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Scanning Electron Microscopy of the Gills of a Freshwater Catfish, *Rita rita*

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Abstract Variations in the gross morphology and surface architecture of the gill filaments and secondary lamellae of a freshwater catfish (*Rita rita*) have been investigated using scanning electron microscopy. Heterogeneity of the gill has been correlated with the distribution of lamellar water-flow at different regions of a gill filament. Higher lamellar water flow (cc/pore/cmH_2O/sec) was estimated for the middle region of the filaments. The filaments are covered with epithelial cells whose surface is provided with well-developed microridges. The lamellae are generally covered with microvillous epithelial cells. The variations in surface architecture of the gill filaments and secondary lamellae have been correlated with their probable functions.

Fish gills are complex structures, the various units of which are covered by different kinds of epithelia which show variations in their surface specializations and the density and distribution of the branchial glands (Hughes, 1979; Dunel and Laurent, 1980; Karlsson, 1983; Hughes and Mondolfino, 1983; Hughes 1984a; Ojha et al., 1987). Munshi (1960, 1964) made a preliminary study of the gills of *Rita rita* (Hamilton) and the role of branchial glands in ionic regulations. However, surface specialization of the gill filaments and secondary lamellae of this fish have received little attention and form the basis of this attempt to correlate the various structural modifications with their functions.

Materials and methods

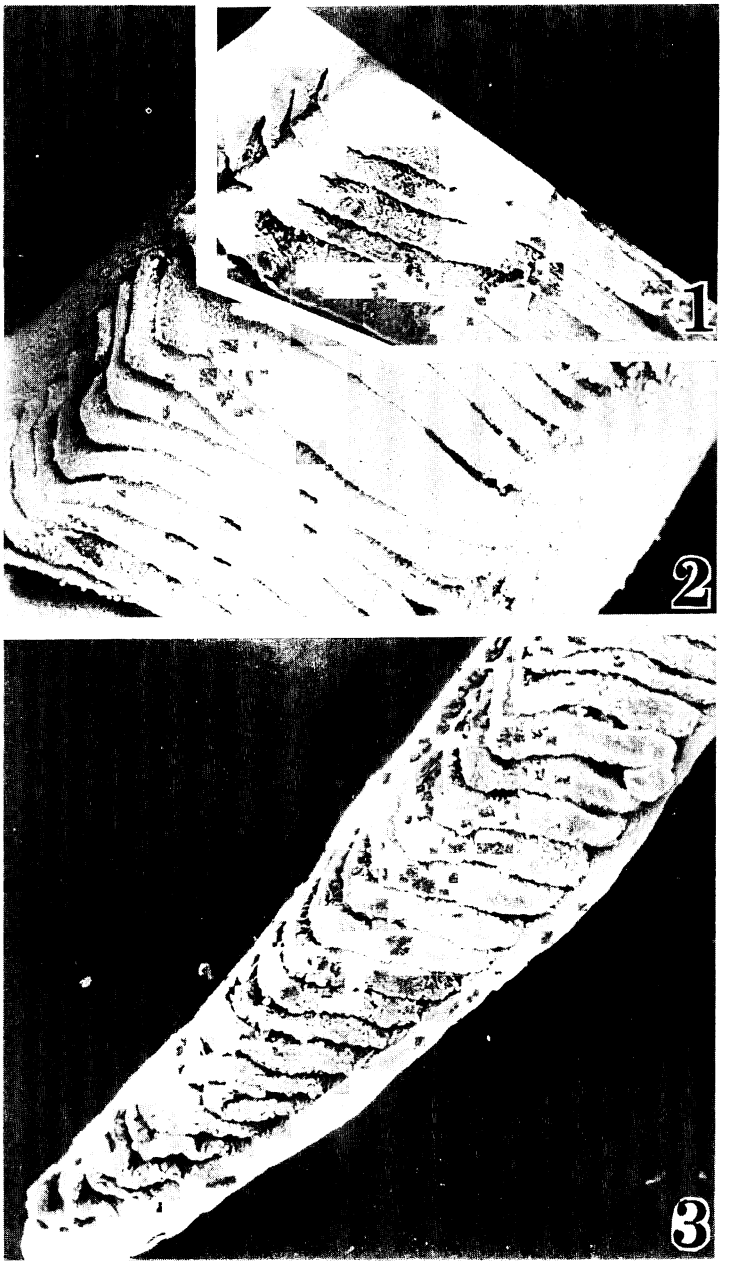
Rita rita is a commercially important catfish of the River Ganges. It belongs to the family Bagridae of the order Siluriformes (Greenwood et al., 1966). It can survive out of water for more than 24 hours if its skin is sprayed with water. Outside water the fish also ventilates its gills with air.

