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# Scanning Electron Microscopy of the Respiratory Organs of Juvenile and Adult Climbing Perch, *Anabas testudineus*

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**Abstract** The gills and air-breathing organs of fingerlings (1–2.5 g) and adult (30 g) *Anabas testudineus* have been studied by scanning electron microscopy. The labyrinthine organs bear only one or two leaf-like plates in fingerlings of 1–2.5 g body weight. In very small finglerlings (1 g) no proper development of respiratory islets could be seen on the labyrinthine plates. There is evidence of the suprabranchial chambers being used for aquatic respiration in juvenile stages. In adults the inhalent and exhalent apertures become well defined with shutters developed on the first pair of gills, when they become obligatory air-breathers.

The climbing perch, *Anabas testudineus* is well known for its varying degrees of bimodal gas exchange in its natural swampy environment, obtaining 54% of its oxygen from the air which is lower than in many other air-breathing fishes (Singh, 1976). It has developed complicated accessory organs for aerial respiration (Munshi, 1968). Studies on the fine structure of its gills and labyrinthine organs established that the latter were not modified gills (Hughes and Munshi, 1973). The morphometry of the gills and accessory organs and their diffusing capacities have also been determined in relation to body weight (Hughes, Dube and Munshi, 1973). Recently the postembryonic development of its gills and accessory respiratory organs has been studied using light microscopy and stereological methods (Hughes, Munshi and Ojha, 1986).

The present paper reports a scanning electron microscopic study of the respiratory surface of the gills and accessory organs of six, eight and twelve month old juvenile and adult fishes.

## Materials and methods

Sexually mature specimens of *Anabas testudineus* were collected from the swamps of North Bihar and maintained in the Ichthyological Laboratory of the Post-Graduate Department of Zoology, Bhagalpur University, India. Male and female specimens were segregated during July (i.e. the rainy season). They were also bred following injection of pituitary extract (heteroplastic pituitary at a dose of 15 mg/100 g body weight). Temperatures were lowered by the addition of iced water and an artificial shower was arranged to cool and mimic rain and hence induce mating. Mating took place at about 3 a.m. and fertilised eggs were reared separately in ten jars. The hatchlings were reared until they began gulping air, about the 13th day. Various developmental stages were fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.4). Some specimens were also post-fixed in a 1% solution of osmium tetroxide in the same buffer. Fixed materials were stored in phosphate buffer and 70% ethanol at 4°C and were transported to Bristol for araldite embedding. One $\mu$m sections were obtained using an LKB ultratome and stained in 1% methylene blue in borax and examined under the light microscope.

Different respiratory organs (gills, labyrinthine organs and respiratory membrane lining the suprabranchial chambers) of 6, 8 and 12 month old specimens were fixed in 2.5 and 25% glutaraldehyde in phosphate buffer, post-fixed for one hour in 2% osmic acid in phosphate buffer (pH 7.4) and stored in 70% ethanol buffer and transported to Bristol and used in the SEM studies.

## Results

Following dissection of the larvae and fingerlings, small portions of material were critically point dried and placed on stubs for examination in a Cambridge stereoscan. Differences in the degree of development of the respiratory organs were observed at both gross morphological level and by observations of the surface architecture of

these organs.

**A. Structure of respiratory organs of six month old (1 g) fish.**

*Gills*: Under low magnification (Fig. 1) the extensive surface of the gill arch of these specimens was seen to be covered with precipitated mucus. At a slightly higher magnification it became clear that the nature of the surface consisted of microridged epithelial cells. Other types of cells present were chloride cells clearly discernible between the large microridged cells. The chloride cells are covered by microvilli. At even greater magnification several minute cells were observed in the region of the chloride cells.

*The first gill arch*: The gill filaments are stumpy and have a variety of shapes and sizes which seem unrelated to their position on the arch. Several of them appear to be dividing into two filaments. The surface of the filament is covered by microridged epithelial cells which are accompanied by characteristic chloride cells. An enlarged view of this field shows the roughened surface of the microridges. Between the microridged cells chloride cells are present in an almost 1:1 ratio and their surface is completely covered with microvilli. The region at the base of the secondary lamellae shows a transition between the microridged surface of the filaments and the microvilli on the lamellar epithelium.

