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Effect of temperature on the development, growth, survival and settlement of green mussel *Perna viridis* (Linnaeus, 1758)

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Abstract

The effect of temperature on the development, growth, survival and settlement of *Perna viridis* was studied under controlled conditions to provide information needed for the development of commercial hatchery technology for green mussel *P. viridis*. Total mortality of the larvae occurred after 24 h at temperatures of 33 °C and 35 °C. At 24 °C, larvae took longer to settle than at temperatures of 27 °C, 29 °C and 31 °C. For optimum larval development (8–13 h), growth (17.2 ± 0.84 μm day^{-1}) and survival ($55.2 \pm 0.84\%$), a hatchery rearing temperature of 31 °C is required. For settlement no significant difference was seen between the percentage settlement at 29 °C ($49.3 \pm 3.34\%$) and 31 °C ($45.8 \pm 1.76\%$). However, the process of settlement began and ended earlier at 29 °C (from 15 to 18 days) than at 31 °C (from 18 to 20 days). Thus for larval settlement a temperature of 29 °C is recommended.

Keywords: *Perna viridis*, green mussel, settlement, temperature, survival

Introduction

Mussel culture in India using *Perna viridis* and *Perna indica* was developed by the Central Marine Fisheries Research Institute (CMFRI) in the mid- and late 1970s. Two decades later the first commercial culture of mussels (green mussel *P. viridis*) was started in late 1995 at Anthakaranazhi (Alleppey district) in Kerala by local fishermen on long lines in the sea with the technical support of CMFRI. Commercial mussel culture activity along the southwest coast of India expanded greatly since 1997 in different parts of the country (Appukuttan, Mohamed, Kripa, Ashokan, Anil, Geetha, Velayudhan, Laxmilatha, Koya, Radhakrishnan, Joseph, Alloycious, Surendranathan, Nagaraja, Jenni & Naik 2001) and cultured mussel production is expected to be about 800 tonnes in 2002 and is projected to be 12 000 tonnes by the year 2007–2008 (K. K. Appukuttan, pers. comm.).

At present seed requirements for mussel farming in India are met mainly from the wild. Widespread mussel spat settlement occurs in the intertidal and subtidal areas during the post-monsoon period. However, substantial quantities of these spat perish due to various adverse ecological conditions (Appukuttan *et al.* 2001). Hence insufficient quantities of seed could become a major bottleneck in the expansion of mussel culture in India. Recent studies indicate that the seed availability for farming is limited and the seed requirement cannot be met only from the wild for commercial-scale mussel production by culture. Thus, the development of commercially viable hatchery technology becomes a priority for long-term sustainable and successful mussel farming in India.

In many invertebrates, the degree of tolerance to various physicochemical factors like temperature, salinity or pH varies during ontogeny. A detailed assessment of the importance of each of these factors based on field studies alone is difficult because of a lack of control and simultaneous changes in more than one variable. Detailed information on

tolerances to changes in various factors can be obtained from laboratory experiments conducted under controlled environmental conditions.

Several investigations have been carried out to elucidate the importance of salinity and temperature in bivalve species, especially for mussel larval settling under natural conditions (Hancock 1973; Bayne 1976; Lutz & Kennish 1992) and under controlled conditions (Loosanoff & Davis 1963; Bayne 1965, 1976, 1983; Widdows 1991; Lutz & Kennish 1992). Generally, two types of experiments are carried out: e.g. monofactorial experiments where the effect of one parameter is studied at a time (Loosanoff & Davis 1963; Bayne 1965; AQUACOP 1979; Sprung 1984a–d; Sreenivasan, Rao, Poovannan & Thangavelu 1988b; Sreenivasan, Rao & Poovannan 1989; Satuito, Natoyama, Yamazaki & Fusetani 1994; Tan & Wong 1996), or multivariate experiments studying the effects of more than one factor at a time (Bayne 1976, 1983; Robert, His & Dinet 1988; His, Robert & Dinet 1989; Hurley & Walker 1997; Doroudi, Southgate & Mayer 1999). Several authors have investigated the effects of temperature independently and in multivariate experiments with salinity and feeding level on the growth and survival of mussel larvae in the laboratory (Bayne 1965, 1976, 1983; Hrs-Brenko & Calabresse 1969; Hrs-Brenko 1973; Lough & Gonar 1973; Widdows 1973; Lough 1974, 1975; Siddall 1979a, b; Beaumont & Budd 1982; Falmagne 1983, 1984; Trevelyan & Chang 1983; Sprung 1984a; Eyster & Pechenik 1987; Pechenik, Eyster, Widdows & Bayne 1990).

