

Tunicate feeding filters

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This review discusses the structure and operation of the fine mesh ‘mucous’ feeding filters of tunicates. The function of the endostyle in producing the feeding filter and the different ways in which the filter is deployed are also described.

The fine structure of the filter includes new data, and the ultrastructural dimensions of the filter mesh and filament thickness are tabulated for the different tunicate groups. Histochemical data suggest that a peptide core is surrounded by a mucopolysaccharide sheath, and endostyle gland cell histochemistry and ultrastructure indicates protein synthesis. The construction of the filter by the endostyle was first considered in ascidians, and has been updated by observations on the simpler endostyle in salps, where there is evidence that secretions of gland cells pass to the bases of a fence of cilia, there to fuse and pass off the ciliary tips as fine filaments composing the filter net. Although all filters that have been examined when deployed have a rectangular mesh, reasons are given for supposing that when formed in the endostyle they have a square mesh in which both longitudinal and transverse filaments are of similar thickness and that the transverse filaments are stretched as the filter is deployed, so becoming thinner.

Finally, some ecological consequences of the filter parameters in the different tunicate groups are considered.

INTRODUCTION

The success of tunicates with almost 2000 ascidian species thus far described and probably 1000 or so yet to be described (P. Mather, personal communication), and over 200 pelagic thaliaceans and appendicularians, perhaps lies not in their eponymous outer covering (lacking in Appendicularia), but rather in the development of a complex and interesting structure, the endostyle.

Although shown on their figures of salps, *Pyrosoma* and some ascidians by several earlier workers including Savigny (1816), the tunicate endostyle was first described from salps collected on his *Rattlesnake* voyage by Huxley (1851). Huxley later observed the same organ in a small ascidian and so was able to confirm his view that the endostyle furnishes ‘a new and very remarkable distinctive character of the Tunicata’.

A modern looking ascidian fossil has been described from the early Cambrian (Shu et al., 2001) but tunicates certainly arose long before then. From the early tunicates, the endostyle is then found in acraniates (linked in both groups to the presence of iodine compounds). Lastly, it appears enlarged and more complex in the lamprey ammocoete larva where (in part) it finally changes at metamorphosis into the vertebrate thyroid.

The function of this new organ in tunicates was only understood 25 years later by Fol (1876), who saw that it secreted a fine filter that trapped food particles. Fol observed the expanded filter of the small salp, *Thalia democratica* by adding carmine particles, and also showed part of the filter of *Doliolum* sp. In both he noted that it was made of *gewundener schleimfaden* (spiral mucous threads or filaments). Since Fol’s paper, the filter has

almost always been termed a mucous filter, without further examination of its structure. Very little is understood of the way in which the endostyle constructs the filter in any tunicate, nor indeed, is much known of the arrangement and composition of the filter itself. In this brief review, only the particle collecting function of the endostylar filter net will be considered, but it should be kept in mind that as well as secreting a food-trapping filter the endostyle secretes enzymes (Godeaux, 1989) as well as containing cells which accumulate iodine (Barrington, 1957) and iodinating peroxidases (Fredriksson et al., 1989). It may well have other functions also.

1. DEPLOYMENT OF THE FILTER FROM THE ENDOSTYLE

Almost all tunicates feed by collecting food particles on a delicate filter of regular mesh constructed in the endostyle. The only exceptions known are the deep sea members of the ascidian families Ascidiidae and Octacnemidae, which have enlarged oral siphons, much reduced endostyles and feed in part or wholly as carnivores hence do not produce a filter net (Monniot & Monniot, 1991). In all other tunicates, a filter net is produced, and in salps, it has been possible to view the net within the endostyle before it has been deployed (Figure 1). In ascidians, salps and appendicularians, ultrastructural observations have been made on the filter when deployed. The endostyle is innervated by four cholinergic nerve branches that run along its length, giving off lateral rami. In salps, each longitudinal nerve lies under the two rows of gland cells on each side of the endostyle. Secretion of the filter net is under nervous control, and can be inhibited, but as a rule

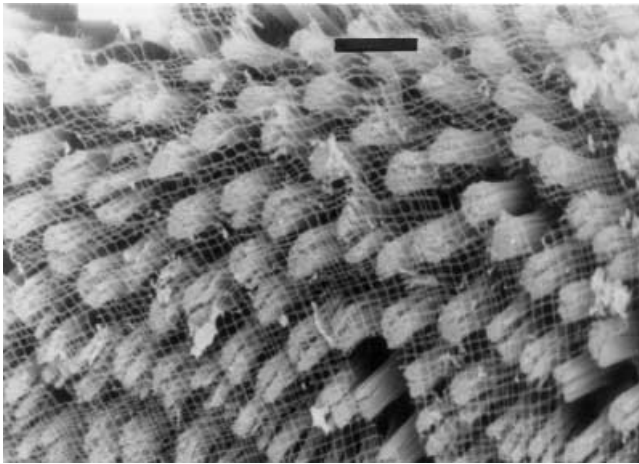


Figure 1. Filter net within the endostyle of *Salpa fusiformis*, overlying columnar cilia. Scale bar: 10 μm . From Bone et al. (2000).

tunicates feed continuously (e.g. Fiala-Medioni, 1978). An exception is provided by the active salp *Salpa cylindrica* where underwater observations by Madin (1974) have shown that the filter net is only deployed for about half the time, in contrast to other salps that he observed where it was always in place. As the net leaves the endostyle to form the particle-trapping filter, it is deployed a little differently in the different groups (Figure 2).

In ascidians (Werner, 1959) and pyrosomes it passes outwards along the length of the endostyle at right angles to its long axis and 'fishes' as it lies draped over the gill slits of a supporting ciliated branchial basket. The cilia of the branchial basket pull water which enters the inhalent siphons across the filter, and towards the exhalent siphon. The filter itself is slowly passed back towards the gut along the dorsal groove, to be rolled up and passed into the oesophagus with the particles captured. A detailed description of the process under different particle concentrations has recently been given by Armsworthy et al. (2001). In doliolids and salps, by contrast, the net is moved forwards inside the endostyle towards its anterior end, only leaving it at the ciliated peripharyngeal bands where it passes upwards around each band to join dorsally forming a conical filter lying freely in the pharynx (Figure 3). Thus it resembles and 'fishes' like a plankton net. Lastly, in appendicularians, particles already collected by a food-concentrating filter in the house are trapped on a free-standing mucous filter lying obliquely in the pharynx, presumably deployed from the endostyle by the peripharyngeal bands.

Water flow through the filter is driven by cilia in pyrosomes, doliolids, ascidians and appendicularians, but by muscular action in salps. Few measurements have been made of the velocity of flow across these filters. In the salp *Pegaea confoederata* (Bone et al., 1991), it is $1.7 \times 10^{-3} \text{ms}^{-1}$, where the pressure drop across the filter is higher than in ciliary driven systems. In doliolids it is between $1.1 \times 10^{-5} \text{ms}^{-1}$ and $4.5 \times 10^{-4} \text{ms}^{-1}$ (Bone et al., 1997; D. Deibel, personal communication). Lastly, in the appendicularian *Oikopleura vanhoeffeni* it is $5.6 \times 10^{-4} \text{ms}^{-1}$ (Acuña et al., 1996). In all tunicates it is sufficiently low that the flow at the filter is in the Reynolds number range $< 10^{-4} \text{ms}^{-1}$, hence flow will be highly viscous and laminar (see Silvester, 1983).

