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

**II. Effects of Eyestalk Removal in Relation to the
State of Ovarian Growth¹⁾**

By

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(With 2 Text-figures, 2 Tables and 1 Plate)

It has been demonstrated by many investigators that in decapod crustaceans both the molting and the ovarian development are regulated by inhibitory hormonal factors which are produced separately in the X organ-sinus gland complex in the eyestalks.

Bilateral eyestalk removal led to acceleration of molting in the crayfish, *Cambarus immunis* (Brown and Cunningham, 1939), the fiddler crab, *Uca pugilator* (Abramowitz and Abramowitz, 1940) and the prawn, *Palaemon serratus* (Drach, 1944), and to precocious ovarian growth in *Palaemon serratus* (Panouse, 1943), *Uca pugilator* (Brown and Jones, 1947), the prawn, *Pandalus kessleri* (Aoto and Nishida, 1956) and the freshwater crab, *Potamon dehaani* (Ôtsu, 1963). In the shore crab, *Carcinus maenas*, the juveniles responded to the operation by proecdysial regeneration of limbs, while mature adults by precocious development of the gonad (Bauchau, 1961; Démeusy, 1965). Mature females of the land crab, *Gecarcinus lateralis* in spring responded to the operation by ovarian enlargement and in autumn they showed proecdysial regeneration of limbs and no ovarian growth (Weitzman, 1964). Study of the different effects in juvenile and adult specimens or in specimens during spring and autumn that had been produced by eyestalk removal would certainly throw light on the problem of endocrine mechanism of molting in these animals.

In the present paper, effects of eyestalk removal on the molting were studied in the freshwater prawn, *Palaemon paucidens*, of different ovarian conditions and different seasons of a year.

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Materials and Methods

The freshwater prawns, *Palaemon paucidens*, used in this experiment were collected in a stream near Sapporo and stocked in aerated aquaria in our laboratory. The experimental animals were placed in individual glass vats (15 cm in diameter and 7 cm in height) and fed small pieces of boiled fish paste on every other day. The water was exchanged on every fourth day. The eyestalk removal was carried out as follows: the optic peduncles were ligated with a fine thread at the basal part 12–24 hours after molt (stage B), and 24 hours later, they were cut off at a level just above the ligation with a pair of Wecker's scissors. All the animals survived through the operation. Intermolt stage of the animals was determined with a method described by Kamiguchi (1968). The dates of successive molts were recorded and the intermolt duration in days was calculated for each animal. To determine the state of ovarian growth the animals were fixed *in toto* with Bouin's fluid after two successive molts. The ovaries were excised and stocked in Bouin's fluid. They were lightly wiped on a filter paper and weighed before further histological use. In compensation for an unavoidable variation in size of the animals a formula was developed: an ovarian factor. It consists of the weight of the ovary in milligrams divided by the cube of the carapace length in centimeters which was measured from a posterior edge of orbit to the most posterior point of the carapace:

$$\text{ovarian factor} = \frac{\text{ovarian weight (mg)}}{\text{carapace length (cm)}^3}$$

On the bases of the body size, the gross ovarian size surveyed with the naked eye through the carapace and other conditions, female prawns employed in the present experiments were classified into five types: I, IIa, IIb, IIIa and IIIb (Table 1). The females of Type IIIb were obtained by keeping them singly in individual glass vats before and after the parturial molt, in which the animals produced unfertilized eggs on the pleopods but dropped them off soon after deposition.

For histological examination, ten ovaries from each experimental group were embedded in paraffin, cut at 8μ and stained with Delafield's hematoxylin and eosin.

Table 1. Classification of females of the freshwater prawn, *Palaemon paucidens*

Type	Body size	Ratio in size of the ovary to a fully grown ovary (Fig. 4) just before egg deposition	Oviposition
I	Immature females (9 mm or smaller in carapace length)	approximately one-eighth or smaller (Fig. 1)	no
IIa	grown females (11 mm or larger in carapace length)	approximately one-fourth or smaller (Fig. 2)	no
IIb	grown females	approximately one-half (Fig. 3)	no
IIIa	grown females	approximately one-fourth or smaller	yes
IIIb	grown females	approximately one-fourth or smaller	yes (laid unfertilized eggs but dropped them off within 24 hr)

