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HORMONAL CHANGES ACCOMPANYING SEXUAL MATURATION IN *DIPLODUS NOCT*, A TELEOST FROM THE SUEZ BAY, RED SEA

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ABSTRACT

The changes in plasma levels of 17β-estradiol, testosterone and gonadotropin hormones of *Diplodus noct* were correlated with gonadal development. In male, estradiol-17β hormone (E_2) was slightly high during the spent stage (12.2 ± 3.58 pg/ml), but there was no significant difference between maturity stages. In female (E_2) has its highest value during the final maturation stage (26.7 ± 5.3 pg/ml), and there was a significant difference in (E_2) concentration between different maturity stages. The male plasma testosterone hormone (T) has its highest level during ripe stage (339 ± 22 pg/ml) and there is a significant difference in its concentration between different maturity stages. In female, (T) concentration was relatively high during final maturation stage (438 ± 102 pg/ml) and no significant difference was detected between different maturity stages. In general, plasma (T) levels in females were higher than males. The male plasma gonadotropin hormone (GtH) reached highest value in mature stage (157.29 ± 31.8 pg/ml) and there was highly significant differences between (GtH) levels in different maturity stages. In female, (GtH) reached highest value during previtellogenesis stage (80.50 ± 12.37 pg/ml) and there was no significant difference with maturity stages.

INTRODUCTION

Family Sparidae is one of the most important families in the Red Sea. Sparidae (seabream) inhabit tropical and temperate coastal waters. In the Red Sea, 11 species have been recorded, namely: *Acanthopagrus berda, Acanthopagrus bifasciatus, Argyrops spinifer, Argyrops filamentosus, Cheimerius nufar, Crenidens crenidens, Diplodus noct, Lithognathus mormyrus, Polysteganus coeruleopunctatus, Rhabdosargus sarba* and *Rhabdosargus haffara*. This family is an important copmponent of the small-scale fisheries (gil net, trammel net and hand line) in the Northern Gulf of Suez (Suez Bay). The main annual total catch during the last ten years was about 300 ton of sparids constitute a mean of about 16.2 % (Mehanna, 2001).*Diplodus noct* is apparently endemic to the Red Sea. This species is very common, especially above sandy bottoms, around coral reefs and in shallow coastal waters. The youngs may form aggregations. They feed on algae and small invertebrates (Fischer & Bianchi, 1984).

Changes in plasma hormone levels that accompany the reproductive process have been investigated in order to elucidate the hormonal control of reproduction in teleost. Most of these studies have focused on the hormone change that occur during annual reproductive cycles (Yeung and Chan 1987, Prat *et al*. 1990, Kime *et al*., 1991, Malison *et*

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al., 1992, Assem 1992 &1995, Carragher and Pankhurst 1993, EL-Gharabawy 1994, EL-Boray 1993 & 1997, Abdo 1996, Hobby and Pankhurst 1997, Haddy and Pankhurst 1998 and Zaki *et al.,* 2001 & 2004). Others studied daily changes in reproductive hormones levels at spawning period (Zohar *et al*., 1988; Matsuyama *et al*., 1990). Also the effect of sex hormones in oocyte maturation were studied by (Venling & pankhurst 1995 and Condeca & Canario 1999).

The purpose of the present investigation was to follow seasonal changes of 17β-estradiol, testosterone and gonadotropin hormones concentrations in plasma of female and male *Diplodus noct* in relation to maturity stages of gonads. The study represents an important subject in applied research particularly in artificially induced spawning.

MATERIALS AND METHODS

A - Collection of fish samples

Monthly samples of living *Diplodus noct* were collected from Suez Bay. Small living fishes were captured by baited hooks and line, while large sized fish were captured by gill net. Species ranging in size from 8.3 to 23.3cm total length and varied from 13.27 to 181.61 gm in total weight were collected during the period from August 1998 to July 1999. Maturity stages identification was carried out through histological techniques. Small pieces of gonads 2 mm thick were fixed in Bouin fluid. After fixation, the specimens were dehydrated in ascending series of ethyl alcohol. Then they were cleared by methyl benzoate over night thereafter placed in Xylene. After clearing, the process of embedding was performed in paraffin wax (m.p: 58-65). After embedding, the specimens were supported by paraffin as a block and then transversely cut by a microtome at thickness of 5 - 6 µm. Sections were stained with Ehrlich haematoxylin and eosin. According to Zaki *et al*. (1985) maturity stages of testis were classified into five stages namely (immature, mature, ripe, spawning and spent). While in ovary they are classified into four stages according to Biesiot *et al.* (1994) namely (previtellogenesis, vitellogenesis, final maturation and postovulation).

