Chapter 4

Adductor and Mantle Musculature

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STRUCTURE AND FUNCTION OF THE ADDUCTOR MUSCLE

The adductor muscle is a dominant feature of the eastern oyster *Crassostrea virginica*, being large and fairly centrally placed (Fig. 1). The eastern oyster belongs to a group, the "surface attached lamellibranchs," that loses the anterior adductor muscle after settlement (Morton 1979). Larval eastern oysters are free-swimming and have two adductors, the "dimyarian" condition. During metamorphosis the larval symmetry is lost as the oyster settles on its flattened right valve. Subsequently, the anterior muscle degenerates, resulting in the "monomyarian" condition in which there is a single adductor (Galtsoff 1964; Morrison and Odense 1973; Elston 1980).

A wide range of muscle types is found in molluscs (Chantler 1983; Hanson and Lowy 1983; Hoyle 1983; Nicaise and Amsellem 1983), and many of them are found in the adductor muscles of bivalves (Morrison 1970). Among the latter, a series of muscle types from transversely striated to obliquely striated to smooth can be found, corresponding to the life style of the mollusc (Morrison and Odense 1973, 1974). Most adductors consist of two types of muscle, one faster-acting than the other. The fast part of the adductor muscle usually has a translucent appearance, whereas the slow portion is opaque. For example, the fast-acting portion of adductor muscle of the sea scallop *Placopecten magellanicus* is transversely striated, which is necessary for closing and releasing the valves rapidly for swimming movements. The slow part of the scallop adductor is smooth with some oblique striation, and is capable of slower, more prolonged contractions (Morrison and Odense 1974).

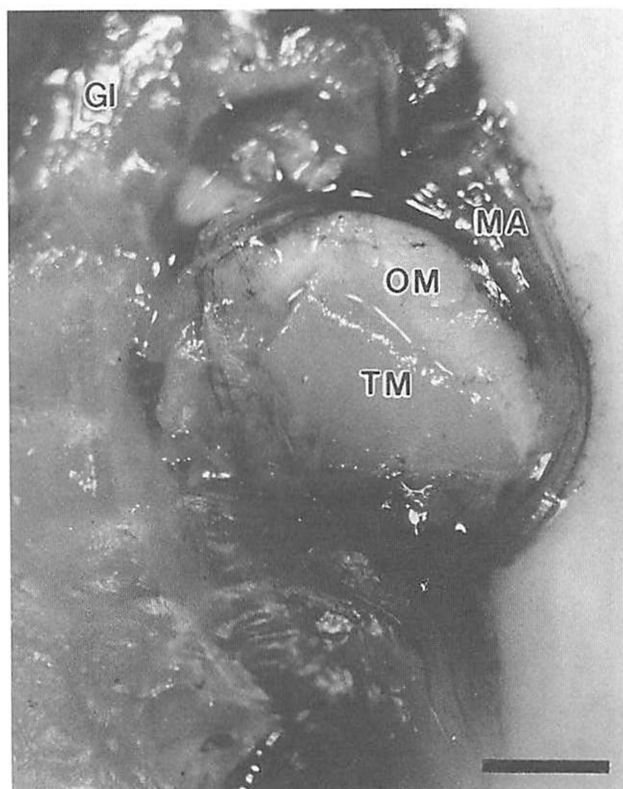


Figure 1. Oyster with one valve of the shell removed to show the position and extent of the fast translucent [TM], and slow opaque [OM] parts of the adductor muscle. The gills [GI] are to one side of the adductor, and the mantle [MA] surrounds the other side. Bar is 5 mm long.

Most of the adductor muscle of the eastern oyster consists of translucent, fast muscle that is obliquely striated. This type of muscle can close the valves quickly in response to disturbances, or as part of a "snapping" action that helps to clear the mantle of pseudofaecal material. Sessile intertidal bivalves such as oysters frequently remain closed for long periods of time, so to one side of the translucent muscle there is a smaller, crescent-shaped portion of opaque smooth muscle; this muscle contracts more slowly but can hold the valves shut against the tension of the hinge ligament for several days with little expenditure of energy, a phenomenon known as "catch" (Millman 1964).

Adductor muscles are composed of long, narrow uninucleate muscle cells, which are called fibers. Those of the translucent part of the adductor are ribbon-like, about 17 µm long and 3 to 4 µm wide, so that in cross-section they have an elongate profile; those of the opaque part are more rounded, about 10 to 20 µm in diameter (Fig. 2). Where the two types of fiber meet, there is a region where they are intermingled. Each fiber is surrounded by the endomysium, which is a thin layer of connective tissue. These fibers occur in groups surrounded by a thicker layer of connective tissue, the perimysium.

Translucent Adductor Muscle

Each fiber contains a central area of thick and thin myofilaments, and fusiform bodies ("dense bodies") that appear dark in electron micrographs, (Fig. 3A, B). Some of the dense bodies are attached to the

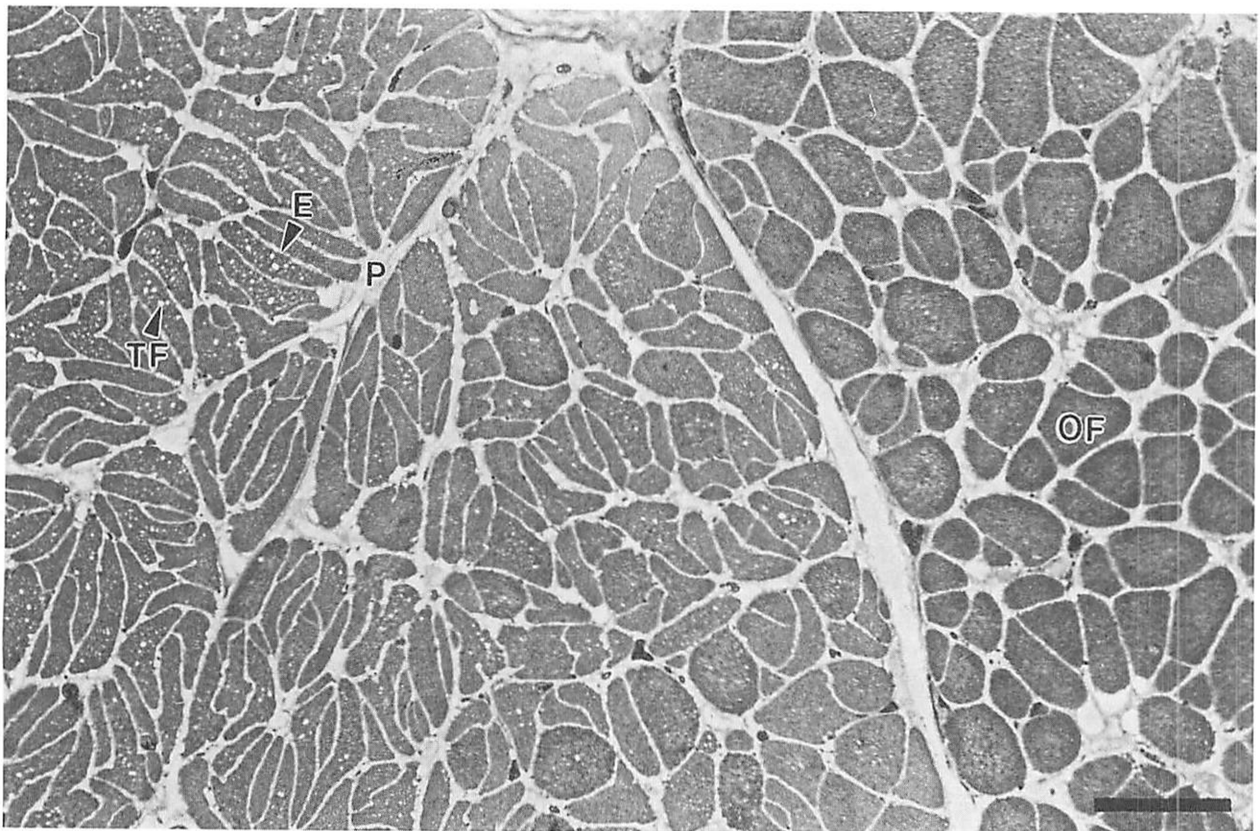


Figure 2. Transverse section of the transition zone between the opaque and translucent parts of the adductor muscle. The specimen was fixed in 2.5% glutaraldehyde in caccodylate buffer, embedded in JB4, and stained in methylene blue/basic fuschin. The rounded fibers [OF] of the opaque part of the muscle are to the right in the micrograph, the flattened fibers of the translucent part [TF] to the left. In the center fibers of both types are intermingled. Each fiber is surrounded by a thin layer of endomysium [E], and groups of fibers are surrounded by perimysium [P]. Bar is 20 µm long.

sarcolemma, forming a half-desmosome or hemidesmosome (Twarog 1967). The contractile apparatus of wider fibers, such as those of the skeletal muscles of vertebrates, is usually sub-divided into myofibrils, but this is not the case with these narrow fibers in molluscan muscles (Hanson and Lowy 1961). Mitochondria, vesicles of sarcoplasmic reticulum, and the nucleus are restricted to the peripheral cytoplasm. A regular system of extensive surface invaginations of the sarcolemma is absent, but the sarcolemma is invaginated where there are hemidesmosomes (Fig. 3B), and in places the sarcoplasm is wide, so these invaginations can be quite long. These invaginations could have a similar transport function to the transverse tubular system of transversely striated muscle. The sarcoplasmic reticulum forms couplings with the sarcolemma, as in some other invertebrates (Nunzi and Franzini-Armstrong 1981), and dense material can be seen between the outer membrane of the sarcoplasmic reticulum and the sarcolemma. The mitochondria and nuclei are elongate in longitudinal section and aligned parallel to the myofilaments, but appear rounded in cross section.

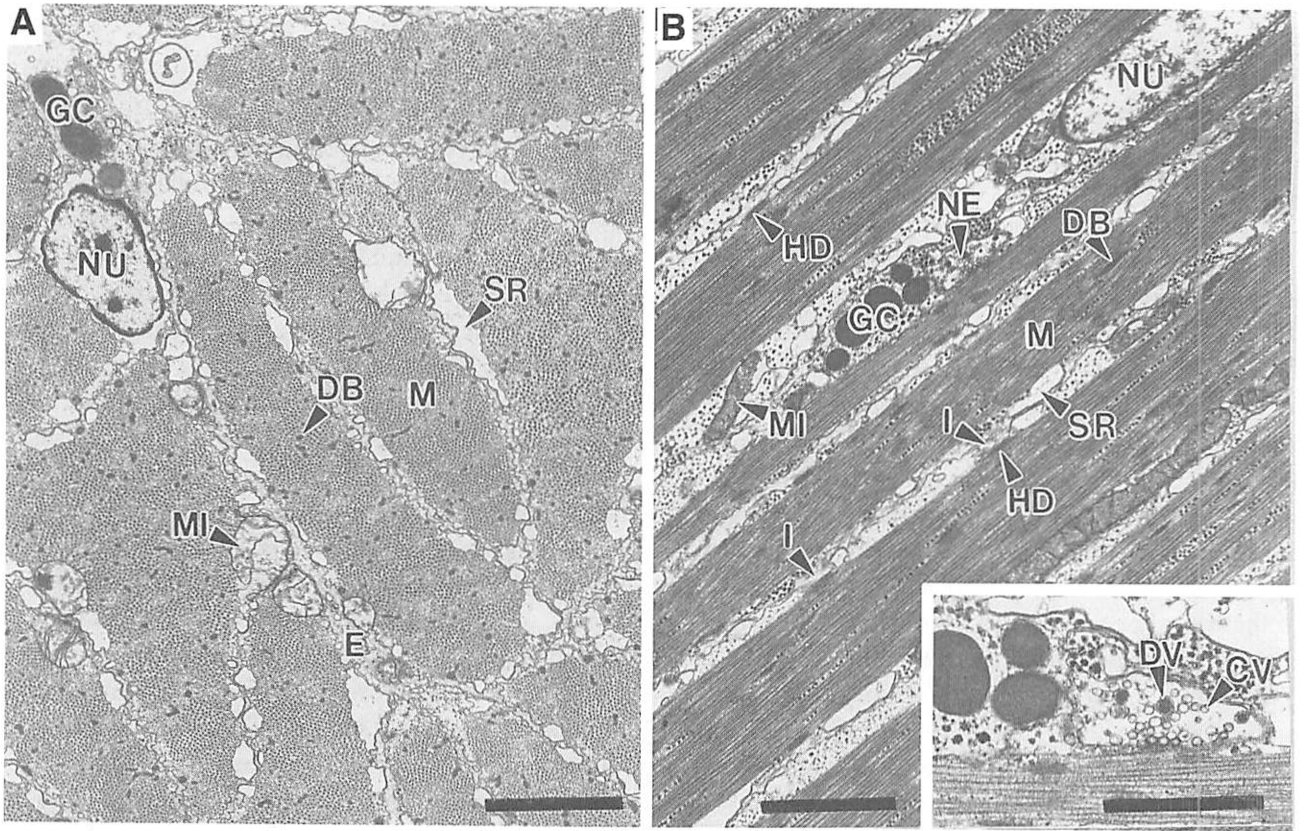
Nerve endings containing vesicles often occur close to the sarcolemma (Fig. 3B, insert). Small, clear vesicles, dense-cored vesicles, and sometimes vesicles with dark contents are present. A variety of vesicles, presumably associated with different types of neurotransmitters, has been reported in other molluscs (Nicaise and Amsellem 1983). Glial cells containing large granules, the gliosomes, are usually closely associated with these nerve-endings, but do not completely surround them. A "glio-interstitial network" has been described in the muscles of various molluscs. Its function is unknown, but it may help to control ion or metabolite exchange between the interstitial tissue and the nerves, or muscles, or both (Nicaise and Amsellem 1983).

The thin myofilaments of *C. virginica* enter the fusiform dense bodies (Morrison 1993b). These are the functional equivalent of the Z-line found in transversely striated muscles such as mammalian skeletal muscle, or the fast part of the adductor of most scallop species (Chantler 1991), but they are probably stronger and can withstand the more sustained tension generated by obliquely-striated and smooth muscles (Nicaise and Amsellem 1983). These dense bodies show regions of oblique alignment (Fig. 3C), as reported for the translucent part of the adductor of the Portuguese oyster, *Crassostrea angulata* (Hanson and Lowy 1961), and as found in obliquely striated muscles in other pelecypod adductors, such as the fast part of the adductor of the ocean quahog, *Arctica islandica* (Morrison and Odense 1974).

Molluscan muscles show a range of organization, so that some are intermediate between striated and obliquely striated muscle, such as the fast parts of the adductor muscles of the thorn oyster, *Spondylus cruentus*, the file shell, *Promantellum hirasei*, and the tellin, *Fabulina nitidula*. There is sometimes, but not always, oblique alignment of the dense bodies in the slow part of the adductor of the sea scallop so that this muscle appears to be intermediate between obliquely striated and smooth muscle (Morrison and Odense 1974). According to Ruegg (1961), across the opaque part of the adductor muscle of *P. magellanicus*, there is a transition in fiber type from obliquely striated to smooth.

Striations usually cannot be seen in sections of obliquely striated muscle with light microscopy and regular illumination, but they can be seen with phase-contrast illumination (Hanson and Lowy 1961). However, oblique striations can often be seen in contracted cells under regular illumination (Bowden 1958; Galtsoff 1964; Salánki and Zs.-Nagy 1966; Morrison and Odense 1974; Morrison 1993b). These are contraction bands, resulting from super-contraction of the muscle fiber. When this happens, the thick myofilaments pass into the regions at the end of the sarcomeres where there are normally only thin myofilaments entering dense bodies. They either overlap with thick myofilaments from the next sarcomere or become folded, so that the dense-body region appears darker in electron and light micrographs.

In cross section, fields of thick and thin myofilaments, with dense bodies among the thin filaments, can be seen (Fig. 3D). Some of the dense bodies are attached to the sarcolemma, forming a hemidesmosome; filaments from the connective-tissue stroma are also connected to the sarcolemma at this point. This arrangement is thought to give structural stabil-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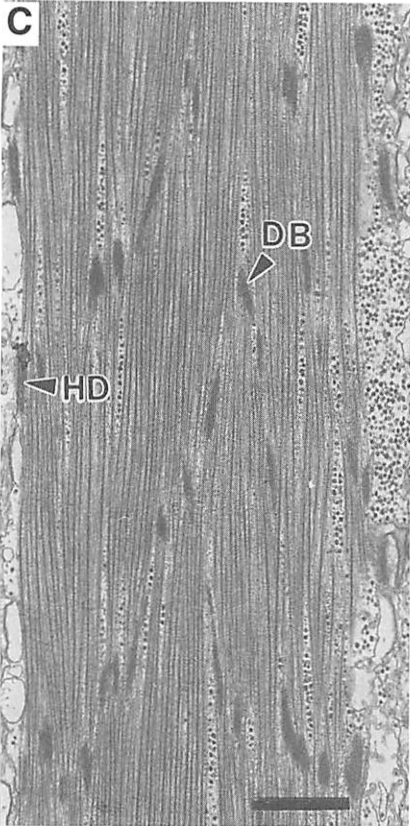
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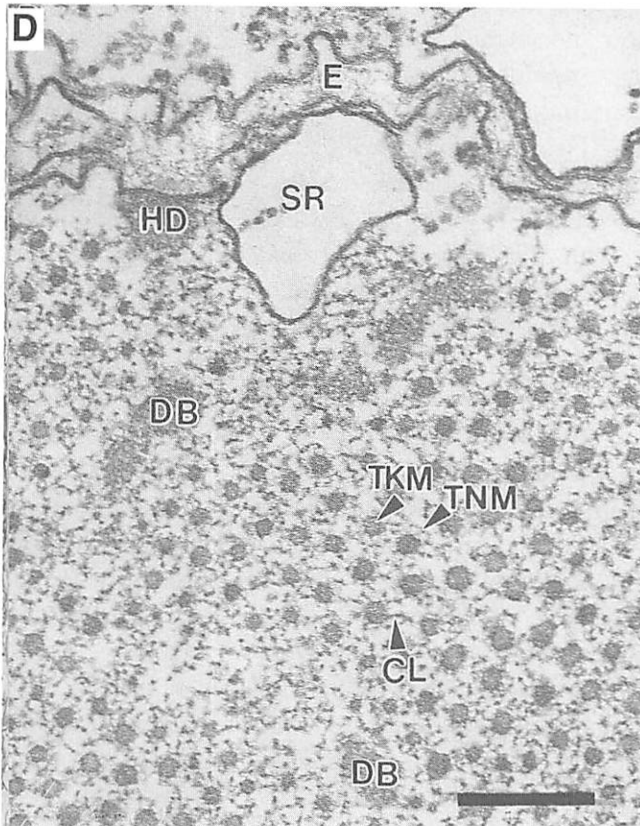
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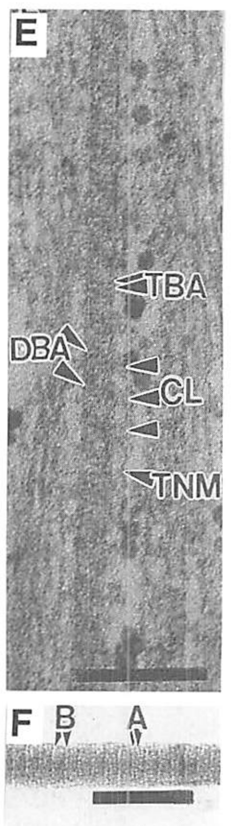
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ity to the muscle (Twarog et al. 1973) and to propagate tension to neighboring cells (Sobieszek 1973).

The thick myofilaments of the translucent part of the adductor of *C. virginica* were found to have a maximum width of about 60 nm and a modal peak at about 32 nm (Fig. 4). These measurements are comparable to those obtained by Philpott et al. (1960), who found that the myofilaments were 20 to 40 nm in width. These myofilaments are therefore wider than those found in the striated muscle of bivalves such as the scallop, *Placopecten magellanicus*, which are 16.5 to 17.5 nm in diameter (Morrison and Odense 1968); but they are similar to those of the obliquely striated muscle of the opaque, slow part of the adductor of *P. magellanicus*, which has a maximum width of about 50 nm and a peak at 36 nm (Morrison and Odense 1974). The thick myofilaments are tapered at the ends, so they appear wider in the center of a sarcomere and become smaller towards the dense bodies between the sarcomeres. They are regularly arranged, like those of the translucent part of the adductor muscle of *C. angulata* (Hanson and Lowy 1961). As in mammalian skeletal muscle, the thick myofilaments are surrounded by a single ring of thin filaments, except in the H-zone at the center of the sarcomere. The number of thin myofilaments surrounding the thick myofilaments in *C. virginica* seems to be about 12 as in *C. angulata* (Hanson and Lowy 1961; Morrison 1993b). Cross links can often be seen between the thick and thin myofilaments.

The thick myofilaments are cross-striated, with a periodicity of about 5 nm (Fig. 3E). Paracrystals of the structural protein paramyosin can be formed from obliquely striated and smooth adductor muscles, and electron micrographs of paramyosin paracrystals from the translucent adductor muscle of *C. virginica* reveal the 5 nm periodicity more clearly than in histological sections (Fig. 3F; Morrison 1970; Morrison et al. 1970; Morrison and Odense 1974). In the paramyosin paracrystals, every third band is accentuated, giving a periodicity of about 15 nm which has been reported as being typical of paramyosin. This periodicity results from the tenden-

Figure 3 (opposite page). Translucent adductor muscle. (A) TEM micrograph of a transverse section of a specimen fixed in 2.5% glutaraldehyde in caccodylate buffer. Elongate profiles of fibers contain myofilaments [M] and dense bodies [DB] that are surrounded by endomysium [E]. A nucleus with a rounded profile [NU], vesicles of sarcoplasmic reticulum [SR] and mitochondria [MI] occur beneath the sarcolemma. There is a glial cell [GC] containing gliosomes in the endomysium. Bar is 3 µm long. (B) TEM micrograph of a longitudinal section of muscle fibers fixed in 1G4F, showing the central core of myofilaments [M] and dense bodies [DB]; and the peripheral cytoplasm containing elongate mitochondria [MI], sarcoplasmic reticulum [SR], and an elongate nucleus [NU]. The sarcolemma is invaginated [I] where there are hemidesmosomes [HD]. Between the muscle fibers, a glial cell [GC] is closely associated with a nerve-ending [NE]. Bar is 2 µm long. (Insert. At a higher magnification, clear [CV] and dense-cored [DV] vesicles can be seen in the nerve ending, which is closely associated with the sarcolemma and a glial cell. Bar is 1 µm long.). (C) TEM micrograph of a longitudinal section of a muscle fiber fixed in 1G4F. The oblique orientation of the dense bodies [DB] is apparent. Hemidesmosomes [HD] are present at the sarcolemma. Bar is 1 µm long. (D) TEM micrograph of transverse section of muscle relaxed in $MgSO_4$, fixed in Karnovsky's fixative. Thick myofilaments [TKM], thin myofilaments [TNM], and dense bodies [DB] are present. Cross-links [CL] can often be seen between the thick and thin myofilaments. There is a vesicle of sarcoplasmic reticulum [SR] and a hemidesmosome [HD] next to the sarcolemma, and thin filaments of endomysium [E] between the sarcolemmas of two adjacent muscle fibers. Bar is 100 nm long. (E) TEM micrograph of a longitudinal section of a specimen fixed in 2.5% glutaraldehyde in caccodylate buffer. There is transverse banding [TBA] at intervals of about 5 nm and diagonal banding [DBA] at intervals of about 35 nm in the thick myofilaments. There are cross-links [CL] between the thick and thin myofilament, also at intervals of about 35 nm. Bar is 100 nm long. (F) This paramyosin paracrystal has narrow bands [A] about 5 nm apart and accentuated bands [B] about 15 nm apart. Bar is 100 nm long.

cy of paramyosin molecules to assemble with a 14.5 nm intermolecular shift as a result of intermolecular ionic interactions (Kendrick-Jones et al. 1969; Castellani and Cohen 1987).

Paramyosin is composed of a coil of polypeptides in a similar manner to tropomyosin B and myosin (Chantler 1983). Paramyosin occurs widely among invertebrates and is present in most molluscan muscles. The proportion of paramyosin in transversely striated muscles such as the fast part of the sea scallop adductor is low (7% by mass; Chantler 1991), but is higher in muscles with thicker myofilaments. The translucent muscle of *C. angulata* contains 16 to 20% paramyosin (expressed as a percentage of total protein in the muscle), whereas the opaque muscle contains 22 to 39% (Chantler 1983).

Obliquely striated muscle contracts more slowly than transversely striated muscle, especially after prolonged periods of stimulation, although not as slowly as smooth muscles such as those in the opaque part of the oyster adductor, or the anterior byssus retractor muscle of the blue mussel *Mytilus edulis* (Millman 1967). The interaction between the thick and thin myofilaments involves a myosin-actin interaction in all muscle types (McLachlan 1984). This topic is reviewed in detail by Chantler (1991) using work on the transversely striated muscle of several species of scallops. Molluscan myosin is similar to vertebrate myosin (Castellani and Cohen 1987), and is present on the surface of the thick myofilament of molluscan smooth muscle, with the paramyosin forming a core (Elliott and Lowy 1970; Szent-Györgyi et al. 1971; Elliott 1974). However, the arrangement of myosin in relation to paramyosin is still unknown. Models have been proposed where the myosin molecules could lie alongside the paramyosin molecules. The rationale for this proposal is that both have a common repeat distance of 72.5 nm; both paramyosin and the rod region or tail of the myosin molecule have similar periodicities in their amino acid sequences (Cohen and Castellani 1988; Chantler 1991), and both consist mainly of an α-helical coil.

The thick myofilaments of the translucent muscle of *C. virginica* also have a diagonal periodicity of about 35 nm, and sometimes cross-links to adjacent

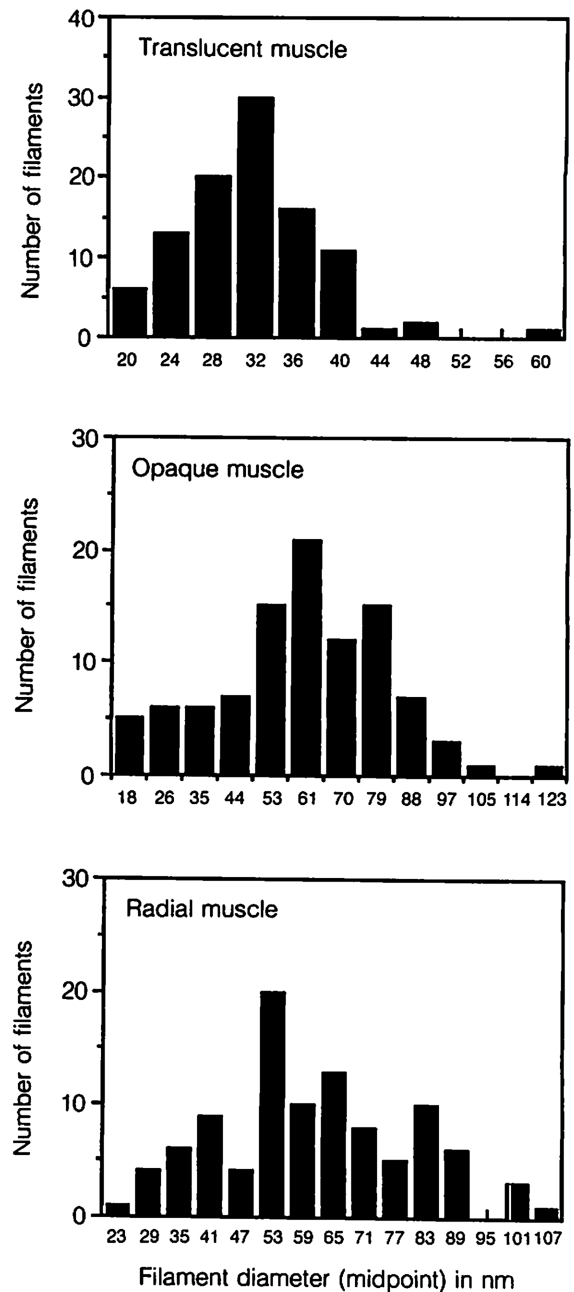


Figure 4. These graphs show the diameters of the thick myofilaments from the translucent and opaque parts of the adductor muscle, and the radial muscle of the mantle. The diameters of 100 myofilaments are grouped in increments of 4 nm for the translucent muscle, 8 or 9 nm for the opaque muscle, and 6 nm for the radial muscle. The mid-point of each group is given in nm on the horizontal axis of each graph.

thin filaments have a similar periodicity (Fig. 3E). This could result from more than one series of myosin-actin cross-links being included in the same section. These patterns are more distinct in the opaque smooth muscle of the adductor, so they will be considered in more detail later.

In the sea scallop, the thin filaments with a diameter of about 5 nm have been shown to be very similar to those in vertebrates, in that they contain actin, tropomyosin, and sometimes troponin (Chantler 1991). The thin myofilaments of other molluscan muscles such as those of the oyster have a similar appearance and diameter, suggesting that they are similar in composition to those found in scallops.

Opaque Adductor Muscle

The arrangement of the cell organelles in opaque adductor muscle is similar to that of the translucent muscle of *C. virginica.* Thick myofilaments containing paramyosin are present, but these are wider than those in translucent muscle, having a modal width of about 61 nm and a maximum width of about 126 nm (Fig. 4). These measurements are in the same range as those obtained by Philpott et al. (1960), who found that the thick myofilaments in *C. virginica* were 30 to 120 nm wide, and Elliott (1964), who found a range of 20 to 150 nm in *C. angulata* with a peak at 70 nm. The central myofilaments and dense bodies are surrounded by sarcoplasmic reticulum and mitochondria, and the nucleus is situated in cytoplasm to one side of the cell (Fig. 5A, B). As in the translucent muscle fiber, some dense bodies are attached to the sarcolemma to form hemidesmosomes.

Opaque muscle is similar in appearance and physiology to the anterior byssus retractor muscle of the blue mussel (Lowy and Hanson 1962; Millman 1964; Twarog 1967; Heumann and Zebe 1968; Sobieszek 1973), but the thick myofilaments are wider (the thick filaments in the blue mussel have a peak width of about 40 nm, and a maximum width of about 60 nm; Sobieszek 1973). Nexal junctions about 15 nm across have been reported between muscle cells in the anterior byssus retractor muscle that may be sites of intercellular conductivity (Twarog et al. 1973); however no similar junctions were found in the adductor muscle of *C. virginica.* The sarcolemmas of adjacent muscle cells often run parallel to each other (Fig. 5B), sometimes for several micrometers, but they are farther apart than in nexal junctions. Similar sarcolemmal junctions have been reported in several molluscs, including the adductor of the freshwater swan mussel *Anodonta cygnea* L. (Zs.-Nagy and Salánki 1970; Nicaise and Amsellem 1983).

The wide myofilaments found in the opaque part of the adductor muscle contain a higher proportion of paramyosin than those in obliquely striated muscle, and are capable of maintaining the "catch" state (Millman 1967). They exhibit cross- and diagonal striations like those of translucent muscle (Fig. 5C), which can be more clearly seen in homogenized preparations (Fig. 5D; Elliott et al. 1968). The diagonal striations are made up of dense patches that extend from one accentuated band to the next, and repeat at intervals of 72.5 nm along the length of the myofilament. The "checkerboard" pattern so formed is characteristic of the paramyosin core (Chantler 1991), and has been described in thick myofilaments of molluscan smooth muscle (Hanson and Lowy 1964). Morrison et al. (1970) used a method for enhancing linear structure on electron micrographs and found the transverse period to be 5.6 nm (so that the accentuated periods are at 16.8 nm) and the diagonal period to be 33.4 nm.

The checkerboard pattern was first shown by Hall et al. (1945) to correspond to a two-dimensional net deduced from low angle X-ray diffraction patterns of mollusc muscles (Bear 1944; Bear and Selby 1956). This pattern became known as the Bear-Selby net. The relationship between the patterns seen in paramyosin and myofilament preparations from "catch" muscle with the electron microscope and the Bear-Selby net are considered in detail by Elliott and Lowy (1970), Cohen et al. (1971), and Elliott (1979). When the thick myofilaments are cut obliquely, a banding can often be seen (Morrison 1993b) which is similar to that produced by tilting the grid (Elliott and Bennett 1982), and may correspond to planes of the Bear-Selby net. The paramyosin molecules are layered to form the core of the

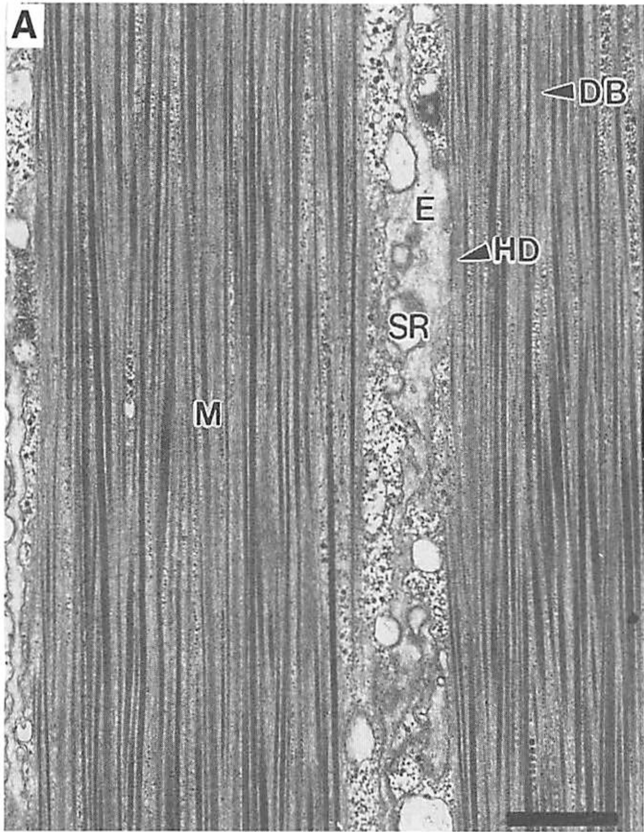
thick myofilaments, but in a sheared fashion. There are usually four molecules between each shear line in the opaque muscle of the European flat oyster *Ostrea edulis* and *C. angulata*, but this is somewhat variable, so that a paracrystalline rather than strict crystalline array is formed (Elliott and Bennett 1984). As in translucent muscle, myosin surrounds the paramyosin core. If the myosin molecules are arranged on the paramyosin core as suggested by Cohen (1982), it can be seen (Fig. 6) how cross-links could appear to be at intervals of about 33.4 nm if more than one layer of myosin molecules is included in a section.

Sometimes only one ring of thin myofilaments surrounds the thick myofilaments in the region of overlap, but more often several thin myofilaments surround each thick one (Fig. 5E). This may occur in a region near a dense body, although clumping of thin filaments has also been reported in muscle when contracted during the "catch" phase. Cross-links can often be seen between the thick and thin myofilaments, and also sometimes between thick myofilaments. Hoyle (1983) has suggested that direct interaction occurs between the thick myofilaments.

Many theories have been put forward to explain "catch" (Galtsoff 1964; Lowy et al. 1964), with recent work reviewed by Cohen and Castellani (1988) and Chantler (1991). The myosin-actin cross-bridges cycle slowly in the presence of ATP in catch muscles (Castellani and Cohen 1987; Cohen and Castellani 1988). Myosin from the anterior byssus retractor muscle from the blue mussel is unusual in that it becomes more soluble when phosphorylated in the rod portion of the molecule; there is evidence that phosphoryation and dephosphorylation are involved in catch (Castellani and Cohen 1987; 1992). As in the translucent muscle, the cross-links appear to have a similar periodicity to the diagonal bands (Hanson and Lowy 1964; Morrison 1993b).

In the opaque as well as the translucent parts of molluscan adductor muscles, the thin myofilaments are attached to dense bodies (Szent-Györgyi et al. 1971; Morrison 1993b) but in the opaque part of the adductor muscle the latter do not show any special arrangement in the muscle fiber. Thus, this type of muscle is classified as smooth (Twarog 1967). Some degree of order in the arrangement of the thick

Figure 5 (opposite page). Opaque adductor muscle. (A) TEM micrograph of longitudinal section of a specimen relaxed in $MgCl_2$, fixed in 2.5% glutaraldehyde in caccodylate buffer. There is a central core of thick myofilaments [M] and dense bodies [DB]. Hemidesmosomes [HD] and vesicles of sarcoplasmic reticulum [SR] are present next to the sarcolemma. The muscle fibers are separated by a thin layer of endomysium [E]. Bar is 1 μm long. (B) TEM micrograph of transverse section of specimen fixed in Karnovsky's fixative. There are myofilaments and dense bodies [DB] in the center of the muscle fiber, and a nucleus [NU] in the cytoplasm to one side of these myofilaments, surrounded by several mitochondria [MI]. Endomysium [E] containing filamentous material is present between the adjoining muscle fibers, except in one region where the sarcolemmas are closely apposed [AS]. Bar is 1 μm long. (C) TEM micrograph of longitudinal section of specimen fixed in 2.5% glutaraldehyde in caccodylate buffer. The thick myofilaments have transverse [TBA] and diagonal [DBA] banding, and sometimes seem to be very close together. Thin myofilaments [TNM] enter a dense body [DB] and glycogen [GL] is present between the myofilaments. Bar is 200 nm long. (D) Thick myofilament from homogenized muscle preparation. There are transverse bands [TBA] at intervals of about 5 nm, with every third band accentuated to give a periodicity of about 15 nm. Diagonal banding [DBA] consists of dense patches [DP] that extend from one accentuated band to the next. Bar is 100 nm long. (E) TEM micrograph of a transverse section of a specimen relaxed in $MgSO_4$ and fixed in Karnovsky's fixative. A single circle of thin myofilaments [TNM] surrounds some of the thick myofilaments [TKM]. Cross-links [CL] are present between thick and thin myofilaments, and between some thick myofilaments. Dense bodies [DB] are present. Bar is 100 nm long. (F) TEM micrograph of a longitudinal section of a specimen fixed in Karnovsky's fixative. Nerve endings containing vesicles are embedded in the peripheral sarcoplasm. Some vesicles are small and clear [CV], some are dense-cored [DV], and others contain varying amounts of dense material [V]. Bar is 500 nm long.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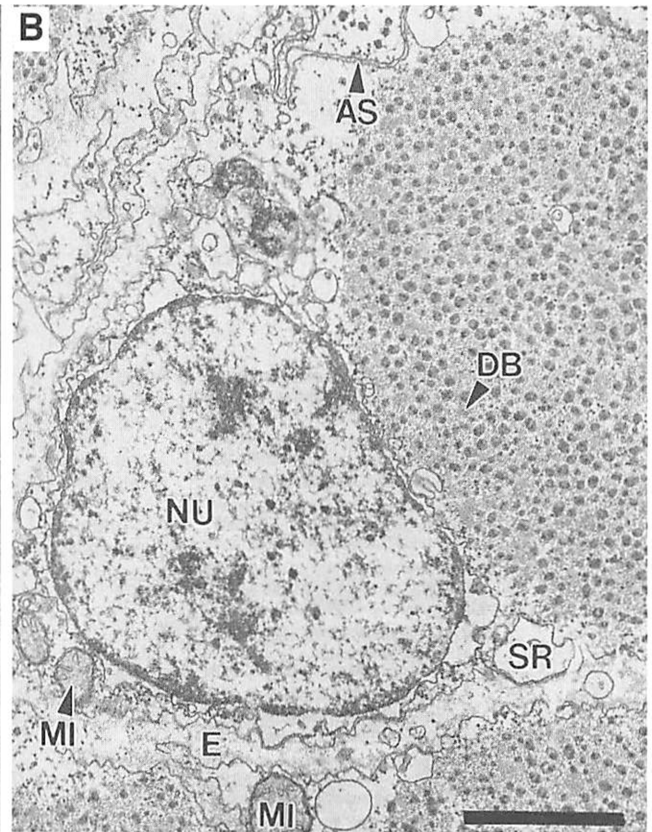
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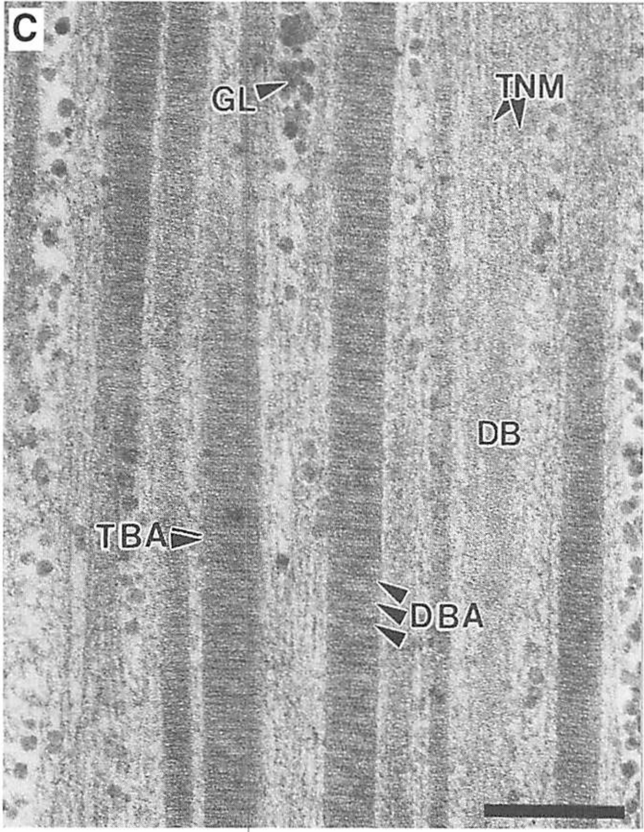
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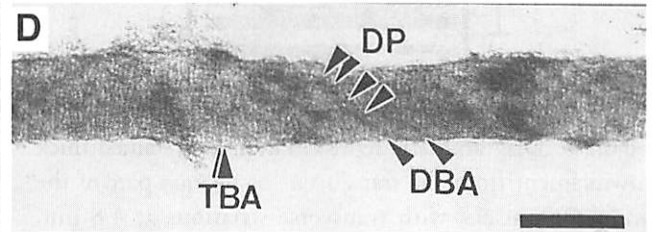
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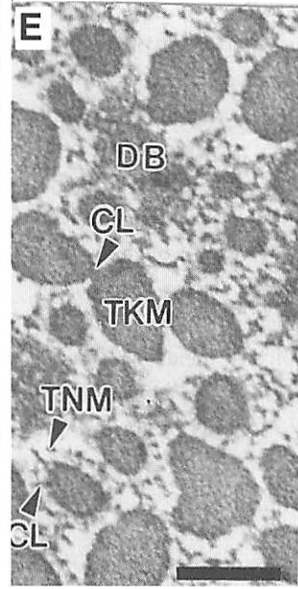
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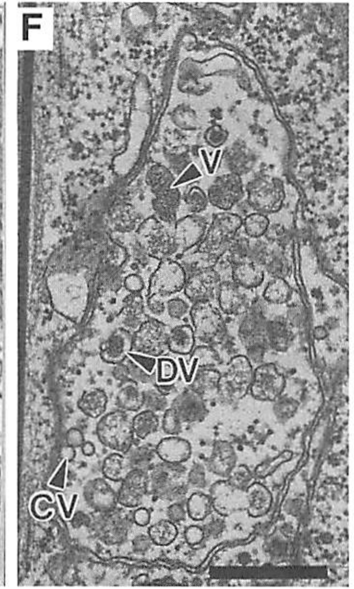
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and thin myofilaments is presumably necessary for the sliding-filament mechanism to work efficiently (Lowy and Hanson 1962), although it may not be as evident as in obliquely striated muscle because the myofilaments are longer. Such an order has been

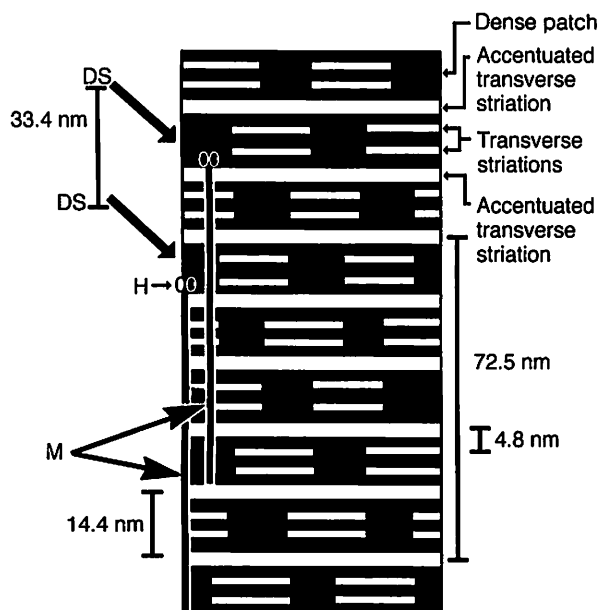


Figure 6. Diagrammatic representation of a stained thick myofilament from the translucent or opaque part of the adductor muscle, with transverse striations at 4.8 nm, with every third striation accentuated to give a periodicity of 14.4 nm. The possible position of two myosin molecules [M] on the surface of the paramyosin core is shown, as suggested by Cohen (1982). Dense patches occur at the ends of the paramyosin molecules, repeating at intervals of about 72.5 nm, and these give the appearance of diagonal striations [DS] at about 33.4 nm. If the myosin molecules are aligned with these paramyosin molecules, the heads, which form the cross-links, are also at intervals of about 66.8 nm, or 72.5 nm as obtained from X-ray diffraction work. If the myosin molecule heads [H] from two adjacent sites are included in the same section, there would appear to be cross links at intervals of about 33.4 nm.

demonstrated by optical diffraction techniques in the anterior byssus retractor muscle from the blue mussel by Sobieszek (1973), who postulated that there are sarcomeres consisting of three to four 25 µm-long thick myofilaments, many 140 µm long thin myofilaments, and two dense body halves. Such long, thin sarcomeres would be difficult to recognize in sectioned material. The thick myofilaments do not show an obvious symmetry (Elliott 1964), but Sobieszek (1973) described a regular, hexagonal arrangement extending for short distances.

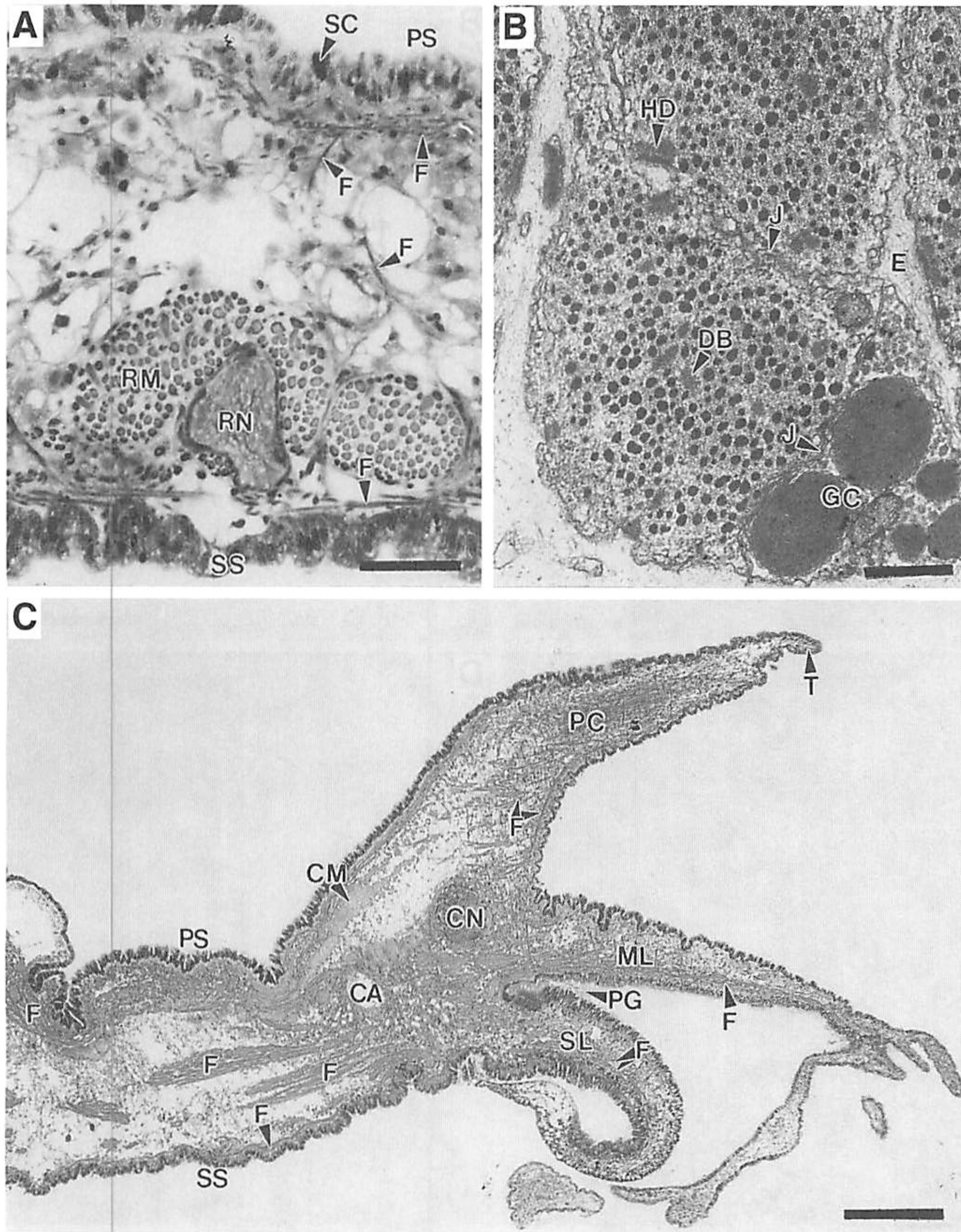
Glial cells occur between the muscle fibers, and there are nerve-endings containing clear vesicles, dense-cored vesicles, and vesicles filled with dense material at the sarcolemma, sometimes embedded in the sarcoplasm (Fig. 5F).

MUSCULATURE OF MANTLE AND MANTLE LOBES

The mantle, including the lobes at the free edge, is very contractile and can be withdrawn inside the shell (Galtsoff 1964; Morrison 1993a). In the mantle, radial muscles extend in a fan-like fashion from the visceral mass to the mantle edge (Fig. 76 in Galtsoff 1964). The radial muscle fibers and nerves are near the surface proximal to the shell, and sometimes a nerve is surrounded by muscle fibers (Fig. 7A). As in the adductor muscles, thick and thin myofilaments and dense bodies can be seen in the center of each muscle fiber, and some dense bodies form hemidesmosomes at the sarcolemma (Fig. 7B). There are junctions between these cells, which also form junctions with glial cells and nerve endings. The thick myofilaments of the radial muscle are of a similar size to those of the opaque part of the adductor, having a modal diameter of 53 nm (Fig. 4).

The radial muscle is thicker at the outer edge of the mantle, so that it extends to the pallial surface, and muscle fibers extend from it into the lobes (Fig. 7C). There is also a layer of circular muscle near the

Figure 7 (opposite page). Muscles of mantle and lobes. (A) Transverse section of mantle from specimen relaxed in $MgSO_4$, fixed in Karnovsky's fixative, embedded in JB4, and stained with chromotrope 2R-methylene blue. The pallial surface [PS] of the mantle with numerous secretory cells [SC] is dorsal, the shell surface [SS] ventral. The radial nerve [RN] is almost



surrounded by radial muscle fibers [RM]. Small subepithelial fibers [F] are present, as well as small muscle fibers crossing the mantle. Bar is 20 µm long. (B) TEM micrograph of transverse section of specimen relaxed in $MgSO_4$, fixed in Karnovsky's fixative. Radial muscle with thick and thin myofilaments, dense bodies [DB] and hemidesmosomes [HD]. Junctions [J] are present between two muscle cells and between one of the muscle cells and a glial cell [GC]. Endomysium [E] is present between the muscle cells. Bar is 500 nm long. (C) Longitudinal section of outer edge of mantle and lobes. Specimen relaxed in $MgSO_4$, fixed in 1G4F, embedded in JB4 and stained in chromotrope 2R-methylene blue. The pallial surface [PS] is dorsal, and the shell surface [SS] ventral. At the periphery, the mantle becomes divided into the pallial curtain [PC], middle lobe [ML], and shell lobe [SL]. The circumpallial nerve [CN] and artery [CA] are at the base of the lobes, and the periostracal groove [PG] is between the middle and shell lobe. Radial muscle fibers [F] extend through the mantle, and some continue into the lobes. There are also individual muscle fibers travelling across the lobes at various angles, and a band of circular muscle [CM] at the base of the pallial curtain. A tentacle [T] extends from the periphery of the pallial curtain. Bar is 0.3 mm long.

