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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Bioinformatics studies and comparison of mRNA transcription of glutathione S-transferase gene in some tissues of common carp (*Cyprinus carpio*) and brown trout (*Salmo trutta*)

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Abstract: Bioinformatics has revolutionized the way we study gene expression and regulation, enabling researchers to analyze large-scale genomic data with unprecedented speed and precision. In this study, we use bioinformatics tools and methods to compare mRNA transcription of glutathione S-transferase (*gstr*) gene in two different fish species: common carp and brown trout. In this study, liver, intestine, muscle, brain, heart, eye, spleen, gill, kidney, stomach, ovary and testis samples were taken from male and female brown trout and common carp, and total RNA was isolated from each tissue to synthesize cDNA from these tissues. Then, the transcript amounts of the *gstr* gene were determined by qPCR from all tissue samples. Gene structures, conserved gene synteny design, phyogenetic tree analyzes and similarity-identity ratios with other vertebrates were determined. When the transcriptional differences between male and female tissues for the brown trout *gstr* gene were examined, it was seen that the intestine, gill, kidney, stomach, muscle and gonads were significantly higher in male fish ($p<0.05$), but the differences between other tissues were not statistically significant. It has been determined that the highest gene expression was liver ($p<0.05$) and brain, eye, spleen, kidney, heart and spleen tissues have significantly lower *gstr* gene expression than other tissues in both male and female in common carp. In addition, the in-silico analysis determined that the brown trout *gstr* gene shared the highest similarity and identity ratio with rainbow trout, and the common carp *gstr* gene shared the highest similarity and identity ratio with goldfish.

Keywords: Brown trout, common carp, in silico analysis, *gstr*, gene expression

INTRODUCTION

Aquaculture is an important industry that helps meet the growing demand for seafood while reducing pressure on wild fish populations (Chen et al., 2021). Brown trout and common carp are two popular species that are extensively farmed for their economic and nutritional benefits (Adamek et al., 2023; Franěk et al., 2021). Common carp farming has been shown to improve glucose metabolism disorder in fish. Carp farming, however, can also contribute to increases in turbidity and internal nutrient load by resuspending sediments, which may eventually reduce the water quality (Arlinghaus and Mehner, 2003). Brown trout is another important aquaculture species that faces challenges due to global warming and a changing climate (Keiz et al., 2023). Inland fisheries, including aquaculture, contribute significantly to food security and economic security by providing primary sources of animal protein, essential for human health and well-being (Lynch et al., 2016).

Glutathione S-transferase (GST) is an enzyme that plays a crucial role in the detoxification of xenobiotics and endogenous compounds by catalyzing the conjugation of glutathione to electrophilic substrates. GSTs are encoded by a large gene family, and their expression is induced by various environmental stressors, including microcystin-LR, cadmium, and weathered polyethylene microplastics. GSTs have been studied in various fish species, including common carp (Chen et al., 2017), Nile tilapia (Liang et al., 2007), and zebrafish (Glisic et al., 2015; Tierbach et al., 2018). The expression of GST genes varies among different tissues and fish species. For example, alpha-class GST gene expression was higher than that of rho-class GST gene in both exposed and control fish of silver carp and grass carp, whereas rho-class GST gene expression was higher than that of alpha-class GST gene in both exposed and control fish of Nile tilapia (Liang et al., 2007). The induction of GST enzyme activity corresponds to *gstr* gene expression at the latter stages of exposure to weathered polyethylene microplastics (Pandi et al., 2022). In cadmium-exposed river pufferfish, seven genes of the GST family were cloned and expressed, and GST1.18 was found to play a critical role in detoxification pathways (Kim et al., 2010). GSTs also play an important role in phase II detoxification of lipid peroxides and demonstrate the functions such as glutathione peroxidase activity towards (Rudneva et al., 2010). Overall, GSTs have an important role in the detoxification of xenobiotics and endogenous compounds in fish (Glisic et al., 2015).

Bioinformatics studies of mRNA transcription in fish have become increasingly popular as they provide insights into the biological mechanisms involved in various physiological processes (Qian et al., 2014). In genetics research, bioinformatics plays a crucial role in studying genetic information, such as DNA and RNA sequences, and their interactions with various biological processes (Bayat, 2002).

In this study, bioinformatics tools and techniques were used to compare the mRNA transcription of the *gstr* gene in the tissues of common carp and brown trout. By employing bioinformatics tools and techniques, we aim to gain a deeper understanding of the expression patterns of the *gstr* gene in different tissues of these two species, and explore potential differences in expression levels between them. Our findings may have significant implications for understanding the role of *gstr* gene expression in aquatic organisms and its potential effects on their health and survival in varying environmental conditions.

MATERIALS AND METHODS

In silico analysis

In-silico analysis for the identification of the *gstr* gene in brown trout and common carp were performed using bioinformatics tools such as Ensembl, NCBI, and UniProt databases. The cDNA sequences of brown trout and common carp *gstr* were obtained from the Ensembl database and their accuracy was confirmed by performing a BLAST search on the NCBI database. It was observed that both brown trout and common carp have one isoform of the *gstr* gene, which was identified through Ensembl database searches. In the study, to determine the mRNA expression of the *gstr* gene in both brown trout and common carp, as well as the reference genes for common carp, actin beta 1 (*actb*) and glyceraldehyde-3-phosphate dehydrogenase (*gapdh*), primers were designed according to the exon-exon junction model (Table 1). The primer sequences used for beta-actin and elongation factor 1a (*eef1a*) genes, which were reference genes for brown trout, were obtained from Özdemir and Bayır (2023) (Table 2). Additionally, genomic primers were designed (Table 3) to amplify the desired regions and obtain the sequence of PCR products from the beginning and end parts of the open reading frame by designing the primers from the closest regions possible to the start and end of the open reading frame. The PCR products obtained from the designed primers for gDNA were placed in three nuclease-free Eppendorf tubes containing 30 µL of PCR product each and sent to a specialized sequencing company for Sanger sequencing.

