

Limits on the bathymetric distribution of keratose sponges: a field test in deep water

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ABSTRACT: The keratose sponges (i.e. those in which the mineral skeleton is replaced by a collagenous skeleton) are generally restricted to shallow-water habitats, but the causes of this distinct bathymetric pattern remain unclear. Sharp pycnoclines at the depth of the upper slope may hinder colonization of deep waters because of thermal stress or reduced light and particulate food below the pycnoclines. It is also possible that oligotrophy and loss of symbiotic cyanobacteria below the pycnocline may lead to a nutritional stress. Using manned submersibles in Exuma Sound, Bahamas, we determined that the pycnocline lies between 70 and 100 m. We transplanted individuals of 2 keratose sponges (*Aplysina fistularis* and *Ircinia felix*) from their natural habitat on a shallow reef (4 m deep) to 3 depths (100, 200, 300 m) within or below the pycnocline to investigate mortality and changes in body size, shape and histology as a function of depth. We also recorded changes in populations of photosynthetic and heterotrophic symbiotic bacteria, as well as the parasitic polychaete *Haplosyllis spongicola*. By transplanting individuals of *A. fistularis* bearing buds for asexual propagation (fistules) and individuals of *I. felix* brooding embryos, we also tested the viability of reproductive propagules in deep-water environments. We found that, although these 2 sponges do not naturally occur at depths below 40 m, 62.5% of *A. fistularis* and 42.8% of *I. felix* survived at 100 m for 12 mo. No *A. fistularis* survived at 200 m, whereas 28.5% of *I. felix* did. All sponges transplanted to 300 m died within 2 mo. Water temperature was the most likely cause of sudden mortality at this depth. There were no significant differences in growth between individuals at the slope and controls on the shallow reef. Cyanobacteria were lost in individuals of *I. felix* that survived at 100 and 200 m, and these sponges repositioned oscules and formed chimney-like processes, probably to enhance water flow through the sponge and compensate for nutritional stress. By contrast, cyanobacteria were still abundant in individuals of *A. fistularis* surviving at a depth of 100 m, and these sponges did not change shape significantly, apart from the loss of fistules. It appears, therefore, that the loss of cyanobacteria and the increasing oligotrophy with depth do not set the lower bathymetric limits of species. Removal of sponge tissues by the parasitic polychaete *H. spongicola* also appears not to aggravate significantly the nutritional stress experienced by sponges transplanted to deep water, at least to the extent that it may restrict the bathymetric distribution of the host. Despite the facts that only the species *I. felix* was heavily parasitized and that parasites survived within hosts at all depths, there was no significant difference in survival with depths between sponge species. A TEM (transmission electron microscope) examination of the mesohyl did not reveal significant cytological differences among sponges transplanted to various depths. At all depths, surviving individuals of both species showed archeocytes engaged in phagocytosis and digestion of cyanobacteria and/or heterotrophic bacteria. Similarly, collencytes and spongocytes were apparently secreting collagen, indicating that temperatures at 100 and 200 m do not inhibit the formation of the skeleton. Sponge recruitment derived from either asexual or sexual propagules was never observed at slope depths. Since adult sponges survived when they were artificially transported to deep waters, the inhibition of larval dispersal or settlement success (perhaps caused by the sharp decrease in temperature with increasing depth) emerges as the most plausible explanation for the shallow-water confinement of these keratose sponges.

KEY WORDS: Keratose sponges · Bathymetric distributions · Sponge ecology · Sponge symbionts · Sponge infauna · Slope megafauna

INTRODUCTION

Sponges are well adapted to a great variety of ecological conditions despite their simple body organization (Bergquist 1978, Vacelet 1979, 1988). There are 3 major groups of sponges, namely Hexactinellida (with triaxonic silica spicules and sincytial organization), Calcarea (with calcareous spicules), and Demospongiae (with either monaxonic or tetraxonic silica spicules, or with a collagenous skeleton replacing silica spicules). Most Hexactinellida live at bathyal depths (Tabachnick 1994), whereas most Calcarea are found in relatively shallow waters, between 0 and 200 m (Reid 1968). Nevertheless, a few shallow-water hexactinellids and deep-sea Calcarea are also known (e.g. Mackie & Singla 1983, Vacelet et al. 1989). To outline the bathymetric distribution of Demospongiae is somewhat more complicated, since this class contains about 95% of the living sponges. Indeed, demosponges are present from freshwater springs high in the mountains to hadal depths in the ocean. Nevertheless, distinct patterns of vertical zonation can be found for a few taxonomic subgroups (reviewed by Vacelet 1988). One of the most striking examples is provided by the so-called keratose demosponges (orders Dendroceratida, Dictyoceratida and Verongida). In these sponges, the silica skeleton has been evolutionarily replaced by collagenous elements, which may be spongin fibers, spongin filaments, or spongin spicules (reviewed by Bergquist 1980). It has long been known that most keratose sponges are only present in shallow waters (Burton 1928), with very few species inhabiting bathyal depths (e.g. Lévi & Lévi 1983, Ilan et al. 1994, Maldonado & Young 1998). Although the causes of this vertical distribution remain unclear, 2 processes are commonly invoked to account for it: (1) the nutritional advantages of a symbiosis between sponges and cyanobacteria within the euphotic zone (Sarà & Vacelet 1973, Cheshire & Wilkinson 1991), and (2) the deleterious effects of cold water on the physiology of keratose sponges (Vacelet 1988).

Many keratose sponges contain photosynthetic symbionts (mostly cyanobacteria) from which the sponge may obtain a substantial part of its nutrition (e.g. Sarà 1971, Vacelet 1971, Wilkinson 1983, Wilkinson & Cheshire 1990). This association is obligate for some species (phototrophic sponges) and facultative for others (mixotrophic sponges). Cheshire & Wilkinson (1991) developed a model describing how the vertical distribution of phototrophic sponges is limited by photosynthetically active radiation. They concluded that these sponges should not survive at depths greater than 30 m. At greater depths, respiration should exceed photosynthetic production over a 24 h period. This argument does not explain, however, why the

lower depth limit in many mixotrophic keratose sponges is similar to that predicted for phototrophic species.

Some keratose sponges are heavily parasitized by sponge-eating polychaetes (e.g. Pawlik 1983, Tsurumi & Reising 1997). This host-parasite relationship appears to be benign in shallow-water habitats, where sponges benefit from symbiosis with cyanobacteria and particulate food is not limiting. However, sponges recruiting at deeper sites on the slope may be nutritionally disadvantaged by the loss of cyanobacteria and lower densities of particulate foods. Under these conditions, we hypothesize that the continuous removal of biomass by the parasite may have fatal consequences for the host sponge. The idea that a counterbalance between parasitic and symbiotic relationships may play some role in determining the vertical distribution has not been investigated.

Many observations suggest that water temperature may limit vertical distributions of keratose sponges. Although a few species are known from cold waters (Burton 1928, Bergquist 1961, Dayton et al. 1974), the importance of the keratose fauna declines dramatically in both latitudes and depths where temperature drops below 18 to 20°C (Vacelet 1988). In a pioneer study on Antarctic sponges, Hentschel (1923) reported that the ratio between spongin and siliceous spicules is lower in sponges living at low temperatures than in congeneric or conspecific sponges living in warmer water. As a result of this work, it is widely believed that low temperatures enhance secretion of siliceous spicules. The fact that these sponges are much better represented in tropical and subtropical areas than in high latitudes is commonly viewed as indirect evidence supporting this idea (Burton 1928, Sarà & Vacelet 1973, Vacelet 1988). The alternative possibility that low temperatures affect negatively the synthesis of collagen-spongin has never been investigated.

If a decrease in irradiance, temperature and particulate food availability with increasing depth actually has deleterious effects on the physiology of keratose sponges, one might expect pycnoclines to be important environmental barriers. Sharp pycnoclines usually involve significant reductions in light availability, temperature and particulate food (Kinne 1971). Moreover, pycnoclines may also hinder larval dispersal. In pycnoclines, numerous physical parameters that are relevant to the physiology of invertebrate larvae, such as dissolved gases, seawater density, viscosity, osmolality, etc., change rapidly over a short vertical distance (reviewed by Kinne 1971). Several studies have suggested that larvae of benthic invertebrates are physically unable to pass through these environmental barriers (e.g. Angel 1968, Vázquez & Young 1996, Metaxas & Young 1998), thereby preventing recruitment of benthic organisms on one side of the pycnocline or

the other. Laboratory experiments with shallow-water tropical sponges indicated that a 5°C decrease in water temperature, from 20 to 15°C, reduces significantly both larval dispersal and settlement success (Maldonado & Young 1996a). There is thus a body of indirect evidence suggesting that sharp pycnoclines may be barriers that hinder colonization of the slope by keratose sponges.

