Marine Biology (2001) 138: 295–309

Suzanne C. Dufour · Peter G. Beninger

A functional interpretation of cilia and mucocyte distributions on the abfrontal surface of bivalve gills

Received: 12 February 2000 / Accepted: 10 September 2000

Abstract The lack of fundamental data on the abfrontal surface of bivalve gills has prompted a comparative study of cilia and mucocytes on this surface, using scanning electron microscopy and histology on eight species of bivalves, representing seven families and the four major gill types: *Mytilus edulis*, *Modiolus modiolus*, *Arca zebra*, *Placopecten magellanicus*, *Crassostrea virginica*, *Spisula solidissima*, *Mercenaria mercenaria*, and *Mya arenaria*. Abfrontal cilia and mucocytes were found in all species studied, with types and densities differing within and between gill types. The three species of homorhabdic filibranchs presented different densities of abfrontal cilia and mucocytes, from very dense in *M. edulis* to sparse in *A. zebra*. The heterorhabdic gills had intermediate cilia and mucocyte densities, with highest concentrations of both abfrontal cilia and mucocytes on the principal filaments. The eulamellibranchs showed low ciliary densities together with high mucocyte densities, especially in *S. solidissima*, where the abfrontal mucocytes were glandular. These results indicate that: (1) the abfrontal surface is a vestigial mucociliary surface; (2) the abfrontal surface cannot participate in water pumping in most species, due to low ciliary densities; and (3) species with high densities of abfrontal mucocytes could utilize abfrontal mucus to reduce drag, especially in the highly fused gills, such as those of the eulamellibranchs. The differing distributions of abfrontal cilia and mucocytes may reflect different selective pressures acting on the gills within the various taxa.

Communicated by S. A. Poulet

S. C. Dufour
Marine Biology Research Division,
Scripps Institution of Oceanography,
University of California, San Diego,
La Jolla, CA 92093-0202, USA

P. G. Beninger (✉)
Laboratoire de Biologie Marine, Faculté des Sciences,
Université de Nantes, 44322 Nantes Cédex France

e-mail: Peter.Beninger@isomer.univ-nantes.fr

Introduction

The gills of suspension-feeding bivalves are complex and structurally diverse organs that capture and process particles in the water column. The elaborate mechanisms through which bivalves of the four main gill types (Fig. 1) capture, transport, and select particles have been studied quite extensively in the past decade (Beninger et al. 1992, 1993, 1997a, b; Ward et al. 1994, 1998; Riisgård et al. 1996; Silverman et al. 1996, 1999). These investigations all dealt with the frontal surface of the gills, which is in direct contact with the particle-laden water drawn into the pallial cavity.

Although the functional features of the frontal gill epithelium are fairly well known, the abfrontal surface, which faces the excurrent flow, has barely been described. This surface is not directly involved in feeding since particle capture occurs on the frontal epithelium. However, mucocytes and cilia, the known effectors of particle processing on the frontal surface of gills (Beninger et al. 1992, 1997b; Ward et al. 1993, Beninger and St-Jean 1997a), are also present on the abfrontal surface, as discussed below.

The data regarding the presence of cilia on the abfrontal surface of bivalve gills are contradictory. In early descriptions of bivalve gills, the presence of abfrontal cilia was noted on all gill types (Lankester 1886; Janssens 1893; Ridewood 1903; Orton 1912; Kellogg 1915; Atkins 1936, 1938). However, several later studies reported that there are no abfrontal cilia on the gills of more evolved protobranchs, of filibranchs, of pseudolamellibranchs, and/or of eulamellibranchs (Yonge 1947, 1959; Cox 1959; Morton 1979; Allen 1985; Jones et al. 1990; Newell and Langdon 1996). Notwithstanding this confusion, the presence or absence of abfrontal cilia has recently been used as a criterion to construct phylogenies (Salvini-Plawen and Steiner 1996; Waller 1998).

Some hypotheses on the function of abfrontal cilia were given in cases where they were found in great abundance. Jones et al. (1990) proposed that, in addi-

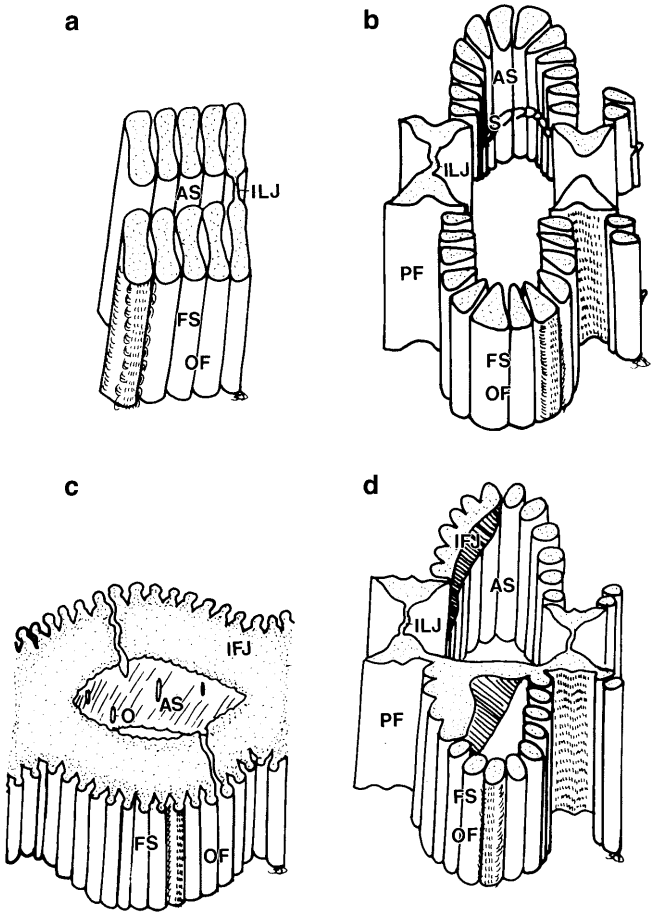


Fig. 1a–d Diagram of the principal gill types in suspension-feeding bivalves. Transversally sectioned demibranchs are illustrated; the *dotted surfaces* are those seen on transverse histological sections. **a** Homorhabdic filibranch. Ordinary filaments (*OF*) connected by interlamellar junctions (*ILJ*). **b** Heterorhabdic filibranch. Principal filaments (*PF*) and ordinary filaments, joined by interlamellar junctions and ciliated spurs (*S*). **c** Homorhabdic eulamellibranch. Ordinary filaments, joined by interfilamentar junctions (*IFJ*). O: ostia. **d** Heterorhabdic pseudolamellibranch. Principal filaments and ordinary filaments, joined by interlamellar junctions and interfilamentar junctions. *AS* Abfrontal surface, *FS* frontal surface

tion to the lateral cilia, the abundant abfrontal cilia of *Mytilus edulis* are involved in water pumping. Similarly, Beninger et al. (1988) suggested that the abfrontal cilia of *Placopecten magellanicus* may create currents on the dorsal respiratory expansions of the gill, although the actual contribution of this structure to respiration has never been investigated (Beninger and Le Pennec 1991). In some cases where the abfrontal cilia were less abundant, as in *Arca tetragona* and *Glycymeris glycymeris*, Atkins (1936) assumed they were sensory in function.

The presence of abfrontal mucocytes has also been noted on bivalve gills; in some cases, the mucopolysaccharide (MPS) secretion type, indicative of the viscosity of mucus (Grenon and Walker 1980), was specified (Owen and McCrae 1976; Eble and Scro 1996; Beninger et al. 1997a). Secretions from the abundant abfrontal mucocytes of *Placopecten magellanicus* might lubricate the gills and protect their delicate structure from hydrodynamic forces during valve clapping, and especially swimming (Le Pennec et al. 1988). Abundant abfrontal mucocytes are also present on the eulamellibranch gill of *Spisula solidissima*; in this species it was suggested that abfrontal mucus lubricates the water channels and canals, facilitating water flow through the gill (Beninger et al. 1997a).

Because our entire knowledge of the abfrontal surface of bivalve gills is limited to a few cursory observations, it is important to establish more rigorously the presence and types of abfrontal mucocytes and cilia on different types of gills, to obtain a more complete picture of gill organization and function. To this end, the present study documents the types and distributions of cilia and mucocytes on the abfrontal gill surfaces of eight species of bivalves. The gills of three homorhabdic filibranchs, *Mytilus edulis*, *Modiolus modiolus*, and *Arca zebra*, and of one heterorhabdic filibranch, *Placopecten magellanicus*, were studied. The gills of one heterorhabdic pseudolamellibranch, *Crassostrea virginica*, and of three homorhabdic eulamellibranchs, *Spisula solidissima*, *Mercenaria mercenaria*, and *Mya arenaria* were also studied. These species were chosen because they represent the four major gill types, cover a broad taxonomic range, and, for some, show taxonomic diversity within a gill type.

The data obtained were used to evaluate previous interpretations of the role of abfrontal cilia and mucocytes in bivalve gills. Specifically, three hypotheses were investigated: (1) the abfrontal cilia as participants in water pumping; (2) mucus secreted by abfrontal mucocytes as a potential gill lubricant; and (3) the abfrontal cilia and mucocytes constituting a mucociliary surface.

Materials and methods

Specimen collection and fixation

Three specimens each of *Mytilus edulis* (shell length: 63.7–65.4 mm), *Crassostrea virginica* (shell length: 80.0–90.2 mm) and *Mercenaria mercenaria* (shell length: 62.5–63.4 mm) were obtained with a rake from Shediac Bay, New Brunswick (46°17′25″N, 64°32′15″W), in September 1996. From the same bay, three specimens of *Mya arenaria* (shell length: 27.0–40.0 mm) were collected from the intertidal zone in November 1996, and two of *Spisula solidissima* (shell lengths: 115.4 and 121.8 mm) were obtained by SCUBA in October 1996. Two *Modiolus modiolus* (shell lengths: 95.4 and 131.2 mm) and four *Placopecten magellanicus* (shell length: 99.5–114.7 mm) were collected by scallop drag in Passamaquoddy Bay, New Brunswick (45°7′10″N, 67°5′10″W) in June 1996 and September 1996. Three specimens of *Arca zebra* (shell length: 54.2–58.4 mm) were obtained from pier pilings at St-George's Bay, Bermuda (32°20′15″N, 64°40′10″W) in July 1996.

