Int. J. Aquat. Biol. (2025) 13(3): 59-67
ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com

Original Article

Sublethal effects of malathion on behavior and hepatic gene expression of HSP70 and CYP1A in Persian Sturgeon (*Acipenser persicus*) fingerlings

Mina Rahbar[*1,5], Masoud Sattari[*1,2], Hamid Alaf Noverian[1,2], Mohaddeseh Ahmadnezhad[3], Hossein Khara[4], Roghieh Safari[5]

[1]Department of Fisheries, Faculty of Natural Resources, University of Guilan, Sowmeh Sara, Guilan, Iran.
[2]Department of Marine Sciences, Caspian Sea Basin Research Center, University of Guilan, Rasht, Iran.
[3]Inland Waters Aquaculture Research Center, Iranian Fisheries Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Bandar Anzali, Iran.
[4]Department of Fisheries, La.C., Islamic Azad University, Lahijan, Iran.
[5]Department of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

Abstract: Malathion, an organophosphate pesticide widely used in agriculture, poses significant risks to aquatic ecosystems. This study investigated the sublethal effects of malathion on hepatic HSP70 and CYP1A gene expression in fingerling Persian sturgeon (*Acipenser persicus*). Fish were exposed to 0.05, 0.1, and 0.2 mg L^{-1} malathion (corresponding to sublethal fractions of the LC_{50}) for 7 days. Liver samples were collected at 1, 2, 4, and 7 days post-exposure, and gene expression was quantified using real-time polymerase chain reaction (PCR). Behavioral responses and mortality were recorded. No mortality occurred in any group; however, fish exposed to 0.2 mg L^{-1} malathion exhibited reduced feeding, impaired swimming balance, and decreased activity, especially toward the end of exposure. HSP70 expression peaked on day 1 at the highest concentration (15.7-fold higher than the control) and then gradually declined. CYP1A expression showed a similar dose-dependent pattern, with the highest induction (19.29-fold) observed on day 4 in the 0.2 mg/L group. Heat map visualization confirmed temporal and dose-dependent expression dynamics. A significant positive correlation (r = 0.73, $P<0.0001$) between the expression levels of HSP70 and CYP1A suggested a coordinated stress response to malathion exposure. These results demonstrate malathion-induced modulation of oxidative stress and detoxification genes in Persian sturgeon, underscoring the utility of these molecular biomarkers for monitoring organophosphate pollution in aquatic species.

Article history:
Received 5 April 2025
Accepted 23 June 2025
Available online 25 June 2025

Keywords:
Organophosphate pesticide
Behavioral responses
Molecular biomarkers
Toxicology

Introduction

The Persian sturgeon, *Acipenser persicus*, an endemic species of the Caspian Sea, is one of the ecologically and economically valuable fish. This species not only plays a crucial role in the Caspian Sea ecosystem but also makes a significant contribution to the global caviar industry. However, its population has dramatically declined in recent decades due to overfishing, habitat degradation, and pollution (Pourkazemi, 2006; Moghim et al., 2016). Among these threats, the contamination of aquatic environments with agricultural pesticides has become a growing concern.

Malathion, a widely used organophosphate pesticide, is known for its effectiveness in pest control but is also highly toxic to non-target aquatic organisms (Suchiang, 2021). Runoff from agricultural areas introduces this compound into freshwater systems, where it can accumulate and adversely affect fish populations (Deka and Mahanta, 2016; Rahbar et al., 2021a). In fish, malathion exposure has been linked to oxidative stress (Rahbar et al., 2021a), disruption of cellular homeostasis (Ghafarifarsani et al., 2023), and altered gene expression (Prathibha et al., 2014; Ullah et al., 2018; Cui et al., 2025). Despite these findings in various species, the molecular responses of Persian sturgeon to malathion exposure remain largely unexplored.

Heat shock proteins (HSPs), particularly HSP70, are highly conserved molecular chaperones involved in protecting cells from stress-induced damage. Their upregulation under chemical or environmental stress

[*]Correspondence: Mina Rahbar, Masoud Sattari
E-mail: m.rahbar27@gmail.com, msattari647@gmail.com
DOI: https://doi.org/10.22034/ijab.v13i3.2541

is a common defense mechanism to preserve protein function and cellular stability (Basu et al., 2002; Safari et al., 2014; Kumar et al., 2022). Similarly, cytochrome CYP1A enzymes are critical for the biotransformation and detoxification of xenobiotics, with CYP1A gene expression often used as a biomarker for chemical exposure (Whyte et al., 2000; Safari et al., 2016; Rahbar et al., 2021b; Cortés-Miranda et al., 2024).

