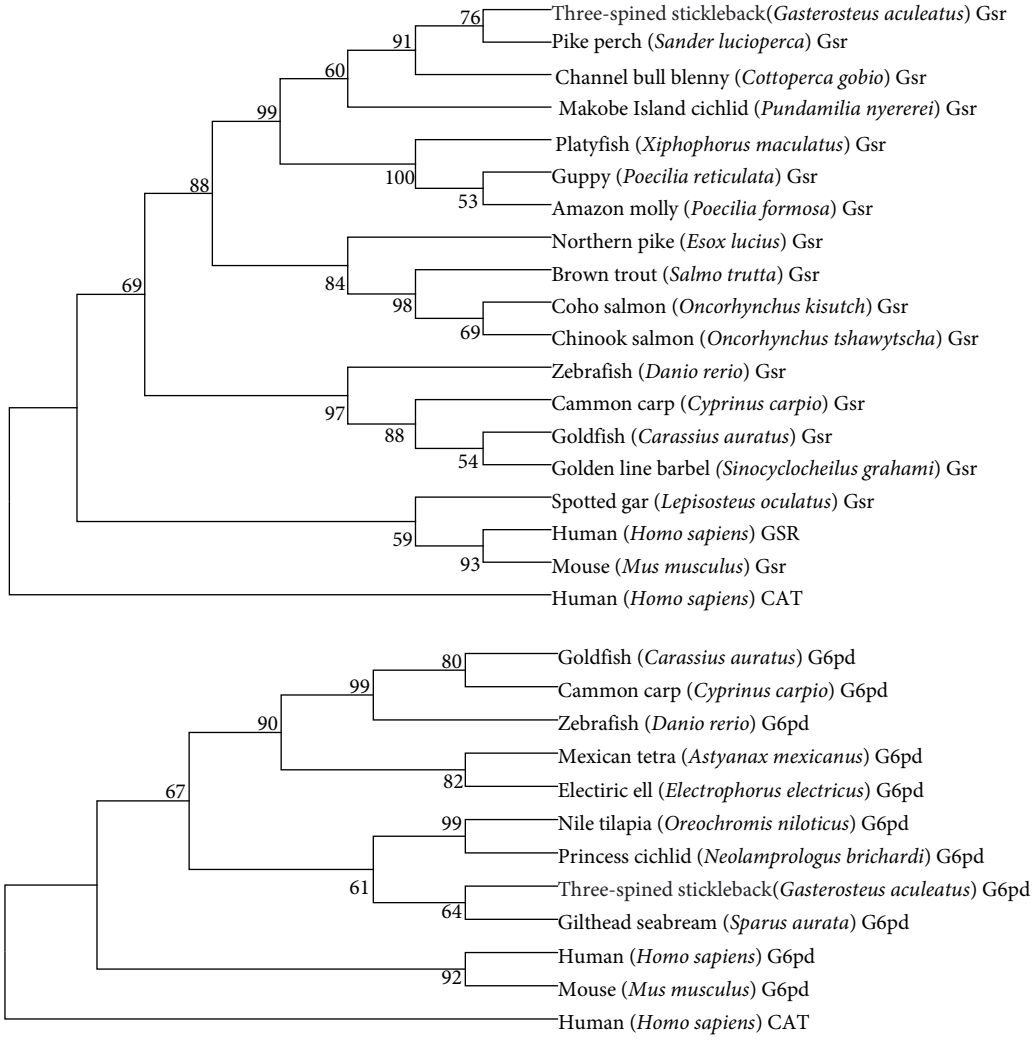

Figure 1. Phylogenetic relationship of zebrafish *gsr* and *g6pd* genes with other vertebrates

Conserved Gene Synteny

To generate a conserved gene synteny map for the zebrafish, medaka, and human *gsr/GSR* and *g6pd/G6PD* genes of, we used the Ensembl database to identify co-localized genes and manually arranged the gene synteny. We selected the relevant regions of the genomes using the region conceptus selection and identified the co-localized genes within those regions. This allowed us to create a conserved gene synteny map that showed the relationship among the *gsr/GSR* and *g6pd/G6PD* genes of zebrafish, medaka, and human (Figure 2).

Statistical Analysis

In our study, we used SPSS Statistics 17.0 software to perform a one-way analysis of variance (ANOVA) and Duncan's multiple comparison tests. We used these statistical tests to compare the levels of expression of the *gsr* and *g6pd* genes across different tissues of both zebrafish. We considered a p-value of less than 0.05 to be statistically significant, which means that there was a significant difference between the groups being compared.

