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Study on Levels of Sex Steroid Hormones of Pike (*Esox lucius* Linnaeus, 1758) in Anzali Wetland

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Abstract: Pike, *Esox lucius* (Linnaeus, 1758) is an economic fish of the Caspian Sea Basin that live in some areas such as the rivers with aquatic plants and wetlands. This fish reproduce in freshwater ecosystems that there is little information available about it. Study of sex steroid hormones in pike is one of the important indicators of reproductive biology. So, the sex steroid hormones in pike broodstocks of Anzali wetland studied from autumn 2010 to summer 2011. Catch of 50 pikes were done randomly at different locations of wetland. Blood samples were collected from the fish after biometry had been done and the measurement of sex steroid hormones was measured. The results of sex steroids levels of plasma showed, the highest of 17β-estradiol hormone levels in autumn, the highest of testosterone hormone levels in winter and the highest of 17α-hydroxy progesterone hormone levels in autumn and winter ($P<0.05$).

Key words: Anzali Wetland · Pike · Testosterone · 17α-Hydroxy Progesterone · 17β-Estradiol

INTRODUCTION

Study of fishes is important in the water ecosystems through various reasons and including review of evolutionary, ecological, behavioral biology, their protection, water resources management, exploitation of stocks and culture of fish resources. The biological study of different fishes is due to keep and rebuild of their stocks in a water ecosystem and in this way, all of economic and noneconomic fishes have great importance and value due to their role in water ecosystems.

With growing pressures due to growth of population on current limited resources, it is felt an urgent need to better understand the characteristics of the aquatics and their environment. Also it is important to correct management of understanding the biology and have of adequate information about the aquatics [1].

Estimation and recognition of fish physiological situation and determination of amount and size is necessary for estimation and keep of economical fishes stocks for artificial reproduction and development of growth condition [2]. There is a significant and positive correlation between estradiol levels in plasma with amount of volume than weight of the yolk [3, 4]. In general, gonadotropins stimulate the theca cell layer for production of testosterone hormone and in continue, stimulate the aromatase granulosa layer for production of steroid hormone such as estradiol. Then estradiol hormone enters into the liver by the blood vessels and stimulates the liver for vitellogenesis. In fact, activity of granulosa layer is very high in period of vitellogenesis [5-8].

Esox lucius (Linnaeus, 1758) is a fish with great economic value [1]. The culture of pike sometimes equal to culture of salmonidae for rebuild of stocks [9]. This fish has average abundance and it need to protected, but the generation of pike decreased due to destruction of its habitat (water pollution and loss of straw) in recent years [10]. The results of studies prove that the share of pikeperch and pike has stabilized in spite of significant stocking efforts. Since both of these species are of interest to anglers and are also key to the proper functioning of fisheries management, it is necessary to study their growth rates [11-14].

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Anzali wetland is placed in the south of the Caspian Sea, the geographical location of 37 degrees and 29 minutes and 13 seconds of north latitude and 49 degrees and 18 minutes and 41 seconds of east longitude. Anzali wetland has special sensitivity due to great importance of ecological, botany, zoology, limnological and biological and also the most important supporter of reproduction economical-fishery fish of the Caspian Sea and has great values correlate with attraction of tourists, control of floods and finally, provide facilities of water connections in transportation of area. Unfortunately, this wetland faces with risk of premature death in recent decades due to water level fluctuations of the Caspian Sea, enter abundant sediment, discharge of pollutants resulting from population growth and industrial development, agricultural development, drainage and change of margin lands and finally, exploitation of stocks and resources of fish and wildlife. Currently, Anzali wetland is about 140 square kilometers. The catchment of wetland is 374,000 hectares [15].

The great studies applied on sex hormones fluctuations during the process of maturation and spawning in teleost fishes by various researchers that can be singed to studies on seasonal changes of thyroid and reproductive steroid hormones in the female channel catfish [16], seasonal cycle of gonadal development and plasma sex steroid levels in grouper (*Epinehelus morio*) [17], assessment of reproductive physiology in yellowtail king fish (*Seriola lalandi lalandi*) [18], seasonal variation of steroid hormone levels in an intertidal-nesting fish, the vocal plainfin midshipman [19], effects of fish size and season changes on sexual maturity stages and gonadal steroid hormones of southern Caspian kutum (*Rutilus frisii kutum*) [20] and changes in sex hormones during stages of ovarian development in grouper (*Epinephelus coioides*) [21].

The purpose of this study was to determination the levels of sex steroid hormones of pike by hormonology studies for appropriate the right strategies of optimum utilization and also preservation of pike stocks.

MATERIALS AND METHODS

Sampling of fish began in autumn 2010 and continued until the end of summer 2011. Catch of 50 pikes were done randomly at different locations of wetland. Samples catch in autumn (November), winter (middle of February and early of March), spring (June) (sampling was conducted in June due to end of the permissive fishing season and don't catch in May) and summer (early of August). In the laboratory, total length was determined using biometric board (accuracy of 1 mm) and weight of fish using balance (accuracy of 1 g).

