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Research Article

Impact of Water Quality at Different Locations of Alexandria Mediterranean Coast on the Pituitary-ovarian Axis of Gilthead Seabream *Sparus aurata*

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Abstract

Environmental pollutants such as metals, pesticides and other organics pose serious risks to aquatic organisms. Impact of pollution of Alexandria coast on gonadotrophs cells (GTC), ovarian structure and steroid hormones level in female *S. aurata* were evaluated in fish collected from four sites along Alexandria coast. The El Max Bay (EMB), Abu Qir Bay (AQB), Eastern Harbor (EH) and Abu Talat Region (ATR) sites were chosen representing regions with variable sources of pollution and compared to reference site (REF). Results showed that the physicochemical characteristics of water from sites EMB and AQB exhibited significant levels of heavy metals (Cd, pb, Cu, Zn and Fe) as well as pesticides that have a toxic impact on fish ovarian structure was revealed in the form of increased percentage of follicular atresia and deformed vitellogenic oocytes. Reduction in level of 17 β-estradiol and progesterone to significant values in both EMB and AQB sites compared to the reference site (REF). Also gonadosomatic index (GSI) of fish in both EMB and AQB sites showed significant decreased values. The gonadotrophs cells number decreases in EMB and AQB sites compared to reference site. The percentage of deformed cells with picnotic nuclei increased in AQB and EMB sites. The present study clearly demonstrated that pollution in EMB and AQB sites exhibits an endocrine disruptive action on the reproduction axis of *S. aurata* affecting quantity of GTC and/or ovaries structure, which could have a detrimental effect on population existence under prolonged exposure to pollution.

Key words: *Sparus aurata*, water quality, ovary, gonadotrophs

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Coastal marine are complex environments due to their location between the land and the sea. They are characterized by high fluctuations in chemical and physical parameters and in most cases there exist a significant occurrence of human activities including industrial, domestic and agricultural waste discharges (Meherm, 2002). Alexandria site is lying on the South East, Egyptian Mediterranean coast. It has about 40% of the total Egyptian industrial activities and is inhabited by about 8 million in habitances. Along with shipping and tourism activities keep the coastal water under constant stresses. As a result, toxic heavy metals and petroleum hydrocarbons (PHC) are accumulated in marine organisms (Aboul Naga and Al Deep, 2005). The El Max bay (EMB), Eastern Harbor (EH) and Abu Qir bay (AQB) are considered as main fishing sources. The ecosystem of Eastern Harbor (Fishing and yachting) has attracted great attention from biological, chemical and physical point of view (Abdel-Halim and Khairy, 2007). While, El Max Bay as a part of West Alexandria is suffering from discharge of huge quantities of agriculture industrial and sewage wastes (El-Rayis *et al*., 1997). Moreover, Abu Qir Bay region of the Mediterranean coast of Egypt, considered as an important underutilized resources of agriculture, tourism and industrial. The extensive pollution in the area is an important factor contributing to deterioration of environmental conditions in general and water quality in particular (Nessim *et al*., 1994).

Although aquatic ecosystems are equipped with a variety of physicochemical and biological mechanisms to eliminate or reduce adverse effects of toxic substances. Un-eliminated toxicants may evoke changes in development, growth and reproduction of water organisms (Palaniappan *et al*., 2010).

Environmental contamination interferes with endocrine system function that had its impact on reproduction of fish (Garcia-Santos *et al*., 2013; Yamaguchi *et al*., 2007; Hontela, 2005). Reproduction in fish is controlled by the Hypothalamic Pituitary Gonadal (HPG) axis. Signals from brain control the hypothalamic secretion of gonadotropin-releasing hormone (GnRH), which stimulates the adenohypophysis to release gonadotropin hormones (GtH). Gonadotropins stimulate gonadal development and production of steroid hormones in gonads, estradiol in females as well as maturation inducing hormone progesterone (Kime, 1993). Any irregularity in the GnRH, GtH or sex steroid levels could lead to a reproductive functions disturbance (Trudeau, 1997). The multitude of hormones controlling HPG axis make from the HPG axis a major target of Endocrine Disturbing Chemicals (EDCs) (USEPA., 1998).

Gonadotropin produced by gonadotrophs is most closely associated with reproduction, stimulating steroid production, uptake of vitellogenin, oocyte maturation and ovulation (Naito *et al*., 1995; Nagahama, 1987). Vongvatcharanon *et al*. (2005) and Mousa and Mousa (1999) indicate the existence of a good correlation between cyclic changes in the secretory activity of GTC and the ovarian cycle, suggesting that climatic factors interact with secretory activity of gonadotrophs that in turn determine ovarian function.

