RESEARCH NOTES AND REPORTS

Commercial spat production of *Pinctada maxima* at Roko Island hatchery, North-East Australia, with *in vitro* fertilisation technique

by Masahiro Ito [1]

Introduction

Most silverlip pearl oyster farms in the Torres Strait are on the verge of collapse and closure because of a shortage of supply of mother-of-pearl (MOP). This shortage is attributed to a poor resource-management strategy, and ignorance by both the public and private sectors. Aquatech Oceania, contributing to a sustainable development of fisheries resources, provided a consultancy service to Roko Pearls in the Torres Strait, NE Australia. We designed a low-cost pearl oyster hatchery system for the silverlip pearl oyster (*Pinctada maxima*) and conducted hatchery staff training during March to June 1997. Our aims were:

1) to develop skilled hatchery technicians through short-term, on-the-job training,

2) to produce a commercially viable number of high quality spat (at least 100 000 two-mm spat per larval run) during the off-peak spawning season (April – September) in this region, and

3) to attain year-round mass production from an inexpensive hatchery system with a minimum number of technicians. During the off-peak spawning season, we successfully produced 200 000 spat from a single larval run with artificially matured ova and activated sperm. Being able to supply spat year-round to the pearl farms is a major breakthrough for the *P. maxima* pearling industry, particularly in the Torres Strait.

Materials and methods

All the broodstock were held in pearl nets, suspended from a surface longline system. They were transferred to a raceway tank before hatchery operation. Donor oysters were washed thoroughly. The gonads were cut off from male and female oysters and kept separately in glass beakers with UV-sterilised and filtered seawater until the gametes were stripped for artificial maturation and activation procedure. Gametes of both sexes were usually immature, so-called 'spent' during this time of year. The solution containing immobile sperms was either stored in the refrigerator for a few days or used for *in vitro* fertilisation straight after stripping the gonads. 0.5×10^{-1} N ammonia solution was used for artificial maturation of ova and *in vitro* fertilisation. Similar techniques have been well documented by various researchers (Wada, 1932; Tanaka & Kumeta, 1981; Hayashi & Seko, 1986; Rose, 1990).

The hatchery was semi-indoors, being a modified workshop. It accommodated 6 larval or spat tanks (1000 litres each). The micro-algae culture room was a similar set-up to that of Tarawa in the Republic of Kiribati, described by the author in an earlier issue of this bulletin (*Pearl Oyster Information Bulletin #9*, pp. 8–11). The larval and spat rearing methodology was basically the same as Ito (1996). Only two 1000 litre tanks were used for rearing 1.5 million larvae. After settlement, the spat were cultured in three tanks each with 60 000–100 000 spat and then later reduced to 40 000–70 000 spat per tank.

[1] Aquatech Oceania, 23 Depper Street, St Lucia, QLD 4067, Australia

Results and discussion

All the D-stage larvae were obtained from *in vitro* fertilisation using artificially matured ova and activated sperms. A total of 1.5 million fertilised eggs were obtained out of 4.3 million ova from 2 females. Although we have found that the sperm can be activated artificially by adding some chemical solution—e.g. sodium metasilicate, green-algae culture medium (Ito, unpublished data), trace metal mix (Ito, 1995)—we used a standard technique with $0.5x10^{-4}$ N ammonia solution for this on-the-job training. Fertilisation rates were 30–50% for 2 batches of stripped ova. The hatching rate of the D-veligers from fertilised eggs was 100% without any deformities.

From this very first hatchery operation, we successfully produced over 200 000 spat (1.5 mm to 3 mm in shell length on day 35, all visible to the naked eye) out of only 1.5 million D-stage veligers. The first batch of 20 000 spat (ranging from 1.5 to 4 mm in shell length), the second batch of 80 000 spat (average 3 mm) and the third batch of 70 000 spat (average 5 mm) were transferred from land-based tank culture to nursery farms for grow-out culture at the end of May, early June and mid June, respectively.

The present success rate from D-stage to 2 mm spat was 13%, which is considered to be very high from a single larval run (Note that the success rate to the 2 mm size has been 1–5% for commercial *P. maxima* spat production (Ito, unpublished data)). Our success rate is similar to that of Akoya oyster (*P. fucata*) with 15–35% success rate in Japanese pearl oyster hatcheries (Hayashi & Seko, 1989) and we believe that the success rate will be improved during the spawning season when using ripe gonads.

Artificial fertilisation techniques have always been considered a last resort, particularly for the silverlip pearl oyster (*P. maxima*) hatchery operations. It is always difficult to access spawning-ripe gonads outside of the spawning season (i.e. around 6 months from April to September in this region), and thus normal spawning induction techniques, using stressing of the broodstock by temperature shock, dry treatment, or such like, are not practical nor economical. To perform constant spat production throughout the year, the techniques for artificial maturation of ova, activation of sperm and fertilisation are the most reliable and economical, saving the hatchery facility labour cost and time.