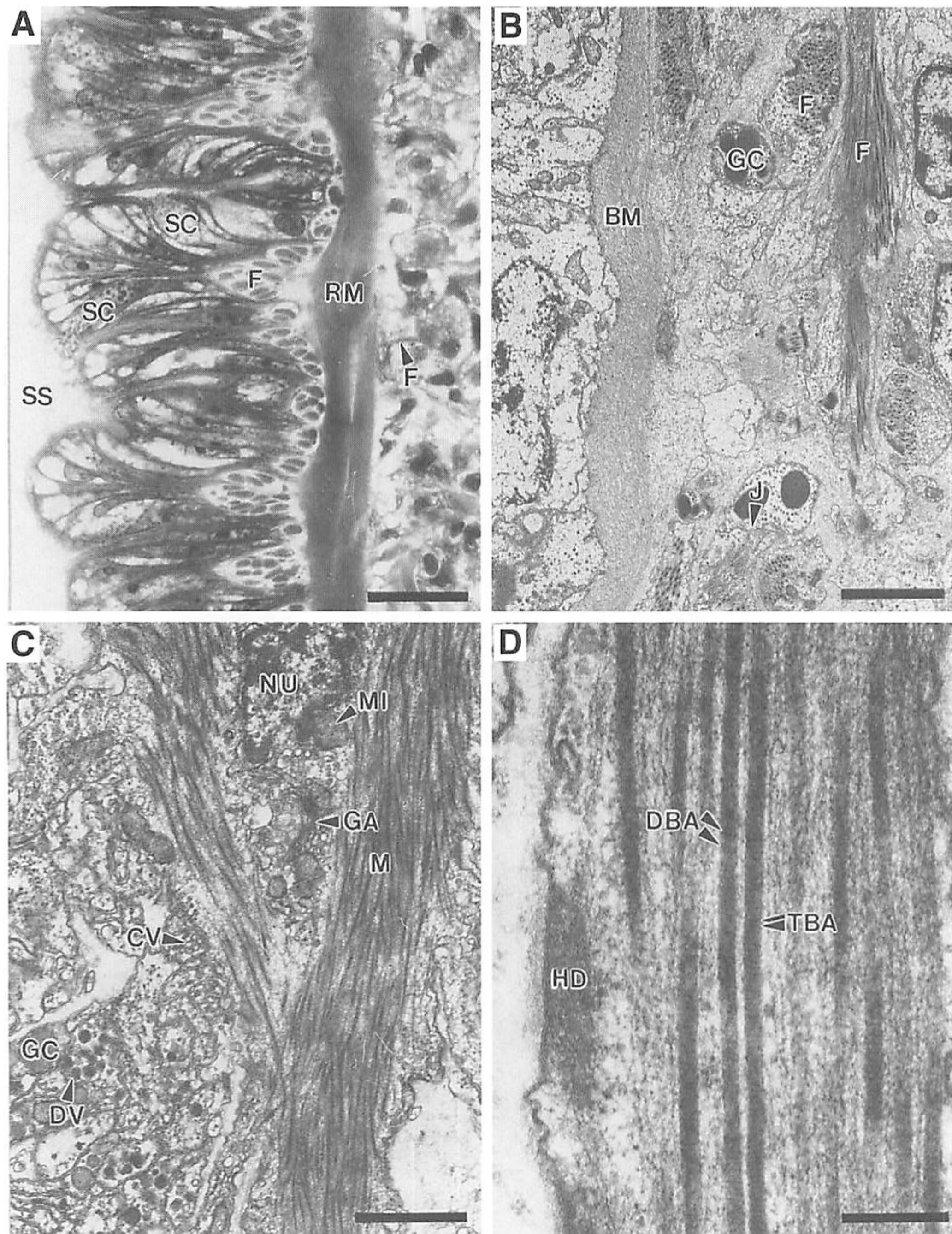


Figure 8. Muscles of mantle and lobes. (A) Longitudinal section of shell surface of mantle, fixed in 1G4F, embedded in JB4, and stained in toluidine blue. The shell surface [SS] is to the left. Subepithelial muscle fibers [F] are in cross-section in folds of the epithelium, and muscle fibers also extend across the mantle. There are longitudinal radial muscle fibers [RM] below the epithelium. Secretory cells [SC] are present in the epithelium. Bar is 20 µm long. (B) TEM micrograph of transverse section of specimen relaxed in $MgSO_4$, fixed in Karnovsky's fixative. Pallial surface of mantle is to the left, with a thick basement membrane [BM] below the epithelium. The subepithelial muscle fibers [F] are oriented in various directions, and form junctions [J] with each other and with glial cells [GC]. Bar is 2 µm long. (C) TEM micrograph of specimen relaxed in $MgSO_4$, fixed in Karnovsky's fixative. Center of middle lobe, with muscle fiber containing myofilaments [M] accompanied by nerve endings containing clear [CV] and dense-cored [DV] vesicles, and glial cells [GC]. There are mitochondria [MI] and a Golgi apparatus [GA] near the nucleus [NU] of the muscle fiber. Bar is 1 µm long. (D) TEM mi-

pallial surface at the base of the lobes. Similar but smaller muscle fibers run in all directions across the mantle and lobes, and are probably equivalent to the fusiform cells described by Galtsoff (1964) because they are difficult to identify as muscle cells without the use of electron microscopy. There is also a layer of small muscle fibers just below the epithelial surfaces (Fig. 7A). These may be the longitudinal muscle fibers described by Galtsoff (1964) in the mantle, but they may also be equivalent to abundant elastic fibers he described as lying just below the epithelium.

When the radial muscle contracts to withdraw the mantle into the shell, the epithelium of the mantle is thrown into folds. The muscle fibers underlying the shell surface are then seen mainly in cross-section in folds of the epithelium (Fig. 8A; Morrison 1993a). Those fibers beneath the epithelium of the pallial surface are not drawn up between the epithelial cells when the radial muscle contracts because there is a thick basement membrane below this epithelium (Fig. 8B).

All the muscle fibers found in the mantle appear to be uninucleate and contain a central group of myofilaments. The nerve-endings, as in the adductor muscle, sometimes contain small clear vesicles, and sometimes dense-cored or larger dark vesicles (Fig. 8C). The thick myofilaments of these muscles are cross-striated, and sometimes show diagonal banding, but the pattern is not as well-defined as that of the adductor muscles (Fig. 8D).

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REFERENCES

Bear, R.S. 1944. X-ray diffraction on protein fibres. Feather rachis, porcupine quill tip and clam muscle, Vol. 2. J. Amer. Chem. Soc. 66:2043-2050.

Bear, R.S. and C.C. Selby. 1956. The structure of paramyosin fibrils according to X-ray diffraction. J. Biophys. Biochem. Cytol. 2:55-70.

Bowden, J. 1958. The structure and innervation of lamellibranch muscle. Int. Rev. Cytol. 7:295-335.

Castellani, L. and C. Cohen. 1987. Rod phosphorylation favours folding in a catch muscle myosin. Proc. Natl. Acad. Sci. USA. 84:4058-4062.

Castellani, L. and C. Cohen. 1992. A calcineurin-like phospathase is required for catch contraction. FEBS 309 (3):321-326.

Chantler, P.D. 1983. Biochemical and structural aspects of molluscan muscle. Pages 78-154 in A.S.M. Saleuddin and K.M. Wilbur (eds.). The Mollusca, Vol. 4 Part 1, Physiology. Academic Press, San Francisco.

Chantler, P.D. 1991. The structure and function of scallop adductor muscles, Pages 225-304 in S.E. Shumway (ed.). Scallops: Biology, Ecology and Aquaculture. Elsevier, Amsterdam.

Cohen, C. 1982. Matching molecules in the catch mechanism. Proc. Natl. Acad. Sci. USA. 79:3176-3178.

Cohen, C. and L. Castellani. 1988. New perspectives on catch. Comp. Biochem. Physiol. 91C:31-33.

Cohen, C., A.G. Szent-Györgyi and J. Kendrick-Jones. 1971. Paramyosin and the filaments of molluscan "catch" muscles. J. Mol. Biol. 56:223-237.

Elliott, A. 1974. The arrangement of myosin on the surface of paramyosin filaments in the white adductor muscle of *Crassostrea angulata.* Proc. Roy. Soc. Lond. B 186:53-66.

Elliott, A. 1979. Structure of molluscan thick filaments: A common origin for diverse appearances. J. Mol. Biol. 132:323-341.

Elliott, A. and P.M. Bennett. 1982. Structure of the thick filaments in molluscan adductor muscle. Pages 11-28 in B.M. Twarog, R.J.C. Levine and M.M. Dewey (eds.). Basic Biology of Muscles: A Comparative Approach. Raven Press, New York.

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crograph of specimen relaxed in $MgSO_4$, and fixed in Karnovsky's fixative. Muscle fiber from middle lobe, near base of mantle. Transverse banding [TBA] and some indication of diagonal banding [DBA] is seen on the thick myofilaments. A hemidesmosome [HD] is present. Bar is 200 nm long.

Elliott, A. and P.M. Bennett. 1984. Molecular organization of paramyosin in the core of molluscan thick filaments. J. Mol. Biol. 176:477-493.

Elliott, A. and J. Lowy. 1970. A model for the coarse structure of paramyosin filaments. J. Mol. Biol. 53: 181-203.

Elliott, A., J. Lowy and J.M. Squire. 1968. Convolution camera to reveal periodicities in electron micrographs. Nature 219:1224-1226.

Elliott, G.F. 1964. Electron microscope studies of the structure of the filaments in the opaque adductor muscle of the oyster *Crassostrea angulata*. J. Mol. Biol. 10:89-104.

Elston, R. 1980. Functional anatomy, histology and ultrastructure of the soft tissues of the larval American oyster, *Crassostrea virginica*. Proc. Natl. Shellfish. Assoc. 7: 65-93.

Galtsoff, P.S. 1964. The American Oyster *Crassostrea virginica* Gmelin. Fish. Bull. 64:1-480.

Hall, C.E., M.A. Jakus and F.O. Schmitt. 1945. The structure of certain muscle fibrils as revealed by the use of electron stains. J. Appl. Phys. 16:459-465.

Hanson, J. and J. Lowy. 1960. Structure and function of the contractile apparatus in the muscles of invertebrate animals. Pages 265-335 in G.H. Bourne (ed.). The Structure and Function of Muscle, Vol. 1. Academic Press, New York

Hanson, J. and J. Lowy. 1961. The structure of the muscle fibers in the translucent part of the adductor of the oyster, *Crassostrea angulata*. Proc. Roy. Soc. London B. 154:173-196.

Hanson, J. and J. Lowy. 1964. The structure of molluscan tonic muscle. Pages 400-409 in J. Gergely (ed.). Biochemistry of Muscle Contraction, Vol. 2. Little, Brown and Company, Boston.

Heumann, H.-G. and E. Zebe. 1968. Uber die funktionsweise glatter muskelfasern. Elektronenmikroskopische untersuchungen am byssusretraktor (ABRM). Z. Zellforsch. 85:534- 551.

Hoyle, G. 1983. Muscles and Their Neural Control. John Wiley and Sons, New York.

Kendrick-Jones, J., C. Cohen, A.G. Szent-Györgyi and W. Longley. 1969. Paramyosin: Molecular length and assembly. Science 163:1196-1198.

Lowy, J. and J. Hanson. 1962. Ultrastructure of invertebrate smooth muscles. Physiol. Rev. 42:34-42.

Lowy, J., B.M. Millman and J. Hanson. 1964. Structure and function in smooth tonic muscles of lamellibranch molluscs. Proc. Roy. Soc. Lond. B. 160:525-536.

McLachlan, A.D. 1984. Structural implications of the myosin amino acid sequence. Ann. Rev. Biophys. Bioeng. 13:167-189.

Millman, B.M. 1964. Contraction in the opaque part of the adductor muscle of the oyster *Crassostrea angulata*. J. Physiol. 273:238-262.

Millman, B.M. 1967. Mechanism of contraction in molluscan muscle. Am. Zool. 7:583-591.

Morrison, C.M.. 1970. A comparison of the adductor muscles of several pelecypods (Phylum Mollusca). Doctoral dissertation. Dalhousie University, Halifax, Nova Scotia, Canada.

Morrison, C.M. 1993a. Histology and cell ultrastructure of the mantle and mantle lobes of the eastern oyster, *Crassostrea virginica* (Gmelin): A summary atlas. Am. Malacol. Bull. 10:1-24.

Morrison, C.M. 1993b. The histology and ultrastructure of the adductor muscle of the eastern oyster *Crassostrea virginica* (Gmelin). A summary atlas. Am. Malacol. Bull. 10:25-38.

Morrison, C.M., M.L. Cameron and P.H. Odense. 1970. Periodicities in the thick filaments of the opaque and translucent parts of the adductor of the oyster, *Crassostrea virginica*. Can. J. Zool. 48: 608-609.

Morrison, C.M. and P.H. Odense. 1968. Ultrastructure of the striated muscle of the scallop (*Placopecten magellanicus*). J. Fish. Res. Board Can. 25:1339-1345.

Morrison, C.M. and P.H. Odense. 1973. Gross structure of the adductor muscle of some pelecypods. J. Fish. Res. Board Can. 30: 1583-1585.

Morrison, C.M. and P.H. Odense. 1974. Ultrastructure of some pelecypod adductor muscles. J. Ultrastruct. Res. 49: 228-251.

Morton, J.E. 1979. The Molluscs. Hutchinson, London.

Nicaise, G. and J. Amsellem. 1983. Cytology of muscle and neuromuscular junction. Pages 1-33 in A.S.M. Saleuddin and K.M. Wilbur (eds.). The Mollusca, Vol. 4. Academic Press, New York.

Nunzi, M.G. and C. Franzini-Armstrong. 1981. The structure of smooth and striated portions of the adductor muscle of the valves in a scallop. J. Ultrastruct. Res. 76:134-148.

Philpott, D.E., M. Kahlbrock and A.G. Szent-Gyørgyi. 1960. Filamentous organization of molluscan muscles. J. Ultrastruct. Res. 3:254-269.

Ruegg, J.C. 1961. On the tropomyosin-paramyosin system in relation to the viscous tone of lamellibranch catch muscle. Proc. Roy. Soc. London. B. 154:224-249.

Salánki, J. and I. Zs.-Nagy. 1966. Comparative histologi-

cal investigations on marine lamellibranch adductors in different functional conditions. Acta Biol. Hung. 17:315-328.

Sobieszek, A. 1973. The fine structure of the contractile apparatus of the anterior byssus retractor muscle of *Mytilus edulis.* J. Ultrastruct. Res. 43:313-343.

Szent-Györgyi, A.G., C. Cohen and J. Kendrick-Jones. 1971. Paramyosin and the filaments of molluscan "catch" muscles. II. Native filaments: Isolation and characterization. J. Mol. Biol. 56:239-258.

Twarog, B.M. 1967. The regulation of catch in molluscan muscle. J. Gen. Physiol. 50:157-169.

Twarog, B.M., M.M. Dewey and T. Hidaka. 1973. The structure of *Mytilus* smooth muscle and the electrical constants of the resting muscle. J. Gen. Physiol. 61: 207-221.

Zs.-Nagy, I. and J. Salánki. 1970. The fine structure of neuromuscular and intermuscular connections in the adductors of *Anodonta cygnea* L. (Mollusca, Pelecypoda). Annal. Biol. Tihany. 37: 131-143.

Chapter 5

Mechanisms and Physiology of Larval and Adult Feeding

ROGER I.E. NEWELL AND CHRISTOPHER J. LANGDON

INTRODUCTION

Planktotrophic larval and benthic life stages of the eastern oyster, *Crassostrea virginica* (Gmelin), obtain food by removing particles from suspension in the water column. Such particulate food is often quantitatively and qualitatively variable in estuarine waters and is composed of complex mixtures of a wide size range of living microorganisms, detritus, and inorganic particles (Soniat et al. 1984; Berg and Newell 1986; Baldwin and Newell 1991). Consequently, feeding mechanisms of larval and adult eastern oysters have evolved to a high degree of specialization necessary to capture and process particulate material.

Here we review current knowledge of these specialized feeding mechanisms of *C. virginica.* Because of the distinctly different feeding processes employed by planktotrophic veliger larvae and the benthic post-metamorphic stage, we have divided our review into two sections that consider each separately. Langdon and Newell in Chapter 6 review information on how food particles are handled by these two life-stages once they pass through the mouth and enter the digestive system. In addition, we review how feeding behavior of larval and adult eastern oysters can be modified by variations in food quantity and composition. Shumway in Chapter 13 reviews the influence of other physical environmental determinants of feeding behavior, including salinity, temperature, and oxygen tension.

LARVAL OYSTERS

Development and Structure of the Velum

Many bivalve species, including *C. virginica,* are oviparous, with both fertilization and larval development occurring entirely outside of the parent and in the plankton. Detailed descriptions of development and larval morphology in *C. virginica* are given by Eble in Chapter 2.

In the eastern oyster, as for many species of bivalve molluscs, the egg yolk contains only sufficient protein and lipid reserves for embryos to develop essential tissues including feeding organs and a gut. Once this functional alimentary system develops, planktotrophic larvae must rely on exogenous food sources in order to grow. Larval *C. virginica* typically feed and grow in the plankton under natural estuarine conditions for about three weeks before reaching the pediveliger stage, when they become competent to metamorphose (see Kennedy, Chapter 10). It has been suggested that this pelagic phase is considerably longer than is needed to ensure adequate dispersal of larvae by estuarine and coastal water currents (Crisp 1976; Strathmann 1987). Therefore, it is believed that the major advantage of producing planktotrophic larvae is that fecundity can be maximized because parental investment of nutrients per egg is minimized (Strathmann 1987).

Nutrients for embryogenesis are supplied by the materials, including lipids and protein, sequestered in the egg by the female during vitellogenesis. The free-floating, fertilized egg develops into the trochophore larval stage. This stage is characterized by a crown of cilia (prototroch) which is used as a swimming organ and by the development of a shell gland, a rudimentary mouth, and a digestive system. Some 24 to 48 h after fertilization, the prototroch expands into two ciliated semicircular lobes that are connected to form the velum (Fig. 1A). The velum functions as an organ for both swimming and capturing food particles; it is also the principal site for absorption of dissolved organic material from seawater (see Langdon and Newell, Chapter 6). The shell is enlarged to cover the larval body, forming the characteristic D-shaped shell of the prodissoconch I veliger (Carriker and Palmer 1979). The mouth and alimentary system develop fully, allowing ingestion and assimilation of food particles. There appears to be little further differentiation of the velum and alimentary system as the bivalve veliger develops to the prodissoconch II stage, although the size of velum and cilia length increase with larval growth.

The following discussion of velum morphology is based in part upon studies on the morphology of larval *C. virginica* (Elston 1980) and the European flat oyster *Ostrea edulis* (Waller 1981). Four bands of cilia encircle the periphery of the velum. Situated on the inner peripheral lobe of the velum is the first and most conspicuous ciliary band (Fig. 1A), called the preoral ciliary band by Erdmann (1935) and Waller (1981), the principal ring by Elston (1980), or the prototroch band by Strathmann (1987). For this review we will use the term, "principal band." In *O. edulis* (Waller 1981) and the hard clam, *Mercenaria mercenaria* (Gallager 1988), this principal band is composed of two concentric orthoplectic[1] groupings of cirri originating from a double row of large cells. It remains to be determined if the principal band in *C. virginica* larvae is also composed of two concentric bands of cilia. In *O. edulis*, each concentric ring consists of cirral units composed of numerous (20 to 80) 50- to 70-μm long cilia originating from a single large cell (Waller 1981). Cilia from a single cell are in contact with each other for nearly their entire length and only splay apart at the tips of each cirrus (Waller 1981). In *M. mercenaria*, each cirrus is composed of fewer cilia than in *O. edulis* (about 10) and the distance between adjacent cirri in the diaplectic[2] rows is about 0.5 μm (Gallager 1988). The principal cirri of larval *C. gigas*, of unspecified age, are about 30 μm long (Strathmann and Leise 1979) and, therefore, are similar to those of *M. mercenaria* which range in length from 32 μm in 2-d old larvae to 45 μm long in 10-d old larvae (Gallager 1988). Thus, both of these planktotrophic species have principal cirri that are about half the length of those found in larviparous *O. edulis*. According to Strathmann and Leise (1979), the length of the principal cirri in various species of molluscan veligers is positively correlated with egg and larval size.

[1] Orthoplectic: parallel to plane of beat and fluid flow (Knight-Jones 1954; Waller 1981).
[2] Diaplectic: perpendicular to plane of beat and fluid flow (Knight-Jones 1954; Waller 1981).

Figure 1 (opposite page). *Crassostrea virginica.* (A) Diagrammatic illustration of prodissoconch II veliger larva viewed from the left × 380. Note that principal and postoral bands are composed of complex cirral units and not single cilia as illustrated. Also, the principal band is composed of a double row of cirri and not the single row illustrated (see text for complete details). Based on drawings by Galtsoff (1964) and Elston (1980); (B) Diagrammatic sketch of the mouth region of the velum with principal and postoral cirral units in various stages of their effective and recovery strokes. The putative direction of the opposing metachronal waves in these two bands is indicated by large black arrows. The movement of food particles in the adoral ciliary tract and the possible anticlockwise movement of a food cluster around the mouth is indicated by broad open arrows, together with the track of particles breaking away and entering the mouth (small black arrow); (C) Diagrammatic sketch of a portion of the velum showing principal and postoral ciliary bands, with cirral units in various stages of their effective and recovery strokes. The direction and arc of the effective stroke are illustrated and the motion of the recovery stoke is indicated by the dashed cirri. The possible tracks of two particles being entrained into the adoral ciliary tract are indicated by the broken lines. Direction of particle movement in the adoral tract is indicated by the broad open arrow.

A

Dorsal

Velar retractor muscles

Visceral cavity

Rectum

Anus

Posterior adductor muscle

Statocyst

Eye spot

Heart

Posterior

Gill rudiment

Stomach

Mantle cavity

Foot

Posterior visceral cavity membrane

Esophagus

Mouth

Adoral ciliary tract

Velum

Principal ciliary band

Ventral

Postoral ciliary band

Inner peripheral lobe

Outer peripheral lobe

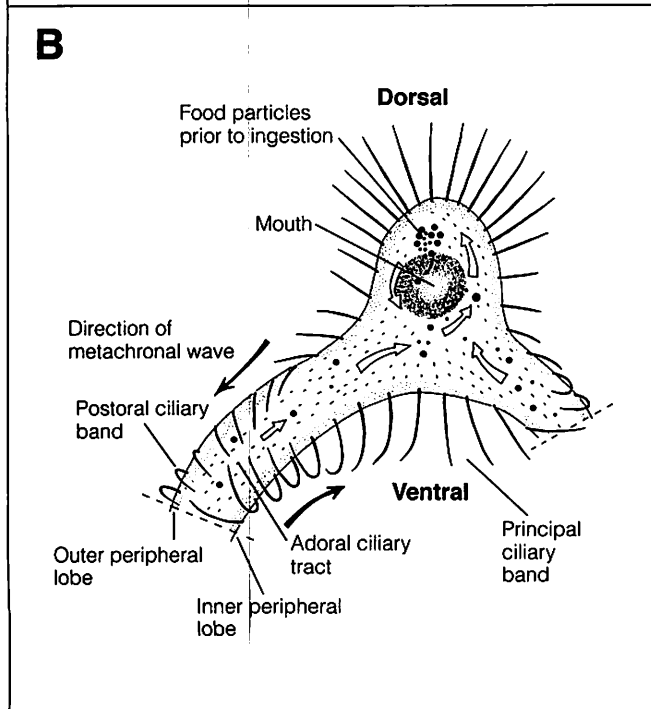
Anterior adductor muscle

Anterior

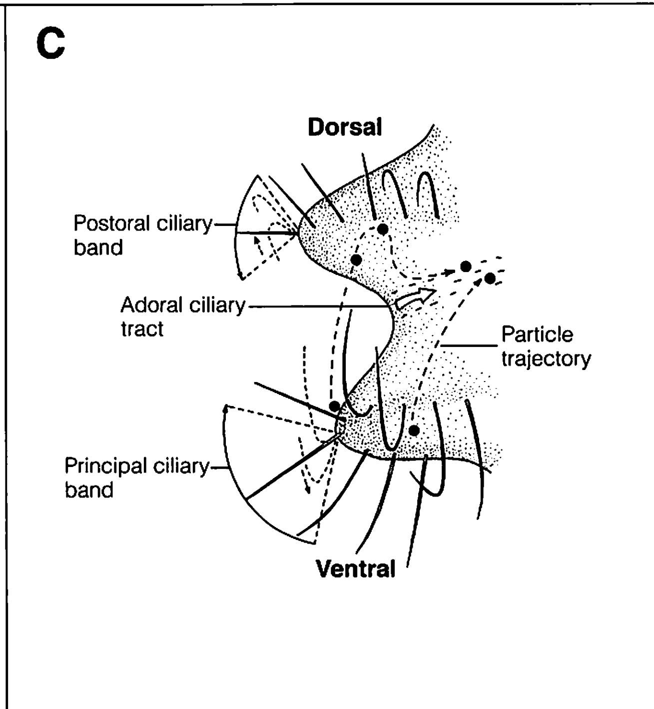
Digestive gland

Intestine

B



C



Generally, rates of particle clearance for molluscan larvae are proportional to the length of cirri on the principal band (Strathmann and Leise 1979). For example, Gallager (1988) reported that the total waterflow through the velal cirri of 10 d-old *M. mercenaria* larvae (234 µm shell length) was 8-fold of that through the cirri of 2 d-old larvae (100 µm shell length). He suggested that this was because older larvae possess a velum of larger circumference and longer cirri. In contrast, Crisp et al. (1985) reported no age related differences in clearance rates of free-swimming *O. edulis* larvae. This rather surprising result is due to the fact that *O. edulis* is larviporous and larvae are not released from the brood pouch until they have grown to a shell length >200 µm. Consequently, larvae available to be studied by Crisp et al. (1985) had a narrow range of shell lengths (214 to 250 µm) and thus the principal cirri of the different age larvae were of a similar length.

The second most distinct band of cilia is located on the outer peripheral lobe of the velum (Fig. 1C). It is commonly called the postoral ciliary band (Erdmann 1935; Elston 1980; Waller 1981) but was also called the metatroch band by Strathmann (1987). This band is composed of a single row of diaplectic cirri, each cirrus being 15 to 20 µm long and composed of a single orthoplectic row of 4 or 5 cilia (Waller 1981).

The third ciliary band lies at the base of the groove formed between the inner and outer peripheral lobes of the velum, and is completely overlapped by the principal and postoral cirral bands. It is called the adoral ciliary tract (Elston 1980; Waller 1981), or food groove (Strathmann 1987). Cilia, randomly arranged within this 20 µm wide tract, are short (about 8 µm in length) and beat towards the mouth.

The fourth ciliary tract, located centrally on the inner peripheral lobe, and encircled by the principal ciliary ring, is the inner preoral ciliary band (Elston 1980; Waller 1981). Cilia within this band are no more than 20 µm long and are randomly arranged (Waller 1981). This band is not illustrated in Fig. 1A.

The mouth merges laterally with the outer peripheral lobe and ventrally with the inner peripheral lobe (Fig. 1B) to form a funnel-shaped opening (Elston 1980). The cilia within the adoral ciliary tract are continuous with cilia lining the alimentary system (Elston 1980). The postoral cirri on the outer peripheral lobe enclose the mouth at the posterior edge of the velum. In this latter region, there has been observed in both *O. edulis* and the blue mussel, *Mytilus edulis*, a tightly clustered group of cilia, called in each species, respectively, the postoral ciliary tuft by Waller (1981) or oral palp by Bayne (1971). Elston (1980) does not mention if such a postoral ciliary tuft is present in *C. virginica*.

Function of Velar Ciliary Bands

Little detailed information is available on feeding processes and ciliary function of *C. virginica* larvae. Such fundamental behavioral and physiological studies have generally not been given as much emphasis as applied feeding studies that have focused on developing algal diets suitable for rearing larvae in commercial hatcheries (see Langdon and Newell, Chapter 6). Fortunately, because there are many similarities in the ontogeny of molluscan planktotrophic larvae, information reported for other bivalve species (including the Ostreidae) can be used to elucidate feeding mechanisms of larval *C. virginica*.

The following discussion of the function of the ciliary bands is mainly based on microscopic studies of preserved larvae. *In vivo* it is the coordinated functioning of these various ciliary bands on the velum that serves to propel the veliger through the water column and concurrently capture and transport food particles to the mouth. However, it only became possible to study such complex motion when traditional microscope techniques were linked to high speed cinematography (Strathmann et al. 1972; Strathmann 1987) and video recorders (Gallager 1988) to follow the motion and fate of particles adjacent to the velum of swimming larvae.

In the principal band, cirri have an effective stroke that is directed dorsally over the edge of the velum (Fig. 1C). This motion generates the thrust that propels the larva velum-first through the water column (Waller 1981). Adjacent cirri are coordinated

into a diaplectic metachronal[3] wave, with adjacent cirri in orthoplectic rows in sequential stages of their effective stroke (Waller 1981). This action produces a continuous ciliary wave propagated around the principal ciliary band. The diaplectic metachronal wave is propagated in a clockwise direction (as viewed looking down onto the ventral surface of the velum) in *O. edulis* and *M. mercenaria.* This clockwise metachronal wave is called the laeoplectic beat (Knight-Jones 1954; Waller 1981). Metachronal beating should cause the veliger to rotate counterclockwise when it swims, i.e., in the direction opposite to the propagation of the metachronal wave (Knight-Jones 1954; Waller 1981). Swimming *O. edulis* larvae, however, slowly rotate clockwise, i.e., in the same direction as the propagated metachronal wave. In these larvae the effective beat of principal cirri is not exactly perpendicular to the velar margin but at an oblique angle which effectively counteracts the rotational forces produced by the metachronal wave (Knight-Jones 1954; Waller 1981). As discussed below, the oblique direction of this beating may have important implications for particle capture.

Cirri in the postoral band have an effective stroke that is directed ventrally toward the velar margin (Fig. 1C) and hence in an opposing direction to the oblique dorsal stroke of the principal cirri (Strathmann and Leise 1979; Waller, 1981). Based on the work of Knight-Jones (1954), Waller (1981) theorized that the metachronal wave of the postoral cirral band should be propagated in the opposite direction around the velum to that of the principal cirri (Fig. 1B). The direction of beat remains to be determined for *C. virginica.* Cirri in the postoral band merge with other cilia around the posterior edge of the mouth to form the post-oral tuft. Although the exact function of this tuft is unknown, it may serve to facilitate the ejection of food particle clusters from the oral region in some veliger larvae (Bayne 1971; Waller 1981).

[3] A metachronal rhythm is an inherent characteristic of ciliated surfaces. It involves the synchronization of the beat of an individual cilium, such that it lags slightly behind adjacent cilia "earlier" in the sequence but slightly precedes adjacent cilia "later" in the sequence.

The adoral ciliary tract, situated in the groove formed between the inner and outer peripheral lobes of the velum, is responsible for carrying food particles to the mouth (Fig. 1B). Adoral cilia on each side of the velum beat in a posterior direction towards the mouth. Little is known concerning the mechanisms and function of the inner preoral ciliary band on the inner peripheral lobe, and Waller's (1981) suggestion that cilia in this band may serve as up-stream tactile receptors has not been tested experimentally.

Mechanisms of Particle Capture

In this section we describe how the ciliary bands on the velum capture and transport food particles to the mouth. A generalized description of larval feeding was developed by Strathmann et al. (1972). They filmed a number of invertebrate larvae and found two basic feeding types: (1) upstream retention in which particles are retained upstream of the current-generating band of cilia, and (2) downstream retention in which particles are retained between the current-generating band and a second parallel row of cilia beating in the opposite direction. Downstream capture of particles occurs in planktotrophic veliger larvae of bivalves (Fig. 1C).

Gallager (1988) used high speed video microscopy to study aspects of particle capture and ingestion in larval *M. mercenaria* fed on the alga *Isochrysis* aff. *galbana* (clone T-Iso). He found that cells from up to 600 µm in front of the velum of *M. mercenaria* were caught up in the flow field generated by the principal cirri. He also confirmed the observation of Strathmann and Leise (1979) that not all particles entrained within the velar flow field have an equal probability of capture. Cells that entered a zone 15 µm wide above the recovery stroke of the principal cirri had a 42 to 64% chance of being captured, whereas those outside this zone were rarely captured.

The original concept that bivalve larvae capture particles by sieving them from suspension on a mesh composed of velar cirri has been shown to be incorrect by Strathmann and Leise (1979). They observed that even though adjacent diaplectic principal cirri are closely spaced [about 0.5 µm in *M. mercenaria*

(Gallager 1988)], the laeoplectic metachrony causes the tips of adjacent cirri to be separated by a large angle, hence creating a gap of up to 20 µm between their effective strokes, resulting in increased space for particles to pass between adjacent cirri. Thus the observed high retention efficiencies of larvae for particles as small as 1 µm (Gallager 1988; Baldwin and Newell 1991) could not occur if particle retention was due to sieving by the principal cirri (Strathmann and Leise 1979).

Strathmann and Leise (1979) performed a micro-cinematographic study of actively feeding veliger larvae of several molluscan species, including the Pacific oyster, *Crassostrea gigas.* They reported that particles entrained in the through-current generated by the effective stroke of the principal cirri were overtaken and pushed forward by beating cirri and sometimes may weakly adhere to the cirri. Although direct evidence for adhesion of particles is currently lacking, this proposed mechanism is similar to the "direct interception" hypothesis of particle capture proposed for suspension-feeding aquatic invertebrates (Rubenstein and Koehl 1977; LaBarbera 1984). Particles adhering to cirral surfaces could be released when principal cirri initiated their recovery stroke, and a proportion of the particles would then be pushed into the adoral food tract (Fig. 1C). It is not known what mechanisms would cause food particles to become attached and then be released from the principal cirri. Strathmann and Leise (1979) speculated that the current generated by the postoral cirral band may help retain particles released from the principal cirri and move them into the food tract.

In the section on feeding mechanisms of adult oysters (page 202), we summarize the hydromechanical mechanism proposed by Jørgensen (1981a, 1983) to explain particle retention by the gill of adult bivalves. We hypothesize that this mechanism of particle retention may also explain particle capture by the larval velum. The oblique through-current generated by the beat of principal cirri will meet an opposing current generated by the opposing beat of the postoral cirral band (Fig. 1C). Steep velocity gradients will be produced above the adoral food tract where the two ciliary currents meet. Particles caught in such velocity gradients will be exposed to transverse forces that could cause them to spin and move perpendicularly to the through current, becoming caught in surface currents generated by adoral cilia in the food tract.

In support of this hypothesis of hydromechanical particle capture in veliger larvae, Strathmann and Leise (1979) reported that particles entering the adoral ciliary tract of molluscan veligers, including *C. gigas,* were not always observed to have been in direct contact with the cilia. Also, although Strathmann and Leise (1979) could find no definitive function for the postoral cirri, Strathmann et al. (1972) determined that particle capture ceased when the postoral band ceased beating, despite continued activity of the principal ciliary band. Gallager (1988) reported that *I.* aff. *galbana* cells captured by veligers of *M. mercenaria* commonly rotated clockwise when in the shearing boundary layer above the return stroke of the principal cirri. This rotation indicates that the algal cells were not attached to cirri but freely suspended in the boundary layer. Obviously these observations would not be expected if particles were being captured by direct contact with cilia, but they are consistent with hydromechanical particle capture. This hydromechanical hypothesis for particle capture in bivalve larvae requires further testing, perhaps by using dye tracer techniques (Gilmour 1986) together with high speed video microscopy (Gallager 1988). It should be noted, however, that the concepts of particle capture by ciliary contact or hydromechanical forces are not mutually exclusive, and both processes may be used by bivalve larvae to capture food particles.

Food Ingestion and Selection

Research by Gallager (1988) has elaborated complex, post-capture, particle-handling mechanisms of larval *M. mercenaria* and his work will be described here because comparable research for oysters is lacking. He observed that captured food particles are transported toward the mouth by the adoral cilia in the food tract. However, as happens in other molluscan veligers (Fretter and Montgomery 1968), particles may not be ingested immediately but first accumulate in loose clusters of 2 to 15 cells at the mouth (Fig. 1B), a process that may depend on gut satiation

(see below). Gallager (1988) observed that cells were periodically separated from clusters at the mouth and were either rejected or ingested while newly captured cells were continually being added to the cluster. The number of cells in the food cluster increased rapidly to about 16 cells in *M. mercenaria* fed on high concentrations of *I.* aff. *galbana* , at which point the entire cluster was often rejected and formation of a new cluster was initiated within a few seconds. Hence, particles were selected in some way from the clusters, and these particles were ingested and carried down the esophagus to the stomach by ciliated epithelial cells lining the esophagus.

It is possible for ingested cells to be regurgitated from the mouth before passing through the ciliated, cellular constriction at the entrance to the stomach (Elston 1980). Particles may be rejected either by rapid constriction of the esophagus associated with contraction of the velum or by cilia lining the esophagus (Gallager 1988). Gallager (1988) suggested that particles were differentially sorted by larvae, perhaps on the basis of size while being rotated in the pre-ingestive food cluster and on the basis of their chemical composition at the esophagus-stomach constriction.

Evidence for the ability of bivalve larvae to discriminate between food particles based on chemical composition is equivocal, mainly because of problems in devising unambiguous experimental protocols. Mackie (1969) reported that larval *C. virginica* preferentially ingested certain species of algae when feeding on natural phytoplankton assemblages. Baldwin and Newell (1991) provided evidence, based on radiotracer studies, that large (20 to 30 μm diameter) rapidly growing mixotrophic dinoflagellates were preferentially ingested by eastern oyster larvae (>200 μm shell height) feeding on natural plankton assemblages. Baldwin (1995) cultured the algae *Thalassiosira pseudonana* strain 3H with low inorganic nitrogen in the media that caused the total carbon to nitrogen content of the cells to increase from the normal value of 7.2:1 to 16.2:1. Eastern oyster larvae detected the differences in composition of these cells as they ingested the more nutritious (i.e., higher proteinaceous nitrogen) low C:N ratio cells at a rate 3.3 times faster than cells with less nitrogen (Baldwin 1995). However, when the two types of cells were fed to eastern oyster larvae in 1:1 mixtures, the larvae did not exhibit the expected response of significant preferential ingestion of the low C:N cells. Wilson (1979) reported that larvae of *O. edulis* actually fed more rapidly on algal cells from cultures in the stationary growth phase than cells from cultures in the log phase. This response was exactly opposite to what would be expected if larvae were capable of selecting particles based on high protein content because cells in stationary phase are presumably less nutritious as a result of having a higher C:N ratio (Davidson et al. 1991).

Further research, using particles of the same size and defined composition, needs to be performed to determine if eastern oyster larvae can selectively ingest particles. Studies of larval *M. mercenaria* indicate that particle surface properties, such as electrostatic charge and hydrophobicity, can influence the capture and post-capture handling of particles (Solow and Gallager 1990; Gallager et al. 1991; Langdon et al. 1991). In addition to obvious nutritional benefits of avoiding filling the digestive system with particles of low nutritional value, rejection of captured mineral particles would prevent an increase in larval mass and consequent higher energetic costs of swimming (Gallager 1988).

There is some evidence that particles in the stomach of larvae undergo at least partial sorting (Robinson 1981; Bayne 1983; Strathmann 1987) with nonnutritious particles "passing through the gut of veligers as quickly as they are ingested" (Gallager 1988). Nutritious algal cells, retained within the style sac and stomach, are subject to digestion that is facilitated by the mechanical swirling action generated by cilia within the style sac (Elston 1980; Gallager 1988). The exact mechanisms underlying such intestinal sorting remain to be fully elucidated, however, because larvae have been observed to retain inert particles in their guts for extended periods. For example, Widdows et al. (1989) found for all larval stages of *C. virginica* that inert polystyrene microspheres coated with an extract of homogenized *I. galbana* cells were ingested and retained within the digestive system for periods of hours. Millar (1955) found no evidence of particle sorting in the stomach of larval *O. edulis.*

Effects of Food Size and Concentration on Larval Feeding

The development of electronic particle counters has allowed rapid estimation of larval retention efficiencies for different sized particles; however, such studies performed in static systems have potential technical problems associated with both increases in the proportion of poorly retained particles in suspension (Williams 1982) and generation of particles from larval feces (Gallager 1988; Baldwin and Newell 1995a). Despite such technical difficulties, results have been remarkably consistent in showing that bivalve veligers have maximum retention efficiencies for particles in the size range of 2 to 9 µm (*O. edulis* [Wilson 1980]; *M. edulis* [Riisgard et al. 1980; Sprung 1984]; *C. virginica* [Baldwin and Newell 1995a]).

Laboratory studies of the nutritional value of various species of unicellular algae for bivalve larvae have demonstrated that species ranging in size from 3 to 10 µm support maximum larval growth (for review see Webb and Chu 1982; Langdon and Newell, Chapter 6) although pediveliger larvae can ingest algal cells up to 30 µm in diameter (Mackie 1969). Fritz et al. (1984) reported that the ingestion rates of eastern oyster larvae feeding on algal cells present in natural water from Delaware Bay were greater for cells smaller than 10 µm in diameter than for larger cells. In an extensive study of the diet and feeding behavior of eastern oyster larvae, Baldwin and Newell (1991) and Baldwin and Newell (1995a) determined that both phototrophic and heterotrophic organisms ranging in diameter from 0.2 to 30 µm were ingested, with maximum retention efficiency for cells between 2 to 4 µm. A large proportion (temporally variable but between 20 to 90%) of the total particulate volume ingested by oyster larvae feeding on natural particulate matter from Chesapeake Bay was derived from picoplankton size (0.5 to 3 µm) cells (Baldwin and Newell 1995a). Under bloom conditions, when the plankton was dominated by large (10 to 30 µm diameter) dinoflagellates, *C. virginica* larvae (>200 µm shell height) selectively ingested 20 to 30 µm diameter mixotrophic dinoflagellates. Under such conditions these large cells provided 20 to 30% of the total ingested volume of particles (Baldwin and Newell 1991).

Crisp et al. (1985) reported that prodissoconch II larvae of *O. edulis* and *C. gigas* (shell length 230 and 213 µm respectively) feeding on mixtures of algae of different sizes [*Tetraselmis suecica* (volume = 268 μm^3), *I. galbana* (23 μm^3), and *Nannochloris* sp. (5.2 μm^3)] did not show selective retention of any of these three species. Gallager (1988) used high speed video microscopy to observe directly that larval *M. mercenaria* captured from suspension with equal efficiency both 0.5 to 1 µm cells (cyanobacteria, *Synechococcus* spp.; 0.19 μm^3) and 4.5 µm algal cells (*I.* aff. *galbana* (clone T. Iso); 47.7 μm^3) when fed to the larvae in a mixture made up of equal cell concentrations (3×10^4 cells ml^{-1}). Unexpectedly, however, cyanobacteria were preferentially ingested compared to *I.* aff *galbana* (ratio of number of cells ingested per hour was 48:1) by 2-d old larvae and also by 10-d old larvae (3:1 cell ratio). Preferential ingestion of *Synechococcus* spp. cells was highest in the young life stages of *M. mercenaria*, perhaps as a consequence of the small size of their esophagus interfering with their ability to ingest larger cells. It is important to note, however, that the biomass of ingested *I.* aff *galbana* was four times greater than that of ingested cyanobacteria for 2-d old larvae and 33 times that of ingested cyanobacteria for 10-d old larvae (Gallager 1988).

Molluscan larvae have a well developed ability to regulate their ingestion rate, defined as the number of particles entering the stomach per unit time, thus avoiding impairing digestive system function at high particle concentrations (Crisp et al. 1985). Strathmann and Leise (1979) suggested that control of ingestion may be effected by cessation of the beating of postoral cirri which causes particle capture to cease without compromising swimming activity. Ingestion rates may also be regulated by controlling the rate at which particles are accepted or rejected at the mouth and entrance to the stomach (Gallager 1988).

The same methodological problems associated with measuring retention efficiencies also affect estimations of ingestion rates when they are determined

from rates of disappearance of particles from suspension. This problem occurs because particles may be cleared from suspension but are rejected at the mouth in clusters that do not break up to release single particles. This potential error may occur when changes in suspended cell concentration are determined microscopically (e.g., Fritz et al. 1984; Crisp et al. 1985) or when electronic particle counters are used for this purpose (e.g., Sprung 1984).

The direct method of determining ingestion rates by microscopic observation and enumeration of cells entering the stomach of tethered larvae (Gallager 1988) provides reliable estimates of ingestion rates. In addition, indirect estimates of ingestion rates can be obtained by feeding radiolabeled algae to larvae for periods of time that are shorter than larval gut passage time (Baldwin and Newell 1991). The advantage of such radiolabeling techniques is that they not only provide information on ingestion rates but also can be used to determine digestion and absorption rates and efficiencies.

Experimental studies of larval ingestion rates are also complicated by factors such as the organism's prior feeding history, which in turn affects the degree of gut satiation (Fretter and Montgomery 1968; Crisp et al. 1985; Gallager 1988) and the rate at which particles are initially ingested to fill the gut. Furthermore, differences in the volume of algal cells can also complicate comparisons among different studies because cell volume determines how many particles can be accommodated in the limited volume of the larval digestive system. Future studies of larval ingestion rates should express ingestion rates in terms of volume of ingested cells.

Baldwin and Newell (1995b) demonstrated a sigmoidal relationship between volume of particles ingested and particle concentration (μm^3 ml^{-1}) when *C. virginica* larvae are fed natural plankton assemblages (Fig. 2). Because maximum ingestion rates were often obtained at below ambient particle concentrations (varying between 20 to 100%), this suggests that larvae of the eastern oyster are not food limited under most conditions when feeding on natural seston in Chesapeake Bay. Crisp et al. (1985) determined that in larval *O. edulis*, clearance rates (volume of water swept clear of particles per unit time) decreased in an hyperbolic manner as cell concentrations increased. Concomitantly, ingestion rates (biomass of food ingested per unit time) increased hyperbolically to a plateau where ingestion rate was at a maximum and dependent on gut capacity and gut residence time. Similarly, Gallager (1988) reported that clearance rates of larval *M. mercenaria* increased hyperbolically with cell concentration to a level of about 10^5 cells ml^{-1} beyond which rates declined, probably due to physical blockage of the velum and food tracts. Gallager (1988) also found for *M. mercenaria* larvae that ingestion rates of particles were proportional to their rate of capture and transport to the mouth at low particle concentrations (<10^3 cells *I.* aff. *galbana* ml^{-1}); however, at higher algal cell concentrations, ingestion rates reached a maximum val-

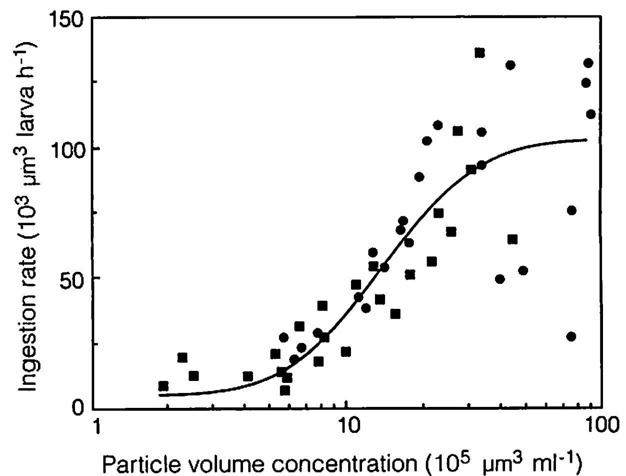


Figure 2. Functional relationship between rate of particle ingestion [volume of particles (μm^3) ingested per hour] by *C. virginica* larvae and concentration of particles in natural assemblages (total volume of particles [μm^3 ml^{-1}]) between 1 to 32 μm) collected from two subestuaries of Chesapeake Bay in August (■ Choptank River; ● Little Choptank River). Particle concentrations were determined using an electronic particle counter (Coulter Multisizer II). Data points represent mean ingestion rates for 40 larvae (shell length 200 to 280 μm) held at a concentration of 1 larva ml^{-1}. Sigmoidal curve fitted by eye. Redrawn from Baldwin and Newell (1995b).

ue that was dependent on larval age and, most likely, larval gut size.

Change in Feeding Associated with Metamorphosis

The process of metamorphosis in bivalve molluscs involves fundamental changes of many organs associated with the transformation from the free-floating pelagic larval stage to a sessile benthic stage. Some of the most dramatic changes involve reorganization of the feeding and alimentary system. The velum is either cast off or reabsorbed within 48 h after settlement (Cole 1938; Galtsoff 1964; Bayne 1971), the mouth is re-oriented, and development of the rudimentary gills, initially present in pediveliger larvae, begins (Menzel 1955; Bayne 1971). In some siphonate bivalves [e.g., the Baltic macoma, *Macoma balthica* (Caddy 1969) and *M. edulis* (Bayne 1971)], cilia on the foot serve to draw food particles into the mantle cavity where they are captured on the developing gill and are transported across the labial palps to the mouth. The rudimentary gill consists of only 2 to 3 filaments at this time, but in *M. edulis* these are fully ciliated with functioning lateral, latero-frontal, and frontal cilia (Bayne 1971).

Because of the disruption to all organs associated with metamorphosis, it is unlikely that the eastern oyster ingests sufficient particulate food to supply all of its nutritive requirements during this period. Thus, lipid reserves, sequestered during larval development, are mobilized to provide energy necessary for the approximately 6 d it takes for oysters to reorganize tissues and fully develop the adult feeding structures (Hickman and Gruffydd 1971). As reviewed by Thompson et al. (Chapter 9), many studies have demonstrated that the viability and growth of juvenile bivalves immediately after metamorphosis is correlated with the amount of lipid sequestered during larval development (Gabbott and Holland 1973; Holland and Spencer 1973; Holland 1978; Gallager and Mann 1986). It is possible that uptake of dissolved organic material may also make an important contribution to oyster nutrition during this non-feeding period at metamorphosis (Manahan and Crisp 1983; Manahan 1989; reviewed by Langdon and Newell, Chapter 6).

ADULT OYSTERS

The objective of this section is to review feeding processes of the post-metamorphic life stage of *C. virginica*. However, in order to facilitate understanding of the oyster's feeding mechanisms, a brief description of the anatomy of the oyster's gill will be presented here. A more detailed account of the anatomy and ultrastructure of the gills and palps is given by Eble in Chapter 2.

Many bivalve classification schemes are based to some degree upon aspects of feeding or digestive systems (Allen 1985; Purchon 1978, 1987). Although there is presently no consensus on a uniform phylogenetic classification of bivalve molluscs, a commonly accepted scheme recognizes two subclasses of bivalves, the Protobranchia and Lamellibranchia (Purchon 1978; Allen 1985). The primitive deposit-feeding condition, in which the gills (=ctenidia) are small and principally used as sites of gaseous exchange, is represented by members of the subclass Protobranchia. In protobranchs the gills are swept clear of trapped particles by ciliated rejection tracts. In the Lamellibranchia these ciliated rejection tracts have evolved to serve as the means of capturing food particles. This has resulted in the enlargement of gill structure to form an efficient organ for collecting and processing food particles captured from currents generated by the activity of highly organized, ciliated gill surfaces. The rigidity of these enlarged gills is enhanced by tissue junctions between the ascending and descending lamellae of each demibranch and by interlocking cilia or tissue connections between filaments.

Lamellibranch bivalves have been divided into two main groups, based primarily on the nature of these interfilament connections. In filibranch species, such as scallops and mussels, adjoining filaments are connected by means of specialized interlocking cilia. In eulamellibranch species, which includes most of the suspension-feeding bivalve superfamilies (Purchon 1978), gill rigidity is enhanced by rigid tissue junctions that replace ciliary interfilamentar discs. The gills of the Ostreacea, including *C. virginica*, are sometimes called pseudoeulamellibranch because even though tissue junctions are present they are not

as extensive as in most superfamilies, such as Cardiacea and Mactreacea.

