

# Seasonal reproductive anatomy and sperm storage in pleurocerid gastropods (Cerithioidea: Pleuroceridae)

Nathan V. Whelan and Ellen E. Strong

**Abstract:** Life histories, including anatomy and behavior, are a critically understudied component of gastropod biology, especially for imperiled freshwater species of Pleuroceridae. This aspect of their biology provides important insights into understanding how evolution has shaped optimal reproductive success and is critical for informing management and conservation strategies. One particularly understudied facet is seasonal variation in reproductive form and function. For example, some have hypothesized that females store sperm over winter or longer, but no study has explored seasonal variation in accessory reproductive anatomy. We examined the gross anatomy and fine structure of female accessory reproductive structures (pallial oviduct, ovipositor) of four species in two genera (round rocksnail, *Leptoxis ampla* (Anthony, 1855); smooth hornsnail, *Pleurocera prasinata* (Conrad, 1834); skirted hornsnail, *Pleurocera pyrenella* (Conrad, 1834); silty hornsnail, *Pleurocera canaliculata* (Say, 1821)). Histological analyses show that despite lacking a seminal receptacle, females of these species are capable of storing orientated sperm in their spermatophore bursa. Additionally, we found that they undergo conspicuous seasonal atrophy of the pallial oviduct outside the reproductive season, and there is no evidence that they overwinter sperm. The reallocation of resources primarily to somatic functions outside of the egg-laying season is likely an adaptation that increases survival chances during winter months.

**Key words:** Pleuroceridae, *Leptoxis*, *Pleurocera*, freshwater gastropods, reproduction, sperm storage, anatomy.

**Résumé :** Les cycles biologiques, y compris de l'anatomie et du comportement, constituent un élément gravement sous-étudié de la biologie des gastéropodes, particulièrement en ce qui concerne les espèces d'eau douce menacées de pleurocérédés. Cet aspect de leur biologie fournit des renseignements importants pour la compréhension de l'incidence de l'évolution sur le succès de reproduction optimal et est essentiel pour éclairer l'élaboration de stratégies de gestion et de conservation. Les variations saisonnières de la forme et de la fonction reproductrices en sont une facette particulièrement sous-étudiée. Par exemple, bien qu'il ait été postulé par certains auteurs que les femelles emmagasinaient du sperme pendant l'hiver ou plus longtemps, aucune étude n'a exploré les variations saisonnières de l'anatomie de reproduction accessoire. Nous avons examiné l'anatomie globale et fine des structures de reproduction accessoires (oviducte palléal, ovipositeur) des femelles de quatre espèces de deux genres (*Leptoxis ampla* (Anthony, 1855); *Pleurocera prasinata* (Conrad, 1834); *Pleurocera pyrenella* (Conrad, 1834); *Pleurocera canaliculata* (Say, 1821)). Des analyses histologiques ont démontré que, bien qu'elles n'aient pas de réceptacle séminal, les femelles de ces espèces peuvent emmagasiner des spermatozoïdes orientés dans leur bourse spermatophore. Nous avons de plus constaté qu'elles présentent une atrophie saisonnière évidente de l'oviducte palléal en dehors de la période de reproduction, et rien n'indique qu'elles emmagasinent du sperme pendant l'hiver. La réaffectation de ressources principalement aux fonctions somatiques en dehors de la période de ponte est probablement une adaptation qui accroît les chances de survie durant les mois d'hiver. [Traduit par la Rédaction]

**Mots-clés :** pleurocérédés, *Leptoxis*, *Pleurocera*, gastéropodes d'eau douce, reproduction, emmagasinage de sperme, anatomie.

## Introduction

How animals partition resources for reproduction and survival is integral to the evolution of different life-history strategies (Roff 2002; Futuyama 2005). Gastropods, in particular, have evolved numerous life-history strategies (e.g., semelparity vs. iteroparity, oviparity vs. viviparity) that have contributed to their success and global diversification (Brusca and Brusca 2003). In temperate zones, food abundance and quality fluctuate seasonally (Fretwell 1972), resulting in variation in invertebrate biomass, reproductive output, and community composition (Anderson and Sedell 1979; Sweeney and Vannote 1986; Richardson 1991; Müller-Navarra and Lampert 1996). As such, allocating resources towards seasonally important somatic functions (e.g., increasing body size for de-

fense vs. sexual functions during periods of reproduction) can be advantageous (Shanley and Kirkwood 2000; Kirkwood and Shanley 2005). Many marine and freshwater gastropods display a cyclical waxing and waning of gametogenesis that often corresponds to increases and decreases in volume of the gonad (e.g., Webber and Giese 1969; McShane and Naylor 1996). This is known to occur not only in temperate species but in those that inhabit the tropics, where seasonal variation in temperature and food availability is much less pronounced, and it reflects synchrony in breeding times to maximize reproductive success. Much less is known about seasonal variation in the development of the accessory reproductive organs, such as the pallial gonoduct, although intuitively these could be expected to also express cyclical activity, as has been documented in some species (e.g., Berrie 1966;

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**Table 1.** Collection localities, month of collection, and National Museum of Natural History (USNM) catalog numbers of four species of Pleuroceridae.

Species	Collection locality	Latitude, Longitude	Collection month	USNM catalog No.
Round rocksnail, <i>Leptoxis ampla</i>	Cahaba River	33.1698°N, 87.0195°W	May	1194241
		32.9578°N, 87.1397°W	August	1194329
Smooth hornsnail, <i>Pleurocera prasinata</i>	Cahaba River	33.1698°N, 87.0195°W	May	1194373
		32.9578°N, 87.1397°W	August	1194386
Skirted hornsnail, <i>Pleurocera pyrenella</i>	Paint Rock River	34.6873°N, 86.3102°W	September	1194454
Silty hornsnail, <i>Pleurocera canaliculata</i>	Tennessee River	34.8531°N, 87.3714°W	October	1249765
		34.6176°N, 86.9391°W	November	1249764

Martel et al. 1986; Vasconcelos et al. 2012). A related issue is whether females store sperm during nonreproductive periods. It is known, for example, that females of some freshwater gastropod species are capable of storing sperm from several months to as long as 2 years (Ankel 1925; Trüb 1990; Albrecht et al. 1999; Oppliger et al. 2003; Dillon et al. 2005; Nakadera et al. 2014), i.e., for at least for the duration of the reproductive season or for much longer.

