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SURFACE MORPHOLOGY OF *Mytella falcata* GILL FILAMENTS FROM THREE REGIONS OF THE SANTOS ESTUARY

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ABSTRACT

Bivalves have been extensively used to monitor aquatic habitats because their sedentary filter feeding habit results in the accumulation of elements from the environment. The genus *Mytella* is of special interest because its habit of burrowing in sediments can provide information about the substrate conditions in estuaries. Gill filaments have a large surface that is in constant contact with the water and therefore are used to monitor aquatic environments. In this study, we used scanning electron microscopy (SEM) to investigate the morphology of *Mytella falcata* gill filaments and to compare the gill structure in specimens from three sites (A, B and C) of the Santos estuary in southeastern Brazil. Site A presents low levels of pollutants, whereas sites C and B were directly affected by industrial discharges and harbor pollutants. The gill filaments of *M. falcata* have three types of cilia, namely, lateral cilia, eu-latero-frontal cirri and frontal cilia. Structures joining the inner and outer lamellae were found on the abfrontal surface, in addition to the presence of composite and simple cilia. A comparison of the filaments of bivalves from the three sites revealed no morphological alterations, but there was an accumulation of mucus on the frontal surface of the gill filaments of specimens collected at site B. This mucus may function as a protective barrier against pollutants. Bivalves from site C showed no such alteration, even though this site had the highest levels of pollutants. This lack of alterations may reflect the fact that these specimens were collected from concrete pillars where there would be less contact with pollutants. Our findings may reflect a long-term adaptation of these mollusks to chronic exposure to pollutants.

Key words: Bivalvia, gill filaments, Mytilidae, pollution, scanning electron microscopy

INTRODUCTION

Mangroves are peculiar ecosystems, mainly because of the presence of seawater and fresh water and their frequent tidal inundation. The high pH and abundance of sulfites in mangrove sediments creates an environment with a high reducing capacity that results in a greater precipitation of metals. In addition, wave action tends to move water and sediment towards the estuary, thereby preventing the escape of sediments and nutrients from the ecosystem [16] and increasing the local concentration of metals.

Bivalve mollusks are sedentary, filter-feeding invertebrates that have been widely used to monitor aquatic habitats [6]. Their use to study the impact of pollutants is of such importance that some countries have adopted an International Mussel Watch program to monitor pollutants using mussels. Most of the studies using this concept have been done in the northern hemisphere, mainly in Europe and the United States. However, recently, some studies have been initiated in the southern hemisphere using *Perna perna*, a common bivalve with a widespread distribution [11]. In Southeast Asia, *Perna viridis*, another mussel typical of that region, has been used [19]. Mussels have also served to monitor the concentration of pollutants in seawater in Brazil [26,27].

The bivalve genus *Mytella* contains a variety of species that live buried in the sediment and can form large colonies interconnected with other specimens by their byssus. In some cases, these mollusks anchor themselves to rocks or the roots of estuarine plants or even concrete pillars. *Mytella* species are of great interest for biomonitoring aquatic environments because they can provide information about the conditions of the estuarine sediments in which they burrow [18]. *Mytella falcata* is distributed along the Atlantic coast of South America, from Venezuela to Argentina, and also along the Pacific coast and

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around the Galapagos Islands [18]. Considered as an important food source, this species is widely consumed in northeastern Brazil, where it is frequently the only source of protein in some regions [4].

Several techniques have been used to evaluate the levels of pollutants in mytilid mussels, including the morphological analysis of gill filaments [1,8,10,11,24,25]. Such analyses are particularly useful since the gill epithelium is in constant contact with the water and its pollutants. In this study, we used scanning electron microscopy (SEM) to describe the morphology of the gill filaments of *M. falcata* in specimens collected from three localities of the Santos estuary in southeastern Brazil. These sites varied in their degree of disturbance and pollution by human activity.

MATERIAL AND METHODS

The specimens of *M. falcata* were collected at three sites in the Santos estuary, on the coast of São Paulo state, in southeastern Brazil. The three sites (site A - 23º55.052'S, 46º26.975'W, site B - 23º54.659'S, 46º20.464W and site C - 23º52.597'S, 46º22.583'W) (Fig. 1) corresponded to sites 12, 6 and 5, respectively, in the CETESB (Environmental Sanitation Technology Company) report on this area [5].

