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# EFFECT OF BILATERAL AND UNILATERAL EYESTALK REMOVAL ON THE MOULT FREQUENCY AND GLYCOGEN IN PENAEUS KERATHURUS (LEACH).

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## ABSTRACT

Bilateral eyestalk removal in **Penaeus kerathurus** (Leach) results in a precocious onest of proecdysis and moulting. A similar but more pronounced effect is observed when unilateral ablation is performed.

The effect of eyestalk removal was studied during the moulting cycle of the experimental animal. The degree of moult acceleration was dependent upon the time of eyestalk removal relative to the age of the prawn and also upon the time of year. Possible mechanisms of moult control in the **Penaeus kerathurus** were discussed.

Both unilateral and bilateral eyestalk removal caused a marked increase in glycogen content of adult female prawn represented by deposition in the muscle and hepatopancreas of destalked animals.

## INTRODUCTION

The physiology and reproduction of crustaceans are linked to the moult cycle. Therefore, in experimental studies it is necessary to be able to identify accurately the stages of the moulting cycle in order to interpret observed biochemical and biological changes.

Earlier studies of the moulting stages of Penaeid prawns have been carried out on several species including **Penaeus duorarum** (Schafer, 1968) **Penaeus indicus** (Read, 1977) **Penaeus californiensis** and **Penaeus stylirostris** (Huner and Colvin, 1979) and **Penaeus merguiensis** (Longmuir, 1983). The moult cycle of **Penaeus esculentus** has been described by (Wassenberg and Hill, 1984).

In crayfishes, eyestalk factors participate in the regulation of the direction of tissue metabolism at various stages in the moult cycle (McWhinnie and Chuce, 1964). On the other hand, there are quite a lot of work on the effect of eyestalk removal on injections of extracts from those organs upon main physiological and biochemical processes in crustacea (McWhinnie et al., 1969; Zerbe et al., 1970; Rangneker and Madhyastha, 1971).

Most current literature concerning moult control in crustaceans concludes that initiation of premoult is controlled by changes in the level of moult

inhibiting hormone (MIH) produced by the eyestalk neurosecretory organs. MIH is thought to act by repressing the secretory activity of the Y-organs, preventing increases in circulating ecdysteriod levels, necessary for moulting (Kleinholz and Keller, 1979). This hypothesis is based upon the fact that eyestalk removal leads to elimination of MIH which accelerates the moulting process (Passano, 1960 and Sochasky et al. 1973). However Sochasky et al., (1973) stated that moulting is not affected by removal of eyestalk.

Webter (1985) examined the effect of eyestalk removal upon moulting, proecdysis and limb regeneration in the prawn **Palaemon elegans** and showed that there is a relationship between sex, size, temperature, photoperiod, season and time of eyestalk removal. He also came to the conclusion that the growth and regeneration of limbs may be stimulated by the removal of neurosecretory tissus.

Crustacea has a large carbohydrate demand for synthesis because of the chitin content of the integument. Since a major part of this is lost at each moult, there is a regular drain on the body carbohydrate pool, especially in those crustaceans with a short moult cycle such as Metapenaeus species (Sceer and Scheer, 1951). Thus the greater part of dietary carbohydrate is likely utilized either for oxidative metabolism or for chitin synthesis.

Scheer and Scheer (1951) found that moult accelerating hormone of the sensory pore-complex may be a hyperglycaemic hormone responsible for mobilization of body carbohydrates.

Decapod eyestalk is known to contain a transglucosylase inhibitor which controls glycogen synthesis (Wang and Scheer, 1963). Schwabe et al. (1962) found that eyestalk ablation has no effect on the glycogen content of the hepatopancreas. They postulated that the decrease in glycogen content of the hepatopancreas after eyestalk ablation would suggest that the equilibrium glycogen plays no role in the sugar metabolism. After bilateral eyestalk removal Monin and Rangneker (1974) observed an acceleration in glycogenesis as a postoperative metabolic effect in the hepatopancreas.

