Italian Journal of Zoology

ISSN: 1125-0003 (Print) 1748-5851 (Online) Journal homepage: https://www.tandfonline.com/loi/tizo20

Necrosis in a population of *Petrosia ficiformis* (Porifera, Demospongiae) in relation with environmental stress

Carlo Cerrano , Giuseppe Magnino , Antonio Sarà , Giorgio Bavestrello & Elda Gaino

To cite this article: Carlo Cerrano , Giuseppe Magnino , Antonio Sarà , Giorgio Bavestrello & Elda Gaino (2001) Necrosis in a population of *Petrosia ficiformis* (Porifera, Demospongiae) in relation with environmental stress, Italian Journal of Zoology, 68:2, 131-136, DOI: 10.1080/11250000109356397

To link to this article: https://doi.org/10.1080/11250000109356397

Ital. J. Zool., 68: 131-136 (2001)

Necrosis in a population of *Petrosia ficiformis* (Porifera, Demospongiae) in relation with environmental stress

CARLO CERRANO
GIUSEPPE MAGNINO
ANTONIO SARÀ

Dipartimento per lo Studio del Territorio e delle sue Risorse, Università di Genova, viale Benedetto XV 5, I-16132 Genova (Italy)

GIORGIO BAVESTRELLO

Istituto di Scienze del Mare, Università di Ancona, via Brecce Bianche, I-60131 Ancona (Italy)

ELDA GAINO

Dipartimento di Biologia Animale ed Ecologia, Università di Perugia, via Elce di Sotto, I-06123 Perugia (Italy)

ABSTRACT

Healthy specimens of the Mediterranean *Petrosia ficiformis* harbour endocellular cyanobacteria (*Aphanocapsa feldmanni*) causing a violet pigmentation of the sponge. Necrosis in *P. ficiformis* can be easily detected by the occurrence of white patches scattered over the surface. Necrotic specimens were examined along the Gallinara Island coasts (Western Ligurian Sea), in coincidence with environmental stress (heavy rainfall, land run-off, high seawater temperature). The appearance of white patches is due to the gradual sloughing of the pinacodermal covering, as evidenced by scanning electron microscopic observations. Sloughing leads to progressive tissue degeneration in the deeper parts. Histological sections showed that, concomitantly with the loss of the superficial layer, internal sponge tissues degenerate and the sponge body becomes exposed to the invasion of ciliates. Spicule bundles of the skeletal network separate damaged tissues from the healthy ones, thereby slowing down spread of necrosis and enabling successful recovery.

KEY WORDS: White-patched sponges - Pinacoderm sloughing - Environmental stress.

ACKNOWLEDGEMENTS

This work was financially supported by Italian MURST funds. Field activity was organized by the diving center "Continente Blu" (Alassio).

(Received 9 November 2000 - Accepted 23 January 2001)

INTRODUCTION

In the late summer of 1999 extensive mortality of sponges (mainly *Spongia officinalis, S. agaricina, Petrosia ficiformis*), gorgonians, and other epi-benthic organisms was observed in the Ligurian Sea (Mediterranean Sea) from the Tuscan Archipelago to Marseille (Cerrano *et al.*, 2000; Perez *et al.*, 2000). This mass mortality episode coincided with a sudden increase of seawater temperature down to more than 50-m depth. Scanning electron microscopic (SEM) analysis revealed that the damaged gorgonian specimens showed an extensive attack by microrganisms (protozoans and fungi), which were interpreted as opportunistic pathogens (Cerrano *et al.*, 2000). Presence of numerous ciliates able to penetrate inwards had been previously reported for corals after bleaching episodes (Williams *et al.*, 1987) and for a sponge species in experimental conditions (Gaino & Pronzato, 1987).

Episodic diseases affecting *P. ficiformis* (Poiret, 1789) specimens were detected by us at the Island of Gallinara (Ligurian Sea) mainly during autumn the last years. Affected specimens were characterized by large white patches scattered over the sponge surface, which is normally of violet pigmentation.

The Mediterranean *P. ficiformis* lives in symbiosis with the cyanobacterium *Aphanocapsa feldmanni* (Sarà & Scalera-Liaci, 1964). Symbiotic associations, especially those with autotrophic organisms, may play an important role in sponge metabolism since symbionts transfer significant amounts of photosynthetates, mainly glycerol, to their hosts (Wilkinson, 1979, 1980, 1983; Arillo *et al.*, 1993). Cyanobacteria are commonly extracellular. However, in a few cases, as in *P. ficiformis,* they fill the cytoplasm of a specific cell type, the bacteriocytes or cyanocytes (Vacelet & Donadey, 1977; Wilkinson, 1978). It is well known that the sponge's surface colour depends on the presence of these autotrophic symbionts, whose abundance is controlled by light intensity (Sarà *et al.*, 1998; Regoli *et al.*, 2000).

