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Improving the quality of pearls from
Pinctada maxima

Thesis submitted by

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for the degree of Doctor of Philosophy
in the School of Marine & Tropical Biology

James Cook University

2009

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Statement of Contribution of Others

Nature of Assistance	Contribution	Names, Titles, and Affiliations of Co-Contributors
Intellectual support	<p>Proposal writing and improving writing skills</p> <p>Statistical support</p> <p>UV-vis spectrophotometry (Chapter 7)</p>	<p>Dr. Gina Curro (JCU)</p> <p>Dr. Laura Castell (JCU)</p> <p>Dr. Rosemary Dunn (JCU)</p> <p>Dr. David Kault (JCU)</p> <p>Prof. Rhondda Jones (JCU)</p> <p>Dr. Snezana Agatonovic-Kustrin (JCU-La Trobe Uni)</p>
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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 (\pm SD) dorso-ventral measurement (DVM) of $125.5 \pm 8.9 \text{ mm}$ 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 (\pm SD) DVM of $151.6 \pm 13.4 \text{ mm}$ 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 secrete 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 ($\chi^2 = 8.990048$, $p = 0.01116441$). 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 ($\chi^2 = 34.01114$, $p = 0$). 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 ($\chi^2 = 112.3091$, $p = 0$). 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 ($\chi^2 = 15.32797$, $p=0.018$). 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 ($\chi^2 = 44.57578$, $p=0$). Mean (\pm 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 ($\chi^2 = 26.99977$, $p=0$). 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 ($\chi^2 = 10.07011$, $p=0.006$). 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.

Contents

Statement of access	ii
Statement of sources	iii
Statement of contribution of others	iv
Acknowledgements	v
Abstract	vii
Contents	xvi
List of figures	xxii
List of tables	xxx
Chapter 1 General Introduction	
1.1 Pearl producing molluscs	3
1.1.1 <i>Molluscs producing non-nacreous pearls</i>	3
1.1.2 <i>Molluscs producing nacreous pearls</i>	3
1.2 <i>Pinctada maxima</i> (Jameson)	4
1.2.1 <i>Taxonomic position and distribution</i>	4
1.2.2 <i>Morphology and anatomy</i>	5
1.2.2.1 <u>The shell</u>	8
1.2.2.2 <u>The mantle</u>	8
1.2.2.3 <u>The gonad</u>	9
1.3 Pearl: history and origin	10

1.3.1	<i>Natural pearls</i>	10
1.3.2	<i>Cultured pearls</i>	13
1.4	Production of cultured pearls	14
1.4.1	<i>Cultured blisters or mabè</i>	14
1.4.2	<i>Cultured round pearl</i>	15
1.4.2.1	<u>Pre operation phase</u>	16
1.4.2.2	<u>Operation phase</u>	16
1.4.2.3	<u>Post operation phase and culture condition</u>	17
1.4.3	<i>Non-nucleated cultured pearls</i>	20
1.5	Pearl formation and pearl characteristics	21
1.6	Pearl grading	22
1.6.1	<i>Pearl quality factors</i>	22
1.6.1.1	<u>Lustre</u>	24
1.6.1.2	<u>Shape</u>	25
1.6.1.3	<u>Surface complexity</u>	26
1.6.1.4	<u>Colour</u>	27
1.6.1.5	<u>Size</u>	28
1.6.1.6	<u>Nacre thickness</u>	30
1.6.2	<i>Pearl grading systems</i>	31
1.6.3	<i>Pearl testing</i>	32

1.7 Problems and potential factors for increasing pearl quality	33
1.7.1 Use of Anaesthetics	33
1.7.2 The potential of regenerated mantle	34
1.8 Major objectives of this study	35
Chapter 2 Use of anaesthetics with <i>Pinctada maxima</i>	
2.1 Introduction	37
2.2 Material and methods	39
2.3 Results	41
2.4 Discussion	46
Chapter 3 Regeneration of excised mantle tissue by <i>Pinctada maxima</i>	
3.1 Introduction	51
3.2 Material and methods	52
3.2.1 Experiment 1 – Survival following mantle excision	53
3.2.2 Experiment 2 – Mantle healing and regeneration following excision	53
3.2.3 Histological analysis of mantle tissue	55
3.3 Results	56
3.3.1 Experiment 1 – Survival following mantle excision	56
3.3.2 Experiment 2 – Mantle healing and regeneration following excision	60
3.4 Discussion	71

