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REPORT

A catastrophic mass-mortality episode of gorgonians and other organisms in the Ligurian Sea (North-western Mediterranean), summer 1999

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Abstract

In the late summer of 1999, an extensive mortality of gorgonians and other epibenthic organisms was observed in the Ligurian Sea (Mediterranean Sea) from the Tuscan Archipelago to Marseille. Quantitative data from Tino Island and Portofino Promontory indicated that the proportion of affected gorgonians ranged from 60% to 100% in populations having a density of 9–27.8 colonies m^{-2}, suggesting that millions of sea fans died along the coast of Liguria. This mass mortality episode coincided with a sudden increase of sea water temperature down to more than 50 m depth. Laboratory analyses showed that the colonies stressed by high temperature also underwent extensive attack by microrganisms (protozoans and fungi), which are interpreted as opportunistic pathogens.

Keywords

Gorgonians, Ligurian Sea, marine benthos, mass-mortality, water temperature.

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INTRODUCTION

We have only recently become aware that mass-mortality may be a relatively frequent event in the sea, and probably plays significant ecological and evolutionary roles (Boero 1996; Harvell *et al.* 1999). In coral communities, for instance, these catastrophic events may serve to maintain diversity, preventing aggressive species from outcompeting other species and allowing persistence of a mosaic of successional stages (Riegl 1999).

There are biases in the types of organisms that attract notice when mortality events occur. As a rule, mass-mortality of small, ephemeral, economically unimportant organisms, probably goes unperceived. In contrast, die-offs of perennial, sessile organisms that shape the underwater seascape are likely to be viewed as major catastrophes. The best recent example of the latter is provided by the extensive bleaching of reef corals in the tropics, which has raised much concern not only among scientists but also among administrators, the media, and the public (Wilkinson 1999; Wilkinson *et al.* 1999). Less attention is given to similar phenomena in temperate seas, such as the Mediterranean, so that their occurrence is mostly anecdotal. In the last decade, several episodes of mass-mortality of sessile epibenthic invertebrates, such as sponges, anthozoans, bivalves and ascidians, have been recorded in the North-western Mediterranean Sea (Rivoire 1991; Gaino *et al.* 1992; Harmelin & Marinopoulos 1994; Mistri & Ceccherelli 1996; Bavestrello *et al.* 1997; Rodolfo-Metalpa *et al.* 2000; Morri & Bianchi, in press). In the late summer of 1999, an extensive die-off of gorgonians and other marine organisms occurred in the Ligurian Sea. This mortality might have significant consequences, since gorgonians, in particular, not only

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characterize the coralligenous biocoenosis (Weinberg 1980; Ros *et al.* 1985; Harmelin 1994), but also contribute greatly to the aesthetic value and attraction to diving tourists of the Mediterranean subtidal seascape (Bianchi *et al.* 1995). The aim of the present paper is to provide a first report of the 1999 mortality event, presenting preliminary quantitative data from selected sites of the Ligurian Sea and discussing possible causes.

METHODS

Evaluation of the mortality event

After the first sign of mortality in the eastern Ligurian Sea, we contacted colleagues in Italy and other countries to ask them for information about the occurrence of mass-mortality in different Mediterranean regions (Fig. 1a). We also re-visited the main Ligurian localities (Fig. 1b) where the sessile epibenthic communities (especially gorgonians) had been described in recent years: Tino Island (Mistri & Ceccherelli 1993; Mistri 1995a; Cocito *et al.* 1997), Mesco Point (Peirano & Tunesi 1989; Tunesi *et al.* 1991; Peirano & Sassarini 1992), Portofino Promontory (Morri *et al.* 1988) and Gallinaria Island (Balduzzi *et al.* 1994). In each locality, one to several surveys were made by SCUBA diving: the species involved were noted, and the incidence of mortality was assessed.

Due to their luxuriant gorgonian populations and easy access, two Ligurian localities were chosen for quantitative estimates of mortality: Tino Island and Portofino Promontory (Fig. 1c, d). The main gorgonian species in these two localities are *Paramuricea clavata*, *Eunicella singularis*, and *Eunicella cavolinii*, which form dense stands on differently sloping rocks below 15 m depth.

In both localities, SCUBA diving was used in September 1999 to count the number of gorgonian colonies in six quadrats (1 m^2 each) along 20 m long transects haphazardly placed in three different sites. Counts were made separately for healthy and affected colonies. Within the latter category, completely dead colonies were distinguished from those showing only partial mortality. Recently affected colonies were easily recognized owing to the clean scleraxis. Usually the first signs of epibiosis are visible after a few days, starting with hydroid polyps (Bavestrello *et al.* 1997). The fracture of gorgonian branches does not occur until before 1 or 2 months after epibiosis (Bavestrello & Boero 1988).

