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Regeneration versus budding in fungiid corals: a trade-off

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ABSTRACT: Following damage, solitary fungiid corals are particularly successful in their ability to repair and regenerate their tissues and skeleton. When repair is impossible, these corals turn to budding as a mode of survival. The present study examines the hypothesis that when repair is not attainable, fungiid corals develop buds from tissue remnants in order to survive, and describes for the first time the mechanism of bud formation in 3 species of fungiids. Dead specimens of the fungiids *Fungia scutaria*, *F. granulosa*, and *F. horrida* collected from under 10 m belt transects from the coral reef in Eilat (Red Sea) were found to contain live buds of various sizes. Bud formation was experimentally induced to confirm the possibility that they may indeed arise from tissue remnants. The development of buds from tissue remnants on treated corals was detected after 4 to 12 mo. Tissue fragments removed from *F. granulosa* developed into planula-like balls, settled, attached, developed new mouths and consequently developed into new anthocauli. These results showed that small viable tissues fragments can reorganize and grow into whole new individuals. Twenty corals were broken into various sized wedge-shaped fragments which were cultured in the lab. Regardless of size, fragments containing no parental mouth tissues developed new mouths within 5 d and fully formed buds were visible 10 to 21 d after breakage. Coral fragments retaining part of the parental mouth regenerated tissues and skeleton around the original mouth but did not develop anthocauli. This suggests the presence of a morphogenetic factor inhibiting the development of additional mouths and thus of new buds in these specimens. The possibility of a trade-off between the processes of regeneration and bud development is discussed.

KEY WORDS: Fungia · Coral · Regeneration · Budding · Morphogenetic factor

INTRODUCTION

Shallow water scleractinian corals are known to suffer damage from wave and wind action, sedimentation, emersion at low tide and immersion in fresh water during flash floods (Loya 1972, Meesters et al. 1992, Rogers 1993). Following trauma many corals undergo rapid tissue and skeletal repair and regeneration (Loya 1976, Bak 1983, Chadwick & Loya 1990, Meesters et al. 1994). The capacity corals have for repair plays an important role in their survival and may affect their growth and reproduction as well as their resistance to disease (Bak & Criens 1981, Bak 1983, Rinkevich & Loya 1989, Meesters et al. 1994). The rate and success of coral regeneration is affected by environmental disturbances, as well as by the corals' innate ability to repair damaged tissues and skeletal elements (Bak & Criens 1981, Rinkevich & Loya 1989, Chadwick & Loya 1990, Meesters et al. 1994). Thus corals that can quickly regenerate damaged tissues and skeleton may be more successful in areas with high disturbances than corals that cannot.

Fungiid corals are free living and are found on unstable substrates such as sand and rocks (Goreau & Young 1968, Hoeksema 1988, Hoeksema & Moka 1989). Their capacity for regeneration plays an important role in their success in these environments (Hoeksema 1991). Coral fragmentation and regeneration is rare in some species, but is common in thin polystomatous species, some of which use fragmentation as a method of reproduction (Yamashiro et al. 1989, Hoek-

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sema 1991, Yamashiro & Nishihara 1994). However, the capacity for regeneration of tissue and skeleton following trauma is common even in species which do not usually fragment naturally (Chadwick & Loya 1990).

There have been many reports on the appearance of buds or anthocauli on dead adult coralla of a number of fungiid species (Boschma 1923, Wells 1966, Veron 1986, Hoeksema 1989). Jokiel et al. (1993) reported an increase in the frequency of dead *Fungia scutaria* with attached anthocaulus-like polyps following freshwater floods in the reef flats of Kaneohe Bay in Hawaii. Krupp et al. (1993) hypothesized that although individual fungiids found under stress of sedimentation and immersion by fresh water may not survive as whole individuals, their residual tissues remained viable, and under favorable conditions differentiated into new polyps. In addition, the fact that the juveniles found on the same parent were phenotypically identical to each other suggested to the authors that the anthocauli were clone mates derived from the same parent tissues (Krupp et al. 1993). Although there is a consensus as to the origins of these buds from residual tissues (Boschma 1923, Wells 1966, Veron 1986, Hoeksema 1989, Krupp et al. 1993), no definitive examination of this process has of yet been presented. Furthermore no description has been given as to the process involved in bud formation. The present study presents experimental and histological evidence that it is indeed the residual tissues which give rise to asexually derived anthocauli, and describes the processes by which these tissues produce the buds.