*Labyrinthine organs*: In these six month old fish the labyrinthine organ is not well differentiated having only one curved saucer-shaped plate. The wrinkled surface of the plate is usually covered with mucus. The characteristic respiratory islets are not seen and the surface is mainly formed of microridged cells with prominent cell boundaries (Fig. 2). The ridges of the microridged surface are rough and provided with peg-like structures which may further aid in the holding of mucus to the surface. The cell boundaries of the microridged and microvillous cells are more well defined in certain areas.

*The respiratory mucosa of the suprabranchial chamber*: The characteristic respiratory islets are visible (Fig. 1). The impression of transverse blood channels is clearly apparent, the surface is highly wrinkled and is mainly covered with microridged cells and mucous glands.

The "lanes" are non-respiratory areas, whose epithelial cells have reticulated microridges on their surface.

**B. Respiratory organs in fish of 2.5 g (8 months).**

*Gills*: The first pair of gill arches is characterised by the attachment of labyrinthine organs (Fig. 3). The arrangement, shape and structure of the gill filaments show that whilst some of them are small, apparently due to stunted growth, others are fairly large and club-shaped. Near the base of the labyrinthine organ there are many gill rakers which bear toothlike structures and others are in the process of development. The secondary lamellae are short and stumpy (Fig. 4). The surface of the secondary lamellae is mainly covered by microridged epithelial cells. At higher magnification three types of cells are recognisable by their surface architecture:

a) cells bearing typical microridges;
b) cells with both microridges and microvilli and
c) microvillous-bearing chloride cells.

*Labyrinthine organs*: At this stage the labyrinthine organs are simple structures with two

Fig. 1. Scanning electron micrograph of gill filaments (GF) with respiratory mucosa of the suprabranchial chamber (MSBC) dissected out from a 1 g fingerling of *Anabas*. Note development of small respiratory islets (RI) on the surface of mucosa lining the suprabranchial chamber. ×17.