While taking up large-scale hatchery production of mussel seed, research and development efforts must be directed towards defining optimal conditions for growth, survival and reducing the duration of larval life. The purpose of the present study was to find out the optimum temperature for the development, growth, survival and settlement of green mussel *P. viridis* larvae reared under laboratory conditions. This work was undertaken with the larger objective of initiation of commercial hatchery techniques for the green mussel *P. viridis* in India and considering the fact that in tropical conditions no work has been done specifically on the temperature requirements of mussel larvae for growth, survival and settlement.

Materials and methods

Larval rearing protocol

All experiments on development and larval rearing were conducted at Tuticorin Research Centre (8°45′N latitude and 78°12′E longitude and is located in Tamil Nadu on the southeast coast of India) of the Central Marine Fisheries Research Institute (CMFRI) from August 1998 to October 1999. In total, four different runs of the same experiment were conducted with different broodstock. In these experiments healthy ripe *P. viridis* were collected and transported from Pondicherry (11°46′–12°30′N latitude and 79°36′–79°53′E longitude), 500 km from Tuticorin. The mussels spawned naturally without any inducement or stimulation.

The effect of temperature on mussel larval rearing was studied by growing the larvae in different temperatures, e.g. 24 °C, 27 °C, 29 °C (control, ambient water temperature), 31 °C, 33 °C and 35 °C (kept constant using a Jumo thermometer, relay and immersion heater system). Each of the experimental units for a temperature consisted of triplicate 5-L glass beakers with 4 L of fresh filtered sterilized seawater kept in 200-L fibreglass-reinforced plastic (FRP) tanks with 150 L of water. The Jumo thermometer, relay and immersion heater system was set for the appropriate temperature, with the silicon-cased immersion heater immersed in the FRP tanks. Even though no aeration was provided to the beakers, the FRP tank water was aerated to keep the temperature uniformly spread among the beakers during the trial. Beakers were covered with a black cloth to prevent contamination with dust and debris and also to prevent the growth of algae.

Larval rearing, sampling and estimation were done according to the protocols of Loosanoff & Davis (1963), with some minor modifications. Briefly, after fertilization the eggs were collected by passing the contents through a 20-μm sieve, thereby retaining the eggs but permitting the debris and damaged eggs to pass through. After estimation of the egg density, the desired number of eggs with a concentration of five eggs mL^{-1} was introduced into each culture beaker. All eggs per trial were taken from the same spawning stock, thus ensuring that all treatments matched closely in egg size. After 28 h, the veliger larvae (82.3 ± 2.12 μm (standard error of the mean in shell length)) were collected by passing the contents through a 70-μm sieve, thereby retaining the veliger larvae and permitting the unfertilized eggs, fertilized eggs, embryos and trochophore larvae to pass through. At this point, the densities of veliger larvae in different experimental beakers were adjusted to two larvae mL^{-1}. Seawater in each of the beakers was changed every 48 h. At each time from each beaker, the larvae were collected on a mesh sieve

(60 μm), washed into a 1-L graduated cylinder and the volume made up to 1 L. From this three 1 mL subsamples were removed to estimate the larval survival. In addition, the shell lengths of 60 larvae in each of the three beakers in the experimental unit were measured, noted and the larvae were released back to the respective beakers. This same procedure was carried out for each of the treatment temperature units. The experiment was terminated after complete settlement of the larvae, and the number of larvae in each beaker was counted. The growth, percentage survival and settlement were separately noted for each of the beakers in each of the treatment units. The same procedure was adopted for each of the four experimental runs.

