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## RESEARCH ARTICLE

### IDENTIFICATION OF PARANEURONAL PSEUDOBRANCHIAL NEUROSECRETORY SYSTEM IN A HILL-STREAM CARP, *SCHIZOTHORAX RICHARDSONII*

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#### ABSTRACT

Pseudobranchial neurosecretory system (PSNS) belongs to the category of “diffused neuroendocrine system” (DNES) and is located in the gill region of certain fishes close to pseudobranch/ carotid gland/ carotid labyrinth. Earlier investigations revealed the presence of PSNS in fishes that are either air-breathing or have an ability to tolerate low oxygen concentrations of water. A recent report revealing the presence of PSNS in the cold water carp *Barilius bendelisis* opened up the possibility of existence of this system in hill-stream carps as well. Therefore, to confirm this it was aimed to investigate the presence of the PSNS in another hill stream carp *Schizothorax richardsonii*. Specimens of snow trout were collected from Alaknanda River, Srinagar, India and dissected on-site to procure the tissues of PSNS close to pseudobranch and processed for paraffin microtomy with Acid Violet staining. The histological observations confirmed the presence of PSNS close to pseudobranch. The PSNS was endowed with different neurosecretory stages of pseudobranchial neurosecretory cells (NSCs) forming neurosecretory cell mass (NSM) along with their numerous cell processes forming thick bundles, entangled with blood vascular structures as neurohaemal complex at several places. This is the first report of the presence of PSNS in *S. richardsonii* and the system has been discussed from a new functional and evolutionary point of view in hyperoxic cold waters to maintain the respiratory homeostasis of the fish.

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## INTRODUCTION

In the recent past, a variety of paraneuronal cells have been reported in the vertebrate body scattered thoroughly and secreting neurochemically active substances typical of endocrine nature (1). These cells belong to the “diffuse neuroendocrine system” (DNES) controlling the complex functioning through paracrine and/or autocrine mode of action (2). This DNES has been divided into two divisions namely central and peripheral division comprising more than 50 peptide amine secreting cells of APUD (Amine precursor uptake and decarboxylation) series (3). The central division of DNES contains neuroendocrine and endocrine cells of the hypothalamo- hypophyseal axis (HPA) including the pineal gland. In contrast, the peripheral division possess all kinds of neuroendocrine cells, and these cells either present as organized glands or diffusely distributed in different organs and tissues (3). In fishes, paraneuronal neuroendocrine (NE) cells are recognized in the skin and gills, and have receptor-secretory functions. This reveals that skin and gills are neuroreceptors or endocrine organs (4-9). The NE cells of gill filaments are recognized as peripheral O<sub>2</sub> chemoreceptors (10-15). These studies have speculated to the existence of similar chemoreceptive cells in the extra-branchial sites in the

gill region such as the orobranchial cavity and pseudobranch (16-17). Henceforth, a new system, i.e., “pseudobranchial neurosecretory system” (PSNS), has been recognized in the gill region, away from the gill filaments in several groups of teleosts of Indian tropical waters (fresh and marine) (18-20). The pseudobranchial neurosecretory system (PSNS) falls in the category of peripheral division of amine precursor uptake and decarboxylation (APUD) series as they are known to secrete various neuropeptides or neurohormones like serotonin, Neuropeptide- Y, neuronal nitric oxide synthase (nNOS), tyrosine hydroxylase (TH), choline acetyltransferase (ChAT) etc., (21-22). The PSNS has also been a significant addition to the list of diffuse neuroendocrine system (DNES) in fishes (19). The PSNS is designated as the “third system of neurosecretion” predominantly present in air-breathing fishes and some other teleosts, apart from the two well-recognized systems namely hypothalamo-hypophyseal and caudal neurosecretory system of fishes (18-19, 23). This system occurs in the orobranchial cavity of fishes close to the first two efferent branchial arteries (EA). The PSNS occurs in the form of jelly-like masses (loosely aggregated) comprising of paraneuronal cells located in the gill region of fishes close to the first two efferent branchial arteries and the pseudobranch in

*Glossogobius giuris* (18, 20, 24), *Channa punctata*, *Mastacembelus armatus* (25) and *Barilius bendelisis* (26), the carotid gland in *Notopterus chitala*, *Notopterus notopterus* (18, 27) and *Leiodon cutcutia* (28); and the carotid labyrinth in most of the Indian catfishes (18-19, 29). Earlier investigations revealed the presence of PSNS in fishes that are either air-breathing or have an ability to tolerate low oxygen concentration of waters (19). The PSNS is uniformly found in all the Indian catfishes belonging to order Siluriformes. However, this system is not found to be well developed in carps of plains belonging to order Cypriniformes, a sister group of catfishes (18). The PSNS has been recently identified in a carp of hill-streams, *Barilius bendelisis* living in a well-oxygenated environment. This recent revelation opened up the possibility of existence of this system in carps belonging to hill-streams (26).

