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● **附：论文报告会获奖名单**

Healing and regeneration of the freshwater pearl mussel *Hyriopsis cumingii* Lea after donating mantle saibos

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Abstract: After excision of mantle tissue from *Hyriopsis cumingii* Lea, survival rates, specific growth rate, healing and regeneration were observed and quantified for three months. High survival rates (94.7%, 92%, 95.3% and 94.7%, respectively) of mussels with small (8–12 mm × 8–12 mm) (S-group)excision taken from the front, middle and back of the ventral mantle margin, and control mussels were observed, and no significant difference was observed among the four groups. The specific growth rates, SGR (length), SGR (thickness) and SGR (weight) of *H. cumingii* with different excision locations did not differ significantly but the SGR (height) of the group with the front excision was significantly lower than that of the other excised groups and controls. Mussels were excised with small, medium (8–12 mm × 28–32 mm) (M-group) or large (8–12 mm × 48–52 mm) (L-group) from the middle ventral mantle margin. The survival rates of mussels with S-, M- and L-group and controls were, respectively, 92%, 90%, 90.7% and 94.7%. The survival rate of the L-group was significantly lower than that of the other three groups. The trends in SGR (length), SGR (height), SGR (thickness) and SGR (weight) among the four groups were similar: controls> S-group> M-group>L-group. Macroscopic observation of mantle tissue showed that the wound area became reduced since 12th d after the excision. To grow to the same level as the surrounding normal tissue, the regenerated tissue needed 40 d, 75 d and 90 d, respectively, in the S-, M-, and L-group. Histological observation of wound tissues showed that haemocytes aggregated at the wound site soon after excision and completely sealed the wound after 12 h. Epithelialization sealed the wound completely after 72 h. The three mantle lobes began to form at 15 d. The regenerated lobes were morphologically different from normal tissues after 25 d but were identical to the normal mantle lobes, in both morphological structure and function, by 90 d. This is the first report on the survival rate, growth rate, wound healing, and regeneration in the freshwater pearl mussel after excision of its mantle tissue. The study demonstrates that donor mussels, without being killed, may donate mantle pieces (saibos) for evaluating the effect of donor mussel on pearl quality. Donor mussels contributing to pearl quality, this provides support for selective breeding of donor mussels in the future.

Keywords: Freshwater pearl mussel; Regeneration; Mantle tissue; Selective breeding; Pearl quality

Abbreviations: SGR (length), specific growth rate of shell length; SGR (height), specific growth rate of shell height; SGR (thickness), specific growth rate of shell thickness; SGR (weight), specific growth rate of body weight.

1. Introduction

Hyriopsis cumingii Lea, which is the most important freshwater pearl mussel in China, produces pearls of high quality[1-2]. The annual output of freshwater pearls from China of 1800 tonnes accounts for more than

95% of world production, of which more than 90% is produced by *H. cumingii*[3]. *H. cumingii* is mainly used to produce non-nucleated pearls through an artificial graft operation. Donor mussels are killed, mantle tissue is removed, and the tissue is divided into a number of small pieces (saibos), which are then grafted into host mussel mantles[4]. The donor mussels have a great effect on pearl colour and quality[5-7]. Therefore, if mantle tissue could be removed and saibos prepared without killing the donor mussels, the donors and hosts could be individually marked[8] so that during harvest each pearl could be traced back to the donor mussel that produced it. In this way, donor mussels producing high-quality pearls could be used in selective breeding.

Regeneration is an important physiological phenomenon in animals[9-12]. There have been a number of studies of regeneration in marine shellfish, including marine pearl oysters[13-15]. *P. fucata martensii*[16], *P. margaritifera*[17] and *P. maxima*[18] were pretreated with a chemical relaxant[19-22], the mantle tissue of which were excised and, after breeding for differing periods, the survival rates, healing and regeneration of wound sites of the oysters were observed histologically. However, it is unknown whether excisions of different sizes, or from different mantle locations, affect survival and growth rates of either marine or freshwater pearl oysters.

In this paper, we report survival rates and growth rates of the freshwater pearl mussel *H. cumingii* after mantle tissue excisions of different sizes and at different locations. Healing and regeneration processes in the mantle tissue were observed by histological methods to provide reference data for selective breeding of donor *H. cumingii*.

2. Material and methods

2.1. Mussels

The experiment was carried out in mid-April 2012. One-year-old *H. cumingii* were produced by artificial culture in Shuangpai Town, Jinhua City, Zhejiang Province, China and transferred to a pond in a freshwater pearl mussel farm in Tangxi Town, Jinhua City two weeks before the experiment.

2.2. Mantle excision, mussels culture and observation

For excision of mantle tissue, the shells of the live *H. cumingii* were prised apart to a width of 1cm using a special shell opener and propped open with a metal spacer. A rectangular piece of mantle tissue was excised from the ventral margin of the mussel with a sterilized #11 scalpel and removed with forceps (Fig. 1), completing these operations within 10 s. The spacer was removed and treated mussels were placed into a bath containing 0.05 g/L chlortetracycline hydrochloride for 2 h, followed by suspended cage culture (cage: 46×46×12 cm) in the pond.

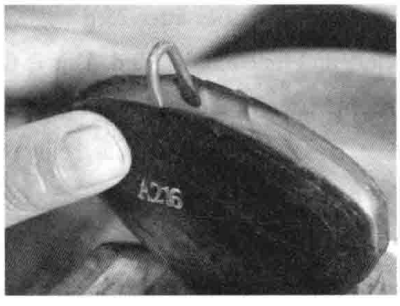

Fig. 1. Opening of the valves of a living *H. cumingii* for excision of mantle tissue.

Two experiments were designed to investigate the effect of the locations and sizes of the excision. The first examined the influence of excision of mantle tissue from different locations on the survival rates and growth rates of mussels. A total of 450 mussels were randomly selected and the initial lengths, heights and thicknesses of the shells were measured (± 0.01 cm) with a vernier calliper[23]. Their body weights were measured (± 0.01 g) as soon as they were taken out of water with an electronic balance. A rectangular piece of

mantle tissue (8–12mm × 8–12mm) was cut from the front of the ventral margin of the mantle of the left shell of 150 of the mussels (Fig. 2). They were then placed into three cages (50 in each cage) as three replicate groups. Ninety days after treatment, the shell lengths, heights and thicknesses, and the body weights, were again measured to calculate the survival rates and specific growth rates of the three replicates. A second and a third set, each of 150 mussels were treated similarly but making the excision at the middle and the back, respectively, of the ventral margin (Fig. 2). A further control group of 150 mussels was randomly selected, placed in the medicated bath for 2 h, divided among three cages with 50 in each, and cultured in the same pond for calculation of the survival rates and specific growth rates of unexcised mussels on completion of the experiment.

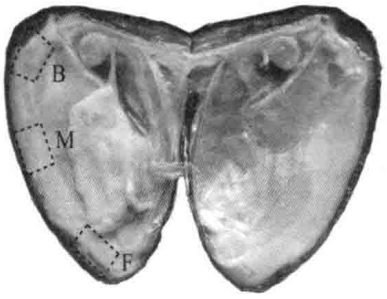

Fig. 2. Opened *H. cumingii* showing the locations of the mantle tissue excisions. B: back; F: front: M: middle of the ventral margin of the mantle of the left shell.