Pleuroceridae is a valuable system for exploring gastropod life-history evolution because of their local abundance and availability and because of their diversity of behaviors. Depending on the species, pleurocerids may be either iteroparous or semelparous (Richardson et al. 1988; Miller-Way and Way 1989; Whelan et al. 2015), and deposition of eggs can occur as single eggs or in a variety of different clutch morphologies and sizes (e.g., Jewell 1931; Winsor 1933; Rosewater 1960; Dazo 1965; Whelan et al. 2015). Pleurocerids are primarily a temperate to subtropical freshwater family with approximately 165 species currently considered valid. The majority of species are distributed in North America east of the Rocky Mountains (Burch and Tottenham 1980; Johnson et al. 2013), but several species just extend into the tropics of northern Mexico (Thompson 2011). They are important primary consumers and prey items (Harvey and Hill 1991; Hill 1992; Huryn et al. 1994) and consequently an important step in the food chain. Sexes are separate, but males are aphyllate and transfer sperm via spermatophores (e.g., Dazo 1965; Glaubrecht and Strong 2004; Strong 2005). Females possess a typical cerithioidean pallial oviduct with a sperm gutter and a sperm-storing pocket hypothesized to be homologous to the spermatophore bursa of other cerithioideans (Strong 2005; Strong et al. 2011). A ciliated egg groove on the right side of the foot conveys eggs to the ovipositor (Houbrick 1988; Strong 2005), which prepares the eggs before they are deposited on solid substrates by the foot. Oviposition occurs between January and July depending on the species (Woodard 1934; Dazo 1965; Whelan et al. 2015). In pleurocerids, it is known from several case studies that gametogenic activity is reduced or ceases and the volume of the gonads shrinks during nonreproductive periods (Magruder 1935; Woodard 1935). However, seasonal variation remains poorly documented in accessory reproductive structures, and only a handful of studies have explored the organization of the pallial gonoduct in detail.

One of the main anatomical characters distinguishing pleurocerids from their close relatives, Semisulcospiridae and Melanopsidae, is the absence of a seminal receptacle (Strong 2005; Strong and Frest 2007; Strong and Köhler 2009; Strong et al. 2011). Generally, the seminal receptacle is the site of long-term storage of orientated sperm (i.e., the tip of the sperm heads are attached to the epithelial cells). These sperm remain in the seminal receptacle until fertilization (Giusti and Selmi 1985; Houbrick 1973, 1993; Ponder and Lindberg 1997; Hodgson 2010). Females of all three families possess a spermatophore bursa, a large pocket in the outer, medial lamina of the pallial oviduct. After copulation, the

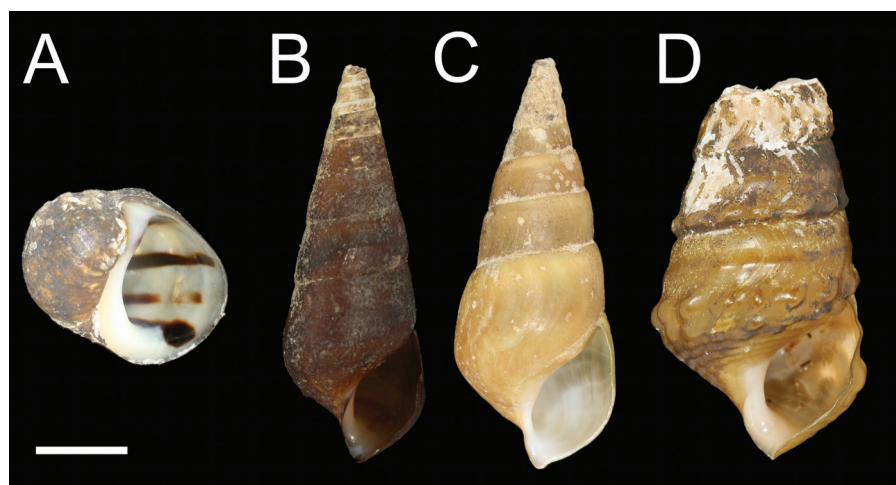
bursa receives the spermatophore whereupon the hardened external casing of male prostatic secretions breaks down and is the site of short-term storage of unorientated sperm. From here, sperm travel along the sperm gutter to the seminal receptacle, if present. However, some gastropods are capable of co-opting different parts of the pallial oviduct for the storage of orientated sperm. For example, in some neogastropods, the copulatory bursa functions in the storage of both unorientated and orientated sperm, and orientated sperm may be found attached to the ciliated ventral channel of the capsule gland (Fretter 1941; Ponder 1972, 1973).

It has been suggested that female pleurocerids store sperm for at least the length of the reproductive season (Woodard 1934; Dazo 1965) or over winter (Dillon 2000: 104) despite not possessing a seminal receptacle, but these hypotheses have never been explicitly tested. Overwintering of sperm in pleurocerids would allow the deposition of fertile eggs as soon as temperatures reached suitable levels, without first requiring mating, although there is anecdotal evidence that mating precedes oviposition (Woodard 1935; N.V. Whelan, personal observation). More generally, however, storage of sperm allows the timing of the production of spawn to coincide with optimal conditions that maximize survival of offspring. Sperm storage from different partners may also be beneficial to the female by allowing multiple paternity and sperm selection (Baur 1998). If pleurocerids do store sperm over winter, they must either be capable of long-term storage of unorientated sperm in the bursa or store orientated sperm somewhere in the oviduct. Neither of these functions has been documented previously in cerithioideans. To examine seasonal partitioning of reproductive resources and assess the potential for overwintering sperm, we examined the anatomy of female accessory reproductive structures of four species in two genera in the late spring, late summer, and fall.

## Materials and methods

We collected females of round rocksnail (*Leptoxis ampla* (Anthony, 1855)) and smooth hornsnail (*Pleurocera prasinata* (Conrad, 1834)) during the egg-laying season (i.e., late winter to mid-summer) and in late August, outside of the egg-laying season (Table 1). We also examined two additional *Pleurocera* species that were collected outside the egg-laying season to confirm seasonal morphological variation. All individuals were collected under appropriate state and federal permits (federal permit TE130300 for *L. ampla*), and animals were treated following the guidelines from the Canadian Council on Animal Care. All species examined are iteroparous (Whelan et al. 2015; N.V. Whelan, unpublished data). The shells of the silty hornsnail (*Pleurocera canaliculata* (Say, 1821)) and the skirted hornsnail (*Pleurocera pyrenella* (Conrad, 1834)) were cracked to remove the body for morphological examination, but representative individuals from collection sites were photographed (Figs. 1A–1D) and deposited at the National Museum of Natural History (USNM) in Washington, D.C. (Table 1); for *L. ampla* and *P. prasinata*, the shell of each individual

**Fig. 1.** Representative shells of species of Pleuroceridae examined: (A) round rocksnail (*Leptoxis ampla*); (B) smooth hornsnail (*Pleurocera prasinata*); (C) skirted hornsnail (*Pleurocera pyrenella*); (D) silty hornsnail (*Pleurocera canaliculata*). Scale bar = 5 mm. Figure appears in colour on the Web.



examined was deposited as a voucher in the USNM. Shell height of all examined *L. ampla* and *P. prasinata* individuals was measured to compare mean size between spring- and fall-collected individuals, to confirm that fall-collected individuals were sexually mature. An independent samples Student's *t* test was performed in SPSS version 20 (IBM corporation, Armonk, New York, USA) to compare mean shell height.

