HATCHERY PRODUCTION OF BIVALVE SEEDS IN SOUTHEAST ASIA: STATE OF THE ART AND FUTURE RESEARCH DIRECTIONS

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ABSTRACT

Since the mid 1980's, adaptive research in a number of laboratories in Southeast Asia has resulted in the successful transfer of bivalve hatchery production technologies pioneered in the United States, Canada and Western Europe to the region, particularly Thailand and Malaysia. Progress in microalgal production, broodstock conditioning, induced spawning, larval culture, setting and other aspects of hatchery operations are highlighted. Economic viability has been demonstrated and currently a few laboratories are poised for commercial level operations. Nevertheless, a number of areas have not been adequately researched. Serious information gaps exist in areas such as the synchronisation of broodstock maturation, larval feeds, diseases of larvae and genetic improvements. These must form the foci of future research to ensure the successful commercialisation of tropical bivalve seed production and culture.

INTRODUCTION

Bivalves have been harvested for food by coastal communities in Southeast Asia for centuries and substantial fisheries for cockles, clams, mussels and oysters still exist in many parts of the region. From the 1950's onwards total production for a number of species have been enhanced by the introduction of culture activities. In its simplest form this involved little more than transplanting juveniles to other areas for growout and harvesting the products when they reached marketable sizes. Subsequent developments have involved efforts to increase seed "catch" (*e.g.* by placing out suitable collectors at appropriate time windows for species such as mussels and oysters in Thailand and the Philippines) or identifying and conserving areas of natural spatfalls for subsequent "harvesting" (*e.g.* blood cockles in Malaysia). Further refinements in seed capture and growout activities have resulted in the establishment of viable culture industries for a number of species, with "cultured" products contributing significant increases to landings from natural fisheries. During the last decade, efforts to further increase culture production have often been constrained by insufficient supply of seeds from natural sources (Angell, 1986; Ng., 1987). This has led local researchers to look at alternative options such as hatchery seed production.

During the late 1970's and early 1980's significant advances were made in bivalve hatchery seed production technology in Western Canada and the west coast states of USA, in particular, the discovery that eyed larvae of the oyster *Crassostrea gigas* could survive out of water for up to a week if kept moist and cool (5°C) and would set if subsequently reimmersed in seawater. This led to the development of "remote setting of the eyed larvae", a technique that tipped the economics in favour of hatchery produced seeds against natural spat collection. Subsequently, hatchery production of oyster seeds became so successful that by the early 1990's, seeds from hatchery sources accounted for more than 90% of the total production of oysters in British Columbia, Canada and the west coast states of USA.

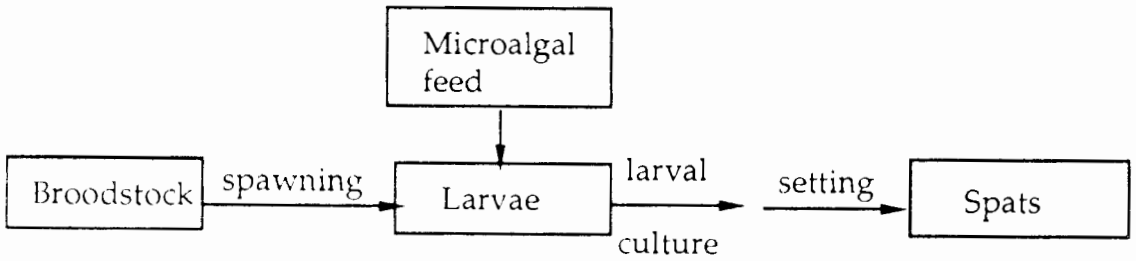

Figure 1. Basic processes in a bivalve seed production operation.

Increased awareness of such advances and adaptive research by regional bivalve researchers with funding and collaborative support of agencies such as GTZ, IDRC and BOBP have led to the successful transfer of hatchery seed production technology to Southeast Asia particularly Thailand and Malaysia and to some extent the Philippines. This paper summarises the basic processes involved in hatchery operations, highlights the progress made in recent years and identifies some areas for future research.