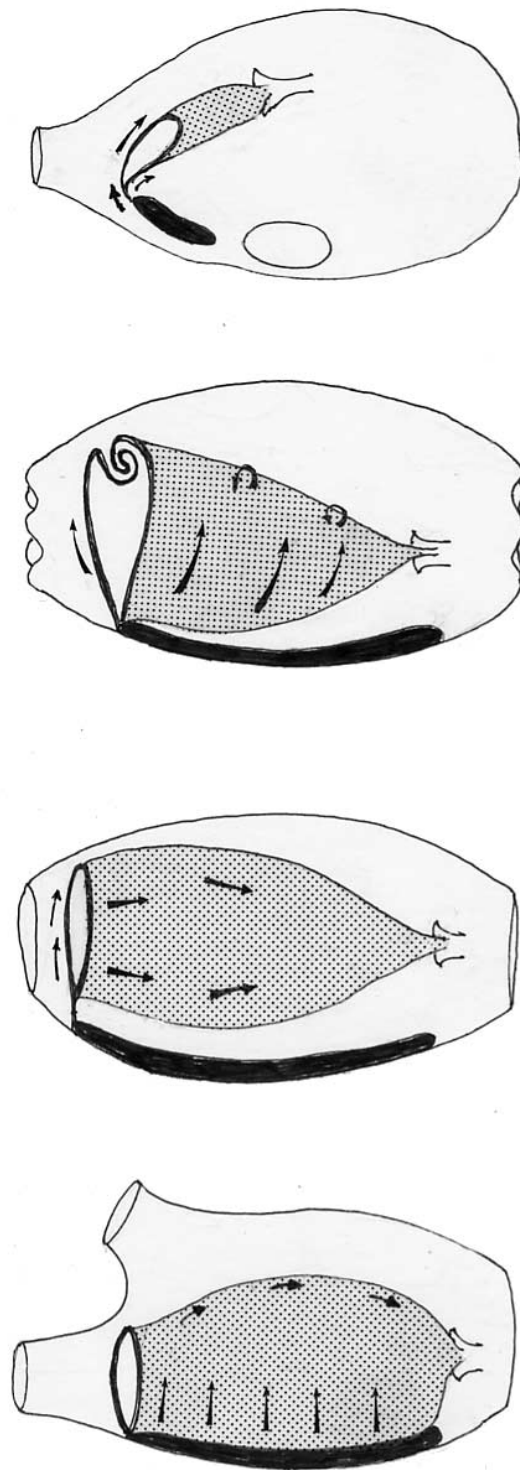


Figure 2. Schematic diagrams showing passage of feeding filter (dotted) from endostyle (black). The peripharyngeal bands are in heavy line, and seem not to be directly concerned with filter deployment in ascidians. From the bottom: ascidian, (*Pyrosoma* is similar); salp; doliolid; appendicularian. Note: to different scales.

2. STRUCTURE OF THE FILTER

The first ultrastructural views of tunicate filters were given by Flood & Fiala-Medioni (1979) in ascidia, their account was extended and amplified subsequently (Flood & Fiala-Medioni, 1981). At about the same time, Monniot

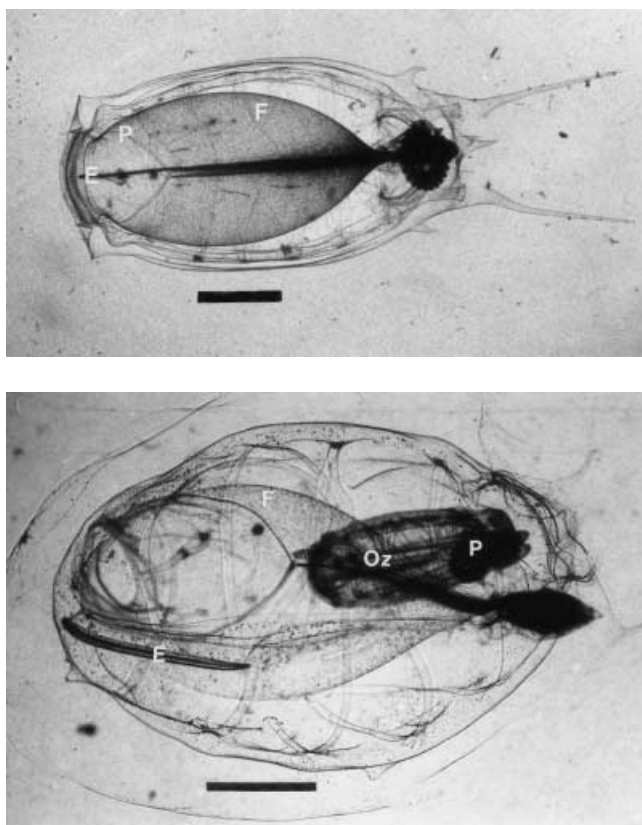


Figure 3. Pharyngeal filters in the salp *Thalia democratica*. (A) Single large oozoid; (B) large blastozoid with young oozoid. Only the filter of the blastozoid is deployed. E, endostyle; F, filter; P, pharynx; oz, oozoid. Scale bar: 5 mm.

(1979) figured the filters in four ascidian species, and Silver & Bruland (1981) obtained fragmentary views of salp filters from faecal pellets. All authors agreed that the filters were composed of fine filaments arranged in a regular rectangular mesh. Different preparatory techniques undoubtedly led to different (unknown) degrees of shrinkage which will be considered in a later section. Flood & Fiala-Medioni (1981) considered that little shrinkage had taken place after ascidian filters were air dried on carbon-coated grids, whilst deep-etched salp filters frozen in the pharynx when deployed (Figure 4) may also be considered to be little shrunken. Table 1 shows the different mesh sizes and filament thicknesses for tunicate pharyngeal filters obtained by direct measurement in electron microscope preparations.

Filament thickness differs between the transverse and longitudinal axes of the mesh, and both in the salp and appendicularian filters is larger than in ascidians, presumably because these unsupported filters have to be stronger than those on the gill bars of ascidians.

In the shadowcast preparations of the ascidian filters made by Flood & Fiala-Medioni (1981), no thickness increase was seen where the filaments crossed each other, suggesting that the two filaments were linked at the level of the core. It is not clear whether this is really the case. For in deep-etched filters fixed in the pharynx of the small salp *Thalia democratica* there is at higher magnification an unexpected substructure, seen in Figure 5. Within both of the types of filaments crossing each other at right angles,

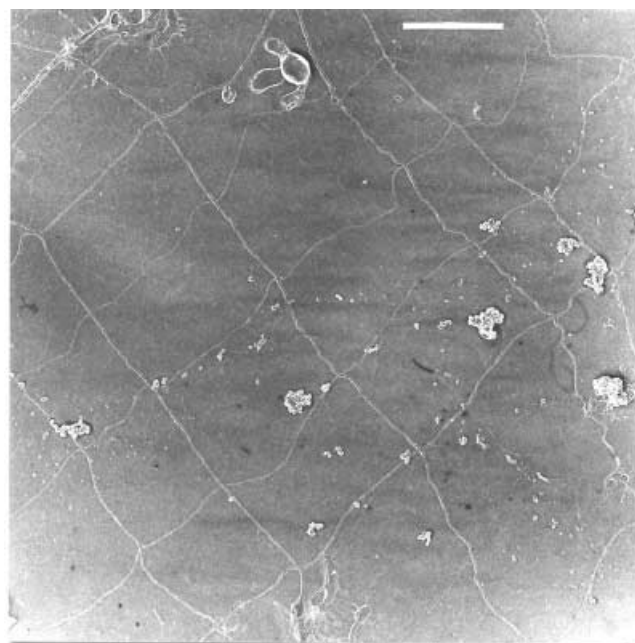


Figure 4. Pharyngeal filter from *Thalia democratica* oozoid, plunge frozen and deep-etched after initial chemical fixation. Apparent thickness of filaments probably related to degree of stretching from square to rectangular mesh. Scale bar: 1.0 μm .

which are apparently tubular, there are transverse internal bridges, spaced some 60–70 nm apart. The whole has to some degree the appearance of sectioned avian bone, the two internal faces separated by ‘columns’ broader at their bases than in the mid-region between them.

