

Initiation, Calcification, and Form of Larval “Archaeogastropod” Shells

R. COLLIN^{1*} AND J. VOLTZOW²

¹Department of Zoology, University of Washington, Seattle, Washington

²Department of Biology, University of Puerto Rico, Rio Piedras Campus, San Juan, Puerto Rico

ABSTRACT The coiled shell of gastropods begins as a cap-shaped lens of organic and calcified material that covers the posterior dorsal side of the larva. During development the cap enlarges to cover the larval visceral mass. Marginal growth then produces the characteristic coiled shell. One model of the initiation of shell coiling in “archaeogastropods” requires that the shell remains flexible and uncalcified until after torsion, and that muscle contraction during torsion deforms the shell. We describe early shell calcification and tested this requirement of the model for the patellogastropod limpets *Tectura scutum* and *Lottia digitalis*, the trochids *Calliostoma ligatum* and *Margarites pupillus* and the abalone *Haliotis kamtschatkana*. We determined the stage of initial calcification by staining larvae with the fluorescent calcium marker calcein and observing them with bright field, crossed polarizing filter, and fluorescence microscopy. In *T. scutum* the earliest observable shell was calcified and calcium was sometimes detected even before the initial shell was visible. Larvae of the other species deposited a noncalcified matrix that was subsequently calcified, and in *C. ligatum* and *M. pupillus* this initial calcification was distinctly spotty. Shells of both patellogastropods and the abalone were demonstrably rigid prior to torsion while the shells of the trochids were not. These results suggest that shell coiling in patellogastropods and abalone is not initiated by contraction of the larval retractor muscle during torsion; in trochids this mechanism is possible. However, analysis of camera lucida drawings of pre- and post-torsional shells of *T. scutum* and *C. ligatum* did not detect shell shape changes during torsion. *J. Morphol.* 235:77–89, 1998.

© 1998 Wiley-Liss, Inc.

Rules governing the coiling and growth of gastropod shells have been of long-standing interest to morphologists and malacologists (reviewed in Illert, '87; Meinhardt, '95). Traditional mathematical models of shell shape use a small number of parameters to describe the shape as simply as possible (e.g., Moseley, 1838; Raup, '61). More recently, biologists have looked to more explicitly ontogenetic conceptual and mathematical models to explain shell shape (Bandel, '82, '86; Løvtrup and Løvtrup, '88; Ackerly, '89; and Morita, '93 and numerous others). Instead of focusing on describing the shape of the finished shell, authors of these models aim to gain insight into the ontogenetic processes that generate shells.

One such conceptual developmental model hypothesizes a mechanistic link between tor-

sion and shell coiling in “archaeogastropod” larvae (Bandel, '82; for a mathematical formalization of this idea, see Morita, '93). “Archaeogastropoda” refers to a paraphyletic assemblage of basal gastropods (Ponder and Lindberg, '96, '97) that usually includes some combination of the monophyletic vetigastropods, patellogastropods, neritids, and sometimes the extinct bellerophonitids (Thiele '29–'31; Hickman, '88; Haszprunar, '93; Ponder

Contract grant sponsor: Pacific Northwest Shell Club; Contract grant sponsor: National Science Foundation; Contract grant number: OCE 9301665; Contract grant sponsor: Conchologists of America and UPR-PIPI.

J. Voltzow is currently at the Department of Biology, University of Scranton, Scranton, PA 18510-4625

*Correspondence to: R. Collin, Committee on Evolutionary Biology, University of Chicago, Culver Hall, 1025 E. 57th Street, Chicago, IL 60637 (current address); E-mail: rcollin@midway.uchicago.edu

and Lindberg, '97). It is unclear which of these groups Bandel ('82) includes in "archaeogastropods." Here, for convenience, we use "archaeogastropod" to refer to patellogastropods and vetigastropods; a paraphyletic grade characterized by several developmental peculiarities. Torsion, the condition in which the adult shell and viscera are rotated 180 degrees with respect to the head and foot, is brought about by an observable ontogenetic process in "archaeogastropods" that is believed to be caused in part by the differential contraction of the larval retractor muscles (Crofts, '37, '55). This mechanism (i.e., the ontogenetic process, *not* the adult condition) is distinct from the process in caenogastropods in which organogenesis and differential growth gradually create a post-torsional arrangement of adult organs (D'Asaro, '69; Demian and Yousif, '73). Based on observations of four species of trochids, *Gibbula adansonii*, *G. drepanensis*, *G. divaricata* (Trochinae: Gibbulini), and *Cantharidus exasperatus* (Trochinae: Cantharidini), Bandel ('82) stated that the portion of the protoconch, or embryonic shell, that is secreted before torsion is composed entirely of flexible organic material. Contraction of the larval retractor muscles, which are anchored to the shell, combined with external pressure from the foot and operculum during torsion (Bandel, '82), is hypothesized to result in an asymmetrical deformation of the flexible organic shell. In this scenario, shell deformation is the first indication of coiling in the formerly symmetrical shell. Subsequent mineralization of the shell fixes this deformation, and marginal shell growth continues using the protoconch as a template for subsequent coiling. Bandel's hypothesis requires that calcification occurs subsequent to torsion, that the shell be flexible during torsion, and that the shapes of pre- and post-calcified shells differ.

Other published studies of molluscan development neither support nor reject this hypothesis. Because they do not focus primarily on calcification, most studies of "archaeogastropod" protoconchs comment on shell calcification superficially. Birefringence when the specimen is viewed between crossed polarizing filters is a criterion often used to determine calcification. Pechenik et al. ('84), however, have demonstrated that larval shells of the caenogastropod *Cymatium parthanopeum* are birefringent but contain negligible amounts of calcium. Organic

shell matrix, which contains ordered arrays of structural proteins, could itself be birefringent. To further add to the confusion, the terms larval shell, embryonic shell, primary shell, secondary shell, and protoconch are used inconsistently in the literature (see Bandel, '82), making it difficult to determine the actual developmental stage at which calcification begins. Finally, published scanning electron micrographs of "larval shells" often do not state when shells were collected relative to torsion, hatching, and other relevant developmental landmarks.

Some evidence supports the hypothesis that "archaeogastropod" shells are pliable at torsion and may be deformed by muscle contraction. Larval shells of the bivalve *Tridacna squamosa* are primarily organic when they are initially secreted. Only after the shells are calcified do the adductor muscles become active (LaBarbara, '74). This has been interpreted as an adaptation to prevent the muscle from deforming the flexible organic shell. Bandel's shell coiling hypothesis (Bandel, '82, '86) is also consistent with our general understanding of adult and larval shell deposition. In most cases of shell growth, an organic layer is deposited before calcification begins, and the leading edge is entirely organic (Wilbur and Saleuddin, '82). Ultrastructural studies suggest that this is also the case for larval shells of *Haliotis kamtschatkana* (Page, '97) and the pulmonate *Biomphalaria glabrata* (Bielefeld and Becker, '91). Additionally, protoconchs of several "archaeogastropod" species show a depression or crease on the lateral sides of the shell (Bandel, '82; Page, '97; R. Collin and J. Voltzow, personal observation). Bandel ('82) suggested that these depressions or folds are formed also by the deformation of the flexible shell at the attachment site of the larval retractor muscles.

