Studies on the Molting in the Freshwater Prawn, *Palaemon paucidens*

I. Some Endogenous and Exogenous Factors Influencing the Intermolt Cycle[1)]

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(With 2 Text-figures and 3 Tables)

In decapod crustaceans, as in other arthropods, growth takes place as the animals shed their old exoskeleton. Various environmental conditions and endogenous physiological factors have been shown to affect the molting; the temperature (Templeman, 1936; Travis, 1954; Passano, 1960a; Bliss and Boyer, 1964), light (Stephens, 1955; Bliss and Boyer, 1964), body size (Hess, 1941; Travis, 1954) and egg-carrying (Hess, 1941; Scudamore, 1948), among others.

In the last two decades it has been demonstrated that the molting in decapod crustaceans is regulated by endocrine factors. The Y-organ was assumed to be the source of the molting hormone which initiates proecdysis (Gabe, 1953; Echalier, 1954) and the molt-inhibiting hormone from the X organ-sinus gland complex was shown to control molting indirectly by inhibiting the Y-organ to produce the molting hormone (Carlisle, 1957; Passano and Jyssum, 1963). More recently, some steroids have been found to possess high molting hormone activity in arthropods. Thus, crustecdysone (=ecdysterone) was effective in initiating molt in various crustacean species (Krishnakumaran and Schneiderman, 1969 in the isopod, *Armadillidium vulgare*; Fukutake *et al.*, 1969 in the crayfish, *Procambarus clarkii*; Matsumoto and Fukutake, 1970 in the marsh crab, *Sesarma intermedia*). However, the origin and controlling mechanism of this substance and its analogues are not completely understood. Then, it may be important to investigate the influence of various environmental and endogenous factors on the molting.

The present study deals with the molting of the freshwater prawn, *Palaemon paucidens*, and its changes under various conditions.

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Materials and Methods

Mature and immature prawns were collected in a stream near Sapporo. The animals were brought to the laboratory and stocked in aerated aquaria. They were fed with small cut pieces of boiled fish paste ("kamaboko") on every other day and the water was renewed on every fourth day. As the laboratory condition was thought to be very much different from that of their natural habitat, all the prawns employed in this study were allowed to acclimatize themselves to the new environment for at least one week before use. For observation, each animal was maintained singly in an aerated glass container (15 cm in diameter and 7 cm in height) covered with a glass plate. For experiments of the effects of light and temperature on the intermolt duration, however, 30–60 prawns were placed together in a large aerated aquarium ($21 \times 36 \times 60$ cm). In the latter case, each specimen was marked with a piece of cotton thread of different colors tied on the rostrum, and the exact dates of more than two successive molts of each animal were recorded. Size of the animals was represented as a carapace length measured from a posterior edge of the orbit to the most posterior point of the carapace. Intermolt stage of each specimen was determined under a dissecting microscope according to the method previously described by Kamiguchi (1968), in which each stage was set based on the morphological characteristics of the setae on the antennal scale.

Observations and Experiments

I. Seasonal variations of the intermolt duration

Four-hundred prawns, ranging 10–15 mm in carapace length, were kept in individual glass containers and their intermolt duration was recorded during the period from October of 1967 to October of 1968. The results are summarized in Fig. 1. Here, the intermolt duration of each specimen was plotted to the month in which the middle day between the two successive molts was included; for instance, the prawn that had molted in October 19 and successively in November 18 was plotted in November 3 with the intermolt duration of 30 days.

During the summer months from July to August, the intermolt duration was the shortest (20.2 days on an average) and the fluctuation among the animals was the least. On the contrary, during the winter months from December to February the intermolt duration was the longest (56.1 days on an average). Fluctuation of the value was also the greatest during the winter months, the duration being 32 days in the minimum and 121 days in the maximum.

The physical conditions in the laboratory were so different between the two seasons: the average water temperature was 22–22.5°C in summer and 13.2–14.2°C in winter, and the day length was 14–15 hours in summer and 9–10 hours in winter. To examine the possible effects of temperature and light on the intermolt duration, the following experiments were carried out.

