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Pathology of tissue loss in three key gorgonian species in the Mediterranean Sea

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ABSTRACT

The Mediterranean is known for its marine biodiversity, especially gorgonian forests. Unfortunately, these are experiencing rapid declines due to climate change, manifested by repeated marine heat waves resulting in mass mortality events since the early 1990 s. To better understand why gorgonians are declining, more systematic approaches to investigate the exact causes are needed, and pathology may aid in this goal.

We described gross and microscopic pathology of tissue loss in three key gorgonian species in the Mediterranean region, *Paramuricea clavata*, *Eunicella cavolini*, and *Leptogorgia sarmentosa*, that were all experiencing various degrees of acute to subacute tissue loss characterized by exposed axial skeleton sometimes partly colonized by epibionts and thinning of adjacent tissues. The most significant variety of lesions was seen in *P. clavata* followed by *L. sarmentosa* and *E. cavolini.* For all species, dissociation of gastrodermal cells was the dominant microscopic lesion followed by necrosis of the gastrodermis. Ciliates invading gastrodermis and associated with necrosis of polyps were seen only in *E. cavolini.* Epidermal tissue loss was seen only in *L. sarmentosa*, while *P. clavata* was distinguished by a prominent inflammatory response and unidentified dark round structures within the tentacle epidermis and gastrodermis with no host response. Further work to understand the cause of death in gorgonians is needed, particularly to elucidate the role of ciliates and environmental co-factors or infectious agents not visible on light microscopy, as well as applications of additional tools such as cytology.

1. Introduction

The Mediterranean Sea is known for its incredible biodiversity notable by gorgonian forests, a complex underwater ecosystem that provides habitat for a significant number of associated organisms (Gori et al., 2017, 2019; Rossi et al., 2017). Unfortunately, marine ecosystems in the Mediterranean have been facing a significant decline in biodiversity and health status over the past decades due to a multitude of threats, such as human impact and pollution, global climate change, and ocean acidification (Jackson et al., 2001; Carpenter et al., 2008; Pandolfi et al., 2011; Rossi, 2013; Hughes et al., 2017; Bevilacqua et al., 2021). Among these threats, climate change stands out as a major contributor to the deterioration of the Mediterranean marine environment (Kim et al., 2019; Pisano et al., 2020; Garrabou et al., 2022). In the region, anthozoans dominate the benthos exemplified by gorgonian forests that are iconic members and ecosystem engineers of Mediterranean benthic communities creating dense monospecific assemblages covering large areas of the coast (Paoli et al., 2017; Gori et al., 2019). This ecosystem is threatened by marine heat waves that appear to be increasingly frequent over the past two decades, beginning with the significant heatwave of

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1999 (Cerrano et al., 2000; Rivetti et al., 2014; Garrabou et al., 2022). These heat waves expose gorgonians to prolonged thermal stress that, in turn, can trigger mass mortality with disrupting effects on entire marine ecosystems and significant implications for benthic communities (Garrabou et al., 2001; Crisci et al., 2011; Oliver et al., 2018; Darmaraki et al., 2019a, 2019b; Garrabou et al., 2019, 2022).

Despite extensive ecological research on the long-term patterns and mortality trends associated with climate change impacts on gorgonians, there remains a knowledge gap in understanding the biological processes responsible for the deterioration of their health and resultant mortality. This scarcity of studies may derive from the challenges in defining the disease etiology, progression, and transmission. Although many studies claim the identification of infectious causes of cnidarian disease (Rosenberg and Ben-Haim, 2002; Sutherland et al., 2004; Sweet and Séré, 2016; Montano et al., 2020), few of those are supported by morphologic evidence at the microscopy level (Work and Aeby, 2006; Work and Meteyer, 2014). Without such descriptions, it becomes difficult to accurately identify the causes of diseases or develop an understanding of disease pathogenesis (Work and Meteyer, 2014; Hawthorn et al., 2023). Therefore, comprehensive and systematic descriptions of lesions at both gross and microscopic levels are gaining importance (Work and Rameyer, 2005; Becker et al., 2023).

We are aware of two studies looking at the histology of gorgonians in the Mediterranean. Carella et al. (2014) examined two sea fans (*Eunicella singularis* and *E. cavolini*) from the Gulf of Naples and saw histologic evidence of thickening of the axial skeleton with molecular signatures of bacteria. In a subsequent paper, Carella et al. (2020) documented nodular lesions interpreted as melanin and amyloid deposits in these same corals. Here, we add to the knowledge of pathology in gorgonians by describing gross and microscopic similar pathology also in two additional species (*Leptogorgia sarmentosa* and *Paramuricea clavata*) from northwest and southwest Italy.