The concentrations of some pollutants found in the sediment of this ecosystem are shown in Table 1. The higlighted values are above the levels considered as "Probable Effect Level" (PEL) by Environment Canada [5]. Site C had large amounts of polycyclic aromatic hydrocarbons (PAHs) followed by site B. High quantities of organochlorine pesticides were present at site A, but the other parameters indicated that this site was less affected than sites B and C.

Two collections were made at each site, with five bivalves being collected in each case, i.e. 10 bivalves per site. At sites A and B, the bivalves were found buried in the sediment and connected by their byssus, while at site C they were found anchored to concrete pillars, at some distance above the sediment surface.

All of the specimens were transported to the laboratory where they were washed and their shells were opened by sectioning the posterior adductor muscle. Undamaged areas of the gill were excised, fixed in Karnovsky fixative [15] and dehydrated in an acetone series. The gill samples were subsequently critical point dried and glued at stubs at different angles to allow comprehensive analysis of the gill filament using a Philips scanning electron microscope operated at 12 kV.

Figure 1. Map of the area studied showing the collection sites.

Table 1. Data from the CETESB report [5] showing the concentrations of some pollutants found in sediment from the three sites studied in the Santos estuary and the values (shaded in grey) considered to be above the Probable Effect Level (PEL).

Chemical group	Substances analyzed	A	B	C	PEL
Heavy metals (μg.g^{-1})	As	0.65	4.50	3.83	41.6
	Cd	0.98	<0.05	1.38	4.21
	Pb	26.33	15.00	219.66	112
	Cu	20.33	12.00	39.33	108
	Cr	34.33	36.33	56.00	160
	Mn	232.66	208.66	967.66	-
	Hg	0.40	0.70	0.66	0.696
	Ni	19.66	12.33	25.00	42.8
	Zn	60.00	55.66	698.33	271
Polycyclic aromatic hydrocarbons (PAHs) (μg.kg^{-1})	Acenaphthene	0.51	5.40	21983.33	88.9
	Acenaphthylene	0.62	17	25000	128
	Anthracene	0.85	32.63	24133.33	245
	Benzo(a)anthracene	12	54	27666.66	693
	Benzo*(a)*pyrene	3.93	91.66	9500	763
	Chrysene	8.6	58.33	28666.66	846
	Dibenz(a,h)anthracene	<1.0	26	3983.33	135
	Phenanthrene	8.4	83.33	39300	544
	Fluoranthene	8.73	126.66	49000	1494
	Fluorene	1.5	16.03	28666.66	144
	Naphthalene	3.5	19.50	25320	391
	Pyrene	6.73	96.66	37333.33	1398
	Total	37.46	620.73	320553.3	-
Aromatic solvents (μg.kg^{-1})	Benzene	1.46	<1.00	<1.00	-
	Ethylbenzene	1.06	<1.00	4.14	-
	op-Xylene	1.73	<1.00	25.52	-
	m-Xylene	1.30	<1.00	9.73	-
	Toluene	5.86	<1.00	<1.00	-
Polychlorinated biphenyls (PCB) (μg.kg^{-1})		4.43	1.08	30.24	189
Organochlorine pesticides (μg.kg^{-1})	Chloroform	23.20	<1.00	<1.00	-
	Alfa BHC	4.80	<1.00	<1.00	0.99
	Beta BHC	<1.0	<1.00	<1.00	0.99
	Delta BHC	1.4	<1.00	<1.00	0.99
	Gama BHC	2.8	<1.00	<1.00	0.99
Phenolic compounds (μg.kg^{-1})	Phenol	33.33	104	633.33	-
	2-methylphenol	1.2	1.4	59	-
	3-methylphenol	<1.0	5.6	100	-
	4-methylphenol	25.33	10.26	823.33	-
	2-chlorophenol	3	<1.0	<1.00	-
	2,4-dimetilphenol	<1.0	<1.0	336.66	-

RESULTS

Mytella falcata gill filaments consisted of two ctenidia, each formed by two V-shaped demibranchs. Each demibranch had inner and outer lamellae connected by projections on their abfrontal surface (arrow in Fig. 2A). All of the filaments have the same homorhabdic shape. When compared with the frontal surface (Fig. 3C), the abfrontal surface had a larger interfilamentar space (Fig. 2A,B) and had scattered composite (arrowhead in Fig. 2B) and simple (arrow in Fig. 2B) cilia.

