AN INVESTIGATION INTO PEARL CULTURE IN THE PINTO ABALONE *Haliotis kamtschatkana* (Jonas) BY EXAMINING ITS REPAIRED SHELL AND FACTORS THAT INFLUENCE ITS REPAIR RATE.

by

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Title of Thesis/Project/Extended Essay

An investigation into pearl culture in the pinto abalone Haliotis kamtschatkana (Jonas) by examining its repaired shell and factors that influence its repair rate.

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ABSTRACT

Development of blister pearls in molluscs imitates the natural process of shell repair. Therefore, an understanding of shell repair and factors influencing it will provide insights into the production of pearls in molluscs. In this study, the repaired shell of the pinto abalone (*Haliotis kamtschatkana*) is described and the influences of photoperiod, seasonality and site of damage are investigated for their influence on the rate of repair. As well, the effects on growth rates of implanting abalone with semi-spherical pearl nuclei was investigated.

The repaired shell in the pinto abalone consists of three layers. The first is an organic membrane, the conchiolin, that is dissimilar in appearance to the organic membrane of the normal growing shell, the periostracum. Onto this, calcitic calcium carbonate crystals are deposited forming a thin prismatic layer. This in turn is covered by a thicker nacreous layer composed of calcium carbonate aragonite crystals deposited in stacks. This organization is compared to that of other gastropods and to other classes of mollusca.

Pinto abalone were found to be very successful at repairing their shells, although showing much individual variation, and showed no significant differences for repair rates between the regions of the shell over the mantle, over the columellar muscle, or by the apical spire. Photoperiod

did not influence repair rate but a seasonal variation was significant, the repair rate decreasing during the late fall and winter. This is hypothesized to be caused by the colder water temperature in the winter months. Abalone shell repair is compared to that of other molluscs.

Abalone implanted with semi-spherical pearl nuclei did not show a decreased growth rate although this could have been masked by the animal's slow growth rate. Animals in a natural photoperiod gained significantly more weight than those kept in a 24 hour dark photoperiod. It is suggested that this is due to the availability of diatoms, the pinto abalone preferred food, growing on the sides of holding tanks kept in a natural photoperiod. Animals kept at 14°C showed no significant difference in growth compared to those kept at 7°C - 8°C, but mortality was much lower for those kept at the higher temperature. These results are compared to other studies on pinto abalone growth.

The results of this study are summarized as advice for abalone pearl cultivators.

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GENERAL INTRODUCTION

The shells of molluscs have been used by humans for millennia. They have been used as tools, for ornamentation and were important in some cultures as barter (Sheratt, 1980). Our fascination with molluscan shells, and their products, continues to the present, most notably in pearl culture which occurs around the world, using a number of different species of molluscs (Fankboner, 1991; Simkiss and Wada, 1980; and Takashi, 1980). It is not surprising that this fascination has spilled over into the scientific community, where the study of shells has a long history (Simkiss and Wada, 1980) and has given us a greater insight into these trinkets that so enrapture us.

The shell of a mollusc develops from its mantle, a sheet of tissue that lies in contact with the inner growing surface of the shell, which in some species extends to cover the outer surface as well. The actual structure of the shell varies considerably among species making it difficult to describe a typical shell. However, shells do share the following in common: an organic, mostly protein, matrix, the periostracum, onto which are deposited layers of calcium carbonate crystals. These crystals are either calcite, aragonite or vaterite, each distinguished by the arrangement of the crystal lattice (Wilbur, 1964).

The calcium carbonate crystals are deposited in identifiable layers, which are divided into subgroups. Watabe

(1988) has reviewed the literature on the structure of these layers and has broken them down into the following seven groups: 1) the prismatic layer, consisting of uniformly oriented calcite or aragonite prisms; 2) the nacreous layer, consisting of thin horizontal layers of aragonite; 3) the foliated layer, consisting of lamellae of
parallel and elongate calcitic crystals; 4) the crossed lamellar layer, consisting of lamellae of aragonite or calcite, with adjacent lamellae inclined in opposite directions; 5) the homogeneous layer, consisting of calcite with no specific structural pattern; 6) the myostracum layer, used by some researchers to describe the distinct shell structure under the area of muscle attachment; and 7) the mosaicostracum, a very thin layer found between the periostracum and the outer shell layers of bivalves. The number and order of layers vary among species, and all the different groups of layers are rarely found in any one species.

The periostracum and each of the calcified layers of the shell are formed by specialized regions of the mantle (Beedham, 1958). The outermost layer, the periostracum is formed from proteins secreted into the extrapallial fluid, found between the mantle and the shell, by the periostracal groove at the mantle edge. The first outer calcified layer of shell, usually the prismatic layer, is deposited by the cells of the mantle adjacent to the periostracal groove. Each succeeding layer is then deposited by mantle cells adjacent to the cells that

deposited the preceding layer.

Abalone shells consist of three layers: the periostracum, an outer prismatic layer and an inner nacreous layer (Dauphin et al., 1989; Mutvei, Dauphin and Cuif, 1985; and Nakahara, Bevelander and Kakei, 1982). The inner nacreous layer in *Haliotis kamtschatkana* is made up of stacks of crystals that grow laterally to form sheets. This is the typical arrangement for gastropod nacre (Mutvei, 1980; and Wise, 1970b). The final nacreous layer is much thicker than the prismatic layer, which is usual among prosobranch gastropods (Wise, 1970b; and Wise and Hay, 1968).

Once the shell has formed, mineralization may not be finished. Molluscs are exposed to different factors that may damage their shells, such as predators, parasites and environmental factors like ocean surf. The repair of this damage involves similar processes as shell growth, the secretion of an organic matrix succeeded by deposition of calcium carbonate crystals, but is not restricted to the mantle edge. Nor is the repaired shell necessarily identical in structure to the original shell.

The organic matrix of repaired shell may be like the periostracum (Meenakshi et al., 1974; Timmermans, 1973; and Beedham, 1965), or not like the periostracum (Blackwelder and Watabe, 1975; Meenakshi et al., 1975; Meenakshi et al., 1974; Saleuddin and Wilbur, 1969; Abolins-Krogis, 1968; and Wagge, 1951). To avoid confusion, the term periostracum is here

reserved for the organic matrix of 'normal' shell and conchiolin is reserved for the organic matrix of repaired shell. The calcified layers may follow the usual arrangement and structure of the normal shell, or, as is more often the case, abnormal layers and/or crystals are present (Watabe, 1983).

How fast a mollusc can repair the damage to its shell varies according to the species (Wagge and Mittler, 1953), the location of the damage (Andrews, 1935; Wagge, 1951; Saleuddin and Wilbur, 1969; Kunigelis and Saleuddin, 1983 and Watabe, 1983) and other factors. For the marine gastropod *Tegula funebralis* a gender difference was found to exist in the repair rates of the shell (Peppard, 1964). Saleuddin and Chan (1969) found that temperature affects shell repair rates.

This present study arises out of the human fascination for pearls. "Pearls are, of course, the layers of aragonite calcium carbonate secreted by molluscs," (Simkiss and Wada, 1980, p32). Since they are not formed at the mantle edge, they are part of the process of shell repair. The purpose of this investigation is to examine the process of shell repair in the abalone *Haliotis kamtschatkana*, presently being used for pearl cultivation in the Canada (Fankboner, 1991), by observing the repair in three regions of the shell. As well, various factors that may affect repair rates were tested, particularly location of damage and seasonal variation.

The first chapter of this thesis examines the repaired shell of *Haliotis kamtschatkana*, which is the layers of calcium

carbonate they secrete in response to damage to the shell. An understanding of the structure of repaired shell is helpful when examining factors that influence the rate of repair, which is the subject of Chapter 2. This thesis will enable abalone pearl cultivators to maximize pearl production by providing a description of the processes involved and explaining the factors that influence them.

CHAPTER ONE

The Repaired Shell of the Pinto Abalone

Haliotis kamtschatkana

INTRODUCTION

The culturing of pearls dates back to the 13th century when miniature, lead casts of Buddha were inserted between the mantle and the shell of the fresh water mussel *Cristaria plicata* (Simkiss and Wada, 1980). However the culturing of marine pearls in North America is a very recent phenomenon and uses either the pinto abalone *Haliotis kamtschatkana* or the red abalone *H. rufescens* as the culture animal (Fankboner, 1991).

Abalone were first used for pearl culture in the late 19th century (Boutan, 1898). By 1991 there were six farms in Japan, Korea, Canada and the United States culturing abalone pearls (Fankboner, 1991); today, the only active farms producing abalone pearls are Korea Abalone Pearl Co. and Pacific Pearl Culture Ltd. (Canada).

This present study attempts to detail the early development of abalone blister pearls, produced by using a similar technique as for the ancient Buddha pearls, by examining the repair of shell in abalone. To ensure adequate understanding of the process, three different regions of the shell were studied: the region over the mantle epithelium, about 1 cm from the growing edge; the region over the site of attachment of the columellar muscle; and the region about 1 cm from the most posterior point on the shell, above the area where the gonads generally can be found, depending on age and ripeness (Figure 1).

Repair of the shell of a mollusc proceeds soon after damage

has occurred, the speed of response varying greatly among species. Generally, marine molluscs respond significantly more slowly than terrestrial molluscs (Wagge and Mittler, 1953). Initially, the tissue underlying the damage responds by secreting a fluid from which an organic membrane, the conchiolin, develops (Uozumi and Suzuki, 1979; Wilbur, 1964). The conchiolin may or may not be like the periostracum. Periostracum or periostracum-like conchiolin has been found in the freshwater gastropod *Lymnaea stagnalis* (Timmermans, 1973), species of the freshwater bivalve *Anodonta* (Beedham, 1965), and the marine bivalve *Mytilus edulis* (Meenakshi et al., 1974). Non-periostracum-like conchiolin has been found in the terrestrial pulmonate gastropods *Helix aspersa* (Wagge, 1951), *H. pomatia* (Saleuddin and Wilbur, 1969 and Abolins-Krogis, 1968), and *Otala lactea* (Meenakshi et al., 1974), and in the freshwater prosobranch gastropod *Pomacea paludosa* (Blackwelder and Watabe, 1975; and Meenakshi et al., 1975).

The conchiolin is only part of the organic matrix of the shell. Calcium carbonate crystals are deposited on the conchiolin within compartments composed of organic membranes (Bevelander and Nakahara, 1969). When the mineral component of shell is dissolved with either HCl or EDTA, the remaining organic matrix can be separated into an insoluble matrix and a soluble matrix. The soluble matrix is believed to be involved in crystal initiation and growth (Wheeler and Sikes, 1984; and Wheeler, George and Evans, 1981).

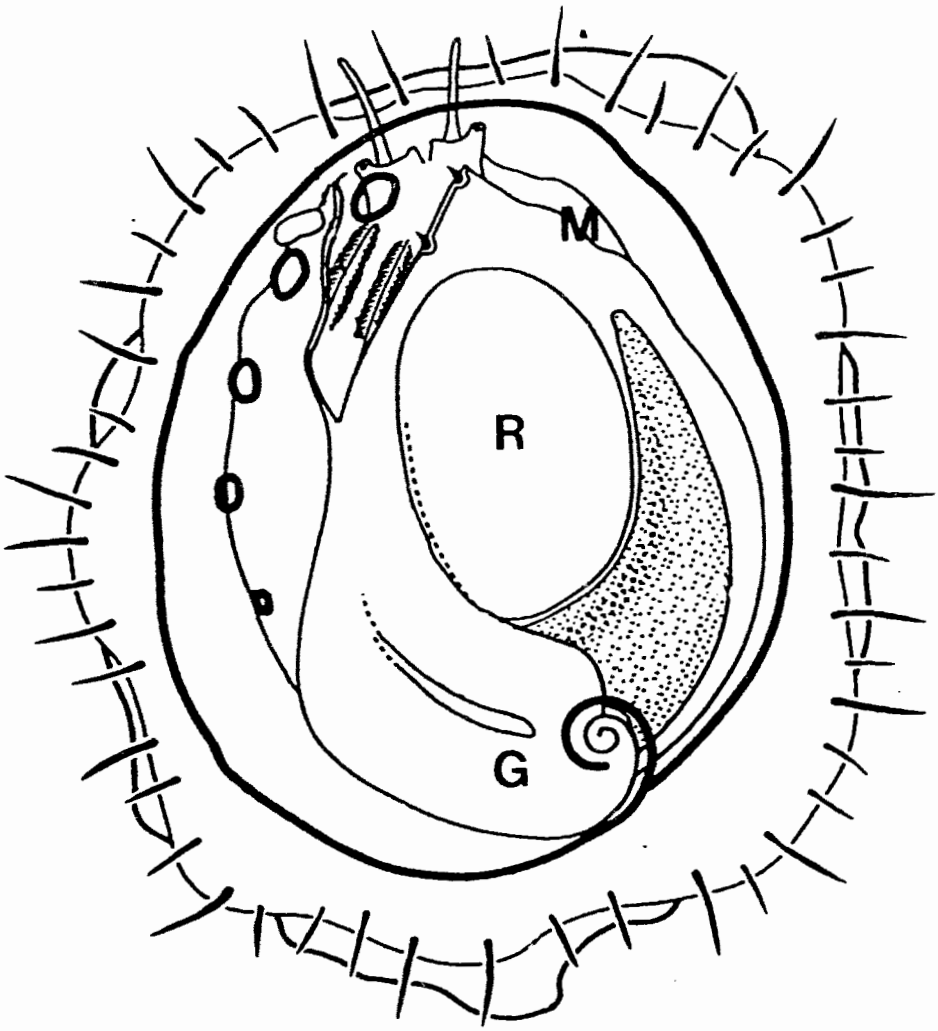


Figure 1: Drawing of abalone (dorsal aspect) with the shell being transparent showing the location of the 5 mm holes (indicated by solid circles) drilled in the shell to study shell repair. One experiment used abalone with only one hole drilled in the shell in one of the three regions and the second experiment used abalone with three holes drilled in the shell, one hole in each region. **R**, Right adductor muscle; **G**, Gonads; **M**, Mantle.

After deposition of the conchiolin is complete, layers of calcium carbonate crystals are deposited. They are either deposited in the manner of the original shell, as seen in species of *Anodonta* where the nacreous layer succeeds the prismatic layer (Tsujii, 1976; and Beedham, 1965), or, as in the majority of the cases, deposited with abnormal layers or

crystals. *H. pomatia*, which normally has a prismatic outer layer, a crossed lamellar middle layer and a nacreous inner layer, all composed of aragonite crystals, deposits 'boat-shaped' aragonite spherulites on the conchiolin followed by calcitic polyhedral or rhombohedral crystals, and, finally, aragonite spherulites are deposited again, sometimes with vaterite crystals as well (Abolins-Krogis, 1963). The repaired shell of *Mytilus edulis* also is structurally different than the normal shell, which is composed of an outer aragonite prismatic layer and an inner aragonite nacreous layer. The repaired shell has aragonite spherulites forming a complex crossed-lamellar outer layer, which is then covered by a calcitic prismatic layer followed by an aragonitic nacreous inner layer (Uozumi and Suzuki, 1979). Meenakshi et al. (1973) found the repaired shell of *M. edulis* to have the same structure of the normal shell, except that a complex prismatic-type layer was often present.

Otala lactea repaired shell is composed of aragonite spherulite crystals and calcitic rhombohedral crystals with an inner nacreous layer of aragonite crystals, which is not like the pure aragonitic prismatic-crossed lamellar-nacreous

composition of the normal shell. The repaired shell grows from aggregates of dumbbell-shaped crystals (Meenakshi et al., 1974). Similar construction has been observed in the marine prosobranchs *Oncomelania formosana* (Davis, 1964) and *Neritina reclinata* (Andrews, 1935).

It is the inner nacreous layer of molluscan shells that, when deposited on the outer surface of a pearl nucleus, makes cultured pearls valuable. Therefore, it is not surprising to find that most studies on abalone shell have emphasized the nacreous layer (Mutvei, 1980, 1978 and 1970; Erben, 1974; Wise, 1970a and 1970b; Gregoire, Duchateaux and Florkin, 1955), although some researchers have dealt with other layers (Suzuki, 1983; Nakahara, Bevelander and Kakei, 1982; and Uozumi and Togo, 1975). No one study has yet detailed the full process (nearly identical to blister pearl culture) abalone undergo while repairing shell.

Shell repair is of ecological importance to abalone. Various parasites of abalone burrow into the shell, including species of *Polydora*. Data indicate that flesh weight of *Haliotis diversicolor aquatilis* decreases significantly with an infestation of more than ten *Polydora* per shell (Kojima and Imajima, 1982). The piddock *Penitella conradi* Valenciennes 1846 (= *Penitella parva* Tyron 1865) is characterized as being usually found boring in the shells of *Haliotis* (Turner, 1955). Octopus have been found to be a major predator of *Haliotis rufescens* in California, attacking by drilling through the shell (Tegner and

Butler, 1985). Abalone shell repair is an important defensive mechanism against predation and parasitic infestation such as these.

It is the purpose of this study to examine the process of wound repair in the shell of the pinto abalone, and by so doing, elucidate the general process of how an abalone reacts to the trauma of perforation shell damage. It is anticipated that the results of this study will also reveal processes which accompany the formation of natural and cultured abalone pearls.

MATERIALS AND METHODS

ABALONE COLLECTION AND HUSBANDRY

Specimens of *Haliotis kamtschatkana* were collected from Barkley Sound around Bamfield, British Columbia (Latitude 48°50'N and Longitude 125°15'W). Specimen size ranged from 5 centimetres to the upper size limit for this species, 15 centimetres. Abalone were held in 120 cm x 60 cm x 20 cm flat bottomed fibreglass trays with aerated flow through sea water.

All animals were fed fresh *Nereocystis leutkeana* and/or

Macrocystis integrafolia every 7 - 10 days which were collected from near shore beds adjacent to the Bamfield Marine Station. In addition to this kelp provided during husbandry, the abalones also had access to resident diatom growth on the sides and bottom of the holding trays.

GENERAL PREPARATIONS FOR OBSERVING THE REPAIRED SHELL

Three regions of the shell of *Haliotis kamtschatkana* were investigated for capability to repair shell. The region over the gonads and the region over the mantle were chosen for their potential as sites for implantation of pearl nuclei. The region over the columellar muscle was chosen to determine if abalone are capable of repairing shell there, something that had not been previously reported for molluscs in the literature. A 5mm hole was drilled in the shell of *Haliotis kamtschatkana* over one of the three regions chosen. Only one hole per abalone was drilled to decrease the probability of increased mortality due to physical stress (Elston and Lockwood, 1983). A 5 mm diameter diamond core drill bit (Crystallite Corporation) mounted in a drill press (500 rpm) was used to bore the holes through the shell. Sea water was used as a lubricant and cooling material during the drilling process. Abalone which were injured during the drilling process were discarded.

OBSERVATIONS ON THE DEPOSITION OF CONCHIOLIN

In order to study the deposition of conchiolin, a plastic cover slip was placed over the hole drilled in the shell. However, the thickness of the shell prevented the coverslip from coming in contact with the soft tissues underneath the hole that are responsible for repair. Therefore animals chosen for this part of the experiment had the region of the shell to be drilled sanded until very thin. This was done by touching the shell to a belt sander secured in a vice. The abalone was briefly touched to the sander then immersed into sea water at < 12°C temperature to prevent heating of the shell and heat stress to the underlying tissues. This was repeated numerous times until the shell was thin enough to appear translucent. The 5 mm hole was then drilled.

The plastic coverslip was secured in place with a hose clamp that was cut with tin snips across its solid section. The two ends formed were bent so to grip the anterior and posterior edges of the shell without causing tissue damage to the mantle edges. Circular rubber slices approximately 1 cm thick were made by cutting down No. 5 rubber stoppers. These biscuit-shaped slices were placed between the plastic cover slip and the altered hose clamp. The hose clamp was then tightened using a screwdriver to prevent slippage of the cover slip.

At intervals of 1, 2, 3 and 4 weeks 50 abalones from the experimental group were sacrificed and the shell removed. The

conchiolin around the wound hole of each specimen was carefully dissected from the shell from the inside to ensure adhesion to the plastic coverslip. Next, the hose clamps were removed and the coverslips were carefully peeled from the shells. The adhering material was fixed in fumes from 4% osmium tetroxide.

After fixation, the conchiolin was tested for proteins using mercuric-bromophenol blue, and for calcium deposits von Kossa's technique was followed. Wound hole samples were also stained with toluidine blue. All histochemistry procedures followed Humason (1979).

LIGHT MICROSCOPY OF REPAIRED SHELL

Experimental *Haliotis kamtschatkana* used to obtain micrographs were sampled at various times from 1 week to 30 weeks. These animals were sacrificed and the shell removed. Light micrographs of the wound area of the shell were obtained on a Zeiss dissecting scope with a camera mount. These pictures were taken at 6x and 12x magnification of the interior and exterior views of the area of repair. Some exterior views of the wound area were taken of live animals.

SCANNING ELECTRON MICROSCOPY OF REPAIRED SHELL

Abalone used for scanning electron micrographs had the repaired area removed from the shell by drilling over the original hole with a 16 mm Crystallite diamond core drill following the technique described above to drill the wound hole. The excised piece was fixed for 15-20 minutes in a 6% solution of glutaraldehyde, made up from 1 part prebuffered (1% calcium carbonate) 12.5% solution of glutaraldehyde to 1 part 0.2 M cacodylate buffer at pH 7.4 (Fankboner, 1978). After rinsing with distilled water, pieces used for examination of calcium carbonate crystals were placed in 5% sodium hypochlorite solution (Javex bleach) over night to remove organic matter, and then rinsed thoroughly with distilled water. Prior to examining, all tissues were critically dried, mounted on stubs and coated with gold (Meenakshi et al., 1973). While research was still undergoing at the Bamfield Marine Station, micrographs were taken at the University of Victoria using a JEOL JSM-35 scanning electron microscope. When research at the Bamfield Marine Station had been completed micrographs were taken at Simon Fraser University using an ETEC Autoscan scanning electron microscope.

