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# Locomotory and feeding effectors of the tornaria larva of *Balanoglossus biminiensis*

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**Abstract**

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The tornaria ciliary bands and oesophagus were examined ultrastructurally to identify the neural components that control larval behaviour. The circumoral ciliary band is known to be innervated in part by fibres from the apical plate and adoral nerve centres. Within the band itself, however, the only neurones we could find were multipolar cells, an unusual cell type with apical processes that traverse the surface of the band. Similar cells occur in the circumoral bands of echinoderm larvae. The tornaria telotroch has a much larger nerve, but no neurones were found either in the band or nearby, so the source of the fibres in the telotroch nerve remains unknown. In addition to having different innervation, the two bands also respond differently to cholinergic agonists, which elicit telotroch arrests but have no visible effect on the circumoral band. The oesophagus has a well-developed musculature and an extensive nerve plexus. During feeding, the oesophagus repeatedly contracts, forcing excess water out along two lateral channels prior to swallowing. These channels are also sites of gill slit formation, so there is evidently a continuity between the water bypass mechanism of the larva and that of the postmetamorphic juvenile.

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## Introduction

The planktotrophic larvae of echinoderms and hemichordates are dipleurula-type larvae of basically similar design (Nielsen 1998) with ciliary bands that function in both feeding and locomotion. Neurociliary control is undoubtedly important for both functions, but is poorly understood. The larval nervous system is diffuse and simple in structure, consisting of sets of ciliary nerves and small ganglion-like apical and adoral concentrations of neurones (Burke 1983; Burke *et al.* 1986; Bisgrove and Burke 1987; Dautov and Nezlin 1992; Moss *et al.* 1994; Chee and Byrne 1999). Past studies of the nervous system have typically relied on electron microscopy (EM) and fluorescence methods for various neurotransmitters, including catecholamines, dopamine, serotonin, or neuropeptides. Correlating the two types of data is often difficult, however, because neither provides a complete picture of the system as a whole. Neurones cannot always be distinguished with certainty from non-neuronal cells using EM data alone and fluorescence studies are limited by the availability of methods for specific transmitters. In addition, consistent results are not always obtained from behavioural studies of neurociliary control because it is inherently difficult to interpret subtle changes in ciliary beat in small, fast-moving larvae.

The enteropneust tornaria larva is especially poorly known. The only recent study of the larval nervous system as a whole is that of Dautov and Nezlin (1992), though Lacalli and West (1993) reported the presence of multipolar cells with apical specializations in the circumoral band. The present study extends previous work with an EM study that includes a detailed examination of a series taken through the midventral region. This allows individual cells to be traced and provides some new conclusions regarding larval innervation patterns. Nevertheless, the results are not sufficiently comprehensive to resolve all outstanding questions, an indication that a good deal of further work is required.

## Methods

Tornaria larvae of *Balanoglossus biminiensis* (Willey) were collected from the plankton in shallow water in front of the Florida State University Marine Station at Turkey Point, FL, where they are generally common in late summer. Two enteropneust species occur locally, *B. biminiensis* and *B. aurantiacus*. Gilmour (1982) raised the former and describes differences between the larvae, which are slight, but can be used for identification.

For transmission EM (TEM), larvae were fixed by the semisimultaneous method: larvae in seawater were first relaxed in a 1 : 1 mixture of seawater and isotonic magnesium chloride. An equal volume of 2% glutaraldehyde in 0.2 M sodium cacodylate with 6% sucrose was added dropwise and, after 2 min, the total volume was doubled again by addition of 4% osmium tetroxide. Specimens were left for 1 h in this mixed fixative, then washed thoroughly in distilled water, stained for 10 h in 2% aqueous uranyl acetate at 65 °C, dehydrated and embedded in Spurr's resin. Sections, cut with diamond knives, were collected on slot grids provided with formvar support films, typically four sections to a grid, and photographed without further staining. Prestaining specimens before embedding is extremely useful for serial work, especially when low-power micrographs free of contamination are required. Two advanced larvae, ≈ 750 μm long, were sectioned, but because of their large size, the blocks were trimmed to include only the ventral surface of the body and the oesophagus. For the most thoroughly examined specimen, sagittal sections were cut at 5-μm intervals until the oesophagus was reached; a serial series was then cut from there to the midline, a distance of about 35 μm.

For scanning EM (SEM), larvae were fixed following a method devised by Quentin Bone (personal communication): initial fixation was for 1 h in a 1 : 1 : 1 : 2 mixture of 4% formaldehyde, 25% glutaraldehyde, 0.5 M sodium cacodylate and seawater. Specimens were then postfixed in 1% osmium tetroxide in seawater, washed in seawater, then distilled water, dehydrated to 70% alcohol, stained in saturated uranyl acetate in 70% alcohol for 1 h, dehydrated further in alcohol, then in acetone, and critical-point dried before being mounted on stubs.

For behavioural observations, larvae were videotaped swimming freely in small chambers or attached to a suction pipette as described by Gilmour (1989); feeding responses were observed by adding *Dunaliella*. Drugs were tested by their addition to tethered larvae during video sessions, or to groups of freely swiming larvae in depression wells.

## Results

The tornaria (Fig. 1A,B) has two main ciliary bands, a highly convoluted circumoral band for feeding and a telotroch for locomotion. The circumoral band divides the ectoderm into separate oral and aboral domains. The former, the oral field, forms a set of concave ciliated grooves that channel food particles to the mouth. The grooves (Fig. 1C) are noticeably deeper than those of echinoderm larvae. The aboral epithelium has a characteristic patchwork appearance; small areas devoid of cilia are surrounded by interconnecting domains containing epithelial cells with short cilia. *Balanoglossus biminiensis* also has two smaller bands: a short postoral ventral band and a secondary telotroch. The ventral band begins below the postoral transverse portion of the circumoral band and stops just short of the telotroch (Fig. 1D). It resumes below the latter and connects with the secondary telotroch (Fig. 1E). Secondary telotrochs have been reported from various tornarias (Burdon-Jones 1957), but their function is not known. Both the ventral band and the secondary telotroch are densely ciliated, with short cilia, and they look essentially identical in external view. In contrast, the telotroch cilia are arranged in circular clusters (Fig. 1D), each arising from a single telotroch cell.

