1 Post-operative care of implanted pearl oysters *Pinctada fucata* in low salinity seawater improves the
2 quality of pearls

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Abstract

Current techniques for culturing the Akoya pearl oyster *Pinctada fucata* result in low yields of blemish-free, round pearls (high quality pearls). We compared the effects of five factors on the proportion of high quality pearls produced during culture using a generalized linear model (GLM). Two factors were physiological, shell-closing strength (SCS) and the whole wet weight of host pearl oysters, and three factors were procedural, post-operative care method, technician, and nucleus diameter. Our results suggest that post-operative care methods have the most significant effect on increasing the proportion of high quality pearls. The proportion of high quality pearls was five-fold higher in the group held in low salinity seawater than in the conventional treatment group. We propose a new post-operative care method in which oysters are immersed in low salinity seawater to increase the production of high quality pearls.

Keywords: *Pinctada fucata*; Pearl quality; Post-operative care; Low salinity; Implantation; Generalized linear model analysis

1. Introduction

Current methods for culturing pearls result in low yields of high-quality pearls (Norton et al., 2000; Kripa et al., 2007). The minimum requirement for a high-quality pearl is that it be round and have no blemishes (Matlins, 1996; Wada, 1999; Akamatsu, 2003; Kripa et al., 2007). The commercial value of pearls is decreased by the presence of deformities and blemishes (Matlins, 1996; Norton et al., 2000; Akamatsu, 2003; Kripa et al., 2007; Ogimura et al., 2012). Deformed pearls have projections or humps. Although blemishes do not affect the shape, they are visible as bluish black or dark brown spots. Minimizing or preventing such deformities and blemishes is one of the most important technical issues currently facing the industry.

Pearl culture consists of four steps (Wada, 1999; chapter 8 by Taylor and Strack, Southgate and Lucas, 2008): 1) pre-operative conditioning, 2) nucleus implantation (hereafter, implantation), 3) post-operative care, 4) culturing and harvest. A number of studies have evaluated methods for implantation and post-operative care and have resulted in improvements in pearl quality (Norton et al., 2000; Ruiz-Rubio et al., 2006; Atsumi et al., 2011). During implantation, nuclei and mantle grafts are embedded into the gonad of host oysters (Cochennec-Laureau et al., 2010). Proper post-operative care minimizes extrusion of the nuclei soon after implantation and reduces the occurrence of abnormal pearls (Uemoto, 1962; Wada, 1999;

Akamatsu, 2003). During post-operative care, the host oysters are placed into small mesh nets that are suspended in the sea in areas that do not experience strong currents or rapid changes in salinity and temperature and held for one to two weeks after implantation.

The quality of pearls is influenced by at least five factors: post-operative care method (Uemoto, 1962; Hayashi, 2008; Atsumi et al., 2011), the competency of the technician performing the implantation (Nava et al., 2000; Atsumi et al., 2011), the shell-closing strength (hereafter, SCS) of the host oyster (Aoki et al., 2010a), the whole wet weight of the host oyster, and the nucleus diameter (Hasuo, 1961; Funakoshi et al., 1991).

We recently developed a new post-operative care method that increased the proportion of blemish-free, round pearls (hereafter, high quality pearls). Immersion in low salinity (25 psu) seawater just after implantation (hereafter, the low salinity treatment) resulted in a significantly higher proportion of high quality pearls than from oysters that were immersed in the sea (hereafter, the conventional treatment) during the post-operative care period (Hayashi, 2008; Atsumi et al., 2011). SCS is the maximum load value needed to open the shell of the pearl oyster to 10 mm with a shell opener. This index is useful as an indicator of an individual oyster's health and physiological condition (Okamoto et al., 2006a; Okamoto et al., 2006b; Aoki et al., 2010b). The unit of SCS is kilogram-force (kgf), where 1 kgf is approximately equal to 9.8 N (SI unit). Aoki et al. (2010a) noted that the proportion of high-quality pearls produced by host oysters with an SCS of 2.0–4.9 kgf was higher than for oysters with an SCS of 1.0–1.9 kgf and 5.0–6.9 kgf. The technician typically decides on the diameter of nucleus to be implanted based on the whole wet weight of host oysters. Some studies suggest that implantation of a larger nuclei is associated with a decrease in the production of high-quality pearls (Hasuo, 1961; Funakoshi et al., 1991). Despite the importance of this issue to the industry, to our knowledge no one has evaluated the combined effects of these factors on the production of high-quality pearls.