B - Blood sampling

The blood was obtained from caudal artery. It was drawn immediately into heparinized vials and centrifuged at 3000 rpm for 10 minutes. Supernatant plasma was pipetted in vials and kept frozen at -20^{o}C for further investigation.

C- Radioimmunoassay (RIA)

Estradiol-17β, testosterone and gonadotropin hormones in the plasma of female and male D. noct were determined by using Orion Diagnostic Kit with gamma counter, Model 600 B Gamma Tec II.

D - Statistical analysis

The computations were used in the analysis of the data obtained in this study were arithmetic mean, standard deviation, standard error and one-way analysis of variance (One-Way ANOVA) using STATISTICA program version 5.

RESULTS

1- Microscopic structure of maturity stages of gonads

A- Male gonad:-

Stage I. Immature (Fig. 1)

In cross section immature testes appears to be composed mainly of spermatogonia which are large cells and grouped in nests. The spermatogonia stain faintly and their diameter ranges from 7 to 12µ. Each spermatogonium has a large nucleus having a diameter ranging from 5 to 8µ. In most spermatogonia a single or rarely two darkly stained nucleoli exist within the nucleus.

Stage II. Mature (Fig. 2)

Sections of the mature testes exhibit incipient active spermatogenesis. The nests of spermatogonia, primary and secondary spermatocytes can be distinguished. Mitotic division of the spermatogonia produces thwe primary spermatocytes. They are smaller than spermatogonia and have a diameter about 8

μ. They are slightly polygonal in shape with a pale cytoplasm. These can be readily distinguished from spermatogonia where the chromatin materials are concentrated at one pole of the nucleus which accordingly acquire a reticulate appearance. This nucleus has about 6 μ in diameter. Secondary spermatocytes nucleus are 3 to 5 μ in diameter. The chromatin in the nucleus is very dense. The secondary spermatocytes exist in large nests extending to the lobule lumen. Few nests of spermatids and spermatozoa can also be detected, where the parachute shape of sperms can be distinguished. The central zone of the testes particularly near the spermatic duct and the vasa efferentia display more active spermatogenesis than the peripheral zone.

Stage III. Ripe (Fig. 3)

Ripe stage displays more active spermatogenesis than in the preceding stage. The different stages of spermatogenesis including spermatocytes, spermatids and spermatozoa can be distinguished throughout the entire section. Spermatid cells were smaller than secondary spermatocytes (2 μ in diameter). These cells had no distinguishable cytoplasm and characterized by dense chromatin in the nucleus which occupied most of the cell. Their nest membrane is no longer apparent although the cells remain in dense clusters. Metamorphosis of spermatids to form spermatozoa occur within the lobules lumen. The spermatozoa have a head with diameter of 1 μ. The seminiferous lobules are distended with spermatozoa and their lumina lead to the vasa efferentia and subsequently to the spermatic duct. The quantity of spermatozoa in the spermatic duct increases with the progression of testis ripeness.

Stage IV. Spawning (Fig. 4)

The spermatic duct contains a fair quantity of sperm cells and the seminiferous lobules became empty of spermatozoa. The discharge of a considerable quantity of sperms during spawning is accompanied with a decrease in the width of the base of the triangular section of the testes.

Stage V. Spent (Fig. 5)

In this stage spermatogenesis has completely stopped and the testes are somewhat thinner. The lobules are vacuolated and contain spermatogonia at the periphery of lobules.

B- Female gonad:-

Stage I. Previtellogenesis (Fig. 6)

This stage contains small oocytes spherical or oval in shape (chromatin cell). They were transparent with vacuole-like nucleoli. The previtellogenic stage comprised also three substages:

(a) Early previtellogenesis, characterised by small oocytes (have a diameter ranging from 15 to 20 μ) in wich the nucleus had a large size (have a diameter from 7 to 12 μ).

