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Impact of excessive sedimentation caused by anthropogenic activities on benthic suspension feeders in the Sea of Marmara

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Abstract

A massive die-off of benthic suspension feeders (BSF) covered by large amounts of sediments was observed along Prince Islands coasts (north-eastern Sea of Marmara) in August 2015. Alcyonarians, pennatulaceans, bivalves and sponges were severely affected. Many BSF probably died from burial and clogging of their feeding and respiratory apparatus. Of the gorgonian colonies, 66 ± 34% (mean ± SD) were dead, while 15 ± 16% (mean ± SD) displayed recent necrosis on the colony surface. In addition, histopathological and microbial examinations of the affected gorgonians and gold corals (*Savalia savaglia*) suggest that stress caused by sedimentation made them vulnerable to exploitation by consistently isolated opportunistic microorganisms. We isolated *Vibrio splendidus* and *Vibrio neptunius* from diseased gold coral colonies, but the bacterial isolates obtained from gorgonians could only be identified to genus *Vibrio* level. The presumably artificially introduced fungus *Mucor circinelloides* was common on both gold coral and gorgonians. This mould and opportunistic bacteria may have colonized BSF by taking advantage of low oxygen levels leading to impaired coral immune responses and thereby exacerbated the BSF mortality. Construction and landfill operations at Yassıada seem to be the greatest contributor to the observed sedimentation, as shown by silicate concentrations in nearby waters. These observations imply that preventive measures are necessary when construction operations take place in the vicinity of sensitive marine habitats.

Introduction

Episodes of mass mortalities and disease outbreaks affecting corals and other benthic sessile suspension feeders (BSF) are recorded with an increasing frequency all over the world (Ward & Lafferty, 2004) including in the Mediterranean Sea (Rivetti *et al.*, 2014). Anomalously high temperature periods and ocean acidification in consequence of climate change seem to be major pressures for BSF in the Mediterranean Sea (Cerrano *et al.*, 2000; Perez *et al.*, 2000; Coma *et al.*, 2006; Garrabou *et al.*, 2009) and also at the global scale (Harvell *et al.*, 2002); local increases of pollutants and sedimentation created by anthropogenic activities are important threats at regional scales (Lohrer *et al.*, 2003; Shahidul Islam & Tanaka, 2004; Thrush *et al.*, 2004; Fabricius, 2005; Maina *et al.*, 2013). Human activities that increase sedimentation as a result of coastal development (dredging, land filling, runoff from coastal construction etc.) threaten 25% of the world's coral reefs (Burke *et al.*, 2011). Increased sedimentation can cause smothering and burial of coral polyps, shading, tissue necrosis and population explosions of bacteria in coral mucus (Erftemeijer *et al.*, 2012). Most studies dealing with the effects of sedimentation are concerned with photosynthetic symbionts bearing scleractinian corals, but octocorals and other heterotrophic BSF are also adversely affected by heavy sedimentation via burial, smothering or clogging of feeding and/or respiratory surfaces (Fabricius *et al.*, 2007; Bell *et al.*, 2015; Hendrick *et al.*, 2016).

The Sea of Marmara is surrounded by seven cities, including Istanbul, Kocaeli and Bursa, which are highly industrialized and overpopulated. Consequently, various anthropogenic disturbances impact the Sea of Marmara, particularly along the Istanbul coasts (Özsoy *et al.*, 2016). This area has undergone significant changes over the last few decades owing to a rapid increase in industrialization and urbanization, particularly along its southern coasts (Kurt *et al.*, 2010). In spite of such anthropogenic disturbances, dense assemblages of endemic gorgonians were reported in the Sea of Marmara very close to southern Istanbul coasts (Topçu & Öztürk, 2015). Together with other BSF, they are important components of the invaluable Mediterranean coralligenous community, with significant effects on the structure, biomass and biodiversity of these communities (Ballesteros, 2006; Bertolino *et al.*, 2013; Ponti *et al.*, 2014).

Recently, a massive die-off of BSF covered by high amounts of sediments was observed along Prince Islands coasts in the north-eastern Sea of Marmara. The purpose of our study was to determine the BSF species affected and to estimate the intensity of the reported incident along Prince Islands coasts. We also aimed to elucidate possible contributing causes and to describe histopathological changes in affected coral soft tissues.

Materials and methods

Study area

The Sea of Marmara is connected to the Aegean Sea by the Çanakkale Strait (Dardanelles) and to the Black Sea by the Istanbul Strait (Bosphorus). Circulation within the Sea of Marmara is characterized by a two-layer stratification, with a brackish upper layer originating from the Black Sea flowing southwards and a lower Mediterranean layer flowing northwards. The two layers are separated by a permanent halocline, located at 15–30 m depth depending on the season and location (Beşiktepe *et al.*, 1994). In the lower layer, the salinity is about 38.5 with temperature stable at 14–15°C throughout the year. The study area is located on the southern coasts of Prince Islands in the north-eastern Sea of Marmara (Figure 1A). Study sites were selected amongst stations that had the highest recorded octocoral density and diversity (Topçu & Öztürk, 2015). The communities on rocky bottoms in the subhalocline layer of southern Prince Islands coasts are dominated by BSF (Figure 1B), represented mainly by tubicolous polychaetes, echinoderms (the most common being *Antedon mediterranea* (Lamarck, 1816), *Ophiothrix fragilis* (Abildgaard in O.F. Müller, 1789) and *Ocnus planci* (Brandt, 1835)), several sponge species and anthozoans (the most common being *Paralcyonium spinulosum* Delle Chiaje, 1822, *Parazoanthus axinellae* (Schmidt, 1862), *Sagartia* spp., *Paramuricea macrospina* (Koch, 1882) and other alyconarians) (Supplementary material 1 and 2; Topçu & Topaloğlu pers. observ.).

Description of the field surveys

Field surveys were conducted from the halocline (where the Mediterranean waters reside, starting from 15–20 m) to a maximum of 40 m depth by scuba diving. Benthic species that showed signs of necrosis or mortality were noted and photographed in August 2015. Quantitative surveys were conducted between October and November 2015. Gorgonians were selected as monitoring models because they constitute key species in the local coralligenous community and as their mortality is easily quantified (Garrabou *et al.*, 2009). The abundances and distributions of four gorgonian species (*Paramuricea clavata* (Risso, 1826), *Paramuricea macrospina*, *Spinimuricea klavereni* (Carpine & Grasshoff, 1975), *Eunicella cavolini* (Koch, 1887)) in the area were recently recorded (Topçu & Öztürk, 2015) and a similar survey was repeated as a part of this study. One m^2 quadrats were placed every metre on either side of the 20 m long transect tape laid on the seabed. In case of vertical walls or large boulders, 20 quadrats were placed haphazardly on the sea bottom. The number of gorgonian colonies in each quadrat were noted according to (1) showing no necrosis (healthy), (2) having recent necrosis on >10% of the colony surface (necrosed) and (3) having a completely denuded skeleton as previously described (Linares *et al.*, 2008*a*; Garrabou *et al.*, 2009). Quantitative surveys at Yassıada (S1 and S2) and Balıkçı Islands (S5 and S6) were repeated at two depths (26 and 35 m) because during qualitative surveys, the distribution of healthy/injured colonies seemed to be different above and below 30 m depth. The percentage of recent/old necrosis (denuded axis or epibiosis by pioneer species/colony overgrowth by more complex organisms such as sponges, bryozoans etc.) (Linares *et al.*, 2005) was noted only for *S. klavereni* as data from previous years were only available for this species.

Sampling of coral material

Sample material was taken from colonies of gorgonians *Paramuricea clavata*, *Spinimuricea klavereni*, *Eunicella cavolini* and the gold coral *Savalia savaglia* showing early stage lesions but no tissue loss. For this purpose, diseased colonies (N = 9 per species) were collected in November (2015) by scuba diving on the study site twice within a 10-day period. Centrifuge tubes containing 50 ml of sterile PBS were brought by the divers to the depth of the coral colonies and then diseased colony samples (~5 cm in length) were collected into separate tubes under water. The tubes were surfaced and they were brought to the laboratory at +4°C where the collected tissues were processed immediately for histopathology and microbiological examination.

Histopathological examination of coral material

Sample material from *S. savaglia* colonies was processed for histopathological examination. Approximately 1 cm^3 of tissue sample was fixed in 10% buffered formalin, dehydrated in ethanol, cleaned in xylene and embedded in paraffin wax. Sections (5 µm thick) were stained with haematoxylin-eosin (HE) following standardized practices (Culling, 1963). The stained tissue sections were examined under the microscope using the image analysis system NIS-Elements BR Microscope Imaging Software (Nikon Instruments).

Microbiological examination of coral material

For microbiological examination, tissue samples (1 cm^3) of each specimen were collected aseptically and rinsed with sterile phosphate-buffered saline solution (pH 7.4) to remove planktonic cells. The tissue samples were then placed onto culture media including Marine Agar 2216 (MA) (Difco) and Mycosel Agar (BD) supplemented with chloramphenicol and cycloheximide (Raymundo *et al.*, 2008). The mucoid layer of each specimen was also sampled by gently touching the coral surface with a sterile loop and streaking onto the same media as described above (Ben-Haim & Rosenberg, 2002; Raymundo *et al.*, 2008). All samples were processed in triplicates and all plates were incubated at 22°C for 72 h. All isolated bacterial pure cultures were characterized using standard protocols to assess motility, Gram staining, oxidase and catalase activity, Oxidative/Fermentative (O/F) reaction, growth on TCBS agar and susceptibility to vibriostatic agent O/129. Tests to characterize fungal isolates included growth at 37°C, sporulation and staining with lacto-phenol cotton blue.

Genomic DNA extraction from microbial isolates

The bacterial isolates which displayed the same morphological and biochemical characteristics were selected and inoculated into Marine Broth 2216 (Difco) and incubated overnight at 22°C in a shaking incubator. For the fungal isolates, samples were taken directly from culture media. Genomic DNA was extracted from all isolates with the GeneJET Genomic DNA Purification Kit (Thermo) according to the manufacturer's instructions and used as the template for PCR.