The Indian hill stream fishes are permanent residents of various rivers of the Himalayan range, where the dissolved oxygen content is always higher than tropical rivers (31). These cold water fishes have adapted and thrived well in the hyperoxic ecosystem, and some morphological and anatomical features were developed in them in due course of evolution (32-33). As oxygen sensing is a very important mechanism in maintaining the respiratory equilibrium within the body (34), these cold water fishes may have also developed some mechanisms to sustain in the hyperoxic environment. In the view of these facts, we hypothesized that pseudobranchial neurosecretory system (PSNS) may be present in these cold water fishes and it could be helping these fishes to cope up with the higher oxygen concentrations of water, as PSNS is a chemosensory system found in the extrabranchial region (orobranchial cavity) of some air-breathing and non air-breathing fishes to counter the hypoxic conditions and other environmental stresses (18-19, 35-37). Therefore, the present histological investigation has been undertaken to confirm the presence of PSNS in a hill-stream carp, *Schizothorax richardsonii* (snow trout) using neurosecretion specific staining technique "Acid Violet" (38). The findings are reported for the first time and the role of the PSNS is discussed in the hyperoxic conditions.

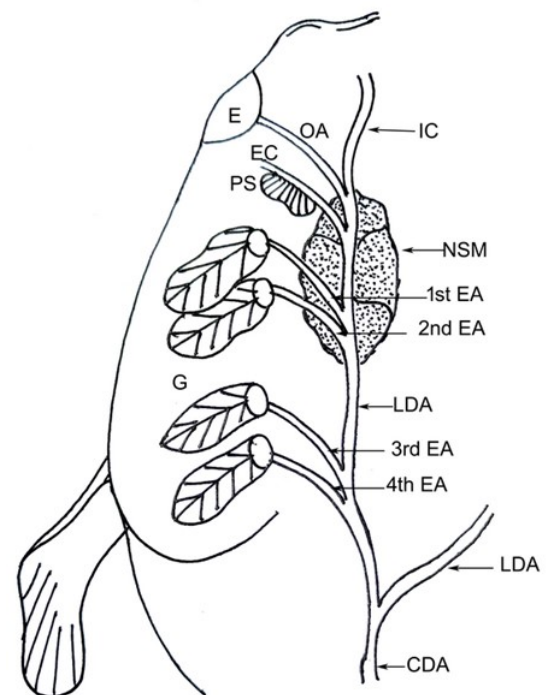
## MATERIALS AND METHODS

Five specimens of *S. richardsonii* (body length 15–20 cm and body weight 50-70 gm) belonging to a single population were procured randomly after active fishing in the local fishing sites of the river Alaknanda at Srinagar, Garhwal (30.22°N, 78.78°E), Uttarakhand, India. The fishes were anesthetized as per protocol for deep hypothermia or cold anesthesia (39) and guidelines for animal experiments were followed. These fishes were on-site dissected to open the palate in order to expose the efferent branchial arteries (EA) in the gill region and the region anterior to it (Fig. 1). The tissue from the region close to the pseudobranch (PS), first two efferent blood arteries (EA), and the lateral dorsal aorta (LDA) connecting first and second efferent arteries were dissected out and fixed in freshly prepared Bouin's fixative for overnight. The tissues were then washed thoroughly with 70% ethanol, dehydrated with ascending grades of alcohol. Paraffin blocks of tissues were prepared for routine paraffin microtomy. Paraffin serial sections were cut at 10 µm thickness and further stained in Acid violet stain (38), which is a specific stain used for staining of neurosecretory cells of

vertebrates. Serial sections of the tissue were studied under a light microscope to understand the topography and detailed structural organization of the pseudobranchial neurosecretory system (PSNS) in relation to the pseudobranch (PS) and efferent branchial arteries (EA) in the gill region, and the size of the neurosecretory cells was calculated using Image J software.

## RESULTS

The histological examination of the serial sections of the tissue from the gill region of *S. richardsonii* revealed the presence of a well developed pseudobranchial neurosecretory system (PSNS) comprising of neurosecretory cells forming jelly-like neurosecretory cell masses (NSM) close to large-sized pseudobranch (PS) and bundles of long cell processes of neurosecretory cells (NSCs) ending close to the blood vascular structures (Figs. 2 A- B). Anterior sections of the gill tissue revealed the pseudobranch (PS), which was large and could be seen easily by naked eyes at the dorsal side of the opercular cavity. The PS was made up of several pseudobranchial lamellae fused or piled with each other and was covered by a thin epithelial membrane (Fig. 2 B). The shape of the NSM was observed to be very irregular in the serial sections. In addition, position of NSM, their cell processes, and blood vessel was observed to be changing in the consecutive sections (Fig. 2).

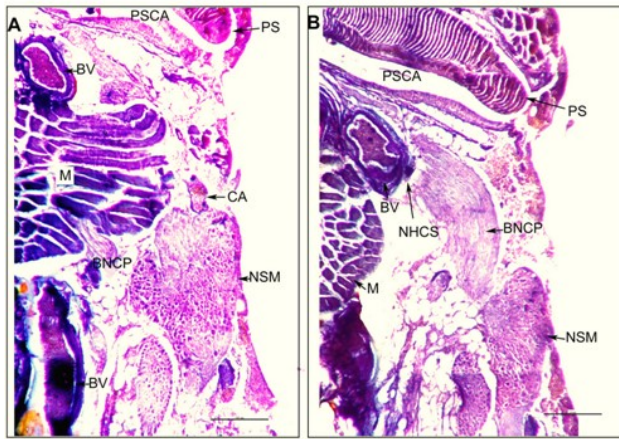


Abbreviation: E- Eye ; OA- ophthalmic artery; EC- external carotid artery; IC- internal carotid artery; PS- pseudobranch; NSM- neurosecretory cell mass; EA- efferent branchial artery; G- gills; LDA- lateral dorsal aorta; CDA- central dorsal aorta).