At times the columns are oblique to the main axis of the filament in both directions, so forming what seems like a strut system comparable to those of structural girders. It is not clear whether these appearances signify a structural system within the filament resisting breakage in use, or whether they are in part at least, artefactual. Where two filaments overlap it is sometimes possible to see that the cores are separate. Unfortunately, such views of the salp filter have not been obtained for other species. In sections of the filter of the ascidian *Phallusia mammillata* Flood & Fiala-Medioni (1981) observed (their figure 11) that the filaments appeared tubular rather than solid, as in the filter of *T. democratica*.

3. COMPOSITION OF THE FILTER

Olsson (1963) examined the endostyles of the appendicularian *Oikopleura dioica* and the ascidian *Corella parallelogramma* in a general histochemical study of endostylar structure including also amphioxus and the lamprey ammocoete. He concluded that the gland cells in both tunicates synthesized and secreted protein and mucopolysaccharides perhaps combined as muco- or glycoproteins. Subsequently, Flood & Fiala-Medioni (1981) concluded from their own histochemical tests, that the strands of the filter were probably composed of a peptide core surrounded by a sheath of polysaccharides, perhaps as an acidic mucoprotein or mucopolysaccharide. No more recent studies have been made of filter composition,

Table 1. *Tunicate pharyngeal filter measurements.*

Species	Mesh width (μm)	Method of measurement	Source	Filament thickness (nm)
Salps				
<i>Salpa fusiformis</i> (within endostyle)	1.3 \times 1.3	SEM	Bone et al. (2000)	200 \times 200
<i>Pegea confederata</i> (in pharynx)	0.7 \times 4.0	SEM	Bone et al. (1991)	100 \times 50
<i>Thalia democratica</i> (in pharynx)	0.9 \times 0.9	TEM on frozen etched replicas	This paper	30–40
<i>S. fusiformis</i> or <i>Pegea socia</i>	0.3 \times 5.4	SEM of faecal pellets	Silver & Bruland (1981)	
Pyrosomas				
<i>Pyrosoma atlanticum</i> (tetrastoid)	0.6 \times 0.6	SEM of cilia in endostyle	Bone et al. (2000)	—
Doliolids				
<i>Doliolum nationalis</i>	0.4 \times 0.45 ?		Bone et al. (1997)	29
Ascidians				
<i>Ciona intestinalis</i>	640.5 \times 415	TEM of air dried	Flood & Fiala-Medioni (1981)	15 \times 22.5
<i>Ascidella aspersa</i>	0.79 \times 297	TEM of air dried	Flood & Fiala-Medioni (1981)	10 \times 20
<i>Phallusia mammilata</i>	0.59 \times 0.31	TEM of air dried	Flood & Fiala-Medioni (1981)	15 \times 25
<i>Microcosmus sabatieri</i>	0.78 \times 0.32	TEM of air dried	Flood & Fiala-Medioni (1981)	10 \times 15
<i>Halocynthia papillosa</i>	0.65 \times 0.17	TEM of air dried	Flood & Fiala-Medioni (1981)	15 \times 40
<i>Styela plicata</i>	1.96 \times 0.5	TEM of air dried	Flood & Fiala-Medioni (1981)	10 \times 20
Appendicularians				
<i>Oikopleura vanhoeffeni</i>	3.26 \times 6.35	TEM of air dried	Deibel & Powell (1987)	203

SEM, scanning electron microscopy; TEM, transmission electron microscopy. Mesh values means, corrected for shrinkage where necessary.



Figure 5. Filament substructure in another region of the same filter as in Figure 4. Scale bar: 0.5 μm .

although, as Godeaux (1989) pointed out, protein secretion was indicated both by the histochemical reactions of the gland cells of the endostyle, and by their ultrastructure. Flood (1981) suggested that the mesh structure of tunicate filters was inherent in their chemical composition, but this view has not been confirmed.

4. CONSTRUCTION OF THE FILTER

The actual construction of the filter within the endostyle has been considered by Holley (1986), and by Bone et al. (2000). Holley (1986) used transmission electron micro-

scopy to examine ciliary spacing within the different zones of the endostyle in the ascidian *Ciona*, and inferred the direction of ciliary beat from the disposition of the basal ciliary apparatus. He concluded that a combination of ciliary combing and organized mucus secretion amongst the very regularly-spaced ciliated cells sufficed to explain the production of the filter. He suggested that a zone of cells with very long cilia at the base of the endostyle secreted the transverse filaments of the net, whilst the longitudinal filaments were superimposed upon these, being secreted by the two very obvious rows of glandular cells. These interpretations have been called in question by a study of the (simpler) salp endostyle using scanning electron microscopy (Bone et al., 2000).

In the salp endostyle the filaments are secreted only by the two rows of glandular cells, whilst the basal cells with long cilia play no part in filament secretion but instead, the cilia keep separate the filters produced on each side of the endostyle. Further, the spacing of the transverse filaments (produced by the upper row of gland cells), is brought about by the ciliary fence immediately above these gland cells. The electron dense granules secreted come to lie on the bases of the ciliary shafts and there fuse to pass down to the tips of the cilia whence they depart to form the transverse filaments. The longitudinal filaments are formed lower in the endostyle and pass over the ciliary fence spacing the transverse filaments, coming to lie at first obliquely then at right angles to the transverse filaments. The filter thus formed passes upwards in the salp endostyle, to lie within it above the cells with columnar cilia (Figure 1), as they do within the ascidian endostyle (Flood & Fiala-Medioni, 1981).

Holley (1986) regarded the difference in thickness between longitudinal and transverse filaments of the filter as implying a qualitative difference between them. It seems more probable, however, that the difference is brought about by different degrees of stretching of identical filaments in the filter along different axes.

5. THE SHAPE OF THE FILTER MESH

It is striking that in both salps and ascidians, the filter has a rectangular mesh when deployed, whilst within the salp endostyle the net has an almost square mesh (Table 1). There seems no particular functional advantage in either arrangement, and we suggest that *within* the endostyles of both (perhaps all tunicate) groups, the filter is made with square meshes of equal filament width. In ascidians the filter moves from the endostyle to become draped over the branchial basket. When it is pulled from the endostyle by the branchial ciliary transport system to cover the branchial basket, the filaments in the direction it is pulled are stretched so that the filter mesh becomes rectangular, and the filaments thinner in the direction of stretch. We have confirmed Werner's (1959) observation in *Clavelina* that the filter is produced directly from the endostyle along its course across the inner surface of the basket (Figure 2), passing out laterally to the dorsal groove where it is rolled up and taken down to the oesophagus. The pressure drop across the filter in ascidians is only around 1–2 Pa (Jorgensen et al., 1953) whilst in salps (where the flow across the filter is produced by muscular rather than ciliary action) it appears to reach at times during the jet cycle at least ten times this value. It seems likely that in both, stretching takes place to make a rectangular mesh net from an elastic net with originally more or less square meshes.

