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The effect of larval brooding on the filtration rate and particle-retention efficiency of *Pyganodon cataracta* (Bivalvia: Unionidae)

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TANKERSLEY, R.A., and DIMOCK, R.V., JR. 1993. The effect of larval brooding on the filtration rate and particle-retention efficiency of *Pyganodon cataracta* (Bivalvia: Unionidae). Can. J. Zool. 71: 1934–1944.

Following fertilization, the outer demibranchs of the unionid mussel *Pyganodon cataracta* undergo extensive morphological changes to accommodate approximately a million shelled glochidia larvae within the ctenidial water tubes which serve as brood chambers. The effect of larval incubation on particle filtration and quantitative selection was determined by comparing clearance rates and particle retention efficiencies of adult mussels during brooding and nonbrooding periods. Particle clearance rates for gravid females were significantly lower than those for nongravid females and were as much as 54% lower than those of males collected simultaneously. Females brooding larvae were less efficient at retaining particles <5 μm than were males or nonbrooding females. Both particle clearance and retention spectra were nearly identical for males and females collected during nonbrooding periods. The effect of larval brooding on the activity of gill cilia on excised gill tissue was assessed by determining the beat frequency of the laterofrontal cirri and the pattern of transport of fluorescently labeled latex microspheres by frontal cilia. The metachronal beat of cirri on marsupial gills was significantly lower than on nonmarsupial gills of both males and females but did not differ between reproductive seasons. Particles were transported faster by frontal cilia on nonmarsupial and nongravid gills vs. gravid marsupial gills, suggesting that the reduction in particle clearance and retention by brooding females resulted from functional changes in the ciliature of the marsupial demibranchs.

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Après la fécondation, les hémibranchies externes de la moule unionidée *Pyganodon cataracta* subissent d'importants changements morphologiques permettant de faire de la place à environ un million de larves glochidies à coquilles à l'intérieur des tubes des cténidies qui servent de chambres d'incubation. L'effet de l'incubation sur la filtration et la sélection quantitative des particules a été déterminé en comparant les taux de clearance et l'efficacité de rétention des particules chez des moules adultes au cours des périodes d'incubation et entre ces périodes. Les taux de clearance des particules chez les femelles gravides sont significativement plus faibles que chez les femelles non gravides et de 54% moins élevées que chez des mâles recueillis au cours des mêmes périodes. Les femelles qui portent des larves sont moins efficaces que les mâles ou les femelles non porteuses à retenir les particules <5 μm. Les spectres des particules qui sont retenues ou éliminées sont presque identiques chez les mâles et les femelles qui sont capturés au cours des périodes non reproductrices. L'incubation affecte l'action qu'ont les cils branchiaux sur le tissu branchial excisé et cet effet a été mesuré en déterminant la fréquence des battements des cirres latérofrontaux et le transport, par les cils frontaux, de microsphères de latex marquées d'une substance fluorescente. Le battement métachronique des cirres des branchies marsupiales est significativement moins rapide que celui des cirres des autres branchies, chez les mâles aussi bien que chez les femelles, et ne diffère pas d'une saison de reproduction à l'autre. Les particules sont transportées plus rapidement par les cils frontaux des branchies non marsupiales non gravides que par ceux des branchies marsupiales gravides, ce qui semble indiquer que la réduction de la clearance et de la rétention des particules chez les femelles reproductrices résulte de changements fonctionnels de la ciliature des hémibranchies marsupiales.

[Traduit par la rédaction]

Introduction

The selection and retention of particles by suspension feeding bivalve molluscs involve the integrated activities of cilia and cirri on the surface of the gills (Dral 1967; Moore 1971; Vahl 1972a; Jørgensen 1975a, 1975b, 1983; Silvester and Sleigh 1984; Silvester 1988). Although it is generally agreed that lateral cilia near the openings of the interfilamentar spaces comprise the "pump" and are responsible for water transport, the details of the fluid mechanics associated with the gill ciliature of lamellibranch bivalves and the mechanisms involved in particle capture and retention are still widely debated (Dral 1967; Jørgensen 1975b, 1981, 1983; Jørgensen et al. 1984, 1986; Silvester and Sleigh 1984; Ward et al. 1993). Traditionally, removal of particles from suspension is thought to be accomplished by the straining of water by rows of laterofrontal cirri that project into the spaces between successive filaments and act as mechanical filters. Trapped particles are then trans-

ported along the surface of the gill to the food grooves, and subsequently to the palps and mouth by the activity of the frontal cilia located on the face of each filament (Moore 1971; Owen 1974; Owen and McCrae 1976; Silvester and Sleigh 1984). Jørgensen (1981, 1983) and Jørgensen et al. (1986) have proposed an alternative model in which laterofrontal cirri do not act as sieves but rather alter particle retention by modifying hydromechanical velocity gradients, which extract suspended particles from the water flowing through the interfilamentar spaces. Recent in vivo observations of suspension feeding in undisturbed bivalves using endoscopy suggest that mucociliary and hydrodynamic mechanisms of particle capture and transport may not be mutually exclusive but might occur simultaneously at different locations on the ctenidia (Ward et al. 1993). Regardless of the mechanisms of particle capture and transport and the specific role of the laterofrontal cirri in filtration, changes in the hydrodynamics of the gill, including the organization, structure, and activity of the gill ciliature, may ultimately affect the mechanical performance of the pump and filtering system.

As for most suspension feeders, the amount of food available to bivalves is determined by the concentration of particles in suspension, the efficiency of particle retention by the gill, and the volume of water processed. Filtration rates have been shown to be modified by a variety of factors including particle size (Haven and Morales-Alamo 1970; Vahl 1972a; Foster-Smith 1975; Hornbach et al. 1984; Paterson 1986) and concentration (Davids 1964; Ali 1970; Morton 1971; Tenore and Dunstan 1973; Winter 1973; Foster-Smith 1975; Schulte 1975; Hildreth and Mallet 1980; Kiørboe et al. 1980; Palmer and Williams 1980; Riisgård and Randløv 1981; Hornbach et al. 1984; Sprung and Rose 1988). Although most lamellibranchs are reported to retain particles larger than 4 μm with 100% efficiency, there appear to be adaptive differences in the ability to capture smaller particles. For most bivalves, particle size selection spectra are seemingly related to the presence and morphology of the laterofrontal cirri (Owen and McCrae 1976; Møhlenberg and Riisgård 1978; Riisgård 1988) and the overall structure of the gills (Møhlenberg and Riisgård 1978). Moreover, particle retention and selective feeding may be influenced by less obvious factors, including electrical charge, shape, and chemical composition of particles, resulting in certain types being retained at higher rates than others (Morton 1971; Rubenstein and Koehl 1977; Kiørboe et al. 1980; Kiørboe and Møhlenberg 1981; Ten Winkel and Davids 1982; Jørgensen 1983; Silvester and Sleigh 1984; Shumway et al. 1985; Ward and Targett 1989).