A second experiment examined the influence of different sizes of the pieces of excised mantle tissue on the survival rates and growth rates of *H. cumingii*. Another 450 mussels were randomly selected and measured as before. A rectangular piece of mantle tissue measuring 8–12 mm × 8–12 mm was cut from the middle of the ventral margin of 150 of the mussels (referred to as the S-group). In a second group of 150 mussels, a rectangular mantle piece measuring 28–32 mm × 8–12 mm was cut from the same location (the M-group), and in a third group of 150, a rectangular mantle piece of 48–52 mm × 8–12mm was removed (L-group). Each group of 150 mussels was divided into three replicate cages of 50 mussels, which were cultured under similar conditions. After 90 d, the shell lengths, heights and thicknesses, and body weights, were measured and the survival rates and specific growth rates were calculated for each replicate set. A control group with 150 mussels was treated like described in the first experiment.

The time course of tissue healing and regeneration in *H. cumingii* following excision of mantle tissue was observed in 100 randomly selected mussels. A rectangular mantle measuring 8–12mm × 8–12mm was excised from the middle of the ventral margin of the mantle of the left shell of each of the mussels, which were then medicated and cultured in the pond as described. To observe wound healing and regeneration, three mussels were killed and photographed 3, 6, 9, 12, 15, 20, 25, 40, 60, 75 and 90 d after the operation. For histological examination, three randomly selected mussels were removed after 1, 3, 6, 12, 24, 36, 48 and 72 h, and after 4, 6, 9, 12, 15, 20, 25, 40, 60, 75 and 90 d following the treatment. Portions of the wound tissues (1 cm^2) were cut off with surgical scissors from mantle tissue, and then immediately fixed in Bouin's solution for 48 h, dehydrated in 70% ethanol for 24h and an ethanol gradient (80% ethanol for 1h, 95% for 0.5h, another 95% for 0.5h, 100%for 0.5h and another 100% for 0.5h), cleared in xylene (mixed solution of 50% ethanol and 50% xylene for 0.5h, 100% xylene for 20min and another 100% xylene for 20min), and embedded in paraffin. Sections (7 μm) were stained using a standard HE method, and observed and photographed with a microscope[24].

2.3 Calculation and statistical methods

Specific growth rates (SGR, % day^{-1}) were calculated using the formulae:

SGR (length) =100 ($\ln L_2$-$\ln L_1$) / t;

SGR (thickness) =100($\ln W_2$-$\ln W_1$) / t;

SGR (height) =100($\ln H_2$-$\ln H_1$) / t;

SGR (weight) =100($\ln w_2$-$\ln w_1$) / t,

where L_1, W_1 , H_1 and w_1 are the mean shell lengths, shell heights, shell thicknesses and body weights, respectively, at the beginning of the experimental period; L_2, W_2 , H_2 and w_2 are the corresponding measurements at the end of the experimental period; and t is the duration of the experiment (days).

Dependent variables were tested for normality by exploratory analysis and descriptive statistics before statistical analysis of survival rates and growth rates using SPSS 17.0. Following one-way ANOVA, the LSD method was used for multiple comparisons. Values of $p<0.05$ and $p<0.01$ were considered to represent significant and highly significant differences, respectively.

3. Results

3.1 Effect of the location of excision of mantle tissue on the survival rate and growth rate of *H. cumingii*

The mean survival rates after making excisions from the front, middle and back regions of the mantle, and in the control group, were, respectively, 94.7%, 92%, 95.3% and 94.7%, and were not significantly different from each other (Fig. 3). The shell lengths, shell heights, shell thicknesses and body weights were not significantly different (p>0.05) among the four groups prior to excision (Table 1). Following excision, SGR (length), SGR (thickness) and SGR (weight) were also not significantly different among the four groups. However, the SGR (height) of the group with the front excision was significantly lower ($p<0.01$) than that of the other two experimental groups and the control group, which did not differ significantly.

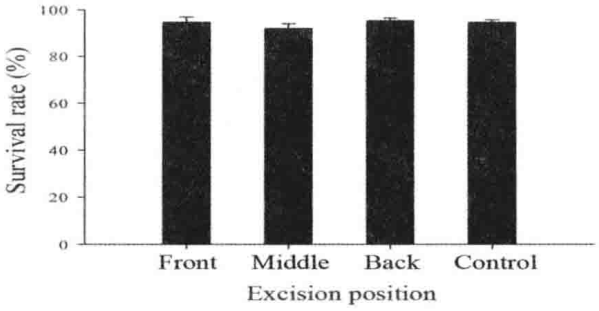

Fig. 3. Survival rates of *Hyriopsis cumingii* after excision of mantle tissue at different locations. All mean values are statistically similar ($p> 0.05$).

Data represent means ± S.D. of three replicates. Values in the same row with different uppercase superscript letters are highly significantly different ($p<0.01$).

Table 1. Growth rates of *H. cumingii* after excision of mantle tissue at different locations.

Location of excision	Initial shell length (cm)	SGR (length) (% day^{-1})	Initial shell thickness (cm)	SGR (thickness) (% day^{-1})	Initial shell height (cm)	SGR (height) day^{-1})	Initial body (% weight (g)	SGR (weight) (% day^{-1})
Front	8.68±0.09	0.18±0.01	1.98±0.15	0.20±0.01	4.13±0.31	0.14±0.01^{A}	54.57±1.34	0.57±0.01
Middle	8.66±0.01	0.17±0.01	1.97±0.01	0.18±0.01	4.10±0.23	0.18±0.02^{B}	55.60±0.14	0.54±0.01
Back	8.71±0.07	0.16±0.01	1.99±0.01	0.20±0.01	4.14±0.20	0.19±0.03^{B}	55.00±0.18	0.55±0.02
Control	8.67±0.42	0.18±0.01	1.98±0.12	0.19±0.02	4.10±0.21	0.19±0.02^{B}	54.83±0.35	0.56±0.04

3.2 Effect of different sizes of mantle tissue excisions on survival rates and growth rates

The survival rates of the S-group, M-group and L-group, and the control group were, respectively, 92%, 90%, 90.7% and 94.7%. The survival rate of the L-group was highly significantly lower than that of the other three groups, which did not differ significantly ($p>0.05$) from each other (Fig. 4). Prior to treatment, the shell lengths, heights and thicknesses, and body weights, were not significantly different among the four groups (Table 2). Following the treatments, SGR (length), SGR (height), SGR (thickness) and SGR (weight) exhibited a similar trend in the four groups, i.e., control group> S-group> M-group> L-group. SGR (length), SGR (thickness) and SGR (weight) in the L-group and M-group were highly significantly different from the corresponding rates in the S-group and the control group. SGR (length), SGR (thickness) and SGR (weight) in the M-group were not significantly different from those in the L-group. The SGR (height) in the L-group was highly significantly different from that in the other three groups.

