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RESEARCH PAPER

Studying Some Blood Parameters of *Otolithes ruber* (Schneider, 1801) in Cold and Warm Seasons as an Indicator of Pollution in Musa Creek

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ABSTRACT
The present study attempts to investigate some blood parameters of *Otolithes ruber* during different seasons in terms of both temperature and pollution. For so doing it uses 10 specimens, for each station and season, collected from 5 polluted stations, including Petrochemical, Ghanam, Zangi, Douragh, Patil, and Sajafi as the control group, away from pollution in Musa Creek. The fish are anesthetized with 1ml of clove extract per liter. Their blood samples are taken immediately from the caudal vein, using a heparinized syringe. Afterwards, the serum is separated in a centrifuge with a speed of 6000 rpm for 2 minutes. The desired factors are measured by the Mindray BS200 auto-analyzer and the total protein level, by Bradford's usual laboratory methods. Results show that AST, ALT, ALP, Glucose, and Triglycerides have increased in more polluted stations ($P \leq 0.05$). In sheer contrast, total protein and Albumin have decreased as pollution grows ($P \geq 0.05$). According to this study, environmental water pollution of the fish has a large impact on the concentration of measured blood parameters, whereas the influence of seasonal changes on most of them is low.

KEYWORDS: Temperature, Heavy metals, Concentrations, Hematological characteristics

INTRODUCTION

Aquatic ecosystems, the largest natural environment, are always faced with such threats as genetic limitations and biodiversity (Koohkan et al., 2014). Musa Creek is one of the most important and valuable marine ecosystems in southern Iran which is strongly affected by industrial pollution (Azarmanesh et al., 2018), making it quite important in terms of contamination. According to conducted studies, environmental pollutants, especially the increasing entry of industrial wastewater with various compounds, persistent pollutants, heavy metals, and pesticides are among the most important factors (Hedayati et al., 2010). The particular impact, this pollution type has, includes its influence on some blood factors of marine biological communities and its potential effects on fish populations and, consequently, on fishing and fisheries activities (Johari et al., 2013; Mahmoud et al., 2019). In marine life, blood as a fluid tissue is one of the most important biological fluids that under the influence of various physiological/pathological conditions and various water pollution, undergoes a change in its compositions. Therefore, diagnosis of many aquatic diseases has always resorted

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to normal values of blood parameters and the way they change in different condition as one of its most important tools (Sayed & Hala, 2017). *O. ruber*, commonly known as tiger tooth croaker, is one of the most distinguished commercial aquatic animals in the south of the country (Eskandari et al., 2019). The fish belong to a demersal species, living in coastal waters up to a depth of 40 meters (Santhoshkumar et al., 2017; Abdi et al., 2007).

The circulatory system is one of the body's organs that works to circulate blood in the body in order to deliver nutrition, all kinds of gases consumed by the body, and to remove waste products from cellular metabolism (Jahan et al., 2017). Researchers believe that plasma biochemical parameters are useful to assess and manage the physiological state of aquatic animals (Wu & Zhou, 2013).

The biochemical parameters of blood in aquatic life depend on various factors such as water pollution of animal environment, seasonal fluctuations, reproductive cycle, age, sex, geomorphological conditions of the habitat, temperature, and day length (Dehghan Madiseh et al., 2009; Bilberg et al., 2012). The hematological characteristics of fish are one of the most important pieces of evidence for physiological stages, reflecting the relation among the characteristics of the aquatic ecosystem and their health (Sayed & Hamed, 2017). For this reason, possession of a natural range of blood parameters in a fish can be used as a biological indicator. Due to its short life span, high growth, adaptation to laboratory conditions, and easy access to blood, fish are used as one of the animal models for monitoring environmental pollution as well as the study of its biological effects on animals (Savari et al., 2016). The aim of the present study is to measure some blood factors, affected by pollution, in the species studied within polluted stations and compare them with far away stations as an indicator of pollution in Musa creek.

