Endoscopic visualization of the functional morphology of the ctenidia of the unionid mussel *Pyganodon cataracta*

RICHARD A TANKERSLEY[1] AND RONALD V. DIMOCK, JR.
Department of Biology, Wake Forest University, Winston-Salem, NC 27109, U.S.A.

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During reproduction, the outer demibranchs of the unionid mussel *Pyganodon cataracta* serve as marsupia, with incubation of the developing shelled glochidia larvae occurring within the water tubes. In this study, recently developed endoscopic video analysis techniques were employed to examine *in vivo* the dynamics of filter feeding and water transport in mussels during gravid and postgravid periods. Particles entering the mantle cavity and retained by the gills were transported to the palps in a complex mucus-bound cord by the ventral food groove of the medial ctenidia. Larval incubation and ctenidial swelling impeded flow around the lateral demibranchs, although marsupial ctenidia were still actively involved in suspension feeding. Cilia on the distended ventral edges of marsupial demibranchs were often observed transporting filtered particles to the frontal surface of the medial gills. Larvae within the brood chambers were morphologically isolated from the surrounding medium by dorsal brood caps on the primary water tubes. Direct observations of the secondary water tubes of marsupial gills constructed during periods of larval incubation confirmed their role as temporary lumina for water transport during gravid periods. Time-lapse video recordings revealed that mature larvae are released from the brood chambers via the suprabranchial cavity and exhalant siphon by rapid adductions of the valves and contractions of the brooding demibranchs.

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Au cours de la reproduction, les hémibranchies externes de la moule unionidée *Pyganodon cataracta* servent de marsupiums et les larves glochidies s'y développent dans leur coquille à l'intérieur de tubes aquifères. Des techniques récentes d'analyse endoscopique sur vidéo nous ont permis d'examiner *in vivo* la dynamique de l'alimentation par filtration et du transport de l'eau chez des moules, au cours des périodes d'incubation et au cours des périodes subséquentes. Les particules qui pénètrent dans la cavité du manteau et sont retenues par les branchies sont transportées vers les palpes en une masse complexe liée par du mucus via le sillon alimentaire ventral des cténidies médiales. L'incubation des larves et le gonflement des cténidies entravent la circulation autour des hémibranchies latérales, mais les cténidies marsupiales restent actives au cours de l'alimentation par filtration. Des cils situés le long des bordures ventrales distendues des hémibranchies marsupiales ont été observés à plusieurs reprises, transportant les particules filtrées à la surface frontale des branchies médiales. Les larves qui se trouvent à l'intérieur des chambres d'incubation sont morphologiquement isolées du milieu ambiant par les couvercles dorsaux des tubes aquifères primaires. L'observation directe des tubes aquifères secondaires des branchies marsupiales formés au cours des périodes d'incubation larvaire confirme leur rôle de citernes temporaires servant au transport de l'eau au cours des périodes d'incubation. Des enregistrements vidéo à temps échelonné ont révélé que les larves à maturité sont libérées dans les chambres d'incubation via la cavité suprabranchiale et le siphon exhalant par des mouvements rapides d'adduction des valves et de contraction des hémibranchies d'incubation.

[Traduit par la rédaction]

Introduction

Early descriptions of the structure of the gills and the morphology of the brood chambers of freshwater unionid bivalves were based upon observations of preserved or freshly dissected tissue (Ridewood 1903; Lefevre and Curtis 1910; Ortmann 1911). More recent studies employing modern histological techniques and electron microscopy also have only been able to provide static evidence of the functional morphology of the bivalve gill, especially in relation to its potentially conflicting roles in ventilation, suspension feeding, and larval incubation (Kays *et al.* 1990; Gardiner *et al.* 1991; Richard *et al.* 1991; Tankersley and Dimock 1992).

While the roles of gill cilia and cirri in producing water currents and capturing and transporting suspended particles over the surface of the gills of bivalve molluscs have been well documented (for review see Ward *et al.* 1993), the opaque shells of adult animals preclude direct observations of the fluid dynamics and structures involved in suspension feeding in intact animals. To date, most *in vivo* observations of gill ciliary activity and particle filtration have been conducted either using juveniles possessing thin and relatively transparent shells (Dral 1967; Jørgensen and Ockelmann 1991; Reid *et al.* 1992) or have involved the removal of sections of the valves or mantle of adults (MacGinitie 1941; Bernard 1974; Foster-Smith 1975*a*, 1978; Jørgensen 1975, 1976; Reid *et al.* 1992). Recently, however, Ward *et al.* (1991) have introduced a new technique employing video endoscopy for *in vivo* observations of the gills and feeding structures of live, intact adult bivalves.

We have previously described and quantified the structural changes associated with larval incubation in the freshwater unionid mussel *Pyganodon cataracta* (Tankersley and Dimock 1992) (formerly *Anodonta cataracta*; Hoeh 1990) and demonstrated that particle filtration, retention, and transport are significantly affected by the presence of developing glochidia in the brood chambers of the lateral gills (Tankersley 1992). However, exactly how particles are processed by the ciliary tracts of gravid marsupial demibranchs under natural conditions is still a matter of inference and conjecture. Furthermore, the construction of secondary water tubes producing the tripartite arrangement of the marsupial gills of anodontine

[1]Present address: Duke University Marine Laboratory, Pivers Island, Beaufort, NC 28516-9721, U.S.A.

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mussels (Ortmann 1911; Richard *et al.* 1991; Tankersley and Dimock 1992) has been assumed to be associated with the maintenance of water transport during larval incubation, but their role as temporary lumina for gill irrigation has never been confirmed. Direct observation of ambient water flow through the mantle cavity and water tubes is necessary in order to relate any changes in suspension feeding and water transport directly to modifications in ctenidial architecture accompanying larval brooding.

