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Regulation of diurnal filter feeding by a novel gill structure in *Amblypharyngodon melettinus* (Teleostei, Cyprinidae)

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Abstract A unique gill structure, apparently associated with filter feeding on phytoplankton and suspended microdetritus, has been found in *Amblypharyngodon melettinus*, an abundant small Cyprinidae of Sri Lanka. The gill lamellae, the site of gas exchange, are bordered by a double row of fine appendices which are spread over the interlamellar gaps during daytime, but folded up at night. A respiratory function of the appendices can be excluded. The changing position of appendices correlates with the diurnal pattern of feeding (day) and swimming (night). The mechanism for movement of the appendices consists of hinge-like joints formed from the basement membranes of pavement cells, driven by variation in lamellar blood pressure. Food collection is based on both an efficient hydrosol filter produced by dense populations of clavate mucous cells of the buccopharyngeal epithelia and the lamellar appendices which cause a slower and more turbulent water current in the buccopharyngeal cavity. This may ensure the proper contact of food particles with the sticky mucous surface before they leave the buccopharyngeal cavity. The uniqueness of this structure is that the filter can be switched off during periodically occurring periods of high oxygen demand (high swimming activity at night) probably benefiting the process of respiration.

Keywords Suspension filter feeding · Hydrosol filter · Gill lamellae · Mucous cells

Introduction

Amblypharyngodon melettinus (Valenciennes, 1844), a member of the Cyprinidae of 8–9 cm adult length, is the dominant species in the pelagic midwater of numerous irrigation reservoirs of Sri Lanka. Its biomass can make up over 50% of the total biomass of Teleostei (Piet and Vijverberg 1998). Analytical studies on the feeding ecology have shown that the diet of *A. melettinus* is composed of a mixture of phytoplankton and bacterial–detrital aggregates (Rott, unpublished observations). While the ecology of *A. melettinus* has been studied in detail (Schiemer 1996; Piet and Vijverberg 1998), nothing is known about the structure of the phytoplankton filter of this species. In fact, filtering mechanisms have been investigated only in a small number of phytoplanktivorous species (Hoogenboezem et al. 1991; Sanderson et al. 1991, 1996, 2001; Goodrich et al. 2000). The filter of the silver carp [*Hypophthalmichthys molitrix* (Valenciennes, 1844)], apparently evolved from the zooplanktivorous type of a mechanical filter, consists of gill arches being equipped with a densely packed double row of exceptionally long and fine gill rakers, leaving slits only 12–26 μm wide (Hampl et al. 1983; Iwata 1986). Their outer surface is coated with a mucus-producing tissue in which numerous pores allow water to pass through the sieve (Jirasek et al. 1981/82; Iwata 1986). Water is pushed through the filter by periodic up and down movements of the palatal organ (Pichler-Semmelrock 1988). This unique feeding apparatus is both a mechanical filter and an efficient hydrosol filter retaining small particles $<10 \mu\text{m}$ (Adamek and Spittler 1984).

Other microphagous species among the Cyprinidae and Cichlidae have only short and widely spaced gill rakers which are unsuitable as a mechanical filter but may help to direct water flow and food particles to the sticky mucous surface of the buccopharyngeal cavity (Hoogenboezem et al. 1991; Sanderson et al. 1991, 1996, 2001). In *Oreochromis niloticus* (Linné, 1758) (Cichlidae) large clavate mucous cells, producing acidic mucus, are located on the trailing edge of gill arches, whereas neutral and

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mixed mucus is secreted by small goblet cells at the anterior face of gill arches and gill rakers (Northcott and Beveridge 1988).

The aim of this study was to investigate the feeding mechanism of *A. melettinus* and, in particular, how this species regulates its diurnal feeding activity with starving at night and filtering during the day (Schiemer 1996).

Materials and methods

Collection of specimens

Amblypharyngodon melettinus were caught in three Sri Lankan reservoirs by exposing gill nets for less than 1 h, and by beach seining, which was successful only at night. Immediately after removing the specimens from the net, they were killed by a blow on the head, the opercular bones were removed and the animals were dipped into fixatives, 5% buffered formaldehyde (pH 7.4), 2.5% glutaraldehyde in 0.01 M cacodylate buffer pH 7.2 or Bouin, respectively. After about 5 min, the heads were cut off and transferred to a fresh fixative.

