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Seasonal variation in the expression pattern of heat shock protein 70 and 90 in Common carp (*Cyprinus carpio*) from Karataş Lake, Burdur, Türkiye

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Abstract

Organisms have evolved defense mechanisms to protect themselves from stressful conditions. The expression of heat shock proteins is considered a valid indication of protection from the adverse effects of hostile conditions. In this study, we used immunohistochemistry to investigate the seasonal effects of some abiotic factors on heat shock protein 70 and 90 (HSP70 and HSP90) expression in the liver, gills, and muscle tissues of 24 Common carp (*Cyprinus carpio*) caught in Karataş Lake (Burdur, Türkiye) using gillnets of various mesh sizes. We also measured some physicochemical parameters *on-site* at sampling time and took water samples for further analyses of other physicochemical parameters and heavy metals. Immunostaining for HSP90 was stronger than for HSP70 in both liver and gill samples. Liver and gill structures exhibited significant seasonal differences in HSP70 and HSP90 immunoreactivity, and the same was true for immunostaining for HSP70 and HSP90 in muscle samples. Some physicochemical properties seemed to vary considerably between seasons, with Fe, Mn, and Zn levels tending to exhibit changes throughout the seasons. However, these levels were considered acceptable for human health. In conclusion, this study suggests that substantial changes in HSP70 and HSP90 expression may be essential for seasonal adaptation and tolerance. Further research on fish HSPs would greatly contribute to aquaculture, which is essential for meeting food requirements.

Introduction

Organisms encounter various challenging environmental conditions that threaten their reproduction and survival.[1,2] To cope with these conditions, they have evolved multiple mechanisms including the formation of the heat shock response, which involves the synthesis of heat shock proteins (HSPs).[3] Under stressful conditions, cells express HSPs constitutively,[4-6] including heat shock protein 70 and 90 (HSP70 and HSP90). The HSP70 family consists of two subtypes: constitutive type HSC70 (heat shock cognate 70) and inducible type HSP70.[7,8] The HSP70 is activated depending on the stress situation, while in the absence of stress, cells express HSC70, which remains unaltered or only modestly expressed when stressed.[9] The HSP90 family consists mainly of four isoforms including two major cytosolic isoforms (HSP90α and HSP90β).[10,11] The HSP90α is involved in stress-induced cytoprotection, while HSP90β is involved in long-term cellular adaptation.[12]

Studies have reported that biotic and abiotic factors can influence HSP expression levels in aquatic poikilotherms.[13-15] Abiotic factors, in particular, can have a substantial impact on fish survival, development, and reproduction.[16] Therefore, it is critical to explore the impact of these parameters on HSP expression in aquatic species that are exposed to abiotic stress factors.[15]

Studies on direct or indirect exposure of fish to various environmental factors have revealed significant alterations in HSP expression.[13,15-21] For example, environmental stressors and varying temperature regimes have led to differential expression of HSP70 and HSP90 in the liver, gill, and muscle tissues of goldfish (*Carassius auratus*).[17] Similarly, in the Kaluga (*Huso dauricus*), HSP70 and HSP90 genes were highly expressed in liver, gill, and muscle tissues during low temperatures, suggesting that these proteins are important for survival during harsh winters.[15] In *Mugil cephalus* hepatocytes, HSP70 was overexpressed indicating that environmental stress and seasonal changes

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may have significant effects on HSP70 expression.[18] However, to the best of our knowledge, no study has investigated the effects of seasonal changes, heavy metals, and physicochemical parameters on HSP70 and HSP90 expression in selected Common carp (*Cyprinus carpio*) tissues in Karataş Lake (Burdur, Türkiye). Intensive agricultural activities around this lake have resulted in the extensive use of agrochemicals, synthetic fertilizers and pesticides,[22] rendering the lake ecosystem polluted and vulnerable considering that heavy metals tend to accumulate in aquatic habitats over time.[23-25] Since heavy metals in these agricultural substances can discharge into aquatic environments and induce the synthesis of cyto-protective proteins such as HSPs,[26] understanding altered HSPs expression is essential for investigating cytoprotection in aquatic organisms, especially fish. Therefore, we hypothesized that seasonal changes in temperature, heavy metal levels, and physicochemical parameters might affect HSP70 and HSP90 expression in Common carp liver, gill, and muscle tissues in Karataş Lake.

