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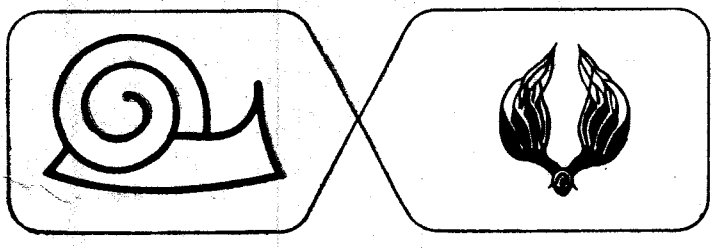

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Review on Controlled Breeding of Bivalves of Aquaculture Importance

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INTRODUCTION

The subject of controlled breeding of bivalves has received considerable attention during the recent years due mainly to the need created for augmenting the supply of quality seed for commercial shellfish culture operations. There have been excellent reviews on the subject during the last two decades. The credit for building up a new school on controlled breeding of molluscs goes to Dr. Victor L. Loosanoff working at the Milford Laboratory in the United States of America. Another scientist who has made outstanding contributions to our knowledge on the subject is Dr. Takeo Imai at the Oyster Research Institute in Miyagi in Japan. There has also been notable progress in the United Kingdom, thanks to the works of Dr. H. A. Cole and Dr. P. R. Walne. Loosanoff and Davis (1963) made an excellent review on the rearing of bivalve molluscs wherein the rearing techniques of 19 species as developed in the Milford laboratory have been discussed. Ino (1972) reviewed aspects of controlled breeding in 31 species belonging to 17 genera of molluscs. Walne (1964) gave a general account of the culture of marine bivalve larvae. Imai (1977) has reviewed in detail artificial culture of shellfish.

Controlled breeding of commercially important bivalves is of strategic importance to India in the present context of development in the culture of the molluscan shellfishes such as the edible oysters, mussels and pearl oysters. Some work has been initiated on controlled spawning of these bivalves with the ultimate objective of developing techniques for the hatchery production of seed. The present paper attempts a review on the controlled breeding of bivalves of aquaculture importance. While it recapitulates briefly the information available in the literature, it indicates the approaches to be made particularly in India where if the technologies developed for the culture of molluscs were to be exploited for commercial production, the development of successful techniques for controlled production of seed at economic levels is the basic prerequisite.

HISTORICAL

The early works on breeding of bivalve molluscs relate to the American oyster *Crassostrea virginica*. Brooks (1880) worked on the development of eggs and early larval stages of *C. virginica*. The attempts of Ryder (1883) and Winslow (1884) to bring the oyster larvae to metamorphosis were not successful. Churchill (1920) discredited artificial rearing of oysters and considered it impossible to have any practical application of rearing techniques to the oyster industry. The silver lining in the development of artificial breeding techniques appeared when Wells (1920) successfully got the settling of oyster larvae in the laboratory. He obtained the gonadal products by stripping the oysters and fertilised them outside. He did not supply any supplementary feed to the larvae but periodically changed sea water. Another important development around this

period has been the work of Prytherch (1924) who for the first time induced oysters to spawn by raising the water temperature. He reared the larvae in running sea water without providing any supplementary feed.

In the United Kingdom, interest on artificial breeding of oysters developed with the work of Cole (1937, 1939) who reared a large number of larvae to metamorphosis in large outdoor tanks. Bruce *et al.* (1940) were probably the first to develop laboratory methods for rearing larvae of *Ostrea edulis*. They fed the larvae with cultures of flagellates.

In Japan, Hori and Kusakabe (1927) were among the first to succeed in raising the larvae of the Japanese oyster *Crassostrea gigas*. They used *Chlorella* as food for the larvae. However, it was Imai *et al.* (1950, 1954) who succeeded in breeding the oyster and rearing the larvae in outdoor tanks by feeding them with the flagellate *Monas*.