The study work aims to give an up to date account on situation of this Egyptian coast and its impact on reproduction of important economic fish, gilthead seabream, *Sparus aurata* as a representative example by assessing the endocrine disturbance potential of pollution of Alexandria coast on ovaries structure, gonadotrophs cells morphology and steroids level of female *S. aurata.*

MATERIALS AND METHODS

Sampling sites, water and fish samples collection: The present study has been carried out along Alexandria coast areas during the study period (September-November, 2015). Four locations were chosen for water and fish sampling.

Location 1: Abu Qir Bay (AQB) (31.2-31.5 N°, 30.0-30.4°E). The water of Abu Qir Bay is affected by 3 main discharges, Boughaz El-Maadya opening, El-Tabia station and the opening of the Rosetta Nile branch represent industrial activities from about 22 factories, domestic sewage discharge and agriculture wastes. The bay is also exposed to oil pollution from fishing boats, the activities of gas production liquefying and export field. Location 2: Eastern Harbor (EH) (32.2-31.22°N, 29.88-29.90°E). The Eastern Harbor is influenced by sewage disposal of central part of Alexandria city beside wastes of the fishing and sailing boats anchoring inside the harbor about 5400 m of sewage per hour. Location 3: El Max Bay (EMB) (31.7-31.19 N°, 29.47-29.89°E). El Max Bay receives a heavy load of waste waters (7×109 m^3 $year^{-1}$) from 6 main industries at rate of 6 million m^3 day^{-1} in addition drainage water mixed with remains of pesticides and fertilizer. As a result, large amount of nutrients, organic compounds, heavy metals and suspended matter were supplied to the bay. Location 4: Abu Talat Region (ATR). This region has no opening of wastes discharge. The only site of pollution in that region, which is about 25 km West of Alexandria derived from urban and beaches activities as well as residues of building construction.

Reference site (REF) is Alam El Room region located on Mediterranean sea, about 290 km West of Alexandria, a small port on the Mediterranean famous for its soft white sand and its transparent water.

Collection of water samples: Surface water samples were collected monthly from the 4 sites and reference site. The pH value is measured by pH meter (model No. 201, serial No. 1880, Orion Research INC), salinity (‰) using Beckman induction salinometer instrument (INC. Cedar Grove, NJ: Model No. R570) and dissolved oxygen by Strickland and Parsons (1975). Heavy metals according to Eaton (1976) using an atomic absorption spectrophotometer (AA-6800 Shimadzu, Germany) and pesticides concentration using Hewlett Packard 5890 according to series II GC gas chromatograph (Duinker, 1993).

Collection of fish samples: Gilthead seabream (*Sparus aurata*) family Sparidea has a great commercial importance in local fisheries and a high potential for aquaculture. Its annual catch from Mediterranean sea about 25.000 t and its catch from Mediterranean sea according to landing sites, El Max Bay (EMB) 11,125 t and Abu Qir Bay (AQB) 7,797 t.

Sexually mature gilthead seabream *S. aurata* fish were collected over the period from September-November, 2015 from the selected sites, transported alive to the laboratory. Sexually mature female fish were identified. Adult females with 246.80±13.20 g of mean body mass and 23.40±0.90 cm fork length were used.

Histological studies: Pituitary gland with brain were removed and fixed in Bouin's solution for 24 h at 4°C, then dehydrated through graded ethanol solution, cleared and embedded in wax (MP: 56-58°C). Consecutive median sagittal sections of the pituitary gland were made at 4 μm thickness according to Conn (1953).

Ovaries were dissected out and weighed to calculate gonadosomatic index, then prepared for histological examination using the same method as for the pituitaries. Gonads and pituitary sections were stained with hematoxylin and eosin as a counter stain.

The number of GTC per unit area (4.2 mm^3) was counted. Four different areas in the sagittal section of the central proximal pars distalis of gland were selected at random and the number of GTC was counted on the photomicrographs (Matsuyama *et al*., 1995) and scoring was repeated for at least 8 fish gland from each site. The incidence ratio for deformed to total number of cells were then calculated (West, 1990) and the mean for each site were obtained.

Hormonal studies: Eight fish from each site were selected. Blood samples from caudal severance were left to clot at 18°C and centrifuged at 1.500×g for 15 min. Serum was collected and stored at 4°C till analyzed using Radio Immune Assay (RIA) Kites for quantifying progesterone and estradiol. The pantex immunocoat catalogue (number 374 M for estradiol and Orion diagnostic spectria SF-02101 for progesterone). The total count of iodine-125 in samples and calibrator control was measured using a gamma counter (LKB, S-75182, 1997).