Gross and fine morphology

Light microscopy

Gill arches removed from formaldehyde-fixed heads, and about 3-mm-thick cross- and longitudinal sections from Bouin-fixed heads were gradually dehydrated in ethanol and embedded in polyethylene glycol-methacrylate. May-Grünwald/Giemsa, PAS and alcian blue staining methods were carried out on 3- μ m microtome sections of gill filaments and head samples. From each sampling period in

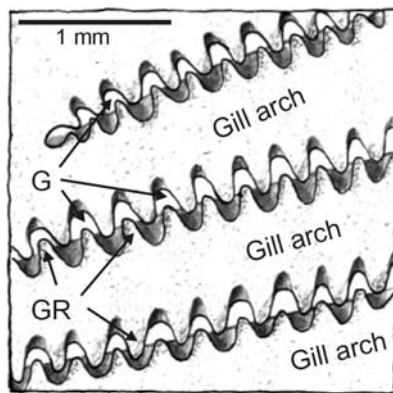
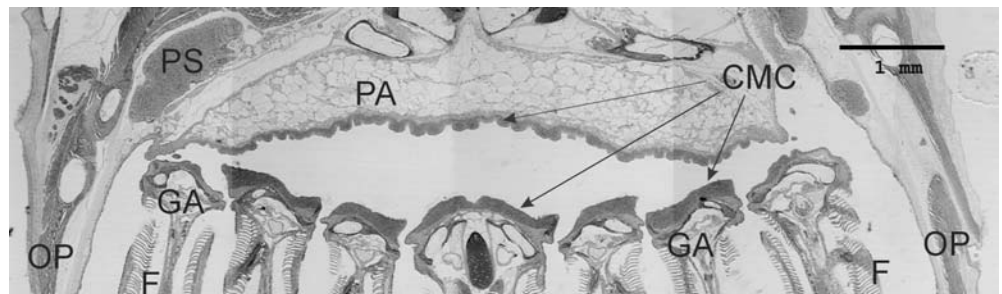


Fig. 1 Gill arches of *Amblypharyngodon melettinus* with interdigitating gill rakers. GR Gill rakers, G gaps

Fig. 2 Cross-section through the branchial cavity of the head of *A. melettinus*. CMC Clavate mucous cells, F gill filaments with lamellae, GA gill arch, OP opercula, PA palatal organ, PS pseudobranch



the morning, afternoon, evening, dawn, early and late night, gills of five to ten specimens were investigated.

Electron microscopy

Gill arches from glutaraldehyde-fixed heads of four specimens caught either during the day or during the night were rinsed several times in 0.01 M cacodylate buffer pH 7.2, and then postfixed in 1% osmium tetroxide and 1.5% potassium ferrocyanide in 0.01 M cacodylate buffer, followed by gradual dehydration in acetone, and embedded in Spurr's low viscosity resin. Semithin and ultrathin sections were cut with a Reichert Ultracut and examined with a Zeiss TEM 902. Osmium fixation for scanning electron microscopy followed the protocol described above. Dehydration was performed in a graduated methanol series. Samples were dried with the critical point method, mounted, sputtered and examined with a Zeiss DSM 950.

Results

Buccopharyngeal cavity

Gill rakers of *A. melettinus* are short and blunt, leaving only small gaps of approximately 100 μ m between the interdigitating elements of neighbouring gill arches (Fig. 1). The epithelia of the anterior faces of the gill arches and gill rakers, and the palatal organ, contain a dense population of elongated mucous cells (clavate mucous cells) with neutral, PAS-positive granules (Figs. 2, 3). Small superficial goblet cells with acidic (alcian blue-positive) or mixed contents cover the entire surface of the buccopharyngeal cavity, including the trailing edges of the gill arches. The fine structure of the clavate mucous cells exhibits a prominent endoplasmic reticulum and Golgi apparatus, and fields of mucous vesicles which are much smaller than those of the goblet cells (Fig. 4).