While it has often been assumed that mature glochidia are released dorsally from gill brood chambers and expelled through the exhalant siphon, most descriptions of larval release have been based upon anecdotal reports of the position of glochidia following their release or the rupture of the gills of freshly dissected or preserved mussels (Ortmann 1911; Matteson 1955; Yokley 1972; Richard *et al.* 1991). Ortmann (1911) reported that most unionids, including some of the Anodontinae, release glochidia via the suprabranchial cavity and exhalant siphon, which he argued was consistent with *a priori* predictions based upon the fluid dynamics within the infra- and supra-branchial cavities and the position of the siphons *in situ*. More recently, Richard *et al.* (1991) concluded, from the results of mechanical stimulation of swollen demibranchs, that mature glochidia of *Pyganodon* (= *Anodonta*) *grandis* are released from the stretched and ultimately ruptured ventral margin of the marsupial gill, a pattern of glochidial exodus that is consistent with the descriptions of larval discharge for several lampsilines (Ortmann 1911). Although the timing and mechanisms involved in glochidial release most likely vary among species with different marsupial morphologies and durations of larval incubation (for review see Kat 1984), the relative isolation of the brood chambers within the marsupial demibranchs has made direct observations of the process of glochidial release in undisturbed mussels impossible.

In this study, we utilize endoscopic techniques and video analysis (Ward *et al.* 1991) to make *in vivo* observations of the morphological features and flow dynamics of the marsupial and non-marsupial gills of *P. cataracta*, including the architecture of the primary and secondary water tubes and brood chambers. The results reported in this study provide the first direct evidence for the function of gravid marsupial gills in particle capture, the role of the secondary water tubes as temporary conduits for gill irrigation during and immediately following larval brooding, and the release of mature glochidia via the suprabranchial chamber.

Materials and methods

Endoscopic examination of the gills and feeding structures of *Pyganodon cataracta* followed the basic procedure recently described by Ward *et al.* (1991). All observations were made using an endoscope 2.7 mm in diameter × 18 cm long (model K27-18-00-62, Olympus Corp., New York) fitted with a fiber-optic light (cold-incandescent) source (Fig. 1A). Video recordings of the pallial organs and ctenidia were made by attaching the ocular of the scope via a C-mount adapter to a Javelin CCD color camera (model JE 3462HR). Most images were recorded on a ½-in. (1 in. = 2.54 cm) VHS video cassette recorder (Mitsubishi model U32; 30 Hz frame rate) and viewed on a color monitor. Long-term monitoring of larval release from the brood chambers of gravid mussels was conducted using a JVC ½-in. time-lapse video recorder (model BR-9000U) at a scanning rate of 1.25 Hz. The camera and endoscope were mounted on a micromanipulator which permitted precise positioning of the endoscope within the mantle and suprabranchial cavities. The tip of the scope provided a 62° field of view, a minimum focal distance of ≈5 mm, and a maximum magnification of ≈150×.[2]

Adult *P. cataracta* (shell length ≈11–15 cm) were collected from Speas' (Yadkin County) and Myer's (Forsyth County) ponds in North Carolina during the brooding (December–January; 9–10°C) and postbrooding (February–March; 12–15°C) periods of their reproductive season. Mussels were mounted stationary in a 20 × 15 × 23 cm Lucite chamber containing 5.7 L of artificial pond water (0.5 mM NaCl, 0.4 mM $CaCl_2$, 0.2 mM $NaHCO_3$, 0.05 mM KCl) by gluing a 1 cm diameter plastic bolt to the center of one valve and attaching it to a 15 × 3.5 cm vertical plastic guide in the middle of the chamber (Fig. 1A). The position of the mussel was adjusted so that the tip of the endoscope could be easily inserted into the mantle or suprabranchial cavities via the pedal gape or exhalant siphon, respectively. Mussels were prevented from closing on the stainless steel shaft (optical insertion tube) of the endoscope by inserting a 1 cm diameter plastic wedge between the valves along the ventral margin near the location of endoscope insertion. Water within the chamber was maintained at collection temperatures by a thermostatically controlled water bath.

Suspension feeding and particle transport by gill ciliature on the frontal surface of the lamellae and the flow dynamics inside the mantle and suprabranchial cavities were monitored by adding fluorescently labeled latex particles, 10 μm in diameter (Duke Scientific Corp., Palo Alto, California), to the suspension inside the chamber. The green dye incorporated into the spheres made them highly refractive and easily distinguishable from background mussel tissue even under standard incandescent illumination. Although the resolution of the endoscope–video camera setup depended somewhat upon the magnification and light intensity, and the reflectance of the particles and tissue, movement of single particles could be easily monitored under most conditions.

Still-image photographs (video micrographs) were made from the video recordings by capturing and enhancing single video frames using a Video Image 1000 frame grabber (Image Systems Technology) and image analysis software (NIH Image) running on a Macintosh IIfx computer. Prints of stored digital images were made from negatives produced using a ColorFast Digital Film Recorder (GCC Technologies, Inc.) and T-max ISO 100 film (Eastman Kodak Co.).

