AquaTips

Improving Pearl Quality by Grafting and Husbandry Methods

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This article was written as part of the work for the project, "Improving Pearl Quality by Grafting Technologies and Husbandry Methods for Development of a Hatchery-based Black Pearl Industry in Pohnpei, the Federated States of Micronesia, Years 1 and 2," which is funded by the Center for Tropical and Subtropical Aquaculture (CTSA) under a grant from the U. S. Department of Agriculture Cooperative State Research, Education, and Extension Service.

The price of a cultured pearl is determined by its quality: shape, size, color, luster, and lack of flaws. Flaws or irregularities, which occur more or less during the natural nacre-layering process of the oyster, were thought to be inevitable, affected apparently by the condition of the host or donor oyster during grafting, or by exogenous physico-chemical and biological stressors. Flawless round pearls fetch far better prices than any other shape, so a primary target of pearl farming is achieving higher rates of roundness in the product. Generally speaking, gem quality perfectly round pearls of the highest luster are rare, being found in only about 5% - 10% of the total output of the grafting operation.

The black pearl industry in the South Pacific nations, the Tahitian black pearl industry in particular, has a history that spans over four decades. Nevertheless, the success rate of marketable pearl production from grafting has been low. While 30% – 40% of marketable quality black pearls are produced from grafted oysters, the majority of the pearls are irregularly shaped (baroques, semi-baroques, and with "circles," "spot" marks, or both), the round shape is found in only 25 pearls out of 150 – 160 oysters from three consecutive operations (i.e., about 15 % roundness rate overall). The overall goal of this CTSA-funded project is to find simpler and more economical ways of improving pearl quality by grafting techniques and husbandry methods to: a) improve shape (roundness); b) reduce flaws; c) improve post-grafting survivorship, and d) transfer pearl aquaculture technology to Micronesia and other regions.

This report describes the post-grafting survivorship from part of the project Year 1 study, together with the findings on flaw improvements (circle reduction) from preliminary experiments in 2006 – 2007.

Materials and Methods

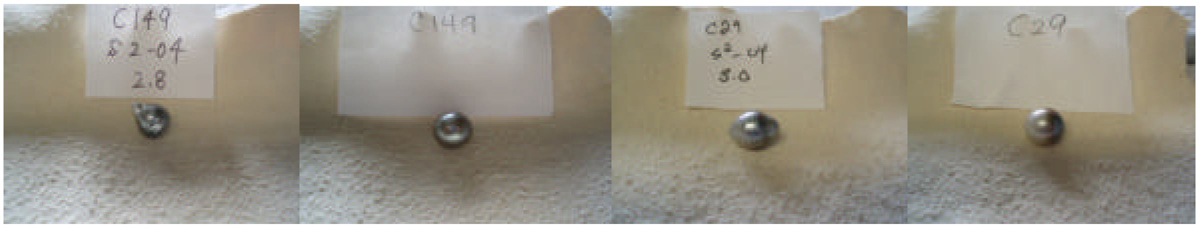
Along with harvesting pearls for a preliminary circle-test, experiments during Year 1 of the project were conducted between July 9, 2007 and August 16, 2007 at a community-based farm at Pakin Atoll, 25 miles east of Pohnpei (Fig. 1). The experimental variables were: nucleus type (chemically treated nuclei using FNC-α, "fibronectine-coated" nuclei, Imai Seikaku Co. Ltd., Japan) and size (2.0-Bu and 2.3-Bu, i.e., 6.06 mm diameter and 6.96 mm diameter, respectively), age of source hosts after fertilization in 2007 (S7 and S10, i.e., 42 months and 33 months, respectively); and grafting techniques (i.e., re-grafting to reduce flaws, such as circle marks). A "circled" pearl in this report refers to a pearl flawed with regular streaks or concave rings perpendicular to an axis of rotation and which occupy over one-third of the surface of the pearl.

Figure 1. Husbandry work (left) and grafting operations (right) at the Pakin Atoll farm.