Following the work of Wells (1920, 1926, 1927) and Prytherch (1924), there was a gap of about two decades when no further progress was made in this field in the U. S. A. Starting from the early forties, Loosanoff and his colleagues in the Milford laboratory made path-finding researches in artificial breeding of molluscs. For the first time, Loosanoff (1945) showed that the American oyster could be induced to spawn outside the spawning period through manipulation of temperature. This enabled obtaining larvae of the oyster on year-round basis against the restricted spawning of 8 to 10 weeks in nature. Through thermal control it was possible to accelerate spawning outside the normal propagation period and also to retard the gonad development and spawning during normal spawning season. The Milford laboratory has since then made tremendous strides in all aspects of larval rearing, including mass culture of suitable food organisms for the different stages of development. Work on hybridisation and genetics has also made considerable progress. Based on the techniques and equipments developed by Wells and supported by the modern developments in shellfish larval rearing in Milford, commercial shellfish producers started to develop oyster hatchery in Long Island. They have gradually evolved into commercial hatcheries which are located along the Atlantic and Pacific coasts of U.S.A. and also the maritime provinces of Canada. On the contrary, although the techniques for the larval rearing for several species of molluscs have been developed and perfected in the Oyster Research Institute and at other centres in Japan, these have not blossomed to commercial hatcheries except on a very moderate scale.

INDUCED SPAWNING

From the time Prytherch (1924) obtained his initial success in spawning of *Crassostrea virginica* through thermal stimulation, there has been a good deal of work on various aspects of induced spawning of molluscs. There have generally been two lines of approach. The first approach has been to induce the mature specimens occurring in nature during spawning season to spawn under controlled conditions in the laboratory; this also led to the application of the reverse process of delaying spawning in the mature specimens. The second aspect deals with conditioning of the molluscs for acceleration of the maturation process irrespective of their maturity stages in their natural environment and spawning them

at critical temperature. Once the fully mature and ripe individuals have been obtained, spawning has been effected through physical, chemical or biological stimulation or a combination of any two of these.

Physical stimulation

Very often spawning is observed in bivalves that have been brought to the laboratory and left in sea water. It occurs under varied conditions such as the change of environment from air to sea water, mere mechanical handling of the molluscs, immersion in sea water after transportation and also changes in water temperature, salinity or pH. In the case of the pearl oyster *Pinctada fucata* such natural spawning has been observed when the oysters are brought from a depth of about 15-20 metres and placed in sea water collected from the surface (Alagarswami *et al.*, 1980). Similarly when the oysters of the farm were brought to the laboratory, spawning occurred. However, successful spawning by any of the above processes is not repeatable as it has not been possible so far to identify the factors responsible for the natural spawning under the conditions mentioned above. Field (1922) found that rough handling of *Mytilus edulis*, such as shaking them in a dish of sea water, would induce spawning within one hour. Exposure to air has been found to be effective in the case of *Haliotis* (Carlisle, 1945, 1962; Ino, 1952). Inoue (1969) found such stimulation to be more effective for the abalone than thermostimulation.

Thermostimulation has been the commonest method adopted in spawning several species of molluscs, particularly those in the temperate and sub-tropical regions. Although Galtsoff (1930, 1932) developed this method for *C. virginica* it was Loosanoff and his colleagues who used the technique on a large number of species of oysters and clams (Loosanoff and Davis 1963). Generally a gradual rise in temperature has been found to yield better results than a quick rise in temperature. Loosanoff and Davis (1963) found that the oyster from the northern Long Island required 15 days of conditioning at 21°C, 8 days at 24°C and 5 days at 27°C. The oysters from the southern New Jersey area were found to require a longer duration of conditioning, *i. e.*, 55 days, 32 days, and 22 days at the above temperature levels. This proved that oysters from different temperature zones require different conditioning periods for maturation and spawning. The conditioned oyster usually spawned around 25°C.

