Mass Mortality of Shallow-Water Temperate Corals in Marine Protected Areas of the North Aegean Sea (Eastern Mediterranean)

Chryssanthi Antoniadou *, Martha Pantelidou, Maria Skoularikou and Chariton Charles Chintiroglou

Department of Zoology, School of Biology, Aristotle University, 54124 Thessaloniki, Greece
* Correspondence: antonch@bio.auth.gr

Abstract: Coral mortality is a global phenomenon of increasing magnitude, correlated with climate change. Prolonged marine heatwaves have particularly affected the north Aegean Sea in summer 2021, threatening shallow-water stony corals, such as *Balanophyllia europaea* and *Cladocora caespitosa*. To assess their population status, ten coastal, rocky-bottom stations dispersed in Natura 2000 sites of Chalkidiki (north Aegean) were surveyed using non-destructive techniques in autumn 2021. At each station, corals’ abundance was estimated in situ, by counting the number of *B. europaea* polyps within randomly placed 50 × 50 cm quadrats, and the number of *C. caespitosa* colonies along three replicate belt transects 1 × 10 m. The status of corals was qualitatively assigned as healthy, bleached (partially or complete), or in necrosis (partial or complete). *B. europaea* was found in 80% of stations; in total, 58.17% of the coral specimens were affected by necrosis. *C. caespitosa* was found in 30% of stations; in total, 27.49% of the coral colonies were partially bleached and 11.32% in necrosis. Another nine sessile invertebrates (sponges, bivalves, and ascidians) were observed in necrosis. These results highlight the need to establish monitoring programs on vulnerable sessile invertebrate populations along the Aegean Sea to assess climate change impacts.

Keywords: benthos; rocky substrata; scleractinia corals; sponges; bivalves; ascidians; marine heatwaves; Natura 2000; *Balanophyllia europaea*; *Cladocora caespitosa*

1. Introduction

Climate change constitute a major threat to marine biodiversity [1,2]. Increasing seawater temperature, acidification, and ice melting degrades the marine environment and cause devastating events, often lethal to a variety of life forms. Among marine biogeographic realms [3], the Mediterranean Sea seems to be especially affected, as is becoming warmer during the past decades and, within the entire basin, temperature is increasing much more rapidly (20%) than global mean [4]. This warming phenomenon is especially relevant for the eastern Mediterranean, which is suffering from the so-called “tropicalization” effect [5–8]. As a result, the frequency of appearance, the magnitude, and the duration of marine heatwaves, i.e., extreme rises in seawater temperature over an extended period, have significantly increased [9]. The Aegean Sea, and especially its northern part, is among the most impacted Mediterranean sub-regions [9]. Mean surface seawater temperature increases by a rate of over 0.60 °C per decade, which is much higher than the that relevant (0.40 °C/decade) for the entire eastern Mediterranean basin [10]. As a result, over 35 marine heatwave events (>300 days) occurred during the recent decade, and accordingly, the area seems to currently constitute a “hot spot” of marine heatwaves [10].

Increased seawater temperature and marine heatwaves severely affect marine biota, long-lived, sessile, epibenthic invertebrates, such as sponges, corals, bivalves, and ascidians, in particular [11–14]. Gorgonians and stony corals are among the most impacted species [15–18]. Focusing on stony corals, bleaching, i.e., the loss of endosymbiotic zooxanthellae from the host coral, is the first signal of thermal distress, which, if prolonged, leads to the necrosis of polyps, and subsequent erosion of corallites. Mass mortality events
of temperate stony corals have been reported from the Mediterranean Sea since the end of 1990s [19–21], and the frequency and severity of such events are steadily increasing over the entire basin [15,21–24]. The most commonly affected species are the tooth coral, *Balanophyllia europaea* (Risso, 1826) and the Mediterranean pillow coral, *Cladocora caespitosa* (Linnaeus, 1767). *B. europaea* is a zooxanthellate ahermatypic solitary coral that thrives on shallow rocks, usually between 2 and 8 m, although it can be found much deeper, up to 40 m depth [25]. It is a Mediterranean endemic species, sensitive to climate change [21,26–28] that has been evaluated as Least Concern by IUCN in 2015, as despite being sensitive to marine warming it is widely distributed in the Mediterranean Sea and forms dense populations in some marine protected areas [28]. *C. caespitosa* is a zooxanthellate hermatypic colonial coral, widespread over the Mediterranean Sea [25]. It is a nearly endemic Mediterranean species, due to its restricted and sporadic presence on the adjacent eastern Atlantic coasts of Morocco and Portugal [28]; environmental conditions seems to prevent further expansion in the latter marine area [29]. *C. caespitosa* is regarded as a fossil relict reef-building coral species [30]. Nowadays, it can be found in different habitats [25,28,31,32] and in different forms, ranging from scattered elliptical suboval colonies attached on rocks to dense beds or large banks developed on rocky or sedimentary bottoms [28,33,34]. However, it is usually found in shallow depths, up to 12 m depth, in the form of small colonies [25,30,35]; beds and banks, however, may be found deeper in dim light conditions [32]. *C. caespitosa* is an endangered (EN) Mediterranean anthozoan species based on IUCN 2015 assessment, as declining populations have been reported from many locations under the main threat of climate change [28]. Both species have suffered from mass mortality events; however, the vast majority of such events has been reported from the western basin (Spanish and French coasts), the Tyrrhenian, and the Adriatic Sea. Surprisingly, too few relevant events have been reported, so far, from the Aegean Sea—all derived from the northern sector [15,24]—despite the fact that the north Aegean Sea is assessed among the most impacted by marine heatwaves sub-regions of the Mediterranean Sea [9,10].

