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## Research Article

# Ultrastructure and histology of reproductive system in the free-living marine nematode (*Oncholaimus campylocercoides*) with reference to polychlorinated biphenyls (PCBs) pollution in Persian Gulf

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**Abstract**

Marine nematodes are the most important sediment living invertebrates. Free living nematodes are considered as bio indicators in coastal areas. Many pollutants such as polychlorinated biphenyls may affect their physiological process. The present study is conducted to describe physiological and histological pattern of marine nematode (*Oncholaimus campylocercoides*) from north Persian Gulf. Three locations with different concentrations of PCBs were selected in Bandar Abbas for sampling. Sex steroid hormones (progesterone, testosterone and 17-B estradiol) were analysed after separation and purification of marine nematodes from sediments. Moreover, fine structures of ovocyte and spermatozoa were studied after histological observation. Results showed that sex ratios were F(1): M(0.97), F(1): M (0.9), F(1): M(0.5) in locations with 0.01, 0.02 and 0.1 µg/mL tissue PCB concentrations, respectively. Level of 17 β-estradiol (1.2±0.07pg/mL) in females at the location with 0.1 µg/mL PCB (power plant) was significantly higher than that in other two sampling locations. Also high level of testosterone (0.4±0.07 pg/mL) was observed in males and females at the polluted sampling site compared with that in other two locations. Histological study showed that in the location with high PCBs, there was an obvious increase in size of oocytes. We concluded that PCBs can affect sex steroid changes as endocride disruptors in *Oncholaimus campylocercoides.* Results of this study supported findings of previous studies on PCB endocrine disrupting roles in marine organisms.

**Keywords:** Sex steroids, Ultrastructure, Nematode, *Oncholaimus campylocercoides,* Persian Gulf, Polychlorinated biphenyls (PCBs)

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## Introduction

Wide variety of chemicals (e.g., organotin compounds, heavy metals, pesticides, xenoestrogens and phytoestrogens) can induce endocrine abnormalities in invertebrates (Rhind, 2009; Frye *et al.*, 2012). Endocrine disrupting compounds (EDCs) usually interfere with endocrine (hormone) systems and may have harmful effects on human and animal health, even at very low exposure levels. Due to their hazard potential, EDCs become a worldwide concern and are considered to be one of the most serious anthropogenic threats to biodiversity and ecosystem health (Hotchkiss *et al.*, 2008; Rhind, 2009; Vethaak and Legler, 2013). In aquatic ecosystems, these compounds can cause developmental and/or reproductive dysfunctions. Adverse effects of EDCs in marine environments are well established (Goksøyr, 2006; Zhou *et al.*, 2009; Bhandari *et al.*, 2015). For example, negative effects of Nonylphenol Ethoxylates on reproductive performance of fresh water fishes are studeid (Maktabi *et al.*, 2014; Amaninejad e*t al.*, 2018).

Polychlorinated biphenyls (PCBs) are a group of EDCs with a global production volume of 1.3–2 million tons in early 2000s (Fiedler, 2001; Breivik *et al.*, 2002). These persistent organic pollutants are of high concern because of their toxicity, bio-accumulative properties and capability of long-range atmospheric transport (Grandjean *et al.*, 2008). Accordingly, PCBs were included in Stockholm Convention list and listed as category 1 carcinogen (Lauby-Secretan *et al.*, 2013; UNEP, 2013). PCBs can enter marine environment following destruction and disposal of industrial plants or emission from construction materials (Kohler *et al.*, 2005) and old electrical equipments. Nematodes are a dominant invertebrate group in freshwater and marine sediments (Heip *et al.*, 1985; Majdi and Traunspurger, 2015) and in soil (Yeates, 1984; Ferris *et al.*, 2001). Marine nematodes are important representatives and ubiquitous members of benthic meiofauna, being the most abundant and diverse taxon in marine sediments. Fundamentally these organisms in benthic food web are recyclers and a trophic link between microorganisms and macrofauna (Gee, 1989; Moens *et al.*, 2013). One of the most important and interesting features of marine nematodes is their potential for environmental monitoring and assessment, as bioindicators for environmental stress (Boyd *et al.*, 2000; Moreno *et al.*, 2011; Alves *et al.*, 2013; Semprucci *et al.*, 2013). As free-living marine nematodes in Iran were unexplored, for the first time Sahraean *et al.* (2017) reported on structure and biodiversity of nematofauna in intertidal soft sediment habitats along coasts of Bandar Abbas. Coastal area is important for artisanal fisheries and ecosystem recruitment as the extended tidal zone offers food web for fish larvae, hence there are few studies dealing with pollution mitigation and sustainable management of Persian Gulf (Khan,

2007; Abuzinada *et al.*, 2008; Sale *et al.*, 2011).

Although endocrine systems in nematodes are not fully understood, there is evidence that many processes are regulated via hormonal pathways (Höss and Weltje, 2007; Köhler *et al.*, 2007; Kostrouchova and Kostrouch, 2015). Accordingly, EDCs may be able to influence nematodes and cause endocrine disruption through xenobiotic effects (Höss and Weltje, 2007). Indeed, there are reports on effects of potential EDCs on nematodes (Novillo *et al.*, 2005; Reichert and Menzel, 2005). The majority of these studies on endocrine regulation and disruption effects in nematodes, are conducted on *Caenorhabditis elegans,* a free-living nematode that is extensively used as a model for developmental biology, genetics and medical sciences (Kaletta and Hengartner, 2006; Leung *et al.*, 2008).

While general anatomy of female reproductive system is widely studied in nematodes (Chitwood and Chitwood, 1950; Coomans, 1964; Hope, 1974; Yushin and Malakhov, 1997; Lorenzen, 1978, 1981; Geraert, 1983; Bird and Bird, 1991; Nisbet *et al.*, 2004), effects of PCBs on gonad structure require more investigation. Hope (1974) reviewed only one detailed light microscopic investigation on histology of female reproductive system in a primitive enoplid nematode, *Deontostoma californicum* (Enoplida, Leptosomatidae).