Results

1. *The annual reproductive cycle in females*

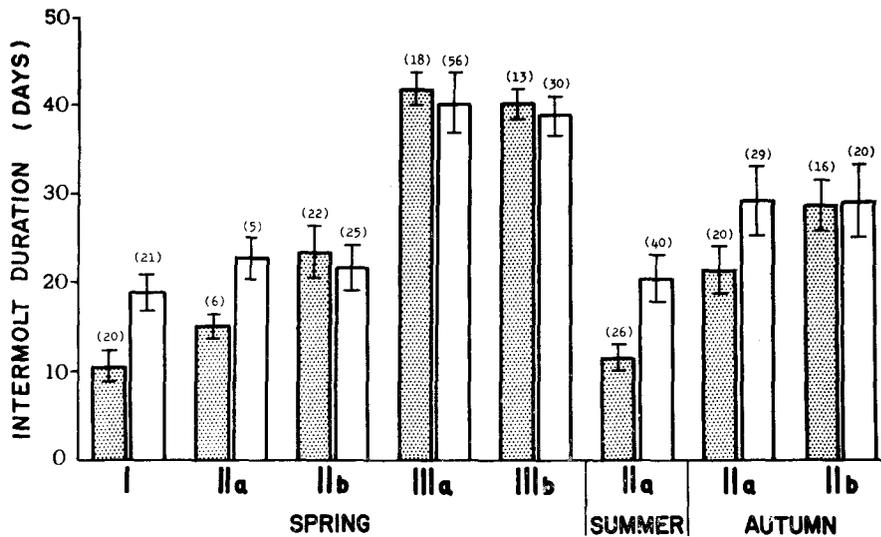
The freshwater prawn, *P. paucidens*, breeds only once a year in spring. Eggs are deposited during May and early June, and the larvae hatch out during late June and early July. In the summer months (July and August) after egg deposition, all the grown females were Type IIa. Ovaries in the immature (Type I) and the Type IIa females contained only small oocytes (80–160 μ in diameter) with no yolk granules (Figs. 5 and 6). During the fall months (September and October) ovaries began to grow with the oocytes (300–350 μ in diameter) increasing in size. Until October the ovaries reached in size approximately one-half of those just before egg deposition (Type IIb), possessing no yolk granules in the oocytes (Fig. 7). At the end of October, most of them already had deposited small amounts of yolk granules in the oocytes (Fig. 8). Yolk deposition was almost completely ceased during the winter (from November to early April). Animals resumed deposition of yolk granules when they emerged from quiescence in mid-April. Thus, in early May most females possessed a considerable amounts of yolk granules in the oocytes (Fig. 9). In mid-May the ovaries reached the maximal size (Fig. 4), containing greenish ova (about 1.0–1.2 mm in diameter). During mid-May and early June females with such ovaries underwent parturial molt and soon laid eggs. In the same months, there were observed a few number of females in corresponding size still being in Type IIb. Only 4 out of twenty-five such females deposited eggs, whereas in the remaining twenty-one animals no oviposition occurred and the oocytes were eventually resorbed (Fig. 10).

2. *Effects of eyestalk removal on the intermolt duration of female prawns*

Effects of eyestalk removal on the intermolt duration were examined in female prawns in various ovarian conditions at different seasons of a year. The results are summarized in Text-fig. 1.

In spring the intermolt duration was shortened in destalked Type I females (10.6 \pm 1.8 days) compared with the intact ones (18.7 \pm 2.0 days). Among the population the Type IIa females were very few in spring, and their intermolt duration was much shorter in the eyestalkless (15.0 \pm 1.3 days) than in the intact animals (22.6 \pm 2.4 days). In the Type IIb females, however, no significant difference in the intermolt duration was seen between the eyestalkless (23.3 \pm 2.9 days) and the intact animals (21.9 \pm 2.6 days). Between the females of Types IIIa and IIIb, not much difference in the intermolt duration was observed in the eyestalkless animals (41.6 \pm 1.8 days and 39.9 \pm 1.7 days, respectively) as well as in the intact, eyestalked, ones (39.9 \pm 3.5 days and 38.7 \pm 2.2 days, respectively). During the summer months all females belonged to Type IIa and their intermolt duration (20.3 \pm 2.7 days) was remarkably shortened when eyestalks were removed bilaterally (11.5 \pm 1.5 days). During the fall the ovaries gradually increased in size. In late

September there were some females still in Type IIa but most females were Type IIb. In the Type IIa females the intermolt duration was shortened considerably in the eyestalkless animals (20.3 ± 2.6 days) compared with that in the intact ones (29.2 ± 4.0 days). In the Type IIb females, on the other hand, the difference in intermolt duration between the eyestalkless (29.0 ± 4.2 days) and the intact animals (28.4 ± 2.9 days) was minor.