MATERIALS AND METHODS

All specimens of dead corals of the species *Fungia scutaria* Lamarck, *F. granulosa* Klutzinger, and *F. horrida* Dana were collected from under 10 m belt transects (10 m^2) (see Loya 1978) at depths of maximum coral density from the reef near the H. Steinitz Marine Laboratory in the Gulf of Eilat (Red Sea). The corals were examined under a Wild stereomicroscope and the number, size and position of buds on each corallum was recorded. The specimens were subsequently returned to the sea. To experimentally induce the development of buds in the corals, a variety of treatments were carried out *in situ* on 8 healthy *F. granulosa* individuals. Three of the corals were covered by a non-toxic putty extending from the mouth and forming wedge shapes on the polyp surface, while the other 5 corals had their surface tissues removed by an air pick. All 8 corals were examined monthly for the appearance of anthocauli. Upon the appearance of polyp buds the corals were taken to the laboratory for further examination.

To determine whether pieces of tissue could indeed produce anthocauli, various-sized wedge-shaped fragments of tissue containing skeleta (septae) were broken off from medium sized live corals of *Fungia granulosa* and kept in petri dishes containing 0.45 (µm filtered sea water. The fragment sizes were classified as follows: (1) large, having a width of more than 5 septae, some with parental mouth and other fragments with no mouth tissues; (2) medium, having a width of 2 to 5 septae; and (3) small, or 'sliver', having a width of less than 2 scleroseptae. In addition, small pieces of tissues were pinched off the corallum using sharp forceps, and kept in petri dishes with 0.45 µm filtered sea water. The dishes were kept in an incubator at a constant temperature of 25°C and under a 12 h light:12 h dark light regime. The water in the dishes was changed daily and the coral and tissue fragments were monitored for morphological changes.

Following evidence of changes in the tissue configuration, i.e. the appearance of mouth, septae and/or tentacles, some of these specimens were relaxed in 4% $MgCl_2$ and fixed in 2.5% glutaraldehyde in filtered sea water. They were then decalcified in saturated EDTA, dehydrated in a series of alcohols, embedded in paraffin, sectioned, stained in hematoxylin eosin and observed under a Nikon light microscope. The remaining specimens were allowed to continue growing until a polyp was formed and a calyx was visible. Buds on excised coral pieces were raised for up to 6 mo in aquaria containing aerated sea water and were subsequently placed in the sea.

RESULTS

At the study sites, 89% of the dead individuals of *Fungia scutaria*, 77% of the dead *F. granulosa*, and 72% of the dead *F. horrida* were found to contain polyp buds (Table 1). Dead specimens of *F. scutaria* contained the highest average number (38 ± 13.6) of

Table 1. Average number (± SD) of buds on dead individuals of *Fungia granulosa*, *F. scutaria* and *F. horrida* collected under 10 m^2 belt transects at Eilat, Red Sea

Fungiid species	No. of individuals collected	Percent dead corals with buds	Average no. of buds per dead individual ± SD	Maximum no. of buds per dead individual
F. granulosa	18	77%	3.83 ± 3.6	16
F. horrida	20	72%	2.20 ± 2.1	7
F. scutaria	28	89%	38.00 ± 13.6	105

anthocauli per parent, while dead individuals of *F. granulosa* and *F. horrida* contained lower averages (3.8 ± 3.6, and 2.2 ± 2.1 respectively; see Table 1). In all cases anthocauli were found on either or both the oral and aboral sides, as well as around the edge of the parent corallum depending on the position of the corallum on the substrate. Those individuals found with oral side face down developed anthocauli on the oral surface, while those found with oral side face up developed buds aborally. No correlation was found between parent coral size and the number of buds found.

Buds that were experimentally induced in adult corals by placing putty on various parts of the adult corallum were visible 7 mo to 1 yr after treatment (Fig. 1a, b). Buds induced by removal of surface tissues developed within 4 mo (Fig. 1c, d). In some cases balled-up tissue remnants were visible between sclerosepta within 1 mo of treatment.

