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Multipurpose gills: effect of larval brooding on the feeding physiology of freshwater unionid mussels

Richard A. Tankersley

Department of Biological Sciences, University of Maryland Baltimore County, Baltimore, MD 21228, USA

Abstract. During reproduction, the lateral (outer) demibranchs of the unionid mussel *Pyganodon cataracta* function in brooding females as marsupia in addition to serving in gas exchange, feeding, and ion transport. Recent studies indicate that glochidial brooding reduces clearance rates and particle retention efficiencies, but the opaque shell prevents direct observations of suspension feeding structures and makes it difficult to identify the underlying causes of the changes in feeding dynamics. In this study, video endoscopic techniques were used to describe and compare, *in vivo,* the feeding structures and dynamics of brooding and non-brooding females. Although circulation within the mantle cavity was slightly altered by the enlarged lateral (gravid) gills of brooding females, both medial and lateral gills continued to retain and process particles. During brooding, circulation through medial gills was maintained by the construction of secondary water tubes near the medial and lateral ends of the brood chambers. *In vivo* monitoring of particles retained by the frontal surface of the gill indicated that transport rates for particles processed by gravid gills of brooders were significantly slower than on lateral gills of non-brooders or on medial gills. Similarly, gravid gills were less efficient at retaining small particles (<6 μm) than medial or non-gravid lateral gills. These findings are consistent with the hypothesis that observed reductions in particle clearance rates and retention efficiencies in brooding female mussels are the result of functional changes in the ciliature and flow dynamics of the marsupial gills. Moreover, similar mechanisms mediating particle capture and processing on medial demibranchs appear to be unaffected by the presence of developing glochidia in the water tubes of the lateral gills.

Additional key words: glochidia, endoscopy, suspension feeding, filtration

Though the gills of suspension feeding bivalves are typically identified as structures for feeding, gas exchange, and ion transport, the ctenidia of many marine and freshwater species also incubate larvae during reproductive periods. Brooding mechanisms vary considerably among species. Most freshwater bivalves, including members of the Corbiculidae and Unionidae, lack planktonic larval stages and incubate developing embryos within internal spaces between gill lamellae (Ortmann 1911; Britton & Morton 1982; Kat 1984; Mackie 1984). In other freshwater and marine bivalve molluscs, developing young are attached to pallial structures or restricted to specialized brood masses, papillae, or sacs (Heard 1977; Bartlett 1979; Richardson 1979; Mackie 1984; Kabat 1985). Conversely, some species, including oysters of the genus *Ostrea,* brood within the infrabranchial cavity but the young are not confined or physically attached to the mother and move about freely in the inter-demibranchial spaces (Chaparro et al. 1993). Although numerous studies have examined the mechanics of the bivalve pump and filter (for review see Jørgensen 1990), the effect of larval incubation on the gills' customary roles as feeding and respiratory structures has been ignored, except for the observation of Walne (1972) that clearance rates of the oyster *Ostrea edulis* are lower during brooding periods.

In the Unionidae, modifications of ctenidia for brooding, including the location and arrangement of larvae (glochidia) within brood chambers, the degree of swelling of the lamellae, and the duration of larval incubation, vary among species and often serve as important taxonomic characters (Ortmann 1911; Heard & Guckert 1971). In most species, the water tubes of either the lateral (outer) demibranchs or all four demibranchs serve as ovisacs. In others, the marsupium is restricted to a portion of the lateral gills, often forming bulges or sulci along the ventral aspect of the demi-

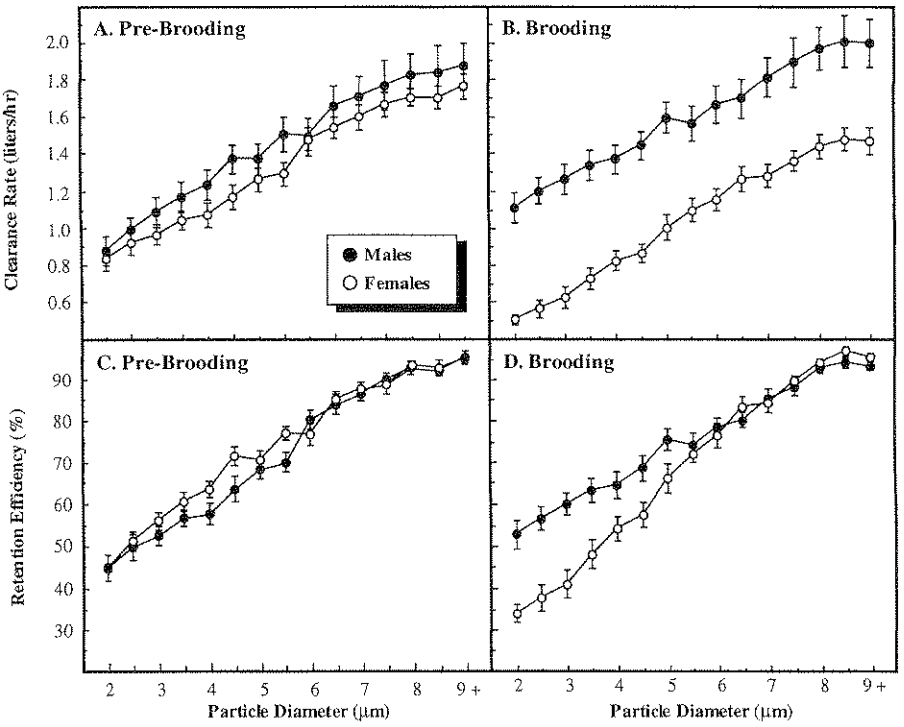

Fig. 1. Comparisons of clearance rates (**A**,**B**) and relative retention efficiencies (**C**,**D**) of males and females of *Pyganodon cataracta* during pre-brooding (**A**,**C**) and brooding (**B**,**D**) periods of their reproductive cycle. Means (±SE) are plotted as a function of particle diameter. Clearance rates were calculated by measuring the rate at which particles were removed from suspension. Particles with the highest clearance rates were assumed to be retained with 100% efficiency and were used to estimate the retention efficiencies for the remaining particle size classes (adapted from Tankersley & Dimock 1993b).

branch (Ortmann 1911; Richard et al. 1991). Shells of these species are frequently sexually dimorphic, as new shell material deposited by the stretched mantle tissue covering the distended marsupia causes the ventral margin of the shells of females to protrude (Burch 1973).

Although brooding by unionid mussels is thought to shelter larvae from unfavorable environmental conditions and possibly aid in dispersal (Wood 1974; Kat 1984), the isolation provided by ctenidial brood chambers might also facilitate the transfer of nutrients from the mother to developing embryos. Wood (1974) reported that the glochidia and interlamellar septa of brooding females of *Anodonta cygnea* contained significant concentrations of ^{14}C after the adults were fed labeled algae. Similarly, maternal calcium reserves in the gills of some members of the Anodontinae and Lampsilinae appear to be mobilized during reproduction and incorporated into the shells of developing glochidia (Silverman et al. 1985, 1987). Although most studies suggest that nutrients or calcium concretions are transferred to developing larvae via secretory cells located in the interlamellar septa, the mechanisms involved are unknown.

