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Studies on the reproductive biology of the female spiny cheek grouper, *Epinephelus diacanthus* (Valenciennes, 1828)

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ABSTRACT

Morphological and histological examination of gonads and detailed classification and description of maturity stages were carried out in the female *Epinephelus diacanthus*. Maximum ova diameter of 650 µm was observed in the ripe ovary. Oocyte size increased with the maturation of gonads. Gonado-somatic Index (GSI) values also increased with the maturation of gonads. Fecundity was in the range of 13.1 x 10^3 to 145.7 x $10^{3.}$

Keywords: *Epinephelus diacanthus*, Gonado-somatic index, Reproductive biology, Spiny cheek grouper

Introduction

Reproduction is a dynamic metabolic activity in most fishes and it involves sequential changes in the germ cells. The pattern of these changes in the gonads is typical for each species. To clearly understand the physiology of fish during reproduction, the study of the seasonal developmental changes of gonads through both macroscopic and microscopic observations is necessary. In the case of hermaphroditic fishes, macroscopic observation may not provide the correct information of the germ cell development during gonadal maturation and has its own limitations. Hence, microscopic observation is considered as important for detailed information on the reproductive mechanism of such fishes. Histological observation will provide information on internal changes in the germ cells.

The success of any fish species is ultimately determined by the ability of its members to reproduce successfully in a fluctuating environment and thereby to maintain the viable population. Information on the reproductive biology of the candidate species is very much essential for the development of aquaculture industry. Tessy (1994) studied biological aspects of *Epinephelus chlorostigma, Epinephelus bleekeri* and *Epinephelus diacanthus,* the three most available captive species of groupers of south-west coast of India. Of these, *E. diacanthus* contributed the major share to the grouper catch along south-west coast of India (Anon, 2000). *E. diacanthus* is a small sized grouper species maturing mostly around 166 mm standard length (SL) and captured in huge quantities in trawls as well as hooks and ines along the Indian coast. The maximum length reported was 502 mm (Tessy, 1994; James *et al*., 1996). Eventhough many studies were carried out on brood stock development of *Epinephelus tauvina* and *Epinephelus malabaricus* (Mathew *et al*., 2002; Mathew, 2005), little information is available on *E. diacanthus* which forms a dominant share in grouper capture fisheries (Chakraborthy, 1994).

In order to proceed with the artificial means of reproduction and to produce good quality eggs, it is necessary to have basic information on reproductive biology of the species. It is against this background that the present study has been taken up on the reproductive biological aspects of female grouper, *E. diacanthus.* The information generated would form a basis for initiating further studies on larger groupers such as *E. tauvina* and *E. malabaricus.*

Materials and methods

The present study was carried out at the Central Marine Fisheries Research Institute, Cochin from September 2002 to August 2004. Live specimens of *E. diacanthus* were collected onboard Fishery Survey of India (FSI) vessel during the cruises off Quilon region (latitude: 8º 55' N and longitude: 76º 30' E) and off Ratnagiri region (Latitude: 15º 42' N and Longitude: 73º 16' E) at the depth of 50 m. Fishes were also collected from local trawlers operated off Quilon region.

Collection methods and preservation

Live fishes collected onboard fishing vessels were measured accurately to nearest millimeter (mm) for total length, standard length and total weight. Each fish was dissected to remove the gonads. The dissected tissues were covered with aluminium foil, and packed in labeled

4 x 5 cm polythene bags. The polythene bags were preserved at -20 ºC until the landing of the vessel. After reaching the shore, all the samples were loaded in an icebox and transferred to the laboratory. The ovaries preserved in polythene bags were taken and their weights were recorded upto milligram (mg) level in an electronic balance (Sartorius) for the determination of Gonado-somatic Index (GSI). The GSI for each fish was calculated using the formula of June (1953) and Yuen (1955). The range and average values of GSI were calculated for each maturity stage. The 'pondreal index' or 'condition factor,' K for each fish was calculated using the formula suggested by Clark (1934). The range and average values of 'K' were determined for each maturity stage. Sex and stage of maturation were determined microscopically.

The gonads were assigned to three different maturity stages as suggested by Qasim (1973). The process of oogenesis was studied by utilizing histological preparations of ovaries from females belonging to different gonad maturity stages with haematoxylin and eosin staining as recommended by Moe (1969) and adopted by Tessy (1994). After dissecting the ovary from fresh fish, the ovary sample was cut into pieces for easy penetration of fixative. The ovary pieces from fresh specimen were fixed in Bouin's fixative and embedded with molten wax (58 °C melting point). The sections (5 mm thick) were stained with Delafield's haematoxylin and counter stained with 1% aqueous eosin.

