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MORPHOLOGICAL CHANGES IN THE DEVELOPMENT OF THE OVARY IN THE EYESTALK ABLATED ESTUARINE CRAB, *SCYLLA SERRATA* (FORSKAL)

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ABSTRACT

The morphological changes in the development of the ovary as a result of eyestalk ablation have been studied in the edible crab, *Scylla serrata* (Forskal) in the laboratory condition. Changes in four stages in the development of the ovary have been described. They are immature, maturing, ripe and spent. It was seen that eyestalk ablation in *Scylla serrata* (Forskal) would speed up the maturation of the ovary. In order to arrive at this conclusion, the eyestalks of the female were ablated. A piece of the ovary was taken out by making a small window on the carapace for knowing the condition of the ovary at the start of the experiment and another piece of the ovary of the same animal was again taken for biopsy at the end of the experiment.

INTRODUCTION

The crustacean eyestalks, especially in brachyurans, contain an endocrine organ, the X–organ sinus gland complex, hormonal secretions of which has been found to be responsible for varied physiological functions of the body. This has been amply supported by the observation that the removal of the eyestalk in crabs bring about many functional disturbances (Abramowitz and Abramowitz, 1939, Abramowitz and Abramowitz, 1940; Kleinholz and Bourquin, 1941; Bliss, 1951; Passano, 1951). One among them is the finding that ablation of the eyestalks result in enhanced ovarian development (Hard, 1942, Brown and Jones, 1949; Vernet Cornubert, 1964; Weitzman, 1964; De Leersnyder, 1967; Adiyodi and Adiyodi, 1970; Kaestner, 1970). This finding undoubtedly aroused the curiosity to find out in *Scylla serrata* (Forskal), a commercially important crab of the estuaries of India, whether ablation of the eyestalks will bring about the growth of the ovary from immature to a mature stage within the shortest time and also whether it is possible to induce spawning. Once spawning is achieved in a short span, by rearing the eggs in the laboratory to the first crab stage, as done by Ong (1966) and further rearing of juveniles, a large scale farming of this edible species can be envisaged.

The experiment described here, is preparatory to further studies on the histology, histochemistry and biochemistry of the ovary of *Scylla serrata* (Forskal).

MATERIAL AND METHODS

Females of *Scylla serrata* (Forskal) of almost uniform size were collected from the Cochin backwaters and were kept in the laboratory for five days for accli-

matization in an aquarium, maintained at a salinity of 29± 1 °/₀₀ at room temperature. After acclimatization, the carapace was cut open on one side and a window was made and a piece of the ovary was taken for biopsy. Later the window was sealed by replacing the cut piece. Using plaster of Paris and cotton plug, sealing was made water tight. Three days after the first biopsy, the animals were taken out, the eyestalks ligatured and subsequently ablated. The animals were kept in the open air to give enough time for the blood to clot, so as to avoid excessive bleeding. Later on the animals were released to the individual aquarium, which was pretreated with the antibiotic, benzyl chloride penicillin, at a ratio of 10,00,000 units: 15 litres, to prevent bacterial action. The water was changed daily. Every day they were fed with fresh prawn meat. Depending upon the initial stage of maturity of the ovary, as decided at first biopsy, the window was again opened, either at the end of second week or third week and a piece of ovary was again taken for biopsy. Appropriate number of crabs were maintained as control animals.

Though the crabs used for this experiment were almost uniform in size, the stages of maturity of the ovary were found to be different. Preliminary studies indicated that in a group of uniform sized crabs, the stages of the ovary may vary from immature to mature or even to spent stage. Hence, the reason for taking a biopsy prior to ablation of the eyestalks to determine the initial stage of the ovary and a final biopsy at the end of the experiment, so that it was possible to determine the growth taken place during the experimental period.

It has also been found that the mortality rate was very high after eyestalk ablation. Of the 20 experimental crabs only five animals survived till the completion of the experiment. The remaining once died at varying intervals, after ablation, making it rather difficult to use the results for analysis. This might be due to excessive bleeding after ablation, despite all the care taken. Another reason for mortality may be the bacterial attack. However, after using the antibiotic, benzyl chloride penicillin, the mortality rate has been found to be reduced though not significantly.

RESULTS

It is seen from the results of the above experiments that in the eyestalk ablated crabs, the food intake increases when compared to that of the controls. Also the rate of oxygen consumption seems to have increased as indicated by the rapid rhythmic movements of the scaphognathites, which is generally taken as an index of oxygen consumption (George, 1968).

The results obtained from these five crabs which lasted until the completion of the experiment, are quite interesting and worth mentioning. In all the five crabs, the ovary although in different stages of development, showed on biopsy, a further rapid growth after eyestalk removal.