The liver, being the central organ for detoxification, is particularly responsive to environmental contaminants (Reynder et al., 2006). However, studies focusing on the hepatic molecular responses of Persian sturgeon to pesticide exposure are scarce. Investigating changes in the expression of HSP70 and CYP1A genes in response to malathion could provide important insights into the stress response mechanisms of this endangered species. Therefore, this study aims to evaluate the effects of sublethal malathion exposure on the hepatic expression of HSP70 and CYP1A genes in fingerlings of Persian sturgeon. The findings are expected to enhance our understanding of pesticide-induced molecular stress in sturgeons and support conservation efforts by identifying potential biomarkers for environmental monitoring in the Caspian Sea basin.

Materials and Methods

Fish and experimental design: A total of 600 Persian sturgeon fingerlings (mean weight: 3±0.31 g; mean total length: 3.6±0.21 cm) were obtained from the Shahid Dr. Beheshti Sturgeon Fish Breeding and Rearing Center (Guilan Province, Iran). Fish were randomly distributed into twelve tanks (120 L each) at a density of 50 fish per tank. During a 14-day acclimation period, the water parameters were maintained at 25±1°C temperature, pH 7.0±0.3, dissolved oxygen 7.8±0.3 mg L^{-1}, and total hardness of 196 mg L^{-1} (as $CaCO_3$). Fish were fed twice daily with frozen bloodworms.

Malathion exposure: After acclimation, fish were exposed to four nominal concentrations of malathion (0 [control], 0.05, 0.1, and 0.2 mg L^{-1}; 57% purity), each in triplicate. Exposure was carried out for 7 consecutive days based on methods adapted from Rahbar et al. (2021a). Actual malathion concentrations in water were measured and recorded as 0, 0.05±0.04, 0.1±0.09, and 0.2±0.06 mg L^{-1}. To maintain consistent exposure, two-thirds of the water in each tank was renewed daily with freshly prepared malathion solution.

Behavioral and mortality observations: Fish were observed daily throughout the exposure period for potential behavioral changes, including appetite, swimming behavior, and general activity. Any clinical signs or mortalities were recorded accordingly.

Sampling and tissue collection: On days 1, 2, 4, and 7 post-exposures, six fish per treatment (24-hour fasted) were randomly sampled and anesthetized using clove powder (0.5 g L^{-1}). Livers were dissected, snap-frozen in liquid nitrogen, and stored at −80°C for molecular analyses.

RNA extraction and cDNA synthesis: Total RNA was isolated using RiboEx™ reagent (QIAGEN, Germany) according to the manufacturer's instructions. RNA quantity and purity were determined spectrophotometrically (Nanophotometer, IMPLEN-P100, Germany), and integrity was checked via 1.5% agarose gel electrophoresis. Genomic DNA was removed by DNase I treatment (Fermentas, France). First-strand cDNA synthesis was performed using oligo(dT) primers and the Fermentas cDNA synthesis kit (France).

Quantitative Real-Time PCR (qRT-PCR): Expression levels of HSP70 and CYP1A genes were quantified using the Maxima SYBR Green qPCR Master Mix (1×, Fermentas) (Safari et al., 2025). Gene-specific primers, melting temperatures, product lengths, and primer efficiency are listed in Table 1. The relative quantification was performed using the $2^{-}\Delta\Delta Ct$ method, with β-actin serving as the internal control. All reactions and melt curve analyses were run on a Bio-Rad qPCR system (version 2.00, Hercules, CA, USA).

Statistical analysis: Data normality was assessed using the Shapiro-Wilk test. Two-way ANOVA was employed to evaluate the effects of malathion concentration and exposure duration on gene

Table 1. Primer sequences, melting temperatures, and product lengths for qRT-PCR analysis of HSP70, CYP1A, and β-actin genes.

Gene	Primer Sequence (5' → 3')	Melting Temperature (°C)	Product Length (bp)	Primer efficiency (%)
HSP70	F: CGCTGGCCTTAATGTTCTCC R: GCGCTTGAACTCTGCAATGA	56	249	98
CYP1A	F: GTCATCTGTGCCATGTGCTT R: TCTTGTCGAAGGAGCGGTAG	56	237	98
β-actin	F: TTGCCATCCAGGCTGTGCT R: TCTCGGCTGTGGTGAA	56	215	98

