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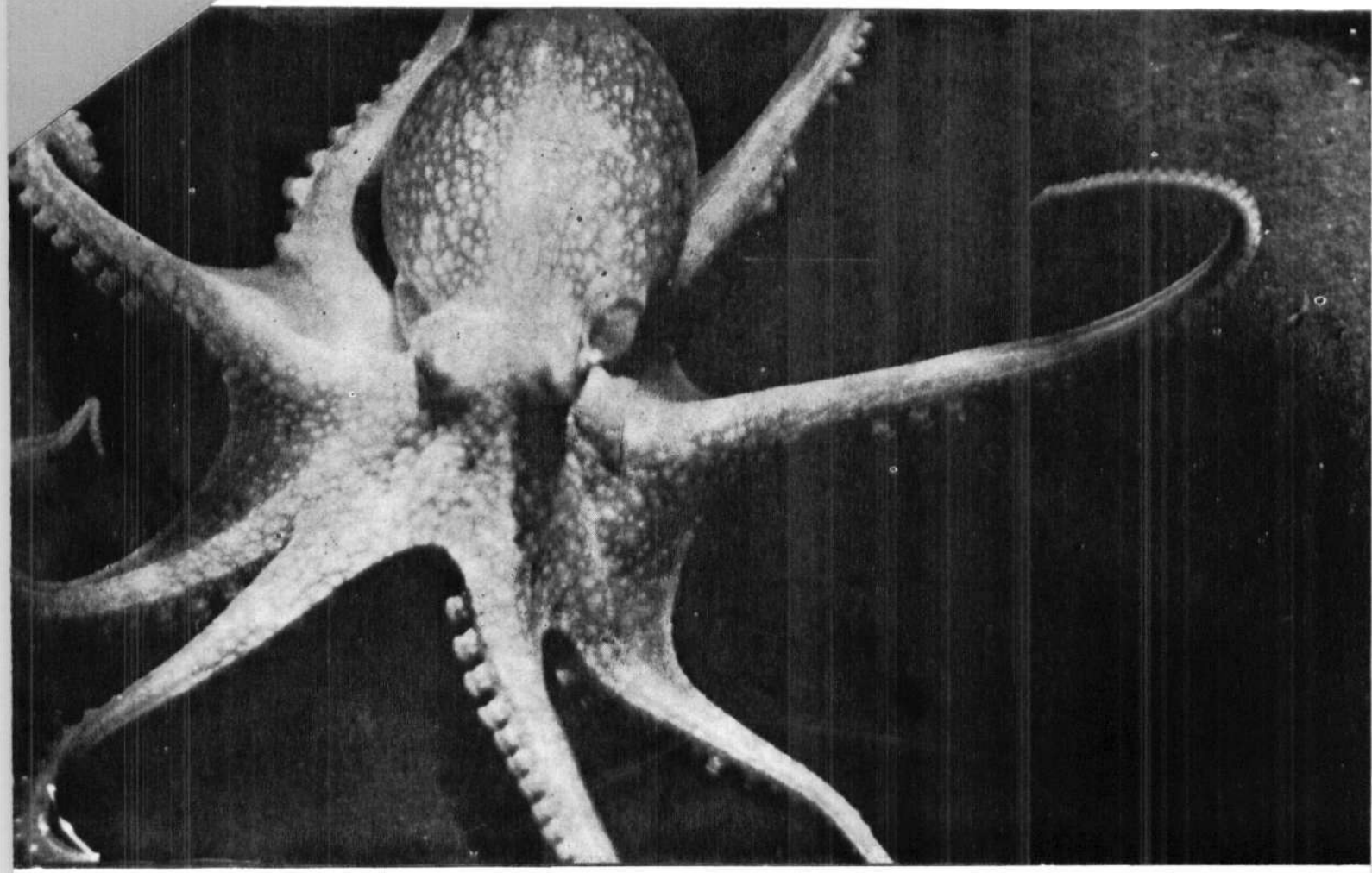

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INSTANCES OF REGENERATION IN THE CUTTLEFISH *SEPIA PHARAONIS* EHRENBERG AND IN THE SQUID *LOLIGO DUVAUCELII* ORBIGNY FROM INDIAN WATERS

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ABSTRACT

Instances of regeneration of the first, third and fourth right arms and both the tentacles of a cuttlefish *Sepia pharaonis*, caught off Waltair, the left tentacle of a cuttlefish of the same species, and the third and fourth right arms and both the tentacles of a squid *Loligo duvaucelii* collected at Vizhinjam are dealt with.