In the north Aegean Sea, algal-dominated communities cover the shallow (i.e., up to 15–20 m depth) sublittoral rocky zone, with various epibenthic sessile invertebrates scattered in-between, mainly, sponges (*Aphelina, Chondrilla, Chondrosia, Cliona, Ircinia, Sarcotragus*), hydrozoans (*Aglaophenia, Eudendrium*), corals (*Balanophyllia, Cladocora*), molusks (*Arca, Spondylus, Vermetus*), and ascidians (*Halocynthia, Microcosmus*) [36,37]. Several of the above mentioned invertebrate species, e.g., sponges [38,39], corals [40,41], and ascidians [42,43], are considered as ecosystem engineers [44] as they provide living habitat of increased complexity to the benthic environment. This coexistence of many species of different architectural structure within a single biotope creates a rather complex and patchy habitat that hosts a large variety of species, enhancing this way local biodiversity [36,43]. This intricate habitat-type extent along the rocky shore of Chalkidiki peninsula (north Aegean Sea), and a large part of this coastal zone is included in the Natura 2000 network as Special Areas of Conservation (SACs).

Marine protected areas (MPAs) have been designated as management tools to address the emerging stressors that, nowadays, are a threat to marine biodiversity. However, they represent rather “local” solutions to “local” threats (e.g., fisheries, pollution, coastal development), and so, their effectiveness to global stressors, such as climate change, is challenging [45]. The growing concern about climate change impacts on MPAs drive the establishment of action plans to address their vulnerability, the development of operational climate change strategies, the definition of physical and biological indicators together with the implementation of relevant monitoring programs, and the setting up of specific quantitative targets and thresholds for adaptive management [46]. These steps are essential to assess the climate change impacts to marine protected areas, as well as the resilience of MPAs to climate change.

As part of the north Aegean Sea, the coastal marine zone of Chalkidiki peninsula, including the Natura 2000 SACs, has been harshly impacted by the especially hot summer 2021. Prevailed climatic conditions were rather unique due to the very high sea surface
temperature associated with a number of intense and prolonged (over 20 days) marine heatwaves [10]. This prolonged warming may have detrimentally affected sessile marine biota, stony corals in particular. Shallow-water stony corals, due to their sensitivity to marine warming [15,16,21,23,24,31,33], may represent appropriate climate change descriptors to assess the resilience of temperate MPAs under ongoing marine warming.

Considering all the above, the present work aims to assess the population status of the most prominent stony-coral species, namely *B. europaea* and *C. caespitosa* in the shallow rocky shores of the five SACs included in Chalkidiki peninsula (north Aegean Sea) [47], right after the particularly hot summer of 2021, by applying non-destructive assessment techniques.

2. Materials and Methods

The study was carried out at the five SACs of the Natura 2000 network (Kassandra: GR1270010 “Akrotirio Pyrgos–Ormos Kypses–Malamo”, GR1270008 “Paliouri–Akrotiri kai Thalassia Zoni”; Sithonia: GR1270007 “Akrotirio Elia–Akrotirio Kastro–Ekvoli Ragoula”, GR1270002 “Oros Itamos–Sithonia”, GR1270009 “Platanitsi–Sykia: Akrotirio Rigas–Akrotirio Adolo”) in Chalkidiki, north Aegean Sea (Figure 1). Chalkidiki peninsula is a part of the Vardar-Axios Zone, and the Serbomacedonian and Rhodope Massif, and covers an area of 2918 km$^2$ with an extended coastline. As part of the north Aegean Sea, the study area has been especially affected by the summer 2021 marine heatwaves [10].