Gross morphology of the gills

The gross morphology corresponds to the basic structure of fish gills: two rows of filaments are attached to each gill arch, and numerous gill lamellae, the site of gas exchange, are located on both sides of the filaments. Water from the buccopharyngeal cavity flows through the narrow gaps between the gill arches (Fig. 1) and passes between the gill lamellae from the outer to the inner side of the filaments, leaving the gills via the interfilamentous space (Fig. 5). The apical ends of the gill filaments are not cone-shaped as in other Teleostei but flattened on their

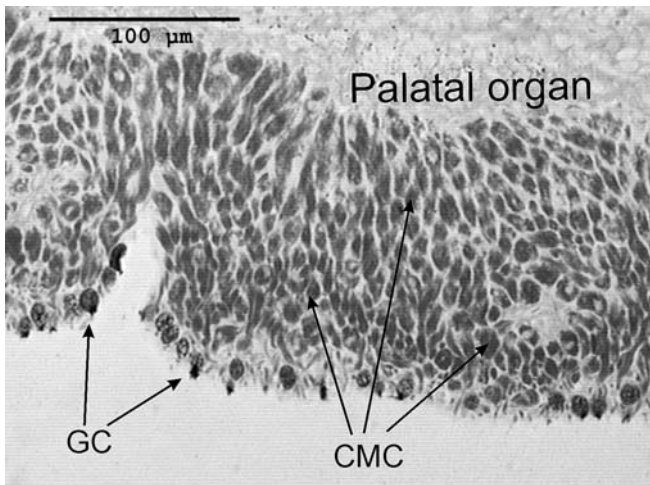


Fig. 3 Section through a branchial arch with elongated clavate mucous cells (CMC) containing neutral mucus (PAS-positive) and superficial goblet cells (GC) with acidic and mixed contents

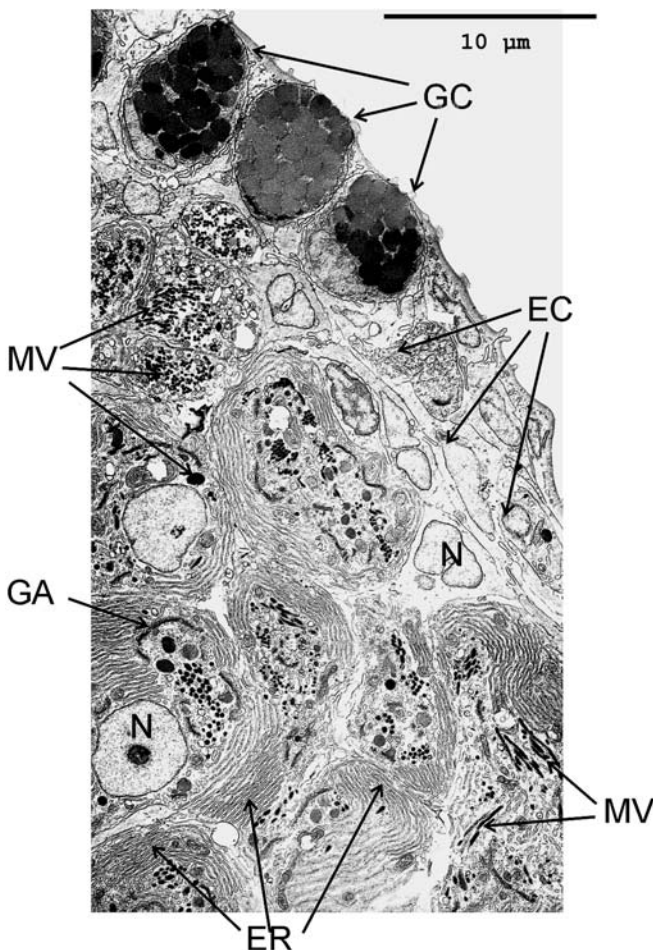


Fig. 4 Ultrastructure of the clavate mucous cells (CMC). EC Epithelial cells, ER endoplasmic reticulum of CMC, GA Golgi apparatus of CMC, GC goblet mucous cells with acidic contents, MV mucous vesicles of CMC with neutral contents, N nucleus of CMC

external side (Fig. 5), ensuring that filaments stay a minimum distance apart. This may force the water current to flow through the interlamellar slits more efficiently than in other fish species.

The shape of the gill lamellae is asymmetric, being small on the outer side of the filaments and becoming gradually broader towards the inner side. A unique structure was found at the lamellar edges which are bordered by double rows of fine appendices beginning at the outer front of the filament and ending where the lamellae attain their largest dimension (Fig. 6). A smaller central spine is not always visible. Lamellar edges facing the inner side of the filaments lack appendices.