### *Ciliary band ultrastructure and innervation*

The circumoral bands (Fig. 2A,B) are formed of closely packed cells that are uniciliate and taper at their apices. The accessory centriole is located on the downstream side of the cilium, as in echinoderm bands (Nielsen 1987), so the direction of cilium beat can be determined from sections. A small ciliary nerve occurs at the base of the band (Fig. 2C), usually as a single tract near the centre. In the regions we searched, the only neurones in the band belonged to a class known as 'multipolar cells', described previously by Lacalli and West (1993). These are flask-shaped with multiple basal processes, a basal body but no cilium, and an array of processes that radiate from the apex and traverse the surface of the ciliary band (Fig. 2D–F). We have examined the distribution of multipolar cells only in the midventral part of the pre- and postoral bands. Here they occur in a single column precisely in the centre on the band, as in Fig. 2(B), and are more numerous in the preoral band, with one cell every 5 μm, compared with one cell every 10–12 μm in the postoral band. Two subtypes occur that differ in overall electron density. The paler subtype (Fig. 2D) was roughly twice as common as the dense subtype (Fig. 2E). The cells are sufficiently numerous for their basal processes, though short, to account for most of the neurites in the ciliary nerves. Scattered vesicles occur in the neurites but no specialized terminals or synapses were observed.

The telotroch (Fig. 3A) differs from the circumoral band in a number of respects. Its cells are multiciliate and the cilia are arranged in circular clusters (Fig. 1D). Much of the volume of each cell is occupied by rootlets, two per cilium, which penetrate deep into the cytoplasm. The ciliary nerve is large and is divided into several tracts by the basal endfeet of the telotroch cells (Fig. 3B). Transverse and tangential sections of the telotroch were examined for other types of cells. None were found, except for mucus cells positioned at

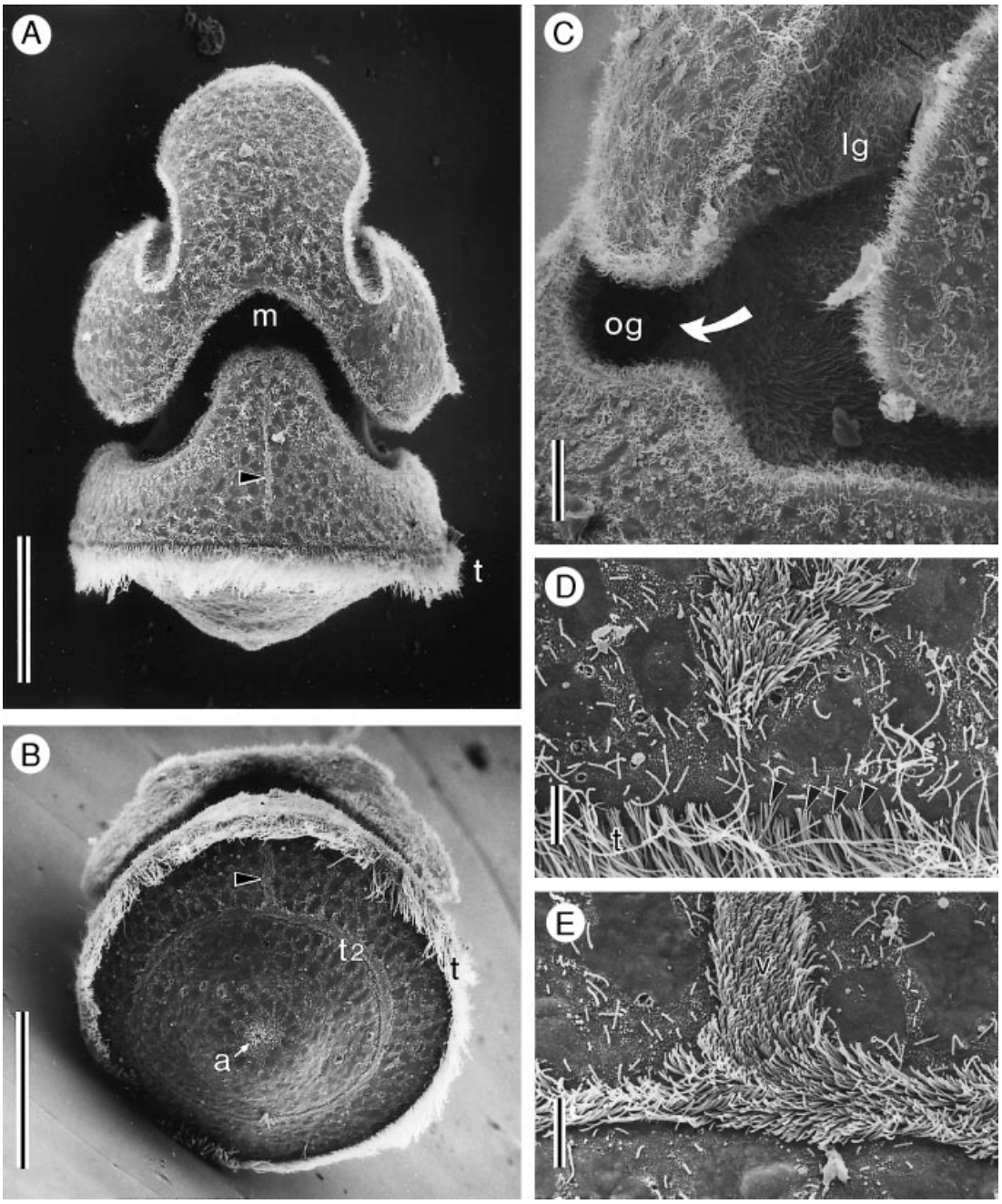


**Fig. 1**—**A**, Ventral view of an advanced *Balanoglossus biminiensis* tornaria. The circumoral ciliary band runs along the margin of the concave grooves (or sulci) of the oral field. These approach the mouth (m) symmetrically from either side, as an inverted V. The pre- and postoral portions of the circumoral band pass, respectively, just above and below the oral region. The telotroch (t) encircles the entire body caudally. The midventral band is indicated by the arrowhead. Scale bar 200 μm. —**B**, Posterior view of a larva of about the same stage as (A) to show the anus (a), telotroch (t), secondary telotroch (t2) and the continuation of the ventral band (arrowhead). Scale bar 200 μm. —**C**, Lateral view of the junction between the longitudinal meridianal groove (lg) and the oral groove (og) of the oral field to show the depth of the latter where it begins its approach to the mouth (in the direction of the arrow). Scale bar 50 μm. —**D**, The midventral band (v) just above the telotroch (t). The latter consists of distinct tufts of cilia (arrowheads), one from each cell. Scale bar 10 μm. —**E**, The connection between the ventral band (v), as it continues caudal to the telotroch, and the secondary telotroch; a detail of (B). Scale bar 10 μm.