OBSERVATIONS AND RESULTS

HISTOLOGICAL STAINS OF THE CONCHIOLIN

The conchiolin on the plastic coverslips, when tested for proteins with mercuric-bromophenol blue, all gave a positive result. The coverslips removed after 4 weeks had a noticeably thicker piece of conchiolin adhering to them. The thickest part of this was too thick to absorb the stain but the thinner edges all tested positive.

The Von Kossa's test for the presence of calcium carbonates gave positive results for the cover slips tested. Controls showed no calcium carbonate. The cover slips removed after 1 week showed only small crystals, measuring about 2 - 4 micrometers in diameter. On the coverslip removed from the region over the columellar muscle, only a very few of these crystals were found. After two weeks some of these crystals appeared to have formed aggregates as large as 30 micrometers in diameter, although the majority of crystals were still the small 2 - 4 micrometer size. The coverslip removed from over the mantle region after two weeks showed streaks of positively staining material that were from 10 - 320 micrometers wide and crossed the diameter of the hole. As well, there was a thick (0.5 mm) ring of crystals circumscribing the hole. By week 4 the conchiolin was once again too thick to see results, although there were still positive staining crystals around the edges.

The coverslips stained with toluidine blue showed reddish violet metachromasia indicating the presence of glycoproteins, most probably mucin (Leeson, Leeson and Paparo, 1985; Humason, 1979). The coverslips from over the foot and the coverslip removed after 1 week from over the mantle had amoebocytes adhering to them. All the coverslips removed after four weeks, and the coverslip removed after three weeks from over the mantle, had conchiolin that was too thick for light penetration.

LIGHT MICROSCOPY

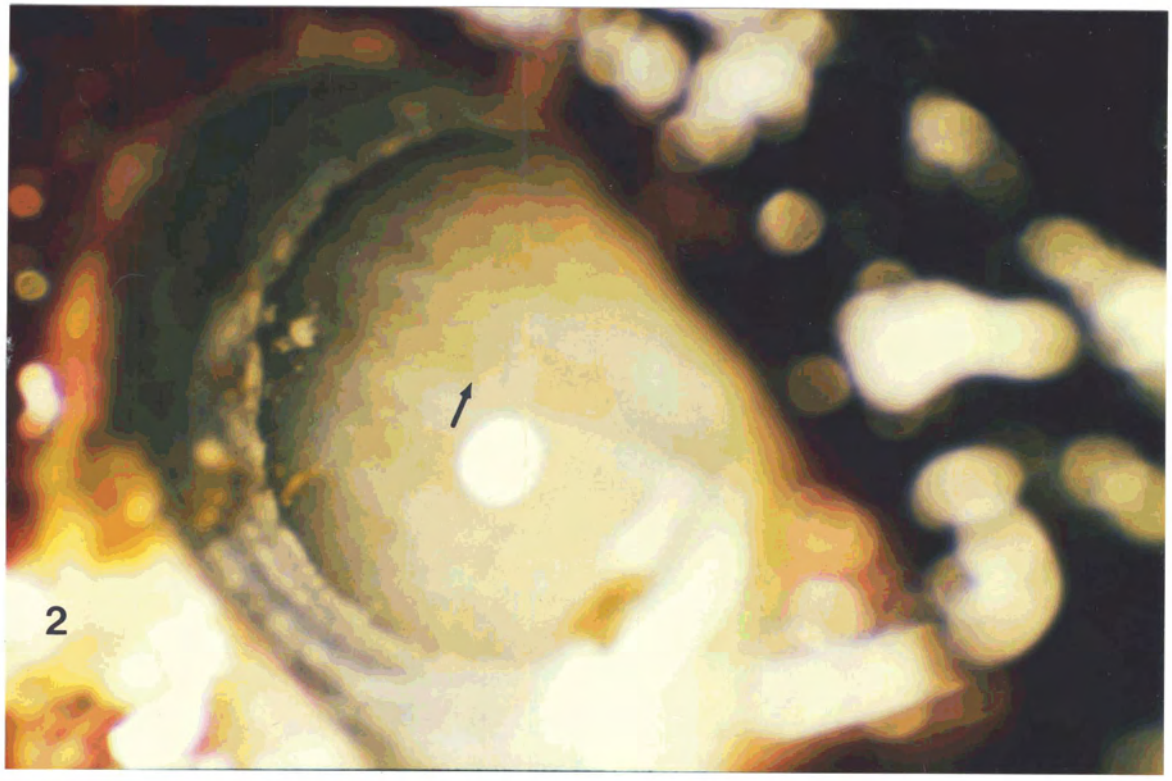
The Mantle Region

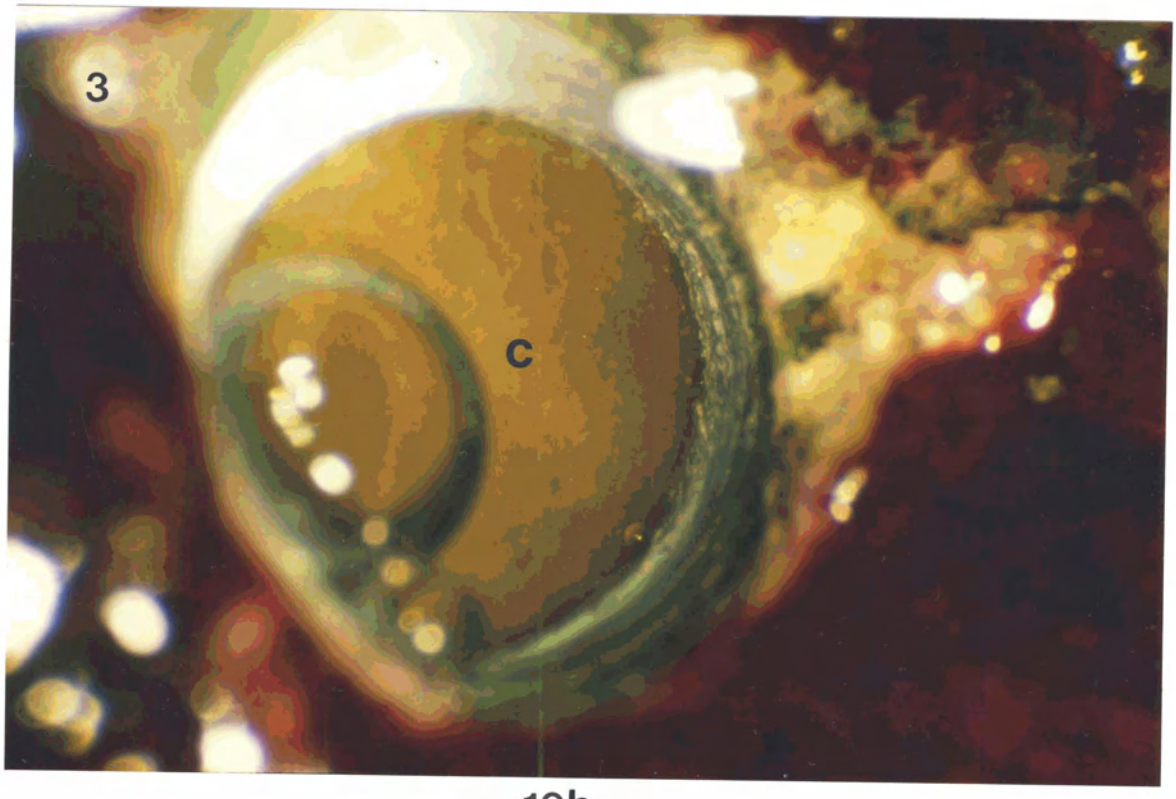
Figure 2 shows the hole in the shell over the mantle region after 1 week of repair. The mantle tissue appeared whitish underneath streaks of conchiolin. By 4 weeks (Figure 3) the hole was completely covered by a sheet of conchiolin. The interior view of repair after 8 weeks (Figure 4) showed an area much larger than the hole covered by conchiolin and some crystallization of the conchiolin around the edges of this area. The conchiolin covering the middle of the hole appeared thinner than that at the edges. After 30 weeks (Figure 5) the conchiolin was completely covered with nacreous aragonite calcium carbonate crystals.

Repair of a 5 mm hole over the mantle region in *Haliotis kamtschatkana*.

Figure 2: Repair of hole drilled over the mantle, 1 cm from the growing edge, of *Haliotis kamtschatkana* at 1 week following wounding.
Abbreviations used: Arrow, conchiolin streaks. x 15

Figure 3: Repair of hole drilled over the mantle, 1 cm from the growing edge, of *Haliotis kamtschatkana* at 4 weeks following wounding.
Abbreviations used: c, conchiolin. x 15

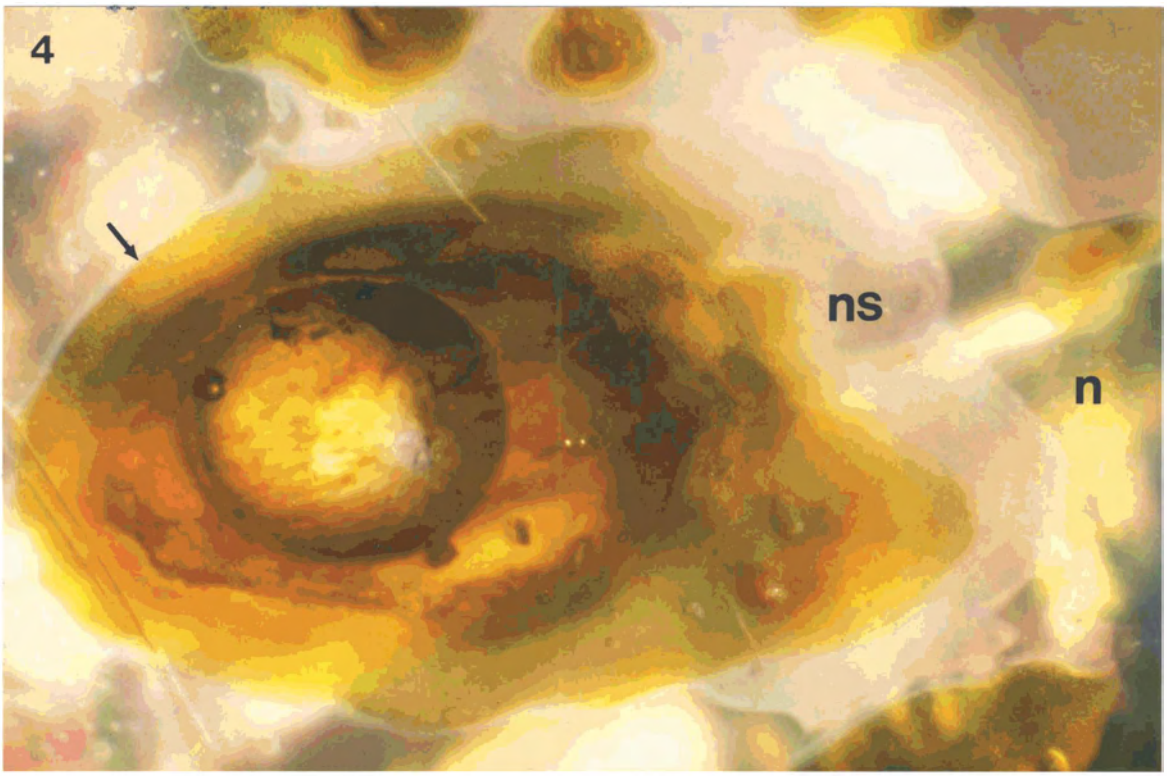




19b

Figure 4: Repair of hole drilled over the mantle, 1 cm from the growing edge, of *Haliotis kamtschatkana* at 8 weeks following wounding.
Abbreviations used: Arrow, edge of conchiolin; n, nacre of unrepaired shell; ns, new shell. x 15

Figure 5: Repair of hole drilled over the mantle, 1 cm from the growing edge, of *Haliotis kamtschatkana* at 30 weeks following wounding.
Abbreviations used: Arrow, edge of repaired shell. x 15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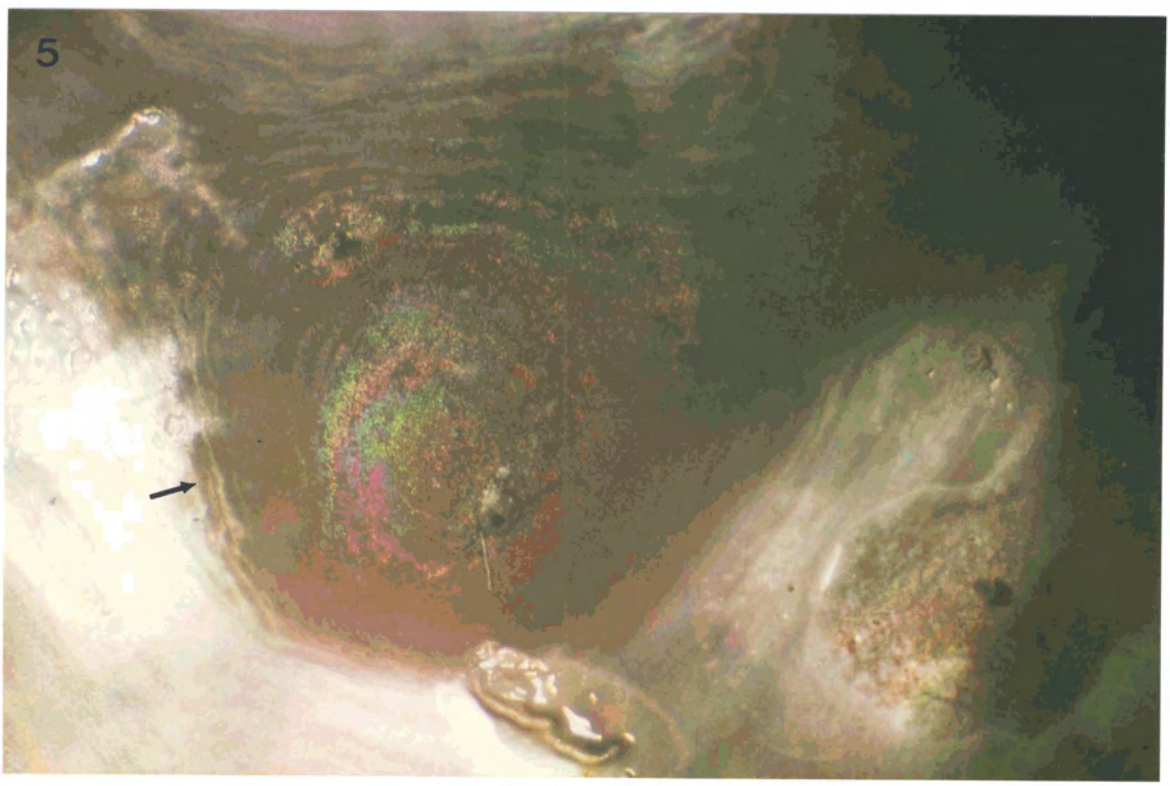
ns
n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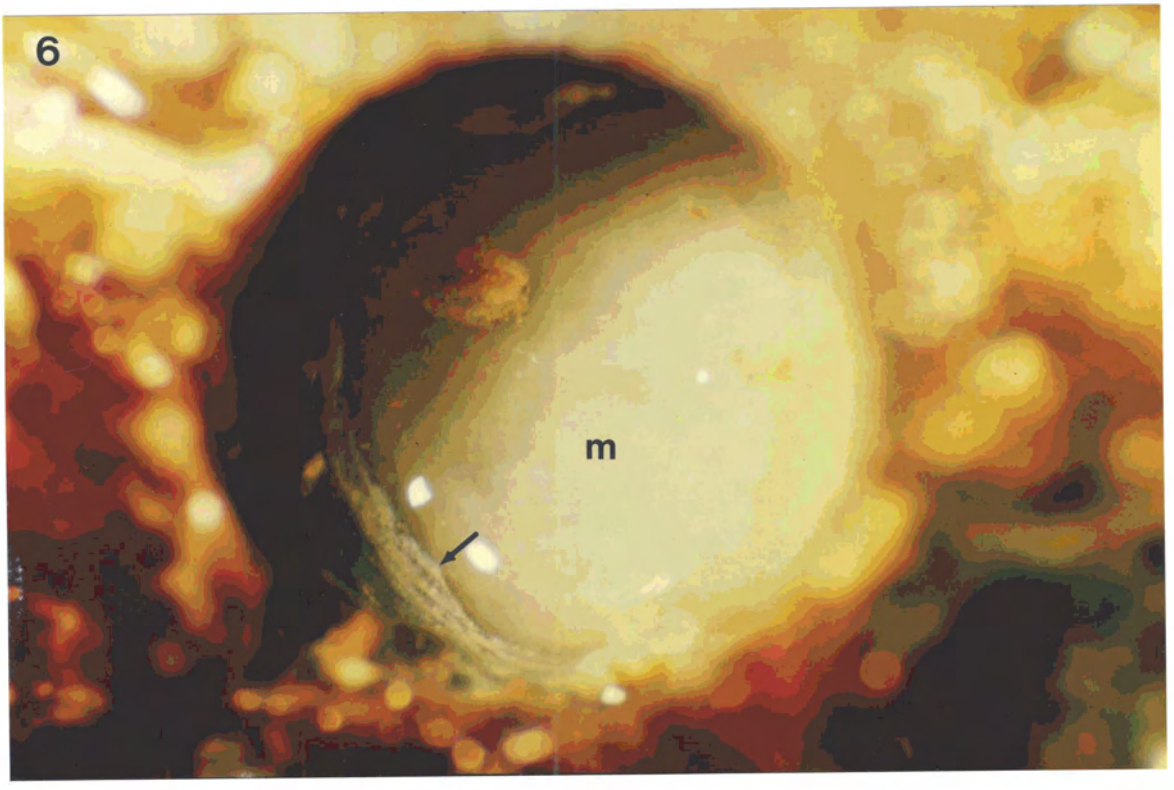
The Columellar Region

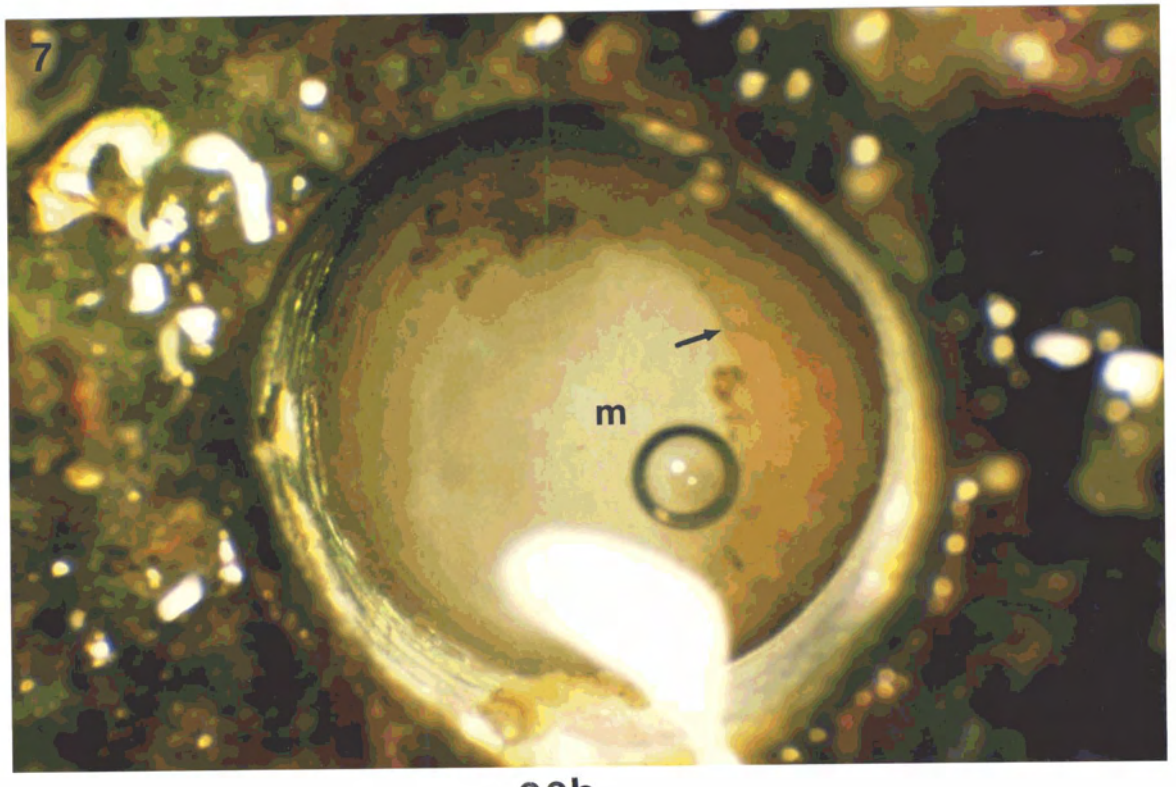
After 1 week (Figure 6), the hole drilled over the region of attachment of the right adductor muscle showed no visible signs of repair. The white exposed muscle could be seen underneath the hole. By week 4 (Figure 7) conchiolin was visibly forming around the edges. Even after 8 weeks the conchiolin secreted by the underlying tissue had not completely covered the hole (Figure 8) although a large area around the hole had been covered (Figure 9). Figure 8 also shows the conchiolin thicker at the edges of the hole. By 10 weeks the hole was completely covered with conchiolin (Figure 10). Despite the slower development of conchiolin compared to the mantle region, after 22 weeks the hole was completely covered with nacre.

Repair of a 5 mm hole over the region of attachment of the right adductor muscle in *Haliotis kamtschatkana*.