The aim of the present study was to investigate comparative effects of PCB congeners on sex ratio and steroid levels (Testosterone, Progesterone and 17-β-estradiol) in marine nematode, *Oncholaimus campylocercoides,* in three locations of coastal zones in Bandar Abbas city, in north Persian Gulf.

## Materials and methods

*Sample collection*

Three sampling locations with triplicate sampling were selected in intertidal zone at Bandar Abbas, Hormozgan Province, North Persian Gulf (Fig. 1).

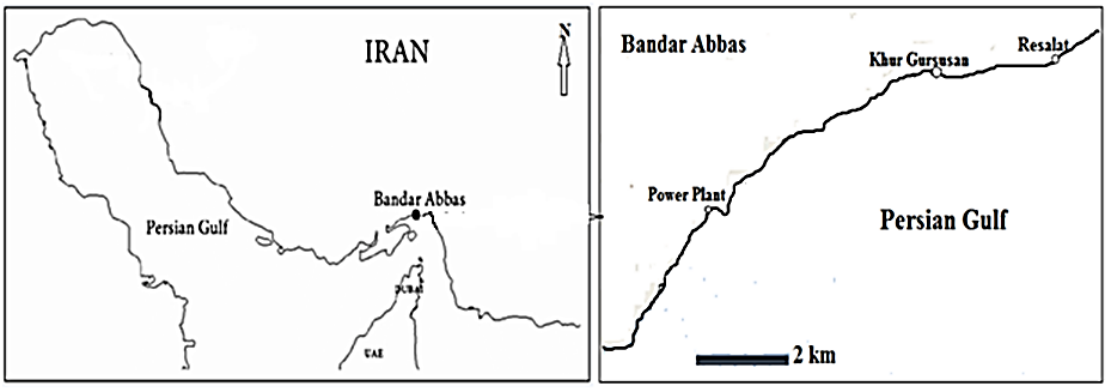


**Figure 1: Sampling locations in the coastline of Bandar Abbas.**

Locations were selected based on difference in level of pollutants, such as polychronated biphenyls (PCBs) according to Mohebbi Nozar *et al.* (2014). The first location, Bandar Abbas power plant is situated next to largest industrial drainage and is by far the most impacted location (Mohebbi Nozar *et al.*, 2014). The second location facing Gursuzan estuary, receives domestic input, pollution level in this location is considerably smaller than the first location. Finally, the third location, Resalat, is considered a comparatively less impacted site, receiving only small amounts of civill pollution; where there is no local sewage or industrial input.

Sediment samples were collected using a PVC hand corer from July to September 2018. Hand corers (10 $cm^2$) were pushed into the sediment to a depth of 10cm. Sediments were immediately preserved in 4% buffered formaldehyde for nematodes extraction, according to Sahraean *et al.* (2017). For biological measurement, samples were kept in -20ºC after washing and raising separated nematodes.

*Nematode extraction and identification*

In laboratory, sediments were rinsed 10 times thoroughly with water and decanted over a 38-µm sieve. Then, nematodes from the retained fraction on the sieve were separated using centrifugation with Ludox© HS40 at specific density of 1.18 (Vincx, 1996) and collected over a 38-µm mesh. This procedure was repeated two times, and the fractions retained on the sieve were then pooled with 4% buffered formaldehyde for identification purpose. Nematode identification was done using pictorial keys (Platt and Warwick, 1983; Warwick and Clarke, 1998) and the NeMys online identification key.

*Sex ratio calculation*

To achieve sex ratio of each sample, 100 randomly selected specimens of *O. campylocercoides* were picked. Each specimen was provided with temporary slides. Then, numbers of males and females were determined using optical microscope according to Platt and Warwick (1983) and Warwick and Clarke (1998). Sex ratios were calculated according to Biswas (1993) using F(1)/M(1) ratio.

*PCBs analysis*

PCBs analysis in nematode tissues was conducted by PCBs congener numbers 101, 138, 151, and 180 following Provini and Galassi (1999). Briefly, samples (1 gram) were extracted by acetone-hexane (1:1) in a Soxhlet apparatus and lipid content was determined by weight after solvent evaporation. Lipids were suspended in 2 mL of n-hexane and digested with concentrated $H_2SO_4$. PCBs were recovered by hexane. The hexane extracts were purified on a Florisil column (4×0.7 cm) and analyzed by a gas chromatograph equipped with an electron capture detector (GC-ECD) as described in Provini and Galassi (1999). The concentration of PCB congeners in exposure water samples were determined after n-hexane extraction (10:1, V:V, Provini and Galassi, 1999).

*Measurements of steroid hormones*

Steroid hormones (Testosterone, 17-Beta Estradiol and Progesterone) of *O. campylocercoides* were measured with ELISA test according to the manufacturer's instructions (ImmunoTech kit -France). The samples and standard solution were prepared and put into micro plate ELISA wells, followed by adding conjugate enzyme, except to blank well. After one hour incubation, each micro plate was washed out using specific wash solution provided with the kit. Then, a chromogenic substrate was added, into each well and incubated for 30 minutes under room temperature. The enzymatic reaction was stopped by adding a stop solution to each well. Reading of absorbance was conducted using ELISA reader at the wavelength of 450 nm.

*Gonad histology*

Several male and female *O. campylocercoides* were randomly picked from samples of each location and fixed in 10% neutral buffered formalin. Five males and females in each location were put into a drop of 0.9% solution of NaCl on a glass slide and then cut with an oculist scalpel, thereby leading to expulsion of the gonads. Tissues were processed following routine histology techniques, embedded in paraffin blocks according to Zograf *et al.* (2008) and Yushin *et al.* (2014). Microscopic sections were taken at 5 µm by Leica RM2255 rotary microtome. The obtained sections were stained with hematoxylin and eosin (H&E). Then they were dehydrated through graded series of alcohol and mounted onto glass slides. The slides were examined under a light microscope (Nikon ECLIPSE E200 microscope) connected with a digital camera (Nikon D5FI1).

