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# Anatomical descriptions and comparison of the reproductive tracts of *Utterbackia imbecillis* and *Villosa iris* (Bivalvia: Unionidae)

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### Summary

The reproductive tracts of dioecious *Villosa iris* (Lea, 1829) and hermaphroditic *Utterbackia imbecillis* (Say, 1829) are described and compared in sections of viscera examined with light and transmission electron microscopy. In both species, gametes are transported via ciliated gonoducts to a collecting gonosinus, connected to a ciliated genital duct by a highly rugated and musculaturized gonopore. This gonopore may be sphinctural and important for retaining gametes in the gonosinus for timely release. The genital duct is separate from the kidney and leads to the suprabranchial chamber. Epithelia of the gonoducts and gonosinuses of *U. imbecillis*, but not *V. iris*, contain secretory cells whose granules stain positive with periodic acid/Schiff (PAS), indicating the presence of carbohydrates or glycoproteins. Examinations of reproductive tracts of other species revealed that those of *Elliptio complanata* (Lightfoot, 1786), *Lexingtonia dolabelloides* (Lea, 1840), *Villosa vanuxemensis* (Lea, 1838), and *Lampsilis fasciola* Rafinesque, 1820 are similar to those of *V. iris* and *U. imbecillis*, except that only the tracts of *U. imbecillis* contain PAS-positive secretory cells. The gonoducts and gonosinuses of all species examined had an abundance of cilia and microvilli. We hypothesize that the numerous microvilli in the reproductive tracts may indicate intra-luminal regulation of important ion and glucose concentrations, and that the PAS-positive secretions of *U. imbecillis* may temporarily inhibit self-fertilization.

***Key words***: Unionidae, reproductive tract, hermaphroditism, reproduction

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## Introduction

Approximately 72% of the 297 species and subspecies of North American freshwater mussels (Unionidae and Margaritiferidae) have been classified as extinct, endangered, threatened, or of special concern (Williams et al., 1993). Declines in species richness and abundance of this faunal group during the past century have been attributed to habitat alteration, commercial harvest, competition from the exotic zebra mussel, *Dreissena polymorpha* (Pallas, 1771), and pollution (Bogan, 1993; Ricciardi, 1998; Neves, 1999). As part of the National Strategy for the Conservation of Native Freshwater Mussels, the National Native Mussel Conservation Committee (1998) identified development of technologies and protocols for captive holding and breeding of threatened and endangered mussels as a priority for mussel conservation. Neves (1997) also identified captive propagation as important to the future of conservation and recovery of this faunal group.

Despite these priorities, little is known of the effects of captivity and holding conditions on gametogenesis in unionid mussels. Gametogenesis in wild mussels has been histologically assessed by various methods to determine timing of reproductive cycles and provide evidence for gamete production and development within acini (Peredo and Parada, 1986; Jirka and Neves, 1992; Woody and Holland-Bartels, 1993; Haggerty et al., 1995; Garner et al., 1999), but a basic understanding of the reproductive anatomy is lacking. Most descriptions of the reproductive tracts of unionid mussels have stemmed from observations during dissections. McMahon and Bogan (2001) stated that spermatozoa are transported from acini to the water column through the exhalant aperture, and fertilized oocytes are delivered to the gills (demibranchs) where they develop as larval glochidia. Heard (1992) reported that gametes are delivered to the suprabranchial chamber via two gonopores, each near the viscera-gill axes of the right and left inner demibranchs. Mackie (1984) described a common gonoduct for transporting both oocytes and spermatozoa to the suprabranchial chambers in hermaphroditic unionids — seven such species of Unionidae have been reported (Sterki, 1898a, 1898b; Bloomer, 1930; Heard, 1970; Smith, 1982; Kat, 1983). However, no comprehensive, histologically-based assessment of unionid reproductive tracts has been published.

The objective of this study was to provide descriptions and comparisons of the reproductive tracts of the dioecious rainbow mussel, *Villosa iris* (Lea, 1929) and hermaphroditic (monoecious) paper pondshell, *Utterbackia imbecillis* (Say, 1829). Our findings are based on observations of histologically-prepared tissues using light and transmission electron microscopy. Descriptions of reproductive anatomy can stimulate future research on reproductive physiology, non-lethal collection of mature gametes for evaluations and experiments, and can help identify cell types associated with the reproductive tracts that may be linked to gamete quality and fertility.

## Materials and Methods

Twelve female and 15 male *V. iris*, and 15 *U. imbecillis* were collected and placed on ice prior to removal of their viscera, which were fixed in chilled, neutral buffered formalin, and processed for thick sectioning (5 µm) and light microscopy. Mean shell lengths of fixed mussels were 38.8 mm (SD = ±4.5), 46.6 mm (±2.4), and 70.7 mm (±3.0) for female and male *V. iris* and *U. imbecillis*, respectively. Before embedding in paraffin, the maximum sagittal width (mm) of each visceral mass was recorded. Nine sections were obtained at approximately regular spatial intervals, including 10 through 90% of the maximum sagittal widths. Sections at 10% of the visceral width were nearest the left valves. For histological comparisons to tissues of *V. iris* and *U. imbecillis*, archived thick sections were examined from previously collected tissues of eastern elliptios, *Elliptio complanata* (Lightfoot, 1786), slabside pearlymussels, *Lexingtonia dolabelloides* (Lea, 1840), wavyrayed lampmussels, *Lampsilis fasciola* Rafinesque, 1820, mountain creekshells, *V. vanuxemensis* (Lea, 1838), Tennessee clubshells, *Pleurobema oviforme* (Conrad, 1834), and threeridges, *Amblema plicata* (Say, 1817). All tissues presented in light microscopy figures were stained with hematoxylin and eosin (H & E), unless otherwise noted. Positive staining with periodic acid/Schiff (PAS) indicates the presence of carbohydrates or glycoproteins (Cook, 1990).