The lamellar appendices are subjected to diurnal movement: they are spread over the interlamellar gaps forming a mechanical filter of about 2 μm mesh size during the day, but are folded up at night and allow the water to pass freely through the interlamellar space (Fig. 7).

Fine morphology of the gill lamellae

The lamellar appendices are formed from the elongated basement membrane of the inner layer of pavement cells. At the apical edge of the lamella the basement membrane is thickened and forms a hinge-like joint, with the terminal cell as a pivot (Fig. 8). At the top of the joint is a shorter central spine which penetrates the epithelia between the lateral appendices (Fig. 9).

Diurnal movements of the appendices are caused by variations in the width of blood spaces in lamellar sections immediately below the joint (Fig. 9). At midday, the blood spaces of all lamellar sections are uniformly narrow, forcing the appendices to spread out. At night, however, the blood spaces below the joints are greatly extended, and as a consequence, the lateral appendices are folded up. The change in position of the appendices is a slow process, taking about 3 h, starting at the beginning and again at the end of the natural photoperiod. No differences in the morphology of the gills were found between specimens caught by beach seining, in which handling stress was not longer than a few minutes, and animals caught by gill netting that suffered severely for half an hour. In starving specimens kept under aquarium conditions the appendices were closed during the day also.

Discussion

There is no doubt that the feeding mechanism of *A. melettinus* is based on an efficient hydrosol filter produced by the dense population of clavate mucous cells at the palatal organ and the gill arches (Figs. 2, 3, 4). But what is the function of the lamellar appendices at the apical end of the lamellae? As the appendices have no contact to the blood space a respiratory function can be excluded (Fig. 8). Spread appendices even severely hinder the water flow along the respiratory surface. There is strong evidence that this unique gill structure among

Fig. 5 **a** Gill filaments of one gill arch with flattened apical ends at their external side which form tight connections with the filament of the neighbouring gill arch. Gill lamellae are situated on both sides of the lamellae. *Arrows* indicate the direction of water flow. **b** Outer surface of gill filaments with their flattened tops

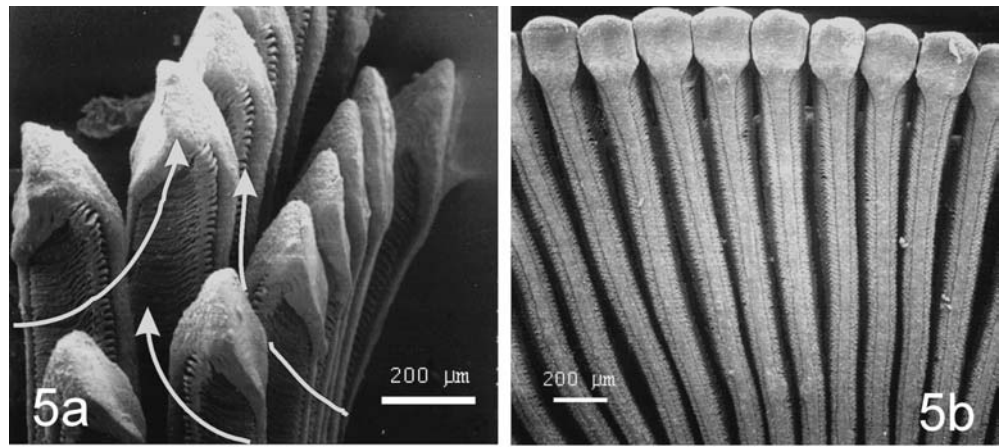


Fig. 6 **a** Outer surface of gill filaments (*F*) with gill lamellae (*L*) bordered with appendices extending up to the filament's front surface. **b** Inner surface of gill filaments. The lamellae are free of appendices. *Arrows* indicate the direction of water flow