Materials and Methods

Study area. Karataş Lake is a shallow lake located in Karamanlı district of Burdur at 37°23'N, 29°58'E. The lake is primarily used for agricultural irrigation and has great potential for fisheries activities. However, the increasing amount of water used for irrigation has caused the lake's level to drop and its area to shrink. Currently, only a few fish species, including Common carp (*Cyprinus carpio*), inhabit this lake.[23]

Sampling and tissue processing. The study was approved by the Animal Experiments Local Ethics Committee of Sivas Cumhuriyet University (proposal No. #65202830-050-04.04-74/06.08.2015) and all animal use was performed in accordance with the guidelines of the International Association for the Study of Pain. A total of 24 live fish samples with an average length of 27.00 cm and an average weight of 460 g) were collected from Karataş Lake by fishermen using multifilament nylon gill nets of different mesh sizes (ranging from 20.00 to 30.00 mm) in the winter (January), spring (April), summer (July), and autumn (October) of 2018, with six fish collected for each season. Common carps were firstly acclimatized to laboratory conditions to avoid transporting stress, before being placed into tanks containing quinaldine sulfate (25.00 mg L^{-1}) and anaesthetized for 1 - 4 min.[27] Liver, gill, and muscle tissue samples were then immersed into 10.00% neutral buffered formalin for 24 hr at room temperature and dehydrated through graded alcohols (70.00%, 80.00%, 96.00%, and 100%). The samples were cleared in xylene and embedded in paraffin wax for immunohistochemical evaluation.[28]

Immunohistochemistry. For immunohistochemistry, the peroxidase-anti-peroxidase method was used[28] following the antigen retrieval process by boiling in 0.01 M citrate buffer (Thermo Fisher Scientific, Fremont, USA), pH 6.00, for 20 min in a microwave oven at full power. After cooling for 20 min, the sections were soaked in 3.00% hydrogen peroxide in absolute methanol for 5 min to deactivate endogenous peroxidase activity. Non-specific binding was blocked with normal goat serum (Sigma-Aldrich, Darmstadt, Germany), and the sections were incubated with primary antibodies, HSP70 (1:300 dilution; Sigma-Aldrich) and HSP90 (1:300 dilution; Sigma-Aldrich) at 4.00 ˚C overnight. The slides were then incubated with anti-rabbit IgG (whole molecule)-peroxidase antibody (1:200 dilution; Sigma-Aldrich) for 30 min. followed by incubation with a peroxidase anti-peroxidase soluble complex antibody (1:200 dilution; Sigma-Aldrich) for an additional 30 min. The resulting signal was developed with 3,3'-Diaminobenzidine (DAB; Thermo Fisher Scientific). The slides were counter-stained with Gill's Hematoxylin and mounted in Entellan™. Negative control slides were prepared by replacing the primary antibody with PBS.

Water physicochemical analysis. We conducted the physicochemical analysis of seasonal water samples by measuring various physicochemical parameters. On-site measurements of temperature, pH, and dissolved oxygen were taken using WTW Universal multi-parameter portable meter ProfiLine pH/Cond 3320 Set 2 (Fisher Scientific, Hampton, USA). For other physicochemical parameters, including PO_4, NO_2, NO_3, NH_4, SO_4, and Cl, water samples were collected in amber glass bottles and analyzed at Süleyman Demirel University Innovative Technologies Application and Research Center. The analysis values are presented in mg L^{-1}.[29]

Heavy metal analysis. For heavy metals analysis, we collected 50.00 mL of water samples into amber glass bottles and added 1.00 mL of nitric acid to the water sample *on-site*. The samples were transported to the measurement laboratory where they were subjected to mineralization and concentrated at 25.00 mL after pouring Kjeldahl flasks. The water samples were analyzed using an Atomic Absorption Spectrophotometer (Optima 5300 DV model ICP-OES; Perkin Elmer, Markham, Canada) by preparing the appropriate standards. The analysis values of heavy metals are expressed as ppm.[29]

Semiquantitative assessment of HSP70 and HSP90 staining. The stained slides were observed under a light microscope (BX51; Olympus, Tokyo, Japan) and photographed using a digital camera (DP26; Olympus, Tokyo, Japan). Immunostaining for HSP70 and HSP90 in carp liver, gills, and muscle was semiquantitatively assessed at 100× and 400× magnification, using a five-point intensity score (IS). The staining intensities were assigned scores as follows: 0 (-), negative; 1 (+), weak staining; 2 (++), moderate staining; 3 (+++), intense staining, and 4 (++++), very intense staining.

Statistical analysis. Statistical analysis was performed using GraphPad Prism (version 8.0.2; GraphPad Software Inc., San Diego, USA). Semiquantitative assessment scores of immunostainings were imported into the software and one-way ANOVA or two-way ANOVA was applied to determine if HSP70 and HSP90 immunostainings in selected tissues changed seasonally, followed by Tukey's post-hoc testing for multiple comparisons. All data are presented as the mean ± SEM and p values less than 0.05 were considered as statistically significant.

