Int. J. Aquat. Biol. (2019) 7(2): 65-70
ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com

Original Article

Plasma 17beta-estradiol and alkali-labile phosphoprotein levels in male and female Tench (*Tinca tinca*) in the Anzali and Amirkolayeh wetlands

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Abstract: Environmental pollutants are potentiate to disturb biological processes such as metabolism, growth and reproduction of aquatic organisms. These compounds are able to cause gonadal abnormalities, biased sex ratios and alteration in reproductive physiology in fish. The aim of this study was to examine plasma 17β-estradiol (E2) and alkali-labile phosphoprotein (ALP) levels in male and female Tench (*Tinca tinca*) from a polluted (the Anzali Wetland) and a non-polluted environments (the Amirkolayeh Wetland). Samples were collected over the maturation season of Tench between May and June 2017. The results revealed significant difference in mean ALP and E2 between genders in the polluted environment. However, the mean plasma ALP concentrations in male Tench of the polluted environment (39.46±1.02 µg/ml) was 45% of the average recorded in female (86.18±2.25 µg/ml) and was two times higher than the amount measured in males in the non-polluted environment (18.68±0.35 µg/ml). High concentrations of E2, were detected in the male samples from the Anzali Wetland. Mean plasma E2 concentrations for male in the Anzali Wetland was almost two times higher than male in the Amirkolayeh Wetland. The results indicate that the reproductive physiology of Tench was affected by contaminants found in the Anzali Wetland, a highly polluted area.

Article history:
Received 2 January 2019
Accepted 2 March 2019
Available online 25 April 2019

Keywords:
ALP
Anzali Wetland
Steroid hormone
Tinca tinca

Introduction

Fish reproductive physiology is controlled by the endocrine system, which is affected by environmental factors such as light, temperature, etc. This system includes various glands, synthesizing and secreting hormones, which in turn regulate fish reproduction (Pait and Nelson, 2003), control and initiate steroidogenesis, vitellogenesis and gametogenesis process mainly through the activation of hypothalamic–pituitary–gonadal (HPG) axis (Pank-hurst and Munday, 2011). In aquatic systems, there are varieties of environmental pollutants causing disturbance in the function of this system (Ogundiran and Fawole, 2018) by simulating or blocking the steroid hormones activity (Mills and Chichester, 2005) and binding to estrogen or androgen receptors (Tapiero et al., 2002). These contaminants are called endocrine disrupting chemicals (EDCs) and include groups of natural and synthetic compounds such as phytoesrogens (Burki et al., 2006), bisphenol-A (Qiu et al., 2015), PCBs (Walczak and Reichert, 2016) and PAHs (Vignet et al., 2016).

Releasing of large quantities of municipal, agriculture and industrial wastewaters into the aquatic ecosystems cause serious impacts on the physiology of aquatic organisms. The Anzali (Sakizadeh et al., 2012) and Amirkolayeh wetlands (Zare Khosh Eghbal and Sajadi Nasab, 2015) are located in the north of Iran and registered as two international wetlands based on 1975 Ramsar Convention. The main water source of the Amirkolayeh Wetland is underground water with no pollution from domestic and industrial wastes, and therefore, is considered as unpolluted and clean environment (Zare Khosh Eghbal and Sajadi

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DOI: https://doi.org/10.22034/ijab.v7i2.596

Table 1. Total length and total weight (mean ±SE) of Tench captured from the Anzali and Amirkolayeh wetlands (n=15 fish for each gender in each wetlands).

	Anzali Wetland		Amirkolayeh Wetland	
	Length (cm)	Weight (gr)	Length (cm)	Weight (gr)
Male	22.50±3.12	33.10±2.20	26.17±2.10	38.45±5.14
Female	25.13±6.41	40.87±4.89	28.32±4.52	43.19±7.26

Nasab, 2015). In contrast, concentrations of EDCs are considerably high in the Anzali Wetland (Mortazavi et al., 2012, 2013) due to releasing large amounts of untreated municipal and industrial wastewater into the wetland; as a result, fish could be exposed to the chemical compounds directly and indirectly in this wetland.