PROGRESS IN HATCHERY SEED PRODUCTION

Overview

There are currently more than 15 species of tropical bivalves for which seeds have been successfully produced, at least at the laboratory scale. These include *Anadara granosa*, *Crassostrea belcheri*, *C. iredalei*, *Ostrea folium*, *Saccostrea* sp, *Paphia undulata*, *Solen brevis*, *S. strictus*, *Perna viridis*, *Pholas orientalis*, *Placuna placenta*, *Tridacna squamosa*, *Pinctada maxima*, *Pteria penguin* and *Amusium pleuronectes*. Amongst these, the production of oyster seeds (*C. belcheri* and *C. iredalei)* has been taken to the pilot commercial scale. Laboratories which are currently involved in bivalve hatchery seed production research are listed in Table 1.

The basic processes involved in bivalve seed production are outlined in Fig. 1. Suitably conditioned or field ripened broodstock are induced to spawn with appropriate stimulation. Fertilized eggs are

Table 1. List of laboratories which are involved in bivalve seed production research in Southeast Asia

Country	Laboratory	Main species
Malaysia		
	Muka Head Station, Universiti Sains Malaysia, Penang	*Anadara granosa* *C. belcheri* *C. iredalei* *Perna viridis* *Paphia undulata* *Solen brevis* *Pholas orientalis*
	Fisheries Research Institute, Penang	*C. iredalei* *Pinctada* sp.
Philippines		
	Bolinao Laboratories, Marine Science Institute, U.P. Diliman	*Tridacna* spp.
	SEAFDEC AQD Iloilo	*Placuna* sp. *C. iredalei*
Thailand		
	Prachuap Khiri Khan Mariculture Unit	*Anadara* spp. *C. belcheri* *Saccostrea lugubris* *Pinna bicolor* *Pinctada maxima* *Pteria penguin* *Solen strictus*
	Ang Sila and Si Chang Laboratories, Institute of Marine Resources, Chulalongkorn University	*C. belcheri* *Saccostrea lugubris*

washed and allowed to undergo embryonic development until the D-hinge stage when feed (in the form of microalgae) is provided at appropriate rations. With good water quality and careful hatchery management practices, the larvae will be developed through the umbo to the plantigrade (eyed larva) stage and subsequently set on appropriate substrate to produce seed (spats).

Research & Development Activities

Fig. 2 outlines some of the R&D activities directed towards maximising the efficient production of bivalve seeds on a mass scale. These will now be discussed using the production of tropical oyster seeds as the model since information on this group appeared most extensive

Broodstock: Numerous studies (Angell, 1973; Broom, 1985; Sahavacharin *et al*., 1988; Wong *et al*., 1990) have shown that in the absence of strong synchronising factors (such as low temperature in the temperate countries), bivalves in the tropics showed more diffuse breeding patterns although a few breeding peaks can often be distinguished. Their frequency and duration drifts from year to year reflecting perhaps variations in local environmental parameters or species specific variations.

From a hatchery production point of view, this diffuse breeding pattern created some problems but also offer interesting challenges. The presence of broodstock at different developmental stages meant that much higher numbers (often hundreds) are required for each spawning attempt. It also means that if the spawning inducer is powerful enough, (*e.g.* serotonin) eggs which are not fully developed are also released. The survival of larvae from these eggs are often low. On the other hand, the asynchrony also means that broodstock for hatchery seed production are available or can be made available all year round, especially with proper "conditioning". Encouraging success has been achieved by conditioning broodstock in waters of high phytoplankton concentrations such as in prawn farms where such facilities are available within convenient distances of the bivalve hatchery. The cropping of phytoplankton by oysters in such farms actually helped improve water quality and are therefore welcomed by prawn farmers. Studies using spawned out *C. iredalei* showed that the percentage of ripe individuals reached a peak of 60% within 30 days of being kept in a prawn farm. (Wong, 1992). Using similiar procedures, laboratories in Thailand, (Prachuap and Ang Sila) were also able to achieve good spawning success as well as improved larval performance.