Figure 6: Repair of hole drilled over the right adductor muscle of *Haliotis kamtschatkana* at 1 week following wounding.
Abbreviations used: Arrow, edge of drill cut; m, right adductor muscle. x 15

Figure 7: Repair of hole drilled over the right adductor muscle of *Haliotis kamtschatkana* at 4 weeks following wounding.
Abbreviations used: Arrow, edge of conchiolin; m, right adductor muscle. x 15

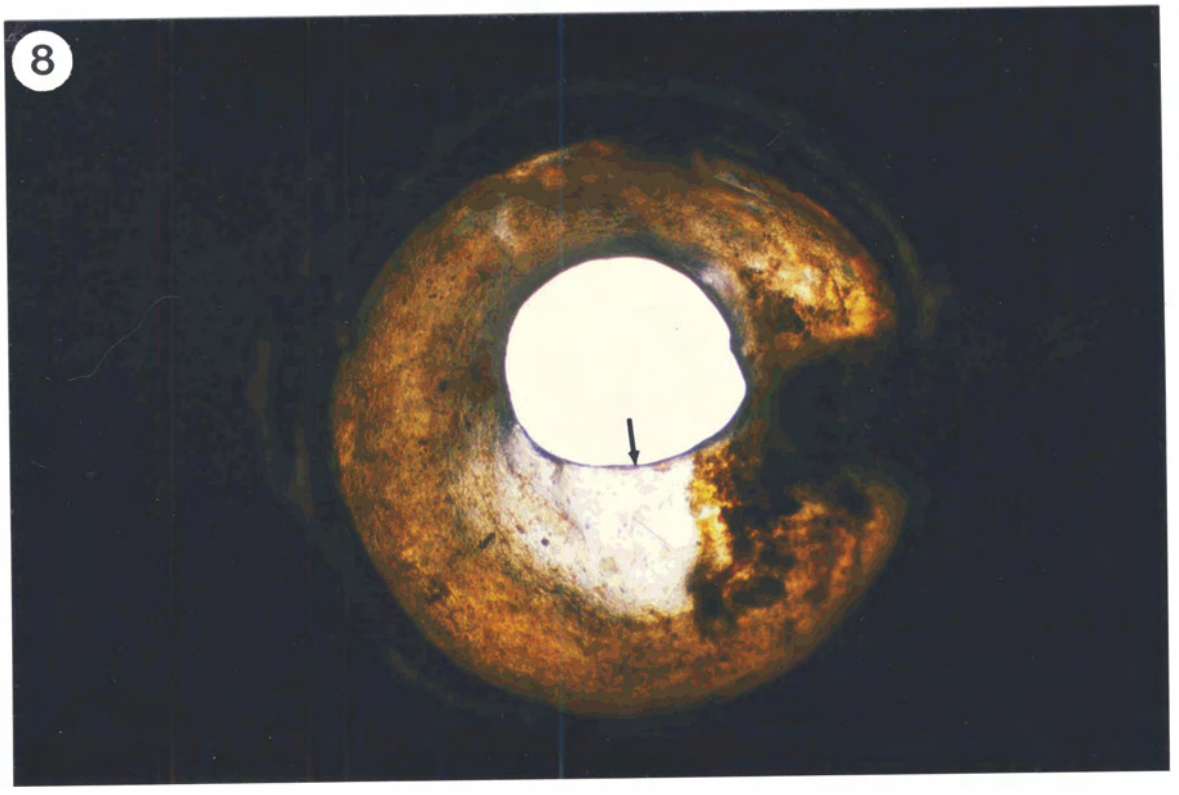




22b

Figure 8: Repair of hole drilled over the right adductor muscle of *Haliotis kamtschatkana* at 8 weeks following wounding. This view is of the exterior of the shell and back lit to emphasize the thickness of the conchiolin. Abbreviations used: Arrow, edge of conchiolin. x 15

Figure 9: Repair of hole drilled over the right adductor muscle of *Haliotis kamtschatkana* at 8 weeks following wounding. This is the same shell as Figure 8 but the view is of the interior of the shell.
Abbreviations used: Arrows, edges of the conchiolin; ms, muscle scar left when shell was removed from the adductor muscle. x 7.5



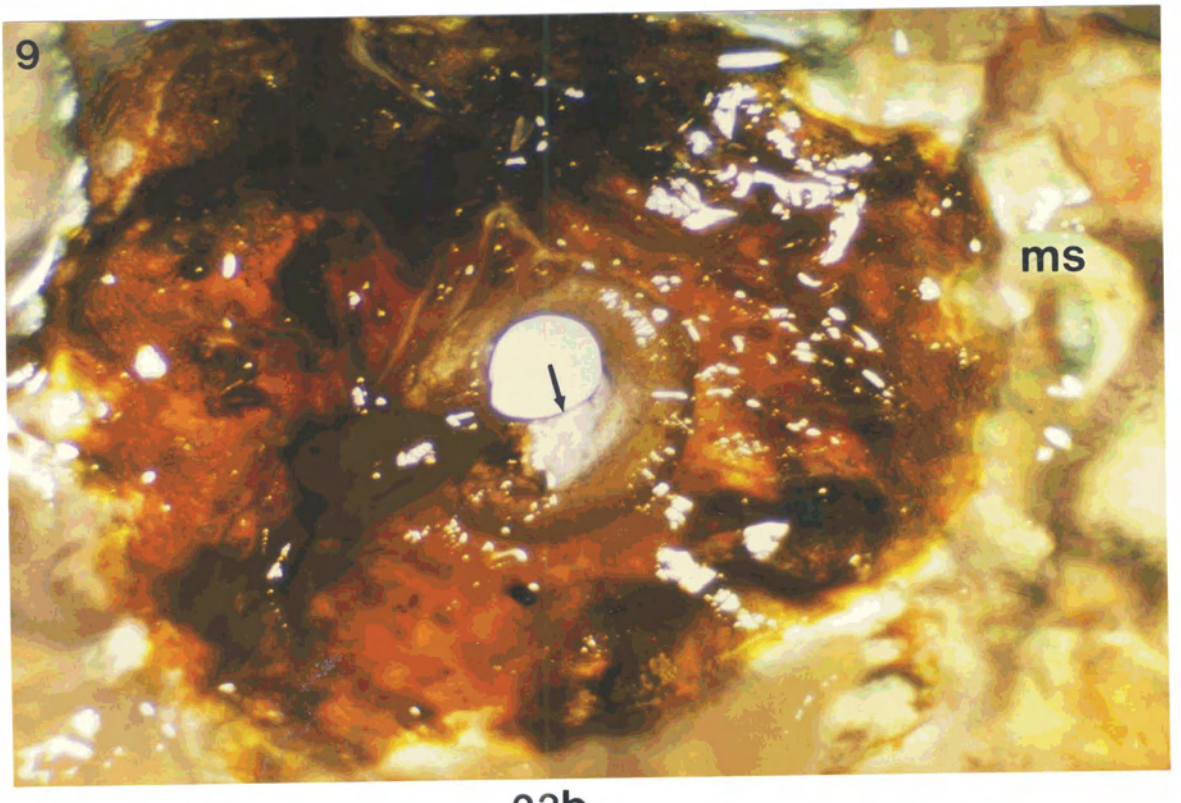


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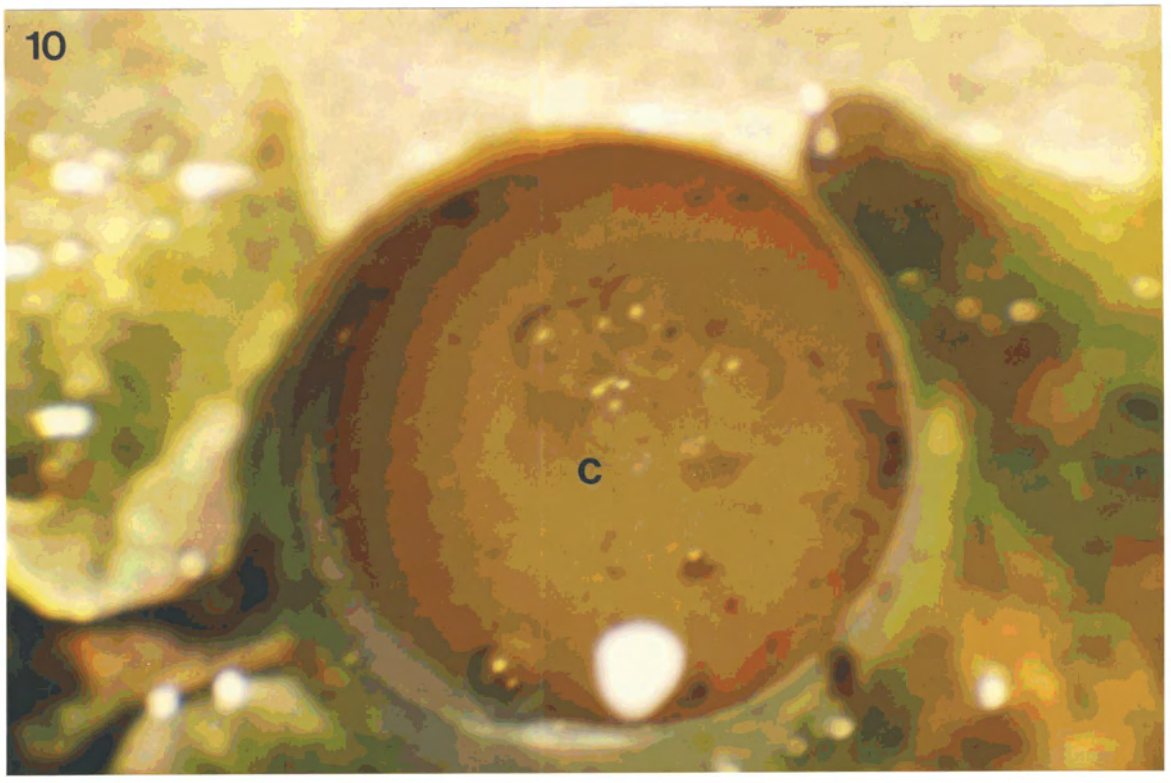
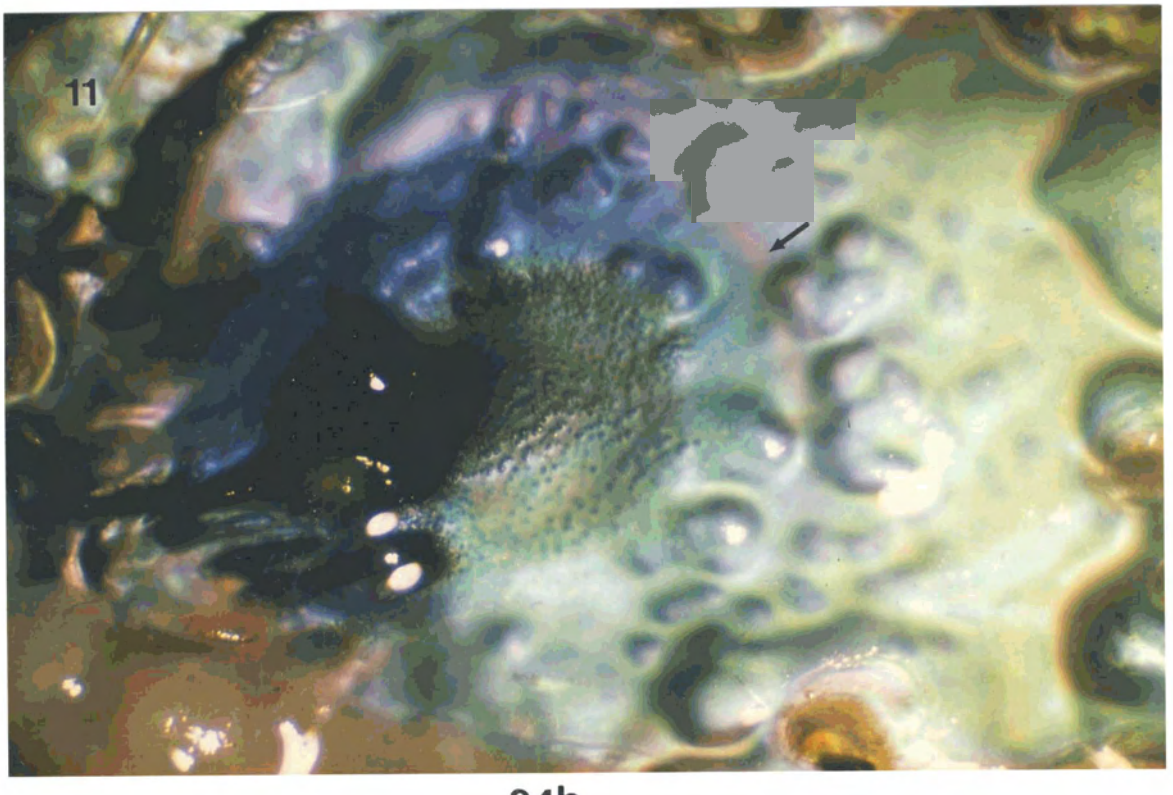
Figure 10: Repair of hole drilled over the right adductor muscle of *Haliotis kamtschatkana* at 10 weeks following wounding. Exterior view.
Abbreviations used: c, conchiolin. x15

Figure 11: Repair of hole drilled over the right adductor muscle of *Haliotis kamtschatkana* at 22 weeks following wounding.
Abbreviations used: Arrow, covered edge of hole. x15





24b

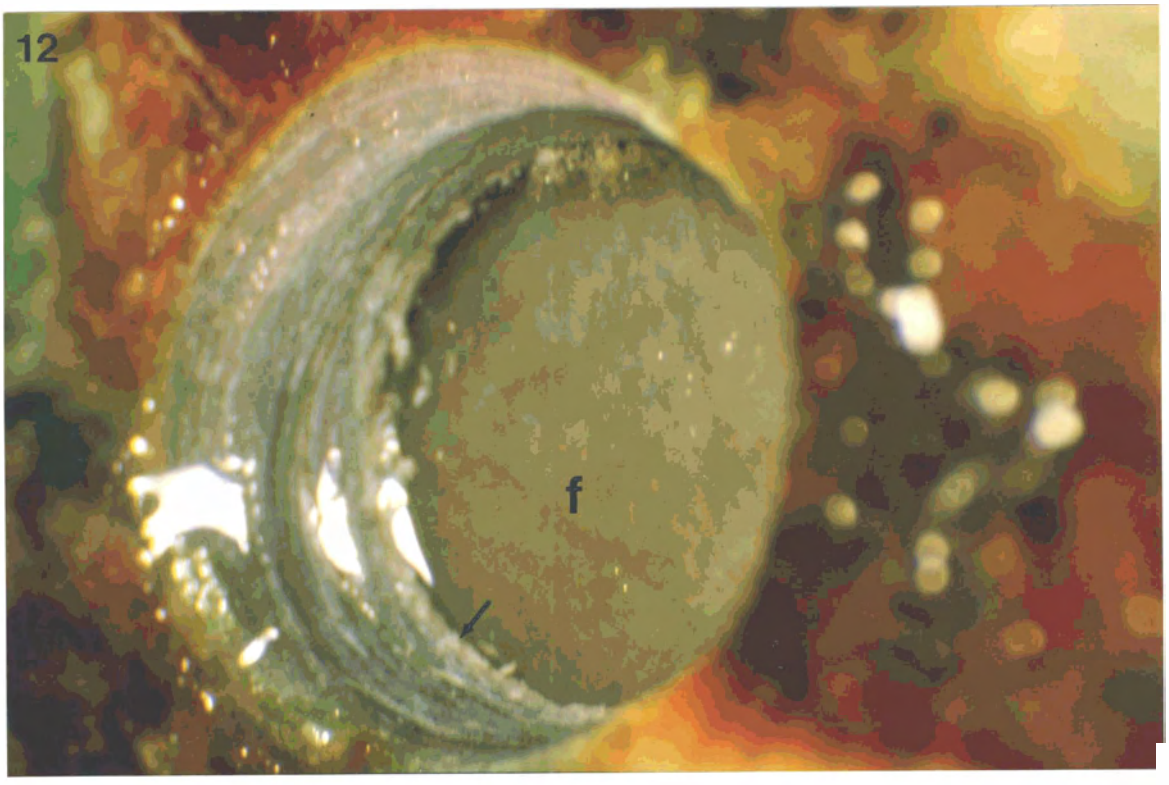
The Gonad Region

After one week, the hole drilled in the shell over the gonad region also showed no evidence of repair (Figure 12). The female gonad was visible below the hole. By week 4 (Figure 13) the hole was completely covered by conchiolin. After 8 weeks the conchiolin could be seen as thinner around the centre (Figure 14) but covered a large area around the hole (Figure 15). The drilled hole was no longer visible after 22 weeks (Figure 16) but the conchiolin still was not completely covered with nacre. This was completed by week 30 (Figure 17).

Repair of a 5 mm hole over the gonad region in *Haliotis kamtschatkana*.

Figure 12: Repair of hole drilled over the gonad region of *Haliotis kamtschatkana* at 1 week following wounding. Abbreviations used: Arrow, edge of drilled hole; f, gonad (female). x 15

Figure 13: Repair of hole drilled over the gonad region of *Haliotis kamtschatkana* at 4 weeks following wounding. The area looks lighter not only because of the conchiolin, but because this is a male, which has cream-coloured gonads. Abbreviations used: Arrow, edge of drilled hole; c, conchiolin. x 15


12
f

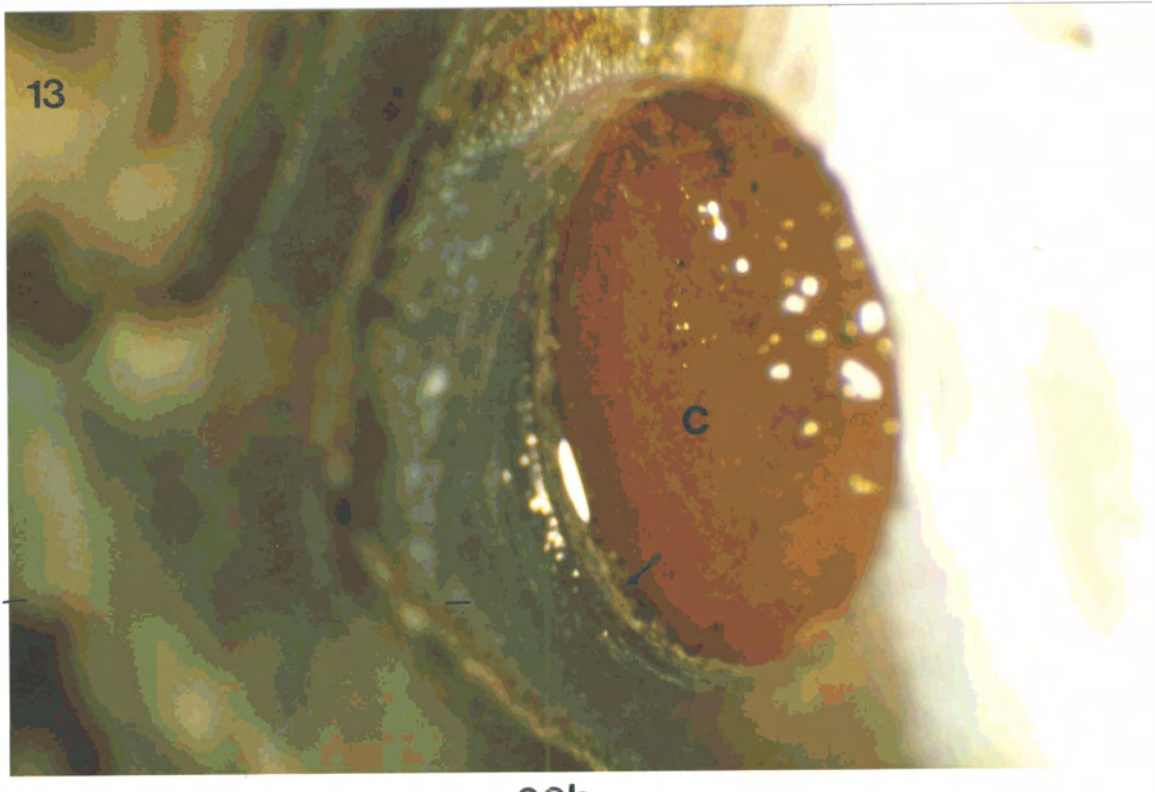

13
c

Figure 14: Repair of hole drilled over the gonad region of *Haliotis kamtschatkana* at 8 weeks following wounding. The hole is back lit to emphasize the difference in the thickness of the conchiolin along the edge of the hole and in the centre of the hole.
Abbreviations used: Arrow, area of very thin conchiolin. x 15

Figure 15: Repair of hole drilled over the gonad region of *Haliotis kamtschatkana* at 8 weeks following wounding. This is an interior view of the same shell seen in Figure 14. Note the conchiolin has covered a greater area than that of the hole.
Abbreviations used: Arrow, edge of conchiolin. x 7.5