The design of the conserved gene synteny manually using the Ensembl database (Figure 1). The chromosomes and regions where the *gstr* gene are found in brown trout and common carp are recorded. The other genes found outside the *gstr* gene in the identified chromosomes and regions, as well as the chromosomal regions of these genes in another organism, rainbow trout, are also identified. A conserved gene synteny is created based on the common genes and their chromosomal locations in the genomes of these three organisms. The CLUSTALW BioEdit program (http://www.mbio.ncsu.edu/bioedit/page2.html) was used to determine the phylogenetic relationship of brown trout and common carp using the *gstr* gene, and to construct a phylogenetic tree (Figure 2). The nucleotide sequences of the *gstr* genes in brown trout and common carp were determined using the Ensembl database. Separate nucleotide sequences were designed for both species, indicating the exons, introns, amino acids synthesized by the exons, 5' and 3' ends, TATA box, poly-A signal, and stop codon of the *gstr* gene (Figure 3, 4). The similarity-identity ratios between the *gstr* genes of common carp and goldfish, zebrafish, rainbow trout, Atlantic salmon, brown trout, and gilthead seabream were calculated using the BioEdit program based on the protein sequences synthesized by these genes (Figure 5, 6).

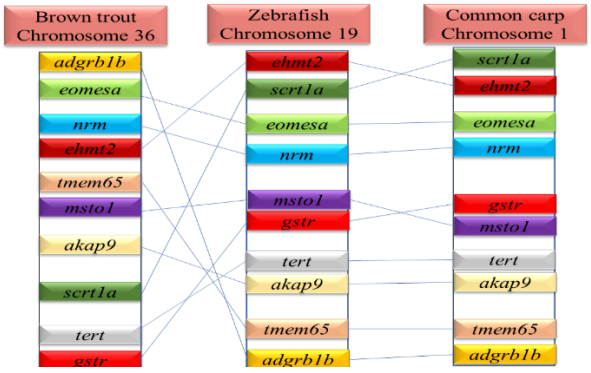

Figure 1. Conserved gene synteny among brown trout, common carp, and zebrafish *gstr* gene

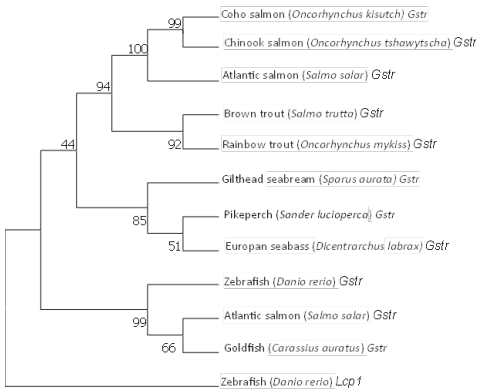

Figure 2. The phylogenetic relationship of brown trout and common carp *gstr* genes with those of other fish species

The protein sequence accession numbers used in the phylogenetic tree, created using the maximum likelihood method (Felsenstein, 1989), are as follows: Atlantic salmon (*Salmo salar*) ENSSSAG00000056150, common carp (*Cyprinus carpio*) ENSCCRG00000013532, brown trout (*Salmo trutta*) ENSSTUG00000037965, rainbow trout (*Oncorhynchus mykiss*) ENSOMYG00000034408, Chinook salmon (*Oncorhynchus tshawytscha*) ENSOTSG00005006610, coho salmon (*Oncorhynchus kisutch*) ENSOKIG00005022094, goldfish (*Carassius auratus*) ENSCARG00000006155, zebrafish (*Danio rerio*) ENSDARG00000042620, gilthead seabream (*Sparus aurata*) ENSSAUG00010003870, European seabass (*Dicentrarchus labrax*) ENSSLUG00000008281, Nile tilapia (*Oreochromis niloticus*) ENSONIG00000034559, European seabass (*Dicentrarchus labrax*) ENSDLAG00005030493.

Table 1. Primer sequences *gstr*, *actb1,* and *gapdh* genes of common carp

Common carp	Forward primer (5´→3´)	Reverse primer (5´→3´)	Tm (°C)
gstr	CCAGAGCTCAGGTTCCAACT	GGTCTCAAACATTCGCTGGT	62
actb1	CCCAGGCATCAGGGAGTGA	TCCATATCATCCCAGTTGGTCA	62.5
gapdh	CAACATGGGGATTTGGCCGT	AGACGGTGATAGCGTGACCA	60

Table 2. Primer sequences for *gstr, actb*, and *ef1α* genes of brown trout

Brown trout	Forward primer (5´→3´)	Reverse primer (5´→3´)	Tm (°C)
gstr	GGACAGCTCCCTGCTTTCAA	CGGGGACACGGTAGTTGTAG	62
b-actin	ATGGAAGGTGAAATCGCC	TGCCAGATCTTCTCCATG	52.1
ef1α	GTCMMTGGAACGCACTCG	CTACTGATTGGCTGCTTCGC	59.45

Table 3. Genomic primers for brown trout and common carp *gstr* genes.

