

## HISTOLOGICAL CHANGES IN THE OOCYTES OF THE ESTUARINE CRAB, *SCYLLA SERRATA* (FORSKAL) AFTER EYESTALK ABLATION

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### ABSTRACT

The effect of eyestalk extirpation on the development of the oocytes in *Scylla serrata* (Forsk.) a commercially important edible crab of the estuaries, brackish and backwaters, has been studied. Histological sections of the ovary, from one and the same crab during the course of the experiment, show that the animals having immature ovary reach the maturing stage and those with mature ovary become ripe or occasionally even spawn in the laboratory within a short time following eyestalk ablation. The size of the oocytes before and after extirpation of the eyestalk has been studied and the sequence of events taking place in the ovary has been shown with the aid of microphotographs.

### INTRODUCTION

The hormonal secretions of the X-organ sinus gland complex, located in the eyestalks of decapods, is responsible for regulating many physiological functions of the body (Adiyodi and Adiyodi, 1970). One among these functions is the inhibitory activity of the hormones on the development of the ovary. (Aoto and Nishida, 1956; Alikunhi, Poernomon, Adisukresne, Budiono and Busman, 1975; Arnstein and Beard, 1975; Carlisle, 1953; Monique, 1972; Seafdec, 1976). Studies on the immature *Scylla serrata* (Forsk.) have shown that eyestalk ablation could induce unseasonal growth of the ovaries (Rangneker and Deshmukh, 1968). However, their studies confined only to immature crabs and the investigation was limited upto the beginning of vitellogenesis. Attention has not been paid to determine whether extirpation of the eyestalks would result in changes in the ovary, leading to spawning in adult *Scylla serrata*. Once these crabs can be made to spawn in laboratory conditions, mass culture of this edible species could be envisaged. With this view in mind, the eyestalk ablation was conducted on adult crabs and the histological changes were studied.

### MATERIALS AND METHODS

Females of *Scylla serrata* (Forsk.) were acclimatised in the laboratory aquarium for five days, kept at room temperature and a salinity of  $29 \pm 1$  ppt. As far as possible, crabs of same size were used for the experiment. Adult crabs of the same size may have the ovary in different stages of development and therefore, prior to the experiment, the condition of the ovary was noted by making a window, as described earlier (Simon and Sivadas, 1978). Pieces of ovary were fixed in Bouin's or Zenker's

fluid. After processing, sections cut at  $5\mu$  thickness were stained in Deiafied's or Heidenhain's iron haematoxylin. An appropriate number of crabs were maintained as control. The stages (immature, maturing and mature) are fixed arbitrarily.

### RESULTS

Although the experiments were repeated with 25 crabs, only three experiments in which the crabs were found to be in three different stages of ovarian development, have been chosen for elucidating the result.

*Table 1.* Changes in the ovary of *S. serrata* after eyestalk ablation.

Initial stage of the ovary	Size of oocyte at the initial stage (mm)		No. of days survived after eyestalk ablation	Stage of ovary at the end of experiment	Size of oocytes at the end of the expt. (mm)	
	Range	Average			Range	Average
Exp. 1. Immature	0.02-0.06	0.038	14 days	Maturing	0.05-0.12	0.082
Exp. 2. Maturing	0.04-0.12	0.074	18 days	Mature	0.16-0.24	0.193
Exp. 3. Mature	0.08-0.17	0.195	17 days	Ripe	0.22-0.28	0.256

From table 1 it is clear that the oocytes of all the three crabs described here showed a remarkable growth after eyestalk ablation.