Results and discussion

Gill morphology and observations of suspension feeding

The large valve gape and unfused mantle margin of *Pyganodon cataracta* permitted insertion of the tip and optical insertion tube of the endoscope between the valves along most of the ventral aspect of the mussel and into the medial cavity (space between the inner demibranchs) and the mediolateral cavities (spaces between the inner and outer demibranchs), facilitating video recordings of the ventral and frontal surfaces of gills and the labial palps (Fig. 1B). Unfortunately, the ridged shaft of the optical insertion tube limited movement within the mantle cavity, and lateral–medial expansion of gravid marsupial gills made observations of the infrabranchial regions, especially the lateral (space between the lateral gills and mantle tissue) and mediolateral cavities, of females difficult (Fig. 1B).

Shortly after insertion of the endoscope between the valves, mussels appeared to resume normal pumping activity (valves were open and siphons extended) and fluorescent particles could be observed entering the mantle cavity in the inhalant water and flowing around both the medial and lateral gills, frequently following a curving path into the infrabranchial spaces

[2]A copy of excerpts from the endoscopic video recordings of *Pyganodon cataracta* suspension feeding and ctenidial morphology may be obtained by sending a blank ½-in. VHS video cassette and return postage to the authors.

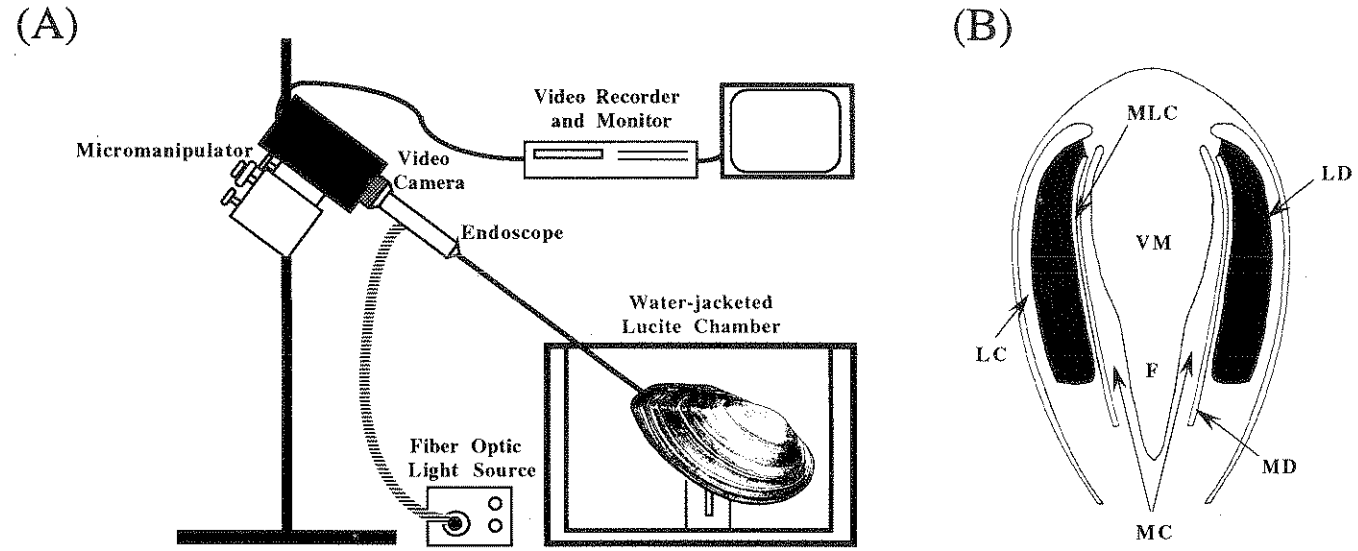


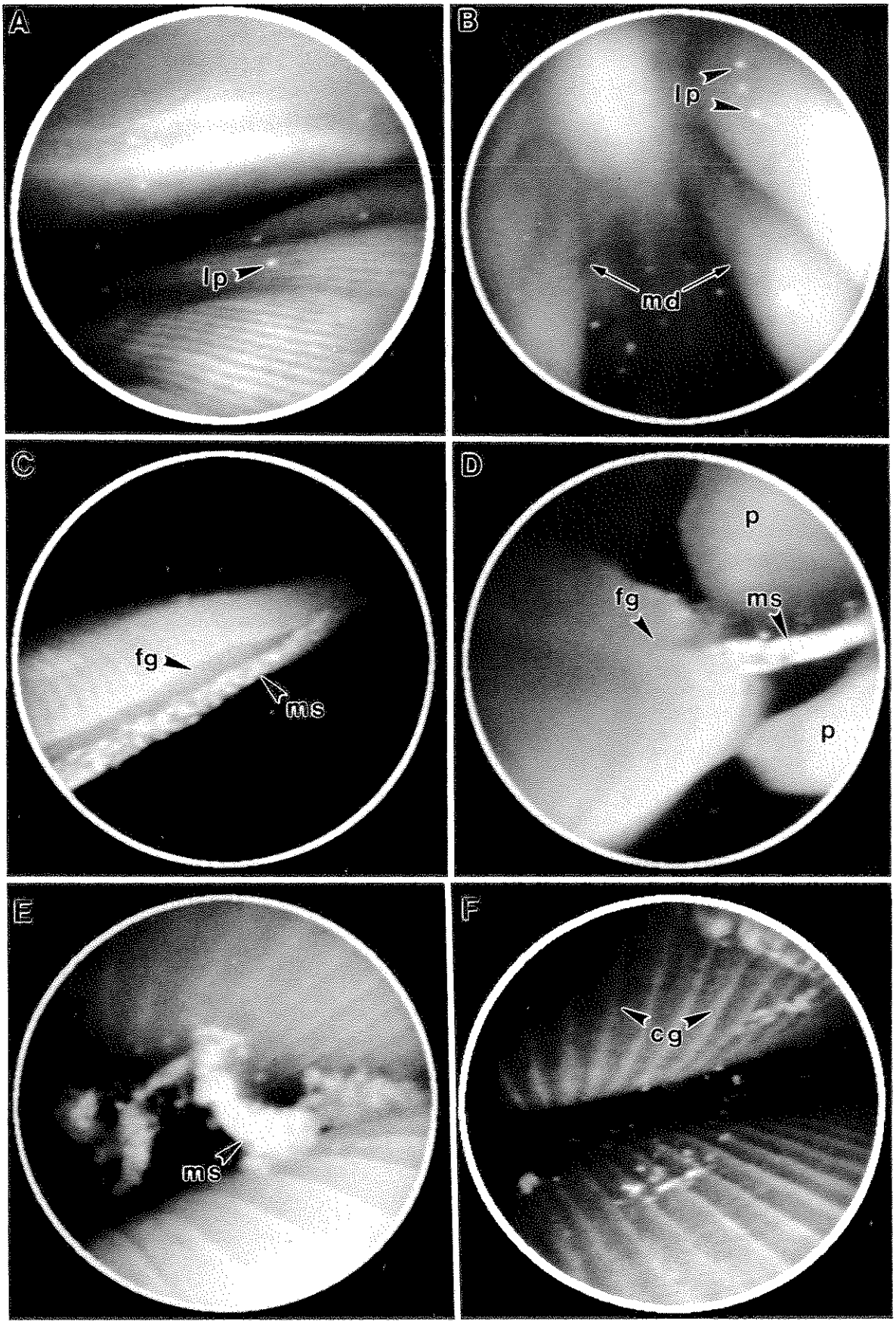
FIG. 1. (A) Diagrammatic representation of the endoscope and video equipment used to monitor suspension feeding in intact *Pyganodon cataracta*. (B) Cross section through a gravid *P. cataracta*, showing the position of the medial (MD) and lateral (LD) demibranchs and the location of the lateral (LC), medial (MC), and mediolateral cavities (MLC). F, foot; VM, visceral mass).