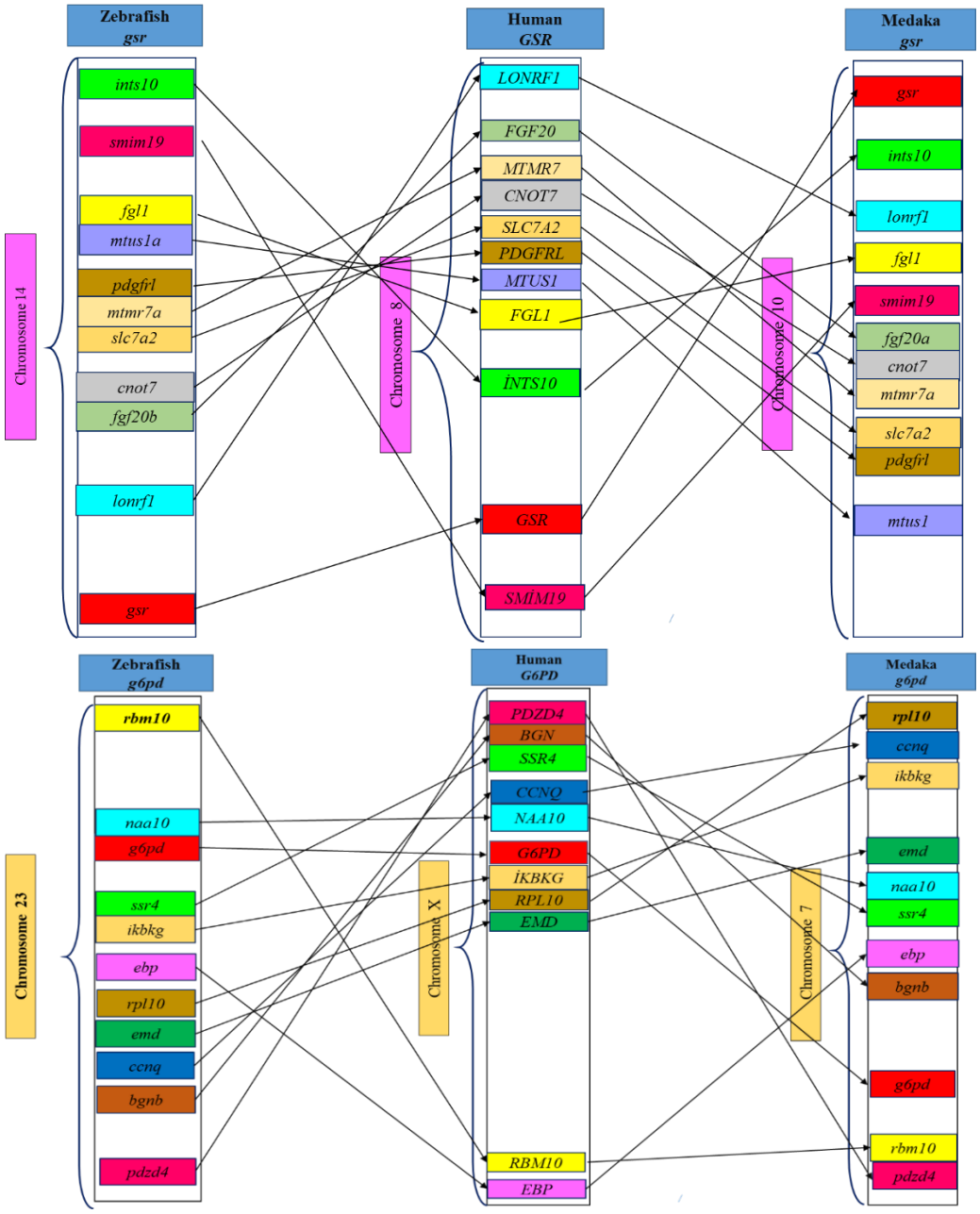

Figure 2. Identification of Conserved Genes of *gsr* and *g6pd* in zebrafish

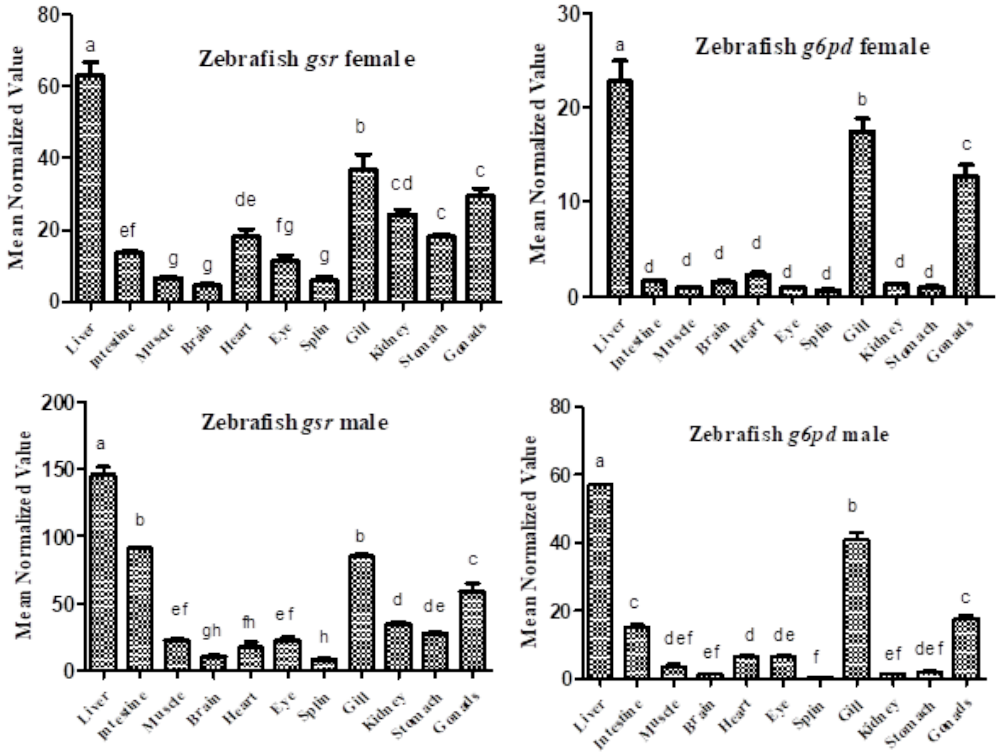

Figure 3. The tissue-specific distribution of zebrafish *gsr* and *g6pd* genes

Results

Bioinformatics Studies of gsr and g6pd Genes in Zebrafish

The characterization and identification of the *gsr* and *g6pd* enzyme genes in zebrafish through *in silico* analysis aims to provide basic data for the development of modern strategies to protect against the harmful effects of oxidative stress in both cultured fish and other vertebrates. The analysis revealed that the zebrafish *gsr* gene has 13 exons and 12 introns, while the *g6pd* gene has 14 exons and 13 introns, both with a highly conserved exon-intron organization (Table 3). Alignment analysis of the zebrafish Gsr/GSR and G6pd/G6PD sequences using CLUSTAL W (Thompson et al. 1994) showed that the polypeptide identity and similarity rates between zebrafish and its such as medaka, stickleback, goldfish, puffer fish, platyfish, mouse, and human were quite high (Table 4). The analysis also revealed that the zebrafish *gsr* gene shared the highest similarity and identity rates with the goldfish, while the *g6pd* gene had the highest similarity and identity rates with the medaka.

Tissue-Specific Transcription of gsr and g6pd Genes in Female and Male Zebrafish (Danio rerio)

