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Maturation and spawning performance of pond-reared *Penaeus merguiensis* in different combinations of temperature, light intensity and photoperiod

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Abstract

The combined effects of temperature (23 and 27 °C), light intensity (1100 and 2 lux or ≈ 21.5 and 0.04 μEinst m^{-2} s^{-1} respectively) and photoperiod (10 h light/14 h dark and 14 h light/10 h dark) on ovarian maturation and spawning performance of ablated pond-reared *Penaeus merguiensis* were investigated in a 51-day experiment. The results showed that temperature was the most influential factor, followed by light intensity, whereas the effect of photoperiod was minor. As the maturation process progressed, the effects of temperature and light intensity became stronger while that of photoperiod became less significant. Temperature significantly affected all the reproductive parameters assessed. Prawns in the 27 °C treatments outperformed those in the 23 °C treatments ($P < 0.05$). The effect of light intensity was found to have a significant effect ($P < 0.05$) only on the proportion of spawners (prawns that spawned) and spawning rate. More prawns spawned at a higher rate under dim light (2 lux) than under strong light (1100 lux). Photoperiod affected only the proportion of prawns reaching stage III of ovarian development ($P < 0.05$). There were interactions between temperature and light intensity affecting the proportion of prawns reaching stage III and, although not significantly, the proportion of spawners ($P = 0.177$), fecundity ($P = 0.134$) and survival ($P = 0.061$). Overall, it is recommended that a combination of 27 °C, 2 lux and 10 h light/14 h dark is suitable for the maturation of pond-reared *P. merguiensis*. There were indications that temperature can be used to control the rate of ovarian maturation. Also, it is possible to increase light intensity up to 1100 lux in *P. merguiensis* hatcheries if prawns are ablated.

Keywords: light intensity, ovarian maturation, *Penaeus merguiensis*, photoperiod, reproduction, spawning, temperature

Introduction

The banana prawn, *Penaeus merguiensis*, is a good candidate for prawn farming and domestication/selective breeding programmes (Hoang 2001). Semi-intensive culture of *P. merguiensis* in south-east Queensland, Australia, in 2000–01 achieved productions of 3–5 tons ha^{-1} per crop (Lobergeiger & Hoang 2001). More importantly, the ability of pond-reared *P. merguiensis* to mature in culture ponds and their acceptable reproductive performance (Lichatowich, Smalley & Mate 1978; Aquacop 1983; Hoang, Lee, Keenan & Marsden 2002a) promise reliable postlarvae production for prawn farming, and will permit genetic selection for growth improvement or development of pathogen-free stocks. Further research on the reproductive biology of this species is therefore needed to assist hatchery production.

Ovarian maturation and spawning of penaeid prawns have long been recognized to be influenced by environmental factors (Primavera 1985; Dall, Hill, Rothlisberg & Staples 1990; Bray & Lawrence 1992). However, disentangling the effects of different factor is difficult as the conditions in which experiments were conducted are not comparable in many cases (Browdy 1992; Benzie 1997). It is difficult to investigate the effect of several factors simultaneously because of the demanding character of prawn reproduction trials, e.g. the need for large replicate tanks under controlled conditions, labour and time required, high operating expense, etc. (Wouters, Lavens, Nieto & Sorgeloos 2001). However, the fact that ovarian maturation of pond-reared *P. merguiensis* is achievable in relatively small tanks (Hoang *et al.* 2002b) provides an opportunity to investigate simultaneously the effects of different key environmental factors such as temperature, light intensity and photoperiod.

Preliminary investigations on pond-reared *P. merguiensis* in south-east Queensland, Australia, revealed that strong light (1100 lux) inhibited ovarian maturation of unablated females (Hoang *et al.* 2002b), but it did not affect maturation and spawning performance of ablated females (Hoang *et al.* 2002c). Spawning performance of prawns was higher at 27 °C than at 23 °C, and was further improved by increasing temperature from 23 to 27 °C (Hoang *et al.* 2002d). Unlike *P. japonicus* (Primavera 1985; Yano 1993) *P. merguiensis* was not very sensitive to photoperiod (T Hoang, unpublished data). Ovarian maturation and spawning performance of prawn broodstock maintained under three different photoperiods: 10 h light/14 h dark (10L:14D) , 12L:12D and 10L:14D were not different. Although the common experimental protocols, e.g. broodstock source, handling and management, maturation diets, have been prestandardized for the aforementioned studies, it still is necessary to investigate the effects of temperature, light intensity and photoperiod in a more comprehensive experiment to (1) identify possible interactions among these factors, (2) evaluate the relative strength of each factor and (3) confirm the findings obtained in the initial studies. Further, this information should be useful to formulate an effective protocol to control reproduction of *P. merguiensis* in captivity and help further understand the physiology and reproductive biology of pond-reared prawn broodstock.