Chapter 4 Shell secretion by regenerated mantle of *Pinctada maxima*

4.1 Introduction	77
4.2 Material and methods	78
4.3. Results	79
4.4 Discussion	87

Chapter 5 Pearl-sac development in *Pinctada maxima* using regenerated mantle as saibo

5.1. Introduction	90
5.2 Material and methods	91
5.3 Results	93
5.3.1 <i>Survival</i>	93
5.3.2 <i>Nucleus retention</i>	94
5.3.3 <i>Histological analysis</i>	94
5.4 Discussion	97

Chapter 6 Cultured pearl production from *Pinctada maxima* (Jameson) using relaxed and regenerated mantle tissue






6.1 Introduction	101
6.2 Material and methods	103
6.2.1 <i>Experimental design</i>	103
6.2.2 <i>First operation</i>	105

6.2.3	<i>Second operation</i>	106
6.2.4	<i>Data analysis</i>	106
6.2.4.1	<u>Pearl shape</u>	108
6.2.4.2	<u>Size and nacre thickness</u>	108
6.2.4.3	<u>Colour</u>	110
6.2.4.4	<u>Surface complexion</u>	110
6.2.4.5	<u>Lustre</u>	110
6.3	Results	113
6.3.1	<i>Oyster survival</i>	113
6.3.2	<i>Nucleus retention</i>	114
6.3.3	<i>Pearl quality</i>	116
6.3.3.1	<u>Pearl shape</u>	117
6.3.3.2	<u>Pearl size and nacre thickness</u>	120
6.3.3.3	<u>Pearl colour</u>	125
6.3.3.4	<u>Surface complexion</u>	128
6.3.3.5	<u>Lustre</u>	131
6.4	Discussion	133
Chapter 7 Assessing pearl quality using Reflectance UV-Vis Spectroscopy: does the same donor produce similar pearl quality?		
7.1	Introduction	139
7.2	Material and methods	140

7.3 Results	144
7.4 Discussion	147
Chapter 8 General Discussion and Conclusions	
8.1 Background to the study	153
8.2 Major findings of this study	154
8.3 Implications of these findings	155
<i>8.3.1 Economic benefits</i>	155
<i>8.3.2 Modification of pearl implanting methods</i>	156
References	159
Appendix 1: Publications resulting from this thesis	167

List of figures

- Fig. 1.1** Geographical distribution of *Pinctada maxima* (Wada & Tëmkin, 2008)5
- Fig. 1.2** The inner surface of two valves of *Pinctada maxima* from different individuals representing gold lip pearl oyster (left) and silver-lip pearl oyster (right); arrows indicate lip colour6
- Fig. 1.3** A pair of valves of *Pinctada maxima* showing shell morphology and orientation; ae, anterior ear (auricle); am, adductor muscle scar; bn, byssal notch; li, ligament; ms, pallial muscle scar; nb, nacre border; nl, nacre layer (mother of pearl=MOP); pl, prismatic layer, and um, umbo7
- Fig. 1.4** Internal anatomy of *Pinctada maxima* (Jameson)9
- Fig. 1.5** Diagrammatic representation of the pearl implantation process: A, Foot and gonad structure of pearl oysters; B, Incision made with a blade; C-D, nucleus insertion into the gonad; E-F, mantle insertion.....19
- Fig. 1.6** Schematic drawings of (a) a three dimensional view of brick-mortar arrangement of aragonite platelets and conchiolin as a coating matrix, and (b) a cross-section view of the brick mortar arrangement of nacre in a pearl (Taylor & Strack, 2008).....23
- Fig. 1.7** Different types of pearl lustre of South Sea pearls from high (left) to low quality (right). Images supplied by The Autore Group.....24
- Fig. 1.8** The various shapes of South Sea pearls considered in pearl grading. Images supplied by The Autore Group25
- Fig. 1.9** Different types of surface complexity of south sea pearls ranging from high (left) to low quality (right). Images supplied by The Autore Group26
- Fig. 1.10** The interference phenomenon on a pearl surface due to the refraction and reflection of light passing through different layers of aragonite and conchiolin