Statistical analysis: Data of gonadosomatic index, hormones level, gonadotrophs number and scoring (mean incidence ratio) as well as physico-chemical water parameters were analyzed using one-way ANOVA test followed by Duncan's test using the SPSS package, version 17 (SPSS Inc, Chicago, IL, USA). Data are presented as Mean±Standard Deviation. Normality and homogeneity of data were confirmed before test.

RESULTS

Water analysis: The physico-chemical characteristics of water samples from the selected sites are represented in Table 1. The results showed a significant decrease ($p<0.05$) in salinity percentage and dissolved oxygen level in EMB and AQB sites compared to REF site with average value of 25.60%, 2.66 mg L^{-1} for EMB site and 24.00%, 3.14 mg L^{-1} for AQB site respectively, compared to average value of 36.0% for salinity and 7.01 mg L^{-1} for dissolved oxygen in reference site (REF).

On the other hand, a significant increase ($p<0.05$) in levels of tested metals (Pb, Cd, Cu, Zn and Fe) were recorded in EMB, AQB compared to REF site (Table 1). Also the average value of total pesticides were significantly increased ($p<0.01$) in EMB, AQB sites (497.46 and 1082.10 ng L^{-1}) compared to average value of 9.25 ng L^{-1} in REF site. Water parameters of Eastern Harbor (EH) site compared to reference site (REF) showed significant decrease in DO value (3.73±0.17 mg L^{-1}) in Eastern Harbor site compared to (7.01±0.32 mg L^{-1}) REF site also a significant increase in total pesticides, Pb, Cu and Zn levels were recorded. Abu Talat region showed a significant increase ($p<0.05$) in Pb, Zn and total pesticides levels compared to reference site.

Gonadotrophic hormones secreting cells (GTC): The total number of gonadotrophic hormone cells (GTC) and incidence ratio of deformed gonadotrophic hormone cells to total cells number are shown in Fig. 1. The total number of GTC cells decreased significantly ($p<0.05$) in EMB and AQB sites (98±3.42, 80±2.71) compared to 130±5.22 in reference site

Table 1: Physicochemical parameters measured in water samples collected from the selected sites (n = 5)

Parameters	Sites EMB	AQB	EH	ATR	REF	WHO (2004)	ANG (2015)
pH	7.90±0.36	8.20±0.37	7.20±0.37	7.37±0.34	6.91±0.31	-	6.5-7.2
Salinity (‰)	25.60±0.71*	24.00±1.09*	37.00±1.68	36.00±1.64	36.01±1.88	-	33-37
DO (mg L^{-1})	2.66±0.08*	3.14±0.14*	3.73±0.17*	6.30±0.29	7.01±0.32	>6	>5
Metal (mg L^{-1})							
Pb	18.12±0.69*	25.20±1.10*	11.47±2.25*	11.12±0.69*	3.24±0.20	0.01	0.001
Cd	0.40±0.03*	0.45±0.04*	0.06±0.01	0.04±0.001	0.01±0.01	0.003	0.005
Cu	36.72±0.92*	28.71±1.22*	25.34±4.28*	19.97±0.62	10.63±0.65	2	0.05
Zn	69.64±5.50*	77.50±4.30*	58.52±4.29*	45.21±2.79*	20.77±1.46	3	<0.05
Fe	47.58±3.75*	92.21±6.10*	32.42±3.95	28.01±1.73	12.23±1.32	0.5	0.01
Pesticides (ng L^{-1})							
TP	497.46±25.40**	1082.10±73.21**	55.66±4.10*	37.80±4.10*	9.25±0.62	---	<0.005

Values are (Means±SD), *Significant at p<0.05, ** Significant at p<0.01, TP: Total pesticides (HCH+TC+DDTs+PCBs), HCH: α HCH+β HCH+γ HCH, TC: Aldrin+Dieldrin +Endrin, DDTs: o, p-DDE+p, p-DDE+o, p-DDD+p, p-DDD+o, p-DDT+p. p-DDT, PCBs: PCBs (28+52+101+118+135+138+180), EMB: El Max Bay, AQB: Abu Qir Bay, EH: Eastern Harbor, ATR: Abu Talat region, REF: Reference site, WHO (2004): Guidelines for water quality, ANG (2015): Australian and Newzealand guidelines for protection of aquaculture species

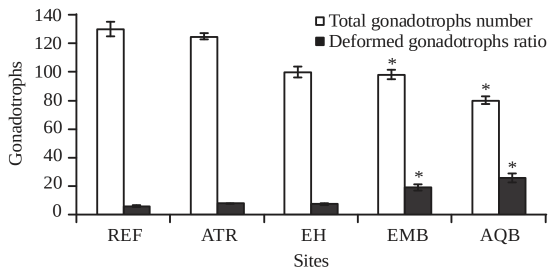

Fig. 1: Total gonadotrophs number (No/4.2 mm^2) and incidence ratio of deformed cells of female *S. auratus* collected from selected sites (n = 10)

(REF). Controversially the incidence ratio of deformed GTCs to total cells number increased significantly (p<0.05) in EMB and AQB sites (16.21±2.22 and 25.8±3.2%, respectively) compared to 5.76±0.82% in reference site (REF) (Fig. 1). Not significant change in total GTC or incidence ratio of deformed to total cells number were recorded in EH and ATR sites compared to REF site (Fig. 1).

