



Histological study of some internal organs and their relationship to reproduction of king soldier bream, *Argyrops spinifer* (Forsskål 1775), Family Sparidae, from Iraqi marine waters

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ABSTRACT

This study was carried out to determine some aspects of reproductive biology of king soldier bream, *Argyrops spinifer* (Forsskål 1775), Family: Sparidae in Iraqi marine waters, Northwest Arabian Gulf. Samples were monthly collected from October 2013 to May 2014 by trawl nets. Variations in gonado-somatic index (GSI) were recorded. It was found that variations in hepato-somatic index (HSI) were related to feeding activity rather than gonadal maturation. Peaks of HIS and GSI were recorded at same period. Six maturity stages were defined for male and female of king soldier bream fish: immature, quiescent, maturing, mature, ripe and spent. Histological examination showed six stages of oocytes for females in this fish: chromatin nucleolus oocytes, perinucleolus oocytes, yolk vesicles, vitellogenic oocytes, hydrated oocytes, atretic oocytes. Five stages for males including: spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa. The gonado-somatic index for males and females reached their peak at ripe stage (2.165) and (3.789) respectively. The peak of hepato-somatic index was in immature stage in males (1.260) and in females (2.409).

INTRODUCTION

Sea-brems belong to the family Sparidae (Order: Perciformes). They are commercially important fishes occurring in shallow coastal waters to deeper offshore reefs (Randall, 1995). The sea-brems of the genus *Argyrops* are represented by two species namely *A. filamentosus* and *A. spinifer* in Omani waters (Al-Abdessalaam, 1995).

Fishes of family Sparidae (commonly known as sea-brems) inhabit both tropical and temperate coastal waters. Whilst large adults tend to be solitary and occur in deeper water, the smaller individuals and young form of aggregations are often found in estuaries (Randall *et al.*, 1997). Many species of this family have been shown to be hermaphroditic, some have both male and female gonads developing simultaneously

(Smale, 1988); whilst others change their sex from male to female (protandrous) or from female to male (protogynous) (Buxton and Garratt, 1990 ; Randall, 1995). Most sea-brems are excellent fish food and are of notable importance to both commercial and recreational fisheries throughout their range (Smale and Buxton, 1985 ; Sommer *et al.*, 1996). Sea-bream diet consists of nekton, molluscs and other benthic invertebrates (Salini *et al.*, 1994). Sea bream fishes are relatively large species attaining maximum size of 70 cm in total length (Randall, 1995). They caught mainly with bottom trawls, handlines and traps (Sommer *et al.*, 1996).

One of these important species is the king soldier bream *Argyrops spinifer* (Forsskål, 1775). The king soldier bream is distributed throughout the Western Indian Ocean extending eastward to the Indo-Malayan archipelago and northern Australia. While it inhabits a wide range of bottom types to 100 m depth, young individuals occur in very shallow waters of sheltered bays (Sommer *et al.*, 1996). Although the commercial importance of this species, a few studies on its biology and fisheries are available (Druzhinin, 1975 and Edwards *et al.*, 1985 in Yemen; El-Sayed and Abdel-Bary, 1995 in Qatari waters; Grandcourt *et al.*, 2004 in the Arabian Gulf, UAE; Al-Mamry *et al.*, 2009 in Omani waters; Raeisi *et al.*, 2011 in the Arabian Gulf, Iran).

The king soldier bream, *Argyrops spinifer* (Forsskål, 1775) is widespread in the Indo-West Pacific, where adults occur mostly between 30–100 m of depth, over a range of bottom types (Randall, 1995). *A. spinifer* is a relatively large sparid fish; it is characterized by a long life span and low growth rate (Ben Meriem *et al.*, 2006 and Al-Mamry *et al.*, 2009). It attains a maximum size of 70 cm total length and age of approximately 25 years (Al-Mamry *et al.*, 2009) and is probably protogynous, undergoing a sex reversal from female to male after a certain age (Grandcourt *et al.*, 2004 and McIlwain *et al.* 2006). This species spawns during the period between October and December and the size at first maturity is around 37 cm total length (Ben Meriem *et al.*, 2006 and Al-Mamry *et al.*, 2009)

Argyrops spinifer has been studied for the reproductive biology from the Arabian Sea of Oman utilizing samples from the trawl catches (McIlwain *et al.*, 2006) and artisanal fisheries (Al-Mamry *et al.*, 2009). Reproductive biology is a key aspect of species' biology and, for exploited species it is important for understanding its life history for stock assessment. It can also be of interest for determining why certain species might be more susceptible to overfishing than others and is of considerable value for conservation and management planning (Coleman *et al.*, 1996). Such information is largely lacking for sparids throughout the vast Asian region, including for those species that occur in the northern South China Sea (SCS) (Law and Sadovy de Mitcheson, 2017). The present objectives were to examine the sexual patterns, sexual maturation and spawning; in addition, the use of the gonado-somatic index (GSI) as a rapid indicator of spawning with histological methods.