INTRODUCTION

Animals have the ability to repair small or sometimes extensive damage caused to their body accidentally or otherwise. This may involve the repair of the damaged tissue of the body or even regeneration of a lost organ. Regeneration exists in almost all groups of the animal kingdom, but it is more common in invertebrates and lower vertebrates.

The first stage of repair, as in salamanders for example (Balinsky, 1981), is that the epidermis from the periphery of the wound starts spreading over the wound and covers the entire open space. This begins to bulge out in more or less a conical shape, and underneath it a mass of cells accumulates and actively proliferates. These cells and the epidermal covering together form what is called the 'regeneration bud' or 'blastema'. Further growth of the lost organ is from this blastema.

In cephalopods, instances of regeneration have been reported by many authors since Steenstrup (1856). Feral (1978), while discussing the post-traumatic regeneration of the arms in young *Sepia officinalis*, has listed the records of regeneration in the following species of cephalopods : *Loligo pealei*, *Ommastrephes illecebrosus* (Verrill, 1881), *Sepioteuthis lessoniana* (Adam, 1937), *Architeuthis harvesi* (Verrill, 1882), *Architeuthis dux* (Aldrich and Aldrich, 1968), *Sepia officinalis*, *Sepiola atlantica* (Feral, 1977), *Eledon aldrovandi* (Parona, 1900), *Eledon moschata* (Hanko, 1913), *Eledon cirrosa* (Lange, 1920), *Octopus fusiformis*, *Octopus inconspicuus*, *Octopus cutieri* (Brock, 1886), *Octopus vulgaris* (Parona, 1900 ; Hanko, 1913 ; Lange, 1920 ; May, 1933 ; Callan, 1940), *Octopus defilippii* (Riggenbach, 1901), *Octopus* sp. (Steenstrup, 1856) and *Tremectopus violaceus* (Portmann, 1952). So far there is no record of regeneration in cephalopods of our waters. The present account deals with instances of regeneration of arms and tentacles in the cuttlefish *Sepia pharaonis* collected off Waltair and Vizhinjam, and in the squid *Loligo duvaucelii* from Vizhinjam area.

One cuttlefish measuring 230 mm in dorsal mantle length was caught in trawl net off Waltair in May, 1982, and this had its first, third and fourth right arms and both the tentacles regenerating (Plate, 1A). This cuttlefish was healthy and robust with well-developed mantle, fins and arms. The regenerated portions of the arms and tentacles are clearly distinguished from the rest of the arms by their subnormal size and thinner colouration due to lack or sparse distribution of chromatophores. Moreover, the suckers, whenever, present, are invariably much smaller than normal.

First Right Arm

This arm has a length of 38 mm including the regenerated portion of 12 mm which is narrow and tapering towards the distal end. There is a small notch-like depression aborally, just like a healed cut, and this is apparently the place where the portion of

the arm was severed off. The suckers on the arm upto the point of regeneration are normal in size but in the regenerated portion they are very small and distributed in four rows as in a normal arm (Fig. 2a).

Third Right Arm

This arm is very short, 44 mm in length including the regenerated portion of 32 mm (Fig. 2b). The suckers on the proximal part of the arm, that is below the regenerated portion are normal in size and arranged in four rows. On the regenerated portion upto about one-third of its length, the suckers are smaller in size and closely packed ; above this upto the distal end they progressively become smaller and are arranged in four rows.

Fourth Right Arm

The regenerated portion of this arm is 14 mm in length and it sharply tapers to the distal end. The suckers are very minute and normally arranged (Fig. 1c).

Right Tentacle

The total length of this regenerating tentacle is only 155 mm, whereas a normal tentacle is much longer. The regenerated portion measures 60 mm (Fig. 2d). The portion below the point of regeneration is more or less conical in outline but the regenerated portion is somewhat cylindrical becoming narrow towards the distal end till it is flattened at about one-third of its length. This flattened portion has the gross shape of a tentacular club but it is much smaller in size. From about one-fourth above the point of regeneration upwards on the inner (oral) side, there are minute suckers and on the flattened distal portion the suckers are larger in size and arranged irregularly. The marginal membrane with crenulate border, present in normal tentacular club, has not yet formed. Chromatophores are faintly distributed on the outer (aboral) side of the regenerated part of the tentacle.

FIG. 1. Regeneration of arms and tentacles of A. *Loligo duvaucelli* (Vizhinjam); B. *Sepia pharaonis* (Waltair) ; C. *Sepia pharaonis* (Vizhinjam).