Colonies of *P. clavata* were counted at Tino Island proper, at the nearby rock called Tinetto, and at a totally submerged rocky shoal (Fig. 1c). *Eunicella singularis* was counted at Tinetto Island, at Tinetto Rock, and at a further nearby rocky shoal. Finally, counts of *E. cavolinii* were made at three different sites of Portofino Promontory, namely Aurora, Altare, and Torretta Point (Fig. 1d).

Laboratory observations

For scanning electromicroscope SEM analysis samples of healthy and affected sea fan tissue were fixed directly underwater in 2.5% glutaraldehyde in artificial sea water for 2 h, washed, and dehydrated in a graded ethanol series. Samples were critical-point dried using a CO_2 Pabish CPD apparatus and then coated with gold-palladium in a Balzers Union evaporator. Samples were examined with a Philips EM 515 SEM.

To isolate filamentous fungi, fresh fragments from both healthy and affected *P. clavata* and *E. cavolinii* were rinsed

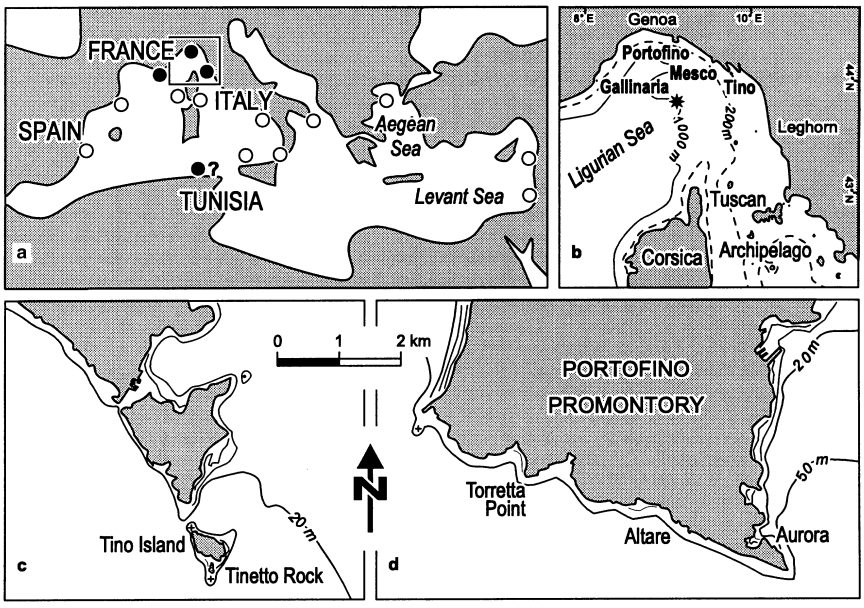

Figure 1 Maps of the sites where information on gorgonian mortality was obtained. (a) Results from an enquiry throughout the Mediterranean Sea: solid circles indicate mass-mortality, open circles (little or) no mortality. (b) Eastern Ligurian Sea, with the localities mentioned in the text; star indicates position of the ODAS buoy (see Methods). (c, d) Sites where quantitative data on the mortality of selected gorgonian species were taken.

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in marine sterile water, soaked in ethyl alcohol for 5 min and then rinsed in sterile water for 5 min. Small pieces of each specimen were placed in Petri dishes containing solid medium PCA (mashed potato 20 g L^{-1}, mashed carrot 20 g L^{-1}, agar 20 g L^{-1}). The Petri dishes were incubated at 22°C under dark conditions and inspected daily for up to 1 month for the development of colonies on the agar. Fungi were isolated exclusively from affected colonies and were identified to the level of genus by microscopic morphology. Nomenclature of fungi follows that listed in Ainsworth *et al.* (1973) and in Kohlenmeyer & Volkmann-Kohlenmeyer (1991).

Ligurian Sea water temperature

We gathered information on water temperature of the Ligurian Sea during the late summer of 1999 from different sources.

Elaboration of temperature and depth data logged in dive computers (a total of 287 dives along the Portofino Promontory and the Tino Island) allowed vertical profiles to be drawn from 0 to about 45 m depth from July to September.

A second data set was provided by the meteo-oceanographic buoy ODAS Italia 1 (Siccardi *et al.* 1998), moored in the centre of the Ligurian Sea (9°6.80′ E, 43°48.90′ N; Fig. 1b). Temperature records at 0 and 32 m depth were averaged weekly for the period 1 August to 20 October. These values were compared with the secular (1909–87) trend calculated on the Ligurian Sea records stored in the hydrological data bank of the Marine Environment Research Centre of La Spezia (Picco 1990).