gstr	**Forward primer (5´→3´)**	**Reverse primer (5´→3´)**	**Tm (°C)**
Brown trout	CCAGAGCTCAGGTTCCAACT	GGTCTCAAACATTCGCTGGT	61
Common carp	TAACACAAGCGCACCACTG	AGACTGTTAATGTGCGCTGC	59

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5’tatacaggtaactagctgagattaggagcacactcttaaagggagtgctcctaatctc
agctcgttacctgtataaaagacacctgggagccagaaatctttctgattgagaggggt
caaatacttatttccctcattaaaatgcaaatcaatttataacatttttgacatgcgttt
ttctggatttttagttgttattctgtctctcagtgttcaaattaacctaccattaaaat
TATAgactgatcatttctttgtcagtgggcaaacgtacaaaatcagcaggggatcaaata
+1
CTTTTTCCCCTCACTGTATATTGGTTCTTAACTTCCCTGAAAGTTGCATATTGCCGGGGC
TATTCGATTCTAATGCGTACTATTTCCATTTTTCTATTTTTCCTGTTTCTTACTTTTTAA
CTGTGCATTGTTTGGAAAGAGCTCGTACTGTAACTAAGCGTTTCACGGTAAAGTCTACAC
CTGTTGTATTCGGCGCAGGTGACAAACACAATTTGATATGACTTCCTTTATGCTGTAGCC
AACATGACTACGCGGAATTCATGTGTTGATAGAAGACCAGTAGAACTGGACTGTCATGAC
   -M--T--T--R--N--S--C--V--D--R--R--P--V--E--L--D--C--H--D-
TCGTACATTAAGgtgac’N361’agcagATTTCGACCATCATGGCCAAGGACATGACACT
-S--Y--I--K-                  -I--S--T--I--M--A--K--D--M--T--L
GCTGTGGGGCTCCGGCTCTCCTCCGTGCTGGCGTGTCATGATCGCTCTGGAGGAGAAGAA
--L--W--G--S--G--S--P--P--C--W--R--V--M--I--A--L--E--E--K--K
ACTGCAGGGATACAATCACAAACTTCTCTCCTTCGAGAAAGCAGAGCACAAGTCTAAAGA
--L--Q--G--Y--N--H--K--L--L--S--F--E--K--A--E--H--K--S--K--E
AGTCCTGGATATCAATCCCAGAGGACAGgtagt’N448’cccagCTCCCTGCTTTCAAAC
--V--L--D--I--N--P--R--G--Q-                  -L--P--A--F--K--
ACGGAGACAACATACTCAACGAGTCATATGCAGCATGCATGTACCTGGAGgtaag’N2045’
H--G--D--N--I--L--N--E--S--Y--A--A--C--M--Y--L--E-
tacagAGCCGGTTCAGGTCCCAGGGACCCCAGTTGATTCCTGAGGGCCAACTAGAGCAGG
     -S--R--F--R--S--Q--G--P--Q--L--I--P--E--G--Q--L--E--Q--
CCCTGATGTACCAGCGCATGTTTGAGATCCTCAACCTCAGTGACAAACTCAgtaag’N415’
A--L--M--Y--Q--R--M--F--E--I--L--N--L--S--D--K--L--
cccagGTAACGTCATCTACTACAACTACCGTGTCCCCGAGGGAGAGAGACATGACTCTGC
     S--N--V--I--Y--Y--N--Y--R--V--P--E--G--E--R--H--D--S--A
TATCAAGAGGAACAAGGAGAACCTGGCCACGGAAATCAAACTGTGGGAGGGATACTTTCA
--I--K--R--N--K--E--N--L--A--T--E--I--K--L--W--E--G--Y--F--Q
GAAGgtgca’N756’tccagATGGAGGTGGGTTCTTACCTGGCAGGAAAAGCCTTCTCAT
--K-                  -M--E--V--G--S--Y--L--A--G--K--A--F--S--
TGGCTGACGTTATTGTCTTCCCTGTGATTGCCTACGCCTTCCGCTTTGGgtaag’N67’t
L--A--D--V--I--V--F--P--V--I--A--Y--A--F--R--F--G
ccagGCTGTCTACGGAGCGTTACCCCAAACTGGGAGCATACTACGATATGATGAAGGAAA
    --L--S--T--E--R--Y--P--K--L--G--A--Y--Y--D--M--M--K--E--
GACCCAGCGTTAAAGCTACCTGGCCCCCACACTGGCTGGAGAACCCTCAGGGAGGGGACG
R--P--S--V--K--A--T--W--P--P--H--W--L--E--N--P--Q--G--G--D--
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A--L--K--E--F--*-
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cctccatataatttctgtgttttgtgtacagaccacatacactggtgtggAATTAAtaaa
aaaaatcataccaag 3’
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Figure 3. Exon-intron organization of the brown trout glutathione S-transferase (*gstr*) gene