PCR amplification and sequence analysis

For bacterial identification, partial 16S rRNA and *gyrB* (DNA gyrase, subunit B) gene products were amplified and sequenced. The

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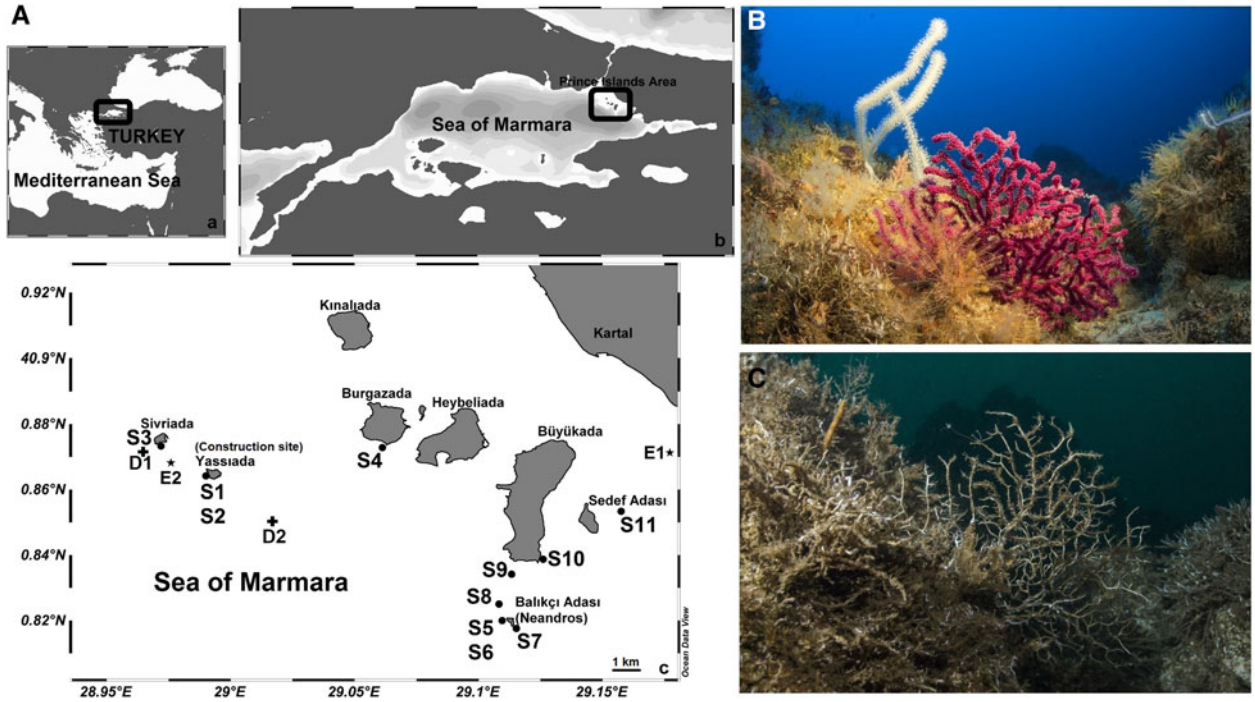

Fig. 1. (A) Location of the study area in the Mediterranean Sea (a), in the Sea of Marmara (b) and location of the sites along Prince Islands coasts (c). Sampling sites for environmental parameters are shown with stars. Dumping sites are shown with crosses. (B) A typical landscape from rocky bottoms in Prince Islands at 30 m photographed on 16.9.2014 showing *Paramuricea clavata* (at the centre), *Spinimuricea klavereni* (at the upper left side), *P. macrospina* (at the left edge and behind *S. klavereni*), *Paralcyonium spinulosum* (at the left side) and several *Antedon mediterranea.* (C) The same location as in B but photographed on 15.11.2015. Photo credit: M. Öztabak.

16S rRNA gene product was amplified using universal bacteria primers S-D-Bact-0008-a-S-20 (5′- AGAGTTTGATCCTGGC TCAG-3′) and S-*-Univ-0536-a-A-18 (5′- GWATTACCGC GGCKGCTG-3′) (Suau *et al.*, 1999). To amplify a part of the *gyrB* gene, the gyrBBAUP2 (5′- GCGGAAGCGGCCNGSNA TGTA-3′) and gyrBBNDN1 (5′- CCGTCCACGTCGGCRTC NGYCAT-3′) primer sets were used (Santos & Ochman, 2004). For fungal identification, the ribosomal internal transcribed spacer (ITS) region was amplified and sequenced using the ITS4 (5′- TCCTCCGCTTATTGATATGC-3′) and ITS5 (5′- GGAA GTAAAAGTCGTAACAAGG-3′) primer set (White *et al.*, 1990). All PCR reactions (50 µl) included ~50 ng template DNA (2 µl), 0.4 µM of each primer (2 × 2 µl), DreamTaq PCR Master Mix (2×) (Thermo Scientific) (25 µl) and nuclease-free water (Thermo Scientific, Arktik) (19 µl). Amplification was performed using a thermal cycler (Thermo Fisher Scientific) with the following parameters for the partial 16S rRNA gene amplification: initial denaturation at 95°C for 3 min, followed by 30 cycles of amplification (denaturation at 95°C for 30 s, annealing at 56°C for 1 min, extension at 72°C for 1 min) and a final extension step of 72°C for 4 min (Suau *et al.*, 1999). For the partial *gyrB* gene amplification the program was: initial denaturation at 95°C for 5 min, followed by 3 cycles of 1 min at 95°C, 2 min 15 s at 55°C, 1 min 15 s at 72°C, 30 cycles of 35 s at 95°C, 1 min 15 s at 55°C, 1 min 15 s at 72°C and final extension at 72°C for 10 min (Pascual *et al.*, 2010). And finally, for the amplification of the fungal ITS region, the program was: initial denaturation at 95°C for 10 min, followed by 35 cycles of amplification (denaturation at 95°C for 15 s, annealing at 52°C for 30 s, extension at 72°C for 20 s) and a final extension step at 72°C for 7 min (Schoch *et al.*, 2012).

After amplification, 10 µl of each PCR reaction was loaded on a 1.5% (w/v) TAE agarose gel containing ethidium bromide (0.5 ug ml^{-1}) and electrophoresis was performed for 40 min at 90 V. PCR products were visualized on a UV transilluminator and fragment sizes were estimated against the GeneRuler 100 bp DNA Ladder (Thermo Scientific). PCR products were purified and sequenced bidirectionally by Medsantek (Istanbul, Turkey). Sequence editing and analysis were performed in Bioedit v7.0.0 (Hall, 1999) using the ClustalX 2.1 (Larkin *et al.*, 2007) and BLASTN 2.2.20 algorithms (Zhang *et al.*, 2000). All obtained nucleotide sequences have been deposited in the GenBank database.

Environmental parameters

Data from other monitoring programmes were retrieved in order to estimate temporal variation of selected environmental parameters in the surrounding waters of Prince Islands. Silicate concentrations provide an indicator of terrestrial sediment and are hence of particular interest. Data series (3 or 4 months/year) of dissolved oxygen (mg l^{-1}) (2000–2006; 2009; 2011) and silicate (µM) (2001–2009) at 40 m depth off the Kartal coast (E1 in Figure 1) were procured from a water monitoring programme conducted by the IU Institute of Marine Science and Management for İSKİ (Istanbul Water and Sewerage Administration) water monitoring programme. The following data were acquired from the integrated marine pollution monitoring project coordinated by TÜBİTAK Marmara Research Center: (1) temperature (°C), salinity, dissolved oxygen (mg l^{-1}) along vertical profiles in August 2015 and 2016 (E2 in Figure 1); (2) silicate (µM) concentrations along vertical profiles at 10 stations in Prince Islands in August 2016; (3) silicate (µM) concentrations in the surface water at 73 stations over the Sea of Marmara except İzmit Bay in August 2016.

Statistical analysis

The differences in the number of dead, necrosed and healthy colonies at two different depths in Yassıada and Balıkçı Islands were compared by χ^2 tests. The relation of the number of damaged colonies with the depth and the slope of sites were analysed by non-

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parametric Spearman rank correlation tests. The percentages of recent and old tissue necrosis on *S. klavereni* colonies before and after the mortality were compared by Kolmogorov–Smirnov tests. All statistical tests were effectuated with GraphPad Prism version 6.01 for Windows.

Results

Qualitative observations

Only organisms that could be identified from the apparent remaining skeletons were determined to species level because mortality had already severely affected the Prince Islands area when observations started in August 2015. The last survey in the area before mortality was in the first week of May 2015 when there were no unusual signs of adverse conditions affecting BSF. Impacts imposed by sedimentation may have started well before the surveys described here. In August 2015, benthic habitats were covered by a brownish deposit. BSF in soft bottom areas, where sea pens were previously recorded, were entirely gone (Supplementary materials 2 and 3). Empty shells of *Pinna* spp. could be observed at almost all sites (Figure 2B and Supplementary material 3). All alcyonarian species were absent after the event. A few colonies of *Paralcyonium spinulosum*, a very common species in the Prince Islands area (Topçu & Öztürk, 2013, 2015), could be found at depths below 32 m near Balıkçı Island. Most gorgonians were dead, but some colonies persisted at some sites (Figure 2, see the section below). Some hexacoral species, such as *Sagartia* spp., *Parazoanthus axinellae* and *Savalia savaglia* were still alive. *Savalia savaglia* colonies showed clear signs of lesions and were covered by a white soft mat which turned brown in October and then disappeared (Figure 3). Almost all massive sponges were absent, sometimes leaving a whitish mat behind (Figure 3). Sponges encrusting beneath other organisms were more abundant. *Raspailia* (*Parasyringella*) *agnata* (Topsent, 1896) and *Stelligera stuposa* (Ellis & Solander, 1786) which are common in the area (Topaloğlu, 2016; Topaloğlu *et al.*, 2016) were the only erect sponges remaining but were showing signs of necrosis (Figure 3). Abundances of tolerant hydrozoans such as *Obelia dichotoma* and *Bougainvillia muscus* had greatly increased whereas other hydrozoans were absent or less prevalent (Topçu *et al.*, 2016). No echinoderms were observed in the area after the sediment deposition event.