In order to gain insight into the cause of the depigmented patches, we examined some affected sponge specimens from Gallinara, and we also considered the possible relationships with environmental parameters, such as seawater temperature, rainfall and hydrodynamic conditions.

MATERIALS AND METHODS

Site description and environmental parameters

The Island of Gallinara (Ligurian Sea - Western Riviera) is located about 1 km off the coast, just in front of the Centa River estuary (Fig. 1). The biological characteristics of the Island have been described elsewhere (Balduzzi *et al.*, 1994). At the end of August 1998, necrotic specimens of *P. ficiformis* were first observed along the rocky cliff of the southern coasts of Gallinara, in 0-5 m depth, where the population reaches a density of 5-10 individuals / 100 m^2. This phenomenon was monitored until December 1998 by underwater

Published online 28 Jan 2009

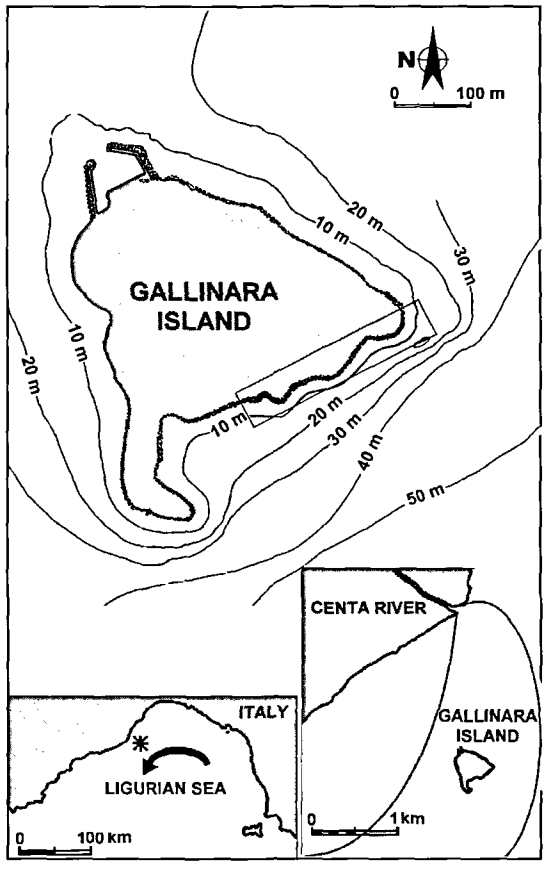

Fig. 1 - Geographic position of the Gallinara Island (asterisk in the left inset). The right inset points out the location of the Island as to the Centa River plume. The shaded area along the southern coasts of the Gallinara Island represents the study site.

photographs, and surveys were carried out in January and February 1999 to estimate the duration of the re-pigmentation process. Data collected from January to December 1998 (Fig. 2), kindly furnished by the Metereological Observatory of Alassio, about wave height, seawater temperature and rainfall were considered.

Specimen collection

At the end of September 1998, portions of tissue both from necrotic specimens and some pigmented ones (as controls) were sampled for histological investigations. Fragments were immediately fixed for 3 h in 2.5% glutaraldehyde in artificial sea water (ASW) buffered to a final pH 7.5.

Preparation of samples for histology and SEM

After fixation, samples were processed for standard histology and for ultrastructural observations. For SEM procedures, selected material was repeatedly rinsed in ASW (used as buffer), dehydrated in graded series of ethanol, and critical point dried using a CO_2 Pabish CPD 750 apparatus. Before dehydration, some specimens were immersed in liquid nitrogen and fractured using a steel blade. The material was mounted on stubs, sputtered with gold using a Balzer Union Evaporator, and observed with a Philips 515 EM.

For routinely used histological techniques, glutaraldehyde-fixed samples were desilified in 4% hydrofluoric acid for 2 h (with two rinsing cycles of 1 h each), dehydrated in graded series of ethanol and embedded in Technovit 8100 resin (Kulzer). Sections of 3-µm thickness were mounted on glass slides, stained with toluidine blue, viewed and photographed at a Leitz diaplan photomicroscope.

