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Histological Study on Reproductive Pattern and Sex Reversal of Dusky Grouper *Epinephelus guaza* in Natural Environment of Antalya Bay of Mediterranean in Turkey

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Abstract

In this study, gonadal changes of dusky grouper *Epinephelus guaza* L.1758 were investigated by histo-anatomically according to sexual maturity in Antalya Bay of Eastern Mediterranean in Turkey. The specimens (*n=104*) were captured monthly by spear-fishing from their natural sites. The gonads of them were dissected as soon as and fixed in 10 % neutral buffered formaldehyde and Bouin's solution. They were embedded in paraffin blocks after several histo-chemical processes. Sectioned tissue samples at 5µm were stained by Haematoxylin-eosin and Mallory trichrome techniques. Mainly four different stages which were immature stage, mature female stages (from F1 to F6), transitional stages (from T1 to T3), and mature male stages (from M1 to M3) were observed according to gonadal development. However GSI (G**onadosomatik indeks)** is the most common used to quantify spawning time in fish; the RGI (Relative gonadal index) was found more appropriate than GSI for the fish. Dusky groupers spawned in June and September in Antalya Bay.

Keywords: Dusky grouper, *Epinephelus guaza*, Reproductive pattern, Protogynous hermaphroditism, Sex reversal.

Akdeniz'in Antalya Körfezindeki Orfoz Balıklarında, *Epinephelus guaza* (Türkiye) Seks Dönüşümü ve Üreme Özellikleri Üzerine Histolojik Çalışma

Özet

Bu çalışmada Doğu Akdeniz'in Antalya Körfezindeki orfoz balıklarının *Epinephelus guaza* L.1758 cinsel olgunluklarına göre gonad değişimleri histo-anatomik olarak araştırılmıştır. Örnekler (n=104) doğal ortamlarında zıpkın ile aylık olarak yakalanmışlardır. Balıkların gonadları hemen kesilerek % 10'luk nötral formaldehit ve Bouin'in solüsyonlarında tespit edilmiştir. Birçok histo-kimyasal işlem aşamalarından geçirildikten sonra alınan dokular kesit alınmak üzere parafin içine gömülmüştür. 5 µm kalınlığında kesitleri alınan doku örnekleri Haematoxylin-eosin ve Mallory trichrome boyama tekniklerine göre boyanmışlardır. Yapılan incelemelerde balıkların gonad gelişimlerimlerinde: olgunlaşmamış gonad evresi, olgunlaşan dişi evreleri (**F1** den **F6 'ya kadar**), dişilikten erkekliğe geçiş evreleri (**T1** den **T3'e kadar**) ve olgun erkek evreleri **(M1'** den **M3'e kadar**) başlıca dört farklı evre gözlenmiştir. Yaygın olarak kullanılan yüzde gonado somatik indeks **(% GSI)** verilerinin araştırmada tespit edilmesine rağmen; relatif gonad indeks bulguları (% **RGI)** bu gibi hermafrodit balıkların üreme dönemlerinin saptanmalarında daha uygun olduğu görülmüştür. Buna göre Antalya körfezinde yaşayan orfoz balıklarının *Epinephelus guaza* L.1758 Haziran ve Eylül ayları arasında yumurtların bıraktıkları tespit edilmiştir.

Anahtar Kelimeler: Orfoz balıkları, *Epinephelus guaza*, Üreme, Protogynous hermafroditizm, Seks dönüşümü.

