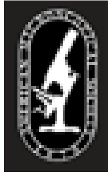


WILEY

Functional Organization of Intrinsic Gill Muscles in Zebra Mussels, Dreissena polymorpha (Mollusca: Bivalvia), and Response to Transmitters in Vitro

Author(s): Scott Medler and Harold Silverman

Source: *Invertebrate Biology*, Summer, 1997, Vol. 116, No. 3 (Summer, 1997), pp. 200-212

Published by: Wiley on behalf of American Microscopical Society

Stable URL: https://www.jstor.org/stable/3226897

REFERENCES
Linked references are available on JSTOR for this article:
https://www.jstor.org/stable/3226897?seq=1&cid=pdf-reference#references_tab_contents
You may need to log in to JSTOR to access the linked references.

JSTOR is a not-for-profit service that helps scholars, researchers, and students discover, use, and build upon a wide range of content in a trusted digital archive. We use information technology and tools to increase productivity and facilitate new forms of scholarship. For more information about JSTOR, please contact support@jstor.org.

Your use of the JSTOR archive indicates your acceptance of the Terms & Conditions of Use, available at https://about.jstor.org/terms

American Microscopical Society and *Wiley* are collaborating with JSTOR to digitize, preserve and extend access to *Invertebrate Biology*

JSTOR

This content downloaded from
149.76.0.33 on Fri, 16 Apr 2021 15:13:37 UTC
All use subject to https://about.jstor.org/terms

Invertebrate Biology 116(3): 200–212.
© 1997 American Microscopical Society, Inc.

Functional organization of intrinsic gill muscles in zebra mussels, *Dreissena polymorpha* (Mollusca: Bivalvia), and response to transmitters *in vitro*

Scott Medler[a] and Harold Silverman

Department of Zoology and Physiology, Louisiana State University,
Baton Rouge, Louisiana 70803, USA

Abstract. Lamellibranch gills are used for various vital functions ranging from food capture to ion regulation. The foundation for many of these functions is the transport of water through the gill. Few studies have examined the role of intrinsic gill muscles and their ability to control water flow by altering the dimensions of the water passageways of the gill. In this report we examine the organization of intrinsic gill muscles and associated connective tissue in zebra mussels, *Dreissena polymorpha.*

Two sets of muscles lie within the hemocoel of the gill and are bathed with hemolymph. In those muscles associated with the connective tissue sheets (which underlie external and internal gill epithelia), the orientation of the muscle fibers is consistent with a role of regulating the external and internal ostial area. Those muscles encased in connective tissue bands and oriented perpendicular to the bases of gill filaments reduce interfilament distance in the gill; these muscles are antagonized by dense connective tissue cross-struts that push the gill filaments apart when the muscles relax. The two sets of muscles are oriented to serve complementary functions in reducing ostial openings, thus affecting water flow through the gill.

The muscles of the gill respond to application of exogenous acetylcholine, FMRFamide, and serotonin. The gill contracts in response to acetylcholine and FMRFamide but relaxes with serotonin application. Microscopic observation of live gill tissues reveals complex and dynamic changes in gill dimensions consistent with a role in regulating water flow.

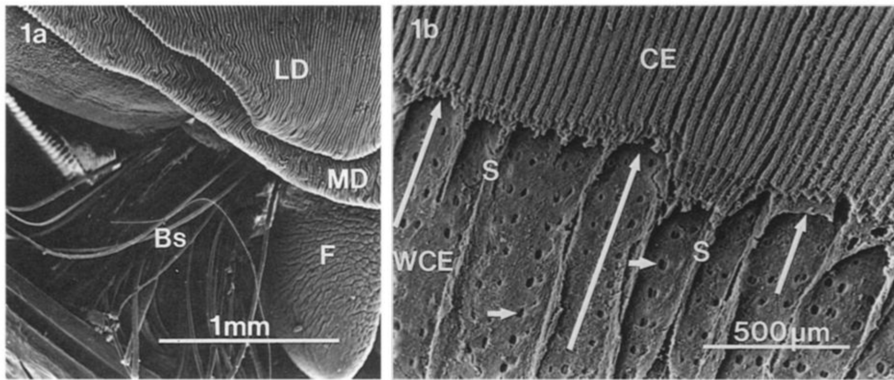
Additional key words: water flow, gill function, muscle fibers, connective tissue

Lamellibranch gills are often the major site of gas exchange, ion transport, and nutrient capture depending on the species of bivalve (Barrington 1979; Dietz 1985; Pearse et al. 1987; Kays et al. 1990; McMahon 1991). In some bivalves, the gill also serves as a reproductive brood chamber (Ortmann 1911; Silverman et al. 1985; Silverman 1989; Tankersley & Dimock 1992; Tankersley 1996) or the organ housing symbiotic organisms that produce nutrients for the bivalve (Southward 1986; Cavanaugh et al. 1987; Dando & Spiro 1993). All of these vital functions can be influenced or regulated by the amount of water flowing through the gill. While there are clearly variations among bivalve families (Ridewood 1903; Atkins 1937), the major force generating water flow in a eulamellibranch is provided by the lateral ciliated cells on adjacent filaments (Jørgensen 1975; Silvester 1988; McMahon 1991). The movement of these cilia propels water into the mantle cavity through an incurrent siphon. Inside the mantle cavity, water is drawn into the gill through external ostia that lead into water canals (Pearse et al. 1987; Gardiner et al. 1991; McMahon 1991). Water moves through these canals into a central water channel via internal ostia, and then travels dorsally through the water channel to the suprabranchial chamber (Barrington 1979; Pearse et al. 1987; Gardiner et al. 1991; McMahon 1991) (see Fig. 1). Water exits the animal through the excurrent siphon.

While the driving force for water movement is ciliary activity, there are several control points in this pathway that are under muscular control. For example, the incurrent and excurrent siphons vary greatly in their diameter in response to the tone of integral muscles. Foster-Smith (1976) characterized the excurrent siphon as the most important adjustable resistance factor influencing water flow through several bivalve species. In their model of the bivalve pump, Jørgensen et

[a] Author for correspondence.
E-mail: smedler@unix1.sncc.lsu.edu

This content downloaded from
149.76.0.33 on Fri, 16 Apr 2021 15:13:37 UTC
All use subject to https://about.jstor.org/terms



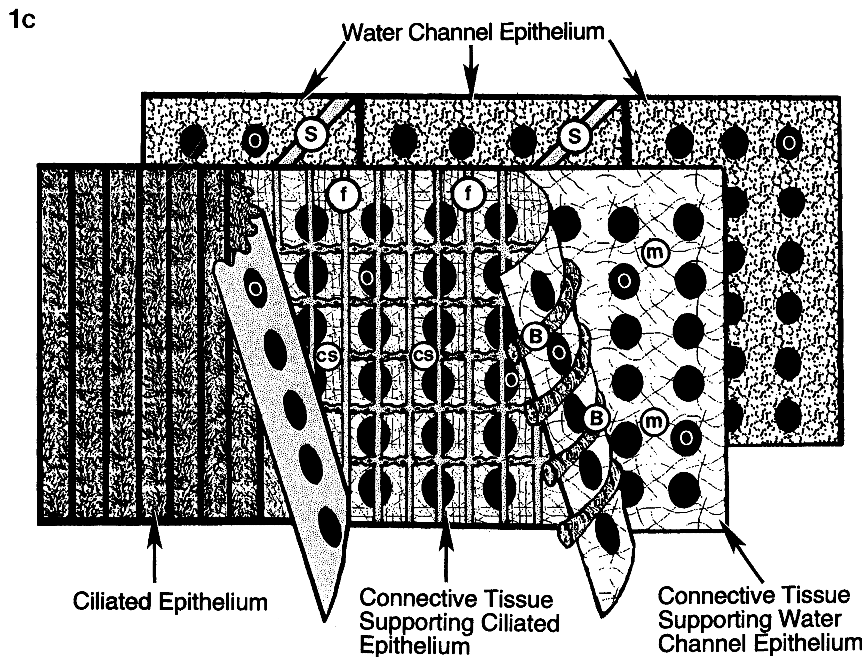


Fig. 1. Gills of *Dreissena polymorpha.* **(a)** Orientation of the gills with respect to the gross morphology of the animal. A lateral demibranch (LD) and a medial demibranch (MD) compose a gill on each side of the animal. Byssus (Bs); foot (F). SEM. **(b)** Higher magnification of a single demibranch showing the outer ciliated epithelium (CE) and the internal structure of the demibranch with the outermost lamella removed. Water moves past the ciliated epithelium and into the central water channel via water canals (not shown). These water canals empty into the central water channel through internal ostia (small arrows) in the water channel epithelium (WCE). The central water channel is partitioned into water tubes by the septa (S) which connect opposing gill lamellae. Water moves dorsally (large arrows) through the water tubes into the suprabranchial chamber (not shown) before exiting through the excurrent siphon (not shown). SEM. **(c)** Diagrammatic composite of the elements of a demibranch (not to scale). The outermost layer is the ciliated epithelium. Supporting the ciliated epithelium is a loose connective tissue sheet, filament supports (f), and cross-struts (cs) between filaments. Bands of connective tissue and muscle fibers (B) lie deep to the base of the filament supports and are continuous with the deep portion of the cross-struts. A second connective tissue sheet supports the water channel epithelium. The hemocoel of the gill lies between these two connective tissue sheets. Associated with both loose connective tissue sheets are arrays of muscle fibers (m) radiating in many directions. External and internal ostia (o) are associated with the external and internal portions of the gill, respectively. Septa (S) connecting the lamellae are continuous with the water channel epithelium and the associated connective tissue sheets.