RESULTS

Environmental data from January to December 1998 (Fig. 2) showed rainfall peaks in April and August-September, coinciding with a fairly long calm period in the summer season. The seawater temperature was highest in August-September, as is usual in this area.

In September 1998 some individuals of *P. ficiformis* (about 25% of 120 specimens) against the typical violet pigmentation (from dark to light violet), stood out owing to the presence of white superficial patches initially dispersed in the pigmented surface (Fig. 3a) which later spread across most of the sponge (Fig. 3b). No completely depigmented specimens were observed. Underwater surveys documented that this phenomenon lasted about two weeks from the first record. Total re-pigmentation was observed in February 1999.

It is well known that Mediterranean *P. ficiformis* is commonly grazed by the nudibranch *Peltodoris atromaculata*. Nevertheless, the white patches present on the sponge surface during the bleaching phenomenon cannot be attributed to the feeding activity of this mollusc. Indeed, mollusc grazing does not cause tissue necrosis (Cattaneo-Vietti, pers. comm.).

Under SEM, affected specimens showed striking differences in the organization of their external surface when white parts were compared with the pigmented regions (Fig. 4a). The latter were bounded by exopinacocytes, typically in form of a uniformly porous flat layer with a tangential orientation of skeletal elements (Fig. 4b). The white regions lacked this covering, and the skeletal spicular network was exposed to the environment (Fig. 4c). Coincidental with the disappearance of the cell covering, spicules of the outermost skeleton were irregularly dispersed. In the superficial regions still delimited by the exopinacodermal layer, the pores were no longer visible (Fig. 4d). The cells were less adherent to one another and acquired the general morphology of mobile elements (Fig. 4e). Some images clearly revealed their detachment from the sponge surface. The cell bor-

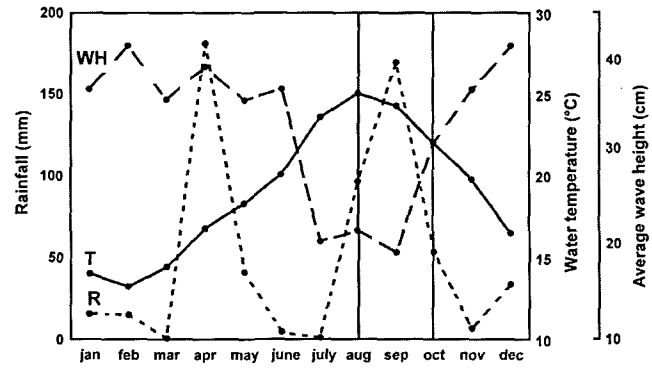

Fig. 2 - Monthly trends of rainfall (R), sea-surface temperatures (T) and wave height (WH). The shaded area shows the period during which the bleaching phenomenon was observed, coincident with high rainfalls, high seawater temperature and calm sea conditions. The heavy rainfalls of April did not influence necrosis owing to the occurrence of cold seawater and rough sea.