A number of biochemical analyses were carried out on the juvenile American lobster, **Homarus americanus** by Mauviot and Castell (1976) to determine whether or not eyestalk ablation had altered its composition. Results of these investigations revealed no significant difference between ablated and control lobsters in the glycogen content of the hepatopancreas.

It is known that metabolic reactions in crustaceans are under the influence of eyestalk hormones (Rangneker and Medhyastha, 1971).

The aim of this study is to investigate the effect of eyestalk removal on moult frequency and glycogen metabolism in the female prawn **Penaeus kerathurus** (Leach) at different stages of maturity.

## MATERIAL AND METHODS

Specimens of **Penaeus kerathurus** (Leach) at different stages of maturity, were caught during winter (from September to May) in the Al-Maadia district of Alexandria. They weighed about 1060 gm each. They were very carefully transported very carefully avoiding rough handling and injuries.

To study the effect of eyestalk removal, adult (mature early and late) female prawns were put in five aquaria (each measuring 30 x 70 x 50 cm volume, with a small amount of circulating sea water created by pumps ) each containing 8 or 10 animals maximum. The first two contained bilateral eyestalkless animals, the third and forth contained unilateral ablated prawns and the normal animals are put in the last aquarium.

Artemia was supplied daily into the aquaria, at which time the water was changed. Each aquarium was examined daily for moulting, spawning, matting or death of the animals.

The animals were acclimatized to laboratory conditions for at least two days before each experiment. The eyestalk were removed by holding the eyes with a forceps and cutting through the flexible membrane using a sterilized pair of scissors. Pencillin was applied to prevent infection.

One eyestalk was removed and the second eyestalk removed 24 h later by the same procedure. After each surgery the prawn was put in filtered sea water for recovery. Prawns were then carefully dried with blotting paper and the total length of each animals was recorded.

After removal of the exoskeleton, each of the muscle, hepatopancreas and ovary was removed, dried and weighed.

The experimental period of observation varied from 5 to 30 days maximum. In general mortality was low (< 10%) and occurred within 24 h of eyestalk removal, in which case death was attributed to the surgical procedure. Examination of ovaries was carried out within 5 days interval.

A summary of experimental schedules and manipulative treatments was shown in Table 1. Manipulative procedures were performed within 24 h of moulting.

Determination of glycogen of the muscle, ovary and hepatopancreas was estimated by Hassid and Abraham method (1957). All the results obtained were statistically treated using Arkin and Colt formulae (1963). Comparison of experimental results with controls was made by student's t-test, and significance was reported at the $P < 0.05$ level.

Ks values (the relationship between moult stage distribution of controls and destalked animals) were obtained according to the formula by Webster (1985) every 3 days intervals.

TABLE 1
Experimental schedules and manipulative treatments.

| | | |
|---|---|---|
| Experiment (1): Eyestalk ablation (ES.A) (bilateral and unilateral ablation) effects on moult cycle. | | |
| a- Untreated control (intact) | initial no. | 25 |
| b- Bilateral eyestalk ablation | | 35 |
| c- Unilateral eyestalk ablation | | 35 |
| Experiment (2): Moult cycle duration during several successive post operative moults following bilateral and unilateral eyestalk ablation (ES.A). | | |
| Group A: Eyestalk removal after the first moult in the laboratory. | | |
| a- Untreated control (intact) | initial no. | 25 |
| b- Bilateral eyestalk ablation | | 35 |
| c- Unilateral eyestalk ablation | | 35 |
| Group B: Eyestalk removal after the second moult in the laboratory. | | |
| a- Untreated control (intact) | initial no. | 25 |
| b- Bilateral eyestalk ablation | | 35 |
| C- Unilateral eyestalk ablation | | 35 |

## RESULTS

### Observational Data

a- Moult cycle duration:

Eyestalk ablated from thirty five prawns which constituted the experimental group. Twenty five intact prawns constituted the control group. At the time of eyestalk removal the majority of the animals are in the intermoult stage. Twenty-four h later the prawns had not shown any further progress into proecdysis (Fig. 1). However, observations after 48, 72 and 96 h the experimentals clearly showed a marked acceleration of the moult cycle i.e. at the end of 48 h 58 percent eyestalkless individuals had initiated proecdysis stages whereas 40% only of control animals begin to prepare themselves to those stages. By 72 h only about 44 percent of the controls were in the ecdysis stages. Whereas all of the eyestalkless animals were in these stages. By 96 h only 6 percent of the controls were in the final stage of metaecdysis whereas more than half of the eyestalkless animals were either in the final stage or had completed the formation of new exoskeleton. The eyestalkless prawns moulted in the third and fourth days after the start of the experiment while in the intact controls they moulted in the fifth and sixth days.

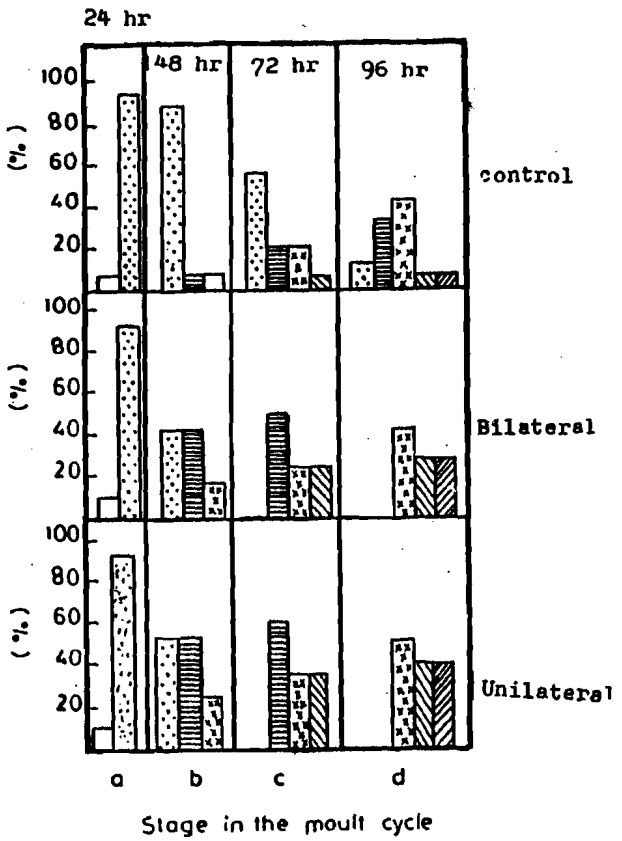


FIG. 1.a
Acceleration of proecdysis prawn by means of bilateral and unilateral eyestalk ablation, the proecdysial changes recorded 24, 48, 72 and 96 hr after operation are shown.
a= intermoult b= proecdysis c= ecdysis, d= metaecdysis.

The present results clearly showed that eyestalk ablation accelerated moult cycle and resulted in precocious ecdysis in these animals. About thirty of the fourth stage prawns that moulted after eyestalk removal survived and moulted again after an interval of a week whereas in the control animals they moulted after 10 days.

Figure 2 shows the effect of both bilateral and unilateral eyestalk removal upon moult cycle duration, compared to untreated controls. Of 35 animals destalked initially, 25 animals survived one complete moult cycle with viable ecdysis. No deaths occurred in other groups. Both manipulative treatments markedly accelerated moulting compared to untreated controls.