*Transmission electron microscope (TEM)*

For transmission electron microscopy, males and females of *O. campylocercoides* were separated from sediment and fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) and 0.25 mg $mL^{-1}$ magnesium chloride ($MgCl_2$) according to Zograf *et al.* (2008), then specimens were cut into small pieces that contain the reproductive system. Males were cut at the base of the pharynx and females at the tail region and then again they were left in the same fixative.

Fixed specimens were stored for 1 week at 4°C. Post fixation was done in 2% osmium tetroxide for 2 h in the same buffer followed by en bloc staining for 1 h in 1% solution of uranyl acetate in distilled water and then the specimens were dehydrated in an ethanol series followed by propylene oxide series and embedded in a Spurr resin (EMS). The block face was trimmed with Leica EM trim and ultra-thin sections were cut with Leica Ultra cut ultra-microtome. Sections were collected form var-coated copper single slot grids (Agar Scientific). Sections were then stained for 30 min in uranyl acetate and 7 min in lead citrate at 20°C using Leica EM AC20 as described by Turpeenniemi (1993). Sections were observed with a

Philips EM 208S transmission electron microscope and pictures were taken by a digital Canon camera.

*Statistical analysis*

Data were presented as mean ± standard deviation. Statistical analysis was done by Statistica 7 software (Statsoft, 2004). Prior to analysis, all data for sex ratio and sex steroids were checked for normality by means of Kolmogorov–Smirnov test and homogeneity of variances using Levene's test. Differences in steroid hormones levels among different locations were analyzed using one-way ANOVA followed by Duncan's multiple range test and significance was set at $p<0.05$. For analysis of sex ratio, Chi-square ($X^2$) test was used with $\alpha=0.05$ (Robards *et al.*, 1999).

## Results

*PCBs concentration*

Concentrations of PCBs in the nematodes are presented in Figure 2. A wide range of PCBs among the locations was observed. These values varied from 0.01 to 0.1 µg/mL fluid tissue. The highest amount of PCB congener in nematode samples was detected in power plant station (0.1±0.02).

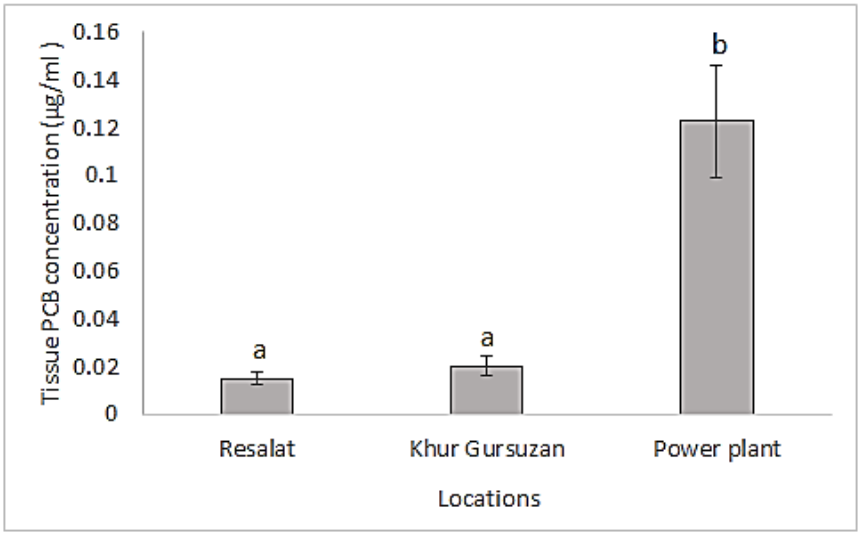


**Figure 2: Mean distribution of ΣCBs concentration in measured tissues from different stations. Different letters indicate significant difference among PCB concentration in of sampling location. Error bars show standard deviation.**

Mean concentration for sum of measured PCBs congeners (101, 138, 151, and 180) for Resalat, Khur Gursuzan and power plant locations were 0.01±0.002, 0.02±0.004, and 0.1±0.02 µg/mL, respectively. Distribution pattern evaluation of PCBs congeners in coastal surface sediment showed that PCB 180 was the dominant congener in tissue fluid of the nematode from studied coastline of Bandar Abbas, Hormozgan Province.

*Sex ratio*

Effects of different amount of PCB on sex-ratio of *O. campylocercoides* is

shown in Figure 3. Sex ratio was F(1): M(0.97), F(1): M(0.9), F(1): M(0.5) in locations with 0.01, 0.02 and 0.1 PCB concentration, respectively. There was significant difference in the total number of male and female specimens in the location with high amounts of PCB ($X^2$=4.3, df=1, α=0.05). The results also showed significant increase of females (57) compared to males (31) in the location with high PCB in comparison with female/male retions of the other two locations ($p$<0.05). While, no significant difference was observed comparing numbers of males and females in medium (0.02) and low (0.01) PCB concentration sites. Sex ratio of *O. campylocercoides* was depending on PCBs concentration levels.

