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Direct observations of feeding structures and mechanisms in bivalve molluscs using endoscopic examination and video image analysis

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Abstract. A new technique is described for observing the structures and mechanisms of suspension feeding in bivalves using endoscopic examination and video image analysis. This method permits direct in vivo observations of whole, intact structures of relatively undisturbed specimens. No surgical alterations of shell or tissue are required for most species. Pallial organ activity can be recorded for future observations and analysis. Using this technique we examined three bivalve species, each with different degrees of mantle fusion: *Mya arenaria* L. *Mytilus edulis* L., and *Placopecten magellanicus* (Gmelin). The specimens were collected between April and September 1990 at various locations in Trinity Bay, Newfoundland, Canada. Particle retention by the gill and transport of material to the palps was observed, and velocity of particles moving on the gill was determined. We demonstrate that the endoscope-video-analysis system is an efficient and affordable technique suitable for studies of pallial organ function and mechanisms of feeding.

Introduction

Research examining the feeding physiology of bivalve molluscs has been hampered by the lack of direct observational techniques for in vivo study of pallial organ structure and function (see Beninger in press for review). Knowledge of these organs has been gained primarily through morphological studies of preserved structures using light and electron microscopy (Drew 1906, Nelson 1960, Moore 1971, Owen 1974, Beninger et al. 1988, 1990a, b, Le Pennec et al. 1988), observations of in vitro freshly dissected structures (Aiello 1970, Moore 1971, Jørgensen 1975, 1976, 1981, Paparo 1985, Beninger et al. 1988), observations of in vivo structures of surgically altered individuals (i.e. through holes cut into the shell, mantle, or both; MacGinitie 1941, Foster-Smith 1975, 1978, Jørgensen 1975, 1976), and observations of in vivo structures of juveniles with transparent shells (Tammes and Dral 1955, Dral 1967). While these types of studies have contributed greatly to the understanding of pallial organ structure and function, all have certain limitations and problems. Artifacts can arise during isolation and fixation of structures, in vitro structures may not function in the same manner as those in whole bivalves, post-operative trauma may alter the function of in vivo structures, pallial structures of juveniles may not have completed development, and active surfaces of in vivo organs can be hidden from view (e.g. ridged palp surfaces, gill arch).

In contrast, endoscopic examination allows direct in vivo observations of whole, intact pallial organs of adult bivalves with minimal disturbance. No surgical alterations of shell or tissue are required for most species. The optical shaft of the endoscope is small enough (1.7 mm diameter) to allow insertion between demibranchs of the gill, or between opposing labial palps. The combination of endoscopy with video recording and image analysis facilitates observation and documentation of events, and allows for post-observational analysis. Despite these advantages, endoscopic observations of bivalve feeding have only been performed in a cursory manner on *Crassostrea gigas* without the benefit of a video camera, recorder, or image analysis system (Bernard 1972, 1974). In this paper we describe an endoscopic video image analysis method to observe, record, and analyze the form and function of feeding structures in bivalves. The complete system is an inexpensive, powerful tool for the study of otherwise inaccessible structures of bivalves and perhaps other invertebrates.

Materials and methods

An endoscope is comprised of a rod lens and a fiber optic light guide sealed within a stainless steel cylinder to form an optical insertion tube (OIT), and a manual or self-focusing ocular. In our technique, the endoscope was connected to a standard charge-coupled-device (CCD) video camera. The performance of color (JE3462RGB, Javelin Electronics, Torrance, California, USA) and black and white (Cohu 4815, Cohu Electronics, San Diego, California, USA) cameras was compared. The camera was connected to a four-head

