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INDUCED SPAWNING OF THE ADULTS AND LABORATORY REARING OF THE LARVAE OF THE EDIBLE OYSTER *CRASSOSTREA MADRASENSIS* (PRESTON)

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ABSTRACT

The paper describes the results of an early experiment on spawning of *Crassostrea madrasensis* and larval rearing in the laboratory. When the gonad was ripe the female readily spawned in the presence of milt from the male oyster in the surrounding water. The results of stimulation by the use of aqueous solutions of chemical substances showed erratic trends.

The fertilised eggs developed into the straight hinge veliger in 12 to 16 hours in a salinity medium of 28 to 30‰ in sea water at a temperature range of 22 to 25°C in the culture bowls. Phytoplankton isolated from the plankton and cultured separately in fingerbowls formed the food of the larvae. The larvae did not show uniform growth. When some of them were well grown to pediveliger stage at the end of 19 days after fertilization, the majority of them remained small and did not grow beyond the early unbonal stage. The fully formed pediveliger after a free swimming existence and occasional creeping at the bottom of the fingerbowl finally settled with the left valve down, cementing it to the glass bowl by a secretion excluding from the foot glands to lead a sedentary life as spat.

INTRODUCTION

INDUCING the adult oyster *Crassostrea madrasensis* to spawn, raising the fertilized eggs to larval stages and rearing the latter to set as spat involves standardisation of techniques which when perfected will ensure increased production to levels to which adequate facilities are built up. At an experimental level, the present writer succeeded in raising fertilized eggs through larval stages to settlement of spat in *Crassostrea madrasensis* for the first time while he was in service at the Central Marine Fisheries Research Institute and this report deals with the methods adopted with brief descriptions of the developmental stages.

MATERIAL AND METHODS

Crassostrea madrasensis were obtained from the Adyar Estuary and Ennur Backwater near Madras during the breeding season (November-December) which has been previously ascertained in the course of separate investigations (Rao, 1951, 1956). Oysters, exceeding 5 cm in long axis and in apparent healthy condition, were cleaned and kept in glass troughs in sea water with salinity adjusted to that of the environment from which they had been collected, by adding required amounts of distilled water. The oysters were fed on an abundance of algal cultures prepared in pastuarised sea water to which soil extract, sodium nitrate and sodium phosphate were added. In the absence of mono-algal stock cultures a few phytoplankters microscopically examined and isolated from inshore plankton were introduced to the culture medium in which they thrived and multiplied in profusion.

Chemical stimulation was tried by injecting the edge of the mantle of the oyster with extremely weak aqueous solutions of potassium chloride, ammonium chloride, ammonium hydroxide and calcium chloride ; of them calcium chloride failed to induce spawning in all cases and the other three solutions produced erratic reactions with majority of the oysters not responding.

Male oysters readily spawned with the increase in water temperature upto 35°C and females only occasionally. It has been observed that the best inducement for the female oyster was afforded by the presence of sperm in the surrounding water. Male oysters have also reacted in a similar manner to the eggs of the oyster introduced into the water. Spontaneous spawning was observed on occasions, soon after the water in the jars was changed.

Spawned out eggs and milt were mixed in fingerbowls in sea water with salinity adjusted to 28 to 30‰ at ambient temperature. It is considered that this is about the optimal level of salinity for this species for larval development (Rao, 1951). Water was changed once a day, filtering it through sintered glass funnels to prevent escape of the larvae. Fresh sea water of adjusted salinity filtered through millipore filter replaced the water removed from the fingerbowls. The developing eggs and larvae were removed at frequent intervals for microscopic examination in cavity slides. When larvae started feeding, about 10 ml of algal culture was added to each fingerbowl every day. Minute microflora like *Chlorella* spp. present in the culture formed the food of the larvae. When the larvae had grown in size, changing the water in the fingerbowls presented no difficulty as they could be spotted under the magnification of a hand lens, picked up with a pipette and transferred to fresh bowls. Settlement of spat took place in the fingerbowls in which they were reared.

