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POSTNATAL DEVELOPMENT OF OLFACTORY APPARATUS IN *LABEO ROHITA* (HAMILTON, 1822)

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ABSTRACT
The microscopical details of time dependent postnatal morphogenesis of olfactory neuroanatomical structures and its cellular components (viz., sensory receptor cell, supporting cell, basal cell, etc.) in Labeo rohita have been studied under light microscope (LM) and scanning electron microscope (SEM) respectively. The development of olfactory apparatus in L. rohita starts from the paired olfactory placode of the hatching embryo at the time of 18hrs. (after hatching) and the multilamella olfactory rosette is primarily differentiated during 22nd day of after hatching. During the morphogenesis of olfactory structures, the cellular components are subsequently differentiated at variable time within the olfactory neuroepithelium. The ciliated sensory receptor cell and ciliated supporting cells appear earlier at 3rd day (after hatching) where as microvillous sensory receptor cells are identified within the olfactory neuroepithelium at the stage of 22nd day after hatching. This study ultimately correlates the function based appearance of distinct sensory receptor cells within olfactory neuroepithelium and suggest to prepare a feeding preference related fish feed formula for the appropriate development of L. rohita (an Indian Major Carp) in indigenous carp-culture system.

KEY WORDS: Labeo rohita, olfactory, placode, neuroepithelium, morphogenesis, *etc*.

INTRODUCTION

Vertebrate possess different modality of sensory organs which can perceive various types of neural information from their external environment by using different receptor cells *viz.,* chemoreceptors, thermoreceptors, mechanoreceptors, photoreceptors, *etc.* (Diaz- Casares *et al.,* 2005). The major sensory organs of head region generally develop from the interaction of neural tube with a series of epidermal thickening, called cranial ectodermal placodes (Brugmann and Moody, 2005). The paired olfactory placodes are one of the important structures which are responsible for the development of olfactory system in vertebrates (von Kupffer, 1894). The placodal cells are apparently homogeneous in nature which differentiates into olfactory organ (Whitlock and Westerfield, 1998). This neurosensory organ is responsible for different biological functions in fish including food searching, mate selection, predator avoidance, *etc.* (Hara, 1971). The olfactory apparatus in fishes is usually comprises of olfactory rosette, olfactory chambers, accessory nasal sacs, olfactory nerve, olfactory bulb and brain (Hamdani and Døving, 2007, De and Sarkar ,2009, Sarkar and De, 2011, Biswas, *et al.,* 2013). The developmental pattern and structural components of the olfactory apparatus shows a wide range of diversity among the teleosts depending on their habitat, species, age, *etc*. (Hansen and Zielinski, 2005, Cox, 2008). The olfactory rosette is externally lined by a neuroepithelium which includes different type of cellular components *viz*., sensory receptor cells, supporting cell and basal cells. These cells are common in all the phyla belonging to the vertebrates (Farbman, 1992). According to Zeiske *et al.,* (2003), the timing of cellular differentiation within the developing olfactory neuroepithelium significantly differs among the teleostean species. Notwithstanding that, the roles of developing and differentiating neurosensory cellular components in various successive embryonic stages of teleosts are also hardly detailed. *Labeo rohia* (Hamilton, 1822) is a common freshwater Indian Major Carp. This species generally spawns during monsoon season but equally respond to induced breeding under favourable conditions (Jhingran and Pullin, 1985). This study is focused on the microscopical details of the postnatal morphogenesis of olfactory apparatus as well as time specific appearance of olfactory neuroepithelial cellular elements in *L. rohia* to correlate their functional relevance in different successive embryonic stages of the said species.

MATERIALS & METHODS

Microanatomy

Different stages of hatchlings of *L. rohita* were collected from Krishi Vignan Kendra (Farm Science Centre), Nimpith Ashram, South 24 Parganas, West Bengal, India. Hatchlings of 18 hrs. to 22nd day [body size ranges from 4.5mm to 20 mm (approx.)] were fixed in aqueous Bouin's solution for 12hours (overnight). After fixation, the fixed tissues were dehydrated in graded ethanol and followed by storing in cedar wood oil for overnight. The tissues were then cleared in xylene and embedded in paraffin wax (56°C–58°C) under a thermostat vacuum paraffin-

embedding bath for a period of 30 minutes. The serial thin longitudinal sections (about 4µm) of the paraffin embedded tissue were cut by using rotary microtome and stretched on Mayer's albuminised glass slide. The tissue sections were stained with Ehrlich's haematoxylin and counter stained with eosin. The slides were examined under light microscope (LM).