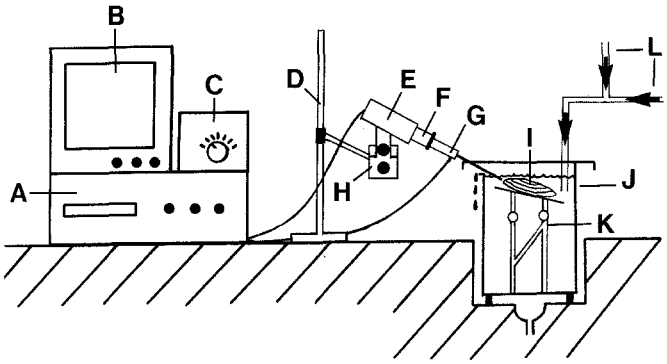


Fig. 1. Instruments and equipment used for bivalve endoscopy. (A) Video cassette recorder, (B) video monitor, (C) halogen light source (150 W), (D) support stand, (E) CCD video camera, (F) zoom lens, (G) endoscope, (H) micromanipulator, (I) bivalve, (J) plastic bucket with lid, (K) adjustable holding stand, (L) seawater and particle inflow

video cassette recorder (VHS, NTSC) and a color or black and white video monitor. A halogen lamp provided cold light to the tip of the optical tube via the incorporated fiber optic light guide. Endoscopes and accessories were obtained from Olympus Corp. (Lake Success, New York, USA) and Schölly Fiberoptic GmbH (W-7819 Denzlinger, Germany). For most of the work we used an SES-1711D (Olympus) endoscope fitted with a 1.7×105 mm rigid OIT (55° angle of view). The endoscope was attached to the camera with a C-mount zoom adapter (Schölly), allowing a maximum magnification of ca. 200× (Fig. 1).

Three species of bivalves were examined using the endoscope-video system: the soft-shelled clam *Mya arenaria* L. (ca. 55 mm shell height, 90 mm shell length), the blue mussel *Mytilus edulis* L. (ca. 40 mm shell height, 91 mm shell length), and the giant scallop *Placopecten magellanicus* (Gmelin) (ca. 44 mm shell height, 148 mm shell length). These species were chosen in order to evaluate the utility of endoscopic examination of bivalves with differing shell shapes and extents of mantle fusion. *Mya arenaria* has a completely fused mantle and two siphons, with openings into the infrabranchial chamber restricted to the incurrent siphon and the pedal gape. *Mytilus edulis* has a fused mantle, a narrow excurrent siphon, and a broad continuous incurrent siphon which allows access to the infrabranchial chamber along the entire ventral margin. *P. magellanicus* has no siphons, a wide gape, and a mantle that is fused only in the dorsal region, allowing access to the pallial cavity along most of the shell margin.

In order to prevent the bivalves from completely closing their valves and damaging the OIT, a small wooden wedge was inserted between the valves of mussels at the anterior-ventral margin near the pedal gape, and a no. 00 rubber stopper was inserted between the valves of scallops at the most posterior-dorsal margin. Specimens plugged in this way were still able to rapidly adduct their valves, and expel jets of water and debris from the mantle cavity. In addition, they produced feces and pseudofeces, and survived in seawater holding tanks as long as did unplugged specimens.

An adjustable PVC plastic stand was constructed to hold the bivalves. This stand fitted into a 25-liter covered plastic bucket, which served as the flow-through seawater holding chamber. Bivalves were secured to the stand using Velcro. The plastic half of the Velcro was epoxied onto the stand, and the fabric half was epoxied onto the shells of bivalves. This attachment method allowed rapid mounting and removal of specimens from the stand. Specimens on the stand could be positioned from horizontal to 45° below horizontal, as well as adjusted vertically in the seawater holding chamber. Two notches were cut into the lip of the bucket. One was contiguous with a notch cut into the lid permitting entry of the OIT. The other notch was for seawater inflow. A hole was also drilled into the lid as a sampling port (Fig. 1).

The video camera with attached endoscope was mounted on a micromanipulator positioned to correspond to the angle of a bivalve secured to the adjustable stand. The OIT was passed through the bucket's notched lip and lid, and then between the valves of the specimen. Ambient seawater (12 to 15 °C) was supplied to the bucket and the bivalve was allowed to adjust to the endoscope for 2 to 12 h, after which observations were made on pallial structures such as gills, palps, and lips. Occasionally, it was necessary to withdraw the OIT from the pallial cavity for cleaning. After removing foreign material, the OIT was reinserted and observations continued.