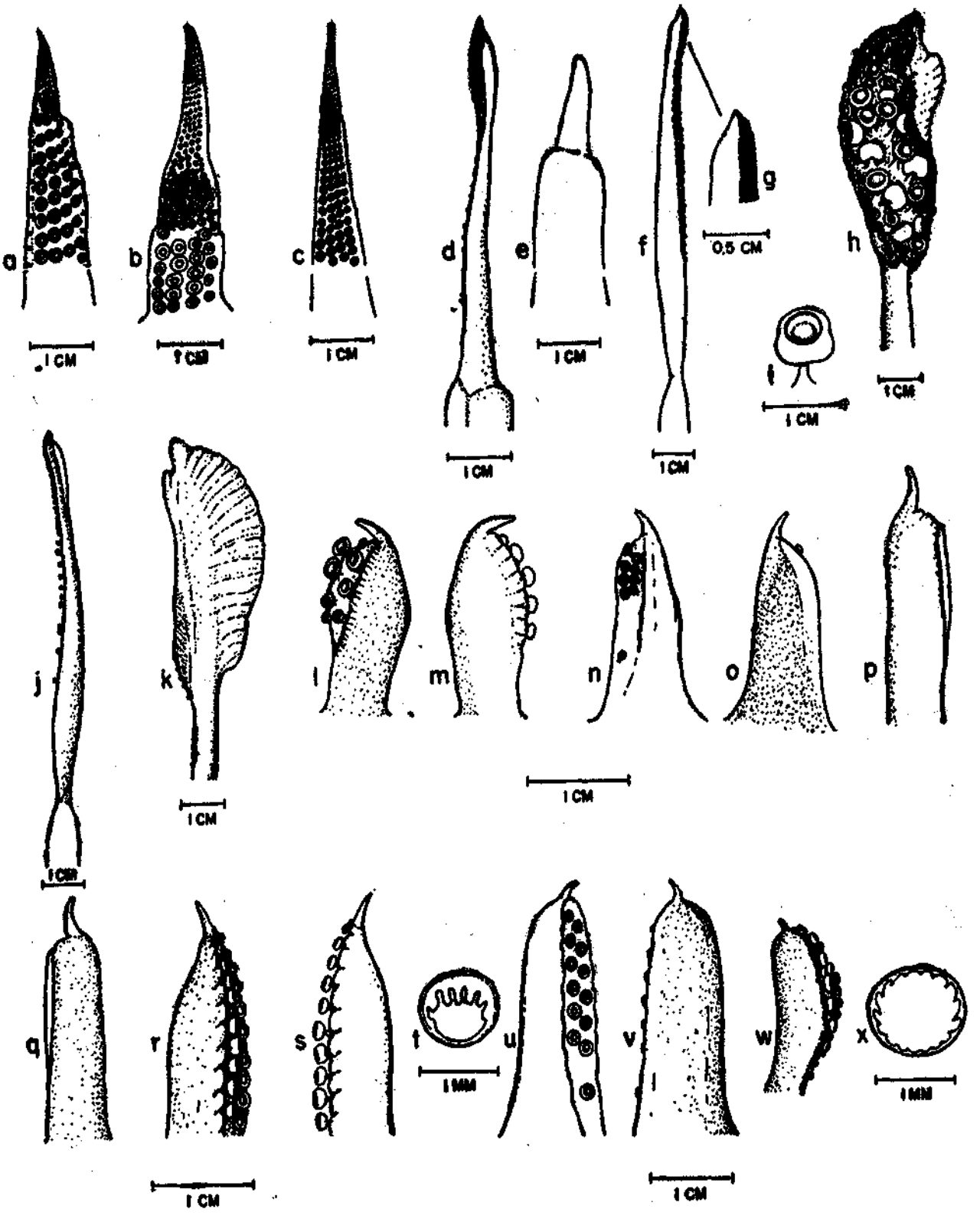

FIG. 2. Regenerated arms and tentacles. (*a-e*) *Sepia pharaonis* (Waltair): (*a*) I right arm; (*b*) III right arm; (*c*) IV right arm; (*d*) right tentacle; (*e*) left tentacle. (*f-k*) *Sepia pharaonis* (Vizhinjam); (*h*) normal right tentacular club oral view: (*i*) largest sucker of right tentacular club; (*j*) left tentacle, aborolateral view; (*k*) normal right tentacular club, aboral view. 1-x *Loligo duvaucelii* (Vizhinjam); (*l*) III right arm, orolateral view; (*m*) aborolateral view of III right arm; (*n*) IV right arm, orolateral view; (*o*) aborolateral view of IV right arm; (*p*) right tentacle's right side; (*q*) right tentacle's left side; (*r*) III left arm, dorsal side; (*s*) ventral side of III left arm; (*t*) sucker ring of III left arm; (*u*) IV left arm, orolateral view; (*v*) aborolateral view of IV left arm; (*w*) left tentacle; (*x*) ring of largest sucker of left tentacle.

