

EFFECT OF EYESTALK EXTIRPATION ON LARVAL DEVELOPMENT OF THE SHRIMP, *MACROBRACHIUM LAMERRII*

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The effect of eyestalk extirpation on larval development of freshwater prawn, *M. lamerrii* was observed. Removal of only one eyestalk at any period during development did not alter the sequence of larval stages or molting frequency. Extirpation of both eyestalks prior to day 2, stage IV resulted in supernumerary larval stages and only the post molt intervals of post larval stages were shortened. The production of supernumerary larval stages and absence of acceleration in molting during larval stages in ablated larvae is discussed.

INTRODUCTION

Crustacean growth involves the interrelationship of molt interval and size increase that occurs from one stage to the next. The development of many crustacean larvae consists of successive molts with gradual morphological changes upto their first post larval (juvenile) stage. Little is known of the development of any endocrine system in crustacean larvae. The mechanisms which regulate crustacean larval molting cycles before and after eyestalk ablation are still poorly known relative to those of post larval.

More recent studies on the effect of eyestalk extirpation during larval development had indicated possible major differences in the larval endocrine system. No effect on larval molting cycle or time of metamorphosis on eyestalk extirpation was observed in larvae of the shrimp, *Palaemonetes* (Hubschman, 1963). Acceleration of metamorphosis to the first crab stage was observed in larvae of the shrimp, *Palaemonetes* (Hubschman, 1963). Acceleration of metamorphosis to first crab stage was observed in *Callinectes sapidus* when both eyestalks were extirpated in megalopa (Costlow, 1963). No effect on the duration of the stages was observed on bilateral eyestalk ablation in zoeal stages of the crabs, *Sesarma reticulatum* (Costlow, 1966a) and *Pisidia longicornis* (Le Roux, 1979). But frequency in molting was increased in megalopa stage.

On eyestalk ablation during larval development intermediate stages had resulted. Extra zoeal stages were observed by many crustacean endocrinologist, on destalking the eyestalks during development of many crabs and shrimps. They proposed the existence of a molt inhibiting hormone in eyestalks of larvae.

Of the larval endocrine regulation studies, much of the work is from Brachyura. The group Natantia is less attempted, may be for its more sensitivity during development. So to cover up the dearth of basic information in larval endocrine regulation, this piece of work was undertaken in larval stages of *Macrobrachium lamerrii* a sensitive freshwater prawn. This present study was attempted to determine how the time of eyestalk extirpation early in larval development effects the molting frequency of the larvae and early post larval stages. If supernumerary larvae could be induced and if so, is their occurrence associated with the time of eyestalk extirpation and any alterations in the normal sequence of molting, development and metamorphosis.

MATERIAL AND METHODS

Berried forms of *M. lamerrii* were procured from Kham river, near Aurangabad. The larvae hatched by 29-30th day of embryonic development were transferred to one liter beakers containing well aerated dechlorinated water. The water changed twice daily and fed with freshwater plankton.

For a single experiment larvae from a single day's hatch were routinely used. The larvae hatched were observed three times daily for the appearance of newly molted stage II larva. By stage II the larvae developed stalked eyes. From this stage onwards at convenient time intervals one or both eyestalks were extirpated. Larvae to be ablated were individually placed on a depression slide and the eyestalks were severed at their bases using an iris scalpel.

Two sets of animals were taken. In the 1st set one eyestalk was ablated on the last day of the II stage and the second eyestalk was severed in the early hours after its ecdysis to the IIIrd stage. In the 2nd set one eyestalk was extirpated at the end (last day) of the stage III and another at the early hours after its ecdysis to stage IV (last larval stage). These larvae were observed 3 times daily to determine the mean molting intervals from III to IV (IV stage molt interval) and IVth to post larval (post larval molt interval) stages.