Introduction

Groupers (*Epinephelus guaza*, L.1758) (Serranidae, sub-family Epinephelidae) more widely known by its synonym, *Epinephelus marginatus* (Lowe) is popular and recreationally important in the Mediterranean Sea (Marino *et al*. 2001). Because of large body, fighting capability and behaviour, this species inhabits shelter-rich, hard substrata on the continental shelf, reaching maximum densities beyond 50 m depth (Reñones *et al*. 2007). They exhibit remarkable diversity of reproductive patterns. Fish migrate to considerable distances and form spawning aggregations at specific sites. Most of spawning activity happens within these aggregations in the evening (Erismann *et al*. 2007). Researching data and gonad histology confirmed that they are protogynous monandric hermaphrodite pathway to

sexual development (Marino *et al*. 2001). Body length of the fish in the first sexual maturity (F3) were measured 39.1cm [Ls] in female and 83.5cm [Ls] in male and body weight in the first sexual maturity (F3) was 1692.5g in female, and 9943.3g in male (M2). Adult sex ratio during the reproductive period was determined 2:1 female to male. It was accounted 19.2% for females and 18.2% males while transitional individual was 4.8% part of the whole adult population. From the results of the present study, it can be concluded that propagation of the dusky grouper (*E. guaza*) has annual cycle and monandric protogynous hermaphroditic characteristics. Reproductive season occur at 20°C in summer time (from June to August) in Eastern Mediterranean of Antalya Bay in Turkey (Özen and Balcı, 2011). They can change sex from female- to- male after exceeding a certain age and body size (Zhou and Gui 2008). The high market prize and low standing stock of dusky grouper caused a prime species for mari-culture and good candidate cultured species (Zabala *et al.* 1997, Glamuzina *et al*. 1998a,b, Marino *et al*. 2001).

Materials and Methods

Sampling Fish and Procedures

Specimens (*n=104*) were collected monthly from July 2001 to April 2003 in Antalya Bay of Eastern Mediterranean in Turkey. Most of fish were caught by professional hook and line in shore waters, at 20-75 m depth and few ones were collected by spearfishing in shallow waters of shore. After sex determination, the fish were weighted and measured and the gonads were removed and weighed.

Histological Examination by Light Microscopy

Tissues sample either, within the abdominal cavity or removed from fishes immediately after sacrifice, were preserved in neutral buffered formalin (10%) and Bouin's solutions and were processed according to routine techniques for microscopy. The fixed samples gradually dehydrated and embedded in paraffin. Sections, 5µm thick and were stained with Harris' haematoxylin- eosin and Mallory's Triple stain (Luna, 1982; Bancroft *et al*. 2007).

All slides was examined and photographed with a Nikon Mikrophot for determination of reproductive states. Periodicity of reproduction was examined by monthly according to relative gonadal index (RGI) and gonadosomatic index (GSI) formulations (Erickson *et al*. 1985; Marino, *et al*. 2001; Avşar, 2005). Age estimation were done by reading opaque and dark band of the scale and operculum of the fish under stereomicroscope. The opaque band were means of summer growing while dark ones were winter's, and both of them was one ages (Avşar, 2005, Türkmen vd. 2010).

Results

Gonadal Morphology

Macroscopically; the gonads of immature fish were seen tiny and thread-like shaped membrane, but the ovarian cells were included small colourless granules initially when they were examined carefully under stereo-microscope, so they were named ovaries (0 - I year old). The ovaries had a pair of unequal lobes which were placed under columna vertebralis longitudinally and also attached to mesentery. Afterwards, the transparent growing ovaries were turned into opaque-white colour in juvenile stage (I-I^+ year old, Figures 1, 2). Female phases were began the F1 stage which were included the immature ovaries were seen pinkie and tiny blood vessels (I-II^+ years old, Figures 1, 2). Reddish colour and densely vascularisation were seen in other phases of the maturing ovaries of female stages. The gonad was covered by thick membrane (F2- F6 stages, I^+ - V

Figure 1. Maturation of female gonads in different stages I; Immature, J; Juvenile, M; Mature arrow; tiny blood vessels, arrowheads; densely vascularisation S; Thick membrane