Whole gills were removed from each specimen by cutting along their dorsal axes. From each animal, one gill was fixed in aqueous Bouin's solution (Martoja and Martoja 1967) for histochemical processing, and the other was placed in 2.5% glutaraldehyde in a hyperosmotic (2,997 mosmol) sodium cacodylate buffer (Beninger et al. 1995a) for scanning electron microscopy (SEM) processing.

Histology

After 48 h, the gills fixed in Bouin's solution were rinsed in running water for 24 h and the demibranchs were dissected into anterior, median, and posterior portions. The latter were then subdivided into dorsal, medial, and ventral portions, which were dehydrated in an ascending ethanol gradient and embedded in paraffin. Next, 7 μm transverse sections of the demibranchs were affixed to slides with fresh egg albumin. Following deparaffination and rehydration, the sections were stained using the alcian blue-periodic acid-Schiff (AB-PAS) protocol described in Beninger et al. (1993), using diastase controls to eliminate non-MPS polysaccharides. The AB-PAS technique stains mucocytes in a range of colors from blue to pink, depending on the proportions of acidic (AB+) and neutral (PAS+) MPS they contain. Abfrontal mucocytes were classified into three groups, the secretions of which are known to have different functions on frontal surfaces (Beninger and St-Jean 1997a): acid (AMPS), blue; neutral (NMPS), pink; and mixed (MMPS), various shades of purple. Distinctions within the MMPS group were not made; terms such as "acid-dominant" and "neutral-dominant," although useful in dealing with a particular species (Beninger et al. 1993), are not readily comparable across species. Numerical values were assigned to different shades of mucocytes using the Pantone Color Formula Guide (Moonachie, N.J.).

Mucocyte counts

Stained transverse sections were used for mucocyte counts rather than typical in toto preparations of separated gill lamellae (Beninger et al. 1993; Beninger and Dufour 1996), because interlamellar fusion in eulamellibranch gills allowed for only small segments of abfrontal epithelia to be exposed. For each species, counting zones ($N = 50$–90) consisting of 10 consecutive ordinary filaments (OF), as well as 10 additional counting zones in heterorhabdic gills, consisting of one principal filament (PF), were chosen at random from all histological sections. Mucocytes in each zone were counted, and means per zone were calculated for each secretion type ($\overline{N}_a$, $\overline{N}_m$, and $\overline{N}_n$ for AMPS, MMPS, and NMPS, respectively) and for all mucocytes combined ($\sum \overline{N}$). In species with more than one mucocyte secretion type, numerical ratios between types were determined from the calculated $\overline{N}_a$, $\overline{N}_m$, and $\overline{N}_n$.

Due to interspecific and intraspecific differences in mucocyte size, numerical means and ratios give only limited information on the potential quantity or viscosity of mucus secreted on a surface. To report comparable results, regardless of mucocyte size variations, volume-corrected values were calculated for both the ratios between mucocyte types in a species and the total mean number of mucocytes in a counting zone ($\sum \overline{N}_v$).

For the different mucocyte types in each species, the approximate mean volume ($\overline{V}$) was calculated from the mean ($N = 35$) maximal mucocyte diameter ($\overline{D}$), as in Eq. 1 (epithelial mucocytes are approximately spherical).

$$\overline{V} = [4\pi(\overline{D}/2)^3]/3 \quad (1)$$

The $\overline{D}$ for each type of mucocyte were obtained from in toto preparations of gill portions (procedure and staining times as in Beninger et al. 1993). Because this technique stains entire mucocytes instead of thin or semi-thin sections, the diameter at the widest part of a mucocyte could be measured, ensuring accuracy and consistency of size measurements. Of the three eulamellibranchs, it was only possible to obtain in toto preparations from *Mercenaria mercenaria*, because interlamellar fusion precluded the dissection of the gills of *Mya arenaria* and *Spisula solidissima*.

Volume-corrected mucocyte ratios were obtained by multiplying values from the numerical ratios with corresponding $\overline{V}$. For each species, volume-corrected total means ($\sum \overline{N}_v$) were calculated by first obtaining mean total diameters ($\sum \overline{D}$, Eq. 2), then mean total volumes ($\sum \overline{V}$, Eq. 3). Ratios of $\sum \overline{V}$ between species were determined, and the values in this ratio ($R_{\sum \overline{V}}$) were multiplied by the $\sum \overline{N}$ for the corresponding species, yielding $\sum \overline{N}_v$ (Eq. 4).

$$\sum \overline{D} = \frac{\overline{N}_a\overline{D}_a + \overline{N}_m\overline{D}_m + \overline{N}_n\overline{D}_n}{\sum \overline{N}}, \quad \text{where } \overline{N}_a, \overline{N}_m \text{ and } \overline{N}_n \text{ are mean maximal numbers and } \overline{D}_a, \overline{D}_m \text{ and } \overline{D}_n \text{ mean maximal diameters for AMPS, MMPS and NMPS, respectively.} \quad (2)$$

$$\sum \overline{V} = \left[4\pi\left(\sum \overline{D}/2\right)^3\right] \Big/ 3 \quad (3)$$

$$\sum \overline{N}_V = \sum \overline{N} \cdot R_{\Sigma\overline{V}} \quad (4)$$

Scanning electron microscopy

After at least 48 h in the fixative, the gill samples were rinsed in sodium cacodylate buffer and demibranchs were divided into anterior, median, and posterior segments. The abfrontal surfaces of filibranch and pseudolamellibranch gill segments were exposed by cutting interlamellar junctions with microsurgical instruments and separating the lamellae; extensive interlamellar fusion precluded a similar dissection for eulamellibranch gills. All samples were dehydrated in an ascending ethanol gradient to spectroscopy-grade ethanol and critical-point dried with "bone-dry" liquid CO_2. At this point, dried pieces of eulamellibranch gill lamellae were removed with forceps, exposing the abfrontal surface. The prepared samples were mounted on stubs, sputter-coated with gold-palladium alloy, and observed with a JEOL JSM 5200 scanning electron microscope. The densities of cilia on abfrontal surfaces were characterized qualitatively from micrographs, and their lengths were approximated using the calibration bar and pieces of string to correct for curve. The cilia were grouped into two major types: simple cilia (including "long" and "short" simple cilia) and composite cilia. The latter are grouped cilia of the same length, originating from a single cell, that, unlike compound cilia, present no organic fusion (Beninger et al. 1999).

Results

Homorhabdic filibranchs

The three species studied had different quantities and types of abfrontal mucocytes (Tables 1, 2). *Mytilus edulis* had the highest number of abfrontal mucocytes. These were predominantly found on the posterior side of the filaments (Fig. 2a) and appeared to be more abundant than the frontal mucocytes. Three types of mucocytes were found in this species, containing NMPS, AMPS, or MMPS. *Modiolus modiolus*, in contrast, had fewer abfrontal mucocytes. In this species, mucocytes were situated in a latero-abfrontal position (Fig. 2b). *Arca zebra* had the fewest abfrontal mucocytes, which contained entirely AMPS (Fig. 2c).

SEM observations revealed that the abfrontal surfaces of the gills of the three homorhabdic filibranchs were structurally similar. Ciliary junctions between filaments, as well as sectioned interlamellar junctions, were visible (Figs. 3, 4). Abfrontal cilia were found in different types and densities in the three species studied (Table 3). *Mytilus edulis* had the highest density of simple abfrontal cilia, which were evenly distributed throughout the gill, on the posterior side of the filaments only (Fig. 4a–c). Some longer composite cilia were either interspersed between simple cilia or present on the anterior side (Fig. 4b, c). *Modiolus modiolus* also had both

Table 1 Types and Pantone correspondence of mucocytes on the frontal and abfrontal surfaces of the bivalve gills studied

Species	Frontal		Abfrontal	
	Secretion type	Pantone value	Secretion type	Pantone value
Mytilus edulis	Acid[a]	2995c[b]	Acid	2995c
	Acid dominant[c]	267c, 266c, 2685c	Mixed	273c
	Neutral dominant[c]	227c, 233c, 234c	Neutral	239c
Modiolus modiolus	Mixed[a]	265c[b]	Mixed	265c
	Neutral[a]	239c[b]	Neutral	239c
Arca zebra	Acid[a]	801c[b]	Acid	801c
	Mixed[a]	2727c[b]	–	–
Placopecten magellanicus	Acid[c]	2995c	Acid	639c
	Mixed[c]	275c	Mixed	2727c
Crassostrea virginica	Acid[d]	639c[e]	Acid	639c
	Neutral[d]	239c[e]	–	–
Spisula solidissima	Acid[a,f]	312c[b]	Acid	312c
Mya arenaria	Acid[a,f]	801c[b]	Acid	801c
Mercenaria mercenaria	Acid[a,f]	313c[b]	Acid	313c

[a] Present study
[b] Pantone values were obtained from the slides used to observe abfrontal mucocytes
[c] Beninger et al. (1993)
[d] Beninger and Dufour (1996)
[e] Pantone values of mucocytes stained with AB-PAS (present study) rather than AB-PAS-fast green (Beninger and Dufour 1996)
[f] Beninger et al. (1997a)

Table 2 Total mean number of abfrontal mucocytes per counting zone and ratios between types of mucocytes for each species studied. *A* AMPS-secreting mucocytes, *M* MMPS-secreting mucocytes, *N* NMPS-secreting mucocytes; $\sum \overline{N}$: volume-corrected total mean per counting zone, *n.a.* not available, *OF* ordinary filaments, *PF* principal filaments, *VC* volume-corrected