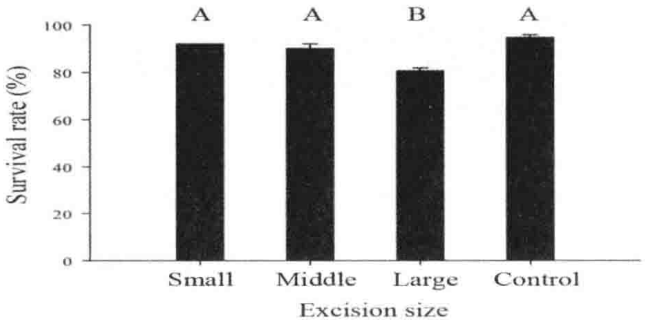

Fig. 4: Survival rates of *Hyriopsis cumingii* after excision of mantle tissue of different sizes. Values with different uppercase superscript letters are highly significantly different ($p< 0.01$).

Table 2. Growth rates of *H. cumingii* after excisions of mantle tissue of different sizes.
Data represent mean ± S.D. of three replicates. Values in the same column with different uppercase superscript letters are highly significantly different ($p<0.01$).

Size of excision	Initial shell length (cm)	SGR (length) (% day^{-1})	Initial shell thickness (cm)	SGR (thickness) (% day^{-1})	Initial shell height (cm)	SGR (height) (% day^{-1})	Initial body weight (g)	SGR (weight) (% day^{-1})
Small	8.88±0.35	0.16±0.01^{A}	2.03±0.01	0.17±0.01^{A}	4.18±0.05	0.18±0.02^{AB}	59.71±0.43	0.52±0.01^{A}
Medium	8.86±0.15	0.12±0.01^{B}	2.05±0.01	0.11±0.01^{B}	4.18±0.06	0.14±0.01^{A}	59.82±1.60	0.36±0.04^{B}
Large	8.89±0.52	0.10±0.02^{B}	2.06±0.03	0.10±0.01^{B}	4.23±0.03	0.10±0.03^{C}	60.40±1.26	0.30±0.03^{B}

3.3 Observation of tissue healing and regeneration at different time after excision of mantle tissue.

The time course of tissue healing and regeneration in *H. cumingii* following excision of a small-sized piece of mantle tissue is illustrated in Fig. 5. Three days after excision (Fig. 5A), visual observation showed little change in the wound, except for a yellow/brown colouration at the wound edge. On the sixth (Fig. 5B) and ninth days (Fig. 5C), this colouration became more conspicuous. After 9 d, the wound size was not significantly diminished but careful observation revealed that it was starting to grow inwards from both sides of the wound by the secretion of a thin layer. After 12 d (Fig. 5D), freshly regenerated tissue could be clearly seen and the area of the wound had diminished. Between 15 d (Fig. 5E) and 25 d (Fig. 5F), regenerated tissue gradually grew towards the ventral margin and, by 40 d (Fig. 5G), the regenerated tissues had almost reached the same level as the normal tissue. However, the background colour was deeper at the wound site and a new

layer of nacre had been laid down on the inner shell at the site of the regenerated tissue. Between 60 d (Fig. 5H) and 90 d (Fig. 5I), the background colour of the wound became progressively lighter and by 90 d, the colour of the regenerated tissue was little different from that of the normal tissue.

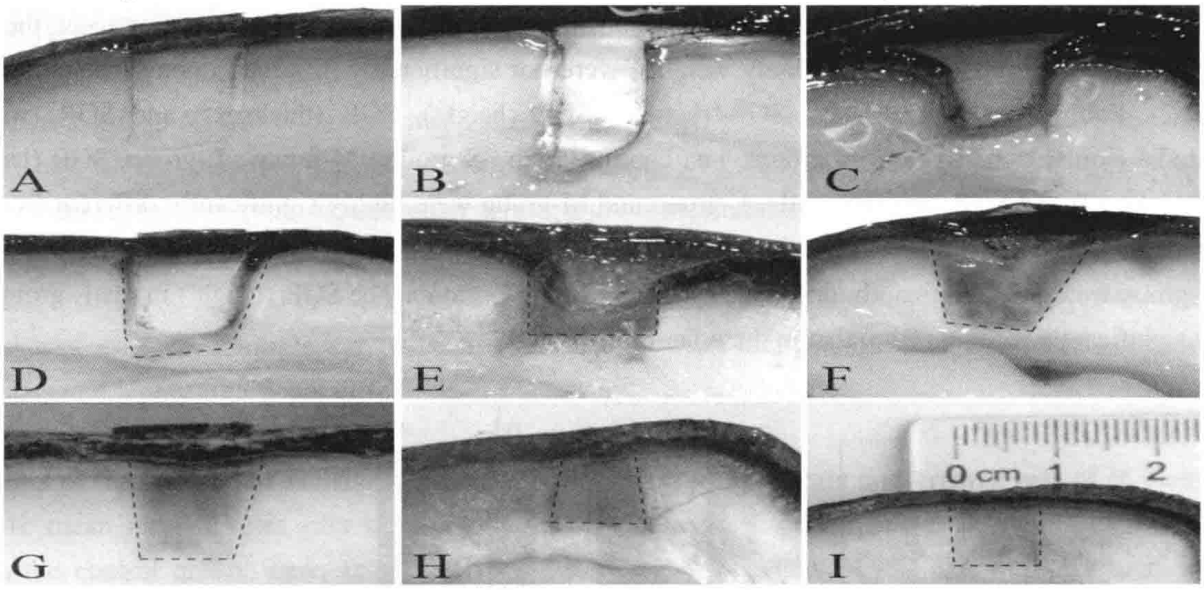

Fig. 5. Tissue healing and regeneration in *H. cumingii* after excision of a small-sized piece of the mantle. A: 3 d; B: 6 d; C: 9 d; D: 12 d; E: 15 d; F: 25 d; G: 40 d; H: 60 d; I: 90 d after excision. The dotted line indicates the excision site.

Healing and regeneration after excision of medium- and large-sized pieces of mantle tissue are illustrated in Fig. 6 and Fig. 7. After 25 d, regenerated tissue was evident in both excision groups (Fig. 6A, Fig. 7A). Between 40 d and 60 d, regeneration and recovery was faster in the M-group (Fig. 6B-C) than in the L-group (Fig. 7B-C), but both were slower than in the S-group over the same period. In the M-group, recovery and regeneration of the wound to the normal tissue level had occurred after 75 d (Fig. 6D), and its background colour was almost the same as that of normal tissues. In the L-group, this level of recovery was not observed until 90 d (Fig. 7D-E).