In this study, we investigated the potential causes of the shallow-water confinement of 2 mixotrophic keratose sponges, *Aplysina fistularis* (Pallas 1766) and *Ircinia felix* (Duchassaing & Michelotti 1864). These 2 species are common in Caribbean lagoons, sand flats and reefs between 1 and 5 m deep (e.g. Wiedenmayer 1977, Van Soest 1978, Zea 1987), but their abundances decrease dramatically with depth. By using manned submersibles, we investigated the location of the pycnocline on a steep Caribbean slope, and confirmed that *A. fistularis* and *I. felix* were not present at or below the pycnocline depth. Individuals of both species were transplanted from their natural habitat in a shallow reef to greater depths along the slope, at and below the pycnocline. We recorded the incidence of mortality over time, and investigated changes in body size, external morphology and histology as a function of depth. Changes in the intra-sponge populations of photosynthetic and heterotrophic symbiotic bacteria, as well as in the population of the parasitic polychaete *Haplosyllis spongicola*, were also examined. By transplanting to the slope individuals of *A. fistularis* bearing buds for asexual propagation and individuals of *I. felix* brooding high numbers of developing larvae, we also tested the hypothesis that a limited dispersal of reproductive propagules may cause the shallow-water confinement of these species. We reasoned that if limited larval dispersal rather than physiological tolerance to deep-water conditions limits colonization of the slope by shallow-water keratose sponges, some short-term recruitment should be expected from these transplants.

MATERIALS AND METHODS

Sponge features. Both *Aplysina fistularis* and *Ircinia felix* are known to be mixotrophic sponges, associated with the cyanobacteria *Aphanocapsa feldmani* (Reiswig 1981, Rützler 1990, Vicente 1990).

Bahamian individuals of *Ircinia felix* (order Dictyoceratida, family Irciniidae) are massive, lobate sponges, reddish brown in color, with purplish or greenish tinges (Wiedenmayer 1977). Oscules are 3 to 6 mm in diameter, often at the top of the lobes, and display a characteristic black rim. The sponge surface is finely conulose, with conules 0.5 to 2 mm high and 1 to

3 mm apart. The skeleton consists of an irregular, coarse network of spongin fibers made of ascending primary fibers connected transversally by secondary fibers. The primaries are grouped in fascicles and heavily filled with debris (exogenous spicules and sand grains), whereas the secondaries are non-fasciculate and contain a moderate amount of debris. Apart from the fiber skeleton, there are numerous spongin filaments permeating the flesh at the internal portion of the sponge (endosome). Spongin filaments are 2 to 5 µm thick and end with an oval knot (8 to 10 µm in diameter). In virtually all individual sponges examined from the natural population, the endosome served as host for numerous parasitic syllid polychaetes of the species *Haplosyllis spongicola*. As in other Dictyoceratida, *I. felix* broods embryos and releases free-swimming parenchymella larvae. Larval release in the studied population takes place in May and June (authors' pers. obs.).

Individuals of *Aplysina fistularis fistularis* (order Verongida, family Aplysinidae) are bright-yellow, vase-shaped sponges with a single oscule at the top provided with a sphincter-like membrane (Wiedenmayer 1977). The apex of the sponge commonly bears numerous thin, digitate processes (fistules). Fistules are 1 to 2 mm in diameter and up to 2 cm long. The sponge skeleton is a regular network of nearly hexagonal meshes made of a single type of fiber. Fibers are amber-colored and pithed, with a laminated bark and lacking exogenous inclusions. Parasitic polychaetes were scarce or absent in most sponges examined. Thus, the effects of parasitism on the host biology were considered to be negligible at the population level. As is typical for Verongida, *A. fistularis* is oviparous, with an unknown larval form. Grafting studies have shown that many local populations are single genets, suggesting that asexual propagation through fistule detachment is the main reproductive pattern (Neigel & Schmahl 1984).

Physical setting and environmental data. Field experiments were conducted near the Caribbean Marine Research Center (CMRC) at Lee Stocking Island, in the southern Exuma Cays, Bahamas (Fig. 1). The Exumas are a group of subtropical islands and cays lining the eastern margin of the Great Bahama Bank. Shallow patch reefs are scattered on the lee sides of the islands and in some of the tidal channels connecting the Bank with Exuma Sound. On the Exuma Sound (windward) side, there is a well-developed fore-reef. The fore-reef at Lee Stocking Island consists of a shallow terrace at 18 m and a deep terrace at 45 m, then cascades to a virtually vertical cliff that angles to a more gentle slope below 200 m.

Water temperature was recorded at shallow sites by using underwater thermometers during SCUBA dives.

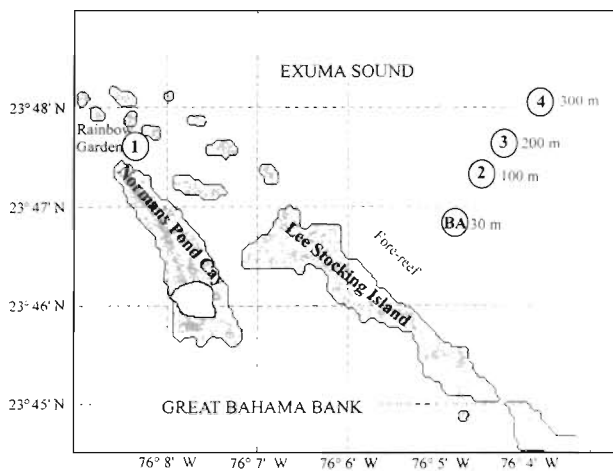


Fig. 1. Map of Lee Stocking Island (Exuma, Bahamas). Study sites: (BA) deep fore-reef site for preliminary block deployment and submersible launch; (1) Rainbow Garden control site; (2–4) study sites on the slope

At greater depths, data were obtained during submersible dives from continuous records provided by a Sea-Bird sensor and a Pisces Design data logger.

Natural depth distribution of sponges. Abundances of *Aplysina fistularis* and *Ircinia felix* (number of individuals) were estimated at the inner reef (4 m deep), fore-reef (10 and 15 m deep), and slope (50, 65, 80, 100, 115, 130 m deep). Counts of individuals at the inner and fore-reef were done by SCUBA diving, using 1 m² random quadrats (N = 30). At greater depths, counts were also done on 1 m² random quadrats (N = 30) obtained by sub-sampling 50 × 1 m videotaped belt transects (N = 3, per depth level) projected on a high-resolution video monitor. Video transects were recorded with an externally mounted video camera on the manned submersible CLELIA. Differences in abundance as a function of sponge species and depth were tested by a 2-way analysis of variance (ANOVA). A *posteriori* pairwise comparisons were made with the Student-Newman-Keuls (SNK) test.

Survival and growth at experimental depths. The effects of depth on sponge survival and growth were assessed by transplanting individuals of *Aplysina fistularis* and *Ircinia felix* from their natural habitat in the reef to unusual depths on the slope. This experiment involved a total of 32 individuals of each sponge species. Individuals of both *A. fistularis* and *I. felix* were collected from a sponge community established in a reef patch (Rainbow Garden) located at a depth of 4 m in a tidal channel, near Lee Stocking Island (Fig. 1). Then, sponges were taken to large aquaculture tanks provided with running seawater, and attached to rectangular limestone blocks (30 × 15 cm) by using Z-spar non-toxic epoxy putty.

A total of 4 sponges were attached to the 4 corners of each of 16 blocks. Individuals of *Aplysina fistularis* and *Ircinia felix* were placed on alternate corners to minimize subsequent differences in survival between species owing to potential effects of within-block position. Sponges were left overnight in aquaculture tanks to allow complete hardening of the putty. Blocks bearing sponges were transferred to a small aquarium and each sponge was photographed using kodachrome transparency film. The camera was always positioned perpendicular to the horizontal face of the block. Blocks were carried in plastic containers to the fore-reef and deployed by SCUBA divers at 30 m depth (BA site; 23° 46.81' N, 76° 04.97' W; Fig. 1). After a 5 d period at this site, sponges were checked for detachment or unhealthy individuals. All sponges were found to be apparently healthy and securely attached to blocks. Random groups of 4 blocks (total of 8 individuals per species) were then transported either back to the sponge natural habitat (sham-transplant controls) or to various depths along the slope (100, 200, 300 m). Control blocks were deployed at Rainbow Garden (4 m deep) by SCUBA diving, whereas treatment blocks were transported along the slope by using a GAMMA submersible equipped with a hydraulic manipulator arm. At depths of 100 and 200 m, where the slope was virtually vertical, blocks were positioned on ledges relatively protected from silt. At 300 m, blocks were deployed on top of a small outcrop to prevent burial by mobile sediments cascading down the slope. Two months after deployment, blocks were checked *in situ* by using the GAMMA submersible. After 1 yr, blocks were finally recovered with the CLELIA submersible and transported to the CRMC laboratory. Surviving sponges were photographed in an aquarium in the same position and angle as at the beginning.

The high cost of submersible operations, as well as inherent difficulties in deployment and retrieval of experiments, compelled us to work with low replication and to make some assumptions for the analyses of survival and growth data. For the statistical analyses, we assumed that within-block and between-block effects on survival and growth at a given depth level were negligible when compared with between-depth and between-species effects on survival and growth. So, each sponge individual was treated as an independent datum for estimating total survival and mean growth. The fact that surviving sponges within a given depth level were more or less scattered among blocks at the end of the experiment indicated that our assumption did not inset an important bias into the statistical analyses.