**Fig. 1. Schematic drawing showing the topographical position of the pseudobranchial neurosecretory cell mass (NSM) in *Schizothorax richardsonii*.**

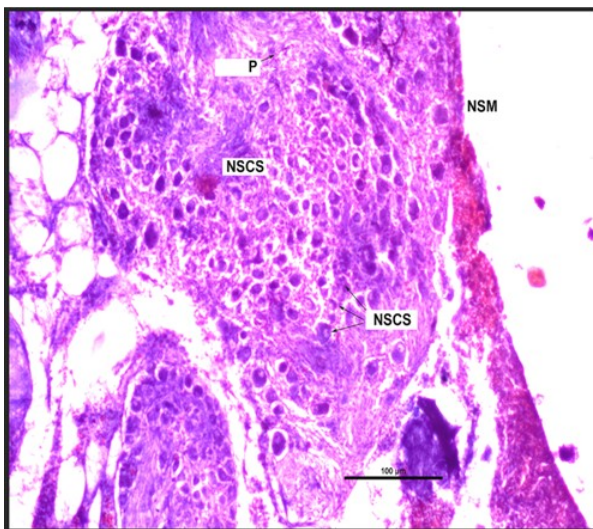
**Table 1. Showing the size range of different secretory stages of NSCs in *Schizothorax richardsonii***

| S.N. | Secretory stage                    | Size range of NSCS (in µm) |
|------|------------------------------------|----------------------------|
| 1.   | Young neurosecretory cells (YNSC)  | 6.02-11.51                 |
| 2.   | Mature neurosecretory cells (MNSC) | 10.62-21.78                |



**Fig. 2.** Photomicrographs of selected sections (A-B) of the tissue from the gill region of *S. richardsonii* showing changes in the shape, structure and position of neurosecretory cell mass (NSM). (M-muscle; BNCP- bundle of nerve cell processes; PS- pseudobranch; PSCA- pseudobranchial cavity; BV-blood vessel; CA- collateral artery; NHCS- neurohaemal contact site) (Magnification=100x. Bar=100µm).

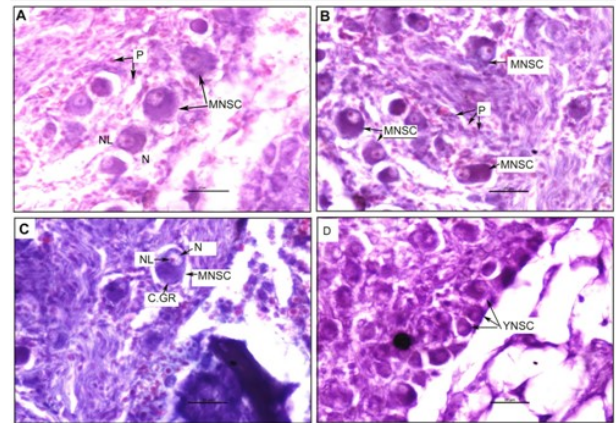
The neurosecretory cell mass (NSM) was comprised of pseudobranchial neurosecretory cells (NSCs) and pericytes (P) (Figs. 3, 4 A and B).



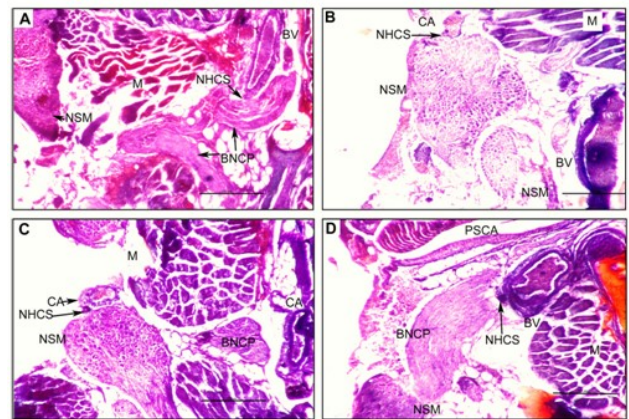
**Fig. 3.** Photomicrograph of neurosecretory cell mass (NSM) of *S. richardsonii* made up of different stages of neurosecretory cells (NSCs) and small pericytes (P) (Magnification= 100x.Bar=100µm)

The pseudobranchial NSCs were observed at different stages of their secretory cycle such as young (YNSC) and mature (MNSC) neurosecretory cells, respectively (Fig. 4). These YNSCs and MNSCs differed from each other in the size of the cell, nucleus (N), and the amount of granular cytoplasm (C.GR) present within cells. Morphologically, YNSCs were similar to the MNSCs but had a relatively smaller size of the cell, nucleus, and granular cytoplasm than MNSCs (Fig. 4 A and D). Mature neurosecretory cells (MNSCs) were observed with a large eccentric nucleus (N) containing one or more nucleoli (NL), and a large amount of granular cytoplasm (C.GR) (Fig. 4 A - C). These neurosecretory cells ranged from 6.02- 21.78µm in size. The size range of YNSCs and MNSCs are given in Table 1.

The pericytes were observed spread over the NSM and could be easily distinguished from the NSCs by their uniform smaller size and appearance (Fig. 4 A and B).