al. (1986) supported this view with the estimation that the excurrent siphon accounts for the largest pressure head in the water pump. Lei et al. (1996) observed changes in excurrent siphon diameter of *Dreissena polymorpha* in response to changes in particle concentration. Water flow may also be controlled by integral gill muscles that control interfilament and ostial dimensions. In the Jørgensen et al. (1986) model of the bivalve pump, the interfilament canals collectively are considered to be the second largest pressure head in the pump, accounting for over 30% of the total pressure in the system. Increases in pressure are inversely related to the square of the interfilament distance. Jørgensen (1989) also indicated that the distance between opposing bands of lateral cilia, controlled by the width of interfilament canals, is "the main factor in determining pump pressure and flow rate in bivalves." Other studies have demonstrated a relationship between ostial dimension and water flow, particle filtration, and filtration efficiency (Nelson & Allison 1940; Dral 1968; Foster-Smith 1975, 1976).

Many studies have documented neural control of the bivalve gill (reviewed by Paparo 1988). Application of exogenous transmitters has been known for some time

This content downloaded from
149.76.0.33 on Fri, 16 Apr 2021 15:13:37 UTC
All use subject to https://about.jstor.org/terms

to affect ciliary activity (Aiello 1960, 1962; Aiello & Guideri 1964). The effects of transmitters on intrinsic musculature (Gardiner et al. 1991) and overall pumping rate (Jones & Richards 1993) have been examined more recently. Previous accounts reported that the gill muscles of *D. polymorpha* respond to exogenous transmitters (Duncan et al. 1994; Medler & Silverman 1994). Acetylcholine, FMRFamide, and serotonin are all physiologically important in a variety of molluscan species. Although the response varies considerably between and even within a species, FMRFamide and acetylcholine are often excitatory neurotransmitters, whereas serotonin is often inhibitory or neuromodulatory (Muneoka & Twarog 1983).

This study examines the role of connective tissue elements, intrinsic gill muscles, and their ability to alter the dimensions of water passageways in a homorhabdic eulamellibranch gill. *Dreissena polymorpha* is a non-native freshwater species recently introduced into North America from the Caspian Sea/Black Sea region of Europe (Ludyanskiy 1993; Smirnova et al. 1993) and represents a phylogenetic group distinct from the previously studied bivalves. We report here that the musculature in the gill of *D. polymorpha* responds to exogenous acetylcholine, FMRFamide, and serotonin. Stimulation by FMRFamide and acetylcholine caused muscle contraction in the gill while serotonin caused muscle relaxation. Observation of live gill tissue revealed complex and dynamic changes in interfilament distance and internal ostial area consistent with regulation of water flow. These changes are related to the contraction of the intrinsic musculature integrated with the associated connective tissue of the gill.

Methods

Animals and maintenance

Zebra mussels, *Dreissena polymorpha* (PALLAS 1771), were collected from Lake Erie at the mouth of the Raisin River in Monroe, Michigan and from the Mississippi River near Plaquemine, Louisiana. The animals were maintained in artificial pondwater (0.5 mM NaCl, 0.4 mM $CaCl_2$, 0.2 mM $NaHCO_3$, 0.05 mM KCl, and 0.2 mM $MgCl_2$) in aerated aquariums under laboratory conditions of ~25°C and 12h:12h light/dark cycles.

General gill preparation

Each gill was freed from its dorsal attachment with forceps, and excised gills were placed in a Ringer's solution designed to approximate blood composition of the animals (Dietz et al. 1994) (5 mM NaCl, 5 mM $CaCl_2$, 5 mM $NaHCO_3$, 0.5 mM KCl, 5 mM $NaSO_4$, 0.5 mM $MgCl_2$; 48 mOsm). Calcium-free Ringer's used in various experiments had the same composition as the above solution except that the $CaCl_2$ was omitted and 4 mM EDTA was added. The osmolality and pH were the same in both solutions. Lateral and medial demibranchs were separated by cutting along their dorsal connection.

Muscle labeling with phallacidin

The f-actin of muscle bands was labeled with phallacidin conjugated to a fluorescent marker, NBD [N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) phallacidin]. Phallacidin is a bicyclic peptide that specifically binds f-actin at nanomolar concentrations in muscle and nonmuscle cells from many different plants and animals (Molecular Probes Inc., Eugene, Oregon). Excised demibranchs were split into single lamellae and stripped of epithelium by placing them in a Ca^{2+}-free Ringer's solution containing 4 mM EDTA and passing them through the end of a small transfer pipette. Phallacidin application procedures were those recommended by Molecular Probes. Briefly, single lamellae were extracted with a solution of −20°C acetone for 3–5 min and then air dried. The lamellae were incubated with phallacidin in Ringer's solution for 20 min at 25°C. Finally, the lamellae were washed twice with Ringer's solution.

Samples were observed on a Nikon Microphot FXA using an excitation filter of 450–490 nm and a barrier filter of 520 nm. Gills were also observed with Nomarski illumination to compare structural correlates of labeled areas.

Preparation for transmission electron microscopy (TEM)

Before gill excision, hemolymph samples were taken from animals by inserting a 26-gauge needle between the valves and into the pericardial space (Fyhn & Costlow 1975; Dietz et al. 1994). Hemolymph osmolality was measured on a Precision Systems freezing point osmometer. A 2% glutaraldehyde solution was adjusted with phosphate buffer to match the hemolymph osmolality. Excised gills were cut along the filaments into strips about 3 mm wide and quickly fixed by immersion in the glutaraldehyde solution for 1 h. Gills were rinsed twice in a phosphate buffer designed to match the hemolymph osmolality and pH (~40 mOsm, pH 7.0) and post-fixed in 1% OsO_4 for 1 h, rinsed twice in phosphate buffer, and dehydrated in a graded ethanol series. Gill strips were embedded in LR White (London Resin Co.) medium grade resin by placing them in a 1:1 mixture of ethanol and resin for 24 h. They were transferred to

This content downloaded from
149.76.0.33 on Fri, 16 Apr 2021 15:13:37 UTC
All use subject to https://about.jstor.org/terms

100% resin for 12 h, and embedded flat in fresh resin at 60°C for 24–48 h.

Gills were sectioned with a Reichert-Jung ultracut E ultramicrotome at 60–90 nm thickness with glass knives. The gills were sectioned in two planes: (1) in a dorso-ventral plane between adjacent filaments, and (2) in frontal sections (*en face*) across gill filaments and through the muscles surrounding internal ostia. Sections were stained with 3% uranyl acetate for 2 min followed by Reynolds' (1963) lead citrate for 2–5 min. The sections were examined with a JOEL 100CX TEM operating at 80 kV.

Preparation of gills for scanning electron microscopy (SEM)

Dissected gills were placed in Ringer's solution as described above. In some instances, gills were exposed to acetylcholine, FMRFamide, or serotonin for 10–20 min before fixation. The solution was carefully drained and quickly replaced with liquid nitrogen in order to prevent muscular contraction of the gills during fixation. After a few seconds, the nitrogen was removed and the frozen gills were immersed in the osmotically balanced 2% glutaraldehyde solution for 1 to 4 h. Gills were rinsed in phosphate buffer (as described above) and post-fixed in 1% OsO_4 for 1 h. After osmication, gills were rinsed in phosphate buffer and dehydrated in a graded ethanol series. Gills were wrapped in lens paper, critical-point dried, and mounted on stubs. Specimens were sputter coated with a mixture of gold and palladium (20 nm) and viewed with a Cambridge S-260 SEM. Measurements of ostial areas were made by cutting out and weighing printed images. These masses were converted into areas by calibrating with a known area.

Confocal microscopy

Optical sections (2 μm) of the connective tissue structures were observed by confocal imaging techniques with a Noran Instruments Odyssey XL Laser Confocal Microscope. Before they were observed, gills were incubated with a horseradish peroxidase-FITC (fluorescein-5-isothiocyanate) conjugate at 1 mg/mL. Initially this methodology was used in an attempt to label neural elements of the gill. However, the marker was absorbed into the highly porous connective tissue elements.