Specimens of *V. iris*, *V. vanuxemensis*, *L. fasciola*, *L. dolabelloides*, and *P. oviforme* were collected in May and June 1999, and August 2002 and 2003 from the North Fork Holston River, Smyth and Washington counties, Virginia; *U. imbecillis* were collected in February 2000 from Haleyville City Lake, Winston County, Alabama; *E. complanata* were collected in August 1997 from the Nottoway River, Dinwiddie County, Virginia; and *A. plicata* were collected in February 2003 from the Clinch River, Scott County, Virginia.

Small replicate tissue blocks (≈1 mm$^2$) from viscera of three each of *V. iris*, *U. imbecillis*, and *L. fasciola* were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at 7.3 pH, post-fixed in a 0.1 M

sodium cacodylate buffered 1.0% osmium tetroxide solution, and embedded with Erlandson's Maraglas, D.E.R. 732 epoxy medium (Caceci, 1984) for thin-sectioning (800 Å). Thin sections were observed using a Zeiss 10CA transmission electron microscope (TEM) at 60 kV. Tissue sections (1 μm) from the epoxy blocks were stained with methylene blue, azure II, and basic fuchsin for light microscopy. Where possible in our descriptions, we follow Mackie (1984) and McMahon and Bogan (2001) for anatomical terminologies.

## Results

After development in acini (Figs. 1 and 2), mature gametes of *U. imbecillis*, *V. iris*, and all other species we examined, exited acini through ciliated primary gonoducts (Fig. 3). Groups or clusters of acini supplied mature gametes via primary gonoducts to secondary gonoducts (Fig. 4) that subsequently formed confluences to create larger gonoducts (Fig. 5). In this way, mature gametes from regional aggregations of acini were transported via ciliated gonoducts to an anatomical structure that appeared to be a gonosinus (Fig. 6). A gonosinus with ciliated epithelium was observed in most *V. iris* and *U. imbecillis* in the dorso-anterior portion of their viscera at approximately 70% of the visceral width, on a tranverse sectional plane that met the posterior intersect of the labial palp-visceral connective axis (Fig. 7). Additionally, these sinuses also were observed in the same relative location in thick sections from viscera of specimens of *E. complanata*, *V. vanuxemensis*, *L. fasciola*, and *A. plicata*. Variance in sizes of the gonosinuses was apparent in all species examined; mean planar surface areas of the gonosinuses in histological thick sections were 1194.5 $\mu m^2$ (±418.4) in *U. imbecillis*, 710.4 $\mu m^2$ (±271.1) in female *V. iris*, and 583.5 $\mu m^2$ (±291.8) in male *V. iris*. In *U. imbecillis*, gonosinuses simultaneously contained sperm and oocytes (Fig. 8); thus, the gonoducts from both male and female acinar regions of the viscera eventually emptied to the gonosinus in this species. Gametogenic acini were abundant in the viscera of all examined species, and all *U. imbecillis* were hermaphrodites, with spermatogenic acini always grouped in the dorso-anterior portion of the viscera.

Gonoducts of *V. iris* and *U. imbecillis* were lined with ciliated, simple, columnar epithelial cells with heterochromatic nuclei (Fig. 9). For reference, epithelia of gonoducts of *V. vanuxemensis*, *E. complanata*, *L. fasciola*, *L. dolabelloides*, *P. oviforme* and *A. plicata* were similar (Figs. 10–12 for *L. dolabelloides*, *E. complanata*, and *L. fasciola*, repectively). Also, the gonosinuses observed in *U. imbecillis*, *V. iris*, *V. vanuxemensis*, *E. complanata*, and *A. plicata* were lined with the same type of epithelium. The putative basal lamina of these epithelia stained weakly for PAS, and the membrane was difficult to observe in some thick sections. Observations using TEM revealed true cilia, with 9+2 axoneme configurations (9 doublets and a central pair of microtubules) and microvilli (Fig. 13). Cilia of the gonoducts and gonosinuses had a seven-sided cross-sectional shape (Fig. 13), and the consistency of this shape among mussels suggests that this was not a distortional artifact of fixation. At the base of each cilium, basal bodies were evident (Fig. 14). Cell membranes were obvious within the ciliated epithelia of gonoducts and gonosinuses of all species, and the cells contained glycogen (rosettes), mitochondria, and occasional lipid droplets (Fig. 14).

The gonoduct and gonosinus epithelia of *U. imbecillis* differed from those of examined dioecious species, because secretory cells were dispersed among the ciliated epithelial cells. Secretory granules of these cells stained positive with PAS (Fig. 15), and therefore contained carbohydrates or glycoproteins. After secretion, thick sections showed that these mucigen droplets seemingly dissolved in the lumen of gonoducts and gonosinuses (Fig. 16). Not all of the secretory cells produce the granules simultaneously; cohorts of these cells seemingly secrete their granules concurrently.

Observations of epoxy-embedded sections using light microscopy revealed greater histological detail than paraffin sections. The epithelia of gonosinuses of *U. imbecillis* specimens were composed of ciliated cells whose nuclei were heterochromatic, intermingled with PAS-positive secretory cells (Fig. 17). On first appearance using low-magnification TEM with thin sections, these secretory cells seemingly possessed densely stained nuclei that were displaced toward the cell membranes (Fig. 18). With higher magnification of these nuclei using TEM, they were contained within membranes of glycogen cells contiguous with the secretory cells (Figs. 17 and 19). The secretory cells resembled goblet cells of intestinal and respiratory tracts in mammals. However, we did not observe displaced nuclei, as expected in true goblet cells. Therefore, the PAS-positive secretory cells in gonoducts and gonosinuses of *U. imbecillis* were termed secretory cells. It should be noted that secretory cells also were observed in the gonoducts and gonosinuses of the dioecious species; however, their secretions did not stain with PAS (Fig. 20).