Algal Production : Larval bivalves require microalgae (5-15µm diameter) as feed. Species that are commonly cultured in the regional laboratories include *Isochrysis galbana* (Tahitian strain T-Iso) *Chaetoceros calcitrans* and *Tetraselmis* sp. Most laboratories practice the batch culture system. Axenic starter cultures are progressively taken up through intermediate cultures (2 l - 5 l scale) to mass cultures (20 l to 10 tons scale). For cultures up to the 50 l scale artificial lighting is normally employed. By enriching the culture media with CO_2, cell concentrations up to 6.0 M cells/ml are regularly achieved. Mass cultures above the 1000 l level are usually conducted outdoors under natural light conditions. Cell concentrations in outdoor culture systems rarely exceed 1.5-2.0 M cells/ml. It is important to note that under the high temperatures prevalent in the tropics bacterial buildup is also very rapid and the use of such feed often introduce pathogenic bacteria into the larval culture tanks. Another point to note is that the nutrient value of the cultured microalgae are best during the log phase of growth. Once the stationary phase is reached, the increasing presence of breakdown metabolites not only diminishes nutrient value but can actually bring about deleterious consequences. Over the last few years, most regional laboratories have developed reasonably reliable systems for algal production based mainly on T-Iso and *Chaetoceros calcitrans*.

Induced spawning: A number of techniques have been successfully employed to induce spawning in tropical bivalve species (Table 2). The chemical serotonin rapidly induces spawning in oysters, carpet shells, mussels and angelwing shells, while temperature, high feed concentration and hydrogen perox-

