Embryonic stages and feeding substances of the South American volutid *Voluta musica* (Caenogastropoda) during intracapsular development

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Abstract: South American volutids are very homogeneous regarding their reproductive patterns. They generally spawn egg capsules with few eggs, the embryos feed on substances contained in the intracapsular fluid, and they hatch as crawling juveniles. *Voluta musica* inhabits soft bottoms between 1 and 2 m depth at Isla Caribe, eastern coast of Venezuela. The spawn consists of a single egg capsule with one to five eggs embedded in a dense, mucous liquid, each egg measuring about 330 µm in diameter. The egg capsules have an internal volume that varies from 500 to 1500 µl. At hatching, between 10 and 310 µl of liquefied fluid remain inside the egg capsule, the rest of the capsule is filled with the crawling juveniles, which measure approximately 7 mm in shell length. The protein and sugar content of the embryos and intracapsular liquid were measured during different stages of development in order to determine the amount of food available for the embryo during early development, and if such contents were enough to account for the totals found in the hatchlings. At the first stage (uncleaved egg stage), the total protein content of the intracapsular liquid varies between 30 and 90 mg among different capsules, including the negligible amount of about 20 µg contained by the four eggs. At hatching, up to 480 µg of protein remain in the intracapsular liquid and the hatchlings contain about 8 mg of protein each. The intracapsular veliger stage is the most important feeding stage and is characterized by a very large velum, a small foot and a non-calcified shell. The total sugar content of the intracapsular liquid at the first stage varies between 3000 and 5000 µg, including less than 40 µg of sugar contained by the four eggs. At hatching, the capsule liquid contains about 100 µg of sugar and each juvenile contains about 1400 µg of sugar. Results indicate that the embryos feed on the intracapsular liquid, the increase in their protein and sugar content being due to the uptake of these substances, which are contained in enough quantity in the intracapsular liquid to account for the amount found in hatchlings. A comparison of the total protein available in the intracapsular liquid and nurse eggs of different species with extraembryonic food sources is given.

Key Words: embryonic development, protein, sugar, Volutidae, hatching size, intracapsular liquid

The tropical volutid *Voluta musica* Linnaeus, 1758 is commonly found in shallow waters of the southern Caribbean, living on mud or sandy bottoms and in *Thalassia testudinum* König, 1805 beds (Flores, 1978). This caenogastropod is distributed from the Greater Antilles to Surinam including the Netherlands Antilles, West Indies and British Guyana (Clench and Turner, 1970; Abbott, 1974). On the Caribbean coast of Venezuela, this species has been reported between the Paraguaná Peninsula (Falcón State) to the west, and Puerto La Cruz (Sucre State) to the east (Coomans, 1958: Gibson-Smith, 1973). It has also been reported at the islands of La Orchila, Los Testigos (Clench and Turner, 1964), Archipiélago de Las Aves (Flores, 1978) and Archipiélago de Los Roques (Work, 1969; Gibson-Smith, 1973).

Clench and Turner (1970), Gibson-Smith (1973), Von Cosel (1976) and Flores (1978) described the spawn and juveniles of *Voluta musica*. The egg capsules are found attached to hard substrata, usually to the internal side of empty bivalve shells. The egg capsules are hemispheric and measure approximately 18 mm in basal diameter. Gibson-Smith (1973) reported three or four embryos inside each egg capsule, all of them developing to the hatching stage.

A summary of the reproductive patterns found in the Volutidae is given by Penchaszadeh *et al.* (1999).The first mode consists of large solitary capsules resting freely on the sea bottom or attached to a hard substrate. This pattern is found in all American volutids up to now studied: *Adelomelon brasiliana* (Lamarck, 1811), *Odontocymbiola magellanica* (Gmelin, 1901), *Zidona dufresnei* (Donovan, 1823), *A. ancilla* (Lightfoot, 1786), *A. beckii* (Broderip, 1836) (Penchaszadeh *et al.*, 1999), *Voluta virescens* Lightfoot, 1786 (Bandel, 1976); *Harpovoluta charcoti* (Lamy, 1910) (Hain, 1992). The second pattern, found in West African volutids consists of the incubation of the egg capsule in a pedal gland: genus *Cymbium* Röding, 1798 (Marche-Marchad, 1968, 1980). The third pattern consists of a composite egg mass with numerous capsules and is

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typical of volutids of the Indo-Pacific and Australian regions: *Melo miltonis* (Griffith and Pidgeon, 1834) (Knudsen, 1993), *M. melo* (Lightfoot, 1786) (Amio, 1963). All three patterns result in a small number of large embryos having direct development. Reported extraembryonic food sources are nurse eggs in the genus *Cymbium* (Marche-Marchad, 1968, 1980) and in *V. virescens* (Bandel, 1976) or the intracapsular fluid of the internal layer of the egg capsule (De Mahieu *et al.*, 1974; Penchaszadeh *et al.*, 1999). In the Indo-Pacific species, no extraembryonic food source has been reported.

