The Egg Capsules, Embryos, and Larvae of *Cancellaria cooperi* (Gastropoda: Cancellariidae)

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ABSTRACT

The egg capsules, and the embryonic and larval development of the cancellariid gastropod *Cancellaria cooperi* Gabb are described. Egg capsules are spatulate in form, having long, narrow stalks that support the eggs above the surrounding sand. Egg capsules contain 4,000–5,000 eggs (165 μm in diameter), which undergo typical prosobranch development to hatch as planktotrophic veligers after 27 days at 15 °C. Larvae in culture grew from 305 μm to 890 μm in shell length over 30 days, but died before metamorphosis. Limited comparative data suggest that long stalked egg capsules are known only in members of the Cancellariinae, that opercula, absent in all adult Cancellariidae, are present or prominent in the late larval stages of at least some species, and that developmental type cannot be inferred from protoconch morphology using the criteria of Shuto (1974) in a majority of cancellariid species.

Key words: Reproduction; development; larvae; eggs; egg capsules; Cancellariidae; *Cancellaria*.

INTRODUCTION

Little is known about the reproductive biology and early development of most of the approximately 200 species that constitute the neogastropod family Cancellariidae. The few published reports (Mörch, 1869; Thorson, 1935, 1944; Knudsen, 1950; MacGinitie, 1955; Kilburn & Rippey, 1982; Bouchet & Warén, 1985) are limited to descriptions of egg capsules and, in some cases, ova or larval shells attributed to cancellariids, usually on the basis of the proximity of living snails.

During studies of the diet and feeding behavior of *Cancellaria cooperi* Gabb, 1865, a species that is attracted to and specifically parasitizes the Pacific electric ray *Torpedo californica* Ayres, 1855 (O'Sullivan *et al.*, 1987), a number of these snails were observed producing egg capsules. The present study supplements our knowledge of the natural history of this cancellariid with descriptions of its egg capsules and embryonic and larval development, and reviews the available data on the reproductive biology and larval development of the Cancellariidae.

MATERIALS AND METHODS

Twenty-three specimens of *Cancellaria cooperi* were collected on the artificial reef "Torrey Pines #1", off San Diego, CA (32°53′12″N, 117°50′50″W) at depths of 20–22 m using SCUBA. The animals were maintained in an aquarium containing sufficient sand for complete snail burial (4–8 cm depth) and supplied with a continuous flow of seawater (12–16 °C). Snails were allowed to feed on electric rays on a bimonthly basis, and had been maintained in this manner for at least 6 months prior to the onset of oviposition.

Individual egg capsules were freed from the aquarium bottom and maintained in beakers containing continuously aerated, 1 μm filtered seawater at 15 °C. Developing embryos were removed with a glass pipette through an incision cut along the narrow edge of the egg capsule. Hatched larvae of *Cancellaria cooperi* were cultured following procedures described by Paige (1986), except that 1 μm filtered natural seawater containing 40 mg/liter each of the antibiotics streptomycin sulfate and sodium penicillin G was used instead of artificial seawater. Larvae were fed a mixture of the green flagellates *Isochrysis galbana* (Park, 1949) and *Pavlova lutheri* (Droop) Green, 1975 at 10^4 cells/ml. Prior to photography, larvae were narcotized in a 1:3 mixture of a saturated aqueous solution of chlorobutanol in seawater.

Eggs and larvae for SEM examination were fixed in 2% formalin in seawater, and stored in 70% ethanol. Specimens were critical-point dried and photographed using a Hitachi S-570 Scanning Electron Microscope.

RESULTS

Beginning April 21 and continuing through May 19, 1986, a total of 15 egg capsules were laid by at least three snails (mean shell length = 62.3 mm), with nine capsules being produced sequentially by a single female.