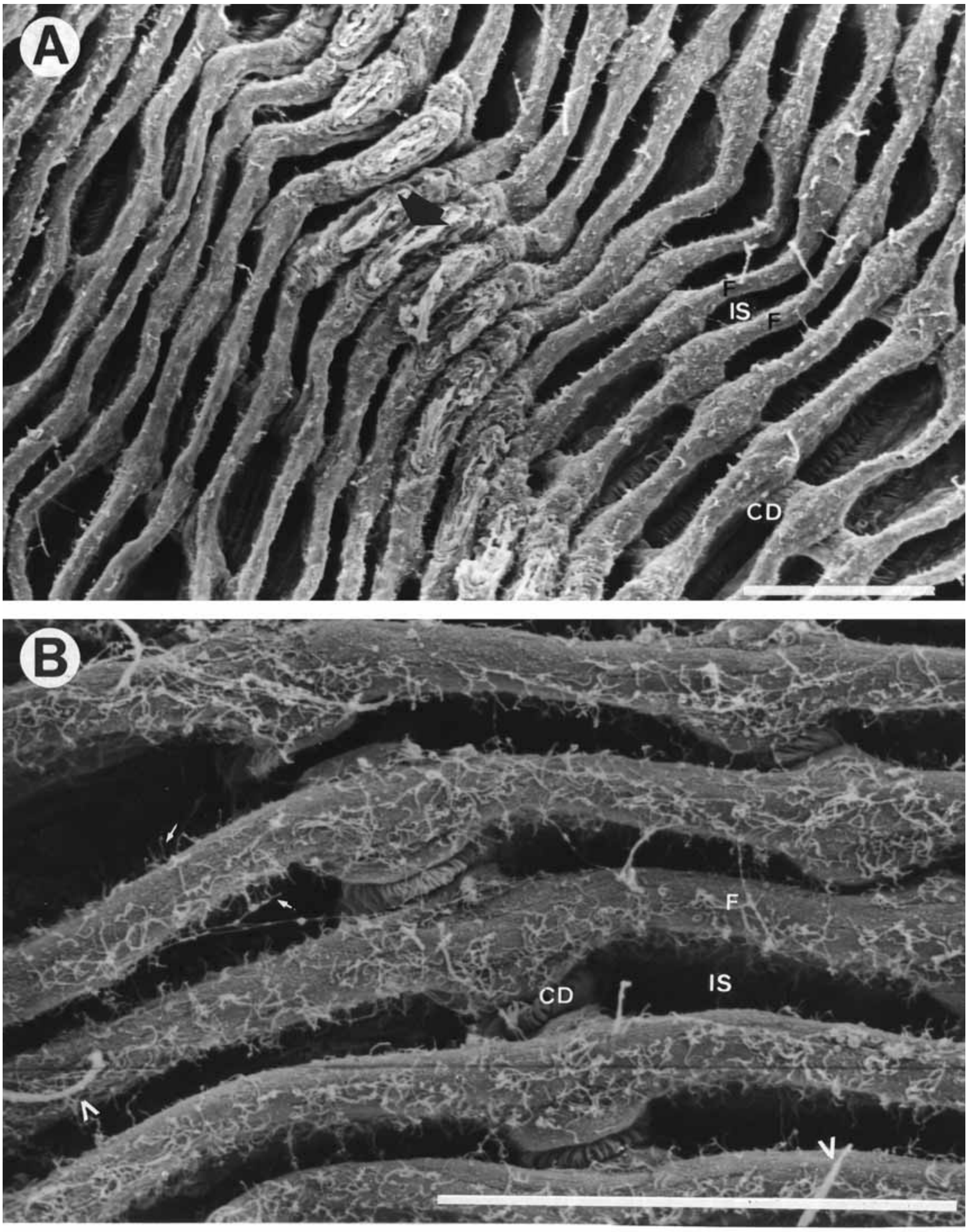

Figure 2. Abfrontal surface of the gill filaments of *M. falcata* from site A. **A.** General view showing the projections that connect the inner and outer lamellae (arrow). B. Detail of the ciliary disks (CD). **F** - filament, **IS** - interfilamentar space, **arrow** - simple cilia, **arrowhead** - composite cilia. Bars: 100 µm.

