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Effect of some environmental indicators on physiological disturbances and endocrine disorders in *Tilapiazillii* (Gervais, 1848) and *Cyprinuscarpio* l, 1758

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Abstract

The pollutants are the effective factors of the presence and spread of aquatic organisms, and the haematological and hormonal parameters are the most common stress indicatorsthat can estimated the fish health. In the present study, some chemical and physical parameters were analyzed from two stations in Basrah province, from Marine Science Centre tanks –BasrahUniversity and the other station in Al-HababaRiver in AbuAl-Khasseebdistrict. An attempt has been made to study the effect of pollutant stressors on haematological [red blood cell count (RBCs), haemoglobin (Hb), packed cell volume (PCV), white blood cell count (WBC) and differential leucocyte count (DLC)] and hormonal (TSH, T3, T4, testosterone, cortisol and glucose) parameters of two types of fish *Tilapiazillii* and *Cyprinuscarpio*. Under the effect of natural stressors, pysico-chemical factors were found to exhibit significant decline in RBC, Hb, PCV and WBCs. DLC when viewed revealed a decrease in lymphocytes, monocytes, eosinophils and basophils whereas neutrophils and thrombocytes exhibited rather an appreciable increase. A significant increase ($P \leq 0.05$) cortisol and glucose levels were observed up. *Tilapiazillii* and *Cyprinuscarpio* also depicted significant decline in TEC, Hb and Hct under the effect of phosphate (6.05 mg/l). TLC depicted significant increase and among Differential leucocyte count (DLC) lymphocytes, monocytes and eosinophils register an increase but neutrophils, basophils and thrombocyte counts exhibit a decline in their numbers, also Cortisol and glucose levels were noticed to increase. Generally, *C. carpio* was more affected than *T.zillii* regarding haematological, biochemical indicators and histopathological examinations.

Keywords: physiological disturbances endocrine, *Tilapiazillii* (Gervais, 1848), *Cyprinuscarpio*L, 1758

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INTRODUCTION

Stress can be described as the physiological response to a stressor. Stress can also be described as an internal hormonal response of a living organism caused by environmental or other external factors that moves organism out of its normal physiological resting state, or homeostasis (Selye, 1973). Stress in fish, a key member of aquatic environment and which also form a valuable commodity for human consumption (proteins, 16-23%) may be induced by various abiotic factors. As well as the stress can also disturb the normal physiological equilibrium or homeostasis of fish.

Beside the natural stressors such as environmental factors (change in water temperature, pH, O2 concentrations, etc.) (Gupta *et al*., 2009 and Raina, 2011; Birhan et al., 2018), heavy metals and xenobiotic are regarded as the serious pollutants which act as major source of stress to fishes (Tavares-Dias and Barcellos, 2005, Buhari and Ismail, 2017, Setyaningrum et al., 2019) which find their entry into waterbodies through industrial, domestic and agricultural discharge system. All these natural and anthropogenic stressors disturb the homeostatic mechanism of fishes besides creating considerable stress to fishes (Vosyliene and Kazlauskiene, 1999). Fish are more sensitive to stressors than many other vertebrates because their physiological homeostasis is intimately bound to and dependent upon the water in the surrounding

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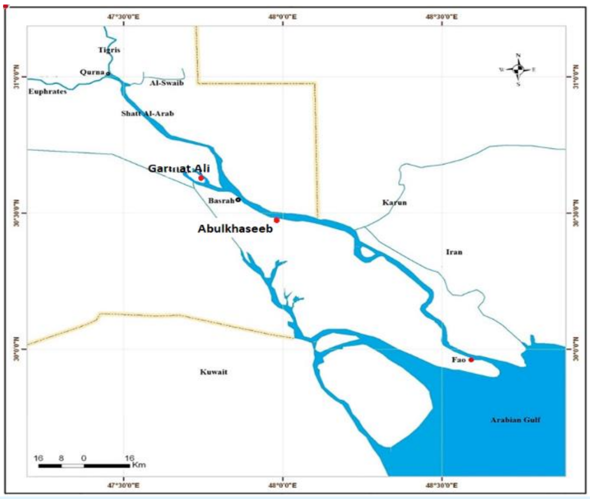

