GROSS AND ULTRASTRUCTURAL DETAILS OF THE SWIM BLADDER (GAS SECRETING COMPLEX IN PARTICULAR) OF TWO MASTACEMBELLIDS- *Mastacembelus armatus* AND *Macrognathus aculeatum*.

Chanda Jha, Abhay kumar and Tapan Kumar Ghosh
Post graduate Department of Zoology,
T.M. Bhagalpur University, Bhagalpur,
812007.

ABSTRACT

The swim bladder of Mastacembelus *armatus* and *Macrognathus aculeatum* (family: Mastacembelidae) are silvery white, elongated and tubular structures. The swim bladder of *Mastacembelus armatus* is semi-transparent while in *Macrognathus aculeatum*, it is absolutely transparent in texture. The gas secreting complex in *M. armatus* is present in the form of five to six curved patches while in *M. aculeatum* it is in the form of a single unit which is an inverted 'U' shaped structure. The surface ultrastructure of the gas secreting complex in both these fishes shows a central depressed area and slightly raised or bulging marginal areas. The region near the middle depressed core are characterized by some raised microvillous cells along with some typical rosette like structure scattered over the surface in *M. aculeatum* whereas the same area in *M. armatus* has some round structures with pores along with a few microvillous cells.

Key Words:- Swim bladder, Gas secreting complex, Ultrastructure, *Mastacembelus armatus, Macrognathus aculeatum*

INTRODUCTION

The occurrence of swim bladder in teleostean fishes is very irregular and shows some extra ordinary modifications in its shape, size and location in the fish. The organ is unique and versatile owing to its variable structure and functionality and has attracted the attention from the past. Many studies of teleostean swim bladder have described the morphological and functional characteristics of the structure along with its gas secreting complex, which is more developed and conspicuous in the physoclist fishes. The swim bladder helps the fish to attain neutral buoyancy, i.e. the ability to use little or no energy to stay at particular levels of water through the expansion (inflation) and contraction (deflation) of the swim bladder due to varying gas pressures (Schmidt and Neilson, 1997). The relationship of the shape and size of the swim bladder with the taxonomy or systematic position of the fish has been observed by Marshall, 1960; Greenwood, 1963; Ahmad, 1971; Evans, 1973; Sujatha and Dutt, 1985; Hemalatha and Rajkumar, 1996. Many studies have correlated the weberian ossicle and inner ear with the swim bladder morphology (Starks, 1908; Evans, 1925 & 1930; Nelson, 1949; Srivastava, 1956; Dehadrai, 1957 & 1959; Jasinski, 1964; Allen *et al.*, 1976; Laming and Morrow, 1981; Barimo and Fine, 1998).

Many studies have described the characteristics of the gas gland and rete mirabile associated with swim bladder (Woodland, 1911, 1913; Scholander, 1956; Dehadrai, 1957; Fahlen, 1959; Wittenberg *et al.*, 1964; Jasinski, 1965) and have revealed marked variability in the morphology of this structure in different teleost species. Inspite of the fact that considerable information on the gas secreting complex and the ultrastructure of swim bladder is available in general but such information is limited about Indian fish species.

The ultastructural details of the swim bladder among different group of fishes have been revealed by several workers (Copeland,1960,1961 & 1969; Jasinski and Kilarski, 1964; Fahlen,1967,1970, 1971 &1973; Brooks,1970; Henderson, 1975 ; Morris & Albright, 1975; Harder, 1975; Green, 1984; Wagner *et al.*, 1987; Zheng and Liu, 1988; Maina *et al.*, 1996; Maina, 2000; Prem *et al.*, 2000) yet the information on surface ultrastructure of the swim bladder epithelium and gas gland area are limited particularly in Indian teleosts.