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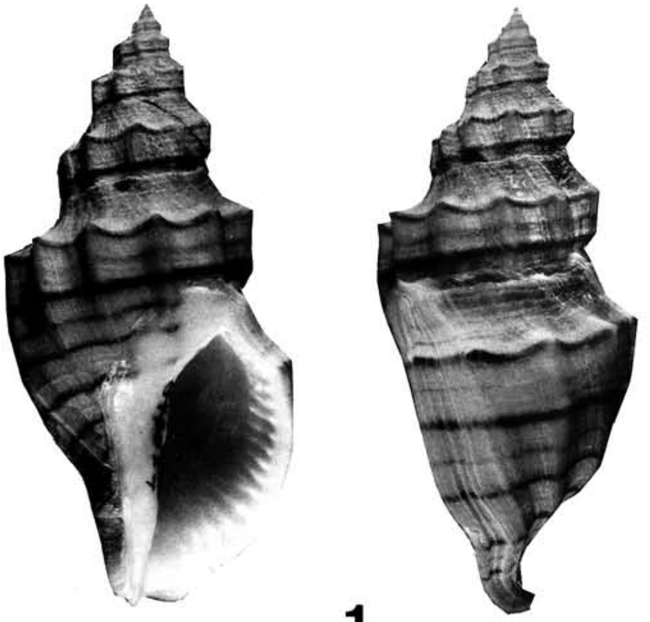

Figure 1. *Cancellaria cooperi* Gabb. Apertural and right side view of female specimen collected by SCUBA in 20–22 m at artificial reef "Torrey Pines #1", off San Diego, CA (32°53′12″N, 117°50′50″W), USNM 846054. 1.0 ×.

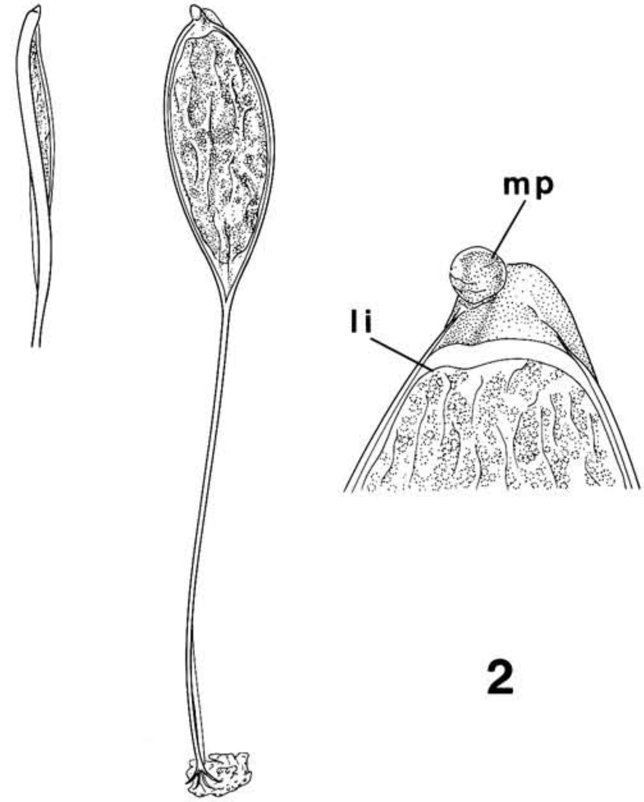

Figure 2. Egg capsule of *Cancellaria cooperi* Gabb. Frontal and side views 1.0 ×, detail of hatching aperture 6.0 ×. mp, mucous plug; li, membranous sack.

Oviposition generally occurred at night or in the morning. Snails emerged partially from the sand, with their raised foot spread anteriorly and enfolded posteriorly, the emerging stalk protruding from the folded portion of the foot. Over the course of several hours, the egg case emerged from the ventral pedal gland and was released, whereupon the stalk straightened and the egg case was supported well above the surface of the sand.

The spatulate capsules (figure 2; table 1) consisted of lenticular egg cases supported on long, narrow (250 μm diameter) stalks. Egg capsules were roughly rectangular in transverse section, with narrow keels running along the outer edge of each corner. A pre-formed hatching aperture, containing a membranous plug (figure 2, mp), was situated at the uppermost end of each egg case. Capsules were affixed to the bottom of the aquarium by holdfasts that spread from the base of the stalk.

Each egg case contained an estimated 4,000–5,000 spherical eggs (figures 3, 4; diameter = 164.5 ± 6.7 μm, N = 10), suspended in a clear, gelatinous matrix, all of which was enveloped in a membranous, transparent sack (figure 2, li). The lenticular walls of the egg case were slightly concave, creating a constriction along the midline of the case that displaced the eggs to either side.

