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Quantitative Analysis of the Structure and Function of the Marsupial Gills of the Freshwater Mussel *Anodonta cataracta*[1]

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Abstract. Gravid females of *Anodonta cataracta* incubate shelled larvae (glochidia) in the water tubes of their outer demibranchs which, in turn, undergo extensive morphological changes in becoming marsupia. In this study, the brooding gills of *A. cataracta* were compared to the non-marsupial demibranchs of females and the gills of males. Scanning electron microscopy and video enhanced light microscopy were used, and computer-generated 3D-reconstructions of gill tissue were also prepared from light micrographs of serial histological sections. Marsupial gills possess a tripartite system of water tubes that are not present in non-marsupial gills and include two secondary water channels and one primary water tube (brood chamber) containing glochidia. The lateral dimension (width) of water tubes of the marsupial gills increases nearly 30-fold during brooding, but the anterior-posterior length of the tubes is unaffected. No apparent changes in the morphology of the non-marsupial inner demibranchs were observed. Glochidia are effectively isolated from the surrounding water by secondary septa, positioned between the primary and secondary water tubes. Secondary septa are present during brooding and immediately after larval release, but are not in evidence among females during non-reproductive periods. Quantification by 3D reconstruction revealed that, although secondary water tubes are smaller than the primary water tubes of non-marsupial gills and non-gravid marsupial gills, collectively they provide about the same cross-sectional area as the primary water tubes that are lost to water transport by occlusion with glochidia. However, considering the fluid dynamics of the ciliary gill pump, net water transport through the lumina of marsupial gills is reduced to only about 16% of that in non-gravid marsupial demibranchs.

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Abbreviations: ANOVA: analysis of variance; AV, arterial vessel; BC, brood chamber; CE, ciliated water tube epithelial cell; F, gill filament; FT, foot; G, glochidium; ID, inner demibranch; IFC, interfilament water canal; ILS, interlamellar septum; LT, lamellar tissue; MP, melting point; N, nerves; O, ostium; OD, outer demibranch; PWT, primary water tube; SEM, scanning electron microscopy; SS, secondary septa; SWT, secondary water tube; 3D, three dimensional; PC1 & PC2, principal components 1 & 2; PCA, principal components analysis; VM, visceral mass.

Introduction

Unlike their marine counterparts, most freshwater bivalve mollusks, including the Sphaeridae and Unionidae, lack a planktonic larva and bypass the trochophore and veliger stages; rather, they incubate their embryos, larvae, or both in their gills. Moreover, the life cycles of the Unionidae are atypical among bivalves in including both a free-living adult and a short-lived obligatory ecto-parasitic larval (glochidial) phase (Coker *et al.,* 1921; Kat, 1984). Following fertilization in the suprabranchial cavity, embryos develop in the water tubes of the female's gills. During reproduction, both outer (lateral) demibranchs of *Anodonta cataracta* (recently reassigned to the genus *Pyganodon* by Hoeh, 1990) serve entirely as a pair of marsupial chambers and undergo pronounced morphological and architectural changes to accommodate nearly a million developing larvae (Fig. 1). *Anodonta cataracta* is a dioecious long-term (bradytictic) brooder that spawns in the late summer, broods throughout the fall and winter, and releases mature glochidia in the early spring (Tankersley, unpub. data).

General descriptions of the gill structure and anatomy of several unionid species, including *A. cataracta,* and the

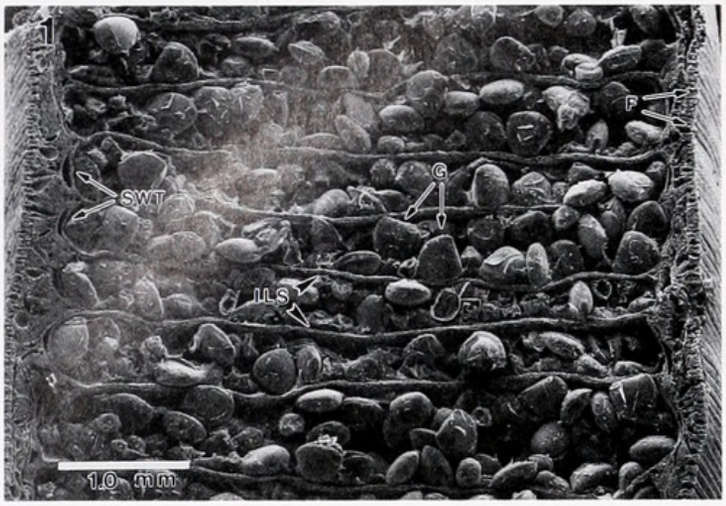


Figure 1. Scanning electron micrograph of a frontal cross section of the marsupial gill of *Anodonta cataracta* showing the position of the glochidia larvae (G) in the brood chambers and the location of the secondary water tubes (SWT) and interlamellar septa (ILS). Additional abbreviations: F, gill filament.

role of demibranchs as sites of larval storage during reproduction have been reported previously (Peck, 1877; Lefevre and Curtis, 1910; Ortmann, 1911; Richard *et al.*, 1991). The non-marsupial gills (outer and inner demibranchs of males and inner demibranchs of females) of *A. cataracta* possess continuous interlamellar septa that run dorso-ventrally at right angles to the gill surface and form evenly spaced, uninterrupted water tubes (Fig. 2) (Ridewood, 1903; Heard and Guckert, 1971). The primary water tubes of the marsupial lamellae are more numerous than those in non-marsupial gills and during gravid periods are divided into three separate compartments: a central brood chamber serving as an ovisac, and two temporary secondary water tubes located on both the lateral and medial ends of the brood chamber parallel to the surface of the demibranchs (Fig. 2). These secondary water tubes are formed from extensions of the interlamellar septa prior to larval incubation, and may be associated with the long brooding period of this species; in particular, they are thought to be responsible for maintaining water transport across the gill surface for respiration, filtration, and aeration of developing larvae (Ortmann, 1911; Heard, 1975; Richard *et al.*, 1991).

