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Limits and constraints: A comment on premises and methods in recent studies of particle capture mechanisms in bivalves

The moving seascape of research into bivalve suspension-feeding mechanisms presents challenges which suffice to redirect many curious biologists toward less choppy waters. The anatomical complexity of the various pallial organs alone leave many potential workers bewildered. The range of techniques employed is also rather spectacular, from clearance studies to endoscopy, flow cytometry, confocal microscopy, new histochemical techniques, and ciliary mapping, each with different constraints and generating different types of information. The scales involved simultaneously span the molecular and the organismic. And then there is the unfortunate tendency of the handful of workers in this field to form rigid schools of thought, periodically circling the wagons and shooting in all directions (e.g., Jørgensen 1996).

The paper by my colleagues Ward et al. (1998*a*) entitled ''A new explanation of particle capture in suspension-feeding bivalve molluscs,'' derives its ''new explanation'' of particle capture exclusively from video endoscopic observations. Many of the basic ideas were presented in Ward (1996), although the present paper concentrates somewhat more on the roles of the laterofrontal cilia/cirri. I would like to point out some questionable preliminary suppositions and generalizations, limitations of the methodology which limit depth of analysis, and indicate the most useful areas of contribution of endoscopy.

Basic suppositions—(1) The authors ''call into question'' (Abstract) and otherwise cast doubt (p. 742) upon vast bodies of information painstakingly built up by many other scientists, simply because the work was done on opened animals or on isolated organs. This echoes the concluding sentence of Ward (1996), and similar remarks in other endoscopy papers. Such broad dismissal does disservice both to these workers and to the field of research, (not to mention the domain of animal physiology as a whole) which has supplied much of modern knowledge in physiology and medicine from such preparations in various species. That circumspection is necessary whenever one makes the leap from in vitro to in vivo is obvious, but this is not the same as ''calling into question''. Several studies have even compared particle behavior on the mantle and palps using both endoscopy and half-shell preparations (Beninger and St-Jean 1997*a*; Beninger et al. 1997*b*), underscoring the similarity between the two. Indeed, the latter paper contained a separate section of the discussion, entitled ''Particle processing in intact vs. dissected specimens'', which specifically made this point. Pseudofeces transport on the mantle is an integral part of particle processing, and it occurs in the same way in both intact and opened specimens. While this does not hold true for aspects of processing on some gill types (notably the heterorhabdic gill), and for gill–palp interactions, a sweeping dismissal or broadly cast doubt is definitely not warranted.

In the following passage, (another dismissal of things not endoscopic), the authors themselves inadvertently provide a cogent example of both the limitations of endoscopy, and the advantages of alternate techniques (based on dissected specimens), to supply the missing information:

''It is more likely that particles are transported in a fine layer of mucus directly in contact with the frontal tracts (i). Particle transport in frontal-surface currents of ordinary filaments is probably an artifact caused by dissection and isolation of ctenidia (ii)'' (Ward et al. 1998*a*, p. 747).

In (i), a recent study clearly demonstrated mucociliary transport on the gill of *Mytilus edulis,* such that their proposition is not just likely, but rather very certain (Beninger et al. 1997*a*). Proposition (ii), (again a dismissal of in vitro studies) is rather incongruous since it was just such a study that allowed the visualization of the mucus layer above the periciliary space using confocal microscopy of gill fragments.

In some cases, such as the section entitled ''Particle Ingestion'' in Ward (1996), endoscopy is used to confirm data already obtained using other techniques. Here the goal was to determine which of two competing hypotheses (H_7 and H_8) of particle ingestion is correct: ingestion in a suspended state, or ingestion in a continuous mucus string. The original debate was whether mucus was involved at all in ingestion. This very point was examined using rapid dissection ($\leq$30 s) and histochemistry in Beninger et al. (1991), who concluded that in the five species studied, mucus always accompanied ingestion. This was called a ''slurry'' in Ward (1996).

(2) The authors base much of their reasoning on the ''low approach angle'' of particles impinging upon a gill surface. There are several problems with this, the first being that as particles approach the gill surface they encounter cilia-generated currents progressively, since the current velocity decreases from the cilia tip outward to approximately 2–3 times the cilia length, or 40–60 μm above the frontal cilia in the homorhabdic filibranch *Mytilus edulis* (Sleigh 1989; Beninger et al. 1997*a*). This means that in the region critical to this interpretation—the immediate vicinity of the epithelium—particles will not move in the straight lines necessary to constitute angles, but rather in curved trajectories which should be readily visible with a stated resolution of 3 μm.

