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Reproductive biology of common silver Biddy, *Gerres filamentosus* (Cuvier)

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ABSTRACT

Common silver biddy Gerresfilamentosus *were collected monthly between November 2002 and November 2005 fromFort Kochiof Kerala. The seasonal reproductive cycle of this species was investigated using histological observation of gonads. On the basis of morphological and histological changes, which the ovaries undergo during maturation, five stages of maturity are identified such as immature, maturing and recovering spent, mature, ripe and spent. From the histological study of Oogenesis, a number of distinct developmental stages were delineated in G.filamentosus, viz. the oogonia,Chromatin nucleolus stage, Early and Late Perinucleolusstages,Lipid droplet stage, Cortical alveoli(Yolk vesicle) stage,Yolk granule stage and Mature oocytes.*

Keywords: *Gerresfilamentosus*, Fort kochi, Histology, Ovary, Oogenesis

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INTRODUCTION

In the endeavour of rational exploitation of fishery resources through the application of biological principles and intensive aquaculture of fishes through selective breeding, brood stock development, domestication and genetic improvement, studies on reproductive biology of fishes have attracted considerable attention.In the study of the biology of fish, maturation process of gonads forms an important aspect as it leads to theunderstanding of the reproductive characteristics and breeding behaviour of the fish. With advances made in the histological and cytological methods, the process of oogenesis and spermatogenesis have been studied in greater detail in recent years. The success or failure of a species in any environment largely depends on its spawning potential. To overcome the various physical and biological hazards in the environment and to attain successful recruitment, fishes have a high reproductive potential. Information on the various aspects of reproductive mechanism and breeding biology of fishes is therefore an essential pre-requisite for the successful management of both capture and culture fisheries.In view of the paucity of works on the histological and cytological studies on gonad development of Indian marine teleosts in general and Gerrids in particular, the present investigation on *Gerresfilanientosus* was carried out.
The reproductive biology of *Gerrids* of Indian waters have been reported by several workers (Jones and Sujansingani, 1954; Jhingran, 1957; PrabhakaraRao 1970, Patnaik,1971, Kurup and Samuel 1984, Sivashanthini and Ajmal Khan, 2004). The histological studies of *G.filamentosus* is still unknown. Little information is available on the reproductive cycles of Gerridae although Austin (1971) and Etchevers (1978) studied the breeding of *Diapterusrhombeus* in the offshore waters of Puerto Rico and Venezuela; Charles (1975) investigated the reproductive cycle of *Eucinostomusgula* in Biscayne Bay, Florida, U.S.A.

MATERIALS AND METHODS

The fishes for the study were collected using different types of fishing gears like gill nets, seines and cast nets from Fort Kochi(Fig.1).

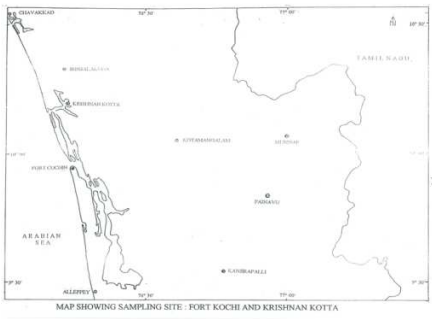

Fig.1 Map showing the collection site

Microscopic examination of the gonad is essential since the sex can be identified macroscopically except for the ripe male and female fishes. The fishes were bought to the laboratory in fresh condition and the total length, standardlength, and weight of each individual fish were noted. For histological studies portions of the anterior, middle and posterior regions of the gonads dissected out, from the freshly killed specimens were fixed in Bouin's fixative.The tissues were then transferred through graded alcohols (70-95 % [v/v] before final dehydration in absolute ethanol. The alcohol contained in the tissues were next eliminated by immersing them in xylene. The tissues were then impregnated with paraffin, which is soluble in xylene, at 60^0C and embedded in paraffin. After processing, 5µm sections were stained using Haematoxylin-eosin staining procedure.

RESULTS

3.1 Gonad Development

Gonads of inactive and immature specimens are thin, transparent tubes, but those of mature and ripe individuals occupy most of the abdominal cavity. Ripe ovaries are the shape of thick, stubby cigars and ripe testes rather flattened with irregularly shaped margins. In males and females both left and right gonads develop and are active throughout gonad development.