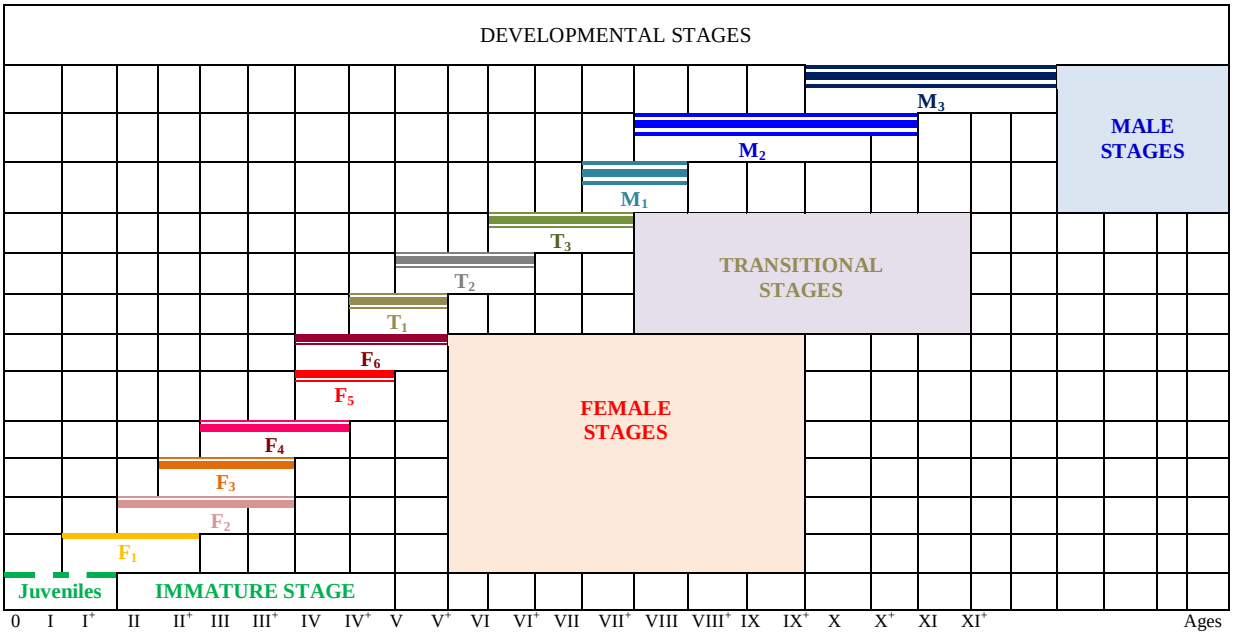

Figure 2. Gonadal Developmental Stages of Dusky grouper.

years old, Figures 1, 2).

Gonadal Differentiations

The grouper was known Protogynous hermaphroditic species. So, gonadal structure underwent transition from ovary to intersexual gonad and then to testis synchronously. These differentiations were included a serial ovarian, transitional and also testicular developing stages. These were mainly four phases, which were immature phases, female stages, transitional periods and male stages (Figure 2). The first of them were involved six different developing stages (F1-F6) which were named Female stages. Ages of the females were varied from I to V years old. The stages were pursued transitional periods which had three stages (T1, T2, T3). Ages of the transitional individuals were changed from IV to VII years old. The last period was male stages which included also three phases (M1, M2, M3). Ages of the male fishes were changed from VI^+ to XI years old (Figure 2).

Immature Female

Anatomically; there were seen translucent bi-lobed gonad adherent to the swim-bladder wall in immature female. Histologically; closely packed gonads were project into the ovarian cavity (Figure 3), [T_L(Total length) = 30.76 cm; mean W_G = 3,1± 1,95g ; RGI = 0,30%].

Mature Stages of Ovaries

These stages were examined six different stages (F1-F6). Due to no difference could be distinguished between the right and left gonadal lobes, only the middle part of the gonad was selected and examined. The ovarian stages have been defined according to presence of the most advanced type oocytes, and its relative abundance. Oogonia and previtellogenic oocyte were present in female ovaries.

Resting Female Stage (F1)

The stage was known Chromatin nucleolus stage. Macroscopically, lamellae and oocytes of the stage couldn't see to naked eye in the translucent and pink-orange colour (no opaque) ovary. Microscopically, oocytes aggregate in the oogonia. Primary germ cells, oogonia, small and densely stained previtellogenic oocytes and chromatin nucleolus oocytes were also characteristics of the stage (Figure 4A), [T_L= 32.24 cm; mean W_G = 10,3± 1,66 g; RGI = 0,41%].

