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EFFECTS OF LOCAL AND GLOBAL STRESSORS ON THE STATUS AND FUTURE PERSISTENCE OF INTERTIDAL CANOPY-FORMING ALGAE

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"*I can only compare these great aquatic forests . . . with the terrestrial ones in the intertropical regions. Yet if in any country a forest was destroyed, I do not believe nearly so many species of animals would perish as would here, from the destruction of the kelp. Amidst the leaves of this plant numerous species of fish live, which nowhere else could find food or shelter; with their destruction the many cormorants and other fishing birds, the otters, seals, and porpoise, would soon perish also; and lastly, the Fuegian[s] . . . would . . . decrease in numbers and perhaps cease to exist.*" Charles Darwin, 1 June 1834, Tierra del Fuego, Chile (Darwin 1909, pp. 256–257).

Effect of local and global stressors on the status and future persistence of intertidal canopy-forming algae

Abstract

Marine canopy-forming seaweeds are among the most important habitat-forming species along temperate and polar rocky coasts. They form diverse, productive and valuable "forest" habitats that play a key role in coastal primary production, nutrient cycling and disturbance regulation, and facilitate abundant algae and animal communities. They are also some of the most heavily impacted coastal habitats, facing increasing pressures from urban sprawl, pollution, overfishing and climatic instabilities. Identifying the type and strength of interactions between multiple anthropogenic and natural stressors can help setting achievable management targets for degraded ecosystems and support ecological resilience through local actions.

My research focuses primarily on understanding the effects of multiple local and global stressors on canopy-forming seaweeds of the genus *Cystoseira*, with and emphasis on field investigation and experimentation. I approached my research integrating different approaches:

1) I investigated which are the factors driving the loss of canopy-forming seaweeds at global level. The results allowed to detect important synergistic interactions between nutrient enrichment, caused mainly by human activities, and different other stressors such as heavy metals, the presence of competitors, low light and increasing temperature. This suggested that local management of nutrient levels would provide the greatest opportunity for preventing the shift from canopy to mat-forming algae;

2) as second step, I analysed the status of the intertidal *Cystoseira* populations around the Italian coast, and explored which factors are most likely to influence it. The results reported severe depletion of intertidal populations of *Cystoseira*, and identified urbanization as one of the main factors related to these poor conditions, confirming the need for urgent management actions to reduce human pressures on these valuable habitat forming species;

3) then, I experimentally investigated the effects of extreme events caused by climate change. I focused my attention on the effects of abrupt increases of air temperature caused by heat-wave events on the intertidal *C. compressa* around the Italian coast. The results indicated that extreme heat-wave negatively affect the photosynthetic activity of *C.*

compressa, and that local biodiversity and thermal history of the alga seem to play a role reducing or increasing respectively the impact of such extreme events.

4) I also explored the possible overlooked role of the epiphytic bacteria growing on *C. compressa*. Bacteria can interact with seaweeds in symbiotic, pathological and opportunistic ways, modulating the health, performance and resilience of their hosts and could, therefore play a critical role on the responses of *Cystoseira* spp. to stress factors. I characterised for the first time the epiphytic bacteria associated to the surface of *C. compressa* using Illumina Miseq sequences of V1-V3 hypervariable regions of 16S rRNA gene, and investigated their seasonal variations and their relationships with the bacterial populations in the surrounding seawater. I found that bacterial populations associated to *C. compressa* were clearly distinct from those in the surrounding media, and identified a clear successional pattern, interestingly characterized by an increase in abundance of potential microbial pathogens associated to older thalli of *C. compressa*;

5) the previous quantitative descriptive work represented an important base-knowledge to further explore experimentally whether surface bacteria could influence the responses of their hosting *Cystoseira* populations to stressors. I analysed experimentally in the field the interacting effects of nutrient enrichment and heat-wave events on *C. compressa* population, and explored whether any resulting changes in the photosynthetic activity of *C. compressa* were associated to changes in the epiphytic bacterial communities. The heat wave caused marked declines of the photosynthetic activity of the intertidal *C. compressa*. These effects persisted for at least 3 hours, while recovery generally occurred after 24 hours. The heat-wave altered the structure of the epiphytic bacteria of *C. compressa*. Thalli exposed to the heat-wave presented an increase of OTUs previously shown to be associated with the natural degradation of the thalli of *C. compressa*, or implied in causing disease or damage to macroalgae. As observed for the photosynthetic activity, these differences decreased over time, suggesting that the microbial community has the ability to recover. Differently from previous work, this experiment did not detect significant effects related to nutrient enrichment, suggesting that the effects of nutrients could be context dependent. These results open new questions concerning the mechanisms by which the epibacterial community could influence the responses and future persistence of these important canopy-forming seaweeds.

EFFECT OF LOCAL AND GLOBAL STRESSORS ON THE STATUS AND FUTURE PERSISTENCE OF INTERTIDAL CANOPY-FORMING ALGAE

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 5

Introduction

Human activities: effect on marine coastal habitats

Coastal marine habitats comprise some of the most productive, diverse and at the same time overexploited marine ecosystems (Airoldi and Beck, 2007). With over 39% of the world population living within 100 kilometres of the coast and exploiting the ecosystem services offered by coastal habitats, it's not surprising that human activities are taking their toll (Cesar et al., 2003). Pollution, overfishing and coastal transformation are just some of the impacts caused by increasing human population and pressures in coastal areas. These impacts are further exacerbated by global climatic changes, such as sea-level rise, ocean acidification, increased sea-surface temperatures and increased frequency of extreme events, amplifying the vulnerability and the loss of marine coastal ecosystems (Airoldi and Beck, 2007; Crain et al., 2009; Coll et al., 2010). Given the multitude of marine threats and the limited resources allocated for conservation, management efforts must prioritize how and where time and money is spent (Crain et al., 2009). In this perspective, efforts should focus on foundation, habitat-forming, species, which are critical for supporting biodiversity and ecosystem functioning (Bruno and Bertness, 2001). Habitat forming species, such as coral reefs, algal forests, seagrass meadows, oyster reefs, mangroves, and salt marshes build the three-dimensional structure that provides habitat for thousands of other species and can also control the physical environmental conditions; the loss of these structures thus reduces marine habitat and causes significant repercussions on the entire ecosystem (Airoldi and Beck, 2007; Duarte et al., 2013; Ferrario et al., 2014). Another important aspect concerns the identification of which stressors, or combinations of multiple stressors, mostly drive the regression and loss of these important habitats, in order to prioritise the most cost-effective management actions.

Multiple stressors affecting canopy-forming macroalgae.

In most cases the deterioration of marine ecosystems is driven by the combined effects of multiple local and global stressors, which can have additive or nonadditive (antagonistic or synergistic) cumulative effects (Halpern et al., 2007; Crain et al., 2008, 2009; Darling and Cote, 2008). The cumulative pressures of multiple stressors can cause declines in foundation species and loss of critical habitats (Ban et al., 2014; Strain et al., 2014). Canopy-forming algae (most often comprising large brown seaweeds from the orders Laminariales or Fucales) are the dominant habitat-forming structures along in many temperate rocky coasts in both intertidal and subtidal habitats (Steneck et al., 2002; Schiel and Foster, 2006; Smale and Wernberg, 2013). When canopy-forming algae are lost they tend to be often replaced by low lying, smaller and structurally less complex turf-forming algae (Gorman, 2009; Gorman and Connell, 2009; Perkol-Finkel and Airoldi, 2010; Smale and Wernberg, 2013; Connell et al., 2014). These habitat shifts have been attributed to a variety of local and global stressors on either canopy-forming algae, or mat-forming algae or both (Steneck et al., 2002; Strain et al., 2014), but the possible interactions between these cumulative impacts had been little explored. The potential interactions among multiple stressors can produce synergistic effects (greater decrease or increase in growth or survival of the target taxa than resulting from the sum of the effects from each separate stressor), or antagonistic effects (lesser decrease or increase in growth or survival than resulting from the sum of the separate stressors) (Crain et al., 2008). Identifying the type of interactions has profound management implications, as synergies accelerate habitat shifts but also provide opportunities for remediation at the local scale, and therefore should be prioritized for management strategies (Brown et al., 2013; Strain et al., 2014). Generally, local stressors are easier to control by well-designed management interventions (e.g. improving water quality, creating marine reserves), while reducing global stressors, such as those resulting from climate change including increased temperatures, heat-wave events, droughts and ocean acidification, requires collaboration among countries or international management bodies, and is less amenable from a management point of view (Brown et al., 2013). Predicting the responses of marine systems to the cumulative effects of environmental and human induced changes remains one of the main challenges for community ecologists and conservation biologists.

Marine canopy forming seaweeds of the genus Cystoseira

Canopy-forming species of genus *Cystoseira* C. Agardh (Fucales, Phaeophyceae) are especially diverse in Mediterranean Sea (Oliveras-Plá and Gómez-Garreta, 1989; Draisma et al., 2010), providing food and protection for rich associated communities, comprising other algae, invertebrates and fish (Mineur et al., 2015). *Cystoseira* spp. are distributed from the infralittoral down to the upper circalittoral zone enhancing the structural complexity and productivity of coastal communities (Ballesteros et al., 2009; Falace and Bressan, 2006;

Bulleri et al., 2002; Giaccone et al., 1994). Environmental parameters such as depth, water temperature, substratum characteristics, wave exposure and seawater nutrients play an important role on the natural distribution of *Cystoseira* species (Giaccone 1971; Ballesteros 1992; Sales and Ballesteros 2009). Further, a variety of human pressures are increasingly reducing the distribution of these species (Chryssovergis and Panayotidis, 1995; Rodríguez-Prieto and Polo, 1996; Soltan et al., 2001; Arevalo et al., 2007; Sales et al., 2011). During the last decades several *Cystoseira* species have retracted their ranges considerably (Airoldi et al. 2014) to the point where several species have been reported to be locally lost (Soltan et al., 2001; Thibaut et al., 2005; Serio et al., 2006; Mangialajo et al., 2007, 2008; Perkol-Finkel and Airoldi, 2010; Thibaut et al., 2015). The loss of *Cystoseira* canopies leads to structurally less complex communities most often dominated by low-lying, turf-forming species (Benedetti-Cecchi et al., 2001; Connell et al., 2014) or sea urchin barrens (Agnetta et al., 2015), causing a general homogenization of the habitats (Airoldi et al., 2008). These evidences prompted ecologists to use *Cystoseira spp*. as biological indicators of water and ecosystem quality (Panayotidis et al., 1999), and some studies (Ballesteros et al., 2007; Mangialajo et al., 2007) are already using these species for the assessment of marine environmental quality according to the Water Framework Directive (2000/60/EC).

Aims, general approach and structure of the thesis.

My PhD research has focused on exploring the effects of multiple local and global stressors on canopy-forming seaweeds, with an emphasis on field experimentation. First, I investigated which are the factors driving the loss of canopy-forming seaweeds at global level. Then, I described quantitatively the status of the intertidal species of *Cystoseira* around the Italian coast, with a particular focus on *C. compressa*, which is the only common and widespread species remaining nowadays along the Italian coasts. Based on the results obtained from this initial work, I carried out manipulative field experiments to test the combined effects of two stressors, excess nutrient levels and extreme heat-wave events (related to climate change). Finally I explored the potential role of the microbial communities in influencing the responses of *C. compressa*. The thesis has been organized in 5 chapters, corresponding to as many stand-alone manuscripts for publication, with possible cross-references.

Chapter one: a meta-analysis was used to identify the type and strength of interactions between local anthropogenic and other stressors, driving the shift from canopy-forming seaweeds towards turf-forming algae. The results showed that nutrient enrichment, caused mainly by human activities, interacts synergistically with different other stressors such as heavy metals, presence of competitors, low light and increasing temperature. This suggested that the management of local nutrient levels, would provide the greatest opportunity for preventing the shift from canopy-forming seaweeds to turf-forming algae.

Chapter two: an extensive field sampling was used to quantify the status of intertidal *Cystoseira* populations around the Italian coasts. An extraction of environmental and anthropogenic parameters from various databases completed the dataset. The aim was to explore which environmental and anthropogenic factors mostly relate to the status of *Cystoseira* populations. Coastal human population density within a 10 km radius, and average annual marine concentrations of chlorophyll-a and nitrates were significantly related to the status of *C. compressa*, explaining 40% of the variations among sites. Wave exposure, distance from urban centre, PAR, salinity, tidal range and seawater temperature did not show to have a significant role. Coastal human population density explained alone about 27% of the variation confirming the need for urgent management actions to reduce human pressures on these valuable habitats forming species.

Chapter three: I used field experiments to analyse the effects of heat-wave events related to climate change on intertidal populations of *C. compressa* at 10 sites around the Italian coast during low tide (emersion phase). I also explored whether local biodiversity and or recent thermal history of *C. compressa* can play a role in mediating responses of *C. compressa* to thermal stress. The results indicate that extreme heat-waves drastically reduced the photosynthetic activity of *C. compressa*. The effect was significant at all sites and persisted also after three hours of recovery. Some recovery occurred during the subsequent 24 hours, but patterns were nit consistent across location. Moreover, local biodiversity and recent thermal history of *C. compressa* seemed to affect the responses observed. In particular a greater local species diversity decreased the impact of the heat wave, while populations of *C. compressa* living close to their thermal limits seemed to be the most impacted.

Chapter four: I characterised for the first time the epiphytic bacteria that grow on *C. compressa* using molecular tools. Bacteria are the first colonizer of algal surfaces capable of metabolizing algae exudates, and interact with seaweeds in symbiotic, pathological and opportunistic ways, modulating the health, performance and resilience of their hosts. I also explored the temporal dynamic of the epiphytic bacteria growing on *C. compressa* over an annual vegetative cycle, and their relationships with the bacterial populations in the surrounding seawater. The results showed a clear distinction between the bacterial communities of *C. compressa* and the surrounding seawater. Moreover, there was a clear successional pattern in the epiphytic bacteria of *C. compressa* over time, characterized by an increase in abundance of potential microbial pathogens associated to older thalli of *C. compressa*. The increase of potential pathogens with seaweeds' senescence leads to hypothesize that bacteria can play a role in the resilience capability of their host and, could therefore potentially affect the responses of *Cystoseira* spp. to stress factors.

Chapter five: I analysed experimentally in the field the interacting effects of nutrient enrichment and heat-wave events on *C. compressa* population, and explored whether any resulting changes in the photosynthetic activity of *C. compressa* were associated to changes in the epiphytic bacterial communities. Heat waves caused a decline of the photosynthetic activity of *C. compressa*. This effect was still evident after 3 hours from the impact, but recovery was observed after 24 hours. The observed effects were paralleled by significant changes in the structure of the epiphytic bacteria associated to *C. compressa*. Thalli exposed to the heat wave presented an increase of bacterial OTUs previously associated with the natural degradation of the thalli of *C. compressa*, or implied in causing disease or damage to macroalgae. As observed for the photosynthetic responses, these differences were not persistent over time, suggesting that the microbial community has the ability to recover. Differently from the results of chapter 1, I did not observe significant effects related to nutrients enrichment, suggesting that the effects of nutrients could be context dependent. These results open new questions concerning the mechanisms by which the holobiont community could influence the responses and future persistence of these important canopy-forming seaweeds.

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Chapter 1

Identifying the interacting roles of stressors in driving the global loss of canopy-forming to mat-forming algae in marine ecosystems[1]

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Abstract

Identifying the type and strength of interactions between local anthropogenic and other stressors can help to set achievable management targets for degraded marine ecosystems and support their resilience by identifying local actions. We undertook a meta-analysis, using data from 118 studies to test the hypothesis that ongoing global declines in the dominant habitat along temperate rocky coastlines, forests of canopy-forming algae and/or their replacement by mat-forming algae are driven by the non additive interactions between local anthropogenic stressors that can be addressed through management actions (fishing, heavy metal pollution, nutrient enrichment and high sediment loads) and other stressors (presence of competitors or grazers, removal of canopy algae, limiting or excessive light, low or high salinity, increasing temperature, high wave exposure and high UV or CO2), not as easily amenable to management actions. In general, the cumulative effects of local anthropogenic and other stressors had negative effects on the growth and survival of canopy-forming algae. Conversely, the growth or survival of mat-forming algae was either unaffected or significantly enhanced by the same pairs of stressors. Contrary to our predictions, the majority of interactions between stressors were additive. There were however synergistic interactions between nutrient enrichment and heavy metals, the presence of competitors, low light and increasing temperature, leading to amplified negative effects on canopy-forming

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algae. There were also synergistic interactions between nutrient enrichment and increasing CO2 and temperature leading to amplified positive effects on mat-forming algae. Our review of the current literature shows that management of nutrient levels, rather than fishing, heavy metal pollution or high sediment loads, would provide the greatest opportunity for preventing the shift from canopy to mat-forming algae, particularly in enclosed bays or estuaries because of the higher prevalence of synergistic interactions between nutrient enrichment with other local and global stressors, and as such it should be prioritized.

Keywords: Anthropogenic stressors, canopy-forming algae, habitat shifts, management, mat-forming algae

Introduction

Marine ecosystems are increasingly being subjected to multiple stressors (Halpern et al., 2007; Crain et al., 2009). The interactions between these stressors can have additive or nonadditive (i.e. antagonistic or synergistic) effects on marine ecosystems (Crain et al., 2008; Darling & Cote, 2008). Stressor interactions can alter food-web complexity, relationships between species, diversity within functional groups, the distribution, range and size of organisms or populations and the biogenic habitat structure (Vinebrooke et al., 2004; Adams, 2005; Crain et al., 2008). In some extreme cases, the nonadditive interactions between multiple stressors can result in shifts between alternative habitats (Scheffer et al., 2001; Folke et al., 2004; Petraitis & Dudgeon, 2004). These newly established habitats typically consist of species of lesser ecological, functional and human value than those that have been replaced, and can persist for decades without management, restoration or intervention actions (Suding et al., 2004; Jones & Schmitz, 2009). Stressor interactions are driven by a range of processes which operate at different scales (Crain et al., 2008; Darling & Cote, 2008). In marine ecosystems, anthropogenic stressors (such as fishing, heavy metal pollution, nutrient enrichment and sedimentation) are predominantly driven by local processes (Knowlton & Jackson, 2008; Cote & Darling, 2010; Brown et al., 2013). These so called local anthropogenic stressors are more easily amendable to management and conservation actions than other types of stressors (i.e. biological, environmental or climatic), which can be driven by a complex suite of indirect triggers or processes (Russell & Connell, 2012; Brown et al., 2013). Thus, there is a growing interest in identifying the type and role of interactions between local anthropogenic and other stressors in driving habitat shifts in marine ecosystems (Carilli et al., 2012; Russell & Connell, 2012; Brown et al., 2013).

Canopy-forming algae or large brown seaweeds (defined as species from the orders Laminariales or Fucales) are the dominant organisms in many temper- ate rocky reefs in both intertidal and subtidal habitats (Steneck et al., 2002; Schiel & Foster, 2006; Smale et al., 2013). These species provide food, habitat, protection and structural complexity, and enhance biodiversity and productivity in coastal ecosystems (Dayton, 1985; Chapman, 1995). There is growing concern about the loss of canopy-forming algae, particularly in many urban areas across the world, e.g. Australia (Coleman et al., 2008; Connell et al., 2008; Smale & Wernberg, 2013), North America, (Steneck et al., 2002), Europe (Benedetti-Cecchi et al., 2001; Eriksson et al., 2002; Thibaut et al., 2005; Perkol-Finkel & Airoldi, 2010) and Japan (Okuda, 2008). Often these forests are being replaced by low lying, smaller and structurally less complex species of persistent turf-forming algae or ephemeral algae which are commonly defined as mat- forming algae (Gorman & Connell, 2009; Perkol-Finkel & Airoldi, 2010; Connell et al., 2013, 2014; Wernberg et al., 2013). Once established, these mat-forming algae can inhibit the recolonization of canopy-forming algae (Kennelly, 1987; Steen, 2004; Raberg et al., 2005; Gorman & Connell, 2009), thus forming an alternative stable state (Petraitis & Dudgeon, 2004; Connell, 2005). In many cases, these habitat shifts have been attributed to the effects on either canopy-forming algae, or mat- forming algae or both of nonadditive interactions between local anthropogenic and other stressors or the cumulative effects of multiple local anthropogenic stressors (Steneck et al., 2002; Petraitis & Dudgeon, 2004; Connell et al., 2008; Forster & Schiel, 2010; Wahl et al., 2011; Russell & Connell, 2012). The nonadditive interactions can result synergistic effects, a greater decrease or increase in growth or survival of the target taxa than the sum of the separate stressors, or antagonistic effects, a lesser decrease or increase in growth or survival than the sum of the separate stressors (Crain et al., 2008). Identifying the type of interaction has pro- found management implications, as synergies accelerate habitat shifts but also provide the greatest opportunity for remediation at the local scale, and therefore should be prioritized for management strategies (Brown et al., 2013).

Previous studies have suggested the key local anthropogenic stressors that could facilitate this habitat shift could include overfishing of higher trophic groups leading to outbreaks of grazers (Duffy & Hay, 2000; Tegner & Dayton, 2000; Steneck et al., 2002), eutrophication (Worm et al., 1999, 2001; Eriksson, 2002; Berger et al., 2004; Gorman & Connell, 2009), excess sediment loads (Devinny & Volse, 1978; Airoldi, 2003; Connell, 2003; Eriksson & Johansson, 2005; Irving et al., 2009), pollution from heavy metals (Andersson et al., 1992; Gledhill et al., 1997; Mayer-Pinto et al., 2010), other point source pollutants such as oil spills, detergents and antifouling paints (Chapman, 1995), and invasive species (Thomsen et al., 2009). These local anthropogenic stressors are thought to negatively interact with environmental stressors or global climatic stressors resulting in declines in canopy algae and increases in mat-forming algae (Connell et al., 2008; Russell & Connell, 2012). Although there have been meta-analyses conducted on the nature and type of interactions between local anthropogenic and other stressors on algal communities (Crain et al., 2008; Darling & Cote, 2008; Wahl et al., 2011), these studies have not specifically considered the effects on both canopy-forming algae and mat-forming algae. Other reviews on canopy-forming algae and mat- forming algae have largely been based on a qualitative rather than quantitative assessment of the literature (Dayton, 1985; Chapman, 1995; Coelho et al., 2000; Airoldi, 2003; Forster & Schiel, 2010). There is a pressing need for quantitative, comprehensive information on the cumulative effects of local anthropogenic stressors and the role of interactions between local anthropogenic and other stressors in driving the shifts between these two habitats.

In this study, we used a meta-analysis approach and a qualitative review to assess the nature and type of interactions between local anthropogenic stressors which are most frequently claimed to play a major role in the declines of canopy-forming algae (i.e. fishing, nutrient enrichment, heavy metal pollution and high sediment loads), and other stressors potentially inter- acting but less amenable to management (presence of competitors or grazers, low light or salinity, high light or salinity, increasing temperature, wave exposure or CO_2 and high UV). Specifically, we tested the hypothesis that nonadditive interactions (either synergistic or antagonistic) between fishing, nutrient enrichment, high sediment loads, and heavy metal pollution and other stressors and the cumulative effects of local anthropogenic stressors will have negative effects on the growth and/or survival of canopy-forming algae and/or positive effects on the growth and/or survival of mat-forming algae (Fig. 1 and references therein).

Materials and methods

We searched the literature using Google Scholar and Web of Science for fully factorial field and laboratory experimental studies in shallow marine systems (either intertidal or subtidal) that manipulated each of our target local anthropogenic stressors in combination with other stressors. We deemed this to be the best approach to evaluate the effects of and interactions between multiple stressors on the responses of canopy-forming and mat-forming algae (Crain et al., 2008; Darling & Cote, 2008).

The search terms included ('effect* or impact*') of local anthropogenic stressors ('nutrient enrichment or eutrophication', 'heavy metal*', 'sediment*', 'fishing or tro- phic cascade*') and other stressors ('competitor*', 'grazer*', 'canopy*', 'light', 'salinity', 'CO_2', 'wave* or exposure', 'ultra- violet radiation') on canopy-forming algae ('canopy*, Fucales or Laminariales') or mat-forming algae ('ephemeral*, bloom*, Ulva, Cladophora, turf* or filamentous*').

We also searched the reference and citation lists of each article identified, using the same search terms. During the initial literature search, we also looked for articles on the effects of other pollutants, oil spills, disease, trampling, invasive species and habitat disturbance on encrusting red algae or nongeniculate coralline algae. However, these terms were excluded because there was insufficient literature for a meta-analysis.

We selected studies for the analyses that manipulated two or more stressors. We only included studies that were conducted between late spring and late summer, during the primary growth period of canopy-forming and mat-forming algae, because there were few experiments conducted in other sea- sons. The studies tested the effects of local anthropogenic and other stressors on population-level metrics (density and/or % survival) and individual metrics (growth: length, width and/or % cover and photosynthesis: maximum electron transport rate (ETR), maximal yield and/or gross P_{max}) on canopy-forming or mat-forming algae. We tested the effects of the stressors on the growth and survival of the two categories of algae, separately.

