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Female Gonadal Histology of Indian River Shad, *Gudusia chapra* (Hamilton, 1822) – A Tactic of Reproductive Biology

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Abstract

The present study deals with histological studies on different maturity stages of female gonad of Indian River Shad, Gudusia chapra collected from the lower reaches of River Brahmaputra, Assam, India during November 2013 to December 2015. The result shows different histological structures of each oocyte developmental stages: The observed seven stages correspond with those described macroscopically for various species of teleost fishes. Stage VI was characterised by ovulated oocyte, which histologically resemble a maturity phase. A high proportion of spawner (55%) was found, together with a relatively low occurrence of juvenile fishes in the each month (22%).

Keywords: *Gudusia chapra*; Histology; Brahmaputra; Oogenesis

Introduction

Gudusia chapra, commonly known as the Indian River shad, is an important small indigenous commercial food fish in Assam, Northeast India. The species represents an important element for many of the subsistence artisanal fisher folks [1-3]. Knowledge on various aspects of fish biology, chiefly reproductive biology, is essential for sound management practices in fishery science. There is no significant data on ovarian histological studies in the clupeid *G. chapra*. Therefore, this report aims to provide information on aspects of oogenesis in *G. chapra* occurring along the lower Brahmaputra River in Assam, India.

Materials and Methods

For the present study, fishes (length: 7.7-11.3 cm; weight: 4.4-13.5 g) were collected from four selected sites viz., Guwahati (26°11′10.2″N 91°45′03.6″E), Nagarbera (26°8′15.7″N 91°41′57.2″E), Pancharatna (26°11′57.4″ N 90°34′18.3″E) and Dhubri (26°01′26.9″ N 89°59′7.6″ E) (Figure 1), along the stretches of lower Brahmaputra on a monthly basis from November 2013 to December 2015. Sampling was done using cast net by taking the help of local fishermen through their traditional expertise. For histological study, fishes were dissected and gonads were removed. The respective length (in cm) and weight (in g) were then taken (Table 1), and examined macroscopically for visual confirmation of different stages of ovary. Ovary were cut into small pieces, preserved in 10% formosaline, and subsequently processed following standard method [4]. The ovarian sections were observed under Bright field microscope (Leica DM3000).

Results and Discussion

The sample represented a high proportion of spawners (55%) compared to juveniles (22%) in each month. The different histological structures for each oocyte developmental stages were classified according to oocyte location and size, staining characteristics, number of nucleoli, presence of the follicular layer, and the distribution of cytoplasmic inclusions. Oogenesis was found to proceed through seven stages namely, Stage I (Immature stage showing empty follicles); Stage II (Virgin stage showing numerous cells in the early perinucleolar stage and a few in the late perinucleolar stage); Stage III (Developing virgin showing early perinucleolar stage oocytes and late perinucleolar stage oocytes); Stage IV (Developing stage showing vitellogenic oocytes); Stage V (Maturing stage showing putative lipid vesicles and yolk granules in early vitellogenic oocytes); Stage VI (Mature stage showing fusion of zona radiata and follicles); and Stage VII (Spent stage showing blood capillaries and cells of degraded follicles). Detailed macroscopic observations and histological characteristics of ovarian development are presented in Table 2. The observed seven stages correspond with those described macroscopically in various species of teleost fishes (Figures 2 and 3). Stage VI was characterized by ovulated oocyte, which histologically resembles a maturity phase. Oocyte growth in this fish species follows a similar pattern in most of the other teleosts like contained oogonia, maturing previtellogenic and matured vitellogenic oocytes [5,6]. Histological studies can determine the peak period of spawning assessment and exploitation of fish, and biological characteristics and life cycle of a species [7,8]. Oogenesis is manifested by a series of changes of the oocytes [9]. Similar changes in the developing oocytes as reported in this study were described for several species of teleosts, including changes in the size of gonad and the oocyte [10]. Accumulation of yolk granules was found in both intraoocytic and extraoocytic spaces. As indirect evidences in support of the hepatic origin of fish vitellogenin was provided by a number of studies, it is generally accepted that the liver is stimulated to synthesize and secrete the same [11]. The yolk materials are transported via blood circulation and formed into yolk granules in the interfollicular spaces, as evident from this study. Yolk granules are taken into the oocyte by pinocytotic uptake. The main histological features of the developmental stages indicate that no immature elements are incorporated into the

Sampling sites	N	Length (cm)	Weight (g)
Guwahati	30	2.4-3.2	0.19-1.13
Nagarbera	30	2.0-3.3	0.15-1.06
Pancharatna	30	1.9-2.9	0.12-0.53
Dhubri	30	2.4-3.6	0.18-1.88

Table 1: Sampling sites, length-weight data of ovary for different developmental stages of *G. chapra*. 'N' is the number of specimens examined.