Detailed descriptions of the morphological differences between marsupial and non-marsupial gills have been reported previously for several unionid mussels and include thinner (stretched) septa joining the ascending and descending gill lamellae, shorter interfilamental canals connecting the infrabranchial chamber with the lumen of the water tubes, and additional primary septa subdividing the water tubes into smaller (antero-posterior dimension) "crowded" channels (Peck 1877; Lefevre & Curtis 1910; Ortmann 1911; Richard et al. 1991; Tankersley & Dimock 1992, 1993a). Many of these structural differences are permanent, enabling marsupial and non-marsupial gills to be distinguished during non-reproductive periods (Ortmann 1911; Richard et al. 1991; Tankersley & Dimock 1992, 1993a). Some of the most striking differences between marsupial and non-marsupial gills occur in members of the Anodontidae. Following fertilization, the water tubes of the lateral gills are subdivided into three separate compartments; a central brood chamber (ovisac) containing developing embryos and a pair of smaller secondary water tubes adjacent to the lamellar walls. These auxiliary tubes are present only during brooding and function to maintain water transport through the marsupial gills for feeding and ventilation (Heard 1975; Richard et al. 1991; Tankersley & Dimock 1992). Moreover, the dorsal openings of the ovisacs are covered by a thin membrane (brood cap) that further isolates and protects the embryos from water circulating through the suprabranchial cavity.

Alterations in the size and hydromechanical design of the marsupial demibranchs of unionid mussels during periods of larval incubation may ultimately affect the mechanical performance of the pump and filtering system. Because filtration efficiency and effectiveness depend upon the properties of the filtering apparatus

and the velocity of flow (Rubenstein & Koehl 1977), changes in the characteristics of the filter or the rate at which gravid ctenidia process water would alter the mussel's ability to filter and retain particles. Although anatomical and ultrastructural studies of unionid gills have provided some insights to the possible effects of ctenidial brooding on the respiratory and feeding physiology of unionid mussels (Richard et al. 1991; Tankersley & Dimock 1992), the opaque shell and narrow valve gape of adult bivalves prevents *in vivo* observations of pallial structures. Thus, negative effects of larval brooding on the feeding dynamics of mussels, including filtration rate and retention efficiency of suspended particles, can only be indirectly linked to alterations in gill structure and mantle cavity hydrodynamics (see next section for a detailed summary of Tankersley & Dimock 1993b).

Development of new, minimally invasive, non-destructive techniques—including video endoscopy—for examining the feeding structures of adult bivalves has rejuvenated the study of suspension feeding processes and prompted a re-evaluation of many of the established paradigms related to the biomechanics of particle capture, retention, and transport (see Beninger et al. 1992; Ward et al. 1993; Levinton et al. and Ward, this issue, for review). The goal of the current study is to examine the adverse effects of brooding on the suspension feeding dynamics of the unionid mussel *Pyganodon* (=*Anodonta*) *cataracta* (SAY 1817), comparing the results of traditional (indirect) methods (Tankersley & Dimock 1993b) with more recent observations using endoscopy (Tankersley & Dimock 1993a). Moreover, I describe how video endoscopy can be used to make *in vivo* measurements of particle transport and retention by individual gill lamellae. Questions to be addressed include: (1) Are flow dynamics within the mantle/gill complex of *P. cataracta* altered during brooding? (2) How are particles processed and transported by the demibranchs? (3) Does brooding influence the retention efficiency of marsupial and non-marsupial gills? (4) Does brooding influence the rate of particle transport on the frontal surface of marsupial gills? (5) What is the arrangement and structure of the water tubes of marsupial and non-marsupial gills *in vivo*?

Effect of Larval Brooding on Suspension Feeding Dynamics

Traditional approaches

Traditional methods for measuring filtration rates of suspension-feeding bivalves involve placing animals in closed or flow-through feeding chambers and monitoring the rate at which particles are removed from suspension. When placed in static chambers containing suspensions of latex beads (2–10 μm) diameter, clearance rates of brooding females of *Pyganodon cataracta* were as much as 50% less than rates calculated for males collected at the same time (i.e., non-brooding controls) (Fig. 1A,B; Tankersley & Dimock 1993b). Moreover, the ability of females of *P. cataracta* to retain small particles (<5 μm diameter) also decreased during brooding (Fig. 1C,D). On the other hand, particle clearance rates and retention efficiencies for male and female mussels collected during non-reproductive periods were not significantly different, suggesting that differences in suspension feeding by brooding and non-brooding females result from the presence of developing glochidia in the water tubes of the lateral gills and not from permanent structural differences between marsupial and non-marsupial gills (Tankersley & Dimock 1992). Lower clearance rates and retention efficiencies in brooding females could represent reductions in the amount of water processed by the gills, alterations in current patterns within the mantle cavity, or modifications of the gill cilia and cirri responsible for particle capture and transport.

Excised marsupial and non-marsupial gills revealed that the rate of particle transport by frontal cilia on the surface of gravid gills is significantly slower than on non-marsupial gills or non-gravid marsupial gills (Tankersley & Dimock 1993b). Similarly, the laterofrontal cirri, thought to be involved in particle capture, beat more slowly than those on the non-marsupial gills of both male and female mussels. Thus, changes in the filtration dynamics of females of *P. cataracta* appear to be linked to functional differences in the activity of the cilia of marsupial gills. Nevertheless, it is unclear whether the reduced capacity and retention efficiency of the filtering system of brooding mussels is due entirely to changes in the marsupial gills associated with brooding or if the presence of embryos in the outer demibranchs also temporarily alters the ability of medial (non-marsupial) gills to capture and retain particles. The contribution of other factors, including modification of complex ventilatory currents flowing through the mantle cavity and gills, to the documented changes in the suspension-feeding dynamics of brooding *P. cataracta* can be verified only by direct observations of the gills, pallial structures, and feeding mechanisms of intact mussels.

Endoscopic methods

Experimental mussels: Adults of *Pyganodon cataracta* (shell length 11.0–14.5 cm) were collected during pre-brooding (May–July 1992–1994; 15–18° C) and brooding periods (November–January 1992–1994; 9–12° C) from Speas' (Yadkin County, North Carolina)

and Myer's (Forsyth County, North Carolina) ponds. The mussels were maintained at ambient collection temperatures for up to 5 weeks in recirculating artificial pond water (APW; 0.5 mM NaCl, 0.4 mM CaCl, 0.2 mM $NaHCO_3$, 0.05 mM KCl) and were fed approximately every other day with a mixed algal culture.

Video endoscopy: The equipment and general procedures for conducting endoscopic video observations of the gills and pallial organs of *P. cataracta* have been described previously (Tankersley & Dimock 1993a) and follow the methods of Ward et al. (1991). The endoscope was fitted with an optical insertion tube (OIT), either 2.7 mm diam. × 18 cm long (model K27-18-00-62, Olympus Corp., New York) or 1.7 mm diam. × 19 cm long (Fibertron Corp., Carrollton, TX), attached to a cold-incandescent (150 W) or xenon (300 W) fiber optic light source (Fig. 2). The standard tip of the OIT provided a 60–62° field of view and a maximum magnification of about 150–180×. Maximum resolution was estimated to be about 4 μm. The viewing direction of the endoscope could be adjusted from 0° (direct-view) to 90° (side-view) by attaching a mirror sleeve to the tip of the OIT. Video recordings of pallial structures were made by attaching the endoscope's ocular to a color CCD video camera (Javelin Model JE3462HR or JE3662RGB). Images were stored for later analysis using a S-VHS recorder (JVC Model HRS 6900). A micromanipulator allowed precise movement of the OIT within the infra- and suprabranchial chambers of mussels.

During endoscopic examinations, mussels were placed in an aerated plastic holding chamber (17.5 cm × 10 cm × 16 cm) containing 2 liters of APW and held stationary in a vertical guide by a nylon bolt cemented to one valve. A rubber plug (9 mm diam.) was inserted between the valves near the incurrent margin of the shell to prevent the mussel from closing on the OIT. Observations of suspension feeding dynamics, including particle capture, retention, and processing by pallial structures, were monitored by adding fluorescently labeled (green) latex particles (10 μm diameter; Duke Scientific Corp., Palo Alto, CA) to the suspension inside the chamber (final concentration about 10^4 beads ml^{-1}).