This paper reports on the combination effects of temperature, light intensity and photoperiod on ovarian maturation and spawning performance of ablated pond-reared *P. merguiensis*.

Materials and methods

Broodstock source

Banana prawns, *P. merguiensis*, were collected from a commercial prawn farm in south-east Queensland, Australia, in mid-March 2001 after 4.5 months of culture (approximately 5.5 months after hatching). These prawns were produced in a hatchery from wild-caught broodstock and were cultured in earthen ponds at an initial stocking density of 40 PL15 m^{-2}. At the time of collection, prawns were relatively uniform in size (16–18 g). Approximately 5% of female prawns possessed spermatophores in their thelycum. The prawns were transferred by road (2 h trip) to Bribie Island Aquaculture Research Centre, stocked in a 200-m^2 pond and grown further for 2 months. Stocking density was 10 prawns m^{-2} with a sex ratio of one female to one male. Prawns were fed twice a day, at 09.00 and 16.30 hours, with commercial pellets (50% crude protein; EBI STAR, Higashimaru, Japan) at a rate of 5% of prawn biomass daily. An algae bloom (diatoms predominated, Secchi disc reading $\approx$ 40–50 cm) was maintained continually during the culture period. Water exchange (up to 30% of pond volume) was conducted twice a week. Basic water quality parameters such as temperature, salinity, pH and dissolved oxygen (DO) were monitored twice a day at feeding times. During this 2-month period, average temperature, salinity, pH and DO were 26.8 °C (23.6–31.3), 33.0 ppt (31.8–33.3), 8.2 (7.7–8.8) and 7.2 mg L^{-1} (5.0–10.9) respectively.

Prior to winter (i.e. in mid-May 2001), female prawns were collected for the experiment. All the selected prawns were healthy in appearance and showed no ovarian development. To monitor individual spawning performance the prawns were tagged with a numbered plastic ring around one eyestalk. Body weight and carapace length were also measured. Prawns were then randomly allocated into 18 250-L experimental tanks (black polythene, measuring 90 × 65 × 50 cm, entirely covered with plastic mesh) at a density of five prawns per tank.

Experimental design and conditions

The experiment had an incomplete factorial design with two temperatures (23 and 27 °C), two light intensities (1100 and 2 lux or ≈ 21.5 and 0.04 μEinst/m^2 s respectively) and two photoperiods (10L:14D and 14L:10D), forming six combination treatments (Table 1). The second level of each factor was considered favourable (F) and the first level was considered unfavourable (U) for ovarian maturation of prawn broodstock. The six treatments were therefore named FFF, FUF, FFU, UFF, UUF and UFU (Table 1). Each treatment had three replicate tanks. Prawn size was not different ($P > 0.05$) among the treatments (Table 1).

The experiment period was 51 days. Prawns were first held in the experimental tanks for 28 days before being unilaterally ablated to induce spawning. Eyestalk ablation involved ligating (tying a thread around the base of the eyestalk), slitting the eyeball and squeezing out the contents. The ablation process took approximately 15 s per prawn. During this handling, the prawns were kept in cool water (16–18 °C) to reduce stress. Ovarian maturation and spawning performance of prawns were monitored for 23 days after eyestalk ablation.