Results

The results of the immunohistochemical analysis showed season-dependent differential HSP70 and HSP90 staining patterns in Common carp liver, gill, and muscle samples. Semiquantitative assessment score results in the liver, gill, and muscle tissues are summarized in Table 1. Figure 1 depicts the statistical analysis results of semi-quantitative HSP70 and HSP90 scoring in the liver, gill, and muscle. Figure 2 demonstrates HSP70 and HSP90 staining patterns in the liver, gill and muscle tissues.

HSP70 expression in liver tissue. Hepatocytes showed a significant difference between winter and the other two seasons (spring and autumn) as demonstrated in Figure 1A ($p < 0.05$). However, bile ducts did not show any seasonal differences in HSP70 immunoreactivity (Fig. 1A, $p > 0.05$). In the hepatopancreas, there was a significant difference between summer and autumn, as well as between spring and winter (Fig. 1A, $p < 0.05$). Endothelial cells showed more significant differences in HSP70 immunoreactivity in the spring and autumn compared to hardly detectable immunoreactivity in winter and summer (Fig. 1A, $p < 0.05$). Notably, HSP70 immuno-reactivity was absent in erythrocytes as shown in the upper panel (liver) of Figures 2A, 2B, 2C, and 2D.

HSP90 expression in liver tissue. Hepatocytes exhibited more HSP90 immunoreactivity in the summer and autumn than in the winter and spring as demonstrated in Figure 1B ($p < 0 .05$). However, there was no significant difference in HSP90 immunoreactivity between summer and autumn, or between winter and spring. Bile ducts had greater autumn immunoreactivity for HSP90 than in the summer and winter, while spring HSP90 immunoreactivity was more prominent than in the winter (Fig. 1B, $p < 0.05$). The hepatopancreas displayed higher autumn HSP90 immunoreactivity than in the winter and spring, with more detectable summer HSP90 immunoreactivity compared to the spring (Fig. 1B, $p < 0.05$). Endothelial cells had more prominent spring HSP90 immunoreactivity than in the summer and winter (Fig. 1B, $p < 0.05$). Winter HSP90 immunoreactivity was higher in erythrocytes than in the spring (Fig. 1B, $p < 0.05$). However, winter HSP90 immunoreactivity was higher in erythrocytes than in summer and autumn, although this difference was statistically insignificant (Figs. 2E, 2F, 2G, and 2H, upper panel: Liver).

HSP70 expression in gill tissues. Chloride cells showed more spring immunoreactivity to HSP70 than in other seasons, with statistically significant seasonal changes as demonstrated in Figure 1C ($p < 0.05$). Epithelial cells displayed no seasonal difference in immunoreactivity, although there was a difference between winter and summer (Fig. 1C, $p < 0.05$). Endothelial cells had a more

Table 1. The results of semiquantitative assessment scores of HSP70 and HSP90 immunoreactivity in carp liver, gill, and muscle tissues from Karataş Lake (Burdur, Türkiye).

Tissue / Protein		Spring	Summer	Autumn	Winter
HSP70 in liver	Hepatocytes	++/+++	++	++/+++	+/++
	Bile ducts	++	++/+++	++	++/+++
	Hepatopancreas	++	+++	+++/++++	++
	Endothelial cells	+	-/+	+	-/+
	Erythrocytes	-	-	-	-
HSP90 in liver	Hepatocytes	+/++	+++	+++	+/++
	Bile ducts	++/+++	++	+++	++
	Hepatopancreas	++	+++	+++/++++	++/+++
	Endothelial cells	+++/++++	++	+++	++/+++
	Erythrocytes	+	+/++	+/++	++
HSP70 in gill	Chloride cells	++/+++	+	+/++	-/+
	Epithelial cells	++	++/+++	++	+/++
	Endothelial cells	-/+	+/++	-/+	-/+
	Erythrocytes	+/++	+/++	+/++	-/+
HSP90 in gill	Chloride cells	+/++	+++	++/+++	+++/++++
	Epithelial cells	+++	+++	++/+++	++++
	Endothelial cells	++/+++	++/+++	++/+++	+++/++++
	Erythrocytes	+/++	+/++	++/+++	+
HSP70 in muscle	Muscle cells	++	+/++	+/++	+/++
HSP90 in muscle	Muscle cells	++/+++	++/+++	+/++	+

The immunoreactivity intensities are as follows: -, negative; +, weak staining; ++, moderate staining; +++, intense staining; ++++, very intense staining.

significant apparent immunoreactivity to HSP70 in summer (Fig. 1C, $p < 0.05$). In the winter, erythrocytes had weaker immunoreactivity to HSP70 than in other seasons (Fig. 1C, $p < 0.05$), (Figs. 2A, 2B, 2C, and 2D, middle panel: Gills).