Five individuals of *L. ampla* and *P. prasinata* from both spring and late summer were dissected using a Leica Microsystems MZ 12.5 stereomicroscope (Leica Microsystems GmbH, Buffalo Grove, Illinois, USA). Ovipositors were dissected, critical point dried, mounted on aluminum stubs with carbon adhesive tabs, coated with gold, and visualized with a Hitachi TM3000 scanning electron microscope (Hitachi, Ltd., Tokyo, Japan) at the USNM Imaging Laboratory. Pallial oviducts were dissected, stained with toluidine blue, and drawn with camera lucida following Strong (2005). Drawings were rendered with Adobe Illustrator version CS5 (Adobe Systems Inc., San Jose, California, USA). For histological examination, oviducts of one individual collected in the spring and two individuals collected in late summer for both *L. ampla* and *P. prasinata* were embedded in paraplast, sectioned at 6  $\mu\text{m}$ , and stained with hematoxylin and eosin–phloxine (Strong 2003). Two individuals of *P. canaliculata* and one of *P. pyrenella* collected in the fall also were prepared for histology. Histological sections were viewed and photographed under a Leica DM LS2 compound microscope with an attached Leica DFC320 digital camera. The contrast of microscopy photographs was modified in Adobe Photoshop version CS5 for ease of viewing.

## Results

The size of females collected in the spring (*L. ampla*: mean shell height = 0.93 cm, SD = 0.2 cm,  $n = 20$ ; *P. prasinata*: mean shell height = 4.0 cm, SD = 0.4 cm,  $n = 19$ ) and of those collected in the fall (*L. ampla*: mean shell height = 1.1 cm, SD = 0.2 cm,  $n = 20$ ; *P. prasinata*: mean shell height = 4.1 cm, SD = 0.4 cm,  $n = 20$ ) were either not significantly different or fall-collected individuals were significantly larger (*L. ampla*: Student's *t* test,  $t_{[38]} = 2.258$ ,  $p = 0.030$ ; *P. prasinata*: Student's *t* test,  $t_{[37]} = -1.055$ ,  $p = 0.298$ ). Given this and known growth rates of pleurocerids (Huryn et al. 1994), animals collected in the fall must have been sexually mature (i.e., they laid eggs the previous season).

All females sampled during the egg-laying season (i.e., spring to mid-summer) had fully developed ovipositors and pallial oviducts. Ovipositors were enlarged and glandular with a deep egg groove (Figs. 2A, 2C). Albumen and capsule glands were opaque, inflated, and heavily glandular (Figs. 3A, 3B). The sperm gutter opened at

the far anterior end of the medial lamina and deepened posteriorly, leading to a short, inflated, and blind spermatophore bursa (Figs. 3A, 3B). The internal epithelium of the bursa was deeply folded and contained large quantities of orientated sperm attached to epithelial cells (Fig. 4A); large quantities of unorientated sperm were found in the center of the lumen (Fig. 4A).

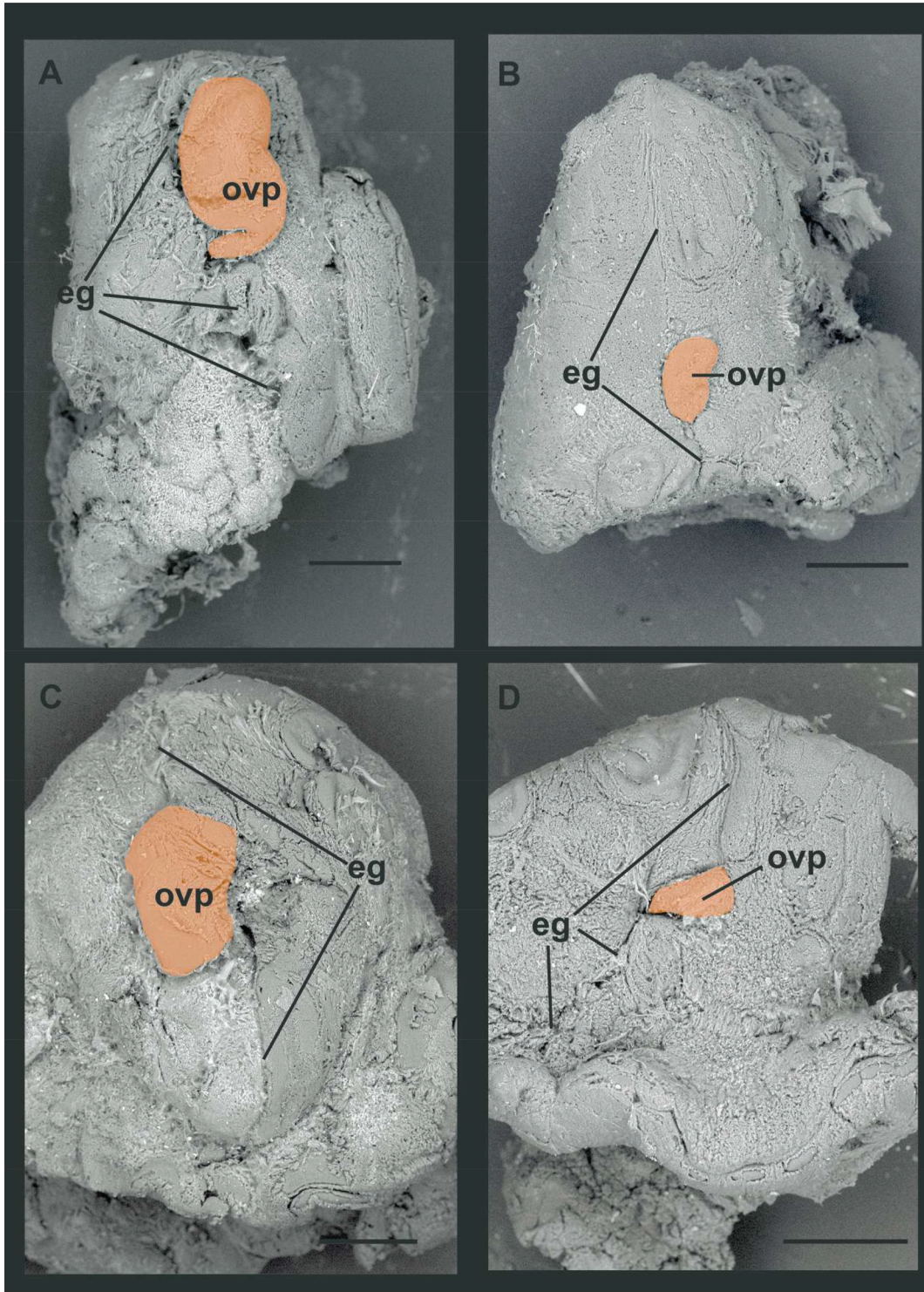
In contrast to females collected in the spring, those collected in the fall revealed highly atrophied accessory reproductive structures. The ovipositor was reduced in size and the egg groove was shallow (Figs. 2B, 2D). The pallial oviduct was conspicuously thin and the glandular development of the albumen and capsule glands was dramatically diminished (Figs. 3C, 3D). At this stage, it was impossible to distinguish macroscopically between the albumen and the capsule glands. The sperm gutter was thin and shallow and the spermatophore bursa was flattened with a simple, unfolded epithelium (Figs. 4B–4D). Only rare, scattered degenerating heads of sperm lacking tails were present; orientated sperm was lacking (Figs. 4B–4D).

