Paddle cilia fixation artefacts in pallial organs of adult *Mytilus edulis* and *Placopecten magellanicus* (Mollusca, Bivalvia)

Peter G. Beninger, Tracy M. Potter, and Sylvie D. St-Jean

Abstract: To ascertain the nature of paddle cilia in adult marine bivalves, three scanning electron microscopic (SEM) fixation protocols were used to fix ciliated organs of the pallial cavity in the blue mussel, *Mytilus edulis*, and the giant scallop *Placopecten magellanicus* (Bivalvia: Mytilidae, Pectinidae). SEM examination concentrated on the various regions of the labial palps and gills in *M. edulis*, with supplementary observations of the osphradium and adjacent regions in *P. magellanicus*. A regular transect approach was used to examine all surfaces, both for thoroughness and to reveal any eventual patterns in distribution. Cilia with paddle-like expansions were observed on all surfaces when both hypotonic glutaraldehyde–cacodylate buffer and hypotonic Sörensen's buffer were used; no intra- or inter-individual pattern was evident. No expansions were present when isotonic glutaraldehyde–cacodylate was employed. We conclude that paddle cilia may be induced in adult marine bivalves by hypotonic fixation; this has wider implications for the other bivalve species in which such cilia have been reported, in addition to the numerous accounts of such structures in other taxa.

Résumé : Afin de mieux comprendre la nature des discocils chez les bivalves marins adultes, trois protocoles de fixation associés à la microscopie electronique à balayage (SEM) ont été employés pour fixer les organes ciliés de la cavité palléale chez la Moule bleue, *Mytilus edulis*, et le Pétoncle géant *Placopecten magellanicus* (Bivalvia : Mytilidae, Pectinidae). Le microscope électronique à balayage a servi surtout à l'examen des diverses régions des palpes labiaux et des branchies de *M. edulis* et des observations supplémentaires ont été faites de l'osphradie et regions voisines chez *P. magellanicus*. Toutes les surfaces ont été examinées le long d'un transect, méthode qui assure un examen complet et qui met en lumière l'existence d'arrangements particuliers s'il y a lieu. Des cils portant des élargissements discoïdes ont été observés sur toutes les surfaces des structures fixées au glutaraldéhyde–cacodylate hypotonique ou du tampon de Sörensen hypotonique; aucun arrangement particulier à un individu ou à un groupe d'individus n'a été observé. Il n'y avait pas d'elargissements visibles après fixation au glutaraldéhyde–cacodylate isotonique. Nous concluons que la présence des discocils peut être provoquée par fixation hypotonique; cela remet en question la présence de tels cils chez d'autres espèces de bivalves de même que chez d'autres taxons où ils ont été signalés dans de nombreux travaux.

Introduction

Numerous electron microscopic studies have reported the existence and appearance of cilia bearing terminal expansions in a variety of marine invertebrate phyla; Haszprunar (1985*a*) summarized these reports in approximately 125 species from 10 marine phyla up to 1985. Since that paper was published, paddle cilia have been described for an additional 9 species of the molluscan classes Caudofoveata and Solenogastres (Haszprunar 1987*a*), 3 species of Gastropoda (Haszprunar 1985*b*), 13 species of Placophora and Bivalvia (Haszprunar 1987*b*), and 1 species of Polychaeta (Laverack 1988). The origin and functions proposed for these cilia range from artefactual through chemoreceptive, locomotor, water movement, and even use as a spatula for the application of byssus threads in *Mytilus californianus* (Tamarin et al. 1974).

Although reports of paddle cilia continued into the recent literature (Laverack 1988), a study in 1978 (Ehlers and Ehlers 1978) showed that formation of these structures could be artificially induced in the cilia of the epidermal sensory cells of marine Turbellaria by subjecting them to altered osmotic or thermal conditions, as well as to common chemicals used in fixation for electron microscopy. In a more recent series of papers, Tamm and colleagues have demonstrated that such cilia may be induced in molluscan veliger larvae under hypotonic fixation conditions, and have deduced fundamental physical and mechanical properties of cilia from the formation of these artefacts. They suggest that paddle

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P.G. Beninger, T.M. Potter,[1] and S.D. St-Jean.
Département de Biologie, Université de Moncton, Moncton, NB E1A 3E9, Canada.

