Journal of Shellfish Research, Vol. 26, No. 1, 65–70, 2007.

LARVAL AND EARLY JUVENILE DEVELOPMENT OF THE VOLCANO KEYHOLE LIMPET, *FISSURELLA VOLCANO*

TEODORO REYNOSO-GRANADOS,[1] PABLO MONSALVO-SPENCER,[1] ELISA SERVIERE-ZARAGOZA[1]* AND SERGIO A. GUZMÁN DEL PRÓO[2]

[1]*Centro de Investigaciones Biológicas del Noroeste (CIBNOR), Mar Bermejo 195, Colonia Playa Palo de Santa Rita, La Paz, Baja California Sur 23090, Mexico;* [2]*Laboratorio de Ecología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional. Prolongación de Carpio y Plan de Ayala s/n, México, D.F. 02800, México.*

ABSTRACT Larval and juvenile development were studied in the volcano keyhole limpet *Fissurella volcano* over 77 days under laboratory conditions at temperatures ranging from 18°C to 20°C. Larvae obtained by spontaneous spawning were fed a mixture of the microalgae *Nannochloropsis oculata* and *Phaeodactylum tricornutum*. Elapsed time from fertilization to veliger larva was 22.5 h. Veliger release occurred on day 3. Formation of the fissure giving rise to the keyhole began after day 16. On days 28 through 33, the keyhole reached its final position and juveniles exhibited adult-like morphological characteristics.

KEY WORDS: *Fissurella volcano*, juvenile, Baja California, limpet

INTRODUCTION

The volcano keyhole limpet *Fissurella volcano* (Reeve 1849) is a prosobranch gastropod of the family Fissurellidae. It is found from northern California through the Baja California Peninsula of Mexico, attaching to rocks of the tidal zone, where it feeds mainly on benthic microalgae, although it may also browse on macroalgal fronds (Hobday 1995). Adults measure 2.5–4.1 cm and have a keyhole-shaped pore on the apex of the conical shell. The decreasing number of these organisms in the southern California coastal region has been used as an indicator of negative environmental impact from human activity (Kaustuv 2004). On the Baja California Peninsula, this limpet occupies the subtidal zone, forming part of the abalone and lobster bank community (Guzmán del Próo et al. 1991).

Fissurella cumingi (Reeve 1849), *F. picta* (Gmelin 1790), and *F. crassa* (Lamarck 1822) (Vega et al. 1996, Bahamondes-Rojas & Bretos 2002, López et al. 2003) are economically important. Interest in commercial species has encouraged studies that describe embryonic and larval development. To this end, various methods to induce spawning have been used, which is most effective in males (Vega et al. 1996, Bahamondes-Rojas & Bretos 2002, López et al. 2003). This study describes development under laboratory conditions of the volcano keyhole limpet *F. volcano* from populations in Baja California Sur, México, from the embryo though juvenile stages, when the specimens attained a length of 3.7 mm.

MATERIALS AND METHODS

Collection and Maintenance of Specimens

From the intertidal zone at Los Morros, Bahía Tortugas, B.C.S., (27.7°N, 114.9°W), 100 adults *Fissurella volcano* (2.0–2.5 cm in length) were collected in May 2004. The specimens were transported in a cooler to our laboratory in La Paz, B.C.S. The specimens were wrapped in several layers of the macroalga *Macrocystis pyrifera* (Linnaeus) C. Agardh, to maintain adequate moisture. Interior temperature was 10°C. In the laboratory, the specimens were placed in 40-L plastic aquariums with water at 18°C to 20°C and under constant aeration. They were fed rehydrated kelp leaves (*Macrocystis pyrifera*) and a mixture of the microalgae *Nannochloropsis oculata* (Droop & Hibberd 1977) and *Phaeodactylum tricornutum* (Bohlin 1897). All water and food was replaced every two days.

Spawning

Of the original specimens, 45 were placed at temperatures ranging from 21°C to 23°C to accelerate development of the gonads. After 40 days, spontaneous spawning occurred in this group, followed by larval development up to juvenile stage before the experiment was brought to an end after 77 days.

