Accepted Manuscript

Effects of the 2015 heat wave on benthic invertebrates in the Tabarca Marine Protected Area (southeast Spain)

Esther Rubio-Portillo, Andrés Izquierdo-Muñoz, Juan F. Gago, Ramon Rosselló-Mora, Josefa Antón, Alfonso A. Ramos-Esplá

PII: S0141-1136(16)30229-X
DOI: 10.1016/j.marenvres.2016.10.004
Reference: MERE 4239

To appear in: Marine Environmental Research

Received Date: 4 July 2016
Revised Date: 20 October 2016
Accepted Date: 24 October 2016


This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Effects of the 2015 heat wave on benthic invertebrates in the Tabarca Marine Protected Area (southeast Spain)

Esther Rubio-Portillo\textsuperscript{a,b,*}, Andrés Izquierdo-Muñoz\textsuperscript{b}, Juan F. Gago\textsuperscript{c}, Ramon Rosselló-Mora\textsuperscript{c}, Josefa Antón\textsuperscript{d}, Alfonso A. Ramos-Esplá\textsuperscript{a,b}

\textsuperscript{a} Department of Marine Science and Applied Biology, University of Alicante, Alicante, Spain.
\textsuperscript{b} Centro de Investigación Marina de Santa Pola (CIMAR), University of Alicante-Santa Pola Town Council, Cabo de Santa Pola s/n, Alicante, Spain.
\textsuperscript{c} Marine Microbiology Group, Department of Ecology and Marine Resources, Mediterranean Institute for Advanced Studies (IMEDEA, CSIC-UIB), Esporles, Spain.
\textsuperscript{d} Department of Physiology, Genetics and Microbiology, University of Alicante, Alicante, Spain.

*Corresponding author: esther.portillo@ua.es

Abstract

In the late summer of 2015, extensive mortality of scleratinian corals, gorgonians, and sponges was observed in the Marine Protected Area of Tabarca (southeast Spain). Quantitative data indicated that at 25 meters depth the sea fan \textit{Eunicella singularis} was the most affected species (50% of colonies affected by partial mortality); while in shallow waters more than 40% of the endemic scleractinian coral \textit{Cladocora caespitosa} population showed tissue lesions that affected more than 10% of their surfaces. Other affected species were the scleractinian corals \textit{Oculina patagonica} and \textit{Phyllangia mouchezii}, the sea fan \textit{Leptogorgia sarmentosa} and the sponge \textit{Sarcotragus fasciculatus}. This mortality event coincided with an abnormal rise in seawater temperature in this region. Microbiological analysis showed a higher abundance of culturable \textit{Vibrio} species in invertebrates exhibiting tissue lesions, which indicated that these opportunistic pathogens could be a key factor in the process.
Key words: Benthic invertebrates, Tabarca Marine Reserve, Mediterranean Sea; Mortality event

Introduction

The Mediterranean Sea is an enclosed miniature ocean, where the effects of global warming are likely to appear earlier and more intensely than in other more open oceans (Coll et al., 2010) and several studies have confirmed such rapid warming of surface seawater. An increase of 0.04 °C ±0.01 °C year$^{-1}$ in sea surface temperature for the whole Mediterranean basin was detected in recent years (1982-2005; Diaz-Almela et al., 2007). A recent study shows that during the last three decades the summer sea surface temperature has increased on average by 1.15°C, and the warming trends range from 0.25°C decade$^{-1}$ in the western basin to 0.65°C decade$^{-1}$ in the eastern basin (Marbá et al., 2015).

Above normal sea temperatures have been linked to mass mortality outbreaks in coastal ecosystems during the past few decades (Harvell et al., 1999). In the Mediterranean Sea, even short duration temperature anomalies have had serious consequences on marine diversity. In the western Mediterranean, positive thermal anomalies during summer 1999 and 2003 resulted in the most catastrophic mass mortality events observed until now. The 1999 episode was the first major regional event recorded and at least 30 benthic species along hundreds of kilometers of the northwest Mediterranean coastline were affected (Cerrano et al., 2000; Perez et al., 2000; Bensoussan et al., 2010; Crisci et al., 2011). The 2003 heat wave struck a much larger area, almost the whole western Mediterranean, Adriatic and central Mediterranean, and 25 benthic species belonging to the Phyla Cnidaria, Porifera, Bryozoa, and Mollusca were affected (Garrabou et al., 2009).