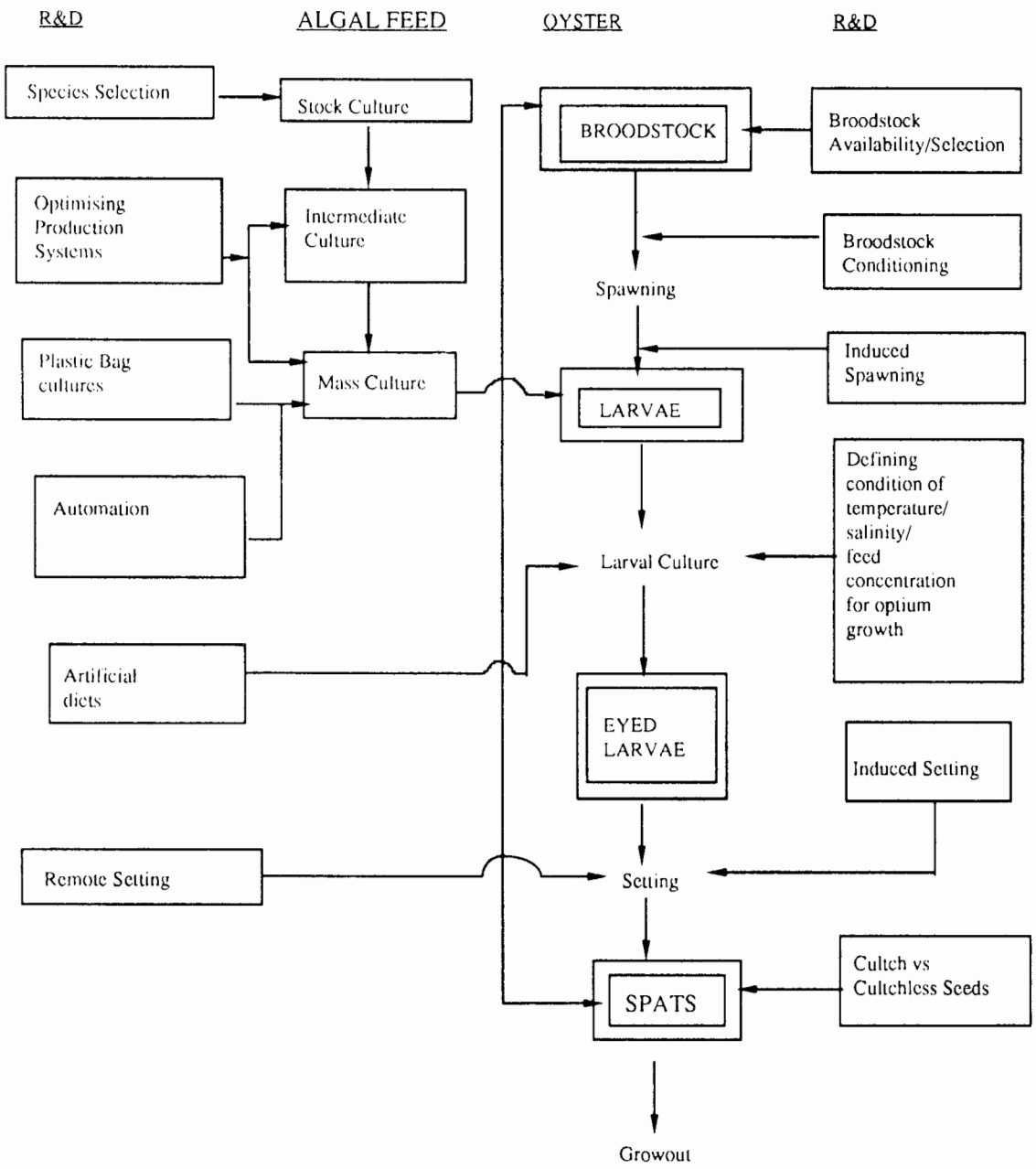

Figure 2. Research & Development activities in bivalve hatchery operations.

Table 2. Bivalve species in Southeast Asia for which seeds have been produced.

Species	Spawning stimulus	Larval duration (days)	Source
Anadara granosa	Alternate exposure to low and high temperature	21 - 22	Wong *et al.*, 1985 Kamal Zaman, 1986
Crassosstrea belcheri	Serotonin Air dry + 35°C seawater	20 - 22 18 - 22	Wong *et al.* 1989 Nugranad (pers. comm.)
Crassostrea iredalei	Serotonin Serotonin	22 18 - 22	Ver, 1986 Tan & Wong, 1990
Ostrea folium *Paphia undulata*	Spontaneous Serotonin	8 - 15 14 - 15	Wong, unpublished Shamsuddin *et al.*, 1987
Solen brevis	High feed concentration	8 - 10	Wong *et al.*, 1986
Pholas orientalis	Serotonin	27 - 29	Wan, 1987
Perna viridis	Temperature and NH_4OH Serotonin	1 29	Sivalingham, 1977 Petrus, 1992
Pinctada maxima	Air dry	21	Nugranad (pers. comm.)
Pteria penguin	Air dry	21	Nugranad (pers. comm.)
Tridacna squamosa	Serotonin	10 - 14	Nugranad (pers. comm.) Gomez (pers. comm.)

ide are effective for the blood cockle, razor clams and fanshells respectively. In Thailand, Nugranad (pers. comm.) reported that ripe oysters can be induced to spawn by overnight exposure to air followed by immersion in running sea water at elevated temperature (35°C). Spontaneous spawning often occur while oysters with very ripe gonads were placed in running UV treated sea water during depuration but this is usually not consistent or predictable and as such has not be adopted as a regular practice.

In order to reduce contamination by microorganisms adhering to the outer surfaces of the shells, broodstock intended for spawning are cleansed by initial scrubbing with a metal brush, immersing in clean freshwater to ensure valve closure and then exposing to a 30 ppm "chlorox dip" for 10-15 minutes to kill off surface microorganisms. Spawning individuals are separated and allowed to complete the spawning process in isolation. Sufficient quantities of sperms are then added to the solution containing the eggs, aerated gently to ensure good mixing and then left to stand for 15-20 minutes for fertilization to be completed. This procedure synchronises the timing of fertilization, post fertilization development and facilitates subsequent handling. Fertilized eggs (40-60 µm) are screened through a 100 -120 µm sieve to filter off spawning debris and then collected in a 20-25 µm screen. After several further washings with filtered and UV treated sea water, the eggs are transferred to large tanks (1-5 tons capacity) and held at a stocking density of up to 10 eggs per ml. Gentle aeration is normally provided but no feeding is required over the first 24 hours.