In the course of this study, two hermaphrodites of *V. iris* were observed. The gonad of one hermaphrodite was characterized by a predominance of male acini, with female acini rarely observed; the opposite situation occurred in the other hermaphrodite. Also, two

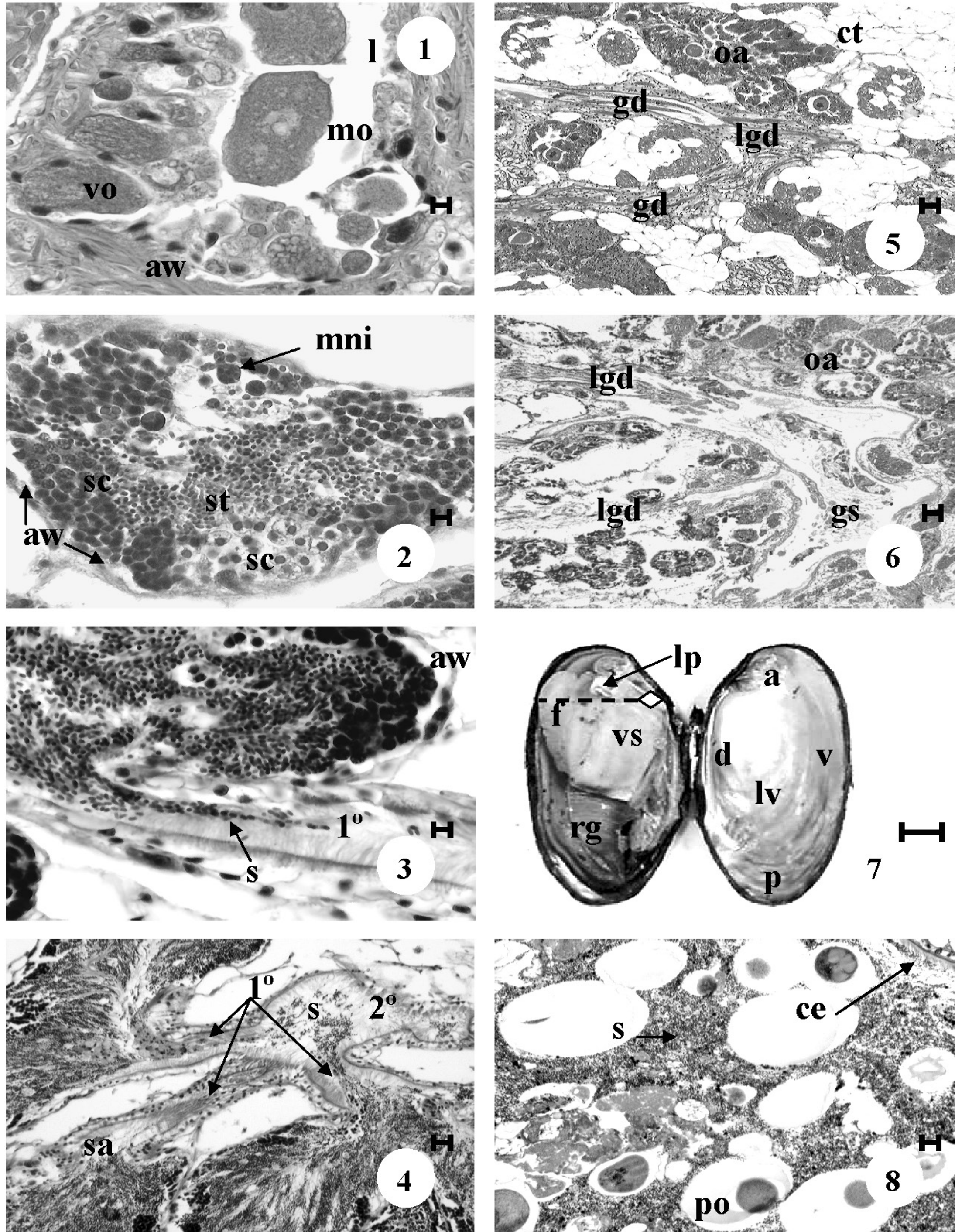


Fig. 1. Development of vitellogenic oocytes (vo) from acinus wall (aw), and mature oocytes (mo) in acinar lumen (l) in *Villosa iris*. Scale bar: 20 µm. Fig. 2. Development of spermatogenic cells from acinar wall (aw) in *Utterbackia imbecillis*. Note spermatocytes (sc) in varied stages of maturation, spermatids (st), and multi-nucleated inclusions (mni). Scale bar: 20 µm. Fig. 3. Anatomical connection of primary gonoduct (1°) from acinus in male *V. iris* with spermatozoa (s) entering gonoduct. Note acinus wall (aw). Scale bar: 20 µm. Fig. 4. Confluences of primary gonoducts (1°) from spermatogenic acini (sa) to secondary gonoduct (2°) in *U. imbecillis*, with spermatozoa (s) in ciliated gonoducts. Scale bar: 40 µm. Fig. 5. Confluence of gonoducts (gd) to larger gonoduct (lgd) in female *V. iris*. Note oogenic acini (oa) within connective tissue (ct). Scale bar: 199 µm. Fig. 6. Anatomical terminations of large gonoducts (lgd) into the gonosinus (gs) in female *V. iris*. Note oogenic acini (oa). Scale bar: 425 µm. Fig. 7. Overview of female *V. iris* showing approximate anatomical position (white diamond) of gonosinus at most dorsal position of the viscera found on a hypothetical dorso-ventral line (dotted line) through the position of the posterior labial palp (lp)-visceral (vs) connective axis. In histological sections of *V. iris* and *U. imbecillis*, gonosinus was observed at this anatomical position at approximately 70% of the visceral width (mm), with measurements obtained from visceral boundary facing the left valve (lv). Note anterior (a), posterior (p), dorsal (d), and ventral (v) orientations, and positions of the foot (f) and right gills (rg). Scale bar: 13 mm. Fig. 8. Presence of spermatozoa (s) and primary oocytes (po) in gonosinus of *U. imbecillis*. Note ciliated epithelium (ce) of the gonosinus at top right corner of the figure. Scale bar: 79 µm.