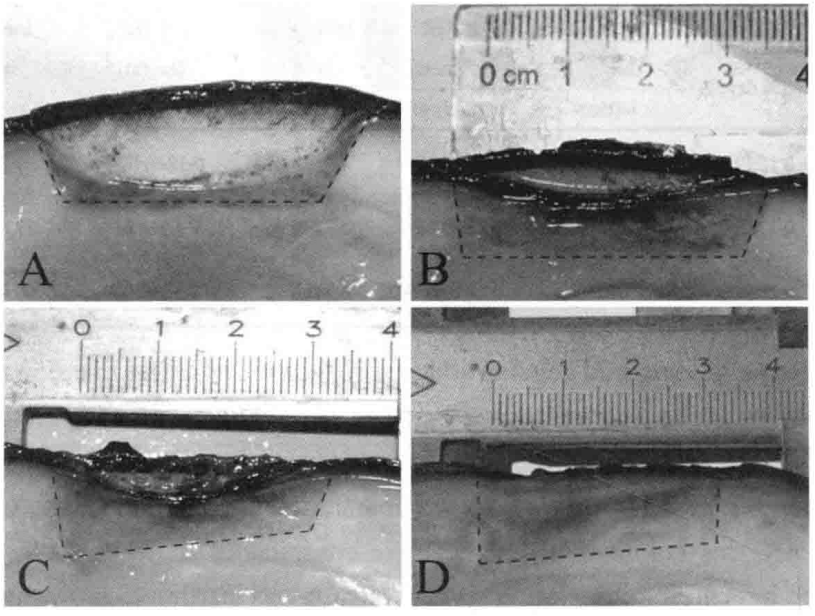

Fig. 6. Tissue healing and regeneration in *H. cumingii* after excision of a medium-sized piece of the mantle. A: 25 d; B: 40 d; C: 60 d; D: 75 d after excision. The dotted line indicates the excision site.

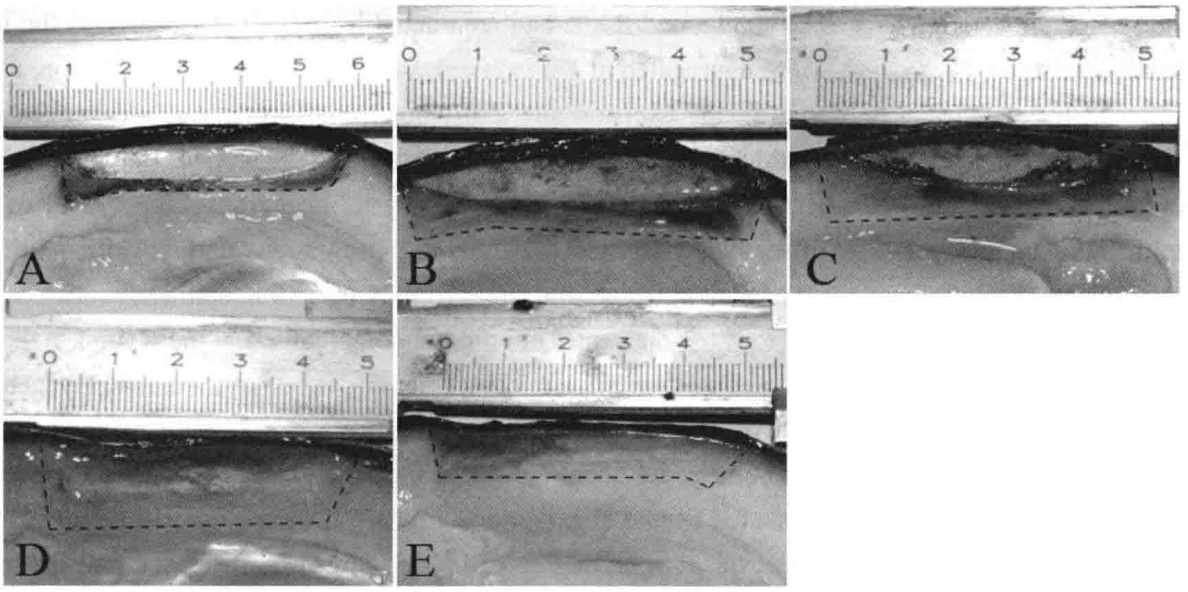

Fig. 7. Tissue healing and regeneration in *H. cumingii* after excision of a large-sized piece of the mantle. A: 25 d; B: 40 d; C: 60 d; D: 75 d; E: 90 d after excision. The dotted line indicates the excision site.

Histologically, the normal (non-regenerated in the absence of an excision) mantle tissue was divided into central, pallial and marginal zones (Fig. 8A). At the ventral margin, there were three obvious lobes, the outer, middle, and inner folds (Fig. 8B)[25]. The outer fold adjacent to the shell had the largest area, length and width at its base. Connective tissue and a little muscle were distributed within the outer fold and its epithelium stained deeply. The middle fold was longer than the inner fold and narrower at the base than the inner fold. Conchiolin secretion was observed in the periostracal groove[26] at the interface between the middle fold and outer fold. The inner fold was the shortest, lancet-shaped in section, and was rich in muscle tissue. Many basophilic secretory cells, blue-stained in HE preparations, were present in the middle and inner folds. Longitudinal and radial muscles were concentrated in the pallial zone near to the inner fold, while loose and compact connective tissues occupied the rest of this zone. The central zone was rich in connective tissue with a few muscle fibres. Adjacent to the shell, the external epithelium was deeply stained and folded into ridges. The internal epithelium on the visceral side was rich in glands associated with secretions. A thick bundle of longitudinal muscle fibre traversed the mantle close to the internal epidermis, alongside dense radial muscles. The annular blood sinus could be clearly observed in the central zone.

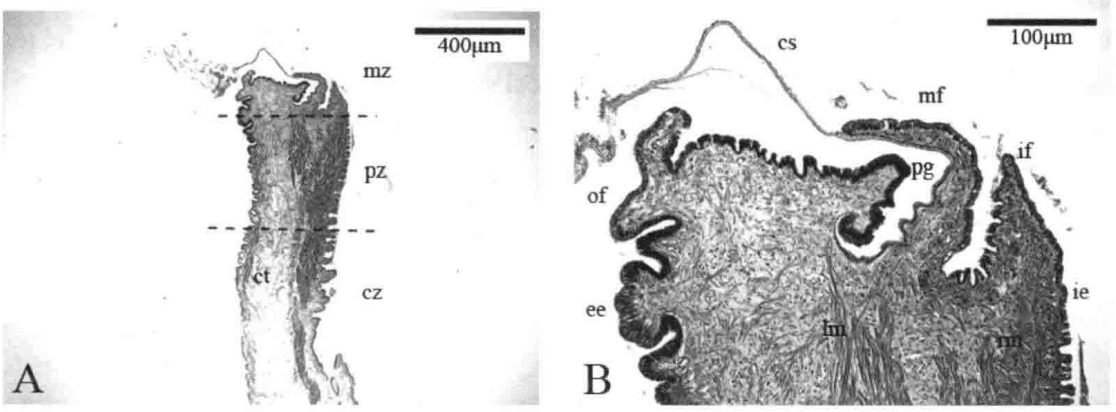

Fig. 8. Histological views of the mantle sectioned through the ventral margin in *H. cumingii*. A: normal tissues without excision treatment; B: enlarged partial view of A; cs: conchiolin secretion; ct: connective tissue; cz: central zone; ee: external epithelium; ie: internal epithelium; if: inner fold; rm: radial muscles; lm: longitudinal muscles; mf: middle fold; mz: marginal zone; of: outer fold; pg: periostracal groove; pz: pallial zone.

