Annual cycle of ovarian development and sex hormones of grey mullet *(Mugil cephalus*) in captivity

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Abstract

The grey mullet fingerling were imported to Iran in 1997 from Hong Kong and in coastal fish pond of north part of Iran were successfully cultured in order to obtain broodstocks and induce artificial reproduction. Seasonal changes in serum concentrations of Testosterone, 17-β Estradiole (E_2), 17-α Hydroxy Progesterone and also the level of calcium, Triglyceride and cholesterol in females grey mullet in captivity were measured by Radioimmunoassay or colorimetry, respectively. Investigating of sex steroids in different sexual maturity indicated that increasing of oocyte diameter and sexual maturity development serum testosterone was increased significantly ($P<0.05$). Although Biochemical parameters concentrations in blood serum of grey mullet diminished significantly until third stage of sexual maturity ($P< 0.05$), their level showed a rising trend at the fourth stage. A significant increase was observed in the serum protein and cholesterol concentrations ($P<0.05$), while, in contrast, serum Calcium ion and triglyceride levels didn't have significant differences in third and fourth stages of sexual development ($P> 0.05$). These results suggest that the seasonal changes in serum lipids and gonadal steroids were associated with reproductive development. The data also support the hypothesis that the shortening photoperiod is a major factor in stimulating reproductive activity in striped mullet.

Keywords: *Mugil cephalus*, Ovarian development, Sexual steroid hormones, Captivity

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Introduction

The grey mullet (*M. cephalus*) is a euryhaline marine teleost widely cultured in brackish and freshwater semi-intensive fishponds due to their herbivorous, fast-growing, and disease resistant. It is considered an efficient bioremediator in aquaculture because of its benthic feeding behavior (Lupatsch *et al*., 2003), and it is often used in polyculture systems to improve sediment quality (Sarig, 1981). The abundance of grey mullets in estuarine and coastal areas of all tropical and sub-tropical regions of the world may be related to their food and feeding habits, as they occupy a relatively low position in the food web (Wright, 1988). However, under captive conditions, mullet brooders do not spawn spontaneously (Lee and Tamaru, 1988; Zohar and Mylonas, 2001; Aizen *et al.*, 2005). Almost all fish reared in captivity exhibit some forms of reproductive dysfunction (Zohar and Mylonas, 2001).

Increasing the demands for the artificial propagation of the fish in captivity became a crucial issue to meet the shortage in seed supply of many cultured species. Hence, great efforts have been directed for the artificial induction of the fish in captivity out of the spawning season. However till now, fingerling supplies for aquaculture have come almost exclusively from the wild. Broodstock management out of the breeding season had a primary concern for any fish hatchery planning to maximize the hatchery fish production. The management should govern two important constraints; the captivity and the control of the timing of reproduction (El-Greisy and Shaheen, 2007).

The grey mullet fingerling were imported to Iran in 1997 from Hong Kong and in coastal fish pond of north part of Iran were successfully cultured in order to obtain brood stocks and induce artificial reproduction. In Iran, cultured mullet males have found to mature and regress earlier than females. Females usually complete vitellogenesis when kept in captivity, but does not undergo final maturation and spawning (Yousefian *et al.*, 2009).

The objective of the present study is to determine the level of sex steroid hormones consists of Testosterone, 17-β Estradiole (E_2), 17-α Hydroxy Progesterone and also the level of calcium, Triglyceride and cholesterol in females grey mullet (*M. cephalus*) during the different seasons and sexual developmental stages in captivity.

Material and methods

Grey mullet specimens used in his study were collected from Gomishan Fish Farm station in south part of the Caspian Sea of Iran. 61 females were collected during a 10 month period from September 2008 to June 2009, and then all samples were weighted, total length, fork length, standard length measured and registered. Blood samples were collected from caudal vessel using an sterile 5 ml syringe and were placed in Eppendorf tubes kept on ice for clotting. After centrifugation (2500 g, 15 min) serum was frozen and stored at -20 ˚C for further analysis. Steroid hormone content was measured by

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Radioimmunoassay (RIA) method. DPC (made in USA), RADIM (made in Italy) and Biosource (made in Belgium) Kits were used to measure the level of testosterone, 17β-estradiol (E_2) and 17α-Hydroxy progesterone hormones, respectively. LKB (made in Finland) Gamma- count was also used for the quantitative essay of these hormones in serum. The levels of Calcium ion, triglyceride and cholesterol in blood serum were determined by Enzymatic Calorimetric method.

