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Histological Characterization of Gonadal Development of Croaker *Otolithes ruber* (Schneider, 1801) from off Paradeep Coast, Odisha, India

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ABSTRACT: Histology is a powerful tool in the study of reproductive health of fishes. It is routinely used for sex verification, identifying stage of development. This study presents the first details on morphological and histological characteristics of gonads, gonadal development stages of the tiger tooth croaker *Otolithes ruber* (Schneider, 1801) Sampling was done fortnightly from January 2018 to December 2019 collecting fresh and live specimen from bottom trawlers fishing off Paradeep fishing harbour, odisha and a total of 196 individuals were collected. The gonads of specimens were removed, their sexes determined and then were fixed in Bouin's solution after checking their morphology and measuring their weights, and lengths. Based on the size, shape and weight of the gonads, degree of occupation of the body cavity, presence or absence of ripe oocytes or milt, diameter of the oocytes in the ovary, and histological observations, six stages of sexual maturation in females were determined by macroscopic and microscopic study. The study on ova diameter and ovary histology of fully ripe females and spent fishes during different months indicates the spawning season is June to October, being a synchronous spawner.

Keywords: Histology, ovary and periodicity, *O. ruber.*

INTRODUCTION

Fish is not only used for human consumption, but also used as a good source of fish meal, provide it with cheap and high quality protein (Anderson and Mitchum, 1974). Croakers is considered as the cheapest source of high quality protein and rich in calcium, phosphate, iodine and vitamins (Dadzie and Wangila 1980). Sciaenids are one of the important demersal fish in the marine landing along the Indian coast accounting for 1.356 lakh tones and second largest group among demersal landing. Sciaenids are generally available throughout the year. Peak landing is during September to March. Trawlers exploit mostly sciaenids, which accounts for more than 20% of total catch in Odisha and to a little extent by mechanized and non-mechanized gill-netters. Dutt and Thankam (1968) reported that along the east coast of India *Otolithus argenteus, Nibea maculata* and *O. ruber* are common in the seas and estuaries of Orissa and West Bengal. Despite expensive and time-consuming procedures being used to determine gonad development, the most accurate technique is histological analysis (West, 1990). The spawning period in teleosts is determined from changes occurring within the gonad throughout the year. The macroscopic or histological study of the gonad (qualitative method) and quantitative estimation of oocyte diameter are commonly applied (Karlou-Riga and Economidis 1997). This technique also helps in detecting the breeding season and establishing breeding characters of mature fishes. In the present study the morphological gonadal development of *O. ruber* was compared with the gonard histological characterization. From this data as an initial input to the population dynamics study and stock assessment investigations and to develop the managing strategies for *O. ruber* fish resources.

MATERIALS AND METHODS

One of the most significant studies of fishery reproductive biology is to determine the gonadal morphology and annual breeding cycle of a fish species. There are a few methods, such as developmental stages based on the external appearance of the gonad and measurements of gonad and histology to evaluate the stage of gonad development of individual fish. Earlier studies revealed that the size at first maturity of *O. ruber* was found to be within the range of 220 mm-240 mm (Vaidya, 1960). Though a

good number of investigations have been done on reproductive biology in a number of sciaenid species, the present study is aimed at histological characterization of gonadal development of sciaenids *O. ruber*.