Gill Anatomy

The two gills of adult oysters together constitute the largest organ of the oyster's body (Fig. 3) and function as a site for food acquisition and gaseous exchange. Gill surface area, and hence feeding efficiency, has been maximized by the elongation and folding of each gill into two structures, termed the inner and outer demibranchs. In a transverse section across the gills, the joined inner and outer demibranchs of each gill give the appearance of a W-shaped structure (Fig. 4). Each demibranch has an ascending and descending fold, termed a lamella. The ascending and de-

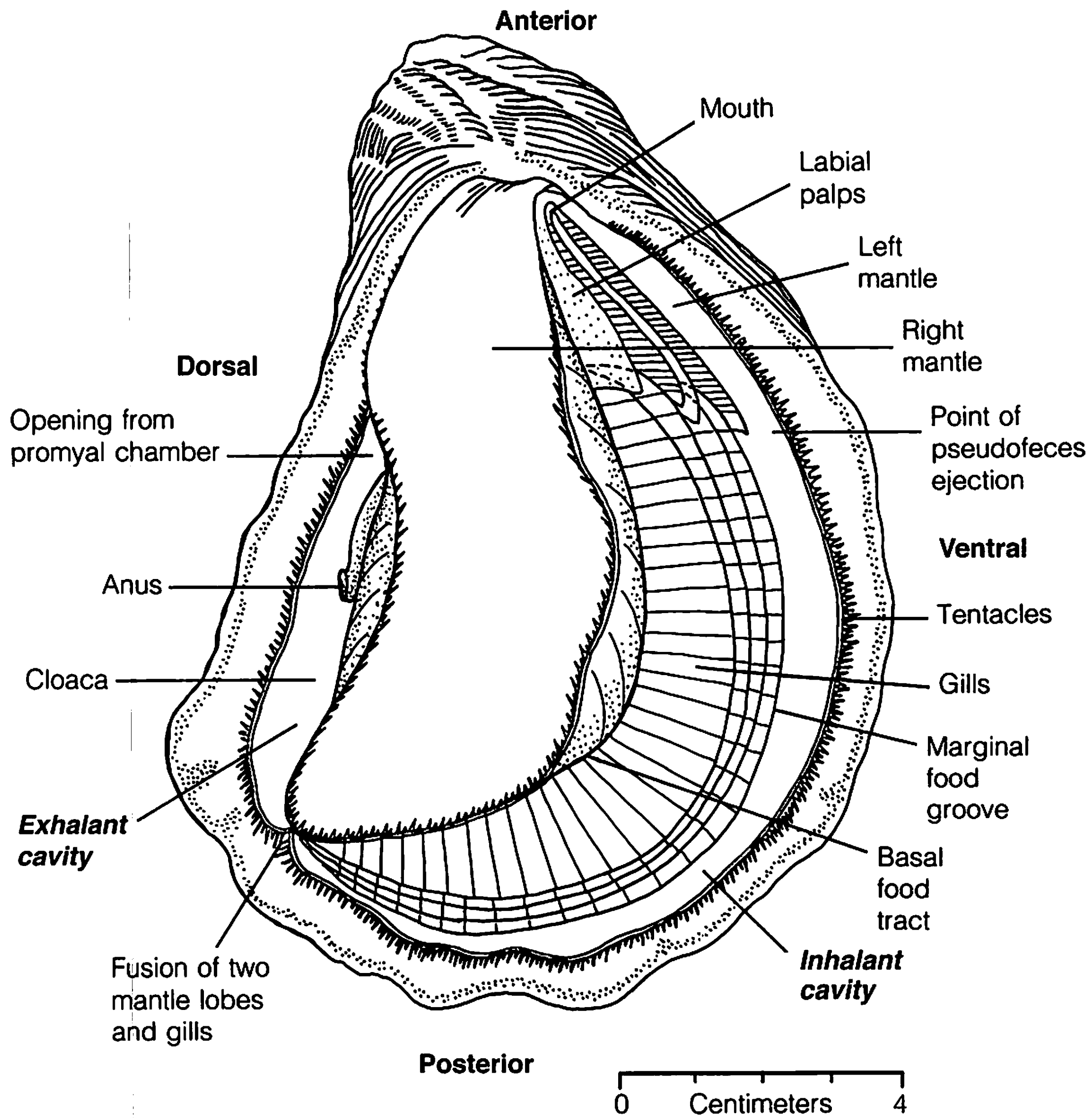


Figure 3. External anatomy of the soft tissues of *C. virginica* after removal of the right valve. Redrawn from Galtsoff (1964).

scending lamellae of each of the four demibranchs are fused along their entire free ventral margin. The dorsal margin of the inner and outer descending lamellae is attached to the visceral mass at the gill axis, which is a ridge of muscle strengthened by a fibrous protein skeletal rod rich in collagen (Brown 1952; Rudall 1955; Le Pennec et al. 1988). Skeletal rods were incorrectly identified as being composed of chitin in original descriptions of the bivalve gill structure (Le Pennec et al. 1988). The ascending lamellae of both outer demibranchs are fused to the mantle. The ascending lamellae of inner demibranchs are fused to each other and the visceral mass along the central axis of the gills. Fusion of lamellae to the mantle and visceral mass (Fig. 4), and the fusion of the two mantle lobes to the gills at their extreme posterior margins, effectively divides the pallial cavity into an inhalant and exhalent cavity (Fig. 3). The only connection between the two cavities is through the numerous small openings between adjacent filaments, called ostia, that open into the plical water chambers, and are, in turn, connected to the water tubes (Figs. 5, 6).

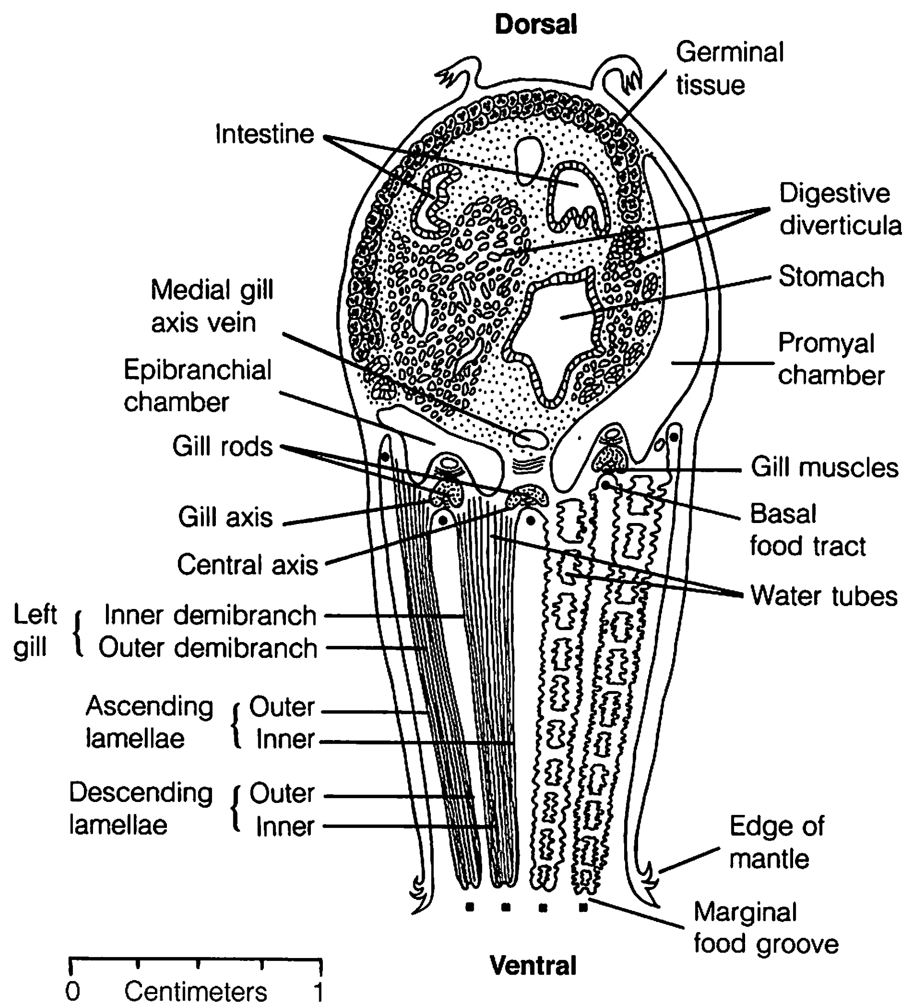


Figure 4. Cross section of body of *C. virginica* drawn from histological sections. The left gill shows the expected parallel lines formed by longitudinally sectioning gill filaments. The right gill illustrates the typical situation found in most real-life preparations where plicae are sectioned obliquely due to minor folds in the gill. The position of the four marginal food grooves is indicated by ■ and the five basal food tracts by ●.

Each lamella is composed of numerous, ciliated, rod-like filaments arranged together in a row, "like pickets in a fence" (Nelson 1960). In some eulamellibranch species, such as *C. virginica*, filaments are structurally differentiated into principal, transitional, or ordinary filaments; a gill composed of such differentiated filaments is called heterorhabdic. Typically 12 [8 to 15 (Nelson 1960) or 10 to 16 (Galtsoff 1964)] ordinary filaments in the gill of *C. virginica* are grouped together to form a plica (= deep fold). A principal filament, which is appreciably larger than the other two filament types (Fig. 5), is located at the base of each plical groove and connects adjoining plicae. Transitional filaments flank each principal filament and separate it from the ordinary filaments. The rather complex gill structure is illustrated as histological sections in Figs. 4, 5 and is represented three-dimensionally in Fig. 6.

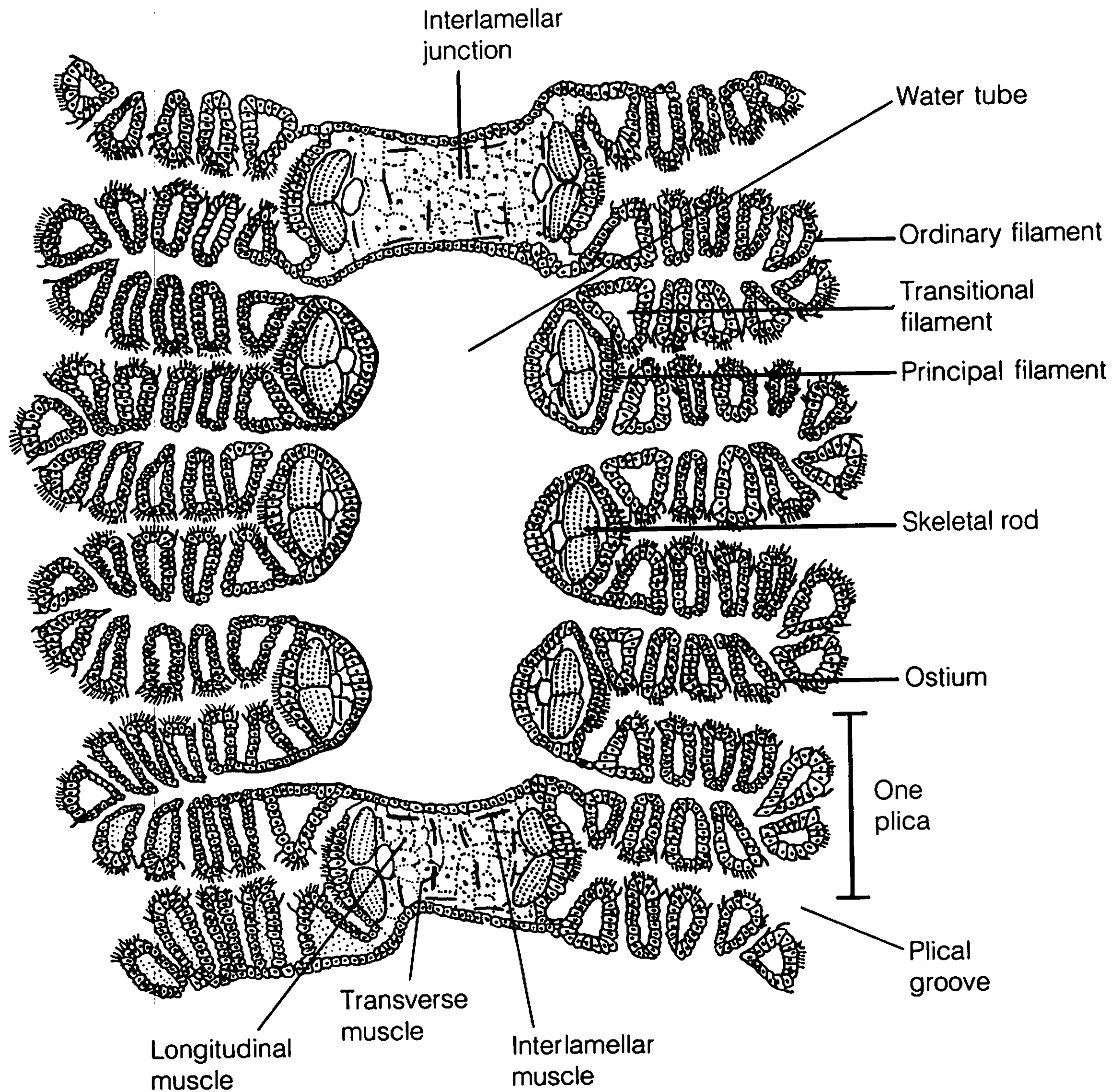


Figure 5. Transverse histological section through the ascending and descending lamellae of one demibranch of *C. virginica*. Illustrated are ordinary, transitional, and principal filaments that comprise a plical unit and their relationship to the ostia and water tubes. × 500. Modified from Galtsoff (1964).

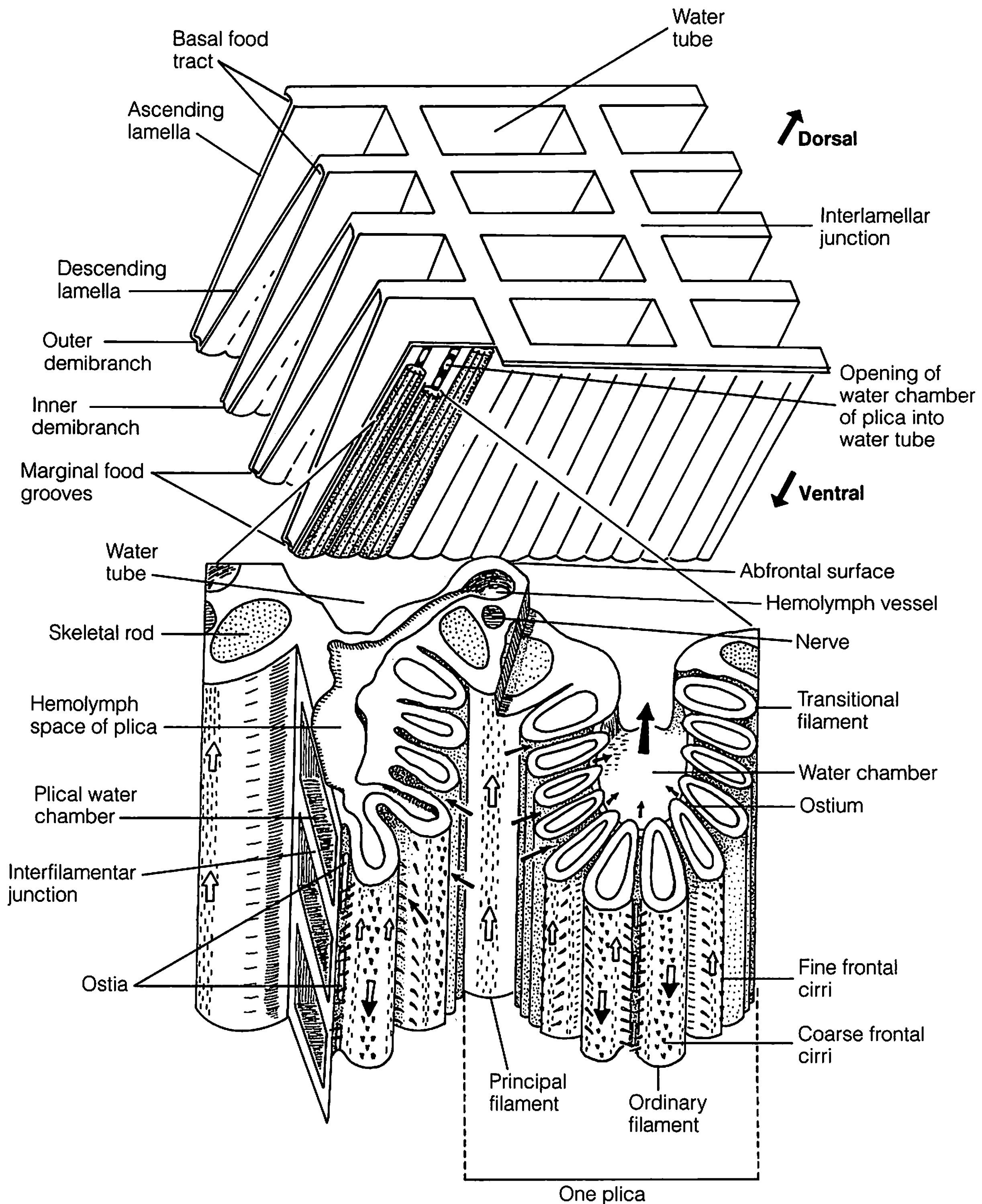


Figure 6. Stereodrawing of part of two gills of *C. virginica* (upper) with expanded detail of a plical fold (lower). The direction of particle movement on the coarse and fine frontal cilia is indicated by the black headed and open arrows, respectively. The direction of water movement between the filaments, through the ostia, and into the water tubes is indicated by the solid black arrows. Based on drawings by M. R. Carriker in Nelson (1960).

Two collagenous skeletal rods run along the axis of each gill filament (Fig. 5) and provide overall rigidity to the complex and fragile gill structure. Abutting filaments are joined by interlocking ciliary junctions that are stiffened by proteinaceous collagen cross-links between the axial rods. These cross-links also provide support for the delicate tissue that frames the 60 by 20 µm ostia between adjacent filaments that allow water to pass through the gills.

Gill rigidity is further enhanced by interlamellar tissue junctions between principal filaments on the ascending and descending folds of each demibranch (Fig. 5). Interlamellar junctions are present between most principal filaments at the distal edge of the gill. Closer to the visceral mass, the frequency of interlamellar junctions gradually decreases so that no interlamellar junctions are present immediately adjacent to the visceral mass; this absence of junctions results in a continuous epibranchial chamber along the center of each of the four demibranchs. The interlamellar space is thus subdivided by the interlamellar junction into a series of compartments, or water tubes (Fig. 5 and 6), that run from the distal edge of the gill and open into the epibranchial chamber (Fig. 4). Water moves dorsally from the plical water chambers to the epibranchial and promyal chambers.

The epibranchial chambers of the four demibranchs are only separate at the anterior region of the gill, adjacent to the stomach. In the mid-section of the gills, close to the heart and adductor muscle, the two epibranchial chambers in each gill fuse to form a single chamber. The chambers from each gill then join to form a common epibranchial chamber that empties into the cloaca, situated immediately adjacent to the posterior side of the adductor muscle (Fig. 3). The epibranchial chambers serve to collect most of the water that is pumped through the ostia and water tubes of the gill. However, some water from the right gill (i.e., the gill closest to the flat valve) is removed from the gill via the promyal chamber (Figs. 3 and 4), a feature unique to oysters in the genus *Crassostrea*.

The promyal chamber is formed as a space between the visceral mass and the mantle (Fig. 4). The chamber starts approximately adjacent to the heart and then curves to open on the anterior side of the adductor muscle, i.e., on the side of the adductor muscle opposite from the cloaca (Fig. 3). For about half the length of the right gill, the water tubes of the inner and outer demibranch empty directly into the promyal chamber and not into the epibranchial chamber (Nelson 1938). Water flow between the promyal chamber and epibranchial chamber of the right gill is minimized by a constriction of the epibranchial chamber adjacent to the adductor muscle (Nelson 1938). The adaptive significance of the promyal chamber is unknown, though Nelson (1938) suggested that water from the promyal chamber may be forcibly ejected by shell adductions and, therefore, may play a role in removing sediments and feces accumulating along the dorsal shell margin.

Ciliation of the Gill Filaments

Cilia on the gills of bivalve molluscs are differentiated into specialized structural and functional units. These units generate the water currents through the gill, remove food particles from suspension, and transport particles from the gills towards the mouth or reject them directly onto the mantle. Early morphological descriptions were inaccurate due to the limited resolving power of light microscopy. It was not until the advent of scanning electron microscopy that the fine details of gill ciliation could be resolved (Moore 1971; Owen 1974; Jørgensen 1975; Owen and McCrae 1976). The following description of ciliation of the gill filaments of *C. virginica* is largely based on a comprehensive study by Ribelin and Collier (1977).

Water movement through the gill is primarily effected by a band of lateral cilia (Figs. 7, 8), present on all filaments, that beat rhythmically to develop a metachronal wave (Nelson 1960). The metachronal waves of lateral cilia on adjacent filaments are opposed (Galtsoff 1964; Sleigh and Aiello 1972). Close to the surface of the filament is a small zone in which the oscillatory beating of each cilium (i.e., the effective and recovery strokes) generates a forward and reverse motion of the water. Beyond this small zone encompassed by the recovery stroke, water movement is unidirectional through the interfilament space

(Jørgensen 1981a) with most water passing distal to the tips of the cilia (Jørgensen 1982).

The supposition that lateral cilia alone are capable of generating the water current through the gill was challenged by Silvester (1988). He calculated that lateral cilia of the mussel *M. edulis* may not be capable of beating rapidly enough to generate the observed current velocity. He suggested that further research was required to determine the hydrodynamics of ciliary beating of the bivalve gill in order to determine the exact contribution of lateral cilia in generating observed water flows. Jørgensen et al. (1988) may have found the mechanism to account for the higher than predicted water velocity. They suggest that the

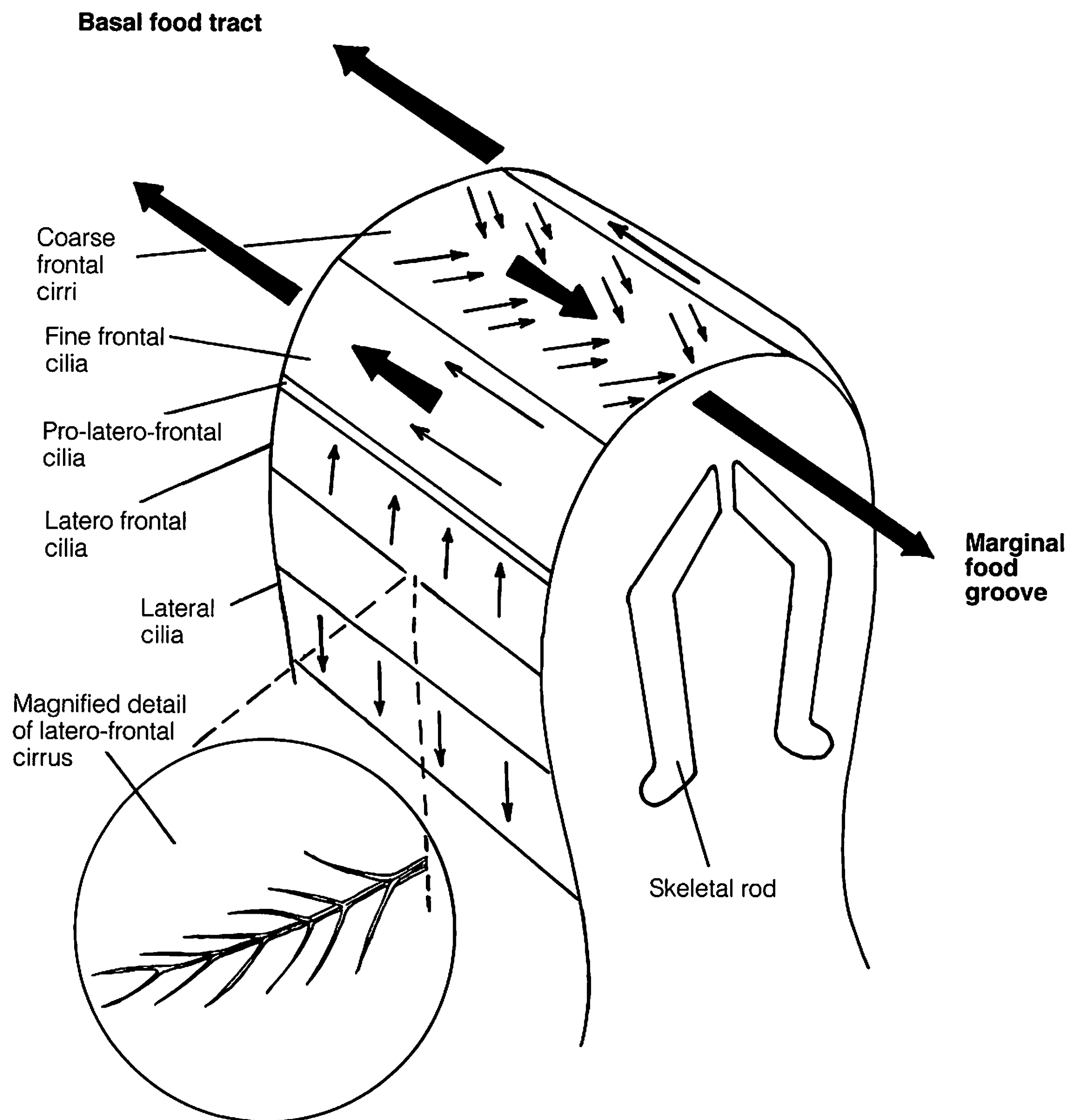


Figure 7. Diagrammatic illustration of the ciliary tracts present on an ordinary filament from the gill of *C. virginica*, with a magnified view of a latero-frontal cirral unit (modified from Ribelin and Collier 1977). Small solid arrows indicate direction of effective stroke of cilia. Broad solid arrows indicate ventral direction of particle transport on the coarse frontal ciliary tracts to the marginal food groove and dorsally on the fine frontal ciliary tract to the basal food tract. Note that the central band of coarse frontal cilia is not present on principal and transitional filaments.

velocity distributions generated by the metachronal beat of lateral cilia on opposite sides of the interfilamentary space can act synergistically in *M. edulis.* This increases the velocity of the generated water current above the theoretical value predicted from calculations based solely on the speed of lateral ciliary beat on one side of the interfilamentary space. Alternatively, Jones et al. (1990) speculated that the through water current in *M. edulis* may not be generated solely by the lateral cilia but may be due in part to the action of abfrontal cilia. Since abfrontal cilia are only present on gills of filibranch species, the lateral cilia must generate all water flow through the eulamellibranch gill of *C. virginica.*

The latero-frontal cirri, sometimes referred to as eulatero-frontal cilia (Atkins 1938; Owen and McCrae 1976), have an effective stroke in the opposite direction to that of the lateral cilia (Figs. 7 and 8). These cirri are complex "feather-like" structures composed of a number of individual cilia (Fig. 7) that branch away from the main axis at a 90° angle along the length of the cirrus. At the point where each cilium branches from the main axis, its diameter is reduced by 40 to 45% from its axial diameter, possibly due to a reduction in the number of microtubules that are responsible for ciliary movement (Satir 1965, 1968). Such a loss in microtubules may mean that the distal portion of the cilium can not move independently of the cirral shaft (Owen 1974). The average distance between branches is about 0.6 μm for cilia on ordinary filaments and 0.54 μm for cilia on the principal filaments (Ribelin and Collier 1977).

The length and number of cilia in each latero-frontal cirrus on both ordinary and principal filaments decreases towards the distal edge of the demibranch. On ordinary filaments, there are about 20 individual cilia per latero-frontal cirrus at the proximal edge of each demibranch but this decreases to only 6 to 8 cilia distally, where latero-frontal cirri terminate about 300 μm from the free edge of the demibranch. Compared to latero-frontal cirri on ordinary filaments, those on principal filaments are closer together (mean intercirral distance of 1.8 compared to 2.28 μm) and composed of a greater number (mean of 22.9 compared to 16.6) of longer cilia (16.0 compared to 13.5 μm) (Ribelin and Collier 1977).

Located adjacent to the frontal side of the latero-frontal cirri of all gill filaments (Fig. 7) is a narrow band of pro-latero-frontal cilia (Owen and McCrae 1976; Owen 1978), originally called para-latero-frontal cilia by Atkins (1938). These cilia are only 5.5 μm long, less than 0.5 μm apart, and arranged in a staggered row (Ribelin and Collier 1977). In contrast to latero-frontal cirri, pro-latero-frontal cirri extend to within a few micrometers of the distal edge of the demibranch (Ribelin and Collier 1977).

The frontal cilia of ordinary filaments can be divided into two types: fine frontal cilia and coarse frontal cirri (Ribelin and Collier 1977). Coarse frontal cirri form a wide ciliary tract in the central portion of the frontal region of each filament (Figs. 6 and 7), and consist of groups of 5 to 8 loosely-associated cilia originating from single cells. These component cilia are arranged in a diagonal row across each cell, pointing towards the center of the tract. It is this grouping of cilia, evident under scanning electron microscopy (Owen and McCrae 1976; Ribelin and Collier 1977), that gives rise to the comparative "coarseness" of coarse frontal cirri compared to fine frontal cilia when observed under light microscopy (Nelson 1960). The effective stroke of the cirri is directed obliquely toward the medial region of the tract (Fig. 7), concentrating particles in the center of the tract and simultaneously moving them ventrally towards the marginal food groove on the edge of the demibranchs.

On each ordinary filament there are two fine frontal ciliary tracts lying between the central tract of coarse frontal cirri and the bands of pro-latero-frontal cilia (Figs. 6 and 7). The effective stroke of fine frontal cilia is directed dorsally, along the longitudinal axis of the filament and towards the basal food tract located in the junction of the demibranch and visceral mass. Localized in the boundary between the tracts of coarse frontal cirri and fine frontal cilia are numerous mucus-producing goblet cells; such cells are less abundant within the coarse cirral tract and are very uncommon within the fine ciliary tract. The frontal cilia on principal and transitional filaments are not differentiated into the coarse or fine elements that are present on ordinary filaments (Ribelin and Collier 1977). Instead, there is only a wide band of dorsally beating fine frontal cilia (Fig. 6).

Mechanisms of Particle Capture

Many hypotheses have been advanced to explain how particles are retained by the gills of bivalve molluscs (for review of the early literature see Jørgensen 1966). A widely accepted hypothesis was that particles larger than the distance between cilia of the latero-frontal cirri were sieved from water flowing between the gill filaments. The latero-frontal cirri were thought to transfer sieved particles to the ciliated frontal tracts of the gills (Dral 1967; Ribelin and Collier 1977; Morton 1983). Although this hypothesis is intuitively satisfactory, it ignores the physical theory applicable to small structures such as cilia moving rapidly through fluids. Under such circumstances low Reynolds numbers (in the range of 10^{-4} to 10^{-3}) apply and, consequently, viscous rather than inertial forces dominate the process of particle capture in suspension-feeding bivalves (LaBarbera 1984; Jørgensen 1990; Vogel 1994). When particles are suspended in a viscous fluid, they tend to remain entrained within the flow and move around obstructions (Rubenstein and Koehl 1977; LaBarbera 1984); therefore, sieving of particles by latero-frontal cirri would be expected to be an inefficient process for removing particles from suspension.[4]

Rubenstein and Koehl (1977) and LaBarbera (1984) postulated that there are a number of general mechanisms whereby aquatic suspension-feeders could capture particles from suspension, even when viscous forces dominate. Of these, only one, called "scan and trap," requires an active biological response to approaching particles. The other mechanisms are primarily associated with physical interactions between particles and feeding organs, viz., (1) inertial impaction, (2) sieving, (3) direct interception/contact, (4) gravitational deposition, (5) motile particle deposition, and (6) electrostatic attraction. In a detailed analysis of the various forces acting on suspended particles, Rubenstein and Koehl (1977) predicted that it was probable that particles were captured by inertial impaction by most aquatic suspension-feeders. In bivalve molluscs, such a capture mechanism requires that particles are somehow pushed onto the frontal ciliary tracts or adhere to latero-frontal cirri upon contact. Such attachment could result from mucus binding (Dral 1967) or either electrostatic or hydrophobic-hydrophilic interactions, which have been found to aid capture of small particles in other aquatic species (LaBarbera 1978; Gerritsen and Porter 1982; Solow and Gallager 1990). If particles are removed from suspension by adherence to latero-frontal cirri, it is unclear how they could be transferred to the frontal ciliary tracts of the gill filaments. Ribelin and Collier (1977) speculated that pro-latero-frontal cilia serve as rakers and remove attached particles from latero-frontal cirri and transfer them to the frontal cilia. This explanation seems unlikely because these cilia are so short (average length 5.5 µm) that it is difficult to imagine how they could reach the ends of the latero-frontal cirri that are over twice as long (13.5 to 16 µm). Although the exact function of the pro-latero-frontal ciliary band has yet to be determined, it is possible that they may serve to help retain particles within the fine frontal ciliary tracts (Atkins 1938; Gibbons 1961).

Particle capture by a sieving action may also be questionable because of the energy costs associated with forcing water through a fine sieve. For example, Gerritsen et al. (1988) estimated that for Cladocerans (*Daphnia* spp.), between 20 to 45% of their metabolic energy expenditures would be required to force water through the setae. It is likely that the energy costs of forcing water through the fine mesh of bivalve's lateral-frontal cirri would be equally high. Indeed, Jørgensen (1983) suggested that the resistance associated with forcing water through latero-frontal cirri may be so high as to overcome the water pressure generated by the lateral cilia (see Davies [1973] for review of the physics associated with viscous flow through filters). Conversely, Silvester and Sleigh (1984) calculated that the lateral cilial "pump" generated sufficient pressure to overcome resistance

[4] A simple analogy of what a low Reynolds number represents to a suspension feeder is to consider how difficult it would be to use a fine tined fork to remove peas from a jar of thick honey. In such a situation the peas would flow around the fork while entrained within the honey and hence would be difficult to capture.

to flow caused by latero-frontal cirri thus allowing water movement through these cirri.

Jørgensen and co-workers (1981a, b, 1982, 1983, 1989) have undertaken an extensive investigation of the function of the filibranch gill of *M. edulis.* Based on this work, they developed a new hydromechanical hypothesis of particle retention by bivalve molluscs that reconciled the observed gill structure with the viscous nature of the water in which the gill functions (for review, see Jørgensen 1990). They suggested that particles are captured by hydromechanical mechanisms that do not depend on the physical sieving (interception) of suspended particles by latero-frontal cirri. According to the explanation of particle capture proposed by Jørgensen and co-workers, a complex three-dimensional pattern of waterflow, characterized by steep shear gradients, is produced at the interface between the through-current generated by lateral cilia, surface currents generated by both frontal and latero-frontal cilia, and oscillatory currents associated with the recovery stroke of lateral cilia (Jørgensen 1981a). A particle entrained in the

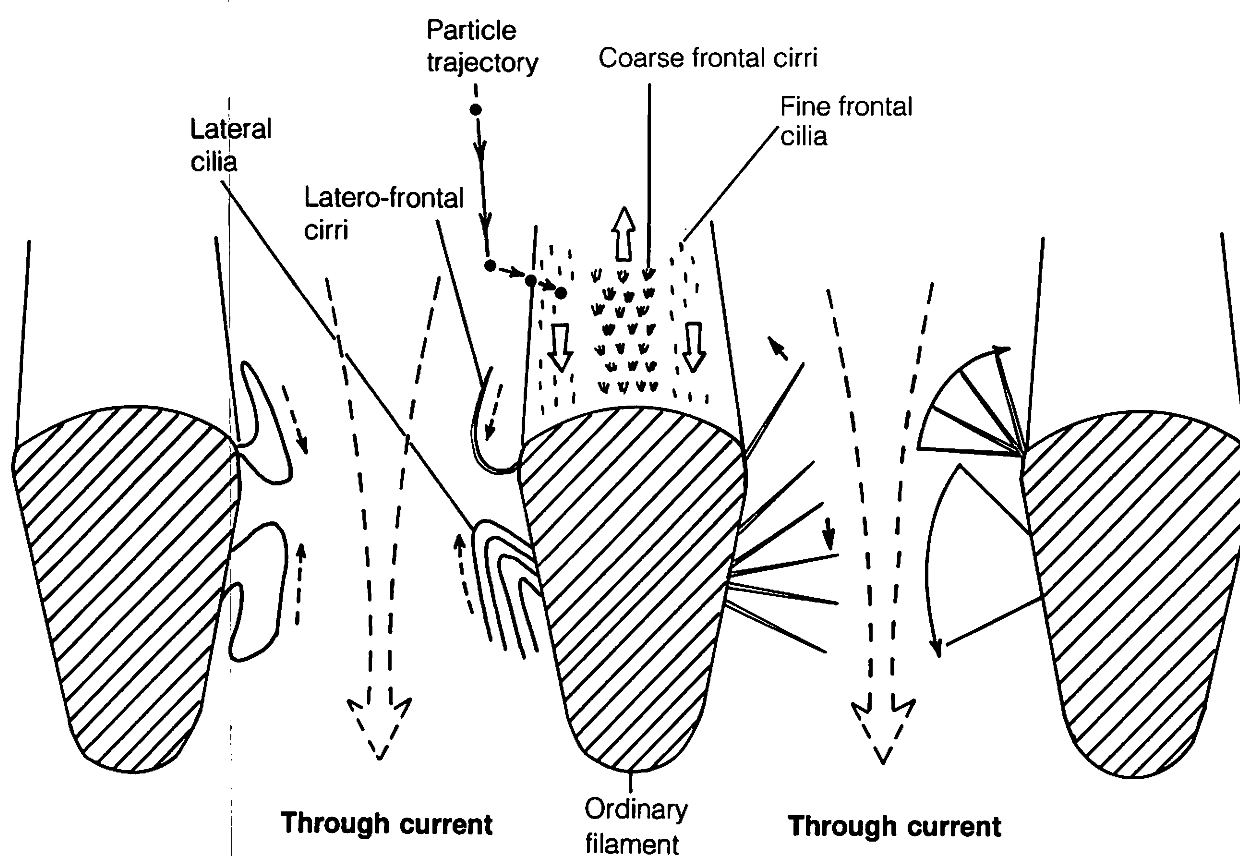


Figure 8. Diagrammatic illustration of a cross section of three ordinary gill filaments of *C. virginica.* On the right side of the middle filament, the directions of the effective strokes of the lateral cilia and latero-frontal cirral units are indicated by two small solid arrows. On the left side, their recovery strokes are shown by two dashed arrows. On the right outer filament the direction of the effective strokes is shown by arcs and on the left outer filament the recovery arcs are illustrated. The direction of the water current through the interfilament space and into the ostia is shown by the broad dashed arrows. The trajectory of a particle caught in the forces generated by velocity gradients at the boundary between the through current and surface currents is indicated by the solid line with arrowheads before it becomes entrained on the fine frontal cilia. Modified from Jørgensen (1981a).

through-current would tend to move perpendicularly to the direction of flow when exposed to these steep shear gradients (Leal 1980; Silvester and Sleigh 1984) and become entrained within the surface current (Fig. 8). Thus, the likelihood of a particle being trapped within the surface current would depend upon the steepness of velocity gradients, particle size, and the probability of the particle encountering such gradients as it passes through the interfilamentar space.

In a closely reasoned review, Silvester and Sleigh (1984) refuted the calculations on which Jørgensen's (1981b) original hypothesis was based. They suggested that lateral shear forces would not be strong enough to extract particles effectively from the through-flow. Jørgensen (1982, 1983) presented a modification of his original hypothesis in which he proposed that the currents associated with the recovery stroke of the lateral cilia contribute to particle capture. In subsequent work, Jørgensen et al. (1986, 1988) presented detailed experimental and theoretical calculations that supported the validity of the calculations upon which the Jørgensen hydromechanical hypothesis was based. They concluded that although latero-frontal cirri do not act as "sieves," they are the key element in particle retention by the gills, especially for particles smaller than about 14 µm in diameter. Further advances in our understanding of the underlying processes of particle retention by the bivalve gill must await a more sophisticated understanding of the nature of the complex three-dimensional flow past the gill filaments and more detailed *in vivo* observations, perhaps using the endoscopic technique developed by Ward et al. (1992, 1994).

Ciliated Tracts on the Gill Surface

Once particles have been removed from suspension they are immediately entrained in either the coarse frontal cirral or fine frontal ciliary tracts on the frontal surface of filaments (Ribelin and Collier 1977). On ordinary filaments, coarse frontal cirri beat ventrally toward the ciliated marginal [= terminal, *sensu* Galtsoff (1964)] food grooves which are located on the free ventral edges of the four demibranchs (Figs. 4, 9). Particles within these marginal grooves have already been bound in mucus on the gill (see below) and are transported anteriorly, by cilia acting on the mucous stream, until the food groove ends. When oysters are feeding at high seston concentrations, some mucus-bound material may be forced from the marginal groove before it reaches the termination point. This material becomes entrained on rejectory ciliary tracts on the surface of the mantle and is then ejected from the mantle cavity as pseudofeces (see page 216).

Ward et al. (1994) observed a previously undetected tract along the most anterior margin of each demibranch that serves to carry excess particles away from the basal gill-palp junction (Figs 10A, B). This tract is formed as a ciliated indentation within which cilia beat posteriorly. This is exactly opposite to the anterior direction of beat of cilia lining the marginal food groove. These two opposing ciliary currents meet approximately at the inflection point where the filaments start to shorten and the demibranch curves sharply in a dorsal direction towards the visceral mass and the basal gill-palp junction (position marked with an X in Fig. 10B).

Frontal cilia on the principal and transitional filaments beat dorsally towards the base of each demibranch as do cilia of the two narrow fine frontal ciliary tracts on each of the ordinary filaments (Ribelin and Collier 1977). Particles in these dorsal-beating ciliary tracts are transported to the junction of the gill lamellae and visceral mass. Located along each of these five junctions between lamellae and the visceral mass is a ciliated basal [=oral] food tract (Figs. 4, 9). This tract carries particles from the gills to the basal junction between the gills and labial palps. Particles within the basal food tract are not tightly bound in mucus but are transported to the palps in a slurry by the beating of cilia lining the food tract (Ward et al. 1994). The fluid mechanical forces associated with this current are sufficient to prevent loss of particles from the food tract even though the particles are not tightly bound within mucus (Jørgensen 1981b, 1989).

Particle Retention on the Gill

Retention efficiencies of the bivalve gill for various-sized particles provide fundamental information

on gill function and ecological information concerning the ability of a species to use the wide size spectrum of particles that comprise natural seston in coastal waters. Experimental studies of particle retention efficiency do not yield data on absolute particle retention efficiencies because samples of water collected from the exhalent siphon are always contaminated with unfiltered water from around the bivalve. Instead, retention efficiencies are generally expressed relative to the retention efficiency for particles larger

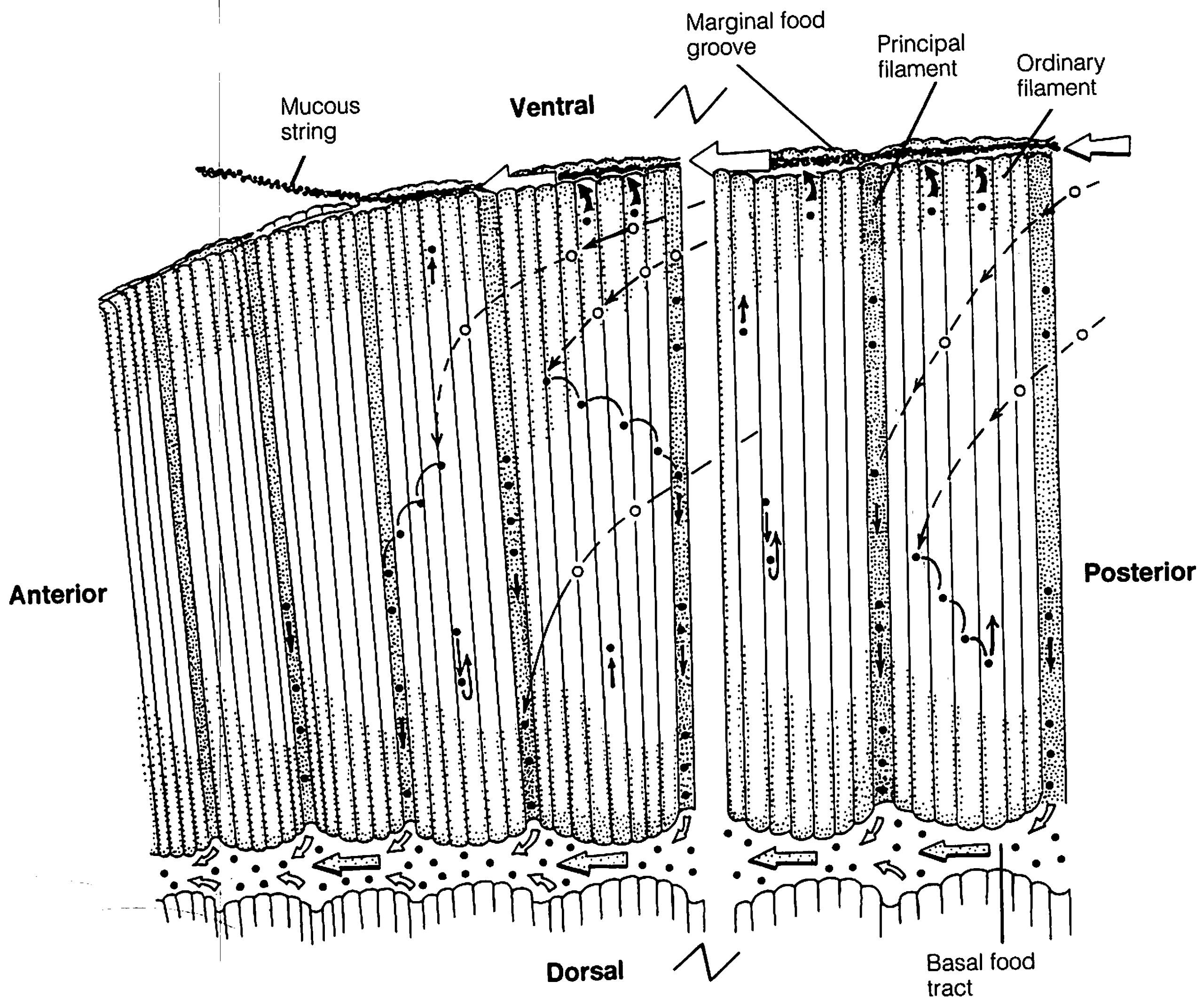


Figure 9. Diagrammatic illustration of a plicate demibranch of *C. virginica* showing particle capture and transport. Broken lines and open circles indicate movement of particles before capture on the gill surface. Solid lines and filled circles indicate particle movement after capture. The "hopping" movement and direction of some particles caught on the ordinary filaments is illustrated as they are moved to the principal filaments at the bottom of each plical grove. Dorsal particle transport on the fine frontal cilia of the principal filaments (continuously stippled filaments) to the basal food tract is shown by small solid arrows. Note that some particles captured on ordinary filaments reverse direction after capture when they are transferred from the fine frontal to coarse frontal ciliary tracts. Particles in the basal tracts are transported anteriorly in a free-suspension (stippled arrows). Particles in the marginal food groove are transported anteriorly in a mucus-bound string (large open arrows). Modified from Ward et al. (1994).

in diameter than 8 to 10 µm. These large particles are generally considered to be captured with high efficiency: absolute efficiencies have been reported to be 86 to 98% for a variety of bivalves (Møhlenberg and Riisgard 1978).

The efficiency with which particles larger than 6 µm in diameter are retained by the gills of *C. virginica* (Fig. 11A) is uniformly high (Haven and Morales-Alamo 1970; Palmer and Williams 1980) whereas particles smaller than 1 µm are poorly retained (Langdon and Newell 1990). This finding is similar to the results obtained for many other species of bivalve molluscs (Møhlenberg and Riisgard 1978; Wilson 1980; Riisgard 1988). However, studies of the efficiency with which *C. virginica* retains particles between 3 and 6 µm have produced conflicting results. Haven and Morales-Alamo (1970) and Palmer and Williams (1980) reported that *C. virginica* can retain particles in this size range with efficiencies as high as for particles larger than 6 µm (Fig. 11A). Conversely, Riisgard (1988) reported that *C. virginica* retains particles smaller than 6 µm with a lower efficiency than found for many other bivalve species.

It is possible that these discrepancies between investigations result from *C. virginica* possessing some degree of control over its retention of small particles. Jørgensen et al. (1986) reported that the efficiency with which *M. edulis* retains particles smaller than about 14 µm progressively declined as the concentration of the nerve transmitter serotonin was increased in the seawater. Because serotonin alters the angle of beat of the latero-frontal cirri, Jørgensen et al. (1986) interpreted these data to indicate that normal functioning of these cirri is essential for the efficient capture of small particles. Small changes in retention efficiency may be effected by varying the angle of beat of latero-frontal cirri, such that the proportion of the interfilamentary space swept by latero-frontal cirri is altered. Consequently, a greater volume of water can pass through the gill without being subject to the shear stress needed for effective capture of small particles. Thus, if *C. virginica* can control the angle of beat of the latero-frontal cirri, it could regulate its ingestion of small particles. Such behavior might be adaptive when there are high ambient levels of inorganic particles that may overload the gill. An alternative explanation for these reported differences in retention of small particles is that experimental conditions, including particle concentrations, differed among studies. For example, Palmer and Williams (1980) reported for *C. virginica* that as algal concentrations increased, retention efficiencies for small particles declined (Fig. 11A). Moreover, the absolute retention efficiency for particles is not constant and can vary by as much as 40% over time spans as short as 0.5 h (Fig. 11B).

The structure of the eastern oyster's plicate gills may also contribute to differential particle retention. The latero-frontal cirri on the ordinary filaments are composed of cilia that are about 2.5 µm shorter and spaced further apart than those on principal filaments (Ribelin and Collier 1977). This spacing may reduce the efficiency with which ordinary filaments capture small particles compared with the principal filaments (Jørgensen 1989). Regulation of retention efficiency could perhaps be further effected by contraction or relaxation of muscles in the gill, which is under hormonal and neural control, causing plical grooves to

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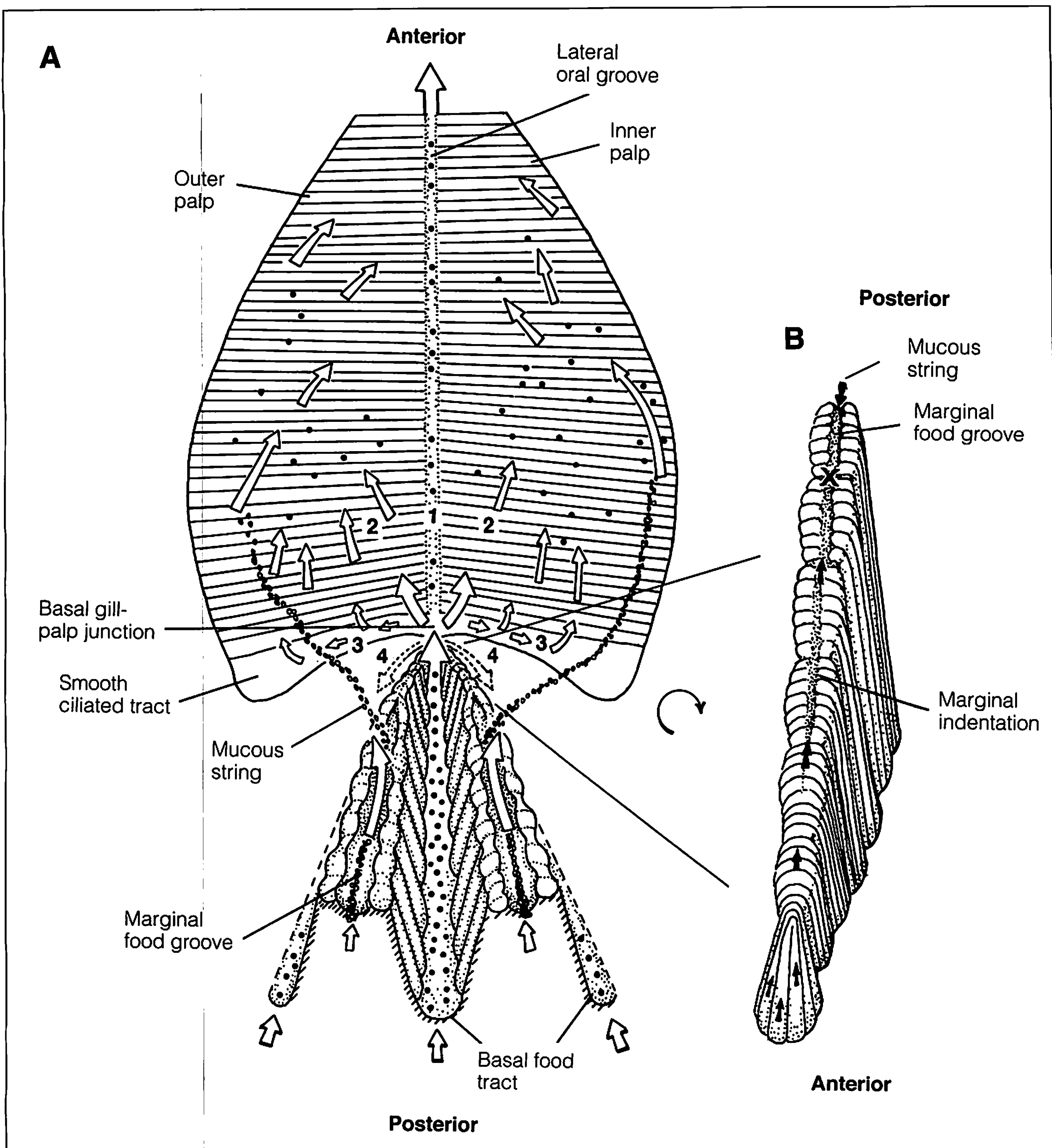
Figure 10 (opposite page). Diagrammatic illustration of anterior portion of two demibranchs of *C. virginica* at the point of insertion into a pair of labial palps at the gill-palp junction. (A) The palps are drawn folded open (i.e., not in life position) to reveal the general direction of particle movement on the smooth ciliated tract and the ridged sorting surface. The movement of particles bound in two mucous strings from the marginal food groove to the inner surfaces of left and right palps is illustrated and the subsequent movement and direction of these particles (solid circles) on the ridged palp surface is indicated by open arrows. Note that the viscosity of the mucous strings is reduced by the mechanical action of the ridged palp surface and hence the entrapped particles are dispersed and can be subject to sorting. The movement of particles in suspension along the basal food tract towards the basal gill-palp junction is indicated by the small solid circles and short open arrows. The subsequent movement of this material either directly into the oral food groove (marked 1), onto the palps (marked 2)



or initially along the smooth ciliated tract and then onto the palp ridges (marked 3) is indicated by broad open arrows. Transport of particles away from the basal gill-palp junction via the marginal indentations on each demibranch is shown by the broad broken arrows (marked 4). (B) An enlargement of the anterior termination of one demibranch illustrating how the marginal food groove becomes narrower and shallower until it forms the marginal indentation tract (note that in order to more clearly illustrate the anterior end of the demibranch the orientation of 10B has been altered by 180° from that illustrated in 10A). The transport of particles posteriorly, away from the gill-palp junction, is indicated by small black arrows. The point where these posteriorly beating cilia meet the anteriorly beating cilia in the main marginal food groove is marked by X. Figure modified from Ward et al. (1994).

narrow or open, hence altering the proportion of smaller particles being trapped by principal filaments. Further research is required to determine if bivalves, including *C. virginica*, respond to changes in the size spectrum of particles in the seston by regulating the size of the smallest particles retained on the gill.