There were no major fluctuations of water quality throughout the experimental period. Water quality parameters during the experiment were the following: salinity, 36 ± 1 ppt, pH 8.2 ± 0.1; dissolved oxygen, 5.2 ± 0.3 ppm; ammonia, 0.0014 ± 0.001 ppm; hydrogen sulphide: nil. Corresponding ambient air and water temperatures were 35 ± 1 °C and 29 °C respectively.

Feeding protocol

The larvae were fed anexic unicultures of the microalgae *Isochrysis galbana*. The unicultures were cultured in 3-L glass haffkine flasks in autoclaved, 0.45-μm-filtered and UV-sterilized seawater using f/2 nutrient medium (Guillard 1983). The microalgae during the exponential growth phase (1×10^6 cells mL^{-1}) were fed to the larvae. The microalgae cell concentration was determined by counting a subsample using a haemocytometer. The larvae were fed daily at 5000 cells larvae^{-1} from the veliger stage to the umbo stage, and at 8000 cells and 10 000 cells larvae^{-1} from the umbo stage to the eyed stage and from the eyed stage to the pediveliger stage respectively. From the pediveliger stage through to the spat stage, the larvae were fed 16 000 cells larvae^{-1}. The required quantity of feed (depending on the larval survival in each of the experimental beakers) was taken from harvested fresh cultures and was acclimatized to the ambient water temperature conditions. It was then sieved through a 40-μm sieve and poured uniformly into the larval rearing beaker just after the release of larvae.

Statistical analyses

Regression analysis using Microsoft EXCEL computer software (licensed to C.M.F.R.I. Cochin, Kerala, India) was performed to determine the relationships between shell lengths and age for each treatment unit (pooled for all the four experimental runs for each experimental unit). The results were presented in scatter diagrams with fitted lines. The significance of the relationships was measured using correlation coefficients (r^2). The fitted lines for each temperature were also tested using pairwise *t*-tests with a significance level of $P < 0.05$.

Statistical analyses of the actual growth rate data, percentage survival and percentage settlement data (pooled for all the four experimental runs for each experimental unit) were arcsine transformed (due to percentages) and analysed through a one-way analysis of variance (ANOVA) using Microsoft EXCEL computer software. In all cases, when the *F*-values of the treatments were significantly different, the best treatment was found out through pairwise (Student's *t*-test $P < 0.05$) comparison of treatment means. Pooled data for each of the treatment units for the percentage survival and percentage settlement were represented in bar graphs.

Results

There were no major differences in the results for the four different runs conducted for the temperature experiments. Hence the results of the four runs were pooled and presented for each of the temperature experiments. For *P. viridis*, 10, 8, 6 and 5 h were required for the fertilized eggs to reach the trochophore stage at 24 °C, 27 °C, 29 °C and 31 °C respectively. Larvae required 30, 22, 14–18 and 8–13 h to become veliger larvae at 24 °C, 27 °C, 29 °C and 31 °C respectively. Development time and larval stage is given (Table 1, Fig. 1). The growth of the larvae increased from a low temperature of 24 °C to a maximum growth at 31 °C. There was total larval mortality at 33 °C and 35 °C after 24 h. Settlement occurred fastest at 29 °C (control temperature). Seven days were required to reach the umbo stage at 27 °C and 31 °C, 5 days at 29 °C, and 8 days at 24 °C. The eyed stage was reached on

Table 1 Development time from fertilized egg to veliger stage in *P. viridis* grown at different temperatures

	Hours to attain the stage			
Stage	**24°C**	**27°C**	**29°C**	**31°C**
Trochophore	10	8	6	5
Veliger	30	22–26	8–13	13–18

