

# Adelphophagy and cannibalism during early development of *Crucibulum auricula* (Gmelin, 1791) (Gastropoda: Calyptraeidae) from the Venezuelan Caribbean

Patricia Miloslavich

Universidad Simón Bolívar  
Departamento de Estudios  
Ambientales  
Apartado Postal 89.000, Caracas 1080  
VENEZUELA

Pablo E. Penschaszadeh

Museo Argentino de Ciencias  
Naturales-CONICET-UBA  
Av. Angel Gallardo 470  
CI405 DJR Buenos Aires  
ARGENTINA  
and  
Universidad Simón Bolívar  
Departamento de Estudios  
Ambientales  
Apartado Postal 89.000, Caracas 1080  
VENEZUELA

## ABSTRACT

*Crucibulum auricula* (Gmelin, 1791) was found living attached to rocky substrates at Isla Caribe, Estado Sucre, Venezuela, between 0.5 and 1 m depth. The shell of sexually mature females ranged from 12 to 23 mm in diameter and 6 to 10 mm in height. These females brooded between 4 and 20 egg capsules in the mantle cavity. Each egg capsule had a stalk and the stalks were joined at the point of attachment to the substrate. Each egg capsule measured between 1.6 and 3.5 mm in length (without the stalk) and between 1.0 and 2.8 mm in width. The number of uncleaved eggs per capsule varied between 55 and 305 and measured approximately 220  $\mu\text{m}$  in diameter. All eggs started development and completed the first four divisions, but only  $7 \pm 4\%$  (between 3 and 24 eggs per capsule) continued to develop. The remainder was ingested by the embryos as nurse eggs. At the end of this period of adelphophagy, the embryos started to cannibalize each other, after which only 1 to 11 embryos were left in each capsule. Juveniles with a well-developed foot and short cephalic tentacles crawled out of the capsule. Ciliary movement was observed in the region where the velum had been resorbed. The shells are brown, calcified, and measure about 730  $\mu\text{m}$  in length. Both intracapsular feeding strategies, adelphophagy and cannibalism have been previously reported in the family Calyptraeidae. Both strategies are here reported for the first time in a species of the genus *Crucibulum*.

*Additional key words:* Caenogastropoda, egg capsules, embryonic nutrition, reproduction, nurse eggs, Venezuela.

## INTRODUCTION

Gastropods of the family Calyptraeidae are characterized by the production of eggs contained in membranous egg capsules that are attached to hard substrates and are

brooded between the neck and propodium of the female. The spawn is composed of several sac-shaped egg capsules joined to each other at the base of a stalk. Each sac is composed of a thin membrane and the stalk is a continuation of this membrane. Within genera, some species produce large eggs with large amounts of yolk that hatch as crawling juveniles. Other species produce smaller eggs that hatch as planktonic veliger larvae and others complete intracapsular development by feeding on nurse eggs (Hoagland, 1986).

The genus *Crepidula* Lamarck, 1799, is the best studied in the family. Hoagland (1986) reviewed encapsulation patterns and brooding of about 20 species of *Crepidula* and found that egg diameter is very variable, ranging from 130 to 440  $\mu\text{m}$ . Hatchlings can be planktotrophic veligers, pediveligers or crawling juveniles. Extra-embryonic nutrition, when present, can be in the form of nurse eggs or cannibalism among sibling embryos. Adelphophagy has been reported in at least 7 *Crepidula* species and cannibalism of damaged or abnormal embryos is widespread in the genus (Hoagland, 1986).

The genus *Crucibulum* Schumacher, 1817, is characterized by cap-shaped shells with a complete cup-like support, which is attached by its base or along one side. Abbott (1974) reported 12 American species, 10 in the Pacific from California to Chile and 2 Atlantic species, *Crucibulum auricula* from South Carolina to Texas and West Indies to Brazil and *Crucibulum striatum* Say, 1824, from Nova Scotia to both sides of Florida. Díaz and Puyana (1994) reported 3 species in the Colombian Caribbean, *C. auricula*, *C. marensis* Weisbord, 1962, and *C. planum* Schumacher, 1817. Reproduction of species in the genus *Crucibulum* is less known. Coe (1938) studied the sexual phases of *Crucibulum spinosum* Sowerby,

