

REPLY COMMENT

Feeding mechanism of the polychaete *Sabellaria alveolata* revisited: reply to Riisgård & Nielsen (2006)

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ABSTRACT: The high-speed videos provided by Riisgård & Nielsen (2006; this volume) allow new insights into *Sabellaria alveolata* feeding processes, but the interpretation of these video observations is compromised both by artifacts of sample preparation and premature dismissal of our findings in Dubois et al. (2005; Mar Ecol Prog Ser 301:159–171). *S. alveolata* appears clearly to be an active suspension-feeder, while not using grouped lateral cilia to catch particles directly from the water column, but by using both the grouped lateral cilia and grouped frontal cilia to modify current patterns around tentacular filaments to entrain suspended particles. Evidence of inter-tentacle variability in particle processing and bi-directional transport of particles make the calculation of the clearance rate based on ciliary band inappropriate by clearly overestimating the actual clearance rate of *S. alveolata*.

KEY WORDS: *Sabellaria alveolata* · Polychaete · Suspension feeder · Ciliary movements · Tentacular filaments · Grouped frontal cilia · Video endoscopy

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The subject of particle capture and processing mechanisms in *Sabellaria alveolata* (Polychaeta: Sabellariidae) was investigated for the first time by Dubois et al. (2005). Using additional electronic micrographs and high-speed video, Riisgård & Nielsen (2006, this volume) re-investigated the feeding mechanisms of this polychaete and re-interpreted ecophysiological data and filtration values previously obtained on this species (Dubois et al. 2003). While high-speed videos provided a new insight and a better understanding of particle capture in *S. alveolata*, we feel that their re-interpretations are not always supported by the available data, and hence do not call into question the previous work.

CILIATION OF TENTACULAR FILAMENTS

As admitted by the authors, the scanning electron micrograph (SEM) photographs provided by Riisgård & Nielsen (2006) display a very obvious fixation artefact, due to hypotonic fixation (Beninger et al. 1995), which could have been avoided by measuring the osmolarity of their specimens' habitat and adjusting their solution accordingly. Hypotonic fixation is responsible for paddle cilia formation and other terminal irregularities, as well as partial disorganization of ciliary structures, leading to ambiguous functional interpretation. A slightly hyperosmotic solution is necessary to fix polychaete ciliated feeding organs while

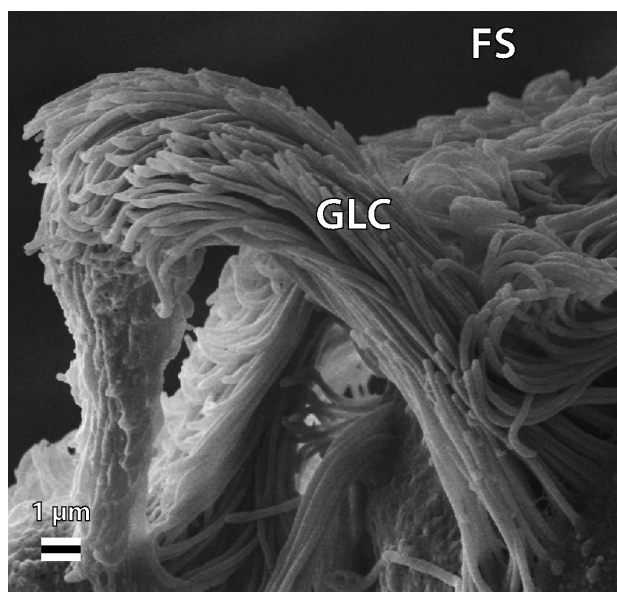


Fig. 1. *Sabellaria alveolata*. SEM of tentacles. Close-up of grouped lateral cilia (GLC). FS: frontal surface. Micrograph after Dubois et al. (2005)

preserving ciliated patterns (e.g. Dauer et al. 2003 and references therein). The ciliary patterns and cilia length measurements proposed by Riisgård & Nielsen (2006) are therefore questionable. In particular, their description of 3 groups of compound lateral cilia (inappropriately called 'spikes') and 3 adjacent cirri is not evident from the micrographs presented, since due to the poor fixation, cilia patterns are too disorganized to be recognizable.