Species	$\sum \overline{N}$ (range)	$\sum \overline{N_v}$	Numerical ratio	VC ratio
Mytilus edulis	24.1 (13–24)	24.1	13N:10A:2M	9A:3N:2M
Modiolus modiolus	10.0 (0–32)	10.3	3N:2M	11N:2M
Arca zebra	4.0 (0–10)	4.6	–	–
Placopecten magellanicus				
OF	5.9 (1–13)	6.9	3A:2M	2A:1M
PF	9.3 (4–19)	10.9	1A:1M	4A:3M
Crassostrea virginica				
OF	6.5 (1–14)	22.2	–	–
PF	3.6 (1–8)	12.3	–	–
Spisula solidissima	Glandular	Glandular	–	–
Mya arenaria	4.8 (0–10)	n.a.	–	–
Mercenaria mercenaria	4.5 (0–17)	18.2	–	–

simple and composite cilia, but in this species, their densities and positions were more variable (Fig. 4d–f): there appeared to be a higher density of simple abfrontal cilia in the dorsal regions of the gills observed. In areas with a higher ciliary density, the cilia were usually in a latero-abfrontal position (Fig. 4d), whereas in areas with a lower density, the cilia tended to be directly abfrontal (Fig. 4f). The position of the abfrontal composite cilia in this species was variable. The gills of *Arca zebra* had simple abfrontal cilia which were found in sparse tufts on all gill regions observed (Fig. 4g, h). Some shorter cilia were also present on the abfrontal surface (Fig. 4g).

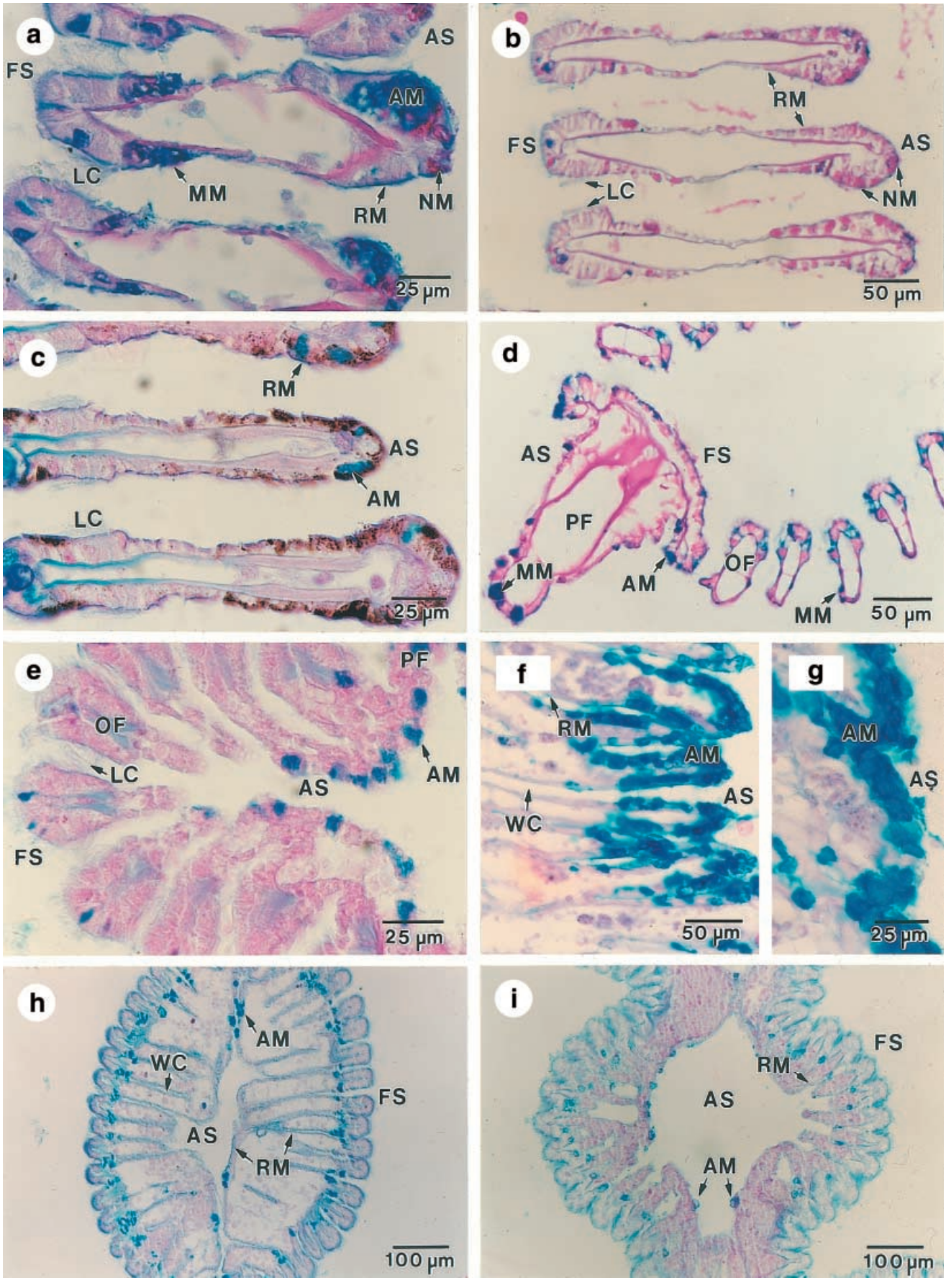
Heterorhabdic filibranch and pseudolamellibranch

The two species in this group had different types of abfrontal mucocytes, which in both cases were more numerous on the larger principal filaments than on the smaller ordinary filaments (Tables 1, 2).

The gills of the heterorhabdic filibranch *Placopecten magellanicus* had both AMPS- and MMPS-secreting mucocytes on their abfrontal and frontal surfaces (Table 1; Fig. 2d). The mucocytes were represented on all abfrontal epithelial surfaces of the principal filaments, from the lateral faces to the afferent vessel, as well as on the dorsal respiratory expansions.

The heterorhabdic pseudolamellibranch gill of *Crassostrea virginica* contained only AMPS-secreting mucocytes on its abfrontal surface; the frontal surface had in addition NMPS-secreting mucocytes (Table 1; Fig. 2e).

Abfrontal surface characteristics are illustrated in Fig. 5 (*Placopecten magellanicus*) and Fig. 6 (*Crassostrea virginica*). Cilia were present on the abfrontal surfaces of ordinary and principal filaments in both species. In *P. magellanicus*, simple abfrontal cilia in the region of the principal filaments ventral to the respiratory expansions were found mainly on the afferent vessel (Fig. 7a). These simple cilia were in high densities in


a
AS
FS
AM
LC
MM
RM
NM
25 µm
b
RM
FS
AS
LC
NM
50 µm
c
RM
AS
AM
LC
25 µm
d
AS
FS
PF
MM
AM
OF
MM
50 µm
e
PF
OF
LC
AS
AM
FS
25 µm
f
RM
AM
AS
WC
50 µm
g
AM
AS
25 µm
h
AM
WC
FS
AS
RM
100 µm
i
FS
RM
AS
AM
100 µm

◄

Fig. 2a–i Transverse sections of bivalve gills, stained with AB-PAS. **a** *Mytilus edulis*. Ordinary filaments, with AMPS-secreting mucocytes (*AM*) and NMPS-secreting mucocytes (*NM*) on the abfrontal surface (*AS*). MMPS-secreting mucocytes (*MM*) are visible under the lateral cilia (*LC*). *FS* Frontal surface, *RM* residual mucus. **b** *Modiolus modiolus*. Ordinary filaments, with NMPS-secreting mucocytes on the abfrontal surface. **c** *Arca zebra*. Ordinary filaments, with AMPS-secreting mucocytes on the abfrontal surface. **d** *Placopecten magellanicus*. Ordinary filaments (OF) and principal filament (*PF*), with AMPS- and MMPS-secreting mucocytes on the abfrontal surface. *Crassostrea virginica*. Ordinary filaments and principal filaments, with AMPS-secreting mucocytes on the abfrontal surface. **f** *Spisula solidissima*. Abfrontal surface, on a section at the ostial level. AMPS-secreting mucocytes and residual mucus in the water canals (*WC*) are visible. **g** *Spisula solidissima*. AMPS-secreting mucocytes on the abfrontal surface, at an inter-ostial tissue level. **h** *Mya arenaria*. Ordinary filaments, with AMPS-secreting mucocytes on the abfrontal surface. Residual mucus can be seen in the water canals. **i** *Mercenaria mercenaria*. Ordinary filaments, with AMPS-secreting mucocytes on the abfrontal surface

anterior regions of the gill (Fig. 7b), whereas in posterior regions they were in tufts (Fig. 7a). On the dorsal respiratory expansions, simple cilia densely covered the afferent vessel, and sparse patches of longer simple cilia were found on the interconnecting vessels (Fig. 7c, d).