MATERIALS AND METHODS

Specimens (males and females) were collected by commercial fishing boats in Iraqi marine waters, Northwest Arabian Gulf. These specimens were caught by standard bottom trawls at a depth of about 10 m from usual fishing grounds during the early

morning hours, and they were brought to the laboratory fresh on ice. Total length (TL) to the nearest 1 mm and body weight (BW) to the nearest 1 g were recorded for the specimens. The gonads and livers were isolated from the fish and weighted to the nearest 0.01 g, while the sex was determined by visual inspection. Gonadosomatic index (GSI) and hepato-somatic index (HSI) were calculated using the following formulas:

$$\text{GSI (\%)} = 100 \times \text{GW} / \text{BW} \quad (\text{Nikolsky, 1963 and Biswas, 1993})$$

$$\text{HSI} = (\text{wet weight of liver} / \text{total wet weight of fish}) \times 100 \quad (\text{Biswas, 1993})$$

Histology of ovarian tissue was used to describe maturation, spawning season, and reproductive periodicity of king soldier bream. For both sexes, the gonads were preserved in Bouin solution for subsequent histological analysis. Ten percent of the individuals from monthly sampling were randomly chosen for the histological studies. Gonads were dehydrated, embedded in paraffin, and sectioned transversely using a rotary microtome at 5–7 μm thickness following Hinton's (1990) conventional processing. Staining was performed using Harris hematoxylin and eosin. Description of the developmental stages of the gonad was based on **Brown-Peterson *et al.* (2011)**.

RESULTS

Gonadosomatic index (GSI) and hepatosomatic index (HSI):

The mean values of gonadosomatic index (GSI) ranged from 0.022931 to 2.164757 and from 0.006527 to 3.789068 for males and females, respectively (**Tab. 1 and 2**). The highest GSI value was observed in ripe stage for both males and females. The lowest GSI value was observed in immature stage for males (**Fig. 1**), while it was recorded in spent stage for females (**Fig. 3**).

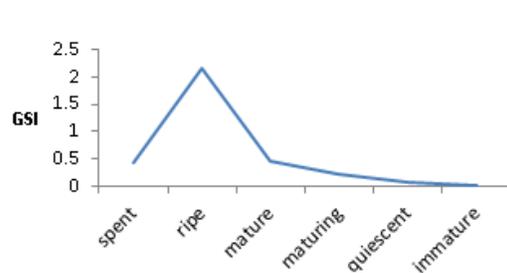
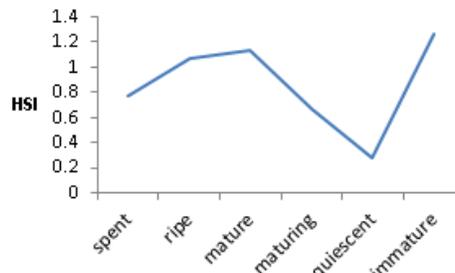
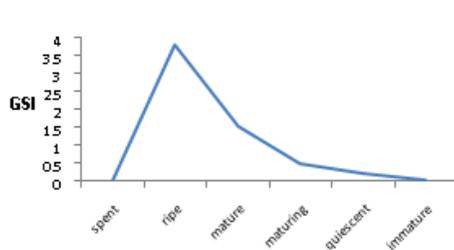
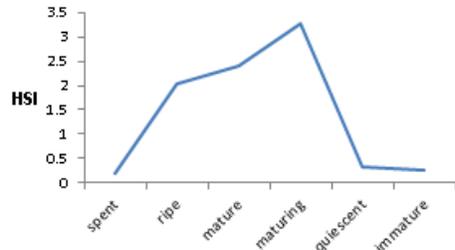
The mean values of hepatosomatic index (HSI) ranged between 0.280713 and 1.260179 (**Tab. 1**) in male specimens and between 0.192209 and 2.409677 (**Tab. 2**) for female specimens. The highest HSI values were observed in immature stage and the lowest was in quiescent stage of males (**Fig. 2**). The highest HSI values were observed in mature stage and the lowest was in spent stage of females (**Fig. 4**).