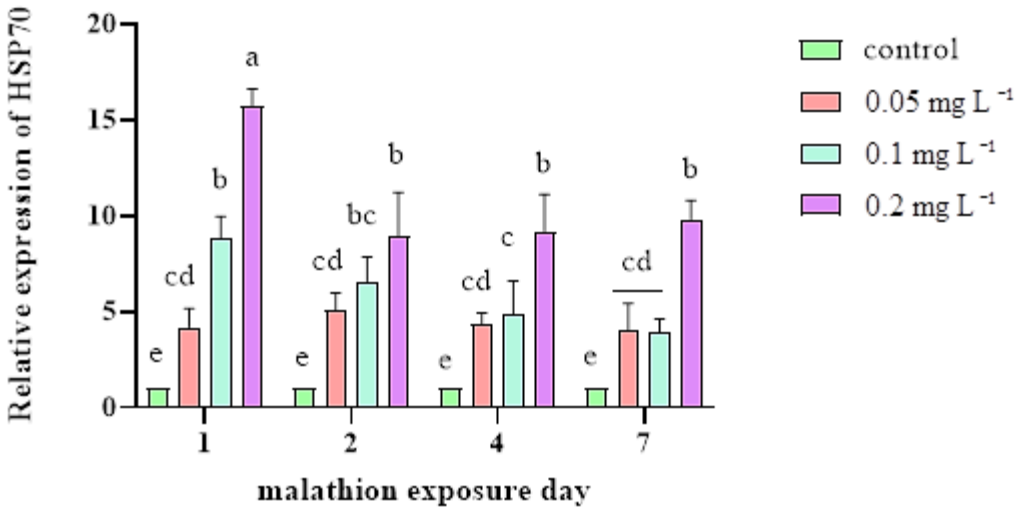

Figure 1. Relative expression levels of the HSP70 gene in the liver of Persian sturgeon (*Acipenser persicus*) exposed to different concentrations of malathion for 7 days. Data are presented as fold change relative to the control group (mean ± SD, n = 3). Different letters indicate significant differences ($P \leq 0.05$).

expression, followed by Tukey's HSD post hoc test for multiple comparisons. Pearson's correlation analysis was used to explore the relationship between HSP70 and CYP1A expression levels. Data are expressed as mean ± SD, and differences were considered statistically significant at $P<0.05$. All analyses and figures were conducted using GraphPad Prism (version 8, GraphPad Software Inc., San Diego, CA, USA).

Results

Behavioral and mortality observations: No mortality was observed in either the control or treated groups during the 7-day exposure period. However, fish exposed to the highest malathion concentration (0.2 mg L^{-1}) exhibited reduced feeding, impaired swimming balance, and decreased activity, particularly during the final days of exposure (4 and 7 days).

Hepatic gene expression: Figure 1 shows the expression of the HSP70 gene on different days following exposure to malathion in the liver. At the concentration of 0.2 mg L^{-1}, the expression level of HSP70 significantly increased on the first day of exposure, peaking at 15.70-fold higher expression compared to the control group. This upregulation declined from days 2 to 7. At the concentration of 0.1 mg L^{-1}, the highest expression of this gene was observed on the first and second days of exposure, gradually decreasing from days 4 to 7. At the 0.05 mg L^{-1} concentration, milder changes were observed, with no significant fluctuations over time. The results revealed significant effects of time ($F(3, 32) = 14.04$, $P<0.0001$), treatment ($F(3, 32) = 149.6$, $P<0.0001$), and their interaction ($F(9, 32) = 6.520$, $P<0.0001$) on HSP70 gene expression in the liver. These results emphasize that the magnitude and persistence of HSP70 induction depend on both malathion dose and exposure time.

Figure 2 illustrates the expression levels of the CYP1A gene in the liver of Persian sturgeon on different days of malathion exposure. At the highest