**Fig. 4.** Photomicrograph of magnified neurosecretory cell mass (NSM) of *S. richardsonii* (A-D) showing Pericytes (P) and neurosecretory cells at different stages of neurosecretion like young (YNSC), mature (MNSC) with eccentric nucleus (N), nucleolus (NL) and granular cytoplasm (C.GR) (Magnification 400x. Bar=20µm)



**Fig. 5.** Selected sections (A-D) of the tissue from the gill region of *S. richardsonii* showing different neurohaemal contact sites (NHCS) observed between the neurosecretory cell mass (NSM), bundle of their nerve cell processes (BNCP), blood vessel (BV), and collateral artery(CA) forming (Magnification=100x. Bar=100µm)

The cell processes arising from neurosecretory cell masses join together to form thick bundles of neurosecretory cell processes (BNCP), which after taking a long tortuous course terminate in close proximity of elements of the circulatory system, i.e., the main blood vessel (BV), collateral artery (CA) or a profuse network of capillaries (Fig. 2 B, 5 A and D). BNCP was observed irregularly intermingled with blood vascular structures (Fig. 5 A and D). Highly diffused types of contacts were observed at several sites with the blood vascular elements (blood vessels and collateral arteries), BNCP and NSM forming multiple neurohaemal contact sites (NHCS) (Fig. 5 A-D). Overall, a very complex type of neurohaemal complex is observed in the system, where all the elements of the neurosecretory system like NSCS, their cell process bundles (BNCP), and neurohaemal contact sites (NHCS) are present in a very diffused pattern intermingled with each other and surrounding muscle fascicles.

## DISCUSSION

The present study has confirmed the existence of a well-developed pseudobranchial neurosecretory system (PSNS) in a hill stream carp, *S. richardsonii*, as observed in other groups of teleosts viz. Cypriniformes, Perciformes (18-20,23-24, 26, 30), Channiformes, Atheriniformes (25), Clupeiformes (18,20,27) and tetradontiformes (28). The PSNS is observed close to the pseudobranch (PS), and its topological placement is similar to catfishes, where the PSNS is seen close to the carotid labyrinth. All the three essential morphological attributes of a neurosecretory system i.e. site for synthesis of the neurosecretory material – the neurosecretory cells (NSCs), mode of transportation – the bundle of nerve cell processes (BNCP), and sites for the release of bioactive substances in the blood vascular elements-neurohaemal contact sites (NHCS) were observed clearly in snow trout, *S. richardsonii*. A neurohaemal organ like urophysis of the caudal neurosecretory system was lacking. A very profuse capillary network was noticed surrounding the neurosecretory cell mass (NSM) and their cell process bundles forming multiple neurohaemal contact sites (NHCs) for the release of bio-active substances secreted by the cells in the blood vascular system swiftly. The pear-shaped mono-polar neurosecretory cells (NSCs) were of varied size representing different stages of the secretory cycle like YNSCs and MNSCs. Co-existence of different neurosecretory stages of NSCs in the same cell mass reflects the presence of a continuous secretory cycle in the cell mass. However, primordial (Pr.NSCs) and spent (SNSCs) neurosecretory cells were not observed in this fish as observed in other fishes during stress conditions like hypoxia (19), photoperiod (25, 36), and pesticides (35, 40). The cell processes (CPR) of NSCs are long and join together to form thick convoluted bundles (BNCP). BNCP show a characteristic tortuosity, interlacing with each other and with the associated muscle fascicles and blood vessels. This tortuosity of cell processes renders it difficult to locate the endings belonging to a certain group of NSCs. The cell process endings are found to be simple and unbranched.

Each ending is blind and represent an abrupt termination of the cell processes without showing any swelling at the end. The endings of cell processes are invariably found to be in close contact with any vascular structure may be main blood vessel (BV) or collateral arteries (CA) fulfilling the anatomical requirements of a neurohaemal mode of release for the neurosecretory product. The cells of this system fulfill all the criteria of paraneurons as proposed by Fujita *et al* (1989) except the developmental pattern which needs to be traced by developmental studies. The pseudobranchial NSCs are similar to NECs of gill filaments (1, 10-11) in their morpho-functional aspects (19, 24). These NSCs secrete several bio-active substances (21-22) similar to NECs of gill filament (8, 41-42). Also, experimental investigations undertaken on two tropical catfishes, namely *Clarias batrachus* and *Heteropneustes fossilis*, have revealed that the pseudobranchial neurosecretory cells (NSCs) of PSNS are chemoreceptive in nature and are seen to respond to stresses like hypoxia by releasing their neurosecretory material (18-19, 36) similar to neuroepithelial cells (NECs) of gill filaments (43-44). As, both the populations of neurosecretory cells show the presence of dense cored vesicles (DCVs) and chemical neurotransmitters in their cytoplasm and receive

