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Research Article

Monitoring anthropogenic pollutants in northern coasts of Hormuz Strait using blood indices and thyroid hormone levels in *Periophthalmus argentilineatus* (Pisces: Gobiidae)

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Abstract

Coastal waters in northern part of Hormuz Strait receive large inputs of anthropogenic pollutants. This study was conducted to determine the effects of environmental contaminants on plasma enzymes, thyroid hormones and biochemical blood parameters of *Periophthalmus argentilineatus* to monitor marine pollution from northern part of Hormuz strait. For this purpose, a total of 90 specimens were collected from three estuarine stations (30 specimens for each station) including Shour-e-aval (first station; St_1), Souro (second station; St_2) and Bustanoo (third station; St_3) and some blood plasma indices, such as aspartate aminotransaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), glucose (GLU), cholesterol (CHOL), triglyceride (TRIG), and thyroid hormones including triiodothyronine (T_3), thyroxine (T_4) and thyroid-stimulating hormone (TSH) were measured. Results showed that the plasma enzymes levels were significantly higher in fish from the polluted location (Bustanoo; station 3). Concentrations of thyroid hormones and also the glucose and cholesterol levels were significantly higher in the samples from the station 3 (p<0.05). Due to the negative impact of pollutants on biochemical and hormonal functions of blood serum of resident species, including *P. argentilineatus,* this species can be used as a bioindicator of pollution in northern part of Hormuz Strait.

Keywords: Hormuz Strait, Anthropogenic pollutants, *Periophthalmus argentilineatus*, Bioindicator, Biochemical blood parameters, Thyroid hormones

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Introduction

Environmental contaminants (such as poly aromatic hydrocarbons (PAHs), pesticides, heavy metals, etc.) are caused by different sources such as industrial, domestic and agriculture effluents and radioactive wastes (Yakan *et al.*, 2015). Several of them have adverse impacts on the health of man and ecosystem (IPCS, 2002; Wu *et al.*, 2009; Yakan *et al.*, 2015) and are capable of causing deleterious effects in aquatic biota (Shailaja and Rodrigues, 2003). For example, PAHs are bioavailable to fish and other marine organisms through the food chain, as waterborne compounds and from contaminated sediments (Mohammadizadeh *et al.*, 2013b).

Fish is widely used in toxicological studies as models to evaluate the health of aquatic ecosystems (Law, 2003). Measurement of biochemical parameters in fish that respond specifically to the degree and type of contamination can be used to evaluate the impact of contaminants on aquatic ecosystems (Petřivalský *et al.*, 1997; Helgason *et al.*, 2008). Concentration of pollutants can change the enzyme activities and often directly induce cell damage in specific organs (Yang and Chen, 2003). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are enzymes used in the diagnosis of the damage caused by pollutants in several fish tissues (De la Torre *et al.*, 2000; Younis *et al.*, 2012). Thyroid gland is the other target that is affected by endocrine disrupting chemicals and environmental contaminants (Schnitzler *et al.*, 2008). Thyroid hormones have many important roles in maintaining proper physiological function and in growth and development of fish (Brown *et al.*, 2004). Environmental contaminants can interfere directly with hormones synthesis in the thyroid gland (Ishihara *et al.*, 2003; Brown *et al.*, 2004; Boas *et al.*, 2006), change their concentrations and have serious effects on their status as shown in some freshwater and marine fish species (Black and Simpson, 1974; Moccia *et al.*, 1986).

The use of fish as a marine pollution monitoring indicator is widely recognized; Marcovecchio (2004) used *Micropogonias furnieri* and *Mugil liza* as bioindicators of heavy metals pollution in La Plata river estuary, Argentina. Oliveira *et al.* (2010) used the relationship between metallothionein concentrations in *Chelon auratus* and environmental metal concentrations in a contaminated coastal system in Portugal to assess the environmental metal contamination. Mohammadizadeh *et al.* (2013b) determined the effects of PAHs concentration on thyroid hormones of *Planiliza klunzingeri* by monitoring marine pollution in Hormuz Strait. The effects of heavy metal pollution on plasma enzymes of *Periophthalmus waltoni* were applied as indicators of marine pollution in northern part of Hormuz Strait (Sarhadizadeh *et al.*, 2014a).