(3) Another basic premise is that the resultant of the three flow vectors is as depicted in Fig. 1 in Ward et al. 1998*a*; this resultant becomes the ''low approach angle'' upon which much of the subsequent reasoning is based. Leaving aside (for the moment) the question of whether most bivalve gills present a functionally flat surface to complete this angle, or whether particles moving close to the gill epithelium do so in the straight lines required to ascribe angles, it should

be noted that this resultant can only be as depicted if the strengths of all three vectors are equal. The authors allow that this may not be quite true for heterorhabdic gill types, which present a strong dorsalward current due to the ciliary beat of the principal filaments (p. 744), but continue to depict this resultant exactly as if all vectors were equal (Fig. 1B). I would submit, that neither the authors nor anyone else knows what the relative strengths of these vectors are, nor is this information likely to be obtained anytime soon, since it will be extremely difficult to selectively de-activate the various ciliated regions. However, it is now known that bivalves have various mantle ciliation patterns which could participate in the creation of pallial currents to greatly different degrees (Beninger et al. 1999*a*, 1999*b*). Even the gill size : mantle cavity volume (which is vastly different in filibranchs, pseudolamellibranchs and eulamellibranchs) would considerably influence the relative strengths of the vectors. In particular, the presence of abundant suprabranchial chamber ciliation in some filibranchs and pseudolamellibranchs contrasts with its near-total absence in the highly-modified eulamellibranch gill. It should be noted that all of this information has been obtained by techniques other than endoscopy.

(4) The approach angle is determined by two lines, the particle trajectory and the gill surface. This angle will doubtless be greater in heterorhabdic species due to the gill plication, as the authors mention. Furthermore, the eulamellibranch gill, while not plicate in the classical sense, is however, undulated due to the interlamellar junctions, thus increasing the approach angle for the very large number of such species (*see below*). The range of possible approach angles will thus depend at least in part on the gill type, and as mentioned below, the homorhabdic filibranch type represents a very small number of bivalve families. With a stated resolution of 3 μm, it should be possible to actually measure the approach angles upon which most of the authors' arguments are based, yet no such measure is presented in either Ward (1996) nor in Ward et al. (1998*a*); although a 30° angle is cited (p. 748), it refers to lines drawn on a schematic diagram (Fig. 4C).

(5) The three types of particle capture kinematics shown in Fig. 2 are said to be for a "typical bivalve ctenidium". If "typical" is to be understood in its usual sense, this is anything but a "typical" gill. Figure 2 depicts a homorhabdic filibranch gill, which is the most simple type found in the nonprotobranchiate bivalves. It is unfortunately not very common; there are 11 such families in the 150 families of the nonprotobranchiate Bivalvia (data from Newell 1965); in other words, 93% of all nonprotobranchiate bivalve families do not present this type of gill organization. The relative percentages of species is likely to be even more biased against the homorhabdic filibranchs due to the extraordinarily large number of species of Heterodonta. Even allowing the reasoning concerning approach angles, it cannot be generalized to all suspension-feeding bivalves, as the title of the paper and conclusion state (Ward et al. 1998*a*); in fact, it would only apply to a small minority of the members of this class.

(6) The literature to date shows that despite some unifying principles, the particle processing systems of homorhabdic and heterorhabdic bivalves are vastly different, yet this distinction is blurred in the present paper, which focuses on the homorhabdic filibranch gill. I have already mentioned several reasons for distinguishing between the two systems (homorhabdic and heterorhabdic); here I would like to clarify a remark which may cause confusion. On p. 747 (Ward et al 1998*a*), the authors state: "Although hydrodynamic entrainment does not seem to occur on the ordinary filaments of bivalves . . . redirection of particles by hydrodynamic forces might operate on the principal filaments of those species with heterorhabdic ctenidia . . .". Besides, the fact that this was indeed demonstrated in Beninger et al (1992), the wording is a bit confusing, since both homorhabdites and heterorhabdites possess ordinary filaments.