innervations from the glossopharyngeal (IX) cranial nerve (19, 45) it can be assumed that these two populations of neurosecretory cells i.e., NECs of gill filaments and pseudobranchial NSCs of the orobranchial cavity may have originated from the same stock of precursor cells and moved to different areas of the gill region to form a complete “gill neuroendocrine apparatus” operating in the gill region. The presence of a well-developed pseudobranchial neurosecretory system (PSNS) in a hill-stream carp, *S. richardsonii* paves the way for the evaluation of the system from a new functional and evolutionary point of view in the fishes of hill-streams. Previously, it was believed that PSNS was commonly possessed by all the catfishes and some other groups of teleosts (18-19). Most of the fish species in which PSNS was recognized were either air-breathing or had the capability to survive in low O<sub>2</sub> concentration of their environment (19). The present investigation refutes the earlier hypothesis for this system that “PSNS is found well developed only in fishes that are air-breathing in habit or have a capacity to tolerate low oxygen concentration and not commonly found well developed in other teleosts, especially the “carps” (18). Therefore, this revelation raises questions regarding the functional significance of the PSNS in *S. richardsonii* living in hyperoxic conditions. Preliminary experimental investigations undertaken on NSCs of PSNS have revealed the role of NSCs in the condition hypoxia (18-19). Immunohistochemical investigations undertaken on the pseudobranchial NSCs of *C. batrachus* and *H. fossilis* revealed the presence of multiple bio-active substances like Serotonin, Neuropeptide Y (NPY), neuronal nitric oxide synthase (nNOS), tyrosine hydroxylase (TH), choline acetyltransferase (ChAT), substance P (SP), vasointestinal peptide (VIP) in these cells (21-22). The soma of the NSCs showed the co-localization of ChAT, nNOS, TH, SP and VIP is seen in the cell processes of the NSCs (22).

These peptides/neurotransmitters/enzymes are known to operate as small intracellular signaling molecules controlling various vital physiological functions in fishes and other vertebrates in their localized area of release (15, 46-47). As far as NPY, nNOS, dopamine and serotonin are concerned, their roles as neurotransmitters or regulatory peptides are well known (42, 47). These bio-active substances, i.e., serotonin, nitric oxide synthase (nNOS), neuropeptide Y (NPY), dopamine produced by neurosecretory cells contribute to oxygen sensing in both fish and mammals by promoting membrane depolarization of these chemosensory cells (15, 47). These studies support and open up the possibility of involvement of PSNS in multiple physiological functions in the biology of fishes belonging to different ecological niche. The pseudobranchial neurosecretory cells (NSCs) may have different functions and respond to different stress regimens according to the need of the fish in their habitat. Plasticity of the chemoreceptive cells may have rendered these cells to take up a different functional role in fishes according to the requirement of the habitat (43). The hill-stream carp *S. richardsonii* belonging to order Cypriniformes inhabit shallow streams of hilly areas, where they are exposed to hyperoxic conditions. The oxygen holding capacity of cold waters is high due to continuous water currents and high density of photosynthetic organisms, because of which cold waters often become supersaturated with oxygen (31, 48-49). Mcarley *et al.*, (2020) in their study stated that “hyperoxia can influence the cardiorespiratory function, acid-base balance, oxidative stress and total

performance of the fish". Hyperoxic conditions in streams can affect the respiratory equilibrium of fish by inducing hypoventilation in gills, increasing the partial pressure of oxygen in arterial and venous blood and retention of carbon dioxide in gills i.e respiratory acidosis (48). As these fishes live in shallow waters they may come across hypoxia (low oxygen concentration) during day by acute warming of waters. These effects of hyperoxia along with the acute warming of shallow waters of streams may act as a stress in the fish inhabiting cold waters. As the pseudobranchial neurosecretory system (PSNS) is considered as a part of complex "Gill Neuroendocrine Apparatus" which work in an orchestrated manner along with other neuroendocrine cells of the gills i.e. NEC to regulate many physiological processes such as rate of aerial and aquatic respiration, ventilatory and cardiovascular responses by 'synaptic or paracrine activities' in certain group of fishes (24, 50). The chemosensory neurosecretory cells of PSNS in *S.richardsonii* may also work in a similar way to regulate the effects caused by the hyperoxic environment along with the acute warming in cold water streams.

The present investigation, though limited in its objective, gives support to the possibility of development of this system much before the development of the bimodal mode of respiration which refute the hypothesis proposed by Gopesh and co-workers (2010) i.e., "pseudobranchial neurosecretory system has developed along with the development of bimodal respiration of fishes". The carotid labyrinth of catfishes has been accepted to be a derived structure of pseudobranch (51-53). The fish species in which the pseudobranch was replaced by carotid labyrinth had been thoroughly studied (19) and topographically the transformation of pseudobranch into carotid labyrinth has been suggested by Srivastava and co-workers in 1988. The transformation had been a gradual process like all evolutionary changes. This notion is supported by the revelation of carotid gland in fishes like *Notopterus notopterus*, *Notopterus chitala* which exactly is the intermediate stage in its morphological details (53). The presence of PSNS in close association of pseudobranch/ carotid gland/ carotid labyrinth is a strong support to this evolutionary feature of fishes on one hand and on the "development of pseudobranchial neurosecretory system in fishes much before the diversification of the environmental niche occupied by different groups of fishes in course of their evolution". Present study supports this view and calls for further investigations in other species of cold water carps to establish the present hypothesis. In-depth investigations on other species inhabiting hill-streams as well as other types of ecological conditions (eco-habitats) are urgently warranted to reveal some conclusive implications for a better understanding of PSNS in the biology of fishes. The bio-active substances which have been identified in the NSCs in *C. batrachus* and *H. fossilis* needs to be confirmed in the hill-stream fishes, and whether these bio-active substances are also co-localized as reported in PNSCs of Indian tropical catfishes including some teleosts and NECs of gill filaments of some European primitive fishes by immunohistochemistry and molecular biology techniques.