(b) Middle previtellogenesis, characterised by the nucleoli (from 5 to 11 in number arranged more or less at the periphery) developing within the nucleus and causing evaginations to form the nuclear envelop. It has a large amount of chromatin material. The oocytes were sourrounded with very thin follicular layer.

(c) Late previtellogenesis (oocytes have a diameter ranging from 43 to 119 μ & nucleus from 20 to 71 μ). The nucleoli increased in number, about 16 and ranging in diameter from 2 to 12 μ. At the beginning of this substage the cytoplasm loses its good affinity to haematoxylin. A small mass, which have been termed yolk nucleus measured about 5 μ is formed in the cytoplasm. With further growth of oocytes by the end of this stage their shape varied between spherical, tetragonal and hexagonal. The yolk nucleus disappeared. The later substage makes the beginning of the transition to stage II.

Stage II. Vitellogenesis (Fig.7)

his stage was characterized by the appearance of lipid vesicles in the cytoplasm of oocytes. The oocytes increased in size (ranged from 158 to 209 μ) as the yolk

material increased. Yolk vacuoles were formed in ooplasm in two regions, one around the nuclus and the other in the outer part of the cytoplasm. With further growth of oocytes, vacuoles around the nucleus and thart at the periphery of the cytoplasm increased in size and number. A layer of unvacuolated ooplasm separates the two regions, the nucleus became irregular in outline, it had a diameter ranging from 79 to 89 µ with projections protruding into the ooplasm. The nucleoli maintained their peripheral position in the nucleus. The zona radiata obviously appeared, it had a thickness of 9 µ. The lipid vesicles appeared and occupied the area around the nucleus. The follicular epithelium increased in thickness to 4 µ. Fine yolk granules appeared in the peripheral region of the cytoplasm; beginning of yolk deposition; the oocytes ranged in diameter from 227 to 399 µ. The formation of yolk granules proceed from outside to the middle part of the ooplasm and left yolk-free area around the nucleus. The nucleus was irregular in shape and the nucleoli lay in peripheral position (about 14, ranged in size from 3 to 12 µ). Furthermore, growth of the cytoplasm was accompanied by centripetal deposition of yolk. Yolk granules were accumulated very rapidly in the inner part of the cytoplasm and also enlarged in size. Some of the lipid vacuoles around the nucleus began to coalesce and others increased in size.

Stage III. Final maturation (Fig. 8)

The ovary in this stage contains oocytes are generally between 442 and 560 µ in diameter. These oocytes are characterized by further increase in number of yolk globules. The nucleus occupied a considerably small part of the egg; 88 µ in diameter. It was somewhat oval in shape. In this stage, the lipid vacuoles around the nucleus continue in coalesces. Yolk globules increased in diameter from 6 to 18 µ. The migration of the nucleus to the animal pole is apparent, and the nucleus amoeboid in shape and without limited boundaries. The zona radiata increased in thickness; the thickness ranged between 18 to 24 µ. The yolk globules form yolk mass and appeared as a homogeneous mass filling the oocytes. No distinct nucleus was observed. The zona radiata thickness was reduced to about 7 µ. The oocyte was no longer surrrounded by the follicular layer indicating that the eggs were ready for spawning. Some atretic oocytes are apparent.

Stage IV. Postovulation (Fig. 9)

Spent ovary in this stage is characterized by thickening of body wall of the ovary, ovigerous folds are distinct and contained perinucleolar oocytes. Plenty of atretic oocytes are observed.

2- Hormonal determination

In the present study, the changes in the concentration of two basic steroid hormones, 17-β estradiol (E_2), Testosterone (T) and gonadotropin (GtH) in the plasma of females and males of *D. noct* were studied in relation to maturity stages of gonads.