Studies of the hydrodynamics of the bivalve pump and filter have focused primarily on marine filibranch species, especially the commercially important mussel *Mytilus edulis* (for reviews see Jørgensen 1989, 1990). Consequently, relatively little is known about the feeding physiology of freshwater bivalves, among which, studies have been confined to a few unionid species (Paterson 1986; Kryger and Riisgård 1988), several pisidiid clams (Hornbach et al. 1984; Burky et al. 1985), and *Corbicula* and *Dreissena* (Morton 1971; Foe and Knight 1986; Kryger and Riisgård 1988; Sprung and Rose 1988; Reeders et al. 1989; Way et al. 1990; Reid et al. 1992). Previous study of the feeding physiology of both freshwater and marine bivalves has largely ignored the effects of larval brooding on the flow mechanics involved in filtration and particle capture, with the notable exception being the observation of Walne (1972) that filtration rates of brooding *Ostrea edulis* are considerably lower than those of nonbrooding oysters.

Alterations in morphology and in the hydromechanical design of bivalve gills, as occur in the marsupial demibranchs of unionid mussels (Ortmann 1911; Richard et al. 1991; Tankersley and Dimock 1992, 1993), might dramatically affect both the activity of the gill ciliature and the consequent patterns of water transport through the mantle cavity/gill complex of brooding females. Changes in ciliary physiology, orientation, or organization accompanying larval brooding might modify the characteristics of the ciliary pump and could ultimately influence the animal's feeding physiology including the filtration rate and the retention and preferential selection of suspended particles. However, any negative changes in the overall performance of cilia on areas of a gill serving as marsupia might be compensated for by concurrent changes in other aspects of the pump-filtration-transport system of nonbrooding demibranchs.

The present study was undertaken to assess the effect of larval brooding on the suspension feeding mechanism of the freshwater unionid mussel *Pyganodon cataracta* (formerly *Anodonta cataracta*; Hoeh 1990) including filtration rate, retention efficiency, and particle selectivity. *Pyganodon cataracta* is classified as a bradytictic (long-term) brooder. Following fertilization in the late summer, larvae (glochidia) are incubated for several months in the water tubes of the female's outer (lateral) gills until they are released in the early spring to begin the parasitic phase of their life cycle (Tankersley 1992; Tankersley and Dimock 1993). Aspects of this mussel's feeding biology were quantified during prebrooding (summer) and brooding (fall – winter) periods using suspensions of latex particles, which simulate their natural diet and prevent the confounding effects of variation in particle quality, including shape, texture, and chemical composition, on particle selection and retention. Under natural conditions, particles retained by the ctenidia are transported ventrally toward the free margin of the gills and become incorporated in mucous threads traveling anteriorly toward the mouth (Tankersley and Dimock 1993). Thus, the activity of the laterofrontal cirri and the pattern of transport of 10 and 16 μm particles by frontal cilia were also assessed using excised gill tissue.

Materials and methods

Collection and maintenance of animals

Pyganodon cataracta were collected from Speas' Pond (Yadkin County, North Carolina) and were maintained for up to 1 week in artificial pond water (APW; 0.5 mM NaCl, 0.4 mM CaCl$_2$, 0.2 mM NaHCO$_3$, 0.05 mM KCl) under fluorescent illumination on a light – dark cycle that approximated the natural photoperiod. All experiments were conducted at collection temperatures to avoid the confounding effects of laboratory acclimation. Therefore, males collected during each time period were used to estimate seasonal effects not attributable to brooding. Since *P. cataracta* lacks external sexually dimorphic features, unequal sample sizes of the two sexes were often used, even though the sex ratio of mussels in the pond was nearly 1:1.

Clearance rate and selectivity spectra

Filtration rates and particle-size selectivity by mussels in both the prebrooding (summer) and brooding seasons (fall – winter) were determined by measuring changes in the particle concentration of a mixed suspension of untreated latex microspheres between 2 and 10 μm in diameter (Duke Scientific Corp.; mean diameter 5.16 μm). The same stock suspension of particles was used for both test periods, assuring that particle concentration and the frequency distributions of the various size classes of particles remained constant for all mussels and did not vary seasonally. Mussels were immersed in 1.5 L of APW in 17.8 × 10.2 × 12.7 cm experimental chambers maintained at collection temperatures by a thermostatically controlled water bath. Mussels were considered to be actively filtering if their valves were open and their siphons were extended. After a 1-h acclimation period, the suspension of latex particles was added to the test chambers (total concentration 1.85 × 10^5 mL^{-1}) and allowed to mix for 2 min before a 10 mL aliquot of the suspension was removed and mixed with an equal amount of Coulter Counter electrolyte. After 30 min the sampling procedure was repeated. Gentle aeration maintained normoxic conditions within the chambers and ensured mixing of the particles. Particle concentration was low enough not to induce the formation of pseudofeces.

Particle concentrations were determined using a Coulter Counter Model ZM particle counter fitted with a 50-μm aperture tube and attached to a Model 256 Channelyzer (Coulter Electronics, Inc). All final calculations of concentration are the means of four separate 50-μL counts from each sample. Particle-size spectra were analyzed using AccuComp II software (Coulter Electronics, Inc.) running on an IBM PS/2 Model 30 computer. For statistical analysis, particle-size distributions were divided into 0.5 μm size classes except those greater than 9 μm, which were pooled. Particles smaller than 2 μm

could not be accurately counted using the described technique and represented less than 2% of the particles in suspension.

Clearance (filtration) rates (CR; $L \cdot h^{-1}$), defined as the volume of water filtered free of particles per unit time, for each of the 15 size classes of particles, were calculated using the formula of Coughlan (1969):

$$[1] \quad CR_i = \frac{v}{t} \ln\left(\frac{C_o}{C_t}\right)$$

where CR_i is the clearance rate ($L \cdot h^{-1}$) for particles in size class i, v is the volume of the experimental chamber (1.5 L), t is the duration of the experiment (0.5 h), and C_o and C_t are the initial and final concentrations of particles in size class i. Settlement was considered negligible, since, according to Stoke's Law, the sinking rate of the largest particles would be approximately $1 \, cm \cdot h^{-1}$. Nevertheless, chambers containing particles and empty mussel shells, or no particles and living mussels, served as controls to adjust the clearance rate measurements.