Although thermostimulation alone has been found sufficient, in several instances addition of sperm or egg suspension has been found to help the process of spawning more quickly (Loosanoff and Davis, 1963). Temperature shock has also been successfully applied to many species of bivalves and also gastropods such as *Haliotis*. The quahog *Mercenaria mercenaria* has been spawned by applying the same techniques as in the case of *C. virginica* and the conditioning period has been found to be 2-3 weeks (Loosanoff and Davis, 1936), Imai and Nishikawa (1969) spawned *Anadara broughtoni* by maintaining them at 23-28°C. Kanno *et al.* (1965) found that *Anadara* requires an integrated water temperature above 20°C for spawning. The scallop *Pecten yessoensis* has been found to have a low effective temperature of 8.0-8.5°C for spawning, and even a rise of 0.5°C was found to be sufficient for inducing spawning (Yamamoto, 1949). Iwata (1951) spawned *Mytilus edulis* by a sudden raise of temperature from 7° to 15°C.

Rao *et al.* (1976) obtained spawning in *Mytilus viridis* by raising the temperature from 26.5-28.0°C to 32-35°. Wada (1963) effected spawning in the pearl oyster *Pinctada martensi* by raising the temperature from 10-15°C to 24-27°C. However, he found this effective only for the males and not for the females. Wada (1976) achieved successful spawning in the same species and by increasing the temperature from 25°C to 30°C and found that more than 700-800 degree-days would be required for the gonads to mature in the Ago Bay. Alagarswami *et al.* (1980) found that raising temperature from 28.5°C to 35°C was effective to induce spawning in *P. fucata* in tropical waters.

The pearl culturists of Japan make use of the thermal stratification in the bay for artificially spawning the oysters to prepare them for nucleus implantation (Alagarswami, 1970). By changing the hanging depth of the oysters, they are subjected to low or high temperature conditions and spawn in the higher range of temperature. This practice is widely applied in artificial breeding of several species of molluscs such sa oysters, scallop and cockle in Maine bay (Imai, 1977).

Electrical stimulation

The Japanese workers have had some amount of success in spawning the mussels by electrical stimulation. Iwata (1949) reported spawning in *Mytilus edulis* by a faradic stimulation (50 cycles). An induction of 20 volts of five seconds duration was found sufficient for the discharge of gametes. However, Loosanoff and Davis (1963) could not succeed in spawning the same species by this method.

Chemical stimulation

Next to thermal stimulation the technique which is widely used for inducing molluscs to spawn is the use of chemicals. Chemical stimulation has been applied through two different ways—by exposing the animals to the sea water medium in which the chemical has been dissolved in different concentrations or by injection of the chemical in the body of the mollusc.

In most cases alkaline sea water medium has been found to be very effective for the purpose. Wada (1942) induced spawning in *Pinctada maxima* by half per cent 0.1 normal NH_4OH sea water. Wada (1947) obtained artificial fertilisation of *P. martensii* by application of 1/10,000 to 1/1,000 N ammoniated sea water for 30 seconds to 1 minute. Setoguchi (1959) spawned *P. margaritifera* using 1.2-1.5 per cent 0.1 normal NH_4OH sea water. Kobayashi and Yuki (1960) found that the rate of fertilisation reached almost 100% by increasing the pH from 8.3 to 8.6 by adding ammoniated sea water. Kinoshita (1963) reported spawning in *Pecten yessoensis* by a change in pH. Iwata (1949, 1951) achieved successful spawning in *Mytilus edulis* by dipping the mantle containing reproductive elements in M/2 KCl solution for five minutes. He found that spawning was accelerated when the solution was made alkaline by the addition of NaOH and that it was inhibited when the medium was acidic by the addition of HCI. Iwata (1951, 1952) conducted some experiments on spawning of mussels by dipping the mantle in M/2 aqueous solution of NH_4Cl, NaCl and LiCl and in M/3 solution of $BaCl_2$, $SrCl_2$, $CaCl_2$ and $MgCl_2$. He found that only NH_4Cl and $BaCl_2$ were effective in inducing spawning. According to him, K, NH_4 and Ba ions appeared to have higher mobility and permeability through the cell membrane than others.