The direct causes of these events in the Mediterranean remain unknown, although climatic conditions were similar during their occurrence, including temperatures 3–4 °C above average and prolonged water column stability and stagnation in late summer. Exposure to high
temperatures causes physiological stress to marine invertebrates under summer low-food conditions and this appears to be the main driver of severe mass mortality events (Coma et al., 2009). These circumstances could make benthic organisms more susceptible to opportunistic and pathogenic microorganisms. Most marine bacterial pathogens (such as Vibrio spp.) are temperature sensitive, with an expected increase in abundance and virulence under long-term high sea-surface temperatures (Vezzulli et al., 2010; Kimes et al., 2012). Therefore, rising global temperatures due to climate change drive a geographical expansion of pathogens and the spread of disease outbreaks (Harvell et al., 1999, 2002, Baker-Austin et al., 2013; Vezzulli et al., 2013).

In the Mediterranean Sea, two Vibrio pathogens linked to Cnidaria diseases have been identified up to now: Vibrio coralliilyticus, involved in mass mortality events of the purple sea-fan Paramuricea clavata (Bally and Garrabou, 2007; Vezzulli et al., 2010), and V. shiloii, a later heterotypic synonym of V. mediterranei (Thompson et al., 2001), identified as the causative agent of mass bleaching events in Oculina patagonica (Kushmaro et al., 1996, 1997). Other recent studies also point to the implication of these two pathogens in O. patagonica bleaching (Mills et al., 2013; Rubio-Portillo et al., 2014a). Although studies on mass mortalities of other benthic invertebrates in the Mediterranean Sea and the involvement of Vibrio pathogens are very scarce, Martin et al., (2002) and Stabili et al., (2012) described a proliferation of Vibrio spp. in diseased specimens of Eunicella cavolonii and Ircinia variabilis, respectively.

In summer 2015, the Spanish Mediterranean suffered an exceptional heat wave with air temperature records 1.5ºC above the seasonal averages from 1981-2010. This was the second hottest summer since 1961, only exceeded by summer 2003. This anomalous event began at the end of June 2015 and lasted through all July. The latter month was the hottest July recorded, with a monthly average of 26.5ºC. In fact, the southeast Iberian Peninsula, where the Tabarca Marine Protected Area (MPA) is located, reached a new all-time record for the July average
temperature (28.2ºC). This was 2.7ºC higher than the monthly average for the early 1900s (State Meteorological Agency, www.aemet.es).

Hence, the main goal of this paper is to assess the effects of extraordinary episodes of high temperatures on marine benthic invertebrates, using as example the 2015 heat wave on the MPA of Tabarca. We provide data on the main affected species, the intensity of the impact and the depth range affected, as well as the presence of Vibrio pathogens in marine invertebrates with signs of tissue loss. Here, even though we cannot provide quantitative data for all species, we confirm that the mortality signs observed during and after that summer are unusual and may well be related to the impact of the heat suffered in July in this region.

Material and Methods

Study Area

This study was carried out during the summer and autumn of 2015 in the Marine Protected Area of Tabarca (south-western Mediterranean Sea, Spain; Fig. 1). This MPA, created in 1986, was the first in Spain (Ramos, 1985) and has been monitored since 2011 within the framework of the Tropical Signals Program (Moschella, 2008), whose aim is to evaluate the effects of tropicalization of the Mediterranean Sea, as a consequence of climate warming, using reliable representative biological macrodescriptors. This study has been conducted in the MPA because this kind of environment is better suited for observing climate change effects than more disturbed regions, where there is normally more interference from other factors such as anthropogenic stressors (Otero et al., 2013).
Fig. 1. Surveyed locations in the Tabarca Marine Protected Area (western Mediterranean) within the Tropical Signals program.