Larval culture : The duration of larval cycles in tropical bivalves are shown in Table 2. It varies from 8-10 days for the razor clam *Solen brevis* to 27-29 days in the angelwing shell *Pholas orientalis*. Within each species, the duration of the larval cycle can vary substantially between batches *e.g.* for the oyster *Crassostrea belcheri* it can be anywhere from 14 to 30 days even when reared under similiar conditions of temperature, salinity and feed rations. In hatcheries where culling is carried out, the fastest growing individuals can set as early as 12 days. Culling on the basis of size is practiced in laboratories involved in pilot scale production.

The most important factors affecting larval survival and growth are water quality, temperature, salinity and feed. Larval culture in most laboratories are conducted in fiberglass tanks of 50 l to 5000 l capacities at initial stocking densities of 10 larvae/ml. This is reduced to 3-5 larval/ml at the eyed larvae stage. Oxygen is seldom a problem as aeration is normally supplied. A complete water change is done every alternate day.

During the first 24 hours of its existence, the zygote develops through the gastrula and trochophore stages into the straight-hinge (D-shape) larva. At this stage the gut and velum are fully developed and feeding begins.

Feed requirements vary from species to species in terms of types of microalgae as well as optimal feed concentrations. Larvae for most tropical bivalves have been successfully cultured using T-Iso and /or *C. calcitrans* though others like *Tetraselmis* sp. has also been used with varying degrees of success. As the larvae grows, feed concentrations need be increased. A typical feed regime for oyster larvae is shown in Table 3. Details of feed types and rations varies with species and laboratories.
The duration of the larval cycle varies with salinity. In the case of *C. belcheri* where the broodstock were conditioned at 30-32 ppt, larvae reared at 18 ppt set at 17 days whereas those reared at 30 ppt only set after 23 days. (Tan, 1993)

Table 3. A typical feed ration used at different stages of larval development in *Crassostrea belcheri* in Universiti Sains Malaysia.

Age of Larva (days)	Concentration of T-Iso (cells/ml)
1 - 2	5000
3 - 4	10000
5 - 6	20000
7 - 12	25000
13 - 14	30000
15 - 18	35000
19 - 23	45000

Setting and Induced Setting : At the end of larval development, the plantigrade stage is reached. In most bivalve larvae, this stage is characterised by the presence of a well developed foot and eyespot (eyespots are however, absent in cockles and clams) while the velum shows progressive resorption. The larvae alternate between swimming and crawling at the bottom and are now ready to set and metamorphose into the juvenile form provided suitable conditions are available. In sessile bivalves such as oysters and mussels, setting is enhanced by the presence of suitably conditioned substrates. In most regional laboratories, the percentage set for each cohort of larvae is reported to vary from 10 - 30% for oysters such as *Crassostrea belcheri* and *C. iredalei*. These figures are comparable to setting rates reported for *C. gigas* in the hatcheries in the west coast of Canada and the USA. (Jones & Jones, 1983). However, given the operational cost of raising larvae to this stage, the "wastage" rate of 70 - 80% appeared rather high and numerous attempts have been made to increase the percentage of setting success. Recent studies at Universiti Sains Malaysia have shown that the percentage set can be increased from 20 - 25% to 36 - 38% by exposure to lower salinities and from 20 - 25% to 70 - 90% by exposure to appropriate concentrations of neuroactive compounds such as epinephrine, norepinephrine, L-Dopa and GABA (Tan, 1993). These results extend to tropical oysters, the findings report for temperate species like *C. gigas* (Coon *et al.*, 1985).