Investigations of the flow dynamics associated with ciliary suspension feeding in bivalves (see Silvester and Sleigh, 1984; Jørgensen *et al.*, 1988; Silvester, 1988) have prompted several studies of the functional anatomy and ultrastructure of the bivalve gill (Moore, 1971; Owen, 1974; Way *et al.*, 1989). Although the reproductive cycles and glochidial morphology of a variety of unionaceans have been examined (see references in Kat, 1984, and Gordon and Smith, 1990), few studies have documented the changes in gill morphology associated with brooding (Ortmann, 1911; Bloomer, 1934; Heard, 1975; Richard *et al.*, 1991) or examined the functional role of the secondary water tubes as structures necessary for sustaining water transport through the lateral demibranchs. Most contemporary examinations of unionid gill structure and function have focused upon the role of gills as sites for ion transport (Kays *et al.*, 1990), and as storage areas for extracellular calcium phosphate concretions (Silverman *et al.*, 1983, 1989) used during reproduction for embryonic shell development (Silverman *et al.*, 1985, 1987).

The objective of the present study was to use both scanning electron microscopy and video enhanced light microscopy to quantify the seasonal changes in the morphology of the marsupial gills of *A. cataracta* females and to compare these changes with those in the non-marsupial inner demibranchs, and with the inner and outer demibranchs of males. The results indicate that, although the marsupial gills swell to more than thirty times their non-brooding thickness when the primary water tubes are modified as brood chambers and are subsequently obstructed by incubating larvae, the construction of secondary water tubes partially compensates for the loss of passageways available for water transport. In addition, these data are used to make theoretical predictions and estimates of the influence of larval incubation on the fluid dynamics of the gills and on their conventional roles as feeding and respiratory organs.

Materials and Methods

Collection and maintenance of animals

Adult *Anodonta cataracta* were collected from Spea's Pond, Yadkin County, North Carolina, and were maintained at ambient collection temperatures in glass aquaria containing artificial pond water (Dietz and Alvarado, 1970). All mussels were sexually mature (average shell length = 12.8 cm; range: 11.2–14.0 cm), were kept on a 12L:12D photoperiod for up to 10 days prior to use, and their collections were scheduled to coincide with pregravid (early July), gravid (October and December), and post-glochidial release (late February) periods. The sex ratio of mussels in the pond was nearly 1:1, and 100% of the females collected during brooding periods possessed gravid marsupia.

Preparation of gills for light microscopy and computerized analysis of serial sections

Lateral and medial gill tissues (approximately 4 cm^2) for histological examination by video enhanced light microscopy were excised from the central part of their respective demibranchs and fixed in Tissue-Fixx (Lerner Laboratories) for 72 h. Specimens were decalcified in Cal-Ex (Fisher Diagnostics) for 24 h, dissolving larval shell