Oysters that had been grafted for a second time (the 2nds) in August 2006 for a preliminary circle-test (n = 260) were harvested in June 2007 to gather data for a preliminary assessment of pearl quality. For Year 1 re-grafting experiments for the circle- test (2007 C-test), 300 each of the oysters that had produced "circled" virgin pearls from the 2005-2007 pearl culture experiments were used. Nuclei between 2.8-Bu (8.48 mm diameter) to 4.3-Bu (13.03 mm diameter) were used for the re-grafting trials. Virgin oysters (n = 1,200) from the S7 and S10 family groups were newly grafted for the FNC- tests. Post-grafting husbandry and data collection were performed bi-monthly and completed in June 2008. Data collection and quality analysis of the harvested pearls from the FNC tests (treated and non-treated) are ongoing at the present time.

All of the oysters were produced from the College of Micronesia (COM) pearl hatchery project, with known dates of fertilization, grow-out locations, and husbandry history. The donor oysters for mantle tissue for the grafts were selected from among younger oysters about 20 months old, such as the S10 or S13 groups. Post-husbandry culture was conducted using the chaplet or "ear-hanging" culture method, by hanging 10 oysters each on a drop-rope at 1 m intervals suspended from the surface line system. The hosts and donors were pre-conditioned by lantern-net culture for two months prior to the grafting operation. Statistical analyses of post-grafting survivorship were conducted using single-factor one-way ANOVA, variances by two-sample Fisheries F-tests, and Students' t-tests were used to determine the effect of the FNC-treated nucleus (FNC vs. nonFNC), nucleus size, source of hosts, and culture methods (P>0.05).

Pearls in this study were categorized as one of four shapes: round (R, less than 1/10 of diameter variation); semi-round (SR, more than 1/10 of diameter variation), drop (D, symmetric with single axis of rotation), and baroque (B, asymmetric and no axis of rotation), and one of four grades: A (high luster with less than 10% of confined surface flaws), B (high to medium luster with less than 1/3 of distributed surface flaws), C (high to medium luster more than 1/3 distributed surface flaws), and D (regardless of luster, with more than 2/3 of the surface or a larger number of visible flaws). See Figures 2 and 3 for examples of various shaped pearls and circle flaws.



A1 **A2** **B1** **B2**

Figure 2. Circle flaws reduced and shape improved, with color pattern inherited (A1-A2, B1-B2) in pearls harvested for the preliminary circle-test in July 2007.

A1 **A2** **B1** **B2**

Figure 3. Circle flaws reduced and shape improved, with color pattern change (A1-A2, B1-B2) in pearls harvested for the preliminary circle-test in July 2007.

Results and Discussion

Duplicated experiments showed that there was no significant difference ($P>0.05$) in the post-grafting survival rate over 10 months period in all experimental treatments between the oysters grafted with a chemically coated nucleus (FNC) or an ordinary nucleus (nonFNC) for the S10 oysters (nucleus size, 2.0-Bu), the S7 oysters (nucleus size, 2.3-Bu), and the Circle-test (C-test) (Fig. 4). Post-grafting survival rates for the FNC test newly grafted oysters over the 10-month period ranged between 69.5% (S10 FNC), and 76.5% (S7 nonFNC). The highest survival (78.7%) was among oysters re-grafted for the circle test (C-test). The C-test oysters also showed the highest survival rate (92.2%) at 2 months after the grafting compared to all of the oysters in the FNC-test groups. All of the oysters recovered from post-grafting trauma during the first two months and became stabilized afterwards, maintaining extremely high survival rates between 95% – 100%. Results indicated that the treating nuclei with FNC did not have a significant effect on post-grafting survivorship[1].

[1] Akiyama et al. (1997) reported that the survival rate of oysters treated with antibiotic (tetracycline hydrochloride, TC-HCl)-coated nuclei (86.7%) was higher than that of the control group (63.3%, inserted with untreated nuclei). They also observed higher survival rates in the group grafted with TC-HCl-coated nuclei than in the control groups in large-scale field experiments, and that nuclei retaining rates were also higher in the treated group (Akiyama et al. 1997). The present results did not indicate whether the FNC-treated groups had a clear advantage over the uncoated (nonFNC) groups, but both S10 and S7 oysters showed similar pearl success rates over the 10-month cultivation period. It is not clear whether FNC-? ® contains an antibiotic coating, because the supplier only describes fibronectine as a coating substance, which functions as a bonding substance in animal cells, and which is also found in human cells.