A healthy matured female specimen of *R. rita* (approximately 560 g, 35.7 cm) was anaesthetized in an aquous solution of MS222 (60 mg/litre) on 15th July, 1982. Its gill filaments were removed carefully and fixed immediately in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 h at 4°C. The gill filaments were then transferred to 12.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 24 h at 4°C. The fixed filaments were rinsed in phosphate buffer and transported to Bristol (England) where they were dehydrated in graded concentrations of ethanol and stored in dried acetone. The gill filaments were critical point-dried and mounted on stubs, coated with gold and viewed in a Cambridge S4 scanning electron microscope (SEM).

Some morphometric measurements were also made with the help of scanning electron micrographs to measure dimensions of the secondary lamellae and spaces between them. These measurements were analyzed with respect to the gaseous exchange and water flow through the gills. Pore dimensions (average interlamellar distance (d), maximum length (l) and average height (b/2) of a secondary lamella) were also determined using unfixed gill filament of a fresh specimen of similar body size. The latter enabled an evaluation to be made of shrinkage due to fixation and other procedures involved in preparation for SEM and the necessary corrections for estimates of water flow through the interlamellar spaces.

Results

Gill filaments of *Rita* are covered with secondary lamellae whose differences in shape are clearly visible in SEM (Figs. 1–3). On the basis of its surface architecture the filament epithelium is divisible into different zones. The leading edge of the filament is mainly covered with epithelial cells (average area, 0.061 μm^2), the plasma mem-



Figs. 1–3. Scanning electron micrographs from the base (Fig. 1; ×235), middle (Fig. 2; ×185), and tip (Fig. 3; ×245) regions of the gill filaments of *Rita rita* showing the shape and arrangement of secondary lamellae on them.

brane of which is folded into smooth microridges (Fig. 4). The distance between two adjacent microridges is about 0.39 μm and height 0.146 μm. At certain points the microridges are connected by microbridges. Y-shaped micropockets are also discernible in the intercellular spaces of some microridged epithelial cells (Fig. 4). Between this zone and the origin of the secondary lamellae there is a region where the surface is reticulated, the epithelial cells being characterized by complex

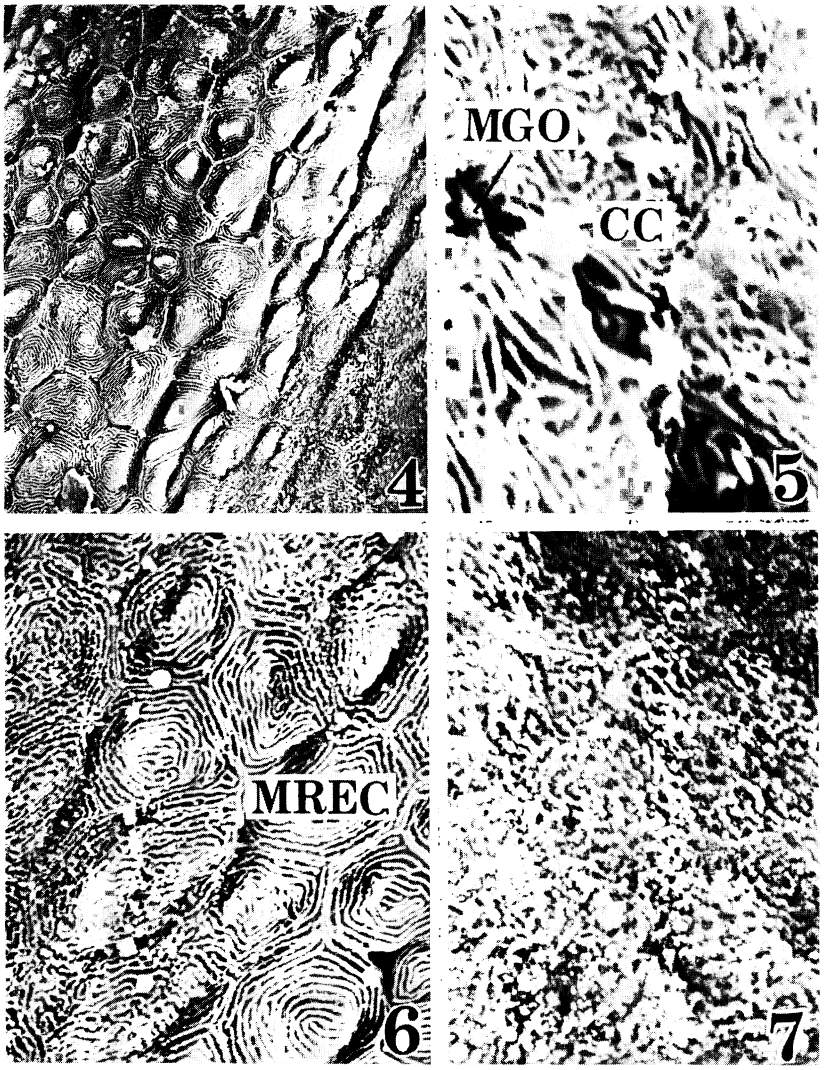


Fig. 4. Scanning electron micrograph of part of the filament showing whorl-like microridged epithelial cells. ×1,180.