Light was provided by daylight fluorescent tubes (Osram 36 W, Australia). The light tubes were wrapped in green 70% shade-cloth (Dindas Lew, cat no. 5c7036 BL) to reduce light intensity in treatments that required an intensity of 2 lux. Light intensity was measured by a photometer (model 185B, LI-COR, USA) at the water surface. Water depth was 40 cm in the experimental tanks. Photoperiod was controlled by timers. Filtered (5 μm) seawater was heated to either 24.5 °C or 28.5 °C in two head tanks and gravity fed to the experimental tanks at a rate of 300% per day, maintaining water temperature in the experimental tanks relatively stable at 23 °C and 27 °C respectively. Water quality was checked daily by an U-10 water checker (Horiba, Japan). A summary of the physical conditions of the experiment is presented in Table 1. During the experimental period, salinity, pH and DO were stable at 33.0 ± 0.2 (SD) ppt, 8.1 ± 0.1 and 5.6–5.8 mg/L, respectively, in 23 °C treatments; and at 33.3 ± 0.2 ppt, 8.0 ± 0.1 and 5.8–5.9 mg/L, respectively, in 27 °C treatments.

Throughout the experiment, prawns were fed with the BIARC moist maturation diet (a mixture of fresh-frozen squid, green mussel, calf liver and other essential nutrients; 54.6% crude protein; Marsden, McGuren, Hansford & Burke 1997) at 10% of prawn biomass, supplemented with 2% of prawn biomass by the EBI STAR pellet. The BIARC moist maturation diet was prepared every 2 weeks and kept in a freezer at −18 °C for daily use. Feeding was conducted twice a day at 10.00 and 17.00 hours. The EBI STAR pellet was given only at 17.00 hours. Uneaten feed and faeces were siphoned out before feeding.

Ovarian maturation (Tuma 1967; Primavera 1985) was checked daily at 17.30 hours, using a submersible flashlight to view the outline of the prawn's ovary. Stage IV females were individually transferred to 150-L spawning tanks and returned to the original experimental tank early the next morning. Temperature in the spawning tank was similar to the original experiment tank. Fecundity was estimated by vigorously agitating water in the spawning tank to ensure a homogeneous suspension of eggs, then quickly taking four 100-mL aliquot samples and counting the number of eggs under a stereomicroscope. No attempt was

Table 1 Treatment conditions and prawn size at the start of the experiment. Data are means ± SD. Prawn size was not significantly different among treatments ($P > 0.05$)

Treatments*	Nominal temperature (°C)	Actual temperature (°C)	Light intensity (lux)	Photoperiod	Carapace length (mm)	Body weight (g)
(1) FFF	27	26.9 ± 0.2	2	14L:10D	37.3 ± 0.6	30.4 ± 2.0
(2) FFU	27	26.9 ± 0.2	2	10L:14D	36.9 ± 0.9	29.8 ± 2.3
(3) FUF	27	27.0 ± 0.2	1100	14L:10D	37.2 ± 0.2	29.4 ± 1.0
(4) UFF	23	22.9 ± 0.2	2	14L:10D	37.8 ± 0.2	31.5 ± 0.8
(5) UFU	23	22.9 ± 0.2	2	14L:10D	38.1 ± 0.4	32.6 ± 0.5
(6) UUF	23	23.0 ± 0.1	1100	10L:14D	37.0 ± 0.7	30.1 ± 1.2

*F, favourable; U, unfavourable.

made to measure hatching rate or survival of prawn larvae. The number of moults per tank per day was also recorded. At the end of the experiment prawns were weighed to assess growth.

Statistical analysis

Variables used to assess ovarian maturation included the proportion of prawns reaching stage III and IV and the proportion of spawners (prawns that spawned).

Spawning performance was assessed by latency period (the interval between eyestalk ablation and the first spawn of a spawner; prawns that did not spawn were assigned a latency period of 23 days, i.e. the maximum interval between eyestalk ablation to the end of the experiment, to facilitate statistical analysis and better reflect treatment effect), spawning rate (number of spawns produced by a female, adjusted by the number of days it survived in the experiment since eyestalk ablation; expressed as spawns per female per day), fecundity (mean number of eggs/spawn; fecundity was calculated using data only from prawns that spawned), egg production per female (averaged for all prawns in the same experimental unit including those did not spawn), spawning interval (number of days between two successive spawns) and time spent at stage IV/spawn (days). Moult interval, growth (% weight gain per day) and survival of prawn broodstock were also assessed.