HSP90 expression in gill tissue. Chloride cells exhibited prominent immunoreactivity to HSP90 in the summer and winter, with lower immunoreactivity in the spring (Fig. 1F, $p < 0.05$). Epithelial cells exhibited seasonal changes in HSP90 expression only during the winter and autumn (Fig. 1F, $p < 0.05$). Endothelial cells displayed lower HSP90 immunoreactivity in the winter, with a significant difference between autumn and winter, but no detectable variation between other seasons (Fig. 1F, $p < 0.05$). Erythrocytes exhibited a significant difference between autumn and other seasons (Fig. 1F, $p < 0.05$), (Figs. 2E, 2F, 2G, and 2H, middle panel: Gills).

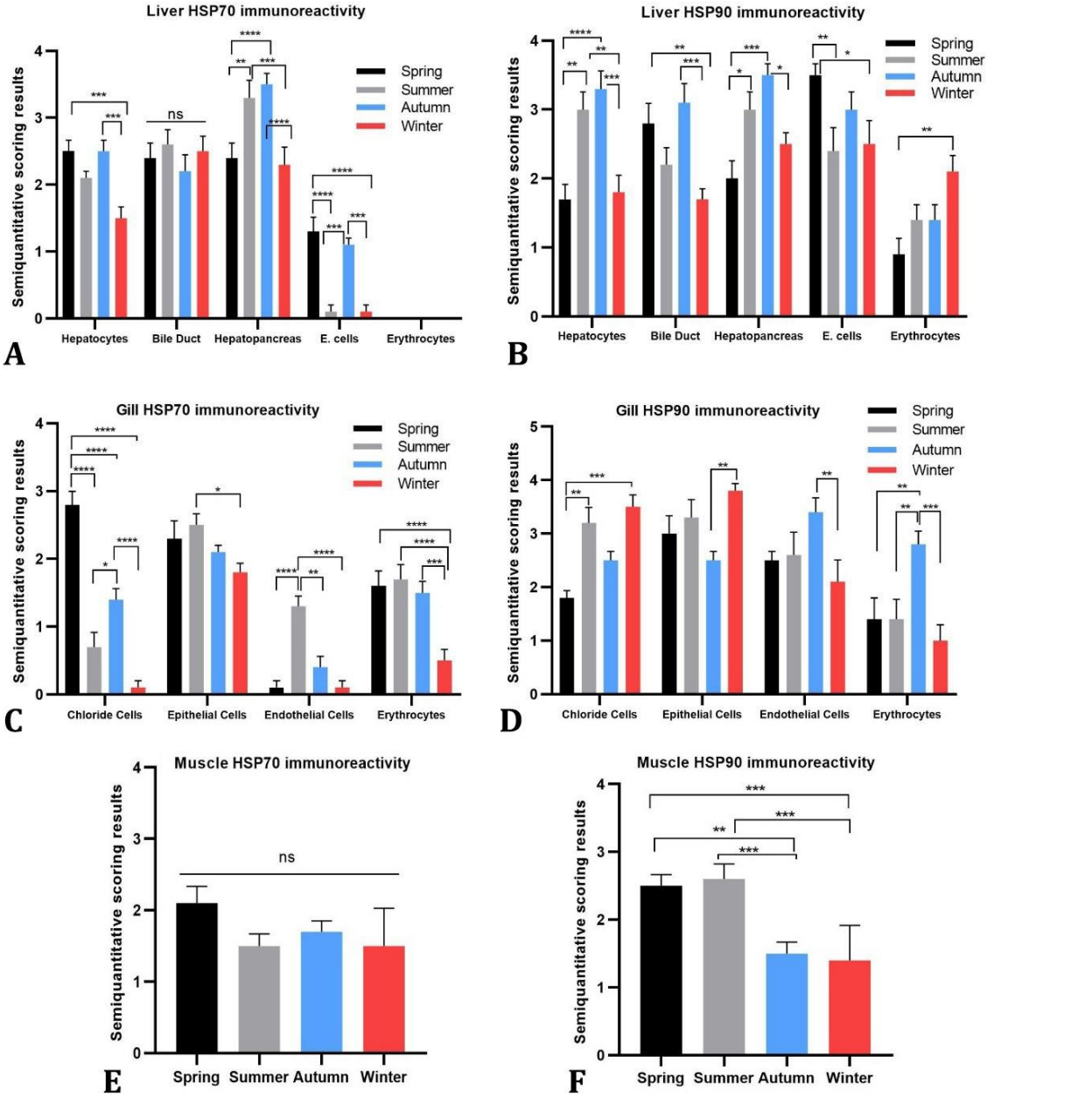

Fig. 1. Statistical analysis results revealed seasonal changes in HSP70 and HSP90 immunoreactivity in Common carp liver, gill, and muscle tissues. **A** and **B)** Seasonal changes in HSP70 and HSP90 immunoreactivity in hepatocytes, bile ducts, hepatopancreas, endothelial cells, and erythrocytes of liver tissue. **C** and **D)** Seasonal changes in HSP70 and HSP90 immunoreactivity in chloride cells, epithelial cells, endothelial cells, and erythrocytes of gill tissue. **E** and **F)** Seasonal changes in HSP70 and HSP90 immunoreactivity in muscle cells. The data shown are representative of ten images from each tissue per season, and the error bars represent the standard error of the mean (SEM). One-way ANOVA or two-way ANOVA with Tukey post-hoc testing was used for seasonal comparisons of each tissue. *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$, ****$p < 0.001$.

