

Growth, Regeneration, and Damage Repair of Spines of the Slate-Pencil Sea Urchin *Heterocentrotus mammillatus* (L.) (Echinodermata: Echinoidea)¹

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ABSTRACT: Spines of sea urchins are appendages that are associated with defense, locomotion, and food gathering. Spines are repaired when damaged, and the dynamics of repair was studied in the slate-pencil sea urchin *Heterocentrotus mammillatus* to provide insights not only into the processes of healing but also into the normal growth of spines and the formation of growth lines.

Regeneration of spines on tubercles following complete removal of a spine was slow and depended upon the size of the original spine. The maximum amount of regeneration occurred on tubercles with spines of intermediate size (1.6 g), which, on average, developed regenerated spines weighing 0.1, 0.3, and 0.7 g after 4, 8, and 12 months, respectively. Some large tubercles, which had original spines weighing over 3 g, failed to develop a new spine even after 8-12 months.

Regeneration of a new tip on a cut stump was more rapid than production of a new spine on a tubercle. Regeneration to original size was more rapid for small spines than for large spines, but large stumps produced more calcite per unit time. In 4 months, a small spine with a removed tip weighing 0.15 g regenerated a new tip weighing 0.09 g, or 63% of its original weight. In the same time, a large spine with 2.35 g of tip removed regenerated 0.40 g of new tip, or 17% of the original weight.

Holes were drilled in spines to serve as bench marks to document the production of new growth lines in response to damage. Blind holes drilled in spines caused 65% loss over 4 months, but holes drilled completely through spines caused only a 6% loss. Cutting a spine near its base did not initiate shedding of the stump. Repair of holes drilled in spines showed that major repair originated from the spine surface, and regeneration into the hole was over obtuse angles. For a slanting hole through a spine, filling of the hole came from the distal edge for the distal opening and from the proximal edge for the proximal opening.

Spines were tagged with tetracycline, but of 46 spines examined 4-12 months following treatment, only 1 displayed evidence that the spine surface had been marked. This suggests that spines usually are not growing.

Abrasion of spines either by wiping with a cloth or sanding with emery paper failed to induce growth lines to form. Of 57 abraded spines and 64 unabraded spines, only 2 in each treatment category developed new growth lines. Results show that some growth lines in spines develop from trauma, but these tend to be local and do not extend from the base to the tip. Spines of *Heterocentrotus mammillatus* usually are not growing but occasionally undergo a growth episode that produces a growth line that extends from the milled ring to the spine tip. Such an episode is not related to damage repair from abrasion or breakage and appears to be endogenous.

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THE SPINES OF A SEA URCHIN are morphological parts that serve as the animal's first defense against assaults from the environment (e.g., Fricke 1974, Strathmann 1981), appendages for gathering and manipulating food (e.g., Ebert 1968, Leighton 1968), and braces for wedging into crevices (e.g., Régis and Thomasin 1982). Spines form an important part of an urchin's maintenance mechanisms (Ebert 1982), and it is no surprise to find that they are repaired rapidly once damaged (Ebert 1967, Heatfield 1971, Mischor 1975).

Spine sizes vary greatly across taxa. For example, in *Strongylocentrotus purpuratus* (Stimpson 1857), calcite of a large primary spine weighs only about 0.03 g. In slate-pencil sea urchins of the genus *Heterocentrotus*, spines are substantially more massive. Calcite in a large *H. mammillatus* (Linnaeus 1758) spine may weigh over 5 g, and calcite of a large primary spine of *H. trigonarius* (Lamarck 1816) may weigh over 11 g.

Although spines from *Strongylocentrotus purpuratus* would be expected to be repaired rapidly (Ebert 1967, Heatfield 1971), it is less clear whether very large spines from *Heterocentrotus* species also would be repaired in just a few months or whether the benefits of large spines carry a potential burden of a long regeneration time.

A second issue associated with damage repair focuses on growth lines in spines. In *Strongylocentrotus purpuratus*, lines form as a consequence of tip regeneration following breakage (Ebert 1967, Heatfield 1971), but growth lines in *Heterocentrotus* spp. have been interpreted as periodic (Dotan and Fishelson 1985, Weber 1969), an interpretation that has been disputed (Ebert 1985, 1987).

The purpose of this paper is to present results of a 1-year study on *Heterocentrotus mammillatus* (family Echinometridae), a species with massive spines. The focus is on the responses of spines to damage, spine repair and regeneration in response to trauma, and the formation of growth lines. Data are presented on (a) regeneration following total removal of spines; (b) repair rates of calcite on cut spine stubs; (c) responses of spines to small holes drilled into or through them; (d) responses of spines to abrasion; and (e) results of tagging spines with tetracycline.

METHODS AND STUDY AREA

Sea urchins were studied at Honaunau Bay (19°26' N, 155°55' W) on the Kona (leeward) coast of the island of Hawaii, which is a site where I previously have studied *Heterocentrotus mammillatus* (Ebert 1971, 1982). During 4–6 November 1983, 100 *H. mammillatus* were collected. Spines were treated, as detailed below, and animals then were returned to the area where they had been collected. Samples of the treated animals were taken during 3–5 March and 26–28 June 1984. At each of these times, 75 additional animals were tagged, and spines were treated. A final collection of treated animals was made on 4 November 1984.

Fourteen spines on each animal were subjected to a variety of treatments to gain insight into the dynamics of calcite deposition, spine repair, and line formation. Data sheets with spine maps had been prepared, and treatments to primary interambulacral spines on the aboral surface were assigned using random numbers.