2. Materials and methods

Samples from three different gorgonian species manifesting tissue loss were collected in three locations off the coast of Italy between August and October 2022 (Fig. 1): three *Paramuricea clavata* from the

Fig. 1. Geographical contextualization of the study area highlighting the sampling site. A) Mediterranean Sea with Liguria and Calabria coasts highlighted; B) Ligurian Sea; C) Scilla area with the sampling site highlighted (38°15′22.5″N 15°42′44.9″E); D) Bergeggi Marine Protected Area with the sampling site highlighted (44°14′04.3″N; 8°26′46.6″E); E) Portofino Marine Protected Area with the sampling site highlighted (44°18′41.0″N; 9°12′47.4″E). Map made from MapTiler, GoogleHybridMap, and GoogleSatellite loaded into QGIS.

Marine Protected Area of Bergeggi (Liguria, Italy, 44°14′04.3″N; 8°26′46.6″E); five *Eunicella cavolini* from the coast of Paraggi in the Marine Protected Area of Portofino (Liguria, Italy, 44°18′41.0″N; 9°12′47.4″E); five *Leptogorgia sarmentosa* and seven *Paramuricea clavata* from Scilla (Calabria, Italy, 38°15′22.5″N; 15°42′44.9″E).

Before sampling, photographs were taken of the individuals displaying varying degrees of tissue loss, exposed axial skeleton, and, in some cases, overgrowth with turf algae (Fig. 2). Based on what is known about gross lesions in corals (Work & Aeby 2006), gross lesions showing overgrowth of skeleton by turf algae or other biota were judged more chronic than those showing bare skeleton alone. The specimens were collected during SCUBA diving activities by removing small fragments of the branches showing normal to abnormal tissue, then placed in zip-lock bags; paired apparently normal tissues were not collected. Upon reaching the surface, each sample was immediately fixed in plastic tubes filled with Z-fix solution (Sigma-Aldrich™, St. Louis, Missouri, USA). During collection, a roving diving technique was used to examine the sampled colonies and the surrounding area for potential predators that could cause similar injuries or tissue loss.

Before processing, each sample was photographed and trimmed into ~ 3 cm long fragments to include both the tissue loss interface and a healthy segment. The samples were then decalcified with Cal-Ex-II Fixative Decalcifier solution (Fisher Scientific™, Hampton, New Hampshire, USA) for 24 to 48 h. Then, the samples were placed in tissue cassettes and embedded in paraffin. The resulting wax blocks were then cut in 4 to 5 µm thick sections with a rotary microtome, mounted on glass microscope slides, and stained with hematoxylin and eosin (H&E). To visualize suspected fungi or melanin in the gorgonin skeleton, the proteinaceous matrix that makes up gorgonin (Ehrlich, 2019), Grocott's Methenamine Silver (GMS) and Fontana-Masson (FM) staining were performed respectively (Prophet et al., 1992). The control for melanin was a melanoma from a dog (Fig. S1), while the control for fungi was a bird lung infected with *Aspergillus* (Fig. S2). The sections were examined with an Olympus BX43 microscope, and photomicrographs were taken using a Lumenera Infinity 3 camera.

3. Results

Based on our analyses during the collection, no evidence was found of predators directly on the colonies or in the surrounding area that could explain this tissue loss. All 20 specimens analyzed had gross evidence of tissue loss.

Grossly, lesions for *P. clavata* and *L. sarmentosa* were similar in that they manifested as abrupt cessation of tissues at branch tips, revealing

