

Developmental stages and potential mariculture for coastal rehabilitation of endangered Pacific angelwing clam, *Pholas orientalis*

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Abstract

Sexually mature Pacific angelwing clams, *Pholas orientalis*, were collected from the wild and spawned and cultured under laboratory conditions to document early developmental stages and to develop culture techniques for coastal rehabilitation of this endangered species.

There was a highly significant difference ($P < 0.01$) found in induced spawning of *P. orientalis* using desiccation method compared with those that were not desiccated. Depending upon the size, spawners released between 1 and 2 million eggs. Wild spawners used in this study were functional simultaneous hermaphrodites that self-fertilized their own eggs (43.0 ± 0.8 μm diameter). First cellular division and motile morula occurred after 46 min and 3 h, respectively. Actively swimming early veliger and crawling pediveliger stages emerged after about 15 h and 22 h, respectively. Approximately 99.7% of straight-hinge larvae (58.3 ± 0.3 μm shell length) appeared after about 23 h. Fed with *Isochrysis galbana*, *Chaetoceros calcitrans*, and *Nannochloropsis* sp., the average survival rate at umbo stage (128 μm shell length; 115 μm shell width) after 10 days was 92% at $30.0 \pm 1.0\%$ salinity, pH 8.04–8.15 and temperature 23.5–29 °C. Survival rate of juveniles (1.046 mm shell length; 0.701 mm shell width) in muddy substrate after 30 days post-spawning was about 16%.

This is the first time that *P. orientalis* has been successfully spawned in captivity and reared through early embryonic, larval, and juvenile stages. Postlarvae of *P. orientalis* were successfully introduced in the muddy cove of San Dionisio, Panay Island, Philippines (11°13'N, 123°04'E) and have produced successive generations that successfully colonized the area. Coastal rehabilitation and mariculture using hatchery-produced seedstock of this species show very encouraging results.

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1. Introduction

The endangered Pacific angelwing clam, *Pholas (Monothyra) orientalis* Gmelin 1790, is a deep burrowing bivalve mollusc found mostly in the tidal flats of Southeast Asia. It has a habit of extending its long siphon

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out of the mud during filter feeding. This clam is one of the most expensive bivalves in the Philippine market because of its large size, tender and juicy meat, and sweet taste. This commercially important clam is exported and highly esteemed in Taiwan and Hong Kong because of its excellent flavor, attractive milky white shell, and the Asian notion that this clam is an aphrodisiac.

Unfortunately, due to unregulated gathering, overfishing and destruction of the natural habitat of this highly prized bivalve, the indigenous population of *P. orientalis* is rapidly vanishing in the Philippine islands of Panay and Negros (Laureta and Marasigan, 2000; Marasigan and Laureta, 2001). The overexploitation of this species through the use of effective fishing and collecting gear in both intertidal and subtidal zones is one factor that has effectively reduced the population of *P. orientalis* in Philippine waters. The unregulated use of mollusc poisons in many shrimp and milkfish fishponds and subsequent flushing and release of these non-biodegradable and organo-metallic pesticides from the fishponds to the coastal environment are also thought to be contributory factors for the disappearance of *P. orientalis* in the estuaries, bays, and coves of the Philippines. There is an urgent need to save this endangered species before it becomes extinct in the Philippine waters.

Since the late 1990s, angelwing clams have been extremely hard to find in the fish markets in the Philippines. In the coastal towns of Negros Occidental along Guimaras Strait, this species has already vanished. Some proposed solutions for reversing this trend are the implementation of sound fishery management to protect the natural population of angelwing clams, and development of hatchery techniques for coastal restocking to augment the declining natural stock of this species (Marasigan and Laureta, 2001).

To date, there is a dearth of basic biological or fishery related literature for *P. orientalis*. The earliest report was that of Ablan (1938) concerning the fishery of this species in Negros Occidental, Philippines. The recent fishery of this species in Southeast Asia was reported by Davidson (1976); in Thailand by Saraya (1982) and Amornjaruchit (1988); and in the Philippines by Young and Serna (1982). Initial attempts to spawn *P. orientalis* in Thailand were reported by Sahavacharin et al. (1988) but no evidence of success was presented. A preliminary description of the reproductive stages of *P. orientalis* maintained in the laboratory was reported by Laureta and Marasigan (2000). Marasigan and Laureta (2001) also compared the effect of mixed algal species of *Chaetoceros calcitrans* and *Tetraselmis suecica* against single-algal species diet to enhance gonadal development and maturation under laboratory conditions.

Although nothing is known of the larval development and larval biology of *P. orientalis*, it is very important to develop a viable hatchery technology and to devise stock enhancement techniques for this species. The objectives of this study were to document and observe the early developmental stages of *P. orientalis*; and, to develop mariculture techniques for mass seed-stock propagation and coastal rehabilitation of this commercially important endangered species.

2. Materials and methods

2.1. Collection, transport and cleaning of spawners

Several *P. orientalis* adults ($N > 500$; total weight = 11.7–29.0 g; shell length = 7.8–21.6 cm) were collected every month during neap tide for a period of about two years from the intertidal muddy flats in Barotac Nuevo, Iloilo (10°52'N, 122°40'E) along Guimaras Strait. Adult angelwing clams were found burrowing in sticky, soft sandy–muddy bottoms rich in silt and fine organic matter but devoid of benthic macrophytes. The ambient water temperature and salinity of the site ranged between 28 and 30 °C and between 30‰ and 35‰ S, respectively. The angelwing clam breeders were collected by digging their burrows (ca. 30–50 cm deep) carefully with bare hands. The collected spawners were placed in plastic buckets with seawater and transported by road to the Science and Technology Park facilities of the University of the Philippines in the Visayas, Miag-ao Campus.