The seven treatments, replicated on the two halves of each test animal, included tagging (1), total removal of spines (2), cutting spines close to the base (3), and drilling small holes into or through spines. Half of the spines with holes were abraded with sandpaper or wiped with a cloth to simulate storm damage (4 and 5), and equal numbers of abraded and unabraded spines were labeled with tetracycline (in November and March only) (6 and 7). After treatment, sea urchins were returned to a single drop site at a depth of about 3 m, which is the same area where animals were studied in 1975–1976 (Ebert 1982). The details of the treatment methods are given below.

1. Tagging

Holes were drilled through one spine on each half of the sea urchin. One spine was tagged on each side of an animal in anticipation of some spine loss during the course of the study. An 0.8- or 1.6-mm ($\frac{1}{32}$ - or $\frac{1}{16}$ -in.) twist drill was arbitrarily selected and mounted on a flexible shaft attached to a battery-operated drill. Holes were made 2 cm from the bases of the spines, or closer, so tags would be

protected by surrounding spines and unlikely to catch on coral or rock edges. Nylon monofilament (0.02-mm diameter) was threaded through each hole, and glass beads were added to the line to form a color code. A small piece of spaghetti tubing (approx. 2 mm) was added at the end of each set of beads. A square knot was tied in the monofilament to form a loop close to the spine, the line was threaded back through the spaghetti tubing, and the knot was pulled inside the tubing to protect it and prevent it from untying.

2. Total Removal

One spine on each side of the animal was totally removed by applying lateral pressure until the muscles and connective tissue holding the spine to the test would tear. The disarticulation was assisted in some cases by using a sharpened probe. Spines were saved and labeled.

3. Partial Removal

Spines were sawed off about 1 cm above the milled ring using a carborundum-edged, saber-saw blade held in a file handle with adjustable jaws. Spines were sawed so the cut was over halfway through the spine shaft, and then the blade was twisted to fracture the spine. Spine tips were saved and labeled.

4. Hole with No Further Treatment

Holes (0.8 or 1.6 mm) were drilled in spines to serve as bench marks to determine whether new growth lines would form between the time of treatment and collection. In November 1983, holes were started at about mid-shaft, and after starting, the drill was tipped up so the hole was drilled down at a steep angle into the spine. Depending on the size of the spine, the holes ranged from 0.5 to 1.5 cm deep and with few exceptions were blind (that is, they did not go entirely through the spines). The procedure for drilling holes was changed both in March and June. In March, holes were drilled perpendicular to the spine surface and entirely through the spine, the same as holes used for tags. In June, holes again were

slanted, but all were drilled entirely through the spine.

5. Hole plus Abrading

Wet-dry sandpaper (220 grit) was used to abrade the surface of spines in November 1983 and March 1984. In June 1984, spines were wiped with cloth to simulate more gentle damage to the spine surface.

6. Tetracycline Tag

Spines were tagged with tetracycline to form a fluorescent mark at growing edges, whether internal or external, and to aid in identifying areas of reworking. Hydrogenated vegetable oil (cooking shortening) was used as a carrier for the tetracycline, because tetracycline is water-soluble and so should remain longer in an oil carrier than if applied in aqueous form. One gram of tetracycline-HCl was mixed with 20 g of hydrogenated vegetable oil; the holes in spines were filled with this mixture, and then the holes were plugged with bleached spines of another echinoid, *Echinometra mathaei* (de Blainville 1825).

Seventeen animals tagged in November 1983 were collected in March, 15 animals (5 tagged in November and 10 in March, 1 of which was damaged and only partially useful) were collected in June, and 22 animals (14 tagged in June, 7 tagged in March, and 2 from the previous November) were collected in November 1984. Animals were air-dried for 1–3 days and then placed in plastic bags for return to San Diego and further preparation. Some spines broke loose from animals during transit and could not be assigned to their original position. As a consequence of this, total numbers of spines in the various analyses will have different values for *N*.

Using the spine maps, spines were removed from the sea urchins and paired with original spines or spine tips, in the cases of total removal or cutting. Spines then were treated with 5% sodium hypochlorite to remove organic matter, soaked in fresh water for 24 hr, and air-dried. When appropriate, spines were sectioned by hand, first on 100-grit, then on 150-grit sandpaper, and finally finished with

600-grit carborundum powder on a glass plate with water as the carrier. The spines were cleaned in a sonic cleaner with tap water as the cleaning medium. When weight determinations were made, cleaned spines were dried at 80°C for about 15 hr before weighing.

RESULTS

Spine Losses Due to Treatments

Recovery of treated spines from tagged animals was dependent upon treatment (Table 1). Of the various treatments reported here, removing most of a spine was the least damaging. Out of 80 spines that were cut (Table 1), only 1 was missing after 4 months, a loss rate of only about 1%. Drilling holes completely through spines, either perpendicular to the spine surface (March treatment) or at an angle through the spines (June treatment), caused more spines to be lost. The difference between the March and June treatments is not significant ($\chi^2 = 1.09$, $df = 1$, $p > 0.05$), but blind holes (November treatment) showed significantly greater loss than the combined March and June treatments ($\chi^2 = 18.63$, $df = 1$, $p < 0.05$). Loss of spines with blind holes was 65%, whereas loss of spines with completed holes was only 15%.

Presence of a monofilament line in the hole through a spine gave an intermediate loss rate. Loss of tagged spines during 4 months was independent of season ($\chi^2 = 2.493$, $df = 2$, $p > 0.05$). This indicates that environmental stresses, which could modify spine loss, were the same during each of the 4-month periods. The overall loss of tagged spines was 30%.