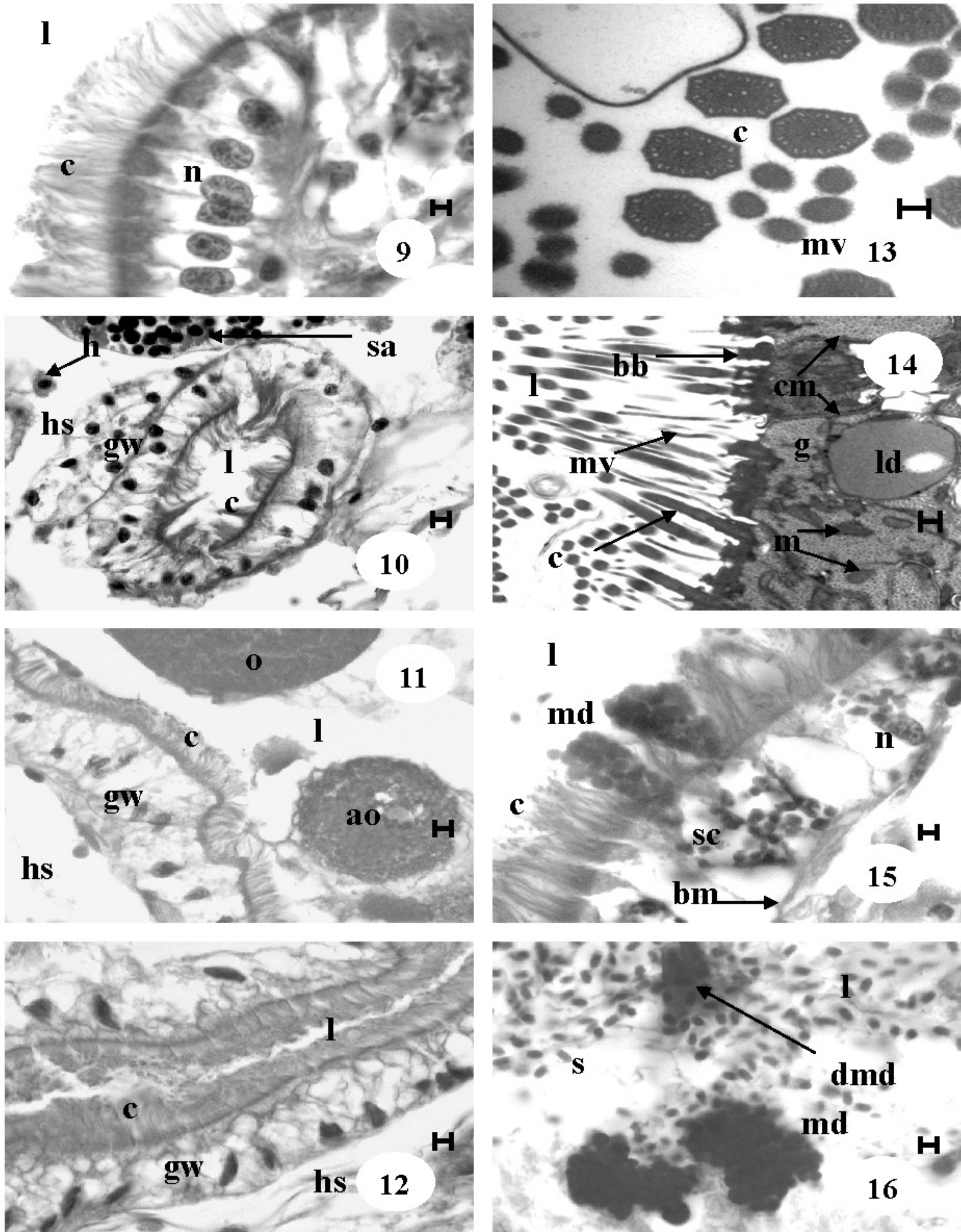


Fig. 9. Simple columnar epithelium of the gonosinus showing heterochromatic nuclei (n) and cilia (c) protruding toward the gonosinus lumen (l) in *Utterbackia imbecillis*. This type of epithelium was observed in gonoducts and gonosinuses of all species examined in this study. Scale bar: 8 µm. Fig. 10. Epithelium of gonoduct wall (gw) in male *Lexintonia dolabelloides* showing cilia (c). Note lumen (l) of gonoduct, spermatogenic acinus (sa), and hemocyte (h) in hemolymph space (hs). Scale bar: 20 µm. Fig. 11. Oocyte (o) and atretic oocyte (ao) in gonosinus lumen (l) of *Elliptio complanata*. Note simple columnar epithelium with cilia (c) and proximity of hemolymph space (hs) to epithelium of gonosinus (gw). Scale bar: 20 µm. Fig. 12. Epithelium of gonoduct wall (gw) with cilia (c) and hemolymph space (hs) in female *Lampsilis fasciola*. Note lumen (l) of gonoduct. Scale bar: 20 µm. Fig. 13. Electron micrograph (TEM) of 9-2 axeoneme in cilia (c) and not in microvilli (mv) of gonoduct epithelium in male *L. fasciola*. Scale bar: 100 nm. Fig. 14. Transmission electron micrograph (TEM) of gonoduct epithelium in male *L. fasciola*. Epithelial cells are bound by cell membranes (cm) and contain glycogen (g), occasional lipid droplets (ld), and abundant mitochrondria (m). Note cilia (c) with basal bodies (bb) and abundant microvilli (mv). Scale bar: 500 nm. Fig. 15. Secretory cells (sc) of ciliated epithelium of gonosinus in *U. imbecillis*. PAS-positive secretory mucigen droplets (md) released from cell crypt at the apical end of secretory cell to lumen (l) of gonosinus. Note cilia (c), heterochromatic nucleus (n), and basement membrane (bm). Tissue stained with PAS. Scale bar: 8 µm. Fig. 16. PAS-positive secretory mucigen droplets (md) and dissolving droplets (dmd) in gonosinus lumen (l) of *U. imbecillis*. Note spermatozoa (s). Tissue stained with PAS. Scale bar: 8 µm.