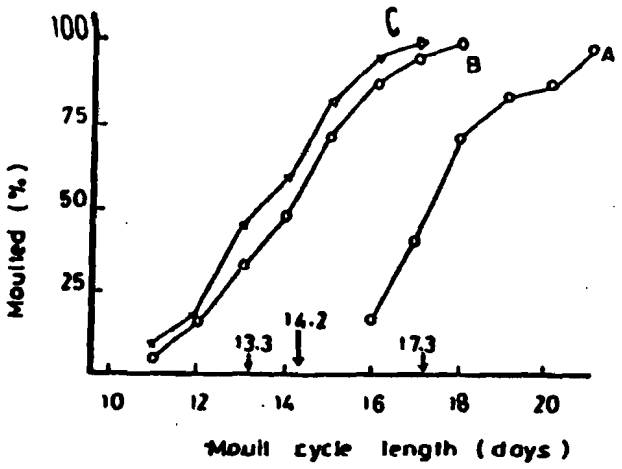


FIG. 1.b
Moulting frequency expressed as a cumulative percentage for control intact prawns (A), Bilateral eyestalk ablation (B) and Unilateral eyestalk removal (C). Median values of moult cycle duration are shown on the ordinate n = 25 in each case.

Moult staging during the moult cycle and statistical analysis using the Ks test (Webster, 1985), (Table 2) demonstrated that moult acceleration was associated with a precocious onest of proecdysis and the effect was more detectable in unilateral eyestalk ablation. Both destalked animals accelerated moulting compared to untreated ones. The moult cycle duration of the unilateral eyestalk ablation group was significantly shorter than that of the bilateral group.

b- Growth rate:

Prawns which completed five moults were measured after each ecdysis with special reference to the mean value of the initial total length, which was measured after the initial laboratory moult (Table 3). As is clear from the table, the rate of increase of total length of the eyestalkless group is more than that of the control. The average percentage of increase in total length premoult was 14.8% for untreated group, 16.3% for bilateral eyestalk ablated individuals and 17.5% for unilateral ablated animals.

c- Moult frequency:

In controls, during September and October there were practically no moults, the moult frequency increased abruptly in November till December, and then dropped from January to April (Fig. 2 A & B). On the other hand

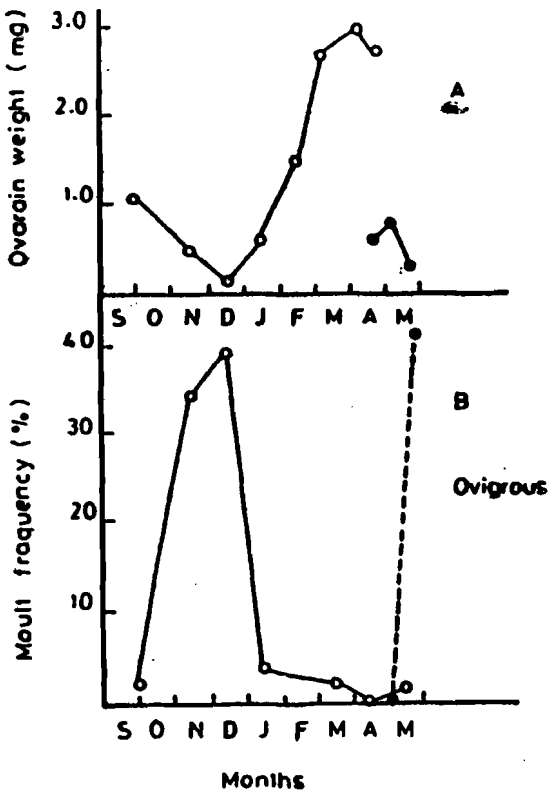


FIG. 2
Mean ovarian weight (A) and frequency of moult (B) for intact prawns as a function of time of year. Open circles represent nonovigers solid circles, April ovigers.

opposite correlation between ovarian weights and moult frequency was observed. Maximal moulting coincides with minimal ovarian weights (December and late May).