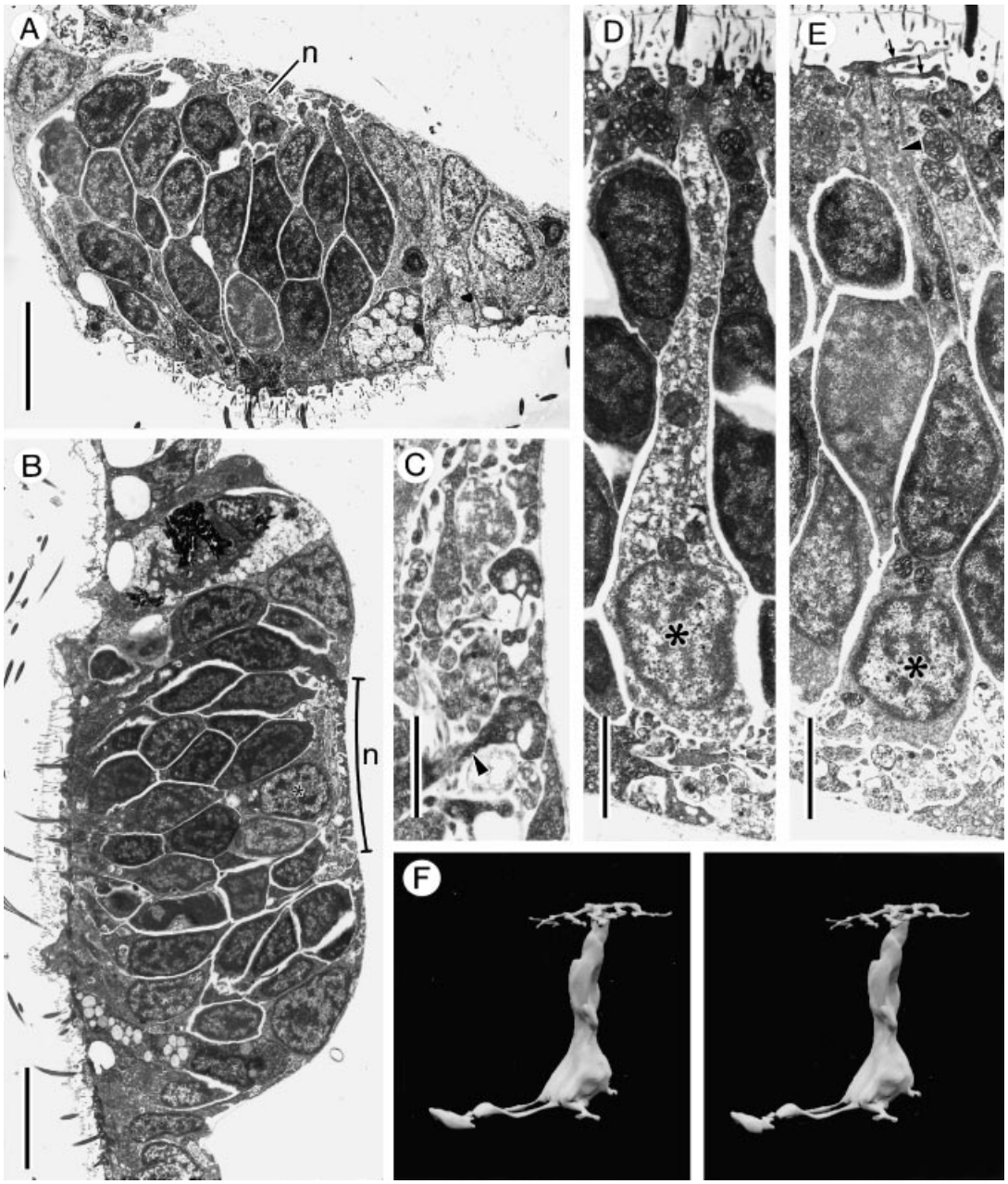


**Fig. 2**—The circumoral ciliary band. —**A**, A section through the preoral transverse band near the ventral midline. The margin of the oral field forms a distinct lip with the band cilia on the inside surface. All the circumoral band cells are uniciliate and of similar type, and there is a small ciliary nerve (n). Scale bar 5 μm. —**B**, The postoral ciliary band, also near the midline. Here the band is flat rather than inwardly curved. It is orientated vertically in the figure, but should be tilted ≈ 45° to the right in order to be correctly orientated relative to the anteroposterior axis of the larva. The cell body of a single multipolar cell (*) is visible adjacent to the nerve (n). Scale bar 5 μm. —**C**, Detail of the ciliary nerve from the postoral transverse band. The lightly stained profiles are neurites. The darker profiles along the basal lamina derive from non-neural ciliary band cells and typically connect to the cell body of the latter via slender processes (arrowhead). Scale bar 1 μm. —**D**, **E**, Two multipolar cells (*) from the preoral transverse band. (D) is an unusually thick section showing an example of the cell type referred to here as 'pale'; see Lacalli and West (1993, Fig. 4) for a similar cell in a section of more normal thickness. (E) An example of a 'dense' multipolar cell. The apex of the cell (arrowhead) has lateral apical processes (small arrows) but connects with the cell body (*) out of the section. Note the basal ciliary nerve in both (D) and (E). Scale bars 2 μm. —**F**, Stereo three-dimensional reconstructions of a multipolar cell of the pale type from the postoral transverse band.