For the convenience of describing the growth, the ovary is classified morphologically into the following four different developmental stages. They are immature, maturing, ripe and spent.

Immature: The ovary at this stage appears as a pair of thin creamy (whitish yellow) filament lying dorsally on either side of the alimentary canal and covered over by a thin peritonial membrane.

Maturing: The maturing ovaries increase in size and begin to extend to the periphery, both laterally and anteroposteriorly, almost occupying the entire space on the dorsal side of the thoracic region. Simultaneously the colour also changes from creamy to golden yellow. The oocytes become fairly well developed.

Ripe: The ripe ovary is packed with fully matured oocytes, bright orange in colour. When the carapace is opened, it would appear that the entire thoracic region contains only the ovaries.

Spent: At this stage, subsequent to spawning, the ovaries shrink in size to a pair of dull orange coloured filaments. These filaments at some places contain a few individual maturing oocytes, which were not released at the time of spawning. The initial stage of the ovary and condition of the same at the end of the experiment is given in Table I.

Table I.

Crab No.	Date of 1st biopsy	Condition of ovary	Date of ablation	Date of 2nd biopsy	Condition of ovary	Remarks
1.	30-9-1974	Ripe	3-10-74	21-10-74	Spent	Spawned on 10-10-1974
2.	24-2-1975	Mature	27-2-75	13-3-75	Ripe	-
3.	1-4-1975	Immature	4-4-75	21-4-75	Mature	-
4.	16-4-1975	Mature	19-4-75	3-5-75	Ripe	-
5.	4-8-1975	Immature	7-8-75	20-8-75	Mature	-

Crab No. 1 when subjected to first biopsy, was found to have a ripe ovary. Three days after the first biopsy, the eyestalks were ablated. The crab spawned after one week from the date of ablation performed. The second biopsy was conducted after 18 days. The ovary was found to be in spent stage. One of the significant observations is that, the eggs after spawning failed to get attached to the pleopods as do in normal ones, and are found to be scattered at the bottom of the experimental tank.

Crab No. 2 on first biopsy, showed a mature ovary. The eyestalks were ablated after three days. The second biopsy was performed after 14 days, following ablation when the condition of the ovary was found to be ripe.

Crab No. 3 was found to have an immature ovary, when subjected to first biopsy. After three days the eyestalks were ablated. The second biopsy was performed 17 days after ablation. The ovary was found to be mature.

Crab No. 4 when subjected to first biopsy, was found to have a mature ovary. After three days the eyestalks were ablated. The second biopsy was conducted

after a period of 14 days of survival following ablation. The ovary was in ripe stage.

Crab No. 5 was found to have an immature ovary at the time of first biopsy. The eyestalks were ablated after three days. The second biopsy was performed after 13 days following ablation. The ovary was found to be mature.

Control animals throughout the period of the experiment, did not show any change in the development of the ovary and remained in the same condition as at the time of the beginning of the experiment.

DISCUSSION

The increased food intake and the increase in the rate of oxygen consumption indicate that in *Scylla serrata* (Forskal), the eyestalk removal brings about an enhanced requirement of energy either for normal continuance of its physiological functions or for certain specific activities which has been triggered as a result of hormonal deficiency due to eyestalk ablation. Marshall and Orr (1960), have pointed out a correlation between the food intake and oxygen consumption. Oxygen consumption must be assumed to be an approximate measure of the metabolism of the animals and thus also of the food absorbed through the alimentary canal.

Subsequent to eyestalk ablation in *S. serrata* (Forskal), the ovary irrespective of the stage shows a tendency to develop further. Visual observations show that the immature ovary will become mature, the mature will become ripe and in some cases where the experimental crab has a ripe ovary, spawning takes place after eyestalk ablation. In *Pachygrapsus marmoratus* (Vernet Cornbert, 1964) eyestalk ablation results in the development of the ovary irrespective of the season. De Leersnyder (1967), while studying the effect of salinity and eyestalk ablation on the moulting and ovarian development in *Eriocheir sinensis*, observed that while salinity variation did not have any effect on the ovarian growth, eyestalk ablation, no doubt, brought about a significant development of the ovary when compared to that of controls.

Cook (1974) has observed that the maturing specimens of *Metapenaeus brevicornis*, when held in aquaria after eyestalk ablation, developed the ovaries to maturity and spawned. Alikunhi, Poernomon, Adisukresno, Budiono and Busman (1975) while studying the effect of eyestalk ablation in *Penaeus monodon* and *Penaeus merguiensis* have observed that the relatively small specimens of *P. monodon* of length 100–110 mm and about 4 months old attain maturity in aquaria in a month after eyestalk removal. In the case of *P. merguiensis*, the female reached full maturity within 8–12 days after operation. Studies conducted by the Prawn Maturity Team of *SEAFDEC* (1976) has, however, reported a longer period for the maturation of the ovary of the eyestalk ablated *P. monodon*.