The frequencies of moulting in destalked prawns relative to the controls are presented in Fig. 3. Maximum effect in inducing moult occurs in September at a time when moult frequency is low in the controls (Fig. 3 A & B). In November, when about 34% of the controls moulted, there were slightly fewer destalked prawns that moulted 27%. In April and early May for ovigerous prawns, the moult frequency reached maximum when no controls moulted.

In late May both destlaked post-ovigerous prawns and their controls moulted at a frequency of about 42%.

TABLE 2

Ks values and significance levels, derived from moult stages distributions, calculated at 3 day intervals for control, unilateral and bilateral eyestalk ablation were calculated and statistically analysed.

| Days moult | Control/unil. ES | Sig. | Control/bil. ES | Sig. |
|---|---|---|---|---|
| 3 | 0.62 | NS | ---- | --- |
| 6 | 3.27 | 0.1 | 1.65 | 1.0 |
| 9 | 3.27 | 0.1 | 3.27 | 0.1 |
| 12 | 4.25 | 0.1 | 2.50 | 0.1 |
| total moult cycle | 3.27 | 0.1 | 2.84 | 0.1 |

Unil. = Unilateral
Bil. = Bilateral
ES = Eyestalk ablation
Sig. = Significance

TABLE 3

The average of total length (Cm) after each moult in Penaeus kerathurus (Leach), ES = eyestalk ablation.

| | Initial | 1 st. | 2 nd. | 3 th. | 4 th. | 5 th. |
|---|---|---|---|---|---|---|
| Control | 12.1 ± 0.2 | 13.5 ± 0.4 | 14.5 ± 0.4 | 15.9 ± 0.5 | 16.2 ± 0.5 | 17.0 ± 0.6 |
| Unilateral ES. | 12.5 ± 0.3 | 14.2 ± 0.3 | 16.3 ± 0.4 | 19.5 ± 0.5 | 20.5 ± 0.5 | 22.2 ± 0.7 |
| Bilateral ES. | 12.4 ± 0.3 | 13.9 ± 0.4 | 15.5 ± 0.3 | 16.8 ± 0.5 | 18.8 ± 0.4 | 20.5 ± 0.7 |

Mean value of control = 14.8
Unilateral = 17.5
Bilateral = 16.3

Although there was no great differnce between controls and destlaked prawns with respect to survival, there were two periods during this study when mortality was high for both groups. The first in December and the second in late May. In these cases the mortality occurred during the stage of ecdysis or just prior to ecdysis. During the other months of the year ecdysis was generally successful both for destalked and intact prawns.

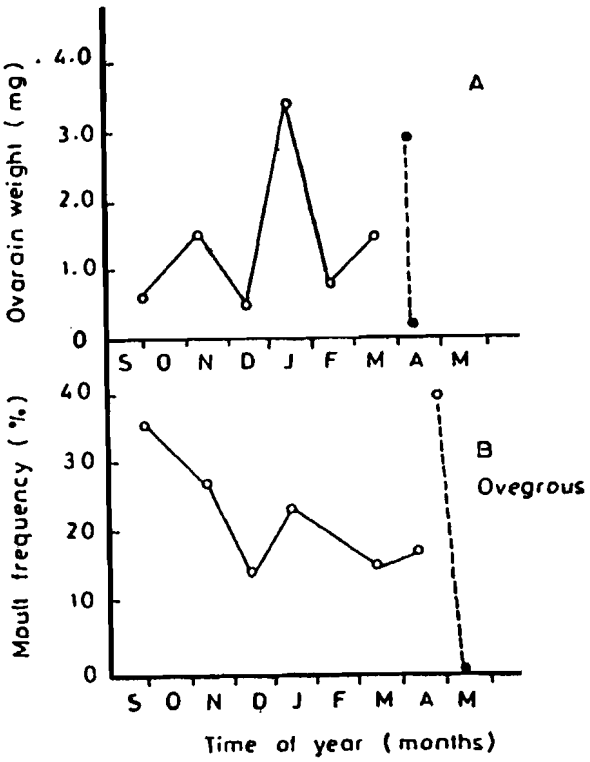


FIG. 3
A change in ovarian weights following eyestalk removal expressed as difference from controls, as a function of time of year: Open circles represent nonovigers; Solid circles, April ovigers.