between adjacent demibranchs (Figs. 2A and 2B). Particles retained by both marsupial and non-marsupial gills were transported ventrally toward the free margin of the ctenidia, most likely either by direct contact with frontal cilia located on the tips of adjacent filaments or by frontal currents generated by the cilia (see Ward *et al.* 1993). The relatively flat homorhabdic lamellae of *P. cataracta* lacked plicae and there were no apparent regional differences over the surface of the gill with respect to either the distribution of active cilia or the portions of the gill engaged in particle capture. Most particles traveled individually along a given gill filament, although mucus-bound clumps of particles were occasionally observed, especially at high particle concentrations.

Although ctenidial swelling limited the circulation of water into the mediolateral cavities of gravid mussels, ciliary tracts on the ascending and descending sides of the lamellae were active and participated in the capture and transport of particles despite the extensive gill modifications associated with larval incubation. Nevertheless, many fewer particles appeared to be retained and processed by gravid marsupial gills than by the lateral gills of males or the similar demibranchs of females following larval discharge.

Latex particles that were "caught" by the medial demibranchs traveled down both the ascending and descending faces of the lamellae toward the free margin of the gill and became trapped and concentrated in mucous threads that moved anteriorly toward the palps and mouth in a well defined and heavily ciliated ventral food groove (Fig. 2C). Under high particle concentrations, the edges of the groove expanded, but larger mucous cords often projected beyond the ventral edge of the relatively shallow furrow. Although particle capture and retention appeared to be the result of cilia-generated currents near the frontal surface of the gill, these densely packed particle–mucous strings moving in the food grooves appeared to be the primary pathway by which filtered particles were ultimately ingested. There was no evidence that the marginal groove served to spatially separate nonmucoid feeding currents inside the furrow from gill and mantle cleaning currents transported outside the furrow (i.e., mucous strings), as suggested by Jørgensen (1981) for *Mytilus* and several other bivalves. Furthermore, very few particles were seen traveling dorsally along the frontal surface of the lateral gills toward the ctenidial axis as has been reported for many other unionid species by Atkins (1937). Unfortunately, the endoscope system did not permit thorough viewing of the dorsal regions of the mediolateral and lateral cavities to determine if alternative pathways of particle transport, such as the anteriorly directed slurries present in the dorsal ciliated tracts of several marine bivalves (Beninger *et al.* 1993; Ward *et al.* 1993), are also involved in delivering captured particles to the palps. In contrast to the inner demibranchs, the free margin of lateral gills of both sexes lacked a well-developed food groove. Particles were frequently observed dropping off the ventral edges of the lateral ctenidia and becoming entrained in currents generated by the cilia on the descending surface of the medial gills.