The present work is an endeavour to determine the morphological and ultrastructural details of the swim bladder and its gas secreting complex in particular of the two Mastacembellid fishes, *Mastacembelus armatus* and *Macrognathus aculeatum.*

MATERIALS AND METHODS

For the study of gross morphology of swim bladder, the two fish species i.e. *Mastacembelus armatus and Macrognathus aculeatum*, belonging to the family Mastacembelidae were taken. The live specimens of both the fishes were first anaesthetized in MS_{222} and then dissected mid ventrally to study the structure, position, attachment and vascular network of the swim bladder. Digital images of fish and the gas secreting complex were taken with a Canon A2000 Camera. For ultrastructural studies, tissues of the swim bladder particularly, from the region of gas secreting complex were fixed with 2.5% glutaraldehyde in phosphate buffer (0.1M, pH-7.4). They were then dehydrated in solutions of mixture of ethanol and acetone and finally stored in anhydrous acetone. The specimens were critical point dried with liquid carbon di-oxide, sputter coated with gold and examined with a Scanning Electron Microscope QUANTA-200 at RSIC, Bose Institute, Kolkata.

RESULTS

Mastacembelus armatus:

In *Mastacembelus armatus* the swim bladder is a single chambered elongated and tubular structure. It is the longest organ in the visceral cavity of the fish occupying its entire length. It originates from below the oesophagus, near the pectoral fin base and extends upto the anal opening above the caudal peduncle. It is whitish in colour and semi-transparent in nature (fig.1).The swim bladder is situated between the vertebral column (dorsally) and alimentary canal (ventrally) of the fish. The bladder is pysostomous as it remains connected to the oesophagus via a pneumatic duct. However, it possesses a well developed gas secreting complex which is characteristic of the physoclistous swim bladder (fig.).

The gas secreting complex is located in the form of several reddish patches on the inner surface of the tunica interna. Though there are a number of gas gland areas associated with the rete mirabile yet the one which is situated near the entrance of pneumatic duct is more prominent and conspicuous. On exposure from the inner side, the gas secreting complex is present in the form of five to six pieces of curved worm like units (fig.). The concavities of these pieces are oriented towards a central region and remain connected through blood vessels. The tufts of blood capillaries (rete mirabile) can be seen radiating from each of the gas gland units.

Macrognathus aculeatum

In *M. aculeatum* the characteristics of the swim bladder is almost similar to that of *M. armatus.* It is tubular and elongated freely lying structure extending into the whole of body cavity. The structure is glossy white in colour and transparent in nature (fig.1,2). The organ acquires maximum diameter in the middle region while both the anterior and posterior ends are pointed. The swim bladder is of physoclistous type and bears no connection with the oesophagus. The gas secreting complex is situated on the ventral wall of swim bladder on its internal side i.e. facing the lumen (fig.3). The region is characterized by a dark red, thick layer of epithelium when exposed and spread. The gas secreting complex is present in the form of an inverted 'U' with rounded ends (fig.4).

ULTRASTRUCTURE

The photomicrographs from the region of gas secreting complex when viewed through a Scanning electron microscope, show some remarkable structures in both of the experimental fishes, *M. armatus* and *M. aculeatum*. The gas gland areas may be distinguished into two separate regions, the central depressed core and the marginal raised areas in both the fishes (fig. 7). The marginal raised areas show numerous parallel striations which ramify from the central depressed core of the gas gland. The striations are the impressions of underlying capillaries. The surface shows epithelial cell margins and mucous gland openings, which are very prominent in this region (fig.6).

The central core of the gas gland epithelium in both the fish species i.e. *M. armatus* and *M. aculeatum* is characterized by well defined hexagonal cells with prominent margins. Some of the cells are raised and covered by distinct microvilli. In *Macrognathus aculeatum* this region bears some rosette like raised structures (fig. 6), scattered near the microvillous cells. While the same region in *Mastacembelus armatus* has some oval structures that are devoid of any microvilli and contain many pocket like openings. They seem to be made up of fine capillaries (fig. 5).