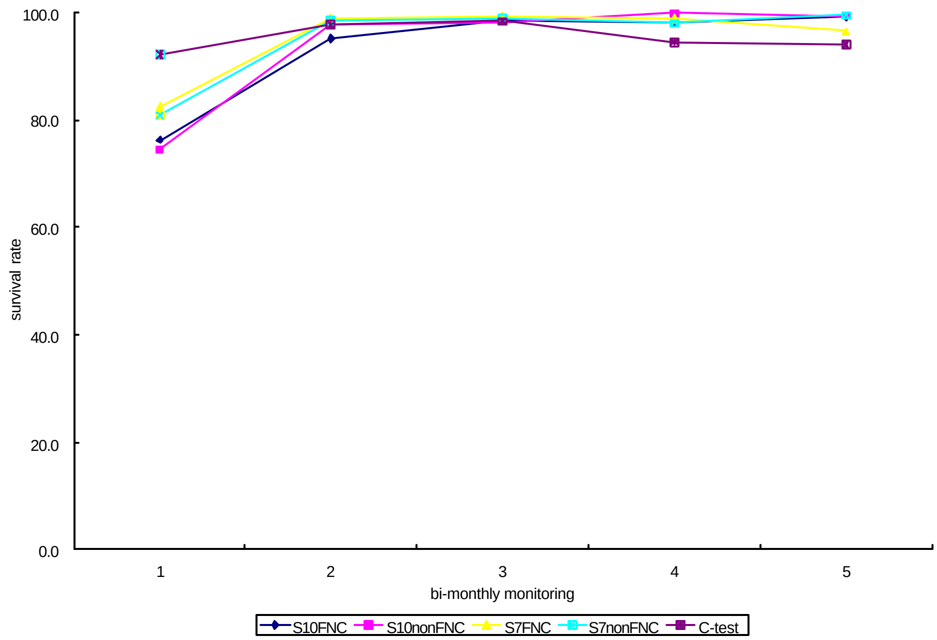


Figure 4. Post-grafting survivorship of the host oysters of the circle-test (C-test) and FNC-test during the Year 1 experiments. Pearls were harvested in June 2008.

Compared to the first grafting (grafting to virgin oysters), re-grafting is generally believed to result in better survival rates, pearl success rates, or nucleus retention rates, because a pearl sac is already formed at the time of the re-grafting operation. Preliminary studies in 2004 – 2006 had indicated that post-grafting survival rates and pearl success rates after a 1-year culture period were similar: among two groups of oysters treated with FNC (78.6% survival rate and 45.1% pearl success rate) and their counterparts that were untreated (78.4% and 42.7%, respectively). The results of the test-harvest experiments in 2005, however, showed some improvements in pearl quality and roundness rate. In particular, higher roundness rates were observed in pearls from the FNC group (18.8%) compared to those of the non-FNC group (4.8%). These findings suggest that the chemically coated nuclei may have a more favorable effect on pearl quality than on post-grafting survivorship.

Grafting Techniques Reduce Circle Flaws and Increase Roundness

In June 2007, 166 pearls (63.8% pearl success rate) were harvested from 216 living oysters out of 260 (83.5% post-grafting survival rate) oysters re-grafted in 2006 that had produced "circled" virgin pearls (Table 1). This preliminary circle-test revealed significant improvements in roundness and reduction in circle flaws among the 2nds compared to the matching pearls from the same oysters that had produced pearls with circle marks the first time. The degree of quality improvement for circle reduction, changes in shape, and the occurrence of spots were given numerical indexes, as shown in Table 2 and Figure 5. Out of 166 matched pearls, 146 (88%) of the 2nd-time pearls had fewer circles, and 68 (41%) were completely free of circle marks. The roundness also increased in 153 (92.2%) of the matched pearls, and 58 (34.9%) had become completely rounded. In contrast, spot marks did not change or improve very much among the 2nd-time pearls (102, or 61.4%); only 61 (36.7%) of the total had slightly improved (i.e., reduced the number of spots). The results suggest that the majority of spots are passed along to subsequent pearls produced by the oysters. Results of these experiments also indicated greater degrees of improvement in roundness (89 out of 166, or 53.6%) from other shapes (Table 3).

Table 1. Ten-month survival rates and pearl success rates from preliminary experiments (2006 – 2007) for the circle test.