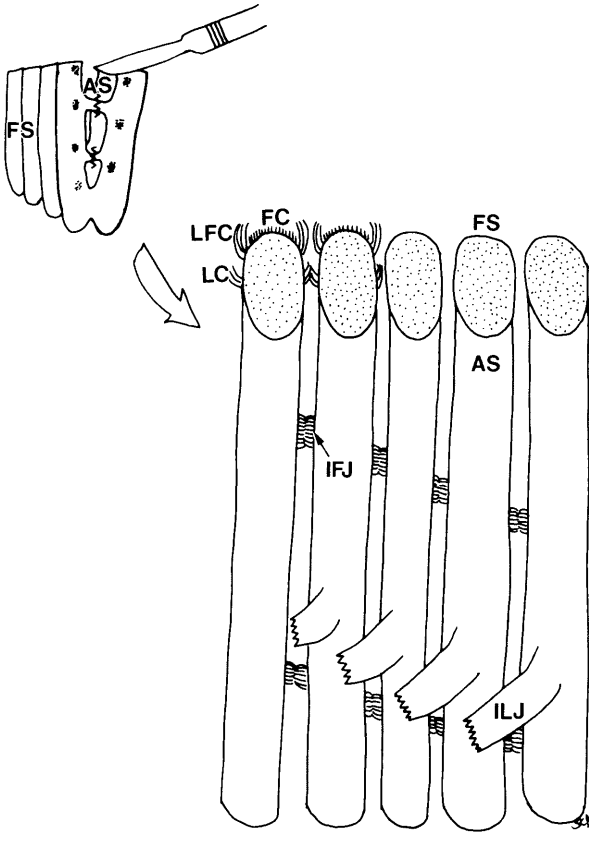


Fig. 3 Drawing of the abfrontal surface of a homorhabdic filibranch gill, as seen with the SEM. The *upper drawing* illustrates the separation of demibranchs to expose the abfrontal surface. The *dotted surface* represents the plane of a transverse section. *AS* Abfrontal surface, *FC* frontal cilia, *FS* frontal surface, *IFJ* interfilamentar junction, *ILJ* interlamellar junction, *LC* lateral cilia, *LFC* latero-frontal cirri

On ordinary filaments, irregular patches of simple cilia were observed (Fig. 7e). There was no apparent pattern in their density throughout the gill. In *C. virginica*, the high degree of abfrontal fusion between principal filaments permits only partial observation of the ordinary filaments with the SEM (Fig. 7f). On visible portions, simple cilia were occasionally seen, as well as rows of short cilia in a latero-abfrontal position (Fig. 7g). The principal filaments showed higher densities of simple abfrontal cilia (Table 3; Fig. 7h), especially in the dorsal-most regions.

Homorhabdic eulamellibranchs

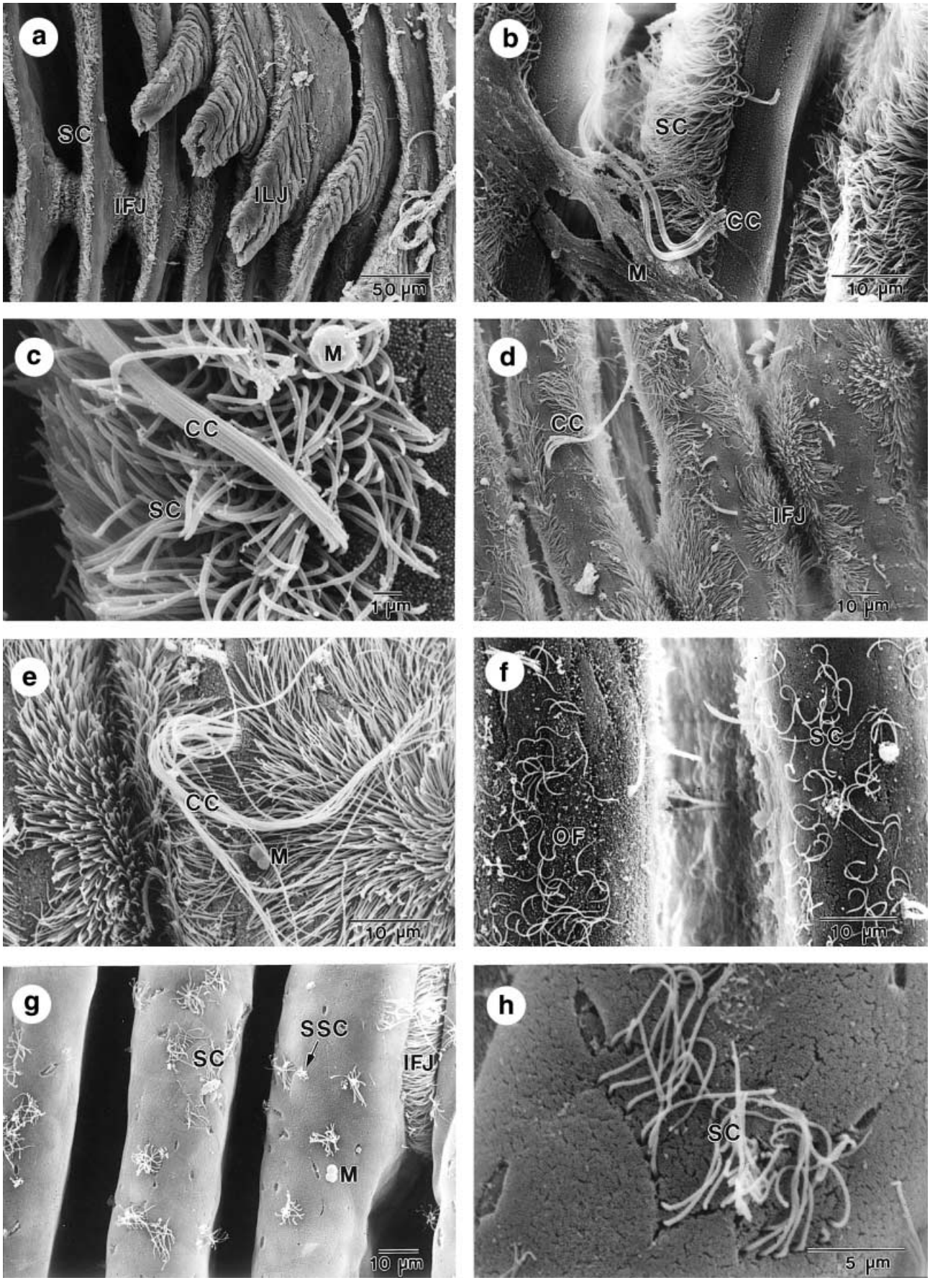
This group had only AMPS-secreting abfrontal mucocytes (Table 1; Fig. 2f–i) and exhibited the most extreme heterogeneity in abfrontal mucocyte density of the gill types studied (Table 2). The abfrontal mucocytes of *Spisula solidissima* were very abundant (Fig. 2f, g); because of the glandular aspect of the mucocytes in this species, counts could not be made. *Mya arenaria* and *Mercenaria mercenaria* had fewer abfrontal mucocytes (Table 2). A volume-corrected mean could not be calculated for *M. arenaria* because the extent of interlamellar fusion was too great in this species for in toto preparations to be made, even for small gill portions.

The three homorhabdic eulamellibranchs studied had a similar abfrontal appearance (Fig. 8), with low densities of abfrontal cilia (Table 3). Tufts of simple cilia were found in relatively similar patterns throughout the gills of *Spisula solidissima* and *M. arenaria* (Fig. 9a–f); the abfrontal cilia *of M. arenaria* were slightly longer than those of *S. solidissima*. After extensive observation of abfrontal surfaces of the gills of three individuals of *Mercenaria mercenaria*, only one tuft of simple cilia was found (Fig. 9g, h).

Discussion

Abfrontal cilia were found, in varying amounts and in different types, on all gills studied. This is consistent with the results of Janssens (1893), Orton (1912), Gray (1931), and Jones et al. (1990) for *Mytilus edulis*, Beninger et al. (1988) and Le Pennec et al. (1988) for *Placopecten magellanicus*, and Nelson (1960) for *Crassortrea virginica*. In *M. edulis*, the grouped abfrontal cilia were previously reported as being compound cilia (Gray 1931; Sleigh and Holwill 1969; Baba and Hiramoto 1970; Baba 1972; Jones et al. 1990). Although they resemble composite cilia through the SEM (Beninger et al. 1999), transmission electron microscopy will be needed to ascertain whether or not these cilia present organic fusion (compound cilia) or adhesion (composite cilia).

The presence of abfrontal mucocytes has already been noted on the gills of some of the species here examined: *Modiolus modiolus* (Atkins 1938), *Placopecten*


a
SC
IFJ
ILJ
50 µm
b
SC
CC
M
10 µm
c
M
CC
SC
1 µm
d
CC
IFJ
10 µm
e
CC
M
10 µm
f
SC
OF
10 µm
g
SSC
SC
IFJ
M
10 µm
h
SC
5 µm

◄

Fig. 4a–h Scanning electron micrographs of the abfrontal surface of homorhabdic filibranch gills studied. **a** *Mytilus edulis*. Simple cilia (*SC*) on the abfrontal surface of ordinary filaments. *IFJ* Interfilamentar junction, *ILJ* sectioned interlamellar junction. **b** *Mytilus edulis*. Simple cilia and composite cilia (*CC*) on the abfrontal surface. *M* Mucus. **c** *Mytilus edulis*. Simple cilia and composite cilia on the abfrontal surface. **d** *Modiolus modiolus*. Ciliated abfrontal surface of a few ordinary filaments. **e** *Modiolus modiolus*. Composite cilia on the abfrontal surface. **f** *Modiolus modiolus*. Abfrontal surface of ordinary filaments (*OF*) in the posterior region of the gill. **g** *Arca zebra*. Abfrontal surface of a few ordinary filaments. *SSC* Short simple cilia. **h** *A. zebra*. Abfrontal surface

magellanicus (Le Pennec et al. 1988), *Crassostrea virginica* (Eble and Scro 1996), *Mya arenaria* (Beninger et al. 1997a) and *Spisula solidissima* (Beninger et al. 1997a). In one case, a different mucocyte secretion type was reported: Eble and Scro (1996), also using AB-PAS, found NMPS-secreting mucocytes on the abfrontal surface of *C. virginica*, whereas in the present study, only AMPS-secreting mucocytes were found.