Measurement of demibranch contraction

Excised demibranchs were placed in Ringer's solution on a glass microscope slide, with the demibranch floating freely in the solution. The solution was then aspirated to leave the demibranch flattened on the surface of the slide. The gill was rapidly immersed in a Ringer's solution containing acetylcholine or FMRFamide at concentrations from 10^{-3} to 10^{-6} M. The transmitter-containing solution was aspirated immediately following its application, again leaving the demibranch flat on the slide. During this procedure, gills were observed at a magnification of about 10× with a dissecting microscope and recorded on VHS videotape. Video images were digitized and measured with Image-1 computer software (Universal Imaging Corp.). Gill area was measured before transmitter exposure and at timed intervals after transmitter application. Changes in gill area over time were expressed either as the relative reduction from the initial area (% of initial area) or as surface area (mm^2).

Observation and measurement of perfused gill tissue

Internal ostial dimensions were observed using a Nikon Diaphot inverted microscope with Hoffman Modulation optics or Nomarski (DIC) optics at magnifications of 400× or 600× and recorded on VHS videotape. Excised demibranchs were split along the interlamellar septa into ascending and descending lamellae. Individual lamellae were placed in a 200-μL perfusion chamber with the water channel epithelium visible and held in place by nylon mesh. The tissue was perfused with 60 or 70% Ringer's solution at a flow rate of 1.5–5 mL/min. The solutions perfusing the lamellae were introduced at the bottom of the chamber and aspirated at the top. Thus, water flow was theoretically unidirectional from bottom to the top of the chamber. Solutions were changed with a valve that switched to a second reservoir.

In addition to control observations with only Ringer's solution bathing the organs, we also observed the effects of serotonin, acetylcholine, and FMRFamide at concentrations from 10^{-4} to 10^{-6} M. In some cases ostial dimensions were measured with Image-1 computer software (Universal Imaging Corp.) as described above. Measured values were calibrated with a stage micrometer in the experimental set-up. Recordings used for measurements were from open ostia flat enough to be within a single plane of focus throughout an experiment and reasonably stable in dimension before experimental manipulation. Images for figures were processed with Adobe Photoshop (Adobe Systems, Inc.) to optimize image brightness and contrast.

In addition to general observations, we compared pre-treatment ostial areas with post-treatment areas for each of the three transmitters at a concentration of 10^{-5} M. In each case, ostial area was measured immediately

This content downloaded from
149.76.0.33 on Fri, 16 Apr 2021 15:13:37 UTC
All use subject to https://about.jstor.org/terms

preceding transmitter application and again after 5 min of perfusion with a transmitter solution. Pre-treatment and post-treatment areas were compared with paired t-tests (n = 10 for each transmitter). The statistical power of the paired comparisons was needed to factor out inherent variability between individual ostia. Statistics were performed with SAS version 6.10 (SAS Institute 1995).

Results

The gill of *Dreissena polymorpha* is of a eulamellibranch form whose gross structure has been described (Ridewood 1903; Morton 1969). Fig. 1 relates the gross structure of the gill and the direction of water movement through it to the complex, layered organization of its tissues, showing the position of the connective tissue elements and intrinsic musculature described below.

Connective tissue skeleton

Stripping the epithelial tissue from the underlying supportive tissue in living gills leaves a "skeleton" that has filaments held apart from one another by connective tissue cross struts (see Fig. 1c, 2b). This skeleton has a compressible yet resilient texture. The gill filaments are supported by a fibrous connective tissue matrix (Fig. 2a) composed of a network of thin fibers several nanometers in diameter. Aside from this rather dense homogeneous matrix, there are no further supportive structures in the filaments. The filaments do not contain calcified rods as are found in the gills of unionids. The cellular elements associated with the filament connective tissue and vascular sinus are located in the central midline of the filament.

The connective tissue matrix supporting individual filaments is continuous with connective tissue struts that cross the filaments at right angles approximately every 30–80 μm (Fig. 2b). This distance can vary with animal size, contractile state, and fixation state. The cross-struts have an elongate figure-8 morphology and have the same fibrous appearance as the filament supports (Fig. 2). The connective tissue supporting the epithelial cells is a thin sheet of loose, but fibrous connective tissue (Fig. 1c, 2d, 3a,b, 4a). One sheet underlies the external ciliated epithelium while a second sheet underlies the internal water channel epithelium. A hemocoel is enclosed between these two sheets of connective tissue and their associated epithelia (see Fig. 1c). In areas where the cross-struts occur, the thin sheet of connective tissue dips under the strut so that the strut lies between the sheet and the outer epithelium (Fig. 2d). The deep regions of the cross-struts form bands of connective tissue perpendicular to the filaments. These bands are interspersed with muscle fibers (see below).

Muscle organization

Muscle fibers were identified structurally using TEM and by their positive labeling with NBD-phallacidin. Groups of obliquely-striated muscle fibers lie in bands at right angles to gill filaments, and muscle fibers are also found in a web-like orientation in association with the loose connective tissue sheet that underlies the external and internal epithelium (Figs. 1c, 3, 4a). The muscle fibers crossing the gill filaments are encased in well-organized connective tissue bands with fiber orientation paralleling the longitudinal axis of the muscle fibers (Fig. 3d). These bands lie at the base of the gill filaments in the hemocoelic space between the outer ciliated epithelium and the inner water channel epithelium. The individual muscle fibers are attached to the connective tissue fibers by peripheral dense bodies (Fig. 3d).

A second major set of muscle fibers is found in the connective tissue sheets underlying the epithelial layers of the gill (Figs. 1c, 3a,b, 4). Contraction of these muscle fibers causes a reduction in the size of the water canal and the ostia leading to the water channel. These individual muscle fibers are small, generally on the order of 1–2 μm in diameter (Fig. 3) and of unknown length. Nervous tissue lies in close association with these fibers, but the overall organization of these nerves has not been formally assessed.

Demibranch contraction

To assess the effects of general intrinsic muscle contraction, we isolated demibranchs and exposed them to the neurotransmitters acetylcholine and FMRFamide. We measured gross area of the demibranch over time, with contraction of the musculature resulting in reduced demibranch area. Gill area is reduced following application of a stimulating transmitter, with the greatest reduction occurring in the first 10–20 seconds and then becoming asymptotic to a level of maximum reduction (Figs. 5, 6). Gills returned to Ringer's solution lacking transmitters were observed to relax after several minutes. Reduction in demibranch area was concentration-dependent following exposure to either acetylcholine or FMRFamide (Fig. 6). Responses to acetylcholine and FMRFamide were similar in magnitude and time course (Fig. 6).

Observation of perfused gill tissue

We made over 20 h of video-taped observations on the internal water channel epithelium and ostia in these

This content downloaded from
149.76.0.33 on Fri, 16 Apr 2021 15:13:37 UTC
All use subject to https://about.jstor.org/terms