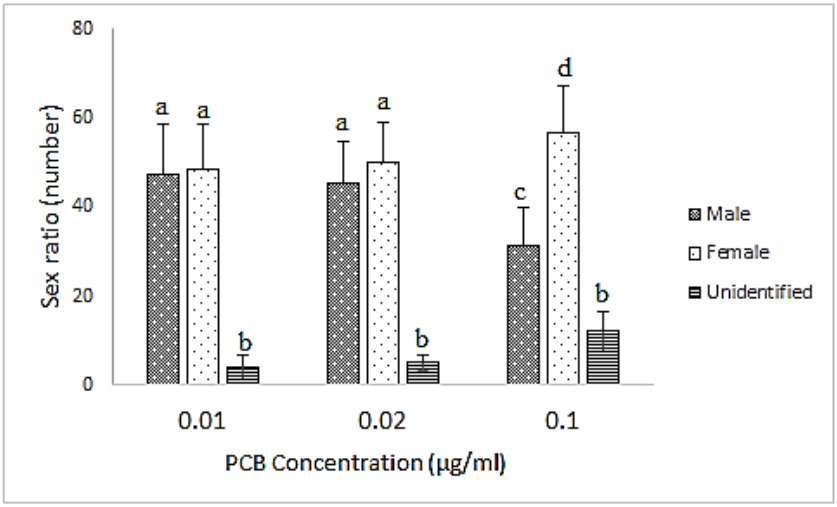


**Figure 3: Sex ratio of *O. campylocercoides* in sampling locations with different tissue PCB concentrations. Different letters indicate significant difference of males and females in sampling locations (PCB 0.01= Resalat; PCB 0.02= Khur Gursuzan; PCB 0. 1= power plant, P<0.05). Error bars show standard deviation.**

*Steroid hormones*

Mean values and standard deviation of steroid hormones (Testosterone, Progesterone and 17-β Estradiol) of *O. campylocercoides* are summarized in Table 1. Results showed that tissue levels of steroid hormones were different between males and females in all sampling locations.

*Female homones*

Level of 17 β-estradiol (1.2±0.07 pg/mL) in females at the location with 0.1 tissue PCB concentration (power plant station) was significantly higher than that in the other two locations ($p$<0.05, Fig. 4). In females, significantly higher level of testosterone (0.4±0.07 pg/mL) was observed in the polluted sampling station than that in the other two locations ($p$<0.05). Also significantly higher tissue level of female progesterone (1.06±0.04 pg/mL) was seen in the location with highest amount of PCB than that found in other locations ($p$<0.05).

**Table 1: Effects of different concentration of polychlorinated biphenyl (PCB) at sampling locations on steroid hormones of tissue fluid of *O. campylocercoides*. Tissue PCB of Resalat = 0.01; PCB of khur Gursuzan = 0.02; PCB of power plant = 0.1 (mean ± standard deviation).**

| | | Resalat | Khur Gursuzan | Power Plant |
|---|---|---|---|---|
| Testosterone pg/mL | Male | 0.06 ± 0.001 | 0.1 ± 0.006 | 0.08 ± 0.006 |
| | Female | 0.06 ± 0.005 | 0.06 ± 0.008 | 0.4 ± 0.07 |
| 17-Beta Estradiol pg/mL | Male | 0.4 ± 0.09 | 0.7 ± 0.03 | 0.5 ± 0.05 |
| | Female | 0.3 ± 0.05 | 0.5 ± 0.05 | 1.2 ± 0.07 |
| Progesterone pg/mL | Female | 0.2 ± 0.09 | 0.4 ± 0.08 | 1.06 ± 0.04 |

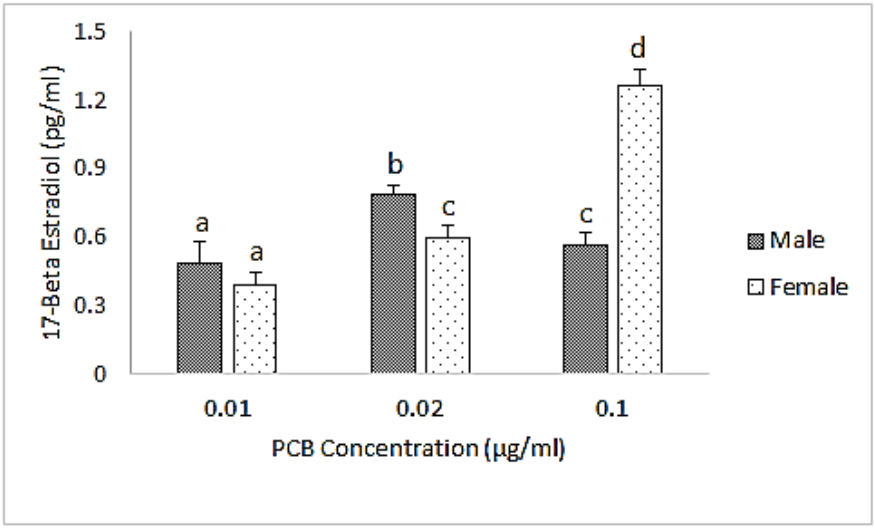


**Figure 4: 17β-Estradiol of male and female *O. campylocercoides* in sampling locations with different concentrations of tissue polychlorinated biphenyl. Different letters indicate significant difference among sampling locations (PCB 0.01=Resalat; PCB 0.02=Khur Gursuzan; PCB 0.1=Power Plant). Error bars show standard deviation.**

*Male hormones*

Tissue level of male testosterone (0.1±0.006 pg/mL) in the location with moderate pollution (Khur Gursusan) was significantly higher than that in the other two sampling locations ($p$<0.05, Fig. 5). Higher value of 17 β, estradiol (0.7±0.03 pg/mL) was obtained in the nematodes sampled in the location with moderate PCBs concentration compared to the other two locations ($p$<0.05, Fig. 4). While the level of male testosterone did not show dose dependent relationship, it significantly increased in sampling locations 1 and 2 ($p$<0.05), then decreased by increasing PCB concentration in location 3. Females showed significant increase in testosterone tissue levels in the location with high PCBs compared with that of the other two locations.