Developmental Stages of Eggs and Larvae

The just spawned out eggs measuring 60 to 63 μm in diameter enclosed in a thin vitelline membrane was spherical, colourless and translucent with granular yolk in the cytoplasm and a large hyaline nucleus at the centre. In some the nucleolus was also clearly seen. Within 20 to 30 minutes after fertilisation the first polar body appeared at the apical pole and it was followed by the extrusion of the second polar body. Segmentation of the egg commenced resulting in two unequal cell stage, a pseudo three cell stage, four cell stage (Pl. I A, B) and an eight cell stage in quick succession within about 45 minutes. At eight cell stage, seven cells were small and one was large. The smaller cells the micromeres dividing had spread over the large cell giving rise to the morula or blastula which developed cilia and began rotatory movements in the water at about two and half hours. This was followed by gastrulation at three to three and half hours, partly by epiboly and partly by invagination, the blastopore appearing initially at the vegetative pole but subsequently shifting ventralwards (Pl. I D). The next stage was the trochophore formed at about five and half hours after fertilisation and it measured about 75 μm with a large preoral lobe destined to develop into the velum and a primordium of a shell in a glandular invagination of the ectoderm. The preoral lobe revealed larger cilia which enabled a more rapid movement than in the earlier stage (Pl. I E).

Between twelve and sixteen hours after fertilisation an equivalve early veliger larva in straight hinge stage was formed with a well developed velum protruding out between gaping valves for rapid movement. An archenteric space was visible without the oesophagus, intestine and digestive gland. The larva was thus still apparently incapable of ingesting food and when disturbed it was withdrawing the velum and settling down at the bottom of the culture bowl (Pl. I F). It measured about 85 μm in length at the hinge. With the formation of the alimentary canal, the veliger larvae started feeding on the organisms contained in the algal cultures introduced into the finger bowls. However, after the early veliger stage, the larvae suffered heavy mortalities.

The growth of the veligers was accompanied by the development of the anterior and posterior