3.2 Classification of gonads or quantification of maturity stages

Based on the colour, size , shape and microscopic observation, teleost gonad can be classified into different maturity stages to quantify gonadal maturation. Different workers have adopted various maturity schemes to classify gonads. The most common one is a five-point maturity scale, ideal for most of the tropical total spawners as developed by Qasim. More or less similar to the scheme given for *Blenniuspholis* L. and *Centronotusgunnnellusgun* (L) (Qasim 1957 a and b), five stages of maturity were drawn on the basis of the general appearance of gonads (Table. 1).

3.2.1 Histology of the ovary

Early in development, longitudinal ridges arise on the ventrolateral surface of the developing ovaries, and fuse to enclose a cavity which is purely coelomic and is lined by the peritoneal epithelium, unlike the ovarian cavities of other vertebrates, which are lined by mesenchyme.

3.2.3 Ovarian histology of *Gerresfilamentosus*

1)Immature virgins :- Oocyte in protoplasmic growth stage, no oocytes beyond a perinucleolar stage of development, Oogonial nests are visible in the epithelium of ovarian lamellae which are strongly basophilic. Ovarian wall thin.
2)Maturing virgins or Recovering spents :- Chromatin nucleolus stage and early perinucleoli stages appear. Larger oocytes with granular cytoplasm and yolk nucleus. Ovarian wall thickens. Basophilia decreases.
3)Maturing/ Ripening :- Oocytes continue to increase in size. Late perinucleolus stage oocytes with less basophilic cytoplasm visible. Endogenous yolk vesicles appear along the periphery of larger oocytes and may reach half way to the nucleus.

4)Ripe :- Yolk vesicles of stage VI oocytes coalesce and become larger in size. Follicular layer of these oocytes becomes clearly visible. Exogenous yolk accumulates and yolk granules appear. ,
5)Spent :- Numerous post ovulatory follicles are present, residual hydrated oocytes may also be present.
Oocytes of *Gerresfilamentosus*followed, the general pattern of development and histology found in other teleosts. Oocyte development and reproductive strategy have been described in many marine teleost species in an effort to understand the time course and energetic consequences of reproductive effort.

Table.1: STAGES OF GONADAL MATURITY

FEMALES	MALES
STAGE I	
IMMATURE VIRGINS '	**IMMATURE VIRGINS**
The ovaries appear as two pinkish translucent jelly like structures united at the posterior end; ovaries very small, elongated and cylindrical, rather oblong in shape; entire gonad occupies 1/4 of the body cavity of fish; Oocytes under magnification appear irregular; transparent and with a centralnucleus.	Testes pinkish and translucent, Tesets very small 0.3 to 0.5cm in length
STAGE II	
MATURING VIRGINS OR RECOVERING SPENTS	**MATURING VIRGINS OR RECOVERING SPENTS**
Ovaries slightly enlarged occupying more than one-third of the body cavity. At this stage each ovarian lobe becomes cylindrical and appears dull reddish. The two lobes will be unequal in size, the right lobe is longer than left. It occupies almost 1/2 of the body cavity. Under microscope, central portion of the eggs appear darker.	Testes pinkish and opaque, still small, Slightly distended but not soft
STAGES III	
MATURIG OR RIPENING	**MATURIG OR RIPENING**
Ovaries enlarged and occupaying ¾ th or more of the body cavity, pinkish yellow in colour. Ovarian will becomes thin, ova round, opaque and appear as dark bodies under microscopes	Testics whitish outer nargin slightly irregular, testis extending 3/4thoft the body cavity
STAGE II	
RIPE	**RIPE**
Ovaries very much enlarged, massive ovarian lobes, fully packed with the ripe eggs, occupying the whole of the body cavity, with numerous blood vessels ramifying over their surface, Yellow and opaque. Some ova visible to the exterior at the vent region.	Testis extending the full length of the body cavity, very soft and creamy white, outer margin irregular with wrinkles
STAGE V	
SPENT	SPENT
Highly shrunken flesh colored and collapsed ovaries occupaying almost ½ of the body cavity. It appears blood-shot, flaccid and loosely packed with primary oocytes. Blood vessels seen on surface. Often some residual eggs are available in the ovarian sac	Testis extending nearly ½ of the bodycavity, dull white and shrunken