Fig. 5. SEM image of part of epithelium from leading edge of the gill filament showing mucous gland openings (MGO) and chloride cell (CC). ×10,400.

Fig. 6. SEM image of part of epithelium on leading edge of filament showing transition from microridged (MREC) to reticulated surface of epithelial cells closer to origin of secondary lamellae. ×2,900.

Fig. 7. SEM image of part of secondary lamellar epithelium showing microvillous epithelial cells. ×7,000.

microridges. Microbridges are more numerous and divide the spaces between ridges into small microchambers. Mucous and chloride cell openings are also visible (Fig. 5). The transition between these two zones of the leading edge is shown in Fig. 6. The trailing edge of the gill filament shows the presence of microridged epithelial cells. The microridges are complex and are also connected with microbridges. However, mucous and chloride cell openings could not be seen in this region.

The surface of the secondary lamellae is quite different from that of the filaments. The cells of the lamellar epithelia are generally characterized by the presence of numerous microvilli (Fig. 7). Intercellular boundaries were not so clearly de-

fined. Mucous and chloride cell openings could not be located on the secondary lamellae.

SEM images provide good three-dimensional views of the base, middle and tip regions of the gill filaments (Figs. 1–3), which are especially helpful in analyzing some morphometric measurements in relation to gill functions.

Lamellar frequencies at these regions are important measurements which indicate heterogeneity of the gill. The tip of *Rita* gill filaments is characterized by a higher lamellar frequency ($1/d' = 30/mm$) than the middle (26/mm) and basal (23/mm) regions. For total gill area measurement lamellar frequency is doubled to obtain the value for the two sides of the filament ($n = 2/d'$).

In addition to lamellar frequency, the bilateral surface area (bl) of a secondary lamella is also an important parameter which is directly proportional to total gill area. Variations in bilateral surface area of secondary lamellae were also measured at different points along the gill filaments from the SEM. The basal regions of gill filaments contain comparatively larger secondary lamellae (0.045 mm^2) than middle (0.0409 mm^2) and tip (0.0229 mm^2) regions.

From the measurements of lamellar area and frequency, lamellar area for unit filament length was calculated ($=n \cdot bl$). For a 1 mm length of filament, total lamellar area was higher in the middle region (2.1284 mm^2) than the basal (2.0792 mm^2) and tip (1.3716 mm^2) regions. These differences are mainly due to the differences in the size of individual lamellae in the three regions.

From SEM attempts were also made to measure the maximum length (l) and average height (b/2) of the sampled secondary lamellae and width of the interlamellar space (d) between two adjacent secondary lamellae. From these measurements pore dimensions and the interlamellar water flow at different (base, middle and tip) regions of the gill have been estimated and are summarized in Table 1. Similar data from the fresh, unfixed gill material are also given for comparison.

Discussion

In course of their evolution, the structure of fish gills has become modified in relation to their mode of life and chemical nature of the water in which they live. Development of secondary lamellae on both sides of the gill filament increases the total effective respiratory surface for gaseous exchange. Frequency and area of secondary lamellae are directly proportional to the gill area. Variations in the lamellar frequency (l/d') at the base (23/mm), middle (26/mm) and tip (30/mm) regions suggest heterogeneity in the gill of *Rita rita*. Heterogeneity in the gill structure is further indicated by areas of the secondary lamellae sampled from base (0.0452 mm^2), middle (0.0409 mm^2) and tip (0.0229 mm^2) of the sampled gill filament. This heterogeneity in the morphology of the various gill components creates problems for quantitative analysis of surface area and distribution of lamellar flow (Hughes, 1984a). Because of these problems due to gill heterogeneity, vari-

Table 1. Pore dimensions and estimates of water flow through the interlamellar spaces of the base, middle and tip regions of gill filaments of *Rita rita*.