DISCUSSION

The swim bladder in both the species i.e. *M. armatus* and *M. aculeatum* is single chambered tubular and elongated structure. It is the longest organ in the visceral cavity of the fish occupying its entire length. The shape is similar to the typical teleost physoclistous condition, particularly to *M. pancalus* of the same family (Jha, 2011) and *Xenentodon cancila* (Day, 1978; Jha, 2011) belonging to the family Belonidae, some of the Hemiramphids and Exocoetids (Tibbets *et al.*, 2007). A single chambered swim bladder are typically regulated by a hypertrophied vascular system including the rete mirabile and the oval body (Prem and Pelster, 2000; Yamada *et al.*, 2004). The structure of the gas secreting complex is very different in both the species, though they belong to a single family Mastacembelidae. The structure of glandular apparatus or the gas secreting complex is essentially of the type described for the eel by (Woodland, 1911). The capillaries emanating from the rete mirabile rejoin to form arterioles and venules which go to the gas gland, where they breakup into capillaries providing a very rich blood supply to the glandular membrane. Every fold of the membrane contains blood vessels and it is probable that each gland cell has access to a blood capillary at its base. Capillary connections are found between arterioles and venules emanating from the same rete as well as between blood vessels emanating from different retia. The scheme of the blood supply is similar to that described in toadfish, *Opsanus tau* (Fange and Wittenberg, 1958) and eel, *Anguilla* (Woodland, 1911).

Woodland (1911), in his classic description of the gas gland, distinguishes three major types of gas gland: those in which the glandular epithelium is composed of a single layer of cells, those in which gland is massive and those in which a primitively single layer of cells is secondarily folded into a massive

structure. The *Macrognathus aculeatum* belongs to the second category (Woodland, 1911) in which "the glandular epithelium is composed of many layers of cells which remains complexly folded...".

In *M. aculeatum* the gas secreting complex is made up of single unit, in the form of 'U' and the ends of this 'U' are rounded or blunt. The area is characterized by a thick epithelium but not as massive as in *M. armatus* and appears to belong to the first category from the description of (Woodland, 1911). According to him, in the first category "the glandular epithelium is composed of a single layer of cells which either remains unfolded or is only simply folded.." .Woodland further subdivides his first class of gas glands on the basis of the extent of the glandular epithelium and the degree of reunion of the blood vessels. The *M. aculeatum* belongs to the Syngnathus subdivision type Ib, similar to that of *Opsanus tau* (Fange and Wittenberg, 1958) and *Anguilla anguilla* (Woodland, 1911; Fange, 1953) in which " the glandular epithelium is restricted in area, not lining the whole of the bladder cavity, and the rete mirabile is contiguous with the gas gland, although a small amount of reunion of the capillaries of the rete may occur before these supply the epithelium".

According to Wittenberg *et al.* (1964) "all gas gland (except in species in which the gas secreting complex is diffused or weakly developed) are intensely vascular. The difference lies in the position of the capillaries". In the majority of species the gas gland capillaries lies below the cells, with which they are intimately associated (Woodland, 1911).

The photomicrographs from the region of gas secreting complex when viewed through the Scanning Electron Microscope, shows some remarkable structures. The gas gland areas may be distinguished into two separate regions, the central depressed core and the marginal raised areas in both the fishes. The marginal raised areas show numerous parallel striations which ramify from the central depressed core of the gas gland. The striations are the impressions of underlying capillaries. The surface shows epithelial cell margins and some mucous gland openings, which are very prominent in this region. Prem *et al.* (2000) has observed similar swim bladder surface with epithelial cells in the non-gas gland region in a perch, *Perca fluviatilis*. Prem *et al.* (2000) has also reported similar openings from the gas gland region, and they have been termed as canal openings in *Perca fluviatilis.*

The central core of the gas gland epithelium in both the fish species i.e. *M. armatus* and *M. aculeatum* is characterised by well defined hexagonal cells with prominent margins. Some of the cells are raised and covered by distinct microvilli. These seem to be impressions of the underlying gas secretory cells, as has been described by Maina *et al*. (1996) in the gas gland region of the swim bladder of a cichlid, *Oreochromis alcalicus alcalicus*. The presence of scattered microvilli in the region of gas gland in *Perca fluviatilis* has also been reported by Prem *et al.* (2000).