3.3 Main stages of oogenesis of *Gerresfilamentosus.*

Stage I: Oogonia

Oogonia were small, round cells with a .relatively narrow zone of clear cytoplasm and a single, prominent nucleolus in the nucleus. They occurred as solitary cells or in small nests in the epithelium of the ovarian lamellae and were always resent . It is strongly stained with haematoxylin. These cells were observed rarely because they are immersed in the stroma; and this tissue was generally lost during processing. (Plate I)

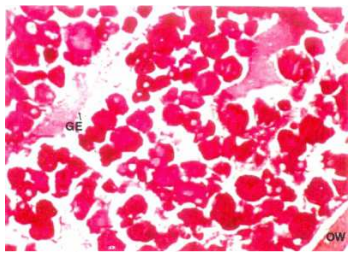

Plate. I: T.S of immature ovary showing the ovarian wall and early developing oocytes X 40

Stage II.Chromatin nucleolus stage

In these primary oocytes, initiation of meiosis had taken place, resulting in the early prophase I. They were

characterized by a nucleus containing one single nucleolus surrounded by chromatin threads. The oocytes had weakly basophilic cytoplasm with a more basophilic nucleus. One or more darkly staining nucleoli were observed within the nucleus (Plate II).

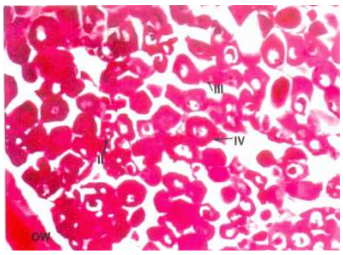

Plate. II: A section of Stage II ovary showing perinucleolus stage oocyte X 100

Stage III: Early perinucleolus stage

This stage was characterized by the first significant increase in size of the oocytes, caused by enlargement of the nucleus as well as the cytoplasm. Numerous; relatively large, basophilic nucleoli appeared at the periphery of the nucleus, indicating increasing nuclear activity. The cytoplasm was homogeneous and strongly basophilic. The first follicular cells started to appear around the oocytes, in close contact with oocyte membrane (Plate II).

Stage IV: Late perinucleolus stage.

The cytoplasm was less basophilic but it was still a uniform shade of purple. A flattered follicular layer surrounding the oocytes could be distinguished at the end of this stage, which is the last one of the first growth phase. In contradiction with the other stages described, this stage was not present throughout the whole year(Plate II).

Stage V. Lipid droplet stage

During this stage of oocytes development yolk Vesicles or'lipid vesicles' (Mayer *et a1.*,1988) appeared in the cytoplasm as unstained `empty' vacuoles. The cytoplasm continued to become less basophilic and no longer stained uniformly. The nucleus began to become irregular in shape(Plate III).

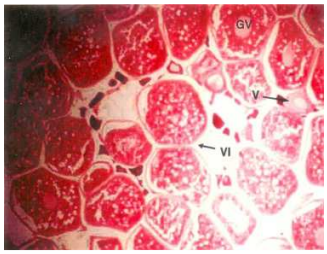

Plate. III: Photomicrograph showing stage V and stage VI oocytes X 40

Stage VI. Cortical alveoli (yolk vesicle) stage

Vacuoles containing glycoproteins form at the cell periphery and increase in number as the oocyte grows. Transparent cortical alveoli, appeared in this first stage of the secondary growth phase, situated at the periphery of the oocyte. The zonaradiata had become visible. On reaching this stage a non-cellular membrane begins to form between the follicular layer(theca and granulosa) and the developing oocyte, this is the zonaradiata. The membrane becomes progressively thicker. This stage was easily distinguished by the acidophilic yolk granules with slightly basophilic cytoplasm. Mayer *et al*., (1988) identified these bright pink structures as protein yolk granules (Plate IV).

