# AUTOTOMY AND REGENERATION IN HAWAIIAN STARFISHES

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## INTRODUCTION

Autotomy or the spontaneous loss of parts has for a long time been known to be a widespread phenomenon among echinoderms. In the Asteroidea this process manifests itself in two general ways, one consisting of the casting off of rays, the other and less common one involving the disk and resulting in the division of the animal into two parts by fission.

As early as 1872 Lutken  $(10)^1$  suggested that in certain starfishes "a radiary division occurs in which cast-off arms formed new rays and a disk". In 1878 Haeckel (7) recorded his observations of "cometoid" spontaneous division and regeneration in species of *Ophidiaster (Linckia)*. He reported that rays were cast off and new arms, disk, mouth, madreporites, etc., were developed from the base of the cast-off parts.

Following the observations of Lutken and Haeckel there was still considerable doubt that rays separated from a starfish had the capacity to regenerate a complete individual. Investigators generally expressed their belief that a portion of the disk along with a severed ray was necessary to bring about the formation of a new starfish.

More recent investigations, however, have amply demonstrated that, in certain starfishes, the basal end of a ray severed some distance from the disk may form a new and complete individual. This method of asexual reproduction, however, seems to have been adopted by a limited number of asteroids, some members of the family Linckiidae showing it to a very high degree.

Kellogg (9) in 1904 observing numerous regenerating rays of starfishes on the reef at Apia, Samoa, inferred that *Linckia diplax* (Müller and Troschel) and *Linckia pacifica* Gray had the capacity

<sup>&</sup>lt;sup>1</sup> Numbers in parentheses refer to the bibliography, p. 20.

<sup>&</sup>lt;sup>2</sup> Fisher (U. S. Nat. Mus., Bull. 76, p. 243, 1911) pointed out that the species Monks studied was not *Phataria* but *Linckia columbiae* Gray.

to regenerate new animals from isolated arms. Although Kellogg did no experimental work with the species at the time, he suggested a possibility that the rays had been thrown off by autotomy instead of being torn off by enemies.

Working with *Linckia columbiae* Gray<sup>2</sup> in 1904 Monks (12) experimentally demonstrated the development of "comets" including new rays and disk from the basal end of isolated arms leaving no doubt about the spontaneous loss of rays and their subsequent regeneration into new individuals in that species.

The probable purpose of self-mutilation resulting in the loss of rays has been suggested by numerous workers. Studer (14) noting the arms of *Labidiaster* were cast off when swollen with mature eggs believed that the phenomenon was associated with sexual propagation. By a separation of the rays near the disk it was thought the eggs might be more rapidly and widely dispersed, thus increasing the possibility of sexual reproduction. Valentine (16) at 'Tortugas, Florida, observed that a large number of specimens of *Linckia* showed regeneration of rays but found no isolated arms. For the absence of separate rays he ascribes two possible reasons, one the mature age of the regenerating specimens and the other that the season was one of sexual inactivity. This investigator apparently correlated the loss of rays with sexual propagation.

Hirota (8), however, has pointed out that since very young specimens of *Linckia multifora* (Lamarck) with rudimentary gonads show evidences of having lost rays, the casting off of arms during the egg-bearing season would not seem to be primarily for fertilization purposes. Observations in Hawaii support the view of Hirota. From the fact that on the reefs of Oahu the loss of rays of *Linckia multifora* is constant throughout the year irrespective of the spawning season, which seems to extend from December to May, it may be assumed that the phenomenon represents a natural method of asexual reproduction.

Spontaneous division involving the disk is of common occurrence among several genera of the family Asteriidae. Fisher (6) records the phenomenon in the genera *Coscinasterias*, *Sclerasterias*, and *Stephanasterias*. He found that in *Sclerasterias* fission through the disk takes place only in very young specimens while in the other two genera fissiparity continues into the adult phase. Fisher noted that in very young specimens of *Sclerasterias*, which normally have

six rays, the madreporites are symmetrically placed, and concluded that their position favors fission of the disk.