The gonadotrophs are found predominantly in the Proximal Pars Distalis (PPD), occupy its entire region. In the pituitary of the mature fish from REF site, the GTC are granulated cells with variable shape and size containing a spherical nucleus. Vacuolation presents a net or sieve like appearance and intracellular vacuoles containing vesicular substances (Fig. 2a and 3a). Pituitary glands of fish from ATR site possess morphological structure similar to that of REF site (Fig. 2b and 3b). Pituitary glands of fish from the other sites have deformed gonadotrophs cells. Gonadotrophs cells with hypertrophied nuclei and fragmented chromatin materials are found in female *S. aurata* from EH site (Fig. 2c and 3c). Decreased in size and granulation of cells and nuclear deformation were seen in GTC of female *S. aurata* from EMB site (Fig. 2d and 3d). In AQB site the GTCs were widely arranged with few granules (intercellular spaces were predominant). The GTC have distinct cell membrane, deformed darkly stained nuclei and large intercellular spaces (Fig. 2e and 3e).

Gonadosomatic index (GSI) and ovary histology: The GSI of female *S. aurata* from the selected sites are represented in Fig. 4. The GSI value decreased in all tested sites compared to reference site. Abu Qir Bay (AQB) has the lowest GSI value. The GSI of EMB and AQB sites decreased significantly (6.68±0.9 and 5.7±0, respectively) compared to REF site (7.56±1.15).

The ovarian activity of female *S. aurata* fish as reflected by GSI and oocyte structure varied with water quality and showed differences in histology (Fig. 5-9). The ovarian histology of fish revealed an increase in deformed oocytes and atretic follicles in ovaries of females from (AQB, EMB and EH) compared to ovaries of females at ATR and REF sites. Ovary of fish of both sites are characterized by the presence of maturing oocytes in different stages of vitellogenesis (Fig. 5 and 6). Ovaries of fish of EH site have deformed vitellogenic oocytes (Fig. 7). While in AQB and EMB sites atretic oocytes were seen in close association with deformed yolk vesicles oocytes with hyper vacuolation (Fig. 8 and 9).

Hormones level: Hormones level of female *S. auratus* from the selected sites are represented in Fig. 10. The estradiol and progesterone values decreased in all tested sites compared to reference site. A significant decrease (p<0.05) in hormones levels in EMB and AQB sites compared to REF site were recorded with maximum decrease in AQB site (0.18±0.01 and 6.38±0.25) compared to values of 0.52±0.03 and 11.32±0.82 for estradiol and progesterone levels in REF site respectively.

