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Histological Alterations through the Period of Reproductive Cycle of Male Rastrelligr Kanagurta Cuvier (Talal Fish) from the Persian Gulf and Oman Sea Coasts

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ABSTRACT

Rastrelligr kanagurta cuvier (Talal), a kind of Scombridae fish species, is considered as a valuable reserve of the southern coasts of Iran and also one of the most valuable bony fishes throughout the world. This study is aimed to determine the gonadosomatic index (GSI), isometric growth and the best timing of spermatogenesis in Talal fish. During this project (July 2009-July 2010), 50 fishes were randomly collected from the Persian Gulf and Oman Sea coasts which salinity and temperature were recorded every month. After morphological and biometrical examinations, males were separated and their testes were used to histological assessment and spawning season determination. A significant increasing in GSI has been observed by increasing the mature spermatozoa production. Accordingly, spawning only once a year (in spring) is performed. The results of our study indicate that the females' frequency is more than males'. As well as, the samples contained isometric growth and spermatogenesis began in November and reached its peak in April. Talal fish follows limited time of reproductive cycle pattern.

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INTRODUCTION

Rastrelliger kanagurta cuvier (Talal, Indian Mackerel, Long-jawed Mackerel, and Rake-gilled Mackerel) is a fish species with blue-green on top, the silver on sides and one or two rows of small dark spots on the upper sides. Below are several gold to dark stripes along the body. Also a dark spot is observed below the pectoral fin. The species grows to 35 cm in length (figure 1)[31].

Talal fish is one of the valuable teleost of anaclitic scombridae family. This fish is the most widespread and epipelagic species in the world [11,22,36]. It grows in tropical marine waters of the Indo-West Pacific. Also the central coast of Western Australia, around the tropical north and south to southern Queensland is recorded as its habitat in Australia [42].

The spawning season around India seems to be extended from March through September and in several batches. Juveniles feed on phytoplankton (i.e. diatoms) and small zooplankton such as cladocerans, ostracods, larval polychaetes, etc. Hence, adults prey primarily on macro-plankton such as larval shrimps and fish. Longevity is believed to be at least 4 years. Small groups have been observed eating Cheilio inermis eggs straight after spawning [24]. Various forms of these fish are used in the market such as fresh, frozen, canned, dried-salted, and smoked ones; also made into fish sauce [9].

Rastrelliger cuvier genus is comprised of three recognized species: Rastrelliger kanagurta cuvier (Indian mackerel), Rastrelliger brachysoma bleeker (Indo Pacific mackerel) and Rastrelliger faughni matsui (island mackerel) [26].

Fisheries scopes:

Rastrelliger kanagurta is the most important commercial and small pelagic fish in the tropical region providing cheap protein source and fish bait [10,14].

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Catches are usually recorded as Rastrelliger spp. or combined with R. brachysoma. In the last 25 years, the world catch for R. kanagurta alone fluctuated between about 96,000 tons in 1975 and a peak of 351,193 tons in 1994; since 1984, catches reported to FAO as Rastrelliger spp. have exceeded 300,000 tons (Figure 2).

Marine and freshwater aquatic environments support more than 20,000 teleost species showing a broad diversity of sexual patterns and reproductive strategies. Although the wide range of gonadal morphologies reflects the complexity of teleost reproduction, basic features are similar [29].

The spermatogenesis of teleost has been studied extensively such as description of the various stages during spermatogenesis (spermatogony development) based on distinct morphological, histological, physiological and/or biochemical cell characteristics [5,6,33,34,39,41]. Knowledge of fish gametogenesis and spawning has played an important role in arrange of commercial applications, including the development of toxicity tests and serve as a basis for experimental research and further comparison among species [35].

The aspect of biology and fisheries of R. kanagurta were studied by Moazzum *et al*. in Pakistani waters [27] . Along west coast of India, Rastrelliger kanagurta is studied by Nair and Rao [30]. Luther [25] studied the season spawning of this species in Andaman Islands [25]. Khayatzadeh *et al*. investigated reproductive biology and ecology of female Talal fish in Iranian southern coast. They showed that the peak of GSI and HIS was in spring for this fish that indicated spawning and vitellogenesis season. Ovary type was possible batch spawner and fish growth was isometric [20]. In another study Khayatzadeh *et al*. showed that GSI had a peak in spring with increasing in testicle weight and fish body length. In third article Khaytzadeh *et al*. demonstrate synchronization of spawning and sperm releasing [21].

Thus the present study aims at the investigation of the reproductive biology and gonadal histology of male R. kanagurta in southern Iranian Coasts**.** Important reproductive parameters of male R. kanagurta fish from southern Iranian Coast at Bandar Abbas, Gheshm and Jusk Iranian harbors were investigated in the present study to clarify the annual reproductive cycle. These parameters are: maturity stages, length at first sexual maturity, Gonadosomatic index, gonadal histology and monthly distribution of the maturity stages.