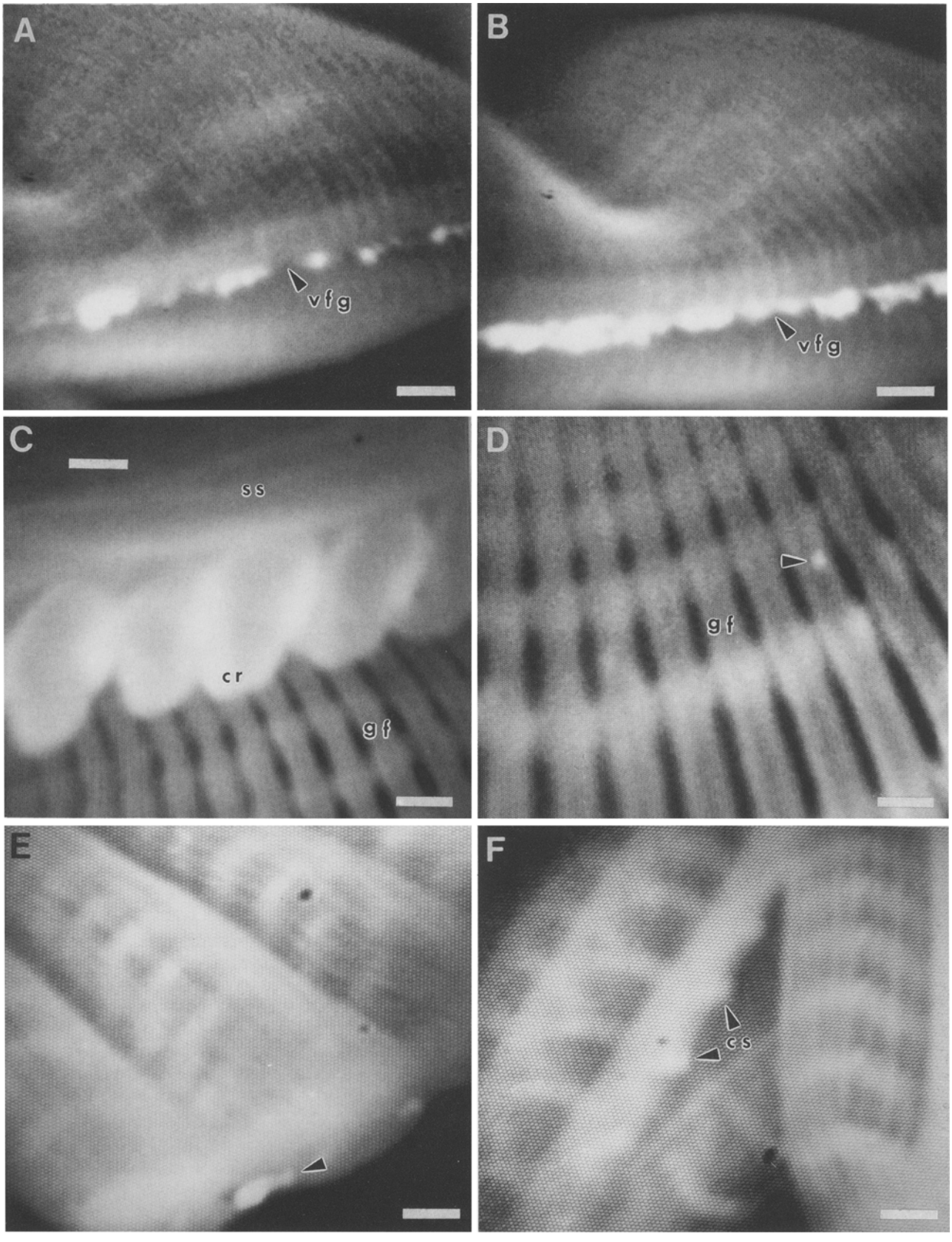
A mixture of the diatom *Chaetoceros muelleri* Lemmermann and red fluorescent plastic particles (both 4 to 7 µm diameter) was delivered to the bucket using a peristaltic pump. Concentration of these particles in the inflowing seawater was determined using an electronic particle counter (Coulter Electronics, Multisizer). Red particles acted as a tracer. They were visible under color conditions and showed up as bright white spots under black and white conditions.