In the laboratory, the percent survival and regeneration of new polyps from experimentally excised fragments was 75 to 88%. Large coral pieces (larger than 5 septae in width) containing remnants of the parental mouth repaired their tissues and began regenerating skeletal elements without developing buds (Table 2). Large fragments with no parental mouth remnants repaired the torn tissues, though no skeletal regeneration occurred. A number of new stomae then developed on the cut edges of the corallum. The new mouth

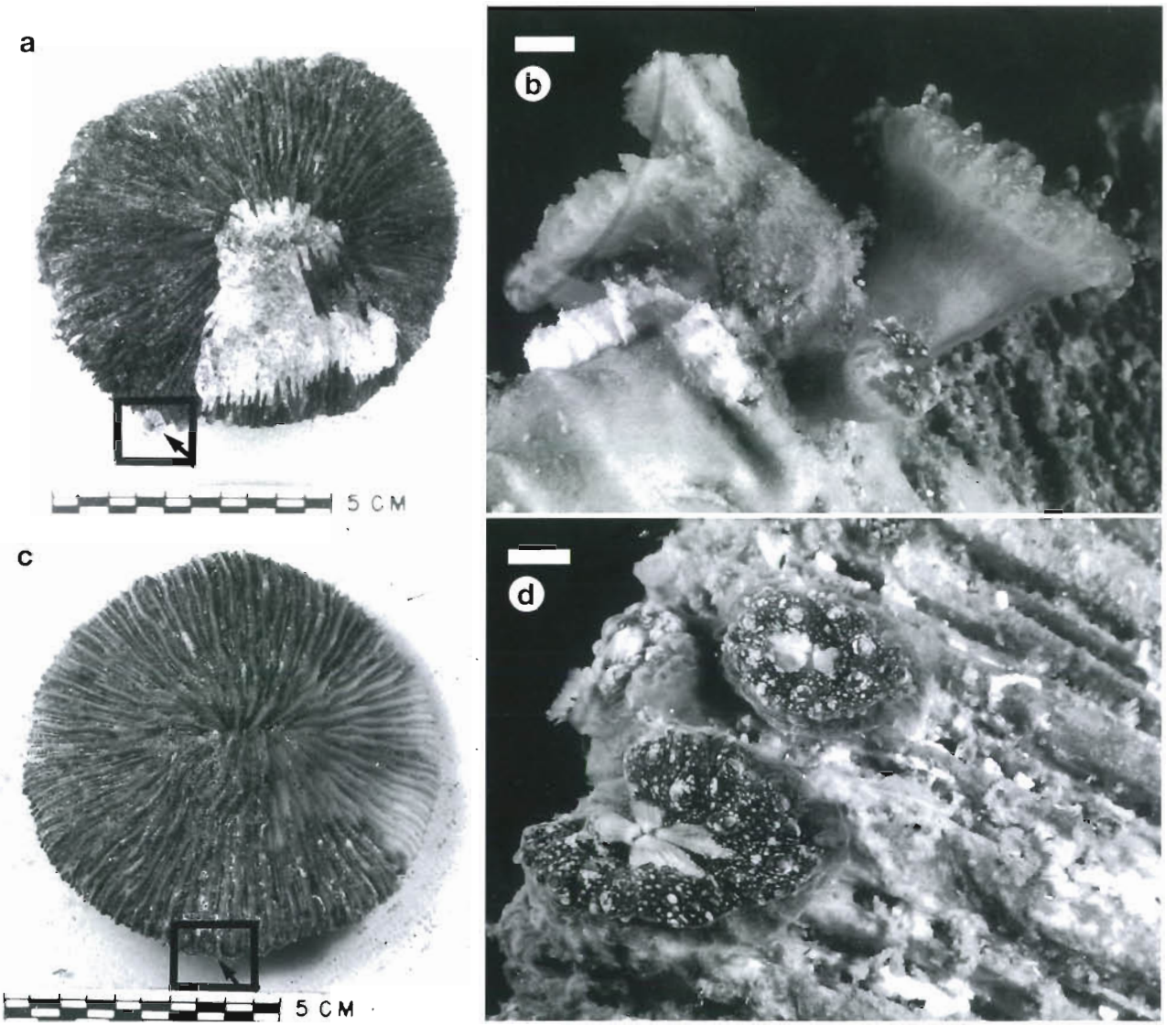

Fig. 1 *Fungia granulosa*. Corallum after experimental treatment: (a) 1 yr after coverage of the polyp's mouth and part of its corallum with putty. Arrow in insert indicates new buds. (b) Close-up of insert of parent polyp showing asexually derived anthocauli; (c) 6 mo after removal of tissue by air pick. Arrow in insert indicates new buds. (d) Close-up of insert of anthocauli developed 6 mo after tissue removal. Scale bar = 1.0 mm

Table 2. *Fungia granulosa*. Characteristics of survival, regeneration, and bud formation, following experimental breakage of various-sized fragments of coral raised under laboratory conditions. Large fragments were greater than 5 septae in thickness, medium-sized fragments were 2 to 5 septae in thickness, and small fragments were 1 septum in thickness. w: with mouth; wo: without mouth

Fragment size w/wo parental mouth	No. of fragments	Tissue state after 2 wk	Average size of buds (mm) after 1 mo ± SD	Average no. of buds after 1 mo ± SD	Maximum no. of buds after 2 mo
Large w mouth	8	Repaired	–	0	0
Large wo mouth	10	Repaired, new mouths	3.28 ± 1.87	2.40 ± 1.35	4
Medium wo mouth	10	Shrunken, balled-up, new mouths	2.15 ± 1.13	6.90 ± 4.58	15
Small wo mouth	32	Shrunken, balled-up, new mouths	1.29 ± 0.71	2.46 ± 1.77	8

or mouths most proximal to the original mouth developed fastest, at times leading to the disappearance of the more distal mouths. The new mouths eventually developed into new buds or anthocauli. Tissue remnants on medium-sized coral fragments (2 to 5 septae) regressed, and formed islands of tissues between the septae (see Table 2, Fig. 3). A number of stomae developed centrally on each tissue island 10 d to 2 wk after breakage. Polyps developed 2 to 3 wk later. Small fragments or slivers gave rise to 1 or more polyps depending on the number of tissue balls remaining on the septae. The tissue began disintegrating and