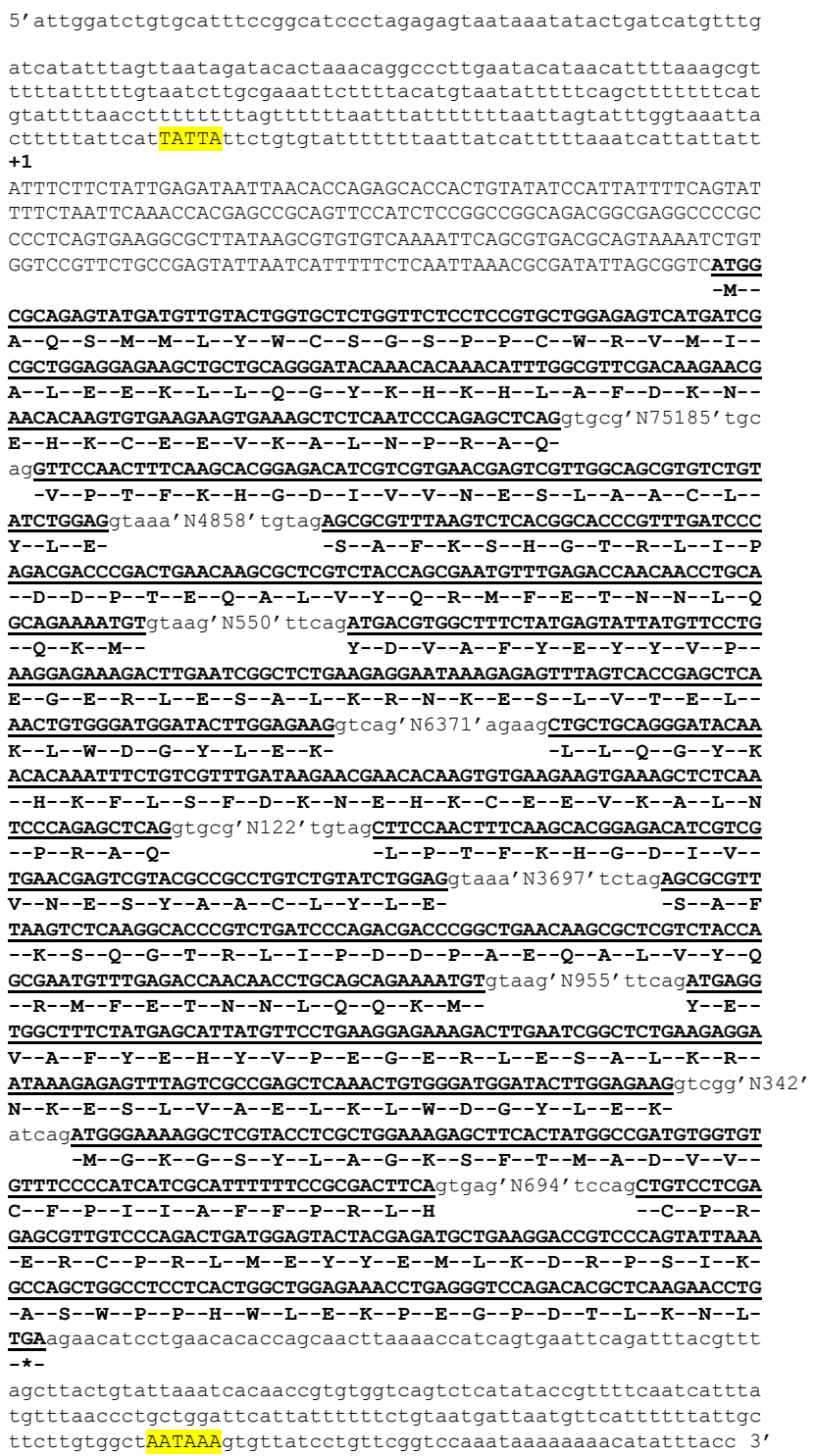

Figure 4. Exon-intron organization of the common carp glutathione S-transferase (*gstr*) gene