Finally, a short time series of temperature measurements off Portofino is available at the University of Genoa. We compared the averaged August–September records for the period 1992–99.

RESULTS

Evaluation of the mortality event

At Tino Island, the early signs of mortality were seen in late August, first on some species of horny sponge at less than 10 m depth and then on gorgonians and other organisms (encrusting coralline algae, the coral *Cladocora caespitosa*, the zoanthid *Parazoanthus axinellae*) down to 27 m (maximum depth explored). The gorgonians *P. clavata* (Fig. 2A–C) and *E. singularis* were severely affected, with nearly all the colonies exhibiting partial mortality and many completely dead. *Leptogorgia sarmentosa* and the rarer *Eunicella verrucosa* were little affected.

At Portofino, gorgonian mortality was first observed on 10 September (A. Tommei, personal communication). At the beginning, it strongly affected gorgonians in shallow water, especially *E. cavolinii* (Fig. 2D), but progressively reached at least 70 m depth in subsequent weeks. *Eunicella singularis*, *E. verrucosa*, *P. clavata* and *L. sarmentosa* also showed mortality. In the well-known local population of the precious red coral *Corallium rubrum* (Cattaneo-Vietti *et al.* 1993), all the colonies were affected at least partially (Fig. 2E), estimated mortality ranging from 50% to more than 80%. Species of horny sponges, *Cladocora caespitosa* and *P. axinellae* experienced mortality also in this locality. Either at Tino or Portofino, the proportion of affected colonies within the local gorgonian populations has been very high, ranging from 60% to 100% (Fig. 3). At Portofino, 40%–60% of the colonies of *E. cavolinii* died off. At Tino, total mortality was around 20% for both *E. singularis* and *P. clavata*. These three species exhibited different population densities: mean ($\pm$ SE) number of colonies per m^2 was 27.8 $\pm$ 0.7 for *E. cavolinii*, 9.0 $\pm$ 0.4 for *E. singularis*, and 19.3 $\pm$ 0.3 for *P. clavata*. However, mortality (partial and total mortalities pooled) was not correlated with population density in any of the three gorgonian species (Fig. 4) and, although based on preliminary observation, there were no differences in severity of damage to tips and base. Similarly, differences among sites within each locality were not significant (Table 1).

Similar situations were seen in the two other Ligurian localities visited over subsequent days. At Gallinaria Island, total mortality was observed on certain sponges (*Spongia*, *Cacospongia* and *Hippospongia*, but neither *Ircinia* nor *Axinella*). *Eunicella verrucosa*, the only abundant gorgonian species there, was little affected. At Mesco Point, mortality affected encrusting coralline algae, species of horny sponge, the zoanthid *P. axinellae*, and gorgonians. About one-fifth of the colonies of *P. clavata*, down to 40 m depth, were dead.

Results from our enquiry throughout the Mediterranean sea (Fig. 1a) indicated that, over the same period, coralline algae, sponges, corals, and gorgonians died near Leghorn, within the Tuscan Archipelago (M. Mistri and G. Santangelo, in litteris; G. Chimenti, personal communication) as well as along the French coast between Nice and Marseilles (J. Harmelin and S. Sartoretto, in litteris). R. Pronzato (personal communication) reported mortality of sponges and gorgonians off the northern coast of Tunisia, but we have had no confirmation by Tunisian colleagues.

No appreciable mortality occurred in Corsica (J. Garrabou and S. Sartoretto, in litteris), at several localities of Southern Italy (F. Barbieri and M. Ferrari, personal communication; G. Corriero and M. Mistri, in litteris), and along the coasts of Spain (J. Garrabou, J.-M. Gili and A. A. Ramos Esplá, in litteris).