## Discussion

Seasonal variation and cyclical reproductive activity is a common phenomenon in invertebrates (Berrie 1966; Webber and Giese 1969; Fankboner and Cameron 1985; Martel et al. 1986; McShane and Naylor 1996), and our data demonstrate pleurocerids undergo a seasonal atrophy of accessory sexual organs. Atrophy of the pallial oviduct and ovipositor occurs in late summer, which likely allows resources to be allocated to somatic functions for surviving colder winter temperatures. However, as rivers begin to warm in the late winter and spring, resources are redirected to replenish the accessory reproductive structures; this surely comes at a cost, but it is also a time of abundant food availability and rapid growth in pleurocerids (Huryn et al. 1994). Experimental studies that manipulate food quantity and quality in the context of other variables such as growth, fecundity, and survivorship are needed to elucidate the precise selective advantages of seasonal reproductive atrophy.

Previous misunderstandings of pleurocerid reproductive anatomy that have been elucidated by this study are best understood in a historical context. Woodard (1934, 1935, 1940) provided a detailed description of the pallial oviduct of panel elimia (*Elimia laqueata* (Say, 1829)) as comprising two laminae that represent a continuation of the (renal) oviduct and that communicate freely with the mantle cavity. He described a posterior folded region of both laminae to which sperm were observed to attach and he referred to this region as the seminal receptacle. Although Woodard (1934) initially believed the ovipositor pore to function as a spermatophore bursa, he later (Woodard 1940) rejected this hypothesis upon

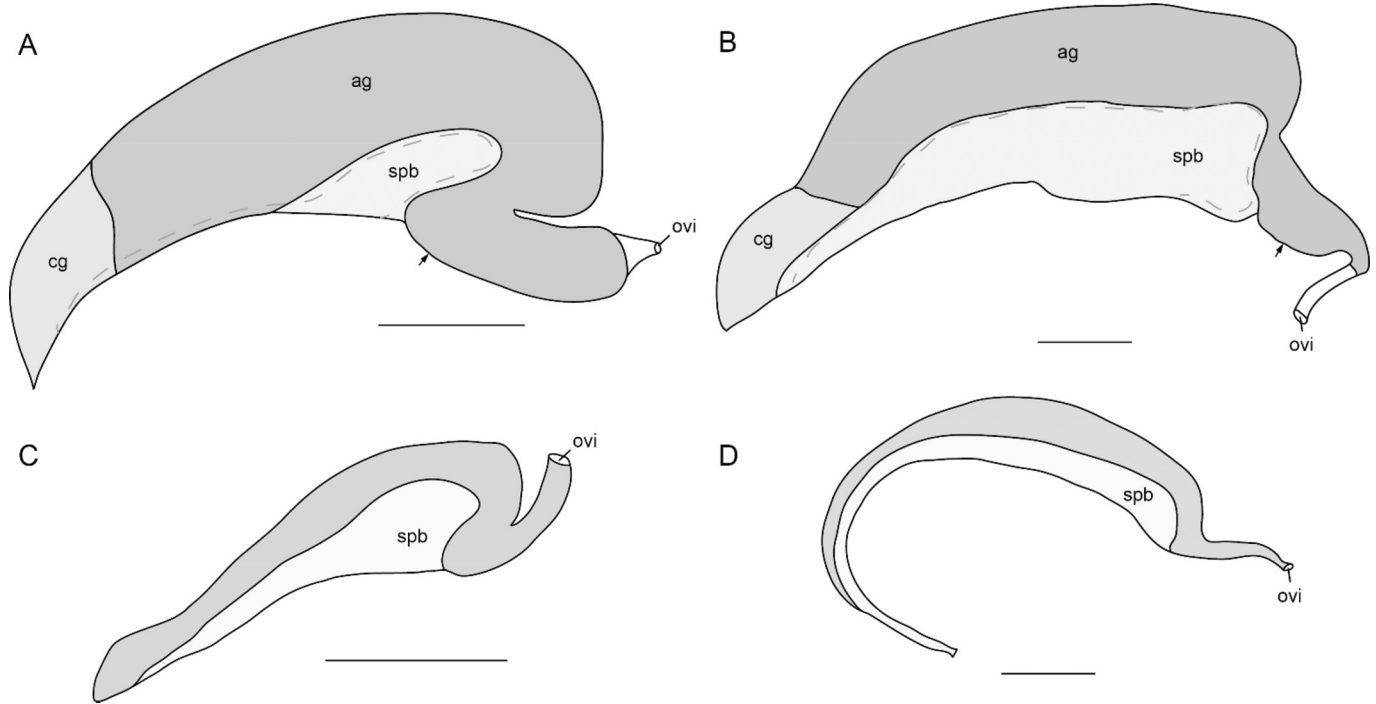
**Fig. 2.** Scanning electron micrographs of ovipositors and egg grooves of species of Pleuroceridae examined: (A) smooth hornsnail (*Pleurocera prasinata*) collected in May; (B) *P. prasinata* collected in late August; (C) round rocksnail (*Leptoxis ampla*) collected in May; (D) *L. ampla* collected in late August. Ovipositors appear as dark gray in print (orange on the Web) for emphasis. Base of the foot is below. eg, egg groove; ovp, ovipositor. Scale bars = 0.5 mm.



the discovery of spermatophores as reportedly from between the laminae. Jones and Branson (1964) and Dazo (1965) perpetuated the notion that the so-called seminal receptacle is formed by a folded posterior region of the gonoduct laminae and did not clarify the earlier confusion that Woodard's description created about the disposition of sperm storage.

