IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 9, Issue 2 Ver. I (Feb. 2016), PP 01-05
www.iosrjournals.org

Study of gonad development using ova diameter analysis in ribbonfish, *Lepturacanthus savala* (Cuvier, 1829)

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Abstract: *Preset paper analyses the changes in ova morphology to assess the reproductive development in ribbonfishes, Lepturacanthus savala. During the study, ova diameter observation showed Stage I (Immature ova) - majority in size range of 0.24-0.26 mm, Stage II - majority in size range of 0.54-0.56 mm, Stage III A - majority in size range of 0.63-0.65 mm, Stage III B - majority in size range of 0.90-0.92 mm, Stage IV A - majority in size range of 1.38-1.40 mm, Stage IV B - majority in 1.57mm, Stage V - majority in size range of 1.59-1.61, Stage VI A - majority in size range of 0.65-0.67 mm, Stage VI B - majority in size range of 0.32-0.34. The stages of oocytes classified according to the diameter were in accordance with the macroscopic analysis of the ovaries and peak spawning season appeared to be from March to May and October to December along the west coast of India.*

I. Introduction

The ribbonfishes (Family: Trichiuridae), also called as the hair tails or cutlass fishes elsewhere, occupy an important place among the food fishes of India. Among the species of ribbonfishes, *Lepturacanthus savala* is an important species contributing the fish catch of Ratnagiri coast of Maharashtra, India. They are abundant and cheap and as such are also preferred by poor people. Ribbonfish form a major and abundant fishery resource among the marine pelagic fin-fishes of the Indian seas. They are distributed in the Atlantic and Indo-Pacific regions as well as all along the Indian coast with abundance in the northwest and central east coasts. Occurrence of *L. savala* in high numbers is recorded during October – December in nearshore waters along Ratnagiri coast.

The ribbon fishes move to the inshore areas of the continental shelf during monsoon, remain close to the shore in areas less than 60 m depth in the post monsoon period and contribute abundantly to the fishery. During maturation period, they move away from the coastal areas to deeper regions (Lazarus et al., 1992). Due to the seasonal availability of this species and occurrence of juveniles in the catches, studies on the reproductive biology were undertaken along this coast from February 2012 to February 2013. Ova diameter gives better information of the maturity and the overall developmental stages as well as the initiation of spawning. Preset paper analyses the changes in ova morphology to assess the reproductive development in *L. savala*.

II. Materials and methods

For ova-diameter studies of intra-ovarian eggs, small pieces of ovaries from the anterior, middle and posterior region were cut and then ova were teased out on to a glass slide. The ova diameter was measured by stage micrometer and occulometer. The process of development of ova from one stage of maturity to another was studied by using Motic Image Plus 2.0 Digital Microscopy Software. For ova diameter study, a total of 300 ova were measured in mm from individual fish in various stages of maturity. Frequency polygons were drawn for all stages of maturity. The maturity stages classified depending only on the macroscopic structure were described as by Brown et al. (2008).

III. Results

During the period of study specimens were randomly selected and their length, weight and the weight of ovaries were also recoded. The total numbers of mature ova in these specimens are given in Table 1. Representative samples of ovaries in different stages of maturity were selected, and 300 ova measured from each of these ovaries were assorted into modal groups and the percentage occurrence of each group was calculated and average values were found. The ova diameter frequency in each stage of maturity is given in Fig. 1.

The maturity stages classified depending only on the macroscopic structure were described as by Brown et al. (2008) as follows.

Stage I — Immature : In this stage, the ovaries appeared very small and transparent. They occupied less than one third of the body cavity. Ova were not visible to the naked eye, were irregular and transparent. Yolk formation had not commenced. Majority of the ova in this stage ranged in size between 0.01 to 0.09 mm and

with the maximum size of the ova up to 0.19 mm. Testes were very slender and thin, occupying about the same space as the ovaries.

Stage II — Maturing : Ovaries in this stage were slightly larger, occupying more than one-third of body cavity. The mode of the maturing group of eggs fell at 0.34 mm, the maximum size of eggs being 0.47 mm. Comparatively testes in this stage occupied lesser space than ovaries. They were narrow and ribbon-like.