Oocyte diameter measurements were taken from ovaries belonging to various developmental stages and oocyte size-frequency profiles were constructed with a view to trace the development of ova from immature stage to ripe condition (Clark, 1934; Prabhu, 1956; Greeley *et al*., 1987). Fecundity estimates were based on subsampling of unbiased samples of ovaries from gravid fish collected during the peak spawning period as recommended by Begenal and Braum (1978). The relationship between the fecundity (F) and total length (L), fecundity and total body weight (W) as well as fecundity and total gonad weight of the fish were determined using regression equations.

Results

The reproductive system of females of *E. diacanthus* includes a pair of ovaries, continued into an oviduct and ends in genital pore. The ovaries are paired egg sacs located behind the stomach and duodenum, below the swim bladder and just above the intestine and connected to it by mesenteries. Each ovary consists of a hollow sac. The right and left lobes are usually unequal in size. Right ovarian lobe is relatively larger than the left, both of which join posteriorly and descend as an oviduct to open in the genital pore immediately behind the anus. The urinary bladder is closely bound to the posterior face of the common oviduct. Supporting mesenteries continue forward from the anterior end of each gonad as ligaments that join a complex of ligaments and mesenteries at the anterior end of swim bladder.

E. diacanthus ovary is of the cystovarian type in which matured eggs will be released into the ovarian cavity during the ovulation; the ova will pass through oviduct on their way to go out at the genital pore. The genital pore is seen as a smaller pore behind the anus which would be bigger and pinkish during spawning season. The wall of the gonad is covered externally with a peritoneal layer. The tunica albuginea has an intermixture of longitudinal, oblique and circular muscle fibres.

Morphological classification of the ovary

Stage I

The ovary in the immature stage I is relatively small, translucent and white pinkish in colour (Fig. 1).

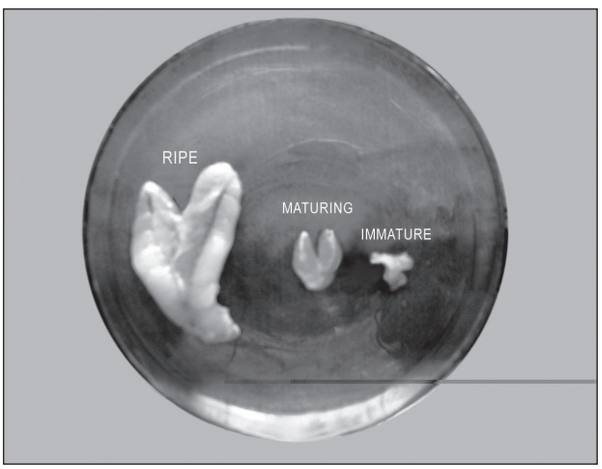

Fig. 1. Morphology of various maturity stages of female *E. diacanthus*

Stage II

Mature resting female / maturing female stage II of *E. diacanthus* is defined as an ovarian stage that had undergone extensive vitellogenesis and recovered into resting state. The ovary is larger than the previous stage and white brownish in colour.

Stage III

Stage III (ripe) is defined as the ovarian stage in which active vitellogenesis takes place in preparation for spawning in the mature active female/ripe female. The ovary occupies 2/3rd of the body cavity and is yellowish in colour. Oocytes are in stages 1, 2, 3 and 4 with stage 3 oocytes dominating during early development of this stage.

Condition factor (K)

The condition factor in *E. diacanthus* was in the range of 1.15 – 1.61. Highest condition factor (1.61) was observed in stage III of gonadal maturation (Fig. 2).

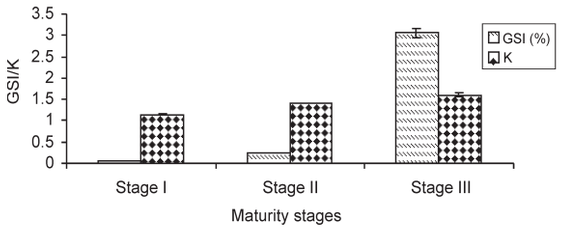

Fig. 2. Trends in GSI and condition factor with the maturation of gonads of female *E. Diacanthus* (Mean ± SD)

Gonado-somatic index (GSI)

In the present study, GSI values of *E. diacanthus* ovary showed correlation with the maturation of gonads (Fig. 2). The immature ovaries in the maturity stage I showed a GSI value of 0.062, the value was 0.234 in the maturing ovaries in the stage II and in the ripe stage ovaries of the maturity stage III, the value was 3.064.