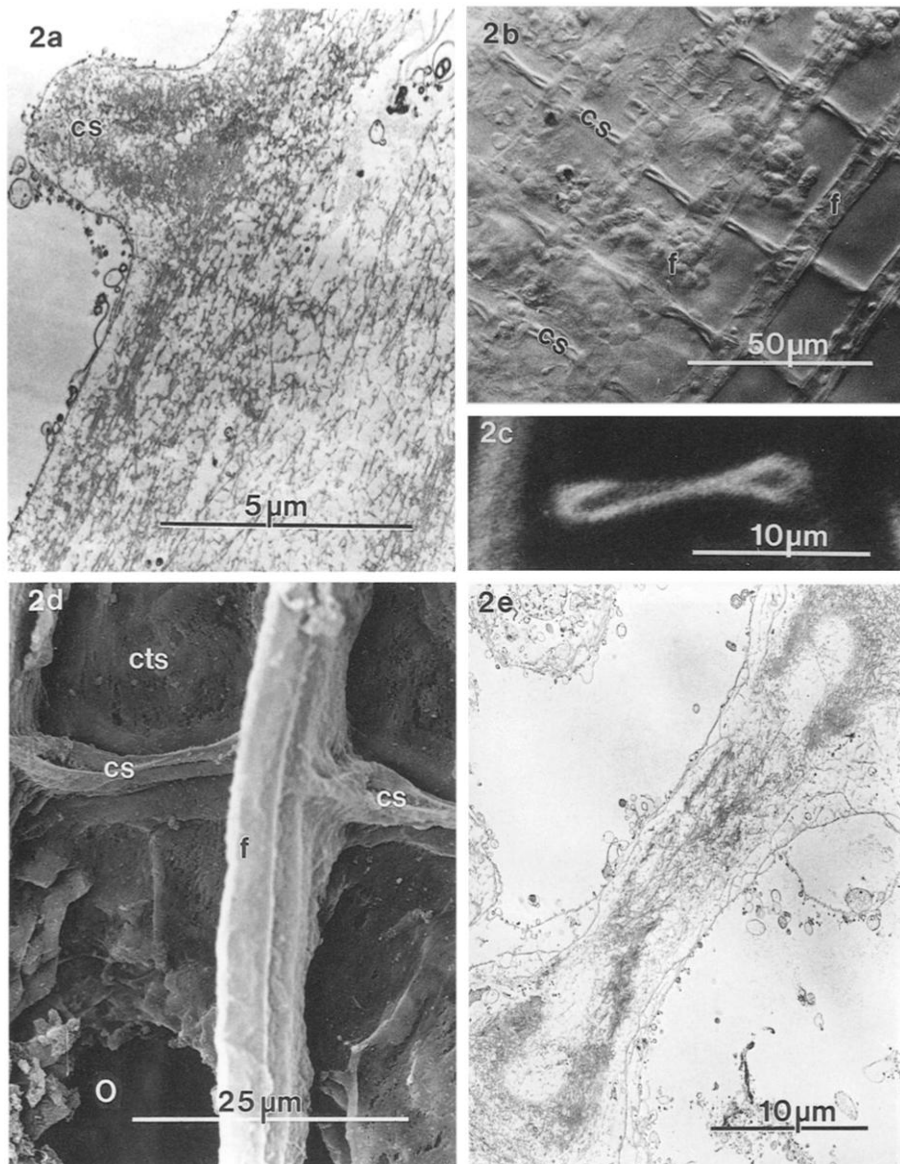


Fig. 2. Connective tissue support of the gill of *Dreissena polymorpha.* **(a)** Filament support demonstrating the fibrous connective tissue of the structure. Tissue forming a cross-strut (cs) is continuous with the filament support. TEM. **(b)** Gill stripped of epithelium to expose underlying connective tissue support. Parallel filaments (f) are separated by perpendicular cross-struts (cs). Nomarski illumination (DIC). **(c)** A 2-µm thick laser confocal optical section of a cross-strut labeled with horseradish peroxidase-FITC. **(d)** Gill stripped of its external ciliated epithelium. The filament supports (f) and cross-struts (cs) overlay the connective tissue sheet (cts) which encloses the hemolymph space. An external ostium (O) is visible. SEM. **(e)** Thin section of a cross-strut showing the filamentous nature of the connective tissue. TEM.

preparations. Generally, ostial area decreased in response to either acetylcholine or FMRFamide, and ostial area increased following serotonin application. The paired comparisons of pre-treatment and post-treatment areas generally support these observations. Ostial area was reduced to an average of 55% of the control value following acetylcholine treatment ($p < 0.01$) and was reduced to an average of 67% of the control value following FMRFamide treatment ($p < 0.02$) (Fig. 7). The ostial area of serotonin-treated preparations was an average of 113% of the control value, but the two values did not differ significantly ($p < 0.1$) (Fig. 7). In the serotonin-treated group, 7 of the 10 ostia increased in size to an average of 120% of the control area.

Overall, we observed conspicuous changes in ostial dimension over time (Figs. 8d–i, 9). While most ostia were fairly stable in dimension during our observations (Fig. 9a), a few ostia were found to be active in their movements even without transmitter application (Fig. 9b). Transmitter-treated preparations showed a wide range of ostial size and shape over short time periods (Figs. 8, 9c–e). Interfilament distance follows the same pattern of change as ostial area, decreasing when the ostial area decreases and *vice versa.*

Discussion

The major muscles in the gill of *Dreissena polymorpha* cross the base of the gill filaments at right angles and radiate within the thin connective tissue sheets supporting the epithelial cells of the gill. While

This content downloaded from
149.76.0.33 on Fri, 16 Apr 2021 15:13:37 UTC
All use subject to https://about.jstor.org/terms

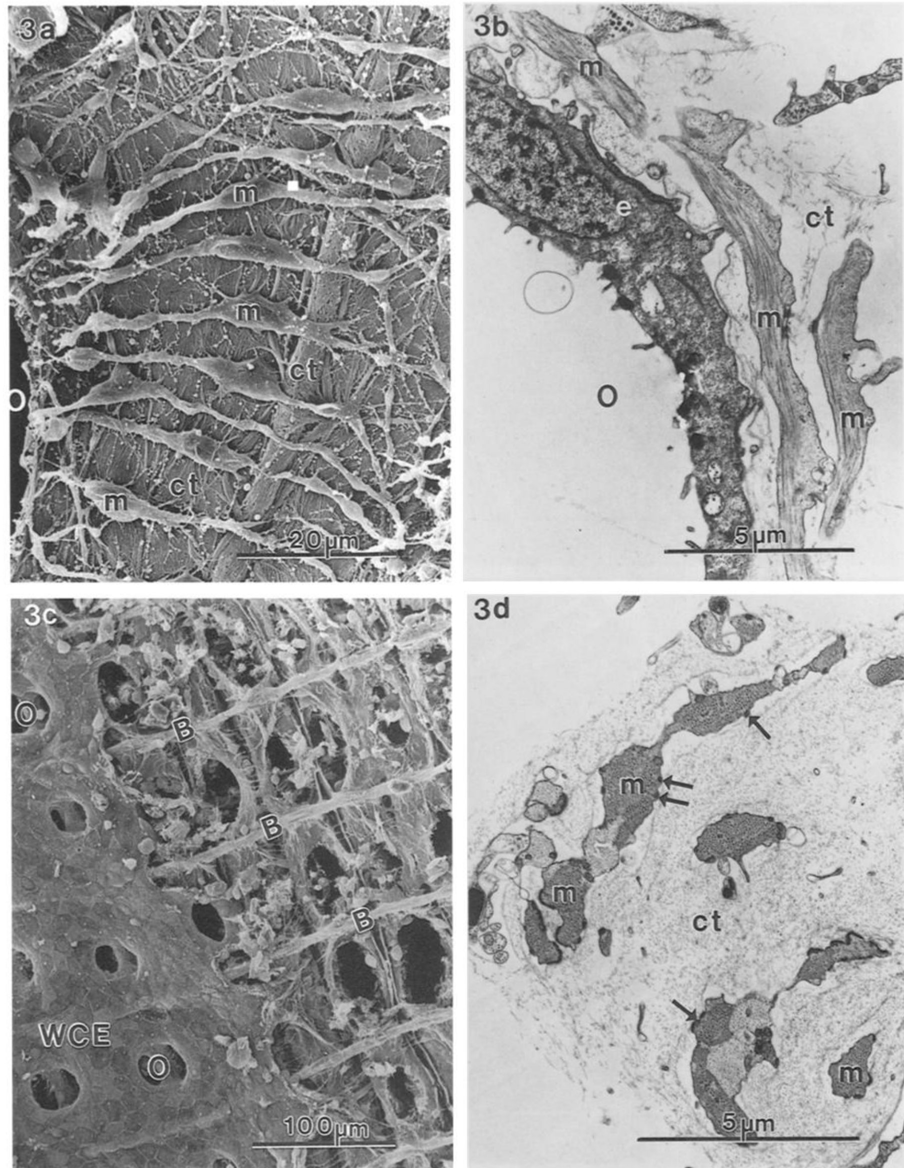


Fig. 3. Intrinsic gill muscles.
(a) Hemocoel side of the connective tissue sheet underlying the internal water channel epithelium. Presumptive muscle fibers (m) are closely associated with the connective tissue (ct). The margin of an internal ostium (O) is just visible. SEM.
(b) Frontal section of a gill at the level of an internal ostium (O). The epithelial tissue (e), muscle fibers (m), and connective tissue (ct) near the ostium are clearly visible. TEM.
(c) Bands (B) of connective tissue and muscle lie in the hemocoel at the base of filaments. Water channel epithelium (WCE) has largely been stripped away to reveal the bands, but some remains with intact internal ostia (O). SEM.
(d) Cross section of the connective tissue (ct) and muscle fiber (m) bands shown in 3c. The connective tissue filaments are arranged in parallel with the muscle fibers and dense bodies (arrows) connect muscle fibers with the surrounding connective tissue. TEM.

neither of these sets is paired with antagonistic muscles, the anatomical relationship between the main supportive connective tissue elements of the gill and the musculature suggests a functional antagonism. In the absence of contraction, the dense interfilament cross-struts serve a mechanical function in keeping the gill filaments apart. During contraction, the interfilament muscle bands pull the filaments together in the concertina-like motion described for many bivalve gills (Setna 1930; Elsey 1935; Atkins 1943; Jørgensen 1976). In addition, the contraction of the fibers surrounding the internal ostia act to close the ostia in a sphincter-like manner. Elsey (1935) first suggested an antagonistic relationship between the muscles and connective tissue skeleton in the bivalve gill. Elsey reported that the cross-struts of *Ostrea lurida* and *O. gigas* are straight when muscles are relaxed but become bent as the contracting muscles draw the filaments together. Intrinsic gill muscles in unionid bivalves have recently been described for *Ligumia subrostrata* and *Anodonta grandis* (Kays et al. 1990; Gardiner et al. 1991). In these species, muscle bands run perpendicular to gill filaments and insert onto calcified (chitinous) rods. Other muscles are associated with the water canals connecting the outside of the gill with the central water channel. Gardiner et al. (1991) concluded that the muscular elements in these unionids were antagonized by the connective tissue skeleton.