Tab. (1): Variations in GSI and HSI of male *A. spinifer* during different maturity stages.

Maturity stages	Average length (mm)	Average weight (g)	No. of fish	GSI (average \pm SD)	HSI (average \pm SD)
Immature	24.193	293.487	9	0.023 \pm 0.0096	0.260 \pm 1.5523
Quiescent	18.133	173.912	6	0.084 \pm 0.1256	0.281 \pm 0.2090
Maturing	23.567	307.443	3	0.222 \pm 0.0213	0.663 \pm 0.1713
Mature	26.340	376.988	5	0.476 \pm 0.1224	1.132 \pm 1.2244
Ripe	34.167	1016.408	6	2.165 \pm 0.9397	1.068 \pm 0.6119
Spent	21.367	229.970	3	0.429 \pm 0.5032	0.765 \pm 0.0425

Tab. (2): Variations in GSI and HSI of female *A. spinifer* during different maturity stages.

Maturity stages	Average length (mm)	Average weight (g)	No. of fish	GSI (average \pm SD)	HSI (average \pm SD)
Immature	24.576	379.147	8	0.023 \pm 0.0173	0.260 \pm 1.5523
Quiescent	22.244	271.999	7	0.154 \pm 0.0765	0.332 \pm 0.1805
Maturing	27.617	472.393	6	0.635 \pm 0.282	0.789 \pm 0.2888
Mature	25.650	418.883	4	1.512 \pm 0.2121	2.410 \pm 2.9712
Ripe	25.117	343.627	6	3.789 \pm 1.7405	2.037 \pm 2.0003
Spent	34.153	842.693	4	0.007 \pm 0.0024	0.192 \pm 0.2322

**Fig.(1):**GSI value during maturation cycle in males of *Argyrops spinifer***Fig.(2):**HSI values during maturation cycle in males of *Argyrops spinifer***Fig.(3):**GSI values during maturation cycle in females of *Argyrops spinifer***Fig.(4):**HSI values during maturation cycle in females of *Argyrops spinifer*

Gonad morphology and histology in male and female *A.spinifer*:

The macroscopic investigation to the gonads of *A. spinifer* showed that both of males and females have 6 maturity stages (immature, quiescent, maturing, mature, ripe and spent) during the maturation cycle. Size, shape and color of the gonads were differed in different maturity stages. At stage I , II and III , the testes are smaller in size and in stage IV and V, the testes are full of semen and a group of spermatozoa was oriented as an umbrella. At stage VI, the testis was wrinkled (**Tab. 3**).

Histological observations showed that male has a lobular type of testes. Lobules are delimited by connective tissue fibers within the lobular wall. The germ cells have been classified as: immature stage has spermatogonia and some primary spermatocytes. Quiescent stage has primary spermatocytes, secondary spermatocytes and spermatids.

Maturing and mature stages have spermatozoa. Spent stage has early spermatogonia which occurred surrounded by Sertoli cells, and were dispersed throughout the immature testes (**Fig. 5**).

Ovaries contained oocytes at various stages of development as confirmed by histological preparations. The oocytes of *A. spinifer* were classified into the following developmental stages: Chromatin nucleolar oocytes found in immature stage; perinucleolar oocytes appear in quiescent stage; cortical alveoli (yolk vesicles) showed in maturing stage; vitellogenic yolk observed in mature stage; nuclear migration appear in ripe stage and hydration in spent stage (**Tab. 3**).

The oocytes of *A. spinifer* were classified into the following developmental stages: Nucleoli distributed around the inner margin of the nuclear membrane (**Fig. 6A**). The oocytes' cytoplasm was stained deeply with hematoxylin. Some large round and basophilic nucleoli were observed in the nucleus. The follicle layer surrounding the oocytes became visible. A large number of yolk vesicles were present within the cytoplasm (**Fig. 6B and C**). Size of the oocytes became larger and less basophilic, but they were still stained with hematoxylin–eosin. The larger follicles became thicker. Yolk globules appeared first in the peripheral cytoplasm of the oocytes as minute granules (**Fig. 6D**). They increased in size and number and occupied most of the cytoplasm. The follicle layer became thicker and the nucleus located in the central area of the oocytes. The entire cytoplasm was then filled with many yolk globules (**Fig. 6E**). The nucleus migrated from the center to the animal pole where the micropyle is located (**Fig. 6F**). At the end of this stage, the nuclear envelope broke down (germinal vesicle breakdown) and the yolk globules almost fused in the peripheral cytoplasm. In spawning stage, the ovaries occupied almost the entire body cavity; yolk globules completely fused together and large eggs were distinguishable (**Fig. 6G**). Oocytes become irregular in shape (**Fig. 6H**).

