



Seasonal Histological Changes in the Olfactory Epithelium of Schilbid Catfish, *Clupisoma garua* (Hamilton, 1822)

S. K. GHOSH

Department of Zoology, Bejoy Narayan Mahavidyalaya, Itachuna, Hooghly-712 147, West Bengal, India

Received 12<sup>th</sup> April 2020 and Revised 2<sup>nd</sup> September 2020

**Abstract:** Olfaction in fish is one of the most important chemosensory modalities driving the essential approaches to interact with the encompassing habitat. The olfactory organ of river catfish, *Clupisoma garua* (Siluriformes; Ailiidae) was studied by employing optical microscopy to delineate the cellular composition in harmony with the annual reproductive cycle. The olfactory epithelium was an intimate folded sheet, sandwiched a thin connective tissue layer, the central core, composed of disposed of connective tissue with nerve fibers and blood vessels through it. A sharp distinction has prevailed between the epithelium and central core by a basement membrane. The thickness of olfactory mucosa with diverse cells was counted attention to their architecture, magnitude, compactness, staining intensity, and distribution patterns throughout altered reproductive phases. In view of the texture of the apical part and outward specialization, the olfactosensory epithelium contained morphologically identified ciliated, microvillous, and rod receptor cells. The non-sensory epithelium was typified by labyrinth cells, mucous cells, mast cells, basal cells, ciliated supporting cells, and non-ciliated supporting cells. They were intermingled in the epithelial lining. The functional emphasis of olfactory cells covering the mucosa was argued with the chemoreception of the fish interested.

**Keywords:** Garua bacha, Olfactory organ, Cellular characteristics, Reproductive cycle, Chemosensory information

## 1. INTRODUCTION

In teleosts, the paired olfactory organs are the significant chemosensory parts of the nasal chamber which are specialized in adaptation to the ecological habitat in which they survive. The sense of olfaction mediates many crucial life processes such as feeding, avoid from enemies, parental behaviour, migration, propagation and reproductive approaches (Nikonov *et al.*, 2017). Such behavioral activities are an exposure of variant processes at cellular and physiological levels, esteemed for characteristic receptor neurons on the olfactory mucosa (Singh *et al.*, 1995). In fish, the olfactory receptor neurons are the foremost integral part of sensory epithelium for sending olfactory information to the brain (Satou, 1992). Characterization of olfactory system in a number of teleosts are draw attention by many workers utilizing light and electron microscopy (Hansen *et al.*, 2003; Liu *et al.*, 2005; Arvedlund *et al.*, 2007; Waryani *et al.*, 2013; Masram and Baile, 2014; Pashchenko and Kasumyan, 2015, Ghosh and Chakrabarti, 2016; Kim *et al.*, 2018). Among the fishes, extreme modifications in the morphology of olfactory organs and cellular components of sensory and non-sensory epithelium of olfactory lamella are recorded by the researchers. Receptor cells can be considered as different functional and structural entities with distinct sensitivities to external stimuli (Yamamoto, 1982).

*Clupisoma garua*, commonly known as butter catfish, is an inhabitant of freshwater rivers, streams, canals, and reservoirs; feeds on insects, shrimps, other crustaceans and other small fishes also (Talwar and Jhingran, 1991; Akter *et al.*, 2019). *C. garua* has well developed olfactory organs with important roles in various aspects of life processes (Ghosh, 2018a; 2019). Perhaps very limited attention has been paid to depict the seasonal alterations of sensory neurons and histological organization of olfactory epithelium in association to reproductive cycles of Indian fishes (Hamdani *et al.*, 2008; Ghosh, 2018b). The present study was undertaken to portray the cyclical changes of sensory and non-sensory cells lining the olfactory epithelium in relation to reproductive activity of bottom dweller catfish, *Clupisoma garua* (Hamilton, 1822) by histological approaches. This study would aid to have information about any variation of the olfactory cell types compared with reproductive cycles.

## 2. MATERIALS AND METHODS

### Collection of specimens

Reproductive adult male (ranged from 10.42-19.67 cm in standard length) and female (varied from 12.24-24.56 cm in standard length) specimens of *Clupisoma garua* were collected from Bhagirathi-Hooghly river at Kalyani surrounding areas of West Bengal using

traditional fishing gear from September 2018 to August 2019. The specimens were deeply anaesthetized with Benzocaine (4 mg/L) and the abdomen of the fishes was cut with scissors for determining their sex, because, it was not conceivable do so by morphological observation of the whole body. The olfactory apparatus were exposed and removed from the olfactory pits.

### **Tissue processing**

Samples of olfactory rosettes fixed in aqueous Bouin's fluid for about 24 hour were washed and dehydrated through ascending series of ethanol (50%-100%), cleared with xylene and infiltrated with paraffin wax (56-58°C melting point) for 1 hour and 30 minute. Transverse section of tissue was cut at 4 µm thickness using Weswax MT-1090A rotary microtome. After routine histological procedure, deparaffinized tissue sections were stained with Delafield's Haematoxylin-Eosin (Fischer *et al.*, 2008), Delafield's Haematoxylin-Phloxin, Romies Azan, and Mallory's Triple stain (Mallory, 1936). The staining slides were examined and photographed under ZEISS Primo Star light microscope with Tucsen 5.0 MP digital microscopy camera at different magnification.

### **Measurement of cellular elements in the olfactory epithelium**

The thickness of olfactory mucosa and the width/tallness of various cells were assessed using an ocular micrometer in a microscope eye piece. The frequency of occurrence of olfactory cells in the interim of reproductive cycle was enumerated with the aid of stage micrometer (Erma).