Sieving of Embryos and Larvae

Embryos were sieved through 236, 160, 140, and 100-μm Nytex mesh to remove organic waste. Sieving was done every four days during embryonic development. Benthic postlarvae were fed a 1:1 mixture of the microalgae *Nannochloropsis oculata* and *Phaeodactylum tricornutum*.

Seawater temperature was kept at 18°C to 20°C in the aquariums where larvae and early juveniles were maintained. Morphological changes were monitored and recorded with a video camera attached to a microscope. Some stages were embedded in liquid nitrogen to study with a scanning electron microscope (Hitachi 5300N). Size data are the mean and standard deviation derived from five observations.

RESULTS

Transportation and laboratory conditions were effective, because all specimens survived the duration of the experiment. The food provided was adequate for the limpet specimens to remain in good condition. Spawning occurred spontaneously during water replacement after 40 days in the laboratory.

*Corresponding author. E-mail: serviere04@cibnor.mx

Developmental Stages and Timing

Day 1

Fertilized eggs are dark green, spherical, with diameter of 205 ± 5 μm (Stage I). The embryo measures 125 ± 5 μm (I-E) and the distance between the embryonic membrane (I-EM) and the external capsule (I-C) is 20 μm. The external capsule is present throughout embryonic development.

First cleavage (Stage II) occurs 25–32 min after fertilization, forming two blastomeres, each with a diameter of 90 μm (II-B). Second cleavage takes place at 50–65 min, resulting in four blastomeres, each with a diameter of 90 μm. Multiple cleavages occur at 1:45–2:05 h (Stage III) and the diameter of the embryo is 125 ± 5 μm. The morula forms at 5:00–5:30 h and embryo diameter is 135 ± 5 μm (Stage IV). The blastula forms at 7:00–7:35 h and has a diameter of 135 ± 5 μm (Stage V). Gastrula formation occurs at 10:00–10:35 h, with a diameter of 145 ± 5 μm. The embryo now exhibits movements of rotation (Stage VI). The trochophore larvae form at 12:45–13:05 h and are 145 ± 5 μm long (Stage VII). The anterior end has a prototrochal girdle and two lateral tufts (VII-LT) that produce spinning movements of the larva about its longitudinal axis. Formation of the early veliger larva, still enclosed in its capsule occurs at 16:05–17:00 h (Stage VIII). The protoconch is present, but does not entirely cover the larva (VIII-P). The velum is apparent and the cephalo-pedal mass becomes prominent. Larvae are 155 ± 5 μm long. The veliger occurs at 21:30–22:30 h. Dimensions remain the same, but the protoconch now covers the larva entirely and withdraws completely inside the protoconch. Larvae are 155 ± 5 μm long (Stage IXa).

Day 2

Velum splits into two lobes (IXa-SV) and there is a fully formed operculum (IXa-O). Larvae with tentacle primordia and eye spot (IXb-ES) are 160 ± 10 μm long. Some specimens exhibit vigorous movement inside the capsule, but are unable to hatch.

Day 3

Hatching begins with the capsule hydrated and increases in volume to a diameter of 360 μm. Larvae move more vigorous and capsule begins to break. Each larva is surrounded only by an embryonic membrane, which eventually breaks, releasing the veliger larva. Free-swimming larvae begin to appear in the water. They remain 160 ± 10 μm long and have tentacles with septa, as well as a fully formed foot (Ixc-F).

Day 4

Larvae swim up and down in the water and some start attaching to the bottom of the tank (Stage X).

Day 5

Benthic postlarvae exhibit a very active foot and strong crawling movements (Stage XI). Some are still swimming. Vestigial velum cilia (XI-VC) and cephalic tentacles with septa and bristles (XI-CT) are prominent. Postlarvae are 165 ± 5 μm long.

Day 6

Branchial filaments are present (Stage XIIa-BF).

Day 7

Postlarvae attach firmly to aquarium bottom. Velum is lost and the teleconch secretion begins (XIIa-T). Postlarvae are 185 ± 5 μm long.

Days 9–12

Postlarvae begin to grow. Gills are conspicuous, and dark brown cells appear on the edge of the foot. Postlarvae are now 330 ± 40 μm long. The fan-shaped teleconch continues to grow, extending elliptically towards the posterior area (XIIb). The protoconch is increasingly limited to the upper part of the postlarvae as an appendix, which will later detach.