The encapsulation of eggs within structures such as egg capsules is a widespread phenomenon among caenogastropods. The egg capsules may provide protection against predation and physical stress, against bacterial attack (because the capsule fluid is axenic in some species, Lord, 1986) and also, they may contain extraembryonic feeding substances such as nurse eggs, proteins and other substances in the capsule fluid and capsule wall material (Miloslavich, 1996a; Pechenik, 1986; Rawlings, 1994). Studies of the biochemical composition of the intracapsular liquid of several caenogastropod species have been carried out: *Adelomelon brasiliana* (De Mahieu *et al.*, 1974), *Busycon carica* (Gmelin, 1791) and *B. canaliculatum* (Linnaeus, 1758) (Harasewych, 1978), *Nucella lapillus* (Linnaeus, 1758) (Stockmann-Bosbach and Althoff, 1989) and *Prunum prunum* (Gmelin, 1791) (Penchaszadeh and Rincón, 1996). Such studies indicate that the intracapsular fluid of these species is composed of proteins and carbohydrates that decrease in concentration throughout development.

In this study, the intracapsular development of *Voluta musica* from the egg to the hatching stage is described. Protein and sugar content of the embryos and the intracapsular liquid is measured in order to determine: (1) the amount of food available for the embryo during early development, (2) ingestion stages, (3) the concentration of these substances throughout development and (4) if the intracapsular liquid contains enough protein and sugar to account for the amount found in the hatchlings.

MATERIALS AND METHODS

Specimens

Egg capsules were collected in April 1994 at Isla Caribe, Chacopata, northern Araya Peninsula, Estado Sucre, Venezuela (10°42`11" N, 63°52`57" W), between 1 and 2 m depth on soft bottoms (sand, mud and *Thalassia testudinum* beds). Egg capsules were usually found attached to the internal side of empty bivalve shells of the genera *Atrina* Gray, 1842, *Pinna* Linnaeus, 1758 and *Trachycardium* Mörch, 1853. A total of 230 egg capsules were collected. Some of the egg capsules were preserved in 5% formalin and some were kept in aquaria at a temperature of 25-27°C in aerated, non-circulating seawater.

Development

The following aspects of the spawn were studied: size of egg capsules, number and size of eggs and developing embryos within egg capsules, observations of the different stages of development and the number of whorls of the embryonic shell. Observations were carried out with fresh and preserved material.

Biochemical Procedures

The egg capsule content consisted of two fractions: embryos and intracapsular liquid. The volume of the intracapsular liquid was measured by extracting it from the capsule with a 20 µl Pipetmann micropipet (precision ± 1 µl). A previous analysis of the eggs was carried out in order to determine their amount of protein and sugar. The eggs were carefully separated from the intracapsular fluid and their protein and sugar content was measured. The protein content varied between 4 and 5 µg per egg and the sugar content was less than 10 µg per egg. These values are overestimated because some of the intracapsular fluid was inevitably still attached to the eggs. For further determinations, embryos at very early stages of development were not separated from the intracapsular liquid because they were embedded in it and separation was difficult without causing harm. Embryos were successfully separated from the liquid once they reached the veliger stage. The material was flash-frozen while fresh in 1.5 ml eppendorfs at -4°C and once frozen, were kept at -70°C for protein and sugar determinations.

Protein was determined following the Bio-Rad protein assay procedure based on the Bradford method (Bradford, 1976). Bovine serum albumin (BSA) was used as a standard. Samples were left overnight in 0.5 N NaOH and thoroughly homogenized.

Total sugar was determined by a modification of the Herbert *et al.* (1971) phenol method. Samples were left overnight and homogenized in citrate buffer (0.1 M, pH 5.0), 50 µl of phenol (80%) were added, mixed with a vortex and incubated at room temperature for 40 minutes. After incubation, 5 ml of concentrated sulfuric acid were added with a fast-flowing pipette and carefully shaken. Readings were taken at 480 nm after the samples were cooled to room temperature (between 22 and 25°C). Analytical saccharose was used as a standard.

RESULTS

Development

The egg capsules of *Voluta musica* were hemispheric, lenticular shaped and attached to the substrate by the flat

side. The convex surface was smooth and it had a preformed exit plug consisting of a suture line on one side of the capsule. The external membrane was thick and resistant. The mean basal diameter of the egg capsule was 18.8 ± 1.9 mm (n = 27, range 15-23 mm) and the mean height was 8.6 ± 0.9 mm (n = 27, range 6-10 mm). The intracapsular fluid was transparent, with a gelatinous consistency at the early stages of development and a liquid consistency at the final stages.