One hour after excision (Fig. 9A), the wound tissue had contracted from both sides towards the middle and a small number of haemocytes were aggregated at the excision site. Three hours after excision, a larger aggregation of haemocytes was present at the excision site and wound contraction was weaker (Fig. 9B). Between 3 h and 12 h, haemocytes continued to aggregate, completely sealing the wound. Between 12 h (Fig. 9C) and 24 h (Fig. 9D) after excision, haemocytes continued to aggregate, forming a distinct layer of blood cells; epithelial cells on both sides of the wound started to extend and grow towards the centre; and freshly generated pink eosinophilic cells were present at the wound site. From 24 h to 36 h, epithelial cells continued to extend from both sides of the wound towards the centre but did not completely seal the wound. After 36 h, freshly generated muscle fibres were seen (Fig. 9E). By 72 h (Fig. 9F) after excision, newly generated epithelial cells completely sealed the wound, and the newly generated epithelial cell possessed oval and cylindrical nuclei, similar to those of columnar epithelial cells, although they had not attained the columnar form. On the fourth day (Fig. 9G), the healed wound began to invaginate, and epithelial cells assumed a more regular shape. Six days (Fig. 9H) after excision, the degree of invagination was increased and the annular blood sinus was visible. After 12 d, the three lobes forming by invagination could be identified (Fig. 10A). On day 15, the outer, middle, and inner folds were obvious (Fig. 10B). The inner fold was the longest at this time; the outer fold was shortest and its top was split into a number of branches. Conchiolin secretion was present in the groove between the middle fold and outer fold. A number of blue-stained oval glands arose in the middle and inner folds near their boundary with each other (Fig. 10C). Twenty-five days (Fig. 10D) after excision, the outer fold had expanded in length and area and contained a few longitudinal muscles and intensely stained columnar epithelial cells with long thin nuclei. The base of the outer fold formed a J-shaped lobe adjacent to the middle fold; in the root of this lobe, irregularly shaped epithelial cells with small oval flat nuclei were formed where conchiolin secretion commenced. The inner fold became the largest in width, and was rich in longitudinal muscles. Epithelial cells in the inner and middle folds were more lightly stained than those in the outer fold, and the columnar epithelial cell nuclei were oval. The epithelial cells in the middle fold adjacent to the outer fold were markedly different in shape from those on the inner fold side, possessing small irregularly shaped nuclei and little cytoplasm. In contrast, the epithelial cells of the middle fold adjacent to the inner fold side were similar to those of the inner fold; i.e., the nuclei of these columnar cells were regularly arranged with secretory goblet cells between the cells and abundant blue-stained glands below the epidermis. From 25 d to 90 d (Fig. 10D-G), the length, base width, and area of the outer fold gradually grew to become the largest of the three lobes. The J-shaped lobe was developed further and enlarged, and conchiolin secretion was seen in the groove at the root of this lobe. The middle fold was developed to become shorter than the outer fold and longer than the inner fold, and with its base narrower than that of the inner fold. After 90 d, the regenerated tissues were similar to normal tissues in both morphological structure and function (Fig. 10H).

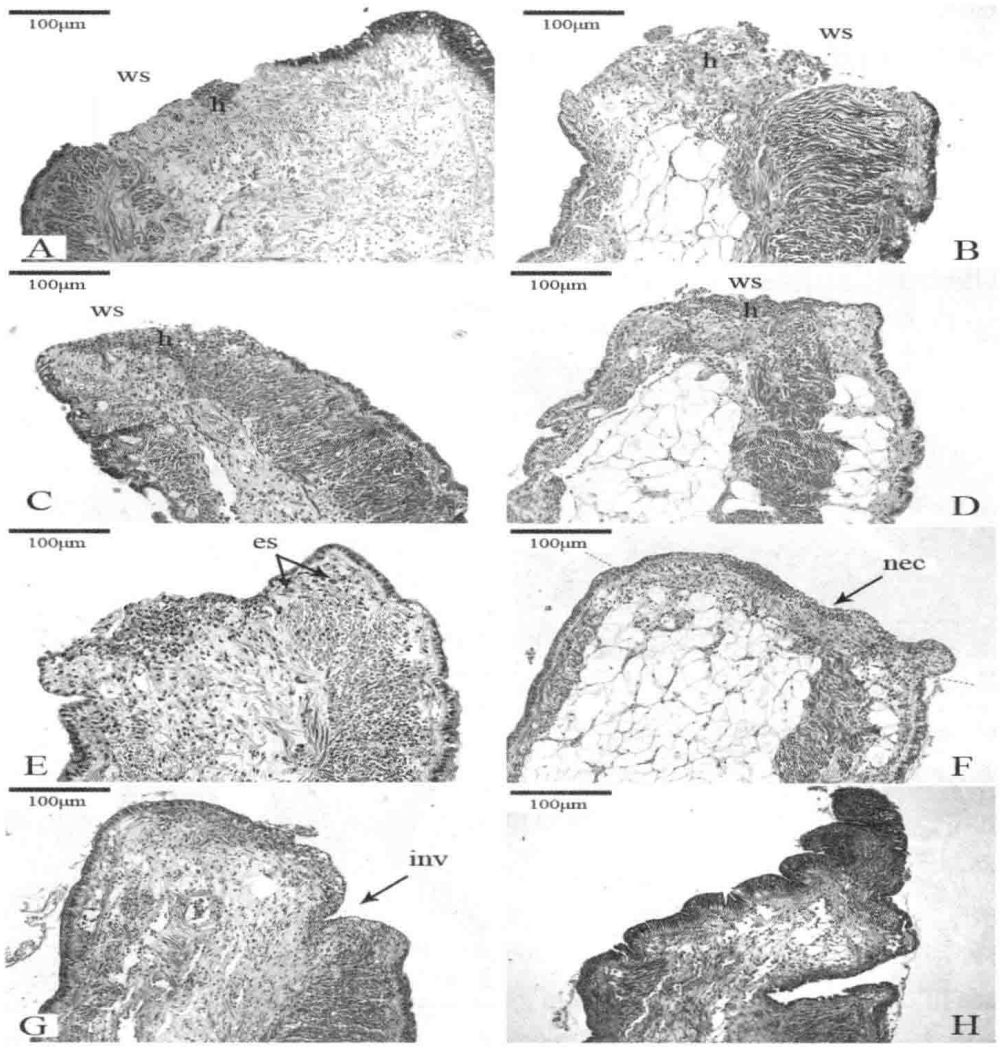

Fig. 9. Histological views of regenerated mantle sections in *H. cumingii* at A: 1 h; B: 3 h; C: 12 h; D: 24 h; E: 36 h; F: 72 h; G: 4 d; H: 6 d after excision. es: eosinophilic cells; h: haemocytes; inv: invagination; nec: newly generated epithelial cells; ws: wound site. The dotted line in F indicates the initial wound site.