Hormones Assay: Blood was taken using a syringe from the caudal peduncle and taken into compartments containing anticoagulant of blood (CBC) and then tubes CBC transferred to lab and their plasma was extracted using a centrifuge and this tubes were transferred to laboratory, University of Agricultural Sciences and Natural Resources, Gorgan. Samples were maintained in freezer-70 °C for hormone analysis.

17β-estradiol, 17α-hydroxy progesterone and testosterone were measured with radioimmunoassay political approach (RIA) by the Specteria Kit of Finland and gamma counter [22].

Statistical Analysis: The SPSS 13 software was used for data analysis. One-way analysis of variance (ANOVA) was employed to camper the means of factors. Where significant F-ratios were calculated by ANOVA, the Duncan test was applied to identify which means was different.

RESULTS

According to the results, the mean hormone concentration of 17β-estradiol was 2038.05±862.40 (minimum 333.28 and maximum 2571.73) in autumn, 410.60±409.77 (minimum 35.59 and maximum 2464.84) in winter, 139.32±80.84 (minimum 54.50 and maximum 309.91) in spring and 356.13±213.40 (minimum 116.43 and maximum 525.66) in summer. The highest of 17β-estradiol was in autumn and the lowest was in spring (Figure 1). There was significant relationship between 17β-estradiol and season ($P<0.05$).

According to the results, the mean hormone concentration of testosterone was 2.46±1.64 (minimum 0.65 and maximum 5.20) in autumn, 2.86±1.40 (minimum 0 and maximum 19.52) in winter, 0.43±0.18 (minimum 0.23 and maximum 0.79) in spring and 1.42±1.34 (minimum 0.38 and maximum 3.32) in summer. The highest of testosterone was in winter and the lowest was in spring (Figure 2). There was significant relationship between testosterone and season ($P<0.05$).

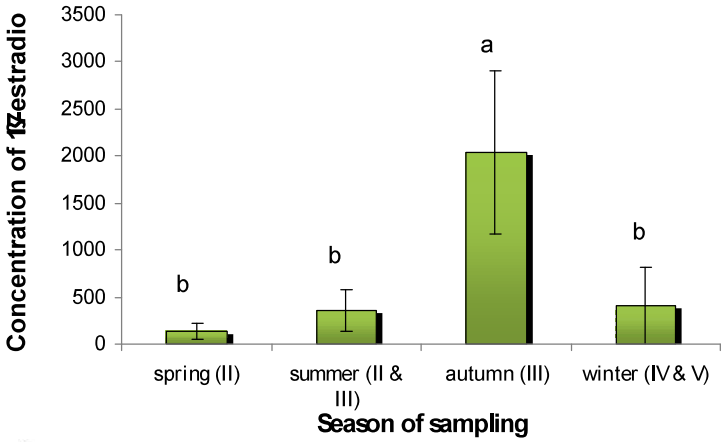

Fig. 1: The changes of 17β-estradiol (mean ± SD) in pike of Anzali wetland

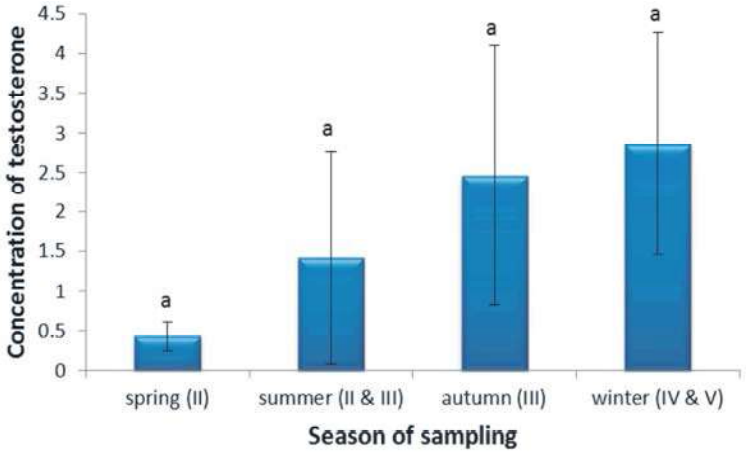

Fig. 2: The changes of testosterone (mean ± SD) in pike of Anzali wetland

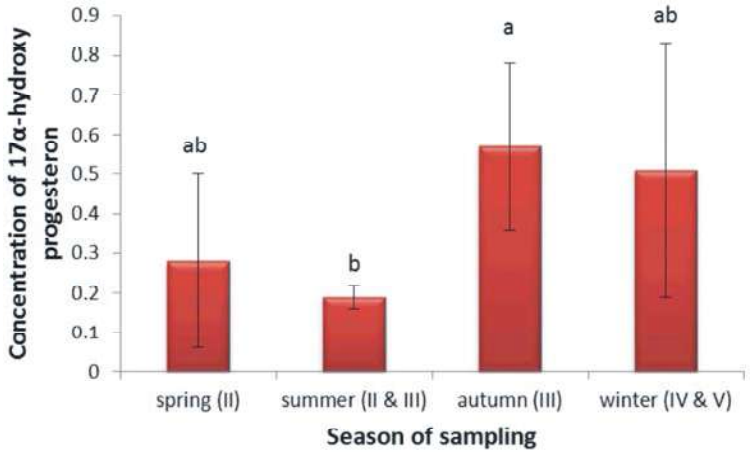

Fig. 3: The changes of 17α-hydroxy progesterone (mean ± SD) in pike of Anzali wetland