Morse *et al.* (1976, 1977, 1978) have recently developed a technique for inducing spawning in abalone, mussel, scallop and mangrove oyster by the addition of hydrogen peroxide to sea water which is normal or alkaline. Morse *et al.* (1978) have indicated that spawning in molluscs may result from a peroxide-induced stimulation of the endogenous enzymatic synthesis of potent hormonelike prostaglandin molecules. They found that the alkaline medium, though not essential for induction of spawning, increases the proportion of animals that will spawn in response to a given concentration of hydrogen peroxide. Alagarswami *et al.* (1980) reported that a concentration of 3-6 mM peroxide was found to evoke some spawning response in *Pinctada fucata*. They also found that Tris-buffered sea water with a pH of 9.0 induced 78.6% of pearl oysters to spawn. Alagarswami *et al.* (1980) obtained 68.4% success in spawning *P. fucata* in the alkaline medium of pH 9.5 prepared by addition of NaOH.

Injection of chemicals has been commonly used by the Japanese workers for inducing spawning in the clams. Iwata (1948) obtained spawning in *Tapes japonica* by injecting 3 ml of neutral potassium salt solutions (M/2) such as KCl, KNO_3, KBr and K_2SO_4 into the visceral cavity. He found that 5 ml of isotonic aqueous solution was sufficient to discharge the gonadal products of *Mactra veneriformis*. Sagara (1958) succeeded in spawning *Tapes* and *Meretrix lusoria* by injection of 2 ml of N/5-N/20 NH_4OH solution into the gonad. Alagarswami *et al.* (1980) found that injection of small doses of 0.2 ml 1/10 normal NH_4OH resulted in the profuse spawning of *P. fucata*.

Biological stimulation

It has been well established that adding of egg or sperm suspension can induce spawning in many molluscs and that it sets up a chain reaction where the whole population could take part in spawning. Galstoff (1930, 1938) was perhaps the earliest worker who induced spawning in *Crassostrea virginica* by adding the sperm in the water around the female. Loosanoff and Davis (1963) have demonstrated this process in a number of molluscs, particularly as a complementary technique to thermostimulation. Miyazaki (1938) experimented with the green alga *Ulva pertusa* and confirmed that it contains an active principle which induces spawning of male oyster *Crassostrea gigas*. He also found similar substance in the algae *Enteromorpha linza, E. intestinalis, E. ramulosa* and *Monostroma* sp. Miyazaki (1938) opined that the algal extract possessed substances similar in nature to that contained in the egg water of the oyster judging from the latency of its reaction, stability to heat and other physico-chemical properties. He had also worked out the minimum dosage which had been found effective.

NEUROSECRETORY CONTROL OF REPRODUCTION

Compared to experimental works on induced spawning of bivalves using external stimuli which have been enumerated above, the mechanism of hormonal and neurosecretory control in the bivalves has been little understood. Lubert (1956, 1957) has suggested neurosecretary control of spawning in *Mytilus edulis* and *Chlamys varia*. He found that the response of these bivalves to other spawning inducers is maximal when there has been a reduction of the neurosecretory products of the cerebropleural and visceral ganglia, after a maximum coincident with gametogenesis (*cf.* Fretter and Graham, 1968). Lubert suggested that the neurosecretion inhibits response to the factors which cause spawning.

Galtsoff (1930, 1938) found that the activating agent produced by gametes is heat stable and dialyzable. The females responded only to sperm of the same species. On the other hand, the male oysters were activated by other males, by a variety of eggs or egg waters, and by thyroxin and glutathione (Galtsoff, 1940). Nelson and Allison (1940) have shown that the receptors in this case are placed on the gills and that their stimulation induces relaxation of a sphincter on the male duct, resulting in spawning.

Nelson (1936) discovered diantlin, a hormone-like protein in the oyster sperm. It has the effect of increasing the ventilation of the mantle cavity by increasing the size of the branchial pores, relaxing the adductors and accelerating the rate of ciliary beat. Young (1942, 1946) recorded that the testicular tissue of *Mytilas californianus* contains a substance causing spawning in females. Wada (1954) found a similar gonadal stimulant affecting the discharge of sperm in *Tridacna.*

Iwata (1952) found that in *Mytilus edulis,* the stimulation of ovarian cells was induced either by penetration of cations of small ionic diameter, such as KNH_4 and Ba or by penetration of NH_4OH. When the interior cells become alkaline, the spawning may be induced. He considered that induced spawning by temperature stimulation may be due to the increased permeability of the ovarian epithelium, which may render the interior of the cells temporarily alkaline, thereby inducing the discharge.

