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Survival Rate and Growth of *Palaemon gravieri* Larvae Reared in the Laboratory (Decapoda: Caridea: Palaemonidae)

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The larvae of *Palaemon gravieri* were reared in the laboratory at three different temperature regimes (15°C, 20°C, and 25°C) with the salinity ranges (28-32 psu) to understand how temperature and body size influence survival rate, and growth components (molt increment and intermolt period). The optimum temperature for the highest survival rate was 25°C. The intermolt periods consistently increased with an increase in size and instar number; however, the molt increments at successive instars generally decreased with an increase in size and instar number. The shortest intermolt period and the highest larval growth rate both occurred at 25°C. Thus, the optimum temperature for larval survival and growth rate was found to be 25°C which was the temperature at which the larvae actually appear in nature.

Key words: *Palaemon gravieri*, Larvae, Molt increment, Intermolt period

Introduction

Larval growth in crustaceans is a discontinuous process through the molt of their exoskeletons (Hartnoll, 1982). Thus, a stepwise growth pattern is obtained, reflecting the sudden increase in body size immediately after each ecdysis. In practice it is difficult to measure larval growth of crustacean in nature. Thus, much less is known about larval growth pattern (Rice, 1968; Gore, 1985; Anger, 1991; Anger and Moreira, 1998), and growth is commonly estimated by indirect methods, such as laboratory rearing experiments (Lebour, 1927; Costlow and Bookout, 1959; Costlow et al., 1960; Hartnoll and Mohamedeen, 1987). Crustacean growth has two components, the intermolt period and the molt increment. These are influenced by external factors, such as temperature, salinity, and food availability, and internal factors, such as size (Rothlisberg, 1979; Hartnoll, 1982; Oh and Hartnoll, 2000). The effect of size on growth was first reported by Olmstead and Baumberger (1923) using grapsid crabs. They observed that, as the crab grew after molting, the molt increment gradually decreased. Many subsequent crustacean growth studies confirmed their observations (Hartnoll and Mohamedeen, 1987; Oh and Hartnoll, 2000). Size also has an effect on the intermolt period. As size increases in decapod crustaceans, the intermolt period generally also increases (Hartnoll, 1982). Lebour (1927) described the early stages of larval development, and the environmental factors affecting larval development were investigated later by rearing individual larvae (Costlow and Bookout, 1959; Costlow et al., 1960). Experiments have been conducted with decapod larvae reared under laboratory conditions to observe the relationship between growth components and internal and external factors (Rothlisberg, 1979; Hartnoll and Mohamedeen, 1987).

Many crustaceans undergo a planktonic period in their early life stage (Sastry, 1983). Growth in this stage is thought to affect many life history traits, such as onset of sexual maturity and growth potential of the adult. Thus, measuring larval growth at the early life stage is very important for understanding subsequent life history traits. In nature, temperature is one of the most important factors known to signifycantly influence decapod larval survival and growth, and many studies have investigated its effect (Hartnoll and Mohamedeen, 1987; Mohamedeen and Hartnoll, 1989). The results confirm the hypothesis that, in most decapod larvae, the molt increment increases as the temperature rises, and the intermolt period shortens; hence, the growth rate increases (Hartnoll and Mohamedeen, 1987; Mohamedeen and Hartnoll, 1989). However, the effects on the molt

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increment vary depending on the species (Hartnoll and Mohamedeen, 1987; Mohamedeen and Hartnoll, 1989).

To understand the effects of internal and external factors on larval growth, a single species should be investigated in the laboratory. The aim of this study was to determine the survival rate of *P. gravieri* larvae and to test how temperature, size, and instar number affect growth (molt increment and intermolt period).

Materials and Methods

Ovigerous females *P. gravieri* were collected around coastal areas of Namhae, Korea, in May, 2000. The temperature and salinity at the sampling site were 20°C and 30 psu, respectively. Samples were transferred to the laboratory and were kept at temperature and salinity of 20°C, 28-32 psu at 12:12 (L:D) for an adaptation period. They were then maintained at three different temperature regimes (15°C, 20°C, and 25°C) in a salinity range (28-32 psu) and 12 h:12 h light:dark cycle. Artificial foods, such as chopped clams and squids, were supplied everyday. Seawater in the container was exchanged every other day, and detritus and excreted materials from the shrimp were siphoned off. Fifty newly hatched larvae were transferred to individual glass bottles for culture. The seawater in the glass bottles was exchanged with filtered seawater everyday. The larvae were fed with newly hatched *Artemia nauplii*.