Quantitative observations

Considering all gorgonian species together, the percentage of colonies with completely denuded skeleton varied among sites from 15.8 to 100.0% and was 65.9 ± 33.8% (mean ± SD) for the Prince Islands area (Figure 4A, C). The percentage of necrosed colonies varied from 0 to 47.1% at sites and was 15.2 ± 16.2% (mean ± SD) for the Prince Islands area, while that of healthy colonies varied from 0 to 59.2% at sites and was 19.0 ± 23.9% (mean ± SD) for the Prince Islands area. Densities of affected and healthy colonies were significantly different between the two depth layers (26 and 35 m) at Yassıada and Balıkçı Islands ($\chi^2 = 291.1$, $P < 0.0001$; $\chi^2 = 42.96$, $P < 0.0001$ respectively). However, for all sites considered together, no significant correlation could be found between the percentage of impacted colonies and the depth or the degree of slope at sites ($r = -0.34$ $P = 0.25$; $r = -0.33$, $P = 0.29$ respectively). Among gorgonians, *P. macrospina* was the most affected species, followed by *S. klavereni*, *P. clavata* and *E. cavolini* (Figure 4B). The percentage of dead colonies in the population of *S. klavereni* after the mortality event increased by 28-fold (Figure 5A). The percentages of recent necrosis on colonies were significantly different before and after the mortality event (Kolmogorov–Smirnov test, $D = 0.96$, $P < 0.05$; Figure 5B). The percentages of old necrosis on colonies were not significantly different before and after the mortality (Kolmogorov–Smirnov test, $D = 0.37$, $P = 0.72$; Figure 5B).

Gross pathological and histopathological findings

External examination revealed that all diseased corals showed discolouration and some degree of tissue loss in the form of focal lesions. An unusual white coloured soft mat was observed on the surface of both diseased and dead colonies. Microscopic examination of this mat suggested that it was an abnormally thick multi-species biofilm containing bacteria and numerous fungal hyphae. HE-stained tissue sections of *S. savaglia* colonies revealed that both the epidermis and gastrodermis were necrotic (Figure 6A, B). Necrosis and exfoliation of mesentery were observed in tissues of diseased colonies (Figure 6C, D) along with sloughed cellular debris (Figure 6E, F).

Microbiological findings

Thirty-nine bacterial and nine fungal isolates were obtained from the diseased coral sample material. All bacterial isolates were observed as Gram-negative curved and motile rods. Three distinguishable isolate variants were recognized according to phenotypic and biochemical characteristics (Table 1). Fungal isolates had branching hyphae producing a filamentous structure, were able to reproduce at 37°C and produced sporangia. Analysis of sequences from bacterial genes (16S rRNA, *gyrB*) and the fungal ITS region in addition to the morphological and phenotypical characteristics of isolates from *Savalia savaglia* identified 13 bacterial isolates as *Vibrio splendidus* (acc. no. KY933309-10), 7 isolates as *Vibrio neptunius* (acc. no. KY933260-61-62) while all fungal isolates were identified as *Mucor circinelloides* (acc. no. KY933391-92). The remaining bacterial isolates obtained from coral species other than *S. savaglia* were identified as members of genus *Vibrio* (acc. no. MF287166-67-68), but could not be further assigned to species level. All obtained sequences showed >99% similarity to sequences in the GenBank database.

Environmental parameters

Dissolved oxygen at 40 m in the period 2000–2011 was 2.3 ± 0.8 mg l^{-1} (mean ± SD) (N = 27) and was generally above 1 mg l^{-1} except in August 2006 and then in 2015. Silicate concentration at 40 m in the period 2001–2009 was 23.52 ± 12.46 µM (mean ± SD) (N = 31) and was generally below 40 µM except in November 2003 and August 2016 (data from 2015 is absent). In August 2015, temperature, salinity and dissolved oxygen were 15.4°C, 38.3 and 0.8 mg l^{-1} at 40 m depth. In August 2016, ~2 months after the constructions had ended, temperature, salinity and dissolved oxygen were 15.5°C, 38.0, 1.43 mg l^{-1} at 40 m depth. The mean silicate concentration in surface waters in August 2016 for 73 stations over the Sea of Marmara was 2.35 µM (with a minimum of 0.017 and a maximum of 11.31 µM) while that for 10 stations along Prince Islands was 34.83 µM (with a minimum of 20.1 and a maximum of 76.16 µM).

Discussion

The effects of sedimentation on benthic suspension feeders

The significant decline of benthic macroorganisms that occurred in Prince Islands coasts appeared to be caused mainly by sediment

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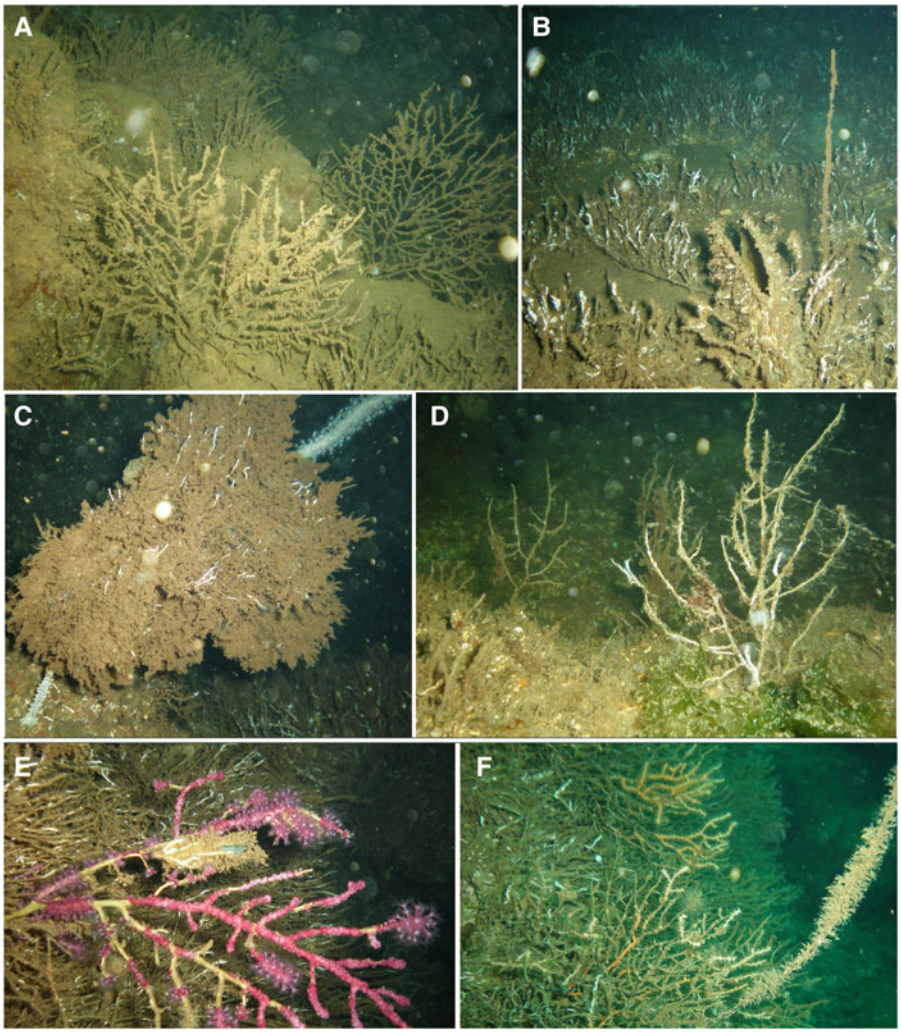

Fig. 2. Gorgonians affected by the mass mortality event: (A) *Eunicella cavolini* colonies that completely lost their tissues, covered with sediment and pioneer epibionts at Yassıada (S1); (B) a dead *Spinimuricea klavereni* colony covered with pioneer epibionts and a dead *Pinna* shell at S11; (C) a dead *Paramuricea macrospina* colony covered with pioneer epibionts and *E. cavolini* colonies at S2; (D) dead *P. macrospina* colonies at S8; (E) *P. clavata* colony showing loss of tissue (denuded axis parts covered by epibionts) at S9; (F) a dead and epibiont covered *S. klavereni* with *E. cavolini* colonies showing partial mortality at S3.

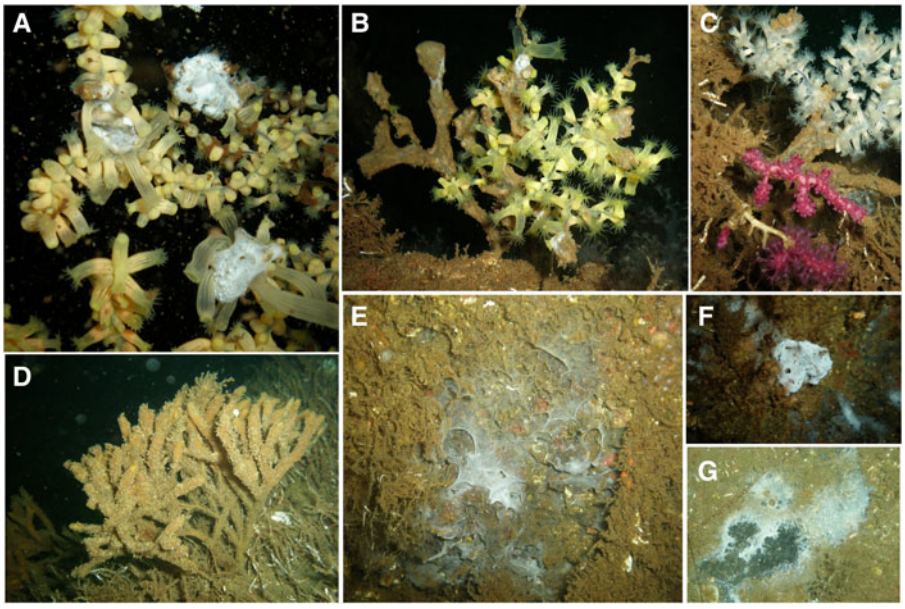

Fig. 3. (A) Colonies of the gold coral, *Savalia savaglia,* covered by a white soft mat (B, C) which turned brown in October at S6; (D) *Stelligera stuposa* sponge covered by epibionts at S8; (E–G) sponges that left a whitish mat behind at S10, S5 and S8.

deposits that resulted in the burial, smothering or clogging of feeding and/or respiratory organs. Burial and smothering by sediments is evidenced by the accumulated deposits on the sea bottom and on gorgonian branches (Supplementary material 4). Similar adverse effects on BSF have been reported following sediment dumping, coastal constructions or dredging operations in the Mediterranean Sea (Nepote *et al.*, 2017) and elsewhere in the world (Li *et al.*, 2013; Haywood *et al.*, 2016).