## CONCLUSION

This is the first report of the pseudobranchial neurosecretory system (PSNS) in the orobranchial cavity of a hill-stream

carp, *Schizothorax richardsonii*. The PSNS is a paraneuronal "diffuse neuroendocrine system" endowed with large-sized pseudobranchial NSCs and neurohaemal complex with nerve plexus which may counter and maintain the respiratory homeostasis in hyperoxic waters in which these cells acts as chemosensory receptors through a down regulated mechanism. Further, in-depth investigations are required in other hill stream carps to reveal the functional and evolutionary significance of PSNS among carps and catfishes, which belong to a similar ancestor or clad line.

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## Glossary of Abbreviations

**APUD:** Amine Precursor Uptake and Decarboxylation

**BNCP:** Bundle of Neurosecretory cell processes

**BV:** Blood Vessel

**C.GR:** Granular cytoplasm

**CA:** Collateral artery

**CPR:** Cell Processes

**DCVs:** Dense cored vesicles

**DNES:** Diffuse Neuroendocrine System

**EA:** Efferent Branchial Artery

**HPA:** Hypothalamo-hypophyseal axis

**LDA:** Lateral Dorsal Aorta

**MNSC:** Mature Neurosecretory Cells

**N:** Nucleus

**NE:** Neuroendocrine

**NECs:** Neuroepithelial cells

**NHCS:** Neurohaemal contact sites

**NL:** Nucleolus

**NSCs:** Neurosecretory Cells

**NSM:** Neurosecretory Cell Mass

**P:** Pericytes

**Pr. NSCs:** Primordial Neurosecretory Cells

**PS:** Pseudobranch

**PSNS:** Pseudobranchial Neurosecretory System

**SNSC:** Spent Neurosecretory Cells

**YNSC:** Young Neurosecretory Cells

## REFERENCES

- Zaccone, G., Mauceri, A., Ainis, L., Fasulo, S., Licata, A., (1999). Paraneurons in the skin and gills of fishes. In: Saksena, D.N. (Ed.), Ichthyology, Recent Research Advances. Science Publishers, Enfield, NH, pp. 417-447.
- Toni, R. (2004). The neuroendocrine system: organization and homeostatic role. Journal of endocrinological investigation, 27(6 Suppl), 35-47.