In the portions of the salp filter seen within the endostyle, the mesh is nearly square and in *Salpa fusiformis* the filaments making up the mesh are equally thick at around 180–200 nm. The deployed filter in the pharynx has been examined in only two species. Here the filaments are thinner than in the endostyle, between 31 and 39 nm in *Thalia democratica* (Figure 3), whilst in the larger *Pegea confoederata* up to 100 nm. In the latter, the portions of the filter in the pharynx show a rectangular mesh with thin and thick filaments, whilst others, perhaps unstretched, have square meshes with thicker filaments (Bone et al., 1991). Interestingly, calculations of the porosity of the filters in both ascidians and salps give a very similar result, although the pressure drop across the two filters is very different since the pump in ascidians is driven by cilia rather than by muscles. Presumably to cope with this pressure drop, and because they are unsupported, the filaments of the salp filter are thicker than those of ascidians (Table 1).

6. TUNICATE FILTERS IN OPERATION

In the sections above, it has been seen that in ascidians, the mesh of the expanded filter is reasonably well-known, as it is in two salp species and a single appendicularian. However, this does not make it clear what range of particle sizes the filter is capable of capturing efficiently in each group. This is an important question, for the size range of particles captured determines the likely ecological effects each group may have.

In all appendicularia there is an external filter on the house (with the curious exception of *Oikopleura longicauda* which lacks it) that prevents very large particles entering the inner food-concentrating filters. Acuña (1994) has suggested that the vertical distribution of *O. longicauda*

results from its ability to feed on larger particles than other species. These external filters vary largely in mesh in different species (see Flood & Deibel, 1998) and in some permit quite large diatoms and dinoflagellates to enter and be collected by the inner filters (Deibel & Turner, 1985).

In other tunicates the upper limit of particle size (provided entry is possible via the inhalent aperture), is set by the dimensions of the oesophagus: larger particles are rejected with the filter enclosing them by 'coughing'. Possibly more significant ecologically are the lower limits set by the performance of the filters of the different tunicates. To answer this question several different approaches have been used, each has merits and difficulties. The earliest experiments were on salps, and the studies on salps conveniently exemplify the different approaches.

a. Particle depletion experiments using algae of different sizes or labelled particles

By feeding a size range of particles such as different-sized algal species or fluorescent microbeads, the minimum particle size that the filter mesh retains can be obtained. Furthermore, it is possible (at least in principle) to use this direct measurement of performance as a check upon preparation artefacts in views of fixed filter nets. As with all techniques there are some problems, signalled by Madin & Kremer (1995) in a useful review of salp feeding rates. Chief amongst these are the requirement to keep the salps in containers, and to use artificially high particle concentrations. As these are gelatinous, delicate and quite large organisms, they are difficult to obtain and maintain in good condition for feeding experiments.

There is general agreement between the four studies that have examined the feeding performance of different salp species using Coulter counter measurements: Harbison & Gilmer (1976), Harbison & McAlister (1979), Caron et al. (1989) and Kremer & Madin (1992). Particles above 3.0–4.0 μm are retained with 100% efficiency, but the retention efficiencies of smaller particles seem different in different species. There are several possible (and quite different) explanations for the capture of small particles with lesser efficiency than particles larger than, say, 2–3 μm . These will be considered later.

In three species Caron et al. (1989) using algae and cyanobacteria of three sizes, subsequently found that 5.0 μm particles were retained with 100% efficiency, those 2.0–2.5 μm were collected at 33% of the efficiency for 5 μm particles, and particles of 0.7 μm only at some 3% of the efficiency for 5 μm particles. Similarly, Kremer & Madin (1992) found that in different species, 1.0 μm beads were retained with around 15% efficiency, and 2.5 μm beads with 60% efficiency. The most recent survey of salp feeding (Madin & Deibel, 1998) concluded that the retention efficiency data indicated that cells smaller than 2 μm were not significantly grazed by most salps, whilst above this threshold size, retention efficiency rose sharply.

It seems reasonable to infer that the smaller the salp, the smaller its endostyle and hence the smaller the mesh of the filter secreted. The question of change in retention efficiency with salp size has been examined by several authors. The data available suggest that different species differ. In the

first study of salp particle retention, Harbison & Gilmer (1976) observed that 'There seems to be no relationship between per cent retention of different particle sizes and size of the salp'. However in later work Harbison & McAlister (1979) found that although for *Cyclosalpa affinis* there was no significant correlation between salp length and particle size retention, such a correlation was found for *Cyclosalpa floridana*.

Kremer & Madin (1992) on the other hand, did not find clear evidence in *Pegea bicaudata* that minimum particle size captured was related to salp length. It may be significant in this regard, that in *Thalia democratica* the young oozoids still attached to the placenta within the pharynx of the mother blastozoid, only deploy their feeding filter when the mother does not (Figure 3B). This suggests that even if the filter of the much smaller young oozoid could trap smaller particles than the filter of the mother blastozoid, this would not be cost-effective since the mother has already finely filtered the water reaching the young oozoid.

b. Determination of effective filter mesh size from examination of particles found in the gut and in faecal pellets

Kremer & Madin (1992) examined the retention efficiencies for different sizes of polystyrene beads and concluded that even small salps (12 mm *Salpa aspera* blastozoids) were incapable of retaining particles of 1.0 μm with more than 15% efficiency, though 1.7 μm beads were retained with around 50% efficiency. As they pointed out, these results are not in good accord with the estimates for the filter mesh of *Pegea confoederata* (4.14 \times 0.7 μm) given by Bone et al. (1991). They considered that the estimates from electron microscopy were probably biased by shrinkage (but note that if the filter operated as a simple sieve, shrinkage of 300% would be required, to produce the retention efficiency calculated). They also suggested that the pressure across the filter might distend the meshes sufficiently to account for the discrepancy. This effect is considered below. Working with the large salp *Cyclosalpa bakeri* in the subarctic Pacific, Madin & Purcell (1992) examined particles in the gut with scanning microscopy, finding unidentified spherical 0.7 μm cells but no cells of the spherical cyanobacterium *Synechococcus* (\sim 0.7 μm) although these were numerically dominant in the water. Presumably those cells in the gut had been shrunken during preparation (see next section). Several workers (e.g. Silver & Bruland, 1981; Matsueda et al., 1986) have collected salp faecal pellets in sediment traps and examined them with scanning microscopy. Matsueda et al. (1986) identified 26 coccolithophorids and 12 larger algal spp. in faecal pellets, ranging in size from 1.3–200 μm . Caron et al. (1989) examined salp faecal pellets using epifluorescence microscopy as well as scanning microscopy and noted significantly that bacteria, chlorococcoid cyanobacteria and nanoplankton were found in the faecal pellets mainly as clumped cells, though some nanoplankton cells were also found singly.

c. Direct measurements of salp filter mesh by electron microscopy

Although views of the general *shape* of the feeding filter (Figure 3) are relatively easy to obtain by feeding dense algal or other suspensions (e.g. in salps Bone et al., 1991,

2000; Madin & Deibel, 1998) it is very much more difficult to obtain well-fixed salp filters to examine mesh size directly. In salps or doliolids the freely suspended filter is extremely hard to obtain in expanded condition to determine operative mesh size, particularly as it is elastic and suffers distortion and shrinkage to a disputable degree upon fixation.