	Re-grafted in 2006	Living at harvest in 2007	Total pearls produced	Total keshi found	Circled pearl[a] to keshi	Keshi to keshi	Keshi to pearl (R/D/C/B[b])
Circle-test	260	217	166	43	35	8	8
Survival rate		83.5%					
Total pearl success rate			63.8%				
Total keshi rate				16.5%			
Pearl to keshi rate					13.5%		
Keshi to keshi rate						50.0%	
Keshi to pearl rate							50.0%

Note: A keshi is a non-beaded pearl formed when the oyster rejects and expels the implanted nucleus before culturing is complete, or the implanted mantle tissue fractures and forms a separate pearl sac that eventually produces a pearl without a nucleus.

[a]Circled pearl: A pearl with circle flaws, regardless of shape.

[b]Types of pearls with a nucleus inside. R: round; SR: semi-round; D: drop; B: baroque.

Table 2. Pearl quality analysis (circle reduction) of pearls from the preliminary circle-test for 166 matched pearls before (virgin pearls) and after (second pearls) the test.

Improvement Index	Circles (Number of pearls)	Roundness (Number of pearls)	Spots (Number of pearls)	Total Points
+2 No circles; Round	68	58	3	+258
+1 Slightly improved	78	95	61	+234
0 Same as before	12	5	39	0
-1 Slightly deteriorated	5	7	56	-68
-2 Deteriorated	3	1	7	-22
Total	166	166	166	+402

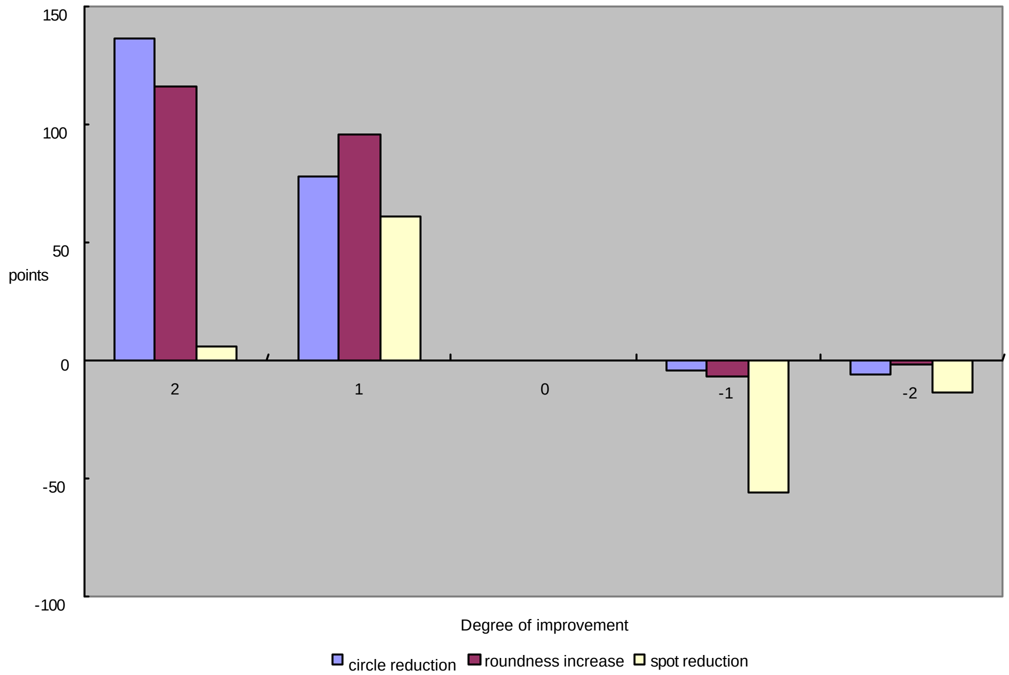


Figure 5. Pearl quality improvement results from the preliminary circle-test in 2006 – 2007.

Table 3. Pearl quality analysis (shape change) of pearls from the preliminary circle-test for 166 matched pearls before (virgin pearls) and after (second pearls) the test.