MATERIAL AND METHODS

The specimen of the present study was gathered in August and January in order to study the warm and cold seasons at five stations in Musa creek, which included Doragh estuaries (adjacent to chloral alkyl factory), petrochemical plants (adjacent to petrochemical industries and urban sewage discharge), Zangi (adjacent to the fishing pier), Patil (adjacent to the shipbuilding industry), Ghanam (adjacent to the dock and the movement of oil-carrying ships), and Sajafi Creek (far from pollution) as control group (Figure 1). A total number of 10 *O. ruber* with similar biometrics were caught by the trawl in each station. The fish were anesthetized with 1ml/l of clove extract (Basir & Abdi, 2016), and their blood was immediately drawn from their caudal veins, using 2ml heparin syringe (Banaee et al., 2014). After separating the serum via blood centrifugation at 6000 rpm for a duration of 2 minutes, the desired factors could be measured with the Mindray BS200 auto-analyzer (Johari et al., 2013; Savari et al., 2016). The level of total protein was measured by Bradford's pars test diagnostic kit, one of the most common methods for so doing (Banaee et al., 2011). In this method, simultaneous with binding the compass color to the protein in the acidic environment, an immediate change occurs in the maximum adsorption, where the sample’s color changes from brown to blue. ALT and AST enzymes were tested by pars azmoon diagnostic kit and by enzymatic method, according to the instructions given by Mahmoud et al. (2019). For AST enzyme test, alphaketoglutaric acid was used as the raw material for the reaction, with the oxaloacetate, formed in response to DNPH, producing hydrazone values that produce a brown color in the alkaline environment (Kavitha et al., 2012). This reaction was detected by colorimetry at a wavelength of 340 nm. As for measuring ALT enzyme, pyruvate reacted with DNPH to produce hydrazone, resulting in a brown color in the alkaline environment (Adedeji et al., 2009). This reaction was detected by colorimetry at a wavelength

of 340 nm (Krasno et al., 2013). Alkaline phosphatase was measured, based on the conversion of nitrophenyl phosphate to nitrophenol and phosphate at a wavelength of 405 nm. Alkaline phosphatase was measured by P-Nitrophenyl phosphate method, usually based on its ability to break down phosphate groups at acidic pH rates. Triglyceride and albumin were measured with Mindray BS200 biochemical analysis, using pars azmoon laboratory kits (Hedayati et al., 2010). Glucose levels were measured enzymatically by using spectrophotometer and Man kit (Man, Iran). All values in two seasons were presented as the mean ± standard error of the mean. To determine data normality, the Shapiro-wilk test was used. Also one-way ANOVA analysis and SPSS software compared the indicators in each fish not only at different stations but also in either season. In case of a significant difference, DUNCAN post-test was employed. The difference was acceptable at the 95% confidence level. Finally the figures got drawn in Microsoft Excel 2010 (Daphedar & Taranath, 2018).

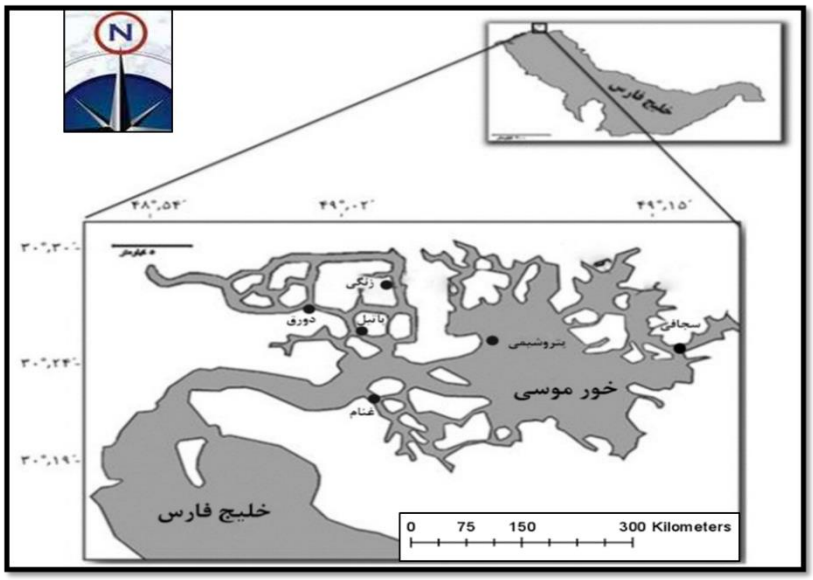

Figure 1. Map of the study area and sampling stations

RESULTS AND DISCUSSION

The highest level of AST was obtained at the Petrochemical Station and the lowest one, at the control and Patil stations. There occurred no significant difference in any of the stations with the change of the seasons. Moreover, in the cold season there was no significant difference in ALT levels in any station, whereas in the warm season, ALT levels in the Petrochemical station were significantly higher than their cold season counterpart. There was no significant difference between Douragh, Patil, and control stations for ALP enzyme in both warm and cold seasons. But in Petrochemical, Ghanam, and Zangi stations this variation in the two seasons stood out. Glucose results showed that it was high in all stations in the warm season, compared to the cold one. Also, only in case of the control and Patil stations, there was no significant difference in the amount of Glucose in either season. The amount of Triglycerides in all stations was higher in warm season than the cold one. Also, the highest amount of Triglycerides was reported at the Petrochemical station with the lowest amount belonging to the control station. There was no significant difference between warm and cold samples at each station. The lowest Total protein was observed at the Petrochemical and the highest, at both Patil and control stations. There was no significant difference between warm and cold samples at any station. In all stations higher Albumin levels could be seen in the warm season than the cold one. In all of the stations, there

