

A preliminary study on the expression level of P₄₅₀ gene in liver and gill tissues of Persian sturgeon (*Acipenser persicus* Borodin, 1987) exposed to water soluble fractions of crude oil

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Abstract

The present study was conducted to investigate the expression level of cytochrome P₄₅₀ enzyme in detoxification of water soluble fractions (WSFs) of crude oil in Persian sturgeon, *Acipenser persicus*. Fish were exposed to WSFs at concentrations of 0 (control), 10, 20, and 50% of the LC₅₀ for 14 days, and then, the expression level of P₄₅₀ gene was assayed in gill and liver samples. According to the results, the expression level of P₄₅₀ gene increased in both the gill and liver tissues of fish when exposed to sub lethal concentrations of WSFs. High level of expression in treatment of 50% WSFs was observed compared to the control ($p < 0.05$). However, no significant differences were observed between treatments of 10%, 20%, and 50% WSFs. Additionally, P₄₅₀ was expressed higher in the gill tissue than the liver. The present research demonstrates that P₄₅₀ gene expression can be used as a molecular biomarker to assay the severity of oil pollution in the Caspian Sea.

Keywords: Crude oil, Cytochrome P₄₅₀, Pollution, Persian sturgeon

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Introduction

The Persian sturgeon is one of the commercial and ecological sturgeon fish composing the majority of sturgeon catch in southern parts of Caspian Sea. Unfortunately, the population of all sturgeon species has declined in Caspian Sea over the last decades (Khodorevskaya *et al.*, 1997; Ivanov *et al.*, 1999; Bahmani *et al.*, 2000; Jabbarzadeh Shiadeh *et al.*, 2000; Khodorevskaya *et al.*, 2009; Nazeri *et al.*, 2019). Moghim *et al.* (2006) have reported a decline of 80–90% for Persian sturgeon populations during last 30–40 years. In many cases, the population decline is attributed to some problems, including loss of habitat and spawning grounds, illegal and overfishing, and also water pollution (De Meulenaer and Raymakers, 1996; Kiabi *et al.*, 1999; Nasrollahzadeh, 2010). It was well recognized that water pollutants adversely affect all aspects of life in sea such as growth, osmoregulation, reproduction, etc. (Hanson *et al.*, 2007; Olsvik *et al.*, 2010; Suvetha *et al.*, 2010; Xing *et al.*, 2012; Katuli *et al.*, 2014). In Caspian Sea, a considerable amount of pollutants such as pesticides, heavy metals, crude oil, and other chemicals are released annually into the water due to agricultural and industrial wastewater activities (Hajirezaee *et al.*, 2016; Hajirezaee *et al.*, 2017).

Over the last decades, crude oil exploration, production, and related industries have caused Caspian Sea to be polluted by oil derivatives, including alkanes, cycloalkanes, and aromatic compounds. Furthermore, oil loading,

transportation, and tankers accidents in sea have intensified the problem (Dumont, 1995; Kaplin, 1995; Dumont, 1998; Abilov *et al.*, 1999; Clauer *et al.*, 2000; Tolosa *et al.*, 2004). The main sources of pollution of the Caspian Sea are considered to be offshore oil production and land-based sources, especially the Volga River (Karpinsky, 1992). The most production of oil in the Republic of Azerbaijan is from offshore resources.

The fate of crude oil in the sea is mainly determined by evaporation, dissolution, emulsification, precipitation, and degradation process. Acute toxicity studies reported that fish are generally the marine organisms susceptible to the water soluble fraction (WSF) of crude oil (Wolfe, 2013). In the present study, we investigated the expression level of cytochrome enzyme, P450 (CYP1) in detoxification of WSFs in Persian sturgeon, *Acipenser persicus* for the first time. The cytochrome P₄₅₀ enzyme not only plays a key role in the degradation of endogenous substrates, such as steroids, fatty acids, and hormone, but also it is important for the metabolism and detoxification of xenobiotics such as drugs, aromatic hydrocarbons, pesticides, and water soluble fractions (WSFs) of crude oil (Goksøyr, 1995; Kennedy and Farrell, 2005; Arukwe *et al.*, 2008; Jager *et al.*, 2016). Therefore, in the present research we examined acute and chronic toxicity of WSF and P450 gene expression as a molecular biomarker in Persian sturgeon.