Temperature datasets

Seawater temperature daily data series (2006-2015) taken at 3m depth from Alicante (2007-2014) and Cabo de Palos buoys, at 60km from Tabarca Island, in the Spanish port network (http://www.puertos.es) were analyzed. Records obtained from the two buoys showed that trends in the thermal anomalies are very similar in both areas, although always somewhat more pronounced off Alicante (Fig. 2). The Cabo de Palos data, showing the highest resolution covering the longest time period of seawater temperature datasets in this region, were used to calculate temperature anomalies throughout summer 2015 in this region (relative to the 2006–2014 baseline).

In situ temperature data were collected daily at 25 m (July-November 2015) depth by a temperature data logger (HOBO ProV2) located at one of the MPA buoys (N 38° 10´ 94” N, 00° 29´60”W). Seawater temperature was also measured each ten minutes at 16 m depth in Alicante bay, at 17 km from Tabarca island, by a salinity and temperature sensor (Alec Electronics COMPACT-CT).
Assessment of impact of the 2015 heat wave on benthic marine organisms

Qualitative and quantitative data on invertebrate health status were acquired to assess the impact of the summer 2015 heat wave. A list of 9 possible affected species was obtained, including mainly sponges, corals and gorgonians that showed tissue necrosis or recent epibiosis (Table 1). The quantitative analysis was focused on the gorgonians *Eunicella singularis* and *Leptogorgia sarmentosa*, the scleractinian corals *Cladocora caespitosa* and *Oculina patagonica*, and the sponge *Sarcotragus fasciculatus* (= *Ircina fasciculata*), since these were the most abundant species affected in the study area. Other species, such as scleractinian coral *Phyllangia mouchezii* and *Balophyllia europaea*, the sponge *Sarcotragus spinulosus* and the bryozoan *Myriapoda truncate*, were also observed during the sampling campaigns. Four sites (S1-S4 in Fig. 1) were studied between 3 and 10m depth to assess the impact on shallow scleractinian corals and sponges, and 2 more sites (S5 and S6 in Fig. 1) were used for gorgonians at 25 meters depth. At each site, surveys were conducted along four 25m long transects and the total number of specimens of each species was recorded. Details were taken of specimens showing necrosis or epibiosis of more than 10% of their surface tissue, and also if the colonies were partially or completely affected (RAC/SPA-UNEP/MAP 2014). Furthermore, results obtained at site 5 during summer 2015 were compared with previous years, 2012 and 2014.

Enumeration of Vibrios and data treatment

Three water samples from the surface and 25 meters depth and four small pieces (~2 cm²) from healthy specimens and those with tissue lesions of *E. singularis*, *L. sarmentosa*, *C. caespitosa*, *O. patagonica* and *S. fasciculatus* were removed from Tabarca MPA in September 2015. Samples were transported to the lab and gently washed three times with 50 ml of sterile filtered seawater (SFSW) to remove non-associated bacteria. Approximately 2 g (wet weight) of tissue of each sample was crushed with 5ml SFSW using a mortar and pestle. For scleractinian corals, after allowing the skeletal matrix to settle, the supernatant (largely containing crushed tissue)
was removed and kept for Vibrio counts. For plate counts of Vibrio spp., 100 µl of 10-fold serial dilutions of seawater samples and crushed tissue were prepared in SFSW, plated on the Vibrio-selective medium thiosulphate citrate bile sucrose (TCBS) agar (Pronadisa, Spain), and incubated at 30ºC for 48 h. For each host species, differences in quantification of Vibrio abundance were tested using one-factor (health status) ANOVA (analysis of variance). Before calculating ANOVA, heterogeneity of variance was tested using Cochran’s C-test.

