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Review

AN OVERVIEW OF THE METHOD, MANAGEMENT, PROBLEM AND THEIR SOLUTION IN THE PEARL OYSTER (*Pinctada margaritifera*) CULTURE

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ABSTRACT

Pearl culture operations can be divided into three categories which are collection/hatchery production, ongrowing and pearl culture. For the hatchery, the pearl oyster industry relies on spat collection at natural production atolls where spat is abundant during the warm season and also from broodstock in the laboratory condition. After that, hatchery grown juveniles are put into the sea on the material which they settle upon. The spat are left to grow for 2 years till an average size of 90 mm. Pearl culture involves the implantation of a spherical nucleus together with a piece of mantle tissue (Saibo) from a sacrificial oyster, into the gonads. Although pearl culture is extensive with little control over weather, the use of good management methods can drastically increase productivity and result in higher profitability. Therefore, management of culture system such as site selection, settlement, feeding, stocking density and pearl culture technique is essential. For example, site selection is the most critical factor affecting pearl oyster productivity and spat collection, as the oysters spend most of their growing time exposed to water elements. Site selection must take into account important water quality parameters like temperature, salinity and turbidity. Moreover, it was identified several problems in pearl oyster culture including predation, disease and biofouling. They can result in massive loss in productivity. However, pearl industries have solution to deal with those problems. For instance, it can be done by cleaning mesh bag, biofouling organisms and pearl oyster regularly. For the future, the genetic approach like to create faster growing oysters, resistance to diseases and production of higher quality pearls has given promising results. Therefore, the productivity of pearl oyster can be improved.

Keywords: pearl oyster ; management ; culture ; *Pinctada margaritifera*

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INTRODUCTION

Pinctada margaritifera is distinguished by the black color of the outer surface of the shell and non-nacreous border. The silver nacre inside the shell is dark towards the distal rim, hence the name black lip (Saville-Kent, 1983). The black lip pearl oyster ranges from Gulf of California, Mexico to the Eastern Mediterranean sea (George, 1978), from French Polynesia to Cook Islands, and across the northern coast of Australia from champion bay in Western Australia to Moreton bay in Queensland. Aquaculture of the species began in 1961 after the French government started an experimental study on the species in collaboration with Nippo Pearls Co. of Japan in Tahiti. The pearl industry grew steadily and by 1998, black-lipped cultured pearls were cultured largely in Tahiti, Cook Islands, Fiji, Marshall Islands and Japan, with Tahiti dominating the market with 95% of total production (USD 143 million) and contributing to 25% of the total pearl production in the world (Hisada and Fukuhara, 1999). This essay will examine methods of culture of the pearl oyster and methods of management across different species which will result in maximum productivity and maximum profits. Moreover, the problem of Pearl oyster

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culture is also reviewed by giving some solution to deal with that obstacle.

METHOD OF CULTURE

Pearl culture operations can be divided into three categories which are (1) collection/hatchery production, (2) ongrowing and (3) pearl culture. Each phrase of production permits a degree of specialization by farmer and allows people of different income and different technical expertise to get involved in cultivation.

Collection/ hatchery production

In French Polynesia, the pearl oyster industry relies on spat collection at natural production atolls where spat is abundant, during the warm season (November to May). Farmers place their collectors, made of black shade mesh and suspended from a longline every 30 cm at a depth of 3 m (Cabral *et al.*, 1985). Spat oysters are left on collectors (**Fig. 1**) for 1 year till 4-5cm in length though some farmers may choose to have them for a year and a half or till they reach 14cm, where they are big enough to be grafted, and skip the ongrowing process altogether. (Le Moullac *et al*., 2003).