Each egg capsule contained between one and five eggs (3.6 ± 0.9, mean ± st. dev., n = 145), all of which developed to the hatching stage. The eggs were pinkish yellow and measured approximately 330 µm in diameter (334 ± 36 µm, n = 56). The first two cleavages produced two and four macromeres respectively, all of equal size. In the third cleavage, each macromere gave rise to a small micromere at the animal pole (Stage 1), this embryo measured about 340 µm in diameter. After gastrulation, the embryo had two distinct regions, a yellow region corresponding to yolk and a pink-orange region corresponding to the visceral mass. The organic matrix of the shell was observed as a transparent grainy layer at the vegetal pole. At the animal pole, the velar cone was observed as well as an incipient velum (Stage 2). This embryo measured approximately 450 µm in length. The early veliger (Stage 3) measured approximately 510 µm lengthwise, it had a small velum (400 µm), and the organic matrix of the shell and the body was pinkish yellow. The second veliger (Stage 4) was characterized by a bilobed velum that was more than 3 mm wide; the shell was very fragile and torsion had begun; this veliger measured about 1200 µm in length. The third veliger (Stage 5), measuring approximately 2300 µm lengthwise was characterized by a very large velum, which was more than 10 mm wide and 8 mm high. The shell was transparent and very fragile. Torsion was complete and a small foot was visible. The veliconch (Stage 6) measured about 4000 µm in shell length and was also characterized by a very large velum, densely ciliated, the cilia measuring between 18.8 and 47 µm. At this stage, calcification of the shell began, and the foot was still small. The pediveliger (Stage 7) measured around 4500 µm in shell length and was characterized by a calcified shell, a large foot and an almost entirely resorbed velum. None of the previous stages survived more than a few hours when excapsulated in sea water. The prehatching stage (Stage 8) measured about 4700 µm; it had a well developed crawling foot, the velum was completely absent, and the shell was pigmented with black spots on an orange background. At the hatching stage (Stage 9), the shell measured about 7100 µm in length; it was orange and had the typical pentagram pattern of the species. The foot and siphon were pigmented with pink spots (Table 1, Figs. 1 and 2).

Biochemical content of embryos and intracapsular fluid

During development, the volume of the intracapsular liquid decreased almost 10 times (Table 2). Between the egg stage and the hatching stage, the total protein content of the intracapsular liquid decreased more than 120 times while the sugar content decreased about 50 times. The protein concentration also decreased three times and the sugar concentration decreased six times (Table 2). The most significant decrease occurred at the third veliger stage (Stage 5) when the velum reached its maximum size.

The protein content of the embryos increased more than 1600 fold from the egg stage to hatching, while the sugar content increased nearly 50 fold to the prehatching stage and then decreased to the hatching stage down to 34 fold in relation to the egg stage (Table 3, Fig. 3). The most significant increases in content occurred from the egg stage to the early veliger stage (70 fold in protein content) and between the third veliger to the veliconch stage (almost 10 fold in protein content and three fold in sugar content). These increases are coincident with foot development and shell calcification. Protein and sugar analysis of the intracapsular liquid at the first and last stages of development indicate that there is enough of these substances in the intracapsular liquid to account for the total found in the hatchlings (Table 4).

DISCUSSION

Development

As a general rule, the South American volutids are very homogeneous regarding their reproductive patterns. Commonly, the egg capsules are attached to hard substrates; the fact that *Adelomelon brasiliana* spawns free egg capsules is a remarkable adaptation to shallow sandy bottoms given that they may be carried away by the currents but never buried in the sand (Penchaszadeh and De Mahieu, 1976). With the exception of *Voluta virescens*, which according to Bandel (1976) spawns egg capsules containing about 200 eggs of which the one or two that develop ingest the rest as nurse eggs, the South American species spawn egg capsules with few eggs; the embryos feed on substances contained in the intracapsular fluid; development is direct (intracapsular metamorphosis); and hatchlings may have a shell length of more than 10 mm (Carcelles, 1944; de Mahieu *et al.*, 1974; Penchaszadeh and De Mahieu, 1976; Penchaszadeh, 1988; Hain, 1992; Penchaszadeh *et al.*, 1999).

The volutids of the West African, Indo Pacific and Australian regions present different reproductive patterns. West African volutids incubate the egg capsule in the pedal gland for a period of up to five months. In the genus