Our objective was to determine which of the five factors exert the most significant effect on the production of high quality pearls. We compared the effects of the factors on the proportion of high quality pearls using a generalized linear model (GLM). We defined “high quality pearls” as round nacreous pearls that have no blemishes or just one blemish smaller than 0.5% of the pearl surface (Inoue et al., 2011, Atsumi et al., 2011).

2. Material and methods

2.1. Pre-operative conditioning

We conducted two experiments to evaluate the effects of five factors (SCS, whole wet weight, post-operative care method, implantation technician, and nucleus diameter) on the proportion of high quality pearls produced in culture. The first experiment was carried out from April to August in 2009. The second experiment was carried out from May to September in 2009. We obtained two-year-old Japanese oysters from the Mie Prefectural Farming Center (Shima, Mie, Japan). The experimental procedure used during the pre-operative conditioning, implantation, and post-operative care is outlined in Figure 1. The pre-operative conditioning in each experiment consisted of the following four steps. 1) Physiological conditioning in the sea: The host oysters were deliberately crowded into a plastic conditioning box with a lid (L×W×H: 355×420×155, 35 plastic conditioning boxes, 70 oysters/box, chapter 8 by Taylor and Strack, Southgate and Lucas, 2008). The host oysters were suspended from a raft in Ago Bay for 10–13 d during the first experiment and 11 d in the second experiment. 2) Physiological conditioning in a tank: the host oysters were moved from the sea to a tank containing 9000 L seawater at 25°C and 33 psu (hereafter, tank 1) for 4 d in the first experiment and 5 d in the second experiment. 3) Spawning induction: the host oysters were moved from tank 1 to a tank containing 9000 L ozonated seawater (Aishinsanki, OA-1) at 25°C and 33 psu (hereafter, ozonated tank) for 7 h in both experiments. The host oysters were moved from the plastic conditioning boxes to round pearl nets (35 round pearl nets, 70 oysters/net) while immersed in the ozonated tank. The host oysters were immersed in tank 1 for 3 d after spawning. 4) Measurement and identification: the SCS and whole wet weight of each host oyster (N = 2022 in the first experiment, N = 1599 in the second experiment) was measured, and each individual was numbered with a marker for identification. The SCS was measured following the method described by Aoki et al. (2010b). The device used for the measurement of SCS consists of a portable load meter and a shell opener. To close the shell, the host oysters were soaked in tap water for 10 min before SCS measurement. Subsequently, the shell opener was inserted into the shell of the closed oyster, and the maximum load value to open the shell of the pearl oyster by 10 mm was measured. The host oysters were divided into two SCS-groups (Group I: SCS of 2.0–4.9 kgf; II: SCS of 5.0–7.9 kgf). Then, the two SCS-groups were placed in plastic conditioning boxes to continue the physiological conditioning and were immersed in tank 1 for 9–10 d in the first experiment and for 10–11 d in the second experiment prior to implantation.

2.2. Implantation

To induce pearl production, a small piece of mantle tissue ($2\text{--}3\text{ mm}^2$) and a small ($6.36\text{--}6.40\text{ mm}$ diameter) or large ($7.73\text{--}7.77\text{ mm}$) nucleus were implanted into the gonad of each host oyster following conventional methods (Cochennec-Laureau et al., 2010). The operation was carried out by two technicians, each with more than 15 years of experience. Approximately equal numbers of host oysters of each SCS-group were implanted with a small and large nucleus by each technician (Fig. 1, Table 1). In the first experiment, a total of 966 individuals (mean wet weight $\pm$ S.D: $57.3 \pm 4.8\text{ g}$, range: $43.2\text{--}73.5\text{ g}$) were implanted on 25–26 May, 2009. In the second experiment, a total of 961 individuals ($58.5 \pm 4.8\text{ g}$ mean wet weight, range: $26.8\text{--}77.1\text{ g}$) were implanted on 15–16 June, 2009.