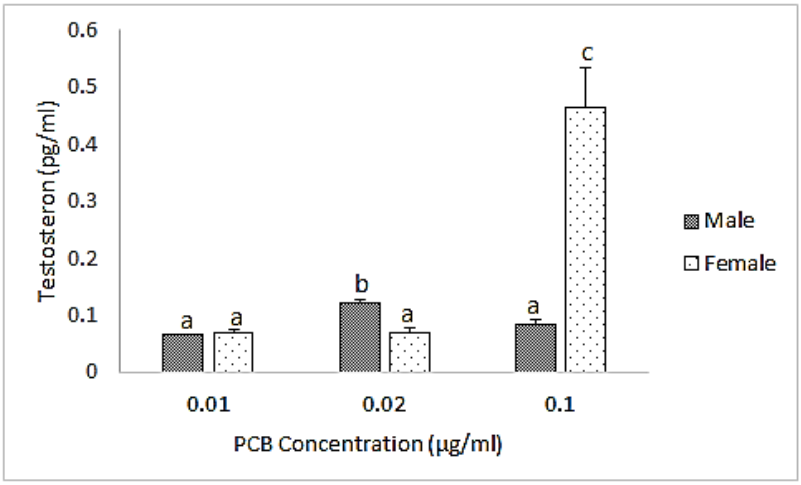


**Figure 5: Testosterone of male and female *O. campylocercoides* in sampling locations with different concentrations of tissue PCB. Different letters indicate significant difference among tissue PCB concentrations (PCB 0.01=Resalat; PCB 0.02=Khur Gursuzan; PCB 0.1=Power Plant). Error bars show standard deviation.**

17-β, estradiol increased in *O. campylocercoides* females with increasing PCB concentration ($p$<0.05), while males did not adopt this pattern and after an increase in location 2 the level of 17- β, estradiol decreased in location 3 (high PCB concentration).

Female progesterone tissue level increased with increasing PCB concentration in sampling locations (Fig. 6). But, in the location with high PCB the level of this hormone was significantly higher than those of the other two stations ($p$<0.05).

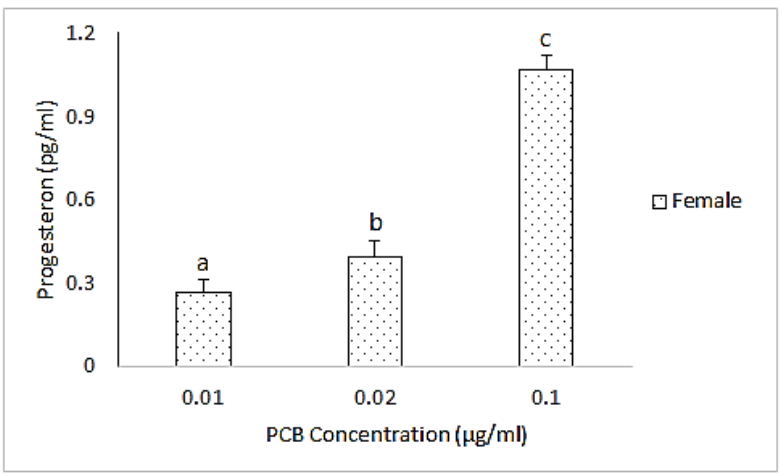


**Figure 6: Progesterone of female *O. campylocercoides* in sampling locations with different concentrations of tissue PCB. Different letters indicate significant difference among sampling locations (PCB 0.01=Resalat; PCB 0.02=Khur Gursuzan; PCB 0.1=Power Plant).**

*Histological findings*

In organism level, elongated, cylindrical, vermifome body had ovary, oviduct and uterus in the mid part. Sexes were separated and males were smaller than females. Male duct opened into the

cloaca. Ovary of *O. campylocercoides* at the location with low amount of PCB (Resalat Station) exhibited normal histomorphology. Results showed oocytes with normal distribution pattern across the ridges. In the location with high PCBs concentration, there was an obvious increase in size of oocytes (Fig. 7).