We found 167 multiple stressors studies and after various exclusions, (i.e. confounding with other factors, no control, data not shown in the article, experiments conducted between late autumn to winter) we extracted data from 118, using GetData Graph Digitizer version 2.25.0.32 (www.getdata-graph-digit- zer.com). There were 65 studies on canopy-forming algae and 53 on mat-forming algae. We tested the effects of low and high levels of stressors, separately (e.g. low light vs. control and high light vs. control). We focused on extracting data from studies which tested the effects of stressors relative to ambient site levels. When studies used multiple levels for each treatment in the design or measured multiple responses of the algae to the same experiment (e.g. growth and photosynthesis or density and survival), we used the level or parameter that was most similar to other studies on the same topic. If the data were reported as a time series, we used data from the final sampling period. In cases where more than two stressors were manipulated in a study, the responses to each stressor pair were extracted at ambient levels of the third stressor. If data were reported on multiple species or at different sites in the same study, we recorded all information.

For the meta-analysis, we defined a study as the measured responses of an individual algal species or broader group, either canopy- or mat-forming algae to the stressors of interest. In some articles, the responses of multiple individual species or groups were measured in separate experiments or at multiple locations. For the purposes of the meta-analysis, these were treated as separate studies. We adjusted for the lack of independence of studies conducted at the same research centre when required (see analysis methods below). For each study, we recorded the means, standard deviations (where reported) and sample sizes for the treatment and the control. For canopy-forming algae, we recorded the life stage in two predefined categories: recruits to early juveniles (<1 year old) and juveniles to adults (≥1 year old), as previous work has suggested algae in these stages tend to be controlled by different factors (Schiel & Foster, 2006). For the mat-forming algae, we recorded their persistence in two predefined categories: ephemeral algae (e.g. *Ulva* spp.) and turf-forming algae (e.g. *Feldmania* spp.), as algae in these groups could be influenced differently by multiple stressors (Connell et al., 2013, 2014). For both canopy-forming and mat-forming algae, we recorded the geographical location of the study, and then we assigned each study a unique number.

The stressor interactions tested in the meta-analysis were:

1. nutrient enrichment and the presence of competitors (either canopy-forming or mat-forming algae), presence of grazers, removal of canopy algae, low light, low salinity, high light, high salinity, increasing temperature, increasing wave exposure, increasing CO_2 or high UV;
2. fishing and the presence of grazers
3. The studies on the effects of fishing and the presence of grazers selected for the meta-analysis focused on a combination of direct and indirect impacts. These studies tested the interaction between excluding predatory or omnivorous fishes or amphipods and the presence of grazers using a 2-factor approach. We used these studies to test whether the indirect effects of fishing (no predators with grazers) were

stronger than the direct effects of the predatory or omnivorous fish or amphipods (predators, no grazers) or the grazers (predators with grazers);

4. heavy metal pollution and low light, low salinity, high light or high salinity;
5. high sediment loads and the presence of competitors (either canopy-forming or mat-forming), presence of grazers, low light, high light, increasing temperature, increasing wave exposure

In addition, we also explored possible interactions between multiple local anthropogenic stressors:

6. NO3 enrichment and PO4 enrichment;
7. nutrient enrichment and fishing, sedimentation or heavy metals;
8. heavy metal pollution (combined effect of two heavy metals)

Data analysis

The effect size of the local anthropogenic and other stressors on the growth and survival of the algae were measured as the Hedge's g standardized mean difference (SMD) (Hedges, 1981),

$$SMD = \frac{\bar{Y}_{stressor(s)} - \bar{Y}_{control}}{S_{pooled}}$$

We chose SMD as opposed to log response ratio, for the effect size of this meta-analysis because our data set contained both negative values (i.e. loss of biomass) and zeroes (i.e. no survival and/or no variance between replicates within the same treatment) (Borenstein et al., 2009). For the analysis, the effects of individual and combined stressors were tested against the control using a random effects model as there was significant heterogeneity between studies (determined by measuring heterogeneity via Cochran's Q, and testing it against a v^2 distribution with n-1 degrees of freedom, where n is the number of studies). The model was fitted using the DerSimonian–Laird random effects estimator (DerSimonian & Laird, 1986). We compared the results from DerSimonian– Laird and the Hedges random effects models and found no detectable differences. For studies that tested the effects of the stressors on more than one species or at different locations, we treated each species/location as a different study. In this case, we tested whether results from the same article were more similar than from different article, by testing the effect of study identity as a moderator in the model (Tables S1, S2, S3 and S4). For canopy-forming algae, we also tested the effect of life stage (recruits to juveniles or juveniles to adults) and for mat-forming algae the effect of persistence (ephemeral or persistent turf-forming algae) as moderators (Tables S1, S2, S3 and S4). Where significant effects were found, we presented the results from the model that included the moderators. For studies that did not report the standard deviation, we substituted the maximum value of the standard deviation from the studies on the same pair of stressors (Furukawa et al., 2006). There were no detectable differences in effect sizes between the studies with and without standard deviations (based on overlapping 95% confidence intervals). We therefore presented results which included studies that did not publish standard deviations. The meta-analysis was only performed on pairs of stressors with three or more studies (see Tables S1–S4 for full details about the number of studies for each stressor pair). However, we also undertook a qualitative review on the effects of pairs of stressors with less than three studies to obtain a more holistic picture of the effects of multiple stressors on the responses of the algae. We checked whether there was a significant correlation between the effect size and sample size, as a measure of publication bias using qualitative tests (weighted frequency histogram, funnel plots and Q–Q normality plots of effect sizes). We also tested whether there were a high number of studies needed to over- turn the results, using the Rosenthal's fail-safe number test (Tables S1, S2, S3 and S4).

We tested whether the interactions between local anthropogenic and other stressors were antagonistic, additive or synergistic based on the methods proposed by Darling & Cote (2008). We focused on the additive model as this is a more conservative estimate of the predicted effect than the multiplicative model (Crain et al., 2008; Darling & Cote, 2008). The formula has been modified from Darling & Cote, for use with the Hedge's g SMD effect size (Hedges, 1981).

$$SMD_{additive} = \frac{\bar{Y}_{stressorA} - \bar{Y}_{control} + \bar{Y}_{stressorB} - \bar{Y}_{control}}{S_{pooled}}$$

We classified the interaction as antagonistic if the actual effect size of Stressor A 9 B was closer to zero than the predicted effect size and synergistic when the actual effect size of Stressor A 9 B was further away from zero than predict effect size. Interactions were nonadditive if the confidence intervals of the actual effect size did not overlap the predicted effect size and additive if the confidence intervals of actual effect size overlapped of the predicted effect size. All analyses were conducted using the Rgui library metafor (Viechtbauer, 2010) and all plots were produced using Rgui (Team RC, 2012).

Results

Of the 118 studies from which data were extracted, the local anthropogenic stressor with the greatest number of experiments was nutrient enrichment (60%), followed by heavy metal pollution (17%), sedimentation (14%) and fishing (8%). The studies were not evenly distributed around the globe, and most of the experiments were conducted in Europe (Figure 2). We studied the effects of 22 pairs of stressors on the growth and survival of canopy-forming algae and the effects of 20 pairs of stressors on the growth and survival of mat-forming algae (see Tables S1, S2, S3, S4, S5 and S6 for full details). Contrary to our hypotheses (Figure 1) across all the pairs of stressors, the majority of interactions were additive (81% growth of canopy-forming algae, 78.57% survival of canopy-forming algae, 68.75% growth of mat-forming algae and 85.72% survival of mat-forming algae) (Tables S5 and 6). There were nota- ble exceptions in the synergistic interactions between nutrient enrichment and other stressors (Figure 3).

Interactions between nutrient enrichment and other stressors

As we hypothesized (Figure 1), nutrient enrichment had synergistic interactions with the presence of competitors and low light leading to amplified negative effects on the growth and survival of canopy-forming algae (Figure 3, Tables S1 and S2). There were also synergistic interactions between nutrient enrichment and increasing temperature with negative effects on the growth of canopy-forming algae (Figure 3, Tables S1 and S2). In general, the effects of the stressor pairs were consistent between both juveniles and adult life stages (Tables S1 and S2). Contrary to our expectations, there was an additive interaction between nutrient enrichment and the presence of grazers, which had a negative effect on the growth of juveniles to adults but no detectable effect on the growth of recruits to juveniles of canopy-forming algae (Figure 3, Tables S1, S2 and S5).

Contrary to our hypotheses (Figure 1), nutrient enrichment had an additive interaction with the presence of grazers and the presence of competitors with positive effects on the growth and/or survival of mat-forming algae (Figure 3, Tables S3, S4, S5 and S6). As we predicted (Figure 1), there was a synergistic interaction between nutrient enrichment and high CO_2 with positive effects on the growth of mat-forming algae (Figure 3, Tables S3 and S5). The qualitative review suggested there could be a synergistic interaction between nutrient enrichment and temperature with positive effects on the growth of mat-forming algae (Table S6). Interestingly most studies on mat-forming algae focused on the responses of ephemeral taxa (Tables S3 and S4).

Interactions between fishing and the presence of grazers

Contrary to our hypotheses (Figure 1), there was an additive interaction between fishing and the presence of grazers with negative effects on the growth of adult canopy-forming algae (Figure 3, Tables S1 and S5). As we expected (Figure 1), there was no detectable effect of the interaction between fishing and the presence of grazers on the growth of mat-forming algae (Table S3).

Interactions between heavy metal pollution and other stressors

There was an additive interaction between heavy metal pollution and low light which resulted in declines in the survival of recruits to juveniles of canopy-forming algae (Figure 3, Tables S1 and S2). The qualitative review also suggested there could be a synergistic interaction between heavy metal pollution and increasing temperature with negative effects on the survival of adult canopy-forming algae (Tables S6). Contrary to our hypotheses (Figure 1), there was a synergistic interaction between the heavy metal pollution and low salinity with negative effects on the growth of mat-forming algae (Figure 3, Table S6).

Interactions between sediment and other stressors

Contrary to our hypotheses (Figure 1), there were additive interactions between high sediment loads and low light with negative effects on the growth of canopy-forming algae (Figure 3, Tables S1 and S2). Similarly, there were additive interactions between high sediment loads and increasing wave exposure with negative effects on the survival of recruits to juveniles of canopy-forming algae (Tables S1 and S2).

Contrary to our hypotheses (Figure 1), only high sediment loads affected the growth of mat-forming algae, and there were no detectable interactions with low light (Table S3).

Interactions between local anthropogenic stressors

There were no detectable effects of PO_4 and NO_3 combined, or nutrient enrichment and fishing combined on the growth or survival of canopy-forming algae (Tables S1 and S2). There was however, a synergistic interaction between nutrient enrichment and heavy metal pollution with negative effects on the growth of adult canopy-forming algae and an additive interaction between heavy metals (two heavy metals combined) with negative impacts on the growth of canopy-forming algae (Figure 4, Tables S1 and S6). The qualitative review suggested there could also be a synergistic inter- action between heavy metal pollution and excess sediment with negative effects on the growth of adult canopy-forming algae (Table S6).

There was an additive interaction between nutrient enrichment (PO_4 and NO_3 combined) and nutrient enrichment and excess sediment with positive effects on the growth of mat-forming algae (Figure 4, Tables S2 and S6). There was also an additive interaction between nutrient enrichment heavy metals with negative effects on the growth of ephemeral algae (Figure 4, Tables S3, S4 and S6). In contrast, there were no detectable effects of the interaction between nutrient enrichment and fishing on the growth of mat-forming algae (Table S3).

Discussion

This meta-analysis represents the first systematic global assessment of the role of the interactions between local anthropogenic and other stressors in driving the ongoing global transitions from forests of canopy-forming algae to mat-forming algae in temperate rocky reef eco- systems. Our results indicate that the interactions between the four dominant local anthropogenic stressors in temperate rocky reef ecosystems (i.e. fishing and outbreaks of grazers, eutrophication, heavy metal pollution and high sediment loads) and other stressors can enhance declines in the growth and survival of canopy- forming algae, at both the recruit to juvenile and juvenile to adult life stages. In contrast, many of the same pairs of stressors had no detectable or positive effects on the growth or survival of mat-forming algae, irrespective of their persistence. These results provide strong evidence to suggest that increasing population growth and development of coastal areas and their associated human activities will have major impacts on the algal community (Coelho et al., 2000; Airoldi & Beck, 2007; Coleman et al., 2008; Mangialajo et al., 2008; Gorman et al., 2009). The information can be used to identify appropriate management actions at a local scale that can help to halt the global loss of canopy-forming algae and their replacement by mat-forming algae.

The identity of the local anthropogenic stressor had a major influence on the nature and type of the interaction and clearly demonstrated the importance of understanding the effects of individual stressors rather than groups or categories of stressors (Claudet & Fras- chetti, 2010; Fraschetti et al., 2011). Contrary to our pre- dictions, the majority of the interactions between local anthropogenic and other stressors were additive, with the notable exception of those interactions involving nutrient enrichment. Nutrient enrichment had synergistic interactions with the presence of competitors, presence of grazers, increasing temperature and heavy metal pollution, leading to much greater negative effects on the growth and/or survival of canopy-forming algae than predicted by the additive model. Conversely, there were synergistic interactions between nutrient enrichment and CO_2 which enhanced the growth and/or survival of mat-forming algae. These results confirm previous suggestions that mat-forming algae are more tolerant or actively benefit from the cumulative anthropogenic stressors that negatively affect the growth and/or survival of canopy-forming algae (Pedersen & Borum, 1996; Amado Filho et al., 1997; Benedetti-Cecchi et al., 2001; Steen, 2004; Eriksson & Johansson, 2005; Gorman & Connell, 2009; Costa et al., 2011). The implications are that management strategies designed to reduce the levels of these four key local anthropogenic stressors, nutrient enrichment, excess sediment loads and heavy metal pollution could help to improve the resilience of canopy-forming algae to other stressors less amendable to local actions, and thereby prevent the shift to mat-forming algae.

Nutrient enrichment was the local anthropogenic stressor with the most frequent nonadditive interactions with other stressors. The input of excess nutrients (primarily nitrate and phosphate) to the marine environment is a global problem associated with a range of human activities. Nutrient enrichment can interact with heavy metals to block carbon storage in canopy-forming algae (Munda & Veber, 1996, 2004). It also increases the palatability of canopy-forming algae to grazers (Worm et al., 1999; Korpinen et al., 2007; Lotze et al., 2001; Korpinen & Jormalainen, 2008), reduces the avail- ability of light or increases turbidity by promoting the growth of epiphytes and algal blooms (Hoffman & Santelices, 1982; Cronin & Hay, 1996; Shivji, 1985), and becomes toxic at high temperatures (Yarish et al., 1990). In contrast, the same synergistic interactions tend to have no detectable or positive effects on the growth of mat-forming algae because of their opportunistic traits which include higher nutrients requirements (Pedersen & Borum, 1996), the ability to assimilate high levels of CO_2 (Gordillo et al., 2001), rapid growth at increased temperatures (Riccardi & Solidoro, 1996) and their posi- tive associations with sediment (Airoldi & Virgilio, 1998; Gorgula &

Connell, 2004). These findings suggest a much stronger potential for shifts in rocky coastal systems with poor water quality, particularly under future scenarios of climate change (Lotze & Worm, 2002; Falkenberg et al., 2012, 2013; Steen, 2004).

The nature of the interactions between nutrient enrichment and other stressors might be also influenced by other factors not covered by the studies identified in this meta-analysis. Experimental tests on multiple stressors, which were the target of this review, are difficult to undertake, and most of the work was carried out in laboratories (85.47% of studies on canopy-forming algae and 54% of studies on mat-forming algae) or in situ in enclosed seas or estuaries (9% of studies on canopy- forming algae and 30.4% of studies on mat-forming algae). This could have enhanced the negative or positive effects of nutrient enrichment because there is very little or no mixing through ocean currents compared with areas along exposed coastlines where algal populations often experience long periods of nutrient depletion or oligotrophic conditions (Russell & Connell, 2012). Some interactions could also vary between sea- sons. For example, while nutrient enrichment and strong warming can worsen the decline of canopy- forming algae by synergistically promoting the growth of epiphytes or increasing their susceptibility to dis- eases during the late spring and summer (Kremer & Munda, 1982; Yarish et al., 1990; da Costa & Valentin, 1994), such effects could be dampened or reversed with moderate warming in the winter and early spring (Yarish et al., 1990). However, there were insufficient experiments to test for seasonal differences in the inter- action between nutrient enrichment and increasing temperature in the meta-analysis. There was sometimes also high variability in the responses of different species of canopy-forming and mat-forming algae to the inter- actions between nutrients and other stressors within the same study (Yarish et al., 1990; Worm et al., 1999, 2001; Steen & Rueness, 2004; Steen & Scrosati, 2004). In the light of these gaps in the literature, a conservative management approach must assume that the interactions between nutrient enrichment and other stressors will have a negative effect on the growth and survival of canopy-forming algae.

Long-term sustainability requires the identification of the causes and interplay between multiple stressors, and the development of stakeholder support for management actions. However, we found significant and striking gaps in research between multiple stressors. The majority of the four local anthropogenic stressors have not been experimentally studied in combination with each other in controlled factorial experiments. There were also very few experiments testing the combined effects of fishing and other stressors on either canopy-forming or mat-forming algae. These gaps were particularly surprising given the large body of literature that suggested that global declines in canopy-forming algae are driven by the combined effects of local anthropogenic stressors (Walker & Kendrick, 1998; Coelho et al., 2000; Connell et al., 2008) or the effects of fishing and other stressors, including rising sea surface temperatures and increasing wave exposure (Tegner & Dayton, 2000; Halpern et al., 2007). For some pairs of stressors, there were no replicate experiments to test the generality of our conclusions in a meta-analysis. The stressors examined in our review have been shown to commonly co-occur in increasingly human-dominated marine systems (Crain et al., 2008, 2009; Darling & Cote, 2008; Cote & Darling, 2010) and research on their cumulative effects is needed for prioritizing management actions (Coelho et al., 2000; Wahl et al., 2011; Harley et al., 2012). There is a particularly urgent need for research on three or more stressors, for which only a hand full of studies could be found (13 studies for canopy-forming algae and 10 studies for mat-forming algae because of the increased probability of nonadditive interactions (Crain et al., 2008; Wernberg et al., 2012).

While the links between local anthropogenic stressor interactions and habitat shifts in algal communities are slowly becoming clearer, there is relatively little empirical evidence about whether reducing or managing these same stressors would be effective for disrupting nonadditive interactions, reversing the spread of alternative habitats, and promoting the recovery of more desirable configurations or species (Russell & Connell, 2012; Brown et al., 2013). Recent experimental research has shown that management of nutrients can suppress the growth of turf-forming algae under future scenarios of increased CO_2 (Russell et al., 2009; Falkenberg et al., 2012, 2013). Similar experimental work is urgently needed to test whether management of water quality can significantly enhance the resilience of canopy-forming algae in areas experiencing rapid increases in sea surface temperature by disrupting the negative synergies between these two stressors. Prioritization of conservation strategies would enormously benefit from experimental manipulations mimicking management actions geared towards recovery in a variety of degraded ecosystems.

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Figures

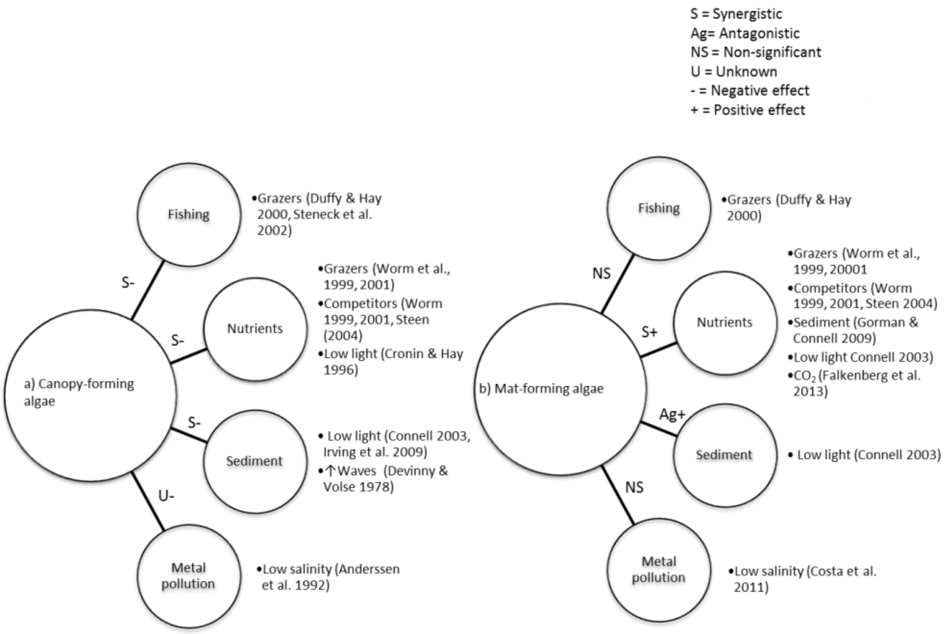

Figure 1: Predicted effects of the interactions between key local anthropogenic and other stressors on (a) canopy-forming and (b) mat-forming algae based on factorial studies from the literature. Lines represent the type of interaction most commonly reported in the literature between the local stressor within the circle and the other stressors listed to its side. Unknown interaction types in the literature are not presented in the figure. Symbols are S = synergistic, NS = nonsignificant, - = negative effects and + = positive effects.

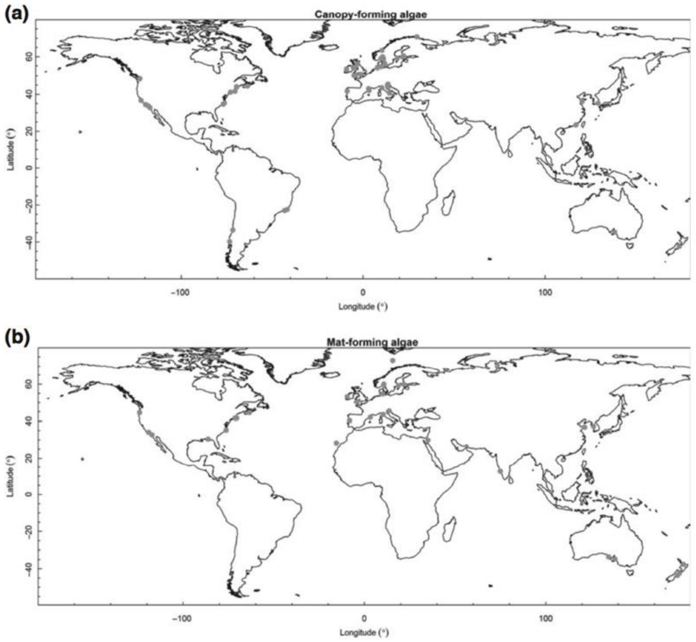

Figure 2 Map showing the geographical location of the studies for (a) canopy-forming algae and (b) mat-forming algae.

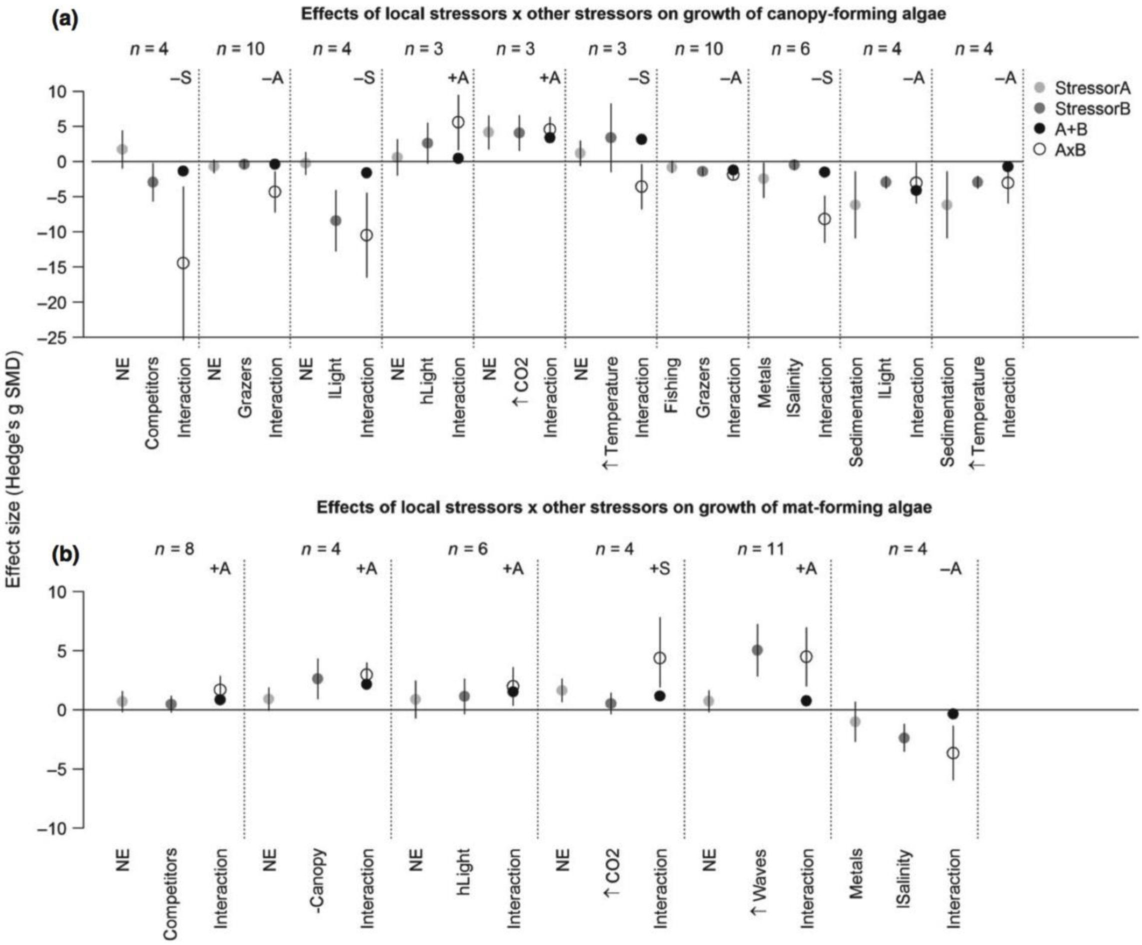

Figure 3 Results of meta-analysis (Hedge's g standard mean difference effect size and 95% confidence intervals) on the effects of local anthropogenic stressors [nutrient enrichment (NE), fishing, heavy metal pollution (metals) and sedimentation] (Stressor A), other stressors (Stressor B), and their combined effect (A + B = predicted effect of the interaction see Eqn (2) and A 9 B = actual effect of the interaction) on the (a) growth of canopy-forming algae and (b) growth of mat-forming algae. Effects are significant if confidence intervals do not overlap zero. Only significant interactions are shown. Interactions are synergistic with negative effects (!S) if the upper 95% confidence interval of the observed interaction is lower than the predicted interaction, synergistic with positive effects (+S) if the observed interaction was less than the lower 95% confidence interval of the predicted interaction, additive with negative effects (!A) if the observed interaction is lower than zero and the 95% confidence interval overlaps the predicted interaction and additive with positive effects (+A) if the observed interaction is higher than zero and the 95% confidence interval overlaps the predicted interaction. Symbols are: l = low, h = high and ! = increasing. Note the differences in the y-axis between (a) and (b).