In this study, while determining the tissue-specific distribution in male and female zebrafish, the transcription of the *gsr* and *g6pd* genes was detected using qPCR (Figure 3). For the *gsr* gene in female zebrafish, the tissue-specific distribution was determined as follows: liver 61.9±4.41; intestine 13.5±0.55; muscle 6.51±0.47; brain 4.56±0.46; heart 19.3±1.57; eye 10.23±1.45; spleen 6.55±0.91; gill 37±4.13; kidney 24.9±1.01; stomach 18±0.89; ovary 30.05±1.8. For the *gsr* gene in male zebrafish, the tissue-specific distribution was determined as follows: liver 145.4±6.03; intestine 91.1±0.31; muscle 22.6±0.88; brain 10.7±0.51; heart 17.5±3.60; eye 23.03±1.95; spleen 8.54±0.48; gill 85.2±0.89; kidney 34.9±1.39; stomach 27.7±1.18; testis 58.98±5.9 (Table 5). For the *g6pd* gene, the tissue-specific distribution in female zebrafish was determined as follows: liver 22.75±2.16; intestine 1.58±0.14; muscle 0.89±0.13; brain 1.50±0.17; heart 0.91±0.095; eye 0.91±0.095; spleen 0.65±0.091; gill 17.37±1.45; kidney 1.20±0.12; stomach 0.99±0.08; ovary 12.63±1.22. For the *g6pd* gene in male zebrafish, the tissue-specific distribution was determined as follows: liver 57±4.29; intestine 15.02±0.01; muscle 3.61±0.76; brain 1.44±0.20; heart 6.57±0.65; eye 6.37±0.78; spleen 0.55±0.091; gill 40.74±2.31; kidney 1.45±0.19; stomach 2.03±0.24; testis 17.91±0.82. While the mRNA transcription of liver, intestine, muscle, brain, eye, gill, kidney, stomach, and gonad tissues for the *g6pd* gene was found to be significantly higher ($P<0.05$) in males than in females, the differences between the other tissues were considered statistically insignificant (Table 6).