Spiral cleavage commenced within 12 hours of oviposition, and the 8 cell stage (figure 5) was reached by the second day. Thereafter, the embryos became increasingly irregular in form (figures 6–8). All the embryos within an egg case underwent development; there were no nurse eggs or unfertilized eggs in the cases examined. By 10 days after deposition, the stomodeal invagination was evident (figure 9). After 12 days, the protoconch and operculum, both of conchiolin, were clearly discernable (figures 10, 11), and torsion was complete after 14 days (figures 12–14). The velar lobes were formed and increasing in size by the 16th day (figures 15, 16). On the

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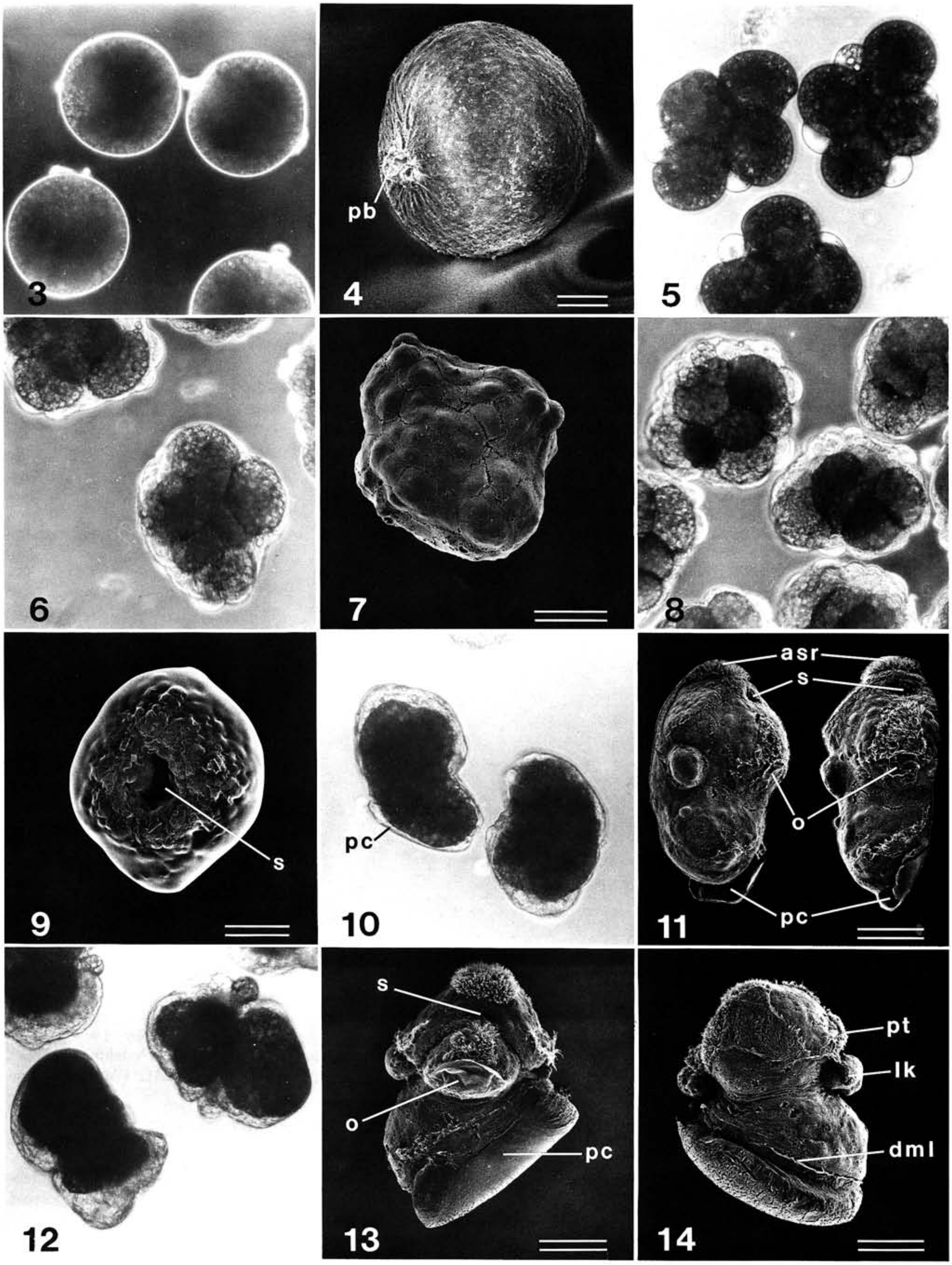
Figures 3–14. Embryonic and larval development of *Cancellaria cooperi* Gabb at 15 °C. **3.** Living embryos, 1 day old. **4.** One-day-old embryo, critical-point dried. Scale bar = 25 μm. **5.** Living embryos, 2 days old. **6.** Living embryo, 4 days old. **7.** Four-day-old embryo, critical-point dried. Scale bar = 50 μm. **8.** Living embryos, 8 days old. **9.** Ten-day-old embryo, critical-point dried. Scale bar = 50 μm. **10.** Living embryos, 12 days old. **11.** Right side and ventral views of same 12-day-old embryo, critical-point dried. Scale bar = 50 μm. **12.** Living embryos, 14 days old. **13, 14.** Ventral (13) and dorsal (14) views of 14-day-old embryos, critical-point dried. Scale bar = 50 μm.