Fig. 1. Map of Shatt Al-Arab river showing the sampling station

environment (Setyaningrum et al., 2019). Disturbance of water and ion homeostasis during stress is due to the very intimate relationship between body fluids in the gills and the ambient water. Physiological responses of fish to environmental stressors have been grouped broadly as primary and secondary. Primary responses, which involve the initial neuroendocrines, include the release of catecholamines from chromaffin tissue (Randall and Perry, 1992; Reid *et al*., 1998) and the stimulation of the hypothalamic-pituitary-interrenal (HPI) axis culminating in the release of corticosteroid hormones into circulation (Donaldson, 1981; Wendelaar Bonga, 1997; Mommsen *et al*., 1999; Martinez-Porchas*et al*., 2009). Secondary responses include changes in plasma and tissue ions and metabolite levels, haematological features, and heat-shock or stress proteins (HSPs), all of which relate to physiological adjustments such as metabolism, respiration, acid-base status, hydromineral balance, immune function and cellular responses (Pickering, 1981; Iwama*et al*., 1997; Gupta, 2012; Nahayo, et al, 2019). Present study designed to evaluate the effect of stressors on haematological and hormonal parameters of fish.

The presence of sublethal concentrations of noxious chemicals in freshwater environments can promote the emergence and development of diseases in fish. It has been suggested that only chronic stress is responsible for impairment of various physiological system of fish viz., blood, reproduction, osmoregulation etc. besides biochemical profile of the fishes. So, in lieu of above fact, investigations were conducted presently to assess the effect of inorganic pollutant, phosphorus, on the haematological and hormonal parameters of the fish.

MATERIAL AND METHODS

Study Areas

The samples of water were collected from two stations (**Fig. 1**), one of them (St. 1) located at Marine

Fig. 2. Sampling area of fish farm at Basrah university camp (St.1)

Fig. 3. Sampling area of Al-Hababah River (St.2). Materials and Methods

Science Centre fish farm at Basrah university camp 30°34′05″N47°44′56″E northwest of Basrah governorate, south region of Iraq. In this pond, (**Fig. 2**) fishes of various sizes were stocked for greater growth and for artificial reproduction. There were some aquatic plants spread on the edges of the pond, in addition to various types of algae. Also, there were many types of amphibian vertebrates such as frogs.

And the second station (St. 2) from Al-Hababah river (**Fig. 3**) at Abu Al-Khaseeb District southern Iraq which is one of the branches of the Shatt al-Arab 30°27’40”N, 47°56’38”E, the river surrounded by trees and grasses. There are various sizes of fishes with aquatic plants, algae and phytoplankton besides some reptiles and amphibians in the river. Throw in the river residues, sewage effluents discharged from houses, waste product from factories in addition to the oils used for motor vehicles and others.

A total of 50 male samples related to Cypriniformes fish (family Cyprinidae) *Cyprinuscarpio*and Perciformes fish (family Cichlidae) *Tilapiazillii* were collected for 5 months in 2018 from two stations (25 fish for each station); Fish farming station of Marine Science Centre - Basrah university (St.1), and Al-Hababah river at Abu Al-Khaseeb District (St.2). Water temperature measured by

Table 1. The mean of some physical factors at study areas

Site	Water Temp.°c	Salinity ‰	pH	EC ms\Cm	Turbidity (NTU)
Station 1	20.5	1.2	7.8	2.72	16.7
Station 2	23.1	17.3	7.1	5.84	47.8

using thermometer. Other physical and chemical factors measured in chemistry laboratory in Marine Science Centre–Basrah University. Fish samples were collected by using hand net and cast net for capture fishes from St1 and St2, and transferred alive in styropore boxes (75 x 40 x 40) cm containing clean river water and provided with a handle aerator in order to maintain the life of the fishes. Fish were brought to the Physiology, Pharmacology and Chemistry laboratory in College of Veterinary Medicine, University of Basrah. The fishes were released at least for 2 to 3 hours for adaptation, after then, fishes were handled gently to avoid stress, and then killed by pithing them with a fine needle. After the completion of blood withdrawal, fish total length, weight and sex were measured. The taxonomy of fishes was done according to Coad, 2010 and www.fishbase.org.