Mudskippers (Gobiidae: Oxudercinae) live in intertidal habitat of the mudflats and in mangrove ecosystems (Murdy, 1989). *Periophthalmus argentilineatus* (Valenciennes, 1837) is one the most abundant mudskipper fish species in estuaries of northern part of Hormuz Strait (Abdoli, 2008). It is a filter and detritus-mud feeder; therefore, it is in contact with pollutants in the water column and sediments. Mudskippers have been used in several studies because they possess several characteristics required as a bioindicators species and, therefore, may be a good tool for monitoring programs. Given this issue and because coastal waters of northern part of Hormuz Strait receive large inputs of anthropogenic pollutants through industrial and urban discharges, atmospheric deposition and terrestrial drainage (Mohammadizadeh *et al.*, 2013a), the purpose of the present study was to determine the effects of environmental contaminants (PAHs and heavy metals) on plasma enzymes, thyroid hormones and biochemical blood parameters of *P. argentilineatus* to monitor marine pollution in northern part of Hormuz Strait.

Materials and methods

Periophthalmus argentilineatus specimens were collected at night at full low tide using a kind of coastal trap net fishing gear named Mushta in local language from three estuaries, including Shour-e-aval as the first station (St_1), Souro as the second station (St_2) and Bustanoo as the third station (St_3), from northern part of Hormuz Strait in March 2012 (Fig. 1). The selection of stations was based on the arrival of pollution, as follows: St_1: No human sewage inlet; St_2: with human sewage inlet; St_3: with human and industrial wastewater inlet. Degree and type of pollution were determined based on the field studies and other previous study plans. A total of 90 specimens (30 specimens for each station) were collected and each was measured to the nearest 0.1 cm body length (TL) and to the nearest 0.01 g body weight (TW). Table 1 illustrates the average body length and weight of fish samples in different stations. All the samples were used for determining the blood collection.

Figure 1: Studied areas and sampling stations, St_1: 60° 39' 18", 25° 15' 20.40", St_2: 60° 34' 23", 25° 18' 59", St_3: 60° 28' 41", 25° 18' 59".

Blood sampling was performed instantly after the fishes were captured and while they were physically restrained, blood samples were collected directly from the heart using 2^{cc} plastic syringes coated with sodium heparin to prevent blood coagulation and then they were placed into dry plastic tubes (Jacobson *et al.*, 1992). For evaluation of blood plasma, a centrifuge system was used and blood tubes were centrifuged for 10 min at 4,000 rpm. Afterward, glass tubes were broken and the resultant blood plasma was emptied into sterile micro tubes and frozen at -20°C for further analysis. Enzyme activity tests, including alkaline phosphatase (ALP), aspartate aminotransaminase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH), were made using an auto-analyzer (Cobas Integra System, France). All the blood determinations were done according to the "National Committee for Clinical Laboratory Standards" (Strik *et al.*, 2007).

Hormonal data tests, including triiodothyronine (T_3), thyroxine (T_4) and thyroid-stimulating hormone (TSH), were performed by Vidas Biomerieux System (Electroimmuno Analyzer, France; Strik *et al.*, 2007). Also biochemical blood parameters determination, including glucose, cholesterol and triglycerides, were performed using an auto-analyzer (Cobas Integra System, France; Strik *et al.*, 2007).

Data were analyzed using SPSS (Version 21). Prior to the analysis, Kolmogorov-Smirnov and Shapiro-Wilk tests were used to examine the normality of data and it was determined that they had a normal distribution. For each analyzed normally distributed parameter, the mean and standard deviation were calculated. For comparison of the levels of different plasma components and also thyroid hormones among samples of different stations, Tukey's test was applied and a 5% significance level was employed throughout (Mohammadizadeh *et al.* 2013b, Sarhadizadeh *et al.*, 2014a,b).

Results

According to the biometry results provided in Table 1, the samples of St_3 showed minimum mean weight and length (7.08±0.21 g; 9.90±3.46 cm, respectively) continued with St_2 and St_1.

Table 1: Average body length and weight (±standard deviation) in *P. argentilineatus* samples in different stations of north coast of Hormuz Strait.

