CULTURE POTENTIAL OF THE PACIFIC CLAM, *ASAPHIS VIOLASCENS* (FORSSKAL, 1775), IN THE MYEIK ARCHIPELAGO

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Abstract

The Pacific clam, *Asaphis violascens* (Forsskal, 1775), locally known as "Gin-Phyu," is a benthic bivalve species native to the Indo-Pacific region. This study demonstrated the potential for aquaculture by successfully breeding the Pacific clam from its embryonic and larval stages to the juvenile stage in a laboratory. While the clams are currently a low-priced seafood, which may limit commercial-scale culture, their production from natural habitats is mainly for local consumption. By studying the clam's life cycle through laboratory culture, we gained insights into its population dispersion and optimum environmental parameters. This culture practice can be used in the future to enhance its abundance in natural beds. The primary objective of this research was to establish suitable rearing conditions for induced spawning, larviculture, and grow-out culture. In this study, adult, mature clams were collected from the sandy beaches of Doris Island. Induced spawning was attempted using emersion and thermal shock methods. Larvae rearing and grow-out culture were investigated in a hatchery setting. Eight species of microalgae (*Chaetoceros simplex* I & II, *Chaetoceros calcitrans*, *Chaetoceros ceratosporum*, *Chaetoceros gracilis* I & II, *Nannochloropsis oculata*, and *Tetraselmis suecica*) were used as food. A sand substrate was found to be essential for the grow-out culture of post-larval clams to reach the adult stage.

Keywords: *Asaphis violascens*, bivalve, clam hatchery, emersion, induced spawning, larviculture, Pacific clam, thermal shock.Keywords: *Asaphis violascens*, bivalve, clam hatchery, emersion, induce spawning, larviculture, Pacific clam, thermal shock.

Introduction

The Pacific clam, *Asaphis violascens* (Forsskal, 1775), is a benthic bivalve in the phylum Mollusca and family Psammobiidae. They are found throughout the Indo-Pacific region, from East Africa to eastern Polynesia and from Japan to central Queensland and New Caledonia. They inhabit intertidal sandy beaches, dwelling in sandy or coarse gravel substrates at depths of 0 to 20 meters (Poutiers, 1998). This species naturally occurs in estuaries and can thrive under a broad range of pH (8.4-7.2) and salinity (10-35‰) conditions. As a dominant species, it can sometimes be found in pure strands within a habitat. One of its notable characteristics is its ability to cope with desiccation and high temperatures during low tide (Subagjo, 1989).

Clams are generally suspension-feeding bivalves that primarily depend on phytoplankton and detritus for nutrition. Microalgae constitute the main food source for most cultured Pacific clams. The average edible weight of the clam is approximately 9 g. They are dioecious (having separate sexes) and reach sexual maturity in about 6 months, at a length of 18-20 mm (Rimmer et al., 2001; Bell, Lober, & Trevor, 2001). Spawning can occur in spring, summer, or fall, with mass spawning typically happening in late summer. The larval stage lasts about 21 days.

While a variety of clam species in Myanmar have been documented, the Pacific clam has been minimally studied despite its wide distribution along the Myanmar coast, especially in the Myeik Archipelago. This study represents the first attempt at induced spawning of the Pacific clam in Myanmar. Samples were collected from Doris Island, Myeik Archipelago, and the induced spawning, larvae rearing, and grow-out culture of the clams were investigated in a

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laboratory. The objective of this research was to determine the optimal conditions for completing the clam's life cycle in a controlled environment.

Materials and Methods

The adult clams were collected from Doris Island (Lat. 11.240930˚N and Long. 98.272172˚E), Myeik Archipelago, Myanmar, in April 2023. As the clams live buried in the sand, they were collected by digging to a depth of 30 cm during low tide. Their location was identified by searching for siphonal openings on the beach. Their habit of living in accessible groups made their collection relatively easy. Approximately 700 individuals, with a size range of 40-80 mm, were collected for induced spawning. The collected clams were cleaned and stocked in a tank containing 30‰ seawater with strong aeration and food. Feeding was observed when the siphons were protruding from their shells.