The carapace length (CL) from the exuviae at molt was measured under a microscope, and the molt increment was calculated with measurement of carapace length of exuviae. In addition to that, The intermolt period was measured during the larval developmental period (from zoea 1 stage to post-larvae) as the daily unit between molts. The molt increment was calculated as: molt increment (%) = [(CL at post-molt−CL at pre-molt)/CL at pre-molt]×100. The growth rate was calculated by daily growth rate between mean intermolt periods. Regression equation was produced to show how instar and carapace length influenced the growth components, intermolt period and molt increment. All regression equations showed the highest goodness of fit. The intermolt period and molt increment values were log transformed owing to the non-homogeneity of their variance. Linear regression equations describing the three different temperature regimes were calculated after performing regression analysis among the intermolt period, molt increment, instar, and carapace length. ANCOVA was performed to obtain the difference between the slopes in the regression equations for the intermolt period and molt increment (Zar, 1996). The intercept of the regression equation was compared if there was no significant difference between the slopes.

Results

Survival rate

The survival rate of the larvae was highest at 25°C (Fig. 1A). The larvae reared at 15°C had a high mortality rate beginning with the early instars, and all of the larvae had died by instar 6. The survival rates of larvae reared at 20°C and 25°C were 96-100% and 100%, respectively, until instar 2. The mortality of the larvae reared at 20°C began to increase at instar 7, later than at other temperature regimes. Some larvae from the early instar survived at 20°C until the post-larval stage. The temporal survival rate of larvae at

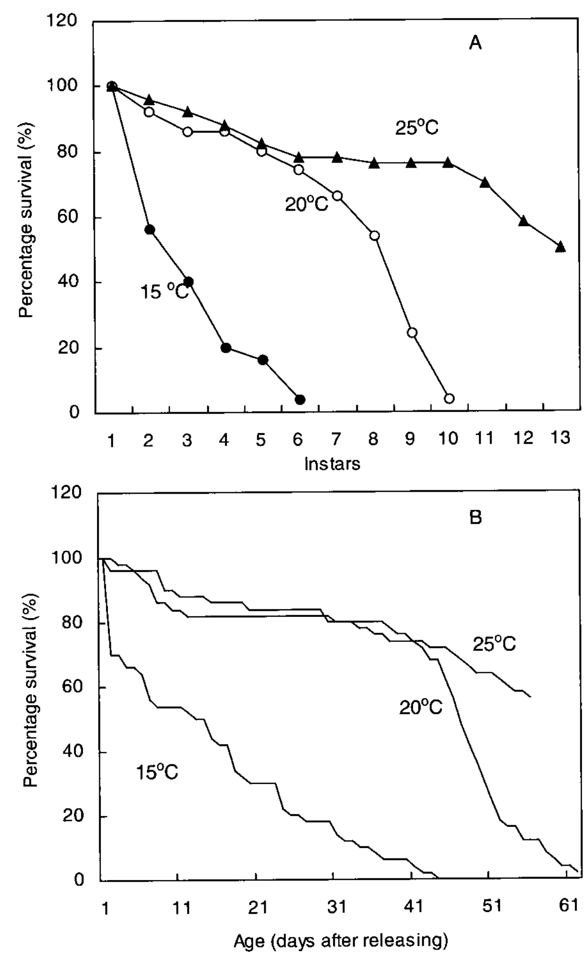

Fig. 1. *Palaemon gravieri.* Survival rate (%) per instar of the larvae reared at 15°C, 20°C, and 25°C (A); Survival rate (%) per age at 15°C, 20°C, and 25°C (B).

15°C rapidly decreased from 70% survival at 2 days (Fig. 1B). At 20°C and 25°C, the initial survival rates of 96% and 100%, respectively, which were higher than at 15°C. Survival rate was gradually decreased as time elapsed. The mortality of larvae reared at 20°C began to increase after 45 days. Survival rate of the larvae was 62% and all larvae died by 61 days. But the larvae reared at 25°C was higher than those of the larvae reared at other temperature regimes. As time went, survival rate was 56% by post larvae.