One up to four sampling stations were randomly established within each SAC; the number of stations was defined according to the cover area of SACs (Figure 1). Overall, two stations (S1, S2) were set in Thermaikos Gulf, four (S3–S6) in Toroneos Gulf, and another four (S7–S10) in Siggitikos Gulf. All the sampling stations were set on the rocky coastline, with the exception of S7, which was set on the small islet of Diaporos. The sea bottom consisted of moderately to strongly inclined rocky cliffs (S3, S5, S7, S8, and S9) or of large boulder reefs (S1, S2, S4, S6, and S10) surrounded by dense meadows of the seagrass species *Posidonia oceanica* (class Magnoliopsida). At all stations, shallow water (>20 m) benthic communities were dominated by algal species with sponges, hydrozoans, corals, mollusks, and ascidians scattered in-between [36,37], as described in the Introduction.

Samplings were carried out in September (Kassandra, 10–13) and October (Sithonia, 20–22) 2021 with SCUBA diving in the shallow sublittoral zone, i.e., up to 15 m depth. Visual census included a combination of belt transects and haphazardly placed quadrats [37] to assess the health status of the populations of the prominent stony coral species in the area, namely *B. europaea* and *C. caespitosa*. Both surveyed species were found between two and 12 m depth (depth standardized according to mean sea level). *B. europaea*, was particularly abundant around 3–6 m, whereas *C. caespitosa* around 7–10 m; therefore, populations were surveyed at these depth levels, respectively.

In the case of *B. europaea*, population density was estimated in situ by counting all individuals present in the randomly placed ten replicate quadrate frames (50 × 50 cm) at each station. The condition of individuals, i.e., alive (normally pigmented polyps), bleached (white or transparent polyps), or dead (white skeleton denude of coral tissue) was also recorded. In the case of *C. caespitosa*, the number of colonies were counted along three replicate transects 1 × 10 m each, at each sampling station. The status of coral colonies was qualitatively assigned in five categories [48], as: (0) healthy, when all the polyps of the colony, or almost all, were normally colored, (1) partially bleached, when several of the polyps of the colony were transparent, (2) completely bleached, when all the polyps of the colony, or almost all were transparent, (3) in partial bleaching and necrosis, when a part of the colony had transparent polyps and the rest was dead, and (4) in recent necrosis, when none, or almost none of the colony’s polyps were alive. In situ recorded data were used to assess the mortality or bleaching rate per sampling station, per SAC, and over the surveyed area.
part of the colony had transparent polyps and the rest was dead, and (4) in recent necrosis, when none, or almost none of the colony’s polyps were alive. In situ recorded data were used to assess the mortality or bleaching rate per sampling station, per SAC, and over the surveyed area.

Figure 1. Map of the Aegean Sea (left) marking the study area (white box) and of the study area (right) indicating the location of sampling stations (S1–S10) within each Natura 2000 SAC (GR1270002–GR1270010) (from Google Earth).

Analysis of variance (ANOVA) was used to test for spatial differences in the health status of stony coral species in Chalkidiki peninsula. More specifically, the effect of SAC (fixed factor) and sampling station (fixed factor nested to SAC) on the abundance of healthy and affected coral polyps or colonies (data were pooled over partially bleached to necrosis categories of corals’ health status) was assessed through a general linear model [49]. The Fisher LSD test was used for post-hoc comparisons. ANOVAs were performed using the SPSS software package (IBM SPSS statistics version 19).

At each sampling site, the presence of different invertebrate species affected by necrosis was also recorded during visual census. Seawater temperature, pH, and dissolved oxygen were also measured in the water column and up to 15 m depth with an autographic recorder (Hach Be Right TM) at the same time as visual census.
3. Results

3.1. Balanophyllia europaea

*Balanophyllia europaea* (Figure 2) was found at eight of the ten surveyed stations (80%), and was not recorded at S2 (GR1270008, Thermaikos) and S5 (GR1270008, Toroneos).