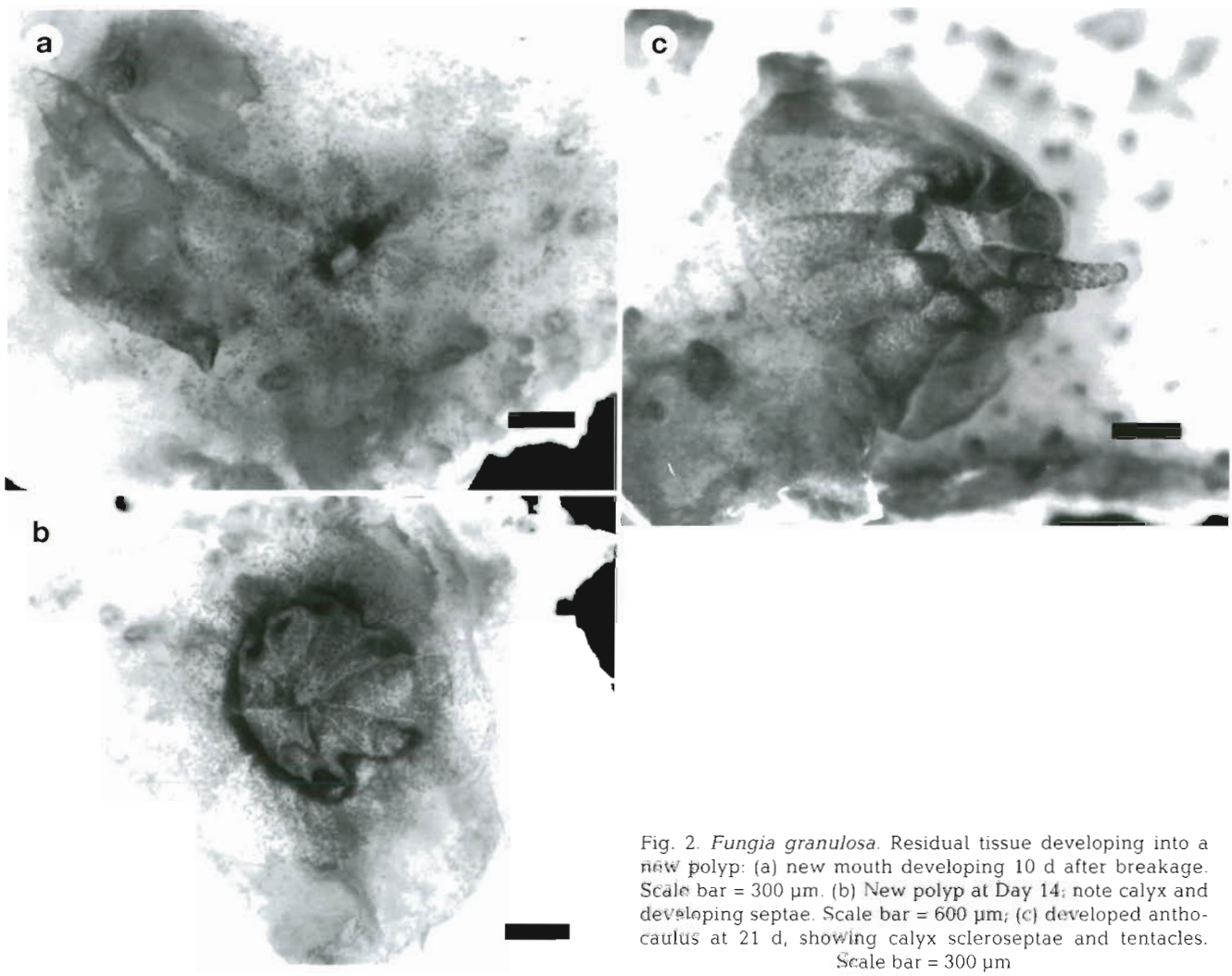

Fig. 2. *Fungia granulosa*. Residual tissue developing into a new polyp: (a) new mouth developing 10 d after breakage. Scale bar = 300 µm. (b) New polyp at Day 14; note calyx and developing septae. Scale bar = 600 µm; (c) developed anthocaulus at 21 d, showing calyx scleroseptae and tentacles. Scale bar = 300 µm

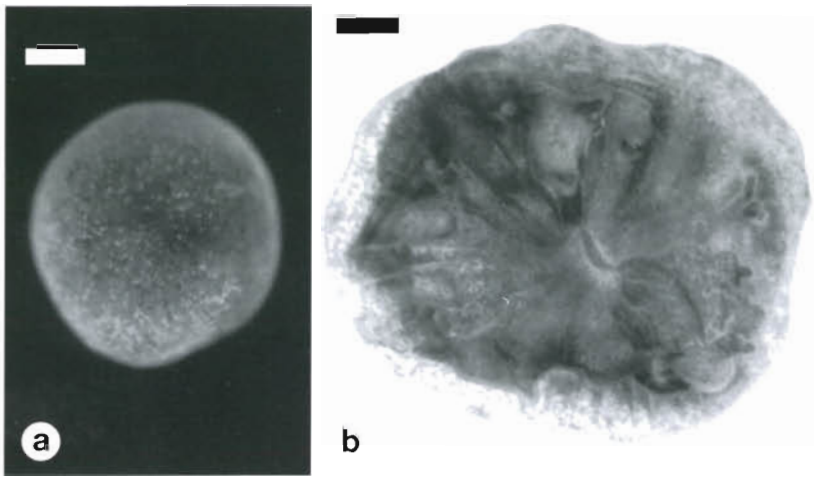

Fig. 3. *Fungia granulosa*. Tissue fragment: (a) planula-like tissue ball 1 d after excision. Scale bar = 100 µm. (b) Tissue ball 21 d after settlement *in vitro*. Note development of mouth, calyx and septae. Scale bar = 300 µm

shrinking within a few days of excision, and the remaining tissue formed a central mouth (Fig. 2a). The theca and septae were visible 10 d after breakage, as were tentacle buds (Fig. 2b). The average time from excision to development of a new polyp with septae was 21 d (Fig. 2c). In some cases tissues formed a ball similar to a settling planula, which developed a mouth within 10 d of excision.