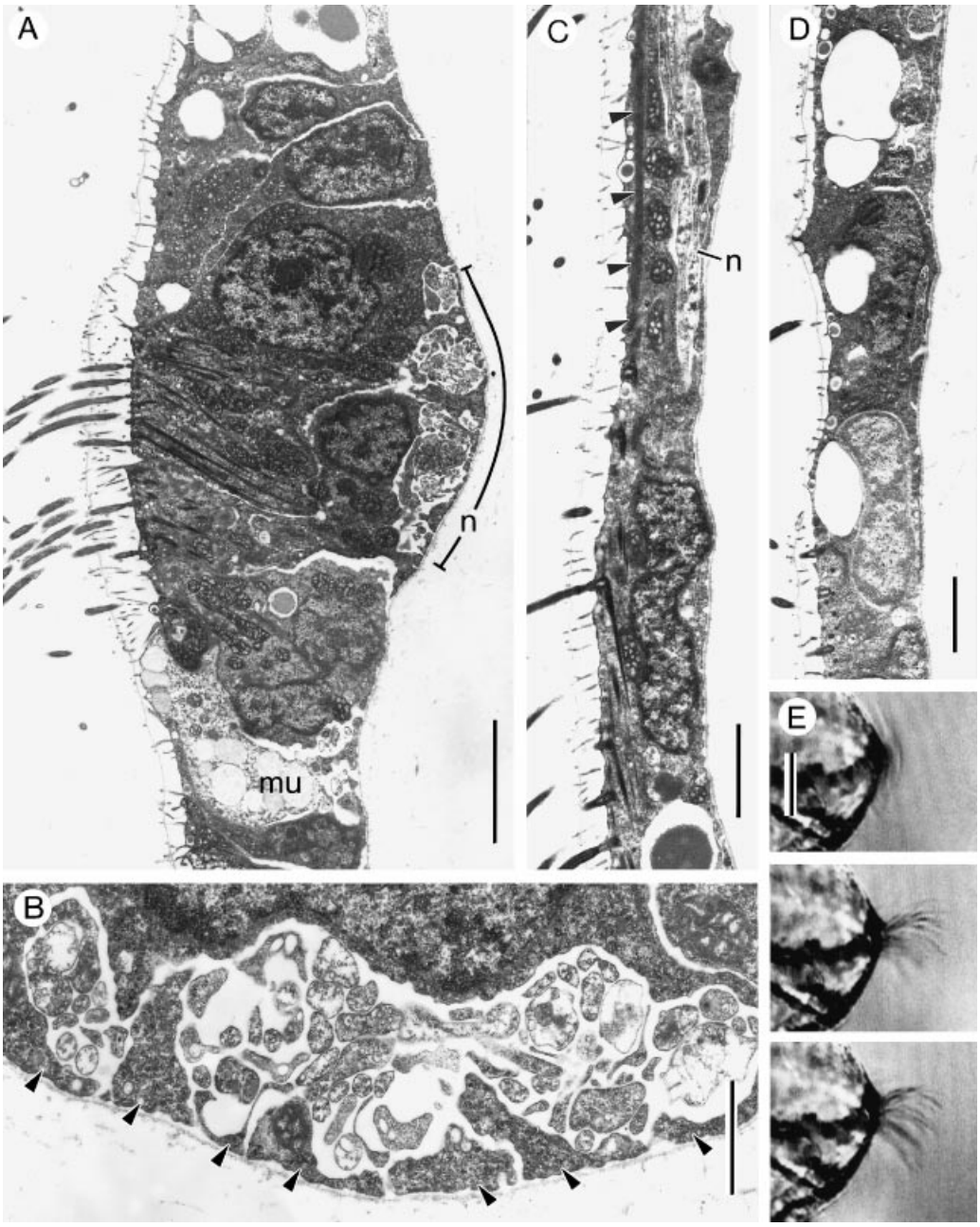


**Fig. 3**—**A**, Longitudinal section through the telotroch near the ventral midline. Note multiple cilia, deep rootlets, the ciliary nerve (n), divided into several separate tracts, and one mucus cell (mu). Scale bar 4 μm. —**B**, Detail of the telotroch nerve. Several types of neurites are evident, some with vesicles. The denser profiles along the basal lamina (arrowheads) belong to the telotroch cells, each of which forms an array of basal attachments to the lamina. Scale bar 1 μm. —**C**, Longitudinal section through the midventral band showing a portion of the nerve (n) that runs beneath it. Rootlets are similar to those of the telotroch, but are much shallower. Note the apical bundle of fibrils (arrowheads), cut along the junction between two cells. It parallels the plane of section for some distance. Such fibre bundles occur quite commonly in the larval epithelium. Scale bar 2 μm. —**D**, A typical region of aboral epithelium showing vacuolate cells interspersed among more normal epithelial cells, which produces the patchwork appearance seen in the SEM images of the body surface. Scale bar 2 μm. —**E**, Single frames from a video sequence showing normal ciliary beat in the telotroch (top) and two consecutive frames (middle and bottom) during a momentary partial arrest. A subset of the cilia continues to beat in metachronal waves during the arrest, but this is not visible except as a slight blurr in single frames. Scale bar 50 μm.

intervals along one side of the band, i.e. no neurones could be identified either in the band or beside it. In consequence, we were unable to identify a source for the fibres in the telotroch nerve.

Cells of the ventral band (Fig. 3C) are also multiciliate, with paired rootlets resembling those of telotroch cells. However, the rootlets are angled so they parallel the surface of the cells more closely. A small nerve runs beneath the band. This may join the telotroch nerve but if it does so, it is too small to account for more than a small proportion of fibres in the latter.

The aboral epithelium (Fig. 3D) consists of vacuolate cells interspersed among normal ciliated epithelial cells. In many cells, both here and in the ciliary band, bundles of filaments run just below the cell surface (Fig. 3D) and attach to junctions between cells. The epithelium may therefore have considerable mechanical strength. In echinoderm larvae, in contrast, the epithelium lacks this structural feature and is generally thinner and more delicate in appearance. Consistent with this, *B. biminiensis* larvae retain their form far better during processing for EM, in our experience, than echinoderm larvae, which tend to collapse. The blastocoel in both types of larvae contains a supporting gel (Strathmann 1989), but tornaria lack subtrochal mesenchyme, which has been proposed as a strengthening element in echinoderm larvae (Lacalli 1996). These observations suggest that there are probably significant differences between the tornaria and other deuterostome larvae in the way the three-dimensional form of the body is maintained.