## **3. RESULTS**

In *C. garua* the lamellae radiating from raphe comprise of sensory and non-sensory regions, consists of two main layers: epithelium (mucosa) and central core containing connective tissues, blood vessels and nerve fibres. The number of olfactory lamella is not constant, but subject to variation according to size/maturity of fish. The sensory epithelium contains structurally noticeable ciliated, microvillous and rod receptor cells where as the non-sensory epithelium has labyrinth cells, mast cells, supporting cells, and mucous cells. Basal cells are buried in the epithelium and presumed to be the ancestor of sensory or supporting cells. The cytoarchitecture of the olfactory mucosa varies throughout the reproductive cycle. The alterations of cellular elements lining the olfactory epithelium are observed by in light of their architecture, staining vigor, distribution patterns, and their nuclei along the thickness of the olfactory mucosa. Each morphotype of sensory receptor cell is portrayed by a cell soma in a distinct layer of the mucosa, versatile extent of dendrite and expansion of axonal process in the direction of

basement membrane. The reproductive period of *C. garua* is conveniently being divided into four phases: preparatory or growth (November to February), pre-spawning or maturation (March to May), Spawning (June to August), and post-spawning or spent (September to October).

### **Growth phase**

The thickness of the epithelium is  $25.34 \pm 0.16$  µm in female (**Fig. 1a**) and  $20.79 \pm 0.64$  µm in male (**Fig. 1b**) respectively. Bending of lamellae is observed in this phase. The sensory epithelium is composed of a primary receptor cells, rod receptor cells ( $12.45 \pm 0.25$  µm length in female and  $11.18 \pm 0.96$  µm in male) and scattered microvillous cells ( $3.89 \pm 0.09$  µm diameter in male and  $4.17 \pm 0.02$  µm in female) with light nuclei, located in more superficial layer in the epithelium (**Figs. 1c-f**). The rod cells are expressed by basally situated vesicular nuclei, and tighten extruded dendrons. The utmost proximal portion close to the basement membrane is occupied by the basal cells ( $6.95 \pm 0.07$  µm diameter in male and  $7.12 \pm 0.02$  µm in female). The nucleus of basal cells stains strikingly with chromophobic cytoplasm (**Figs. 1c, e-f**). The primary receptor cells ( $12.05 \pm 0.15$  µm length in male and  $13.18 \pm 0.86$ µm in female) (**Figs. 1c-f**) are characterized by profoundly stained oval shaped nuclei and thin lengthy dendrites grasping up to epithelial surface. The middle portion of epithelium contains round shaped mast cells ( $6.14 \pm 0.17$  µm diameter) having centrally placed nuclei (**Fig. 1d**). Labyrinth cells ( $6.96 \pm 0.12$  µm diameter) are diffused in the superficial layer of the olfactory epithelium. They are ovoid or rounded in shape bearing discernible nuclei. The supporting cells are polygonal with central nuclei, spread throughout the mucosa (**Figs. 1c-e**) and the tip of the some supporting cells bear faintly visible cilia connected with the basal bodies, defined as ciliated non-sensory cells (**Fig. 1c**). The mucous cells ( $6.18 \pm 0.27$  µm diameter in male and  $7.37 \pm 0.02$  µm in female) are profusely distributed along the free surface of the olfactory epithelium (**Figs. 1d, f**) and occasionally observed in the deeper region of the epithelium. The broad central core is separated from mucosa by basement membrane (**Figs. 1d, f**) and composed of loose connective tissue which is invaded by nerve fibers and blood vessels (**Figs. 1a-f**).

### **Maturation phase**

During this phase spreading trend in the elevation of olfactory mucosa is observed, around  $33.08 \pm 0.12$  µm in male and  $41.63 \pm 0.43$  µm in female specimens (**Figs. 2a-c**). The incidence of secondary folds on olfactory lamellae is noticed due to raise of surface mucosa as well as the susceptibility of receptor cells. An

expansion in the height of primary receptor cell ( $20.07 \pm 0.06 \mu\text{m}$  length in male and  $21.15 \pm 0.12 \mu\text{m}$  in female) is observed (**Figs. 2d-f**). The apical part of receptor cell is extended as a narrow cylindrical process up to the free epithelial margin where it enlarges into a small knob like structure (**Fig. 2f**). In male specimens, the dendrite end of the receptor cells are comparatively more extended to the surface of the epithelium contrary to female. Rod cells ( $14.07 \pm 0.36 \mu\text{m}$  length in male and  $16.15 \pm 0.22 \mu\text{m}$  in female) with firm rod like cilia occupy almost the entire free margin (**Figs. 2d-g**). Microvillous cells ( $5.07 \pm 0.17 \mu\text{m}$  diameter in male and  $5.89 \pm 0.37 \mu\text{m}$  diameter in female) are few in number and left out cilia (**Figs. 2d, g**). Basal cells ( $7.46 \pm 0.16 \mu\text{m}$  in diameter) are small in number, oblong in shape containing a prominent round central nucleus spotted superior to the basement membrane (**Figs. 2e, g**). The epithelial surface is comprised of supporting cells ( $6.85 \pm 0.24 \mu\text{m}$  diameter) with conspicuous nuclei and small number of mucous cells ( $6.98 \pm 0.47 \mu\text{m}$  diameter) (**Figs. 2d-e**). Dispersed labyrinth cells ( $7.36 \pm 0.47 \mu\text{m}$  diameter) are ovoid or rounded in appearance with prominent nuclei situated more basally (**Fig. 2d**). The distal limb of ciliated supporting cells is broad and supported by stubby cilia (**Figs. 2d-e**). Mast cells are roughly rounded in shape ( $6.04 \pm 0.06 \mu\text{m}$  diameter in male  $6.54 \pm 0.51 \mu\text{m}$  diameter in female) with comparably smaller amount of cytoplasm and polymorphous nuclei (**Figs. 2e, g**). The central core is narrow and contained huge blood vessels and few pigment cells (**Figs 2a-g**).