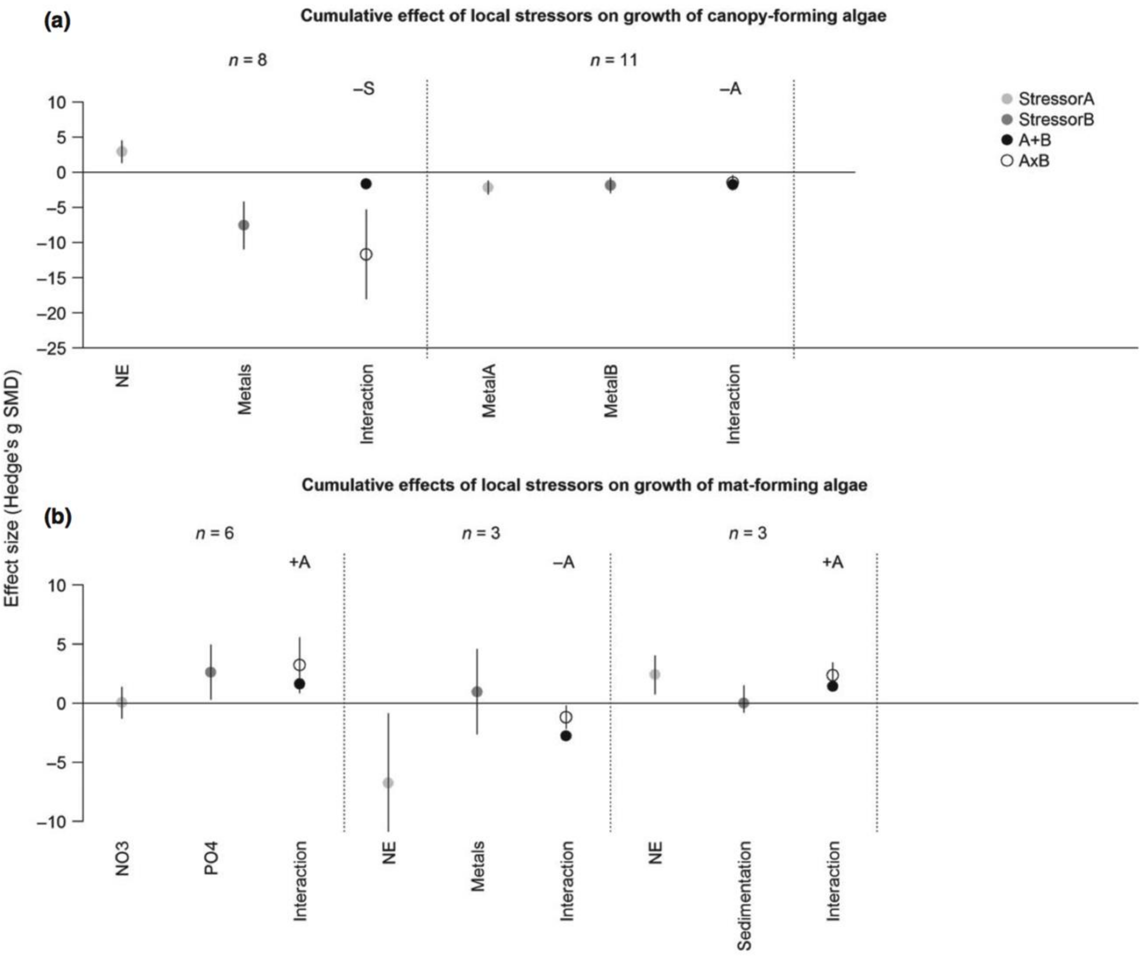

Figure 4 Results of meta-analysis (Hedge's g standard mean difference effect size and 95% confidence intervals) on the cumulative effects of local anthropogenic stressors [NO3, PO4, heavy metal A (metal A), heavy metal B (metal B) and sedimentation], Stressor A or Stressor B, and their combined effect (A + B = predicted effect of the interaction see Eqn (2) and A 9 B = actual effect of the interaction) on the (a) growth of canopy-forming algae and (b) growth of mat-forming algae. Effects are significant if confidence intervals do not overlap zero. Only significant interactions are shown. Interactions are synergistic with negative effects (!S) if the upper 95% confidence interval of the observed interaction is lower than the predicted interaction, additive with negative effects (!A) if the observed interaction is lower than zero and the 95% confidence interval overlaps the predicted interaction and additive with positive effects (+A) if the observed interaction is higher than zero and the 95% confidence interval overlaps the predicted interaction. Note the differences in the y-axis between (a) and (b).

Supplementary materials

Table S1. Effects (Hedges g standard mean difference) of (a) local anthropogenic [fishing, nutrient enrichment (NE), heavy metal pollution (Metal) and high sediment loads (Sediment)] and other stressors (presence of competitors, and grazers, removal of canopy algae, low light and salinity, high light and salinity, and increasing temperature, wave exposure and CO_2) and (b) multiple local anthropogenic stressors [NO_3 and PO_4 enrichment, heavy metals A and B, nutrient enrichment (NE), heavy metal pollution (metals)] on the growth of canopy-forming algae. Results are the overall estimate of effect size (overall estimate, 95% lower confidence interval (LC), higher confidence interval (HC), Q_E *P*-value (the test of the residual heterogeneity), and the effects of the moderators, study identity [estimate, 95% lower confidence interval (LC), higher confidence interval (HC)], and life stage (estimate, 95% lower confidence interval (LC), higher confidence interval (HC). The number of experiments with standard deviations reported (N with SD), without standard deviations reported (N no SD), the number of experiments at each life stage (Stage 1 = recruits to juveniles and Stage 2 = juveniles to adults) and the Rosenberg fail-safe number of experiments required to overturn the results (Fail safe no). Effects are significant if confidence intervals do not overlap zero. Significant effects are shown in bold print.

Test	**Overall (estimate)**	**LC**	**HC**	**Q_E (P-value)**	**Study (estimate)**	**LC**	**HC**	**Life stage (estimate)**	**LC**	**HC**	**N (with SD)**	**N (no SD)**	**Stage 1**	**Stage 2**	**Fail Safe No**
a) Effects of local anthropogenic and other stressors															
NE	1.159	-0.602	2.919	>0.05	0.681	-1.997	0.636	NA	NA	NA	3	1	4	0	NA
Competitors	**-2.949**	**-5.631**	**-0.267**	**<0.0001**	3.459	-0.089	7.007	NA	NA	NA					10
Interaction	**-14.478**	**-25.37**	**-3.586**	**<0.0001**	**5.301**	**0.109**	**10.493**	NA	NA	NA					10
NE	**-0.681**	**-1.61**	**0.25**	**<0.0001**	-0.043	-0.541	0.456	**2.211**	**0.118**	**4.304**	9	1	3	7	NA
Grazers	**-1.317**	**-2.419**	**-0.215**	**<0.0001**	0.083	-0.191	0.356	0.805	-0.519	2.128					NA
Interaction	**-1.693**	**-2.957**	**-0.428**	**<0.0001**	-0.043	-0.541	0.456	**2.673**	**0.593**	**4.753**					27
NE	-0.275	-1.857	1.306	0.008	1.592	-1.208	4.392	-0.571	-5.185	4.042	3	1	3	1	NA
lLight	**-8.426**	**-12.74**	**-4.116**	**0.002**	**2.297**	**0.855**	**3.738**	-0.571	-5.185	4.042					13
Interaction	**-10.474**	**16.464**	**-4.485**	**0.004**	**2.905**	**0.923**	**4.886**	-3.768	-11.67	4.131					13
NE	0.133	-0.522	0.788	>0.05	-0.211	-1.371	0.95	NA	NA	NA	2	1	3	0	NA
hLight	0.6	-0.249	1.449	>0.05	-0.936	-2.214	0.342	NA	NA	NA					NA
Interaction	**5.55**	**1.671**	**9.429**	>0.05	**-1.929**	**-3.578**	**-0.28**	NA	NA	NA					5
NE	**4.129**	**1.75**	**6.507**	**0.039**	-2.808	-8.817	3.2018	NA	NA	NA	3	0	0	3	24
↑CO_2	**4.062**	**1.553**	**6.571**	>0.05	-2.172	-4.047	-0.298	NA	NA	NA					5

Interaction	**4.552**	**2.814**	**6.289**	>0.05	-1.59	-6.293	3.114	NA	NA	NA					25
NE	1.158	-0.602	2.92	**0.004**	NA	NA	NA	NA	NA	NA	3	0	0	3	NA
↑Temperature	3.359	-1.473	8.194	**<0.001**	NA	NA	NA	NA	NA	NA					NA
Interaction	**-3.602**	**-6.786**	**-0.418**	**<0.001**	NA	NA	NA	NA	NA	NA					19
Fishing	-0.83	-1.571	0.089	**<0.001**	-0.533	-0.871	1.197	NA	NA	NA	10	0	0	10	NA
Grazers	**-1.448**	**-1.981**	**-0.915**	**0.02**	-0.341	-0.783	0.11	NA	NA	NA					184
Interaction	**-1.867**	**-2.529**	**-1.205**	**<0.001**	-0.340	-0.753	0.073	NA	NA	NA					224
Metal	**-2.804**	**-5.414**	**-0.195**	**<0.001**	-1.367	-4.559	1.824	NA	NA	NA	3	1	0	3	12
hLight	0.422	-1.605	2.449	**<0.001**	-0.439	-3.299	2.421	NA	NA	NA					NA
Interaction	-4.122	-10.02	1.774	**<0.001**	3.459	-3.872	10.79	NA	NA	NA					NA
Metal	**-2.474**	**-5.156**	**-0.195**	**0.0003**	3.459	-3.872	10.79	-1.938	-7.173	3.295	3	3	1	5	7
lSalinity	-0.513	-1.195	0.167	<0.05	0.721	-0.062	1.504	-0.087	-1.885	1.712					NA
Interaction	**-8.21**	**-11.51**	**-4.91**	**0.0013**	**2.726**	**1.522**	**3.93**	-1.61	-6.245	3.044					NA
Sediment	-0.0427	-0.89	0.805	<0.05	-0.933	-2.634	0.768	-0.933	-2.634	0.768	3	0	2	1	NA
Competitors	-0.116	-1.267	1.034	<0.05	-1.548	-3.295	0.199	-1.548	-3.295	0.199					NA
Interaction	-0.2364	-2.126	1.652	**0.017**	-2.647	-4.512	0.782	-2.647	-4.512	0.782					NA
Sediment	**-6.159**	**-10.89**	**-1.43**	**<0.001**	6.576	-6.455	6.697	NA	NA	NA	4	0	4	0	6
lLight	**-2.963**	**-3.832**	**-2.096**	<0.05	-1.309	-3.132	0.513	NA	NA	NA					43
Interaction	**-3.07**	**-5.917**	**-0.224**	**<0.001**	-4.466	-11.25	2.323	NA	NA	NA					22
Sediment	**-0.852**	**-1.579**	**-0.126**	>0.05	-0.445	-2.09	1.199	NA	NA	NA	3	0	3	0	6
↑Temperature	-0.305	-1.21	0.591	>0.05	1.019	-1.035	3.074	NA	NA	NA					NA
Interaction	**-1.496**	**-2.899**	**-0.092**	>0.05	-0.055	-4.19	4.079	NA	NA	NA					6
Sediment	-0.522	-1.454	0.409	<0.05	NA	NA	NA	NA	NA	NA	4	0	4	0	NA
↑Waves	-0.1995	-1.493	1.094	**0.03**	NA	NA	NA	NA	NA	NA					NA
Interaction	-0.7714	-2.089	0.546	**0.03**	NA	NA	NA	NA	NA	NA					NA
b) Effects of multiple local anthropogenic stressors															
NO_3	-0.332	-1.016	0.353	>0.05	-0.204	-1.16	0.753	-0.308	-2.079	1.464	3	1	3	1	NA
PO_4	-0.177	-0.863	0.509	>0.05	-0.375	-1.335	0.585	-0.166	-2.073	1.741					NA
Interaction	0.321	-1.141	1.781	>0.05	1.069	-1.058	3.195	-3.332	-5.949	0.714					NA
NE	**2.9456**	**1.372**	**4.518**	**<0.001**	3.004	-5.039	11.047	NA	NA	NA	8	0	0	8	61
Metal	**-7.5683**	**-10.94**	**-4.2**	**<0.001**	1.9017	-0.212	4.015	NA	NA	NA					26
Interaction	**-11.694**	**-18.05**	**-5.343**	**<0.001**	1.8607	-2.696	3.0253	NA	NA	NA					17
Metal A	**-2.189**	**-3.148**	**-1.229**	**0.0008**	**0.685**	**0.285**	**1.086**	NA	NA	NA	12	3	0	15	212
Metal B	**-1.882**	**-2.964**	**-0.8**	**0.003**	**0.571**	**0.075**	**1.067**	NA	NA	NA					213
Interaction	**-1.483**	**-2.528**	**-0.438**	**0.007**	0.465	-0.006	0.937	NA	NA	NA					200

Table S2. Effects (Hedge g standard mean difference) of local anthropogenic [nutrient enrichment (NE), heavy metal pollution (Metal) and high sediment loads (Sediment)] × other stressors (presence of competitors and grazers, removal of canopy algae, low light and salinity, high light and salinity, and increasing temperature, wave exposure and CO_2) on the survival of canopy-forming algae. Results are overall estimate of effect size [overall estimate, 95% lower confidence interval (LC), higher confidence interval (HC), Q_E the test of the residual heterogeneity], and the moderators, study identity [overall estimate, 95% lower confidence interval (LC), higher confidence interval (HC)], and life stage [life stage estimate, 95% lower confidence interval (LC), higher confidence interval (HC)]. The number of experiments with standard deviations reported (N with SD), without standard deviations reported (N no SD), and the Rosenberg fail-safe number of experiments required to overturn the results (Fail safe no) on the density and survival of canopy-forming algae. Effects are significant if confidence intervals do not overlap zero. Significant effects are shown in bold print.

Test	**Overall (estimate)**	**LC**	**HC**	**Heterogeneity (P-value)**	**Study (estimate)**	**LC**	**HC**	**N (with SD)**	**N (no SD)**	**Stage 1**	**Stage 2**	**Fail Safe No**
NE	-0.734	-1.574	0.105	<0.05	0.849	-0.981	2.8	3	0	3	0	NA
Competitors	-0.84	-1.89	0.19	<0.05	-0.923	-3.566	1.72					NA
Interaction	**-6.180**	**-11.67**	**-0.69**	**0.001**	-1.923	-1.566	1.72					15
NE	-0.693	-2.738	1.351	**0.01**	-2.052	-4.001	0.103	3	0	3	0	NA
Grazers	-0.544	-3.625	2.536	**0.001**	-2.919	-4.316	1.523					NA
Interaction	-1.325	-3.809	1.159	**0.003**	-2.376	-3.729	1.022					NA
NE	-1.466	-3.389	0.457	**0.003**	-2.322	-7.772	3.128	3	1	4		NA
lLight	0.269	-0.438	0.976	>0.05	0.3637	-1.248	1.975					NA
Interaction	-0.434	-1.375	0.507	>0.05	-0.629	-3.177	1.919					NA
NE	-0.982	-2.778	0.814	**0.004**	-1.717	-6.98	3.55	3	1	4		NA
hLight	-1.338	-3.23	0.554	**0.003**	-2.219	-7.647	3.208					NA
Interaction	-1.775	-3.764	0.213	**0.003**	-2.813	-8.108	2.482					NA
NE	-0.754	-1.637	0.129	**0.01**	NA	NA	NA	8	0	8	0	NA
↑Temperature	-0.724	-2.702	1.252	**<0.001**	NA	NA	NA					NA
Interaction	**-3.474**	**-5.988**	**-0.957**	**<0.001**	NA	NA	NA					26
Metals	-0.886	-2.082	0.308	>0.05	0.832	-0.634	2.298	3	0	3	0	NA
lSalinity	-9.565	-19.417	0.289	**<0.001**	5.82	-11.203	22.844					NA
Interaction	**-10.965**	**-21.803**	**-0.128**	**<0.001**	9.062	-12.203	30.327					4
Sediment	**-6.122**	**-9.205**	**-3.04**	**<0.001**	2.878	-1.27	7.02	4	0	4	0	50
lLight	**-1.828**	**-3.597**	**-0.06**	**<0.001**	-3.433	-5.096	1.772					51
Interaction	**-6.750**	**-10.19**	**-3.305**	**<0.001**	2.210	-4.887	9.308					52
Sediment	**-0.852**	**-1.579**	**-0.126**	>0.05	-0.445	-2.09	1.19	3	0	3	0	6
↑Temperature	-0.305	-1.2	0.59	>0.05	1.019	-1.035	3.073					NA
Interaction	**-1.495**	**-2.899**	**-0.092**	>0.05	-0.055	-4.19	4.079					5
Sediment	**-2.119**	**-3.932**	**-0.306**	**0.001**	-1.282	-2.863	0.31	7	0	7	0	8

↑Waves	-0.57	-1.86	0.723	**0.004**	-1.43	-2.504	0.37					NA
Interaction	**-2.7**	**-4.53**	**-0.87**	**0.001**	-2.014	-3.998	0.032					17

Table S3. Effects (Hedges g standard mean difference) of (a) local anthropogenic [fishing, nutrient enrichment (NE), heavy metal pollution (Metal) and high sediment loads (Sediment)] × other stressors (presence of competitors and grazers, removal of canopy algae, low light or salinity, high light or salinity, and increasing CO_2, temperature or wave exposure) and (b) multiple local anthropogenic stressors [NO_3 and PO_4 enrichment, nutrient enrichment (NE), fishing, heavy metal A and B, heavy metal pollution (Metal) and high sediment loads (sediment)] on the growth of mat-forming algae. Results are the overall estimate of effect size using the standard mean difference [overall estimate, 95% lower confidence interval (LC), higher confidence interval (HC), Q_E p-value (the test of the residual heterogeneity)], and the effects of the moderators, study identity [estimate, 95% lower confidence interval (LC), higher confidence interval (HC)], and persistence [estimate, 95% lower confidence interval (LC), higher confidence interval (HC)], the number of experiments with standard deviations reported (N with SD), without standard deviations reported (N no SD), the number of experiments for each functional group (ephemeral or turf-forming algae) and the Rosenberg fail-safe number of experiments required to overturn the results (Fail safe no). Effects are significant if confidence intervals do not overlap zero. Significant effects are shown in bold print.

Test	**Overall (estimate)**	**LC**	**HC**	**Heterogeneity (P-value)**	**Study (estimate)**	**LC**	**HC**	**Functional group (estimate)**	**LC**	**HC**	**N (with SD)**	**N (no SD)**	**Ephermal**	**Turf**	**Fail Safe No**
a) Effects of local anthropogenic and other stressors															
NE	0.704	-0.168	1.575	**0.003**	-0.35	-0.956	0.257	0.274	-2.298	2.846	7	1	7	1	NA
Competitors	0.4687	-0.209	1.147	**0.04**	0.346	-0.196	0.889	-0.236	-2.275	1.803					NA
Interaction	**1.691**	**0.542**	**2.841**	**<0.001**	0.139	-0.765	1.043	-1.193	-4.926	2.541					19
NE	0.897	-0.077	1.873	<0.05	NA	NA	NA	-1.63	-4.112	0.852	4	0	1	2	NA
-Canopy	**2.614**	**0.928**	**4.301**	**0.02**	NA	NA	NA	2.584	-0.663	5.83					25
Interaction	**2.96**	**1.942**	**3.978**	<0.05	NA	NA	NA	-1.261	-4.45	1.928					22
NE	-0.098	-1.163	0.967	**0.001**	-0.265	-0.265	0.533	-1.585	-4.639	1.4693	6	1	6	1	NA
Grazers	0.187	-1.25	1.624	**<0.001**	0.0912	-1.056	1.238	2.485	-1.326	6.297					NA
Interaction	0.5183	-0.606	1.643	**0.001**	-0.139	-1.033	0.7548	1.357	-1.889	4.603					NA
NE	0.869	-0.707	2.445	**0.001**	-0.994	-2.551	0.564	NA	NA	NA	7	0	7	0	NA
lLight	-0.794	-1.771	0.182	>0.05	-0.036	-0.991	0.9176	NA	NA	NA					NA

Interaction	-0.058	-1.224	1.106	0.005	-0.191	-1.346	0.964	NA	NA	NA					NA
NE	0.869	-0.707	2.445	**0.001**	-0.994	-2.551	0.564	NA	NA	NA	5	1	6	0	NA
hLight	1.135	-0.347	2.616	**0.001**	0.723	-0.347	2.616	NA	NA	NA					NA
Interaction	**1.9766**	**0.3745**	**3.5788**	**0.001**	-0.228	-1.892	1.436	NA	NA	NA					10
NE	**0.7525**	**0.0951**	**1.401**	>0.05	0.17	-0.841	1.181	NA	NA	NA	4	1	5	0	13
lSalinity	**-2.938**	**-5.139**	**-0.737**	**0.03**	**1.058**	**0.117**	**1.999**	NA	NA	NA					13
Interaction	0.354	-0.241	0.949	>0.05	0.237	-0.612	1.088	NA	NA	NA					NA
NE	**1.631**	**0.656**	**2.606**	>0.05	0.423	-1.242	2.088	NA	NA	NA	4	0	0	4	13
↑CO_2	**0.5226**	**-0.364**	**1.4094**	>0.05	-1.0705	-2.145	0.005	NA	NA	NA					NA
Interaction	**4.359**	**1.9201**	**7.798**	**<0.001**	-1.725	-7.924	4.474	NA	NA	NA					15
NE	0.7158	-0.181	1.612	**0.018**	0.154	-0.716	1.024	0.485	-2.698	3.669	8	1	8	1	NA
↑Waves	**5.035**	**2.861**	**7.211**	**0.001**	-1.56	-2.507	-0.613	-2.910	-7.349	1.528					39
Interaction	**4.474**	**2.003**	**6.946**	**0.006**	1.109	-2.206	-0.013	-1.276	-5.694	3.142					54
Fishing	0.053	-2.135	2.242	**0.002**	-0.754	-4.493	2.984	-0.502	-7.424	6.418	3	3	2	1	NA
Grazers	-0.596	-1.397	0.203	>0.05	-0.246	-1.507	1.015	0.895	-0.802	2.593					NA
Interaction	-0.255	-1.419	0.907	>0.05	-0.442	-2.339	1.454	1.463	-0.689	3.616					NA
Metal	-1.012	-2.691	0.665	>0.05	NA	NA	NA	NA	NA	NA	4	0	4	0	NA
lSalinity	**-2.384**	**-3.533**	**-1.235**	>0.05	NA	NA	NA	NA	NA	NA					10
Interaction	**-3.659**	**-5.954**	**-1.365**	>0.05	NA	NA	NA	NA	NA	NA					13
Sediment	-0.107	-1.447	1.2318	**<0.001**	0.074	-1.924	2.072	2.645	-0.454	4.836	7	0	3	4	NA
Competitors	-1.363	-3.263	0.536	**<0.001**	1.699	-1.265	4.663	-0.124	-4.262	4.012					NA
Interaction	-1.286	-2.849	0.277	**<0.001**	0.9602	-1.362	3.282	0.788	-2.617	4.193					NA
Sediment	0.4136	-0.138	0.965	>0.05	0.022	-0.715	0.7603	NA	NA	NA	6	0	0	6	NA
Grazers	-0.131	-2.135	1.873	**<0.001**	1.022	-0.715	1.7603	NA	NA	NA					NA
Interaction	-0.261	-1.236	0.713	**<0.001**	-0.067	-1.402	1.267	NA	NA	NA					NA
Sediment	-0.224	-1.848	1.399	**0.013**	-1.136	-3.104	0.832	NA	NA	NA	4	0	0	4	NA
lLight	0.2992	-0.426	1.024	>0.05	-0.077	-0.97	0.817	NA	NA	NA					NA
Interaction	-0.138	-0.86	0.586	>0.05	-0.459	-1.349	0.432	NA	NA	NA					NA
b) Effect of multiple local anthropogenic stressors															
NO_3	0.05	-1.276	1.376	**0.001**	NA	NA	NA	NA	NA	NA	5	1	6	0	NA
PO_4	**2.63**	**0.3205**	**4.939**	**<0.001**	NA	NA	NA	NA	NA	NA					15
Interaction	**3.216**	**0.876**	**5.556**	**<0.001**	NA	NA	NA	NA	NA	NA					26
NE	**1.374**	**0.406**	**2.343**	>0.05	0.305	-1.499	2.111	0.7909	-1.424	3.006	6	0	4	2	15
Fishing	0.387	-0.52	1.294	>0.05	-0.439	-2.044	1.166	0.44	-1.601	2.481					NA
Interaction	0.773	0.348	1.894	>0.05	0.949	-0.902	2.799	0.198	-2.508	2.904					NA
NE	0.9687	-2.624	4.561	**<0.001**	NA	NA	NA	NA	NA	NA	3	0	3	0	NA
Metal	**-6.762**	**-12.64**	**-0.884**	**<0.001**	NA	NA	NA	NA	NA	NA					17
Interaction	**-1.189**	**-2.162**	**-0.217**	**<0.001**	NA	NA	NA	NA	NA	NA					4
NE	-0.011	-0.803	1.476	>0.05	NA	NA	NA	3.084	-0.831	5.34	3	0	1	2	NA
Sediment	**2.4014**	**0.787**	**4.015**	>0.05	NA	NA	NA	-2.975	-6.282	0.332					10
Interaction	**2.367**	**1.309**	**3.425**	**<0.001**	NA	NA	NA	-0.657	-2.014	0.699					13

Table S4. Effects (Hedges g standard mean difference) of (a) local anthropogenic [fishing, nutrient enrichment (NE), heavy metal pollution (Metal) and high sediment loads (Sediment)] × other stressors (presence of competitors and grazers, removal of canopy algae, low light and salinity, high light and salinity, increasing temperature, wave exposure, CO_2 and high UV) and (b) multiple local anthropogenic stressors [fishing and nutrient enrichment (NE)] on the survival of mat-forming algae. Results are the overall estimate of effect size using the standard mean difference [overall estimate, 95% lower confidence interval (LC), higher confidence interval (HC), Q_E the test of the residual heterogeneity], and the moderators, study identity [overall estimate, 95% lower confidence interval (LC), higher confidence interval (HC)], and persistence [life stage estimate, 95% lower confidence interval (LC), higher confidence interval (HC)], the number of experiments with standard deviations reported (N with SD), without standard deviations reported (N no SD), the number of experiments of each functional group (ephemeral or turf-forming algae) and the Rosenberg fail-safe number of experiments required to overturn the results (Fail safe no). Effects are significant if confidence intervals do not overlap zero. Significant effects are shown in bold print.