II. Experiments on the effect of environmental factors on the intermolt cycle

a) Light. Effect of the light condition on the intermolt duration was studied in March, using the prawns of 12–15 mm in carapace length regardless of the

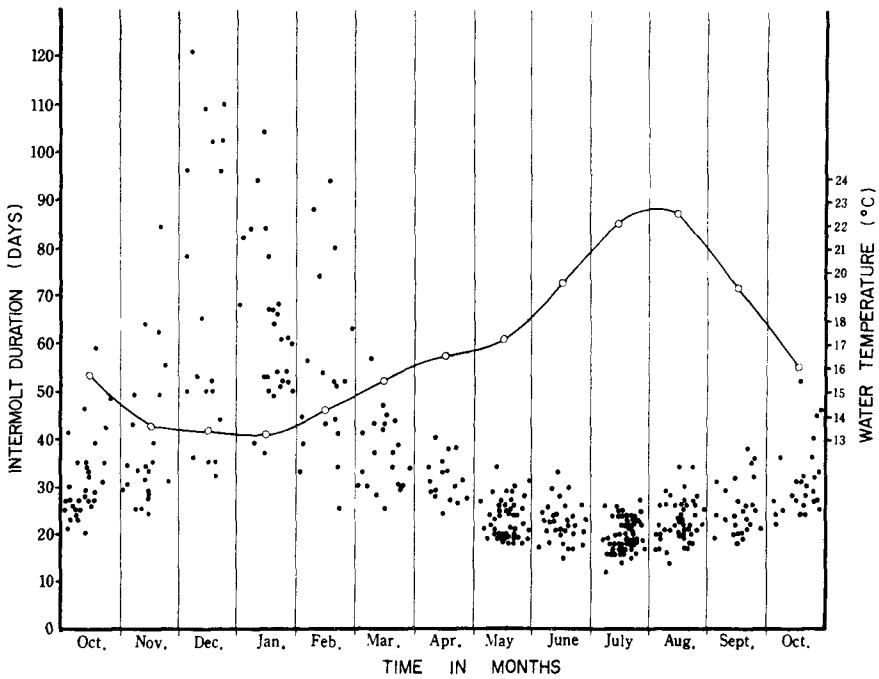

Fig. 1. Seasonal variation of the intermolt duration in the freshwater prawn, *Palaemon paucidens*. Open circles, average monthly water temperature in aquaria; dots, intermolt duration in days of each animal.

sexes. Four experimental groups of 30 animals each were exposed to different light conditions: the constant light, constant darkness, long-day (16L:8D) and short-day (8L:16D) conditions. Thirty control animals were subjected to normal day-night condition (11.13L:12.47D-12.12L:11.48D) throughout the experiment which extended for 30 days. Water temperature was kept at 24°C. For experimental groups artificial photoperiod was provided with a fluorescent lamp (20W) hung above the aquarium, with the light intensity of about 800 lux. Daily inspection, feeding and water exchange obliged the animals in "constant darkness" to the exposure to light for about 10 minutes every day. The results are given in Table 1. Apparently, there was no significant difference in intermolt duration among the five groups.

b) *Temperature.* In order to test the possible effects of temperature on the molting, 360 prawns ranging 12–15 mm in carapace length were divided into six groups of 60 each, and they were placed in aquaria maintained at 10°C, 16°C, 20°C, 24°C, 28°C, and 32°C, respectively. The time required for the two successive molt was measured. The prawns that had been kept at 10°C or cooler did not molt at

Table 1. Intermolt duration of the freshwater prawn, *Palaemon paucidens*, that had been exposed to different light conditions

Group	Light condition	No. of animals used	No. of animals survived	Intermolt duration (days)
I	Constant light	30	27	15.7±1.6*
II	Long-day (16L: 8D)	30	25	15.8±2.0
III	Short-day (8L: 16D)	30	26	15.7±1.8
IV	Constant darkness	30	27	15.6±1.9
Control	Natural light (roughly 12L: 12D)	30	28	15.7±1.9

* Mean±standard deviation

Table 2. Intermolt duration of the freshwater prawn, *Palaemon paucidens*, kept in different water temperatures

Temperature (°C)	No. of animals used	Mortality (%)	Intermolt duration (days)
16	60	6.7	28.1±3.3*
20	60	15.0	21.8±2.3
24	60	18.3	15.7±1.9
28	60	41.7	10.7±1.4

* Mean±standard deviation

least during the experiment which lasted for 5 months. Also, the animals kept at 32°C had a mortality so high that no decisive data were available, showing that the temperature was too high for this species. The data concerning the remaining four experimental groups are summarized in Table 2.

A parallelism between the temperature and the intermolt duration was evident. The animals maintained in low temperature (16°C) took approximately twice as much time as the ones kept in high temperature (28°C). A high mortality was noticed among the animals kept in high temperature. Twenty-five prawns that had been maintained at 28°C died without performing the molt, whereas only four out of sixty animals that had been kept at 16°C died without molting.