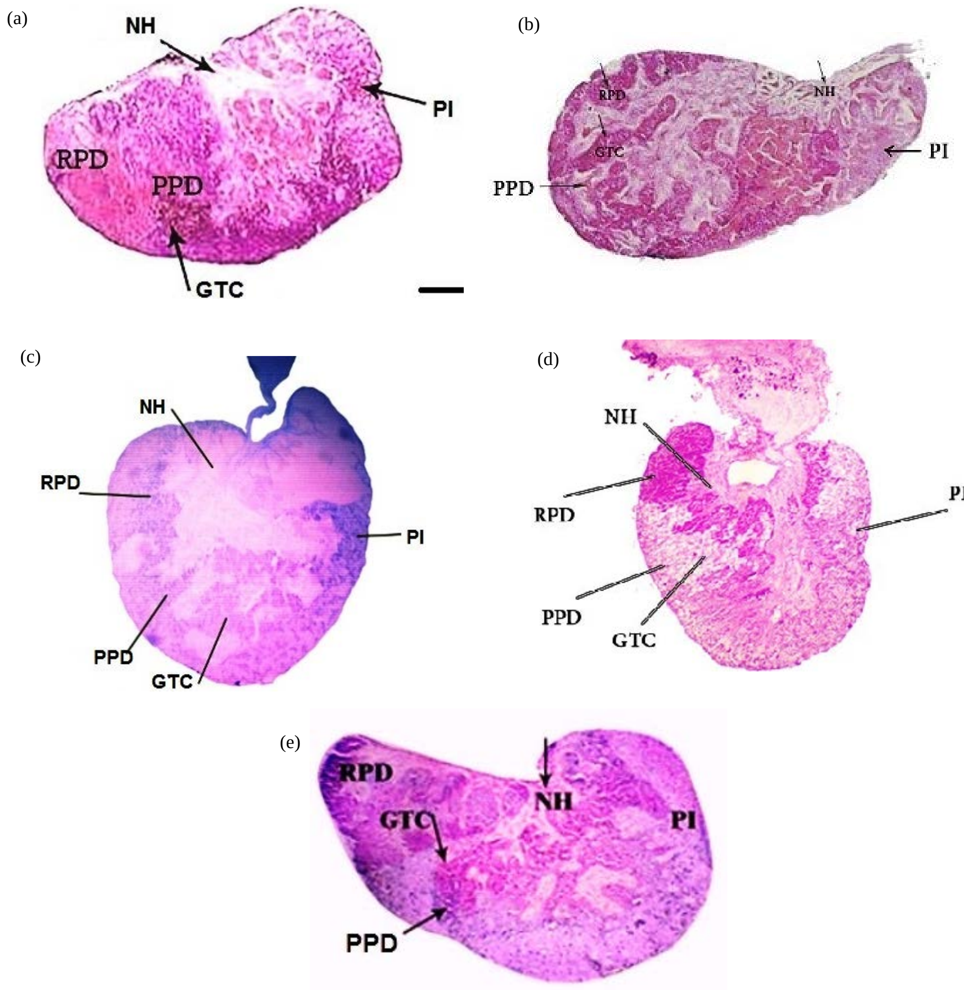

Fig. 2(a-e): Sagittal section of pituitary gland of female *S. aurata* from tested sites stained with Hematoxylin and Eosin (H and E), showing gonadotrophscells (GTC) located at PPD (bar = 150 µm), (a) REF site, (b) ATR site, (c) EH site, (d) EMB site and (e) AQB site, NH: Neurohypophysis, PI: Pars intermediata, PPD: Proximal pars distalis and RPD: Rostral pars distalis

DISCUSSION

Few researches on impact of environmental pollution in EH and El Max Bay on fish reproduction were recorded. Ghanem (2012) and Moharram *et al.* (2011) reported disrupted gonadal indices and endocrine haemostasis in *Sardinella aurita* and *Siganus rivulatus* in Eastern and Western Egyptian Mediterranean coast. Also El-Naggar (2009), Khaled (2009) and Said *et al.* (2008) reported high level of metals, pesticides and organochlorine contaminants in fish of economic interest in Egyptian Mediterranean sea. The present results indicated that the physico-chemical characteristics of water from studied sites were above the allowed and desired baseline levels for public safety (WHO., 2004). Water analysis of selected sites revealed higher levels of Endocrine Disrupting Chemicals (EDCs) as total pesticides (DDT metabolites, phenolic compounds and fungicides), sewage and trace metals (Cd, Cu and Pb). These EDCs interfere with synthesis, transport and/or degradation of endogenous hormones and/or inducing structural alteration (Thomas, 1990; Bulger and Kupfer, 1985). Gonadal growth disturbance was noted in Atlantic croaker after exposure to PCBs is related to disruption of gonadotropin synthesis (Khan and Thomas, 2000). Cadmium also was found to disrupt the hypothalamus-pituitary-gonadal axis at multiple levels acting centrally to disrupt steroidogenesis at pituitary level or acting on gonads (Radhakrishnan and Hemalatha, 2010), alters calcium homeostasis and disrupts signal transduction processes responsible for gonadal and pituitary hormones secretions (Thomas, 1999; Blazquez *et al.*, 1998). Fish samples