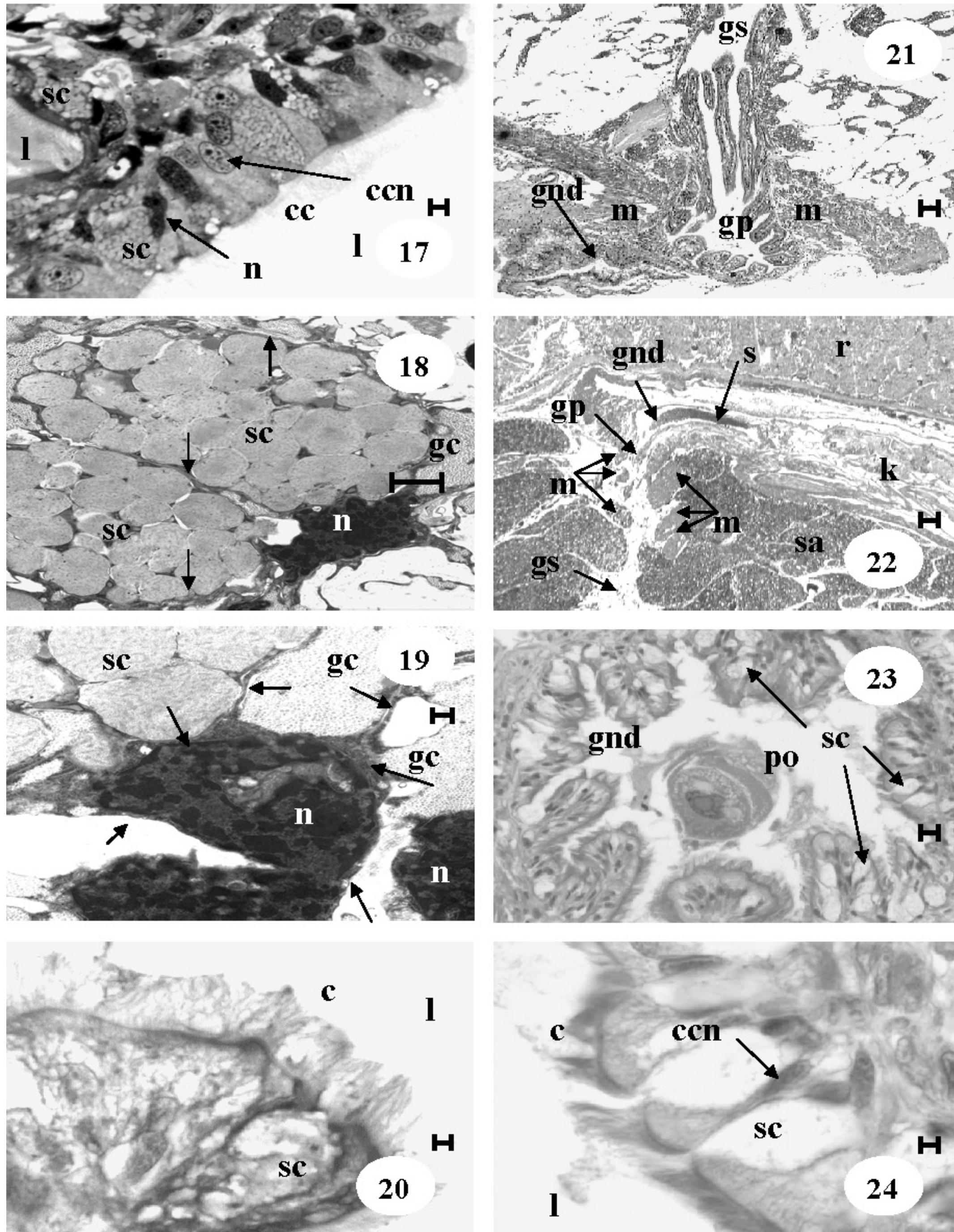


Fig. 17. Epoxy embedded ciliated epithelium of *Utterbackia imbecillis* gonosinus, with heterochromatic nuclei (ccn) of ciliated cells (cc), and densely stained nuclei (n) in apparent association with secretory cells (sc). Since the gonosinus is highly rugated, its lumen (l) appears is different areas of the section. Tissue stained with a combination methylene blue, azure II, and basic fuchsin. Scale bar: 10 µm. Fig. 18. TEM of exocrine secretory cells (sc) and densely stained nucleus (n) in gonosinus epithelium of *U. imbecillis*. Note glycogen cell (gc) contiguous with secretory cell. Arrows indicate cell membranes of secretory cells. Scale bar: 8 µm. Fig. 19. TEM of densely stained nucleus (n) of glycogen cell (gc) contiguous, but separate, from secretory cell (sc) in gonosinus of *U. imbecillis*. Arrows indicate cell membrane of glycogen cell also enclosing nucleus. Scale bar: 500 nm. Fig. 20. Secretory cell (sc) in ciliated (c) epithelium of gonosinus in female *Villosa iris*. Secretion stained negative for PAS. Tissue stained with PAS. Scale bar: 8 µm. Fig. 21. Gonosinus (gs) anatomically communicates with rugous gonopore (gp) in female *V. iris*. Note musculature (m) surrounding gonopore that may indicate a sphincter, and continuation of reproductive tract from gonopore via a genital duct (gnd). Scale bar: 79 µm. Fig. 22. Anatomical communication of gonosinus (gs), gonopore (gp), and genital duct (gnd) in male *V. iris*. Note musculature (m) of putative sphincter is proximate to gonopore, spermatozoa (s) in genital duct, kidney (k), rectum (r), and spermatogenic acini (sa). Scale bar: 425 µm. Fig. 23. Cross-section of rugous genital duct containing primary oocyte (po) in female *V. iris*. Note secretory cells (sc) in columnar ciliated epithelium of genital duct (gnd). Secretory products did not stain PAS-positive. Tissue stained with PAS. Scale bar: 40 µm. Fig. 24. Epithelia of genital duct of *V. iris*, and all other species, composed of ciliated cells with prominent nuclei (ccn) with cilia (c). Note that secretory cells (sc) have secreted their contents to genital duct lumen (l). Scale bar: 8 µm.

hermaphroditic *P. oviforme* were discovered in archived thick sections. The gonoducts of the hermaphroditic *V. iris* and *P. oviforme* specimens were similar to those of dioecious specimens examined; however, the PAS-positive secretions found in the gonoducts and gonosinuses of *U. imbecillis* were not observed in hermaphroditic specimens of normally dioecious species, including *V. iris* and *P. oviforme*.