Marine Science and Technology Bulletin

Table 4. Similarity-identity rates between *gsr* and *g6pd* gene sequences of zebrafish and other vertebrates' *gsr/GSR* and *g6pd/G6PD* gene sequences

```
                      10        20        30        40        50        60
                ....|....|....|....|....|....|....|....|....|....|....|....|
Zf Gsr   1      ------------------------------------------------------------
Gf Gsr   1      ----------------------------------------------MEILSPLARLR
St Gsr   1      ------------------------------------------------------------
Pf Gsr   1      ----------------------------------------------MQLLKIRR
Me Gsr   1      ----------------------------------------------MLLKKICR
Mo GSR   1      ----------------------MALLPRALGVGAAPSLRRAAR---------------
Fu Gsr   1      HLIFPGYTPLLQFIMTQQWLKFGPPTRASELRARPASASFLVMAEVILRTRVQLLFSSKQ
Hu GSR   1      ----------------------MALLPRALSAGAGPSWRRAARAFRGFLLLLPEPAAL

                      70        80        90       100       110       120
                ....|....|....|....|....|....|....|....|....|....|....|....|
Zf Gsr   1      --------------------MASGSVSRFDFLVVGGGSGGLAGARRAAELGATTAVIESH
Gf Gsr   12     ASFVTLGSSLSSSRRLFGRS...AT.T......I..................A......
St Gsr   1      ---------------MAS---.DPQTT.L....I.............S....SA......
Pf Gsr   9      LLCVSLRRHELVRRSMASNRASAAETT......I..................N.......
Me Gsr   9      LLPSVSLSFPRLRVLRRSSMASESDAT......I..................NA......
Mo GSR   21     --ALTCAMASPGEPQPPAP-----DT.S..Y..I........S.........RA..V...
Fu Gsr   61     RLFSAFCRQDVVRRSMASD-PS.TDIT......I.............S....SA......
Hu GSR   37     TRALSRAMACRQEPQPQGPPP.A.A.ASY.Y..I........S.........RA..V...

                     130       140       150       160       170       180
                ....|....|....|....|....|....|....|....|....|....|....|....|
Zf Gsr   41     RLGGTCVNVGCVPKKVMWNTSTHAEYLHDHEDYGFEGAKAHFSWQIIKHKRDAYVSRLNQ
Gf Gsr   72     K.....................................N..L..R...........
St Gsr   43     ...................AAV........S.....VESVR...EAL.A.....I.H..R
Pf Gsr   69     K..................AAV........S....ATE.V....ETL.A..........H
Me Gsr   69     K..................AAV........C.....TGSVR...EAL.A.....IAH..R
Mo GSR   75     K...................AV.S.FM...V....QSCEGK...HV..Q..........T
Fu Gsr   120    K..................AAV........S.....VGNVR...EAL.T.....I.H..R
Hu GSR   97     K...................AV.S.FM...A....PSCEGK.N.RV..E..........A

                     190       200       210       220       230       240
                ....|....|....|....|....|....|....|....|....|....|....|....|
Zf Gsr   101    IYRSNLEKGKIEFIHGYARFTDDPEPTVEVNGKKYTATHILISTGGHPSTVSEDDVPGSS
Gf Gsr   132    ...N..D.A...S.........................P....A..........N....A.
St Gsr   103    ...N..D.A.VQN.Q.H....N........D.R....P....A...Q.TVL.DA.I..GN
Pf Gsr   129    ...N..D.A...T.Q.F..............R.L..P....A...Q..VL.DEE...A.
Me Gsr   129    ...N..D.A.VT..Q........A.............P........Q..VL.DEE...A.
Mo GSR   135    ..QN..T.SH..I.....T.A.G.R.........F..P....A...V.TVPH.SQI..A.
Fu Gsr   180    ...N..D.A..QT.Q.H....N..............P....A...Q..VL.DTE...A.
Hu GSR   157    ..QN..T.SH..I.R.H.A..S..K..I..S......P....A...M...PH.SQI..A.

                     250       260       270       280       290       300
                ....|....|....|....|....|....|....|....|....|....|....|....|
Zf Gsr   161    LGITSDGFFELESCPKRSVIVGAGYIAVEMAGILSTLGSKTSIIIRQGGVLRNFDALISS
Gf Gsr   192    ............................................................
St Gsr   163    ............TL...T.........................M....S.......SFL.T
Pf Gsr   189    ............TL.....................A.......LV...T........F..A
Me Gsr   189    ...N........TL.............................L....T...........A
Mo GSR   195    .........Q..DL.S.............I.....A......LM..HDK......S....
Fu Gsr   240    ............L.....V.....................L....T.......S...T
Hu GSR   217    .........Q..EL.G...................A......LM..HDK...S..SM..T
```


