Improving the quality of pearls from *Pinctada maxima*

Thesis submitted by

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DECLARATION

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 Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

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Statement of Contribution of Others

Nature of Assistance	Contribution	Names, Titles, and Affiliations of Co-Contributors
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Abstract

The method used for cultured round pearl production was developed in the 1920's and has changed little since. It utilises mantle tissue grafts ('saibo') from donor oysters which are killed. The quality of resulting pearls is highly influenced by the nacre quality of the donor and, because of this, a pearl farm's best oysters are sacrificed for pearl production. This is potentially a major constraint to the pearling industry which, unlike most livestock industries, cannot use its highest quality animals in breeding programmes to improve the stock quality. Recent research has shown that saibo tissue can be removed from donor pearl oysters using anaesthetics, without killing the oyster, and that excised mantle tissue is regenerated within three months. Potential benefits to the cultured pearl industry from these results include the use of donor oysters producing high quality pearls as broodstock to improve farmed oyster stock, and potential multiple saibo donation by high quality donors. These benefits, however, assume that the mantle tissue of anaesthetised pearl oysters and regenerated mantle tissue perform in a similar manner to 'normal' mantle when used as saibo for cultured pearl production. Assessing this new approach and testing this assumption was the basis of this study which was conducted with the silver- or gold-lip pearl oyster *Pinctada maxima*.

The experiment reported in Chapter 2 assessed seven anaesthetics for their efficacy with *P. maxima*: 3 mL L^{-1} 2-phenoxyethanol, 500 mg L^{-1} and 1200 mg L^{-1} of benzocaine, 1.5 mL L^{-1} clove oil, 0.25 mL L^{-1} and 0.4 mL L^{-1} menthol liquid, and 2.5 mL L^{-1} propylene phenoxetol. Of the 27 oysters exposed to each treatment, the highest proportion of relaxed oysters (96.3, 88.9 and 88.9%) and the shortest exposure times required for anaesthesia (13.8 ± 6.4, 10.5 ± 7.9 and 15 ± 7.1 min), were recorded for the treatments of 3 mL L^{-1} 2-phenoxyethanol, 1200 mg L^{-1} of benzocaine, and 2.5 mL L^{-1} propylene phenoxetol,

respectively. In contrast, none of the oysters exposed into 0.25 mL L^{-1} menthol liquid became relaxed and most oysters exposed into 1.5 mL L^{-1} clove oil died during the experiment. Oysters exposed to 3 mL L^{-1} 2-phenoxyethanol and 1200 mg L^{-1} benzocaine remained relaxed for up to 30 min while the number of relaxed oysters exposed to 2.5 mL L^{-1} propylene phenoxetol decreased during that time. With the exception of oysters exposed to clove oil, all relaxed oysters recovered within 2 h of being placed back into seawater and there was close to 100% survival after one month.

The capacity for regeneration of excised mantle tissue by *P. maxima* was investigated in Chapter 3. Oysters were anaesthetised with 2.5 mL L^{-1} propylene phenoxetol prior to a piece of tissue (approximately 10 mm x 30 mm) being excised from the ventral region of the mantle. In the first experiment, 56 oysters with mean (± SD) dorso-ventral measurement (DVM) of 125.5 ± 8.9 mm had tissue excised from either the right mantle lobe, left mantle lobe or both mantle lobes. Following a further three-month period in suspended culture, oyster survival was recorded and two oysters were selected arbitrarily from each group to be sacrificed for histological examination of healed mantle. In the second experiment 36 oysters with mean (± SD) DVM of 151.6 ±13.4 mm were used for excision of the distal part of the ventral region of the left mantle lobe. Two oysters were sampled at 1, 3, 6, 12, 24, 36, 48, 72 and 120 h (5 days) after mantle excision, and then at 12, 24, 45, 72 and 90 d after mantle excision for histological and histochemical analysis of mantle regeneration. There was almost 100 percent survival in both experiments. Healing and regeneration of mantle tissue in oysters subject to excision from the left, right or both mantle lobes was evident, with regenerated mantle appearing similar to normal mantle. All external and internal components of normal mantle were present in regenerated mantle tissue. Epithelization signifying wound healing occurred within 36 to 72 hours and was characterised by a reduced wound area,