Test	Overall (estimate)	LC	HC	Heterogeneity (P-value)	Study (estimate)	LC	HC	Functional group (estimate)	LC	HC	N (with SD)	N (no SD)	Ephermal	Turf	Fail Safe No
a)	**Effects of local and other stressors**														
NE	0.704	-0.168	1.575	**0.003**	-0.35	-0.956	0.257	0.274	-2.298	2.846	7	1	7	1	NA
Competitors	0.4687	-0.209	1.147	**0.04**	0.346	-0.196	0.889	-0.236	-2.275	1.803					NA
Interaction	**1.691**	**0.542**	**2.841**	**<0.001**	0.139	-0.765	1.043	-1.193	-4.926	2.541					19
NE	3.141	1.935	4.348	**0.004**	**-0.688**	**-1.148**	**-0.228**	-0.344	-1.976	1.287	10	0	8	2	72
Grazers	-0.464	-1.115	0.188	>0.05	0.118	-0.483	0.719	0.417	-1.274	2.109					NA
Interaction	0.117	-0.753	0.987	**0.002**	0.279	-0.509	1.068	0.711	-1.554	2.975					NA
Metal	**-3.113**	**-5.149**	**-1.077**	**<0.001**	NA	NA	NA	NA	NA	NA	4	0	4	0	75
lSalinity	-0.404	-0.878	0.0706	>0.05	NA	NA	NA	NA	NA	NA					NA
Interaction	**-7.847**	**-12.01**	**-3.693**	**<0.001**	NA	NA	NA	NA	NA	NA					90
Metal	**-3.113**	**-5.149**	**-1.077**	**<0.001**	NA	NA	NA	NA	NA	NA	3	0	3	0	45
hSalinity	-0.404	-0.878	0.071	>0.05	NA	NA	NA	NA	NA	NA					NA
Interaction	-1.117	-2.447	0.213	**<0.001**	NA	NA	NA	NA	NA	NA					NA
b)	**Effect of local anthropogenic stressors**														
NE	0.384	-0.758	1.528	>0.05	NA	NA	NA	NA	NA	NA	3	0	3	0	NA
Fishing	0.108	-1.026	1.241	>0.05	NA	NA	NA	NA	NA	NA					NA
Interaction	0.178	-1.536	1.893	>0.05	NA	NA	NA	NA	NA	NA					NA

Table S5. Predicted effect (PE) and actual effect (AE) (Hedge g standard mean difference) of the interactions between (a) local anthropogenic [fishing, nutrient enrichment (NE), a heavy metal pollution (Metals) and high sediment loads (Sediment)] × other stressors (presence of competitors or grazers, removal of canopy algae, low light or salinity, high light or salinity, and increasing CO_2, temperature or wave exposure and high UV) and (b) multiple local anthropogenic stressors [NO_3 and PO_4, fishing, heavy metal A and B, nutrient enrichment (NE), heavy metal pollution (Metals) and high sediment loads (Sediment)] on the growth of canopy-forming algae and mat-forming algae and survival of canopy-forming algae and mat-forming algae. Results are the overall estimate of effect size using the standard mean difference [overall estimate, 95% lower confidence interval (LC), higher confidence interval (HC)]. Effects are antagonistic if the actual effect size of Stressor A × B was closer to zero than the predicted effect size and synergistic when the actual effect size of Stressor A × B was further away from zero than predict effect size. Interactions were nonadditive if the confidence intervals of the actual effect size did not overlap the predicted effect size and additive if the confidence intervals of actual effect size overlapped of the predicted effect size. Antagonistic and synergistic interactions are shown in bold print.

Test	**Canopy-forming algae growth**						**Mat-forming algae growth**					
	PE	**LC**	**HC**	**AE**	**LC**	**HC**	**PE**	**LC**	**HC**	**AE**	**LC**	**HC**
a) Interactions between of local anthropogenic and other stressors												
NE,-Canopy	NA	NA	NA	NA	NA	NA	2.152	1.323	2.982	2.96	1.942	3.978
NE,Competitors	**-1.359**	**-2.806**	**0.089**	**-14.478**	**-25.37**	**-3.586**	0.841	0.087	1.595	1.691	0.542	2.841
NE,Grazers	-0.881	-1.647	-0.114	-1.693	-2.957	-0.428	0.596	-0.334	1.527	0.5183	-0.606	1.643
NE,lLight	**-1.647**	**-3.81**	**0.516**	**-10.474**	**-16.464**	**-4.485**	0.149	-1.21	1.504	-0.058	-1.224	1.106
N,hLight	0.432	-0.417	1.282	5.55	1.671	9.429	1.507	0.182	2.832	1.9766	0.3745	3.5788
NExlSalinity	-2.863	-6.031	0.307	-1.0308	-6.617	4.556	0.372	-0.354	1.097	0.354	-0.241	0.949
NExhSalinity	-1.013	-3.659	1.634	-0.3524	-1.24	0.535	NA	NA	NA	NA	NA	NA
NE,↑CO_2	3.357	1.379	5.335	4.552	2.814	6.289	**1.147**	**0.435**	**1.859**	**4.359**	**1.9201**	**7.798**
NE,↑Temperature	**3.132**	**-1.149**	**7.415**	**-3.603**	**-6.787**	**-0.418**	NA	NA	NA	NA	NA	NA
NE,↑Waves	NA	NA	NA	NA	NA	NA	1.715	0.755	2.674	4.474	2.003	6.946
Fishing,Grazers	-1.245	-1.849	-0.64	-1.867	-2.529	-1.205	-0.486	-3.641	2.669	-0.255	-1.419	0.907
Metals,lLight	**-1.647**	**-3.811**	**0.516**	**-12.174**	**-16.05**	**-8.301**	NA	NA	NA	NA	NA	NA
Metals,hLight	-1.093	-3.409	1.223	-4.122	-10.019	1.773	NA	NA	NA	NA	NA	NA
Metal,lSalinity	-1.543	-3.049	-0.038	-1.819	-3.542	-0.097	**0.372**	**-0.353**	**1.097**	**-3.659**	**-5.954**	**-1.365**

Sediment,Competitors	-0.036	-1.103	1.031	-0.2364	-2.126	1.652	-1.141	-2.908	0.626	-1.286	-2.849	0.277
Sediment,Grazers	-0.328	-2.819	2.163	-0.261	-1.236	0.713	0.265	-2.484	3.015	-0.261	-1.236	0.713
Sediment,lLight	-4.112	-6.833	-1.389	-3.07	-5.917	-0.224	0.157	-0.566	0.882	-0.138	-0.86	0.586
Sediment,↑Temperature	-0.751	-1.472	-0.03	-1.495	-2.899	-0.092	NA	NA	NA	NA	NA	NA
Sediment,↑Waves	-0.417	-1.09	0.255	-0.7714	-2.089	0.546	NA	NA	NA	NA	NA	NA
b) Interactions between local anthropogenic stressors												
NO_3,PO_4	-0.219	-0.905	0.469	0.321	-1.141	1.781	1.616	-0.284	3.518	3.216	0.876	5.556
NE,Metala	**-1.655**	**-3.266**	**-0.044**	**-11.694**	**-18.05**	**-5.343**	-2.774	-5.528	-0.021	-1.189	-2.162	-0.217
NE,Sediment	NA	NA	NA	NA	NA	NA	1.417	0.476	2.358	2.367	1.309	3.425
MetalA,MetalB	-1.772	-3.251	-0.294	-1.483	-2.528	-0.438	NA	NA	NA	NA	NA	NA
Test	**Canopy-forming algae survival**						**Mat-forming algae survival**					
a) Interactions between of local anthropogenic and other stressors												
NE,Competitors	**-0.979**	**-1.844**	**-0.115**	**-6.180**	**-11.67**	**-0.69**	0.735	-1.632	3.101	1.691	0.542	2.841
NE,Grazers	-0.573	-3.134	1.986	-1.325	-3.809	1.159	1.326	-0.665	1.987	0.117	-0.753	0.987
NE,lLight	-1.085	-2.511	0.341	-0.434	-1.375	0.507	NA	NA	NA	NA	NA	NA
NE,hLight	-1.347	-3.257	0.563	-1.775	-3.764	0.213	NA	NA	NA	NA	NA	NA
NE,↑Temperature	**-0.871**	**-2.02**	**0.279**	**-3.474**	**-5.988**	**-0.957**	0.906	-1.24	3.053	1.929	-1.853	5.713
NE,UV	NA	NA	NA	NA	NA	NA	6.094	0.649	11.539	4.422	-2.372	11.218
Metal,lSalinity	-5.874	-12.172	0.423	-10.965	-21.8	-0.128	-2.959	-5.003	-0.915	-7.847	-12.01	-3.693
Metal,hSalinity	-2.4	-6.223	1.42	-7.086	-21.94	7.774	-2.959	-5.003	-0.916	-1.117	-2.447	0.213
Sediment,Competitors	0.466	-0.681	1.614	0.336	-0.803	1.476	NA	NA	NA	NA	NA	NA
Sediment,Grazers	-2.581	-4.135	-1.027	-5.639	-13.82	2.539	NA	NA	NA	NA	NA	NA
Sediment,lLight	-4.171	-6.658	-1.683	-6.750	-10.19	-3.305	NA	NA	NA	NA	NA	NA
Sediment,hLight	**-1.509**	**-2.474**	**-0.544**	**-5.169**	**-9.593**	**-0.747**	NA	NA	NA	NA	NA	NA
Sediment,↑Temperature	-1.668	-2.702	-0.635	-1.495	-2.899	-0.092	NA	NA	NA	NA	NA	NA
Sediment,↑Waves	-1.518	-3.006	-0.031	-0.7714	-2.089	0.546	NA	NA	NA	NA	NA	NA
b) Interactions between local anthropogenic stressors												
NE,Fishing	NA	NA	NA	NA	NA	NA	0.362	-0.778	1.504	0.178	-1.536	1.893

Table S6. Qualitative review of nature of interactions between multiple local anthropogenic stressors [fishing, nutrient enrichment (NE), heavy metal pollution (M) and high sediment loads (Sed)] and local anthropogenic and other stressors (presence of competitors and grazers, removal of canopy algae, low light and salinity, high light and salinity, increasing CO_2, temperature, and waves and high UV) on the (i) growth of canopy-forming algae (ii) growth of mat-forming algae (iii) survival of canopy-forming algae and (iv) survival of mat-forming algae. Effects are additive (Add) if the actual effect size overlaps with the predicted effect size, antagonistic (A) if the actual effect size of Stressor A × B were closer to zero than the predicted effect size, synergistic (S) if the actual effect size was further away from zero than the predicted effect size and not significant (NS) if the actual effect overlaps zero. The numbers of experiments for each interaction type are indicated in brackets. Pairs of stressors in which the majority of experiments show antagonistic interactions are shaded in light grey and synergistic interactions are shaded in dark grey.

Test	**NE**	**M**	**Sed**	**Competitors**	**Grazers**	**-Canopy**	**lLight**	**lSalinity**	**↑CO_2**	**hLight**	**↑Temp**	**hSalinity**	**↑Waves**	**hUV**
i) Growth of canopy-forming algae														
Fish	NA	NA	NA	Add (1)	**S (1)** Add (5) NS(4)	NA	NA	NA	NA	NA	NA	NA	NA	NA
NE	NS(3) Add(1)	**S(6)** Add(1) NS(1)	NS(1)	**S(3)** NS(1)	**S(5)** Add(3)	Add(1)	**S(3)** Add(2)	NS(2)	Add(3)	Add(2)	**S(3)**	NS(2)	NS(2)	NA
M		Add(12)	**S(1)**	NA	NA	NA	**S(1)** Add(1)	**S(4)** Add(2)	NA	Add(3) **S(1)**	**S(1)**	Add(4) **S(1)**	NA	NA
Sed				Add(4)	Add(2)	NA	**S(2)** Add(2)	NA	NA	Add(2)	Add(3)	NA	Add(4)	NA
ii) Growth of mat-forming algae														
Fish	NS(2) **S(1)**	NA	NA	NA	NS(3)	NA	NA	NA	NA	NA	NA	NA	NA	NA
NE	Add(4) NS(2)	Add(1) NS(2)	Add(3)	Add(4) NS(3)	**A(1)** NS(6)	**S(1)** Add(3)	Add(1) NS(5)	NS(6)	**S(3)** NS(1)	**A(1)** Add(1) NS(4)	**S(1)**	NA	**A(2)** Add(3) NS(4)	NA
M		Add(1)	NA	NA	NA	NA	NA	**S(2)** Add(1)	NA	NA	Add(1)	Add(1)	NA	NA
Sed			NA	**S(1)** Add(2) NS(4)	**S(1)** Add(2) NS(4)	NA	NS(3)	NA	NA	Add(1)	NA	NA	NA	NA
iii) Survival of canopy-forming algae														
Fish	NS(2)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
NE	**S(1)** Add(1)	NA	NA	**S(2)** Add(1)	**S(1)** Add (1) NS (1)	NA	**S(1)** NS(3)	NA	NA	**S(1)** NS(3)	**S(3)** NS(1)	NA	NA	NA
M		**S(1)**	NA	NA	NA	NA	NS(1)	**S(2)** NS(1)	NA	NS(1)	**S(1)**	**S(1)** NS(1)	NA	NA
Sed			NA	NS(2)	**S(1)** NS(1)	NA	**S(4)**	NA	NA	**S(2)**	**S(1)** Add(1)	NA	**S(2)** Add(2) NS(2)	NA
iv) Survival of mat-forming algae														
Fish	NS (2)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
NE		Add(1)	NA	Add(1) NS(1)	NS(8) **S(1)** Add(1)	NS(2)	NA	NA	NA	NA	Add(1) NS(1)	NA	NA	Add(2)
M		Add(1)	NA	NA	NA	NA	NA	Add(3) **S(1)**	NA	NA	NA	Add(3) NS(1)	NA	NA

Sed					NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Chapter 2

Status of depleted intertidal populations of *Cystoseira* spp. along the Italian coasts and relationships with environmental factors and anthropogenic pressures.

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Abstract

Canopy-forming seaweeds of the genus *Cystoseira* are one of the main biogenic habitats along Mediterranean rocky intertidal shores, but they are retracting considerably to the point where several species have been reported as locally lost in many areas. We investigated the status of intertidal *Cystoseira* spp. at thirteen rocky sites around the Italian coastline. We documented a general degradation of intertidal stands of *Cystoseira* spp., with low percentage covers and density at most locations. Only eleven sites supported some populations of *Cystoseira* spp., with average percentage cover less than 38%. Most populations comprised monospecific stands of *Cystoseira compressa* (Esper) Gerloff & Nizamuddin, 6 sites also supported some specimens of either *Cystosera amentacea* (C.Agardh) Bory v. *stricta* Montagne, or *Cystoseira brachycarpa* J. Agardh emend. Giaccone v. *brachycarpa*, while no site comprised all three species. We analysed whether the status, described as a combination of cover, density, morphological characters and amount of epiphytes, of the most common *C. compressa* was related to 11 relevant environmental and anthropogenic factors. Coastal human population density within a 10 km radius, and average annual marine concentrations of chlorophyll-*a* and nitrates were significantly related to the status of *C. compressa*, explaining 40% of the variations between sites. Conversely, wave exposure, distance from urban centre, PAR, salinity, tidal range and seawater temperature did not have a significant relationship. Coastal human population density explained alone about 27% of the variation confirming the important role of human pressures on intertidal rocky shore systems. Thallus width and height, and branches diameters of *C. compressa* varied across sites and were affected by high coastal human population and seawater nitrate concentration. Epiphytes were abundant at sites characterized by high seawater chlorophyll-a concentration. Overall the results indicated a severe depletion of intertidal populations of *Cystoseira*, and identified urbanization as one of the main factors related to these poor conditions, confirming the need for urgent management actions to reduce human pressures on these valuable habitats forming species.

Keywords: Canopy-forming seaweeds, Fucales, *Cystoseira* spp., *Cystoseira compressa*, rocky intertidal, morphology, environmental variables, anthropogenic pressures, habitat loss.

Introduction

Coastal marine habitats comprise some of the most productive, diverse and at the same time overexploited marine ecosystems (Airoldi and Beck, 2007). Pollution, overfishing and coastal transformation are just some of the impacts caused by increasing human population and pressures in coastal areas. These impacts are further exacerbated by global climatic changes, such as sea-level rise, ocean acidification, increased sea-surface temperatures and increased frequency of extreme events, amplifying the vulnerability and the loss of marine coastal ecosystems (Airoldi and Beck, 2007; Crain et al., 2009; Coll et al., 2010). Many of these coastal ecosystems are built by foundational, habitat-forming species, which are critical for supporting biodiversity and ecosystem functioning. In the Mediterranean Sea intertidal rocky shores host some of the most diverse, productive and valuable macroalgal beds (Coll et al., 2010), which are usually composed by erect red and brown macroalgae. Loss of these species usually leads to a decline of biodiversity, and a shift towards less productive systems (Benedetti-Cecchi et al., 2001; Airoldi et al., 2008).

Canopy-forming species of the genus *Cystoseira* C. Agardh (Fucales, Phaeophyceae) are especially diverse in the Mediterranean Sea (Oliveras-Plá and Gómez-Garreta, 1989; Draisma et al., 2010), providing food and protection for rich associated communities, comprising other algae, invertebrates and fish (Mineur et al., 2015). *Cystoseira* spp. are distributed from the infralittoral down to the upper circalittoral zone (Giaccone et al., 1994; Bulleri et al., 2002; Falace and Bressan, 2006; Ballesteros et al., 2009), and their distribution is controlled by several environmental parameters including depth, water temperature, substratum characteristics, wave exposure and nutrient concentrations (Giaccone 1971; Ballesteros 1992; Sales and Ballesteros 2009). Further, human pressures are increasingly limiting the distribution of these species (Chryssovergis and Panayotidis, 1995; Rodríguez-Prieto and Polo, 1996; Soltan et al., 2001; Arevalo et al., 2007; Sales et al., 2011). During the last decades several *Cystoseira* species have retracted their ranges considerably (Airoldi et al., 2014) to the point where several species have been reported to be locally lost (Soltan et al., 2001; Thibaut et al., 2005; Serio et al., 2006; Mangialajo et al., 2007, 2008; Perkol-Finkel and Airoldi, 2010; Thibaut et al., 2015). The loss of *Cystoseira* canopies leads to structurally less complex communities most often dominated by low-lying, turf-forming species (Benedetti-Cecchi et al., 2001; Connell et al., 2014) or sea urchin barrens (Agnetta et al., 2015). These shifts are attributable to the interactive effects of different local and global stressors (Asnaghi et al., 2013; Strain et al., 2014, 2015) causing a general homogenization of the habitats (Airoldi et al., 2008). For these reasons *Cystoseira spp*. have been used as biological indicators of water and ecosystem quality (Panayotidis et al., 1999), including the assessment of environmental quality according to the Water Frame-work Directive (2000/60/EC) (Ballesteros et al., 2007; Mangialajo et al., 2007)

Assessing the status of threatened populations is an important step to develop adequate protection and prevent further losses. In this study we quantified the distribution and abundance of intertidal species of *Cystoseira* at 13 locations along the Italian coastline, covering a range of biogeographic location, environmental characteristics and levels of anthropogenic pressures. We also characterised and compared the morphology and general status of the only species that was still relatively common at most locations, specifically *C. compressa*. Despite severe regressions, this species is relatively resistant to some environmental and anthropogenic factors compared to other species of *Cystoseira*, and still persists at many otherwise depleted localities of the Mediterranean Sea (Thibaut et al., 2005; Mangialajo et al., 2008), offering an opportunity for comparisons across localities. We quantified different descriptors (including percentage cover, density, morphological characteristics, and cover and number of epiphytes) and tested whether any variations were related to differences in key environmental and anthropogenic factors, including wave exposure, photosynthetic active radiation, salinity, tidal range, seawater temperature, marine chlorophyll-*a*, nitrate and phosphate concentrations, distance from urban centre and coastal human population density.

Materials and methods

Study area

Italy is a long peninsula extending more than 950km from north to south into the Mediterranean basin, with a coastline of more than 7600km. Flat, sandy shores and high rocky coasts alternate along the peninsula that is surrounded by four different seas (Figure 1). Peculiar environmental and geological characteristics allow the establishment of rich and diverse marine ecosystems. Despite the presence of several Marine Protected Areas (MPA) the Italian coast is generally overexploited. The long and narrow shape of the Italian peninsula allows coastal access to the majority of the population. Most of the largest Italian cities are located along the coastline, and millions of people rely on the services provided by marine ecosystems. Tourism and industry impose additional threats to the coast.

Sampling was carried out at 13 locations (Figure 1; Table S1), hereafter names sites. All sites were well or moderately exposed, with an average tidal range of ≈ 20-40 cm, with the exception of Trieste, in the northern Adriatic Sea, with average tides of ≈ 89 cm (Table 1). At all sites, the intertidal system was formed by rocky platforms of different inclinations with crevices and ponds, or boulders. Assemblages generally comprised mixed stands of *Cystoseira* spp. and other macroalgae (Pp, Kr, LI, PA, Ob, Pn, TA, Ot and Mz), with the exception of 4 localities where assemblages were dominated by mussels (comprising either *Mytilus galloprovincialis* Lamarck or *Mytilaster minimus*) (Ga, Or, AN and TS). Only TS site was located into a MPA, while all the other sites were characterized by a variety of human pressures (Table 1). The main environmental and anthropogenic characteristics for each site are summarised in Table 1, and described in the results.