Marine Science and Technology Bulletin

Table 4. (continued)

```
                      310       320       330       340       350       360
                 ....|....|....|....|....|....|....|....|....|....|....|....|
Zf Gsr   221 NCTKELQNNGIDLRKNTQVKSVKKNGKGLSITLVTKDPDDKDSQEKFDTHUDVDCLLWAI
Gf Gsr   252 ........H.......S..R....TDQ...V........E..A...Y...HE........
St Gsr   223 ........S.V..W..S..T..R.TE...EV.V....QEK.NDE..TS..QE........
Pf Gsr   249 ............W..S.....S.TD...EV.I.....EK--ND..IS..EE.E......
Me Gsr   249 .....I.......W..S.....C.TE...EV.I......KT-ND..ISV.EE........
Mo GSR   255 ...E..E.A.VEVL.F....E...TSS..ELQV..SV.GR.---PTTTM.P.........
Fu Gsr   300 ........S....W..S..R..C.TD...EV.IA.R..ER.NEE..LR..QE........
Hu GSR   277 ...E..E.A.VEVL.FS...E...TLS..EVSM..AV.GRL---PVMTM.P.........

                      370       380       390       400       410       420
                 ....|....|....|....|....|....|....|....|....|....|....|....|
Zf Gsr   281 GREPNTAGLNLSQIGVKLDERGHIVVDEFQNTSRPGVYAVGDVCGRALLTP---------
Gf Gsr   312 ...........RS........EQ.........T.A................VAIAAGRKL
St Gsr   283 ..Q...S...VAAM.LEM..................I........K.....---------
Pf Gsr   307 ..Q...S...IGSM.LDT......IA..........I........K.....VAIAAGRKL
Me Gsr   308 ..Q.......IGAM..DT.D....I..D....T.S.I........K.....VAIAAGRKL
Mo GSR   312 ..D..SK....NKV.IQT..K...L.......NVK..........K.....VAIAAGRKL
Fu Gsr   360 ..Q..IT...IGHLN.DT..K.............A.I..............VAIAAGRKL
Hu GSR   334 ..V...KD.S.NKL.IQT.DK...I.......NVK.I........K.....VAIAAGRKL

                      430       440       450       460       470       480
                 ....|....|....|....|....|....|....|....|....|....|....|....|
Zf Gsr   331 ----------------------------------DEAVKTYGKDKVKVYTTSFTPMYYA
Gf Gsr   372 AHRLFEGKADSKISYDNIPTVVFSHPPIGTVGLTE...I..W...N..............
St Gsr   333 ---------------------------------E..IRSR..EN....K.......H.
Pf Gsr   367 AHRLFEGKKDSKLDYSSIPTVVFSHPPIGTVGLTEE....SH..EN..I.K.......H.
Me Gsr   368 AHRLFEGKKDSKLDYSCIPTVVFSHPPIGTVGLTEE.....K..EN..I.K.......H.
Mo GSR   372 AHRLFECKQDSKLDYDNIPTVVFSHPPIGTVGLTE....HK....N..I.S.A.....H.
Fu Gsr   420 AHRLFEGKKDSKLDYSTIPTVVFSHPPIGTVGLTEE...RSN..EN..I.K.......H.
Hu GSR   394 AHRLFEYKEDSKLDYNNIPTVVFSHPPIGTVGLTE...IHK..IEN..T.S.......H.

                      490       500       510       520       530       540
                 ....|....|....|....|....|....|....|....|....|....|....|....|
Zf Gsr   357 ITTRKSQCIMKLVCAGENEKVVGLHMQGFGCDEMLQGMOVAVNMGATKADFDRTIAIHPT
Gf Gsr   432 ................K.............................E..........
St Gsr   359 ..S...........E.KE.........L............IK.........K.V.....
Pf Gsr   427 ..S.R.P.......V.KE.........L............IK.........N.V.....
Me Gsr   428 ..R...........V.KE.........L............IK.....E...K.V.....
Mo Gsr   432 V....TK.V..M...NKE.....I....I............K.........N.V.....
Fu Gsr   480 ..N.....V.....V.KE.........L.........S..IK.........K.V.....
Hu GSR   454 V.K..TK.V..M...NKE.....I....L............K.........N.V.....


                 ....|....          Similarity (%)     Identity(%)
Zf Gsr   417 S-EELVTLR              100                100
Gf Gsr   492 .S.......              76                 81
St Gsr   419 .S..F..M.              73                 87
Pf Gsr   487 .S..F..M.              64                 75
Me Gsr   488 .S..F..M-              64                 73
Mo GSR   492 .S.......              58                 68
Fu Gsr   540 .S..F..M.              58                 67
Hu GSR   514 .S.......              54                 66
```


Marine Science and Technology Bulletin

Table 4. (continued)

```
                                                10   20   30   40   50   60
                    ....|....|....|....|....|....|....|....|....|....|....|....|
Zf G6pd    1    ---------------------------------MMGSRASADKMSLPLSRSEVFGQLRK
Me G6pd    1    -------------------------------------------MNR.........E...
Pf G6pd    1    ---------------------------------------------.T.........E..Q
Gf G6pd    1    MLSLSAFDSAVYIQLSTKFSFYLYFLLCISCECDALHEII.KL.............E...
Fu G6pd    1    ----------------------------------------M.NIP---........E...
St G6pd    1    ----------------------------------------FGSSP.HQQRH.....E...
Hu G6PD    1    -------------------------------------------MAEQVA...TQ.C.I..E
Mo G6PD    1    -------------------------------------------MAEQVT...TQ.C.I..E

                         70        80        90       100       110       120
                    ....|....|....|....|....|....|....|....|....|....|....|....|
Zf G6pd   27    ELHDDTAFHQSDVHIFIIMGASGDLAKKKIYPTLWWLFRDGLLPEQTYFVGMORSDLTVD
Me G6pd   18    ..Y..EE.....................................S.F............
Pf G6pd   17    ..Y..EK.....................................E.............
Gf G6pd   61    ...E.AE.....................................................
Fu G6pd   18    ...E.KE.......V.....................................A....
St G6pd   21    ...EVEE....-A.V.............................................
Hu G6PD   19    ..FQGD......T.................I...........N.FI..Y...R...A
Mo G6PD   19    ..YQND....A.T.................I..........KE.FI..Y...Q....

                        130       140       150       160       170       180
                    ....|....|....|....|....|....|....|....|....|....|....|....|
Zf G6pd   87    AIRIACMPYMKVVDNEAERLAAFFSRNSYISGKYVEESSFSDLNTHLLSLPGGAEANRLF
Me G6pd   78    ...TG.......A.T..D..SV............AD.....K..S.I......N......
Pf G6pd   77    ...T....F...TEM.....S...A.........A.DA...K..A.M.A....P......
Gf G6pd   121   ...A..L.....T---....T.............D.....N............G.....
Fu G6pd   78    ...TS....L..TET.SD..S...........N.TAGG...E..A.IM....ASD.....
St G6pd   80    ...T..T..L..T.KDT...S...K..T......TD.....K..S.MT.....PA.....
Hu G6PD   79    D..KQSE.FF.ATPE.KLK.ED..A....VA.Q.DDAA.YQR..S.MNA.HL.SQ.....
Mo G6PD   79    D.QKQSE.FF.ATPE.RPK.EE..T....VV.Q.DDPA.YKH..SYHUA.HQ.MQ..H..

                        190       200       210       220       230       240
                    ....|....|....|....|....|....|....|....|....|....|....|....|
Zf G6pd   147   YLALPPSVYHDVTKNIKHQCMSTK--GWNRVIVEKPFGRDLQSSEELSSHLSSLFTEEQI
Me G6pd   138   ......T........L.L......--............H.................A.D..
Pf G6pd   137   ......T..........QH.....--............H.........T......S.D..
Gf G6pd   178   ..................H.....--....I................T.........D..
Fu G6pd   138   ......TI..S..E....F...A.--............H.........T........D..
St G6pd   140   ......T..........DC...A.YR..T.........H..K......T........D..
Hu G6PD   139   ......T..EA.....HES...QI--....I.............DR..N.I....R.D..
Mo G6PD   139   ......T..EA.....QET...QT--.F..I.............NQ..N.I....R.D..

                        250       260       270       280       290       300
                    ....|....|....|....|....|....|....|....|....|....|....|....|
Zf G6pd   205   YRIDHYLGKEMVQNLMVLRFGNRIFGPIWNRDSVACVVLTFKEPFGTQGRGGYFDDFGII
Me G6pd   196   ............................................................
Pf G6pd   195   ............................................................
Gf G6pd   236   ..........................................................M.
Fu G6pd   196   ..............................N.............................
St G6pd   200   ..............................L.............................
Hu G6PD   197   ....................A...........NI...I.........E.......E....
Mo G6PD   197   .......D............A.........G.NI...I.........E.......E....
```