Excised tissue fragments balled up and became planula-like in appearance (Fig. 3a). These planula-like balls of tissue settled on the petri dishes and secreted mucus strings by means of which they attached themselves to the dish. Ten days after attachment a mouth and tentacles were visible, and septae and a calyx were beginning to develop (Fig. 3b). The bud size depended on the amount of residual tissues left on the coral (Table 2). Those coral fragments with larger tissue remnants developed larger buds. In anthocauli developing from larger pieces of tissue, remnants of 'parent tissues' were visible surrounding the new bud (Fig. 4a). Histological sections of these young buds showed all tissue layers found in normal juveniles (Fig. 4b). These tissues differentiated from adjacent parental tissue remnants, in which very thin ectoderm mesoglea and gastroderm were present and which formed a continuum with the bud tissue.

DISCUSSION

Fungiid corals are abundant in volatile environments uninhabited by other coral species, and are able to with-

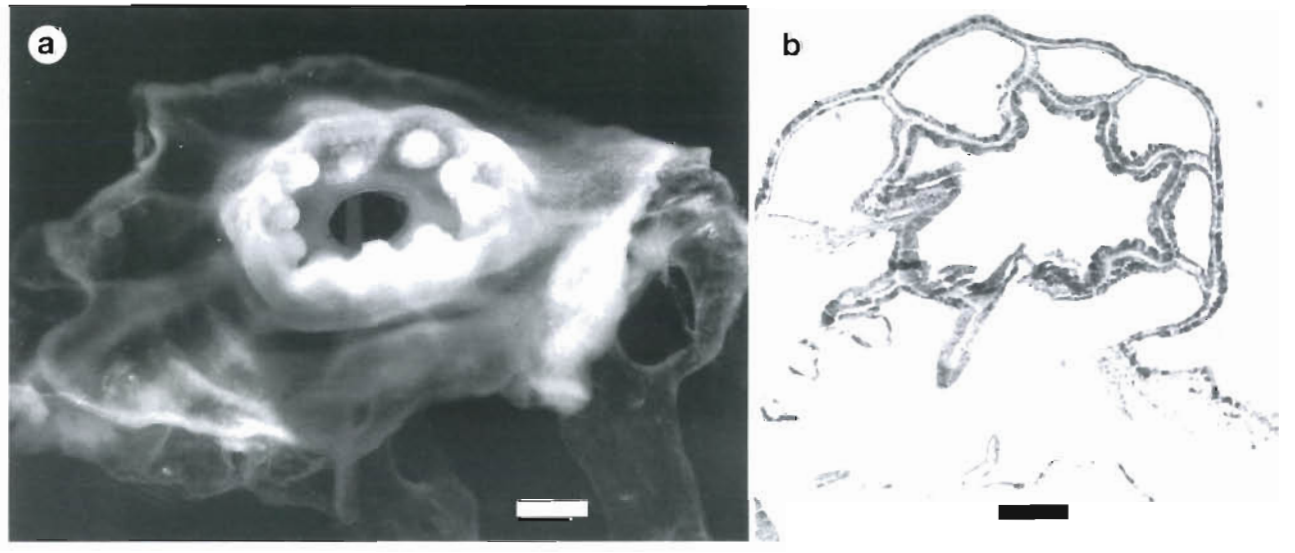

Fig. 4. *Fungia granulosa*. Residual tissue: (a) decalcified bud developed from residual tissue, surrounded by remnant parental tissue. Scale bar = 300 µm. (b) Histological section of the bud revealing tissue formation from parental tissues. Remnant parental tissues at bottom of photograph. Scale bar = 200 µm

stand sedimentation, breakage and immersion by fresh water for short periods of time (Hoeksema 1989, Yamashiro et al. 1989, Chadwick & Loya 1990, Jokiel et al. 1993). To survive in these areas, fungiids developed mechanisms of quick regeneration or of budding which allow them to repopulate these areas following catastrophes. In addition, our results show that following trauma in which the corallum is broken small pieces of tissue may be shed from the parent corallum. These tissue shreds may settle, attach and redevelop into new polyps. This ability of the fungiid tissues to undergo regression and to redevelop into new polyps may hint at a reaction to stress similar to the polyp bail-out found in *Seriatopora hystrix* (Sammarco 1982). Thus, the fungiid's capacity to survive and resurge after a variety environmental hazards may be a mechanism of rapid recruitment in unstable environments (Krupp et al. 1993).