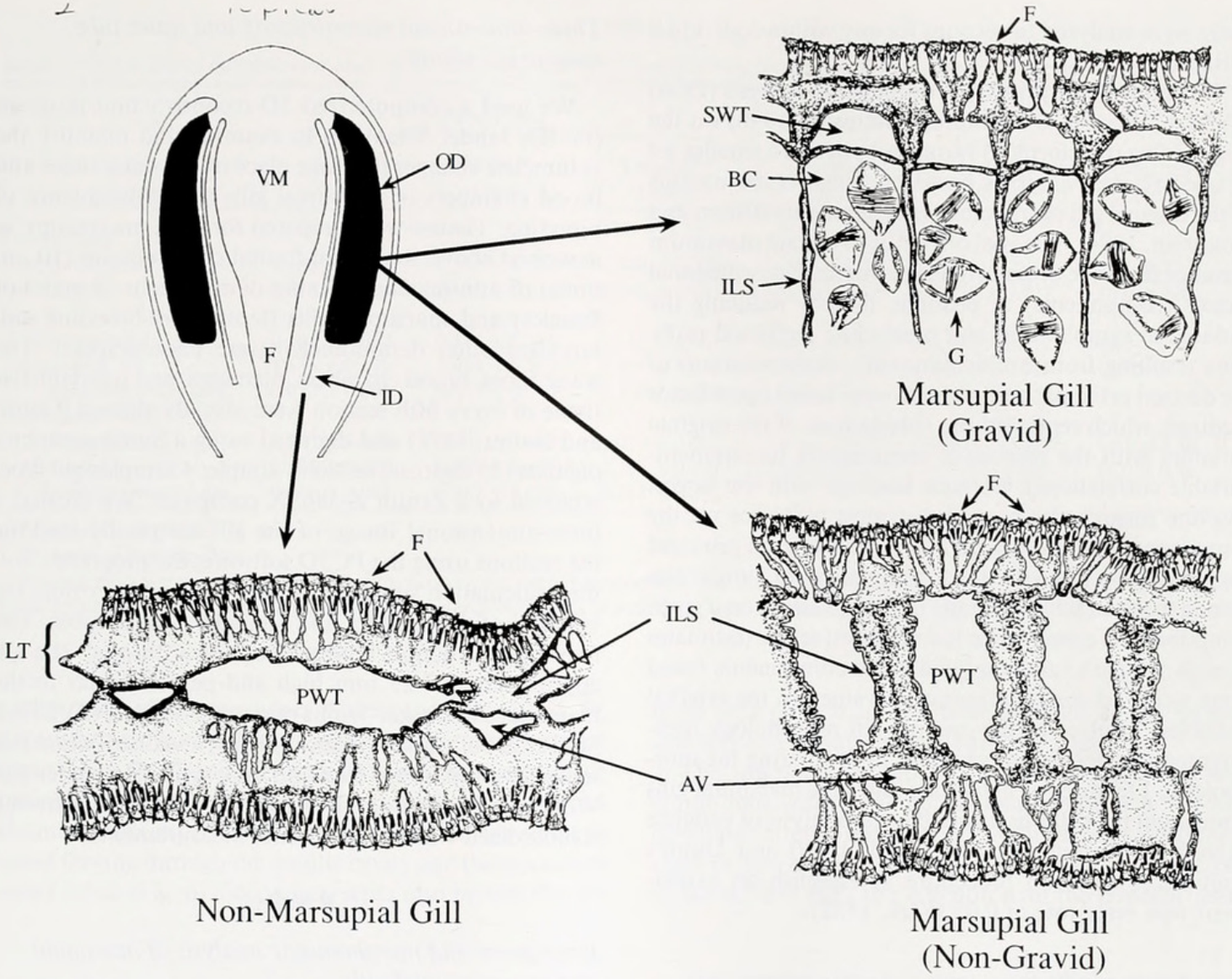


Figure 2. Schematic illustration of a cross section through a gravid female *Anodonta cataracta* showing the position of the lateral and medial demibranchs, and the arrangement of the lamellar tissue (frontal sections) of non-marsupial and marsupial demibranchs during gravid and non-gravid periods. Abbreviations: AV, arterial vessel; BC, brood chamber; F, gill filament; FT, foot; G, glochidium larvae; ID, inner demibranch; ILS, interlamellar septum; LT, lamellar tissue; OD, outer demibranch; PWT; primary water tube; SWT, secondary water tube; VM, visceral mass.

and extracellular calcium concretions that might have interfered with sectioning (Silverman *et al.*, 1985; Richard *et al.*, 1991), and were then dehydrated in ethanol and embedded in paraffin (Paraplast; MP 56°C) by vacuum infiltration (Lipshaw Manufacturing Co.). Serial frontal sections (7–8 μm thick) were mounted on glass slides and stained with hematoxylin and eosin according to the procedures outlined in Humason (1979).

Morphometric measurements ($\pm$2.0 μm for linear and 4.0 μm^2 for area measurements) included the area (frontal cross section), length (maximum anterior-posterior axis distance), and width (maximum left-right axis distance) of the primary water tubes (brood chambers in gravid and post-release marsupial gills) and secondary water tubes (gravid and post-release marsupial gills only); the gill thickness (maximum distance between the filaments of opposing lamellae); the thickness of lamellar tissue (maximum distance from the base of filaments to the lumen of the primary or secondary water tubes); and the number of filaments per interlamellar septum (including filaments present on both ascending and descending lamellae). These measurements were made with an Image-1 Video Image Analyzer (Universal Imaging Corp.) and a Hamamatsu C2400 video camera and Javelin color video camera attached to a Zeiss Axiophot microscope and a Nikon SMZ-2T dissection microscope, respectively. Three sets of measurements on every fourth section, for a total of 12 sets per specimen (5–6 specimens/sex/collection pe-

riod), were analyzed to account for any within-individual variation.

We performed a principal components analysis (PCA) (SYSTAT Statistical Software; Wilkinson, 1990) on the original (log transformed) variables to derive a smaller set of uncorrelated variables based on linear combinations of the original gill morphology measurements (Dillon and Goldstein, 1984). The goal of PCA is to extract maximum variance from the original data set with as few orthogonal factors (components) as possible, thereby reducing the variable to sample ratio and precluding statistical problems resulting from multicollinearity. Interpretations of the derived principal components were based upon factor loadings, which represent the correlations of the original variables with the respective components (component-variable correlations). Because loadings with the largest absolute magnitudes have the greatest influence on the components, the subsequent description of each principal component was based upon an appraisal of similarities among those variables with the highest loadings on a given component. We used factor (component) scores (estimates of each sample's value on the derived components based upon weighted combinations of its values on the original variables) in place of the original gill morphology measurements as dependent variables in comparing the morphological features of marsupial and non-marsupial gills throughout the collection period using analysis of variance (ANOVA) (Tabachnick and Fidell, 1989) and Dunn's multiple comparison procedure to establish an experiment-wise error rate of 0.05 (Kirk, 1982).

Preparation of gills for scanning electron microscopy (SEM)

Dissected gill specimens for scanning electron microscopy were fixed in 2% glutaraldehyde in 0.2 *M* Sorenson's sodium phosphate buffer (pH 7.2) at 4°C for 2 h, post-fixed in 2% cacodylate buffered (pH 7.4) osmium tetroxide for an hour, and rinsed with several changes of buffer. A vibratome (Lancer Model 1000) was used to section the gill specimens into 2–8 mm thick segments that were either parallel or perpendicular to the dorsal-ventral axis. This procedure exposed the frontal surface of the gill lamellae and allowed us to examine the arrangement and morphology of the water tubes and the position of the glochidia in the brood chambers. Specimens were later dehydrated through a graded ethanol series, dried in a Pelco CPD-2 critical point drier, mounted on aluminum SEM stubs, and sputter-coated with gold-palladium (Pelco Model SC-4). External gill features, primary and secondary water tubes, brood chambers, and lamellar tissues exposed by sectioning were examined and photographed with a Philips 515 scanning electron microscope operating at 15 kV.

Three-dimensional reconstruction and water tube volume calculation

We used a computerized 3D-reconstruction program (PC3D, Jandel Scientific) to examine and quantify the volumetric changes that take place in the water tubes and brood chambers of marsupial gills as a consequence of brooding. Tissues were prepared for light microscopy as described above, and serial frontal cross sections (10 μm thick) of non-marsupial (inner demibranchs of males or females) and marsupial gills (female pre-brooding and brooding outer demibranchs) were photographed. The water tubes, brood chambers, filaments, and interlamellar tissue of every fifth section were visually aligned (Gaunt and Gaunt, 1978) and digitized using a Summagraphics digitizer (25 digitized sections/sample; 4 samples/gill type) attached to a Zenith Z-386SX computer. We created a three-dimensional image of the gill sample by stacking the sections using the PC3D software; the program's volume calculation subroutines were used to determine the volumes of the primary and secondary water tubes. The final reconstruction represented a slice through the gill approximately 1.25 mm high and perpendicular to the filaments. A Kruskal-Wallis one-way ANOVA (SYSTAT Statistical Software; Wilkinson, 1990) and distribution free multiple comparisons based on rank sums (Hollander and Wolfe, 1973) were used to test for differences between standardized water tube volume measurements.