Limitations of endoscopy

(1) Optical range and image quality: Endoscopy was first used to study bivalve suspension-feeding by Bernard (1974). The arrival of video technology allowed Dr. Ward and myself to refine the technique and determine its usefulness (Ward et al. 1991). It is an optical method of observation, and as such it possesses a particular useful range. Its resolution is initially limited by the diameter of the optical insertion tube, decreasing with small diameters. Although particles as small as 7 μm can be seen, especially if they scatter light strongly, the picture is often much less than optimal. It is not clear whether the authors use the accepted definition of resolution (Ward et al. 1998*a*), which is the minimum distance between two points at which they may be seen to be separate, given by one of the forms of Abbe's equation: $d_0 = 0.6\ \mu/n \sin \alpha$, where d_0 is the minimum distance, λ is the wavelength of the light source, n is the refractive index of the medium between the specimen and the lens (here the fiber optic bundle), and α is half the angle of acceptance. Thus d_0 depends not on magnification but on the numerical aperture of the lens ($n \sin \alpha$) when the specimen is in focus; the greater the distance between the lens and the specimen, as is often the case in the endoscope-pallial cavity environment, the smaller the numerical aperture, and the larger the value of d_0 (hence the lower the resolution). There are several boroscopes on the market currently used as endoscopes, and the authors should present the technique used to determine the stated resolution of 3 μm for their system.

It should also be noted that resolution alone cannot determine perception of detail in an image; contrast is also required (ergo highly scattering or colored particles), and singularly lacking in the uniform colors of most pallial organs. Hence, resolution values obtained under ideal calibration conditions would not completely translate to actual detail perception in endoscopic images of pallial organs.

In practice, it has even been difficult to determine whether particles are on the same or different filaments, or whether the same particle stays on one filament or crosses to another, or even to visualize the position and movements of the labial palp ridges (oyster tapes, Ward et al. 1994; Newell and Langdon 1996)—structures which measure several tens of μm in width. Such problems are totally incompatible with a stated resolution of 3 μm.

In addition to inherent optical limitations, the lens unit tends to accumulate mucus (which is omnipresent, but rarely

visible) when it is close to pallial epithelia (i.e., when resolution is greatest), obscuring the optics and necessitating frequent withdrawal for cleaning and re-positioning. However, the endoscope does allow the unique opportunity to manoeuver in a limited way within the pallial cavity, and to observe certain delicate phenomena which can only take place in an intact animal, such as gill-palp transfer of mucus-particle cords and palp processing (Ward et al. 1997; Beninger et al. 1997*b*)—something unattainable with any other current technique.

(2) Visual access: There are many crucial aspects of particle processing that simply cannot be observed using endoscopy in its present configuration. Ward et al. 1998*a* reports on a ''new explanation'' of particle capture in bivalves, but particle–cilia interactions were not and could not be observed using endoscopy. It is thus largely based on inference (dye and particle movement without visualized cilia), in contrast to its domain of maximum utility—particle transport—in which observations are direct. One reason is the image quality, as explained above, and the other is the low recording speed of the video systems used to date in endoscopy. Confocal microscopy of isolated gill fragments allows latero-frontal cilia—particle interactions to be seen (Silverman et al. 1996), and the associated high-speed video recordings allow the process to be documented and decomposed. Before calling into question such studies on isolated gill fragments, it would be best to recall that current video endoscopy cannot visualize either cilia or this process at all.

Another example of a crucial aspect of particle processing that cannot be accessed to any significant extent by endoscopy is the role of the various mucopolysaccharides. Beninger and St-Jean (1997*b*) present a unifying pattern in this aspect of particle processing by the very different systems. This information was obtained from 'altered' specimens: indeed, they were dead, fixed, stained, and at times sectioned on a microtome! It is unlikely that mucocytes change position or content as a result of any of the above . . . Similarly, different nonfused cilia types are now known to perform distinct processing functions (Beninger et al. 1999*a*, 1999*b*), yet none of them are visible using endoscopy.