Species	Stations	Total length (cm)	Weight (g)
	St_1	10.30±3.45	8.20±0.27
P. argentilineatus	St_2	10.20±2.56	8.26±0.26
	St_3	9.90±3.46	7.08±0.21

The plasma enzyme levels (in units per liter (U/L)) were higher in fish from the St_3 than in samples from two other stations and statistical analysis showed significant difference for AST among the three stations ($p<0.05$). About other plasma enzymes (ALT, ALP and LDH), significant differences were found between St_1 and two other stations, St_2 and St_3 (p<0.05). AST levels in St_1 and St_2 showed a significant difference with ALP levels in the relevant stations (p<0.05), whereas no significant differences were found for ALT and LDH (p>0.05) (Table 2).

Table 2: Average plasma enzymes levels (±standard deviation) (U/L) of *P. argentilineatus* samples in different stations of north coast of Hormuz Strait, N=30.

Stations	Plasma enzymes			
	AST	ALT	ALP	LDH
St_1	226.66±9.56 $^{c\,(*)}$	4.06±0.82 ^{b}	103.50±4.14 $^{b\,(*)}$	686.66±18.95 ^{b}
St_2	251.11±6.94 $^{b\,(*)}$	5.56±0.86 ^{a}	115.16±4.46 $^{a\,(*)}$	717.16±6.81 ^{a}
St_3	268.88±8.65 ^{a}	5.71±0.80 ^{a}	113.33±5.02 ^{a}	750.50±12.29 ^{a}

* p<0.05

Measurement of thyroid hormones for all samples in the three stations showed that concentrations (in nanomoles per liter (nmol/L)) of T_3, T_4 and TSH were higher in St_3 compared with the two other stations. According to the statistical analysis, concentrations of T_3 and TSH in St_1 showed significant differences with the two other stations (p<0.05); which were lesser than other stations; concentration of T_4 showed significant difference in all three stations (p<0.05), which was higher in St_3. Concentration of T_4 in St_1 and St_2 showed significant difference with the values of T_3 and TSH (p<0.05, Table 3).

Table 3: Average thyroid hormones concentrations (±standard deviation) (nmol/l) of *P. argentilineatus* samples in different stations of north coast of Hormuz Strait, N=30.

Stations	Thyroid hormones concentrations		
	T_3	T_4	TSH
St_1	1.17±0.17 ^{b}	78.76±3.58 $^{c\,(*)}$	0.37±0.08 ^{b}
St_2	1.50±0.19 ^{a}	88.73±3.81 $^{b\,(*)}$	0.61±0.11 ^{a}
St_3	1.58±0.22 ^{a}	94.23±5.56 ^{a}	0.61±0.14 ^{a}

* p<0.05

The levels (mg/dl) of measured biochemical blood parameters of *P. argentilineatus* are shown in Figures 2 and 3. The biochemical blood parameters, glucose and cholesterol, varied considerably between St_3 and the two other stations; as they were higher in collected samples from St_3 (p<0.05). About triglyceride, its concentration was significantly higher in St_2 compared with St_1 (p<0.05); whereas this difference with St_3 was not significant (p>0.05).

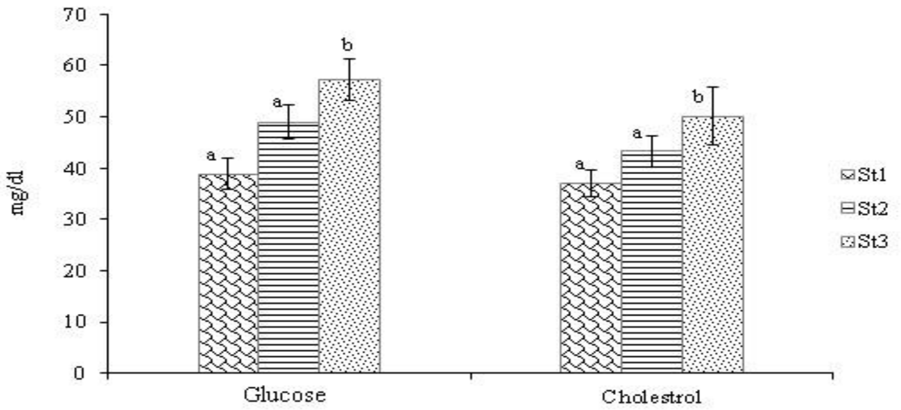

Figure 2: Comparison of mean biochemical blood parameters (glucose and cholesterol, mg/dl) in *P. argentilineatus* sampled at different stations. Error bars show standard deviation.