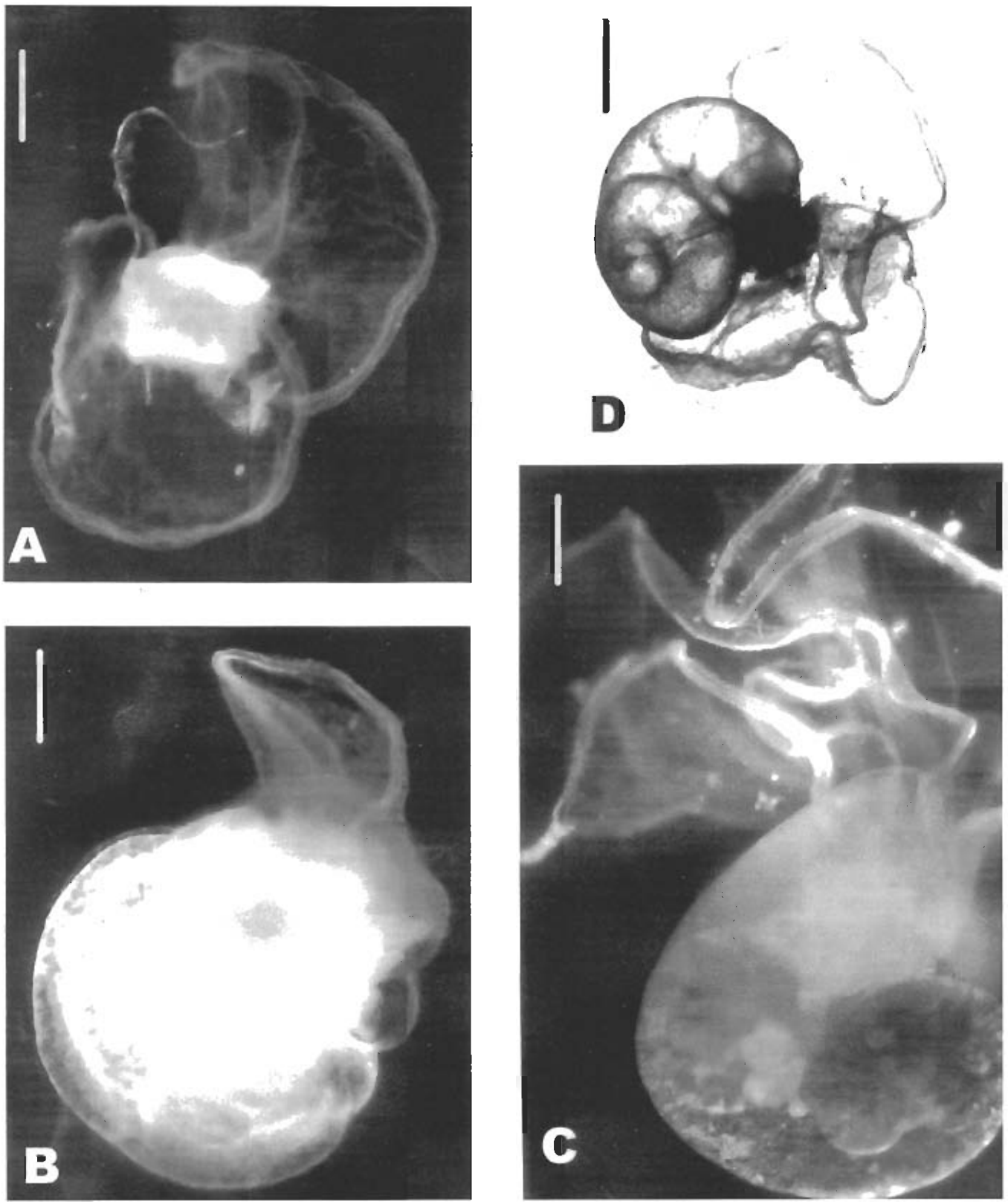

Figure 1. Intracapsular veliger stages of *Voluta musica*. **A.** First veliger, scale bar: 120 µm. **B.** Second veliger, scale bar: 280 µm. **C.** Third veliger, scale bar: 500 µm. **D.** Veliconch, scale bar: 1500 µm.

Table 1. Summary of the intracapsular development of *Voluta musica*. For embryo size, values indicate mean ± standard deviation; numbers in parenthesis indicate range.

STAGE	Size of embryos (µm)	Number of volutions	Remarks
0 Egg	339.3 ± 36.4 (250 - 450) n = 56		Yellow - pinkish uncleaved eggs
1 2, 4, 8 cells	340.2 ± 29.8 (288 - 384) n = 18		Early segmentation, first 3 cleavages
2 Embryo	442.9 ± 50.3 (360 - 550) n = 21		Gastrulation Organic matrix of the shell starts Origin of the velar cone Incipient velum Posterior yellow region with yolk Anterior pink region with visceral mass
3 First veliger	511.4 ± 126.8 (332 - 780) n = 58		Small velum (400 µm wide) Organic matrix of shell Body is yellow-pinkish
4 Second veliger	1163.8 ± 206.3 (860 - 1620) n = 21	< 1	Development of the velum (bilobed, > 3 mm wide) Very fragile shell Torsion begins
5 Third veliger	2285.5 ± 816.5 (1120 - 4160) n = 43	1 - 2	Very large velum (> 10 mm wide and > 8 mm high) Fragile shell Torsion completed Small foot
6 Veliconch	3939.6 ± 880.4 (1680 - 5610) n = 95	1 - 2+338°	Very large velum Calcification of shell begins (still transparent) Small foot
7 Pediveliger	4490.3 ± 272.1 (3960 - 4950) n = 15	3 - 3+158°	Velum absorption Calcified shell Large foot
8 Prehatching	4712.0 ± 678.1 (3280 - 5920) n = 40	3+45° - 3+348°	Velum absent Shell shows pigmentation (black spots on orange background) Well developed crawling foot
9 Hatching	7173.6 ± 610.6 (6400 - 8640) n = 22	4	Orange shell with pentagram pattern Pigmented foot and siphon (pink spots)

Cymbium, there are two extraembryonic food sources, nurse eggs (several thousand) and substances contained in the intracapsular fluid (Marche-Marchad, 1968, 1980). Hatching takes place as a veliconch, which measures up to 50 mm in shell length.

The *Melo* species from the Indo Pacific, southern Japan and Australia present a composite egg mass, in a pineapple or cylindrical shape, the number of egg capsules is variable and they are arranged in a spiral. Only one embryo per capsule develops and hatches as a crawling juvenile (Tokioka, 1962; Amio, 1963; Knudsen, 1993). No nurse eggs have been reported, however, the intracapsular liquid may contain proteins given the observations provided by Knudsen (1993), who described that the intracapsular liquid of capsules with embryos almost ready to hatch was a clear fluid that was transformed into a filamentous mass some time after preservation in 10% formalin.