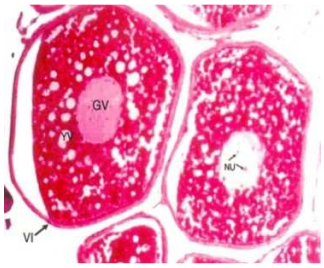

Plate. IV: Magnified portion of stage VI X 100

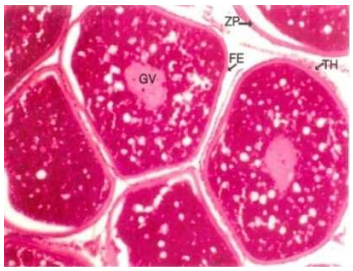

Plate. V: Photomicrograph showing the details of follicular layer of stage VI oocyte X 100

Stage VII. Yolk granule stage

The progression of stage VII development in *Gerres*is illustrated by the early, peripheral development of the secondary yolk component which appears as small red-staind granules. The yolk component is now found throughout the cytoplasm although numerous primary yolk granules are still present. The cytoplasm is almost completely filled with secondary yolk and only a few primary granules remain (Plate VI).

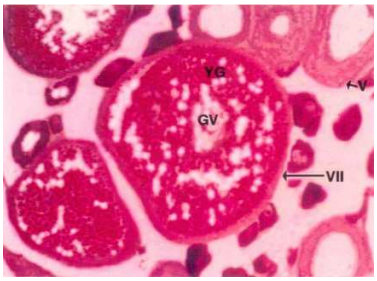

Plate. VI: Photomicrograph of vitellogenic follicle corresponding to stage VII . Note the stage V oocyte lying adjacent X 100

Stage VIII. Mature oocytes

The envelope layers were clearly observed at this stage, and the zonaradiata was greatly enlarged; the theca interna had small blood vessels to each oocyte. The vitellogenic granules were very conspicuous and well distributed. The nucleus was central, surrounded with cortical alveoli. Although final oocyte maturation is restricted to a limited period, all other phases did not occur at a fixed moment in the annual cycle (Plate:VII).

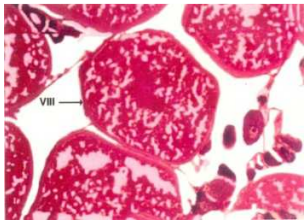

Plate. VII: T.S of ovary of spawning phase showing ripe eggs (stage VIII) ready for ovulation X 40

DISCUSSION

A perusal of the various reports available on the maturity and spawning of *Gerres* of different regions of India indicate that there is no uniformity in respect of the time of spawning at different places. The observations in this aspect by different authors are inconsistent. However, the monthly distribution of the maturity stages of *Gerresfilamentosus* from two different localities shows one specific period of availability of spent specimens in a year. This suggests the probability of one spawning period in the local population. The fully mature group of ova were not sharply differentiated from maturing group and hence it could well be inferred that the process of maturation was continuous and the spawning might take place within a prolonged tune. Since there was only one group of ova with advanced stage of maturation in the fully ripe ovary, it can also be presumed that individual fish spawned only once within a definite spawning period in an year. These observations agree well with that of PrabhakaraRao (1970) and Patnaik (1971) who noticed that *Gerresoyena*sand *Gerressetifer* spawn only once in an year in Pulicat and Chilka lakes respectively and the spawning period was a prolonged one.

In *G.filamentosus* a single protracted period of relativity high percentage of fully ripe male and females are met with from Oct to Feb indicating an extendedperiod of spawning. The presence of spent fishes throughout this period further supports this inference. This observation is in agreement with the study made by (Kurup and Samuel,1983). An investigation of the reproductive state and gonad development of *Gerridae* in Natal(Cyrus andBlaber 1984) showed that the mature or ripe gonads were observed in *G. acinaces* during all four seasons and in*G.filarnentous* and *G.rappi*in summer, autumn and winter.

Results from this study have shown that *Gerres* do not breed in estuaries and this supports the statement by Wallace(1975). Blaber and Blaber(1980) however, recorded recruitment of *G. Oyena* only during the winter and *G. ovatus* only during summer and autumn, in Moreton Bay, Queensiand, Austraila In Puerto Rico, Austin(1971) found that the *GerridDiapterusrhombeus* spawned in the outer margins of muddy bays and that the fry moved into mangrove areas and shallow muddy bays, which acted as the nursery grounds.