According to the results, the mean hormone concentration of 17α-hydroxy progesterone was 0.57±0.21 (minimum 0.31 and maximum 0.94) in autumn, 0.51±0.32 (minimum 0.05 and maximum 1.46) in winter, 0.28±0.22 (minimum 0.13 and maximum 0.99) in spring and 0.19±0.03 (minimum 0.16 and maximum 0.23) in summer. The highest of 17α-hydroxy progesterone was in autumn and winter and then declined and the lowest was in summer (Figure 3). There was significant relationship between 17α-hydroxy progesterone and season ($P<0.05$).

DISCUSSION

To obtain scientific findings in recent decades, endocrinology enter into the new phase with basic changes, so that endocrinology of fish has special importance and position to control the reproduction and development of the reproduction and aquaculture process now and hormonal control to be used as an efficient tool for reproduction and aquaculture [23]. Growth and maturation of eggs involves different stages in teleost fishes, which these stages are under the control of different hormones such as gonadotropin, testosterone, progesterone and estradiol [24]. During eggs development, gonadotropin hormones cause to stimulate the ovaries and production of 17β-estradiol, also testosterone is as a precursor of 17β-estradiol [25]. Studies have shown that annual fluctuations of hormones related to reproductive, feeding and growth cycles in fishes [21]. Annual rhythm of hormones closely related to with factors such as temperature, environment, species of fish, length of day and gonadal sex steroids [26]. Several factors including season, temperature, social circumstance, age, genetics [27], physiology and biochemical status of fish [28] and other variables related to reproductive capacity and opportunity.

Environmental factors also influencing developing of gonad and reproduction behavior [29]. Vitellogenesis and final maturation of egg is regulated by steroid hormones in fish [24]. A positive correlation was observed between estradiol, progesterone and testosterone with fertilization rate, relative fecundity and gonadosomatic index [30].

Fluctuations in steroid hormones during vitellogenesis and the maturation phase depend on spawning strategy. The pattern of steroid secretion in species with synchronous gamete development is typified by one or two main peaks, as seen in some salmonids and cyprinids where there is annual or bi-annual spawning and production of a single ovulatory clutch [31, 32]. Based on these results, pike has once spawning.

Estradiol stimulated vitellogenesis in teleost and changed the levels of sex hormones during gonadal maturation [33]. Based on the results, changes in levels of 17β-estradiol showed difference. Levels of 17β-estradiol are higher in autumn than other seasons and 17β-estradiol increases in vitellogenesis stage. This result coincided with other studies such as study on southern Caspian kutum (*Rutilus frisii kutum*) [20] and grouper (*Epinephelus coioides*) [21], that its peak is March for Southern Caspian kutum (*Rutilus frisii kutum*) and early of May for grouper (*Epinephelus coioides*) (before spawning). In these studies, levels of estradiol had difference during year and its peak was before spawning. These studies express the role of steroid hormones in reproductive of fish. In fishes, the amount of estradiol increased with start the vitellogenesis of eggs and it reaches to the highest of amount in the third stage of egg growth (vitellogenesis) and decreases after vitellogenesis and spawning [17]. Present study confirmed these results. In this study, hormone 17β-estradiol increased before ovulation.

According to the results, testosterone in winter was more than other seasons (the stage of sexual maturation), which coincides with after vitellogenesis. This result is in agreement with previous reports on channel catfish [16], grouper [17], perch [34], toadfish and midshipman [19] and grouper [21].

Studies on the toadfish and midshipman [19] and grouper (*Epinephelus coioides*) [21] stated the relationship between testosterone and estradiol. As testosterone increase coincident with of estradiol before spawning. While levels of estradiol reduced in the time of final maturation but levels of testosterone increased. Present study on pike, confirmed these results. Also, a peak of estradiol appeared during the periovulatory period whereas level of testosterone diminished [34], which is consistent with the results of pike. Levels of testosterone and estradiol increased in the early stages of egg maturation coincident with increase GTH-II and levels of estradiol decreased in the late stages of oocyte final maturation [18].

The results of this study indicate that highest of progesterone is in autumn and winter. Level of progesterone remained low and is relatively constant. Peak of progesterone was observed during the periovulatory period [34]. Levels of progesterone significantly increased in stage of V (0.89±0.06 ng/ml) and it can be used as one indicator for distinguishing stage of V from other stages [35].

So, pike has once spawning and gonads began to grow again in spring and granules of yolk with vacuoles seen in summer, that these observations coincident with results of study on pikeperch and pike in Tresna reservoir [36]. Vitellogenesis was complete in the middle of autumn and spawning occurs in late of winter. The highest of the estradiol was in the time of vitellogenesis and before spawning, the highest of testosterone was after vitellogenesis and the highest of 17α-hydroxy progesterone was at the time of sexual maturation and spawning.

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