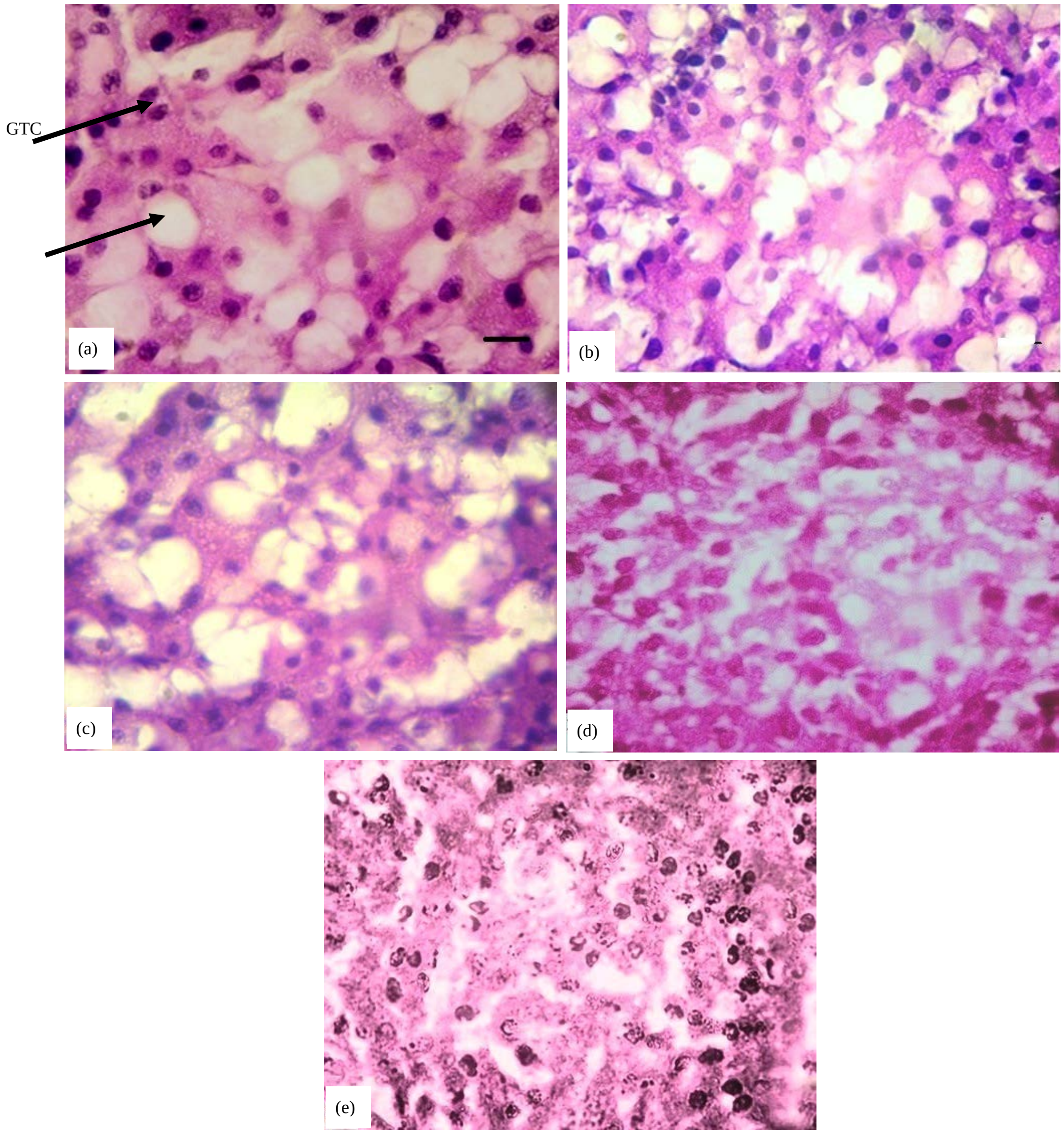

Fig. 3(a-e): Hyper-magnified part of PPD region of pituitary gland of female *S. aurata* from tested sites stained with Hematoxylin and Eosin (H and E), (bar = 15 µm), (a) REF site, showing normal shaped granulated gonadotrophs cells (GTC) with intracellular vacuoles (arrow), (b) ATR site, showing granulated gonadotrophs with intracellular vacuoles, (c) EH site gonadotrophs with hypertrophied nuclei and fragmented chromatin materials, (d) EMB site, showing deformed gonadotrophs with decreased staining affinity and nuclear deformation and (e) AQB site, showing hypertrophied gonadotrophs with darkly staining nuclei and intercellular spaces

from tested sites (AQB, EMB and EH) in the present study possess gonad and gonadotrophs structural alteration and decreased hormones level due to higher pollutants level.

In the present study, the water quality of tested sites affect the activity of the hypophysial-gonadal axis of *S. auratus* and in turn on its reproduction. The hypothalamic-pituitary gonadal axis is a prime target of heavy metals toxicity (Lafuente, 2013; Henson and Chedrese, 2004). Change in water quality recorded in the present study in AQB and EMB sites caused a significant decrease in GTCs number. Similar result was recorded by Mousa *et al*. (2015) and Mousa and Mousa (1999) on exposing *Oreochromis niloticus* to change in water quality and mercury exposure reduce GTCs number in cat fish *Clarias batrachus*

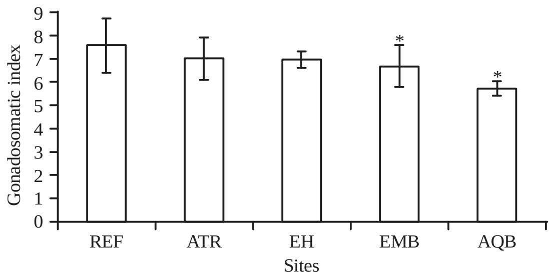

Fig. 4: GSI of female *S. auratus* collected from selected sites (n =10)