Blood Samples were Used in Measure

The haematological parameters viz. RBC, Hb, PCV, WBC and DLC and cortisol and glucose levels of fishes were studied by collecting blood samples with the help of disposable insulin syringes by making puncture through the heart .RBC and WBC were counted with the help of improved Neubauerhaemocytometer (Dethloff *et al*.,1999). DLC was counted by methodology adopted by (Maule and Schreck, 1990). Hb was estimated by using Sahlihaemometer (Tort *et al*., 1998). PCV was determined by centrifugation method (Wintrobe *et al*., 1967). For the estimation of cortisol and glucose blood was collected in plastic Eppendrof tubes. After centrifugation, blood plasma was removed and the samples were then analyzed for measuring the levels of TSH, T3, T4, testosterone and cortisol by ELISA Kit following the methodology adopted by (Anderson, 2003). Glucose was estimated following the methodology followed by Correland Langley (1956).

Statistical Analysis

The results obtained were analyzed (Barton, 1997). The results obtained were analyzed statistically by one way analysis of variance (ANOVA) by SPSS software for determining the significance of change from control.

RESULTS

The Environmental Factors

The environmental analysis showed a variations in the measurements of both physical and chemical factors, where the station 2 showed a significant increases in both temperature, salinity, electrical conductivity and Turbidity compared to its counterparts in station 1 and on the contrary, a measurement of hydrogen ions was in station 2 less than in the station 1 (**Table 1**). While **Table 2** indicates that all chemical measurements showed a significant increase in station 2 compared to the same in Station 1.

Table 2. Mean values of some chemical parameters in the study areas

Chemical parameter mg\L	Station 1	Station 2
PO_4	0.426	6.05
Ca	75	400
Mg	96	252
Cl	428	1359
TDS	174	37370

Station 1 represent water sample from marine Centre science ponds. Station 2 represent water sample from Al- HababahRiver.

Table 3. Effect of environmental stresses on Length and Weight in *T.zillii* and *C. carpio* (Mean±SD. N=50)

Groups / Parameters	St.1		St.2	
	T. zillii	*C. carpio*	*T. zillii*	*C. carpio*
Length (cm)	32.25±5.73a	31±2.92a	27.5±8.24b	21±2.95c
Weight (g)	412.5±29.37a	400±38.75a	280±22.69c	325±26.59b

N=number of fish, small letters denote differences between groups,P≤0.05 vs. unstressed

Table 4. Effect of environmental factors on Haematological Parameters in *T.zillii* and *C.carpio*: (Mean±SD, N=50)

Groups / Parameters	St.1		St.2	
	T.zillii	*C. carpio*	*T.zillii*	*C.carpio*
RBC (×106 / cmm)	4.83±0.15a	4.79±0.18a	2.47±0.12b	2.32±0.05 7b
WBC (×103 /cmm)	13.23±0.27a	13.02±0.11a	11.01±0.21b	10.47±0.28b
Hb (mg\dl)	9.6±0.23a	8.9±0.37a	6.2±0.14b	5.1±0.24b
PCV%	41.3±0.16a	41.2±0.13a	33.0±0.13b	30.0±0.22b
Neutrophiles%	39.11±0.61b	35.3±0.20 b	55.8±0.37a	56.8±0.12a
Eosinophiles%	3.2±0.29 a	3.1±0.36a	0.2±0.03b	0.2±0.01b
Basophiles%	2.5±0.25a	3.4±0.39a	0.3±0.015b	0.1±0.47b
Lymphocyte%	40.2±0.02a	42.8±0.16a	33.3±0.81b	31.6±0.16b
Monocyte%	15.3±0.22a	14.1±0.1a	9.2±0.34b	7.9±0.012b

N=number of fishes, Small letters denote differences between groups, P≤0.05 vs. station 1

Effect of Environmental Stresses on Length and Weight of Fishes

In comparison with their fish from station 1 counterparts, the results in **Table 3** showed a significant decrease (P≤0.05) in length and weight in fish living in Al-Hababah River (station 2).