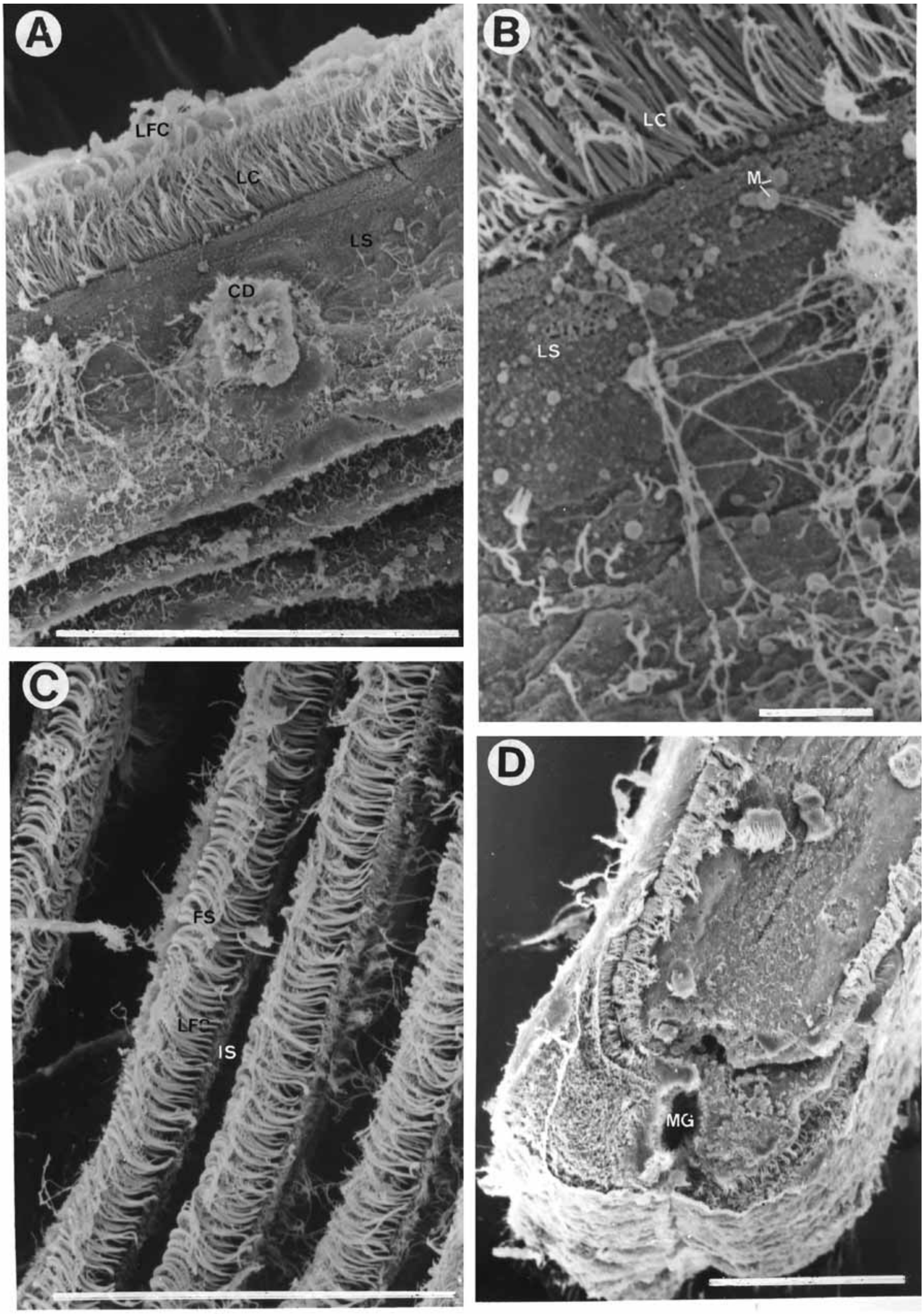

Figure 3. Lateral (**A**,**B**) and frontal (**C**) surface of a gill filament of *M. falcata* from site A. **D.** Detail of the marginal groove (**MG**) located in the ventral region of the filament. **CD** - ciliary disk, **FS -** frontal surface, **IS** - interfilamentar space, **LC -** lateral cilia, **LFC** - eu-latero-frontal cirri, **LS** - lateral surface, **M** - mucus. Bars: A, C and D = 100 µm, B = 10 µm.