Tanks were used as the observation unit for statistical analysis. Experimental data were analysed with Genstat (version 4.1 for Windows) using a generalized linear model (McCullag & Nelder 1989; Payne, Lane, Digby, Harding, Leech, Morgan, Todd, Thompson, Wilson, Welham & White 1993). The significance level (α) value was set at 0.05. Growth data did not have a normal distribution and therefore were log transformed for statistical analysis. The presented means of weight gain and 95% confidence limits were backtransformed from the logarithmic values (Sokal & Rohlf 1981). Data for both the actual and adjusted latency periods are presented.

Results

The observed data are summarized in Table 2, while a summary of the results of statistical tests on these data are presented in Table 3.

Ovarian maturation

Temperature strongly affected both the proportion of prawns reaching stage IV of ovarian maturation ($P = 0.015$) and the proportion of spawners ($P < 0.001$), but it had a minor effect on the proportion of prawns reaching stage III of ovarian maturation ($P = 0.08$) (Table 3). Further, the maturation rate was high and consistent at 27 °C, while it was lower and progressively reduced at 23 °C (Fig. 1). Overall, at 27 °C 90% of the prawns reached stage III, 88% reached stage IV and 87% spawned. In contrast, at 23 °C, 82% of the prawns reached stage III, but only 62% reached stage IV and 35% spawned (Fig. 1).

The effect of light intensity increased as the maturation process progressed. Light intensity had a minor effect on the proportion of prawns reaching stage IV ($P = 0.078$), but it significantly affected the proportion of spawners ($P = 0.036$) (Table 3). Dim light favoured ovarian maturation. The proportion of spawners was 67% at 2 lux, significantly higher than 44% at 1100 lux (Fig. 2).

As the ovarian maturation process progressed, the effect of photoperiod became less significant (Table 3). Photoperiod affected only the proportion of prawns reaching stage III ($P = 0.017$). About 89% of the prawns reached stage III of ovarian maturation at 14L:10D compared with 77% at 10L:14D.

An interaction between temperature and light intensity was found for the proportion of prawns reaching stage III ($P = 0.048$). When temperature was favourable (i.e. at 27 °C) light intensity had no effect, but when temperature was unfavourable (i.e. at 23 °C) strong light intensity (1100 lux) significantly reduced the proportion of prawns reaching stage III to 53%, compared with 91% under dim light (2 lux) (Fig. 3). Although not significant, there was evidence of interaction between temperature and light intensity affecting the proportion of spawners ($P = 0.177$). At 23 °C, only 7% of the prawns spawned at 1100 lux, compared with 44% spawned at 2 lux, while the proportion of spawners was similar between the two light intensities at 27 °C (80% and 89% respectively).

Spawning performance

Temperature strongly influenced spawning performance of the prawns (Table 3). Spawning rate,

Table 2 Summary of maturation and spawning performance of pond-reared *Penaeus merguiensis* in different combinations of water temperature, light intensity and photoperiod