Left Tentacle

This tentacle is very short in size, only 60 mm long, including 17 mm of regenerated portion which is just a stub with blunt distal end (Fig. 2e), and has no tentacular club, suckers or chromatophores.

In another male cuttlefish (*Sepia pharaonis*) measuring 225 mm in dorsal mantle length and weighing 910 g taken in hooks and line off Vizhinjam on 17th May, 1982, the left tentacle was regenerating (Fig. 1c). The tentacle is only 290 mm long including 90 mm of regenerated portion, as against the normal right tentacle measuring 435 mm. Figs. 2f, g, h, i, j show the oral and aboral sides of the normal tentacular club and orolateral and aborolateral sides of the regenerating tentacular club. The regenerated portion which begins from a constriction on the tentacular stalk is flattened laterally tapering to the distal end (Fig. 2f). Minute suckers cover the upper two-thirds of the inner (oral) side of the regenerated portion (Fig. 1g). Towards the distal end the suckers become progressively smaller in size and are very closely arranged in four rows. The largest sucker on this tentacle is only 1.5 mm in diameter, whereas the largest one of the normal right tentacle is 7.5 mm (Fig. 2h, i). There are no chromatophores on the tentacular stalk below the constriction but they are thinly distributed on the aboral side of the regenerated portion (Fig. 2j). The marginal membrane of the normal tentacular club (Fig. 2k) is not present.

The second right arm and the first left arm are cut off; the remaining proximal portions of these arms measure 23 mm and 30 mm respectively. It appears that these arms were lost just before the capture of the cuttlefish, since the wounds were still fresh and did not show any sign of healing. All other arms and the remaining right tentacle were normal and well developed.

A sample of squids of the species *Loligo duvaucelii* collected on 28th July, 1983 from boat seine landings at Vizhinjam contained a male squid measuring 125 mm in dorsal mantle length and weighing 54 g, and this squid showed signs of regeneration on its third and fourth right arms and both tentacles (Fig. 1a). The specimen was otherwise normal and healthy.

In all the arms and tentacles the regenerated portions are in the form of buds which look almost similar in size and shape. They measure only 1.5-3 mm in length and do not possess any suckers. Most of these buds have very small chromatophores on the aboral side.

Third Right Arm

This measures 19 mm including 2 mm of regenerated portion. There are only 8 suckers on the aboral side of the arm, arranged in two longitudinal rows (Fig. 2l) and the largest sucker has a diameter of 1.5 mm. The aboral keel is feeble and it is restricted to the proximal part of the arm. The marginal membrane is present on both sides of the oral plane of the arm and it is more prominent on the ventral side. The regeneration bud is small, pointed and bent towards the oral side. The outer skin of the arm continues on to the bud on which there are a few minute chromatophores aborally. The chromatophores are distributed along the entire dorsal side but on the ventral side they are restricted to the aboral part (Fig. 2m).

Fourth Right Arm

The total length of this arm is 21.5 mm including the regenerated bud which measures 1.5 mm. On the distal half of the arm there are 8 suckers arranged in two rows besides a single sucker on the proximal half (Fig. 2n), and these are smaller in size than those on the third right arm. The aboral keel is well developed. The marginal membrane is restricted to the middle portion of the arm. There are no chromatophores on the dorsal side but on the opposite side they are well distributed, becoming sparse towards the distal end with none on the regeneration bud (Fig. 2o).

Right Tentacle

This has a total length of 75 mm including 3 mm of the regeneration bud. The tentacular club is entirely absent, and what remains is only the tentacular stalk (Fig. 2p). Proximal to the regeneration bud the oral side is slightly flat. Chromatophores are present on the distal half of the tentacular stalk on the left side (Fig. 2q) but on the right side they are very few, restricted to the aboral side upto the tip of the regeneration bud.

Third Left Arm

Of the total length of 41 mm, the regeneration bud measures 3 mm (Fig. 2r). The suckers on the ventral side of the arm are slightly larger than those on the right side (Fig. 2s). There are 24 suckers arranged in two longitudinal rows, their size becoming smaller towards the distal end. The diameter of the largest sucker is 1.75 mm and that of the horny ring is 1.25 mm. The inner margin of the sucker ring bears on its upper side seven broad plate-like teeth and the lower side is smooth (Fig. 2t). The aboral keel is present upto the tip of the arm and the outer skin continues on to the regeneration bud. The marginal membrane is well developed on the ventral side and the distribution of chromatophores is more pronounced on the opposite side. There are very few small chromatophores on the aboral side of the regeneration bud.