[1] Present address: Marine Chemistry Division, Bedford Institute of Oceanography, Darthmouth, NS B2Y 4A2, Canada.

Table 1. Fixatives and osmolarities used for scanning electron microscopic preparation of surfaces studied.

Fixative	Osmolarity (mosmol)	Species	Surface
1	868	*Mytilus edulis*	Labial palps
			Smooth*†
			Ridged*†
			Gills
			Frontal*†
			Abfrontal*†
		Placopecten magellanicus	Osphradium
			Ridge*
			Adjacent regions*
2	576	*M. edulis*	Labial palps
			Smooth*†
			Ridged*†
3	1058	*M. edulis*	Labial palps
			Smooth*†
			Ridged*†
			Gills
			Frontal*†
			Abfrontal*†
		P. magellanicus	Osphradium
			Ridged*
			Adjacent regions*

*Osmolarity of seawater from collection site = 960 mosmol.
†Osmolarity of seawater from collection site = 1058 mosmol.

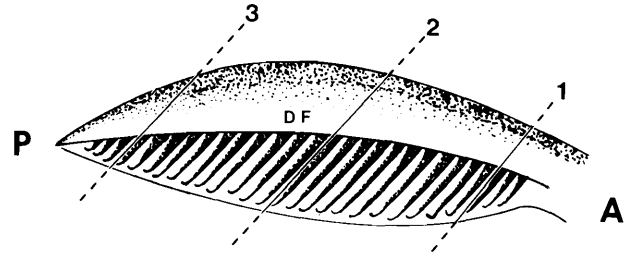
cilia may be artefacts in other taxa in which the fixative osmolarity was not rigorously adjusted to that of the medium in which the animals lived (Short and Tamm 1989, 1991; Deiner and Tamm 1991; Deiner et al. 1993).

The purpose of the present study was to investigate the presence and eventual distribution of paddle cilia on various ciliated structures of the adult bivalve pallial cavity under conditions of hypo- and iso-tonic fixation, to determine whether such structures are artefacts. Given the range of functions suggested for paddle cilia, the labial palps of the blue mussel, *Mytilus edulis*, seemed to constitute ideal test structures: the cilia transport particles, and some may also be sensory, since particle selection is known to occur somewhere between the labial palps and mouth (see Beninger et al. 1993). Similarly, such cilia have previously been reported from the osphradia of marine bivalves (Haszprunar 1987*b*), so observations of this structure are also warranted. The present study thus concentrated on the ciliation of the labial palps and gills in *M. edulis*, with additional observations on the osphradium and gill of the giant scallop *Placopecten magellanicus*.

Material and methods

Three different fixatives were used for the various organs dissected out of the pallial cavity in these two species, as shown in Table 1. The composition of fixative 1, used with two adult specimens of *M. edulis* and *P. magellanicus*, was as follows: hypotonic glutaraldehyde-cacodylate buffer, pH 7.2, consisting of 2 volumes 6% glutaraldehyde in distilled water, 1 volume 0.4 M sodium cacodylate, and 1 volume 4% NaCl, final osmolarity 868 mosmol. The scallops were sampled in May 1993 from Passamaquoddy Bay, where the seawater osmolarity was 1058 mosmol, while the mussels were sampled from Lameque Bay in April 1993 and maintained in an open-circuit aquarium for 1 month at an osmolarity of 960 mosmol.

Fig. 1. An example of the transects used for SEM observations: the ridged (oral) surface of the labial palp of *Mytilus edulis*. A, anterior extremity; DF, dorsal fold; P, posterior extremity; 1, anterior transect; 2, median transect; 3, posterior transect. Identical transects were performed on the smooth (aboral) surface (not visible).



