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Effect of temperature and photoperiod on growth, molting and survival of marron *Cherax tenuimanus*

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Abstract. Growth in crayfish consists in a series of molting and it is influenced by environmental factors. Among those, temperature and photoperiod are considered as major factors. The experiment to study effect of temperature and photoperiod on growth, molting and survival of juvenile marron (*Cherax tenuimanus*) was conducted over a period of three months. Three temperature ranges (19, 22 and 25°C) and two photoperiods (long day, 10h dark : 14h light and short day, 14h dark: 10h light) treatments with three replicates were applied in the experiment. The results showed that temperature significantly affected growth, molting and survival of marron whereas photoperiod as well as interaction between temperature and photoperiod had no effect on those parameters. Specific growth rate and molt increment were significantly higher at higher temperature compared to the lower temperature. In addition, the number of molt was higher and intermolt period was shorter at higher temperature. On the contrarily, survival was higher at lower temperature.
Key Words: Crayfish, intermolt period, crawfish, crawdads, freshwater lobsters, mudbugs.

Introduction. Marron *Cherax tenuimanus* is a large freshwater crayfish, endemic to Western Australia. This species is high quality and has the highest value of freshwater crayfish farmed in Australia. Although rapid expansion of marron farming occurred since 1990's in Western Australia and South Australia, the current production of marron in Australia is less than 100 tones year^{-1} and has remained relatively stable over the past decade (Lawrence 2007). The major problem in the culture of marron is the difficult growing process.

Growth in crustacean including marron consists of a series of molts and separated by what are known as intermolt periods. A number of environmental factors affect growth and molt of crustacean. Among those, temperature and photoperiod are considered as the major factors in regulating molt cycle in crustacean (Stephens 1955; Aiken 1969; Armitage et al 1973; Aiken & Waddy 1992). Temperature affects the growth of all poikilothermic animals including crayfish. In order to grow, crayfish have species-specific temperature threshold (Morrissy 1990; Jones 1995). Temperatures below the optimum normally inhibit growth while the temperatures above optimal cause stress and result in increased mortality (Morrissy 1976; Rouse & Kartamulia 1992). The lower and upper limits of temperature for marron (*C. tenuimanus*) were 12°C and 30°C, respectively (Morrissy 1990). Photoperiod can significantly affect growth and determine molt cycle of freshwater crayfish. Several studies have been prformed to examine the effect of photoperiod on growth and molting of freshwater crayfish but the results are contradictory. Sáez-Royuela et al (1996) reported that photoperiod has minimal effect on growth. However, other studies showed that growth and survival can be improved with increasing photoperiod (Taugbølt & Skurdal 1992).

There are several studies of the relationship between temperature and growth (Morrissy 1990; Rouse & Kartamulia 1992) or between photoperiod and growth (Taugbølt & Skurdal 1992; Sáez-Royuela et al 1996). However, up to now, there is no study to

examine combined effect of temperature and photoperiod on growth and survival of crayfish. Therefore, this experiment was conducted to investigate the effect of temperature and photoperiod on growth, molting and survival of marron (*C. tenuimanus*).

Material and Method. Juvenile (0 + year old) marron of mixed parentage to study the effect of temperature and photoperiod on growth, molting and survival was obtained from Kangabbie farm, Adelaide Hills, South Australia. Juvenile marron (mean weight 4.43 ± 0.11 g, orbital carapace length 18.31 ± 0.14 mm) used in this project are assumed to be free from the effect of reproduction on growth (Hammond et al 2006). Marrons were acclimatized for one week prior to the experiment. During the experiment, the subjects were fed with commercial marron pellets (21% protein, 0.9% calcium) produced by Westfeeds Pty Ltd, Bentley, Western Australia at 1-2% of wet body weight per day.

Three temperature ranges (19, 22 and 25° C) and two photoperiods i.e. long day (10 hour dark : 14h light) and short day (14h dark: 10h light) were applied in this experiment with three replicates.