Ridewood (1903) and Elsey (1935) both described the soft skeleton of bivalves as elastic and resistant in nature. Our experience with the connective tissue skeleton of *D. polymorpha* is consistent with these descrip-

This content downloaded from
149.76.0.33 on Fri, 16 Apr 2021 15:13:37 UTC
All use subject to https://about.jstor.org/terms

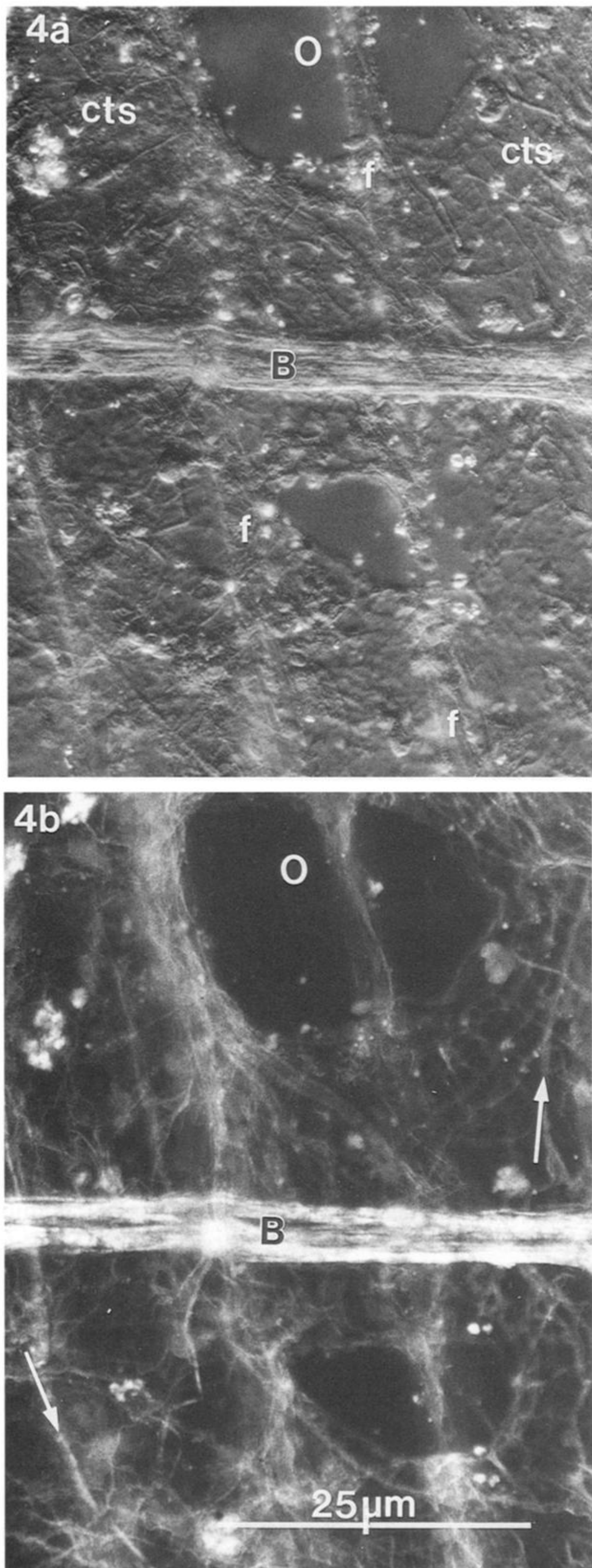


Fig. 4. Gill stripped of its epithelium. **(a)** Filament supports (f), the connective tissue sheet (cts), ostia (O), and bands (B) crossing the filaments at right angles are visible with Nomarski illumination. **(b)** Same gill labeled with phallacidin to reveal f-actin filaments in presumptive muscle bands. ← Large bands of muscle fibers lie at right angles to the filaments, while fibers (arrows) associated with the connective tissue sheet form a complex web-like pattern around the ostia.

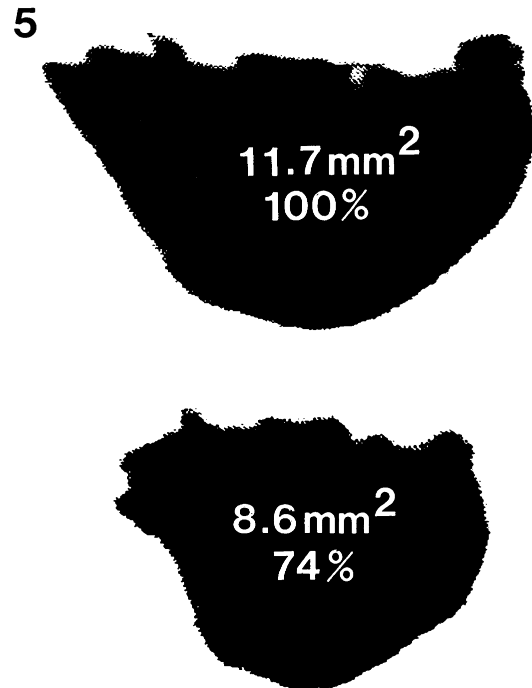


Fig. 5. Digitized image of a single demibranch just before acetylcholine application (above) and at 60 seconds after the addition of acetylcholine (below). For both time points, the absolute area (mm^2) and the relative reduction in gill area (% of initial area) are given.

tions. While the connective tissue support of gill filaments has often been termed chitinous (Ridewood 1903; Elsey 1935), preliminary data indicate that this connective tissue is similar to other connective tissues, containing collagen fibers embedded in a ground substance (Medler, unpubl.). The individual fibrils composing larger vertebrate collagen fibers are on the order of 20–200 nm in diameter, but those composing the reticular fibers of vertebrate connective tissue can be similar in size to those seen here (Kelly et al. 1984).

Experiments with whole demibranchs show that, as muscles contract, the demibranch shortens mainly in an anterio-posterior direction, thereby decreasing the demibranch area (Fig. 5). This reduction proceeds in a dose-dependent manner with either acetylcholine or FMRFamide application (Fig. 6). Since the degree of muscle activation is a function of intracellular calcium concentration (Ruegg 1986), it is likely that the differing levels of contraction are correlated with the

This content downloaded from
149.76.0.33 on Fri, 16 Apr 2021 15:13:37 UTC
All use subject to https://about.jstor.org/terms

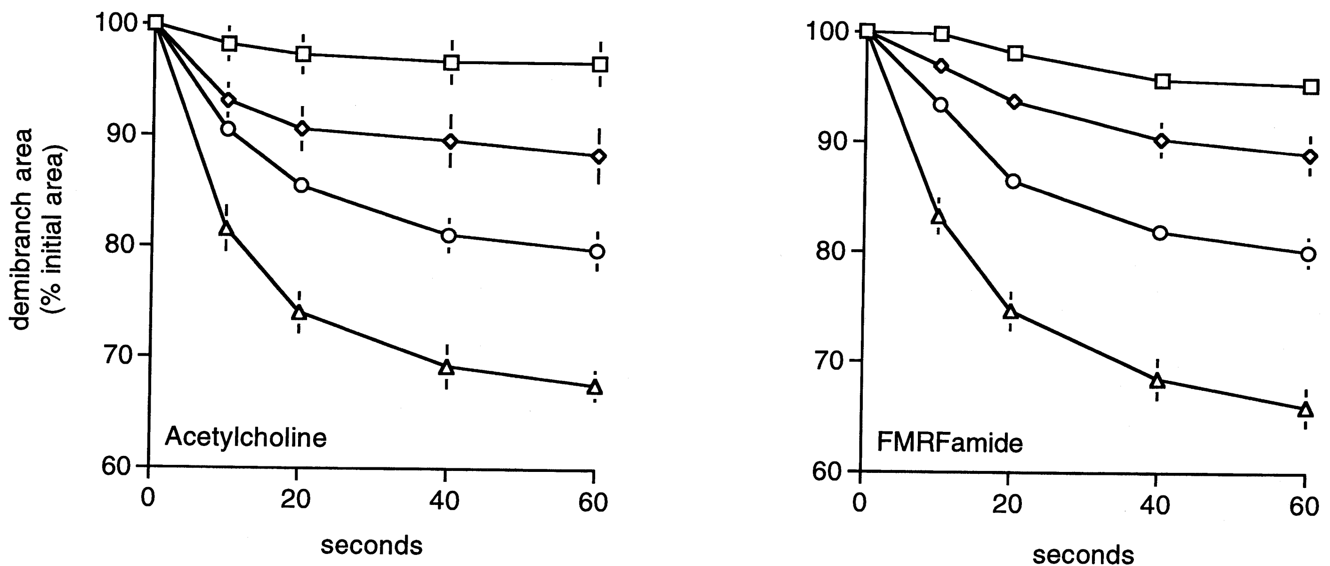


Fig. 6. Reduction in gill area (% of initial area) as a function of time in response to exogenous acetylcholine and FMRFamide. Dose-dependent responses to 10^{-6} M (squares), 10^{-5} M (diamonds), 10^{-4} M (circles), and 10^{-3} M (triangles) acetylcholine or FMRFamide are shown. Both experiments used 10 animals, with each of the four demibranchs per animal randomly distributed to a transmitter concentration. (Each point is the mean ± SE).