Histological study of the ovary

Stage I - Immature

In this stage, the diameter of oocyte ranged between 17 and 50 µm. The cytoplasm becomes strongly basophilic. A thin follicular layer surrounds the oocyte at this stage. The ovary contains stage 1 and stage 2 oocytes (Fig. 3). Chromatin nucleolus stage oocytes are more abundant than perinucleolus stage oocytes. Primary stage oogonial cells observed in this stage are embedded in the ovigerous tissue and usually found along the periphery of the ovarian lamellae.

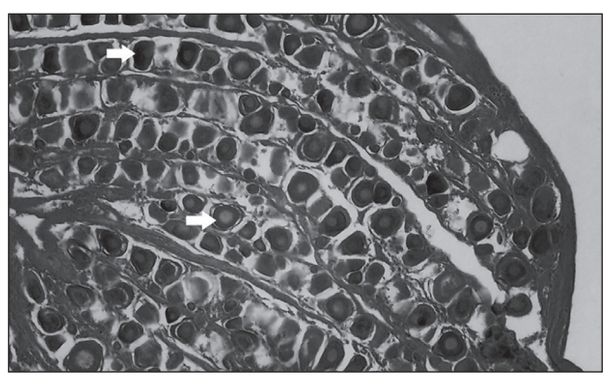

Fig. 3. Light micrograph of gonad development stage I, immature ovary of female *E. diacanthus*. Upper arrow indicates stage 1 and lower arrow indicates stage 2 oocytes (10X)

Stage II - Maturing

During this stage, the oocyte diameter size varied from 60 to 250 µm. This stage occurs before vitellogenesis. The ovary contains stage 3 oocytes abundantly (Fig. 4). The cytoplasm is strongly and evenly basophilic. A thin follicular layer surrounds the oocyte.

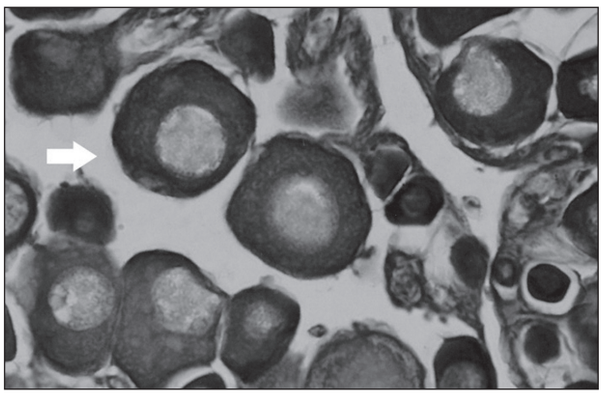

Fig. 4. Light micrograph of maturing ovary of female *E. diacanthus*. Arrow indicates stage 3 oocytes (40X)

Stage III - Mature active/ripe

The size range of oocyte diameter in this stage was from 80 to 520 µm. The oocyte expands generally and regains its rotundity. The nucleus also increase in relation to its size. The ovary contains early and late vitellogenic oocytes. The stage 4 oocytes are abundant in the ovary (Fig. 5).

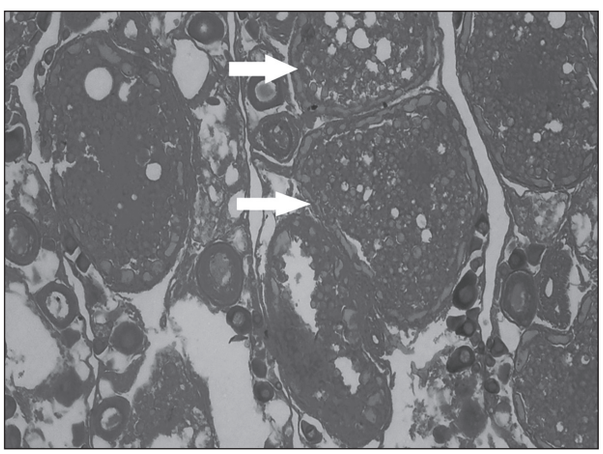

Fig. 5. Light micrograph of female *E. diacanthus* ripe ovary. Above arrow indicates early vitellogenic oocytes. Below arrow indicates late vitellogenic oocyte (10X)