A total of 18 glass aquaria (H x W x L: 30 x 30 x 40 cm, area: 0.12 m^2) was used in this study. Each aquarium was provided with a rack shelters. Four PVC water pipes (length: 10 cm, diameter: 3 cm) were tied at the bottom of the rack while fly screen net was placed at its surface. Each aquarium was connected to a sump tank for each temperature treatment. Higher water temperature was maintained with immersion heater placed in the sump tanks while lower water temperature was maintained by setting room temperature at 19°C. Photoperiod was regulated via clock controlled fluorescent light. Black plastic sheet was used to separate the long photoperiod aquaria from the short period aquaria. Water was re-circulated by a submerged pump and let flow freely back to the sump tank via outlet pipe. Continuous aeration for each aquarium was provided via charcoal filters. Uneaten food and solid waste as well as exuviae (molted exoskeletons) were siphoned and approximately 10% of water in the system was replaced everyday in the morning before feeding. Water temperature and pH were recorded daily. Data of ammonium, and nitrite was taken once a week.

Nine marron individuals were placed in each aquarium. The marrons were tagged individually with color polish nail. The aquaria were checked daily for molted animals. A newly molted individual was retagged 3–5 days after molting. Dead marrons were replaced to maintain constant density during the experiment. However, the replacement animal was not included in the final analysis.

Orbital carapace length was measured from the mid-posterior edge of the carapace to the stem of the eyestalk to the nearest millimeter using vernier caliper. An electronic balance was used to weigh animals to nearest milligram. Prior to weighing, animals were placed on absorbent paper to removed excess water. To study specific growth, marrons were weighed at the beginning and at the end of the experiment, while for intermolt increment 3-5 days after ecdysis. The experiment was conducted for three months (March to May 2010) at Animal House, Flinders University, Adelaide, South Australia.

Growth of marrons was measured as molt increment (MI), specific growth rate (SGR) and intermolt period (Tim) using following formula proposed by Jussila & Evans (1998) and Hammond et al (2006):

$$MI\% = (Wa - Wb) \times 100\ Wb^{-1}$$

where: Wa - weight after molt (g)
Wb - weight before molt (g)

$$SGR = (\ln Wf - \ln Wi) \times 100\ T^{-1}$$

where: Wf - final weight (g)
Wi - initial weight (g)
T - study period (days)

$$Tim = Tn+1 - Tn$$

where: Tn+1 - date of n+1 molt
Tn - date of n molt

Survival (S) was estimated as the percentage of animal remaining, excluding replacements at the end of the experiment for each treatment:

$$S = (Nf\ Ni^{-1}) \times 100$$

where: Nf - final number of marron
Ni - initial number of marron

Data was processed with SPSS v 18. Initial weight and orbital carapace length of marron in each tank was analyzed using one way ANOVA. Differences among treatments were analyzed using two-way ANOVA. Subsequent analysis using Tukey HSD test was conducted when there are significant differences among treatments. The level of significance is $P < 0.05$.

Results and Discussion. During the the experiment, water pH value average for the three temperature treatments (25, 22, 19°C) were 8.45 ± 0.1, 8.33 ± 0.1 and 8.22 ± 0.1, respectively with the corresponding average temperatures 25.08 ± 0.1, 22.04 ± 0.1 and 18.95 ± 0.1°C (mean ± SE). Nitrite level was <0.1 mg L^{-1} while ammonia was undetectable during the experiment. The water condition in this experiment in relation to temperature, pH and ammonia are suitable for growth of marron. The temperature range chosen is expected to cover the thermal optimum for growth of marron. According to Morrissy (1990), over 50% of maximum growth of marron can be achieved when water temperature ranged from 17°C to 27°C. pH has an important role in the uptake of calcium, and thus affects calcification. Low pH conditions have been reported to inhibit carapace mineralization and growth of freshwater crayfish (Aiken & Waddy 1992). Ammonia is toxic to freshwater crayfish in the gaseous form. To minimize inhibition of growth of crayfish, unionized ammonia should be less than 0.01 mg L^{-1} (Lourey & Mitchel 1995).

Average initial and final weight for each treatment ranged from 3.84 ± 0.20 g (long day, 22°C) to 4.91 ± 0.31 g (long day, 19°C) and from 6.77 ± 0.63 g (long day, 19°C) to 8.21 ± 0.85 g (short day, 22°C) respectively (Table 1). Mean initial orbital carapace length for the treatment group ranged from 17.82 ± 0.32 mm (long day, 22°C) to 18.66 ± 0.34 (long day, 19°C) while its final from 20.29 ± 0.44 mm (long day, 19°C) to 21.45 ± 0.62 mm (short day, 25°C) (Table 1). Initial and final weight as well as orbital carapace length of marron among tanks were not significantly different ($P > 0.05$).