Intermolt period (IP) and molt increment (MI)

At 15°C, the intermolt period lasted 7-11 days for instars 1 to 5 (average, 9 days); at 20°C, the intermolt period was 4-10 days for instars 1 to 9; at 25°C, it was 3-7 days for instars 1 to 13 (average, 5 days) (Fig. 2A). The longest intermolt period occurred at the lowest temperature (15°C), while the shortest intermolt period occurred at the highest temperature (25°C). For the relationship between instar number and carapace length, the molt increments per instar were 16.32%, 11.31%, 11.29%, 9.05%, and 6.25% at 15°C; 14.57%, 12.23%, 11.63%, 11.09%, and 10.08% at 20°C; and 32.57%, 24.09%, 16.69%, 10.38%, and 9.58% at 25°C (Fig. 2B). As the instar number increased, the molt increment decreased by 10% at 15°C. At 20°C, the molt increments were 14.57% for instar 1 and 9.58% for instar 9, and the standard deviations were 10.52% and 4.12%, respectively. The molt increments ranged from 32.57% for instar 1 to 2.35% for instar 12; the standard deviation was 16.78, and it gradually decreased by 0.99%. Of the three different temperature regimes, 25°C produced the maximum molt increment.

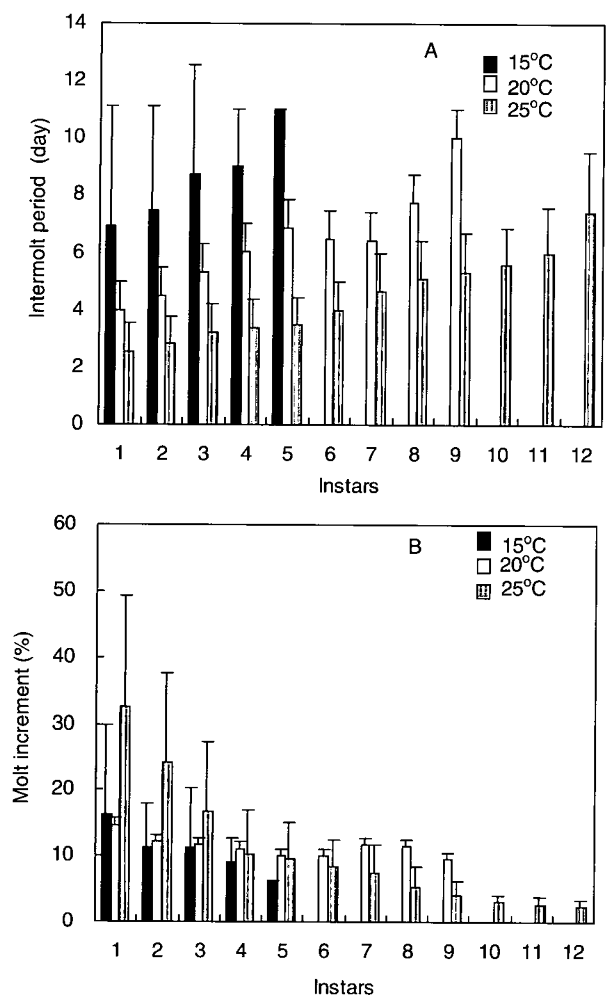

Fig. 2. *Palaemon gravieri.* Intermolt period plotted against instar number (A) and molt increment plotted against instar number (B) for larvae reared at 15°C, 20°C, and 25°C.

The regression relationships between the intermolt period and the instar number (Fig. 3A) at the three different temperatures are shown in the logarithmically transformed linear equations below:

15°C: $\ln IP = 0.1116IT + 1.8066$
($r^2 = 0.96$, $n = 5$, $p < 0.01$),
20°C: $\ln IP = 0.09621IT + 1.3354$
($r^2 = 0.90$, $n = 9$, $p < 0.05$), and
25°C: $\ln IP = 0.0906IT + 0.855$
($r^2 = 0.98$, $n = 12$, $p < 0.05$).

These regression equations show high deterministic coefficients ($r^2 > 0.9$) and strong linear regression relationships. At 15°C, 20°C, 25°C, the slopes of the linear regressions showed significant positive relationships, indicating that the intermolt period gradually increases as the instar number increases. ANCOVA found no significant difference among the slopes of the regression lines for the instar number and the intermolt period. This indicates that the intermolt period decreases as the instar number increases with a temperature increase.