Day 16

A fissure, appearing on the anterior edge of the teleconch, will give rise to the keyhole (XIIc-KF), which characterizes the family Fissurellidae. Postlarvae are 470 ± 10 μm long.

Days 23 to 25

The fissure closes and forms a pore on the anterior edge of the shell (XIId-KH). Reddish-brown spots appear on the posterior region of the shell (XIIe-RP). Postlarvae are 645 ± 35 μm long.

Day 26

Juveniles (Stage XIIIa). Pigmentation occurs on anterior part of shell. Reddish-brown spots on surface of foot increase in

TABLE 1.

Development time in *Fissurella volcano* from embryo to juvenile under laboratory conditions at temperatures ranging from 18°C to 20°C.

Stage	Time
Fertilized egg	0
First cleavage	25–32 min
Second cleavage	50–65 min
Multiple cleavages	1:45–2:05 h
Morula	5:00–5:30 h
Blastula	7:00–7:35 h
Gastrula	10:00–10:35 h
Trochophore	12:45–13:05 h
Early veliger	16:05–17:00 h
Veliger	21:30–22:30 h
Splitting of velum (Formation of operculum, eye spot and tentacle primordium).	2 days
Veliger larvae released.	3 days
Benthic-planktonic behavior.	4 days
Benthic postlarvae exhibit tentacles and bristles.	5 days
Branchial filaments appear.	6 days
Onset of teleconch secretion; velum cilia lost.	7 days
Dark-brown pigmented cells appear on edge of foot. Protoconch increasingly limited to upper part of organism.	9–12 days
Formation of fissure from which keyhole arises.	16 days
End of keyhole formation. Reddish-brown spots appear on posterior of shell.	23–25 days
Anterior region of shell pigmented. Base of foot exhibits reddish brown spots ventrally. The shell grows around the keyhole.	26 days
Keyhole at center of shell. Protoconch still present.	28–33 days
Detachment of protoconch and calcification of its former area.	77 days

number. The shell continues to grow around the keyhole (60-μm diameter), giving the impression that the hole is migrating toward the center of the shell (XIIIa-AK). Juveniles are 715 ± 15 μm long and are adult-like in appearance.

Days 28 to 33

Keyhole is centered at the top of the shell and is 100 μm in diameter. The vestigial protoconch is present toward the posterior end of the shell (XIIIb-VP), which is now 1,030 ± 20 μm long.