*Experiment 1.* The oocytes are immature at the beginning of the experiment (Fig. 1A). The follicular epithelium, covering the oocytes is not very distinct. Each oocyte is surrounded by a thin outer cell membrane. The cytoplasm is feebly stained in the periphery, where minute yolk vesicles appear, marking the commencement of vacuolization. However, the cytoplasm immediately surrounding the nucleus appears denser, finely granular and more darkly stained as though pushed from the periphery to the centre, as a result of the development of yolk vesicles. The central nucleus occupies the major portion of the oocyte and is seen separated from the surrounding denser cytoplasm by the nuclear membrane. Both the cell membrane and the nuclear membrane are deeply stained. The nucleolus appears as a dark spheroid body. The chromatin material is granular and stains deeply. In some of the oocytes, a darkly stained spherical body, the yolk nucleus, is found embedded in the periphery of the cytoplasm in a clear space (Fig. 1B). In some others, the yolk nucleus is found close to the nuclear membrane (Fig. 1B). In none of the oocytes, mitosis could be observed.

At the end of the experiment, after eyestalk ablation, the oocytes show further development (Fig. 1C). The cell membrane appears much thicker. The follicular epithelium surrounding the oocytes is clearly seen with distinct nuclei. The cytoplasm has moved further into the centre and even in the densely granulated central cytoplasm layer, described earlier, vacuolization has begun. Yolk globules of different size appear in the yolk vesicles. At this stage, the distribution of the yolk globules does not show any specific pattern. In the interstices of these maturing oocytes, a few immature oocytes are also seen, each having a dense cytoplasm, a prominent nucleus and a darkly

stained nucleolus (Fig. 1D). The yolk nucleus is not clearly seen. The nucleus is distinct. The chromatin material appears like strands and the nucleolus is darkly stained. Mitosis is not observed in the sections examined.

*Experiment 2.* At the commencement of the experiment, the oocytes are maturing (Fig. 1E). The yolk vesicles, which made their initial appearance at the periphery of the cytoplasm during immature stage, are now seen laden with yolk, the smaller yolk globules have now moved centripetally and as a result, the cytoplasm moves further towards the centre and also into the interstices of these yolk globules. The yolk globules appear as dark spheroid granules, when stained in Heidenhain's iron haematoxylin. Larger yolk granules are found more at the periphery than at the centre of the oocyte. The nuclear membrane and the chromatin material are poorly stained while the nucleolus is deeply stained. The yolk nucleus is not observed at this stage. Follicular epithelium is seen clearly, enclosing nuclei and nucleoli. Mitosis is not observed in any of the sections. At the culmination of the experiment, the oocytes show further deposition of yolk globules and most of the oocytes seem to have laden with yolk, excepting at the centre, where a small quantity of cytoplasm is seen enclosing a shapeless nucleus (Fig. 1F). The nucleolus is not clearly visible. In some of the oocytes, the accumulation of the yolk is so large that, even the cytoplasm is not visible perhaps having dispersed in the interstices of the yolk globules. Follicular epithelium is thick and conspicuous. Mitosis stages are not observed in the oocytes.