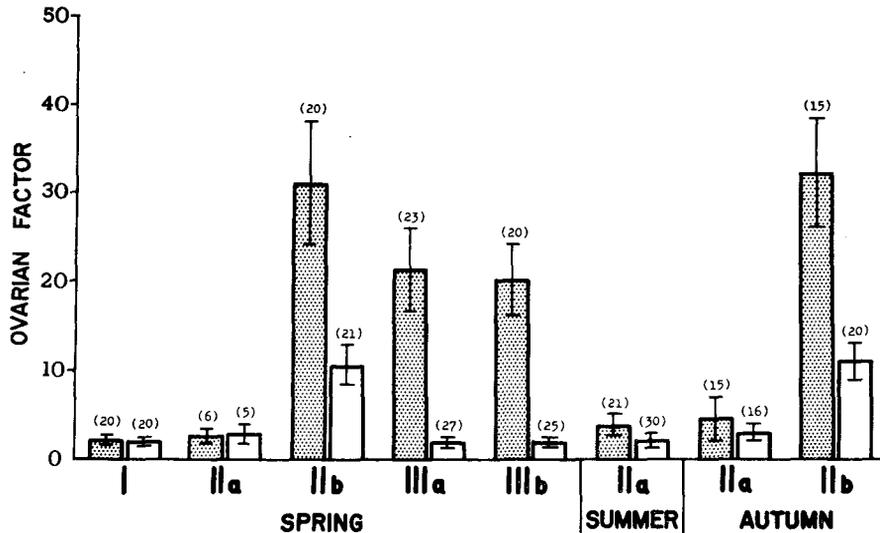


Text-fig. 1. Effects of bilateral eyestalk removal on the intermolt duration of the freshwater prawn, *Palaemon paucidens*, in various ovarian conditions at different seasons of a year. The number of animals used per column on the graph is indicated in parenthesis. Longitudinal lines on the columns represent the standard deviation of the arithmetic mean. White columns, intact animals; stippled columns, eyestalkless animals. For other abbreviations, see Table 1.

3. Effects of eyestalk removal on the ovarian growth

Effects of eyestalk removal on the ovarian growth were examined in terms of the ovarian factor. The results are given in Text-fig. 2.

In the spring, the difference between the intact and eyestalkless animals was minor in the Type I females (2.0 ± 0.5 and 2.2 ± 0.5 , respectively) as well as in the Type IIa females (3.0 ± 1.1 and 2.8 ± 0.8 , respectively). In the Type IIb females, however, the ovarian factor was markedly greater in the eyestalkless (31.1 ± 6.9) than in the intact animals (10.6 ± 2.3). Actually not a single destalked female failed to undergo the parturial molt and subsequent egg deposition, whereas only 4 out of 25 intact animals (about 16%) laid eggs. Of the Type IIIa females the ovarian factor was far greater in the eyestalkless (20.1 ± 4.0) than in the intact



Text-fig. 2. Effects of bilateral eyestalk removal on the ovarian growth of the freshwater prawn, *Palaemon paucidens*, in different seasons. Abscissa represents the ovarian factor: ovarian weight (mg) \times 1/carapace length (cm³). For abbreviations, see legend for Text-fig. 1 and Table 1.

animals (2.0 ± 0.5). The same situation was observed among the Type IIIb females: values for the ovarian factor were 21.3 ± 4.6 in the eyestalkless animals and 1.9 ± 0.6 in the intact animals. The viability was low in destalked animals of Types IIIa and IIIb: of sixty operated animals, 29 animals died just before (St. D₃-D₄), during (St. E) or immediately after (St. A) the molting. However, all remaining animals underwent parturial molt and deposited eggs. Thus, these destalked prawns laid eggs twice successively, in contrast to the normal stalked ones which bred only once a year. If a destalked mother prawn had been mated to a male soon after the parturial molt, these secondarily deposited eggs developed normally. Not much difference was seen in the ovarian factor of the Type IIa females between the intact and eyestalkless animals: during the summer the value was 2.1 ± 0.7 for the intact prawns and 3.9 ± 1.3 for the eyestalkless ones, and in the fall it was 3.2 ± 0.9 for the intact and 4.5 ± 2.4 for the destalked animals. Among the Type IIb females, on the contrary, the ovarian factor was much greater in the eyestalkless (32.2 ± 6.1) than in the intact ones (10.9 ± 2.2) during the fall. The eyestalk removal performed during the fall inevitably produced extraseasonal parturial molt and subsequent egg-laying in the Type IIb females.