It is clear that massive damage to a spine in the form of cutting most of it away does not cause the remaining stump to be shed. In contrast, a hole in the spine, which appears to be minor damage, initiates events that lead to spine shedding. The presence in a sea urchin spine of mechanisms that are sensitive to relative damage and that can lead to spine retention and repair or to shedding, together with evidence of damage and repair occurring naturally (see Figure 3H), suggest that these responses to damage are part of the adaptive machinery associated with maintenance of the body wall and, hence, with modifying survival rates.

Regeneration on Tubercles Following Complete Removal of Spines

Following spine removal, some tubercles were able to initiate spine regeneration during a 4-month period, but others were not (Figure 1). After 8 months (November–June), 3 out

TABLE 1

SUMMARY OF SPINES RECOVERED FROM SLATE-PENCIL SEA URCHINS, *Heterocentrotus mammillatus*, FOLLOWING TREATMENT AT HONAUNAU BAY, HAWAII

TREATMENT	NOV.—MAR.		MAR.—JUNE		JUNE—NOV.	
	PRESENT	ABSENT	PRESENT	ABSENT	PRESENT	ABSENT
Tagged	26	8	10	8	20	8
Cut	33	1	18	0	28	0
Drilled	12	22	17	1	22	6
Drilled + abraded	8	26	14	4	19	9
Tetracycline	5	29	13	5	—	—
Tetracycline + abraded	4	30	9	9	—	—

NOTE: In November 1983, blind holes were drilled into spines at a steep angle; in March 1984, holes were drilled perpendicular to spine shaft and completely through spines; in June 1984, holes were drilled at a steep angle and through spines. Values in the table are number of spines recovered after 4 months; cut spines had $\frac{3}{4}$ of tip removed.

of the 10 tubercles that were examined had not produced a new spine. All 13 tubercles from the 8-month period March–November showed some regeneration. In November 1984, 1 tubercle out of the 4 that had been treated the previous November still did not have a new spine developing on it.

The scatter diagram in Figure 1 shows a maximum in regeneration weights at inter-

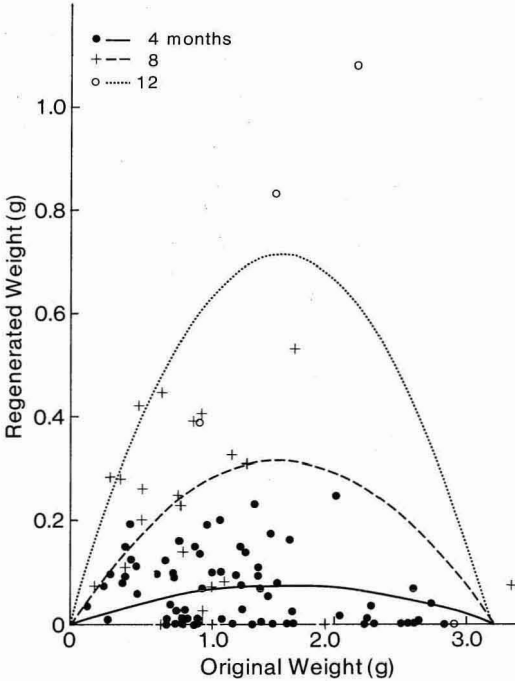


FIGURE 1. Weight (in grams) of regenerated spines on tubercles of *Heterocentrotus mammillatus* following complete removal of original spines; curves are least-squares fits of second-order functions obtained by multiple regression.

mediate original weights and small amounts of regeneration on tubercles that had both very small and very large spines. This suggests that the data may be described by a second-order equation. Curves must pass through the origin, and examination of Figure 1 indicates that the maximum spine size with zero regeneration is the same for all time periods. A reasonable model for regenerated weight (W_R) as a function of original spine weight (W_O) and time (t), in months, with b_1 , b_2 and k as fitted constants, is

$$W_R = b_1 W_O^2 t^k + b_2 W_O t^k \quad (1)$$

Analysis (Table 2) was by multiple regression (BMDP1R, Dixon 1981). Integer values from 1 to 3 were tried for the exponent k , and $k = 2$ provided the largest multiple correlation coefficient: $R = 0.83$ and $R^2 = 0.69$. The original spine size that is associated with maximum regeneration was found by examining the derivative of Equation 1 with respect to W_O :

$$\frac{dW_R}{dW_O} = 2b_1 W_O t^2 + b_2 t^2 \quad (2)$$

Setting the derivative equal to zero and solving for W_O gives the size of an original spine that is associated with maximum regeneration:

$$W_O = \frac{-b_2}{2b_1} \quad (3)$$

Equation 3 is solved by using the constants b_1 and b_2 that were estimated by multiple regression (Table 2). According to this analysis, the maximum amount of regeneration would take place on a tubercle that had an original spine, W_O , weighing 1.603 g.

Using Equation 1 and the fitted constants in Table 2, curves were drawn through the

TABLE 2

REGENERATION OF SPINES ON TUBERCLES FOLLOWING COMPLETE REMOVAL OF SPINES

VARIABLE	COEFFICIENT	SE	t VALUE	p
$W_O^2 t^2$	$b_1 = -0.1933E-2$	0.2088E-3	-9.258	<0.001
$W_O t^2$	$b_2 = 0.6196E-2$	0.4967E-3	12.476	<0.001

NOTE: Model, $W_r = b_1 W_O^2 t^2 + b_2 W_O t^2$; W_r = regenerated weight (g); W_O = original spine weight (g); t = time (months); correlation coefficients, $R = 0.8284$, $R^2 = 0.6863$.

scatter of data points for 4, 8, and 12 months (Figure 1). For an original spine, W_0 , weighing 1.603 g, regenerated weights after 4, 8, and 12 months would be 0.079 g, 0.318 g, and 0.715 g respectively. These regenerated weights are 4.9%, 19.8% and 44.6% of original weight.