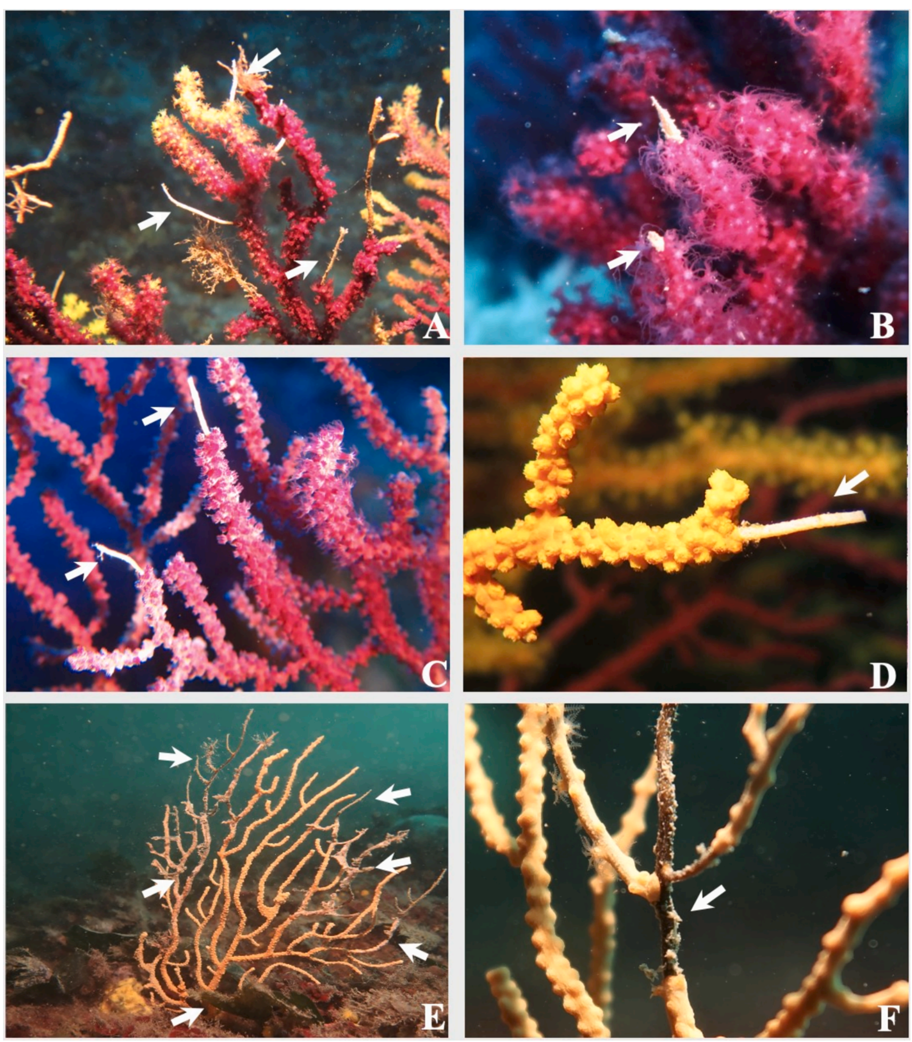

Fig. 2. Tissue loss overview in wild Mediterranean anthozoan colonies. A) colony-wide view and B) close-up of tissue loss at apical branches *P. clavata*; note exposed axial skeleton (arrow); C) colony-wide and D) close-up of *L. sarmentosa* showing tissue loss at apical branches; note bare axial skeleton (arrow); E) colony-wide view of extensive tissue loss on *E. cavolini* colony both basally and apically, showing axial skeleton with overgrowth by amorphous material and suggesting a more chronic process (arrows); F) close-up detail depicting tissue loss in *E. cavolini*; note segmental area of tissue loss exposing axial skeleton with overgrowth by black material (arrow).

mostly unfouled axial skeleton, suggesting that tissue loss was a recent event (Fig. 2A-D). We concluded this because bare substrates in marine environments tend to become colonized by turf algae and other epibionts quickly; examples include subacute tissue loss in corals (Work & Aeby, 2006). In contrast, tissue loss in *E. cavolini* was a more diffuse process encompassing branch tips and bases, leading to exposure of the axial skeleton with deposition of amorphous unidentified dark material on the bare skeleton, suggesting a more chronic process. Moreover, intact tissue adjacent to areas of tissue loss manifested varying degrees of indistinct pink discoloration (Fig. 2E and F). All species had an abrupt transition from healthy to tissue loss.

On histology, several tissue changes were observed. The apparently normal polyps consisted of tentacles over a mesoglea penetrated by solenia lined by gastrodermis and bare cavities (sclerites) atop an axial