Recent investigations have suggested that exposure of wild fish to EDCs causes gonadal abnormalities and intersex (Scholz and Klüver, 2009), biased sex ratio (Larsson and Forlin, 2002) and induction of vitellogenin in males (Tyler et al., 2002). Vitellogenin is egg yolk protein precursor, which is synthesized in the liver of female fish (Jobling et al., 1996). Although there is vitellogenin gene in male, it is not expressed under normal conditions. However, when fish are exposed to EDCs, they may respond to these compounds by increasing the level of plasma vitellogenin. A considerable increase in the level of vitellogenin has been reported in male *Squalius cephalus* (Randak et al., 2009) and *Carassius auratus* (Li et al., 2009) inhabited in aquatic ecosystems contaminated by EDCs. Vitellogenin is dominant in plasma after the onset of vitellogenesis and the protein is heavily phosphorylated. This enables indirect quantification through measurement of alkali-labile protein bound phosphate (ALP) as alternative to the more expensive ELISA assay (Hallgren et al., 2009).

In this study, Tench (*Tinca tinca*) was selected as target species, being an indicator of water quality, used as a model species for studies of physiology (Martin et al., 1999), and lives in wetlands of Iran north, including the Anzali and Amirkolayeh wetlands. The present study started with hypothesis that reproductive physiology of Tench in the Anzali wetland could be affected by environmental pollution, and the Amirkolayeh wetland was considered as reference site.

Materials and Methods

Sixty male and female tench were collected during the reproduction season (between May and June 2017) by fyke nets (mesh size, 34-54 mm) at two sites: the Anzali Wetland (Pirbazar region), as a polluted environment and the Amirkolayeh Wetland as a non-polluted environment or reference site. Mean total length, sex, and number of collected samples are given in Table 1.

The fish were anesthetized in 150 mg/l clove powder solution and blood samples were immediately collected on site from the caudal vine using a heparinized 2.5 ml syringe. The collected blood from every fish was immediately placed in 2 ml plastic tubes, stored on ice, and transferred to the laboratory for further analyses. Blood samples were centrifuged at 1500 g, 4°C for 3 min (Scan Speed, 1730 R, Denmark). The separated plasma was stored at -80°C until analysis. Total length of fish were measured to the nearest millimeter, and weighed to the nearest gram. Gonads of each fish were observed macroscopically.

Plasma vitelloginine concentrations were measured indirectly by determination of plasma ALP (Negintaji et al., 2018; Matthiessen et al., 2018). In this method, vitelloginine-related phosphate released into plasma and was measured by spectrophotometer with the wavelength at 630 nm according to Hallgren et al. (2009). The levels of E2 were measured by Estradiol II kit (Biomereux, France) and with enzyme linked fluorescent assay (ELFA) method at 450 nm (Diver, 1987).

For calculating the effects of sex and location on measured parameters (ALP and E2), two-way analysis of variance (ANOVA) was applied. All data were tested for normality and homogeneity of variance using the Kolmogrov–Smirnov test and the Levene test, respectively. Statistical analyses were conducted

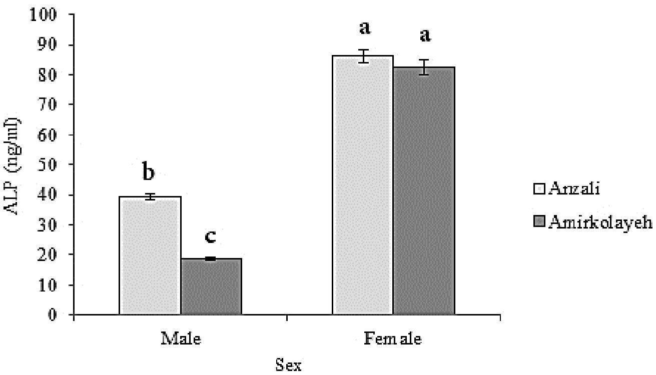

Figure 1. Concentrations of ALP for male and female Tench captured from the Anzali and Amirkolayeh wetlands (n = 15). Data are presented as the mean ± standard error. Different letters above the bars show significant differences.