As stated earlier, the hypotheses concerning the functional aspects of the abfrontal surface here explored are (1) the abfrontal cilia create currents improving

Table 3 Types, approximate lengths (μm), and relative density of abfrontal cilia on the bivalve gills studied. *OF* Ordinary filaments, *PF* principal filaments. Density scale: −: absent; +: scattered tufts; + +: 25–50% abfrontal surface covered; + + +: more than 50% abfrontal surface covered

Species	Filament type	Cilia type	Length (μm)	Relative density
Mytilus edulis	OF	Simple	5	+ + +
		Composite	40	+ +
Modiolus modiolus	OF	Simple	7	+ +
		Composite	60	+ +
Arca zebra	OF	Simple	7–10	+
		Short simple	2	+
Placopecten magellanicus	OF	Simple	5	+
		Short simple	2	+
	PF	Simple	5–15	+ + +
		Long simple	15–20	+ +
Crassostrea virginica	OF	Short simple	1–3	+
	PF	Simple	5	+
Spisula solidissima	OF	Simple	5–8	+
Mya arenaria	OF	Simple	8–10	+
Mercenaria mercenaria	OF	–	–	–

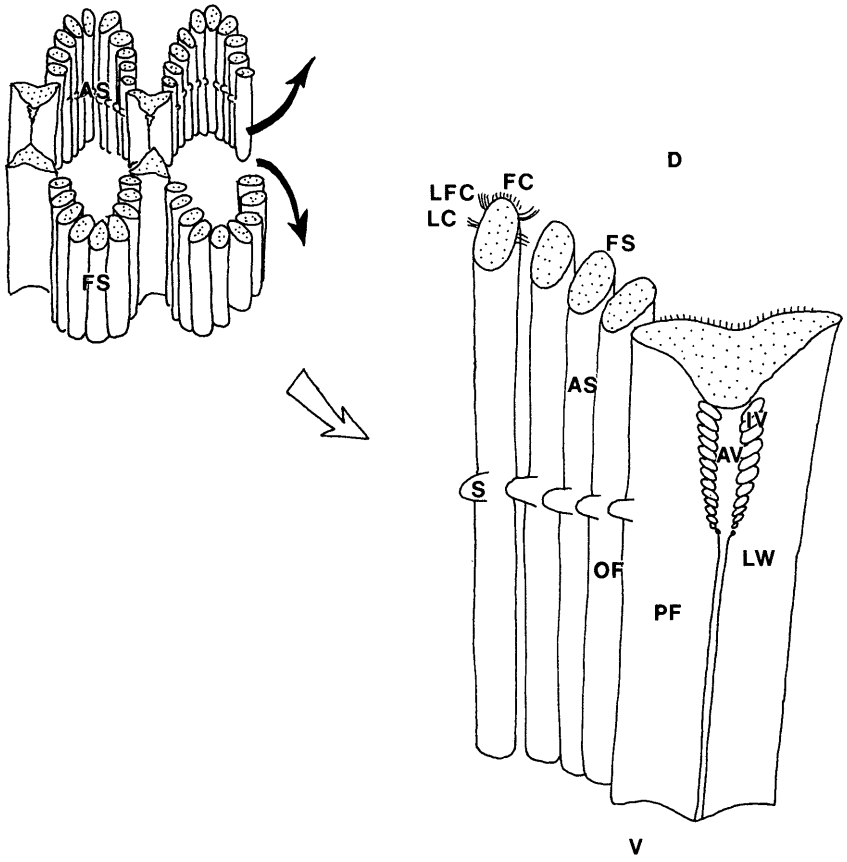


Fig. 5 Drawing of the abfrontal surface of a heterorhabdic filibranch gill, as seen with the SEM. The *upper drawing* illustrates the separation of demibranchs to expose the abfrontal surface; interlamellar junctions are only found in the ventral-most regions. The *dotted surface* represents the plane of a transverse section. Dorsal respiratory expansions (*AV* afferent vessel, *IV* interconnecting vessels) are illustrated on the dorsal portion (*D*) of the principal filament (*PF*). *AS* Abfrontal surface, *FC* frontal cilia, *FS* frontal surface, *LC* lateral cilia, *LFC* latero-frontal cilia, *LW* lateral wall, *OF* ordinary filament, *S* ciliated spur, *V* ventral

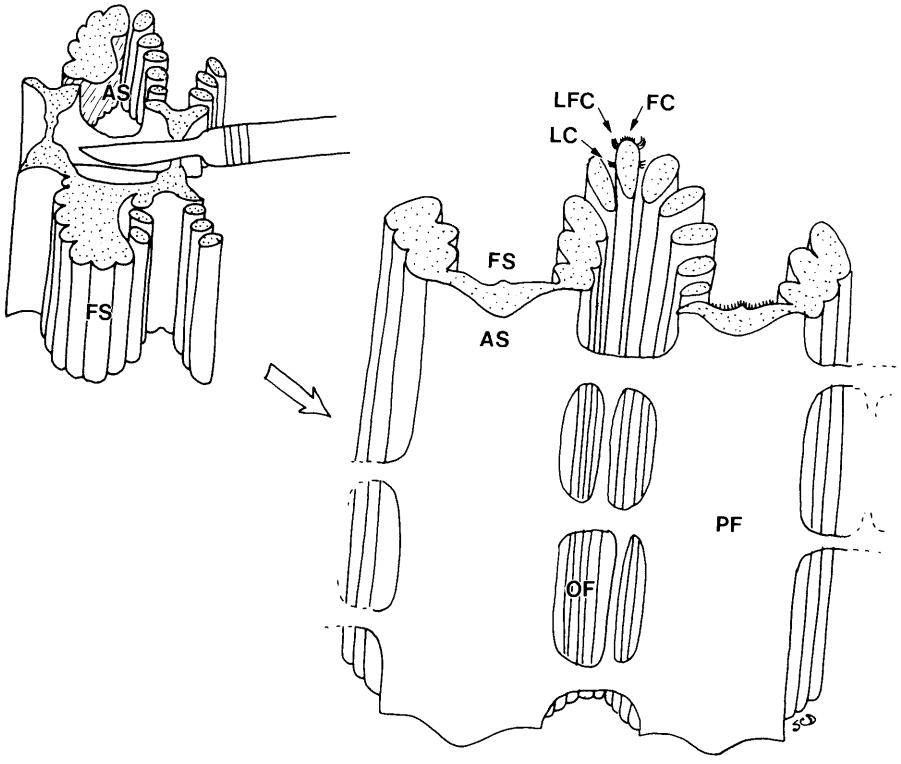


Fig. 6 Drawing of the abfrontal surface of a heterorhabdic pseudolamellibranch gill, as seen with the SEM. The *upper drawing* illustrates the separation of demibranchs to expose the abfrontal surface. The *dotted surface* represents the plane of a transverse section. Ordinary filaments (*OF*) are visible between the tissular expansions of the principal filaments (*PF*). *AS* Abfrontal surface, *FC* frontal cilia, *FS* frontal surface, *LC* lateral cilia, *LFC* latero-frontal cilia

water flow through the gills; (2) mucus secreted by abfrontal mucocytes lubricates the abfrontal surface; and (3) the abfrontal cilia and mucocytes together form a mucociliary surface previously or currently adapted for particle transport.

Hypothesis 1 – abfrontal cilia help pump water through gills

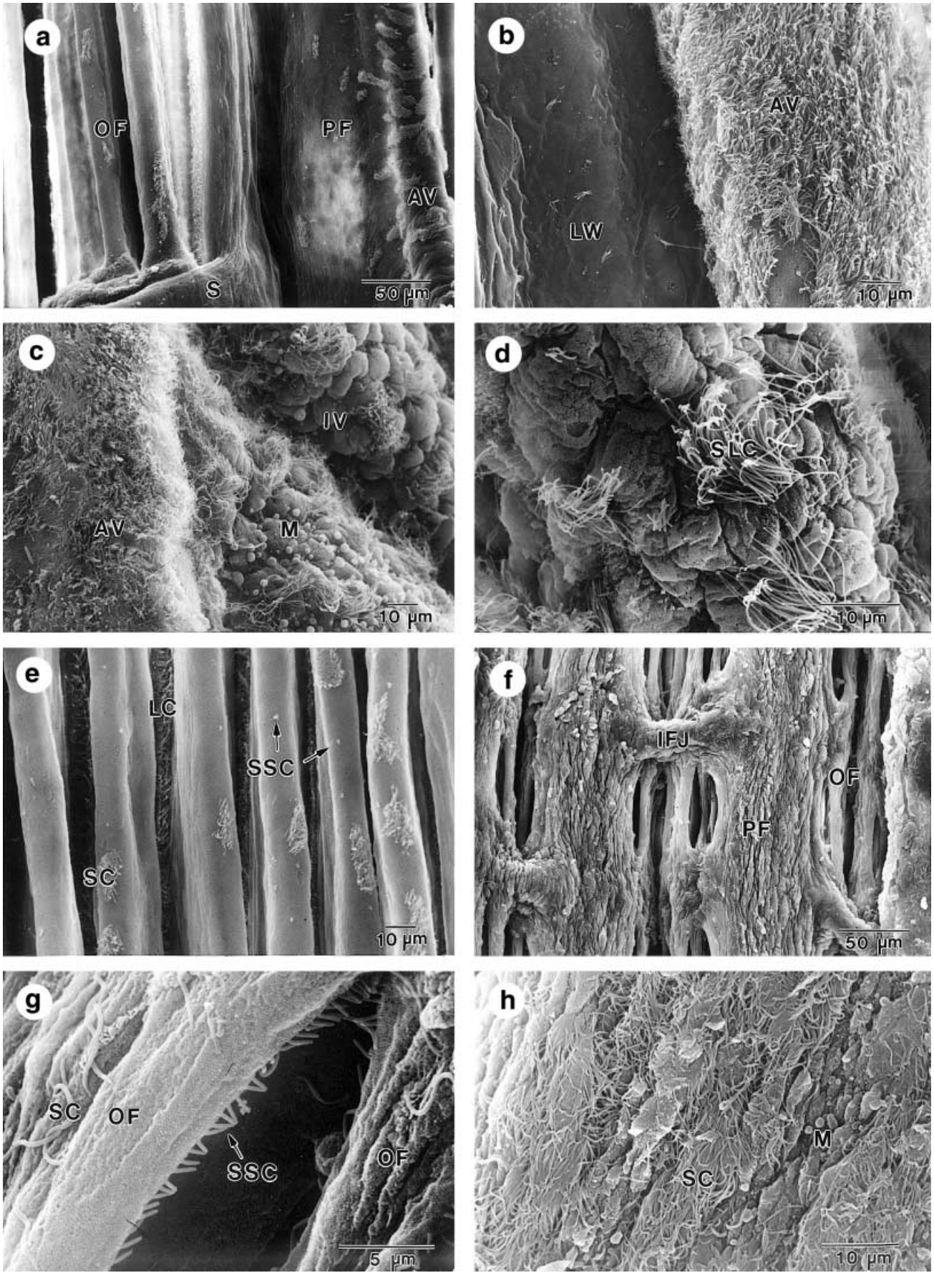
This hypothesis can be evaluated by comparing the abfrontal cilia of the gills studied with cilia known to be involved in water pumping, that is, lateral cilia. Only the simple abfrontal cilia will be used in this comparison because the composite abfrontal cilia of *Mytilus edulis* and *Modiolus modiolus* are too sparse to function in water pumping. The short simple cilia (1–3 μm) are also unlikely to propel water; although sensory functions have previously been ascribed to short cilia (Atkins 1936; Galtsoff 1964; Dwivedy 1973; Owen and McCrae 1976), no reliable electrophysiological studies exist to support this, and their function is yet unknown.