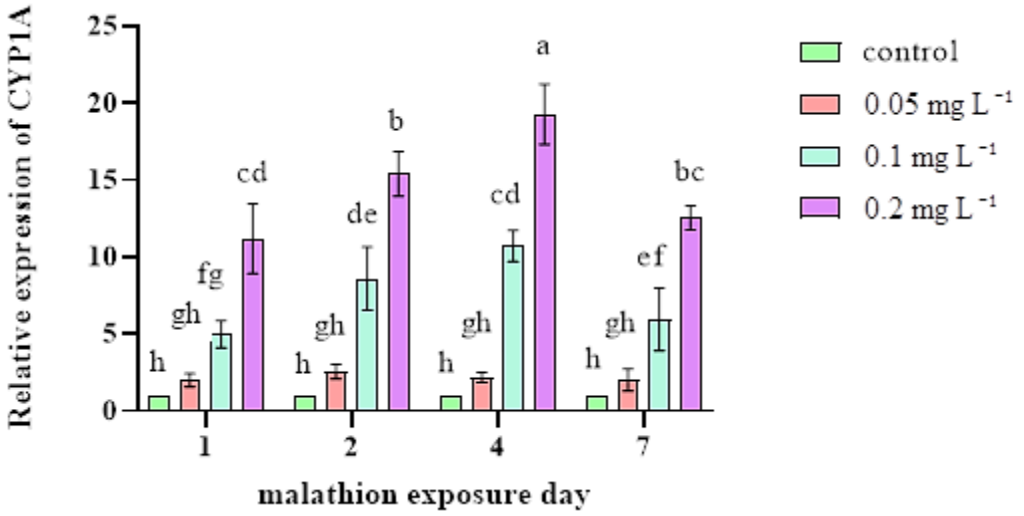

Figure 2. Relative expression levels of the CYP1A gene in the liver of Persian sturgeon (*Acipenser persicus*) exposed to different concentrations of malathion for 7 days. Data are presented as fold change relative to the control group (mean ± SD, n = 3). Different letters indicate significant differences ($P \leq 0.05$).

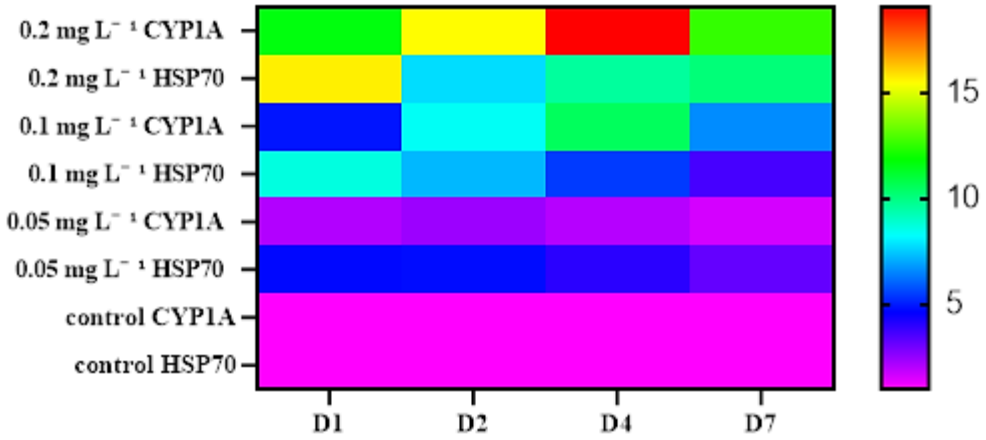

Figure 3. Heatmap of relative expression levels of HSP70 and CYP1A genes in liver tissues of Persian sturgeon (*Acipenser persicus*) exposed to malathion over time. Color gradient indicates expression intensity (red: high; purple: low). D1, D2, D4, and D7 refer to days of exposure to malathion.

concentration (0.2 mg L^{-1}), a significant increase in CYP1A expression was observed up to day 4 (19.29-fold higher expression compared to the control group), after which the expression level declined by day 7. At the concentration of 0.1 mg L^{-1}, the trend of gene expression was similar to that of 0.2 mg L^{-1}, but the expression level was lower. In contrast, no significant differences were observed between the control group and the 0.05 mg L^{-1} treatment throughout the exposure period. The results revealed a significant effect of time ($F(3, 32) = 20.91$, $P<0.0001$), treatment ($F(3, 32) = 324.8$, $p < 0.0001$), and their interaction ($F(9, 32) = 6.830$, $P<0.0001$) on CYP1A gene expression in the liver. These findings indicate a dose- and time-dependent induction of the CYP1A gene in response to malathion, particularly at higher concentrations.

Figure 3 presents a heat map overview of the relative expression levels of HSP70 and CYP1A genes across different treatment concentrations and time points. The color gradient visualizes the magnitude of gene expression, with warmer colors (yellow to red) representing higher expression values. As shown, both genes exhibited distinct temporal and dose-dependent expression patterns. Notably, the highest expression levels for both HSP70 and CYP1A were observed at 0.2 mg L^{-1}, particularly on days 1 and 4, respectively.

