EFFECT OF SALINITY ON HATCHING, LARVAL GROWTH, AND SURVIVAL IN THE GREEN MUSSEL *PERNA VIRIDIS* (LINNAEUS)

Shau-Hwai Tan
Muka Head Research Station, Centre for Marine and Coastal Studies, Universiti Sains Malaysia, 11800 Penang, Malaysia

ABSTRACT

The effect of salinity (0-30 ‰) on egg development, growth, and survival of the D-larvae to the plantigrade stage in *Perna viridis* were studied. Eggs were able to develop into D-larvae from 6 to 30 ‰ salinity. Optimum development of eggs to the D-larvae occurred at salinities between 24 and 30 ‰, where more than 80 % of D-larvae were formed. The D-larvae were able to survive to the settling stage when cultured in salinities from 18 to 30 ‰, with the best survival occurring in 24 ‰ (25.5-31.5 %). In 24 ‰ salinity, first settling occurred on the 21st day compared to 24th day for larvae cultured in 30 ‰; and 28th day for those cultured in 18 ‰.

INTRODUCTION

The green mussel *Perna viridis* (Linnaeus, 1758) is the second most important bivalve species in Malaysia as far as culture production is concerned. Culture of this species developed in the early 1980's following the successful demonstration of culture techniques as well as economic feasibility by personnel from the Fisheries Research Institute (Choo 1979). Though confined to southern Johore in the early 1980's, mussel culture has now spread to Melaka, Perak, Kedah and Perlis where transplantation of seeds have proved successful (Ong & Rabihah 1989); and there is every indication that mussel production will continue to rise in the near future. Studies have been undertaken at Universiti Sains Malaysia to characterise its basic breeding biology, spawning and larval culture with the objective of producing seeds via hatchery techniques.

The green mussel, *P. viridis* is a prominent member of the littoral fauna of estuaries, and is exposed to extreme fluctuations in salinity. The duration of the larval life of mussels typically ranges from about one to four weeks and is contingent upon salinity, temperature, available ration, and other factors (Bayne 1976; Sprung 1984).

Numerous studies have been conducted on the effect of salinity on the embryonic and larval development of temperate mussels, *Mytilus edulis* (Hrs-Brenko & Calabrese 1969) and *Adula californiensis* (Lough & Gonor 1971). However, relatively little has been published on green mussel *P. viridis*. This paper reports on the salinity tolerance of embryos and larvae of *P. viridis* during the planktonic phase (D-larvae stage till the settling stage).

MATERIALS AND METHODS

Mussel larvae

Mature *P. viridis* were collected from Merbok River, Kedah. The Merbok River site experienced salinity as low as 15 ‰ during the rainy season and as high as 30 ‰ during the dry season. The mussels collected were acclimatised in sand filtered sea water (30 ‰) in the laboratory for a few days. During this period, they were fed an algal mixture containing *Chaetoceros calcitrans* and *Isochrysis galbana* (Tahitian strain). The mussels were induced to spawn with serotonin (Gibbons & Castagna 1984). Egg handling and fertilisation were as described by Loosanoff & Davis (1963). All experiments were conducted with pooled progeny from a number of parent broodstock.

Algae

The unicellular algae, *Isochrysis galbana* (T-

Iso) and *Chaetoceros calcitrans* were batch cultured in 1 mm filtered sea water (salinity 30-32 ‰) using F/2 medium (Guillard 1972). Algal cultures were produced in 20 litre glass carboys at 28±2 °C. Only cultures in the log phase of growth were used. A haemocytometer was used to determine cell concentrations.

Test salinities

Test salinities (0, 6, 12, 18, 24, and 30 ‰) were prepared by mixing 1 µm filtered sea water with distilled water (0 ‰). Salinities were determined by a refractometer (ATAGO Ltd.) precalibrated with distilled water. Salinity was tested based on the range of salinity occurring in the natural environment of *P. viridis* (25-30 ‰). Salinity of 0 ‰ was tested because salinity may drop sharply to 15 ‰ in Merbok River, and sometimes even as low as 3 ‰, coinciding with the North-East monsoon which brings rain to the catchment at the source of the river.

Experiments

Development of embryos: fertilised eggs were gently aerated for 15-20 minutes. The first polar body was observed. The fertilised eggs were then transferred to different test salinities in 1 litre plastic aquaria at a stocking density of 10 embryos per ml (10,000 fertilised eggs in 1000 ml). Slow aeration was supplied via air diffusers. Temperature was not controlled and varied from 27-29 °C during the experimental period (24 hours). All experiments were conducted in triplicate.