Besides a 2 - 3 fold increase in setting success both epinephrine and norepinephrine also induces the formation of a high percentage of free or cultchless spats, a desirable feature in the production of single oysters for the half-shell market. The post set survival of spats exposed to chemical induction are comparable to those setting naturally while the additional cost of neuroactive compounds is negligible relative to the total cost of rearing larvae to the plantigrade stage.

Remote Setting : Prior to the 1980's, the economics of commercial scale production of oyster seeds via hatchery techniques was under constraint by the need to set spats on cultch (oyster shells) before sale. As a result , when spats were sold, the transport of the bulky oyster shells on which spats have setted posed serious cost problems especially when large orders need be sent to growout sites hundreds of kilometers away.

This problem disappeared during the early 1980's with the discovery that plantigrade or eyed larvae of *C. gigas* not only survived for up to a week when kept moist at low temperature (5°C) but subsequently were able to set and metamorphose into healthy spats (*e.g.* Jones & Jones, 1983). These findings effectively eliminated the problem of transporting cultched seeds since large numbers of eyed larvae (10 milion eyed larvae has the same volume as 4 golf balls!) can be airfreighted across great distances to be set on sites where subsequent growout can take place. Subsequent refinements led to the widespread application of this "remote setting" technique which resulted in the economics of hatchery seed production being so competitive relative to collection of natural seeds that by the 1990's, hatchery produced seeds constitute 90% of all oysters cultured in British Columbia and in the west coast of the United States.

Initial attempts by local laboratories to adapt this technique for *C. belcheri* was not successful as eyed larvae kept at 5°C performed worse than controls. Subsequent studies showed that the optimum holding temperature for this tropical species was at 15°C at which temperature, the performance of eyed larvae held for up to 72 hours was either equal or better than controls at 0 hour. (Wong & Tan, 1992). The optimum conditions for holding *C. iredalei* are currently being studied at Universiti Sains Malaysia.

Nursery Systems : Two types of oyster spats are produced in the regional hatcheries, cultch or cultchles (singles). In both cases, attempts to transfer the early spats to growout sites (with the aim of eliminating feed costs) have proved unsuccessful as high mortality were often recorded due to the excessive sedimentation common to tropical estuaries. As a consequence, most laboratories have to set up nursery systems in one form or another. Cultched seeds are normally nursed in raceways through which filtered seawater is recirculated. Feed is added to the system either once or twice a day. For single spats upwelling systems are normally used. These consist of hollow PVC segments with appropriately meshed nylon screens partitions. Large number (thousands) of single spats can be held in each upweller. A strong water current containing the microalgal feed is forced upwards through the thick bed of spats. Unused feed and waste is then discharged over the top of the upweller.

Most local laboratories have found that nursing the spats to at least 0.8-1.0 cm before sending them to the growout sites resulted in much higher survival rates.

INFORMATION GAPS AND FUTURE RESEARCH DIRECTIONS

Over the past few years most of the regional laboratories have reported increased production and/or the successful production of seeds of additional bivalve species. Nevertheless, a detailed analysis, complemented by discussions with fellow researchers in the region has indicated that serious information gaps exist. These will now be discussed.

Broodstock

Although some success has been achieved by conditioning oysters in nutrient rich waters such as

prawn farms, available data concerning the relationship between breeding cycles and environmental parameters have not shown clear trends. There is a need for more experimental studies to develop in depth understanding on what and how extrinsic or intrinsic factors trigger gonadal maturation and spawning in the commercially important tropical bivalve species. Knowledge and improved capabilities to manipulate final gonadal maturation as well as spawning without the need for such powerful spawning agents as serotonin are likely to yield more fully matured eggs thereby ensuring better larval performance. Synchronisation of final maturation of broodstock will also reduce the number of spawners required on each occasion as well as ensuring a consistent supply of spawners throughout the year.

Larval Feed

Algal production often accounts for more than 50% of a hatchery's operational costs. Hence improvements in this part of the operation will have very significant impacts on cost savings. Yet, most regional laboratories have basically adopted on a wholesale basis, classical techniques of culture developed 20-30 years ago. In most laboratories, insufficient emphasis has been placed on the algal section beyond establishing a unit capable of producing required quantities of algae on a reasonably consistent basis.