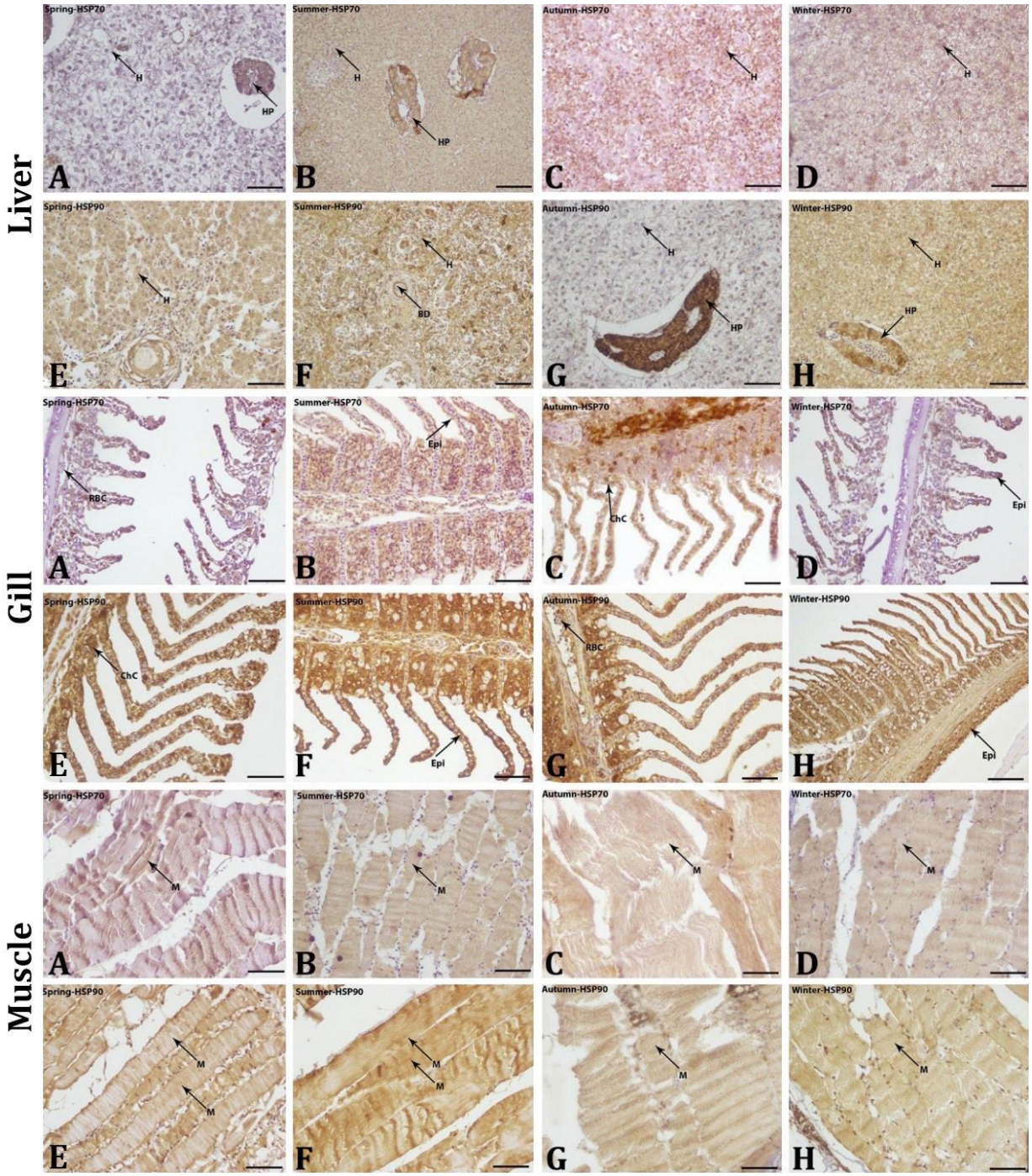

Fig. 2. Upper panel: Seasonal HSP70 (A-D) and HSP90 (E-H) immunoreactivity in Common carp liver tissue. **A)** Weak to moderate reaction in hepatocytes; moderate to strong reaction in hepatopancreas. **B)** Moderate to strong reaction in hepatocytes and hepatopancreas. **C)** Strong to very strong reaction in hepatocytes. **D)** Moderate to strong reaction in hepatocytes. **E)** Moderate reaction in hepatocytes. **F)** Moderate and strong reaction in bile ducts and hepatocytes. **G)** Very strong reaction in hepatopancreas. **H)** Strong reaction in the hepatopancreas and weak reaction in hepatocytes. Abbreviations: BD, bile duct; H, hepatocytes; HP, hepatopancreas. Middle panel: Seasonal HSP70 (A-D) and HSP90 (E-H) immunoreactivity in carp gill tissue. **A)** Moderate reaction in the epithelial cells. **B)** Moderate reaction in red blood cells. **C)** Strong reaction in the epithelial cells. **D)** Strong reaction in the chloride cells. HSP90 immunoreactivity in gills. **E)** Strong reaction in the epithelial cells. **F)** Strong reaction in the chloride cells. **G)** Strong reaction in the epithelial cells. **H)** Strong reaction in epithelial cells. RBC: red blood cells, Epi: epithelial cells, ChC: chloride cells. Lower panel: Seasonal HSP70 immunoreactivity in carp muscle tissue. **A** and **C)** Moderate reaction in muscle cells. **B** and **D)** Weak reaction in muscle cells. HSP90 immunoreactivity in muscle. **E** and **F)** Moderate to strong reaction in muscle cells. **G** and **H)** Weak to moderate reaction in muscle cells (M): M, muscle cells. For all panels above, arrows indicate immunoreactivity (Immunohistochemistry staining, bars = 50.00 µm).

HSP70 expression in muscle tissue. Spring HSP70 immunoreactivity was more prominent than in the other seasons, as shown in Figure 1E, although this difference was statistically insignificant ($p > 0.05$), (Fig. 2A, B, C, and D, lower panel: Muscle).