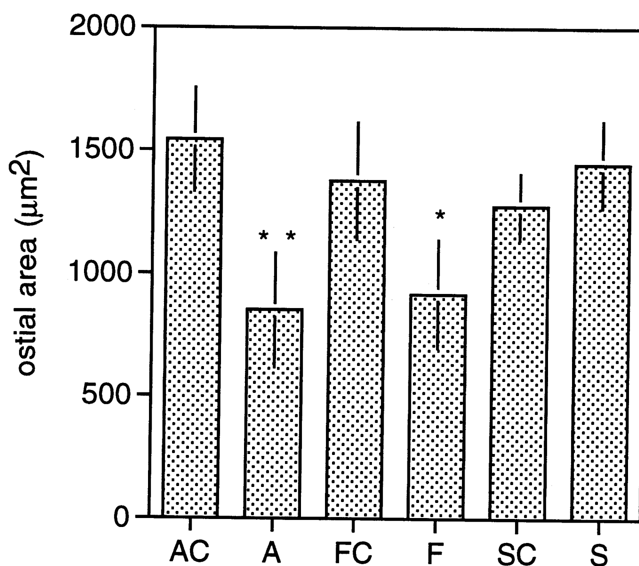


Fig. 7. Pretreatment control ostial areas (μm²) for acetylcholine (AC), FMRFamide (FC), and serotonin (SC) and the corresponding ostial areas (A, F, and S) following 5 min of exposure to the respective transmitter. Paired comparisons of pretreatment control ostia with corresponding post-treatment ostia were made for each of the three transmitters. Transmitter concentration in each case was 10^{-5} M. (Each value is mean ± SE, n = 10 preparations. ** $p < 0.01$; * $p < 0.02$; for the serotonin group, $p < 0.1$).

amount of calcium entering the muscle. Preliminary experiments with calcium-free Ringer's solutions suggest that external calcium is required for muscle contraction to proceed. This dependence on external calcium is not surprising given the small size of these fibers and the apparent lack of a developed intracellular membrane system (Fig. 4b,d). These changes in whole demibranch area are consistent with contraction of the muscle fibers in connective tissue-associated bands that run across the base of the filaments at right angles.

Observation of the water channel epithelium and internal ostia also reveals consistent responses to exogenous transmitter application. Changes in ostial dimension in response to acetylcholine, FMRFamide, and serotonin are rapid and reversible (Fig. 9). Acetylcholine and FMRFamide both cause ostial area to diminish. Serotonin generally causes the ostia to open more widely. Apparently, acetylcholine and FMRFamide cause the muscle fibers to contract while serotonin causes the muscle fibers to relax. While the experimental examination of the serotonin effect was not statistically significant, the response is biologically important but variable. The response to serotonin is generally an increase in ostial area, but in a few cases ostia clearly decrease in size. Elsey (1935) observed dramatic changes in the ostial dimensions of the actively functioning gills of *O. lurida* and Gardiner et al. (1991) demonstrated statistically significant increases in ostial dimensions of *Ligumia subrostrata* following serotonin (10^{-5} M) application. Further work is needed

This content downloaded from
149.76.0.33 on Fri, 16 Apr 2021 15:13:37 UTC
All use subject to https://about.jstor.org/terms

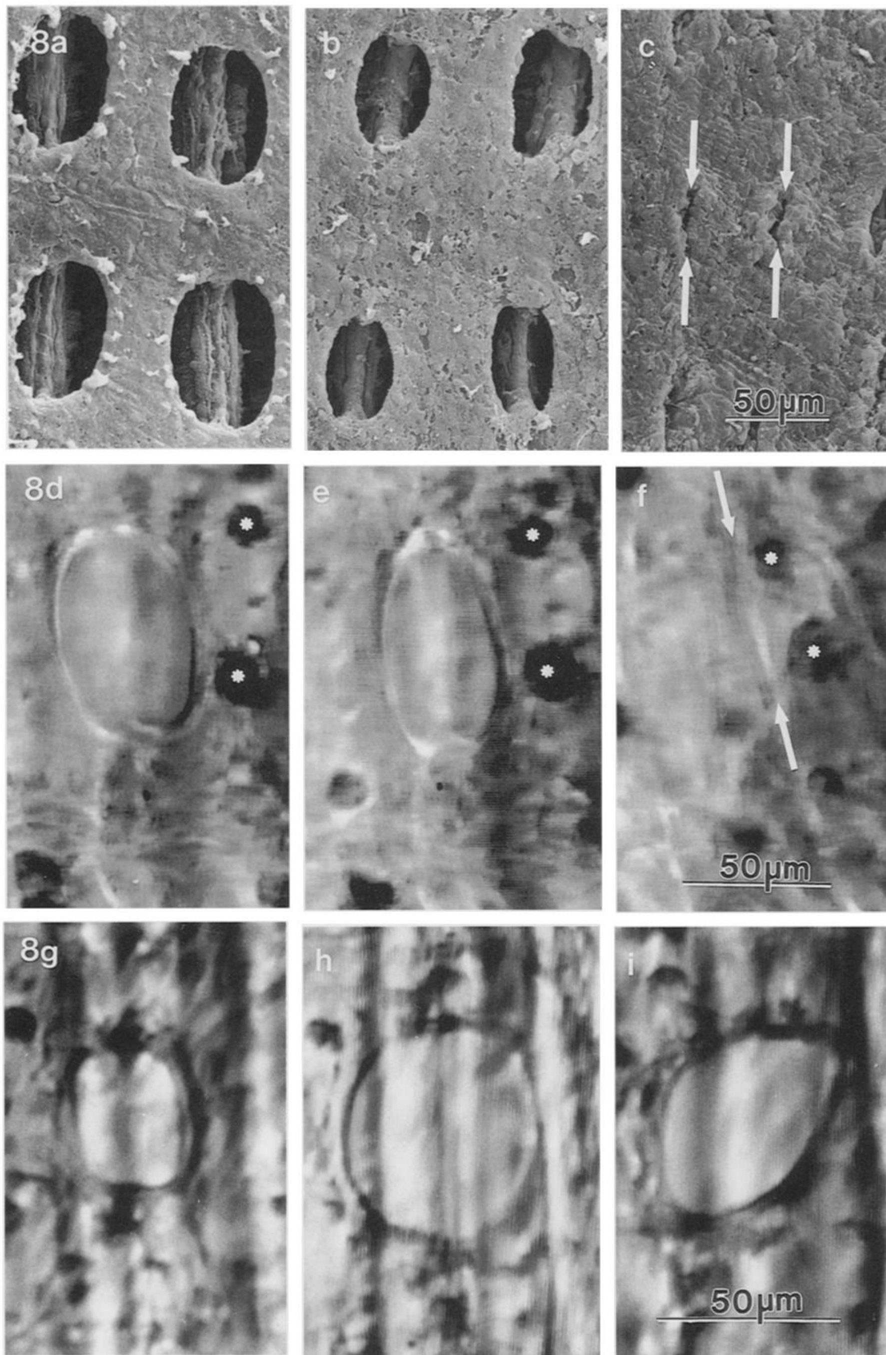
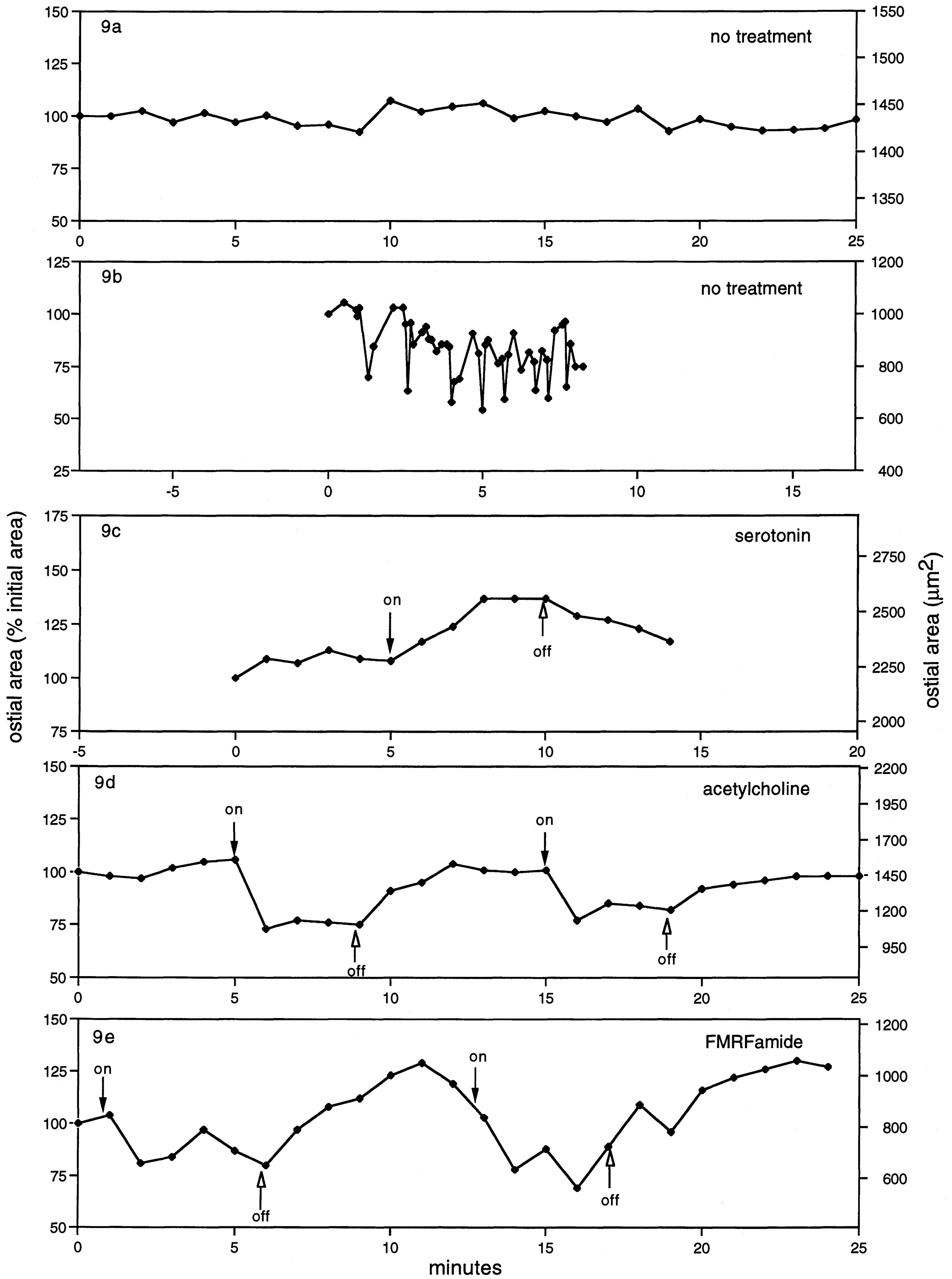