At the end of 24 hours, twenty 1 ml samples were collected and the numbers of embryos which had developed into normal, D-hinge larvae were recorded. Normal larvae are defined here as being perfectly D-shaped at the prodissoconch-I stage while abnormal larvae had irregular, or misshapen shells, either completely, or incompletely formed. Shell heights of 30 larvae from each salinity were measured using a compound microscope (Olympus; phase-contrast BH) fitted with a micrometer eyepiece, precalibrated with a 50 x 2 micron graticule.

Table 1. Feeding used in salinity experiments.

DAY	CONCENTRATIONS OF T-ISO (cell ml^{-1})
1 - 2	5,000
3 - 4	10,000
5 - 6	20,000
7 - 12	25,000
13 - 14	30,000
15 - 18	35,000
19 - 23	40,000
24 - 29	50,000

Larval growth and survival: D-hinge larvae were reared in each test salinity in 15 litre glass carboys at a stocking density of 10 larvae ml^{-1} (150,000 D-hinge larvae in 15 litre). Aeration was supplied via air diffusers. All cultures were covered with Perspex™ sheets to prevent evaporation. The larvae were fed T-Iso with an initial feed concentration of 5,000 cell ml^{-1}. This was raised progressively as the larvae developed (Tab. 1). All test cultures received the same feed rations.

The culture media were completely changed every 2 days, during which samples were taken for microscopic examination, larval count, and morphometric measurements. The shell heights of 30 larvae from each treatment were measured using a compound microscope. Three 1.0 ml samples were collected and the mean value for larval density calculated. When the larvae reached 300 µm length and 280 µm height; a polyethylene rope with diameter of 2 mm was suspended in each culture vessel to act as cultch. The experiments were terminated when settling of pediveligers was observed.

Statistical analysis

Data were analysed with a one-way ANOVA to examine the effects of salinity on hatching, growth, and survival. If significant interactions were present, the data were then tested with the Duncan's Multiple Range Test to determine which treatments were significantly different.

RESULTS

Development of embryos

Tab. 2 shows the percentage of *P. viridis* eggs that developed into normal D-larvae at different salinities. Eggs could develop into D-larvae in 6 to 30 ‰. In 0 ‰, there was 100 % mortality. Development of eggs was normal in 18 to 39 ‰. Salinities between 24 and 30 ‰ yielded the highest percentages of normal D-larvae (84.2 % - 90.8 %) which did not differ significantly ($p < 0.01$).

In 12 and 6 ‰, survival of embryos decreased significantly to 45.8 % and 14.2 %, respectively. In 6 ‰ numerous ciliated trochophores were still seen after 24 hours whilst some of the D-larvae appeared deformed.

The mean shell height and length of normal D-larvae in different salinities are shown in Tab. 3. Growth (both shell height and shell length) was highest in 30 ‰ though the differences in growth between larvae in 18, 24, and 30 ‰ were not significant. The size of D-larvae decreased significantly in 6 and 12 ‰ salinity.

Table 2. Percentages of *Perna viridis* eggs that developed into normal D-veligers in different salinities.

Salinity	Percentage of normal D-veligers
0	0
6	14.2±5.7
12	45.8±12.8
18	69.1±7.1
24	84.2±12.3
30	90.8±7.2

Table 3. The mean shell length of 24 hours *Perna viridis* in different salinities.

Salinity (‰)	Shell length (µm)
0	0
6	88±4
12	93±4
18	96±4
24	98±6
30	104±5

Larval growth and survival

Tabs. 4 and 5 show the percentage survival and shell growth (length) respectively, of *Perna viridis* larvae in different salinities. In 0 ‰ (freshwater), 100 % mortality was noted during observations made on day 3. In 6 ‰, the percentage survival was reduced to 16 % by day 3 and 8 % by day 5; and 100 % mortality by day 11. In 12 ‰, the larvae suffered fairly high mortality (35 to 80 %) during the first 7 days. The larvae continued to show progressive die-off until mortality reached 100 % by day 17.

Survival till settling occurred in 18, 24, and 30 ‰ with the best survival (28.5 %) in 24 ‰. A one way ANOVA showed that the differences between 24 and 18 or 30 ‰ were significant ($P > 0.01$), while the difference between 18 and 30 ‰ was not significant ($P < 0.05$). The first settling occurred on day 21 in 24 ‰. First settling was recorded from larvae grown in 30 ‰ on day 24 whilst larvae grown in 18 ‰ only set on day 28.

Growth was highest in 24 ‰, followed by larvae cultured in 30 ‰ salinity and then by 18 ‰. The differences in growth rate between 24 ‰ and those of other salinities were statistically significant ($P > 0.01$). Larvae grown in 12 ‰ were stunted and by day 15 had only reached 164 µm compared to the 211-325 µm achieved by larvae grown under more favourable salinities.