Water-pumping cilia in metazoans are typically 10–20 μm in length (Sleigh 1982): the water-pumping lateral cilia on the gills of *Mytilus edulis* measure 15 μm (Aiello and Sleigh 1972; Silvester 1988). Efficient pumping of water occurs when cilia are in high densities, such as in the bands of lateral cilia on the gill filaments of *M. edulis* (Silvester 1988).

The simple abfrontal cilia in the species studied are not typical of water-pumping cilia: they are shorter (most are 5–15 μm) and occur mostly in densities too low to be effective in water flow. More importantly, the abfrontal cilia are always associated with mucocytes. This precludes a predominant role in water pumping because cilia cannot primarily propel water if they are at least partly covered with a layer of mucus; lateral cilia, for instance, are never found in association with underlying mucocytes. However, it was suggested that the dense abfrontal cilia of *Mytilus edulis* (which are accompanied by numerous abfrontal mucocytes) assist in water pumping (Jones et al. 1990; Jones and Richards 1993). This hypothesis has not yet been tested by in vivo observation of abfrontal cilia or by noninvasive flow-measuring techniques; thus the role of abfrontal cilia in *M. edulis* is still unclear. The abfrontal cilia of the other species studied, being much less abundant than those of *M. edulis*, are probably not involved in water pumping.

Hypothesis 2 – mucus is used as a lubricant

Mucus in molluscs serves many purposes, one of which is lubrication (Prezant 1985; Simkiss 1988; Davies and Hawkins 1998). In the context of bivalve gills, the secretion of mucus on the abfrontal surface might be adaptive if it reduces friction between water and the epithelium, in a manner similar to the drag reduction produced by fish epithelial mucus (Hoyt 1975; Daniel 1981). Abfrontal surface lubrication could be of particular importance in eulamellibranchs because water pumped through their gills is directed into abfrontal chambers of reduced volume, where frictional drag be-


a
OF
PF
AV
S
50 µm
b
AV
LW
10 µm
c
IV
AV
M
10 µm
d
SLC
10 µm
e
LC
SSC
SC
10 µm
f
IFJ
OF
PF
50 µm
g
SC
OF
SSC
OF
5 µm
h
M
SC
10 µm

◄

Fig. 7a–h Scanning electron micrographs of the abfrontal surface of the heterorhabdic filibranch and pseudolamellibranch gills studied. **a** *Placopecten magellanicus*. Abfrontal surfaces of a principal filament (*PF*) and a few ordinary filaments (*OF*), situated ventrally to the dorsal respiratory expansions. Ciliary tufts are visible on the afferent vessel (*AV*). *S* Ciliated spur. **b** *P. magellanicus*. Principal filament, situated ventrally to the dorsal respiratory expansions. Simple cilia are present on the afferent vessel and on the lateral wall (*LW*). **c** *P. magellanicus*. Dorsal respiratory expansion, with simple cilia on the afferent vessel and the interconnecting vessels (*IV*). *M* Mucus. **d** *P. magellanicus*. Tufts of simple long cilia (*SLC*) on an interconnecting vessel. **e** *P. magellanicus*. Simple cilia (*SC*) and short simple cilia (*SSC*) on ordinary filaments. *LC* Lateral cilia. **f** *Crassostrea virginica*. View of the abfrontal surface, with principal filaments connected by interfilamentar junctions (*IFJ*), and a few ordinary filaments in the background. **g** *C. virginica*. Short simple cilia on ordinary filaments. **h** *C. virginica*. Abfrontal surface of a principal filament, with simple cilia and mucus

needed for the same reason in other gill types having much larger abfrontal water chambers (and therefore less frictional drag). The filibranch *Mytilus edulis* is an example of a bivalve having such a gill type, but also having a high density of abfrontal mucocytes. Another explanation for the presence of abundant mucocytes is needed for such bivalves.

It was suggested that the abfrontal mucus of *Placopecten magellanicus* facilitates the dorso-ventral and anterio-posterior contractions of the gill during valve clapping, and notably during the escape swimming response, protecting filaments from hydrodynamic and mechanical forces (Le Pennec et al. 1988). This interpretation is plausible, since in *P. magellanicus* the abfrontal mucocytes were abundant on the principal filaments, which are the only ones in contact with the

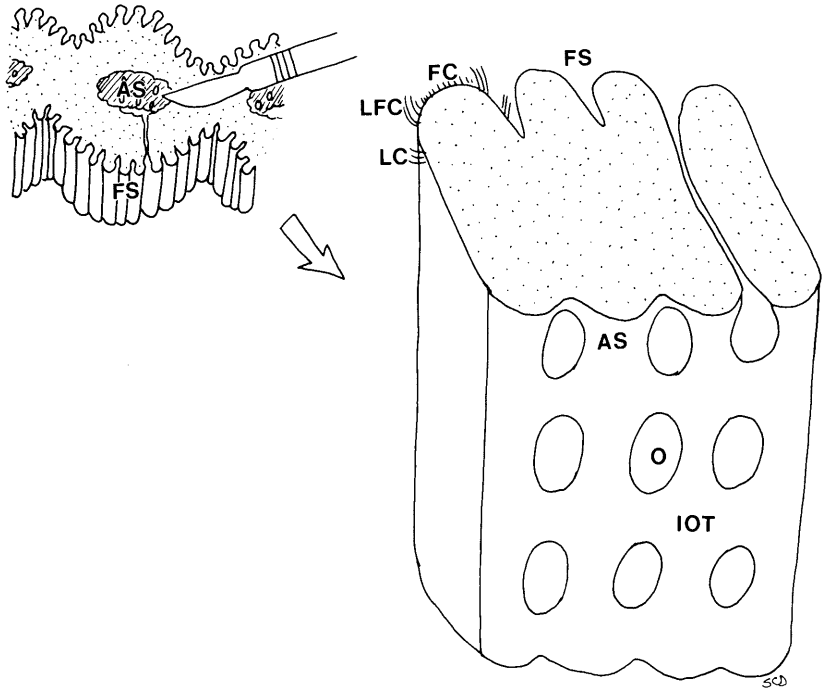


Fig. 8 Drawing of the abfrontal surface of a homorhabdic eulamellibranch gill, as seen with the SEM. The *upper drawing* illustrates the separation of demibranchs to expose the abfrontal surface. The *dotted surface* represents the plane of a transverse section. Ostia (*O*), surrounded by inter-ostial tissue (*IOT*), are visible on the abfrontal surface (*AS*). *FC* Frontal cilia, *FS* frontal surface, *LC* lateral cilia, *LFC* latero-frontal cilia

tween water and the epithelium is probably important (Beninger et al. 1997a).