Upon arrival, the spawners were placed in white plastic basins (20-L capacity) and the shells were scrubbed with fine brush to remove dirt, potential parasites, and fouling organisms. Samples were measured using Vernier calipers and weighed using a digital balance. The length–weight correlation equation for this species was derived and plotted with the aid of statistical package Sigmaplot (SPSS Science, Chicago, IL, USA). Temperature, pH, salinity and D.O. of spawning and culture water were checked using a clinical thermometer, Hanna digital pH meter, Atago refractometer, and YSI dissolved oxygen meter, respectively. The seawater (30.0 ± 1.0‰ S) used in the experiment was pumped directly from the sea (3–5 m depth), passing through a sand–gravel filter box with activated charcoal and finally through a 1 µm filter bag.

2.2. Spawning experiments

To determine the most efficient way of spawning gravid angelwing clams by natural means, the following

experiments were conducted. In the first experiment, a set of about 20 newly collected breeders (shell length=15.5–21.6 cm; weight=15–29 g) was exposed to air and placed in covered pails without water for 10–12 h during transport to the hatchery. Another equal set of breeders was continuously soaked in seawater from the time of collection until they were used in the spawning experiment. For each treatment, randomly selected spawners were placed in four white plastic basins (20-L) at a stocking density of 0.25 individual L^{-1} . Each spawning basin was supplied gently with aeration and filtered seawater (30‰ S). The comparison experiment was repeated three times every month. The spawning results in percentages were transformed into arcsines and evaluated using paired comparison *t*-test.

In the second experiment, there were three replicates for each treatment. Five randomly selected spawners were placed in each of the three basins (20-L) assigned for each treatment. Wild spawners that were acclimated in broodstock tanks for about a week were used in this experiment. The following treatments were used: (1) desiccated spawners; (2) thermally stimulated spawners; and (3) unstressed spawners (control). In Treatment 1 (desiccation method), 15 spawners were taken from the broodstock tank, placed in covered basins without water, and left undisturbed. After about 8 h, the desiccated spawners were equally distributed in 3 white plastic basins (20-L capacity) and supplied with mild aeration and filtered seawater (30‰ S) at ambient temperature (29 ± 0.5 °C). In Treatment 2 (thermal stimulation method), the temperature of the mildly aerated spawning water was adjusted and maintained at 37 °C with boiled seawater for about one hour. In Treatment 3 (control), unstressed spawners from broodstock tanks were used and stocked directly in spawning basins with water conditions just like in Treatment 1. The percentage data were transformed into arcsine values and analyzed using one-way ANOVA; then, treatments were subjected to Duncan's multiple range test to determine significant differences (Gomez and Gomez, 1984).

2.3. Broodstock development and burrowing habit observation

For future use in other studies, spent spawners and sexually immature *P. orientalis* were acclimated and placed in concrete tanks (250- to 500-L capacity) with 30 cm of sterilized muddy substrate. Some were stocked in concrete drainage canals (width=50 cm) with muddy substrate (depth=ca. 20 cm) and slow flowing water (height \geq 20 cm). The mud used as substrate in this

study was collected from Barotac Nuevo, Iloilo and sterilized by boiling for one hour in big pots, then sun-dried for about a week. The concrete tanks and canals holding the breeders were provided with flow-through unfiltered seawater. An equal ratio of phytoplankton (*C. calcitrans*, *Skeletonema costatum*, *Tetraselmis* sp., and *Nannochloropsis* sp.) by volume, cultured separately in 10,000-L capacity canvas and concrete tanks, was fed ad libitum to the breeders. Mixed phytoplankton cultured in canvas-lined earthen ponds (200 m^2 ; depth \geq 1.5 m) were pumped continuously to the breeders maintained in canals at a flow rate of about 500-L day^{-1} using plastic tubing (4.8 mm diameter).

The burrowing habit of angelwing clams was observed in glass aquaria with mild aeration. Nine adults (shell length=15.5–21.6 cm; weight=15–29 g) were placed in glass tanks (100-L capacity) with about 25 cm of sterilized sticky, soft sandy–muddy substrate. Three adult angelwing clams were placed randomly on their sides on the surface of the substrate. Three others were inserted in the mud vertically and the rest were inserted obliquely (45° inclined) with the foot and protoplax buried 4–5 cm below the mud. Mixed phytoplankton from 10,000-L capacity canvas and concrete tanks were provided ad libitum to experimental clams for about 30 days. About 30–50% of the culture water volume was changed daily.

2.4. Algal culture

Algal inocula were bought from the Phycology Laboratory of the Aquaculture Department of Southeast Asian Fisheries Development Center (SEAFDEC AQD) in Tigbauan, Iloilo, Philippines. Before the start of spawning operations, unicellular algae were cultured in 1-L dextrose bottles, Erlenmeyer flasks, and 4-L carboys. *Tetraselmis* sp. and *Nannochloropsis* sp. were cultured using Yashima medium (Hirata, 1980). *C. calcitrans*, *S. costatum*, and *Isochrysis galbana* were cultured by enriching the culture medium with 300 $mg L^{-1}$ of KNO_3 , 30 $mg L^{-1}$ of $NaH_2PO_4 \cdot 2H_2O$, 15 $mg L^{-1}$ of Na_2SiO_3 and 30 $mg L^{-1}$ of Clewat-32 (Ronquillo and Saisho, 1993, 1994, 1995, 1997; Ronquillo et al., 1997, 1999). The uni-algal culture in 10,000-L capacity canvas tanks and mixed algal culture in canvas-lined earthen ponds (200 m^2 ; depth \geq 1.5 m) were fertilized with agricultural fertilizers at the rate of 40 $g ton^{-1}$ ammonium phosphate, 20 $g ton^{-1}$ ammonium sulfate, and 20 $g ton^{-1}$ urea. Algal cultures, which reached the exponential phase, were harvested and fed to the larvae at a density described below (Section 2.6).

2.5. Sex determination and estimation of fecundity

Newly collected sexually mature angelwing clams ($N=20$) were randomly selected and shucked to investigate sexuality and gonadal condition. Half were preserved in formalin and processed for histological preparation (Bancroft and Stevens, 1990). A research microscope (Nikon Labophot-2), that was interfaced with GLOBAL LAB® Image software (Scientific Imaging Software, I-Cube Inc.), was used to analyze fresh smears of gonads from newly sacrificed specimens ($N=10$). The same microscope was used to examine newly released gametes during spawning process, and to document the embryonic and post-embryonic developmental changes. Fecundity was determined by aliquot sampling. At least 5 samples from each spawning tank were counted using a Sedgewick-Rafter counting chamber under a Nikon dissecting microscope. To estimate the total number of spawned eggs of individual spawners, the average number of eggs from 5 aliquot samples was multiplied with the total volume of the spawning tank.