### *Ciliary arrest and drug effects*

During normal swimming, contact with obstacles by the apex or sides of the larva generates momentary flicks of the telotroch cilia. Because the metachronal wave is interrupted, these events are easily visible at low power. They are abolished when the larvae are placed in a 50% mixture of seawater and isotonic magnesium chloride, which is consistent with the involvement of nerves. Videotapes of tethered larvae, at higher power, show that cilia along the entire band are suddenly arrested in mid-beat (Fig. 3E), for a duration of one-third to one-half of a second. Some of the cilia continue to beat normally during this time, however, and the metachronal wave continues. From the video images, it appears to be predominantly those cilia in the lower (caudal) half of the band that arrest but we cannot be certain because the cilia that continue to beat are not resolved in single frames except as a faint blur. The cilia of the circumoral band are unaffected by these events and continue to beat normally, so far as could be determined. Reversals of cilial beat were not observed in the telotroch, nor in the circumoral band. The latter was more difficult to observe, however, so we cannot be certain that reversals never occur.

The most pronounced drug effects were obtained with two cholinergic agonists, carbachol and nicotine. The threshold for response in both freely swimming and tethered larvae was extremely variable, however. Both induced arrests of the telotroch similar to the normal arrest (i.e. some cilia continued to beat) in some larvae, typically for 10–20 s. at concentrations of 5–15 μM. Some larvae were unaffected at doses of 20 μM and even those affected by the agonists typically recovered and resumed swimming after 20–30 s. A possible explanation for both the variable threshold and the recovery is that cholinergic control acts upstream in a multistep process, and that the downstream transmitters are depleted in the presence of drugs and, in some larvae, during normal behaviour. If this is the case, once the larvae resume swimming, and so long as the drug is still present, they should also fail to show normal arrests, which they did.

Concentrations of both carbachol and nicotine greater than 20 μM typically blocked ciliary beat entirely in most larvae, such that all cilia were fully arrested on the downstroke. This is probably not a normal physiological effect, as opposed to a non-physiological, toxic effect, since it was never observed in untreated larvae. Ciliary beat in the circumoral band was entirely unaffected by drugs so far as we could determine. Similar behavioural tests using epinephrine and serotonin failed to affect either the circumoral band or the telotroch in consistent ways.

### *Oesophageal structure, food collection and swallowing*

The oesophagus (Fig. 4A) tapers gradually to the valve separating it from the stomach (Fig. 4B). Bands of muscle encircle the oesophagus and the first group of these, located at the opening to the oesophagus, effectively defines the mouth. Mucus cells lie in a roughly U-shaped pattern just in front of this point, i.e. they are outside the mouth and both ventral and lateral in position. Nerves course beneath the oesophageal epithelium, just above the basal lamina, forming a series of small rings around the oesophagus and a major large tract along its base (Fig. 4C,D). The epithelial cells are multiciliate, and two types could be distinguished based on their electron densities. Dense cells were most numerous but a number of pale cells were scattered among them (for examples, see Fig. 4C). Both types have multiple basal endfeet. Those from the dense cells are clearly attachment structures, and resemble the basal endfeet of the telotroch cells. Those from the pale cells look more like neurites; they contain microtubules and run laterally for short distances in the nerve tract before contacting the basal lamina. Some form specialized contacts with the basal lamina adjacent to muscle cells, as indicated in Fig. 4(D). Whether any of these represent functional neuromuscular junctions is not clear. Repeated searching failed to reveal any obvious synapse-like junctions between putative neurites and any of the oesophageal muscle cells. Uniciliate cells occur at several places within the multiciliate epithelium, notably just inside the lower margin of the mouth. No neurites could be traced from these cells, however, and their basal contacts were not synaptic in

