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SEED PRODUCTION AND HATCHERY DEVELOPMENT

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It is fundamental to culture of any aquatic organism that the right type of seed is available at the right time. Man, from the time he developed aquaculture interest, has depended on the seed available in the wild for stocking the farms and even today this dependence is absolute in most cases. However, techniques have been evolved for "catching" the seed on collectors or cultches to reduce labour, to ensure quantity and to increase operational efficiency. But fluctuations are common upsetting the plans of the culturists.

During the last two decades, tremendous interest has been generated in the field of artificial breeding of molluscs, following the success of Dr. Victor Loosanoff and his colleagues at the Milford Laboratory in the U.S.A. in the early sixties. This has led to the establishment of commercial hatcheries, particularly in the U.S.A., for oysters and clams.

SEED PRODUCTION IN NATURE

Reproductive strategy

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The general reproductive strategy of molluses, particularly the bivalves, is the production of a large number of eggs, in millions, external fertilisation, a pelagic phase of the larval stages and ultimate settlement of the young ones in suitable substratum. The American oyster <u>Crassostrea virginica</u> releases an average 54.1 million eggs and the clam <u>Mercenaria mercenaria</u> discharges an average 24.6 million eggs. The mortality rates at different stages of the development are so high that the number of viable young ones finally settling on the beds is very very low. The very high fecundity is nature's mechanisms for the propagation of species against all odds of a dynamic environment. The fertilised eggs pass quickly through the early developmental stages and reach the typical veliger stage within 24 - 48 hrs when the larvae are able to feed upon microalgal food in the environment. The veliger subsequently passes through umbo and other stages before they metamorphose to the young stage closely resembling the parent and settle down on the substratum. The term "spat" is used to denote the stage at settlement in the case of species such as oyster, pearl oyster and mussel where the young ones attach themselves permanently or temporarily as the case may be to the substratum with cementing substance or byssal threads. It is seen generally that the areas suitable for settlement of spat are not always good for growth of the molluscs. This had led to the development of exclusive seed collection centres and production centres in many cases. For example, in mussel farming in France, the seed collection centres are located in the southern France in La Rochelle, whereas the production centres are in the north coast of Brittany.

Seed collection

Most of the aquaculture systems are semi-culture systems in the sense that seed is collected from the wild and transplanted in suitable areas for achieving higher and quicker yields through manipulation of the culture system. Several methods have been developed for the collection of spat of molluscs.

a) <u>Ceramic tiles</u>: Perhaps the earliest and efficient collectors of spat of oyster <u>Crassostrea</u> angulata were developed in the Bay of Arcachon. Slightly curved ceramic tiles are used as collectors (cultches). They are first thinly coated by bathing them in a mixture of lime and water. The treated tiles are piled up in crates and laid in the spat collection beds. The spat can be easily removed from the tiles which are used over and over again. The tile collectors are used extensively in European oyster culture with subtle variations.

b) <u>Shells</u>: The use of molluscan shells as collectors of spat is highly developed in the oyster culture industry of Japan. Scallop shells are strung on galvanised wire and are suspended as "rens" from racks. A good set is considered to consist of about 200 spat per shell of which 50 - 60 survive to the seed oyster size of 1 to 1.5 cm. The shells of oysters and mussels either broadcast on the bed or placed in bags are used as collectors of oyster spat in Europe, U.S.A. and Japan. Shells of mussels are spread on the bottom in the Oosterchelde of the Netherlands for the collection of spat of oyster.

c) <u>Ropes</u>: Ropes are standard spat collectors in mussel culture in Spain and France. Loosely woven and heavily tarred ropes of 12 - 15 mm diameter are suspended from rafts or racks. Although natural fibres such as coco and esparto grass ropes are in use, these are being fastly replaced by non-toxic synthetic ropes.

d) <u>Poles, racks, sticks, twigs etc</u>: These wooden structures are commonly used for the collection of oyster spat in Japan and Australia. Rows of poles called "Bouchots" are used for the collection of mussel spat in France. Cedar springs are extensively used for the collection of pearl oyster spat in Japan. Branches of red mangrove and wooden planks coated with bitumen are used for the collection of mangrove oyster spat in Venezuala and Cuba. Split bamboo frames have proved very successful for the collection of <u>Pinctada margaritifera</u> seed in Dongonab Bay in Sudan.