Developing Female Stage (F2)

Macroscopically; pinky ovaries with several elogated ovigerous folds projected into the ovarian cavity. Vitellogenesis began in the stage. In microscopic description; Oocyte membrane and oocytes with lipid globules in the cytoplasm were present. Chromatin nucleus and male germ cysts were rearly observed. Lipid vesicle oocyte atresia was variable (Figure 4B), [T_L = 35.79 cm; mean W_G = 39,33± 4,53 g ; RGI = 0,94%].

Maturing Female Stage (F3)

Anatomically; the oocytes were observed

opaque and granular structure besides a little vascularisation. Histologically; at the beginning small and spherical yolk globules and later the section is occupied by vitellogenic oocyte and largely lipid and yolk globules; in early and late oocyte growing. These were named by Yolk granules I (YI), Yolk granules

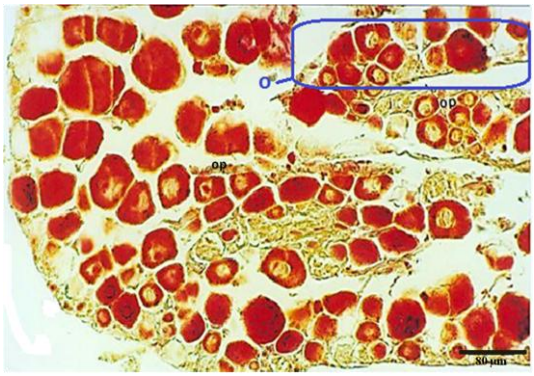

Figure 3. The packet cell of the immature oocytes, Mallory's Triple Stain; Scale bar=80 µm. Op: The packet cell of the immature oocytes, O: Oogonia.