Sponge survival was estimated as percentage of sponges surviving at a given depth after a given period of time (2 and 12 mo). Some sponges detached from

blocks while being transported by submersible, so that only 7 individuals of *Aplysina fistularis* were deployed at 200 m, 7 individuals of *Ircinia felix* were transplanted to 100 and 200 m depths, and 5 *I. felix* were used as controls.

We constructed a 3-factor frequency table by recording the number of sponges that died and survived after 12 mo (survival factor: live vs dead) as a function of transplantation depth (depth factor: 4, 100, 200, 300 m) and sponge species (species factor: *Aplysina fistularis* vs *Ircinia felix*). Then, we tested the independence of survival (response variable or dependent variable), sponge species and depth factors using log-linear models (Bishop et al. 1975). We approached the final model step by step, adding and eliminating terms hierarchically (main effects and interactions) to examine the partial and marginal association of each term in the model. The partial association between, for example, survival and depth factors was computed by comparing the fit (i.e. evaluating the chi-square difference) of the model that includes all 2-way interactions with the model that excludes the interaction under consideration. Alternatively, the marginal association between, for example, survival and depth was computed by comparing the fit of the model that excludes all main effects (i.e. all effects of lower order than the one of interest) with the model including the interaction under consideration. A term was retained or excluded from the model on the basis of the significance of its partial and marginal association. The statistical significance of the goodness of fit of a particular model, that is, whether or not the expected cell frequencies under the respective model were significantly different from the observed cell frequencies, was evaluated by using the Pearson chi-squared (χ^2) statistic. When significant differences were found ($p < 0.05$), the model was rejected.

As the log-linear analysis revealed that sponge survival was not dependent on the species factor; the 3-way table was collapsed across levels of the species factor and became a 4×2 table (depth factor: 4, 100, 200, 300 m; survival factor: live vs dead). Subsequently, we tested the association between sponge survival (both species pooled) and particular pairs of depth levels by decomposing the table in a total of six 2×2 sub-tables. Each sub-table was evaluated using the Yates-corrected Pearson chi-squared statistic (χ^2_{y}). Analyses of frequency data were done using Statistica software (StatSoft Inc, OK, USA).

Sponge growth after 12 mo was assessed by analyzing photographs taken at the beginning and the end of the transplantation experiment. Growth was expressed as the positive or negative increment in the area as viewed from the side. We did not attempt to obtain an accurate quantitative value for sponge growth, but

rather assessed the relative magnitude of positive versus negative growth in 2 dimensions. Other authors have used 1-dimensional measurements of linear horizontal or vertical growth to evaluate total growth in similar reef sponges (e.g. Hoppe 1988). Changes in body form during the 12 mo period at the experimental depths were assessed using the circularity index (C). Circularity was calculated by dividing the sponge area by the area of a circle with a perimeter equivalent to that of the sponge (Turon & Becerro 1992). All parameters needed to estimate sponge growth and re-shaping were measured on digitized pictures using SigmaScan software. Differences in sponge growth (projected area) and shape (circularity of projected area) in the species *Aplysina fistularis* as a function of depth were tested by the non-parametric Mann-Whitney rank sum (MWRs) test, as sponges survived at just 2 depths. In *Ircinia felix*, differences were tested by the Kruskal-Wallis non-parametric 1-way ANOVA, as there were survivors at 3 depth levels. Non-parametric tests were used, because data were either non-normally distributed or heteroscedastic.

Recruitment at experimental depths. To assess the viability of asexual propagation at different experimental depths, we used the individuals of *Aplysina fistularis* transplanted to depths of 4, 100, 200 and 300 m, as described in the section above. These sponges bore numerous fistules, a type of bud for asexual propagation. The presence of sponge recruits on blocks and rocks near the deployment sites (within an approximately 5 m^2 quadrat around the block), as well as the formation of new fistules by transplanted sponges, was checked after 2 and 12 mo.

To determine the success of larval recruitment at experimental depths, we conducted a second transplantation experiment using the method explained above. Nevertheless, we used only brooding individuals of *Ircinia felix* this time. A total of 4 brooding sponges were attached to the 4 corners of each block. Random pairs of blocks were deployed at a depth of 15 m in the fore-reef (controls), and at 100 and 200 m on the slope (i.e. 8 sponges per depth level). After 3 mo, blocks deployed on the slope were retrieved and the surrounding bottom (an approximately 5 m^2 quadrat around the block) was checked for young recruits by using the GAMMA submersible. Unfortunately, controls blocks were lost.

In both experiments, we reasoned that any recruitment by short-lived larvae or buds would support the hypothesis that limited dispersal hinders colonization rather than physiological tolerance of adults or juveniles.

Sponge histology, symbionts and parasites. We investigated depth-related changes in the symbiotic populations of cyanobacteria and heterotrophic bac-

teria, as well as in cytological aspects of sponge feeding and collagen-spongin secretion, by using transmission electron microscopy (TEM). Material for TEM was prepared according to the methodology described by Rützler (1990). Although Rützler recommends that tissue samples should not be held in fixative solution longer than 2 to 4 h, an emergency hurricane evacuation forced us to retain material in the fixative for 4 d. This extended fixation did not seem to compromise the cells or tissues in any way. Sections were viewed with a Philips EM-301 electron microscope. The proportion of cyanobacteria and heterotrophic bacteria (mean \pm SE %) with respect to the peripheral sponge tissue was estimated on semi-thin (1 μ m) stained sections. Counts were done using a Weigel graticule inside the ocular of a Zeiss Axioplan microscope with a 100 \times oil immersion objective. We examined 10 sections per species and depth level.

The presence of parasitic syllid polychaetes *Haplo-syllis spongicola* in sponges was determined by using dissecting and compound stereomicroscopes. Estimates of polychaete abundance in the control sponges were based on counts of organisms (mean \pm SE) present in 1 cm³ endosomal blocks (N = 5). However, counts of polychaetes in the few sponges surviving at depths of 100 and 200 m were based on examination of very small pieces of tissue (≤ 0.5 cm³), as a significant fraction of the material was preserved for histological and chemical study. Therefore, estimates on polychaete abundance should be considered tentative.

RESULTS

Physical setting and environmental data

Mean water temperature at a shallow site on the inner reef (4 m deep, Rainbow Garden; Fig. 1) ranged from 26–27°C during January and February to 31–32°C from July through September. During winter, the water in Exuma Sound was homogeneous in both temperature (24 to 25°C) and salinity (36.7 ppt) between depths of 3 and about 100 m (Fig. 2). A progressive warming of the upper water during spring and summer produced a sharp thermocline with an associated halocline positioned between 75 and 100 m. Below 100 m, water temperature did change seasonally, but was characterized by a progressive decrease with increasing depth. In summary, yearly values of water temperature at depths of 100, 200 and 300 were 24 to 26, 20 to 22 and 18 to 19°C, respec-

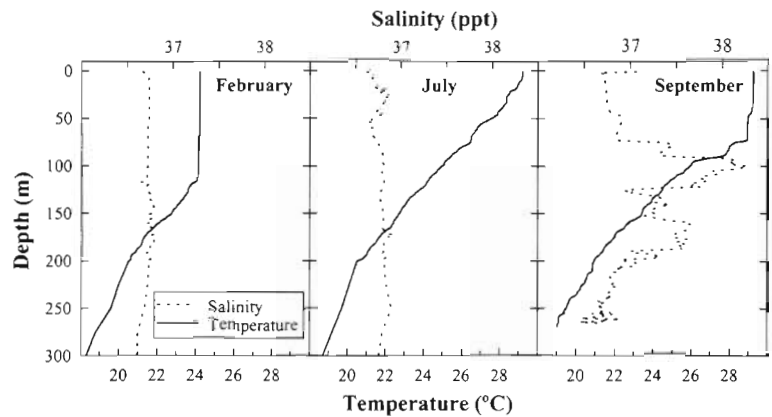


Fig. 2. Temperature and salinity profiles of Exuma Sound over time

tively. By contrast, water temperature at depths shallower than 100 m ranged from 24 to 32°C, depending upon season and depth (Fig. 2).

Natural depth distribution of sponges

The sponges *Aplysina fistularis* and *Ircinia felix* were common organisms in the reef communities near Lee Stocking Island, but both species were absent at depths of 50, 65, 100, 115 and 130 m. Submersible observations indicated that the absolute lower limit of vertical distribution for both species is about 35 to 40 m deep. The abundance of both species (individuals m⁻²) decreased significantly with increasing depth even between 4 and 15 m (Fig. 3, Table 1). The depth effect was most dramatic in *I. felix*, which was relatively rare in fore-reef quadrats taken at 11 and 15 m.