Osmotically inadequate fixation for SEM is also inadequate for transmission electron micrographs (TEM), as evidenced by the extraordinarily distended basal lamina (the 'basal membrane' of their Fig. 3A), diminishing the credibility of the TEM data furnished by these authors. Based on well-fixed material, we observed cohesive groups of long lateral cilia, (Dubois et al. 2005, their Fig. 2f; Fig. 1), and called them grouped lateral cilia (GLC) to avoid the existing terminological pitfalls with respect to cilia types. In effect, apart from the V-shaped component ciliary distribution of compound cilia (not found in either Riisgård & Nielsen or our own SEMs), to our knowledge, no ultrastructural distinction has been made between compound and non-compound grouped cilia in marine organisms; the greater cohesiveness of compound cilia is unexplained to date. However, our well-fixed SEM of the GLC argues for a less structured and cohesive grouping than that normally assigned to compound cilia; we have previously used the term 'composite cilia' to designate such groupings

(Beninger & Veniot 1999, Beninger et al. 1999). Pending the demonstration of a concrete distinguishing ultrastructural feature of these cilia types, accounting for the differences in cohesiveness, we feel it is prudent to use the more generic term 'grouped cilia'. In any event, the term 'spike' is very misleading, since even its biological meaning is unclear (a botanical term referring to flower structure; cf. Henderson 1989). We maintain that the GLC terminology should be preferred to describe the grouping of several ciliated cells (at least 6, as shown in their video observation, see our comments in the section 'Particle capture'), resulting in the association of several groups of ca. 40 μm long cohesive cilia. These GLC form a ciliated band perpendicular to the frontal cilia band and thus range from latero-frontal position to lateral position along each tentacle, evenly spaced every 25 or 30 μm.

Riisgård & Nielsen's (2006) SEM figures show a smooth frontal ciliary band on tentacular filaments, whereas Dubois et al. (2005) previously showed that long grouped cilia (their Fig. 2d; Fig. 2), thinner than those of the GLC, were actually widely scattered in a median or marginal position, among the frontal ciliary band. These grouped frontal cilia (GFC) were difficult to observe *in vivo* and their role was unsolved in the Dubois et al. (2005) paper. Riisgård & Nielsen's (2006) high-speed videos now enable a better understanding of the function of these GFC (see our interpretation of their videos in the next section).

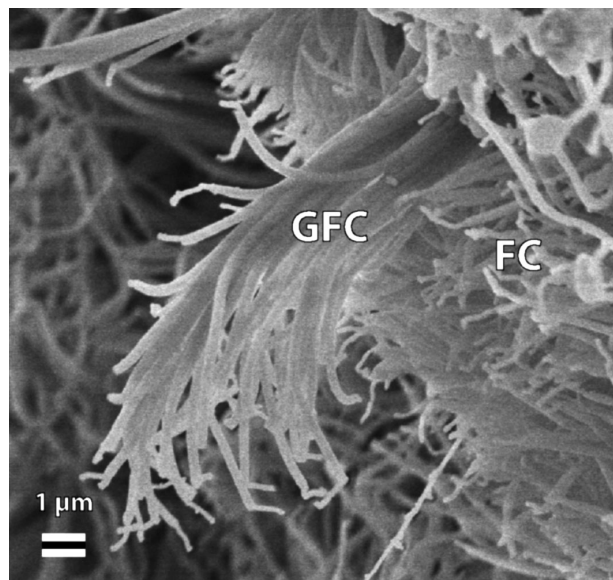


Fig. 2. *Sabellaria alveolata*. SEM of tentacles. Close-up of the frontal ciliary band showing frontal cilia (FC) and longer grouped frontal cilia (GFC). Micrograph after Dubois et al. (2005)