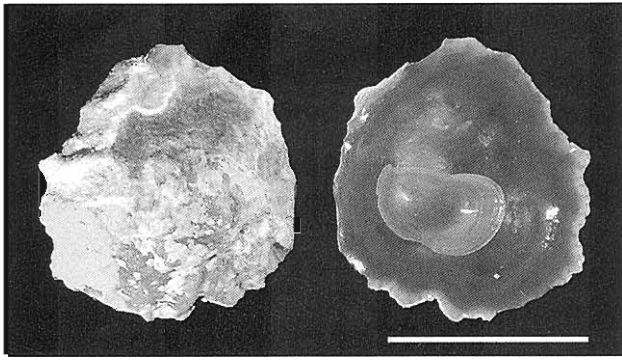


Figure 1. Adult shell of *Crucibulum auricula*. Dorsal and ventral view. Scale bar = 13 mm.

1824, from the coast of Southern California. Development of *Crucibulum scutellatum* Wood, 1828, from the Persian Gulf was studied by Thorson (1940). Bandel (1975) described the protoconch of *Crucibulum auricula* (Gmelin, 1791) from the Colombian Caribbean. Penchaszadeh (1984) studied some aspects of the intracapsular development of *Crucibulum mareense* Weisbord, 1962, from Venezuela. Hoagland (1986) gave information on some reproductive aspects of *Crucibulum personatum* Keen, 1958, *Crucibulum scutellatum*, *Crucibulum spinosum*, and *Crucibulum umbrella* Deshayes, 1830, from the Pacific coast of Central America.

Most *Crucibulum* species mentioned above have planktonic development and hatch as veliger larvae (*C. personatum*, *C. scutellatum*, *C. spinosum* and *C. umbrella* reviewed by Hoagland, 1986) or veliconch (*C. auricula*, according to Bandel, 1975). Up to now, the only species known to have non-planktonic direct development is *C. mareense* (Penchaszadeh, 1984). Neither nurse eggs nor cannibalism among embryos have been reported in any of the *Crucibulum* species.

Herein we describe the egg capsules, intracapsular development of the embryos, and hatching mode of *C. auricula* from Isla Caribe, located on the northeastern coast of Venezuela. The relationship between female size and the number and size of egg capsules, size of the eggs and size of hatchlings was also determined.

## MATERIALS AND METHODS

### SPECIMENS

Brooding specimens were collected in March 1994 and February 1995 at Isla Caribe, Chacopata, northern Araya Peninsula, Estado Sucre, Venezuela (10°42'11"N, 63°52'57"W) between 0.5 and 1 m depth. There, individuals of *Crucibulum auricula* were found attached to large flat rocks set in a patchy habitat of sand and turtle grass (*Thalassia testudinum*). They were easily identified by the edge of the inner cup, which is entirely free (figure 1) and the outer shell, which is beige with coarse ribbons (descriptions in Abbott, 1974, Díaz and Puyana, 1994). Some of the individuals were fixed in a glucamine-acetate (GA) buffer containing 4–6 % formalin (Mil-

Table 1. *Crucibulum auricula*. Characteristics of embryos during intracapsular development. Values represent mean  $\pm$  SD, numbers in parentheses indicate range.

Stage	Characteristics	Size ( $\mu\text{m}$ )
1 Egg	Uncleaved yellow egg.	218.1 $\pm$ 16.0 (195–236) n = 99
2 Embryo	Developing embryos begin to ingest nurse eggs.	362.4 $\pm$ 82.4 (236–472) n = 99
3 Embryo	Nurse egg ingestion ends. Cannibalism among sibling embryos begins.	615.7 $\pm$ 82.9 (472–786) n = 105
4 Early veliger	Cannibalism ends. Early veliger with round velum, small foot, eyes, short and thick cephalic tentacles, organic matrix of shell.	701.0 $\pm$ 78.5 (511–786) n = 69
5 Veliger	Veliger with velum measuring 250 $\mu\text{m}$ in diameter, pigmented with white spots, non-calcified protoconch, enlargement of foot.	726.1 $\pm$ 61.2 (629–825) n = 64
6 Pediaveliger	Prehatching with reduced velum (less than 200 $\mu\text{m}$ ), long and thin cephalic tentacles, long foot with edge pigmented (measures 1 mm), calcification of shell (yellowish) begins. Packed yolk in the posterior region of the animal.	740.5 $\pm$ 69.6 (629–825) n = 13
7 Hatching	Crawling juvenile, absence of velum, cilia remain, foot with two lobes in the anterior part, calcified brown shell, little to no yolk reserves.	728.0 $\pm$ 70.0 (680–840) n = 10

oslavich and Penchaszadeh, 1997) and others were kept alive in tanks at 25–27° C and 35 ‰ salinity with aerated, non-circulating seawater.