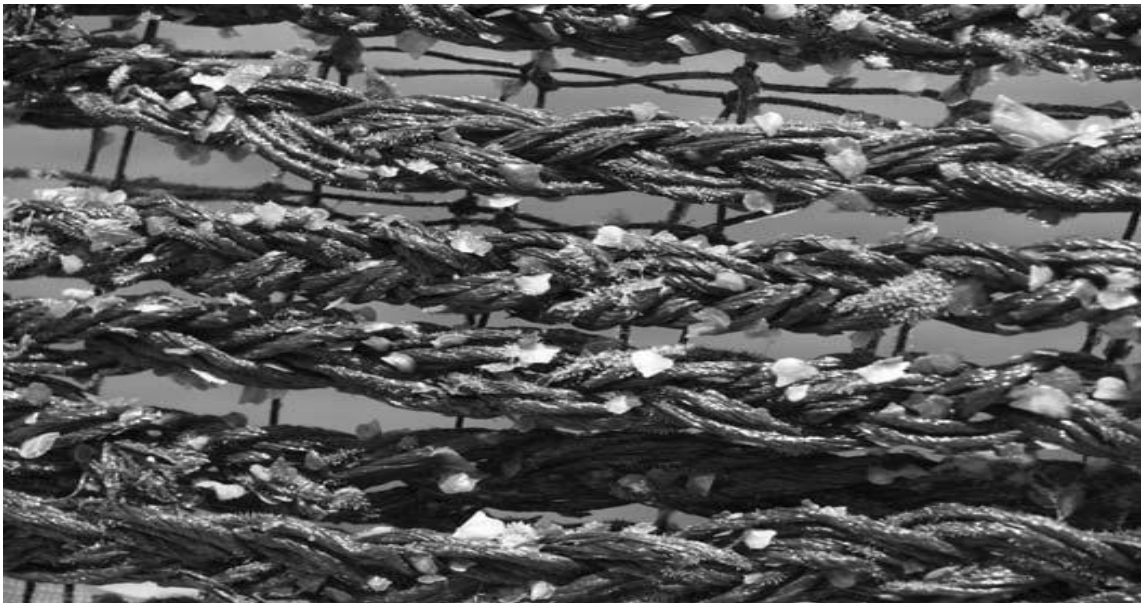

Fig. 1. Spat pearl oyster attached to spat collector (Southgate and Lucas, 2008)

In Australia, however, some of the spat are produced in hatcheries, where broodstock are first conditioned on 50–100 x 10^3 cells/ml of a 1:1 mixture of *Isochrysis galbana* and *Chaetoceros simplex* for 6 weeks. To induce spawning, oysters are held overnight at 20 ^{0}C, and then transferred to a raceway with the seawater temperature at 30–32 ^{0}C. The first spawning male triggers spawning by other oysters and this occur within a few minutes to 1 hour (Southgate and Beer, 1997). Spawning oysters are removed individually into individual containers. Larvae are reared in static water exchange systems where water is changed every 2-3 days. *Isochrysis galbana and Pavlova lutheri* were given once a day concentrations from 5 - 15 cells/µl until metamorphosis (Doroudi *et al.*, 1999). At day 20, when most of them reach 150µm, they are transferred to seawater settlement tanks.

On growing

Hatchery grown juveniles are put into the sea on the material which they settled upon. The material is hung from longlines in areas of calm water (**Fig. 2**). Longline are horizontal ropes which are supported by floats and anchored at the final part of ropes to maintain position. A protective mesh covers the material, and mesh size is increased as spat grow. The spat are left to grow for 2 years till an average size of 90 mm. A hole is then drilled through the posterior ear and the oysters are hung down in pairs on a down line.

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Fig. 2. Longline used for Pearl oyster culture. Note the panel nets containing pearl oyster juveniles suspended from the longline (Southgate and Lucas, 2008)

Pearl culture

Pearl culture involves the implantation of a spherical nucleus together with a piece of mantle tissue (Saibo) from a sacrificial oyster, into the gonads. The mantle tissue grows around the nucleus and secretes nacreous deposits to form a pearl. At a minimum size of 100mm, the preoperative pearl oyster undergoes a weakening process for 40 days for the muscular and gonadal epithelium to disintegrate. They are either crowded together, limiting the oxygen and food supply or moved to deeper water and then moved up the water column to create stress, which causes the oyster to spawn.

Implantation takes place during the cooler months, when the conditioned oysters are wedged open, an incision is made in the gonads and the nucleus and Saibo is added. The wound is cleaned with antiseptic. The oysters are then returned to the sea and placed in calm conditions. Cells in the pearl sac secretes nacre onto the outer surface of the nucleus and cultured pearls are harvested when nacre are 1 mm thick after 18-24 months (Gervis and Sims, 1992).