After a one-day conditioning period, the clams were subjected to various induced spawning methods, including thermal shock, salinity shock, emersion, hormone induction, sperm activation, and stripping. Seawater filtration systems, hatchery facilities, spawning and larvae rearing tanks, aeration systems, and food culture were prepared before spawning. The following phytoplankton species were used: *Chaetoceros simplex* I & II, *Chaetoceros calcitrans*, *Chaetoceros ceratosporum*, *Chaetoceros gracilis* I & II, *Nannochloropsis oculata*, and *Tetraselmis suecica*. A sand substrate was also required for the grow-out culture of the clams in the laboratory.

Figure 1. A) Pacific Clam, *Asaphis violascens*: B) Siphon opening

Results

Induced Spawning

Broodstock with a shell length of 60-80 mm were acclimated in 500 L tanks containing UV-filtered seawater at a temperature of 29°C and a salinity of 31‰. Spawning induction was attempted using thermal shock, salinity shock, emersion, hormone induction, sperm activation, and stripping. After testing several methods, it was observed that the emersion method was the most effective for spawning.

Approximately 30 individuals were used for each treatment. For the emersion method, clams were removed from the water and exposed to air for 2 hours (at an air temperature of 33°C). Afterward, they were placed in 200 L spawning tanks with UV-filtered seawater at 29°C. A large amount of food was provided with gentle aeration during this period. Additional stimuli, such as stripped eggs or sperm from a opened clam, were also used. The process was repeated once the clams started to spawn.

Pacific clams have two short siphons through which they filter water. The inhalant siphon, which takes in water, is located on the ventral side, while the exhalant siphon, which

expels water, is on the dorsal side. During spawning, gametes are ejected through the exhalant siphon. Males began to expel sperm like smoke, and females released eggs next to the males. Based on observations of different spawning designs, most males were moved to a separate tray during spawning to prevent an excess number of sperm for fertilization. When the release of gametes stopped, all broodstock were removed, and the remaining liquid was left undisturbed for approximately 1 hour to allow fertilization to occur.

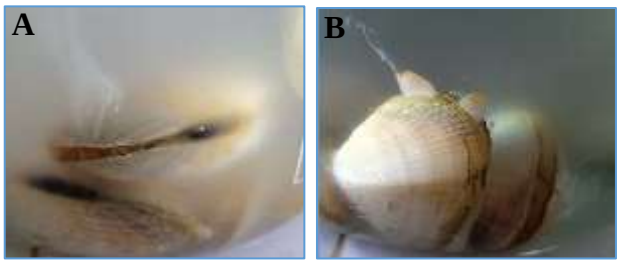

Figure 2: Pacific Clams: A) Spawning female; B) Spawning male

Fertilization and early larval stage

After spawning, the fertilized eggs were sieved through a 20-µm polyethylene screen and transferred to 200 L tanks filled with filtered seawater. Gentle aeration was provided to enhance survival. Through repeated cell division, the fertilized eggs transformed into D-hinged veliger larvae within 20 hours. After 20 hours, the larvae were transferred to 500 L larvae rearing tanks using a 40-µm screen, and the water was renewed.