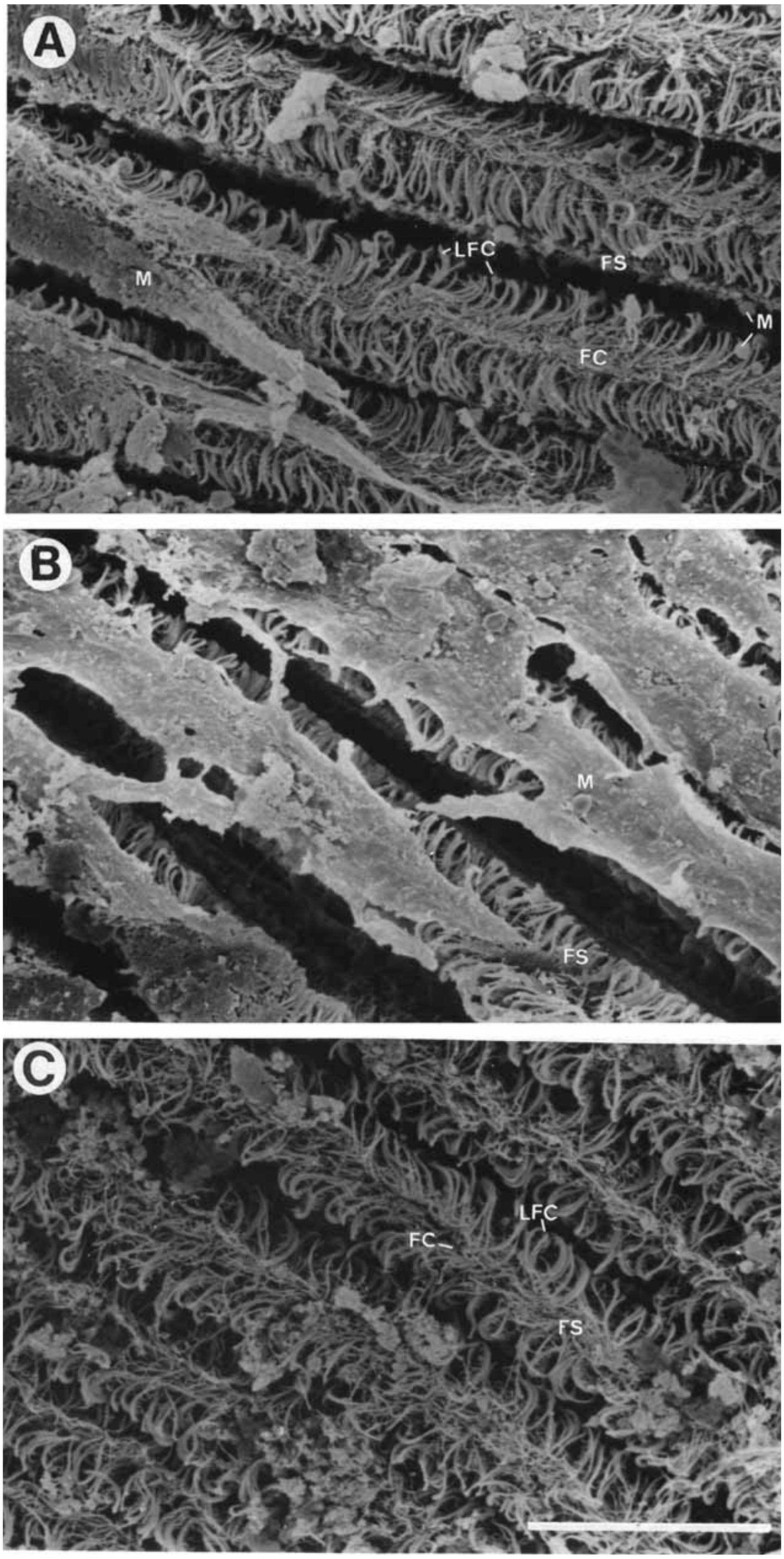

Figure 4. Frontal surface of *M. falcata* gill filaments. **A,B - s**ite B, **C -** site C. **FC -** frontal cilia, **FS** - frontal surface, **LFC -** eu-latero-frontal cirri, **M** - mucus. Bar = 0.1 mm

The lateral surface (Fig. 3A,B) showed no ciliation, but ciliary disks were observed at regular intervals (Figs. 2B and 3A). These ciliary disks were located at the top of small elevations of the lateral epithelium and were responsible for connecting the filaments. Drops of mucus were seen on the abfrontal and lateral surfaces (Fig. 3B). All the particles captured are conducted to a marginal groove (Fig. 3D) in the ventral region of the gill filament and then directed to the oral region.