Macroanatomy

Live, adult, healthy, sex-independent specimens of *L. rohita* (total length: 20 cm -24 cm) were collected from the local markets of North 24 Parganas, West Bengal, India and brought to the laboratory. The specimens were acclimatized in laboratory conditions for 72 hours at room temperature and then anaesthetized by MS-222 (dose: 100 mg/l – 200 mg/l). The macroanatomical detail of the head, nostrils, solitary chemosensory cells and olfactory structures was studied under a binocular light microscope.

Scanning electron microscope (SEM) study

For scanning electron microscope (SEM) study, the dissected olfactory rosette of *L. rohita* was fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2- 7.4) at 4°C for 2 hours. After rinsing in phosphate buffer, the samples were dehydrated in graded chilled ethanol. The specimens were critical point dried, mounted on aluminum stubs and coated with gold. The olfactory structures were observed under a scanning electron microscope [SEM: LEO - 435] operated at 15 kV in 0° tilt angle.

RESULTS

The olfactory placodes are paired structures and appear at the ventrolateral part of the head just anterior to the eye at the period of 18hrs. after hatching of the embryo of *L. rohita* (average body size of 4.5 mm) (Fig. 1). The olfactory placode invaginates from the surface to form the olfactory pit at the period of 30hrs. after hatching. The entire pit is covered with epithelial lining (Fig. 2). This epithelium is composed of multilayer epithelial cell viz., columnar cells, undifferentiated cells at the apical and basal part (Fig. 3). The undifferentiated cells are present at the middle and basal part of the olfactory placode and possess large, spherical nucleus (Fig. 3).

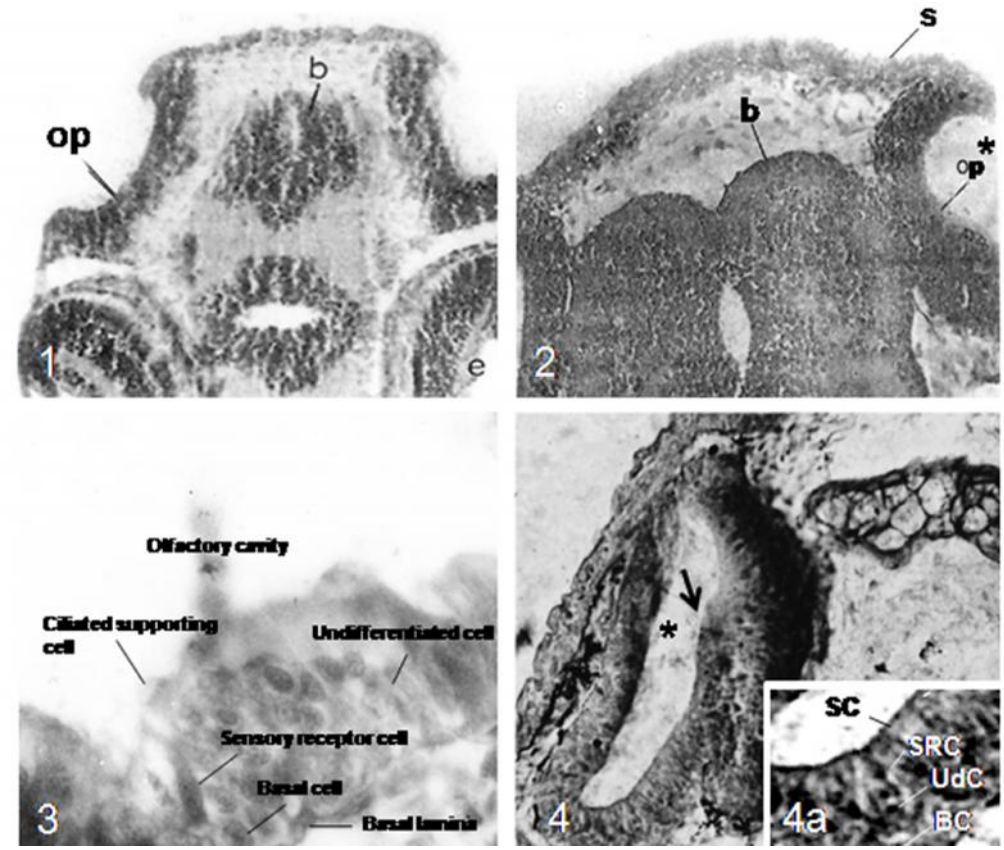

FIGURE 1: The olfactory placode (op) is formed just anterior to the eye (e) and lateral part of the brain (b) at the stage of 18hrs. of embryo (after hatching) of *L. rohita*. [Mag. x 100 (approx.)]