Endoscopic video micrographs were made from video recordings by capturing (digitizing) single video frames using a Scion LG-3 frame grabber (Scion Corp., Frederick, MD) and image analysis software (NIH-Image version 1.55)[1] running on a Macintosh PowerPC 7100 computer. Digitized images were further enhanced using Adobe Photoshop 3.0 software (Adobe System, Inc.).

Particle retention and transport speed: Retention of particles by the lateral and medial gills of prebrooding and brooding females of *P. cataracta* were determined by comparing the frequency distributions of particles present in the infrabranchial (pre-filter) and suprabranchial (post-filter) cavities. Mussels were fed a mixed suspension of fluorescently labeled latex microspheres, 2–18 μm diam. (Duke Scientific Corp., Palo Alto, CA). The endoscope was inserted into the suprabranchial cavity via the excurrent siphon and positioned above either the lateral or medial gill (Fig. 2A). Because the excurrent siphons were relatively large (12–18 mm^2 cross sectional area) compared to the endoscope's OIT (2 or 6 mm^2), the presence of the tip of the scope within the suprabranchial cavity had no apparent effect on the pumping activity of the mussels or on the flow of water traveling through the ctenidia and exiting the siphon. After the mussels became acclimated to the experimental conditions and resumed apparently normal feeding activity, particles were added to the test chamber and mixed by gentle aeration (final concentration 2.3×10^4 particles ml^{-1}). Once particles were observed entering the suprabranchial cavity from the dorsal openings of the water tubes, a 300 μl sample of the filtered water was collected using a syringe connected to a cannula (200 μm diam.) attached to the tip of the OIT.

Particle-size (diameter) distributions were determined using hemocytometer counts and computerized image analysis software (PrismView, Analytical Vision, Raleigh, NC) performed at 40× on a Zeiss Axiolab Microscope (Carl Zeiss, Germany). For statistical analysis, particles were separated into 2 μm size classes and the relative frequency of particles in each size class (i) was calculated. Because particle size classes were not equally represented in test chamber water, differential retention was quantified using a modified version of Chesson's α (Chesson 1978):

$$\alpha_i = 1 - \frac{\frac{r_i}{p_i}}{\sum_i \frac{r_i}{p_i}}, \qquad i = 1, \ldots, m$$

where r is the relative frequency of size class i in the particles retained by the gill (i.e., not present in the samples aspirated from the suprabranchial cavity), p is the relative frequency of size-class i in the water entering the mantle cavity (i.e., particles added to the test chamber), and m is the number of particle-size classes ($m = 10$). The index varies between 0 and 1 with

[1] NIH Image is a public domain software program written by Wayne Rasband at the U.S. National Institutes of Health and available from the Internet by anonymous ftp from zippy.nimn.nih.gov or on floppy disk from NTIS, 5285 Port Royal Rd., Springfield, VA 22161, part number PB93-504868.

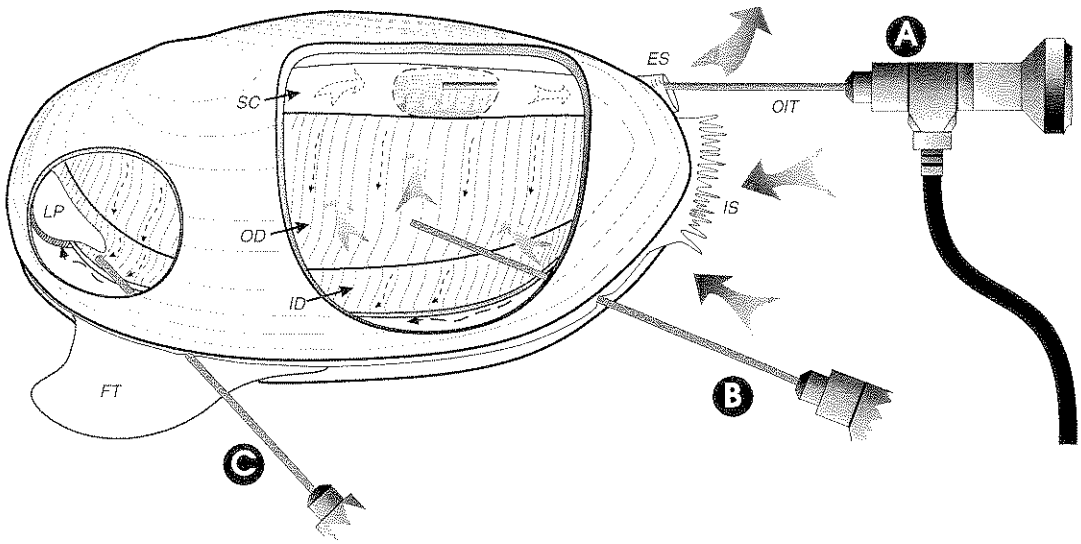

Fig. 2. Diagrammatic representation of the placement and orientation of the optical insertion tube (OIT) of the endoscope for recording the suspension feeding activities of *Pyganodon cataracta.* Observations of ctenidial water tubes and marsupial brood chambers were recorded by inserting the tip of the scope through the excurrent siphon (ES) so that it came to rest within the suprabranchial cavity (SC) (position A). Particle capture, transport, and processing were analyzed by placing the OIT within the inter-demibranchial spaces between gill lamellae (position B) or near the palp-gill junction (position C). Large arrows indicate the general flow of water currents entering the mantle cavity via the incurrent siphon (IS) and pedal gape and exiting through the excurrent siphon. Suspended particles retained by the gills (dashed arrows) are transported ventrally toward the margin of the inner (medial) demibranch (ID) and incorporated in a mucous string traveling anteriorly toward the labial palps (LP) and mouth. Foot (FT), outer demibranch (OD).

values above and below $1/m$ (0.1 in this case) indicating disproportionate "retention" and "loss," respectively. To facilitate interpretation, α was rescaled to ϵ following the procedures described by Chesson (1983) to yield values between -1 (loss) and 1 (retention), with 0 indicating that the relative frequency of retained particles was equal to their contribution to the suspension of particles entering the mantle cavity. The calculated ϵ for each size class thus provides an unbiased estimate of its contribution to the total quantity of particles retained by the gill. Retention-efficiency curves for marsupial and non-marsupial gills of females of *P. cataracta* were compared during brooding and non-brooding periods using repeated measures analysis of variance (ANOVAR) (SYSTAT Statistical Software; Wilkinson 1990). All relative frequencies were arcsine square-root transformed before analysis to normalize the data and remove heteroscedasticity (Zar 1984).

Particle transport speeds were calculated by fitting the endoscope with a 90° side-view mirror and placing it within the inter-demibranchial space between the medial and lateral gills (mediolateral cavity), parallel to the lamellar surface (Fig. 2B). Video recordings of fluorescent beads (10 μm diameter) traveling on the frontal surfaces of the gills were digitized (Scion LG-3 frame grabber; 20 frames sec^{-1}) and the paths of individual particles reconstructed by plotting their positions in successive frames. Images were calibrated by dissecting the ctenidia and measuring the distance between filaments. Average speeds (μm s^{-1}) were calculated for 25 particles that were followed for at least 1 sec. Marsupial and non-marsupial gills of five female mussels were examined during each period (brooding and non-brooding) and the results were compared using ANOVAR, with one between-subject factor (reproductive season) and one within-subject factor (gill type).