14

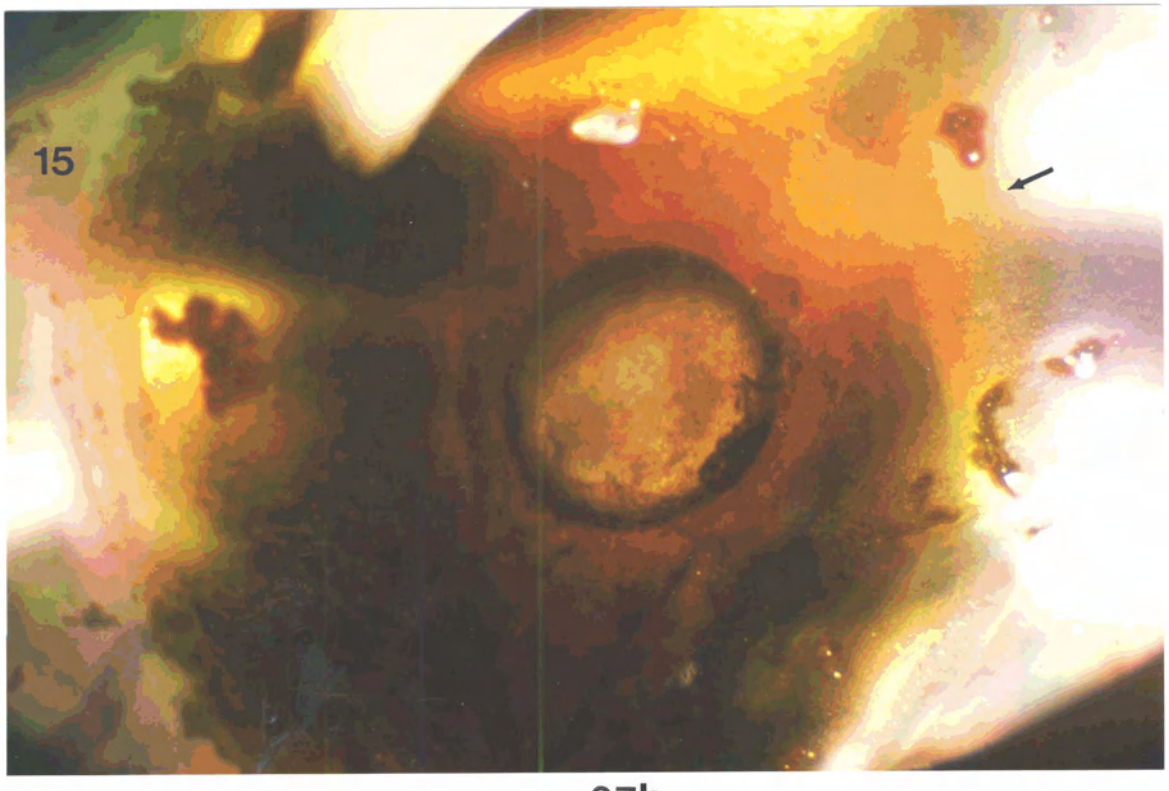
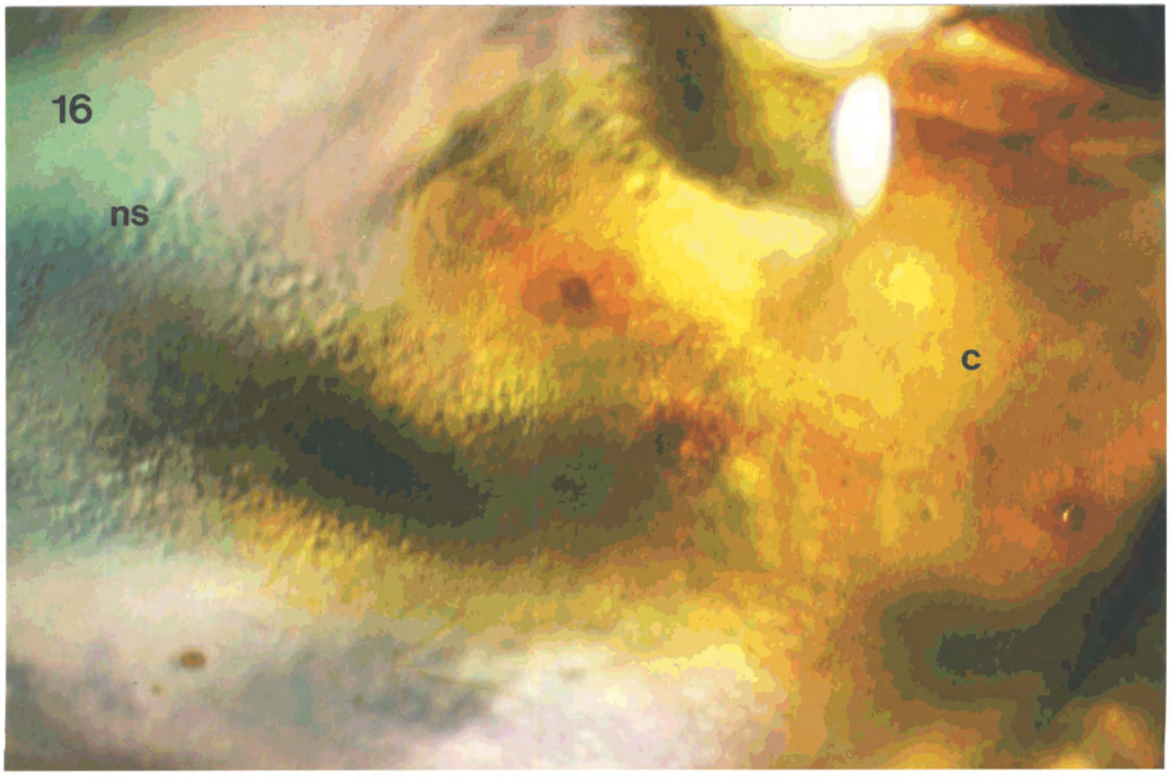
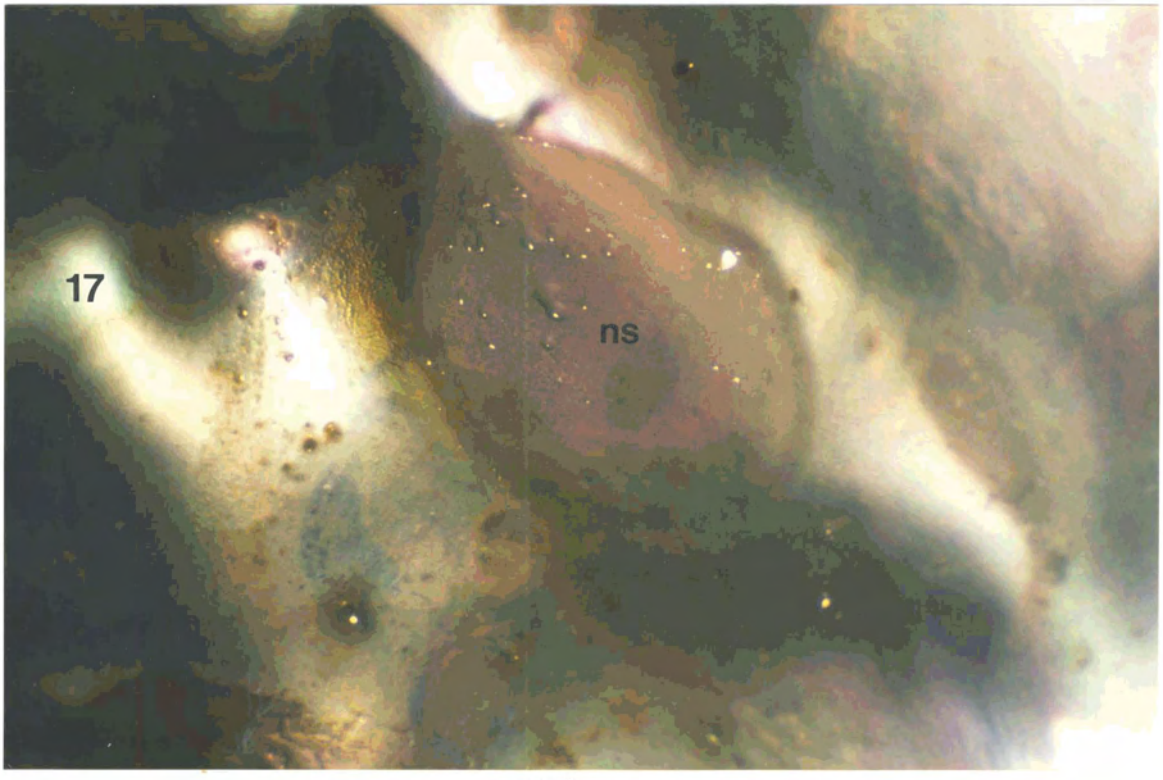

15

Figure 16: Interior view of repair of hole drilled over the gonad region of *Haliotis kamtschatkana* at 22 weeks following wounding. Much of the conchiolin has been covered with calcium carbonate crystals but it is still in view.
Abbreviations used: c, conchiolin; ns, new shell. x 7.5

Figure 17: Interior view of repair of hole drilled over the gonad region in *Haliotis kamtschatkana* at 30 weeks following wounding.
Abbreviations used: ns, new shell. x 7.5





28b

SCANNING ELECTRON MICROSCOPY

The general organization of repaired shell can be seen in Plate 1, Figures 1 and 2. The conchiolin was laid down onto the preexisting shell, onto which calcium carbonate crystals were deposited. The new calcified layer followed the contours of the conchiolin just as the conchiolin followed the contours of the pre-existing shell. The lamellar construction of the conchiolin is readily visible in Plate 1, Figure 3.

Once the conchiolin had stopped being secreted, irregular prismatic calcium carbonate crystals began to accumulate on it (Plate 2, Figures 1 and 2). On top of these, nacreous crystals were deposited (Plate 2, Figure 3). Eventually, the stacks of nacreous crystals (Plate 2, Figure 4) grew together forming a solid brick-wall like construction (Plate 2, Figure 5).

Scanning electron micrographs of the repaired shell of *Haliotis kamtschatkana*.

Plate 1

Figure 1: Cross section of shell repair of drilled hole over the gonads in *Haliotis kamtschatkana* after 25 weeks following wounding. Note how the conchiolin initially follows the contours of the original shell but becomes irregular. The new calcium carbonate crystals deposited on the conchiolin follows these contours.
Abbreviations used: c, conchiolin; n, new shell; o, original shell. x 100

Figure 2: Cross section of shell repair of drilled hole over the gonads in *Haliotis kamtschatkana* after 25 weeks following wounding. Same as Figure 1 but at greater magnification.
Abbreviations used: c, conchiolin; n, new shell; o, original shell. x 275

Figure 3: Cross section of the conchiolin of a drilled hole over the mantle in *Haliotis kamtschatkana* after 10 weeks following wounding. Note the lamellar construction of the conchiolin. x 950

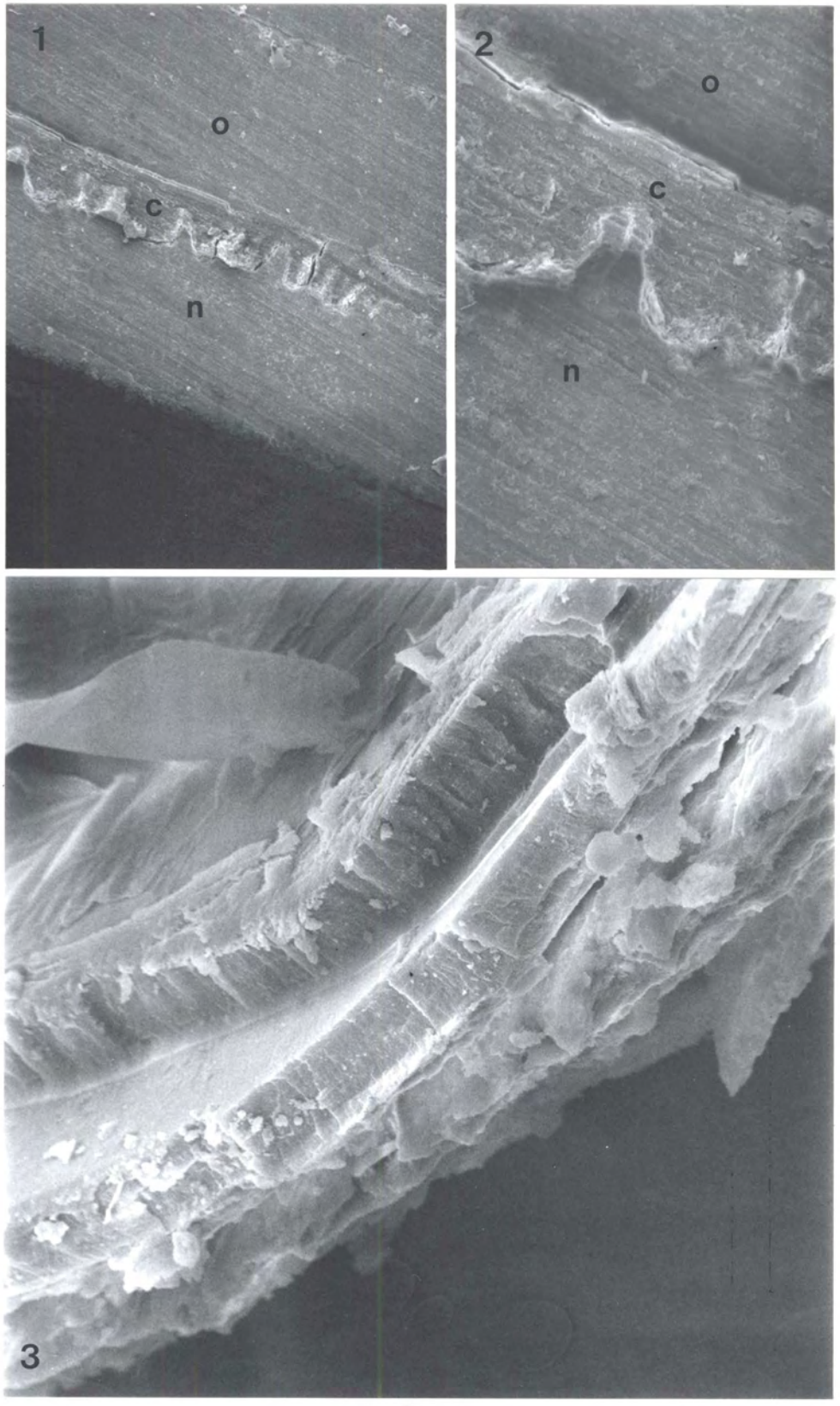


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Plate 2

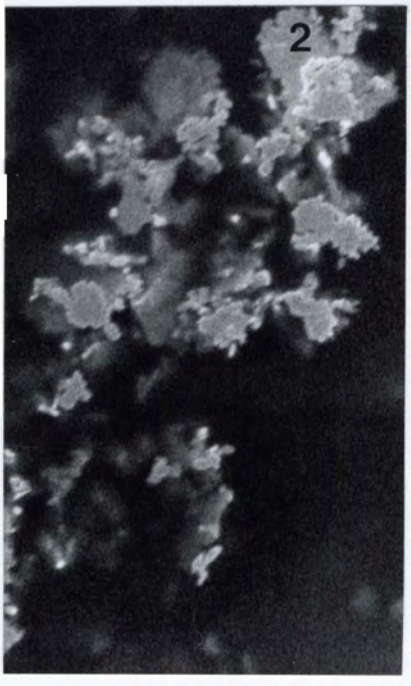
Figure 1: Plan view of conchiolin of a drilled hole in the shell of *Haliotis kamtschatkana* after 4 weeks following wounding. Note the presence of many small prismatic calcium carbonate crystals (arrow). x 200

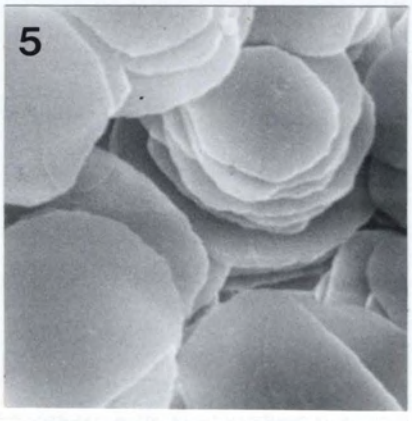
Figure 2: Plan view of conchiolin of a drilled hole in the shell of *Haliotis kamtschatkana* after 4 weeks following wounding. Same as Figure 1 but at a higher magnification. x 1600

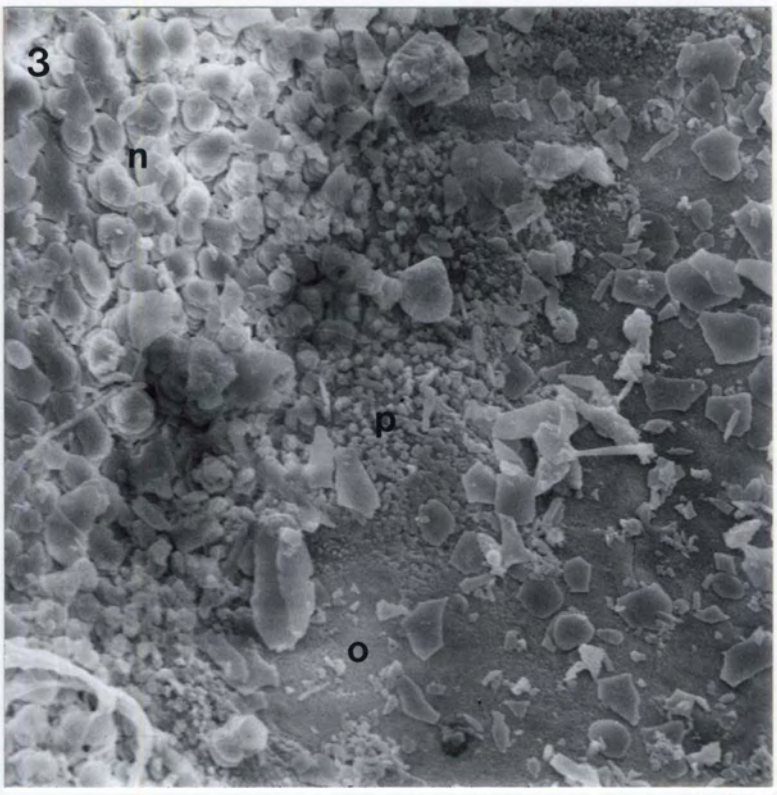
Figure 3: Plan view of repaired shell of a hole drilled over the right adductor muscle of *Haliotis kamtschatkana* after 26 weeks following wounding. Organic material was removed by soaking in Javex. This is the boundary between the original shell and the new repaired shell. Abbreviations used: n, nacre; o, original shell; p, prismatic calcium carbonate crystals. x 1100

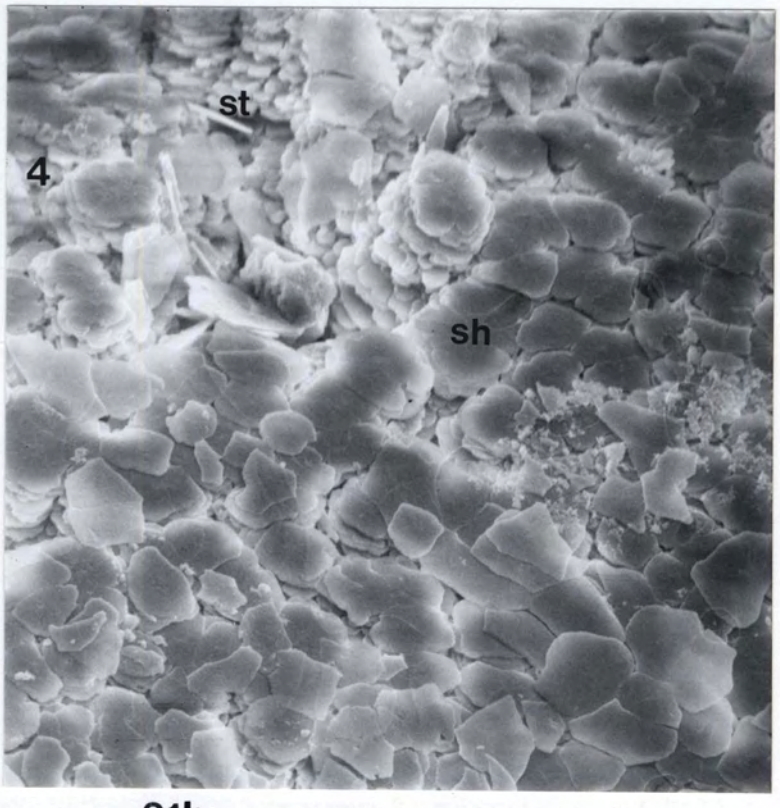
Figure 4: Plan view of repaired shell of a hole drilled over the right adductor muscle of *Haliotis kamtschatkana* after 26 weeks following wounding. Organic material was removed by soaking in Javex. Nacreous stacks can be seen as well as the sheets formed as the calcium carbonate crystals join through lateral growth.
Abbreviations used: sh, nacreous sheets; st, nacreous stacks. x 1100

Figure 5: A nacreous stack of calcium carbonate crystals in repaired shell of a hole drilled over the mantle in *Haliotis kamtschatkana* after 26 weeks following wounding. x 6000









31b

DISCUSSION

Shell repair by *Haliotis kamtschatkana* follows the general pattern of calcification observed in marine molluscs described by Bevelander and Benzer (1948): (1) the formation of an organic membrane (the conchiolin), and (2) the deposition of mineral salts (calcium carbonate crystals). The first step occurs within 1 - 2 weeks after the initial damage when conchiolin is observed forming, starting at the edges of the hole and spreading across it to form a continuous, homogeneous sheet. Repair commencing at the margin of the damaged site was also reported in species of *Anodonta* (Beedham, 1965). A few *Oncomelania formosana* had conchiolin start from an arc of the hole and then grew across it (Davis, 1964). The latter sounds similar to the pattern observed in Figure 7. Saleuddin and Chan (1969) observed the conchiolin of *Helix pomatia* appearing as either homogeneous sheets or fibres running crisscross or parallel. This was for a repair of a hole over the mantle epithelium. In the repair of a similarly placed hole in abalone, there also appears to be parallel streaks of conchiolin forming across the opening (Figure 2).

That conchiolin covers a larger area than just the area of injury is not often mentioned in the literature. The obvious advantage of this is to provide an anchor for the new shell so it will not fall off. It may also be the result of the underlying tissues sensing some type of microfractures in the

area around the wound hole caused by the original damage. This response would ensure that the repair is complete and fractures would not develop later after the repair process had ended. It would also mean that the area covered by the repair material would not be related to the size of the hole but to the extent of the wound, which would ensure the abalone does not expend energy repairing an area that does not need it.