The relative retention efficiency (RE; expressed as a percentage) for each particle-size class was calculated by standardizing the clearance rate for each size class (CR_i) by the clearance for the one size class that was most completely removed by each mussel (i.e., highest clearance rate or CR_{max}). CR_{max} was also used as an indirect and conservative estimate of ventilation (pumping rate; assuming 100% retention of particles) for comparisons between treatments. Selectivity by mussels for particles based upon size (quantitative selection) was quantified using Chesson's α (Chesson 1983):

$$[2] \quad \alpha_i = \frac{CR_i}{\sum\limits_{j=1}^{m} CR_j} \quad , \, i = 1, \ldots, m$$

where CR_i is the clearance rate of particles in size class i and m is the number of food types (particle-size classes) present ($m = 15$). The index varies between 0 ("rejection") and 1 ("preference") with values near 0.067 (i.e., $1/m$) indicating nonselective feeding or "no preference". Therefore, the calculated α for each particle-size class provided an estimate of its contribution to the total quantity of particles filtered by the mussel. The index has the further advantage of being insensitive to differences in particle concentration between size classes (Chesson 1983). Since animals were subjected to the same size particles at similar concentrations, differences in the selectivity curves represent size-selective feeding or preferential clearance by the ctenidia. For interpretation and display purposes, α was rescaled to ε using the procedures outlined by Chesson (1983) to yield values ranging between -1 and 1 with 0 representing random feeding.

Gill areas were determined by excising all four demibranchs and digitizing their outlines using a computerized video analysis program (JAVA; Jandel Scientific) and digitizing board (PCVision Plus, Imaging Technology) running on a Zenith PC/AT IBM-compatible computer. Area measurements were doubled to account for opposing gill surfaces. The shell length (maximum anterior−posterior dimension), soft tissue dry mass (DW; dried at 60°C), and ash-free dry mass (AFDW; DW minus ash weight after combustion at 400−425°C) were calculated for each mussel. Outer demibranchs were analyzed separately from the remaining soft tissue to assess the contribution of glochidia to the total mass of brooding females.

Filtration rates and retention-efficiency curves of males and females between reproductive seasons (prebrooding and brooding) were compared using repeated measures analysis of variance (ANOVAR) (SYSTAT Statistical Software; Wilkinson 1990) following the procedures outlined by Potvin et al. (1990). Retention efficiencies were arc sine square-root transformed prior to analysis to normalize the data and remove heteroscedasticity. Analysis of covariance (ANCOVA) could not be used to remove the confounding effect of mussel size on filtration rate (see Packard and Boardman 1988) because the relationship between traditional body size measurements (soft tissue dry and ash-free masses, shell length, and gill area) and clearance rate varied

considerably between sexes and reproductive periods (violation of homogeneity of regression). Since bivalve pumping rates have been shown to scale closely with shell length (Jones et al. 1992), size differences were accounted for by placing animals into blocks based upon shell size (<12.5, $12.5-13.25$, $13.3-14$, >14 cm) as recommended by Tabachnick and Fidell (1989). Pumping-rate estimates (CR_{max}) were analyzed using a two-factor analysis of variance (ANOVA). A priori contrasts between treatment combinations were conducted using SYSTAT SPECIFY procedures at an experimentwise error rate of 0.05 (SYSTAT Statistical Software; Wilkinson 1990). Although not used for statistical analysis, estimates of size-specific ventilation rates (CR_{max}) are reported for comparison with other published studies.

Activity of laterofrontal cirri

The metachronal beat frequency of the laterofrontal cirri present on isolated marsupial and nonmarsupial gills of male and female *P. cataracta* was determined stroboscopically using a Chadwick-Helmuth Slip-Sync Strobex Lamp (Chadwick-Helmuth Co., Inc.) attached to a Zeiss Axiophot Microscope (Carl Zeiss, Germany). Since the gills of *P. cataracta* are relatively opaque, especially female lateral demibranchs containing glochidia, observations of the activity of the cirri on gill filaments required the use of incident illumination and modified Epi-DIC (Differential Interference Contrast) at $40-65\times$ rather than transmitted stroboscopic light as is traditionally used to observe the cilia of bivalve molluscs.

Preparations of gill sections for light microscopy followed those described by Gosselin (1961). The valves of the mussels were opened by cutting the anterior and posterior adductor muscles; rectangular sections (2×4 cm) of gill tissue were removed from the center of the medial and lateral demibranchs near the ventral margin, placed on glass microscope slides, and transferred to APW. Each gill piece was gently restrained on a slide by securing small glass rods over each end of the gill, thus preventing excessive movement of the sections that otherwise results from the continued beating of frontal cilia in contact with the glass slide. Mounted gill sections were placed in a 5 cm diameter circular water-jacketed lucite microscope stage containing 30 mL of APW maintained at the mussel's collection temperature. The beat frequencies (beats $\cdot$ s^{-1}) of cirri exhibiting stable metachronal activity were recorded from five separate regions of the demibranch by adjusting the frequency of the stroboscope until the cirri appeared to stop beating. All measurements were completed within 30 min of gill excision, and the order of examination of the demibranchs for each mussel (medial or lateral) was random.

To test for any influence of larval brooding on the activity of cirri, comparisons of the beat frequencies of the cirri on medial and lateral gills (gill type; within subjects factor) of male and female *P. cataracta* (sex) during prebrooding and brooding periods (reproductive season) were made using ANOVAR (SYSTAT Statistical Software; Wilkinson 1990).

Particle transport by frontal cilia

The efficiency of particle transport by frontal cilia was monitored by videotaping the movement of 10 and 16 μm green fluorescent latex microspheres (Duke Scientific Corp., Palo Alto, California) on the surface of excised gills. Although a variety of inert particles and algae have been used to monitor and quantify particle transport by gill cilia (Gosselin and O'Hara 1961; Lagerspetz and Dubitscher 1966), latex particles are particularly effective because they are uniform, become monodispersed in suspension, and are available in a variety of sizes. Furthermore, the spheres are highly refractive and when labeled with a fluorescent dye are easily isolated from background gill tissue using epifluorescence microscopy, which enables the observation of single moving particles.

