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NOTA BREVE

CULTURE OF BRACHYURAN (CRUSTACEA : DECAPODA) EGGS *IN VITRO*

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Culture of Brachyura under laboratory conditions from egg through the different stages of larva to the juvenile stage has been successfully carried out in several parts of the world utilizing different culture methods (Sandifer *et al*. 1974; Roberts, 1975). In most of these cases, the experiment involves an ovigerous female maintained in an aquarium under laboratory conditons until the eggs hatch into zoea larvae. Though this method may be adequate for some of the species, it is often difficult to furnish appropriate food and simulate adequate environmental conditions for the maintanence of the adult. Besides, some of the female crabs and lobsters have a tendency to shed their eggs under laboratory conditions (Provenzano, 1967).

Costlow and Bookhout (1960) succeeded in hatching the eggs *in vitro* maintaining them in individual compartment of plastic trays which were kept constantly shaken by a variable speed shaker. Experiments carried out here with a special incubator has given promising results. The incubator has the added advantage of not having to handle the larvae once the experiment is set. The system has a constant supply of air from a simple aeration pump which also prevents the eggs from settling down.

The present experiment was designed to determine the efficacy of the incubator to hatch eggs *in vitro*; further study to determine its suitability in rearing the larvae to juvenile stage will be attempted eventually.

Description of incubator (Fig. 1)

The incubator is in the form of a square tray made of perspex sheets (6 mm in thickness) with a depth of 10 cms. The bottom of the incubator is provided with two rows of 3 holes through which a tube (F) is introduced. The incubator has an inlet tube (D) on one side near the bottom for entrance of water and an exit tube (E) near the top for overflow of excess water. Six containers (C) are utilized in each incubator. A polythene bottle of 100 ml capacity is utilized as containers; the botton of these bottles are cut off and the cap of the bottle (J) with a hole in the middle is fixed above each hole of the incubator with araldite. A piece of plankton net (K) is placed inside the cap. The body of the container is provided with two windows on opposite sides, each covered with a piece of plankton net (G). The container is screwed on to the fixed cap inside the incubator. The incubator receives water by gravity from a reservoir (10 litre capacity) placed at a higher level. The pipes (F) provide a steady flow of air controlled by a stopper (H). The incubator is covered with a lid (B) of perspex sheet.

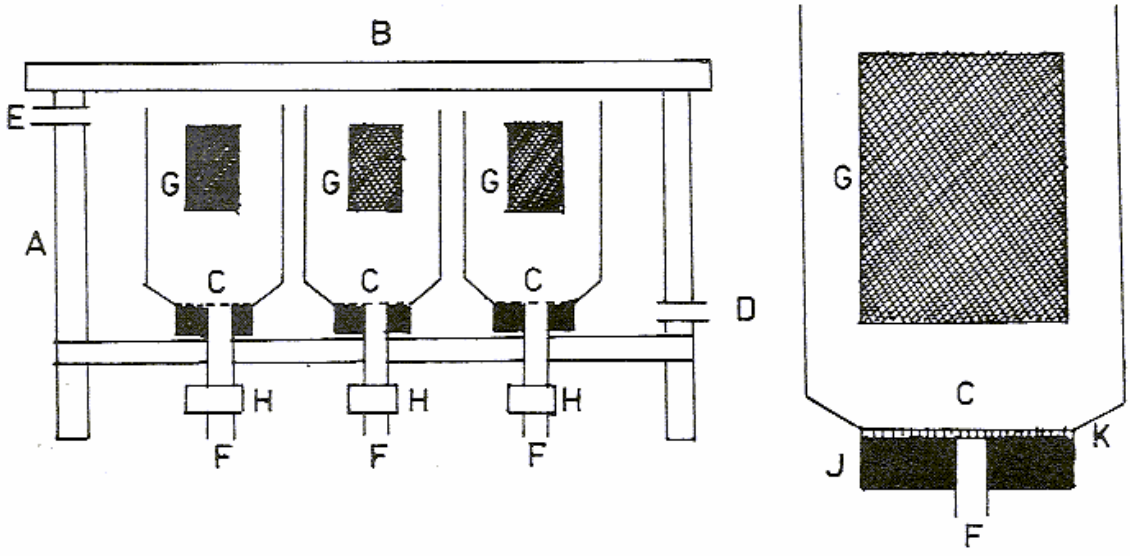

Figure 1. Incubator used in the experiments. A. Incubator tray; B. Lid of incubator; C. Container; D. Water inlet; E. Water outlet; F. Air inlet; G. Windows of container covered with nylon gauze; H. Air control ; J. Cap of container fixed to the bottom of incubator; K. Nylon gauze.

METHODOLOGY

The eggs removed from the females were placed in each container (50 in each). The incubator was set up by filling them with water drawn from the larger container through the pipe (D) and excess water drains off through outlet (E). The windows (G) permits exchange of water while retaining the eggs within the container. The air supplied through the tube (F) keeps the eggs constantly aerated and agitated within the container and prevents the eggs from settling at the bottom. The bolting silk (K) at the bottom of the container retains the eggs within the container. The water inside the incubator was renewed once every day.