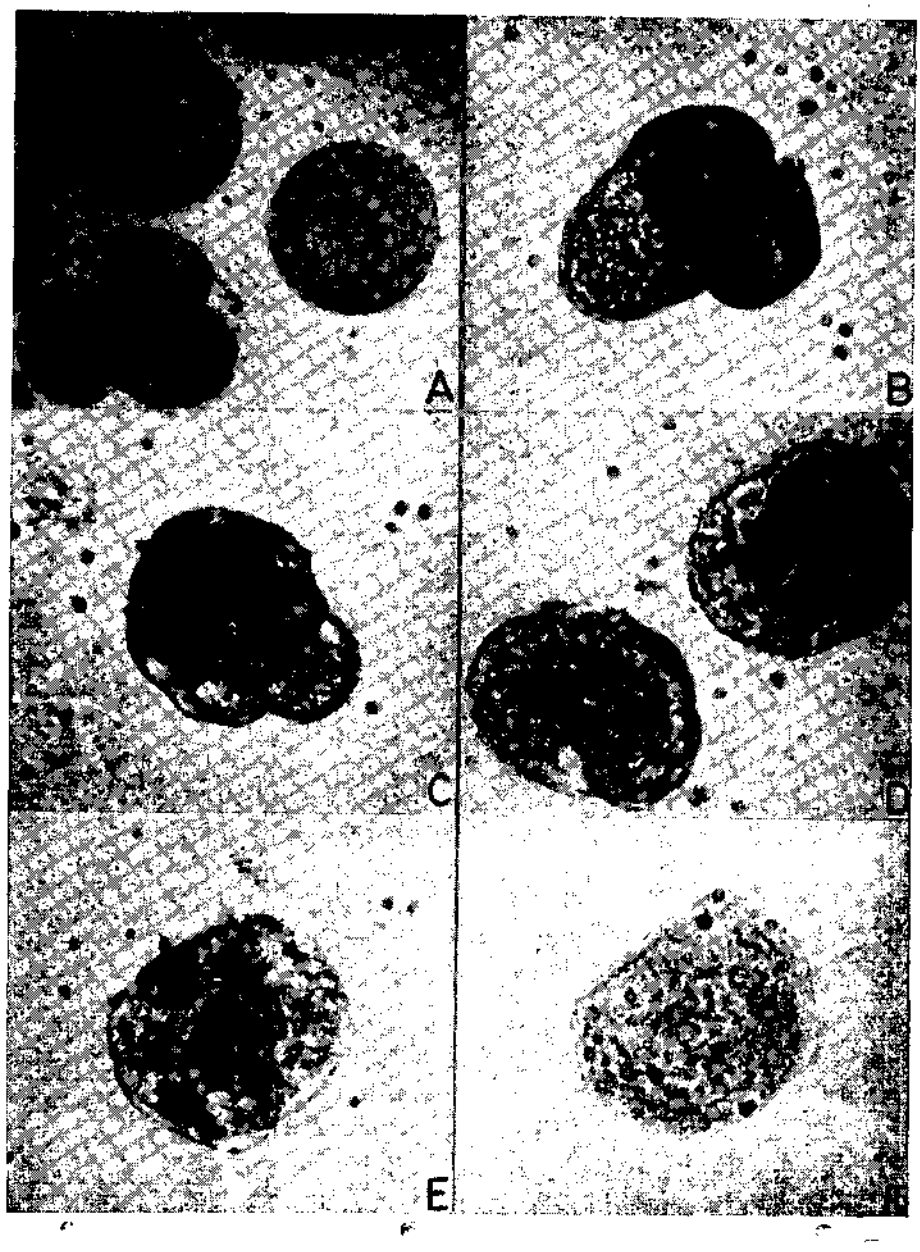

PLATE I. Developmental stages of *Crassostrea madrasensis* : A. Dividing and undivided eggs. Actual size of undivided eggs 60-63 μm, B. 4 Cell stage. Actual size 65-68 μm, C. The smaller cells spreading over the larger cells giving rise to morula stage. Actual size about 70 μm, D. Gastrula stages. Actual size about 70 μm, E. Trochophore stage. Actual size about 75-80 μm and F. Straight-hinge stage of veliger larva. Actual size about 85 μm.