Excised lateral and medial gill tissues were prepared for light microscopy as described above (see *Activity of laterofrontal cirri*) except that two sections of each gill were prepared separately and randomly assigned to either the 10 or 16 μm particle treatment. Gill sections were immersed in 30 mL of APW containing approximately 2.5×10^4 particles $\cdot$ mL^{-1} and were viewed under low magnification
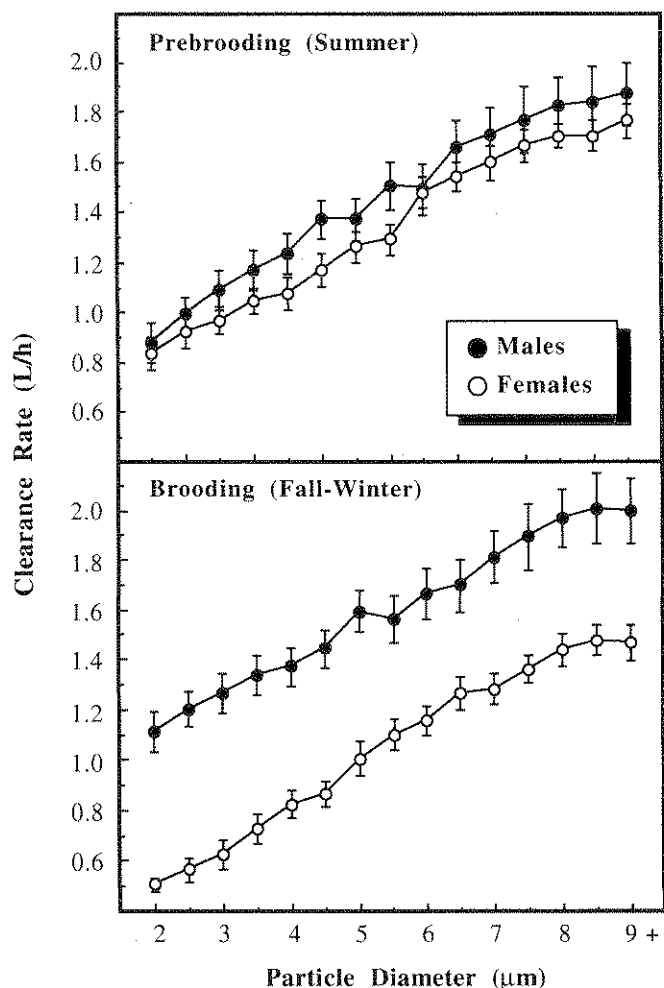
FIG. 1. The mean ($\pm$SE) clearance rates (CR; $L \cdot h^{-1}$) plotted as a function of particle diameter for male and female *Pyganodon cataracta* during prebrooding (upper panel) and brooding periods (lower panel).

FIG. 2. The mean ($\pm$SE) maximum clearance rates (CR_{max}; $L \cdot h^{-1}$) of male and female *Pyganodon cataracta* during prebrooding and brooding periods. Bars with the same letter are not significantly different at $P < 0.05$.

(5$\times$) using a Zeiss Axiophot microscope fitted for fluorescence microscopy (excitation $\lambda = 450$ nm). Particles transported by frontal cilia along the filaments were isolated from those in suspension by restricting the plane of focus to the gill surface. Particles were video-taped using a Hamamatsu C2400 video camera attached to a computerized image analysis system (Image-1 Video Image Analyzer, Universal Imaging Corp.) to enhance the image and improve the contrast between the gill tissue and the fluorescent particles.

Video-taped recordings (30 frames $\cdot$ s^{-1}) were analyzed using a computerized motion analysis system (Expertvision, Motion Analysis Corp.). The centroids of individual particles were tracked at 10 Hz and were used to reconstruct the paths of the particles as they traveled vertically across the screen representing approximately a 1.45 $\times$ 1.55-mm section of the gill surface. The average speed (mm $\cdot$ s^{-1}), rate of change of direction (absolute value of the angular velocity in degrees $\cdot$ s^{-1}), and linear acceleration (rate of change in speed; first derivative of the speed in mm $\cdot$ s^{-2}) were calculated for 50 particles that were followed for at least 1 s using the software's analysis subroutines. Since the particles were traveling in the plane of focus of the microscope, movement was considered to be two dimensional and no adjustment was made to include any upward or downward component of their paths. Particles that traveled as clumps (usually bound by mucus) or became trapped by gill cilia or filaments were eliminated from the analysis.

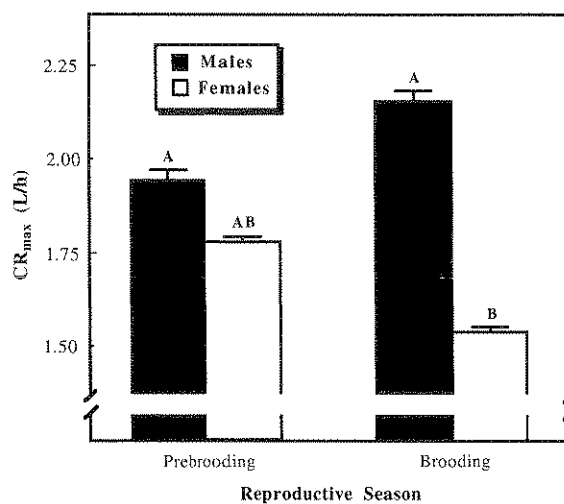Twenty mussels were examined during each season (prebrooding,

brooding, and postbrooding) and the results for each dependent variable (speed, rate of change of direction, and linear acceleration) were analyzed using ANOVAR with two within-subject factors (particle size (10 or 16 μm) and gill type (lateral and medial)) and two between-subject factors (reproductive season and sex).

Results

Clearance rate and selectivity spectra

Clearance rates (filtration rates; $L \cdot h^{-1}$) for male and female mussels during brooding and nonbrooding periods are summarized in Fig. 1. The filtration rate for all mussels increased with particle diameter and reached a maximum for beads between 8$-$9 μm, a pattern that did not differ significantly between sexes or reproductive seasons (Table 1). Rates for male and female *P. cataracta* varied differently depending upon reproductive season (i.e., significant sex-by-season interaction; Table 1) with rates for females decreasing during periods of larval incubation. The CRs of gravid females were 13$-$39% lower than those of nongravid levels and were 25$-$54% lower than those of males collected during the same period. The filtration rates of males remained relatively constant between seasons and resembled those of females during prebrooding periods (Fig. 1).

Figure 2 shows the mean ventilation rate (pumping; $1 \cdot h^{-1}$; CR_{max}) as calculated from the maximum clearance rate, assuming 100% retention of the size class of particles that were retained most completely. Although the ventilation rates of females did not differ significantly between seasons, their rate of pumping was significantly lower than that of males during the winter (i.e., brooding period) (Fig. 2). There were no significant differences among males between seasons nor were the rates of males and females collected in the summer significantly different (Fig. 2).

Size-specific CR_{max} values for mussels during each reproductive season are summarized in Table 2. Comparison of these values is misleading, since seasonal differences in available food and the presence of glochidia caused significant variation in the dry and ash-free mass and gill-area values used to normalize these data (Table 3). During nonbrooding periods, lateral demibranchs comprised approximately 10.9 and 7.6%

TABLE 1. Repeated measures analysis of variance (ANOVAR) testing the effects of sex (male and female) and season (prebrooding and brooding) on the clearance rate (CR; $L \cdot h^{-1}$) and retention efficiency (RE) of *Pyganodon cataracta*

Source of variation	Clearance rate					Retention efficiency				
	df	MSE $\times 10^6$	F-ratio	P	H–F P	df	MSE	F-ratio	P	H–F P
Between subjects										
Sex (S)	1	35.22	16.56	<0.001		1	0.567	6.72	<0.025	
Season (R)	1	0.14	0.07	ns		1	0.064	0.76	ns	
S × R interaction	1	12.30	5.78	<0.025		1	0.165	1.96	ns	
Block (shell size)	3	5.60								
Error	73	2.13				76	0.084			
Within subjects										
Particle size (P)	14	5.69	161.00	<0.001	<0.001	14	4.008	266.00	<0.0001	<0.001
P × S interaction	14	0.03	0.71	ns	ns	14	0.063	4.16	<0.001	<0.001
P × R interaction	14	0.02	0.58	ns	ns	14	0.021	1.42	ns	ns
P × S × R interaction	14	0.04	0.99	ns	ns	14	0.039	2.65	<0.001	<0.005
Error	1022	0.04				1064	0.015			

NOTE: The Huynh–Feldt epsilon (ε) was significantly less than 1 ($\varepsilon = 0.356$ for CR and 0.766 for RE) indicating a violation of compound symmetry of the covariance matrix. Therefore, the Huynh–Feldt corrected significance values (H–F P) are also presented. (ns = not significant, $P > 0.05$). MSE, mean square error.