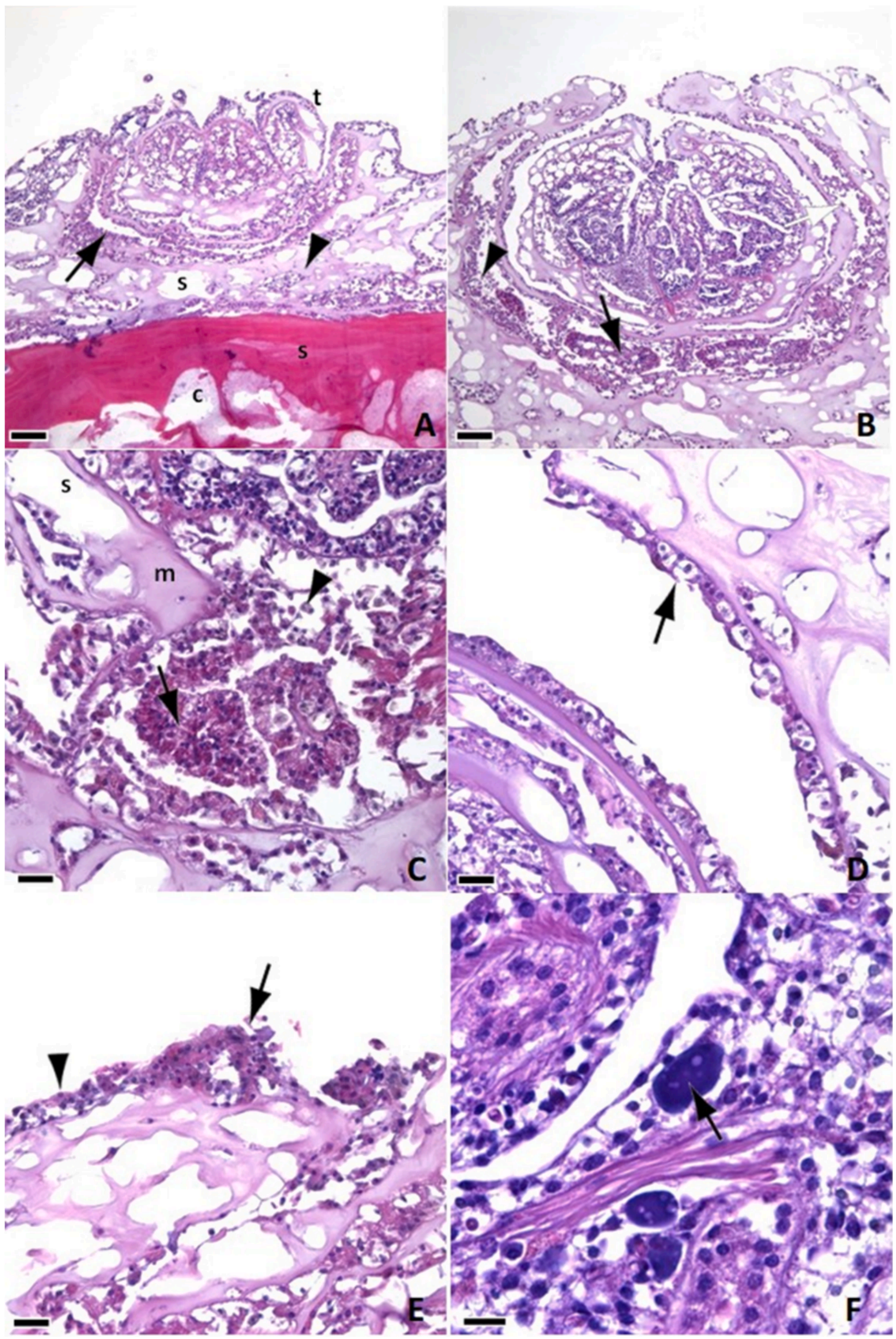

Fig. 3. Histology of sea fans collected from Italy showing acute to subacute tissue loss, hematoxylin and eosin. A) *L. sarmentosa* apparently normal polyp atop axial skeleton (s) containing cavities (c); note tentacle (t), sclerites (s) within mesoglea (arrowhead) and gastrodermis (arrow) lining solenia; bar = 50 µm. B) *L. sarmentosa* polyp; note necrotic gastrodermis (black arrow) characterized by clumps of hypereosinophilic material within solenia, dissociating (arrowhead) and necrotic (arrow) gastrodermal cells; bar = 50 µm. C) Details of necrotic gastrodermis sloughing into solenia; note clumps of hypereosinophilic debris with karyorrhectic and pyknotic nuclei (arrow) along with dissociating gastrodermal cells (arrowhead); (m) mesoglea, (s) sclerite; bar = 10 µm. D) *P. clavata*; note vacuolation of gastrodermis (arrow); bar = 10 µm. E) *L. sarmentosa*; note epidermal necrosis (arrow) with exposure of underlying mesoglea and contrast with more intact epidermis (arrowhead); bar = 20 µm. F) *P. clavata*; note unidentified amorphous dark bodies with variably sized cavities within the gastrodermis and epidermis of the tentacle (arrow); bar = 10 µm.

skeleton composed of gorgonin (Elrich, 2019) with variably sized cavities (Fig. 3A). The most common lesion across all species was dissociation (Fig. 3B) or necrosis (Fig. 3B-C) of gastrodermis. In less severe changes, the gastrodermis showed vacuolation (Fig. 3D). Occasionally, necrosis of the epidermis with tissue loss (Fig. 3E) was seen in *L. sarmentosa*. Additionally, unidentified dark amorphous structures were seen in the epidermis and gastrodermis of tentacles of *P. clavata*, with no associated host response (Fig. 3F).