MALDI-TOF MS analyses of Vibrio strains

A recent study has demonstrated that MALDI-TOF MS profiling can be used for rapid identification of Vibrio environmental isolates (Erler et al., 2014). Consequently we carried out an initial screening for Vibrio strains with MALDI-TOF. A total of 100, isolated from the 5 invertebrate species (see above), were selected on the basis of their color, size and morphology in order to identify the presence of possible Vibrio pathogens in marine invertebrates affected by the 2015 heat wave, using whole cell biomass as previously published (Viver et al., 2015). For pathogen identification, three reference strains (V. mediterranei = V. shiloi AK-1 = CECT 7873, V. mediterranei CECT 623, and V. coralliilyticus LMG 20984<sup>T</sup>). The matching scores of these strains with the reference strains were divided into ranges: highly probable species identifications (>2.3), probable species identifications (2.0–2.3), reliable genus identifications (1.7–2.0) and unreliable identifications (<1.7), and the profiles of Vibrio isolates with matching scores up to 2 with these reference strains were grouped into clusters.

Results and discussion

Thermal anomalies

The mass mortality events observed in the Mediterranean Sea were likely triggered by two types of thermal anomaly (Crisci et al., 2011). The first type is characterized by short episodes (up to 5 days in duration) with mean seawater temperatures around 27 ºC and high intra-day
variability, while the second presents long periods (up to 40 days in duration) with a sea
temperature of up to 24 °C and low intra-period variability. In the region of southeast Spain,
coastal waters underwent a significant warming event in the summer of 2015, staying at 24°C
for over 80 days and the maximum temperature observed during this heat wave reached
28.23°C. Compared with the preceding 9 years, the warming anomaly during July and August
was of 2°C or more and persisted for approximately 6 weeks (Fig. 2).

At 16 meters depth, seawater temperature was at 24°C for 29 consecutive days and at 25m
temperature was above 24°C at the beginning of September (Fig. 3). Usually, 25m corresponds
to the seasonal intermediate thermocline level but the fact that seawater temperature at this
depth was similar to the shallow water temperature indicates that the thermocline remained
below 25m depth during the whole summer. Therefore, the region of southeast Spain showed a
long-term anomaly in 2015 with warm mean seawater temperatures of approximately 24°C
associated with low intra-period variability.

Fig. 2. Monthly temperature anomalies during 2007-2015 for Alicante and Cabo de Palos (°C
with respect to the mean calculated for the entire period) and summer temperature anomalies for
2015 (°C relative to means of the preceding 9 years) generated from Cabo de Palos buoy series.
Fig. 3. Seawater temperature at 16m depth in Alicante bay and at 25m in the Tabarca Marine Protected Area.

After the summer of 2015 a mortality event occurred, affecting coralline algae at depths ranging from 0 to 30m depth in the northwestern Mediterranean Sea, which has been related to positive seawater thermal anomalies (Hereu and Kersting, 2016). Within Tabarca MPA, some important hard-bottom bioengineering species belonging mainly to Class Anthozoa (Table 1) showed signs of mortality in the depth range between 3 and 25m. Although our shallow temperature data are too incomplete to draw any firm conclusions, together with data recorded at 25m depth they indicate the mortality event observed in Tabarca was registered concomitantly with the occurrence of temperatures higher than average.

Table 1. Species affected during the 2015 mass mortality in the Tabarca Marine Protected Area. (A): Species affected by tissue lesions; (NA) species not affected, with only two or three colonies affected; (NO) not enough observations