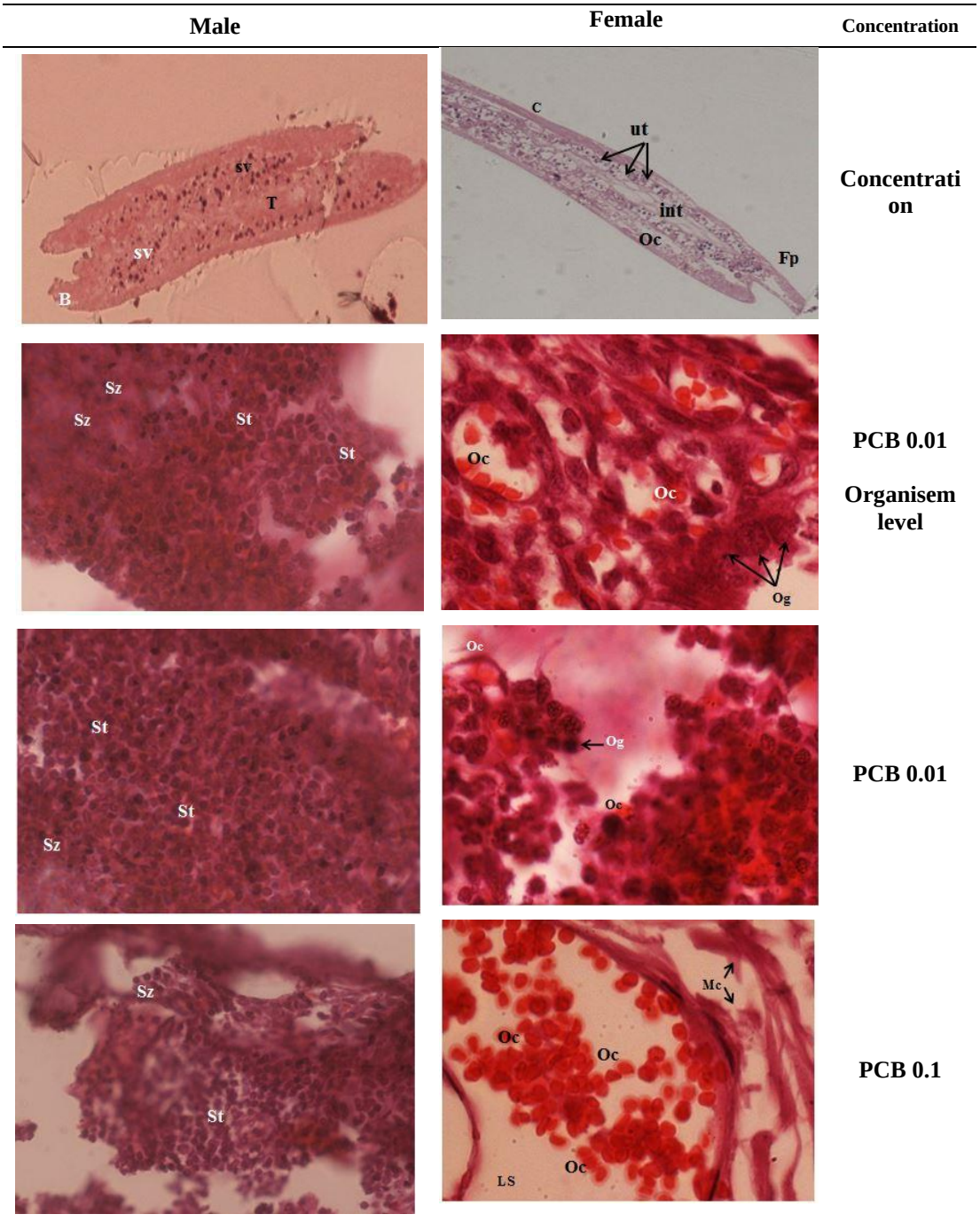


**Figure 7: Gonad sections in male and female nematodes exposed to different PCB concentrations at sampling locations. Bursa=B, Seminal vesicle=Sv, Testis=T, Uterus=ut, Intestin=int, Oocytes=Oc., Oogonia=Og, Spermatozoa=Sz, Spermatid=St, Myoepithelial cells=Mc., Lumen space=Ls., Seminiferous lobule=Sl., Cuticul=C., Front part=Fp., (H&E, X 1200).**

In high PCB concentration location, females showed significant differences in oocyte size, frequency and density. In locations with low and medium PCB concentration area (Resalat=0.01 mg/L and Khur Gursusan=0.02 mg/L) oocytes had orange round shape in tube like oviduct staining with H & E. While oocytes in the high PCB concentration area (power plant=0.1 mg/L) had dense bigger size with frequent yellow colour. Small amount of oocytes were observed with irregular shape and oviduct wall shrinkaged with scattered muscle fibers. Ovaries in the location with high pollution of PCB showed a decrease in size and number of oocytes. The results of LM exhibited normal structure in the ovary of *O. campylocercoides* at locations with low and medium amount of PCB (Fig. 8).