was no significant difference between warm and cold season samples, with the exception of Ghanam and Petrochemical stations. Also, the highest amount of albumin was observed in the control and the lowest amount at the Petrochemical station (Figure 2)

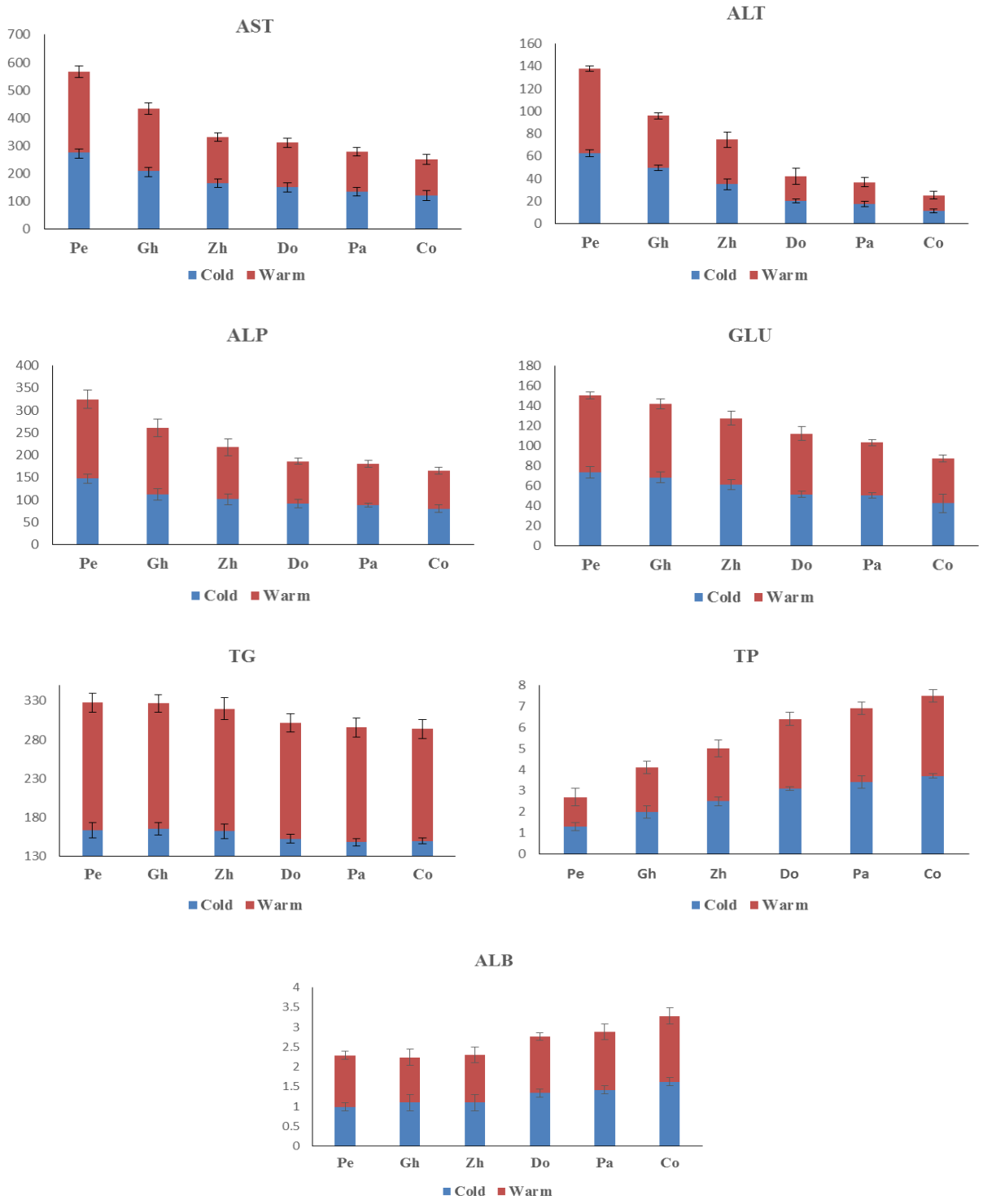

Figure 2. The amount of AST (U/L), ALT (U/L), ALP (U/L), GLU (mg/dl), TG (mg/dl), TP (g/dl), and ALB (g/dl) enzyme in *O. ruber* at different stations in both cold and warm seasons as ($P<0.05$) (Pe: Petrochemi, Gh: Ghanam, Zh: Zangi, Do: Douragh, Pa: Patil, Co: control)