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Table 4. (continued)

```
                        310        320        330        340        350        360
                   ....|....|....|....|....|....|....|....|....|....|....|....|
Zf G6pd     265 RDVMQNHLLQMLSLVAMEKPASTSSDDVRDEKVKVLKCIEPVTLSDVVLGQYVGDPDGEG
Me G6pd     256 ............C.............................A...M........M.N.....
Pf G6pd     255 ............C.............................T..SM..........N.E...
Gf G6pd     296 ............C................................S................

Fu G6pd     256 .......M....C..........N................V.ASM............E...
St G6pd     260 ............C.............................T..SI............E...
Hu G6PD     257 ............C..........N................SE.QANN.........N.....
Mo G6PD     257 .....S......C.........T.D.....N.......R.SE.ETDN.I......N.N...

                        370        380        390        400        410        420
                   ....|....|....|....|....|....|....|....|....|....|....|....|
Zf G6pd     325 EAKLGYLDDKTVPKGSTQATMOTAVLYVKNERWDGVPFILRCGKALNERKAEVRLQFTDV
Me G6pd     316 .........P...................H..............................
Pf G6pd     315 D........P...................H..............................
Gf G6pd     356 ............E.................................................
Fu G6pd     316 D........P............V....H..............................
St G6pd     320 .........P...........T......H..............................
Hu G6PD     317 ..TK.....P...R...T....AV....E............................H..
Mo G6PD     317 ..AN.....P...R...T....A..................................R.I

                        430        440        450        460        470        480
                   ....|....|....|....|....|....|....|....|....|....|....|....|
Zf G6pd     385 PGDIFSSQCRRNELVVRVQPNEAIYAKMMSKKPGVYFSPEETELDLTYHSRYRDVKLPDA
Me G6pd     376 .....GN..Q.....................................K...K.......
Pf G6pd     375 .....DNK.Q.....................................K...K.......
Gf G6pd     416 .......H.....................................K.......
Fu G6pd     376 .....RN..Y.........................T..........K...K.......
St G6pd     380 .....GN..............V.....................K...K.......
Hu G6PD     377 A....HQ..K.....I.......V.T...T....MF.N...S......GN..KN......
Mo G6PD     377 .....HQK.K.....I.M.....V.TT..T....MF.N...S......GNK.KN....G.

                        490        500        510        520        530        540
                   ....|....|....|....|....|....|....|....|....|....|....|....|
Zf G6pd     445 YERLILDVFCGSQMHFVRSDELREAWRIFTPLLHQIESEKTPPIKYKYGSRGPAEADELV
Me G6pd     436 ..................................DK..PK..P..............
Pf G6pd     435 ..............................I....DK..PK..P........S...D..
Gf G6pd     476 ..................................K.....................
Fu G6pd     436 ..................................K..PK..P..............
St G6pd     440 ................................H.DK..PK..P.I............A
Hu G6PD     437 ..................................L..PK..P.I......T.....M
Mo G6PD     437 ...........C......T.....G.........K..R..PQ.FP.V......T.....M

                        550
                   ....|....|....|....
Zf G6pd     505 QKVGFRYEGTYKWVNPHKL
Me G6pd     496 .R..................
Pf G6pd     495 KR..................
Gf G6pd     536 K.................P
Fu G6pd     496 KR...............R.
St G6pd     500 K...............R.
Hu G6PD     497 KR...Q.............
Mo G6PD     497 RR...Q.K....GTHK.--
```

	Similarity (%)	Identitiy(%)
Zf G6pd	100	100
Me G6pd	89	93
Pf G6pd	87	93
Gf G6pd	87	89
Fu G6pd	85	91
St G6pd	85	89
Hu G6PD	74	85
Mo G6PD	69	82

Note: * The dots in the figure represent the similarity between amino acid sequences, while the short dashes indicate the missing amino acids. Similarity rate refers to the percentage of nucleotides in the sequence that are identical or similar between the two species, while identity rate refers to the percentage of nucleotides that are identical. "Me" represents medaka (*Oryzias latipes*), "St" represents stickleback (*Gasterosteus aculeatus*), "Gf" represents goldfish (*Carassius auratus*), "Fu" represents fugu (*Fugu rubripes*), "Pf" represents platy fish (*Xiphophorus maculatus*), "Hu" represents human (*Homo sapiens*), and "Mo" represents mouse (*Mus musculus*) in the table.