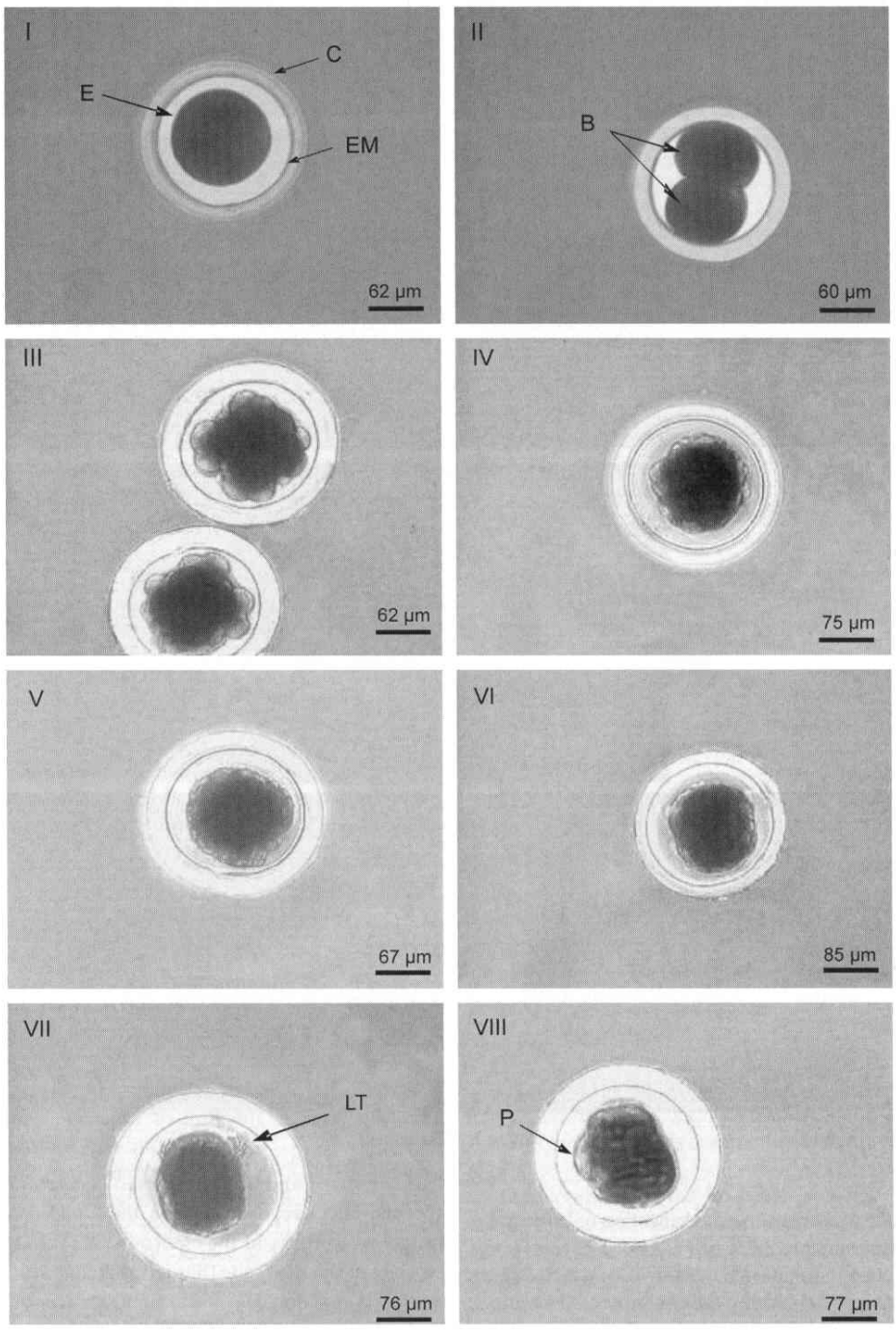

Figure 1. (I) Fertilized egg: C = capsule, EM = embryonic membrane, E = embryo; (II) First cleavage: B = blastomere; (III) Multiple cleavage; (IV) Morula; (V) Blastula; (VI) Gastrula; (VII) Trochophore: LT = lateral tufts; (VIII) Early veliger: P = protoconch; (IX a, b, c) Late veliger: SV = splitting of velum, O = operculum, ES = eye spot, F = foot; (X) Benthic-planktonic larva; (XI) Postlarva: VC = vestigial velum cilia: CT = cephalic tentacles with septa and bristles; (XII a, b, c, d, e) Postlarva: BF = branchial filament, T = teleconch, KF = keyhole fissure, KH = keyhole, RP = reddish pigment; (XIII a, b, c) Juvenile: AK = apical keyhole, VP = vestigial protoconch, CP = calcification of protoconch area.