Results

Description and morphometric analysis of marsupial and non-marsupial gills

The nomenclature and terminology used to describe the gills of *A. cataracta* in the present study are similar to those of previous descriptions of lamellibranch gills by Ridewood (1903) and Ortmann (1911). Compared to non-marsupial demibranchs, the marsupial gills of all female mussels collected throughout the study were subdivided by additional interlamellar septa, resulting in shorter (anterior-posterior axis) water tubes and a lower mean filament/septum ratio (15.2 *vs.* 48.9) (Table I). Water tubes (brood chambers) containing larvae were swollen to more than 30 times their original non-brooding width (medial-lateral axis), producing nearly a 24-fold increase in cross-sectional area and causing the ventral edge of the demibranch to expand into a thin, non-ciliated connection between opposing lamellae. Conversely, brooding had little effect on the spacing and length (anterior-posterior distance) of the water tubes of marsupial gills (Table I). The lamellar tissue of marsupial gills was slightly thinner than in non-marsupial gills, especially during periods of larval incubation, but still possessed well-developed interfilament water canals leading to ostial openings in the la-

Table I

Results (mean ± SE) of morphometric analysis of male and female demibranchs during brooding and non-reproductive periods

Collection period & gill type	Water tube Area (mm^2)	Water tube Width* (mm)	Water tube Length** (mm)	Gill thickness (mm)	Lamellar thickness (mm)	Filaments/ Septum	n
Pre-brooding							
Non-marsupial	0.158 ± 0.021	0.193 ± 0.017	1.028 ± 0.078	0.974 ± 0.034	0.385 ± 0.018	46.5 ± 3.12	15
Marsupial	0.078 ± 0.014	0.135 ± 0.021	0.548 ± 0.041	1.052 ± 0.039	0.273 ± 0.027	16.0 ± 0.89	5
Gravid							
Non-marsupial	0.193 ± 0.022	0.186 ± 0.015	1.153 ± 0.061	0.840 ± 0.033	0.333 ± 0.012	46.2 ± 1.68	32
Marsupial	1.846 ± 0.179	3.938 ± 0.205	0.457 ± 0.032	4.550 ± 0.187	0.186 ± 0.205	13.7 ± 0.77	10
Secondary water tube	0.020 ± 0.005	0.063 ± 0.010	0.370 ± 0.026				
Post-release							
Non-marsupial	0.233 ± 0.032	0.267 ± 0.032	1.077 ± 0.055	1.130 ± 0.076	0.391 ± 0.032	51.1 ± 2.46	15
Marsupial	0.185 ± 0.050	0.768 ± 0.169	0.298 ± 0.097	1.589 ± 0.075	0.240 ± 0.169	16.0 ± 0.86	5
Secondary water tube	0.011 ± 0.002	0.082 ± 0.010	0.215 ± 0.032				

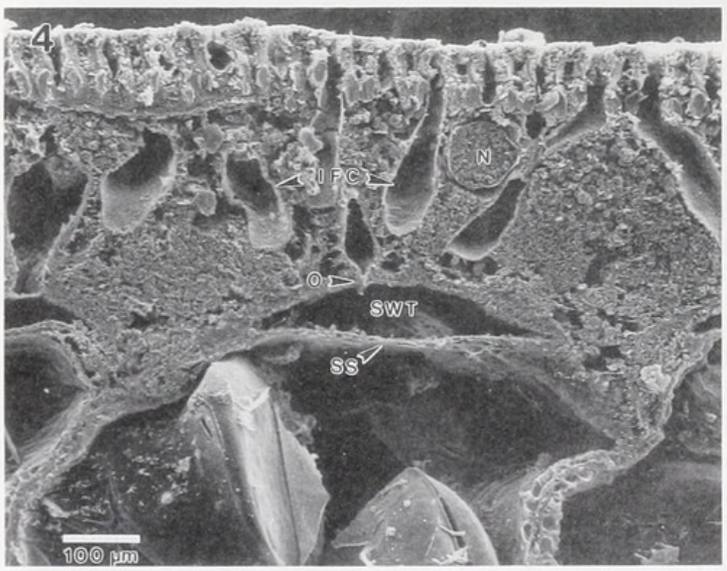
* Medial-Lateral axis.

** Anterior-Posterior axis.

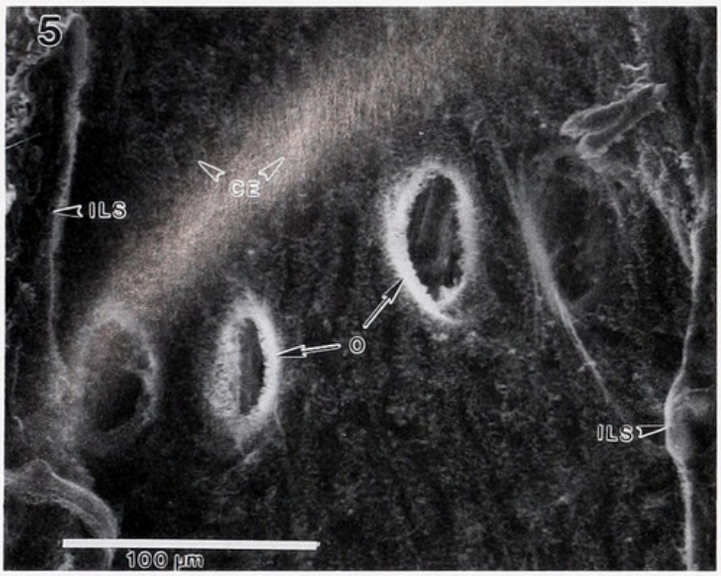
Because there were no significant differences between the respective morphometric characters of all non-marsupial demibranchs, the data for the inner demibranchs of females, and the inner and outer demibranchs of males, for each collection period have been pooled.

mellar walls of the secondary water tubes (Figs. 3, 4, 5). Secondary septa were continuous with the interlamellar septa (Figs. 3, 4) and lacked any apparent openings or ostia leading from the secondary water tubes to the brood chambers, effectively isolating the developing larvae from water flowing through the mantle cavity and the secondary water tubes (Fig. 6). Secondary septa also lacked the ciliated cells present on the lamellar walls of the primary and secondary water tubes (compare Figs. 5, 6).