Filament region	Pores in 1 mm	Pore dimensions			Water flow/ pore (q) (cc/cmH_2O/ sec)	Water flow/ mm gill filament (cc/cmH_2O/ sec)
		Average inter-lamellar distance (d) cm	Maximum length of average sec. lam. (l) cm	Average height of sec. lam. (b/2) cm		
Unfixed gill filament						
Base	18	0.0022	0.0615	0.0057	2.1×10^{-5}	3.6×10^{-4}
Middle	15	0.0024	0.0569	0.0092	4.6×10^{-5}	6.9×10^{-4}
Tip	19	0.0023	0.0392	0.0096	6.2×10^{-5}	1.2×10^{-3}
Fixed and critically point dried gill filament						
Base	23	0.0012	0.0387	0.0038	3.7×10^{-6}	8.5×10^{-5}
Middle	26	0.0019	0.0454	0.0045	1.4×10^{-5}	3.7×10^{-4}
Tip	30	0.0011	0.0202	0.0031	4.2×10^{-6}	1.3×10^{-4}

ous sampling procedures (Hughes, 1966, 1984a, b; Muir and Hughes, 1969; Hughes and Morgan, 1973; Hughes and Ojha, 1985) have been employed for gill area measurements in order to reduce errors.

Gill resistance was first defined in relation to water flow by Hughes and Shelton (1958). Later data on the measurements of the average height (b/2) of the lamellae, their length (l) across a gill filament, and the distance (d) between them were applied (Hughes, 1966) to a modified Poiseuille's equation:

$$q=\frac{P_1-P_2}{\eta}\cdot\frac{5d^3b}{24L}$$

for the quantitative measurement of water flow through rectangular lamellar channels. Similar measurements were also made on the various sampled regions of gill filaments of *R. rita*. Comparatively higher interlamellar flow (cc/pore/cmH_2O/sec.) of water in the middle part (1.4×10^{-5}) than the base (3.7×10^{-6}) and tip (4.2×10^{-6}) regions of the gill filament is obviously due to larger morphological components (d, l and b/2). However, these flow rates are lower than values reported for *Micropterus dolomieu* and *Calli-onymus lyra* (Hughes, 1966). Lower water flow rate of *Rita*'s gill is perhaps due to the measurements made on SEM photographs. Various gill dimensions will be reduced because of greater shrinkage as a result of fixation and critical point drying in SEM preparations. Under such circumstances it is suggested that measurements of various morphological gill components should also be made on unfixed or recently-fixed specimens. When this was done values of 2.1×10^{-5}, 4.6×10^{-5} and 6.2×10^{-5} were obtained for flows through the base, middle and tip regions.

In addition to the adaptations of the gill filaments and secondary lamellae at a gross morphological level, modifications are also present on the outer surface of the epithelium. In *Rita rita* the gill filaments and lamellae are covered with epithelial cells whose surface is covered with microridges and microvilli of varied dimensions and configurations, as in the rainbow trout (Hughes, 1979) and other fishes. On the basis of their surface structure, different zones of each gill filament may be differentiated. The leading edge is convex and provided with surface epithelial cells with well developed whorl-like microridges. At certain points the microridges are connected by microbridges. The microgrooves and pockets formed by the microridges and microbridges help to hold mucus and moisture which may protect the surface of the filaments from desiccation when the fish is out of water and ventilates the gills with air. Concentration of mucous gland openings towards the leading edge of the filament is interesting. Water first comes in contact with the leading edges during gill ventilation. Mucus secreted by branchial glands helps to remove sediments from the ventilating water and they keep the epithelium free from deposites which might interfere with their effective functioning. Some chloride cell-pits are also discernible near the leading edge of the filaments. These chloride cells are associated with ionic regulation. Near the trailing edges the epithelial cells are provided with complex microridges and microbridges. The microridges are again provided with villouslike expansions. Similar structures have also been reported in the gills of *Trachurus mediterraneus* (Hughes and Mondolfino, 1983). These microvilli in association with the microridges may help to increase the surface area of the cell. The microridged epithelial cells of the gill filaments may serve to retain mucus and moisture to protect the gill surface from desiccation when the fish is out of water.

The secondary lamellae have a different surface structure. The cells are generally provided with microvilli. The secondary lamellar epithelium has a comparatively rigid surface due to the presence of many blood channels underlying them. This may be one of the reasons for the flattening of the microridged surface into a covering for the secondary lamellae of *R. rita*. When a fish is out of water the small microvilli may entrap water molecules which may serve as a medium that is regularly oxygenated during gill ventilation with air. This oxygen-rich film may provide a regular source of oxygen to the fish when it is out of water.

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淡水産ナマズ *Rita rita* の鰓の走査電子顕微鏡的研究

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淡水産ナマズの一種 *Rita rita* の鰓弁および二次鰓弁の形態と表面構造の変異を，走査電子顕微鏡を用いて調べた．鰓弁の部位による形態の変異は，二次鰓弁を通過する水の量の変異を示すものと考えられ，計算によればこの流水量は鰓弁の中央部分で最大である．鰓弁の上皮細胞の表面には微小堤がよく発達しており，二次鰓弁の上皮細胞は一般に微絨毛におおわれている．鰓弁と二次鰓弁の表面構造の違いと，それぞれの機能との関連性を考察した．