Several episodes of mass mortality involving mainly benthic suspension feeders have been recorded in the Mediterranean

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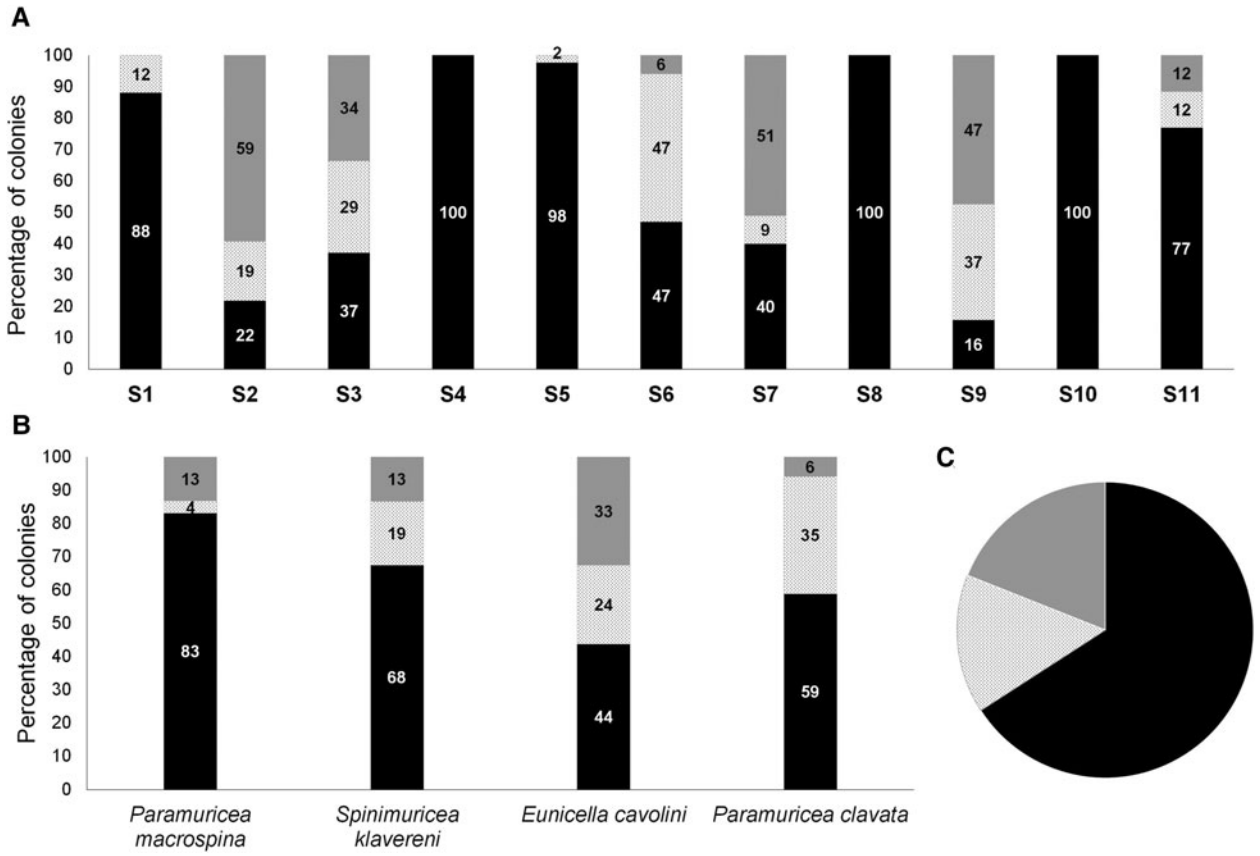

Fig. 4. (A) Percentages of entirely denuded, necrosed and healthy colonies at study sites. (B) Percentages of entirely denuded, necrosed and healthy colonies for each species at the study area. (C) Percentages of entirely denuded, necrosed and healthy colonies, all species combined at Prince Islands.

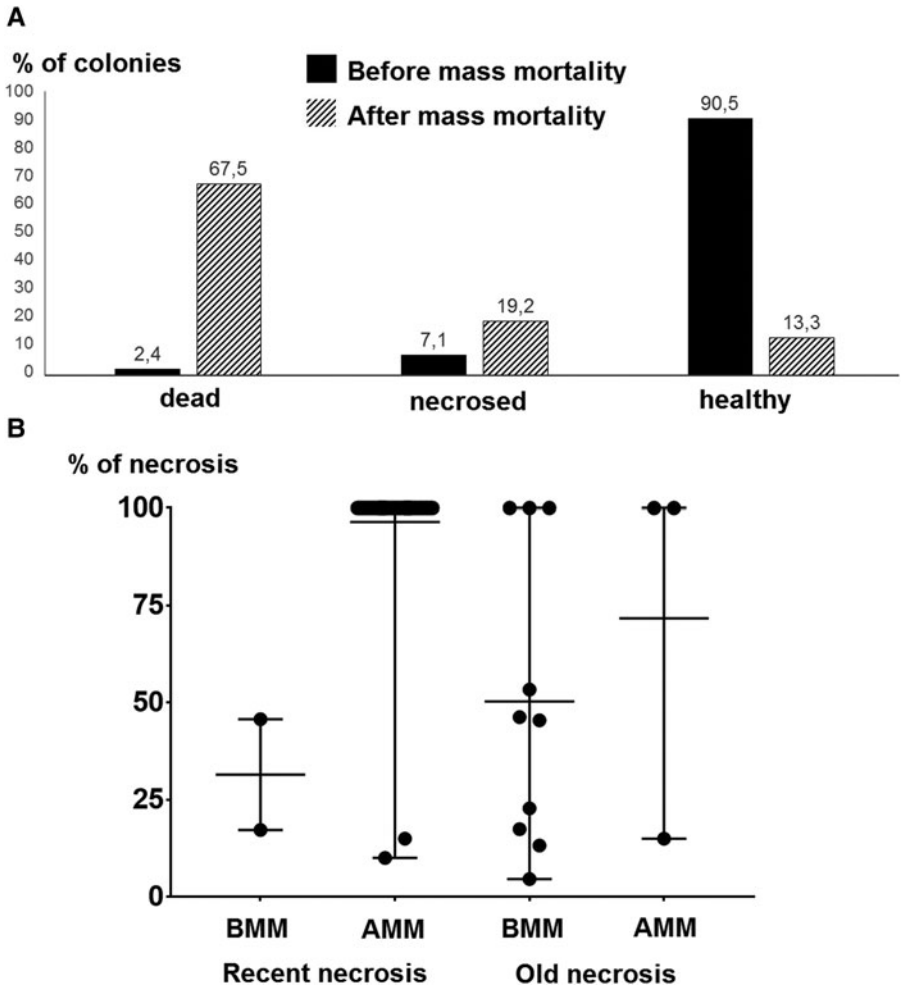

Fig. 5. (A) Percentages of entirely denuded, necrosed and healthy *Spinimuricea klavereni* colonies. (B) Percentages of necrosis (mean with range) for *S. klavereni* colonies; before mass mortality (BMM), after mass mortality (AMM).

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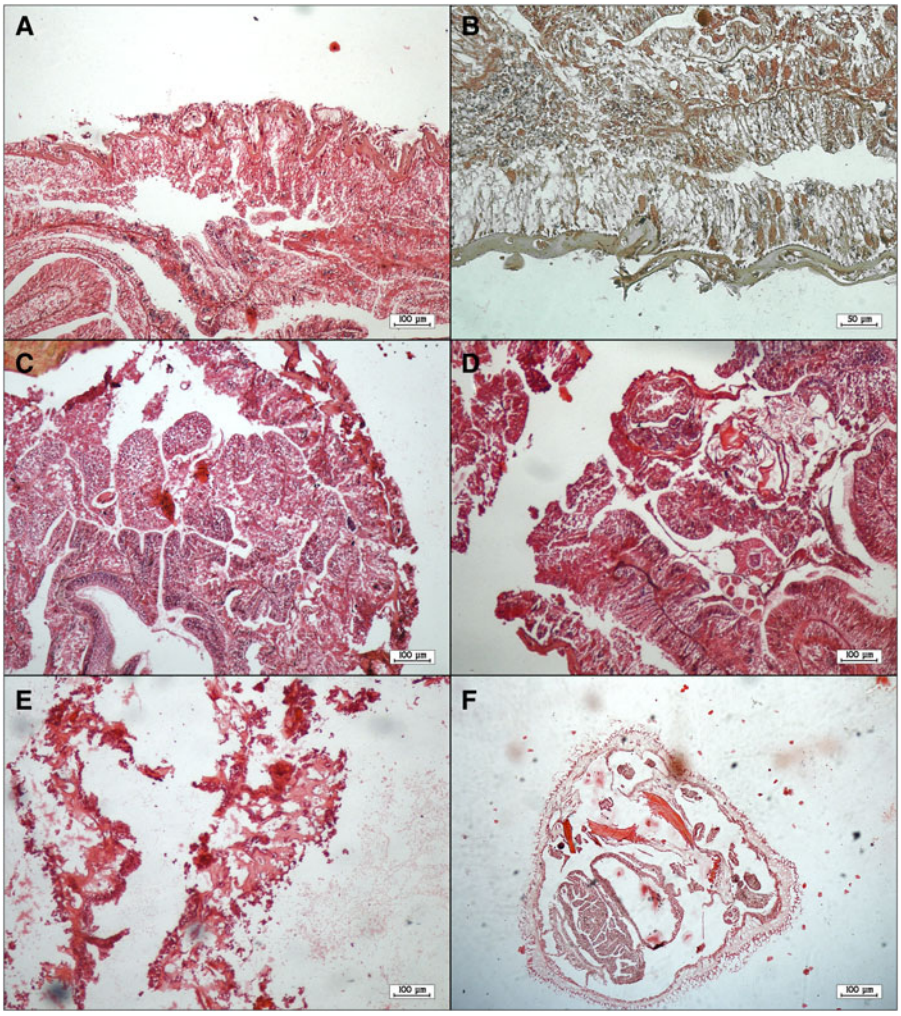