(3) Subjectivity: The interpretation of events on endoscopy tapes is necessarily subjective, and should be understood as such. The extent to which each viewer is conversant with the concepts of anatomy, flow, ciliary beat, etc. will obviously affect his/her interpretations. Conclusions are often reached by consensus, in which the persuasiveness of some viewers can alter the interpretations of others. In addition, for some events which are only seen clearly several times, subjective selection of such sequences is necessary but not without danger. It is often necessary to edit many hours of tape to produce several minutes of clear images which actually show what the observers have determined to be ''typical'' aspects of particle processing. Recent studies underscore the subjective nature of the interpretation of video sequences of suspension feeding (Hart 1996).

It is important that those who have never viewed endoscopy tapes realize that one never sees images such as Fig. 3 in Ward et al. (1998*a*) and in Ward (1996). As mentioned in the figure legends, large white circles were placed over gill images; although they ''represent'' particles, they are not particles. Real images show a multitude of much smaller particles, exhibiting a range of behaviors, entering and exiting the visual field. The use of an endoscopic micrograph as background for these figures belies their schematic nature; they have the appearance of actual data, when in fact they are reconstructions of recorded observations.

Conclusion

The authors state that ''our model should be considered an overall principle for the mechanisms of particle capture in suspension-feeding, lamellibranchiate bivalves''. Yet they go on to say that the types of cilia and cirri will affect the ''small-scale dynamics of particle encounter and retention''. In the words of the melancholic Prince of Denmark, ''there's the rub''. Endoscopy cannot in fact access the underlying mechanisms for the processes of particle capture, transport, and selection, although it can (subject to the caveats presented above) observe the net result of the processes and suggest where and what techniques to use to seek the sequence. The underlying effector mechanisms are cilia and mucus, and calling the filament the ''capture unit'' does not change this; neither of them is readily accessible using endoscopy. Endoscopy is thus not a ''silver bullet'', but rather one useful technique, with its own intrinsic limitations, in the formidable array necessary to elucidate the complex and diverse phenomena of particle processing in bivalves. Some of the authors themselves have demonstrated the utility of combining endoscopy with other techniques, as in their confirmation and extension of Atkins' (1937) observation of the site of particle selection in bivalves with a heterorhabdic gill (Ward et al. 1997, 1998*b*), and other workers have shown how endoscopy can improve the reach of their own observations of aspects inaccessible to endoscopy (Beninger et al. 1993; Beninger and St-Jean 1997*a,b*). Information obtained from a host of techniques can provide different, and indispensable, pieces of the puzzle. This type of synergy among techniques, and open, thoughtful exchange among researchers, is the only approach which will, at some point in the future, allow a complete ''explanation'' of particle processing in bivalves.

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In vitro studies of particle capture and transport in suspension-feeding bivalves

Contribution of various techniques to our understanding of particle transport and selection by ctenidial organs of suspension-feeding bivalves—Particle processing mechanisms in suspension feeding bivalves can be divided into the following components: encounter, capture, transport, selection, and finally ingestion. Recent development of a number of new research tools or approaches has advanced understanding of the overall process. Three recent technical advances leading to better insights into particle processing have been the use of (1) endoscopic techniques (Bernard 1974; Ward et al. 1991, 1998*b*; Beninger et al. 1992, 1993; Ward 1996); (2) confocal laser microscopy (Silverman et al. 1996; Beninger et al. 1997); and (3) advanced modeling of isolated elements based on experimental video recording of the pallial organ system (Nielsen et al. 1993; Riisgård et al. 1996). These advances have driven modern reexaminations of pallial organ structure (Beninger and St. Jean 1997) and of ciliary movements (Silverman et al. 1997). In addition, numerical modeling (Nielsen et al. 1993) coupled with clearance studies provide a better understanding of the mechanisms underlying each of the component steps.

Visualization of the process in living, intact, bivalves has been impeded by the shell and specifically in the case of particle capture by the magnification and resolution necessary to critically observe the interaction of particles with gill elements or water currents created by those elements. The advent of endoscopy using a small-bore fiber-optics tube has provided valuable insight into the particle transport and particle selection mechanisms. Endoscopy allows visualization of net processes that are occurring. Endoscopy reveals the end result of some types of mucus secretions without observing the source of the secretion. Endoscopy also shows water currents and vectors produced by ciliary activity without seeing any particular cilia or cirral movement or their potential interactions with a particle (Beninger et al. 1992, 1993).

Endoscopy has afforded observations of large mucus strands (sometimes the width of a gill filament, 50–70 μm),