Materials and methods

Preparation of water soluble fractions (WSFs) of crude oil

The crude oil was mixed with well-filtered water (the same water source used for fish) in a special container at ratio of 1:9. Then, the emulsion was shaken for 23 h by shaker and remained for 1 h until the soluble phase was separated. The lower part, which is soluble phase or WSFs, was isolated, and used as stock solution over the course of the experiment (Anderson *et al.*, 1974).

Determination of acute toxicity and mean lethal toxicity (LC₅₀) of WSFS

Fish were obtained from Shahid Beheshti Sturgeon Hatchery Center, Rasht. The experiments were conducted in Sturgeon Reference Centre (Gene Bank), Rasht. A semi-static method was used to determine the acute toxicity and toxicity limit of crude oil on Persian sturgeon (mean weight: 5±0.5 g). In this method, fish were kept in 30×30×40 cm aquariums (10 fish per aquarium) and exposed to different concentrations of WSF solution including 27, 29.25, 31.5, 33.75, 36, and 38.25 mg L⁻¹, and the last group was considered as control. The experiment was carried out in 3 replications. During the experiment, all the aquariums were aerated and the temperature was kept in the range of 18 to 20 °C. In each treatment, the WSF solution of the aquariums was renewed every 24 h with a fresh solution at same concentration. Additionally, the dead fish were removed from the aquarium and counted at the same time. After 96 h, the total number of dead fish in each

aquarium was counted. Finally, using the obtained numbers, a LC₅₀ value of 33.95 mg L⁻¹ was calculated via Probit regression analysis with SPSS software, version 20 (Finney, 1952).

Exposure experiment

The experiment was conducted in 4 experimental treatments with three replicates. Persian sturgeon with stocking density of 10 fish per aquarium were exposed to WSFs at concentrations of 0 (control), 10, 20, and 50% of LC₅₀ for 14 days and after this period, the expression level of P450 gene was assayed in gill and liver samples. During the 14 days experiment, the water quality parameters (e.g., temperature, dissolved oxygen, pH, turbidity, etc.) were held in normal range.

Gill and liver sampling

Liver and gill tissue samples were obtained after killing fish using high concentration of MS-222 solution (anesthetic agent made in SIGMA factory). Then samples were placed into RNAase-free microtubes and stored at -80 °C until the onset of RNA extraction and gene expression analysis.

RNA extraction and cDNA synthesis

RNA extraction of gill and liver tissues was carried out using acid guanidinium thiocyanate-phenol-chloroform method described by Chomczynski and Sacchi (1987). For total RNA extraction, 50-100 mg of each tissue was homogenated in 1.0 ml Biozole reagent (Bio flux; China) for 15 min at room temperature until the cell digestion was

completed and the nucleoproteins became separated. Then, the chloroform was added to homogenized solution at ratio of 1:5. After 15 times shaking, the homogenate was incubated at room temperature for 5 min., and then centrifuged at 12000 rpm at 4 °C. In the next step, 100 µl Phenol-Chloroform-Isoamyl alcohol (5:1:1) was added to supernatant and after shaking the solution for 15s, it was centrifuged at 12000 rpm at 4 °C. The supernatant was decanted and the residual was washed with 75% alcohol. The concentration of RNA was measured by spectrophotometer (DR 2800, HACH Germany) at 260/280 nm. Moreover, the RNA quality was evaluated by electrophoresis on a 1.5% agarose gel and stained by ethidium bromide (Miandare *et al.*, 2013).

First-strand cDNAs was synthesized from 4 µl of total RNA using a Fermentas cDNA synthesis Kit (Bio RI, Bioflux-Bioer) for RT-PCR, following the manufacturer's instructions. The quality of synthesized cDNA was evaluated by PCR using the primers of β -actin gene.