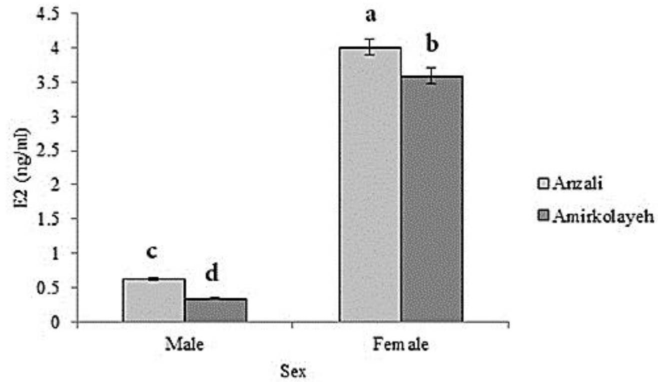

Figure 2. Concentrations of E2 for male and female of Tench captured from the Anzali and Amirkolayeh wetlands (n = 15). Data are presented as the mean ± standard error. Different letters above the bars show significant differences. The bars show significant differences.

using SPSS (Version 16, Inc., Chicago, IL, USA). All statistics were performed with a critical α of 0.05.

Results

The males from the Anzali Wetland had significantly higher (~2 folds) ALP and E2 compared to those of the Amirkolaye Wetland (Figs. 1, 2). There were no significant differences in the female's plasma ALP levels between the sampling sites (Fig. 1), but plasma E2 levels of the females from the Anzali Wetland were significantly higher than the females from the Amirkolayeh Wetland (Fig. 2). In both wetlands, females had significantly higher plasma ALP (Fig. 1) and E2 compared to the males (Fig. 2).

Discussions

There are increasing concerns for the presence of varieties of environmental pollutants with endocrine systems disrupting ability in aquatic organisms. EDC act by mimicking or interrupting hormone function, inducing vitellogenin synthesis in males, biased sex ratio and etc., which may affect natural reproductive and developmental processes (Mills and Chichester, 2005).

In the present study, the plasma ALP levels of the female fish from the Anzali Wetland were significantly different from males. Vitellogenin is a significant source of nutrition for developing embryos and larvae that synthesized in female fish liver in response to circulating E2 hormone (Monson et al., 2017). However, during the reproductive cycle of Tench in the Anzali Wetland, synthesis vitellogenin was found in the male fish. There are vitellogenin genes in male fish and are not expressed under normal conditions, but might be induced when exposed to estrogen (Juin et al., 2017) or estrogen like compounds in the aquatic environment (Folmar et al., 2000). Field studies on wild *C. auratus* (Lu et al., 2010) and *Rhinichthys cataracte* (Jeffries et al., 2008) showed high concentrations of vitellogenin levels in the fish from EDCs-contaminated environment.

In this study, ALP levels of the males from the Anzali wetland were 45% of the females. Fossi et al. (2002) studied *Xiphias gladius* and *Thunnus thynnus* from the Mediterranean Sea, and found plasma vitellogenin concentrations of the males were 28% and 11% of the females, respectively. Plasma ALP levels in the males from the Anzali Wetland were higher than the males from the Amirkolayeh Wetland, which is similar to previous reports by Abbasi (2013) that suggested increased plasma ALP levels in the male pike (*Esox Lucius*) from the Anzali Wetland more than from Pike males captured from Amirkolayeh Wetland. Other researchers also found similar results over different species and in different regions (Hashimto et al., 2000; Folmar et al., 2001; Dick Vethaak et al., 2002).