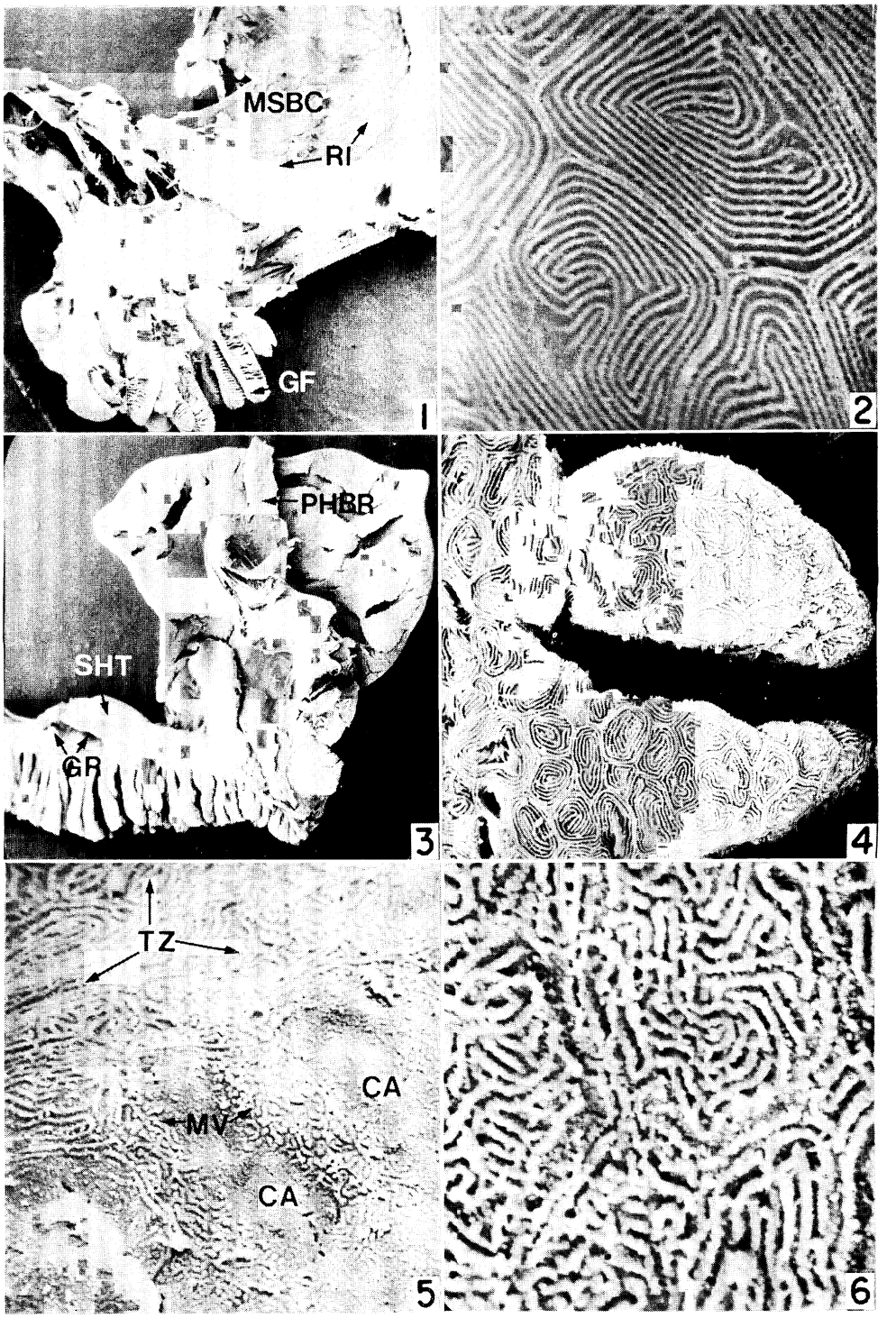
Fig. 2. Non-respiratory surface of minute labyrinthine organ of *Anabas* fry, 1 g body weight. Large MR cells with prominent cell boundaries. ×3910.

Fig. 3. *Anabas* fingerling (2.5 g) SEM of developing labyrinthine organ, two wing-shaped plates and first gill arch with shutter (SHT) and stumpy gill filaments dissected out. Note the rod-shaped pharyngobranchial (PHBR), and outlines of respiratory islets. ×18.

Fig. 4. View of two secondary gill lamellae of first gill of *Anabas* fingerling (2.5 g body weight). Note the stumpy nature of the lamellae covered by MR cell. ×1640.

Fig. 5. SEM of part of a respiratory islet of labyrinthine organ of a fingerling (2.5 g). Note the central smooth surface (CA), surrounded by microvilli (MV) in between the vascular respiratory epithelial surface and transitional non-respiratory zone (TZ). ×4340.

Fig. 6. View of the non-respiratory surface (lanes) of labyrinthine organ of a fish of 2.5 g. Note the reticulate microridged surface. ×8700.


MSBC
RI
GF
1
2
PHBR
SHT
GR
3
4
TZ
CA
MV
CA
5
6

leaf-like plates (Fig. 3). The axis of each organ is mainly formed of epi- and pharyngobranchial cartilages. The pharyngobranchial is a small knob-like structure attached to the centre of the labyrinthine organ which thereby remains attached to the skull and maintains the whole structure in position. The respiratory islets are slightly raised structures situated between the non-vascular lanes as can be seen in views of the entire labyrinthine organ. Characteristic lanes and respiratory islets are also distinguishable at the margin of the labyrinthine plates. The respiratory islets are covered by cells mainly bearing microvilli at their margins (Fig. 5). A transitional zone (TZ) between the respiratory and non-respiratory surfaces is shown in Fig. 5.