The composition of fixative 2, used with one adult mussel from Passamaquoddy Bay sampled in March 1994, was as follows: hypotonic glutaraldehyde – Sörensen's buffer, consisting of a final concentration of 2% glutaraldehyde in Sörensen's buffer, pH 7.2 (57.5 mL 0.2 M dibasic sodium phosphate (NaH_2PO_4), 192.5 mL 0.2 M monobasic sodium phosphate (Na_2HPO_4), osmolarity 576 mosmol). The composition of fixative 3, used with two adult mussels and one scallop sampled from Passamaquoddy Bay in April 1994, was as follows: isotonic glutaraldehyde – sodium cacodylate buffer consisting of a final concentration of 2.5% glutaraldehyde in 0.1 M sodium cacodylate, pH 7.2, adjusted to 1058 mosmol (identical with seawater from the site), with 23 mg NaCl for each millilitre of buffered fixative. The fixation media and osmolarities are summarized in Table 1. Osmolarities were determined using an Advanced Digimatic Osmometer Model 32D (Advanced Instruments Ltd., Needham Heights, Mass.).

The specimens were then dehydrated in an ascending ethanol series, critical-point dried using liquid CO_2, mounted on stubs, and sputter coated with gold–palladium. The specimens were observed using a JEOL 5200 scanning electron microscope; preliminary trials indicated that best results were obtained at 15 kV.

The surfaces were scanned using a transect approach. For both surfaces of the mussel labial palps, three transects were performed in the ventrodorsal direction: one at the posterior extremity, one at the anterior extremity, and one in the median region (Fig. 1). Detailed examinations were performed at every 100 μm along each transect, and photomicrographs were taken at every 500 μm. For both frontal and abfrontal surfaces of the gills of *M. edulis*, four transects were scanned along the dorsoventral axis. Photomicrographs were taken at 500-μm intervals, including the frontal and laterofrontal cilia (frontal surface), as well as the abfrontal cilia. For the scallop osphradium and adjacent zones, a transect was performed in a dorsoventral direction at both extremities and in the median region, encompassing a distance of 1 mm on either side of the osphradial ridge. Photo-