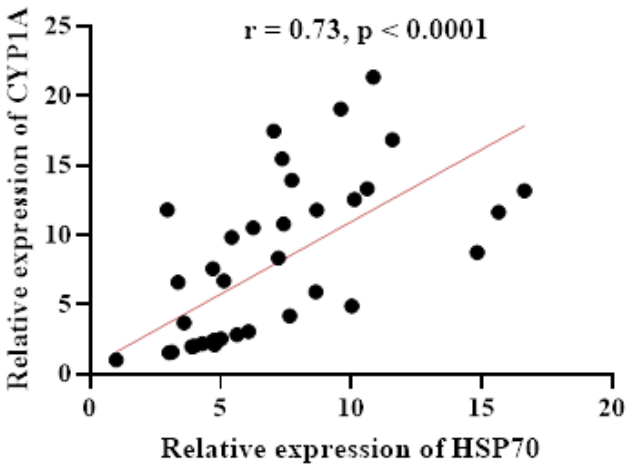

Figure 4. Pearson correlation between the expression levels of HSP70 and CYP1A genes across all treatments and time points.

The control and 0.05 mg L^{-1} groups consistently displayed the lowest expression levels, confirming the minimal impact of lower malathion concentrations. This visualization reinforces the results and highlights the coordinated transcriptional response of these stress-related genes under toxic exposure. A significant positive correlation was observed between the relative expression levels of HSP70 and CYP1A across all treatments and time points ($r = 0.73$, $P<0.0001$) (Fig. 4).

Discussions

The present study provides the first comprehensive evidence of malathion-induced modulation of hepatic HSP70 and CYP1A gene expression in Persian sturgeon, highlighting the molecular stress responses of this endangered species to organophosphate exposure. Our findings reveal a dose- and time-dependent upregulation of both genes. The observed gene expression patterns suggest a coordinated molecular response to pesticide-induced cellular stress.

Behavioral responses are among the earliest and most sensitive indicators of sublethal toxic effects in aquatic organisms. In the present study, although no mortality was observed in either the control or treated groups throughout the 7-day exposure period, fish exposed to the highest malathion concentration (0.2 mg L^{-1}) displayed notable behavioral changes, particularly on days 4 and 7. These changes included reduced feeding activity, impaired swimming balance, and overall lethargy. Such responses are commonly associated with neurotoxicity, especially due to the inhibition of acetylcholinesterase (AChE), a known primary target of organophosphate pesticides (Aroniadou-Anderjaska et al., 2020). Previous studies have reported similar behavioral impairments—such as erratic swimming, hypoactivity, and loss of equilibrium—in fish species exposed to various organophosphates (Velisek et al., 2009; Deka and Mahanta, 2016). These sublethal effects, while not immediately fatal, can significantly reduce survival chances in natural environments by impairing essential functions such as feeding, predator avoidance, and migration. Notably, the onset of behavioral symptoms in our study coincided with molecular stress responses such as increased expression of HSP70 and CYP1A, suggesting a link between neurotoxic effects and cellular stress pathways. These findings underscore the ecological relevance of behavioral endpoints and their potential role as early-warning biomarkers in aquatic toxicology, particularly for vulnerable species like Persian sturgeon.

The early and marked upregulation of HSP70 suggests an acute cellular response to malathion exposure, particularly at the highest concentration (0.2 mg L^{-1}) on day 1. The upregulation of HSP70 gene expression in rainbow trout (*Oncorhynchus mykiss*) exposed to 2,4-dichloroaniline (Kilemade and Mothersil, 2001), in the liver and kidney of snakehead fish (*Channa punctatus*) exposed to arsenic (Roy and Bhattacharya, 2006), in rainbow trout exposed to deltamethrin (Atamanalp and Erdogan, 2010; Ceyhun et al., 2010), in the liver of white cloud mountain minnow (*Tanichthys albonubes*) after 96-hour exposure to cadmium and copper (Jing et al., 2013), and in Persian sturgeon (*A. persicus*) exposed to cadmium (Safari et al., 2014) has been reported. HSP70 is a dominant protein that shows increased expression following exposure to pollutants, and the intensity of its response varies among species depending on the type of contaminant, concentration,

and duration of exposure (Singer et al., 2005; Safari et al., 2014; Wilhelm et al., 2017). The increase in HSP70 may be attributed to its chaperone role in preventing improper protein interactions (misfolding and aggregation), as well as cellular protection and repair (Kim et al., 2020). Additionally, the HSP70 gene may be among the early-response genes that induce the expression of other genes (Whitley et al., 1999; Eder et al., 2007). In the present study, the observed decrease in HSP70 gene expression from day 2 to day 7 could be due to stress adaptation and acclimation (Eder et al., 2007), tissue damage or necrosis caused by malathion toxicity over time, or possible gene silencing. Stressors can limit the synthesis of this gene over time by inducing cellular damage (Chen et al., 2012), and their effect on HSP70 expression levels may vary depending on the type of stressor and the tolerance threshold of the species (Singer et al., 2005). It can be concluded that HSP70 has significant potential for monitoring chronic stress in aquaculture environments.