3. Pearse A.G.E. (1982) The diffuse neuroendocrine system: an extension of the APUD concept. In: Cuello A.C. (eds) Co-Transmission. Palgrave Macmillan, London.
4. Icardo, M.J., Colvec, E., Kuciel, M., Lauriano, R.E., Zaccone, G., (2017). The lungs of *Polypterus senegalus* and *Erpetoichthys calabaricus*: Insights into the structure and functional distribution of the pulmonary epithelial cells. *Journal of Morphology*; 278, 1321–1332.
5. Jonz, G. M., Buck, L. T., Perry, F. S., Schwerte, T., Zaccone, G., (2016). Sensing and surviving hypoxia in vertebrates. *Annals of the New York Academy of Sciences*, 1365, 43–58.
6. Lauriano, E.R., Capillo, G., Icardo, J.M., Fernandes, J.M.O., Kiron, V., Kuciel, M., Zuwala, K., Guerrero, M.C., Aragona, M. and Zaccone, G. (2021). Neuroepithelial cells (NECs) and mucous cells express a variety of neurotransmitters and neurotransmitter receptors in the gill and respiratory air-sac of the catfish *Heteropneustes fossilis* (Siluriformes, Heteropneustidae): a possible role in local immune defence. *Zoology*, 125958.
7. Porteus, S.C., Pollack, P., Tzaneva, V., Kwong, M.W.R., Kumai, Y., Abdallah, J.S., Zaccone, G., Lauriano, R.E., Milsom, K.W., Perry, F.S., (2015). A role for nitric oxide in the control of breathing in zebrafish (*Danio rerio*). *Journal of Experimental Biology*; 218, 3746-3753.
8. Zaccone, G., Lauriano, E. R., Kuciel, M., Capillo, G., Pergolizzi, S., Alesci, A., Dugo, P. and Icardo, J. M. (2017). Identification and distribution of neuronal nitric oxide synthase and neurochemical markers in the neuroepithelial cells of the gill and the skin in the giant mudskipper, *Periophthalmodon schlosseri*. *Zoology*, 125, 41-52.
9. Zaccone, G., Maina, J., Germanà, A., Montalbano, G., Capillo, G., Aragona, L., Kuciel, M.J., Lauriano, E.R. and Icardo, J. M. (2019). First demonstration of the neuroepithelial cells and their chemical code in the accessory respiratory organ and the gill of the sharptooth catfish, *Clarias gariepinus*: A preliminary study. *Acta Zoologica*, 100(2), 160-166.
10. Bailly, Y., Dunel-Erb, S. and Laurent, P., (1992). The neuroepithelial cells of the fish gill filament: Indolamine-immunocytochemistry and innervation. *The Anatomical Record*, 233(1), 143-161.
11. Dunel-Erb, S., Bailly, Y. and Laurent, P., (1982). Neuroepithelial cells in fish gill primary lamellae. *Journal of Applied Physiology*, 53(6), 1342-1353.
12. Jonz, M. G., Zachar, P. C., Da Fonte, D. F., and Mierzwa, A. S. (2015). Peripheral chemoreceptors in fish: a brief history and a look ahead. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, 186, 27-38.
13. Milsom, W.K. and Bursleson, M.L., (2007). Peripheral arterial chemoreceptors and the evolution of the carotid body. *Respiratory physiology and neurobiology*, 157(1), 4-11.
14. Perry, S. F., Jonz, M. G., and Gilmour, K. M. (2009). Oxygen sensing and the hypoxic ventilatory response. In *Fish physiology*, 27, 193-253. Academic Press.
15. Zachar, P. C., and Jonz, M. G. (2012). Neuroepithelial cells of the gill and their role in oxygen sensing. *Respiratory physiology and neurobiology*, 184(3), 301-308.
16. Florindo, L. H., Leite, C. A., Kalinin, A. L., Reid, S. G., Milsom, W. K., and Rantin, F. T. (2006). The role of branchial and orobranchial O<sub>2</sub> chemoreceptors in the control of aquatic surface respiration in the neotropical fish tambaqui (*Colossoma macropomum*): progressive responses to prolonged hypoxia. *Journal of experimental biology*, 209(9), 1709-1715.
17. McKenzie, D. J., Bursleson, M. L., and Randall, D. J. (1991). The effects of branchial denervation and pseudobranch ablation on cardioventilatory control in an air-breathing fish. *Journal of Experimental Biology*, 161(1), 347-365.
18. Gopesh, A., (1983). Studies on peculiar pseudobranchial neurosecretory cells in certain teleostean fishes. D. Phil Thesis, Allahabad University, Allahabad (UP), India.
19. Gopesh, A. (2009). Carotid labyrinth and associated pseudobranchial neurosecretory Cells in Indian catfishes. In: Zaccone, G., Cutz, E., Adriaensen, D., Nurse, C., Mauceri, A. (Eds.), *Structure, Evolution, and Function of the Airway Chemoreceptors in the Vertebrates*. Science Publishers, Enfield (NH), 31–60.
20. Srivastava, C.B.L., Gopesh, A. and Singh, M., (1981). A new neurosecretory system in fish, located in the gill region. *Experientia*, 37(8), 850-851.
21. Gopesh, A., Jaiswal, U., and Prakash, S. (2003). Immunocytochemical location of neurophysin, neuropeptide Y, and serotonin in the pseudobranchial neurosecretory cells of two catfish species *Clarias batrachus* and *Heteropneustes fossilis*. *Proceedings National Academy Science India*, 73(III and IV), 275-281.
22. Zaccone, D., Gopesh, A., Anastasi, G., Favalaro, A., Sfacteria, A. and Marino, F., (2012). Localization of neurotransmitters, peptides, and nNOS in the pseudobranchial neurosecretory cell system and associated carotid labyrinth of the catfish, *Clarias batrachus*. *Acta histochemica*, 114(1), pp.62-67.
23. Srivastava, C.B.L., Gopesh, A., (1987). A third system of neurosecretion in fish—the pseudobranchial neurosecretory system. In: Bhatnagar, V.S. (Ed.), *Advances in Cytology and Genetics*. Zoology Department, University of Allahabad, India, 122–128.
24. Tripathi, S., Sengar, M., and Gopesh, A. (2020c). Paraneuronal pseudobranchial neurosecretory system in tank goby *Glossogobius giurus* with special reference to novel neurohaemal contact complex. *Respiratory Physiology and Neurobiology*, 278, 103440, 1-9.
25. Devi U. (1987) Studies on the pseudobranchial neurosecretory system of certain teleosts with particular reference to its function. D. Phil Thesis, University of Allahabad, Allahabad (UP), India.
26. Tripathi, S., Sengar, M., Gopesh, A. (2020b) Presence of Pseudobranchial Neurosecretory System in *Barilius bendelisis*: A Paraneuronal Neuroendocrine System. *National Academy Science Letters*, 44(2), 97-100.
27. Gopesh, A., Sengar, M. and Tiwari, S., (2010). Presence of paraneuronal pseudobranchial neurosecretory system in the gill region of two air-breathing clupeids, *Notopterus chitala* and *Notopterus notopterus*. *Respiratory physiology and neurobiology*, 171(2), 135-143.
28. Tripathi, S., Sengar, M., Gopesh, A., et al. (2020a) The paraneuronal gill neuroendocrine system in ocellated pufferfish *Leiodon cutcutia*. *Microscopy Research and Technique*, 83(6), 627-635.