Fig. 6. Tissue sections of diseased colonies of *Savalia savaglia* staining with haematoxylin-eosin: necrotic epidermis and gastrodermis and sloughing of epithelium (A, B); necrosis and exfoliation of mesentery (C, D); sloughed cellular debris and denuded mesoglea (E, F).

Sea during recent decades (Cerrano *et al.*, 2000; Garrabou *et al.*, 2009; Rubio-Portillo *et al.*, 2016). These mortality events coincided with thermal anomalies in seawater temperature that have been causally linked with observed invertebrate mass mortalities (Rivetti *et al.*, 2014, 2017). In the Sea of Marmara, the seawater temperature in the lower layer which would be experienced by the BSF studied here varies between 13.1 and 16.6°C according to monthly long-term data (Kayisoglu, 2016). Because most anthozoans and sponges in the Mediterranean Sea have an upper limit of thermal tolerance around 24°C (Garrabou *et al.*, 2013) temperature thus appears not to be a cause of the mass mortality reported here. Overall, it is possible to exclude additive effects deriving from global climate change on this event.

Nevertheless, low oxygen levels may have contributed to mortality. Oxygen levels in the lower layer of Prince Islands coast are low in general, being around 1–2 mg l^{-1}. In fact, the lowest oxygen levels recorded during the last 16 years coincided with the reported BSF die-off. These low oxygen conditions might have become even more severe after the heavy sedimentation and caused extreme stress in suspension feeder invertebrates as explained further in the second part of the discussion. Before the observation of massive die-off in late summer 2015, several events that might have increased sedimentation occurred simultaneously in the Prince Islands area. Extensive red tide events developed in May 2015 and lasted for months (Türkoğlu, 2016). In July–August 2015, during the Kurbağalıdere stream rehabilitation process, dredged stream sediments were transported by ships and dumped near Yassıada and Sivriada. This dumping operation was confirmed by media coverage and there were rumours of stream mud dumping taking place near Büyükada (Kemiklidere). In addition, several building constructions and landfill operations began at Yassıada during May 2015 (Figure 7A, B). An aerial picture during construction clearly shows the large amount of terrestrial sediments spreading from the coasts of Yassıada in surrounding seawater (Figure 7D). Although there is no silicate data related to the construction work, it is reasonable to attribute high concentrations in both surface waters and at 40 m depth to these human activities on Yassıada. Such silicates in the seawater are always of terrestrial origin. Surface seawater in particular had almost 15 times higher concentrations of silicates, than other locations in the Sea of Marmara, which clearly illustrate the scale of input from Yassıada. Indeed, three separate sources have likely contributed to the excessive sedimentation in the study area (red-tides, stream sediment dumping and constructions/land filling) and their cumulative effect might have rendered BSF communities even more vulnerable to successive damages.

The degree of damage to benthic macroorganisms by heavy sedimentation in various locations of Prince Islands may have depended on several factors acting simultaneously, including species composition, distance to the construction and dumping sites, local currents, the slope and depth of affected sea bottom areas. Sivriada, despite being located very close to the main construction site, was less affected by heavy sedimentation in comparison to other sites probably because the rocky substrates are characterized by vertical walls or a steeply sloping seabed. In fact, vertical

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Table 1. Morphological and phenotypic characteristics of the bacterial isolates obtained from diseased corals

Characteristics	*Vibrio neptunius*	*Vibrio splendidus*	*Vibrio* sp.
Morphology	R	R	R
Motility	+	+	+
Gram staining	–	–	–
Catalase	+	+	+
Cytochrome oxidase	+	+	+
O/129 resistance (150 µg)	S	S	S
Growth on TCBS agar	G	Y	G
O/F	NS	F	F
Indole	–	–	–
Voges Proskauer reaction	–	–	–
Methyl red	–	–	–
Nitrate reduction	+	+	+
Arginine dihydrolase	–	+	–
Lysine decarboxylase	–	–	–
Ornithine decarboxylase	–	–	–
Citrate	–	–	–
Degradation of urea	–	–	–
H_2S production	–	–	–
ONPG	+	+	+
MacConkey	–	–	–
Glucose	+	+	+
Sucrose	–	+	–
Maltose	–	+	–
Lactose	–	–	–
Arabinose	–	–	–

R, rods; –, negative; +, positive; F, fermentative; G, green; NS, non-saccharolytic; S, sensitive; Y, yellow; TCBS, Thiosulphate-Citrate-Bile-Sucrose; O/F, Oxidative/Fermentative test.

substrates do not allow sediment deposition and the communities are less affected with respect to an area with more levelled sea bottom (Nepote *et al.*, 2016).

Soft corals (mainly *Paralcyonium spinulosum*), *Paramuricea macrospina* and *Spinimuricea klavereni* were the least tolerant and the first to respond to heavy sedimentation, exhibiting rapid mortality. Partially alive or healthy colonies of *Eunicella cavolini* were encountered in some locations where other gorgonians were dead (Supplementary material 3). The rapid mortality of *S. klavereni* and *P. macrospina* was surprising because these species are considered to be more tolerant to sedimentation due to their higher abundance on horizontal seafloors (Bo *et al.*, 2010; Topçu & Öztürk, 2016*a*). Excessive sediment deposits seem to have surpassed their tolerance limit.

Four gorgonians encountered in the region all have different life history traits and their recovery patterns might proceed distinctly in space and time. *Spinimuricea klavereni*, for example, grows relatively fast and has a high reproductive output throughout the year in the Sea of Marmara (Topçu & Öztürk, 2016*a*, 2016*b*). The confamilial gorgonian *P. clavata* is, on the other hand, a long-lived, slow-growing species, with high reproductive output but low recruitment (Coma *et al.*, 1995; Linares *et al.*, 2008*b*). Despite slow dynamics, in the north-western Mediterranean Sea where they have been decimated by mass mortalities, populations of *P. clavata* seem to show a recovering trend (Cerrano *et al.*, 2005; Cupido *et al.*, 2008) but not everywhere (Bonhomme *et al.*, 2003; Linares *et al.*, 2008*c*). On the positive side, a recovery trend in populations of two octocorals with distinct life history traits, *P. clavata* and *Corallium rubrum* (Linnaeus, 1758), was observed even after a drastic mortality increase off the Italian coast (Santangelo *et al.*, 2015). The conditions of the Sea of Marmara are very different from the north-western Mediterranean Sea and recovery patterns of the gorgonians may proceed faster at the former location, supported by the mesotrophic-eutrophic level of productivity, if anthropogenic sources of sedimentation and other potential disturbances are successfully discontinued.

Histopathological and microbial findings in/on diseased gorgonians and gold corals

Although the link between anthropogenic stressors and disease susceptibility are poorly understood and the exact mechanisms remain unknown, coral diseases associated with water pollution and anthropogenic activities, such as increases in nutrient runoff (Bruno *et al.*, 2003), sewage inputs (Pastorok & Bilyard, 1985; Coles & Ruddy, 1995) and dredging (Sheppard *et al.*, 2010; Hanley, 2011), have been reported from all over the world. To date, more than 20 distinct coral diseases have been described (Sheridan *et al.*, 2013), however, primary pathogens have been reported in only a few cases (Peters, 2015). Instead, most cases have been attributed to infections by opportunistic microorganisms in corals where the immune system has been weakened by a variety of stress factors (Vezzulli *et al.*, 2013; Peters, 2015).

The gross pathological and histopathological findings we observed such as tissue loss, discolouration and necrosis of mesentery and sloughed cellular debris are similar to previous reports on *Vibrio*-related disease outbreaks in other coral species (Kushmaro *et al.*, 2001; Ben-Haim *et al.*, 2003; Raymundo *et al.*, 2003; Luna *et al.*, 2007). The family Vibrionaceae contains Gram-negative facultative anaerobic organisms that are ubiquitous in marine environments. As previously reported, some vibrios can even be associated with healthy tissues of common Mediterranean gorgonians that under extraordinary conditions act as a triggering mechanism of mass mortality events in the coastal NW Mediterranean Sea (Vezzulli *et al.*, 2010). Although members are found in the normal microbiota of numerous organisms (Thompson *et al.*, 2004) including corals (Krediet *et al.*, 2013), the Vibrionaceae contains highly pathogenic species for many marine organisms (Thompson *et al.*, 2004; Stabili *et al.*, 2012; Blanquer *et al.*, 2016). To date, members of genus *Vibrio* associated with coral mortalities, in corals other than *Savalia savaglia*, include *Vibrio shiloi* (*V. mediterranei*) (Kushmaro *et al.*, 2001; Rubio-Portillo *et al.*, 2016), *Vibrio coralliilyticus* (Ben-Haim *et al.*, 2003; Bally & Garrabou, 2007; Vezzulli *et al.*, 2010; Huete-Stauffer *et al.*, 2011), *Vibrio harveyi* (Luna *et al.*, 2010), *Vibrio owensii* (Ushijima *et al.*, 2012) but also unidentified members of genus *Vibrio* (Cervino *et al.*, 2004). Both *Vibrio splendidus* and *Vibrio neptunius*, isolated from diseased corals in the present study, have been shown to cause disease in a variety of organisms such as clams (Gómez-León *et al.*, 2005), oysters (Lacoste *et al.*, 2001; Prado *et al.*, 2005), sea cucumbers (Zhao *et al.*, 2012), corals (Hall-Spencer *et al.*, 2007) and fish (Gatesoupe *et al.*, 1999; Austin *et al.*, 2005). However, to our knowledge, these bacteria have not been associated with or reported as contributing to disease in *Savalia savaglia* corals until now.