Stage III A — Early mature: Ovaries occupied about half of the body cavity and appeared distinctly yellow in colour. The ovarian wall was thick. Ova were spherical, opaque and with a full deposition of yolk. The mode of the largest group of ova fell at 0.62 mm, the maximum size of the ova being 0.79 mm. Testes in this stage appeared creamy white, long and ribbon-shaped. They extended up to half the length of body cavity.

Stage III B — Late mature : Ovaries in this stage occupied about three fourths of the body cavity and retained the distinctly yellowish colour of the previous stage. The ovarian wall became thin. The mode of the largest group of ova in this stage fell at 0.81 mm, the maximum size of the ova being 1.14 mm.

Stage IV A — Early ripe : Ovaries were enlarged and occupied almost the entire body cavity. They lost the yellow colour of Stage III. The ovarian wall became almost transparent and the intra-ovarian eggs appeared light yellow in colour. Ovaries were fully packed with large eggs and the largest common egg diameter fell at 1.38 mm, the maximum size of the eggs being 1.61 mm.

Stage IV B — Late ripe : Ovaries occupied the entire body cavity. Eggs turned semi-transparent and the oil-globule was fully formed measuring 0.53 mm. The largest common egg diameter in this stage fell at 1.57 mm.

Stage V — Spawning : The mode of the largest ova was above 1.57 mm, and the maximum size of the eggs above 1.71 mm.

Stage VI A — Partially spent : In this stage the ovaries occupied nearly half of the body cavity. The stage resembled stage III but differed from it in the relatively smaller size and the loosely packed nature of the ovaries, which were occupied by the residual eggs. In fresh condition some blood patches were also seen. The common egg diameter fell at 0.62 mm and the maximum size being 0.85 mm.

Stage VI B — Fully spent: In this stage, ovaries appeared quite shrunken and flaccid. Majority of the ova were small, transparent, invisible to the naked eye and belonged to the immature stock. Scattered amongst them, a few large whitish granular ova were visible to naked eye. The mode of the largest group of eggs fell at 0.34 mm with the maximum size up to 0.47 mm.

Following trend in ova was observed:

Stage I (Immature ova) - majority in size range of 0.24-0.26 mm, Stage II - majority in size range of 0.54-0.56 mm, Stage III A - majority in size range of 0.63-0.65 mm, Stage III B - majority in size range of 0.90-0.92 mm, Stage IV A - majority in size range of 1.38-1.40 mm, Stage IV B - majority in 1.57mm, Stage V - majority in size range of 1.59-1.61, Stage VI A - majority in size range of 0.65-0.67 mm, Stage VI B - majority in size range of 0.32-0.34.

Stage I oocytes were transparent and pear- or round- shaped. The nucleus occupies most of the cell and a thin follicle layer surrounds the oocyte. In stage II, oocytes were round and had multiple nucleoli. Both lipid bodies and the yolk vesicles (cortical alveoli) were difficult to identify. In stage IIIA, yolk granules were found in peripheral regions and appeared as small opaque spheres when examined under a light microscope. Follicular layers composed of theca cell layer, granulosa cell layer and zona radiata increased in thickness. Nuclei were no longer observed because the yolk granules conceal them.

As stage IIIA advanced further, more yolk granules were seen. Yolk globules then aggregated and occupied the entire central area of the oocytes. Follicular layers further increased in thickness. In stage IIIB, centripetal yolk globules started to fuse together and oocytes became more transparent. Larger droplets of lipids were also seen. Stage IV was seen as lipid bodies fused with one another to form one large oil droplet. Oocytes become transparent and nuclei disappear (germinal vesicles breakdown). Oocytes became hydrated and reached maximum size. Stage V showed hydrated.

IV. Discussion

James (1967) reported that the ova diameter frequency polygon of a mature ovary of *L. savala* showed certain amount of similarity with that of a mature ovary of E. intermedius. While this fact suggests that spawning in these two species takes place at about the same time, the duration and the frequency of spawning may be variable in the species. A total of 750 ova were measured at random from the anterior, middle and posterior regions of the ovary. It was found that the distribution of ova in these three regions of the ovary was uniform. Ova measuring less than 0.11 mm represented immature stock. Ova measuring less than 0.11 mm were immature, transparent, and invisible to naked eye. The next group of maturing ova, visible to naked eye, yellowish with deposition of yolk, ranged in size between 0.20 to 0.47 mm with a mode at 0.34 mm. The third group of mature ova ranged in size between 0.87 to 1.42 mm with a mode at 1.10 mm. The last group of ova was large and opaque which were destined to transform into ripe eggs and be shed in the ensuing spawning

