CMFRI

Course Manual

Winter School on

Recent Advances in Breeding and Larviculture of Marine Finfish and Shellfish

30.12.2008 -19.1.2009

Compiled and Edited by

Dr. K. Madhu, Senior Scientist and Director, Winter school

&

Dr. Rema Madhu, Senior Scientist and Co-ordinator

Central Marine Fisheries Research Institute

Central Marine Fisheries Research Institute

(*Indian Council of Agricultural Research*)

P.B.No.1603, Marine Drive North Extension,

Ernakulam North ,P.O.

Cochin, KERALA – INDIA - 682018

EDIBLE OYSTER SEED PRODUCTION AND REMOTE SETTING

34

V.Kripa,
Principal scientist, Tuticorin Research Centre of CMFRI, Tuticorin.
E -mail : kripa_v@yahoo.com

Introduction

Throughout the world natural spat collection forms the basis of most oyster culture industries. Along the Pacific Northwest USA coast, hatchery produced seed are used in the cultivation of the Pacific oyster *Crassostrea gigas*. Natural seed collection is cheaper when compared to the cost of seed produced in the hatchery but is unpredictable. However, in the recent years, more efficient methods of setting the oyster seed in remote areas have been developed and this method is widely practiced now.

Natural Spat Collection

The substrate provided to the oyster larvae for attachment is known as 'cultch' or 'collector'. It should be clean and hard. In the temperate waters, oyster or scallop shell is widely used as cultch. In India, empty oyster shells are used by farmers for spat collection. The selection of the cultch material depends upon the type of culture. For example, in the production of individual oysters (unattached) for the half-shell market, lime-coated tiles give good results as it is easy to remove the oyster spat from the tiles for further rearing. For the ren method of culture, strings made of oyster shells with spacers inserted between the shells are hung usually from racks. The grow out culture is often carried by using the same rens.

Seed Production in the Hatchery

Attempts to raise oyster seed under controlled conditions were made towards the end of the 19th century. Later studies have standardized the oyster seed production technology in hatcheries. This development has paved the way not only for the commercial production of oyster seed for oyster culture but also for researches in oyster genetics. The selection of suitable site for oyster hatchery is of utmost importance and many factors are to be taken into consideration. Uninterrupted supply of good quality seawater, free from industrial and sewage pollution is required. The suspended particles and silt load in the water should be low. Sites close to river mouths should be avoided, since during monsoon, flooding dilutes the seawater salinity, rendering it unsuitable for seed production. It is advantageous to select a site which is close to the oyster farm and natural oyster beds. The site should be easily accessible for the transport of men and materials throughout the year.

Selection and Conditioning of Broodstock

The word conditioning is used to denote the process by which the gonad maturation of the oysters is hastened so that the gametes become ripe for spawning. The process involves manipulation of environmental conditions and nutrition. In India conditioning the oysters about 5^0 C below the ambient water temperature with suitable algal diets accelerated the gonad development, resulting in sexually ripe oysters. The oysters are selected based on the condition factor and age. Selection of a mixed and heterogeneous stock of oysters from several areas will give better results. It is also desirable that the salinity regime of the area from where the oysters were collected is comparable to that of the water salinity in the hatchery. Otherwise the oysters should be acclimatized before they are conditioned.

C. madrasensis of the length range 60-90 mm are considered as ideal and it is preferable that 30 % of them belong to '0' age group (60-75 mm) in order to be assured about the presence of males in the broodstock. The maturity stage of oysters is ascertained by the examination of gonad tissue smears under a microscope. Oysters which show dominance in 'maturing stage' of gonad development are preferred since the conditioning period will be relatively short when compared to the spent/ indeterminate stage oysters. The selected oysters are cleaned thoroughly with wire brush to remove the plants and animals adhering to the shell. The oysters are conditioned by holding them in the conditioning room at about 5^0 C below the ambient water temperature. They attain full maturity in 10-20 days. The raw seawater used for broodstock conditioning contains supplementary food and in a subsequent study gave mixed microalagae at the rate of 3 liter / oyster / day.