Lutken (10) observed the result of spontaneous division in Asterias tenuispina Lamarck, now Coscinasterias tenuispina (Lamarck), noting the disparity in the size of the rays. If a specimen had 6 rays these were separated into two groups of 3 approximately equal rays, those of the two groups being unequal, and if the specimen possessed 7 rays these fell into two divisions of 4 and 3 rays each. Farquhar (5) described a species in New Zealand, Stichaster insignis, now Allostichaster insignis (Farquhar), which exhibited fission through the disk. The normal number of rays was 6 but their irregularity was very pronounced as the author showed by a large number of figures.

More recently Bennett (1) in discussing autotomous reproduction in certain New Zealand starfishes suggested that the number of rays exceeding five is connected with the capacity of multiplication by transverse division, especially in *Allostichaster insignis* (Farquhar), *Allostichaster polyplax* (Müller and Troschel), *Coscinasterias calamaria* (Gray), and perhaps *Asterina regularis* (Verrill).

Crozier (3) in a critical examination of *Coscinasterias tenuispina* (Lamarck) found the usual number of rays to be 7 and that these fall into groups of 3 and 4 rays, as was noted by Lutken, the difference in the two groups being due to regeneration of parts of spontaneously divided disks. In more recent work on the same species Crozier (4) observed that fission alternated, to a large extent, with sexual reproduction, which occurred during the months of January and February.

Although the cause initiating transverse fission in starfishes is by no means clear, the position of the madreporites has been most frequently proposed as a possible stimulating factor. Since it has been shown that in starfishes with unequal rays usually the longest ray, or a long ray near the madreporite, is the "directive ray" in movement, Crozier (4) suggests that as madreporites multiply in number each new one may furnish an additional "physiological anterior point" and thereby provide conditions favorable to fission.

In order to determine the extent of the phenomena of autotomy among Hawaiian starfishes and to follow the processes involved in the regeneration after natural or artificial injury a series of investigations extending over a period of approximately three years was con-

ducted with the species of local asteroids most available. Specimens of Linckia multifora were obtained from Kaneohe Bay, Oahu, and Linckia diplax from Honaunau, Hawaii, and Kaneohe Bay, Oahu. A small race of Coscinasterias acutispina Stimpson was taken from Maalaea Bay, Maui, and larger specimens from Kaneohe Bay, Oahu. Specimens of Pentaceros hawaiiensis Fisher were collected at Maile Point, Oahu, and Dactylosaster cylindricus pacificus Fisher and a small undetermined form, probably Nepanthia species, at Black Point, Oahu. Experimental work on regeneration was conducted on the leeward and windward reefs of Oahu as well as at the Marine Biological Laboratory at Waikiki. I am indebted to Ray J. Baker for assistance in the pursuance of this investigation.

#### OBSERVATIONS

Linckia multifora (Lamarck) and Linckia diplax (Müller and Troschel).

Both Linckia multifora and Linckia diplax are plentiful in certain localities about the Hawaiian islands. Kaneohe Bay, Oahu, is a favorable habitat of these species, Linckia multifora being more abundant here. At Honaunau Bay, Hawaii, Linckia diplax was found to be the predominating starfish in 1931.

That autotomy of rays in these two species is a widespread phenomenon, as in other members of the genus, has been the observation of Kellogg (9) at Samoa and Hirota (8) in Japan. This is equally true of the species in Hawaii and especially of *Linckia multifora*. In Kaneohe Bay seldom may a perfectly symmetrical example of this species be found on the reef, one or more rays almost invariably showing evidences of regeneration.

Typically the species has 5 rays. In a lot of 85 specimens two had 6 rays and seven had 4 rays. In a lot of 52 animals 107 rays showed evidence of autotomy at some previous time; in another group of 85 specimens 238 breaks in rays were counted and in a third lot of 137 animals the number of breaks indicating autotomy was 298. In the latter lot 17 of the breaks were 40 mm or more from the disk and 216 were 25 mm or less from the disk. These breaks, in some instances at least, having occurred several years previous to the time of observation, actually took place somewhat nearer the disk than is indicated by the above data as some account must be taken of the linear growth of the basal portion of the ray.