The great scatter of data points in Figure 1 and the large number of tubercles with zero regeneration indicate the difficulties that tubercles of all sizes have in reconstituting spines. Also, the fact that t^2 provides a better fit than t probably is related to a lag phase during the early stages of regeneration, which means that regeneration is accelerating over the 12-month period. The adequacy of the second-order relationship can be interpreted as meaning that large tubercles have longer lag times than small tubercles. In the long run of several years, it is reasonable to expect the second-order relationship to disappear and a linear relationship to be appropriate; small tubercles will have small spines and large tubercles will have large spines.

Regeneration on Stumps Following Cutting

Figures 3A and 3B show longitudinal sections through spines that were cut in November 1983. Both spines show substantial regeneration with new calcite added to the top of the cut stump and extending proximally toward the milled ring. My approach to estimating calcite mass was to use linear measurements that can be made without cutting off the regenerated calcite and to use the original tips to establish a relationship between volume (estimated from linear measurements) and weight.

The lengths of both the original and regenerated tips were measured, as well as the greatest diameter of the tip and the diameter of the regenerating spine at the level of the break (which frequently was not the greatest diameter). To approximate volume, the diameters of original and regenerated tips were squared and multiplied by the appropriate lengths (Figure 2). Because a double logarithmic plot was used, the slope is a good indication of whether there is a simple proportional relationship between weight (W) and

diameter² × length (d^2l). The appropriate test is whether the slope is significantly different from 1.0, which would indicate a simple proportional relationship between actual weight and the linear measurements.

The estimated slope is 0.982 and SE = 0.159. The t value is 0.11 with 54 df, which cannot be rejected at $\alpha = 0.05$. It is reasonable to conclude that there is a simple proportional relationship between W and d^2l , which is an estimate of volume (V). The reduced major axis or geometric mean functional regression equation (Ricker 1973) is

$$W = 0.464V \quad (4)$$

where weight is in grams and linear measurements are in centimeters.

The relationship between linear measurements and weight is important because analyses can be conducted using the linear measurements, but the biological interpretation can be made in terms of weight and so can be compared with weights of regenerated spines on tubercles following complete removal.

Results of spine regeneration on cut stumps are summarized in Table 3. The fraction of the original length that was regenerated increased

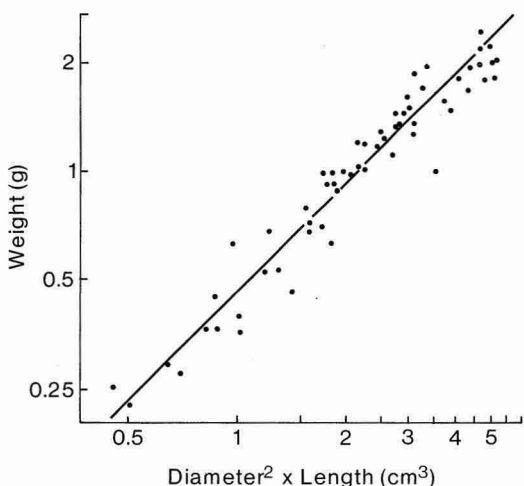
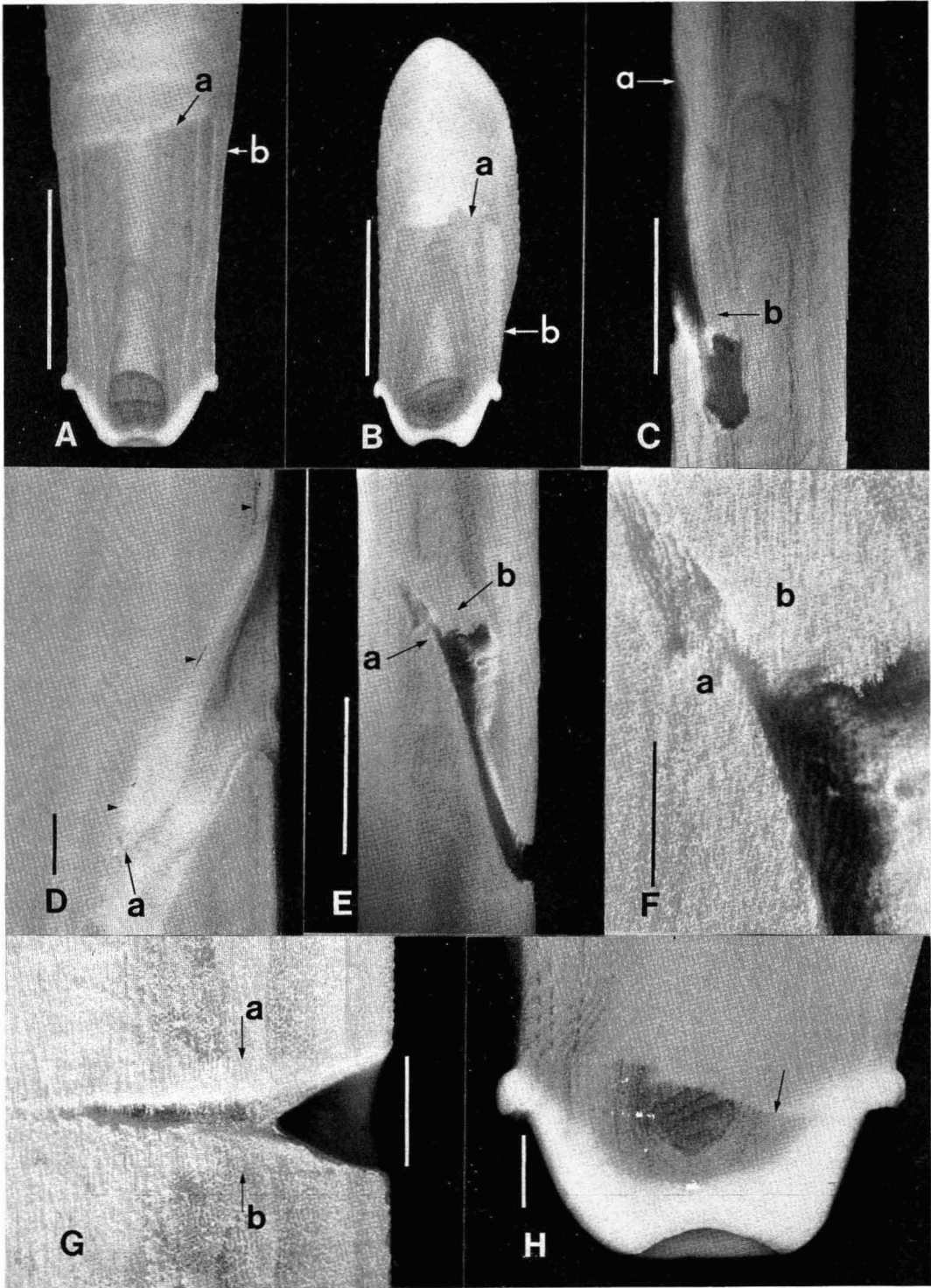