2.3. Post-operative care and culture

Approximately half of the oysters in each of the four subgroups (high and low SCS and large and small nuclei) that were implanted by each technician ($N = 492$ in the first experiment, $N = 476$ in the second experiment) were immersed in low salinity seawater ($24.9 \pm 0.1\text{ psu}$ and $25.2 \pm 0.3^\circ\text{C}$ in the first experiment, $25.0 \pm 0.0\text{ psu}$ and $25.0 \pm 0.3^\circ\text{C}$ in the second experiment) in a closed recirculation tank for 14 d (low salinity treatment) (Fig. 1). The low salinity (25 psu) seawater was obtained by adding tap water to ambient seawater (33 psu). The oysters were not fed during the low salinity treatment. The remaining implanted oysters ($N = 474$ in the first experiment, $N = 485$ in the second experiment) were suspended from a raft in the sea, exposed to gentle currents (depth: 3 m ; $33.8 \pm 0.2\text{ psu}$ and $21.6 \pm 0.8^\circ\text{C}$ in the first experiment; $32.9 \pm 0.3\text{ psu}$ and $24.7 \pm 0.9^\circ\text{C}$ in the second experiment) in Ago Bay for 14 d (conventional treatment). Following the post-operative care period, the oysters in both the low salinity and conventional treatment groups were cultured for 60 d in Ago Bay.

2.4. Harvest and pearl quality criteria

Oysters are typically grown for 1 year and harvested between December and January. However, our focus was on the presence of deformities and blemishes in the early stage of pearl formation. Thus, the pearls in this study were harvested from host oysters between 10 and 11 August 2009 in the first experiment and between 31 August and 1 September 2009 in the second experiment. Therefore, we note that pearls obtained in this study had incomplete nacre deposition, luster, and tint compared with commercial pearls. The pearls harvested in the present study were classified as nacreous, prismatic, organic, or nucleus without deposition (Atsumi et al., 2011). Nacreous pearls have nacreous luster and color of white or whitish. Prismatic pearls have no nacreous luster and a yellowish green color, like mung

beans. Organic pearls are completely opaque and are bluish black or dark in color. Prismatic and organic pearls have no commercial value. We focused only on nacreous pearls, which were visually classified as round or deformed (Matlins, 1996). Furthermore, the quality of the round nacreous pearls was assessed based on the surface condition, i.e., the proportion of blemished surface. High quality pearls were defined as round nacreous pearls that had no blemishes, or just one blemish that was <0.5% of the pearl surface (Fig. 2, Inoue et al., 2011, Atsumi et al., 2011). The diameter of high quality pearls was measured using digital vernier calipers.

2.5. Data analysis

We used a GLM to evaluate the effects of the five factors on the proportion of high quality pearls (Crawley, 2005). The proportion of high quality pearls produced (H) was modeled as follows:

$$\log(H/(1-H)) = \beta_0 + \beta_1 PO + \beta_2 \text{technician} + \beta_3 SCS + \beta_4 \text{weight} + \beta_5 \text{nucleus} \quad (1)$$

where β_0 is the intercept, and $\beta_1, \beta_2, \beta_3, \beta_4$ and β_5 are the coefficients for PO , *technician*, SCS , *weight*, and *nucleus*, respectively. The proportion of high quality pearls was calculated by dividing the number of high quality pearls by the total number of harvested pearls to exclude bias resulting from extrusion of nuclei and death of the host oyster. PO represented the post-operative care methods where the low salinity treatment = 0 and the conventional treatment = 1. The factor “*technician*” represented the two technicians responsible for implantation: technician 1 = 0 and technician 2 = 1. SCS represented the SCS of the host oyster: a two-level categorical variable (SCS of 2.0–4.9 kgf: 0, and SCS of 5.0–7.9 kgf: 1). The factor “*weight*” represented the whole wet weight of the oysters and was modeled as a continuous variable. The factor “*nucleus*” represented the nucleus diameter (large = 0, small = 1). We used a logistic model with a binomial distribution and parameter estimation was performed by the maximum likelihood method (*glm* in R ver.2.15.1, <http://cran.ism.ac.jp/>).

The percentage of oysters with an SCS of 5.0–7.9 kgf was higher in the first experiment (64%) than in the second experiment (50%), suggesting the oysters in the two experiments differed from each other (Table 1). Therefore, the data from the two experiments were analyzed separately. First, we conducted parameter estimation for each of the variables using a univariate analysis. Then, we conducted parameter estimation for all variables using a multivariate analysis. These analyses were performed to check for confounding factors that may have affected the results of the study. Last, we used a stepwise forward regression for parameter estimation. We calculated Akaike’s information criteria (AIC) and the model

with the lowest AIC (hereafter, the optimum model) was chosen. A significance level was set at 0.05.