haemocyte infiltration and accumulation, and cell dedifferentiation. Within 48 hours of mantle excision, the latero-ventral edges of the wound flexed dorsally and attached to the dorsal edge of the wound reducing the wound area. Between five and twelve days after excision, the distal part of the mantle had divided into three small lobes which developed into the outer, middle and inner mantle folds two weeks later. Ninety days after excision the mantle had completely regenerated with histological observations indicating no difference in epithelial structure or in other internal mantle accessories when compared to normal mantle. Shell material began to be secreted onto the shell by regenerating mantle twelve days after excision. Initially this occurred in a position dorsal to the non-injured mantle edge. However, forty-five days after mantle excision, regenerated mantle had extended ventrally to a position similar to that of non-injured mantle. Nacre deposition by regenerated mantle had now reached the same position ventrally as that of non-injured mantle indicating full acquisition of nacre secreting abilities by regenerated mantle. Complete regeneration of the mantle had occurred 90 days after excision when no differences in epithelial structure or other internal mantle accessories were evident when regenerated and normal mantle were compared.

The results of Chapters 2 and 3 showed that appropriate anaesthetics can be used to relax *P. maxima* to allow mantle excision, and that excised mantle tissue could regenerate with secretory functions within 3 months of excision. On this basis, it would be possible to obtain saibo from living anaesthetised donor oysters and from regenerated mantle tissue. However, no prior study had investigated whether regenerated mantle had the ability to secrete the same quality nacre as normal mantle or whether anaesthetised and regenerated mantle were able to proliferate to form a functional pearl-sac when implanted into a recipient oyster. In Chapter 4, regenerated mantle from *P. maxima* was shown to produce shell material with the same structure as normal mantle. The nacre produced by both types of mantle appeared identical in

both the size and structure of nacre platelets. Regenerated mantle tissue appeared to secreted nacre at a more rapid rate than normal mantle tissue which was indicated by the greater thickness of nacre adjacent to the mantle wound site. These results confirmed that regenerated mantle has the ability to secrete nacre and the potential to secrete nacre of similar quality to normal mantle tissue. Chapter 5 investigated the ability of relaxed saibo, and saibo from regenerated mantle, to form a pearl-sac following implantation into a recipient oyster. Survival of recipient oysters implanted with relaxed, regenerated or normal saibo ranged from 90% to 100% and did not differ significantly between treatments (p-value= 0.2333). Nucleus retention was much poorer than expected with a total of only 15 oysters retaining nuclei (of 191 nucleated oysters) and showing pearl-sac development. Eight nuclei (53% of the total) were retained by oysters in the control treatment (normal saibo x normal recipient), 4 (26.7%) were retained by the anaesthetised saibo x anaesthetised recipients oysters, 2 (13.3%) by the regenerated saibo x normal recipients treatment and 1 (6.7%) by the anaesthetised saibo x normal recipients treatment. Pearl-sacs from seven of these were used for histological analysis: four from oysters in the control treatment (normal saibo x normal recipient), one from the anaesthetised saibo x anaesthetised recipient treatment, and two in the regenerated saibo x normal recipient treatment. The six-week duration of this study allowed complete pearl-sac development in oysters implanted with relaxed, regenerated or normal saibo. However, the thickness of the pearl-sac epithelium varied, indicating differences in the degree of pearl-sac maturity. Pearl-sacs in all treatments had cell accessories: epithelium and mucous cells. In the control treatment which used normal saibo, greater nacre deposition was evident compared to that produced by both relaxed and regenerated saibo. Despite variation in the thickness of the epithelium produced by each type of saibo, each pearl-sac produced approximately the same thickness of matrix or mineral deposition. This experiment confirmed the results of Chapter 4 in showing that regenerated

mantle tissue from *P. maxima* apparently regains full secretory function and showed that saibo from relaxed oysters and from regenerated mantle tissue is able to form a pearl-sac capable of mineral secretion onto an implanted nucleus.