Effect of Environmental Stresses on Haematological Parameters on Fishes

Compared to station 1, the results in **Table 4** confirmed that the fish captured from station 2 recorded a significant decline (P≤0.05) in values of each RBC counts, Hb, PCV% and WBC counts. Also, the differential leucocyte countsshowed decrease in lymphocytes, monocytes, eosinophils and basophils. In contrast, neutrophils and thrombocytes recorded increasing in their values.

Effect of Environmental Stresses on Glucose and Hormonal Parameters in *T.Zillii* and *C.Carpio*

The results in **Table 5** indicate a significant (P≤0.05) decrease in the values of both T3, T4, and testosterone, and on the contrary there is a significant increase in the

Table 5. Effect of Environmental Factorson Glucose and Hormonal Parameters in *T.zillii* and *C. carpio* (Mean±SDN=50)

Parameters \ Group	St.1		S.t2	
	T.zillii	*C.carpio*	*T. zillii*	*C.carpio*
Glucose(mg/dl)	110± 18.06b	100± 20.21b	143± 31.08a	137±25.11a
TSH(μIU/ml)	2.05±0.10b	2.16±0.12b	3.60±0.11a	3.08±0.52a
T3(ng/ml)	1.91± 0.08a	2.01 ± 0.14a	1.42 ± 0.51b	1.03 ± 0.37b
T4(μg/dl)	11.08 ± 4.72a	10.29± 4.72a	7.40±1.60b	5.61±0.38b
Testosterone (ng/ml)	0.78±0.011a	0.62±0.031a	0.42±0.015b	0.40±0.021b
Cortisol(ng/ml)	4.47±0.016b	4.47±0.016b	7.86±0.012a	8.39±0.011a

N=number of fishes, Small letters denote differences between groups, ($P \leq 0.05$ vs. st.1.)

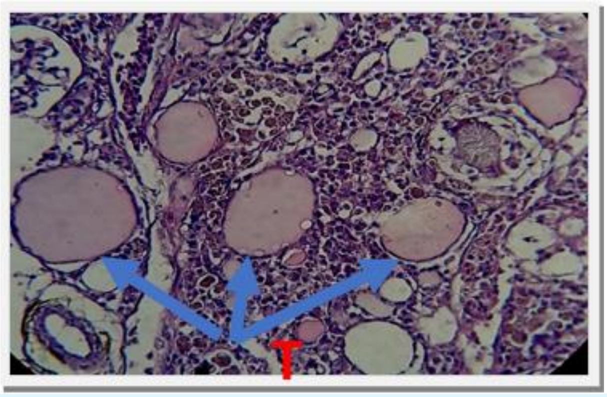

Fig. 4. Renal tissue showed glomarular and present thyroid follicles (TF) different size in *T. zillii*

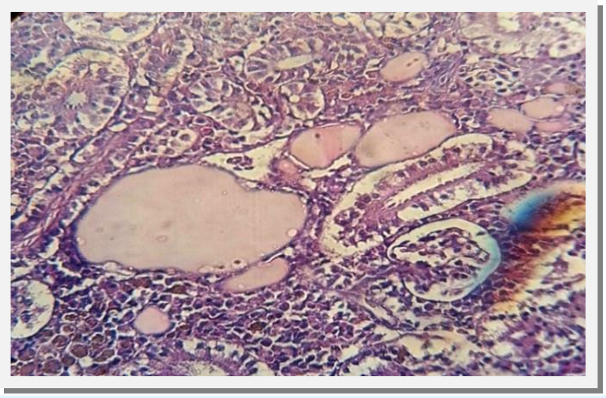

Fig. 5. Renal tissue showed glomarular vacuolation in renal tubular and present thyroid follicles (TF) different size in *C. carpio*

values of glucose, TSH and cortisol in fish caught from station 2 compared to fish captured from station 1.