	Round-after	Semi-round-after	Drop-after	Baroque-after	Total
Semi-round-before	9	4	2	3	18
Drop-before	66	27	15	16	124
Baroque-before	14	3	2	5	24
Total	89	34	19	24	166

The project is a part of a process of understanding the mechanisms of the formation of flaws or blemishes, particularly circles and "spot" marks, which are commonly found in a significantly large proportion (i.e., 60% - 95%) of total pearl production in black pearl farming, which has been a bottleneck for profitability in farming operations. In the past, many oysters used for the first grafting were wasted without further pearl production--as much as 40% – 50% of oysters did not produce a pearl after the first grafting. An additional 50% – 70 % of the oysters that produced pearls are usually discarded after the pearls are harvested without a re-grafting attempt. If we effectively prove that the quality of pearls can be improved by re-grafting oysters that produced lower grade pearls (because of flaws, such as circle marks and/or shapes that are not round), farm business could be more profitable than they are currently.

A widely believed theory of the mechanism of circle formation in pearls is that these types of flaws are formed because the pearl rotates inside the pearl sac, and that circled pearls occur more frequently in younger and muscular oysters. Our observations, however, indicated that all of the circle marks were directional, perpendicular to the axis of rotation, and parallel to each other. Recently, Fang, et al. (2008) proposed a hypothetical model for the proliferation and differentiation of the mantle of *Pinctada fucata*, in which a proliferation ‘‘hot spot’’ exists in the outer epithelial cells of the central zone, and the ability to proliferate decreases progressively from this ‘‘hot spot’’ towards the marginal zone. Differentiation of the entire mantle was proposed to occur continuously, with its growth and direction from the proliferation ‘hot spot’ (central zone) towards the marginal zone. The proliferation rate was thought to decrease from the center to the margin, which almost loses the ability to proliferate. This would suggest that it is highly likely that an implanted piece of mantle propagates directionally, so that the newly formed pearl sac would be a collective of directionally-formed epithelial cells. Thus, linear formations of groups of cells could have similar or the same functions in terms of nacre (pearl) secretion, which develop perfectly lined circle marks from perfectly aligned groups of cells (Ito, ms. in preparation).

A widely believed theory of mantle proliferation and differentiation is based on an assumption that the mantle has numerous growth centers all over the mantle epithelium, with the same proliferative activity throughout the entire outer epithelial cells of the mantle. The existing theory, however, cannot explain the linear formation of perfect circle/ring marks. The findings of Fang et al. (2008), on the other hand, support the hypothesis of a circle formation mechanism by the author of this article. No sinuous or irregularly lined marks have been found among the circled pearls examined by the author of this article, except for a few pearls with “90 degree crossed-circles”, doubled/overlapped-circles” and “diagonally crossed-circles” out of several thousands of pearls examined, all of which still show perfectly “ringed” or linear forms. Thus, it is possible that the circle marks were formed by groups of lined epithelial cells functioning with or without normal nacre secretion (Ito, ms. in preparation).

Acosta-Salmon et al. (2004) reported that the outer epithelium cells of these slices propagated rapidly to regenerate after a small portion of mantle was removed and secreted nacre onto the pearl shell. Their report, however, did not explain clearly how the mantle tissue regenerations related to the pearl formation, particularly to the circle formation (Acosta-Salmon et al. 2004). Both the preliminary and the project’s Year 1 studies showed that the majority of the circle flaws were apparently formed during the first/virgin pearl formation, but the spots tended to recur in the pearls from the 2nds (the re-grafted oysters), indicating a different formation mechanism (Ito, ms. in preparation).

In June 2008, 307 pearls, with an additional 196 Keshi pearls (a non-beaded pearl formed when the oyster rejects and expels the implanted nucleus before culturing is complete) were produced from 472 living oysters from the original 600 oysters which had produced “circled pearls” in 2007 for the Year 1 circle-test (Fig. 6). The 472 matched pearls (first pearl and the 2nd from the same oyster) are being assessed for pearl quality. Pearl quality assessments are also underway for 850 pearls produced by FNC-treated oysters (447 pearls) and non FNC-treated oysters (403 pearls). The results will be incorporated into Year 2 progress reports on the project.