Strong's (2005) anatomical study clarified the configuration of the pallial oviduct and established the presence of a sperm gutter and sperm-storing pouch in the outer, medial lamina. Owing to the presence of unorientated sperm and the similarity in position and structure, Strong (2005) concluded the structure to be homologous to the spermatophore bursa of other cerithioideans. Given

**Fig. 3.** Internal anatomy of female reproductive system of species of Pleuroceridae examined: (A) round rocksnail (*Leptoxis ampla*) collected in May; (B) smooth hornsnail (*Pleurocera prasinata*) collected in May; (C) *L. ampla* collected in late August; (D) *P. prasinata* collected in late August. Note that the albumen and capsule glands were not externally differentiable in individuals collected in late August. ag, albumen gland; cg, capsule gland; ovi, oviduct; spb, spermatophore bursa; broken line, sperm gutter. Scale bars = 1 mm.



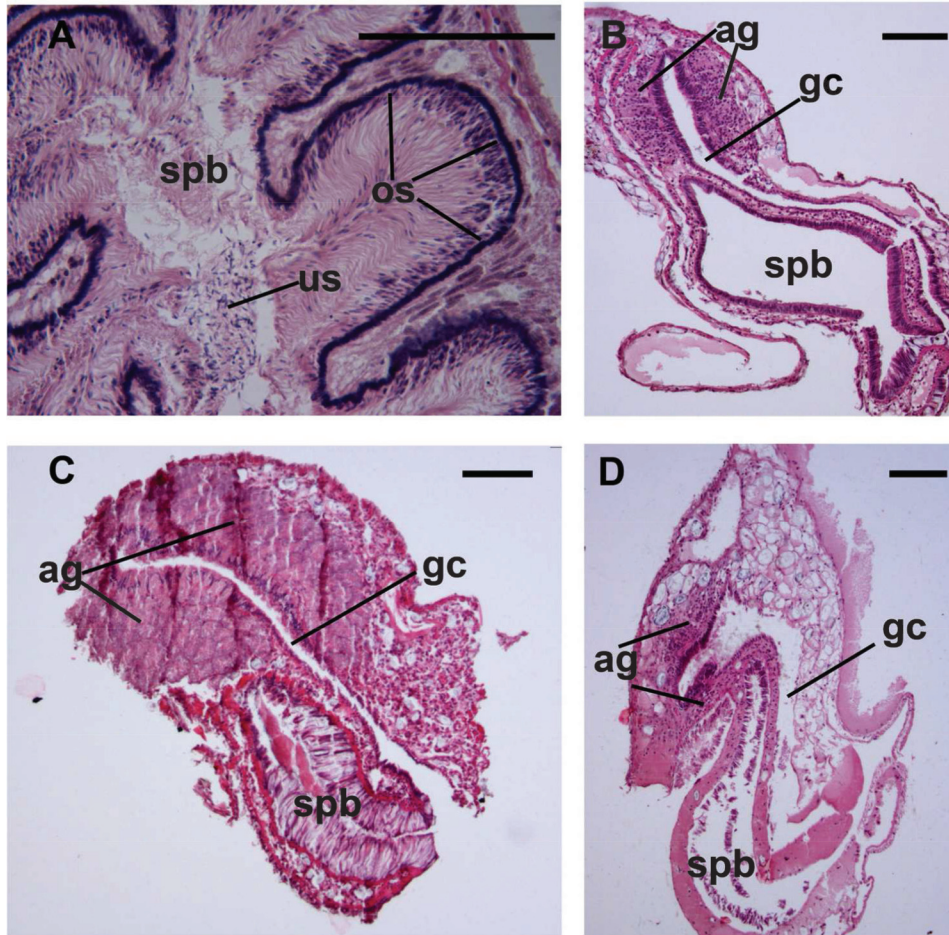
that the oviductal groove has been found to contain orientated sperm in other gastropods (e.g., Fretter 1941), the report of the presence of orientated sperm by Woodard (1934, 1935, 1940) was dismissed owing to its apparent presence within the gonoductal groove and the propensity in some of the historical literature to use the terms “bursa” and “receptaculum” interchangeably or indiscriminately. It is now clear, however, that the pocket in the medial lamina of pleurocerids is structurally homologous to the spermatophore bursa of other cerithioideans, but functionally fulfills the role of both a bursa and a seminal receptacle. Although it is not common for cerithioideans to lack seminal receptacle(s), all that do so (e.g., species of the genus *Tiphobia* Smith, 1880 (Paludomidae), some Pachychilidae, many Thiariidae) are currently understood to store only unorientated sperm (Starmühlner 1976; Köhler and Glaubrecht 2001, 2007; Strong and Glaubrecht 2006; Gomez et al. 2011). However, not all studies of cerithioidean reproductive anatomy are explicit as to the nature of sperm storage or it was not examined histologically; occasionally, the sperm pockets are depleted and do not allow an assessment of their contents. Certainly, given the present result, this bears closer scrutiny.

It is not clear what may have been the selective advantage of using the spermatophore bursa for the storage of orientated sperm versus retention of the seminal receptacle for this function. Furthermore, it is impossible to say if the seminal receptacle was lost first, followed by acquisition of the ability to store orientated sperm in the spermatophore bursa, or alternatively, if the facultative storage of orientated sperm in the bursa arose before the loss of the seminal receptacle. The bursa of semisulcospirids apparently functions only in receiving the spermatophore and in storing unorientated sperm (e.g., Nakano and Nishiwaki 1989; Rasshepkina 2000, 2009; Prozorova and Rasshepkina 2005, 2006), so is not informative in inferring how this transformation may have occurred. Rarely, small amounts of orientated sperm have been reported in the bursa of some other cerithioidean species

(Marcus and Marcus 1963, 1964; Houbriek 1993; Ponder 1993). At least in some cases, these seem to be localized patches in the bursa canal (the closed distal portion of the sperm gutter) and regions of the bursa adjacent to the entry of the sperm gutter (Marcus and Marcus 1964; Houbriek 1993; E.E. Strong, unpublished data). The relative scarcity of such observations suggests that this may be a transient occurrence that happens a short time after the breakdown of the spermatophore as a consequence of the “clumping” behavior observed by Woodard (1940). On the other hand, this also suggests that it is not unusual for the bursa to contain small quantities of orientated sperm, and thus, that the transition to utilizing the bursa for orientated sperm storage may not be difficult to achieve.