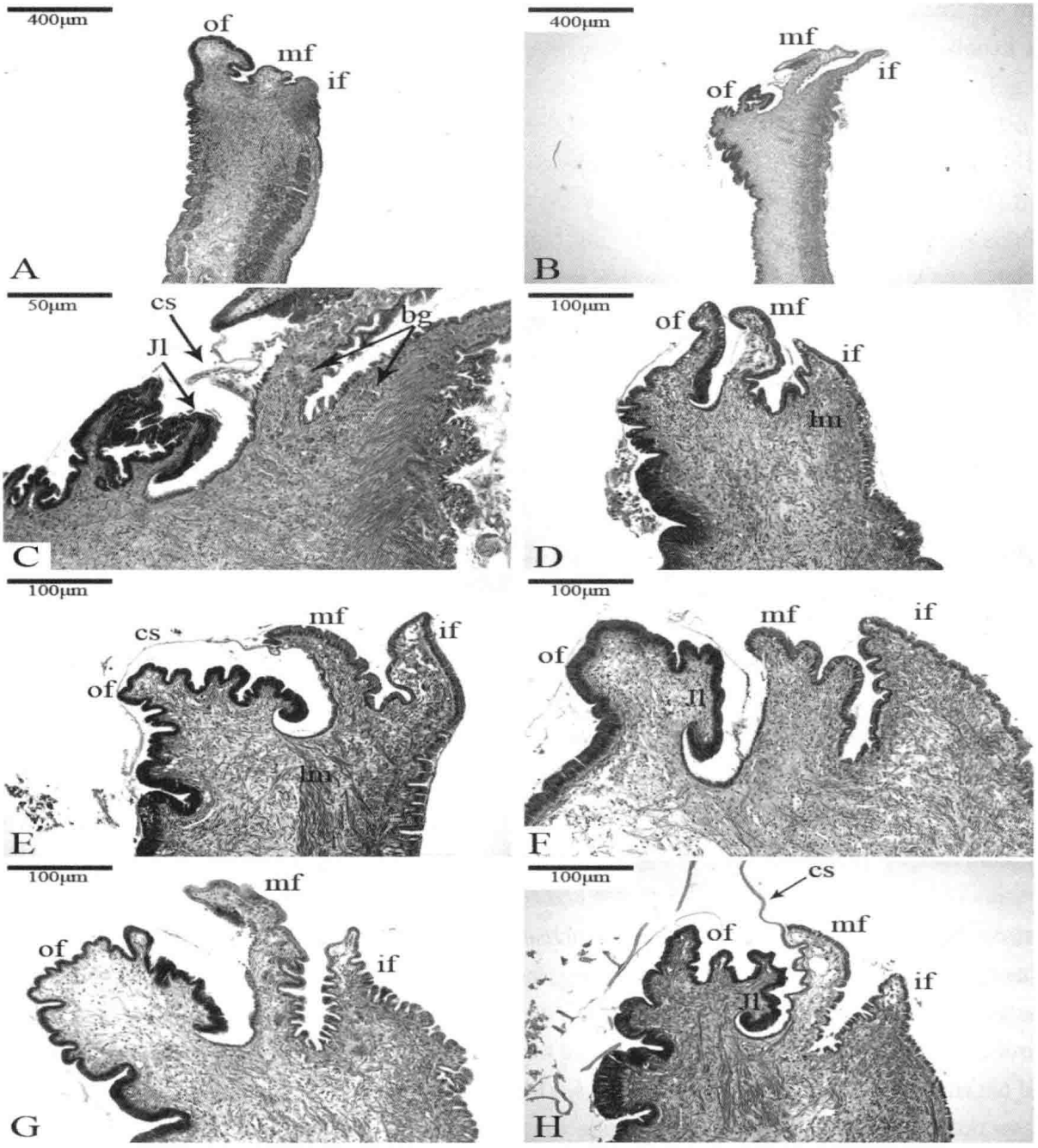

Fig. 10. Histological views of regenerated mantle sections in *H. cumingii* at A: 12 d; B: 15 d; C: enlarged partial view of B; D: 25 d; E: 40 d; F: 60 d; G: 75 d; H: 90d after excision. cs: conchiolin secretion; bg: blue-stained glands; if: inner fold; Jl: J-shaped lobe; lm: longitudinal muscles; mf: middle fold; of: outer fold.

4. Discussion

The influence of mantle saibos on pearl quality has been reported in studies of pearl cultivation in both marine[5-6, 27]and freshwater[7] species. Acosta-Salmón studied survival rates of *Pinctada fucata* after excision of mantle tissue from live oysters and proposed a new approach to pearl oyster broodstock, as follows: 1) cut mantle saibos from living donor oysters for pearl culture during grafting; 2) culture donor oysters after excision treatment for survival; 3) select donor oysters producing high-quality pearls as the broodstock for future selective breeding[16]. During the seawater pearl culture, usually only one (for *Pinctada maxima* and *Pinctada margaritifera*) or a few pearl nuclei (for *Pinctada martensii*) are grafted into each host oyster[28-29] so it is very difficult to evaluate whether the high-quality pearl is attributed to the host oyster or donor oyster. However, during freshwater pearl culture with *H. cumingii*, typically 30 to 40 mantle saibos are excised from

one donor mussel and 26 to 32 saibos are grafted into one host mussel. Thus, it is more accurate to evaluate the influence of donor mussels on pearl quality using more donated saibos from the same donor mussel. Since larger size excision of mantle tissue could provide more saibos, we carried out the research about different excision sizes. Moreover, it is considered that the locations of mantle used for preparing saibos of donor mussels have an effect on pearl quality, so we conducted the experiment about different excision locations.

This is the first report on the survival of freshwater pearl mussels after excising mantle tissue from their living bodies. The survival rates of *H. cumingii* cultured for 3 months after excision of small-sized pieces of mantle tissue (8–12 mm × 8–12 mm) at three different locations were not significantly different from those of unoperated mussels. Medium-sized medial excisions were similarly non-lethal. However, survival after large-medial excisions was significantly impaired, showing that mussel viability was affected by mantle wound size but not its location. Such viability may allow *H. cumingii*, after taking mantle saibos from living mussels, to survive for a long period for the future use as described above. Besides, *H. cumingii* used as donor mussel could also be used as a host mussel after recovery for 3 months to determine whether donor mussels that produce high-quality pearls also produce high-quality pearls when used as hosts. The survival of pearl oysters is affected by many factors, including disease, culture conditions, and postoperative disinfection[28, 30]. Which period is the crucial period for death after excision and how to reduce the mortality of pearl oysters after taking saibos from live bodies is worthy of further study.