III. Variations in the intermolt duration among animals of different sizes and sexes

Variations in the intermolt duration among animals of different sizes and sexes were examined in 314 prawns in July and August when the fluctuation of the duration among individuals was supposed to be the least throughout a year (Fig. 2). It was evident that the smaller animals molted more frequently than the larger ones, especially among the immature animals (males smaller than 8 mm or females smaller than 10 mm in carapace length). In sexually mature specimens

(males ranging 8–12 mm or females raging 10–15 mm in carapace length), however, the duration of a cycle was almost the same for both sexes (about 20 days). In the extremely large specimens (males larger than 12 mm or females larger than 15 mm in carapace length) the intermolt duration again tended to become longer as they grew. Thus, apparently three different patterns of molting process could be distinguished throughout the life cycle of a prawn.

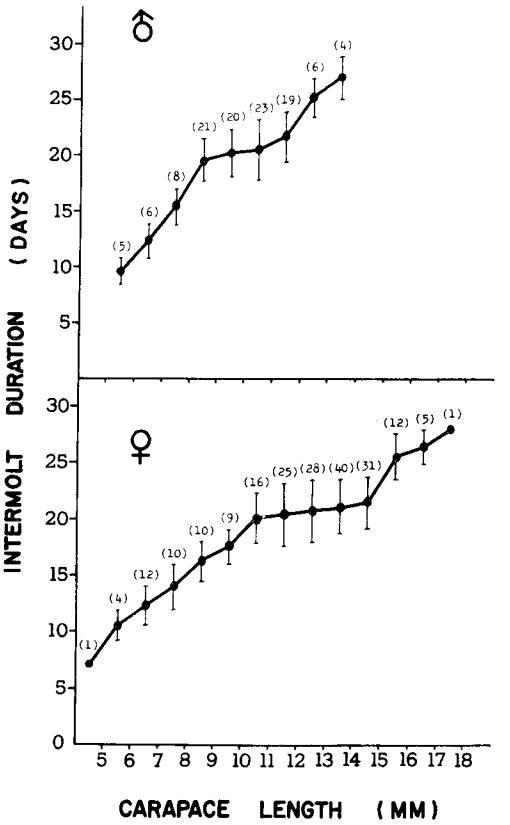

Fig. 2. Intermolt duration in the freshwater prawn, *Palaemon paucidens*, of different sizes and sexes. The number of animals used per point on the graphs is indicated in parentheses. All the points respresent the arithmetical mean with standard deviation.

Among the animals ranging 8–10 mm in carapace length females had the intermolt duration shorter than in males; for instance, ten females, 8–9 mm in carapace length, molted in a cycle of 16.2 days on an average, whereas twenty-one males of the same size required 19.5 days for molting. These results reflect the fact that

the males of this size are already sexually mature, while the females reach maturity only when they become 10 mm or larger in carapace length.

IV. Intermolt duration in ovigerous females

Intermolt duration in ovigerous females is closely correlated to the embryonic development in *P. paucidens*. Eggs are deposited by females when they are in stage B of the intermolt cycle and the larvae hatch out when the mother pwawns are in stage D_2-D_4. Usually it takes about 38 days from egg-laying to the hatching at 17–19°C. Within approximately three days after the hatching, mother prawns usually perform molting. As the "post-parturition" molting takes much longer time than the ordinary molt, it was tested to see whether the presence of deposited eggs on the pleopods somehow affect the intermolt duration of the mother. Eighty-six mature females were selected in May and were kept singly in the glass container. They were divided into two groups (groups I and II) and their molting behavior was observed. In group I, a male was put into the same container immediately after the female performed the parturial molt and, after copulation, the male was removed away. Mated females deposited fertilized eggs on the pleopods when they are at stage B of an intermolt cycle, about 24–48 hours after the parturial molt. In group II, the females remained solitarily through and after their parturial molt. All the 'virgins' deposited eggs on the pleopods as mated females did, but their unfertilized eggs dropped off within 24 hours. The intermolt duration of these animals was compared with that of non-ovigerous controls (group III) during the same period in May and June.