Primer design for qPCR

The qPCR primers were designed based on gene sequence of P450 gene for

Persian sturgeon in GenBank of NCBI database. Table 1 displays the sequence of designed primers. The β -actine gene was used as reference gene in order to standardize the expression levels of gene.

Quantitative real-time PCR (qPCR)

Real-time PCR analysis was carried out by an iCycler [(Bio-Rad, USA) CFX] using commercial kit (Bio flux-Bioer Technology Co., China) following the manufacturer's instructions. The obtained data were analyzed using Bio-Rad CFX Manager software version 1.6. The relative gene expression was calculated by the $2^{-\Delta\Delta ct}$ method (Livak and Schmittgen, 2001).

Data analysis

All data were presented as mean \pm standard error of mean (SEM). The Kolmogorov–Smirnov test was used to evaluate the normality of the data. As data were not normally distributed, Levene's test was used to compare P450 gene expression in gill and liver tissues relative to β -actine. All statistical analyses were performed using SPSS software V.20. The significant differences between groups were considered at $p < 0.05$.

Table 1: Name, sequence, melting temperature (Tm) and product length of primers used in the present study to quantify P450 1A transcript of Persian sturgeon *Acipenser persicus* through Real-time PCR.

Gene	Tm	qPCR primers Forward/Reverse	Product length (bp)
P450	56	GTCATCTGTGCCATGTGCT TCTTGTCGAAGGAGCGGTAG	237
Reference gene (β - actin)	56	TTGCCATCCAGGCTGTGCT TCTCGGCTGTGGTGAA	2015

Results

According to the results of qPCR, P₄₅₀ gene was expressed in both gill and liver tissues. It was found that the level of expression varied slightly depending on the type of the tissue ($p < 0.05$) (Fig. 1). The P₄₅₀ gene showed significantly different expression levels in all treatments ($p < 0.05$). All WSFs treatments showed higher P₄₅₀ gene expression compared to the control ($p < 0.05$). The highest P₄₅₀ gene

expression in both liver and gill tissues was observed in treatment of 50% WSFs compared to the control ($p < 0.05$). Also, the expression level of P₄₅₀ in treatment of 50% WSFs was higher in gill than in liver tissue ($p < 0.05$). There were no significant differences in P₄₅₀ gene expression between the liver and gill tissues in three treatments of 10%, 20%, and 50% WSFs ($p > 0.05$).