3. Results

The numbers of high quality pearls and the total number of harvested pearls are given in Table 1. The proportion of high quality pearls was at least 4.9 times higher in the low salinity treatment than the conventional treatment group, even though the proportion of harvested pearls was lower in the former group. The proportion of high quality pearls was at least 2.2 times higher in oysters with an SCS of 2.0–4.9 kgf than in those with an SCS of 5.0–7.9 kgf. The proportion of high quality pearls was at least 1.4 times higher for technician 2 than technician 1. Similarly, the proportion of harvested pearls was higher for technician 2 than technician 1, particularly in the second experiment. The proportion of high quality pearls was at least 1.9 times higher in the group implanted with a large nucleus than in the group implanted with a small nucleus. The proportion of harvested pearls was higher in the group implanted with a small nucleus in experiment 1, whereas the opposite was true in experiment 2. The proportion of high quality pearls tended to decrease with an increase in wet weight. Conversely, the proportion of harvested pearls tended to increase with weight. The mean diameter of high quality pearls from oysters implanted with a large nucleus was 7.99 ± 0.30 mm ($\pm$ S.D.) in experiment 1 and 8.17 ± 0.20 mm in experiment 2. The mean diameter of high quality pearls from oysters implanted with a small nucleus was 6.75 ± 0.10 mm and 6.79 ± 0.16 mm in experiments 1 and 2, respectively. Total survival rate was 85.7% in experiment 1 and 70.7% in experiment 2.

In both experiments, the simple models (models i-v) and the multiple model (model vi) yielded positive and negative variable estimates that were similar (Tables 2 and 4). Model vii in the first experiment, including four parameters (*PO*, *SCS*, *weight* and *nucleus*), had the lowest AIC (Table 2). All four parameters in model vii exerted a significant effect on the proportion of high quality pearls ($p < 0.05$, Table 3). The AIC of model vi in the second experiment, including all parameters (*PO*, *technician*, *SCS*, *weight* and *nucleus*), was the lowest (Table 4). Three parameters exerted a significant effect in model vi, post-operative care method, technician, and SCS ($p < 0.05$, Table 5). There was good correspondence between the measured and predicted values from the optimum models.

The results of the GLM analysis for the optimal models in experiment 1 and 2 are given in Table 3 and Table 5. Of the factors we evaluated, the post-operative care method had the most significant effect on the production of high quality pearls, followed by SCS. The effects of the post-operative care method on the

proportion of high quality pearls was >2 times than that of SCS (Tables 3 and 5). The adjusted odds ratios (hereafter, AORs) for the post-operative care method in the first and second experiments were 0.181 and 0.046, indicating that the proportion of high quality pearls produced by the low salinity treatment was 5.52 times and 21.74 times higher than for the conventional treatment, respectively. The AOR for the technician in the second experiment was 2.546, indicating that the proportion of high quality pearls produced by oysters handled by technician 2 was 2.55 times higher than technician 1. The AORs for SCS in experiment 1 and 2 were 0.419 and 0.385, indicating that the proportion of high quality pearls produced was 2.39 times and 2.60 times higher in oysters with an SCS of 2.0–4.9 kgf than in those with an SCS of 5.0–7.9 kgf. The AORs for the nucleus in the first and second experiments were 0.407 and 0.532, indicating that the proportion of high quality pearls produced was 2.46 times and 1.88 times higher when implanted with a large nucleus versus a small nucleus. The AORs for whole wet weight in the first and second experiments were 0.886 and 0.923, indicating that the proportion of high quality pearls decreased 1.13 times and 1.08 times for every 1 g increase in wet weight in each experiment, respectively. The wet weight ranged from 40 to 70 g in each experiment. The proportion of high quality pearls obtained from 40 g wet weight oysters was 38.17 times and 10.93 times higher than for 70 g wet weight oysters in experiments 1 and 2, respectively.

The proportion of high quality pearls was highest when a large nucleus was implanted into the gonad of host oysters that were 40–50 g wet weight in experiment 1 and 50–60 g wet weight in experiment 2.

4. Discussion

The post-operative care method and SCS had the most significant effect on the production of high quality pearls after 60 d of culture in Ago bay (Tables 3 and 5). We recommend that pearl farmers use the low salinity treatment post-operation and only select host oysters with an SCS of 2.0–4.9 kgf. These measures will yield higher numbers of high quality pearls after two months of culture in the sea relative to conventional procedures.