Liver histology in *A. spinifer*:

The liver of *A. spinifer* is composed of a parenchyma covered by a thin capsule of connective tissue. It is divided into irregular lobules by the exocrine pancreas or hepatopancreas, associated to connective tissue. Even though they cannot be considered true hepatic triads, bile ducts associated to blood vessels are often found, to which that name is given. Within the parenchyma, the hepatocytes are radially arranged in cords around a central sinusoid. The lumen of the sinusoids contains mainly erythrocytes and macrophages. Large cells resting on the luminal surface of the sinusoid endothelium are present; these cells are known as Kupffer cells. Sinusoids are covered by typical endothelial cells with flatten nucleus (**Fig. 7**). With argentic impregnation, a mesh of reticular fibers between the sinusoids and the trabecules of hepatocytes was observed. Hepatocytes vary from polyhedral to round shape. Each hepatocyte contains a large, round, and central nucleus with a prominent dark nucleolus. Veins are scattered through the liver parenchyma without a well-defined arrangement, and they are surrounded by hepatic parenchyma or pancreatic tissue, sometimes accompanied by an artery or a bile duct (**Fig. 7**).

Tab. (3): Macroscopic and microscopic structure of maturity stages of *Argyrops spinifer*

Maturity stages	Male		Female	
	Macroscopic	Microscopic	Macroscopic	Microscopic
I Immature	Small, transparent, pale, occupying a very small portion to 1/3 of body cavity	Abundance of spermatogonia, some primary spermatocytes.	Small, transparent, pale, occupying a very small portion to 1/3 of body cavity ova not visible to naked eye	Chromatin nucleolar: very small oocytes. Clear, spherical nucleus surrounded by a thin layer of purple stained cytoplasm. No nucleolus visible.
II Quiescent	Whitish, translucent occupying about 1/2 of body cavity	Primary spermatocytes predominate, presence of secondary spermatocytes and spermatids	Pale yellow, granular ova visible to naked eye occupying about 1/2 of body cavity	Perinucleolar: oocyte size increases because of thick cytoplasm around a light nucleus, containing few to many peripheral nucleoli.
III Maturing	Creamy white, occupy about 3/4 of body cavity	Increasing number of secondary spermatocytes, presence of spermatids and spermatozoa.	Pale yellowish, blood vessels visible on dorsal side, ova clearly visible occupying about 3/4 of body cavity	Cortical alveoli: appearance of yolk vesicles in cytoplasm, thick & pink stained zona radiata distinguishable.
IV Mature	Creamy white, soft, occupying about full length of body cavity	Predominance of spermatids and spermatozoa	Pinkish yellow, blood vessels prominent, large ova prominently visible occupying about full length of body cavity	Vitellogenic yolk: Marked increase in oocyte size. Cytoplasm filled with yolk granules, oil vesicles and yolk vesicles. Peripheral nucleolus around the nuclear membrane.
V Ripe	Milt freely flowing when cut,	Spermatozoa predominate, mature sperm present in spermatid ducts	Hyaline eggs free running expressed when express	Nuclear migration: Migration of nucleus to periphery of cytoplasm, fusion of oil vesicles into the oil droplet, coalescence of yolk granules to form uniform plate
VI Spent	Flabby, slightly reddish, occupying slightly more than 1/2 of body cavity	Residual spermatozoa. spermatogonia present towards testis margin	Flaccid, reddish, occupying about 1/2 of body cavity	Hydration: Yolk granules fused into few plates. Thecal cells appear like astring.