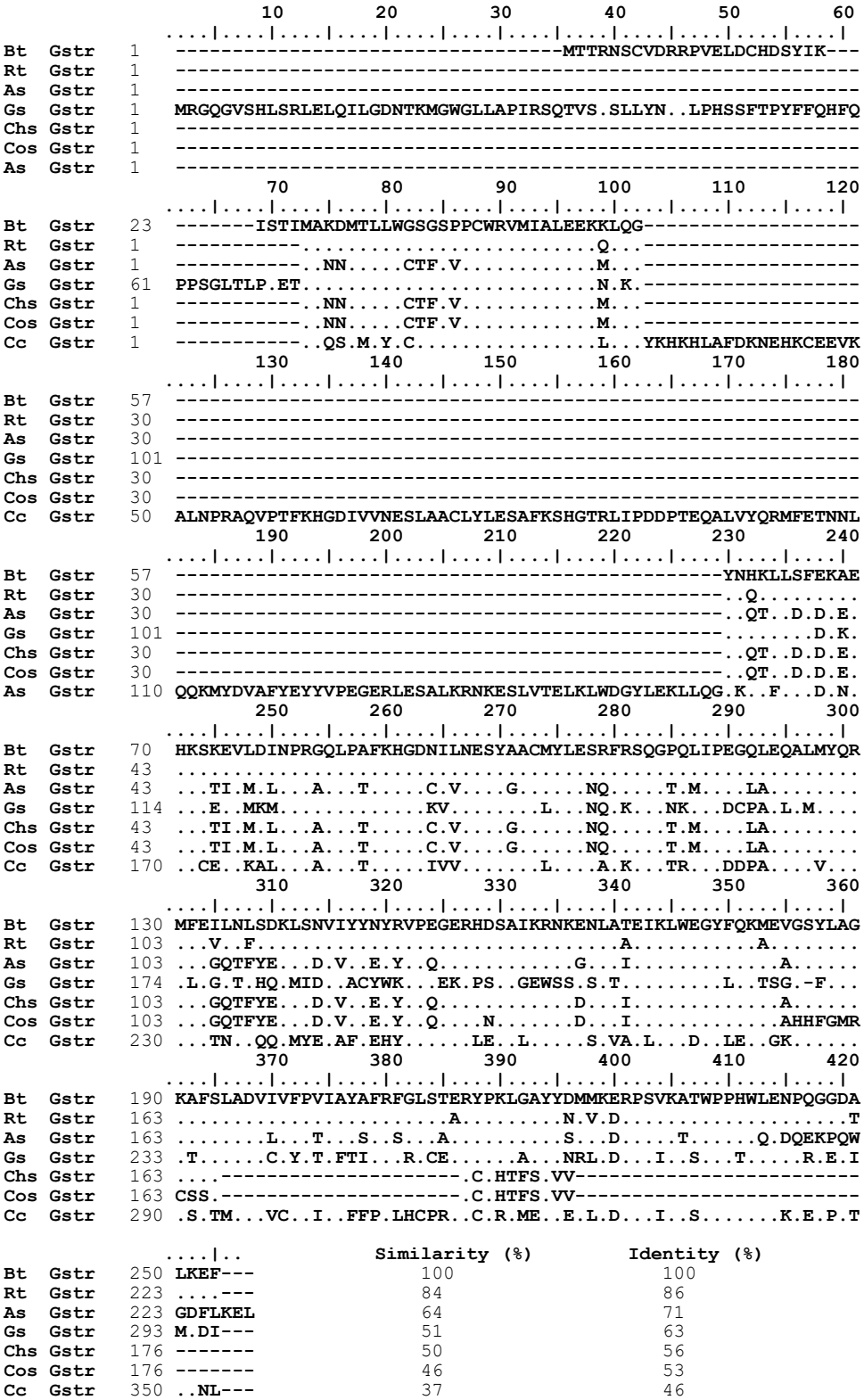

Figure 5. Similarity-Identity rates among *gstr* genes of brown trout (Bt) and rainbow trout (Rt), Atlantic salmon (Ats), gilthead seabream (Gs), chinook salmon (Chs), coho salmon (Cos), and common carp (As)

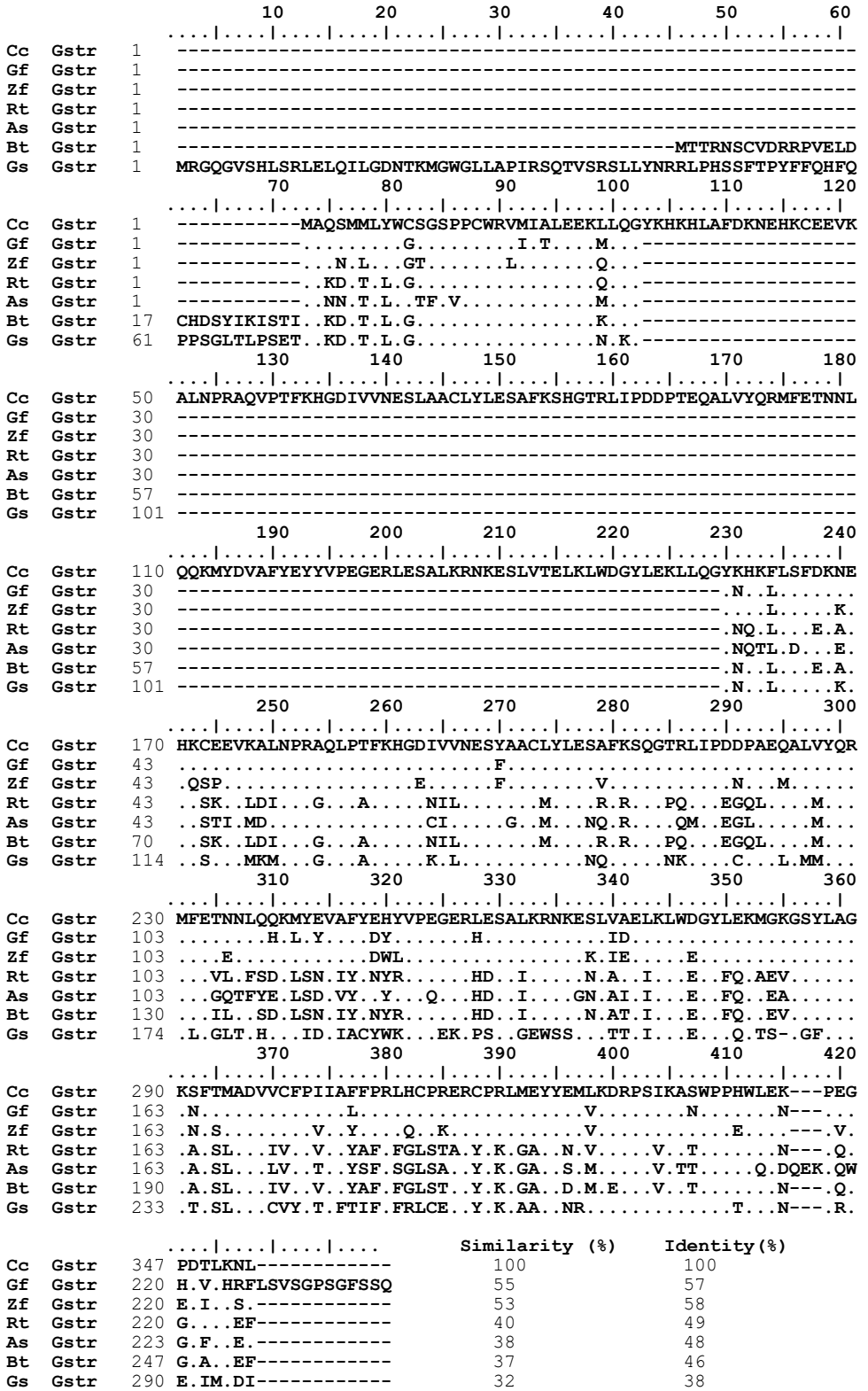

Figure 6. Similarity-identity rates between the brown trout (Bt) and the rainbow trout (Rt), Atlantic salmon (As), Gilthead seabream (Gs), zebrafish (Zf), and common carp (Cc), goldfish (Gf) *gstr* genes.