e) <u>Plastic meshes</u>: Rubber-like plastic not material called "Netron" is gaining importance as collector of oyster spat in Japan although it is about five-times costlier than shell collectors. Plastic sheets of 3 mm thickness made of polyethylene or poly-propylene have shown promise for the collection of oyster spat. The French oyster culturists have tried rubber-like plastic mesh material, of the size and shape same as that of ceramic tiles, coated with cement but have found it uneconomical.

f) <u>Others</u>: Cement-coated egg carons have proved successful as oyster spat collectors in Prince Edward Island. Coconut shells are also used as cultches in some cases. g) <u>Collection of clam seed</u>: The clam seed are collected using fine mesh wire scoops. The density of seed population of <u>Anadara granosa</u> is as high as $10,000/m^2$ or more in the Malaysian beds.

h) <u>Cultchless spat</u>: A more recent development in hatchery production of oyster seed is the cultchless spat. Calcium carbonate particles are used for collecting the spat. When spat settle on plastic sheets they can be removed. These free spat are desired for growing regular shaped oysters for market.

Factors deciding successful seed collection

Besides employing suitable cultches for the collection of seed, several factors decide the success of seed collection. The areas for good spat settlement should be identified. In many parts of the world only a small percentage of oyster beds are suitable for spat collection. The timing of laying the collectors is very crucial. If the collectors are laid a little too early, settlement of barnacles and other fouling organisms will take place and the collectors will not be useful for seed collection. If they are laid late, they will miss the spatfall. In all commercial seed collection operations such as the oyster soed collection in Gulf of Morbihan in France, Long Island Sound in U.S.A. and Miyagi Prefecture in Japan, the Government biologists monitor the abundance of planktonic larvae and guide the farmers on the time for laying the collectors and predict spatfalls. Another crucial factor is the level at which the spat collectors are suspended. This depends on the layer where most of the advanced stage larvae of the particular species are found. This would also enable avoiding layers susceptible for barnacle settlement.

Seed trade

The seed collection and supply has developed into an industry as such. Generally those engaged in culture for production are not involved in seed collection and they purchase seed for planting in the farm. International seed trade has also become popular with the introduction of species native to a region to other areas. The Pacific oyster <u>Grassostrea gigas</u> has been introduced along the Pacific coast of U.S.A. and Ganada and in Spain and France. The Japanese culturists grow spat on oyster shells as required by their foreign buyers and ship them to those countries. This has lod to introduction of pests, predators and parasites also to the new areas with the attendant problems of control.

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HATCHERY PRODUCTION OF SEED

Interest in the artificial breeding of oysters dates back to the eighties of the nineteenth centry. W.K. Brooks of John Hopkins University had in 1880 worked on the development of eggs and early larval stages of the American oyster <u>Crassostrea virginica</u> and J.A. Ryder in 1883 and F. Winslow in 1884 made an unsuccessful attempt to bring oyster larvae to metamorphosis. It is in 1920 that W.F. Wells of the New York Conservation Commission succeeded in rearing the oyster larvae to setting which opened the door for further development in this direction. Wells also succeeded in rearing the larvae of the mussel <u>Mytilus edulis</u>, the clams <u>Mercenaria mercenaria</u> and <u>Mya</u> <u>arenaria</u> and the scallop <u>Pecten irradians</u>. His method which is popularly known as Wells-Glancy method used the food naturally present in the sea water for the rearing of larvae and naturally spawning adults were used as parents.