In gravid lateral demibranchs, the ventral margin of the gill is greatly distended as a consequence of the extensive swelling of the occupied brood chambers, but lacks any external features (i.e., indentations or sulci) identifying the position of the internal septa and brood chambers (Fig. 3A). However, particles retained by these lateral demibranchs were also transported on the stretched epithelium of the ventral surface, indicating that this region of the gill possessed active cilia. On several occasions, the extensive stretching of the ventral epithelium permitted the dark calcified shells of developing glochidia to be observed within the semitransparent water tubes. Immediately following larval release, the distended ventral surface of marsupial demibranchs becomes deflated and convoluted (Fig. 3B). Glochidia are released sequentially from the brood chambers in posterior–anterior order. Several mussels collected during the release period possessed marsupia that were only partially full, which is consistent with patterns of larval discharge reported for other anodontine mussels (Ortmann 1911). Although particle retention and transport appeared to be hampered in brooding demibranchs, filtration activity increased in regions of marsupial gills containing empty brood chambers, and particles were often seen moving along the convoluted ventral gill margin toward the frontal surface of the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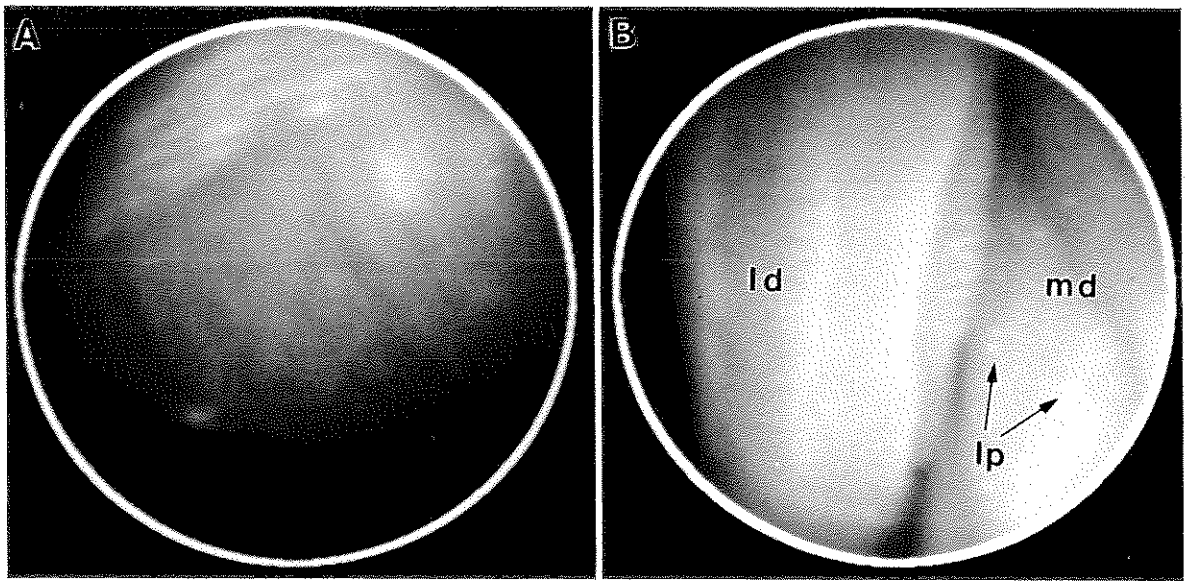


FIG. 3. Video micrographs of marsupial gills, showing the stretched epithelium of the ventral edge during periods of larval incubation (A) and the convoluted appearance of the same region following larval release (B). *ld*, lateral demibranch; *lp*, latex particles; *md*, medial demibranch.

descending lamella of the underlying medial gill (Fig. 3B). Furthermore, there was no indication that the ventral tissue of marsupial gills had been damaged or ruptured to facilitate larval release, as has been suggested for *Pyganodon* (=*Anodonta*) *grandis* by Richard *et al.* (1991).

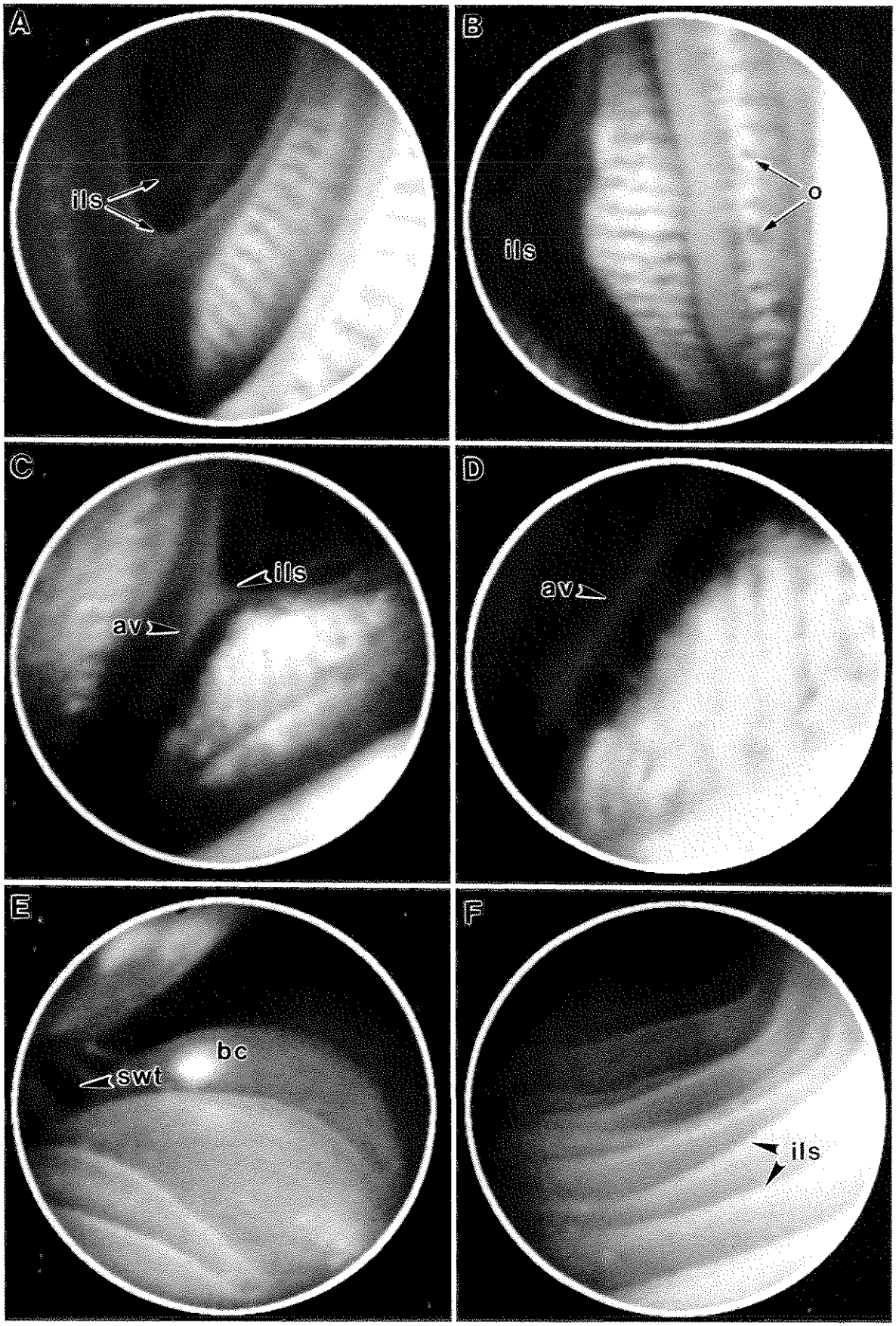
Anteriorly, the ventral edge of the medial demibranchs inserts between the inner ridged surfaces of the paired palps located on each side of the mussel's body (category I association as outlined by Stasek (1963)) (Fig. 2D). Mucus-bound particles traveling along the medial food groove were transferred as continuous strands to the opposing surfaces of the labial palps at the point of branchial contact (Figs. 2D and 2E), a pattern that is consistent with the observations of Ward *et al.* (1993) for *Crassostrea virginica*, *Mytilus edulis*, and *Mya arenaria*. The outer surface of the palps is smooth but small mucous threads containing particles could occasionally be seen moving (most likely propelled by cilia) across the outer surface into the space between the appressed palp lamellae. Placement of the endoscope between the lamella of the palps revealed the intense metachronal activity of the ciliary bands and the deep grooves located on the inner surfaces (Fig. 2F). Unfortunately, the slow scan rate of the video recorder (30 Hz) did not permit accurate quantification of the beat frequency of the large undulating cilia.