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Maturity Stage	Macroscopic description	Histological description
Immature stage Stage I	Ovary transparent with no eggs visible. Gonad small and tucked inside body cavity (Figure 2a).	Late peri-nucleus (Figure 3a) most advanced oocyte stage present.
Virgin Stage II	Ovary is small, thread-like and translucent and clearly visible inside the body cavity (Figure 2b).	Oogonia (Figure 3b) with early perinucleolar oocytes (small size) and few in the late perinucleolar stage (larger size) were observed.
Developing virgin Stage III	Colour is reddish white or creamy white and Increase in volume and weight (Figure 2c).	Early perinucleolar stage oocytes (empty arrows) and late perinucleolar stage oocytes (dark arrows) were observed (Figure 3c).
Developing Stage IV	Ovaries translucent to opaque; the left side is slightly longer than the right and about half the length of body cavity (Figure 2d).	Vitellogenic stage (Figure 3d) most advanced oocyte stage present. Early and late peri-nuclear stages were present. No yolk formation. No postovulatory follicles.
Maturing Stage V	Ovaries in this stage are with slight granular appearance (Figure 2e).	Peri-nucleus, primary, secondary and tertiary yolked (Figure 3e), nuclear migration and prematuration stages present. No postovulatory follicles
Mature Stage VI	Stage VI can be distinguished clearly and it occupies 70% of the visceral cavity (Figure 2f).	Ovulation has occurred. Peri-nucleus, yolked, nuclear migration, prematuration, and maturation stages present. Postovulatory follicles present (Figure 3f).
Spent Stage VII	Ovaries uniformly tubular and flaccid (Figure 2g).	Histological sections reveal irregular, convoluted ovigerous folds containing large numbers of ruptured, postovulatory follicles, as well as atretic follicles. (Figure 3g).

Table 2: Macroscopic and Histological description of various stages of ovary development in *G. chapra.*

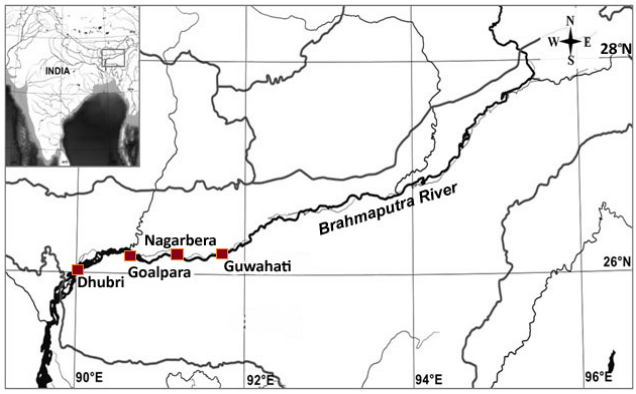

Figure 1: Map showing the collection sites of *G. chapra* in lower reaches of the River Brahmaputra.

Figure 2: Macroscopic stages of Gonads of female *G.chapra*; a. Stage I; b. Stage II; c. Stage III; d. Stage IV; e. Stage V; f. Stage VI; and g. Stage VII.

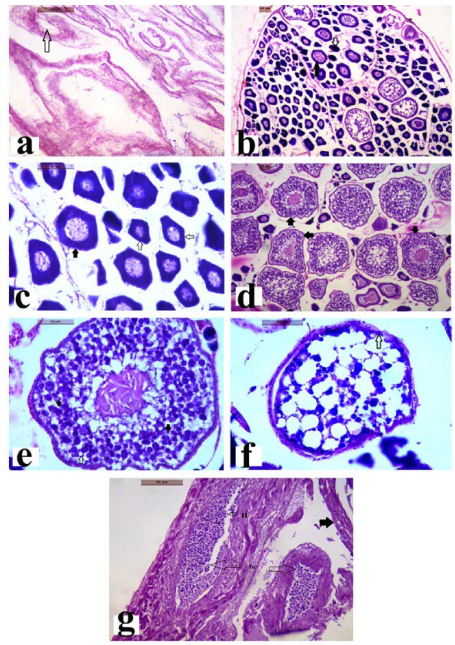

Figure 3: TS of female gonads of *G. chapra*; a. Stage I showing immature oocytes (empty arrow shows empty follicles) (400X); b. Stage II showing virgin oocytes (dark arrow shows early perinucleolar stage and empty arrow shows a few in the late perinucleolar stage) (100X); c. Stage III showing developing virgin oocytes (empty arrow shows early perinucleolar and dark arrow shows late perinucleolar stage) (400X); d. Stage IV showing developing oocytes (dark arrow shows vitellogenic oocyte) (100X); e. Stage V showing maturing oocytes (empty arrow shows yolk vesicles and dark arrow shows yolk granules) (400X); f. Stage VI showing matured oocytes (empty arrow shows fusion of zona radiata and follicles) (400X); g. Stage VII showing spent stage (empty arrows shows n=nucleus; bc=blood capillaries and dark arrow shows cells of degrading follicles) (400X).

cohort of yolky oocytes that was evacuated during the current spawning season [12,13].

This is the first report of a detailed histological study on early ovarian growth, development and maturity in *G. chapra* with the help of light microscopy. Gonadal development and reproductive strategies have been studied in details in many teleost fish species which is referred to an effort to understand the time course and energetic consequences of their reproductive effort by many authors. However, visual determination of reproductive maturity is not feasible enough as structures within the ovary, such as oocytes at different stages or interstitial tissue with accumulation of yolk materials, is not evident to the naked eye. Direct observation through histological architecture is the most accurate and appropriate tactic to know the exact stage of first maturation which the ovary undergoes [14]. The present study on *G. chapra* reveals the basic histological architecture and identified the oocytes found within the ovary, which would provide a basic knowledge for future studies on reproductive biology, reproductive toxicoloy and histopathology in this clupeid fish.

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