In the mid-1940s Dr. V.L. Loosanoff, H.C. Davis and other colleagues in the U.S. Bureau of Commercial Fisheries Laboratory at Milford, Connecticut, U.S.A. developed techniques for induced spawning and rearing of larvae using laboratory-reared algal culture. Subsequently the Milford team developed techniques for out-of-season maturing and spawning of a number of commercially important molluscs; production of selected micro-algal food for the larvae; and disease control. The hatchery technology of this team is known as the Milford method. These developments have lad to the establishment of commercial hatcheries along both the Pacific and Atlantic coasts of U.S.A. and the Maritime Provinces of Canada. The hatcheries are so versatile that they can switch over from the production of seed of oyster to that of clam or abalone.

Model operation of commercial oyster hatchery

- Selection of mature oysters based on size, shape and growth rate.
- Hold at 10.°C in the hatchery.
- Condition for spawning by slowly raising the temperature to 18°C or more.
- Hold the oysters at the above temperature for 2- 4 weeks.
- Induce spawning of oysters in glass trays by raising temperature to 25 °C.
- Spawning and fertilisation.
- Transfer fertilised eggs to 120-gallon conical rearing tanks.
- Larval development.
- Grade larvae by screening. Retain only those above 0.3 mm (20%) and discard others (80%). This step is to select only the fast growing ones.
- Transfer selected larvae to Larval Rearing Tank. Sea water pumped from the bay and centrifuged to remove larger plankton. Water carrying only small algal cells suitable as food for oysterflarvae is stored in 20,000 litre tanks in greenhouse. Algae allowed to grow for 24 hours. If numbers are insufficient, 200-litre algal culture is inoculated. The resultant culture of microorganisms is used to fill the Larval Rearing Tanks.
- Larvae ready to set after 10 15 days.
- Transfer to Settling Tanks. Fach plastic settling tank of 3600-litres capacity contains 10 bushels (1 bushel = 35.2 litres) of specially selected oyster shells spread at the bottom.
- Setting occurs in 24 48 hours.

- Transfer shells with spat to Mursing Tanks. The Nursing Tanks of 27,000-litres capacity each are located in a greenhouse and supplied with water containing algal bloom. Shells with spat are transferred to half-bushel plastic mesh bags and 200 such bags are suspended in the Mursing Tank from wooden beams.
- Maintain spat in Nursing Tanks for 4-7 days or more.
- Transfer wooden beams with shell bags by chain-hoist and overhead rail to floating rafts moored outside near the dock.
- Spat reach fingernail size (1 2 cm) in 2 3 weeks.
- Plant the spati on the oyster beds.

Total duration of hatchery operation is 4 - 6 weeks.

Basic requirements for hatchery production

Controlled spawning: Techniques for the controlled reproduction of the species must be available. The Milford Laboratory has developed techniques for maturation of gonads and spawning of several species of bivalves at any part of the year irrespective of the reproductive condition of the organisms under wild conditions. Several methods have been developed for the induced spawning of molluscs. The commonest technique is conditioning the molluscs for accelerated development of gonad through thermal stimulation and spawning them by a quick rise in temperature to the optimum level and adding egg or sperm suspension. This method has been particularly successful for the species in the sub-tropical and temperate regions. The Japanese workers have mostly relied on chemical stimulation for spawning molluscs. The methods include spawning the animals in ammoniated sea water or injection of neutral potassium salts or ammonium hydroxide. Stripping the gonad and treating the eggs with a weak solution of ammonium hydroxide also gives good results in some cases. Methods such as giving a mild electric shock and pricking or severing the adductor muscle have proved useful for spawning the mussels. Addition of hydrogen peroxode to alkaline sea water has been effective in a number of molluscs including abalones. Thus a range of physical,

chemical and biological induction methods are available for spawning the molluscs and those suitable for particular species should be developed.

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Water quality

Water quality is one of the critical factors in determining the success of hatchery production of seed. Temperature, salinity and pH must be maintained at the required level. Water should be relatively pure from pollutants, particularly metallic salts, pesticides and detergents. Silt will have an adverse effect and should be removed by filtration. The water should be treated with antibiotics, sulpha drugs or untraviolet radiation. Areas where intensive algal blooms appear frequently should be avoided.

Larval food

Food for the different stages of larval forms is another important aspect of hatchery operation. The right type of food in right concentration should be supplied. The algae must be of size suitable to be consumed by the larvae and must not have a cell wall and must not produce toxic metabolites.