PARTICLE CAPTURE

Uncertainty remains in Riisgård & Nielsen's (2006) description of the feeding organs, probably related to their terminology. As described in Dubois et al. (2005), a clear distinction has to be made between palps and tentacular filaments (also called tentacles). The palps of *Sabellaria alveolata* (Fig. 3F in Dubois et al. 2005) are functionally very different from tentacular filaments. The palps collect large particles (e.g. shell fragments used to build the tube), and clean the cephalic floor and the principal grooves of tentacular filaments under high particle concentration. The ciliated pattern of palps has not yet been fully studied, but these feeding organs are morphologically different from tentacular filaments, since their width increases from 60 µm at the distal part to 120 µm at the proximal part, close to the mouth. The width of tentacular filaments is ca. 60 µm and is almost constant along the tentacle length. Inconsistencies are found in Riisgård & Nielsen (2006), who described an 'increase in width (measured on the frontal side) from about 40 µm near the tip, to about 60 µm in the middle, and up to 90 µm near the base.' However, their figures do not show any change in tentacular filament width (e.g. Figs. 4, 5 & 10). Hence, there might be some confusion in video observations and SEM photographs between the palps and the tentacular filaments. A previous investigation (Riisgård et al. 2000, see p. 46 paragraph 'other data related to particle capture') already confused the movements of cilia from tentacular filaments with the movement of the cilia of *S. alveolata* gills, which are not involved in particle capture.

Contrary to what is implied in Riisgård & Nielsen (2006), we did not report that sabellids and serpulids bare resemblances to sabellariids, but suggested that the pattern of tentacular filaments exhibits similarities with the lophophore of lophophorids (McKinney 1990, Riisgård & Manríquez 1997). Nevertheless, high-speed video observations provide a new insight into sabellariid particle capture. We agree with Riisgård & Nielsen's (2006) interpretation that the GLC beat by successively involving a different number of grouped cilia, from 1 to 6 (see the uppermost right GLC in Riisgård & Nielsen's Video clip #1; Video clips #1 to #3 of Riisgård & Nielsen [2006] are available in MEPS Supplementary Material at www.int-res.com/articles/suppl/m328p295_videos/). The movement of the different groups of cilia is described in Riisgård & Nielsen's Fig. 9 and, as first mentioned by Dubois et al. (2005), GLC may remain stuck to the tentacular filament before their recovery stroke. The movement of the GLC is nevertheless not parallel to the tentacle, nor obviously perpendicular as for other suspension feeders such as the entoproct *Loxosoma pectinaricola*

(Riisgård et al. 2000). The movement is rather obliquely oriented toward the frontal surface and probably contributes to water flow. By analogy with some spionids exhibiting complex ciliary bands on their palps, GLC may create lateral vortices that potentially entrain suspended particles (Dauer et al. 2003). However, GLC do seem to be involved in the direct capture of particles, such as performed by the catch-up process in *Fabricia stellaris* (Riisgård & Nielsen's Video clip #3)

As shown by our own high-speed videos, numerous GFC beat in a more constant manner than the GLC. Contrary to what we previously hypothesized (Dubois et al. 2005), GFC likely play a role in generating currents at the filament surface. Observations of a microalgal particle trajectory at the vicinity of a tentacular filament (Video clip #1; our Video clips #1 to #5 available in MEPS Supplementary Material at www.int-res.com/articles/suppl/m328p307_videos/) and of water motion on its frontal surface (Video clip #2) suggest that *Sabellaria alveolata* is not a passive suspension feeder completely dependent on ambient currents that bring suspended food particles into contact with the tentacles. Video clip #3 provides 2 examples of particle capture by the current generated by the tentacles; particles in the through current passing close to a tentacle may enter a counter-flow current before being stuck to the frontal cilia.

Subjective visual observation of stomach contents cannot be used to determine whether an organism is a deposit rather than a suspension feeder, nor does such a distinction make ecological sense in an intertidal zone surrounded by mudflats and subjected to constant tidal and wind-driven resuspension of 'deposited' matter. Such observations cannot be used to determine whether an organism is capable of pre-ingestive selection. Even species such as oysters, proven to be selective feeders in numerous studies using various techniques (Newell & Jordan 1983, Shumway et al. 1985, Ward et al. 1998, Cognie et al. 2003), present stomach contents qualitatively resembling 'unsorted matter', especially in high-turbidity habitats (P. Beninger pers. obs. and an open invitation to anyone who wishes to verify). Previous observations on juveniles of *S. alveolata* (S. Dubois pers. obs.) have shown that newly settled juveniles use their 2 palps in the same way as spionids, until the formation of the first tentacular filaments. Prior to the developmental completion of the tentacular crown, the palps can play a role in the feeding process by lapping the surface of sediment; however, according to our observations, this function is not activated in adults. Further research is needed to understand the ontogenetic changes in the feeding processes of *Sabellaria alveolata*.