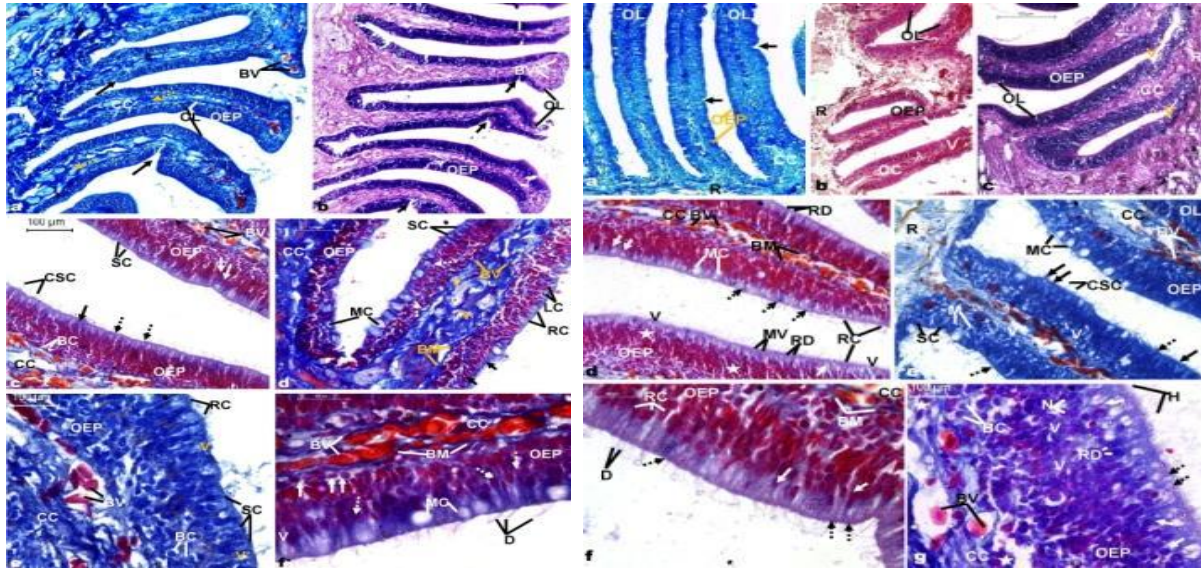
#### **Spawning phase**

The olfactory lamella is comparatively lingering with well furnished mucosa containing closely arranged cellular elements (**Figs. 3a-b**). The olfactory mucosa is  $44.03 \pm 0.34 \mu\text{m}$  height in female and  $39.67 \pm 0.26 \mu\text{m}$  in male; composed of a large number of long primary neurons ( $22.56 \pm 0.25 \mu\text{m}$  in male and  $22.07 \pm 0.04 \mu\text{m}$  in female) (**Figs. 3b-f**) and secondary neurons (**Fig. 3d**). Primary receptor cells are columnar, contained posteriorly placed cell body and a thin long dendrite up to the free epithelial surface. In male the primary receptor cells are arranged in a single row and the dendrite bulged out to form a little flask shaped swelling end, the olfactory knob supported with apical hairs (**Figs. 3d, f**). In some regions of sensory epithelium, the receptor cells are typical bipolar neurons (**Fig. 3d**). In female ciliated supporting cells are intermingled among the receptor cells (**Figs. 3c, e**). Secondary receptor cells

are mostly located under the primary receptor cells and identified by their profoundly stained protracted nuclei. In the male mucosa, the axons of scanty primary receptor cells terminate in synaptic relations to the dendritic consequences of the secondary receptor cells (**Fig. 3d**). Neighboring to ciliated receptor cells, scattered microvillous cells ( $5.76 \pm 0.55 \mu\text{m}$  in diameter) are well marked (**Figs. 3d, f**). In female, rod receptor cells ( $19.02 \pm 0.15 \mu\text{m}$  in height) are outlined by a lance like dendrites and eminently basophilic nuclei which buried in the epithelium (**Figs. 3c, e**). Tiny basal cells ( $7.81 \pm 0.05 \mu\text{m}$  in diameter) are ovoid in shape, having curled central nuclei, lying over the basement membrane (**Figs. 3c, f**). Non-ciliated supporting cells ( $6.88 \pm 0.24 \mu\text{m}$  of cell body) are elliptical to columnar in texture with grainy cytoplasm and basophilic nuclei (**Fig. 3c**). Mucous cells are less ( $5.64 \pm 0.07 \mu\text{m}$  in diameter) in number and dispersed in the epithelial lining. Mast cells ( $6.59 \pm 0.06 \mu\text{m}$  diameter) are spherical in shape and submerged in the midst portion of mucosa (**Figs. 3c, f**). The labyrinth cells are fairly larger, ( $7.84 \pm 0.21 \mu\text{m}$  in diameter) imparted with extrusive nuclei and restricted to margin of epithelium (**Figs. 3c-d, f**). Attenuated central core consists of loosely arranged connective tissue. Nerve fibers and blood vessels exist in this portion (**Figs. 3a-f**).