The population density of the solitary *B. europaea* ranged from 2.8 polyps/m² (S6) to 23.2 polyps/m² (S7) with an overall mean 9.5 ± 8.3 polyps/m². The density of healthy corals ranged from 1.2 polyps/m² (S6) to 9.6 polyps/m² (S7) with an overall mean 3.4 ± 2.2 polyps/m². The density of corals in recent necrosis ranged from 1.6 polyps/m² (S6) to 18.4 polyps/m² (S4) with an overall mean 6.05 ± 4.9 polyps/m², whereas no bleached polyps were observed. Accordingly, mortality ranged from 45.45% (S10) to 82.14% (S4) with an overall mean at 58.17%. The lowest mortality was observed at GR1270009 stations, whereas the highest at Kassandra GR1270008 (Table 1, Figure 3), mostly due to the very dense and highly affected *B. europaea* population at Agios Nikolaos Bay (S4).

Nested ANOVAs revealed significant spatial differences in the abundance of both healthy and affected *B. europaea* polyps (Table 2), with significantly increased abundance of impacted corals in GR1270008 and of healthy corals in GR1270002 (Figure 4). This pattern was mainly due to the effect of S4 in GR1270008 and of S7 in GR1270002.

![Figure 2](image-url). Healthy (left) and in necrosis (right) specimens of *B. europaea* in Chalkidiki peninsula during autumn 2021 samplings.

**Table 1.** Total number of polyps of the affected polyps (in necrosis) and percent mortality of *Balanophyllia europaea* per sampling station at the surveyed SACs in Chalkidiki peninsula in autumn 2021.

<table>
<thead>
<tr>
<th>Chalkidiki</th>
<th>Natura 2000 SAC</th>
<th>Station</th>
<th>Number of Polyps</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>Affected</td>
</tr>
<tr>
<td>Kassandra</td>
<td>GR12700010</td>
<td>S1</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>S2</td>
<td>0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>S3</td>
<td>17</td>
<td>9</td>
<td>52.94</td>
</tr>
<tr>
<td></td>
<td>S4</td>
<td>56</td>
<td>46</td>
<td>82.14</td>
</tr>
<tr>
<td></td>
<td>S5</td>
<td>0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>S3–4</td>
<td></td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sithonia</td>
<td>GR1270008</td>
<td>S6</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>S7</td>
<td>58</td>
<td>34</td>
<td>58.62</td>
</tr>
<tr>
<td></td>
<td>S8</td>
<td>16</td>
<td>9</td>
<td>56.25</td>
</tr>
<tr>
<td></td>
<td>S7–8</td>
<td></td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>GR1270009</td>
<td>S9</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>S10</td>
<td>11</td>
<td>5</td>
<td>45.45</td>
</tr>
<tr>
<td></td>
<td>S9–10</td>
<td></td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
Nested ANOVAs revealed significant spatial differences in the abundance of both healthy and affected *B. europaea* polyps (Table 2), with significantly increased abundance of impacted corals in GR1270008 and of healthy corals in GR1270002 (Figure 4). This pattern was mainly due to the effect of S4 in GR1270008 and of S7 in GR127002.

**Figure 3.** Percentage of estimated mortality and bleaching of the stony corals *Balanophyllia europaea* and *Cladocora caespitosa* per sampling station in Chalkidiki peninsula during autumn 2021 samplings.

**Table 2.** ANOVA results on the effect of SAC (5-level fixed factor) and sampling station (8-level fixed factor nested in SAC) on the abundance of *Balanophyllia europaea* healthy and affected (in necrosis) polyps in Chalkidiki peninsula in autumn 2021.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Abundance of <em>B. europaea</em> Polyps</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy</td>
</tr>
<tr>
<td>df</td>
<td>F</td>
</tr>
<tr>
<td>SACs</td>
<td>4</td>
</tr>
<tr>
<td>Sampling stations (nested on SACs)</td>
<td>7</td>
</tr>
</tbody>
</table>

**Figure 4.** Mean abundance ± Fisher LSD of healthy (upper graphs) and affected by mortality (lower graphs) *Balanophyllia europaea* polyps per SAC and sampling station in Chalkidiki peninsula in autumn 2021 samplings.
**Table 2.** ANOVA results on the effect of SAC (5-level fixed factor) and sampling station (8-level fixed factor nested in SAC) on the abundance of *Balanophyllia europaea* healthy and affected (in necrosis) polyps in Chalkidiki peninsula in autumn 2021.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Abundance of <em>B. europaea</em> Polyps</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
<td>p</td>
<td>df</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>SACs</td>
<td>4</td>
<td>13.61</td>
<td>&lt;0.0001</td>
<td>7.21</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Sampling stations (nested on SACs)</td>
<td>7</td>
<td>23.21</td>
<td>&lt;0.0001</td>
<td>17.72</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

**3.2. Cladocora caespitosa**

*Cladocora caespitosa* (Figure 5) was only found at three of the ten surveyed stations (30%, Table 3).