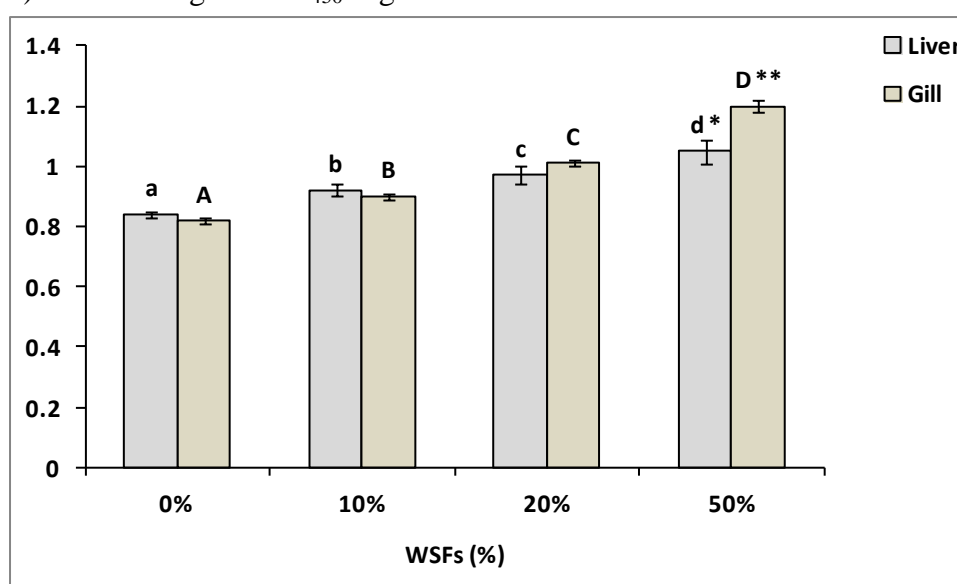


Figure 1: Relative P₄₅₀ gene expression in liver and gill of Persian sturgeon, *Acipenser persicus* exposed to 0%, 10%, 20% and 50% WSFs for 14 days. In each tissue, bar assigned with different letters denote separately the significant differences during 14 days WSFs exposure ($p < 0.05$). In each WSFs treatment, the significant differences in gene expression between gill and liver tissues are indicated with different symbols. Values are presented as the mean ± SD.

Discussion

In order to assess the impact of pollutants in fish species, P₄₅₀ gene expression alterations in response to xenobiotic were demonstrated (Zhang *et al.*, 2012; Kumar and Denslow, 2016; Kühnert *et al.*, 2017). The results obtained in this study showed that P₄₅₀ gene is up-regulated in both the liver and gill tissues in response to WSFs exposure. This confirms that P₄₅₀

enzyme is among the responses of the fish body to modify metabolic effects of WSFs. Moreover, it may be concluded that P₄₅₀ is an early gene with the ability to induce the expression of other genes. Furthermore, our results were in agreement with those of the studies conducted by Safari *et al.* (2016) on Persian sturgeon, indicating the higher expression of P₄₅₀ in response to endosulfan. Being transported to the

nucleus, the receptor contaminant complex leads to expression of the gene (Billard *et al.*, 2002). It has been shown that pollutant toxicity is related to its affinity to AhR. In addition, high affinity of pollutants to AhR induce P450 (Billiard *et al.*, 2002). According Safari *et al.* (2016) P450 might be one of the first genes that activate later response genes, which can be considered as one more reason for a higher up-regulation on the primary day (Waisberg *et al.*, 2003). Moreover, Paknejad *et al.* (2016) were evaluated cadmium impacts in Persian sturgeon. Their results were incontestable that cadmium causes some changes in two angiogenesis connected genes' expression in Persian sturgeon. Lari *et al.* (2015) were proposed that WSF of crude oil interrupt the systema respiratorium in Caspian roach (*Rutilus caspicus*) that lead to asphyxia can be a principal mechanism that concludes in fatal impact of WSF. Given the above, the P₄₅₀ CYP1A enzyme can be considered as an effective candidate for assessment of environmental stresses, and several studies have indicated an induction of this enzyme activities with regard to the presence of anthropogenic sources of pollution, such as industries, urban wastewater, and harbors (Payne and Penrose, 1975; Stegeman, 1992; Stegeman, 1993; Sjøfteland *et al.*, 2010; Zhang *et al.*, 2012; Huang *et al.*, 2014; Safari, 2014; Safari *et al.*, 2016).

In this study, higher expression level of P₄₅₀ was observed for WSFs treatment, showing the catalytic role of this protein in modulating the effects of various exogenous or endogenous

agents, including WSFs in Persian sturgeon (Goksøyr and Förlin, 1992). Some studies have reported the increased expression level of P₄₅₀ gene after exposure to environmental pollutants (Goksøyr, 1995; Dar *et al.*, 2017; Zhang *et al.*, 2017). In fact, the expression of P450 1A is adjusted by the Ah receptor (AhR). The transported receptor contaminant complex to the nucleus leads to expression of P₄₅₀ gene (Billard *et al.*, 2002). It has been shown that pollutant toxicity is related to its affinity to AhR. In addition, high affinity of pollutants to AhR induces P450 (Billard *et al.*, 2002). Given the above, the P₄₅₀ up-regulation in the contaminated Persian sturgeon may be mediated by WSF' high affinity to Ah receptor. Other studies have found decreases in P₄₅₀ gene expression in fish exposure to high concentrations of pollutants (Sjøfteland *et al.*, 2010; Zhang *et al.*, 2012; Huang *et al.*, 2014). These studies have concluded that the lower expression level of P₄₅₀ gene may be due to the interaction of P₄₅₀ protein with cortisol, as well as liver and gill tissue damages caused by high concentrations of PAHs and subsequent silence of P₄₅₀ gene (Sjøfteland *et al.*, 2010). In *Gambusia affinis* exposure to high concentrations of cadmium decreased the expression level of P450 gene (Huang *et al.*, 2014). Moreover, the lower expression level of P450 gene was reported in *Ruditapes philippinarum* in response to 40 µg L⁻¹ cadmium compared to 10 µg L⁻¹ treatments (Zhang *et al.*, 2012). It can be concluded that, P₄₅₀ gene may be