HSP90 expression in muscle tissue. Both spring and summer HSP90 immunoreactivity were more prominent than in winter and autumn (Fig. 1F, $p < 0.05$). Furthermore, spring HSP90 immunoreactivity was similar to that of summer and the same was true for winter and autumn results (Figs. 2E, 2F, 2G, and 2H, lower panel: Muscle).

Water physicochemical analysis. The upper part of Table 2 summarizes the physicochemical analysis results of water samples. Some parameters exhibited considerable seasonal differences, which were reflected in the immunohistochemical results.

Heavy metal analysis. The lower part of Table 2 demonstrates the heavy metal analysis results of water samples. Based on the reference values provided by World Health Organization (WHO), levels of heavy metals were found to be below the permissible level.[30] The levels of most heavy metals did not appear to differ significantly, except for Fe, Mn, and Zn. However, Fe levels exhibited seasonal variation, with higher levels observed in autumn. Additionally, Mn and Zn levels tended to be higher in autumn compared to other seasons.

Table 2. Analysis results of physicochemical parameters and heavy metals in Karataş Lake (Burdur, Türkiye).

Parameters	Spring	Summer	Autumn	Winter
Physicochemical analysis				
Dissolved oxygen (mg L^{-1})	5.75	4.90	6.55	9.20
Temperature (˚C)	14.70	28.90	19.40	7.60
pH	9.15	9.65	9.02	8.70
PO_4 (mg L^{-1})	0.12	0.07	0.05	0.005
NO_2 (mg L^{-1})	0.05	0.04	0.007	0.015
NO_3 (mg L^{-1})	0.45	0.9	25.198	0.45
NH_4(mg L^{-1})	0.28	0.66	5.093	0.22
SO_4 (mg L^{-1})	546	552	170.2	170
Cl (mg L^{-1})	21.30	67.45	65.53	10.65
Heavy metals analysis				
Cd (228.802 nm)	<0.005	<0.005	<0.005	<0.005
Cr (267.716 nm)	<0.006	<0.006	0.008	<0.006
Cu (327.393 nm)	<0.004	<0.004	<0.004	<0.004
Fe (238.204 nm)	0.520	< 0.008	0.473	0.203
Mn (257.610 nm)	< 0.003	< 0.003	<0.012	< 0.003
Mo (202.031 nm)	<0.010	<0.010	<0.010	<0.010
Pb (220.353 nm)	<0.008	<0.008	<0.008	<0.008
Zn (206.200 nm)	<0.004	<0.004	<0.022	<0.004