The interlamellar septa of non-marsupial gills were continuous with the lamellar tissue and contained well-developed arterial vessels (Fig. 7). Comparable vessels were rarer in marsupial gills and were positioned at the base of the septum near the junction with the lamellar tissue.



Figures 3 & 4. Frontal section of a marsupial demibranch (only one lamella is shown) containing mature glochidia (G). Brood chambers (BC) are separated by thin interlamellar septa (ILS) which connect the ascending and descending sides of the demibranch. Figure 4 is a higher magnification view of the highlighted area in Figure 3 showing the position of the secondary septa (SS) forming the secondary water tubes (SWT). Well-developed interfilament canals (IFC) are located between adjacent filaments (F) and lead to ostial openings in the lamellar tissue (walls) of the water tubes. Nerves (N) located in the lamellar tissue are also present.



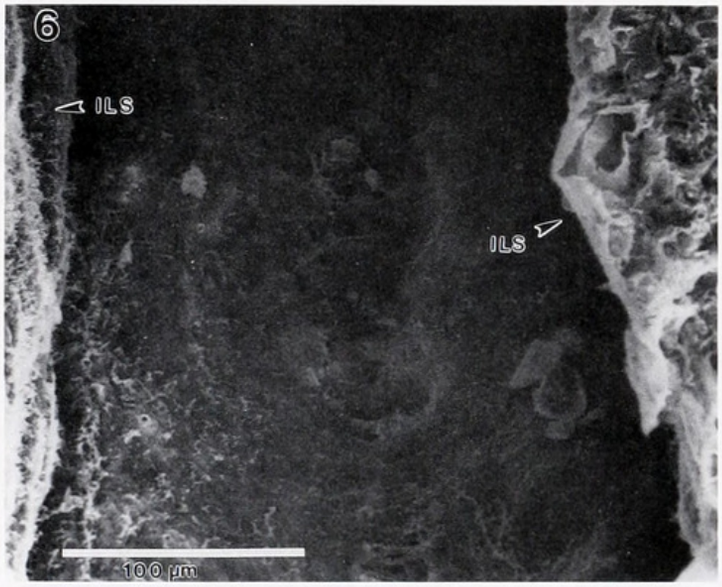


Figure 5. Scanning electron micrograph of the inner lamellar surface of a secondary water tube of a gravid marsupial gill of *Anodonta cataracta*. Water pumped through the interfilament canals by lateral cilia enters the secondary water tubes through well-defined ostia (O). Lamellar walls consisted primarily of ciliated epithelial cells (CE). The edges of the interlamellar septa (ILS) connecting the opposing lamellae and forming the anterior and posterior walls of the secondary water tube are also visible.

Figure 6. Lateral view of the inner surface (brood chamber side) of a secondary septum of a gravid marsupial demibranch (glochidia removed). Note the absence of ciliated epithelial cells and ostia (see Fig. 5) leading from the secondary water tubes. Secondary septa are formed by outgrowths of the interlamellar septa (ILS) prior to larval incubation.

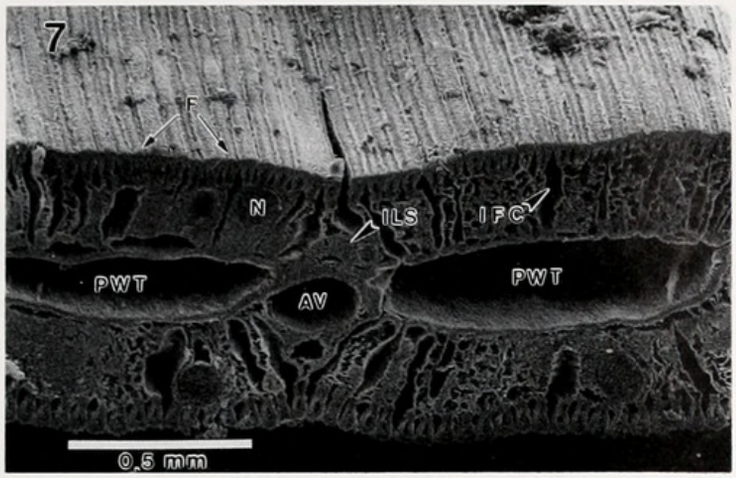
Distended ovisacs and numerous secondary water tubes were still present in the gills of females collected just after glochidial release (Fig. 8), but were not present in gills prior to brooding (Fig. 9). Brooding had no apparent effect on the presence or distribution of frontal cilia or laterofrontal cirri, which were visible on the surface of the gill, and all gills lacked the frontal cirri recently reported found in some other freshwater species (Way *et al.*, 1989). Moreover, there were no seasonal differences between males and females in the morphology of their non-brooding demibranchs.

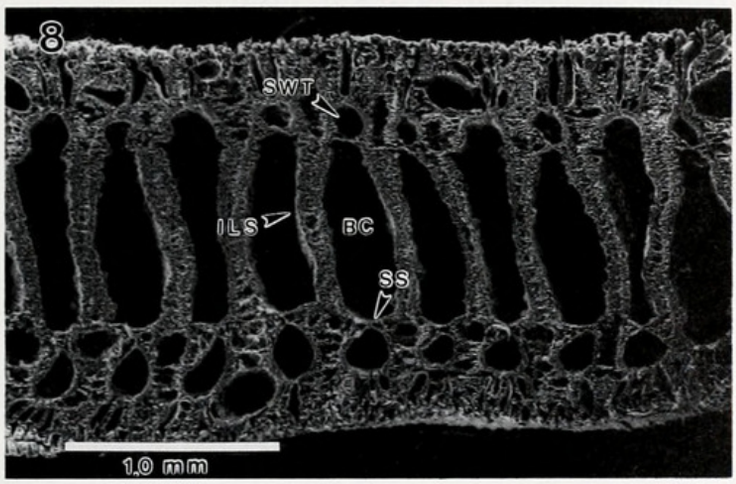
Principal component analysis on the six gill morphology variables resulted in two components (PC1 & PC2) being retained (eigenvalues > 1) that explained approximately 89% (PC1 = 49%; PC2 = 40%) of the total variance. An orthogonal rotation (varimax) was performed on the extracted factors (components) to improve their interpretability while still maintaining independent factor scores. Water tube area and width and gill thickness all had high loadings on PC1 and represented morphological features associated with the gill's left-right axis dimension (Fig. 10). The remaining three variables, water tube length, filaments/septum ratio, and lamellar tissue thickness, had high loadings on PC2 and characterize features associated with the arrangement, spacing, and number of water tubes. Therefore, PC1 and PC2 were respectively labeled "left-right axis thickness" and "water-tube compactness."