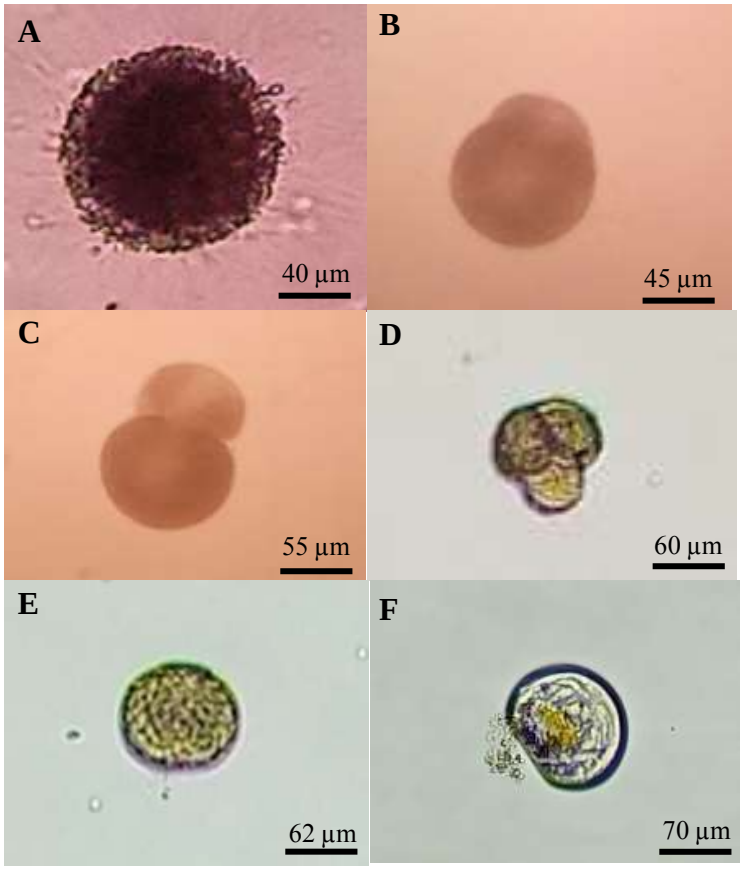

Figure 3. Early development of fertilized egg: A) sperm around egg; B) first polar body; C) second polar body; D) four cell stage; E) trochophore larva; F) D-hinged veliger larva

Larval Rearing

The 'D' larva was actively swimming. Larvae were not fed until 48 hours. after fertilization and then they were fed with phytoplankton twice daily. Seawater was renewed every alternate day. On the third day, the larva was slightly oval and started to reach early umbo stage. On the seventh day, the umbo becomes distinct. The late umbo stage was reached on 12-13 days. Eye spots formation stage was reached on 14-16 days. At this stage, the larvae were transferred into a transparent glass tank containing cleaned sand for grow up culture. During larval rearing stage, Antibiotics viz., mixed penicillin and streptomycin, were used to avoid bacterial infection and increase survival rates. Eight species of microalgae, *Chaetoceros simplex* I and II, *Chaetoceros calcitrans*, *Chaetoceros ceratosporum*, *Chaetoceros gracilis* I and II, *Nannochloropsis oculata* and *Tetraselmis suecica*, were used as food.

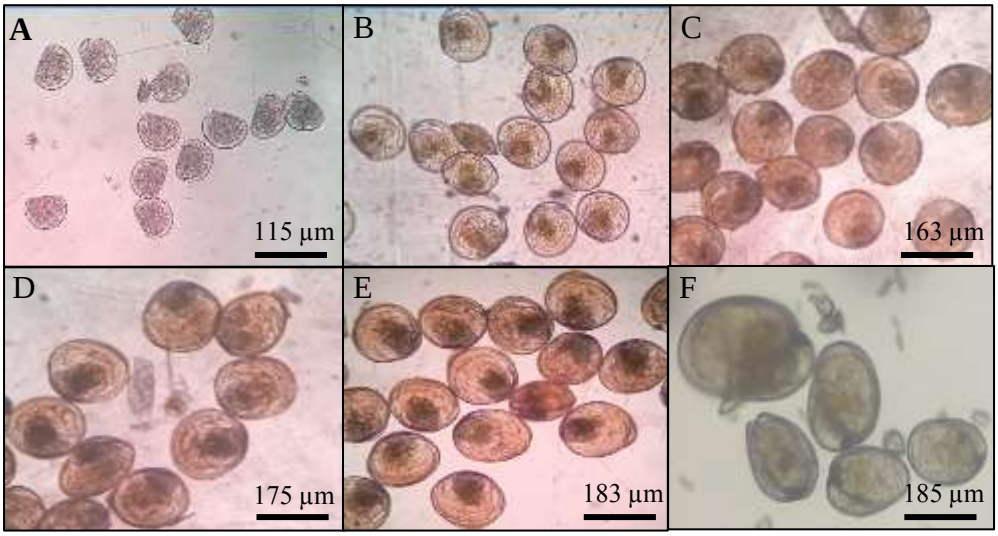

Figure 4. Larval development: A) 3 days-old; B) 7 days-old; C) 10 days-old; D) 12 days-old; E) 14 days-old; F) 16 days-old

Grow up culture

After 16 days, the larvae developed ciliated velum, which is used for locomotion and feeding. After 21 days, the veliger larvae develop a foot and become pediveliger stage. Soon after, the clam larvae developed into juveniles in which their swimming organs will lost and develop siphons, which are used for respiration and feeding. At this stage, the juveniles were already settling to the bottom and buried in the sand substrate of the tanks. The grow up culture has been conducted in the sand tanks up to 162 days-old in which the clam reached 30.76 mm in trial 1 and 27.88 mm in trial 2. In this stage, the water can be changed once a week and no need to add the antibiotics. Moreover, the phytoplankton which were directly collected from natural water can be fed to young clams for food. The growth of larvae and juveniles of Pacific clam was shown in Table 1.