Table 1. Number of mature ova in individuals of *L. savala* along the Ratnagiri coast

Sr. No.	Total length of fish (cm)	Weight of fish (g)	Weight of ovary (g)	Total no. of mature ova in ovary
1	67	264	3.7	7555
2	65.7	280	17.58	22619
3	64	238	14.9	21783
4	64	184	3.54	16184
5	63	154	4.12	13628
6	62.8	178	6.91	10752
7	62.5	218	50.6	25535
8	62	184	25.1	14809
9	61.5	184	6.25	8612
10	61.5	170	8.75	14233
11	61	184	11.8	16307
12	60.8	168	8.31	15384
13	60	200	3.95	10380
14	60	184	4.95	11919
15	60	180	24.58	15370
16	60	174	11.99	14859
17	59	130	6.21	8395
18	57.8	146	5.83	9417
19	57.5	136	7.3	10794
20	57.4	148	7.53	12394
21	57.2	120	3.49	1421
22	57	128	8.87	8603
23	55	122	6.27	9354
24	54.5	104	8.56	8719
25	49.5	78	7.57	9916

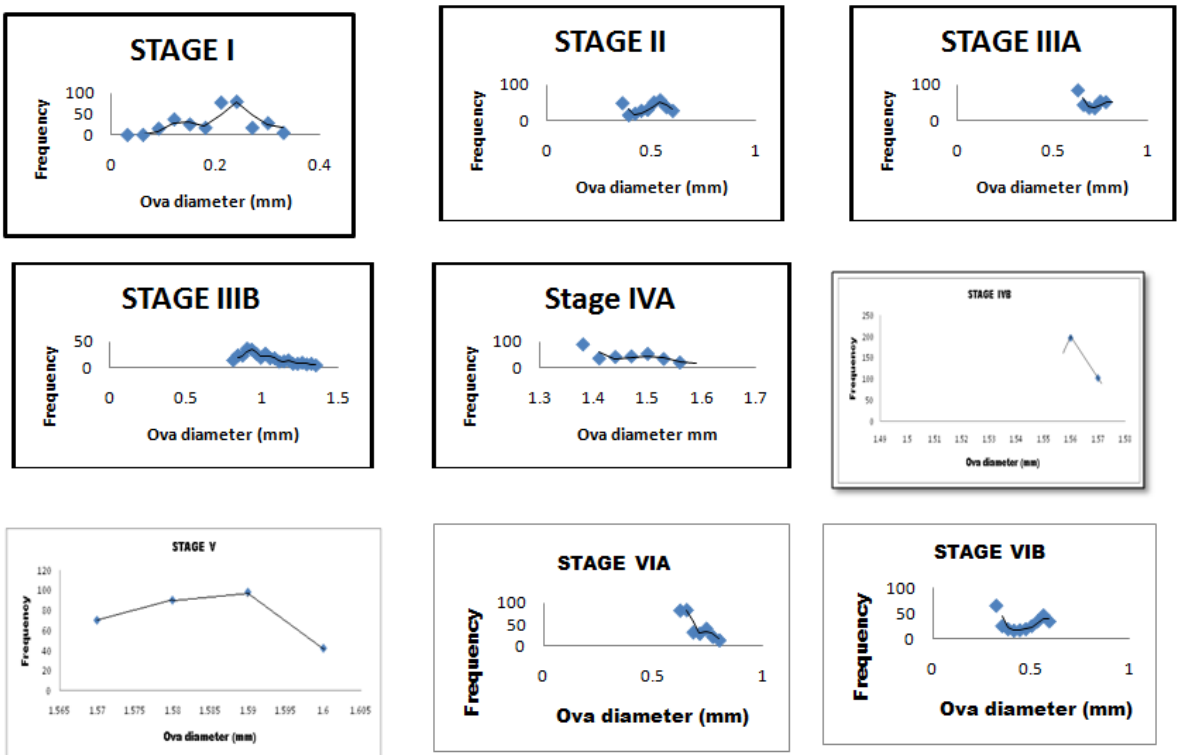
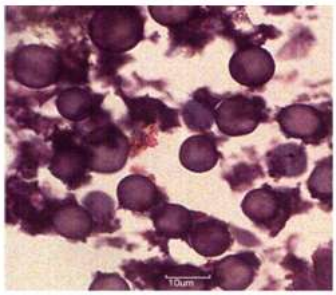
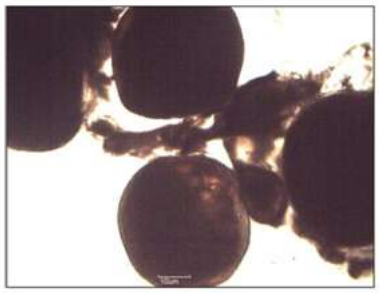