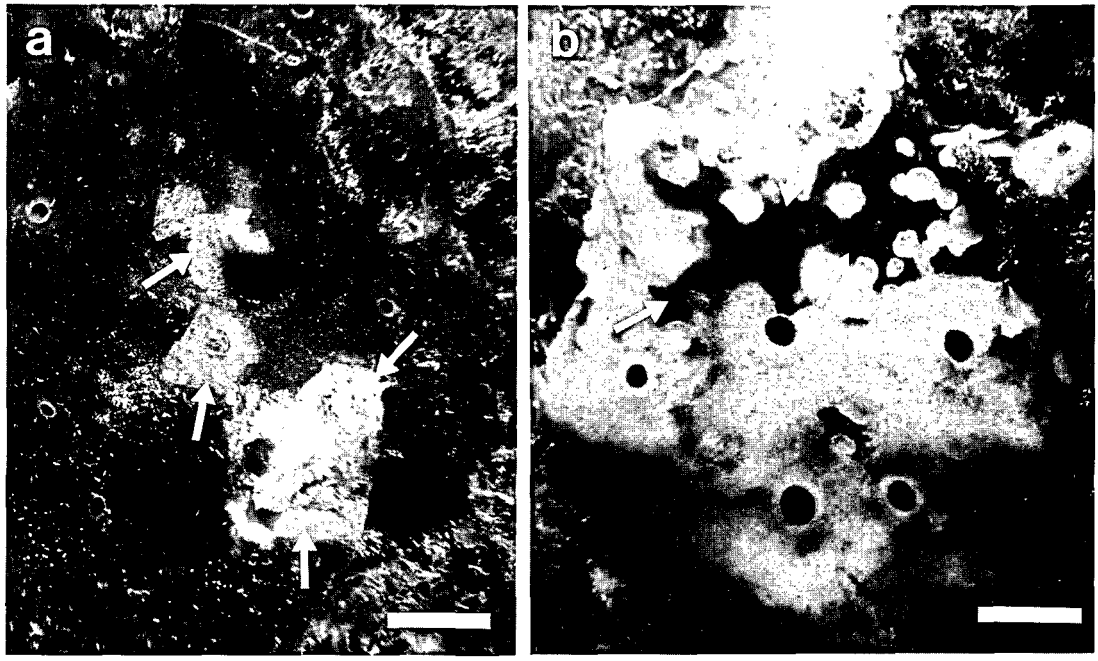

Fig. 3 - Specimens of *Petrosia ficiformis* showing (**a**) the initial phase of the formation of white superficial patches (arrows) within the pigmented surface and (**b**) a progressive phase during which only few pigmented areas (arrows) are present (bar = 1 cm).

der was slightly uplifted allowing the collagen fibrillar matrix to be seen underneath (Fig. 4f).

Fractured specimens examined under SEM showed that the deeper sponge regions had a different organization depending on the presence or absence of a superficial exopinacoderm covering. In the latter condition, sponge structure was completely disorganized, and spicule bundles were the only persisting structure of the sponge body, sometimes interposed between functional tissues and no longer active parts (Fig. 5a). Histological sections confirmed that pigmented areas corresponded to healthy tissue (Fig. 5b), whereas the white regions exhibited a loose matrix containing rare scattered cells (Fig. 5c). No particular aggregations of bacteria were associated with such degenerating tissues (Fig. 5d). However, loss of the covering that protects inner sponge regions from environment allows sediments and various organisms to penetrate into the sponge body. Histological sections demonstrated that ciliates constituted an important fraction of degenerating sponges. They formed a superficial mat (Fig. 5e and detail in inset) from which they penetrated inwards into the dead part of the sponge body (Fig. 5f). Recovery was probably archived by elimination of the damaged parts, which allowed sponges to recover. In the survey of February 1999, five months after the necrosis episode, only pigmented sponges were observed.

DISCUSSION

The necrosis phenomenon observed in *P. ficiformis* is the result of the detachment of the exopinacocytes, which cover the sponge surface. The loss of this protective layer causes tissue degeneration in the deeper parts, which then leads to contamination with cilates and an encapsulation of healthy regions by spicule bundles reinforced by collagen and arranged in layers to delimit polygonal spaces. This necrosis is not the primary consequence of bacterial and fungal infection, because no sign of a massive invasion by these organisms was detected.

Exopinacoderm detachment exhibits similarities to the elimination of coral endodermal cells containing zooxanthellae when the animals were experimentally exposed to both cold and heat stress (Gates *et al.*, 1992), an event that also occurs in the field in advanced state of bleaching (Brown *et al.*, 1995). To date sponge bleaching has been recorded exclusively from coral reef specimens. This phenomenon was observed during field sponge populations surveys (Williams *et al.*, 1987; Vicente, 1990), but no histological studies have been carried out to ascertain the reason for the alteration.

The anomalies recorded in environmental parameters during August-September 1998 may explain the 'bleaching' in *P. ficiformis*. Indeed, the western Ligurian coast experiences regular heavy rainfalls during the autumn season, but in 1998 these and the related land run-off occurred at the end of the summer, coincidental with the highest seawater temperature values and with calm seas. The mouth of the Centa River near the studied area remarkably contributed to fresh water and sediment input. Such a synergism may be responsible for the necrosis phenomenon in *P. ficiformis*. The sponge recover detected five months later may be related to the restoration of environmental parameters.