In Kaneohe Bay a considerable number of recently detached rays may be found on the reef at all seasons of the year as well as "comets" in all stages of development (fig. 1, c). The number of free rays and comets, however, is by no means commensurate with the number of regenerated rays of living specimens. This may be explained by a high rate of mortality of freshly isolated rays together with their increased facility, because of small size, for concealment among branching corals and porous rocks on the reef.

My observation is that in Kaneohe Bay Linckia diplax shows less tendency toward autotomy of rays than does Linckia multifora. Large symmetrical specimens of L. diplax with 5 or 6 rays and no indication of autotomy having taken place are not uncommon in this locality and few detached rays or comets of this species are to be seen. Experimental evidence, however, indicates that severed rays of Linckia diplax regenerate new individuals quite as freely as do those of Linckia multifora. At Honaunau Bay, Hawaii, in 1931 a series of small forms of Linckia diplax was discovered, each individual showing autotomy of one or more rays. It is quite likely that the young of this species exhibit self-mutilation to a greater degree than do older and more mature individuals.

The actual process of the spontaneous separation of a ray from a starfish has seldom been observed. MacBride (11) states: "Even a single arm may regenerate the whole starfish. Now in some cases (Astropectinidae, Linckiidae) starfish will readily snap off their arms on irritation." The casting off of a ray in *Linckia multifora*, as I have observed it, is not a snapping process but a slow pulling apart. It begins on the ventral surface by a transverse separation of adjacent ambulacral ossicles, the break then spreading laterally and dorsally. A steady pull of the animal and ray in opposite directions by the action of the tube feet finally completes the separation, the areolar tissue sometimes stretching for a distance of two inches before parting (fig. 1, a). The entire process may be accomplished in about 60 minutes, the time varying, however, with the activity of the animal.

There seems to be no definite point of weakness in the arm of *Linckia multifora* where autotomy usually takes place, although local observations indicate that most fractures occur about 1 inch or less from the disk. Crozier (4) noted a "breaking joint" in the arm of *Asterias tenuispina* Lamarck, now *Coscinasterias tenuispina* (Lamarck), at the fifth ambulacral ossicle.



FIGURE 1.—Linckia multifora (a, b, c, g, j); Linckia diplax (d-f, h, i): a, autotomy nearing completion, the areolar tissue stretching as the ray is separated; b, "germinal ridge," gr, formed about ambulacral groove at injured end of a ray; c, a "comet" taken from the reef; d, e, basal and distal segments, respectively, of the same ray showing gradation of regeneration; f, a severed ray with madreporite, mp, after 10 months' regeneration; g, segment of an arm with rays developing at both ends; h, i, regeneration of an artifically severed ray after 10 months; j, six-rayed specimen with a branched arm.

The slow pulling apart of the arm results in irregular and ragged injured surfaces both of the stump of the ray and the proximal end of the separated portion. Closure of the open ends is accomplished by an infolding of the tissue of the dorsal, lateral, and, to a lesser degree, of the ventral walls, this overgrowth requiring about 10 days for completion. A smoother and more rapid closure occurs in rays artificially severed than in those naturally pulled apart.

The rapid proliferation of the integument resulting in a closure of the injured end may possibly be due to an activating agent which speeds up cell division. Discussing regeneration in nemerteans Coe (2) says: "Cut nerve cords liberate some influence, not improbably a growth-stimulating substance which acts superficially upon dormant cells of the neighboring parenchyma, transforming them into active regenerative cells and directing their movements forward."