FIGURE 2: At the stage of 30hrs. (after hatching), the olfactory placode (op) is gradually invaginates from the surface layer (s) and forms a prominent olfactory pit (*). The primary subdivisions of the brain (b) are also appears. The placodal epithelium or olfactory epithelium includes multicellular layers with few columnar cell and numerous undifferentiated cells. [Mag. x 400 (approx.)]

FIGURE 3: The olfactory epithelium of 3 rd day of embryo (after hatching) possess sensory receptor cell, ciliated supporting cell, basal cells, etc. that are present on the basal lamina. [Mag. x 1000 (approx.)]

FIGURE 4a: The epithelial folding (→) is first noted within the olfactory pit at the stage of 7 th day of embryo (after hatching) in *L. rohita*. [Mag. x 400 (approx.)]

FIGURE 4b – The olfactory epithelium [7 th day of embryo (after hatching)] of *L. rohita* possess large number of sensory receptor cells (SRC), supporting cells (SC), undifferentiated cell (UdC), basal cell (BC), etc. [Mag. x 1000 (approx.)]

The placode elongates in the longitudinal axis. Basal lamina is not well demarcated. In the embryo of 3rd day (after hatching) when the embryo attains 7.5 mm body length (approx.), the sensory receptor cell with prominent dendron is appears within the epithelium (Fig. 3). The cilia are easily recognizable at the apical part of the neuroepithelium, directing towards the nasal cavity. The ciliated supporting cells and basal cells are also identified. Basal lamina is prominent. No epithelial folding is observed within the olfactory neuroepithelium (Fig. 3). At the stage of 7 th day after hatching (the average size of the embryo ranges from 7.5 mm to 9.5 mm), the primary epithelial folding of the olfactory epithelium is noted at the floor of the olfactory chamber (Fig. 4).

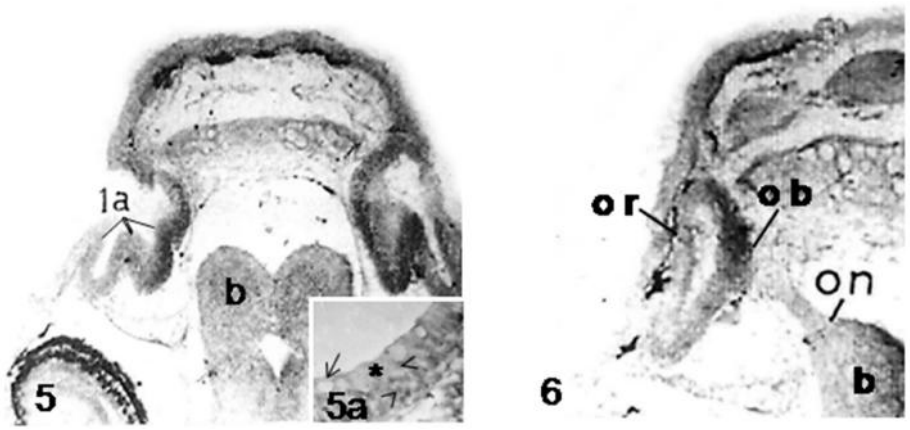

FIGURE 5a: At the stage of 22 nd day of embryo (after hatching)] of *L. rohita*, several folding of the olfactory neuroepithelium is found and forming multilamellar structure (la). [Mag. x 100 (approx.)]

FIGURE 5b: The olfactory neuroepithelium shows different sensory receptor cells (arrow heads), microvillous supporting cell (*), mucous cells (→), etc. [Mag. x 1000 (approx.)]

FIGURE 6: The primary olfactory rosette (or) is formed at the end of 22 nd day of embryo (after hatching). The olfactory bulb (ob) is attached at the distal part of the olfactory rosette (or) and short olfactory nerve (on) is associated with olfactory bulb (ob) and proximal part of the brain (b) respectively. [Mag. x 100 (approx.)]