The hypothesis that pleurocerids store sperm from one reproductive season to the next (Dillon 2000; Hodgson 2010) is rejected by our data. Although relatively few species were investigated, there is no reason to suspect that this pattern is not broadly applicable to other temperate and subtropical species, but it should be explored further in tropical species. Sperm transfer from males to females has not been observed in pleurocerids, but Jones and Branson (1964) observed close female and male contact in the pyramid elimia (*Elimia potosiensis* (Lea, 1841)) that they hypothesized to be the mating process, shortly before females deposited eggs. This behavior has also been observed in captive populations of other species in the spring only weeks before egg-laying commenced (P.D. Johnson, personal communication, 2014). Given these observations and the evidence presented here that show motile, functional sperm appear to be absent from pleurocerid oviducts by early September and at least through November (Figs. 4B–4D), it is clear that females must obtain sperm from males each year. Our findings reveal important aspects of reproduction in a critically imperiled group of freshwater gastropods and future descriptive or comparative work on pleurocerids will

**Fig. 4.** Histological cross sections of internal female reproductive system of species of Pleuroceridae examined: (A) smooth hornsnail (*Pleurocera prasinata*) collected in May; (B) *P. prasinata* collected in late August; (C) round rocksnail (*Leptoxis ampla*) collected in late August; (D) skirted hornsnail (*Pleurocera pyrenella*) collected in November. ag, albumen gland; gc, gonoductal groove; os, orientated sperm; spb, spermatophore bursa; us, unorientated sperm. Scale bars = 100  $\mu$ m. Figure appears in colour on the Web.



be best done during the reproductive season to capture the full elaboration of pleurocerid reproductive anatomy.

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### References

- Albrecht, E.A., Carreño, N.B., and Castro-Vazquez, A. 1999. A quantitative study of environmental factors influencing the seasonal onset of reproductive behaviour in the South American apple snail *Pomacea canaliculata* (Gastropoda: Ampullariidae). *J. Mollusc. Stud.* **65**(2): 241–250. doi:10.1093/mollusc/65.2.241.
- Anderson, N.H., and Sedell, J.R. 1979. Detritus processing by macroinvertebrates in stream ecosystems. *Annu. Rev. Entomol.* **24**: 351–377. doi:10.1146/annurev.en.24.010179.002031.
- Ankel, W.F. 1925. Zur Befruchtungsfrage bei *Viviparus viviparus* L. *Beobachtungen über die erste Reifungsteilung des Eies*. *Senckenbergiana*, **7**: 37–54.
- Baur, B. 1998. Sperm competition in molluscs. In *Sperm competition and sexual selection*. Edited by T.R. Birkhead and A.P. Møller. Academic Press, London. pp. 255–306.
- Berrie, A.D. 1966. Growth and seasonal changes in the reproductive organs of *Lymnaea stagnalis* (L.). *J. Mollusc. Stud.* **37**(2): 83–92.
- Brusca, R.C., and Brusca, G.J. 2003. *Invertebrates*. 2nd ed. Sinauer Associates, Inc., Sunderland, Mass.
- Burch, J.B., and Tottenham, J.L. 1980. North American freshwater snails, species list, ranges, and illustrations. *Walkerana*, **3**: 1–215.
- Dazo, B.C. 1965. The morphology and natural history of *Pleurocera acuta* and *Goniobasis livescens* (Gastropoda: Cerithiacea: Pleuroceridae). *Malacologia*, **3**(1): 1–80.
- Dillon, R.T. 2000. *The ecology of freshwater molluscs*. Cambridge University Press, Cambridge.
- Dillon, R.T., Jr., McCullough, T.E., and Earnhardt, C.E. 2005. Estimates of natural allosperm storage capacity and self-fertilization rate in the hermaphroditic freshwater pulmonate snail, *Physa acuta*. *Invert. Reprod. Dev.* **47**(2): 111–115. doi:10.1080/07924259.2005.9652151.
- Fankboner, P.V., and Cameron, J.L. 1985. Seasonal atrophy of the visceral organs in a sea cucumber. *Can. J. Zool.* **63**(12): 2888–2892. doi:10.1139/z85-432.
- Fretter, V. 1941. The genital ducts of some British stenoglossan prosobranchs. *J. Mar. Biol. Assoc. U.K.* **25**(1): 173–211. doi:10.1017/S0025315400014375.
- Fretwell, S.D. 1972. *Populations in a seasonal environment*. Princeton University Press, Princeton, N.J.
- Futuyma, D.J. 2005. *Evolution*. 1st ed. Sinauer Associates, Inc., Sunderland, Mass.
- Giusti, F., and Selmi, M.G. 1985. The seminal receptacle and sperm storage in *Cochlostoma montanum* (Issel) (Gastropoda: Prosobranchia). *J. Morphol.* **184**(2): 121–133. doi:10.1002/jmor.1051840204.
- Glaubrecht, M., and Strong, E.E. 2004. Spermatophores of thalassoid gastropods (Paludomidae) in Lake Tanganyika, East Africa, with a survey of their occurrence in Cerithioidea: functional and phylogenetic implications. *Invertebr. Biol.* **123**(3): 218–236. doi:10.1111/j.1744-7410.2004.tb00157.x.
- Gomez, M.I., Strong, E.E., and Glaubrecht, M. 2011. Redescription and anatomy of the viviparous freshwater gastropod *Hemisinus lineolatus* (W. Wood, 1828)