Most laboratories in the region have used the batch culture system with varying degree of success. Final mass culture systems are often conducted outdoors using natural light. Bacterial and fungal contamination occurs regularly and in fact contaminated algal feed is the most common source of bacterial/fungal contamination of larval cultures leading to the resultant "crashes".

Recent developments in high intensity sterile cultures inside large plastic bags, coupled with the setting up of automatic systems for monitoring and maintaining nutrient levels as well as cropping of such continuous culture systems have yet to be transferred to any of the regional bivalve laboratories. There is a need for more intensive research efforts in this area since improvements in this aspect of the production operation will obviously result in very substantial lowering of overall production costs.

Another area where exciting developments are taking place is the use of microencapsulated diets. Developed primarily to cater for feed requirements as well as to provide an effective micronutrient/vitamin delivery system for the prawn seed production industry in the 1980's, suitably sized microcapsules (5-20 μm diameter) have been successfully used for rearing larval of giant clams to the settlement stage (Braley, 1992). The importance of research in this exciting field cannot be overemphasised. The successful development of suitable microencapsulated diets for raising larvae from the D-stage to the settlement stage will constitute a major breakthrough in bivalve hatchery seed production. It will significantly simplify hatchery operations as the need to synchronise algal production cycles to larval requirements (the single most important bottleneck in the production process) as well as the considerable resources put onto microalgal production can be much reduced if not eliminated entirely. Likewise the problem of bacterial/fungal contamination of larval cultures via algal feed. In addition, species and/or stage specific diets can be developed together with the necessary vitamin supplements to ensure optimal growth and survival of the larval stages. Research in this critical area must be given the highest priority.

Larval Culture

Although successful larval culture has been reported for numerous species, nevertheless the percentage survival from egg - settlement stage could be further improved. Most laboratories in the region report that a 30-40% survival would constitute a good batch. Percentage of survival varies tremendously between batches and complete "crashes" occur frequently. While adequate information defining temperature and salinity conditions as well as feed rations for optimal growth and survival are available for some species of oysters and mussels, little information exist for many other commercially important tropical bivalve species. More studies need to be conducted.

Another area where serious information gaps exist is disease and infections of bivalve larvae. Currently, most laboratories in the region attempt to prevent diseases and infections via hatchery management

procedures. Where an infection occurred, the whole batch is simply discarded and the system disinfected before subsequent use. None of the laboratories in the region have adequate facilities or personnel for histopathological research. There is an obvious need for studies in this area for it is only through an understanding of the disease development and transmission processes that more effective prophylactic measures can be implemented.

Induced and Remote Setting

Current information on the effectiveness of physical and chemical factors for onsite and remote setting is only known for a single oyster species, *i.e. Crassostrea belcheri*. While there is ongoing studies at Universiti Sains for *C. iredalei*, little is known for other bivalve species. As indicated earlier, the use of external inducers can result in significantly enhanced setting success. Knowing the appropriate temperature to transport larvae for remote setting can also greatly increase setting performance. The ability to define physico-chemical conditions for optimal setting will greatly enhance production efficiencies since considerable effort has already been expended to raise the larvae to this settlement stage. There is a need for similar information for other important bivalve species.

Genetics

In most of the laboratories, spawners are usually obtained from the wildstock. While a few cross-breeding experiments have been attempted in Prachuap Khiri Khan and Ang Sila, no sustained genetic studies have been programmed. There is an obvious need for genetic selection studies to develop strains that are fast growing and disease resistant so that quality broodstock can be supplied to the commercial hatcheries.

CONCLUSIONS

Progress in bivalve hatchery technology in Southeast Asia has reached the point where commercial scale operations will soon become a reality. It is anticipated that with continued research efforts into a few critical areas, bivalve hatchery seed production will soon reach a level where they can supply the amount of quality seeds required to expand existing culture industries in the same way prawn hatcheries drove the expansion of prawn culture in the 1980's.

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