2.6. Incubation and larval rearing

After spawning, the newly released gametes were taken out of the spawning tank, and filtered through layers of 1000 μm , 500 μm and 250 μm plankton nets to remove dirt and potential contaminants. They were then placed in big white basins (20-L capacity) with seawater filtered using 1- μm filter bags, at a stocking density of

100–200 eggs mL^{-1} . Egg incubation was done at room temperature (27–29 °C) with gentle aeration applied. After about 15 h, the veliger larvae were transferred to two 1000-L fiberglass tanks without substrate and to a fabricated canvas tank (0.5 m \times 0.3 m \times 5 m) with sterilized muddy substrate at a stocking density of 2–4 ind. mL^{-1} or a total of about 2.25×10^6 larvae. After about 24 h, when the larvae reached the settlement stage, they were fed daily with a mixed algal diet consisting of $25\text{--}50 \times 10^3$ cells mL^{-1} *I. galbana*, $20\text{--}30 \times 10^3$ cells mL^{-1} *C. calcitrans*, and $30\text{--}50 \times 10^3$ cells mL^{-1} *Nannochloropsis* sp. The survival rate of postlarvae and juveniles was determined by counting the average number of survivors in five randomly selected areas (2.54 cm \times 2.54 cm dimensions) in the culture tanks; then multiplying the average with the total area of the tank.

2.7. Monitoring of developmental stages

As the spawners released gametes, samples were taken directly from the siphons and monitored using a research microscope (Nikon Labophot-2). Others were placed in 500-mL glass beakers and in 100-L aquaria. Using GLOBAL LAB® Image (GLI) software, every stage of embryonic and larval development was observed on a TV monitor. Images were saved for embryonic and larval measurements; and then electronic images were processed using Corel Draw and Adobe PhotoShop software. Changes in embryonic and post-embryonic development were monitored and recorded.

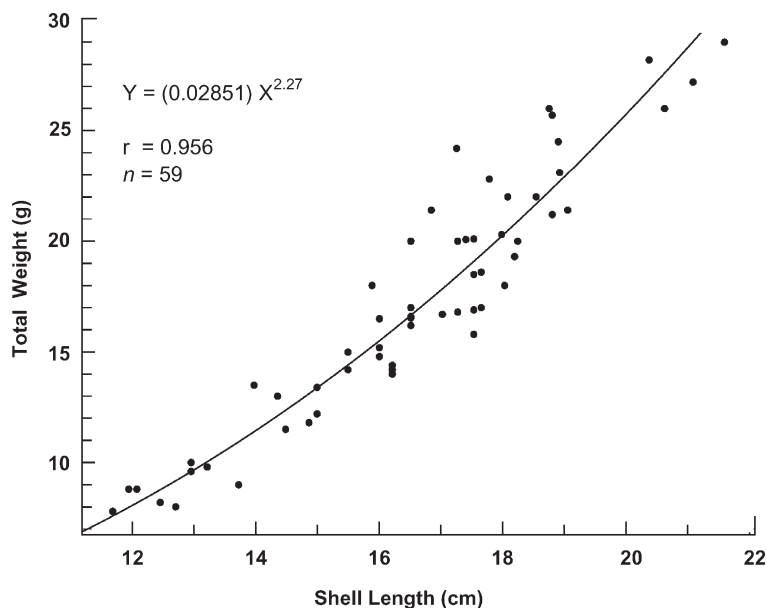


Fig. 1. Length–weight relationship of *Pholas orientalis* broodstock.

2.8. Initial field stocking

For field stocking, several spent adults and hatchery-produced postlarvae of *P. orientalis* were initially stocked in the cove of San Dionisio, Iloilo (11°13'N, 123°04'E). The cove was selected because it is protected from strong waves and current by the surrounding small islands. Seawater salinity of the area was 28–33‰ S and there were no large fishponds operating in the vicinity. The patchy mangrove trees along the coast were dominated with mixed stands of *Rhizophora*, *Avicennia*, and *Bruguiera*. The cove was rich in phytoplankton like diatoms and chlorophytes, and several unidentified nanoplankton. The bottom contained the sticky, muddy substrate preferred by *P. orientalis*. Based on field surveys and interviews with coastal community residents, there was no historical record or evidence that *P. orientalis* had ever existed in this area prior to this study.

Adult *P. orientalis* ($N=10$; weight= 17.3 ± 5.4 g; length= 16.6 ± 2.3 cm), that were introduced in the cove to test the suitability of the area for survival and growth, were enclosed by a net and bamboo poles for ease of monitoring and as protection against predators. After determining the viability of the area, the postlarvae or early umbo stage organisms ($N\geq 3,000,000$; $127.5\pm$

0.5 μm shell length; 115.1 ± 0.5 μm shell width) were scattered throughout the cove using a motorized boat in a trial test to determine whether they would be able to develop and colonize the cove.

3. Results

3.1. Size and morphology

There was no external sexual dimorphism observed among the spawners. The length–weight regression relationship of *P. orientalis* breeders (total weight= 11.7 – 29.0 g; shell length= 7.8 – 21.6 cm; $N=59$) is shown in Fig. 1. The derived correlation equation is expressed as

$$Y = (0.02851)X^{2.27} \quad r = 0.956$$

where Y is the total weight of live clam in grams (g); and X is the shell length in centimeters (cm).