FIGURE 2. Weight of spine tips that were cut near the milled ring as a function of diameter (d) at the cut and length (l); slope of the line is not significantly different from 1.0, so there is a simple proportional relationship between weight and the two linear measurements.



with time. After 4 months, approx. 25% of the original length had regenerated; after 8 months, approx. 52% had been regenerated, and this rose to 78% after 12 months. There is a hint that regeneration is accelerating because the percent regenerated per month is 6.31, 6.46, and 6.52% for the 4-, 8-, and 12-month intervals, respectively.

The estimate of volume, d^2l , was used to compare calcite regeneration on cut spines by an ANCOVA (Table 4). The slopes of \log_e -transformed data are homogeneous ($F = 2.07$, $df = 5, 93, p > 0.05$). The common functional slope is 0.535 ± 0.039 , which means that there is proportionally less of a large spine replaced during any time interval than for a small

spine. Biologically, the analysis shows that (a) large spines that are cut regenerate more calcite per unit time than small spines (the positive and significant common slope for the regression); and (b) small spines, even though they are adding less calcite than large spines, are adding a greater fraction of the weight lost (the slope is less than 1.0). The equation relating regenerated volume (V_{rt}) to original volume (V_O) and time (t , in months) is

$$V_{rt} = 0.0923tV_O^{0.5347} \quad (5)$$

Because there is a simple proportional relationship between the approximation of volume and actual calcite weight, a direct comparison can be made between regeneration of

TABLE 3

SUMMARY STATISTICS OF REGENERATION OF SPINES FOLLOWING REMOVAL OF THREE-QUARTERS OR MORE OF TIP

TIME INTERVAL	MEAN VALUES				SD				N	PERCENT REGENERATION*	PERCENT REGENERATION PER MONTH†
	a	b	c	d	a	b	c	d			
4 months											
Nov.–Mar.	4.48	0.71	1.17	0.71	1.33	0.08	0.23	0.08	32	26.1 } 20.3 } 27.8 }	6.31
Mar.–June	5.02	0.70	1.02	0.68	2.12	0.12	0.29	0.12	19		
June–Nov.	5.07	0.69	1.41	0.68	1.70	0.11	0.37	0.12	26		
8 months											
Nov.–June	4.11	0.62	2.73	0.62	0.99	0.10	0.46	0.10	10	66.4 } 41.9 }	6.46
Mar.–Nov.	5.46	0.70	2.29	0.67	1.63	0.13	0.56	0.11	15		
12 months											
Nov.–Nov.	3.92	0.70	3.07	0.70	1.50	0.20	0.83	0.16	4	78.3	6.52

NOTE: All measurements are in centimeters; a = length of tip removed; b = maximum diameter of original tip; c = length of regenerated tip; d = diameter at site of regeneration; mass of original tip is proportional to b^2a and regenerated mass is proportional to d^2c .

* Percent regeneration is $c/a \times 100$.

† Percent regeneration per month is the weighted mean for each time period.