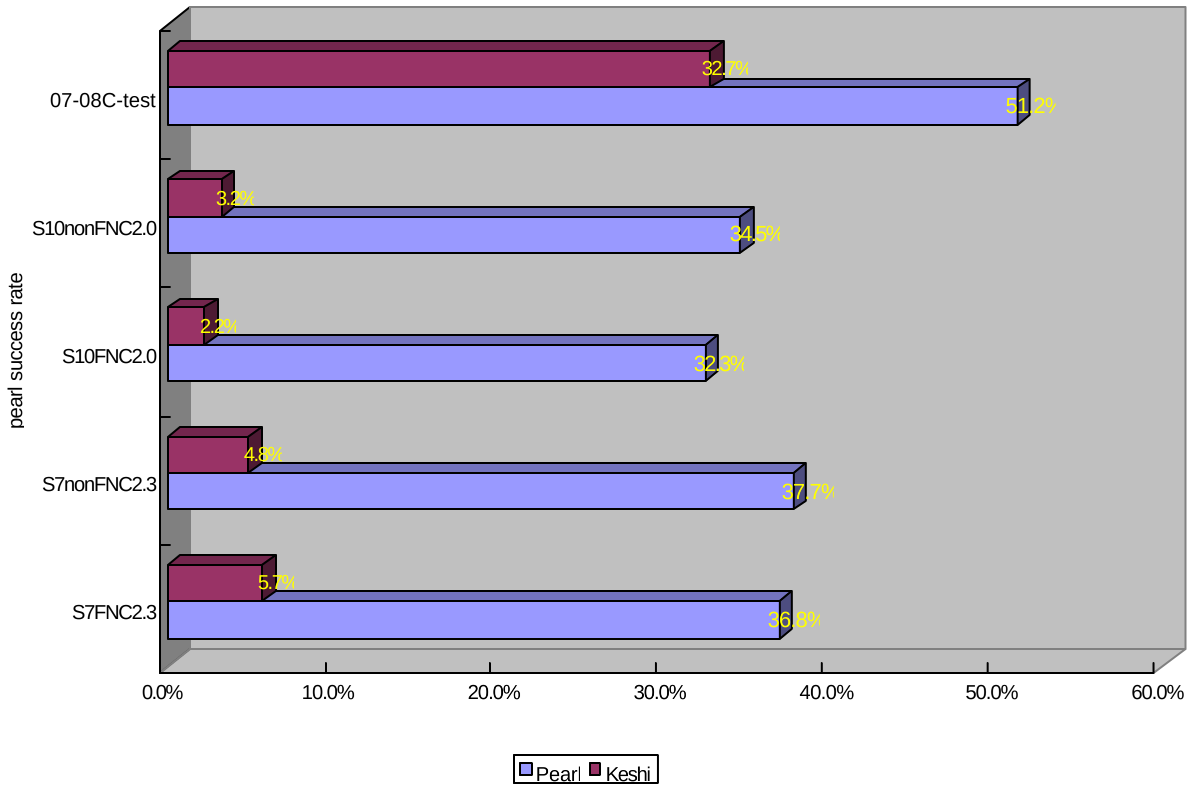


Figure 6. Pearl production success rates from the circle-test and FNC-test from the Year 1 harvest in June 2008. "Pearl" indicates a true pearl, with the nucleus being retained. "Keshi" indicates a non-nucleated pearl formed after the oyster rejects and expels the nucleus.

References

Akiyama, N., Itoh, A., Morohoshi, H., Asahina, K., Hirose, H., Mano, N., Fukushima, N., Taniyama, J., and Itoh, J. 1997. Effect of tetracycline hydrochloride (TC-HCl) coating to nucleus on pearl oyster (*Pinctada fucata martensii*) survival after nucleus insertion. Aquaculture Science 45(4): 457-466.

Acosta-Salmon, H., Martinez-Fernandez, E., and Southgate, P.C. 2004. A new approach to pearl oyster broodstock selection: Can saibo donors be used as future broodstock? Aquaculture 231: 205–214.

Fang, Z., Feng, Q., Chi, Y., Xie, L., and Zhang, R. 2008. Investigation of cell proliferation and differentiation in the mantle of *Pinctada fucata* (Bivalve, Mollusca). Marine Biology 153: 745–754.

Acknowledgements

This project was funded by the Center for Tropical and Subtropical Aquaculture. The preliminary circle-test in 2006-2007 was conducted by the College of Micronesia Land Grant Program under UDSA-HATCH Research Grant 2003-2008. Special thanks are extended to the College of Micronesia pearl project staff.