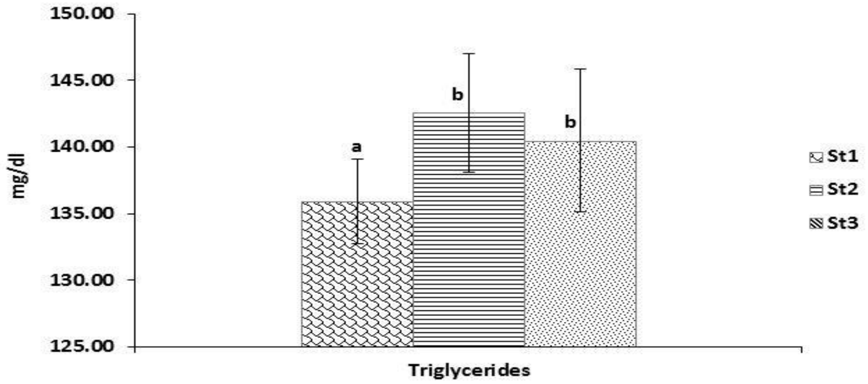

Figure 3: Comparison of mean biochemical blood parameter (triglyceride, mg/dl) in *P. argentilineatus* sampled at different stations. Error bards show standard deviation.

Discussion

Based on the results of measurement of plasma enzymes, including AST, ALT, ALP and LDH, the levels of all these plasma enzymes (U/L) were higher in the samples from St_3 than those in samples from the two other stations. Svoboda (2001), Van der Oost *et al.* (2003) and Palanivelu *et al.* (2005) stated that increase in any of these enzymes could be an indication of cellular or tissue damage that may have occurred because of the presence of a stressor in the living environment. In this study, the increment of plasma enzymes in St_3 indicates changes in protein metabolism and cellular damage in the samples of this region. The increase in the enzymes activity in this station indicates a type of cellular damage, which could be due to the presence of stressors in this region, which has led to cellular and tissue damage in this fish. According to our studies on this region, which have been published previously, it is determined that St_3 in Hormuz Strait receive more pollution (poly aromatic hydrocarbons (PAHs) and heavy metals) than the two other stations (Sarhadizadeh *et al.*, 2014a,b). Our results are in accordance with the results of other studies; Levesque *et al.* (2002) investigated the activity of AST and ALT in the blood plasma of *Perca* *flavescens* in environmental condition and Zikić *et al.* (2001) in the blood plasma of *Carassius auratus* in laboratory condition. They observed similar results based on the increment of enzymes activity due to the presence of heavy metals such as copper, zinc and cadmium. Also, Folmar *et al.* (1992) and Harvey *et al.* (1994) concluded that in the presence of environmental pollutants such as different heavy metals, the activity of enzymes in blood plasma would increase.

Thyroid hormones as effective factors in physiological functions, affect early development, growth, nutritional processes and reproduction in fish species (Brown *et al.*, 2004; Khetan, 2014). The mechanism of production of these hormones is affected by the activation of neuroendocrine hypothalamic-pituitary-thyroid (HPT) axis (Blanton and Specker, 2007; Zoeller, 2007). Anterior pituitary gland secretes thyroid-stimulating hormone (TSH); then TSH primarily activates and controls synthesis of thyroid hormones (T_3 and T_4). Adequate amount of thyroid hormones in fish results in faster growth and better health condition. High sensitivity of thyroid system to some biotic and abiotic environmental factors can lead to variation in secretion of thyroid hormones (T_3 and T_4) with both physical and chemical origin (Deane *et al.*, 2001; Howdeshell, 2002; Peter *et al.*, 2007). For example, environmental contaminants can directly affect hormone synthesis in the thyroid gland (Brown *et al.*, 2004; Peretz *et al.*, 2014); in fact, they can intervene in the hypothalamic–pituitary–thyroid axis in various ways. Also they can alter the metabolism of thyroid hormones (Wade *et al.*, 2002; Boas *et al.*, 2006). Disruption of thyroid action under unfavorable conditions could have severe consequences in fish. In this study, concentrations of different thyroid hormones in St_3 were higher than concentrations of these hormones in St_1 and St_2. Statistical analysis showed significant difference among the concentration of T_4 in the three stations. In this regard, it is reported that pollutants induce a hyperactivity of the thyroid follicles witch results in an increment of T_4 concentration (Brown *et al.*, 2004). Generally, according to the results, pollution conditions had an important role in increment of T_3, T_4 and TSH levels. Studies showed that exposure to acute and chronic pollution can change T_3 and T_4 levels in blood plasma of some teleost fishes (Hontela *et al.*, 1995; Zhou *et al.*, 1999, 2000). According to the results of this study, based on higher concentrations of all of the three hormones in $St_{3;}$ and also according to the studies performed on the levels of thyroid hormones of *P. waltoni* in the same region based on high concentrations of heavy metals, such as nickel, zinc, vanadium, lead and the contamination of PAHs in St_3 (Sarhadizadeh *et al.*, 2014a,b), it could be declared that there was a positive relationship between increment of pollutants and levels of thyroid hormones. In addition, sensitivity of the thyroid axis and activities of related hormones in fish physiological processes confirm the role of these hormones in response to stressors in *P. argentilineatus*. Our results are in agreement with other studies such as the study carried out by Bleau *et al.* (1996); they found that when *Oncorhynchus mykiss* samples expose to mercury for 4h, the level of T_4 increases and return to control level after 72 and 168 h. Kirubagaran and Joy (1994) observed a gradual reduction in plasma T_3 and T_4 of *Clarias batrachus* after 1-week exposure to methyl mercury. Oliveira *et al.* (2010) examined the effects of 5 infected areas off the coast of Portugal using *Chelon auratus* as a bioindicator; results showed that in contaminated areas TSH level was higher, while T_3 and T_4 levels were lower; these results about TSH is similar to our results but about T_3 and T_4 is contradictory.