plays main role in the diverse environmental pollutants metabolizing.

In the current study, the P₄₅₀ gene was expressed more in the gill tissue than the liver. These results are contrary to what were obtained for three-spined stickleback (*Gasterosteus aculeatus*) (Gao *et al.*, 2011), killifish (*Fundulus heteroclitus*) (Zanette *et al.*, 2009), Zebrafish (*Danio rerio*) (Jönsson *et al.*, 2007), Rainbow trout (*Oncorhynchus mykiss*) (Jönsson *et al.*, 2010) and brazillian guppy (*Poeciliavivipara*) (Dorrington *et al.*, 2012), where higher expression of the gene was observed in the liver.

In conclusion, we reported the P₄₅₀ gene expression induction in Persian sturgeon fingerlings in response to WSFs, indicating the key role of this gene in detoxification of WSFs in this species. Thus, P₄₅₀ gene expression can be used as a molecular biomarker of crude oil pollution for Persian sturgeon.

References

- Abilov, F.A., Orudjev, A.G. and Lange, R., 1999. Optimization of oil-containing wastewater treatment processes. *Desalination*, 124(1-3), 225-229.
- Anderson, J., Neff, J., Cox, B., Tatem, H. and Hightower, G., 1974. Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. *Marine biology*, 27(1), 75-88.
- Arukwe, A., Nordtug, T., Kortner, T.M., Mortensen, A.S. and Brakstad, O.G., 2008. Modulation of steroidogenesis and xenobiotic biotransformation responses in zebrafish (*Danio rerio*) exposed to water-soluble fraction of crude oil. *Environmental research*, 107(3), 362-370.
- Bahmani, M., Oryan, S., Pourkazemi M.O. and Vosoughi G., 2000. Ecophysiological indicators of stress in female Persian sturgeon, *Acipenser persicus*. *Iranian Journal of Fisheries Sciences*, 2(1), 37-52.
- Billiard, S.M., Hahn, M.E., Franks, D.G., Peterson, R.E., Bols, N.C. and Hodson, P.V., 2002. Binding of polycyclic aromatic hydrocarbons (PAHs) to teleost aryl hydrocarbon receptors (AHRs). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 133(1), 55-68.
- Chomczynski, P. and Sacchi, N., 1987. Reagent for the single step simultaneous isolation of RNA. *Analytical Biochemistry*, 162, 536-7.
- Clauer, N., Chaudhuri, S., Toulkeridis, T. and Blanc, G., 2000. Fluctuations of Caspian Sea level: Beyond climatic variations? *Geology*, 28(11), 1015-1018.
- Dar, S.A., Gora, A.H., Bhat, I.A., Kumar, S., Srivastava, P., Muralidhar, P. and Gupta, S., 2017. Studies of anthelmintic benzimidazole derivatives on cytochrome P450 1A (CYP1A) dependent detoxification mechanism in *Labeo rohita*. *Aquaculture*, 481, 79-84.
- De Meulenaer, T. and Raymakers, C., 1996. Sturgeons of the Caspian Sea and the international trade in caviar. A TRAFFIC Network report. Cambridge, England.
- Dorrington, T., Zanette, J., Zacchi, F.L., Stegeman, J.J. and Bainy, A.C., 2012. Basal and 3-methylcholanthrene-induced expression of cytochrome P450 1A, 1B and 1C genes in the Brazilian guppy, *Poecilia vivipara*. *Aquatic Toxicology*, 124, 106-113.