A- 17-β estradiol (E_2) in plasma of male:

Immature stage was not represented here because the blood drawnfrom small sized fishes was insufficient for (E_2) determination purpose. Table (1) shows the changes in concentration of plasma (E_2) in male, it varied between 2.3 and 17.0 Pg/mL with an average 10.3 $\pm$4.26 Pg/mL in mature stage. The (E_2) concentration showed a gradual decrease during the ripe stage, ranging from 2.7 to 20.0 Pg/mL with an average value of (7.4 $\pm$4.21 Pg/mL). In spawning stage the value of (E_2) raised to be as average value (10.9 $\pm$4.60 Pg/mL). In spent stage (E_2) showed a slight increase, ranging from 8.6 to 15.8 Pg/mL with an average value 12.2 $\pm$3.58 Pg/mL. The concentration of (E_2) in male was not significantly different ($P>0.05$) among the maturity stages.

C- Testosterone (T) in plasma of male:

Table (3) shows changes of plasma (T) in male *D. noct.* Plasma (T) of male showed a maximum average value in immature stage of 360 $\pm$ 15 Pg/mL. Thereafter, it decreased

from immature stage to mature stage, (161 ±80 Pg/mL). In ripe stage the average concentration of plasma (T) increased again to attain a high value; 339 ± 22 Pg/mL. At spawning stage the concentration of (T) rapidly decreased to reach an average value; 118 ±10 Pg/mL which undergoes a little rise to be on the average 140 ± 17 Pg/mL in spent stage. The concentration of (T) in male showed a significant difference (P<0.05) among the maturity stages.

Table (1): Concentration of plasma 17 β-estradiol (pg/ml) for male *Diplodus noct* in relation to different maturity stages.

Maturity stages	No. of fish	Minimum	Maximum	Average ± S. E.
Immature	--	--	--	--
Mature	3	2.3	17.0	10.3 ± 4.26
Ripe	4	2.7	20.0	7.4 ± 4.21
Spawning	3	6.3	15.5	10.9 ± 4.6
Spent	3	8.6	15.8	12.2 ±3.58

(P < 0.05)

Table (2): Concentration of plasma17β-estradiol (pg/ml) for female *Diplodus noct* in relation to different maturity stages.

Maturity stages	No. of fish	Minimum	Maximum	Average ± S. E.
Previtellogenesis	3	5.8	8.8	7.4 ±0.88
Vitellogenesis	3	6.0	9.8	7.9 ± 1.90
Final maturatiom	7	15.0	53.0	26.7 ± 5.30
Postovulation	7	6.0	16.5	9.8 ± 1.79

(P < 0.05)

Table (3): Concentration of plasma testosterone (pg/ml) for male *Diplodus noct* in relation to different maturity stages.

Maturity stages	No. of fish	Minimum	Maximum	Average ± S. E.
Immature	3	345	375	360 ± 15
Mature	3	44	420	161 ± 80
Ripe	15	240	460	339 ± 22
Spawning	3	92	140	118 ± 10
Spent	3	123	157	140 ± 17

(P < 0.05)

D- Testosterone (T) in plasma of female:

The data which represent the changes of plasma (T) during different maturity stages of ovaries of *D. noct* are presented in Table (4). The concentration of (T) in female was not significantly different (P>0.05) among the maturity stages. Plasma (T) in females showed a gradual increase during previtellogenesis, vitellogenesis and final maturation stages. The average values of those stages were 200 ± 50, 253 ± 30 and 438 ± 102 Pg/mL, respectively. In postovulation

stage plasma (T) concentration decreased to reach an average of about 300 ± 40 Pg/mL.

E- Gonadotropin (GtH) in plasma of male:

The plasma (GtH) hormone in male *D. noct* was represented in Table (5)**.** The average concentration showed an increase from 76.50 25.50 Pg/mL in immature to 157.29 ± 31.80 Pg/mL in mature stage. Thereafter, a progressive decrease recorded

during ripe and spawning stages, its value of 61.32 ± 2.13 and 56.00 ± 2.02 Pg/mL, respectively. While in the spent stage, the average concentration of (GtH) hormone increased to 76.30 ±12.97 Pg/mL. The concentration of (GtH) in male exhibited high significant difference (P<0.01) among the maturity stages.