DISCUSSION

In the present study the salinity range is 6-30 ‰ for possible development of eggs of *P. viridis* to D-larvae. The optimum salinities for both growth and survival of *P. viridis* embryos were between 24-30 ‰ (84-91 % survival). Similar responses were reported for the same species, *Mytilus* (*Perna*) *viridis* by Lim (1992) (24-30 ‰), and Tham *et al.* (1972) (26-29 ‰); and also other Mytilidae such as *Mytilus edulis* (30 ‰) (Bayne 1965); and *M. californianus* (28-35 ‰) (Young 1941). However, higher salinity (40 ‰) was reported to be optimal salinity for development of eggs in *M. galloprovincialis* (Hrs-Brenko 1974). It is apparent that the green

Table 4. Percentage survival of *Perna viridis* larvae cultured in different salinities.

Age (days)	0 ‰	6 ‰	12 ‰	18 ‰	24 ‰	30 ‰
1	100	100	100	100	100	100
3	0	15.7±3.1	65.3±9.8	80.5±11.6	87.1±10.4	83.7±10.8
5		8.3±1.5	36.4±5.7	63.3±13.9	78.9±10.5	72.5±11.5
7		1.6±0.4	19.8±3.5	50.8±8.8	71.5±15.1	63.1±16.3
9		0.8±0.2	11.2±2.7	32.7±14.8	65.3±11.6	58.3±13.4
11		0	5.8±1.3	26.8±5.1	60.7±9.7	50.6±10.0
13			1.2±0.3	24.1±8.4	58.1±8.5	43.8±12.2
15			0.6±0.2	22.8±9.0	50.9±5.7	31.7±10.0
17			0	19.5±7.6	46.5±7.6	26.9±5.0
19				18.1±9.5	33.0±6.5	24.1±6.7
21				16.7±6.3	28.5±3.0	22.1±8.8
23				16.3±8.8		20.0±5.1
25				16.0±9.5		18.9±3.2
27				15.8±2.6		

Table 5. Shell length (μm) of *Perna viridis* cultured in different salinities.

Age (days)	0 ‰	6 ‰	12 ‰	18 ‰	24 ‰	30 ‰
1	102±3	102±3	102±3	102±3	102±3	102±3
3	0	107±5	109±5	108±6	118±6	115±6
5		113±6	115±6	110±8	129±6	126±8
7		121±5	124±5	137±9	145±12	138±11
9		135±7	137±18	169±10	187±12	168±10
11			143±21	177±15	235±10	211±14
13			156±18	203±19	297±19	258±19
15			164±26	211±14	325±17	272±17
17				242±14	341±21	286±19
19				251±16	357±16	297±12
21				263±18	381±15	315±17
23				280±17		337±24
25				309±21		345±21
27				325±24		

mussels generally require almost full strength sea water for optimal development of eggs to the D-larvae. The number of abnormal larvae increased substantially as the salinity deviated from the optimal range for embryonic development.

Tham *et al.* (1972) also reported that *Mytilus* (*Perna*) *viridis* eggs were not able to tolerate salinities out of the 26-29 ‰ range. However, the embryos in this study were able to develop into normal D-larvae in a salinity as low as 6 ‰ (14 %), and showed fair percentages of D-larvae in 12 ‰ (46 %) and 18 ‰ (68 %).

Previous studies have shown that performance during embryo development is influenced by the salinity at which the broodstock is conditioned (Davis 1958; Loosanoff & Davis 1963). Broodstock used in this study were conditioned in 30 ‰. Further investigations are needed to determine whether the effect of broodstock conditioning salinity on

embryo development also applies to *P. viridis*.

The results show that *P. viridis* larvae were capable of tolerating a salinity range of 6-30 ‰ for extended periods. However, complete larval development to metamorphosis only occurred between 18-30 ‰. Optimal growth rates were seen in 24 ‰ (27-29 °C). This was accompanied by the earliest set (21 days) while in 30 ‰, first settling occurred on day 24, and on day 28 in 18 ‰. The survival of *M. edulis* larvae cultured at 30 °C with salinities between 15-40 ‰ showed 100 % mortality at 30 °C (Hrs-Brenko & Calabrese 1969). However, larval growth rates were optimal at 20 °C in cultures with salinities between 25 and 30 ‰, but decreased when temperatures of cultures were lowered to 10 °C or raised to 25 °C (Hrs-Brenko & Calabrese 1969).

The duration of the planktonic larval stages of *P. viridis* cultured in 24 ‰ was within the range of the 19-24 days recorded by Juario & Benitez (1988); and comparable to *M. edulis* (20 days) (Bayne 1965; Sprung 1984); but was faster compared to the 28-29 days reported by Lim (1992) for *P. viridis*; and *M. californianus* (35 days) reported by Trevelyan & Chang (1983).