**Total Glycogen Content**

As shown from Table 4, it is clear that there are striking differences in total glycogen of the ovary tissue between eyestalkless adult female prawns and their matching controls. The levels of total glycogen content are drastically lower in both operated animals at 5 and 15 days than those obtained for normal organs.

The levels of glyogen content showed a significant progressive increase in both muscle and hepatopancreas after ablating . After 30 days from the bilateral eyestalk ablation, the levels of glycogen content in muscle and hepatopancreas reached 39.81 and 26.93 mg glucose equivalent/100 mg tissue, while in untilateral, the levels were 43.5 and 34.6 mg glucose equivalent/100 mg tissue (Table 4).

TABLE 4

Average values of tissue glycogen (muscle, ovary and hepatopancreas) of mature individuals of Penaeus kerathurus (Leach) under normal and eyestalkless conditions. Each mean result corresponds to mean value of 5 experiments, ± standard deviation expressed in mg glycogen/100 mg tissue.

| Period after treatment days: | Muscle | | Ovary | | Hepatopancreas | |
|---|---|---|---|---|---|---|
| | unilateral mean ± S.D. | bilateral mean ± S.D. | unilateral mean ± S.D. | bilateral mean ± S.D. | unilateral mean ± S.D. | bilateral mean ± S.D. |
| Control | 18.3 ± 1.09 | | 11.8 ± 1.27 | | 14.1 ± 0.46 | |
| 5 | 22.1 ± 1.13 | 20.7 ± 1.1 | 10.8 ± 1.01 | 11.3 ± 0.9 | 16.21 ± 1.3 | 14.97 ± 0.19 |
| 10 | 25.3 ± 1.17 | 23.7 ± 0.13 | 13.73 ± 1.6 | 12.6 ± 1.3 | 20.31 ± 1.21 | 16.34 ± 0.18 |
| 15 | 28.7 ± 1.25 | 25.97 ± 1.19 | 10.20 ± 1.8 | 9.35 ± 1.5 | 21.52 ± 1.62 | 18.52 ± 0.36 |
| 20 | 35.9 ± 2.36 | 29.45 ± 1.15 | 14.92 ± 2.0 | 13.53 ± 1.9 | 24.51 ± 1.91 | 21.31 ± 0.39 |
| 25 | 38.5 ± 2.44 | 33.68 ± 2.31 | 16.34 ± 2.3 | 14.98 ± 0.8 | 29.32 ± 2.31 | 25.46 ± 1.2 |
| 30 | 43.5 ± 2.52 | 39.81 ± 2.60 | 16.10 ± 2.0 | 14.25 ± 1.1 | 34.63 ± 2.50 | 26.93 ± 1.7 |

## DISCUSSION

In the present study, both bilateral and unilateral eyestalk ablation of **Penaeus kerathurus** (Leach) accelerated the onest of proecdysis and moulting. Similar results have been reported by Humbert (1965) and Kenneth et al.(1977) on **Palaemon serratus** following selective ablation of X-organs in the species and Webster (1985) on the bilateral eyestalk ablation of **Palaemon elegans.** Comparable results have also been obtained for other crustaceans exhibiting "diecdysic" moult cycle patterns (Freeman and Bartell, 1975). Contradictory reports showing failure to accelerate moulting in eyestalk ablated **Palaemon** have been published by Carlisle (1953) and Sochasky et al. (1973) on the grounds that many factors such as number, size and time of eyestalk removal were not rigorously controlled.

Carlisle (1953) used female prawn **Palaemon** came to the conclusion that eyestalk removal accelerated gonad development coincident with inhabiting somatic growth and retarded moulting.