Haven and Morales-Alamo (1970) noted that even though the retention efficiency of *C. virginica* for particles smaller than 3 µm was less than 50% of that for particles larger than 7 µm, small particles might be of significance in the nutrition of oysters because of the abundance of suspended material <3 µm in size in estuarine waters. As discussed on page 193, for larvae feeding on natural seston, however, such statements concerning particle numbers ignore the volume of material being ingested from each size fraction. Thus, in order to determine the nutritional significance of any particular size class of particles, future studies should express results in terms of volume and not simply numbers of particles ingested.

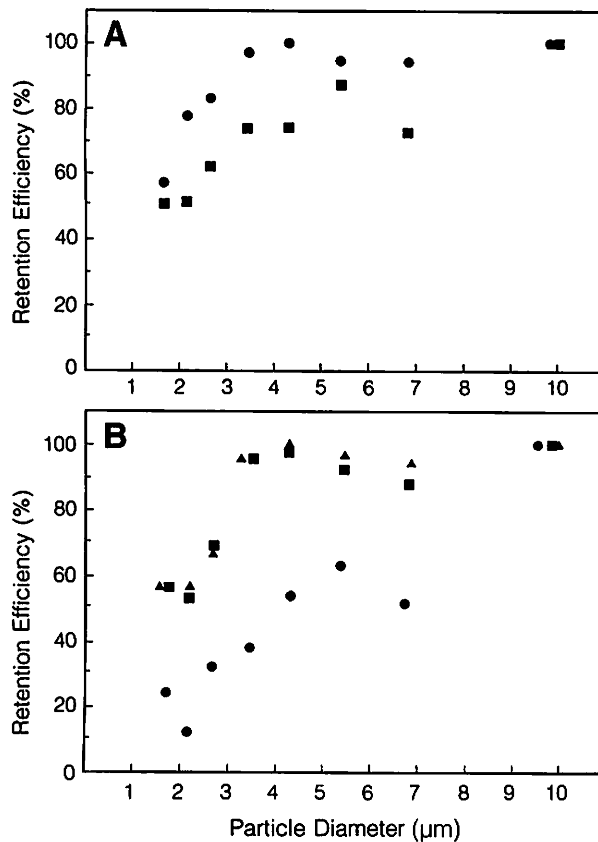


Figure 11. Retention efficiency of adult *C. virginica* for algal cells of various diameters. (A) Feeding at high (■ 6.64 mg L^{-1}) and low (● 1.45 mg L^{-1}) algal concentrations, and (B) temporal variability in retention efficiency measured over a 2 h period (■ time 0; ● 0.5 h; and ▲ 2 h) for eastern oysters fed algae at 6.64 mg L^{-1}. Graphs redrawn from Palmer and Williams (1980).

Although it is clear that particle retention by the bivalve gill is dependent on particle size, the possibility of differential retention of similarly sized particles by the gills of *C. virginica* has not been extensively studied. Shumway et al. (1985) reported that *O. edulis* was able to preferentially retain the dinoflagellate *Prorocentrum minimum* on its gills when the dinoflagellate was mixed with two other algal species of similar cell size (the diatom *Phaeodactylum tricornutum* and the cryptomonad flagellate *Chroomonas salina*). In contrast, *C. virginica* showed no differential retention of the same three algal species. Jørgensen (1990) cautions, however, that the bivalves studied by Shumway et al. (1985) may have been disturbed because they were filtering particles at less than 15% of their maximum capacity. These experiments need to be repeated in order to confirm the findings of Shumway et al. (1985) and determine if differential retention or selection of similar-sized particles observed in *O. edulis* was based on chemical composition, surface properties, or other characteristics of particles.

Post-Capture Sorting of Particles

It has long been recognized that bivalve molluscs can sort food particles before ingestion, perhaps on the basis of size (Yonge 1926) or chemical composition (Loosanoff 1949; Menzel 1955). By comparing the biochemical composition of food, feces, and pseudofeces (material rejected from the gills and palps before ingestion), Newell and Jordan (1983) confirmed that *C. virginica* was capable of preferentially ingesting organic material and rejecting inorganic particles. Their results not only showed that the eastern oyster could distinguish between algal cells and inorganic particles but also that eastern oys-

ters feeding on natural seston preferentially ingested nitrogen-rich particles compared to carbon-rich particles. This observation suggests a more highly developed selection process than one that simply differentiates between organic and inorganic particles. This selection process does not result in the total elimination of inorganic particles from the ingested ration. Indeed, there is experimental evidence (reviewed by Langdon and Newell in Chapter 6) that inorganic particles added to a pure algal diet can stimulate the growth of bivalve molluscs, perhaps by increasing digestion efficiencies.

Shumway et al. (1985) studied post-capture sorting of unicellular algae in five species of bivalves concurrently with an examination of differential particle capture and retention by the gills (discussed in previous section). They found that four bivalve species, including *O. edulis* but not *C. virginica*, could distinguish between three algal species, such that the diatom *P. tricornutum* was rejected in the pseudofeces while the dinoflagellate *P. minimum* and the cryptomonad flagellate *C. salina* were preferentially ingested. Ward and Targett (1989) demonstrated that *M. edulis* ingested or rejected test particles as pseudofeces on the basis of the type of algal extract adsorbed onto particle surfaces. These results demonstrate that at least some bivalve species are capable of selecting particles before ingestion. It is still uncertain from these studies whether particle selection is taking place primarily on the surfaces of the gill or labial palps.

Particle Selection on the Gill

There has been much speculation about the possible role of the heterorhabdic plicate gill of the eastern oyster in particle selection (Atkins 1937; Menzel 1955; Ribelin and Collier 1977). It was thought that smaller, and perhaps more nutritious, particles may enter deeper into the plical groove and, hence, be more likely to be retained on the fine frontal ciliary tracts of the principal and transitional filaments. As a consequence of the lack of mucus-producing goblet cells in these tracts (Ribelin and Collier 1977), particles are not incorporated in large quantities of mucus (Menzel 1955; Ward et al. 1994). Particles in these tracts are carried dorsally to one of the basal food tracts (Figs. 6, 9). In the basal food tract, particles are transported in suspension to the labial palps where they can be transferred directly to the oral groove that carries particles directly to the mouth with no additional sorting (Yonge 1926; Jørgensen 1966; Ward et al. 1994). Larger particles would not penetrate to the bottom of the plical groove and hence would be more likely to be captured on the ordinary filaments. Some of these particles may be transferred directly to the two fine frontal ciliary tracts and thence to the basal food tract (Fig. 6). Based on observations of pieces of isolated gill and SEM photomicrographs, Ribelin and Collier (1977) hypothesized that some particles, although initially transferred to the fine frontal cilia, cause the numerous goblet cells located in the boundary between coarse frontal cirri and fine frontal cilia to coat the particle with mucus. Such mucus-bound particles could be transferred from the fine frontal cilia to the coarse frontal cirri tract which then carries particles ventrally toward the marginal food groove.

All particles in the basal food tract reach the labial palps where they are either transported directly by the oral groove to the mouth or are subjected to sorting on the palp ridges (Fig. 10; Ward et al. 1994). Particles in mucous strings within the marginal food grooves can also be carried to the palps for sorting (Figs. 10, 12A). However, under some circumstances, such as when *C. virginica* is feeding on high concentrations of particles, the gills may become "overloaded" and excess mucus-bound material may be rejected directly from the marginal food grooves (Fig. 12B). Rejection occurs when transverse muscle fibers extending between the two sides of the marginal tract contract, forcing mucus-entangled particles directly onto the mantle surface, from where they are rejected as pseudofeces (see p. 216). Therefore, it is possible that at higher seston concentrations particle sorting on the gill may be involved in regulating the composition of food particles being transferred to the palps, and hence available for ingestion.

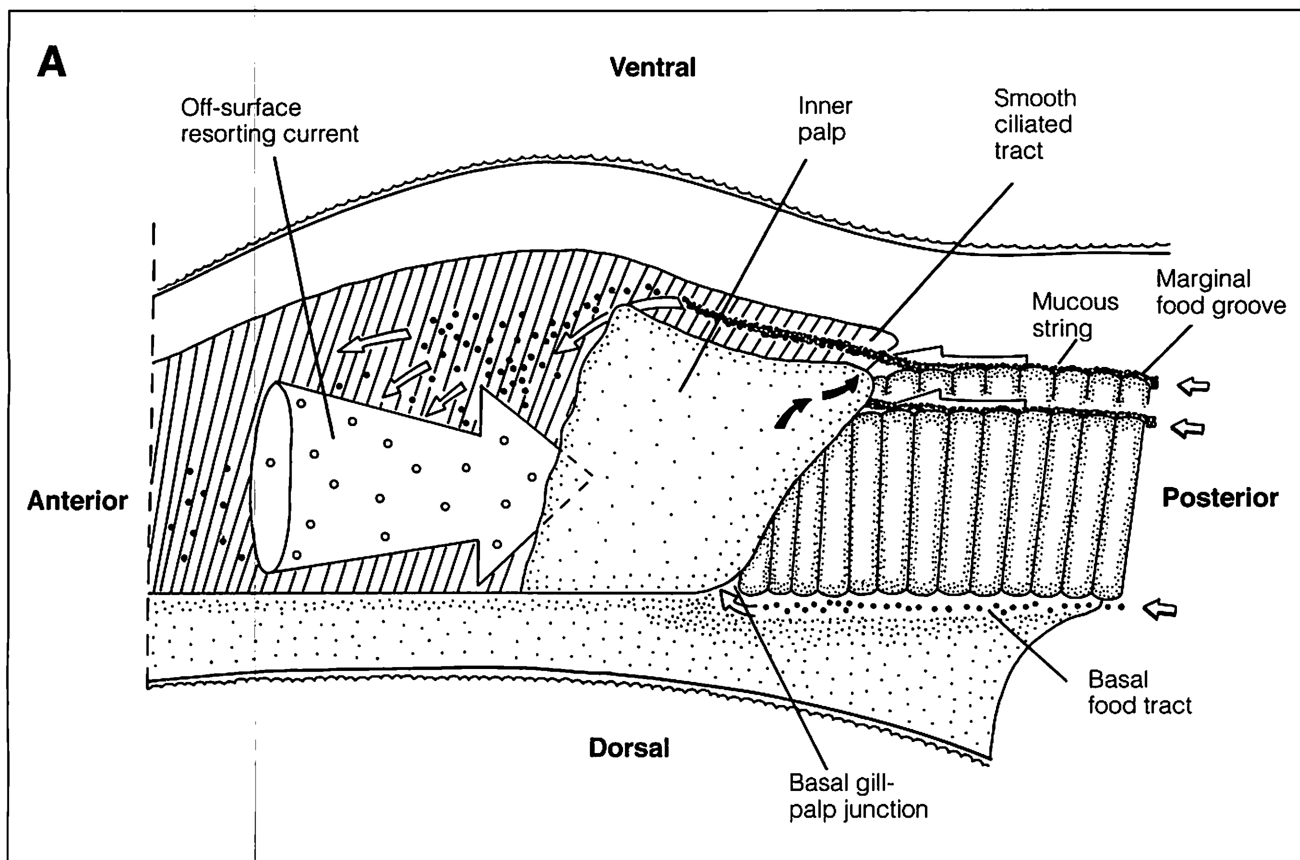
Ward et al. (1994) endoscopically observed the gill of living *C. virginica* and confirmed that particles initially moving dorsally in the fine frontal ciliary tracts were transferred onto the coarse frontal cirri and directed ventrally (Fig. 9). However, they observed in preliminary experiments no differences in selection between differently sized polystyrene microspheres when coated or not coated with algal ectocrines. This observation suggests that the gill of the oyster may not have a major role in particle selection. A more comprehensive study using particles with well-determined physical and chemical characteristics is required to elucidate fully the ability of the gill to process particles differentially based on biochemical composition.

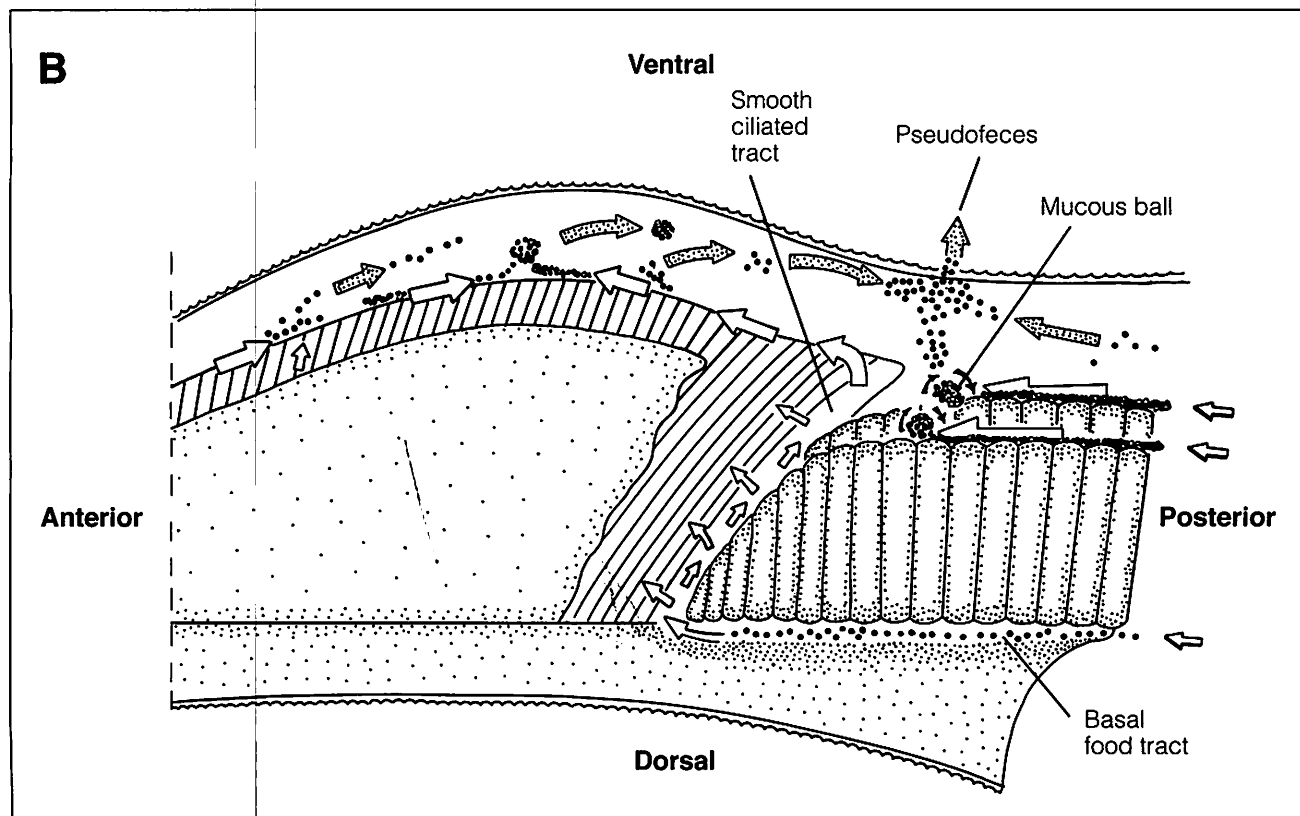
Particle Sorting on the Labial Palps

The labial palps are a specialized organ (Fig. 13) known to control the total amount of food ingested (Menzel 1955; Foster-Smith 1978), but they may also sort food before ingestion, perhaps on the basis of particle size (Yonge 1926) or chemical composition (Loosanoff 1949; Newell and Jordan 1983). The possibility that the labial palps serve to sort and select individual particles was questioned by Foster-Smith (1975a, b) because he observed that particles on the gills and palps of *M. edulis*, the cockle, *Cardium edule*, and clam, *Venerupis pullastra*, were bound together in mucus. Consequently, he concluded that it would be impossible for individual particles to be sorted on the palp surface. Instead, Bernard (1974) and Foster-Smith (1978) suggested that the primary function of the palps was to control the total volume of mucus and mucus-bound particles entering the mouth. However, Newell and Jordan (1983) hypothesized that mucus viscosity could be reduced as a consequence of mechanical agitation associated with the ciliary action and muscular pressure of the apposed surfaces of the palps. This reduction in mucus viscosity would facilitate sorting of particles by the complex ciliary currents associated with the ridged and grooved surfaces of the palps.

Because of technical difficulties in observing the inner ridged surfaces of the palps, early researchers could not obtain reliable information on palp function in undisturbed living bivalves. Consequently, much of the evidence for the function of the palps was based on histological and anatomical examination of dissected specimens (Allen 1958). Direct evidence for the role of the labial palps in particle sorting was first obtained by Menzel (1955). He observed through the left valve of juvenile *C. virginica* attached to glass slides that inorganic particles were rejected from the palps while organic particles were passed to the mouth. Unfortunately this method did

Figure 12 (opposite page). Diagrammatic illustration of interaction between demibranch and palps of *C. virginica*. Note that the outer palp is shown partly cut-away to reveal the ridged sorting surface of the inner palp surfaces. (A) This illustrates the situation when palp apices are in contact with the demibranch and particles entrapped in mucous strings are being transported from the marginal food groove to between the pair of palps. On the ridged sorting surfaces, the viscosity of the mucus-bound strings is reduced such that entrapped particles can disperse (solid circles). Subsequent movement and direction of these particles on the ridged palp surface is indicated by open arrows. When the appressed palp surfaces part slightly, some particles are moved posteriorly in an off-surface current towards the ciliated smooth tract (open circles within columnar arrow). Cleaning ciliary currents on the smooth outer surface of the palp carry particles toward the free ventral margin (broad black arrows). (B) This illustrates the situation when the palp apices are retracted away from the demibranch. Particles that have reached the end of the marginal food groove (point X marked in Figure 10B) are forming a ball that slowly rotates. The rejection of these balls onto the mantle and their subsequent ejection as pseudofeces is indicated by broad stippled arrows. At this time, only particles from the basal food tract are transported onto the palp surface. Particles rejected from the ridged palp surface are moved to the palp margin where they aggregate. This material is either rejected directly on to the mantle or moved along the palp margin (broad open arrows) to a point where two opposing ciliary currents meet. At this point the rejected material leaves the palp and moves onto the mantle's ciliated rejection tracts. Also indicated by stippled arrows is the movement along the mantle of particles that sediment out within the mantle cavity or are forced out of the marginal food groove at high seston loads. Figure modified from Ward et al. (1994).


A
Ventral
Off-surface
resorting current
Inner
palp
Smooth
ciliated
tract
Mucous
string
Marginal
food groove
Anterior
Posterior
Basal
food tract
Dorsal
Basal gill-
palp junction


B
Ventral
Smooth
ciliated
tract
Pseudofeces
Mucous ball
Anterior
Posterior
Basal
food tract
Dorsal

not provide details on processes occurring between the palps. Similarly, the technique of cutting a hole through the shell and mantle to form a glass observation window (Foster-Smith 1975a, b, 1978) does not allow the apposed ridged surface of the palp to be observed. In addition, this procedure may cause excess mucus production and, hence, create experimental artifacts (Jørgensen 1990).

These technical problems were overcome by Ward et al. (1991) who developed an endoscopic technique that has allowed an extensive study of feeding and palp function in *C. virginica* (Ward et al. 1994). The

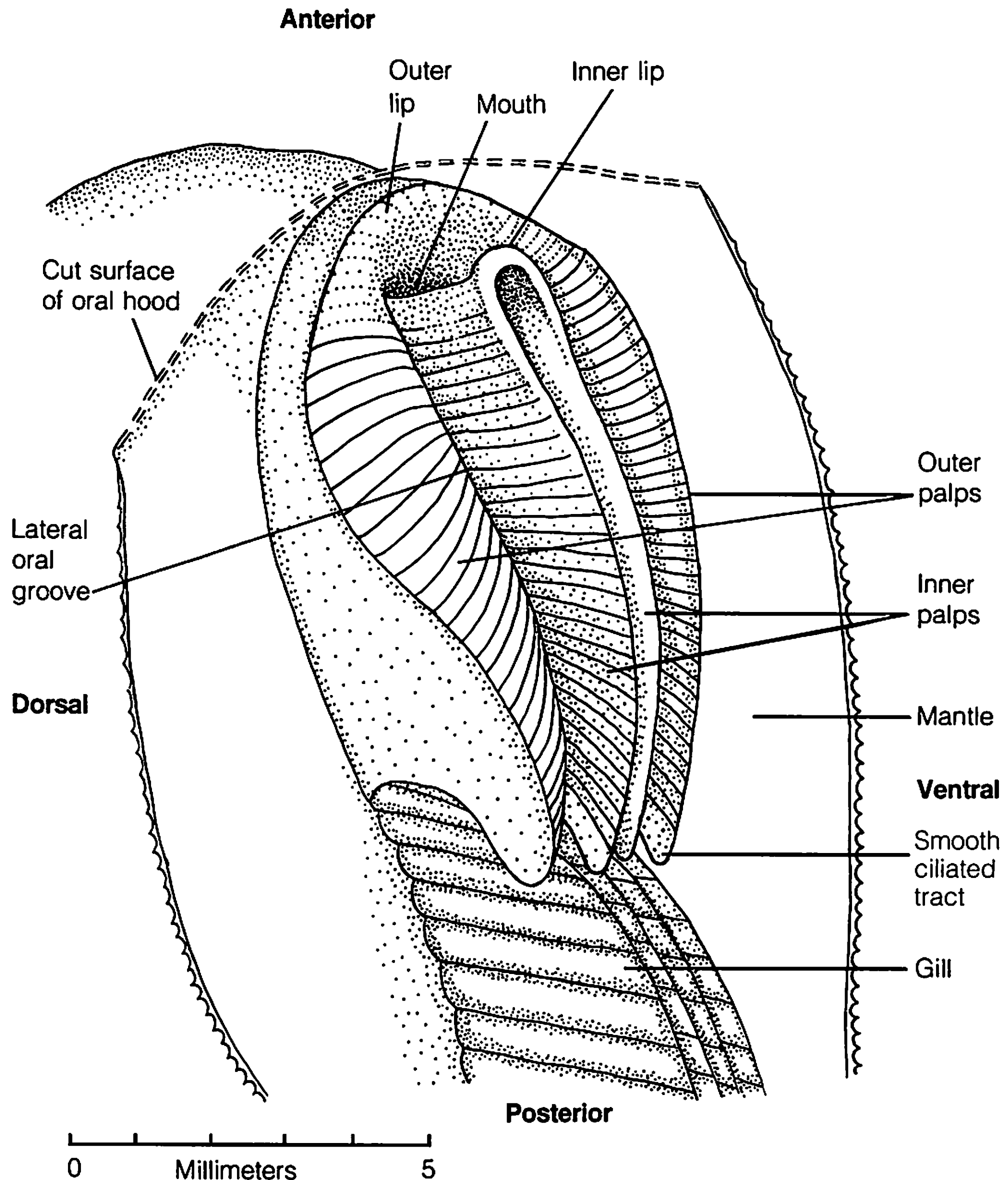


Figure 13. Diagrammatic anatomy of the palp and oral region of *C. virginica.* The mantle hood has been removed along the dashed line and pulled open to reveal the lips and mouth. Modifications to diagram in Galtsoff (1964) based on results from Ward et al. (1994).

results from that study form the basis for much of the following discussion. Additional evidence for the important role of the palps in particle sorting was obtained by Kiørboe and Møhlenberg (1981) who found a positive correlation between the efficiency of particle selection in ten bivalve species and the surface area of their labial palps.

Beninger et al. (1990) performed a detailed histological examination of palp structure in two species of scallop, the sea scallop, *Placopecten magellanicus*, and the variegated scallop, *Chlamys varia*. Based on an examination of the palp epithelium, Beninger et al. (1990) postulated that the palps may play an accessory nutritive role by absorbing dissolved and colloidal organic matter resulting from the breakdown of mucus as well as any organic molecules released from food particles. The palp epithelium, in conjunction with the gill epithelium in these species (Beninger et al. 1988), may also play a role in absorbing dissolved organic material directly from seawater. The ability of eastern oysters to absorb organic material through gill and palp epithelial layer, and the nutritional significance of any net uptake, remains to be determined (see Langdon and Newell, Chapter 6).

Palp Structure. The four triangular palps of *C. virginica* are attached to the visceral mass along their longest edge (Figs. 3, 13) on either side of the mouth. The shortest posterior edge is held in close proximity to the anterior margin of the gill. The point where the gills and palps are attached to the visceral mass and where they meet is a roughly triangular space called the basal gill-palp junction (Fig. 10A; Ward et al. 1994). Thus, there are two basal gill-palp junctions and each accepts particles from one inner and one outer basal food tract; material from the medial basal food tract is directed toward either palp junction (Ward et al. 1994). The third palp edge is unattached and curves slightly from the mouth to the ventral margin of the gill. Numerous muscle fibers run longitudinally and transversely through the palp (Galtsoff 1964). The mantle is fused at the ventral end to form a hood over the anterior portion of the palps that protects the mouth from direct contact with the pallial cavity (Figs. 3, 13).

On each side of the mouth (Fig. 13), an inner and outer palp form a functional unit, with the two apposed ridged "inner" surfaces accepting material from the inner and outer demibranchs, respectively (Fig. 10A). The bases of the two external palps are joined at the anterior end of the mouth to form an "outer" lip; an "inner" lip is formed by a similar junction of the two internal palps (Fig. 13). The inner lip is shorter and thicker than the outer lip (Galtsoff 1964).

The outer surfaces of each pair of inner and outer palps are sparsely covered mainly with small cilia and with occasional tufts of long cilia (see Eble, Chapter 2). These cilia are not involved in feeding but serve a cleaning function by transporting particles that impinge on this palp surface towards the palps' ventral free edge (Fig. 12A). Particles along these outer palp surfaces become entrained in the rejectory ciliary current that runs along the distal edges of the palps and are carried onto the mantle to be rejected as pseudofeces (Fig. 12B).

In contrast to the smooth outer surfaces of each pair of palps, the inner opposing surfaces possess an extremely complicated and highly ciliated surface which is folded into a series of transverse, (dorsal/ventral) orientated ridges and grooves about 300 µm deep (Galtsoff 1964) The angle that the palp ridge forms to the palp surface is not fixed but can be adjusted by muscular action to control the number of particles moving toward the mouth. When the ridges are held perpendicular, no particles can be transported toward the mouth; when they are slanted at an acute angle toward the mouth, most particles can be passed oralward (Foster-Smith 1975b). The cilia covering the inner surfaces of the palps are stout and range in length from 8 µm in the grooves to 20 µm on the ridges. These cilia are organized into four different functional tracts (Fig. 14; Purchon 1955; Allen 1958; Nelson 1960; Foster-Smith 1975b, 1978):

1. deep rejection tracts at the base of grooves that transport particles ventrally to the free ventral margin of each palp;

2. crest rejection tracts that transport particles ventrally to the palp margin;

3. oral acceptance tracts on the crests of ridges that beat anteriorly towards the mouth; and
4. resorting and accessory resorting tracts on the sides of grooves that either move particles out of grooves, deeper into grooves, or dorsally along the sides of grooves.

In addition to these fine-scale ciliary tracts associated with the ridged surface of the palps, a ciliated oral groove is located at the basal junction of each pair of palps that carries particles directly to the mouth. Slightly ventral to this oral groove is an off-surface aboral water current, located within the fluid trapped between the two palps, that carries particles posteriorly toward the smooth ciliated tract (Fig. 12A; Ward et al. 1994). Both the oral groove and off-surface current collect particles that have been subject to sorting in the tracts that run along the sides of the grooves on the surface of the palps. At the extreme ventral edge of the palps, there is a tract that collects particles from rejection tracts of the grooves and helps carry particles off the palp and onto the

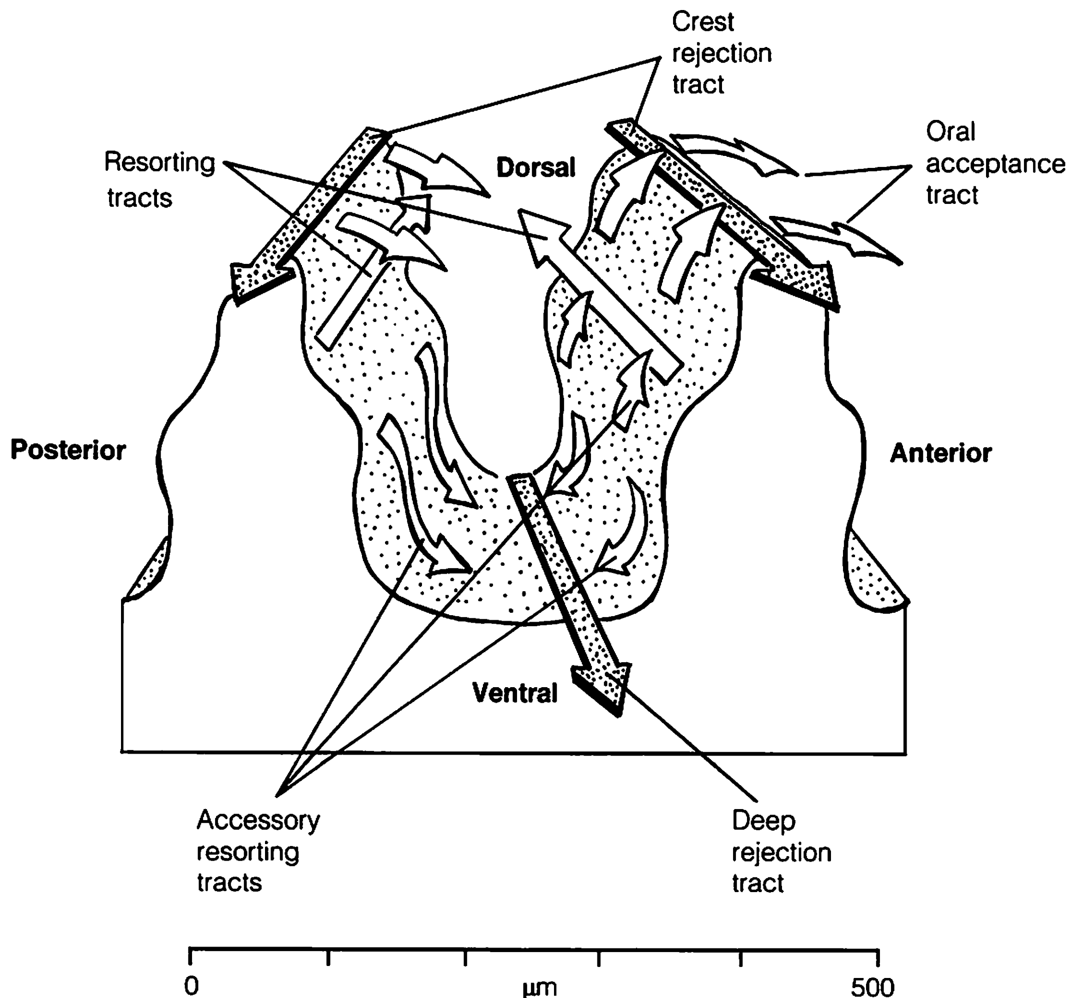


Figure 14. Diagrammatic representation of two folds on the inner surface of a generalized eulamellibranch palp showing the direction of the effective stroke of cilia on the various rejection, sorting, and acceptance tracts. Based on Allen (1958) and Foster-Smith (1978).

mantle (Fig. 12B); this rejection tract was described above for the smooth, outer surfaces of each palp pair.

Palp Function. Ward et al. (1994) determined that particles in the basal food tract of *C. virginica* are transported in a slurry toward the basal gill-palp junction. Particles leave this junction along one or more of four routes (numbers annotated in Fig. 10A correspond to the following four routes):

1. into the oral groove of the palp and thence directly to the buccal region;
2. onto the ridged sorting surface of the palp;
3. onto a smooth ciliated tract on the posterior palp edge and then in a ventral direction toward the palp apex; and
4. onto the anterior margin of each demibranch to be transported posteriorly.

The actual disposition of particles appears to be under the animal's behavioral control and is highly dependent on the number of particles being transported to the gill. Thus, under most conditions particles are directed either into the oral groove or onto the palp ridges. At some elevated suspended particle concentration, the rate of particle capture by the gill, exceeds the animal's ingestive capacity or the palps sorting capacity. Thus, when the numbers of particles reaching the gill-palp junction increases to the point where the junction becomes overloaded, particles are rapidly removed via the smooth tract. Particles in this smooth tract can either be introduced onto the ridged palp surface ventrally (Figs. 10A, 12B) or be transported to the palp apex and then rejected as pseudofeces (Fig. 12B). As particle concentrations increase further, particles are removed from the basal gill-palp junction by being directed onto the marginal ciliated indentations located along the anterior margin of each demibranch (Fig. 10A). Particles are transported within this indentation posteriorly until they meet mucus-bound particles being transported anteriorly in the main ciliated marginal food groove (point X in Fig 10B).

Mucus-entrapped particles carried from the gills in the marginal food grooves are transported anteriorly until the marginal food groove terminates (Figs. 9, 10B). At this position the mucus-bound particles coalesce with any particles being transported posteriorly away from the basal gill-palp junction, to form a ball that slowly revolves as it builds up mass (Fig. 12B). This ball can be picked off from each demibranch by the highly mobile apex of the corresponding palp, a process that establishes a mucus stream from the marginal food groove to the palp (Figs. 10A, 12A). Alternatively, a ball may grow so large that it is swept off by pallial currents onto the mantle where it is rejected as pseudofeces, whereupon a new ball starts to form (Fig. 12B). The transfer of the mucous string to the palps is not continuous because the palps may contract and move their posterior edges away from the anterior end of the gills, hence breaking the mucous string bridging the gap between gill and palp.

Labial palps exhibit alterations in muscular and ciliary activity that are correlated with the total number of particles being transported to the palps (Ward et al. 1994). The rheological properties of mucus are such that its viscosity is reduced when subject to sufficient shear stress, such as is perhaps generated by the combined ciliary and muscular action of the apposed ridged surfaces of the palps (Newell and Jordan 1983). This may explain the observation of Ward et al. (1994) that mucus bound aggregates of material entering between the palps from the marginal food groove are broken down into individual particles. Consequently, despite arguments to the contrary by Jørgensen (1990), individual particles are subject to selection at the numerous palp ridges before they either enter the oral groove or are rejected. If a particle is rejected it may be immediately removed from the palp via a ventrally beating crest or deep rejection tract (Fig. 14). Alternatively, particles may be subjected to sorting by tracts along the sides of grooves before being rejected or passed to the mouth. Ward et al. (1994) also observed an off-surface aboral water current (Fig. 12A) that carries particles towards the smooth tract on the posterior palp margin. This current may allow particles to be recy-

cled through the palp sorting process a number of times before being ingested or rejected.

There is no compelling evidence to suggest that the sorting process on the palp surface involves any type of feedback from sensory cells. A detailed histological examination of the palps of two species of scallop by Beninger et al. (1990) did not reveal any cell types that were characteristic sensory cells. An early neurophysiological study of the palps of *C. virginica* performed by Dwivedy (1973) has been criticized by Beninger et al. (1990) for two reasons: because of possible recording artifacts from the use of silver electrodes and because the few putative sensory cells detected were located on the smooth outer palp surfaces rather than the ridged inner surface. More detailed histological, biochemical, and neurophysiological studies of palp function in bivalves, including eastern oysters, will be required in order to fully understand the mechanisms of particle selection.

The position and muscular movements of the ridges of the palps in living eastern oysters are still uncertain because of limits on the magnification of the endoscope used by Ward et al. (1994). Palp ridges are thought to be inclined towards the mouth at an oblique angle to the palp's surface, with successive ridges overlapping and covering ciliary tracts within the grooves (Foster-Smith 1978). Muscular action can also pull the crest rejection tract down between the palp ridges, such that only the orally directed ciliary tracts are exposed (Foster-Smith 1978). In this position, particles would be transferred on the oral acceptance tract across the palp ridges and moved anteriorly toward the mouth. Only when the muscles of the palps contract (Nelson 1923a, b) or relax (Foster-Smith 1975a) would particles be exposed to the rejection and accessory resorting ciliated tracts of the palps' grooves and the crest rejection tract (Fig. 14).

Particles that have been subject to some degree of sorting on the palp can be transported to the free edge of each palp in rejection tracts within each palp ridge (Ward et al. 1994). These rejected particles form loose aggregations that may either disintegrate, with some of the material re-entering between the palps to be re-sorted, or may be rejected from the palps and then be carried onto the surface of the mantle to be rejected as pseudofeces (Fig. 12B). At high particle concentrations, mucus strings leaving the marginal food groove on each demibranch may not enter between the palps but can be moved directly from the demibranch anteriorly along the ventral margin of the palp. There they combine with particles rejected by the palps and these mucus-bound aggregations are transferred to pseudofeces rejection tracts on the mantle (Ward et al. 1994).

Pseudofeces Production

Large dense particles that settle out from the inhalant water current fall onto the mantle in the pallial cavity and are carried by cilia covering the mantle surface to the mantle margin (Bernard 1974). This ciliary motion also collects mucus-coated material rejected from the palps and the marginal food groove of the gill (Figs 12B). This combination of rejected particles, called pseudofeces, is transported to the ventral free edge of the mantle adjacent to the labial palps (Fig. 3). At this position, they are either pushed out between the mantle margins by ciliary action or forcibly ejected by rapid closure and opening of the valves.

The voiding of excess particles as pseudofeces is the major mechanism whereby *C. virginica* regulates the amount of material ingested. Thus, pseudofeces are composed not only of material rejected by the palps based on particle composition, but also particles in excess of the gut's processing capacity. In addition, even though particle capture on the gills and sorting on the palps is an almost continuous process, particle ingestion is discontinuous. Such fine control of ingestion is effected by either starting or stopping cilia on the lips, which are responsible for transporting particles from the palps through the mouth and into the digestive system. Cilia of the lip were observed to be under separate control from cilia on the gill and palps (Ward et al. 1994). Such regulation of the amount of material ingested presumably serves to prevent the digestive system from becoming overloaded. The exact nature of this control still remains to be determined, one of the questions being whether or not neural feedback from the stomach or digestive diverticula is involved.

When eastern oysters are feeding under conditions of high concentrations of suspended particles, copious amounts of mucus are produced by the gills and most of the material captured by the gills is transferred via the coarse frontal cirri to the four marginal food grooves. These food grooves can then be closed by muscles, forcing material from the tracts directly onto the mantle surface, hence by-passing the palps entirely (Menzel 1955; Nelson 1960; Jørgensen 1966). Removal of mucus-bound pseudofecal material from the gill can also be aided by vigorous muscular contractions of the entire demibranch (Ward et al. 1994).

Measurement of Clearance Rates

As described in detail above, *C. virginica* is an active suspension feeder, removing particulate food from water pumped through the gills. The flow rate of water through the gills is termed the ventilation (= pumping) rate, whereas the volume of water totally cleared of suspended particles per unit time is called the clearance (= filtration) rate (Bayne et al. 1985). Ventilation and clearance rates are equal only if particle retention efficiency is 100%. Because this efficiency is rarely attained, measured clearance rates usually underestimate ventilation rates.

Ventilation rates have been technically difficult to measure directly because of problems associated with measuring water flow from the exhalent siphon without affecting flow rates. For example, Galtsoff (1926) devised an isolating collar attached to the shell of oysters to directly measure water flow from the exhalent cloaca of *C. virginica.* Unfortunately, this method imposed a positive hydrostatic head pressure on the water downstream of the lateral cilia which inhibited pumping activity (Hildreth 1976; Famme et al. 1986). While the laser-doppler method of Famme et al. (1986) allowed non-intrusive measurement of the ventilation rates of *M. edulis,* this technology has not yet been applied to determining ventilation rates of *C. virginica.*

Clearance rates can be measured by monitoring decreases in particle concentration with an electronic counter (e.g., Coulter Counter). Such measurements can be performed in closed systems where the logarithmic decrease in particle concentration is used to calculate clearance rate (Coughlan 1969). Alternatively, the rate at which particles must be added to the closed system in order to maintain a constant particle concentration or changes in turbidity can be determined (e.g., Winter 1973). Measurements of clearance rates of animals in closed systems may be biased because more efficiently captured particles are likely to be removed first. Such differential capture would lead to a progressive increase in the proportion of smaller, less efficiently retained particles, and thus result in an apparent decline in filtration rate with time (Williams 1982).

Maintaining bivalves in flowing water allows feeding activity to be measured under ambient conditions, including concentrations of natural particles (Bayne et al. 1977). Instantaneous clearance rates can be calculated from the difference in particle concentration between inflow and outflow together with measurements of the flow rate through the apparatus (Hildreth and Crisp 1976; Bayne et al. 1985). Clearance rates of eastern oysters can also be calculated by dividing the total amount of inorganic or indigestible material in the biodeposits produced per unit time (feces and pseudofeces combined) by the total concentration of the same refractory particulate material in the seston (Jordan 1987). The accuracy of this latter method depends on negligible absorption of the refractory particulate material by the eastern oyster.

The clearance rate of *C. virginica* is not constant but infinitely variable between zero and a size-dependent maximum value depending on the balance between endogenous factors and a wide variety of exogenous factors (e.g., particle concentration, temperature, salinity, etc.). The maximum clearance rate is partly determined by the gill size of the oyster and is related to tissue weight by the standard allometric equation (Newell 1979; Bayne and Newell 1983):

$$CR = aW^b$$

where CR = maximum clearance rate; W = dry tissue weight; a = constant; and b = weight exponent.

Because gill area is not directly proportional to tissue weight, the weight exponent is less than unity and has a theoretical value of 0.67. Thus the weight-

specific maximum clearance rate is higher for small bivalves than for large individuals. For *C. virginica* (Newell, unpubl. data) and many other bivalve species, the weight exponent is frequently lower than the theoretical value of 0.67 (Bayne et al. 1976; Winter 1978; Bayne and Newell 1983). This discrepancy is perhaps due to a relatively larger than expected reduction in gill area per unit body weight in large eastern oysters, in which sequestered nutrient reserves and germinal tissue comprise a large fraction of total tissue mass. In addition, reported rates for large eastern oysters may be underestimated due to re-filtration of seawater in volume-limited experimental chambers (Hildreth and Crisp 1976; Jørgensen 1976; Winter 1978; Newell 1979). A weight exponent for *C. virginica* of 0.73 + 0.22 (SE) was reported by Riisgard (1988). The standard error of this weight exponent is three times larger than that reported by Riisgard (1988) for the ribbed mussel, *Geukensia demissa*, and *M. mercenaria* measured in the same study, which is again indicative of the high variability of eastern oyster feeding activity even under controlled laboratory conditions.

Endogenous Regulation of Ventilation and Clearance Rates

Many experimental studies of the clearance rates of *C. virginica* have been conducted with specimens fed on cultured unicellular algae under laboratory conditions (e.g., Tenore and Dunstan 1973; Epifanio and Ewart 1977; Higgins 1980a, b; Palmer 1980; for review see Epifanio 1982). Unfortunately, it may be inaccurate to extrapolate clearance rates obtained from these laboratory studies to the estuarine situation where eastern oysters feed on natural seston made up of high proportions of inorganic particulate material. For example, Widdows et al. (1979) reported that blue mussels feeding on natural seston maintained elevated clearance rates even at particle concentrations previously found to inhibit feeding when unicellular algae had been the sole food source. Thus, in the following discussion on the endogenous and exogenous factors affecting feeding behavior of *C. virginica*, emphasis will be given to studies that used natural seston as a food source. Information on the feeding behavior of bivalve molluscs in artificial aquaculture systems has been comprehensively reviewed by Winter (1978).

Shell closure is an important mechanism for protecting the eastern oyster from short-term adverse environmental factors, including abrupt changes in salinity, high turbidity, etc. Limited periods of shell closure can also occur, however, even when eastern oysters are maintained sub-tidally under optimum conditions with a plentiful supply of food. Loosanoff and Nomejko (1946), for example, reported that eastern oyster valves were closed 6% of the time when maintained in unfiltered, flowing seawater with ambient seston concentrations. Changes in ambient conditions are detected by means of a row of sensory and highly contractile tentacles on the middle and inner lobes of the mantle (see Eble, Chapter 2). Tentacles on the middle lobe are mostly short (ca. 0.3 mm) and slender except for every fourth or sixth tentacle which is stouter and longer (ca. 0.5 mm). The inner mantle lobe bears only long (ca. 0.8 mm) and stout tentacles. Responses of tentacles to specific stimulants have not been defined, although tentacles have been reported to respond to changes in salinity, concentration of cations (Hopkins 1932a), and various organic compounds, including extracts of known predators (Hopkins 1932b; Galtsoff 1964). Ward et al. (1992) demonstrated that *P. magellanicus* can detect both intra- and extracellular organic compounds from phytoplankton, and the presence of these compounds stimulated the sea scallops' filtration rate and increased particle ingestion rates. The specific sensory organ(s) involved still remains to be identified and although it is likely that organic compounds were detected via chemoreception of dissolved molecules, the exact mode of detection requires further investigation.

Crassostrea virginica is also capable of responding to changes in ambient water conditions by adjusting its ventilation rate. As mentioned previously, a common observation in studies of the feeding of adult *C. virginica* is the high degree of short-term variability in clearance rate, even under constant conditions. This variability is not associated with tidal or diurnal cycles (Loosanoff and Nomejko 1946; Palmer 1980). Mechanisms whereby bivalves control the flow of water through the gills are still not fully understood. Ventilation rate in bivalves may possibly be regulated

by adjusting the activity of the lateral cilia of the gill. However, Jørgensen et al. (1986, 1988) reported that the relationship between lateral ciliary beating and pumping rate was not linear in *M. edulis* and they concluded that control of the beating rate of lateral cilia is not a primary means of controlling water flow.

The eastern oyster can exert fine control over ventilation activity by adjusting the space between opposing mantle margins, either where water enters the mantle cavity or leaves through the cloaca. Similarly, many bivalve species respond to changes in ambient conditions, such as seston concentration and salinity fluctuations, by expanding or contracting the exhalent siphon opening, changing the degree of valve gape, and retracting the mantle margin (Foster-Smith 1976; Jørgensen et al. 1986, 1988). Such muscular contractions result in the shortening of the entire demibranch axis and a consequent reduction in the space between adjacent filaments. Consequently, water velocity gradients generated by the metachronal beat of lateral cilia on opposite sides of the interfilamentary space no longer act synergistically, resulting in a reduction in water flow through the gill (Jørgensen et al. 1986, 1988; Jørgensen 1989).

In the eulamellibranch-type gill of eastern oysters, it is possible that water flow through the gill is also subject to direct control via the extensive musculature within this type of gill (Fig. 5; Eble, Chapter 2). Contraction of muscles associated with the skeletal gill rods at the point where lamellae are attached to the visceral mass adjusts the relative proximity and degree of extension of each demibranch. Such adjustments control both the width of the interfilamentar space and the size of the ostia. As in *M. edulis*, the interfilament width will control the distance between lateral cilia and hence the efficiency with which they can move water. Ostia size, regulated by muscles running along the axis of each filament, may also serve to control the resistance of the gill to water flow. In addition, muscles at interlamellar junctions (Fig. 5) are used to control the space and size of water tubes between ascending and descending lamellae, resulting in control of the water flow into the epibranchial and promyal chambers.

Powell et al. (1992) reviewed the literature on clearance rates in a number of different species of bivalve, including the eastern oyster. They suggested that most species of bivalve have two characteristic clearance rates that they called low and high gear. When the high gear rates were integrated with other literature estimates of physiological rate functions into a simulation model of energy flux, the predicted rates of growth were over-estimated compared with measurements of actual eastern oyster growth rates in the Gulf Coast. The low gear rate, however, gave predicted growth rates that were closer to the actual growth of natural populations of eastern oysters. Consequently, Powell et al. (1992) cautioned against assuming that the high gear rates are representative of bivalves feeding undisturbed in the field (see Jørgensen 1990). Conversely, data obtained by Jordan (1987) from eastern oysters held under close to ambient conditions in a flume indicated that they maintained high clearance rates (ca. 10 L $h^{-1}g^{-1}$ dry tissue weight) for extended periods of 12 h or more. This controversy on the magnitude of clearance rates can be resolved by obtaining concurrent estimates of the physiological rate functions (e.g., clearance rate, assimilation efficiency, metabolic rate and nitrogen excretion) required to construct a balanced energy budget (Bayne et al. 1985) under close to ambient conditions. In this way the consistency of the various rate functions can be examined within the context of the eastern oyster's balanced energy budget.

Exogenous Factors Affecting Feeding Activity

Water currents can exert a strong influence on eastern oyster populations because they serve to remove biodeposits and prevent self-silting of the eastern oyster reef (Lund 1957a, b). The relationship between water flow and growth of bivalves is more complex, however, and needs further study. For example, some studies (e.g., Incze et al. 1981; Manzi et al. 1986; Grizzle and Lutz 1989) have demonstrated a positive relationship between the velocity of water currents and growth of bivalves in the field. Conversely, Grizzle et al. (1992) found that the growth of *C. virginica* in laboratory flumes was negatively correlated with tested current speeds between 2 to 9 cm s^{-1}. Growth was highest at a current speed of 1 cm s^{-1} (about three times higher than the average growth

measured at the faster flows) but almost ceased at zero water flow. Similarly, in two species of scallop studied in laboratory flumes, there is either no apparent relationship between water flow and growth (Cahalan et al. 1989) or a negative relationship once currents exceed a certain flow velocity (Kirby-Smith 1972; Wildish et al. 1987; Eckman et al. 1989).

The basis of the positive relationship between current speed and bivalve growth is likely an increased rate of supply of particulate food (seston fluxes), thereby reducing local food depletion associated with feeding. Frechette et al. (1989) demonstrated the importance of vertical and horizontal seston fluxes in affecting feeding activity of *M. edulis*. And horizontal seston fluxes have been postulated as a major control on individual growth, and possibly feeding, of bivalves generally (Grizzle and Lutz 1989; Grizzle and Morin 1989). Certainly, for most species of bivalve, when water velocities exceed the critical erosional velocity for a particular sediment grain-size (generally above 10 cm s^{-1} [Jordan 1987; Grizzle and Lutz 1989]), growth decreases, perhaps due to bedload transport increasing seston concentrations to the point where feeding inhibition occurs (Wildish et al. 1987; Grizzle and Lutz 1989).

Evidence for direct effects of water flow on the feeding activity of suspension-feeding bivalve molluscs is equivocal. Walne (1972) suggested that there is a positive relationship between clearance rate and water flow rate. However, on the basis of their own experiments and a revised analysis of Walne's (1972) data, Hildreth and Crisp (1976) concluded that there was no direct evidence for any bivalve species that clearance rates were directly dependent on water flow. Further experimental work by Hildreth (1976) demonstrated no effect of water flow between 2 to 42 L h^{-1} on the feeding activity of *M. edulis*. Wildish et al. (1987) showed a strong negative relationship between filtration rate and water current velocities above 10 cm s^{-1} for *P. magellanicus*. In contrast, Jordan (1987) reported a positive relationship at low flows (<2 cm s^{-1}) between water velocity and clearance rate of *C. virginica* held in a flume supplied with unfiltered seawater at current speeds ranging from 0.8 to 10 cm s^{-1}. Jordan (1987) did not attribute this effect of low flow on eastern oyster clearance rate to localized depletion of seston in the experimental flume at the low flow rates. However, his experiments designed to explicitly test for the direct effect of water flow on filtration activity were inconclusive.

Under typical estuarine conditions, field studies often cannot discriminate between effects of particle concentration and water velocity on bivalve feeding and growth. This is because seston concentrations are often positively related to water velocity due to increasing benthic boundary layer turbulence resuspending surficial particles, including organic material (e.g., benthic diatoms, microheterotrophs, etc.) and inorganic sediments. Because so few studies have been undertaken in which these possible confounding effects have been adequately controlled it is not possible to come to a definite conclusion concerning the effects of water flow on bivalve feeding activity. Grizzle et al. (1992) observed, however, that it is generally siphonate bivalve species that exhibit a positive effect of water flow on feeding activity whereas non-siphonate species, such as the eastern oyster, show a negative response to flow above a certain velocity. They hypothesized that siphonate species require a higher degree of water flow to prevent localized seston depletion compared with non-siphonate species. This topic warrants rigorous examination in laboratory flumes. Eckman et al. (1989) discuss some of the pertinent hydrodynamical factors that must be considered in such future research.