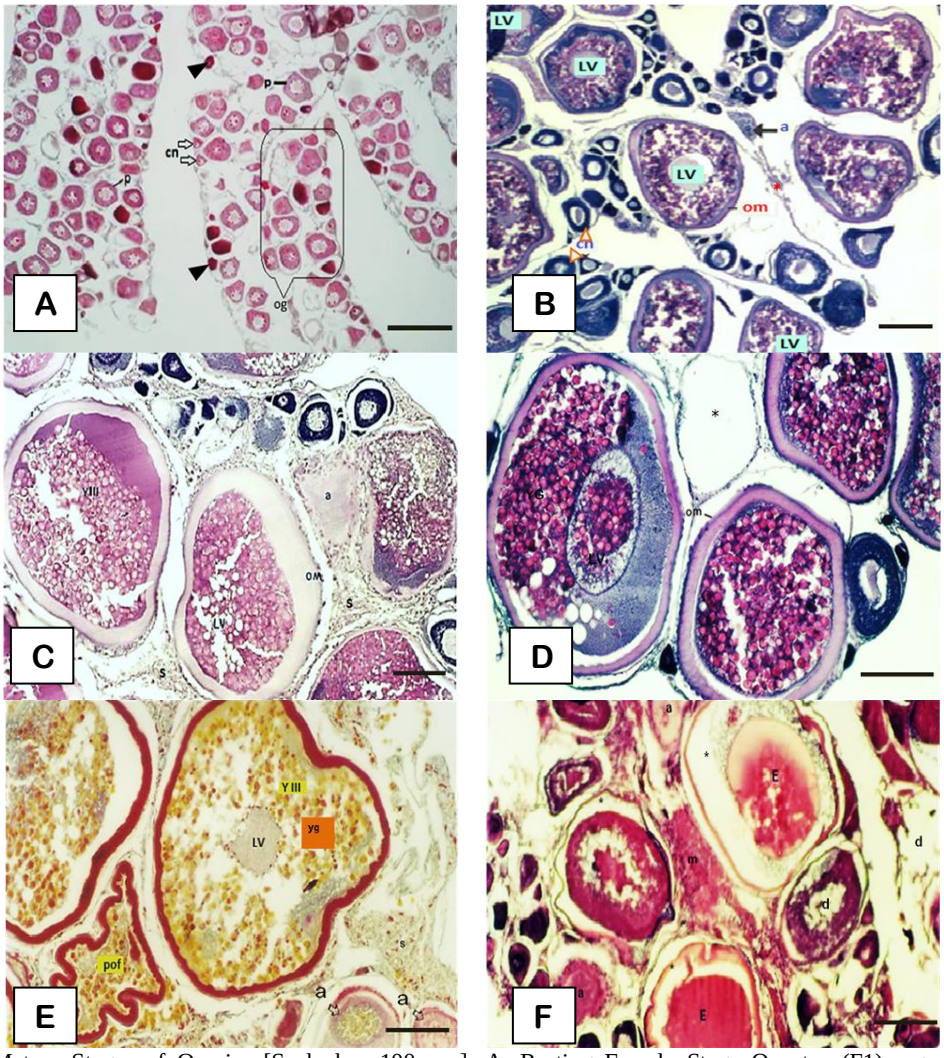

Figure 4. Mature Stages of Ovaries [Scale bar=100 µm]. A. Resting Female Stage Oocytes (F1): og; Oogonia, p; Perinucleolus, cn; Chromatin nucleolus, arrowhead; previtellogenic oocytes (H&E), B. Developing Female Stage (F2): a; atretic follicle, om; ooytes membrane, s; male germ cells cysts, LV; Lipid vesicles (H&E). C. Maturing Female Stage (F3) : YIII; oocytes III. stage in yolk, (H&E), D. Mature Female Stage (F4) : YG; Yolk globules, om: Oocytes membrane, ho; Hydrated oocyte (H&E), E. Partially Running Female Stage (F5) : pof; post-ovulatory follicle (Mallory's Triple), F. Post-Spawning Female Stage (F6) : E; eosinophilic oocytes, dc; degenerated cells, do; discharging of oocyte (H&E).

II (YII), Yolk granules III(YIII). Atretic follicles and cycts of male germ cells were seen occasionally (Figure 4C), [T_L = 38.31 cm; mean W_G = 358,9± 6,06 g ; RGI = 3,90%].

Mature Female Stage (F4)

Macroscopically; swollen and asymmetric ovary filled in body cavity in the stages. So, large and rippened gonad were clear with naked eye. Eggs were released with application of a bit abdominal pressure. But microscopically; abundant atretic vitellogentic oocytes and all the oocyte developmental stages were present but hydrated follicles and oocytes were very few (Figure. 4D), [T_L = 41.62 cm; mean W_G = 621,8± 41,49 g ; RGI = 5,71%].

Ripe and Partially Running Female Stage (F5)

Macroscopically; the ripe gonads were resembles the other stages of gonadal devleopments (from F4 to F6). Histolgical features of the running stage gonads; spawning activity were displayed by the post-ovulatory follicles amongst new oocytes groups in different stages of vitellogenesis. The number of one batch of YIII oocytes were seen. In addition to a few of atretic oocyte, vitellogenic globules in oocytes and some cysts of male germ cells also observed (Figure. 4E), [T_L = 45.57 cm; mean W_G = 225,9± 19,29 g ; RGI = 3,40%].

Post-Spawning Female Stage (F6)

Macroscopically; pink colour of the flaccid ovaries were seen to turn into reddish. Their membran was thickened and with a few opaque residuel oocytes were discerned in the ovaries. Over and above their blood vessel were displayed to enlarge and filled of the blood (Figure.1). Histologically; in the ovigerous folds were empty and a few residual oocytes which were named eosinophilic oocytes also observed there. The eosinophilic oocytes were signed beginning of cellular degeneration and also displayed to the end of the stage in the area. Furthermore, the expasion of the begining of the testicular tissue at the former were seen more widespread in the gonads of the stage (Figure. 4F), [T_L=50.75cm; mean W_G = 39,1± 1,86 g; RGI = 0,82%].