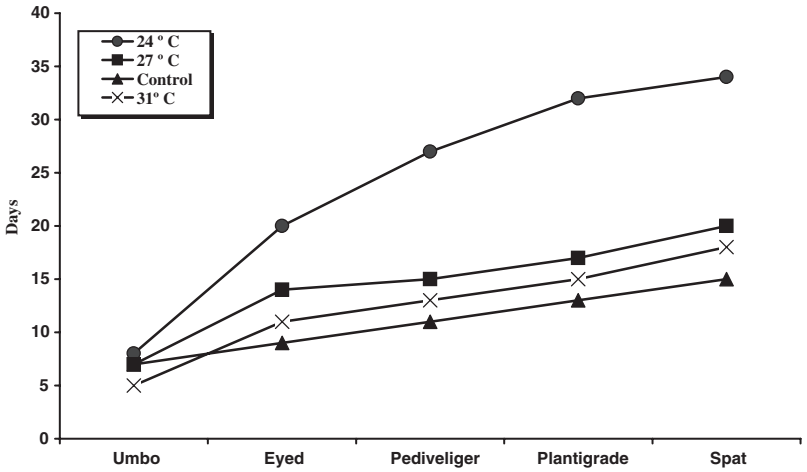

Figure 1 Days to attain different larval stages in *P. viridis* grown in different temperatures.

days 9, 11, 17 and 20 at 29 °C, 31 °C, 27 °C and 24 °C respectively. The pediveliger stage was observed on days 11, 13, 15 and 27 at 29 °C, 31 °C, 27 °C and 24 °C respectively. The plantigrade stage was observed on days 13, 15, 17 and 32 at 29 °C, 31 °C, 27 °C and 24 °C respectively. Settlement was observed at 29 °C from days 15–18, whereas it took 18–20 days at 31 °C. Settlement required 20–24 days at 27 °C. Spat settlement was slowest at 24 °C, requiring 34–41 days.

The fitted regression lines and scatter plots between the shell length (*Y*) and days (*X*), with the corresponding fitted equation and the r^2 values for each temperature treatment, are given in Fig. 2. Overall larval growth increased with increased water temperatures. Growth rates were 6.01 ± 0.19, 10.44 ± 0.895, 12.08 ± 0.56 and 17.13 ± 0.84 µm day^{-1} for 24 °C, 27 °C, 29 °C and 31 °C respectively (Fig. 3). The growth rates for days 1–10 showed a significant difference in larvae reared at 24 °C (3.85 ± 0.57 µm day^{-1}) and 27 °C (9.08 ± 0.27 µm day^{-1}). Growth was nearly four times faster at 29 °C (13.26 ± 0.62 µm day^{-1}) and at 31 °C (15.9 ± 0.39 µm day^{-1}) (Fig. 3). From days 10 to 21 growth rate doubled at 24 °C (6.19 ± 0.22), whereas it was steady at 27 °C

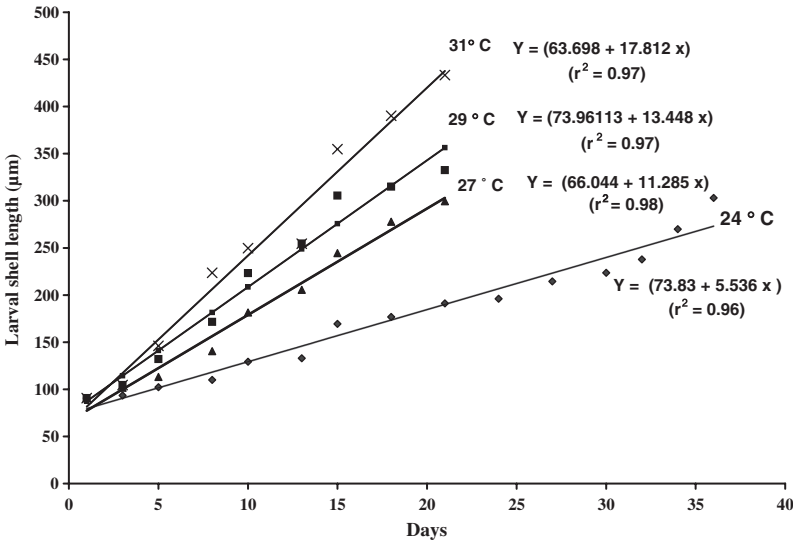

Figure 2 Regression lines of shell length for the effect of temperature on *P. viridis* larval growth.