In order to determine the sexual maturity of mullet females in captivity, apical, central and caudal portions of the ovaries were analyzed and preserved in Phosphate buffer solution, monthly. Small pieces of gonads were taken from preserved samples. These pieces were dehydrated by a graded ethanol series, embedded in paraffin wax, cut into 5 μm sections, and then stained with haematoxylin-eosin.

All data were subjected to one-way ANOVA to determine the sex steroid hormones and biochemical factors of blood serum in the different stages of sexual maturity using statistical software of SPSS version 11.0. Duncan's multiple range tests was used to estimate the differences among means at 5% level of significance (Duncan, 1995).

Results

During the study period, the average of water temperature ranged between 28.7 ˚C in July and 5.0 ˚C in December (Fig. 1).

Figure 1: Thermal fluctuations in Gomishan during 2008 to 2009

Histological observations

According to the histological parameters, 5 main stages of oocyte development described as below:

The first step of the oocyte development is named as "primary oocyte stage" or chromatin nucleolus stage. At the beginning phase of this stage, relatively small oocytes were mostly spherical in shape. Oocytes diameter in this stage was 20.17 ± 6.37 μm. As oocytes were enlarged, the nucleoli could be easily seen to form a line oriented peripherally and chromatin materials relevant to the nucleoli were clearly visible in both of the small and relatively large oocytes.

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Cytoplasm was basophile and hematoxilin was shown with dark blue (Fig. 2A). “perinuleolus stage” is the second step of the oocyte development. While the diameter of the oocytes were increased (87.83 ± 9.14 µm).The extent of nucleus and the numbers of nucleoli were increased, Nuclei were irregular in shape, chromatin materials could be seen at the central zone of nucleus and Most of the nucleoli were attached to inner border of nuclear membrane (fig. 2B). The end of the stage was characterized by the migration of the nucleus to the animal pole, just beneath the oocyte surface and follicular layers were thickened and the most prominent one was zona radiata. In the third step which is named as “yolk vesicle stage”, the oocytes were more enlarged and their means reached to 70-200 µm. Yolk vesicles appeared in the border of the oocytes and lipid vesicles were accumulated around the nucleus (fig. 2C). Spawning following the rupture of the layers would occur at the end of the stage. Next step which is named as “yolk globule stage“, the means of oocyte diameter was 489.92±9.016 µm. At the first of this stage yolk globules appeared gradually and the end of stage Oovoplasm was completely filled with yolk globules (fig. 2D).

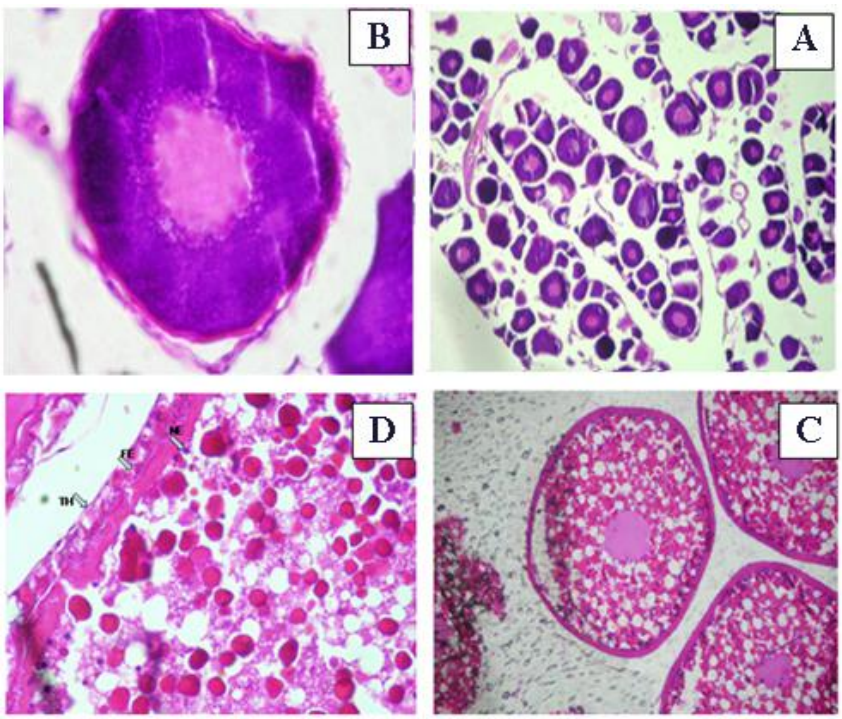

Figure2: Histological observations of oocytes and determination of development stages. A) primary oocyte stage” or chromatin nucleolus stage, B) perinuleolus stage (second step), C) yolk vesicle stage, D) yolk globule stage.