The impressive mass mortality of gorgonians in autumn 1993 (Bavestrello *et al.*, 1994) and of gorgonians

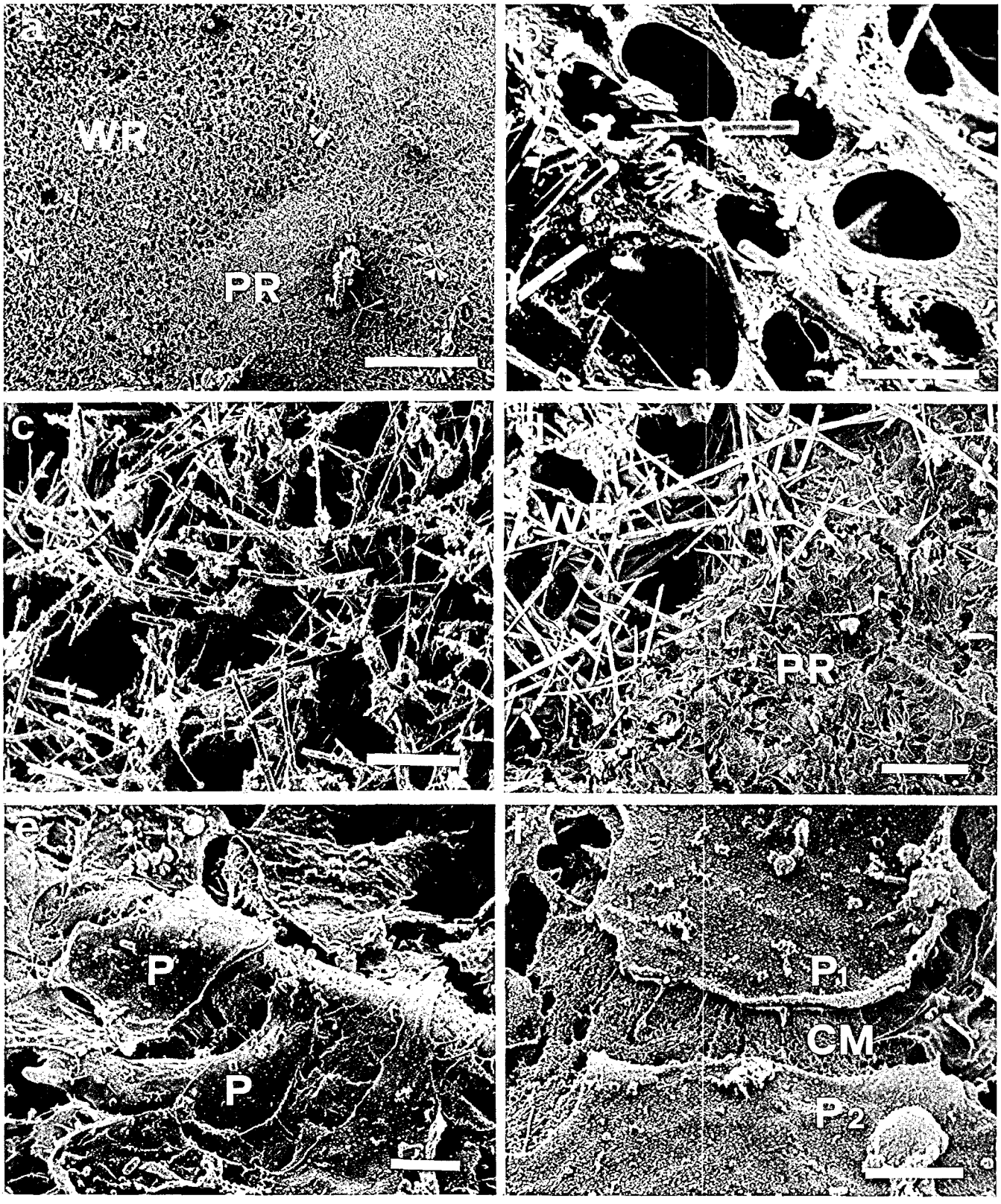

Fig. 4 - SEM views of necrotic specimens of *Petrosia ficiformis* characterized by superficial white spots. **a**, coexistence of a white region (WR) with a pigmented one (PR) (bar = 1 mm). **b**, detail of a uniformly pigmented specimen (used as control) with porous surface (bar = 5 µm). **c**, detail of a white area with irregular arrangement of spicule bundles lacking epithelial covering (bar = 50 µm). **d**, superficial region including a white (WR) and a pigmented zone (PR). Note that the exopinacocytes of the latter lack pores (bar = 50 µm). **e**, enlarged view showing the pinacodermal sloughing (P) (bar = 5 µm). **f**, the lost of cell adhesion between two pinacocytes (P1, P2) allows the collagenous matrix (CM) to be seen underneath (bar = 5 µm).