Environmental and anthropogenic factors

For each of the 13 study sites, we collected data relative to a total of 11 environmental and anthropogenic parameters (Table 1) to be used in the subsequent analyses. Estimates of annual mean sea surface temperature (SST), standard deviation of SST (as a proxy for seasonal variation) and tidal range were derived from the ISPRA (Istituto Superiore per la Protezione e Ricerca Ambientale, http://www.mareografico.it.) buoy closest to the site of interest (temporal range 2000-2012). Annual means of marine chlorophyll-*a* concentration, photosynthetic active radiation (PAR) and salinity at each site were obtained from the

Environmental Marine Information System (EMIS, http://mcc.jrc.ec.europa.eu/emis/, temporal range 2003-2012) using the "EMIS" R package (Copyright (c) 2014 ldbk). Annual means of nitrate and phosphate concentrations were obtained from the "biogeochemical model and data assimilation of surface chlorophyll concentration" OPATM-BFM (Generated using CMEMS products, http://marine.copernicus.eu/, temporal range 2002-2012). Wave fetch was calculated as the sum of fetch values in the cells defined by 16 angular sectors (22.5° each), and expressed as log10 (Burrows, 2012). Finally, to explore the effects of anthropogenic pressures, we derived the coastal population density in a radius of 10km from the site of interest ISTAT (Istituto Nastionale di Statistica, data series 2011), and calculated the distance along a straight line to the nearest town or urbanization centre by geographical information system (GIS). To detect possible skewness and/or strong correlation among pairs of environmental and anthropogenic factors we used the "*chart.Correlation*" function in the "PerformanceAnalytics" R package (Peterson and Carl, 2014). We corrected for right-skewness by applying a log(x+1) transformation to nitrate, phosphate and chlorophyll-*a* concentrations, and a square-root transformation on distance to the nearest urban centre and coastal human population density. Due to the high correlation of phosphate concentration with both tidal range (cor.=0.96) and nitrate concentrations (cor.=0.93), we removed phosphate concentrations from the subsequent analyses (Figure S2).

Cover, density morphology and epibiota of **Cystoseira** *spp.*

Sampling was carried out from 24 June to 23 July 2013, when vegetative growth of *Cystoseira* spp. was at the peak. We sampled populations occurring between -10 cm to +10 cm relative to Mean Low Water Level; MLWL. Percentage cover and density of intertidal *Cystoseira* spp. at each site were estimated in six, randomly located quadrats. Sampling quadrats (30 x 30cm) were divided into 25 equal squares, and the percentage cover of *Cystoseira* spp. was quantified by giving a score ranking from 0 to 4 in each square, and then adding up scores for all the smaller squares as described by Dethier et al (1993). Density of each species of *Cystoseira* was also estimated as number of individuals in the same six, quadrats. Morphological characters were described only for *C. compressa*. Ten thalli were randomly collected and fixed in a 4% formalin seawater solution until laboratory measurements of: average length and diameter of primary and secondary branches (n=4), thallus height and thallus width (Falace et al, 2004) and ash-free dry weight (AFDW). Moreover, for each thallus epibionts were carefully removed with a pair of tweezers, weighted and identified to the lowest taxonomic level possible (Table 2).

The matrix of data containing the mean values of cover, density, morphometric characteristics and amount of epiphytes per each study site was used to describe the status of *C. compressa* and test its relationship with key local environmental and anthropogenic factors (Table 2). Due to the different types of measurements of the descriptors the matrix was pretreated as it is normally done with matrices of environmental data. We used the "*chart.Correlation*" function in the "PerformanceAnalytics" R package (Peterson and Carl, 2014) to detect strong correlations or possible skewness among pairs of status descriptors. Because high collinearity was found between branches length and thallus high, and between branches diameter, we only retained thallus height and diameter of first order branches as response descriptors. We also retained density but excluded cover percentage of *C. compressa* because the high autocorrelation. We applied a square-root transformation to weight of epiphytes and AFDW to correct for right-skewness (Figure S3). Finally, we created a Euclidean distance matrix based on the normalized data and used it to test the relationships between the matrix of status variables and the environmental variables by using the DistLM analysis (Legendre and Andersson, 1999).

Data analyses

We tested differences in cover and density of *Cystoseira* species among sites (13 levels, random factor) by performing univariate permutational analysis of variance (PERMANOVA), with PERMANOVA+ (Anderson et al., 2008) for PRIMER v.6 (Clarke and Gorley, 2006). The analysis was based on Bray-Curtis distance matrix made on square-root transformed data, with type III of sum of squares, 9999 permutations and unrestricted permutation of raw data.

Distance-based linear model routine (DistLM) (Legendre and Andersson, 1999) was performed to model relationships between the status, described as a combination of cover, density, morphological characters and amount of epiphytes, of *C. compressa* and the environmental and anthropogenic factors. The analysis was performed using with PERMANOVA+ (Anderson et al., 2008) for PRIMER v.6 (Clarke and Gorley, 2006), selection procedure and selection criterion used were step-wise and adjusted R^2 respectively, with 9999 number of permutations. We used distance-based redundancy analysis (db-RDA) triplot to visualize relationships between status of *C. compressa* and environmental and anthropogenic factors presenting significant correlation in the predicting model. Db-RDA was conducted using the "*capscale*" function in the "*vegan*" R-package (Oksanen et al., 2015).

Results

Environmental and anthropogenic parameters

All sites presented moderate wave exposures, with fetch values > 3 $\log_{10}$(cells). For most sites average annual tides were ≈ 20-30 cm, with the exception of TS with average tides of ≈ 89 cm. Annual average seawater salinities were generally 38 psu, with only three sites, Or, AN and TS, that presented values < 37 psu. Greatest average annual chlorophyll-*a* concentration were found in AN (1.9 mg/m^3), followed by Or (1.3 mg/m^3) and TS (1.1 mg/m^3), while the other sites presented values below 0.8 mg/m^3 , with the lowest values of 0.2 mg/m^3 recorded at Pn. Nitrate concentrations were highest at Or, AN and TS, with 2.51 mmol/m^3, 6.14 mmol/m^3 and 23.12 mmol/m^3 respectively. The other sites presented nitrate concentrations < 0.99 mmol/m^3, with the minimum value of 0.12 at Pp. Phosphate concentration ranged from 0.04 mmol/m^3 at Pp to 2.01 mmol/m^3 at TS. PAR values decreased from southern to northern sites ranging from ≈ 38 E/m^2/day at Pp to ≈ 30 E/m^2/day at TS. The same trend was observed for annual average seawater temperatures, that decreased from 20°C at PA to 17°C at AN and TS. An opposite trend was observed for the annual standard deviation of seawater temperature, that increased at northern compared to southern sites.

Concerning the anthropogenic factors, six sites, KR, Mz, Ob, PA, Pp, and TA were very close to urban centres, with the extremes of LI and Ot which were < 1000 m distant. In the remaining sites the distance from urban centres increased, reaching the maximum values of 6,858 m for the site of AN. The coastal human population densities within a radius of 10km from the sites ranged from 50 n°ind/Km^2 at LI to 529 n°ind/Km^2 at TS.

Cover, density morphology and epibiota of Cystoseira *spp.*

At the 13 study sites we found only 3 species of *Cystoseira*: *Cystoseira amentacea* (C.Agardh) Bory v. *stricta* Montagne, *Cystoseira brachycarpa* J. Agardh emend. Giaccone v. *brachycarpa* and *Cystoseira compressa* (Esper) Gerloff & Nizamuddin. The three species were never simultaneously observed at any sites. Seven sites (KR, Ga, LI, Ot, Pn, Pp and Mz) presented combinations of two species, four sites (AN, Or, Ob and PA) presented one species only, while at two sites (TA and TS) we could not find any longer any *Cystoseira* species. *C. compressa* was the most widespread species, detected at eleven sites. Percentage cover and density largely differed among sites (Figure 2 A- B, Table S2). *C. amentacea* had the highest average percentage cover at Mz (83%), *C. brachycarpa* at Pp (68%), while the average percentage cover *C. compressa* was never > 38% (Figure 2 A). On the other hand, the average density was never > 5 individuals (900 cm^2), reached by *C. compressa* at two sites, KR and LI, followed by *C. brachycarpa* with 4 individuals (900 cm^2) at Pp and LI, while *C. amentacea* reached 3 individuals only at KR (Figure 2 B).

The morphological characteristics of *C. compressa* differed among locations (Table 2). For example, thallus length ranged from 35.4±5.8 mm to 222.3±7.8 mm at Pn and Or, respectively. Branches length of first order ranged from 27.5±4.7 at PA to 81.1±24.7 at Or, while second order branches ranged from 13.3±3.3 at PA to 37.1±7 at Or. Only two sites, Pb and Pn, presented individual of *C. compressa* with branch diameter > 2 mm, while little variations were found between the other sites with average values about 1.5 mm. Finally, the biomasses were highest at Mz, Or and Pp, with 3.39±1.3 gr, 3.43±1.9 gr and 3.61±0.8 gr respectively and lowest at Ot, Pn and PA, with 0.49±0.3 gr, 0.47±0.1 and 0.3±0.1 gr respectively.

In total 22 epibionts were identified (Table S3). However, some of these were not identified at the level of species, mostly because of their small sizes or absence of characters facilitating their identification (e.g. presence of reproductive structures). The epibionts mostly comprised red algae (Rhodophyta). Animal epibionts mostly comprised mussels (*Mytilus galloprovincialis*) and were present only at few locations (LI, AN, GA and Or). AN, GA and Or presented higher values of epibiota weights (Table 2).

Relationship between **Cystoseira** *status and environmental and anthropogenic parameters*

The marginal DistLM test showed that density of coastal human population, tidal range and wave fetch, when taken alone, had a significant relationship with the matrix of variables describing the status of *C. compressa*, explaining 27%, 23% and 20% of *C. compressa* status variability respectively (Table 3A). The sequential test, based on a stepwise procedure, selected density of coastal human population, chlorophyll-*a* concentration, wave fetch, distance from urban centre, PAR and standard deviation of seawater, which together explained a total variation of 56% (Adj. R^2, Table 3B). However, only density of coastal human population, chlorophyll-*a* concentration and nitrate concentration were statistically significant ($p<0.05$) and therefore the model should only retain the first 3 factors explaining approximately 40% (Adj. R^2) of data variation (Table 3B).

The dbRDA ordination triplot showed the relationships between the descriptors of the status of *C. compressa* and environmental and anthropogenic factors (Figure 3). The first two dbRDA axes explained 99.5% of the fitted model variation that is about the 52% of the total variation in the status of *C. compressa*. The variable most closely related with the first dbRDA axis was coastal human population density. Chlorophyll-*a* seawater concentration was the variable most related with the second dbRDA axis. Nitrate seawater concentration was significantly related with the first axis but presented a good relationship also with the second dbRDA axis (Figure 3). When looking at the different descriptors of the status of *C. compressa* most of them had a negative relation with density of coastal human population and nitrate concentration. Moreover, abundance of epiphytes was positively related with chlorophyll-*a* seawater concentration (Figure 4).

Discussion

Our results highlight a general degradation status of the intertidal *Cystoseira* populations at the thirteen study sites along the Italian coast. We found only three species, *C. amentacea*, *C. brachycarpa* and *C. compressa*, and none of the investigated sites presented the three species simultaneously. *C. compressa* was the most common species found at eleven sites. However, the low percentage cover and density values generally recorded suggest that even this relatively tolerant species is in a depleted status. Coastal human population density, used as proxy of urbanization, explained the major part of the variation of the status of *C. compressa*, confirming the severe human pressures on this species. Wave fetch and chlorophyll-*a* concentration were the two environmental parameters most related to the status of *C. compressa.*

The loss of *Cystoseira* species around the Mediterranean Sea has been documented at numerous locations (Airoldi et al., 2014). Several factors have been suggested to drive the loss of *Cystoseira* spp., including urbanisation and eutrophication (Benedetti-Checchi et al., 2001; Thibaut et al., 2005; Mangialajo et al., 2008; Sales and Ballesteros, 2009; Villalonga, 2010; Mineur et al., 2015), increase in water turbidity and sedimentation (Airoldi, 2003; Perkol-Finkel and Airoldi, 2010; Strain et al., 2015), over-grazing (Agnetta et al 2015) and climate change (Asnaghi et al., 2013). We found *C. compressa* at most sites, confirming that this species is relatively tolerant to human pressures (Thibaut et al., 2005; Mangialajo et al., 2008), which nevertheless remains the major determinant on its status.

We also found a positive relationship between chlorophyll-*a* concentrations, usually used as proxy of eutrophication, and increase of epiphytes of *C. compressa*. Algae of the genus *Cystoseira* generally host epiphytic assemblages of considerable amount on the thalli (Belegratis et al., 1999; Faucci and Boero, 2000). For other intertidal seaweeds it has been suggested that epiphytes could be beneficial because they can limit grazing by potential herbivores (Harlin, 1975), reduce desiccation stress during low tide (Stewart, 1982) or reduce photoinhibition at high light levels by providing shade (Wiencke and Davenport, 1988). On the other hand, epiphytes can constitute an important source of stress for seaweeds. They can reduce the light levels at the surface of the alga, reducing the growth rates (Makarov et al., 2013) and chemical defences of the algae (Karez et al., 2000). Moreover, epibionts could increase the drag of the fronds and cause blade/fronds loss under wave action (Krumhansl et al., 2011). In this study the major part of epiphytes found on *C. compressa* comprised red algae. However, major epibiota weights were associated with presence of mussels. Growth of little mussels on *C. compressa* could weigh down and facilitate the tear of branches under wave action. However, our results didn't support this hypothesis. Nevertheless, observation in the field highlights that when the bottom are covered entirely by mussels the branches of *C. compressa* can be cutted by rubbing on mussel's shell. This aspect was not documented before and should more investigation.

The increasing coastal human population combined with the effect caused by climate change suggest that conditions for populations of *Cystoseira* spp will be worst in future years. A recent study highlighted that management of local human pressures could alleviate some of the impacts and facilitate the persistence of populations of *Cystoseira* spp despite ongoing climatic changes, and therefore should be prioritized for management (Strain et al., 2014). Our results support the idea that management actions aiming at preserving *Cystoseira* habitats, should be focused on those impacts correlated to coastal urbanization.

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Tables and Figures

Table 1 Environmental and anthropogenic variables at the study sites. Time data series 2000-2012. **CHLA**= average annual value of chlorophyll-a seawater concentration; **PAR**= average annual value of Photosynthetic Active Radiation; **SBOT**= average annual value salinity; **fetch**= wave fetch expressed as log10 of numbers of cells of fetch values; **wt. temp**= average annual value of seawater temperature; **sd. wt**= annual standard deviation of seawater temperature; **tide**= average annual value tidal range; **N**= average annual value of nitrate seawater concentration; **P**= average annual value of phosphate seawater concentration; **Dens.pop**= coastal human population density expressed as number of residents per Km^2 ; **Dist.ab**= distance in meter from urban centre .

	Environmental parameter									**Anthropogenic factors**	
Site	**CHLA**	**PAR**	**SBOT**	**fetch**	**wt. temp**	**sd. wt**	**tide**	**N**	**P**	**Dist.ab**	**Dens.pop.**
AN	1.901	31.183	36.759	3.581	16.8	6.2	31.7	6.14	0.305	6858	67
Ga	0.787	34.149	38.178	3.62	19.3	4.2	33.8	0.59	0.091	2629	402
KR	0.284	34.947	38.394	3.503	19.3	5	24.6	0.66	0.09	0	68
LI	0.447	32.479	38.154	3.568	18.9	4.8	31.4	0.51	0.087	673	50
Mz	0.232	37.09	38.215	3.62	19.2	3.1	19.7	0.35	0.09	0	213
Ob	0.283	34.389	38.104	3.11	18.9	2.3	26.5	0.54	0.088	0	137
Or	1.281	32.756	37.513	3.575	17.7	6.6	32.6	2.51	0.245	4321	54
Ot	0.309	35.155	38.294	3.532	19.2	5.1	39.1	0.84	0.115	460	52
PA	0.263	36.108	38.113	3.602	20.2	4.5	29.7	0.42	0.09	0	188
Pn	0.174	35.468	38.201	3.413	19.8	4.4	31.7	0.55	0.092	5684	99
Pp	0.221	38.5	38.392	3.529	18.9	3.1	23.7	0.15	0.038	0	68
TA	0.291	35.006	38.375	3.449	18.9	5.2	30.6	0.99	0.12	0	393
TS	1.125	30.658	36.76	3.244	16.9	6.3	89.1	23.12	2.01	3749	529

Table 2 Descriptors of the status of *C. compressa* used in the study. Values are means ± SD (n= 6-10). **Cover**= percentage cover of *C. compressa*; **Density**= density of *C.compressa*; **AFDW**= ash free dry weight; **Th_h**= thallus height; **Th_w**= thallus width; **B1_l**= first order branch length; **B2_l**= second order branch length; **B1_d**= first order branch diameter; **B2_d**= second order branch diameter; **w_epi**= weight of epiphytes; **n_epi**= number of epiphyte species.

Site	**Cover**	**Density**	**AFDW**	**Th_h**	**Th_w**	**B1_l**	**B2_l**	**B1_d**	**B2_d**	**w_epi**	**n_epi**
AN	25.0±20.0	3±2	0.72±0.2	92.9±22.8	28.2±7.1	53.7±17.7	28±3.4	1.7±0.2	0.9±0.1	8.02±0	2
KR	37.7±22.3	5±3	1.67±0.9	147.6±33.4	57.6±13.2	76.5±15.8	24.5±5.9	1.3±0.2	0.8±0.1	0±0	0
Ga	15.5±18.0	2±2	1.23±0.6	95±15.6	37.8±12.9	61.8±16.9	23.4±2.1	1.6±0.1	1.1±0.1	4.02±0	2
LI	18.3±7.8	5±2	1.62±0.4	96.1±13.9	46.7±8.3	54.5±10	22.6±2.8	1.7±0.2	1.2±0.2	0.26±0	1
Or	23.0±11.4	3±0	3.43±1.9	222.3±7.8	43.2±7.1	81.1±24.7	37.1±7	1.6±0.1	1.2±0.2	3.79±0	2
Ot	12.8±17.4	1±2	0.49±0.3	53.1±17.8	39.1±11.2	36.4±11	17.8±4.1	1.3±0.1	0.9±0.1	0±0	0
Ob	0.5±0.8	0±1	0.52±0	43.8±1.7	48±12.6	43±4.4	17.4±4.5	2.6±0.5	2±0	0±0	0
Pn	0.3±0.5	0±1	0.47±0.1	35.4±5.8	50.1±9	35.9±4.4	15.1±2.8	3±0.8	2.6±0.5	0±0	0
Pp	6.6±8.4	1±2	3.61±0.8	107.9±27.2	63±6.8	61.1±11.3	27.5±4.2	1.4±0.1	1±0.1	0.09±0	1
Mz	27.1±22.3	3±1	3.39±1.3	165.3±23.1	47.1±6.9	77.2±9.1	27.7±4.4	1.5±0.1	1.1±0.1	0.31±0	1
PA	7.5±8.4	2±1	0.3±0.1	59±13	47.9±11.1	27.5±4.7	13.3±3.3	1±0.2	0.6±0.1	0.03±0	1
TA	0	0	0	0	0	0	0	0	0	0	0
TS	0	0	0	0	0	0	0	0	0	0	0

Table 3 DistLM analysis of the status of *C. compressa*

(A) MARGINAL TESTS				
Variable	**SS(trace)**	**Pseudo-F**	**P**	**Prop.**
Log(CHLA+1)	11.043	1.9929	0.1089	0.15338
PAR	8.4807	1.4687	0.223	0.11779
SBOT	7.6232	1.3026	0.2606	0.10588
fetch	14.72	2.8268	**0.0366**	0.20444

wt.temp	7.5805	1.2944	0.2563	0.10528		
sd.wt	8.8563	1.5428	0.1964	0.123		
tide_range	16.437	3.2541	**0.0202**	0.22829		
Log(N+1)	12.122	2.2269	0.0734	0.16836		
Sqr(Dist.ab)	6.1326	1.0242	0.3869	8.52E-02		
Sqr(dens.pop.10km)	19.141	3.9834	**0.0188**	0.26585		
(B) SEQUENTIAL TESTS						
Variable	**Adj R^2**	**SS(trace)**	**Pseudo-F**	**P**	**Prop.**	**Cumul.**
+Sqr(dens.pop.10km)	0.19911	19.141	3.9834	**0.0176**	0.26585	0.26585
+LOG(CHLA+1)	0.30171	10.961	2.6162	**0.0407**	0.15224	0.41809
+LOG(N+1)	0.40369	9.6966	2.7102	**0.0454**	0.13468	0.55277
+fetch	0.43945	5.2941	1.5741	0.2325	7.35E-02	0.6263
+Sqr(Dist.ab)	0.47285	4.7662	1.5069	0.2522	6.62E-02	0.69249
+PAR	0.50324	4.2571	1.4283	0.2538	5.91E-02	0.75162
+sd.wt	0.51083	3.2081	1.093	0.3541	4.46E-02	0.79618
-LOG(N+1)	0.55695	1.2747	0.43432	0.7001	1.77E-02	0.77847

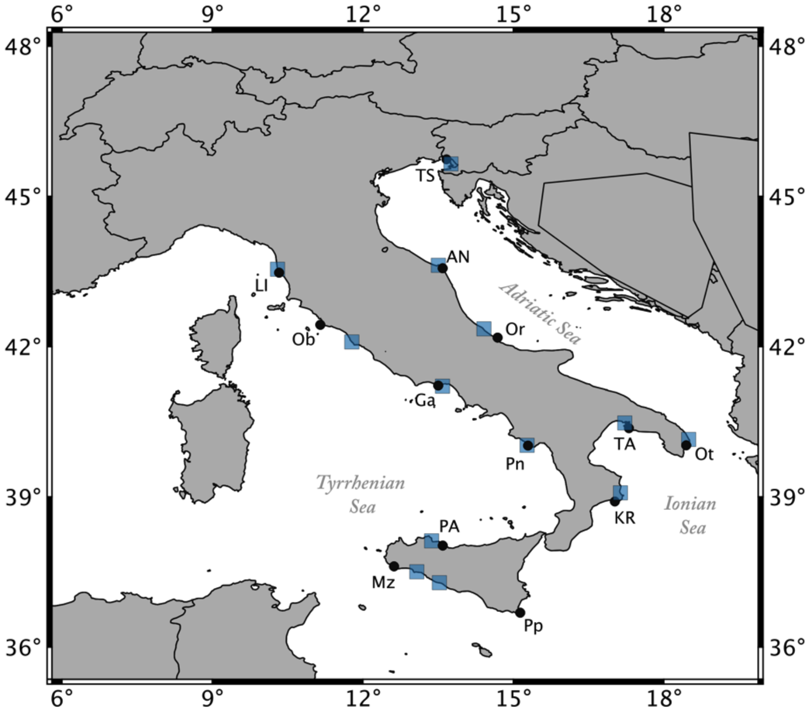

Figure 1 Location of the 13 rocky-intertidal study sites along the Italian coastline (black dots). **TS**: Trieste, **AN**: Ancona, **LI**: Livorno, **Ob**: Orbetello, **Or**: Ortona, **Ga**: Gaeta, **Pn**: Palinuro, **Ot**: Otranto, **KR**: Crotone, **PA**: Palermo, **Mz**: Mazara del vallo, **Pp**: Portopalo di capo passero. The position of the ISPRA buoys is indicated by the blue squares. Geographic coordinates of the sites and ISPRA buoys are reported in Table S1. Map projection WGS84.

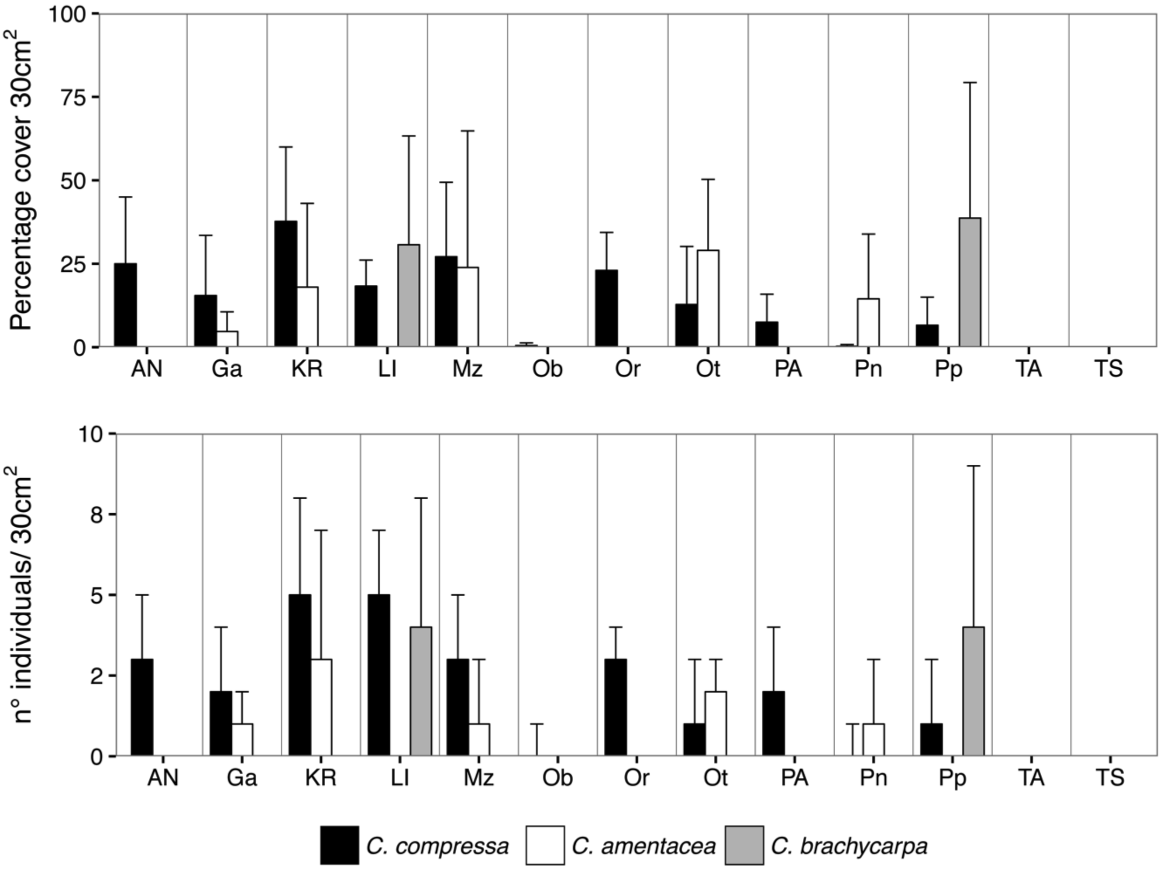

Figure 2 Percentage cover (A) and density (B) of the 3 intertidal species of *Cystoseira* found at the 13 study sites. Data are averages ± 1 SE (n= 6). Symbols of study sites are as in Figure 1.