Voucher adult material of *C. auricula* (entire individuals fixed in formalin and preserved in ethanol) has been deposited in the Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, catalogue number MCZ 304128.

### DEVELOPMENT

We studied 30 females of *C. auricula*. In each spawn we observed: (1) the number and size of egg capsules brooded per female, (2) the number and size of eggs and developing embryos within the capsule, (3) the different stages of development, and (4) the hatching mode. The 30 females produced a total of 373 egg capsules; of these we randomly chose 125 egg capsules (between 1 and 10 from each female). Egg capsules were separated from the cluster, classified according to stage of development, and kept in 30 ml beakers inside tanks

with aerated and Millipore-filtered (Whatman GF B) seawater. Filtered seawater was renewed daily. Egg capsules were incubated in the dark at 26 °C in a Precision (818) incubator.

Observations of live and preserved material was done with a ZEISS dissecting microscope and a ZEISS compound microscope; measurements were taken with an ocular micrometer. Results are reported as mean  $\pm$  standard deviation.

## RESULTS

The shell diameter and height of sexually mature females of *C. auricula* ranged from 12 to 23 mm ( $18.4 \pm 2.6$  mm,  $n = 30$ ) and from 6 and 10 mm ( $7.6 \pm 1.4$  mm,  $n = 16$ ) respectively. A significant correlation was found between female shell diameter and capsule size (Pearson correlation coefficient  $r = 0.54$ ,  $p < 0.01$ ), that is, larger females brooded larger egg capsules. However, no significant correlation was found between female diameter and the number of capsules, eggs per capsule, number of hatchlings, nor hatchling size.

## DEVELOPMENT

Females brooded between 4 and 20 egg capsules in the mantle cavity ( $12.4 \pm 4.4$ ,  $n = 30$ ). Egg capsules had a triangular shape, with one corner extending to form a stalk, and were attached to the substrate at the base of this short stalk. The stalks are flattened only at the base of the capsule and the rest is thread-like. The capsule walls were thin, fragile, transparent (figure 2), and the egg capsule was turgid. No exit plug was observed. Egg capsules measured between 1.6 and 3.5 mm lengthwise (without the stalk) ( $2.5 \pm 0.3$  mm,  $n = 125$ ) and 1.0 to 2.8 mm wide ( $2.1 \pm 0.3$  mm,  $n = 125$ ).