Individual and mucus-embedded clumps of latex beads could be seen in the furrows of the inner surface of the palps, but most were associated with the compact mucus–particle strings which remained relatively intact as they were transported anteriorly toward the mouth, aided by cilia beating on the opposing surfaces of the palps (Figs. 2E and 2F). Although the morphological characteristics of the grooved and ciliated structures of the palps suggested that they may be involved in mechanical processing and handling of individual particles, there was no apparent breakdown in the viscosity of the mucous cords by the palp cilia (Newell and Jordan 1983) and no pre-ingestive sorting of the entrapped particles was noted even at high particle concentrations. These observations are consistent with those of Foster-Smith (1975*a*, 1975*b*, 1978), who proposed that palps primarily function to control ingestion by either transporting mucus-bound material to the mouth or rejecting it as pseudofeces. Although the ridges of the palps most likely lead to a proximal oral groove and mouth, the tip of the endoscope could not be inserted more than a few millimetres past the margin of the palps without becoming fouled by particles and mucus. Therefore, it is unclear whether particles were ingested as mucus-bound cords, as suggested by Morton (1960) and Beninger *et al.* (1991), or free in suspension (Jørgensen 1981; Kiørboe and Møhlenberg 1981; Jørgensen *et al.* 1984).

Water tube morphology of non-marsupial gills

Once inserted into the suprabranchial cavity through the exhalant siphon, the tip and optical insertion tube of the endoscope could be navigated to rest within the suprabranchial space between the ascending and descending lamellae of the ctenidia. In *P. cataracta*, the suprabranchial cavity is a relatively large and straight opening running anterioposteriorly along the dorsal margin of the demibranch, allowing the endoscope to be positioned at almost any point along its entire length. There were no apparent differences in the arrangement of the water tubes of non-marsupial demibranchs of males (inner and outer demibranchs) and females (inner demibranchs), and the suprabranchial cavities of each ctenidium were morphologically isolated from one another except at their posterior

FIG. 2. Video micrographs of the frontal surface of a medial demibranch (*md*) (A) and the medial cavity (space between medial demibranchs) (B) of an active mussel, showing the movement of fluorescent latex particles (*lp*) within the infrabranchial region and on the gill filaments. Particles reaching the ventral edge of the medial gills become bound and concentrated in a mucous string (*ms*) traveling anteriorly along the ventral food groove (*fg*) toward the palps and mouth (C). At the ventral margin of the palps (*p*), mucus-bound particles are transferred as continuous strings (*ms*) to the inner surfaces of the palps (D and E), which consists of bands of deep, heavily ciliated grooves (*cg*) (F).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end, where they opened into a common atrium just anterior to the exhalant siphon. In non-marsupial gills, interlamellar septa connecting the opposing sides of the gill formed relatively equally spaced and parallel water tubes running dorsoventrally parallel to the gill surface (Figs. 4A and 4B). During periods of active pumping, the ctenidia became inflated and the V-shaped terminal connections of the thin septa permitted the water tubes and the suprabranchial cavity to expand along the gill's medial–lateral axis to accommodate the water traveling toward the exhalant siphon. In general, the distended parallel water tubes of non-marsupial gills appeared more cylindrical in cross section than previously observed in fixed tissue (Tankersley and Dimock 1992). Large blood vessels with periodic branches extending horizontally into the lamellar tissue (Figs. 4C and 4D) were located in the center of each septum and ran dorsoventrally down the gill. The gills of several individual mussels appeared to expand and contract rhythmically ($\approx 6-8$ beats/min; $N = 6$) in a pattern that was independent of valve activity and was most likely linked to either the cardiac rhythm and subsequent blood transport through the vascularized interlamellar septa or to muscular activity of the water canals and ostia (Gardiner *et al.* 1991). This relatively large scale rhythmic motion of the demibranchs may aid water transport through the gills or assist particle capture and transport by changing or optimizing the position or shape of the ctenidia.