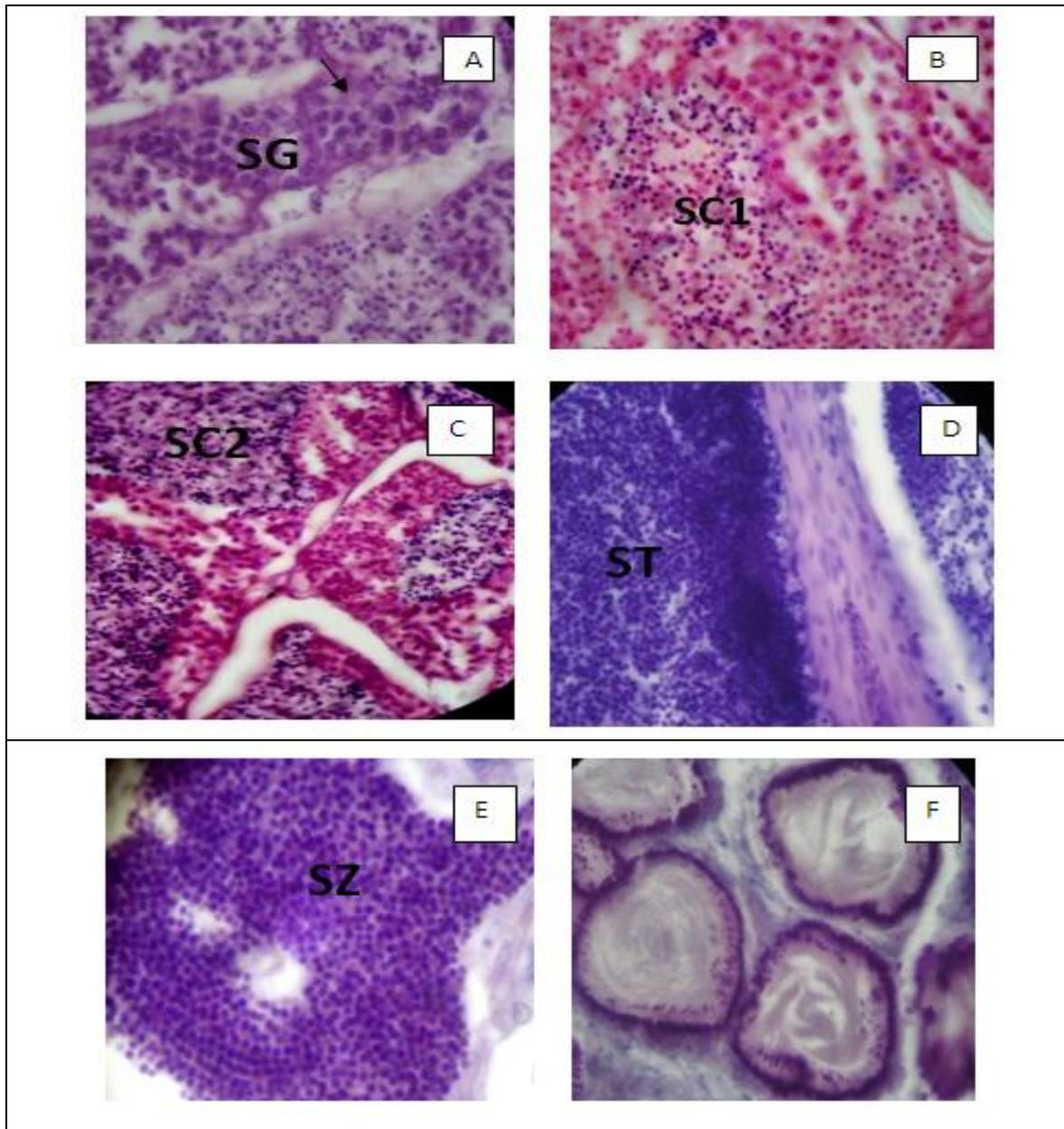


Fig. (5): Testicular developmental stages in testis of male *Argyrops spinifer* from developing to spawning. A) SG: Spermatogonia B) SC1: Primary spermatocytes. C) SC2: Secondary spermatocytes. D) ST: Spermatids. E) Spawning stage with spermatozoa (SZ). F) Testis in the spawning capable phase with visible sperm bundles arranged near the periphery (H & E; x 400 in A-D and x 1000 in E-F).