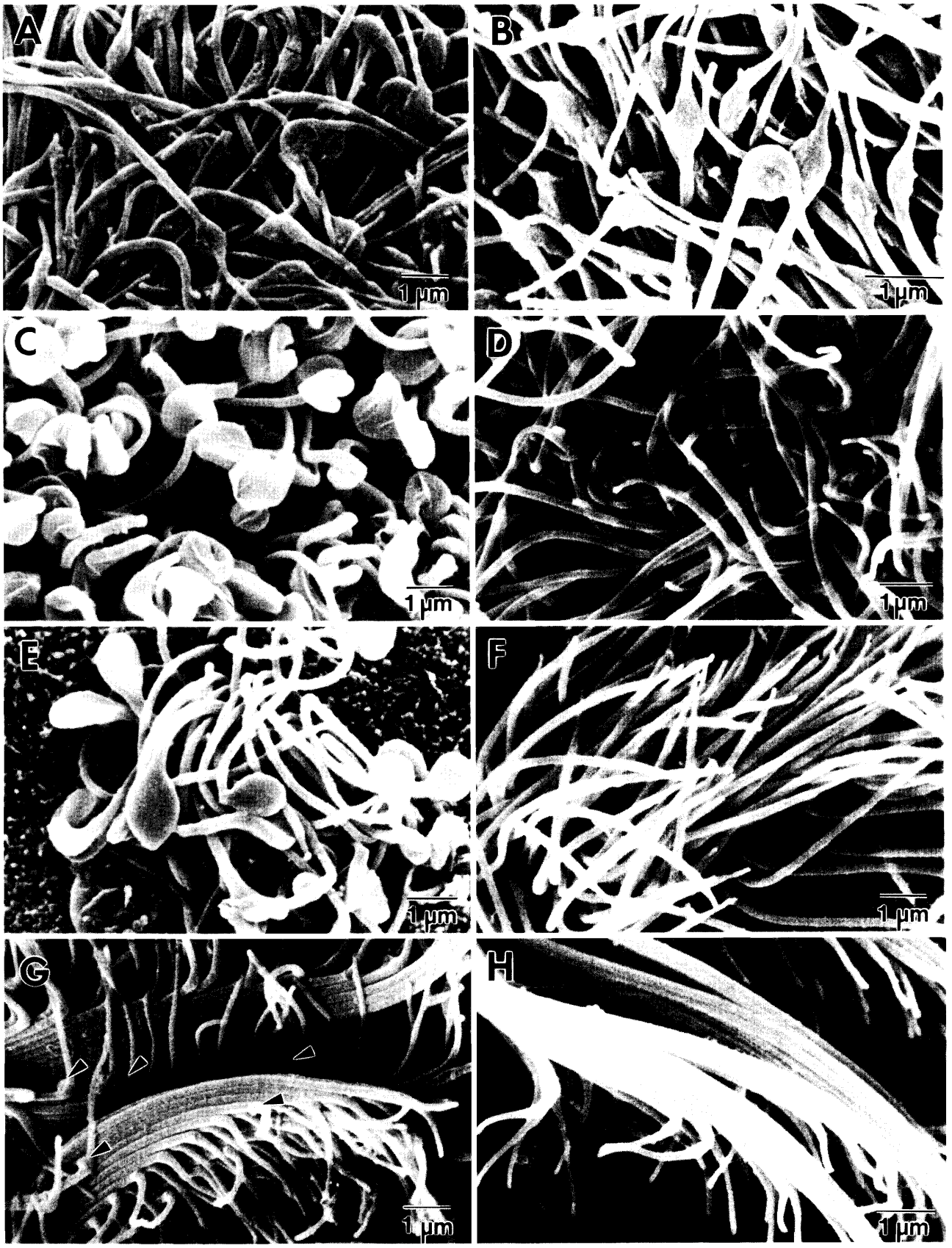


Fig. 2. Scanning electron micrographs of cilia from pallial organs of *Mytilus edulis*, fixed in solutions of different osmolarities. (A) Labial palp fixed in hypotonic glutaraldehyde–cacodylate buffer, showing the ridged surface, anterior transect, on the ventral margin of the dorsal fold. Note the expansions at midlength of the cilia. Osmolarity of fixative 868 mosmol; osmolarity of seawater at fixation 960 mosmol. (B) Detail of A, showing bent axonemes and stretched ciliary membranes. (C) Labial palp fixed in hypotonic Sörensen's buffer, oral surface, median transect in the midregion of the dorsal fold. Note the terminal position of the expansions. Osmolarity of fixative 576 mosmol; osmolarity of seawater at fixation 1058 mosmol. (D) Ridged surface of labial palp fixed in isotonic glutaraldehyde–cacodylate buffer, posterior transect, ventral rim of the dorsal fold. No expansions of any type are present, although some cilia show a tendency to roll at the distal extremity. (E) Labial palp fixed in hypotonic Sörensen's buffer, smooth surface, posterior transect, 400 μm dorsal to the ventral margin. Note the terminal expansions on all cilia. Osmolarity of fixative 576 mosmol; osmolarity of seawater at fixation 1058 mosmol. (F) Labial palp fixed in isotonic glutaraldehyde–cacodylate buffer, smooth surface, median transect, ventral margin. No terminal expansions are present on any cilia. Compare the background microvilli to those in (E). (G) Descending gill filament fixed in hypotonic glutaraldehyde–cacodylate buffer, frontal surface, transect station 1 (see Fig. 1). Note the expansions at the bases of cilia diverging from fused cirri (arrowheads). Osmolarity of fixative 868 mosmol; osmolarity of seawater at fixation 960 mosmol. (H) Descending gill filament fixed in isotonic glutaraldehyde–cacodylate buffer, frontal surface, transect station 1 (see Fig. 1). No expansions are present.

micrographs were taken at every 100 μm. The entire data set thus consisted of approximately 1000 observations and 280 photomicrographs.