Andrews (1935) reports that the conchiolin is firmly fastened to the inner side of the old shell in *Neritina virginea* like an inner patch, but gives no measurements. Davis (1964) reported that the conchiolin would attach to the shell 0.5 mm from the edge of the hole if the body did not make contact with the shell. The conchiolin would then be deposited to encircle the body tube. In the case of *Haliotis kamtschatkana* with a hole of 5 mm in diameter, the conchiolin covers an area on the inside of the shell 2 - 3 times the diameter of the hole (Figures 4, 9, 15 and 16). It also did not matter whether the body pressed against the shell or not. Over the right adductor muscle, the body is attached to the shell. Still the surrounding area becomes detached and secretes material to form conchiolin over a much larger area. Tompa and Watabe (1976) offer an explanation of how muscle insertion can occur with the growth of the animal, which would also explain how the columellar muscle of the abalone would reattach itself to the repaired shell. However, it is not understood how the area surrounding the damage in this region undergoes the

disintegration of the organic fibres that anchor the muscle to the shell matrix. If the underlying tissues were responding to microfractures, this would explain how the cells would be stimulated to start a process that would lead to repair. Disintegration of the organic fibres could be accomplished by amoebocytes with lysosomes.

Amoebocytes, 30-40 micrometers in diameter, were seen in the early stages of repair on a few coverslips, most notably on the coverslips from over the columellar region. They correspond to Wagge's (1951) Type B amoebocytes. Their purported role in shell repair has been traditionally controversial. For instance, amoebocytes have been reported to transport materials needed for repair to the repair site (Tsujii, 1976; Kapur and Gupta, 1970; Abolins-Krogis, 1963a; Dunachie,1963; Wagge and Mittler, 1953; and Wagge, 1951). Pan and Watabe (1989) reported amoebocytes phagocytizing shell debris in the early stages of repair in the Brachiopod *Glottidia pyramidata*. Wilbur (1964) and Beedham (1965) believe that amoebocytes only play a limited role in the early stages of repair, which would explain their limited presence in my preparation. Other than the region over the columellar muscle, however, amoebocytes were only present on one other sample. The coverslip removed after one week from over the mantle region had amoebocytes adhering to it.

Amoebocytes were never present in the region of the gonads of *Haliotis kamtschatkana*. Drilling the hole over the columellar muscle resulted in injury to the muscle, since the

shell is directly attached to the underlying tissue. And, when drilling the hole over the mantle, there was a greater risk of damage to underlying tissue because the mantle rests against the underside of the shell. The presence of amoebocytes, then, could be a response to injury of the soft tissues and not to damage of the shell. Saleuddin (1970) found amoebocytes present only when the mantle was injured when removing a piece of the shell. Amoebocytes responding to soft tissue injury has been reported in gastropods for the freshwater snail *Lymnaea stagnalis* (Sminia, Pietersma and Scheerboom, 1973) and the abalone *Haliotis cracherodii* (Armstrong et al., 1971).

All histochemical reactions with conchiolin have revealed the presence of proteins, glycoproteins or mucoproteins (Degens, 1976; Meenakshi et al., 1975; Kapur and Gupta, 1970; Saleuddin and Chan, 1969; Beedham, 1965; Abolins-Krogis, 1963b; and Durning, 1957). This also seems to be the case with *Haliotis kamtschatkana*. Mucoproteins and glycoproteins are thought to be involved in nucleation of calcium carbonate crystals (Wada, 1980; Weiner and Hood, 1975; and Saleuddin and Chan, 1969). Very small calcium carbonate crystals were seen on the conchiolin early in the repair process. This could have been a result of some cells that had continued to secrete calcium carbonate for a while after the injury had occurred. However, in shell repair of the freshwater snail *Poamacea paludosa* initial crystals of 1 - 3 micrometers were deposited after the formation of the conchiolin (Blackwelder and Watabe, 1977).

Despite an initial deposition of calcite crystals on the conchiolin, a mineralized layer does not develop quickly, and more conchiolin is continued to be laid done in lamellar fashion (Plate 1, Fig. 3,). There is also variation among and within individual abalone regarding the thickness of the conchiolin (cf. ref Chapter 1). This could be a result of the soluble organic matrix of the conchiolin regulating the nucleation and growth of calcium carbonate crystals rather than just initiating them (Wheeler, George and Evans, 1981). In their scenario, the growth of the mineralized layers would not start until the secretion of the soluble matrix had been controlled to allow for greater crystallization. The controlling factors for soluble matrix are not known.

Abalone shells consist mainly of two layers: an outer prismatic layer and an inner nacreous layer (Dauphin et al., 1989; Mutvei, Dauphin and Cuif, 1985; and Nakahara, Bevelander and Kakei, 1982). The outer prismatic layer is not seen well here because of the preparation technique used. However, prismatic crystals can be seen forming on the conchiolin in Plate 2, Figures 1 and 2 (Appendix II). This meets the first requirement of Nakahara and Bevelander (1971) for the formation of a prismatic layer. That is, the formation of a lamella that serves as the internal boundary of the future prisms. Prismatic crystals can also be seen at the base of the nacreous layer in Plate 2, Figure 3 (Appendix II).

The inner nacreous layer in *Haliotis kamtschatkana* is made

up of stacks of crystals that grow laterally to form sheets (Appendix II, Plate 2, Figures 4 and 5). This is the typical arrangement for gastropod nacre (Mutvei, 1980; and Wise, 1970b). The final nacreous layer is much thicker than the prismatic layer (Appendix II, Plate 2, Figure 3) which is usual among prosobranch gastropods (Wise, 1970b; and Wise and Hay, 1968).

Suzuki (1983) reports an inner prismatic layer on the normal shell of *Haliotis discus*. This was not always seen in repaired shell, however. Only an outer prismatic layer was found here for *Haliotis kamtschatkana*, the nacreous layer being the inner most layer.

SUMMARY

The shell repair process was studied for the archeogastropod *Haliotis kamtschatkana*. After initial injury by drilling a 5 mm hole in various regions of the shell, a protein membrane, called conchiolin, composed of glycoproteins is laid down as a homogeneous sheet covering the hole and an area 2 - 3 times the hole's diameter. Calcium carbonate crystals are deposited on the conchiolin but a truly mineralized layer does not form until conchiolin secretion is controlled. The first crystals of a mineralized layer are prismatic. On top of these are deposited stacks of calcium carbonate crystals forming a thicker nacreous layer. The structure of the repaired shell of *Haliotis kamtschatkana* is compared to that of other mollusca

reported in the literature.

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CHAPTER TWO

Factors that Influence the Rate of Shell Repair in *Haliotis kamtschatkana*

INTRODUCTION

The human obsession for the beauty of pearls has led to seeking a methodology in producing them at will rather than finding them by happenstance (Joyce and Addison, 1993). As early as the 13th century artificial pearl production was underway (Simkiss and Wada, 1980). Interest in pearls was not restricted to those found in the best known of the pearl producing mollusca, the pearl oyster *Pinctada fucata*, for natural abalone pearls were collected over 1200 years ago (Shirai, 1970), and abalone pearls were first cultured in the late nineteenth century (Bouton, 1898), continuing to the present (Takashi, 1980; and Fankboner, 1991). Still, no data are found on parameters that might affect abalone pearl production. With the idea of developing a pearl culture industry in Canada, the pinto abalone, *Haliotis kamtschatkana*, readily found along the west coast of Canada and already explored for aquaculture (Paul, Paul, Hood and Nerve, 1976), was chosen as a possible pearl producer. This present study examines parameters that might enable the cultivator to maximize the development of pearls in this species.

The use of abalone in pearl culture has concentrated on blister pearls (Shirai, 1970). These are produced by affixing a pearl nucleus between the mantle and the shell (Fankboner, 1991). The abalone treats this intrusion as an irregularity in the shell's inner surface and reacts by covering the nucleus

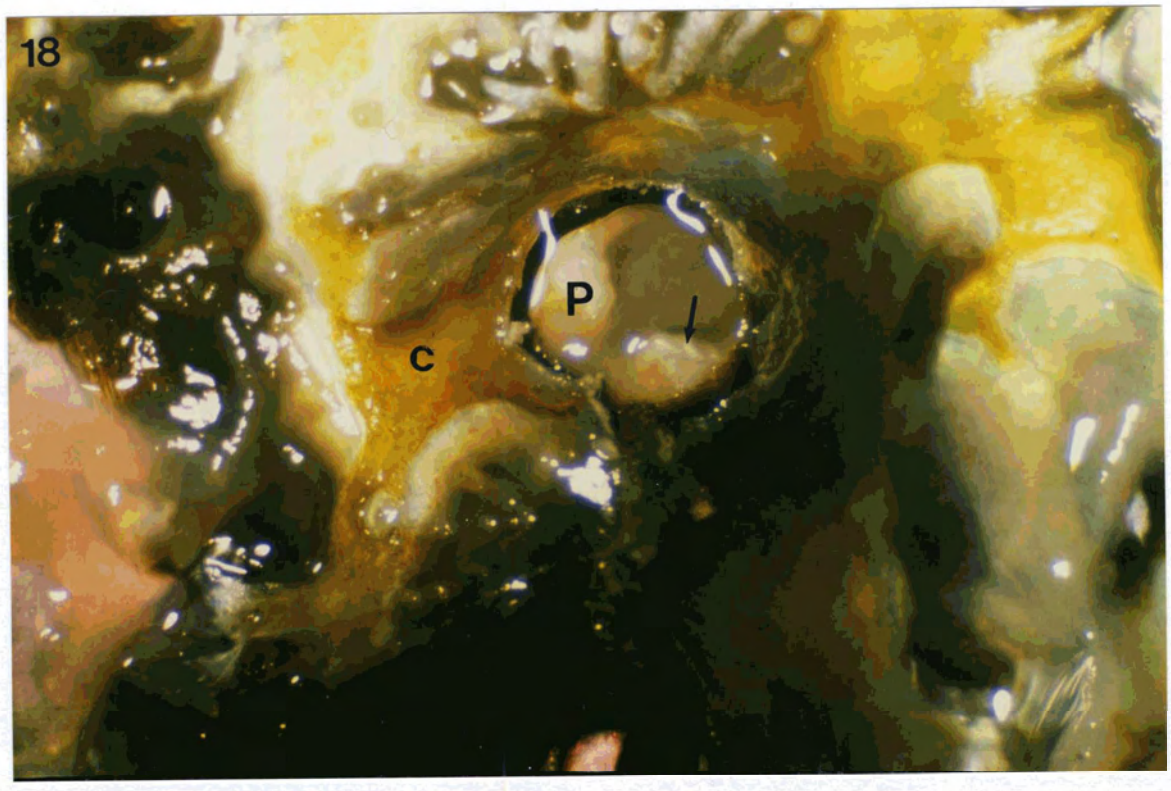
with nacre. This response is identical to the response of abalone to the shell-boring bivalve *Penitella conradi* (Cox, 1962; Figures 18, 19 and 20). After settling on an abalone shell, *P. conradi* is believed to use its sculpted anterior shell and especially its umbonal reflections to erode a pit into the host's shell (Turner, 1955). Once the pit breaks through to the inner side of its host's shell, the abalone's epithelium is stimulated to begin a process of repair which is initiated by laying down an organic membrane (the conchiolin) over the area of the intrusion. Later calcium carbonate crystals will be deposited onto the conchiolin, initially irregular prismatic crystals succeeded by pyramidally stacked nacreous crystals, embedded in an organic membraneous envelope. When the bivalve erodes away this shell repair, the abalone responds by laying down a new layer of conchiolin and repair shell. Often however, this catch up is unnecessary because of the death or decreased shell growth of the bivalve. If the bivalve has penetrated deeply into the shell when this occurs, the result is a naturally formed blister pearl. The development of secretions of nacre over a pearl nucleus, then, would likewise be considered shell repair. Therefore, observing the process of shell repair should provide insight into the process of natural blister pearl production and may lead to ways to maximize production of cultured blister pearl.

The following account examines two parameters that may affect the rate of shell repair. The first parameter is the

Formation of a natural blister pearl in *Haliotis kamtschatkana* by invasion of *Penitella conradii*.

Figure 18: *Penitella conradii* shown intruding through the shell of *Haliotis kamtschatkana*. The sculpted edges of the *Penitella conradii* are visible. Note that conchiolin is visible around the opening the bivalve has made. Abbreviations used: Arrow, ridges of the umbos; P, *P. conradii*; c, conchiolin. x 7.5

Figure 19: *Penitella conradii* shown intruding through the shell of *Haliotis kamtschatkana*. The abalone has successfully covered the hole and the intruding bivalve shell with conchiolin.
Abbreviations used: Arrow, umbos of *P. conradii*; c, conchiolin. x 7.5


18
P
C

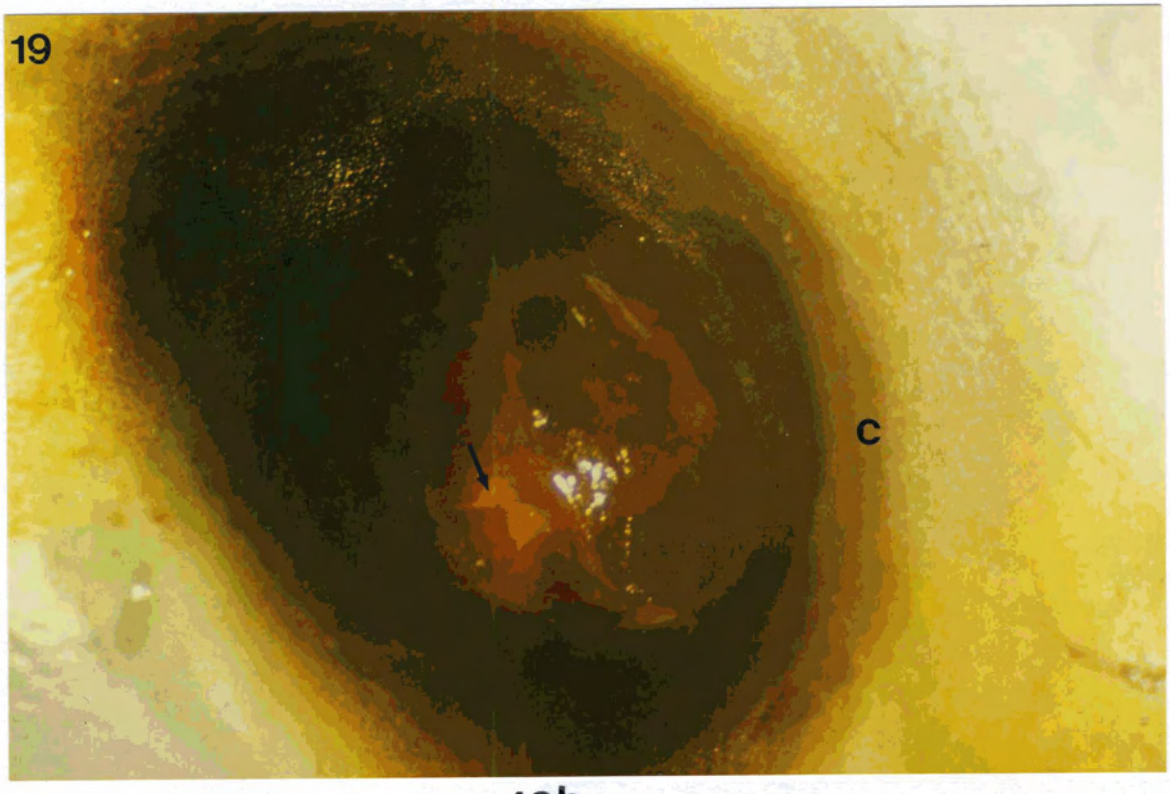
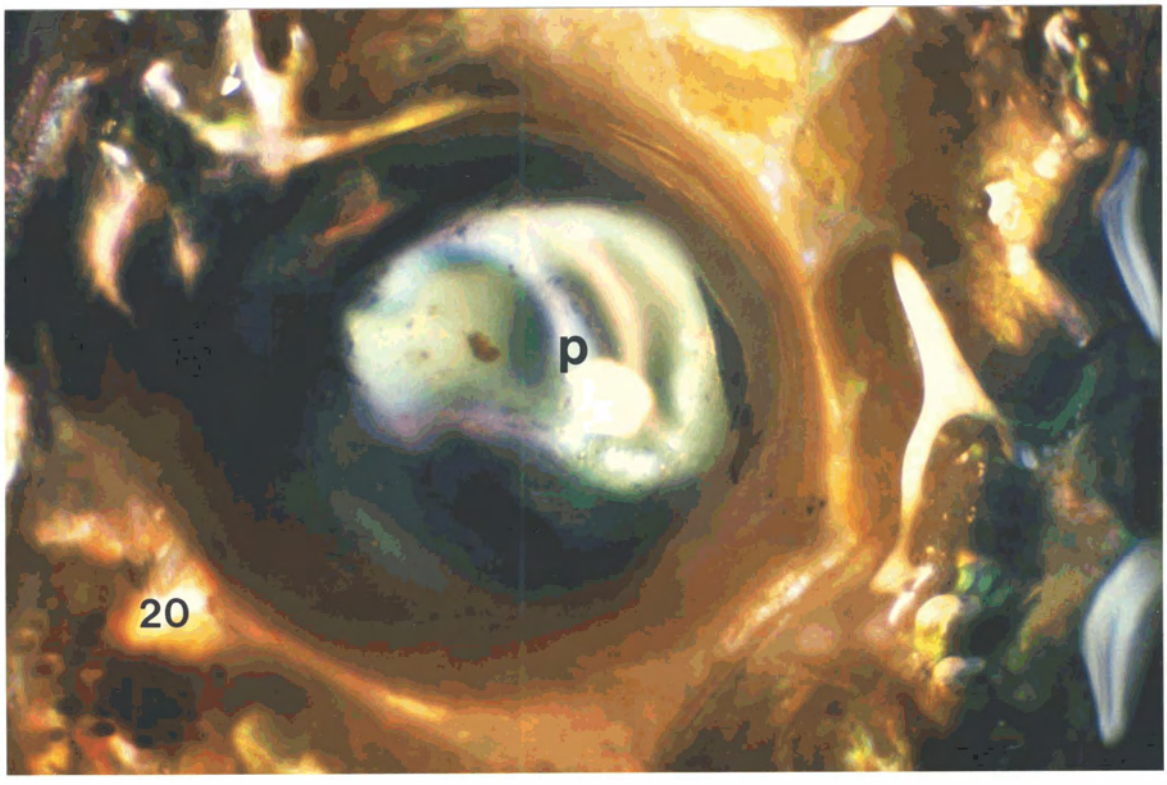

19
C

Figure 20: Naturally formed blister pearl on the inside of the shell of a *Haliotis kamtschatkana*. This was formed over the intruding shell of a *Penitella conradii* which was still visible from the exterior.
Abbreviations used: p, natural blister pearl. x 7.5



49b

influence of the location of the damage on repair rate, as has been found with other mollusca (Andrews, 1935; Wagge, 1951; Saleuddin and Wilbur, 1969; Kunigelis and Saleuddin, 1983; and Watabe, 1983). To maximize the rate of return to the cultivator, the area of the shell that is most rapidly repaired would be the optimal location for pearl nucleus implantation. The second parameter is a possible relationship between growth rate and repair rate. If there is a positive correlation between these, it will benefit the cultivator to use faster growing animals and to hold them under conditions that will foster maximal growth rates. Ebert and Houk (1984) found that raised temperatures and complete darkness increased the growth rates for *Haliotis rufescens*. These same conditions are investigated to see if they influence the growth of *H. kamtschatkana*. Finally the effects on growth rates of implanting pearl nuclei into abalone are investigated.

MATERIALS AND METHODS

ABALONE COLLECTION AND HUSBANDRY

Specimens of *Haliotis kamtschatkana* were collected by divers from Barkley Sound around Bamfield, British Columbia (Latitude 48°50'N and Longitude 125°15'W). Specimen size ranged from 5 centimetres to the upper size limit for this species, 15 centimetres. Because of the number of abalone being held and

the limited number of containers available, it was necessary to use containers of two different sizes: 120 cm x 60 cm x 20 cm flat bottomed fibreglass trays and 120 cm x 120 cm x 60 cm flat bottomed fibreglass tanks, all with flow through sea water.

The mean abalone size was not statistically different between the tanks. This permitted a technique to keep initial densities of abalone equal for both types of containers. By dividing the submerged surface area of each tank by the number of abalone in that tank an area of substrate per abalone was calculated and could be adjusted by the addition or subtraction of individuals. This area was approximately 300 cm^2 of substrate per abalone for each container.

Abalone were kept in two different photoperiods, natural light or complete darkness, the latter resulting in increased feeding activity for *Haliotis rufescens* (Ebert and Houk, 1984). Those kept in complete darkness were in trays with plywood covers.

All animals were fed fresh *Nereocystis leutkeana* and/or *Macrocystis integrafolia* collected every 7 - 10 days from areas around the Bamfield Marine Station. Containers in natural photoperiod also had noticeable diatom growth on the sides and bottom which was not seen in tanks with covers. Tanks and trays were cleaned at the time of feeding. All tanks and trays were checked daily and dead animals were removed when found.

EFFECT OF LOCATION OF DAMAGE ON RATE OF REPAIR

The effect of location of damage on the rate of shell repair was investigated by drilling a 5mm hole in the shell either over the mantle, about 1 cm from the growing edge; or over the site of attachment of the shell muscle; or over the gonads (Figure 1). Only one hole per abalone was drilled to decrease the probability of increased mortality due to physical stress (Elston and Lockwood, 1983). A Crystallite 5 mm diamond core drill bit mounted in a drill press with a steady stream of sea water washing the bit during drilling was used to bore the holes through the shell. Abalone which were injured during the drilling process were discarded. To distinguish individual abalone, each animal was tagged with a numbered plastic Floytag disc cemented to the shell with an epoxy putty. Each abalone was weighed and measured for shell length at both the beginning and the end of the repair period. The abalone were split into two groups of equal numbers to investigate the effect of photoperiod on repair rate. One was kept in a natural photoperiod and the other in complete darkness.