The excision treatment influenced the growth of *H. cumingii*. While the excision of 8–12mm × 8–12mm pieces at different mantle locations did not affect growth of shell length, shell thickness or weight, excision of the front mantle tissue did retard growth in shell height. Presumably, this is because the front excision location is located at one of the points from which shell height growth takes place, and excising mantle with epithelial cells, which are responsible for shell growth and nacre secretion, slows down the shell formation at this location. Modifying the size of excisions had significant effects on the speed of growth in shell length, shell height, shell thickness and body weight. In each case, the order of growth rates was: L-group< M-group< S-group< control group. The bivalve mantle functions as an energy reserve[31-32]. Therefore, excision of more mantle tissue would be expected to have a greater influence on the pearl oyster's energy reserves, and on growth. In addition, the tissue regeneration process after excision must consume nutrition and energy reserves of the body[15, 17]. Larger wounds require more tissue to be regenerated, requiring the consumption of more nutrition and energy. A similar conclusion was reached during research into wound regeneration in *Scrobicularia plana*[14]. Seasonal factors also affect the growth of *H. cumingii* in the recovery period. The breeding seasons in spring and summer are critical for shellfish energy reserves[33]. The present study was carried out during the peak reproduction period for *H. cumingii* and the sampling did not exclude some sexually mature individuals. Gonadal development processes, which consume much energy, might have exacerbated the effect of excision treatment on *H. cumingii* growth. Further research into the following problems is necessary. Would augmentation of the nutrition supplied in the water reduce the adverse effect of healing and regeneration on the mussel growth? What is the influence of different seasons on the mussel growth in the regeneration period?

Repair of lost tissues in organisms involves at least two steps: wound healing and regeneration. Before morphogenesis occurs, wound healing is characterized by complete coverage of the epidermis[11]. Structures rebuilt in the regeneration process allow the new body section to be identical to the original part in structure and function[10]. Three months after excision, no differences from the control group were detected in the regenerated mantle with respect to structure (e.g., muscle fibre distribution, connective tissue distribution, shapes of epidermal and secretory cells, size and shape of the three lobes) or function (conchiolin secretion). The time required for regeneration to normal state depended on excision size: L-group> M-group> S-group.

Histological observation showed that recovery went through several phases: muscle contraction, which reduced the wound area; blood cell aggregation, epithelization and morphogenesis, which restored the normal morphology; and recovery of secretory capacity. After excision of mantle tissue from *H. cumingii*, rapid muscular response at the wound site caused tissues to contract from both sides of the wound towards the centre. This phenomenon was also observed in studies of the marine pearl oysters *P. fucata*, *P. margaritifera* and *P. maxima*[17-18, 34], and was believed to reduce the wound area and blood loss. This was followed by haemocyte aggregation. After 3 h, the contracted muscles were relaxed and haemocytes aggregated at the wound site to completely seal the wound until 12 h. This process occurred within 6 h in *P. fucata*[34], and within 12 h in *P. maxima*[18]. Epithelization is important for wound healing and regeneration in organisms. It prevents infection and provides a barrier against osmotic imbalance in aquatic animals[11]. *H. cumingii* required 72 h to complete this process. This was similar to the time required by *P. maxima* [18] but slower than that of *P. fucata* (48 h)[34].

Epithelization, which heals the wound, was followed by morphogenesis, in which the mantle tissue was gradually restored to its shape before excision. Visually, the most obvious manifestation of this was the occurrence and development of the mantle lobes. On day 12, the shapes of the three lobes forming on the wound through invagination could be recognized. By day 15, the lobes had formed but the outer fold was significantly smaller than the middle and inner folds. After 25 d, the shapes of the regenerated lobes were a little different from the normal tissues, and muscles were densely distributed in the pallial zone and lobes. *P. fucata* required 30 d to complete this process[17], and *P. maxima* 45 d[18]. After 15 d, a lobe formed in the basal zone of the outer fold extending towards the middle fold. This J-shaped lobe has not been reported in studies of marine pearl oysters. At present, the relationship between this lobe and secretory function is unknown. Conchiolin secretion arose from the periostracal groove between the J-shaped lobe and middle fold and the recovery of this function was faster than in *P. fucata* [17] and *P. maxima*[18]. At 90 d, it can be observed that the morphology of columnar epithelial cells between the root of the J-shaped lobes and the tip of the middle fold (outer epidermis side) changed drastically with their nuclei became smaller. This process has not been reported in marine pearl oysters. The outer periostracum was secreted close to these cells in the root of the J-shaped lobe, usually separating from the proximal end of the middle fold and gradually thickening after the separation. This process was similar to reports of periostracum formation in *Unionidae*[35]. The anatomical deformities in the proximal area of the regeneration inner fold illustrated in studies of *P. maxima*[18] were not observed in this study.

The rate of healing of *H. cumingii* in this study was close to that of the marine pearl oysters *P. fucata*[17] and *P. maxima*[18, 36]. However, regeneration was faster than in the latter two species, perhaps related to species diversity and culture temperature[37]. In this study, the speeds of healing and regeneration were generally similar between individual *H. cumingii* but a few individuals showed extremely slow or fast recovery. This may have been caused by errors in the excision size, which is reported to affect the wound recovery speed[13]. Another possible explanation could be physical difference between individual *H. cumingii* or hereditary factors. The development of saibos from donor mussels within host mussels during the process of pearl formation is essentially a process of healing and regeneration of excised mantle tissues. This is similar to the process observed here with respect to epidermal cell and connective tissue proliferation and secretory cell formation[38]. It would be interesting to determine whether the speed of recovery of *H. cumingii* after excision of its mantle affects the quality, such as colour, shapes, sizes and lustre, of pearls formed by the saibos provided by these donor mussels. The growth rate and survival rate of host mussels that also have more than twenty wounds after grafting saibos are also worthy of further exploration. If there is a correlation between these factors, then the healing and regeneration speed of pearl mussels after excision of partial mantle

tissue could be used as a new index for selective breeding.

This is the first report on the influence of excisions of different sizes, and from different locations on the mantle, on the survival rate and growth rate of *H. cumingii.* It is also the first description of the process of tissue healing and regeneration in this species after excision of mantle tissue from the living body. The technology of taking saibos of mantle tissue from living *H. cumingii* is simple and practicable. Furthermore, 3 months after excision, the mussel is still alive with completely restored structure and physiological function. Based on these observations, we provide new ideas for the selective breeding of donor *H. cumingii.* Mantle saibos can be taken from living donor mussels and then grafted into host mussels for pearl culture; the donor mussels producing high-quality pearls can then be evaluated for directed selective breeding.

References

[1] Hua D,Gu R. Freshwater pearl culture and production in China[J]. Aquaculture Asia, 2002, 7(1):6-8.

[2] Li J L. Exploitation and protection of genetic resources of freshwater pearl mussel[J]. Sci Fish Farming, 2007, 6:1-2.

[3] Li J L,Li Y S. Aquaculture in China—Freshwater pearl culture[J]. World Aquaculture, 2009, 40(1):60-62.

[4] Hua D,Neves R J. Captive survival and pearl culture potential of the pink heelsplitter *Potamilus alatus*[J]. N. Am. J. Aquac., 2007, 69(2):147-158.

[5] Taylor J. Producing golden and silver south sea pearls from Indonesian hatchery reared *Pinctada maxima*[M], World Aquaculture 2002, World Aquaculture Society, Baton Rouge LA, USA (2002), p. 754 Beijing, PR China, Apr. 23- 27: 2002.