The regression equations (Fig. 3B) below were obtained after regression analyses of the carapace length and the intermolt period:

15°C: $\ln IP = 3.0293CL + 0.8303$
($r^2 = 0.93$, $n = 5$, $p < 0.05$),
20°C: $\ln IP = 1.3588CL + 0.7607$
($r^2 = 0.92$, $n = 9$, $p < 0.05$), and
25°C: $\ln IP = 1.1029CL + 0.3115$
($r^2 = 0.93$, $n = 12$, $p < 0.05$).

The highly significant and positive regression relationships given by these equations indicate that the intermolt period increases as the carapace length

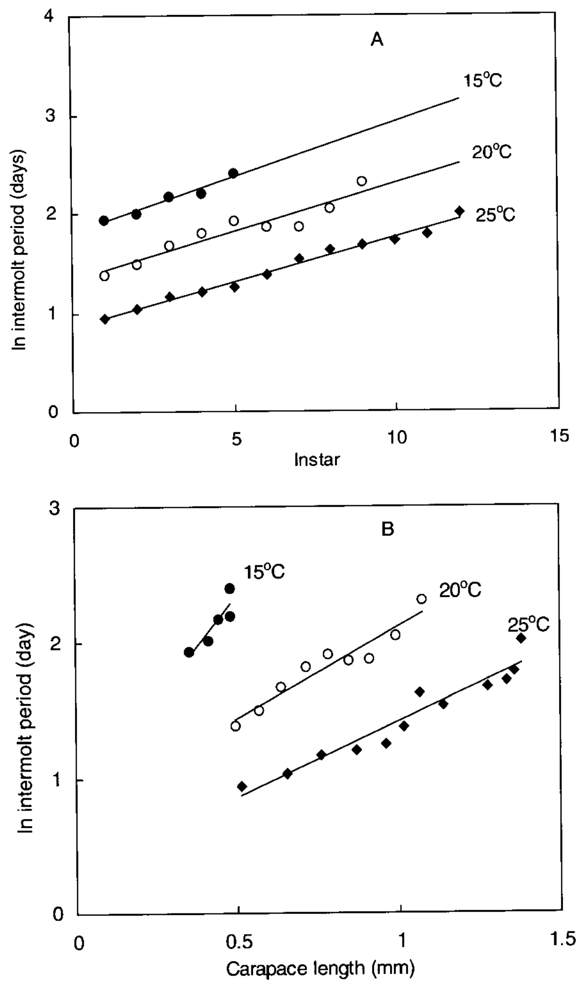

Fig. 3. *Palaemon gravieri.* Regression relationship between log intermolt period and instar (A) and between log intermolt period and carapace length (mm) (B).

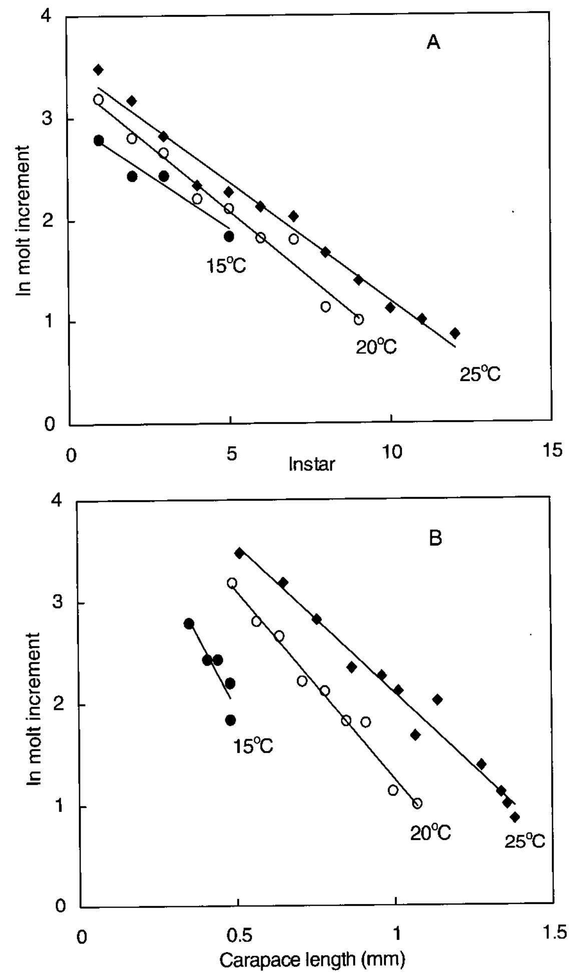

Fig. 4. *Palaemon gravieri.* Regression relationship between log molt increment and instar (A) and log molt increment and carapace length (mm) (B).

increases. An ANCOVA for the slopes of the intermolt period revealed no significant differences among the slopes, but a comparison of intercepts among the three temperature regimes revealed a significant difference. This result indicates that the average intermolt period decreases at high temperatures and that the intermolt period increases as carapace length increases.