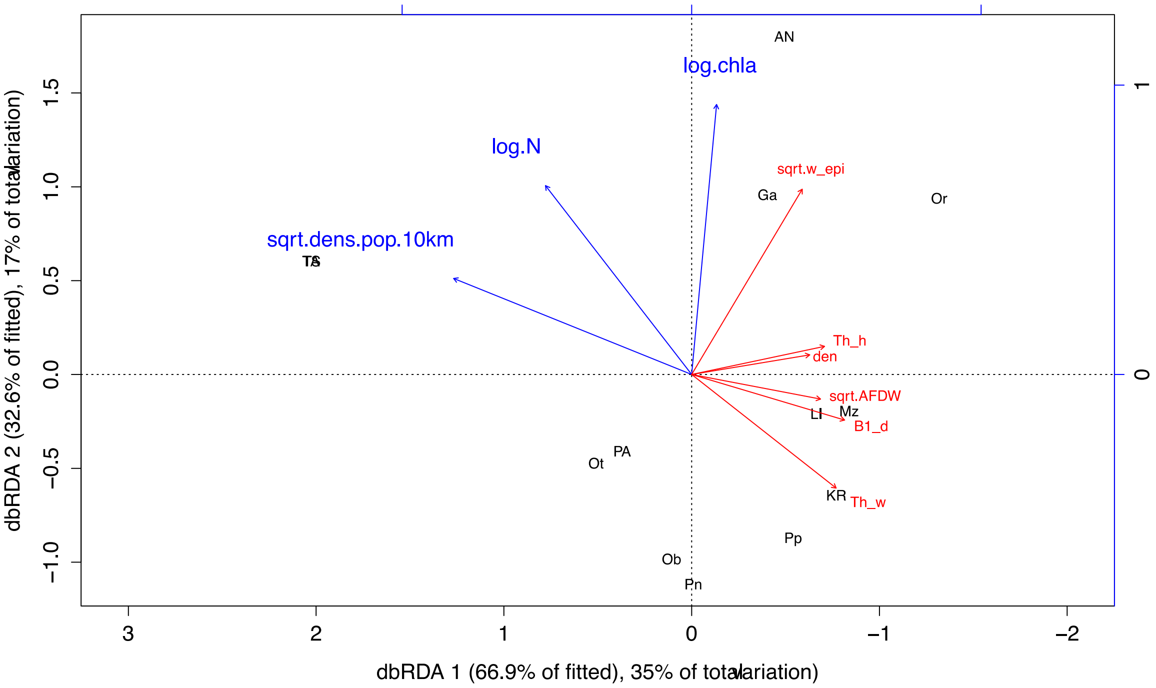

Figure 3 Distance-based redundancy analysis (dbRDA) showing relationship between the descriptors of the status of *C. compressa* (red lines) and the environmental and anthropogenic factors (blue lines) selected by DistLM model (scaling 2). **Sqrt.dens.pop.10km**= coastal human population density within a 10 km radius; **log.N**= nitrogen seawater concentration; **log.Chla**= chlorophyll-a seawater concentration; **sqrt.w_epi**= weight of epiphytes; **Th_h**= thallus height; **dens**= density; **sqrt.AFDW**= ash-free dry weight; **B1_d**= branches diameter; **Th_w**= thallus width.

Supplementary materials

Table S1 Geographical coordinates and id codes of the 13 sampling sites.

		Sites coordinates		**ISPRA buoys coordinates**	
Site	**ID**	**Lat.**	**Long.**	**Lat.**	**Long.**
Portonovo (Ancona)	**A**	43° 33' 57.780"	13° 35' 31.250"	43° 37'	13° 30'
Torre San Vito (Gaeta)	**G**	41° 13' 23.570"	13° 30' 11.966"	41° 12'	13° 35'
Le castella (Crotone)	**K**	38° 54' 26.597"	17° 1' 32.135" E	39° 04'	17° 08'
Calafuria (Livorno)	**LI**	43° 28' 39.119"	10° 19' 48.626"	43° 32'	10° 17'
Mazara del Vallo (Trapani)	**M**	37° 36' 44.136"	12° 37' 20.140"	37° 30'	13° 04'
Santo Stefano (Orbetello)	**O**	42° 26' 10.478"	11° 9' 8.842" E	42° 05'	11° 47'
Punta Aderci (Ortona)	**O**	42° 10' 49.926"	14° 41' 14.964"	42° 21'	14° 24'
Santa Cesarea Terme (Otranto)	**Ot**	40° 1' 45.592" N	18° 26' 56.036"	40° 08'	18° 29'
Altavilla (Palermo)	**P**	38° 1´ 37.412"	13° 35´ 36.366"	38° 07'	13° 22'
Faracchio (Palinuro)	**Pn**	40° 1' 25.813" N	15° 17' 39.516"	40° 01'	15° 16'
Portopalo di Capo Passero	**Pp**	36° 41' 10.244"	15° 8' 17.653" E	37° 17'	13° 31'
Leporano Marina (Taranto)	**T**	40° 22' 14.398"	17° 18' 23.670"	40° 28'	17° 13'
Grignano (Trieste)	**T**	45° 44' 27.575"	13° 40' 7.043" E	45° 38'	13° 45'

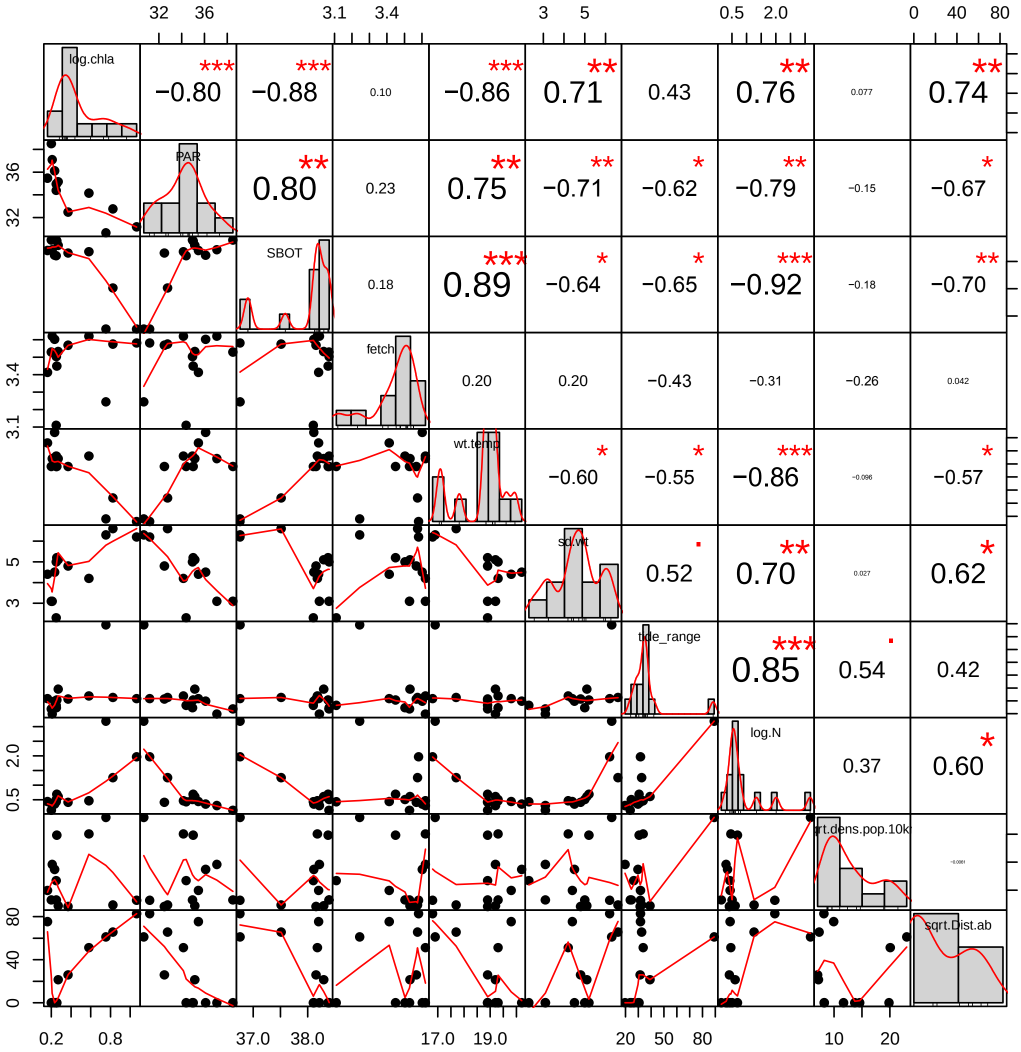

Figure S1 Correlation plot of the selected environmental and anthropogenic factors retained in the analysis. **log.chla**=chlorophyll-a seawater concentration; **PAR**= Photosynthetic Active Radiation; **SBOT**= salinity; fetch= wave fetch; **wt.temp**= seawater temperature; **sd.wt**= standard deviation of seawater temperature; tide= tidal range; **log.N**= nitrate seawater concentration; **sqrt.dens.pop.10km**= coastal human population density within 10km of site; **sqrt.dist.ab**= distance of site from urban centre.

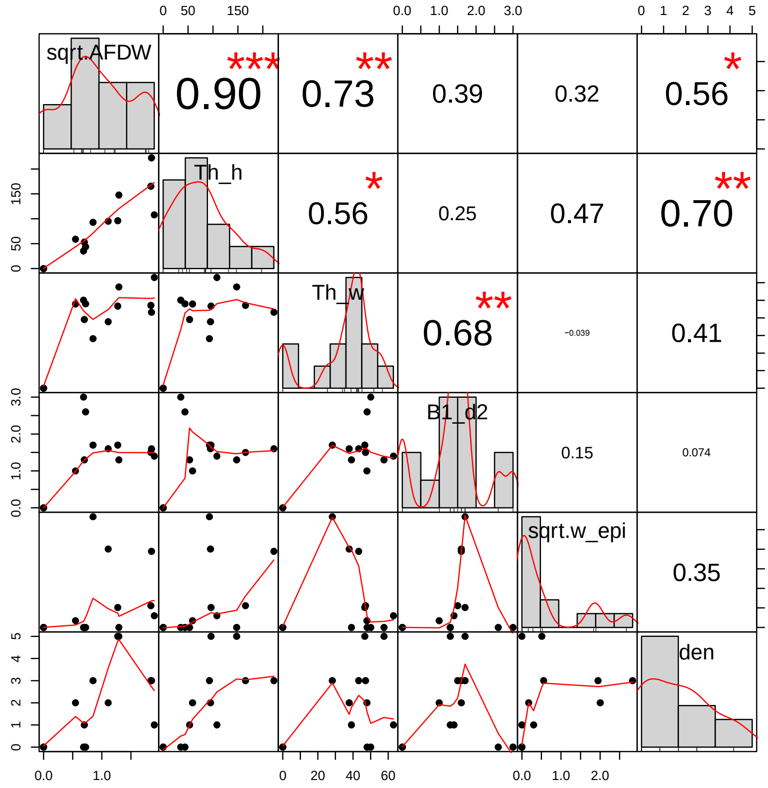

Figure S2 Correlation plot of the selected descriptors of the status of *C. compressa*. **sqrt.AFDW**= ash free dry weight; **Th_h**= thallus height; **Th_w**= thallus width; **B1_d2**= first order branch diameter; **sqrt.w_epi**= weight of epiphytes; **den**= density.

Table S2 PERMANOVA results. Differences in cover and density of species of *Cystoseira* among the 13 sites.

Factors

Name	Abbrev.	Type	Levels
site	si	Random	13

PERMANOVA table of results: ***Cystoseira* spp. cover percentage**

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
si	12	81022	6751.8	6.8993	**0.0001**	9904
Res	67	65568	978.63			
Total	79	1.47E+05				

PERMANOVA table of results: ***Cystoseira* spp. Density**

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
si	12	44063	3671.9	6.905	**0.0001**	9897
Res	67	35629	531.77			

Total	79	79692

Table S3 Epiphytes species recorded on *C. compressa*. x= presence. Sites codes as in Table S1.

Species/localities	**KR**	**Ot**	**Or**	**AN**	**LI**	**Ga**	**Pp**	**PA**	**Mz**	**Pn**	**TS**	**TA**	**Ob**
Aglaothamnion sepositum						x							
Aglaothamnion sp.									x				
Ceramium botryocarpum			x										
Ceramium ciliatum									x				
Ceramium diaphanum				x					x				
Ceramium flaccidum								x					
Ceramium pallidum				x									
Ceramiales		x	x	x		x							
Corallina elongata							x						
Corallina officinalis							x						
Encrusting algae							x	x	x				
Dasya sp.			x	x	x								
Jania rubens rubens													
Laurencia pyramidalis							x						
Mytilus galloprovincialis			x	x	x	x							
Polysiphonia fucoides													
Polysiphonia simulans													
Rhizoclonium sp.		x											
Sphacelaria sp.					x								
Stypocaulon scoparium													
Ulva rigida			x										
Wrangelia penicillata			x	x	x								

Chapter 3

Effects of extreme heat-wave events on the intertidal canopy-forming seaweed *Cystoseira compressa* and factors enhancing resilience

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Abstract

Global warming and human activities are boosting the odds of heat-wave events. However, the effects of heat waves have received little attentions, especially in marine coastal environments. Intertidal rocky shores are among the ecosystems mostly exposed and vulnerable to such extreme events. In the Mediterranean Sea intertidal rocky shores host diverse but at the same time threatened canopy-forming seaweeds of the genus *Cystoseira*. We carried out field experiments to investigate the effects of simulated heat waves on intertidal *Cystoseira compressa* populations from 10 localities along the Italian coast. We quantified the responses of *C. compressa* before and at the end of the heat wave and after 3h and 24h of recovery by measuring in vivo chlorophyll *a* fluorescence of photosystem II with a portable pulse amplitude modulation fluorometer (PAM). We used linear mixed model to test whether the response of *C. compressa* to the heat wave and its subsequent capability of resilience were affected by the characteristics of each individual alga (length, biomass, and pigment content), by the status of *C. compressa* local populations (percentage cover and density), by the local species richness (number of the dominant species of the assemblage), or by the thermal history of *C. compressa* (based on a biophysical model). Heat waves had clear impacts on *C. compressa* photosynthetic activity. The effects were detectable at all sites, independently of locations, and generally persisted during the first three hours after cessation of the impact. Some recovery occurred during the subsequent 24 hours, but patterns were not consistent across locations. A greater species richness of the local assemblage was moderately related to less severe immediate responses of *C. compressa*. This trend was still evident, even though no longer significant, after 3 hours recovery, while no relationship was found after 24 hours recovery. The recent thermal history of *C. compressa* was also related to the responses, as populations living close to their thermal limits were the most severely affected by the heat wave. The limited recovery observed after the impact of even a short heat-wave suggests that prolonged or repeated heat waves would severely impact this species. Further work should explore the longer-term effects of these extreme events particularly in populations subjected to other stressors, to better understand and predict the future risks for these important habitat-forming species.

Keywords: Global warming, climate change, heat wave, biodiversity, thermal history, *Cystoseira compressa,* intertidal, emersion phase, impact, resilience, photosynthetic stress.

Introduction

Climate change is one of the greatest threats to biodiversity and ecosystem functioning in marine ecosystems, having wide-ranging community-level impacts on species' abundances, and distributions (Walther et al., 2002; Sorte et al., 2010; Sala et al., 2011; Wernberg et al., 2012). One of the impacts of climate change is related to the projected increase of extreme events such as hurricanes, heavier rainfalls and flooding, wildfires and heat waves (Hegerl et al., 2011; Coumou and Rahmstorf, 2012).

Heat waves are one of the most dangerous aspects of global warming. A recent study has estimated that human influence at least doubled the chances of these extreme heat-wave events (Christidis et al., 2015). These events are typically defined as periods characterized by mean or maximum temperatures of at least 3-5°C above normal conditions (Meehl, 2004). Extreme heat waves, related to abrupt increases of water temperature, have been reported to cause mass mortality, changing species' distribution and altering the structure of different marine ecosystems, including seagrasses (Winters et al., 2011; Thomson et al., 2015), corals (Garrabou et al., 2009) invertebrates and macroalgae (Wernberg et al., 2012; Smale and Wernberg, 2013) and fish (Wernberg et al., 2012). The impacts of heat waves related to abrupt increases of air temperature, have received comparatively less attention, particularly in coastal marine habitats (Bell, 1993; Helmuth et al., 2002).

Coastal marine habitats comprise some of the most productive, diverse and at the same time threatened marine ecosystems (Airoldi and Beck, 2007). In the Mediterranean Sea intertidal rocky shores support valuable canopy-forming seaweeds of the genus *Cystoseira* C. Agardh (Fucales, Phaeophyceae). These species support rich communities and increase the structural complexity and productivity of the system (Giaccone et al., 1994; Bulleri et al., 2002; Falace and Bressan, 2006; Ballesteros et al., 2009; Mineur et al., 2015). Every day they cope with natural selective pressures such as wave exposure (Burrows, 2012), salinity, desiccation (Flores-Molina et al., 2014; Kumar et al., 2014), nutrient limitation, high irradiation, high and low temperature and osmotic stress (Zaneveld, 1969; Davison and Pearson, 1996). Moreover, human pressures are increasingly limiting the range of intertidal *Cystoseira* species (Chryssovergis and Panayotidis, 1995; Rodríguez-Prieto and Polo, 1996; Soltan et al., 2001; Arevalo et al., 2007; Sales et al., 2011) to the point where several species have been reported to be locally lost (Soltan et al., 2001; Thibaut et al., 2005; Serio et al., 2006; Mangialajo et al., 2007, 2008; Perkol-Finkel and Airoldi, 2010;Thibaut et al., 2015). Climate-change will further exacerbate the risks, potentially interacting with other stressors (Strain et al., 2014). In this perspective, assessing the vulnerability of *Cystoseira* species to abrupt climate events is necessary to better predict the future persistence of this important habitat. Although there is a good knowledge of factors that cause the regression of canopy-forming algae, information on environmental or biotic factors that could enhance their resilience is limited (Perkol-Finkel and Airoldi, 2010; Strain et al., 2015). Biodiversity is known to be a major determinant of ecosystem dynamics and functioning (Tilman et al., 2014), and can increase the stability and resistance of communities to climate events (Loreau and de Mazancourt, 2013; Isbell et al., 2015). However, it is not known if local biodiversity can also enhance the resilience of habitat-forming species. Another potentially important factor is the thermal history of a species. The thermal history of a species (i.e. body temperature) sums the evolutionary history and the thermal events recently experienced by individuals, including short-term acclimation to environmental variations (Helmuth, 1998; Sarà et al., 2011; Giomi et al., 2016). Thermal history represents one of the most important drivers of physiological performance of a species. Evidence that the thermal history determines the expression of heat shock proteins in some macroalgae has been already found (Li and Brawley, 2004; Henkel and Hofmann, 2008). However, the relationship between thermal history and tolerance to heat wave events has not been examined in intertidal *Cystoseira* species.

We experimentally investigated the effects of a simulated heat wave event on intertidal populations of *Cystoseira compressa* (Esper) Gerloff & Nizamuddin. We estimated the degree of impact and the resilience capacity of *C. compressa* on both short- and long-term recovery after the heat wave simulation. Moreover, we used linear mixed model to test whether the responses of *C. compressa* to the heat wave were affected by the characteristics of each individual alga (length, biomass, and pigment content), by the status of *C. compressa* local populations (percentage cover and density), by the local species richness (number of the dominant species of the assemblage), or by the thermal history of *C. compressa* (based on a biophysical model).

Materials and methods

Study area

This study was carried out at 10 sites along the Italian coast between the 22nd of June and the 24th of July 2013. In 2014 a longer-term experiment was repeated at 3 sites between the 22nd and the 28th of July (Figure 1, Table S1). The 10 sites were randomly chosen between a series of sites located nearby the ISPRA (Istituto Superiore per la Protezione e Ricerca Ambientale, http://www.mareografico.it) meteomarine buoys, in order to have access to good temporal environmental data. All sites were well or moderated exposed with an average tidal range of ≈ 20-30 cm. The intertidal system was formed either by rocky platforms of different inclinations with crevices and ponds, or fields of boulders of various sizes. Assemblages generally comprised mixed stands of *Cystoseira* spp. and other macroalgae (Pp, LI, PA, Ob, Pn, Ot and Mz), with the exception of 3 localities where assemblages were dominated by mussels (comprising either *Mytilus galloprovincialis* Lamarck or *Mytilaster minimus*) (Ga, Or, and AN). Data derived from the ISPRA buoys and from EMIS (Environmental Marine Information System, http://mcc.jrc.ec.europa.eu/emis/) between 2000-2012, indicate that photosynthetic active radiation (PAR) decreases with increasing latitude ranging from ≈ 38 E/m^2/day at Pp to ≈ 31 E/m^2/day at AN. The same trend was observed for annual average seawater temperature that ranged from 20°C of PA to the 17°C of AN, while an opposite trend was observed for the annual standard deviation of seawater temperature that tends to increase at the northern sites. The environmental data were also used to simulate realistic heat wave conditions at each site. This was done by simulating the maximum recorded air temperature reached in the last 10 years at each site. These values were obtain by the nearest ISPRA buoy to the site (Table S2, Figure S1-S10).

Measurements of relevant environmental and biological parameters

During aerial exposure at low tide, body temperature BT is influenced by multiple environmental factors, including the morphology of the organism (Helmuth, 1998). We used a biophysical heat budget model (Kearney et al., 2010; Helmuth et al., 2011; Sarà et al., 2011, 2013) to get a proxy of the mean BT of *C. compressa* before the start of each heat-wave simulation. Data for the heat budget model (hourly air temperature, tide amplitude, wind speed) were obtained from the ISPRA buoy network for the 2 days preceding the beginning of each experiment; daily irradiance data were downloaded from the European Commission Joint Research Centre (2012; http://re.jrc.ec.europa.eu/pvgis/apps4/pvest.php). Although the model was originally designed for invertebrates, the variables involved in the heat budget of algae are similar to those affecting the BT of intertidal ectotherms providing a starting exploratory proxy in the absence of a validated model for algae and specifically for intertidal *C. compressa*.

Percentage cover and density of intertidal *Cystoseira* spp. at each site were estimated in six, randomly located quadrats. Sampling quadrats (30 x 30cm) were divided into 25 equal squares, and the percentage cover of *Cystoseira* spp. was quantified by giving a score ranking from 0 to 4 in each square, and then adding up scores for all the smaller squares as described by Dethier et al (1993). Density of each species of *Cystoseira* was also estimated as number of individuals in the same six, quadrats.

At the end of each experiment we also measured the biomass of each individual of *C. compressa* used in the tests. Thalli were dried to constant weigh for 48 h at 60 °C and weighed, then ignited in a muffle furnace for 4 h at 500 °C to obtain ash free dry weights (AFDW) (Stein-Taylor et al., 1985). On the same thalli we collected and stored at -20°C algal tissue for the pigments analysis. We used spectrophotometrically analysis to calculate the content of Chlorophyll *a* (Chl-a) and of Carotenoids (Car) of *C. compressa*. Pigments were extracted following the modified protocol of Pusceddu et al (2003). Briefly, we placed algal tissue inside a test tube with 0.1g of magnesium carbonate ($MgCO_3$), to avoid rapid degradation of chlorophyll-a. Then, 3ml of 90% acetone was added and the sample was homogenized for 30 sec using a glass stick. After that other 3 ml of 90% acetone were added and the tube was closed with parafilm. The samples were vortexed for 30sec and left at 4°C overnight. Chl-a and Car contents were determined in accordance with formulas provided by Jeffrey and Humphrey (1975) and Parsons & Strickland's (1963).

At each site, we also estimated the species diversity, as average number of species occurred in 6 (30 x 30cm) randomly located quadrats.

Experimental design

The experiment was designed to measure the responses of the intertidal *Cystoseira compressa* to a simulated heat wave at low tide (Figure 2). In 2013 the experiment was designed to explore a short-term recovery of 3h. At each sites we randomly collected, with hammer and chisel, 10 individuals of *C. compressa* at high tide from the sublitoral fringe (-10 cm to + 10 cm relative to Mean Low Water Level; MLWL). The individuals were left into two plastic boxes filled with natural seawater for one hour of acclimation and then randomly assigned to either control or heat wave treatments (n=5). We simulated the low tide by removing the seawater from the plastic boxes, and then we started the heat wave simulation (Figure 2). Air exposure lasted 3 hours; maximum air temperature was reached after 1 hour and constantly maintained for the remaining 2 hours. The air temperature was increased using infrared lamps of 100 watt (Trixie). Temperature was constantly monitored with two thermometers placed near the algal surface. Control samples were also exposed to the simulated low tide but were left at the natural air temperature. Incident photosynthetic radiation was maintained at 200 μ mol quanta m^{-2} s^{-1} by 4× GreenPower LED lights. Light intensity in the spectral range from 400 to 700 nm was measured with measured by the fibre quantum sensor of the Diving-PAM (Waltz). At the end of the air exposure we simulated the rise of tide, slowly filling the plastic box with natural seawater. Then, we investigated if the physiological activities of *C. compressa* recovered during the subsequent three hours (Figure 2).