Notably, *P. clavata* was also distinguished by an inflammatory response consisting of eosinophilic granular cells infiltrating mesoglea, sometimes associated with algae growth in the axial skeleton (Fig. 4A) or vacuolated gastrodermis (Fig. 4B). In *E. cavolini* ciliates were found colonizing the epidermis (Fig. 4C) or gastrodermis (Fig. 4D), occasionally associated with necrosis of the gastrodermis (Fig. 4E) or the entire polyp (Fig. 4F).

Microscopic changes in the axial skeleton were generally limited to the presence of inflammatory cells adjacent to the deposition of gorgonin (Fig. 5A) that stained negative with Fontana-Masson (Fig. 5B), as

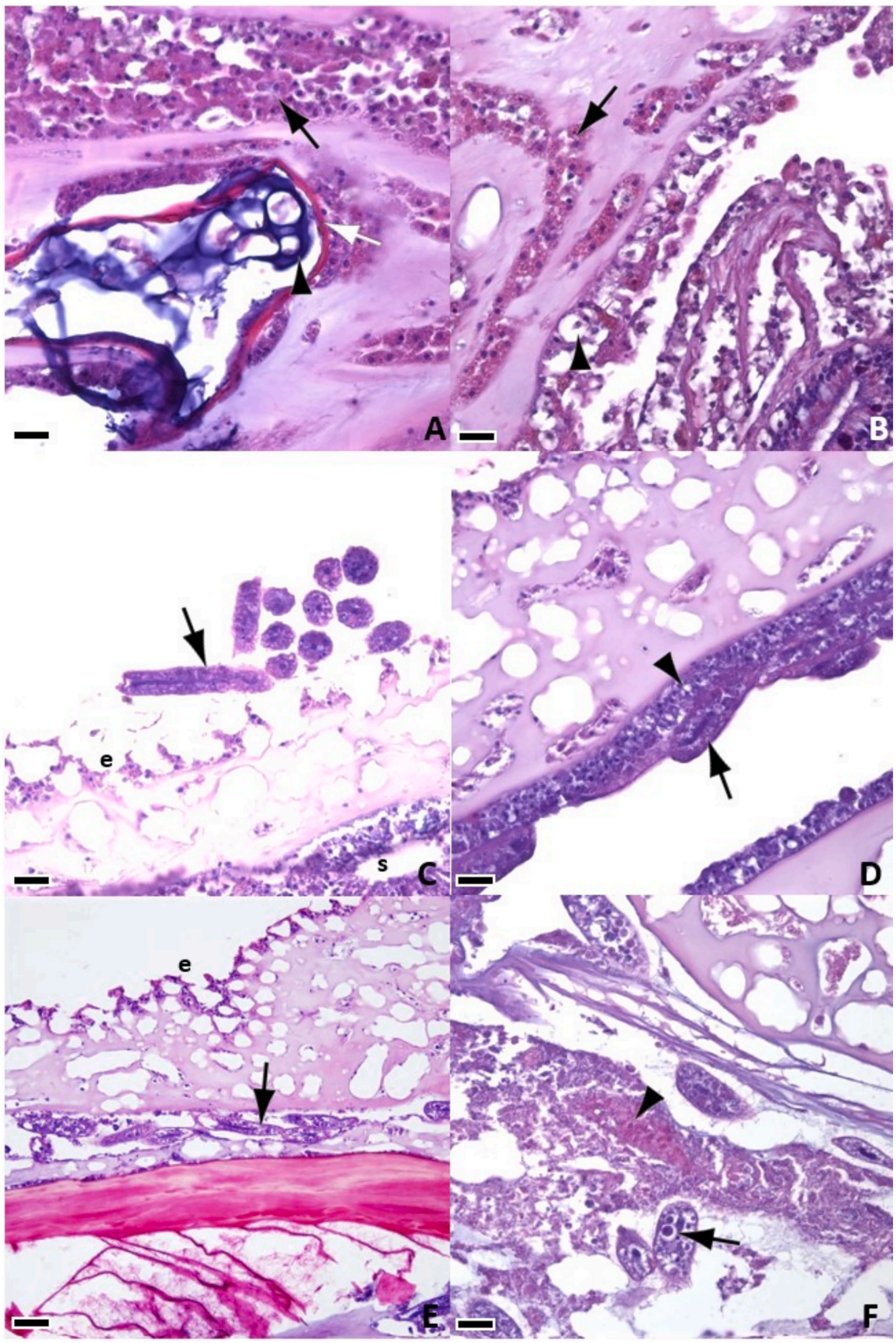

Fig. 4. Histology of inflammation and ciliates in sea fans from Italy with tissue loss, hematoxylin and eosin; bar = 10 µm all plates. A) *P. clavata*; note infiltrates of eosinophilic granular cells in mesoglea (arrow) adjacent to infiltrates of algae with cell walls (arrowhead) with deposition of gorgonin (white arrow). B) *P. clavata*; note infiltrates of eosinophilic granular cells in mesoglea (arrow) and vacuolation and dissociation of gastrodermis (arrowhead). C) *E. cavolini*; note ciliates (arrow) on top of the intact epidermis (e) and solenia (s) surrounded by gastrodermis. D) Close-up of ciliates (arrow) invading intact gastrodermis (arrowhead). E) *E. cavolini*; note ciliates (arrow) among gastrodermal cell debris within solenia and intact epidermis (e). F) Necrotic polyp with ciliates; note clumps of eosinophilic debris (arrowhead) associated with ciliates one of which has ingested material (arrow).