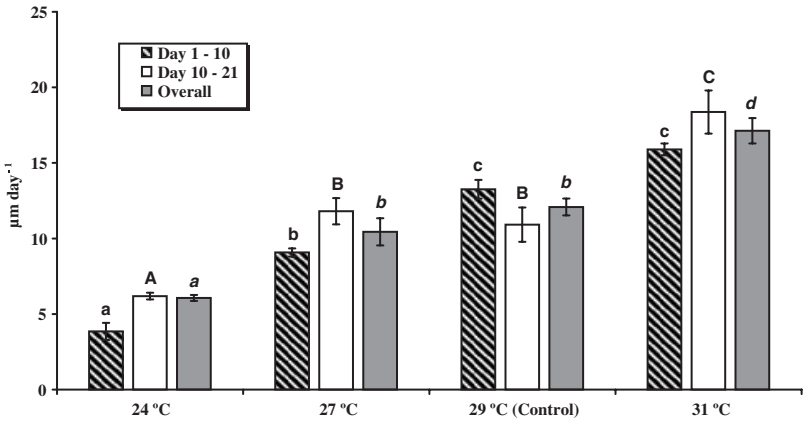

Figure 3 Growth rate of *P. viridis* larvae reared on different temperatures till settlement (mean ± SE; $n = 4$). Means with the same subscripts are not significantly different ($P < 0.05$).

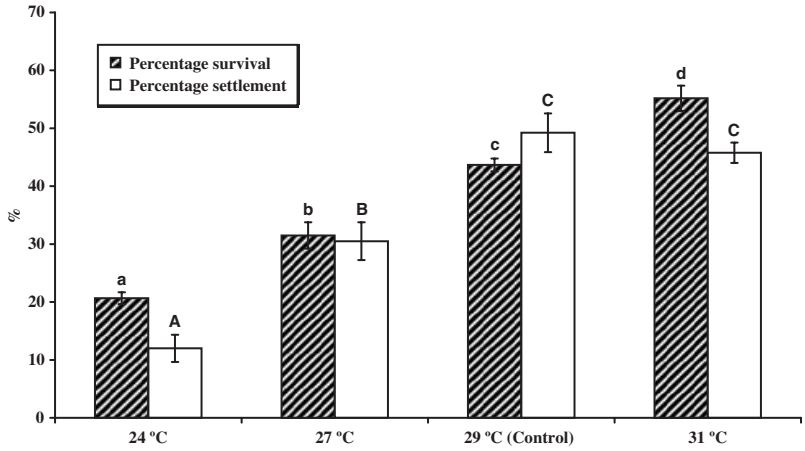

Figure 4 Effect of different temperatures on the percentage of larval survival and settlement in *P. viridis* (mean ± SE; $n = 4$). Means with the same subscripts are not significantly different ($P < 0.05$).

(11.08 ± 0.87 µm day^{-1}) and slightly decreased at 29 °C (10.91 ± 1.14 µm day^{-1}). The greatest growth occurred at 31 °C (18.37 ± 1.43 µm day^{-1}) (Fig. 3). The growth of larvae at 24 °C was slower (7.46 ± 0.41 µm day^{-1}) between days 21 and 36 (Fig. 3).

The percentage survival was 20.7 ± 1.02%, 31.5 ± 2.26%, 43.7 ± 1.11% and 55.2 ± 2.19% at 24 °C, 27 °C, 29 °C and 31 °C respectively (Fig. 4). The percentage settlement was 12 ± 2.35%, 30.5 ± 3.26%, 49.3 ± 3.34% and 45.8 ± 1.76% at 24 °C, 27 °C, 29 °C and 31 °C respectively (Fig. 4).

One-way ANOVA revealed a statistically significant difference among the temperature treatments. When the growth regression lines were tested (r^2 values given in Fig. 2), there were significant differences ($P < 0.05$) among the treatments, with a temperature of 31 °C yielding the highest growth. This was confirmed when the overall growth rate data were examined with a pairwise *t*-test. The overall growth rate at 31 °C differed significantly from the other temperatures, including the control. The control differed significantly from 24 °C but not with 27 °C. In the initial days 1–10, a temperature of 31 °C was significantly different from all the other temperatures except for the control. However, for the rearing cycle (from days 10 to 21), the growth rate at 31 °C differed significantly from all the other temperatures including the control. The control differed significantly from 24 °C but not with 27 °C. Overall, the growth of larvae was highest at 31 °C.