Small outgrowths of different shapes and sizes are seen bulging from this surface and remains scattered all over in both the fishes. In *M. aculeatum*, on higher magnification these impressions appear as typical rosette like structures. These rosettes demonstrate the presence of guanine crystals on the surface of gas secreting complex. The swim bladder wall is made impermeable by the presence of these guanine crystals to prevent gas loss from its surface. In *M. armatus* the gas gland surface from the marginal raised area is worked with two distinct types of structures, one having microvillous surface while the other is round structure made of capillary network.

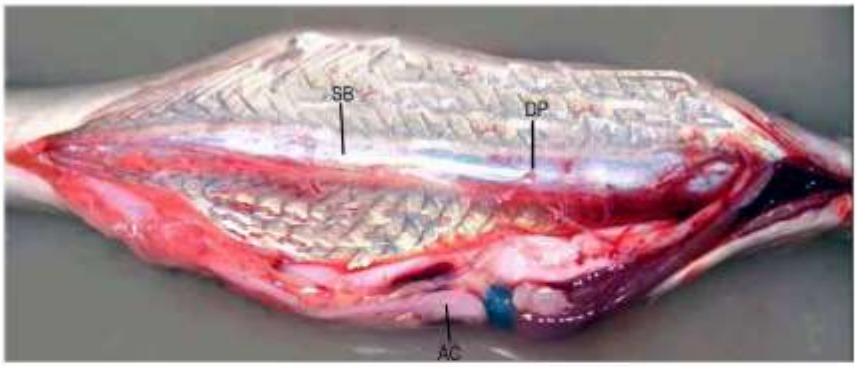

Fig. 1

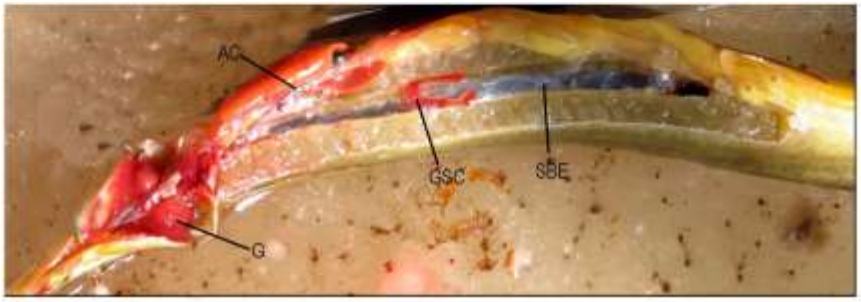

Fig. 2

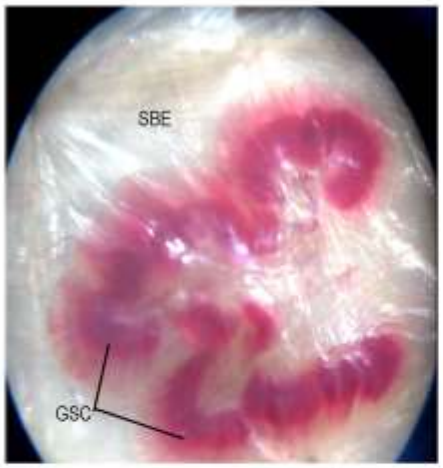

Fig. 3

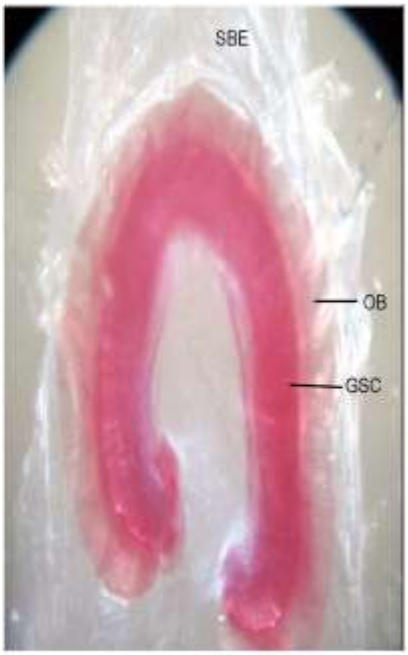

Fig. 4

Fig. 1,2 : Ventral view of body cavity showing the swim bladder with associated structures in situ. Nat. size