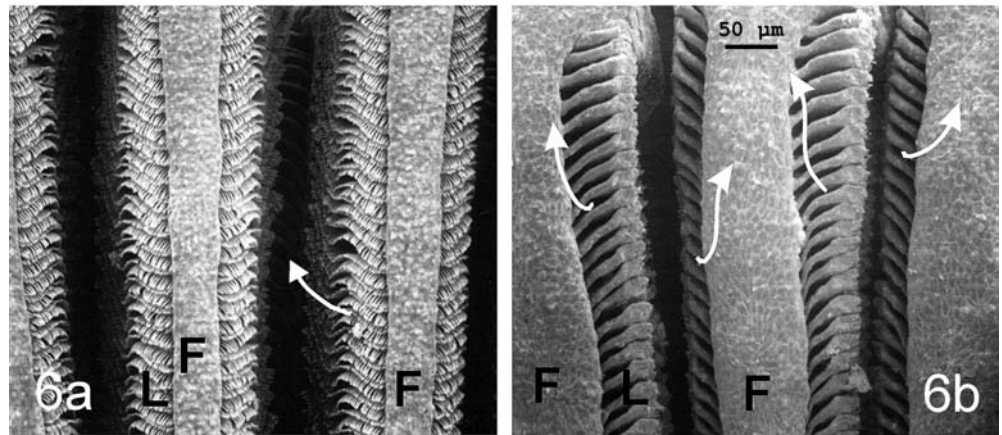
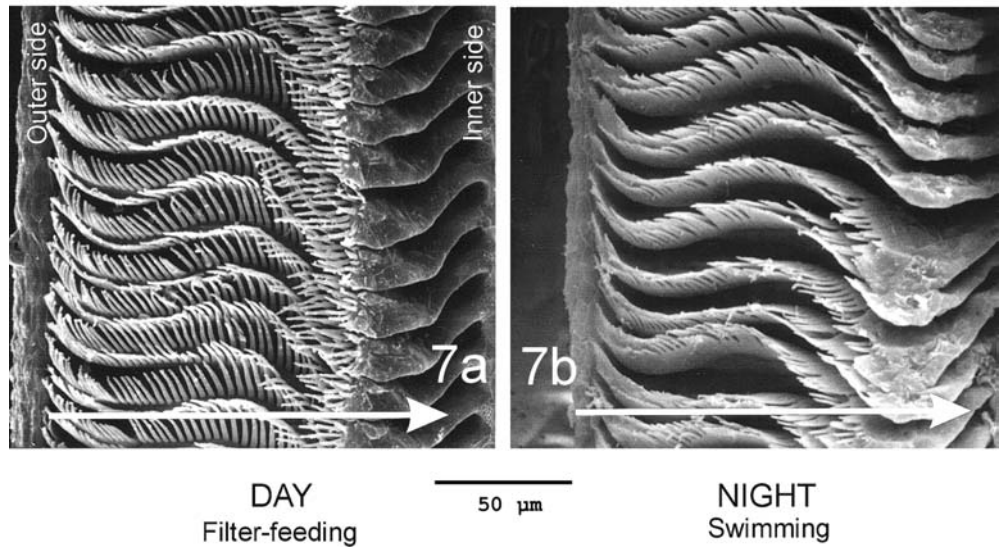


Fig. 7 **a** During the day, the appendices are widely spread covering the interlamellar gaps. The distance between the appendices is about 2 µm. **b** At night, the appendices are folded allowing the water to flow unhindered through the lamellae. *Arrows* indicate the direction of water flow



Teleostei plays a specific role in accumulating food items. However, the position of a mechanical filter would be expected at the gill arches which separate the buccopharyngeal cavity from the gill cavity (as seen in *H. molitrix*), but not close to the respiratory surfaces of the gill lamellae. *Amblypharyngodon melettinus* may combine the effects of two different filtering strategies described in other Teleostei, a cross-flow filter and the formation of turbulent water flow. Filtration in zooplanktivorous species and in the phytoplanktivorous silver carp (*H.*

molitrix) is based on a typical cross-flow filter. As the water to be filtered flows parallel to the surface of the gill rakers, only the fluid passes through the pores but food particles remain suspended becoming more concentrated towards the oesophagus. This ensures that the filter does not become clogged (Sanderson et al. 2001). In *A. melettinus* the filtering surface is shifted from the level of gill arches downwards to the outer lamellar surface and thus is probably less efficient than a simple mechanical filter (Figs. 6, 7). However, both the high water resistance

Fig. 8 Ultrastructure of the gill lamella. *A* Appendices, *BM* basement membrane, *BS* blood space with erythrocytes (*E*), *HLJ* hinge-like joint, *LE* lamellar epithelia cells, *PC* pillar cell, *TS* terminal cell