The nature of the stimulus or stimuli initiating autotomy in starfishes is not clear. Various external factors in the form of irritants seem to be effective in some instances and a general alteration of ecological conditions has at times apparently aroused a marked increase in spontaneous casting off of rays. In a lot of 50 specimens of *Linckia multifora* brought from the reef and placed in laboratory tanks of circulating sea water, 18 had lost one or more rays within 18 hours. This change was doubtless one affecting the general metabolism of the animals. Crozier (3) noted that the autotomy of rays in *Coscinasterias tenuispina* was induced by localized stimuli such as injury, holding the ray, or an application of acid. I have caused arms of *Linckia multifora* to be cast off at different levels by lightly binding the ray with fine copper wire.

After having been naturally or artificially separated from the disk the rays have a tendency, as Monks (12) observed, to cling to the sides of the aquarium with the injured end down. Following the closure of the injured end the ray moves about more freely, usually seeking the surface of the water and frequently creeping out of the uncovered aquarium entirely.

Under laboratory conditions and assumably under natural conditions on the reef the mortality rate of isolated rays is very high during the first few days following either autotomy or artificial severance. Apparently bacterial infection is responsible for a large percentage of the early losses. Rays artificially sectioned two or more times are doubly exposed to the inroads of infection by reason

of injuries at both extremities. If the severed ray survives until the end is closed by overgrowing tissue the chances are much greater for the formation of a new individual.

In a detached arm the approaching development of new rays at the proximal end is forecast by the appearance of a crescent-shaped ridge of tissue about the ambulacral groove. This ridge, which I have called the "germinal crescent," is usually sharply contrasted in color from the surrounding tissue, being of a yellowish tint. The crescent is first observed after the ray has been isolated for six or seven weeks (fig. 1, b). Soon the crescent becomes marked transversely by furrows which develop into ambulacral grooves, and a mouth is formed at the point from which the grooves radiate (fig. 1, d, e). As growth continues, the rays, at first very broad for their length, become longer and thicker with their boundaries more completely differentiated. For some time the rays are confined to the end of the arm of which they are an outgrowth but after about three months they begin to extend beyond the boundaries of the parent ray, this extension being the beginning of the formation of a disk. In six months' time the new rays are about 8 mm long, and in 10 months they have reached a length of 10 mm (fig. 1, h, i). These growth rates are for laboratory-controlled specimens. My experiments show that the rate of regeneration in the sea is approximately the same as in the laboratory.

Although tube feet appear in the newly formed ambulacral grooves before the rays are fully outlined, madreporites are not observed until much later. In *Linckia diplax* a madreporite first made its appearance after six months' regeneration, being indicated by a shallow depression in the newly formed discal tissue of the dorsal surface. After 10 months the madreporite becomes functional as is indicated by its reticulated surface. At this time the new rays have a length of 10 mm and a "half-disk" has developed (fig. 1, f).

Rays of *Linckia multifora* and *Linckia diplax* were artificially severed into 2, 3, and 4 sections to determine the smallest segment capable of regenerating a new individual and also to determine, if possible, the existence of a gradient development. Rarely in these experiments have even three segments of an arm survived to the point of formation of new rays. Frequently, however, two out of three segments survive. The results clearly show a gradation of development from proximal to distal extremity of a ray. In *Linckia diplax* 

in a basal segment 40 mm long the initial rate of regeneration of new rays is much more rapid than in a distal section of the same arm and of equal length, the ratio of development being 2:1 (fig. 1, d, e). Here the middle section of the severed arm was lost before regeneration of rays occurred. Similar results were obtained for Linckia multifora. A basal segment 20 mm long was compared with a distal section of the same length. The initial development of new rays was more rapid in the basal segment. At the end of two months the rays at the proximal end of the basal segment are twice the length of those of the distal section. The disparity in volume between proximal and distal segments of equal length is, however, an appreciable amount. To compensate for the difference in volume basal segments of known length were compared with slightly longer distal sections. The ratio of initial rate of regeneration still remained 2:1 in favor of the basal segments. It should be noted, however, that the initial ratio is gradually altered until at the end of four months' development the rays of the distal segment equal in length those of the basal segment.