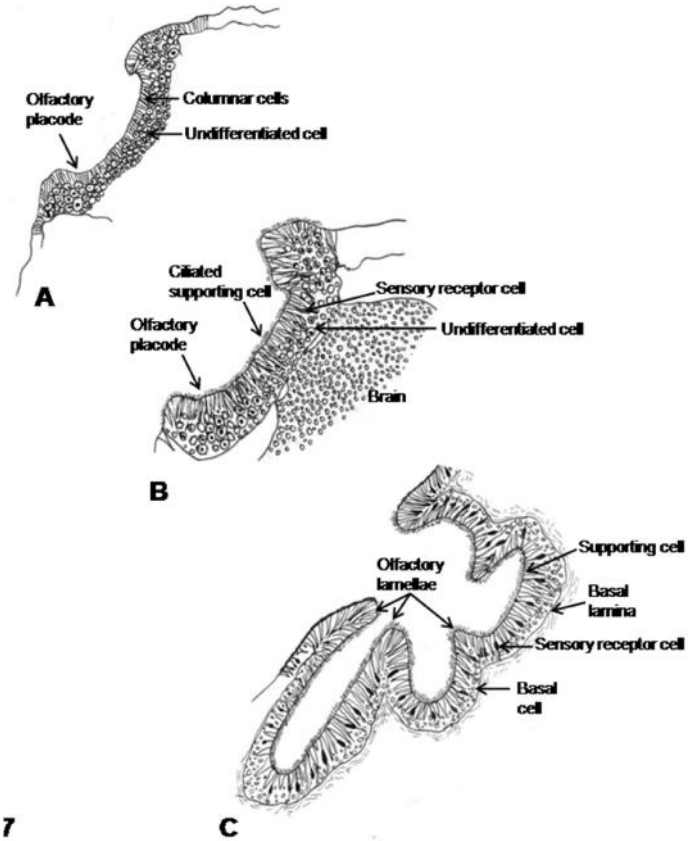

FIGURE 7: The diagrammatic representation of the time dependent post–natal development and differentiation of olfactory neuroepithelium and its cellular components from olfactory placode to primary olfactory rosette in *L. rohita* (A-C). [Not to Scale]

Sensory receptor cell, undifferentiated cells and basal cells are also present at the various depth *i.e*., middle and lower part of this epithelium (Fig. 4b). The olfactory rosette with multiple olfactory lamellae is primarily formed at the stage of 22nd day after hatching (average 15 mm – 20 mm) (Fig. 5). Each lamella has a central core, which is composed of loose connective tissue and bears epithelial lining on both side of it (Fig. 5a). The olfactory epithelium shows different type of cellular element and constitutes the multilayer pseudostratified structure. In this neuroepithelium, sensory receptor cells (*i.e*., ciliated and microvillous sensory receptor cell), supporting cells (*viz*., ciliated and microvillous supporting cell), undifferentiating cells, goblet cell and basal cells are present (Fig. 5b). Goblet cell in the olfactory neuroepithelium is not found in early embryonic stage (Fig. 5b). The nasal cavity is extended towards the apical surface of the outer ectoderm to communicate with the nostril. The olfactory bulb, single accessory nasal sac and olfactory nerve tract is prominent at the stage of 22nd day of embryonic period (Fig. 6). The initial olfactory pit of 18hrs embryo is transformed into nasal cavity at 22nd day of embryo (Figs. 7A – 7C).

The macroanatomical details on the completely differentiated olfactory apparatus of *L. rohita* (total body length about 20 cm– 24cm.) shows distinct olfactory chambers, olfactory rosette, accessory nasal sac, olfactory bulbs, olfactory nerve tracts and brain (Fig. 8). The nostrils *i.e*., anterior and posterior nostril are closely associated with each other (Fig. 9a). The nostrils are roughly oval in shape. A skin like nasal flaps is also present in between the nostrils and may guards the posterior nostril (Fig. 9b). A cup shaped olfactory chamber is present just beneath the nostril and shows distinct arrangement of multiple olfactory lamellae (number ranges from 46 to 56), radiating from central raphe (Fig. 10). The orientation of the olfactory lamella at the either side of groove like nasal cavity may forms an oval shaped olfactory rosette (Fig. 10). The rosette is well communicated with the nostrils through nasal cavity. Spherical olfactory bulbs are attached with the posterior part of the olfactory chamber at either side of the head (Fig. 8). The olfactory nerve tracts are originate from the olfactory bulb and connected with the fore brain of *L. rohita* (Fig. 8)