![Figure 5](image.png)

**Figure 5.** Colonies of *Cladocora caespitosa* in Chalkidiki peninsula during autumn 2021 samplings: healthy (left), bleached (middle), and in old necrosis (right).

**Table 3.** Total number of colonies, healthy colonies (0), affected colonies (1 = in partial bleaching, 2 = in complete bleaching, 3 = in partial bleaching and partial necrosis, 4 = in recent necrosis), and percent of partial bleaching and of mortality (colonies in partial and complete necrosis) of *Cladocora caespitosa* per sampling station at the surveyed SACs in Chalkidiki peninsula in autumn 2021.

<table>
<thead>
<tr>
<th>Chalkidiki</th>
<th>Natura 2000 SAC</th>
<th>Station</th>
<th>Number of Colonies</th>
<th>Partially Bleached</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total 0 1 2 3 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kassandra</td>
<td>GR1270010</td>
<td>S1</td>
<td>0 – – – – – –</td>
<td>– – – – – – – – – –</td>
<td>– – – – – – – – – –</td>
</tr>
<tr>
<td></td>
<td>GR1270008</td>
<td>S2</td>
<td>0 – – – – – –</td>
<td>– – – – – – – – – –</td>
<td>– – – – – – – – – –</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S3</td>
<td>13 10 2 0 0 1</td>
<td>15.38</td>
<td>7.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S4</td>
<td>0 – – – – – –</td>
<td>– – – – – – – – – –</td>
<td>– – – – – – – – – –</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S5</td>
<td>0 – – – – – –</td>
<td>– – – – – – – – – –</td>
<td>– – – – – – – – – –</td>
</tr>
<tr>
<td>Sithonia</td>
<td>GR1270007</td>
<td>S6</td>
<td>0 – – – – – –</td>
<td>– – – – – – – – – –</td>
<td>– – – – – – – – – –</td>
</tr>
<tr>
<td></td>
<td>GR1270002</td>
<td>S7</td>
<td>29 13 11 0 1 4</td>
<td>37.93</td>
<td>13.79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S8</td>
<td>24 13 7 0 1 3</td>
<td>29.16</td>
<td>12.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S7–8</td>
<td>33.54</td>
<td>13.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GR1270009</td>
<td>S9</td>
<td>0 – – – – – –</td>
<td>– – – – – – – – – –</td>
<td>– – – – – – – – – –</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S10</td>
<td>0 – – – – – –</td>
<td>– – – – – – – – – –</td>
<td>– – – – – – – – – –</td>
</tr>
</tbody>
</table>
The population density of *C. caespitosa* ranged from 1.3 colonies/m$^2$ (S3) to 2.9 colonies/m$^2$ (S7) with a mean at 2.2 ± 0.82 colonies/m$^2$ (considering only the sites where the species were recorded) and an overall mean at 0.66 ± 1.13 (considering zero-coral sites, as well). The density of healthy corals (category 0) ranged from 0.8 colonies/m$^2$ (S3) to 1.3 colonies/m$^2$ (S7 and S8) with an overall mean 1.2 ± 0.3 colonies/m$^2$. The density of partially bleached corals (category 1) ranged from 0.2 colonies/m$^2$ (S3) to 1.1 colonies/m$^2$ (S7) with an overall mean 0.7 ± 0.4 colonies/m$^2$. No completely bleached colonies (category 2) were observed, and the density of colonies in partial bleaching and necrosis (category 3) was particularly low (0.06 ± 0.07 colonies/m$^2$ detected in S7 and S8). Finally, the corals in recent necrosis (category 4) ranged from 0.1 colonies/m$^2$ (S6) to 0.3 polyps/m$^2$ (S7 and S8) with an overall mean 0.2 ± 0.1 colonies/m$^2$. Accordingly, about one-third (27.49%) of the observed colonies were partially bleached and 11.32% were in partial or in complete necrosis. A very dense population of *C. caespitosa* exist in the marine protected area of Vourvourou (GR1270002), which seems to have suffered from increased levels of bleaching and successive mortality (Table 3, Figure 3). The presence of colonies in old necrosis with strongly eroded corallites was also noticed during samplings (see Figure 5).

Nested ANOVAs revealed significant spatial differences in the abundance of partially bleached and of affected by necrosis *C. caespitosa* colonies, in contrast with healthy ones (Table 4). Significantly increased abundance of impacted corals were observed in both stations of GR1270002 (Figure 6).