The first ultrastructural views of salp filters were obtained by Silver & Bruland (1981) who examined faecal pellets by scanning microscopy, finding clear views of remnants of the filter nets. They examined faecal pellets caught in particle traps that came from either *Pegea socia* or *Salpa fusiformis* (both species being abundant at the time), and found regular-spaced nets whose dimensions were 1.9 \times 0.2 μm . Subsequently, Bone et al. (1991) were able to fix the filter of *Pegea confoederata* as it was deployed in the pharynx, obtaining a rectangular mesh of 3.3 \times 0.57 μm .

Naturally the crux here is what correction to apply for shrinkage during preparation. Silver & Bruland (1981) used critical point drying following chemical fixation and dehydration to acetone but did not apply any correction for shrinkage. Flood & Fiala-Medioni (1981) observed around 38% shrinkage in critical-point dried ascidian filters, compared with those directly air dried on electron microscope grids, which were presumably little shrunken. Other workers (on other material) have variously estimated shrinkage up to 65% (see Robards & Sleytr, 1985). Bone et al. (1991) assumed 20% shrinkage for their filters which were plunge frozen from 70% ethanol after chemical fixation and then critical point dried, giving corrected values of 4.14 \times 0.71 μm .

Deibel & Powell (1987) showed that the mucous filter in *Oikopleura vanhoeffeni* faecal pellets had shrunk by some 40–50% compared with filters that had been taken from the pharynx and air dried on grids. This suggests that Silver & Bruland's values should be increased to the same degree, giving fairly similar values to Bone et al. (1991) viz 3.8 \times 0.4 μm . Note that even after having been increased to correct for shrinkage, these values are apparently still too small (if the filters are regarded as simple sieves) to accord with the Coulter counter data discussed above.

Possibly the least shrunken images for the salp filter net yet obtained have been given by deep-etched replicas of plunge frozen filters rapidly chemically fixed (by injection of osmium tetroxide solutions into the filter) whilst it was deployed within the pharynx. In the small salp *Thalia democratica* this gave mean side length 0.923 μm (N=29) for the mesh (Figure 6). Mostly the mesh appears to be rectangular, in what seem to be the least deformed of the meshes, 0.74 \times 1.23 μm . Note that the longer mesh sides vary between 0.66 μm and 3.2 μm , strongly suggesting that the filaments are elastic, and that the mesh size does increase as the filter is expanded by incurrent flow. Measurements of the circumference of the expanded filter in life however, place limits on this lateral size increase in the filter normally deployed.

d. An indirect method

The filter within the endostyle, and the spacing of ciliary fences

Some information about filter mesh dimensions can be obtained from scanning microscope studies of the endostyle. It is sometimes possible to see the pharyngeal filter

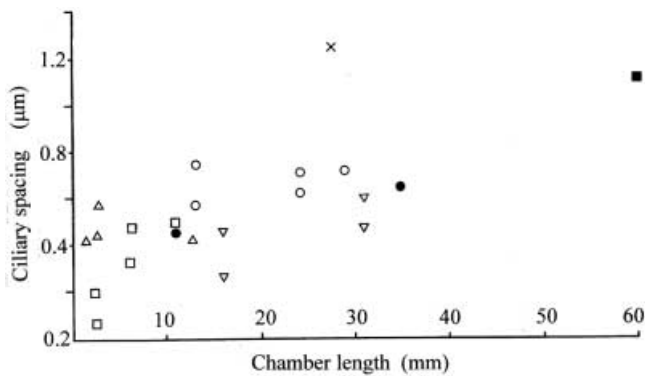


Figure 6. Filter mesh size inferred from outer ciliary fence spacing in salps of various species and sizes. Where two symbols are given from salps of the same species and chamber length, they represent two separate measurements of ciliary spacing at different points along the endostyle. Filled symbols: measurements from portions of net within the endostyle. Δ , *Ihleia punctata*; ∇ , *Thalia democratica*; \square , *Salpa fusiformis*; \circ , *Pegea confederata*; \times , *Cyclosalpa pinnata*.

within endostyles that are secreting it, as a well-formed square net. However, even if this is not possible, since it is the spacing of the cilia within the outer (upper) ciliary fence in the endostyle that determines the distance between the vertical filaments of the net (Bone et al., 2000), this spacing can be measured. It is usually a relatively simple matter (in salps) to dissect the critical-point dried endostyle and to measure the spacing of the outer ciliary fence. What is more, this can be done even upon museum specimens long fixed in formalin, though of course, the problem of the amount of shrinkage remains. We have examined the ciliary spacing that sets the mesh width in a variety of salp species, and in different stages and sizes of several species. Our results are seen in Figure 5.

Although we have only examined a few sizes of a few species, it seems that the larger the salp, the greater the ciliary spacing, and the larger the filter mesh. However, both in the ascidians examined by Flood & Fiala-Medioni (1981) and in the two salp species examined, the mesh in the functioning filter is rectangular, rather than square, with the thicker filaments forming the shorter sides. The ratios of the two sides of the filter mesh varied from around 5:1 in *Pegea* to almost square at 3:2 in *Thalia democratica*.

The filter in the endostyle, next to its upper margin, has (in *Salpa fusiformis*) a mesh of $1.3 \mu\text{m}$, corresponding to a similar spacing of the outer fence cilia (Bone et al., 2000). Assuming 38% shrinkage after glutaraldehyde or formalin fixation followed by critical point drying, this would indicate a $1.79 \mu\text{m}$ mesh *in vivo*.

In *Pegea confederata* of approximately the same chamber length, the square mesh was $0.75 \mu\text{m}$ and making the same assumption about shrinkage would have been *in vivo* some $2.09 \mu\text{m}$. It is not clear how the net is constructed within the endostyle, moved forwards, and passed around the peripharyngeal bands, where the two halves join up dorsally and ventrally to form the filter. Nevertheless, it seems reasonable to suppose that it leaves the endostyle to form the filter at the same mesh size as it is in the endostyle. The filter when formed is certainly elastic, and it

increases in circumference at its widest point in the pharynx by some 20–25% of the value at the peripharyngeal bands as water flows through it. Here then, the mesh would be expected to be 125% or so of the net in the endostyle. Accepting $2.09 \mu\text{m}$ for the endostylar value after correction for 38% shrinkage during preparation, some $2.61 \mu\text{m}$ would represent the mesh width over most of the length of the filter deployed in the pharynx.

7. RETENTION EFFICIENCY IN OTHER TUNICATE GROUPS

a. Pyrosomas

Neither pyrosomas nor doliolids have been studied in as much detail as salps. No retention experiments have been carried out on pyrosomas, and a single measurement only has been made of ciliary spacing in the tetrazoid endostyle, suggesting a filter mesh spacing of $0.6 \mu\text{m}$ (Bone et al., 2000). Since tetrazoids are closely similar to sessile ascidians in their feeding mechanism and might be expected to have a similar mesh to those described by Flood & Fiala-Medioni (1981) in several ascidian species this seems a reasonable value. Even if particle capture by the tetrazoid filter is considered simply as a sieving process, and assuming the same mean filament thickness as in ascidians, and the longer side of the mesh as $0.8 \mu\text{m}$, this gives a reasonable porosity of around 95%.