	Treatments*					
Variable	**(1) FFF**	**(2) FFU**	**(3) FUF**	**(4) UFF**	**(5) UFU**	**(6) UUF**
Ovarian maturation						
Proportion of prawns reaching stage III (%)	93.3 ± 5.6	80.0 ± 9.0	93.3 ± 5.6	100	73.3 ± 10.0	53.3 ± 11.3
Proportion of prawns reaching stage IV (%)	93.3 ± 7.5	80.0 ± 12.1	86.7 ± 10.3	80.0 ± 12.1	60.0 ± 14.8	26.7 ± 13.4
Proportion of spawners (%)	93.3 ± 6.4	80.0 ± 10.3	80.0 ± 10.3	46.7 ± 12.9	40.0 ± 12.6	6.7 ± 6.4
Spawning performance						
Latency period (days)†	10.72 ± 1.16	9.5 ± 0.38	10.32 ± 1.41	15.22 ± 0.62	17.17 ± 0.73	10.00
Adjusted latency period‡	11.47 ± 1.64	12.2 ± 0.31	12.53 ± 2.61	19.53 ± 0.84	20.73 ± 0.68	22.13 ± 0.87
Spawning rate (spawn per prawn per day)	0.114 ± 0.009	0.086 ± 0.005	0.081 ± 0.016	0.026 ± 0.009	0.020 ± 0.008	0.006 ± 0.006
Mean fecundity ($\times 10^3$ eggs)†	70.2 ± 2.4	76.3 ± 5.6	81.1 ± 5.3	60.4 ± 14.8	40.8 ± 11.1	82.6
Egg production per female ($\times 10^3$ eggs)§	146.0 ± 19.6	145.8 ± 14.0	152.7 ± 41.2	30.6 ± 7.2	21.9 ± 11.2	11.0
Egg production per treatment ($\times 10^3$ eggs)	2190.7	2186.5	2290.9	458.9	328.3	165.1
Spawning interval (days)	4.61 ± 0.96	4.14 ± 0.52	5.44 ± 0.40	15	4	11
Time spent at stage IV/spawn (days)	1.68 ± 0.24	1.36 ± 0.18	1.95 ± 0.10	5.72 ± 1.64	4.78 ± 1.30	4.00
Moulting, growth and survival						
Moult interval (days)	17.21 ± 1.52	17.45 ± 0.87	15.98 ± 0.58	24.02 ± 3.01	22.42 ± 0.59	21.71 ± 1.45
Specific growth rate (% weight gain/day)	0.363 ± 0.104	0.313 ± 0.045	0.314 ± 0.037	0.257 ± 0.015	0.141 ± 0.005	0.205 ± 0.041
Survival of prawns (%)	73.3 ± 11.4	73.3 ± 11.4	86.7 ± 8.8	100	93.3 ± 6.4	80.0 ± 10.3

*F, favourable; U, unfavourable.
†Data from spawners only.
‡Adjusted by assigning the period from eyestalk ablation to the end of the experiment (23 days) as the 'latency period' for prawns that did not spawn.
§Averaged for all prawns including those did not spawn. Data are means ± SE.

Table 3 Summary of results of statistical tests (using the generalized linear model)

	Treatment effects				
Variable	***T***	***I***	***P***	***T*** × ***I***	***T*** × ***P***
Ovarian maturation					
Proportion of prawns reaching stage III	0.080	0.101	0.017	0.048	0.172
Proportion of prawns reaching stage IV	0.015	0.078	0.180	0.202	0.877
Proportion of spawners	< 0.001	0.036	0.352	0.177	0.492
Spawning performance					
Adjusted latency period*	< 0.001	0.282	0.498	0.597	0.869
Spawning rate	< 0.001	0.047	0.093	0.939	0.253
Mean fecundity	0.025	0.134	0.474	0.260	0.185
Egg production/female	< 0.001	0.819	0.832	0.550	0.842
Time spent at stage IV/spawn	< 0.001	0.930	0.444	0.365	0.702
Spawning interval†	< 0.001	–	–	–	–
Moulting, growth and survival					
Moult interval	< 0.001	0.316	0.674	0.954	0.572
Log SGR	0.006	0.992	0.087	0.862	0.180
Survival of prawns	0.103	0.838	0.719	0.061	0.281

*Adjusted by assigning the period from eyestalk ablation to the end of the experiment (23 days) as the latency period for prawns that did not spawn.
†Owing to limited data, only temperature effect was assessed. The experimental design allowed only interactions between water temperature (*T*) and light intensity (*I*) and between water temperature and photoperiod (*P*) to be assessed. Data are *P*-values.

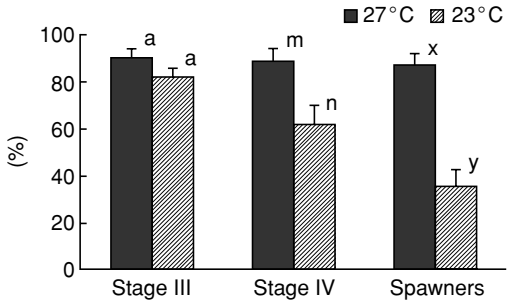

Figure 1 Effect of water temperature on ovarian maturation of pond-reared *Penaeus merguiensis*. Data are adjusted means ± 1 SE. Stage III, the proportion of prawns reaching stage III of ovarian maturation; stage IV, the proportion of prawns reaching stage IV; spawners, the proportion of prawns that spawned. Within categories bars with different letters are significantly different ($P < 0.05$).