- from Jamaica (Cerithioidea, Thiariidae). *Malacologia*, **53**: 229–250. doi:10.4002/040.053.0203.
- Harvey, B.C., and Hill, W.R. 1991. Effects of snails and fish on benthic invertebrate assemblages in a headwater stream. *J. N. Am. Benthol. Soc.* **10**(3): 263–270. doi:10.2307/1467599.
- Hill, W.R. 1992. Food limitation and interspecific competition in snail-dominated streams. *Can. J. Fish. Aquat. Sci.* **49**(6): 1257–1267. doi:10.1139/f92-141.
- Hodgson, A.N. 2010. Prosobranchs with internal fertilization. In *The evolution of primary sexual characters in animals*. Edited by J. Leonard and A. Cordoba-Aguilar. Oxford University Press, Oxford. pp. 121–145.
- Houbrick, R.S. 1973. Studies on the reproductive biology of the genus *Cerithium* (Gastropoda: Prosobranchia) in the western Atlantic. *Bull. Mar. Sci.* **23**(4): 875–904.
- Houbrick, R.S. 1988. Cerithioidean phylogeny. *Malacol. Rev.* **4**(Suppl.): 88–128.
- Houbrick, R.S. 1993. Phylogenetic relationships and generic review of Bittitiinae (Prosobranchia: Cerithioidea). *Malacologia*, **35**(2): 261–313.
- Hury, A.D., Koebel, J.W., and Benke, A.C. 1994. Life history and longevity of the pleurocerid snail *Elimia*: a comparative study of eight populations. *J. N. Am. Benthol. Soc.* **13**(4): 540–556. doi:10.2307/1467850.
- Jewell, D.D. 1931. Observations on reproduction in the snail *Goniobasis*. *Nautilus*, **44**(4): 115–119.
- Johnson, P.D., Bogan, A.E., Brown, K.M., Burkhead, N.M., Cordeiro, J.R., Garner, J.T., Hartfield, P.D., Lepitzki, D.A.W., Mackie, G.R., Pip, E., Tarpley, T.A., Tiemann, J.S., Whelan, N.V., and Strong, E.E. 2013. Conservation status of freshwater gastropods of Canada and the United States. *Fisheries* (Bethesda), **38**(6): 247–282. doi:10.1080/03632415.2013.785396.
- Jones, W.C., and Branson, B.A. 1964. The radula, genital system, and external morphology in *Mudalia potosienis* (Lea) 1841 (Gastropoda: Prosobranchiata: Pleuroceridae) with life history notes. *Trans. Am. Microsc. Soc.* **83**(1): 41–62. doi:10.2307/3224840.
- Kirkwood, T.B.L., and Shanley, D.P. 2005. Food restriction, evolution and ageing. *Mech. Ageing Dev.* **126**(9): 1011–1016. doi:10.1016/j.mad.2005.03.021. PMID: 15893805.
- Köhler, F., and Glaubrecht, M. 2001. Toward a systematic revision of the Southeast Asian freshwater gastropod *Brotia* H. Adams, 1866 (Cerithioidea: Pachychilidae): an account of species from around the South China Sea. *J. Mollusc. Stud.* **67**: 281–318. doi:10.1093/mollus/67.3.281.
- Köhler, F., and Glaubrecht, M. 2007. Out of Asia and into India: on the molecular phylogeny and biogeography of the endemic freshwater gastropod *Paracostoma* Cossmann, 1900 (Caenogastropoda: Pachychilidae). *Biol. J. Linn. Soc.* **91**: 627–651. doi:10.1111/j.1095-8312.2007.00866.x.
- Magruder, S.R. 1935. The anatomy of the fresh water prosobranchiate gastropod, *Pleurocera canaliculatum* (Say). *Am. Midl. Nat.* **16**: 883–912. doi:10.2307/2420225.
- Marcus, E., and Marcus, E. 1963. Mesogastropoden von der Küste São Paulos. *Abh. Math.-Naturwiss. Klasse*, **1**: 5–103.
- Marcus, E., and Marcus, E. 1964. On *Cerithium atratum* (Born, 1778) (Gastropoda: Prosobranchia). *Bull. Mar. Sci. Gulf Caribb.* **14**: 494–509.
- Martel, A., Larrivière, D.H., Klein, K.R., and Himmelman, J.H. 1986. Reproductive cycle and seasonal feeding activity of the neogastropod *Buccinum undatum*. *Mar. Biol.* **92**(2): 211–221. doi:10.1007/BF00392838.
- McShane, P.E., and Naylor, R. 1996. Variation and recruitment of *Haliotis iris* (Mollusca: Gastropoda). *N.Z. J. Mar. Freshw. Res.* **30**(3): 325–332. doi:10.1080/00288330.1996.9516719.
- Miller-Way, C.A., and Way, C.M. 1989. The life history of *Leptoxis dilatata* (Conrad) (Prosobranchia: Pleuroceridae) from the Laurel Fork River, West Virginia. *Am. Midl. Nat.* **122**(1): 193–198. doi:10.2307/2425695.
- Müller-Navarra, D., and Lampert, W. 1996. Seasonal patterns of food limitation in *Daphnia galeata*: separating food quantity and food quality effects. *J. Plankton Res.* **18**(7): 1137–1157. doi:10.1093/plankt/18.7.1137.
- Nakadera, Y., Blom, C., and Koene, J.M. 2014. Duration of sperm storage in the simultaneous hermaphrodite *Lymnaea stagnalis*. *J. Mollusc. Stud.* **80**(1): 1–7. doi:10.1093/mollus/eyt049.
- Nakano, D., and Nishiwaki, S. 1989. Anatomical and histological studies on the reproductive system of *Semisulcospira libertina* (Prosobranchia: Pleuroceridae). *Venus*, **48**: 263–273.
- Oppliger, A., Naciri-Graven, Y., Ribi, G., and Hosken, D.J. 2003. Sperm length influences fertilization success during sperm competition in the snail *Viviparus ater*. *Mol. Ecol.* **12**(2): 485–492. doi:10.1046/j.1365-294X.2003.01748.x. PMID:12535098.
- Ponder, W.F. 1972. The morphology of some mitriform gastropods with special reference to their alimentary and reproductive systems (Mollusca: Neogastropoda). *Malacologia*, **11**(2): 295–342.
- Ponder, W.F. 1973. The origin and evolution of the Neogastropoda. *Malacologia*, **12**(2): 295–338. PMID:4788271.
- Ponder, W.F. 1993. A new cerithiid from south Western Australia (Mollusca: Gastropoda: Caenogastropoda: Cerithiidae). In *Proceedings of the Fifth International Marine Biological Workshop: the Marine Flora and Fauna of Rott-*
- nest Island, Western Australia, Rottmest Island, January 1991. Vol. 1. Western Australian Museum, Perth. pp. 267–277.