b. Doliolids

Knowledge of the doliolid filter is only indirect, as with pyrosomas, although it is known that it operates rather differently to the salp filter, since particles are trapped between two layers of filter (like the Hardy plankton recorder) which are rolled up to enter the oesophagus (Bone et al., 1997). The doliolid filter has not been successfully fixed for electron microscopy, but distorted views of an *Isochrysis galbana* cell trapped within the filter of a 2.5 mm *Doliolum nationalis* phorozooid suggested a mesh size of some $0.62 \times 0.74 \mu\text{m}$ (corrected for 38% shrinkage during preparation). The filter fibres were 29 nm thick (Bone et al., 1997). However, assuming (as seems reasonable) that the pressure drop across the filter is similar to that in ascidians (i.e. some $1.2\text{--}1.3 \text{ Pa}$) calculations from the modified Tamada–Fujikawa equation (Silvester, 1983) suggest a larger filter mesh of some $1.2 \times 1.5 \mu\text{m}$ (Bone et al., 1997). Deibel (1985) found that areas of doliolid abundance were poor in particles $0.2\text{--}0.5 \mu\text{m}$ in diameter, suggesting a smaller mesh or a different way in which the filter captures particles. Although Tebeau & Madin (1994) found that *Dolioletta gegenbauri* trophozooids only captured $1.0 \mu\text{m}$ plastic beads at 30% of the efficiency with which they could capture $2.5 \mu\text{m}$ beads, it seems possible that smaller doliolid stages at least are capable of trapping sub-micron particles efficiently.

c. Ascidians

In eight ascidian species, the ultrastructure of the filter nets deployed on the inner surface of the branchial basket were described by Flood & Fiala-Medioni (1981).

They showed that these filters are made of very fine filaments 10–40 nm thick and have rectangular meshes 0.2–0.5 μm wide and 0.5–2.2 μm long. In the species examined, the ratio of longer to shorter mesh sides varied from around 17:1 to 2:1. The longer sides of the rectangular mesh (disposed at right angles to the axis of the endostyle) consisted of thinner filaments than the shorter sides. They pointed out that the porosity (open area) of such filters is extremely high, up to the remarkable level of 98%! Unfortunately, in view of the recent recognition of the relationship between appendicularia and aplousobranchs (Stach & Turbeville, 2002), the single aplousobranch species examined (*Clavelina*) was not well enough fixed for measurement of the filter mesh, nor have any aplousobranchs been examined subsequently. Although Flood & Fiala-Medioni found that in *Styela plicata* the longer sides of the mesh were more than twice as long as in the other species they examined, the shorter sides were a little longer, making the mesh still small enough to trap bacteria. Particle clearance experiments (Randlov & Riisgård, 1979) on four species (including two of those examined by Flood & Fiala-Medioni) showed that more or less spherical algae around 2 μm diameter were retained with 100% efficiency, and 1 μm particles with around 70% efficiency. Jorgensen & Goldberg (1953) found that ascidian filters retained 1–2 μm graphite particles with 100% efficiency. These values are in quite good agreement with the expected retention (on a sieving model) from the measurements made by Flood & Fiala-Medioni suggesting that their measurements are approximately correct for the mesh dimensions *in vivo*. At present, no experiments have been made on the retention of sub-micron particles by ascidians, although Bishop & Bone (unpublished) have found that several species retain *Sepia* ink particles.

The melanin particles of *Sepia* ink were used as a tracer for appendicularian particle capture by Flood et al. (1990, 1992). They used transmission electron microscopy of resin embedded melanin to determine the mean diameter of the melanin spheres to be 0.102 μm (SD: $\pm 0.021 \mu\text{m}$). It is not clear what degree of shrinkage may have taken place (the authors suggest a possible 30%, thus mean diameter *in vivo*: 0.146 μm). It is possible however, that it may have been greater than 30%. Our own initial measurements on *Sepia* ink air dried on stubs gave a mean value of 0.216 μm , assuming the lesser value of 20% shrinkage this would be some 0.26 μm *in vivo*. Since the ink failed to pass a 0.25 μm filter, but passed readily through a 0.45 μm filter, around 0.3 μm is likely to be near the *in vivo* diameter. It is therefore scarcely surprising that ascidian filters capture such ink particles. Ink particles from a second (smaller) *Sepia* appeared to be of lesser diameter, and passed a 0.2 μm filter: they were not captured by the *Ciona* filter.

We have so far been unable either to obtain views of the filter within the endostyle, or of any fence of endostylar cilia comparable to those spacing the filter seen in salps. The construction of the filter in ascidians is unclear. The similarity of the endostyles of ascidians with those of salps, however, suggests that they must operate in a similar way, even if not all ciliary zones can be equated in both groups. For example, the uppermost (outermost) rows of cilia in the endostyles both of *Ciona* and salps consist of peculiar

columns from large multiciliated cells, it seems to be these which move the net out of the endostyle, or along within it. Further attempts to examine the secretion of the ascidian filter are continuing.

d. Appendicularians

Of the three appendicularian families, something is known of retention efficiencies in two oikopleurids, a very little of feeding in fritillariids, and nothing at all of the Kowalewskiidae (since these lack an endostyle, they must feed differently to almost all other tunicates, and fall outside this review). Appendicularians are capable of collecting sub-micron particles or even colloids (Flood et al., 1992) but to do so, it seems that their filters must operate in a somewhat different way to those of other tunicates. Compared with other tunicates, appendicularians have relatively small endostyles and pharyngeal filter dimensions. Presumably this reflects the efficient pre-filtration by the food-concentrating filters within the house, driven by muscular movements of the tail.

Oikopleurids

Filter feeding in the large oikopleurid *Oikopleura vanhoeffeni* has been examined in some detail by Deibel and his colleagues. Deibel & Powell (1985) succeeded in the difficult task of fixing the unsupported pharyngeal filter *in situ* and placing it on electron microscope grids for measurement. The filter was made up of a more or less rectangular mesh, much less regular than in other tunicates examined. This irregularity was perhaps hardly astonishing considering the difficulty of extracting the unsupported filters from the pharynx. What was surprising, however, was that not only were mesh width and length much larger than in other tunicates at mean values of 6.35 μm wide and 3.26 μm long, but also, that all fibres forming the net were similar in diameter and much thicker than in other tunicate nets examined at some 176–294 nm (Table 1; mean for four individuals: 203 nm). Despite the fibres being so thick, the porosity of the filter in *O. vanhoeffeni* was similar to that of other tunicates (91%) since the meshes were large.

The retention efficiency of this (relatively) large mesh filter has been examined by Deibel & Lee (1992), who obtained a surprising result. Using mixtures of 0.6 μm , 1 μm , 3 μm and 7 μm fluorescent beads and sampling the water and the beads in the gut and faecal pellets, they determined that the smallest were trapped with an efficiency around 45% of the 3 μm and 7 μm beads, which had a retention efficiency of near 90%.

Plainly, as Deibel & Lee pointed out, if the filter was simply acting as a sieve, some explanation is called for to account for the ability of the animal to collect particles smaller than the mesh size. Two kinds of explanation have been suggested.

First, oikopleurids live in complex houses that they secrete, and before entering the pharynx to pass across the pharyngeal filter, the particle passes first across a coarse inlet filter and thence into a three-layered structure with two filtering screens. This filter concentrates the particles in the inhalent flow and at intervals,

these are then sucked into the pharynx by the activity of the spiracular cilia. Fenaux (1986) and Flood (1991) have given descriptions of the structure of the house and the process of particle collection is summarized together with new information in Flood & Deibel (1998). In *O. vanhoeffeni* the food concentrating filter screens have a rather precise rectangular mesh of only $0.22 \times 0.104 \mu\text{m}$ (Deibel et al., 1985), whilst in *Oikopleura dioica* the food concentrating filter has a mesh of $0.15 \times 0.98 \mu\text{m}$ (Flood, 1978).