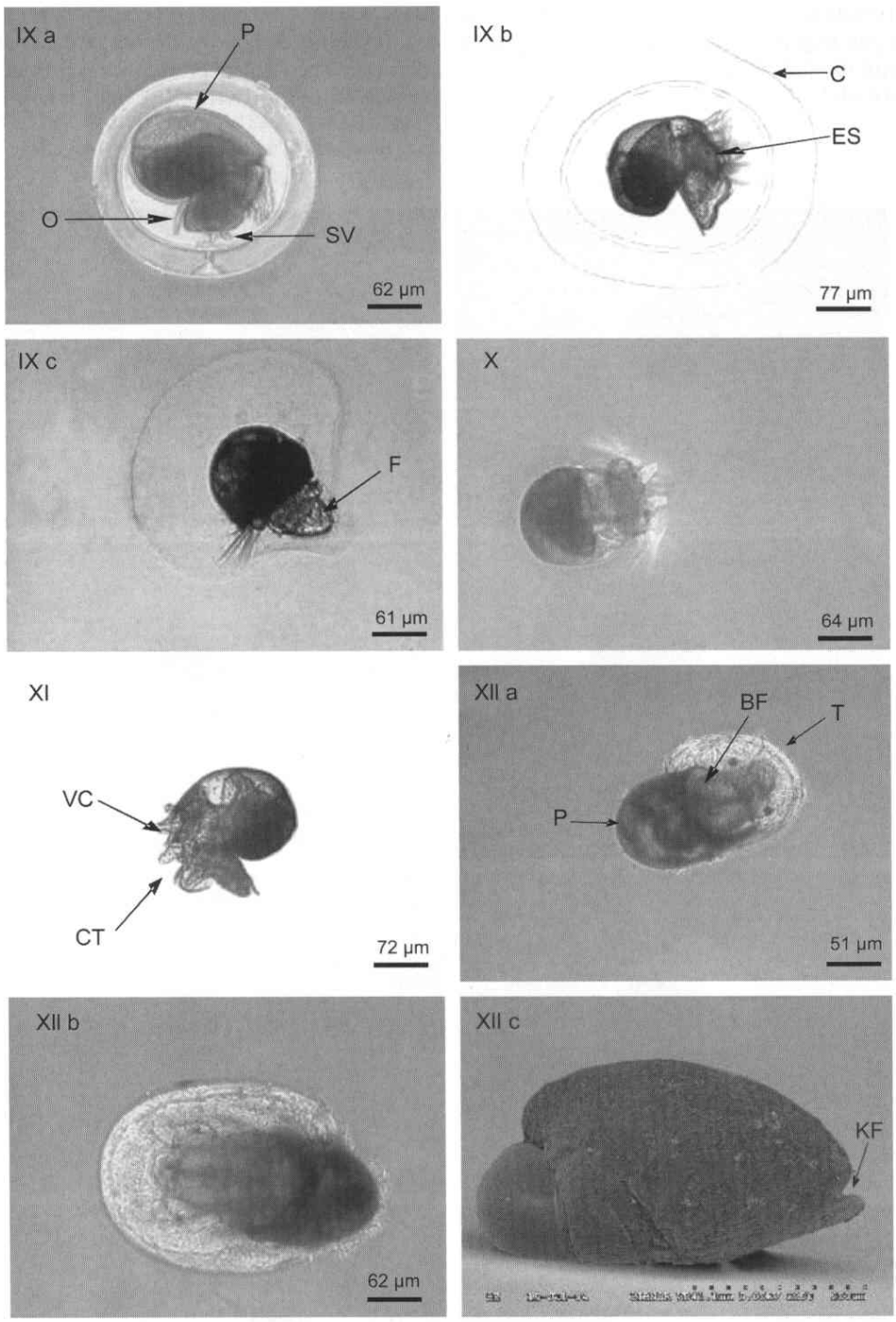

Figure 1. (Continued)

Day 77

Detachment of the protoconch and calcification of its site takes place (XIIIc-CP). Juveniles are 3,320 µm (3.32 ± 0.03 mm) long.

Table 1 and Figure 1 summarize the stages of development. Figure 2 summarizes growth during the experiment. The rate of growth was about 43 µm·day^{-1}.

DISCUSSION

Laboratory maintenance of original adult limpets posed no problems. Feeding the specimens on rehydrated kelp leaves (*M. pyrifera*) and a mixture of *N. oculata* and *P. tricornutum* proved effective, even though these microalgae are not found attached to rocks in the environments inhabited by this limpet species at the study site (Siqueiros-Beltrones 2002). The diet was suitable, not only for adult maintenance, but also for maturation of the gonads, as evidenced by spontaneous spawning after 40 days in the laboratory. Other species, such as *F. cumingi* (Vega & Osorio 1995, Vega et al. 1996), *F. crassa* (Huaquín et al. 1998) and *F. picta* (Bahamondes-Rojas & Bretos 2002), required different methods to induce spawning to obtain gametes under