The frontal surface of the filament was totally covered by cilia classified as frontal cilia, eu-latero-frontal cirri and lateral cilia. The first of these formed a single row and were partially concealed by eu-latero-frontal cirri (Fig. 3C). The eu-latero-frontal cirri were longer than the frontal cilia, ramified at their tip and arranged in two rows immediately below the frontal cilia (Fig. 4A,C). This arrangement of the eu-latero-frontal cirri formed a fine mesh for particle retention. The lateral cilia were numerous and were inserted in the filament below the eu-latero-frontal cirri (Fig. 3A).

There were no morphological differences among bivalves from the three sites. The ciliation showed the same distribution and the ciliary disks had the same appearance. The only difference was that at site B there was an increase in the number of mucus drops that accumulated in the frontal region of the filament (Fig. 4A,B); this phenomenon was not seen in specimens from site C (Fig. 4C).

DISCUSSION

The position and distribution of the cilia seen here in the frontal region of *M. falcata* gill filaments were the same as reported in other Mytilidae species such as *Perna perna* [9] and *Mytilus edulis* [20,22]. The ramifications of the eu-latero-frontal cirri are apparently important for particle retention by filter feeding bivalves, as discussed elsewhere [17,20]. The lateral cilia are the only cilia responsible for pumping water into the bivalve shell. However, based on a study of the role of abfrontal cilia in *M. edulis* and other eulamelibranchs, Jones *et al.* [14] concluded that the abfrontal cilia have an important role in pumping water towards the exhalant siphon immediately after passing through the interfilamentar space.

The abfrontal ciliation of *M. falcata* was scattered and very similar to that described for the abfrontal surface of ordinary filaments in the posterior region of *Modiolus modiolus* gills by Dufour and Beninger [7]. According to these authors, this ciliation is not involved in water pumping. Silvester [23] stated that efficient pumping of water occurs when the cilia occur in high densities, such as in the band of lateral cilia on the gill filaments of *M. edulis*. Dufour and Beninger [7] stated that the abfrontal surface is a vestigial mucociliary epithelium and that the different distributions of cilia and mucocytes seen on the abfrontal surfaces of bivalve gills result from selective forces that vary in nature and degree. Our findings demonstrate a distribution of cilia similar to other Mytilidae [7] and indicate that this arrangement is characteristic of this type of gill.

The role of mucus in food transportation has been extensively studied. Beninger and St-Jean [2] and Beninger *et al.* [3] concluded that mucus has an important role in selecting particles that will be ingested and those that will be rejected. Studies with fish exposed to metals have reported an increase in the production of the mucus that covers the body and gills [12,13,28]. This increased mucus production may serve as a protective barrier to pollutants, as a binding site to capture heavy metals before they can damage the tissue, or as a means of expelling pollutants absorbed by secretory and other cells.

The CETESB (Environmental Sanitation Technology Company) report [5] for this region showed that sites B and C have higher levels of pollutants when compared to site A (Table 1), and this could explain the increased mucus production seen in specimens from site B (with the mucus providing protection against the pollutants). However, specimens from site C, the most polluted site studied here that had high concentrations of PAHs [5], did not show a similar increase in mucus production. This lack of alterations may reflect the fact that these specimens were collected from concrete pillars at a considerable distance above the substrate surface where there would be less contact with pollutants, since they are present in higher levels near the sediment or directly bound to its particles.

In a study of *Placopecten magellanicus*, Potter *et al.* [21] found that an elevated water temperature resulted in the exfoliation of epithelial cells as a consequence of increased cell turnover. In contrast, no exfoliation of epithelial cells caused by pollutants was seen here. Gregory *et al.* [10] studied the morphology of *P. perna* gill filaments exposed to different concentrations of mercury and found some alterations that included loss of abfrontal cilia and increase in the number of cilia on the lateral surface. These authors suggested that this increase in cilia was an attempt to enhance the circulation of water, thereby increasing the rate of filtration and gill oxygenation.

In conclusion, there were no significant morphological alterations in the gill morphology of bivalves from different sites of the Santos estuary. This lack of variation may reflect a long-term adaptation of these mollusks to chronic exposure to pollutants. The effects of acute exposure remain to be determined. The increased mucus production seen in bivalves from site B reflects the presence of pollutants near or directly bound to substrate particles and the absence of such alteration at site C shows that these response is related to the burrowing habit of specimens from site B that are more exposed to pollutants present at the sediment.

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