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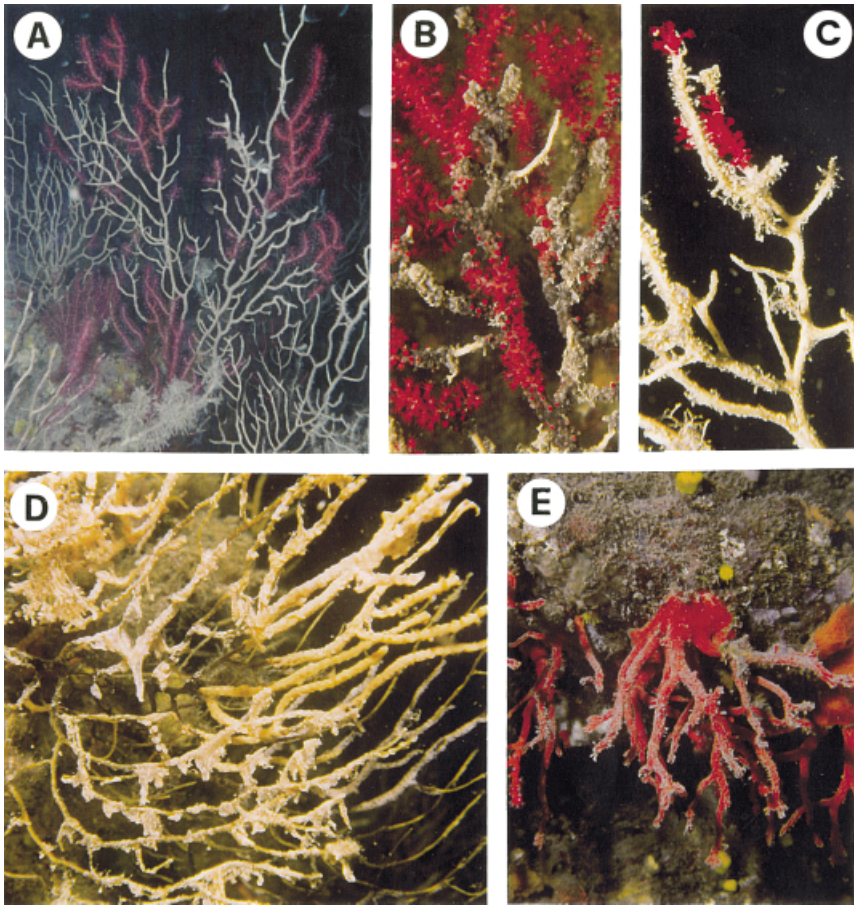

Figure 2 Aspects of gorgonian mortality. Coenenchime necrosis and detachement in *P. clavata* (A–C), *E. cavolinii* (D), and *C. rubrum* (E).

Severe mortality of sponges and/or corals, but not of gorgonians, has been observed in the Eastern Mediterranean (C.-C. Chintiroglou, G. Bitar and M. Fine, in litteris). In the Levant Sea, however, gorgonians are rare to absent or thrive deeper than the usual range of SCUBA diving (G. Bitar and M. Fine, in litteris). In the north Aegean Sea, colonies of *E. singularis* suffered from an unexplained infestation of epiphytic algae (C.-C. Chintiroglou, in litteris).

Although we have no information from other Mediterranean localities, it is apparent that this mortality event has touched principally the Ligurian Sea at large, from the Tuscan Archipelago to Marseilles, and perhaps northern Tunisia. According to satellite data collected by the U.S. Naval Research Laboratory in late October, these two areas showed positive anomalies of sea surface temperature up to 4°C.

Field observations and laboratory analyses

On the focal gorgonian species the first sign of stress was an unusual mucus secretion, followed after a few days by a progressive loss of pigmentation: the coenenchyme became greyish in *P. clavata* (Fig. 2A–C) and whitish in *E. cavolinii* (Fig. 2D) and was colonized by fungi (Fig. 2D). The damaged portions of coenenchyme detached from the skeletal axis (Fig. 2C, D), red coral (*C. rubrum*) colonies quickly lost their coenenchyme (Fig. 2E), and filamentous algae and campanulariid hydroids rapidly colonized the naked skeletal axis (Fig. 2C). This mortality affected both the base and the apexes of the colonies with the same intensity. The most common species (the sponge *Pleraplysilla spinifera,* the hydroid *Garveia grisea*, the bivalve *Pteria hirundo,* the bryozoan *Turbicellepora avicularis*) forming the typical species assemblage epibiotic on *P. clavata* (Bavestrello *et al.* 1997) were not affected.

The SEM analyses showed remarkable differences occurring between healthy and damaged portions in all the examined gorgonians (Fig. 5A, C, D). In the normal coenenchyme, the spicules were covered by a thin epithelium composed of large and flattened cells and exhibited a regular spatial arrangement (Fig. 5C), while in the damaged portions the epithelium was completely lacking and spicules disorganized (Fig. 5D). The damaged polyps were retracted and around them it was possible to observe a dense concentration of protozoan ciliates that