Fresh live specimens of all the three species were collected fortnightly from the bottom trawlers fishing off Paradeep coast, odisha, Orissa. To study the annual ovarian cycle through developmental stages of ovary, histology procedure was carried out at fishery biology laboratory of College of Fisheries (OUAT), Berhampur. Gonads were weighed to the nearest 0.001g. Samples of ovary were preserved in 10 % formalin for ova diameter studies. A total of 195 numbers of ovaries of different maturity stages of *O. ruber,* were examined for maturity studies both by quantitative and qualitative study. According to Prabhu (1956) measurement of at least 500 eggs was necessary to mitigate the probable errors in the representation of various groups of eggs in different stages of maturity. The diameters of ova were measured in straight line under a compound microscope magnified 100 times with eyepiece fitted with an ocular micrometer. In the present study, ova samples from the middle part of both the ovary lobes were measured to the nearest micrometer (1µm division = 0.016mm) ova measuring above 5 µm division and above were considered for evaluating percentage of frequencies. The measured ova were grouped into 5 micrometer division class intervals and their frequency polygons drawn as followed by Clark (1934); Palekar and Karandikar (1952); Prabhu (1956). In order to study the maturity and spawning season, 195 females and 134 males of *O. ruber* were observed. The maturity stages were classified exclusively depending on the stages, size and other observations of the ovary. The spawning period was ascertained from the occurrence of ripe females. The length at first maturity was determined based on the examination of the ovaries. The females in stage III and above were considered as mature for the determining the length at first maturity. The data collected for twelve months were and percentage of cumulative frequency were plotted against the size to determine the size at which 50% fish mature. The fecundity was determined using the formula, Fecundity = (No of ova in the sample/ weight of sample) × weight of paired ovaries.

For gonad histology, the ovarian samples were taken from anterior, middle and posterior regions and fixed in freshly prepared aqueous Bouins fluid for overnight, then washed with flowing water to remove the excess stain. The fixed tissues were processed according to the suggestions of Epple (1967) using tetrahydrofurane as cleaning agent, embedded in paraffin wax (59-60°C) and cut into 4-5 µm thick sections and stained with the haematoxilin and eosin, as suggested by Humason (1967). Developmental stages were determined by the scale of ICES (Wood 1930) as Immature, Early maturing, Maturing, Late maturing, Mature, Ripe and Spent. The gross histological feature of ovary in different developmental stages was followed as per Yadava (1995).

RESULTS AND DISCUSSION

The spawning periodicity study (Fig. 1) reveals that ovary 'A' in addition to germinal stock line, there are ova of 0.36 mm which is immature, designated as 'a' and 'b' as 0.60 mm and 'c' as 0.68 mm. In ovary 'B' the ova are of 'a', 'b' and 'c' mode of which 'a' is having ova of the diameter of 0.36 mm, 'b' is of 0.60 mm and 'c' of 0.68 mm. The difference between 'b' and 'c' is very less. In ovary 'C' apart from the germinal stock maturing eggs at 'a', having a diameter of 0.60 mm and also maturing eggs at 'b' with ova of 0.76 mm. Apart from that there is a mode at 'c' and 'd' having ova of 0.84 mm and 0.92 mm. These are mature ova, some of oil globules in them. In the ovary 'D', the ova belong to immature stock with modes 'a' at 0.36 mm and maturing stock 'b' at 0.48 mm. Similarly, in ovary 'E' also two different modes are there designated as 'a' and 'b' have modes at 0.36 mm and 0.44 mm. respectively. The eggs of ovary 'F' belong to early maturing category with modes 'a' and 'b' at 0.28 mm and 0.36 mm respectively. The ovary 'G' indicates the eggs belonging to second stage of maturation or partly spent category with modes 'a' at 0.20 and 'b' 0.36 mm. In ovary 'H' a distinct mode of more than 50% eggs are found at 0.76 mm. Apart from these other eggs are of minor significance. In ovary 'I', ova belong to the maturing stock where three modes 'a' 0.28 mm, 'b' 0.36 mm and 'c' 0.44 mm are available. In ovary 'J', 'a' and 'b' modes of eggs were at 0.20 mm and 0.36 mm. From the mature ovaries in 'A', it appears that the modes b'' and 'c' at 0.60 mm and 0.68 mm are very close where as mode 0.36 mm are widely separated.

Thus, there is evidence of single spawning as modes 'b' and 'c' form large percentage of eggs. Ovary 'B' also indicates similar distribution of eggs modes 'b' and 'c' lying in close proximity. Ovary 'C' perhaps belongs to the advanced mature and probably oozing type of eggs with mode 'c' and 'd' reaching up to 0.80 and 0.92 mm respectively. It is probable that the fishes release its eggs in only one batch at a time and the interval between each batch is rather long. Largest ovum measured for this species was 0.928 mm. Ova diameter frequencies for different stages of maturity for this species are shown in the Fig. 1.