**Table 4.** ANOVA results on the effect of SAC (2-level fixed factor) and sampling station (3-level fixed factor nested in SAC) on the abundance of *Cladocora caespitosa* healthy, partially bleached, and affected (in partial or complete necrosis) colonies in Chalkidiki peninsula in autumn 2021.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Healthy</th>
<th>Partial Bleached</th>
<th>Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>SACs</td>
<td>df</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SACs</td>
<td>1</td>
<td>1.04</td>
<td>0.34</td>
</tr>
<tr>
<td>Sampling stations</td>
<td>2</td>
<td>0.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

**Figure 6.** Mean abundance ± Fisher LSD of partially bleached (upper graphs) and affected by mortality (lower graphs) *Cladocora caespitosa* colonies per SAC and sampling station in Chalkidiki peninsula during autumn 2021 samplings.
Table 4. ANOVA results on the effect of SAC (2-level fixed factor) and sampling station (3-level fixed factor nested in SAC) on the abundance of *Cladocora caespitosa* healthy, partially bleached, and affected (in partial or complete necrosis) colonies in Chalkidiki peninsula in autumn 2021.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Abundance of <em>C. caespitosa</em> Colonies</th>
<th></th>
<th></th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Healthy</td>
<td>Partial Bleached</td>
<td>Affected</td>
<td>Healthy</td>
<td>Partial Bleached</td>
<td>Affected</td>
<td></td>
</tr>
<tr>
<td></td>
<td>df</td>
<td>F</td>
<td>p</td>
<td>F</td>
<td>p</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>SACs</td>
<td>1</td>
<td>1.04</td>
<td>0.34</td>
<td>33.38</td>
<td>&lt;0.001</td>
<td>18.29</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Sampling stations (nested on SACs)</td>
<td>2</td>
<td>0.00</td>
<td>1.00</td>
<td>7.45</td>
<td>&lt;0.034</td>
<td>0.86</td>
<td>0.39</td>
</tr>
</tbody>
</table>

3.3. Various Other Affected Sessile Invertebrates

Apart from the two stony coral species described above, another nine (9) sessile invertebrate species exhibited signs of partial or complete necrosis within the study area. These were the sponges *Aplysina aerophoba* (Nardo, 1833), *Chondrilla nucula* Schmidt, 1862, *Chondrosia reniformis* Nardo, 1847, *Ircinia variabilis* (Schmidt, 1862), *Sarcotragus foetidus* Schmidt, 1862 and *Spongia officinalis* Linnaeus, 1759, the bivalves *Spondylus gaederopus* Linnaeus, 1758 and *Pinna nobilis* Linnaeus, 1758, and the sea squirt *Microcosmus sabatieri* Roule, 1885 (Figure 7). Although no quantitative data were collected for the above species, *S. foetidus*, *I. variabilis*, and *S. gaederopus* were by far the most severely affected over the study area.

![Figure 7](image-url)

**Figure 7.** Sessile invertebrates in complete or partial necrosis observed in Chalkidiki peninsula during autumn 2021 samplings; *Aplysina aerophoba* (A), *Chondilla nucula* (B), *Ircinia variabilis* (C) *Spongia officinalis* (D), *Microcosmus sabatieri* (E), *Chondrosia reniformis* (F), *Sarcotragus muscarum* (G), *Spondylus gaederopus* (H), *Pinna nobilis* (I).

3.4. Seawater Temperature, pH, and Dissolved Oxygen

The seawater temperature was homogenous at vertical (0–15 m depth) scale. In horizontal scale (i.e., across the spatially dispersed sampling stations), water masses were also homogenous at all the sites sampled in the same time, i.e., Kassandra stations, sampled in 10–13 September, and Sithonia stations, sampled about a month later, in 20–22 October. Mean temperature was 24.75 °C in September and 20.06 °C in October samplings. The pH was extremely stable at both studied spatial scales, varying around 8.22. Dissolved oxygen ranged from 8.14 mg/L to 9.14 mg/L; accordingly, seawater masses were highly saturated.
4. Discussion

The rocky shores of the north Aegean Sea constitute a highly diverse habitat that host a large number and variety of benthic invertebrate species [36,37], justifying their inclusion in Natura 2000 network as SAC. These SACs have been implemented in the study area (Chalkidiki peninsula) since 2011, and yet, no active management plans have been established. Marine biodiversity remains largely understudied, as only very recently (2021–2023) the relevant Management Unit completed the first basal surveys on these SACs.

In the shallow sublittoral zone of Chalkidiki’s SACs, the most prominent macroscopic invertebrates are sponges and stony corals, among which are several of the currently reported species in partial or complete mortality. By focusing on stony corals, *B. europaea* and *C. caespitosa* are by far the most common species [37,47], as also observed during the performed underwater surveys.