Transitional Gonadal Stages and Differentiations

Intersexual or Transitional stages were defined three phases which were 1st. Early transitional stage (T1), 2nd Bisexual stage (T2), and 3st Late transitional stage (T3). Gonad wall became thick and more fibrous, and vascularisation was intense during sex reversal in the gonads. The prior ovarian cavity and lamellar structure of the gonad were still maintained in all the stages of differentiations.

Early transitional stage (T1)

Anatomically; at the beginning of intersexual stage, the gonads were similar to an inactive ovary due to asymmetric shape and compact lobes. Later stages gonadal size became small, so the ovaries and testes could not be recognized. Histologically; testicular tissues were become widespread while previtellogenic oocytes were degenerated gradually. There were also seen a few atresia of vitellogenic oocyte (Figure 5A).

Bisexual Stage (T2)

The gonads which were in secondary step of transitional stage were determined both ovarian and testicular tissues. But no lobular testicular organisation had not yet been although the male germ cells were commonly present. Spermatogenic cells cycts and previtellogenic cells were intermingled. Melenaphore macrophage center, a few mature and atretic oocytes were also seen in preparations of the stages (Figure 5B).

Late transitional stage (T3)

The last phase of the transional gonads was T3 stage. Testicular tissues were mostly formed amongst severeal degenerating and previtellogenic oocytes (Figure 5C).

Male gonads and Testicular Differentiations

Immature Male Stage

Anatomically; whitish colours gonads were placed in the swim-bladder wall like immature female (Figure 5D). Histologically; there was seen onset of lobulation in the stage of gonad.

Developing Male Stage (M1)

Formation of testicular lobulation was observed clearly in the developing male stage. The testicular tissue were boundered by interlobular septa all of the gonad. The lobes were filled by male reproductive cells. There was seen no ovarian remainder anymore in the area (Figure 6A), [T_L=70.00cm; mean W_G = 65,1± 8,34 g; RGI = 0,46%].

Mature Male Stage (M2)

Macroscopilacally; milt released with light abmodinal pressure. The greyish colour gonad of the stage was well vascularisated and covered by whitish thick membran. (Figure 6B).

Histologically; it was seen that spermatogenesis was began in the mature testes, and different stages of