An outstanding characteristic during the embryonic development of *Voluta musica* is the presence of a large velum. According to Giese and Pearse (1977), "prosobranchs" with direct development usually have a small velum. The velum could have several functions in species

Table 2. Total protein and sugar in the intracapsular liquid of *Voluta musica*. Values indicate mean ± standard deviation; numbers in parentheses indicate range.

STAGE	VOLUME OF INTRACAPSULAR LIQUID (µl)	TOTAL PROTEIN (mg)	µg PROTEIN / µl	TOTAL SUGAR (mg)	µg SUGAR / µl
Egg (Stage 0)	879.4 ± 270.8 n = 18 (400 - 1500)	57.1 ± 18.0 n = 9 (28.5 - 89.5)	69.5 ± 14.5 n = 9 (49.7 - 88.6)	4.9 ± 0.8 n = 9 (3.9 - 6.4)	6.0 ± 2.8 n = 9 (2.9 - 10.0)
Early Veliger (Stage 3)	861.2 ± 239.5 n = 26 (450 - 1400)	41.1 ± 13.6 n = 8 (13.3 - 54.4)	53.3 ± 15.6 n = 8 (27.2 - 73.1)	5.4 ± 1.4 n = 18 (2.6 - 7.5)	6.1 ± 1.3 n = 18 (3.7 - 8.5)
Third Veliger (Stage 5)	493.8 ± 182.3 n = 16 (150 - 750)	13.4 ± 5.0 n = 11 (7.8 - 25.4)	33.9 ± 13.3 n = 11 (15.6 - 56.8)	3.4 ± 2.0 n = 5 (1.6 - 6.4)	5.4 ± 2.2 n = 5 (2.7 - 8.6)
Veliconch (Stage 6)	307.4 ± 145.4 n = 19 (50 - 715)	9.6 ± 4.0 n = 8 (3.3 - 17.4)	26.2 ± 10.7 n = 8 (5.1 - 40.9)	1.1 ± 0.4 n = 9 (0.4 - 1.5)	5.2 ± 2.2 n = 9 (2.9 - 10.0)
Prehatching (Stage 8)	285.7 ± 171.4 n = 7 (130 - 600)	5.2 ± 3.0 n = 5 (2.1 - 9.0)	22.5 ± 11.2 n = 5 (5.1 - 32.6)	0.9 ± 0.3 n = 2 (0.7 - 1.2)	3.6 ± 2.3 n = 2 (1.9 - 5.2)
Hatching (Stage 9)	94.0 ± 127.4 n = 5 (10 - 310)	0.45 ± 0.04 n = 2 (0.42 - 0.48)	22.5 ± 1.9 n = 2 (21.2 - 23.9)	0.1 ± 0.1 n = 3 (0.008 - 0.3)	0.9 ± 0.1 n = 3 (0.8 - 0.9)

with intracapsular metamorphosis, the first is for handling and ingesting nurse eggs (Fioroni, 1967; Hadfield and Iaea, 1989) and the second is for respiration through gelatinous egg masses (Hunter and Vogel, 1986). The velum of *V. musica* is densely ciliated and characterized by small cilia. According to Hadfield and Iaea (1989), the velum of the vermetid *Petaloconchus montereyensis* Dall, 1919 is highly modified for the ingestion of nurse eggs during intracapsular development, lacking the necessary structures for swimming and feeding during planktotrophic life (pre- and postoral ciliary bands and food groove). Excapsulated veligers of *V. musica* are also unable to swim, and the large velum is probably used either for feeding (which occurs mostly at this stage of development) or for respiration. Resorbtion of the velum begins at the pediveliger stage (Stage 7), when more than 80% of the protein and sugar content has been consumed from the intracapsular liquid. Hadfield and Iaea (1989) proposed that velar resorbtion is nutritionally triggered, that is, it occurs in response to the absence of an external food supply, which in this case, could be the low contents of protein and sugar within the egg capsule fluid.

Biochemical content of the embryos and intracapsular liquid

Gastropod egg capsules are very complex structures; the microstructure is composed of three or four layers (D'Asaro, 1988; Rawlings, 1990, 1994; Tamarin and