Four sets of experiments were conducted with *Uca leptodactyla* Rathbun, *U. cumulanta* Crane and *Callinectes danae* Smith. An inicial experiment to verify the efficacy of the incubator was done by placing some egg mass from the female (*U. leptodactyla*) in different containers of the incubator while maintaing the ovigerous adult in an aquarium. In subsequent experiments with *Uca* spp. and *Callinectes danae* while employing same methodology, a fixed number of 50 eggs was kept in each container

of the incubator to determine the percentage of hatching. The experiment conducted at room temperature was not pursued beyond the hatching.

RESULTS

The results obtained were interesting enough to form the body of this paper. In the initial experiments when we attempted to find out the functioning of the incubator, the eggs hatched successfully in the incubator and aquaria simultaneosly or a day to three days ahead in the incubator. In subsequent experiments with 15 females of both *Uca* spp. and *Callinectes danae*, the hatching time in the incubator eas reduced up to 2 days, except in one case it was delayed by 2 days.

In three instances involving *Callinectes danae*, the crabs released all the eggs within a few hours of introducing them in the aquaria.

An attempt was also made to determine the hatching efficiency of the incubator (Fig. 2). In experiment 1 with Uca spp., it can be seen that of the five series of studies conducted there was a hatching rate of 24% to 84% between containers of the same incubator. Experiments 2 and 3 were done with eggs of *Callinectes danae* obtaining a hatching rate between 2% and 86%. The wide fluctuation in the hatching rate is attributed to the fact that there was no uniform aeration of all the containers of the incubator. This defect needs to be rectified to improve the efficiency of the incubator.

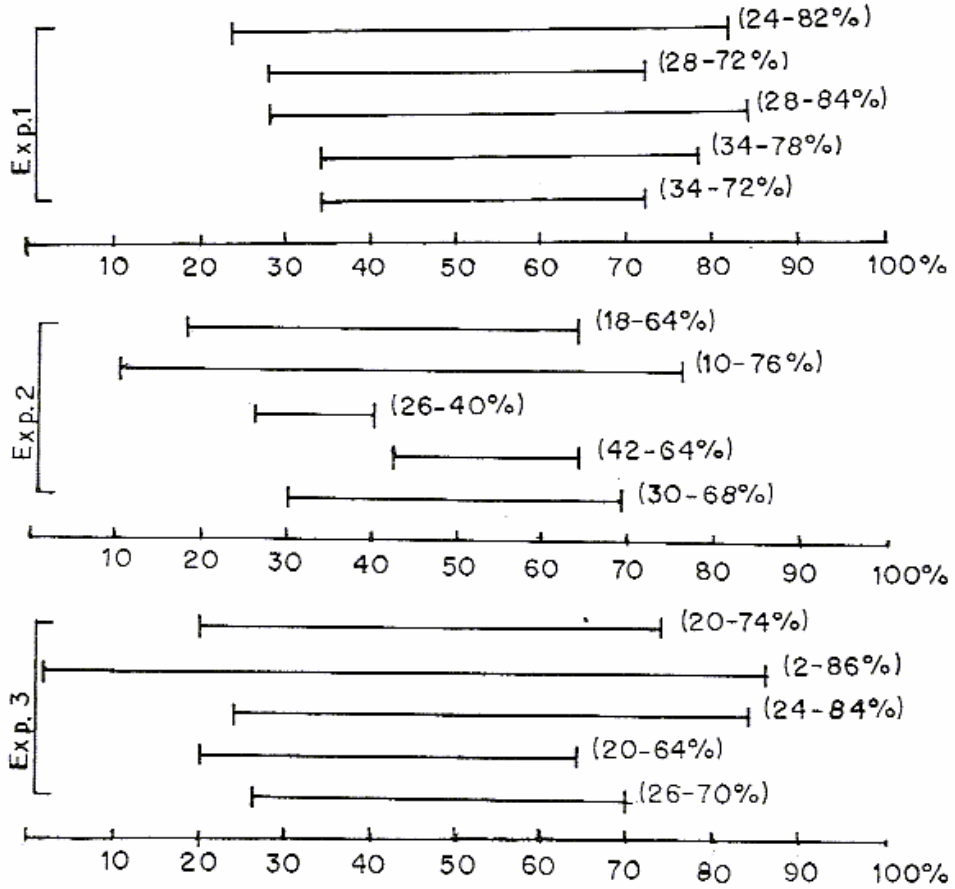

Figure 2. Hatching rate of eggs of *Uca leptodactyla, U. cumulanta* and *Callinectes danae* in each incubator. Exp. 1. With eggs of *U. leptodactyla* and *U. cumulanta*. Exp. 2 and 3. With eggs of *C. danae*.

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