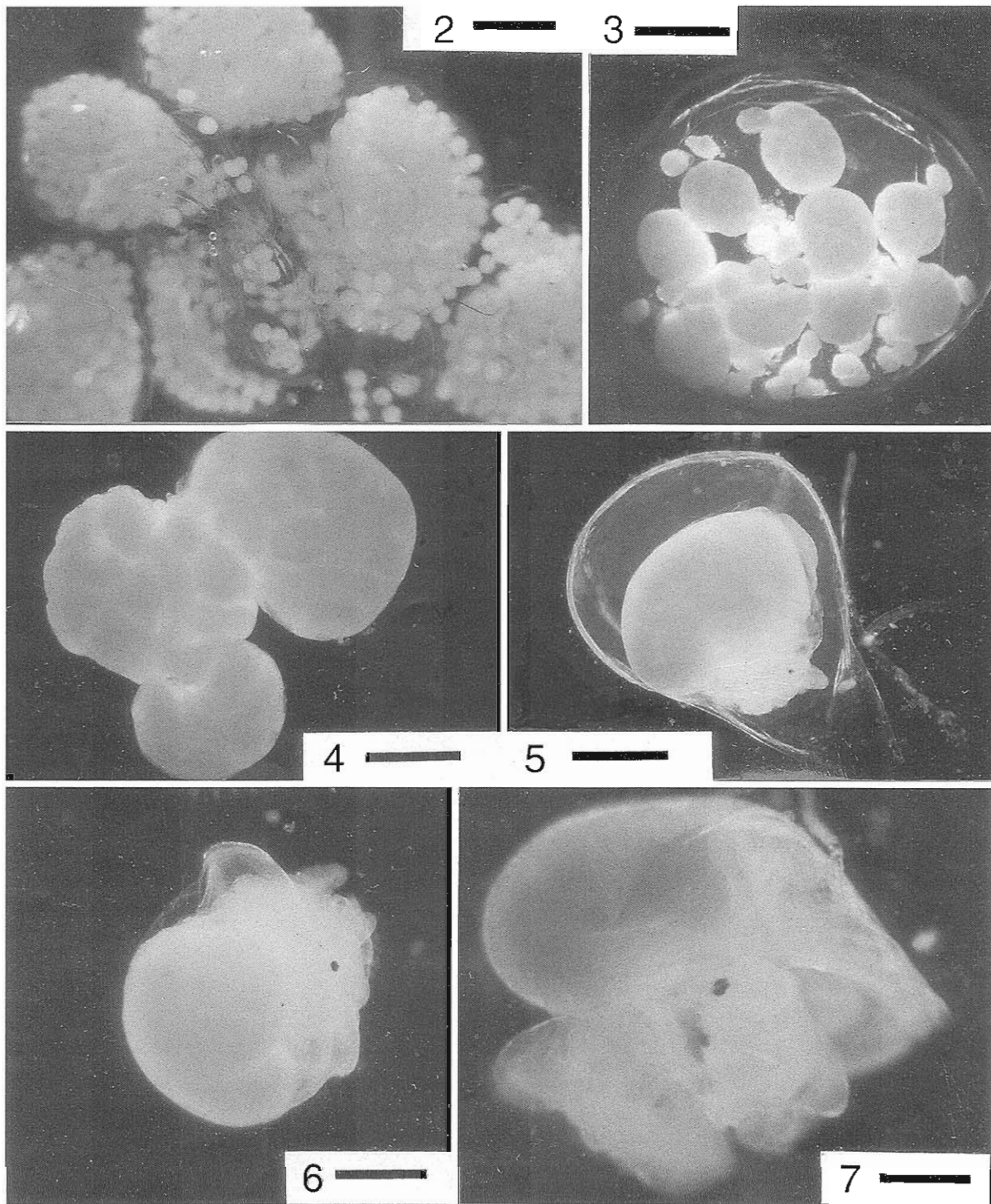
The number of eggs per capsule varied from 55 to 305 ( $161 \pm 56$ ,  $n = 56$ ). Uncleaved eggs were yellow and measured approximately 220  $\mu$ m in diameter (figure 2). All eggs underwent the first four cleavages, 93% arrested development at this stage and were ingested by the remaining developing embryos (between 3 and 24 per egg capsule,  $11.0 \pm 6.0$ ,  $n = 21$ ) as nurse eggs. Within the capsules from a single female, embryos developed synchronously. Gastrulation is accomplished when the ectoderm undergoes epiboly from the animal pole and envelops the other cells of the embryo. Developing embryos measured about 362  $\mu$ m when they started to ingest the whole nurse eggs, they were oval-shaped, yellow, with no velar lobes nor shell (figure 3). At the end of the ingestion stage, these embryos measured about 616  $\mu$ m in length; they were yellow and the nurse eggs were tightly packed in their interior. At this point of their development, the embryos started to cannibalize each other. It was common to find two embryos feeding upon a third one (figure 4). After the cannibalism stage, 1 to 11 embryos remained in each capsule ( $7.0 \pm 3.0$ ,  $n = 21$ ) and developed the velar lobes. The early intracapsular veliger measured about 700  $\mu$ m and