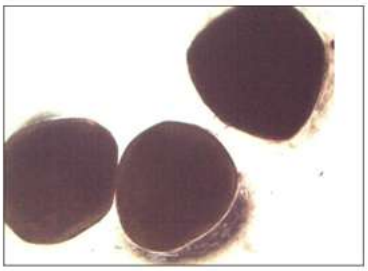
Fig.1. Ova diameter frequency in each stage of maturity in *L. savala*



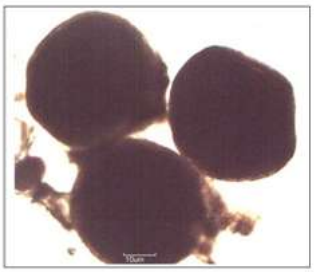
Stage I: Diameter 0.11 to 0.33 mm



Stage II: Diameter 0.34 to 0.61 mm

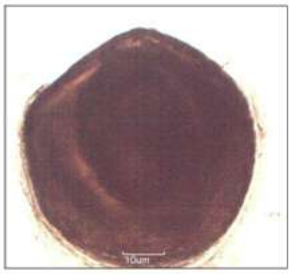


Stage IIIA: Diameter 0.62 to 0.80 mm



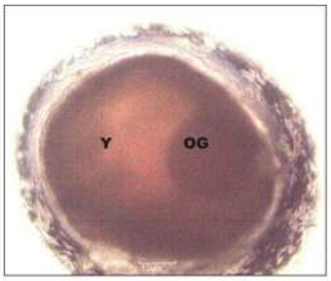
Stage IIIB: Diameter 0.81 to 1.37 mm

Stage IVA: Diameter 1.38 to 1.56 mm



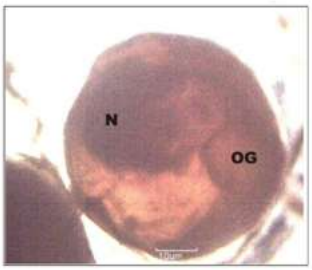
Stage IVB: Diameter 1.57 mm

Plate1. Variation in ova diameter of *L. savala*



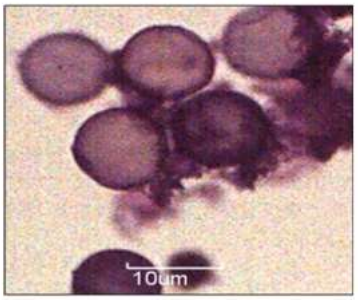
Stage V: Diameter 1.57 to 1.62 mm

Y- Yolk, OG - Oil globule

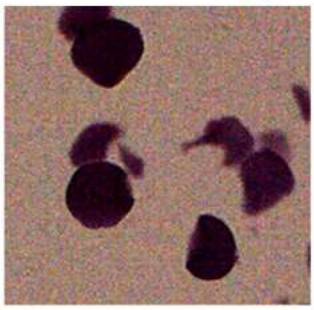


Stage V: Spawning

N - Nucleus, OG - Oil globule



Stage VIA: Diameter 0.62 to 0.80 mm



Stage VIB: Diameter 0.32 to 0.62 mm

Plate 2. Variation in ova diameter of *L. savala*

Season. He also reported the classification of maturity stages based on the macroscopic appearance and the microscopic structure of ova in the females.