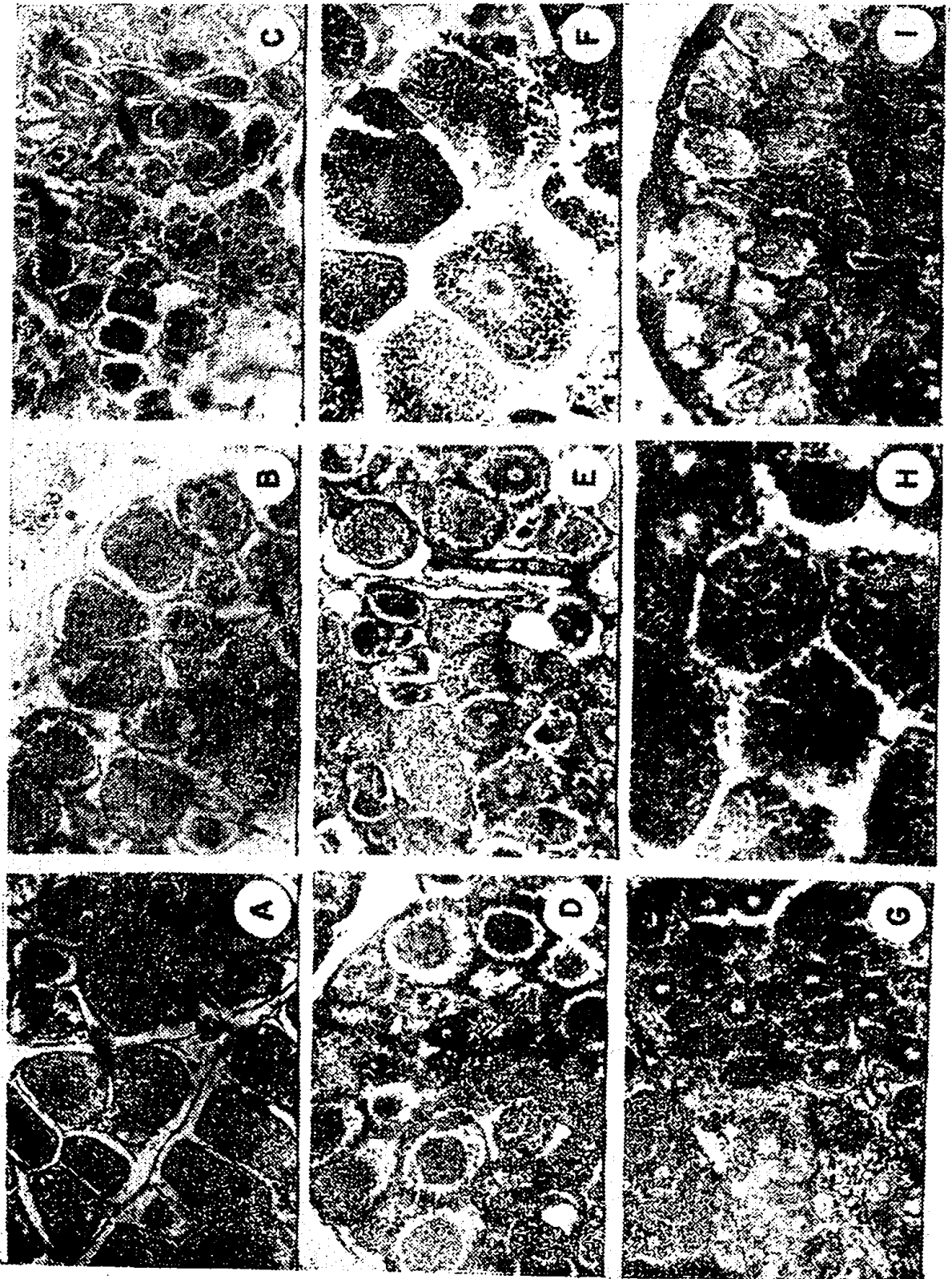
*Experiment 3.* The oocytes at the beginning of the experiment are seen to be in the mature stage (Fig. 1G). The darkly stained yolk granules, which were initially at the periphery in the immature ovary are now seen occupying the entire cytoplasm. The cell membrane and the nuclear membrane are also stained. The nucleus with the nucleolus is prominently seen. There is no indication of yolk nucleus.

The major changes, that could be seen at the end of the experiment, is the total deposition of the yolk globules in the oocyte (Fig. 1H). Each yolk globule has enlarged considerably in size with the result no cytological details excepting the cell membrane of the oocyte is visible. This stage could be described as the ripe stage since further developmental stages could not be seen because the experimental crabs spawned in the laboratory soon after this. Here again none of the oocytes show mitosis.

During spawning, most of the fully matured eggs are shed. The liberated eggs do not get attached to the pleopods (Simon and Sivadas, 1978). The eggs have close resemblance to those of *Neptunus sanguinolentus* (George, 1963). Sections of the ovary during the spent stage show mostly immature oocytes (Fig. 1I). Occasionally, a few oocytes having one or two yolk globules are visible. Some of the oocytes also show the onset of vacuolization. The oocytes do not show any breakup or reabsorption, indicating atresia. The control animals do not show any visible histological changes during the experimental period.