	(Taylor & Strack, 2008).....	28
Fig. 1.11	Various colours of south sea pearls in pearl grading. Images supplied by The Autore Group	29
Fig. 2.1	Changes in the number of suitable <i>Pinctada maxima</i> donors within 30 minutes of exposure to the following anaesthetics:  3 mL L ⁻¹ 2-phenoxyethanol;  500 mg L ⁻¹ benzocaine;  1200 mg L ⁻¹ benzocaine;  0.4 mL L ⁻¹ menthol liquid;  2.5 mL L ⁻¹ propylene phenoxetol. The total number of oysters per treatment was 27.....	43
Fig. 2.2	Survival (%) of <i>Pinctada maxima</i> one month after exposure to the following anaesthetics: 2-P, 3 mL L ⁻¹ 2-phenoxyethanol; B500, 500 mg L ⁻¹ benzocaine; B1200, 1200 mg L ⁻¹ benzocaine; CO, 1.5 mL L ⁻¹ clove oil; ML0.25, 0.25 mL L ⁻¹ menthol liquid; ML0.4, 0.4 mL L ⁻¹ menthol liquid; PP, 2.5 mL L ⁻¹ propylene phenoxetol; and control, without anaesthetic.	45
Fig. 3.1	<i>Pinctada maxima</i> with one shell valve removed showing approximate area (dotted line) of excised mantle tissue. Inset: the excised mantle.....	54
Fig. 3.2	Histological view of regenerated mantle tissue, three months after excision (A-D) and normal mantle tissue (E, F) of <i>Pinctada maxima</i> . A. regenerated left mantle following excision from both mantle lobes; B. regenerated right mantle following excision from both mantle lobes; C. regenerated mantle following excision of left mantle lobe; D. regenerated mantle following excision of right mantle lobe; E. normal left mantle; and F. normal right mantle (if: inner fold, mf: middle fold, of: outer fold, cs: conchiolin secretion, mus: muscle, and pec: pigmented epithelium cells. A, E stained with Haematoxilin eosin– erythrosin technique, and B, C, D, F with MSB trichrome technique. Bar scale 200 μm).	57
Fig. 3.3	Photomicrograph of the distal part of the outer fold and part of middle fold of regenerated mantle three months after mantle excision showing haemolymph vessels (hv), connective tissue (ct) and both basophilic (bc) and eosinophilic cells (ec) distributed along the epithelium and at the subepithelial region of the	

- folds. Columnar epithelial cells (cec) are the main cell type of the epithelium.
 Stained with MSB trichrome. Bar scale 20 μm 58
- Fig. 3.4** Stricture on the inner fold of the regenerated mantle tissue of *Pinctada maxima* three month excision. Bar scale 200 μm59
- Fig. 3.5** Early wound healing following mantle excision in *Pinctada maxima*: A. 3 hour, B. 2 days, and C. 5 days after excision. Note the invagination of both latero-ventral edges of the wound area (arrows) in B and C.....61
- Fig. 3.6** Development of regenerated mantle (rm) and shell material deposition (*) on A. 12 days, B. 24 days and C. 45 days after excision of mantle in *Pinctada maxima*. Notice the border between nacreous secretion and the periostracum (b) is reduced from A to B due to material deposition (*), and finally in (C) the establishment of a new area of material deposition with a new border (b1); the area is characterised by darker background due to a thin cover of the deposition. Normally developed mantle (nm) is on the left and right of the picture62
- Fig. 3.7** Transverse view of the mantle wound site of *Pinctada maxima*, A: 1 hour, B: 3 hours, C: 6 hours, and D: 12 hours after excision. Scale bars 100 μm . Notice the contraction of muscle (mus) that squeeze the connective tissue (ct) in B and accumulation of haemocytes (h) at the wound site (ws) and finally the haemocytes seal the wound after 12 hours (D).....63
- Fig. 3.8** Photomicrographs of sections of the mantle tissue of *Pinctada maxima*, 24 hours after excision. A. Formation of the wounded area of the mantle tissue (Scale bar 200 μm) B. Detailed view of the inset at A (Scale bar 20 μm)64
- Fig. 3.9** Sections of the mantle tissue of *Pinctada maxima* at 36 hours (A, B) and 72 hours after excision (C). B is the enlargement of the inset in A, showing dedifferentiating muscle fibres (dmf) among hemocytes (h). A, C Scale bars 200 μm , B Scale bar 20 μm . The dashed line in C indicates the initial wound site.....65
- Fig. 3.10** Histological sections showing formation of regenerated mantle folds in *Pinctada maxima* at A: four days, B: twelve days and C: twenty-four days after