Recorded images from the endoscope were analyzed using video analysis software (JAVA, Jandel Scientific, Corte Madera, California, USA) run on an IBM AT compatible computer with an installed video digitizing board (TARGA, Truevision, Inc., Indianapolis, Indiana, USA). This system allowed us to freeze an image on screen, enhance and clarify that image, and perform morphometric measurements. Calibrations for morphometric measurements were performed by dissecting the pallial organs and measuring distances between adjacent gill filaments and adjacent palp ridges with a compound microscope and calibrated ocular micrometer. The velocity at which particles moved along gill structures was determined by counting the number of frames required for a given particle clump to traverse a known distance. Recording speed was a standard 30 frames s^{-1}.

Results and discussion

Endoscopic observations gave us a novel perspective of pallial organs in actively feeding bivalves. We observed and recorded movements of gills and palps, and measured the velocity of particles moving on these structures. The precision of velocity measurements was ± 1 frame, or $\pm 3.3\%$. Accuracy of these measurements depends upon microscopic estimates of the size of the pallial organ, which is a function of magnification and ocular micrometer calibration.

Fig. 2. *Mya arenaria* (A, B), *Mytilus edulis* (C, D) and *Placopecten magellanicus* (E, F). Endoscope micrographs of whole, in vivo pallial structures. (A, B) Frontal surface of ventral bend of one plicate gill demibranch (*M. arenaria*) showing fluorescent particles (bright material) in ventral food groove (vfg); individual filaments show up on micrograph as parallel lines; (A) specimen exposed to 19 600 particles ml^{-1}, (B) specimen exposed to 80 000 particles ml^{-1} (scale bars = 100 µm). (C) Ventral margin of labial palp (foreground) overlying frontal surface of one gill demibranch (background; *M. edulis*); note smooth surface (ss) and ciliated ridges (cr) of palp, and individual filaments of gill (gf) (scale bar top/foreground = 240 µm; scale bar bottom/background = 150 µm). (D) Frontal surface of one gill demibranch (*M. edulis* showing fluorescent particle clump (arrow) moving along gill filament (gf) toward ventral margin (bottom of micrograph; scale bar = 100 µm). (E) Frontal surface of ventral bend of one plicate gill demibranch (*P. magellanicus*) showing fluorescent particles (arrow) on ventral margin; ordinary filaments show up on micrograph as parallel lines running the length of each plica (scale bar = 210 µm). (F) Frontal surface of one plicate gill demibranch (*P. magellanicus*); abnormal separation of two ordinary filaments showing ciliated spurs (cs) which connect adjacent ordinary filaments and give gill a banded appearance


A
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A resolution of ca. 10 μm was achieved using the black and white system. This resolution was better than that obtained with the color camera, partly due to the 150 W maximum output of the light source which was insufficient for the color camera we tested (min. 20 lx; see Inoué 1986 for discussion). Use of a stronger light source or lower-lux camera is therefore required for color recordings. One advantage of color is the ability to use different-colored particles as tracers in feeding studies.

Mya arenaria presented the greatest challenge to this technique, due to its great degree of mantle fusion. Entering the infrabranchial chamber via the incurrent siphon was possible, but specimens were sensitive to the smallest movements of the endoscope. After insertion, clams became accustomed to the OIT, extending their siphons, opening incurrent apertures, and flaring out guard tentacles. During this period it was possible to observe the posterior portion of gill lamellae; however, any movement of the endoscope resulted in siphon withdrawal. Attempts to enter the pallial cavity via the pedal gape were impeded by the clam's foot, which blocked the lens and coated it with mucus. The best observations were made by passing the OIT through a small (2 to 3 mm) mid-ventral, cauterized incision in the mantle. At this location, *M. arenaria* were not sensitive to movements of the endoscope, and we could observe individual gill filaments, particles moving ventrally along lamellae, and particle clumps and strings moving anteriorly in ventral food grooves (Fig. 2A, B). Mean velocities of particles in these food grooves were 438 ± 64 (SD; range 375 to 562) μm s^{-1} ($n=10$ observations), and 277 ± 10 (SD; range 259 to 292) μm s^{-1} ($n=10$ observations) at concentrations of 19 600 and 80 000 particles ml^{-1}, respectively. The lower velocity of particles at the higher concentration could have been caused by the increased amount of material in the ventral food groove (Fig. 2A, B).