FIGURE 3. Ground, longitudinal midsections through spines of *Heterocentrotus mammillatus* that were cleaned with sodium hypochlorite to remove tissue; proximal is at the bottom for all figures; scale is 5 mm for A, B, C, and E, 1 mm for D, F, G, and H. A, B: a shows surface exposed by cut in November 1983, b is the proximal extent of regeneration along the spine shaft (A was collected in June and B in March 1984); C: regeneration into blind holes, a is the distal extent of regeneration, b indicates the proximal end of regenerated calcite in the hole, spine had been tagged with tetracycline in November 1983 (collected in June 1984); D: regeneration into blind holes, a is the proximal extent of regeneration, arrows mark the boundary between regenerated calcite and original spine, drilled in November 1983, no other treatment (collected in March 1984); E, F: regeneration in hole drilled entirely through spine, a shows the distal extent of regeneration that entered the proximal edge of the proximal entrance, b indicates the proximal extent of regeneration that entered the distal edge of the distal entrance, tagged with tetracycline in November 1983 (collected in March 1984); G: regeneration into hole drilled at right angle through the spine, a and b show the proximal and distal edges of regeneration in the hole, spine was abraded with sandpaper in March (collected in June 1984); H: untreated spine, arrow shows where a natural break, which extended below the milled ring, occurred, followed by regeneration of the entire tip.

TABLE 4

ALLOMETRY ANALYSIS OF REGENERATION OF CALCITE VOLUME (d^2l) ON CUT SPINES OF *Heterocentrotus mammillatus*

TIME INTERVAL	SSX	SXY	SSY	b	RESIDUAL SS	df	C	$\exp(C)$	$\exp(C)/t$
4 months									
Nov.-Mar.	8.3101	2.9254	3.3764	0.3520	2.3466	30	-1.0513	0.3495	} 0.0929
Mar.-June	9.9207	1.8902	1.5026	0.1905	1.1425	16	-1.1139	0.3283	
June-Nov.	9.3839	3.2325	2.5548	0.3445	1.4414	24	-0.8474	0.4285	
8 months									
Nov.-June	2.4732	1.5398	1.1711	0.6226	0.2124	8	-0.1792	0.8360	} 0.0912
Mar.-Nov.	7.5724	3.2755	1.9377	0.4326	0.5209	13	-0.4178	0.6585	
12 months									
Nov.-Nov.	2.2888	1.3861	0.8770	0.6056	0.0375	2	0.0691	1.0715	0.0893
Pooled					5.7011	93			
Common					6.3369	98			
Total					18.2283	103			

NOTE: $X = \log_e$ of volume of the tip that was removed; $Y = \log_e$ of volume of regenerated calcite; intercepts C and $\exp(C)$ are for a common functional slope of 0.5347; $\exp(C)/t$ = regeneration per month on a stump that had an original tip volume of 1 cm^3 ; weighted mean $\exp(C)/t$ for all time periods is 0.0923. Differences among slopes: $F_{5,93} = 2.07$, $p > 0.05$; therefore, accept a common slope.

tips on stumps and regeneration of spines on tubercles following complete removal. The stump from which a small spine tip (2 cm long with a base diameter of 0.4 cm and estimated weight of 0.15 g) had been cut would regenerate 0.09 g, or 63% of its original weight, in 4 months. During the same time period, a stump from which a large tip had been cut (7 cm long with a base diameter of 0.85 cm and estimated weight of 2.35 g) would regenerate 0.40 g, or 17% of its original weight. For comparison, regeneration on tubercles following removal of spines weighing 0.15 g and 2.35 g would be 0.014 (9%) and 0.062 (3%), respectively (equation 1).

Repair of Holes in Spines

Figures 3C and 3D both show repair of blind holes that had been drilled into spines. For both of these spines, the holes were being filled from the distal edge of the opening; regeneration proceeds from the distal side and moves into the hole, coating the more distal surface of the cavity before extending around to the proximal surface.

Figure 3E shows how regeneration proceeds when a hole passes through a spine. At the distal opening of the hole, regeneration enters over the distal edge of the hole, but

for the proximal hole opening, regeneration enters the hole over the proximal edge. Figure 3F is a detail of the central portion of the spine shaft and shows the region where regenerated calcite formed by cells entering from the distal opening nearly meets the calcite regenerated by cells entering the proximal opening. When a hole is drilled perpendicular to the spine surface, regeneration is approximately equal from proximal and distal edges of the hole (Figure 3G).

Growth and Repair of Spines Following Abrasion

The original purpose of drilling holes in spines and tagging with tetracycline was to create bench marks that could be used to determine whether growth lines would form during repair following abrasion. A total of 136 spines were examined (Table 5). With respect to the original expectations, the results were very disappointing because only 4 spines showed the presence of a new growth line over the hole that had been drilled. All 4 of these spines had been drilled in March and collected 8 months later, in November 1984. All 4 spines had been tagged with tetracycline. Two spines had been abraded with sandpaper and 2 had

TABLE 5
NEW GROWTH LINES ADDED OVER HOLES DRILLED
IN SPINES

TIME INTERVAL	TREATMENT							
	d		d + a		t		t + a	
	l.	n.l.	l.	n.l.	l.	n.l.	l.	n.l.
4 months								
Nov.–Mar.	—	12	—	5	—	5	—	4
Mar.–June	—	10	—	8	—	10	—	4
June–Nov.	—	21	—	15	—	—	—	—
8 months								
Nov.–June	—	2	—	1	—	1	—	—
Mar.–Nov.	—	2	—	9	2	10	2	7
12 months								
Nov.–Nov.	—	1	—	3	—	—	—	1
Totals	0	48	0	41	2	26	2	16

NOTE: Numbers in body of table are numbers of spines with lines (l.) or with no lines (n.l.); treatments are drilled (d), drilled and abraded (d + a), treated with tetracycline (t), and treated with tetracycline and abraded (t + a); total number of spines $N = 136$; spines were abraded with sandpaper in November 1983 and March 1984; in June 1984, spines were wiped with a cloth to simulate more gentle abrasion.

not. Based on these results, it is reasonable to conclude that abrasion does not induce the development of lines.