Fig. 8. Ostial areas between gills and for individual ostia over time. **(a–c)** Internal ostia and water channel epithelium. SEM. **(a)** Serotonin-treated gill (10^{-5} M): average ostial area = 2760 μm^2. **(b)** Untreated gill: average ostial area = 1444 μm^2. **(c)** FMRFamide-treated gill (10^{-5} M): ostia completely closed (arrows denote dorsal and ventral margins). **(d–f)** Digitized video images of a single ostium from a perfused live gill preparation (DIC optics). Asterisks mark two reference cells visible throughout the series. The preparation was briefly treated with serotonin ($\sim 10^{-3}$ M) a few minutes before setup in the chamber. **(d)** Ostium as it is perfused with a control Ringer's solution 1.25 min before treatment with FMRFamide (10^{-5} M for 1.5 min). The ostial area is 2525 μm^2. **(e)** Same ostium 1.16 min after the start of FMRFamide treatment. The ostial area has been reduced to 2013 μm^2 (80% of the area in 8d). **(f)** Same ostium 2.5 min after the initial exposure to FMRFamide. The ostium in this figure is completely occluded (arrows denote dorsal and ventral margins). **(g–i)** Digitized video images of a single ostium from a perfused preparation (Hoffman modulation optics). **(g)** Single ostium 2 min before treatment with serotonin (10^{-4} M for 1.33 min). The ostial area is 912 μm^2. **(h)** Same ostium after 1.33 min of serotonin treatment. The ostial area is 2017 μm^2 (221% of the area in 8g). **(i)** Same ostium 3.67 min after return to control Ringer's. The ostial area is 1450 μm^2 (159% of the area in 8g).

to understand which transmitters are physiologically important for direct muscular control.

Recent *in vivo* observations have documented what seems to be muscular control of gill function. Tankersley (1996) made endoscopic observations from the suprabranchial chamber of the unionid *Pyganodon cataracta* and found that the internal ostia were more visible and less constricted during active water pumping. These results were interpreted to be consistent with the muscular control of ostia reported by Gardiner et al. (1991). The rhythmic expansion and contraction of the gills of *P. cataracta* have also been observed; these were thought to result from cardiac rhythm and subsequent blood movement or from the muscular activity of water canals and ostia (Tankersley & Dimock 1993). Endoscopic observations of *Placopecten magellanicus* indicated that concertina-like movements of the gill are important in regulating ingestion volume (Beninger et al. 1992). Ward et al. (1994) observed movements in *Crassostrea virginica,* including expan-

This content downloaded from
149.76.0.33 on Fri, 16 Apr 2021 15:13:37 UTC
All use subject to https://about.jstor.org/terms



This content downloaded from
149.76.0.33 on Fri, 16 Apr 2021 15:13:37 UTC
All use subject to https://about.jstor.org/terms

sion and contraction of the plicae caused by movements of the ordinary filaments. Observing through the transparent shell of small specimens of *Dreissena polymorpha,* Sprung & Rose (1988) found gill expansion following shell opening.

The size of the passageways for water flow are important parameters for bivalve gill function. Models of the bivalve gill have used fixed estimates of interfilament distance or ostial dimension to determine other values like pump capacity and interfilament flow velocity (Foster-Smith 1976; Silvester & Sleigh 1984; Jørgensen et al. 1986; Jones et al. 1993). Our observations on *D. polymorpha* and those from other studies on oysters (Elsey 1935) and freshwater unionids (Gardiner et al. 1991) show that the interfilament distance and ostial dimensions are variable and under muscular control. Our study demonstrates that muscular alteration of the water passageways through the gill can be both rapid and dramatic in an excised gill preparation. The importance of muscle structure and function across bivalve phylogenies and gill types, as well as the extent of gill movements *in vivo,* remain to be determined.

Acknowledgments. Thanks to Ron Bouchard for technical assistance and for photographic suggestions. Thanks to Drs. Thomas H. Dietz and John W. Lynn for laboratory support and suggestions during this study. Thanks to Julie Cherry for editorial assistance. This work was supported by Louisiana Sea Grant R/ZM-1-PD and National Sea Grant NA46RG0096 Project R/ZMM-1.

References

Aiello EL 1960. Factors affecting ciliary activity on the gill of the mussel *Mytilus edulis.* Physiol. Zool. 33: 120–135.

——— 1962. Identification of the cilioexcitatory substance present in the gill of the mussel *Mytilus edulis.* J. Cell. Comp. Physiol. 60: 17–21.

Aiello E & Guideri G 1964. Nervous control of ciliary activity. Science 146: 1692–1693.

Atkins D 1937. On the ciliary mechanisms and interrelationships of lamellibranchs. Part III: Types of lamellibranch gills and their food currents. Q. J. Microsc. Sci. 79: 375–420.

——— 1943. On the ciliary mechanisms and interrelationships of lamellibranchs. Part VIII. Notes on gill musculature in Microciliobranchia. Q. J. Microsc. Sci. 84: 187–256.

Barrington EJW 1979. Invertebrate Structure and Function, 2nd edition. John Wiley and Sons, New York. 765 pp.

Beninger PG, Ward JE, MacDonald BA, & Thompson RJ 1992. Gill function and particle transport in *Placopecten magellanicus* (Mollusca: Bivalvia) as revealed using video endoscopy. Mar. Biol. 114: 281–288.

Cavanaugh CM, Levering PR, Maki JS, Mitchell R, & Lidstrom ME 1987. Symbiosis of methylotrophic bacteria and deep-sea mussels. Nature 325: 346–348.

Dando PR & Spiro B 1993. Varying nutritional dependence of the thyasirid bivalves *Thyasira sarsi* and *T. equalis* on chemoautotrophic bacteria, demonstrated by isotope ratios of tissue carbon and shell carbonate. Mar. Ecol. Prog. Ser. 92: 151–158.