Mortazavi et al. (2012, 2013) stated that the levels of EDCs (such as nonylphenol, octylphenol and bisphenol-A) at the Anzali Wetland were higher compared to some other parts of the world such as rivers in China (Jin et al., 2010) and Japan (Isobe et al., 2001). When xenoestrogens present in water, they may changes aromatase activity (the enzyme converts androgens to estrogens) in males by increasing plasma E2 concentrations (Solé et al., 2003) and induction of vitellogenin.

E2 is one of the most important sex steroids that are produced by ovarian follicles (Monson et al., 2017) and regulates sexual processing and reproductive processes (Martyniuk et al., 2006). Our results demonstrated that the males captured from the Anzali Wetland had a doubled E2 levels compared to the males from the Amirkolayeh Wetland, indicating that EDCs compounds at the Anzali Wetland interrupted reproduction system.

Conclusion

These results suggest that EDCs compounds in the Anzali Wetland might increase synthesis vitellogenin compared to the Amirkolayeh Wetland. The male Tench from the Anzali Wetland have increased plasma ALP and E2, suggesting disruption of reproduction system. However more studies will need to approve our results.

Acknowledgements

We thank Mr. Ghorbanzade, Ghane and Darand for sampling.

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Int. J. Aquat. Biol. (2019) 7(2): 65-70
E-ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com

چکیده فارسی

سطوح ۱۷بتا-استرادیول و آلکالین-فسفوپروتئین پلاسما در نر و ماده لای‌ماهی (*Tinca tinca*) در تالاب‌های انزلی و امیرکلایه

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چکیده:
آلاینده‌های زیست محیطی که به درون اکوسیستم‌های آبی وارد می‌شوند توانایی مختل کردن فرایندهای متابولیسم، رشد و نمو و تولیدمثل یک موجود آبزی را داشته و می‌توانند موجب ناهنجاری‌های گنادی، انحراف نسبت‌های جنسی و تغییر فیزیولوژی تولیدمثل ماهی شوند. هدف از این مطالعه ارزیابی برخی پارامترهای بیوشیمیایی در پلاسمای خون لای ماهی نر و ماده از یک محیط آلوده (تالاب انزلی) و یک محیط غیرآلوده (تالاب امیرکلایه) می‌باشد. نمونه‌ها در طول فصل بلوغ لای ماهی بین ماه‌های اردیبهشت و خرداد ۱۳۹۶ جمع‌آوری شدند. فسفوپروتئین متصل به آلکالین (ALP) به‌عنوان یک شاخص غیرمستقیم از مقدار ویتلوژنین پلاسما اندازه‌گیری شد. همچنین ۱۷-بتا استرادیول (E2) در نمونه‌های پلاسما اندازه‌گیری شد. نتایج، اختلاف معنی‌داری در میانگین ALP و E2 بین جنس‌ها در محیط آلوده را نشان داد. با این حال غلظت‌های ALP در لای ماهی نر محیط آلوده (۱/۰۲±۳۹/۴۶ میکروگرم/ میلی لیتر) ۴۵٪ میانگین ثبت شده در ماده‌ها (۲/۲۵±۸۶/۱۸ میکروگرم/ میلی لیتر) و ۲ برابر مقدار اندازه گیری شده در لای ماهی‌های نر محیط غیر آلوده (۰/۳۵±۱۸/۶۸ میکروگرم/ میلی لیتر) بود. غلظت‌های بالایی از E2 در نمونه‌های نر تالاب انزلی تعیین شد. میانگین غلظت‌های E2 پلاسما در تالاب انزلی تقریباً دو برابر نرهای تالاب امیرکلایه بود. نتایج نشان داد که فیزیولوژی تولیدمثلی لای‌ماهی توسط آلاینده‌های تالاب انزلی، یک محیط به شدت آلوده، تحت تاثیر قرار گرفته است.
کلمات کلیدی: ALP، تالاب انزلی، هورمون استروئیدی، *Tinca tinca*.