F- Gonadotropin (GtH) in plasma of female:

The changes in concentration of plasma (GtH) hormone in female *D. noct* during the ovaries development are demonstrated in Table (6). Concentration of plasma (GtH) showed a decreasing trend from previtellogenesis stage; 80.50 ±12.37 Pg/mL to vitellogenesis stage, 63.40 ±6.75 Pg/mL. The plasma (GtH) increased to be 65.46 ±3.79 Pg/mL in final maturation stage, while in postovulation stage it was 66.63 ±4.97 Pg/mL. Therefore it was not significantly different (P>0.05) among the maturity stages.

Table (4): Concentration of plasma testosterone (pg/ml) for female *Diplodus noct* in relation to different maturity stages.

Maturity stages	No. of fish	Minimum	Maximum	Average ± S. E.
Previtellogenesis	7	60	320	200 ± 50
Vitellogenesis	3	210	310	253 ± 30
Final maturatiom	10	90	960	438 ± 102
Postovulation	7	50	970	300 ± 40

(P < 0.05)

Table (5): Concentration of plasma gonadotropin (GtH) (pg/ml) for male Diplodus noct in relation to different maturity stages.

Maturity stages	No. of fish	Minimum	Maximum	Average + S. E.
Immature	**3**	**51**	**102**	**76.50 + 25.50**
Mature	**9**	**51**	**285**	**157.29 + 31.80**
Ripe	**25**	**51**	**77**	**61.32 + 2.13**
Spawning	**3**	**52**	**64**	**56.00 + 2.02**
Spent	**5**	**51**	**130**	**76.30 + 12.97**

(P < 0.05)

Table (6): Concentration of plasma gonadotropin (GtH) (pg/ml) for female Diplodus noct in relation to different maturity stages.

Maturity stages	No. of fish	Minimum	Maximum	Average ± S. E.
Previtellogenesis	15	49	170	80.50 ± 12.37
Vitellogenesis	3	56	90	63.40 ± 6.75
Final maturatiom	14	52	91	65.46 ± 3.79
Postovulation	12	50	110	66.63 ± 4.97

(P < 0.05)

DISCUSSION

The informations obtained from the study of hormonal secretion during the natural spawning process represent an important subject in applied research particularly in artificially induced spawning. Delineated hormonal changes that are accompany spawning provide fish culturist with required hormone levels to simulate the levels at which it may intervene.

Male *D. noct* has a moderate level of (E_2) in mature stage which decreased in ripe stage, but the high levels of (E_2) were observed during spawning and spent stages. The later results are compatible with (Abdo, 1996) for *Dicentrarchus labrax* and (EL-Boray, 1997) for *Rhabdosargus haffara.* Also, Rinchard *et al*. (1993), in *Gobio gobio,* found that all steroids during the post-spawning were high levels. In contrast, Assem (1995), in *Solea vulgaris* and *Solea aegyptiaca,* found that plasma (E_2) reached a maximum value in the pre-spawning period, then decreased throughout the spawning season to reach minimum value in the spent male.

The plasma 17-β estradiol (E_2) levels of female *D. noct* during the reproductive cycle, in previtellogenesis and vitellogenesis stages are low. This is in agreement with findings in other teleosts (Pankhurst and Conroy, 1987; Berlinsky and Specker, 1991). The highest level of (E_2) was observed during the final maturation stage. Similar patterns have also been observed in multiple spawning fishes as *Carassius auratus* (Kagawa *et al*., 1983), *Sparus aurata* (Kadmon *et al*., 1985), *Dicentrarchus labrax* (Prat *et al*., 1990) and *Diplodus sargus* (Zaki *et al.,* 2004). These teleosts have an asynchronous ovarian development and after ovulation, their ovaries contain both protoplasmic oocytes and oocytes at various stages of vitellogenesis. These remaining vitellogenic oocytes were able to produce this steroid after ovulation (Kagawa *et al*., 1984). The later interpretation explain the drop in (E_2) level in postovulation stage but not lesser than the (E_2) level in the early maturity stages for *D. noct.*