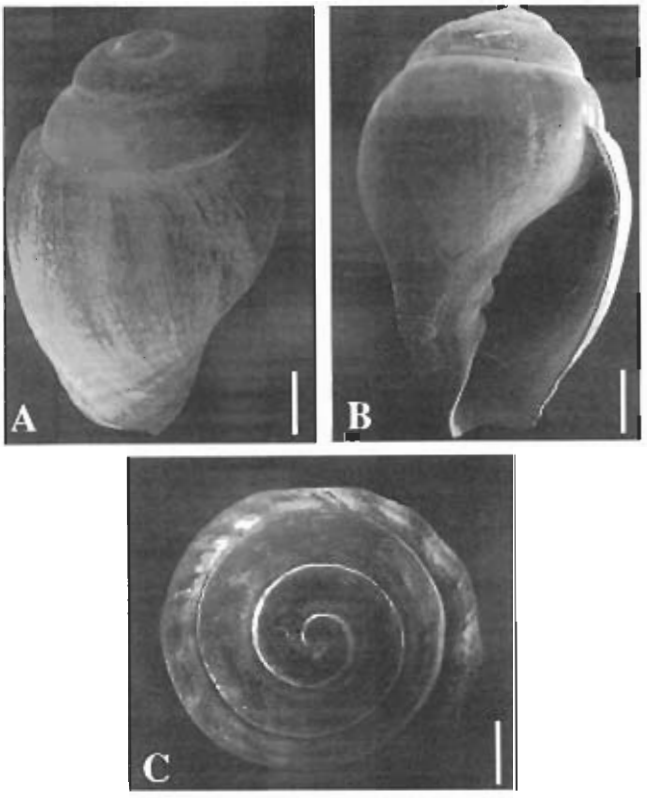

Fig. 2. SEM of the hatching shell of *Voluta musica*. **A.** Dorsal view. **B.** Aperture. **C.** Spire. Scale bar: 1 mm.

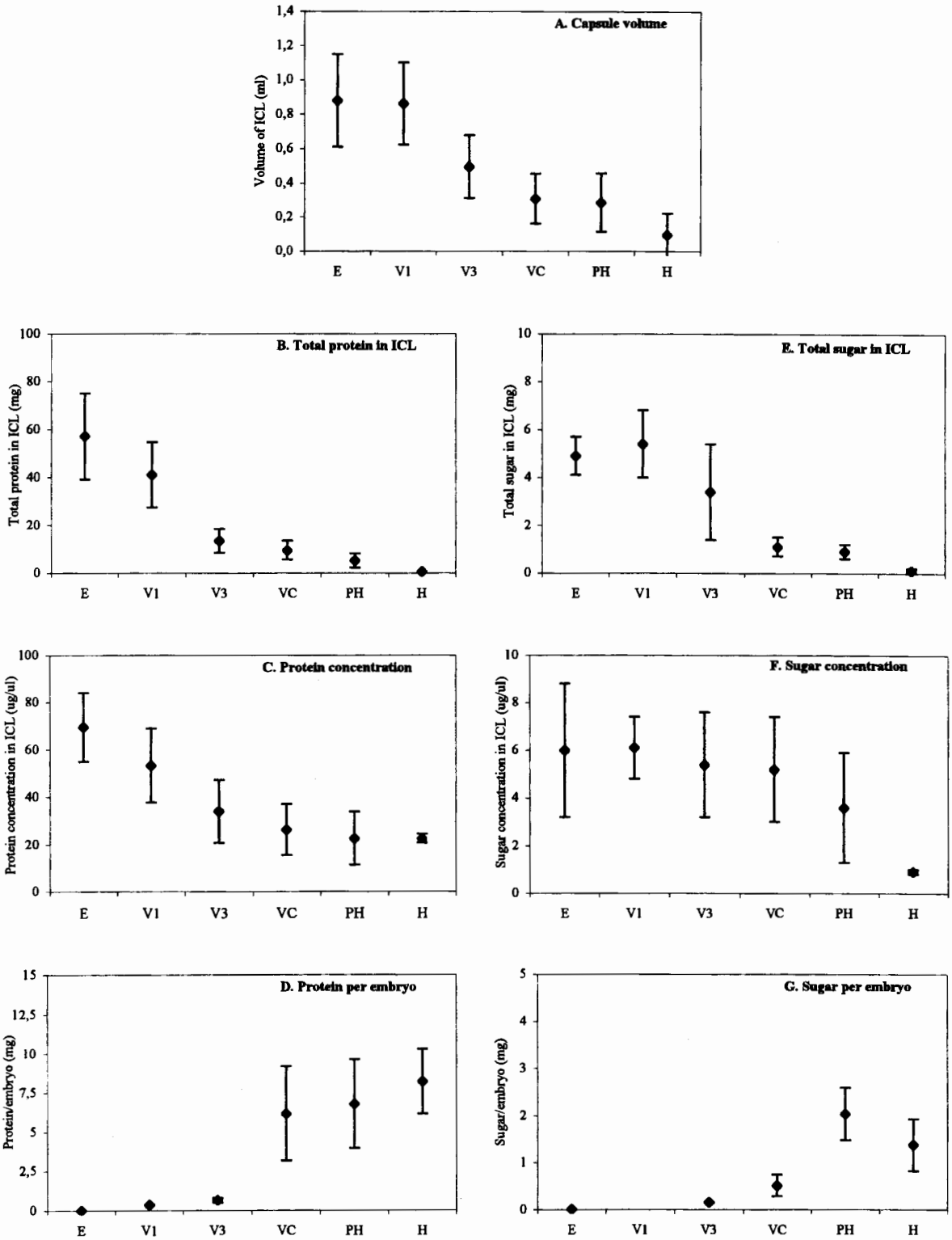

Figure 3. Protein and sugar in the intracapsular liquid (ICL) and in embryos of *Voluta musica* at different stages during intracapsular development. **A.** Volume of intracapsular liquid. **B.** Total protein in the intracapsular liquid. **C.** Protein concentration in the intracapsular liquid. **D.** Total protein per embryo. **E.** Total sugar in the intracapsular liquid. **F.** Sugar concentration in the intracapsular liquid. **G.** Total sugar per embryo. Error bars are standard deviation. Abbreviations: E: egg (stage 0), V1: First veliger (stage 3), V3: third veliger (stage 5), VC: veliconch (stage 6), PH: prehatching (stage 8), H: hatching (stage 9).