Histological Examination

See **Table 5** and **Figs. 4-9**.

DISCUSSION

Shatt al-Arab is an important water resource for the province of Basrah, where all human, industrial and agricultural uses depend on it. Shatt al-Arab waters are affected by the water coming from the Tigris and Euphrates rivers (Moyel, 2014). The physical, chemical and hydrological characteristics of the Shatt al-Arab

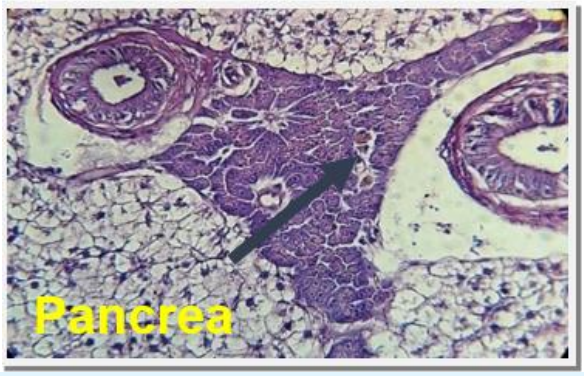

Fig. 6. Hepatic tissue showed vaculation of hepatocyte, note portal traid (PT) (portal vein, bile ducts and hepatic artery)in the liverand present Pancreas also perihepatic adipose tissue with foci of pancreatic tissue in *T. zillii*.Stained with H&E

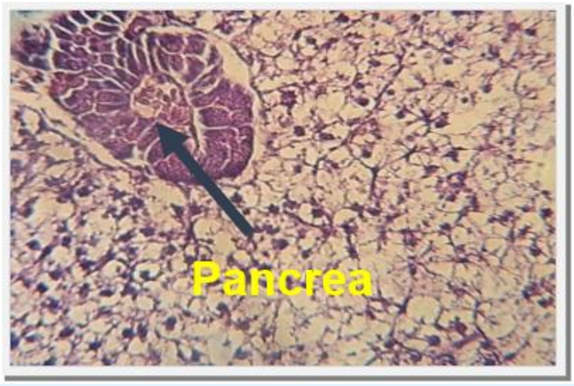

Fig. 7. Hepatic tissue showed vaculation and present Pancreas in*C. carpio* Stained with H&E 100X

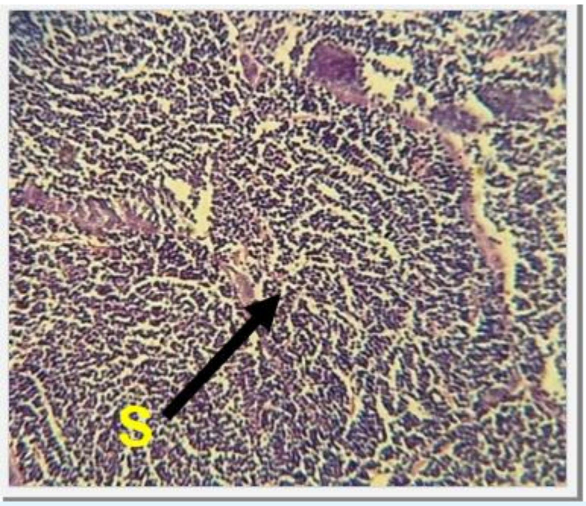

Fig. 8. Testis tissue showed present sperms (S) appeared as small dot-like the testis with different lobules with the sperms and no present seminiferous tubulesstructure in *T. zillii.* stained with H&E 100X

waters are affected by the quality of water coming from the tributariesdepending on the conditions related to rain and groundwater recharge and storage conditions above the river basin as well as the salt front and tidal energy coming from the Arabian Gulf (Al-Mahmood*et al.*, 2008).