The vitellogenic oocytes continue to expand and reach maximum attainable size before ovulation. The nucleus is well defined in early stage 4 (Fig. 6). Yolk vesicles are prominent and usually surround the nucleus in early stage 4 and coalesce towards the centre, when nucleus loses its

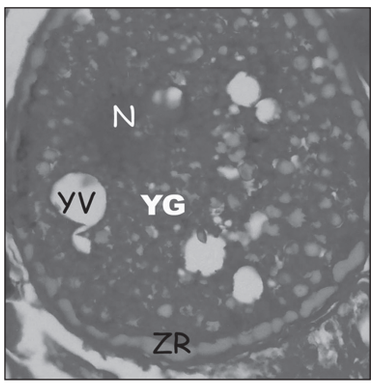

Fig. 6. Light micrograph of late vitellogenic oocyte which is showing migration of nucleus to the periphery of the oocyte (20X). N- Nucleus, YV- Yolk vesicle, YG- yolk globule, ZR – zona radiata

definition. These yolk vesicles are usually evident in late stage 4 near the oocyte periphery. Acidophilic yolk globules largely replace the basophilic cytoplasm in early stage 4 and become large and well developed in mid stage. The yolk globules coalesce in late stage 4 and present a smooth acidophilic appearance.

Distribution of ova in the ovary

Stage I

Ova in this stage are between 0 -150 µm size range. Majority of oocytes are in 0 - 50 µm size. The ova are immature with modes at 0 - 50 µm and 51 - 100 µm diameter (Fig. 7a).

Stage II

Maturing group of ova with a mode shifting to 201 - 250 µm are dominant. The size range of the ova at this stage was from 51 to 300 µm (Fig. 7b).

Stage III

Largest group of ova with a mode at 501 - 550 µm and with secondary modes at 351 - 400 µm, 401 - 450 µm, 451 - 500 µm, 551 - 600 µm and 601- 650 µm. In the stage III of *E. diacanthus* ovary, 650 µm was the maximum ova diameter recorded (Fig. 7c).

Fecundity

Fecundity of fishes is usually determined from the number of ova of the mature group in the ovary. In the present stuy, fecundity of *E. diacanthus,* was determined from the examination of 25 specimens. The fecundity of *E. diacanthus* varied from 13.1 x 10^3 to 145.7 x 10^3 with an average of 75, 547ova.

Relationship between fecundity and weight of ovary

The number of eggs was plotted against the weight of ovary in a scatter diagram (Fig. 8a). It is found that the

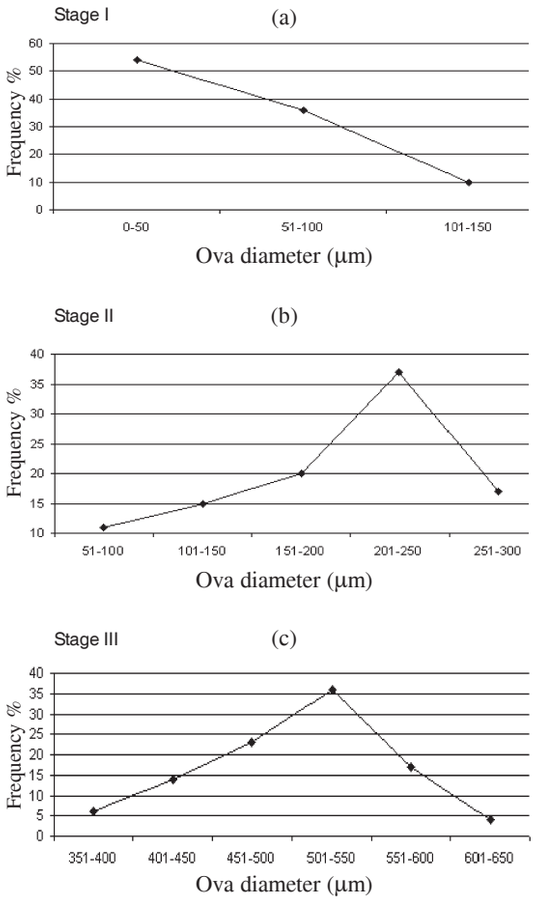

Fig. 7. Distribution of ova in the ovary of *E. diacanthus*

fecundity generally increases with increase in weight of the ovary. The relationship between fecundity and gonad weight in *E. diacanthus* was linear (Fig. 8a). The regression of fecundity on gonad weight (GW) can be expressed as F= 9387.9GW+34026 with an r^2 value of 0.5723. The values indicated that the correlation was significant.