Results and Discussion

Suspension feeding dynamics and water tube morphology

Preliminary endoscopic observations of the feeding biomechanics and ctenidial structure of *Pyganodon cataracta* have been reported previously by Tankersley & Dimock (1993a). To document the processes involved in particle capture and transport, the endoscope was inserted between the valves of the mussel near the incurrent aperture and positioned to rest within the inter-demibranchial spaces or just below the ventral edge of the ctenidia (Fig. 2B). During both non-brooding (summer) and brooding (fall–winter) periods, the frontal surfaces of the marsupial and non-marsupial gills

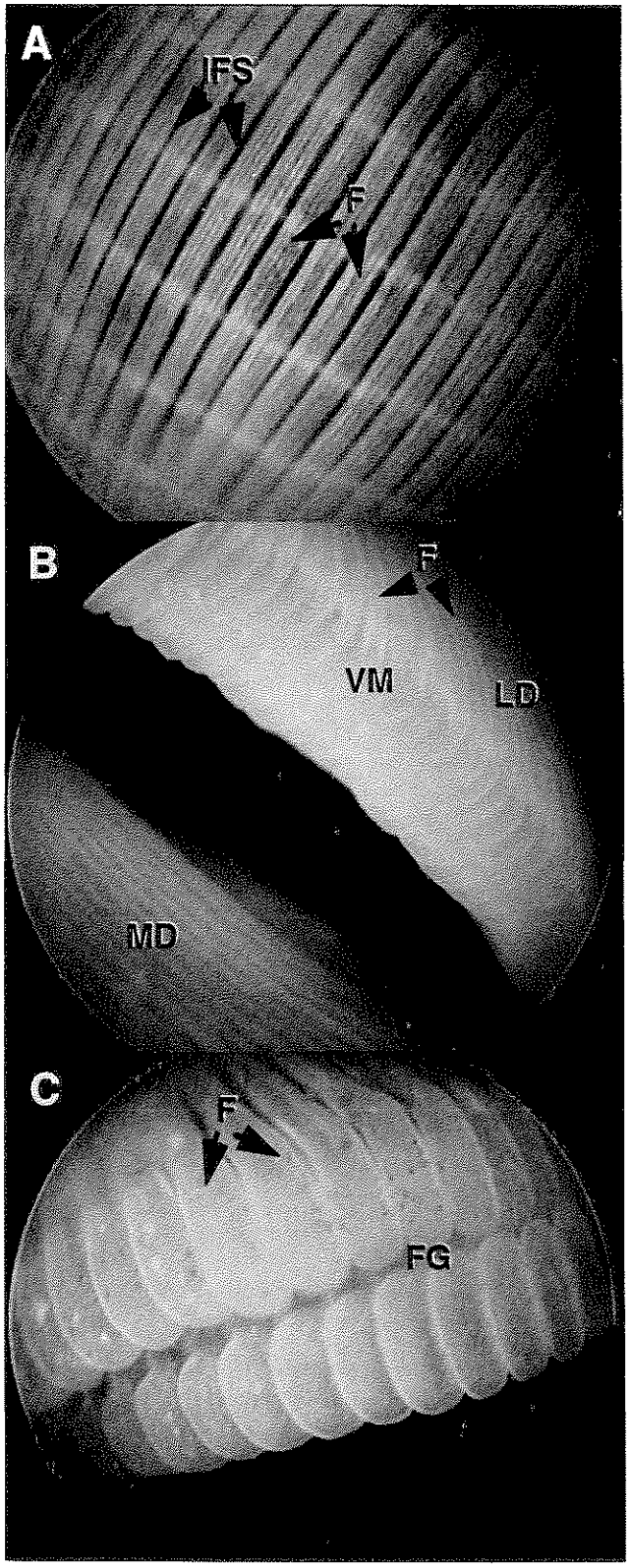

Fig. 3. Video micrographs of the demibranchs of female of *Pyganodon cataracta*. **A.** Frontal surfaces of non-gravid lateral demibranchs consist of rows of parallel filaments (F) separated by dark interfilamental spaces (IFS) leading to ostia. **B.** At the ventral margin (VM) of the lateral demibranchs (LD), ascending and descending lamellae fuse to form a smooth, rounded raphe. Medial demibranchs (MD). **C.** The ventral tip of the medial demibranchs possesses a deep, ciliated food groove (FG) that transports strings of mucus-bound particles anteriorly to the palps and mouth.

were similar and consisted of evenly spaced rows of parallel filaments separated by interfilament grooves leading to external ostia (Fig. 3A). Lamellae of both types of gills were flat and relatively homogeneous (homorhabdic) and lacked any apparent ridges, indentations, or plicae marking the location of internal septa.

During periods of active pumping, suspended particles entered the infrabranchial cavity via the incurrent siphon and initially became entrained in pallial currents flowing anteriorly before being deflected dorsally toward the gills and inter-demibranchal spaces. Although flow around the lateral gills was limited during periods of glochidial incubation, overall patterns of particle transport did not differ between brooding and non-brooding periods. Particles retained by both the lateral and medial demibranchs moved ventrally along the ascending and descending sides of the lamellae. Most particles traveled individually or in small mucus-bound clumps near the apices of the filaments or within the interfilamental spaces. Particles rarely traveled dorsally toward the gill arches; in fact, beads reaching this region of the infrabranchial cavity often reversed direction or became resuspended and entrained in pallial currents flowing ventrally parallel to the gill surface. Thus, the dorsal gill arch of *P. cataracta* does not appear to be an important conduit for delivering food particles to the palps and mouth as reported for several marine species including *Placopecten magellanicus, Crassostrea virginica,* and *Mytilus edulis* (Beninger et al. 1992; Ward et al. 1993; Ward, this issue). Previously published descriptions of ctenidial food currents for a variety of unionid mussels indicated that particles retained by the outer demibranchs are transported dorsally by frontal currents (see Atkins 1937 for summary). As early observations of particle transport on the surface of the gills of unionid mussels were conducted on excised tissue or specimens with all or portions of one valve removed, discrepancies between previous descriptions and those presented here using intact mussels most likely result from artifacts induced by the preparation or dissection of specimens for observation. Thus, many original accounts of particle transport on the surface of the gills of unionid mussels should be re-examined using video