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Because of the continuous accumulation of yolk sac, the area occupied by ooplasm was very limited in the last step of the oocyte development which is named as "maturation stage". The oocytes were more enlarged and reached at their maximal size (approximately 930 μm). Oocyte layers were folded irregularly. Spawning following the rupture of the layers would occur at the end of the stage.

Changes in steroid hormone level

Changes in serum levels of testosterone, Estradiol and Progestrone in females are shown in Figs 3 and 4. The maximum serum testosterone level in the fish during the different month was found in summer. A marked increase in the level of testosterone occurred in April through September. Since sampling was not performed in October, its level was extremely decreased between November and February in which the level of mentioned hormone was approximately zero (Fig. 3). Investigating of changes in Estradiol hormone level in females showed that this hormone was considerably increased in spring and its means reached to 3.1 ng/ml in June, also serum Estradiol level in some samples was more than 6ng/ml. In spite of insignificant estradiol changes in summer, a significant increase was noted in October and averaged 3.6ng/ml in November. Besides, its level was decreasing within colder weather and also water temperature in which its means was declined in to 1.8ng/ml (Fig. 4).There were not observed any high fluctuations in serum Progesterone level until October, followed by a rapid increase during October and November that in which reached to the its peak value with 0.42ng/ml then declined its minimum (approximately zero) in February (Fig. 4). Investigating of sex steroids in different sexual maturity indicated that increasing of oocyte diameter and sexual maturity development serum testosterone was increased significantly ($P<0.05$). Although there were not any significant differences in Serum estradiol in second and third stages of sexual development ($P>0.05$), its level was increased in the fourth stage, considerably ($P<0.05$). Serum progesterone was decreased in the third stage whereas a subsequent six fold rise was observed in the fourth stage ($P< 0.05$) (Table 1). Although Biochemical parameters concentrations in blood serum of grey mullet diminished significantly until third stage of sexual maturity ($P< 0.05$), their level showed a rising trend at the fourth stage. A significant increase was observed in the serum protein and cholesterol concentrations ($P<0.05$) (Figs. 5 and 6), while, in contrast, serum Calcium ion and triglyceride levels didn't have significant differences in third and fourth stages of sexual development ($P> 0.05$) (Figs. 5 and 7).

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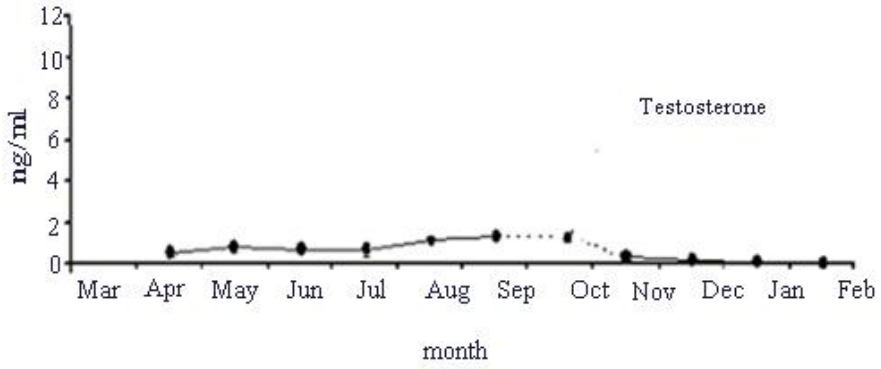

Figure 3: Changes in testosterone hormone level in female grey mullet *(M. cephalous*) in different months