Table 1

Initial and final sizes of marron *Cherax tenuimanus*

Treatment	*Weight (g)*		*Orbital carapace length (mm)*	
	Initial	*Final*	*Initial*	*Final*
Long day, 25°C	4.10 ± 0.22	7.11 ± 0.71	18.04 ± 0.36	20.98 ± 0.56
Long day, 22°C	3.84 ± 0.20	7.10 ± 0.63	17.82 ± 0.32	21.01 ± 0.55
Long day, 19°C	4.91 ± 0.31	6.77 ± 0.63	18.66 ± 0.34	20.29 ± 0.44
Short day, 25°C	4.31 ± 0.26	8.00 ± 0.97	18.17 ± 0.32	21.45 ± 0.62
Short day, 22°C	4.54 ± 0.21	8.21 ± 0.85	18.64 ± 0.32	20.91 ± 0.47
Short day, 19°C	4.89 ± 0.36	6.89 ± 0.71	18.53 ± 0.36	20.33 ± 0.41

Values are mean ± SE.

One hundred and ten individuals (67.9%) marron molted during the experiment with the total number of molting were 146 (Table 2). Thirty two of those individuals molted two (16.7%) or three (3.1%) times.

Table 2

Total number of molt of marron *Cherax tenuimanus*

Photoperiod	*Temperature (°C)*			*Total*
	25	22	19	
Long day	28 (1.04 ± 0.13)	33 (1.22 ± 0.15)	17 (0.63 ± 0.12)	78 (0.96 ± 0.08)
Short day	30 (1.11 ± 0.15)	24 (0.89 ± 0.16)	14 (0.52 ± 0.12)	68 (0.84 ± 0.09)
Total	58 (1.07 ± 0.10)	57 (1.06 ± 0.11)	31 (0.57 ± 0.09)	146 (0.90 ± 0.06)

Values in bracket are mean frequency ± SE.

There was highly significant effect of the three temperature treatments ($P < 0.001$) on number of molt whereas photoperiod ($P > 0.28$) or interaction between photoperiod and temperature ($p > 0.35$) had no significant effect. Post-hoc test showed significant differences between pairwise of 19 and 22°C, and 19 and 25°C, but not between 22 and 25°C. The total number of molt by temperature treatments ranged from 31 molts (mean frequency 0.57 ± 0.09) at 19°C to 58 molts (mean frequency 1.07 ± 0.10) at 25°C (Table 2). Overall mean frequency of the number of molt was 0.90 ± 0.06 (n=146). The number of molt in crayfish appears to vary according to temperature and availability of food (Lowery 1988). Marron kept at higher temperature in this experiment fed more actively and consumed higher quantities of food than those kept at lower temperature. The result in the present study is similar to the previous studies on marrons (Rousse & Kartamulia 1992) and other crayfish (Verhoef & Austin 1998; Kozak et al 2009). According to Rousse & Kartamulia (1992) higher temperature resulted on average of higher number of molts compare to those growths at lower temperatures. Mean frequency of molt in the present study (0.90) is higher than those observed by Morrissy (1990) i.e. 0.5 and Rousse & Kartamulia (1992) i.e. 0.59. This difference could be caused by the size/age of the animal and length of experiment. Morrissy (1990) conducted his trial for 50 days while Rousse & Kartamulia (1992) for six weeks only.

Molt increment of individual marron during the experiment varied from 11.23 to 50.51% with the corresponding average for each treatment ranged from 19.93 ± 2.02% (long day, 19°C) to 32.10 ± 2.19% (long day, 25°C) (Table 3).

Table 3

Molt increment (%) of marron *Cherax tenuimanus*

Photoperiod	*Temperature (°C)*			*Average*
	25	22	19	
Long day	29.19 ± 2.01	28.72 ± 1.67	19.93 ± 2.02	26.70 ± 1.20
Short day	32.10 ± 2.19	30.08 ± 1.62	24.41 ± 2.69	29.57 ± 1.30
Average	30.58 ± 1.48	29.31 ± 1.68	21.92 ± 1.66	-

Values are mean ± SE.