The development of an unusual white mat-like biofilm on holobiont surfaces may have been enabled by low oxygen levels leading to a compromised immune system in affected corals.

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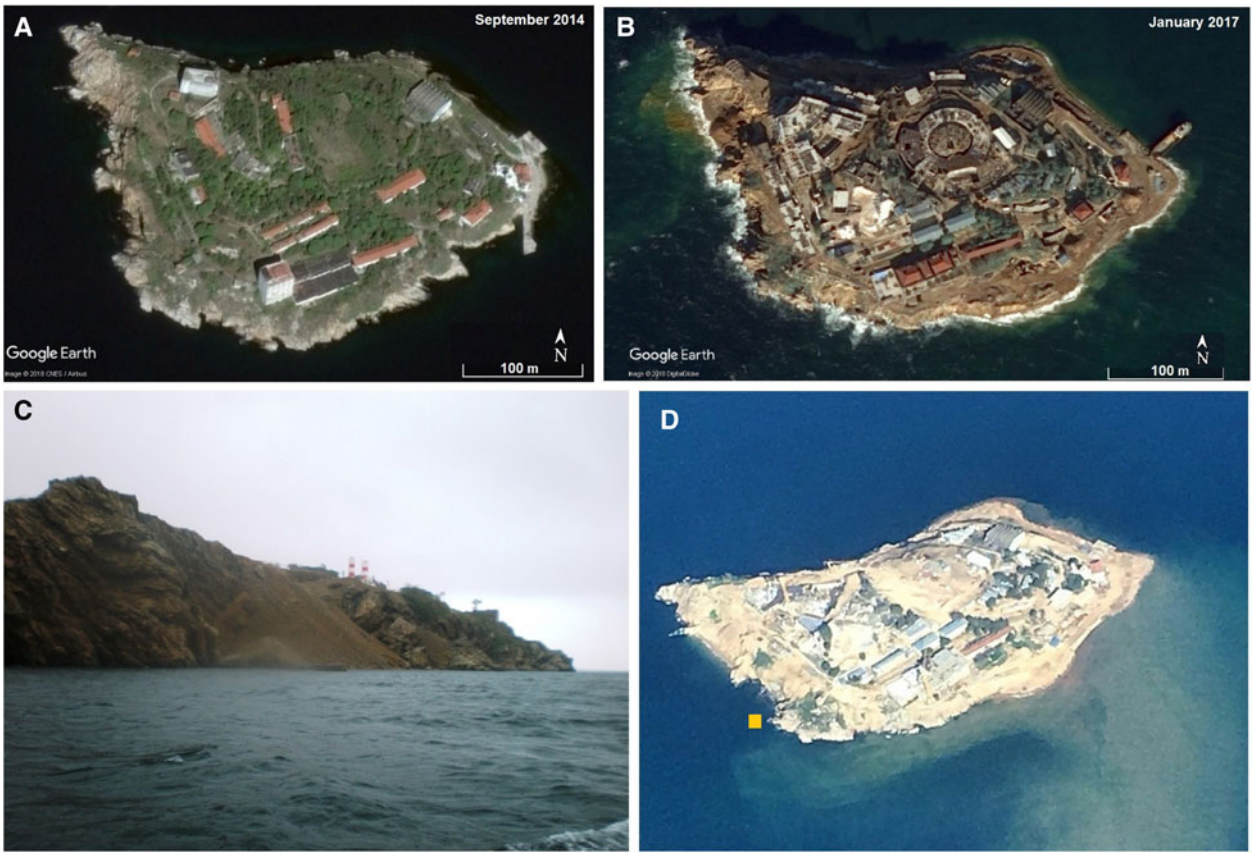

Fig. 7. Google Earth pictures of Yassıada (A) before and (B) after land filling operations. (C) Yassıada coast (S1) in October 2015 during fieldwork. (D) An aerial photo of Yassıada taken by a flight passenger in February 2016, photo credit: Levon Bağış.

Our microbial examination of affected corals also revealed the presence of a fungus identified as *Mucor circinelloides*. Like the two said *Vibrio* species, this fungus was consistently isolated also from coral mucous and soft tissues showing clear signs of necrosis. *Mucor circinelloides* is considered an opportunistic mould responsible for tissue necrosis in yellow catfish (Ke *et al.*, 2009) as well as cases of mucormycosis (previously called zygomycosis) in immunocompromised humans (Iwen *et al.*, 2007; Dizbay *et al.*, 2009; Karan *et al.*, 2014). According to the scientific literature, moulds in order Mucorales are ubiquitous in nature, but associated mostly with soil/terrestrial habitats, more specifically plants, processed food, dung, compost piles and decaying fruit (Richardson, 2009). It is therefore reasonable to assume that the fungus was artificially introduced to the marine environment and the affected corals either through the Prince Islands runoffs or with the off-shore dumping of highly polluted stream sediments. Our *Mucor circinelloides* isolates appear to be salt tolerant and able to grow in the marine environment like strains of a common soil fungus *Aspergillus sydowii*, an emerging opportunistic sea fan pathogen in the Caribbean (Smith *et al.*, 1996; Nagelkerken *et al.*, 1997; Geiser *et al.*, 1998). Artificial dissemination of pathogens or opportunistic microorganisms into new habitats, as a result of human activities, is a potential problem that should not be overlooked or underestimated. One would expect indigenous corals to be less resistant to infection and disease caused by such 'alien' microorganisms.

Conclusion

A significant decline of BSF, mainly comprising octocorals and sponges, was observed in the Prince Islands region of the northeastern Sea of Marmara and likely caused primarily by heavy sedimentation. The bulk of observed sedimentation was clearly a direct result of the construction and landfill operations that took place at Yassıada. BSF suffered from burial, clogging or smothering of feeding and respiratory apparatus, and necrosis on body surfaces. However, numerous bacteria belonging to genus *Vibrio* and an opportunistic mould *Mucor circinelloides*, whose presence most likely was the result of either off-shore dumping of polluted stream mud or landfill run-offs, were consistently isolated from an unusual white mat-like biofilm covering the surface of diseased corals as well as from necrotic soft tissues. This overgrowth of opportunistic microorganisms may have been made possible by low oxygen levels leading to an impaired immune response in highly stressed corals and thus exacerbated the described BSF die-off. These results suggest that preventive measures are necessary when construction operations occur in the vicinity of sensitive marine habitats.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0025315418001066.

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References

Austin B, Austin D, Sutherland R, Thompson F and Swings J (2005) Pathogenicity of vibrios to rainbow trout (*Oncorhynchus mykiss*, Walbaum) and *Artemia* nauplii. *Environmental Microbiology* **7**, 1488–1495.

Ballesteros E (2006) Mediterranean coralligenous assemblages: a synthesis of present knowledge. *Oceanography and Marine Biology* **44**, 123–195.

Bally M and Garrabou J (2007) Thermodependent bacterial pathogens and mass mortalities in temperate benthic communities: a new case of emerging disease linked to climate change. *Global Change Biology* **13**, 2078–2088.

Bell JJ, McGrath E, Biggerstaff A, Bates T, Bennett H, Marlow J and Shaffer M (2015) Sediment impacts on marine sponges. *Marine Pollution Bulletin* **94**, 5–13.

Ben-Haim Y and Rosenberg E (2002) A novel *Vibrio* sp. pathogen of the coral *Pocillopora damicornis*. *Marine Biology* **141**, 47–55.

Ben-Haim Y, Thompson F, Thompson C, Cnockaert M, Hoste B, Swings J and Rosenberg E (2003) *Vibrio coralliilyticus* sp. nov., a temperature-dependent pathogen of the coral *Pocillopora damicornis*. *International Journal of Systematic and Evolutionary Microbiology* **53**, 309–315.

Bertolino M, Cerrano C, Bavestrello G, Carella M, Pansini M and Calcinai B (2013) Diversity of Porifera in the Mediterranean coralligenous accretions, with description of a new species. *ZooKeys* **336**, 1–37.

Beşiktepe ŞT, Sur Hİ, Özsoy E, Latif MA, Oğuz T and Ünlüata Ü (1994) The circulation and hydrography of the Marmara Sea. *Progress in Oceanography* **34**, 285–334.

Blanquer A, Uriz MJ, Cebrian E and Galand PE (2016) Snapshot of a bacterial microbiome shift during the early symptoms of a massive sponge die-off in the Western Mediterranean. *Frontiers in Microbiology* **7**, 1–10.

Bo M, Bavestrello G, Canese S, Giusti M, Angiolillo M, Cerrano C, Salvati E and Greco S (2010) Coral assemblage off the Calabrian Coast (South Italy) with new observations on living colonies of *Antipathes dichotoma*. *Italian Journal of Zoology* **78**, 231–242.

Bonhomme D, Garrabou J, Pérez T, Sartoretto S and Harmelin J (2003) Impact and recovery from a mass mortality event of the gorgonian *Paramuricea clavata* populations on the French Mediterranean coasts. *Geophysical Research Abstracts* **5**, 10676.

Bruno JF, Petes LE, Drew Harvell C and Hettinger A (2003) Nutrient enrichment can increase the severity of coral diseases. *Ecology Letters* **6**, 1056–1061.