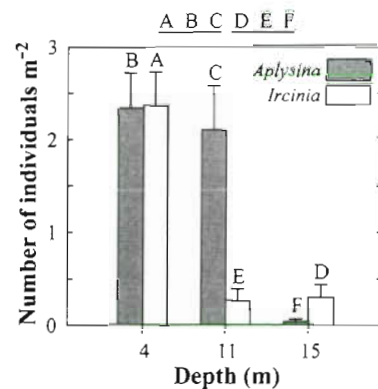


Fig. 3. *Aplysina fistularis* and *Ircinia felix*. Abundances of sponges on the reef (inner-reef site: 4 m; fore-reef sites: 11 and 15 m). Bars and error lines indicate mean values and standard errors, respectively. A–F: mean values arranged in descending order. Groups of underlined letters indicate non-significant differences between pairs of means according to SNK tests ($p > 0.05$) following a 2-way ANOVA (see Table 1)

Table 1. One-way ANOVA of sponge abundance (ind. m⁻²) on the reef as a function of sponge species (*Aplysina fistularis*, *Ircinia felix*) and depth (4, 11, 15 m). See Fig. 3 for a posteriori pairwise comparisons

| Source | df | MS | F | p |
|-----------------|-----|-------|-------|--------|
| Depth | 2 | 71.62 | 25.67 | <0.001 |
| Species | 1 | 11.76 | 4.21 | 0.041 |
| Depth × Species | 2 | 19.87 | 7.12 | 0.001 |
| Error | 174 | 2.79 | | |

Sponge survival and growth

After a 2 mo period, 100% of sponges survived at depths of 4, 100 and 200 m, but all sponges deployed at 300 m died (Fig. 4). Clean fiber skeletons were still in place on the blocks at the latter depth, but no remains of decaying tissue were found. After a 12 mo period, all individuals of both species survived on blocks deployed at 4 m depth. Apparently, some species-specific differences in survival occurred on blocks deployed at depths of 100 and 200 m. At 100 m, survival was 62.5% in *Aplysina fistularis* and 42.8% in *Ircinia felix*. At 200 m, no *A. fistularis* survived, whereas 28.5% of *I. felix* individuals did. However, the best-fitting log-linear model ($\chi^2 = 2.853$, df = 8, p = 0.943) included just

Table 2. Tests of partial and marginal association for main effects and interactions of a potential 3-way log-linear model estimating the observed frequencies of individuals that survived and died as a function of sponge species and depth

| Effect | df | Partial association | | Marginal association | |
|--------------------|----|---------------------|--------|----------------------|--------|
| | | χ^2 | p | χ^2 | p |
| Species | 1 | 0.242 | 0.622 | 0.242 | 0.622 |
| Depth | 3 | 0.303 | 0.959 | 0.303 | 0.959 |
| Survival | 1 | 2.194 | 0.138 | 2.194 | 0.138 |
| Species × Depth | 3 | 0.313 | 0.957 | 0.420 | 0.936 |
| Species × Survival | 1 | 0.010 | 0.920 | 0.117 | 0.732 |
| Depth × Survival | 3 | 35.171 | <0.001 | 35.278 | <0.001 |

the interaction between depth and survival factors (Table 2), indicating that the observed frequencies of sponge survival were dependent on the depth factor, but not on the species factor. Subsequent tests of association between sponge survival and particular depth levels indicated that sponge mortality (both sponge species pooled) was significantly greater at all slope depths compared to controls (Table 3). Sponge mortality did not significantly increase with depth between 100 and 200 m, but it was significantly higher at 300 m (Table 3).

The analysis of growth data (increment of the exposed area in mm²) revealed that mean growth was positive in individuals of *Aplysina fistularis* that survived at a depth of 100 m, whereas it was slightly negative in control sponges (Figs. 5 & 6). Nevertheless, this difference was not statistically significant (MWRS, p = 0.682). Similarly, mean growth was positive in individuals of *Ircinia felix* transplanted to depths of 100 and 200 m, whereas it was negative in control individuals (Figs. 5 & 7). Again, differences in mean growth among depth levels were not statistically significant (Kruskal-Wallis ANOVA, p = 0.436).

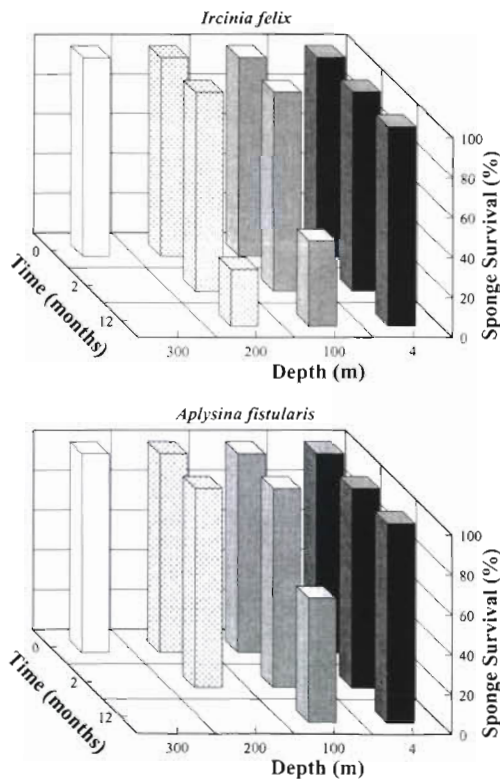


Fig. 4. *Aplysina fistularis* and *Ircinia felix*. Survival of sponges after 2 and 12 mo at depths of 4, 100, 200 and 300 m

Table 3. Two-way 4 × 2 table containing frequencies of sponges (both species pooled) that survived and died as a function of transplantation depth. Subsequent 2 × 2 partitions (df = 1) of the table are evaluated by the Yates-corrected Pearson chi-squared statistic (χ^2_y) to test the effect of particular depth levels on sponge survival

| Depth (m) | Survival | | χ^2_y | p |
|-----------|----------|------|------------|--------|
| | Alive | Dead | | |
| 4 | 13 | 0 | 5.791 | 0.016 |
| 100 | 8 | 7 | 16.735 | <0.001 |
| 200 | 2 | 12 | 25.097 | <0.001 |
| 300 | 0 | 16 | 3.311 | 0.068 |
| | | | 8.884 | 0.002 |

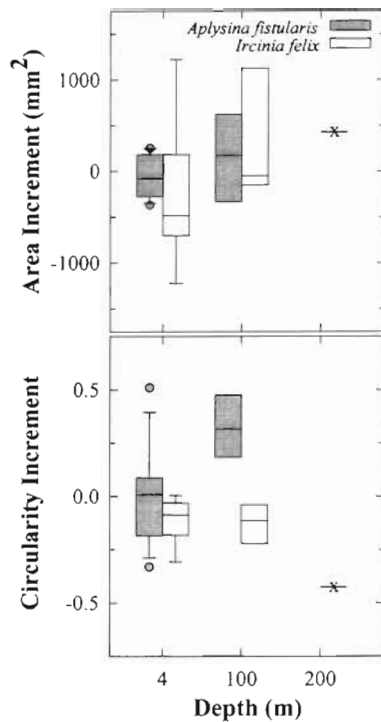


Fig. 5. *Aplysina fistularis* and *Ircinia felix*. Box plots showing positive or negative increment in contour area and circularity experienced by sponges that survived 12 mo at depths of 4, 100 and 200 m. Lower and upper boundaries of boxes indicate 25th and 75th percentiles, respectively. Vertical lines above and below boxes indicate 10th and 90th percentiles. (○) Data outside the latter percentiles. The line within the box indicates median. A single datum (x) is available for *I. felix* from 200 m, since other surviving individuals were lost during the retrieval operation

The analysis of sponge shape (circularity) revealed that controls of *Aplysina fistularis* did not reshape, whereas a statistically significant positive increment in circularity values (MWRs, $p = 0.028$) took place in sponges surviving at greater depths (i.e. 100 m). This difference in contour circularity reflected the loss of fistules in all sponges that survived at 100 m. The opposite trend was noted in circularity values of *Ircinia felix*, as individuals transplanted to 100 m developed lobes and those surviving at 200 m deep formed long chimney-like processes (Figs. 5 & 7). The Kruskal-Wallis ANOVA indicated that differences in circularity among sponges surviving at 4, 100 and 200 m were not statistically significant ($p = 0.404$). However, we suspect a significant type II error as a consequence of the low number of sponges surviving at some depth levels. Indeed, the morphological changes in *I. felix* were dramatic from a qualitative point of view (Fig. 7). It is noteworthy that some oscules were closed or consistently repositioned at the apices of lobes and chimneys in most individuals transplanted to the slope. Indi-

viduals that survived at a depth of 200 m developed oscular chimneys that were 3 to 8 cm in height and 0.3 to 0.5 cm in diameter (Fig. 7E, F). Chimneys were hollow, with thin walls, externally covered with very small conules (smaller than 1 mm in height) in a dense arrangement. Chimney walls were internally supported by a network made of newly formed spongin fibers and spongin filaments. Primary fibers were mostly tangential to the axis of the chimney and connected by short secondary fibers. Both fiber types contained debris. The external surface of the basal part of the sponge body had developed abnormally high conules (4 to 6 mm in height) in some areas, resulting from an intense retraction of the ectosome around protruding primary fibers. Although all sponges surviving at depths of 100 and 200 m faded to a whitish color, the black rim persisted around all oscules (Fig. 7D).