TABLE 2. Mean ($\pm$SE) size-specific maximum clearance rates (CR_{max}) for male and female *Pyganodon cataracta* during prebrooding and brooding periods of the year

Season and sex	$CR_{max} \cdot$ dry mass^{-1} (mL $\cdot$ h$^{-1} \cdot$ g^{-1})	$CR_{max} \cdot$ ash-free mass^{-1} (mL $\cdot$ h$^{-1} \cdot$ g^{-1})	$CR_{max} \cdot$ gill area^{-1} (mL $\cdot$ h$^{-1} \cdot$ cm^{-2})	N
Prebrooding				
Male	160.75±9.74	266.77±16.45	16.13±1.03	20
Female	179.53±10.94	294.45±21.03	13.90±0.55	20
Brooding				
Male	269.24±19.37	415.93±22.77	18.74±1.09	24
Female	138.75±10.09	265.17±21.91	10.31±0.66	16

NOTE: N is sample size.

TABLE 3. Soft-tissue mass (dry and ash-free), gill area, and shell length measurements of mussels used in testing the effects of larval brooding on the filtration rate and particle selectivity of *Pyganodon cataracta*

Reproductive season and sex	Dry mass (g)		Ash-free mass (g)		Gill area (cm²)		Shell length (cm)	N
	Lateral gills	Total	Lateral gills	Total	Lateral	Medial		
Prebrooding								
Male	1.21±0.06	12.28±0.43	0.50±0.04	7.48±0.32	51.6±1.3	70.6±1.6	13.29±0.12	20
Female	1.13±0.06	11.06±0.77	0.47±0.04	6.92±0.53	57.2±1.3	76.6±1.9	13.47±0.12	20
Brooding								
Male	0.83±0.05	8.08±0.25	0.33±0.02	5.07±0.27	48.3±1.3	64.9±1.6	12.91±0.15	24
Female	4.91±0.33	11.43±0.52	1.76±0.15	6.07±0.33	80.0±2.5	71.6±1.8	13.26±0.22	16

NOTE: N is sample size. Values are means ± SE.

of the females' DW and AFDW, respectively, compared with 42.4 and 28.9% during brooding periods. The contribution of the lateral demibranchs to the total tissue weight for males remained relatively constant between seasons (Table 3).

In addition to effecting pronounced changes in the mass of lateral demibranchs of females during brooding, the presence of glochidia in the water tubes of females was accompanied by substantial swelling of the outer gills (e.g., a 30-fold increase in gill thickness (Tankersley and Dimock 1992)). These morphological changes resulted in a shift in the ratio of the area of lateral to medial demibranchs from 0.75 ± 0.01 ($\bar{X} \pm$ SE; $N = 20$) prior to incubation, to 1.13 ± 0.03 ($N = 16$) when

brooding. Unfortunately, without direct observations of particle capture by the gills, we were unable to determine if this difference in overall shape of the brooding demibranch resulted in an increase in the total surface area of gill utilized for filtration and water transport.

Relative retention efficiency spectra for males and females mimicked the patterns found for filtration rates, with maximum retention for all mussels occurring with particles around 8 μm in diameter (Fig. 3). The nearly linear relationship between relative retention efficiency and particle diameter suggested that the largest spheres in the mixture most likely represented the minimum size particles that could be retained
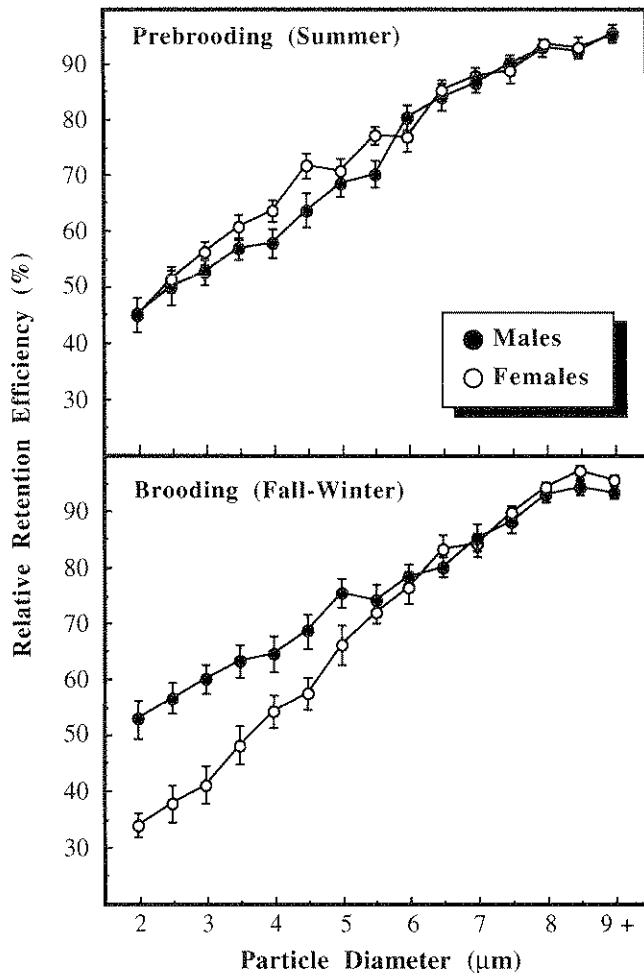
FIG. 3. Relative retention efficiency curves (relative clearance) for male and female *Pyganodon cataracta* during prebrooding (upper panel) and brooding (lower panel) reproductive periods. ($\overline{X} \pm$ SE).

FIG. 4. Chesson's electivity index α (rescaled to ε to vary between -1 (selection against) and 1 (selection for) with 0 representing non-selective or random feeding) plotted as a function of particle diameter for male and female *Pyganodon cataracta* during prebrooding (upper panel) and brooding periods (lower panel).

with 100% efficiency. All mussels, regardless of sex and reproductive status, were able to retain particles larger than 4 μm with greater than 50% efficiency (Fig. 3). Retention of larger particles was similar in males and females during brooding periods, but the efficiency of retention became increasingly disparate between the sexes for particles less than 5 μm (Fig. 3), resulting in significant interactions between particle size and sex and among particle size, sex, and season (Table 1).