mantle excision. Scale bars 200 μm . a: artery, cec: columnar epithelium cells, cuc: cuboidal epithelium cells, cs: conchiolin secretion, if: inner fold, mf: middle fold, of: outer fold, pg: periostracal groove, pn: pallial nerve, and sec: squamous epithelium cells67

Fig. 3.11 Sections of regenerated mantle tissue of *Pinctada maxima* 45 days after excision, A: overall structure of mantle development with two insets, characterised by: outer fold (of), middle fold (mf), inner fold (if), conchiolin secretion (cs), pallial nerve (pn) and artery (a); B: enlargement of the inset covering part of outer fold area in A, and C: enlargement of the inset covering part of middle and inner folds area in A. Note the distribution of muscle (mus), haemolymph vessels (hv), basophilic (bc) and eosinophilic (ec) cells as well as columnar epithelium cells (cec) and cuboidal epithelium cells (cuc). Scale bar 200 μm 68

Fig. 3.12 Development of the mantle folds of *Pinctada maxima* at day 72 days after excision, cs: conchiolin secretion, if: inner fold, mf: middle fold, of: outer fold, pa: pallial artery, pg: periostracal groove, pn: pallial nerve. Scale bar 200 μm 69

Fig. 3.13 Structure of the mantle folds of *Pinctada maxima* 90 days after excision, cs: conchiolin secretion, if: inner fold, mf: middle fold, of: outer fold, pa: pallial artery, pn: pallial nerve. Scale bar 200 μm 70

Fig. 3.14 A parallel section of mantle three days after excision showing a wound site covered with hemocytes (h) and an early development of connective tissue (ct). Scale bar 100 μm 73

Fig. 4.1 Two areas of shell sections (solid line) representing regenerated (Reg) and normal (Nor) shell. The dashed line indicates the area of shell where nacre was secreted by regenerated mantle79

Fig. 4.2 A photograph of two sections of a shell of *Pinctada maxima* showing: A,B, the development of new layer of mineral depositions from regenerated mantle over an assemblage of biofouling and C, the development of normal shell. bio,

biofouling assemblages; bor, border between first and second deposition; enl-1, eroded nacreous layer; nl, nacreous layer; nl-1, nacreous layer of first deposition; nl-2 nacreous layer of second deposition by regenerated mantle; pl, prismatic layer; pl-1, prismatic layer of first deposition; and pl-2, prismatic layer of second deposition by regenerated mantle. Arrow heads indicate growth direction81

Fig. 4.3 The SEM structure of a shell of *Pinctada maxima* showing A. shell formed by regenerated mantle and B. by normal mantle of the same oyster . na, nacreous layer; pr, prismatic layer, and tr, transition area; while arrow heads indicate the direction towards the internal surface of the shell83

Fig. 4.4 SEM of regenerated shell of *Pinctada maxima* showing the whole structure representing prismatic (pr) and nacreous (na) layers of the shell: B, the edge of the prismatic layer from the top inset of A; C, the bottom part of the prismatic layer of the medium inset of A showing a loose attachment of the prismatic and nacreous layers; and D, the transition area (tr) of prismatic and nacreous layers of the bottom inset of A84

Fig. 4.5 An intermediate development of nacre platelets of the inner surface of the shell of *Pinctada maxima* showing: A, nacreous layer constructed by the regenerated mantle; and B, by the normal nacre of the same animal. Insets are the wide view of the surface, arrowheads indicate the directions of growth85