Apart from loss of fistules, no noticeable morphological changes were noted in individuals of *Aplysina fistularis* that survived at a depth of 100 m (Fig. 6D). Sponges lost some of their green pigmentation and their yellow coloration became brighter.

Sponge recruitment

All sponges surviving at depths of 100 and 200 m still bore asexual buds (fistules) after 2 mo, but fistules were lacking in all sponges surviving 12 mo at these depths. Unless complete resorption occurred, some detachment of fistules should have taken place. However, no young individuals of *Aplysina fistularis* were found on or near blocks deployed on the slope. Young sponges did not recruit on control blocks at 4 m either, but recruits were found in the adjacent bottom area. A comparison between photographs taken at the beginning and the end of the experiment indicated that many fistules were detached and replaced by new ones in control sponges (Fig. 6A, B). By contrast, lost fistules were not replaced in sponges transplanted to the slope. This suggests that the asexual propagation process, which is the main reproductive mode of this species in reef habitats, is inhibited at slope depths.

Three months after deployment of brooding individuals of *Ircinia felix*, no recruitment was found either on blocks at depths of 100 and 200 m or on adjacent bottoms. The information to be provided by controls was lost, as control blocks were missing. During this period, 100 and 37.5% of sponges survived at depths of 100 and 200 m, respectively. Examination of these surviving individuals revealed no sign of developing embryos in the endosome. Therefore, embryos that were present in the sponges at the time of deployment had been either expelled as larvae or resorbed.

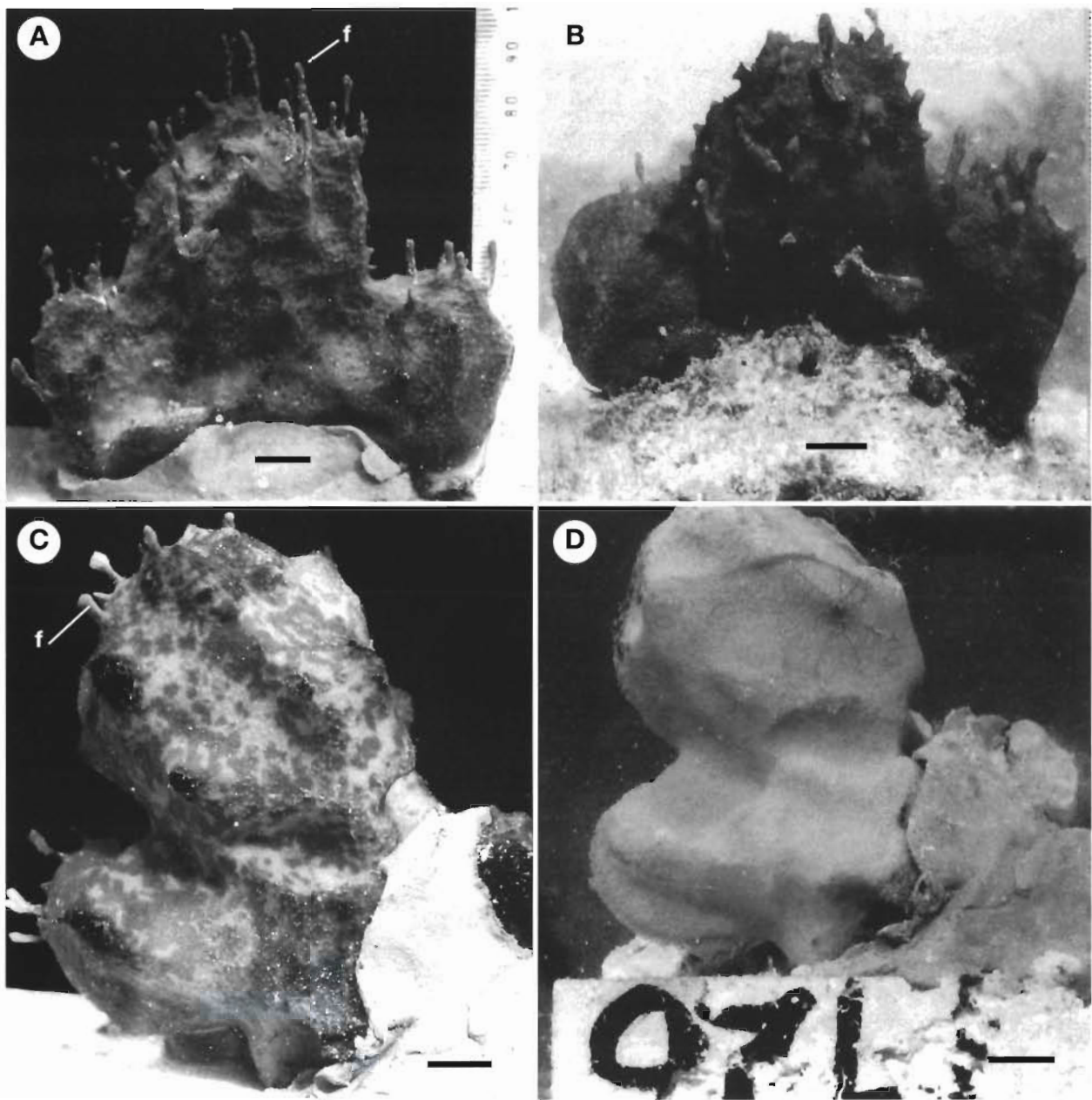


Fig. 6. *Aplysina fistularis*. Examples of shape changes experienced by individuals that survived 12 mo at depths of (A, B) 4 m and (C, D) 100 m. (A, C) Sponges at the beginning of the transplantation experiment; (B, D) sponges at the end of the experiment. Note that individuals transplanted to 100 m lost all fistules (f). Scale bars = 1 cm

Sponge histology and symbionts

The peripheral tissues of control individuals of both sponge species contained abundant *Aphanocapsa feldmani* cyanobacteria (Figs. 8–10). Cyanobacteria were completely lost in individuals of *Ircinia felix* surviving 1 yr at depths of 100 and 200 m, but they were still abundant in individuals of *Aplysina fistularis* surviving at a depth of 100 m. In all cases, the cyanobac-

teria were found only in a narrow subectosomal tissue layer about 2 to 3 mm thick in *I. felix* and about 5 mm thick in *A. fistularis*. Within this subectosomal layer, they represented $20 \pm 5\%$ of the tissue volume in control sponges, but only about $10 \pm 3\%$ in individuals of *A. fistularis* that survived at a depth of 100 m.

Cyanobacteria were oval, measuring $1.1\text{--}1.45 \times 1.8\text{--}2.5 \mu\text{m}$ in transverse and longitudinal sections, respectively (Figs. 8A–C & 10A). Most cyanobacteria