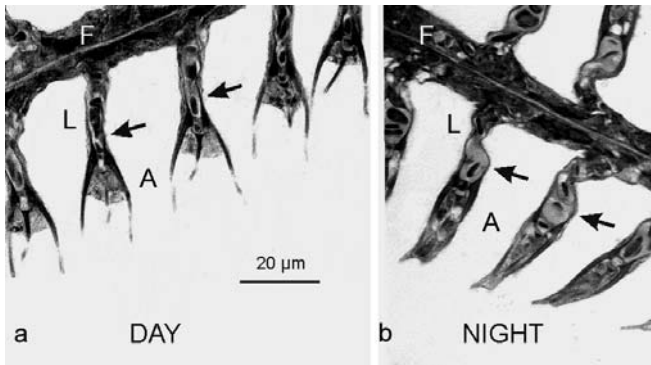
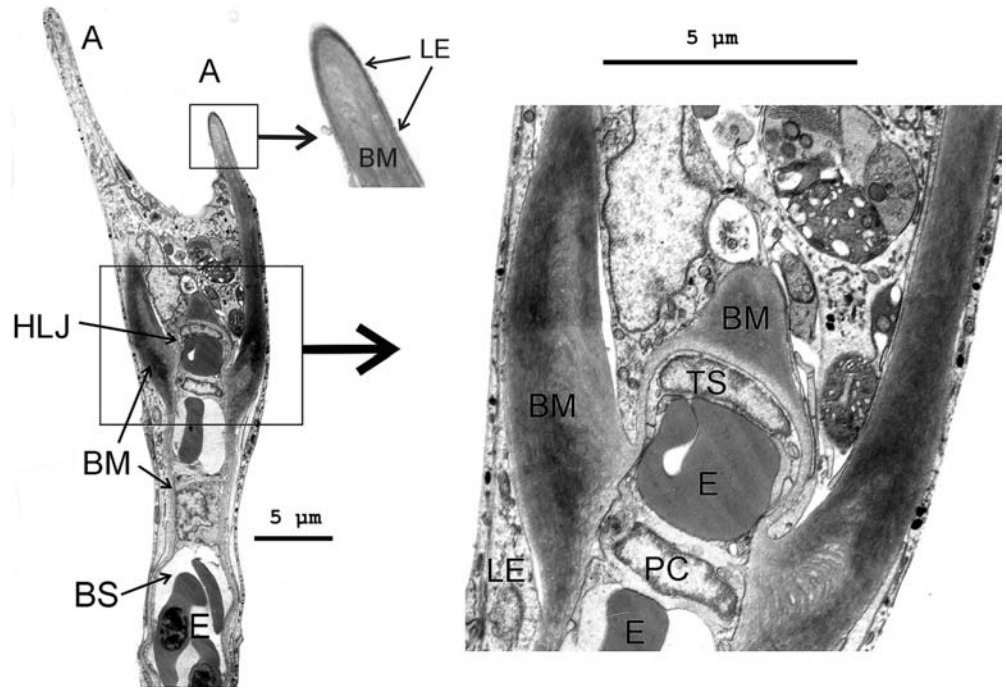


Fig. 9 **a** Gill lamellae with appendices spread during day. **b** Gill lamellae with appendices folded during the night. Note the width of the blood space (*arrowhead*). *F* Filament, *L* lamella, *A* appendices

caused by the extended lamellar appendices and the tooth-like gill rakers must produce a highly turbulent water current. This may ensure the proper contact of food particles with the sticky mucous surface before they leave the buccopharyngeal cavity. It has been demonstrated that small structures on the gill arches of *Orthodon microlepidotus* (Ayres, 1854) (Cyprinidae) direct water flow to the mucous surface (Sanderson et al. 1991). In *A. melettinus* a portion of food particles may also enter the interlamellar slits and clog the filter surface. This can be prevented by periodic flow reversal which transports the food particles back to the buccopharyngeal cavity. Such a behaviour is well known for the microphagous *O. niloticus*. Sanderson et al. (1996) used a fiberoptic endoscope for observing the water flow in the buccopharyngeal cavity of this species. During feeding the normal flow pattern from anterior to posterior was interrupted periodically by flow reversal. Two to three normal pumping movements were followed