asr, apical sensory region; dml, dorsal mantle lip; lk, larval kidney; o, operculum; pb, polar body; pc, protoconch; pt, prototroch; s, stomodeum.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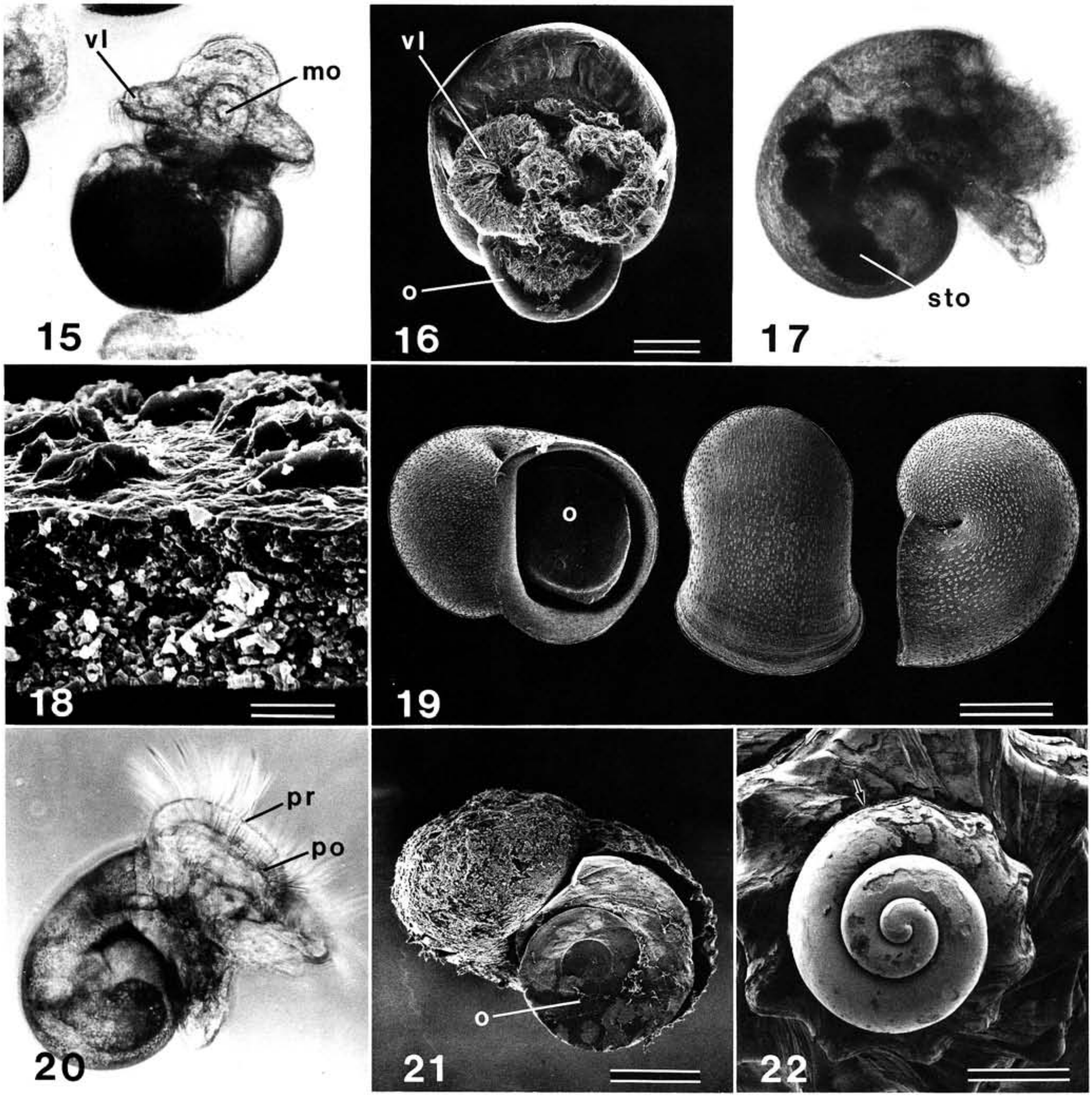