Measuring blood parameters is mainly used to diagnose the physiological condition and determine the general state of fish health. Biochemical changes in various tissues of the body reflect alterations in the metabolism and biochemical processes of living organisms, which can be affected by many different factors such as the animal's contact with toxins and pollutants (Alishahi et al., 2011; Savari et al., 2020). The results of the present study show that the highest levels of phosphatase, glucose, and triglycerides and the lowest amount of total protein and albumin belonged to the Petrochemical station, compared to the other stations as well as the control. Due to proximity to pollutant sources, such as discharge of effluents and the chlorine alkali factory, the presence of heavy metals seems reasonable. In line with this finding, researchers believe that the level of enzymes in fish is a good indicator of severe stress, providing information about organ dysfunction (Aluru & Vijayan, 2009; Mekkawy et al., 2019). Knowing the extent of changes in fish hematology can be a useful tool to predict the level of environmental pollution (Bastami, 2014). Changes in the ratio of fish blood factors may indicate the presence of a disease or exposure to chemical materials. Laboratory studies can indicate the potential risk of toxins in aquatic environments (Davis et al., 2008; Murugesan et al., 2013). Result analysis in the present study depends not only on the relation between environmental pollution and sediments of each stations, but on the close relation between fish biology and seasonal differences. Data and information from toxicology experiments in the field of toxicology ecosystem shows the effects of these toxins on fish populations. These changes in the activity of liver enzymes are probably related to the destruction of liver tissue due to stress caused by pollutants like heavy metals (Dogan and Can, 2011). AST and ALT enzymes are found in various tissues such as the liver, heart, skeletal muscle, kidneys, pancreas, spleen, red blood cells, and fish gills. Most of these enzymes are located inside liver cells in the mitochondria of hepatocytes; therefore, any slight damage, inflammation, or necrosis of liver cells will cause these enzymes to be released and their plasma levels, increased (Kazemi et al., 2013). Significant increase in AST enzyme levels was reported in a similar situation in *Channa punctatus* freshwater fish in contact with trophic aphorbias (Sayed & Hala, 2017). The results of total protein and albumin measurement in the studied stations showed that the amount of these two factors in both cold and warm seasons in stations with more pollution such as petrochemical and Ghannam stations was higher, compared to Patil and control stations. This reaction, along with the increase in protein decomposition to bind to the toxic agent, is one of the mechanisms for adapting aquatic organisms, exposed to stressors (Singh et al., 2018; Safahieh et al., 2011). Determining blood plasma factors such as protein can be a good indicator of ecological and physiological stress as well as pollution in aquatic animals. Changes in protein synthesis are one of the most common responses to cell damage. Therefore, measuring the amount of protein can indicate a degree of cell damage. With continued stress and reduced carbohydrates and lipids, protein can be used as a source of energy and decline. According to the researchers who have found the destruction of the renal tubules and liver tissue, changes in the activity of enzymes involved in protein biosynthesis reduce the amount of protein synthesis (Krasno et al., 2013). Decrease in total protein of various fish has been previously reported, e.g. *Astacus leptodactylus* exposed to endosulfan (Banaee & Ahmadi., 2011), *Labeo rohita* exposed to malathion (Patil & David., 2008), *Oreochromis mossambicus* exposed to endosulfan (Kumar et al., 2011), *Alburnus mossulensis* under the influence of Fanpro Patrin (Banaee et al., 2014), *Oncorhynchus mykiss* exposed to diazinon (Banaee et al., 2011), *Cyprinus carpio* exposed to Lindan (Saravanan et al., 2011), and *Oreochromis niloticus* exposed to cypermethrin (Korkmaz et al., 2009).

CONCLUSION

Blood parameters, affected by pollution, were under the influence of contamination amount, accompanied by an increase or decrease in the number of these factors in the blood of the *O. ruber*. Also, the change of seasons had less impact on these parameters than pollution. In general, measuring these parameters in blood of *O. ruber* can be mentioned as bioindicator and pollution index of Musa creek.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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