Induced Spawning, Fertilization and Early Development

Fully matured bivalves can be induced to spawn by giving different kinds of stimuli like raising water temperature, addition of sperm suspension to a container holding females, mechanical stress, and addition of chemicals such as Hydrogen peroxide, Ammonium hydroxide, Sodium hydroxide and Tris buffer. In India, *C. madrasensis* is induced to spawn by thermal stimulation. Approximately 25 oysters, conditioned for about 10-20 days at about 5- 10^0 C below the ambient temperature are induced to spawn by transferring them to 100 l Perspex tank containing 50 l filtered seawater with temperature range of 2-4^0 C above the ambient. A silica immersion heater and a Jumo thermometer are used to raise and monitor the water temperature in the spawning tank. Aeration is provided in the tank. The sudden change of water temperature (thermal shock) induces spawning during the first one hour. If spawning is not achieved, fresh sperms stripped from a sexually ripe male are added to the broodstock spawning tank and the sperm suspension induces spawning. The spawning oysters are immediately transferred to separate spawning trays (one oyster in each 3 L glass tray) containing filtered seawater at ambient temperature. On completion of the spawning the oysters are removed from the trays. If the female is a heavy spawner and the water becomes highly murky, it is transferred to another tray to complete the spawning. It is essential to remove the oysters, on completion of spawning in the trays to prevent the oysters from filtering the gametes. The egg suspension from each spawning tray is filtered through a 100 mm stainless steel or nylobolt sieve into a container. The sperms obtained from individual trays are mixed and the pooled sperm suspension is added to each tank containing eggs. This results in greater heterozygosity of the progeny. The gametes are mixed and mild aeration is provided. Most of the eggs are fertilized within 60 minutes of spawning. The fertilized eggs settle at the bottom and aeration is suspended. The supernatant water, containing sperms, unfertilized eggs and debris is removed. Fresh filtered seawater is added and decanting is carried 3-4 times. This is followed by addition of fresh seawater and mild aeration. The fertilized eggs undergo first cleavage within 45 minutes.

Larval Rearing

At the end of 4 hrs after fertilization, as a result of rapid cell divisions, the morula stage is reached and at the end of 20 hrs the straight-hinge, also called D-shelled larva or veliger larva stage is reached. The 'D' larvae actively swim and are siphoned from the tank and reared in 1 t FRP tank filled with filtered seawater and aerated. The D-larvae are semi-transparent; velum protrudes out and creates strong ciliary current which directs minute particles of food into the stomodaeum. The actively swimming larvae are separated by siphoning, leaving the sluggish in the tank. This culling process is continued for the first 2 days. The straight hinge larvae are stocked at a density of 5 larvae / ml of seawater in 1 t tank for further rearing. The larvae are fed with phytoflagellates, *Isochrysis galbana* and *Pavlova lutheri* at the end of 24 hrs from fertilization. During larval rearing, the water in the rearing tanks is changed daily with fresh filtered seawater and then food given. Aeration is provided. The sequence of the development of the larvae from the straight hinge stage to the pediveliger stage is given below.

Stage	Size mm	Hours / Days
Straight-hinge	60-70	20 hrs
Early umbo	100	3rd day
Mid umbo	150	7th day
Advanced umbo	260 to 270	12th to 15th day
Eyed larva	280 to 290	13th to 17th day
Pediveliger	330 to 350	14th to 18th day

The rearing density of the larvae at various growth stages and the feeding protocol with flagellates are given below. The nannoplankters measuring up to 10 mm pass through the sand filter used for seawater filtration. As a result additional food is available to the larvae.

Stage of larvae	Number of larvae/ml	Algal cell concentration in nos/larva/day
Straight-hinge	5	3,000-4,000
Umbo	3	4,000-5,000
Advanced umbo	2	5,000-8,000
Eyed stage	2	8,000-10,000
Pediveliger	2	10000-12000