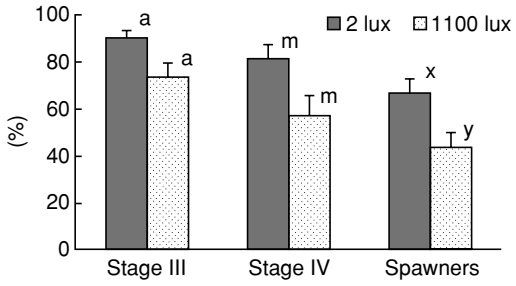

Figure 2 Effect of light intensity on ovarian maturation of pond-reared *Penaeus merguiensis*. Data are adjusted means ± 1 SE. Stage III, the proportion of prawns reaching stage III of ovarian maturation; stage IV, the proportion of prawns reaching stage IV; spawners: the proportion of prawns that spawned. Within categories bars with different letters are significantly different ($P < 0.05$).

fecundity and egg production per female were all significantly higher ($P < 0.05$), whereas latency period, spawning interval and number of days spent at stage IV/spawn were significantly ($P < 0.001$) lower at 27 °C than at 23 °C (Table 4). On average, prawns produced 0.099 spawns per prawn per day, with an average egg production per female of 147.6×10^3 at 27 °C, fivefold higher ($P < 0.001$) than 0.019 spawns per female per day with an average egg production per female of 23.5×10^3 at 23 °C. At 27 °C, prawns responded more quickly to eyestalk ablation, i.e. latency period was shorter, and had a shorter rematuration period, i.e. shorter spawning interval. Their ovarian maturation also progressed faster as they spent significantly ($P < 0.001$) less time at stage IV/spawn (1.6 days) than at 23 °C (5.1 days) (Table 4).

Light intensity had a significant effect only on the spawning rate ($P = 0.047$). At 2 lux, prawns produced 0.064 spawns per female per day on average, compared with 0.044 spawns per female per day for those maintained at 1100 lux.

In addition, there were some possible effects of light intensity on fecundity ($P = 0.134$), and of photoperiod on spawning rate ($P = 0.093$). The fecundity of prawns improved at 1100 lux (81.7×10^3 compared with 64.0×10^3 at 2 lux), while 14L:10D favoured spawning rate (0.061 spawns per female per day compared with 0.053 spawns per female per day at 10L:14D).

Survival, growth and moulting

Temperature significantly influenced moult interval ($P < 0.001$) and growth ($P = 0.006$), but it did not significantly affect survival of prawns ($P = 0.103$) (Table 3). The average moult interval of prawns was significantly shorter at 27 °C than at 23 °C (Table 4). Similarly, prawn growth was significantly higher at 27 °C (SGR = 0.319% per day; 95% confidence limits: $L_1 = 0.191$, $L_2 = 0.532$) than at 23 °C (weight gain = 0.206% per day; $L_1 = 0.124$, $L_2 = 0.343$). However, survival of prawns tended to be lower at 27 °C (77% compared with 93% at 23 °C).

No effects of light intensity and photoperiod were found on the survival, growth and moulting of the prawns (Table 3). However, there was a possible interaction between temperature and light intensity affecting survival of prawns ($P = 0.061$). The difference in survival of prawns between the two temperatures at 1100 lux was only 7%, but increased to 24% at 2 lux (Fig. 3). Lower survival of prawns at the favourable temperature (27 °C) and favourable light intensity (2 lux) appeared to be associate dwith their higher spawning rate.

Discussion

Overall, this study demonstrated that water temperature, light intensity and photoperiod affected ovarian maturation and spawning performance of pond-reared *P. merguensis* differently and to some extent independently. That difference was reflected in the number of reproductive parameters affected by each of these factors and its corresponding magnitude. Temperature had a significant effect on all variables except the proportion of prawns reaching stage III. Light intensity was had a significant effect