In *V. iris* and *U. imbecillis*, the genital duct was anatomically connected to a rugous gonopore (Figs. 21 and 22). This gonopore was surrounded by an investment of musculature that may indicate a sphincter complex (Fig. 21). The epithelia of the gonopores of these species were composed of cells similar to those found in their gonoducts and gonosinuses; namely, ciliated simple, columnar cells with interspersed secretory cells in *V. iris*, and PAS-positive secretory cells in *U. imbecillis*. The epithelial cells of the genital ducts were simple columnar, ciliated cells with an abundance of intermingled secretory cells; however, it is important to note that the PAS-positive secretory cells were absent in the genital ducts of *U. imbecillis*. The secretory cells found in the genital ducts of *V. iris* and *U. imbecillis* were characterized by secretions that did not stain with PAS (Figs. 23 and 24).

The genital ducts proceeded in a posterior direction from the gonopore (Fig. 22). Dorsal-to-ventral viewing of sagittal histological thick sections showed the sequential tissue occurrence to be kidney, genital duct, visceral-inner gill axis, and epibranchial chamber (Fig. 25). In other histological sections, the pericardial cavity, heart, and rectum were evident dorsal to the kidney. Our histological sections may have been askew from the visceral center-line (dorso-ventral, sagittal axis), since a gill and an epibranchial chamber were evident in many sections (Fig. 25). However, the genital duct was proximal and dorsal to the visceral-gill axis, and was contiguous but distinct from the kidney. This distinction was substantiated by histological differences between the epithelia of the genital duct and kidney. Whereas the genital duct was of simple columnar, ciliated epithelium with exocrine secretory cells, the kidney was composed of convoluted diverticula and tall columnar cells with microvilli (brush border) (Fig. 26). In the kidney, serous apocrine secretions were observed from the distal edges of the diverticula (Fig. 26). In the interiors of the diverticula, hemocoels contained circulating hemocytes (Fig. 26).

Observations of archived transverse thick sections of *E. complanata* indicated that the kidney was a paired organ, with left and right lobes (Fig. 27). In sections from dorsal to ventral, the rectum passed through the ventricle within the pericardial cavity (Fig. 27). Ventral to the pericardial cavity were the left and right lobes of

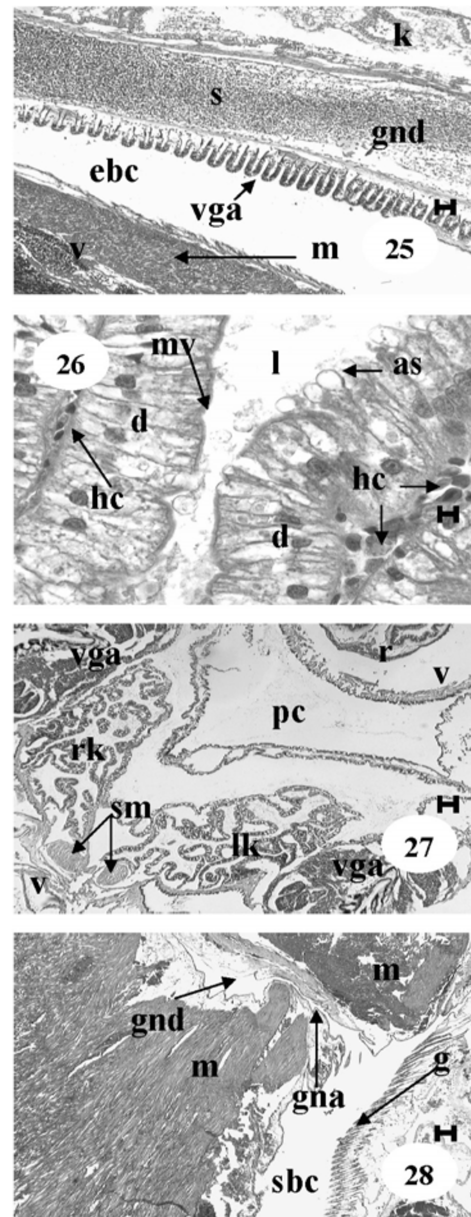


Fig. 25. Genital duct (gnd) of male *Villosa iris* containing spermatozoa (s) is ventral to kidney (k) and dorsal of the viscera-inner gill axis (vga). Note epibranchial chamber (ebc) and muscles in viscera (v). Scale bar: 199 μm. Fig. 26. Diverticula (d) of kidney in *V. iris* are composed of tall columnar epithelial cells with microvilli (mv) (brush border). Hemocoels of diverticula contain circulating hemocytes (hc); serous apocrine secretions (as) are observed from the distal edge of the diverticulum. Scale bar: 20 μm. Fig. 27. Cross-section of heart-kidney complex in male *Elliptio cpmplanata*. From dorsal to ventral, rectum (r) passes through ventricle (v) in pericardial cavity (pc). Kidney is bi-lobed, with left (lk) and right (rk) lobes, with smooth muscles (sm) in ventral portion of each lobe at viscera (v)-kidney connective axes. Note visceral-gill axes (vga). Scale bar: 425 μm. Fig. 28. Termination of genital duct (gnd) to the suprabranchial chamber (sbc) via a putative genital aperture (gna) in male *V. iris*. Note widening of genital duct proximal of genital aperture, gill (g), and musculature (m) surrounding the genital aperture suggestive of a sphincter. Scale bar: 425 μm.