Table 3. Total protein and sugar content per embryo at different stages of development of *Voluta musica*. Values indicate mean ± standard deviation; numbers in parenthesis indicate range.

STAGE	µg protein / embryo	µg sugar / embryo
Egg (Stage 0)	Less than 5 µg / egg	Less than 40 µg / egg
Early veliger (Stage 3)	354.5 ± 26.2 n = 2 (335.9 - 373.0)	----------
Third veliger (Stage 5)	641.9 ± 176.3 n = 5 (352.0 - 803.0)	151.2 ± 5.1 n = 2 (147.6 - 154.8)
Veliconch (Stage 6)	6164.2 ± 3019.8 n = 21 (1173.0 - 10357.2)	505.7 ± 230.2 n = 11 (193.1 - 816.9)
Prehatching (Stage 8)	6777.8 ± 2836.5 n = 10 (1992.0 - 11583.0)	2031.3 ± 560.3 n = 7 (1147.8 - 2653.9)
Hatching (Stage 9)	8213.1 ± 2071.7 n = 9 (4521.6 - 11719.2)	1371.9 ± 556.7 n = 8 (603.3 - 2349.5)

Carriker, 1967) and they are biochemically composed of proteins, amino acids, carbohydrates, acid mucopolysaccharides, COOH groups and lipids (Hunt, 1966; Miloslavich, 1996b, Sullivan and Maugel, 1984). There are very few studies that test the permeability of the capsule wall to different substances. Pechenik (1982) reported that the capsule walls of three muricid species are permeable to water, salts and to small carbohydrate molecules (glucose) and Hawkins and Hutchinson (1988), reported that the egg capsules of the muricid *Ocenebra erinacea* (Linnaeus, 1758) are permeable to water and salts at all stages of development. Moreover, Pechenik (1983), reported that the egg capsules of another muricid species are permeable to NaCl and water, but substantially less permeable to small organic molecules such as amino acids, glucose and sucrose, and that the existing solute molecules are inorganic ions. These studies seem to indicate that substances such as proteins and other large molecules remain in the egg capsule throughout development and therefore can be used by the embryos as a nutrition source.

During the intracapsular development of *Voluta musica*, protein and sugar are incorporated mostly between the third veliger (Stage 5) and veliconch (Stage 6) stages, when there is a marked decrease in the total content of these substances in the intracapsular liquid, and the embryos show a marked increase of both protein and sugar. At these stages, the velum of the embryos is very large and shell calcification and foot development begin. De Mahieu *et al.* (1974) reported that the embryos of *Adelomelon brasiliana* also incorporate most of the protein from the intracapsular liquid during shell calcification. Penchaszadeh and Rincón (1996) have found similar results in *Prunum prunum*. As pointed out by Wilbur (1972), shell formation integrates a series of physiological, biochemical and crystallization processes, which result in a highly organized structure of calcium carbonate crystals in an organic matrix (composed mainly of proteins, free amino acids and mucopolysaccharides, Grégoire, 1972).

The uncleaved egg size of *Voluta musica* (250 to 450 µm) is the largest recorded yet among the South American volutids, comparable only to that of *Odontocymbiola magellanica,* which measures between 280 and 300 µm (Penchaszadeh and De Mahieu, 1976). The importance of egg size and the amount of extraembryonic food sources during development has been discussed by several authors (Fioroni,1982). Fioroni (1988) has

Table 4. Biochemical (protein and sugar) balance between the egg and hatching stage of *Voluta musica*. Values at the hatching stage were calculated for a mean number of four (4) developing embryos per capsule.

	TOTAL IN EGG CAPSULE AT EGG STAGE (mg) (eggs + liquid)	TOTAL IN 4 EMBRYOS AT HATCHING (mg)	TOTAL IN LIQUID AT HATCHING STAGE (mg)	TOTAL IN EGG CAPSULE AT HATCHING STAGE (mg) (embryos + liquid)	BALANCE
PROTEIN	60 (*)	32.8	0.45	32.8 + 0.45 = 33.25	Positive
SUGAR	5 (**)	5.6	0.10	5.6 + 0.1 = 5.7	± Equal
TOTAL (Protein + sugar)	65	38.4	0.55	38.4 + 0.55 = 38.95	Positive

(*) Includes 20 µg of protein contained by the four eggs.
(**) Includes 40 µg of sugar contained by the four eggs.

Table 5. Comparison of the total protein available in the intracapsular liquid (ICL) and nurse eggs (NE) of different species with extraembryonic food sources at the first stage of development (uncleaved egg).