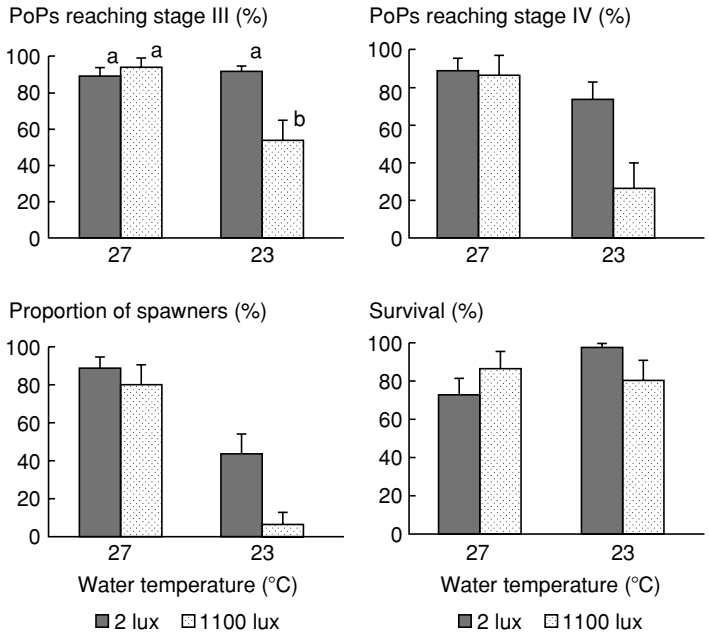

Figure 3 Interactions between water temperature and light intensity on maturation rate and survival of prawns. Data are adjusted means ± 1 SE. Bars with different letters are significantly different ($P < 0.05$). PoPs, proportion of prawns.

Table 4 Spawning performance of pond-reared *Penaeus merguiensis* at two different temperatures regardless of light intensity and photoperiod

	Water temperature (°C)	
Variable	**27**	**23**
Latency period (days)*	10.3 ± 0.6^{a}	14.9 ± 0.6^{b}
Adjusted latency period (days)†	11.9 ± 0.8^{a}	20.5 ± 0.8^{b}
Spawning rate (spawns per female per day)	0.099 ± 0.006^{a}	0.019 ± 0.006^{b}
Mean fecundity ($\times 10^3$ eggs)*	73.9 ± 5.6^{a}	58.1 ± 6.1^{b}
Egg production/female ($\times 10^3$ eggs)‡	147.6 ± 12.7^{a}	23.5 ± 12.7^{b}
Time spent at stage V (days)	1.63 ± 0.50^{a}	5.13 ± 0.53^{b}
Spawning interval (days)	4.7 ± 0.4^{a}	11.2 ± 0.7^{b}

*Data from spawners only.

†Adjusted by assigning the period from eyestalk ablation to the end of the experiment (23 days) as the 'latency period' for prawns that did not spawn.

‡Averaged for all prawns including those did not spawn. Data are means ± SE. Within rows means with the same superscripts are not significantly different ($P > 0.05$).

only on the proportion of spawners and spawning rate, while photoperiod affected only the proportion of prawns reaching stage III. As the key parameters that determine the success of broodstock maturation are the proportion of spawners, latency period, spawning rate, fecundity and larval production, it can be concluded that, within the scope of this study, temperature appeared to be the most influential factor, followed by light intensity and then photoperiod.

As temperature, light intensity and photoperiod appeared to act rather independently, the suitability of each combination was weighed by the number of favourable against unfavourable factors and their relative magnitude. The unfavourable temperature (23 °C) in the UFF, UUF and UFU treatments

strongly impaired maturation and spawning performance of prawns. The trend of reducing maturation and spawning performance from the UFF to UFU and to UUF suggests that maturation and spawning performance of prawns were further compromised by the presence of more unfavourable factors. In this respect, the effect of light intensity was stronger than that of photoperiod. The interactions between temperature and light intensity also followed this pattern. At 27 °C, maturation and spawning performance of prawns were similar at the two light intensities. However, at 23 °C, the strong light intensity in the UUF treatment clearly reduced the maturation and spawning performance of prawns. However, the favourable temperature (27 °C) could override the presence of unfavourable light intensity in the FUF treatment or unfavourable photoperiod in the FFU treatment.