Relationship between fecundity and total weight

The observed values of fecundity for 25 specimens were plotted against the weight of fish in Fig. 8b. The relationship between fecundity and weight of fish in female *E. diacanthus* was linear and it showed a gradual increase of fecundity with increase in total weight. The regression equation of fecundity on total weight can be expressed as F = 11.586TW + 72163 (F= fecundity; TW = Total weight) with an r^2 value of 0.0115.

Relationship between fecundity and total length

The number of eggs produced by individuals of *E. diacanthus* was plotted against the length of fish (Fig. 8c). In the present study, fecundity showed low correlation coefficient with the total length of the fish. The

regression of fecundity and total length can be expressed as F = 677.14TL + 56947 (TL = Total length) and r^2 value was 0.0217.

Relationship between fecundity and standard length

The relation between fecundity and standard length of fish was tested by plotting the observed values in a scatter diagram (Fig. 8d). In *E. diacanthus,* it showed a linear regression. The regression of fecundity and standard length can be expressed as F = 556.21SL + 63141 (SL = Standard length) and r^2 value was 0.01.

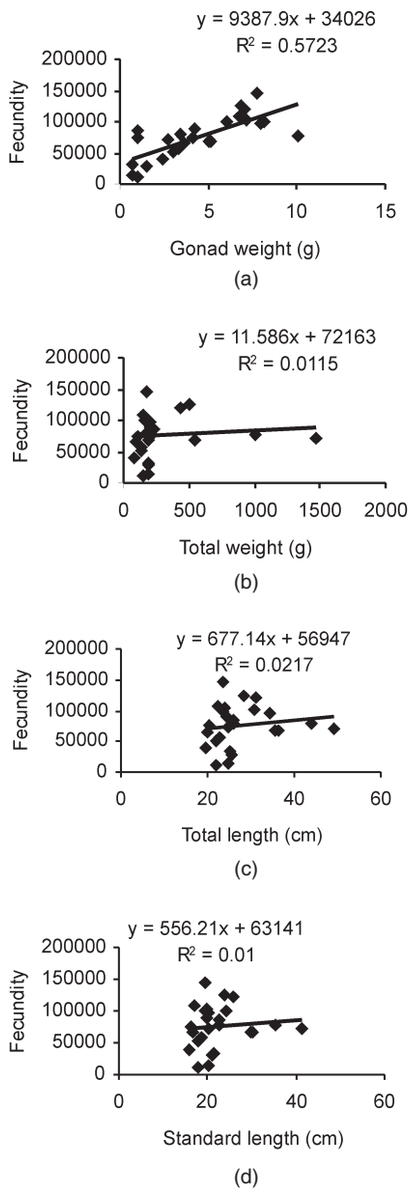

Fig. 8. Fecundity relationship with total length, standard length, total weight and gonad weight of *E. diacanthus*

Discussion

The observations in the present study on histological changes in the ovary with the different gonadal development stages were comparable to the observations made earlier in *E. diacanthus* by Tessy (1994).

Reproduction involves changes in growth and development of oocytes during the process of gonad maturation. With the advancement of maturation, oocytes accumulate energy reserves and enlarge for further need for the onset of embryogenesis. In the present study, *E. diacanthus* oocyte size increase from stage I to stage III of gonad maturation. Yashiro *et al*. (1993) observed that oocyte size increased with the progression of gonad maturation in *E. malabaricus*. They have reported that the oocyte size increased from 0.28 to 0.41mm with the advancement of vitellogenesis. The above results are similar to the observations made in the present study on *E. diacanthus*. Yeh *et al*. (2003) noticed that in *Epinephelus tukula,* oocyte diameter increases from immature stage (120 μm) to ripe stage (552 μm), which is very similar to *E. diacanthus*. The egg diameter of *Epinephilus morio* was found to be less than1mm by Moe (1969). Thompson and Munro (1978) found that in *Epinephelus guttatus,* with the maturation of gonads, the egg diameter varied between 0.70 and 0.90 mm. Brule and Deniel (1996) also observed similar trend in oocyte cyclic development in the immature oocyte (54 μm) to ripe oocytes (897 μm) in red grouper, *E. morio*. In the present study, largest oocyte diameter was 650 μm in ripe stage ovary of *E. diacanthus*. Tessy (1994) also has observed the largest oocyte diameter as 600 μm in *E. diacanthus*. Powell and Tucker (1992) reported eggs of 0.92 mm diameter in *E. striatus*.