Large numbers of Juvenile marine teleosts occur in Indo-Pacific estuaries and for many species a period in the estuarine environment is an obligate phase of the life history(Wallace,1975;Blaber and Blaber,1980). Few marine fish species spawn in estuaries but fry enter estuaries shortly after hatching in adjacent coastal waters: (Wallace, 1975).

However, the increase in the frequency of spawning fish during some months suggest that there is a period of peak spawning. *Gerressetifer* and other species have been seen in catches m large numbers during the rainy season specially in the central and southern sectors, though according to Chaudhuri (1923) *G. Setifer* is a dry weather visitor and does not breed in the lake.

A study of the major environmental parameters of the Cochin back water during this period-.revealed very little fluctuation in temperature . However, the dissolved oxygen content and salinity varied over a relatively wide range during the period of the study. These hydrological parameters agree with those recorded by earlier workers (George and Kartha,1963; Ramamritham and Jayaraman,1963).

A comparison of the distribution of different maturity stages and the temperature in the study area indicates that temperature has not any profound influence on the reproductive cycle under natural condition in *Gerresfilamentosus*. This may be due to the relatively small range of temperature which is found to vary from 27-30 °C.

However, higher GSI was observed; either when the temperature of the surface water was seen decreasing from the maximum peak or increasing from the lower values recorded during the monsoon months. It may also be observed here that since the area chosen for the study does not exhibit great fluctuations in temperature or photoperiod, it' is very likely that the reproductive cycles of the *Gerres* population in this region might not have been timed as per environmental cue alone. It is highly probable therefore that the timing of the reproductive cycle in the *Gerres* of the indigenous population of Vembanad lake is endogenously controlled under the natural conditions.

All records on *Gerres* spawning in nature show a strong preference for oceanic water as the medium of incubation. A number of species of *Gerres* are known to exhibit spawning migrations towards sea-The breeding biology of fishes of the family Gerridae of Pulicat and Chilka lakes was investigated by PrabhakaraRao (1970) and Patnaik (1971). In

Chilka lake, Jones andSujansingani (1954) observed the male specimens of *G. Setifer*in ripe condition with the flowing milt and female with ovary almost in ripe condition but they were notable to say whether the species bred in the lake or not. PrabhakaraRao (1970) stated that *G. oyena* with oozing gonads were not encountered from Pulicatlake and so he presumed that the final stage of maturity of this species was attained only in the sea. A similar observation was also reported by Cyrus andBlaber (1984) in *Gerres*of Natal estuaries. Gonad histology revealed that no females from the kosi system had oocytes beyond stage V (red staining yolk stage), but males completed sperm development in the system. *Gerres* leave Kosi on attaining these stages of development and completion of female gonad development probably take place in the sea. Further evidence of *Gerres* leaving the estuarine environment to spawn was provided by the presence of large shoals of adult *G. rappi* at the estuary mouth during the summer and autumn of 1979 and 1980. *Gerresacinaces* also migrate to the marine environment along the east coast of southern Afiica. Results from this study have shown that *Gerres* do not breed in estuaries and this supports the statement by Wallace (1975).

The effect of dissolved oxygen on gametogenesis has not been studied in detail by many authors. Low dissolved oxygen is known to prevent spawning in *Pimphalespromelas* (Brungs,1971) and in *Proximusnigromaculates* (Carlson and Herman, 1978). Gillet*et al*., (1981) found that in temperate gold fish, low levels of dissolv6d oxygen caused gonadal regression. This is probably true of tropical gold fish also (Lam,1983).Dissolved oxygen level was not considered as a limiting factor for gametogenesis in *Gerres.*

4.1 Oocyte growth

The development of oocytes in all teleosts is basically the same, with slight differences in yolk composition, yolk deposition, rapidity of growth and surrounding membranes .The early oocyte has a large nucleus and small amount of basophilic cytoplasm . Growth is due mainly to an increase in non-yolky cytoplasm until primary and secondary yolk are laid down.