Eastern oysters can modulate their feeding activity in response to ambient seston concentration. Higgins (1980a) reported that fed eastern oysters opened their shell 94% of the time whereas animals held in particle-free water only opened 35% of the time. *Crassostrea virginica* is not stimulated to feed continuously when exposed to particle concentrations below 1 mg L^{-1}. At particle concentrations of between 1 and 5 mg L^{-1}, eastern oysters open their valves and clearance rates rapidly increase toward the maximum size-dependent rate. At seston concentrations between 5 and 10 mg L^{-1}, clearance rates are maximal and particle capture starts to exceed the rate of particle ingestion. Ingestion rates are finite and dependent on gut volume (which is a function of body size) and gut

residence time (Bayne et al. 1984). Once maximum ingestion rates are attained, any further increase in seston concentration results in increasing amounts of material being rejected as pseudofeces (Haven and Morales-Alamo 1966). Clearance rates do not decline unless particle concentrations exceed about 25 mg L^{-1} and only cease at particle concentrations greater than about 75 mg L^{-1}.

Crassostrea virginica exhibits a complex feeding response when exposed to ambient variations of temperature and seston. Over an annual cycle, Jordan (1987) measured the biodeposition rate (fecal and pseudofecal production rates combined) of *C. virginica* held in a flume supplied with ambient estuarine water. He then used these data to develop a multiple regression model to predict biodeposition rates from a quadratic function of temperature, seston concentration, and their interaction. Jordan's (1987) model predicted that eastern oyster clearance rates (calculated by dividing biodeposition rate by seston concentration) would increase until seston concentrations reached about 25 mg L^{-1} and then would decline at higher seston concentrations at all temperatures (Fig. 15). However, an increase in seston concentration had less apparent effect on biodeposition rates at temperatures below 20°C than at temperatures above 20°C. Clearance rates of over 10 L $h^{-1}g^{-1}$ dry tissue weight measured by Jordan (1987) for *C. virginica* were higher than many clearance rates reported for this species in laboratory studies using algal diets (e.g., Higgins 1980b, Palmer 1980) but similar to those recorded by Loosanoff and Nomejko (1946) for eastern oysters feeding in flowing unfiltered seawater.

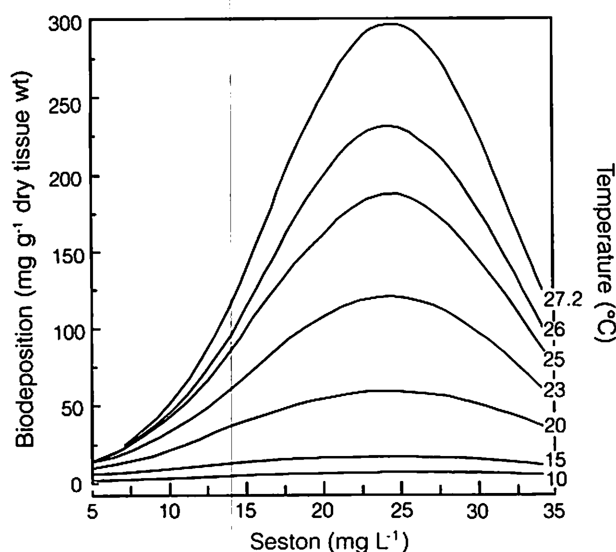


Figure 15. Production (predicted by a multifactorial regression model) of feces and pseudofeces (biodeposition) by adult *C. virginica* as functions of temperature and seston concentration. Redrawn from Jordan (1987).

The well-documented ability of *C. virginica* to maintain high clearance rates at elevated seston concentrations (Nelson 1938; Menzel 1955; Lund 1957a; Galtsoff 1964; Jordan 1987) has also been reported for *M. edulis* (Foster-Smith 1975; Widdows et al. 1979; Kiørboe et al. 1981). This response differs from that of some other bivalve species, in which filtration rates decline as seston concentrations increase (Foster-Smith 1975; Bricelj and Malouf 1984). The nutrition of *C. virginica* is most likely improved by its ability to maintain a high clearance rate even though its gut cannot process all of the captured particles and a large proportion of them are rejected as pseudofeces. In the estuarine environments inhabited by *C. virginica*, seston is composed of a high proportion of inorganic particles, and relatively refractory plant detritus comprises a seasonally variable proportion of the particulate organic material (Widdows et al. 1979; Soniat et al. 1984; Berg and Newell 1986; Crosby et al. 1989; Grizzle and Lutz 1989). By maximizing the number of particles captured and subjected to its efficient pre-ingestive sorting and selection processes, *C. virginica* can maximize ingestion of nutritive particles. Also, the energetic costs of pumping water and capturing particles are low, being less than 2% of metabolic energy expenditures in *M. edulis* (Jørgensen et al. 1986). In contrast, the energetic demands of subjecting material to the digestive process are much higher, comprising about 17% of energy expenditure in *M. edulis* (Widdows and Hawkins 1989). Thus, in energetic terms, efficiency can be maximized by ensuring that only the most nutritive particles are ingested and subjected to the digestive processes.

In addition to the short-term behavioral compensations for changes in food availability discussed above, mussels and possibly other bivalve species such as *C. virginica* can compensate for dietary changes by adjusting ingestion rates, absorption efficiencies, and efficiencies of the digestive process to help offset changes in the organic content of the food (Bayne et al. 1987, 1989). However, Bayne et al. (1989) demonstrated that an acclimation period of about two weeks is necessary for such compensation mechanisms to be fully invoked. Therefore, such compensatory mechanisms are likely to be more important in mediating responses to seasonal changes in food quality, rather than short-term diurnal changes.

EPILOGUE

Since publication of Galtsoff's (1964) monograph summarizing the literature on the eastern oyster, significant advances have been made in understanding suspension feeding in bivalve molluscs. Considerable new information on structure and patterns of ciliation in larval and adult eastern oysters has been obtained from anatomical studies that have employed scanning electron microscopy (e.g., Ribelin and Collier 1977; Elston 1981). This work has extended the earlier meticulous histological and anatomical studies that still form the basis of our chapter. Perhaps the most notable insights gained over the last three decades, however, have been improved understanding of how the various feeding structures of bivalves actually function *in vivo*. These advances have primarily been a result of the application of new microcinematographic techniques. Such photographic studies started with the pioneering work of Strathmann and coworkers (e.g., Strathmann et al. 1972; Strathmann and Leise 1979) studying particle capture in veliger larvae. Since then the field has rapidly advanced with technological developments in high speed video techniques allowing unparalleled observations of larval feeding mechanisms (Gallager 1988). In adult oysters the use of video endoscopy has improved our ability to understand how the pallial organs capture and transport particles (Ward et al. 1991, 1994).

These advances in observational techniques have been matched by increased understanding of the physics of particle motion at low Reynolds numbers, when viscous rather than inertial forces dominate (e.g., Rubenstein and Koehl 1977; Leal 1980; Vogel 1994). Such insights have been further developed by Jørgensen and coworkers, who have made substantial contributions to developing the hydromechanical hypothesis of particle capture in bivalve molluscs (summarized in Jørgensen 1990).

Although much work still remains to be done in elucidating the basic processes of particle capture in bivalve molluscs, it is important that more effort be directed to understanding physical and biochemical mechanisms of particle sorting and selective ingestion. Such research is needed in order to elucidate sensory processes that both larvae and adults may possess to help optimize their nutrition. Future research should also include quantitative measurements of feeding, ingestion, and digestion of natural seston with its complex mixture of plankton assemblages. Only by taking some of these questions into the natural environment can we attain the ultimate goal of understanding the role of nutrition in regulating reproduction and growth of bivalve mollusc populations in nature.

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REFERENCES

Allen, J.A. 1958. On the basic form and adaptations to habitat in the Lucinacea (Eulamellibranchia). Phil. Trans. R. Soc. B.241:421-484.

Allen, J.A. 1985. The recent Bivalvia: their form and evolution. Pages 337-403 in E.R. Truman and M.R. Clarke (eds.). The Mollusca, volume 10. Academic Press, New York.

Atkins, D. 1937. On the ciliary mechanisms and interrelationships of lamellibranchs. Part II. Sorting devices on the gills. Quart. J. Microsc. Sci. 79:339-373.

Atkins, D. 1938. On the ciliary mechanisms and interrelationships of lamellibranchs. Part VII. Latero-frontal cilia of the gill filaments and their phylogenetic value. Quart. J. Microsc. Sci. 80:345-436.

Baldwin, B.S. 1995. Selective particle ingestion by oyster larvae (*Crassostrea virginica*) feeding on natural seston and cultured algae. Mar. Biol. 123:95-107.

Baldwin, B.S. and R.I.E. Newell. 1991. Omnivorous feeding by planktotrophic larvae of the eastern oyster *Crassostrea virginica.* Mar. Ecol. Prog. Ser. 78:285-301.

Baldwin, B.S. and R.I.E. Newell. 1995a. Relative importance of different size food particles in the natural diet of oyster larvae (*Cassostrea virginica*). Mar. Ecol. Prog. Ser. 120:135-145.

Baldwin, B.S. and R.I.E. Newell. 1995b. Feeding rate responses of oyster larvae (*Crassostrea virginica*) to seston quantity and composition. J. Exp. Mar. Biol. Ecol. 189:77-91.

Bayne, B.L. 1971. Some morphological changes that occur at the metamorphosis of the larvae of *Mytilus edulis.* Pages 259 - 280 in D.J. Crisp (ed.). Fourth European Marine Biology Symposium, Cambridge University Press, Cambridge.

Bayne, B.L. 1983. Physiological ecology of marine molluscan larvae. Pages 299-343 in N.H. Verdonk, J.A.M. van den Biggelaar and A.S. Tompa (eds.). The Mollusca, Vol 3. Academic Press, New York.

Bayne, B.L., D.A. Brown, K. Burns, D.R. Dixon, A. Ivanovici, D.R. Livingstone, D.M. Lowe, M.N. Moore, A.R.D. Stebbing and J. Widdows. 1985. The Effects of Stress and Pollution on Marine Animals. Praeger, Greenwood Press, Westport, Connecticut. 384 pp.

Bayne, B.L., A.J.S. Hawkins and E. Navarro. 1987. Feeding and digestion by the mussel *Mytilus edulis* L. (Bivalvia: Mollusca) in mixtures of silt and algal cells at low concentrations. J. Exp. Mar. Biol. Ecol. 111:1-22.

Bayne, B.L., A.J.S. Hawkins, E. Navarro and I.P. Iglesias. 1989. Effects of seston concentration on feeding, digestion, and growth in the mussel *Mytilus edulis.* Mar. Ecol. Prog. Ser. 55:47-54.

Bayne, B.L., D.W. Klumpp and K.R. Clarke. 1984. Aspects of feeding, including estimates of gut residence time, in three mytilid species (Bivalvia, Mollusca) at two contrasting sites in the Cape Peninsula, South Africa. Oecologia 64:26-33.

Bayne, B.L. and R.C. Newell. 1983. Physiological energetics of marine molluscs. Pages 407-515 in A.S.M. Saleuddin and K.M. Wilbur (eds.). The Mollusca, Volume 4. Physiology, Part 1. Academic Press, New York.

Bayne, B.L., R.J. Thompson and J. Widdows. 1976. Physiology I. Pages 121-206 in B.L. Bayne (ed.). Marine Mussels: Their Ecology and Physiology. Cambridge University Press, New York.

Bayne, B.L., J. Widdows and R.I.E. Newell. 1977. Physiological measurements on estuarine bivalve molluscs in the field. Pages 57-68 in B.K. Keegan, P. O'Ceidigh and P.J.S. Boaden (eds.). Biology of Benthic Organisms. Pergamon Press, Oxford.

Beninger, P.G., M. Auffret and M. Le Pennec. 1990. Peribuccal organs of *Placopecten magellanicus* and *Chlamys varia* (Mollusca: Bivalvia): structure, ultrastructure and implications for feeding. I. The labial palps. Mar. Biol. 107:215-223.

Beninger, P.G., M. Le Pennec and L. Salaun. 1988. New observations of the gills of *Placopecten magellanicus* (Mollusca: Bivalvia), and implications for nutrition. I. General anatomy and surface microanatomy. Mar. Biol. 98:61-70.

Berg, J.A. and R.I.E. Newell. 1986. Temporal and spatial variations in the composition of seston available to the suspension feeder *Crassostrea virginica*. Estuar. Coast. Shelf Sci. 23:375-386.

Bernard, F.R. 1974. Particle sorting and labial palp function in the Pacific oyster *Crassostrea gigas* (Thunberg). Biol. Bull. 146:1-10.

Bricelj, V.M. and R.E. Malouf. 1984. Influence of algal and suspended sediment concentrations on the feeding physiology of the hard clam *Mercenaria mercenaria*. Mar. Biol. 84:155-165.

Brown, C.H. 1952. Some structural proteins of *Mytilus edulis*. Quart. J. Micros. Sci. 93:487-502.

Caddy, J.F. 1969. Development of mantle organs, feeding and locomotion in post-larval *Macoma balthica* (L.) (Lamellibranchiata). Can. J. Zool. 47:609-617.

Cahalan, J.A., S.E. Sidall and M.W. Luckenbach. 1989. Effects of flow velocity, food concentration and particle flux on growth rates of juvenile bay scallops *Argopecten irradians*. J. Exp. Mar. Biol. Ecol. 129:45-60.

Carriker, M.R. and R.E. Palmer. 1979. Ultrastructural morphogenesis of prodissoconch and early dissoconch valves of the oyster *Crassostrea virginica*. Proc. Natl. Shellfish. Assoc. 69:103-128.

Cole H.A. 1938. The fate of the larval organs in the metamorphosis of *Ostrea edulis*. J. Mar. Biol. Assoc. U.K. 22:469-484.

Coughlan, J. 1969. The estimation of filtering rate from the clearance of suspensions. Mar. Biol. 2:356-358.

Crisp, D.J. 1976. The role of the pelagic larva. Pages 145-155 in P.S. Davies (ed.). Perspectives in Experimental Biology. Pergamon, New York.

Crisp, D.J., A.B. Yule and K.N. White. 1985. Feeding by oyster larvae: the functional response, energy budget and a comparison with mussel larvae. J. Mar. Biol. Assoc. U.K. 65:759-783.

Crosby, M.P., C.J. Langdon and R.I.E. Newell. 1989. Importance of refractory plant material to the carbon budget of the oyster *Crassostrea virginica*. Mar. Biol. 100:343-352.

Davidson, K., K.J. Flynn and A. Cunningham. 1991. Relationships between photopigments, cell carbon, cell nitrogen and growth rate for a marine nanoflagellate. J. Exp. Mar. Biol. Ecol. 153:87-96.

Davies, C.N. 1973. Air Filtration. Academic Press, London.

Dral, A.D.G. 1967. The movements of the latero-frontal cilia and the mechanism of particle retention in the mussel (*Mytilus edulis* L.). Neth. J. Sea Res. 3:391-422.

Dwivedy, R.C. 1973. A study of chemo-receptors on labial palps of the American oyster using microelectrodes. Proc. Natl. Shellfish. Assoc. 63:20-26.

Eckman, J.E., C.H. Peterson and J.A. Cahalan. 1989. Effects of flow speed, turbulence, and orientation on growth of juvenile bay scallops *Argopecten irradians concentricus* (Say). J. Exp. Mar. Biol. Ecol. 132:123-140.

Elston. R. 1980. Functional anatomy, histology and ultrastructure of the soft tissues of the larval American oyster *Crassostrea virginica*. Proc. Natl. Shellfish. Assoc. 70:65-93.

Epifanio, C.E. 1982. Phytoplankton and yeast as foods for juvenile bivalves: a review of research at the University of Delaware. Pages 292-304 in G.D. Pruder, C.J. Langdon and D.E. Conklin (eds.). Second International Conference on Aquaculture Nutrition: Biochemical and Physiological Approaches to Shellfish Nutrition. Louisiana State University, Baton Rouge.

Epifanio, C.E. and J. Ewart. 1977. Maximum ration of four algal diets for the oyster *Crassostrea virginica* Gmelin. Aquaculture 11:13-29.

Erdmann, W. 1935. Untersuchungen uber die Lebensgeschichte der Auster. Nr. 5. Uber die Entwicklung und die Anatomie der "ansatzreifen" Larve von *Ostrea edulis* mit Bermerkungen uber die Lebensgeschichte der Auster. Wiss. Meeresunters., Abt. Helgoland. 19:1-25.

Famme, P., H.U. Riisgard and C.B. Jørgensen. 1986. On direct measurement of pumping rates in the mussel *Mytilus edulis*. Mar. Biol. 92:323-327.

Foster-Smith, R.L. 1975a. The effect of concentration of suspension on the filtration rates and pseudofaecal production for *Mytilus edulis* L., *Cerastoderma edule* (L.) and *Venerupis pullastra* (Montagu). J. Exp. Mar. Biol. Ecol. 17:1-22.

Foster-Smith, R.L. 1975b. The role of mucus in the mechanism of feeding in three filter-feeding bivalves. Proc. Malac. Soc. Lond. 41:571-588.

Foster-Smith, R.L. 1976. Some mechanisms for the control of pumping activity in bivalves. Mar. Behav. Physiol. 4:41-60.

Foster-Smith, R.L. 1978. The function of the pallial organs of bivalves in controlling ingestion. J. Moll. Stud. 44:83-99.

Frechette, M., C.A. Butman and W.R. Geyer. 1989. The importance of boundary-layer flow processes in supplying phytoplankton to the benthic suspension feeder, *Mytilus edulis* L. Limnol. Oceanogr. 34:19-36

Fretter, V. and M.C. Montgomery. 1968. The treatment of food by prosobranch veligers. J. Mar. Biol. Assoc. U.K. 48:499-520.

Fritz, L.W., R.A. Lutz, M.A. Foote, C.L. Van Dover and J.W. Ewart. 1984. Selective feeding and grazing rates of oyster (*Crassostrea virginica*) larvae on natural phytoplankton assemblages. Estuaries 7:513-518.

Gabbott, P.A. and D.L. Holland. 1973. Growth and metabolism of *Ostrea edulis* larvae. Nature 241:475-476.

Gallager, S.M. 1988. Visual observations of particle manipulation during feeding in larvae of a bivalve mollusc. Bull. Mar. Sci. 43:344-365.

Gallager, S.M. and R. Mann. 1986. Growth and survival of larvae of *Mercenaria mercenaria* (L.) and *Crassostrea virginica* (Gmelin) relative to broodstock conditioning and lipid content of eggs. Aquaculture 56:105-121.

Gallager, S.M., C.J. Langdon, L. Davis, and D.K. Stoecker. 1991. High-speed video analysis of particle capture by mollusc larvae (Abstract). J. Shellfish Res. 10:282.

Galtsoff, P.S. 1926. New methods to measure the rate of flow produced by the gills of oysters and other molluscs. Science 63:233-234.

Galtsoff, P.S. 1964. The American oyster *Crassostrea virginica* (Gmelin). Fish. Bull. 64:1-480.

Gerritsen, J. and K.G. Porter. 1982. The role of surface chemistry in filter feeding by zooplankton. Science 216: 1225-1227.

Gerritsen, J., K.G. Porter and J.R. Strickler. 1988. Not by sieving alone: Observations of suspension feeding in *Daphnia*. Bull. Mar. Sci. 43:366-376.

Gibbons, I.R. 1961. The relationship between the fine structure and direction of beat in gill cilia of a lamellibranch mollusc. J. Biophys. Biochem. Cytol. 11:179-205.

Gilmour, T.H.J. 1986. Streamlines and particle paths in the feeding mechanisms of larvae of the sea urchin *Lytechinus pictus* Verill. J. Exp. Mar. Biol. Ecol. 95:27-36.

Grizzle, R.E., R. Langan, and W.H. Howell. 1992. Growth responses of suspension-feeding bivalve molluscs to changes in water flow: differences between siphonate and nonsiphonate taxa. J. Exp. Mar. Biol. Ecol. 162: 213-228.

Grizzle, R.E. and R.A. Lutz. 1989. A statistical model relating horizontal seston fluxes and bottom sediment characteristics to growth of *Mercenaria mercenaria*. Mar. Biol. 102:95-105.

Grizzle, R.E. and P.J. Morin. 1989. Effect of tidal currents, seston and bottom sediments on growth of *Mercenaria mercenaria*: results of a field experiment. Mar. Biol. 102:85-93.

Haven, D.S. and R. Morales-Alamo. 1966. Aspects of biodeposition by oysters and other invertebrate filter feeders. Limnol. Oceanogr. 11:487-498.

Haven, D.S. and R. Morales-Alamo. 1970. Filtration of particles from suspension by the American oyster *Crassostrea virginica*. Biol. Bull. 139:248-264.

Hickman R.W. and L.D. Gruffydd. 1971. The histology of the larva of *Ostrea edulis* during metamorphosis. Pages 281 - 294 in D.J. Crisp (ed.). Fourth European Marine Biology Symposium, Cambridge University Press, Cambridge.

Higgins, P.J. 1980a. Effects of food availability on the valve movements and feeding behavior of juvenile *Crassostrea virginica* (Gmelin). I. Valve movements and periodic activity. J. Exp. Mar. Biol. Ecol. 45:229-244.

Higgins, P.J. 1980b. Effects of food availability on the valve movements and feeding behavior of juvenile *Crassostrea virginica* (Gmelin). II. Feeding rates and behavior. J. Exp. Mar. Biol. Ecol. 46:17-27.

Hildreth, D.I. 1976. The influence of water flow rate on pumping rate in *Mytilus edulis* using a refined direct measurement apparatus. J. Mar. Biol. Assoc. U.K. 56:311-319.

Hildreth, D.I. and D.J. Crisp. 1976. A corrected formula for calculation of filtration rate of bivalve molluscs in an experimental flowing system. J. Mar. Biol. Assoc. U.K. 56: 111-120.

Holland, D.L. 1978. Lipid reserves and energy metabolism in the larvae of benthic marine invertebrates. Pages 85-123 in D.C. Malins and J.R. Sargent (eds.). Biochemical and Biophysical Perspectives in Marine Biology. Academic Press, New York.

Holland, D.L. and B.E. Spencer. 1973. Biochemical changes in fed and starved oysters *Ostrea edulis* L. during larval development and growth, metamorphosis and early spat growth. J. Mar. Biol. Assoc. U.K. 53:287-298.

Hopkins, A.E. 1932a. Chemical stimulation by salts in the oyster *Ostrea virginica*. J. Exp. Zool. 61:13-28.

Hopkins, A.E. 1932b. Sensory stimulation of the oyster

Ostrea virginica by chemicals. U.S. Bur. Fish. Bull. 47:249-261.

Incze, L.S., R.A. Lutz and E. True. 1981. Modeling carrying capacities for bivalve molluscs in open, suspended-culture systems. J. World Maric. Soc. 12:143-155.

Jones, H.D., O.G. Richards and S. Hutchinson. 1990. The role of ctenidial abfrontal cilia in water pumping in *Mytilus edulis* L. J. Exp. Mar. Biol. Ecol. 143:15-26.

Jordan, S.J. 1987. Sedimentation and remineralization associated with biodeposition by the American oyster *Crassostrea virginica* (Gmelin). Doctoral dissertation. University of Maryland, College Park. 200 pp.

Jørgensen, C.B. 1966. Biology of Suspension Feeding. Pergamon Press, Oxford. 357pp.

Jørgensen, C.B. 1975. On gill function in the mussel *Mytilus edulis.* Ophelia 13:187-232.

Jørgensen, C.B. 1976. Growth efficiencies and factors controlling size in some mytilid bivalves, especially *Mytilus edulis* L.: A review and interpretation. Ophelia 15:175-192.

Jørgensen, C.B. 1981a. A hydromechanical principle for particle retention in *Mytilus edulis* and other ciliary suspension feeders. Mar. Biol. 61:277-282.

Jørgensen, C.B. 1981b. Feeding and cleaning mechanisms in the suspension feeding bivalve *Mytilus edulis.* Mar. Biol. 65:159-163.

Jørgensen, C.B. 1982. Fluid mechanics of the mussel gill: The lateral cilia. Mar. Biol. 70:275-281.

Jørgensen, C.B. 1983. Fluid mechanical aspects of suspension feeding. Mar. Ecol. Prog. Ser. 11:89-103.

Jørgensen, C.B. 1989. Water processing in ciliary feeders, with special reference to the bivalve filter pump. Comp. Biochem. Physiol. 94A:383-394.

Jørgensen, C.B. 1990. Bivalve Filter Feeding: Hydrodynamics, Bioenergetics, Physiology, and Ecology. Olsen and Olsen, Fredensborg, Denmark. 140 pp.

Jørgensen, C.B., P. Famme, H.S. Kristensen, P.S. Larsen, F. Møhlenberg and H.U. Riisgard. 1986. The bivalve pump. Mar. Ecol. Prog. Ser. 34:69-77.

Jørgensen, C.B., P.S. Larsen, F. Møhlenberg and H.U. Riisgard. 1988. The mussel pump: properties and modelling. Mar. Ecol. Prog. Ser. 45:205-216.

Kiørboe, T. and F. Møhlenberg. 1981. Particle selection in suspension-feeding bivalves. Mar. Ecol. Prog. Ser. 5:291-296.

Kiørboe, T., F. Møhlenberg and O. Nøhr. 1981. Effect of suspended bottom material on growth and energetics in *Mytilus edulis.* Mar. Biol. 61:283-288.

Kirby-Smith, W.W. 1972. Growth of the bay scallop: the influence of experimental water currents. J. Exp. Mar. Biol. Ecol. 8:7-18.

Knight-Jones, E.W. 1954. Relations between metachronism and the direction of ciliary beat in the metazoa. Quart. J. Microscop. Sci. 95:503-521.

LaBarbera, M. 1978. Particle capture by a Pacific brittle star: Experimental test of the aerosol suspension feeding model. Science 201:1147-1148.

LaBarbera, M. 1984. Feeding currents and particle capture mechanisms in suspension feeding animals. Amer. Zool. 24:71-84.

Langdon, C.J. and R.I.E. Newell. 1990. Utilization of detritus and bacteria as food sources by two bivalve suspension-feeders, the oyster *Crassostrea virginica* and the mussel *Geukensia demissa.* Mar. Ecol. Prog. Ser. 58:299-310.

Langdon, C.J., V.L. Shaffer, S.M. Gallager and D.K. Stoecker 1991. Surface properties of particles effect their selection by clam larvae. (Abstract) J. Shellfish. Res. 10:517.

Leal, L.G. 1980. Particle motion in a viscous fluid. Ann. Rev. Fluid Mech. 12:435-476.

Le Pennec, M., P.G. Beninger and A. Herry. 1988. New observations of the gills of *Placopecten magellanicus* (Mollusca:Bivalvia), and implications for nutrition. II. Internal anatomy and microanatomy. Mar. Biol. 98:229-237.

Loosanoff, V.L. 1949. On the food selectivity of oysters. Science 110:122.

Loosanoff, V.L. and C.A. Nomejko. 1946. Feeding of oysters in relation to tidal stages and to periods of light and darkness. Biol. Bull. 90:244-264.

Lund, E.J. 1957a. A quantitative study of clearance of a turbid medium and feeding by the oyster. Publ. Inst. Mar. Sci. Univ. Texas 4:296-312.

Lund, E.J. 1957b. Self-silting, survival of the oyster as a closed system, and reducing tendencies of the environment of the oyster. Publ. Inst. Mar. Sci. Univ. Texas 4:313-319.

Mackie, G. 1969. Quantitative studies of feeding in the oyster, *Crassostrea virginica* (Abstract). Proc. Natl. Shellfish. Assoc. 59:6-7.

Manahan, D.T. 1989. Amino acid fluxes to and from sea-

water in axenic veliger larvae of a bivalve (*Crassostrea gigas*). Mar. Ecol. Prog. Ser. 53:247-255.

Manahan, D.T. and D.J. Crisp. 1983. Autoradiographic studies on the uptake of dissolved amino acids from seawater by bivalve larvae. J. Mar. Biol. Assoc. U.K. 63:673-682.

Manzi, J.J., N.H. Hadley and M.B. Maddox. 1986. Seed clam, *Mercenaria mercenaria*, culture in an experimental-scale upflow nursery system. Aquaculture 54: 301-311.

Menzel, R.W. 1955. Some phases of the biology of *Ostrea equestris* Say and a comparison with *Crassostrea virginica* (Gmelin). Publ. Inst. Mar. Sci. Univ. Texas 4:69-153.

Millar, R.H. 1955. Notes on the mechanism of food movement in the gut of the larval oyster *Ostrea edulis*. Quart J. Microsc. Sci. 96:539-544.

Møhlenberg, F. and H.U. Riisgard. 1978. Efficiency of particle retention in 13 species of suspension feeding bivalves. Ophelia 17:239-246.

Moore, H.J. 1971. The structure of the latero-frontal cirri on the gills of certain lamellibranch molluscs and their role in suspension feeding. Mar. Biol. 11:23-27.

Morton, B. 1983. Feeding and digestion in Bivalvia. Pages 65-147 in A.S.M. Saleuddin and K.M. Wilbur (eds.). The Mollusca, Volume 5. Academic Press, New York.

Nelson, T.C. 1923a. On the feeding habits of oysters. Proc. Soc. Exper. Biol. Med. 21:90-91.

Nelson, T.C. 1923b. The mechanism of feeding in the oyster. Proc. Soc. Exper. Biol. Med. 21:166-168.

Nelson, T.C. 1938. The feeding mechanism of the Oyster. I. On the pallium and the branchial chambers of *Ostrea virginica*, *O. edulis*, and *O. angulata*. J. Morph. 63:1-61.

Nelson, T.C. 1960. The feeding mechanism of the Oyster. II. On the gills and palps of *Ostrea edulis*, *Crassostrea virginica* and *C. angulata*. J. Morph. 107:163-191.

Newell, R.C. 1979. Biology of Intertidal Animals. Marine Ecological Surveys Ltd., Faversham, U.K. 781 pp.

Newell, R.I.E. and S.J. Jordan. 1983. Preferential ingestion of organic material by the American oyster, *Crassostrea virginica*. Mar. Ecol. Prog. Ser. 13:47-53.

Owen, G. 1974. Studies on the gill of *Mytilus edulis*: the eulatero-frontal cirri. Proc. R. Soc. Lond. B. 187:83-91.

Owen, G. 1978. Classification and the bivalve gill. Phil. Trans. R. Soc. Lond. B. 284:377-386.

Owen, G. and J.M. McCrae. 1976. Further studies on the latero-frontal tracts of bivalves. Proc. R. Soc. Lond. B. 194:527-544.

Palmer, R.E. 1980. Behavioral and rhythmic aspects of filtration in the bay scallop, *Argopecten irradians concentricus* (Say), and the oyster, *Crassostrea virginica* (Gmelin). J. Exp. Mar. Biol. Ecol. 45:273-295.

Palmer, R.E. and L.G. Williams. 1980. Effect of particle concentration on filtration efficiency of the bay scallop *Argopecten irradians* and the oyster *Crassostrea virginica*. Ophelia 19:163-174.

Powell, E.N., E.E. Hofmann, J.M. Klinck, and S.M. Ray. 1992. Modelling oyster populations. I. A commentary on filtration rate. Is faster always better? J. Shellfish Res. 11:387-398.

Purchon, R.D. 1955. The structure and function of the British Pholadidae (rock-boring lamellibranchia). Proc. Zool. Soc. Lond. 124:859-911.

Purchon, R.D. 1978. An analytical approach to a classification of the Bivalvia. Phil. Trans. R. Soc. Lond. B. 284:425-436.

Purchon, R.D. 1987. The stomach in the Bivalvia. Phil. Trans. R. Soc. Lond. B. 316:183-276.

Ribelin, B.W. and A. Collier. 1977. Studies on the gill ciliation of the American oyster *Crassostrea virginica* (Gmelin). J. Morph. 151:439-450.

Riisgard, H.U. 1988. Efficiency of particle retention and filtration rate in 6 species of northeast American bivalves. Mar. Ecol. Prog. Ser. 45:217-223.

Riisgard, H.U., A. Randlov and P.S. Kristensen. 1980. Rates of water processing, oxygen consumption and efficiency of particle retention in veligers and young post-metamorphic *Mytilus edulis*. Ophelia 19:37-47.

Robinson, W.E. 1981. Quantification of ingestion by *Mercenaria mercenaria* (L) veligers feeding on mixed suspensions of inert material and algae using microspectrofluorimetry. J. Moll. Stud. Suppl. 12A:167-171.

Rubenstein, D.E. and M.A.R. Koehl. 1977. The mechanisms of filter-feeding: some theoretical considerations. Amer. Nat. 111:981-994.

Rudall, K.M. 1955. The distribution of collagen and chitin. Symp. Soc. Exp. Biol. 9:49-71.

Satir, P. 1965. Studies on cilia. II. Examination of the distal region of the ciliary shaft and the role of filaments in motility. J. Cell. Biol. 26:805-834.

Satir, P. 1968. Studies on cilia. III. Further studies on the cilium tip and a sliding filament model of ciliary motility. J. Cell. Biol. 39:77-94.

Shumway, S.E., T.L. Cucci, R.C. Newell and C.M. Yentsch. 1985. Particle selection, ingestion, and absorption in filter feeding bivalves. J. Exp. Mar. Biol. Ecol. 91:77-92.

Silvester, N.R. 1988. Hydrodynamics of flow in *Mytilus* gills. J. Exp. Mar. Biol. Ecol. 120:171-182.

Silvester, N.R. and M.A. Sleigh. 1984. Hydrodynamic aspects of particle capture by *Mytilus*. J. Mar. Biol. Assoc. U.K. 64:859-879.

Sleigh, M.A. and E. Aiello. 1972. The movement of water by cilia. Acta Protozool. 11:265-277.

Solow, A.R. and S.M. Gallager. 1990. Analysis of capture efficiency in suspension feeding: application of nonparametric binary regression. Mar. Biol. 107:341-344.

Soniat, T.M., S.M. Ray and R.J. Zimmerman. 1984. Components of the seston and possible food available for oysters in Galveston Bay, Texas. Contr. Mar. Sci. 27:127-141.

Sprung, M. 1984. Physiological energetics of mussel larvae (*Mytilus edulis*). I. Shell growth and biomass. Mar. Ecol. Prog Ser. 17:283-293.

Strathmann, R.R. 1987. Larval feeding. Pages 465-550 in A.C. Giese, J.S. Pearse, and V.B. Pearse (eds.). Reproduction of Marine Invertebrates, Volume IX. General Aspects: Seeking Unity in Diversity. Blackwell Scientific, Palo Alto.

Strathmann, R.R., T.L. Jahn and J.R.C. Fonseca. 1972. Suspension feeding by marine invertebrate larvae: clearance of particles by ciliated bands of a rotifer, pluteus, and trochophore. Biol. Bull. 142:505-519.

Strathmann, R.R. and E. Leise. 1979. On feeding mechanisms and clearance rates of molluscan veligers. Biol. Bull. 157:524-535.

Tenore, K.R. and W.M. Dunstan. 1973. Comparison of feeding and biodeposition of three bivalves at different food levels. Mar. Biol. 21:190-195.

Vogel, S. 1994. Life in Moving Fluids: The Physical Biology of Flow. Second edition. Princeton University Press, Princeton.

Waller, T.R. 1981. Functional morphology and development of the veliger larvae of the European oyster *Ostrea edulis* Linne. Smithsonian Contrib. Zool. 328:1-70.

Walne, P.R. 1972. The influence of current speed, body size and water temperature on the filtration rate of five species of bivalves. J. Mar. Biol. Assoc. U.K. 52:345-374.

Ward, J.E., P.G. Beninger, B.A. MacDonald and R.J. Thompson. 1991. Direct observations of feeding structures and mechanisms in bivalve molluscs using endoscopic examination and video image analysis. Mar. Biol. 111:287-291.

Ward, J.E., H.K. Cassell, and B.A. MacDonald. 1992. Chemoreception in the sea scallop *Placopecten magellanicus* (Gmelin). I. Stimulatory effects of phytoplankton metabolites on clearance and ingestion rates. J. Exp. Mar. Biol. Ecol. 163:235-250.

Ward, J.E., R.I.E. Newell, R.J. Thompson and B.A. MacDonald. 1994. Endoscopic observations of particle capture and transport in the eastern oyster *Crassostrea virginica* Gmelin. Biol. Bull. 186:221-240

Ward, J.E. and N.M Targett. 1989. Influence of marine microalgal metabolites on the feeding behavior of the blue mussel *Mytilus edulis*. Mar. Biol. 101:313-321.

Webb, K.L. and F.E. Chu. 1982. Phytoplankton as a food source for bivalve larvae. Pages 272-291 in G.D. Pruder, C.J. Langdon and D.E. Conklin (eds.). Second International Conference on Aquaculture Nutrition: Biochemical and Physiological Approaches to Shellfish Nutrition. Louisiana State University, Baton Rouge.

Widdows, J., P. Fieth and C.M. Worrall. 1979. Relationships between seston, available food and feeding activity in the common mussel *Mytilus edulis*. Mar. Biol. 50:195-207.

Widdows, J. and A.J. S. Hawkins. 1989. Partitioning of rate of heat dissipation by *Mytilus edulis* into maintenance, feeding, and growth components. Physiol. Zool. 62:764-784.

Widdows, J., R.I.E. Newell and R. Mann. 1989. Effects of hypoxia and anoxia on survival, energy metabolism and feeding by oyster larvae (*Crassostrea virginica*). Biol. Bull. 177:154-166.

Wildish, D.J., D.D. Kristmanson, R.L. Hoar, A.M. DeCoste, S.D. McCormick and A.W. White. 1987. Giant scallop feeding and growth responses to flow. J. Exp. Mar. Biol. Ecol. 113:207-220.

Williams, L.G. 1982. Mathematical analysis of the effects of particle retention efficiency on determination of filtration rate. Mar. Biol. 66:171-177.

Wilson, J.H. 1979. Observations on the grazing rates and growth of *Ostrea edulis* L. larvae when fed algal cultures of different ages. J. Exp. Mar. Biol. Ecol. 38: 187-199.

Wilson, J.H. 1980. Particle retention and selection by larvae and spat of *Ostrea edulis* in algal suspensions. Mar. Biol. 57:135-145.

Winter, J.E. 1973. The filtration rate of *Mytilus edulis* and its dependence on algal concentration, measured by a continuous automatic recording apparatus. Mar. Biol. 22:317-328.

Winter, J.E. 1978. A review on the knowledge of suspension feeding in lamellibranchiate bivalves, with special reference to artificial aquaculture systems. Aquaculture 13: 1-33.

Yonge, C.M. 1926. Structure and physiology of the organs of feeding and digestion in *Ostrea edulis.* J. Mar. Biol. Assoc. U.K. 14:295-386.

Chapter 6

Digestion and Nutrition in Larvae and Adults

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INTRODUCTION

In the period since Galtsoff's (1964) monograph on the eastern oyster *Crassostrea virginica*, our understanding of bivalve digestion and nutrition has increased significantly because of the availability of new research techniques. For example, modern biochemical techniques have been applied to the study of the enzymology of bivalve digestion (see review by Reid 1982). Advances in our understanding of the ultrastructure of the digestive system were made possible by the use of the electron microscope (Owen 1970; Pal 1971, 1972). In the 1980s, the use of microencapsulated diets (see review by Langdon et al. 1985) and application of high performance liquid chromatography techniques in studying uptake of dissolved organic matter (Manahan et al. 1982) have enhanced our understanding of the nutrition of bivalve molluscs.

Here we review current knowledge of digestion and nutrition of *C. virginica*, as well as information provided by Galtsoff's (1964) monograph on this species. Reviews that provide information on general bivalve digestion and nutrition include those by Purchon (1968), Owen (1974), Epifanio (1982), Reid (1982), Webb and Chu (1982), Bayne (1983), Bayne and Newell (1983), and Morton (1983).

ADULT OYSTERS

Stomach

Ingested particles are transported through the mouth and short (ca. 5 mm in adult oysters) esophagus into the stomach (Fig. 1). Mucous threads and mucous-coated food particles have been observed on the head of the crystalline style dissected from bivalve molluscs. Some researchers thought that the rotation of the style pulled mucous-wrapped strings of particles into the stomach (Nelson 1933; Owen 1955; Purchon 1968). In contrast, others suggested that cilia of the esophagus and stomach epithelium were more important in bringing food into the stomach (Reid 1965). A detailed histological examination by Beninger et al. (1991) of five species of bivalves, including *Crassostrea virginica*, indicated that particles in the esophagus were bound in mucous and transported by ciliary action.

In contrast, Kiørboe and Møhlenberg (1981) observed that particles in the esophagus of the blue mussel *Mytilus edulis* were generally free and not trapped in mucus. In support of this latter finding, Ward et al. (1994) endoscopically observed food processing in living eastern oysters and found that algal cells were transported individually through the mouth and esophagus. Ward et al. (1994) also observed that particles in the stomachs of living eastern oysters appeared to be suspended in non-viscous mucus; however, mucous viscosity greatly increased and particles became bound in mucous strings in stomach samples from dissected oysters. This latter observation supports the hypothesis of Newell and Jordan (1983) that mucous viscosity in undisturbed oysters is reduced by the mechanical action of cilia associated with epithelial surfaces of the oyster's feeding and digestive system.

The stomach is irregularly shaped, with discernible anterior and posterior chambers (Fig. 2). A blind-ending pouch, the caecum, arises from the anterior chamber. Food particles are initially sorted in the caecum according to size (Galtsoff 1964). It remains to be determined if sorting mechanisms also select particles on the basis of their surface or chemical characteristics. An intestinal groove runs along the wall of the caecum and transports rejected material from the caecum and stomach to the opening of the mid-gut.

Purchon (1957, 1987) classified bivalve molluscs into five main divisions on the basis of position of the intestinal groove and associated typhlosoles in relation to duct openings of the digestive diverticula. The eastern oyster is classified as being a member of the Gastrotriteia (Type III stomach). The diagnostic feature of this stomach type is that the tongue of the

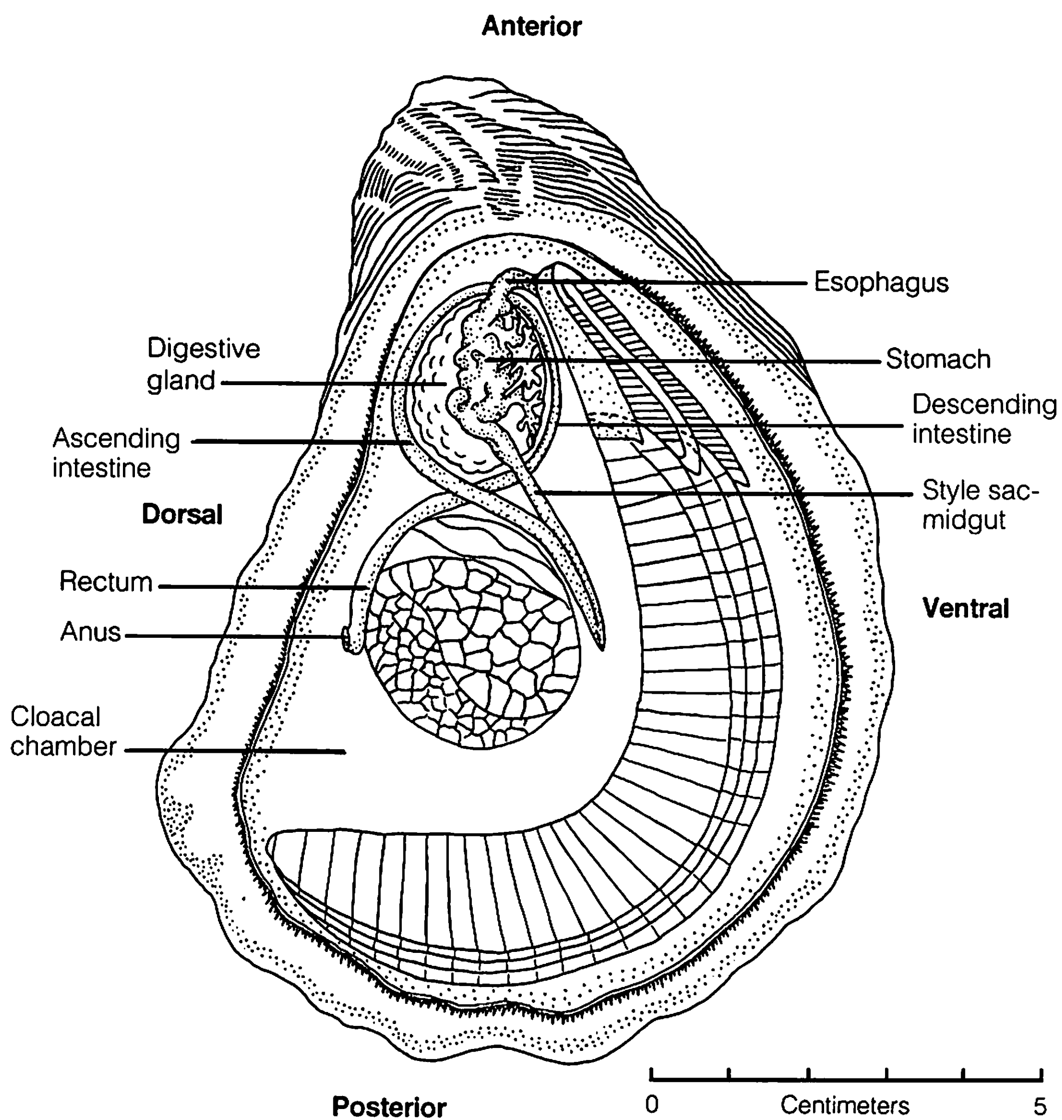


Figure 1. Digestive system of *Crassostrea virginica.* Parenchymal tissues have been removed to expose the stomach and intestine. Redrawn from Galtsoff (1964).

major typhlosole and the intestinal groove penetrate the food-sorting caecum at its apex (Purchon 1957, 1987).

Mixing of food particles within the stomach cannot occur by peristalsis because there is no well developed muscular layer surrounding the stomach. However, R.G.B. Reid (University of Victoria, Canada, pers. comm.) has observed undulating contractions of the stomach walls of many bivalve species which he suggests have the effect of exposing rejection grooves to large-sized particulate material that is then rejected from the stomach. Ciliated ridged areas on the stomach wall of the anterior chamber are involved in sorting of particles as well as mixing of particles with digestive enzymes. In the posterior stomach chamber, further mixing of ingested particles and gastric juices occurs by the action of the ciliated epithelium and rotation of the crystalline style.

Crystalline Style and Style Sac

The crystalline style is a flexible rod of muco-protein that protrudes from the style sac and impinges onto the gastric shield that covers the left ventral wall of the posterior stomach chamber (Fig. 3; Shaw and Battle 1957; Halton and Owen 1968; McQuiston 1970). The style is made up of numerous concentric, longitudinally-orientated lamellae surrounding an inner core of granular material (Dean 1958; Kristensen 1972a). Doyle (1966) found that about 50% of style organic matter of the cockle, *Cardium edule,* and soft clam, *Mya arenaria,* was proteinaceous. Polymers of neutral sugars (mannose, galactose, fucose, xylose, deoxyribose, glucose) and amino sugars (glucosamine, galactosamine) were also present in the carbohydrate fraction of the styles of these bivalve species.

Bernard (1973) reported that the style of the Pacific oyster, *Crassostrea gigas,* was formed along the entire length of the style sac. The epithelium of the style sac of oysters is made up of three cell types (A, B, and C; Bernard 1973; Mathers 1973a). According to Bernard (1973), type-A cells are the most common in the style sac of *C. gigas* and consist of ciliated cells with basal nuclei. Type-B cells are elongated with fewer and longer cilia than those of the type-A cells, and are found overlying the typhlosoles on either side of the communication between style sac and midgut. Type-C cells are similar in appearance to

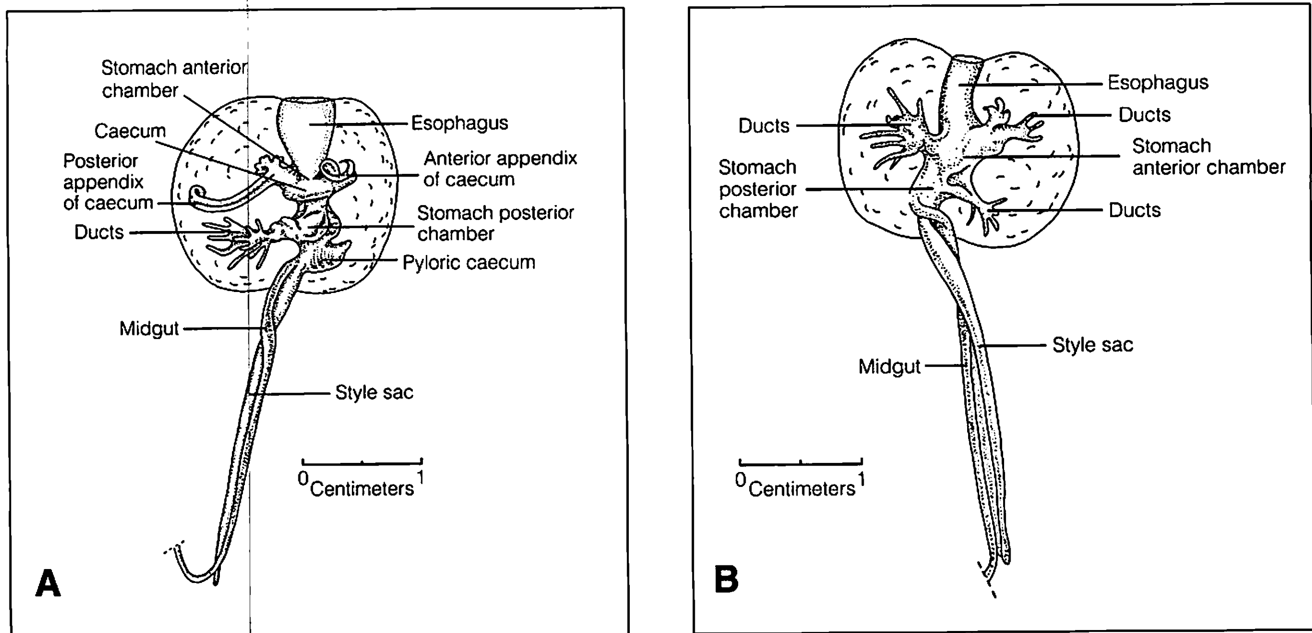


Figure 2. Digestive system of *Crassostrea virginica* drawn from latex molds and viewed from (A) left side and (B) right side. Esophagus is slightly distended by latex injection process. Redrawn from Galtsoff (1964).

type-A cells and Bernard (1973) suggests that they may in fact be the same cell type.

Histochemical, PAS (periodic acid schiff)-staining of style and mid-gut sections of Pacific oysters showed pronounced secretion of mucopolysaccharides by type-B cells of the typhlosole (Fig. 4), indicating that these cells were responsible for style formation (Bernard 1973). Cell types A and C cause the style to rotate on its longitudinal axis in a clockwise direction, as seen from the stomach, and cilia may also advance the style into the stomach (Nelson 1933; Giusti 1970). The action of the rotating style against the gastric shield is thought to facilitate digestion by mechanically breaking down fragile algal cells (Nelson 1925; Yonge 1926a; Owen 1974) and mixing ingested food with gastric enzymes. In addition, Kristensen (1972a) reported dissolved style material was effective in emulsifying lipids and consequently increasing the surface area of lipid droplets exposed to extracellular lipases and esterases.

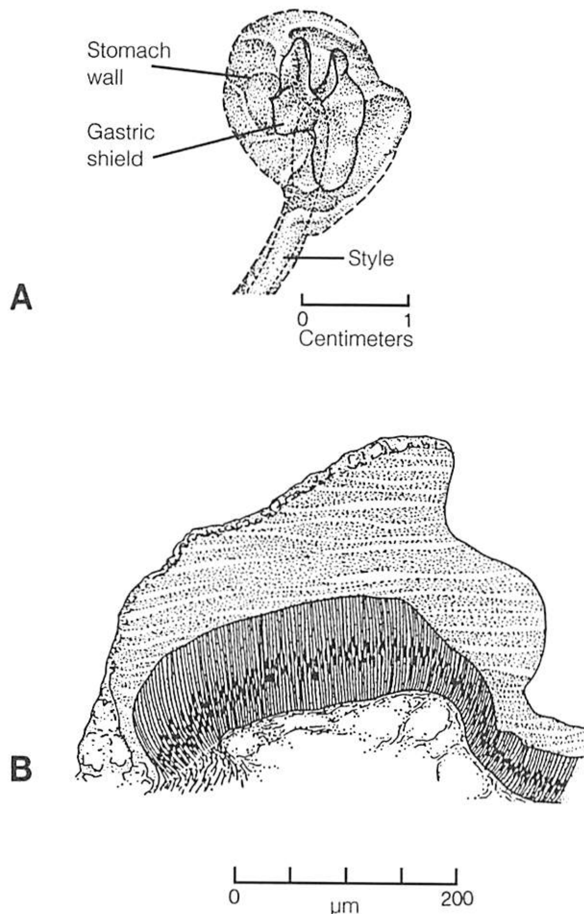


Figure 3. Gastric shield of *Crassostrea virginica.* (A) Position of the shield and crystalline style. Rod-shaped outline of the style shown by dotted line. (B) Cross-section of shield at its thickest point. From Galtsoff (1964).