3.2. Gonads and gametes

Gonadal colors of freshly shucked angelwing clam spawners were whitish for testis and light beige for

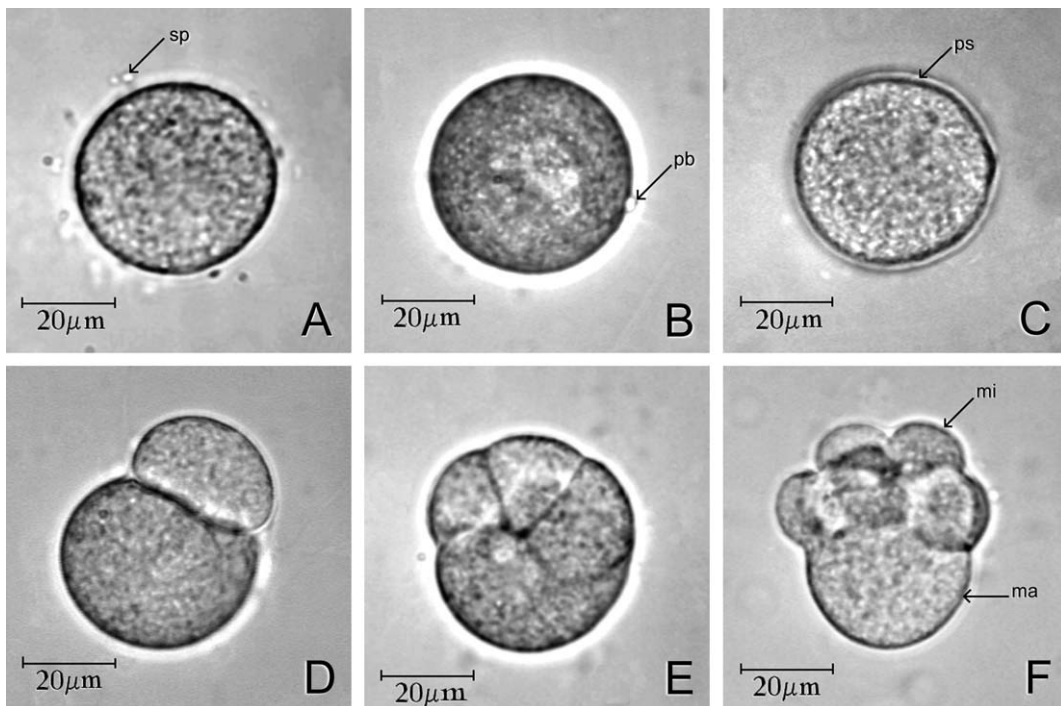


Fig. 2. Early developmental stages of *Pholas orientalis*: (A) unfertilized egg with attached sperm cells (00:00); (B) formation of first polar body (00:20); (C) fertilized egg showing perivitelline membrane (00:38); (D) first cleavage (00:46); (E) second cleavage (01:17); (F) third cleavage (01:20). Abbreviations: ma, macromere; mi, micromere; pb, polar body; ps, perivitelline space; sp, sperm.

ovaries. Histological analysis of *P. orientalis* gonads showed fused ovary and testis (ovotestis) enclosing the entire visceral region. Follicles revealed multi-flagellated spermatids. Motile mature spermatozoa had a globular head ($1.0 \pm 0.07 \mu\text{m}$) and possessed one long flagellum ($35.3 \pm 3.5 \mu\text{m}$ long) and four short flagella ($10.0 \pm 0.5 \mu\text{m}$ long). The average diameter of newly released matured eggs was $43.0 \pm 0.8 \mu\text{m}$ (Fig. 2A). Presence of both eggs and sperm cells were detected in gonoducts and both emerged simultaneously from the siphons during spawning with numerous sperm cells attaching to each released egg (Fig. 2A).

3.3. Spawning and fertilization

For the first spawning experiment, the paired *t*-test showed that there was a highly significant difference ($P < 0.01$) found in the induced spawning of *P. orientalis* using the desiccation method compared with the other treatment method. The spawners desiccated for about 8–10 h released gametes 5–30 min after being placed in a plastic basin filled with new filtered seawater. Similarly for the second spawning experiment on induced spawning of acclimatized breeders, there was a highly significant difference found ($P < 0.01$) using ANOVA in the use of the desiccation method (Treatment 1) compared with the thermal stimulation spawning method (Treatment 2) and the control (Treatment 3). No spawning was observed in the control and 0–20% spawning was observed in thermal stimulation.

During spawning, functional simultaneous hermaphrodite *P. orientalis* slowly released both sperm and eggs from exhalant and inhalant siphons, but very seldom from the posterior opening. Depending upon the size, each spawner has a fecundity ranging between 1 and 2 million eggs. Microscopic analysis of gametes coming out of the siphons of each individual spawner showed that both eggs and sperm were simultaneously released into the water column. As gametes emerged out of the siphons, sperm were already attached to the eggs (Fig. 2A). As observed in situ, self-fertilization in *P. orientalis* was ectosomatic.

3.4. Embryonic and early larval development

Table 1 summarizes the embryonic and larval development of *Pholas orientalis*. Figs. 2–5 show the series of embryonic and larval development; while Fig. 6 illustrates the constructed life cycle. During the sperm penetration stage, swarms of spermatozoa surrounded an egg. The eggs (Fig. 2A) and early embryos were

Table 1
Early developmental stages of *Pholas orientalis* at 29.0‰ S and pH 8.04–8.15

Stage	Temperature (°C)	Cumulative developmental time (h:min)	Cumulative degree-days (D-D)
Spawning	29.0	0:00	0.00
Sperm attachment	29.0	0:00	0.00
First polar body formation	28.9	0:20	0.40
Fertilization membrane formation	28.8	0:38	1.16
First cleavage	28.8	0:46	2.08
Second cleavage	28.6	1:17	3.62
Third cleavage	28.5	1:20	5.20
Fourth cleavage	28.0	1:41	6.78
Fifth cleavage	27.0	2:36	9.00
Sixth cleavage	26.5	2:55	11.33
Seventh cleavage	26.5	3:08	13.26
Blastula	25.0	4:40	19.26
Gastrula	24.8	8:02	24.61
Early trochophore	24.0	10:07	31.65
Late trochophore	23.5	11:02	39.11
Early veliger	23.8	15:37	49.87
Late veliger	24.5	20:55	65.97
Crawling pediveliger	25.3	21:50	80.29
D-Hinge/Settlement	26.0	23:10	96.21
Umbo	29.0	168:25	288.80

denser than seawater, immotile and settled at the bottom of incubation tanks. About 20 min after spawning, formation of the first polar body ($4 \mu\text{m}$) was observed (Fig. 2B) and the egg became motionless. This was followed by the formation of perivitelline membrane and very narrow (ca. $1 \mu\text{m}$) perivitelline space (Fig. 2C) within 38 min after spawning.