#### DISCUSSION

It has been observed that there is an accelerated development of the ovary in *Scylla serrata* (Forsk.) when the eyestalks are removed. (Rangneker and Deshmukh, 1968; Simon and Sivadas, 1968). Due to the extirpation of the eyestalks, the immature ovary reaches the maturing stage and the maturing becomes ripe faster. This is indicated



by the increase in size of the oocytes and in the quantity and size of the yolk granules deposited. Therefore, there is an indication that the vitellogenesis is very precocious after eyestalk ablation. Rangneker and Deshmukh (1968) have also observed an increase in weight of the ovary along with the increase in the size of the oocytes of immature *S. serrata*, after bilateral eyestalk ablation. Brown and Jones (1949) have observed that in fiddler crab, *Uca pugilator*, the average fresh weight of the destalked animals increased approximately linearly with time. In the shrimp, *Palaemon serratus*, the ovaries are quiescent during the summer in normal specimens and vitellogenesis proceeds rapidly in October. However, when the eyestalks are removed in them, during the quiescent summer period, vitellogenesis onsets soon (Panouse, 1943). Alikunhi, Poernomon, Adisukresne, Budiono and Busman (1975) have observed rapid development of the ovary in *Penaeus monodon* and *Penaeus merguensis*, when both eyestalks were ablated. Unilateral eyestalk ablation did not show any visible effect in them. Seafdec technical report (1976) pointed out that in *Penaeus monodon*, the development of the ovary was more rapid in bilaterally ablated females than in the unilaterally ablated ones.

From the beginning of the maturation of the ovary till it is completely laden with yolk, the nucleus is visible in the oocyte of *S. serrata* unlike the case in the development of some fishes. In none of the stages during the developmental process of the oocytes, the nucleolus has either extruded as such or dispersed through the nuclear membrane as fine granules, though such a phenomenon has been reported by Gresson (1929) in *Tenthredinids*, Wheeler (1924) and Narain (1937) in the fish *Pleuronectes* and *Saccobronchus*, *Clarius* and *Auabas* respectively; Swift, Rebhun, Rasch and Woodard (1956) in clam oocyte and Venugopalan (1962) in the developing oocytes of the fish *Ophiocephalus striatus* have observed that the nucleoli increase in their size from the early stage upto the commencement of vitellogenesis, indicating the possibility of their participation in yolk deposition. In *S. serrata*, however the participation of the nucleolus in the formation of the yolk could not be conclusively proved, as nucleolus is found to be present right from the immature to the mature stage. In the ripe oocytes these are not visible perhaps, being overlaid with yolk.

In *S. serrata*, it is to be presumed that after eyestalk ablation, the proliferation of new crop of oocytes is rapid since in experimental crabs kept in the laboratory aquaria, no trace of atresia could be observed after spawning. On the contrary, the ovary of the spent crab contained immature and maturing oocytes; the latter perhaps being in the process of development while the spawning took place.

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← Fig. 1. A Section of the ovary prior to the experiment, showing the immature oocytes. Note the formation of minute yolk vesicles at the periphery. X320. B Section of the ovary showing the yolk nucleus, one at the periphery and another close to the nuclear membrane. X320. C Section of the ovary showing the onset of yolk deposition after eyestalk ablation. X100. D Section of the ovary of the same crab showing a few immature oocytes in the interstices of the maturing oocytes. X320. E Section of the ovary showing the maturing oocytes before eyestalk ablation, Note the deposition of yolk globules in the periphery. X420. F Section of the ovary at the end of the experiment showing maturing oocytes, laden with yolk globules. Note the shapeless nucleus. X400. G Section of the ovary before the commencement of the experiment showing oocytes, heavily laden with yolk. X100. H Section of the ovary showing ripe oocytes. The yolk globules are so densely packed that even the nucleus is not seen. X400. I Section of the spent ovary showing the immature and the maturing oocytes. X 20.

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