In 2014 we repeated the same experiment at three sites, Pp, PA and Or, following a long-term recovery phase of 24h. Moreover, we increased the number of replicates from 5 to 8 individuals of *C. compressa*.

During the experiment, the photosynthetic activity of each alga was quantified by measuring in vivo chlorophyll-*a* fluorescence of photosystem II (PSII) with a portable pulse amplitude modulation fluorometer (Diving-PAM, Waltz). The samples were dark adapted for 30 minutes, after which we measured the maximal quantum yield (F_v/F_m) which is an indicator of physiological stress (Maxwell and Johnson, 2000). For each replicate, three measures of Fv/Fm were made on randomly surface areas of *C. compressa*. The mean of the three values was used for the subsequent statistical analyses.

Data analysis

We calculated impact of heat wave and the resilience capacity of *C. compressa* with the following equations. The impact was defined as:

$$I = \frac{Y_i - Y_{hw}}{Yi} \qquad (1)$$

and resilience as:

$$\Delta = \frac{Y_i - Y_{rc}}{Y_{i-}Y_{hw}} \qquad (2)$$

where Y_i, Y_{hw} and Y_{rc}were respectively the photosynthetic activity before, at the end of air exposure and after a recovery period of 3 hours (in 2013) or 3 and 24 hours (in 2014). Impact (*I*) indicates the proximity of photosynthetic activity to normal levels after the heat event. It can range from 0 (no changes), to 1 (maximum impact hypothetically occurring if no photosynthetic activity is maintained). Impact values lower that 0 indicate greater photosynthetic activity compared to initial conditions. Resilience (Δ) estimates the rate of return towards normal physiological levels after impact ceases. If there is complete recovery of photosynthetic activity then $\Delta = 0$, while $\Delta = 1$ indicate no variation of photosynthetic activity compared to the end of air exposure. As for the impact, negative values of Δ indicate greater photosynthetic activity compared to the end of air exposure. In contrast, values of Δ major than 1 indicates a worst condition of *C. compressa* at the end of recovery phase compared the end of air exposure. Resilience was estimated only for those thalli exposed to the heat wave that had shown a negative response to the heat-wave impact ($I > 0$). In 2013, both impact (*I*) and resilience (Δ) were estimated at the end of air exposure (hw) and at the end of 3h recovery (rc), while in 2014 we also estimated responses at the end of 24h recovery (rc.24). Differences in the photosynthetic responses of *C. compressa* between treatments (Tr: control or simulated heat wave) and among sites (Si: random 10 levels in 2013, 3 levels in 2014) were tested by using univariate permutational analysis of variance (PERMANOVA), with PERMANOVA+ (Anderson et al., 2008) for PRIMER v.6 (Clarke and Gorley, 2006).

The analysis was based on a Euclidean distance matrix of raw data, using type III sum of squares, and 9999 unrestricted permutation of raw data. Separate PERMANOVAs were performed at the end of air exposure (hw) and at the end of 3 h (rc) and 24 hours (rc.24).

We used two distinct linear mixed-effects models (LMM) to test whether impact and resilience depend on species diversity and or on the body temperature of *C. compressa*. Biodiversity LMM (Bio-LMM) included species diversity (quantified as number of species of each site), latitudinal gradient, cover and density of *C. compressa*, AFDW, Chorophyll-a and Carotenoids content and all interactions terms. Otherwise, body temperature LMM (BT-LMM) included body temperature (average of body temperature of the 2 days before the heat wave), latitudinal gradient, cover and density of *C. compressa*, AFDW, Chorophyll-a and Carotenoids content and all interactions terms.

Best model selection was obtained following the protocol suggested by Zuur et al. (2009). We started with a complete model containing all factors and interactions. First of all, we tested the random structure, comparing model with no random term and with random intercept model using sites. Akaike's Information Criteria (AIC) was use to retain the optimal random structure. Then, we search the optimal fixed structure. We dropped the every terms of the model each in turn and applied the maximum likelihood (ML) estimation. The final selected model was presented using restricted maximum likelihood (REML) estimation. P values less than 0.05 were considered significant. Models were fitted with the "lme" function in the "nlme" R-package (Pinheiro et al., 2016).

Results

Impact of the simulated heat wave

In 2013, there was a clear effect of the heat wave on *C. compressa* photosynthetic activity at the end of the air exposure and after 3 hours of recovery (Figure 3 A-B, Table 1). At the end of air exposure thalli of *C. compressa* subjected to the heat wave had an average decrease of PAM values by 66% compared to thalli left at natural air temperature condition. These differences were still significant at the end of recovery with an average decrease by 47% in treated thalli (Figure 3 A-B). At the end of 3h recovery there was a reduction of impact-effect on AN, PA, Mz and Or while Ga and Ob increased (Figure 3 A-B). In 2014, we also observed a marked effect of the simulated heat wave on *C. compressa* at the end of the air exposure and after 3 hours of recovery. However, no impact was found in Pp site after 24h of recovery, while Or and PA showed slightly and higher levels of impact respectively, probably coinciding with the different heat wave temperature applied (Figure 4 A-B, Table 2, Table S2).

At the end of air exposure ($\boldsymbol{I}_{\mathbf{hw}}$), Bio-LMM selected a model including species diversity as fixed effect + random effects of site ($\boldsymbol{I}_{\mathbf{hw}}$, AIC= -17.72, t= -2.98, p= 0.0176; Table 3 A). Species diversity was moderately but significantly related to lower impacts of the heat wave on *C. compressa* (Figure 5A), as individuals of *C. compressa* from more diverse communities exhibited smaller proportional changes in photosynthetic activity at the end of the heat wave. These mitigative effects were observed irrespective of the other factors that were discarded during model selection ($p>0.05$). The same model was selected for impact after 3 hours of recovery ($\boldsymbol{I}_{\mathbf{rc}}$), but the relationships with species diversity was no longer significant ($\boldsymbol{I}_{\mathbf{rc}}$, AIC= -17.72, t= 5.29, p= 0.074; Table 3 B, Figure 5B).

BT-LMM simplification selected a model characterized by fixed effects, body temperature + random effects of site ($\boldsymbol{I}_{\mathbf{hw}}$, AIC= -13.56, t= 2.34, p= 0.047; Table 3 A). An increase in body temperature was significantly related to a greater impact of the heat wave on *C. compressa* at the end of air exposure (Figure 6A), irrespective of other factors whose were discarded during the model selection ($p>0.05$). Also in this case, BT-LMM was not significant for the impact at the end of recovery ($\boldsymbol{I}_{\mathbf{hw}}$, AIC= 10.04, t= 1.03, p= 0.33; Table 3 B, Figure 6B).

For 2014 it was not possible to include the interaction terms in the models due the few numbers of sites investigated. Results obtained from data of 2014 showed no effect of species diversity on the impact of *C. compressa* either at the end of air exposure ($\boldsymbol{I}_{\mathbf{hw}}$, AIC= -21.28, t= 2.29, p= 0.261; Table 4) or after 3h and 24h of recovery ($\boldsymbol{I}_{\mathbf{rc}}$, AIC= 8.37, t= 0.66, p= 0.627; $\boldsymbol{I}_{\mathbf{rc.24}}$, AIC= -5.99, t= 0.28, p= 0.827; Table 4A). Similar results were obtained for BT-LMM either at the end of air exposure ($\boldsymbol{I}_{\mathbf{hw}}$, AIC= -14.53, t= -0.545, p= 0.6821; Table 4B) or after 3h and 24h of recovery ($\boldsymbol{I}_{\mathbf{rc}}$, AIC= 12.56, t= 0.226, p= 0.858; $\boldsymbol{I}_{\mathbf{rc.24}}$, AIC= -3.83, t= 1.889, p= 0.309; Table 4B).

Resilience of* C. compressa *after simulated heat wave

In 2013 no recovery was observed for thalli of *C. compressa* exposed to simulated heat wave at the end of 3 hours. Eight sites, AN, Ga, LI, Mz, Ob, Ot, Pn and Pp, presented no variation or worst condition compared to the end of air exposure, while PA and Or showed a slight and good recovery respectively (Figure 7). The results of 2014, confirmed the no recovery after 3 hours. However, after 24 hours we observed a clear different degree of recovery denoting various resilience capacities between sites (Figure 8 A-B). Bio-LMM selection selected a model characterized by fixed effects, species diversity + random effects of site, while BT-LMM simplification selected a model characterized by fixed effects, body temperature + random effects of site. However, there was not relationship of species diversity and body temperature on the resilience of *C. compressa* after 3h of recovery ($\mathbf{\Delta_{rc}}$, AIC= 77.85, t= -0.003, p= 0.9978, Table 3A; $\mathbf{\Delta_{rc}}$, AIC= 72.28, t= -0.5577, p= 0.592, Table 3B).

The same models were selected in 2014, where, after 3h and 24h of recovery, no relationships were found either for species diversity ($\mathbf{\Delta_{rc}}$, AIC= 21.18, t= 0.019, p= 0.9881; $\mathbf{\Delta_{rc.24}}$, AIC= 15.43, t= 0.227, p= 0.8574; Table 4A), ad body temperature ($\mathbf{\Delta_{rc}}$, AIC= 28.87, t= 0.1648, p= 0.896; $\mathbf{\Delta_{rc.24}}$, AIC= 16.87, t= 2.159, p= 0.2761; Table 4B) with the resilience of *C. compressa*.

Discussion

We investigated for the first time the potential effects of heat-wave events on the intertidal *C. compressa* along the Italian coast. Heat waves had significant impacts on the photosynthetic performances *C. compressa*. This impact was significant at all localities independently of the local heat wave intensity, and persisted for > 3 hours after cessation of the heat wave, while partial recover was observed at some localities after 24 hours. Linear mixed model showed that greater species diversity of the assemblage was related to smaller impacts of the heat wave. This tendency was still evident, but not significant, at the end of 3 hours recovery, while no relationships were found after 24 hours. The recently thermal history was also related to the response of *C. compressa*, as thalli with higher body temperature were the most impacted by the heat wave. Also in this case the relationship was still evident but not significant at the end of 3 hours recovery, while no relationships were found after 24 hours.

On a global scale, species are responding to thermal stress with phenological changes, latitudinal contraction usually towards high latitude, changing vertical distribution and in some cases with local extinctions (Pearson et al., 2009; Wernberg et al., 2012; Smale and Wernberg, 2013; Pereira et al., 2015; Thomson et al., 2015). This is even more evident for species living on coastal rocky shore that are more exposed to selective pressures from environmental and anthropogenic factors (Airoldi and Beck, 2007). Moreover, species living in intertidal zone are often considered an early warning signal of climate change (Somero 2010). Our results showed that the heat-wave events caused a drastic impact on intertidal *C. compressa* regardless its geographical position. The fact that after 3 hours of recovery thalli of *C. compressa* showed even higher levels of impact and no resilience capability, leads to hypothesize that repeated heat wave events would increase the vulnerability of this species.

A number of recent studies highlight the relationship between diversity and the stability of ecosystem properties (Loreau and de Mazancourt, 2013; Tilman et al., 2014; Isbell et al., 2015). It has been observed that biodiversity increased the resistance of ecosystem productivity also to extreme climate events (Isbell et al., 2015). Our results show that species diversity was significantly related to lower impacts of the heat wave on *C. compressa.* That is, individuals of *C. compressa* in more diverse communities exhibited smaller proportional changes at the end of a heat wave event, suggesting that biodiversity could mitigate he individual responses. This aspect is of considerable interest as it suggests that more diverse assemblages could favour the long-term sustainability of *C. compressa* population in the face of environmental and anthropogenic changes as well as variation caused by climate change. This aspect needs to be more investigated using field experiments specifically manipulating the species numbers.

In this study we also found that the body temperature of *C. compressa* can play a role on the impact caused by the heat wave. Body temperature, shaped by environmental conditions experienced by species in natural habitat, is an important characteristic to keep in mind to formulate reliable predictions about the adaptive potential of a species (Kearney et al., 2010; Giomi et al., 2016). Thalli of *C. compressa* characterized by higher thermal body temperature, likely near to their thermal limits, were more affected by the heat wave. This should suggest that species living closer to their thermal limits are more vulnerable to extreme heat wave events. The rapid rise in water and air temperature caused by global warming is happening too quickly for species to adapt. This will bring more *C. compressa* populations to live near their thermal limits with consequent increase of their vulnerability to stochastic extreme events as heat wave.

C. compressa is one of the few still relatively widespread species of *Cystoseira* along the Italian coast. Even this relatively tolerant species is retracting, particularly in urban areas (chapter thesis n°2). As it is an important intertidal key species, the disappearance of this seaweed will likely trigger major ecological changes in the entire associated ecosystem. The results of this study showed that extreme heat wave event could negatively affect *C. compressa.* Future studies should explore the effects of repeated heat wave events under various scenarios of selected environmental and human factors already identified as major driver in the status of *Cystoseira*. Moreover, molecular responses in terms of induction of heat shock proteins and genotypes characterization should be also taken into account in order to have a clearer picture of organism's response.

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Tables and figures

Table 1 PERMANOVA results assessing the effects of the simulated heat wave at the end of air exposure (I_{hw}), and after a recovery period (I_{rc}) in 2013. **Si**= Site; **Tr**= Treatment. Significant p values (p<0.05) are in bold.

Factors

Name	Abbrev.	Type	Levels
Site	Si	Random	10
Treatment	Tr	Fixed	2

PERMANOVA table of results

			I_{hw}						I_{rc}			
Source	df	BIO	MS	Pseudo-F	P(perm)	Unique perms	df	BIO	MS	Pseudo-F	P(perm)	Unique perms
Si	9	1.4784	0.16427	8.7442	**0.0001**	9944	9	1.1052	0.1228	3.8297	**0.0004**	9936
Tr	1	5.1707	5.1707	65.762	**0.0002**	9841	1	4.1923	4.1923	18.577	**0.0031**	9829
SixTr	9	0.70826	7.87E-02	4.189	**0.0002**	9941	9	2.033	0.22589	7.0449	**0.0001**	9935
Res	79	1.4841	1.88E-02				79	2.5331	3.21E-02			
Total	98	8.8432					98	9.8418				

Table 2 PERMANOVA results assessing the effects of the simulated heat wave at the end of air exposure (I_{hw}), after 3h (I_{rc}) and 24h ($I_{rc.24}$) of recovery in 2014. **Si**= Site; **Tr**= Treatment. Significant p values (p<0.05) are in bold.

Factors

Name	Abbrev.	Type	Levels
Site	Si	Random	3
Treatment	Tr	Fixed	2

PERMANOVA table of results

	I_{hw}						I_{rc}						$I_{rc.24}$					
						Unique						Unique						Unique
Source	df	BIO	MS	Pseudo-F	P(perm)	perms	df	BIO	MS	Pseudo-F	P(perm)	perms	df	BIO	MS	Pseudo-F	P(perm)	perms
Si	2	5.01E-02	2.51E-02	1.9588	0.1532	9951	2	5.70E-02	2.85E-02	0.85442	0.4368	9953	2	1.0125	0.50626	34.22	**0.0001**	9962
Tr	1	0.40003	0.40003	5.8443	0.1329	60	1	0.50409	0.50409	4.7741	0.229	60	1	0.48182	0.48182	2.341	0.1848	60
SixTr	2	0.1369	6.84E-02	5.3492	**0.0079**	9954	2	0.21118	0.10559	3.1645	**0.0495**	9949	2	0.41164	0.20582	13.912	**0.0001**	9942
Res	42	0.53743	1.28E-02				42	1.4014	3.34E-02				42	0.62135	1.48E-02			
Total	47	1.1245					47	2.1737					47	2.5273				

Table 3 Linear mixed models fitted by "lme" function for the effect of biodiversity (A, BIO-LMM) and body temperature (B, BT-LMM) on both impact of heat wave and resilience of *C. compressa* at the end air exposure (hw) and after 3h of recovery (rc) on data of 2013. Si= sites; Bio= biodiversity; BT= body temperature.

(A) BIO-LMM		
Impact		**Resilience**
I_{hw}	I_{rc}	Δ_{rc}
Linear mixed-effects model fit by REML	Linear mixed-effects model fit by REML	Linear mixed-effects model fit by REML
AIC BIC logLik	AIC BIC logLik	AIC BIC logLik
-17.72204 -10.23724 12.86102	5.294384 12.77919 1.352808	77.85678 85.34159 -34.92839
Random effects:	Random effects:	Random effects:
Formula: ~1 | Si	Formula: ~1 | Si	Formula: ~1 | Si
(Intercept) Residual	(Intercept) Residual	(Intercept) Residual

StdDev: 0.09552498 0.1561096	StdDev: 0.1385675 0.1950555	StdDev: 0.3184665 0.4109688
Fixed effects: list(Form5)	Fixed effects: list(Form5)	Fixed effects: list(Form5)
Value Std.Error DF t-value p-value	Value Std.Error DF t-value p-value	Value Std.Error DF t-value p-value
(Intercept) 1.3631753 0.25009253 40 5.450684 0.0000	(Intercept) 1.2487701 0.3461003 40 3.608116 0.0008	(Intercept) 0.9250750 0.7772121 40 1.1902478 0.2410
Bio -0.1068016 0.03583738 8 -2.980174 0.0176	Bio -0.1018647 0.0495950 8 -2.053932 0.0741	Bio -0.0003238 0.1113718 8 -0.0029077 0.9978
Correlation:	Correlation:	Correlation:
(Intr)	(Intr)	(Intr)
Bio -0.989	Bio -0.989	Bio -0.989
Standardized Within-Group Residuals:	Standardized Within-Group Residuals:	Standardized Within-Group Residuals:
Min Q1 Med Q3 Max	Min Q1 Med Q3 Max	Min Q1 Med Q3 Max
-2.77425433 -0.67881791 0.08753324 0.75994193 1.58874651	-2.3387572877 -0.5522183751 -0.0002321326 0.9772099265 1.4916136730	-2.9781387 -0.5160708 -0.1214465 0.4451067 2.5602610
Number of Observations: 50	Number of Observations: 50	Number of Observations: 50
Number of Groups: 10	Number of Groups: 10	Number of Groups: 10

(B) BT-LMM

I_{hw}	I_{rc}	Δ_{rc}
Linear mixed-effects model fit by REML	Linear mixed-effects model fit by REML	Linear mixed-effects model fit by maximum likelihood
AIC BIC logLik	AIC BIC logLik	AIC BIC logLik
-13.56031 -6.075509 10.78016	10.04189 17.52669 -1.020944	72.2888 79.9369 -32.1444
Random effects:	Random effects:	Random effects:
Formula: ~1 | Si	Formula: ~1 | Si	Formula: ~1 | Si
(Intercept) Residual	(Intercept) Residual	(Intercept) Residual
StdDev: 0.1125668 0.1561095	StdDev: 0.1689513 0.1950557	StdDev: 0.2664315 0.4109687
Fixed effects: list(Form5)	Fixed effects: list(Form5)	Fixed effects: list(Form5)
Value Std.Error DF t-value p-value	Value Std.Error DF t-value p-value	Value Std.Error DF t-value p-value

(Intercept) -0.17119150 0.3435395 40 -0.4983168 0.6210	(Intercept) 0.04178083 0.4931437 40 0.0847234 0.9329	(Intercept) 1.3971763 0.8567776 40 1.6307340 0.1108
BT 0.02889773 0.0123564 8 2.3386830 0.0475	BT 0.01826858 0.0177374 8 1.0299488 0.3332	BT -0.0171891 0.0308165 8 -0.5577894 0.5922
Correlation:	Correlation:	Correlation:
(Intr)	(Intr)	(Intr)
BT -0.993	BT -0.993	BT -0.993
Standardized Within-Group Residuals:	Standardized Within-Group Residuals:	Standardized Within-Group Residuals:
Min Q1 Med Q3 Max	Min Q1 Med Q3 Max	Min Q1 Med Q3 Max
-2.62490858 -0.63728163 0.04794261 0.76900406 1.48513331	-2.38984715 -0.54297052 0.01676688 0.86079010 1.45700191	-2.99677106 -0.59006249 -0.08762274 0.38479280 2.63497707
Number of Observations: 50	Number of Observations: 50	Number of Observations: 50
Number of Groups: 10	Number of Groups: 10	Number of Groups: 10

Table 4 Linear mixed models fitted by "lme" function for the effect of biodiversity (A, BIO-LMM) and body temperature (B, BT-LMM) on both impact of heat wave and resilience of *C. compressa* at the end air exposure (hw) and after 3h (rc) and 24h (rc.24) of recovery on data of 2014. Si= sites; Bio= biodiversity; BT= body temperature.

(A) BIO-LMM		
	Impact	
I_{hw}	I_{rc}	$I_{hw.24}$
Linear mixed-effects model fit by REML	Linear mixed-effects model fit by REML	Linear mixed-effects model fit by REML
AIC BIC logLik	AIC BIC logLik	AIC BIC logLik
-21.28192 -16.91775 14.64096	8.375526 12.7397 -0.1877628	-5.998776 -1.634606 6.999388
Random effects:	Random effects:	Random effects:
Formula: ~1 | Si	Formula: ~1 | Si	Formula: ~1 | Si
(Intercept) Residual	(Intercept) Residual	(Intercept) Residual
StdDev: 0.008608484 0.1085228	StdDev: 1.761971e-06 0.2131742	StdDev: 0.3926392 0.1398953

Fixed effects: list(Form5)	Fixed effects: list(Form5)	Fixed effects: list(Form5)
Value Std.Error DF t-value p-value	Value Std.Error DF t-value p-value	Value Std.Error DF t-value p-value
(Intercept) 0.3095320 0.19595585 21 1.579601 0.1291	(Intercept) 0.14197699 0.3755840 21 0.3780166 0.7092	(Intercept) -0.25456588 1.972104 21 -0.1290834 0.8985
Bio 0.0638086 0.02780518 1 2.294846 0.2616	Bio 0.03531784 0.0532935 1 0.6627040 0.6274	Bio 0.07797914 0.279832 1 0.2786641 0.8270
Correlation:	Correlation:	Correlation:
(Intr)	(Intr)	(Intr)
Bio -0.993	Bio -0.993	Bio -0.993
Standardized Within-Group Residuals:	Standardized Within-Group Residuals:	Standardized Within-Group Residuals:
Min Q1 Med Q3 Max	Min Q1 Med Q3 Max	Min Q1 Med Q3 Max
-2.07031453 -0.56702337 0.02440601 0.72649754 1.37632040	-1.2347068 -0.7460451 -0.2063677 0.4401290 2.2933310	-1.68386000 -0.46195034 -0.09688759 0.50385163 2.25441785
Number of Observations: 24	Number of Observations: 24	Number of Observations: 24
Number of Groups: 3	Number of Groups: 3	Number of Groups: 3
	Resilience	
Δ_{rc}	**$\Delta_{rc.24}$**	
Linear mixed-effects model fit by REML	Linear mixed-effects model fit by REML	
AIC BIC logLik	AIC BIC logLik	
27.17769 31.54186 -9.588843	15.42981 19.79398 -3.714907	
Random effects:	Random effects:	
Formula: ~1 | Si	Formula: ~1 | Si	
(Intercept) Residual	(Intercept) Residual	
StdDev: 6.369601e-06 0.3268271	StdDev: 0.5826664 0.228657	
Fixed effects: list(Form5)	Fixed effects: list(Form5)	
Value Std.Error DF t-value p-value	Value Std.Error DF t-value p-value	
(Intercept) 0.5257223 0.5758251 21 0.9129895 0.3716	(Intercept) -0.26449155 2.9314193 21 -0.09022645 0.9290	

Bio 0.0015326 0.0817068 1 0.0187572 0.9881	Bio 0.09473335 0.4159541 1 0.22774951 0.8574
Correlation:	Correlation:
(Intr)	(Intr)
Bio -0.993	Bio -0.993
Standardized Within-Group Residuals:	Standardized Within-Group Residuals:
Min Q1 Med Q3 Max	Min Q1 Med Q3 Max
-1.1346191 -0.7381231 -0.1214127 0.4039217 2.7212449	-1.53693242 -0.61505101 0.01987046 0.43663107 2.76277506
Number of Observations: 24	Number of Observations: 24
Number of Groups: 3	Number of Groups: 3