During the study, oocytes of smaller diameters were also encountered in developing ovaries. Microscopic analysis of the ova was also done during the present study. The stages of oocytes classified according to the diameter were in accordance with the macroscopic analysis ovaries (plate 1 and 2). Stage I oocytes were transparent and pear or round shaped. The nucleus occupies most of the cell and a thin follicle layer surrounds the oocyte. In stage II, oocytes were round and had multiple nucleoli. Both lipid bodies and the yolk vesicles (cortical alveoli) were difficult to identify. In stage IIIA, yolk granules were found in peripheral regions and appeared as small opaque spheres when examined under a light microscope. This marked the beginning of vitellogenesis. Follicular layers composed of theca

cell layer, granulosa cell layer and zona radiata increased in thickness. Nuclei were no longer observed because the yolk granules conceal them. As stage IIIA advanced further, more yolk granules were incorporated, they coalesced to form yolk globules. Yolk globules then aggregated and occupied the entire central area of the oocytes. Follicular layers further increased in thickness. In stage III B, centripetal yolk globules started to fuse together and oocytes became more transparent. Lipid bodies began to fuse to form larger droplets and resulting in few numbers. Stage IV was seen as lipid bodies fused with one another to form one large oil droplet. Oocytes become transparent and nuclei disappear (germinal vesicles breakdown). Hydrated oocytes reached maximum size and were ready to be spawned. Stage V showed hydrated ova reached to maximum size and spawning was commenced. Empty follicles occurred after spawning and they were difficult to identify by whole oocyte examination because of the rapid degeneration of post - ovulatory follicles. During stage VI, oocytes were seen in atresia (degeneration). Atresic oocytes were characterized by separation of different follicular layers (e.g. zona radiata separates from granulosa cell layer), irregular shapes of oocytes, change in yolk appearances, and the breakdown of outer membranes. The degree of formation of yolk in maturing and mature ova was taken as a reliable guide in judging the stages of maturity of the intra-ovarian eggs.

Chacko (1950) mentioned that the eggs of *L. savala* measure 2.15-2.40 mm with a 0.7 mm yellowish oil globule. According to James (1967) ova measuring less than 0.11 mm represented immature stock. Ova measuring less than 0.11 mm were immature, transparent, and invisible to naked eye. The next group of maturing ova, visible to naked eye, yellowish with deposition of yolk, ranged in size between 0.20 to 0.47 mm with a mode at 0.34 mm. The third group of mature ova ranged in size between 0.87 to 1.42 mm with a mode at 1.10 mm. The last group of ova was large and opaque which were destined to transform into ripe eggs and be shed in the ensuing spawning season.

The presence of oil globules in fish eggs and larvae is ecologically and phylogenetically widespread. Prior to feeding the embryo and yolk - sac larva rely solely on endogenous energy sources--those contained in the yolk and oil globule (Eldridge et. al., 1977). The oil globules present in the eggs of *L. savala* may be used as a source of energy to the developing embryo and the larvae.

Kwok and Ni (1999) classified the oocytes in two *Trichiurus spp*. They found that in comparison with macroscopic ovary observations, the whole oocyte examination was more accurate in staging the development of ovaries. The accuracy is attributed to the characteristics of the oocytes of these species-for example the clarity of the oocyte and its content, fusion of yolk globules and subsequent increased transparency, an ultimate large oil droplet and oocyte expansion induced by hydration.

In the present study, peak spawning season appeared to be from March to May and October to December. There was a brief resting period between the two spawning peaks, during which the ovaries and testes continued the gametogenesis. The microscopic analysis of the oocytes classified according to the diameter was in accordance with the macroscopic analysis of the gonads. The fully developed and ready to spawn ova showed a large oil globule. The gametogenesis progressed from January onwards with a first peak from March to April. The gonads showed a brief resting phase and the oocytes retained in the ovary in stage III and IV progressed further and spawning again commenced from October to December.

V. Conclusion

Present study indicated that *L. savala* had two spawning peaks and the ova in all the stages were present in the ovary. Oil globules were present in the maturing ova which may be used as energy source by the embryo and the lavae.

Reproduction is the process by which species are perpetuated. It is also a continuous developmental process throughout ontogeny, requiring energetic, ecological, anatomical, biochemical and endocrinological adaptations. An understanding of the reproductive biology of a species is a central aspect of providing sound scientific advice for fisheries management. Study of the ova diameter provides an insight about the reproductive developments and the spawning season in an organism. Present work analyses the ova diameter of ribbonfish, as this would give a better idea about the reproductive development in *L. savala.*

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