All values for heavy metals are in ppm. According to WHO (2017), maximum allowable limits of heavy metal in water are Cd: 0.01, Cr: 0.55, Cu: 0.017, Fe: 0.50, Mn: 0.20, Pb: 0.065, Zn: 0.20.[30]

Discussion

This study has demonstrated considerable heterogeneous HSP70 and HSP90 expression in selected Common carp tissues in Karataş Lake. Differential expression patterns of HSP70 and HSP90 could be attributed to seasonal changes in temperature, heavy metal levels, and physicochemical characteristics of the lake. These findings highlight the potential role of HSPs as biomarkers for monitoring the health status of fish populations in aquatic ecosystems.

The liver is known to be affected by contaminant concentrations in the water,[31,32] with heavy metal exposure increasing concentration-dependent HSP70 expression in liver cell line studies.[33] However, other studies have reported no significant difference in HSP70 expression of either juvenile or adult rainbow trout liver samples after exposure to heavy metals.[34] Pollution has been shown to increase HSP70 expression in the liver of milkfish[35] and exposure to sublethal doses of $CdCl_2$ has been found to increase HSP70 mRNA expression in the liver of Persian sturgeon.[36] Spatiotemporal changes in heavy metal levels across aquatic systems may be caused by various factors, including atmospheric deposition, erosion of the geological matrix, and anthropogenic sources such as industrial effluents and mining waste. During rainy seasons, there may be a significant influx of water that contributes substantially to the release of pesticides, agrochemicals, fertilizers, and heavy metals into the water systems.[37-40]

The tendency to increase Fe levels in the winter, spring, and autumn, as well as Mn and Zn levels in the autumn, could have led to increased HSP70 and HSP90 expression in the liver of fish in Karataş Lake. The complex interaction of heavy metals, especially Fe, Mn, and Zn, may also affect HSP70 and HSP90 expression in the carp liver. Furthermore, even if concentrations of heavy metals are generally low, the exposure to their complex mixtures might lead to toxic effects that induce HSP production.

Previous studies have reported a time-dependent increase in HSP70 expression in the liver of *Cirrhinus mrigala* exposed to 37.00 ˚C for different periods.[41] A significant winter upregulation of HSP70 was detected in goldfish liver, whereas spring upregulation was insignificant.[17] However, their findings are inconsistent with our results, which reflect alterations in HSP expression in a species, habitat, and temperature-dependent fashion. They also found that HPS70 expression was noticeably, progressively augmented in grey mullet liver in response to environmental stressors with higher expression during summer (April-September) than during the monsoon season (October-March).[18] In our study, we observed a significant difference in HPS70 findings in the winter and autumn, which are negatively correlated.[18]

Authors reported that short-term exposure augmented more HSP70 and HSP90 expression in the liver of murrel compared with long-term exposure.[14] They concluded that HSP70 and HSP90, respectively, are needed for the long-term and immediate survival of fish at high temperatures. In a study examining the impacts of temperature and

salinity on HSP70 and HSP90, investigators reported that, Kaluga (*Huso dauricus*) liver, HSP70 and HSP90 expression increased gradually by cold- and heat stress, and plateaued at 4.00 ˚C and 28.00 ˚C, respectively.[15] They also noticed that liver HSP70 expression maximized at 40.00 ppt salinity while liver HSP90 expression plateaued at 20.00 ppt salinity.

A study found heat-induced HSP70 expression in rainbow trout liver to be higher at 24.00 ˚C than at 18.00 ˚C revealing tissue-specific expression profiles of HSP70.[16] Variations in HSP expression might indicate cellular alterations at molecular levels making HSPs as a good mediator of cellular insult due to their variable expression nature. The presence of HSP70 and HSP90 expression showed that the stress caused by contaminants in Karataş Lake could induce their synthesis. Furthermore, extreme temperatures could induce HSP70 and HSP90 expression, suggesting that HSP synthesis protects cells against damage that may occur at these extremes. Our finding of HSP70 and HSP90 expression in the liver appeared to be positively correlated with winter temperatures, which is consistent with the findings of the Kaluga liver.[15] However, their expressions have a negative correlation with summer temperatures. The reason for these contradictions could be the different responses of HSP70 and HSP90 proteins to diverse stressful factors in the ecosystems. As mentioned above, the literature reports conflicting results about HSP70 and HSP90 expression across different fish species.