Table 1. Average length (mm) of growth of Pacific clam, *Asaphis violascens*

Age (days)	Trial 1		Trail 2	
	No. of measured individuals	Average larval length	No. of measured individuals	Average larval length
5	44	119.4 µm	28	119 µm
6	25	127 µm	32	127 µm
7	22	142 µm	104	134 µm
8	32	150 µm	128	147 µm
9	60	150.8 µm	99	151 µm
10	80	163 µm	59	162.6 µm
11	64	171.4 µm	56	167.8 µm
12	81	175.2 µm	70	174.6 µm
14	36	183.8 µm	94	180.4 µm
16	65	185.2 µm	136	182.4 µm
44	16	3.12 mm	18	2.52 mm
58	15	4.02 mm	16	2.92 mm
65	20	4.94 mm	17	3.12 mm
72	24	5.88 mm	15	3.46 mm
80	20	8.42 mm	15	7.12 mm
94	22	12.5 mm	12	9.26 mm
112	20	15.2 mm	6	13.4 mm
162	8	30.76 mm	5	27.88 mm

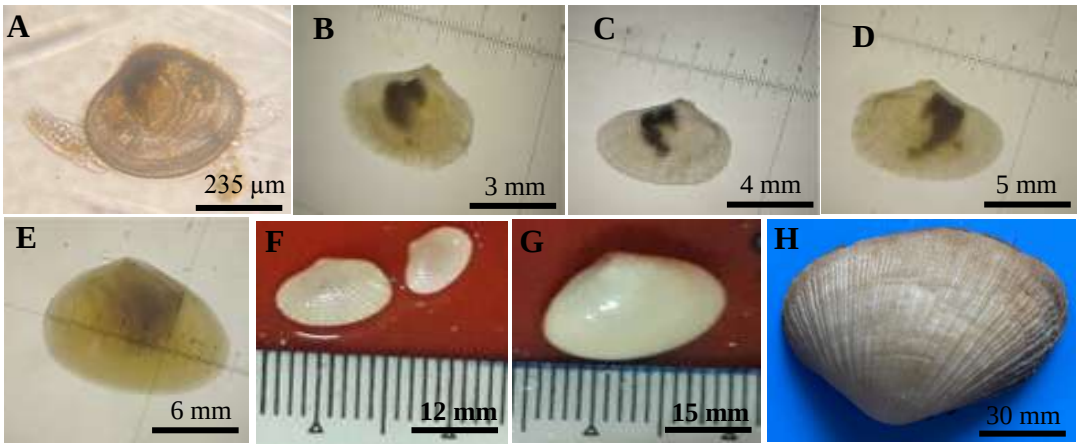

Figure 5. Juveniles' development: A) 20 days-old; B) 44 days-old; C) 58 days-old; D) 65 days-old; E) 72 days-old; F) 94 days-old; G) 112 days-old; H) 162 days-old

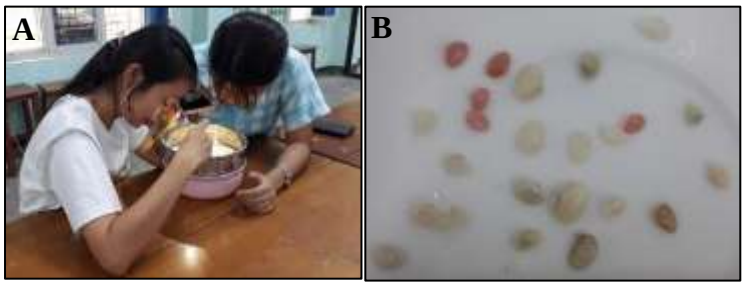

Figure 6. Numerical assessment of juvenile clam: A) select the clam from the sand; B) counting and measuring the size in the laboratory

Discussion

Induced spawning and grow up culture of Pacific clam, *Asaphis violascens*, have been investigated in the laboratory. The natural habitats of the clams are intertidal sandy beaches and they are dwelling buried in the upper layer of the sand. For the grow up culture of juveniles, the sand substrate was prepared in the rearing tanks. In the present study, the emersion method was reached successful spawning. Some other induced methods were also conducted; however; no spawning occurred in the study period.