Studies designed specifically to observe calcification often find that mineralization occurs earlier than expected based on other descriptions of development. Eyster and Morse ('84) and Eyster ('86) demonstrated that larval and embryonic shells of some nudibranchs and caenogastropods calcify as early as the "pre-veliger" stage. Bielefeld and Becker ('91) also found measurable calcium levels in shells of pulmonate embryos. This does not bear directly on Bandel's hypothesis because "archaeogastropods" are not closely related to these other groups (Ponder and Lindberg, '97), and nudibranchs, pulmo-

nates, and many caenogastropods are generally considered to have derived development compared to "archaeogastropods" (but see Page, '94, for an alternate view).

We examined the early events of larval shell secretion, calcification, and growth in several "archaeogastropods": two lottiid patellogastropods, two trochids, and a haliotid. The three main objectives of this study were to determine when shell calcification begins, when the shell becomes rigid, and if the shell changes shape during torsion. Our broader taxon sampling allows us to determine if Bandel's scenario based on his interpretation of the development of *Gibbula* spp. could apply to other "archaeogastropod" species as he suggests (Bandel, '82).

MATERIALS AND METHODS

Although "lower" prosobranch relationships are not resolved clearly, we used representatives of what are generally believed to be three distinct clades (see Fig. 1, for one suggested phylogeny) (Haszprunar, '88; Tillier et al., '94; Ponder and Lindberg, '96, '97). Adult patellogastropod limpets (*Tectura scutum* (Rathke, 1833) and *Lottia digitalis* (Rathke, 1833); Patellogastropoda: Lottiidae), topshells (*Calliostoma ligatum* (Gould, 1849) and *Margarites pupillus* (Gould, 1841); Vetigastropoda: Trochidae) and abalone (*Haliotis kamtschatkana* Jonas 1845; Vetigastropoda: Haliotidae) were collected from shallow water around San Juan Island, Washington, during 1995 and 1996. *Haliotis kamtschatkana*, *C. ligatum*, and *T. scutum* were spawned in the summers of 1995 and 1996 and *L. digitalis* and *M. pupillus* were spawned in the winter of 1995–1996. *Haliotis kamtschatkana* were induced to spawn using hydrogen peroxide according to the protocol suggested in Strathmann ('87). The other snails were spawned by placing them individually in dishes of filtered sea water and allowing them to warm to room temperature (Strathmann, '87). Eggs were collected and fertilized with a dilute sperm suspension. After fertilization, developing embryos were cultured at ambient sea temperature (8°–11°C in the winter and 11°–14°C in the summer) in small glass dishes according to the method outlined in Strathmann ('87). Embryos and larvae were observed periodically throughout development and at approximately 2-hr intervals during shell formation. All observations were repeated for at least 10 individuals from each of several cultures, from several females of each spe-

cies. The timing of events varied considerably with even small fluctuations in temperature, so our descriptions of calcification rely more on ontogenetic stages than absolute time after fertilization as landmarks for comparing relative timing of events.

Shell development and mineralization were observed with Nikon Optiphot-2 and Olympus BHS microscopes using bright-field, crossed polarizing filters, and fluorescence microscopy, and were photographed with TMAX 400 film. The fluorescent stain calcein (Molecular Probes; Eugene, Oregon, USA) was used to test for the presence of calcium in the shells. Calcein acts similarly to tetracycline by binding to calcium and becoming incorporated into calcium carbonate structures (Sun et al., '92; Managhan, '93). Because it is almost entirely specific to calcium and has fewer harmful effects than tetracycline (Managhan, '93), calcein is preferable for studies of development. Live embryos and larvae were incubated for at least 1 hr in finger bowls to which several drops of a stock solution of 0.2 mg/ml calcein in seawater had been added (approximately 1,000-fold dilution). They were then rinsed several times in filtered seawater and viewed as live whole mounts under a compound microscope with an ultraviolet (UV) light source and blue filters (excitation maximum of 495 nm). To compare different techniques for visualizing the shell, each animal was viewed with bright field, crossed polarizing filters, and fluorescence. Individual animals were photographed in the same orientation with each light source.

Because the presence of calcium in the shell does not necessarily imply that the shell is rigid, we independently tested for rigidity. Shell rigidity before and after torsion was determined by preparing wet mounts of live larvae and applying gentle pressure to the coverslip while observing the larvae through the microscope. Shells were considered to be rigid if they cracked or shattered. Those that crumpled and deformed before tearing were scored as flexible and potentially pliable.

To compare the shapes of pre- and post-torsional larval shells to determine if the shell shape had indeed changed during torsion, larvae of *Tectura scutum* and *Calliostoma ligatum* were drawn with a camera lucida. As a result of seasonal reproduction or scarcity of adults, we were unable to perform this analysis on larvae of the other