The bivalve style is not a permanent structure but has been reported to vary in size according to environmental and gastric conditions and perhaps endogenous rhythms. Bernard (1973) estimated that styles of subtidal *C. gigas* were completely renewed every 8 to 9 h at 16°C. Styles of intertidal eastern oysters have been reported to dissolve on the ebbing tide (Nelson 1918, 1925). According to Langton and Gabbott (1974), maximum length and protein content of styles of intertidal European flat oysters, *Ostrea edulis,* occurred 3 h after high tide and decreased to a minimum 3 h after low tide.

There is some controversy over the mechanism responsible for fluctuations in style size. Mathers (1974) reported that as *in vitro* pH increased from 4.0 to 7.8, the time required for complete dissolution of the style of *O. edulis* decreased from 170 min to 55 min; however, the style remained intact for 4 to 5 d at pH 9.0. In contrast, Morton (1977) found that *in vivo* style volume of *C. gigas* was not correlated with pH of stomach fluids (measured pH range: 5.8 to 7.7) and he suggested that style dissolution was due to the action of proteolytic enzymes associated with spherules released from the diverticula. Accelerated *in vitro* dissolution of bivalve styles by diverticula extracts has been reported by Yonge (1926a) and Kristensen (1972a). Furthermore, Reid and Sweeney (1980) found that the crystalline style of *C. gigas* was susceptible to proteolytic digestion by trypsin; however, although style reconstitution could be inhibited by gastric proteases, gastric proteolytic activity was considered by Reid and Sweeney (1980) to be insufficient to cause complete style dissolution.

Extracellular digestion in *C. virginica* reduces particle size and releases soluble material from ingested food material, hence facilitating absorption and intracellular digestion by the diverticula. Dean (1958) observed that cells of the alga *Cryptomonas* sp.

disintegrated on contact with the style of *C. virginica*, whereas cells of *Pavlova* (*Monochrysis*) sp. were only immobilized and cells of *Isochrysis* sp. were unaffected. Nelson (1933) observed that stomach juices of *C. virginica* caused bursting of the integument of nematodes, releasing material that was small enough to be endocytosed by diverticular cells. It was not clear whether these effects of style and gastric extracts on ingested living organisms were due to physical factors, such as reductions in surface tension (Kristensen 1972a), or to enzymatic digestion.

Enzymatic breakdown of cell walls of ingested living organisms is an important initial step in digestion. Cellulose is a common structural component of algal cell walls. It is, therefore, not surprising that *C. virginica* possesses extracellular cellulolytic activity (Lucas and Newell 1984; Brock et al. 1986; Newell and Langdon 1986; Brock and Kennedy 1992) that presumably facilitates digestion of algal cells in the stomach. Newell and Langdon (1986) demonstrated that the style of *C. virginica* possessed C_x cellulase (poly-β glucosidase) activity, enabling the oyster to break down non-crystalline cellulose into soluble oligomers; however, these authors reported that neither C_1 cellulase, capable of solubilizing crystalline cellulose nor cellobiase (β-glucosidase) were present in style extracts (Table 1). Newell and Langdon (1986) also reported that C_x cellulase activity was endogenous and was not dependent on the presence of a bacterial gut flora.

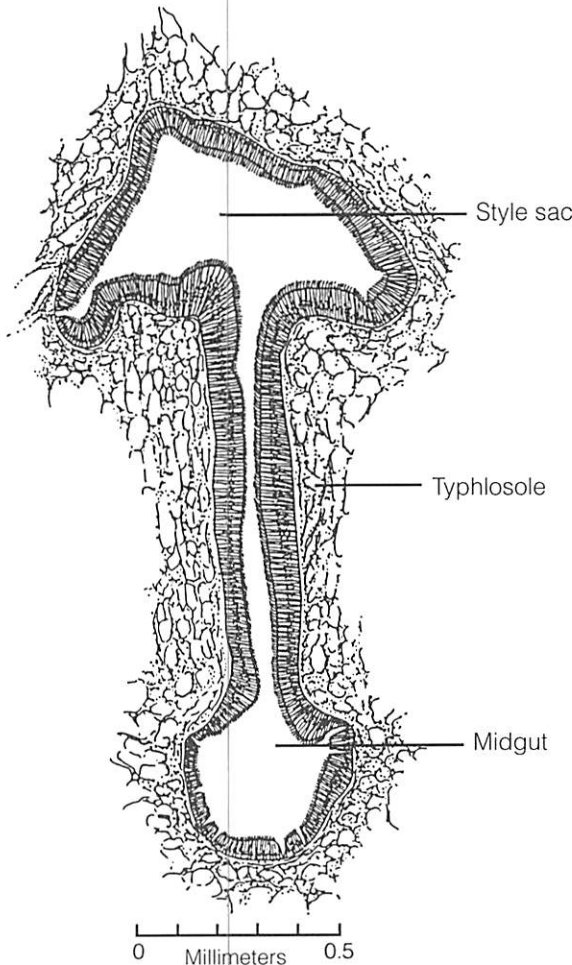


Figure 4. Transverse section of the style sac and midgut. Crystalline style is absent. From Galtsoff (1964).

Crosby and Reid (1971) compared poly-β glucosidase activities of a variety of bivalve species and found that suspension-feeding species, such as *C. gigas* and *Mytilus edulis*, possessed stronger activities than deposit feeders, such as Whitesand macoma, *Macoma secta*, and the nuculanid, *Yoldia ensifera*. In contrast, Brock and Kennedy (1992) did not observe any significant differences among the poly-β glucosidase activities of style extracts of the suspension-feeders *C. virginica*, *Geukensia demissa*, and *Rangia cuneata* and the deposit feeders *Macoma balthica* and *Macoma mitchelli*.

Chitin is an important structural carbohydrate of some diatoms (Jeuniaux 1982); for example, McLachlan et al. (1965) reported that up to 38% of the total dry organic weight of the diatom, *Thalassiosira fluviatilis*, was composed of chitin. Smucker and Wright (1984, 1986) found high chitinase activity (which depolymerizes chitin to the dimer chitobiose) in extracts from the crystalline style of C. *virginica*. Smucker and Wright (1986) reported that maximum chitinase activity of style extracts occurred at pH 4.8 and at 34°C. However, pH values of the style sac of *Ostrea edulis* and the Portuguese oyster, *C. angulata*, are higher than pH 4.8 (Mathers 1974), suggesting that the pH of the style sac of *C. virginica* may also be higher than 4.8, the optimum pH for chitinase activity. Birkbeck and McHenery (1984) reported that style chitinase of *M. edulis* was specific for chitin

Table 1. Digestive enzymes of oyster species, *Crassostrea angulata, C. gigas, C. virginica,* and *Ostrea edulis* in various organs, tissues, or in stomach contents. D (diverticula); H (hemocytes); M (midgut); Sc (stomach contents); St (style); SS (style sac); Sw (stomach wall).

Enzyme	Species	Organ	Authority
		Polymerases	
Amylase	*C. virginica*	St	Lucas and Newell 1984
	C. virginica	D	Brock et al. 1986
	O. edulis	St,D	Yonge 1926a
	O. edulis	H	Takatsuki 1934
	O. edulis	St,Sc,Sw,D,M	Mathers 1973b
	O. edulis	St	Langton and Gabbott 1974
C_x cellulase	*C. virginica*	St	Lucas and Newell 1984
(Poly-Glucosidase)	*C. virginica*	D	Brock et al. 1986
	C. virginica	St	Newell and Langdon 1986
	C. virginica	St,Sc,Sw,D	Mathers 1973b
Chitinase	*C. virginica*	St	Smucker and Wright 1984,1986
Laminarinase	*C. virginica*	D	Brock et al. 1986
	C. gigas	D	Onishi et al. 1985
Protease	*C. gigas*	Sc,D	Reid 1978
	O. edulis	D	Yonge 1926a
	O. edulis	H	Takatsuki 1934
Xylanase	*C. gigas*	D	Onishi et al. 1985
Alginase	*C. gigas*	D	Onishi et al. 1985
Fucosidase	*C. gigas*	D	Onishi et al. 1985
		Oligomerases	
Acid phosphatase	*C. angulata*	St,Sw,D,M,H	Mathers 1973a
	O. edulis	St,Sw,D,M,H	Mathers 1973a
Alkaline phosphatase	*C. angulata*	SS,Sw,D,M,H	Mathers 1973a
	O. edulis	SS,Sw,D,M,H	Mathers 1973a
	C. virginica	D	Eble 1966
Arylamidase	*C. angulata*	SS,Sw,D,M,H	Mathers 1973a
	O. edulis	SS,Sw,D,M,H	Mathers 1973a
Cellobiase	*C. virginica*	D	Mayasich and Smucker 1986
	O. edulis	Sw,Sc,D,M	Mathers 1973b
Chitobiase	*C. virginica*	St,D	Mayasich and Smucker 1986
Dextrin-1, 6-glucosidase	*O. edulis*	Sw,Sc,D,M	Mathers 1973b
Fructosidase	*O. edulis*	D	Mathers 1973b
Galactosidase	*O. edulis*	D	Mathers 1973b
	O. edulis	Sc,D	Yonge 1926a
Galactosidase	*O. edulis*	St,Sw,Sc,D,M	Mathers 1973b
	O. edulis	H	Takatsuki 1934
Glucosidase	*O. edulis*	Sw,Sc,D,M	Mathers 1973b
	O. edulis	Sc,D	Yonge 1926a
	O. edulis	H	Takatsuki 1934
Glucosidase	*O. edulis*	D	Mathers 1973b
	O. edulis	H	Takatsuki 1934
Maltase	*C. virginica*	D	Mayasich and Smucker 1986
	O. edulis	Sw,Sc,D,M	Mathers 1973b
	O. edulis	H	Takatsuki 1934
	O. edulis	Sc,D	Yonge 1926a
Trehalase	*O. edulis*	Sw,D	Mathers 1973b
Esterase	*C. angulata*	Sw,SS,D,M,H	Mathers 1973a
	O. edulis	Sw,SS,D,M,H	Mathers 1973a
	C. virginica	D	Eble 1966
Lipase	*C. virginica*	St	George 1952
	O. edulis	D	Yonge 1926a
	O. edulis	H	Takatsuki 1934

and, unlike chitinases of some gastropod species, had no lysozyme activity.

Lysozymes break down bacterial cell walls by hydrolyzing peptidoglycan, present in cell walls of both Gram positive and Gram negative bacteria (Brock and Madigan 1988). McHenery et al. (1979) found lysozyme activity primarily in style and diverticula extracts of a variety of bivalve species and suggested that lysozyme could play a major role in the utilization of bacteria as a food source. In support of this hypothesis, McHenery and Birkbeck (1985) demonstrated using radiotracer techniques that several bivalve species, including *Ostrea edulis*, could digest *Escherichia coli*. Lysozyme activity was demonstrated in mucus collected from the gills of *C. virginica* (McDade and Tripp 1967), but its presence in the digestive system of oysters has not been reported; however, Crosby et al. (1990) reported that *C. virginica* digested and absorbed carbon and nitrogen from ingested cellulolytic bacteria with efficiencies of 52 and 57%, respectively, suggesting the presence of lysozymes associated with digestion in *C. virginica*.

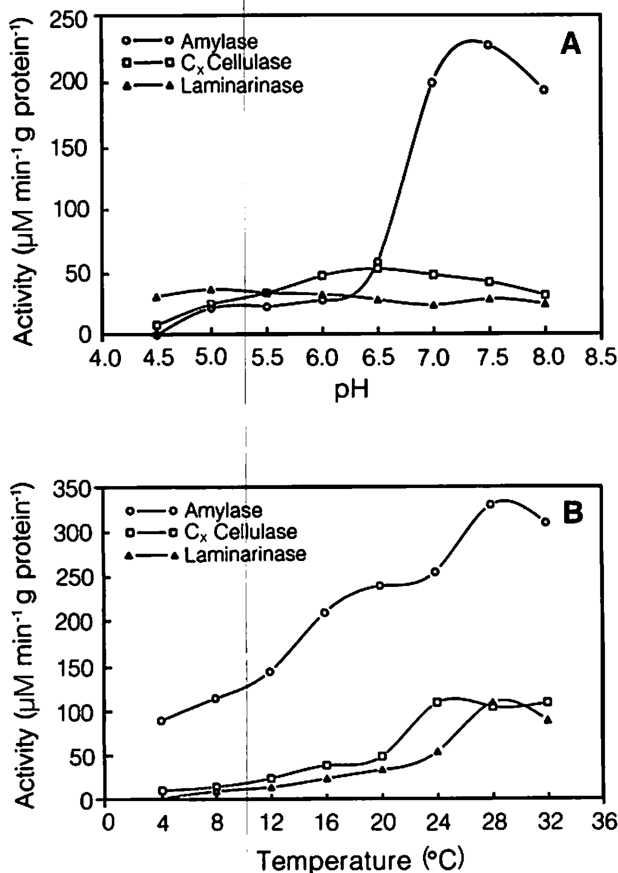


Figure 5. Effect of (A) pH and (B) temperature on the activity of carbohydrases from the diverticula of *Crassostrea virginica*. Effect of pH on enzyme activities measured at 18°C. Effect of temperature on enzyme activity measured at pH 7.0 (amylase), pH 6.5 (C_x cellulase), or pH 6.0 (laminarinase). Enzyme activity expressed as µM maltose equivalents released per min per g protein in the tissue homogenate. After Brock et al. (1986).

Alginic acid and its derivatives are structurally important in algae of the class Phaeophyceae. These polysaccharides are largely polymers of manuronic and guluronic acids containing 1,4 and possibly 1,3 linkages. Kristensen (1972b) reported that alginase activity was absent in style extracts of a variety of marine bivalve species and only weakly present in diverticula extracts. In contrast, Favorov and Vaskovsky (1971) found that style extracts of the mactrid clams, *Spisula sachalinensis* and *Mactra sulcatoria*, possessed strong alginase activities during the summer season. There are no reports of tests for alginase activity in *C. virginica*, although Onishi et al. (1985) reported weak alginase activity in diverticula extracts of *C. gigas*.

In addition to extracellular digestion of structural carbohydrates, enzymatic breakdown of many storage polysaccharides also occurs extracellularly in bivalves. Amylose, amylopectin and glycogen are 1,4-linked α-glucans that serve as common reserve polysaccharides in living organisms. The presence of amylase has been reported in style extracts of many bivalve species, including *C. virginica* (Lucas and Newell 1984; Table 1). Mathers (1973b) reported α-amylase activity in the epithelium and lumen of the stomach of *Ostrea edulis* and concluded that the stomach wall secreted amylase.

Maximum amylase activity of style extracts of *O. edulis* has been reported at pH values of 5.9 (Yonge 1926a) and 6.1 (Mathers 1973b). Mathers (1974) reported that extracellular pH values for the style and style sac of *O. edulis* varied between 5.5 and 7.2, which suggests that extracellular gastric pH is not always optimal for maximum amylase activity. However, the pH-activity peak for style amylase appears to

be broad for *O. edulis* and pH differences of ± 0.2 units from the optimum pH have little effect on amylase activity (Mathers 1973b). Maximum amylase activities of extracts of the diverticula of *C. virginica* occurred between pH 7.0 and 7.5 (Brock et al. 1986; Fig. 5).

Newell et al. (1980) extracted and fractionated amylases from the crystalline styles of black mussels (*Choromytilus meridionalis*) acclimated for 18 d to 8°C or 22°C. They found that four fractions were present in style preparations of both groups of mussels; however, specific amylase activities varied among fractions depending on acclimation temperature. Highest specific amylase activity was present in fraction IV of styles from mussels acclimated to 8°C whereas fraction I possessed the highest specific activity in styles from mussels acclimated to 22°C. Furthermore, the specific amylase activity of fraction IV isolated from mussels at 8°C was 3.6 times greater at an assay temperature of 8°C than at an assay temperature of 22°C, whereas fraction I from mussels acclimated at 22°C possessed a specific activity at 22°C that was about 2.9 times greater than that at 8°C. These results suggest that inducible digestive isoenzymes of bivalve species may be adapted to environmental conditions. No reported studies have been carried out on induction of digestive isoenzymes in oysters.

Amylase hydrolyses amylose to the dimer maltose; therefore, both amylase and maltase are necessary for the complete breakdown of amylose to glucose. The principal product of amylopecten hydrolysis is isomaltose (glucose-1, 6-α-glucoside) which is broken down by dextrin-1,6-glucosidase to produce glucose. Interestingly, Mathers (1973b) reported that neither maltase nor dextrin-1,6-glucosidase were present in style extracts of *O. edulis* (Table 1), although these enzymes were present in the diverticula where, he suggested, breakdown of amylose and amylopecten to glucose is completed.

Laminarin contains both β-1,3 and β-1,6 linkages, the ratio varying from 1:6 to 1:3 depending on the origin of the substrate. According to Kristensen (1972b), laminarin is a common storage product of the Phaeophyta and β-1,3-linked glucans are found in the Chrysophyta, diatoms, and euglenoids. The true nature of laminarinase activity is not known, but it is thought to be due to the action of an enzyme complex that includes both exo- and endo-hydrolytic β-1,3-gluconases and β-glucosidases (Kristensen 1972b). Laminarinase activity has been reported in style extracts and, to a lesser extent, in diverticula extracts of a wide range of bivalve species (Sova et al. 1970). Brock and Kennedy (1992) reported high laminarinase activity in style extracts of *C. virginica*, whereas low laminarinase activities were reported in oyster diverticula extracts (Brock et al. 1986; Fig. 5).

The presence of lipases in bivalves may be very important for digestion of waxes and triglycerides which are ubiquitously present, mainly as reserve compounds, in both plants and animals. In contrast to Yonge's (1926a) earlier view that lipase activity in bivalves was entirely intracellular, George (1952) found that the style of *C. virginica* was a major source of extracellular lipase. Furthermore, Mathers (1973a) reported both lipase and esterase activity in histological sections of the epithelium of the stomach and style sac of *O. edulis* and *C. angulata*. Patton and Quinn (1973) have found that style lipases of the surf clam, *Spisula solidissima*, showed specificity for the primary position of triglycerides and were capable of hydrolyzing wax esters.

Yonge (1926a, b) first recognized that protein digestion mainly occurs intracellularly in bivalve molluscs, although Reid (1968) reported that the proportion of extracellular versus intracellular protein digestion varies phylogenetically. Extracellular proteolysis takes place primarily by the action of endopeptidases, such as trypsin (Reid 1977; Reid and Rauchert 1970, 1972). Only weak extracellular proteolytic activities have been reported in oysters (*C. gigas*; Reid 1978).

Digestive Diverticula

Two of the primary ducts of the digestive diverticula of *C. virginica* open from the anterior stomach chamber and the third from the posterior stomach chamber (Fig. 2B). According to Owen (1955), the ciliated epithelium of the stomach of *O. edulis* pro-

duces a current that sweeps across the entrances of the ducts of the diverticula, from the non-ciliated inhalant side to the ciliated exhalant side. Therefore, fresh fluid and particles are continuously drawn into the non-ciliated portion of the ducts, whereas waste material is carried away from the ciliated portion (Fig. 6). Furthermore, Purchon (1968) suggested that one of the main functions of the ciliated sorting areas of the bivalve stomach is to prevent all but the smallest particles from gaining access to the ducts of the diverticula because any blockage of the ducts would interfere with the bivalve's capacity to perform intracellular digestion.

The primary ducts of *C. virginica* are made up of a dorsal, non-ciliated half that is partly divided from a ciliated ventral half by typhlosoles projecting into the lumen of the ducts (Fig. 6; Owen 1955). Owen (1955) suggested that cilia of the ventral part of the primary ducts remove undigested material from the diverticula. These celia also create counter-currents that carry food particles along the non-ciliated dorsal half of the ducts into non-ciliated secondary ducts and finally to the blind-ending tubules of the diverticula. The existence of a counter-current in the primary ducts of *O. edulis* has been confirmed by Mathers (1972) using ^{14}C-tracer techniques.

Movement of particles into the short secondary ducts is thought to be brought about by absorption of fluid by tubule cells (Owen 1955; Purchon 1968). According to Owen (1974), particle selection also occurs at this stage because only particles remaining in suspension in the fluid medium can enter the secondary ducts. There is some disagreement over whether or not tubules are surrounded by musculature that could influence fluid movement by tubule contractions. Nakazima (1956) observed that tubule walls of a variety of bivalve species do not possess longitudinal or circular smooth muscle fibers. Galtsoff (1964) reported the absence of muscle fibers surrounding the tubules of *C. virginica.* Owen (1955), however, has identified circular and longitudinal smooth muscle fibers surrounding the tubules of *O. edulis.* According to Owen (1955), occasional contractions of circular and longitudinal smooth muscle fibers balance weak pressures resulting from absorption of tubule fluid, whereas contractions of longitudinal fibers may empty tubule contents into the secondary ducts of the diverticula.

Cells lining the tubules of the diverticula of bivalves can be categorized as either digestive cells or basophil cells (Fig. 7). Many researchers have shown that digestive cells endocytose material from the lumen of the tubules (e.g., Yonge 1926b; Owen 1955; Pal 1972; Weinstein 1995). Intracellular digestion of the material occurs in a lysosomal system of vesicles containing digestive enzymes. Waste products of intracellular digestion are contained in residual bodies that are released from digestive cells into the lumen of the tubules (Fig. 6B; McQuiston 1969). Using ^{14}C-tracer techniques, Mathers (1972) noted that excretory sphericules were nipped off from the tips of digestive cells of *O. edulis* and passed out of the diverticula 60 to 90 min after oysters were fed algae.

The second cell type lining tubules of the diverticula is the basophil cell. Basophil cells have been described in *C. virginica* (Weinstein 1995), and they have been observed in the crypts of the tubules of eulamellibranchs and in clusters between the digestive cells of filibranch species (McQuiston 1969; Sumner 1969; Owen 1970; Pal 1971; Mathers 1972). Two types of basophil cells occurs in *C. virginica* (Weinstein 1995). Type I basophil cells are pyramidal in shape and exhibit a well defined endoplasmic reticulum together with features normally associated with the synthesis and secretion of protein (Fig. 7). Type II basophil cells differ in that they are slender, columnar, and flagellated and are thought to be a precursor stage of digestive cells (Pal 1971). Although Type I basophil cells are thought to be secretory, there is little evidence that the products are digestive enzymes. Sumner (1969) reported that very few of the secretory granules present in basophil cells of *Mytilus edulis* stained positively for acid phosphatase, aryl sulfatase, β-glucuronidase or N-acetyl-β-glucosaminidase; however, non-specific esterase activity was found throughout the cytoplasm of Type I cells.

Several investigators have observed synchronized cyclic changes in the structure of digestive cells of many bivalve species and at least four phases of cell activity have been described: holding, absorption, disintegration, and reconstitution (Fig. 8; Morton 1977, 1983). Many researchers have reported that

cyclic, structural changes of diverticula cells are synchronized according to the tidal cycle (see review by Morton 1983). Furthermore, Morton (1977) reported that the digestive cell cycle can vary seasonally, with the absorptive phase of digestive cells of *C. gigas* occupying a greater proportion of the cell cycle in winter than in summer (Fig. 8). Seasonal changes in the digestive tubules of *C. virginica* collected from Canada (Shaw and Battle 1957) and New Jersey (Eble 1966) have been reported. No such changes were observed, however, by Weinstein (1995) in oysters collected from the more southern state of South

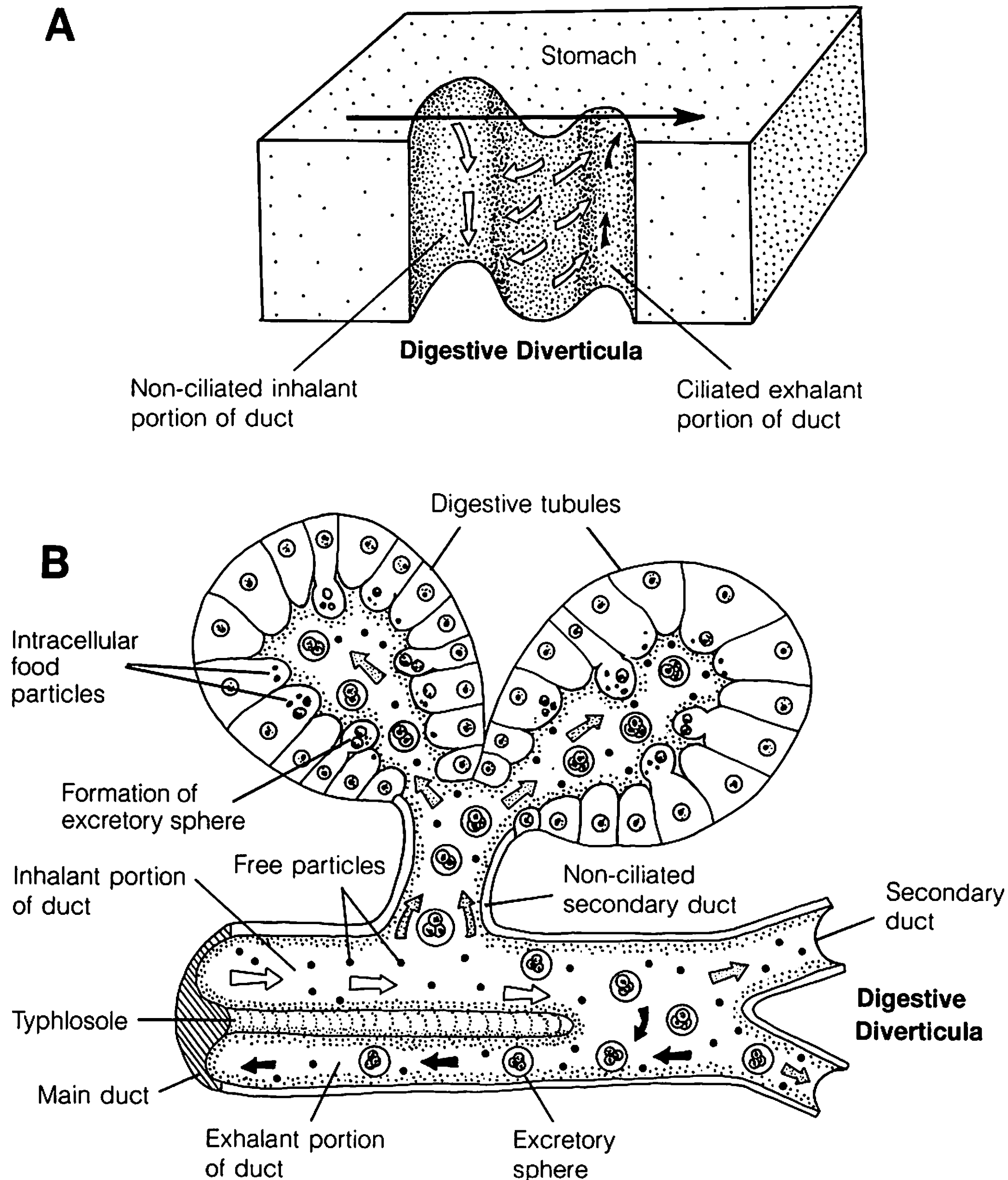


Figure 6. (A) Diagrammatic representation of the junction between the stomach and the main duct of the digestive diverticula. (B) Probable circulation of fluid and food particles within the digestive diverticula. Solid arrows represent ciliary currents; open arrows represent the inhalant, countercurrent; stippled arrows represent movement of fluid due to absorption of fluid by tubule cells. Redrawn from Owen (1955).

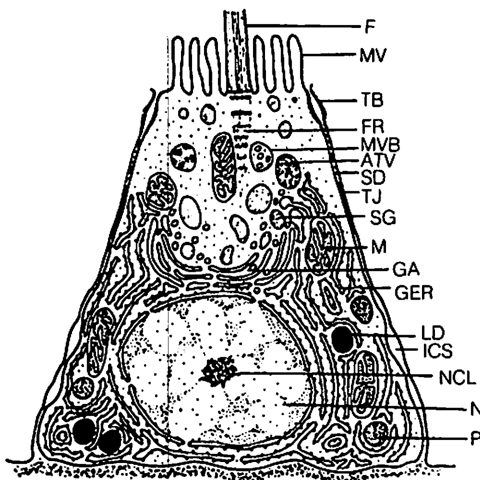
Carolina where seasonal variation in seawater temperature and food supply are likely to be less extreme.

Purchon (1971) and Morton (1973) suggested that extracellular digestion in the stomach and intracellular digestion in the diverticula are organized in strictly alternating phases as a result of tidal influences on digestive rhythms. Owen (1974) suggested that synchronized structural changes of diverticula cells are controlled by the availability of food rather than by tidal cycles per se. Indeed, Nakazima (1956) found that digestive cell height increased after food was ingested in several bivalve species and Winstead (1995) experimentally demonstrated that starvation significantly reduced digestive cell height in *C. virginica.*

In contrast, Langton (1975) observed no well-defined synchronized changes in the structure of the digestive cells of intertidal *M. edulis.* Furthermore, Robinson et al. (1981) found no quantitative evidence for synchronous cycles in the abundance of absorptive cells in either subtidal hard clams, *Mercenaria mercenaria,* low-intertidal *O. edulis,* or mid-intertidal *M. edulis.* Future studies on digestive rhythms of bivalves should include appropriate statistical analysis of digestive cell type frequencies so that it will be possible to come to some definitive conclusions regarding this controversial subject.

Brock et al. (1986) reported the presence of amylase, C_x cellulase, and laminarinase in diverticula extracts of *C. virginica* (Fig. 5), indicating that breakdown of polymers into soluble oligomers is not an exclusively extracellular process. Furthermore, Mayasich and Smucker (1986) reported the presence of the glycosidic enzymes maltase, cellobiase, and chitobiase in the diverticula of *C. virginica,* indicating that com-

A



B

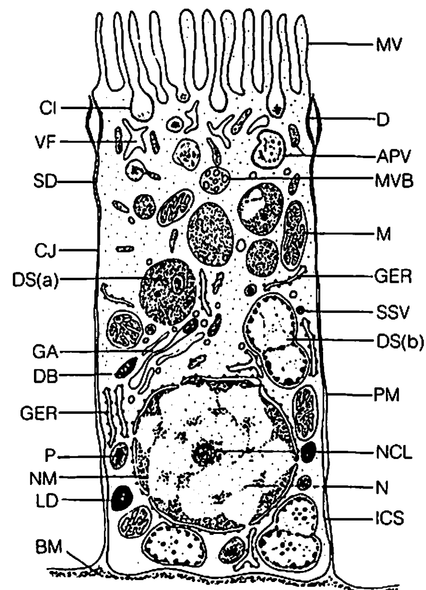
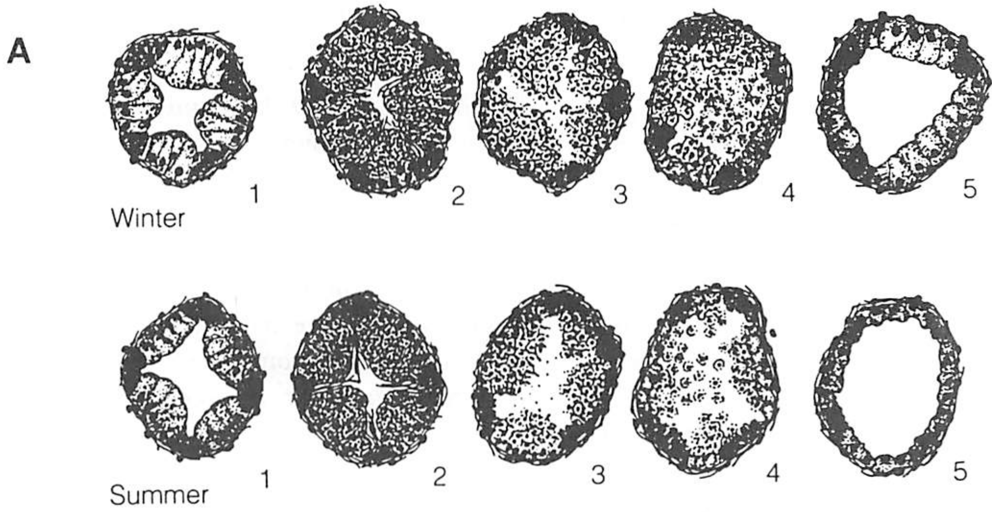


Figure 7. Diagrammatic representation of the ultrastructure of a Type I basophil cell (A) and a digestive cell of the diverticula (B). (A) After Pal's (1971) description for the basophil cell of *Mya arenaria* and (B) after Pal's (1972) description for the digestive cell of *M. arenaria.* [APV] apical vesicles, [ATV] autophagic vacuole, [BM] basement membrane, [CI] pinocytotic invaginations, [CJ] close junction, [D] desmosome, [DB] dense body, [DS (a), DS (b)] cytoplasmic digestive spheres, [F] flagellum, [FR] flagellary rootlet, [GA] Golgi apparatus, [GER] granular endoplasmic reticulum, [ICS] intercellular space, [LD] lipid droplet, [M] mitochondrion, [MV] microvilli, [MVB] multivesicular body, [N] nucleus, [NCL] nucleolus, [NM] nuclear membrane, [P] peroxisome, [PM] plasma membrane, [SD] septate desmosome, [SG] secretory granule, [SSV] smooth-surfaced microvesicle, [TB] terminal bar, [TJ] tight junction, [VF] apical vacuolar field.



B

Winter

Summer

Tidal level (meters)

Digestive cell height (μm)

Tubule lumen diameter (μm)

Time (hours)

Winter: Absorption; Digestion; Breakdown; Reformation; Preparation; Absorption; Digestion; Breakdown; Reformation; Preparation; Absorption

Summer: Digestion; Breakdown; Reformation; Preparation; Absorption; Digestion; Breakdown; Reformation; Preparation; Absorption

plete breakdown to glucose of starch, cellulose, and chitin, respectively, can occur in this organ. Mathers (1973b) also reported that the diverticula of *O. edulis* possessed poly-β glucosidase, cellobiase, and other oligosaccharidases that were less active or absent in style and stomach fluids (Table 1).

There is some evidence that cellulolytic activity in the diverticula of *C. gigas* is inducible by exposure of the oyster to algal food. Brock (1989) reported that diverticula extracts from *C. gigas* maintained on an algal diet of *Phaeodactylum tricornutum* and *Tetraselmis sueica* possessed significantly higher cellulolytic activities, as measured by algal cell lysis, than those in extracts from starved oysters. Further studies are required to examine the effects of diet on induction of other digestive enzymes.

Reid (1978) reported low gastric proteolysis and intermediate diverticula proteolysis in *C. gigas*, with peaks of activity at pH 3, 6, and 7.5. Therefore, *C. gigas*, and probably *C. virginica*, are similar to most other suspension-feeding bivalve species in that extracellular proteolytic activity is weak and protein digestion is primarily intracellular (Reid and Rauchert 1972, 1976).

Proteolytic digestive enzymes of *C. virginica* have not been characterized; however, weak chymotrypsin activity is characteristic of diverticula extracts of members of the Gastrotriteia (Reid 1968; Reid and Rauchert 1970) and Reid and Rauchert (1972) suggested that chymotrypsin activity may be characteristic of the diverticula of bivalves. Reid and Rauchert (1970) reported the presence of weak trypsin, carboxypeptidase A, and leucine aminopeptidase activity in diverticula extracts of the scallop, *Chlamys hericius*, and acid endopeptidases or cathepsins were found in diverticula extracts of several species of *Macoma*. Reid and Rauchert (1976) performed a more intensive study of cathepsins in the horse clam, *Tresus capax*. They found endopeptidase cathepsins B and D and exopeptidase cathepsins A and C in diverticula extracts of the clam, although they suggested that cathepsin D had a non-digestive role in protein metabolism. Cathepsin B activity, together with tryptic activity, was also evident in gastric extracts of this clam species.

Extensive investigations of aminopeptidase activity in *Mytilus edulis* (see review by Koehn 1983) have shown that these enzymes hydrolyse di-, tri-, and tetrapeptides. Maximum aminopeptidase activity in *M. edulis* occurs with oligopeptide substrates possessing neutral or aromatic amino acids at the N-terminal position (Young et al. 1979). Aminopeptidase activity is present in the epithelial lining of the intestine, the stomach, and the ducts (inhalant) and tubules of the digestive diverticula of *M. edulis* (Moore et al. 1980). Most of the activity in the diverticula is found in the apical border of microvilli lining the ducts, but about 10 to 15% of the total activity is located in lysosomes (Moore et al. 1980). Lysosomal aminopeptidase activity is thought to be involved in the regulation of intracellular concentrations of amino acids in response to salinity fluctuations (Koehn 1983). The function of aminopeptidases associated with the epithelia lining the gut is less likely to be associated with osmoregulation because salinity fluctuations do not appear to have an effect on activity of these enzymes (Moore et al. 1980). It is possible that non-lysosomal aminopeptidases are important in protein digestion and catabolism.

Mid-Gut and Rectum

The mid-gut of *Crassostrea virginica* begins on the ventral wall of the stomach next to the opening of the crystalline style sac and runs parallel to the sac

Figure 8 (opposite page). (A) Diagrammatic representation of cyclic changes in tubule structure of *Crassostrea gigas* over tidal cycles in winter and summer. (1) swelling of cells in holding stage; (2) absorptive phase, food particles in cells; (3) absorptive phase, some cell disintegration; (4) disintegration; (5) reconstitution. (B) The height of digestive cells and lumen diameter of tubules of *Crassostrea gigas* over tidal cycles in winter (February) and summer (July). Note taller digestive cells and longer absorption phase in winter than in summer. Both A and B redrawn from Morton (1977).

as far as its distal end. It then turns sharply back on itself, loops around the stomach and passes to the rectum and anus (Fig. 1). A well-developed typhlosole and intestinal groove extends along the entire length of the mid-gut. The mid-gut epithelium is made up of both columnar ciliated cells and mucus-secreting cells (Giusti 1970). Galtsoff (1964) observed no muscular layer surrounding the mid-gut. Movement of material through this region of the digestive system must, therefore, be caused by the action of cilia and be lubricated by mucus produced from mucus-secreting cells.

The rectum extends along the dorsal side of the heart and possesses a similar structure to the mid-gut, except that near the anus the lining epithelium is thrown into numerous folds and the typhlosole disappears. A circular layer of smooth muscle surrounds the rectum but no anal sphincter was observed by Galtsoff (1964; Fig. 9).

There is some controversy over the role of the mid-gut in the digestive process. Yonge (1926a) attributed the role of absorption of water and consolidation of feces to the mid-gut. However, Giusti (1970) found no epithelial absorptive cells in the "terminal" intestine (section from the distal end of the style sac to the anus) of the Mediterranean mussel, *Mytilus galloprovincialis*, although undetermined proteinaceous secretions from ciliated cells were observed. In contrast, *in vitro* hydrolysis of glycogen as well as weak trehalase, α-galactase, maltase and cellobiase activities of the mid-gut epithelium of *Ostrea edulis* (Mathers 1973b), together with esterase activity (Mathers 1973a), suggest that enzymatic digestion of food can occur in the mid-gut of this oyster species. Absorption of amino acids, D-glucose, and sodium acetate by mid-gut preparations of *Mya arenaria* against a concentration gradient (Stewart and Bamford 1976) suggests that the mid-gut could also be important in the absorption of nutrients released from food digested in the stomach.

Hemocytes

Blood cells of bivalve molluscs are termed hemocytes (Farley 1968), and their morphology is reviewed by Cheng in Chapter 8. Two general types of hemocytes have been described in oysters (Takatsuki 1934; Feng et al. 1971). One type is granular, yellowish-green or yellow in appearance, varying in diameter from 9 to 13 µm. These granular hemocytes are amoeboid and sometimes produce bristle-like

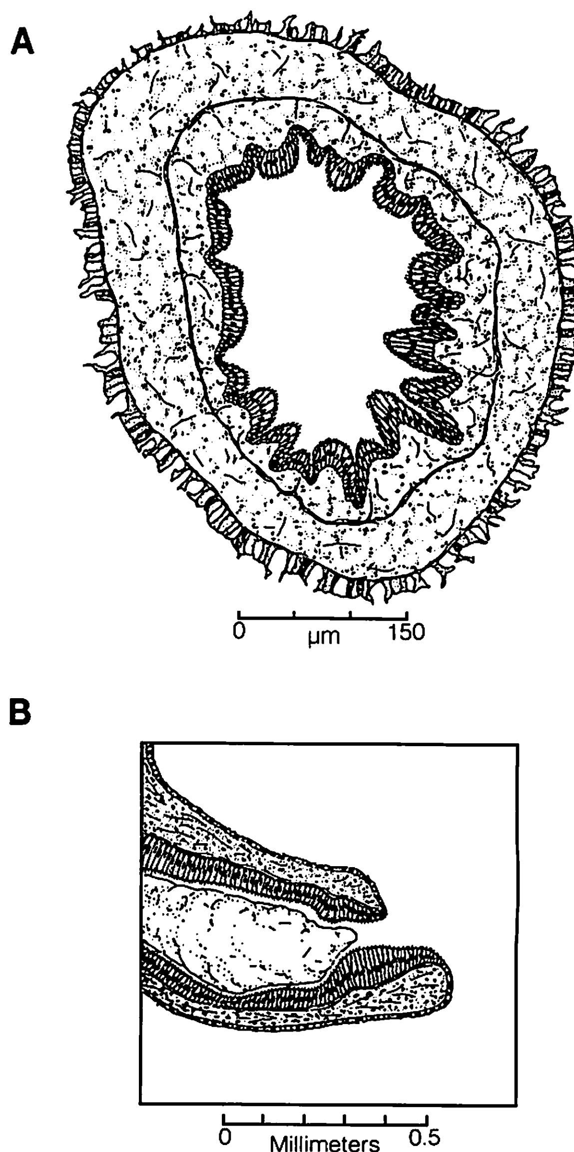


Figure 9. (A) Cross-section of the rectum near the anal region of *Crassostrea virginica.* (B) Longitudinal section of the anus and adjacent portion of the rectum of *Crassostrea virginica.* From Galtsoff (1964).

pseudopodia. Feng et al. (1971) further sub-divided granular hemocytes into three types, based on observations from electron microscopy and Giemsa-staining techniques. The non-granular type of hemocyte is more hyaline in appearance, 5 to 15 μm in diameter and is less amoeboid than granular hemocytes (Takatsuki 1934; Galtsoff 1964; Feng et al. 1971).

The granular hemocytes are commonly found in the alimentary canal of adult oysters (Yonge 1926a, b; Takatsuki 1934; Nakazima 1956; Galtsoff 1964; Giusti 1970; Mathers 1972) and oyster larvae (Yonge 1926a; Elston 1980a). Granular hemocytes possess a wide range of digestive enzymes, including amylases, lipases, esterases, and proteases (Yonge 1926a, b; Takatsuki 1934; Mathers 1973a). Yonge (1926a) suggested that the principal function of hemocytes in bivalves is to endocytose and digest food particles from the lumen of the gut and transport digested material to tissues lining the gut where the material is assimilated. Several researchers have reported the occurrence of large numbers of hemocytes containing digested material in the hemocoel surrounding the digestive tubules of oysters (Yonge 1926a, b; Morton 1971). In contrast, Mathers (1972) reported that hemocytes present in the ducts of the diverticula of *O. edulis* did not transport ^{14}C labelled material from the lumen to the epithelial tissue. Mathers (1972) found that food vacuoles formed in the epithelial cells lining the ducts were liberated into the hemocoel where ingestion by hemocytes took place. Further studies are required to better understand the function of granular hemocytes in bivalve digestion (Auffret 1988).

In addition to granular hemocytes, Elston (1980a) reported large cuboidal cells to be present in greatest concentrations in the umbone region and adjacent dorsal regions of the visceral cavity of larval *Crassostrea gigas* and *C. virginica.* These cells contained high concentrations of smooth endoplasmic reticulum (SER), and Elston (1980a) referred to these cells as SER cells because of this feature. A possible function of SER cells in larvae might be the processing, conversion, and re-secretion of dissolved substances that have been secreted into the visceral cavity by the absorptive cells of the diverticula (Elston 1980a).

Gut Microflora

Perhaps the most commonly reported enteric bacteria occurring in oysters are those associated with the style. *Cristispira* spp. are colorless gram negative spirochetes 0.3 to 0.8 μm in diameter and 30 to 150 μm in length that live in the matrix of the style of oysters (Dimitroff 1926; Yonge 1932). Morphologically, *Cristispira* spp. can be distinguished from other spirochetes by the presence of a ridge or crest-like structure called the "crista" (Tall and Nauman 1981). A smaller spirillum, *Spirillum ostreae,* may also be present in styles of *C. virginica* (Dimitroff 1926). It has been postulated that these *Cristispira* spp., as well as other types of bacteria, contribute to the style's production of enzymes (Kuhn 1978). Mayasich and Smucker (1987) tested this hypothesis by assaying cellulase, chitinase, and chitobiase activity in crystalline styles removed from oysters in which style bacterial levels had been regulated by antibiotic treatment. Their results indicate that enzymes in the crystalline style of oysters are produced endogenously and that *Cristispira* spp. and other bacteria do not contribute significantly to the production of these enzymes.

There have been several studies on the bacterial gut flora of oysters that indicate the presence of proteolytic bacteria and types capable of fermenting glucose under anaerobic conditions (Colwell and Liston 1960, 1961; Murchelano and Bishop 1960). Prieur (1981) performed a microscopic examination of bacteria present in the gut of *Mytilus edulis* and reported that some bacteria, particularly species of *Vibrio,* could live and multiply in the hind gut of the mussel. However, Garland et al. (1982) observed no bacteria attached to the gut epithelium of *C. gigas* and very few bacteria attached to mucus layers covering the epithelium.

Crosby and Reid (1971) suggested that the gut microflora of *C. gigas* play a significant role in extracellular digestion of cellulose, but they were not able to determine the relative importance of endogenous versus exogenous cellulases of bacterial origin. Newell and Langdon (1986) and Crosby et al. (1989) found no significant reduction in carbon absorption efficiencies of *C. virginica* fed on ^{14}C-labelled cellulose

in the presence of antibiotics, even though the antibiotic treatment reduced concentrations of stomach bacteria to below detectable levels.

Seiderer et al. (1987) reported the presence of a bacteriolytic agent produced by bacteria associated with the crystalline style of *M. edulis*. Transmission electron micrographs indicated the presence of bacteria-like cells in the laminated outer layers of the style of *M. edulis* which Seiderer et al. (1987) suggested could be responsible for bacteriolytic activity. It is unknown whether bacteriolytic bacteria are associated with styles of other bivalve species.

Digestion and Absorption Efficiencies

Digestion and absorption efficiencies of bivalve molluscs have been reviewed by Bayne and Newell (1983). *Crassostrea virginica* is efficient in using many algal species as a source of nutrition for its metabolism and growth. For example, Romberger and Epifanio (1981) reported assimilation efficiencies of 72 and 74% for *C. virginica* fed the diatom, *Thalassiosira pseudonana*, and the flagellate, *Isochrysis galbana*, respectively. However, some algal species are poorly utilized as a food source by *C. virginica*; for example, *Tetraselmis suecica* was reported to be digested and absorbed with an efficiency of only 6% (Romberger and Epifanio 1981). Many chlorophyte algal species are thought to be of poor food value because of their characteristically thick cellulosic cell walls (Webb and Chu 1982). For example, Peirson (1983) reported that the bay scallop, *Argopecten irradians*, absorbed carbon with an efficiency of only 17% when fed on ^{14}C-labelled *Chlorella autotrophica* and Walne (1976) found the chlorophytes *Chlamydomonas coccoides*, *Dunaliella tertiolecta*, and *Dunaliella euchlora* to be among the poorest algal foods that he tested for *Ostrea edulis*.

Penry and Jumars (1986, 1987) have applied digestion models, couched in terms of chemical reactor theory, to their analyses of the effects of through-put time (the time it takes for one gut volume of food to be processed) on digestion efficiencies in marine invertebrates. The longer that ingested material is held in the gut and subjected to digestive processes, the greater the proportion that is likely to be digested and absorbed (Calow 1975, 1977). Bayne et al. (1984) reported that absorption efficiencies of three species of mytilid bivalves (black mussel, *Choromytilus meridionalis*, brown mussel, *Perna perna*, and ribbed mussel, *Aulacomya ater*) fed on natural seston were positively correlated with gut residence time and that gut residence time increased as diet quality decreased. There has been little research on the effects of gut through-put times on digestion efficiencies of oysters.

Crassostrea virginica may optimize through-put time and digestion efficiency by controlling ingestion rate. Thompson and Bayne (1974) and Foster-Smith (1975) reported that absorption efficiencies of bivalves fed on algae were inversely proportional to ingestion rates. Ingestion rate may be controlled by either adjusting filtration rate (Winter 1973; Navarro and Winter 1982), or the proportion of filtered material rejected by the palps, or both.

According to Palmer (1980), there was no adjustment of filtration rate when *C. virginica* was fed *Isochrysis galbana* at concentrations that ranged from 1.72 to 6.80 mg wet weight of algae L^{-1}, although some reduction in filtration rate was observed when oysters were fed on high concentrations (4.38 mg wet weight of algae L^{-1}) of the diatom *Thalassiosira pseudonana*. Epifanio and Ewart (1977) found that the effect of algal concentration on oyster filtration rate varied according to species of alga. Adjustment in the filtration rate of *C. virginica* fed on either *T. pseudonana* or *Carteria chuii* at concentrations ranging from 1 to 12 mg dry weight L^{-1} resulted in only slight differences in rates of removal of algal biomass per oyster. In contrast, the rate of removal of both *I. galbana* and *Chroomonas salina* increased as algal concentration increased because compensatory reductions in oyster filtration rate did not occur. In addition, Epifanio and Ewart (1977) noted the onset of pseudofecal production by *C. virginica* when exposed to any of the four algal species at concentrations greater than 10 mg dry weight L^{-1}. In summary, it appears that *C. virginica* is capable of regulating ingestion rate of some algal diets by adjusting its filtration rate, although production of pseudofeces is a more general mechanism for controlling ingestion rates, through-put times, and digestion efficiencies (see Newell and Langdon, Chapter 5).

Through-put time in intertidal oysters can also be increased as a result of tidal exposure, possibly resulting in higher digestion and absorption efficiencies (Calow 1975, 1977). Langton and McKay (1974, 1976) found that growth rates of discontinuously fed (6 h fed:6 h unfed) juvenile *Crassostrea gigas* were greater than those of continuously fed oysters. Gillmor (1982) reported that intertidal *C. virginica* exposed for about 20% of the tidal cycle grew faster than subtidal oysters, and he suggested that periodic exposure may impose a digestive rhythm on intertidal oysters, resulting in increased absorption efficiencies. In support of Gillmor's hypothesis, Kreeger et al. (1991) reported that absorption efficiencies of intertidal (6 h exposed:6 h submerged) ribbed mussels, *Geukensia demissa*, fed on cellulose were significantly greater than those of mussels exposed to a subtidal regime. Further studies are required to determine the effects of exposure on digestion in intertidal oysters.

Additions of inorganic silt or clay particles to algal or artificial diets have been reported to improve bivalve growth (Murken 1976; Winter 1976; Kiørboe et al. 1980, 1981; Møhlenberg and Kiørboe 1981; Langdon and Siegfried 1984; Urban and Langdon 1984). Murken (1976) suggested that digestion efficiency was improved by the action of inorganic particles grinding food in the bivalve's style sac. In support of this hypothesis, Ewart and Carriker (1983) reported that the addition of silt in combination with low concentrations (1×10^4 cells ml^{-1}) of the alga *Phaeodactylum tricornutum* reduced the proportion of intact algal cells present in the feces of *C. virginica*, compared with the proportion occurring in feces of oysters fed on algae alone.

Bayne et al. (1987) studied the effects of adding various concentrations of silt to a diet of *Phaeodactylum tricornutum* on the feeding and digestive processes of *Mytilus edulis*. These researchers reported that *M. edulis* showed long-term compensatory adaptations that allowed mussels to maintain a positive scope for growth when fed diets with high inorganic content by increasing ingestion rates, absorption efficiencies, and gut capacities. In a subsequent paper, Bayne et al. (1989) reported that absorption efficiencies for *M. edulis* fed on diets composed of different proportions of silt and algae showed no significant relationship with diet quality (mg particulate organic matter [POM] mm^{-3} diet) or concentration of diet in suspension, even though gut passage time varied inversely with POM concentration (mg POM L^{-1}). Bayne et al. (1989) suggested that *M. edulis* is able to maintain constant absorption efficiencies for the mixed diets over a range of gut passage times by adjusting the amounts or activities of digestive enzymes. Because absorption efficiencies remained constant over the range of diets that Bayne et al. (1989) tested, absorption rates increased proportionally with ingestion rates. Furthermore, there was only a small increase in energy expenditure by *M. edulis* when feeding on and ingesting food material at higher rates and estimated metabolic costs of feeding and digestion only increased from 16 to 22% of the absorbed ration. Therefore, scope for growth was linearly proportional to absorbed ration, reaching a maximum value at the highest concentrations of POM tested (1.79 mg L^{-1}). According to Bayne et al. (1989) the growth of *M. edulis* is primarily limited by the maximum rates of digestion and absorption that are possible, given the morphological constraints of the feeding and digestive systems in bivalves.