Discussion

The longer intermolt period in ovigerous females than in non-ovigerous ones, which is naturally a necessity for the attached embryos for survival, has been noticed in many crustacean forms, but the mechanism by which they attain it is still obscure. For such a phenomenon in the crayfish, *Cambarus propinquus*, Scudamore (1948) surmised that the presence of eggs or embryos on mother's pleopods induces prolonged production of the molt-inhibiting hormone in the X organ-sinus gland complex. In the freshwater prawn, *Palaemon paucidens*, bilateral removal of eyestalks did not alter the intermolt duration of ovigerous females. Also, without available males after parturial molt the female prawns laid unfertilized eggs that dropped off soon, but their intermolt duration in the absence of brooding eggs was not changed. Thus, it may be concluded that the prolonged intermolt duration in ovigerous *Palaemon* is caused by a factor other than the molt-inhibiting hormone that functions in the crayfish.

Results of the bilateral eyestalk removal in female *Palaemon* clearly showed that there is an antagonism between its effects on the molting and the ovarian growth (Table 2). The eyestalk removal induced in grown females shortening of the intermolt duration when the ovarian growth was not accelerated, and the

Table 2. Effects of bilateral eyestalk removal on the molting and ovarian growth in the freshwater prawn, *Palaemon paucidens*.
For abbreviations, see Table 1.

Season	Group	Molt acceleration	Ovarian growth
Spring	I	+	-
	IIa	+	-
	IIb	-	+
	IIIa	-	+
	IIIb	-	+
Summer	IIa	+	-
Autumn	IIa	+	-
	IIb	-	+

animals responded to the operation by precocious egg-maturation only when molting was not accelerated in spite of the absence of molt-inhibiting hormone from the eyestalks. The data obtained in the present study seemed to support Passano's (1960) model of the molt-controlling endocrine system in crustaceans. He postulated that a hypothetical gland not only stimulates the egg maturation but also inhibits the production and release of molting hormone by the Y-organ, and that the gland, in turn, is inhibited by eyestalk hormone. Somewhat different results obtained in immature, eyestalkless *Palaemon* may be explained as that this hypothetical gland in juveniles was functionless yet so that only precocious molting

was induced by a sudden loss of molt-inhibiting hormone from the eyestalks. Whether such an endocrine gland does exist and functions to mediate the molt-controlling effect from the X organ-sinus gland complex to the epidermis and other tissues of *Palaemon* remains to be elucidated.

Summary

1. Bilateral eyestalk removal was performed in the female freshwater prawns, *Palaemon paucidens*, in different states of ovarian growth at different seasons of a year, and its effects on the molting and ovarian maturation were studied.

2. It shortened the intermolt duration in immature females (Type I) and in the grown females (Type IIa) with the ovaries approximately one-fourth or less in size of the fully grown ovaries just before egg deposition, whereas the operation produced no change in the duration in ovigerous females (Type IIIa) and in the grown females (Type IIb) with the ovaries approximately one-half of those just before deposition.

3. The operation resulted in precocious ovarian growth in Type IIb females and ovigerous females, while it induced no ovarian growth in immature females and Type IIa females.