- Dumont, H., 1995.** Ecocide in the Caspian Sea. *Nature*, 377(6551), 673-674.
- Dumont, H., 1998.** The Caspian Lake: History, biota, structure, and function. *Limnology and Oceanography*, 43(1), 44-52.
- Finney, D.J., 1952.** *Statistical method in biological assay*: Charles Griffin: London, England.
- Gao, K., Brandt, I., Goldstone, J.V. and Jönsson, M.E., 2011.** Cytochrome P450 1A, 1B, and 1C mRNA induction patterns in three-spined stickleback exposed to a transient and a persistent inducer. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, 154(1), 42-55.
- Goksøyr, A., 1995.** Use of cytochrome P450 1A (CYP1A) in fish as a biomarker of aquatic pollution. *Toxicology in Transition*. Springer, Berlin, Germany. 95 P.
- Goksøyr, A. and Förlin, L., 1992.** The cytochrome P-450 system in fish, aquatic toxicology and environmental monitoring. *Aquatic Toxicology*, 22(4), 287-311.
- Hajirezaee, S., Mirvaghefi, A.R., Farahmand, H. and Agh, N., 2016.** Effects of diazinon on adaptation to sea-water by the endangered Persian sturgeon, *Acipenser persicus*, fingerlings. *Ecotoxicology and Environmental Safety*, 133, 413-423.
- Hajirezaee, S., Mirvaghefi, A., Farahmand, H. and Agh, N., 2017.** NMR-based metabolomic study on the toxicological effects of pesticide, diazinon on adaptation to sea water by endangered Persian sturgeon, *Acipenser persicus* fingerlings. *Chemosphere*, 185, 213-226.
- Hanson, R., Dodoo, D., Essumang, D., Blay, J. and Yankson, K., 2007.** The effect of some selected pesticides on the growth and reproduction of fresh water *Oreochromis niloticus*, *Chrysiichthys nigrodigitatus* and *Clarias gariepinus*. *Bulletin of Environmental Contamination and Toxicology*, 79(5), 544-547.
- Huang, G.Y., Ying, G.G., Liang, Y.Q., Liu, S.S. and Liu, Y.S., 2014.** Expression patterns of metallothionein, cytochrome P450 1A and vitellogenin genes in western mosquitofish (*Gambusia affinis*) in response to heavy metals. *Ecotoxicology and Environmental Safety*, 105, 97-102.
- Ivanov, V., Vlasenko, A., Khodorevskaya, R. and Raspopov, V., 1999.** Contemporary status of Caspian sturgeon (Acipenseridae) stock and its conservation. *Journal of Applied Ichthyology*, 15(4,5), 103-105.
- Jabbarzadeh Shiadeh, S.M., Abtahi, B., Mojazi Amiri, B. and Nazari, R.M., 2000.** Study on the changes of some physiological factors during osmoregulation of juvenile Persian sturgeons (*Acipenser persicus*). *Iranian Journal of Fisheries Sciences*, 2(1), 61-74.
- Jager, T., Altin, D., Miljeteig, C. and Hansen, B.H., 2016.** Stage- dependent and sex- dependent sensitivity to water- soluble fractions of fresh and weathered oil in the marine copepod *Calanus finmarchicus*. *Environmental Toxicology and Chemistry*, 35(3), 728-735.
- Jönsson, M.E., Orrego, R., Woodin, B. R., Goldstone, J.V. and Stegeman, J.J., 2007.** Basal and 3, 3', 4, 4', 5-pentachlorobiphenyl-induced expression of cytochrome P450 1A, 1B and 1C genes in zebrafish. *Toxicology and Applied Pharmacology*, 221(1), 29-41.
- Jönsson, M.E., Gao, K., Olsson, J.A., Goldstone, J.V. and Brandt, I., 2010.** Induction patterns of new CYP1 genes in environmentally exposed rainbow