We also demonstrated that long term (for 1 to 2 months after settlement) high-density spat culture (50 000 to 100 000 spat/1000 litre tank) was possible using a simple, low-cost, partial flow-through culture system with a minimum algae culture facility with about 500 litre indoor and 1000 litre outdoor capacity. Roko Pearls has now two skilled hatchery technicians trained by us and expects to produce 200 000 spat (5 to 10 mm size) per run throughout the year.

Table 1: **Summary of hatchery run**

Days	Eggs/larvae/spat in 1000-litres tanks	Size (shell length)	Success rate *
0	4.5×10^6 ova		30 to 50% fertilisation rate (total of 1.5×10^6 fertilised eggs
1	1.5×10^6	85 µm	100% hatching rate (total of 1.5×10^6 D-stage)
7	8.2×10^5	105 to 115 µm	
9	4.6×10^5	130 µm ≈	Umbo stage
14	3.1×10^5		
16		240 µm ≈	30% eyed veliger (total of 1×10^5)
17	3.1×10^5		50% eyed veliger (total of 1.5×10^5)
18	Spat collectors in settling tanks		80% eyed veliger (total of 2.5×10^5)
24	3.0×10^5	300 µm ≈	Crawling pediveliger & spat
27	2.6×10^5		
30		0.5 mm ≈	All spat, no swimming larvae
33	2.0×10^5	0.5 to 2 mm	13% success rate from D-stage
37	2.0×10^5	1.5 to 4 mm	
38	2.0×10^5	1.5 to 4 mm (ave. 2 mm)	10% (20 000 spat) transferred to other farm
39	1.7×10^5	2 to 4 mm (ave. 3 mm)	40% (80 000 spat) transferred to nursery farm grow-out
54	7×10^4	3 to 7 mm (ave. 5 mm)	30% (40 000 spat) transferred to other farms
61	3×10^4	5 to 15 mm (ave. 10 mm)	20% (30 000 spat) transferred to other farm

* Success rate here does not represent survival rate from day 1, where some live larvae were discarded from the culture system during tank cleaning and size selection process

Aquatech Oceania owns expertise in hatchery and grow-out system design, staff training and commercial production for pearl oysters (*P. maxima, P. margaritifera, P. fucata* and *Pteria penguin*) and decapod crustaceans (prawns, crabs and spiny lobsters).

We emphasise our commitment to sustainable resource development and environmental awareness in business practices. We do not use any antifungal chemicals nor antibiotics for controlling water quality, but we adopt careful hatchery system management through continual technical improvement.

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Major success for TMRC blacklip pearl oyster hatchery, Penrhyn Atoll, Northern Cook Islands

by Dr Rick Braley

During mid-1997 the Tongareva Marine Research Centre hatchery had achieved its goal of successfully mass producing blacklip pearl oyster (*Pinctada margaritifera*) spat. An Asian Development Bank project, the Outer Islands Marine Resources Management Training Project, which started in July 1996, substantially modified the existing hatchery and algal lab (original infrastructure resulting from the USAID project, 1991-1995) and continued with in-house training of staff. New seawater systems were constructed, one from the ocean-side of the atoll for the hatchery and one from the lagoon for the land nursery culture of spat. New reservoir and culture tanks were obtained, the flow-through system of larval culture was used and a specific protocol developed for TMRC on hygiene, feeding, and algal production.

The first substantial spat settlement was about 30 000. This was followed by what is perhaps a world record batch of 250 000 spat [estimate in August 1997 by the senior hatchery technician, Mataora Bill Marsters] for this species. After about 60 days the spat were transferred from the settlement tanks to land nursery raceways where they receive subsand-intake filtered lagoon seawater and are fed mass microalgae produced under the capable direction of senior algal technician Lolongi Taime.

A third batch of spat was earlier estimated to be >100 000 but in early November 1997 volumetric count estimates of spat on sides and bottom of tanks were about 200 000, while those on collectors (black boxes and PVC slats as described in *Pearl Oyster Information Bulletin # 10*, pp. 12–14) were estimated to be about 100 000.

Spat are being placed in the lagoon nursery in trays. An indication of growth rates and survival of small spat [caught on 3.6 mm mesh versus those on 6 mm mesh] placed out in trays after 1 month was: 3.6 mm grade: mean 120% shell DVM increase and mean 55% survival; 6 mm grade: mean 60% shell DVM increase and mean 60% survival. Improved trays and regular checks and cleaning should improve survival rates.