MANAGEMENT METHODS

Although pearl culture is extensive with little control over weather, the use of management methods can drastically increase productivity and result in higher profitability. Therefore, management of culture system such as site selection, settlement, feeding, stocking density and pearl culture technique is essential.

Site selection

Site selection is the most critical factor affecting pearl oyster productivity and spat collection, as the oysters spend most of their growing time exposed to water elements. Site selection must take into account important water quality parameters like temperature, salinity and turbidity. Temperature affects transparency level, absorption efficiency of suspension feeding, respired energy and excreted energy. Temperature was found to significantly increase respiratory energy of *P. margaritifera* at high temperatures reducing potential for growth (Yukihira *et. al.*, 2000). Compared to *P. maxima* (32 ^{0}C), optimum growth for *P. margaritifera* occurred at 23-28 ^{0}C and is therefore suitable for cooler climates. High temperatures were also found to increase oxygen consumption and ammonia excretion in another species *P. mazatlanica* (Saucedo *et. al*, 2004). The effect of ammonia excretion can be mitigated through co cultivation with red alga *Kappaphycus alvarezii*, which can remove nitrogenous waste and CO_2 released by oysters and provide O_2 from photosynthesis (Wu *et al.*, 2003). Temperature also has a pronounced effect on larval development in nursery culture. At 18-26^{0}C, *P. imbricata* increased rate and number of embryo development into D-stage veliger with increasing temperature, under 14 ^{0}C, larvae did not develop into veliger.

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Temperature also affects byssal attachment, with the highest rate at 18^0C (O'Connor and Lawler, 2004). Most importantly, temperature controls quality of nacre. Higher temperatures accelerate nacre deposition, however, lower temperatures decrease the deposition rates but increasing the quality of the nacre (Shirai, 1970). Colder temperatures have a positive influence on lustre (O'Sullivan *et al*., 1998) .Therefore, seeding of pearls is always done at the coldest times of the year.

Salinity also plays an important role in larvae development. Survival of *P. imbricata* larvae was highest at 32 and 35 ppt and at 29-35ppt, percentage of embryo developing to D-stage larvae increases significantly with increasing salinity (O'Connor and Lawler, 2004). Turbidity also has an influence on growth and survival of oysters. Turbidity is caused by suspended particulate matter (SPM), and is inversely proportional to organic particulate matter (Yukihira *et al.*, 1999), of which oysters feed from. *P. margaritifera* do not survive and grow well in areas of high SPM (Yukihara *et al*., 2006). Furthermore, *P. margaritifera* has develop a trophic strategy involving clearance of large amount of water to gain sufficient nutrients in nutrient poor water (Pouvreau *et al.*, 2000), and this process is energy consuming and reduces the scope of growth.

Settlement

A major part of a successful pearl venture depends on collecting maximum numbers of spat, both in the hatchery and from the sea. Settlement of larval spat is affected by various factors. Firstly, use of natural biofilm induces settlement in *P. maxima* larvae (Zhao *et. al*., 2003). Types of substrate also influence larvae settlement. Deep colour material like red and blue attracted significantly more larvae from *P. martensii* than lighter coloured green and yellow material. In addition, rougher material also promoted more settlement, so does coating of substrate with tissue extract from the same species (Su *et. al.*, 2007).

Depth and chemical cues also affect spat recruitment, with an intermediate depth of 60-90cm collecting the most *P. Mazatlanica* (Saucedo *et al.*, 2005). High settlement of larvae occurred in the presence of GABA at concentrations of 10^{-3} and 10^{-4} M. However, mortality rate was also high (32%) at a concentration of 10^{-3} M (Doroudi and Southgate, 2002), suggesting unsuitability of use. Other less invasive chemicals include excess potassium as well as 50mM calcium chloride (Zhao *et al*., 2003b).

In hatcheries, larvae tend to settle on side of settlement tanks and removal is by scraping (Rose and Baker, 1994) which is time consuming and may damage shells. Exposing the oysters to hypersaline conditions of 45 ppt for one hour or lowering the pH of water to 4 can cause voluntarily detachment of spat from substrate without any mortality (Taylor *et al.*, 1997a), saving time in the process. This also has implications for transport of spat, as spat will detach if transported under conditions that cause them to dehydrate.