- trout. *Aquatic Toxicology*, 98(4), 311-321.
- Kaplin, P., 1995.** Implications of climate change and water-level rise in the Caspian Sea Region. *United Nations Environment Programme*.
- Karpinsky, M., 1992.** Aspects of the Caspian Sea benthic ecosystem. *Marine Pollution Bulletin*, 24(8), 384-389.
- Katuli, K.K., Amiri, B.M., Massarsky, A. and Yelghi, S., 2014.** Impact of a short-term diazinon exposure on the osmoregulation potentiality of Caspian roach (*Rutilus rutilus*) fingerlings. *Chemosphere*, 108, 396-404.
- Kennedy, C.J. and Farrell, A.P., 2005.** Ion homeostasis and interrenal stress responses in juvenile Pacific herring, *Clupea pallasii*, exposed to the water-soluble fraction of crude oil. *Journal of Experimental Marine Biology and Ecology*, 323(1), 43-56.
- Khodorevskaya, R., Pavlov, D. and Ruban, G., 2009.** Behaviour, migrations, distribution, and stocks of Sturgeons in the Volga-Caspian Basin: BoD–Books on Demand.
- Khodorevskaya, R.P., Dovgopol, G.F., Zhuravleva, O.L. and Vlasenko, A.D., 1997.** Present status of commercial stocks of sturgeons in the Caspian Sea basin. *Sturgeon biodiversity and conservation*. Springer, Dordrecht, Netherland. 219 P.
- Kiabi, B.H., Abdoli, A. and Naderi, M., 1999.** Status of the fish fauna in the South Caspian Basin of Iran. *Zoology in the Middle East*, 18(1), 57-65.
- Kühnert, A., Vogs, C., Seiwert, B., Aulhorn, S., Altenburger, R., Hollert, H., Küster, E. and Busch, W., 2017.** Biotransformation in the zebrafish embryo–temporal gene transcription changes of cytochrome P450 enzymes and internal exposure dynamics of the AhR binding xenobiotic benz [a] anthracene. *Environmental Pollution*, 230, 1-11.
- Kumar, G. and Denslow, N.D., 2016.** Gene expression profiling in fish toxicology: A review. *Reviews of Environmental Contamination and Toxicology*, 241, 1-38.
- Lari, E., Abtahi, B., Hashtroudi, M.S., Mohaddes, E. and Døving, K.B., 2015.** The effect of sublethal concentrations of the water- soluble fraction of crude oil on the chemosensory function of Caspian roach, *Rutilus caspicus* (Yakovlev, 1870). *Environmental Toxicology and Chemistry*, 34(8), 1826-1832.
- Livak, K.J. and Schmittgen, T.D., 2001.** Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*, 25(4), 402-408.
- Miandare, H.K., Farahmand, H., Akbarzadeh, A., Ramezani, S., Kaiya, H., Miyazato, M., Rytönen, K.T. and Nikinmaa, M., 2013.** Developmental transcription of genes putatively associated with growth in two sturgeon species of different growth rate. *General and Comparative Endocrinology*, 182, 41-47.
- Moghim, M., Kor, D., Tavakolieshkalak, M. and 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, 99-107.
- Nasrollahzadeh, A., 2010.** Caspian Sea and its ecological challenges. *Caspian Journal of Environmental Sciences*, 8(1), 97-104.
- Nazeri, S., Amiri, B.M., Farahmand, H. and Raine, J.C., 2019.** Triiodothyronine reduces toxic effects of diazinon in Persian sturgeon (*Acipenser persicus*) embryos.

- Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, 224, 1085-1097.
- Olsvik, P.A., Kroglund, F., Finstad, B. and Kristensen, T., 2010.** Effects of the fungicide azoxystrobin on Atlantic salmon (*Salmo salar* L.) smolt. *Ecotoxicology and Environmental Safety*, 73(8), 1852-1861.
- Paknejad, H., Niknejad, M., Shabani, A. and Safari, R., 2016.** Exposure of Persian sturgeon (*Acipenser persicus*) to cadmium results in biochemical, histological and transcriptional alterations. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, 181, 1-8.
- Payne, J. and Penrose, W., 1975.** Induction of aryl hydrocarbon (benzo [a] pyrene) hydroxylase in fish by petroleum. *Bulletin of Environmental Contamination and Toxicology*, 14(1), 112-116.
- Safari, R., Kolangi Miyandareh, H. and Jafernade, A., 2014.** Effect of cadmium chloride on p450 gene expression Persian Sturgeon (*Acipenser persicus* Borodin, 1897). *Journal of Applied Ichthyological Research*, 2(3), 43-57.
- Safari, R., Khalili, M., Reza Imanpour, M. and Pourkazemi, M., 2016.** The effects of endosulfan on P450 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.
- Søfteland, L., Holen, E. and Olsvik, P.A., 2010.** Toxicological application of primary hepatocyte cell cultures of Atlantic cod (*Gadus morhua*)—effects of BNF, PCDD and Cd. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, 151(4), 401-411.
- Stegeman, J., 1993.** Cytochrome P450 forms in fish. Cytochrome P450. Springer, Berlin, Germany. 291 P.
- Stegeman, J.J., 1992.** Nomenclature for hydrocarbon-inducible cytochrome P450 in fish. *Marine Environmental Research*, 34(1-4), 133-138.
- Suvetha, L., Ramesh, M. and Saravanan, M., 2010.** Influence of cypermethrin toxicity on ionic regulation and gill Na⁺/K⁺-ATPase activity of a freshwater teleost fish *Cyprinus carpio*. *Environmental Toxicology and Pharmacology*, 29(1), 44-49.
- Tolosa, I., de Mora, S., Sheikholeslami, M.R., Villeneuve, J.P., Bartocci, J. and Cattini, C., 2004.** Aliphatic and aromatic hydrocarbons in coastal Caspian Sea sediments. *Marine Pollution Bulletin*, 48(1-2), 44-60.
- Waisberg, M., Joseph, P., Hale, B. and Beyersmann, D., 2003.** Molecular and cellular mechanisms of cadmium carcinogenesis. *Toxicology*, 192(2-3), 95-117.
- Wolfe, D.A., 2013.** Fate and effects of petroleum hydrocarbons in marine ecosystems and organisms: Proceedings of a symposium. USA, Washington, November, 2013.
- Xing, H., Li, S., Wang, Z., Gao, X., Xu, S. and Wang, X., 2012.** Histopathological changes and antioxidant response in brain and kidney of common carp exposed to atrazine and chlorpyrifos. *Chemosphere*, 88(4), 377-383.
- Zanette, J., Jenny, M.J., Goldstone, J.V., Woodin, B.R., Watka, L.A., Bainy, A.C. and Stegeman, J.J., 2009.** New cytochrome P450 1B1, 1C2 and 1D1 genes in the killifish *Fundulus heteroclitus*: Basal expression and response of five killifish CYP1s to the AHR agonist PCB126. *Aquatic Toxicology*, 93(4), 234-243.

Zhang, L., Dong, X., Wang, C., Zuo, Z. and Chen, M., 2017. Bioaccumulation and the expression of hepatic cytochrome P450 genes in marine medaka (*Oryzias melastigma*) exposed to difenoconazole. *Journal of Environmental Sciences*, 52, 98-104.

Zhang, L., Gan, J., Ke, C., Liu, X., Zhao, J., You, L., Yu, J. and Wu, H., 2012. Identification and expression profile of a new cytochrome P450 isoform (CYP414A1) in the hepatopancreas of *Venerupis (Ruditapes) philippinarum* exposed to benzo [a] pyrene, cadmium and copper. *Environmental Toxicology and Pharmacology*, 33(1), 85-91.