Fig. 3,4 : Internal view of gas secreting complex. Nat. x 12

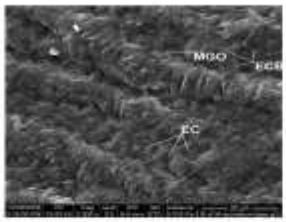

Fig. 5

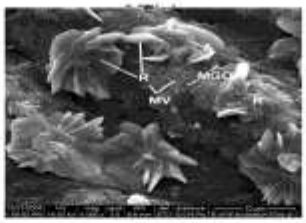

Fig. 6

Fig. 5 : View of the gas gland surface showing transverse striations along with the epithelial cell margins x 400

Fig. 6 : enlarged view (higher magnifications) from the same region with some rosette like structure adhering to the surface x 4000

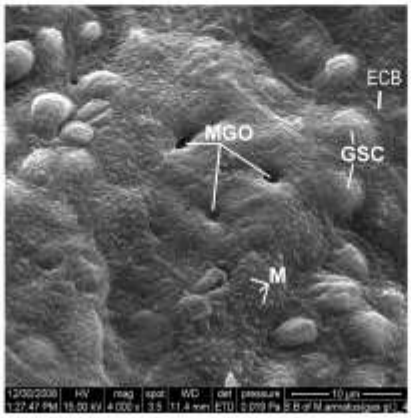

Fig.7

Fig. 7 : View of the adjacent middle depressed core of the gas gland region showing microvillous structure and round structures with capillary network

REFERENCES

Ahmad, M.F. (1971): Morphology of the air bladder of Sciaenoid fishes and its significance in the classification. *Rec. Zool. Surv. Pakistan*, **2:** 131-143.

Allen, J.M., Blaxter, J.H.S. and Denton, E.J. (1976): The functional anatomy and development of the swimbladder-inner ear-lateral line system in herring and sprat. *J. Mar. Biol. Ass. U.K.*, **56**: 471-486.

Allen, J.M., Blaxter, J.H.S. and Denton, E.J. (1976): The functional anatomy and development of the swimbladder-inner ear-lateral line system in herring and sprat. *J. Mar. Biol. Ass. U.K.*, **56**: 471-486.

Barimo, J.F. and Fine, M.L. (1998): Relationship of swim-bladder shape to the directionality pattern of underwater sound in the Oyster toadfish. *Can. J. Zool*. **76(1)**: 134-143.

Brooks, R.E. (1970): Lung alveolar and swimbladder epithelial cell cytosomes:A consideration of their significance. *Z. Zellforschung.* **106**: 484-497.

Brooks, R.E. (1970): Ultrastructure of the physostomatous swimbladder of Rainbow trout (*Salmo gairdneri*). *Z. Zellforsch. Mikrosk. Anat.*, **106**: 473-483.

Copeland, D.E. (1960): Secretory epithelium of the swim-bladder in Fundulus. *Biol. Bull.*, **119**: 311-312.

Copeland, D.E. (1961): Ultrastructure (sic) of Swim-bladder. *Anat. Rec.*, **139**: 298.

Copeland, D.E. (1969): Fine structural study of gas secretion in the physoclistous swim bladder of *Fundulus heteroclitus* and *Gadus callarias* and in the euphysoclistous swim bladder of *Opsanus tau*. *Z. Zellforsch.*, **93**: 305-331.

Day (1978): *The fishes of India being a natural history of the fishes known to inhabit the seas and freshwater of India, Burma and Ceylon*. **Vol. I & II**, pp. 778

Dehadrai, P.V. (1957): On swim bladder and its relation with the internal ear in genus *Notopterus*. *J. Zool. Soc. India*, **9 (1)**: 1-10.