The difference in number of buds found on the 3 species of fungiids in this study may be due to the fact that they are found under different degrees of environmental disturbances. *Fungia scutaria* is a shallow water species and may be subjected to greater environmental stresses than the other 2 fungiids in this study, which, although found in shallow water, are in greater abundance in deeper waters (Kramarsky-Winter pers. obs.). Therefore, the number of buds found on individuals of *F. scutaria* may be an indication of the instability of the shallow water environment in which they are found.

Our results indicate that there is a physiological tradeoff (see Stearns 1992) between regeneration and bud formation in these corals. If repair of tissues and skeleton is possible, regeneration occurs, and the individual coral continues growing. However, if repair is not possible, coral tissues regress into a larval-like state, and eventually redevelop into new buds. This mechanism allows for the survival of these corals even when they are very badly damaged. The mouth tissues may be seen as the limiting resource directing tissue morphogenesis, controlling repair versus bud formation.

The driving force for regeneration is the confluence of peripheral tissues with the mouth, which evidently act as an organizing center directing repair. Absence of the parental mouth, or its detachment from peripheral tissues, results in the reorganization of these tissues which develop into new polyps. These results are consistent with previous works (Boschma 1923, Chadwick & Loya 1990, Jokiel & Bigger 1994). As early as 1923, Boschma observed that when the mouth and tissues of the fungiid *Fungia fungites* were covered by putty the coral formed new buds. He hypothesized that some isolated tissues in the overall decaying tissues somehow remained alive and produced new buds. Jokiel & Bigger (1994) found that, similar to our results for *F. granulosa* and *F. horrida*, broken pieces of *F. scutaria* containing parental mouths did not regenerate additional mouths and continued growing in the coral's original axis, while mouthless sections formed new mouths which later formed anthoblasts which grew on a new axis. In addition, we found that tissue remnants, whether on the parent calyx or severed from it, developed into planula-like balls. These planula balls then developed a new mouth around which a new axis of growth was oriented.

Although in previous works Krupp et al. (1993) succeeded in maintaining pieces of excised mesenterial filaments of the fungiid *Fungia scutaria* for as long as 2 mo, no settlement of these planula-like balls was observed. This may have been due to the fact that mesenterial filaments are endodermal in origin with no ectodermal elements. It is likely that those tissues alone are not capable of forming new anthocauli. The use of both tissue elements, i.e. endoderm and ectoderm, in the excision used in the present work ensured the presence of all tissue elements used in rebuilding the new polyp. In addition it is possible that the presence of the mesoglea found between the 2 tissue layers is necessary for development of new polyps. Indeed the importance of the mesoglea in development and regeneration has been widely studied in hydrozoa (Plickert 1987, Schmid et al. 1991, 1992). Schmid et al. (1991, 1992) found that transdifferentiation from muscle cells into nerve cells in jellyfish occurs when isolated striated muscle cells come in contact with a cell-free extracellular matrix, and concluded that mechanochemical interactions between the muscle cells and their substrate are responsible for the activation and inhibition of the process of transdifferentiation. It is likely that a similar mechanism occurs during bud formation from the tissue remnants in fungiids, and it is conceivable that the directional cue for differentiation and body form is a diffusable morphogen found in the mouth tissue.

The presence of morphogenetic factors which inhibit or promote regeneration has been widely studied for hydrozoa (Wolpert et al. 1974, Berkin 1991, Sato et al. 1992, Meinhardt 1993), and has been inferred here for fungiids. Our results suggest the presence of a mouth-inhibiting factor, which when prevented from reaching the coral tissues allows these tissues to develop into anthocauli. Thus, even large mouthless pieces of the fungiids which at first underwent tissue repair developed new mouths which then developed into new buds. These morphogenetic factors and their role in regeneration and bud formation, as well as the environmental and physiological cues responsible for recuperation, are currently being studied in fungiid corals and are of special interest, as it is likely that similar factors are involved in recuperation and regeneration in other coral species.

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