One possibility might be that the particles collected by the extremely small mesh of the food concentrating filter become aggregated in the process, thus forming large enough aggregations to be collected by the pharyngeal filter. Whilst it seems likely that at least some degree of aggregation takes place in the food concentrating filter, Flood et al. (1992) observed that oikopleurids were capable of filtering colloidal melanin particles from *Sepia* ink (determined by electron microscopy to have a mean diameter of $0.17 \mu\text{m}$). A large degree of aggregation would have to take place if the pharyngeal filter were to sieve out such particles.

However, aggregation by the food-concentrating filter does not suffice to explain collection of sub-micron particles by the pharyngeal filter. Oikopleurids will continue to feed outside their houses after light anaesthesia with MS222 (Q. Bone, personal observation), when the only particles collected are therefore those trapped directly by the pharyngeal filter. Under these conditions *Sepia* ink is readily collected by the pharyngeal filter.

Thus it appears that the simple sieving model is inappropriate for oikopleurids, as Acuña et al. (1996) have indicated (see next section).

There is a further complication, examined by Bedo et al. (1993) in feeding experiments with *O. dioica*. They found that this (smaller) species could capture $0.2 \mu\text{m}$ spheres as efficiently as $0.75 \mu\text{m}$ beads, and in this study, Bedo and his colleagues were able to determine the subsequent fate of the particles collected by the food-concentrating filter. A proportion was trapped by the pharyngeal filter, and thence passed to the gut and faecal pellets, whilst others passed the filter and exiting the pharynx via the spiracles entered the house, either to become attached to its inner walls, or to be recycled back to the food-concentrating filter. Beads of $0.2 \mu\text{m}$ as well as $0.75 \mu\text{m}$ beads were easily collected by the pharyngeal filter, and appeared in the faecal pellets. However, the ratio of the $0.2 \mu\text{m}$ beads to those of $0.75 \mu\text{m}$ in the faecal pellets was between 34% and 62%. Interestingly, although larger individuals filtered a greater quantity of water than the smaller, no evidence was obtained for the smaller individuals feeding more efficiently on the $0.2 \mu\text{m}$ beads than the larger.

Fritillariids

Flood et al. (1992) using diluted *Sepia* ink found that *Fritillaria borealis* could feed efficiently on particles smaller than $0.45 \mu\text{m}$, but neither the food concentrating nor the pharyngeal filters have yet been visualized in detail (see Flood & Deibel, 1998).

8. THE CAPTURE OF SUB-MICRON PARTICLES

Not all tunicates are capable of capturing sub-micron particles efficiently. No salps are known to be able to capture sub-micron particles or colloids, although doliolids perhaps can, and oikopleurid appendicularians certainly can do so. These differences evidently influence the roles that the different pelagic tunicates play in the marine food web. They are discussed here.

How do appendicularians and (perhaps) doliolids manage to capture sub-micron particles? Various suggestions have been made by different authors. Earlier work was based on the view that the filters acted as simple sieves, so that where the filter mesh was known, (for example in several species of sessile ascidians) it was a simple matter to decide the minimum particle size that would not pass the mesh. In five of the species examined by Flood & Fiala-Medioni (1981) this means that the filter should retain particles above $0.32 \mu\text{m}$, whilst in the sixth species, *Styela plicata*, the filter should retain particles above $0.5 \mu\text{m}$. Using a particle spectrum between $1.0 \mu\text{m}$ and some $7 \mu\text{m}$, Randlov & Riisgård (1979) found experimentally that $1.0 \mu\text{m}$ particles were retained with about 70% of the efficiency of $2\text{--}3 \mu\text{m}$ particles, which were completely retained. Neither they nor other workers on ascidian filtering have examined the retention of sub-micron particles. Recent studies by Bishop & Bone (unpublished) are attempting to do so, using *Sepia* ink and various sub-micron sizes of polystyrene beads.

Later work has been much influenced by theoretical papers on the hydrodynamics of filter feeding in fluids by Rubenstein & Kohl (1977), Silvester (1983) and Loudon & Alstad (1990). These workers followed Wallengren (1905) who first pointed out that if the fibres making up the filters were sticky, then even particles much smaller than the mesh of the filter could be captured when they struck the fibres and stuck to them.

Direct interception and attachment of particles to the filter fibres is not the only mechanism involved, as LaBarbera (1984) pointed out. Different 'aerosol' particle capture mechanisms may occur if the particles are for various reasons (e.g. density difference from the fluid) able to cross streamlines through the filter thus increasing their chance of striking a filter strand. All such mechanisms favouring the capture of particles smaller than the mesh size naturally depend on the attachment of the particles to the filter strands.

Evidently, the velocity of flow through the filter, its mesh size, and the diameter of the fibres making up the mesh will all have significant effects on such particles, and these parameters can be measured, though not without difficulty. But a most important factor, the degree of adhesivity or 'stickiness', must evidently also have a large effect on the capture of particles small enough to pass through the filter mesh. This is by no means an easy quantity to measure, or even guess! Silvester (1983) assumed that any particle striking a filter fibre immediately became attached to it, i.e. 100% adhesion. It is important to notice that a later test of filtering by caddis fly nets (Loudon, 1990) concluded that this adhesion value was a very large overestimate, and that theory and practice could best be reconciled by assuming that only some 0.2% of particles

striking the filter stuck to it. Alternatively, as she remarked, '... adhesion is very strong and the theory does not adequately describe particle capture'.

Silvester (1983), Loudon (1990) and Loudon & Alstad (1990), were concerned with the feeding of caddis fly larvae (Trichoptera: Hydropsychidae) which deploy filters constructed of silk, with a much larger mesh size and larger fibre thickness than those of any tunicate. In different species, hydropsychid meshes range from some 40 μm to over 500 μm , with fibres up to nearly 30 μm thick. The tunicate filter is apparently made of mucopolysaccharides surrounding a protein core, and it is not yet known whether it is similar in all tunicates or even whether it is sticky or not.

It is certainly striking that the data available from ascidians and salps suggests that their filter performance is adequately described (so far as we know) by simple sieving alone. Direct observation of feeding in doliolids shows that (algal) particles 5 μm in diameter touching the inner surface of the filter in the pharynx do not necessarily stick to it, since it is often possible to see such particles rebounding from the filter surface.

Deibel & Lee (1992) found experimentally that the appendicularian pharyngeal filters could trap much smaller particles than its relatively coarse mesh, as the analysis of *Oikopleura vanhoeffeni* by Acuña et al. (1996) later indicated. In their analysis they followed Silvester (1983) in assuming 100% adhesion. With the important provisos that the sub-micron particles are not much aggregated by the food-concentrating filter upstream of the endostylar filter (see above), and that the dimensions of the pharyngeal filter are correct, it seems clear that the fibres of the appendicularian pharyngeal filter must be relatively sticky, and that particle capture is by an effective combination of sieving and attachment to the fibres of the filter.

According to the results of particle retention experiments no salp seems capable of collecting significant quantities of sub-micron particles. Even if sieving alone accounted for the performance of the salp filter, it would be able with much lesser efficiency to capture particles smaller than the mesh over the main body of the expanded filter. Indeed, it does so. This is because mesh size along the length of the filter decreases (see below). It seems difficult therefore to avoid the conclusion that the filters of ascidians and salps are composed of slightly different materials to that of appendicularians and that the former (if sticky at all) are not so sticky as are the latter.