LARVAL OYSTERS

The digestive system of bivalve larvae has received less attention than that of adult stages. The system is simple in structure because the organs are generally formed by either a single cell layer or stratified epithelium bounded by a thin enveloping cell layer separating the organs from the visceral cavity (Elston 1980b). Horst (1884), Yonge (1926a), Erdmann (1935), and Millar (1955) made some of the earliest observations on the internal anatomy of the larvae of *Ostrea edulis*, and Stafford (1913) defined some histological structures of larval *Crassostrea virginica*. Most of the following description of the larval digestive system is based on the work of Elston (1980b) who used both light and electron transmission microscopy to study the ultrastructure of the soft tissues of *C. virginica*. (For additional details of the anatomy of the larval digestive system see Eble, Chapter 2.)

According to Lucas and Rangel (1983), there is a delay before larval *C. gigas* begins to ingest algal cells after reaching the D-stage of development of about 8 to 9 h at 21°C or 6 h at 24°C. This delay, which is likely typical for all planktotrophic veliger larvae, is probably due to incomplete development of the larva's organs for feeding and digestion (see Newell and Langdon, Chapter 5).

Particles captured by velar cilia are transferred to the ciliated adoral food tract that in turn transfers particles along the edge of the velum to the mouth (Fig. 10). The mouth of the young oyster veligers is small and estimated to be no more than 10 µm wide in larvae with shell lengths of 75 µm (Ukeles and Sweeney 1969). The ciliated mouth is funnel-shaped and opens into the ciliated esophagus (Figs. 10, 11). At the base of the esophagus, Elston (1980b) observed a valve-like constriction that is important in both controlling entry of food particles into the stomach and in preventing mucus and food particles from being lost from the stomach into the esophagus (Fig. 11A).

The stomach is a bell-shaped, partly ciliated organ. The anterior and lateral luminal aspects of the stomach wall are partly covered by the gastric shield which is made up of intertwining microvilli embedded in a mucinous matrix (Figs. 11B, C). A deep, cup-shaped, ciliated, style sac protrudes from the dorsal part of the stomach (Fig. 11B). Elston (1980b)

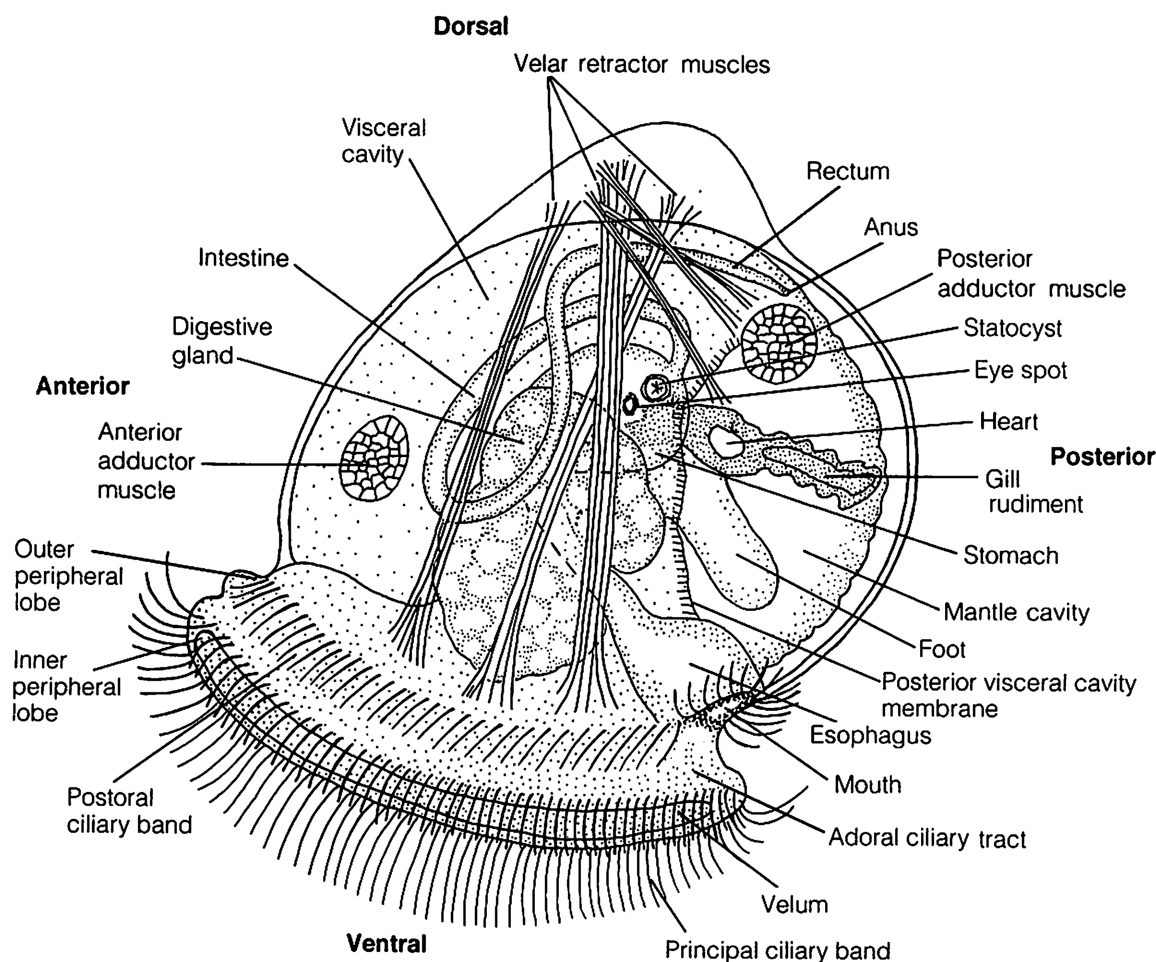


Figure 10. *Crassostrea virginica.* Diagrammatic illustration of prodissoconch II veliger larva viewed from the left. × 380. Note that principal and postoral bands are composed of complex cirral units and not single cilia as illustrated. Also, the principal band is composed of a double row of cirri and not the single row illustrated. Based on drawings by Galtsoff (1964) and Elston (1980).

did not observe a crystalline style in *Crassostrea virginica*. Yonge (1926a) and Millar (1955) observed in larvae of *Ostrea edulis*, a rapidly rotating style (36 to 90 rpm) that generally rotated in a clockwise direction as viewed from the anterior end of the larvae. Ciliary activity and rotation of the style mixed stomach contents.

The larval digestive gland consists of two pairs of lobed outgrowths of the stomach (Figs. 10, 12A). Undifferentiated cells at the tips of the lobes divide as the larva grows and enlarge the size of the diverticula. The walls of the diverticula are made up of two other cell types that appear to be primarily involved in digestion (Fig. 12B). One cell type is absorptive and is capable of endocytosing Indian ink particles (Elston 1980b). The other cell type possesses ultrastructural features, including abundant rough endoplasmic reticulum, Golgi complexes and apical condensation vacuoles, that suggest that it is involved in enzyme secretion. The presence of ciliated cells in the diverticula of European flat oyster larvae has been inferred by Millar (1955), but ciliated cells were not observed by Elston (1980b). Contractions and expansions of the lobes of the diverticula (Millar 1955; Elston 1980b) probably move material to and from the diverticula and stomach.

A fecal groove is present at the base of the larval stomach and appears to have the same function as the intestinal groove of adults in that it transports rejected material from the stomach to the intestine (Fig. 10). The larval intestine makes a series of loops before joining the anus (Fig. 10). A post-anal ciliary tuft is thought to facilitate defecation by wafting feces away from the larva (Waller 1981).

Bayne (1983) has reviewed digestion and absorption efficiencies of marine molluscan larvae. There are difficulties in determining these efficiencies because it is not usually possible to measure larval fecal production directly. Walne (1965) fed larvae of *Ostrea edulis* on ^{32}P-labelled algal cells and estimated assimilation efficiencies of 29% (range 15 to 45%), although these estimates are likely to be conservative (Bayne 1983). Other researchers have calculated assimilation efficiencies indirectly by considering components of the energy budget of larvae. From data of Gabbott and Holland (1973), Bayne (1983) recalculated assimilation efficiencies for larval *Ostrea edulis* (178 to 219 µm in shell length) fed on the alga *Isochrysis galbana* to be between 28 to 52%. This estimate is consistent with a direct measurement of assimilation efficiency of 29% reported by Walne (1965).

DISSOLVED ORGANIC MATTER AS A SOURCE OF NUTRITION

The possibility that dissolved organic matter (DOM) could contribute to the nutrition of marine animals was suggested by Pütter 1909 (see review by Jørgensen 1976, 1983; Stephens 1982a). In this process, soluble organic nutrients present in seawater are directly absorbed and used by the animal for metabolism and growth. Concentrations of dissolved organic carbon (DOC) in seawater are sometimes comparable to concentrations of particulate organic carbon and generally range from 0.3 to 3.0 mg C L^{-1} (25 to 250 µmol C L^{-1}; Williams 1975; Wangersky 1978). However, most of the DOC is refractory and not available to organisms as a source of nutrition; only about 10% of the total DOC of seawater is in the form or dissolved carbohydrates, organic acids, proteins and other identified compounds (Williams 1975).

Use of radiotracer techniques has allowed researchers to determine both the sites and kinetics of uptake of dissolved nutrients by soft-bodied marine invertebrates (Stewart 1979; Stephens 1982b; Wright 1988; Manahan 1990a, b). The gill and perhaps labial palps are the main sites of uptake of amino acids in adult bivalves (Beninger et al. 1990), whereas the velum is the primary site of uptake in bivalve larvae (Manahan and Crisp 1983).

Uptake kinetics for DOM can be described by Michaelis-Menten kinetics, indicating that carrier-mediated transport mechanisms are involved. Values for K_t (the substrate concentration at which uptake is half the maximum uptake rate [V_{max}]) range from 1 to 10 µM for amino acids in adult bivalves actively pumping water across their gills (Wright and Stephens 1978; Jørgensen 1983) and larvae (Manahan 1983). Values for V_{max} range from 0.6 to 5.0 mg dissolved amino acid absorbed per g dry weight per h for adult California mussels (*Mytilus californianus*)

and veliger blue mussels, respectively (Manahan and Crisp 1982). Greater V_{max} values for bivalve veligers reflect their higher surface area-to-volume ratios compared to those of adults. Manahan (1990a) reported that mass coefficients and exponents for amino acid transport rates and metabolic rates for larval *Crassostrea gigas* are not significantly different, indicating that as growth occurs, larvae are able to increase their uptake of amino acids in direct proportion to increases in their metabolic requirements.

One of the major challenges in determining the nutritional importance of DOM is determination of concentrations of dissolved nutrients that bivalve larvae experience in their natural habitat. According to Manahan (1989), larvae of *C. gigas* (shell length 70 µm) could meet all their metabolic requirements if exposed to amino acid concentrations of about 10 µM. However, reported amino acid concentrations for water-column samples are usually much less than 10 µM (Henrichs and Williams 1985), although micro-scale and sediment amino acid concentrations may be closer to 10 µM (Henrichs and Farrington 1979). The potential contribution of other soluble organic molecules to the nutritional requirements of bivalves has not been fully determined, although the presence of a carrier-mediated uptake system for D-glucose has been demonstrated in the gill of *C. gigas* (Bamford and Gingles 1974). Uptake of glucose, maltose, cellobiose, and cellotriose has been demonstrated by larval *C. gigas* (Welborn and Manahan 1990).

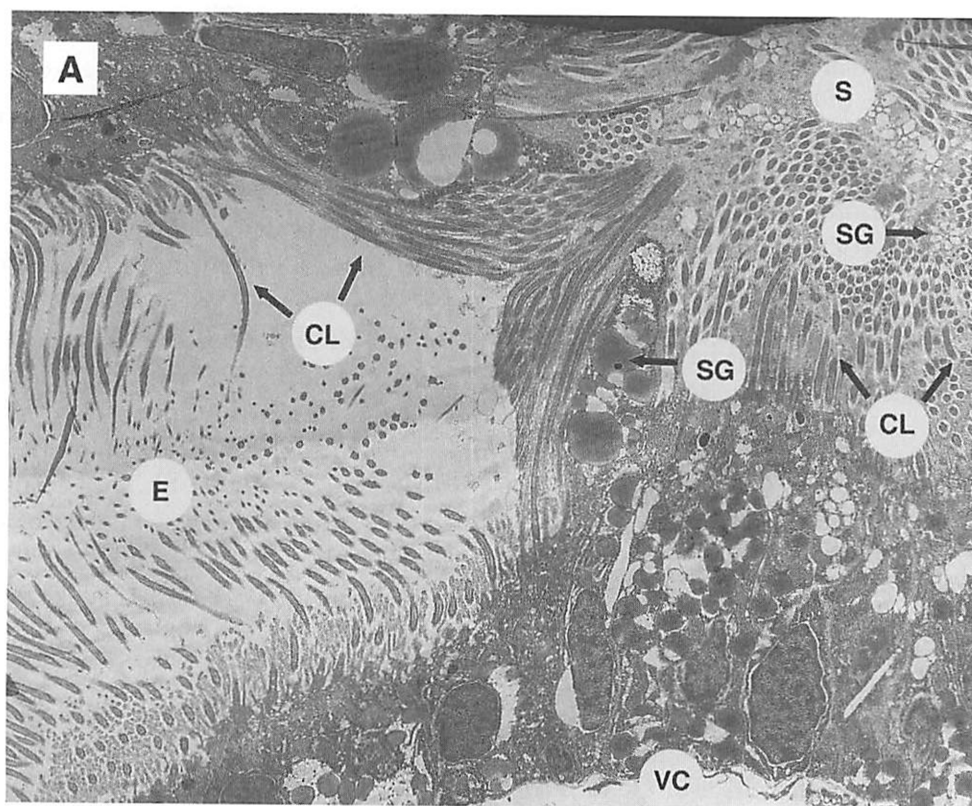
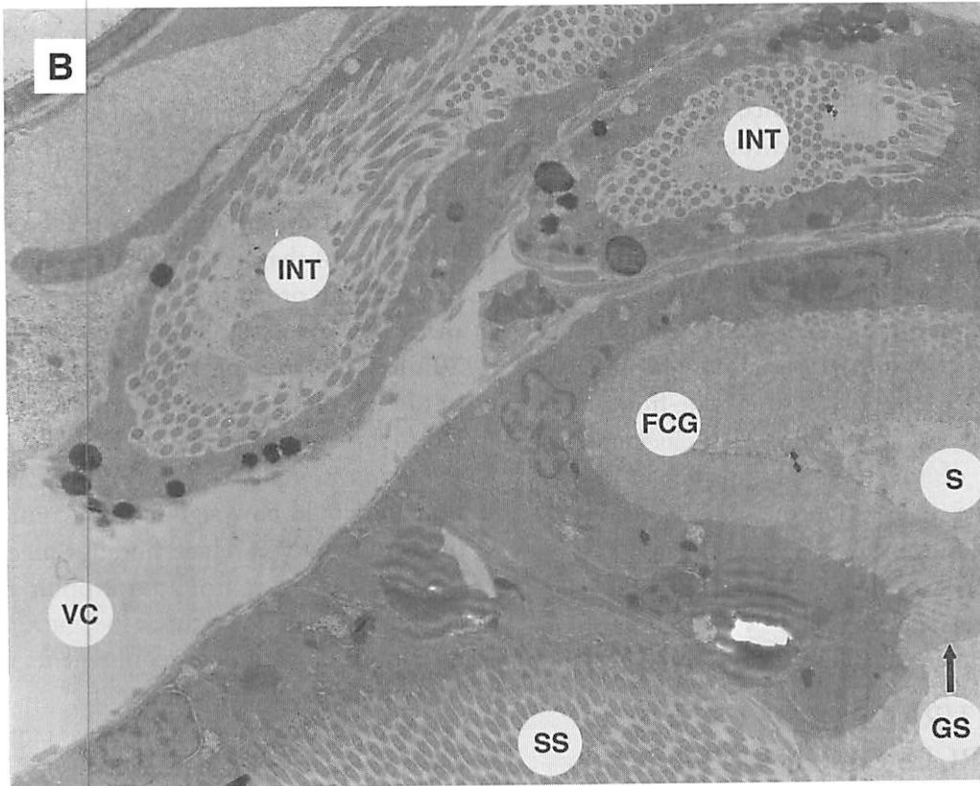


Figure 11 (above and opposite page). Transmission electron micrographs of the larval stomach of *Crassostrea virginica.* (A) The funnel-shaped constriction between the esophagus [E] and stomach [S]. × 4760. (B) Anterior view of the fecal groove [FCG] formed by the ridge between the stomach [S] and style sac [SS]. × 4080. (C) The gastric shield [GS] of the stomach formed by densely intertwined microvilli [MV] and mucoid secretions. × 17,000. After Elston (1980b). [CL] cilia, [INT] intestine, [L] lumen of the stomach, [SC] stellate crystals, [SG] secretion granules, [VC] visceral cavity.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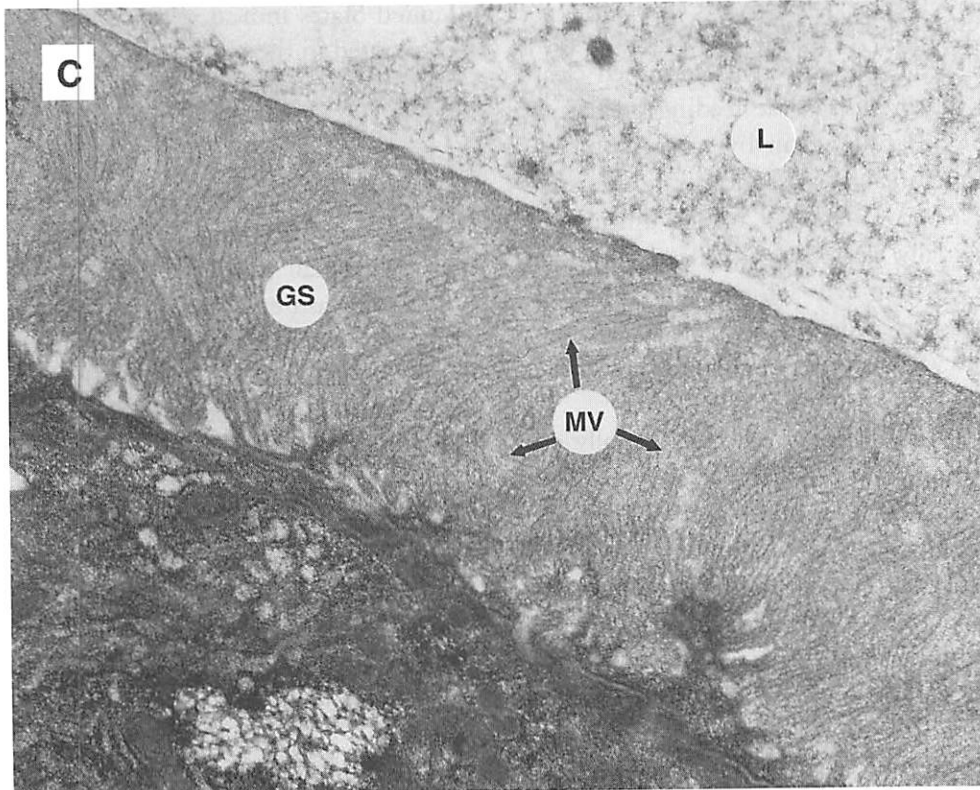
C
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The use of high performance liquid chromatographic techniques has enabled researchers to determine that rates of loss of amino acids from bivalves were negligible compared with rates of uptake from ambient concentrations of amino acids as low as 10 nM (Manahan et al. 1982; 1983). However, Manahan (1990b) reported that there was a net loss of taurine from Pacific oyster larvae, although the larvae had a capacity to actively absorb taurine from seawater. Wright and Secomb (1986) have elucidated the important role of integumental transport in recovering amino acids, such as taurine, that leak from the gill surface of adult bivalves. They estimated that between 30 and 50% of the taurine lost from the gill of *Mytilus edulis* may be recovered by the action of taurine transporters on the gill surface.

NATURAL PARTICULATE SOURCES OF NUTRITION

Phytoplankton has been considered to constitute the principle food source of oysters from the earliest studies on bivalve nutrition (Dean 1887; Lotsy 1895; Moore 1908; Stafford 1913; Kellogg 1915). More recently, Fritz et al. (1984) directly determined clearance rates for *C. virginica* larvae feeding on natural assemblages of phytoplankton by microscopically measuring changes in algal cell concentration in samples grazed by larvae. Larvae (71 to 136 μm in shell length) preferentially cleared algal cells smaller than 10 μm compared with cells larger than 10 μm, although there was no evidence for selective clearance from among the various types of algae present in the seston samples.

Crisp et al. (1985) estimated that naturally occurring concentrations of phytoplankton, larger than 2 μm, were not sufficient to meet the energy requirements of larval *Ostrea edulis*; therefore, they concluded that oyster larvae must use additional sources of food, such as phytoplankton cells smaller than 2 μm, nanozooplankton, detritus, bacteria, or dissolved organic matter. In support of the hypothesis of Crisp et al. (1985), Baldwin and Newell (1991) used dual $^{14}C/^{3}H$ labelling techniques to demonstrate that *C. virginica* larvae are omnivorous and can feed on a wide range of types of suspended particulate material present in the natural seston, including bacteria, protozooplankton and photoautotrophs. Interestingly, 87% of the total particulate ^{14}C ingested by prodissochonch II oyster larvae (mean shell length ± S.D.; 179 ± 5 μm) that fed on 0.2 to 30 μm plankton was derived from organisms 20 to 30 μm in size and, furthermore, larvae selectively ingested particles in the 20 to 30 μm size range. Microscopic examination of seston used in the feeding studies indicated that heterotrophic protozoans and dinoflagellates (*Gyrodinium uncatenum*) dominated the 20 to 30 μm size fraction and could have significantly contributed to ^{14}C ingested by larvae. These findings suggest that other food sources apart from autotrophic nanoplankton (Sieburth et al. 1978) may be more important in meeting the nutritional requirements of bivalve larvae in their natural environment.

Many early European workers suggested that European flat oysters were mainly detritus eaters as adults (Peterson and Jensen 1911; Blegvad 1914; Savage 1925). Stable isotope analyses of adult *C. virginica* inhabiting detritus-rich marshes of the eastern United States indicated that most of the carbon incorporated in the oyster's tissues is derived from phytoplankton and not from detritus originating from marshgrass (Haines 1977; Haines and Montague 1979; Hughes and Sherr 1983). The contribution of detritus and bacteria to the nutrition of *C. virginica* inhabiting a salt marsh in Delaware was also estimated to be small (Langdon and Newell 1990) on the basis of measurements of the oyster's potential filtration and assimilation of bacteria and cellulosic detritus. Langdon and Newell (1990) estimated that suspended cellulosic detritus and bacteria combined would contribute only 6% to the metabolic carbon requirements and 27% to the metabolic nitrogen requirements of oysters inhabiting this salt marsh in the summer. However, Crosby et al. (1990) estimated that detrital complexes (non-living organic matter and attached bacteria) may provide up to 60% of the carbon requirements of *C. virginica*, under conditions where bacterial concentrations are high (10^{-7} cells ml^{-1}) and up to 30% of suspended bacteria are attached to particles.

Some of the first recorded attempts at rearing oyster larvae on diets of cultured algae were made by

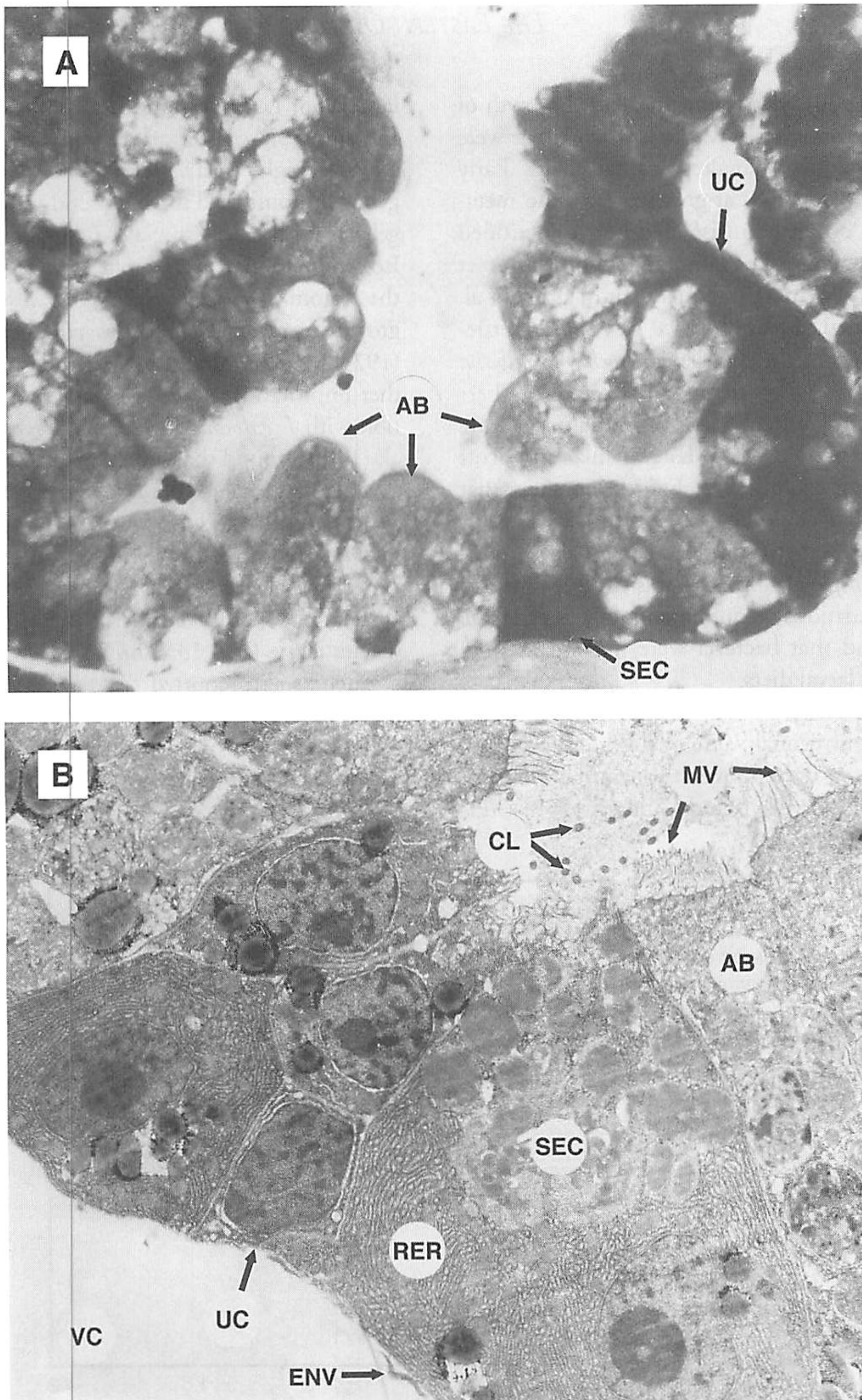

Figure 12. The larval digestive gland of *Crassostrea virginica.* (A) Histological section through the lobe of the digestive gland. × 2040. (B) Transmission electron micrograph of the digestive gland. × 7650. From Elston (1980b). [AB] absorptive cell, [CL] cilia, [ENV] enveloping cell layer, [MV] microvilli, [RER] rough endoplasmic reticulum, [SEC] secretory cell, [UC] undifferentiated cell, [VC] visceral cavity.

Cole (1937, 1938) who found that larval growth of *O. edulis* in outdoor tanks was best when larvae were provided with 2 µm or smaller algal flagellates. Early attempts by Cole (1938) at growing larvae to metamorphosis on a diet of an isolated but unidentified flagellate under controlled laboratory conditions met with some success. In a subsequent study, Bruce et al. (1939) succeeded in rearing larval *O. edulis* to settlement on isolates of the flagellates, *Isochrysis galbana* and *Pyraminomonas grossi*. These latter researchers were the first to develop successful laboratory culture techniques for bivalve larvae. Later, Millar and Scott (1967) used axenic culture techniques to show that larvae of *O. edulis* could be grown to the "eyed" pediveliger stage on a diet of *Pavlova lutheri* alone, indicating that a single algal species was capable of meeting all the nutritional requirements of oyster larvae for growth and that bacteria were not an essential component of larval diets.

Many researchers have continued to test and compare the nutritional value of different algal species as foods for oysters over the last half century (Davis 1950, 1953; Davis and Guillard 1958; Guillard 1958; Loosanoff and Davis 1963; Walne 1970; Epifanio 1976, 1979b, 1982; Babinchak and Ukeles 1979; Enright et al. 1986a). These researchers have generally confirmed the high food value of naked flagellates for oysters, especially *Isochrysis* aff. *galbana* (T-Iso) and *Pavlova lutheri*. Epifanio (1976) reported that the diatom, *Thalassiosira pseudonana*, supported good growth of juvenile *Crassostrea virginica* and Epifanio (1979b, 1982) found that oyster growth could be further improved if *T. pseudonana* was fed in combination with *I. galbana* or *Tetraselmis suecica*. Enright et al. (1986a) found that several diatom species were superior foods for juvenile *Ostrea edulis* compared to *I.* aff. *galbana* (clone T-Iso; Fig. 13). Growth of juvenile *O. edulis* fed on a diet consisting of equal cell numbers of five algal species was superior to the growth of oysters fed on full rations of the component algal species alone (Fig. 13; Enright et al. 1986a). Other researchers also reported that mixed-species algal diets were superior to single-species diets for bivalve culture (Davis 1953; Davis and Guillard 1958; Walne and Spencer 1968; Calabrese and Davis 1970; Helm 1977).

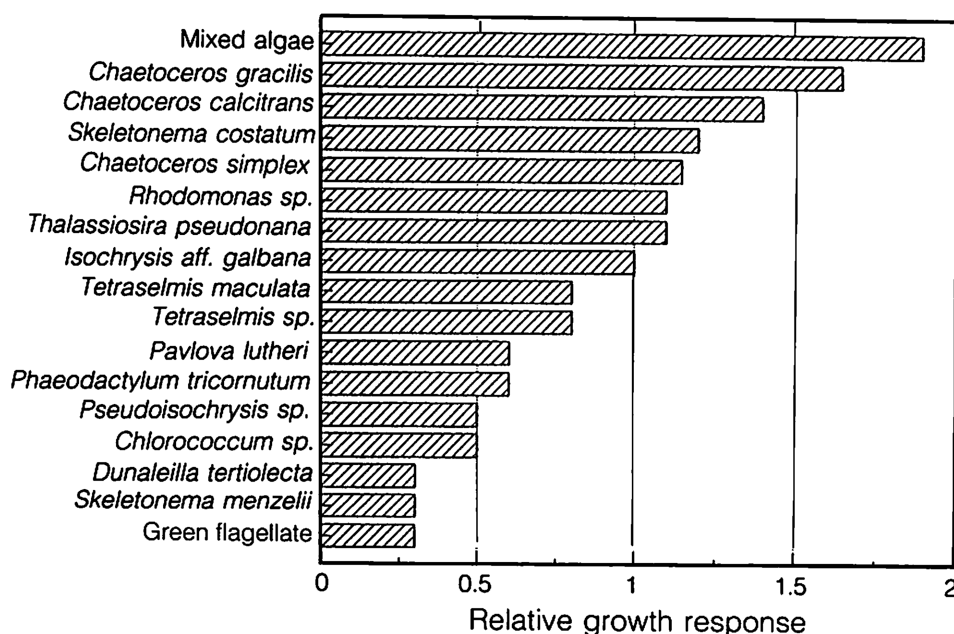

Figure 13. Growth of juvenile *Ostrea edulis* fed on different algal diets. Growth rates are expressed relative to those obtained with a diet of *Isochrysis* aff. *galbana* (T-Iso). The mixed diet consisted of equal cell numbers of *Chaetoceros gracilis*, *C. simplex*, *Isochrysis* aff. *galbana* (T-Iso), *Pavlova lutheri*, and *Phaeodactylum tricornutum*. From Enright et al. (1986a).

Algal species with indigestible cell walls, such as those belonging to the genera *Chlorella*, *Chlorococcum*, *Platymonas*, as well as *Phaeodactylum tricornutum*, have been shown to be poor foods for oysters. Digestion of *Monochrysis lutheri* and *Chlorella autotrophica* was directly observed in the stomachs of larval *C. virginica* by Babinchak and Ukeles (1979) using an epifluorescence microscope. These researchers observed that 54% of ingested cells of *M. lutheri* were lysed within 1.5 h after feeding had begun, whereas cells of *C. autotrophica* were not digested and were voided intact from larvae.

Oysters in their natural habitat are exposed to wide fluctuations in the biochemical composition of available food as the algal and detrital composition of the seston changes over tidal, seasonal, and annual cycles. Mayzaud et al. (1989) reported that protein-to-carbohydrate ratios varied from 0.6:1 (June, 1976) to 2.6:1 (July, 1976) in Bedford Basin, Nova Scotia, Canada (Fig. 14). In addition to seasonal variations in seston composition, annual and site specific variations in patterns of primary production alter the biochemical quality and quantity of available food (Berg and Newell 1986).

Wide variations in the gross biochemical composition of algal diets have been reported due to differences both between species within the same genus and within strains of the same species (Flaak and Epifanio 1978; Bourne et al. 1989, Patterson 1992; Fig. 15). Variation can also arise because cultured algal species may be misidentified (Patterson 1992) or have been in culture for so long that a strain with a different biochemical composition to that of the initial isolate may have arisen. Biochemical composition can also be affected by specific laboratory culture conditions such as light regime (Morris 1981) and the availability of nutrients in the algal culture medium (Gallager and Mann 1981; Enright et al 1986b; Utting 1986). The growth phase of the culture at the time of harvest can also alter the carbon:nitrogen (C:N) ratio and, hence, biochemical composition (Chu and Dupuy 1980; Whyte 1987; Whyte et al. 1989). All of these possible sources of variation indicate that it is advisable that periodic biochemical analyses of algae cultured as food sources for marine invertebrates should be undertaken. Only in this way can it be assured that the algal strains and culture conditions used are producing a uniform, if not optimum, diet.

There have been many attempts to determine the effects of variations in gross biochemical composition of algal diets on the growth of bivalves (Flaak and Epifanio 1978; Gallager and Mann 1981; Enright et al. 1986b; Utting 1986, Wikfors et al. 1991). Despite the widely reported importance of lipid as an energy reserve of bivalve larvae (see reviews by Holland 1978 and Gabbott 1983), Waldock and Nascimento (1979) found no correlation between the proportion of lipid in algal diets and the growth of larval *Crassostrea gigas*. Whyte et al. (1989) also found no correlation between the proportion of protein or lipid in algal diets and either growth or total energy content of Japanese scallop, *Patinopecten yessoensis*, larvae; however, growth and energy content of larvae were correlated with the proportion of carbohydrate in the diet. In contrast, Chu et al. (1982) found no correlation between the nutritional value of algal diets and their carbohydrate content. Utting (1986) ex-

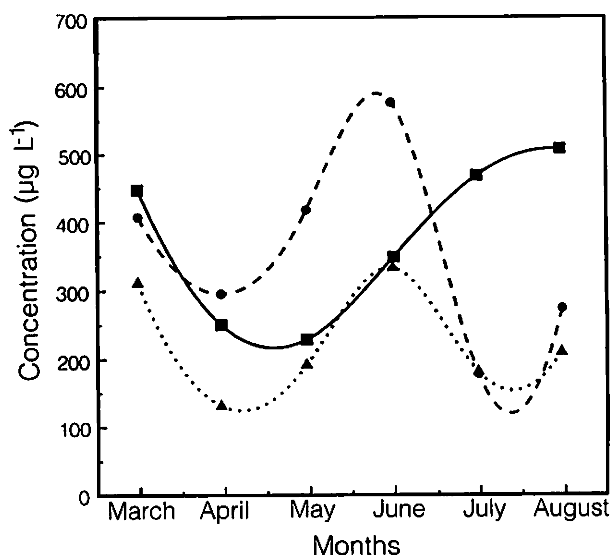

Figure 14. Seasonal fluctuations in the concentration (µg L^{-1}) of protein (■), carbohydrate (●), and lipid (▲) associated with particles in the 1.2 to 163 µm size fraction from water samples collected (1976) from Bedford Basin, Nova Scotia, Canada. Redrawn from data in Mayzaud et al. (1989).

amined the effect of algal composition on settlement of larval *C. gigas* and reported that protein content of algal diets was positively correlated with successful settlement.

In laboratory experiments, growth of juvenile *C. virginica* has been found to be positively correlated with C:N ratios of cultured algal diets i.e., diets with low protein content support better growth than diets with high protein content (Flaak and Epifanio 1978). In support of this finding, Utting (1986) reported a negative correlation between algal protein content and growth of juvenile *C. gigas*. Furthermore, growth of both juvenile *Ostrea edulis* (Enright et al. 1986b) and *C. virginica* (Wikfors et al. 1984) could be increased by feeding oysters on algae with high carbohydrate content.

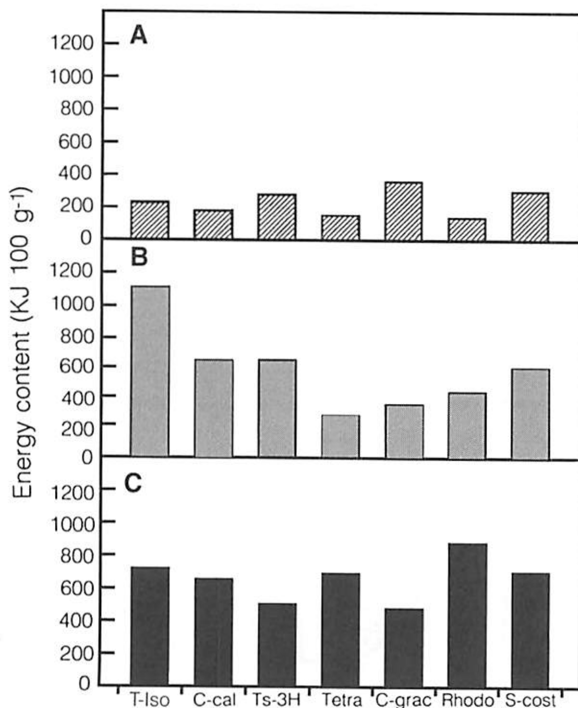

Figure 15. Variation in energy content of various biochemical fractions (KJ 100 g^{-1} dry weight algal biomass) of algal species commonly used in the culture of bivalves (A) Carbohydrate. (B) Lipids. (C) Protein. Energy content calculated as described by Whyte (1989). [T-Iso] *Isochrysis* aff. *galbana* (Tahitian), [C-cal] *Chaetoceros calcitrans*, [Ts-3H] *Thalassiosira pseudonana*, [Tetra] *Tetraselmis suecica*, [C-grac] *Chaetoceros gracilis*, [Rhodo] *Rhodomonas* sp., [S-cost] *Skeletonema costatum*. Redrawn from data in Bourne et al. (1989).

Research by Hawkins and co-workers on the protein and energy requirements of *Mytilus edulis* (reviewed by Hawkins and Bayne 1991) indicates that blue mussels are able to efficiently recycle protein within their body tissues, albeit with high energetic costs. These authors predicted that blue mussels and other sedentary filter-feeders are more likely to be growth-limited by dietarily available energy rather than by protein. They estimated that the average protein requirements for maintenance in blue mussels is 87.3 ± 29.1 mg protein N dry weight $kg^{-0.75}d^{-1}$, a value that is similar to those reported for fish and mammals.

Very little is known about the amino acid requirements of oysters and other bivalve species. Manahan (1990a) reported that axenic *C. gigas* larvae could take up dissolved ^{14}C-glucose from seawater and incorporate ^{14}C into the "essential" amino acids valine, isoleucine, leucine, lysine, histidine, and arginine. However, it is not known whether rates of synthesis are sufficient to meet larval requirements for these amino acids. Future work on amino acid requirements of oysters depends on the development of defined artificial diets.

Amino acid compositions of commonly used algal food species have been reviewed by Webb and Chu (1982), with additional analyses provided by Enright et al. (1986b) and Whyte et al. (1987). On the basis of these reports, algal species appear to possess all the amino acids commonly incorporated in protein and, therefore, should supply all the essential amino acid requirements of oyster s for tissue growth. A possible exception to this generalization is the reported deficiency of tryptophan in *Phaeodactylum tricornutum* which Epifanio et al. (1981) suggested could explain the poor food value of this algal species for *C. virginica*. However, this reported lack of tryptophan may be due to the loss of this labile amino acid during protein hydrolysis and amino acid analysis.

Webb and Chu (1982) attempted to determine if the food value of various algal species could be explained by the balance of amino acids present in algal protein, assuming that the nutritional value of the protein was greatest when it was made up of essential

amino acids in similar proportions to those of the bivalve's body tissues. They found a correlation between overall food value and predicted dietary protein quality of algal species. Therefore, the quantitative amino acid composition of algae may be a factor in determining algal food quality for bivalve molluscs.

Analyses of unicellular algae indicate that nutritionally satisfactory species for bivalve molluscs possess significant levels of eicosapentaenoic (20:5ω3) and docosahexaenoic (22:6ω3) fatty acids (Fig. 16; Ackman 1982; Webb and Chu 1982; Volkman et al. 1989; Thompson et al. 1990). Algal species belonging to the class Chlorophyceae are generally poor foods for bivalve molluscs and are deficient in or possess low concentrations of 20:5ω3 and 22:6ω3 fatty acids.

Langdon and Waldock (1981) fed juvenile *Crassostrea gigas* on the chlorophyte alga, *Dunaliella tertiolecta* (which is deficient in the higher polyunsaturated fatty acids 20:5ω3 and 22:6ω3), either with or without encapsulated supplements of 22:6ω3. Growth rates of oysters fed on 22:6ω3-supplemented algal diets were greater compared to those of animals fed on *D. tertiolecta* alone. Furthermore, no improved oyster growth was observed when Pacific oysters were fed on *D. tertiolecta* supplemented with a calorically equivalent amount of encapsulated triolein, indicating that the observed higher growth of individuals fed on algae supplemented with 22:6ω3 was not simply due to an increase in the caloric content of the diet. In addition, fatty acid analyses of *C. gigas* fed on *D. tertiolecta* alone indicated no capability for synthesis of 20:5ω3 or 22:6ω3 fatty acids. Later studies using ^{14}C-tracer techniques have supported the conclusion that *C. gigas* and *C. virginica* have a dietary requirement for these polyunsaturated fatty acids by demonstrating limited synthesis of both 20:5ω3 and 22:6ω3 from ^{14}C-la-

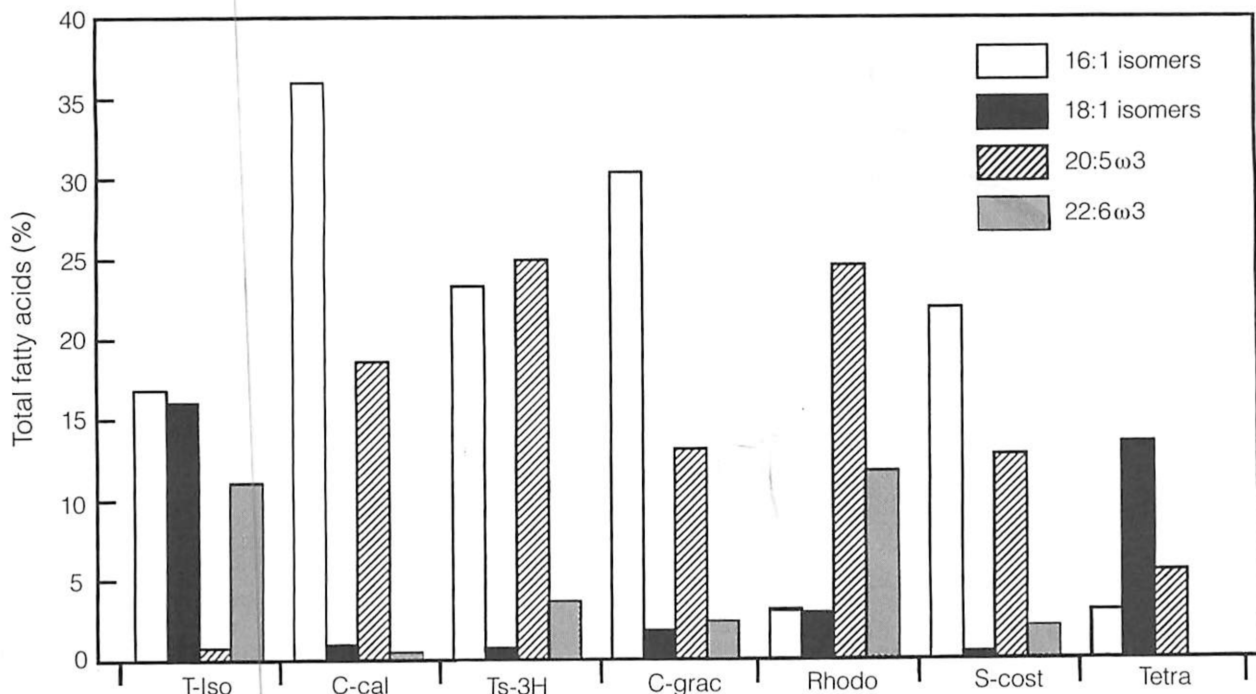

Figure 16. Percent concentrations of 16:1 and 18:1 isomers, eicosapentaenoic (20:5ω3) and docosahexaenoic (22:6ω3) fatty acids in algal species commonly used in the culture of bivalves. Abbreviations for algal species the same as those for Fig. 15. Redrawn from data in Bourne et al. (1989).

belled precursors (Waldock and Holland 1984; Chu and Greaves 1991).

Because of a lack of information concerning the amount of polyunsaturated fatty acids required by bivalves to sustain balanced germinal and somatic growth, it is unknown whether oyster growth is limited by the availability of fatty acids in nature. Mayzaud et al. (1989) reported that both 20:5ω3 and 22:6ω3 fatty acids were present from March to August, 1976, at concentrations ranging from 0.53 to 11.10% (20:5ω3) and 0.94 to 9.46% (22:6ω3) of the total fatty acids extracted from seston collected from Bedford Basin. These amounts may or may not be sufficient to allow bivalves in this locality to meet their nutritional requirements for these two fatty acids.

Sterols are necessary elements in the nutrition of all animals, although many invertebrate species only possess a limited capacity for their synthesis (Nes and McKean 1977). Bivalve molluscs appear incapable of the *de novo* synthesis of sterols and, therefore, dietary sources of sterols are essential (Tamura et al. 1964; Teshima 1982). For example, Salaque et al. (1966) reported that the oyster, *Ostrea gryphea,* did not synthesize sterols from mevalonate, and similar findings were reported by Walton and Pennock (1972) for both *Mytilus edulis* and *Cardium edule.* Voogt (1975) showed that bivalves alkylate pre-existing sterols with ^{14}C from a ^{14}C-acetate precursor and that *de novo* sterol synthesis is limited. Initial reports by Teshima and Patterson (1981b) that *Crassostrea virginica* could synthesize four sterols from ^{14}C-labelled acetate were found to be inaccurate by Holden and Patterson (1991). Apparently, aquatic fungi contaminating the original oyster tissue incubations performed by Teshima and Patterson (1981b) were most likely responsible for synthesizing the observed sterols (Holden and Patterson 1991). Although it is thought that bivalves do have some capacity for the transformation by dealkylation of dietary algal sterols (Teshima 1982), the quantitative significance of such transformations in supplying the essential sterols for bivalves has yet to be determined. The importance of sterols to the nutrition of *C. virginica* was investigated by Wikfors et al. (1991) in growth studies in which juvenile eastern oysters were fed 16 microalgal diets. A detailed analysis of these data (G. Patterson, University of Maryland, pers. comm.) indicates that the amount and composition of the algal sterols accounted for 35% of the difference observed in oyster growth between diets. The amount of two essential fatty acids (20:5ω3 and 22:6ω3) in each diet accounted for a further 28% of the observed growth differences.

Mayzaud et al. (1989) measured seasonal fluctuations in the concentrations of sterols in Bedford Basin from March to August and found that cholesterol and 24-methylenecholesterol were the major sterols present (Fig. 17), whereas nor-24-cholesterol, coprostanol, 22-dehydrocholesterol, brassicasterol, desmosterol, sitosterol, and fucosterol where always minor components. Cholesterol is also commonly present in small quantities in algal food species used in oyster culture (Lin et al. 1982). The sterol composition of oysters tends to reflect that of the diet (Idler and Wiseman 1972; Teshima et al. 1980; Berenberg and Patterson 1981; Teshima and Patterson 1981a; Gordon and Collins 1982; Wikfors et al. 1991). Cholesterol is the most abundant sterol in the tissues of most marine bivalve species (Idler and Wiseman 1972).

ARTIFICIAL DIETS

Some progress has been made towards the development of non-algal artificial diets for bivalves. Initially these studies were designed to "fatten" oysters by feeding them on carbohydrate supplements. Haven (1965) reported significantly higher wet tissue weights of *Crassostrea virginica* held in flowing seawater and fed on supplements of starch or dextrin, compared with those of starved controls. Similar studies have confirmed that it is possible to increase tissue weight and glycogen content of *C. virginica* with carbohydrate supplements (Gillespie et *al.* 1965; Sayce and Tufts 1967; Dunathan et al. 1969; Turgeon and Haven 1978).

Epifanio (1979a, 1982) fed juvenile *C. virginica* on partial algal rations supplemented with yeast but reported that they did not benefit from yeast additions and that growth was simply dependent on the proportion of algae in the diet. Urban and Langdon (1984) performed a series of growth studies in which *C. virginica* was fed on partial algal rations supple-

mented with various non-algal foods. They found that up to 50% of an algal ration could be substituted with a mixture of yeast, rice starch, and kaolin without a significant reduction in oyster growth.

There have been several attempts to develop diets that would completely replace algae as a food for cultured oysters. Claus and Adler (1970) reported that adult *C. virginica* fed on formalin-cured, mammalian red blood cells grew at equal or greater rates compared with growth rates of oysters fed on living algal diets; however, these results have not been confirmed by other researchers (Masson 1977). Castell and Trider (1974) performed a series of experiments in which formulated artificial diets were fed to juvenile *C. virginica.* They reported that oysters fed on the artificial diets grew at about one tenth the rate of oysters grown in nature.

Further progress in the development of artificial diets for oysters has resulted from the application of microencapsulation techniques for nutrient delivery (Langdon et al. 1985). Toner (1978) encapsulated algae within starch microspheres and observed an increase in the ash-free weight of juvenile *C. gigas* fed on these capsules. Gabbott et al. (1976) and Langdon (1982) encapsulated a diet of hemoglobin, starch, and cholesterol in capsules with walls composed of cross-linked, nylon-protein; the growth of C. *gigas* fed on this encapsulated diet was only about one fifth that of Pacific oysters fed on an algal diet, possibly because the diet was not nutritionally complete.

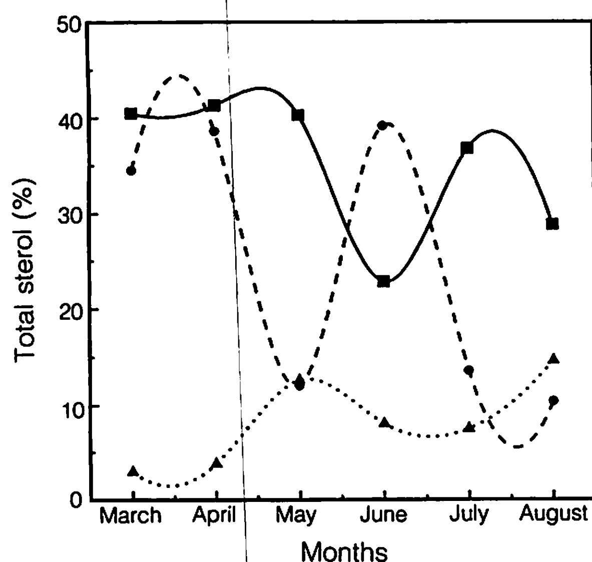

Figure 17. Seasonal fluctuations in the relative concentrations of sterols (■ cholesterol; ▲ 24-methylenecholesterol; ● brassicasterol) associated with particles in the 1.2 to 163 µm size fraction from water samples collected (1976) from Bedford Basin, Nova Scotia, Canada. Redrawn from data in Mayzaud et al. (1989).