29. Gopesh, A. and Srivastava, C.B.L., (1997). Paraneuronic nature of pseudobranchial neurosecretory cells. *Journal of Freshwater Biology*, 9(2), 78-82.
30. Gopesh, A., Prakash, S. and Jaiswal, U., (2002). Carotid labyrinth and associated pseudobranchial neurosecretory system in two marine catfish species *Plotosus canius* and *Osteogeneiosus militaris*. *National Academy Science Letters*, 25(9-10), 298-301.
31. Valentina, T., Singh, H., Tamuli, A., and Teron, R. (2015). Assessment of Physico-Chemical Characteristics and Fish Diversity of Hill streams in Karbi Anglong district, Assam, India. *International Research Journal of Environmental Science*, 4(5), 6-11.
32. Helfman, G., Collette, B.B., Facey, D.E. and Bowen, B.W., (2009). *The diversity of fishes: biology, evolution, and ecology*. John Wiley and Sons. 323-325.
33. Jayaram, K.C., (2010). *The Freshwater Fishes of the Indian Region*, 2nd edition, Delhi, Narendra Publishing House.
34. Pan, W., Scott, A. L., Nurse, C. A., and Jonz, M. G. (2021). Identification of oxygen-sensitive neuroepithelial cells through an endogenous reporter gene in larval and adult transgenic zebrafish. *Cell and Tissue Research*, 384(1), 35-47.
35. Mishra, A. K., Gopesh, A., Singh, K. P., (2020). Acute toxic effects of chlorpyrifos on pseudobranchial neurosecretory system, brain regions, and locomotory behavior of an air-breathing catfish, *Heteropneustes fossilis* (Bloch1794). *Drug and Chemical Toxicology*; doi:10.1080/01480545.2020.1762631.
36. Singh, K. P. (1992). Studies on surfacing behavior of certain fishes. D. Phil. Thesis, University of Allahabad, India.
37. Singh, K. P., Srivastava, C. B. L., Srivastava, D. and Srivastava, S. (1993). Diel pattern of surfacing activity of an air-breathing catfish, *Clarias batrachus*. *Journal of Fresh Water Biology*, 5 (4): 331-342.
38. Takasugi N, Bern HA (1962) Experimental studies on the caudal neurosecretory system of *Tilapia mossambica*. *Comparative biochemistry and physiology*, 6(4) 289-303.
39. Coyle S D, Durborow R M, Tidwell JH (2004) *Anesthetics in aquaculture* (No. 3900). Texas: Southern Regional Aquaculture Center.
40. Mishra, A. K., Gopesh, A. and Singh, K. P. *et al.*, (2021). Acute toxic effects of chlorpyrifos and indoxacarb on dorsal pars medialis of telencephalon and swimming behavior of a catfish, *Heteropneustes fossilis*. *Journal of Applied Biology and Biotechnology*, 9(03), 137-143. DOI: 10.7324/JABB.2021.9317.
41. Jonz, M. G., Zachar, P. C., Da Fonte, D. F., and Mierzwa, A. S. (2015). Peripheral chemoreceptors in fish: a brief history and a look ahead. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, 186, 27-38.
42. Zaccone, G., Mauceri, A., and Fasulo, S., (2006). Neuropeptides and nitric oxide synthase in the gill and the air-breathing organs of fishes. *Journal of Experimental Zoology*, 3054, 428-439.
43. Jonz, M. G., and Nurse, C. A. (2009). Oxygen-sensitive neuroepithelial cells in the gills of aquatic vertebrates. In *Airway chemoreceptors in vertebrates: structure, evolution and function* (Ed. Zaccone, G., et al.) 1-30 (Science Publishers).
44. Regan, K.S., Jonz, M.G. and Wright, P.A. (2011). Neuroepithelial cells and the hypoxia emersion response in the amphibious fish *Kryptolebias marmoratus*. *Journal of Experimental Biology*, 214(15), 2560-2568.
45. Yadav, L., Sengar, M., Zaccone, D., and Gopesh, A. (2013). Paraneuronal pseudobranchial neurosecretory cells in scorpion catfish *Heteropneustes fossilis*: an environment scanning electron microscope and transmission electron microscope study. *Acta Zoologica*, 94(1), 58-65.
46. Porteus, S.C., Pollack, P., Tzaneva, V., Kwong, M.W.R., Kumai, Y., Abdallah, J.S., Zaccone, G., Lauriano, R.E., Milsom, K.W., Perry, F.S., (2015). A role for nitric oxide in the control of breathing in zebrafish (*Danio rerio*). *Journal of Experimental Biology*; 218, 3746-3753.
47. Reed, M., Pan, W., Musa, L., Arlotta, S., Mennigen, J. A., & Jonz, M. G. (2024). A role for dopamine in control of the hypoxic ventilatory response via D2 receptors in the zebrafish gill. *Journal of Comparative Neurology*, 532(2), e25548.
48. McArley, T. J., Sandblom, E., & Herbert, N. A. (2021). Fish and hyperoxia—From cardiorespiratory and biochemical adjustments to aquaculture and ecophysiology implications. *Fish and Fisheries*, 22(2), 324-355.
49. Rucker, R.R. (1972). Gas-Bubble Disease of Salmonids: A Critical Review. Technical Paper, 58, 1-10. Bureau of Sport Fisheries and Wildlife, Washington, DC.
50. Sengar, (2012). Structure and organization of gill neuroendocrine system of certain teleostean fish. D. Phil Thesis, Allahabad University, Allahabad (UP), India.
51. Munshi, J.S.D., Hughes, G.M., (1981). Gross and fine structure of the pseudobranch of the climbing perch: *Anabas testudineus* (Bloch). *Journal of Fish Biology*, 19, 427-438.
52. Tripathi, S., (1985). Studies on the carotid labyrinth in teleosts. D. Phil. Thesis, University of Allahabad, Allahabad.
53. Srivastava, C.B.L., Tripathi, S., Panday, K.N., Singh, M., (1988). Fish carotid labyrinth—a transformed pseudobranch. *Arch. Biol. (Bruxelles)* 99, 33-50.

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