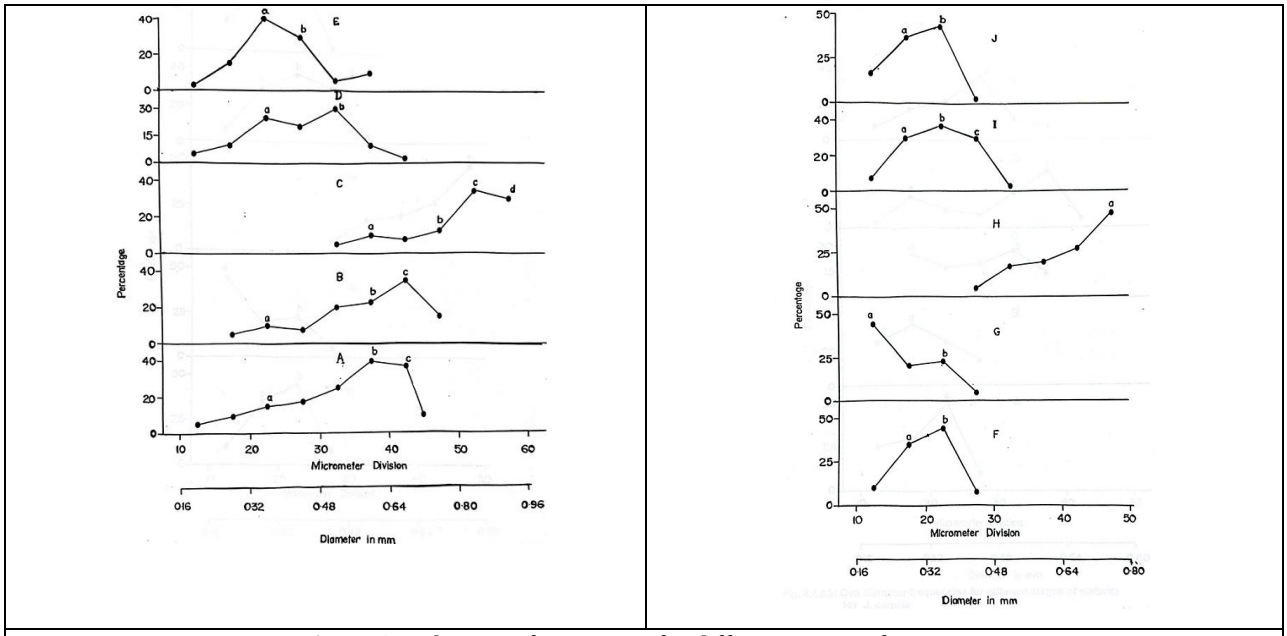

Fig. 1. Ova diameter frequencies for different stages of maturity.

Chacko (1949) reported the presence of larvae and post larvae of O. *ruber* during August to September in Gulf of Mannar. Devadoss (1969) reported the length at first maturity for *O. ruber* from Bombay waters as 170 mm and a majority of the species maturing at 200 mm. He also stated that It breeds once in a year, from January to October. According to Devadoss (1969) the absolute fecundity varied between 44,621 to 1, 79,659 eggs and diameter between 0.018 to 0.87mm and he also reported that prior to spawning males were more abundant as compared to females. In Porto Novo water, Pillai (1983) observed that *O. ruber* breeds for a short period between July-October with fecundity ranging from 43,810- 7,93,242 eggs. In the present investigation the ova ranged from 0.20 –0.92 mm. The study confirms the single spawning of this species as observed by Devadoss (1969); Pillai (1983). The average size of this species recorded from Paradeep is high. This may be one of the reason for getting a higher length at maturity as compare to that reported by Devadoss (1969). The average size of ovary is also bigger; consequently, the fecundity reported here is more in compared to earlier studies.