Burke L, Reytar K, Spalding M and Perry A (2011) *Reefs at Risk Revisited*. Washington, DC: World Resources Institute.

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

Cerrano C, Arillo A, Azzini F, Calcinai B, Castellano L, Muti C, Valisano L, Zega G and Bavestrello G (2005) Gorgonian population recovery after a mass mortality event. *Aquatic Conservation: Marine and Freshwater Ecosystems* **15**, 147–157.

Cervino JM, Hayes RL, Polson SW, Polson SC, Goreau TJ, Martinez RJ and Smith GW (2004) Relationship of *Vibrio* species infection and elevated temperatures to yellow blotch/band disease in Caribbean corals. *Applied and Environmental Microbiology* **70**, 6855–6864.

Coles SL and Ruddy L (1995) Comparison of water quality and reef coral mortality and growth in southeastern Kane'ohe Bay, O'ahu, Hawai'i, 1990 to 1992, with conditions before sewage diversion. *Pacific Science* **49**, 247–265.

Coma R, Ribes M, Zabala M and Gili J-M (1995) Reproduction and cycle of gonadal development in the Mediterranean gorgonian *Paramuricea clavata*. *Marine Ecology Progress Series* **117**, 173–183.

Coma R, Linares C, Ribes M, Diaz D, Garrabou J and Ballesteros E (2006) Consequences of a mass mortality in populations of *Eunicella singularis* (Cnidaria: Octocorallia) in Menorca (NW Mediterranean). *Marine Ecology Progress Series* **327**, 51–60.

Culling CFA (1963) *Handbook of Histopathological Techniques*, 2nd edition. London: Butterworth and Co.

Cupido R, Cocito S, Sgorbini S, Bordone A and Santangelo G (2008) Response of a gorgonian (*Paramuricea clavata*) population to mortality events: recovery or loss? *Aquatic Conservation: Marine and Freshwater Ecosystems* **18**, 984–992.

Dizbay M, Adisen E, Kustimur S, Sari N, Cengiz B, Yalcin B, Kalkanci A, Gonul II and Sugita T (2009) Fungemia and cutaneous zygomycosis due to *Mucor circinelloides* in an intensive care unit patient: case report and review of literature. *Japanese Journal of Infectious Diseases* **62**, 146–148.

Erftemeijer PLA, Riegl B, Hoeksema BW and Todd PA (2012) Environmental impacts of dredging and other sediment disturbances on corals: a review. *Marine Pollution Bulletin* **64**, 1737–1765.

Fabricius KE (2005) Effects of terrestrial runoff on the ecology of corals and coral reefs: review and synthesis. *Marine Pollution Bulletin* **50**, 125–146.

Fabricius KE, Golbuu Y and Victor S (2007) Selective mortality in coastal reef organisms from an acute sedimentation event. *Coral Reefs* **26**, 69.

Garrabou J, Bensoussan N, Pairaud I, Garreau P, Somot S, Linares C, Kersting D, Cebrian D, de Caralt S, Kipson S, Ledoux JB and Frleta-Valić M (2013) Assessing climate change impacts on marine biodiversity conservation: the case study of mass mortality events in the NW Mediterranean basin. *CLIMCARES CLimate Impacts on Mediterranean Coastal AREaS Project Final report*, June 2013, 49 pp.

Garrabou J, Coma R, Bensoussan N, Bally M, Chevaldonné P, Cigliano M, Diaz D, Harmelin JG, Gambi MC, Kersting DK, Ledoux JB, Lejeusne C, Linares C, Marschal C, Pérez T, Ribes M, Romano JC, Serrano E, Teixido N, Torrents O, Zabala M, Zuberer F and Cerrano C (2009) Mass mortality in Northwestern Mediterranean rocky benthic communities: effects of the 2003 heat wave. *Global Change Biology* **15**, 1090–1103.

Gatesoupe F-J, Lambert C and Nicolas J-L (1999) Pathogenicity of *Vibrio splendidus* strains associated with turbot larvae, *Scophthalmus maximus*. *Journal of Applied Microbiology* **87**, 757–763.

Geiser DM, Taylor JW, Ritchie KB and Smith GW (1998) Cause of sea fan death in the West Indies. *Nature* **394**, 137–138.

Gómez-León J, Villamil L, Lemos M, Novoa B and Figueras A (2005) Isolation of *Vibrio alginolyticus* and *Vibrio splendidus* from aquacultured carpet shell clam (*Ruditapes decussatus*) larvae associated with mass mortalities. *Applied and Environmental Microbiology* **71**, 98–104.

Hall TA (1999) Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Proceedings of the Nucleic Acids Symposium Series* **41**, 95–98.

Hall-Spencer JM, Pike J and Munn CB (2007) Diseases affect cold-water corals too: *Eunicella verrucosa* (Cnidaria: Gorgonacea) necrosis in SW England. *Diseases of Aquatic Organisms* **76**, 87–97.

Hanley J (2011) Environmental monitoring programs on recent capital dredging projects in the Pilbara (2003–10): a review. *Australian Petroleum Production & Exploration Association (APPEA)* **51**, 273–294.

Harvell CD, Mitchell CE, Ward JR, Altizer S, Dobson AP, Ostfeld RS and Samuel MD (2002) Climate warming and disease risks for terrestrial and marine biota. *Science* **296**, 2158–2162.

Haywood MDE, Dennis D, Thomson DP and Pillans RD (2016) Mine waste disposal leads to lower coral cover, reduced species richness and a predominance of simple coral growth forms on a fringing coral reef in Papua New Guinea. *Marine Environmental Research* **115**, 36–48.

Hendrick VJ, Hutchison ZL and Last KS (2016) Sediment burial intolerance of marine macroinvertebrates. *PLoS ONE* **11**, e0149114.

Huete-Stauffer C, Vielmini I, Palma M, Navone A, Panzalis P, Vezzulli L, Misic C and Cerrano C (2011) *Paramuricea clavata* (Anthozoa, Octocorallia) loss in the Marine Protected Area of Tavolara (Sardinia, Italy) due to a mass mortality event. *Marine Ecology* **32**(s1), 107–116.

Iwen PC, Sigler L, Noel RK and Freifeld AG (2007) Mucor circinelloides was identified by molecular methods as a cause of primary cutaneous zygomycosis. *Journal of Clinical Microbiology* **45**, 636–640.

Karan MA, Tunçcan ÖG, Kalkancı A, Arman D and Kuştimur S (2014) Widespread colonization of *Mucor circinelloides* in a patient with type 2 diabetes and colon cancer: case report. *Turkiye Klinikleri Journal of Case Reports* **22**, 186–190.

Kayisoglu M (2016) Investigation of the Interaction Between the Black Sea and the Sea of Marmara with Monthly Data Series of Temperature and Salinity (Master thesis). Istanbul University Institute of Marine Sciences and Management, Turkey.

Ke X, Wang J, Li M, Gu Z and Gong X (2009) First report of *Mucor circinelloides* occurring on yellow catfish (*Pelteobagrus fulvidraco*) from China. *FEMS Microbiology Letters* **302**, 144–150.

Krediet CJ, Ritchie KB, Paul VJ and Teplitski M (2013) Coral-associated micro-organisms and their roles in promoting coral health and thwarting diseases. *Proceedings of the Royal Society of London B: Biological Sciences* **280**, 20122328.

Kurt S, Karaburun A and Demirci A (2010) Coastline changes in Istanbul between 1987 and 2007. *Scientific Research and Essays* **5**, 3009–3017.

Kushmaro A, Banin E, Loya Y, Stackebrandt E and Rosenberg E (2001) *Vibrio shiloi* sp. nov., the causative agent of bleaching of the coral

https://doi.org/10.1017/S0025315418001066 Published online by Cambridge University Press

Oculina patagonica. *International Journal of Systematic and Evolutionary Microbiology* **51**, 1383–1388.

Lacoste A, Jalabert F, Malham S, Cueff A, Gelebart F, Cordevant C, Lange M and Poulet S (2001) A *Vibrio splendidus* strain is associated with summer mortality of juvenile oysters *Crassostrea gigas* in the Bay of Morlaix (North Brittany, France). *Diseases of Aquatic Organisms* **46**, 139–145.

Larkin MA, Blackshields G, Brown N, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A and Lopez R (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* **23**, 2947–2948.

Li X, Huang H, Lian J, Yang J, Ye C, Chen Y and Huang L (2013) Coral community changes in response to a high sedimentation event: a case study in southern Hainan Island. *Chinese Science Bulletin* **58**, 1028–1037.

Linares C, Coma R, Diaz D, Zabala M, Hereu B and Dantart L (2005) Immediate and delayed effects of a mass mortality event on gorgonian population dynamics and benthic community structure in the NW Mediterranean Sea. *Marine Ecology Progress Series* **305**, 127–137.

Linares C, Coma R, Garrabou J, Diaz D and Zabala M (2008*a*) Size distribution, density and disturbance of two Mediterranean gorgonians: *Paramuricea clavata* and *Eunicella singularis*. *Journal of Applied Ecology* **45**, 688–699.

Linares C, Coma R, Mariani S, Díaz D, Hereu B and Zabala M (2008*b*) Early life history of the Mediterranean gorgonian *Paramuricea clavata*: implications for population dynamics. *Invertebrate Biology* **127**, 1–11.

Linares C, Coma R and Zabala M (2008*c*) Effects of a mass mortality event on gorgonian reproduction. *Coral Reefs* **27**, 27–34.

Lohrer A, Hewitt J, Thrush S, Lundquist C, Nicholls P and Liefting R (2003) *Impact of Terrigenous Material Deposition on Subtidal Benthic Communities*. Hamilton: Auckland Regional Council, Technical Publication No. 217.

Luna G, Biavasco F and Danovaro R (2007) Bacteria associated with the rapid tissue necrosis of stony corals. *Environmental Microbiology* **9**, 1851–1857.

Luna GM, Bongiorni L, Gili C, Biavasco F and Danovaro R (2010) *Vibrio harveyi* as a causative agent of the White Syndrome in tropical stony corals. *Environmental Microbiology Reports* **2**, 120–127.