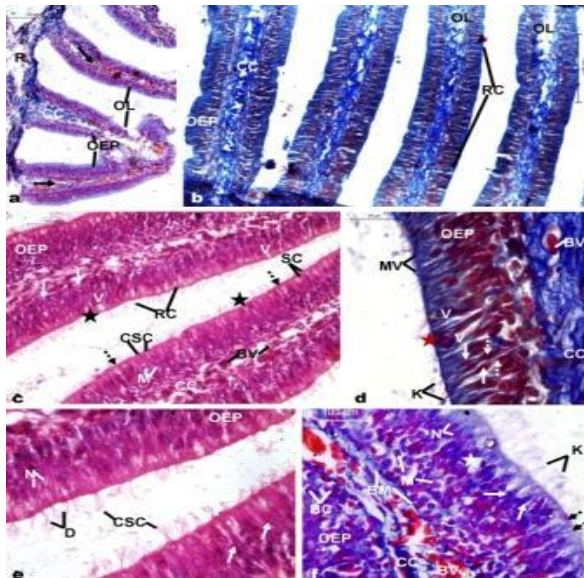
#### **Resting phase**

The olfactory mucosal lining in both sexes attenuates in width (ranging  $34.04 \pm 0.06 \mu\text{m}$  in female and  $28.14 \pm 0.41 \mu\text{m}$  in male) (**Figs. 4a-c**) which is separated from broad stromal sheet by distinct basement membrane. The central core consists of loosely disposed fibrous connective tissue. Nerve fibres and blood capillaries pass through this territory (**Figs. 4a-f**). Adjoining to basement membrane is occupied by the basal cells then succeeded by mast cells (**Figs. 4e-f**). The surface epithelium contains primary receptor cells, rod receptor cells, labyrinth cells and ciliated supporting cells having basally settle nuclei at their broad distal limbs (**Figs. 4b-e**). Dissipated primary receptor cells ( $14.17 \pm 0.07 \mu\text{m}$  tallness in female and  $13.12 \pm 0.02 \mu\text{m}$  in male) are marked in between supporting (**Figs. 4c, f**). In male, constrict cylindrical end of the receptor cells are bedding with bulge like structure at their apical side (**Fig. 4e**). Oval shaped mucous cells with secreted mucin are fairly larger ( $11.87 \pm 0.31 \mu\text{m}$  in female and  $11.04 \pm 0.21 \mu\text{m}$  in male), having profuse granules, distributed at the free margin of the mucosa intermingle with different epithelial cells (**Figs. 4b-f**).

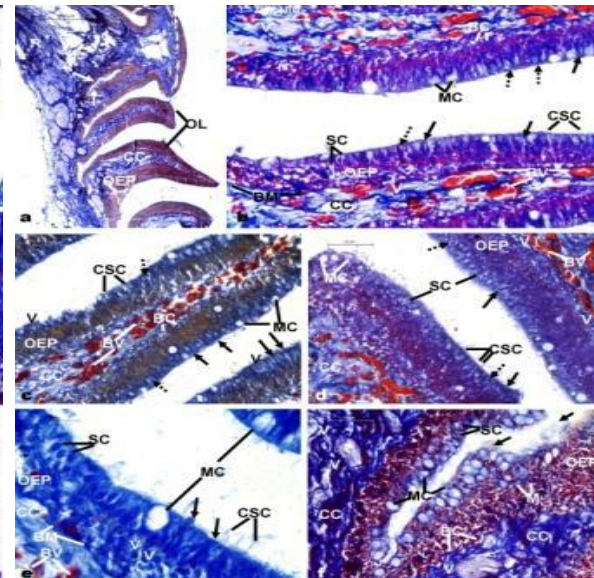


**Fig. 1: Growth phase**

**Fig. 2: Maturation phase**



**Fig. 3: Spawning phase**



**Fig. 4: Resting phase**

**Figure legends**

**Fig. 1:** Photomicrographs of the histoarchitecture of olfactory lamellae of female and male *C. garua* during growth phase stained with Mallory’s Triple (MT), Delafield’s Haematoxylin-Phloxin (HP) and Romies Azan (RA) stain:

(a) - Female olfactory lamellae (OL) based on raphe (R) showing olfactory epithelium (OEP) separated by wide central core (CC) having connective tissue (broken arrows), and blood vessels (BV). Solid arrows indicated the infoldings of OL (MT)  $\times 100X$ ; (b) - Male OL radiated from R shows infoldings (arrows); contained OEP and C with BV (HP)  $\times 100X$ ; (c) - Male OEP

composed of primary receptor cells (RC) (solid arrows), rod cells (RD) (broken arrows), supporting cells (SC), and ciliated non-sensory cells (CSC). Noted the presence of basal cells (BC) above the CC which contains BV (RA)  $\times 400X$ ; (d) - Female OEP showed mucous cells (MC), labyrinth cells (LC), mast cells (broken arrows), SC, and RC. Noted the heap of connective tissue (arrow heads) and BV in CC which was distinguished from OEP by basement membrane (BM) (RA)  $\times 400X$ ; (e) - High magnification of Female OEP showing primary RC, microvillous cells (arrow heads), and BC. CC contained BV (MT)  $\times 1000X$ ; (f) - Male OEP typified with RC (broken arrows) with long dendrites (D) towards epithelial surface, microvillous

cell (arrow head), BC (solid arrows), and MC. Noted the presence of BM which separated CC from OEP. BV indicated blood vessels (RA)  $\times$  1000X.

