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Endocrine regulation of larval ecdysis and metamorphosis of the prawn *Macrobrachium lamerrii*

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Abstract
Macrobrachium lamerrii passes through four larval stages and a juvenile stage before metamorphosing into the adult prawn. The larvae undergo six to eight molts during development before metamorphosis to the adult stage. Various physiological activities such as molting and reproduction are controlled by Neuroendocrine centres like eyestalk, brain and thoracic ganglion. Exposure of the larvae to extracts of eyestalks, brain, or thoracic ganglia brought about significant alternations in the molt frequency. The extracts of the brain and thoracic ganglia reduced the duration of the intermolt period while eyestalk extracts increased the intermolt duration. It was further observed that exposure of larvae to extracts of brain and thoracic ganglia brought about increase in size and weight of the larvae. So the eyestalk of *M. Lamerrii* is found to be composed of growth inhibiting hormones where as thoracic ganglion and bra in are composed of molt / growth accelerating hormones. The hormones of the adult *Macrobrachium lamerrii* are also found to be more potential than that of the juvenile *M. Lamerrii*.

Keywords: Juvenile, adult, neuro endocrine centres, extract effect, intermolt period, growth performance

1. Introduction
The crustacean body is unsheathed by a thick outer covering of cuticle called exoskeleton. Due to the presence of a rigid integument, somatic growth in crustacean can be achieved only through periodic shedding and reformation of cuticular sheath. This shedding of exoskeleton or molting forms the most important metabolic event which dominates the life cycle of these specific group of animals (Highman, K.C. and Hill, L. 1979) [5]. In adults a great deal of information has been accumulated and reviewed time to time describing the hormonal basis of molting in crustaceans. (Nagabhushnam, R. and Sarojini, R. 1986, Fingerman, M. 1987 and Vijayan, K.K. 1988) [7, 4, 11].
Relatively research on hormones and hormonal processes in decapods larvae is very scarce compared with what is known on these subjects in adult decapods. Decapods molt several times during their larval life. The larvae of decapods crustaceans are usually free swimming planktonic forms which hatch from eggs and go through several molts before metamorphosing to the juvenile stages.
Drach, P. (1939) [3] subdivided the molt cycle of adult decapods into five major stages or phases based on the hardness of the exoskeleton. Skinner, D.M. (1962) [9] divided this subdivision according to the structural changes in integumentary tissues. The primary event which characterize the molt cycle include completion of the new exoskeleton during post molt (stage A and B) and inter molt (stage C) and preparation for the next ecdycis is premolt (Stage D). The primary events of premolt are separation of the epidermis from the old cuticle (apolysis) and secretion of new exoskeleton. Stage E is ecdysis.
The duration of molt cycle is species specific. Decapods crustacean larvae undergo a series of molts culminating in metamorphosis. Ample of literature dealing with different metabolic processes by various endocrinological manipulations is there in adults. But crustacean larvae remained unexplored to such neuroendocrinological manipulations. Therefore the following study is undertaken to cover up the lacuna. Eyestalk, brain and thoracic ganglion extract addition experiments were carried out to determine the presence of morphogenetic hormones in the neuroendocrine centres and their regulation in molting.

Materials and Methods
Berried forms of *Macrobrachium lamerrii* were procured from Khan river, near Aurangabad.

By 29-30th day of embryonic development the larvae hatch out. These larvae were separated into 4 groups of 20 each. Each group was maintained in acrated glass beakers containing one liter of dechlorinated water.

Eyestalk, brain and thorasic ganglion were isolated from 20 intermolt mature (adult) *M. lamerrii* with the assumption of a molt inhibiting hormone was present, it would be present in greatest titter during this period of molt. Their extracts were prepared with a few ml of distilled water and centrifuged at 2000 rpm for ten minutes.

Four beakers were selected and named as control, experimental i.e. eyestalk, brain and thoracic ganglion. The prepared extracts were added right from the day of their release to post larval stage (15 days old). During this tenure, water was changed daily and larvae were fed with freshwater plankton.

Molting records were noted twice daily and dead animals if any were removed regularly. On 16th day they were fixed in 4% formalin. The weight and length of animals were recorded.

The experiment was carried out with both juvenile (immature) and adult (mature) stage prawn neuroendocrine center extracts. The difference in the neuroendocrine center potentialities of juvenile (immanure) and adults (mature) were recorded.

Results and Discussion

Larvae of *Macrobrachium lamerrii* molt very frequently upto metamorphosis. After the metamorphic molt the frequency in molting was decreased. The 6th molt was the metamorphic molt in these larvae of *M. lamerrii*. The results are tabulated in Tables 1 – 5.

Table I: Effect of different Neuroendocrine center extracts on the intermolt duration of larvae of freshwater prawn *M. lamerrii*.