The mean factor scores for both PC1 and PC2 for each type of gill are plotted in Figure 11. There were no significant differences between the inner and outer demibranchs of males (PC1: $F = 5.03$; PC2: $F = 4.58$; d.f. = 1, 20; $P > 0.05$) or between the inner demibranchs of males and females (PC1: $F = 7.63$; PC2: $F = 4.83$; d.f. = 1, 39; $P > 0.05$). Because there were no seasonal differences in the factor scores of any of these non-marsupial gills (PC1: $F = 1.28$; PC2 = 1.36; d.f. = 2, 59, $P > 0.05$), all scores for each type of gill were pooled to simplify the analysis. Marsupial gills containing larvae had higher PC1 factor scores than pre- or post-brooding gills ($F = 81.40$; d.f. = 2, 17; $P < 0.01$). The arrangement of the water tubes (PC2) of marsupial gills differed significantly from that of non-marsupial gills ($F = 70.22$; d.f. = 1, 36; $P < 0.01$) but remained consistent throughout the collection period (*i.e.*, exhibited no significant seasonal variation; $F = 2.57$; d.f. = 2, 17; $P > 0.05$).

3D reconstructions and comparison of primary and secondary water tube volumes

The mean volumes of 1-mm sections of each type of water tube are listed in Table II. Because the total number of tubes present in each demibranch varied with the type of gill (marsupial or non-marsupial) and the mussel's reproductive condition (*i.e.*, marsupial gills had two secondary water tubes/septum during brooding periods), the volume measurements are also expressed as the volume of water tube/100 gill filaments (counted as filaments present on both the ascending and descending lamellae). Although the volume of the primary water tubes of non-marsupial gills, expressed as ml/mm of gill tissue, was significantly larger than that of either the primary or secondary canals of marsupial gills ($H = 9.85$; d.f. = 2; P





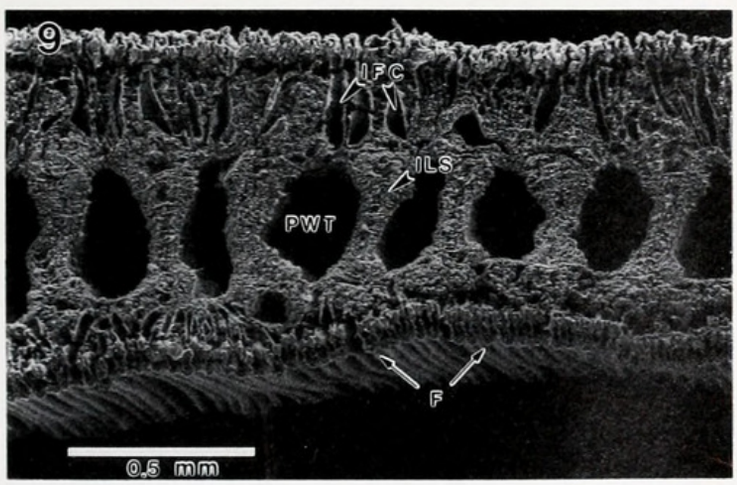


Figure 7. Frontal view of the lamellar surface of a non-marsupial demibranch. Water pumped by lateral cilia present on the gill filaments (F) enters the interfilament canals (IFC), which empty into the primary water tubes (PWT). Arterial vessels (AV) are located between water tubes in the interlamellar septa (ILS). Nerves (N) situated in the lamellar tissue are also visible.

Figure 8. Frontal section of a distended post-brooding marsupial demibranch showing the empty brood chambers (BC) and the stretched interlamellar septa (ILS). Most brood chambers still possessed well-developed secondary septa (SS) forming secondary water tubes on both the lateral and medial sides of the gill, but some secondary septa are lost by this post reproductive stage.

Figure 9. Representative section through a non-gravid marsupial gill showing the crowded organization of the water tubes (PWT). Following larval release, secondary septa and water tubes disappear, the interlamellar septa (ILS) become thickened, and the interfilament water canals (IFC) channel water through ostia located in the walls of the primary water tubes.

< 0.01), differences in water tube volumes standardized by filament number were not significant ($H = 4.88$; d.f. $= 2$; $P = 0.09$). These data suggest that, although the secondary water tubes of marsupial gills were significantly smaller than the primary water tubes of non-marsupial gills, the tripartite arrangement of the brooding demibranchs partially compensated for the blockage of the brood chambers by developing larvae by supplying approximately the same total volume for irrigation.

Discussion

Ortmann's (1911) early descriptions of the anatomical features of the gills of *Anodonta* emphasized both the tripartite morphology of the marsupia during reproductive periods, and the compact arrangement of their primary water tubes, relative to the inner demibranchs of females and all four demibranchs of males. The permanent differentiation in the architecture of the marsupial gills of *A. cataracta* is represented by the second principal component (PC2) in the current study and remains one of the few sexually dimorphic features of this species. The majority of changes in gill morphology associated with larval incubation occurred within the left-right axis (represented by PC1) but were only transient changes associated specifically with brooding. The unidirectional swelling of the outer gill was accompanied by comparable alterations in the size of the water tube walls and interlamellar septa, but no changes in the spacing or arrangement of the filaments. Furthermore, the presence, in gravid marsupial gills, of well-developed interfilament water canals leading to the secondary water tubes suggests that the marsupial gills continue to transport water and presumably filter particles despite striking changes in their morphology. Brooding caused no corresponding changes in female medial gills, such as an increase in water tube area, that might offset changes in the lateral marsupial demibranchs.