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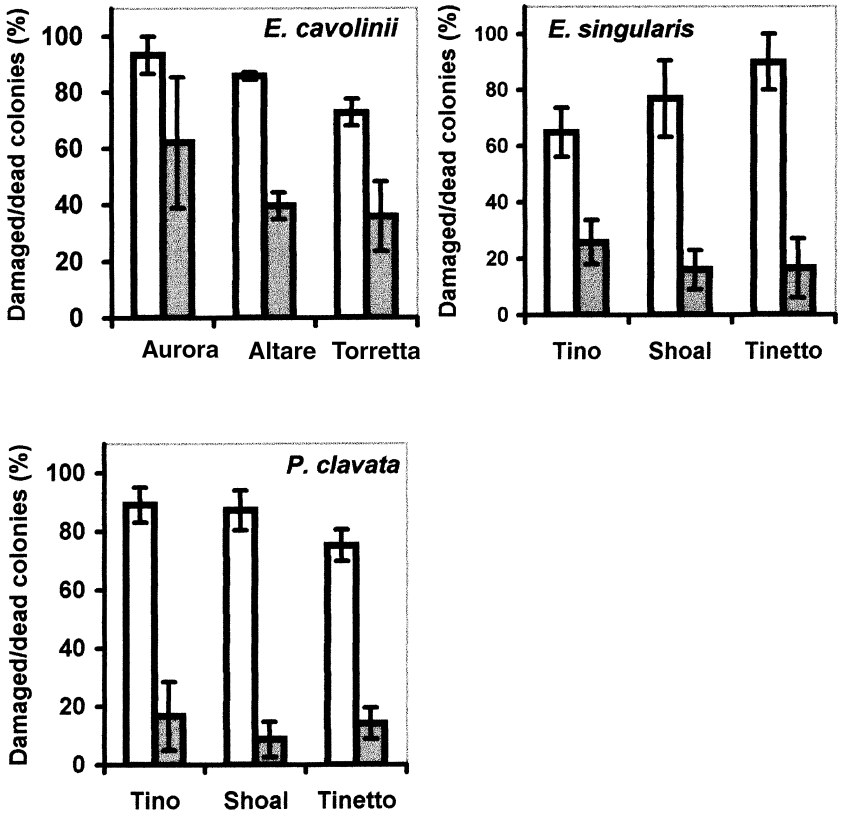

Figure 3 Percentage of damaged (white bars) and completely dead (black bars) colonies in three species of sea fan, in six quadrats (1 m^2 each) along 20 m long transects.

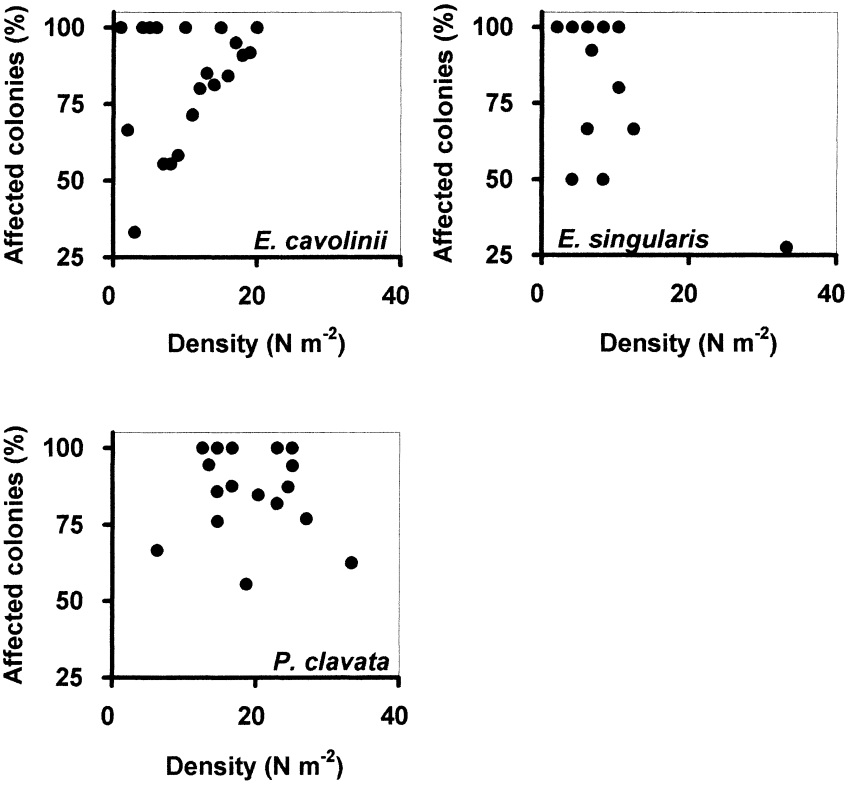

Figure 4 Percentage of affected colonies *versus* population density in three species of sea fan.