was characterized by a small round velum, a small foot, eyes, very short and thick cephalic tentacles and surrounded by an organic matrix (figure 5). A fragile shell measuring around 726  $\mu$ m developed from the organic matrix. The developed velum measured 250  $\mu$ m in diameter and had white spots (figure 6). The intracapsular pediveliger stage had a reduced velum measuring less than 200  $\mu$ m in diameter, had elongated and thin cephalic tentacles, and a long foot (about 1 mm) with brown lines along the edges. When calcification started (figure 7), the pediveliger shell measured about 740  $\mu$ m. Pediveligers were not able to swim when excapsulated into seawater. A few days prior to hatching (6 to 7 days), yolk was observed inside of the pediveligers. These yolk reserves disappeared almost completely by hatching, and only a small yellow spot in the posterior region of the animal remained. Hatching occurred as crawling juveniles with a brown calcified shell measuring 730  $\mu$ m in length, the shell was relatively smooth with thin horizontal lines separated 20  $\mu$ m from each other, juveniles had a well-developed foot with a small and transparent operculum, short cephalic tentacles and no velum. However, ciliary movement was observed in the region where the velum had been resorbed (table 1).

## DISCUSSION

The relationship between female size and some reproductive parameters such as capsule size, number of eggs and developing embryos within each capsule, and hatching size of the juveniles has been reported in several families of marine gastropods (Spight *et al.*, 1974, in Muricidae; Robertson, 1983, in Epitoniidae; Miloslavich and Dufresne, 1994, in Buccinidae). These studies indicate that larger females produce bigger egg capsules that contain more eggs and more and bigger hatchlings than smaller females. In *Crucibulum auricula* we found that larger females brood larger egg capsules than small females; however, these egg capsules do not contain more eggs nor produce more or bigger hatchlings, therefore the reproductive outcome of both small and large females is the same. Chaparro *et al.* (1999) found no relationship between female size and the number of developing embryos of *Crepidula dilatata* Lamarek, 1822, however, the mean size of the juveniles at hatching did increase with the shell length of the female, because more nurse eggs were available to an embryo from a larger female than to an embryo from a smaller one.

In calyptraeids, the number of egg capsules per brood is usually related to female size (see table 3 in Hoagland, 1986). *Crucibulum auricula* is one of the smallest species of *Crucibulum*. The number of egg capsules (mean 12 egg capsules per brood) in this species is one of the lowest among the five species of the genus for which there is information on reproduction published. It is similar to that of *C. mareense* (about 14 egg capsules) but it is lower than *C. scutellatum*, *C. spinosum* (both with a mean of 20 capsules), and *C. personatum* (30 capsules). The production of hatchlings of *C. auricula* is also



**Figures 2–7.** Intracapsular development of *Crucibulum auricula*. **2.** General view of the egg capsules with uncleaved eggs (stage 1). Scale bar = 1.5 mm. **3.** Egg capsule containing both developing embryos (large) and nurse eggs (small) (stage 2: adelphophagy). Scale bar = 600  $\mu\text{m}$ . **4.** Cannibalism among sibling embryos (stage 3). Scale bar = 200  $\mu\text{m}$ . **5.** Early veliger inside egg capsule (stage 4). Scale bar = 650  $\mu\text{m}$ . **6.** Veliger with non-calcified protoconch (stage 5). Scale bar = 250  $\mu\text{m}$ . **7.** Prehatching (stage 6). Scale bar = 150  $\mu\text{m}$ .

**Table 2.** Brood characteristics and total production of hatchlings within the genus *Crucibulum*. (NA = not available)

Species	Egg size ( $\mu\text{m}$ )	No. capsules	No. hatchling/ capsule	Total hatchling production	Hatchling size ( $\mu\text{m}$ )/mode	Source
<i>C. mareense</i>	NA	14	14.5	203	1020–1060 Crawler	Penchaszadeh, 1984
<i>C. personatum</i>	NA	39	275	8250	320 Veliger	Hoagland, 1986
<i>C. scutellatum</i>	NA	20	200	4000	NA Veliger	Hoagland, 1986
<i>C. spinosum</i>	NA	20	200	4000	280 Veliger	Hoagland, 1986
<i>C. umbrella</i>	NA	31	150	4650	440 Veliger	Hoagland, 1986
<i>C. auricula</i>	218	12	7	84	728 Crawler	This work

very low (about 84 crawling juveniles), followed by *C. mareense* with a total production of 200 crawling juveniles. The other *Crucibulum* species produce veliger larvae in numbers over 4000 (Hoagland, 1986) (table 2).