The potential use of saibo from relaxed oysters and from regenerated mantle tissue for pearl production was investigated in Chapter 6, in an experiment conducted using 1,520 oysters at a commercial pearl farm in north Bali, Indonesia. Two pearl implanting operations were conducted three months apart. In the first, donor oysters were anaesthetised to provide saibo and then allowed to regenerate excised mantle tissue before the second operation which used regenerated mantle tissue as saibo. Pearls were harvested 24 months after the operations and graded into categories using commercial grading schemes for the following pearl quality criteria: size and nacre thickness, shape, colour, surface complexion and lustre. Pearl oyster survival varied from 90% (normal saibo) to 92% (regenerated saibo) and 95% (relaxed saibo). These values differed significantly (χ^2= 8.990048, p=0.01116441). Overall nucleus retention varied from 27% for oysters implanted with relaxed and normal saibo to 37% for those implanted with regenerated saibo. There was a very significant effect of types of saibo on nucleus retention (χ^2= 34.01114, p=0). The total number of pearls produced by oysters implanted with relaxed, regenerated and normal saibo was 240, 165 and 19, respectively, and the proportion of these that were considered to be of acceptable commercial quality was 99%, 62% and 53%, respectively. There was a highly significant difference between these values (χ^2= 112.3091, p=0). The majority of pearls were graded into the ‘round’ shape category (34.8% of a total of 425 pearls) and the majority of these were produced by oysters implanted with relaxed saibo (47% of category total). Pearls in the ‘drop’ category made up 20.2% of the total number of pearls produced and again, the majority were produced by oysters implanted with relaxed saibo (24% of category total). There was a highly significant effect of

saibo type (relaxed and regenerated) on pearl shape (χ^2= 15.32797, p=0.018). Pearls produced by relaxed saibo ranged from 3-14 mm in size with the highest proportion in the 10-11 mm category which collectively made up 46% of the total. Pearls produced by relaxed saibo attained a larger size than those resulting from both regenerated and normal saibo; those in the 12.5 mm to 14 mm size ranges made up 3% of the total number of pearls produced by relaxed saibo. Pearls produced by regenerated saibo ranged from 4 mm to 12 mm with the majority (64.2%) in the 8-9 mm size class. The largest pearls produced by regenerated saibo were in the 12 mm size class but only 3.6% of the total number of pearls fell into the 10.5 mm to 12 mm size categories. Pearl produced by normal saibo ranged from 8 mm to 11 mm and did not attain the larger sizes of pearls produced by relaxed and regenerated saibo. The majority (57.9%) of pearls produced by normal saibo were in the 8.5 mm to 9 mm size category. There was a very significant effect of type of saibo on pearl size (χ^2= 44.57578, p=0). Mean (± SE) sizes of pearls produced by relaxed, regenerated and normal saibo were 10.3 ± 0.14 mm, 8.7 ± 0.11 mm and 9.2 ± 0.33 mm, respectively. The average nacre thickness on pearls was 3.4 ± 0.12 mm for relaxed saibo, 1.8 ± 0.11 mm for regenerated saibo and 2.4 ± 0.30 mm for normal saibo. Relaxed saibo produced significant greater nacre thickness than both regenerated (p = 0.000) and normal saibo (p = 0.013), while nacre thickness of pearls produced by regenerated saibo did not differ significantly from that of normal saibo (p = 0.120). There was a weak correlation between pearl size and nucleus size (r = 0.31, n = 146) but a strong correlation between pearl size and nacre thickness (r = 0. 95, n =146). White/silver colours were dominant in pearls produced by oysters implanted with white/silver donor saibo making up 96% of total pearls produced by relaxed saibo, and 83.1% and 84.2% of the total for regenerated and normal saibo, respectively. There was much greater variability in pearl colour produced by yellow/gold oysters implanted with relaxed, regenerated and normal saibo from yellow/gold donors, when compared to pearls produced