- Ponder, W.F., and Lindberg, D.R. 1997. Towards a phylogeny of gastropod molluscs: an analysis using morphological characters. *Zool. J. Linn. Soc.* **119**(2): 83–265. doi:10.1111/j.1096-3642.1997.tb00137.x.
- Prozorova, L.A., and Rasshepkina, A.V. 2005. On the reproductive anatomy of *Semisulcospira* (Cerithioidea: Pleuroceridae: Semisulcospirinae). *Byulleten' Dal'nevostochnogo Malakologicheskogo Obshchestva*, **9**: 123–126.
- Prozorova, L.A., and Rasshepkina, A.V. 2006. On the radula and pallial gonoduct morphology of the gastropod *Biwamelania decipiens* and *B. multigranosa* (Cerithioidea: Pleuroceridae: Semisulcospirinae). *Byulleten' Dal'nevostochnogo Malakologicheskogo Obshchestva*, **10**: 130–132.
- Rasshepkina, A.V. 2000. Anatomy of the pallial oviduct of the genus *Hua* Chen (Pachychilidae, Cerithioidea). *Byulleten' Dal'nevostochnogo Malakologicheskogo Obshchestva*, **4**: 99–100.
- Rasshepkina, A.V. 2009. Reproductive anatomy of genus *Juga* (Gastropoda: Cerithioidea: Pleuroceridae) from South Korea. *Kor. J. Soil Zool.* **13**(1–2): 6–9.
- Richardson, J.S. 1991. Seasonal food limitation of detritivores in a montane stream: an experimental test. *Ecology*, **72**(3): 873–887. doi:10.2307/1940589.
- Richardson, T.D., Scheiring, J.F., and Brown, K.M. 1988. Secondary production of two lotic snails (Pleuroceridae: *Elimia*). *J. N. Am. Benthol. Soc.* **7**(3): 234–245. doi:10.2307/1467423.
- Roff, D. 2002. Life history evolution. Sinauer Associates, Inc., Sunderland, Mass.
- Rosewater, J. 1960. The egg mass and gross embryology of *Pleurocera canaliculata* (Say, 1821). *Annu. Rep. Am. Malacol. Union*, **26**(1): 10–12.
- Shanley, D.P., and Kirkwood, T.B.L. 2000. Calorie restriction and aging: a life-history analysis. *Evolution*, **54**(3): 740–750. doi:10.1111/j.0014-3820.2000.tb00076.x. PMID:10937249.
- Starmühlner, F. 1976. Beiträge zur Kenntnis der Süßwasser-Gastropoden pazifischer Inseln. *Ann. Naturhist. Mus. Wien B*, **80**: 473–656.
- Strong, E.E. 2003. Refining molluscan characters: morphology, character coding and a phylogeny of the Caenogastropoda. *Zool. J. Linn. Soc.* **137**(4): 447–554. doi:10.1046/j.1096-3642.2003.00058.x.
- Strong, E.E. 2005. A morphological reanalysis of *Pleurocera acuta* Rafinesque, 1831, and *Elimia livescens* (Menke, 1930) (Gastropoda: Cerithioidea: Pleuroceridae). *Nautilus*, **119**(4): 119–132.
- Strong, E.E., and Frest, T. 2007. On the anatomy and systematics of *Juga* from western North America (Gastropoda: Cerithioidea: Pleuroceridae). *Nautilus*, **121**: 43–65.
- Strong, E.E., and Glaubrecht, M. 2006. The morphology and independent origin of ovoviviparity in *Tiphobia* and *Lavigeria* (Caenogastropoda: Cerithioidea: Paludomidae) from Lake Tanganyika. *Organ. Divers. Evol.* **7**: 81–105. doi:10.1016/j.ode.2006.02.003.
- Strong, E.E., and Köhler, F. 2009. Morphological and molecular analysis of '*Melania jacqueti* Dautzenberg and Fischer, 1906: from anonymous orphan to critical basal offshoot of the Semisulcospiridae (Gastropoda: Cerithioidea). *Zool. Scr.* **38**(5): 483–502. doi:10.1111/j.1463-6409.2008.00385.x.
- Strong, E.E., Colgan, D., Healy, J., Lydeard, C., Ponder, W.F., and Glaubrecht, M. 2011. Phylogeny of the gastropod superfamily Cerithioidea using morphology and molecules. *Zool. J. Linn. Soc.* **162**(1): 43–89. doi:10.1111/j.1096-3642.2010.00670.x.
- Sweeney, B.W., and Vannote, R.L. 1986. Growth production of a stream stonefly: influences of diet and temperature. *Ecology*, **67**: 1396–1410. doi:10.2307/1938695.
- Thompson, F.G. 2011. An annotated checklist and bibliography of the land and freshwater snails of México and Central America. *Bull. Fla. Mus. Nat. Hist.* **50**: 1–299.
- Trüb, H. 1990. Züchtung von Hybriden zwischen *Viviparus ater* und *V. contectus* (Mollusca, Prosobranchia) im Zürichsee und ökologische Untersuchungen in einer gemischten Population im Gardasee. University of Zürich, Zürich, Switzerland.
- Vasconcelos, P., Moura, P., Barroso, C.M., and Gaspar, M.B. 2012. Reproductive cycle of *Bolinus brandaris* (Gastropoda: Muricidae) in the Ria Formosa lagoon (southern Portugal). *Aquat. Biol.* **16**(1): 69–83. doi:10.3354/ab00434.
- Webber, H.H., and Giese, A.C. 1969. Reproductive cycle and gametogenesis in the black abalone *Haliotis cracheroidii* (Gastropoda: Prosobranchiata). *Mar. Biol.* **4**(2): 152–159. doi:10.1007/BF00347041.
- Whelan, N.V., Johnson, P.D., and Harris, P.M. 2015. Life history traits and shell morphology in the snail genus *Leptoxis* Rafinesque, 1819 (Gastropoda: Cerithioidea: Pleuroceridae). *J. Moll. Stud.* In press. doi:10.1093/mollus/eyu058.
- Winsor, C.P. 1933. The eggs of *Goniobasis virginica* Gmelin and *Anculosa carinata* Bruguière. *J. Wash. Acad. Sci.* **23**(1): 34–36.
- Woodard, T.M. 1934. Anatomy of the reproductive system of *Goniobasis laqueata* (Say). *J. Tenn. Acad. Sci.* **9**(4): 243–259.
- Woodard, T.M. 1935. Spermic dimorphism in *Goniobasis laqueata* (Say). *J. Morphol.* **57**(1): 1–29. doi:10.1002/jmor.1050570102.
- Woodard, T.M. 1940. The function of the apyrene spermatozoa of *Goniobasis laqueata* (Say). I. The behavior of the apyrene and eupyrene spermatozoa under natural and artificial conditions. *J. Exp. Zool.* **85**(1): 103–125. doi:10.1002/jez.1400850106.