In like manner stumps of rays severed close to the disk show a more rapid rate of regeneration than do those cut farther from the disk. In an example of *Linckia diplax* with one ray, several 15 mm and another 42 mm from the disk, twice as much regeneration occurred in 60 days in the shorter arm as in the longer one.

Mid-ray sections of *Linckia multifora* 10 mm long regenerate rays and a disk, but tip-end segments 12 mm long seldom live more than 10 days. Sections 20 mm long from the tips of rays, however, are able to develop new rays 2 mm long in 113 days.

It is remarkable that a section of a ray less than 1 inch long will live indefinitely with the nerve cord severed and the water tube cut off from the natural supply. The behavior of the segment indicates that the nervous system functions normally and that the tube feet carry on their activities as usual. The demonstration by Paine (15) of nerve cells comparable to a nerve net in the tube feet of starfishes would explain in part at least the maintenance of life in a small section of a ray. It is probable that organic matter is absorbed through the softer tissues exposed to the water in sufficient quantity to effect growth.

The formation of rays and a disk at both extremities of a segment of an arm is an unusual phenomenon. Only twice have I observed it

to take place among several hundreds of experimental specimens. The segment was each time from the middle of a ray and one involved in its distal half a section of a previous regeneration (fig. 1, g).

Other abnormalities may modify the form of the arms. Nusbaum and Oxner (13) describe and figure *Echinaster sepositus* Lamarck with branched rays. In a specimen of *Linckia multifora* from Kaneohe Bay, Oahu, two small rays are developing laterally from near the tip of a regenerating arm (fig. 1, j). The aberrant rays have their source several millimeters from the distal extremity of the arm from which they branch and probably were activated by an injury. To supply the needs of these abnormal rays a madreporite has developed dorsally and a mouth ventrally.

## Coscinasterias acutispina Stimpson.

This species was first described from Japanese waters by Stimpson. It was collected in the central Pacific area at Kure Island and Pearl and Hermes Reef by the Tanager Expedition in 1923 and later discovered in Kaneohe Bay, Oahu. A dwarf race of the species was located along the rocky shore of Maalaea Bay, Maui, in 1931. Here large numbers of small specimens with rays ranging from 6 mm to 30 mm in length are to be found on the under surface of stones near shore. In Kaneohe Bay specimens with rays 90 mm long are not uncommon.

The dwarf race of Maui consists of fragmented and irregular individuals with rays ranging in number from 3 to 9 (fig. 2). Although the number of rays in normal specimens may vary, seven seem to be typical. The larger and more symmetrical specimens in Kaneohe Bay usually possess this number; a few, however, have 6 and some 8 rays.

A careful examination of asymmetrical specimens having from 6 to 8 rays indicates two unequal groups of rays. One group consists of 3 or 4 rays of nearly uniform size while opposite is another group of 3 or 4 rays of about equal size but larger or smaller than the first. That this asymmetry is a result of fission through the disk cannot be doubted and seems to follow the process as observed in other members of this genus and related genera by Crozier (4), Fisher (6), Farquhar (5), and others.

Typically the number of madreporites possessed by this species is four. It will be seen that a line bisecting the asymmetrical specimen, separating the groups of longer and shorter rays, falls between the two pairs of madreporites (fig. 3, a). The position of the madreporites evidently determines the direction which the line of fission takes. This is further supported by an examination of triradiate specimens (fig. 2, a, c, f) where two madreporites are seen to lie in a plane of the two opposite rays. The other two madreporites have been removed with the other portion of the disk during the process of fission.

From triradiate fragments (fig. 2, a, c, f) asymmetrical individuals with a full complement of rays are developed by regeneration (fig. 2, b, g, k, l). The additional madreporites appear as the disk is filled out on the side of the regenerating rays.

Fission seems to be the universal rule among members of this dwarf race and evidence of it is also seen among small specimens taken in Kaneohe Bay. In this locality, however, large symmetrical specimens with rays more than 3 inches long showing no signs of fission or regeneration are to be found. The asymmetrical arrangement of the madreporites is suggested as an explanation for the absence of fissiparity in large specimens (fig. 3, b).