The gills are one of the first organs directly exposed to harmful stressors in aquatic environments and are vulnerable to those stressors owing to their large surface. Therefore, the gills are considered the most suitable indicator of water pollution levels.[42] It has been reported that a combination of heavy metals taken up via the diet and water significantly increased HSP70 expression in the gills of juvenile rainbow trout, and high levels of heavy metals in gills might be a physiological indicator of stress.[34] A study showed that the black sea bream gills expressed much HSP70 protein depending on increased water salinity.[43] An investigation revealed a time-dependent increase in HSP70 expression in the gills of *Cirrhinus mrigala* fish exposed to 37.00 ˚C for different periods.[41] It has been shown that hypersalinity leads to the over-expression of HSP70 in the gills of black-chinned tilapia.[13] The HSP70 expression in the gills of fish from polluted areas was more prominent than in less polluted areas.[35] Exposure to sublethal doses of $CdCl_2$ increased HSP70 mRNA expression in the gills of Persian sturgeon.[36] In our study, high levels of Fe in the winter and autumn and high levels of Mn and Zn in autumn could reduce the production of HSP90. The essential roles of those metals in biological systems might explain this reverse correlation between HSP90 and Fe, Mn, and Zn. However, differential- and seasonal HSP90 expression may also arise from other factors; therefore, it would not be an appropriate approach to generalize the results of different fish species and regions to our findings.

A study reported that, in the Kaluga gill, HSP70 and HSP90 expression was augmented gradually by cold stress, with a peak at 4.00 ˚C.[15] Heat stress caused a gradual rise in HSP70 and HSP90 expression in gills, with a maximal expression of HSP70 at 28.00 ˚C. However, heat stress trials did not significantly change HSP90 expression in the gills. They also observed an undulating expression pattern of HSP70 and HSP90 in gills, of which Hsp70 expression reached a peak at 40.00 ppt salinity and HSP90 expression at 20.00 ppt salinity. In our study, concentrations of parameters (PO_4, NO_2, NO_3, NH_4, SO_4, and Cl) with the potential to form salts varied seasonally. The seasonal changes in their concentrations might only explain the differential expression of HSP90 in gills. However, different outcomes regarding HSP70 and HSP90 expression among fish species pose a significant challenge in drawing general inferences about their distribution.[13,15,35,36,41] Thus, whether these parameters impact HSP70 and HSP90 proteins expression alone, combined, or cumulatively merits further research.

The muscles are the major edible parts of fish that are important for the human diets. Therefore, determination of the levels of heavy metals accumulating in fish muscle via the food chain and water is essential for human health. The number of positively stained muscle cells increased in polluted areas and HSP70 expression was more noticeable in the muscle of fish from more polluted areas than in less polluted areas.[35] Levels of both HSPs tended to be higher in Karataş Lake in the autumn, consistent with their results.[35] The high levels of HSP70 and HSP90 in Karataş Lake in the autumn could be due to the high levels of Fe and Zn. Interplay between heavy metals, especially in the autumn, could also lead to variable HSP70 and HSP90 expression. Gradually increasing water temperature caused a significant increase in HSP70 mRNA expression in the muscle of Iberian fishes, with a species-specific difference in muscle HSP70 expression.[2] However, our HSP70 findings are inconsistent with their results.[2] This difference in HSP70 expression could be a species-specific or adaptational situation.

A study found that, in the Kaluga muscle, HSP70 and HSP90 expression increased gradually by cold stress and reached a peak at 4.00 ˚C.[15] Under heat stress, HSP70 was more expressed in the Kaluga muscle at 25.00 ˚C than at 28.00 ˚C. However, muscle HSP90 expression did not significantly differ from the control. They also observed that muscle HSP70 and HSP90 expression showed a gradual increment, with a plateau at 40.00 ppt salinity, in a concentration-dependent manner. However, there are some differences between our results and their findings.[15] The Kaluga fish inhabits its natural habitat from 0.00 ˚C to 25.00 ˚C, at which muscle HSP70 and HSP90 expressions peak.[15] However, carp has better adapted to its habitat

between 27.00 ˚C and 31.00 ˚C.[44] These observations suggest that these two species have adapted to different temperature intervals, with adaptable differences in their HSP70 and HSP90 expressions. HSP70 expression in muscles of *Fundulus grandissimus* and *Fundulus persimilis* varies in a species, habitat, and temperature-dependent manner.[20] They concluded that HSP70 expression could affect the habitat preference of *Fundulus* species.[20]

In conclusion, this study shows that there could be considerable differences in HSP70 and HSP90 expressions in carp liver, gill, and muscle according to season, temperature, and tissue, with enormous differences reported by previous studies regarding the expression of HSP70 and HSP90. Therefore, further investigations into the roles of HSPs would deepen our understanding of their successful adaptation to extreme environmental conditions.

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Conflict of interest

Authors have no conflict of interest to declare.

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