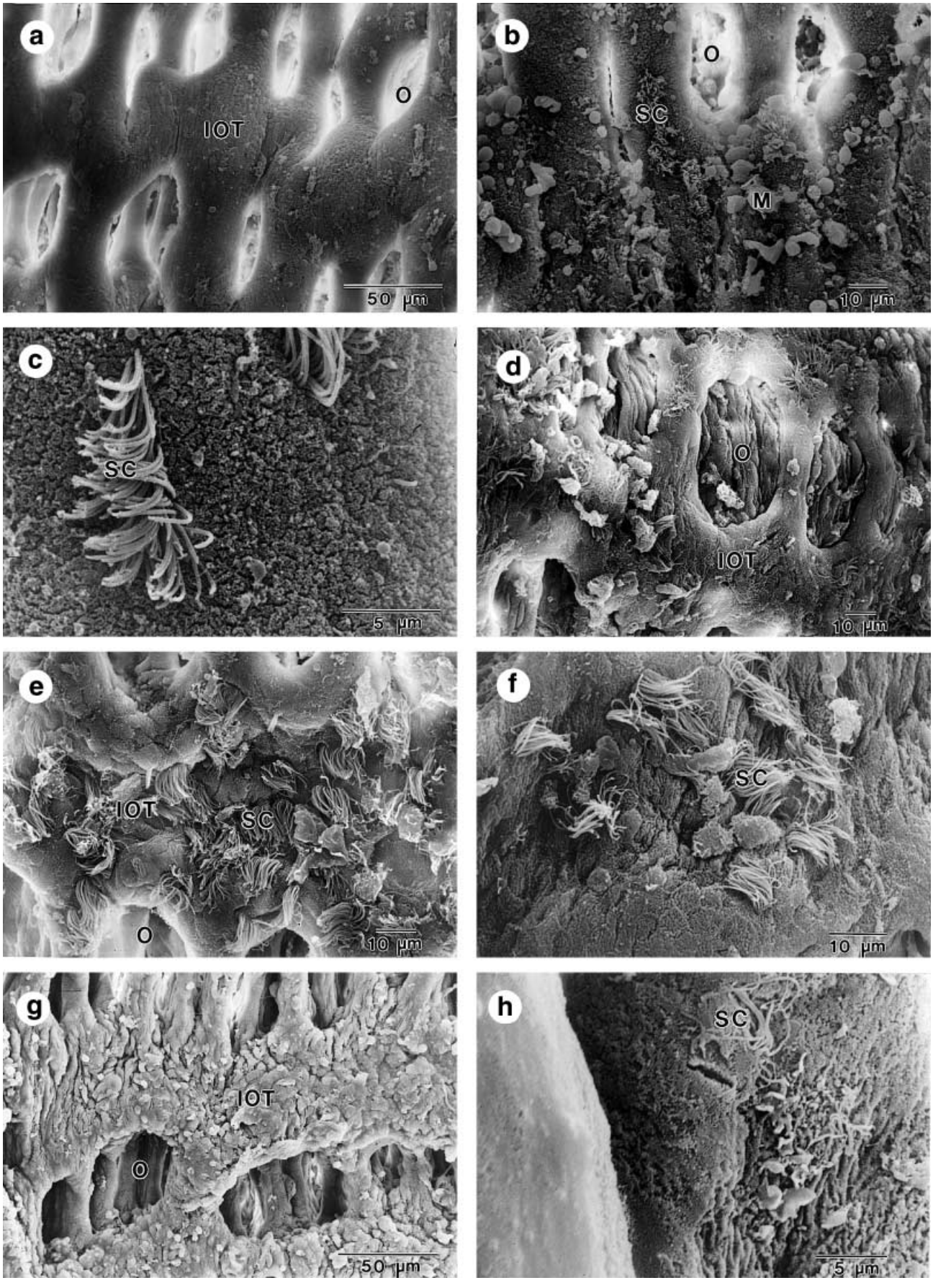
In the species studied, abfrontal mucocyte density was greatest in the eulamellibranch *Spisula solidissima*. The two other eulamellibranchs, although they had few abfrontal mucocytes, can still secrete a substantial amount of mucus because the diameter of those mucocytes was large (Fig. 2h, i; Table 2). The presence of residual mucus shows that AMPS are secreted on the abfrontal surface of the gills of these eulamellibranchs. Acidic mucus is highly viscous and is a good lubricant because it is not easily hydrated or removed from the epithelium (Hunt 1970; Faillard and Schauer 1972).

It is therefore possible that mucus on the abfrontal surface of eulamellibranch gills is important in lubrication. However, it is questionable that abfrontal mucus is

mantle (Fig. 2d; Table 2). About two-thirds of the abfrontal length of the descending filaments faces the mantle; abfrontal lubrication is probably advantageous in these regions.

Hypothesis 3 – the abfrontal surface is a mucociliary surface

Mucociliary surfaces, characterized by a dense covering of simple cilia with an overlying layer of mucus, are common in metazoans (Sleigh 1982, 1989; Meyer and Silberberg 1978; Beninger et al. 1997b; Blake et al. 1998). In bivalves, such surfaces exhibit a diversity of function. On the gills of the more primitive protobranchs such as *Nucula* sp. (Drew 1901), mucociliary


a
IOT
O
50 µm
b
O
SC
M
10 µm
c
SC
5 µm
d
O
IOT
10 µm
e
IOT
SC
O
10 µm
f
SC
10 µm
g
IOT
O
50 µm
h
SC
5 µm

◄

Fig. 9a–h Scanning electron micrographs of the abfrontal surface of homorhabdic eulamellibranch gills. **a** *Spisula solidissima*. View of the ostia (*O*) and inter-ostial tissue (*IOT*). **b** *S. solidissima*. Simple cilia (*SC*) and mucus (*M*) on the abfrontal surface. **c** *S. solidissima*. Tufts of simple cilia on inter-ostial tissue. **d** *Mya arenaria*. View of the ostia and inter-ostial tissue. **e** *Mya arenaria*. Tufts of simple cilia on the inter-ostial tissue. **f** *Mya arenaria*. Tufts of simple cilia on the inter-ostial epithelium. **g** *Mercenaria mercenaria*. Inter-ostial tissue and ostia. **h** *M. mercenaria*. Tuft of simple cilia on the inter-ostial tissue

transport is used to clean these solely respiratory organs of deposited particulate matter (Atkins 1936, Yonge 1947). This cleaning function has evolved into a feeding function on the gills of more evolved protobranchs such as *Yoldia ensifera* and *Solemya velum* (Kellogg 1915; Stasek 1965; Krueger et al. 1992), and of suspension-feeding bivalves.

Given that the abfrontal surface of bivalve gills is isolated from suspended particles, it would seem unlikely that mucociliary transport occurs on this surface. However, the presence of both cilia that are characteristic of mucus-transporting simple cilia (shorter than water-pumping cilia, Sleigh 1982) and mucocytes on this surface, even in low densities, suggests at least an original functional interdependence.

The capacity for mucociliary transport on the abfrontal surface of *Mytilus edulis* gills has been demonstrated by Jones et al. (1990): after depositing graphite particles on the abfrontal surface of dissected gills, they observed mucus-particle masses being transported ventro-dorsally on individual filaments. Although such mucociliary transport has not been observed in the other species studied, it would be possible in species where ciliary densities are high.

The differences in abfrontal cilia and mucocyte densities observed between species, together with the facultative particle transport observed on *Mytilus edulis* abfrontal surfaces and the near-absence of particles in the supra-branchial chamber, lead us to believe that the abfrontal surface is a vestigial mucociliary surface. Beninger et al. (1995b) and Beninger and St-Jean (1997b) have previously documented the presence of vestigial mucociliary surfaces lining blind tubes in *Mytilus edulis* labial palps: this mucociliary epithelium is histologically functional, but endoscopic observations confirm that there is no particle transport in this area. This interpretation requires that mucociliary transport on the abfrontal surface of bivalve gills served a purpose in ancestral forms, and that this purpose was subsequently lost as the gills evolved. After this mucociliary surface became vestigial, abfrontal cilia and mucocytes in some species decreased in number (and possibly in diversity), leading to the present condition.

The present understanding of bivalve gill evolution offers an explanation to the presence of vestigial cilia and mucocytes on the abfrontal surface. Contemporary suspension-feeding gills are thought to have evolved from a protobranch-like deposit-feeding ancestor whose simple, unfolded gill filaments elongated, then reflected vertically, with the dorsal tips of the ascending filaments eventually attaching to the mantle or visceral mass (Yonge 1947). This caused the separation of the mantle cavity into an infrabranchial and suprabranchial chamber, and the isolation of the abfrontal surface from particles in the incurrent flow of water. Therefore, the abfrontal surface of the contemporary gills studied derives from the abfrontal surface of primitive protobranch-like gills that used mucociliary transport to prevent gill fouling by suspended matter.

We thus conclude that the different distributions of cilia and mucocytes observed on the abfrontal surfaces of bivalve gills result from selective forces different in nature or degree, acting on this vestigial mucociliary epithelium. Future studies could usefully investigate functional correlates of the abfrontal surface, using direct observational techniques such as endoscopy, as well as high-resolution in vitro techniques such as confocal laser scanning microscopy. It would also be of interest to study the ontogeny of gill mucocytes and cilia in different species, to determine whether selective loss occurs at early stages.

Acknowledgements We thank G. Bacon for providing us with specimens of *Spisula solidissima*, the St. Andrews Biological Station for the use of ship time and scallop trawls, the Bermuda Biological Station for assistance with *Arca zebra* sampling, as well as L. Blanchard for excellent photographic work. This work was funded by an NSERC postgraduate scholarship to SCD and an NSERC operating grant to PGB.

References

Aiello E, Sleigh MA (1972) The metachronal wave of lateral cilia of *Mytilus edulis*. J Cell Biol 54: 493–506

Allen JA (1985) The recent Bivalvia: their form and evolution. In: Trueman ER, Clarke MR (eds) The Mollusca, vol 10. Evolution. Academic Press, London, pp 337–397

Atkins D (1936) On the ciliary mechanisms and interrelationships of lamellibranchs. I. New observations on sorting mechanisms. Q J Microsc Sci 79: 181–308

Atkins D (1938) On the ciliary mechanisms and interrelationships of lamellibranchs. VII. Latero-frontal cilia of the gill filaments and their phylogenetic value. Q J Microsc Sci 80: 346–430

Baba SA (1972) Flexural rigidity and elastic constant of cilia. J Exp Biol 56: 459–467

Baba SA, Hiramoto Y (1970) A quantitative analysis of ciliary movement by means of high-speed microcinematography. J Exp Biol 52: 675–690

Beninger PG, Dufour SC (1996) Mucocyte distribution and relationship to particle transport on the pseudolamellibranch gill of *Crassostrea virginica* (Bivalvia: Ostreidae). Mar Ecol Prog Ser 137: 133–138

Beninger PG, Le Pennec M (1991) Functional anatomy of scallops. In: Shumway SE (ed) Scallops: biology, ecology and aquaculture. Elsevier, Amsterdam, pp 133–224

Beninger PG, St-Jean SD (1997a) The role of mucus in particle processing by suspension-feeding marine bivalves: unifying principles. Mar Biol 129: 389–397