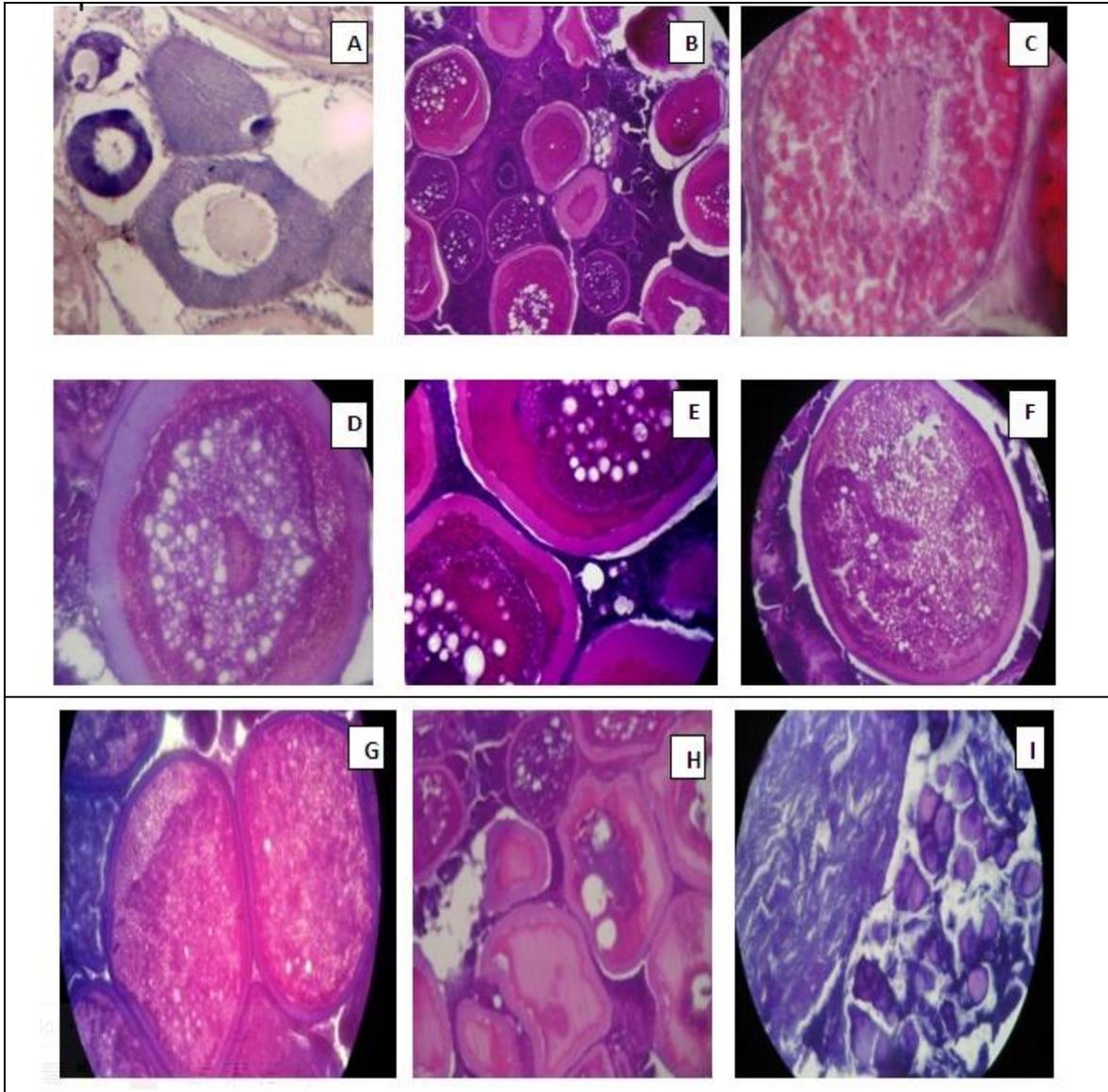


Fig. (6): The histological structure of the ovary in *Argyrop spinifer* showing ovarian development in female A) Perinucleolus stage. B) Yolk vesicle stage. C) Cortical alveoli. D) Yolk globule stage. E) Vitellogenic stage. F) Migratory nucleus stage. G) Hydrated oocytes. H) Atretic oocytes. I) Spawning capable male with bisexual gonad (H & E; 400 x).