The lower filtration rates for brooding females were also accompanied by changes in particle selectivity. Both male and female *P. cataracta* preferentially removed the larger particles from the mixed suspension during nonbrooding periods, but the lower retention efficiencies of gravid females resulted in a marked shift in their preference (i.e., higher Chesson's α values) for larger sized particles and a subsequent increase in their rejection of smaller beads (Fig. 4). Moreover, the particle size preferences of males were relatively indistinguishable between collection periods. Differences in the electivity indices of males and females during brooding periods represented changes in preingestive discrimination based on particle size alone, given that beads were inert and had the same chemical surface and adhesive properties (i.e., "taste"), eliminating differentiation based upon chemical cues. Additional processing of particles may take place at the labial palps, as
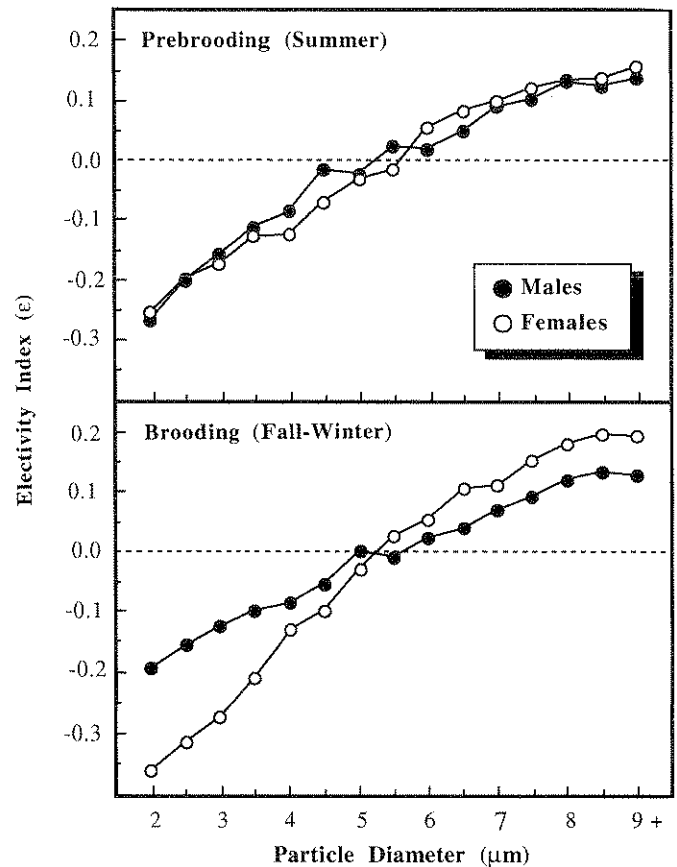
suggested by several researchers (for review see Beninger et al. 1990), further altering the distribution of particles actually ingested.

Activity of laterofrontal cirri

Laterofrontal cirri on all gills, including gravid marsupial demibranchs, were active after excision and exhibited stable metachronal motion in APW, indicating that tissue manipulation and preparation did not seriously alter the physiology of the ciliated epithelium. Cirri on isolated gill sections remained active for up to 24 h after excision without the addition of cilioexcitatory substances and neurotransmitters such as 5-hydroxytryptamine.

Since adjoining filaments of eulamellibranch bivalve gills are fused by interlamellar tissue, only surface views of the cirri were possible, making the depth of focus limited and observations of large groups of cirri difficult. Mean beat frequencies ranged between 7.9 and 13.4 beats $\cdot$ s^{-1} and were significantly lower for the lateral (marsupial) demibranchs of females during both brooding and prebrooding periods than for the medial demibranchs of females or either demibranch of males (Table 4 and Fig. 5). Cirri activity on marsupial gills showed a slight but not statistically significant decrease during brooding compared with preincubation levels (Fig. 5). Acclimation temperature and time of the year had no apparent effect on the activity of the cirri, and there were no significant inter-

TABLE 4. Results of a repeated measures analysis of variance (ANOVAR) on the effect of sex (male and female), reproductive season (prebrooding and brooding), and gill type (inner or outer demibranch) on laterofrontal cirri beat frequency

Source of variation	df	MSE	F-ratio	P
Between subjects				
Sex (S)	1	7.23	6.40	<0.025
Season (R)	1	1.35	1.20	ns
S × R interaction	1	3.85	3.40	ns
Error	56	1.13		
Within subjects				
Gill type (G)	1	23.95	71.25	<0.001
G × S interaction	1	20.64	61.38	<0.001
G × R interaction	1	0.19	0.55	ns
G × S × R interaction	1	0.42	1.26	ns
Error	56	0.34		

NOTE: ns, not significant; $P > 0.05$. MSE, mean square error.

actions between gill type and season or among all three factors (Table 4). Cirri activity on female medial gills during prebrooding periods was significantly greater than on comparable gills of males (Fig. 5). Unfortunately, the stroboscopic technique did not permit determining if differences in cirrus beat frequency resulted from changes in metachronal coordination or disruption of the beat pattern, including the lengths of the effective (power) and recovery strokes.

Particle transport by frontal cilia

Under fluorescent microscopy, dye labeled latex beads appeared as bright spheres on a dark background. Most particles could be seen moving along the frontal surface of the filaments although some were most likely influenced by the activity of the laterofrontal cirri, since their trajectories were positioned in the spaces between adjacent ctenidial filaments. The proximity of the particles to the cilia could not be accurately judged, although the pattern of movement suggested that latex spheres were not always in direct contact with frontal cilia and appeared to be entrained in currents produced by gill cilia and cirri. Both particle sizes were adequately retained by the gills although occasionally the 10 μm diameter beads were observed entering the interfilament canals and water tubes through open ostia.