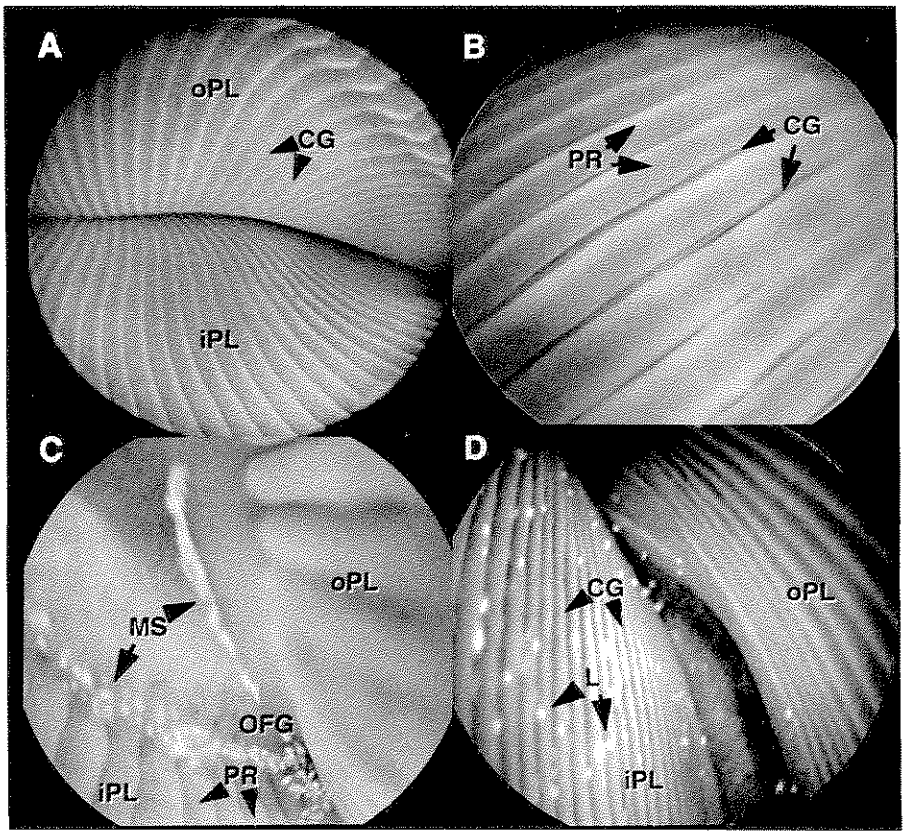

Fig. 4. Endoscopic micrographs of the labial palps of *Pyganodon cataracta*. **A,B.** The opposing surfaces of the palps consist of parallel bands of ridges (PR) separated by heavily ciliated grooves (CG) (higher magnification in **B**). **C.** Mucus-bound particles are transferred from the marginal food groove of the medial gills to the oral food groove (OFG) and inner surface of the palps as continuous mucous strings (MS). **D.** Once between the palp lamellae, the viscous mucous cords are often broken down by the action of large cilia present in the furrows, causing particles to disperse on the palp surface. Inner palp lamellae (iPL), outer palp lamellae (oPL), latex particles (L).

endoscopy or other minimally invasive techniques to determine if normal particle trajectories were altered or disrupted by changes in feeding currents, ciliary activity, or the position of pallial structures.

Ascending and descending lamellae of the medial and lateral gills intersect at the ventral edge (Fig. 3B,C), but the raphae formed by the filaments differ. As described below, the free margin of the medial gills possesses a heavily ciliated food groove that transports concentrated mucus-bound particles anteriorly toward the mouth (Fig. 3C). Conversely, the edge of the lateral gill is rounded and lacks any channels or folds (Fig. 3B). During reproduction, developing embryos within the brood chambers cause the ventral margin of the marsupial gills to become stretched (left-right axis) and distended. After several weeks of development, the dark, calcified shells of the embryos within the brood chambers were visible through the thin translucent membrane. Although Richard et al. (1991) provided evidence suggesting that *Anodonta grandis* releases mature larvae by rupturing the stretched ventral edge of the marsupial gills, in *P. cataracta* the raphe remains intact and larvae are discharged through dorsal openings in the brood chambers (Tankersley & Dimock 1993a; see description below). Immediately following larval release, the ventral epithelium of the marsupial gill becomes deflated and creased. After several weeks, the gill returns to its pre-brooding morphology, and the raphe resembles that of the lateral demibranchs of male mussels.

Because the lateral gills of *P. cataracta* are shorter (dorso-ventral axis) than the medial gills, particles reaching the rounded edge of the lateral demibranchs are transported to the frontal surface of the adjacent medial gill and continue to travel ventrally toward the food groove. In gravid gills, particle transfer to the medial demibranchs was often disrupted by the stretched ventral edge of the marsupium and latex beads transported by the gills frequently became resuspended in pallial currents rather than continuing ventrally on the descending lamellae. Thus, disruption of particle delivery to the medial demibranchs and ventral food groove from the gravid marsupial gill may partially explain the lower filtration rates of brooding females (Tankersley & Dimock 1993b; see summary above).

Upon reaching the margin of the medial gill, particles were immediately incorporated in a viscous mucous string traveling anteriorly in the ciliated food groove. As the mucous string approached the palps, the diameter of the cord increased and often extended beyond the shallow furrow. Nevertheless, the high vis-

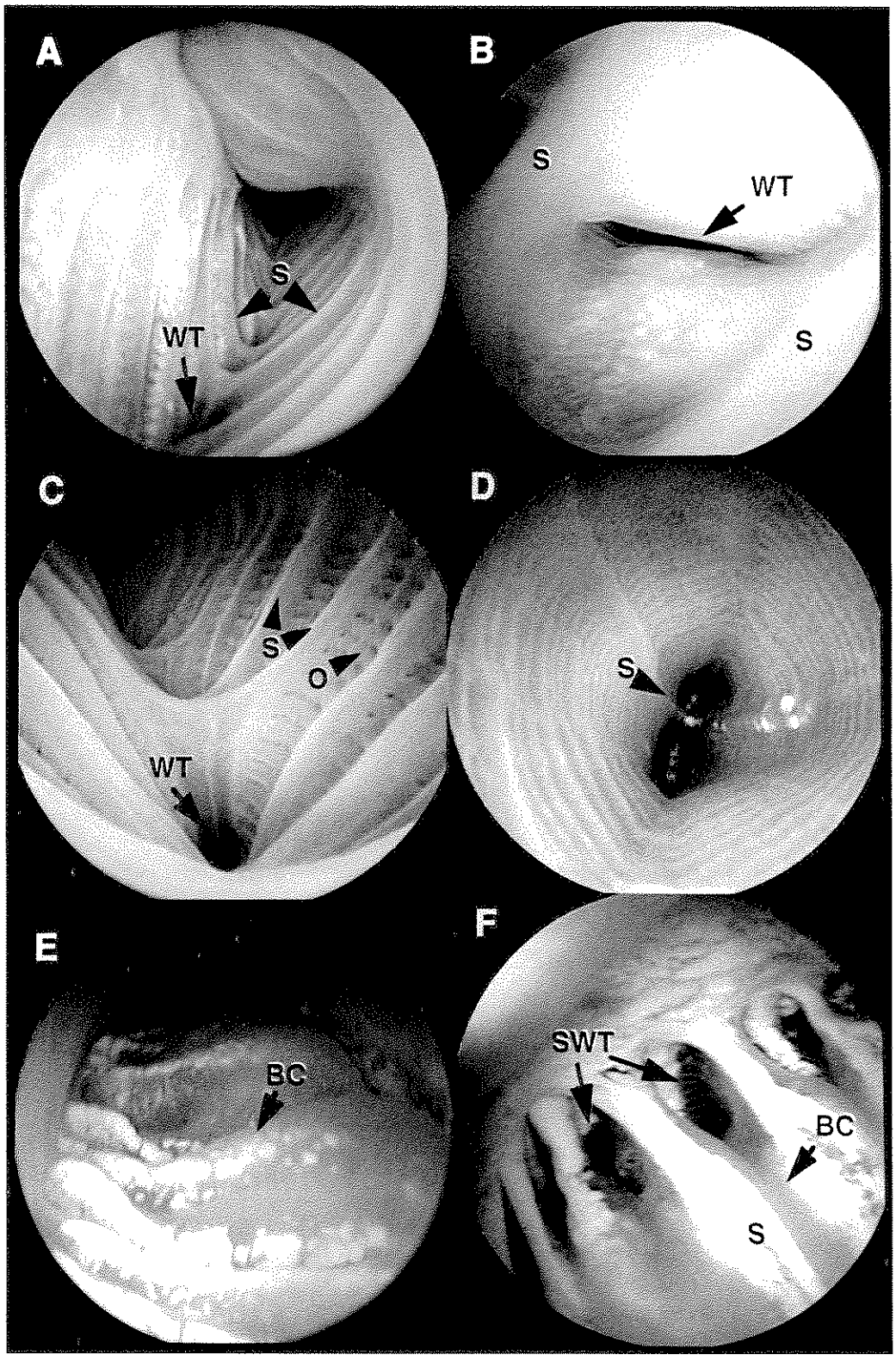

Fig. 5. Transverse (left panels) and frontal (right panels) endoscopic views of the suprabranchial cavities of the medial (**A–D**) and lateral (**E,F**) gills of females of *Pyganodon cataracta*. Water tubes (WT) run dorso-ventrally parallel to the gill surface and are formed by septa (S) connecting the ascending and descending lamellae. **A,B.** During inactive (non-pumping) periods, the water tubes collapse and opposing sides of the canals often come in contact with one another. **C,D.** When