Examinations of the pallial organs of *Mytilus edulis* were easier to perform. The OIT could be inserted at any point along the broad incurrent siphon, allowing for observations of the entire frontal surface of gills. Mussels were most sensitive to movements of the endoscope along the posterior portion of the mantle margin where guard tentacles were present. If disturbed by movements, however, mussels adjusted to the new position of the OIT within minutes, showing signs of normal pumping activity. The relatively small gape of even large *M. edulis* (91 mm shell length) reduced to some degree our freedom to manipulate the OIT within the infrabranchial chamber. Nevertheless, observations could be made of gill filaments and labial palps (Fig. 2C), particles moving ventrally along lamellae (Fig. 2D), and particle clumps and strings moving anteriorly in ventral food grooves. Movements of the latero-frontal cirri were also recorded; however, these structures could not be clearly resolved because of their size (ca. 12.0×0.5 μm; Owen 1974). Mean velocity of particles moving ventrally on gill lamellae was 320 ± 65 (SD; range 267 to 400) μm s^{-1} ($n=4$ observations) at 6500 particles ml^{-1}. In ventral food grooves, mean velocities were 399 ± 41 (SD; range 333 to 482) μm s^{-1} ($n=8$ observations) and 421 ± 33 (SD; range 377 to 465) μm s^{-1} ($n=8$ observations) at 7600 and 10 000 particles ml^{-1}, respectively.

Placopecten magellanicus was the easiest bivalve to examine. The OIT could be inserted anywhere along the unfused mantle margin, and, after a period of adjustment, scallops were minimally disturbed by minor movements of the endoscope. Reactions to excessive movements of the endoscope included gill retraction and rapid valve adductions. Scallops disturbed in this way, however, recovered within minutes and resumed normal feeding behavior. The large valve gape of *P. magellanicus* afforded maximal freedom in OIT movement, which facilitated observations of ascending and descending lamellae of the inner and outer demibranch, as well as the ventral bend, dorsal bend, gill arch, palps, and lips. During observations of the gill, we could clearly identify major morphological features of the lamellae (e.g. ordinary filaments, ciliated spurs; Fig. 2E, F). Particles were routinely observed streaming into the infrabranchial chamber and being intercepted on the frontal surface of gill lamellae. In addition, particles were observed streaming along the gill arch and dorsal bend food tracts. Mean velocity of particles travelling along the arch was 7000 ± 2000 (SD; range 5652 to 10 000) μm s^{-1} ($n=4$ observations) at a concentration of 11 000 particles ml^{-1}. Mean velocities of particles travelling along the dorsal bend were 9000 ± 2000 (SD; range 6500 to 13 000) μm s^{-1} ($n=8$ observations), and 6000 ± 1000 (SD; range 5652 to 7647) μm s^{-1} ($n=8$ observations) at concentrations of 2000 and 20 000 particles ml^{-1}, respectively.

During observations of the posterior portion of labial palps, we recorded intermittent particle clumps moving from the gill lamellae onto the palp surface. These clumps could be followed as they moved from the gill-palp junction to a region between opposing palps. We did not, however, survey the entire palp surface. Activity of the palps was observed, including undulations of the palp ridges, and waving of cilia. Detailed analysis of particle movements on the pallial organs of *Placopecten magellanicus* is now in progress (Beninger et al. in preparation).

The linking of fiber optics with video and computer technology provides a powerful tool that will allow researchers to make direct observations of otherwise inaccessibie structures of bivalves and perhaps other invertebrates. The real power and impact of this method lies in the ability to monitor and measure dynamic processes of intact pallial organs under near-natural conditions. Such observations will give further insight into the form and function of pallial structures, and will facilitate study of particle capture, transport, selection, and ingestion in bivalves.

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