Results of separate ANOVARs for the three particle-transport parameters are summarized in Table 5. Particle movement was predominantly unidirectional, and beads rarely crossed filaments or reversed direction. Transport speeds were significantly influenced by demibranch type, but the effect of gill type depended upon both sex and season (Table 5). In females, latex beads travelled between 23 and 113% faster on nonmarsupial than on marsupial gills (Table 6). Velocities of particles on medial gills of females during brooding periods were similar to those recorded for both gill types of males (Table 6). Comparisons of 10 and 16 μm beads revealed that none of the movement parameters were significantly influenced by particle diameter (Table 5). Mean linear accelerations for all factor combinations were close to 0 (Range: $1.4 \times 10^{-2} - 8.7 \times 10^{-4}$ mm · s^{-2}) indicating that the velocities of the particles were relatively constant. Rates of change of direction (RCD) of beads were significantly influenced by gill type, with particles on medial gills having significantly straighter paths (i.e., lower RCD values; Table 6), whereas the effects of sex and
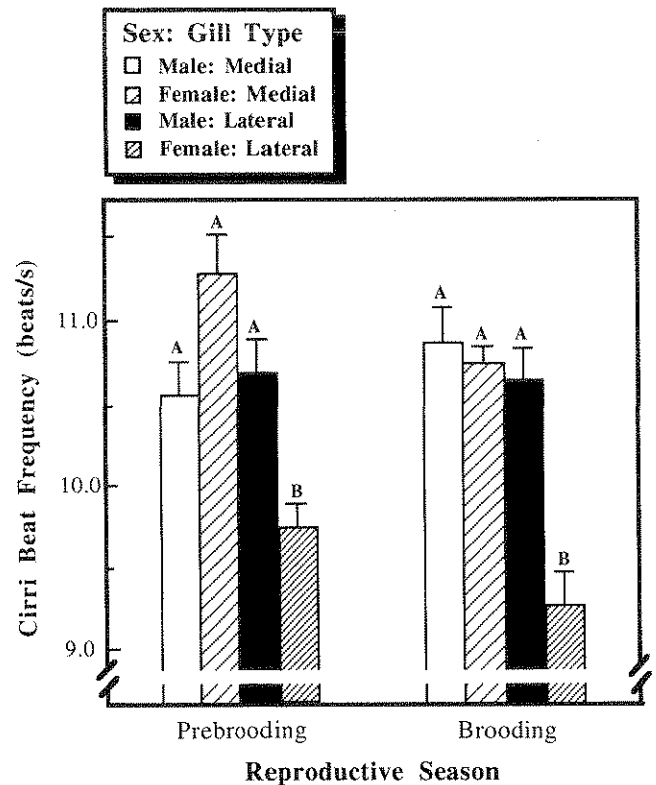

Sex: Gill Type
☐ Male: Medial
▨ Female: Medial
■ Male: Lateral
▨ Female: Lateral

FIG. 5. The mean (±SE) beat frequency (beats · s^{-1} or Hz) of the laterofrontal cirri present on the medial and lateral gills of male and female *Pyganodon cataracta* plotted as a function of reproductive season. Bars with the same letter are not significantly different at $P < 0.05$.

reproductive season and their interaction were non-significant (Table 5).

Discussion

The maximum clearance rates (CR_{max}) for both brooding and nonbrooding *Pyganodon cataracta* reported in Fig. 2 are in close agreement with filtration rates previously reported for most unionid mussels, as determined with comparable experimental techniques (see Kryger and Riisgård 1988 for review), including *Elliptio complanata* (Paterson 1986), and *Anodonta anatina*, *Unio pictorum*, *U. tumidus*, and *U. crassus* (Kryger and Riisgård 1988). Although size-specific values reported by other researchers for different unionid species vary slightly from the values presented in Table 2, our results support the conclusions of Kryger and Riisgård (1988) that filtration rates in unionid mussels tend to be considerably lower (up to 8 times) than those of marine bivalves of similar size.

Filtration rates of particles between 2 and 10 μm for male *P. cataracta* remained relatively constant between prebrooding and brooding periods, indicating that seasonal changes, including differences in temperature and ambient food supplies, had little effect on their feeding physiology (Fig. 1). As a result, the observed reduction in the clearance rates of females during brooding periods when compared with rates prior to larval incubation (Fig. 1) suggest that the presence of larvae within the brood chambers of the lateral demibranchs has a significant impact on both the rate of water processing and the pattern and efficiency of the retention of particles by

TABLE 5. Results of separate analysis of variance with repeated measures (ANOVAR) on parameters of particle transport (dependent variables) by frontal cilia with two between-subjects factors (reproductive season and sex) and two within-subjects factors (gill type and particle size)

	Factors				
	Between subjects		Within subjects		
Parameter	Season (R)	Sex (S)	Gill (G)	Particle (P)	Interactions
Speed	ns	ns	***	ns	G × R*, G × S***
Rate of change of direction	ns	ns	**	ns	
Linear acceleration	ns	ns	ns	ns	

NOTE: R, prebrooding, brooding, and post-release. S, male and female. G, gill type; lateral and medial. P, particle size; 10 and 16 μm. Only significant interactions ($P < 0.05$) are given. ns, not significant.
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

TABLE 6. Results of particle transport parameters for latex beads transported by the frontal cilia of excised *Pyganodon cataracta* gills

	Parameter and gill type						
	Speed[a]		Rate of change of direction[b]		Linear acceleration[c] ($\times 10^{-2}$)		
Season and sex	Lateral	Medial	Lateral	Medial	Lateral	Medial	N
Prebrooding							
Male	0.39±0.02	0.42±0.03	15.5±1.3	13.8±1.4	−0.4±0.55	−0.5±0.62	10
Female	0.39±0.04	0.48±0.03	14.5±0.8	12.7±1.0	−1.3±0.32	−0.2±0.43	10
Brooding							
Male	0.42±0.03	0.46±0.04	16.2±1.6	12.3±0.7	−1.1±0.43	−1.4±0.59	10
Female	0.21±0.01	0.46±0.04	19.5±2.6	13.4±1.2	1.3±0.98	−0.8±0.76	10
Postrelease							
Male	0.45±0.04	0.51±0.05	17.6±1.8	18.9±3.1	−0.7±0.69	−1.5±1.29	11
Female	0.29±0.03	0.54±0.06	21.3±5.3	13.0±1.3	−1.0±0.36	−0.7±0.77	9

NOTE: Values are means ± SE. Since particle size had no effect on any of the parameters (see Table 5), reported values represent the average of the 10 and 16 μm particles. (N is sample size).
[a]mm · s^{-1}.
[b]Degrees · s^{-1}.
[c]mm · s^{-2}.

marsupial gills. Moreover, comparable maximum clearance rates (CR$_{max}$) for males and females prior to larval incubation (Fig. 2) imply that permanent architectural differences between marsupial and nonmarsupial gills, including the spacing and number of primary water tubes (Tankersley and Dimock 1992), do not, in themselves, alter the pumping kinetics of female mussels. Although larval brooding resulted in a marked increase in the total gill area of females (Table 3), the actual extent of gill surface utilized for suspension feeding and water transport, as well as the amount of water reaching the frontal surfaces and entering the interfilamental openings of the lateral gills, is unknown. Nevertheless, direct observations of the hydrodynamics and fluid mechanics of suspension feeding in intact *P. cataracta* using video endoscopy indicate that marsupial gills continue to participate in particle filtration and transport during periods of larval incubation (Tankersley and Dimock 1993).

Because the rates of water renewal within the mantle cavity determine the availability of particles for capture, the lower clearance rates of brooding females could represent both reductions in the amount of water processed by the gills and modifications of the circulation of water currents within the mantle cavity. Jørgensen et al. (1988) reported that the pumping rate of *Mytilus* is influenced more by valve gape, mantle cavity area, and the geometry of the interfilament canals than by lateral cilia activity. While the specific hydrodynamic consequences of brooding remain unclear, the presence of swollen lateral gills in the infrabranchial cavity alters the patterns of water movement around the frontal surfaces of the ctenidia and retards current flow into the regions surrounding the lateral gills, including the mediolateral cavities between the inner and outer demibranchs (Tankersley and Dimock 1993). Glochidial brooding presumably influences not only the rate at which the gills encounter particles suspended in water transported through the interfilamentar canals but may also affect other potential retention mechanisms, including those acting on particles carried in currents within the mantle cavity (shunt flow), which are thought to enhance particle filtration in *Mytilus edulis* (Famme and Kofoed 1983).