Recovery of host oysters in low salinity seawater resulted in a five-fold increase in the proportion of high quality pearls relative to the conventional treatment (Tables 3 and 5). This is consistent with results in our previous study using Japanese–Chinese hybrid pearl oysters (Atsumi et al., 2011). Thus, it is clear that the post-operative care in low salinity seawater increases the proportion of high quality pearls regardless of the host oyster strain.

In experiments 1 and 2, the proportion of high quality pearls produced by oysters with an SCS of 2.0–4.9 kgf was more than twice as high as for oysters with an SCS of 5.0–7.9 kgf (Tables 3 and 5). Our observations are consistent with those of our earlier study using Japanese–Chinese hybrid pearl oysters (Aoki et al. 2010a). Thus, culturists wanting to maximize production of high quality pearls should select host oysters with an SCS of 2.0–4.9 kgf, regardless of the host oyster strain.

The effect of the technician was minimal in our study. During the first experiment this parameter was excluded from the optimum model (Table 2). This is interesting as it is generally thought that the skill level of an experienced technician has a large effect on the production of high quality pearls (Nava et al., 2000).

Our observations suggest that the proportion of high quality pearls is negatively correlated with whole wet weight. A decrease in whole wet weight of 3.75 g was associated with a 1.6-fold increase in the proportion of high quality pearls produced in the first experiment (Table 3) and a 1.3-fold increase in the second experiment (Table 5). Prior studies suggest that the proportion of high quality pearls decreases when larger nuclei are implanted (Hasuo, 1961; Funakoshi et al., 1991). In contrast, our results indicate there is an optimal combination of whole wet weight of the host oyster and nucleus diameter at which the proportion of high quality pearls produced will be maximal. There is room for further investigation determining the optimal combinations of these factors.

Given the magnitude of effects of the post-operative care method and SCS, further attention should be given to understanding the mechanism by which pearl production is improved in the low salinity treatment and selection of host oysters.

Examination of a cross-section through the anomalies revealed black or brown inclusions located just above the nucleus surface at the affected site (Ogimura et al., 2012). There were large numbers of dead inflammatory cells within the cone-shaped extensions called ‘tails’ of the initial secreted layers in all the deformed pearls (Norton et al., 2000). The tails attached to many of the nuclei in the ‘dead’ space above the nucleus; i.e., the passageway made by the pearl knife. These observations indicate that almost all the deformities and blemishes are formed in the space adjacent to the nucleus during the early stage of pearl formation. Atsumi et al. (2011) noted that the production of high quality pearls after a typical period of culture was significantly higher at harvest (December–January) in oysters that immersed in 25 psu seawater than in the sea. Taken together with our current results, the improvements in the production of

high quality pearls from the low salinity treatment post-operation appear to be maintained throughout the culture period until harvest time. Thus, the effect of a given treatment on the formation of deformities and blemishes can be evaluated during the early stages of pearl formation. We did not grade the pearls recovered in our study to assess their market value, as did Norton et al. (2000). However, oysters should be cultured for more than six months after implantation if pearl attributes besides deformities and blemishes (e.g., luster, tint, surface perfection, and color) are to be evaluated.

Deformities and blemishes are due to accumulations of organic substances (e.g., hemolymph, inflammatory cells, germ cells, or bacteria) in the space between the pearl sac and the nucleus (Wada, 1999; Norton et al., 2000; Ogimura et al., 2012). There is an inflammatory reaction, manifested by a hemocytic infiltration resulting in black to brown pigmentation making contact with the nucleus (Norton et al., 2000; Ogimura et al., 2012), and leading to the periostracum layer and a fibrous aragonite-like formation at the accumulation site (Ogimura et al., 2012). Thus, the formation of deformities and blemishes is the result of a physiological reaction of the host oyster to the implantation process. Given this, we hypothesize that a decrease in the formation of blemishes and deformities results from suppression of these physiological responses immediately after implantation.

Immersion in low salinity seawater affects a number of physiological processes in oysters. For example, Alagarswami and Victor (1976) demonstrated that the filtration rate in Indian pearl oysters, *Pinctada fucata*, tended to decrease with a decrease in salinity. Furthermore, Wada (1969) noted that the crystalline style, an indicator of an individual physiology and health, was decreased in size or absent in Japanese pearl oysters, *Pinctada fucata*, held in diluted seawater (salinity 10.3–12.9 psu) for 57–114 h compared with those held in normal seawater (salinity 31.1–33.7 psu). In American oysters, *Crassostrea virginica*, acute changes in salinity affected the rate of hemocyte locomotion and prolonged the time of hemocyte spreading (Fisher and Newell, 1986). Characteristics of hemocyte of pearl oysters on acute changes in salinity are probably similar to those of American oysters.