The logarithmically transformed regression equations (Fig. 4A) below demonstrate the linear relationships between instar number and molt increment:

15°C: lnMI = -0.2343IT + 3.5438
($r^2 = 0.98$, n = 5, $p < 0.05$),
20°C: lnMI = -0.2143IT + 2.9783
($r^2 = 0.93$, n = 9, $p < 0.05$), and
25°C: lnMI = -0.2641IT + 3.4
($r^2 = 0.97$, n = 12, $p < 0.05$).

The slopes of the linear regressions for all three temperature regimes showed significant negative values, which implies that the molt increment tends to decrease as the instar number increases (Fig. 4A). After ANCOVA on difference of the slopes in the linear regression of the instar number and molt increment among the three different temperatures there was a significant difference (ANCOVA, $p < 0.001$).

The logarithmically transformed equations (Fig. 4B) below show the linear relationship between carapace length and molt increment:

15°C: lnMI = -5.9517CL + 4.9112
($r^2 = 0.81$, n = 5, $p < 0.05$),
20°C: lnMI = -3.7061CL + 4.9663
($r^2 = 0.98$, n = 9, $p < 0.05$), and
25°C: lnMI = -2.9214CL + 5.021
($r^2 = 0.97$, n = 9, $p < 0.05$).

The regression equations between carapace length and molt increment showed significant negative relationships, similar to the regression analyses between instar and molt increment. The ANCOVA result for the differences between the slopes in the regression equations of carapace length and molt increment showed that, as carapace length increases at all three temperatures, molt increment decreases, but the decreasing rate exhibits no constant tendency among three different temperatures.

The regression equations (Fig. 5) below showed significantly positive linear regression relationships between the instar number and carapace length at three different temperatures:

15°C: CL = 0.0295IT + 0.3424
($r^2 = 0.93$, n = 6, $p < 0.05$),
20°C: CL = 0.0744IT + 0.411
($r^2 = 0.99$, n = 10, $p < 0.001$), and
25°C: CL = 0.0741IT + 0.5377
($r^2 = 0.96$, n = 13, $p < 0.001$).

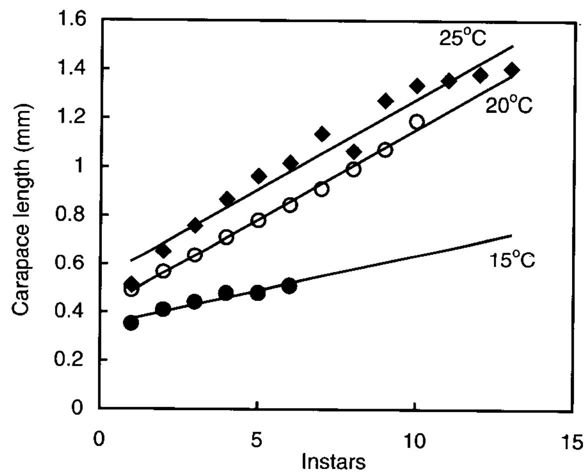

Fig. 5. *Palaemon gravieri.* Regression relationship between carapace length (mm) and instar.

Growth rate

The growth rate was calculated on the basis of growth components such as molt increment and intermolt period. Daily larval growth showed that daily larval growth rate was highest at 25°C and lowest at 15°C (Fig. 6). The highest growth rate for the intermolt period (0.0087 mm/day) occurred between day 1 and 6; the lowest growth rate (0.0001 mm/day) occurred between days 13 and 20. Larvae reared at 20°C survived for 60 days, and the growth rate during this period was 0.0199 mm/day, with the lowest growth rate (0.0090 mm/day) occurring between days 27 and 34. The first zoea grew to the post-larval stage in 56 days. The period showing the highest growth rate (0.0544 mm/day) was from day 1 to day 3; the lowest growth rate (0.0031 mm/day) occurred between days 49 and 56.