Percentage survival was significantly different among the treatments ($P < 0.05$). Subsequent testing revealed that a temperature of 31 °C gave the highest larval survival. One-way ANOVA with the percentage settlement data showed that there was a significant difference ($P < 0.05$) in the percentage of larvae settling at the different test temperatures. Pairwise *t*-tests indicated that the settlement percentages did not differ significantly ($P < 0.05$) between 31 °C and 29 °C. However they differed significantly from 27 °C and 24 °C. Settlement was best in the temperature range of 29–31 °C.

Discussion

The results of the experimental larval rearing at six different temperatures indicated a direct relationship between temperature and larval growth, survival settlement and spat production. There was complete mortality after 24 h of the larvae reared at 33 °C and 35 °C, showing that high temperatures are not favourable for larval rearing of *P. viridis.* Bivalve larval growth rate increases with an increase in temperature to an optimum and then decreases (Bayne 1983; Widdows 1991). There are several reports of increase in the growth rates of larvae with increased temperature in mussels (Bayne 1965; Siddall 1979a, b; His *et al.* 1989; Trevelyan & Chang 1983; Sprung 1984a). On the other hand, low temperature has also been reported to be preferable for the growth and survival of some bivalve larval species (Hrs-Brenko 1978; His *et al.* 1989; Lemos, Nascimento, De Araujo, Pereira, Bahia & Smith 1994; Hurley & Walker 1997), while Loosanoff (1954) found that temperature did not influence bivalve larval growth.

In the present study we observed that higher water temperatures gave better growth results than lower ones. This could be due to better feed assimilation at higher temperatures as reported by Loosanoff & Davis (1963). The effect of temperature on the growth rate of bivalve larvae is primarily reflected on feeding, the metabolic processes and the rapid growth of the shell. The increased growth and survival of bivalve larvae at high temperature is primarily due to better assimilation of enzyme for feed digestion. According to Widdows (1991), the precise maximum growth rate varies throughout the geographic range of the species, with maximum growth rates occurring at high temperatures for larvae originating from lower latitudes. His *et al.* (1989) reported that discounting nutrition, temperature was clearly the dominant factor influencing the larval growth of bivalves.

The mortality of larvae at temperature > 33 °C is in agreement with the findings of most bivalve larval studies. There are no reports of larvae surviving at more than 32 °C. Davis & Calabrese (1964) found that although the larvae of *C. gigas* grew well and set at a high temperature, growth was reduced at 33 °C and the larvae died at 35 °C. Bayne (1965) attributed mortality of *Mytilus edulis* larvae at 30 °C to the destruction of algal cells, leading to a bacterial buildup in the rearing system. Optimum temperature for bivalve larval rearing has been identified for the following temperate and tropical species (Table 2).

In *P. viridis* there has not been extensive research exclusively on the temperature of larval rearing except for Siddall (1979a, b), who found 26.4 °C as the best temperature for larval rearing carried out under high-latitude temperature conditions. Routine larval rearing has been conducted in this species at 25–28 °C (Rao, Kumari & Qasim 1976; Sivalingam 1977; AQUACOP 1979, 1983; Sreenivasan *et al.* 1988a,b; Tan 1997) under tropical conditions.

The results from the present study indicated that mussel larvae are sensitive to low temperatures as indicated by poor growth, settlement and spat production. The inability of the larvae to grow at low temperatures could be due to their inability to digest ingested feed at those temperatures. Moreover, the results obtained in this study also contradict the results of Tan (1975) and Rao *et al.* (1976), who were able to settle the larvae at 23–25 °C and 25–27.4 °C in 8–12 and 16–19 days respectively, whereas it took 34–45 days to settle the larvae in the present study at 24 °C in *P. viridis*. Generally, percentage settlement is positively correlated with an increase in temperature up to a point in most of the bivalves. Table 3 identifies the optimum temperature for mussel larval settlement for thetemperate and tropical species .