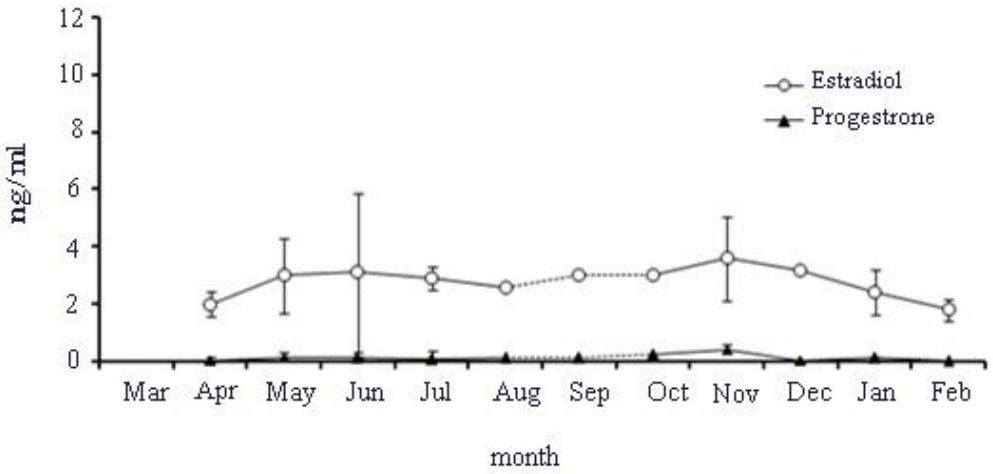

Figure 4: Changes in Estradiol and Progesterone levels in female grey mullet *(M. cephalous*) in different months

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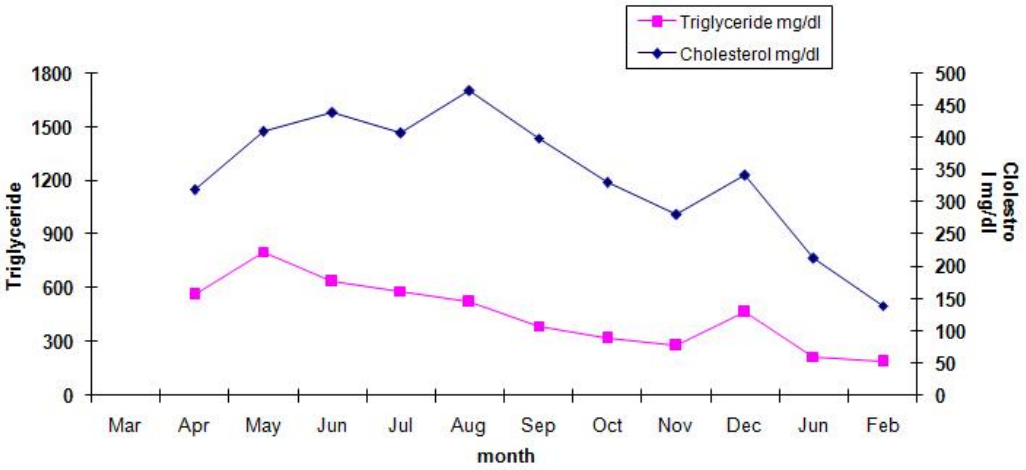

Figure 5: Changes in Triglyceride and cholestrol levels in female grey mullet *(M. cephalous*) in different months

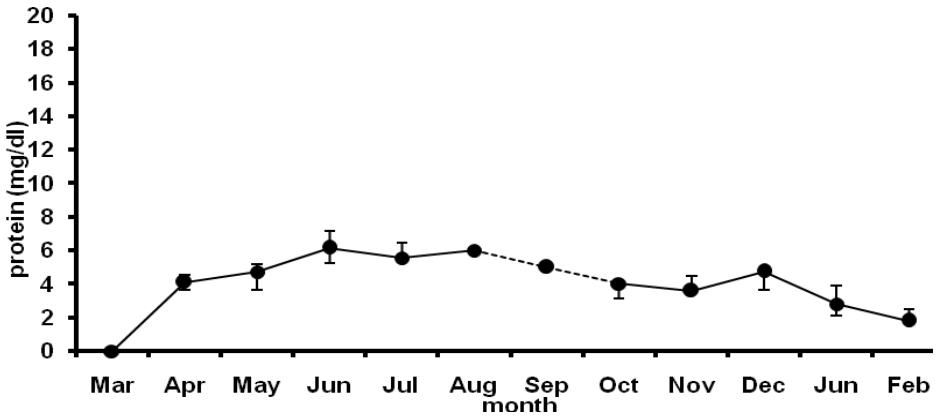

Figure 6: Changes in protein level in female grey mullet (M. cephalous) in different months