**Fig. 2:** Photomicrographs of the histological organization of olfactory lamellae of female and male *C. garua* during maturation phase stained with Mallory's Triple (MT), Delafield's Haematoxylin-Eosin (HE), Delafield's Haematoxylin-Phloxin (HP) and Romies Azan (RA) stain: **(a)** - Male olfactory lamellae (OL) attached with raphe (R) showing compact olfactory epithelium (OEP), and central core (CC). Arrows indicated folding of OL (MT)  $\times$  40X; **(b)** - Female OL based on R exhibited organized OEP separated by CC which contained blood vessels (arrow heads) (HE)  $\times$  40X; **(c)** - Female OL showing infoldings (arrow heads), radiated from R contained OEP on either side CC (HP)  $\times$  100X; **(d)** - Male OEP showing primary receptor cells (RC), rod receptor cells (RD), microvillous cells (MV), mast cells (M) (asterisks), labyrinth cells (broken arrows), supporting cells (SC) (arrow heads), and ciliated supporting cells (solid arrows). Noted large number of blood vessels (BV) in CC which separated from OEP by basement membrane (BM) (RA)  $\times$  400X; **(e)** - Female OL exhibited primary RC (solid arrows), RD (broken arrows), SC, ciliated supporting cells (CSC), M, and basal cells (arrow heads) in the deeper part of OEP. Noted presence of BV in CC (MT)  $\times$  400X; **(f)** - Magnifying male sensory OEP was distinguished from CC by BM; contained RD (solid arrows) and long primary RC with knob like structures (broken arrows), and protruding dendrites (D) (RA)  $\times$  1000X; **(g)** - Female OEP showing basal cells (BC), mast cells (arrow heads), RD, MV (broken arrows), and RC (solid arrows) characterized with basophilic nuclei (N), and sensory hairs (H). Noted pigment cells (asterisks) and BV in CC (MT)  $\times$  1000X.

**Fig. 3:** Photomicrographs showing the transverse section of olfactory epithelium of female and male *C. garua* during spawning phase stained with Romies Azan (RA), Mallory's Triple (MT) and Delafield's Haematoxylin-Eosin (HE) stain: **(a)** - Female olfactory lamellae (OL) based on raphe (R) exhibited well grown olfactory epithelium (OEP) separated by the central core (CC). Arrows marked blood vessels (BV) in CC (RA)  $\times$  40X; **(b)** - Male OL showing OEP having large number of primary receptor cells (RC) on either side of narrow CC (MT)  $\times$  100X; **(c)** - Female OEP typified with primary RC, rod cells (broken arrows), ciliated supporting cells (CSC), non-ciliated supporting cells (SC), mast cells (M), mucous cells (arrow heads), labyrinth cells (asterisks), and basal cells (solid arrows). CC packed with BV (HE)  $\times$  400X; **(d)** - Male sensory OEP illustrated microvillous cells (MV), labyrinth cells (asterisks), synaptic contact in between primary RC (solid arrows) and secondary RC (broken arrows).

Noted knob like (K) structure of ciliated RC, bipolar neurons (arrow heads) in OEP and BV in CC (MT)  $\times$  1000X; **(e)** - Higher magnification of female OEP illustrated CSC, rod cells (arrows) and RC with long dendrites (D) and basophilic nuclei (N) (HE)  $\times$  1000X; **(f)** - Magnified male OEP showing labyrinth cell (asterisk), MV (broken arrows), M, basal cells (C), RC (solid arrows) supported with deeply stained nuclei (N) and knob like (K) like structures. Noted well vascularised CC with BV distinguished from OEP by basement membrane (BM) (RA)  $\times$  1000X.

**Fig. 4:** Microphotographs of transverse section of olfactory lamella of female and male *C. garua* during post-spawning phase stained with Mallory's triple (MT) and Romies Azan (RA) stain: **(a)** - Female olfactory lamellae (OL) radiated from raphe (R) showing olfactory epithelium (OEP) and middle central core (CC). Arrows marked series of mucous cells (MT)  $\times$  100X; **(b)** - Male OEP lined with primary receptor cells (RC) (solid arrows), rod cells (broken arrows), ciliated supporting cells (CSC), supporting cells (SC), mucous cells (MC), and basal cells (BC) CC contained connective tissue (arrow heads) and blood vessels (BV). Noted presence of basement membrane (BM) in between CC and OEP (RA)  $\times$  400X; **(c)** - Part of male OEP typified with RC (solid arrows), labyrinth cells (LC) (broken arrows), CSC, SC (arrow heads), BC, and MC. Noted the presence of blood vessels in CC (MT)  $\times$  400X; **(d)** - Female OEP furnished with scattered RC (solid arrows), LC (broken arrows), CSC, SC, BC (arrow heads) and series of MC. CC with BV (RA)  $\times$  400X; **(e)** - Magnified male OEP exhibited knob like structure of RC (solid arrows), mast cells (arrow heads), granulated MC, CSC, and SC. OEP distinguished by BM from CC which contained BV (MT)  $\times$  1000X; **(f)** - Female OEP illustrated aggregation of MC with secreted mucin (solid arrows), SC, BC, and mast cells (M). Broad CC was richly supplied with fibrous connective tissue (arrow heads) (MT)  $\times$  1000X.