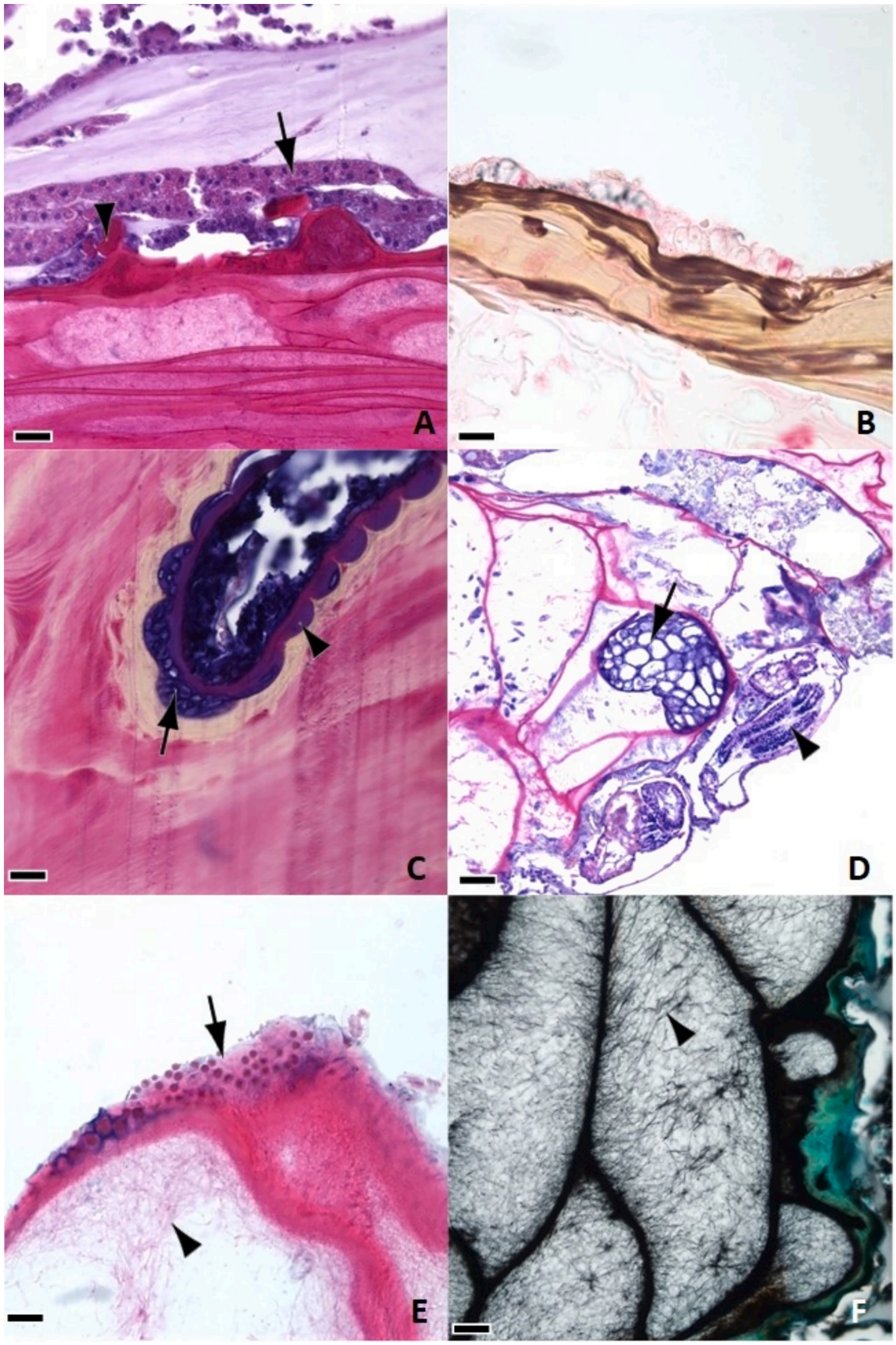

Fig. 5. Histology of axial skeleton of sea fans with tissue loss collected from Italy showing acute to subacute tissue loss, hematoxylin and eosin on all panels, except B (Fontana-Masson) and F (Grocott's Methenamine Silver). A) *P. clavata*; note infiltrates of granular mesogleal cells (arrow) and deposition of gorgonin (arrowhead); bar = 10 μm. B) Fontana-Masson stain of deposition of gorgonin; note lack of staining for melanin (compare with Fig. S1); bar = 10 μm. C) *L. sarmentosa*; algal infiltrates in axial skeleton overlaid by live tissue; note structures with cell walls (arrow) and deposition of gorgonin (arrowhead); bar = 10 μm. D) *L. sarmentosa*; deposition of epibionts on the bare axial skeleton; note algae in the skeletal matrix (arrow) and unidentified metazoan (arrowhead) on the surface; bar = 50 μm. E) *L. sarmentosa*; note microbial mat of unicellular structures on the surface of the bare axial skeleton (arrow) along with finely fibrillar material within space in the axial skeleton (arrowhead); bar = 10 μm. F) *L. sarmentosa*; note silver stain of filaments in E that do not fit the morphology of fungi characteristic of parallel walled branching structures with septa (Fig. S2); bar = 10 μm.

compared with Fig. S1. In cases where live tissue overlaid the axial skeleton, algae infiltration was accompanied by deposition of gorgonin (Fig. 5C), whereas this was not evident in areas of bare axial skeleton colonized by epibionts (Fig. 5D) or microbial mats (Fig. 5E). Within the cavities of the axial skeleton, finely fibrillar material was found in both bare and live tissue-overlaid areas (Fig. 5E), staining positive with silver stain (Fig. 5F), but not matching the size or morphology expected for fungi (Fig. S2).