The SCS tends to be positively correlated with the nutritional condition of the oysters (Aoki et al., 2010b). Thus, we assume that the physiological processes of host oysters with an SCS of 2.0–4.9 kgf is lower than for oysters with an SCS of 5.0–7.9 kgf. Host oysters are forced open during the implantation process and the ease of opening a shell is inversely correlated with the SCS. Thus shells that have a low SCS are easily opened and the nucleus is smoothly implanted into the gonad with a mantle graft. The ease

of opening a shell is therefore directly correlated with the production of higher quality pearls.

Taken together, these observations suggest that the decrease in deformities and blemishes following post-operative immersion in low salinity water is due to suppression of physiological processes in the oysters. Furthermore, host oysters with low physiological processes can be selected by measuring SCS. Interestingly, the use of relaxants to aid shell opening is associated with the production of significantly smaller high-quality pearls than from untreated oysters (Norton et al., 2000). This suggests that the effect of physiological processes suppression on pearl quality is not related to muscle relaxation pathways. Indeed, the mechanisms by which low salinity treatment and changes in SCS influence pearl quality remain unknown at present, but are worth evaluating in future. In addition, it is also interesting that adhesive use significantly reduces the prevalence of pearls with the tails (Norton et al., 2000).

We evaluated the effects of five factors on the production of blemish-free, round pearls. We hypothesize that improvements in pearl quality are due to a reduction in the accumulation of organic substances because of suppression of physiological processes immediately after implantation. In a follow-up study we compare the physiological processes of pearl oysters (e.g., pearl sac epithelium extension, time to hemocyte spreading, the rate of hemocyte locomotion, healing time after implantation) in oysters held in low salinity seawater and normal seawater. We also investigate the SCS of pearl oysters and the relationship between SCS and maturity or the rate of hemocyte locomotion.

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Table 1 Numbers of implanted oysters and harvest statistics by treatment

Experiment	Category	Numbers of total samples	Numbers of sample for variables												
			Post-operative care method		Shell-closing strength		technician		Nucleus diameter		Oyster whole wet weight (g)				
			The low salinity treatment	The conventional treatment	2.0-4.9 kgf	5.0-7.9 kgf	1	2	Large 7.73-7.77 mm	Small 6.36-6.40 mm	<40	40≤,<50	50≤,<60	60≤,<70	70≤
1st	Implanted oysters	966	492	474	350	616	486	480	487	479	0	66	606	291	3
	Harvested pearls	634	302	332	228	406	311	323	307	327	-	31	385	215	3
	High quality pearls	27	22	5	16	11	11	16	18	9	-	5	17	5	0
	^a Proportion of harvested pearls (%)	65.6%	61.4%	70.0%	65.1%	65.9%	64.0%	67.3%	63.0%	68.3%	-	47.0%	63.5%	73.9%	100.0%
	^b Proportion of high quality pearls (%)	4.3%	7.3%	1.5%	7.0%	2.7%	3.5%	5.0%	5.9%	2.8%	-	16.1%	4.4%	2.3%	0.0%
2nd	Implanted oysters	961	476	485	484	477	487	474	479	482	1	30	576	346	8
	Harvested pearls	474	210	264	244	230	207	267	246	228	0	5	269	193	7
	High quality pearls	27	25	2	19	8	8	19	18	9	0	0	21	6	0
	Proportion of harvested pearls (%)	49.3%	44.1%	54.4%	50.4%	48.2%	42.5%	56.3%	51.4%	47.3%	0.0%	16.7%	46.7%	55.8%	87.5%
	Proportion of high quality pearls (%)	5.7%	11.9%	0.8%	7.8%	3.5%	3.9%	7.1%	7.3%	3.9%	0.0%	0.0%	7.8%	3.1%	0.0%

a) represents the total number of pearls harvested divided by the number of oysters that were implanted.

b) represents the number of high quality pearls divided by the total number of pearls harvested.