Successive mortality events of growing magnitude have affected *B. europaea* populations in several Mediterranean locations [17,20,21,26,27] causing localized declines and reducing fitness [28]. However, no relevant events have been reported in the past from the Aegean Sea. The population of *B. europaea* has been thoroughly studied in the Mljet MPA of the Adriatic Sea for over a decade with annual surveys and seawater temperature monthly records [17]. In Mljet, the reported mortality of the species ranged from 80% to 100% and it was highly correlated to thermal anomalies and marine heatwaves during the summertime. The southern sector of the MPA was particularly affected; the coral has been assigned as locally extinct from several sites, and its recovery is uncertain [17].

According to the presented results, the 2021 mass mortality event had severely affected the population of *B. europaea* in Chalkidiki peninsula, as, overall, 58% of the observed coral specimens suffered from necrosis. The species mortality was especially high in Toroneos Gulf (GR1270008, Kassandra), where necrosis affected locally over 80% of the surveyed population. Within the same SAC, the condition of *B. europaea* was much better in S3, which is the most exposed site over the surveyed stations. S3 is located at the edge of Kassandra peninsula, at cape Kalogria (“meeting point” of Thermaikos and Toroneos gulfs), an area of very steep bathymetry, influenced by strong currents; this marine area has been less affected by the 2021 marine heatwaves due to its geomorphology and prevailing hydrological conditions [10]. On the contrary, S4, which is located rather close to S3, but in a shelter and rather shallow bay of Kassandra peninsula having front to Toroneos Gulf, was extremely affected. The SACs of Sithonia were impacted in a similar degree, as mortality affected around 57% of the coral’s population in GR1270002 and GR1270007; in GR120009, less than half of *B. europaea* population were assessed in necrosis. The latter SAC, which is located close to the edge of Sithonia peninsula and in the front of Siggitikos Gulf, represents an exposed marine area, less impacted by the 2021 marine heatwave [10].

Severe bleaching and subsequent mass mortality events of *C. caespitosa* have been widely reported from the Mediterranean Sea [13,15,16,19,20,28,50–53] since 1997 [14]. Focusing into the Aegean Sea, only one mass mortality event has been previously reported from the North Turkish coasts, where about 15% of the coral colonies were in necrosis [54]. Due to the long lifespan and slow dynamics, the recovery abilities of affected *C. caespitosa* populations were low [28]. Furthermore, the species exhibit different physiological responses, according to the maximum values of the seawater temperature and the duration of the thermal anomaly, as well as to habitat features and other abiotic factors [16,31]. For example, in the Ligurian Sea [20] and the Spanish Columbretes Islands [31], *C. caespitosa* colonies were not bleached but died due to progressive tissue necrosis, and this response has been attributed to the thermal tolerance of its symbiotic zooxanthellae of the genus *Symbiodinium* [31,55]. On the contrary, bleaching of colonies has been observed in the Adriatic Sea [16]. Totally bleached colonies failed to recover in Mljet MPA, in contrast with the Piran MPA, where the majority of bleached colonies did not suffer from tissue necrosis and recovered, a response attributed to the higher food availability in the latter area [16].

The 2021 mass mortality event has considerably affected *C. caespitosa* as, overall, 27.49% of the observed colonies were partially bleached and another 11.32% showed partial or
complete necrosis. Mortality rate was higher in GR1270002 SAC (Sithonia peninsula). This marine area forms a kind of “interrupted atoll” due to the presence of several islets in irregular crescent shape to the shoreline. Due to the peculiar geomorphology, currents are rather strong ensuring food availability. Dense C. caespitosa population has been reported within this SAC, both as scattered colonies and as a large bank of over 2 m high, which is unique in the Aegean Sea [41,56]. Therefore, this marine area represents a biotope of local importance for the survival of the species. Moreover, considering that C. caespitosa colonies create a complex biogenic habitat that hosts a highly diverse assemblage of macrobenthic invertebrates, over 286 invertebrate species have been reported to live in association with the coral colonies [41]. Since algae rapidly cover the dead coral colonies [16], mass mortality events may have detrimental cascade effects on the biodiversity of associated invertebrate communities, as well.