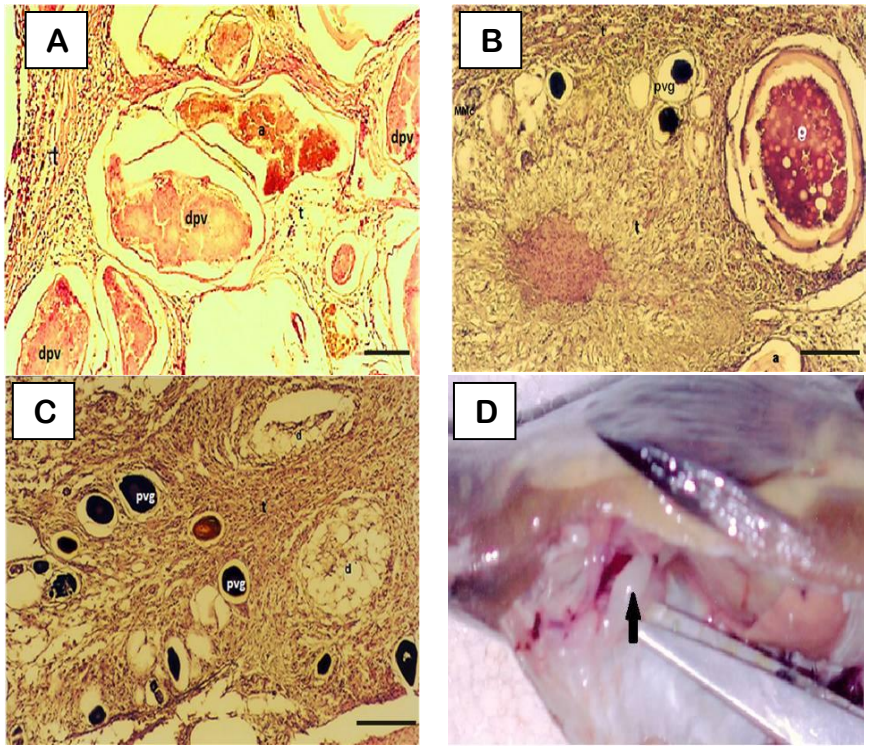

Figure 5. Transitional Gonadal Stages and Differentiations (H&E), [Scale bar=100 µm]. A. Early transitional stage (T1): t: testicular tissue, a: atresia of vitellogenic oocyte, dpv: degenerated previtellogenic oocytes, B. Bisexual Stage (T2): pvg; previtellogenic, o: mature cell, mmc: melano-macrophage centres, C. Late transitional stage (T3): d: degenerated oocytes, D. Immature Male Stage (arrow).

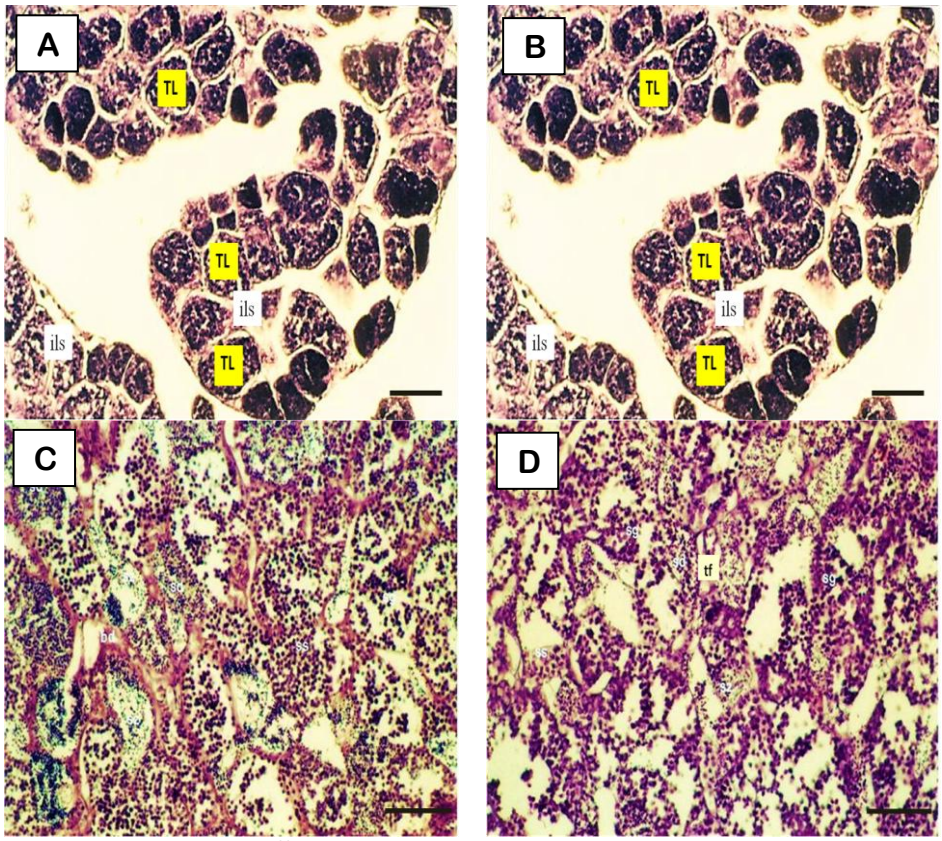

Figure 6. Male gonads and testicular differentiations. (H&E), [Scale bar=100 µm]. A. Developing Male Stage (M1); TL: testicular lobules, ils: Inter-lobular septa, B. Mature Male Stage (M2); t; testes, C. Mature Male Stage (M2); sz: Spermatozoa, ss: Spermatocytes, sd: spermatids, sg: Spermatogonia, tf: testicular folded, D. Ripened Male Stage (M3)

developing sperm cells was also remarkable. Testicular foldeds of spermatogonium were divided the testicular tissue into several productional lobes. Spermatogonia, spermatozoa, spermatocytes and spermatids were diversely cells (Figure 6C), [T_L=83.53cm; mean W_G = 83,2± 1,97 g; RGI = 0,49 %].