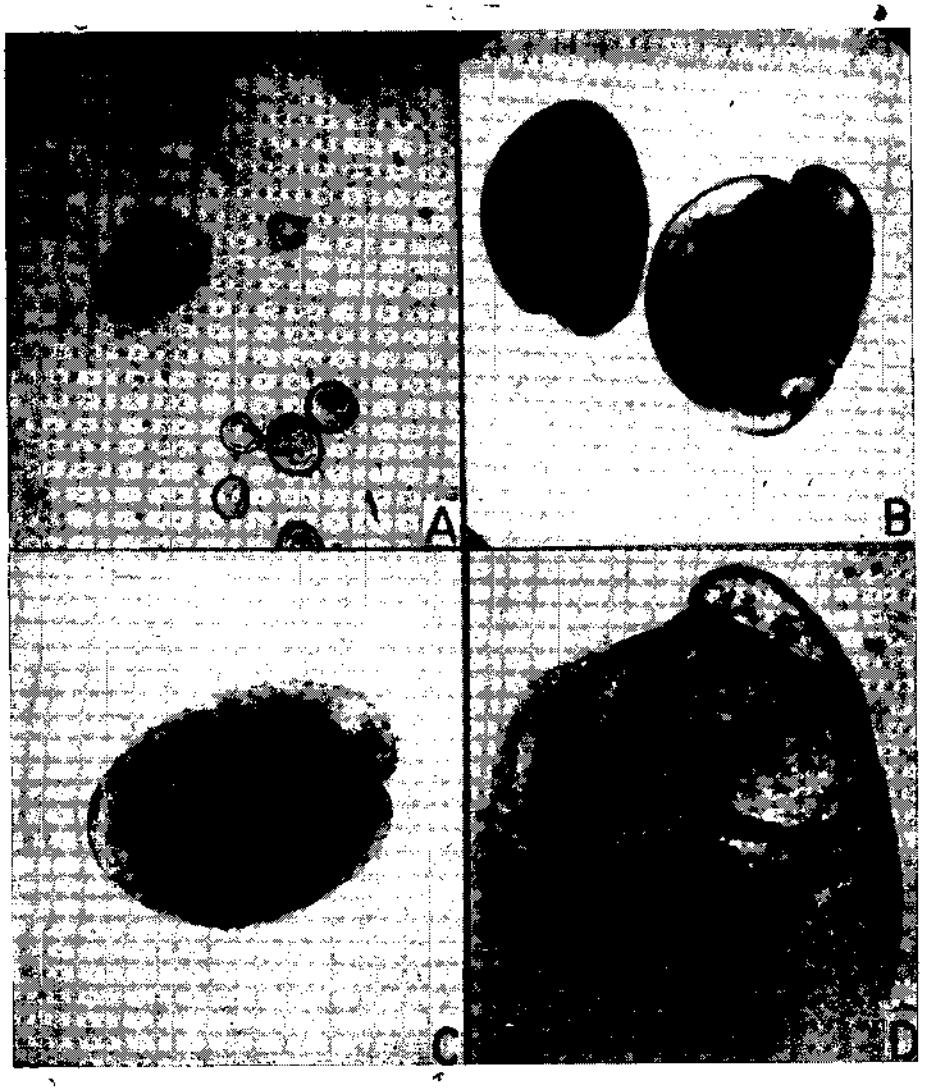

PLATE II. Developmental stages of *Crassostrea madrasensis*: A. Unequal development of the larvae of the same brood, B. Pediveliger stages of larvae. Actual size about 300μm, C. Pediveliger stage about to set. Actual size over 311 μm and D. Early spat, one day after setting. Actual size about 1 mm in long axis.

adductor muscles, the formation of the umbo at the anterior middle region of the hinge, the inequivalve development of the shell, the origin of a rudiment of a gill, the appearance of the statocyst and the pigmented eye spot. The velum had developed into a fairly large structure for locomotion and food capture and a small foot was in the process of formation. By the nineteenth day after fertilization some of the larvae had nearly grown upto 298 μm with a distinct inequivalve shell, broad at the base and narrow and bluntly pointed at umbo acquiring a brownish colouration (Pl. I E, Pl. II C). The foot had grown into a finger shaped or strap-like structure with the help of which the larva now known as the pediveliger was occasionally creeping at the side or at the bottom of the finger bowls. There was no uniformity in the growth of the larvae. While some had grown to pediveliger stage others remained smaller even at the early umbonal stage (Pl. II A).

The largest of the pediveligers observed was 302 μm in its long axis. After occasional creeping with the foot, the pediveliger had settled at the bottom with the left valve down. With the exudation from the foot glands, the shell valve was cemented to the surface of settlement. This brought about a shift from the free-living habit to one of sedimentary life as spat (Pl. II D) which involved rapid structural changes, they being absorption of the velum and the foot, the disintegration of the eye spot, elaboration of the gills, development of the labial palps and a change in position of the adductor muscle very nearly to a central place between the two valves. The anterior adductor muscle disappeared but the statocyst remained. The original larval shell was retained as prodissoconch in the spat and at the edges of the valves fresh shell material was continuously laid down by the secretion from the mantle edges.

Remarks

Some of the techniques developed for induced breeding in large number of molluscs have been reviewed by Ino (1973). According to him they involved physical, electrical, mechanical, chemical and biological stimulations. In general they are not universal in their application, the responses varying with the species concerned. The present writer has tried some of the methods in inducing the green mussel *Mytilus viridis* to spawn with varying degrees of success (Rao *et al.*, 1976). Perhaps, the oysters can more readily be induced to spawn than most other groups of molluscs. In the American oyster *Crassostrea virginica* Galtsoff (1938) reported spawning reaction of female in the presence of sperm in the water. Loosanoff and Davis (1963) reported that the suspension of sex products along with increase in water temperature helped *Crassostrea virginica* and *Ostrea edulis* to initiate spawning. In the present observations *C. madrasensis* could be induced to spawn with the suspension of sperm in the water without increasing the temperature as the prevailing water temperature are always generally high.

C. madrasensis is a dioecious species in which the sexes are separate with hermaphrodite forms occurring occasionally (Rao, 1956). In the open tanks the volume of water required for successful larval culture is to be ascertained by experimentation. The present paper reports only the possibility of initiating tank culture experiments and the details have to be worked out. Mono-algal cultures used for feeding are bound to result in more rapid growth of the late veliger larvae for setting of the spat to take place in a shorter period of time.

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