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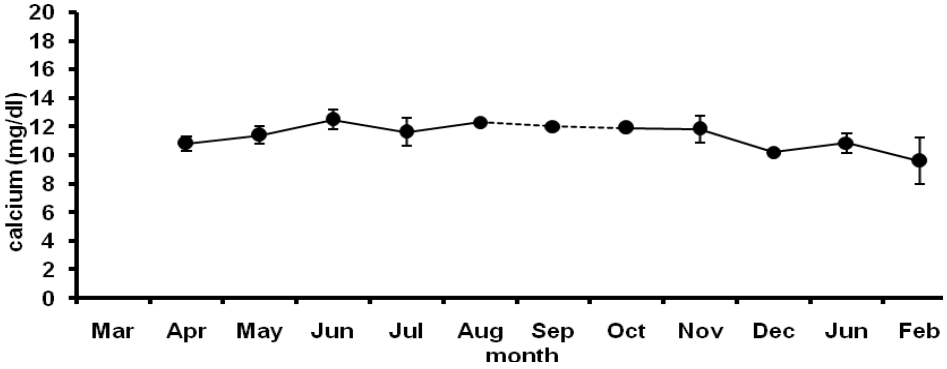

Figure 7: Changes in calcium level in female grey mullet *(M. cephalous*) in different months

Table1: Steroid hormones level and blood serum parameters in female grey mullet (*M. cephalous*) during the sexual development stages.

	Stage 2	Stage 3	Stage 4
Oocyte diameter (µm)	132^{c}	566^{b}	720^{a}
Gonado Somatic Index (GSI)	0.07^{c}	0.10^{b}	0.17^{a}
Testosterone (ng/ml)	0.011^{c}	0.070^{b}	0.370^{a}
Estradiol(ng/ml)	2.60^{b}	2.260^{b}	4.20^{a}
Progestrone(ng/ml)	0.11^{b}	0.04^{c}	0.66^{a}
Cholestrol (mg/dl)	743.0^{a}	210.60^{c}	314.20^{b}
Ttriglyceride(mg/dl)	522^{a}	254^{b}	301^{b}
Protein(mg/dl)	6.00^{a}	2.80^{c}	3.99^{b}
Calcium (mg/dl)	12.30^{a}	10.60^{b}	11.39^{b}

Discussion

Studies of stripped mullet and other fall spawning fishes indicate that shortening day length is the key stimulus of annual reproductive development and migration (Kuo *et al*. 1974; McQuarrie *et al.* 1978; Whitehead *et al.* 1978). Kuto *et al.* (1974) reported that a short photoperiod (6 h light: 18 h dark) stimulated ovarian development in striped mullet, while temperature regulated the rate of vetilogenesis. Our

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observations were the same and consistent with this finding. The start of rapid gonadal development and migration coincides with decreasing natural photoperiod (to less than 12 h) and with a drop in water temperature (to ≤20 °C).The rapid increase in serum gonadal steroids in mullets is highly correlated with the increase in gonadosomatic index. Also, water temperature fluctuations regulate the vetilogenesis.

In this study, with dropping the water temperature to ≤20 °C at the end of November, oocyte diameter raised up to 500 μm. This function was occurred during short period which is consistent with mentioned studies.

Jeffry *et al.* (1995) assessed reproductive development of striped mullet *M. cephulus* from the coastal waters of Louisiana. Maturation of primary oocytes began in September of each year, and by October, a synchronous group of homogeneous, vitellogenic oocytes was observed (isochronal development). Based on indirect evidence, spawning occurred from November through early January. Our observations confirmed oocyte development of striped mullet *M. cephulus* in their investigations. Seasonal variations in gonadal growth, serum gonadal steroids and their correlations with sexual development stages described our results. We found that a rapid rise in oocyte diameter associated with shortening of light hours, furthermore, gonadal steroids levels such as testosterone and estrogens were increased during autumn. It should be considerate that the peak value of gonadal steroids resulted in this study were lower than the other studies though hormone fluctuations were similar to them. It is supposed that the observed changes in steroids levels were caused by sever drop in water temperature and glacial appearance in the ponds containing the mullets resulted which considered as a stress factor in aquatic environment. The weather temperature averaged 5 °C in December while it reached to below zero during nights. The effects of stress on reproductive steroids and the developing oocytes were investigated in female wild snapper *Pagrus auratus* in captivity by Cleary *et al.*, (2000). Stress resulted in an increase in plasma cortisol and concomitant decreases in 17β-estradiol and testosterone. In other study, Seasonal changes in serum concentrations of the testosterone in males, 17b-estradiol in females and triglyceride and cholesterol in both sexes of *Capoeta capoeta umbla* were determined. Results suggest that seasonal changes in both serum lipids and steroid hormones were associated with reproductive activity in *C. c. umbla* (Erdogan *et al.*, 2002). Assessment of annually Specific growth rate (%), average daily food intake (%) and gross conversion efficiency in a population of European sea bass (*Dicentrarchus labrax L.*) showed relatively higher values in the warm spring and summer months. The specific growth rate correlated better with the seawater temperature and the average daily food intake than with the photoperiod. The concentrations of plasma total lipids, triglycerides and glucose were higher in summer. Total plasma cholesterol concentrations were higher during the pregametogenesis and gametogenesis periods. Plasma triglycerides correlated better with photoperiod and plasma glucose than with the seawater temperature