The size of the uncleaved egg is very important because it determines the amount of yolk available for the embryo to develop when other extraembryonic food sources such as nurse eggs, cannibalism among siblings, or intracapsular fluid, are not available. The egg of *Crucibulum auricula* is within the size range of calyptraeid species in the genus *Crepidula*, which vary between 150 and 420  $\mu\text{m}$  (Hoagland, 1986). Within the genus *Crucibulum*, the egg size has been previously reported only for *C. spinosum* (from 170 to 190  $\mu\text{m}$ ; Coe, 1938).

Within calyptraeids and particularly in the genus *Crepidula*, two feeding modes during intracapsular development have been described: adelphophagy, or nurse egg ingestion, and cannibalism: both were observed in *C. auricula*. Hoagland (1986) reviewed the patterns of encapsulation and brooding in calyptraeids and reported no nurse eggs in the five *Crucibulum* species for which we have data (*C. personatum*, *C. scutellatum*, *C. spinosum*, *C. umbrella*, and *C. mareense*). However, Coe (1938) reported that a considerable proportion of the eggs of *C. spinosum* fail to complete development, as the number of veliger larvae that hatch is much lower than the original number of eggs. This difference between number of eggs and number of hatching veligers could be due not only to nurse eggs but also to embryos ingested. Thorson (1940) reported that the large size of the embryos at hatching (0.7 mm) in *C. scutellatum* from the Persian Gulf was probably due to cannibalism and that nurse eggs do not seem to occur. If properly identified by Thorson, this species would have a very wide geographic range even for a species with planktonic development (Hoagland, 1986), since Keen (1971) and Abbott and Dance (1982) reported it as a tropical western American species ranging from Mexico to Ecuador. Penchaszadeh (1984) stated, for *C. mareense*, that presumably neither nurse eggs nor cannibalism are involved during development given the uniformity of shell size at

hatching and because undeveloped eggs were not observed.

Bandel (1975; 1976) reported that *C. auricula* from the Colombian Caribbean produces 10 to 12 yellowish eggs and that all develop into a veliconch possessing a functional foot and a large velum. His observations were probably carried out after the developing embryos finished eating the nurse eggs, which could have led him to misinterpret the embryos as eggs and to not observe the 150 nurse eggs per capsule we observed (93 % nurse eggs). Another possibility is that he worked with a different species. In the Colombian Caribbean, Díaz and Puyana (1994) have reported three *Crucibulum* species: *C. auricula*, *C. mareense*, and *C. planum*, the first two are relatively common and the third one is uncommon. The shell of *C. mareense* is similar in size to *C. auricula* (15 mm in diameter) but is characterized by a curved apex; the shell of *C. planum* is much larger (40 mm in diameter) and is characterized by brown reddish spots. Given that the taxonomy of all calyptraeids, especially *Calyptraea* Lamarck, 1799, and *Crucibulum* is in need of major revision, Bandel probably misidentified the species. However, if Bandel's observations are correct and he properly identified the species, this would be a unique case of poecilogony among gastropods, which, we suggest, is unlikely to happen (see reviews by Bouchet, 1989, and Hoagland and Robertson, 1988).

Embryos of *C. auricula* develop synchronously within the capsules of a single female. However, they reach variable sizes (between 680 and 840  $\mu\text{m}$ ), probably depending on the number of nurse eggs and embryos ingested. Hoagland (1986) reported that, for most Calyptraeidae, larvae develop synchronously, except in those species with nurse eggs; and in *Crucibulum spinosum*, there is asynchrony between egg capsules, implying that the brood does not hatch all at once.

Most *Crucibulum* species herein mentioned (*C. personatum*, *C. scutellatum*, *C. spinosum*, *C. umbrella*) hatch as free-swimming veligers (Hoagland, 1986; Coe, 1949). Penchaszadeh (1984) reported crawling juveniles for *C. mareense* and Bandel (1975; 1976) reported veli-

conchs for *C. auricula* in the Colombian Caribbean. In this study, we found that *C. auricula* hatches as crawling juveniles, with some velar (ciliar) remains but are unable to swim. Even in the absence of a free-swimming stage, this species has a wide geographic distribution in the western Atlantic, from the coast of South Carolina south to the Caribbean to tropical Brazil (Abbott, 1974; Díaz and Puyana, 1994).