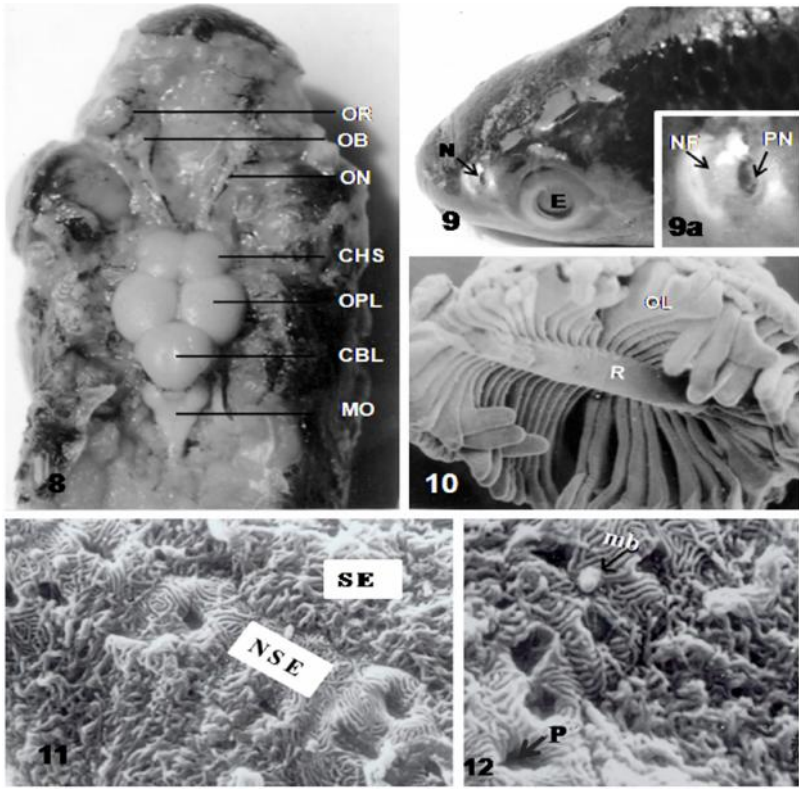

FIGURE 8: The morphoanatomy of the olfactory apparatus having total body length about (20– 24) cm is comprises of olfactory rosette (OR), olfactory bulb (OB), olfactory nerve tract (ON), cerebral hemisphere (CHS), optic lobe (OPL), cerebellum (CBL), medulla oblongata (MO), etc. [Not to Scale]
FIGURE 9a: The olfactory rosette is situated in between the nostrils (N) located just anterior to the eye (E). [Not to Scale]
FIGURE 9b – The photomicrograph shows a skin like nasal flap (NF) is present in front of the posterior nostril (PN). [Not to Scale]
FIGURE 10: The scanning electron micrograph shows olfactory rosette which is comprises of multiple olfactory lamella (OL) radiating from central raphae (R). [Mag. x 50 (approx.)]
FIGURE 11: The surface structure of each olfactory lamella indicate distinct arrangement of sensory (SE) and nonsensory (NSE) epithelial components. [Mag. x 2500 (approx.)]
FIGURE 12: Several pores (P) and mucous balls (mb) are identified in the nonsensory epithelium of the olfactory lamella in *L. rohita* under scanning electron microscope (SEM). [Mag. x 2800 (approx.)]

The olfactory rosette of *L. rohita* is oval in shape, observed under scanning electron microscope (SEM) (Figs. 8 and 10). It has a median depression where an elongated raphe is present (Fig. 10). The lamellae are arranged pinnately along with the raphe almost parallel to each other with approximately 6 µm gap in between them. The size and shape of the lamella vary according to its position. The dorsal margin of lamella is provided with linguiform process (Fig. 10). The raphe has a spongy appearance with uneven surface. The sensory zones are separated by the presence of narrow area of fingerprint like polygonal microridges (Fig. 11). The nonsensory epithelial components are characterized by the presence of polygonal microridges, mucous secretory pits, *etc*. (Figs. 11 and 12). The entire lateral part of the raphe is covered with fingerprint like microridges with randomly distributed pits. The diameter of the pit is approximately 0.3 µm. Mucous balls are also noted under SEM study (Fig. 12). The sensory epithelial components are mostly covers the dorsal and median surface of the olfactory lamella.