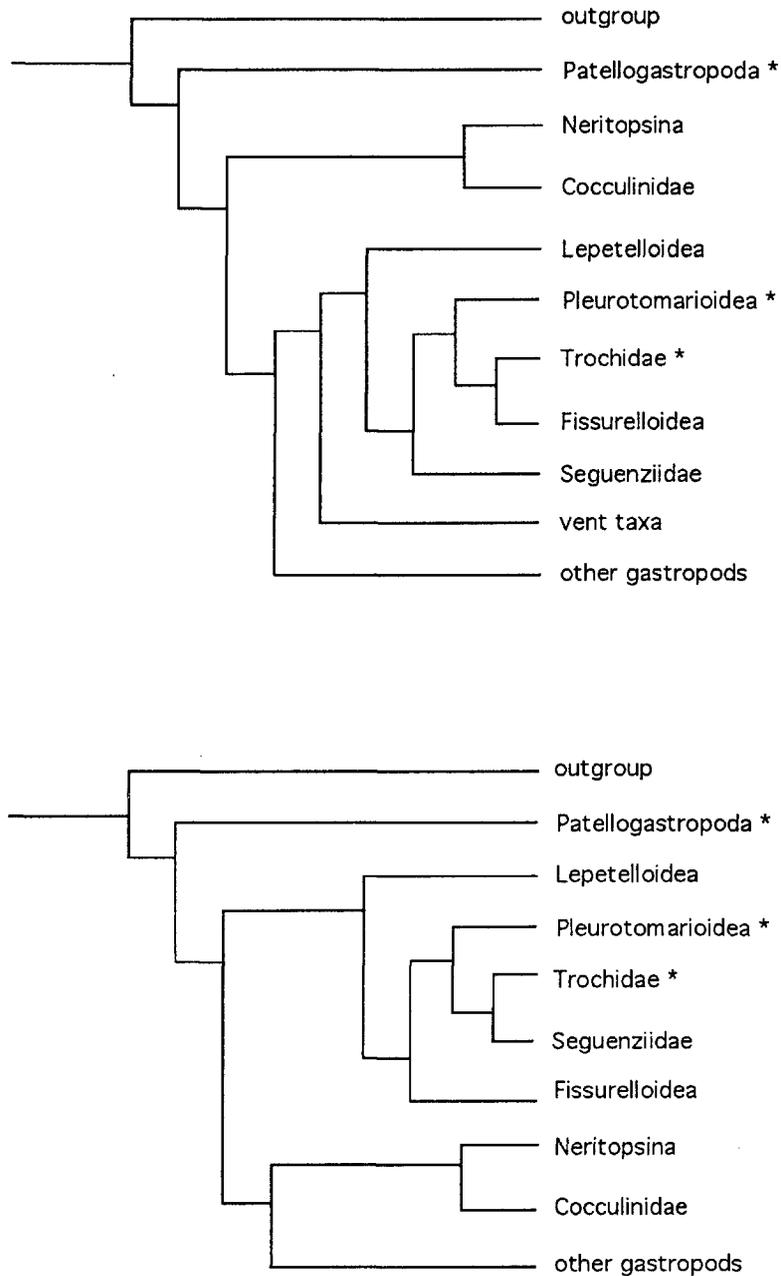


Fig. 1. Cladograms illustrating phylogenetic hypotheses of basal gastropod clades adapted from a strict consensus tree from the literature-based morphological analysis in Ponder and Lindberg ('96). *, clades represented in this study.

three species. Drawings of left, right, dorsal, and ventral orientations were digitized using a scanner. Outlines of shells of the same and of different stages were compared by

overlaying the digitized outlines using MacDraw II 1.1. Because there were no homologous landmarks between pre- and post-torsional shells, and because post-torsional

shells were larger, the shell apices were aligned by eye.

RESULTS

All of the larvae observed shared some general similarities in the pattern of shell secretion. Prior to shell secretion, the cells of the shell field invagination (SFI, see Eyster and Morse [84] for discussion of terminology) were visible as a ring of cells on the posterior dorsal side of the larva (Fig. 2). We first could detect the shell as a small, rounded, smooth lens or cap appressed to the larval body over the area of the SFI (Figs. 3, 4). The cap was birefringent and stained obviously with calcein (Figs. 3, 4), although it was difficult to detect with bright field microscopy. This initial cap steadily expanded around the edges and continued to grow until 1 or 2 days after torsion. As it expanded, the shell also thickened; calcein staining and birefringence grew stronger, sculpture became more obvious, and the shell became more opaque (Fig. 3). This thickening was generally more advanced on the older parts of the shell and calcification appeared to increase even in parts of the shell that were not connected to the mantle. All events except some of the thickening and sculpture development, but including substantial calcification over the entire shell, occurred before torsion, over the course of 1 or 2 days. Shells of the patellogastropods and abalone were rigid and cracked well before torsion, but the trochids' shells did

not become rigid until shortly (within a day) after torsion (Fig. 5). Specific differences in the development of the early larval shells in the five species observed are detailed below and summarized in Figure 6.

Trochophore larvae of *Tectura scutum* hatched before any sign of shell secretion was observed. Calcein staining was visible occasionally before the shell could be detected with either bright field or crossed polarized light. However, the initial shell cap was usually birefringent and visible with fluorescence beginning at about 28 hr after fertilization at 10°–12°C. This first shell showed the outline of the shell field cells (Fig. 4), giving the impression that either the edge of the shell lay below the cells of the shell field or calcium deposition corresponded to cell boundaries. Throughout subsequent shell growth, the area that stained with calcein was the same as the birefringent area. The edge of the fluorescence was sharp and distinct, and staining was uniform. The shell was demonstrably rigid and could be cracked easily prior to torsion, which began about 3.5 days after fertilization.

As in *Tectura scutum*, the trochophore larvae of *Lottia digitalis* hatched before shell secretion began. The earliest detected calcein staining (40–48 hr), which occurred when there was already a birefringent cap-shaped shell over the SFI, was slightly uneven and spotty. The most intense staining was at the center of the shell cap. The outlines of the cells of the SFI were not visible at the edge of the shell and calcein staining never occurred without birefringence. Subsequent events were as in *T. scutum*.

All the pre-torsional and much of the post-torsional development of *Calliostoma ligatum* occurred in the egg capsule; the details of initial shell secretion were quite different from those of the patellogastropods. When first observed, the cap of shell covering the SFI was evenly birefringent, but staining with calcein revealed about 20–30 large distinct spots (Fig. 3). Some areas stained brightly while others were dim and some did not stain at all. Calcein staining subsequently became uniform, but the area that stained with calcein clearly did not extend to the edge of the shell, which was birefringent and also visible with bright field illumination. This suggests that the proteinaceous portion of the shell extended past the area of calcification. Shell sculpture was very faintly visible well before torsion if the edge of the

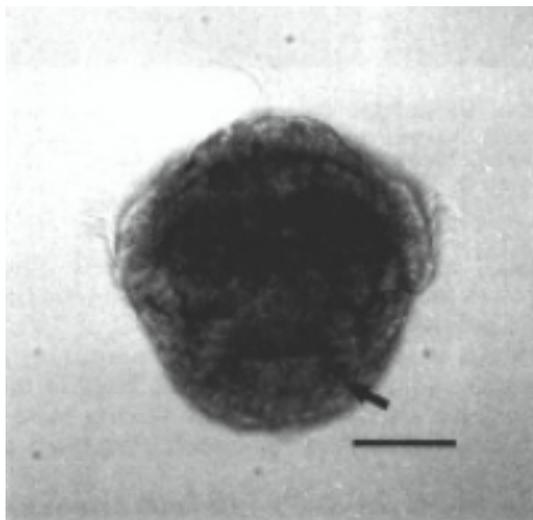


Fig. 2. Trochophore of *Tectura scutum*. Arrow, shell field invagination. Scale bar = 55 μ m.