Ripened Male Stage (M3)

The stage was the last one of the male reproduction phases. The seminiferous lobules were mostly empty or contained very little spermatogonia or mature spermatozoa, and testicular folds were thinned and broken down (Figure 6D), [T_L=98.52cm; mean W_G = 86,3± 1,99 g; RGI = 0,49%].

Discussion

The grouper (*Epinephelus guaza*, L.1758) has been reported that inhabits most frequently rocky bottom, hard substrata and shelter rich on shelf from shallow waters up to 80 m depth, maximum density (Irigoyen *et al.* 2005; Reñones *et al.* 2005). They were caught through their cavern and rocky bottom up to 30 m depth by harpoon in Antalya Bay of the southern Mediterranean in Turkey. As illustrated by Marino *et al*. (2001) and Irigoyen *et al.* (2005), Özen and Balcı (2011), and also observed in the present study *Epinephelus guaza* had shown protogynous monandric hermaphrodite pathway among the member of the Serranidae family. The dusky grouper ovulated in ambient seawater Southern Adriatic during half of August and early September. Whereas these fish spawn in June and July in the warm water in Tunisia, and during early August in Spain waters (Glamuzina *et al.* 1998a). Spawning of the study fish was observed in July to September in Antalya Bay of the southern Mediterranean in Turkey. Histological demographic data also support a monandric pattern of development (Marino *et al.* 2001). It was provided that accurate information on gonadal development and sex reversal based on microscopic examination of gonads. According to data, sexual life of the fish begun at first female characteristic and underwent sex inversion and turned into male characteristic in the end. Marino *et al.* (2001) had depicted gonadal morphology of dusky grouper (*Epinephelus marginatus*) which captured from Pelagie Islands of Mediterranean. Some previous researches have stated mainly on descriptions of gonad histology at different developmental stages of the grouper (Glamuzina *et al.* 1998 a,b, Marino *et al.* 2001, Bertoncini *et al.* 2003, Irigoyen *et. al.* 2005, Erismann *et. al.* 2007). In the study, mainly four phases and their related stages were notified alike of them. Immature, female, transitional and male were main phases, while six female stages (F1 to F6), three transitional stages (T1 to T3), and three male stages (M1 to M3) were their related steps. They declared that first sexual maturity of length of female was 43.8 cm and while male was 81.3 cm (n=432). First mature males were observed at he age of VII years (M2), while females were II years (F3). It was determined in the search that total body length (T_L) of the fish (n=104) in the first sexual maturity were measured T_L=38.31 cm; (mean weight) W_G = 358,9± 6,06 g; in maturing female stage (F3) and T_L=83.53cm; mean W_G =83,2±1,97 g; mature male stage (M2). Consequently, it was observed that the grouper *Epinephelus guaza* was protogynous monandric hermaphrodite live in Antalya Bay of the southern Mediterranean in Turkey and environmental and demographic factors could effect sex change and also timing. According to some authors the fish is a prime species for marinculture and for restocking fished-out ecosystems in Mediterranean (Glamuzina *et al.*1998 a, Zhou and Gui 2008). Nowadays it is known that some protogynous stocks may be vulnerable to fishing, so the grouper stocks as in other serranids, were typically reef fish stocks to collapse in response to increasing fishing pressure (Bertoncini *et al.* 2003). Due to their popularity, long life-span and delicious flesh and also recreationally important, restocking of the fish must be encourage. So economical value of this species become raise day by day. They will be good candidate for rearing in saltwater resirculation systems. The reproductive background as well as knowledge of farming conditions leads the way to farmers.

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