Figures 15–22. Embryonic and larval development of *Cancellaria cooperi* Gabb at 15 °C. **15.** Living embryo, 16 days old. **16.** Sixteen-day-old embryo, critical-point dried. Scale bar = 50 μm. **17.** Living, newly-hatched larva with carmine particles in the gut. **18.** Shell ultrastructure of newly-hatched larva, plane of fracture parallel to growing edge. Scale bar = 2 μm. **19.** Apertural, dorsal and apical views of shells of newly-hatched larvae. Scale bar = 100 μm. **20.** Living larva, 30 days after hatching. **21.** Shell and operculum of larva, 30 days after hatching. Scale bar = 250 μm. **22.** Apical portion of adult shell (LACM 40-95.1), arrow indicating transition from protoconch to teleoconch, scale bar = 500 μm.

mo, mouth; o, operculum; po, posterior ciliary band; pr, preoral ciliary band; sto, stomach; vl, velar lobe.

20th day after capsule deposition, embryos began to move more freely within the egg case. The gelatinous matrix in which the developing embryos had been suspended became less viscous, and the swimming embryos aggregated randomly within the case. After an average of 27 days at 15 °C the membranous plug occluding the hatching aperture dissolved, and swimming larvae escaped. Shells of the newly hatched veligers (figures 18, 19) had a mean diameter of 304.6 μm (N = 10, SD = 3.5), and were 3.9 μm thick. The veligers possessed an operculum

Table 1. Measurements of egg capsules of *Cancellaria cooperi* Gabb. All measurements in mm (N = 10).

Character	Mean	Range	SD
Total length	79.6	70.5–83.6	4.8
Capsule length	30.1	27.8–32.8	1.9
Capsule width	11.4	10.7–12.4	0.6
Capsule thickness	1.8	1.6–2.0	0.2
Stalk length	49.5	37.7–54.0	6.0
Maximum diameter of holdfast	9.1	7.4–10.8	1.2

(figure 19, o), and a fully functional alimentary system. When added to a small dish of seawater containing suspended carmine particles, the veliger larvae rapidly filled their guts with these particles (figure 17, sto).

Attempts to rear these veliger larvae through metamorphosis were unsuccessful. At 20 °C, larvae grew rapidly, reaching a mean shell diameter of 644 μm (figure 20) 22 days after hatching. Thereafter, the larvae began to die, with only a single larva surviving 30 days after hatching (shell diameter = 890 μm, figure 21). Larvae that had survived more than 22 days after hatching developed propodia, but did not metamorphose in the presence of fresh or frozen *Torpedo* mucus, or in the presence of sand from an aquarium containing adult snails. No crawling or search behavior associated with the onset of metamorphic competence was observed. Protoconchs of adult shells of *Cancellaria cooperi* indicate that the larval shell reaches 3.3 whorls (shell diameter = 1,160 μm) prior to metamorphosis (figure 22; table 2).