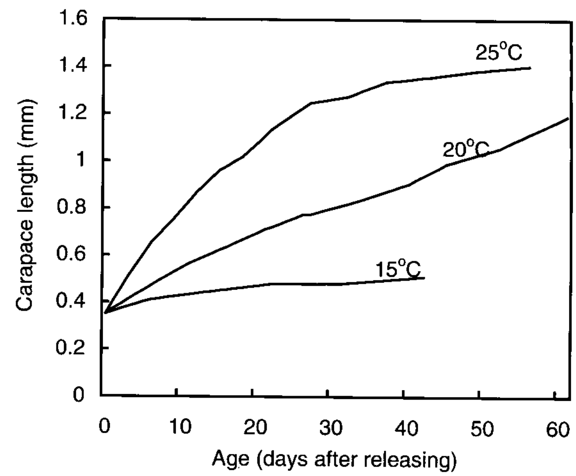

Fig. 6. *Palaemon gravieri.* Daily growth rate.

Discussion

Survival rate

To estimate the survival rate requires distinguishing the difference between survival rate related with time and that for the period reaching specific instar. From a species perspective, the number of larvae reaching the post-larval stage successfully is the most important measure of recruitment success. In general, internal (size) and external factors (temperature) have the most influence on the larval survival rate (Hartnoll, 1982; Sastry, 1983). Most larvae are hypothesized to have evolved to favor an increased survival rate and thus are thought to be most abundant when larval food sources, such as phytoplankton, are sufficiently available for their survival (Thorson, 1950; Bauer, 1992). In the warm temperate environment, spring is the period of phytoplankton blooms, and water temperature gradually increases to the optimal level for larval survival. In this study, *P. gravieri* larvae occurred from May to July at the sampling site, at which water temperatures ranged between 20°C and 25°C (Kim and Hong, 2004).

Those temperature ranges were similar to those shown in th result of the experiments for the larvae reared in the laboratory for the survival. Similar results were obtained by Rochanaburanon and Williamson (1976). They reported higher survival rates for *Crangon crangon, Palaemon elegans,* and *Processa nouveli* reared at 20°C than for those reared at 15°C. A similar result was also shown for the crabs

Inachus dorsettensis and *Pilumnus hirtellus* (Hartnoll and Mohamedeen, 1987). In the case of *Palaemon serratus*, the temperature range for optimal survival was between 22°C and 26°C (Reeve, 1969), which indicates that more larvae seem to survive in laboratory conditions for temperature and other external factors than in the natural environment. The optimal temperature range in *P. gravieri* larvae for maximum survival was much overlapped with that for the larvae of other species.

Intermolt period, molt increment, and growth rate

This study yielded two consistent results on intermolt period. First, intermolt period increases as size and number of instar stages increase. Second, the intermolt period was longest at the lowest temperature (15°C) and shortest at the highest temperature (25°C). The intermolt period increased steadily with the pre-molt size at all temperatures, which is typical of crustacean growth (Hartnoll, 1982). The longer intermolt period reflects the longer time needed for the larvae to accumulate sufficient nutrition (Mauchline, 1976, 1977; Hartnoll, 1982). The results of this study show that the intermolt period of larvae is influenced by temperature and size.

Intermolt period consistently increased with size at all three different temperatures, whereas the molt increment decreased with instar number and size Molt increment was high at the highest temperature (25°C), which is inconsistent with the intermolt period. Hartnoll (1982) suggested that temperature influences molt increment in many crustaceans. Temperature is known to have a greater effect on molt increment than on intermolt period (Oh and Hartnoll, 2000). In this study, molt increment increased per instar and carapace length with a temperature increase. On the other hand, in several species (*Orconectes limnosis, Carcinus maenas,* and *Phronima sedentaria*) the temperature did not influence the molt increment within the range of different temperature regimes (Kracht, 1974; Larval, 1975). Other studies have shown that the molt increment increases as temperature increases in *Eriocheir sinensis* (Leersnyder, 1972) and *Rhithropanopeus harrisii* (Hartnoll, 1978) at the temperature ranges of 15-20°C and 20-30°C, respectively. These findings are consistent with the molt increment pattern of *P. gravieri* larvae. In conclusion, the growth rate determined by growth components was found to be contingent on change in the water temperature, which is supported by studies on the growth and survival rates of other Palaemonid species such as *P. elegans* and *P. serratus* (Salama and Hartnoll, 1992; Reeve, 1969; Forster, 1973).

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