The ovary of *O. ruber* are paired in structure, lying in the posterior abdominal cavity. The ovaries are equal in size, suspended in the body cavity by the membrane 'mesovarium'. Each ovaries is elongated in structure, tapering at the ends and swollen in the middle. Anteriorly the ovaries are blunt and free, posteriorly they form oviducts, which are united to form median oviduct and open out through gonophores.

Examination of the morphology and histological features of the ovary for stages of maturity in *O. ruber* to study the annual ovarian cycle, suggested that different stages of maturity are more or less same in the three species except colour of the ovary and diameter of ova and orientation of eggs in different stages (Plate 1).

Stage I: Ovary is short tube like structure, with pale cream colour is red and occupying less than ¼ of the body cavity. Ova are not visible with naked eye. A thin peritoneal layer covers the ovary. The diameter of the immature ova is 0.20 mm. Histological slide was observed under microscope, which revealed innumerable tiny, transparent, round oogonia. The centrally placed nucleus is distinctly seen. Nucleoli were restricted to the center of nucleus in small oogonia, whereas nucleoli were arranged at periphery of nucleus in large oogonia. The nucleoli are small in size and many of them occur closely adhering to inner surface of the nuclear membrane while some are centrally placed in the nucleus. The stage I with only immature oocytes in the month of December.

Stage II: Ovary appears as thick tube like structure, colour varies from wine red in *O. ruber,* and extends less than 1/3 of body cavity, Ova are invisible with naked eyes. The diameter of the early maturing ova is 0.32 mm. In this stage the size of nucleus has increased. Small nucleoli are found in addition to primary nucleoli that are in close association with nuclear membrane. The yolk nucleus appears in the cytoplasm and is deeply stained. The stage II with early maturing oocytes in the month of December.

Stage III: Ovary is slightly enlarged structure, ovary pink cream to yellow and ovary occupies 1/2 to 2/3 of body cavity. Ova are visible with naked eyes. The diameter of the ova is 0.44 mm. The important feature of oocytes is the appearance of dense round body, the yolk nucleus just in the vicinity of nucleus lying

adjacent to nuclear membrane and later moves to periphery in this stage. Accumulation of yolk vesicles in the cytoplasm has started and a large number of clear vacuoles called yolk vesicles are visible. The cytoplasm shows a difference in staining, dark staining for bigger granules and light stained for small granules. Nucleus extrusion has also been observed. The stage III and IV with late maturing oocytes in the month of February.

Stage IV: Ovary is thick tube like structure, ovary cream to yellow Ovary occupies 2/3 of body cavity. Ova are easily visible with naked eyes. The diameter of the ova is 0.60mm. In mature stage along with mature ova, some maturing oocytes are also visible. The mature ova are characterized by their large size, spherical shape, small nucleus and cytoplasm with large amount of yolk vesicles and yolk granules. The follicular layers are well differentiated into zona radiata and zona granulosa. The stage IV with only late maturing oocytes in the month of March

Stage V: Ovary occupies 3/4 to 4/5 of body cavity, colour reddish yellow, Ova are round, transparent and blood vessels are prominent in the ovary. The diameter of the ova is 0.76mm. Along with ripe ova, few immature ova are seen in this stage. Ripe ova are large and granular, characterized by large quantity of yolk. Small nucleoli with irregular margin and absence of yolk vesicles are observed. Intra vesicular and inter vesicular yolk deposition initiated in this stage with increase in size. The yolk granules then move centripetally. The stage V with mature oocytes in the month of May.

Stage VI: Ovary occupied entire length of the body cavity, Plum pudding appearance; ripe ova are visible from the wall of the ovary. The diameter of the ova *is* 0.92mm. In this stage the oocytes show increased size as intra vesicular yolk granules becomes larger forming yolk particles of various shapes. The nucleus migrates towards periphery. The follicular layer has under gone hypertrophy and the zona radiata becomes thin. The nuclear wall whose out line is oblong, breaks and disintegrate. The stage VI with ripe oocytes in the month of July.