After about 46 min, the first indeterminate cleavage (Fig. 2D) was observed. The first daughter cell was seen protruding gradually and enlarging to about $20 \mu\text{m}$ in diameter. Then, after 1 h 17 min post-spawning, the second cleavage (Fig. 2E) was observed: the micromere divided equally and the macromere produced a daughter cell similar in size with the other two micromeres. As cleavage continued, the micromeres became concentrated in the animal pole. Embryos showed modified spiral cleavage and protostomic development.

When the embryo reached sixth cleavage stage (Fig. 3C), cilia appeared from the external surface of individual blastomeres and became motile, i.e. spinning very fast like a top. During the seventh cleavage, the embryo had a centrally located depression (Fig. 3D), $49 \mu\text{m}$ long, very motile but it swam in a random, graceful, and vacillating motion. During the closing of the blastopore (Fig. 3E), the shape of the embryos became rounded and the motion changed to a rapid

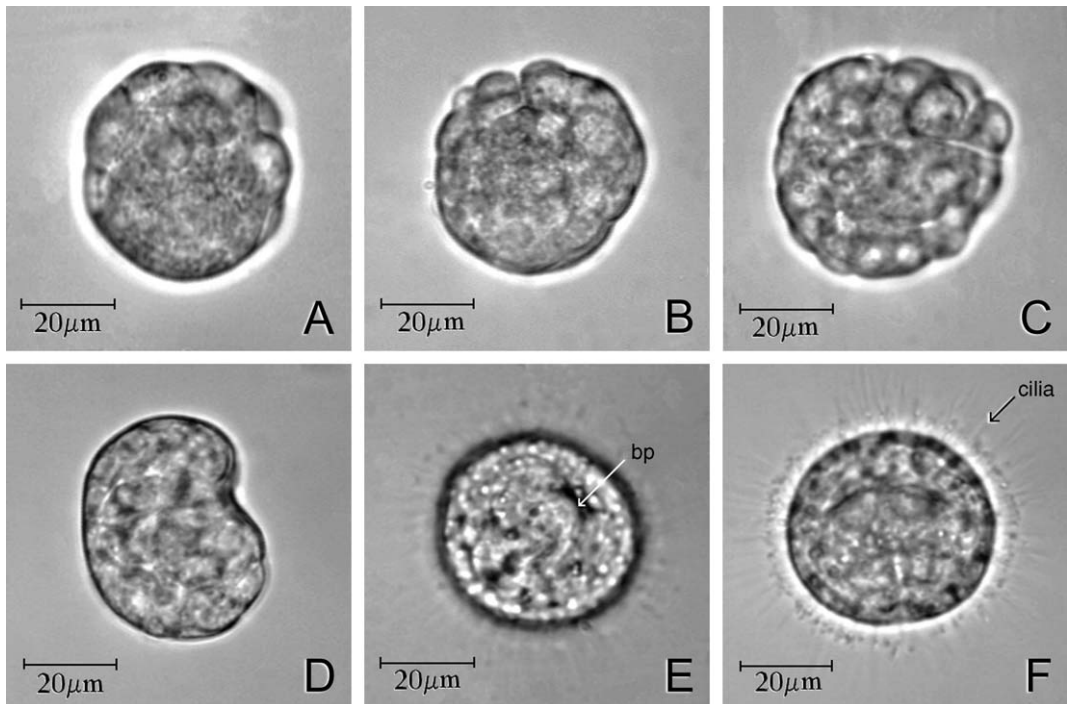


Fig. 3. Early developmental stages of *Pholas orientalis*: (A) fourth cleavage (01:41); (B) fifth cleavage (02:36); (C) sixth cleavage (02:55); (D) seventh cleavage (04:40); (E) blastula (08:02); (F) gastrula (09:25). Abbreviations: bp, blastopore; c, cilia.