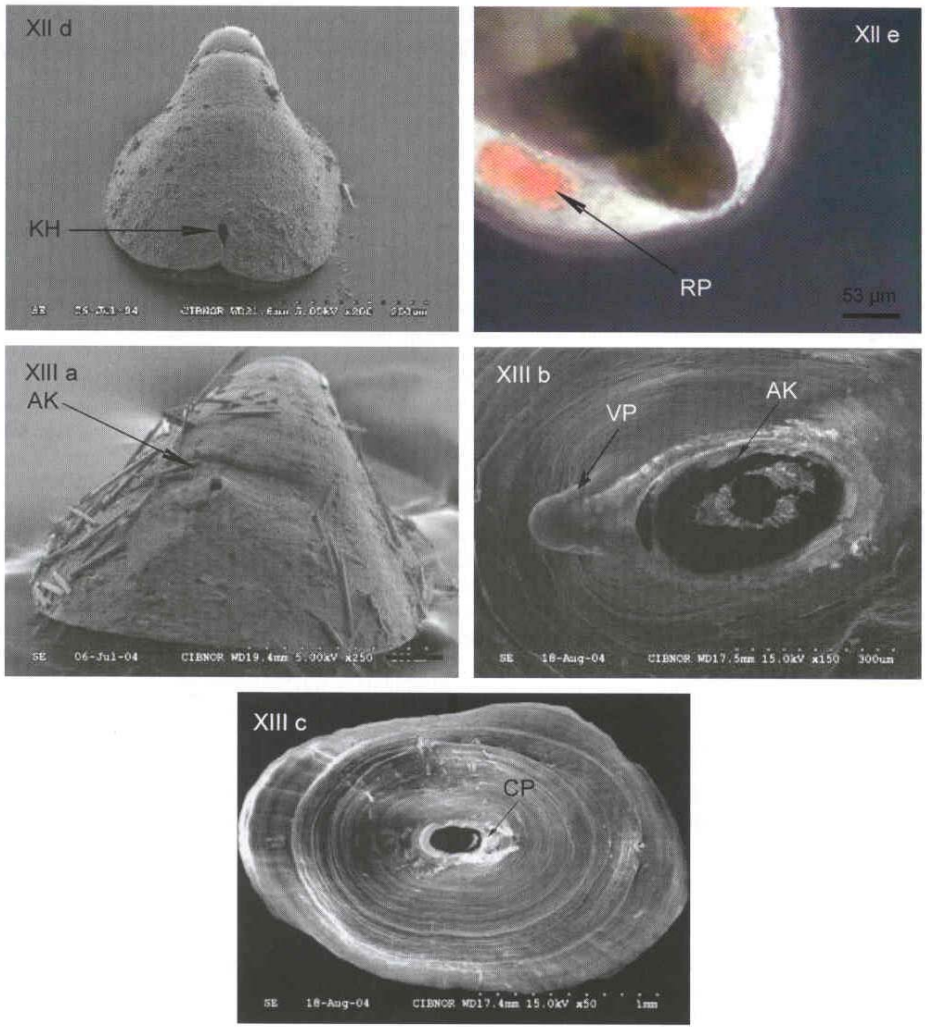

Figure 1. (Continued)

laboratory conditions, such as potassium chloride injected into the mantle cavity and added to the seawater, maceration of gonads along with exposure to ambient air, hydrogen peroxide injections or prostaglandin added to seawater exposed to UV radiations. In *F. picta*, artificial techniques are also reported to have failed and spawn had to be obtained by collecting ripe specimens (González et al. 1999). In our case, spawning may have been induced by specimen handling during water replacement, which implied exposure to ambient air and minimal changes (±2°C) in aquarium temperature.

Mean diameter of *F. volcano* oocytes (125 ± 5 µm) is smaller, as well as more homogeneous, than other species of the genus. For example, oocyte diameter in *F. picta* is 117–327 µm with a mean of 178 µm (Bahamondes-Rojas & Bretos 2002) and *F. crassa* is 229 ± 22 µm (Huaquín et al. 2004). A gelatinous membrane surrounding the oocyte has been described in *F. picta* (González et al. 1999), but not observed in our study.