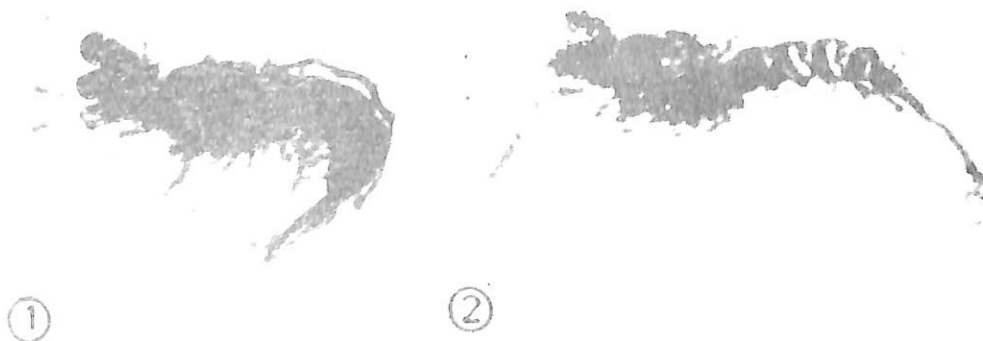
RESULTS

In three of the four replicate experiments the IV and post larval molt intervals were not shortened. But extra-intermediate forms were obtained with characters neither of their former nor later and showed increment in size.

In the first set an extra (intermediate) IV stage occurred. They had smaller antennae and chelaé as that of the III stage. The body and carapace length in extra (intermediate) IV stage increased (Fig. 1; Table I).

From the normal IV stage (Fig. 2; Table I), the exopods of pleopods and the lateral spines of the telson were as that in normal IV stage. They were observed to be consistently in a curled position with the abdomen flexed under the cephalothorax.

In the second set, when the eyestalks were ablated between III and IV stages, an extra post larval form occurred. Compared to the normal post-larva (5.8 and 1.5 mm) its body length and carapace (6.5 and 1.8 mm) increased. Its antenna and chelae length were as that in normal post larvae. The exopods of pleopods and the lateral spines of the telson were more reduced, as like in post larval stages. Some fringe setae were also apparent on the telson



Figs. 1-2. Effect of eyestalk ablation on development. 1. Normal stage IV larva $\times 100$; 2. Intermediate (extra) stage IV larva, carapace and body length increased $\times 100$.

as in post-larvae. But they were as numerous as in normal IVth stage. These extra post larval forms after 1 to 2 molts depicted an increase in molting, simultaneously decreasing the intermolt period. So only the post molt intervals of post larval stages were shortened.

Table I. Morphological changes in normal, unilateral and bilatera^l eyestalk extirpated larvae of *Macrobrachium lamerrii* at different stages of development.

Treatment	Carapace length (mm)	Body length (mm)
One eyestalked	4.8	1.2
Normal III stage	4.8	1.2
Extra stage	5.8	1.4
Normal IV stage	5.3	1.3
Extra stage	6.5	1.8
Normal postlarval stage	5.8	1.5

The intermediate stages were obtained to a greater % when the eyestalk extirpation was done in the early hours of a molt (Table II); when eyestalk extirpation was done within ten hours after a molt then almost all of them entered intermediate stage. As the time of extirpation went on increasing, a gradual decrease in the intermediates and a concomitant increase in their respective normal (succeeding stages was observed).

Table II. Morphological characteristics at the 4th stage of *M. lamerrii* larvae after eyestalk ablation at different times.

Time of extirpation (hrs)	One eyestalked III stage larvae (%)	IV stage (%) intermediate	Normal IV stage
2	12 (0)	12 (100)	0 (0)
5	14 (0)	14 (100)	0 (0)
8	15 (0)	14 (93)	1 (7)
12	16 (0)	14 (88)	2 (12)
24	16 (0)	6 (38)	10 (62)
36	15 (0)	3 (20)	12 (80)
48	15 (0)	1 (7)	14 (93)
Intact controls	25 (100)	0 (0)	25 (100)

Removal of only one eyestalk at any period during development did not alter the sequence of the larval stages nor accelerate the molting frequency of larval stages post larval stages. Removal of both eyestalks prior to the 2nd day of IVth (last larval stage) stage resulted in supernumerary larval stages. But acceleration of larval molts was not observed regardless of the time of extirpation. Only the post molt intervals of post larval stages were shortened.