In the present study, the changes in the concentration of plasma testosterone in male *D. noct* shows a positive correlation with the development of the testes (from mature to ripe stage) which decrease sharply before spawning stage, then attained higher level in spent stage. Fostier *et al.* (1982) showed that in male teleosts, growth and development of the testes is associated with rising plasma levels of testosterone and 11-Ketotestosterone. Also, Marte and Lam (1992) observed that testosterone levels were positively correlated with GSI in male milkfish. Serum testosterone levels increased as spermatogenesis progressed with peak levels obtained in spermiating males. Also, they suggested that testosterone play a role in the initiation of spermiation in addition to the recruitment of succeeding waves of spermatocytes during the breeding season. The results of plasma (T) of male *D. noct* in disagreement with findings of Assem (1992) in *Oblada melanura* and Assem (1995) in *Solea vulgaris* and *S. aegyptiaca* where she reported that plasma testosterone content was at its maximum value in both sexes during spermiation or ovulation period.

In the present study, the plasma testosterone (T) concentration of female *D. noct* increased with the early maturation of the ovary and reached to the highest level in final maturation stage, then returned to decrease in adequate level during postovulation stage. This observation is in agreement with what has been observed for female *Diplodus sargus* (Zaki *et al.,* 2004). The highest level of (T) in final maturation is consistent with the role of (T) as a precursor for (E_2) synthesis as reported by Hobby and Pankhurst (1997). The last result came in accordance with Haddy and Pankhurst (1998) who recorded that plasma (T) concentration of black bream, *Acanthopagrus butcheri* females were elevated at midday in association with final oocyte maturation.

Fostier *et al*. (1983) suggested that high concentrations of (T) might have vitellogenic

actions on the liver. Plasma (T) levels increase at the end of vitellogenesis. This acute rise in (T) indicates that oocytes are fully mature in the ovary and ready to ovulate (Kobayashi *et al*., 1987). However, Nagler and Idler (1992) suggested a role for (T) during the final maturation-ovulation process in winter flounder, as highest levels of (T) occurred during the pre-spawning and spawning periods. After ovulation, high levels are maintained since the ovary contains oocytes at different stages of maturity.

Kobayashi *et al*. (1987) in gold fish showed that after ovulation testosterone levels decreased. This may be due to the degeneration of postovulatory follicles which had mainly produced testosterone and 17alpha, 20β-di oH-P. The rising of testosterone with a commencement of the gonadotropin (GtH) surge seems to be produced by the mature follicles of the oocytes.

Although there is a little information about the possible role of testosterone in female reproduction, many authors proposed several roles for it. Scott *et al*. (1980) proposed the possibility that testosterone may involve in the maintenance of social behavior. Grim *et al*. (1981) reported that it can have direct vitellogenic action or indirectly as a substrate for aromatization to estrogen. It may be important in regulating (GtH) secretion through a positive feed back mechanism. Young *et al*. (1982) suggested that testosterone in the plasma of female teleosts may represent a substrate pool for estradiol-17β production. While Mart and Lam (1992) in milkfish, *Chanos chanos,* observed that testosterone levels in female were considerably higher than 17β-estradiol at all stages and are about ten-fold higher in mature fish, this is in accordance with the present finding.

In the present study, most of plasma levels of (T) in *D. noct* are higher in females than in males. This agrees with Prat *et al*. (1990) in Sea bass and Nakamura *et al*. (1994) in anemone fish. It has been stated that androgens are the precursors of estrogens and the former is released into the plasma when no longer needed for aromatization (Campbell *et al*., 1976).

On the other hand, male of *D. noct* recorded a high level of plasma (GtH) in mature and prior to spawning stages. These results are in conformity with the observation of Kobayashi *et al*. (1986) who stated that plasma (GtH) levels in males showed a marked increase that was synchronous with the preovulatory surge in females. Low levels of male (GtH) throughout the breeding period except for a very short period around spawning are in agreement with Resink *et al*. (1987a) and Van Oordt *et al*. (1987). This (GtH) surge which is most likely required for spermiation and for oocyte maturation and ovulation (Donaldson and Hunter, 1983; and Zaki *et al.,* 2001). In the present study, plasma gonadotropin (GtH) concentration of female *D. noct* shows a little increase with the maturity stages from vitellogenesis stage to final maturation stage.

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