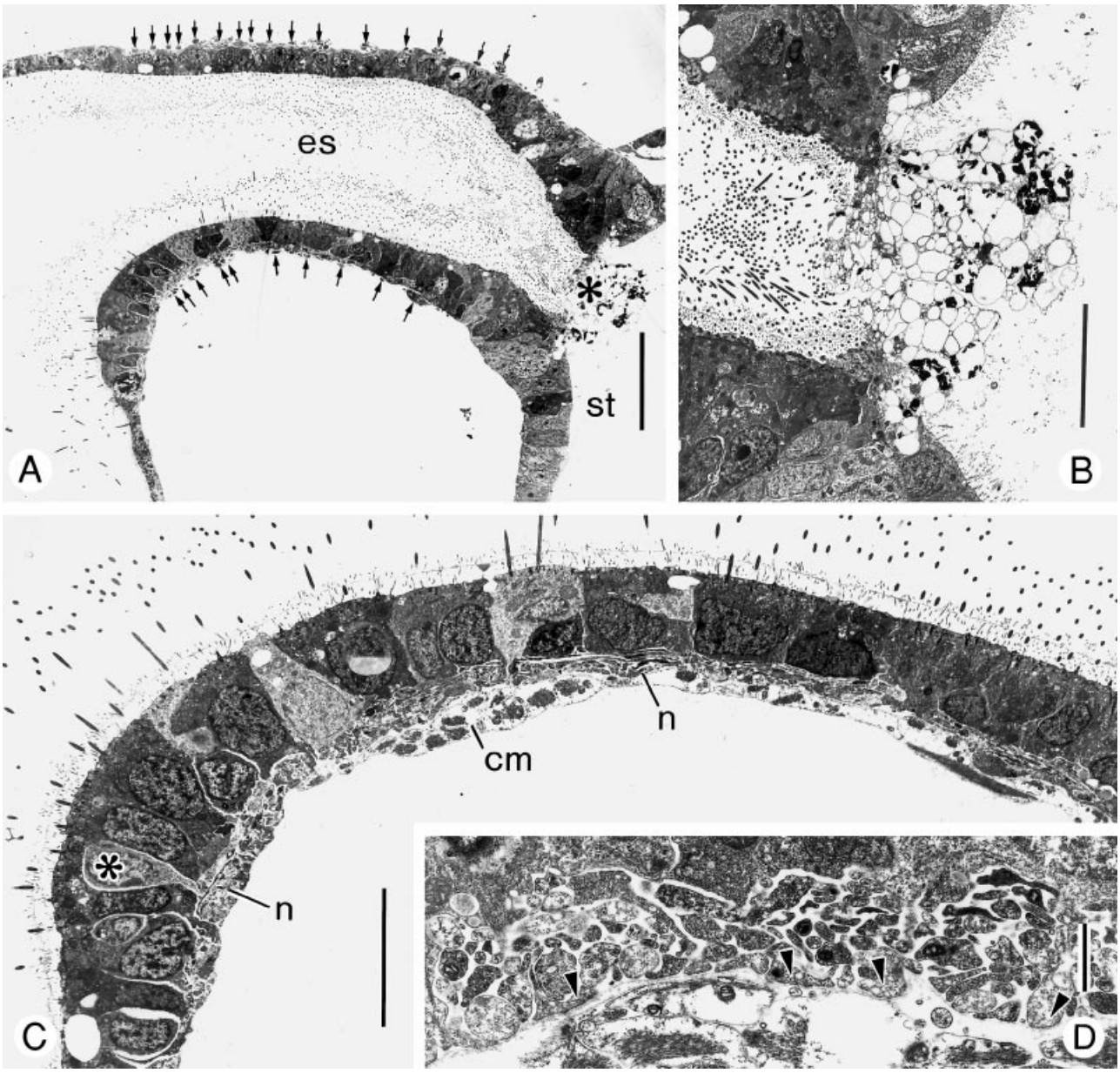


**Fig. 4**—**A**, Midsagittal section through the oesophagus (es), which opens into the stomach (st) through a valve (*). Muscle fibres, either individually or in small groups, encircle the oesophagus (small arrows). In this specimen the mouth is open; see Gilmour (1982; Fig. 13) for a comparable section with the mouth partially closed. Scale bar 20 μm. —**B**, Detail of the valve separating the oesophagus and stomach, formed by a ring of cells with highly vacuolate apical extensions. Cilia are absent on the stomach side. Scale bar 10 μm. —**C**, The midventral floor of the oesophagus. Note circular muscles (cm), cut in transverse section, and the extensive intraepithelial nerve plexus (n). Both dense and pale multiciliated epithelial cells occur, as well as a few flask-shaped uniciliate cells (e.g. *) that may be neurones. Scale bar 10 μm. —**D**, Detail of the nerve plexus. Repeated searches failed to reveal any obvious synapses, but specialized attachments to the basal lamina adjacent to muscle cells occur (arrowheads). Scale bar 1 μm.

appearance. We are thus unable to account for the many fibres in the oesophageal plexus or provide morphological evidence for what must certainly be its main function, co-ordinating the contraction of the oesophageal muscles. An additional section series through the oesophagus is currently being analysed and this may resolve some of these issues.

Tornaria feed on suspended algae that are carried along the grooves of the oral field towards the mouth, as described

 

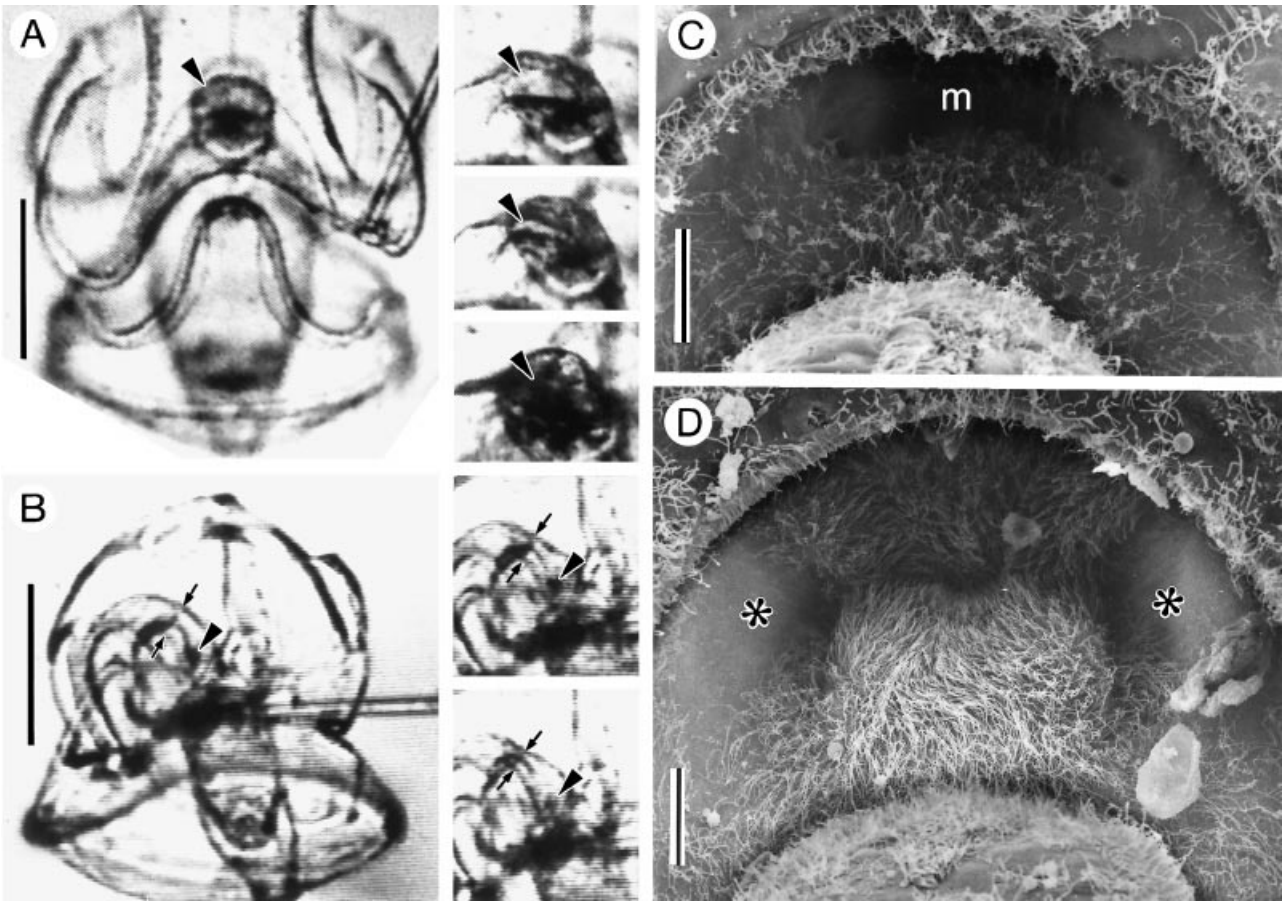


**Fig. 5**—**A**, Single frames from a video sequence of a tethered larva feeding on *Dunaliella*, in ventral view. The mouth (arrowhead) is fully open in the main image. Varying degrees of closure are shown in the insets, from partly open (top) to full closure (bottom). Scale bar 200 μm. —**B**, Side view of a younger larva showing a food bolus of about a dozen *Dunaliella* cells (arrowhead) accumulating in the lower oesophagus. The insets show two steps in the swallowing process. Partial contraction (top) does not dislodge the bolus, but full contraction (bottom) does, propelling it through the oesophageal valve. Note that the oesophagus is more fully compressed (between the small arrows) in the bottom image. Scale bar 200 μm. —**C**, Ventral view into the open mouth (m) of a late-stage larva. The sides of the oral opening are sparsely ciliated, in contrast with the roof and floor of the vestibule leading into the oesophagus. Scale bar 25 μm. —**D**, The mouth in a partially closed condition. This raises the densely ciliated floor of the oesophagus to meet the roof, leaving open channels (*) on either side. Scale bar 25 μm.