Highly significant differences were found among the three temperatures levels ($P < 0.01$), but not between photoperiods ($P > 0.09$) or interaction between temperature and photoperiod ($P > 0.77$). Post-hoc test showed significant difference of molt increment occurred between pairwise comparison of 19 and 25°C, and 19 and 22°C but not between 22 and 25°C. Weight gain at molt in the present study was comparable to the result of Jussila & Evans (1996) but lower than weight gain observed by Morrissy (1990). Jussila & Evans (1996) reported 23.4–32.1% of weight gain of juvenile marron at 22-24°C while Morrissy (1990) observed a 52% molt increment. The higher molt increment

of Morrissy (1990) could be attributed to individual reared condition in the trial. According to Aiken & Waddy (1992), communal rearing may increase social interaction and frequent encounter that could cause stress resulting in slower growth.

Average specific growth rate of marron ranged from 0.25 ± 0.03% (long day, 19°C) to 0.63 ± 0.08% (short day, 22°C) (Table 4).

Table 4

Specific growth rate (% day^{-1}) of marron *Cherax tenuimanus*

Photoperiod	*Temperature (°C)*			*Average*
	25	*22*	*19*	
Long day	0.57 ± 0.05	0.59 ± 0.05	0.26 ± 0.03	0.45 ± 0.03
Short day	0.61 ± 0.08	0.63 ± 0.08	0.25 ± 0.05	0.48 ± 0.05
Average	0.59 ± 0.05	0.61 ± 0.05	0.25 ± 0.03	-

Values are mean ± SE.

Highly significant differences were found among the three temperature levels ($P < 0.001$), but not between photoperiods ($P > 0.58$) or interaction between temperature and photoperiod ($P > 0.89$). Subsequent analysis using Tukey's HSD test indicated significant differences in specific growth rate between the pairwise comparisons of 19 and 22°C, and 19 and 25°C, but not between 22 and 25°C. Average specific growth rate at 22 and 25°C was more than twice than at 19°C. According to Reynolds (2002), growth rate of crayfish is a product of molt increment and frequency of molt. Since marron at higher temperature molt more frequent than those at lower temperature, it is not surprising that their specific growth rate is higher. Specific growth rate in this study were comparable to those reported by other researchers on marron. Morrissy et al (1995) observed specific growth rate ranged from 0.4 to 0.5 while Jussila & Evans (1998) reported a ranged from 0.20 to 0.68.

Average intermolt period of marron during the experiment ranged from 28.25 ± 4.34 days (short day, 25°C) to 52.50 ± 0.50 days (long day, 19°C) (Table 5). Overall mean of intermolt period was 35.32 ± 1.81 days (n=32).

Table 5

Intermolt period (day) of marron *Cherax tenuimanus*

Photoperiod	*Temperature (°C)*			*Average*
	25	22	19	
Long day	35.25 ± 1.65	37.70 ± 3.01	52.50 ± 0.50	38.94 ± 2.32
Short day	28.25 ± 4.34	36.50 ± 2.31	38.50 ± 7.50	32.63 ± 2.61
Average	30.58 ± 3.04	37.25 ± 2.02	45.50 ± 5.07	-

Values are mean ± SE.

Significant difference was evident among temperature treatments but not between photoperiods. Tukey's test revealed significant differences in intermolt period between pairwise comparisons of 25 and 19°C but not between 25 and 22°C as well as between 22 and 19°C. Morrissy (1976) reported that no molt occurred over a period of four months when water temperature was less than 12°C for marron weighing 30–50 g. However, when marron at the same weight is held at 16-20°C, their intermoult period ranged from 2 to 3 months (Morrissy 1979). Westin & Gydemo (1986) showed that low temperature affected the delay but not the inhibition of molting. Jussila & Evans (1996, 1998) observed intermolt period between 32 and 62 days for juvenile marron held at 22–24°C. For other crayfish, *Paranephros zealandicus,* Hammond et al (2006) reported a reduction for greater than 90 days at 14° to 40 days at 20-22°C. Increasing water temperature tends to induce molting by reducing intermolt period (Morrissy 1990; Hammond et al 2006). The authors suggested that rise in temperature increases metabolic rate and food intake of the animals making them ready to molt.