Balanophyllia europaea was the most widespread stony coral species over the studied area, as it has been found in eight out of the ten surveyed stations; on the contrary, C. caespitosa was recorded at only three sites. The tooth coral (B. europaea) was not found at S5—not even as eroded remnants of polyps in necrosis—a station where it has been previously recorded as abundant (authors’ personal data concerning the period 2000–2010). The Mediterranean pillow coral (C. caespitosa) was absent from the same station (S5), as well as from another two stations (S4 and S9) where it has been previously reported as abundant [36,37], and authors’ personal data; there are no data for its presence on the other four surveyed stations. This result implies the local extinction of B. europaea and C. caespitosa from one and three stations, respectively, during about the last decade raising concerns on their future viability. These stations are located on rocky areas of increasing touristic development and are highly frequented by SCUBA divers, and recreational and professional fishers. Although it is difficult to assess possible anthropogenic impacts, mainly due to the absence of systematic surveys, the collection of C. caespitosa colonies for decorative reasons has been witnessed, whereas the detachment and mechanical damage (i.e., fragmentation) of colonies from small-scale fisheries has been documented in the North Aegean Sea [57,58].

Both coral species were also absent from S2 (located in Loutra Agia Paraskevi Kassandra). S2 constitutes a particular case due to the presence of gas emission vents in shallow depth (0.5–1 m and 5 m), with diffuse bubbling and aligned bubble trains of low or medium flux [59]. White deposits are visible around the orifices and the substrata contains consolidated sand and stones, which are indicative of hot-water emission. The vents contain H2S, which is, however, below the analytical detection limit (<10 ppm) [59]. Although gas exchange between bubbles and seawater seems to be limited [59], the peculiar facies of hydrothermal oozes with impoverished fauna and dense populations of nematodes dominate the benthic community [60]. Therefore, environmental conditions may be unfavorable for the development of stony corals in S2.

This is the first documented mass mortality event of stony corals in the Hellenic Seas, as only anecdotal reports on affected colonies exist. According to the presented results, the mass mortality event has largely affected the population of B. europaea—58% of coral specimens suffered from necrosis—and to a lesser degree of C. caespitosa—27.49% and 11.32% of coral colonies suffered from partial bleaching and partial or complete necrosis, respectively. Persisting marine heatwaves that have affected the north Aegean Sea during summer 2021 [10] have probably caused the reported event [15], which seems to have distress the entire Chalkidiki peninsula. It is also worth noting that the species were absent from sites where they were previously reported as abundant [36,37], indicating that further threats, such as the collection for decorative purposes or from gillnet fisheries, apart from climate change affect their survival. Coastal topography (shelter bays and depth) combined with seawater circulation patterns and mixing may explain the observed differences in mortality rates between the sampling Natura 2000 sites, as healthier populations were recorded at the most exposed sites. Differences in mortality rates of temperate corals in small spatial scales have been attributed to habitat variability with temperature being the triggering factor [16].
Overall, 11 long-live sessile invertebrates were affected over the study area, with stony corals and sponges (S. muscarum, in particular) being among the most impacted species, as previously reported from other Mediterranean regions [14]. The 2021 event was rather unique for the study area, which was severely affected by persistent marine heatwaves [10]. These findings highlight the need for implementing a strategic monitoring plan over SACs to safeguard their management utility in conserving fragile marine habitats and biodiversity. Obviously, the temperate stony corals B. europaea and C. caespitosa represent ideal descriptors for monitoring the impacts of marine warming and the resilience of MPAs under predicted climate crisis.

5. Conclusions

This is the first report of B. europaea mass mortality event and the second of C. caespitosa from the Aegean Sea. Our results demonstrated that this intense and prolonged marine heatwaves that affected the north Aegean Sea, had detrimental effect on shallow water stony corals by devastating 58% and 39% of B. europaea and C. caespitosa populations, respectively. Therefore, both the endemic Mediterranean solitary tooth-coral B. europaea and the colonial pillow coral C. caespitosa seem to be under serious threat in the study area, especially considering the predicted warming trend. Due to their sensitivity to warming, the species may serve as appropriate climate-change descriptors over the Mediterranean Sea to assess the resilience of MPAs. Moreover, seeing that other benthic species, such as sponges (S. muscarum, I. variabilis), bivalves (S. gaederopus) and ascidians (M. sabatieri) suffered as well, recursive monitoring using standard protocols are needed to detect future changes and impacts in larger spatial scales under climate crisis to safeguard the marine biodiversity.

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Institutional Review Board Statement: Ethical review and approval were waived for this study due to the absence of a relevant framework for the usage of stony corals in marine research; moreover, the study was solely based on non-destructive sampling techniques (in-situ visual census and photography of coral specimens) without collecting or performing any experimental treatment that could torture the animals.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy reasons.

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