Oocyte development and reproductive strategy have been described in many marine teleost species in ` an effort to understand the time course and energetic consequences of reproductive effort. Oocyte growth follows a similar general pattern in most teleosts. Oogonia give rise to immature oocytes with multiple peripheral nucleoli. The perinucleolar oocytes then undergo primary vitellogenesis the accumulation of mucopolysaccharides in cortical alveoli(Khoo 1979). The oocyte enlarges and its zonaradiata thickens as vitellogenic yolk is deposited .The nucleus migrate to the animal pole prior to the break down of the nuclear membrane (Yamamoto, 1956). ,

Hydration precedes ovulation and the appearance of these hyaline oocytes is an indication of imminent spawning.The follicle collapses after the oocyte has been released to form; structures called post ovulatory follicles(POFs) which are indications of recent spawning and are not thought to persist for a long time.Oocytes of *G. filamentosus*followed, the general pattern of development and histology found in other teleosts.

4.2 Histology of oogenesis

The primordial germ cells of *G. filamentosus* are ovoid and larger than the somatic cells. The oogonia which represent the stem cell population; giving rise to oocytes are found in ovaries of *G.filamentosus*. The dynamics of early oogenesis in *G.filamentosus* are, in general, in agreement with previous observations, on other teleosts(Yamamoto 1965; Brusle, 1981; Begovacand Wallace 1988). The present results provide evidence for the cytological changes of great interest during the first fundamental steps of oogenesis, which are little known than the terminal development of eggs. The early differentiation of an oogonium into a primary oocyte in *G.filamentosus* is characterized by the highly undulating or wavy nuclear envelope, which increases the surface area of nucleus. A progressive increase in the diameter of the nucleolus was observed in accordance with the growth of early oocytes, which was followed by an increase in the nurnber of nucleoli. A prominent feature of the perinuclear stage is the formation of a rather amorphous `yolk nucleus' within the ooplasm. The functional significance of the yolk nucleus is not fully understood but it is presumed that yolk nucleus is intimately involved in the metabolic activities related to the formation, multiplication, accumulation, and distribution of cytoplasmic organelles and inclusions, which are needed within the oocyte prior to yolk formation(Gurayaet *al*., 1986).

Histoligical studies carried out during the present investigation show that three type of inclusions are formed during vitellogenesis in *G. filamentosus.* These three types of inclusions viz.Lipid droplets, protein yolk globules and cortical alveoli, differ distinctly in their, morphology, staining properties and chemical nature and they are deposited sequentially although considerable overlap occurs.

The first type of yolk inclusion to accumulate in the developing oocytes of *G.filamentosus is* the lipid yolk(triglycerides) in the form of distinct lipid droplets, the appearance of which can be considered to mark, the start of endogenous vitellogenesis(Shackleyand King, 1979). It has been opined that these lipid bodies arise either from dictyosomes or mitochondria in the ooplasmShackley and King (1979) suggested, the synthesis of lipid droplets is probably endogenous (occurring in the perinuclearooplasm).

Protein yolk accumulation (yolk globule stage) occurs after, and concomitant to lipid yolk accumulation *in G. filamentosus*. Small yolk globules first appear in the cortical region of the oocytes and later they fill the entire ooplasm in the form of larger globules. It is now generally accepted that the protein yolk is hepatically produced(exogenous in origin), the yolk precursor having been identified as the female-specific serum, glycoprotein complex, vitellogenin(vtg)(Gurayae*t al.,* 1986). The Vtg. is specifically sequestered through elathrin coated micropinocytic vesicles, under gonadotropin control by the developing oocyte (Wallace, 1985). The third and quantitatively minor type of inclusion is the cortical alveoli(carbohydrate yolk or yolk vesicles,) which release their contents in the perivitelline space during cortical reaction. For this reason, they can not be considered as yolk in strict sense. This inclusion appears as a narrow zone below the zonaradiata after both lipid yolk and protein yolk formation have stared in*G.filamentosus*; whereas in majority of teleost species, the cortical alveoli formation occurs prior to both lipid and protein yolk formation (Khoo,1979; Gurayae*t al.,* 1986). Cortical alveoli.containmucopolysaccharides, which are now believed to be synthesized endogenous under the control of gonadotropim. (Gurayae*t al.,* 1986). This have been recorded form the fishes like *G.filamentosus* (Cyrus and Blaber, 1984), *D.1abrax*(Mayer *et al.,* 1988) .The formation of the thick zonaradiata givea mechanical protection to the oocyte and its plasticity. allows the increase in size of the oocyte without preventing nutrition(Brusle, 1981).

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