As reported by many researchers, analysis of biochemical blood parameters is considered as one of the most valuable diagnostic tools, because physiological values, which are obtained from these analysis are species-specific and age-dependent (Celik, 2004; Patriche *et al.*, 2011), and also these parameters can provide warnings in early stages of infection (Mohammadizadeh *et al.*, 2013b). According to the results, glucose and cholesterol concentrations were higher in samples from St_3 than those in samples from the two other stations and this difference was statistically significant ($p<0.05$). Since glucose and cholesterol are sensitive to stressors such as pollution (Zikić *et al.*, 2001), so higher concentrations of these parameters in St_3 could be due to contaminants in this station. Some studies concluded that contaminants can induce hyperglycemia and hypercholesterolemia in different fish species (Zikić *et al.*, 2001). Our results are consistent with the results of Cicik and Engin (2005) based on increment of blood glucose levels in under stress fish. This increment may be due to disorder of carbohydrate metabolism as a result of increased glycogen degradation by liver; increased liver glycogen degradation may also be due to increased secretion of hormones such as glucagon and adrenocorticotropic or decreased insulin function (Raja *et al.*, 1992). Our data is compatible with a toxicant stress inducing higher glycaemia and cholesterolemia. Oliveira *et al.* (2010) studied *Chelon auratus* in the coat of Portugal showing that glucose and lactate levels increased in areas contaminated with heavy metals and PAHs. In general, the process of glucose production in fish is done through the glycogenesis to provide the needed energy against the stressor; therefore, glucose reaches the cells through the blood cycle and enters the cells through function of insulin (Lehninger *et al.*, 1993). Typically, fish affected by contaminated water need more glucose to adapt and repair the damage (Palermo *et al.*, 2008).

Furthermore, other factors such as sampling techniques, analysis methods, age, habitat, and diet may affect biochemical blood parameters of fish species (Sakamoto *et al.*, 2001).

Overall, coastal waters in northern part of Hormuz Strait receive a great number of anthropogenic pollutants; Bustanoo station (St_3), which was one of the studied coasts here, is considered as a high-polluted area, which according to previous studies receive large inputs of anthropogenic pollutants through different resources, including industrial and urban discharges, human effluents and vessel activities. These pollutants lead to many environmental changes; so organisms of this region are also strongly affected by these changes. In fact, waters of this region have a high potential for negative impact on biochemical and hormonal functions of blood serum of species living in this region, including *P. argentilineatus*, which is found in the estuaries of the region; so that this species can be used as a bioindicator of pollution in this region. The results of this study presented useful information on performance and impact of contaminants on fish health and ecology of the region. Due to this, pollutant control in the area to prevent environmental deterioration and thus prevent serious damage to living organisms is essential. Finally, it is suggested that studies related to environmental pollution using suitable bioindicators should be done continuously in the future for environmental management of the coasts of this region.

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