Because the effectiveness of the filters of suspension feeders depends upon the characteristics of the filter and the velocity of flow (Rubenstein and Koehl 1977), differences in the rate at which the gills of brooding *P. cataracta* process water (maximum clearance rate) would influence the efficiency of

particle retention. The majority of filtration studies on both marine and freshwater bivalves report that most species can retain particles greater than 4 μm in diameter with nearly 100% efficiency (Haven and Morales-Alamo 1970; Vahl 1972a, 1972b; Mathers 1974; Jørgensen 1975a; Møhlenberg and Riisgård 1978; Palmer and Williams 1980; Paterson 1984, 1986). However, the retention efficiency spectra of smaller size classes of particles for nonbrooding P. cataracta (Fig. 4) were lower than those reported for most marine species (Jørgensen and Goldberg 1953; Vahl 1972a; Jørgensen 1975b) and for several other unionid bivalves including Elliptio complanata (Paterson 1986), Anodonta cygnea and Unio pictorum (Jørgensen et al. 1984). This discrepancy can probably be attributed to differences in the experimental conditions employed in the various studies (including the nature and concentration of the particles) and in the methods of calculation of retention efficiency rather than fundamental differences in the structure of the gill or presence of laterofrontal cirri (Møhlenberg and Riisgård 1978; Williams 1982).

The lack of an asymptote or any decline in the relative retention efficiency for larger particles by both male and female P. cataracta (Fig. 4) suggests that the largest particles in the suspensions used in the current study (≈10 μm) are near or below the upper size limit that this species is capable of filtering. Furthermore, because filtration rates and retention efficiencies of male P. cataracta did not differ between brooding and non-brooding periods, particle selection in this species apparently is not affected by seasonal changes in the availability of natural seston or by "algal conditioning" as has been suggested for some copepods (Donaghay and Small 1979) and the marine bivalve Crassostrea virginica (Epifanio and Ewart 1977). Therefore, the significant reduction in the ability of female P. cataracta to retain small particles during brooding periods (Fig. 4) indicates that the presence of glochidia within the brood chambers of the outer demibranchs alters the particle-size spectrum that gravid mussels can retain and most likely reflects a temporary distortion in the hydrodynamic mechanisms of suspension feeding.

Although the specific mechanism of particle capture by lamellibranch gills is not completely understood for any species of bivalve mollusc, the laterofrontal cirri may either directly sieve particles from suspension (Silvester and Sleigh 1984) or establish velocity gradients near the surface of the gill which are necessary for extracting particles (Jørgensen 1981, 1982). The beat frequencies of laterofrontal cirri of both brooding and non-brooding P. cataracta (Fig. 6) generally were higher than those reported by Dral (1967) for Mytilus edulis (between 4 and 9 Hz) but were comparable to more recent rates reported for M. edulis by Sanderson et al. (1985) (range 6–14 Hz) who used a slit camera and photodetection technique. The slower beat frequencies of cirri on lateral gills of female P. cataracta throughout the year suggest that sex-specific differences in the architecture of marsupial gills include features that influence the activity of the cirri and ultimately reduce the retention efficiencies of these demibranchs. Dral (1967) reported that the efficiency of retention of small particles by intact young Mytilus edulis was altered by changes in the width of the ostia and shifts in amplitude of the cirral beat, thus increasing the distance between the tips of cirri on adjoining filaments. Similarly, removal of the laterofrontal cirri from the path of the currents entering the interfilamentar space of Mytilus edulis by stopping them in their effective stroke with high concentrations of serotonin results in a significant

reduction in the retention of smaller particles (≤6 μm) (Jørgensen et al. 1986). The lower retention efficiency of smaller particles by M. edulis during spawning is thought to be the result of increased activity in the serotonergic nervous system (Jørgensen et al. 1990). A fuller understanding of the role of laterofrontal cirri in particle retention by P. cataracta will await detailed examination of the spacing, morphology and beat cycle of the laterofrontal cirri of gravid marsupial gills compared with nonmarsupial demibranchs.

Variation in the relative "porosity" of the marsupial demibranchs of brooding female P. cataracta could involve changes in the openings of the ostia and interfilamentar water canals associated with modification of the resistance to flow through the outer demibranchs (Jørgensen et al. 1986). Because marsupial gills may be less efficient at retaining smaller particles when the primary route for water transport is through the smaller secondary water tubes of gravid marsupial demibranchs (Tankersley and Dimock 1992, 1993), the lower retention efficiencies for brooding females may represent a compromise between the demands of suspension feeding and of ventilation of the gills and brood chambers. Comparative studies of particle retention by brooding vs. nonbrooding unionids that utilize all four demibranchs for larval incubation (e.g., Quadrula spp.) or that lack secondary water tubes (e.g., Elliptio and other lampsillines) may reveal even more pronounced functional differences between male and female mussels.

Differences in the activity of the ciliature of brooding demibranchs is further evidenced by the reduction in the speed of particles traveling in frontal currents (generated by the laterofrontal cirri and frontal cilia) on the surface of the gills during brooding and immediately following larval release compared with nonbrooding conditions (Table 6). Significant differences in the rates of change of direction of particles on lateral and medial gills (Table 5) probably reflect pronounced differences in the patterns of frontal currents on the two types of demibranchs (Atkins 1937). While it is unlikely that the slower transport speed of particles on lateral gills would significantly inhibit particle filtration or selection per se, the reduced particle velocity may influence ingestion by limiting the efficiency and rate by which particles are delivered to the palps and mouth. However, the absence of any seasonal difference in the activity of the laterofrontal cirri and of transport of particles on the surface of the nonmarsupial (medial) gills of females indicates that there are no compensatory changes in the physiology of medial demibranchs that might counteract the observed changes in the activity of the ciliature of the lateral gills.

Detailed analysis of the concentrations and size–frequency distributions of natural seston, especially during periods of larval incubation, are essential in order to determine if the observed changes in retention efficiency and filtration rates of females during brooding periods represent real changes in the quality and quantity of food filtered and subsequently ingested. Additional studies are also needed to determine if changes in quantitative filtration (selection and retention based on size) by females are compensated for by changes in qualitative selection (Grave 1916; Loosanoff 1949; Ten Winkel and Davids 1982; Shumway et al. 1985; Ward and Targett 1989), or if female P. cataracta utilize alternative modes of feeding, including the ingestion of substrate particles using pedal ciliary tracts as has been proposed for Corbicula fluminea and Mysella bidentata (Way et al. 1990; Reid et al. 1992) to sup-

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