Overall, the morphometric measurements reported in the present study probably represent conservative estimates of gill alterations associated with brooding, because sample preparation, including fixation and dehydration, caused some shrinkage of tissue (Humason, 1979; Gabriel, 1982). Furthermore, water tube measurements may only approximate *in vivo* conditions, because pressure differences maintained by the cilia as they pump water between the mantle cavity and the lumen of the demibranch cause the demibranchs and water tubes to be inflated compared to newly excised tissue (Jørgensen *et al.*, 1986).

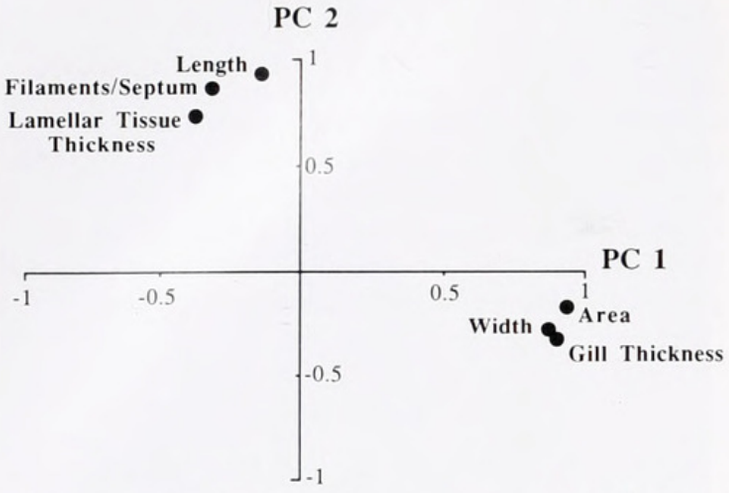


Figure 10. Pairwise plot of the factor loadings (PC1 & PC2) for the six morphometric variables following orthogonal (varimax) rotation.

Many taxonomic schemes established for unionid mussels (for example Ortmann, 1911, and Heard and Guckert, 1971) rely heavily upon reproductive characteristics associated with the marsupial demibranchs, including the number (2 or 4) and location (inner or outer) of the marsupia, the proportion of the demibranch used for brooding, the location of developing larvae within the gills, the arrangement of the brood chambers including the presence of secondary water canals, the magnitude of swelling of the lamellae, and the duration of the incubation period. The bradytictic, ectobranchous, tripartite marsupial arrangement of anodontine mussels, including *A. cataracta,* is considered to be more specialized ("advanced") than that of other mussels, which characteristically have shorter incubation periods, more marsupial demibranchs (tetragenous), and no secondary water tubes (Ortmann, 1911; Heard and Guckert, 1971). The tripartite arrangement of the marsupia of anodontine mussels has long been linked to the lengthy incubation phase of their breeding cycle, because it permits isolation of larvae while providing passageways for the maintenance of water transport. However, the assumption by most researchers, including most recently Richard *et al.* (1991), that secondary water tubes serve as lumina for irrigation during brooding periods has not been confirmed.

Construction of temporary secondary septa and a thin membrane at the dorsal end of the ovisacs of the lateral gills of female *A. cataracta* provides formal barriers to the circulation of water from the mantle cavity through the marsupial gills and effectively isolates and protects the larvae from the surrounding medium. Larval isolation has also been documented in unionids that lack secondary septa, including members of the Lampsilinae, and is thought to be accomplished by the contraction of the ostia that lead from the interfilamentar canals which, in turn, restricts the flow of water into the water tubes (brood chambers) (Richard *et al.,* 1991). Transport of water in these species is probably limited to only the demibranchs or portions of demibranchs not containing glochidia. The mechanism by which tetragenous mussels sustain water transport during brooding is less obvious, because all four demibranchs are used for larval incubation and retain the marsupial morphology. In lampsilines, only a portion of the gill is used for brooding; the remainder possesses water tubes that are similar to those of non-marsupial demibranchs. This may be an alternative mechanism for satisfying the conflicting demands of water transport required for filtration, respiration, and larval incubation, and more specialized than the tripartite arrangement of *A. cataracta* (Heard and Guckert, 1971; Kat, 1984).

In addition to serving as shelters for larval development, brood chambers and the glochidial isolation they provide might also facilitate the transfer of nutrients from the female to the developing larvae. As reported for other unionids (Heard, 1975; Silverman *et al.,* 1987; Richard *et al.,* 1991), the epithelia of the secondary and interlamellar septa of *A. cataracta* lack openings or ostia leading to the secondary water canals and thereby limit direct nutrient or ion exchange with external pond water. Investigations of the maternal investment in larval nutrition and development have been restricted primarily to examinations of the use of maternal calcium reserves for the formation of larval shells (Silverman *et al.,* 1985, 1987). Although the mobilization of calcium concretions in the gills of females and their subsequent incorporation in the shells of brooded embryos is well documented, the mechanism of transfer is still unknown. Wood (1974) re-

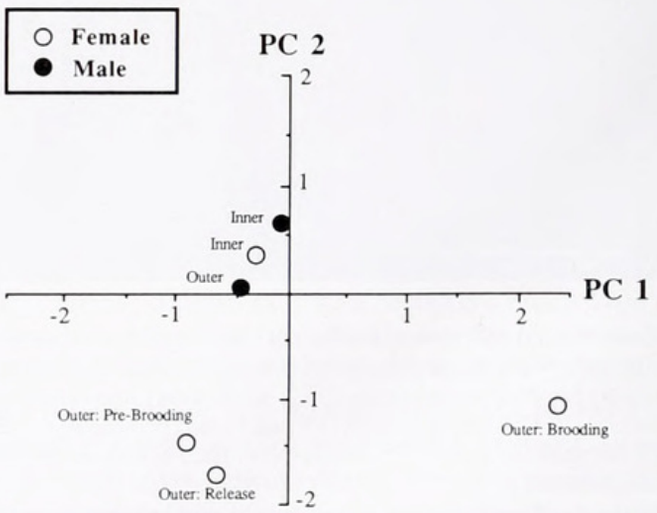


Figure 11. Pairwise plot of the mean factor scores (see Fig. 10: PC1 describes changes in the lateral-medial axis of the demibranchs and brood chambers; PC2 describes the number and arrangement of water tubes and filaments) for male and female inner and outer demibranchs throughout the study. Because there were no significant differences in the factor scores of female inner demibranchs and male inner and outer demibranchs among the collection periods, the scores for each type of gill were pooled to simplify the analysis.