Although the fixed viewing angle of the endoscope limited observations to the distal ends of the water tubes and associated epithelium, numerous ostia, arranged in parallel horizontal bands and leading from interfilament canals, were clearly visible on the inner surface of the lamellar tissue that essentially comprises the "floor" and "walls" of the suprabranchial cavity (Fig. 4B). Fluorescent beads not retained by the gills could be seen moving vertically upward within the water tubes and becoming entrained in the faster flowing water traveling posteriorly through the suprabranchial cavity, indicating that particles approximately 10 μm in diameter are not retained with 100% efficiency by *P. cataracta*. Although flow rates within the suprabranchial chamber could not be determined, flow appeared relatively laminar and unaltered by the presence of the endoscope. The trajectories of particles traveling near the walls of the water tubes were often influenced by the beating of ciliated epithelial cells lining the inner lamellar surface, which may aid in cleansing the inner surfaces of the lamellae and maintaining flow through the water tubes as suggested for the abfrontal cilia of *Mytilus* (Jones *et al.* 1990). Frequently, particles suspended in the suprabranchial cavity were observed to abruptly change direction and move back into the water tubes more anteriorly in the suprabranchial cavity. This rapid change in flow velocity most likely represented either counterflow equalizing pressure differences between the infra- and supra-branchial chambers after lateral cilia stopped beating (Foster-Smith 1976; Jørgensen 1982; Silvester and Sleigh 1984; Jones and Allen 1986), or pressure differences produced by rapid valve adduction, thought to be used for exchanging mantle fluid as well as the ejection of pseudofeces (Morton 1983).

Brood chamber morphology and observations of glochidial release

The presence of developing larvae in the water tubes of marsupial gills caused the suprabranchial cavity to be significantly wider than that of non-marsupial gills (compare Figs. 4A and 4E). Brood chambers packed with glochidia were capped at their distal (dorsal) end by formal extensions of the interlamellar septa connecting the opposing lamellae (Fig. 4E), providing additional evidence for the physical isolation of developing larvae within the brood chambers from the surrounding water (Tankersley and Dimock 1992). Secondary water tubes located on the lateral and medial ends of the brood chambers were open, and particles could be seen streaming from these openings and entering the suprabranchial cavity (Fig. 4E). Nevertheless, the number and speed of particles were substantially less than in non-marsupial gills of mussels exposed to the same particle concentration, suggesting that flow through gravid marsupial gills is markedly reduced relative to flow in non-marsupial demibranchs. This observation is consistent with predictions based upon morphometric and hydromechanical analyses of water tubes and brood chambers of marsupial and non-marsupial gills (Tankersley and Dimock 1992).

Following larval release, marsupial gills remained distended (mediolaterally) and the thin interlamellar septa forming the anterior–posterior walls of the brood chambers were clearly visible (Fig. 4F). Although direct *in vivo* observations of the secondary water tubes were not possible, particles were seen only entering the suprabranchial cavity from locations near the left and right ends of the brood chambers, indicating that the secondary septa were still present and prevented water flow into the empty central brood chambers (primary water tubes).

Figure 5 is a sequence of frames from a time-lapse video recording of the release of glochidia via the suprabranchial cavity. Glochidia discharged from the uncapped brood chambers into the suprabranchial chamber were initially bound together by mucus and larval threads and many could be observed gaping widely and rapidly adducting their valves as they progressed posteriorly toward the exhalant siphon. The posterior region of marsupial gills of several mussels collected during the season of larval release contained empty but laterally distended brood chambers, with the anterior portion of the ctenidia possessing only partially filled and uncapped brood chambers, thus providing additional evidence for the sequential posteroanterior release of glochidia. Although Richard *et al.* (1991) provide indirect evidence for the release of larvae through the ventral margin of the gill in *P. grandis*, the swollen septa of the uncapped brood chambers of *P. cataracta* (Fig. 5A) resemble descriptions of the enlarged terminal ends of the brood chambers of *P. grandis* (Richard *et al.* 1991), suggesting that the previous reports may have been based upon regions of gravid ctenidia which had already initiated glo-

FIG. 4. Endoscopic views of the dorsal openings of the primary water tubes of non-marsupial gills, showing the location of the interlamellar septa (*ils*) (A and B). Large arterial blood vessels (*av*) are located in the center of each septum (C), with branches entering the lamellar tissue (D). Rows of ostia (*o*) (B) leading from interfilament canals are apparent on the inner surfaces of the water tubes. Similar views of the suprabranchial cavities of marsupial gills during the brooding (E) and post larval-release periods (F) reveal that the distal openings of the expanded brood chambers (*bc*) of gravid marsupia are capped by extensions of the interlamellar septa (*ils*), but secondary water tubes (*swt*) located on the left and right ends of the brood chambers open directly into the suprabranchial cavity and are used for water transport (E). Brood caps were absent in marsupial gills immediately following larval release (F), but secondary septa were still present, as evidenced by the flow of latex particles into the suprabranchial cavities only from locations near the left and right edges of the brood chambers.