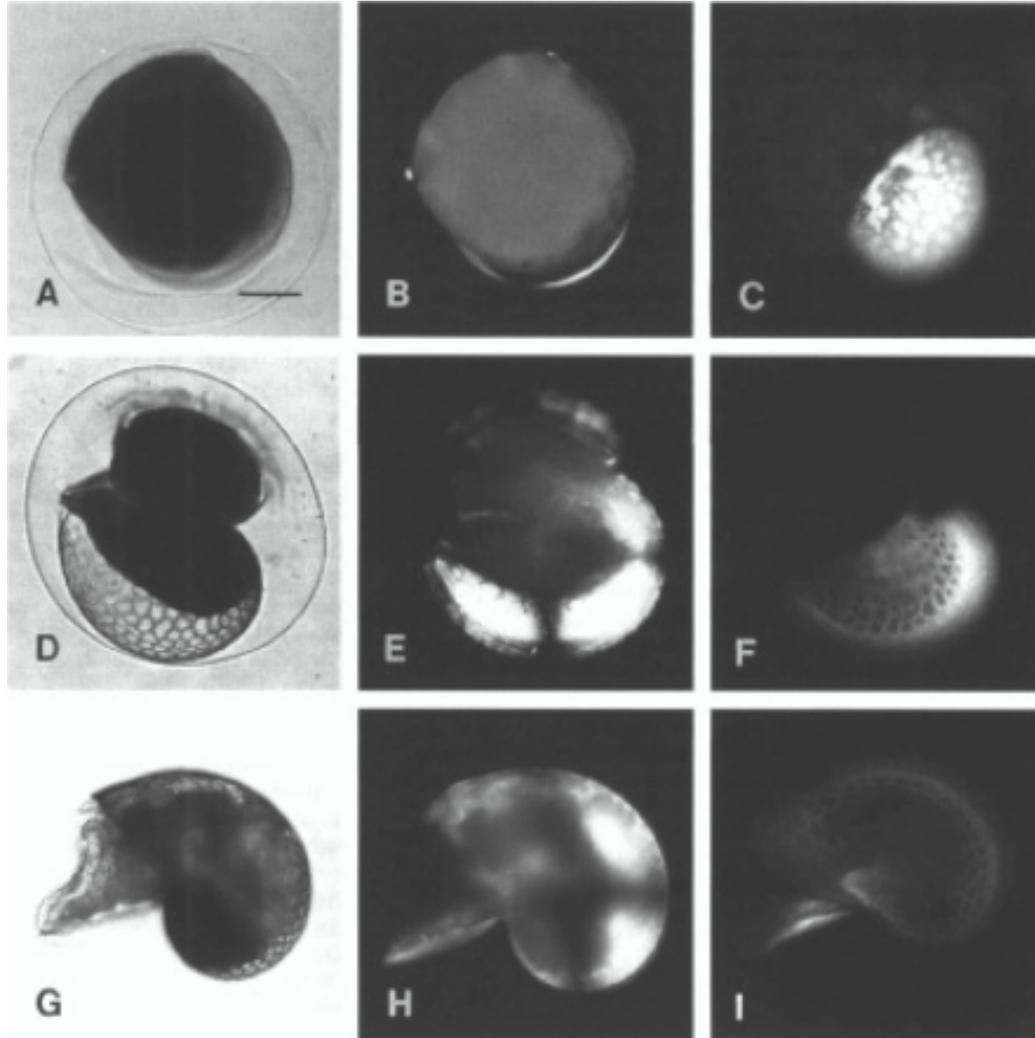


Fig. 3. Stages in the development of *Calliostoma ligatum*. A–C: Initial shell calcification. D–F: Late pre-torsional veliger. G–I: Post-torsional veliger viewed with

bright-field (A,D,G), polarized light (B,E,H), and fluorescence (C,F,I) microscopy. Photographs in each row are of the same individual larva. Scale bar = 75 μ m.

shell was viewed with polarizing filters. When pressure was applied to pre-torsional and mid-torsional shells (about 48–52 hr at 20°C, 3.5 days at 12°C), they often wrinkled or crumpled, and sometimes ripped along the edges of the reticulate sculpture (Fig. 5). Within a day of the completion of torsion, however, the shells cracked or shattered when compressed (Fig. 5).

Veligers of *Margarites pupillus* hatched prior to torsion, but shell secretion began before hatching. The initial shell was de-

tected after about 48 hr at 8–9°C. Shell growth and calcification were similar to that of *Calliostoma ligatum* except that initial calcein staining showed more numerous smaller spots. The shell was flexible and crumpled easily prior to and during torsion (72 hr) but became rigid soon afterward, as in *C. ligatum*.

Larvae of the abalone *Haliotis kamtschaticana* varied in the stage at which they hatched. Animals in most cultures hatched as trochophores, but a few hatched after

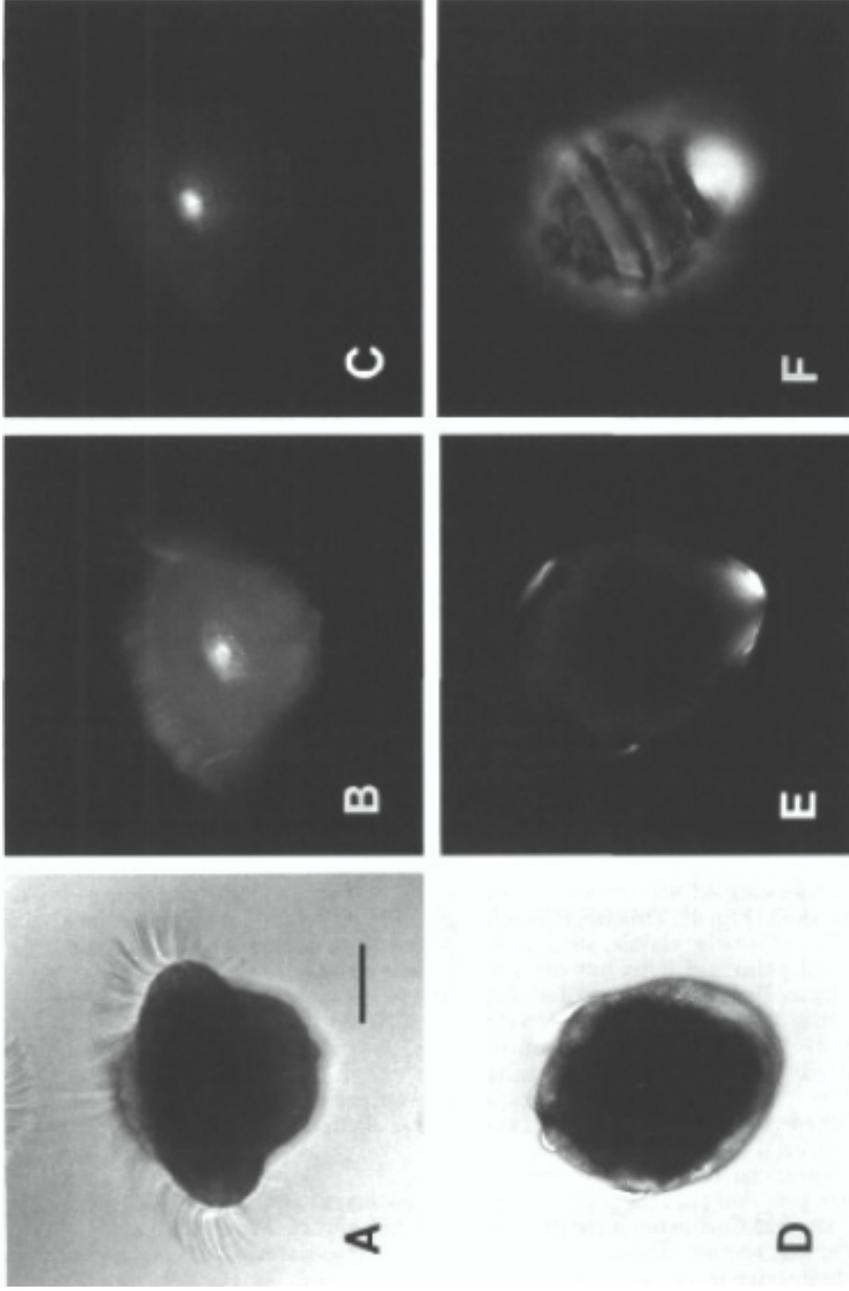


Fig. 4. Initial shell calcification in *Tectiura scutum* (A-C) and *Haliotis kamitschatkana* (D-F), viewed with bright-field (A,D), polarized light (B,E), and fluorescence (C,F) microscopy. Larvae of *H. kamitschatkana* autofluoresce. Photographs in each row are of the same larva. Scale bar = 60 μm .