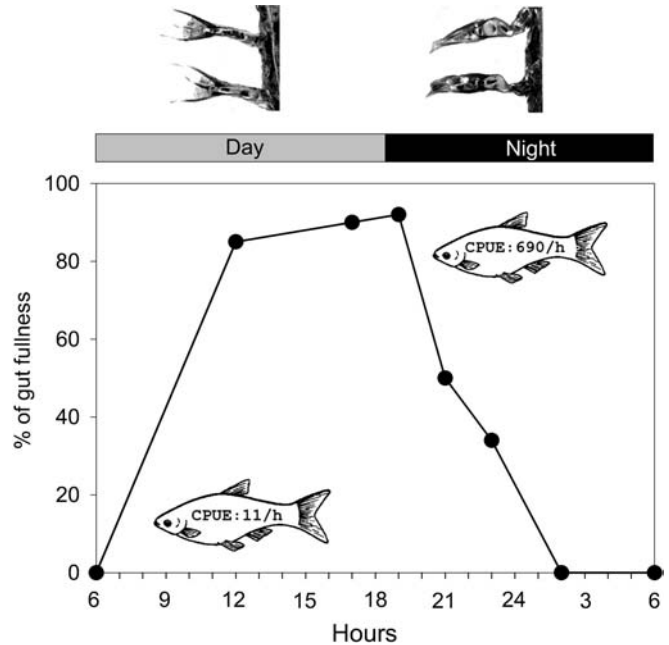


Fig. 10 Diurnal cycles of relative gut fullness (percent of gut length filled with diet indicating feeding activity) and the catch per unit effort (*CPUE*; indicating swimming activity) of *A. melettinus* in one of the irrigation reservoirs of Sri Lanka (data from Schiemer 1996 and Schiemer and Hofer, unpublished observations). Means of *CPUE* (number of specimens caught per hour) were obtained by gill nets (25 m², 10 mm mesh size) exposed at 10-min intervals during several 24-h periods

by one or two reversals which caused the mucous layers to be lifted from the branchial arches. Sibbing et al. (1986) have described a similar "back-wash" behaviour in the suspension feeding carp (*Cyprinus carpio* Linné, 1758) and concluded that back-washing served to re-suspend food particles from the branchial sieve. We never observed accumulations of algal material worth mentioning at the lamellar surface of *A. melettinus* but all specimens investigated during their feeding period were obtained from gill nets and thus were heavily disturbed.

Due to the dominance of planktonic algae in the intestinal contents (Schiemer and Hofer 1983; Rott, personal communication) *A. melettinus* is a true seston filtering species. The fine detritus found in the intestine probably derives from floating material and not directly from the sediment. The filtering apparatus of *Oreochromis* spp. is relatively simple which prevents these species from meeting their basic maintenance requirement by filter-feeding in the open water (Demster et al. 1995). The uptake of algal aggregations near the bottom compensates for the lower efficiency of their branchial structure. The same feeding habit has been observed in several less specialised microphagous Cyprinidae [*Eso-mus danrica* (Hamilton, 1822), *Henicorhynchus siamensis* (Souvage, 1881), *Dangila delineatus* (Souvage, 1878), Hofer, unpublished observations].

Whereas in *H. molitrix* the branchial apparatus of zooplanktivorous species is further developed by narrowing the pores of the filter, *A. melettinus* evolved a completely new system. However, in both cases a barrier in front of the respiratory surface increases water resistance and thus the cost of pumping water through the gills. In contrast to *H. molitrix* with their rigid gill rakers, *A. melettinus* fold up their filter during starving periods which provides an unhindered water flow through the respiratory surface (Figs. 7, 8, 9). *Amblypharyngodon melettinus* divide their diurnal behaviour into two periods differing in activities of locomotion and feeding (Schiemer 1996; Schiemer and Hofer, unpublished results). The animals stay almost stationary (extremely low catch per unit effort) in the midwater of deeper parts of the lakes, their intestines being completely filled with phytoplankton (Fig. 10). In all specimens investigated the lamellar appendices were totally extended. Beginning at dusk *A. melettinus* shifted towards the water surface and the littoral zone of the lake accompanied by a large increase in the catch per unit effort (indicating high swimming activity; Fig. 10) and the folding up of their lamellar appendices. During this time the long coiled intestine evacuated gradually and was completely empty after 2 a.m.

When freshly caught animals were kept in the aquarium under stressed conditions the appendices retained their folded position even during the day. This and the slow movement of the appendices indicate a hormonal driven regulation of the width of the lamellar blood space which turns the appendices on the hinge-like joint at the apical lamellar ends.

The ability of *A. melettinus* to fold up their filter during diurnal periods of high oxygen demand is a unique

strategy among Teleostei probably benefiting the process of respiration.

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