Originally, the number of abalone with holes drilled in each region of the shell was approximately equal. Due to mortality, more abalone had to be prepared to keep the group sizes similar. The date of preparation was recorded as was the date of final measurement of the repair. At the time of sampling, the shell around the repair area was removed from the animal by drilling

around the original hole using a Crystallite 16mm diamond core drill and the technique described above to drill the original hole. The shell fragment removed was then cut in half, bisecting the 5 mm hole, using a lapidary saw. The thickness of the repair material was magnified 50 times with a Zeiss dissecting microscope and measured using an ocular micrometer. Three measurements were taken; thickness of the total repair; thickness of the conchiolin; and the thickness of the calcium carbonate crystals. It was not possible to distinguish the prismatic layer from the nacreous layer using a dissecting microscope so these two layers were measured together. For a measurement to be taken, a complete layer of material had to cover the hole and be at least 0.008 mm thick (0.5 of an ocular unit at 50x magnification).

The time period the abalone underwent repair varied from 69 to 249 days. A rate of repair was determined by dividing the thickness of the repair by the number of days since the hole was made. The final analysis was done on 49 abalone with holes over the mantle region, 36 abalone with holes over the shell muscle region and 48 abalone with holes over the gonad region, totalling 133 abalone. The size of abalone was determined by the ability to remove an individual from a tank. When abalone are disturbed they cling to the substrate so effectively that "it is easier to tear the shell from the animal than to release the foothold" (Croft, 1929, p. 18). Therefore animals were selected that allowed safe removal from the tank.

EFFECT OF LOCATION OF DAMAGE ON REPAIR RATE (CONTROLLING FOR INDIVIDUAL DIFFERENCES)

Due to variation in repair rates among individual animals (Kunigelis and Saleuddin, 1983), a second experiment was designed to control for individual differences between repair sites. This involved drilling a 5 mm hole in the shell over each of the three regions mentioned above resulting in each abalone having three holes bored in it. Holes were made in the same way as in the first experiment. The abalone were prepared on September 27, 1989 and the amount of repair was measured on December 18, 1989, a total of 82 days. Approximately 20 animals were prepared but only fourteen survived to be analyzed. The abalone were approximately 9 centimetres long. Abalone were weighed and measured at the beginning and at the end of the experiment. Results were determined as per the first experiment.

GROWTH OF PEARL-NUCLEATED ABALONE

To determine the effects on growth due to implantation of pearl nuclei, abalone were implanted with a 16 mm semispherical polyester resin pearl nucleus between the shell and gonadal epithelium. Each animal was tagged in the same manner as the shell repair animals. After implantation and at approximately monthly intervals the abalone were wet weighed to the nearest

0.1 gram on a balance and their greatest length measured to the nearest 0.1 millimetre using Vernier callipers. The abalone were separated into two groups; one kept in a natural photoperiod and the other in complete darkness. Implantation took place from May to August, 1988. Five hundred animals were implanted but, due to mortality and sampling, data from only 215 were used in the statistical analysis.

GROWTH OF PEARL-NUCLEATED ABALONE AND TEMPERATURE

The effects of warmed water on the growth of nucleated *H. kamtschatkana* was investigated between January 24, 1989 and April 30, 1989. One hundred abalone were kept in a tank at ambient temperature and starved for one month before being implanted with a 16 mm semispherical polyester resin nucleus and tagged as in the first growth experiment. They were then separated into two groups of 50 each. One group was held in a tray at ambient water temperature (7°-8° C) and the other group was held in a tray where the water temperature was slowly increased over a period of days to 14° C. The water flow for both trays was approximately 40 litres per hour. The abalone were measured and weighed at the beginning and at the end of the experiment.

STATISTICAL ANALYSIS

Statistical analysis was done on IBM compatible personal computers using the Number Cruncher Statistical System (NCSS)(Hintze, 1989). The repeated measures ANOVA and the corresponding Neuman-Keul's test for differences between paired means were done using NCSS and a hand calculator following the method described by Winer (1962).

RESULTS

EFFECT OF LOCATION OF DAMAGE ON RATE OF REPAIR

Data gathered from the experiment using abalone with one hole drilled in either the mantle region of the shell, the shell muscle region or the gonad region are summarized in Table A.1. For comparison to the experiment using abalone with three holes per animal, data from abalone that were in the experiment for 69 and 83 days are summarized in Table A.2.

Variable Name	Count (n)	Mean	Standard Deviation	Minimum Value	Maximum Value
DAYS	133	171	52.1	69	249
INITIAL WEIGHT	124	163.4 g	88.28391	25.8 g	487.8 g
FINAL WEIGHT	128	170.8 g	90.30388	26.6 g	492.9 g
% WEIGHT CHANGE	120	12.31	14.23711	-9.528	73.333
INITIAL LENGTH	124	9.72 cm	1.674022	5.57 cm	13.93 cm
FINAL LENGTH	128	9.79 cm	1.6385	5.62 cm	13.87 cm
% LENGTH CHANGE	120	2.61	5.090375	-8.883	37.343
MEMBRANE	133	0.1457 mm	0.1389	0 mm	.672 mm
NACRE	133	0.1244 mm	0.1435	0 mm	.64 mm
REPAIR	133	0.2701 mm	0.2011	0 mm	.752 mm
REPAIR/DAY	133	1.455 Um	0.9584	0 mm	3.672 Um
M-MEMBRANE	49	0.1496 mm	0.1616	0 mm	.672 mm
M-NACRE	49	0.1146 mm	0.1289	0 mm	.4 mm
M-REPAIR	49	0.2642 mm	0.1845	0 mm	.672 mm
M-REP/DAY	49	1.534 Um	1.005	0 mm	3.672 Um
S-MEMBRANE	36	0.1342 mm	0.1575	0 mm	.672 mm
S-NACRE	36	0.07556 mm	0.1442	0 mm	.64 mm
S-REPAIR	36	0.2098 mm	0.2360	0 mm	.752 mm
S-REP/DAY	36	1.119 Um	1.034	0 mm	3.241 Um
G-MEMBRANE	48	0.1503 mm	0.09415	0 mm	.448 mm
G-NACRE	48	0.171 mm	0.1456	0 mm	.56 mm
G-REPAIR	48	0.3213 mm	0.1785	0 mm	.72 mm
G-REP/DAY	48	1.627 Um	0.7922	0 mm	3.435 Um

TABLE A.1 Summary of data of the one hole per abalone experiment. Differences in the count (sample size) result from missing data. MEMBRANE refers to the conchiolin partition of the hole repair. NACRE refers to the calcium carbonate crystals partition of the hole repair. REPAIR refers to the total hole repair (MEMBRANE+NACRE). Variable REP/DAY is REPAIR divided by DAYS for that abalone (unit value is micrometers/day). A letter in front of the variable indicates the region of the abalone where the hole was drilled: M = the mantle region; S = the shell muscle region; G = the gonad region.

Variable Name	Count (n)	Mean	Standard Deviation	Minimum Value	Maximum Value
M-MEMBRANE	6	0.02933mm	0.02563	0mm	.064mm
M-NACRE	6	0	0	0mm	0mm
M-REPAIR	6	0.0293mm	0.2563	0mm	.064mm
M-REPAIR/DAY	6	0.4251Um	0.3715	0Um	.927Um
S-MEMBRANE	11	0.04654mm	0.04200	0mm	.122mm
S-NACRE	11	0mm	0	0mm	0mm
S-REPAIR	11	0.04654mm	0.04200	0mm	.122mm
S-REPAIR/DAY	11	0.6177Um	0.5760	0Um	1.623Um
G-MEMBRANE	7	0.04800mm	0.04525	0mm	.128mm
G-NACRE	7	0mm	0	0mm	0mm
G-REPAIR	7	0.04800mm	0.04525	0mm	.128mm
G-REPAIR/DAY	7	0.6398Um	0.6419	0Um	1.855Um

TABLE A.2 Summary of data for abalone that repaired for 69 and 83 days in the experiment using abalone with one repair site. A letter in front of the variable indicates the region of the abalone where the hole was drilled: M = the mantle region; S = the shell muscle region; G = the gonad region. MEMBRANE refers to the conchiolin partition of the hole repair. NACRE refers to the calcium carbonate crystals partition of the hole repair. REPAIR refers to the total hole repair (MEMBRANE+NACRE).

To test if photoperiod, position of the hole or the date the hole was drilled influenced the repair rate, a General Linear Model Analysis of Variance (GLM ANOVA) was done on the main effects of the position of the hole (POSITION), the photo period the abalone was exposed to (PHOTO) and the date the hole was drilled in the shell (START) against the daily repair rate (REPAIR/DAY). Interactions of these effects were not tested due to empty cells. The analysis of variance report is shown in Table A.3 (Appendix I). The results show the photoperiod had no significant effect on the rate of repair at the 95% level (p=0.5708). There was also no significant difference among repair rates between positions of the hole at the 95% level

(p=0.0590). However, the difference in repair rates due to the date the abalone was originally drilled was highly significant (p=0.0000). The highest mean repair rate was for abalone drilled on the second earliest date, September 18, 1988 and the lowest mean repair rate was for abalone drilled on the latest date, February 28, 1989.

Neuman-Keul's post-hoc multiple comparison test was computed on the adjusted means of variable START to determine significant differences among repair rate adjusted means of abalone with different start dates for the repair process. The results (Table A.4, Appendix I) show that the repair rate means can be grouped in three categories; those drilled on September 18, 1988 and November 11, 1988; those drilled on September 15, 1988 and November 19, 1988; and those drilled on February 28, 1989; November 17, 1988; November 20, 1988; and November 18, 1988.

Scatter plots and correlations were done on the start date versus the repair rate. A total of four plots were made; one for all the repair rates, and one each for the repair rates of the holes over the mantle, of the holes over the shell muscle, and of the holes over the gonads (Figures 21, 22, 23, and 24, Appendix I). All the correlations were negative (-0.4596, -0.4199, -0.5023, and -0.4429 respectively) indicating that the repair rate for all holes decreased the later in the fall or winter the hole was drilled.

A stepwise regression analysis of the drilling date

(START), the initial weight of the abalone (INITIAL WEIGHT), the percent change in the abalone weight (% WEIGHT CHANGE), the initial length of the abalone (INITIAL LENGTH) and the percent change in abalone length (% LENGTH CHANGE) against the daily repair rate (REPAIR/DAY) was done to determine if any linear relationships exist that could provide a predictor for determining a maximum repair rate reconfirm the relationship between the start date and repair rates (Table A.5, Appendix I), showing that this was the only linear relationship among these variables. The start date was regressed against the repair rate. The results (Table A.6, Appendix I) show that the linear relationship was highly significant ($p=0.0000$) and that the date of injury accounts for 20% of the variation in the repair rate (adjusted $R^2=0.2006$).

EFFECT OF LOCATION OF DAMAGE ON REPAIR RATE (CONTROLLING FOR INDIVIDUAL DIFFERENCES)

The data from the experiment using abalone each drilled with three holes is summarized in Table B.1. Since the number of days the abalone underwent repair was the same, the thickness of the repair indicates the speed of the repair. Measurable repair was seen in all animals for the three regions except in one abalone that failed to lay down a complete layer of conchiolin over the shell muscle. Each region had abalone that failed to deposit a measurable amount of calcium carbonate

crystals. The maximum thickness for the conchiolin for every region was 0.16 mm. The similarities between these results raise the question of repair in one region relating to repair in other regions.

Variable Name	Count (n)	Mean Thickness	Standard Deviation	Min. Thick.	Max. Thick
M-MEMBRANE	14	0.09029 mm	0.03692	0.048 mm	0.16 mm
M-NACRE	14	0.02514 mm	0.03421	0 mm	0.096 mm
M-REPAIR	14	0.1154 mm	0.06187	0.048 mm	0.256 mm
S-MEMBRANE	14	0.07886 mm	0.05039	0	0.16 mm
S-NACRE	14	0.01029 mm	0.02315	0	0.08 mm
S-REPAIR	14	0.08914 mm	0.05844	0	0.192 mm
G-MEMBRANE	14	0.08343 mm	0.03086	0.032 mm	0.16 mm
G-NACRE	14	0.04229 mm	0.04886	0	0.16 mm
G-REPAIR	14	0.1257 mm	0.05357	0.032 mm	0.224 mm
MEMBRANE	42	0.08419 mm	0.03952	0	0.16 mm
NACRE	42	0.02590 mm	0.03838	0	0.16 mm
REPAIR	42	0.1101 mm	0.05873	0	0.256 mm

TABLE B.1 Summary of the data used in the statistical analysis of the three hole per abalone experiment. The definitions of the variable names are as follows. A letter in front of the variable indicates the region of the abalone where the hole was drilled: M = the mantle region; S = the shell muscle region; G = the gonad region. MEMBRANE refers to the conchiolin partition of the hole repair. NACRE refers to the calcium carbonate crystals partition of the hole repair. REPAIR refers to the total hole repair (MEMBRANE+NACRE). The variables with counts of 42 are the combined data of the three regions.

Correlations (Table B.5, Appendix I) between conchiolin thicknesses, calcium carbonate crystal thicknesses and total repair thicknesses show extreme variation and only moderate relationships. The strongest relationships were between the thicknesses of the calcium carbonate crystals over the mantle and over the gonads (r=0.5285); and the thicknesses of the conchiolin and the calcium carbonate crystals over the mantle (r=0.5123).

The mean thickness of the total repair material was thicker

for the hole over the gonads (0.1257 mm) than the hole over the mantle (0.1154 mm), which was thicker still than the hole over the shell muscle (0.08914 mm). However, the difference between the three thicknesses were not statistically significant at the 95% level ($2.09_{0.05,\ 3.37}<F$) as found by a repeated measures ANOVA (Table B.3, Appendix I).

When the repair material was partitioned into conchiolin and calcium carbonate crystal components similar differences were found. In the gonad region both these partitions had thicker mean results than in the mantle region or in the shell muscle region; but the only significant difference at the 95% level is found between the thickness of the calcium carbonate crystals over the gonads (0.04229 mm) and the thickness of the calcium carbonate crystals over the shell muscle (0.01029 mm)(Tables B.4 and B.5, Appendix I).

GROWTH OF PEARL-NUCLEATED ABALONE

A summary of the data from the growth experiment with abalone implanted with semi-spherical pearl nuclei is found in Table C.1. Since the sizes of the abalone differed the percent weight change of abalone was calculated to allow for comparisons. The mean percent weight change for implanted abalone was 14.41%. The minimum value for the length change in abalone was -0.24 cm. It was observed that no abalone broke the marginal edge of their shell and no shrinkage of shell length in

abalone appears in the literature. Therefore, this minimum must have been part of the measurement error, which must have varied by as much on the positive side of the scale. Since the maximum

Statistical Summary

Variable Name	Count (n)	Mean	Standard Deviation	Minimum Value	Maximum Value
TOTAL DAYS	215	270	37.53331	148	342
INITIAL LENGTH	215	10.15 cm	1.437611	6.92 cm	14.01 cm
FINAL LENGTH	215	10.34 cm	1.331805	7.39 cm	13.95 cm
LENGTH CHANGE	145	0.0349 cm	0.1136	-0.24 cm	0.24 cm
INITIAL WEIGHT	215	167.6 g	78.44608	37.5 g	448.7 g
FINAL WEIGHT	215	185.4 g	77.05953	59.2 g	435.4 g
% WEIGHT CHANGE	215	14.41	15.09564	-11.11	77.07
% WT CHANGE/DAY	215	0.05404	0.05676	-0.04227	0.2769

TABLE C.1 Summary of data used in statistical analysis in the pearl nucleated abalone experiment.

length change was also 0.24 cm, no mean change in length can be concluded.

A GLM ANOVA was done to determine the effects of photoperiod (PHOTO), sex (SEX), the implantation date (DATE), and the interaction that sex and photoperiod have on the daily growth rate measured by the per cent change in weight (% WEIGHT CHANGE). The results (Table C.2, Appendix I) show no significant growth differences between sexes (p=0.3780) or the interaction of sex and photoperiod (p=6333), but highly significant growth differences between photoperiod (p=0.0000) and date of implantation (p=0.0000). Abalone raised in a normal photoperiod gained significantly more weight than abalone kept in darkness.

Neuman-Keul's post-hoc multiple comparison report on pairs

of adjusted means was done on the results of the implantation date (DATE) from the GLM Anova in Table C.2. The results (Table C.3, Appendix I) show that the only significant differences (at the 95% level) were between abalone implanted on May 22, 1988 or May 11, 1988 and those implanted on May 29, 1988, June 6, 1988 or August 20, 1988.

A stepwise regression was performed to determine if the implantation date (DATE), the initial abalone weight (INITIAL WEIGHT), the initial abalone length (INITIAL LENGTH) and the total number of days the abalone were implanted (TOTAL DAYS) were linearly related to abalone growth as measured by the per cent weight change (% WEIGHT CHANGE). The results (Table C.4, Appendix I) indicate implantation date and initial weight were related to the percent weight change ($p=0.0236$ and $p=0.0000$ respectively). A scatter plot of percent weight change versus implantation date (Figure 25, Appendix II) shows the relationship may not be linear while a similar plot between initial weight and percent weight change (Figure 26, Appendix II) reinforces a linear relationship.

A linear regression was performed on the percent weight change and the initial weight, the results (Table C.5, Appendix I) showing a highly significant ($p=0.0000$) linear relationship between the two. The initial weight of abalone explained 23% of the variation in the percent weight change (adjusted $R^2=0.2297$).

GROWTH OF PEARL-NUCLEATED ABALONE AND TEMPERATURE

A summary of the data from the experiment investigating the relationship between water temperature and the growth of nucleated abalone is found in Table D.1. Due to the time required to implant abalone with pearl nuclei, it was not possible for all abalone to have the same start date. Nucleation dates were from January 24 to January 31, 1989. To account for this small difference in time, the percent weight change was divided by the number of days to give a daily rate of percent weight change. This was the variable used in the statistical analysis.

A GLM ANOVA to test the main effects of water temperature (TEMPERATURE) on the growth of abalone measured as the percent daily increase in weight (%WEIGHT/DAY) (Table D.2, Appendix I) show that abalone kept at ambient temperature gained more weight than did the ones in the heated tray but the gain was not statistically significant at the 95% level ($p=.0823$). However significantly more abalone died in the tray at ambient temperature (22) than the heated tray (9).

Statistical Summary

Variable Name	Count	Mean	Standard Deviation	Minimum Value	Maximum Value	
(Kept at 14°C)						
INITIAL LENGTH	50	10.15 cm	1.161745	7.88 cm	12.92	cm
INITIAL WEIGHT	50	164.0 g	67.59291	64.1 g	342.7 g	
FINAL LENGTH	41	10.16 cm	1.122707	7.86 cm	12.55 cm	
FINAL WEIGHT	41	172.0 g	64.96255	62.6 g	359.5 g	
% WT CHANGE/DAY	41	0.03622	0.07199	-0.09178	0.1629	
(Kept at 7°-8°C)						
INITIAL LENGTH	50	9.55 cm	1.290501	7.32 cm	11.97	cm
INITIAL WEIGHT	50	138.7 g	59.41165	46.2 g	271.0 g	
FINAL LENGTH	28	9.75 cm	1.190317	7.42 cm	11.52 cm	
FINAL WEIGHT	28	151.4 g	55.23031	61.7 g	266.1 g	
% WT CHANGE/DAY	28	0.06843	0.07804	-0.09584	0.2475	
(Abalone that died during the experiment)						
INITIAL LENGTH	31	9.58 cm	1.452397	7.36 cm	12.92	cm
INITIAL WEIGHT	31	137.4 g	72.93187	46.2 g	320.4 g	
(Abalone that survived the experiment)						
INITIAL LENGTH	69	9.97 cm	1.153979	7.32 cm	12.57	cm
INITIAL WEIGHT	69	157.6 g	59.97157	58.0 g	342.7 g	

TABLE D.1 Statistical summary of the data used in the statistical analysis of the heat and nucleated abalone growth experiment.

DISCUSSION

EFFECT OF LOCATION OF DAMAGE ON RATE OF REPAIR

Repair rate in *Haliotis kamtschatkana* is highly variable, as has been found in *Helisoma duryii* (Kunigelis and Saleuddin, 1983). Some factors were found that had little influence on that variability. Unlike *Tegula funebralis* (Peppard,1964) male and female abalone had similar repair rates. A difference was not found due to a normal or 24 hour dark photoperiod, as was found for the onset of repair in *Helix pomatia* (Saleuddin and

Chan, 1969).

The size, by weight or length, or growth rate of an abalone showed no evidence of influencing repair rate. Similar results were found by Palmer (1983) and were reported by other 2researchers in Watabe (1983). Although Zischke, Watabe and Wilbur (1970) found calcium deposition to increase toward the shell aperture with shell length in *Ampullarius glaucus*, this was studied for normal shell growth and not shell repair. Their explanation for increased shell thickness, however, may explain why size does not affect shell repair. As the snail shell grows longer, the linear growth rate decreases. So as *Ampularius* grows longer the secreting areas of the mantle are over any particular location for a longer period of time, because of the decreased growth rate, and, therefore, have more time to thicken the shell in that area. In shell repair the location of the secreting membrane remains in position under the damaged site depositing calcium carbonate crystals until the repair is completed, regardless of where the damage has occurred. It is not reliant on the moving mantle periphery and, therefore, should be less depended on than calcium carbonate deposition during growth.

However, the amount of calcium carbonate deposited was not found to be constant. This may be a result of not all animals having finished repairing. Abalone, like most marine snails, repair much more slowly than fresh water or terrestrial snails (Andrews, 1935; Wagge and Mittler, 1953; and Watabe, 1983). Therefore, comparing thicknesses of the nacre deposited to

examine a consistency in amou t of repair, when nacre deposition may not have been finished, would be misleading. Shell formation, though, is composed of two distinct phases. The first is the laying down of the conchiolin and the second is the crystallization of mineral salts on this membrane (Bevelander and Benzer, 1948). Once calcium carbonate crystals begin to appear, then the thickening of the conchiolin is completed. Comparing the thicknesses of conchiolin might indicate similarities in repair. This seems to be the case. In the three hole per abalone experiment the mean thicknesses of the organic membranes are not statistically different, and their maximum thicknesses are numerically equal. The mean thicknesses of the membranes in the one hole per abalone experiment varied by only 10% between thickest and thinnest.