Seventy three (66%) of molted marrons were survived the experimental period. Most of the molted marron (81%) died after the first molt. Average molting success (survival of molted animals) rate among treatment ranged from 52 ± 10.6% (long day, 25°C) to 87 ± 9.1% (long day, 19°C) (Table 6). There were no significant effect of temperature ($P > 0.11$) and photoperiod ($P > 0.53$) on molting success of marron. The finding in the present study is similar with those reported by Rouse & Kartamulia (1992). Molting is critical time in the life of crayfish. Recently molted marron is vulnerable because its new exoskeleton is still soft and easy to be broken. In addition, because newly molted marron can move only slowly, it is very vulnerable to be attacked by others. Even though marrons are not aggressive like yabbies, they are cannibalistic and thus the weak and injury molted marron are the easy target for other individuals.

Table 6

Molting success (%) of marron *Cherax tenuimanus*

Photoperiod	*Temperature (°C)*			*Average*
	25	*22*	*19*	
Long day	52.0 ± 10.6	73.0 ± 9.7	87.0 ± 9.1	68.0 ± 6.1
Short day	57.0 ± 11.1	71.0 ± 11.4	67.0 ± 14.2	64.0 ± 6.9
Average	55.0 ± 7.6	72.0 ± 7.3	78.0 ± 8.2	-

Values are mean ± SE.

Survival of marron by treatment ranged from 48 ± 9.8% (long and short days, 25°C) to 81 ± 7.6% (long day, 19°C) (Table 7). Temperature had significant effect ($P < 0.02$) on survival of marron while photoperiod ($P > 0.32$) and interaction between temperature and photoperiod ($P> 0.72$) had no effect. Tukey's HSD test showed significant difference in survival between pairwise comparisons of 25°C and 19°C but not between 25°C and 22°C as well as between 22°C and 19°C. Rouse & Kartamulia (1992) showed that there was significant and negative effect of temperature on survival and concluded that the best temperature for survival of marron were 22°C and below. Survival of marron at 25°C in the present study was higher than the survival of marron held at 24°C (27.8 ± 13.7%) reported by Rouse & Kartamulia (1992) but lower than those reported by Jussila & Evans (1998) i.e. 88.9–100% survival for juvenile marron reared at 22-24°C. Lower survival in the present study compared to the result of Jussila & Evans (1998) may be due to differences in density i.e. 75 individuals m^{-2} in this study compared to 5–25 individuals m^{-2}. Verhoef & Austin (1998) stated that during molting in communal rearing, the higher density, the more vulnerable are crayfish to cannibalism. Because elevated temperature tended to increase molting frequency, the chances of mortality of newly molted marron by cannibalism may increase too and thus reduced survival significantly.

Table 7

Survival (%) of marron *Cherax tenuimanus*

Photoperiod	*Temperature (°C)*			*Average*
	25	22	19	
Long day	48.0 ± 9.8	70.0 ± 9.0	81.0 ± 7.6	67.0 ± 5.3
Short day	48.0 ± 9.8	63.0 ± 9.5	67.0 ± 9.2	59.0 ± 5.5
Average	48.0 ± 6.9	67.0 ± 6.5	74.0 ± 6.0	-

Values are mean ± SE.

In the present study, photoperiod did not show any significant effect on growth, molting and survival of marron. Similar results were observed in a previous study by Sáez-Royuela et al (1996) who reported no significant effect of photoperiod on growth and survival of juvenile signal crayfish (*Pacifastacus leniusculus*).

Conclusions. The present study reveals that temperature significantly affected growth, molting and survival of marron (*C. Tenuimanus*) whilst photoperiod as well as

combination of temperature and photoperiod had no effect on those parameters. High temperature improved marron growth but reduced survival rate. At higher temperature, specific growth rate and molt increment as well as number of molt were higher and intermolt period was shorter. Contrarily, survival was higher at lower temperature. The results of this study indicate that the best temperature range is needed to optimize growth, molting and survival in order to improve production of marron.

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