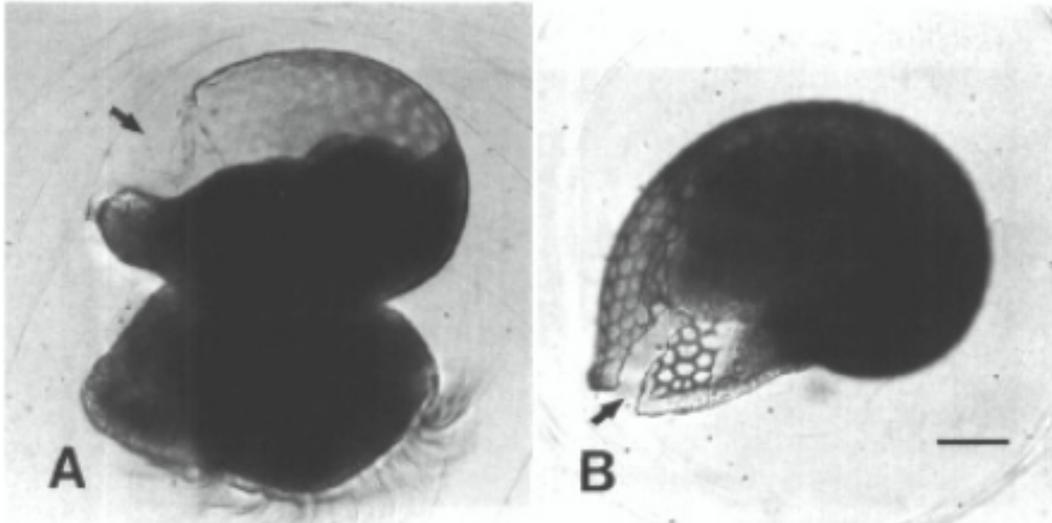


Fig. 5. Crumpled and cracked larval shells of *Calliostoma ligatum*. **A**: Crumpled shell of a mid-torsional animal. **B**: Cracked shell of a post-torsional animal. Scale bar = 50 μ m.

considerable shell growth. However, events of shell formation were the same, regardless of the stage at hatching. Unlike the other species studied, live, unstained abalone larvae autofluoresced when viewed with fluorescence microscopy (Fig. 4F). The SFI of an abalone larva was a large open depression that was more obvious than in the other species. After 40 hr at 11°–12°C, when the first shell material was visible, the SFI appeared to have everted. Instead of being a depression, the area of the SFI bulged slightly and was capped with the earliest portion of the shell (Fig. 4). This initial shell over the SFI was easily visible with both bright-field and polarized light but did not stain with calcein. By the time it had doubled in diameter the entire shell stained with calcein (Fig. 4). Unlike the case of *Tectura scutum*, the edges of the calcein staining were indistinct. The shell was also rigid and could be cracked prior to torsion, which occurred after about 3 days.

There appeared to be no differences in shape between pre- and post-torsional shells of either the trochid *Calliostoma ligatum* or the limpet *Tectura scutum* (Figs. 7, 8). Comparisons of shell shape within a stage showed little variation for either lateral view of *C. ligatum* (pre-torsional: right, $n = 7$; left, $n = 5$. post-torsional: right, $n = 13$; left, $n = 13$) or *T. scutum* (pre-torsional: dorsal, $n = 5$; ventral, $n = 8$; right, $n = 6$; left, $n = 6$;

post-torsional: right, $n = 8$; left, $n = 4$; dorsal, $n = 9$; ventral, $n = 6$). Drawings of dorsal and ventral views of *C. ligatum* were so highly variable within a stage that subsequent among-stage comparisons were meaningless. This variation was probably due to the difficulty of positioning slightly laterally flattened larvae with dorsal or ventral sides up.

Comparisons of pre- and post-torsional shapes showed that post-torsional shells were larger and that, except for newly grown shell, the shapes had not changed (Figs. 7, 8). Late post-torsional shells appeared more coiled than earlier stages because they had grown considerably around the aperture. They also appeared to be more strongly coiled because the lip became elaborated and thickened. For all views of both species, however, the range of variation among outlines of pre-torsional shells was coincident with the range of post-torsional shells.

DISCUSSION

The larval shells described here calcified at much earlier stages than previous studies of "archaeogastropod" development have suggested (Iwata, '80; Bandel, '82). Shells of all five species contained calcium not only before torsion, but also before the larvae had clearly identifiable feet or velar lobes. Although the literature suggests that calcification occurs later than this in "archaeogastro-

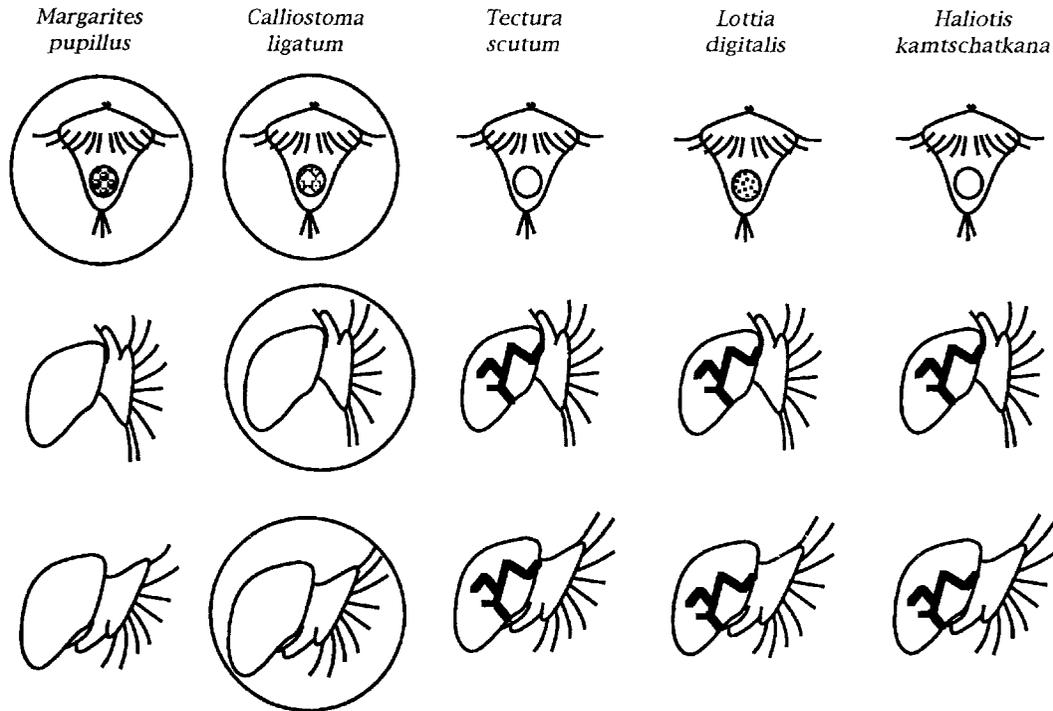


Fig. 6. Diagram summarizing the stage at hatching and the stage at which the shell became rigid in the five species observed in this study. The upper row represents the trochophore stages, the middle row pre-torsional veligers, and the bottom row post-torsional veligers. Stippling on the early shell at the trochophore stage

represents larvae that showed blotchy calcein staining of the early shell. The circle around a larva indicates that it is still in the egg capsule. A thick jagged line across the shell indicates that the shell at that stage cracked when pressure was applied.