by Gilmour (1982). Contacts between food particles and the band are infrequent and do not appear to be essential for food capture. In their study of feeding *Ptychodera* larvae, Strathmann and Bonar (1976) concluded that such contacts were part of the capture mechanism, but they also observed that particles could reach the mouth without them. In *Balanoglossus* larvae, the distance between the bands and the deepest parts of the food groove is greater, which may be why particle encounters with the band are less frequent.

Once at the mouth, particles slip over the lower lip and lodge in the back of the oesophagus, where a bolus of up to 15–20 algal cells typically accumulates. During this process, the mouth performs a series of partial contractions, ranging in frequency from once every few seconds to twice a second during active feeding. This involves the contraction of the muscles nearest the opening to the oesophagus, which reduces the mouth to a thin slit (Fig. 5A). Uncontracted, the mouth has the form of an inverted crescent, rounded at the ends. Cilia cover the floor and roof of the oesophagus quite densely, very much as Garstang (1939) describes for a tentaculate tornaria. In contrast, the lateral surfaces (i.e. the ends of the crescent) are very sparsely ciliated (Fig. 5C). Thus, when partially closed, the centre of the oesophagus is effectively blocked (Fig. 5D) but channels remain open along the sides as described by Gilmour (1982). Muscle contraction reduces the volume of the oesophageal lumen and the lateral channels provide the only obvious means of escape for the water contained in it. This provides, in principle, a way of separating excess water from food particles before

swallowing. Since particles typically do not escape from the mouth during such contractions, we can only infer the path that excess water would take. However, so long as there is no possibility of escape along the midline, there appears to be no other alternative.

Swallowing occurs periodically and is due to a more pronounced contraction that closes the mouth completely (lower inset, Fig. 5A). This is accompanied by a less forceful contraction of the rest of the oesophagus. With the mouth closed, the food bolus is forced through the valve into the stomach (Fig. 5B). Twenty *Dunaliella* cells seems to be about the maximum that can accumulate before swallowing is initiated, but smaller numbers will also induce it.

## Discussion

Identifying neurones and nerve fibres in invertebrate larvae from EM sections is often difficult. The neurones are seldom of conventional type so ultrastructural features, such as neurite structure or the presence of vesicles, are not particularly reliable as diagnostic criteria. In the case of the tornaria, the ciliary band cells have slender connections to their basal endfeet, so it is easy to mistake them for neurones with axons. Preparing three-dimensional reconstructions of the ciliary band cells at the EM level is therefore useful, because both the ultrastructure and the overall morphology of a given cell can be examined together. The method has limitations, since only a small proportion of a larva as large as the tornaria can be sampled. So, for example, reconstructions of small parts of ciliary band are meaningful only if the cell types encountered are distributed uniformly along its length. Furthermore, there is a good chance that key concentrations of neurones, if their location is not already known, will be missed. The results of the present study need to be interpreted with these limitations in mind.

In their study of *Balanoglossus proterogonius* larvae, Dautov and Nezlin (1992) identified clusters of putative neurones containing monoamines near the apical plate and in the suboral region. Fibres from the apical cells entered adjacent apical parts of the circumoral band (see also Hay-Schmidt 2000), while those from the adoral centres could be traced into the pre- and postoral bands. Based on this account, we expected to find comparatively large tracts of fibres travelling along the bands, as in bipinnaria larvae (Lacalli *et al.* 1990). Instead, the majority of fibres in the areas we examined were short processes of largely local extent and most belonged to multipolar cells. Longer fibres originating elsewhere may well be present, but if so, it seems unlikely that there are more than a few such fibres.

Multipolar cells were first identified in tornaria bands by Lacalli and West (1993). Similar cells occur in the circumoral bands of echinoderm larvae. However, as in the case of the tornaria telotroch, they appear to be absent from bands chiefly concerned with locomotion, e.g. epaulettes in pluteus larvae and holothurian doliolaria bands (Lacalli and West 2000, and unpublished). Multipolar cells are evidently not aminergic, since they have never been reported in fluorescence studies. Their function is not known, nor that of their apical processes, nor even whether the latter are afferent or efferent. Nevertheless, their presence in the circumoral bands and absence from other ciliary effectors suggest that their role, whatever it is, is more concerned with feeding than with locomotion.

We have also failed to obtain any evidence as to the source of nerve fibres in the telotroch nerve. Dautov and Nezlin (1992) found individual neurones at various points around the body below the telotroch, all with forward-projecting axons. The latter cross the telotroch, but do not enter its nerve. Fibres from the midventral nerve probably do enter the telotroch nerve (L. P. Nezlin, personal communication), but in our specimens the former is too small to account for more than a fraction of the fibres in the latter. The source of the majority of fibres in the telotroch nerve is thus still uncertain.

The enzyme cholinesterase is distributed along the circumoral band in the tornaria, but is absent in the telotroch according to Dautov and Nezlin (1992). One might expect, from this, that one or both of the multipolar cell types would be cholinergic. This has not been tested, and there is a complicating factor, in that the circumoral band fails to respond visibly to cholinergic agonists. In contrast, the telotroch, which lacks multipolar cells, does. Acetylcholinesterase is also found along the circumoral band of pluteus larvae (Gustafson *et al.* 1972; Ryberg 1973). It is not known whether cholinergic neurones are present, however, or where they are located, despite the fact that cholinergic agonists have dramatic effects on pluteus behaviour (Lacalli and Gilmour, 1990). In fact, in no case among the various echinoderm and hemichordate larvae has it been possible to attribute these types of cholinergic effects to an identified class of neurones. Furthermore, the transmitters that can be localized to specific cell types generally have less marked effects on larval behaviour. It is thus clear that a great deal more research is needed before ciliary band innervation and function is fully understood.