The lanes are covered with microridged epithelial cells, whereas the surface of the respiratory islets is covered with cells having a smooth central area (CA) surrounded by microvilli towards the cell boundaries (Fig. 5). The microridges show a variety of configurations (Fig. 6).

*Respiratory membrane lining suprabranchial chamber*: No material available for a specimen of this size.

**C. Structure of the respiratory organs of adult fish (30 g, age—more than one year).**

*Gills*: The first pair of gills (Fig. 7) are better developed than those of the third and fourth (Fig. 8). The fourth gills have become very much atrophied bearing only a few small bud-like filaments (Fig. 8). Some of the gill rakers of the first gill near the base of the labyrinthine organ have been modified to form the so-called shutter used for closing the inhalent apertures of the suprabranchial chambers of the adult fish (Fig. 7). The gill rakers are stout and bear pointed tooth-like structures used in manipulating prey. The gill filaments of the first and second gill arches have many chloride cells situated in between the MR cells in the ratio of 1 : 3 (Fig. 9).

*Labyrinthine organs*: At this stage these organs are complicated structures having many curved saucer-shaped plates. The characteristic respiratory islets are discernible with lanes between them. The surface of a respiratory islet may be recognised by the presence of parallel sets of blood channels with their typical bulging surfaces (Fig. 10). At low magnification the respiratory islets show the typical parallel arrangement of these channels and their beaded nature is apparent (Fig. 10). The characteristic bi-serial arrangement of the blood channels of the respiratory islet has been observed. Microvilli are mainly distributed on both sides of the surface of these capillaries, the region directly over the centre of the channels being devoid of microvilli. The bulges are due to the presence of endothelial cells, with their large nuclei, situated along the course of individual capillaries. In Fig. 11 the epithelial covering of the surface of a part of the respiratory islet has been removed and shows the course of the capillaries containing red blood cells. The non-vascular lanes are covered by reticulate microridged cells.

*Suprabranchial chamber*: The suprabranchial chambers have well defined inhalent and exhalent apertures. The inhalent aperture is guarded by shutter-like structures formed by the modification of a few gill rakers of the first gill arch (Fig. 7). The mucosal surface lining the chambers is very much folded and bears respiratory islets (Fig. 12).

## Discussion

Aquatic respiration has been found to be obligatory in the young stages of *Anabas*. The

Fig. 7. SEM view of part of the first gill arch with the shutter (SHT) (modified gill rakers) and filaments (GF) of an adult fish of 30 g. ×48.

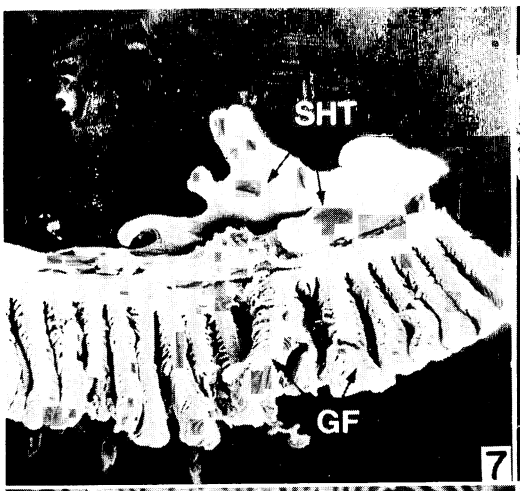
Fig. 8. View of III and IV gill arches of an adult fish (30 g body weight). Note minute bud-like gill filaments of IVth arch (GF) and comparatively large gill rakers (GR) bearing teeth. ×16.

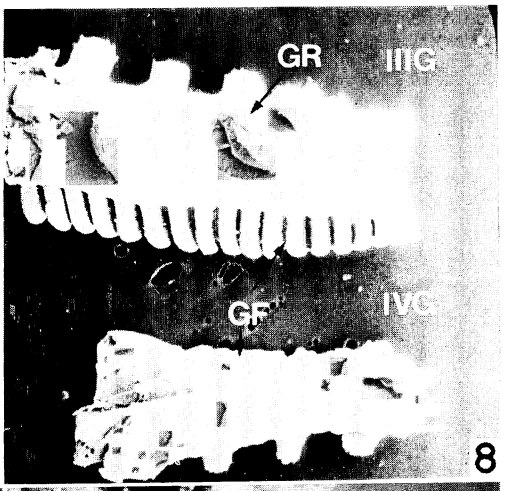
Fig. 9. SEM of gill filament surface showing the chloride cells (CLC) bearing microvilli situated in between microridged epithelial cells. Note some precipitated mucus on the surface (30 g body weight). ×4880.