#### 4. DISCUSSION

The olfactory mucosa of *C. garua* is conspicuously thrown up into folds whose surfaces distinguished into sensory and non-sensory regions based on ecological niche. The folding of olfactory lamella enhances the surface territory of the mucosa as well as the acuteness and efficiency of olfactory system (Zeiske *et al.*, 1976). The sensory epithelium of *C. garua* contains structurally evident ciliated, microvillus, and rod receptor cells. It is substantiated that various types of sensory receptor cells existing on sensory epithelium are able to judge the chemical characteristics in the surroundings.

The ciliated receptor cells harmonize to type I cell of Yamamoto and Ueda (1978), though microvillous receptor cells to type II cells of Muller and Marc (1984)

and rod receptor cells of those type IV cells of Ichikawa and Ueda (1977). Receptor cells bearing odorant receptors expose the stimuli and turn over the message to the olfactory bulb (Hansen and Zeiske, 1998). *C. garua*, a bottom dweller, the protracted and well grow dendrite of the ciliated receptor cell facilitates organism to smell and encounter the environmental stress. The observant aspect of the present work is the finding of secondary receptor cells and the occurrence of synaptic contacts in between primary and secondary receptor cells in the olfactory mucosa. The axons of the secondary receptor cells continue to the central core of the olfactory lamellae, suggests that the impulses perceived by the dendrite ends of primary neurons finally address impulses to the stromal sheet and to the cerebral hemisphere ultimately. This finding is similar with the observation of secondary neurons in the olfactory lining of *Labeo rohita* (Ojha and Kapoor, 1973). Graziadei and Metcalf (1971) added that commencing neurons substitute the aged and degenerating ones and put fresh synaptic connection in the olfactory bulb. The microvillous receptor cells recognize and communicate with pheromone, which is a momentous proceeding of breeding in *Labeo rohita* (Bhute and Baile, 2007). On contrary, Bakhtin (1977) and Bannister (1965) stated that microvillous neurons in the olfactory mucosa of *Squalus acanthias* and teleosts are prototype of ciliated receptor cells. Datta and Bandyopadhyay (1997) reported that rod cell is not a usual subtype, the forming of rod probably due to fusion of cilia of ciliated region. Hernadi (1993) reported that the presence of the rod cell due to habitat of a new physiological climate.

The non-sensory epithelium is typified with ciliated supporting cells have no sensory function but they perhaps help in mechanical dissociation. The beating of cilia helps in driving out the mucin mass poured out from the mucous cells (Bandyopadhyay and Datta, 1998). The cilia perform as guides facilitating dissolved chemicals to contact tips of sensory neurons bearing receptor sites. The non-ciliated supporting cells have been suggested to perform several functions: secretory, absorbing, and glial (Theisen, 1972; Yamamoto and Ueda, 1978; Hernadi, 1993; Hansen and Zeiske, 1998). The labyrinth cells cell type related to chloride cells which presumably are involved in electrolyte transport in fish gills and pseudobranch (Bertmer, 1982). Mast cells in the olfactory epithelium are having to perform a crucial role in reproduction of *Labeo rohita* (Bhute and Baile, 2007) and Baltic trout (Bertmer, 1982). They can innovate metabolic function of sensory neurons and thereby the sensitivity of olfactory epithelium. The basal cells are conceited to be the progenitor cells of the receptor and supporting cells (Zeiske *et al.*, 1992). The occurrence of basal cells buried in the epithelium abets

to sustain the mucosa during normal cell turn over or necrobiosis. Mucous cells secrete mucus to shield the mucosa from mechanical injury and helps in binding the microscopic debris to keep the sensory receptor accessible for new odorants. Hornung and Mozell (1981) advocated that the discharge of mucous cells assists in promoting the odorant discharge.

Reproductive behavior of *C. garua* is cyclic and periodic, spawning occurs in monsoon season (June-August) (Pasha *et al.*, 2019; Serajuddin and Singh, 2019). Breeding consists of a continuance of circumstances linked to both pre-spawning and spawning phases. The ecological and specific environmental factors are of great concern in controlling the spawning and the annual reproductive cycle. The experimental fish shows the course of maturation and depletion of gonads in synchronic with the growth and development of varied receptor cells on the olfactory lining in the distinct phases of reproductive cycle. Occurrence of receptor cells displays seasonal variations in their shape, size, distribution pattern, and specialize aspect in the epithelial surface. Receptor cells are arranged in compact masses during maturation and spawning phases. Noticeable differences are observed in the outline of the lamella. They are elongated in spawning phase. The presence of well organized lamellae is due to their pronounced olfactory sense. Folding of the lamella is an adaptation for adequate usage of the major space in nasal cavity (Bertmer, 1972). Surface area of lamella increases by folds. Rana *et al.* (1978) reported that the inclusion of new receptor cells takes place with the expansion in region of olfactory epithelium. The occurrence of more receptor cells is thought to boost the effectiveness of olfactory organ to interact with the surrounding habitat and possibly involving in reproduction. Spawning phase signifies the peak maturity of sensory ciliated receptor cells and microvillous receptor cells, showing expansion of the olfactory mucosa at the time breeding takes place in population. The proliferation of dendrite tips in receptor cells commence with the incipience of maturation phase. The ciliated receptor cells have apical swelling provided with sensory hairs in spawning season. The aggregation of bipolar neuron is well also well marked. The dendrite tip of sensory neurons contains receptor sites and commences the sense of olfaction (Hansen *et al.*, 2004). The olfactory vesicle with sensory hairs and microvilli of receptor cells implies various functional activities and skill for recognition of chemical nature of odorants (Mokhtar and Abd-Elhafeez, 2014). These sensory components mediate reproductive behavior of the fish. Hamdani *et al.* (2008) mentioned that in salmon the size of the olfactory bulb, related to that of the telencephalon and the reveal volume of the input layer of the bulb both go

through a marked, continued expansion. The reform morphology of the olfactory bulb probably increases in the number of sensory neurons which mediates ecological-feeding habits associated to reproductive behaviour. The his to architecture of the assorted neurons gently diminishes coming the regressive period and compactness of the olfactory lining also appreciably shorten at resting phase of reproductive cycle.