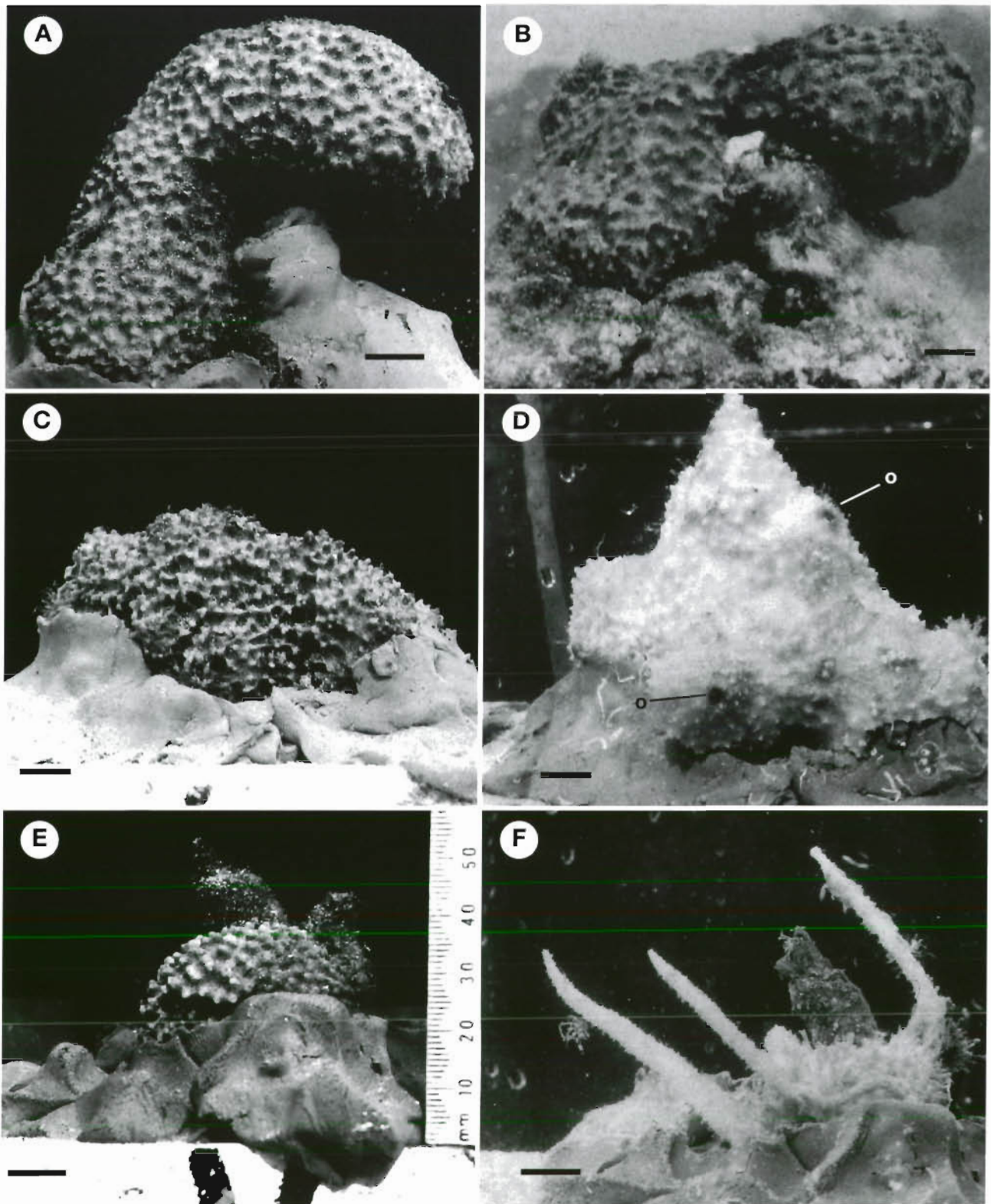


Fig. 7. *Ircinia felix*. Example of shape changes experienced by individuals that survived 12 mo at depths of (A, B) 4 m, (C, D) 100 m and (E, F) 200 m. (A, C, E) Sponges at the beginning of the transplantation experiment; (B, D, F) sponges at the end of the experiment. Sponges transplanted to depths of 100 and 200 m formed oscular lobes and chimneys. Although sponges faded at these depths, the black rim around oscules (o) persisted. Scale bars = 1 cm

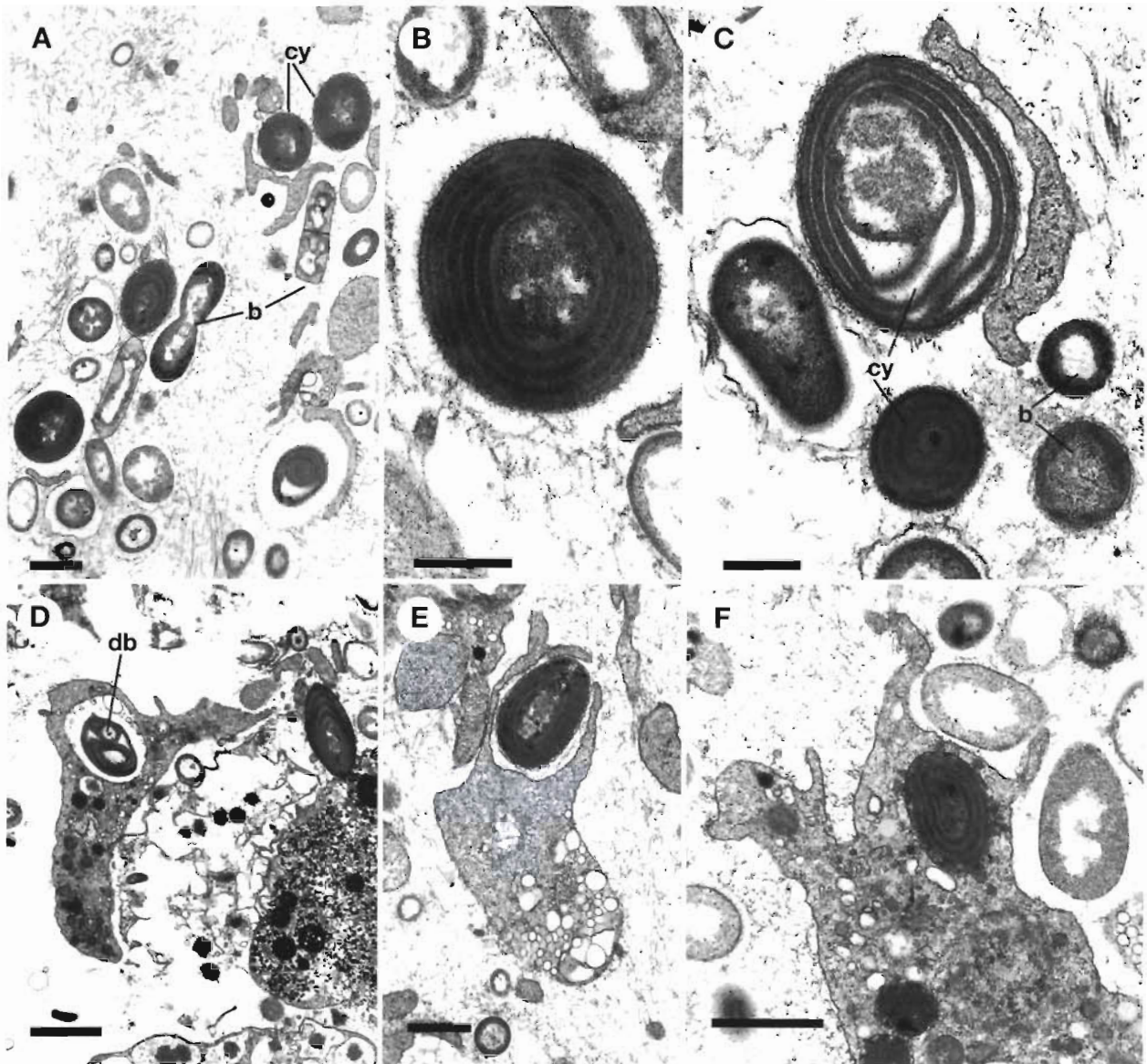


Fig. 8. Cytology of *Aplysina fistularis*. (A) Abundance of cyanobacteria (cy) and heterotrophic bacteria (b) in the peripheral tissue of a control sponge. (B) Cyanobacteria in a control sponge. (C) Cyanobacteria in a sponge that survived at 100 m. (D) Intracellular cyanobacterium parasitized by a *Bdellovibrio*-like bacterium (db) in a sponge that survived at 100 m. (E) Cyanobacterium being phagocytosed by a sponge cell in a sponge that survived at 100 m. (F) Archeocyte of control sponge phagocytosing a bacterium and containing cyanobacteria at different states of digestion. Scale bars: (A) 1 μm , (B, C) 0.5 μm , (D) 2 μm , (E, F) 1 μm

were extracellular in both sponge species, though they were also found in intracellular vacuoles. Intracellular cyanobacteria were found in an apparent living state and also at different states of digestion (Figs. 8D–F & 9A, B). In *Aplysina fistularis*, cyanobacteria were occasionally observed dividing, both in control sponges and in individuals that survived at a depth of 100 m. The thylakoid of most cyanobacteria showed between 4 and 6 spirals. The number of spirals was not found to be correlated with either depth in the sponge tissue or depth at which sponges lived. Indeed, individuals of *A. fistu-*

laris surviving at a depth of 100 m showed some cyanobacteria with thylakoid somewhat less developed than those of cyanobacteria in control sponges (Fig. 8B, C). The only noticeable difference between cyanobacteria populations living in control and 100 m deep *A. fistularis* sponges was the occurrence of a low percentage of cyanobacteria parasitized by a *Bdellovibrio*-like bacterium at the greater depth (Fig. 8D).

Heterotrophic bacteria were well represented in both sponge species. At a depth of 5 mm in the sponge tissue, bacteria accounted for about $20 \pm 6\%$ and $30 \pm$