cosity and cohesiveness of the mucous string helped to maintain the integrity of the cord. These findings support the suggestion of Ward et al. (1993) that incorporation of captured particles in viscous mucus increases feeding efficiency by preventing potential food items from being lost or resuspended by strong pallial currents. Other recent endoscopic examinations of the gills of suspension feeding bivalves have described similar mucus-bound particle strings in the ventral food grooves of bivalves from a variety of families (Ward et al. 1993, 1994; Ward, this issue). Thus, these complex particle strings appear to be the dominant or sole route by which many species, including *P. cataracta,* deliver captured food particles to the palps and mouth for ingestion.

At the anterior margin of the lateral gill, the ventral edge of the demibranch and the food groove contact the free edge of the palp. At the gill-palp junction, mucus-particle strings were transferred to the inner ridged sorting surfaces of the palp lamellae (Fig. 4). Although earlier descriptions of the transfer and processing of mucous strings at the palp margin suggested that the mucous cords remain intact as they travel toward the mouth (Tankersley & Dimock 1993a), more recent observations of the inner surface of the palps using a smaller diameter OIT (1.7 mm vs. 2.7 mm) revealed that mucus-bound material between the opposing palp lamellae was frequently degraded by the activity of large cilia within the grooves separating the palp ridges (Fig. 4B,D). The presence of particles on the surface of the palps was often accompanied by an increase in the activity of the ciliary bands. Moreover, high ciliary activity often declined when the observation chamber was flushed of particles. Ward et al. (1994) reported a similar activation/deactivation in the cilia of the palp ridges and lips of the oyster *Crassostrea virginica* when animals were subjected to changes in particle concentration. Although an increase in ciliary activity most likely serves to reduce the viscosity of particle-bearing mucous strings and facilitate the sorting of food particles by the palp ridges (Newell & Jordan 1983; Ward, this issue), factors mediating these changes in palp cilia are unknown.

To observe the internal structure of the ctenidial water tubes and brood chambers, the tip of the endoscope was inserted through the excurrent siphon and into the suprabranchial spaces between the ascending and descending lamellae of the gills (Fig. 2A). In non-marsupial gills, the V-shaped primary septa connecting the opposing sides of the demibranch are continuous with the lamellar tissue and subdivide the gill into water tubes about 1 mm wide (anterior-posterior axis) (Fig. 5A–D). Each septal division corresponds to about 20–25 external filaments (Tankersley & Dimock 1992). During inactive periods, the water tubes were deflated and laterally compressed (Fig. 5A–B), but quickly expanded and became more cylindrical in cross-section (Fig. 5C–D) when pumping resumed. The water tube epithelium was lined with horizontal rows of internal ostia leading from interfilament canals in the lamellar walls. Histological analysis of the musculature associated with the water tubes and ostia of *Anodonta grandis* and *Ligumia subrostrata* indicated that these openings are controlled by dorso-ventrally oriented muscles near the internal openings of the interfilament water canals (Gardiner et al. 1991). Continuous endoscopic monitoring of ostia indicated that they are more visible and less constricted during periods of active pumping, suggesting that their size (diameter) is closely tied to the flow of water through the suprabranchial cavity. These observations support the suggestion of Gardiner et al. (1991) that water currents passing through the mantle cavity/gill complex are regulated, at least in part, by changes in the dimensions of the ostia and interfilament canals as well as the activity of cilia on the lamellar surface.

In gravid lateral demibranchs, the septa connecting the ascending and descending lamellar tissue are greatly distended due to the extensive swelling of the occupied brood chambers (Fig. 5E–F). Thus, the suprabranchial cavity was significantly wider (medial-lateral axis) and the terminal connections of the septa were flat rather than V-shaped (Fig. 5E). During brooding, the dorsal openings of the brood chambers were capped by thin membranes extending from the interlamellar septa, effectively isolating the larvae and shutting down water flow through the brood chamber. Conversely, the terminal ends of the secondary water

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pumping resumes, the water tubes inflate and become more cylindrical. The inner lamellar surfaces of the gills are lined with horizontal rows of internal ostia (O) leading from interfilament canals. The tissue separating these bands forms conspicuous concentric rings on the inner surface of the water tubes (**D**). The water tube in **D** appears to be split into two smaller tubes by an additional septum near the center of the micrograph. **E,F.** Comparable views of gravid marsupial gills reveal the tripartite arrangement of the brood chambers (BC) and secondary water tubes (SWT). The enlarged distal ends of the septa and the thin brood caps isolate developing larvae within the brood chambers. Secondary water tubes (**F**) empty into the suprabranchial cavity and possess bands of ostia similar to those lining the water tubes of non-marsupial gills.

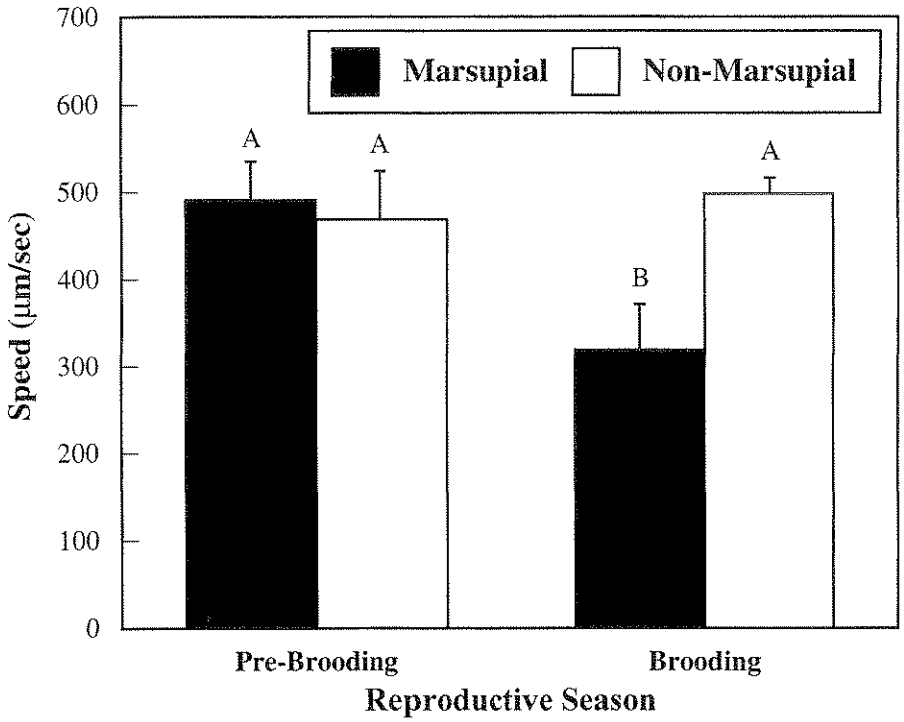

Fig. 6. Transport speeds (μm sec^{-1}) of 10 μm latex particles traveling on the frontal surface of the marsupial (lateral) and non-marsupial (medial) gills of females of *Pyganodon cataracta*. Mean (±SD) values are plotted as a function of reproductive season. Bars with the same letter are not significantly different at $P < 0.05$ (ANOVAR; N = 5).

tubes, located on the medial and lateral ends of the brood chambers (Fig. 5F), open directly into the suprabranchial cavity. Because the water tubes of marsupial demibranchs are shorter (about 400–500 μm, anterior-posterior dimension), each secondary water tube corresponds to about 6–7 external filaments (Tankersley & Dimock 1992). During periods of active pumping, fluorescent beads not retained by the gills were seen entering the water tubes through internal ostia lining the lamellar wall, flowing dorsally into the suprabranchial cavity, and becoming entrained in water currents flowing posteriorly toward the excurrent siphon. These observations provide direct visual confirmation that the temporary secondary water tubes are used to maintain water flow through the marsupial gills and ventilate the brood chambers of *P. cataracta* during periods of glochidial incubation.