DISCUSSION

In general, eyestalk removal induces precocious molting in decapod crustaceans. But this was not the case in larval stages. Removal of eyestalks at early hours of any larval molt did not depict acceleration of molting in larval stages but an acceleration in molting was observed in the post larval stages. When eyestalk extirpation was done only within 12 hours after the previous ecdysis, then a significant acceleration in molts of megalopa was observed in *Callinectes sapidus* (Costlow, 1963) and *Rithropanopeus harrisii* (Freeman & Costlow,

1980). The frequency in molting was accelerated in megalopa of *Sesarma reticulatum* (Costlow, 1960a), the anomuran *Pisidia longicornis* (Le Roux, 1979) and post larval stages of *Homarus americanus* (Mart *et al.*, 1986).

In addition to precocious molting, eyestalk removal also lead to the development of intermediate stages. 11nd megalopa stage occurred when larvae were destalked early in 11nd zoeae stage of *Rhithropanopeus harrisi* (Costlow, 1966a) and *Sesarma reticulatum* (Costlow, 1966b). 2nd to 4th larval periods in *Palaemon macrodactylus* (Little, 1969) and IV stage in *Homarus americanus* (Charmantier *et al.*, 1985; Mark *et al.*, 1986). Our results are in concurrence with the above, when destalking was done in the early hours of an ecdysis. An extra larval form was attained as a result of 4th molt when eyestalks were severed, one at the last day of II stage and the other at early hours of ecdysis to III stage. Another extra form was obtained when one eyestalk was severed at the end of III stage and the other in early hours of ecdysis to IV stage. After 1 to 2 molts these extra post larval animals depicted the shortening of the molt interval.

Costlow (1965) commented that these extra zoeal stages late in development were associated with a partial malfunction of the endocrine systems which control molting during zoeal development. Acceleration of molting in the later post larval stages of these eyestalkless larvae suggest that the X organ sinus gland complex contained in the eyestalks may be first activated. In keeping view with this concept removal of one eyestalk was done prior to activation of the X organ sinus gland complex (stage III larvae) and the 2nd was done before a regulatory level of molt inhibiting hormone could be attained (early hours of IV stage).

The products of this X organ sinus gland complex which is present within the eyestalks determine the rate of morphological changes associated with consistent number of larval stages and the rate of transition from final larval stages to the post larval stage and also the changes involved in metamorphosis. The accumulation (activity of this site of hormone to a certain level of activity in this site appeared to begin during the final larval stage (possibly day 2 of stage IV in *M. lamerrii*). If removal of both eyestalks occur prior to this point, normal transition, from final larval stage to post larval and its later stages were not possible. So consequently additional larval forms resulted at the time of the next molt, rather than the normal postlarval stage. Later these eyestalkless larvae also attained a degree of morphological development and were metamorphosing as in normal larvae. It can be suggested that the actual source of hormone is not located within the eyestalk but rather that the hormone is produced elsewhere and stored and released from a point within the eyestalk. So removal of this storage organ does not prevent but delays development and metamorphosis until a sufficient titre of hormone is again attained to permit the resumption of the normal pattern of development.

The absence of any acceleration of molting in larval stages suggests that not only is the X organ sinus gland complex nonfunctional during larval development but does not activated in this species until the late stages of post larval stages. The increase in carapace width of eyestalkless larvae during the first two post larval molts, without accompanying acceleration in molting frequency, is further substantiating the concept that molting and size increase are under the control of different mechanisms.

Removal of only one eyestalk at any point in development never resulted in extra forms or accelerated the molting rates upto the post larval stages.

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