Results and discussion

Both hypotonic fixatives (glutaraldehyde–cacodylate and Sörensen's buffers) produced cilia with disclike expansions. These expansions were terminal in the case of the ospradium and adjacent regions of *P. magellanicus* fixed in slightly hypotonic glutaraldehyde–cacodylate buffer, and the *M. edulis* labial palps fixed in the greatly hypotonic Sörensen's buffer, but occurred at the bases of the diverging cilia in the laterofrontal cirri of the gill of *M. edulis*, fixed in slightly hypotonic glutaraldehyde–cacodylate buffer, and at midlength in the labial palp cilia of this species fixed in slightly hypotonic buffer (Fig. 2). Fixation with isotonic glutaraldehyde–cacodylate buffer adjusted with NaCl (as suggested by Short and Tamm 1991) eliminated all of these expansions. The osphradial tissue is known to be extremely sensitive to fixation osmolarity (Beninger et al. 1995). These results suggest that for most tissues, slight hypotonic fixation (approximately 100 mosmol) results in nonterminal expansions, whereas greatly hypotonic fixation results in terminal expansions; extremely sensitive tissues such as the osphradium, however, will yield terminal expansions with slight hypotonic fixation.

The present study is the first confirmation of the artefactural nature of paddle cilia in adult marine bivalves, extending the findings of Short and Tamm (1989), Deiner and Tamm (1991), Short and Tamm (1991), and Deiner et al. (1993) in molluscan veliger larvae. Haszprunar (1985*a*) states, from his own work and that of others, that paddle cilia are found ony in marine organisms. It is interesting to note that of the eight species of bivalves studied by Haszprunar (1987*b*), osphradial paddle cilia were reported in all but two, *Anodonta cygnea* and *Dreissena polymorpha*; these are both freshwater species for which standard electron microscopy fixatives would be hypertonic and thus not provoke the formation of paddle cilia. This observation may have wider application to the other marine groups studied.

Although the rigorous transect approach followed in this study would have revealed consistent patterns of distribution of paddle cilia were they related to sensory areas as suggested by Davis and Matera (1981) and Haszprunar (1985*a*), no such patterns emerged. The appearance and distribution of paddle cilia were primarily dependent on the osmotic concentration of the fixatives; the position of the paddles within the cilia differed between the two hypotonic fixatives used. Moreover, the aboral palp surface of *M. edulis* is not suspected of being any more sensory than other nondifferentiated pallial surfaces (no particles are handled on this surface), yet virtually all of its cilia presented paddles when fixed with hypotonic Sörensen's buffer (Fig. 2E). It thus seems unlikely that paddle cilia could even represent sensory cilia whose particular composition results in a conformational change when they are exposed to hypotonic fixatives (see Haszprunar 1985*a*, 1987*a*, 1987*b*; Deiner et al. 1993). Indeed, it may be that the sensory cilia of the labial palps are indistinguishable from ordinary kinocilia, as suggested by Laverack for *Echinus* sp. (Echinodermata; Laverack 1968, 1988). The fact that in some regions of some surfaces, not all the cilia presented paddles does, however, attest to differences in response to the hypotonic glutaraldehyde–cacodylate fixative; again, given the nonpatterned character, this is not related to the functional specificity of the regions. As indicated by Deiner et al. (1993), some cilia present a tendency to "roll" in the distal region, which may facilitate the formation of artefactual paddles. Such a tendency was also observed in the present study (Fig. 2D). Much more complex and refined investigation would be required to determine the reasons for such differential responses, but it should be remembered that all cilia fixed in the most hypotonic solution (Sörensen's) presented terminal paddles.

The experimental evidence presented here thus asserts the artefactual nature of paddle cilia in two species of adult marine bivalve. It would be of interest to extend this verification to the ciliated organs of other species, especially the osphradium of other marine molluscs. Extensive studies have imputed a chemosensory and ultimately reproductive function for these structures (Haszprunar 1985*a*, 1985*b*, 1987*b*), with the presence of paddle cilia as a major proof. While the osphradia may indeed be chemosensory, as shown in the electrophysiological work of Sokolov and Zaitseva (1982), the presence of paddle cilia cannot be regarded as an indicator of such a function in this organ or any other. As a general recommendation, authors reporting the presence of paddle cilia should indicate the exact osmolarities of both fixative and medium at the time of fixation.

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