Dehadrai, P.V. (1959): The swim bladder and its connection with the internal ear in family Cichlidae. *Proc. Nat. Inst. Sci. India*, **25 B** : 254-261.

Evans, H.M. (1925): A contribution to the anatomy and physiology of the air-bladder and the weberian ossicles in Cyprinidae. *Proc. Roy. Soc. Lond.* **B, 97**: 545-575.

Evans, H.M. (1930): The swimbladder and weberian ossides and their relation to hearing in fishes. *Proc. Roy. Soc. Med.*, **23**: 11.

Evans, R.R. (1973): The swim bladder and associated structures in Western Atlantic sea robins (Triglidae). *Copeia*, **1973(2):** 315-321.

Fahlen, G. (1959): Rete mirabile in the gas bladder of *Coregonus lavaretus*. *Nature* (London), **184**: 1001.

Fahlen, G. (1967): Morphological aspects on the hydrostatic function of the gas bladder of *Clupea harengus. L. Acta. Univ. Lund.* Sect. **2 no. (1)**: 1-49.

Fahlen, G. (1967): Morphology of the gas bladder of *Coregonus lavaratus. L. Acta. Univ. Lund*. Sect. **2 no. (28)**: 3-36.

Fahlen, G. (1970): The gas bladder of *Argentina silus L.* with special reference to the ultrastructure of the gas gland cells and the countercurrent vascular bundles. *Z. Zellforsch*., **110**: 350-372.

Fahlen, G. (1971): The functional morphology of the gas bladder of the genus Salmo. *Acta. Anat.* **78**: 161-184.

Fange, R. (1953): The mechanism of gas transport in the euphysoclist swimbladder. *Acta Physiol. Scand.*, **30:** 1-133.

Fange, R. and Wittenberg, J.B. (1958): The swimbladder of the toadfish (*Opsanus tau L.*). *Biol. Bull.*, Woods Hole, **115**: 172-179.

Green, S.L. (1984): Ultrastructure and innervation of the swim-bladder of *Tetractenos glaber* (Tetraodontidae). *Cell Tissue Res.* **237**: 277-284.

Greenwood, P.H. (1963): The swim bladder in African Notopteridae (Pisces) and its bearing on the taxonomy of the family. *Bull. Brit. Mus. Nat. Hist. Zool.* **II**; **5**: 377-412.

Harder, W. (1975): *Anatomy of Fishes*. **Vol. I**, E. Schweizerbart'sche Verlagsbuchhandlung Stuttgart. pp.1-612.

Hemalatha, K.K. and Rajkumar, R. (1996): Taxonomic relationship of air-bladder in six families of Cypriniformes (Teleostei). *J. Inland Fish. Soc. India*, **28(2)**: 62-74.

Jasinski, A. (1964): Weberian apparatus and swim bladder of the pond loach (*Misgurnus fossilis L.*) *Acta Biol. Cracov.*, (*Ser. Zool.*), **7**: 11-20.

Jasinski, A. (1965): The vascularization of the air-bladder in fishes. II. Sevruga (*Acipenser stellatus* Pallas), grayling (*Thymallus thymallus*), pike (*Esox lucius*) and Umbra (*Umbra Krameri* Walbaum) *Acta Biol. Gacoviensia Zool.*, **8**: 199-210.

Jasinski, A. and Kilarski, W. (1964): The gas gland in the swimbladder of the burbot (*Lota lota L.*) and stone perch (*Acerina cernua L.*) its macro and microscopic structure based on observations of electron microscopy. *Acta Biol. Cracov.* (*Ser. Zool.*). **7**: 111-125.

Laming, P.R. and Morrow, G. (1981): The contribution of the swim bladder to audition in the roach (*Rutilus rutilus*). *J. Comp. Biol. Physiol.*, **69(3)**: 537-541.

Maina, J.N. (2000): Functional morphology of the gas gland cells of the air-bladder of *Oreochromis alcalicus grahami* (Teleostei : Cichlidae) : an ultrastructural study on a fish adapted to a severe, highly alkaline environment. *Tissue and Cell*, **32(2)**: 117-132.