MATERIALS AND METHODS

Samples of R. kanagurta used in the present study were collected from the southern Iranian Coasts, at Bandar Abbas, Ghesm and Jusk harbors. The sampling was done monthly from July 2008- June 2009.

Total Length (to the nearest mm) and total weight (to the nearest gm) of the fish were recorded before its dissection. Then the gonads were examined to determine the sex and maturity stage, after that they were removed and weighed (to the nearest mg). The gonadosomatic index was calculated as the percentage weight of the gonad to the gutted weight of the body.

For histological examination, pieces of the testes were fixed in Bouin's fluid or 4% Formal saline. Then they were washed with 70% ethyl alcohol prior to dehydration with an ascending series of Ethanole. After that they were cleared in Zylen and embedded in Paraffin wax (m.p. 58-62 C) [35].

Sections of $6^{\mu m}$ thickness from the middle portion of these blacks were cut using a microtome (microtome, HM 325). Slides were stained with Hematoxylin & eosin. Then, the clearest sections were analyzed, [16,32].

All slides of gonad sections were examined by light microscope for sex determination and photographed by use of Zeiss model of digital camera. Maturity stages were classified according to earlier studies of this species gametogenesis [35]. In this study, histological examination of gonad development and gonad index were documented.

Results:

1. Maturity stages:

Maturity stages were detected through a morphological examination of the gonads by the naked eye. A scale of four stages [1,7,43] was adopted as follows:

Stage I (immature or recovery stage):

The testes are thin, flattened, and semi-transparent which occupy small proportion of the body cavity. They were milky white in appearance with evidence of vascularization.

Stage II (maturation stage):

The testes increased in size to occupy one third of the body cavity which appeared swollen, milky white, well vascularized and readily released milt under gentle Pressure of the abdomen.

Stage III (spawning stage):

The testes slightly decreased in size due to the discharge of milt during the spawning process. So, they become slightly flaccid and flabby. They have a white color**.**

Stage IV (spent stage):

The testes are highly reduced in size, completely shrunken and collapsed. There were empty testes with evidence of hemorrhage.

2. Length at first sexual maturity:

According to Gombang *et al*., the length at which 50% of a fish population reaches sexual maturity (L50) is considered to be the length at first sexual maturity [17]. In present study, fish of different length groups were classified to either immature or mature individuals. Fish of stages I (immature) are considered to be immature.

Fish of stages II to IV (mature to spend) gonads are designated as mature individuals. Figure 3 shows that all male R. kanagurta fish with total length less than 20 cm are immature, while those longer than 24 cm are mature. By adopting L50 value, it was found that male R. kanagurta fish reach first sexual maturity at 24.5 cm.

3. Gonadosomatic Index (GSI):

Monthly distribution of males GSI is shown in Figure 4 and table 1. It is obvious that the GSI was increased from March to reach the peak in May and then decreased. This suggests that onset of spawning in male R. kanagurta occurred in annual cycle, [8,18].

Histological characteristics of the testes during spermatogenesis:

Spermatogenesis in the testes of R. kanagurta was demonstrated in the present study through five stages of male germ cells [2,28,29]. These stages are described as follows:

1. Spermatogonia:

Recovering or Spermatogonia stage is more frequent in the immature testes. However, it could be rarely found in maturing stages. Along this stage the lobules of testis are small and they have a thick wall lining with Spermatogonia (Figure: 5A).

Spermatogonia are the largest period of male germ cells which could be divided into two forms: Mother Spermatogonia (A) larger and fainter cells; and Daughter Spermatogonia (B) smaller and darker ones. The chromatin material in the nucleus of Spermatogonia B looked lobular with irregular boundary.

2. Slow Spermatogenesis:

While, testis enters slow Spermatogenesis stage, the lobules of testes increased in sizes which Spermatogenic cells is developed through three stages: Spermatogonia, Spermatocyte and spermatid (Figure: 5B).

Primary spermatocytes are produced by mitotic division of the Spermatogonia. They have smaller size than the Spermatogonia. The chromatin material is condensed in one pole of the nucleus and became of a crescent shape. Secondary spermatocytes are produced by a meiotic division of the primary spermatocytes. They are smaller and darker than the primary spermatocytes.

3. Rapid Spermatogenesis:

The lobular style can be seen in all cell stages, especially in spermatids in various stages of their development as well as spermatozoa (Figure: 5C). Spermatids are produced by mitotic division of the secondary spermatocytes which are smaller and more condensed than the secondary Spermatoytes.

4. *Spawning:*

During this period Spermatids undergo a Spermiogenesis process to produce the sperms. This process includes nuclear condensation, tail formation and casting of the residual body. Spermatozoa have the smallest size among the male germ cells. They are formed by the differentiation of the spermatids and placed in clusters at the center lumen of each lobule (Figure: 5D).