Fecundity information of a species is essential for estimating seed production capacity and spawning population of the species concerned. Fecundity of the individual fish is determined from the total number of mature ova that are destined to be shed at the ensuing spawning season. In the present study, *E. diacanthus* gonad weight in relation to the total fecundity showed a significant linear relationship. Tessy (1994) has also observed similar relationship between gonad weight and fecundity in *E. diacanthus* and *E. bleekeri*. Bouain and Siau (1983) have reported that the fecundity is very closely related to the weight of the gonads in *E. aeneus*. The total body weight of *E. diacanthus* showed a low correlation coefficient with the fecundity. Yashiro *et al*. (1993) also observed similar relation with total body weight and fecundity in *E. malabaricus*. It may be due to the fact that weight of the ripe gonads in relation to the total body weight of the fish is small.

Fecundity in *E. diacanthus* showed linear relationship with total length and standard length of the fish. It has shown

low correlation coefficient, r^2 of 0.0217 and 0.01 respectively compared to the gonad weight (r^2 = 0.5841). Tessy (1994) made similar observations in *E. diacanthus* and *E. bleekeri*. Bouain and Siau (1983) have also observed low coefficient of correlation with the fecundity and standard length in the grouper *E. aeneus*. However, Chen *et al*. (1980) found correlation with the standard length and fecundity in *E. diacanthus* from the Pacific Ocean.

In the present study, the average fecundity of *E. diacanthus* estimated was 75,547. Highest fecundity recorded in the present study was 1,45,755. Tessy (1994) reported that the average fecundity of *E. diacanthus* was 57,458 and the highest fecundity was 1,65,000. Chen and Hsieh (1980) have found that fecundity of *E. diacanthus* in the Pacific Ocean ranged from 63,000 to 2,33,000. Bouain and Siau (1983) reported that for equal sizes (standard length = 44 cm), *Epinephelus aeneus* (Fecundity = 0.64 million) was more fecund than *Epinephelus guaza* (F = 0.60 million) and *Epinephelus alexandrinus* (F = 0.43 million). Estimates of potential fecundity in *Epinephelus tauvina* ranged from 0.85 million for a fish of 35.1cm long to 2.9 million for a fish of 62.3cm long (Abu-Hakima, 1987). Selvaraj and Rajagopalan (1973) estimated the total potential fecundity in *E. tauvina*, as 258.9 million.

The condition factor (K) is a measure of fish energy reserves. Condition factor values follow interannual variations and seasonal cycles (Lambert and Dutil, 1997). In the present study, condition factor values are in the range of 1.15 to 1.61 in *E.diacanthus*. Condition factor has increased in *E. diacanthus* from stage I to stage III of gonad maturation. Gopalakrishnan (1991) reported increase of condition factor with the advancement of maturation in *Mugil cephalus*. Hernandez *et al*. (2003) have also observed increase of condition factor with the progress of reproductive season in the fish, *Diplodus puntazzo*.

The state of maturity of a fish may be determined by the size of ovaries. Gonado-somatic index (GSI) indicates the stage and readiness of the ovary for maturation and spawning. Throughout maturation, the GSI values of *Dentex dentex* females were much higher than males implying a greater proportion in body reserves were allocated to the gonads (Chatzifotis *et al*., 2004). Gonado-somatic index has been used by many earlier investigators like Htun-Han (1978) to explain the degree of ripeness of ovary in a number of fishes. In the present study, the values of GSI for *E. diacanthus* have showed increasing trend from immature (0.06%) to ripe stage (3.06%). Yashiro *et al*. (1993) have also observed GSI values increasing from 0.43% to 5.2% with the maturation of gonads in *E. malabaricus*. The GSI values obtained in the present study correlated well with the GSI values observed by Tessy (1994) in various size groups of *E. diacanthus*. Brule *et al*. (1999) noticed greatest variations in the mean gonado-somatic index of female red grouper, *E. morio* from 0.27% to 2.14 % in maturing and ripe running stages.

Histological changes in the ovary of several species of groupers have been shown to correspond well with changes in the GSI. In the present study, the GSI values of female *E. diacanthus* have also showed similar increasing trend associated with histological changes. GSI value increase with corresponding histological changes were also noticed in *E. morio* (Johnson *et al*., 1998) and in *E. merra* (Lee *et al*., 2002).

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