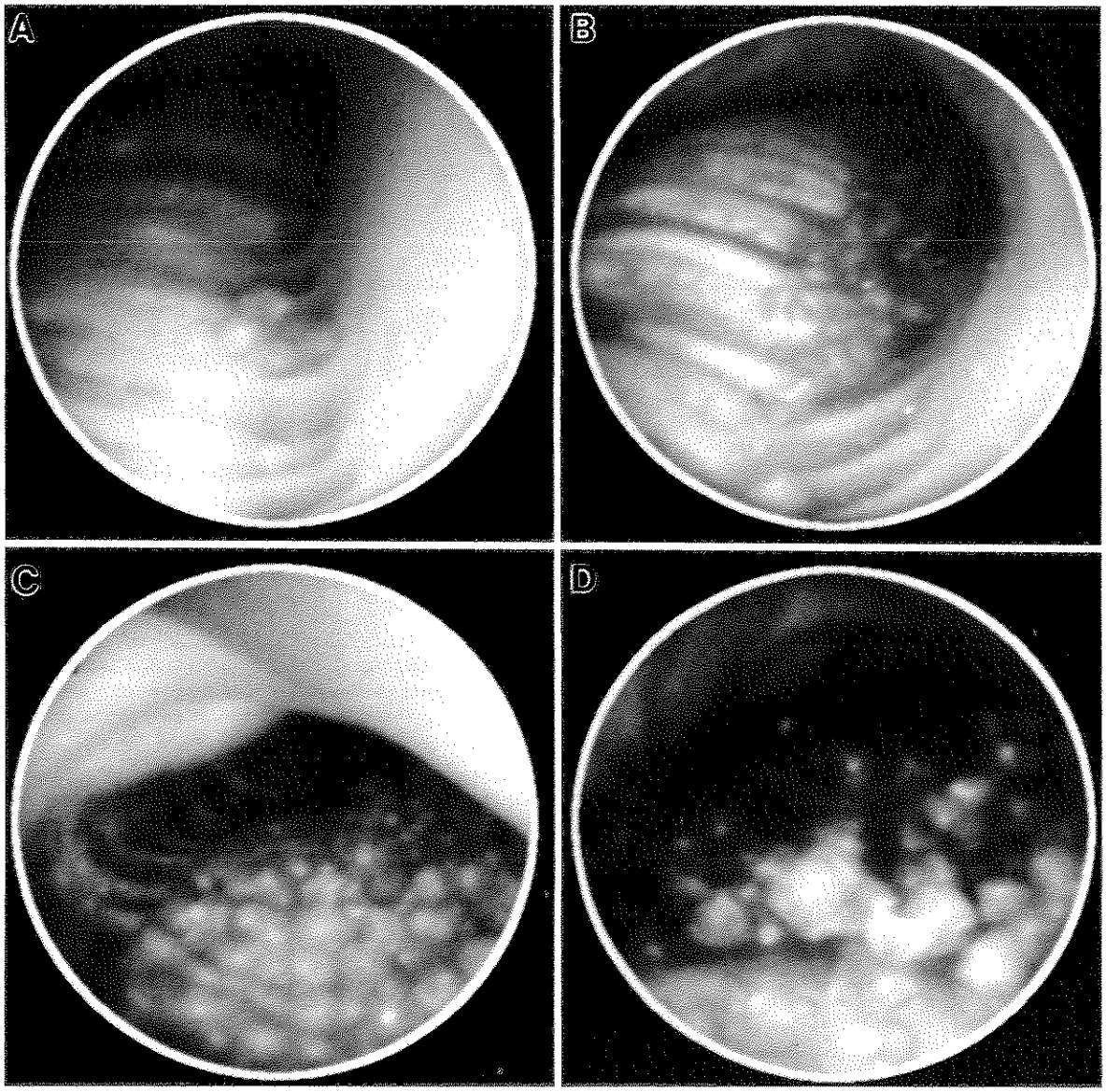

FIG. 5. Video micrograph sequence of the release of glochidia via the suprabranchial cavity and exhalant siphon. Larvae were discharged from the brood chambers by the rapid adduction of the valves and the muscular contraction of the gills. Most glochidia were active following release, as evidenced by the movement of their valves (D).

chidial release through dorsal openings in the brood chambers. Time-lapse recordings of *P. cataracta* showed quite dramatically that glochidia were expelled from the brood chambers by the muscular contraction of gill lamellae and transported posteriorly by the forceful flow of water within the secondary water tubes and suprabranchial chamber. Flow rates within the suprabranchial cavity of gravid marsupia appeared to be highest during periods of larval release, suggesting that in addition to their providing lumina for gill irrigation and particle filtration, the secondary water tubes may have an important role in larval release. Because latex particles suspended within the suprabranchial chamber frequently reversed direction, the observed pulses of high velocity flow through the marsupial gills probably result from rapid valve adductions rather than from abrupt changes or interruptions in the activity of lateral cilia (Jørgensen 1982; Jones and Allen 1986; Silvester 1988).

The present observations provide the first evidence directly linking changes in the suspension feeding of females during gravid periods (Tankersley 1992) to the dramatic alterations in gill morphology and architecture associated with larval incubation (Tankersley and Dimock 1992). Furthermore, endoscopy provides a unique perspective of the processes of particle capture and transport and the fluid dynamics associated with the eulamellibranch gill. Future studies using endoscopic techniques should permit quantification of the patterns of water movement through the mantle cavity and interlamellar spaces that influence suspension feeding (Famme and Kofoed 1983) and are most likely altered during periods of larval incubation. Moreover, additional observations are needed to identify the mechanisms of larval release in unionid mussel species possessing different marsupial morphologies, and to document the process of embryo deposition into the brooding chambers.

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