Husbandry and dissection of fish

The study obtained three adult female and three adult male brown trout, in addition to three female and three male common carp from the Faculty of Fisheries at Atatürk University.These fish were housed in a 100-liter aquarium at temperatures maintained at 29 ± 1°C for common carp and 9 ± 1°C for Brown trout. They were fed a commercial diet twice daily until they were fully satiated. The stocking density was set at 100 fish per cubic meter, and the pH level was maintained at 7.5 for common carp and 7 for Brown trout. A diurnal light: dark cycle of 12:12 hours was provided by fluorescent lighting. Molecular analyses were conducted at the Agricultural Biotechnology Laboratory. In the study, liver, intestinal muscle, brain, heart, eye, spleen, gill, kidney, stomach, and gonad samples were taken from all fish. The samples were placed in 2 ml Eppendorf tubes containing 1 ml of RNA later and stored at +4°C for 24 hours and then at -80°C until the day of analysis. Prior to sample collection, the fish were anesthetized using clove oil. Before the dissection process, the dissecting instruments and the work area were sterilized and cleaned using RNase ZAP (Invitrogen™). The entire study was conducted in accordance with the rules of the Local Ethics Committee for Animal Experiments at Atatürk University (29.04.2021/E-75366018-000-2100117626).

RNA isolation and reverse transcriptase (RT) and real-time PCR (qPCR) analysis

To extract total RNA, liver and gill tissue samples were taken out of RNAlater and homogenized using trizol reagent (Life Technologies). The concentration of RNA was measured using a Nanodrop 8000 spectrophotometer, and the quality of the total RNA was assessed through agarose gel-electrophoresis. For cDNA synthesis, 2 µg of RNA from each tissue was utilized. The RNA underwent DNase treatment (DNase I, Amplification Grade, Life Technologies) and was then converted into cDNA using the High-Capacity cDNA Reverse Transcription Kit (Life Technologies). After RNA isolation, the isolated RNA samples were quantitatively analyzed using nanodrop measurements. RNA samples with quantities ranging from 800 to 1000 ng/µl and OD260/OD280 ratio between 1.8-2 were used. In cases where the RNA concentrations were high, dilutions were performed.

The quantity of brown trout and common carp *gstr* transcript (copy number/µL) was determined using the SYBR Green PCR Kit method on a qPCR instrument. Each qPCR tube contained 10 µL SYBR Green, 5 µL DNAse/RNAse-free water, 2 µL forward primer, 2 µL reverse primer, and 1 µL cDNA. For each sample, two replicates were performed, and a negative control was included in each analysis. The qPCR procedure consisted of an initial denaturation at 95°C for 15 minutes, followed by 40 cycles of denaturation at 95°C for 20 seconds, annealing at the optimum temperature determined for each gene for 30 seconds, and elongation at 72°C for 30 seconds.

Statistical analysis

The statistical analyses were conducted using GraphPad Prism 9 software in the United States. The data underwent one-way ANOVA, and significance was determined using Duncan's multiple range post hoc test. These statistical tests were used to compare the levels of *gstr* gene expression in different tissues of both brown trout and common carp. All data are presented as mean ± SEM. Values were considered statistically significant when $p < 0.05$.

RESULTS

Bioinformatics studies of *gstr* gene in brown trout and common carp

The *gstr* gene and other genes such as *adgrb1b*, *eomesa, nrm, tmem65, msto1, akap9, scrt1a,* and *tert* which are conserved among these organisms, were found on chromosome 36 in brown trout, chromosome 1 in common carp, and chromosome 19 in zebrafish.

The in-silico analysis of the *gstr* gene in brown trout and common carp aimed 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 brown trout *gstr* gene has 7 exons and 6 introns, while common carp *gstr* gene has 6 exons and 5 introns, both with a highly conserved exon-intron organization. Alignment analysis of the brown trout and common carp Gstr/GSTR sequences using CLUSTAL W revealed that the polypeptide identity and similarity rates between brown trout and other species, such as rainbow trout, Atlantic salmon, sea bream, Chinook salmon, Coho salmon, and common carp, were quite high. Similarly, the polypeptide identity and similarity rates between common carp and goldfish, zebrafish, rainbow trout, Atlantic salmon, brown trout, and sea bream were also quite high. The analysis also revealed that the brown trout *gstr* gene shared the highest similarity and identity rates with rainbow trout, while common carp *gstr* gene had the highest similarity and identity rates with goldfish.