During larval release, the brood caps rupture and glochidia are sequentially discharged from the distal (dorsal) ends of the water tubes in a postero-anterior fashion. Mature larvae are expelled into the suprabranchial cavity by rhythmic contractions of the musculature of the gill lamellae and adductions of the valves. Clumps of entangled glochidia, held together by mucus and larval threads, were transported toward the excurrent siphon by swift currents flowing through the suprabranchial cavity. Moreover, pressure changes caused by rapid valve adductions caused the water flowing through the gill and suprabranchial cavity to reverse direction, often forcing newly expelled glochidia back into the uncapped brood chambers. Similar changes in the pattern of water moving through the suprabranchial cavity may be involved in the deposition of newly fertilized embryos in the brood chambers at the onset of brooding.

Particle retention and transport speed

Particle transport rates on lateral gills declined during brooding (Fig. 6). Rates calculated for latex beads moving on the surface of non-marsupial (medial) gills and non-gravid marsupial gills were similar but were 47–56% higher than values calculated for particles moving on brooding demibranchs. Although *in vivo* measures of particle transport speeds were higher (2–52%) than rates previously reported for similar particles traveling on excised gills (Tankersley & Dimock 1993b; see summary above), these findings support the hypothesis that the adverse effects of larval brooding are confined to the lateral marsupial demibranchs. Moreover, particle velocities calculated for beads traveling on the frontal surfaces of both marsupial and non-marsupial gills (245–567 μm s^{-1}) are consistent with values typically associated with mucociliary transport (Ward et al. 1993; Ward, this issue). Since particles are most likely transported on or near the gill surface by direct contact with frontal cilia or by entrainment in a layer of low-viscosity mucus covering the ctenidial filaments and cilia, slower transport rates for particles traveling on gravid lateral demibranchs

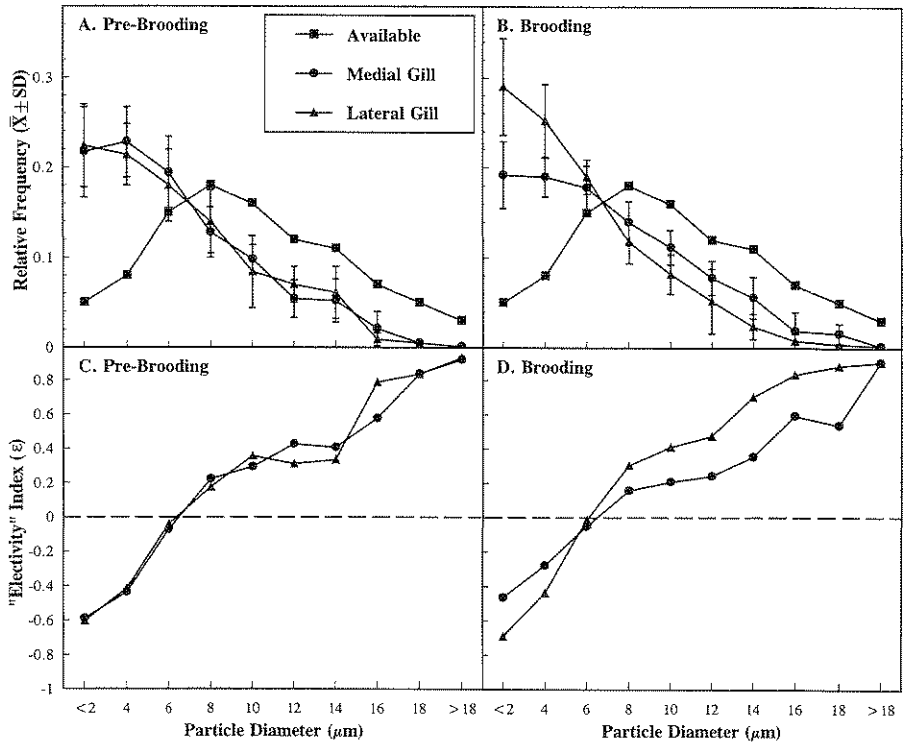

Fig. 7. Size distributions of latex particles in water samples from the suprabranchial cavities (medial and lateral) of females of *Pyganodon cataracta* during pre-brooding (**A**) and brooding (**B**) periods. (Mean ± SD; N= 5). Proportion of particles in each size class not retained by the gills and the distribution of particles present in the test chamber ("available") were used to calculate (**C,D**) Chesson's "electivity" index α—transformed to ε so that values range between −1 (disproportionate loss) and 1 (disproportionate retention); 0 represents retention of particles in proportion to their availability.

may reflect changes in the activity of frontal cilia, the pattern of frontal ciliary currents, or the quantity and viscosity of the mucous sheet covering the lamellar surface. Nevertheless, elucidation of the underlying causes of slower transport rates of particles on gravid gills will await a more detailed understanding of the hydrodynamic and mechanical mechanisms involved in particle capture and mucociliary transport.

Particle retention by medial and lateral gills of females of *P. cataracta* during brooding and non-brooding periods is summarized in Fig. 7. In general, the proportion of unretained particles increased significantly with decreasing particle size (significant effect of particle size; Table 1 & Fig. 7). Thus, the calculated retention indices (Fig. 7C–D) represent pre-ingestive, quantitative, differential retention based on size alone, since the density, biochemical composition, and surface chemistry of the particles were identical. During brooding periods, marsupial gills were less efficient at retaining small particles (<6 μm; significant interactions between particle size and gill type and among particle size, gill type and reproductive period; Table

Table 1. Results of repeated measures analysis of variance (ANOVAR) testing the effects of gill type (marsupial and non-marsupial) and reproductive period (pre-brooding and brooding) on the particle retention efficiency of *Pyganodon cataracta*. (ns = not significant at $P < 0.05$).

Source of variation	df	MS × 10^4	F-ratio	*P*
Between Subjects				
Reproductive Period (R)	1	0.3	0.01	ns
Gill Type (G)	1	28.0	1.02	ns
G × R Interaction	1	7.2	0.26	ns
Error	16	27.5		
Within Subjects				
Particle Size (P)	9	5,949.4	256.0	<0.001
P × R Interaction	9	18.2	0.78	ns
P × G Interaction	9	53.7	2.31	<0.05
P × R × G Interaction	9	64.9	2.79	<0.005
Error	144	23.2		

1 and Fig. 7B) than non-marsupial medial gills. The result was a significant shift in their retention of larger particles (i.e., higher Chesson's α values; Fig. 7D), relative to the size spectrum available. During non-brooding periods, particle distributions within the suprabranchial cavities of the lateral and medial gills were nearly identical (Fig. 7A,C). Thus, particle retention mechanisms appear to be unaffected by permanent differences in the morphology and architecture of the gills of female mussels. These findings are consistent with the results of earlier studies indicating that the gills of males and females of *P. cataracta* have similar retention efficiencies during non-brooding periods (Tankersley & Dimock 1993b; see summary above). Moreover, the retention indices of non-marsupial (medial) gills of brooding and non-brooding females are similar, suggesting that no compensatory changes in the suspension feeding dynamics of the medial gills offset the observed reduction in particle retention by the lateral gills.