During stage I (immature stage), presence of Spermatogonia is obvious. Spermatocytes have also high frequency; especially through primary spermatocytes they are abundant. Throughout stage Π (maturation) secondary Spermatocytes increase in frequency, Spermatids and spermatozoa appear in high amount. Over the stage ΙΙΙ (spawning) lobules of testes are mainly full of Spermatozoa, but in comparison with the time passed from the onset of the spawning process, they are less congested.

According to Zaki *et al*. and Abdel *et al*., the testes of R. Kanagurta were classified into four stages based on its maturity and morphology [1,43] Histologically, Spermatogenesis process may be demonstrated in four stages of male germ cell according to Nagahama and Abdel *et al [1,29]*.

5. Depletion:

The testes contain a few amounts of the spermatozoa, which remained in the testes and failed to be spawned. Thus, the testes looked flaccid and flabby. Spermatogonia become abundant gradually (Figure: 5E)

Monthly distribution of maturity stages:

The percentage distribution of different maturity stages of male R. Kanagurta in southern Iranian Coastal Water is given in (Figure 6). It is obvious that the recovering (immature) stages were recorded with the highest percentage during the period during seven months of year.

Slow spermatogenesis phase was recorded at the highest percentage during the period from November to December. The rapid spermatogenesis period was recorded throughout the winter and spring seasons at the maximum levels through March, April and May. Spawning stage was recorded throughout 6 months of the year with the peak form May to June. Depletion period appeared in April recorded the peak in June [35].

Discussion:

There are four maturity stages for the testes in R. kanagurta based on their morphology can be recognized by the naked eyes in the present study:

- Stage I (immature)
- Stage II (maturation)
- Stage III (spawning)
- Stage IV (spent)

The same scheme was used by Zaki *et al*. for Clarias lazera Cuv & Val (Valenciennes & Cuvier) (Zaki *et al*., 1985); Assem for solea species [3] El-Greisy for Diplodus sargus Linnaeus [13] Claereboudt *et al*. for king fish [8] Chellapa *et al*. for Cichla monoculus Spix and Agassiz and Abdel , El-Graesy for lizard fish, but some of these researcher determined additional stages of maturity for their fish [7,13]. Abdel *et al*. determined some stages like: nearly ripe, ripe, before spawning and spawning [1].

Since knowledge about the length at first sexual maturity is important for fishery management, the present study investigated the range of length at first sexual maturity for male R. kanagurta was 24.5 cm total length. The same studies were recorded by Claereboudt *et al*.[8] for Scomberomorus commerson Lacepede (Scombridae) in the Oman Sea, Timalina and Romanov for Katsu wonus Linnaeus (Scombridae), Talebzadeh for Gidar fish (scombridae) in the Persian Gulf recorded 84.7, 40 and 81 cm, respectively [8,38].

Differences in the length of first sexual maturity from one region to another could be depended on environmental factors being specific for each species. The length of the spawning season and the breeding activity could be demonstrated through the monthly distribution of the GSI values.

These values started to increase from February to reach the maximum in May (spring season). After that, they decreased rapidly due to the spawning process. This rapid decrease in GSI values indicated that this species has short time spawning season. However, Nair and Roa recorded summer monsoon months in west coast of India [30] Venkataraman recorded summer month in the west coast of India [40] Luther recorded winter months (October to April) in Andaman Islands [25] and Moazzum *et al*. recorded Summer in Pakistan water [27].

Difference in season spawning from one region to another could be attributed to the difference in environmental conditions. In these studies GSI value indicated annual spawning one time each year. Thus, it seems that R. Kanagurta species demonstrated some reproductive annual patterns of spawning throughout the region of distribution that is confirmed in other studies from this point.

Histologically, the course of male germ cells was divided into five stages starting by the spermatgonia and ending by the spermatozoa according to Nagahama and Agrawal [2,29]. As spermatogenesis and spermiogenesis proceed, the cyst expands and eventually ruptures, liberating sperms into the lobular lumen, which are continuous with the sperm duct.

The histological features of the testes of R. kanagurta throughout the spawning season showed that the discharge of the spermatozoa from the seminiferous lobules proceeds rapidly and highly. Thus, the spawning season is short and expands nearly a month round. Despite of these results in Abdel *et al* research, male Lizard fish spawning season is very long and discharge proceeds slowly [1].

Demonstration of the monthly distribution of the maturity stages of R. kanagurta showed that the Recovering stage was recorded during the fall and winter with a peak during October and November.

Maturation of the testes increased to record the maximum percentage of slow spermatogenesis during October, after that rapid Spermatogenes stage starts to appear and increase to record the maximum in December. Spawning stage starts at late January and extends until June while the maximum amount observes in May. Depletion stage occurs in April. Such a these results can be extracted from Shahri article [35]. Since gonad indices generally increase prior to spawning event and decrease during the event this data is consistent with the pattern shown by gametogenesis data from microscopic and morphological studies.