Tissue-specific transcription of gstr gene in brown trout and common carp

In this study, the tissue-specific distribution of the *gstr* gene was determined in female and male brown trout and common carp using qPCR (Figure 7). For female brown trout, the tissue-specific distribution of the *gstr* gene was determined as follows: liver 25.66 ± 1.49, intestine 13.68 ± 0.61, muscle 0.42 ± 0.02, brain 1.73 ± 0.39, heart 2.89 ± 0.43, eye 2.35 ± 0.18, spleen 0.96 ± 0.21, gill 14.27 ± 0.82, kidney 0.98 ± 0.17, stomach 1.25 ± 0.15, and ovary 7.39 ± 0.32. For male brown trout, the tissue-specific distribution was determined as follows: liver 32.60 ± 1.70, intestine 22.08 ± 0.59, muscle 4.01 ± 0.25, brain 1.38 ± 0.15, heart 4.79 ± 0.25, eye 1.84 ± 0.13, spleen 1.33 ± 0.08, gill 24.70 ± 1.14, kidney 1.83 ± 0.08, stomach 2.74 ± 0.13, and testis 15.86 ± 0.83. The results showed that the liver had higher gene expression than all other tissues, and the intestine and gill

had significantly higher gene expression than the liver in both female and male brown trout.

The ovary and testis tissues had the third-highest *gstr* gene expression. When the transcriptional differences between male and female tissues were examined, it was observed that the intestine, gill, kidney, stomach, muscle, and gonads had significantly higher expression in male brown trout, while other tissues did not show significant differences between male and female brown trout.

The tissue-specific distribution of the *gstr* gene in common carp (Figure 8) was also determined, and the *gstr* gene in female common carp showed the following expression levels: liver 39.06 ± 3.63, intestine 29.48 ± 2.98, muscle 19.32 ± 1.32, brain 4.07 ± 0.50, heart 5.96 ± 0.39, eye 2.11 ± 0.08, spleen 1.09 ± 0.093, gill 8.43 ± 0.33, kidney 1.79 ± 0.21, stomach 3.00 ± 0.43, and ovary 12.11 ± 0.62.

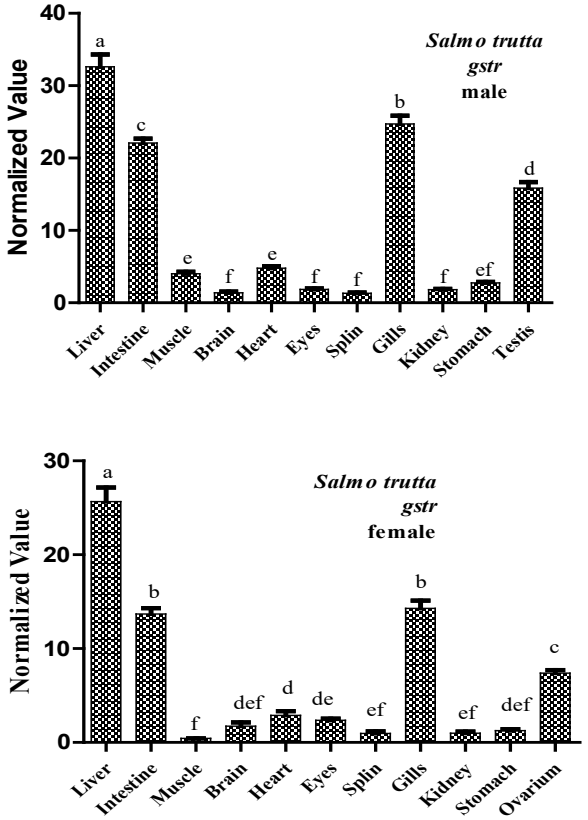

Figure 7. The tissue-specific distribution of brown trout *gstr* gene

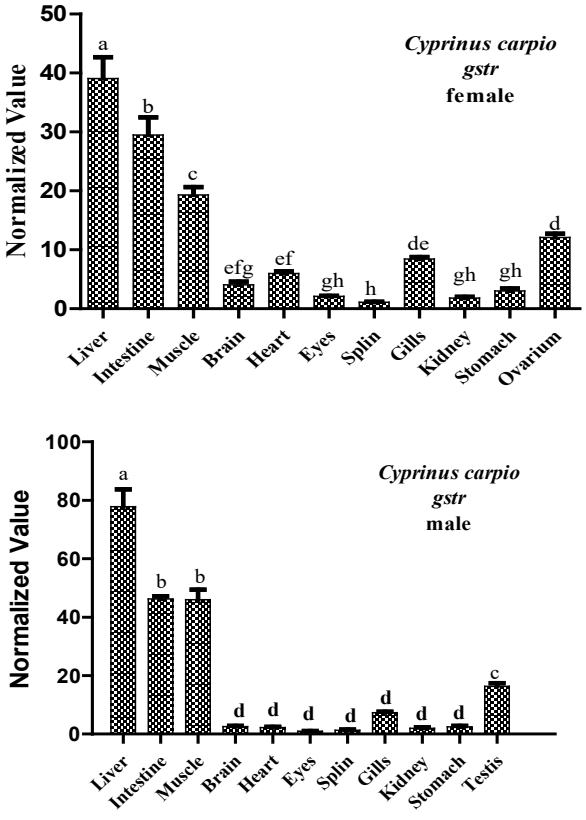

Figure 8. The tissue-specific distribution of common carp *gstr* gene

In male common carp, the tissue-specific distribution showed the following expression levels: liver 77.81 ± 5.95, intestine 46.25 ± 0.91, muscle 45.95 ± 3.42, brain 2.56 ± 0.23, heart 2.20 ± 0.25, eye 0.92 ± 0.081, spleen 1.32 ± 0.18, gill 7.27 ± 0.37, kidney 1.99 ± 0.27, stomach 2.46 ± 0.33, and testis 16.29 ± 1.16. The highest gene expression was observed in the liver for both female and male common carp, while the second-highest gene expression in females was in the intestine, and in males, it was in both the intestine and muscle. The brain, eye, spleen, kidney, heart, and gill tissues showed significantly lower *gstr* gene expression in both female and male common carp. In brown trout, the *gstr* gene exhibits the highest gene expression in the liver tissue in both females and males ($p<0.05$), while the intestine and gills are identified as tissues with the second-highest gene expression.