PROTEIN AVAILABLE PER EMBRYO IN SPECIES THAT FEED ON THE INTRACAPSULAR LIQUID:							
SPECIES (Family)	Total protein in ICL	Total volume of ICL	Protein concentration in ICL	Mean number of embryos	Protein available per embryo (µg)	Hatching mode	Reference
Nucella lapillus (Muricidae)	0.23 mg	31.6 ± 8.9 µl	7.4 mg/ml (gastrula stage)	34 + 966 NE	220 + 30 NE each	Crawling	Stockmann-Bosbach and Althoff, 1989
Urosalpinx cinerea (Muricidae)	"albumen"	5.4-19.8 µl	Not determined	1-19 8.12 ± 3.0	2 µl of albumen	Crawling	Rivest, 1986
Engoniophos unicinctus (Buccinidae)	100-150 µg	10 µl	12.5 mg/ml	4-5	30	Crawling + velar remains	Miloslavich and Penchaszadeh, 1994 Miloslavich, 1999
Busycon carica (Melongenidae)	1.9 mg	500 µl	3.8 mg/ml	55	30	Crawling	Harasewych, 1978 Conklin, 1907
Busycon canaliculatum (Melongenidae)	4.35 mg	500 µl	8.7 mg/ml	110	40	Crawling	Harasewych, 1978
Prunum prunum (Marginellidae)	0.14-0.26 mg	6-11 µl	23.4 ± 6.4 mg/ml	1	200	Crawling	Penchaszadeh and Rincón, 1996
Adelomelon brasiliana (Volutidae)	1.54 g	77 ml	20 mg/ml	22	70,000	Crawling	DeMahieu *et al.*, 1974 Penchaszadeh and DeMahieu, 1976
Voluta musica (Volutidae)	60 mg (*)	800 µl	69.5 ± 14.5 mg/ml	4	8,200	Crawling	Present work

(*) Includes 20 µg of protein contained by the four eggs.

PROTEIN AVAILABLE PER EMBRYO IN NURSE EGG FEEDING SPECIES:						
SPECIES (Family)	Total protein in egg capsule content (Eggs + NE) (mg)	Ratio (NE: embryo)	µg of protein per egg	Protein available in NE for developing embryo (µg)	Hatching mode	Reference
Eualetes tulipa (Vermetidae)	0.54 (289 eggs)	1.5:1	1.9	2.85	Veliger	Miloslavich, 1996
Buccinum undatum (Buccinidae)	2.78 (918 eggs)	92:1	3.0	276	Crawling	Miloslavich, 1996
Buccinum cyaneum (Buccinidae)	1.25 (567 eggs)	189:1	2.2	415.8	Crawling	Miloslavich, 1996
Fasciolaria tulipa hollisteri (Fasciolariidae)	6.36 (3119 eggs)	446:1	2.0	892	Crawling	Miloslavich, 1996
Fusinus closter (Fasciolariidae)	0.66 (291 eggs)	17:1	2.3	39.1	Crawling + velar remains	Miloslavich, 1996

focused on species that complete development by ingesting nurse eggs and Miloslavich (1996a) has studied the biochemical value of the eggs and hatchlings of some nurse egg feeding species. The nourishing value of the intracapsular liquid was determined for a few caenogastropod species by De Mahieu *et al.* (1974), Harasewych (1978), Stockmann-Bosbach and Althoff (1989) and Penchaszadeh and Rincón (1996). The total protein available in the intracapsular liquid and nurse eggs of different species with extraembryonic food sources is summarized in Table 5.

This amount varies from 2.85 to 70,000 µg per embryo for *Eualetes tulipa* (Chenu, 1843) (Miloslavich, 1996a) and *Adelomelon brasiliana* (De Mahieu *et al.*, 1974) respectively. The hatching mode of *E. tulipa* (species with less than 3 µg available for each embryo) is as a veliger larva, while embryos from other species that ingested more than 30 µg of protein during intracapsular development hatch as crawling juveniles. The highest values are found in the Volutidae with 8,200 µg (*V. musica*) and 70,000 µg (*A. brasiliana*) of protein available per embryo.

The egg capsules found in the Volutidae are among the largest recorded for caenogastropods (Penchaszadeh *et al.*, 1999), and possibly contain more protein than other species. Other egg capsules comparable in size and morphology to those of Volutidae are found in some Buccinidae of the northern Pacific (subfamily Volutopsiinae). The egg capsules of the Volutopsiinae are very large (up to 66 mm in diameter), single laid and nearly hemispherical, an unusual morphology for Buccinidae. They are lined in the inside with a thick layer of albuminous substance on which the embryos feed after they have ingested the nurse eggs (very few). The size of the hatching embryos is very large, up to 20 mm in *Volutopsius norvegicus* (Gmelin, 1791) (Kantor, 1990). Gonor (1964) described two egg capsules of *Pyrolofusus deformis* (Reeve, 1847) from Alaska. These are globular, with a flat base firmly cemented to the substrate and measured 27 mm in diameter. One contained "a colorless, thick, jelly-like material in the basal portion and some soft yellowish material above this" and the other contained "three small snails which virtually filled the inside". These observations indicate that the embryos probably consumed this thick material. A biochemical study of the intracapsular liquid of these egg capsules would be interesting to determine if not only the morphology but also the protein content is similar to volutids. In this way, more comparisons could be made about the diversity of maternal investment in the embryos of several species as well as how different families within the caenogastropods have resolved the problem of nutrition during intracapsular development.

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