Morse *et al.* (1978) presumed that hormone-like prostaglandins may also control physiological processes governing reproduction in many invertebrates, including molluscs. They found that mammalian prostaglandins in very low concentration in sea water could specifically induce spawning in abalones. Morse *et al.* (1978) succeeded in control and synchronous induction of broadcast spawning in males and females of abalones (*Haliotis rufescens, H. corrugata* and *H. fulgens*), mussels (*Mytilus edulis* and *M. californianus*), scallop (*Hinnites giganteus*) and the mangrove oyster (*Crassostrea rhizophora*). Enzymological and physiological studies carried out by them indicated that spawning may result from a peroxide-induced stimulation of the endogenous enzymatic synthesis of potent hormone-like prostaglandin molecules. Morse *et al.* (1976) claimed that hydrogen peroxide induction is an inexpensive and easily controlled chemical method for a large number of animals used in mariculture.

The neurosecretory functions of visceral and cerebral ganglia and their effect on spawning of mussels and oysters have been demonstrated (Lubert, 1856, 1959; Nagabhushanam, 1963, 1964, 1980; Desai and Nimavat, 1980). The annual neurosecretory cycle and the gametogenic cycle appear to be closely correlated. Ablation of the cerebral ganglion at the end of gametogenes is, but before spawning, hastens maturation process and gamete release. However, Lubert (1956, 1959) remarks that it is impossible to decide if the mechanism is nervous or hormonal in nature. Lubert and Choquet (1971) have associated a 'hormone', which is linked with the spermatozoa, with spawning in *M. edulis.* Nelson and Allison (1940) had demonstrated the presence of a hormone active in inducing spawning in *C. virginica.*

GENERAL CONSIDERATIONS

Starting from Prytherch (1924) and Galtsoff (1932), a great deal of work has been done on the controlled breeding of almost all the commercially important species

of molluscs, particularly those species which are used in aquaculture. The approaches to the problems have been pragmatic and result oriented. However there is a big gap in our understanding on the hormonal and neurosecretory mechanism of control of reproduction which is necessary for a thorough understanding of the reproductive physiology. The species on which some detailed work on hormonal control of reproduction has been done appears to be *Octopus vulgaris* (Wells and Wells, 1959) which is a highly evolved species among the molluscs. The few works on bivalves have already been mentioned. It is important that directed investigations on hormone and neurosecretory control of reproduction of all species of economic value are taken up.

The review has shown that different techniques of induced spawning have been evolved for different species of molluscs. Most of the species respond to more than one method, singly or in combination of two or more methods. Commercial aquaculture operations would require methods which are efficient for application on a large scale, which are inexpensive and which do not produce any after-effects on the population used for spawning. Morse *et al.* (1978) have indicated that the hydrogen-peroxide technique would meet these requirements. There is scope for further work on this aspect to evolve or standardise techniques suitable for species and regions.

Hatchery technology for the seed production of molluscs has been well developed in the U. S. A (Davis, 1969) and Japan (Imai, 1977). The wide ambient temperature range in the sub-tropical and temperate waters has made it possible to use nearly identical techniques in the hatcheries in the above two countries. Although the principles of the technology could be adopted in the tropical region, the actual techniques and operations would be distinct. Tropical hatchery for molluscs is a new development which needs a good deal of attention for making the culture of molluscs, particularly food oyster, pearl oyster and mussel in which seed availability in nature is unpredictable. Some work on controlled spawning and larval rearing of these species has already been carried out (Alagarswami *et al.* 1980; Kuriakose, 1980; Samuel, 1980; Desai *et al.*, 1980). Preliminary work on establishment of an experimental molluscan hatchery has been taken up (Nayar and Easterson, 1980).

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