Broodstock Management and Egg Quality of the Pearl Oysters *Pinctada margaritifera* and *Pinctada fucata*

Thesis submitted by
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Abstract

Marine pearl culture is one of the most valuable aquaculture industries in the world with a total estimated value of about US$500 million. The major pearl producing nations are Australia and French Polynesia and, although reliable methods for hatchery culture of pearl oysters were developed in the 1980's and 1990's, pearl production in these countries still relies primarily on oysters collected from the wild. Generally, the cultured pearl industry, particularly the 'black' pearl industry, has been slow to adopt important advances in aquaculture and other relevant disciplines and is still based on 'traditional' methods. Nevertheless, over recent years there has been increasing interest in research relating to general culture methods for pearl oysters and pearl oyster genetics (particularly relating to pearl quality), and there has been increasing reliance on hatchery production. This study addressed important issues relating to broodstock selection, breeding cycle and egg quality in pearl oysters.

Pinctada margaritifera and *P. fucata* were exposed to propylene phenoxetol at a concentration of 2.5 mL L^{-1} and benzocaine at concentrations of 250, 500 and 1200 mg L^{-1}. Once relaxed, oysters were observed every 5 minutes to evaluate the condition of the mantle and gills. Oysters were classified as either 'suitable saibo' or 'non-suitable saibo' depending on their suitability for use as saibo donors for pearl production. Survival of oysters in all treatments was 100%. With the exception of oysters relaxed with 250 mg L^{-1} of benzocaine, where no relaxation was recorded, oysters exposed to all other treatments became relaxed and showed good condition and acceptable characteristics to be used as saibo donors.

To determine whether mantle tissue could be removed from oysters without mortality, *P. margaritifera* and *P. fucata* were anaesthetised with 500 mg L^{-1} of

benzocaine and had the ventral margin of either their left, right or both left and right mantle lobes removed. Survival after 4 weeks was 100% for all treatments and oysters showed regeneration of excised mantle tissue. Mantle border grew back to almost its original extent within 60 days after excision. Muscular fibres within the new tissue were not seen until 30 days after mantle excision. Functional (secretory) abilities were presumably recovered before day 15 when conchiolin secretions and secretory cells were seen in the newly regenerated epithelia. Mantle regeneration in *P. margaritifera* up to 90 days after mantle excision was similar to that for *P. fucata*. Anaesthetised oysters can provide mantle tissue for pearl seeding and be kept alive for future uses which may include receptors for pearl production (following seeding season) as broodstock (only those providing mantle that produced high quality pearls) and possibly as multiple saibo donors.

A biopsy technique to obtain gonad tissue was assessed in *P. margaritifera*. Prior to biopsy, oysters were anaesthetised with 2 mL L^{-1} of propylene phenoxetol. Three different 9 cm long biopsy needles (16, 18 and 20 gauge) with a 10 mm sample notch, were compared as a means of obtaining gonad tissue from 20 oysters. Samples were removed from each oyster using each of the 3 biopsy needles. Following the biopsy procedure, each oyster was killed and the gonad sectioned for standard histological preparation. Samples were observed microscopically to assess gonad condition and to compare samples taken using biopsy with those taken using destructive sampling. Oysters showed 100% recovery from the anaesthetic and biopsy procedure after 2 weeks. Non-destructive biopsy sampling was an accurate means of assessing gonad condition in male pearl oysters. However, the use of thicker biopsy needles (e.g. 14 or 12 gauge) may allow better interpretation of gonad stage,

particularly in female oysters for which results showed that 16 gauge biopsy needles (the thickest used in this study) were unsatisfactory.

Collection of oysters from culture stock held at Magnetic Island and Orpheus Island was conducted from August 2003 to February 2004 to describe biochemical and histological changes associated to gamete development. Six oysters from each site were collected every month for seven months. Samples of mantle, adductor muscle, gonad and digestive gland were obtained for biochemical analyses. Two spawning peaks (winter and summer) were confirmed for *P. margaritifera* in north Queensland. The adductor muscle played an important role in storage of protein and carbohydrate during gonad development.

To analyse changes in micronutrient composition during embryological development of *P. margaritifera*, samples of eggs, embryos and larvae were taken for determination of carotenoid, ascorbic acid (vitamin C), α-tocopherol (vitamin E) and fatty acid content 0, 4, 8, 12, 16, 24 and 46 h after fertilisation. Carotenoids were found only in trace amounts in *P. margaritifera* eggs and probably do not have an important role in embryo development. The vitamin C and vitamin E contents of *P. margaritifera* embryos increased with time and may not be limiting nutrients for embryological development. The fatty acids 14:0, 16:0 and most unsaturated C18s were highly utilised during embryological development of *P. margaritifera* as demonstrated by their decline during development. These fatty acids may be good indicators of egg quality.

Much of the research in this study was conducted for the first time with pearl oysters. This study describes new and novel information relating to the breeding cycle, broodstock selection and egg quality of pearl oysters. The results of this study provide a basis for more efficient culture methods and may facilitate significant changes to traditional aspects of pearl oyster culture and pearl production.

Statements of contribution of others

The following people in addition to my supervisor provided support, which aided in the preparation of my thesis:

The project team led by Dr. Malcolm Brown (CSIRO, Marine Research Laboratories, Hobart, Tasmania) undertook the analyses of micronutrients reported in Chapter 8 of this thesis. Sections of the scientific report prepared by Dr. Brown were used in the writing of Chapter 8 of this thesis.

Erika Martinez-Fernandez provided technical cooperation in a number of field trips and during the biochemical analyses reported in Chapter 7 of this thesis.

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Statement of sources

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

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