Table 2 Estimates of the variables and Akaike information criteria (AIC) values for each model in the first experiment

^a Model	Estimate of the variables						^d AIC
	β_0 [<i>Intercept</i>]	β_1 [^b <i>PO</i> ‘the conventional treatment’ (relative to ‘the low salinity treatment’)]	β_2 [<i>technician</i> ‘technician 2’ (relative to ‘technician 1’)]	β_3 [^c <i>SCS</i> ‘5.0–7.9kgf’ (relative to ‘2.0–4.9kgf’)]	β_4 [<i>weight</i>]	β_5 [<i>nucleus</i> ‘small’ (relative to ‘large’)]	
i	-2.544	-1.637					213.5
ii	-3.306		0.352				226.5
iii	-2.584			-0.997			221.0
iv	3.929				-0.125		218.0
v	-2.776					-0.789	223.5
vi	4.990	-1.721	0.523	-0.874	-0.123	-0.953	201.4
vii	5.143	-1.708	ns	-0.870	-0.121	-0.900	201.0

a) Model i-v represent simple models each containing a single variable; Model vi represents a multiple model that incorporates all variables; Model vii represents an adjusted model that incorporates all variables except "technician". b) *PO* = post-operative care. c) *SCS* = shell closing strength. d) AIC = Akaike information criteria. Blanks indicate the variables was not selected.

Table 3 Estimate, standard error (S.E.), p value, and adjusted odds ratio (AOR) for each of the factors in the optimum model in the first experiment

Variable	Estimate	S.E.	p value	AOR
β_0 [<i>Intercept</i>]	5.143	2.369	0.030	
β_1 [<i>PO</i> ‘the conventional treatment’ (relative to ‘the low salinity treatment’)]	-1.708	0.511	0.001	0.181
β_3 [<i>SCS</i> ‘5.0–7.9kgf’ (relative to ‘2.0–4.9kgf’)]	-0.870	0.417	0.037	0.419
β_4 [<i>weight</i>]	-0.121	0.043	0.004	0.886
β_5 [<i>nucleus</i> ‘small’ (relative to ‘large’)]	-0.900	0.431	0.037	0.407

Table 4 Estimates of the variables and Akaike information criteria (AIC) values for each model in the second experiment

^a Model	Estimate of the variables						^d AIC
	β_0 [<i>Intercept</i>]	β_1 [^b <i>PO</i> ‘the conventional treatment’ (relative to ‘the low salinity treatment’)]	β_2 [<i>technician</i> ‘technician 2’ (relative to ‘technician 1’)]	β_3 [^c <i>SCS</i> ‘5.0–7.9kgf’ (relative to ‘2.0–4.9kgf’)]	β_4 [<i>weight</i>]	β_5 [<i>nucleus</i> ‘small’ (relative to ‘large’)]	
i	-2.002	-2.874					180.8
ii	-3.214		0.645				208.8
iii	-2.472			-0.852			206.9
iv	0.438				-0.056		209.6
v	-2.539					-0.653	208.6
vi	2.747	-3.085	0.934	-0.956	-0.080	-0.631	175.4

a) Model i-v represent simple models each containing a single variable; Model vi indicates the multiple model obtained using all variables and the adjusted model. b) *PO* = post-operative care. c) *SCS* = shell closing strength. d) AIC = Akaike information criteria. Blanks indicate the variables was not selected.

Table 5 Estimate, standard error (S.E.), p value, and adjusted odds ratio (AOR) for each of the factors in the optimum model in the second experiment

Variable	Estimate	S.E.	p value	AOR
β_0 [<i>Intercept</i>]	2.747	3.000	0.360	
β_1 [<i>PO</i> ‘the conventional treatment’ (relative to ‘the low salinity treatment’)]	-3.085	0.753	0.000	0.046
β_2 [<i>technician</i> ‘technician 2’ (relative to ‘technician 1’)]	0.934	0.463	0.043	2.546
β_3 [<i>SCS</i> ‘5.0–7.9kgf’ (relative to ‘2.0–4.9kgf’)]	-0.956	0.453	0.035	0.385
β_4 [<i>weight</i>]	-0.080	0.052	0.128	0.923
β_5 [<i>nucleus</i> ‘small’ (relative to ‘large’)]	-0.631	0.442	0.154	0.532

Fig. 1 Outline of experimental procedure from the pre-operative conditioning to the post-operative care in this study. Numbers of oysters implanted were indicated how all groups were split up in each experiment.

Fig. 2 Criteria for designation of high quality pearls. Only round, nacreous pearls with no blemishes or just one blemish that was $<0.5\%$ of the pearl surface (Solid square) were designated as being of high quality pearls.