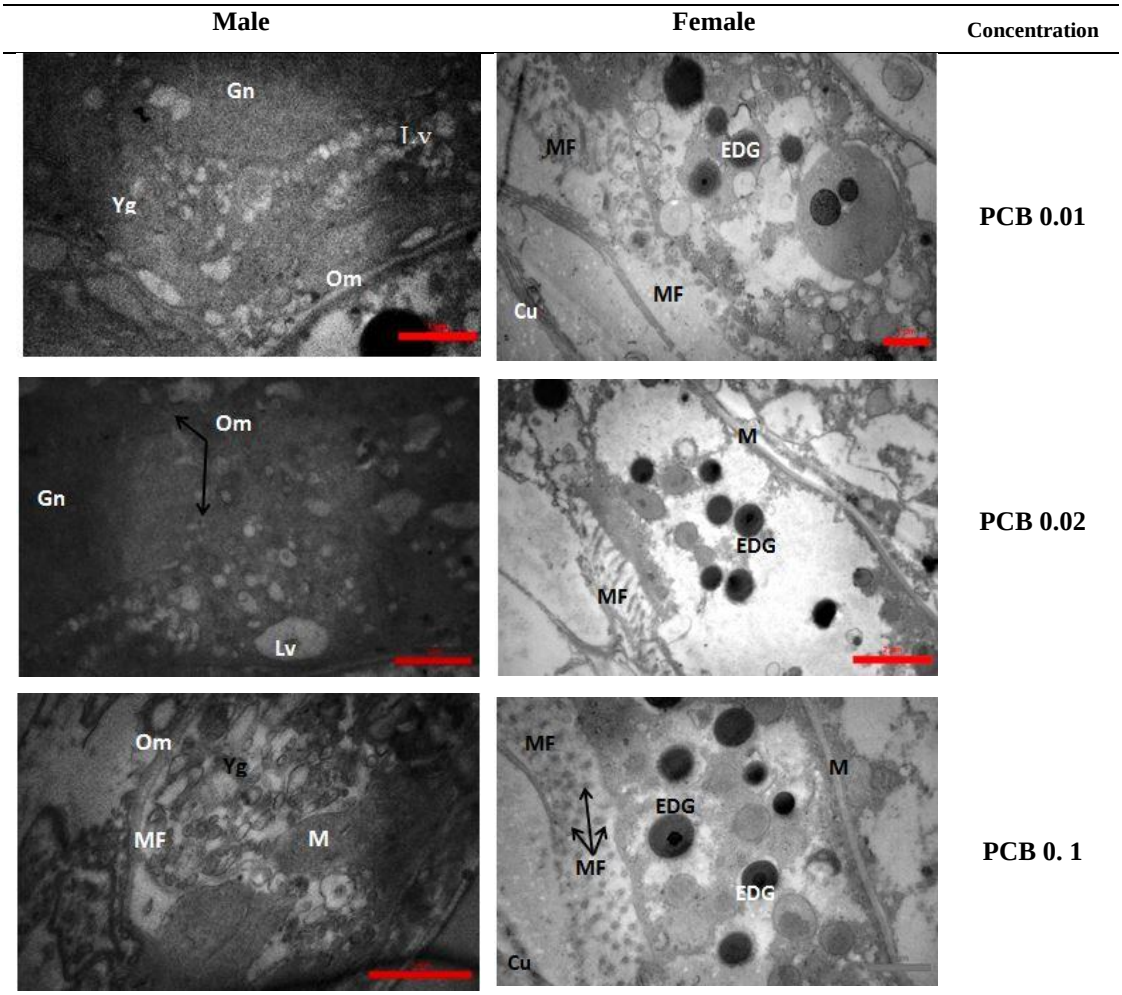


**Figure 8: Transmission electron micrograph of gonad of male and female nematode exposed to different PCB concentrations at sampling locations. Membrane=M, Mayofilaments=MF, Oocytes=Oc, DG=Distal gonad, GL=Gonadal loop, GN=Giant nuclei, Om=Oocyte membrane, Yg=Yolk granules, Lv=Lipid vesicle, M=Mitochondri , EDG=Electron dense granules, Cu=Cuticle, x 1200.**

Wall of the ovary consisted of thin epithelial cells. Several swellings oocytes were observed in the proximal region of the ovary. Ovarian wall in the

germinal zone was made up of flattened or pyramidal epithelial cells. Several of these cells surrounded distal tip of the ovary.

Wall of the oviduct was lined with high columnar cells with dense cytoplasm. Oviduct wall had numerous longitudinal muscle processes running of the myoepithelial cells of the ovary sac. The oviduct was connected to the middle part of the ovary at the growth zone and was followed by a short narrow sphincter, myoepithelial cells with bundles of the myofilaments running parallel to longitudinal axes of the cells.

Testis of nematodes was present in the location with low amount of PCB and contained spermatids, spermatozoa, lumen space, seminiferous lobule, and interlobular connective tissue in the histological structure. Decrease in spermatids and spermatozoa, degeneration of cells were observable in sections obtained from nematodes present in the location with high pollution of PCB (Fig. 7). Males showed small irregular shape spermatozoa situated between boundles of muscular fibers in low and medium PCB concentrations. Irregular spermatocytes and ducts were observed in high PCB concentration location (power plant=0.1). Nucleus was enclosed by a single membrane, with an appearance similar to that of plasma membrane.

*Transmission electron microscopy (TEM)*

Oocytes with dense cytoplasm containing extensive rough endoplasmic reticulum (RER), numerous darkly stained Golgi bodies, mitochondria, and vesicles of various sizes and contents were observed in SEM microscopy. Apical cytoplasm facing the lumen contained Golgi bodies, vesicles, and sparse flocculent material. Lumen of the oviduct was filled with homogeneous dark-stained substance (Fig. 8). In 0.01 PCB concentration, nucleus membrane of oocyte disappeared and unclear cytoplasmic reticulum was visible.

Amoeboid spermatozoa containing mitochondria, membranous organelles, filaments, microtubules were observed in electron microscopy. Plasma membrane of the spermatozoa was smooth, the electron-dense granules observed in the testicular cells appeared to be absorbed by spermatozoa in the location with high concentration of PCB (power plant). Cytoplasm of spermatozoa was characterized by many mitochondria and an extensive network of small tubules in two other sampling locations rather than polluted area.

**Discussion**

While our study focused on *O. campylocercoides* in Persian Gulf area (Hormozgan), nematodes served as models in studies on aging (Tissenbaum, 2015), behavior (Dusenbery, 1992) and environmental pollutants (Edwards, 2002; Höss *et al.*, 2011) and were on advanced edge of genetic research (Sugi, 2016; Weinhouse *et al.*, 2018). Higher levels of steroid hormones (Testosterone, Progesterone and 17-β-estradiol) were observed in higher PCBs concentration area (Table 1). Highly sensitive technique was employed in a

wide range of experiments to demonstrate binding of steroid hormones and estrogen receptor in nematodes, such as *Panagrellus redivivus* and *C. elegans*, which contained gene encoding for the estrogen receptors (Kostrouch *et al.*, 1995). Influence and occurrence of steroid hormones in invertebrates had received less attention. All invertebrates have a hormone system based on internal signaling, although complexity of that system varies greatly across invertebrate phyla. Mammalian sex steroid hormones were detected from free living nematode *Turbatrix aceti* by chromatography (Lee *et al.*, 1990).

In this study we found that *O. campylocercoides* was sensitive to environmental PCB pollution. Reproductive system of the nematode was affected by PCBs via endocrine disruptive pathways. Results showed significant differences ($p$<0.05) in sex steroid hormones in different sampling locations with different PCBs concentration. Research supports the hypothesis that some endocrine disruptors, such as dieldrin, nonylphenols and toxaphene may mimic estrogen, altering normal pathways of estrogen metabolism (Hood *et al.*, 2000). Some reproductive studies suggested that lipophilic or steroid hormones influence *C. elegans* lifespan (Hsin and Kenyon, 1999; Dumas *et al.*, 2013). This lifespan extension requires DAF-12 (Hsin and Kenyon, 1999), a nuclear hormone receptor (Antebi *et al.*, 2000) as well as cytochrome P450-like protein DAF-9 (Gerisch *et al.*, 2001). Nematodes such as *C. elegans* contain steroids, such as progesterone, allopregnanolone, epiallopregnanolone and etiocholanolone (Broué *et al.*, 2007). A number of sterols (which contain 27-carbon atoms) are identified in *C. elegans* (Chitwood, 1999; Held *et al.*, 2006; Motola *et al.*, 2006) and some of them, such as dafachronic acids (Motola *et al.*, 2006) and cholestenoic acids (Held *et al.*, 2006) were described as ligands of DAF-12.