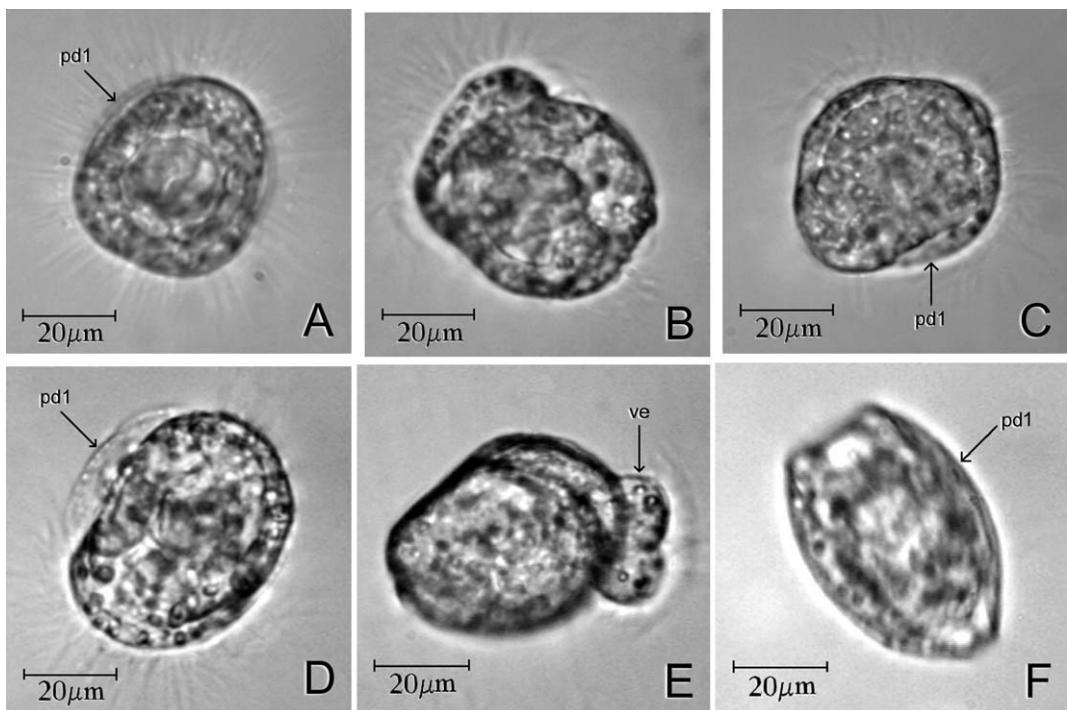


Fig. 4. Early developmental stages of *Pholas orientalis*: (A) late gastrula (10:07); (B) early trochophore (11:02); (C) late trochophore (12:30); (D) trochophore transforming into veliger (14:15); (E) early veliger showing bilobed velum with cilia (15:37); (F) ventral view of swimming veliger (17:41). Abbreviations: pd1, prodossoconch I; ve, velum.

spinning movement that continued through gastrula stage. The rate of embryonic development was faster at earlier cleavage stages than in the later blastula and gastrula stages.

After about 11 h from spawning, the embryo shed off posterior cilia; the early trochophore stage was barrel-shaped with anterior velum lined with cilia (Fig. 4B) and it started swimming randomly. After about 15 h 37 min, the early veliger developed a D-shaped shell (prodissoconch I) with bilobed velum lined with cilia (Fig. 4E). The pediveliger with extended siphon and foot (Fig. 5C) was observed crawling after 21 h and 50 min after spawning. After 23 h 10 min, the larvae entered the settlement stage. 99.7% of D-hinge larvae ($58.3 \pm 0.3 \mu\text{m}$ shell length) appeared after about 25 h (Fig. 5D). After about 10 days from spawning (Fig. 5F), the average survival rate to umbo stage ($127.5 \pm 0.5 \mu\text{m}$ shell length; $115.1 \pm 0.5 \mu\text{m}$ shell width) was $92 \pm 0.8\%$ at $30 \pm 1\%$ S, pH 8.04–8.15 and a temperature range of 23.5–29.0 °C. Without substrate, settled larvae were covered with benthic algae (e.g. *Navicula*) and were not able to survive beyond 30 days. However, the larvae provided with substrate were free of benthic algae and were able to develop to early juvenile stage

(1.046 mm shell length; 0.701 mm shell width) after 30 days with an estimated survival rate of about 16%.

3.5. Burrowing habit

P. orientalis adults were able to dig their own burrows when placed in glass aquaria (100-L capacity) with 30-cm-thick muddy substrate. Angelwing clams were able to dig their own holes by the combined action of the short foot, that was continuously extending downwards, and the scooping action of protoplax (accessory plate covering the exposed postero-dorsal end of the angelwing clam) that shoved mud towards its posterior opening. With the quick suction from the posterior opening, the mud was spewed out vigorously from both the inhalant and exhalant siphons. This action was repeated several times until the angelwing clam was able to pull itself down the burrow. Angelwing clams that were placed vertically on the substrate took about 10–20 min to create their own burrow or completely hide under the muddy substrate. When the whole length of the shell was covered with mud, the siphon of each individual was extended out for filter feeding. Those placed horizontally and diagonally were able to create

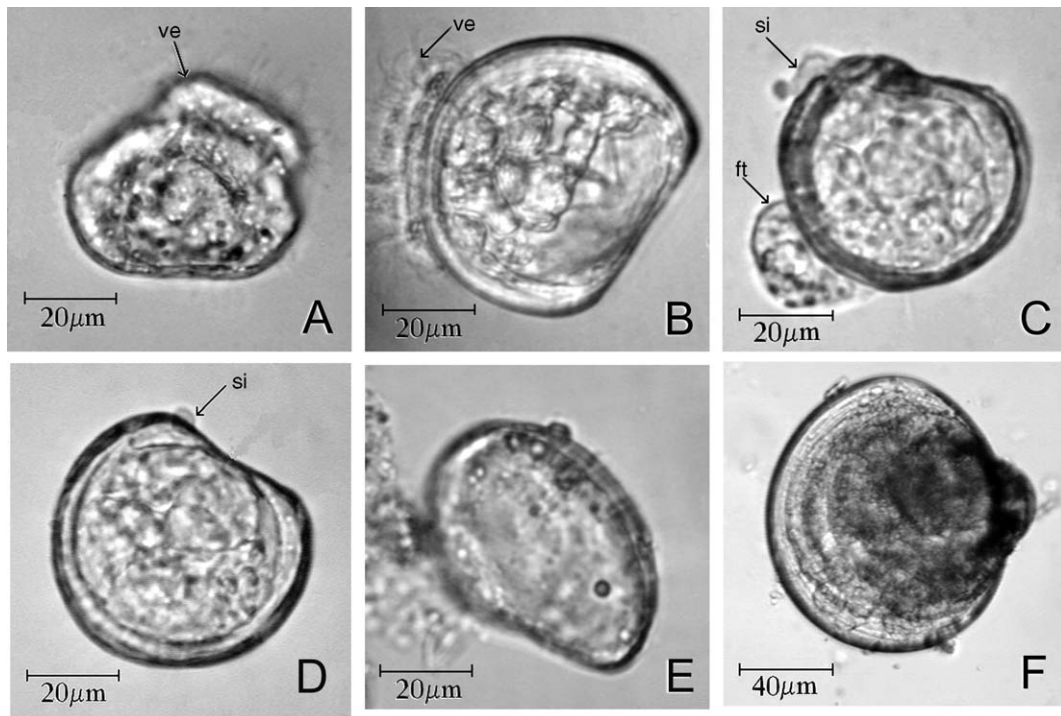


Fig. 5. Early developmental stages of *Pholas orientalis*: (A) early veliger (19:37); (B) straight-hinge late veliger (20:55); (C) crawling pediveliger (21:50); (D) settlement stage with developing umbo (76:05); (E) isometric view of postlarva (120:00); (F) late umbo (168:25). Abbreviations: ft, foot; si, siphon; um, developing umbo; ve, velum.

only shallow burrow, with about half of their shells still exposed.