Changes in the "leakiness" of marsupial demibranchs most likely represent alterations in the dimensions of the ostial openings and interfilamental water canals connecting the infrabranchial cavity to the secondary water tubes. Recent morphological analysis of the gills of *P. cataracta* indicated that changes in the size and morphology of the marsupial gills during incubation periods should increase the flow resistance of the ctenidial water tubes and interfilament canals and hamper ventilation (Tankersley & Dimock 1992). Results of indirect and direct measurements of ventilation rates of *P. cataracta* are conflicting (see Tankersley & Dimock 1993b,c). Coupling endoscopic methods with techniques and instrumentation designed to directly measure flow through the water tubes and suprabranchial cavities may help confirm suspected changes in the ventilation rate of marsupial gills during brooding.

Adoption of endoscopic techniques for observing the feeding structures of intact bivalve molluscs has proved to be an invaluable tool for examining the intricacies of suspension feeding and the fluid dynamics associated with the eulamellibranch gill. Moreover, video endoscopy has helped eliminate many of the methodological constraints that have prevented researchers from elucidating the processes responsible for particle capture, retention, and transport. Future studies will benefit from rapidly developing technologies for digital image acquisition and analysis that should provide improved temporal and spatial resolution and enable real-time tracking and analysis of particle movements.

Moreover, efforts are currently underway to expand the capabilities of endoscopic analysis by coupling it with other imaging tools. For example, optical filters used for fluorescent microscopy have recently been incorporated into the endoscope's light path to permit *in vivo* identification of algae and other particles based upon their fluorescent signatures. Thus, endoscopy is a promising complement to traditional histological techniques for examining fundamental questions about the morphology and function of the multipurpose gills of unionid mussels. Questions remaining to be addressed include: (1) What mechanisms are involved in fertilization and glochidial deposition into the brood chambers? (2) How is water transport maintained during brooding by other species with different marsupial morphologies, especially those that lack secondary water tubes or that use all four demibranchs as brood chambers? (3) Are the suspension feeding dynamics of other unionid species adversely affected by the presence of marsupia? (4) Are unionid mussels capable of selective filtration? (5) What roles do the gills and palps play in particle sorting and differential retention? (6) How is ctenidial ventilation regulated?

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References

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: 375–421.

Bartlett BR 1979. The possible role of gill filament papillae in the development of the brooding bivalve *Parastarte triquetra*. Am. Zool. 19: 957.

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.

Britton JC & Morton B 1982. A dissection guide, field and laboratory manual for the introduced bivalve *Corbicula fluminea*. Malacol. Rev. 1982, Sup. 3: 1–82.

Burch JB 1973. Freshwater unionacean clams (Mollusca: Pelecypoda) of North America. Water Pollution Control Research Series, 11, Environmental Protection Agency, Washington, DC. 176 pp.

Chaparro OR, Thompson RJ, & Ward JE 1993. *In vivo* observations of larval brooding in the Chilean oyster, *Ostrea chilensis* Philippi, 1845. Biol. Bull. 185: 365–372.

Chesson J 1978. Measuring preference in selective predation. Ecology 59: 211–215.

———— 1983. The estimation and analysis of preference and its relationship to foraging models. Ecology 64: 1297–1304.

Gardiner DB, Silverman H, & Dietz TH 1991. Musculature associated with the water canals in freshwater mussels and

response to monoamines *in vitro*. Biol. Bull. 180: 453–465.

Heard WH 1975. Sexuality and other aspects of reproduction in *Anodonta* (Pelecypoda: Unionidae). Malacologia 15: 81–103.

——— 1977. Reproduction of fingernail clams (Sphaeriidae: *Sphaerium* and *Musculium*). Malacologia 16: 421–455.

Heard WH & Guckert RH 1971. A re-evaluation of the Recent Unionacea (Pelecypoda) of North America. Malacologia 10: 333–355.

Jørgenson CB 1990. Bivalve Filter Feeding: Hydrodynamics, Bioenergetics, Physiology and Ecology. Olsen and Olsen, Fredensborg, Denmark. 140 pp.

Kabat AR 1985. The allometry of brooding in *Transennella tantilla* (Gould) (Mollusca: Bivalvia). J. Exp. Mar. Biol. Ecol. 91: 271–279.

Kat PW 1984. Parasitism and the Unionacea (Bivalvia). Biol. Rev. 59: 189–207.

Lefevre G & Curtis WC 1910. The marsupium of the Unionidae. Biol. Bull. 19: 31–34.

Mackie GL 1984. Bivalves. In: The Mollusca, vol. 7, Reproduction. Tompa AS, Verdonk NH, & Biggerlaar JVD, eds., pp. 351–418. Academic Press, Orlando, FL.

Newell RIE & Jordan SJ 1983. Preferential ingestion of organic material by the American oyster *Crassostrea virginica*. Mar. Ecol. Prog. Ser. 13: 47–53.

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

Peck RH 1877. The miniature structure of the gills of lamellibranch Mollusca. Q. J. Microsc. Sci. 17: 43–66.

Richard PE, Dietz TH, & Silverman H 1991. Structure of the gill during reproduction in the unionids *Anodonta grandis, Ligumia subrostrata,* and *Carunculina parva texasensis*. Can. J. Zool. 69: 1744–1754.

Richardson MG 1979. The ecology and reproduction of the brooding antarctic bivalve *Lissarca miliaris*. Br. Antarct. Surv. Bull. 49: 91–115.

Rubenstein DI & Koehl MAR 1977. The mechanisms of filter feeding: some theoretical considerations. Am. Nat. 111: 981–994.

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

Silverman H, Kays WT, & Dietz TH 1987. Maternal calcium contribution to glochidial shells in freshwater mussels (Eulamellibranchia: Unionidae). J. Exp. Zool. 242: 137–146.

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

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

——— 1993b. The effect of larval brooding on the filtration rate and particle-retention efficiency of *Pyganodon cataracta* (Bivalvia: Unionidae). Can. J. Zool. 71: 1934–1944.

——— 1993c. The effect of larval brooding on the respiratory physiology of the freshwater unionid mussel *Pyganodon cataracta*. Am. Midl. Nat. 130: 146–163.

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

Ward JE, Beninger PG, MacDonald BA, & Thompson RJ 1991. Direct observations of feeding structures and mechanisms in bivalve molluscs using endoscopic examination and video image analysis. Mar. Biol. 111: 287–291.

Ward JE, MacDonald BA, Thompson RJ, & Beninger PG 1993. Mechanisms of suspension feeding in bivalves: resolution of current controversies by means of endoscopy. Limnol. Ocean. 38: 265–272.

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.

Wilkinson L 1990. SYSTAT: The System for Statistics. SYSTAT, Inc., Evanston, IL. 676 pp.

Wood EM 1974. Development and morphology of the glochidium larva of *Anodonta cygnea* (Mollusca: Bivalvia). J. Zool. 173: 1–13.

Zar JH 1984. Biostatistical Analysis, 2nd edition. Prentice-Hall, Englewood Cliffs, NJ. 718 pp.