<table>
<thead>
<tr>
<th>Species</th>
<th>Depth in meters</th>
<th>Nº transects</th>
<th>Nº colonies</th>
<th>Species status (% of colonies affected mean ±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcyonacea</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Eunicella singularis</em></td>
<td>20-25</td>
<td>8</td>
<td>73</td>
<td>A (53.82 ± 4.36)</td>
</tr>
<tr>
<td><em>Leptogorgia sarmentosa</em></td>
<td>20-25</td>
<td>8</td>
<td>38</td>
<td>A (41.11 ± 8.40)</td>
</tr>
<tr>
<td>Scleractinia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cladocora caespiosa</em></td>
<td>3-10</td>
<td>12</td>
<td>25</td>
<td>A (47.22 ± 11.01)</td>
</tr>
<tr>
<td><em>Oculina patagonica</em></td>
<td>3-10</td>
<td>12</td>
<td>44</td>
<td>A (30.09 ± 6.27)</td>
</tr>
<tr>
<td><em>Phyllangia mouchezii</em></td>
<td>20-25</td>
<td>8</td>
<td>3</td>
<td>A, NO</td>
</tr>
<tr>
<td><em>Balanophyllia europaea</em></td>
<td>3-10</td>
<td>12</td>
<td>21</td>
<td>NA</td>
</tr>
</tbody>
</table>
At 3 meters depth, *S. fasciculatus* was affected by the rise in seawater temperature in summer 2015, the first record of disease signs was noted at the beginning of July and by the end of September more than 40% of the population (Table 1) was partially affected by tissue lesions, evident from the disappearing ectosome and the sponge skeleton becoming visible (Fig. 4a). Furthermore, skeletons of completely dead specimens were occasionally observed on the beaches of the MPA (personal observations), likely as a result of this event. Extensive mortalities in *S. fasciculatus* populations have been previously related to thermal anomalies, with similar characteristics to those reported here, for locations off southern Spain (Maldonado et al., 2010), the Balearic Islands and Corsica (Cebrian et al., 2011), the southern Adriatic and Ionian Sea (Stabili et al., 2012), and the northern Adriatic Sea (Di Camilo et al., 2013).
Fig. 4. Tissue lesions in affected invertebrate species during the 2015 mass mortality event in the Tabarca Marine Protected Area. a) Sarcotragus fasciculatus; b) Cladocora caespitosa; c) Oculina patagonica; d) Eunicella singularis; e) Leptogorgia sarmentosa; f) Phyllangia mouchezii.

Among the shallow scleractinian corals present in the MPA, the endemic C. caespitosa (Fig. 4b) was the shallow species most affected by the mortality event, resulting in the total or partial death of almost 50% of its population (Table 1). This species also showed tissue necrosis signs in other areas along the east coast of Spain, such as Columbretes MPA (Kersting, pers. comm.), where this species has suffered recurrent tissue necrosis since 2003, following summers in which positive thermal anomalies have been recorded (Kersting et al., 2013). The scleratinian coral O. patagonica showed lower percentages of tissue damage than C. caespitosa (Table 1). These were similar to those detected in the same study area in 2011 (Rubio-Portillo et al., 2014b), when temperatures over 26°C were maintained for 54 consecutive days (Rubio-Portillo et al., 2014b). However, in the 2015 mortality event, O. patagonica showed tissue necrosis (Fig. 4c) instead of the bleaching observed in 2011.

Nevertheless, the solitary scleractinian coral Balanophyllia europaea is a common species in shallow waters in Tabarca MPA and was not affected by this event, since all its colonies showed normal coloration (n=21). However, this species has undergone mass bleaching followed by tissue necrosis in previous years during thermal anomalies, such as those observed off the coast of Genoa and Spain in 2003 (Garrabou et al., 2009; Kersting and Templado, 2006), and in the Adriatic Sea in 2003, 2009 and 2012 (Kružić and Popijač, 2014). These differences are probably because populations exposed to contrasting temperature regimes can exhibit different degrees of resilience to global warming (Hughes et al., 2003), as demonstrated in other Mediterranean species (Linares et al., 2005).
At 25 meters depth, the most affected species were the gorgonians *E. singularis* and *L. sarmentosa* (Table 1). These two species displayed a 4 to 8-fold increase in affected colonies compared to previous years (Table 2), which is an excellent descriptor to quantify the impact of the mass mortality event in Tabarca MPA. Both species firstly showed tissue necrosis that resulted in denuded axes, and finally at the end of September and early October their axes began to show epibiosis (Fig. 4d and 4e). Such a pattern could result from exposure to high temperatures that reduce the fitness of Mediterranean gorgonians as a consequence of a reduction in their metabolic activity, as previously observed in experimental aquaria by Previati *et al.* (2010).