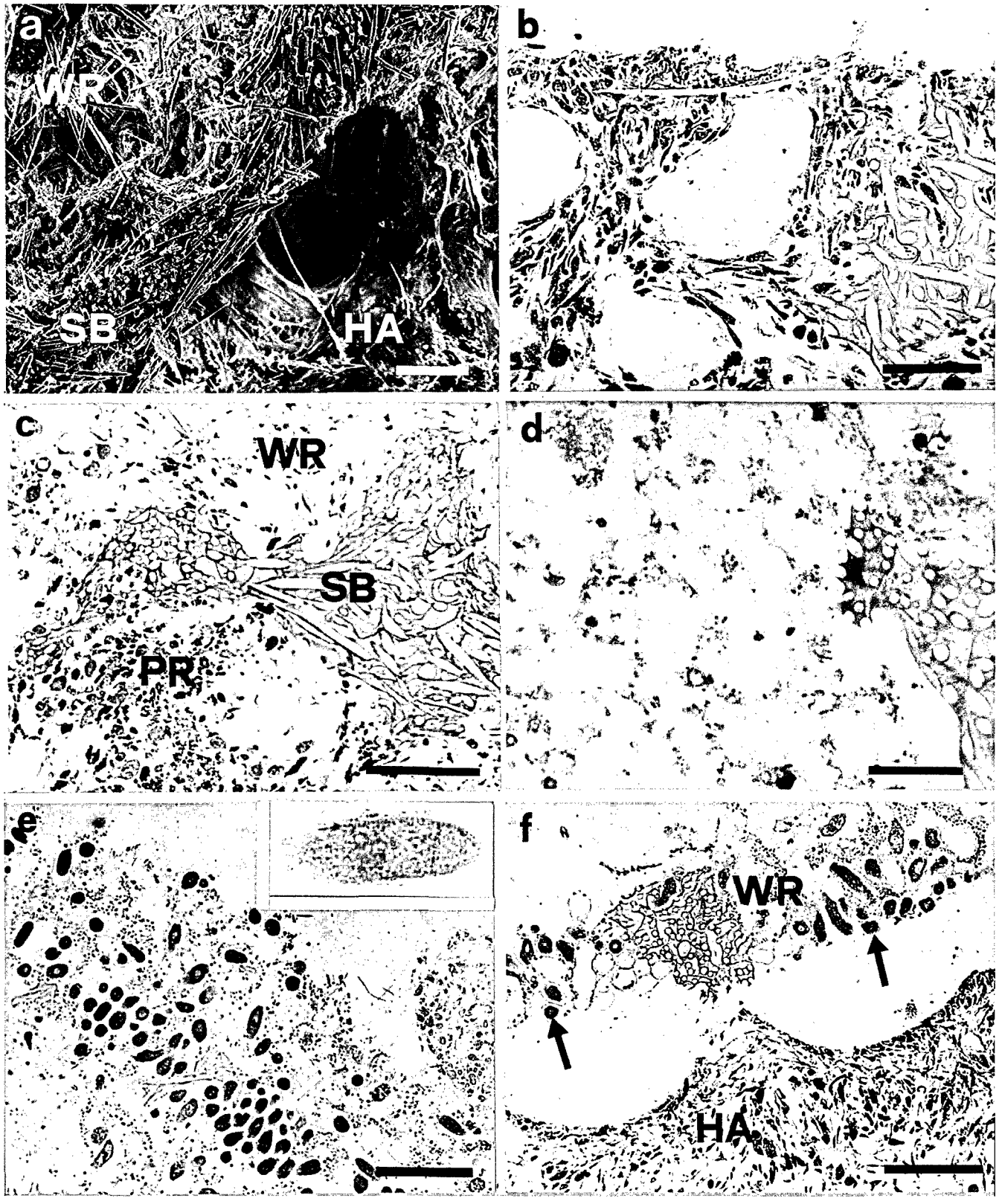

Fig. 5 - SEM view (a) and histological sections (b-f) of necrotic specimens of *Petrosia ficiformis*. **a**, a fractured specimen showing spicule bundles (SB) interposed between a non-functional upper white region (WR) and a healthy lower area (HA) below the pigmented region (bar = 100 μm). **b**, ectosome organization of a pigmented specimens used as control (bar = 100 μm). **c**, a spicule bundle (SB) separates the white region (WR) from the pigmented one (PR). The former shows a loose matrix with rare scattered cells, which contrasts with the cellular organization of the latter (bar = 100 μm). **d**, detail of the degenerating tissue without any particular aggregation of bacteria (bar = 50 μm). **e**, a mat of ciliates (arrows) (one of them in the inset) settled on the white region of the sponge (bar = 100 μm). **f**, bound region between the lower functional part (HA) located below the pigmented region and the degenerating white region (WR) filled with ciliates (arrows) (bar = 100 μm).

and sponges in summer 1999 (Cerrano *et al.*, 2000; Perez *et al.*, 2000) in the Ligurian Sea were attributed to stress due to changes in the environmental parameters (decrease of salinity consequent to autumnal rainfalls and increase of seawater temperature respectively).