DISCUSSION

The olfaction is the most primitive type of chemosensory modality of vertebrates (Buck, 2000, Dominy *et al.,* 2004). This sense is regarded as the first chemical sense which appears during the ontogenetic development of fish (Kotrschal *et al.,* 1997). The developmental events of the olfactory apparatus are variable among teleosts (Whitlock 2004, Katoh *et al*., 2011). In *Acipenser baerii* (Siberian sturgeon) and *Acipenser ruthenus* (sterlet), this event is initiated at the different time than *L. rohita* during embryonic development (Zeiske *et al.,* 2003). The cone shaped olfactory placode in *Polypterus senegalus* appears at the embryonic stage of 40hrs – 52 hrs. after fertilization (Zeiske *et al*., 2009). The present study indicates that the olfactory placode in *L. rohita* is appears little earlier (at 18 hrs. after hatching) than *A. baerii*, *A. ruthenus* and *P. senegalus.* Does these variations are reflecting species-specific time dependent diversities (?). The detail on this time dependent variation regarding the appearance of olfactory placode is still little known to us. Possibly, the physical factors (specifically temperature) may influence embryonic development of teleost in aquatic ecosystem (Herzig and Winkler, 1986, Blaxter, 1988, Nwosu and Holzlohnev, 2000, Morehead and Hart, 2003) which may reflects the time dependent variation in comparative analysis of the ontogeny of olfactory apparatus. After development of the olfactory placode, the placodal cells are subsequently differentiated to various neuroepithelial components within the olfactory neuroepithelium of *L. rohita*. Ciliated sensory receptor cell and ciliated supporting cells in the olfactory neuroepithelium of *L. rohita* are also developed at the time of 3 rd day (after hatching), earlier than *P. senegalus* (at the stage of 5th day after fertilization) and *A. ruthenus* (at 108hrs. - 126 hrs. after fertilization) respectively (Zeiske *et al.,* 2003, Zeiske *et al.,* 2009). The olfactory pit of *L. rohita* is communicated with the nostrils. Nostrils are important anatomical structure for incurrent and excurrent of water over the olfactory rosette (Nevitt, 1991). The epithelial folding of the olfactory neuroepithelium may leads to form the olfactory rosette in *L. rohita*. The distribution pattern of the sensory and non-sensory neuroepithelial components is depending on the cellular organization during the development of the olfactory neuroepithelium in respective species. In *L. rohita*, the sensory components are mostly found on the medial and dorsal part of the olfactory lamella that also indicates the probable interaction site with water soluble chemicals. The surface structures of the sensory neuroepithelial components of *L. rohita* probably involves in the perception and transduction of neural information through their axonal projection into the olfactory bulb. At the time of hatching, the larvae of *L. rohita* possess neither a mouth nor the gut and obtain their nourishment from the yolk-sac (Kamal, 1966). The mouth develops a day after hatching, but it took some days for becoming functionally adapted for gulping the food items from the water. The food item were found for the first time within the gut of *L. rohita* fry at the 5th day (after hatching) and shows variety of zooplanktons (Alikunhi, 1952). At the 19 th day (after hatching), the gut contains comparatively less amount of zooplankton than phytoplankton (Alikunhi, 1957, Kamal, 1967). According to Hamdani *et al.* (2001), the feeding behaviour in fish is mediated through microvillous sensory receptor neurons that forms lateral olfactory tract within the olfactory bulb. Sato & Suzuki (2001), described the ciliated sensory receptor cells as functionally 'generalists' that can respond to wide range of odorants including feeding related stimuli but the microvillous sensory receptor neurons are 'specialist' only for recognition of feeding related chemical cues. This study reveals that the sensory receptor cells specifically ciliated sensory receptor cell within the olfactory neuroepithelium are appeared during the 3 rd day after hatching, which may help to initiate extrinsic feeding behaviour in *L. rohita* but the olfactory apparatus become fully developed during the period of 19th to 22nd day after hatching that probably involve to take preferable food materials from the external environment. Therefore, this study suggests to prepare a prospective fish feed formulation which will be useful for the nourishment of developing embryo of *L. rohita* in indigenous carp-culture system.

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