3.6. Initial field trial

The spent spawners and sub-adults of *P. orientalis* introduced in the field were able to burrow by themselves to about 20–25 cm depth in muddy substrate and showed shell growth of about 2.0 ± 0.03 cm after 30 days of transplantation with 100% survival rate. This positive result led to the second phase of introducing hatchery-produced postlarvae to the cove. Twelve months after the initial introduction of settlement larvae, numerous appearances of adult *P. orientalis* were reported in San Dionisio cove, indicating the colonization by introduced angelwing clam of the area. Several adult angelwing clams were poached and harvested from San Dionisio cove and were discovered being sold in local fish markets. The initial introduction of *P. orientalis* in San Dionisio cove has been deemed to be so successful that other municipalities in Negros

Occidental requested replication of the experiment in their own coastal communities to rehabilitate the vanished population of angelwing clam.

4. Discussion

This paper reports for the first time the successful spawning, larval rearing, and seeding of a cove with hatchery-produced postlarvae of the endangered *P. orientalis* for coastal rehabilitation. This is also the first documentation of the early developmental stages of *P. orientalis*. The schematic illustration of the life cycle of this species as a functional simultaneous hermaphrodite is presented in Fig. 6.

4.1. Sexuality

The absence of external sexual dimorphism to identify the sexuality of *P. orientalis* (Laureta and Marasigan, 2000) has frustrated the attempts of many researchers to develop hatchery technology for its

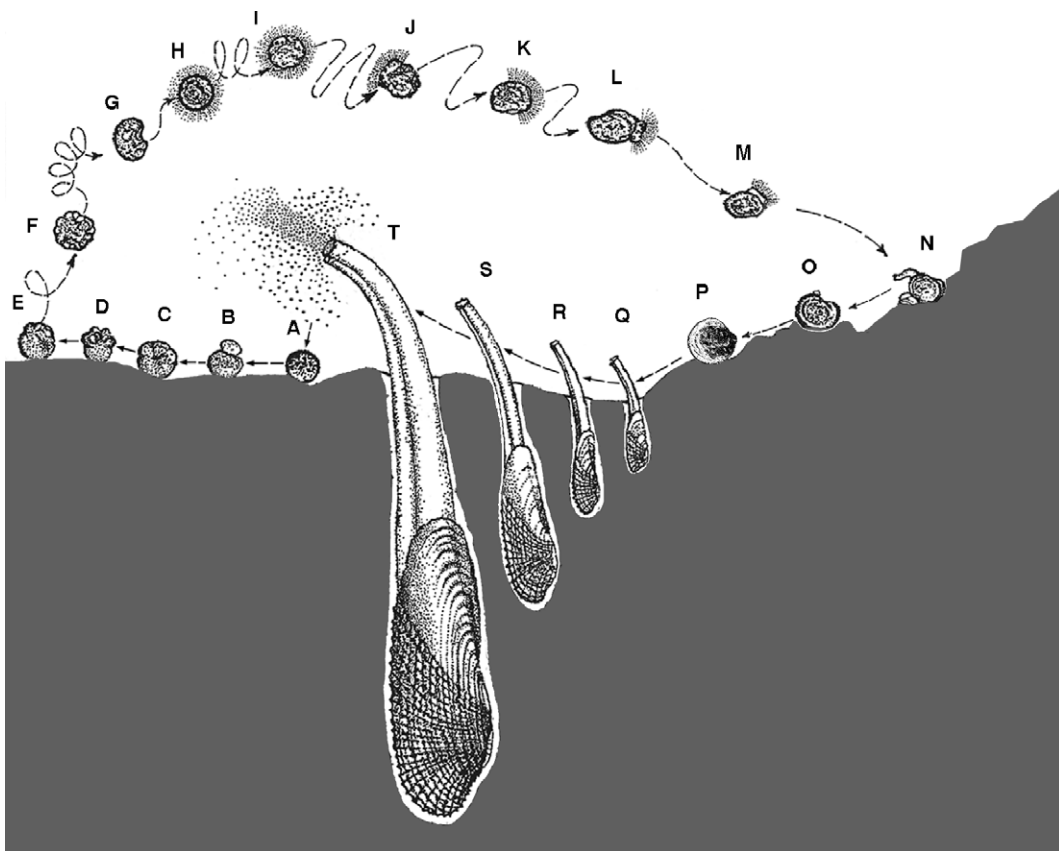


Fig. 6. Life cycle of functional simultaneous hermaphrodite *Pholas orientalis*: (A) fertilized egg; (B) first cleavage; (C) second cleavage; (D) third cleavage; (E) fifth cleavage; (F) sixth cleavage; (G) seventh cleavage; (H) blastula; (I) gastrula; (J) early trochophore; (K) late trochophore; (L) early veliger; (M) late veliger; (N) pediveliger; (O) postlarva; (P) umbo; (Q) early juvenile; (R) late juvenile; (S) immature adult; (T) spawner.

coastal rehabilitation. Laureta and Marasigan (2000) reported that this species is dioecious but failed to spawn them naturally. This present study reports that *P. orientalis* spawners collected from the wild were functional simultaneous hermaphrodites and could be spawned naturally in the laboratory. Histological analyses of gonads of newly collected wild *P. orientalis* spawners showed the occurrence of previtellogenic oocytes, spermatoocytes and spermatids in the ovotestis. Presence of both matured ova and spermatozoa on gonoducts and the emergence of ova with attached spermatozoa from the siphons of wild spawners during actual spawning proved the functional hermaphroditism of this species. Self-fertilization seemed to be the rule on all the spawnings of wild *P. orientalis* spawners. However, there might be a possibility for cross-fertilization to occur through a difference in timing of the male and female portion of the gonad to prevent extended self-fertilization, just like in some hermaphroditic bivalve species. This needs further verification.