Within larva rearing period, the antibiotic Chloramphenicol was used for trail 1 and mixed Streptomycin and Penicillin was used for trial 2 and observed that Chloramphenicol was better than the rest for the growth of larva. Hidu & Tubiash (1963) reported that the larval culture of clam *Mercenaria mercenaria*, usually resulted in a significant increase in growth rate of larvae by adding "Combi-strep" (dihydrostreptomycin-streptomycin sulfates) antibiotic.

Fertilized eggs of most mollusks are transformed into D-shaped larvae within 18 hours. after fertilization. However, Duisan *et al*. (2022) observed that fertilized eggs of hard clam, *Meretrix meretrix* changed into the D-shaped larvae were first observed after 24 hours of fertilization. From the results, the fertilized eggs of *A. violascens* reached the D-shaped lava 20 hr. after fertilization.

Owing to its low price, culture of Pacific clam in Myanmar is very rare. However, mass production of it was first developed in Palau, a Pacific island nation, by Mr. Gerald Heslinga in 1970s, mainly to produce its adductor muscle for overseas markets, particularly in Asian countries (Okada 1997). Pacific clams can have optimal reproduction at any salinity within the range 20-30‰. Peak gonad development is between April and May in Myanmar.

For the larva development, types of diets have a strong influence on their growth and mortality. Among these species, *Chaetoceros* sp and *Tetraselmis* sp are the most popular feeds for Pacific clam culture (Chícharo & Chícharo, 2001). In the present study, *Chaetoceros simplex* I and II, *C. calcitrans*, *C. ceratosporum, C. gracilis* I and II were fed to larval stage whereas a mixture of *Nannochloropsis oculata*, *Tetraselmis suecica* and *Chaetoceros* sp were fed to juveniles.

It was observed that sand substrate is ideal for the growth and survival of the Pacific clam, *A. violascens.* Several trials were tested for the grow up culture of juveniles in the tanks without sand, observing that they can survive only for a few days. High survival was observed in the tanks with sand substrate in which the post larvae was stocked when they needed to settle on the substrate.

Larval development stages of *A. violascens* are similar to other tropical bivalves; however, planktonic period was observed 16 days in the present study. Bolasina (2022) reported that *A. violascens* has a 10-day planktonic period. The present study observed the larval stage from induced spawning to adulthood, achieving a length of 2.5-3 cm over a period of about 5 months in the laboratory.

The implementation of small-scale rural aquaculture of Pacific clams may be an interesting option for improving household food security and supplementing family income of the rural poor (Bolasina, 2019). Besides this, bivalve aquaculture can bring ecosystem services, improve water quality, and provide wildlife habitat, bringing positive results on coastal habitat conservation and restoration (Theuerkauf *et al*., 2021). In the majority of cases, bivalve farming needs the collection of seeds from the sea (Chávez *et al*., 2002). Being the basis of their diet, the phytoplankton species occurring (Bricelj & MacQuarrie, 2007) and their abundance (Chícharo & Chícharo, 2001) have a strong influence on their growth and mortality, thus limiting the supply of seeds from the wild.

Conclusion

According to the literature, Pacific clam, *Asaphis violascens*, can be successfully induced to spawn using various methods. Emersion method is the best method for the induced spawning in the present study. Broodstock was collected in April when the gonad is fully developed. Peak spawning season in natural habitat is in late summer. Induced spawning and an attempt to culture the life cycle of clams over a period of five months have been conducted for the first time in Myanmar. Among the several experiments, two trials (1000 indv. /10L each) using different antibiotics and sandy substrate were successfully cultured. The sizes reached about 30.76 mm and 27.88 mm in two experiments. It was observed that sandy substrate is the main factor for the survive in the laboratory. Food availability, water quality maintenance and aeration are the important factors that influence the growth and survival of the clams.

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