Table II

Water tube volume calculations (mean ± SE; n = 4) based upon 3D reconstructions of non-marsupial and marsupial gills of Anodonta cataracta *during brooding and pre-brooding (non-gravid) periods*

	Non-marsupial gill	Marsupial gill		
		Pre-brooding	Brooding	
	PWT	PWT	BC	SWT
ml/mm of gill ($\times 10^{-5}$)	17.3 ± 2.0	5.3 ± 0.8	161.1 ± 23.6	1.5 ± 0.18
ml/mm/100 filaments ($\times 10^{-5}$)	35.8 ± 4.2	35.6 ± 5.4	1090 ± 158	20.5 ± 2.39*

* Includes two secondary water tubes per brood chamber.

Abbreviations: PWT, primary water tube; BC, brood chamber; SWT, secondary water tube.

ported that gravid *Anodonta cygnea* that were fed ^{14}C-labelled algae incorporated significant concentrations of the label in both the glochidia and interlamellar septa; Wood also suggested that nutrients could have been transferred from the female to the developing larvae via mucus secreted by cells located in the interlamellar septa. Although the lateral swelling of marsupial gills and the packing of the brood chambers with larvae may isolate many of the glochidia from direct contact with the lateral and medial inner surfaces of the gill, the narrow (anterior-posterior dimension) arrangement of the individual brood chambers of *A. cataracta* keeps the larvae in close contact with the interfilamentar septa and may facilitate the transfer of nutrients from the female to developing larvae.

Gill irrigation and suspension feeding in bivalves are dominated by the viscous forces characteristic of low Reynolds numbers (<1), producing a laminar flow of water through the demibranchs (Jørgensen, 1982, 1983). The pump, generated by the beating of lateral cilia present on the gill filaments near the entrance to the interfilament water canals, is influenced by, among other parameters, the velocity of water passing through the interfilament water canals and pressure heads produced by the lateral cilia (Jørgensen *et al.*, 1988). Jørgensen *et al.* (1986) established the following equation for analysis of pump and system characteristics:

$$\Delta H_p = \Delta H_{12} + \Delta H_f + \Delta H_{ex} + \Delta H_{lf}$$

where ΔH_p = pressure difference produced by the pump, ΔH_{12} = back pressure, ΔH_f = frictional resistance of the canal system (including the interfilamentary water canals, the water tubes and exhalent siphon), ΔH_{ex} = exit loss generated by the kinetic energy of the water leaving the exhalent siphon, and ΔH_{lf} = active resistance produced by the beating of the latero-frontal cirri located on the gill filaments. Indirect estimates of the various components of the ciliary pump of the marine mussel *Mytilus edulis* revealed that interfilament canals constituted about 32% of the total resistance in the system (Jørgensen *et al.*, 1986), but the frictional resistance produced by the lumen of the water tubes was assumed to have only a negligible effect on the pump. Although the morphology of the eulamellibranch gill of *A. cataracta* differs markedly from the filibranch gill of *Mytilus*, and detailed comparisons of the properties and energetics of the ciliary pumps of both types of gills are unavailable, larval incubation may have a greater impact on the molluscan pump of brooding eulamellibranchs than is indicated by the current simplified model based on non-brooding, filibranch bivalve characteristics.

Because the flow resistance of a fluid passing through a cylinder is extremely sensitive to reductions in bore size (Vogel, 1981), the use of a series of smaller diameter tubes (secondary water tubes *vs.* primary water tubes) by gravid marsupial gills most likely has a significant impact on the resistance to flow and the cost of pumping. If the primary and secondary water tubes are treated as a series of parallel cylinders, according to Poiseuille's equation, volume flow rate would vary with the fourth power of the tube's radius. Therefore, even if the combined cross-sectional area of the two secondary water tubes were equal to that of a single primary water tube during non-reproductive periods, the overall flow rate in the two smaller tubes would be only one-half that of the larger tube for a given pressure change (Vogel, 1981). Consequently, estimates of flow rates through secondary water tubes based upon the present volume calculations (Table II) would predict that the total flow in marsupial gills during brooding would only be approximately 16% and 4% of that in primary water tubes of non-gravid marsupial and non-marsupial gills, respectively.

Maintenance of flow rates through the secondary water tubes that are comparable to those through unobstructed primary water tubes also would be energetically costly. Because the power required to generate flow through a cylinder is inversely related to the square of its radius, the pressures that would be required to irrigate the smaller diameter secondary water tubes likely exceed the capa-

bility of a ciliary pump (Jørgensen *et al.*, 1986). Thus, the flow rates within brooding marsupial gills are probably much lower than those produced within non-gravid marsupial gills or non-marsupial gills, even though the volume of lumina available for water transport is compensated for by the construction of the secondary water tubes. Changes in total gill volume (swelling during brooding) that modify the flow dynamics within the mantle cavity and additionally restrict water transport, may further reduce the effectiveness of the marsupial gill. It is unclear whether the extensive reorganization of marsupial gill tissue following larval release permits the demibranchs to assume functional characteristics of non-marsupial gills after the brooding season. Investigations are currently underway in our laboratory to assess the impact of larval incubation on the pumping and feeding physiology of *A. cataracta*.

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