There is no positive evidence that single rays in this species spontaneously separate from specimens at some distance from the disk as in the genus *Linckia* and regenerate new individuals at the proximal end. Arms of large specimens show no evidence of segmentation and regeneration and, among several hundred specimens taken on the reef, I have found no single rays and few if any fragments which may be considered "comets". Certain specimens (fig. 2, h) have the general appearance of comets but the presence of two welldeveloped madreporites in close proximity to the base of the large ray may indicate that a fragment of the disk remained with this arm as it separated. If this interpretation is correct the other two madreporites have not yet made their appearance.

Verrill (17) calls attention to the addition of rays in certain starfishes by budding, a phenomenon which in *Pycnopodia helianthoides* (Brandt) may continue until late in life. Such a condition is apparent in *Coscinasterias acutispina*, where occasionally young specimens with nine rays show one or more of these budded from the disk in a dorsal position instead of from the margin (fig. 2, d).



FIGURE 2.—Coscinasterias acutispina (a-l), 12 specimens of a small race from Maui showing results of fission through the disk. Natural size.

Repeated tests were made to determine whether rays of this species artificially severed have the capacity to regenerate new individuals from the proximal ends. The results were all negative, the severed rays dying without signs of regeneration. The several rays generally died within a few days but occasionally survived 30 days or longer. A ray severed 1 inch from the disk on August 19 lived until October 25, or 67 days, without showing regeneration. On several occasions under laboratory conditions single rays have spontaneously detached themselves at their junction with the disk and survived for a few days in an isolated condition. In this species the skin is very thin and delicate, which may partially account for an apparently low resistance in severed rays.

Autotomy in *Coscinasterias acutispina* follows a course of fission with the line of breakage penetrating the disk between two pairs of madreporites. The phenomenon is comparable to that occurring in *Coscinasterias tenuispina*, and in species of *Sclerasterias* and *Allostichaster* as recorded by Crozier (4), Fisher (6), Farquhar (5), and Bennett (1). The position of the madreporites as a favoring factor in spontaneous division, suggested by numerous investigators, is supported by my observations on the Hawaiian species.

# Dactylosaster cylindricus pacificus Fisher.

The type specimen of this subspecies was collected at Laysan Island by the Tanager Expedition and other specimens were taken by the same expedition at Kure Island and from an unrecorded locality. More recently this species has been found to occur in considerable numbers on the Kahala side of Black Point, Oahu, where it is concealed in crevices of porous rocks in shallow water.

The species normally has five quite rigid, cylindrical rays and one madreporite. It differs from the typical species, *Dactylosaster cylindricus* (Lamarck) of the Indian Ocean, primarily in lacking pedicellariae.

Of the many specimens taken at Black Point most of them are quite symmetrical in form with rays of nearly equal length. Occasionally a specimen is found with one or more rays undergoing regeneration (fig. 3, c). No "comet" forms in which single rays exhibit regeneration have been observed under natural conditions and no isolated rays or fragments of rays have been found on the reefs.



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FIGURE 3.—Coscinasterias acutispina (a, b); Dactylosaster cylindricus pacificus (c); Nepanthia species? (d-g); Pentaceros hawaiiensis (h): a, an eight-rayed specimen with symmetrical arrangement of madreporites, dotted line indicates line of previous fission through disk; b, disk of a symmetrical adult with asymmetrical arrangement of madreporites; c, a regenerating specimen taken from the reef; d, a specimen cut into three sections as indicated by dotted lines; e-g, ventral view of sections (d) after two months' regeneration; h, a specimen with four rays severed close to the disk.

Autotomy, however, has been observed to take place under laboratory conditions. On these occasions the spontaneous separation of rays occurred within a few hours after specimens were transferred from the reef to circulating sea water of the laboratory. Spontaneous subdivision of artificially severed rays has also been observed under controlled conditions. When such rays fragment themselves the small sections all die within a few days.