Feeding

Feeding of spat cannot be controlled for farming in the sea. However, survival of larvae and juvenile oysters in hatchery tanks requires management of feed. Optimum diet for *P. margaritifera* larvae was found to be 1:1 mixture of *Pa*vlova *salina* and *Chaetoceros simplex* and dried *Tetraselmis suecica* and commercial yeast L-10, Microfeast (Southgate *et al.*, 1998). Recent results showed that addition of another diatom *C. muelleri* resulted in better growth for D and umbone stage larvae (Martinez-Fernandez and Southgate, 2007). Apparently, microalgae diets vary with species, *as Isochrysis aff. galbana* (T-ISO) and *Pavlova lutheri* provide for optimum growth of *P. sterna* larvae (Matinez-Fenandez *et al*., 2004).

Stocking density

Stocking density has been found to affect larvae, juvenile and spat growth of oysters. Taylor *et al.*, (1997b) found the maximum rate of survival and growth in terms of wet weight, shell length and height of *P. maxima* juveniles at 1.3 juvenile per $100cm^3$. Higher stocking density tends to affect water flow, which directly affects food availability. Under prolonged low food availability, oysters partition energy into increasing shell weight and thickness over body tissue growth (Wilson, 1987). This effect is also determined by stage of culture. Juveniles of *Pteria sterna* are better able to withstand higher stocking density. An

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optimum protocol has been worked out to start nursery culture at more than 100 individuals/ tray and perform density reduction until there are 100 individuals/ tray after 3 months. Then reduce individuals to 50/tray from the 4th month onwards (Monteforte *et al.,* 2005). Moreover, self-thinning will also occur in oyster populations at high density (Frechette *et al.,* 1996). For juveniles on suspended box culture, a 30% stocking density is the optimum method for culturing *P. margaritifera* (Southgate and Beer, 2000).

Pearl culture techniques

During seeding operation, high numbers of oysters will reject the nucleus and the rest will produce poor quality pearls. Time of implantation is of importance. As the oyster grows, there is a progressive reduction in growth rate. This implies that the sooner the nucleus is implanted, the greater is the rate of nacreous deposition, and the shorter is the time to obtain a marketable pearl (Pouvreau *et al*., 2000). Implantation of pearl when temperatures are low also resulted in high quality mabe pearls of the oyster *P. sterna* (Ruiz-Rubio *et al.,* 2006).

Use of a relaxant will allow relaxation of abductor muscle and opening of shell valve so that the muscles will not be damaged from overstretching during operation. It also decreases bead rejection. Propylene phenoxetol is effective at concentrations of 2.0 to 3.0 ml/l result in rapid induction and 100% recovery and survival after 7 days (Norton *et al*., 1996). Use of 1:50 aqueous dilution of 10% povidone iodine antiseptic also reduces mortality and promotes recovery. Pearl shape was improved when the surgical incision was closed with a flexible cyanoacrylate adhesive (Norton *et al.,* 2000).

Anaesthetic can also enable donor oysters to recover their mantle tissue after excision. Therefore, donor oysters need not be sacrificed and genetic selection can take place. *P. fukata* can grow the excised tissue within 105 hours (Acosta-Salmón and Southgate, 2006) and *P. margaritifera* when exposed to 1200 mg/l of benzocaine resulted in 30 minute relaxation time and rapid recovery with no mortality after operation (Acosta-Salmón *et al.,* 2005).

PROBLEM AND THEIR SOLUTION IN PEARL OYSTER CULTURE

Predation

Predation of oyster spat and juveniles can result in massive loss in productivity. Predators include excavating sponges of the family *Clionaidae (Porifera: Demospongiae)* (Fromont *et al.,* 2005), gastropods *Cymatium* (Family: Ranellidae), and Crustacea. There are some ways to deal with the problem. Firstly, spat can be removed from collectors at a shorter period 3-4 months instead of 6 months, when mean size is 15mm DVM, and reared in panel nets (Friedman and Bell, 2000). Alternatively, the spat can be covered with mesh, and a mesh size of 3 mm improved spat survival of *P. maxima* while not fouling so easily (Taylor *et al.*, 1998). However, in environments like Solomon Islands, mesh bags should not be used, as they trap larvae of predators such as *Cymatium* spp, gastropods and portunid crabs and fouled easily, impeding water flow to the collector (Friedman and Bell, 1996). For juvenile culture, ear hanging and 24 pocket juvenile panel nets were the preferred method, showing highest growth and lowest fouling and mortality from predators (Southgate and Beer, 2000).