Among the species analyzed, the greatest variety of lesions was seen in *P. clavata* followed by *L. sarmentosa* and *E. cavolini*. Finally, the inflammatory response was seen only in *P. clavata*, epidermal necrosis was seen exclusively in *L. sarmentosa*, and ciliates were present only in *E. cavolini* (Table 1).

Table 1
Enumeration of presence/absence of each type of lesion (n.d. = non-detected).

LESION TYPE	*Eunicella cavolini* (n = 5)	*Leptogorgia sarmentosa* (n = 5)	*Paramuricea clavata* (n=10)	TOTAL
Gastrodermal dissociation	5	5	10	20
Gastrodermal necrosis	3	3	6	12
Ciliates	4	0	0	4
Round bodies tentacles	0	0	4	4
Epidermal tissue loss	0	3	0	3
Gastrodermal vacuolation	0	0	3	3
Granular cell infiltrates mesoglea	0	0	3	3
Algae in skeleton	0	2	1	3
Total	12	14	51	38

4. Discussion

Similar tissue loss on the tips of gorgonians branches has been reported in the last decades during prolonged thermal stress periods and mass mortalities events, where, in some cases, some recovery has been noted both in terms of population health and individuals affected by tissue loss (Cerrano et al., 2000; Carella et al., 2014; Turicchia et al., 2018; Canessa et al., 2023). Indeed, this type of lesion on the tissue of gorgonians has been observed and potentially associated with thermal stress in previous research (Carella et al., 2014; Carella et al., 2020; Garrabou et al., 2022). Here, we wanted to understand better whether the observed patterns of tissue loss across different species could be attributed solely to thermal stress or if other factors, such as pathogens or predation, might also contribute to this phenomenon.