Adiyodi and Adiyodi (1970) and Kenneth et al. (1977) working on **Palaemon** reported that a moult acceleration hormone in addition to the moult inhibiting hormone are involved in the control of moulting in diecdysic crustaceans.

In the present work the major factor influencing the degree of moult acceleration following eyestalk removal was mainly the time of year the operation was performed. Eyestalk removal was more effective in accelerating moulting when performed during the summer and after the second laboratory moult. This is in agreement with the work done by Fingerman and Fingerman, (1976) and Webster (1985), in female **Uca pugilator,** but this was probably due to seasonal difference in gonad inhibiting

hormone levels, antagonising the action of moult inhibiting hormone. An explanation of the differential response to eyestalk removal related to age and season can be proposed by consideration of Aiken's (1969) hypothesis that temperature and photoperiod intract to maintain high levels of moult inhabiting hormone during the winter when moulting normally ceases (Webster, 1985). It can be postulated that thermal shock, associated with transfer to laboratory conditions, produce a precipitous decline in moult inhibiting levels, allowing rapid moulting. This would explain why eyestalk removal after the first moult in winter is ineffective.

Present results confirm the hypothesis that moult inhibiting hormone produced by eyestalk neurosecretory tissue controls the moult cycle in **Penaeus kerathurus** (Leach). However, it is apparent that season and age of the animals are of major importance upon the moulting endocrinology of crustaceans in the laboratory.

These results suggest that the removal of eyestalks (bilateral and unilateral) stimulates moulting in **Penaeus kerathurus** (Leach). The results also support the findings of the authors studying the eyestalkless land crabs **Gecarcinus lateralis** (Skinner and Graham, 1972), walking legless crayfish **Procambarus clarkii** (Bittner and Kopanda, 1973) and fresh water shrimp **Palaemonetes kadiakensis** (Stoffel and Herbschman, 1974).

In the present experiments, it was found that the average growth rate of total length in the eyestalkless prawns after every moult was about twice than that of the intact animals, and that the duration of the moult cycles of the former was about one fourth of that of the latter. From these data, the mean weight increase of the eyestalkless animals was larger than the corresponding weight of the control animals 30 days after the operation. Thus, it is clear that growth was accelrated by both bilateral and unilateral eyestalk ablation and that the secretion of moulting hormone was induced by the growth of the body.

The effect of eyestalk ablation on growth and moult of **Macrobrachium rosenbergii** was studied by Huang et al. (1981) during 105 days for aquaculture application. One or both eyestalks were removed and carapace length was measured every 15 days. In the group of bilateral eyestalk ablation, the moult frequency and growth were greatly accelerated than that of the unilateral eyestalk ablation group, and this in turn is higher than that of the controlled group.

Ponnuchamy (1981) studied the bilateral eyestalk ablation in the fresh water prawn **Macrobrachium lanchesteri** and showed that there was a high mortality in the unilateral eyestalk ablated prawns exhibited a high survival rate. He also observed that there was marked increase in the growth of bilateral eyestalk ablated prawns.

Rajeswari et al. (1982) investigated the effect of bilateral ablation of eyestalks on the moulting and neurosecretory activity in the crab **Uca annulipes**. He found that in immature crab, eyestalk ablation results in shortening of the intermoult period, initiation of unseasonal moulting,

increased activity in neurosecretory material in the neurosecretory cells and their concentration at optic stubs. Shortening of the intermoult period and initiation of moulting is due to the induction of the Y-organ to secrete moulting hormone.

Recent reviews on the metabolic events of decapod crustacea, and on the hormonal control of these events, have emphasized the fragmentary nature of our present knowledge. The present investigation is based on a study of laboratory population of the prawn **Penaeus kerathurus** (Leach), in which the content of glycogen was determined in samples drawn at intervals from the population.