Disease control

Cleanliness of all tanks, utensils and other materials should be maintained regorously. Growth of pathogenic bacteria, fungi, ciliates etc. should be controlled. To a large extent these could be controlled with antibiotics, sulpha drugs and ultraviolet treatment of incoming sea water.

Closed sycle shallfish factory

Success in commercial hatchery operation has led to the concept of controlled culture of the full life cycle of the molluscs. A closed cycle shellfish factory is being tested at the University of Delaware in U.S.A. for production of oysters and clams from egg release to market size. Although technical feasibility has been established, a substantial amount of research and development is yet to be done to make the project economically viable.

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LARVAL NUTRITION

Larval nutrition has received much attention simultaneous with the development of hatchery techniques. The stored food in the fertilised eggs lasts only for a few hours and thereafter availability of appropriate food decides the growth of the larvae. Live algal food has been found to be the best for the larvae of most of the molluscs. studied. But certain species of algae produce metabolites toxic to bivalve large and they should be avoided. Those which contain a cell wall are also not so suitable as food of larvae. The naked flagellates Isochrysis galbana and Monochrysis lutheri have been found to be exceptionally good for oysters and clams. The food value of microorganisms also depends, in part, upon how completely they meet the food requirements of larvae. It has been found by several workers that a mixture of suitable species such as I. galbana, M. lutheri, Platymonas sp., Dunaliella euchlora, all naked flagellates, induce better growth rate of larvae than when they are used singly. The feeding density varies from 5000 to 15,000 algal cells per larvae twice a day.

Dried algal food has been used successfully in the case of some species of oysters but has not been useful in most other cases. Artificial feedforeparations have also not been useful.

The success of larval food production in hatcheries is often dependent on an adequate supply of good stock cultures to ensure continuance of the strain and consistent results. Stock cultures are best maintained in small volumes of an enriched sea water medium. Several media for algal culture have been developed by scientists and composition varies based on the requirements of algal species. Convenient culture vessels are 120 or 150 mm screw-capped testtubes filled with 10 ml of media or 125 ml screw-capped flasks filled with 60 ml of media. Cultures may also be maintained in solid media, such as sea water agar slants.

Pyrex carboys of 20-litre capacity or more are used for culturing foods either in batch or semicontinuous culture. The advantages of this size vessel are that moderately large volumes of several species can be made simultaneously available and that cultures may be discarded if they are not satisfactory foods. In semicontinuous culture, the cultures are harvested as needed and volume removed is made up with sterile media. Where extensive hatchery operations are carried out, a much larger volume of food may be needed and outdoor tank culture is resorted to. In open tank culture complete control of the system is not possible. Mass culture of algal food is one of the essential functions of the shellfish hatcheries. While the stock cultures and carboy cultures are done under illumination from flourescent lights, tank culture is done in greenhouses.

GENETIC IMPROVEMENT OF STOCKS

Studies on the genetic remources of the cultivable species, particularly the American oyster <u>C</u>. <u>virginica</u>, Pacific oyster <u>C</u>. <u>gigas</u> and the quahog clam <u>M</u>. <u>mercenaria</u> have received some attention and cross-breeding has been experimentally successful. But a lot of work remains to be done yet in this field for upgradation of stocks. At the Virginia Institute of Marine Sciences, strains of oysters which are resistant to oyster-diseases have been developed using the survivors of the Chesapeako Bay disease as parents. The Oyster Research Institute at Kesemurn, Japan, has carried out extensive cross-breeding experiments on oysters. Hatchery production will become truly beneficial when our knowledge on the genetic resources of the cultivated species of molluses has improved and practical application becomes possible for evolving strains or upgrading stocks with desirable characteristics.

Men's increasing interference with the foreshore environment and the estuaries for recreational, industrial and other purposes is

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affecting the ecosystem of natural production of molluscs. Dependence on nature for seed requirements will be more and more unpredictable in future. Hatchery production of seed willgain further importance and will perhaps be the only means of sustaining culture operations in the distant future.

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