The expression of the CYP1A gene, a key component of the phase I detoxification system, showed a dose- and time-dependent induction in Persian sturgeon exposed to malathion, with the highest expression (19.29-fold) observed on day 4 in the 0.2 mg L^{-1} group. This temporal peak suggests a maximal activation of biotransformation pathways during acute exposure, possibly reflecting a critical threshold of metabolic stress. However, the observed decrease in expression by day 7 may be attributed to several factors, including cellular exhaustion, oxidative stress feedback, or hepatic tissue damage. This biphasic response is consistent with previous findings that prolonged or high-level activation of CYP1A enzymes can result in overproduction of reactive oxygen species (ROS), causing oxidative damage and initiating downregulation mechanisms (Whyte et al., 2000; Safari et al., 2016; Shen et al., 2020). Similar upregulation patterns of CYP1A have been reported in various fish species exposed to organophosphates, such as zebrafish (*Danio rerio*) (Cui et al., 2025) and rainbow trout (Safari et al., 2016). Shen et al. (2020) demonstrated significant transcriptional changes in oxidative stress and detoxification-related genes—including cytochrome CYP1A family members—in embryonic zebrafish exposed to malathion alone or in combination with other pesticides. Similarly, Ortiz-Delgado et al. (2021) observed structural hepatic damage in *Solea senegalensis* larvae exposed to sublethal doses of malathion, although CYP1A expression was not significantly altered. These findings underscore that CYP1A gene regulation may be modulated not only by concentration and duration of exposure but also by species-specific sensitivity, developmental stage, and tissue condition. The elevated expression of CYP1A in higher malathion concentrations observed in our study reflects increased metabolic demand. It supports the applicability of this gene as a sensitive molecular biomarker for pesticide exposure in vulnerable species such as *A. persicus*. Overall, this research highlights the dual role of cytochrome CYP1A enzymes in both detoxification and potential toxicity through ROS generation, emphasizing the importance of monitoring their expression dynamics in ecotoxicological studies.

The dose- and time-dependent nature of gene expression, visualized effectively in the heat map, indicates that even short-term sublethal exposure (0.1-0.2 mg L^{-1}) can elicit measurable transcriptional changes. Notably, no significant alterations were observed at 0.05 mg L^{-1}, suggesting a possible threshold below which the molecular response is minimal or absent. The sharp expression gradient at higher doses—paired with the clear threshold behavior—not only underscores the risk of agricultural runoff during peak application periods but also highlights the urgent need to define environmentally relevant exposure levels for sensitive species like *A. persicus*, calling for stricter regulatory monitoring.

The significant positive correlation observed between HSP70 and CYP1A gene expression levels suggests a coordinated molecular response to malathion-induced stress. HSP70, as a molecular chaperone, plays a critical role in stabilizing proteins and preventing aggregation during cellular stress, while cytochrome CYP1A enzymes metabolize

xenobiotics and initiate detoxification cascades. Their synchronous upregulation implies that the fish mount an integrated response involving both cytoprotective (HSP70-mediated) and detoxification (CYP1A-mediated) mechanisms to counteract the cellular damage caused by malathion. This finding is consistent with previous studies that showed concurrent activation of stress-related and detoxification genes under chemical exposure (Singer et al., 2005; Safari et al., 2016; Ardeshir et al., 2018; Shen et al., 2020; Cui et al., 2025). The temporal pattern observed in our study—an early spike in HSP70 (Day 1) followed by a delayed peak in CYP1A (Day 4)—also reflects a logical physiological sequence in which acute stress is first buffered at the protein level, followed by activation of biotransformation pathways to eliminate the toxicant. Such coordinated regulation enhances the fish's resilience against environmental pollutants and highlights the potential of using multigene panels for ecotoxicological assessment.

Conclusion

Given the ecological and economic importance of Persian sturgeon and their declining populations in the Caspian basin, identifying sensitive molecular biomarkers is essential for conservation and management efforts. The responsiveness of HSP70 and CYP1A genes to malathion exposure in this study highlights their utility as early-warning indicators of chemical stress in endangered species. Further research should expand on these findings by examining other organs (e.g., gills, brain), evaluating long-term and recovery-phase gene expression, and incorporating proteomic and enzymatic analyses for a more comprehensive understanding.

Funding

This study was supported by the Caspian Sea Basin Research Center, University of Guilan, under the grant No. 2195167.