Elapsed time before attaining the various stages of embryonic development, up to veliger larva, differed from reported times in *F. picta* (Table 2). Higher temperature may accelerate mollusc development (Acosta et al. 2000). However, differences in developmental time in *F. volcano* and *F. picta* (Bahamondes & Bretos 2002) may be attributed to characteristics unique to the species itself, because tank temperature was similar (18°C to 20°C and 17.5 ± 1°C, respectively). Variations among populations of *F. picta* may result from differences in the temperatures at which the assays were performed, such as, 10.0°C versus 17.5 ± 1°C (González et al. 1999, Bahamondes-Rojas & Bretos 2002). Comparisons are not possible beyond the planktonic veliger larval stage because the available information about other *Fissurella* species does not include the later developmental stages.

Development of the keyhole, as a fissure on the anterior region of the shell, is a characteristic of the Fissurellidae, which begins on day 16. This is similar to the process in the gastropod *Diodora aspera* (Rathke 1833) (Pernet 1997).

Embryonic and larval development in the volcano keyhole limpet, up to the veliger larval stage, is morphologically similar to development in the gastropods *Megastraea undosa* and *Tegula funebralis*, which was described in earlier studies, but differs until secretion of the teleconch occurs (Guzmán del Próo et al. 2003, 2006). In the volcano keyhole limpet, the teleconch is

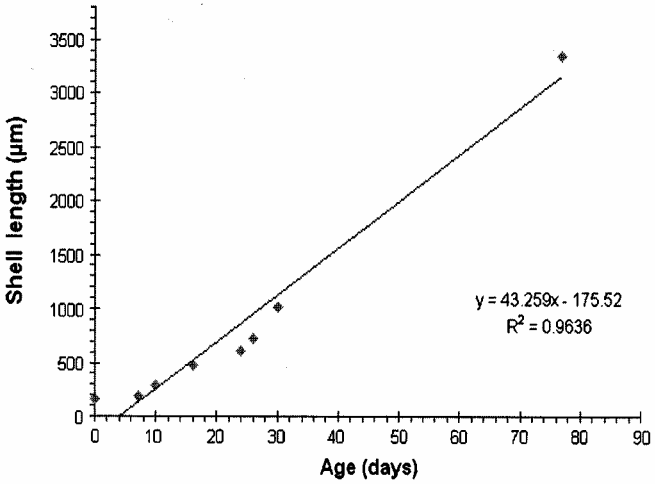

Figure 2. Postlarval and juvenile growth of *Fissurella volcano* over 77 days. Day 0 corresponds to veliger stage. Specimens were fed a 1:1 mixture of *Nannochloropsis oculata* and *Phaeodactylum tricornutum*. ♦ = Average of 5 measurements.

fan-shaped and extends toward the posterior region, whereas in *M. undosa* and *T. funebralis* it is tubiform, spiral-shaped, and extends toward the anterior part of the shell (Guzmán del Próo et al. 2003, 2006).

TABLE 2.

Comparison of embryonic development in *Fissurella volcano* and *F. picta*.

Stage	*Fissurella picta*[1]	*Fissurella picta*[2]	*Fissurella volcano*[3]
Temperature (°C)	10	17.5 ± 1	18–20
Salinity (psu)	32	29–30	38
First polar body	1 h	0.2–0.25 h	NO
Multiple cleavages	>12 h	>7 h	1:45–2:05 h
Trochophore	72 h	15 h	12:45–13:05 h
Early veliger	NO	21 h	16:05–17:00 h
Free-swimming veliger and first torsion	NO	40 h	21:30–22:30 h

[1] González et al. (1999), [2] Bahamondes-Rojas & Bretos (2002), [3] This study. NO = Not observed.

ACKNOWLEDGMENTS

The authors express their appreciation to Centro de Investigaciones Biológicas del Noroeste (CIBNOR Grant AC5.1) and Instituto Politécnico Nacional (IPN Grant CGEPI 200494) for financial support of our bi-institutional study. Jorge Belmar and Jorge Carrillo helped procure broodstock, Alejandra Mazariegos assisted in broodstock maintenance, and Ira Fogel provided editing services. The microalgae laboratory at CIBNOR supplied the microalgae used as food. Cooperativa de Producción Pesquera Bahía Tortugas and its technicians, Alejandro Villa Bastida and Alberto Castro provided support during fieldwork; and Centro Regional de Investigaciones Pesqueras in La Paz allowed us to use their facilities in Bahía Tortugas. Permiso de Pesca de Fomento SAGARPA.123.05.1836/2003.