Cold temperature stress may cause denaturation of proteins involved in cell adhesion (Watson & Morris, 1987; Suzucki & Choi, 1990). The effect of heat shock on cell adhesion and cytoskelatal organization has been investigated in various systems and there is compelling evidence that influx of ions may alter cell adhesiveness, causing the collapse of actin and intermediate filaments (Cress *et al.*, 1990; Walter *et al.*, 1990). As cytoskeletal elements and cell adhesion proteins function as a whole to maintain the integrity of the epithelia, disruption of the former affects the functionality of the latter (Takeicki, 1988).

In the studied necrosis episode, the first evidence of sponge stress is the closure of the superficial pores along with pinacoderm detachment. Closure of the pores could reduce freshwater inflow and preclude sediments from penetrating inwards into the sponge aquiferous system, thereby avoiding the clogging of the sponge canals. In addition, tissue death in *P. ficiformis* seems to differ from the diseases occasionally reported in sponges (Vacelet & Gallissian, 1978) and mainly noticed in commercial sponge populations (Storr, 1964; Gaino & Pronzato, 1989, 1992; Pronzato & Gaino, 1991; Vacelet *et al.*, 1994).

In conclusion, the present investigation on *P. ficiformis* proved that specimens can undergo a necrotic process, coincidental with environmental stress. The first marker is represented by white superficial patches followed by marked alteration of functionality

REFERENCES

Arillo A., Bavestrello G., Burlando B., Sarà M., 1993 - Metabolic integration between symbiotic cyanobacteria and sponges: a possible mechanism. Mar. Biol., 117: 159-162.

Balduzzi A., Bianchi C. N., Cattaneo-Vietti R., Cerrano C., Cocito S., Cotta S., Degl'Innocenti F., Diviacco G., Morgigni M., Morri C., Pansini M., Salvatori L., Senes L., Sgorbini S., Tunesi L., 1994 - Primi lineamenti di bionomia bentica dell'isola Gallinaria (Mar Ligure). *In*: G. Albertelli, R. Cattaneo-Vietti & M. Piccazzo (eds), Atti 10° Congr. A.I.O.L. Lang, Genova, pp. 603-617.

Bavestrello G., Bertone S., Cattaneo-Vietti R., Cerrano C., Gaino E., Zanzi D., 1994 - Mass mortality of *Paramuricea clavata* (Anthozoa, Cnidaria) on Portofino Promontory cliffs, Ligurian Sea, Mediterranean Sea. Mar. Life, 4: 15-19.

Brown B. E., Le Tissier M. D. A., Bythell J. C., 1995 - Mechanisms of bleaching deduced from histological studies of reef corals sampled during a natural bleaching event. Mar. Biol., 122: 655-663.

Cerrano C., Bavestrello G., Bianchi C. N., Cattaneo-Vietti R., Bava S., Morganti C., Morri C., Picco P., Sara G., Schiaparelli S., Siccardi A., Sponga F., 2000 - A catastrophic mass-mortality episode of gorgonians and other organisms in the Ligurian Sea (North-western Mediterranean), summer 1999. Ecol. Lett., 3: 284-293.

Cress A. E., Majida J. A., Glass J. R., Stringer D. E., Gerner E. W., 1990 - Alteration of cellular adhesion by heat shock. Exp. Cell Res., 190: 40-46.

Gaino E., Pronzato R., 1987 - Ultrastructural observations of the reaction of *Chondrilla nucula* (Porifera, Demospongiae) to bacterial invasion during degenerative processes. Cah. Biol. mar., 28: 37-46.

Gaino E., Pronzato R., 1989 - Ultrastructural evidence of bacterial damage to *Spongia officinalis* fibres (Porifera, Demospongiae). Dis. aquat. Org., 6: 67-74.