References

Ardeshir R.A., Zolgharnein H., Movahedinia A., Salamat N., Zabihi E. (2018). CYP1A gene expression as a basic factor for fipronil toxicity in Caspian kutum fish. Toxicology Reports, 5: 113-124.

Aroniadou-Anderjaska V., Figueiredo T.H., Apland J.P., Braga M.F. (2020). Targeting the glutamatergic system to counteract organophosphate poisoning: A novel therapeutic strategy. Neurobiology of Disease, 133: 104406.

Atamanalp M., Erdogan O. (2010). Alternations of HSP70 gene expression in rainbow trout (*Oncorhynchus mykiss*) exposed to deltamethrin. Turkish Journal of Veterinarian and Animal Science, 34: 359-363.

Basu N., Todgham A.E., Ackerman P.A., Bibeau M.R., Nakano K., Schulte P.M., Iwama G.K. (2002). Heat shock protein genes and their functional significance in fish. Gene, 295(2): 173-183.

Ceyhun S.B., Senturk M., Ekinci D., Erdogan O., Ciltas A.A., Kocaman E.M. (2010). Deltametrin attenuates antioxidant defense system and induces the expression of heat shock protein 70 in rainbow trout. Comparative Biochemistry and Physiology C, 152: 215-223.

Chen X., Zhu Y.H., Cheng X.Y., Zhang Z.W., Xu S.W. (2012). The protection of selenium against cadmium-induced cytotoxicity via the heat shock protein pathway in chicken splenic lymphocytes. Molecules, 17: 14565-14572.

Cortés-Miranda J., Rojas-Hernández N., Muñoz G., Copaja S., Quezada-Romegialli C., Veliz D., Vega-Retter C. (2024). Biomarker selection depends on gene function and organ: the case of the cytochrome CYP1A family genes in freshwater fish exposed to chronic pollution. PeerJ, 12: e16925.

Cui J., Xiao S., Guo H., Wei Y., Shi X., Zhao F., Liu X., Zhou Z., Liu D., Wang P. (2025). Insights into organophosphorus insecticide malathion induced reproductive toxicity and intergenerational effect in zebrafish (*Danio rerio*). Science of The Total Environment, 959: 178188.

Deka S., Mahanta R. (2016). Malathion toxicity on fish – a review. International Journal of Current Research, 8(12): 44120-44128.

Eder K.J., Kohler H.R., Werner I. (2007). Pesticide and pathogen: Heat shock protein expression and acetylcholinesterase inhibition in juvenile Chinook salmon in response to multiple stressors. Environmental Toxicology and Chemistry, 26: 1233-1242.

Ghafarifarsani H., Raeeszadeh M., Hajirezaee S., Ghafari Farsani S., Mansouri Chorehi M. (2023). The effect of

malathion concentration and exposure time on histopathological changes in the liver and gill of rainbow trout. Aquaculture Research, 1: 3396066.

Jing J., Liu H., Chen H., Hu S., Xiao K., Ma X. (2013). Acute effect of copper and cadmium exposure on the expression of heat shock protein 70 in the Cyprinidae fish (*Tanichthys albonubes*). Chemosphere, 91: 1113-1122.

Kilemade M., Mothersil C. (2001). Heat shock protein 70 levels in rainbow trout primary epidermal cultures in response to 2,4-dichloroaniline exposure: a novel in vitro aquatic toxicology marker. Environmental Toxicology, 16: 253-259.

Kim J.Y., Barua S., Huang M.Y., Park J., Yenari M.A., Lee J.E. (2020). Heat shock protein 70 (HSP70) induction: chaperonotherapy for neuroprotection after brain injury. Cells, 9(9): 2020.

Kumar V., Roy S., Behera B.K., Das B.K. (2022). Heat shock proteins (Hsps) in cellular homeostasis: a promising tool for health management in crustacean aquaculture. Life, 12(11): 1777.

Moghim M., Kor D., Tavakolieshkalak M., Khoshghalb M.B. (2006). Stock status of Persian sturgeon (*Acipenser persicus* Borodin, 1897) along the Iranian coast of the Caspian Sea. Journal of Applied Ichthyology, 22.

Ortiz-Delgado J.B., Funes V., Albendín G., Scala E., Sarasquete C. (2021). Toxicity of malathion during Senegalese sole, *Solea senegalensis* larval development and metamorphosis: Histopathological disorders and effects on type B esterases and CYP1A enzymatic systems. Environmental Toxicology, 36(9): 1894-1910.

Pourkazemi M. (2006). Caspian Sea sturgeon Conservation and Fisheries: Past present and Future. Journal of Applied Ichthyology, 22.