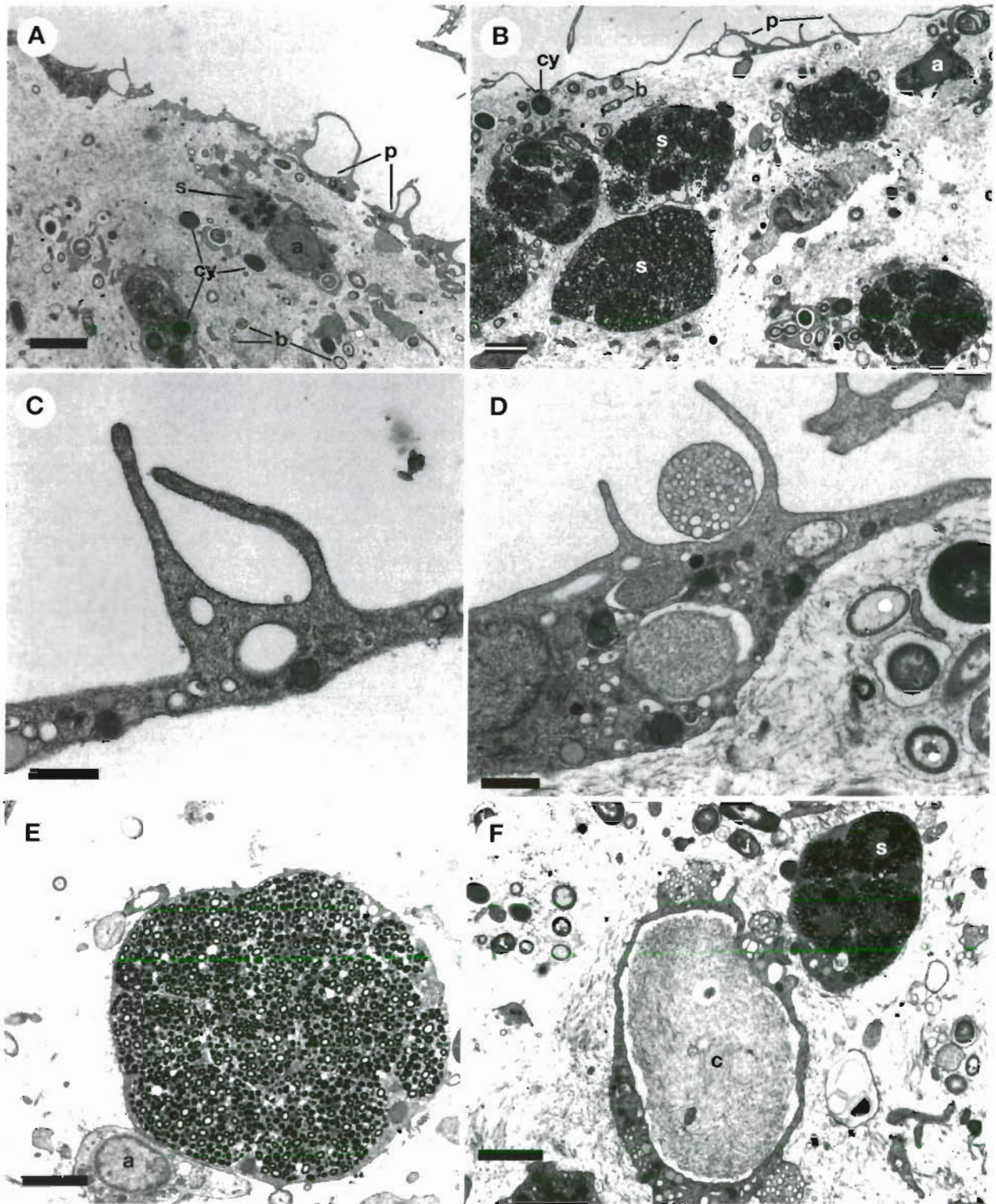


Fig. 9. Cytology of *Aplysina fistularis*. (A) Tissue beneath the endopinacothelium of a peripheral aquiferous channel in a control sponge. Note the presence of extra- and intracellular cyanobacteria (cy) and bacteria (b), spherulous cell (s), archeocytes (a), and endopinacocytes (p) with large empty vacuoles. (B) Tissue beneath the endopinacothelium of a peripheral channel in a sponge that survived at 100 m. Note the presence of all cell and bacterial types shown in (A). (C, D) Details of pinacocytes with abundant inclusions and pseudopod-like protrusions. (E) Detail of a spherulous cell contacting an archeocyte (a). (F) Collencyte secreting collagen (c) within a sponge that survived at 100 m. Scale bars: (A, B) 5 μ m, (C) 0.5 μ m, (D) 1 μ m, (E, F) 2 μ m

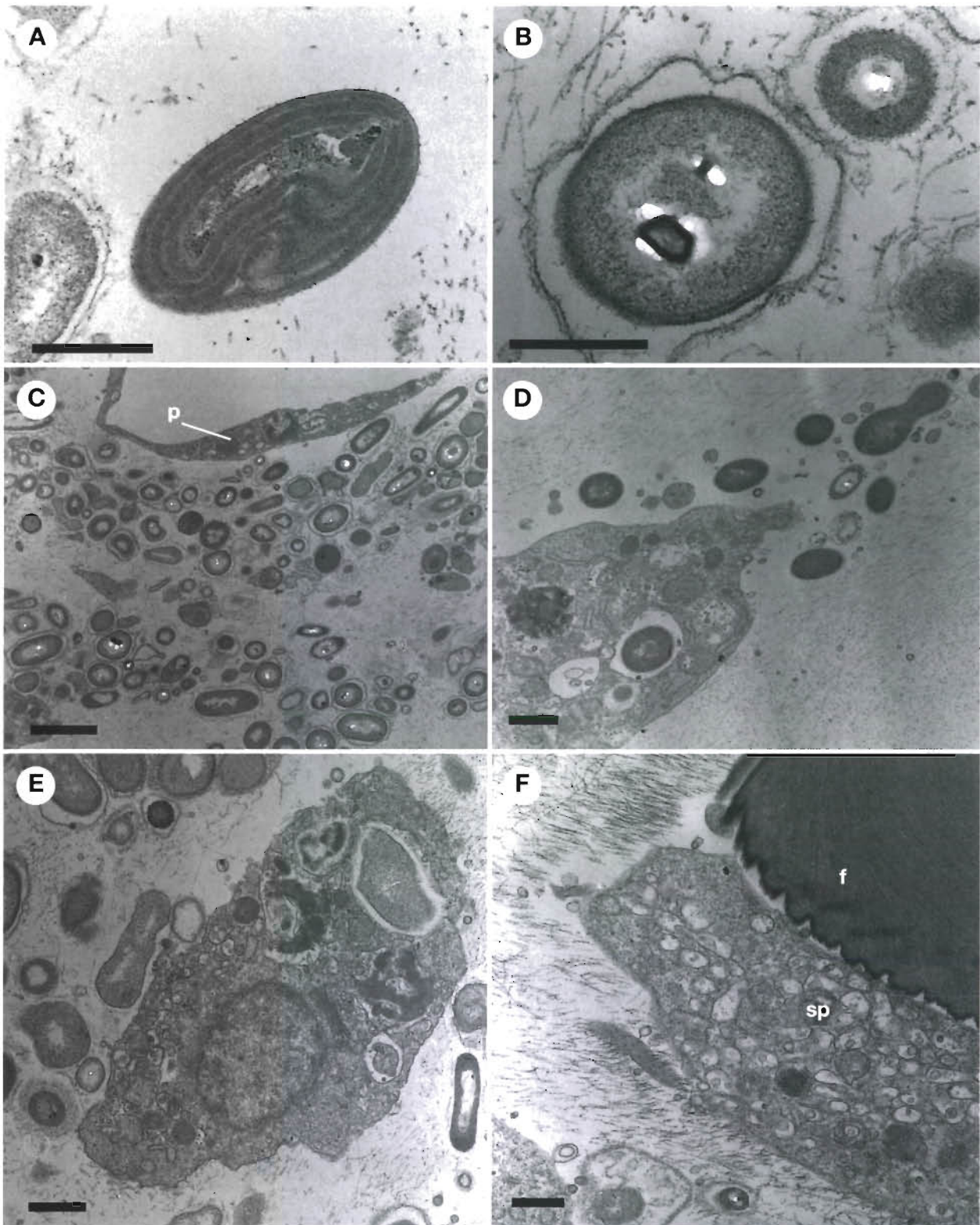


Fig. 10. Cytology of *Ircinia felix*. (A) Cyanobacterium in a control sponge. (B) Two common morphological types of heterotrophic bacteria. (C) Abundance of heterotrophic bacteria beneath the endopinacothelium (p) of a peripheral channel in a sponge that survived at 100 m deep. (D, E) Archeocytes with phagocytosed bacteria in sponges that survived at depths of 200 and 100 m, respectively. (F) Spongocyte (sp) secreting collagen around a spongin filament (f) in a sponge that survived at 200 m. Scale bars: (A, B) 0.5 μ m, (C) 2 μ m, (D) 1 μ m, (E) 0.5 μ m, (F) 1 μ m

8% of the sponge tissue volume in control individuals of *Aplysina fistularis* and *Ircinia felix*, respectively. Similar proportions were found in sponges surviving at 100 m, whereas the proportion was lower ($20 \pm 8\%$) in individuals of *I. felix* surviving at 200 m. The bacterial population of both sponge species was dominated by 3 major morphological bacterial types (Figs. 8A, C & 10B, C) that were comparable to the B, C and D types described by Vacelet (1975) from 2 Mediterranean species of *Aplysina*.