A total of 46 spines that had been tagged with tetracycline were sectioned to examine internal morphology. Only 1 spine showed the presence of a tetracycline mark, and this spine is illustrated elsewhere (Ebert 1985). Two new growth lines (including the outer line) had been added from the base of the spine starting at the milled ring, but these lines did not extend to the hole and so are not included as new lines in Table 5. The poor success of tagging probably means that most spines were not growing at the time they were tagged.

DISCUSSION

Spine repair in sea urchins is part of body wall maintenance and so can be considered to be an adaptive feature of echinoid biology. It is reasonable to expect that repair should follow pathways that minimize the time between injury and complete recovery and that

responses to damage should be appropriate to the damage.

The differences between rates of repair of tips on stumps and regeneration on tubercles following complete removal of spines probably is a reflection of the problems associated with first healing the surface of a tubercle compared to healing the surface of a stump. The amount of regeneration on both stumps and tubercles, though impressive in terms of calcite deposition, is not great in terms of rate of return to the original condition. A broken spine of *Strongylocentrotus purpuratus* regenerated 74% of its original mass in just 2 months (Ebert 1967) compared with 9–31% regeneration (depending on size) in *Heterocentrotus mammillatus* (Equation 4). Heatfield (1971) showed rapid growth of regenerating *S. purpuratus* spines with decreased growth after 2 months as spines approached maximum size. The rate of repair is highly temperature-sensitive (Davies et al. 1972), which clearly complicates comparisons even within a species.

Some large tubercles of *Heterocentrotus mammillatus* showed no new spines even after 8–12 months, but small tubercles were more likely to show spine formation. For comparison, in three species of *Strongylocentrotus* (Swan 1952), after 2 months, 15 out of 15 tubercles were regenerating new spines in *S. franciscanus* (Agassiz 1863), 18 out of 18 in *S. purpuratus*, and 21 out of 21 and 17 out of 17 for two *S. droebachiensis* (O. Fr. Müller 1776). Whether very small spines in *H. mammillatus* would show similar high success rates is unknown.

Two direct comparisons of growth of *Heterocentrotus mammillatus* spines have been made in other studies. Dotan and Fishelson (1985) give 10 months as the length of time for repair of cut spines, but do not show size-dependence of repair rate. Régis and Thomasin (1982) show growth of spine tips (their Figure 7) of *H. mammillatus* held in a closed-circulation seawater system. Growth of non-regenerating tips averaged about 0.03 cm^3 in 3 months, which would be only about 0.014 g of calcite (equation 4). This growth rate is very low, compared with results at Honaunau Bay, but may be explained because measurements were on nonregenerating spines that were

close to maximum size. However, more important as a determinant of spine growth rate in their study is that culture conditions appeared not to favor calcification. The effect of these conditions is a major point of their paper, which is on structural anomalies of *H. mammillatus* spines.

Return to original state is slower for the large spines of *Heterocentrotus mammillatus* than for the much smaller spines of *Strongylocentrotus purpuratus*, but it is clear that severe damage does occur under natural conditions (Figure 3H) and spines can be found that have been totally regenerated. Large spines carry a burden of slow rates of repair, so it is significant that some forms of damage can initiate spine loss and hence the additional burden of reorganizing a tubercle prior to development of a new spine.

Shedding spines in response to a blind hole but not to a major break may be a manifestation of an adaptation or may represent historical baggage and no longer be adaptive. It is unlikely that shedding is a consequence of bacterial infection because spines treated with the antibiotic tetracycline had a higher loss rate than untreated spines. Certain snails (*Pel-seneeria* spp.) are spine parasites in cidaroids where they induce galls (Hyman 1955). Shedding spines would be a way of eliminating such parasites that bore into the spines; however, I have never observed parasites in spines of *Heterocentrotus mammillatus*, or any other echinoid. This may indicate the effectiveness of spine shedding in keeping these animals free from parasites or that there are no animals capable of boring into echinoid calcite that has a living epidermis. (Cidaroids lack a spine epidermis.)

The significant change in loss rate from blind holes to holes that are drilled completely through a spine suggests that whatever sensory system is monitoring spine well-being is highly sensitive to subtle changes. The spine properties that are being sensed and the cells that are involved are unknown.

The lack of spine shedding in response to cutting certainly decreases the time needed to repair a spine compared to the length of time needed for total replacement. In cidaroids, if

a spine is cut, it is shed from the cut tip down to the collar, which is a short region above the milled ring that has an epidermis. An abscission zone (Prouho's membrane) forms and the spine breaks at this zone; then a new tip is regenerated on the stump (Märkel and Röser 1983, Prouho 1887).

The asymmetrical repair of slanting holes in spines, the symmetrical repair when the hole is perpendicular to the spine surface, and the shape of the regenerated calcite inside holes suggest that regeneration begins not with single cells moving into the holes but rather with a blastema formed in the initial stages of wound repair (F.-S. Chia, personal communication). Heatfield (1971) did not observe formation of a blastema during spine regeneration on a cut stump, and although Shimizu and Yamada (1976) describe a blastema containing sclerocytes in test regeneration, they do not specifically refer to a blastema in spine regeneration (Shimizu and Yamada 1980).

The overall picture of spine growth and repair that emerges is that spines of *Heterocentrotus mammillatus* usually are not growing but occasionally have a growth episode that produces a new growth line (see also Ebert 1987). Trauma, in the form of cutting or drilling a hole, initiates a local growth line as part of repair, as demonstrated here and also by Dotan and Fishelson (1985); however, abrasion does not initiate growth line development.