Fourth Left Arm

This has a total length of 26.5 mm, including the regeneration bud which measures 2.5 mm. There are 13 small suckers on the oral surface with marginal membrane on either side (Fig. 2u). The aboral keel is well developed. The right side of the arm is devoid of any chromatophores but on the opposite side they are well distributed extending on to the regeneration bud (Fig. 2v).

Left Tentacle

This tentacle is 92 mm in total length including the tentacular club of 21 mm (Fig. 2w). At the distal end of the club there is a regeneration bud measuring 1.5 mm. At the proximal part of the tentacular club the suckers are very small but beyond the middle portion they are larger in size. The largest sucker measures 2 mm and its horny ring 1.25 mm in diameter (Fig. 2x). The ring has 17 marginal teeth, the lateral ones being pointed with broad bases ; those on the anterior and posterior margins are blunt. The marginal protective membrane is present on both sides of the club. Chromatophores are sparsely distributed on the distal half of the tentacular stalk and the left side of the club, but on the right side they are thickly distributed extending on to the aboral side of the regeneration bud.

Since all the regeneration buds on the arms and tentacles of this squid are in the same stage of growth and as there is not much difference in their sizes (1.5-3 mm), it is evident that all these appendages were cus off at the same time.

There are many morphological characteristics associated with the regenerated arms and tentacles. These appendages are always shorter and thinner than the normal arms and tentacles. The outer skin and chromatophores (whenever present) smoothly continue on to the regenerated portion eventhough there may be a constriction or healed cut between the original and the regenerated portions. Sometimes the regenerated portions will be a protrusion (blastema) without suckers and chromatophores or with the latter alone. The suckers when present on the regenerated portions are invariably smaller in size, often a few in number and irregular in distribution. The marginal membrane are either poorly developed or absent.

These characteristics have been observed in the oceanic squid *Ommastrephes bartrami* with regenerated tentacles and arms by Murata *et al.* (1981), who have found that a large number of squids with missing or regenerating tentacles survive normally. According to these authors the tentacles do not always seem to constitute an indispensable organ for preying. There were no noticeable differences in the mantle length composition and the mantle length—body weight relationship between squids with regenerated arms and tentacles and those with normal appendages. The reasons for the loss of arms and tentacles are not clearly understood. According to these authors the tentacles of *Ommastrephes bartrami* are easily dismembered at the stalk portion when they fall off the jig lines operated by automatic squid jigging machines. This may be the reason why there occur large number of squids of this species with missing or regenerating tentacles in the catch taken by jigging ; their incidence is upto 15% of the total number of squids. However, in all cephalopods it is possible that the appendages are lost either in encounters with prey animals (mainly fish, crustaceans and other cephalopods) or in escape bids made from enemies.

Feral (1978) has observed different stages of regeneration in the arms and tentacles of the squids *Loligo vulgaris* and *Loligo forbesi*. He has also performed experiments on the young ones of the cuttlefish *Sepia officinalis* in the laboratory by amputating the arms and tentacles, and based on the results, he has recognised six stages in the continuous process of regeneration : (1) wound healing ; (2) formation of a bud ; (3) growth of regenerating arm ; (4) formation of the suckers ; (5) appearance of the first chromatophores and (5) recovery of motor function.

While most of these stages were met with in the regenerating arms and tentacles of the present material, some characters of the different stages seem to co-occur, or the order of occurrence of these stages shows some exceptions. The regenerating portions of the first, third and the fourth right arms and the right tentacle of the Waltair specimen, and the left tentacle of the Vizhinjam specimen (both cuttlefish) are in the growing stages in having developing suckers and chromatophores. As they measure 12-32 mm (arms) and 60-90 mm (tentacles), in all probability they must have motor function, moving along with the remaining part of the respective arm or tentacle. According to Feral's stages, appearance of the first chromatophores is the fifth stage, but in the case of the squid collected at Vizhinjam the chromatophores are present even on the blastema which is in the very early stage of regeneration. However, these variations are to be expected, as the comparison is among totally different species and between organisms obtained from the wild and those subjected to experiments in the laboratory.

Suffice it to say that these damages do not seem to have any adverse effect on the animals. What will be interesting is to know how soon in nature this healing and regeneration process takes place.

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