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(Kavadias *et al*, 2003). The rapid elevation in serum estrogen in stripped mullet appears to cause rapid ovarian follicular growth and may result from photoperiodically controlled gonadal stimulation. Seasonal variations of serum total lipids and cholesterol (Fig. 5) suggest a period of rapid deposition of lipids during the summer and mobilization during the fall migration. Broadhead (1956) and Anderson (1957) noted heavy premigratory feeding in striped mullet. Striped mullet, like salmon, do not feed during migration (De Silva and Perera 1976); thus they require the accumulation of large fat stores prior to migration and spawning (Hoar 1960; Woodhead 1975). Perera and De Silva (1987) suggested that striped mullet don't store carbohydrates for energy utilization, thus we must consider this point to determine the dietary feeding. Measurement of cholesterol and triglyceride concentrations in blood serum of *M. cophalus* confirmed this matter. Seasonal changes in serum lipids and gonadal steroids were associated reproductive development. The data also support the hypothesis that the shortening photoperiod is a major factor in stimulating reproductive activity in striped mullet (Dindo and Mac Greogor, 1981) with the same finding in this investigation. The results from this study showed that the mullet rise in to the fourth stage of oocyte development although the stress condition. The level of cholesterol and triglyceride were high at the second stage while their level decreased rapidly at the third stage with reproductive development. This is because of cholesterol and triglyceride consumption during maturity and gonadal development especially in November and December. This process is coincides with the natural conditions. It is probable that because of thermal stress gonadal development has been improved until the beginning of fourth step then it has been stopped with confront of stress, therefore, fish starts normal feeding. More studies are needed to confirm these recent mentioned result.

Acknowledgments

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چرخه سالیانه تکامل تخمدان و هورمون‌های جنسی ماهی کفال خاکستری (*Mugil cephalus*) در شرایط اسارت

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چکیده

ماهیان انگشت قد کفال خاکستری در سال ۱۹۹۷ از کشور هنگ‌کنگ به ایران وارد شد. به منظور استحصال مولدین و انجام تکثیر مصنوعی این ماهیان به طور موفقیت امیزی در استخرهای خاکی پرورش داده شدند. تغییرات فصلی در میزان تستوسترون، استرادایول، ۱۷ آلفا هیدروکسی پروژسترون سرم و همچنین سطوح کلسیم، کلسترول و تری گلیسرید سرم در ماهیان کفال خاکستری به ترتیب با روش رادیوایمونواسی (RIA) و رنگ سنجی آنزیمی اندازه گیری شد. بررسی استروئیدهای جنسی در مراحل مختلف رسیدگی جنسی افزایش قطر تخمک را نشان داد و همچنین میزان تستوسترون سرم در مراحل مختلف جنسی نیز به طور معنی داری افزایش یافت($P<0.05$). با وجود اینکه غلظت پارامترهای بیوشیمیایی در سرم خون کفال ماهیان تا مرحله سوم رسیدگی جنسی پایین بود($P< 0.05$)، سطح آنها در مرحله چهارم رسیدگی جنسی روند افزایشی داشت. افزایش معنی داری در میزان پروتئین و کلسترول مشاهده شد ($P<0.05$) در حالی که به عکس، سطوح کلسیم و تری گلیسرید در مراحل سوم و چهارم رسیدگی جنسی تفاوت معنی داری نشان ندادند($P> 0.05$). نتایج پیشنهاد می کند که تغییرات فصلی در لیپیدهای سرم استروئیدهای تخمدان با مراحل رسیدگی جنسی مرتبطند. این تحقیق، فرضیه کوتاه شدن طول روز عامل اصلی در تحریک فعالیت تولید مثلی ماهی کفال خاکستری است را تایید می کند.

واژگان کلیدی: ماهی کفال خاکستری، تکامل گنادی، هورمون استروئیدی جنسی، اسارت

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