However, the thicknesses of the conchiolin between the two experiments are not similar. Abalone repairing at only one site have conchiolin with mean thicknesses 166 to 179 percent thicker than the mean thicknesses of membranes of corresponding sites of the abalone with three holes. This could be due to the energy involved in repairing multiple sites. Palmer (1983) showed that the production of the organic matrix in shell repair was more demanding metabolically than the crystallization of calcium carbonate. Since the end purpose of repair is to restore the integrity of the shell by calcium carbonate deposition, not by thickening the conchiolin, animals undergoing the more metabolically costly multiple repair may compensate for the

extra energy loss by laying down a thinner conchiolin, without compromising the final integrity of the repaired shell.

The thicknesses of the conchiolin in the experiments were highly variable. Correlations for the three holes per abalone experiment were determined to see if abalone that repaired thickly at one site did so at other sites. The thickness of the conchiolin at one site did not show a relationship to the thickness at any other site. Nor did the thickness of the conchiolin show a consistent correlation with the thickness of the corresponding nacre. Although Palmer (1983) found that the amount of additional shell material produced decreased with increasing skeletal organic matrix content for 15 species from the three suborders of prosobranch gastropods, there was no significant association for any one species. Nor is there one for *Haliotis kamtschatkana*.

A seasonal influence was noticed in the repair rate, which decreases from summer to winter. Also, abalone undergoing repair for the same time frame in the Winter as abalone in the Fall failed more often to show any measurable signs of repair. Since photoperiod has shown not to statistically significant at the 95% level, this difference could have been due to temperature, the repair rate being slower in the colder winter waters than in the warmer fall waters. This would concur with the results of Saleuddin and Chan (1969) for *Helix*, and Kobayashi (1951, in Watabe, 1983) for *Pinctada martensii*.

Variation in repair rates due to location of injury are

more complicated. When only one hole was drilled in each abalone, the differences in repair rates were not statistically significant. This may be due to the interference of other variables such as individual variation between animals (Peppard, 1964; Saleuddin and Chan, 1969; and Kapur and Gupta, 1970) and the season the damage was done (Miyauti, 1970 in Watabe, 1983). For these reasons the second experiment was done with three holes per abalone, all drilled around the same time. Once again there was no statistically significant difference in repair rates until the thickness of the nacre was examined.

The repaired nacre over the gonads was significantly thicker than that over the shell muscle. The columellar muscle of gastropods does not insert directly into the shell. It terminates at the base of an epithelium composed of a single layer of tendon cells. The apical end of these cells attach to a tendon sheath whose processes insert directly into the shell (Tompa and Watabe, 1976). When the piece of shell is removed from over the shell muscle, this layer of tendon cells that is intimately attached to the shell is also removed, leaving no epithelial cells with an ability to excrete the materials needed to repair the damage. Thus, before repair can be undertaken, an epithelium capable of repairing must be grown (unpublished data). By the time the epithelium has developed to the stage when it can repair, the epithelium over the mantle and over the gonads are already repairing. These two sites have a head start. It is interesting that this head start results in a

significant difference at only one site, and disappears when the total amount of repair is considered. This is an indication of how rapidly abalone can grow an epithelium, particularly in relation to how slowly they repair shell.

Kunigelis and Saleuddin (1983) summarized molluscan shell repair rate investigations by categorizing them into the following three groups according to location of the injury: Type I injury occurred along the shell margin or growing edge and is repaired in a manner similar to normal shell growth. Type II injury, the most commonly studied type, occurs away from the growing edge but over the mantle's dorsal epithelium. In this study, the hole over the mantle is a Type II injury. Type III injury occurs behind the reach of the mantle. This category would include the hole over the gonads, and, strictly speaking, the hole over the shell muscle. However the studies of this type of injury (Davis, 1964; Peppard, 1964; and Dillaman and Ford, 1982) are over areas with an epithelium. Since repair over an epithelium is considerably different than repair over exposed muscle, injuries resulting in the latter should belong to a new category, Type IV injury. That abalone can successfully repair shell over their shell muscle is unique in the literature. The only other mention of such repair is by Boutan (1923) who successfully removed the shells of several *Haliotis tuberculata*. The abalone were able to regrow shells but left a gaping hole over the shell muscle, only sometimes being able to cover it with a yellowed, deformed mass that was

poorly adhered to the muscle even after four to five months.

It has been established that *Haliotis kamtschatkana* can repair shell due to Type II, Type III and Type IV injuries and do so at similar rates, the repair requiring months to complete. This is not true for all molluscs. *Helix* species can repair damage to their shell within a few hours (Wagge, 1951; Durning, 1957; Abolins-Krogis, 1958; and Saleuddin and Wilbur, 1969), Type I injury being repaired more slowly than Type II injuries, which only took place if part of the visceral hump was left unprotected when the snail was fully retracted (Wagge, 1951). *Euplecta indica* (Kapur and Gupta, 1970) and *Otala lactea* (Chan and Saleuddin, 1974), both terrestrial snails, repair Type II injuries as quickly as *Helix*. The freshwater snails *Pomacea paludosa* (Meenakshi, Blackwelder, Hare, Wilbur and Watabe, 1975) and *Lymneae stagnalis* (Timmermans, 1973) and the amphibious snail *Oncomelania formosana* (Davis, 1964) all repair Type II injuries in about two weeks, although *L. stagnalis* can repair a Type I injury more quickly. The marine snail *Tegula funebralis* could repair Type I injuries faster than its normal growth rate and took weeks to repair Type II injuries. However, only one animal of ten with Type III injuries managed a successful repair, which bulged out like a bubble (Peppard, 1964). The bivalve *Mytilus edulis* (Meenakshi, Blackwelder and Wilbur, 1973) and the cephlapod *Nautilus nacromphalus* (Meenakshi, Martin and Wilbur, 1974) take six or more weeks to repair a Type II injury. These time frames concur well with those of Wagge and Mittler

(1953) for terrestrial, freshwater and marine molluscs, except that they found most marine species were unable to repair holes in their shells without an artificial covering for assistance. *Haliotis kamtschatkana* repairs slowly, as would be expected of a marine species, but seems to repair more successfully than most other species.

GROWTH OF PEARL-NUCLEATED ABALONE

Measuring abalone with Vernier callipers resulted in an error of measurement of about 0.35%. Unfortunately, pinto abalone of the mean size used in the study grow so slowly that this error was large enough to mask any real growth, making it necessary to disregard all the length measurements for statistical analysis. The error arises from not measuring the abalone at exactly the same spot on the shell each time and/or from not holding the abalone exactly parallel to the callipers. Both errors can be overcome by using other measuring methods. Fournier and Breen (1983) used a measuring board which would prevent both errors but would be time consuming. The method used by some researchers (Zischke, Watabe and Wilbur, 1970; Paul, Paul, Hood and Nerve, 1976; and Kunigelis and Saleuddin, 1978) might be initially more time consuming, but would save time whenever other measurements were taken, and would yield the most accurate results. This is to mark or etch the growing edge of the shell and measure the shell growth beyond the mark.

Despite the errors in the results collected in this study, some abalone did show growth of shell, albeit very small; the new growth was a different colour than the older shell.

Fournier and Breen (1983) found growth of 3-7 mm/year in wild populations in Barkley Sound of *H. kamtschatkana* of similar size as those used here. Mean growth of abalone in Barkley Sound from June 1984 to March 1985 was 2.9 mm for untransplanted abalone and 7.1 and 7.2 for two transplanted populations (Emmett and Jamieson, 1989). Quayle (1971) found growth rates of pinto abalone with a mean size of 10 cm to be 5-9 mm/year depending on site. Paul, Paul, Hood and Nerve (1976) found that *H. kamtschatkana* in captivity in Alaska grew from 5-6 mm for 65-75 mm long abalone to 1-2 mm for abalone up to 110 mm long over a 200 day period beginning in May 1975. It appears that abalone implanted with pearl nuclei grow less than abalone studied by others. However, the average size of abalone in this study was greater than 100 mm. The growth reported by others for abalone of this size would be masked by the measurement error that occurred in this study. The implantation of a pearl nucleus does not automatically mean that growth will be compromised. Stahl and Lodge (1990) found a positive relationship between shell damage along the aperture and growth. The effects of pearl nucleus implantation on growth should be studied more.

Unfortunately, most growth studies centre on shell length and not on body weight, the parameter of most interest to growers. Quayle (1971) found the greatest weight growth

increment for the pinto abalone to be 69 grams and the smallest 0 grams, but does not discuss growth rates in terms of weight, probably due to the complications caused by seasonal variations, onset of spawning, osmotic differences, etc. Quayle (1971), Paul, Paul, Hood and Nerve (1976) and Fournier and Breen (1983) all found that shell growth decreases with increasing shell length in *H. kamtschatkana*, which has been found in other molluscs (Ziscke, Watabe and Wilbur, 1970; Zipser and Vermeij, 1980; and Tutschulte and Connell, 1988)). Although these researchers were looking at shell length, the same pattern for weight has been found in three *Haliotis* species in California. *H. corrugata*, *H. fulgens* and *H. sorenseni* all showed decreasing relative growth in soft body weight with increasing size (Tutschulte and Connell, 1988). This pattern has been found here with *H. kamtschatkana*. Larger abalone have a lower percent increase in weight than smaller abalone. That some abalone showed no increase in weight only shows agreement with the results of Quayle (1971). Abalone that lost weight may be animals in physical stress. Mortality rate for implanted abalone was around 50%, many of those showed dramatic weight losses before dying. But it was also noticed that weights rose and fell for some animals during the study. A decrease in weight at any one time may only indicate a weight fluctuation within a normal range for that animal.

Growth and photoperiod

No data are available regarding the effects of photoperiod on growth for *H. kamtschatkana*. Increased growth in the dark has been found for *Ampularrius glaucus* (Ziscke, Watabe and Wilbur, 1970) and *Helix duryi* (Kunigelis and Saleuddin, 1978). Photoperiod had no effect on the absolute growth rates of *Mytilus edulis* and *M. californianus* (Dodd, 1969).

The difference in growth in complete darkness of the red abalone (increased growth) and the pinto abalone (decreased growth) can be explained by their eating habits. Red abalone feed on macroalgae, primarily *Nereocystis leutkeana* (Cox, 1962). Paul, Paul, Hood and Nerve (1976) found that pinto abalone prefer diatoms to macroalgae and this was also theorized by Cox (1962) based on the coloration of their shells. All abalone are considered nocturnal (Imai, 1978). Therefore, red abalone kept in the dark and fed macroalgae will have more time for eating than ones in a continuous light or a 12:12 photoperiod, and a better opportunity for growth. But pinto abalone in continuous dark will have less of their preferred food available because diatoms need light to grow. Since pinto abalone in a normal photoperiod gained significantly more weight than their counterparts in a dark photoperiod, they must eat more, and do so because of the light-dependent availability of their preferred food.

Seasonal variation in growth

Statistical analysis was done on the percent weight change per day to compensate for the different nucleation dates for abalone. Despite this a significant difference in weight change rates exists due to the date of implantation. A seasonal variation in growth, which has been seen in other abalone (Tutschulte and Connell, 1988), explains this. Tutschulte and Connell (1988) hypothesize that abalone shift energy allocation from growth to reproduction during the season of rapid gonad enlargement. If this is true for the pinto abalone, individuals implanted during the spawning season would show a lower relative weight gain than animals implanted before, which have their relative weight gain increased by the faster growth before gonad development, and abalone implanted afterwards, which do not have their rates penalized by the spawning season. The spawning season for *H. kamtschatkana* is late May to August (Strathmann, 1987) and the hypothesized trend is seen in these data for this period.

Growth and Temperature

Warmed water has been shown to increase the growth rate of *H. discus hannai* (Sakai,1962) and *H. fulgens* (Leighton, Byhower, Kelly, Hooker and Morse, 1981). This was not found with the pinto abalone. The demonstrated slow growth of this species may

not allow for a difference to appear in the relatively short time period the experiment was run. Or, raising the temperature may have caused the abalone to start developing its gonads for spawning, which is hypothesized to decrease the growth rate. No spawning was observed, however, among the abalone.

Temperature and mortality

Most studies on shell repair state that shell damage does not increase mortality (Wagge, 1951; Davis, 1964; Zipser and Vermeij, 1980; Geller, 1990 and Stahl and Lodge, 1990). Peppard (1964) found that some *Tegula* died after having a hole drilled in their shell because of tissue rupture due to rubbing against the hole's sharp edge. Kunigelis and Saleuddin (1983) found Type III injury (areas beyond the reach of the mantle) often resulted in high mortality. Elston and Lockwood (1983) found that excessive stress increases mortality in abalone due to decreasing the abalone's resistance to *Vibrio* bacteria. The high mortality sufferd by abalone implanted with a pearl nucleus in this study has been attributed to the stress the abalone is exposed to during the implantation procedure. The data obtained from holding nucleated abalone at elevated temperatures indicate that placing the abalone in heated water after implantation drastically reduces mortality. The warmer water may increase the abalone's metabolism allowing it to more effectively combat the effects of the induced stress.

SUMMARY

Since no significant difference was found for repair rates between three different injury sites on the pinto abalone, the site of implantation of pearl nuclei can be decided by ease of implantation or other factors decided by the grower. The abalone should be implanted either in the Spring or early Fall. This would avoid the slow growing rates due to spawning in the summer months, and allow for some pearl development before the onset of the cold water months. Pinto abalone should be placed in water of less than or equal to 14° Celsius after nucleus implantation to improve survivability. All abalone should be kept in a normal photoperiod to encourage diatom growth which results in increased rates of weight gain.

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APPENDIX I

Statistical Tables

APPENDIX I

A. Repair Rate, Location and Photoperiod

ANOVA table for response variable REPAIR/DAY

Source	DF	Sum of Squares	Mean Square	F-ratio (p)	Prob>F	Error Term
A (POSITION)	2	4.003149	2.001575	2.90	0.0590	ERROR
B (PHOTO)	1	0.223354	0.223354	0.32	0.5708	ERROR
C (START)	7	30.84855	4.406936	6.38	0.0000	ERROR
ERROR	122	84.319	0.6911393			
TOTAL (Adj)	132	121.2402				

Adjusted Means and Standard Errors for Y = REPAIR/DAY

Term	Count	Mean (mm)	Standard Error
ALL	133	1.55129	
A: POSITION			
MANTLE	49	1.9659	0.118764
SHELL MUSCLE	36	1.3996	0.138558
GONAD	48	1.2883	0.119995
B: PHOTO			
NORMAL	58	1.4763	0.109161
DARK	75	1.6263	0.09599
C: START DATE			
SEPTEMBER 15, 1988	7	1.9077	0.31422
SEPTEMBER 18, 1988	18	2.2841	0.195951
NOVEMBER 11, 1988	14	2.1961	0.222187
NOVEMBER 17, 1988	19	1.0871	0.190724
NOVEMBER 18, 1988	18	1.2258	0.195951
NOVEMBER 19, 1988	12	1.9571	0.239990
NOVEMBER 20, 1988	21	1.1527	0.181415
FEBRUARY 28, 1989	24	0.5998	0.169698

TABLE A.3 General linear model analysis of variance of the main effects between the daily repair rate (REPAIR/DAY) and the position of the hole (POSITION), the photoperiod the abalone was kept in (PHOTO), and the Julian date the hole was drilled in the shell (START). For ease of reading the Julian dates have been converted to Gregorian calender dates.

Neuman-Keul's multiple comparison report

Response Variable: REPAIR/DAY
Factor: C (START) Error Term: ERROR

START DATE	A	B	C	D	E	F	G	H
A (2/28/89)		-	-	-	**	**	**	**
B (11/17/88)			-	-	**	**	**	**
C (11/20/88)				-	**	**	**	**
D (11/18/88)					-	-	**	**
E (9/15/88)						-	-	-
F (11/19/88)							-	-
G (11/11/88)								-
H (9/18/88)								

** indicates a significant (alpha = 0.05) difference between the means.

TABLE A.4 Neuman-Keul's post hoc multiple comparison report on the difference of the adjusted means of repair rates (REPAIR/DAY) between abalone that had their shells drilled on different dates (START). As an example, the table shows that the repair rate mean for abalone started on November 18, 1988 (D) is significantly different from those started on November 11, 1988 (G) and September 18, 1988 (H); but not from those started on February 2, 1989 (A); November 17, 1988 (B); November 20, 1988 (C); September 15, 1988 (E); and November 19, 1988 (F).

Stepwise Regression Report

Dependant Variable: REPAIR/DAY

IN	Variable	S-Est	R^2-add	R^2-Xs	T-Value	Prob	%RMSE
Yes	START	-0.38	0.142	0.000	-4.4	0.0000	7.5
No	INITIAL WEIGHT		0.012	0.001	1.3	0.1979	-0.3
No	% WEIGHT CHANGE		0.001	0.162	0.4	0.6805	0.4
No	INITIAL LENGTH		0.009	0.013	1.1	0.2799	-0.1
No	% LENGTH CHANGE		0.000	0.190	0.1	0.9189	0.4

R-Squared: 0.1419 Root Mean Square (RMSE): 0.891839

TABLE A.5 Stepwise regression report to determine if there is a linear relationship between the daily repair rate (REPAIR/DAY) and the Julian date the hole was made (START) and the initial abalone weight (INITIAL WEIGHT), the percent weight change of abalone (% WEIGHT CHANGE), the initial length of abalone (INITIAL LENGTH), and the percent length change in abalone (% LENGTH CHANGE).

Linear Regression Report

Dependent Variable: REPAIR/DAY

Independent Variable	Parameter Estimate	Standardized Estimate	Standard Error	t-value	Prob. Level
Intercept	20996.73	0.0000	3594.036	5.84	0.0000
START	-0.0858	-0.4546	0.01468	-5.84	0.0000

Correlation: -0.4546

Analysis of Variance Report

Dependent Variable: REPAIR/DAY

Source	df	Sum of Squares (Sequential)	Mean Square	F-Ratio	Prob. Level
Constant	1	281.6	281.6		
Model	1	25.056	25.056	34.13	0.0000
Error	131	96.184	0.7342		
Total	132	121.24	0.91849		

Root Mean Square Error	0.85687
Mean of Dependent Variable	1.4551
Coefficient of Variation	0.5889
R Squared	0.2067
Adjusted R Squared	0.2006

TABLE A.6 Linear Regression Report and its Analysis of Variance Report for testing the possibility of a linear relationship between the daily repair rate for abalone (REPAIR/DAY) and the Julian date the repair began (START).

ANALYSIS OF VARIANCE

Source of variation	SS	df	MS	F
Between abalone	0.06939	13	0.005338	
Within abalone	0.07203	28	0.002572	
Position	0.06207	2	0.004980	2.09
Residual	0.06207	26	0.002387	
Total	0.1414	41		

TABLE B.2 Repeated measures analysis of variance report for the difference in the thickness of the total repair material between the hole over the mantle region, the hole over the shell muscle region and the hole over the gonad region. The critical value for $F_{(2,26)}$ with an alpha value of 0.05 is 3.37.

ANALYSIS OF VARIANCE

Source of variation	SS	df	MS	F
Between abalone	0.02614	13	0.002011	
Within abalone	0.03786	28	0.001352	
Position	0.0009265	2	0.0004632	0.326
Residual	0.03693	26	0.001421	
Total	0.06400	41		

TABLE B.3 Repeated measures analysis of variance report for the difference in the thickness of the organic partition of the repair between the hole over the mantle region, the hole over the shell muscle region and the hole over the gonad region. The critical value for $F_{(2,26)}$ with an alpha value of 0.05 is 3.37.

ANALYSIS OF VARIANCE

Source of variation	SS	df	MS	F
Between abalone	0.02814	13	0.002164	
Within abalone	0.03225	28	0.001152	
Position	0.007180	2	0.003590	3.72
Residual	0.02507	26	0.0009641	
Total	0.06038	41		

TESTS ON DIFFERENCES BETWEEN PAIRS OF MEANS

(i)

Position		Shell Muscle	Mantle	Gonad
	Totals	0.144	0.352	0.592
Shell Muscle	0.144	--	0.208	0.448
Mantle	0.352		--	0.240
Gonad	0.592			--

(ii)

r	2	3
$q_{.95}(r,26)$	2.91	3.52
$nMS_{res}q_{.95}(r,26)$	0.3381	0.4089

(iii)

	Shell Muscle	Mantle	Gonad
Shell Muscle		--	**
Mantle			--

** indicates a significant difference between a pair

TABLE B.4 Repeated measures analysis of variance and the Neuman-Keuls method for tests on differences between pairs of means for determining the difference in the thickness of the inorganic partition of the repair between the hole over the mantle region, the hole over the shell muscle region and the hole over the gonad region. All tests are for an alpha level of 0.05. A difference between two pairs in (i) greater than the respective $nMS_{res}q_{.95}(r,26)$ value in (ii) indicates a significant difference and is indicated as such in (iii).

CORRELATIONS AMONG THICKNESSES OF REPAIR

	M-MEM.	S-MEM.	G-MEM.	M-NACRE	S-NACRE	G-NACRE
M-MEMBRANE	--	.2503	-.0333	.5123	.2353	.3080
S-MEMBRANE		--	.1040	.2693	.1459	.0691
G-MEMBRANE			--	-.0506	-.2461	-.1558
M-NACRE				--	.1954	.5285
S-NACRE					--	.1430
G-NACRE						--

	M-REPAIR	S-REPAIR	G-REPAIR
M-REPAIR	--	.3556	.4006
S-REPAIR		--	.1015
G-REPAIR			--

TABLE B.5 Correlations between thicknesses of repair for abalone undergoing repair over the mantle, over the shell muscle and over the gonads simultaneously. Variable names are the same as in Table A.1.