## 5. CONCLUSION

The well developed olfactory organs of *C. garua* are primarily concerned with odour perception for exploring the surroundings in which they live. Structural forgoing of the olfactory organ is necessary for olfaction. Specialized receptor cells in the epithelial lining manifest the olfactory stimuli to assess the encompassing aquatic habitat. The sensory epithelium containing ciliary cells, microvillous cells and rod cells endure structural variations during the reproductive cycle concerned with seasonal requirements. Further studies should be conducted to investigate the specific components of olfaction in relation to ecological and reproductive behaviour of fish.

## 6. ACKNOWLEDGEMENTS

Financial support from Department of Higher Education, Science & Technology and Biotechnology, Government of West Bengal [Memo Number: 275 (Sanc.)/ST/P/S&T/1G-37/2017 dt. 27/03/2018].

## REFERENCES:

Akter, Y., H. A. Hosen, I. Miah, Z. F. Ahmed, M. S. Chhanda and S. I. Shahriar (2019). Impact of gonad weight on the length-weight relationships of river catfish (*Clupisoma garua*) in Bangladesh. *Egypt. J. Aquat. Res.*, 45, 375-379.

Arvedlund, M., P.L. Munday and A.Takemura (2007). The morphology and ultra structure of the peripheral olfactory organ in newly metamorphosed coral-dwelling gobies, *Paragobiodon xanthosomus* Bleeker (Gobiidae, Teleostei). *Tissue and Cell*, 39, 335-342.

Bandyopadhyay, S. K. and N. C. Datta (1998). Surface ultra structure of the olfactory rosette of an air-breathing catfish, *Heteropneustes fossilis* (Bloch). *J. Biosci.*, 23, 617-622.

Bakhtin, E. K. (1977). Peculiarities of the fine structure of the olfactory organ of *Squalus acanthias*. *Tsitol.* 19, 725-731.

Bannister, L. H. (1965). The fine structure of the olfactory surface of teleostean fishes. *Q. J. Micr. Sci.*, 106, 333-342.

Bertmar, G. (1972). Scanning electron microscopy of olfactory rosette in sea trout. *Z. Zellforsch. Mikrosk. Anat.*, 128, 336-346.

Bertmer, G. (1982). Structure and function of the olfactory mucosa of migrating Baltic trout under environmental stresses, with special reference to water pollution. In: Hara, T. J. (Eds.), *Fish chemoreception*. Elsevier, Amsterdam, 395-422.

Bhute, Y. V. and V. V. Baile (2007). Organization of the olfactory system of the Indian Major Carp *Labeo rohita* (Hamilton): a scanning and transmission electron microscopic Study. *J. Evol. Biochem. Physiol.*, 43, 342-349.

Datta, N. C. and S. Bandopadhyay (1997). Ultrastructure of cell types of the olfactory epithelium in a catfish, *Heteropneustes fossilis* (Bloch). *J. Biosci.*, 22, 233-245.

Fischer, A. H., K. A. Jacobson, J. Rose and R. Zeller (2008). Hematoxylin and eosin staining of tissue and cell sections. *C.S.H. Protoc.*, 3, 1-3.

Ghosh, S. K. and P. Chakrabarti (2016). Histomorphological and microanatomical characteristics of the olfactory organ of freshwater carp, *Cirrhinus reba* (Hamilton). *Arch. Pol. Fish.*, 24, 201-208.

Ghosh, S. K. (2018a). Histological Characterization of the Olfactory Organ in Schilbid Catfish, *Clupisoma garua* (Hamilton, 1822). *Int. J. Aquat. Biol.*, 6, 281-287.

Ghosh, S. K. (2018b). Cellular organization of the olfactory epithelium during growth, maturation, spawning and post-spawning phases of freshwater catfish, *Eutropiichthys vacha* (Hamilton, 1822) (Teleostei: Siluriformes). *Iran. J. Ichthyol.*, 5, 126-138.

Ghosh, S. K. (2019). Histology and surface morphology of the olfactory epithelium in freshwater teleost, *Clupisoma garua* (Hamilton, 1822). *Fish. Aqua. Life*, 27, 122-129

Graziadei, P. P. C. and J. F. Metcalf (1971). Autoradiographic and ultrastructural observations on the frog's olfactory mucosa. *Z. Zellforsch.*, 116, 305-318.

Hamdani, E. H., S. Lastein, F. Gregersen and K. B. Døving (2008). Seasonal variations in olfactory sensory neurons-fish sensitivity to sex pheromones explained. *Chem. Senses*, 33, 119-123.

Hansen, A. and E. Zeiske (1998). The peripheral olfactory organ of the zebrafish, *Danio rerio*: an ultrastructural study. *Chem. Senses*, 23, 39-48.