Gaino E., Pronzato R., 1992 - Epidemia e pesca intensiva minacciano la sopravvivenza delle spugne commerciali del bacino Mediterraneo. Boll. Mus. Ist. biol. Univ. Genova, 56-57: 209-224.

Gates R. D., Baghdasarian G., Muscatine L., 1992 - Temperature stress causes host cell detachment in symbiotic cnidarians: implications for coral bleaching. Biol. Bull., 182: 324-332.

Perez T., Garrabou J., Sartoretto S., Harmelin J. G., Francour P., Vacelet J., 2000 - Mortalité massive d'invertébrés marins: un événement sans précédent en Méditerranée nord-occidentale. C. R. Acad. Sci. Ser. III Sci. Vie, 323: 853-865.

Pronzato R., Gaino E., 1991 - La malattia delle spugne commerciali: considerazioni storico-economiche. Boll. Mus. Ist. biol. Univ. Genova, 54-55: 1-26.

Regoli F., Cerrano C., Chierici E., Bompadre S., Bavestrello G., 2000 - Susceptibility to oxidative stress of the Mediterranean demosponge *Petrosia ficiformis*: role of endosymbionts and solar irradiance. Mar. Biol., 137: 453-461.

Sarà M., Scalera-Liaci L., 1964 - Associazione fra la cianoficea *Aphanocapsa feldmanni* e alcune Demospongie marine. Boll. Zool., 31: 55-65.

Sarà M., Bavestrello G., Cattaneo-Vietti R., Cerrano C., 1998 - Endosymbiosis in Sponges: Relevance for epigenesis an evolution. Symbiosis, 25: 57-70.

Storr J. F., 1964 - Ecology of the Gulf of Mexico commercial sponges and its relation to the fishery. U.S. Fish Wild. Serv. spec. Sci. Rep. Fish., 466: 1-73.

Suzucki M., Choi B. H., 1990 - The behavior of extracellular matrix and the basal lamina during the repair of cryogenic injury in the adult rat cerebral cortex. Acta Neuropathol., 80: 355-361.

Takeicki M., 1988 - The cadherins: cell-cell adhesion molecules controlling animal morphogenesis. Development 102: 639-655.

Vacelet J., Donadey C., 1977 - Electron microscope study of the association between some sponges and bacteria. J. exp. mar. Biol. Ecol., 30: 301-314.

Vacelet J., Gallissian M. F., 1978 - Virus-like particles in cells of the sponge *Verongia cavernicola* (Demospongiae, Dictyoceratida) and accompanying tissues changes. J. Invertebr. Pathol., 31: 246-254.

Vacelet J., Vacelet E., Gaino E., Gallissian M. F., 1994 - Bacterial attack of spongin skeleton during the 1986-1990 Mediterranean sponge desease. *In*: R. W. M. van Soest, Th. M. G. van Kempen & J. C. Braekman (eds), Sponges in time and space. Balkema, Rotterdam, pp. 355-362.

Vicente V. P., 1990 - Response of sponges with autotrophic endosymbionts during the coral-bleaching episode in Puerto Rico. Coral Reefs, 8: 199-202.

Walter M. F., Petersen N. S., Biessman H., 1990 - Heat shock causes the collapse of the intermediate filament cytoskeleton in *Drosophila* embryos. Dev. Genet., 11: 270-279.

Watson P. F., Morris G. J., 1987 - Cold shock injury in animal cells. Symp. Soc. exp. Biol., 41: 311-340.

Wilkinson C. R., 1978 - Microbial associations in sponges. III. Ultrastructure of the *in situ* associations in coral reef sponges. Mar. Biol., 49: 177-185.

Wilkinson C. R., 1979 - Nutrient translocation from symbiotic cyanobacteria to coral reef sponges. *In*: C. Levi & N. Boury-Esnault (eds), Biologie des spongiaires. CNRS, Paris, pp. 373-380.

Wilkinson C. R., 1980 - Cyanobacteria symbiotic in marine sponges. *In*: W. Schwemmler & H. E. H. Schenk (eds), Endocytobiosis, endosymbiosis and cell biology, vol. I, Walter de Gruyter Co., Berlin, New York, pp. 553-563:

Wilkinson C. R., 1983 - Net primary productivity in coral reef sponges. Science, 219: 410-412.

Williams E. H., Goenega C., Vicente V. 1987 - Mass bleaching on Atlantic coral reefs. Science, 238: 877-878.