Fig. 10. Higher magnification of part of the respiratory islet showing the beaded nature of transverse capillaries, separated by epithelial cell boundaries bearing microvilli (30 g body weight). ×950.

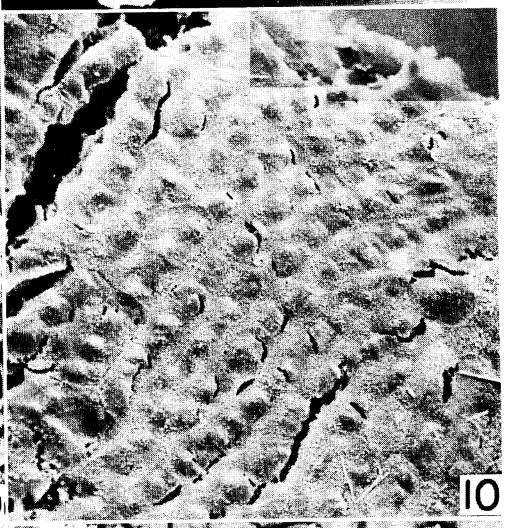
Fig. 11. Higher magnified view of beaded transverse capillaries (TRC) of respiratory islet after peeling-off of the surface epithelium (RE). Note the position of microvilli on the surface of torn off respiratory epithelium (30 g body weight). ×5080.

Fig. 12. View of the respiratory mucosa of suprabranchial chamber showing the nature of respiratory islets (RI) separated by furrow-like lanes (30 g body weight). ×56.


SHT
GF
7


GR
IIIG
GF
IVG
8


CLC
9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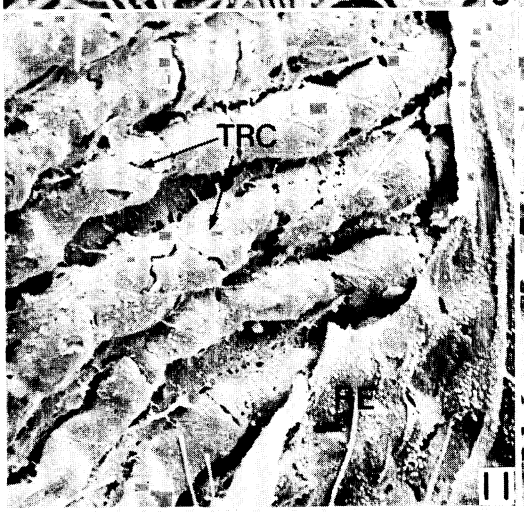
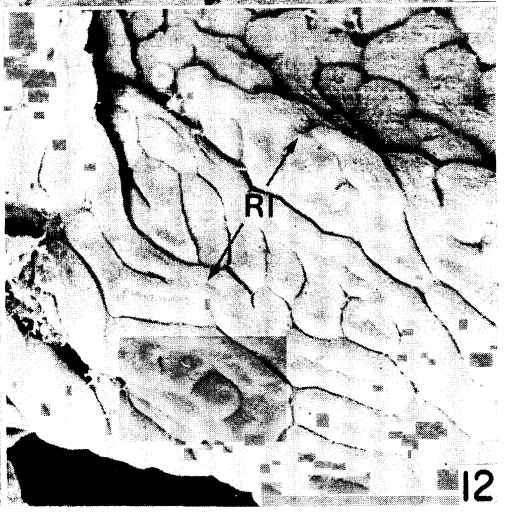