- Hansen, A., S. H. Rolen, K. Anderson, Y. Morita, J. Caprio and T. E. Finger (2003). Correlation between olfactory receptor cell type and function in the channel catfish. *J. Neurosci.*, 23, 9328-9339.
- Hansen, A., K. Anderson and T. E. Finger (2004). Differential distribution of olfactory receptor neurons in goldfish: structural and molecular correlates. *J. Comp. Neurol.*, 477, 347-359.
- Hernádi, L. (1993). Fine structural characterization of the olfactory epithelium and its response to divalent cations  $Cd^{2+}$  in the fish *Alburnus alburnus* (Teleostei, Cyprinidae): a scanning and transmission electron microscopic study. *Neurobiol.*, 1, 11-31.
- Hornung, D.E. and M. M. Mozell (1981). Accessibility of odorant molecules to the receptors. In: Cagan, R. H. and M. R. Kare (Eds.), *Biochemistry of taste and olfaction*, New York, Academic Press, 33-45.
- Ichikawa, M., and K. Ueda (1977). Fine structure of the olfactory epithelium in the goldfish, *Carassius auratus*. A study of retrograde degeneration. *Cell Tiss. Res.*, 183, 445-455
- Kim, H. T., Y. J. Lee, H. S. Kim and J. Y. Park (2018). Structure and histological characters of the olfactory organ in Korean endemic fish, *Microphysogobio yaluensis* (Cypriniformes, Cyprinidae). *Korean J. Ichthyol.*, 30, 161-166.
- Mallory, F. B. (1936). The aniline blue collagen stain. *Stain Technol.*, 11, 101-102.
- Masram, S. C. and V. V. Baile (2014). Ultrastructure of the olfactory organ in the striped snakehead *Ophiocephalus striatus* (Bloch). *Neurobiol.*, 5, 955-964.
- Mokhtar, D. M. and H. H. Abd-Elhafeez (2014). Light and electron-microscopic studies of the olfactory organ of red-tail shark, *Epalzeorhynchus bicolor* (teleostei: cyprinidae). *J. Microsc. Ultrastruct.*, 2, 182-195.
- Muller, J. F. and R. E. Marc (1984). Three distinct morphological classes of receptors in fish olfactory organs. *J. Comp. Neurol.*, 222, 482-495.
- Nikonov, A. A., J. M. Butler, K. E. Field, and J. Caprio (2017). Reproductive and metabolic state differences in olfactory responses to amino acids in a mouth brooding African cichlid fish. *J. Exp. Biol.*, 220, 2980-2992.
- Ojha, P. P. and A. S. Kapoor (1973). Structure and function of the olfactory apparatus in the Freshwater *Labeo rohita* (Ham. Buch.) *J. Morphol.*, 140, 77-85.
- Pasha, R. H., M. Z. Anjum, I. Ullah, M. A. Khan, A. Ali, S. Rehaman, S. Batool and A. Emmanuel (2019). Seasonal variation in the microscopic anatomy of gonads and gonadosomatic index of *Clupisoma garua*. *Pak. J. Agric. Res.*, 32, 670-674.
- Pashchenko, N. I. and A. O. Kasumyan (2015). Scanning electron microscopy of development of the olfactory organ in ontogeny of grass carp *Ctenopharyngodon idella*. *J. Ichthyol.*, 55, 880-899.
- Rana, A. K., J. Ojha and J. S. D. Munshi (1978). Morphometrics of the olfactory rosette in relation to body weight in a fresh water murrel *Channa punctatus* (Bloch). *Arch. Biol.*, 89, 403-417.
- Satou, M. (1992). Synaptic organization of the olfactory bulb and its central projection. In: Fish Chemoreception, Hara, T. J. (Eds.). Chapman & Hall, London, 40-59.
- Serajuddin, M. and A. Singh (2019). Gangetic catfish, *Clupisoma garua*: fishery and biology, Lambert Academic Publishing, Germany.
- Singh, N., K. C. Bhatt, M. K. Bahuguna and D. Kumar (1995). Fine structure of olfactory epithelium in *Schizothorachthys progastus* McClelland and *Schizothorax richardsonii* Gray (Cyprinidae: Teleostei) from Garhwal Himalaya (India). *J. Biosci.*, 20, 385-396.
- Talwar, P. K. and A. G. Jhingran (1991). Inland Fishes of India and Adjacent Countries, Vol.- 2, Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi-Calcutta.
- Theisen, B. (1972). Ultrastucture of the olfactory epithelium in the Australian lungfish, *Neoceratodus forsteri*, *Acta Zool.*, 53, 205-218.
- Waryani, B., R. Dai, Y. Zhao, C. Zhang and A. R. Abbasi (2013). Surface ultra structure of the olfactory epithelium of loach fish, *Triplophysa dalaica* (Kessler, 1876) (Cypriniformes: Balitoridae: Nemacheilinae), *Ital. J. Zool.*, 80, 195-203.
- Yamamoto, M. (1982). Comparative morphology of the peripheral olfactory organ in teleosts. In: Hara T. J. (Eds.), *Chemoreception in fishes*. Elsevier, Amsterdam, 35-59.
- Yamamoto, M. and K. Ueda (1978). Comparative morphology of fish olfactory epithelium-III. Cypriniformes. *Bull. Jpn. Soc. Sci. Fish.*, 44, 1201-1206.
- Zeiske, E., R. Mellinkat, H. Breucker and J. Kux (1976). Ultrastructural studies on the epithelia of the olfactory organ of Cyprinodonts (Teleostei, Cyprinodontoidae). *Cell Tiss. Res.*, 172, 245-267.
- Zeiske, E., B. Theisen and H. Breucker (1992). Structure, development and evolutionary aspects of the peripheral olfactory system. In: Hara T. J. (Eds.), *Fish chemoreception*. Chapman and Hall, London, 13-39.