LITERATURE CITED

Acosta, V., L. Freites & C. Lodeiros. 2000. Densidad, crecimiento y supervivencia de juveniles de *Lyropecten* (*Nodipecten*) *nodosus* (Pteroidea: Pectinidae) en cultivos suspendidos en el Golfo de Cariaco, Venezuela. *Rev. Biol. Trop.* 48:799–806.

Bahamondes-Rojas, I. & M. Bretos. 2002. Induction of spawning and early development in *Fissurella picta* (Mollusca: Archaeogastropoda) from southern Chile. *J. Shellfish Res.* 21:185–191.

González, M. L., M. Pérez, D. A. López, J. M. Uribe & C. A. Pino. 1999. Early development of *Fissurella picta* (Gmelin 1791). *Veliger* 42:275–277.

Guzmán del Próo, S. A., P. S. R. Mille, G. R. Guadarrama, G. S. de la Campa, L. J. Carrillo, C. A. Pereira, P. J. Belmar, A. M. J. Parra & G. A. Luque. 1991. La comunidad bentónica de los bancos de abulón (*Haliotis* spp. Mollusca: Gastropoda) en Bahía Tortugas, Baja California Sur, México. Anales de la Escuela Nacional de Ciencias Biológicas, México. *D. F.* 36:27–59.

Guzmán del Próo, S. A., T. Reynoso-Granados, P. Monsalvo-Spencer & E. Serviere-Zaragoza. 2003. Larval and early juvenile development of the wavy turban snail *Megastraea undosa* (Wood 1828) (Gastropoda: Turbinidae). *Veliger* 46:320–324.

Guzmán del Próo, S. A., T. Reynoso-Granados, P. Monsalvo-Spencer & E. Serviere-Zaragoza. 2006. Larval and early juvenile development in *Tegula funebralis* (Adams 1855) (Gastropoda: Trochidae) in Baja California Sur, México. *Veliger* 48:116–120.

Hobday, A. 1995. Body-size variation exhibited by an intertidal limpet: influence of wave exposure, tidal height and migratory behavior. *J. Exp. Mar. Biol. Ecol.* 189:29–45.

Huaquín, L., R. Guerra & M. Bretos. 1998. Identificación del sexo y morfología de gametos de la lapa *Fisurella crassa* Lamarck 1822 (Mollusca: Archaeogastropoda). *Revista de Biología Marina y Oceanografía* 33:223–339.

Huaquín, L., R. Guerra & A. del Campo. 2004. Ovary cell differentiation and morphology of mature oocytes in *Fisurella crassa* Lamarck 1822 (Mollusca: Archaeogastropoda). *Invert. Rep. Dev.* 46:103–110.

Kaustuv, R., A. G. Collins, B. J. Becker, E. Begovic & J. M. Engle. 2004. Anthopogenic impacts and historical decline in body size of rocky intertidal gastropods in southern California. *Ecol. Lett.* 6: 205.

López, D. A., M. L. González & M. C. Pérez. 2003. Feeding and growth in the keyhole limpet, *Fissurella picta*, (Gmelin 1791). *J. Shellfish Res.* 22:165–169.

Pernet, B. 1997. Development of the keyhole and growth rate in *Diodora aspera* (Gastropoda: Fissurellidae). *Veliger* 40: 77–83.

Siqueiros-Beltrones, D. 2002. Diatomeas bentónicas de la península de Baja California; Diversidad y Potencial Ecológico, Instituto Politécnico Nacional y Universidad Autónoma de Baja California Sur. Ed. Instituto Politécnico Nacional-Centro Interdisciplinario de Ciencias Marinas. México, 102 pp.

Vega, G. & C. Osorio. 1995. Evaluación del potencial de cultivo de *Fissurella maxima* y *Fissurella cumingi*. In: XV Jornadas Ciencias del Mar. Coquimbo, Chile. May.

Vega, G., C. Osorio, J. Reemonsellez & A. Von Marées. 1996. Avances en el cultivo de *Fissurella cumingi* (Mollusca:Archaeogastropoda). In: IX Congreso Latinoamericano de Acuicultura, Coquimbo, Chile. October 15–18.