The increase of the glycogen content in the hepatopancreas of operated prawns may be derived ultimately either from protein or carbohydrate or both. The fact that in ablated animals, the utilization of glucose is also increased, might lead one to place the site of presumed endocrine effect in the process of glycogenesis, rather than in that of glyconeogenesis (Scheer and Scheer, 1951). Therefore, it may be postulated that, after bilateral eyestalk removal there is a gradual transition from carbohydreate oxidation to glycogen synthesis as a major pathway of carbohydrate metabolism.

Previous results have given support to this work, conclusive evidence is lacking, that some endocrine hormone secretion in the eyestalk regulates glycogen metablism and that secretion of the above hormone stops after the operation of eyestalk amputation. This proposal was in agreement with that of Wang and Scheer (1963) who have emphasized that decapod eyestalk is known to contain a transglucosylase inhibitor which controls glycogen synthesis.

The results of the present investigation agreed with that of Menon and Sivdas (1967) for the crab, **Scylla serrata** (Forskal), they reported an acceleration in glycogenesis in the hepatopancreas after bilateral eyestalk extirpation. For the same species, Monin and Rangneker (1974) have also noted an increase in the glycogen content of the hepatopancreas after the operation.

It is reasonable to suppose that the demonstration of an increased glycogen content following eyestalk removal of the prawn **Penaeus kerathurus** (L) muscles may come as a definite addition to the long list of metabolic and other changes which are consequences of this operation.

The increase in the glycogen content as the animal approaches a moult was already known from the study of Renaud (1949) on **Cancer pagurus** and Schwabe et al. (1952) on spiny lobsters. Their data suggest that eyestalk removal in the intermoult period increases the total glycogen of the body. While the same operation in the premoult period result in no change. Neiland and Scheer (1953) suggested that the eyestalk acts to retrain intermediary metabolism in general. They postulated that the hormone tends to shift metabolism toward increased anabolism.

In contrast to the data presented in this study, Schwabe et al. (1952) reported that eyestalk ablation has no effect on the glycogen content of the hepatopancreas of crustaceans. A similar results was obtained by Mauviot and Castell (1976) showing that their tests on juvenile and adult American lobster Homarus americanus revealed no significant difference between ablated and control lobster in the glycogen content of the hepatopancreas.

Eyestalk ablation caused a reduction in carbohydrate out-put with an apparent tendency to glycogen. At the same time there was a significant reduction in blood sugars below the starvation death point (Scheer and Scheer, 1951). These results indicate that eyestalk ablation interferes with the mobilization of blood sugars from body reserves, and also with their utilization.

Naga and Kulkarni (1980) have studied the role of eyestalk hormone in the carbohydrate metabolism of a marine Penaeid prawn Parapenaeopsis hardwickii and they reported that bilateral eyestalk ablation has brought about a significant (P> 0.01) fall and rise in glycogen content in the hepatopancreas and abdominal muscle respectively. There was an obvious change in glycogen content of the hepatopancreas and abdominal muscle of normal prawns when injected with eyestalk extracts from the prawns in different moulting stages. Eyestalk extract from intermoult prawns caused a significant (P> 0.05) decrease and increase in the glycogen quantity in the hepatopancreas and abdominal muscle respectively. Eyestalk extract from premoult animals caused a decrease in the utilization of glycogen content in the hepatopancreas, while in postmoult prawns it leads to an increase in this utilization (Naga and Kulkarni, 1980).

Previous results (Abd El-Hamid, 1988) on Penaeus kerathurus (Leach) in relation to lipid and protein contents after eyestalk ablation together with the present data on glycogen on the same animal are in agreement with Kulkarni, et al. (1981), who showed that there was a significant (P> 0.05) augmentation in total glycogen and fat content and decrease in protein quantity in ovaries of prawn treated with brain and thoracic extracts as compared to the ovaries of control prawns. Variations in organic reserves of ovaries of eyestalkless prawns did not significantly (P> 0.05) differ from those in the control prawn Parapenaeopsis hardwickii.

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