The most significant lesion seen in all sea fans was necrosis and vacuolation of the gastrodermis, which likely led to tissue sloughing and gross lesions observed in the field in this study. Necrosis of gastrodermis is a common host response in other cnidaria with tissue loss of unexplained origin, and it has been seen in corals with tissue loss from the Caribbean (Landsberg et al., 2020) and the Pacific (Work and Aeby, 2011). In Caribbean Sea fans (*Gorgonia ventalina*) with purpling lesions, Becker et al. (2023) saw gastrodermal necrosis and amoebocytic infiltrates along with deposition of gorgonin around algae invading skeleton similar to changes seen here. Inflammation in gorgonians is also well documented (Mydlarz et al., 2008). Carella et al. (2014) examined *E. cavolini* and *E. singularis* in the Gulf of Naples using histology, which showed inflammation in tissues and cyanobacteria presence detected by molecular assays and histology. In subsequent investigations, Carella et al. (2020) concluded that nodules found in sea fans were the result of infection with cyanobacteria, leading to the deposition of melanin in the axial skeletons; however, the organisms they showed in their figures were more compatible with plant cells with cell walls (algae), something we observed here invading the axial skeleton of gorgonians. We were unable to convincingly show staining of melanin in sea fan axial skeletons here, at least, compared to positive controls. Becker et al. (2023) stated they saw melanin in Caribbean Sea fans but without a confirmatory stain, while Carella et al. (2020) showed staining of the axial skeleton with Fontana-Masson that was not all that different from our specimens and did not reflect the staining expected in our positive control. Although melanin deposition can play an important role in the defense mechanisms of invertebrates (Nappi and Christensen, 2005), whether melanin plays such a role in sea fans remains to be confirmed.

The only foreign organisms associated with host changes at the cellular level were infiltrates of algae in the axial skeleton associated with varying degrees of gorgonin deposition. Similar changes were seen in Caribbean Sea fans (Becker et al., 2023), and based on the presence of what appears to be macroalgae in the figures of Carella et al. (2020), nodules in *E. cavolini* and *E. singularis* from the Gulf of Naples may be responses to algal overgrowth of axial skeleton. Indeed, algal overgrowth of the gorgonian axial skeleton leading to nodule formation has been known since the early 1980 s (Goldberg et al., 1984; Morse et al., 1981). Unlike Becker et al. (2023), we did not observe convincing evidence of fungal infection in gorgonians. The presence of cavities in the axial skeleton with a framework of finely fibrillar material that, here, stained positive with silver, seems to be a normal part of the skeletal structure of Mediterranean gorgonians; similar cavities have been seen in other species from the region (Carella et al., 2014; Carella et al., 2020). The unidentified dark bodies in the tentacles of *P. clavata* were similar to those seen by Carella et al. (2020), who judged them to be cells; however, we were unable to visualize nuclei in the structures seen here, thus their identity remains uncertain.

The presence of ciliates exclusively in *E. cavolini* was intriguing, particularly in cases where ciliates were seen colonizing intact epidermis and invading intact gastrodermis and associated with necrosis of polyp tissue and gastrodermis (Fig. 4). Invasion of intact tissue layers suggests that ciliates are not mere detritivores and could play a potential role in the pathogenesis of tissue loss in this species. However, apparently normal tissues were not collected in this study, so we cannot be certain that these are pathogenic. Becker et al. (2023) saw ciliates in apparently normal and lesioned tissues of Caribbean Sea fans and saw ciliates settling on the epidermis; however, they did not have any conclusions regarding their role in causing lesions. This should be addressed in future work, because ciliates invading tissues and associated with tissue loss have been seen in other corals like *Montipora capitata* (Work et al., 2012) and *Acropora sp.* (Bourne et al., 2008).

Future studies of histopathology in Italian gorgonians would benefit from additional histology investigations, including examination of apparently normal tissues to aid interpretation of lesions. Other methods, such as cytology, may also aid in understanding the morphology and the pathogenesis of lesions in this important group of animals (Work et al., 2024).

As gorgonian forests and other marine ecosystems face increasing threats, gaining a deeper understanding of the processes and causes leading to tissue damage becomes imperative for the effective conservation of this fragile ecosystem, which is fundamental for the Mediterranean benthic community.

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CRediT authorship contribution statement

Jacopo Gobbato: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **Thierry M. Work:** Writing – review & editing, Validation, Supervision, Investigation, Formal analysis. **Martina P. Facchinelli:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Federica M. Siena:** Writing – review & editing, Formal analysis. **Enrico Montalbetti:** Writing – review & editing. **Davide Seveso:** Writing – review & editing. **Yohan D. Louis:** Writing – review & editing. **Paolo Galli:** Supervision. **Simone Montano:** Writing – review & editing, Validation, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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The use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the US government.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jip.2024.108197.

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