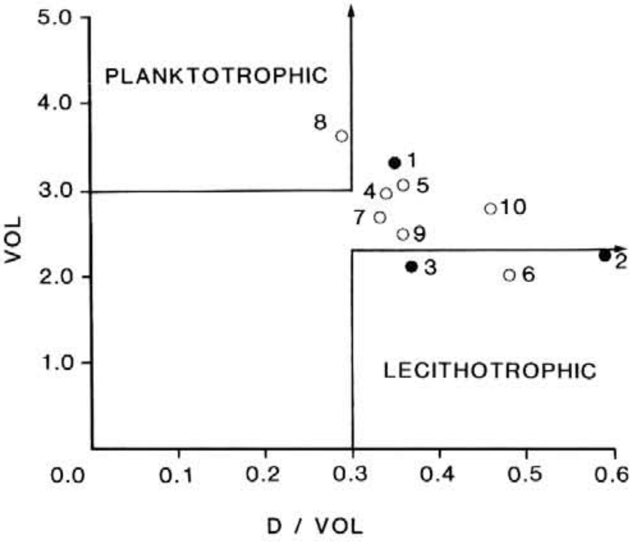

Figure 23. Relationship of the number of whorls (Vol) and the ratio of maximum diameter to number of whorls (D/Vol) of cancellariid protoconchs. Solid circles denote species for which type of development is known or inferred on the basis of number of ova per capsule. Open circles denote species for which type of development is unknown. 1, *Cancellaria cooperi*; 2, *Trigonostoma foveolata*; 3, *Admete viridula*; 4, *Cancellaria reticulata*; 5, *Cancellaria spengleriana*; 6, *Trigonostoma scalare*; 7, *Olssonella smithii*; 8, *Narona mitraeformis*; 9, *Scalptia obliquata*; 10, *Cancellaria similis*.

DISCUSSION

Egg capsules of *Cancellaria cooperi* resemble those of many neogastropods (*e.g.*, D'Asaro, 1970; Radwin & Chamberlin, 1973; Bandel, 1976), with notable modification in the length of the supporting stalk. This elongation of the stalk appears to be an adaptation for unstable sediments in which capsules are deposited. Under natural conditions, egg capsules are most likely attached to buried stones or shells, with the long stalk supporting the egg case well above the sand surface, preventing its burial, and possibly protecting the eggs from small, bottom-dwelling predators. The compressed, spatulate form of the egg case may serve to increase the surface area available for diffusion of gases and waste products between the egg case and the surrounding seawater. Capsules were most frequently laid with the broad face of the case oriented into the stream of flowing seawater.

The egg cases of *Cancellaria cooperi* are most similar in morphology to those reported by Knudsen (1950: fig. 18) for *Cancellaria* sp., and to his account of the egg cases of *Cancellaria spengleriana* (Deshayes, 1830). All have the characteristic long stalk, but the latter two are described or figured as being hemi-elliptical rather than lenticular in profile, and triangular rather than rectangular in transverse section. Egg cases of *Trigonostoma foveolata* (Sowerby, 1848), a species occurring "in sand or gravel among rocks in low-tide pools", are similar to those of *Cancellaria cooperi* in shape, but are smaller in size, and have a proportionally shorter stalk (Kilburn & Rippey, 1982:115). Capsules of *Admete viridula* (Fabricius, 1780), a subtidal boreal species, are attached directly to the substrate and lack a stalk [Thorson, 1935: fig. 71

Table 2. Protoconch measurements of the three cancellariid species for which the mode of development is known or inferred based on number of ova per capsule. Measurements are presented in the format mean/standard deviation. D = diameter in mm; Vol = number of whorls or volutions, measured according to Jablonski and Lutz (1980:332).

Species	D	Vol	D/Vol
Cancellaria cooperi: LACM 40-95.1; LACM 39-94.1; LACM 39-116.2 (N = 4)	1.16/0.02	3.29/0.02	0.35/0.01
Trigonostoma foveolata: NM C8393; NM C8394; NM B7651 (N = 5)	1.33/0.21	2.24/0.21	0.59/0.08
Admete viridula: USNM 189720 (N = 10)	0.78/0.05	2.12/0.10	0.37/0.01

Table 3. Protoconch measurements for cancellariid species for which mode of development is not known. Data are presented in the format of mean/standard deviation. D = diameter in mm; Vol = number of whorls or volutions, measured according to Jablonski and Lutz (1980:332).