Pods" (Iwata, '80) many studies employed polarized light to look for calcium and may have overlooked weak birefringence (Bandel, '82; Hadfield and Strathmann, '90). Our results are consistent with Eyster and Morse ('84), and Eyster ('86) in that calcification occurred at the "pre-veliger" stage. Their results are particularly convincing because they used observations made with polarized light as well as scanning electron microscope analysis to examine a variety of nudibranchs (*Dendronotus frondosus*, *Aeolidia papillosa*, and *Hermisenda crassicornis*), caenogastropods (*Crepidula fornicata*, *Crepidula convexa*, and *Ilyanassa obsoleta*), and the bivalve *Spisula solidissima*. Bielefeld and Becker ('91) also combined polarized light microscopy and energy-dispersive X-ray analysis to detect calcification in early embryos of *Biomphalaria glabrata*.

Vagueness in the literature regarding the stage of initial calcification of larval shells also may be due to the different methods

used to assess calcification. We found that those structures that stained with calcein were not always birefringent under polarized light. For example, the shell gland of *Tectura scutum* sometimes stained with calcein before birefringence could be detected. Birefringence of the initial shell was often faint and depended on the orientation of the larva. Additionally, structures that were birefringent under polarized light did not always stain with calcein. The initial shell lenses of *Haliotis kamtschatkana* and *Calliostoma ligatum* showed areas that were clearly birefringent but did not stain with calcein. Additional confusion may be due to terminological ambiguity, when referring to stages of development. For example, Iwata ('80) figures larvae of the abalone *Haliotis discus* that are clearly pre-torsional but states in his developmental timetable that they had already undergone torsion.

The results reported here can be interpreted in the context of previous studies of

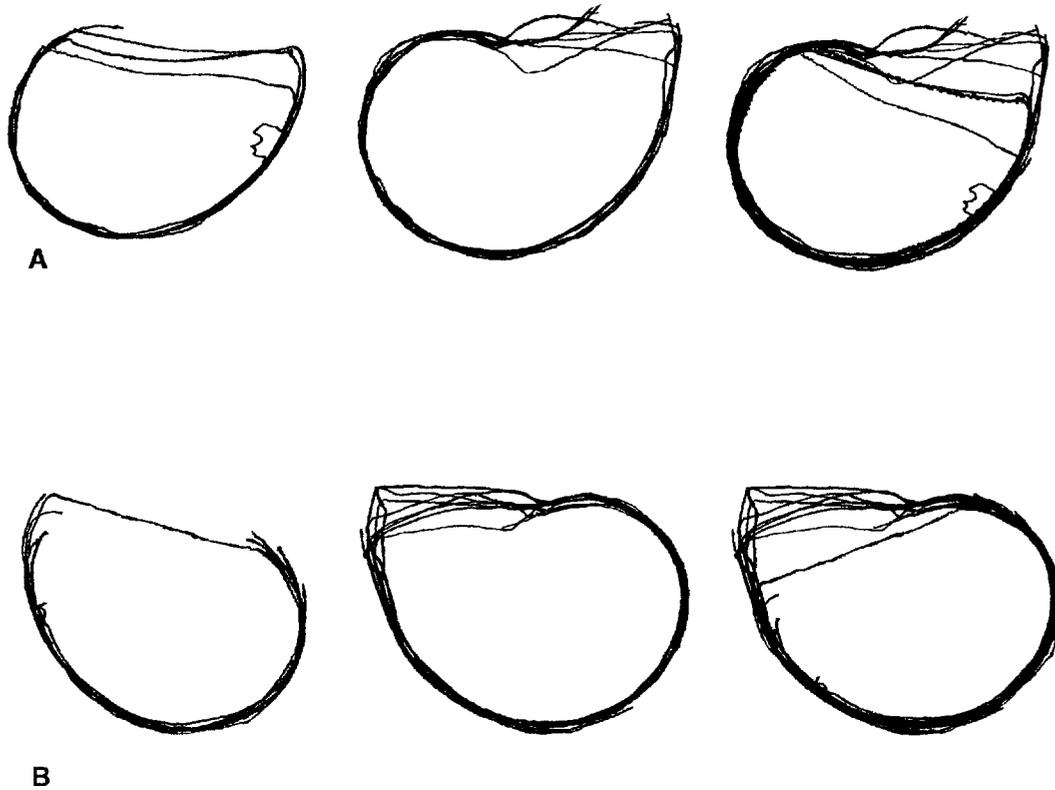


Fig. 7. Comparison of pre- and post-torsional larval shell shapes for larvae of *Calliostoma ligatum*. Overlays of right (A) and left (B) views of, from left to right, pre-torsional shells, post-torsional shells, and combined

pre- and post-torsional shells. All outlines were drawn at the same magnification (pre-torsional: right, $n = 7$; left, $n = 5$; post-torsional: right, $n = 13$; left, $n = 13$).

early shell calcification. Scanning electron microscope studies of early calcification in *Haliotis discus*, *H. kamtschatkana*, and *Neptunea arthritica* (a buccinid) (Iwata, '80; Togo et al., '91; Page, '97) show that calcification does not extend to the very edge of the organic shell. Our observation that the area of shell that stained with calcein was noticeably smaller than the visible shell in *Calliostoma ligatum* is consistent with this result. Additionally, Iwata ('80) and Togo et al. ('91) found that calcification was initiated on organic spherules on the inside of the organic shell. In *H. discus* these spherules are less than 2 μm (usually 0.5–1.0 μm) in diameter and appear to be densely distributed over the organic shell cap (Iwata, '80). Because neither Iwata's study nor this study combined SEM and calcein staining it is difficult to know how the same structure would appear when observed with these different methods. However, it seems unlikely that a

densely distributed layer of 1- μm spherules could be easily resolved with fluorescence microscopy on whole mounts. Therefore it is not surprising that calcein staining of initial larval shells of *H. kamtschatkana* and *Tectura scutum* appeared uniform. It does suggest, however, that the distinctly spotty staining of early shells of the trochids *C. ligatum* and *Margarites pupillus* represents large, widely separated centers of calcification. Detailed scanning electron microscopy studies are necessary to determine whether this is in fact the case. Bielefeld and Becker ('91) detected spotty early calcification of the pulmonate *Biomphalaria glabrata* using energy-dispersive X-ray analysis. However they reported that their fixation protocol seemed to "dislocate" calcium in the shell.