Stage VII: Ovary is blood shot, shrunken, flaccid and with few residual eggs. The ovigerous lamella is hollow and their undulating walls appear shrunken. Follicules are contracted, folded and tunica wall appears increased in diameter. The stage VII with oocytes of stage III in the month of July.

The morphological and histological study of this fish concludes that ova diameter and ovary histology of fully ripe females and spent fishes during different months indicates the spawning season is June to October, being a synchronous spawner. The average percentage composition of male and female indicates that the females out numbered the males. The length at first maturity (50%) is estimates as 260.5 mm, The fecundity reveals that the number of eggs produced was found to be increase with increase in total length; total weight and ovary weight and fecundity shared a good correlation with body weight and ovary weight. The diameter of fully ripe ova 0.92mm and there are six different developmental stages of ovary.

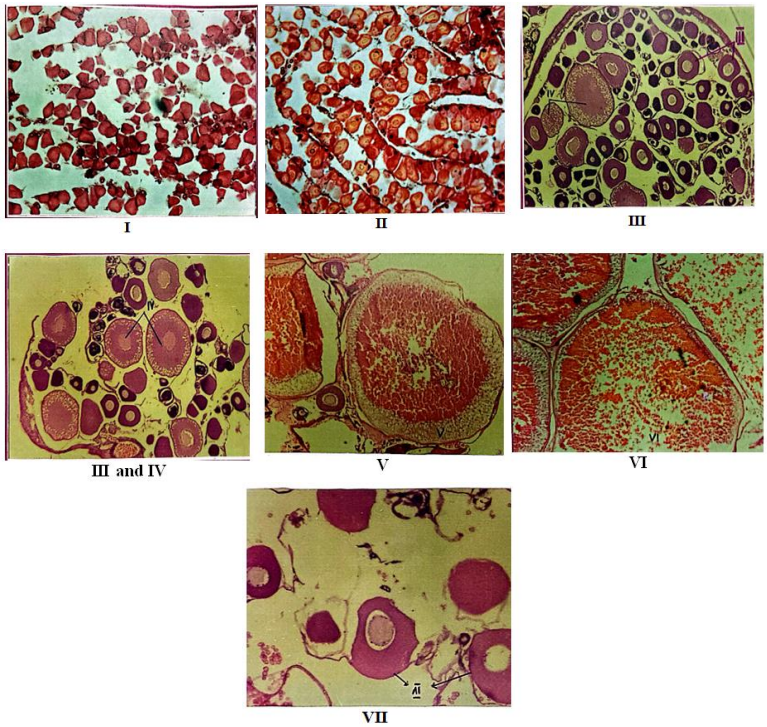

Plate 1. C.S of ovary showing different stages of development I –VII.

It is observed from frequency of ova diameter study that *O. ruber* spawns in one distinct period as the mature eggs are far separated from the immature stock. This type of behavior has also been reported on this species by Chacko (1949) from Gulf of Mannar, Devadoss (1969) from Bombay, Pillai (1983) from Port Novo water. Walsh *et al.* (2003) was also found almost a similar opinion about the lobes of ovary, their shape, location, commencing and ending position in case of Australian long finned river eel, *Anguilla reinhardtii*. Colombo *et al.* (1984) observed the ovaries of European eel, *Anguilla anguilla*, to the naked eye as frilled ribbon organs of various widths. The analysis of histological sections of the ovaries of *O. ruber* during annual cycle shows that in the month of September the specimens that will take part in spawning the following years, both immature and early maturing oocytes can be distinguished and there is rapid growth in diameter of oocytes which begins in December as a consequence of exogenous vitellogenesis, and later endogenous vitellogenesis in February which is in accordance with the studies of synchronous spawner like *Colisa faciata* (Bhatti and Javid 1973), *Liza subviridis* (Chan and Chua 1980), Pike *Esox lucius* (Treasurer, 1990) and Coho *Oncorhynchus kisutch.* In addition to this, ripe ovary with thin and hypertrophied follicular layer was observed during the month of July to August confirmed the single spawning season of *O. ruber*. The spent and atretic oocytes are observed in the ovary during the month of July to September.

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