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Table 1 One-way analysis of variance of mortality incidence (proportion of damaged colonies, partial and total mortalities pooled) in the three gorgonian populations selected

Sources of variation	SS	d.f.	s^2	F	p
P. clavata at Tino					
Among sites	684.3	2	342.17	1.574	0.240
Within sites	3261.7	15	217.44		
Total	3946.0	17			
Cochran's test				$C = 0.418$	0.874
E. singularis at Tino					
Among sites	1833.9	2	916.97	1.116	0.352
Within sites	13149.4	16	821.83		
Total	14983.4	18			
Cochran's test				$C = 0.556$	0.473
E. cavolinii at Portofino					
Among sites	1539.2	2	769.60	0.790	0.470
Within sites	16559.1	17	974.06		
Total	18098.3	19			
Cochran's test				$C = 0.475$	0.847

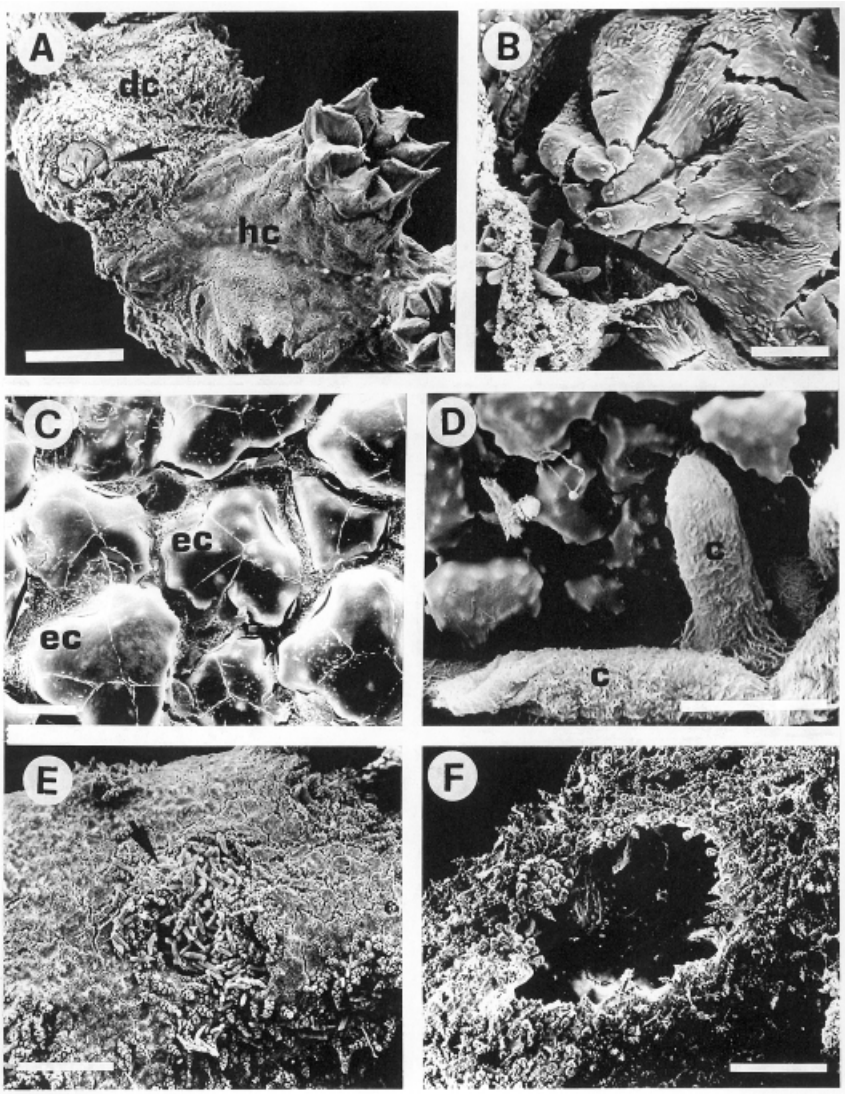

Figure 5 SEM photographs of the damaged tissues of sea fans. A, comparison between a healthy (hc) and a damaged (dc) portion of the coenenchime of *P. clavata.* Arrow indicates a retract polyp in the damaged zone. (B) Magnification showing a group of ciliates (c) penetrating inside the polyp. (C) Normal ectodermic surface of *E. cavolinii* showing the spicules regularly arranged and covered by a thin epithelium composed by large and flattened cells (ec). (D) Damaged portions of the ectodermic surface of *E. cavolinii* where the epithelium was completely lacking due to the feeding activity of large ciliates (c). (E) A group of ciliates (arrow) eating on a polyp of *L. ceratophyta.* (F) At the end of ciliate activity, the polyp is completely removed as shown in this portion of *E. cavolinii.* Scale bars: (A) 1 mm; (B) 100 μm; (C) 10 μm; (D) 50 μm; (E, F) 500 μm.