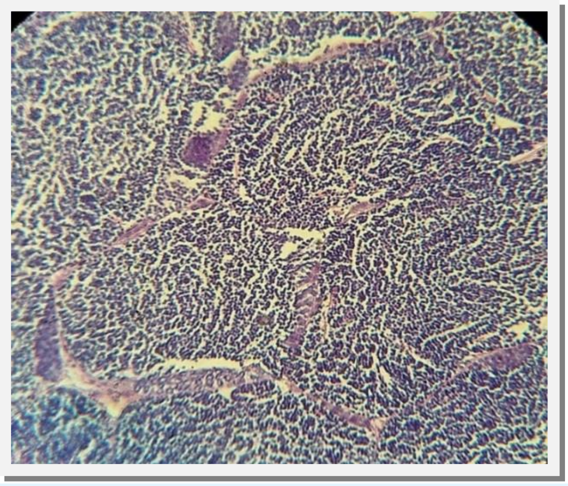

Fig. 9. Testis tissue showed present sperms and no present seminiferous tubules in *C. carpio* Stained with H&E 100X.

Physical and chemical properties of water are the most important characteristics that must be relied upon to determine the validity of water for human, agricultural and industrial uses (CO SQC, 2000). Assessing these traits periodically gives a clear impression of how well water is improving or deteriorating (Al-Mahmood*et al*., 2008). Water temperature plays an important role in the control of behavior an organism such as reproduction, feeding, migration, metabolism process and activity as well as on the solubility and, consequently, it also affects the toxicity of some chemicals in water systems as well as the sensitivity of living organisms to toxic substances (Holker, 2003). In the present study **Table 1** showed the mean of temperature ranging 20.5 to 23.1 in station 1 and station 2 respectively.

Turbidity is widely considered as an important parameter for water quality assessment, it's a measure of suspended particles (inorganic and/or organic matters) in water systems (Obi *et al*., 2007)**.** Turbidity can also limit the growth of phytoplankton and macrophytes when light penetration within the water column is restricted. In this study, turbidity was generally higher in the St.2 compared to st.1, which could be attributed to decrease river volume and increase pollutants. The observed values in st.2 exceeded the permissible level recommended by the WHO (2017) for drinking water.

The coming tide energy rates from the Arabian Gulf, the human activities, all are important factors that increase the incursion of salt, accompanied with the high salinity of the Shatt al-Arab have increased due to declining revenue stream Shatt al-Arab River and the rise of the Arabian Gulf water level due to natural causes and human activities are the main causes in elevating the values of salt in the al- hababah river compared to tanks water in station 1this result agreed with Moyel and Aboud Hussain, (2015) who stated that the reduction of freshwater flow with the intrusion of a marine salt wed ge front from Arabian Gulf increases the concentration of salinity. The diversion of the Karun River towards Iran, which was a significant source of freshwater entering the Shatt al-Arab River, reduced the rivers capacity to act as a natural barrier for the intrusion of the marine salt wedge front from the Arabian Gulf.

The pH is an important parameter that determines the suitability of water for different purposes, and its values are considered acidic or alkaline water. The obtained data noted from **Table 1** indicated obvious decreasing values of pH in St. 2 (7.1) compared to St. 1 (Randall and Perry, 1992**;** Reid *et al*., 1998). This is due to the regulatory viability of the water resulting from its high phosphate, carbonate and bicarbonate content(Moyel and AboudHussain, 2015),the present study consistent with previous studies made on aquatic ecosystems in Southern Iraq whom suggest the acceptable range of 6.5 to 8.5 (Al-Mahmood *et al*., 2008; Al Hejuje, 2014).