The innervation of the oesophagus is equally puzzling. Large numbers of nerve-like fibres occur, but no recognizable synapses. Yet larvae clearly have a complex and well-controlled swallowing response. Similar adoral and circumoral nerves and neural centres occur in echinoderm larvae, and it would be surprising if they were not homologous features that operate in approximately similar ways. The adoral cell clusters identified by Dautov and Nezlin (1992) presumably provide some of the fibres, but the very diversity of fibres in the plexus indicates that other cells, as yet to be identified, also contribute to the plexus.

The oesophagus exhibits two types of contractile behaviour: partial contractions, that we infer are a means of expelling water from the oesophagus without dislodging the food bolus, and full contractions, that propel the bolus into the stomach. We interpret the first of these as a mechanism

 

for concentrating the food and removing excess water via sparsely ciliated lateral channels, as proposed by Gilmour (1982). The sides of the oesophagus are later the sites of gill slit formation, so there appears to be a direct continuity between the water bypass mechanism used by the larva and that used by the juvenile.

Our examination of oesophagus structure was undertaken in part to confirm the report by Ritter (1894) of ciliated ridges in the floor of the oesophagus that he interpreted as precursors of an endostyle. The pharyngeal filter of chordates develops from a region of the anterior gut roughly comparable with the oesophagus of larval hemichordates, so Ritter's proposal could be correct. We did indeed find ciliated zones in the floor of the oesophagus that form ridge-like structures when contracted, as in Fig. 5(D). However, there is nothing specific about these to link them unequivocally with endostyles. Therefore Ritter's claim, in our view, remains unconfirmed. It would nevertheless be a very useful exercise to follow oesophageal differentiation further, through metamorphosis, so as to understand better the relationship between the larval oesophagus and the juvenile pharyngeal structures, including gill slits, that develop from it.

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## References

Bisgrove, B. W. and Burke, R. D. 1987. Development of the nervous system of the pluteus larva of *Strongylocentrotus droebachiensis*. – *Cell and Tissue Research* **248**: 335–343.

Burdon-Jones, C. 1957. Hemichordata: Enteropneusta. – *Fiches d'Identification de Zooplancton* **70**: 1–6.

Burke, R. D. 1983. The structure of the larval nervous system of *Pisaster ochraceus*. – *Journal of Morphology* **178**: 23–35.

Burke, R. D., Brand, D. G. and Bisgrove, B. W. 1986. Structure of the nervous system of the auricularia larva of *Parastichopus californicus*. – *Biological Bulletin* **170**: 450–460.

Chee, F. and Byrne, M. 1999. Development of the larval serotonergic nervous system in the sea star *Patiriella regularis* as revealed by confocal imaging. – *Biological Bulletin* **197**: 123–131.

Dautov, S. S. and Nezlin, L. P. 1992. Nervous system of the tornaria larva. A histochemical and ultrastructural study. – *Biological Bulletin* **183**: 463–475.

Garstang, W. 1939. Spolia Bermudiana II. The ciliary feeding mechanism of tornaria. – *Quarterly Journal of Microscopical Science* **81**: 347–365.

Gilmour, T. H. J. 1982. Feeding in tornaria larvae and the development of gill slits in enteropneust hemichordates. – *Canadian Journal of Zoology* **60**: 3010–3020.

Gilmour, T. H. J. 1989. A method for studying the hydrodynamics of microscopic animals. – *Journal of Experimental Marine Biology and Ecology* **133**: 189–193.

Gustafson, T., Ryberg, E. and Treufeldt, R. 1972. Acetylcholine and contractile activity in the echinopluteus. – *Acta Embryologiae Experimentalis* **2**: 199–223.

Hay-Schmidt, A. 2000. The evolution of the serotonergic nervous system. – *Proceedings of the Royal Society* **B267**: 1071–1079.

Lacalli, T. C. 1996. Mesodermal pattern and pattern repeats in the starfish bipinnaria larva, and related patterns in other deuterostome larvae and chordates. – *Philosophical Transactions of the Royal Society* **B351**: 1737–1758.

Lacalli, T. C. and Gilmour, T. H. J. 1990. Ciliary reversal and locomotory control in the pluteus larva of *Lytechinus pictus*. – *Philosophical Transactions of the Royal Society* **B330**: 391–396.

Lacalli, T. C., Gilmour, T. H. J. and West, J. E. 1990. Ciliary band innervation in the bipinnaria larva of *Pisaster ochraceus*. – *Philosophical Transactions of the Royal Society* **B330**: 371–390.

Lacalli, T. C. and West, J. E. 1993. A distinctive nerve cell type common to diverse deuterostome larvae: comparative evidence from echinoderms, hemichordates and amphioxus. – *Acta Zoologica* **74**: 1–8.

Lacalli, T. C. and West, J. E. 2000. The auricularia-to-doliolaria transformation in two aspidochirote holothurians, *Holothuria mexicana* and *Stichopus Californicus*. – **119**: 421–432.

Moss, C., Burke, R. D. and Thorndyke, M. C. 1994. Immunocytochemical localization of the neuropeptide S1 and serotonin in larvae of the starfish *Pisaster ochrceus* and *Asterias rubens*. – *Journal of the Marine Biological Association of the U.K.* **74**: 61–71.

Nielsen, C. 1987. Structure and function of metazoan ciliary bands and their phylogenetic significance. – *Acta Zoologica* **68**: 205–262.

Nielsen, C. 1998. Origin and evolution of animal life cycles. – *Biological Reviews* **73**: 125–155.

Ritter, W. E. 1894. On a new *Balanoglossus* larva from the coast of California, and its possession of an endostyle. – *Zoologischer Anzeiger* **17**: 24–60.

Ryberg, E. 1973. The localization of cholinesterases and non-specific esterases in the echinopluteus. – *Zoologica Scripta* **2**: 163–170.

Strathmann, R. R. 1989. Existence and functions of a gel filled primary body cavity in development of echinoderms and hemichordates. – *Biological Bulletin* **176**: 25–31.

Strathmann, R. R. and Bonar, D. 1976. Ciliary feeding of tornaria larvae of *Ptychodera flava*. – *Marine Biology* **34**: 317–324.