Fig. 5: Ovary of *S. auratus* from REF site, showing normal oocytes at different developmental stages, stained with Hematoxylin and Eosin (H and E), x300

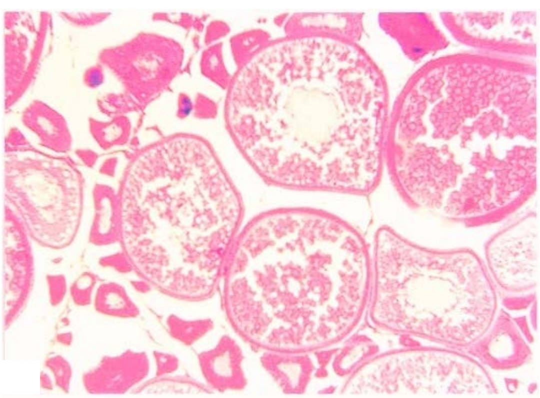

Fig. 6: Ovary of *S. auratus* from ATR site, showing normal shaped oocytes at different developmental stages stained with Hematoxylin and Eosin (H and E), x250

(Crump and Trudeau, 2009). Cypermethrin showed decreases in size of gonadotrophs in *Heteropneustes fossilis* (Singh and Singh, 2008), change in water salinity decrease the number and diameter of gonadotrophs in *Rhabdosargus haffara* fish (Assem and El-Boray, 2001) and also

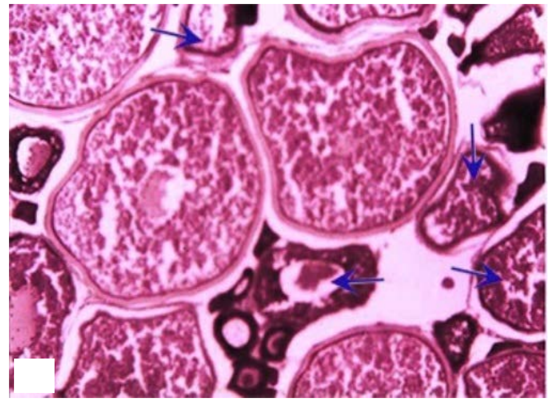

Fig. 7: Ovary of *S. auratus* from EH site, showing deformed vitellogenic oocytes (arrows) stained with Hematoxylin and Eosin (H and E), x300

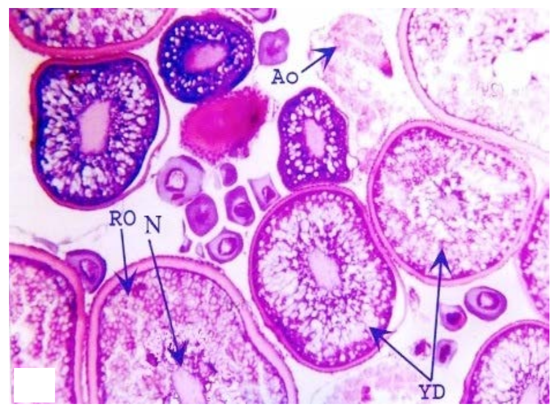

Fig. 8: Ovary of *S. auratus* from EMB site, showing Atretico Ocytes (AO), hyper-vacuolated vitellogenic oocytes (arrows) stained with Hematoxylin and Eosin (H and E), x300

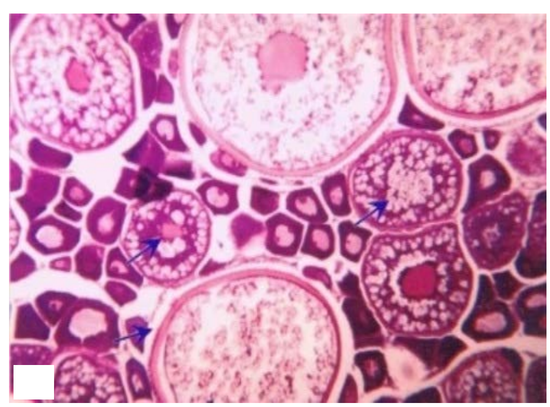

Fig. 9: Ovary of *S. auratus* from AQB site, showing hyper- vacuolated vitellogenic oocytes (arrows) stained with Hematoxylin and Eosin (H and E), x300

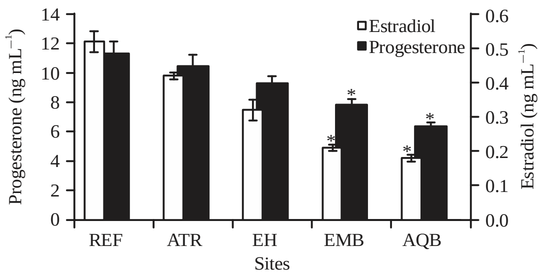

Fig. 10: Hormones level in female *S. aurata* collected from selected sites (n = 10), REF: Reference site, ATR: Abu Talat region, EH: Eastern Harbour, EMB: El Max Bay and AQB: Abu Qir Bay

Puntius ticto treated for 30 days with 7.7 mg L^{-1}. Cadmium showed reduction in number of gonadotrophs cells and increase of deformed cells (Pundir and Saxena, 1992).