FILTRATION RATES AND FEEDING PROCESSES

Riisgård & Nielsen (2006) support the hypothesis that *Sabellaria alveolata* is a passive suspension feeder by interpreting the individual clearance rate (CR) of 0.75 ml h^{-1} obtained by Dubois et al. (2003). They compared this value using that of active suspension-feeding serpulids and sabellids based on length-specific CR (per cm of ciliary band) multiplied by the estimated total length of ciliary band (presumably active) on the tentacular filaments of *S. alveolata* (500 cm), to obtain individual CR value (e.g. Riisgård et al. 2002). Their calculation, which shows that CR should theoretically be 250 to 900 times higher for an active suspension feeder, disregards some important aspects of the *S. alveolata* feeding processes:

(1) There is great inter-tentacle variability in particle processing, some particles being transported on the tentacles frontal surfaces directly toward the mouth, while others are transported toward the tentacle tips before being released into the water column (Dubois et al. 2005; Video clips #4 and #5). Hence, a bi-directional transport of particles makes the calculation of CR based on ciliary band inappropriate, and clearly overestimates the actual CR of *Sabellaria alveolata*.

(2) The estimations of *Sabellaria alveolata* CR (Dubois et al. 2003) were done with blocks containing ca. 1000 individuals, under the assumption that 100% of the individuals were filtering. Recent experiments based on photographic analysis of worm activity (S. Dubois & L. Barillé unpubl.) shows that ca. 50% of the worms are out of their tubes. Our preliminary CR value is thus under-estimated by a factor 2.

(3) Since the ecophysiological experiments were conducted on 225 cm^2 reef blocks, the CR estimations including inter-individual disturbance between tentacle crowns (Dubois et al. 2003) were probably lower than the optimal clearance rate of a single individual.

A more disturbing problem is the unsupported statement by Riisgård & Nielsen (2006; p.304) that the reef blocks used in our ecophysiological experiments may have been 'contaminated' by the sabellid *Fabricia stellaris* and that the CR data of Dubois et al. (2003) could be attributed solely to this sabellid. Apart from the fact that such a statement could only be made if the authors had access to the blocks used, or were themselves present during the experiments, Dubois et al. (2002, 2006) have unequivocally shown that only 'degraded' reefs, i.e. reefs with low abundances of *S. alveolata* tubes, numerous crevices and high surface heterogeneity, were colonized by high abundances of *F. stellaris*. The ecophysiological experiments described in Dubois et al. (2003, see their Fig. 2) used reef blocks extracted from 'platform' reefs, where absolutely no *F. stellaris* was found (Dubois et al. 2002). In addition, platform

stages generally have a very low abundance of other associated species that were carefully removed from each block.

CONCLUSION

The high-speed videos provided by Riisgård & Nielsen (2006) allow new insights into *Sabellaria alveolata* feeding processes, but the interpretation of these video observations is compromised both by SEM-TEM fixation artefacts and the sweeping dismissal of our findings in Dubois et al. (2005). Taken together, we conclude that the findings of both studies show *S. alveolata* to be an active suspension feeder, using both the GLC and GFC to modify current patterns around tentacular filaments and to collect particles from the water column. Particles collected on the frontal surfaces of tentacles are transported toward the mouth embedded in mucus, which plays a crucial role in the feeding process, as evidenced by our mucocyte distribution mapping.

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Editorial responsibility: Otto Kinne (Editor-in-Chief), Oldendorf/Luhe, Germany

*Submitted: November 10, 2006; Accepted: November 13, 2006
Proofs received from author(s): November 29, 2006*