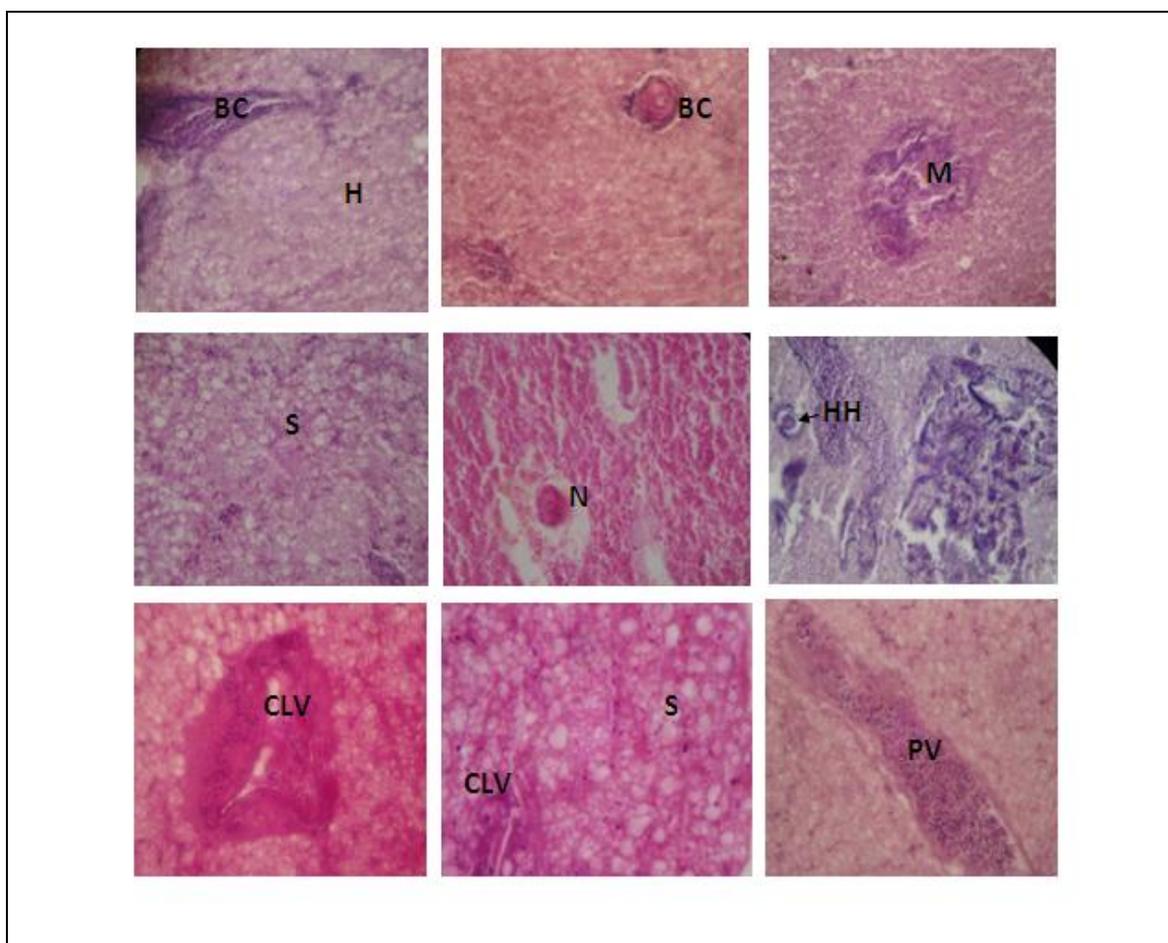


Fig. (7): Photomicrographs of liver of *A. spinifer*, Blood congestion (BC), hepatocytes (H), melanomacrophage aggregates (M), hepatic sinusoids (S), nucleus (N), hepatocytes hypertrophy (HH), hepatopancreas (HP), centerlobular vein (CLV), portal vein (PV) (H & E; x 200).

DISCUSSION

Sea breams or sparids (Family: Sparidae) have long been important components of coastal fisheries in temperate and sub-tropical regions. Estimated and reported global sparid catches increased from 100000 t in 1950 to 693732 t in 2006 (**Law and Sadovy deMitcheson, 2017**). *Argyrops spinifer* inhabits a wide range of bottoms in depths to 400 m, usually occurring between five and 100 m (**Sommer et al., 1996**).

Al-Mamry *et al.* (2009) studied age determination of this species in the Arabian Sea, they found that, age of female and male *A. spinifer* ranged between 2 and 25 years for total length ranged from 20 to 68 cm for males and from 25 to 70 cm for females. The maximum age for *A. spinifer* is 25 years with oldest fish being females (**Al-Mamry et al., 2009**). Young fish occur in very shallow waters of sheltered bays (**Sommer et al., 1996**).

while larger individuals occur in deeper water. It feeds on benthic invertebrates, mainly molluscs (**Salini *et al.*, 1994**).

The occurrence of spent gonads indicated that the spawning in *A. filamentosus* was between August and March; however, peak spawning was during September–January as evidenced by the incidence of higher percentages of mature females during these months (**Jayabalan *et al.*, 2011**). In study of *A. spinifer* higher GSI values of males and females between December and February coincided with the peak spawning season of the fish. While, higher HSI values during March–January showed better nutritional status prior to and during initial spawning months. The presence of mature and spent gonads during various months was considered as the direct evidence of spawning period in the population. The microscopic observation of ovaries showed clear differences among the immature, maturing and mature groups of ova. In both males and females, generally higher HSI values indicating better nutritional status of the fish for conservation of the energy required for that spawning. In the Arabian Sea, female fish maturing was earlier than males have been reported in several species of demersal fishes (**McIlwain *et al.*, 2006** and **Al-Marzouqi *et al.*, 2009**).