Analysis of Variance Report

ANOVA Table for Response Variable: % WT CHANGE/DAY

Variable	DF	Sum of Squares	Mean Square	F-Ratio	Prob. Level	Error Term
A (SEX)	1	0.002070	0.0020703	0.78	0.3780	ERROR
B (PHOTO)	1	0.05232	0.05232	19.73	0.0000	ERROR
AB	1	0.0006054	0.0006054	0.23	0.6333	ERROR
C (DATE)	10	0.1325	0.1324	5.00	0.0000	ERROR
ERROR	199	0.5276	0.002651			
TOTAL(Adj)	212	0.6629				

Adjusted Means & Standard Errors for Y = % WT CHANGE/DAY

Term	Count	Adjusted Mean	Standard Error
ALL	213	0.05691	
A: SEX			
FEMALE	103	0.06018	0.005073
MALE	110	0.05363	0.004909
B: PHOTO			
NORMAL	83	0.09216	0.005651
DARK	130	0.02165	0.004516
C: DATE			
MAY 11, 1988	26	0.1158	0.01009
MAY 22, 1988	46	0.1058	0.007591
MAY 29, 1988	37	0.001235	0.008465
JUNE 6, 1988	1	0.01522	0.05149
JUNE 7, 1988	21	0.06323	0.01123
JUNE 9, 1988	5	0.08271	0.02302
JUNE 28, 1988	8	0.06144	0.01820
JULY 9, 1988	16	0.05030	0.01287
JULY 10, 1988	7	0.06522	0.01946
AUG. 4, 1988	23	0.05690	0.01073
AUG 10, 1988	23	0.008052	0.01073
AB: SEX,PHOTO			
FEMALE,NORMAL	45	0.09720	0.007675
FEMALE,DARK	58	0.02316	0.006761
MALE,NORMAL	38	0.08710	0.008352
MALE,DARK	72	0.02015	0.006068

TABLE C.2 General Linear Model Analysis of Variance of the main effects of sex (SEX), photoperiod (PHOTO) and implantation date (DATE), and the interaction of sex and photoperiod (AB) on the daily growth rate of implanted abalone measured as the percent change in weight per day (% WT CHANGE/DAY).

Newman-Keul's multiple comparison report

Response Variable: % WT CHANGE/DAY
Factor: C (DATE) Error Term: ERROR

IMPLANT DATE	A	B	C	D	E	F	G	H	I	J	K
A (5/29/88)		-	-	-	-	-	-	-	-	**	**
B (8/20/88)			-	-	-	-	-	-	-	**	**
C (6/6/88)				-	-	-	-	-	-	**	**
D (7/9/88)					-	-	-	-	-	-	-
E (8/4/88)						-	-	-	-	-	-
F (6/28/88)							-	-	-	-	-
G (6/7/88)								-	-	-	-
H (7/10/88)									-	-	-
I (6/9/88)										-	-
J (5/22/88)											-
K (5/11/88)											

** indicates a significant (alpha = 0.05) difference between the means.

TABLE C.3 Newman-Keul's post hoc multiple comparison report on the difference of the adjusted means of the rate of growth of implanted abalone measured by the daily per cent change in weight (% WT CHANGE/DAY) and the date the abalone were implanted (DATE). For example, the table shows that the repair rate mean for abalone implanted on May 11, 1988 (K) is significantly different from those implanted on May 29, 1988 (A), August 20, 1988 (B), and June 6, 1988 (C); but not from those implanted on July 9, 1988 (D), August 4, 1988 (E), June 28, 1988 (F), June 7, 1988 (G), July 10, 1988 (H), June 9, 1988 (I), and May 22, 1988 (J).

Stepwise Regression Report

Dependant Variable: % WEIGHT CHANGE

IN	Variable	S-Est	R^2-add	R^2-Xs	T-Value	Prob	%RMSE
Yes	DATE	-0.14	0.018	0.006	-2.3	0.0236	1.5
Yes	INITIAL WEIGHT	-0.47	0.221	0.006	-7.9	0.0000	13.6
No	INITIAL LENGTH		0.001	0.913	0.6	0.5520	0.2
No	TOTAL DAYS		0.003	0.557	0.9	0.3946	0.1

R-Squared: 0.2515 Root Mean Square (RMSE): 13.02372

TABLE C.4 Stepwise regression report to determine if there is a linear relationship between the per cent change in weight since implantation (% WEIGHT CHANGE) and the Julian date the abalone was implanted (DATE) and/or the initial abalone weight (INITIAL WEIGHT), the initial length of abalone (INITIAL LENGTH), and the total number of days since implantation (TOTAL DAYS).

Linear Regression Report

Dependent Variable: % WEIGHT CHANGE

Independent Variable	Parameter Estimate	Stndized Estimate	Standard Error	t-value (b=0)	Prob. Level	Simple R-Sqr
Intercept	29.72705	0.0000	2.124193	13.99	0.0000	
INITIAL WEIGHT	-0.0922	-0.4830	0.01149	-8.03	0.0000	0.2333

Correlation: -0.4830

Analysis of Variance Report

Dependent Variable: % WEIGHT CHANGE

Source (Sequential)	df	Sums of Squares	Mean Square	F-Ratio	Prob. Level
Constant	1	43577.31	43577.31		
Model	1	11153.28	11153.28	64.50	0.000
Error	212	36658.52	172.9176		
Total	213	47811.81	224.4686		

Root Mean Square Error	13.14981
Mean of Dependent Variable	14.26998
Coefficient of Variation	.9215019
R Squared	0.2333
Adjusted R Squared	0.2297

TABLE C.5 Linear Regression Report and its Analysis of Variance Report for testing the possibility of a linear relationship between the percent weight change in implanted abalone (% WEIGHT CHANGE) and the initial weight of the abalone (INITIAL WEIGHT).

ANOVA Table for Response Variable. % WT CHANGE/DAY

Variable	DF	Sum of Squares	Mean Square	F-Ratio	Prob. Level	Error Term
TEMPERATURE	1	0.01726	0.01726	3.11	0.0823	ERROR
ERROR	67	0.3717	0.005548			
Total(Adj)	68	0.38896				

Means and Standard Errors for % WT CHANGE/DAY

Term	Count	Mean	Standard Error
ALL	69	0.0523309	
TEMPERATURE			
1(14°C)	41	0.03622	0.01163
2(Ambient)	28	0.06843	0.01407

TABLE D.2 GLM Anova report to test the difference in the daily percent change in weight of abalone kept at 14°C and abalone kept at ambient water temperature (7-8°C).

APPENDIX II

Scatter Plots

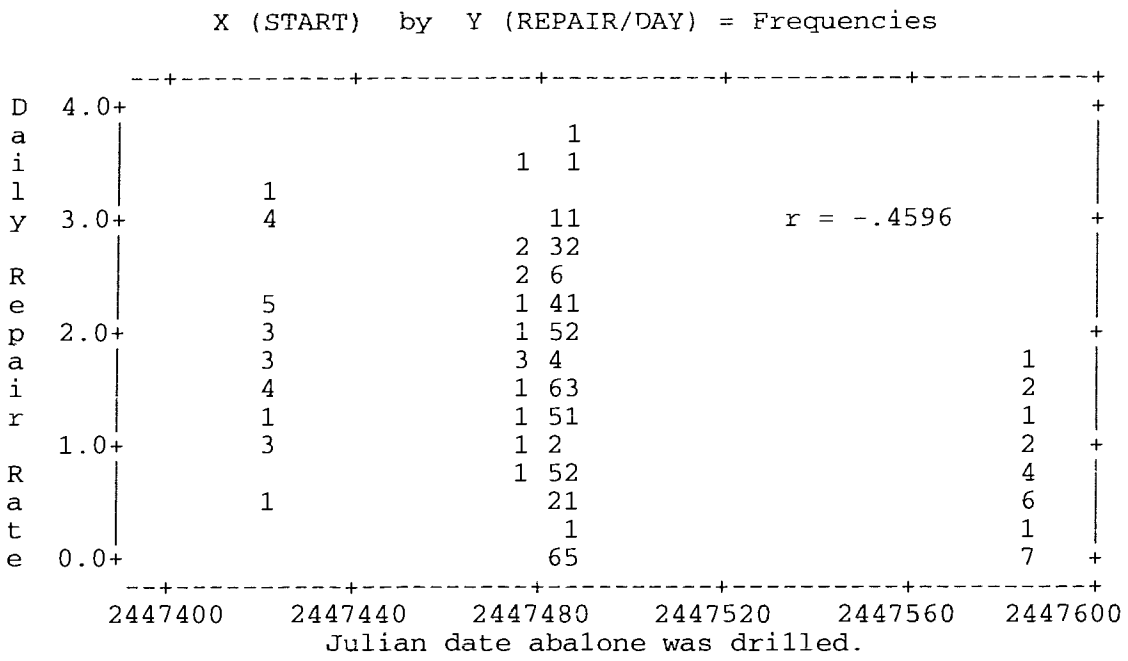

FIGURE 21. Scatter plot using frequency of observation showing the relationship between the date abalone had a hole drilled in their shell (all sites) and the rate of repair of the hole (in micrometers per day).

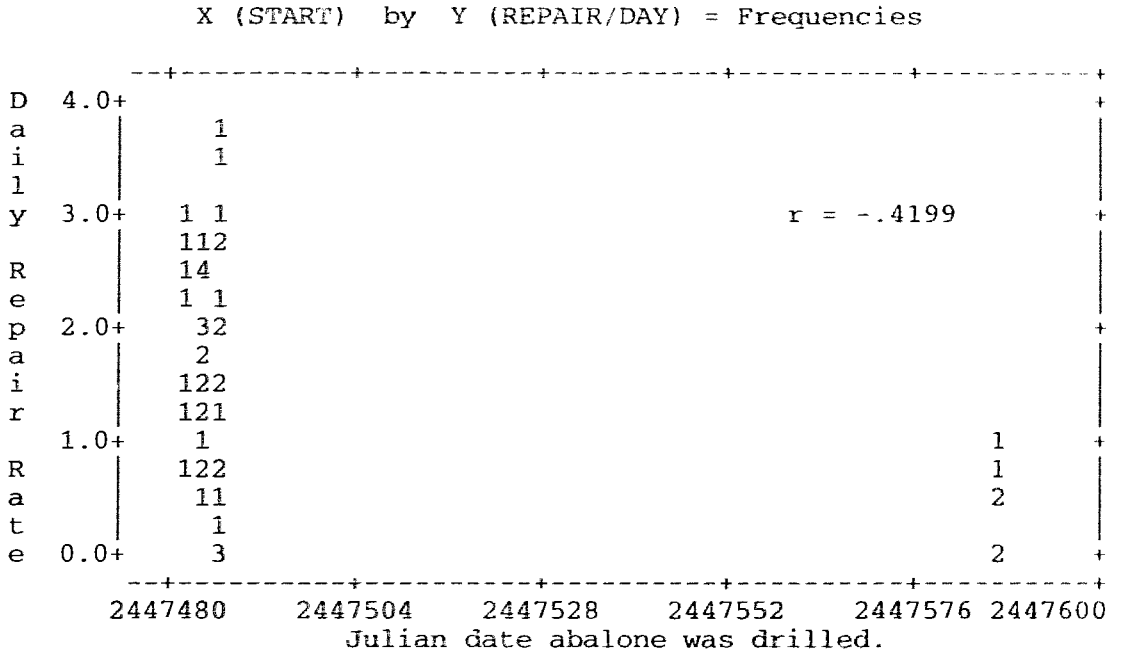

FIGURE 22. Scatter plot using frequency of observation showing the relationship between the date abalone had a hole drilled in their shell over the mantle and the rate of repair of the hole (in micrometers per day).

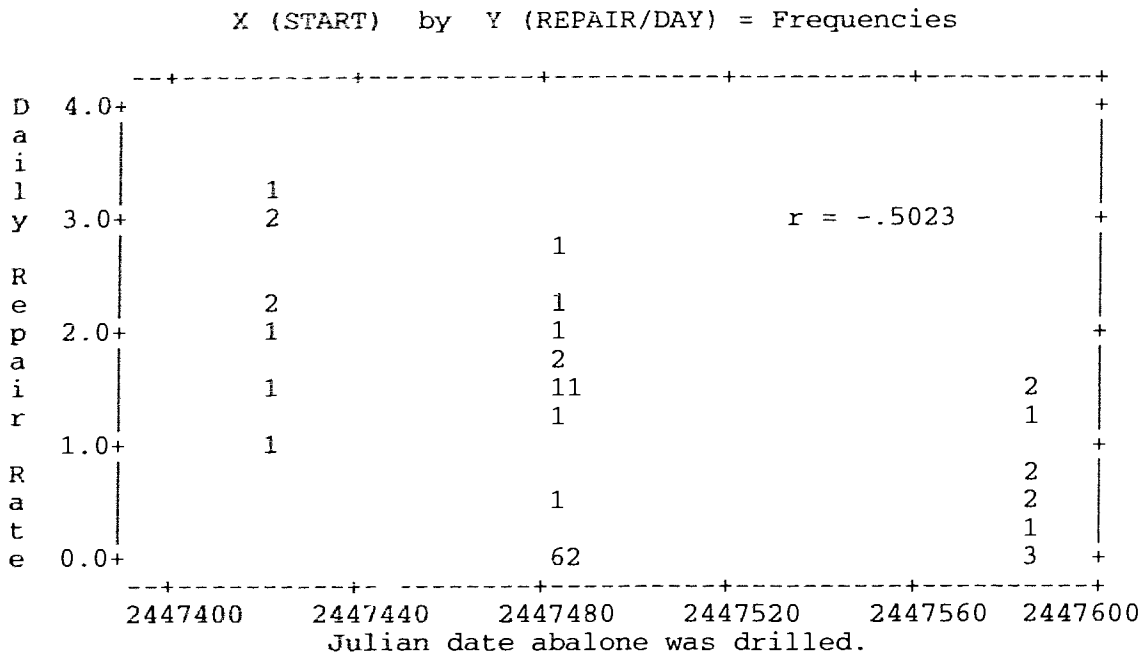

FIGURE 23. Scatter plot using frequency of observation showing the relationship between the date abalone had a hole drilled in their shell over the shell muscle and the rate of repair of the hole (in micrometers per day).

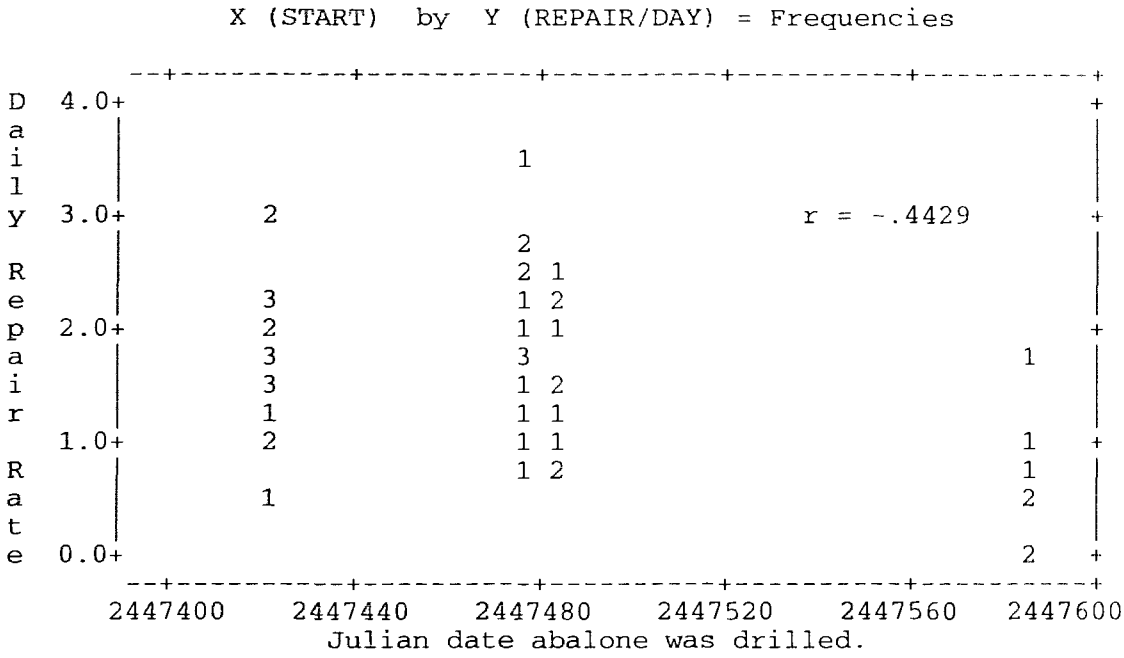

FIGURE 24. Scatter plot using frequency of observation showing the relationship between the date abalone had a hole drilled in their shell over the gonads and the rate of repair of the hole (in micrometers per day).

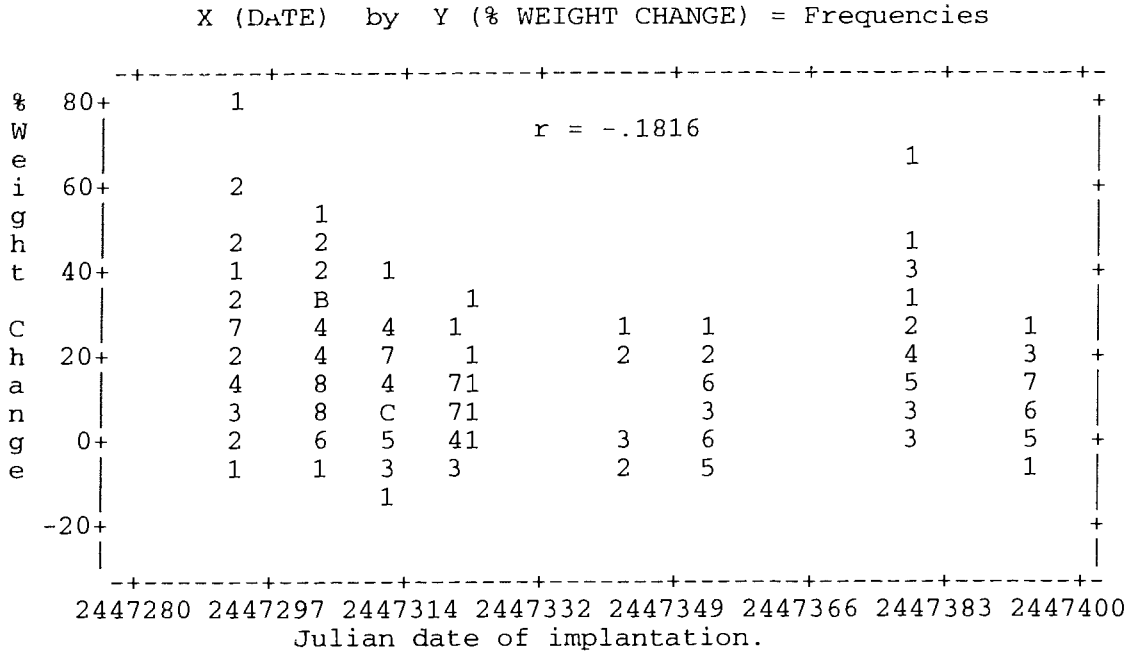

FIGURE 25. Scatter plot using frequency of observation to see if a linear relationship exists between the date of implantation of a pearl nucleus and the per cent weight change in abalone.

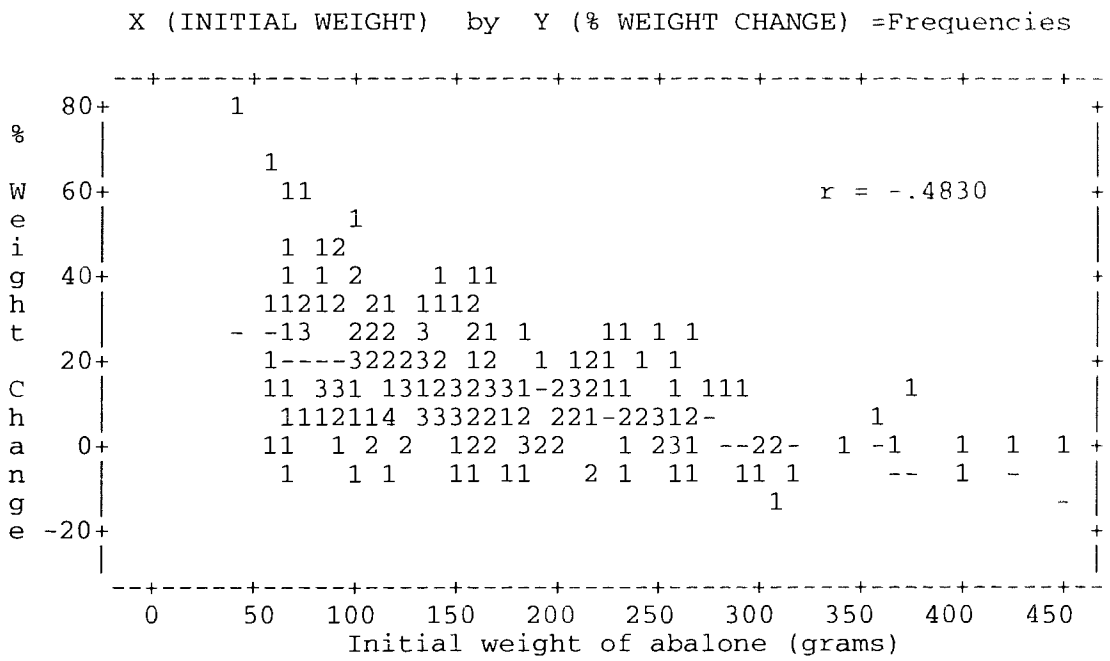

FIGURE 26. Scatter plot using frequency of observation to see if there is a linear relationship between the initial weight of abalone and their percent weight change. Dashes in the plot represent the best fit least squares line.