This study also showed that, as the maturation process progressed, the effect of temperature and light intensity became stronger whereas that of photoperiod became less significant (Table 3). Thus, it is suggested that from stage III onwards it is crucial to maintain temperature and light intensity at appropriate levels for the completion of ovarian maturation and spawning. One finding of this study, that light intensity at 1100 lux significantly ($P < 0.05$) affected the proportion of spawners and spawning rate, might appear contrary to the result of our previous study (Hoang *et al.* 2002c), which found that the proportions of ablated *P. merguiensis* spawners held at 1100 lux and 2 lux were not different at 27 °C. However, this is explainable. In that study, the prawns were ablated only 3 days after being exposed to these light intensities, whereas the prawns in this study were maintained at these light intensities for 28 days. Given the fact that 1100 lux inhibited ovarian maturation of unablated *P. merguiensis* (Hoang *et al.* 2002b), it was expected that the response of the prawns in this study to light intensity would fall between those findings. In other words, there was probably a residual effect of strong light intensity from that 28-day period contributing to the overall effect of light intensity on the proportion of spawners. Furthermore, the difference in the proportion of spawners between the two light intensities derived mainly from treatments with the unfavourable temperature (Fig. 3). This may suggest that the effect of light intensity on ovarian maturation of ablated *P. merguiensis* becomes observable when temperature is unfavourable.

Between the two temperatures tested in this study, treatments with a temperature of 27 °C clearly outperformed those with a temperature of 23 °C. This result confirms our previous finding that spawning performance of pond-reared *P. merguiensis* at 27 °C was superior than at 23 °C (Hoang *et al.* 2002d). However, the maturation and spawning performance of the prawns used in that study were lower than in this study. This was probably because of their smaller size (10.5 g on average at the start of the 60-day experiment) compared with the prawns used in this study (30.5 g on average at the start of this 51-day experiment). That difference implies that the magnitude of the temperature effect could be modified by some other factors, among which prawn size is one possible factor. Generally, fecundity and egg production per female are known to increase with prawn size (Motoh 1981; Primavera 1985; Menasveta, Sangpradub, Piyatiratitivorakul & Fast 1994; Cavalli Scardua & Wasielesky 1997; Crocos & Coman 1997).

It is interesting to note that, in this study, 82% of the prawns reached stage III and 62% reached stage IV of ovarian maturation, which is relatively high, but only 35% spawned at 23 °C. Further, the development rate of prawn's ovaries at this temperature was slow. Those who spawned at 23 °C spent significantly more time at stage IV as well as between two successive spawns (Table 4). As the prawns used in this study were at the appropriate size for hatchery production, these findings suggests that temperature can be used to control the maturation rate and spawning of *P. merguiensis*. Using temperature to stimulate or delay spawning has been reported in other species (Robertson, Bray & Lawrence 1991; Li 1995). However, as hatching rate and larval survival were not included in this study, it is not known whether these can be affected by maintaining prawn broodstock at 23 °C for a period of time. Kelemec & Smith (1984) reported that short-term cooling at 10–15 °C for up to 21 days did not affect fertilization and hatching rate in wild-caught *P. plebejus*. Further research are recommended to address this concern. The trend towards reduced survival of prawns at 27 °C appeared to be the result of reproductive exhaustion, i.e. the significantly higher spawning rate. This has been observed consistently in *P. merguiensis* and is discussed by Hoang *et al.* (2002c).

One should also consider the option of raising the light intensity (if the broodstock are ablated) to

avoid the inconvenience caused by working in dim conditions. In this study, when temperature and photoperiod were favourable, strong light in treatment FUF, although slightly reducing the proportion of spawners and spawning rate, improved fecundity of prawns, thus resulting in a total egg production similar to treatment FFF (Table 2). As the ultimate goal of hatchery production is high production of eggs and larvae, increasing the light intensity to facilitate easy broodstock management may be a desirable option for *P. merguiensis* hatcheries. The improvement in the fecundity of *P. merguiensis* observed at 1100 lux, although not statistically significant, has been consistently obtained in our laboratory (Hoang *et al*. 2002b, c, e). Further work at molecular level may shed the light on the mechanism(s) through which light intensity influences fecundity. Finally, the results of this study suggest that the maturation environment for pond-reared *P. merguiensis* needs to satisfy the requirements for, first, temperature, second, light intensity and, third, photoperiod. A combination of 27 °C, 2 lux and 14L:10D appeared to be suitable for ovarian maturation of *P. merguiensis* in addition to the application of eyestalk ablation and the provision of appropriate maturation diets.

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