Marine Science and Technology Bulletin

Table 5. The differences in transcription of the *gsr* gene between genders in zebrafish

Tissue	Sex	N	Average±Std. Error	t	P
Liver	Female	6	61.49±4.41	-11.172	0.000**
	Male	6	145.4±6.03	-11.172	0.000**
Intestine	Female	6	13.5±0.55	-123.065	0.000**
	Male	6	91.1±0.31	-123.065	0.000**
Muscle	Female	6	6.51±0.47	-16.146	0.000**
	Male	6	22.6±0.88	-16.146	0.000**
Brain	Female	6	4.56±0.46	-8.948	0.000**
	Male	6	10.7±0.51	-8.948	0.000**
Heart	Female	6	19.3±1.57	0.458	0.657
	Male	6	17.5±3.60	0.458	0.661
Eye	Female	6	10.23±1.45	-5.267	0.000**
	Male	6	23±1.95	-5.267	0.000**
Spleen	Female	6	6.55±0.91	-1.936	0.082
	Male	6	8.54±0.48	-1.936	0.091
Gill	Female	6	37±4.13	-11.411	0.000**
	Male	6	85.2±0.89	-11.411	0.000**
Kidney	Female	6	24.9±1.01	-5.772	0.000**
	Male	6	34.9±1.39	-5.772	0.000**
Stomach	Female	6	18±0.89	-6.484	0.000**
	Male	6	27.7±1.18	-6.484	0.000**
Gonad	Female	6	30.05±1.8	-4.611	0.001**
	Male	6	58.98±5.9	-4.611	0.004**

Note: **: P<0.05

Table 6. The differences in transcription of the *g6pd* gene between genders in zebrafish

Tissue	Sex	N	Average±Std. Error	t	P
Liver	Female	6	22.75±2.16	-7.126	0.000**
	Male	6	57±4.29	-7.126	0.000**
Intestine	Female	6	1.58±0.14	-13.116	0.000**
	Male	6	15.02±0.01	-13.116	0.000**
Muscle	Female	6	0.89±0.13	-3.521	0.006
	Male	6	3.6±0.76	-3.521	0.015
Brain	Female	6	1.50±0.17	0.189	0.854
	Male	6	1.44±0.20	0.189	0.854
Heart	Female	6	0.91±0.095	-8.603	0.000**
	Male	6	6.6±0.65	-8.603	0.000**
Eye	Female	6	0.91±0.095	-6.939	0.000**
	Male	6	6.4±0.78	-6.939	0.001**
Spleen	Female	6	0.65±0.091	0.742	0.475
	Male	6	0.55±0.092	0.742	0.475
Gill	Female	6	17.37±1.45	-8.561	0.000**
	Male	6	40.08±2.32	-8.561	0.000**
Kidney	Female	6	1.20±0.12	-1.096	0.299
	Male	6	1.45±0.19	-1.096	0.303
Stomach	Female	6	0.99±0.08	-4.163	0.002
	Male	6	2.03±0.24	-4.163	0.006
Gonad	Female	6	12.63±1.22	-3.585	0.005**
	Male	6	17.91±0.82	-3.585	0.005**

Note: **: P<0.05

Discussion

Tissue-Specific Transcription of gsr and g6pd Genes in Female and Male Zebrafish (Danio rerio)

Glutathione reductase plays an important role among the biomarkers of oxidative stress (Puppel et al., 2015; Nandi et al., 2019). During the body's response to oxidative stress, *gsr* is responsible for maintaining the homeostasis of reduced glutathione, which is the most important substrate for the synthesis of bioproteins together with glutathione peroxidase, as well as the main antioxidants such as ascorbic acid and α-tocopherol (Szudrowicz et al., 2022). This study was conducted in zebrafish, which serves as a model organism in science, to perform detailed bioinformatic and molecular analyses of the *gsr* and *g6pd* enzyme genes, in order to better understand the molecular mechanism of oxidative stress damage in vertebrates, and to determine which gender and tissues these genes are dominant in. It has been determined that the *gsr* and *g6pd* enzyme genes are dominant in the liver tissue of both male and female fish. Therefore, we can say that the liver is the most suitable tissue for gene expression studies on stress-related diseases in the future, based on tissue-specific analyses. The gill tissue gene expressions are significantly (P<0.05) lower than liver tissue gene expressions, although it is the second tissue where the *gsr* and *g6pd* genes are dominant, and significantly (P<0.05) higher than other tissues. The *gsr* and *g6pd* mRNA transcription identified in the ovary and testis tissues are significantly (P<0.05) lower than the transcription of gill and liver tissues, but it is seen that the gonads have significantly (P<0.05) higher gene expression than other tissues. Therefore, the most dominant tissues in both *gsr* and *g6pd* genes are the liver, gill, and gonads in order. When the tissue-specific distribution differences between male and female fish were examined, it was seen that the gene expression of *gsr* and *g6pd* in males is significantly higher than in females in these three