Maina, J.N., Wood, C.M., Narahara, A.B., Bergman, H.L., Laurent, P. and Walsh, P.L. (1996): Morphology of the swim (air) bladder of a cichlid teleost: *Oreochromis alcalicus grahami* (Trewavas, 1983), a fish adapted to a hyperosmotic, alkaline and hypoxic environment. pp.179-192. In: *Fish Morphology : Horizon of New Research* (Eds. J.S.D. Munshi and H.M. Dutta), Science Publishers, Inc., USA.

Marshall, N.B. (1960): Swim bladder structure of deep sea fishes in relation to their systematics and biology. *Discovery Rep.* **31**:11-22.

Morris, S.M. and Albright, J.T. (1975): The ultrastructure of the swimbladder of the Toadfish, *Opsanus tau* L. *Cell Tissue Res.* **164**: 85-104.

Nelson, E.M. (1949): The swim bladder and weberian apparatus of *Raphiodon vulpinus* Agassiz, with notes on some additional morphological features. *J. Morphol.*, **84**, 495-523.

Prem, C., Salvenmoser, W., Wurtz, J. and Pelster, B. (2000): Swim bladder gas gland cells produce surfactant: in vivo and in culture. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **279(6)**: R2336-R2343.

Schmidt-Nielson, K. (1997): Movement, Muscle, Biomechanics. pp. 395-463 In: *Animal Physiology: Adaptation and Environment*, 5th ed. Cambridge University Press, U.K.

Srivastava, P.N. (1956): Morphology and histology of air-bladder of *Hilsa ilisha*, *Gadusia chapra* and their connection with internal ear. *Proc. Nat. Inst. Sci., India*, **22, B (1)**: 28-33.

Sujatha, K. and Dutt, S. (1985): Shape of the swim bladder in family Sillaginidae (Pisces) and its taxonomic value. *Mahasagar Bull. Nat. Inst. of Oceanogr.*, **18(3)**: 429-431.

Starks, E.C. (1908): On a communication between the air-bladder and the ear in certain spinyrayed fishes. *Science*, **28**: 613-614.

Tibbetts, I.R., Collette, B.B., Issac, R. and Kreiter, P. (2007): Functional and phylogenetic implications of the vesicular swimbladder of *Hemiramphus* and *Oxyporhamphus convexus* (Beloniformes : Teleostei) *Copeia*, **2007(4)**: 808-817.

Wagner, R.C., Froehlich, R., Hossler, F.E. and Andrews, S.B. (1987): Ultrastructure of capillaries in the red body (rete mirabile) of the eel swim bladder. *Microvasc. Res*. **34(3)**: 349-362.

Wittenberg, J.B., Schwend, M.J. and Wittenberg, B.A. (1964): The secretion of oxygen into the swim bladder of fish. III. The role of carbon dioxide. *J. Gen. Physiol.*, **48**: 337-355.

Woodland, W.N.F. (1911): On the structure and function of the gas glands and retia mirabilia associated with the gas bladder of some teleostean fishes, with notes on the teleost pancreas. *Proc. Zool. Soc. London*, **1911**:183-248.

Woodland, W.N.F. (1913): Notes on the structure and mode of action of the 'oval' in the pollack (*Gadus pollachius*) and mullet (*Mugil chelo*). *J. mar. biol. Ass*. U.K. **9**: 561-565.

Yamada, Y., Okamura, A., Tanaka, S., Mikawa, N., Horie, N., Utoh, T., Akazawa, A., and Oka, H.P. (2004): Monthly changes in the swim bladder morphology of the female japanese eel *Anguilla japonica* in the coastal waters of Mikawa Bay. *Ichthyol. Res. Pub.* **51(1)**: 52-56.

Zheng, W.B. and Liu, W.S. (1988): Morphology and histology of the swim bladder and infrastructure of respiratory epithelium in the air-breathing catfish, *Pangasius sutchi* (Pangasiidae), *J. Fish Biol.*, **33(1)**: 147.