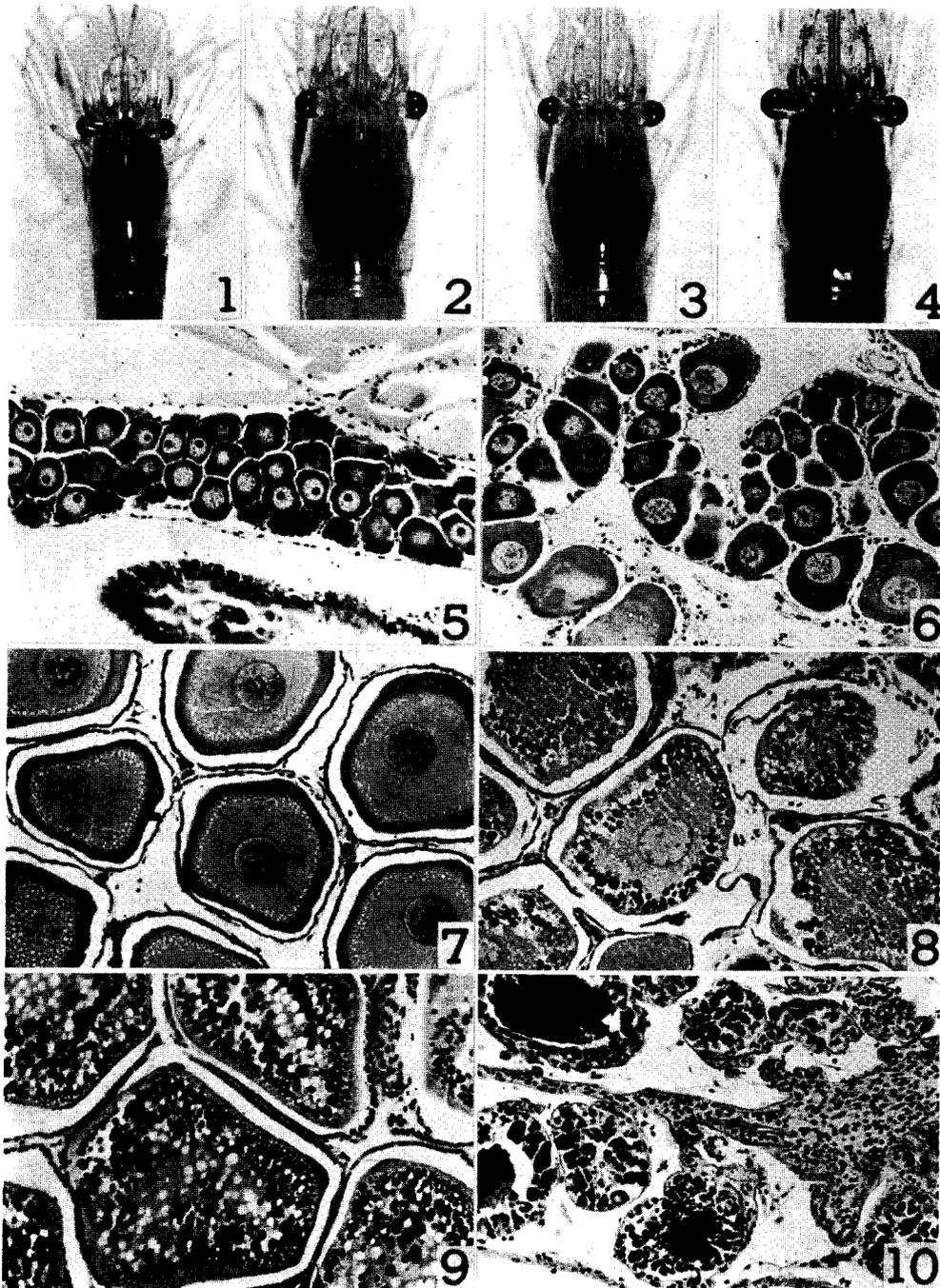
4. Mature females, when kept from mating by isolation during and soon after the parturial molt, produced unfertilized eggs which, however, dropped off from pleopods within 24 hours after deposition. Such 'virgin' prawns had a long intermolt duration comparable to that of ovigerous females, whether they were destalked or not.

5. These results were discussed in relation to the data obtained by previous investigators.

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Explanation of Plate VII

Figs. 1-4. The female freshwater prawns, *Palaemon paucidens*, in different states of ovarian growth. Live specimens. $\times 2$.

Fig. 1. An immature female (Type I).

Fig. 2. A grown female (Type IIa) with an ovary approximately one-fourth in size of that (Fig. 4) just before egg deposition.

Fig. 3. A grown female (Type IIb) with an ovary approximately one-half of that (Fig. 4) just before egg deposition.

Fig. 4. A grown female just before egg deposition with a fully grown ovary.

Figs. 5-10. Microphotographs of the sections of ovarian tissue of prawns in different seasons. Delafield's hematoxylin and eosin stain. $\times 75$.

Fig. 5. The ovary of an immature female in May (Type I), possessing only small oocytes, 80μ in diameter.

Fig. 6. The ovarian tissue of a Type IIa female in July, consisting mainly of young oocytes, ranging $80-160 \mu$ in diameter.

Fig. 7. The ovarian tissue of a Type IIb female in late September, containing oocytes in advanced stage, ranging $300-350 \mu$ in diameter.

Fig. 8. The ovarian tissue of a Type IIb female in late October, showing some oocytes with yolk granules deposited on the periphery.

Fig. 9. The ovarian tissue of a grown female in early May, containing oocytes with abundant yolk granules.

Fig. 10. The ovarian tissue from a Type IIb female in June, showing resorption of oocytes and enlarged connective tissue.