#### ACKNOWLEDGMENTS

We wish to thank Oliver Contreras for his research assistance, both in the field and in the laboratory. We are also indebted to Claudio Paredes and Ana Karinna Carbonini for their valuable help in the laboratory. We sincerely thank Dr. Diarmaid Ó Foighil, Museum of Zoology, University of Michigan, and Dr. Roberto Cipriani, Universidad Simón Bolívar, for their comments on an earlier version of the manuscript. This work was supported by a grant of the Decanato de Investigación y Desarrollo to the Grupo de Ciencias Marinas (Universidad Simón Bolívar). We also thank Dr. José H. Leal, The Bailey-Matthews Shell Museum, for the photographs in figure 1.

#### LITERATURE CITED

- Abbott, R. T. 1974. American seashells. 2nd ed. Van Nostrand Reinhold, 663 pp.
- Abbott, R. T. and S. P. Dance. 1982. Compendium of seashells. Odyssey Publishing, Hong Kong, 411 pp. (1998 printing)
- Bandel, K. 1975. Das Embryonalgehäuse karibischer Meso- und Neogastropoden (Mollusca). Akademie der Wissenschaften und der Literatur, Mainz, 1: 1-133.
- Bandel, K. 1976. Observations on spawn, embryonic development and ecology of some Caribbean lower mesogastropoda. The Veliger 18: 249-271.
- Bouchet, P. 1989. A review of poecilogony in gastropods. Journal of Molluscan Studies, 55: 67-78.
- Chaparro, O. R., R. F. Oyarzun, A. M. Vergara and R. J. Thompson. 1999. Energy investment in nurse eggs and egg capsules in *Crepidula dilatata* Lamarck (Gastropoda, Calyptraeidae) and its influence on the hatching size of the juvenile. Journal of Experimental Marine Biology and Ecology 232: 261-274.
- Coe, W. R. 1938. Sexual phases in the gastropod *Crucibulum spinosum*. Journal of Morphology 63: 345-361.
- Coe, W. R. 1949. Divergent methods of development in morphologically similar species of prosobranch gastropods. Journal of Morphology 84: 383-399.
- Díaz, J. M. and M. Puyana. 1994. Moluscos del Caribe Colombiano. Un catálogo ilustrado. 1 era ed., Colciencias-Fundación Natura-INVEMAR, 291pp.
- Hoagland, K. E. 1986. Patterns of encapsulation and brooding in the Calyptraeidae (Prosobranchia: Mesogastropoda). American Malacological Bulletin 4: 173-183.
- Hoagland, K. E. and R. Robertson: 1988. An assessment of poecilogony in marine invertebrates: phenomenon or fantasy? Biological Bulletin, 174: 109-125.
- Keen, M. 1971. Sea-shells of tropical west America (Marine mollusks from Baja California to Peru). 2nd. Edition, Stanford University Press, Stanford, 1094 pp.
- Miloslavich, P. and L. Dufresne. 1994. Development and effect of female size on egg and juvenile production in the neogastropod *Buccinum cyaneum* from the Saguenay fjord. Canadian Journal of Fisheries and Aquatic Science 51: 2866-2872.
- Miloslavich, P. and P. Penchaszadeh. 1997. Spawn and development of *Fusinus closter* Philippi, 1850 (Gastropoda: Prosobranchia) from the Venezuelan Caribbean. The Veliger 40: 93-100.
- Penchaszadeh, P. E. 1984. Direct development in *Crucibulum marensis* Weisbord, 1962. (Gastropoda; Calyptraeidae) from Golfo Triste, Venezuela. Journal of Molluscan Studies 50: 237-238.
- Spight, T. M., C. Birkeland and A. Lyons. 1974. Life history of large and small murexes (Prosobranchia: Muricidae). Marine Biology 24: 229-242.
- Robertson, R. 1983. Observations of the life history of the wattletrap *Epitonium albidum* in the West Indies. American Malacological Bulletin, 1:1-12.
- Thorson, G. 1940. Studies on the egg masses and larval development of gastropods from the Iranian Gulf. Danish Scientific Investigations in Iran, part II: 159-238.