Sub-lethal concentration of lead acetate (3.0 mg L^{-1}) for 6 weeks produces alteration in gonadotrophs cells structure in *Heteropneustes fossilis* (Renu, 2015). The gonadotrophs cells of female *S. auratus* from AQB site showed hypertrophic changes and nuclear deformation, same effects have been reported in pituitary gland of *O. niloticus* from lake Manzalah (Mousa *et al*., 2015) and also in guppy exposed to xenobiotics (Wester *et al*., 2004). Hypertrophy of GTC and nuclei with extensive intercellular spaces were reported by Narayanaswamy and Mohan (2014) after treatment with neem oil and by Assem and El-Boray (2001) in haffara fish reared in brackish water and after malathion exposure (Jagadeesh and Sahai, 1986, 1989). Vacuolization of gonadotrophs and damaged nuclei were observed in endosulfan treated tilapia, *Oreochromis mossambicus* exposed to both (DDT, BHC) and malathion at 4 mg L^{-1} for 20 days (Shukla and Pandey, 1986). Atrophied gonadotrophs reported in the present study in pituitary of female *S. auratus* from EMB site were also recorded in *Channa punctatus* exposed to 2, 4 mg L^{-1} potassium dichromate for 1 and 2 months (Mishra and Mohanty, 2012).

The gonadotropic hormones LH, FSH released from fish pituitary affect recrudescence of gonad, ovulation and production of sex steroids (Weltzien *et al*., 2004; Kamei *et al*., 2005), so disruption of gonadotropin secretion has a great impact on fertility. Therefore, pollution induced inhibition of gonadotropic activity was correlated with impaired gonadal development (Crump and Trudeau, 2009). This may be one of the reasons of the observed low values of GSI. Decreased GSI is indicative of decreased hypothalamic, pituitary or gonadal activity (Kime 1999; Ansari and Ansari, 2011). Cadmium significantly decrease ovarian GSI in *Labeo bata*, hamilton and winter flounder (Das, 1988; Pereira *et al*., 1993). Similarly lowered GSI values and increased atretic follicles in female *Oreochromis niloticus* were reported by Mousa *et al*. (2015) and Mousa and Mousa (1999) due to change in water quality and fish habitat. Atretic follicles were observed in fish from polluted sites of Bizerta lagoon (Louiz *et al*., 2009). Reduced GSI, damaged ovarian follicle were observed in perch exposed to leachate from Swedish refuse dumps (Noaksson *et al*., 2005). Increased atretic oocytes in white perch in areas affected by domestic and industrial effluents (Kavanagh *et al*., 2004) and in *Siganus rivulatus* exposed to Copper Works Company effluent (Wahbi, 2004a). These findings agreed with the present study since GSI values and altered ovarian structure were observed in ovaries of *S. auratus* fish from EMB, AQB and EH sites.

Steroid hormones play an essential role in maintaining reproduction function (Kime, 1995). In present study progesterone and estradiol showed reduced values in the more contaminated sites (EMB, AQB). A reduction in progesterone level was found in cyprinid fish from polluted sites along the Kor river (Ebrahimi and Taherianfard, 2011). Plasma steroids levels reduced in female zebrafish exposed to contaminated sediment (Linderoth *et al*., 2006) and in cod and turbot exposed to crude oil and alkyl phenols (Martin-Skilton *et al*., 2006). Also white seabream exposed to lowered pH values (Wahbi, 2004b). Plasma estradiol level was lowered in lake white sucker collected below Kraft pulp mills (McMaster *et al*., 1991; Munkittrick *et al*., 1992). Phenol and sulphide exposure decrease conversion of injected radiolabeled cholesterol to progesterone in carp ovaries (Mukherjee *et al*., 1991). Controversy estradiol level in English sole is positively related to ovarian PCB concentration (Collier *et al*., 1992).

CONCLUSION

Sparus auratus collected from tested sites (AQB, EMB and EH sites) showed degrees of deformation in oocytes and gonadotrophic cells structure, positively correlated with level of pollution of these sites compared to reference site (REF). Fish in EMB and AQB sites have the highest incidence of gonadal and gonadotrophs cells abnormalities and the lowest gonad size and plasma steroids level.

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