**Table 2.** Mean percentage of colonies with more than 10% of colony surface showing tissue lesions from the same gorgonian populations (study site S5, see Fig. 1) in different years. (NA) not affected

<table>
<thead>
<tr>
<th>Species</th>
<th>Survey years</th>
<th>Nº colonies</th>
<th>% Affected colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Eunicella singularis</em></td>
<td>2012</td>
<td>25</td>
<td>6.60 ± 3.88</td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td>35</td>
<td>7.88 ± 5.14</td>
</tr>
<tr>
<td></td>
<td>2015</td>
<td>31</td>
<td>49.87 ± 3.65</td>
</tr>
<tr>
<td><em>Leptogorgia sarmentosa</em></td>
<td>2012</td>
<td>10</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td>13</td>
<td>8.9 ± 3.52</td>
</tr>
<tr>
<td></td>
<td>2015</td>
<td>12</td>
<td>41.66 ± 4.81</td>
</tr>
</tbody>
</table>

Two specimens of the scleractinian coral *Phyllangia mouchezii* also showed tissue loss during the heat wave (Fig. 6f), but this species was rare at the sampling sites (n=3) and data was insufficient to determine the degree of injury in this species as a consequence of heat stress. Tissue necrosis of *P. mouchezii* was not observed in the severest mass mortality events that affected the western Mediterranean basin in 1999 and 2003, nor in 2012 in the Levantine area (Jimenez *et al.*, 2014), which suggests that this species is probably more thermotolerant than other Mediterranean coral species.

Detection of *Vibrio spp.* in the affected invertebrates
Besides anomalous temperature conditions, mortality due to thermo-dependent pathogens cannot be ruled out, considering that experimental and field data confirm that the rise of seawater temperature promotes pathogen virulence and/or favors host susceptibility (Martinez-Urtaza et al., 2010; Vezzulli et al., 2010; Kimes et al., 2012; Gallana et al., 2013; Maynard et al., 2015). In marine habitats, the spread of Vibrio-related diseases has been identified as an emerging global concern correlated to rising seawater temperatures (Cervino et al., 2004; Martinez-Urtaza et al., 2010; Vezzulli et al., 2012, 2013; Baker-Austin et al., 2013). Furthermore, Vibrio species were recently identified as specific etiological agents in mass mortalities of benthic invertebrates in the Mediterranean. V. coralliilyticus has been implicated in diseases affecting the gorgonian Paramuricea clavata (Bally and Garrabou 2007) and V. mediterranei in O. patagonica bleaching (Kushmaro et al., 1996; 1997; Rubio-Portillo et al., 2014a). Vibrios have also been related to the appearance of disease signs in the genus Ircinia (=Sarcotragus) in the Mediterranean Sea (Stabili et al., 2012), although in this case Koch’s postulates were not satisfied to prove etiology.