Beninger PG, St-Jean SD (1997b) Particle processing on the labial palps of *Mytilus edulis* and *Placopecten magellanicus* (Mollusca: Bivalvia). Mar Ecol Prog Ser 147: 117–127

Beninger PG, Le Pennec M, Salaün M (1988) New observations of the gills of *Placopecten magellanicus* (Mollusca: Bivalvia), and implications for nutrition. I. General anatomy and surface microanatomy. Mar Biol 98: 61–70
Beninger PG, Ward JE, MacDonald BA, Thompson RJ (1992) Gill function and particle transport in *Placopecten magellanicus* (Mollusca: Bivalvia) as revealed using video endoscopy. Mar Biol 114: 281–288
Beninger PG, St-Jean S, Poussart Y, Ward JE (1993) Gill function and mucocyte distribution in *Placopecten magellanicus* and *Mytilus edulis* (Mollusca: Bivalvia): the role of mucus in particle transport. Mar Ecol Prog Ser 98: 275–282
Beninger PG, Potter TM, St-Jean SD (1995a) Paddle cilia fixation artefacts in pallial organs of adult *Mytilus edulis* and *Placopecten magellanicus* (Mollusca: Bivalvia). Can J Zool 73: 610–614
Beninger PG, St-Jean SD, Poussart Y (1995b) Labial palps of the blue mussel *Mytilus edulis* (Bivalvia: Mytilidae). Mar Biol 123: 293–303
Beninger PG, Dufour SC, Bourque J (1997a) Particle processing mechanisms of the eulamellibranch bivalves *Spisula solidissima* and *Mya arenaria*. Mar Ecol Prog Ser 150: 157–169
Beninger PG, Lynn JW, Dietz TH, Silverman H (1997b) Mucociliary transport in living tissue: the two-layer model confirmed in the mussel *Mytilus edulis* L. Biol Bull 193: 4–7
Beninger PG, Veniot A, Poussart Y (1999) Principles of pseudofeces rejection on the bivalve mantle: integration in particle processing. Mar Ecol Prog Ser 178: 259–269
Blake JR, Sleigh MA, Liron N (1998) Roles of cilia in filter feeding and mucous transport. In: Baum GL, Priel Z, Roth Y, Liron N, Ostfeld E (eds) Cilia, mucus, and mucociliary interactions. Dekker, New York, pp 119–126
Cox LR (1959) The geological history of the Protobranchia and the dual origin of taxodont Lamellibranchia. Proc Malacol Soc Lond 33: 200–209
Daniel TD (1981) Fish mucus: in situ measurements of polymer drag reduction. Biol Bull 160: 376–382
Davies MS, Hawkins SJ (1998) Mucus from marine molluscs. Adv Mar Biol 34: 1–71
Drew GA (1901) Life-history of *Nucula delphinodonta*. Q J Microsc Sci 44: 313–391
Dwivedy RC (1973) A study of chemo-receptors on labial palps of the American oyster using microelectrodes. Proc Natl Shellfish Assoc 63: 20–26
Eble AF, Scro R (1996) General anatomy. In: Kennedy VS, Newell RIE, Eble AF (eds) The eastern oyster *Crassostrea virginica*. Maryland Sea Grant, College Park, pp 19–73
Faillard H, Schauer R (1972) Glycoproteins as lubricants, protective agents, carriers, structural proteins and as participants in other functions. In: Gottschalk A (ed) Glycoproteins. Their composition, structure and function, part B, vol 5. Elsevier, Amsterdam, pp 1246–1267
Galtsoff PS (1964) The American oyster *Crassostrea virginica* Gmelin. Fish Bull US Bur Comm Fish 64: 571–588
Gray J (1931) The mechanisms of ciliary movement. VI. Photographic and stroboscopic analysis of ciliary movement. Proc R Soc Lond B 107: 313–332
Grenon JF, Walker G (1980) Biochemical and rheological properties of the pedal mucus of the limpet, *Patella vulgata* L. Comp Biochem Physiol B 66: 451–458
Hoyt JW (1975) Hydrodynamic drag reduction due to fish slimes. In: Wu TVT, Brokaw CJ, Brennen C (eds) Swimming and flying in nature. Plenum Press, New York, pp 653–673
Hunt S (1970) Polysaccharide-protein complexes in invertebrates. Academic Press, London
Janssens F (1893) Les branchies des acéphales. In: Carnoy JB, Gilson G, Denys J (eds) La cellule: recueil de cytologie et d'histologie générale. Joseph van In, Lier, Belgium, pp 7–91 and four out of text plates
Jones HD, Richards OG (1993) The effects of acetylcholine, dopamine and 5-hydroxyltryptamine on water pumping rate and pressure in the mussel *Mytilus edulis* L. J Exp Mar Biol Ecol 170: 227–240
Jones HD, Richards OG, Hutchinson S (1990) The role of ctenidial abfrontal cilia in water pumping in *Mytilus edulis* L. J Exp Mar Biol Ecol 143: 15–26
Kellogg JL (1915) Ciliary mechanisms of lamellibranchs with descriptions of anatomy. J Morphol 26: 625–701
Krueger DM, Gallager SM, Cavanaugh CM (1992) Suspension feeding on phytoplankton by *Solemya velum*, a symbiont-containing clam. Mar Ecol Prog Ser 86: 145–151
Lankester ER (1886) On green oysters. Q J Microsc Sci 26: 71–94
Le Pennec M, Beninger PG, Herry A (1988) New observations of the gills of *Placopecten magellanicus* (Mollusca: Bivalvia), and implications for nutrition. II. Internal anatomy and microanatomy. Mar Biol 98: 229–237
Martoja R, Martoja M (1967) Initiation aux techniques de l'histologie animale. Masson, Paris
Meyer FA, Silberberg A (1978) Structure and function of mucus. In: Respiratory tract mucus. Ciba Foundation Symposium no. 54. Elsevier, Amsterdam, pp 203–218
Morton JE (1979) Molluscs, 5th edn. Hutchinson, London
Nelson TC (1960) The feeding mechanism of the oyster. II. On the gills and palps of *Ostrea edulis*, *Crassostrea virginica*, and *C. angulata*. J Morphol 107: 163–191
Newell RIE, Langdon CJ (1996) Mechanisms and physiology of larval and adult feeding. In: Kennedy VS, Newell RIE, Eble AF (eds) The eastern oyster *Crassostrea virginica*. Maryland Sea Grant, College Park, pp 185–229
Orton JH (1912) The mode of feeding of *Crepidula*, with an account of the current-producing mechanism in the mantle cavity, and some remarks on the mode of feeding in gastropods and lamellibranchs. J Mar Biol Assoc UK 9: 444–478
Owen G, McCrae JM (1976) Further studies on the latero-frontal tracts of bivalves. Proc R Soc Lond B 194: 527–544
Prezant RS (1985) Molluscan mucins: a unifying thread. Am Malacol Bull (Spec Issue) 1: 35–50
Ridewood WG (1903) On the structure of the gills of lamellibranchia. Philos Trans R Soc Lond B 195: 147–284
Riisgård HU, Larsen PS, Nielsen NF (1996) Particle capture in the mussel *Mytilus edulis*: the role of latero-frontal cirri. Mar Biol 127: 259–266
Salvini-Plawen Lv, Steiner G (1996) Synapomorphies and plesiomorphies in higher classification of Mollusca. In: Taylor J (ed) Origin and evolutionary radiation of the Mollusca. Oxford University Press, Oxford, pp 29–51
Silverman H, Lynn JW, Dietz TH (1996) Particle capture by the gills of *Dreissena polymorpha*: structure and function of latero-frontal cirri. Biol Bull 191: 42–54
Silverman H, Lynn JW, Beninger PG, Dietz TH (1999) The role of latero-frontal cirri in particle capture by the gills of *Mytilus edulis*. Biol Bull 197: 368–376
Silvester NR (1988) Hydrodynamics of flow in *Mytilus* gills. J Exp Mar Biol Ecol 120: 171–182
Simkiss K (1988) Molluscan skin (excluding cephalopods). Trueman ER, Clarke MR (eds) The Mollusca, vol XI. Form and function. Academic Press, London, pp 11–35
Sleigh MA (1982) Movement and coordination of tracheal cilia and the relation of these to mucus transport. Cell Motil [Suppl] 1: 19–24
Sleigh MA (1989) Adaptations of ciliary systems for the propulsion of water and mucus. Comp Biochem Physiol A 94: 359–364
Sleigh MA, Holwill MEJ (1969) Energetics of ciliary movement in *Sabellaria* and *Mytilus*. J Exp Biol 50: 733–743
Stasek C (1965) Feeding and particle sorting in *Yoldia ensifera* (Bivalvia: Protobranchia) with notes on other nuculanids. Malacologia 2: 349–366
Waller TR (1998) Origin of the molluscan class Bivalvia and a phylogeny of major groups. In: Johnston PA, Haggart JW (eds) Bivalves: an eon of evolution-paleobiological studies honoring

Norman D. Newell. University of Calgary Press, Calgary, pp 1–45

Ward JE, MacDonald BA, Thompson RJ, Beninger PG (1993) Mechanisms of suspension-feeding in bivalves: resolution of current controversies by means of endoscopy. Limnol Oceanogr 38: 265–272

Ward JE, Newell RIE, Thompson RJ, MacDonald BA (1994) In vivo studies of suspension-feeding processes in the eastern oyster, *Crassostrea virginica* (Gmelin). Biol Bull 186: 221–240

Ward JE, Levinton JS, Shumway SE, Cucci T (1998) Particle sorting in bivalves: in vivo determination of the pallial organs of selection. Mar Biol 131: 283–292

Yonge CM (1947) The pallial organs in the aspidobranch Gastropoda and their evolution throughout the Mollusca. Philos Trans R Soc Lond B 232: 443–518

Yonge CM (1959) The status of the Protobranchia in the bivalve Mollusca. Proc Malacol Soc Lond 33: 210–214