Species	D	Vol	D/Vol
Cancellaria reticulata: USNM 619108 (N = 10)	0.99/0.11	2.90/0.06	0.34/0.01
Cancellaria spengleriana: USNM 664965; USNM 344417 (N = 6)	1.08/0.07	3.02/0.09	0.36/0.02
Trigonostoma scalare: USNM 846304 (N = 1)	0.96	2.0	0.48
Olssonella smithii: USNM 806986; USNM 450577; USNM 667720 (N = 5)	0.85/0.04	2.62/0.16	0.33/0.02
Narona mitraeformis: Petit collection (N = 3)	1.06/0.03	3.60/0.15	0.29/0.00
Scalptia obliquata: USNM 629063 (N = 4)	0.89/0.03	2.45/0.09	0.36/0.01
Cancellaria similis: USNM 664967 (N = 5)	1.26/0.06	2.75/0.00	0.46/0.02

(as *Velutina undata* Brown, see Thorson, 1944); Bouchet & Warén, 1985: fig. 687]. The egg capsules of all cancellariids studied to date have roughly elliptical, parallel sides with strongly to weakly keeled margins, and a medial, dorsal hatching aperture. Capsules with very long stalks appear to be restricted to the subfamily Cancellariinae.

Development of *Cancellaria cooperi* is similar to that of *Thais haemastoma floridana* as described by D'Asaro (1966), although *C. cooperi* takes about 80% longer to reach comparable developmental stages, and does not produce a noticeable sinusigeral ridge. It is interesting to note the presence of an operculum, particularly prominent in the planktotrophic larval stage (figure 21), in a family noted for the absence of opercula in adults.

The present study comprises the first direct observation of oviposition and development of any species of cancellariid, although the mode of development can be deduced in several cases from previously published data on capsule contents. Thus, *Cancellaria* sp. (30–40 ova/capsule, 500 μm in diameter; Knudsen, 1950:109), *Trigonostoma foveolata* (16 larvae/capsule; Petit & Harasewych, in preparation), *Admete viridula* (6–7 larvae/capsule; Thorson, 1935:67) and *Admete* sp. (6 larvae/capsule, capsule referred to by MacGinitie, 1955:51, USNM 664468) all likely undergo lecithotrophic development, as indicated by the low number of large larvae or ova per capsule. To date, *Cancellaria cooperi* is the only cancellariid known to undergo planktotrophic development.

In the absence of direct information, gastropod larval development may be inferred from the morphology of the protoconch at the apex of the adult shell. Thorson's "apex theory" (Thorson, 1950; Jablonski & Lutz, 1980, 1983) asserts that a large, rounded, paucispiral protoconch indicates non-planktotrophic larval development, while a narrow, polygyrate, sculptured protoconch suggests planktotrophic development. In more quantitative studies of this relationship, Shuto (1974) found that the ratio of the maximum protoconch diameter (D) to the number of protoconch whorls (Vol) was a reasonable indicator of developmental type. Values greater than 1.0 were indicative of lecithotrophic larvae, while values below 0.3 were more characteristic of planktotrophic larvae, especially if the protoconch consisted of three or more whorls. Species with D/Vol ratios between 0.3 and 1.0 usually have lecithotrophic larvae if protoconchs consist of less than 2¼ whorls. A plot of Vol *vs.* D/Vol values for 10 species of cancellariids (figure 23) indicates that although protoconch morphology appears to be an accurate indicator of the mode of larval development for those species of Cancellariidae that fall within the diagnostic regions proposed by Shuto (1974), developmental type cannot be inferred for the majority of cancellariid taxa using these criteria.

As more information on the life histories and diets of additional species of cancellariids becomes available, the relationship between the mode of larval development and the mobility and patchiness of prey species distribution may prove fruitful ground for investigation.

ACKNOWLEDGEMENTS

We thank R. R. McConnaughey for assistance in collecting and maintaining animals, and R. E. Petit for providing information on the Cancellariidae, and for critical review of the manuscript. Susann Braden took the Scanning Electron Micrographs.

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