[6] Wada K T,Komaru A. Color and weight of pearls produced by grafting the mantle tissue from a selected population for white shell color of the Japanese pearl oyster *Pinctada fucata martensii* (Dunker)[J]. Aquaculture, 1996, 142(1-2):25-32.

[7] Zhu W B. Study of the effect of two shell nacre colors on the color of pearls produced by Hyriopsis Cumingii (in Chinese). [D]. Shanghai, China: Shanghai Ocean University, 2011.

[8] Fu L L,Bai Z Y,Jin W,*et al.*. Evaluation of laser labeling on growth and survival of the freshwater pearl mussel, *Hyriopsis cumingii*[J]. Aquaculture Int, 2011, 20:431-441.

[9] A Sanchez Alvarado. Regeneration in the metazoans: why does it happen?[J]. Bioessays, 2000, 22:578-579.

[10] Alvarado A S. Regeneration and the need for simpler model organisms[J]. Philos. Trans. R. Soc. Lond. B Biol. Sci., 2004, 359(1445):759-763.

[11] Carlson B M. Principles of regenerative biology[M], Elsevier Inc., London 2007.

[12] Alagarswami K. Pearl culture in Japan and its lessons for India[C]. India: Proceedings of the symposium on mullusca. Marine biological association of India, 1969: 975-993.

[13] Armstrong D A,Armstrong J L,Krassner S M,*et al.*. Experimental wound repair in the black abalone, *Haliotis cracherodii*[J]. J. Invertebr. Pathol., 1971, 17(2):216-227.

[14] Hodgson A. Studies on wound healing, and an estimation of the rate of regeneration, of the siphon of *Scrobicularia plana* (da Costa)[J]. J. Exp. Mar. Biol. Ecol., 1982, 62(2):117-128.

[15] Pekkarinen M. Regeneration of the inhalant siphon and siphonal sense organs of brackish-water (Baltic Sea) *Macoma balthica* (Lamellibranchiata, Tellinacea)[J]. Ann. Zool. Fennici, 1984, 21:29-40.

[16] Acosta-Salmón H,Martinez-Fernandez E,Southgate P C. A new approach to pearl oyster broodstock selection: can saibo donors be used as future broodstock?[J]. Aquaculture, 2004, 231(1-4):205-214.

[17] Acosta-Salmón H,Southgate P C. Mantle regeneration in the pearl oysters *Pinctada fucata* and *Pinctada margaritifera*[J]. Aquaculture, 2005, 246(1-4):447-453.

[18] Mamangkey N G F,Southgate P C. Regeneration of excised mantle tissue by the silver-lip pearl oyster, *Pinctada maxima* (Jameson)[J]. Fish Shellfish Immunol, 2009, 27(2):164-174.

[19] Acosta-Salmón H,Martinez-Fernandez E,Southgate P C. Use of relaxants to obtain saibo tissue from the blacklip pearl

oyster (*Pinctada margaritifera*) and the Akoya pearl oyster (*Pinctada fucata*)[J]. Aquaculture, 2005, 246(1-4):167-172.

[20] Kanjanachatree K,Piyathamrongrut K,Kaewteen P. Effects of relaxants before embedding nucleus for the survival rate and pearl qualities in *Pinctada fucata*[J]. Songklanakarin J. Sci. Technol, 2006, 28(1):87-97.

[21] Norton J H,Dashorst M,Lansky T M,*et al.*. An evaluation of some relaxants for use with pearl oysters[J]. Aquaculture, 1996, 144(1-3):39-52.

[22] Norton J H,Lucas J S,Turner I,*et al.*. Approaches to improve cultured pearl formation in *Pinctada margaritifera* through use of relaxation, antiseptic application and incision closure during bead insertion[J]. Aquaculture, 2000, 184(1-2):1-17.

[23] Jin W,Bai Z,Fu L,*et al.*. Genetic analysis of early growth traits of the triangle shell mussel, Hyriopsis Cumingii, as an insight for potential genetic improvement to pearl quality and yield[J]. Aquaculture International, 2012, 20(5):927-933.

[24] Culling C F A,Allison R,Barr W. Cellular pathology technique[M], Butterworths, London, 1985.

[25] Fang Z,Feng Q,Chi Y,*et al.*. Investigation of cell proliferation and differentiation in the mantle of *Pinctada fucata* (Bivalve, Mollusca)[J]. Mar Biol, 2008, 153(4):745-754.

[26] Jabbour-Zahab R,Chagot D,Blanc F,*et al.*. Mantle histology, histochemistry and ultrastructure of the pearl oyster *Pinctada margaritifera* (L.)[J]. Aquat. Living Resour., 1992, 5(4):287-298.

[27] Arnaud-Haond S,Goyard E,Vonau V,*et al.*. Pearl formation: persistence of the graft during the entire process of biomineralization[J]. Mar. Biotechnol., 2007, 9(1):113-116.

[28] Gervis M,Sims N A. The biology and culture of pearl oysters (Bivalvia Pteriidae)[M], ICLARM Stud.Rev., Manila, Philippines, p.34-37, 1992.

[29] Kripa V,Mohamed K,Appukuttan K,*et al.*. Production of Akoya pearls from the Southwest coast of India[J]. Aquaculture, 2007, 262(2):347-354.

[30] Bondad-Reantaso M G,McGladdery S E,Berthe F C J. Pearl oyster health management: a manual[M], Roma: Food and Agriculture Organization of the United Nation: 2007: 8-15.

[31] Berthelin C,Kellner K,Mathieu M. Storage metabolism in the Pacific oyster (*Crassostrea gigas*) in relation to summer mortalities and reproductive cycle (West Coast of France)[J]. Comp Biochem Physiol B, 2000, 125(3):359-369.

[32] Mathieu M,Lubet P. Storage tissue metabolism and reproduction in marine bivalves - a brief review[J]. Inv. Repr. Dev., 1993, 23(2-3):123-129.

[33] Barber B J,Blake N J. Energy storage and utilization in relation to gametogenesis in Argopecten irradians concentricus (Say)[J]. J. Exp. Mar. Biol. Ecol., 1981, 52(2-3):121-134.

[34] Acosta-Salmón H,Southgate P C. Wound healing after excision of mantle tissue from the Akoya pearl oyster, *Pinctada fucata*[J]. Comp Biochem Physiol A, 2006, 143(2):264-268.

[35] Checa A. A new model for periostracum and shell formation in Unionidae (Bivalvia, Mollusca)[J]. Tissue Cell, 2000, 32(5):405-416.

[36] Mamangkey N. Improving the quality of pearls from Pinctada maxima[D]. James Cook University, 2009.

[37] Pilditch C,Grant J. Effect of temperature fluctuations and food supply on the growth and metabolism of juvenile sea scallops (*Placopecten magellanicus*)[J]. Mar Biol, 1999, 134(2):235-248.

[38] Awaji M,Suzuki T. The pattern of cell proliferation during pearl sac formation in the pearl oyster[J]. Fisheries Science (Tokyo), 1995, 61(5):747-751.