Sl. No.	Experimental condition	Intermolt period in hours
1.	Control	40-46
2.	Eyestalk extract	45-54
3.	Brain extract	30-32
4.	Thoracic ganglion extract	20-24

The larvae *Macrobrachium lamerrii* molt very frequently upto metamorphosis. After the metamorphic molt, the frequency in molting was decreased. The sixth molt was the metamorphic molt in these larvae of *M. lamerrii.* The intermolt duration in controlled animals was 40-46 hours (Table 1.). As indicating in Table 1. the intermolt duration in thorasic ganglion (20-24 hours) and brain (30-32 hours) extract added animals, was considerable decreased. The decrease was more significant in thorasic ganglion (20-24 hours) extract added larvae than the brain (30-32 hours) extract added larvae. Whereas it was the reverse in the case of eyestalk extract added larvae. As tabulated in the table 1.the eyestalk extract added larvae exhibited increase (45-54 hours) in their intermolt duration.

Table 2: Effect of various neuroendocrine center extracts from juveniles (immature) of *M. lamerrii* on the body length in larvae of *M. lamerrii*.

Sl. No.	Experimental condition	Length (mm)
1.	Control	5.3
2.	Eyestalk extract	5.0
3.	Brain extract	5.7
4.	Thoracic ganglion extract	5.9

Table 3: Effect of various Neuroendocrine center extracts from adults (mature) of *M. lamerrii* on the body length in larvae of *M. lamerrii.*

Sl. No.	Experimental condition	Length (mm)
1.	Control	5.3
2.	Eyestalk extract	4.9
3.	Brain extract	6.0
4.	Thoracic ganglion extract	6.4

Table 4: Effect of various Neuroendocrine center extracts from juveniles (immature) of *M. lamerrii* on the body weight (mgs) in larvae of *M. lamerrii.*

Sl. No.	Experimental condition	No. of animals taken	Weight (mgs)
1.	Control	10	2.5 ± 0.11
2.	Eyestalk extract	6	2.35 ± 0.04
3.	Brain extract	8	2.50 ± 0.08
4.	Thoracic ganglion extract	10	2.65 ± 0.035

Table 5: Effect of various Neuroendocrine center extracts from adults (mature) of *M. lamerrii* on the body weight (mgs) in larvae of *M. lamerrii.*

Sl. No.	Experimental condition	No. of animals taken	Weight (mgs)
1.	Control	10	2.5 ± 0.11
2.	Eyestalk extract	6	2.30 ± 0.02
3.	Brain extract	8	2.70 ± 0.03
4.	Thoracic ganglion extract	10	2.79 ± 0.02

It is a well known fact that molting in crustacean decapods is under endocrine and neuroendocrine regulations. There are many reports for this evidence. Existence of molt inhibitory factor in eyestalk was demonstrated by various authors, using eyestalk ablation process (Snyder, M.J. Chang, E.S. 1986 and Vijayan, K.K. 1988) [10, 11]. Anilakumar, G. and Adiyodi, K.Q. (1980) [1], Hiroshi, T. Yoshihiro, Y. And Naokuni (1986) [6] and Nagabhushnam, R. and Sarojini, R. (1988) stated that there is a stimulating hormone in central nervous system (brain and thoracic ganglion). Our results are also in concurrence with these above statements. In our results the eyestalk extracts inhibited the frequency of molting and growth (size and weight); whereas the brain and thoracic ganglion extracts exhibited the acceleration in frequency of molting and growth (size and weight), thus indicating that the central nervous organs outside the eyestalks are source of molt (growth) accelerating substances, Sangvikar, P. (1980) in *Caridina rajadhari*, Challa, V.R. 1984 in *M. kistnensis* and Vijayan, K.K. (1988) [11] in *Penaeus indicus* declared the same pattern.

Increase in length and weight (molting frequency i.e. growth) of larvae treated with thoracic and brain extracts of juveniles (Table 2 and 4) and adults (Table 3 and 5) were revealing the presence of morphogenetic hormones.

Decrease in length and weight of larvae treated with eyestalks extracts of juveniles (Table 2 and 4) and adults (Table 3 and 5) were revealing that growth inhibiting hormones are existing in eyestalk.

Molt acceleration (growth) of thoracic ganglion extract was higher than that of brain extracts, which is again revealing that more potential morphogenetic hormones or more number of morphogeneric hormones are present in thoracic ganglion than in brain (Table 2 to 5).

The results of neuroendocrine extracts of juveniles

Macrobrachium lamerrii are similar to that of the results of adult neuroendocrine extracts but the results of adults (Table 3 and 4) neuroendocrine extracts were more significant than that of juvenile extracts (Table 2 and 4). This may be due to the fact that neuroendocrine hormones or factors in matured forms are more potential than that of juveniles (immature).

Conclusion

From the above results, it may be concluded that the multiregulatory complex (eyestalk) is composed of inhibitory morphogenetic hormones. Brain and thoracic ganglion are having stimulatory morphogenetic hormones or factors. And also that the thoracic ganglion of adult (mature) is having more potential or more number of morphogenetic hormones. Lastly it is also concluded at the nueroendocryne hormones are factors in matured forms are more potential than that of juveniles.

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