the kidney. In the ventral areas of each lobe of the kidney, smooth muscle was always observed in transverse sections (Fig. 27). Because the kidney is a paired, lobulated organ, it may be that 2 genital ducts occur in *V. iris*, *U. imbecillis*, and other unionid species, with one proximal to each lobe of the kidney. In sagittal sections, the genital duct terminated at the suprabranchial chamber by an opening surrounded with musculature (Fig. 28). For descriptive purpose, we label this terminus of the genital duct to be the genital aperture (Mackie, 1984). The musculature surrounding this pore may indicate that it is a sphincter. Proximal to the genital aperture, the genital duct increased in internal volume (Fig. 28). This increase in volume, in combination with a putative sphincter, implies a storage capacity for retaining gametes for timely release to the suprabranchial chamber.

## Discussion

The reproductive tracts of species examined in this study shared many common anatomical characteristics. Through confluences, progressively larger ciliated gonoducts transport gametes from acini to a gonosinus. Oocytes and spermatozoa were observed simultaneously in the gonoducts and gonosinuses of *U. imbecillis*, and this agrees with the descriptions of gonoducts by Mackie (1984) for hermaphroditic unionids, and Hoeh et al. (1998) for *U. imbecillis*. The commonality of gonosinuses in examined specimens leads us to presume that they are regular anatomical features in *V. iris* and *U. imbecillis*. This is probably the case in other species of freshwater mussels, because we also observed gonosinuses in the same approximate visceral positions (dorso-anterior area of viscera) in *A. plicata*, *E. complanata*, and *V. vanuxemensis*. If further research substantiates that gonosinuses are regular anatomical features of unionid mussels, then non-lethal collection by needle and syringe of mature gametes from these sinuses may be possible. However, before such procedures can be used reliably, the positions of gonosinuses in target species must be determined.

The epithelial linings of gonoducts and gonosinuses of our specimens were ciliated, and ciliary action presumably aids in the transport of gametes along the reproductive tract. However, Thompson and Bebbington (1969) noted peristalsis in freshly dissected reproductive tracts of gastropods. From our results, we cannot substantiate the hypothesis that peristalsis occurs in *V. iris* or *U. imbecillis*, since we did not observe smooth musculature associated with the epithelia of their gonoducts. It may be that contractions of the smooth muscles that we observed in the ventral portions of the kidney lobes facilitate both urine passage and movement of gametes.

From the gonosinus, gametes entered the genital duct through a gonopore. The formidable musculature that surrounds the gonopores of *U. imbecillis* and *V. iris* suggests that the pore may serve as a sphincter to retain gametes for controlled release. In fact, gonosinuses swollen with gametes were observed in *U. imbecillis* and *V. iris*, while no gametes were seen in genital ducts. We hypothesize that gametes were being stored in the gonosinuses of these specimens, as in seminal vesicles and carrefour complexes of pulmonate and opisthobranch gastropods (Runham, 1988). Indeed, transport of gametes from the carrefour in the gastropod genus *Tritonia* is controlled by a sphincter (Thompson, 1976). Induced release of gametes in bivalves is initiated by exposure to serotonin, sperm, increased food concentration, or water and temperature changes (Sivalingam, 1977; Smith and Strenhlow, 1983; Pong, 1998; Spencer, 2002; Gosling, 2003). For controlled spawning to become a viable component of captive propagation of unionid broodstock, research on cues linked to release of unionid gametes would be beneficial.

The genital tracts of *V. iris* and *U. imbecillis* continued from the gonopore to a genital duct that was ventral, contiguous, and distinct from the kidney. There may be two genital ducts, each associated with corresponding lobes of the kidney. Coe (1932) found that genital canals in Olympia oysters, *Ostrea lurida* (Carpenter, 1865), deliver gametes to two genital pores positioned ventral to the adductor muscle. Heard (1992) observed two pores that open to the suprabranchial chamber above the left and right demibranchs. Additionally, our finding that the genital and urinary tracts are distinct agrees with the description of Mackie (1984) for Unionidae. However, we did not observe a nephridiopore for delivery of urine to the suprabranchial chamber. Hoeh et al. (1998) observed stored spermatozoa in kidneys of giant floaters, *Pyganodon grandis* (Say, 1829), but we found no evidence of gametes in any sections containing kidney tissue.

Discussion of the anatomy of the reproductive tracts of freshwater mussels should be guided by consideration of their necessary mechanical and physiological functions. At the most simplistic level, these tracts must maintain suitable physiological environments for gametes in transport from acini to the suprabranchial chamber. In this context, the occurrence of cilia, microvilli, and secretory cells may be considered anatomical components of ductile epithelia for the maintenance of appropriate physiological conditions for gametes during intra-visceral transport. Ciliated reproductive tracts have been reported for bivalves and other molluscs, including the eastern oyster, *Crassostrea virginica* (Gmelin,

1791), soft-shell clam, *Mya arenaria* (Linnaeus, 1758), great scallop, *Pecten maximus* (Linnaeus, 1758), marsh pond snail, *Stagnicola elodes* (Say, 1821), and the conch genus *Strombus* (Stickney, 1963; Kennedy and Battle, 1964; Rudolph, 1983; Reed, 1995; Widowati et al., 1995). However, no research has been conducted on the role of microvilli in the reproductive tracts of molluscs. The notion that the anatomical presence of microvilli is indicative of physiological need for increased cell surface area may be correct but simplistic. Increased surface area could be accomplished merely by rugation of gonoducts. Therefore, we question why an anatomical need for increased surface area in reproductive tracts necessitates an abundance of microvilli.