The development and elaboration of calcified sculpture on "archaeogastropod" protoconchs does not seem to depend on a close association of the mantle and the shell. The

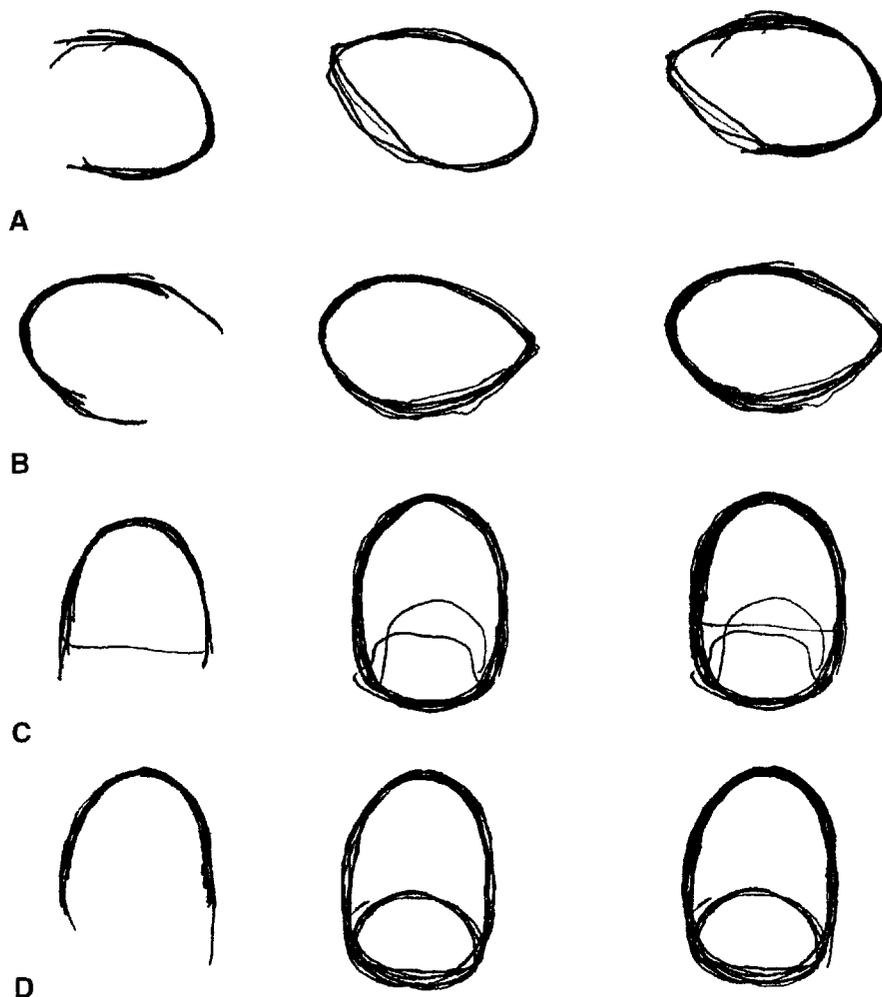


Fig. 8. Comparison of pre- and post-torsional larval shell shapes for *Tectura scutum*. Overlays of left (A), right (B), dorsal (C), and ventral (D) views of, from left to right, overlays of pre-torsional, post-torsional, and combined pre- and post-torsional shells. All outlines

were drawn at the same magnification (pre-torsional: dorsal, n = 5; ventral, n = 8; right, n = 6; left, n = 6; post-torsional: right, n = 8; left, n = 4; dorsal, n = 9; ventral, n = 6).

shell is obviously closely connected to the mantle at the shell aperture, and the visceral lobe and larval retractor muscles attach to the apical portion of the shell. However, during all but the earliest phase of shell formation, most of the shell is free of the larval body (Fig. 3). Electron microscopic studies have shown that there is not even a thin epithelial lining inside the larval shell of the abalone *Haliotis kamtschatkana* (Page, '97; L. Page, personal communication). This implies that the development and thickening of shell sculpture visible on the shell

body and apex in all the species observed in this study is independent of the mantle edge. This suggests that the organic shell matrix may act as a template, and that calcium is secreted into the space between the larval body and the shell and then passively precipitates onto the inside of the organic shell. The mechanisms of sculpture formation for both larval and adult gastropod shells are almost entirely unknown.

Our observations show that Bandel's ('82) hypothesis that deformation of the flexible larval shell initiates shell coiling is not appli-

cable to all "archaeogastropods." However, the shells of the trochids *Calliostoma ligatum* and *Margarites pupillus* were flexible at torsion, despite being calcified. This agrees with observations of four other trochid species (Bandel, '82) and suggests some explanations for the variation among "archaeogastropod" taxa observed in this study. Based on his observations of freshwater gastropods, Riedel ('93) suggested that gastropods that have extended encapsulated or viviparous development tend to calcify relatively late in development. Thus, the extent and timing of calcification may depend on developmental type and stage at hatching. *Calliostoma ligatum*, *M. pupillus*, and three of the trochid species studied by Bandel ('82) hatch at a later developmental stage than either the patellogastropods or abalone observed in this study. *Calliostoma ligatum* was the only species in this study whose larvae hatch after torsion and *Gibbula adansonii*, *G. drepanensis*, and *Cantharidus exasperatus* hatch either as pediveligers or juveniles (Bandel, '82). This could explain Bandel's observations of the three species with direct development. However, he also observed a flexible larval shell in *G. divaricata*, in which trochophores hatch prior to shell secretion (Bandel, '82). Increased sampling among "archaeogastropod" species with early and late hatching larvae is necessary to test for an association between stage at hatching and stage at which the shell becomes rigid.

Bandel ('82) extrapolated from his observations of development of two trochid genera to all "archaeogastropods" and even ammonites (Bandel, '86). In the present study we increased the breadth of taxonomic sampling and showed that representatives of three distinct "archaeogastropod" clades had calcified larval shells prior to torsion. However, the larval shell of the two trochid species remained flexible until the completion of torsion. Our data on *C. ligatum* and *M. pupillus* combined with Bandel's ('82) observations of *Gibbula* spp. and *Cantharidus exasperatus* suggest that the presence of larval shells that remain flexible until after torsion may be widespread within the Trochidae. *Calliostoma* and *Margarites* represent trochid tribes that are more basal and more derived than the tribes containing *Gibbula* and *Cantharidus* (Hickman, '96). Our observations, however, do not include representatives of the most derived trochid clades. Turbinids (basal trochoideans; see cladistic

analysis in Hickman, '96) and more derived trochids need to be examined to determine whether shells that remain flexible until torsion are characteristic of the Trochidae. Wider taxonomic representation including turbinids, fissurellids, and non-lottiid patellogastropods, as well as less well known "archaeogastropod" groups is necessary to identify clades for which the conditions of Bandel's ('82) hypothesis are met.

ACKNOWLEDGMENTS

We thank T. Anderson, N. Phillips, and C. Hammerstrom for collecting the abalone, G. and M. Koch for allowing us access to their property, and A. Beckmann for helping translate Bandel's work. This manuscript benefited from the comments of A. Kohn, B. Pernet, and R. Strathmann. We are grateful to the faculty and staff of Friday Harbor Laboratories for providing the use of their facilities, and L. Page (Department of Biology, University of Victoria, Victoria, BC V8W 2Y2) for allowing us to cite a personal communication. Support for this research was provided by a Pacific Northwest Shell Club grant and by a National Science Foundation predoctoral fellowship (to R.C.) NSF grant OCE 9301665 to R. Strathmann, and by awards from the Conchologists of America and UPR-FIPI (to J.V.).