In the present experiment, when the eyestalk ablation was performed in crab having a ripe ovary, spawning took place. However, the released eggs failed to get attached to the pleopods. Brown and Jones (1964) have also shown a similar phenomenon in *Uca pugilator*, where after eyestalk ablation, eggs of the spawned crab did not get attached to the pleopods. It is quite possible to presume that the

liberation of the entire lot of eggs into the water, without being attached to the pleopods might be due to the lack of a certain specific hormone contained in the X-organ sinus gland complex of the eyestalk, which the eyestalkless crabs are deprived of. However, it has been occasionally observed that during spawning in normal *S. serrata* (Forskal), some of the eggs remain unattached to the pleopods and sink to the bottom of the experimental tank. Ong (1966) observed that of about two million eggs liberated by *S. serrata* (Forskal), only $\frac{1}{3}$ got attached to the pleopods. The remaining that have been fallen into the aquarium, were found to develop and hatch when placed in aerated sea water.

Various methods have been chosen to indicate the stage of the ovary. Brown and Jones (1949) have taken the weight of the ovary as the index of maturity in the fiddler crabs of same size. De Leersnyder (1967) has taken the dimension of the ovary as the maturity index in *Eriocheir senensis* of the same size. In the present experiment on the eyestalk ablated *S. serrata* (Forskal), since the ovary has been watched right from the initial stage to the end of the experiment in one and the same crab by making the window, visual observation was found to be sufficient to indicate the rapid growth of the ovary.

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REFERENCES

Abramowitz, R. K. and A. A. Abramowitz, 1939. Moulting and viability after removal of the eyestalk in *Uca pugilator*. *Biological Bulletin*, Woods Hole, **77**: 326-327.

Abramowitz, R. K. and A. A Abramowitz, 1940 Moulting, growth and survival after eyestalk removal in *Uca pugilator*. *Biological Bulletin*, Woods Hole, **78**: 179-188.

Adiyodi, K. G. and Rita G. Adiyodi, 1970. Endocrine control of reproduction in decapod crustacea. *Biological Review*, **45**: 121-165.

Alikunhi, K. H., A. Poernomon, S. Adisukresno, M. Budiono and S. Busman, 1975. Preliminary observations on induction of maturity and spawning in *Penaeus monodon* Fabricius and *Penaeus merguiensis* De Man by eyestalk extirpation. *Bulletin of Shrimp Culture Research Centre*, **I** (1): 1-11.

Bliss, D. E., 1951. Metabolic effect of sinus gland or eyestalk removal in the land crab, *Gecarcinus lateralis*. *Anatomical Record*, **111**: 502.

Brown, F. A. and G. M. Jones, 1949. Ovarian inhibition by a sinus gland principle in the fiddler crab. *Biological Bulletin*, Woods Hole, **96**: 228-232.

De Leersnyder, M., 1967. Influence de la salinité et de l' ablation des pédoncules oculaires sur la mue et sur le dévelopement ovarien d' *Eriocheir sinensis* H. Milne Edwards. *Cahiers De Biologie Marine*, **8**: 421-435.

George, M. J., 1968 The effect of salinity changes on the weight and respiratory rate of the crab, *Portunus sanguinolentus* (Herbst). *Crustaceana*, **14**(2): 164-168.

Hard, W. L., 1942. Ovarian growth and ovulation in the mature blue crab, *Callinectes sapidus* Rathbun. *Chesapeake Biological Laboratory Publication*, **46**: 3–17.

Kleinholz, L. H. and E. Bourquin, 1941. Effects of eyestalk removal on decapod crustaceans. *Proceedings of National Academy of Sciences*, Washington, **27**: 145–149.

Ong, K. S., 1966. Observations on the post larval life history of *Scylla serrata* Forskal, reared in the laboratory. *The Malaysian Agricultural Journal*, **45**(4): 429–443.

Passano, L. M., 1951. The X-organ sinus gland neurosecretory system in crabs. *Anatomical Record*, **111**: 86-87.

SEAFDEC, 1976. Development of a brood stock of the tiger prawn, *Penaeus monodon* Fabricius. Technical Report No. 1, February; 1–16.

Vernet Cornubert, C., 1964. Comparaison des effets exercés par l' ablation des pédoncules oculaires et par la régénération intensive sur la ponte et les caractéres sexuels externes de *Pachygrapsus marmoratus* (Fabricius). *Bulletin de la societe zoologique de France pour l' annee*, p. 243–253.

Weitzman, M. C., 1964. Ovarian development and moulting in the tropical land crab, *Gecarcinus lateralis* (Freminville). *American Zoology*, **4**(3): 329–330.