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actively ate the polyp soft tissues and the epithelium covering the coenenchime (Fig. 5B, D, E). At the end of the ciliates' activity, the polyps were completely eliminated (Fig. 5F) and the spicules, no longer retained by the soft tissue, progressively detached from the scleraxis. The pattern of tissue loss followed similar steps in all the studied species.

Fungi grew profusely on the damaged gorgonian tissues. The most common fungi isolated belong to the saprophitic genus *Trichoderma,* but *Cladosporium, Penicillum* and *Humicola* were also commonly present. All these genera are known to include opportunistic pathogens that have been reported to cause disease in plants and in immuno-compromised humans.

Ligurian Sea water temperature

During the late summer of 1999, there was a sudden warming of the sea water below 15 m depth (Fig. 6A). Data from September show that the temperature remained above 20°C in the whole water column down to 40 m depth. Confirmation comes from the short time series available at the University of Genoa (Fig. 6B). These last data indicate that the temperatures of the period August–September 1999 and 1993 (the year of another mass-mortality of sea fans) are greater than the average values of the period 1992–98 (excluding 1993).

Records of the ODAS buoy (Fig. 6C) indicate that sea water temperature in the late summer of 1999 was higher than normal by 2–4°C, which is in good accordance with satellite imagery data. Information is still too poor for drawing any firm conclusions, but suggests the following pattern. Surface water was warmer than usual throughout the period in consideration, with perhaps a major peak at the end of August. Temperatures at 32 m depth were normal, or even a little colder than average, until late September, when a sharp peak nearly reached 23°C and then decreased, remaining 1 or 2°C higher than normal.

DISCUSSION

The observations and data presented above clearly indicate that the summer 1999 mortality event was the largest, in terms of both geographical extent and intensity, ever recorded in the Ligurian Sea. Based on quantitative data on *E. cavolinii*, *E. singularis*, and *P. clavata* and considering the area involved we estimate that millions of sea fans probably died along the Ligurian coast. At Portofino, the best studied locality until now, Bavestrello & Boero (1988) described mortality of *E. cavolinii* in February 1985. Eight years later, in October 1993, Bavestrello *et al.* (1994) recorded a mass-mortality of *P. clavata* that caused the disappearance of about 10 000 sea fans between 20 and 35 m depth in an area of about 800 m^2. In the same period, Harmelin & Marinopoulos (1994) documented mortality of *P. clavata* in Provence (France) where a similar event had already occurred in the 1980s (Rivoire 1991).

To explain these mortalities, different physical, chemical and biological causes have been invoked, according to the period, the locality and the species affected (Bavestrello *et al.* 1994). In the case of the summer 1999 event, the sudden appearance and concomitance with sea water temperature anomalies suggests a meteorological cause. Records of the Meteorological Observatory of Genoa indicate an unusual period of calm winds, but no particular anomalies in terms of runoff or rainfall. Air temperature was higher as compared to most previous years. Coincidentally or not, another temperature high was recorded in 1993 when a gorgonian mortality also occurred, even if in this case heavy rainfalls were thought to have been involved (Bavestrello *et al.* 1994). In the tropics, massive coral mortality has also been attributed to elevated temperatures (Ware 1997; Strong *et al.* 1998; Berkelmans & Oliver 1999; Sheppard 1999; Stone *et al.* 1999), but other instances have also been documented (Brown 1997). Apparent spread with the Ligurian Current, regularly flowing westward along the coast (Astraldi *et al.* 1995), suggests that a disease may have been involved. However, gorgonian mortality was not density dependent nor did it exhibit a patchy distribution, as could be expected if disease were involved. In previous Ligurian Sea mortality events, which were less severe than that in 1999, bacterial infection of gorgonian tissues was excluded (Bavestrello *et al.* 1994). In recent years, mortality of the coral *Oculina patagonica* in the SE Mediterranean has been caused by the pathogenic bacterium *Vibrio shiloi* (Kushmaro *et al.* 1996, 1997; Rosenberg & Loya 1999), whereas Caribbean gorgonians died because of the pathogenic fungus *Aspergillus sydowii* (Smith *et al.* 1996; Nagelkerken *et al.* 1997a, b; Geiser *et al.* 1998). Clearly, elevated temperature and bacterial or fungal infection are not mutually exclusive. Many pathogens have the capacity to cause disease only in a host under stressful conditions: increased seawater temperature (or other environmental stress) can trigger mortality by inducing a microbe to be more virulent or the host more vulnerable (Kushmaro *et al.* 1998; Toren *et al.* 1998).