by saibo from white/silver donors. White/silver colours shared about the same percentage in both relaxed and regenerated saibo (53.1 and 52.6%, respectively) but the proportion of pearls in the yellow/gold colour range was greater from relaxed saibo (22.5%) than from regenerated saibo (11%). There was considerable variation in the proportion of pearls in each of the major categories of surface complexion. Only 12.9% of the pearls produced in this study were graded within the A1 category characterised by no blemishes or a small blemish that can be removed by drilling. Within the A1 category, pearls produced by relaxed, regenerated and normal saibo made up 11.7%, 15.1% and 10.5% of the total, respectively. Pearls in category B1 made up 13.9% of the total pearls produced, composed of 17.1%, 8.4% and 21.1% of the pearls produced by relaxed, regenerated and normal saibo, respectively. Most of pearls produced from both relaxed (45.4%) and normal (47.4%) saibo were graded within the B2 category. There was a highly significant effect of saibo type (relaxed and regenerated saibo only) on surface complexion of pearls produced (χ^2= 26.99977, p=0). Only 10.6% of the pearls produced in this study were graded within the highest category for lustre. Pearls produced by relaxed saibo made up the majority (14.2%) of these. The majority of the pearls produced from all treatments were placed into lustre category 2 and were characterised as being bright pearls with a slightly blurred reflection. Pearls from regenerated saibo made up the majority of pearls in this category which contained 69.9% of pearls from regenerated saibo, 57.9% of pearls from normal saibo and 54.6% of those from relaxed saibo. The effect of saibo type (relaxed and regenerated only) on pearl lustre within categories 1-3 was very significant (χ^2= 10.07011, p=0.006). Based on the major criteria used to assess the performance of both oysters and pearls after implanting with relaxed, regenerated and normal mantle, the results indicate that relaxed saibo from anaesthetised pearl oysters performed better than both regenerated and normal saibo.

The final research chapter of this thesis used UV-visible (UV-Vis) spectrophotometry to analyse some of the pearls produced in Chapter 6. Of particular interest was a comparison between pearls from the same saibo donors and pearls with various colours (from white/silver to gold) and overtones. Three pearls with different colours resulting from the same gold donor showed different absorption spectra. Cream and gold coloured pearls showed a wide absorption from 320 to about 460 nm while there was just slight reflectance around 400 nm by the white pearl with a pink overtone. Cream and gold pearls reached a reflectance peak at 560 to 590 nm while the white pearl with pink overtone showed slightly wider absorption in this region. Both cream and gold pearls showed an absorption peak after the reflectance peak; at about 700 nm for the cream pearl and 750 nm for the gold pearl. Two other pearls produced by the same gold saibo donor (white with cream overtone and cream with various overtones) showed similar spectra which differed in their intensity. One of these pearls had very high lustre and its spectrum showed a much higher % reflectance than the second pearl with inferior lustre. This result may indicate that reflectance is a useful quantitative indicator of pearl lustre. The spectra of two white pearls resulting from different silver nacre donors showed a reflectance at 260 nm, followed by absorption at 280 nm and another reflectance peak at 340 nm. After this peak the spectra for these pearls remained flat until a slight absorption peak around 700 nm. Throughout the visible region, all white pearls used in this study showed similar reflectance spectra although there were differences in reflectance intensity. Unlike the spectral results from white pearls, the results from yellow and gold pearls varied according to colour saturation of the pearl. The results of this study show that similarities between absorption and reflectance spectra of cultured pearls resulting from the same saibo donor are negligible and could not be detected with UV Vis spectrophotometry. Nevertheless, this technique could have a role to play in developing less subjective methods

of assessing pearl quality and in further studies of the relationships between pearl quality and that of the donor and recipient oysters.

This study has confirmed that *Pinctada maxima*, like *P. margaritifera* and *P. fucata*, is able to be anaesthetised to allow mantle excision without mortality, and that excised mantle can regenerate within a period of 3 months. Relaxed and regenerated mantle were shown to possess secretory function, similar to normal mantle tissue, and the ability to proliferate to form a pearl-sac when used as saibo. Indeed, relaxed mantle from anaesthetised oysters was shown to produce pearls of superior quality to those produced by normal mantle tissue when used as saibo. This result has major implications for the pearling industry and indicates that minor changes to the pearl seeding process (i.e., use of relaxed mantle as saibo) could bring about improvements in pearl yield and pearl quality.

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