A TEM examination of the mesohyl of surviving sponges did not reveal significant differences in sponge cytology as a function of transplantation depth. At depths of both 4 and 100 m, individuals of *Aplysina fistularis* showed archeocytes engaged in phagocytosis and digestion of cyanobacteria and heterotrophic bacteria (Figs. 8D–F & 9A, B). Similarly, endopinacocytes showed pseudopod-like protrusions, suggesting active capture of particulate material. These cells were also characterized by the presence of large, empty vacuoles (Fig. 9A–D). The significance of these structures remains unclear. Immediately below the endopinacothelium, spherulous cells were abundant and a few, scattered chromocyte-like cells were also found (Fig. 9A, B, E, F). Interestingly, we observed collencytes apparently secreting collagenous fibrils in both controls and individuals transplanted to 100 m (Fig. 9F). Similarly, we found apparently active spongocytes in close contact with spongin filaments in *Ircinia felix* individuals that survived at depths of 100 and 200 m (Fig. 10F). These observations indicate that spongin synthesis was not inhibited at these depths. Active capture and digestion of bacteria by archeocytes was also observed in individuals of *I. felix* surviving at control, 100 and 200 m depth levels (Fig. 10D, E).

Parasitic polychaetes, *Haplosyllis spongicola*, were present in the endosomal tissues of all surviving individuals of *Ircinia felix*, irrespective of sponge transplantation depth. Polychaetes measured 1000–4000 \times 300–350 μm and appeared at mean (\pm SE) densities as high as 35 ± 15 syllids cm^{-3} . Casual inspection suggested that neither polychaete size nor abundance was significantly different among populations inhabiting control sponges and sponges that survived at depths of 100 and 200 m.

DISCUSSION

The keratose sponges *Aplysina fistularis* and *Ircinia felix* were common in shallow habitats on the reef, but their abundance decreased significantly with increasing depth along the outer reef, and both were completely absent from the upper slope. This vertical distribution agrees with data from the abundant literature

available for these 2 species (e.g. Wiedenmayer 1977, Zea 1987 and literature therein). Nevertheless, 3 deep-water specimens collected from Puerto Rico (Piscadera, 500 m deep), Barbados (100 m) and Venezuela (72 m) were tentatively assigned to the species *I. felix* by Van Soest (1978), although the author did not exclude the possibility that they belong to a separate deep-water taxon. Our numerous submersible observations at Lee Stocking Island and throughout other parts of the Bahamas (e.g. Maldonado & Young 1996b) are consistent with the large body of the literature indicating that these species do not occur naturally at depths much greater than 35 to 40 m. However, both species survived at greater depths when artificially transplanted, and some of the transplanted individuals even experienced a positive mean growth after 1 yr. Therefore, these results suggest that limited larval dispersal or unsuccessful settlement at deep habitats may be responsible for the confinement of this species to shallow water. We found no sign of recruitment from either sexual or asexual origin at experimental depths, but cannot discard the possibility that we failed to observe recruitment because detached buds or expelled larvae dispersed and recruited out of the area we surveyed. Nevertheless, our field experience with other littoral sponges indicates that at least a few recruits should be found on blocks or the nearby bottom if buds or brooded larvae were actually viable at these depths (e.g. Uriz et al. 1998).

It is not surprising that sponges transplanted to bathyal depths showed a tendency to grow better than at control depths. At bathyal depths, some reproductive processes (i.e. formation of asexual buds) were inhibited, and many embryos probably either were resorbed or did not develop at all. Therefore, the energy usually allocated for reproduction may have been reallocated to somatic growth. Wilkinson & Vacelet (1979) found a similar effect on the growth of other sponges. In their study, *individuals* of the sponges *Aplysina cavernicola* and *Chondrilla nucula* transplanted to different light conditions often grew better than control individuals that remained in their natural habitat.

Symbiosis with cyanobacteria does not appear to play an important role in determining the lower limit of depth distribution for either sponge species. *Aplysina fistularis* does not occur deeper than 40 m, but transplanted sponges survived to at least a depth of 100 m. At this depth, symbiotic cyanobacteria were still abundant in the peripheral sponge tissue. Therefore, the absence of this species in deep fore-reef and upper-slope habitats is not directly caused by loss of cyanobacteria. *Ircinia felix* survived even deeper (200 m), despite the fact that they lost cyanobacteria at a depth even shallower than 100 m. The fact that symbiotic

cyanobacteria were present in *A. fistularis* but lost in *I. felix* under virtually identical environmental conditions (same block) suggests that the relationship between *Aphanocapsa feldmani* and its host varies with sponge species, especially in the case of mixotrophic sponges. On the other hand, the presence of symbiotic cyanobacteria in sponges living as deep as 100 m is not probably an exceptional condition. This observation is consistent with studies reporting that zooxanthellate scleractinians occur to depths of about 100 m in the Caribbean area (Lang et al. 1988).

Sponge survival results do not support the hypothesis that parasitism by *Haplosyllis spongicola* may hinder the colonization of deeper habitats by these species. The log-linear analyses indicated that there was no significant difference in survival between both sponge species with depth, despite the fact that only *Ircinia felix* was heavily parasitized at all depths. Furthermore, some individuals of this latter species survived at greater depths than non-parasitized individuals of *Aplysina fistularis*. Further studies are needed to understand this interesting polychaete-sponge association. The large number of polychaetes still present in sponges that survived at 200 m, where oligotrophy should also be a problem for the sponge, suggests that the sponge-polychaete relationship is mutualistic or commensalistic rather than parasitic. Otherwise, one cannot easily understand how sponges under nutritional stress in deep-sea conditions can counterbalance the effects of grazing by such a high concentration of parasites.

Cytological observations indicate that both feeding and collagen-spongins secretion proceed normally in sponges surviving at depths greater than the lower natural limit of these species. The positive growth experienced by some sponges at the experimental depths was the strongest evidence for this (Fig. 10D–F). The abundant population of symbiotic heterotrophic bacteria still present in the endosome of *Aplysina fistularis* and especially *Ircinia felix* at these depths may contribute to palliate the negative effects derived from the loss of cyanobacteria and the impoverishment of available particulate food in deep waters. The reshaping process that took place in individuals of *I. felix* with increasing depth was probably triggered by the need to enhance filter feeding and compensate for lower food levels. Vogel (1978) showed how sponges may exploit ambient currents by inducing passive flow with chimney-like extensions of the oscules. Indeed, the reshaping of *I. felix* that survived at depths of 100 and 200 m may be one of the most clear empirical confirmations of Vogel's theories about the significance of body form in sponges. Oscular chimneys similar to those formed by *I. felix* were described in specimens of the Mediterranean species *Ircinia pipetta* (Schmidt)

collected from an undetermined depth between 70 and 120 m (Uriz & Maldonado 1993). The species *A. fistularis* did not reshape, possibly because its body form was already appropriate for exploiting ambient currents (Vogel 1978).

Although lower food with increasing depth appeared to have an important impact on the shape of transplanted sponges, food does not emerge as the key factor hindering the colonization of the slope by these sponges. The fact that individuals of both species died relatively quickly (within 2 mo) when transplanted to a depth of 300 m suggests that sponge physiology was dramatically affected at that depth. A process of slow shrinking and delayed mortality should be expected in the case of death by starvation or parasitism. Predation is unlikely to play a significant role in determining the lower limit of depth distribution of these sponges. The fiber skeleton of individuals of both sponge species remained intact even after death in both species transplanted to bathyal depths. There was no sign of predation in any individual. In our opinion, water temperature was most likely the reason for sudden mortality after transplantation, since it is the environmental factor, apart from light, that changes most dramatically with depth. Temperature at a depth of 300 m (18°C) was about 10°C lower, on average, than on the reef (27 to 32°C). Our observations agree with Vacelet's (1988) suggestion that a temperature below 18 to 20°C has a deleterious effect on keratose sponges. Nevertheless, the negative effects of low temperature may not affect adult sponges as severely as larvae, asexual buds or early recruits. Some information in the sponge literature suggests that adult individuals of some keratose species are little affected by temperature. For example, Ilan et al. (1994) have recently described 2 keratose sponges living at a depth of 830 m in the Red Sea, where water temperature is around 10°C. These individuals were tentatively regarded as conspecifics of 2 sublittoral sponge species, *Ircinia* cf. *retiderma* and *Sarcotragus* cf. *muscarum*. Both sponges served as host for an interesting invertebrate infauna that included sublittoral polychaetes. Although the authors did not offer any explanation for these unexpected deep-water records of shallow sublittoral sponges and polychaetes, one cannot exclude the possibility that sponges have been transported from the shelf to the slope by fishing trawling-nets. This fishing practice has been shown to affect significantly both the biogeographical and bathymetric distribution of some bathyal sponge species (Uriz 1990). This mechanism would also explain some unexpected deep records of typical shallow-water keratose sponges in Mediterranean areas that are subject to intense exploitation by trawling-fishing nets (Pansini & Musso 1991). This hypothesis is also consistent with the results of our transplantation exper-

iments. Thus, if adult keratose sponges artificially transported to great depths can survive in these new environmental deep-sea conditions, the hypothesis that a inhibition of larval dispersal or settlement success (probably by a decreasing temperature with increasing depth) emerges as the most plausible explanation for the shallow-water confinement of most keratose sponge.

It is widely thought that the keratose orders are the most modern demosponges (e.g. Lévi 1973). Our experimental results appear to be consistent with the ideas that they evolved from ancestors inhabiting shallow, warm waters, and that they would have to modify some of their physiological processes (e.g. reproduction) in order to colonize successfully slope habitats.

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