The open area fraction or porosity of the filter nets in the ascidians examined by Flood & Fiala-Medioni (1981), was over 90%, and (in *Styela*) reached the astonishing value of 97–98%. These filters are supported on the branchial basket, but even in the salp *Pegea confoederata* where the filter is unsupported and the filaments thicker, porosity is 91% (Bone et al., 1991). In the smaller *Thalía democratica* it is around 95% for much of the filter in the pharynx. This will naturally decrease as the filter narrows approaching the oesophagus, so increasing the resistance to flow across it, and decreasing the relative volume of the total flow across it. For some 68% of its length in *Salpa fusiformis*, the filter has the same or larger circumference than at the peripharyngeal bands, and then it narrows fairly abruptly so that the mesh size is narrower than at

its origin at the peripharyngeal bands. Similarly, in *T. democratica* 61% of the filter length has the same or larger circumference before narrowing to the oesophagus. Not enough is known of the different parameters of the filter needed to calculate flow velocity accurately. But making reasonable assumptions in *P. confoederata*, where porosity over the larger part of the filter is 91%, as it begins to narrow so that the mesh is half the size, porosity declines to 75% and mean flow velocity is under half that of the main part of the filter. Thus, change in mesh size and flow across it would alone account for the capture of low percentages of particles under 2–3 μm by the posterior region of the filter. No doubt even under natural conditions of particle abundance, the last part of the filter mesh just before the oesophagus will be more or less choked by particles collected earlier, and flow through its reduced meshes will be so reduced as to fail to trap sub-micron particles. As noted earlier, Caron et al. (1989) examined salp faecal pellets collected from salps feeding in natural seawater, and found that the smaller items (cyanobacteria, bacteria and nanoplankton) were clumped together as would be expected if they had been collected in the last part of the filter.

9. OPERATION OF TUNICATE FILTERS AND THEIR ROLE IN THE ECOSYSTEM

In more recent years, there has been increased appreciation of the importance of gelatinous animals in the planktonic ecosystem (Briand, 2002), and the importance of gelatinous filter feeders such as salps in oligotrophic areas has been recognized (Acuña, 2001). The filtering capacity of pelagic tunicates has a fundamental and important impact on the marine food web. Not only do pelagic tunicates filter large volumes of water, but they are often very abundant, forming blooms that may cover many tens of km^2 . All are able to reproduce rapidly, either by asexual budding in the Thaliacea (see Gibson & Paffenhöfer, 2002), or by very rapid generation time (for some appendicularian species around 24 h in tropical waters, Hopcroft & Roff, 1995), hence all may have a significant effect on particulates in the water they graze.

Estimates of the resident water volume filtered by doliolids suggest this may be 100% or over d^{-1} , so leading to an inverse relationship between doliolid abundance and that of heterotrophic and autotrophic flagellates (see Deibel, 1998).

Acuña (2001) suggested that large gelatinous oceanic salps are adapted by their watery bodies and large filtering surfaces to oligotrophic conditions. The vast amounts of seawater filtered by the salps as they cruise slowly along permits them to exist in oligotrophic oceans, where picoplankton predominate (Chisholm, 1992). Although no salps are able to capture picoplankton efficiently, as far as is known, there may be a trade-off between reducing mesh size and being able to filter large volumes of water, such that even relatively inefficient retention of sub-micron particles will be a successful strategy. Large specimens of *Cyclosalpa bakeri* are able to filter no less than 101h^{-1} (Madin & Purcell, 1992). Such large scale filtration has important effects on the ecosystem. Not only do salp populations directly affect the phytoplankton, but they are important in recycling material and exporting it to

deeper layers (see, e.g. Zeldis et al., 1995). An extended analysis by Li (2002) has shown that small nanoplankton (accessible to oceanic salps) form a more or less stable background to the decrease of picoplankton and increase in larger nanoplankton as phytoplankton biomass increases.

Sommer & Stibor (2002) discussed the role of tunicates in pelagic food webs, in comparison with copepods and cladocerans. They supposed that salps and doliolids filtered the entire size range of particles from very small colloids to large phytoplankton chains, whilst appendicularians were usually considered to be microphagous.

Consideration of what is known of salp filter retention efficiency suggests that it is only appendicularians that are capable of filtering down to colloidal levels. The experiments with *Oikopleura dioica* by Bedo et al. (1993) have demonstrated unequivocally that in this species filtration is non-selective down to 0.2 µm, as in the larger *O. vanhoeffeni* (Flood et al., 1992). As Gorsky & Fenaux (1998) observed, the high retention efficiency for picoplankton allows appendicularians to use a food resource not available to other filter feeders (such as copepods or salps). What is more, their discarded houses act as bacterial 'greenhouses', providing an important source of organic carbon for other organisms (Alldredge, 1976; Silver & Gowing, 1990). Appendicularians themselves are consumed by fish and especially by fish larvae, in some cases forming the sole diet.

As Sommer & Stibor (2002) remark, it is unclear why tunicates do not entirely dominate the zooplankton, given their ecophysiological advantages. In fact, as these authors emphasize, traditional sampling gear heavily underestimates tunicates and renders their numbers in plankton surveys unreliable.

Salps are consumed by a wide variety of fish and also by chelonians (see Harbison, 1998), but their most important role in the oceans is probably the production of faecal pellets, which export biogenic carbon directly to deeper layers (Madin, 1982; Caron et al., 1989; Fortier et al., 1994). Salp faecal pellets are dense, packaged within a delicate membrane, and are often very abundant in sediment traps.

Attempts to estimate the amount of this vertical organic carbon flux are uncertain, but range from 6–100% of benthic carbon demand. Where salps are feeding on small coccolithophores in oligotrophic waters, a significant contribution may be made to carbonate flux from the surface to deeper layers (see Andersen, 1998).

Sessile ascidians are not usually considered to play an important role in the marine food web as a result of their filtering capacity, yet in certain situations they may do so. For instance, Petersen & Riisgård (1992) have examined the filtering capacity of a population of *Ciona intestinalis* in a shallow fjord, concluding that in late summer, the ascidian was potentially a key organism controlling phytoplankton in a bay at the head of the fjord.

10. CONCLUDING COMMENTS

Filter feeding in tunicates has been examined from a variety of aspects, but whilst in several sessile ascidians, the operating mesh size of the pharyngeal filter is known

with some accuracy, as are its functional capabilities, this is only partially true for pelagic tunicates. Sessile ascidians are easier to obtain and to maintain in physiological condition in small aquaria, and both experiments on them, and the determination of the mesh of their filters spread out on and supported by the branchial basket, are of course both much easier. Nevertheless, even in sessile ascidians, neither the way in which the filter is produced by the endostyle, nor the lower size limit of efficient particle capture are yet well understood. Also it is still not clear whether their filters are able to trap colloids. The observations on pelagic tunicates briefly considered in this review have involved patient and difficult work on exceptionally delicate structures within delicate and difficult to culture animals. It is little wonder that direct observations on the pharyngeal filters of pelagic tunicates are few.

Molecular evidence (Swalla et al., 2000) now seems to show clearly that salps, doliolids and pyrosomes (the three groups of the Thaliacea) were separately derived from phlebobranch ascidians. Most recently, appendicularians have been indicated as a sister group to aplousobranch ascidians, all other tunicates whether pelagic or sessile forming a clade comparable to the appendicularians + aplousobranchs (Stach & Turbeville, 2002). Studies of appendicularians have suggested that their filters may operate rather differently to those of other tunicates. If appendicularian filters are really different in properties to those of phlebobranch ascidians and their thaliacean derivatives, it would be reasonable to examine aplousobranch ascidians, before concluding that appendicularians are uniquely different.

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