In order to assess the possible relationship between rising seawater temperature, presence of Vibrio spp. and the appearance of tissue lesions in marine invertebrates, we collected water and tissue samples from healthy and diseased invertebrates and used them to inoculate a Vibrio-specific culture medium. In seawater samples, Vibrio spp. reached 3.39x10^4 ± 4.01x10^3 CFU l^-1 and 4.45x10^3 ± 1.95x10^2 CFU l^-1 at 3 and 25 meters depth, respectively. The cultivable Vibrio counts at 3 meters were about 10-fold higher than values detected in the same area in 2011, when a bleaching event in O. patagonica was recorded (Rubio-Portillo et al., 2014a). The higher occurrence of Vibrio spp. in shallow waters is probably correlated with temperature, as observed in previous studies that showed Vibrio spp. abundance in seawater was higher during warmer months (Vezzulli et al., 2010). Vibrio counts in invertebrate samples collected during the studied mortality event were higher in specimens that showed tissue lesions in all species, but abundances were significantly higher in E. singularis, S. fasciculatus, O. patagonica and C. caespitosa (ANOVA, p<0.005), with a 5 to 15-fold increase in affected colonies compared to
specimens that did not show signs of disease (Fig. 5). Therefore, the increase in Vibrio numbers appears to be correlated with the health status of the benthic invertebrates studied in this work. It is therefore likely that these bacteria were involved as etiological agent in the mortality event observed in the Tabarca MPA during summer 2015. In fact, among the 100 selected Vibrio isolates analyzed by MALDI-TOF, 11 isolates from diseased specimens of E. singularis, S. fasciculatus and O. patagonica showed high similarities with V. mediterranei, with scores ranging from 2 to 2.4 (Fig. 6 and Table 3). The identification of the majority of the isolates as V. mediterranei is in accordance with the type strain AK-1 of the species being previously identified as the causative agent of mass bleaching events in O. patagonica (Kushmaro et al., 1997). Another 4 strains were also thus related to the coral pathogen V. coralliilyticus, although all were isolated from apparently healthy organisms (Table 3).

** Fig. 5. ** Concentration of culturable vibrios in healthy and diseased colonies of marine invertebrates. ** p = 0.05
Figure 6. Dendrogram of relatedness between *Vibrio* isolates from marine invertebrates and type strains LMG20984 *V. coralliilyticus*, CECT 623 *V. mediterranei*, CECT 7873 *V. mediterranei* AK-1 as reference.

Table 3. Strains exhibiting high MALDI-TOF mass relatedness together with type strains LMG20984 *V. coralliilyticus*, CECT 623 *V. mediterranei*, CECT 7873 *V. mediterranei* AK-1.

<table>
<thead>
<tr>
<th>Strain ID</th>
<th>Invertebrate specie</th>
<th>Health status</th>
<th>Reference strain</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>IrU_323</td>
<td><em>S. fasciculatus</em></td>
<td>Tissue lesions</td>
<td>CECT 7873 <em>V. mediterranei</em></td>
<td>2.51</td>
</tr>
<tr>
<td>IrU_328</td>
<td><em>S. fasciculatus</em></td>
<td>Tissue lesions</td>
<td>CECT 7873 <em>V. mediterranei</em></td>
<td>2.471</td>
</tr>
<tr>
<td>EuE_334</td>
<td><em>E. singularis</em></td>
<td>Healthy</td>
<td>CECT 7873 <em>V. mediterranei</em></td>
<td>2.461</td>
</tr>
<tr>
<td>IrH_312</td>
<td><em>S. fasciculatus</em></td>
<td>Healthy</td>
<td>CECT 7873 <em>V. mediterranei</em></td>
<td>2.437</td>
</tr>
<tr>
<td>IrU_303</td>
<td><em>S. fasciculatus</em></td>
<td>Tissue lesions</td>
<td>CECT 7873 <em>V. mediterranei</em></td>
<td>2.349</td>
</tr>
<tr>
<td>IrU_324</td>
<td><em>S. fasciculatus</em></td>
<td>Tissue lesions</td>
<td>CECT 623 <em>V. mediterranei</em></td>
<td>2.299</td>
</tr>
<tr>
<td>EuU_333</td>
<td><em>E. singularis</em></td>
<td>Tissue lesions</td>
<td>CECT 7873 <em>Vibrio mediterranei</em></td>
<td>2.247</td>
</tr>
<tr>
<td>EuH_343</td>
<td><em>E. singularis</em></td>
<td>Healthy</td>
<td>LMG 20984 <em>V. coralliilyticus</em></td>
<td>2.187</td>
</tr>
<tr>
<td>CIH_377</td>
<td><em>C. caespitosa</em></td>
<td>Healthy</td>
<td>LMG 20984 <em>V. coralliilyticus</em></td>
<td>2.163</td>
</tr>
<tr>
<td>IrU_309</td>
<td><em>S. fasciculatus</em></td>
<td>Tissue lesions</td>
<td>CECT 7873 <em>V. mediterranei</em></td>
<td>2.134</td>
</tr>
<tr>
<td>OcH_375</td>
<td><em>O. patagonica</em></td>
<td>Healthy</td>
<td>LMG 20984 <em>V. coralliilyticus</em></td>
<td>2.08</td>
</tr>
<tr>
<td>OcH_376</td>
<td><em>O. patagonica</em></td>
<td>Healthy</td>
<td>LMG 20984 <em>V. coralliilyticus</em></td>
<td>2.072</td>
</tr>
<tr>
<td>OcU_367</td>
<td><em>O. patagonica</em></td>
<td>Tissue lesions</td>
<td>CECT 7873 <em>V. mediterranei</em></td>
<td>2.059</td>
</tr>
<tr>
<td>OcU_368</td>
<td><em>O. patagonica</em></td>
<td>Tissue lesions</td>
<td>CECT 7873 <em>V. mediterranei</em> AK-1</td>
<td>2.032</td>
</tr>
<tr>
<td>OcU_371</td>
<td><em>O. patagonica</em></td>
<td>Tissue lesions</td>
<td>CECT 7873 <em>V. mediterranei</em></td>
<td>2.006</td>
</tr>
</tbody>
</table>
Conclusion