Although the majority of bivalve molluscs are dioecious, some show specialized features such as functional hermaphroditism, sex reversal, incubation of developing embryos and young larvae within the ctenidium, and even development of a part of the ctenidium into a special brood pouch or marsupium (Purchon, 1977). Ganapati and Nagabhushanam (1953) reported protandry with sexual reversal in closely related pholad clam, *Martesia striata*. Transition from uni-sexuality to hermaphroditism or vice-versa in a species is possible and depends on environmental conditions (Purchon, 1977). Self-fertilization among hermaphrodite molluscs is commonly observed. As Ibarra et al. (1995) documented self-fertilization of functional hermaphrodites among *Argopecten circularis* under laboratory conditions, Navarte and Pascual (2003) observed self-fertilization of *Argopecten tehuelchus* under natural conditions. Beninger and Le Pennec (1991) and Ibarra et al. (1995) pointed out that self-fertilization in hermaphrodite pectinids is probably the rule more than the exception. For future studies, an accurate examination of gonads from all period of the life cycle of *P. orientalis* is necessary to determine the stage when they are dioecious and the phase when they become functional simultaneous hermaphrodites.

4.2. Spawning

Based on this study, the simplest and most efficient way of spawning *P. orientalis* is by desiccation method. Further research on induced spawning of this species under laboratory controlled conditions should also be

investigated, including the effect of mechanical and thermal stimuli (Loosanoff and Davis, 1963; Sastry, 1979), use of UV sterilized seawater (Eversole, 1989), overnight refrigeration (Castagna and Manzi, 1989), addition of large amounts of food and raising the pH for induced spawning (Breese and Robinson, 1981), and the administration of neurotransmitters like serotonin 5-HT (Gibbons and Castagna, 1984; Gustafson et al., 1991; Hamida et al., 2004).

Laureta and Marasigan (2000) reported that *P. orientalis* peak spawnings occurred in the months of June and October, at the onset of rainy seasons but clarified their cyclical reproductive pattern cannot be related to temperature and salinity. This present study reports that the monthly laboratory spawning of newly collected wild spawners was spread over the whole year coinciding with new moon and spring tide. Korringa (1947) also reported the correlation of spawning with lunar periodicity on larviparous *Ostrea edulis*.

4.3. Early development and seedstock production

Although the newly spawned eggs were heavy and stayed at the bottom until the sixth cleavage stage, the embryos from the seventh cleavage stage 3 h post-spawning became motile and swam in the water column. The late embryos and lecithotropic larvae from trochophore to veliger stage are planktonic for 18 h. This is the phase when the larvae are dispersed in great distance by currents, waves, and tides. These planktonic stages of *P. orientalis* are susceptible to vigorous predation from filter feeders, raptorial zooplankton, and planktotrophic invertebrate and fish larvae. After 22 h post-spawning, a crawling pediveliger stage appeared. This stage started probing the bottom to find suitable substrate to hide from predators, commenced digging burrows to establish their permanent niche, and started feeding with their developing siphons. Several studies pointed out that larval stage of clams obtain their nourishment by actively feeding and by absorbing dissolved organic materials through certain body tissue (Sorokin and Wyshkwarzev, 1973; Stephens, 1981; Manahan, 1983; Stephens and Manahan, 1984). It is presumed that once the larval siphons of *P. orientalis* are developed, they start filter-feeding on nanoplankton and microparticulate organic matter. A mixed microalgal diet like *I. galbana*, *C. calcitrans*, and *Nannochloropsis* sp. proved to be effective in producing viable seedstock. These minute unicellular algal diets are commonly used in clam hatcheries (Helm et al., 2004). Production of settlement stage or postlarvae of this species is relatively easy to maintain. However, when

the postlarvae start developing into early juvenile stage, suitable substrate is needed. Without this basic necessity, postlarval mass mortality was observed.

4.4. Coastal rehabilitation

The concept of sea farming as practiced in Japan (Oshima, 1984; Matsuoka, 1989) has been incorporated in this study to develop a technique that would reintroduce hatchery-produced seedstock of endangered in the natural environment for coastal rehabilitation. After determining the suitability of the area as manifested by the good health, faster growth, and high survival rate of transplanted adults, the hatchery-produced postlarvae were used in seeding the muddy cove of San Dionisio instead of the early juveniles produced in the nursery tanks.

The postlarvae were observed to be the transition stage when the early larval stages ceased to be planktonic and started assuming benthic existence by burrowing in the mud to escape predators and to establish their permanent niche. It is thought that the survival chance of actively burrowing postlarvae is greater than that of large juveniles that are susceptible to handling and could easily become prey to various predators. This is contrary to most clam “seeding” practices of using larger seed to avoid predation (cf. Helm et al., 2004). Further research is needed to establish the soundness of this hypothesis.

The growth of angelwing clam in the wild with high primary productivity was rapid with additional shell length growth of 2.54 ± 0.03 cm after 30 days; however, those maintained in the laboratory had an average additional shell length growth of 0.20 ± 0.01 cm during the same period. It took about a year to determine the success of the initial seeding when numerous adult angelwing clams appeared en masse throughout the cove. Many coastal fisher folks started poaching and harvesting them. Some were even sold to nearby local fish markets. Despite this problematic development, which was rectified with regulation from municipal government, the newly established population of *P. orientalis* started to produce succeeding generations that colonized the whole area.

The positive results of the study inspired the Office of Provincial Agriculturist in Negros Occidental to use the stock enhancement experience in San Dionisio as a model to rehabilitate the vanishing *P. orientalis* and other threatened mollusc species in several coastal municipalities in Negros Island along Guimaras Strait.

The success of coastal rehabilitation of *P. orientalis* and other threatened mollusc species is dependent not

only on the application of newly developed seedstock hatchery technology but also on the integration of coastal resource management that will involve the active participation of coastal communities, local government, and technical personnel (cf. Oshima, 1984; Matsuoka, 1989; Liao, 1997).

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