EC estimates the amount of total solids or amount of total dissolved ions in water, the current study shows a significant increase in the EC values in station 2 (5.84) compared to station No. 1 (2.72). This is due to the apparent increase in the concentration of dissolved salts (TDS) in st.2 (37370) compared to st.1. (174). and where the EC of water generally increases as the levels of dissolved pollutants and salinity with correlated ions (such as PO4, Cl-, Ca+2 and Mg+2) increases hardness as shown in **Tables 1** and **2**.The parameters on this component indicate fluctuation and reduction in water flow and increase in soluble salts from Arabian Gulf (Al Maliky, 2012; Moyel and AboudHussain, 2015).

The rise in all these valuescompared with station 1indicating that the level of nutrient contamination at Al-HababahRiver station was significant due to subjected to several pollution activities, mostly from domestic waste discharge, industrial waste and agricultural and as put this waste directly into the river without any handle them, and confirms the deterioration of water and the lack of validity for human uses, especially drinking. This waste be loaded mostly pollutants may pose serious health and can adversely affect the use beneficial to the river (Al-Mahmood*et al*., 2008) which led to rely on other sources to meet the need of Basrah province and expensive prices or follow desalination methods, as confirmed by study of Al-Imarah *et al*. (2001); Al-Mahmood *et al*. (2008); Hameed and Aljorany (2011); AlHejuje, 2014; Yaseen *et al*. (2016).

Haematological evaluation of fish provides valuable facts concerning the physiological response of fish to changes in the external environment. Study of haematological of the fish (Barton, 1997; Barton *et al*., 2005; Raina, 2011). Blood is a sensitive indicator of stress and any physiological dysfunctioning in fish's body get reflected as alterations in its blood constituents. Blood being the medium of intercellular and intracellular transport, comes in contact with various organs and tissues of the body and thus can pose a direct threat to physiological functions of the fish. Xenobiotics (like heavy metals/ pesticides) rapidly bind to the blood proteins and thus may induce haematological changes on one hand and histopathological on the other. In fishes like mammals, the glucocorticoids are important in regulating a number of functions that enable them to respond to stress and to resist stressors (Begg and Pankhurst, 2004). Glucocorticoid steroid hormones regulate the production and functioning of a great many proteins and are important not only in regulation of homeostatic functions like metabolism and osmoregulation but also in their capacity to affect immune functions. Stress has been reported to elevate plasma cortisol which is one of important glucocorticoid (Rottmann *et al*., 1992; Das, 1998; Devi*et al*., 2008; and Sachar, 2011) and many researchers consider it as a "rule of thumb" that fishes undergoing stressful situations exhibit plasmatic increase in cortisol levels. Cortisol not only activates glycogenolysis and gluconeogenesis in fish but also activates the chromaffin cells to increase the release of catecholamines which further increase glycogenolysis and modulate cardiovascular and respiratory function (Santhakumara *et al*., 2000). This whole process increases the substrate levels (glucose) to produce enough energy as per the demand and thus prepare the fish for an emergency situation (Singh and Tandon, 2009; Gupta, 2012). Thyroid gland produces mainly prohormonethyroxine (T_4). In tissues type I and type II monodeiodinases catalyze it's conversion into physiologically active form – triiodothyronine (Stein-Behrens and Sapolsky, 1992; Tavares-Dias and Barcellos, 2005).Main trends of investigation in thyroidology relate to study of controlling mechanisms of hormonogenesis and tissue metabolism of thyroid hormones (TH). These mechanisms are based on delicately organized interactions of TH receptor's group (Torres *et al*., 1984) deiodinases (Vosyliene and Kazlauskiene, 1999), transport complex of carrier proteins, providing TH entry into cell through plsmalemma (Wedemeyer*et al*., 1990; Wendelaar Bonga, 1997; Vosyliene *et al*., 2003) and mechanisms, maintaining systemic levels of T_3. They are realized due to conversion of T_4 into T_3 with subsequent T_3 release into extracellular fluid (Al-Mahmood*et al*., 2008; Moyel, 2014).

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