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tissues. The study discussed in the previous sections provides valuable information about the tissue-specific distribution of *gsr* and *g6pd* genes in zebrafish. The results show that the liver is the most suitable tissue for gene expression studies on stress-related diseases, as *gsr* and *g6pd* genes are dominant in this tissue for both male and female fish. This finding is consistent with previous studies that have shown that the liver plays a critical role in antioxidant defense mechanisms and is sensitive to oxidative stress. This finding suggests that liver, gill and gonads tissues may be a useful target for future studies on the effects of environmental stressors on fish health. The study also identified significant differences in gene expression between male and female fish in the liver, gill, and gonads, with male fish showing significantly higher levels of gene expression in these tissues. Furthermore, the significantly higher transcription values of the *gsr* and *g6pd* genes in male tissues, compared to females, suggest that males may experience more stress in fish, as in many other animals in nature, resulting in increased gene expression related to stress. Many environmental challenges can lead to the accumulation of ROS, which are fundamental cell signaling molecules in living organisms (Apel & Hirt, 2004; Schieber & Chandel, 2014). Under normal conditions, the balance of ROS in tissues can be disrupted by the presence of stress, which can lead to a decrease in male fertility (Dowling & Simmons, 2009). This can result in male fish exhibiting higher levels of gene expression related to oxidative stress compared to females, as a way of coping with the greater levels of stress they experience. In this study, the expression of *gsr* and *g6pd* were analyzed in liver, gill, and gonads tissues that serve as biochemical and molecular indicators of the antioxidant system, with the genes being found to be more highly expressed in males suggesting that males may be trying to cope with stress. Parallel to this study, Ozdemir & Bayır (2022) reported on transcriptional differences in the antioxidant enzyme gene *sod1* between male and female brown trout. Ozdemir & Bayır (2022) suggested that the higher expression of antioxidant enzyme genes in males may be related to hypotheses about the role of antioxidants in mating performance, which is based on the male's efforts to attract females, known as sexual ornamentation (Blount, 2004; Catoni et al., 2008). This finding highlights the importance of considering sex-specific differences in gene expression in future studies on oxidative stress in fish. Further research is needed to determine how these gene expression patterns relate to fish health and the effects of environmental stressors on fish populations.

Bioinformatics Studies of gsr and g6pd Genes in Zebrafish

The identification and characterization of the *gsr* and *g6pd* genes in zebrafish provide valuable information on the evolution and function of these genes across different species. This information can be used to develop modern strategies to protect against the harmful effects of oxidative stress in both cultured fish and other vertebrates. The synteny designed to identify conserved genes between zebrafish, medaka, and humans suggests that the zebrafish *gsr* and *g6pd* genes are the result of teleost whole genome duplication (TWGD). Zebrafish genome mapping studies (Barbazuk et al., 2000; Postlethwait et al., 2000) and phylogenetic analyses of zebrafish genes (Meyer & Schartl, 1999; Taylor et al., 2001) also support the hypothesis that an early genome duplication event occurred in ray-finned fish (Taylor et al., 2003). Taylor et al. (2001) estimated that the fish-specific duplication event occurred more than 300 million years ago. However, it is impossible to determine the exact ages of zebrafish gene copies because the third codon positions used to estimate their ages have reached saturation (Taylor et al., 2003). Previous studies have reported that teleost fish have two copies of many genes that other vertebrates have only one (Postlethwait et al., 1998; Meyer & Schartl, 1999; Braasch & Postlethwait, 2012). However, this study found that the zebrafish genome contains only one copy of the *gsr* and *g6pd* genes, and it is believed that the reason for their presence as single copies is due to functional redundancy, which is a common occurrence in duplicated genes (Glasauer & Neuhauss, 2014). Functional redundancy occurs when one copy of a duplicated gene maintains the original function, while the other copy may acquire new functions or undergo functional divergence (Glasauer & Neuhauss, 2014). In the case of the *gsr* and *g6pd* genes, it is possible that the single copies in zebrafish have retained the original function of their duplicated counterparts, while the duplicates may have undergone functional divergence or been lost in the course of evolution. Further studies are needed to investigate the functional significance of the single copies of the *gsr* and *g6pd* genes in zebrafish and their potential roles in protecting against oxidative stress.

Conclusion

In conclusion, this study provides important information on the bioinformatics and tissue-specific distribution of *gsr* and *g6pd* genes in zebrafish. The results show that the liver is the

dominant tissue for both genes in both female and male zebrafish. The study also reveals sex differences in the expression of these genes, with higher levels of *gsr* and *g6pd* transcripts found in various tissues of male zebrafish compared to female fish. *In silico* analyses demonstrate that the *gsr* gene of zebrafish has high similarity and identity ratios with goldfish and stickleback, while the *g6pd* gene shows the highest similarity and identity ratios with medaka fish and platy fish. These findings provide a foundation for further molecular studies of *gsr* and *g6pd* genes in zebrafish and related species, and could contribute to a better understanding of their roles in physiological and pathological processes.

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Compliance With Ethical Standards

Authors' Contributions

BNU: Literature review, Drafting, Writing, Laboratory, Data analysis and management experiments

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

Both 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

The research adhered to all relevant international, national, and institutional guidelines for the ethical care and use of animals.

Data Availability Statement

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

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