In conclusion R. Kanagurta has a defined breeding season in spring with short and rapid spawning peak during April that is consistent with GSI peak in April. It is hoped that these results can help us in breeding and fisheries activity for economic uses.

Table 1: Monthly distribution of GSI (N=10) of male R. kanagurta in southern Iranian Coasts.

SD	Month
0.2030	October
0.2625	November
0.2383	December
0.2283	January
0.2489	February
0.7900	March
0.7870	April
3.9707	May
2.7600	June

Legend to Figures:

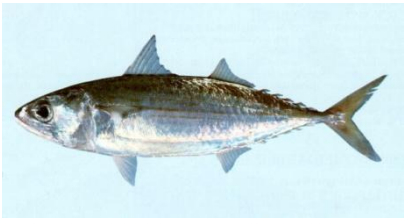

Fig. 1: Rastrelliger kanagurta (Talal) by Iranian Fisheries Research Organization (IFRO) (rakan_u0.jpg) [3].

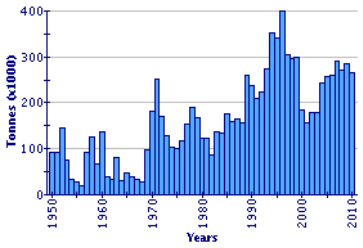

Fig. 2: Global Capture production for Rastrelliger kanagurta (FAO Fishery Statistic) [15].

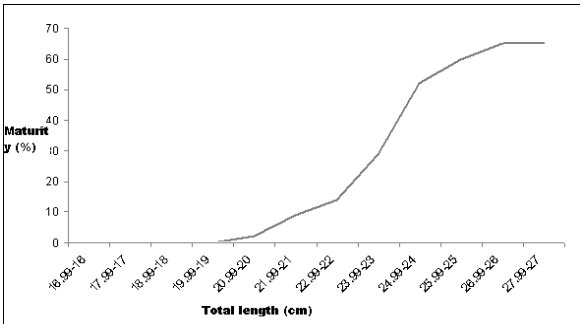

Fig. 3: The percentage distribution of mature R. kanagurta in southern Iranian Coasts.

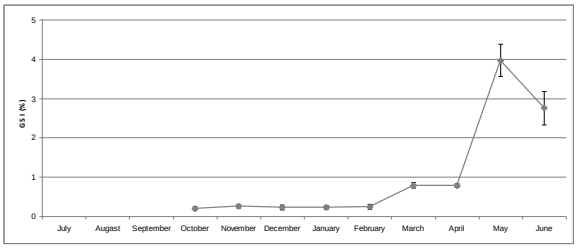

Fig. 4: Monthly distribution of males GSI R. kanagurta in southern Iranian Coasts.

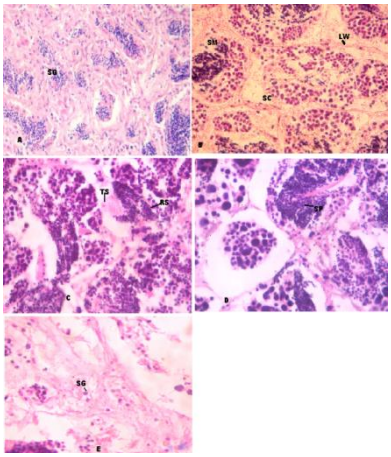

Fig. 5: Histologic texture of testis (A-E) of R. kanagurta, showing the five maturity stages (by Hematoxylin-Eosine staining). (A) Recovering (x 400), (B) Slow Spermatogenesis stage (x 400), (C) Rapid Spermatogenesis stage (x100), (D) Spawning stage (x100), (E) Deplation stage (x400) .SG: Spermatogononia , Sc: Spermatocyte , LW: Lobular Wall, SM: Spermatid, RS :Sperm , TS: Tail Sperm , SP: Spermatozoed.

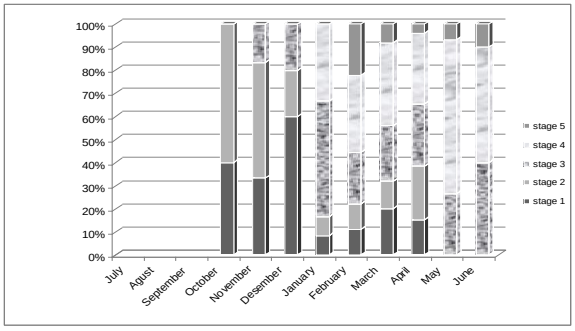

Fig. 6: Monthly distribution of different maturity stages of male R. kanagurta in southern Iranian Coasts.

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