Prathibha Y., Murugananthkumar R., Rajakumar A., Laldinsangi C., Sudhakumari C.C., Mamta S.K., Dutta-Gupta A., Senthilkumaran B. (2014). Gene expression analysis in gonads and brain of catfish *Clarias batrachus* after the exposure of malathion. Ecotoxicology and Environmental Safety, 102: 210-219.

Rahbar M., Sattari M., Alaf Noverian H., Ahmadnezhad M., Khara H., Safari R. (2021a). Biochemical and histopathological alterations in Persian sturgeon, *Acipenser persicus* exposed to malathion. Toxin Reviews, 40(4): 1383-1395.

Rahbar M., Sharifian M., Masaeli S., Safari R. (2021b). A review of the importance of cytochrome oxidase CYP1A in fish. Journal of Ornamental Aquatics, 8(1): 37-47. (in Persian)

Reynder H., Campenhout K.W., Bervoet L., Coen W.M.D., Blust R. (2006). Dynamics of cadmium accumulation and effects in common carp (*Cyprinus carpio*) during simultaneous exposure to water and food (*Tubifex tubifex*). Environmental Toxicology and Chemistry, 25(6): 1558-1567.

Roy S., Bhattacharya S. (2006). Arsenic-induced histopathology and synthesis of stress proteins in liver and kidney of (*Channa punctatus*). Ecotoxicology and Environmental Safety, 65: 218-229.

Safari R., Hoseinifar S.H., Shabani A., Ghafarifarsani H., Raissy M., Khaleghi S.R., Van Doan H., Yazici M., Rahbar M., Nouri M. (2025). Dietary administration of green macroalgae (*Ulva intestinalis*) on growth performance, serum immune parameters, and gene expression in common carp (Cyprinus carpio). Annals of Animal Science, 25(1): 317-327.

Safari R., Khalili M., Reza Imanpour M., Pourkazemi M. (2016). The effects of endosulfan on CYP1A 1A gene expression, antioxidant enzymes activity and histopathological alterations in liver of Persian sturgeon (*Acipenser persicus* Borodin, 1987). Journal of Applied Ichthyology, 32(4): 636-642.

Safari R., Shabani A., Ramezanpour S., Imanpour M.R., Rezvani S. (2014). Alternations of heat shock proteins (hsp70) gene expression in liver and gill of Persian sturgeon (*Acipenser persicus* Borodin, 1987) exposed to cadmium chloride. Iranian Journal of Fisheries Sciences, 13(4): 979-997.

Shen W., Lou B., Xu C., Yang G., Yu R., Wang X., Li X., Wang Q., Wang Y. (2020). Lethal toxicity and gene expression changes in embryonic zebrafish upon exposure to individual and mixture of malathion, chlorpyrifos and lambda-cyhalothrin. Chemosphere, 239: 124802.

Singer C.H., Zimmermann S., Sures B. (2005). Dreissena polymorpha following exposure to platinum group metals (platinum, palladium and rhodium): Comparison with lead and cadmium exposure. Induction of heat shock proteins (HSP70) in the zebra mussel. Aquatic Toxicology, 75: 65-75.

Suchiang P. (2021). A review on toxicity of pesticides in catfishes: reproductive, haematological and biochemical aspects. Annual Research & Review in Biology, 36(9): 47-59.

Ullah S., Li Z., Hasan Z., Khan S.U., Fahad S. (2018). Malathion induced oxidative stress leads to histopathological and biochemical toxicity in the liver of rohu (*Labeo rohita*, Hamilton) at acute concentration. Ecotoxicology and Environmental Safety, 161: 270-280.

Velisek J., Wlasow T., Gomulka P., Svobodova Z., Dobsikova R., Novotny L. (2009). Effects of subchronic exposure to diazinon on biochemical, haematological and histopathological parameters of common carp (*Cyprinus carpio* L.). Neuroendocrinology Letters, 30 (Suppl 1): 236-241.

Whitley D., Goldberg S.P., Jordan W.D. (1999). Heat shock proteins: a review of the molecular chaperones. Journal of Vascular Surgery, 29: 748-751.

Whyte J.J., Jung R.E., Schmitt C.J., Tillitt D.E. (2000). Ethoxyresorufin-O-deethylase (EROD) activity in fish as a biomarker of chemical exposure. Critical Reviews in Toxicology, 30(4): 347-570.

Wilhelm S., Henneberg A., Köhler H.R., Rault M., Richter D., Scheurer M., Suchail S., Triebskorn R. (2017). Does wastewater treatment plant upgrading with activated carbon result in an improvement of fish health? Aquatic Toxicology, 192: 184-197.