Recent research has established that microvilli are integral epithelial features involved with maintenance of intra- and extra-cellular physiological environments (Lange, 1999, 2000a, 2000b). It is the dynamic interactions among the cytoplasmic compartment, entrance compartment (tip of microvillus), and actin filaments in the shaft region of a microvillus that allow detection of concentration gradients, and facilitation of intra- and extra-cellular solute exchange (Lange, 2000a). Ion exchange ($Na^+/H^+$, $Ca^{2+}/Na^+$, $Ca^{2+}/H^+$, and $Mg^{2+}/Na^+$), NaCl transport, cation/phosphate co-transport, and $K^+$, $Cl^-$, glucose, and water regulations are processes accomplished in microvilli (Lange, 2000a, 2000b, 2001). The widespread distribution of microvilli on the epithelia of reproductive tracts in our specimens may indicate regulation of solute concentrations in luminal fluid, important to gametes during their intra-visceral transport.

In mammals and invertebrates, extracellular ionic concentrations regulate sperm development and function. Sufficient extracellular concentrations of $Ca^{2+}$, $Na^+$, $K^+$, and $H^+$ must be maintained, so that requisite intracellular ionic changes can occur to initiate functionality of sperm (Fraser, 1995). Acrosomal exocytosis in both invertebrates and mammals is dependent upon activation of $Ca^{2+}$ channels to permit influx of $Ca^{2+}$, and levels of extracellular energy substrates such as glucose (Fraser, 1992, 1995). *In vitro* fertilization rates of human oocytes are related to incubation concentrations of glucose, as are sperm motility and movement characteristics (Mahadevan et al., 1997). In addition, composition of luminal fluids from reproductive tracts vary among species (Lahnsteiner et al., 1995). Although no research has been conducted on the importance of luminal solute concentrations to gametes in reproductive tracts of freshwater mussels, the abundance of microvilli indicates that regulation of ions and glucose is probably a feature of the tracts. The effects of reproductive tract intra-luminal fluids of unionid mussels on *in vitro* fertilization rates and sperm motility and functionality could be investigated by comparing these responses using gametes collected directly from acini and gametes from gonosinuses, after they have been exposed to the environments of the reproductive tracts.

The observed secretory products of epithelial cells may be important to sperm maturation, motility, agglutination, survival in the water column before fertilization, and elimination of non-viable sperm in reproductive tracts. Janna (1982) reported that secretions of male reproductive tracts of *Tubifex* worms are polysaccharides responsible for the formation of spermatozeugma. Heard (1975) reported the presence of sperm morulae, and although we have not been able to substantiate this observation, it is possible that the secretory products of the reproductive tracts contribute to their formation in Unionidae. A male-associated polypeptide (MAP) has been detected throughout the reproductive tracts of male Mediterranean mussels, *Mytilus galloprovincialis* (Lamarck, 1819), and was linked to sperm agglutination, survival in the water column, functional maturation, and motility (Torrado et al., 2003). Furthermore, a glycoprotein (HEP64) from epididymal secretory cells in hamsters reportedly coats dead and non-viable spermatozoa (NagDas et al., 2000).

Secreted glycoproteins are also important in lubrication of luminal gametes in reproductive tracts, and may create disease-resistant and non-adhesive cell surfaces on gametes (Lagow et al., 1999). Related to this, mucin coats of mammalian sperm may reduce sperm adhesion to reproductive tract walls during transport (Lagow et al., 1999). Zona pellucida glycoproteins from the ovary surround and infiltrate ova, and are important to gamete recognition (Lagow et al., 1999). These recognition glycoproteins appear to have a species-specific effect. In fact, Schmidt et al. (1997) showed that sperm-oocyte binding was inhibited when oocytes and sperm were exposed to oviductile fluids from other species.

It is interesting that the PAS-positive secretory cells seen in the gonoducts and gonosinuses of hermaphroditic *U. imbecillis* were not observed in those of the dioecious species. In *U. imbecillis*, the PAS-positive secretory cells only occurred in the gonoducts and gonosinuses, whereas the secretory cells in their genital ducts did not stain positive with PAS. In all dioecous specimens, only secretory cells with secretions unstained by PAS were observed throughout their entire reproductive tracts. An hypothesis to explain the exclusive occurrence of the PAS-positive granules in the gonoducts and gonosinuses, where spermatozoa and oocytes of *U. imbecillis* can intermingle, is that this secretion may provide a cell-surface coating that temporarily inhibits self-fertilization. Hoeh et al. (1998) noted that rates of self-fertilization among populations

of *U. imbecillis* varied, with some populations exhibiting high levels of selfing. It would be of interest to determine whether the occurrence and abundance of the PAS-positive exocrine cells varies among populations of *U. imbecillis*, and whether these secretory cells are characteristic of unionid species that are normally hermaphroditic. If these secretory granules are linked to temporary inhibition of self-fertilization, then characterization of possible glycoproteins would be important to document the mechanism for regulation of self-fertilization in hermaphroditic freshwater mussels.

It is well established for marine bivalves that dietary conditioning of female adults can affect subsequent larval and juvenile condition through oocyte quality (Bayne et al, 1978; Robinson, 1992a, 1992b), and that oocyte quality is related to larval and juvenile metamorphosis, development, growth, and survival (Helm et al., 1973; Bayne et al., 1975; Gallager and Mann, 1986). The nutritional status of adult bivalves is important to oocyte quality, because intra-visceral transfer of lipids, proteins, and carbohydrates for oocyte investment is dependent on available energy stores in adult females (Barber and Blake, 1985; Pipe, 1985). We have found no research on the effects of diet on sperm quality and functionality in bivalves, although this link has been established in mammals, especially with regard to lipid and antioxidant intake (Strzezek et al., 2004; Eskenazi et al., 2005). No research on the effects of dietary status on adult mussels and characteristics of reproductive luminal fluids is available. It may be that diet quality also influences physiological environments of unionid reproductive tracts, and may affect gamete quality, functionality, and fertilization rates.

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