Subsequently, improvements have been made in methods for preparing cross-linked, protein-walled capsules that have resulted in both the elimination of potentially toxic detergents (Tweens) from the encapsulation process, and the elimination of nylon from the cross-linked wall (Jones et al. 1987; Langdon 1989; Langdon and DeBevoise 1990). Langdon and DeBevoise (1990) reported that juvenile *C. gigas* assimilated ^{14}C-protein from protein-walled capsules with an efficiency of 39%, which was significantly greater than an efficiency of 29% for oysters fed on glyceride-coated, nylon-protein walled capsules; however, the latter retained ^{14}C-protein more efficiently (86%) than protein-walled capsules (62%) when suspended in non-sterile seawater at 25°C for 24 h. Therefore, selection of capsule type to maximize use of encapsulated protein by cultured marine suspension-feeders should depend on both the susceptibility of capsules to leakage losses when suspended in the culture medium as well as the ability of the cultured suspension-feeder to digest and assimilate encapsulated material.

Cross-linked, protein-walled capsules have been commercially prepared ("Frippak," Mars U.K. Ltd) for the delivery of diets to penaeid shrimp larvae (Jones et al. 1987) and bivalves (Laing 1987). Laing (1987) used a "Frippak" diet in growth experiments with juvenile *C. gigas* and reported that up to 60% of an algal ration could be substituted with the encapsulated diet without significant reductions in growth, compared with the growth of oysters fed a full algal ration.

Various lipid-walled, artificial particle types have also been use to deliver dietary ingredients to oysters. Parker and Selivonchick (1986) demonstrated that liposomes (phospholipid-walled vesicles) delivered lipids, protein, amino acids, and glucose to juvenile *C. gigas* and that individuals were able to digest and metabolize these encapsulated nutrients. Langdon (1982) re-

ported improved growth of juvenile *C. virginica* fed on algae with supplements of lipid-walled capsules containing water-soluble vitamins, compared with that of oysters fed algae alone. Chu et al. (1987) also used lipid-walled capsules to deliver a diet of protein, dextrose, and B vitamins to larval *C. virginica*. They reported that in some experiments, capsule-fed larvae grew to settlement and metamorphosis, whereas no larval settlement was observed in other cultures. Chu et al. (1987) speculated that the observed variation in the performance of capsule-fed larvae was due to differences in bacterial populations present in larval cultures.

Langdon and Bolton (1984) described a microencapsulated artificial diet for juvenile *C. virginica* which supported growth that was equal to that of oysters fed on a full algal ration. However, growth of oysters fed on the microencapsulated diet was reduced by about 50% when antibiotics were added to the culture medium, suggesting that bacteria were important in the nutrition of oysters fed on the microencapsulated diets.

Further definition of the nutritional requirements of oysters and other suspension-feeders will be dependent on axenic culture techniques in order to eliminate the effects of bacterial contamination on oyster growth (Ukeles 1971). Langdon (1982, 1983) described a simple technique for obtaining axenic oyster larvae without the use of antibiotics. He performed a series of growth experiments in which axenic larvae of *C. gigas* were fed on a biphasic artificial diet of dissolved and particulate nutrients. The growth of the larvae on the artificial diet was limited, possibly due to difficulties in delivering the nutrients to the larvae. Future application of improved encapsulation techniques may overcome problems in the delivery of artificial diets to axenic bivalve larvae and lead to a better understanding of bivalve nutrition.

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REFERENCES

Ackman, R.G. 1982. Fatty acid metabolism of bivalves. Pages 358-376 in G.D. Pruder, C.J. Langdon and D.E. Conklin (eds.). Second International Conference on Aquaculture Nutrition: Biochemical and Physiological Approaches to Shellfish Nutrition. Louisiana State University, Baton Rouge.

Auffret, M. 1988. Bivalve hemocyte morphology. American Fisheries Society Special Publication 18:169-280.

Babinchak, J. and R. Ukeles. 1979. Epifluorescence microscopy, a technique for the study of feeding in *Crassostrea virginica* veliger larvae. Mar. Biol. 51:69-76.

Baldwin, B.S, and R.I.E. Newell. 1991. Omnivorous feeding by planktotrophic larvae of the eastern oyster *Crassostrea virginica*. Mar. Ecol. Prog. Ser. 78:285-301.

Bamford, D.R. and R. Gingles. 1974. Absorption of sugars in the gill of the Japanese oyster, *Crassostrea gigas*. Comp. Biochem. Physiol. 49:637-646.

Bayne, B.L. 1983. Physiological ecology of marine molluscan larvae. Pages 299-343 in N.H. Verdonk, J.A.M. van den Biggelaar and A.S. Tompa (eds.). The Mollusca, Volume 3. Academic Press, New York.

Bayne, B.L., A.J.S. Hawkins and E. Navarro. 1987. Feeding and digestion by the mussel *Mytilus edulis* L. (Bivalvia:Mollusca) in mixtures of silt and algal cells at low concentrations. J. Exp. Mar. Biol. Ecol. 111:1-22.

Bayne, B.L., A.J.S. Hawkins, E. Navarro and I.P. Iglesias. 1989. Effects of seston concentration on feeding, digestion and growth in the mussel *Mytilus edulis*. Mar. Ecol. Prog. Ser. 55:47-54.

Bayne, B.L., D.W. Klumpp and K.R. Clarke. 1984. Aspects of feeding, including estimates of gut residence time, in three mytilid species (Bivalvia, Mollusca) at two contrasting sites in the Cape Peninsula, South Africa. Oecologia 64:26-33.

Bayne, B.L. and R.C. Newell. 1983. Physiological energetics of marine mollusca. Pages 407-515 in A.S.M. Saleuddin and K.M. Wilbur (eds.). The Mollusca, Volume 4. Academic Press, New York.

Beninger, P.G., M. Auffret and M. Le Pennec 1990. Peribuccal organs of *Placopecten magellanicus* and *Chlamys*

varia (Mollusca: Bivalvia): structure, ultrastructure and implications for feeding. I. The labial palps. Mar. Biol. 107:215-223.

Beninger, P.G., M. Le Pennec and A. Donval. 1991. Mode of particle ingestion in five species of suspension-feeding bivalve molluscs. Mar. Biol. 108:255-261.

Berenberg, C.J., and G.W. Patterson. 1981. The relationship between dietary physosterols and the sterols of wild and cultivated oysters. Lipids 16:276-278.

Berg, J.A. and R.I.E. Newell. 1986. Temporal and spatial variations in the composition of seston available to the suspension feeder, *Crassostrea virginica*. Est. Coastal Shelf Sci. 23:375-386.

Bernard, F.R. 1973. Crystalline style formation and function in the oyster *Crassostrea gigas* (Thunberg, 1785). Ophelia 12:159-170.

Birkbeck, T.H. and J.G. McHenery. 1984. Chitinase in the mussel, *Mytilus edulis* (L.). Comp. Biochem. Physiol. 77B:861-865.

Blegvad, H. 1914. Food and conditions of nourishment among the communities of invertebrate animals on or in the sea bottom in Danish waters. Rept. Dan. Biol. Sta. to the Board of Agric. 22:43-78.

Bourne, N., C.A. Hodgson and J.N.C. Whyte. 1989. A manual for scallop culture in British Columbia. Can. Tech. Rep. Fish. Aquat. Sci. 1694. 215 pp.

Brock, T.D. and M.T. Madigan. 1988. Biology of Microorganisms. Prentice Hall, Englewood Cliffs, New Jersey. 835 pp.

Brock, V. 1989. *Crassostrea gigas* (Thunberg) hepatopancreas-cellulase kinetics and cellulolysis of living monocellular algae with cellulose walls. J. Exp. Mar. Biol. Ecol. 128:157-164.

Brock, V. and V.S. Kennedy. 1992. Quantitative analysis of crystalline style carbohydrases in five suspension- and deposit-feeding bivalves. J. Exp. Mar. Biol. Ecol. 159:51-58.

Brock, V., V.S. Kennedy and A. Brock. 1986. Temperature dependency of carbohydrase activity in the hepatopancreas of thirteen estuarine and coastal bivalve species from the North American east coast. J. Exp. Mar. Biol. Ecol. 103:87-101.

Bruce, J.R., M. Knight and M.W. Parke. 1939. The rearing of oyster larvae on an algal diet. J. Mar. Biol. Assoc. U.K. 24:337-374.

Calabrese, A. and H.C. Davis. 1970. Tolerances and re quirements of embryos and larvae of bivalve molluscs. Helgol. Wissen. Meersunters. 20:553-564.

Calow, P. 1975. The feeding strategies of two freshwater gastropods, *Ancylus fluviatilus* Mull. and *Planorbis contortus* Linn. (Pulmonata), in terms of ingestion rates and absorption efficiencies. Oecologia 20:33-49.

Calow, P. 1977. Ecology, evolution and energetics: a study in metabolic adaptation. Adv. Ecol. Res. 10:1-62.

Castell, J.D. and D.J. Trider. 1974. Preliminary feeding trials using artificial diets to study the nutritional requirements of oysters (*Crassostrea virginica*). J. Fish. Res. Board Can. 31:95-99.

Chu, F.-L. E. and J.L. Dupuy. 1980. The fatty acid composition of three unicellular algal species used as food sources for larvae of the American oyster (*Crassostrea virginica*). Lipids 15:356-364.

Chu, F.-L. E., J.L. Dupuy. and K.L. Webb. 1982. Polysaccharide composition of five algal species used as food for larvae of the American oyster, *Crassostrea virginica*. Aquaculture 29:241-252.

Chu, F.-L. E. and J. Greaves. 1991. Metabolism of palmitic, linoleic and linolenic acids in adult oysters, *Crassostrea virginica*. Mar. Biol. 110:228-236.

Chu, F.-L. E., K.L. Webb, D.A. Hepworth and B.B. Casey. 1987. Metamorphosis of larvae of *Crassostrea virginica* fed microencapsulated diets. Aquaculture 64:185-197.

Claus, G. and C. Adler. 1970. Optional conditions for oysters grown in closed environments. Proc. Mar. Tech. Soc. 1:671-679.

Cole, H.A. 1937. Experiments in the breeding of oysters (*Ostrea edulis*) in tanks, with special reference to the food of larvae and spat. Fish. Invest., Min. Agric. Fish., Lond., Ser II, 15:1-24.

Cole, H.A. 1938. A system of oyster culture. J. Cons. Perm. Internat. Explor. Mer. 13:221-235.

Colwell, R.R. and J. Liston. 1960. Microbiology of shellfish: bacteriological study of the natural flora of Pacific oysters (*Crassostrea gigas*). Appl. Microbiol. 8:104-109.

Colwell, R.R. and J. Liston. 1961. A bacteriological study of the natural flora of Pacific oysters (*Crassostrea gigas*) when transplanted to various areas in Washington. Proc. Natl. Shellfish. Assoc. 50:181-188.

Crisp, D.J., A.B. Yule and K.N. White. 1985. Feeding by oyster larvae: the functional response, energy budget and a comparison with mussel larvae. J. Mar. Biol. Assoc. U.K. 65:759-783.

Crosby, M.P., C.J. Langdon and R.I.E. Newell. 1989. Importance of refractory plant material to the carbon budget of the oyster *Crassostrea virginica*. Mar. Biol. 100:343-352.

Crosby, M.P., R.I.E. Newell and C.J. Langdon. 1990. Bacterial mediation in the utilization of carbon and

nitrogen from detrital complexes by *Crassostrea virginica*. Limnol. Oceanogr. 35:625-639.

Crosby, N.D. and R.G. Reid. 1971. Relationships between food, physiology and cellulose digestion in Bivalvia. Can. J. Zool. 49:617-622.

Davis, H.C. 1950. On the food requirements of larvae of *Ostrea virginica*. Anat. Rec. 108:132-133.

Davis, H.C. 1953. On the food and feeding of larvae of the American oyster, *C. virginica*. Biol. Bull. 104:334-350.

Davis, H.C. and R.R. Guillard. 1958. Relative value of ten microorganisms as foods for oyster and clam larvae. Fish. Bull. 58:293-304.

Dean, B. 1887. The food of the oyster: its conditions and variations. Second Rep. Oyster Invest., State of New York, Albany.

Dean, D. 1958. New property of the crystalline style of *Crassostrea virginica*. Science 128:837.

Dean, H.K. 1980. Microstructure of the crystalline style of *Spisula solidissima* (Bivalvia: Mactridae). The Nautilus 94:54-58.

Dimitroff, V.T. 1926. Spirochaetes in Baltimore market oysters. J. Bacteriol. 12:65-135.

Doyle, J. 1966. Studies on the chemical nature of the crystalline style. Pages 256-263 in H. Barnes (ed.). Some Contemporary Studies in Marine Science. Allen and Unwin, London.

Dunathan, J.P., R.M. Ingle and W.K. Havens. 1969. Effects of artificial foods upon oyster fattening with potential commercial applications. Florida Res. Lab. Tech. Ser. 58:1-38.

Eble, A.F. 1966. Some observations on the seasonal distribution of neglected enzymes in the American oyster as revealed by enzyme histochemistry. Proc. Nat. Shellfish. Assoc. 56:37-42.

Elston, R. 1980a. Functional morphology of the coelomocytes of the larval oysters (*Crassostrea virginica* and *Crassostrea gigas*). J. Mar. Biol. Assoc. U.K. 60:947-957.

Elston, R. 1980b. Functional anatomy, histology and ultrastructure of the soft tissues of the larval American oyster, *Crassostrea virginica*. Proc. Natl. Shellfish. Assoc. 70:65-93.

Enright, C.T., G.F. Newkirk, J.S. Craigie and J.D. Castell. 1986a. Evaluation of phytoplankton as diets for juvenile *Ostrea edulis* L. J. Exp. Mar. Biol. Ecol. 96:1-13.

Enright, C.T., G.F. Newkirk, J.S. Craigie and J.D. Castell. 1986b. Growth of juvenile *Ostrea edulis* L. fed *Chaetoceros gracilis* Schutt of varied chemical composition. J. Exp. Mar. Biol. Ecol. 96:15-26.

Epifanio, C.E. 1976. Culture of bivalves mollusks in recirculating systems: nutritional requirements. Pages 173-194 in K.S. Price, W.N. Shaw and K.S. Danberg (eds.). Proceedings of the First International Conference on Aquaculture Nutrition. University of Delaware Press, Newark, Delaware.

Epifanio, C.E. 1979a. Comparison of yeast and algal diets for bivalve mollusks. Aquaculture 16:187-192.

Epifanio, C.E. 1979b. Growth in bivalve mollusks: nutritional effects of two or more species of algae in diets fed to the American oyster *Crassostrea virginica* (Gmelin) and the hard clam *Mercenaria mercenaria* (L.). Aquaculture 18:1-12.

Epifanio, C.E. 1982. Phytoplankton and yeast as foods for juvenile bivalves: a review of research at the University of Delaware. Pages 292-304 in G.D. Pruder, C.J. Langdon and D.E. Conklin (eds.). Second International Conference on Aquaculture Nutrition: Biochemical and Physiological Approaches to Shellfish Nutrition. Louisiana State University, Baton Rouge.

Epifanio, C.E. and J. Ewart. 1977. Maximum ration of four algal diets for the oyster *Crassostrea virginica* Gmelin. Aquaculture 11:13-29.

Epifanio, C.E., C.C. Valenti and C.L. Turk. 1981. A comparison of *Phaeodactylum tricornutum* and *Thalassiosira pseudonana* as foods for the oyster, *Crassostrea virginica*. Aquaculture 23:347-353.

Erdmann, W. 1935. Untersuchungen über die Lebensgeschichte der Auster. Nr. 5.Über die Entwicklung und die Anatomie der "ansatzreifen" Larve von *Ostrea edulis* mit Bermerkungen über die Lebensgeschichte der Auster. Wiss. Meeresunters., Abt. Helgoland 19: 1-25.

Ewart, J.W. and M.R. Carriker. 1983. Characteristics of fecal ribbons from juveniles of *Crassostrea virginica* (Gmelin) fed *Phaeodactylum tricornutum* Bohlin with and without the addition of silt: preliminary observations (Abstract). J. Shellfish Res. 3:90.

Farley, C.A. 1968. *Minchinia nelsoni* (Haplosporida) disease syndrome in the American oyster, *Crassostrea virginica*. J. Protozool. 15:585-599.

Favorov, V.V. and V.E. Vaskovsky. 1971. Alginases of some marine invertebrates. Comp. Biochem. Physiol. 38: 689-696.

Feng, S.Y., J.S. Feng, C.N. Burke and L.H. Khairallah. 1971. Light and electron microscopy of the leucocytes of *Crassostrea virginica* (Mollusca:Pelecypoda). Z. Zellforsch. 120:222-245.

Flaak, A.R. and C.E. Epifanio. 1978. Varying protein levels in the diet of the oyster *Crassostrea virginica* (Gmelin). Mar. Biol. 45:157-163.

Foster-Smith, R.L. 1975. The effect of concentration of suspension and inert material on the assimilation of algae by three bivalves. J. Mar. Biol. Assoc. U.K. 55:411-418.

Fritz, L.W., R.A. Lutz, M.A. Foote, C.L. Van Dover and J.W. Ewart. 1984. Selective feeding and grazing rates of oyster (*Crassostrea virginica*) larvae on natural phytoplankton assemblages. Estuaries 7:513-518.

Gabbott, P.A. 1983. Developmental and seasonal metabolic activities in marine mollusks. Pages 165-217 in P.W. Hochachka (ed.). The Mollusca, Volume 2. Academic Press, New York.

Gabbott, P.A. and D.L. Holland. 1973. Growth and metabolism of *Ostrea edulis* larvae. Nature 241:475-476.

Gabbott, P.A., D. A. Jones and D.H. Nichols. 1976. Studies on the design and acceptability of microencapsulated diets for marine particle feeders. II. Bivalve mollusks. Pages 127-141 in G. Persoone and E. Jaspers (eds.). Proc. 10th Europ. Symp. Mar. Biol. Universa Press, Wetteren, Belgium.

Gallager, S.M. and R. Mann. 1981. The effect of varying carbon/nitrogen ratio in the phytoplankter *Thalassiosira pseudonana* (3H) on its food value to the bivalve *Tapes japonica*. Aquaculture 26:95-105.

Galtsoff, P.S. 1964. The American oyster *Crassostrea virginica* Gmelin. Fish. Bull. 64:1-480.

Garland, C.D., G.V. Nash and T.A. McMeekin. 1982. Absence of surface-associated microorganisms in adult oysters (*Crassostrea gigas*). Appl. Environ. Microbiol. 44:1205-1211.

George, W.C. 1952. The digestion and absorption of fat in lamellibranchs. Biol. Bull. 102:118-127.

Gillespie, L., R.M. Ingle and W.K. Havens. 1965. Glucose nutrition and longevity in oysters. Quart. J. Florida Acad. Sci. 27:279-288.

Gillmor, R.B. 1982. Assessment of intertidal growth and capacity adaptations in suspension-feeding bivalves. Mar. Biol. 68:277-286.

Giusti, F. 1970. The fine structure of the style sac and intestine in *Mytilus galloprovincialis* (Lamellibranchia). Proc. Malac. Soc. Lond. 39:95-111.

Gordon, D.T. and N. Collins. 1982. Anatomical distribution of sterols in oysters (*Crassostrea gigas*). Lipids 17: 811-817.

Guillard, R.R. 1958. Some factors in the use of nanoplankton as food for larval and juvenile bivalves. Proc. Natl. Shellfish. Assoc. 48:134-142.

Haines, E.B. 1977. The origins of detritus in Georgia salt marsh estuaries. Oikos 29:254-260.

Haines, E.B. and C.L. Montague. 1979. Food sources of estuarine invertebrates analyzed using $^{13}C/^{14}C$ ratios. Ecology 60:48-56.

Halton, D.W. and G. Owen. 1968. The fine structure and histochemistry of the gastric cuticle of the protobranchiate bivalve, *Nucula sulcata*. Proc. Malac. Soc. Lond. 38:71-81.

Haven, D.S. 1965. Supplemental feeding of oysters with starch. Chesapeake Sci. 6:43-51.

Hawkins, A. J. S. and B. L. Bayne. 1991. Nutrition of marine mussels: factors influencing the relative utilizations of protein and energy. Aquaculture 94:177-196.

Helm, M.M. 1977. Mixed algal feeding of *Ostrea edulis* larvae with *Isochrysis galbana* and *Tetraselmis suecica*. J. Mar. Biol. Assoc. U.K. 57:1019-1029.

Henrichs, S.M. and J.W. Farrington. 1979. Amino acids in the interstitial waters of marine sediments. Nature 297:955-959.

Henrichs, S.M. and P.M. Williams. 1985. Dissolved and particulate amino acids and carbohydrates in the sea surface microlayer. Mar. Chem. 17:141-164.

Holden, M. and G.W. Patterson. 1991. Absence of sterol biosynthesis in oyster tissue cultures. Lipids 26:81-82.

Holland, D.L. 1978. Lipid reserves and energy metabolism in the larvae of benthic marine invertebrates. Pages 85-123 in D.C. Malins and J.R. Sargent (eds.). Biochemical and Biophysical Perspectives in Marine Biology. Academic Press, New York.

Horst, R. 1884. The development of the oyster (*Ostrea edulis* L.). Rep. of the Commissioner for 1884, U.S. Comm. on Fish and Fisheries 12:891-911.

Hughes, E.H. and E.B. Sherr. 1983. Subtidal food webs in a Georgia estuary: ^{13}C analysis. J. Exp. Mar. Biol. Ecol. 67:227-242.

Idler, D.R. and P. Wiseman. 1972. Molluscan sterols: a review. J. Fish. Res. Bd. Can. 29:385-398.

Jeuniaux, C. 1982. La chitine dans le règne animal. Bull. Soc. Zool. Fr. 107:363-386.

Jones, D.A., K. Kurmaly and A. Arshard. 1987. Penaeid shrimp hatchery trials using microencapsulated diets. Aquaculture 64:133-288.

Jørgensen, C.B. 1976. August Pütter, August Krogh, and modern ideas on the use of dissolved organic matter in aquatic environments. Biol. Rev. 51:291-328.

Jørgensen, C.B. 1983. Patterns of uptake of dissolved amino acids in mussels (*Mytilus edulis*). Mar. Biol. 73: 177-182.

Kellogg, J,L. 1915. Ciliary mechanisms of lamellibranchs with descriptions of anatomy. J. Morph. 26:625-701.

Kiørboe,T. and F. Møhlenberg. 1981. Particle selection in suspension-feeding bivalves. Mar. Ecol. Prog. Ser. 5: 291-296.

Kiørboe, T., F. Møhlenberg and O. Nøhr. 1980. Feeding, particle selection and carbon absorption in *Mytilus edulis* in different mixtures of algae and resuspended bottom material. Ophelia 19:193-205.

Kiørboe, T., F. Møhlenberg and O. Nøhr. 1981. Effect of suspended bottom material on growth and energetics in *Mytilus edulis.* Mar. Biol. 61:283-288.

Koehn, R.K. 1983. Biochemical genetics and adaptation in mollusks. Pages 305-330 in P.W. Hochachka (ed.). The Mollusca, Volume 2. Academic Press, New York.

Kreeger, D.A., R.I.E. Newell and C.J. Langdon. 1991. Effect of tidal exposure on utilization of dietary lignocellulose by the ribbed mussel *Geukensia demissa* (Dillwyn) (Mollusca: Bivalvia). J. Exp. Mar. Biol. Ecol. 144:85-100.

Kristensen, J.H. 1972a. Structure and function of crystalline styles of bivalves. Ophelia 10:91-108.

Kristensen, J.H. 1972b. Carbohydrases of some marine invertebrates with notes on their food and on the natural occurrence of the carbohydrases studied. Mar. Biol. 14:130-142.

Kuhn, D.A. 1978. The genus *Cristispira.* Pages 555-563 in M.P. Starr, H. Stolp, H.G. Truper, A. Balows, and H.G. Schlegel (eds.). The Prokaryotes, Vol. 1. Springer-Verlag, New York.

Laing, I. 1987. The use of artificial diets in rearing bivalve spat. Aquaculture 65:243-249.

Langdon, C.J. 1982. New techniques and their application to studies of bivalve nutrition. Pages 305-320 in G.D. Pruder, C.J. Langdon, and D.E. Conklin (eds.). Second International Conference on Aquaculture Nutrition: Biochemical and Physiological Approaches to Shellfish Nutrition. Louisiana State University, Baton Rouge.

Langdon, C.J. 1983. Growth studies with bacteria-free oyster (*Crassostrea gigas*) larvae fed on semi-defined artificial diets. Biol. Bull. 164:227-235.

Langdon, C.J. 1989. Preparation and evaluation of protein microcapsules for a marine suspension-feeder, the Pacific oyster *Crassostrea gigas.* Mar. Biol. 102: 217-224.

Langdon, C.J. and E.T. Bolton. 1984. A microparticulate diet for a suspension-feeding bivalve mollusc *Crassostrea virginica* (Gmelin). J. Exp. Mar. Biol. Ecol. 82:239-258.

Langdon, C.J. and A.E. DeBevoise. 1990. Effect of microcapsule type on delivery of dietary protein to a marine suspension-feeder, the oyster *Crassostrea gigas.* Mar. Biol. 105:437-443.

Langdon, C.J., D.M. Levine and D.A. Jones. 1985. Microparticulate feeds for marine suspension-feeders. J. Microencapsulation 2:1-11.

Langdon, C.J. and R.I.E. Newell. 1990. Utilization of detritus and bacteria as food sources by two bivalve suspension-feeders, the oyster *Crassostrea virginica* and the mussel *Geukensia demissa.* Mar. Ecol. Prog. Ser. 58:299-310.

Langdon, C.J. and C.A. Siegfried. 1984. Progress in the development of artificial diets for bivalve filter-feeders. Aquaculture 39:135-153.

Langdon, C.J. and M.J. Waldock. 1981. The effect of algal and artificial diets on the growth and fatty acid composition of *Crassostrea gigas* spat. J. Mar. Biol. Assoc. U.K. 61:431-448.

Langton, R.W. 1975. Synchrony in the digestive diverticula of *Mytilus edulis* L. J. Mar. Biol. Assoc. U.K. 55:221-229.

Langton, R.W. and P.A. Gabbott. 1974. The tidal rhythm of extracellular digestion and the response to feeding in *Ostrea edulis.* Mar. Biol. 24:181-187.

Langton, R.W. and G.U. McKay. 1974. The effect of continuous versus discontinuous feeding on the growth of hatchery reared spat of *Crassostrea gigas* Thunberg. J. Cons. Intl. Explor. Mer. 35:361-363.

Langton, R.W. and G.U. McKay. 1976. Growth of *Crassostrea gigas* (Thunberg) spat under different feeding regimes in a hatchery. Aquaculture 7:225-233.

Lin, D.S., A.M. Ilias, W.E. Connor, R.S. Caldwell, H.T. Cory and G.D. Davis. 1982. Composition and biosynthesis of sterols in selected marine phytoplankton. Lipids 17:818-824.

Loosanoff, V.L. and H.C. Davis. 1963. Rearing of bivalve mollusks. Adv. Mar. Biol. 1:1-136.

Lotsy, J.P. 1895. The food of the oyster, clam, and ribbed mussel. Rept. U.S. Comm. Fish. and Fisheries for 1893, 19:375-386.

Lucas, A. and C. Rangel. 1983. Detection of the first larval feeding in *Crassostrea gigas,* using the epifluorescent microscope. Aquaculture 30:369-374.

Lucas, M.I. and R.C. Newell. 1984. Utilization of saltmarsh detritus by two estuarine bivalves: Carbohydrase activity of crystalline style enzymes of the oyster *Crassostrea virginica* (Gmelin) and the mussel *Geukensia demissa* (Dillwyn). Mar. Biol. Lett. 5:275-290.

Manahan, D.T. 1983. The uptake and metabolism of dis-

solved amino acids by bivalve larvae. Biol Bull. 164: 236-250.

Manahan, D.T. 1989. Amino acid fluxes to and from seawater in axenic veliger larvae of a bivalve (*Crassostrea gigas*). Mar. Ecol. Prog. Ser., 53:247-255.

Manahan, D.T. 1990a. Adaptations by invertebrate larvae for nutrient acquisition from seawater. Amer. Zool. 30:147-160.

Manahan, D.T. 1990b. Amino acid fluxes to and from seawater in axenic veliger larvae of a bivalve (*Crassostrea gigas*). Mar. Ecol. Prog. Ser. 53:247-255.

Manahan, D.T. and D.J. Crisp. 1982. The role of dissolved organic material in the nutrition of pelagic larvae: amino acid uptake by bivalve veligers. Amer. Zool. 22:635-646.

Manahan, D.T. and D.J. Crisp. 1983. Autoradiographic studies on the uptake of dissolved amino acids from sea water by bivalve larvae. J. Mar. Biol. Assoc. U.K. 63:673-682.

Manahan, D.T., S.H. Wright and G.C. Stephens. 1982. Transport of dissolved amino acids by the mussel *Mytilus edulis*: demonstration of net uptake from natural seawater. Science 215:1253-1255.

Manahan, D.T., S.H. Wright and G.C. Stephens. 1983. Simultaneous determination of net uptake of sixteen amino acids by a marine bivalve. Am. J. Physiol. 244: R832-R838.

Masson, M. 1977. Observations sur la nutrition des larves de *Mytilus galloprovincialis* avec des aliments inertes. Mar. Biol. 40:157-164.

Mathers, N.F. 1972. The tracing of natural algal food labelled with a carbon 14 isotope through the digestive tract of *Ostrea edulis* L. Proc. Malac. Soc. Lond. 40: 115-124.

Mathers, N.F. 1973a. A comparative histochemical survey of enzymes associated with the process of digestion in *Ostrea edulis* and *Crassostrea angulata* (Mollusca:Bivalvia). J. Zool. 169:169-179.

Mathers, N.F. 1973b. Carbohydrate digestion in *Ostrea edulis* L. Proc. malac. Soc. Lond. 40:359-367.

Mathers, N.F. 1974. Digestion and pH variation in two species of oysters. Proc. Malac. Soc. Lond. 41:37-40.

Mayasich, S.A. and R.A. Smucker. 1986. Glycosidases in the American oyster, *Crassostrea virginica* Gmelin, digestive tract. J. Exp. Mar. Biol. Ecol. 95:95-98.

Mayasich, S.A. and R.A. Smucker 1987. Role of *Cristispira* sp. and other bacteria in the chitinase and chitobiase activities of the crystalline style of *Crassostrea virginica* (Gmelin). Microb. Ecol. 14:157-166.

Mayzaud, P., J.P. Chanut and R.G. Ackman. 1989. Seasonal changes of the biochemical composition of marine particulate matter with special reference to fatty acids and sterols. Mar. Ecol. Prog. Ser. 56:189-204.

McDade, J.E. and M.R. Tripp. 1967. Lysozyme in the oyster mantle mucus. J. Invertebr. Pathol. 9:581-582.

McHenery, J.G and T.H. Birkbeck. 1985. Uptake and processing of cultured microorganisms by bivalves. J. Exp. Mar. Biol. Ecol. 90:145-163.

McHenery, J.G., T.H. Birkbeck and J.A. Allen. 1979. The occurrence of lysozyme in marine bivalves. Comp. Biochem. Physiol. 63B:25-28.

McLachlan, J., A.G. McInnes and M. Falk. 1965. Studies on the chitan (chitin:poly-n-acetylglucosamine) fibers of the diatom *Thalassiosira fluviatilis* Hustedt. Can. J. Bot. 43:707-713.

McQuiston, R.W. 1969. Cyclic activity in the digestive diverticula in *Lasaea rubra* (Montagu). Proc. Malac. Soc. Lond. 38:483-492.

McQuiston, R.W. 1970. Fine structure of the gastric shield in the lamellibranch bivalve, *Lasaea rubra* (Montagu). Proc. Malac. Soc. Lond. 39:69-75.

Millar, R.H. 1955. Notes on the mechanism of food movement in the gut of the larval oyster *Ostrea edulis*. Quart. J. Micros. Sci. 96:539-544.

Millar, R.H. and J.M. Scott. 1967. Bacteria-free culture of oyster larvae. Nature 216:1139-1140.

Møhlenberg, F. and T. Kiørboe. 1981. Growth and energetics in *Spisula subtruncata* (Da Costa) and the effect of suspended bottom material. Ophelia 20:79-90.

Moore, H.F. 1908. Volumetric studies of the food and feeding of oysters. Bull. U.S. Bur. Fish. 28:1297-1308.

Moore, M.N., R.K. Koehn and B.L. Bayne. 1980. Leucine aminopeptidase (aminopeptidase-I) N-acetyl-hexoaminidase and lysosomes in the mussel, *Mytilus edulis* L. in response to salinity changes. J. Exp. Zool. 214:239-249.

Morris, I. 1981. Photosynthetic products, physiological state, and phytoplankton growth. Pages 83-102 in T. Platt (ed.). Physiological Bases of Phytoplankton Ecology. Can. Bull. Fish. Aquat. Sci. 210.

Morton, B.S. 1971. The diurnal rhythm and tidal rhythm of feeding and digestion in *Ostrea edulis*. Biol. J. Linnean Soc. Lond. 3:329-342.

Morton, B.S. 1973. A new theory of feeding and digestion in the filter-feeding Lamellibranchia. Malacologia 14:63-79.

Morton, B.S. 1977. The tidal rhythm of feeding and di-

gestion in the Pacific oyster, *Crassostrea gigas* (Thunberg). J. Exp. Mar. Biol. Ecol. 26:135-151.

Morton, B.S. 1983. Feeding and digestion in Bivalvia. Pages 65-147 in A.S.M. Saleuddin and K.M. Wilbur (eds.). The Mollusca, Volume 5. Academic Press, New York.

Murchelano, R.A. and J.L. Bishop. 1960. Bacteriological study of laboratory reared juvenile American oysters (*Crassostrea virginica*). J. Invertebr. Pathol. 14:321-322.

Murken, J. 1976. Feeding experiments with *Mytilus edulis* L. at small laboratory scale. III. Feeding of waste organic products from the fish industry of Bremerhaven as a means of recycling bio-degradable wastes. Pages 273-284 in G. Persoone and E. Jaspers (eds.). Proc. 10th European Symposium of Marine Biology. Universa Press, Wetteren, Belgium.

Nakazima, M. 1956. On the structure and function of the midgut-gland of Mollusca, with a general consideration of the feeding habits and systematic relations. Jap. J. Zool. 11:467-566.

Navarro, J.M. and J.E. Winter. 1982. Ingestion rate, assimilation efficiency and energy balance in *Mytilus chilensis* in relation to body size and different algal concentrations. Mar. Biol. 67:255-266.

Nelson, T.C. 1918. On the origin, nature, and function of the crystalline style of lamellibranchs. J. Morph. 31: 53-111.

Nelson, T.C. 1925. Recent contributions to the knowledge of the crystalline style of lamellibranchs. Biol. Bull. 49:86-99.

Nelson, T.C. 1933. On the digestion of animal forms by the oyster. Proc. Soc. Exp. Biol. Med. 30:1287-1290.

Newell, R.C., I. Parker and P.A. Cook. 1980. A possible role of α-amylase isoenzymes from the style of the mussel *Choromytilus meridionalis* (Krauss) following thermal acclimation. J. Exp. Mar. Biol. Ecol. 47:1-8.

Newell R.I.E. and S.J. Jordan. 1983. Preferential ingestion of organic material by the American oyster *Crassostrea virginica*. Mar. Ecol. Prog. Ser. 13:47-53.

Newell, R.I.E. and C.J. Langdon. 1986. Digestion and absorption of refractory carbon from the plant *Spartina alterniflora* by the oyster *Crassostrea virginica*. Mar. Ecol. Prog. Ser. 34:105-115.

Nes, W.R. and M.L. McKean. 1977. Biochemistry of Steroids and Other Isopentenoids. University Park Press, Baltimore. 629 pp.

Onishi, T., M. Suzuki and R. Kikuchi. 1985. The distribution of polysaccharide hydrolase activity in gastropods and bivalves. Bull. Jap. Soc. Sci. Fish. 51: 301-308.

Owen, G. 1955. Observations on the stomach and digestive diverticula of the lamellibranchia. I. The Anisomyaria and Eulamellibranchia. Quart. J. Microsc. Sci. 96:517-537.

Owen, G. 1970. The fine structure of the digestive tubules of the marine bivalve *Cardium edule*. Phil. Trans. R. Soc. Ser. B 258:245-260.

Owen, G. 1974. Feeding and digestion in the bivalvia. Pages 1-35 in O. Lowenstein (ed.). Advances in Comparative Physiology and Biochemistry. Academic Press, New York.

Pal, S.G. 1971. The fine structure of the digestive tubules of *Mya arenaria* L. I. Basiphil cell. Proc. Malac. Soc. Lond. 39:303-309.

Pal, S.G. 1972. The fine structure of the digestive tubules of *Mya arenaria* L. II. Digestive cell. Proc. Malac. Soc. Lond. 40:161-169.

Palmer, R.E. 1980. Behavioral and rhythmic aspects of filtration in the bay scallop, *Argopecten irradians concentricus* (Say), and the oyster, *Crassostrea virginica* (Gmelin). J. Exp. Mar. Biol. Ecol. 45:273-295.

Parker, R.S. and D.P. Selivonchick. 1986. Uptake of lipid vesicles from seawater by juvenile Pacific oysters (*Crassostrea gigas*). Aquaculture 53:215-228.

Patterson, G.W. 1992. Sterols of algae. Pages 118-159 in G.W. Patterson and W.D. Nes (eds.). Physiology and Biochemistry of Sterols. American Oil Chemists Society, Champaign, Illinois.

Patton, J.S. and J.G. Quinn. 1973. Studies on the digestive lipase of the surf clam, *Spisula solidissima*. Mar. Biol. 21:59-69.

Peirson, W.M. 1983. Utilization of eight algal species by the bay scallop *Argopecten irradians concentricus* (Say). J. Exp. Mar. Biol. Ecol. 68:1-11.

Penry, D.L. and P.A. Jumars. 1986. Chemical reactor analysis and optimal digestion. BioScience 36:310-315.

Penry, D.L. and P.A. Jumars. 1987. Modeling animal guts as chemical reactors. Amer. Nat. 129:69-97.

Petersen, C.G.J. and P.B. Jensen. 1911. Valuation of the sea. Animal life of the sea-bottom, its food and quantity. Rep. Danish Biol. Sta. 20:1-81.

Prieur, D. 1981. Experimental studies of trophic relationships between marine bacteria and bivalve molluscs. Kieler Meersforsch. 5:376-383.

Purchon, R.D. 1957. The stomach in the Filibranchia and Pseudolamellibranchia. Proc. Zool. Soc. Lond. 129: 27-59.

Purchon, R.D. 1968. The Biology of the Mollusca. Pergamon Press, Oxford. 560 pp.
Purchon, R.D. 1971. Digestion in filter feeding bivalves - a new concept. Proc. Malac. Soc. Lond. 39:253-261.
Purchon, R.D. 1987. The stomach in the Bivalvia. Phil. Trans. R. Soc. Ser. B 316:183-276.
Pütter, A. 1909. Die Ernährung der Wassertiere und der Stoffhaushalt der Gewässer. Fisher, Jena.
Reid, R.G.B. 1965. The structure and function of the stomach in bivalve mollusks. J. Zool. 147:156-184.
Reid, R.G.B. 1968. The distribution of digestive tract enzymes in the Lamellibranchiate bivalves. Comp. Biochem. Physiol. 24:727-744.
Reid, R.G.B. 1977. Gastric protein digestion in the carnivorous septibranch *Cardiomya planetica* Dall, with comparative notes on deposit and suspension feeding bivalves. Comp. Biochem. Physiol. 56A:573-575.
Reid, R.G.B. 1978. The systematic, adaptive and physiological significance of proteolytic enzyme distribution in bivalves. Veliger 20:260-265.
Reid, R.G.B. 1982. Aspects of bivalve feeding and digestion relevant to aquaculture nutrition. Pages 231-251 in G.D. Pruder, C.J. Langdon and D.E. Conklin (eds.). Second International Conference on Aquaculture Nutrition: Biochemical and Physiological Approaches to Shellfish Nutrition. Louisiana State University, Baton Rouge.
Reid, R.G.B. and K. Rauchert. 1970. Proteolytic enzymes in the bivalve mollusc *Chlamys hericius* Gould. Comp. Biochem. Physiol. 35:689-695.
Reid, R.G.B. and K. Rauchert. 1972. Protein digestion in members of the genus *Macoma* (Mollusca: Bivalvia). Can. J. Zool. 47:649-657.
Reid, R.G.B. and K. Rauchert. 1976. Catheptic endopeptidases and protein digestion in the horse clam *Tresus capax* (Gould). Comp. Biochem. Physiol. 54B:467-472.
Reid, R.G.B. and B. Sweeney. 1980. The digestibility of the bivalve crystalline style. Comp. Biochem. Physiol. 65B:451-453.
Robinson, W.E., M.R. Pennington and R.W. Langton. 1981. Variability of tubule types within the digestive glands of *Mercenaria mercenaria* (L.), *Ostrea edulis* L. and *Mytilus edulis* L. J. Exp. Mar. Biol. Ecol. 54:265-276.
Romberger, H.P. and C.E. Epifanio. 1981. Comparative effects of diets consisting of one or two algal species upon the assimilation efficiencies and growth of juvenile oysters, *Crassostrea virginica* (Gmelin). Aquaculture 25:89-94.
Salaque, A., M. Barbier and E. Lederer. 1966. Sur la biosynthèse des stèrols de l'huître (*Ostrea gryphea*) et de l'oursin (*Paracentrotus lividus*). Comp. Biochem. Physiol. 19:45-51.
Savage, R.E. 1925. The food of the oyster. Fish. Invest., Min. Agric. Fish., Lond., Ser. II, 3:1-50.
Sayce, C.S. and D.S. Tufts. 1967. The supplemental feeding of *Crassostrea gigas* in the laboratory (Abstract). Proc. Natl. Shellfish. Assoc. 58:14.
Seiderer, L.J., R.C. Newell, K. Schultes, F.T. Robb and C.M. Turley. 1987. Novel bacteriolytic activity associated with the style microflora of the mussel *Mytilus edulis*. J. Exp. Mar. Biol. Ecol. 110:231-224.
Shaw, B.L. and H.I. Battle. 1957. The gross and microscopic anatomy of the digestive tract of the oyster *Crassostrea virginica* (Gmelin) Can. J. Zool. 35. 325-347.
Sieburth, J. McN., V. Smetacek and J. Lenz. 1978. Pelagic ecosystem structure: Heterotrophic compartments of plankton and their relationship to plankton size fractions. Limnol. Oceanogr. 23:1256-1263.
Smucker, R.A. and D.A. Wright. 1984. Chitinase activity in the crystalline style of the American oyster *Crassostrea virginica*. Comp. Biochem. Physiol. 77A:239-241.
Smucker, R.A. and D.A. Wright. 1986. Characteristics of *Crassostrea virginica* crystalline style chitin digestion. Comp. Biochem. Physiol. 83A:489-493.
Sova, V.V., L.A. Elyakova and V.E. Vaskovsky. 1970. The distribution of laminarinases in marine invertebrates. Comp. Biochem. Physiol. 32:459-464.
Stafford, J. 1913. The Canadian Oyster, its Development, Environment and Culture. The Mortimer Co., Ltd., Ottawa, Canada. 159 pp.
Stephens, G. S. 1982a. Recent progress in the study of "Die Ernahrung der Wassertiere und der Stoffhaushatt gewaser." Amer. Zool. 22:611-619.
Stephens, G. S. 1982b. Dissolved organic material and the nutrition of marine bivalves. Pages 338-357 in G.D Pruder, C.J. Langdon and D.E. Conklin (eds.). Second International Conference on Aquaculture Nutrition: Biochemical and Physiological Approaches to Shellfish Nutrition. Louisiana State University, Baton Rouge.
Stewart, M.G. 1979. Absorption of dissolved organic nutrients by marine invertebrates. Oceanogr. Mar. Biol. Ann. Rev. 17:163-192.
Stewart, M.G. and D.R. Bamford. 1976. Absorption of soluble nutrients by the mid gut of the bivalve *Mya arenaria*. J. Molluscan Stud. 42:63-73.
Sumner, A.T. 1969. The distribution of some hydrolytic

enzymes in the cells of the digestive gland of certain lamellibranchs and gastropods. J. Zool., Lond. 158: 277-291.

Takatsuki, S. 1934. On the nature and functions of the amoebocytes of *Ostrea edulis.* Quart. J. Microsc. Sci. 76:379-431.

Tall, B.D. and R.K. Nauman. 1981. Scanning electron microscopy of *Cristispira* species in Chesapeake Bay oysters. Appl. Environ. Microbiol. 42:336-343.

Tamura, T., B. Truscott and D.R. Idler. 1964. Sterol metabolism in the oyster. J. Fish. Res. Board Can. 21: 1519-1523.

Teshima, S. 1982. Sterol Metabolism. Pages 205-216 in G.D Pruder, C.J. Langdon and D.E. Conklin (eds.). Second International Conference on Aquaculture Nutrition: Biochemical and Physiological Approaches to Shellfish Nutrition. Louisiana State University, Baton Rouge.

Teshima, S. and G.W. Patterson. 1981a. Delta 5, 7-sterols of the oyster, *Crassostrea virginica.* Comp. Biochem. Physiol. 65B:177-181.

Teshima, S. and G.W. Patterson. 1981b. Sterol biosynthesis in the oyster, *Crassostrea virginica.* Lipids 16:234-239.

Teshima, S., G.W. Patterson and S.R. Dutky. 1980. Sterols of the oyster. *Crassostrea virginica.* Lipids 12: 1004-1011.

Thompson, P.A., P.J. Harrison and J.N.C. Whyte. 1990. Influence of irradiance on the fatty acid composition of phytoplankton. J. Phycol. 26:278-288.

Thompson, R.J. and B.L. Bayne. 1974. Some relationships between growth, metabolism and food in the mussel *Mytilus edulis.* Mar. Biol. 27:317-326.

Toner, M.A. 1978. Preliminary studies on the development of a synthesized diet for juvenile oysters, *Crassostrea gigas.* Proc. Natl. Shellfish. Assoc. 68:91-92.

Turgeon, K.W. and D.S. Haven. 1978. Effects of cornstarch and dextrose in oysters. Veliger 20:352-358.

Ukeles, R. 1971. Nutritional requirements in shellfish culture. Pages 43-64 in K.S. Price and D.L. Mauer (eds.). Proceedings of the Conference on Artificial Propagation of Commercially Valuable Shellfish. University of Delaware, Newark.

Ukeles, R. and B.M. Sweeney. 1969. Influence of dinoflagellate trichocysts and other factors on the feeding of *Crassostrea virginica* larvae on *Monochrysis lutheri.* Limnol. Oceanogr. 14:403-410.

Urban, E.R. and C.J. Langdon. 1984. Reduction in costs of diets for the American oyster, *Crassostrea virginica* (Gmelin), by the use of non-algal supplements. Aquaculture 38:277-291.

Utting S.D. 1986. A preliminary study on growth of *Crassostrea gigas* larvae and spat in relation to dietary protein. Aquaculture 56:123-138.

Volkman, J.J., S.W. Jeffrey, P.D. Nichols, G.I. Rogers and C.D. Garland. 1989. Fatty acid and lipid composition of ten species of microalgae used in mariculture. J. Exp. Mar. Biol. Ecol. 128:219-240.

Voogt, P.A. 1975. Investigations of the capacity of synthesizing 3β-sterols in Mollusca - XIV. Biosynthesis and composition of sterols in some bivalves (Eulamellibranchia). Comp. Biochem. Physiol. 50B:505-510.

Waldock, M.J. and D.L. Holland. 1984. Fatty acid metabolism in young oysters, *Crassostrea gigas*: polyunsaturated fatty acids. Lipids 19:332-336.

Waldock, M.J. and I.A. Nascimento. 1979. The triacylglycerol composition of *Crassostrea gigas* larvae fed on different algal diets. Mar. Biol. Lett. 1:77-86.

Waller, T.R. 1981. Functional morphology and development of veliger larvae of the European oyster, *Ostrea edulis* Linne. Smithson. Contr. Zool. 328:1-70.

Walne, P.R. 1965. Observations on the influence of food supply and temperature on the feeding and growth of the larvae of *Ostrea edulis* L. Fish. Invest., Lond. Ser. 2, 24:1-45.

Walne, P.R. 1970. Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera *Ostrea, Mercenaria,* and *Mytilus.* Fish. Invest., Lond. Ser 2, 25:1-62.

Walne, P.R. 1976. Factors affecting the relation between feeding and growth in bivalves. Pages 169-183 in O. Devik (ed.). Harvesting Polluted Waters. Plenum Press, New York.

Walne, P.R. and B.E. Spencer. 1968. Some factors affecting the growth of larvae of *Ostrea edulis.* International Council for the Exploration of the Sea, K15:1-7.

Walton, M.J. and J.F. Pennock. 1972. Some studies on the biosynthesis of ubiquinone, isoprenoid alcohols, squalene and sterols by marine invertebrates. J. Biochem. 127:471-479.

Wangersky, P.J. 1978. Production of dissolved organic matter. Pages 115-220 in O. Kinne (ed.). Marine Ecology, Volume 4. Academic Press, New York.

Ward, E.J., R.I.E. Newell, R.J. Thompson and B.A. MacDonald. 1994. Endoscopic observations of particle capture and transport in the eastern oyster, *Crassostrea virginica* Gmelin. Biol. Bull. 186:221-240.

Webb, K.L. and F.E. Chu. 1982. Phytoplankton as a food

source for bivalve larvae. Pages 272-291 in G.D. Pruder, C.J. Langdon and D.E. Conklin (eds.). Second International Conference on Aquaculture Nutrition: Biochemical and Physiological Approaches to Shellfish Nutrition. Louisiana State University, Baton Rouge.

Weinstein, J.E. 1995. Fine structure of the digestive tubule of the eastern oyster, *Crassostrea virginica* (Gmelin, 1791). J. Shellfish Res. 14:97-103.

Welborn, J.R. and D.T. Manahan. 1990. Direct measurements of sugar uptake from seawater into molluscan larvae. Mar. Ecol. Prog. Ser. 65:233-239.

Whyte, J.N.C. 1987. Biochemical composition and energy content of six species of phytoplankton used in mariculture of bivalves. Aquaculture 60:231-241.

Whyte, J.N.C., 1989. Fatty acid profiles from direct methanolysis of lipids in tissue of cultured species. Aquaculture 75:193-203.

Whyte, J.N.C., N. Bourne and C.A. Hodgson. 1987. Assessment of biochemical composition and energy reserves in the larvae of the scallop *Patinopecten yessoensis*. J. Exp. Mar. Biol. Ecol. 113:113-124.

Whyte, J.N.C., N. Bourne and C.A. Hodgson. 1989. Influence of algal diets on biochemical composition and energy reserves in *Patinopecten yessoensis* (Jay) larvae. Aquaculture 78:333-347.

Wikfors, G.H., J.W. Twarog and R. Ukeles. 1984. Influence of chemical composition of algal food sources on growth of juvenile oysters, *Crassostrea virginica*. Biol. Bull. 167:251-263.

Wikfors, G.H., P.K. Gladu and G.W. Patterson. 1991. In search of the ideal algal diet for oysters: Recent progress, with emphasis on sterols (Abstract). J Shellfish Res. 10:292.

Williams, P.J.le B. 1975. Biological and chemical aspects of dissolved organic material in sea water. Pages 301-363 in J.P. Riley and G. Skirrow (eds.). Chemical Oceanography. Academic Press, New York.

Winstead, J.T. 1995. Digestive tubule atrophy in eastern oysters, *Crassostrea virginica* (Gmelin, 1791), exposed to salinity and starvation stress. J. Shellfish. Res. 14: 105-111.

Winter, J.E. 1973. The filtration rate of *Mytilus edulis* and its dependence on algal concentrations, measured by a continuous automatic recording apparatus. Mar. Biol. 22:317-328.

Winter, J.E. 1976. Feeding experiments with *Mytilus edulis* L. at small laboratory scale II. The influence of suspended silt in addition to algal suspension on growth. Pages 585-602 in G. Persoone and E. Jaspers (eds.). Proc. 10th Europ. Symp. of Mar. Biol. Universa Press, Wetteren, Belgium.

Wright, S.H. 1988. Amino acid transport in the gill epithelium of a marine bivalve. Comp. Biochem. Physiol. 90A:635-641.

Wright, S.H. and T.W. Secomb. 1986. Epithelial amino acid transport in marine mussels: role in net exchange of taurine between gills and seawater. J. Exp. Biol. 121:251-270.

Wright, S.H. and G.C. Stephens. 1978. Removal of amino acids during a single passage of water across the gill of marine mussels. J. Exp. Zool. 205:337-352.

Yonge, C.M. 1926a. Structure and physiology of the organs of feeding and digestion in *Ostrea edulis*. J. Mar. Biol. Assoc. U.K. 14:295-386.

Yonge, C.M. 1926b. The digestive diverticula in the Lamellibranchs. Trans. R. Soc. Edinb. 54:703-720.

Yonge, C.M. 1932. The crystalline style of the Mollusca. Sci. Prog. 104:643-653.

Young, J.P.W., R.K. Koehn and N. Arnheim. 1979. Biochemical characterization of "LAP", a polymorphic aminopeptidase from the mussel, *Mytilus edulis*. Biochem. Genet. 17:305-323.

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