The spawning season for *A. spinifer* in the Arabian Sea was observed from September to January, immediately after the monsoon period, with a peak in spawning around October–November. In the southern Persian Gulf, spawning occurs from January to April (**Grandcourt *et al.*, 2004**). **McIlwain *et al.* (2006)** suggested that there are two spawning seasons for *A. spinifer* in the Arabian Sea: a small peak in July during the monsoon season and a main spawning season between November and March. There is some evidence of large aggregations during autumn in the Gulf of Aden (**Druzhinin, 1975**). Gonad condition of male and female fish indicated that the spawning season occurred between September and January (**Al-Mamry *et al.*, 2009**). *A. spinifer* is known to spawn over an extended period and so the differences seen between **Al Mamry *et al.* (2009)** study and the **McIlwain *et al.* (2006)** study in both the length of the spawning season and the peak of spawning may be due to regional differences between different stocks or regional differences in the temporal changes in surface water temperatures which appear to influence the timing of spawning in sparid species (**Sarre and Potter, 1999** and **Al Aisary *et al.*, 2004**). *A. spinifer* has been identified as a probable protogynous hermaphrodite (**Buxton and Garrett., 1990**). Recent studies in Arabian coastal waters have shown that the larger size-classes contain more male fish than females (**Grandcourt *et al.*, 2004**; **McIlwain *et al.*, 2006** and **Al-Mamry *et al.*, 2009**). However, as **McIlwain *et al.* (2006)** highlight, it is not known whether the observed differences in sex ratios and length-frequency distributions for *A. spinifer* in Omani coastal waters representing true hermaphroditism or reflecting sex-dependent differences in migration patterns or susceptibility to the hand-line fishery or spatio-temporal separation of sexes. Further research is needed to explain these results.

One consequence of protogynous hermaphroditism can be differences in size-at-age between the sexes. The sex change process, hard to detect with histology, as in protandric *Acanthopagrus berda* (Forsskål 1775), which is difficult to differentiate transitional from post-spawning individuals (**Tobin, 1998**). Therefore, the percentage of individuals undergoing sexual transition may be higher than can be detected histologically. In sparids, in general and in protandric species in particular, unlike many

protogynous species, little is known of the control of adult sex change. Changes in social structure have experimentally induced adult sex change in many protogynous species on the other hand, sex change it seem do not depend on the presence or absence of males suggesting that non-social factors (such as age or body size) might control the timing of sexual transition in such species (**Sunobe *et al.*, 2016**). Certain life-history characteristics may confer greater resilience to fishing than others, such as rapid growth and sexual maturation, periods of unavailability to fishing gears and high rates of reproduction (**Cheung *et al.*, 2005**). On the other hand, possible spawning aggregations or schooling habits could make such species more vulnerable to fishing if these are heavily exploited (**Sadovy de Mitcheson and Erisman, 2012**).

The surface of the liver is covered with serous membrane, and some connective tissue from this capsule extends inward into the parenchyma. Lobular structures containing a small vein in the center are present in the liver of higher vertebrates. In fishes, however, these structures vary depending on the species and are generally obscure. The histology of fish liver differs from the mammalian in that there is far less tendency for disposition of the hepatocytes in cords or lobules. Sinusoids, which are irregularly distributed between the polygonal hepatocytes, are fewer in number and lined by endothelial cells with very prominent nuclei. The function of the liver as a digestive gland is to secrete bile. Bile is secreted by the hepatic cells into the intracellular bile canaliculi which it is carried into the extracellular bile canaliculi. Bile canaliculi join to form the bile duct, which subsequently joins with the hepatic duct. The latter leaves the liver and opens into the duodenum. In stomach-less fishes such as carp and goldfish, the bile duct opens into the anterior portion of the intestinal bulb. The liver can differ in weight, size and volume according to body weight and length of each animal. The liver of *A. spinifer* was yellow-brown. We believe that variation in liver color may be related to different dietary habits. There are other factors associated with liver color such as health conditions, vascularization and hepatocyte content (**Bruslé and Anadon, 1996**). Fish liver is described as multifunctional organ acting in detoxification, production of vitellogenin as well as act on the deposition and metabolism of carbohydrates and fat. In most teleost fish, the liver is divided into lobes located cranially and ventrally in the body cavity with a reddish-brown color (**Bruslé and Anadon, 1996**).

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