We found that females showed significantly higher level of testosterone (0.4±0.07), 17β-estradiol (1.2±0.07) and progesterone (1.06±0.04) in the location with high PCBs concentration ($p$<0.05). Our results showed dose-dependent relationship, ie. hormone levels were increased with increasing PCBs concentrations in different sampling locations for females. While level of male testosterone did not show dose-dependent trend. In this case, the highest value of testosterone (0.1±0.006) was observed in the location with moderate PCBs concentration. Levels of androgens (testosterone, 5α-DHT or androstenedione) and progesterone were in the range of 0.1–10 ng/g in tissues from most invertebrate species tested, whereas levels of pregnenolone (only investigated in crustacean ovaries) ranged from 10 to 100 ng/g.

Alterations in sexual characteristics or reproduction, due to exposure to exogenous testosterone, are observed in invertebrates such as molluscs (Takeda, 1979; Spooner *et al.*, 1991; Sakr *et al.*, 1992; Bettin *et al.*, 1996). Many EDCs alter endocrine homeostasis by

interfering with steroid synthesis or metabolism and consequently altering steroid levels (Van der Kraak *et al.*, 2001; Santos *et al.*, 2012). Change in enzyme activities after in vivo exposure to chemicals may result from direct effect of the chemical on that metabolic pathway (Janer and Porte, 2007).

Tributyltin (TBT) and Bisphenol A (BPA) are another endocrine disruptors that may affect biological systems. Quang *et al.*, (2017) reported the effect of TBT on morphometry and biomass of nematode communities in different harbours in Vietnam. They reported that nematodes in contaminated sediments from Saigon River harbours were characterised by slender morphotypes. Individual nematode biomass was generally low, especially in wet seasons. There was significant correlation between TBT and nematode morphometrics in wet seasons, but not for dry seasons. Essid *et al.* (2013) reported the effects of 17-α-estradiol on community structure of free-living marine nematodes. Four concentrations of 17-α-estradiol, such as 0.15, 0.31, 0.62 and 1.24 ppm, were tested and their effects were examined after 30 days. They reported that there were significant differences between nematode assemblages in the control and those in 17-α-estradiol treatments. Total nematode abundance, Shannon–Wiener index and evenness were affected by 17-α-estradiol contamination, but species richness was unaffected. Some marine nematodes, such as *Chromadorina metulata* and *Ascolaimus elongatus* seemed to be intolerant to estradiol and were omitted at all tested doses. *Craspodema octogoniata* decreased at all doses and could be categorized as sensitive to estradiol contamination. *Spirinia gerlachi* increased at all tested doses and seemed to be an opportunistic species.

Several hormonal factors highlight the importance of invertebrate endocrine disruption studies. Hoshi *et al.* (2003) investigated the effects of 17-β-estradiol (E2), bisphenol A (BPA) and TBT on germ cells of *C. elegans*. Two estrogenic compounds of E2 and BPA increased the number of germ cells at concentrations as low as 0.1 and 1.0 nM. But the androgenic compound like TBT is shown to reduce those cells. Nitrates which are commonly used as fertilizers are implicated in disruption of animal physiology (Edwards *et al.*, 2006) Although they are chemically very different from compounds that are normally of concern, EDCs can have different physiological effects, such as altering (inhibiting or stimulating) secretion of hormones. It is well known that corticosteroids and sex steroids participate in differentiation, and protein synthesis, in essential metabolic pathways and in immune system function of vertebrates and invertebrates (Milla *et al.*, 2011; De Loof *et al.*, 2016; Subramoniam, 2017). Researchers reported presence of sex steroid receptors for progesterone, testosterone, and 17-flestradiol in an extract from the ruminant nematode, *Trichostrongylus colubriformis* (Kiser *et al.*, 1986; Lee *et al.*, 1989).

Our results showed changes in sex ratio pattern with increasing PCBs concentration. Sex ratio was F (57): M (31) in the location with high PCBs concentration (power plant Station) while in other two locations (Khur Gursuzan and Resalat) there was no significant difference between male and female ratio. Investigation of sex ratio in nematode populations is used to test the effects of xenobiotic chemicals (Prahlad *et al.*, 2003; Leung *et al.*, 2008; Jitjaroen *et al.*, 2017). Primary sex ratio of animals is expected to be balanced (50:50) as proposed by (Fisher, 1930). However, endocrine disrupting substances significantly affect this index (Martinović *et al.*, 2007; Fazio *et al.*, 2008; Liang *et al.*, 2015). According to the results of our work, sex ratio was shifted to females (F(57):M(31) in the high PCBs concentration location. There was no effect on sex ratios, but a reduction in fecundity occurred in *P. Redivivus* (Hood *et al.*, 2000). Some researchers noted that sex ratio was affected in invertebrates when exposed to EDCs (Tankoua *et al.*, 2012; Kang *et al.*, 2019). Several studies revealed that sex steroids and their derivatives affect reproductive performance in nematodes. Their results showed significant increase in oocyte size and density in high concentrated PCBs area. Effects of endocrine disruptors on gonad histology in koi carp (*Cyprinus rubrofuscus*) were also reported increasing oocyte maturation and proliferation in high nonylphenol treatments (Hosseinzadeh Sahafi *et al.*, 2017). Although striated sperms are described in *Tobrilus spp*., *Syringolaimus spp*., and in some members of trichodorids (Chitwood, 1931; Riemann, 1983; Bird and Bird, 1991; Bernard, 1992). The ultrastructure of spermatozoon in *O. campylocercoides* indicates that it was aflagellate. Each ovary has a well-developed ovarian sac where the oocytes terminate their growth. Based on our results fluctuations in sex steroids, as well as sex ratio, were observed in three locations with 0.01, 0.02 and 0.1 µg/mL tissue PCB concentration. It is concluded that *O. campylocercoides* can play role as a key component of intertidal ecosystems and a bio-indicator for PCBs in pollution evaluation.

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