LITERATURE CITED

- Ackerly, S.C. (1989) Kinematics of accretionary shell growth, with examples from brachiopods and molluscs. *Paleobiology* 15:147-164.
- Bandel, K. (1982) Morphologie und Bildung der frühontogenetischen Gehäuse bei conchiferen mollusken. [Translation by A. Beckmann and R. Collin.] *Facies* 7:1-198.
- Bandel, K. (1986) The ammonitella: a model of formation with the aid of the embryonic shell of archaeogastropods. *Lethaia* 19:171-180.
- Bielefeld, U., and W. Becker (1991) Embryonic development of the shell in *Biomphalaria glabrata* (Say). *Int. J. Dev. Biol.* 35:121-131.
- Crofts, D.R. (1937) The development of *Haliotis tuberculata* with special reference to organogenesis during torsion. *Philos. Trans. R. Soc. Lond. B* 228:219-268.
- Crofts, D.R. (1955) Muscle morphogenesis in primitive gastropods and its relation to torsion. *Proc. Zool. Soc. Lond.* 125:711-750.
- D'Asaro, C.N. (1969) The comparative embryogenesis and early organogenesis of *Bursa corrugata* Perry and *Distorsio clathrata* Lamarck (Gastropoda: Prosobranchia). *Malacologia* 9:349-389.
- Demian, E.S., and F. Yousif (1973) Embryonic development and organogenesis in the snail *Marisa cornuarietis* (Mesogastropoda: Ampullariidae). I. General outlines of development. *Malacologia* 12:123-150.
- Eyster, L.S. (1986) Shell inorganic composition and onset of shell mineralization during bivalve and gastropod embryogenesis. *Bio. Bull.* 170:211-231.

- Eyster, L.S., and M.P. Morse (1984) Early shell formation during molluscan embryogenesis with new studies on the surf clam *Spisula solidissima*. *Am. Zool.* 24:871–882.
- Hadfield, M.G., and M.F. Strathmann (1990) Heterostrophic shells and pelagic development in trochoideans: Implications for classification, phylogeny and palaeoecology. *J. Moll. Stud.* 56:239–256.
- Haszprunar, G. (1988) On the origin and evolution of major gastropod groups, with special reference to the Streptoneura. *J. Moll. Stud.* 54:367–441.
- Haszprunar, G. (1993) The Archaeogastropods: a clade, a grade or what else? *Am. Malacol. Union Bull.* 10:165–177.
- Hickman, C.S. (1988) Archaeogastropod evolution, phylogeny and systematics: A re-evaluation. *Malacol. Rev., Supplement* 4:17–34.
- Hickman, C.S. (1996) Phylogeny and patterns of evolutionary radiation in trochoidean gastropods. In J.D. Taylor (ed): *Origin and Evolutionary Radiation of the Mollusca*. Oxford: Oxford University Press, pp. 177–198.
- Illert, C. (1987) Formulation and solution of the classical seashell problem. I. Seashell geometry. *Il Nuovo Cimento* 9:791–813.
- Iwata, K. (1980) Mineralization and architecture of the larval shell of *Haliotis discus hannai* Ino (Archaeogastropoda). *J. Fac. Sci. Hokkaido Univ. Ser. IV* 19:305–320.
- LaBarbara, M. (1974) Calcification of the first larval shell of *Tridacna squamosa* (Tridacnidae: Bivalvia). *Mar. Biol.* 25:233–238.
- Løvtrup, S., and M. Løvtrup (1988) The morphogenesis of molluscan shells: A mathematical account using biological parameters. *J. Morphol.* 197:53–62.
- Managhan, J.P. (1993) Comparison of calcein and tetracycline as chemical markers in summer flounder. *Trans. Am. Fish. Soc.* 122:298–301.
- Meinhardt, H. (1995) *The Algorithmic Beauty of Sea Shells*. Berlin: Springer-Verlag.
- Morita, R. (1993) Development mechanics of retractor muscles and the “dead spiral model” in gastropod shell morphogenesis. *Neues Jahr. Geol. Palaontol. Abh.* 190:191–217.
- Moseley, H. (1838) On the geometrical forms of turbinated and discoid shells. *Philos. Trans. R. Soc. Lond.* 1838:351–370.
- Page, L.R. (1994) The ancestral gastropod larval form is best approximated by hatching-stage opisthobranch larvae: Evidence from comparative developmental studies. In W.H. Wilson, S.A. Stricker, and G.L. Shinn (eds): *Reproduction and Development of Marine Invertebrates*. Baltimore: Johns Hopkins University Press, pp. 206–223.
- Page, L.R. (1997) Ontogenetic torsion and protoconch form in the archaeogastropod *Haliotis kamtschakana*: Evolutionary implications. *Acta Zool.* 78:227–245.
- Pechenik, J.A., R.S. Scheltema, and L.S. Eyster (1984) Growth stasis and limited shell calcification in larvae of *Cymatium parthenopeum* during trans-Atlantic transport. *Science* 224:1097–1099.
- Ponder, W.F., and D.R. Lindberg (1996) Gastropod phylogeny—Challenges for the 90s. In J.D. Taylor (ed): *Origin and Evolutionary Radiation of the Mollusca*. Oxford: Oxford University Press, pp. 135–154.
- Ponder, W.F., and D.R. Lindberg (1997) Towards a phylogeny of gastropod molluscs: An analysis using morphological characters. *Zool. J. Linn. Soc.* 119:83–265.
- Riedel, R. (1993) Early ontogenetic shell formation in some freshwater gastropods and taxonomic implications of the protoconch. *Limnologica* 23:349–368.
- Raup, D.M. (1961) The geometry of coiling in gastropods. *Proc. Natl. Acad. Sci. USA* 47:602–609.
- Strathmann, M. (1987) *Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast*. Seattle: University of Washington Press, 670 pp.
- Sun, T.C., S. Mori, J. Roper, C. Brown, T. Hooser, and D.B. Burr (1992) Do different fluorochrome labels give equivalent histomorphometric information? *Bone* 13:443–446.
- Thiele, J. (1929–31) *Handbuch der Systematischen Weichtierkunde*. Vol. 1. Jena: Gustav Fischer Verlag.
- Tillier, S., M. Masselot, J. Guerdoux, and A. Tillier (1994) Monophyly of major gastropod taxa tested from partial 28S rRNA sequences, with emphasis on Euthyneura and hot-vent limpets Peltospiroidea. *Nautilus, Suppl.* 2:122–140.
- Togo, Y., S. Suzuki, K. Iwata, and S. Uozumi (1991) Larval shell formation and mineralogy in *Neptunea arthritica* (Bernardi) (Neogastropoda: Buccinidae). In S. Suga and H. Nakahara (eds): *Mechanism and Phylogeny of Mineralization in Biological Systems*. Tokyo: Springer-Verlag, pp. 151–155.
- Wilbur, K.M., and A.S.M. Saleuddin (1983) Shell formation. In A.S.M. Saleuddin and K.M. Wilbur (eds): *The Mollusca*. Vol. 4: Physiology. Part 1. San Diego: Academic Press, pp. 235–287.