Repeated experiments have been conducted to learn whether artificially severed rays of this species have the capacity of regenerating new rays at the injured end. The results have always been negative. Usually the rays die within a week and in the few which have lived for 30 or even 40 days no appearance of new rays has been indicated.

Although *Dactylosaster* belongs to the same family as *Linckia* it seems evident that autotomy and the regenerative capacity of isolated rays may be specific generic adaptations.

#### Nepanthia species?

A minute starfish, probably representing a small race of the genus *Nepanthia*, has recently been discovered in Hawaiian waters. It has been collected at Black Point and Maile Point, Oahu, and at Maalaea Bay and on the Maliko coast of Maui.

Few symmetrical specimens are found, nearly all having rays of unequal size. The number of rays varies, seven usually being present in the more symmetrical individuals. Large specimens have a total breadth of about 15 mm but tiny fragments 3 mm across, consisting of a part of a disk and two or three small rays, may be found. The surface is covered with irregular-shaped, imbricating plates which bear on the upper face of the free ends tufts of minute spinelets. A dull gray color renders the starfish inconspicuous as it clings to the under surface of stones in shallow water.

That this small form almost universally undergoes transverse fission there can be no doubt since nearly all show a loss of one quarter to one third of the disk together with several rays. The line of fracture is apparently quite straight and always excentric to the oral aperture. From the injured margin as many as five new rays may appear in regeneration.

Multiple madreporites are present in the species, although it is difficult to determine their number and position because of the imbri-

cating surface plates. In some fragmental specimens two madreporites are distinctly observed, in others three. They stand upon rounded tubercles at the height of the surface plates and are covered by spinelets. Their position in some specimens would suggest that fission took place parallel with the line of the madreporites and that a corresponding pair was removed by division. In other specimens the unsymmetrical arrangement of the madreporites cannot be related to the apparent line of discal fission.

A specimen with three visible madreporites was cut into three sectors each including a portion of the oral aperture (fig. 3, d). After 60 days each sector presented a reformed boundary of the mouth and regenerating rays in various phases of development (fig. 3, e-g). The section including 4 rays and 2 madreporites (fig. 3, e) made the greatest growth.

Single rays of this species have lived for more than 60 days without showing evidence of the formation of new rays at the basal end.

#### Pentaceros hawaiiensis Fisher.

This species was first collected by the Albatross Expedition in Hawaiian waters at depths ranging from 32 to 73 fathoms. Recently it has been found in considerable numbers on the reef at Maile Point, Oahu, close to shore. Here the specimens are small to medium-sized, the larger ones having a radius of about 85 mm. The species is typically five-rayed, very symmetrical, with a large disk and relatively short rays, the entire surface being covered with a thick leathery skin. One madreporite is present.

Although most specimens taken about Oahu are symmetrical in outline, the rays varying but little in size and length, occasionally an individual is seen with one or more rays very much smaller than the others. Here the short arm has a narrow base and shows no evidence of being a regenerated portion of a once longer ray. Among specimens examined I have found no rays presenting abrupt constrictions indicating mutilation and subsequent regeneration. No isolated rays or comets of this species have been found on the reef and neither autotomy of rays nor fission occurs under laboratory conditions.

Individual rays artificially severed close to the disk have lived for periods up to 58 days without signs of regeneration except for the closing over of the injured surface by the thick skin, which is accomplished in about 10 days. Of four arms artificially separated from an individual (fig. 3, h) on August 2, one died on September 18, one on September 20, one on September 22, and one survived until September 29. None of the isolated arms showed evidence of the formation of new rays at the proximal end.

Regeneration of the stumps of arms severed close to the disk is exceedingly slow in this species. In a specimen thus mutilated no appreciable regeneration of the rays occurred after a period of four months.

It seems clear that the phenomenon of autotomy has not been adopted by *Pentaceros hawaiiensis* and that the isolated rays have no capacity to regenerate new individuals. Rays may regenerate following accidental loss, which apparently rarely occurs, but regrowth takes place very slowly.

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