P. viridis larvae cultured in 18 ‰ were capable of completing larval development to metamorphosis although at a slower rate compared to those cultured in 24 ‰. The ability of *P. viridis* larvae to tolerate salinities as low as 6 ‰ for short periods appears to provide an adaptive advantage for life in estuaries characterised by high daily salinity fluctuations. It is interesting to note that concordant with larvae developing from embryo to the pediveliger stage, there is a decrease in tolerance to lower salinities. It suggests the possibility of the *P. viridis* embryonic stage adapting itself to lower salinity when triggered to spawn during the rainy season. Exposure to low salinity has been reported to induce spawning in *P. viridis* (Lim 1992).

From the viewpoint of hatchery production, the results indicate that for the optimal production, larvae of *P. viridis* should hatch in 24-30 ‰ and be cultured in 24 ‰.

ACKNOWLEDGEMENTS

This study was carried out at Muka Head Research Station, Universiti Sains Malaysia, with funding support from Centre For Marine and Coastal Studies (File No.: 132/0131/0102). I record my sincere thanks to all project staff for technical support. I thank DANIDA (TMMP Denmark) for travel support and the 7th TMMP Organising Committee for generous local hospitality.

REFERENCES

Bayne, B.L. 1965. Growth and the delay of metamorphosis of the larvae of *Mytilus edulis* (L.). - Ophelia **2**(1): 1-47.

Bayne, B.L. 1976. Marine mussels: Their ecology and physiology. - International Biological Programme 10. Cambridge University Press.

Choo, P.S. 1979. Culture of the mussel, *Mytilus viridis* L., in the Straits of Johore, Malaysia. Ministry of Agriculture of Malaysia. - Malaysia Agricultural Journal **52**(1): 68-76.

Davis, H.C. 1958. Survival and growth of clam and oyster larvae at different salinities. - Biological Bulletin **14**: 296-307.

Gibbons, M.C. & M. Castagna. 1984. Serotonin as an inducer of spawning in six bivalve species. - Aquaculture **40**: 189-191.

Guillard, R.R.L. 1972. The culture of phytoplankton for feeding marine invertebrates. Pages 29-60 *in* Smith, W.L. & Chanley, M.H. (eds.). The Culture of Marine Invertebrate Animals. Plenum Press, New York and London.

Hrs-Brenko, M. 1974. Temperature and salinity requirements for embryonic development of *Mytilus galloprovincialis* Lmk. - Thalassia Jugoslavica **10**: 131-138.

Hrs-Brenko, M. & A. Calabrese. 1969. The combined effects of salinity and temperature on larvae of the mussel *Mytilus edulis*. - Marine Biology **4**: 224-226.

Juario, J.V. & L.V. Benitez. 1988. Perspectives in aquaculture development in Southeast Asia. - Development in Southeast Asia, Iloilo City, Philippines 8-12 Sept. 1987. Aquaculture Department Southeast Asia Fisheries Development Center Tigbaun, Iloilo, Philippines.

Lim, P.A.C. 1992. Pembiakan aruhan dan kesan saliniti terhadap penetasan telur, tumbesaran serta kemandirian larva siput sudu, *Perna viridis* (Linnaeus). - Universiti Sains Malaysia, 148 pp.

Loosanoff, V.L. & H.C. Davis. 1963. Rearing of bivalve molluscs. - Advances in Marine Biology **1**: 1-136.

Lough, R.C. & J.J. Gonor. 1971. Early embryonic stages of *Adula californiensis* (Pelecypoda: Mytilidae) and the effect of temperature and salinity on development rate. - Marine Biology **8**: 118-125.

Ong, K.S. & Rabihah Mahmood. 1989. Status and problems of molluscs production, sanitation and marketing in Malaysia. - Paper presented at FAO - French Molluscs Workshop, France 16-28th Oct. 1989.

Sprung, M. 1984. Physiological energetics of mussel larvae *Mytilus edulis* 1. Shell growth and biomass. - Marine Ecology Progress Series **17**: 283-293.

Tham, A.K., S.L. Yang & W.H. Tan. 1972. Experiments in coastal aquaculture in Singapore: Cultivation of *Mytilus viridis*. Pages 379-382 *in* Coastal Aquaculture in the Indo-Pacific Region.

Trevelyan, G.A. & E.S. Chang. 1983. Experiments on larval rearing of the California mussel (*Mytilus californianus*). - J. World Maricul. Soc. **14**: 137-148.

Young, R.T. 1941. The distribution of the mussel (*Mytilus californianus*) in relation to the salinity of its environment. - Ecology **22**: 379-386.

ISSN 0858-3633