Dietz TH 1985. Ionic regulation in freshwater mussels: a brief review. Amer. Malac. Bull. 3: 233–242.

Dietz TH, Lessard D, Silverman H, & Lynn JW 1994. Osmoregulation in *Dreissena polymorpha:* the importance of Na, Cl, K, and particularly Mg. Biol. Bull. 187: 76–83.

Dral ADG 1968. On the feeding of mussels (*Mytilus edulis* L.) in concentrated food suspension. Neth. J. Zool. 18: 440–441.

Duncan J, Ram JL, Fong PP, & Snow V 1994. Zebra mussel gills: long term culture and contractile responses. Amer. Zool. 34: 35A.

Elsey CR 1935. On the structure and function of the mantle and gill of *Ostrea gigas* and *O. lurida.* Trans. Roy. Soc. Canada, sec. 5: 131–158.

Foster-Smith RL 1975. 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.

——— 1976. Some mechanisms for the control of pumping activity in bivalves. Mar. Behav. Physiol. 4: 41–60.

Fyhn HJ & Costlow JD 1975. Anaerobic sampling of body fluids in bivalve molluscs. Comp. Biochem. Physiol. 52A: 265–268.

←

Fig. 9. Changes in area of representative internal ostia of perfused gill tissues. **(a)** Ostium receiving continuous Ringer's without transmitter. Overall, ostial area is stable but does vary over time. Movements demonstrated by this ostium are typical of what we observed in control ostia. **(b)** Ostium receiving continuous Ringer's without transmitter was observed to pulsate with a sphincter-like contraction. This ostium's movement is atypical but not unique. **(c–e)** Responses in ostial area after application of exogenous transmitters at a concentration of 10^{-6} M. In each case, "on" arrows indicate start of perfusion with the transmitter-containing Ringer's solution and "off" arrows indicate a return to control Ringer's solution. **(c)** Average changes in 2 adjacent ostia in response to serotonin application. **(d)** Average changes in 3 adjacent ostia in response to acetylcholine application. **(e)** Average changes in 2 adjacent ostia in response to FMRFamide application. In each case, the net changes appear to result from the response to the applied transmitter and from the spontaneous movements of the gill.

This content downloaded from
149.76.0.33 on Fri, 16 Apr 2021 15:13:37 UTC
All use subject to https://about.jstor.org/terms

Gardiner DB, Silverman H, & Dietz TH 1991. Musculature associated with water canals in freshwater mussels and response to monoamines *in vitro*. Biol. Bull. 180: 453–465.

Jones HD & Richards OG 1993. The effects of acetylcholine, dopamine, and 5-hydroxytryptamine on water pumping rate and pressure in the mussel *Mytilus edulis* L. J. Exp. Mar. Biol. Ecol. 170: 227–240.

Jones HD, Richards OG, & Southern TA 1993. Gill dimensions, water pumping rate, and body size in the mussel *Mytilus edulis* L. J. Exp. Mar. Biol. Ecol. 170: 213–237.

Jørgensen CB 1975. On gill function in the mussel, *Mytilus edulis* L. Ophelia 13: 187–232.

——— 1976. Comparative studies on the function of gills in suspension feeding bivalves, with special reference to effects of serotonin. Biol. Bull. 151: 331–343.

——— 1989. Water processing in ciliary feeders, with special reference to the bivalve filter pump. Comp. Biochem. Physiol. 94A(2): 383–394.

Jørgensen CB, Famme P, Kristensen HS, Larsen PS, Møhlenberg F, & Riisgård HU 1986. The bivalve pump. Mar. Ecol. Prog. Ser. 34: 69–77.

Kays WT, Silverman H, & Dietz TH 1990. Water channels and water canals in the gill of the freshwater mussel, *Ligumia subrostrata:* ultrastructure and histochemistry. J. Exp. Zool. 254: 256–269.

Kelly DE, Wood RL, & Enders AC 1984. Bailey's Textbook of Microscopic Anatomy, 18th edition. Williams and Wilkins, Baltimore. 909 pp.

Lei J, Payne BS, & Wang SY 1996. Filtration dynamics of the zebra mussel, *Dreissena polymorpha.* Can. J. Fish. Aquat. Sci. 53: 29–37.

Ludyanskiy ML 1993. Recent introductions of *Dreissena* and other forms to North America—the Caspian Sea/Black Sea connection. In: Zebra Mussels: Biology, Impacts, and Control. Nalepa TF & Scholesser DW, eds., Chapter 41. Lewis Publ., Boca Raton, FL.

McMahon RF 1991. Mollusca: Bivalvia. In: Ecology and Classification of North American Freshwater Invertebrates. Thorp JH & Covich AP, eds., Chapter 11. Academic Press, San Diego, CA.

Medler S & Silverman H 1994. Organization of gill musculature in the zebra mussel, *Dreissena polymorpha.* Amer. Zool. 34: 96A.

Morton B 1969. Studies on the biology of *Dreissena polymorpha* Pall. I. General anatomy and morphology. Proc. Malacol. Soc. London 38: 301–321.

Muneoka Y & Twarog BM 1983. Neuromuscular transmission and excitation-contraction coupling in molluscan muscle. In: The Mollusca, Vol 4. Saleuddin ASM & Wilbur KM, eds., Chapter 2. Academic Press, New York.

Nelson TC & Allison JB 1940. On the nature and action of diantlin: a new hormone-like substance carried by the spermatozoa of the oyster. J. Exp. Zool. 85: 299–338.

Ortmann AE 1911. A monograph of the Najades of Pennsylvania. Mem. Carnegie Mus. 4: 279–347.

Paparo AA 1988. Ciliary activity on the ctenidium of bivalve molluscs. Comp. Biochem. Physiol. 91C: 99–110.

Pearse V, Pearse J, Buchsbaum M, & Buchsbaum R 1987. Living Invertebrates. The Boxwood Press, Pacific Grove, CA. 848 pp.

Reynolds ES 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17: 208–213.

Ridewood WG 1903. On the structure of the gills of the Lamellibranchia. Phil. Trans. Roy. Soc. London 195: 147–284.

Ruegg JC 1986. Calcium in Muscle Activation: a Comparative Approach. Springer Verlag, Berlin. 300 pp.

SAS Institute 1995. SAS release 6.10 edition. SAS Institute, Campus Drive, Cary, NC 27513.

Setna SB 1930. The neuro-muscular mechanism of the gill of *Pecten.* Q. J. Microsc. Sci 73: 365–392.

Silverman H 1989. Form and function of calcium concretions in unionids. In: Origin, Evolution, and Modern Aspects of Biomineralization in Plants and Animals: Proc. Fifth Internatl. Symp. on Biomineralization. Crick R, ed., pp 367–384. Plenum Press, New York.

Silverman H, Steffens WL, & Dietz TH 1985. Calcium from extracellular concretions in the gills of freshwater unionid mussels is mobilized during reproduction. J. Exp. Zool. 236: 137–147.

Silvester NR 1988. Hydrodynamics of flow in *Mytilus* gills. J. Exp. Mar. Biol. Ecol. 120: 171–182.

Silvester NR & Sleigh MA 1984. Hydrodynamic aspects of particle capure by *Mytilus.* J. Mar. Biol. Assoc. UK 64: 859–879.

Southward EC 1986. Gill symbionts in thyasirids and other bivalve molluscs. J. Mar. Biol. Assoc. UK 66: 889–914.

Smirnova NF, Biochino GE, & Vinogradov GA 1993. Some aspects of the zebra mussel (*Dreissena polymorpha*) in the former European USSR with morphological comparisons to Lake Erie. In: Zebra Mussels: Biology, Impacts, and Control. Nalepa TF & Scholesser DW, eds., Chapter 13. Lewis Publ., Boca Raton, FL.

Sprung M & Rose U 1988. Influence of food size and food quantity on the feeding of the mussel *Dreissena polymorpha.* Oecologia 77: 526–532.

Tankersley RA 1996. Multipurpose gills: effect of larval rooding on the feeding physiology of freshwater unionid mussels. Invertebr. Biol. 115(3): 243–255.

Tankersley RA & Dimock RV 1992. Quantitative analysis of the structure and function of the marsupial gills of the freshwater mussel *Anodonta cataracta.* Biol. Bull. 182: 145–154.

——— 1993. Endoscopic visualization of the functional morphology of the ctenidia of the unionid mussel *Pyganodon cataracta.* Can. J. Zool. 71: 811–819.

Ward JE, Newell RIE, Thompson RJ, & MacDonald BA 1994. *In vivo* studies of suspension-feeding processes in the eastern oyster, *Crassostrea virginica* (Gmelin). Biol. Bull. 186: 221–240.

This content downloaded from
149.76.0.33 on Fri, 16 Apr 2021 15:13:37 UTC
All use subject to https://about.jstor.org/terms