The differences between these two tissues are statistically insignificant in both female and male fish. The results indicate that the liver has the highest gene expression among all tissues, the intestine and gills have significantly lower *gstr* gene expression compared to the liver, and ovaries and testes have the third-highest *gstr* gene expression. When examining transcriptional differences between genders, the intestine, gills, kidney, stomach, muscle, and gonads show significantly higher gene expression in males ($p<0.05$), while the differences among other tissues are statistically not significant.

In common carp, the *gstr* gene shows the highest gene expression in the liver tissue in both females and males ($p<0.05$). In females, the intestine has the second-highest gene expression, while in males, both the intestine and muscle tissues exhibit the highest gene expression.

DISCUSSION

Gene expression analysis to determine the effects of various sources of stress on cells compared to healthy cells is commonly used in the diagnosis and treatment of disease

(Aubrecht and Caba, 2005). This approach can also be used to develop compounds that bind to expressed proteins and to identify transcriptional regulators that cause changes in expression levels. The common carp (*Cyprinus carpio*) and brown trout (*Salmo trutta*) will be used in this study to identify and characterize *gstr* gene which is antioxidant enzyme (AE) gene and to determine the biological significance of a signaling pathway. The tissue-specific distribution of the glutathione s transferase (*gstr*) gene in common carp and brown trout will be studied, and the results will be used as essential and fundamental precursor data for other studies. Antioxidant enzymes play a vital role in the antioxidant defense system in biological systems. Therefore, this study will be important for developing gene therapy for stress-induced diseases in the future.

Bioinformatics Studies of of *gstr* gene in brown trout and common carp

The designed conserved gene synteny indicates that the *gstr* gene in brown trout and common carp resulted from teleost whole-genome duplication (TTGD). Based on the synteny, it can be said that the conservation rate of the *gstr* gene is quite high (Figure 1). After the teleost-specific genome duplication in teleost fish, many genes have duplicate copies (Braasch and Postlethwait, 2012). However, it was determined that both brown trout and common carp have only one copy of the *gstr* gene. Therefore, it is suggested that this gene underwent duplication first and then one of the copies was lost.

In brown trout and common carp, in-silico analyses were conducted to characterize and identify the *gstr* gene. Especially, valuable data for developing molecular strategies to protect against the effects of reactive oxygen species in cultured fish were obtained and presented to the scientific community. In this study, the *gstr* gene in the brown trout and common carp genomes was found to have 7-6 and 6-5 exon-intron counts, respectively, based on Ensembl database searches. Alignment analyses of the *gstr* gene of brown trout and common carp with their orthologs in rainbow trout, Atlantic salmon, sea bream, sea bass, Coho salmon, and common carp, and Japanese medaka, zebrafish, rainbow trout, Atlantic salmon, brown trout, and sea bream, respectively, using CLUSTAL W (Thompson et al., 1994) revealed that brown trout has high identity and similarity rates with rainbow trout (Figure 5). On the other hand, common carp was found to have the highest identity and similarity rates with Japanese medaka (Figure 6).

Tissue-specific transcription of *gstr* gene in brown trout and common carp

Genetic expression changes are primary responses in fish, making genomic analyses a valuable advantage for research, and measurements of gene expression could facilitate the early detection and assessment of adverse effects on fish caused by various stressors (Larsen et al., 2010; Rojas-Hernandez et al., 2019). Approaches to gene expression have the potential to identify sensitive, mechanism-based biomarkers that can also reveal long-term harmful effects (Voelker et al., 2007). When examining the applications of genomic analysis in aquaculture, it has been observed that responses to stress factors might involve not only small changes in gene expression but also a series of gene interactions (Guo et al., 2023). Core genes generally regulate metabolic pathways, and alterations in these core genes can lead to various outcomes observable through genomic responses (Papin et al., 2003).

CONCLUSION

In conclusion, genomic analysis and measurements of gene expression are valuable tools for assessing the effects of stressors on fish and identifying sensitive, mechanism-based biomarkers that can reveal long-term harmful effects. The *gstr* gene exhibits the highest gene expression in the liver tissue of both brown trout and common carp, with statistically significant differences observed between tissues. Additionally, transcriptional differences between genders were observed in several tissues. The importance of examining gene interactions and alterations in core genes that regulate metabolic pathways when examining responses to stress factors in fish. Overall, the use of genomic analysis and gene expression measurements can provide valuable insights into the health of aquatic ecosystems and the effects of environmental contaminants on fish populations.

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AUTHOR CONTRIBUTIONS

The manuscript, produced from Badrul Islam Elsevar's master thesis, involves collaborative contributions from the authors. Badrul Islam Elsevar has taken on responsibilities such as literature review, drafting, writing, laboratory experiments, and data analysis and management. Meanwhile, the role of another author, referred to as Mehtap Bayır, includes conceptualization, drafting, writing, review, editing, and supervision. It is important to note that all authors have collectively reviewed and endorsed 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. Approval was granted by the Local Ethics Committee for Animal Experiments of Atatürk University (27.05.2021/No:127)

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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