The mass mortality event in the summer of 2015 is the first recorded in the Marine Protected Area of Tabarca. As in other mortality events in the Mediterranean Sea during recent decades, cnidarians and sponges were the most affected groups. However, it cannot be ascertained whether the rise in seawater temperature had only a direct effect on invertebrates or has a potential multifactorial origin, as suggested previously (Garrabou et al., 2009; Kersting et al., 2013, 2015; Rodolfo-Metalpa et al., 2014). Probably these events are combined with the presence of potential microbial pathogens, which considerably increase their abundance with temperature. This work shows a relationship between seawater temperature, presence of *Vibrio* coral pathogens and the appearance of benthic invertebrate tissue lesions. Considering the present warming trend in the Mediterranean, new mass mortality events may occur in the near future. Therefore, programs monitoring vulnerable populations of gorgonians, corals and sponges would be best combined with physical and microbial parameters. These programs should be established in Marine Protected Areas, which are potentially resilient sites (or refugia) for these threatened species.

Acknowledgments

The authors are grateful to the staff at the Department of Marine Sciences and Applied Biology, especially Carlos Valle for his assistance in the sea, and we wish to acknowledge the friendly cooperation received from the Fisheries Secretary of the Spanish Ministry of Agriculture, Food and Environment, and Tabarca Marine Reserve guards (particularly Felio Lozano). We also thank Jose Luis Sánchez-Lizaso and Yolanda Fernández-Torquemada for the data on sea water temperature at 16 meters depth in Alicante bay. Thank also to Guido Jones for reviewing the English version. This work has been performed within the framework of the CIESM (Mediterranean Science Commission) International Program 'Tropical signals' coordinated by
Paula Moschella and co-supported by Prince Albert II of Monaco Foundation. RRM and JFG acknowledge the financial support of Deep Blue Sea Enterprise for the mass spectrometry identification.

References


Mills E, Shechtman K, Loya Y, Rosenberg E (2013) Bacteria appear to play important roles in both causing and preventing the bleaching of the coral *Oculina patagonica* *Mar Ecol Progr Ser* 489:155–162.


**Formatting of funding sources**

This work has been performed within the framework of the CIESM (Mediterranean Science Commission) International Program 'Tropical signals', co-supported by Prince Albert II of Monaco Foundation. RRM and JFG acknowledge the financial support of Deep Blue Sea Enterprise for the mass spectrometry identification.
Highlights

In the summer of 2015 a mass mortality event was recorded in the Marine Protected Area of Tabarca.

This mortality event coincided with an abnormal rise in seawater temperature in the region.

Opportunistic *Vibrio* pathogens were detected in invertebrates tissue lesions.