Our laboratory analyses suggest the involvement of microrganisms, such as protozoans and fungi in the 1999 event. The environmental factors related to high water temperature may stress the cells that were then unable to produce antifouling substances (e.g. Pawlik *et al.* 1987), which otherwise completely inhibit the attack of predator or saprophitic organisms. The attack of ciliates probably

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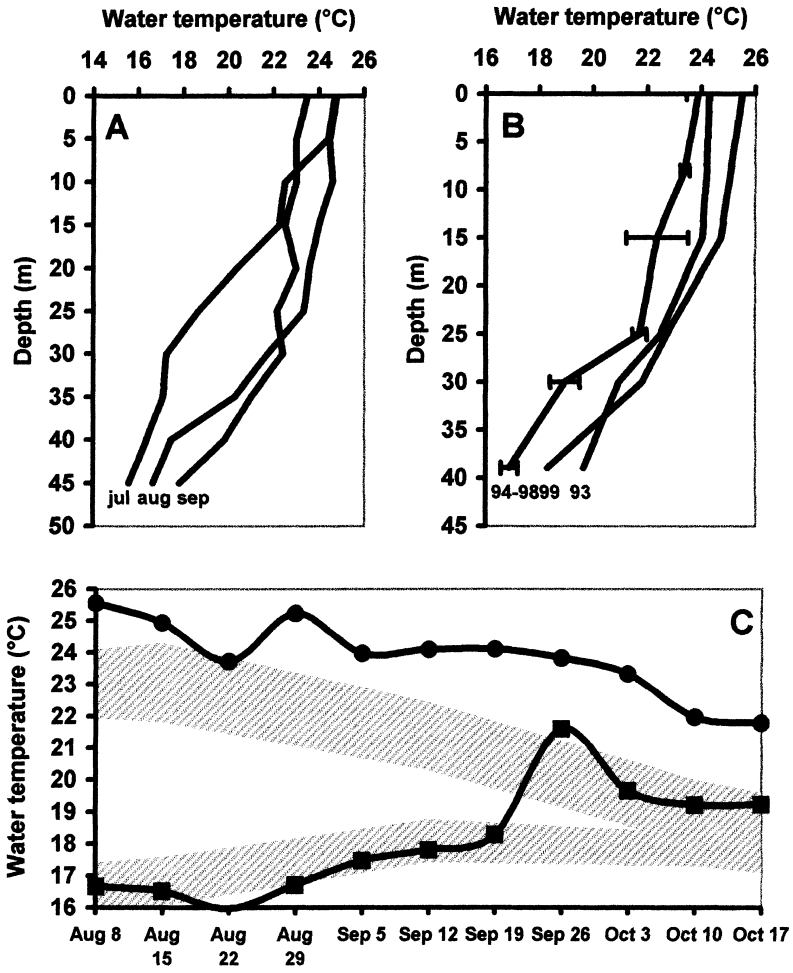

Figure 6 Ligurian Sea water temperature during the late summer of 1999. (A) Vertical profiles obtained by elaboration of temperature and depth data logged on dive computers; July, August, September 1999. (B) Vertical profiles obtained from data of the period August–September available at the University of Genoa; average values (± SE) of the period 1992–98 (excluding 1993), 1993, and 1999. (C) Trends of water temperature (weekly averages) recorded by the ODAS buoy at the surface (circles) and 32 m depth (quadrats). The grey bands indicate the corresponding confidence limits (±95%) obtained from the secular (1909–87) trend calculated on Ligurian Sea records.

plays a crucial role in determining coenenchyme disaggregation and its subsequent detachment. As such, this mortality event may be regarded as a consequence of the interaction of an environmental stress and opportunistic pathogens. A similar interpretation was also given by Gaino *et al.* (1992) for the horny sponge mortality.

Lack or scarcity of historical, baseline data not only on mortality, but even on density and population structure of Ligurian Sea gorgonians, makes it difficult to determine if the apparent increased frequency of mortality events in the last few decades is a novelty, possibly linked to rapid environmental change, or simply a reflection of intensified investigation (Harvell *et al.* 1999; ISRS 1999). We believe that the role of mass-mortality events on the structure and functioning of marine ecosystems should be a research priority in the years to come. Gorgonians and other large sessile invertebrates grow slowly (Mistri 1995b), live for many years, and are thought to reproduce sporadically. Repeated mass-mortality events may have far reaching consequences for populations and communities and urgently requires attention from marine scientists and environmental managers.

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