Table 2 Optimum temperatures for larval rearing reported for different bivalve species

Species	Optimum temperature (°C)	Reference
Mya arenaria	17.2–23.2	Stickney (1964)
Crassostrea gigas	15–20	Helm & Millican (1982)
Saccostrea ehinata	29	Coeroli *et al.* (1984)
Pinctada fucata	28–32	Krishnan (1987)
Mytilus edulis	15–20	Hrs-Brenko & Calabrese (1969)
Perna perna	27.7	Siddall (1979a, b)
Perna viridis	26.4	
Perna viridis	31	Present study

Table 3 Optimum temperatures for larval settlement reported for different mussel species

Species	Temperature (°C)	Days for settlement/ settlement stage	Reference
Mytilus edulis	16	16–20	Bayne (1965)
	11	34–38	
	22.5	20	Hrs-Brenko (1973)
	10	42	
	17	32	Beaumont & Budd (1982)
	18	20	Sprung (1984a)
	16	31	Eyster & Pechenik (1987)
	16	22–32	Pechenik *et al.* (1990)
Mytilus galloprovincialis	20–26	12	Hrs-Brenko (1978)
	18	26–32	Satuito *et al.* (1994)
Mytilus californianus	20	22	Trevelyan & Chang (1983)
Perna perna	27.2	11–13	Siddall (1979a, b)
Perna viridis	26.4	10–12	
Perna viridis	29	15–18	Present study

No work has been carried out specifically on the temperature requirements of mussel larvae for growth, survival and settlement under tropical conditions. We observed that although 31 °C was the best temperature for growth and survival, 29 °C was the best for settlement. Settlement was obtained on day 15 at this temperature and ended on day 18, while it took 18–21, 20–24 and 34–41 days for complete settlement at temperatures 31 °C, 27 °C and 24 °C respectively.

The results of this study are in agreement with those of other routine larval rearing experiments with the same or related mussel species. AQUACOP (1979, 1983) obtained spat settlement by 15–26 days at 24–28 °C for *P. viridis*, while Appukuttan, Nair & Thomas (1984) and Appukuttan, Mathew & Thomas (1988) obtained total spat settlement by 20–29 days for *P. indica*, whereas Rao *et al.* (1976) and Sreenivasan *et al.* (1988a, b) obtained total spat settlement by 15–19 and 16–20 days for *M. viridis* and *P. viridis* respectively. Tan (1997) obtained settlement by 18–30 days at 27–29 °C at varying salinities with optimum settlement at 24 ppt by the 18th day. Only the results of Tan (1975) contradict the majority of these results. He obtained the quickest larval settlement by 8–12 days at a temperature of 23–25 °C and at an optimum salinity of 28–30 ppt for *M. viridis*. For *P. viridis* it took 34–41 days in the present study to obtain complete settlement at 24 °C. Increased settlement at high temperature has been reported for other bivalves, mainly oyster and clams (Loosanoff, Miller & Smith 1951; Loosanoff & Davis 1963; Davis & Calabrese 1964, 1969; Stickney 1964; Helm and Millican, 1982; Henderson 1982; Coeroli, De Gaillande, Landret & Coatanea 1984; Krishnan 1987), while no effect was observed by some others (Prytherch 1934; Bayne 1969).

According to Bayne (1983) and Strathmann(1987), at low temperatures larvae delay metamorphosis for longer periods than at high temperatures, and the duration of the free swimming larval period depends primarily on temperature. The percentage of larvae completing metamorphosis decreased progressively with each decrease in temperature (Davis & Calabrese, 1964). They also mentioned that survival and growth of the larvae increased with increased temperature but within the limits of salinity and food concentration. Analysis of salinity and feed parameters of larval rearing of the related larval rearing experiments in *P. viridis*, *P. indica*, and *M. viridis* larvae reveal that salinity and food concentration (even though there was some variation in the feed concentration) were nearly the same. Variations in the results may be due to various reasons like species-specific variations, nutritional content of the diet or due to variations in the geographical ranges where the experiments were conducted.

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