On the third day the larval shell is slightly oval in shape and the early umbo stage is reached. They are filtered through 80 mm sieve. On the seventh day the umbo on the shell is distinct and pronounced concentric rings are seen on the larval shell. Between 12 and 15 days the late umbo stage is reached. In 13 to 14 days the eyed stage is reached with the appearance of characteristic eye spot. The pediveliger larvae start setting within 24 hrs or sometimes it is prolonged by 2 to 6 days depending on the availability of favourable substratum. Before metamorphosis, the oyster larvae permanently cement themselves to a suitable substrate and this is called "settlement". During larval rearing, mortality of 2 to 3 % per day was considered as normal by Nayar *et al.* (1984). The D-larvae of C*. gigas* can also be grown at densities of 15-20/ml but growth and survival have improved considerably at densities below 10/ml. They stated that mixed algal diets are preferred and a suitable diet for the D-shelled larvae is a mixture of *Chaetoceros* and *Isochrysis*; the most suitable cell densities are 125 cells/ ml and 50 cells/ml respectively. The number of algal cells/ larva/day is higher than that reported from India. They suggested that with high densities of larvae, it is necessary to add the total daily ration in two or more feeding sessions. The larval culture is carried in static water systems (i.e., flow-through system avoided).

Preparation of Cultch Materials and Spat Production

The cultch materials used in the hatchery must be non-toxic and clean. They should be compact to allow sufficient water circulation in the rearing tank and hard enough to withstand handling. They should not alter the water quality. The most common materials used for the setting of oyster spat in the hatchery are oyster shells, shell grit and polythene sheet; the most preferred are oyster shells. A hole is drilled at the center of the shell, brushed well, washed in chlorinated water and pretreated by soaking and repeated washings in seawater. By this process the pH of the water in rearing tanks will not be affected. These shells are spread uniformly at the bottom of FRP tanks containing filtered seawater and several rows of shell rens are also suspended in order to increase the surface area for settlement in the tank, when majority of the larvae pass off the eyed stage (300-350 mm). In a 200 x 100 x 50 cm tank 400-500 oyster shells are laid. The larvae are released into the tanks at a concentration of 2 larvae/ ml and the setting tank is well aerated. The larvae are fed with *Isochrysis* at the rate of 10,000-12,000 cells/ larva/ day. During the next few days the larvae set on the shells and majority of the larvae settle on the concave side of the shells. The production of attached spat for a 1 t tank holding 400 oyster shells is 15,630.

Oyster shell grit and polyethylene sheets are used for the production of cultch less spat. Oyster shell grit of 0.5 mm in size are washed thoroughly, sterilized in 10 ppm chlorine, washed once more in running filtered seawater and dried. The shell grit are uniformly spread at the bottom of one tonne capacity FRP tank and the larvae at setting stage are released. For setting on the polyethylene sheet the bottom and sides of the tank are lined with pretreated polyethylene sheet and the released larvae settle on the sheet. In the larval setting tanks, before feeding, water is completely changed on alternate days and half the water is changed on the other days. It takes 5-6 days for larval setting. The spat are reared for three weeks and are fed with mixed phytoplankters such as *Chaetoceros* sp, *Skeletonema costatum, Thalassiosira subtilis, Nitzschia* spp. etc. Average setting on polyethylene sheet is 4 spat/ cm^2. In a study on the rate of spat setting, it was observed that the D-larvae of *C. madrasensis*, reared at 29.1 to 32.4^{0}C in the hatchery gave spat production of 2.6 to 7.9 % of the initial larval stock.The chemicals epinephrine and nor-epinephrine added at concentrations of 10^{-4} M - 10^{-5} M are said to induce oyster larvae to settle and metamorphose for the production of cultch less spat. For spat rearing, nursery upwelling systems are suitable for oysters, immediately after settlement. Spat growth is largely dependent by the quantity of food available for feeding.

Remote setting

Remote setting is the method by which eyed or pediveliger larvae are transported without water, in moist condition to distant places where they are set on the cultch material. The use of this technique has revolutionized oyster culture on the west coast of the USA where seed production is no longer a problem. Significant results were obtained in larval transport and distant setting of Crassostrea gigas and in *C.virginica*. In the USA, farmers usually get 20-30% of the larvae of *C.gigas* setting as spat; in some cases as high as 80% spat set on cultch material has been reported. In India, success has been achieved in remote setting of *Crassostrea madrasensis* larvae produced in the Shellfish Hatchery of CMFRI, Tuticoin.

☆☆☆☆☆