TRC
11


RT
12

labyrinthine organs start developing at 5th, but the hatchlings do not take air-breaths until they reach 13/14 days (Hughes, Munshi and Ojha, 1986). It has been observed that the time for asphyxiation of *Anabas*, when not allowed to breathe air, varies with the age and size of the fish. This again supports the previously discussed view that the capacity of the gills to take up oxygen decreases as the fish grow in size (Hughes, Dube and Munshi, 1973). Hughes and Singh (1970) reported that specimens of *Anabas* weighing 29–51 g obtain about 53.6% of their total oxygen requirement through the air-breathing organs. The observed ability of smaller fishes (1–25 g), depending upon gill breathing alone, to survive for longer periods than larger fish has been related to the decrease in oxygen diffusing capacity of gills as fishes grow in size (Munshi and Dube, 1973). At lower temperatures (17–20°C) specimens of *Anabas* weighing up to 30 g survived very well for a few months without taking air, but at 30–31°C even smaller fishes (10–15 g) asphyxiate within 24 hours (Dube, 1972). As in other fish this is due to two factors (Hughes, 1963) (i) at 17–20°C the concentration of dissolved oxygen in tap water generally varies from 5.0 to 5.5 ml/l which is reduced to 3.0 to 3.5 ml/l at 30–32°C; and (ii) the general metabolism increases with temperatures.

A main objective of this work was to see if there is any difference in structure of the respiratory surface of young and adult fishes.

At six month old (1 g) fish the gills are well differentiated but covered mostly with microridged epithelial cells. Mucous glands and chloride cells bearing microvilli are found in good numbers. The labyrinthine organs consist of only one curved saucer-shaped plate and respiratory islets are not seen on the surface. The microridged cells have reticulated surfaces to which mucus adheres rather well. Only at higher magnification is the microvillous surface visible and which may be respiratory in function.

The presence of chloride cells on the labyrinthine plate is interesting. Perhaps the suprabranchial chamber together with the labyrinthine organ is used in aquatic respiration at this young stage of six months. Ventilation of the suprabranchial chambers with water persists in adult anabantoids and represents a special mechanism only found in fishes (Hughes, 1978; Peters, 1978).

In 8 month old fishes the labyrinthine organs have developed into two leaf-like plates and respiratory islets are fully differentiated. By 12 months there are many saucer-shaped plates added to the labyrinthine organ. The labyrinthine organs of an 8 month old fish are comparable to fully grown specimens of *Colisa* (=*Trichogaster*) *fasciatus* which have only two leaf-like plates (Munshi, 1965).

The respiratory surface of the islets is always smooth and bears microvilli only at their margins. This confirms our earlier observations (Hughes and Munshi, 1973) where under TEM very minute microvilli could be seen at the margins of the respiratory islets.

It may be concluded that during juvenile stages the accessory respiratory organs of *Anabas* remain undeveloped with ill-defined respiratory islets. Consequently it remains a facultative air-breather depending more on aquatic breathing, perhaps ventilating its suprabranchial chambers with water in addition to the gills. The fishes first attain maturity when they grow to a length of 23 mm and 8.5 g weight (Mookerjee and Mazumdar, 1946; Banerjee and Prasad, 1974). By this time they have well developed labyrinthine organs and become obligatory air-breathers.

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## キノボリウオ *Anabas testudineus* の稚魚および成魚の呼吸器官の走査電子顕微鏡による観察

**J. S. Datta Munshi and G. M. Hughes**

キノボリウオの稚魚 (1–2.5 g) および成魚 (30 g) の鰓と空気呼吸器官（上鰓腔内の迷路器官）を走査電子顕微鏡によって研究した．稚魚 (1–2.5 g) では，迷路器官は 1–2 個の葉状板を有するのみであった．非常に小さい稚魚 (1 g) では，迷路板上に島状呼吸組織の発達が認められなかった．稚魚期には上鰓腔が水呼吸に用いられる証拠がある．空気呼吸が必須となる成魚では，第 1 鰓弓にシャッターが発達して，上鰓腔の吸入口と呼出口が明瞭になる．