Table 3. Comparison of the average intermolt duration in days among three groups of females

Group	Condition of animals	No. of animals used	Intermolt duration (days)
I	Ovigerous females	56	39.9±3.5*
II	Unmated females that produced unfertilized eggs	30	38.6±2.2
III	Non-ovigerous females	25	21.5±2.6

* Mean±standard deviation

The results are shown in Table 3. The average intermolt duration was almost the same in groups I and II, approximately twice as long as that in group III. It may be concluded, therefore, that the extremely long intermolt duration in ovigerous females is not brought about by some physical stimuli from deposited eggs, but by some endogenous factors, possibly hormonal ones. It is suggested that the hormonal situation which brought about the ovarian maturation and deposition of eggs also functioned as the molt-inhibitor in ovigerous females.

Discussion

The light condition has been shown to affect molt in several crustanceans (for reference, see Passano, 1960b). However, the role of light in the molting process is still controversial. Thus, both constant darkness and constant light lengthened the the intermolt period (Stephens, 1955; Bliss and Boyer, 1964). In the present study, the intermolt duration was almost the same in the four experimental groups that had been subjected to different light conditions and in the normal control. At least in *Palaemon paucidens*, no evidence was available in favor of the view that light can influence molt.

On the other hand, temperature has been found to have a definite effect on the molting in many crustaceans (Templeman, 1936; Travis, 1954; Bliss and Boyer, 1964), showing that in a certain range of temperature the animals molt more frequently in higher temperature than in lower temperature. The results obtained by the present writer in *Palaemon* are in agreement with those obtained by previous investigators. Apparently, the molting process is accelerated by higher temperature in this species: in temperature ranging 16–28°C the intermolt duration of the prawn was shortened by 20–30% as temperature was raised by 4°C.

It has been pointed out by several investigators that the small individuals molt more frequently than the larger ones (Hess, 1941; Travis, 1954). In *Palaemon* not only the body size but the state of sexual maturity affect the length of an intermolt cycle. Thus, apparently, three phases of the molting pattern were distinguished among animals. The duration was distinctively shorter in immature animals when compared with sexually mature specimens. They took progressively longer time for a molt (7–17 days) as they come near maturation. In sexually mature specimens the duration of their intermolt cycle was more or less consistent (about 20 days) despite of the great diversity of their body size. Extremely large specimens required yet longer time for the next molt. In this connection, it is of interest to note that Inagaki (1969) described three phases of growth during the life cycle of the isopod, *Ligia oceanica*, from the comparison of the ratio of containing Ca and Na in the exuvia. But, the relationship between the two phenomena remains to be elucidated.

No evident difference in cycle length was noticed between the ovigerous and non-ovigerous females of *Palaemon xiphias* (Scheer, 1960). And Kajishima (1949), removing the eggs from the pleopods of ovigerous *P. pacificus*, failed in shortening the intermolt duration. On the other hand, Hess (1941) in *Crangon* and Scudamore (1948) in *Cambarus* reported that the similar operation shortened the duration. They concluded that prolongation of intermolt cycle in ovigerous females was dependent upon attachment of the embryos to the females. In *Palaemon paucidens*, the intermolt duration in ovigerous females (39.9±3.5 days on an average) was approximately twice as long as that in non-ovigerous females (21.5± 2.6 days), and this prolonged duration was maintained even in unmated females whose unfertilized eggs dropped off from the pleopods soon after deposition.

Therefore, it may be concluded that the long intermolt duration in ovigerous *P. paucidens* is brought about by some endogenous factors other than physical stimuli from eggs.

Summary

The influence of various physiological and environmental conditions on the intermolt duration in the freshwater prawn, *Palaemon paucidens*, was studied, and the following results were obtained.

1. There was a seasonal variation in the intermolt duration: it was the shortest during the summer (20.2 days on an average) and the longest during the winter (56.1 days). Moreover, fluctuation of the duration among individual specimens was the least during the summer and the greatest during the winter.

2. No evidence was available to support the view that light condition directly affects the intermolt duration.

3. In a certain range of temperature, the prawns molted more frequently in high temperature than in low temperature.

4. Three different patterns of molting process were distinguished throughout the life cycle of a prawn in relation to the state of maturity of the animal. In sexually immature specimens, molting frequency was high and dependent on the size of animals, whereas in sexually mature specimens, molting occurred more or less in constant intervals (about 20 days) regardless of their size. Extremely large individuals required yet more time for an intermolt cycle.

5. Ovigerous females in May and June had a long intermolt duration (39.9 ± 3.5 days on an average), whereas non-ovigerous females of similar size during the same period had a much shorter duration (21.5 ± 2.6 days). In unmated females that had dropped off the unfertilized eggs after parturial molt, the intermolt duration was 38.6 ± 2.2 days, almost corresponding to that of ovigerous females.

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