Regeneration of a spine on a tubercle, following complete removal of a spine, is slow, and there appear to be problems associated with reorganizing the tissue of a tubercle to form a new spine. The problem of reorganization seems particularly significant on large tubercles.

Regeneration is much more rapid if a spine is cut off to form a stump rather than completely ripped from the test. Regeneration rate is a function of the size of the tip that was removed and the length of time since breakage.

The rate of return to original size is slower than it is in *Strongylocentrotus purpuratus*, a species with much smaller spines, so it is reasonable to conclude that the benefits of large spines carry a burden of slow repair.

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LITERATURE CITED

- DAVIES, T. T., M. A. CRENSHAW, and B. M. HEATFIELD. 1972. The effect of temperature on the chemistry and structure of echinoid spine regeneration. *J. Paleo.* 46:874–883.
- DIXON, W. J., ed. 1981. BMDP statistical software 1981. Univ. California Press, Los Angeles.
- DOTAN, A., and L. FISHelson. 1985. Morphology of spines of *Heterocentrotus mammillatus* (Echinodermata, Echinoidea) and its ecological significance. Pages 253–260 in B. F. Keegan and B. D. S. O'Connor, eds. Proc. 5th Internat. Echinoderm Conf. (Galway). A. A. Balkema, Rotterdam.
- EBERT, T. A. 1967. Growth and repair of spines in the sea urchin *Strongylocentrotus purpuratus* (Stimpson). *Biol. Bull.* 133: 141–149.
- . 1968. Growth rates of the sea urchin *Strongylocentrotus purpuratus* related to food availability and spine abrasion. *Ecol.* 49: 1075–1091.
- . 1971. A preliminary quantitative survey of the echinoid fauna of Kealakekua and Honaunau bays, Hawaii. *Pac. Sci.* 25: 112–131.
- . 1982. Longevity, life history, and relative body wall size in sea urchins. *Ecol. Monogr.* 52: 353–394.
- . 1985. The nonperiodic nature of growth rings in echinoid spines. Pages 261–267 in B. F. Keegan and B. D. S. O'Connor, eds. Proc. 5th Internat. Echinoderm Conf. (Galway). A. A. Balkema, Rotterdam.
- . 1987. A new theory to explain the origin of growth lines in sea urchin spines. *Mar. Ecol. Progr. Ser.* 34: 197–199.
- FRICKE, H. W. 1974. Möglicher Einfluss von Feinden auf ad Verhalten von *Diadema*-Seeigeln. *Mar. Biol.* 27: 59–62.
- HEATFIELD, B. M. 1971. Growth of the calcareous skeleton during regeneration of spines of the sea urchin, *Strongylocentrotus purpuratus* (Stimpson): A light and scanning electron microscopic study. *J. Morphol.* 134: 57–90.
- HYMAN, L. H. 1955. The invertebrates: Echinodermata. McGraw-Hill, New York.
- LEIGHTON, D. L. 1968. A comparative study of food selection and nutrition in the abalone *Haliotis rufescens* Swainson, and the sea urchin, *Strongylocentrotus purpuratus* (Stimpson). Ph.D. Thesis, University of California, San Diego. 197 pages.
- MÄRKEL, K., and U. RÖSER. 1983. Calcite-resorption in the spine of the echinoid *Eucidaris tribuloides*. *Zoomorphol.* 103: 43–58.
- MISCHOR, B. 1975. Zur Morphologie und Regeneration der Hohlstacheln von *Diadema antillarum* Philippi und *Echinothrix diadema* (L.) (Echinoidea, Diadematidae). *Zoomorphol.* 82: 243–258.
- PROUHO, H. 1887. Recherches sur le *Doricidaris papillata* et quelques autres échinides de la Méditerranée. *Arch. Zool. Exp. Gén.*, Paris 15: 213–380.
- RICKER, W. E. 1973. Linear regressions in fishery research. *J. Fish. Res. Bd. Can.* 30: 409–434.
- RÉGIS, M.-B., and B. A. THOMASSIN. 1982. Ecologie des échinoïdes réguliers dans les récifs coralliens de la région de Tuléar (S. W. de Madagascar): Adaptation de la microstructure des piquants. *Ann. Inst. Océanogr.*, Paris 58: 89–98.
- . 1983. Anomalies de structure des radioles *Heterocentrotus mammillatus* (Echinodermata: Echinoidea) en microcosme *in vitro*. *Mar. Biol.* 75: 89–98.
- SHIMIZU, M., and J. YAMADA. 1976. Light and electron microscope observations of the regenerating test in the sea urchin, *Strongylocentrotus intermedius*, Pages 261–281 in N. Watabe and K. M. Wilbur, eds.

The mechanisms of mineralization in the invertebrates and plants. University of South Carolina Press, Columbia.

———. 1980. Sclerocytes and crystal growth in the regeneration of sea urchin test and spines. Pages 169–178 in M. Omori and N. Watabe, eds. The mechanisms of biomineralization in animals and plants. Tokai Univ. Press, Tokyo.

STRATHMANN, R. R. 1981. The role of spines in preventing structural damage to echinoid tests. *Paleobiol.* 7:400–406.

SWAN, E. F. 1952. Regeneration of spines by sea urchins of the genus *Strongylocentrotus*. *Growth* 16:27–35.

WEBER, J. N. 1969. Origin of concentric banding in the spines of the tropical echinoid *Heterocentrotus*. *Pac. Sci.* 23:452–66.