Fig. 4.6 A full development of nacre platelets of the inner surface of the shell of *Pinctada maxima* showing: A, nacreous layer constructed by the regenerated mantle; and B, by the normal nacre of the same animal. Insets are the wide view of the surface, arrowheads indicate the directions of growth86

Fig. 4.7 Mean (\pm SD, n = 28) thickness of nacre platelets from *Pinctada maxima* shell secreted by normal and regenerated mantle.....87

Fig. 5.1 Survival of *Pincatda maxima* following seeding for pearl production using various combinations of donor and recipient oysters.94

Fig. 5.2	Two areas (left and right) of epithelial regions of the pearl-sac of A. regenerated saibo, B. relaxed saibo and C. normal saibo from <i>Pinctada maxima</i> six weeks after pearl implantation. det, detachment; e, epithelium; ms, mucous cells; md, mineral deposition; o, oocytes; om, organic matrix; sc, stem cells; and, sp, spermatozoa. Scale bars 20 μ m. All was stained with MSB trichrome	96
Fig. 6.1	A scheme showing the use of donor pearl oysters in two pearl operations conducted in this study	107
Fig. 6.2	Survival (%) of <i>Pinctada maxima</i> two years after operation by five technicians (recorded two weeks before harvest). Tech 1 (n= 570 oysters), Tech 2 (n= 401 oysters), Tech 3 (n= 82 oysters), Tech 4 (n= 183 oysters) and Tech 5 (n=179 oysters).....	113
Fig. 6.3	Survival (%) of <i>Pinctada maxima</i> two years after implantation with relaxed saibo (n = 753 oysters), regenerated saibo (n = 572 oysters) and normal saibo (control)(n = 90 oysters)(recorded two weeks before harvest). (Chi Square= 8.990048, p=0.01116441).....	114
Fig. 6.4	Percentage of nucleus retention by <i>Pinctada maxima</i> implanted by five technicians in (A) all surviving oysters, (B) surviving oysters implanted with relaxed saibo and (C) surviving oysters implanted with regenerated saibo	115
Fig. 6.5	Percentage of nucleus retention by <i>Pinctada maxima</i> implanted with different types of saibo (relaxed, regenerated and normal). Relaxed saibo (n = 206 pearls), regenerated saibo (n = 214 pearls) and normal saibo (control)(n = 24 pearls)(Chi Square= 34.01114, p=0)	116
Fig. 6.6	The percentage of accepted pearls as a proportion of total pearls produced by <i>Pinctada maxima</i> implanted with three types of saibo (relaxed, regenerated and normal). Relaxed saibo (n = 240 pearls), regenerated saibo (n = 166 pearls) and normal saibo (control)(n = 19 pearls) (Chi Square= 112.3091, p=0)	117
Fig. 6.7	The proportions of various pearl shape categories produced by <i>Pinctada</i>	

maxima. A: total pearls (n= 425), B: pearls from oysters implanted with relaxed saibo (n = 240 pearls), C: pearls from oysters implanted with regenerated saibo (n = 166 pearls) and, D: pearls from oysters implanted with normal saibo (n = 19 pearls).....119

Fig. 6.8 Size-frequency distributions of pearls produced by *Pinctada maxima* implanted with (A) relaxed, (B) regenerated and (C) normal saibo. Relaxed saibo (n = 239 pearls), regenerated saibo (n = 165 pearls) and normal saibo (n = 19 pearls)..121

Fig. 6.9 Proportions of nucleated pearls produced by *Pinctada maxima* within three different sizes classes following implantation with (A) relaxed, (B) regenerated and (C) normal saibo. Relaxed saibo (n = 204 pearls, regenerated saibo (n = 147 pearls) normal saibo (n = 19 pearls)122

Fig. 6.10 Mean (mm ± SE) pearl size and nacre thickness of round nucleated pearls produced by *Pinctada maxima* implanted with relaxed, regenerated and normal saibo123

Fig. 6.11 Correlation between pearl size and nacre thickness ($r = 0.9460512$) of round nucleated pearls from *Pinctada maxima*.....125