Maina J, de Moel H, Zinke J, Madin J, McClanahan T and Vermaat JE (2013) Human deforestation outweighs future climate change impacts of sedimentation on coral reefs. *Nature Communications* **4**, 1986.

Nagelkerken I, Buchan K, Smith G, Bonair K, Bush P, Garzon-Ferreira J, Botero L, Gayle P, Harvell C and Heberer C (1997) Widespread disease in Caribbean sea fans: II. Patterns of infection and tissue loss. *Marine Ecology Progress Series* **160**, 255–263.

Nepote E, Bianchi CN, Morri C, Chiantore M and Montefalcone M (2016) Pattern and intensity of human impact on coral reefs depend on depth along the reef profile and on the descriptor adopted. *Estuarine Coastal and Shelf Science* **178**, 86–91.

Nepote E, Bianchi CN, Morri C, Ferrari M and Montefalcone M (2017) Impact of a harbour construction on the benthic community of two shallow marine caves. *Marine Pollution Bulletin* **114**, 35–45.

Özsoy E, Çağatay MN, Balkıs N, Balkıs N and Öztürk B (eds) (2016) *The Sea of Marmara; Marine Biodiversity, Fisheries, Conservation and Governance*. Istanbul: Turkish Marine Research Foundation (TUDAV). Publication No: 42.

Pascual J, Macián MC, Arahal DR, Garay E and Pujalte MJ (2010) Multilocus sequence analysis of the central clade of the genus *Vibrio* by using the 16S rRNA, recA, pyrH, rpoD, gyrB, rctB and toxR genes. *International Journal of Systematic and Evolutionary Microbiology* **60**, 154–165.

Pastorok RA and Bilyard GR (1985) Effects of sewage pollution on coral-reef communities. *Marine Ecology Progress Series. Oldendorf* **21**, 175–189.

Perez T, Garrabou J, Sartoretto S, Harmelin J-G, Francour P and Vacelet J (2000) Mortalité massive d'invertébrés marins : un événement sans précédent en Méditerranée nord-occidentale. *Comptes Rendus de l'Académie des Sciences – Series III – Sciences de la Vie* **323**, 853–865.

Peters EC (2015) Diseases of coral reef organisms. In Birkeland C. (ed.), *Coral Reefs in the Anthropocene*. Dordrecht: Springer, pp 147–178.

Ponti M, Perlini RA, Ventra V, Grech D, Abbiati M and Cerrano C (2014) Ecological shifts in Mediterranean coralligenous assemblages related to gorgonian forest loss. *PLoS ONE* **9**, e102782.

Prado S, Romalde JL, Montes J and Barja JL (2005) Pathogenic bacteria isolated from disease outbreaks in shellfish hatcheries. First description of *Vibrio neptunius* as an oyster pathogen. *Diseases of Aquatic Organisms* **67**, 209–215.

Raymundo L, Couch C and Harvell CD (eds) (2008) *Coral Disease Handbook: Guidelines for Assessment, Monitoring and Management*. Melbourne: Currie Communications.

Richardson M (2009) The ecology of the Zygomycetes and its impact on environmental exposure. *Clinical Microbiology and Infection* **15**(s5), 2–9.

Rivetti I, Fraschetti S, Lionello P, Zambianchi E and Boero F (2014) Global warming and mass mortalities of benthic invertebrates in the Mediterranean Sea. *PLoS ONE* **9**, e115655.

Rivetti I, Boero F, Fraschetti S, Zambianchi E and Lionello P (2017) Anomalies of the upper water column in the Mediterranean Sea. *Global and Planetary Change* **151**, 68–79.

Rubio-Portillo E, Izquierdo-Muñoz A, Gago JF, Rosselló-Mora R, Antón J and Ramos-Esplá AA (2016) Effects of the 2015 heat wave on benthic invertebrates in the Tabarca Marine Protected Area (southeast Spain). *Marine Environmental Research* **122**, 135–142.

Santangelo G, Cupido R, Cocito S, Bramanti L, Priori C, Erra F and Iannelli M (2015) Effects of increased mortality on gorgonian corals (Cnidaria, Octocorallia): different demographic features may lead affected populations to unexpected recovery and new equilibrium points. *Hydrobiologia* **759**, 171–187.

Santos SR and Ochman H (2004) Identification and phylogenetic sorting of bacterial lineages with universally conserved genes and proteins. *Environmental Microbiology* **6**, 754–759.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Bolchacova E, Voigt K and Crous PW (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences USA* **109**, 6241–6246.

Shahidul Islam M and Tanaka M (2004) Impacts of pollution on coastal and marine ecosystems including coastal and marine fisheries and approach for management: a review and synthesis. *Marine Pollution Bulletin* **48**, 624–649.

Sheppard C, Al-Husiani M, Al-Jamali F, Al-Yamani F, Baldwin R, Bishop J, Benzoni F, Dutrieux E, Dulvy NK and Durvasula SRV (2010) The Gulf: a young sea in decline. *Marine Pollution Bulletin* **60**, 13–38.

Sheridan C, Kramarsky-Winter E, Sweet M, Kushmaro A and Leal MC (2013) Diseases in coral aquaculture: causes, implications and preventions. *Aquaculture* **396**, 124–135.

Smith GW, Ives LD, Nagelkerken IA and Ritchie KB (1996) Caribbean sea-fan mortalities. *Nature* **383**, 487–487.

Stabili L, Cardone F, Alifano P, Tredici SM, Piraino S, Corriero G and Gaino E (2012) Epidemic mortality of the sponge *Ircinia variabilis* (Schmidt, 1862) associated to proliferation of a *Vibrio* bacterium. *Microbial Ecology* **64**, 802–813.

Suau A, Bonnet R, Sutren M, Godon J-J, Gibson GR, Collins MD and Doré J (1999) Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut. *Applied and Environmental Microbiology* **65**, 4799–4807.

Thompson FL, Iida T and Swings J (2004) Biodiversity of vibrios. *Microbiology and Molecular Biology Reviews* **68**, 403–431.

Thrush SF, Hewitt JE, Cummings VJ, Ellis JI, Hatton C, Lohrer A and Norkko A (2004) Muddy waters: elevating sediment input to coastal and estuarine habitats. *Frontiers in Ecology and the Environment* **2**, 299–306.

Topaloğlu B (2016) Sponges of the Sea of Marmara with a new record for Turkish sponge fauna. In Özsoy E, Çağatay MN, Balkıs N, Balkıs N and Öztürk B (eds), *The Sea of Marmara; Marine Biodiversity, Fisheries, Conservation and Governance*. Istanbul: Turkish Marine Research Foundation (TUDAV). Publication No. 42, pp. 418–450.

Topaloğlu B, Evcen A and Çınar ME (2016) Sponge fauna in the Sea of Marmara. *Turkish Journal of Fisheries and Aquatic Sciences* **16**, 51–59.

Topçu NE and Öztürk B (2013) Octocoral diversity of Balıkçı Island, the Marmara Sea. *Journal of the Black Sea/Mediterranean Environment* **19**, 46–57.

Topçu NE and Öztürk B (2015) Composition and abundance of octocorals in the Sea of Marmara, where the Mediterranean meets the Black Sea. *Scientia Marina* **79**, 125–135.

Topçu NE and Öztürk B (2016*a*) First insights into the demography of the rare gorgonian *Spinimuricea klavereni* in the Mediterranean Sea. *Marine Ecology* **37**, 1154–1160.

https://doi.org/10.1017/S0025315418001066 Published online by Cambridge University Press

Topçu NE and Öztürk B (2016*b*) Reproduction in the Mediterranean endemic gorgonian *Spinimuricea klavereni* (Anthozoa, Octocorallia, Plexauridae). *Invertebrate Biology* **135**, 13–19.

Topçu NE, Martell LF, Yılmaz İN and İşinibilir M (2016) Hydrozoans as pioneer colonizers after a mass mortality event in the Sea of Marmara. *Rapp. Comm. int. Mer Médit.* **41**, 369.

Turkoglu M (2016) Bloom dynamics of Emiliania Huxleyi (Lohmann) Hay & Mohler, 1967 in the Sea of Marmara: a review. In Thajuddin N and Dhanasekaran D (eds), *Algae, Organisms for Imminent Biotechnology*. IntechOpen. Published online 29 June 2016.

Ushijima B, Smith A, Aeby GS and Callahan SM (2012) *Vibrio owensii* induces the tissue loss disease Montipora white syndrome in the Hawaiian reef coral *Montipora capitata*. *PLoS ONE* **7**, e46717.

Vezzulli L, Previati M, Pruzzo C, Marchese A, Bourne DG and Cerrano C (2010) Vibrio infections triggering mass mortality events in a warming Mediterranean Sea. *Environmental Microbiology* **12**, 2007–2019.

Vezzulli L, Pezzati E, Huete-Stauffer C, Pruzzo C and Cerrano C (2013) 16SrDNA pyrosequencing of the Mediterranean gorgonian *Paramuricea clavata* reveals a link among alterations in bacterial holobiont members, anthropogenic influence and disease outbreaks. *PLoS ONE* **8**, e67745.

Ward JR and Lafferty KD (2004) The elusive baseline of marine disease: are diseases in ocean ecosystems increasing? *PLOS Biology* **2**, e120.

White TJ, Bruns T, Lee S and Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: a Guide to Methods and Applications* **18**, 315–322.

Zhang Z, Schwartz S, Wagner L and Miller W (2000) A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology* **7**, 203–214.

Zhao Y, Zhang W, Xu W, Mai K, Zhang Y and Liufu Z (2012) Effects of potential probiotic *Bacillus subtilis* T13 on growth, immunity and disease resistance against *Vibrio splendidus* infection in juvenile sea cucumber *Apostichopus japonicus*. *Fish and Shellfish Immunology* **32**, 750–755.

https://doi.org/10.1017/S0025315418001066 Published online by Cambridge University Press