Biofouling

Removal of biofouling organisms is done by manual labour (**Fig. 3**) and contributes to most of the expenses of a pearl oyster farm. *P. maxima* left uncleaned for 4 weeks or more had stunted growth and shell deformities were observed after 8 weeks, caused by biofouling organisms like barnacles and polychaete worms (Taylor *et al*., 1997). However, cleaning also affect survival rates, and frequent cleaning every 4 weeks resulted in high mortality for *P. margaritifera*. Handling oysters will stress them and therefore optimum cleaning rate for this species is once every 8 weeks (Pit and Southgate, 2003). Age of oyster may also affect biofouling rate as the periostracum, a thin, flexible, fibrous and sclerotinized protein layer covering the calcified shell functioning as a physical defence against fouling organism, wears off over time (Scardino *et al.*, 2003). A novel way of dealing with biofouling involves painting them with anti biofouling chemicals

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but as evident in the research done by de Nys and Ison (2000), the coating only last for 3 months in the northern territory and therefore it is more economical to perform manual cleaning of oysters.

Fig. 3. Manual removal of fouling from pearl oyster culture nets using knives (Southgate and Lucas, 2008).

Diseases

Oyster diseases have the potential to affect productivity. Pearl oyster *P.maxima* suffered high mortality in Australia in 1996 due to the bloom of algae *Trichodesmium erythraeum*. The oyster exhibit dilated digestive gland lumens, sloughing of epithelial cells and large numbers of residual bodies and with increased numbers of brown cells and granulocytes under the epithelial layer, indicative of an inflammatory response. This is due to the algae producing a paralytic shellfish toxin, saxitoxin (Negri *et al.*, 2004) and this cannot be prevented. However, another commonly occurring oyster disease, the reddening of abductor muscle caused by infection in *P. fucata* can be prevented by lowering the temperature of water to less than 19 ^{0}C (Nagai *et al.*, 2007).

Genetics

The future of pearl oyster aquaculture lies in genetic selection, which can create faster growing oysters, resistance to diseases and production of higher quality pearls. This first step in doing so is to ensure quality gametes from selected broodstock are kept at their optimum conditions. Cryptopreservation of sperm can ensure the preservation of desirable genes and good motility of sperms. For *P. fucata*, 10% METmethanol plus 90% diluents comprising 80% seawater and 20% FBSfetal bovine serum, cooled to 50 ^{0}C at a cooling rate between 15 and 20 ^{0}C/min is a good method of cryptopreservation (Kawamoto *et al*., 2007). *P. margaritifera* has a different method of cryptopreservation- 0.45 M trehalose with 0, 0.64, 1.02 or 1.53 M Me2SO and frozen using slow to medium-rapid cooling rates (2.1–5.2 ^{0}C/min) (Acosta-Salmon *et al.*, 2007). When needed, sperms can be activated using 2.0 mM NH3 solution (Ohta *et al.*, 2007).

Selection in *P. fucata* has already shown effect. By selectively crossing white male and female oysters, frequency of inferior yellow coloured pearls was reduced (Wada and Komaru, 1996). One of the worries of selection through inbreeding is loss in genetic diversity, which may lead to inferior growth and survival (Wada and Komaru, 1994). Fortunately, in

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French Polynesia, no evidence has been found to indicate a change in the number of alleles and heterozygosity for current farming methods (Arnaud-Haond *et al.*, 2003). Caution needs to be exercised to prevent genetic loss from happening in future.

CONCLUDING REMARKS

Pearl oyster cultures have several constraints that can affect their productivity such as predation, biofouling and disease. On the other hand, pearl farming has gone a long way in establishing control of production through various management methods. The future scope for expansion in pearl farming is in genetics, where more valuable quality pearls can be produced in a shorter time in higher quantity and pearl oysters can improve resistance to diseases. However, genetics is a double edged sword and future management decisions must ensure the preservation of genetic diversity as a buffer against unforeseen circumstances. Therefore, for the future research which improves pearl oyster production especially through genetic approach is important.

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