Fig. 6.12 Correlation between pearl size and nacre thickness ($r = 0.9460512$) of round nucleated pearls from *Pinctada maxima*.....127

Fig. 6.13 Percentage of pearls from *Pinctada maxima* implanted with yellow/gold saibo into yellow/gold oyster. A: implanted with relaxed saibo and B: implanted with regenerated saibo (see Table 6.3 for colour categories and their abbreviations)128

Fig. 6.14 Proportions of pearls within five surface complexion categories (see Table 6.4). A: total pearls (n = 425), B: pearls produced by relaxed saibo (n = 240), C: pearls produced by regenerated saibo (n = 166) and D: pearls produced by normal saibo (control)(n = 19).....130

Fig. 6.15 The proportion of pearls produced by *Pinctada maxima* based on lustre category

(see Table 6.5). A: total pearls produced (n=425), B: pearls produced by relaxed saibo (n = 240), C: pearls produced by regenerated saibo (n = 166) and D: pearls produced by normal saibo (control, n = 19).....132

Fig. 7.1 Pearls from *Pinctada maxima* analysed in this study. Above (left to right): RD 2-2, RD 7-7, RDG 5-3, and RDG 5-4; bottom (left to right): RDG 5-6, RDG 12-6, RDG 13-2, and RDG 13-6. Five pearls were from two donors, RDG 5-3, RDG 5-4 and RDG 5-6 were from the first donor and RDG 13-2 and RDG 13-6 were from the second donor.....142

Fig. 7.2 UV-Vis spectral data (reflectance) of three pearls from the same gold donor (top) and two pearls from another gold donor (bottom) from *Pinctada maxima*146

Fig. 7.3 UV-Vis spectral data (reflectance) of two white pearls from different silver donors and a rejected pearl from *Pinctada maxima*, and a pearl nucleus.....147

Fig. 8.1 Proposed modification to the method for cultured pearl production based on the results of this study157

List of tables

Table 1.1	Production and the value of world cultured pearl production at the pearl farm level in 2005 according to Golay's estimates	2
Table 1.2	Size distribution of both shells and pearls of major commercially cultured pearl oyster species	30
Table 2.1	Mean (\pm SD) time required for <i>Pinctada maxima</i> to relax and the numbers of oysters that became relaxed when exposed to seven anaesthetic treatments ...	42
Table 2.2	A summary of the effectiveness of three anaesthetics, 3 mL L ⁻¹ 2-phenoxethanol, 1200 mg L ⁻¹ benzocaine and 2.5 mL L ⁻¹ propylene phenoxetol	47
Table 2.3	Induction time (min \pm SD) for different species of molluscs when exposed to various anaesthetics.....	48
Table 5.1	Four implantation combinations conducted in this study	92
Table 5.2	Pearl-sac development in <i>Pinctada maxima</i> (Scoones, 1996)	97
Table 6.1	Oysters used in the first and second operations in this study	103
Table 6.2	Categories based on shape characters in grading of <i>Pinctada maxima</i> pearls modified from Taylor (2007).....	109
Table 6.3	Categories based on colour in grading pearls of <i>Pinctada maxima</i> (Taylor, 2007).....	111
Table 6.4	Categories based on surface complexion used for grading pearls of <i>Pinctada maxima</i> (Taylor, 2007).....	112
Table 6.5	Categories based on lustre used for grading pearls of <i>Pinctada maxima</i> (Taylor, 2007).....	112
Table 6.6	Effect of types of saibo (normal, relaxed, regenerated) on nacre thickness	

	tested with One-Way Analysis Variance (ANOVA).....	124
Table 6.7	Numerical ranking of the performance of oysters and pearls resulting from implantation of <i>Pinctada maxima</i> with three types of saibo (relaxed, regenerated and normal) against standard criteria where 1 = most favourable and 3 = least favourable.....	136
Table 7.1	Characteristics of tested South Sea pearls produced by <i>Pinctada maxima</i> implanted with relaxed saibo.....	143
Table 8.1	Total and average values of pearls produced in this study using relaxed, regenerated and normal saibo calculated using the Atlas Pacific Ltd ‘retail price matrix’ based on the grading criteria outlined in Chapter 6.....	156