(B) BT-LMM

Impact		
I_{hw}	**I_{rc}**	**$I_{\mathrm{rc.24}}$**
Linear mixed-effects model fit by REML	Linear mixed-effects model fit by REML	Linear mixed-effects model fit by REML
AIC BIC logLik	AIC BIC logLik	AIC BIC logLik
-14.53147 -10.54854 11.26574	12.56604 16.54897 -2.28302	-3.833767 0.149162 5.916884
Random effects:	Random effects:	Random effects:
Formula: ~1 | Loc	Formula: ~1 | Loc	Formula: ~1 | Loc
(Intercept) Residual	(Intercept) Residual	(Intercept) Residual
StdDev: 0.0479104 0.1083803	StdDev: 0.04552707 0.2165796	StdDev: 0.1832781 0.1354966
Fixed effects: list(Form3)	Fixed effects: list(Form3)	Fixed effects: list(Form3)
Value Std.Error DF t-value p-value	Value Std.Error DF t-value p-value	Value Std.Error DF t-value p-value
(Intercept) 0.8998251 0.28929417 19 3.1104157 0.0058	(Intercept) 0.5017540 0.4268553 19 1.1754660 0.2543	(Intercept) -1.344604 0.8783648 19 -1.530803 0.1423
M2.BT -0.0055277 0.01013541 1 -0.5453861 0.6821	M2.BT -0.0033702 0.0149044 1 -0.2261227 0.8584	M2.BT 0.058369 0.0308861 1 1.889811 0.3098
Correlation:	Correlation:	Correlation:
(Intr)	(Intr)	(Intr)

M2.BT -0.992	M2.BT -0.992	M2.BT -0.992
Standardized Within-Group Residuals:	Standardized Within-Group Residuals:	Standardized Within-Group Residuals:
Min Q1 Med Q3 Max	Min Q1 Med Q3 Max	Min Q1 Med Q3 Max
-2.3779965 -0.5977054 0.1081220 0.8945750 1.2010187	-1.2473428 -0.6714754 -0.2257022 0.4648927 2.2406199	-1.6421151 -0.5504088 -0.2407772 0.4964066 2.3015707
Number of Observations: 22	Number of Observations: 22	Number of Observations: 22
Number of Groups: 3	Number of Groups: 3	Number of Groups: 3
	Resilience	
Δ_{rc}	**$\Delta_{rc.24}$**	
Linear mixed-effects model fit by REML	Linear mixed-effects model fit by REML	
AIC BIC logLik	AIC BIC logLik	
28.87016 32.85309 -10.43508	16.87357 20.85649 -4.436783	
Random effects:	Random effects:	
Formula: ~1 | Loc	Formula: ~1 | Loc	
(Intercept) Residual	(Intercept) Residual	
StdDev: 7.166138e-06 0.3277795	StdDev: 0.2356877 0.2302723	
Fixed effects: list(Form3)	Fixed effects: list(Form3)	
Value Std.Error DF t-value p-value	Value Std.Error DF t-value p-value	
(Intercept) 0.4718380 0.5613238 19 0.8405807 0.411	(Intercept) -2.0677349 1.158873 19 -1.784264 0.0904	
M2.BT 0.0032236 0.0195558 1 0.1648427 0.896	M2.BT 0.0879339 0.040729 1 2.159000 0.2761	
Correlation:	Correlation:	
(Intr)	(Intr)	
M2.BT -0.992	M2.BT -0.992	

Standardized Within-Group Residuals:	Standardized Within-Group Residuals:
Min Q1 Med Q3 Max	Min Q1 Med Q3 Max
-1.26027605 -0.74193229 0.01032288 0.38988708 2.58438428	-1.55110488 -0.61156132 -0.04715738 0.41609848 2.71844195
Number of Observations: 22	Number of Observations: 22
Number of Groups: 3	Number of Groups: 3

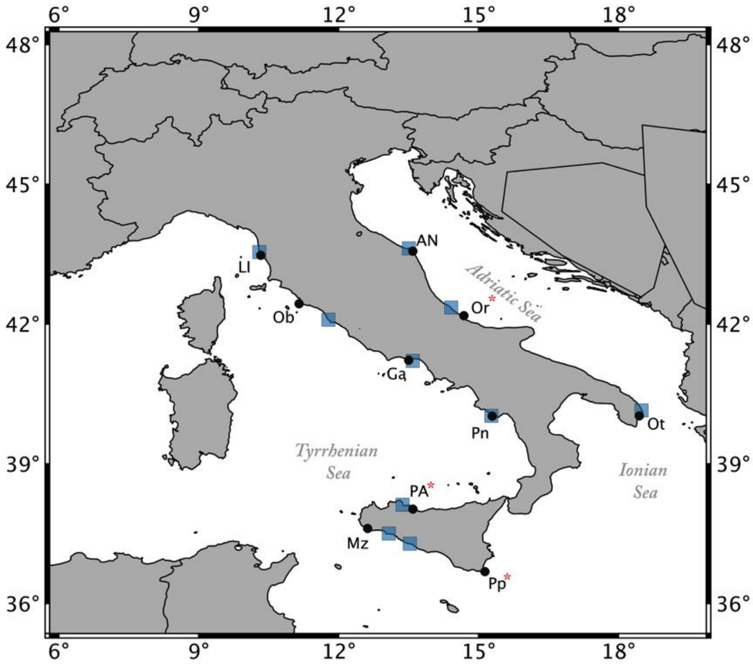

Figure 1 The 10 rocky-intertidal study sites used for the experiments in 2013 along the Italian coastline (black dots). Red asterisks show the three study sites where the experiments were repeated in 2014. **AN**: Ancona, **LI**: Livorno, **Ob**: Orbetello, **Or**: Ortona, **Ga**: Gaeta, **Pn**: Palinuro, **Ot**: Otranto, **PA**: Palermo, **Mz**: Mazara del vallo, **Pp**: Portopalo di capo passero. The position of the ISPRA buoys is indicated by the blue squares. Geographic coordinates of the sites and ISPRA buoys are reported in Table S1. Map projection WGS84.

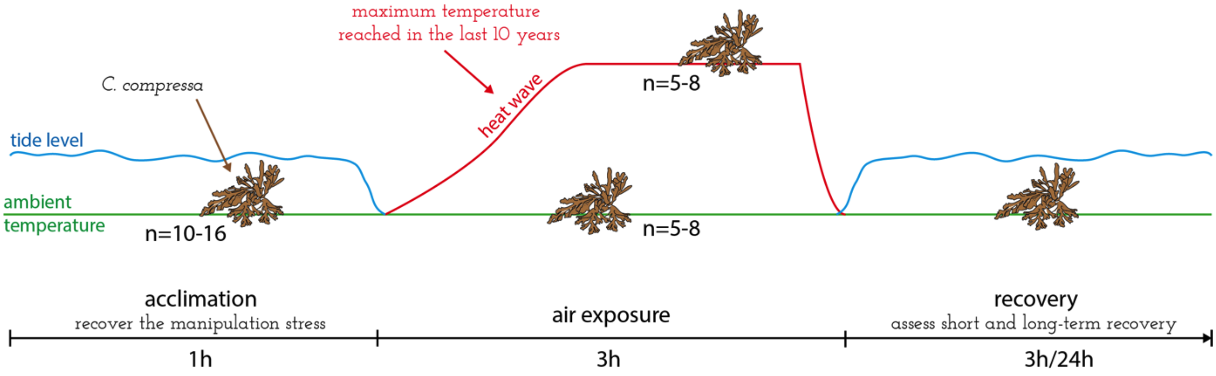

Figure 2 Schematic diagram of the heat wave experiment. The experiment was designed to investigate the effect of a heat-wave on *C. compressa* during the low tide. The individuals of *C. compressa* were collected and left into natural seawater for one hour of acclimation. We simulated the low tide and then we started the heat wave simulation. Air exposure lasted 3

hours; maximum air temperature was reached after 1 hour and constantly maintained for the remaining 2 hours. Control samples were also exposed to the simulated low tide but were left at the natural air temperature. At the end of the air exposure we simulated the rise of tide, slowly filling the plastic box with natural seawater. Then, we investigated the recovery of the physiological activities of *C. compressa*. In 2013 the experiment was carried out in 10 sites (AN, LI, Ob, Or, Ga, Pn, Ot, PA, Mz, Pp) and designed to explore a short-term recovery of 3h. Ten individuals of *C. compressa* were randomly assigned to either control (ambient temperature) or heat wave treatments (n=5). In 2014 the same experiment was repeated at three sites, Pp, PA and Or, following a long-term recovery phase of 24h. Moreover, the number of replicates was increased from 5 to 8 individuals of *C. compressa*.

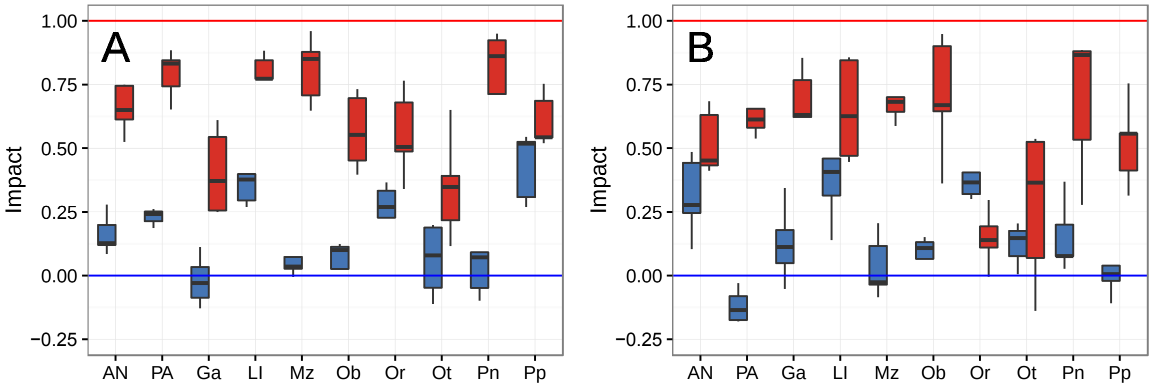

Figure 3 Short-term responses of *C. compressa* to a simulated heat wave at 10 intertidal study sites in 2013. Plotted are impacts (estimated variations in PAM measurements of photosynthetic activity relative to start conditions) for thalli subjected to the heat wave (red box) compared to controls (blue box) at the end of air exposure (A) and after 3h of recovery (B). Blue and red lines indicate minimum (no variations in photosynthetic activity relative to start) and maximum (no photosynthetic activity) responses respectively. Values lower than 0 indicate greater photosynthetic activity compared to initial values.

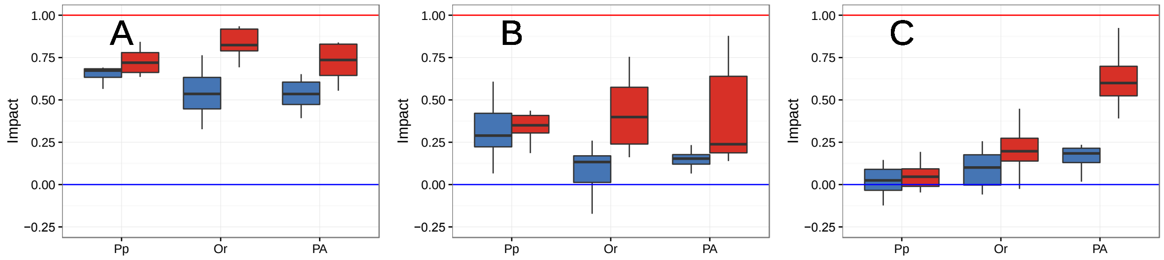

Figure 4 Short and long-term responses of *C. compressa* to a simulated heat wave at 3 intertidal study sites in 2014. Plotted are impacts (estimated variations in PAM measurements of photosynthetic activity relative to start conditions) for thalli subjected to the heat wave (red box) compared to controls (blue box) at the end of air exposure (A) and after 3h of recovery (B). Blue and red lines indicate minimum (no variations in photosynthetic activity relative to start) and maximum (no photosynthetic activity) responses respectively. Values lower than 0 indicate greater photosynthetic activity compared to initial values.

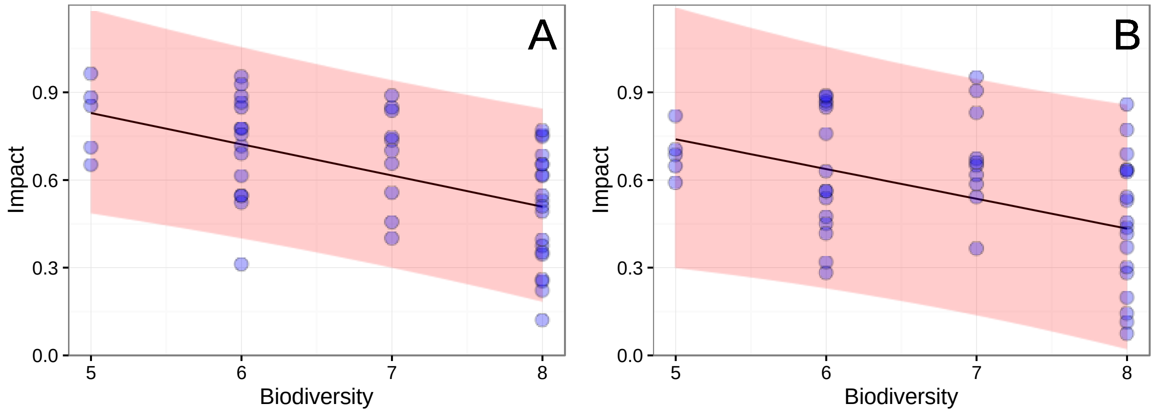

Figure 5 Relationships between measured impact of heat wave on *C. compressa* photosynthetic activity and local species diversity (species number) at the end of air exposure (A) and after 3h of recovery (B). Lines are BIO-LMM model fits across all sites (thick lines with predicted intervals).

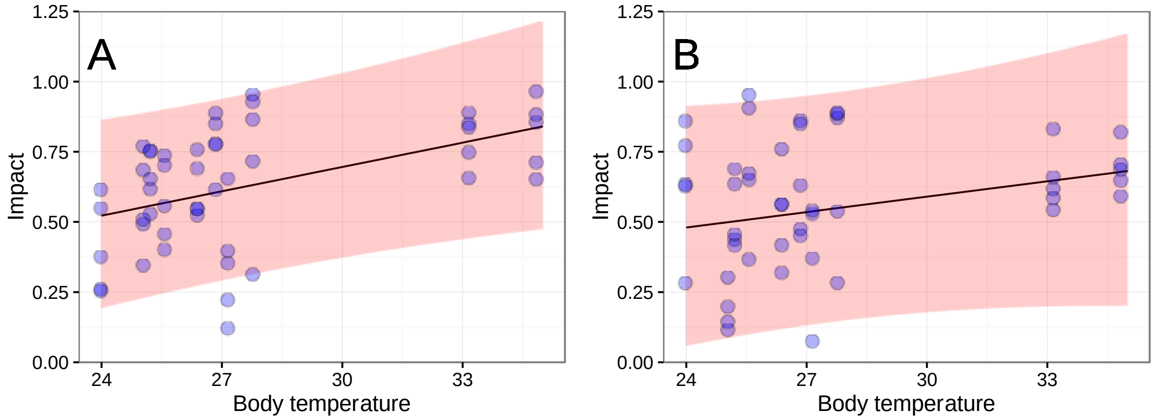

Figure 6 Body temperature effects on the heat wave impact on *C. compressa* at the end of air exposure (A) and after 3h of recovery (B). Lines are BT-LMM model fits across all sites (thick lines with predicted intervals).

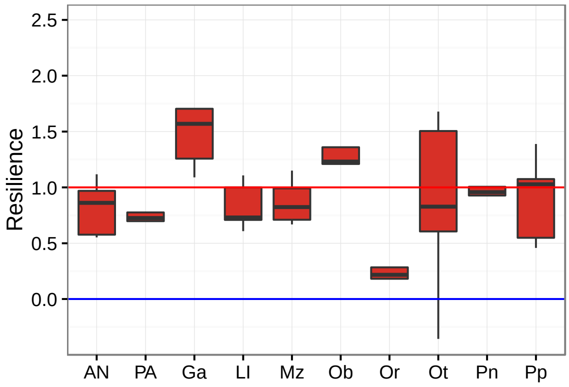

Figure 7 Resilience of *C. compressa* calculated after 3h of recovery (red boxes) from 2013 data. Blue and red lines indicate complete and no recovery respectively. Values lower than 0 indicate an improvement compared before the experiment, while values major than 1 indicates a worst condition of *C. compressa* at the end of recovery phase compared the end of air exposure.

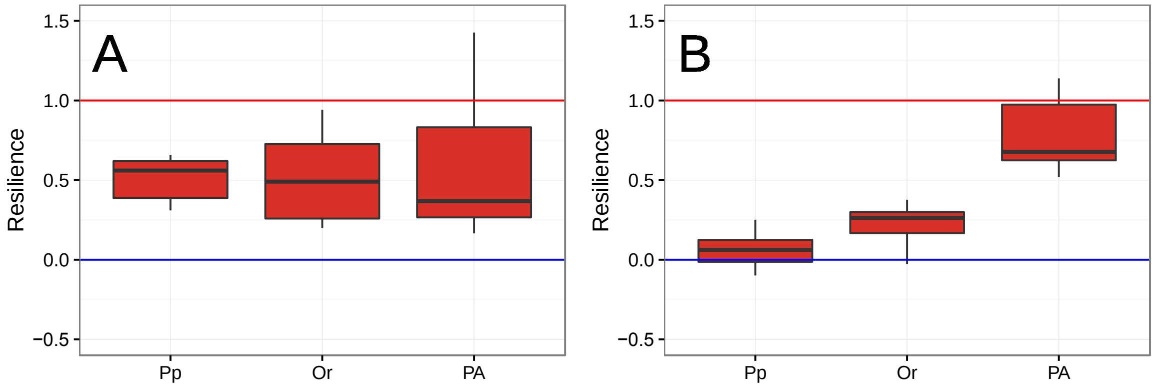

Figure 8 Resilience of *C. compressa* calculated after 3h (A) and 24h (B) of recovery (red boxes) from 2014 data. Blue and red lines indicate complete and no recovery respectively. Values lower than 0 indicate an improvement compared before the experiment, while values major than 1 indicates a worst condition of *C. compressa* at the end of recovery phase compared the end of air exposure.

Supplementary materials

Table S1 Geographical coordinates and id codes of the 10 sampling sites.

Site	ID	Lat	Long
Portonovo (Ancona)	**AN**	43° 33' 57.780" N	13° 35' 31.250" E
Torre San Vito (Gaeta)	**Ga**	41° 13' 23.570" N	13° 30' 11.966" E
Calafuria (Livorno)	**LI**	43° 28' 39.119" N	10° 19' 48.626" E
Mazara del Vallo (Trapani)	**Mz**	37° 36' 44.136" N	12° 37' 20.140" E
Santo Stefano (Orbetello)	**Ob**	42° 26' 10.478" N	11° 9' 8.842" E
Punta Aderci (Ortona)	**Or**	42° 10' 49.926" N	14° 41' 14.964" E
Santa Cesarea Terme (Otranto)	**Ot**	40° 1' 45.592" N	18° 26' 56.036" E
Altavilla (Palermo)	**PA**	38° 1´ 37.412" N	13° 35´ 36.366" E
Faracchio (Palinuro)	**Pn**	40° 1' 25.813" N	15° 17' 39.516" E
Portopalo di Capo PaBioero (Siracusa)	**Pp**	36° 41' 10.244" N	15° 8' 17.653" E

Table S2 Maximum air temperature recorded in the last ten year in the sites investigated in this study.

Site	Lat	Long	Heat wave	Date hw
AN	43° 33' 57.780" N	13° 35' 31.250" E	37	18/08/03
Ga	41° 13' 23.570" N	13° 30' 11.966" E	38	24/08/07
LI	43° 28' 39.119" N	10° 19' 48.626" E	35	04/08/03
Mz	37° 36' 44.136" N	12° 37' 20.140" E	40	08/08/12
Ob	42° 26' 10.478" N	11° 9' 8.842" E	35	26/08/07
Or	42° 10' 49.926" N	14° 41' 14.964" E	42	29/06/05
Ot	40° 1' 45.592" N	18° 26' 56.036" E	41	24/07/07
PA	38° 1´ 37.412" N	13° 35´ 36.366" E	45	25/06/07
Pn	40° 1' 25.813" N	15° 17' 39.516" E	38	24/07/07
Pp	36° 41' 10.244" N	15° 8' 17.653" E	40	08/08/12

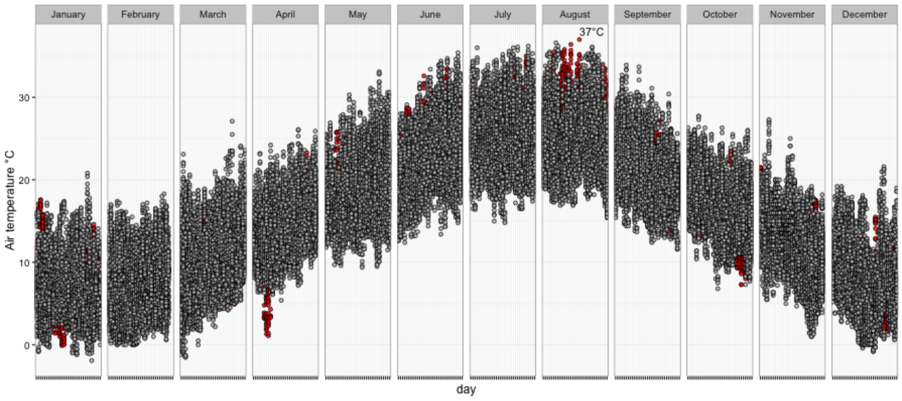

Figure S1 Oscillation of the air temperature oscillation in the last ten years in Portonovo site (AN). Red dot highlights the year when maximum temperature recorded**.**

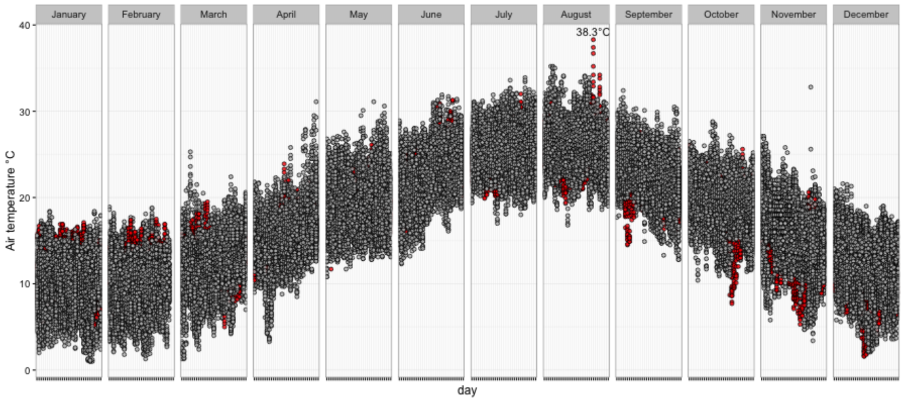

Figure S2 Oscillation of the air temperature oscillation in the last ten years in Torre San Vito site (Ga). Red dot highlights the year when maximum temperature recorded**.**

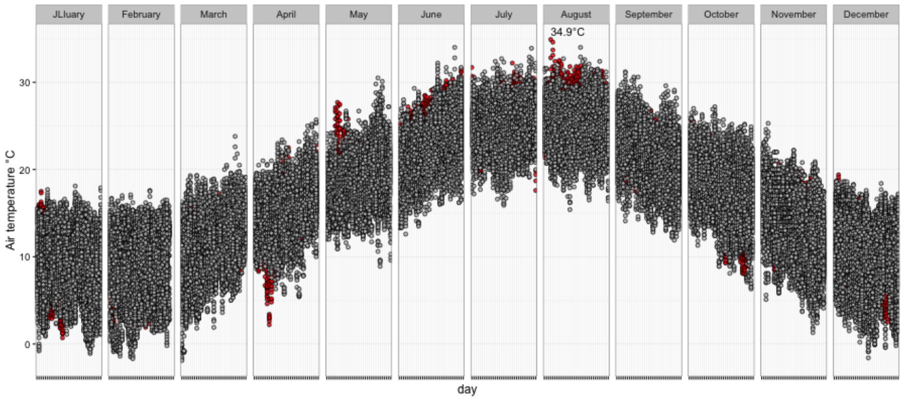

Figure S3 Oscillation of the air temperature oscillation in the last ten years in Calafuria site (LI). Red dot highlights the year when maximum temperature recorded**.**

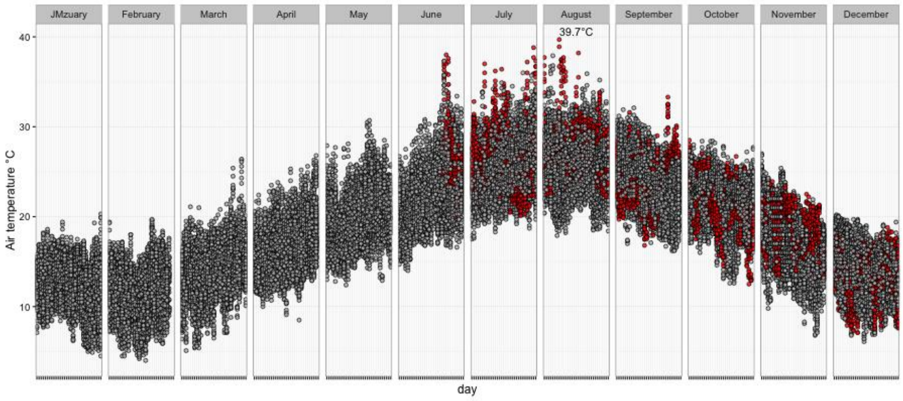

Figure S4 Oscillation of the air temperature oscillation in the last ten years in Mazara del Vallo site (Mz). Red dot highlights the year when maximum temperature recorded**.**

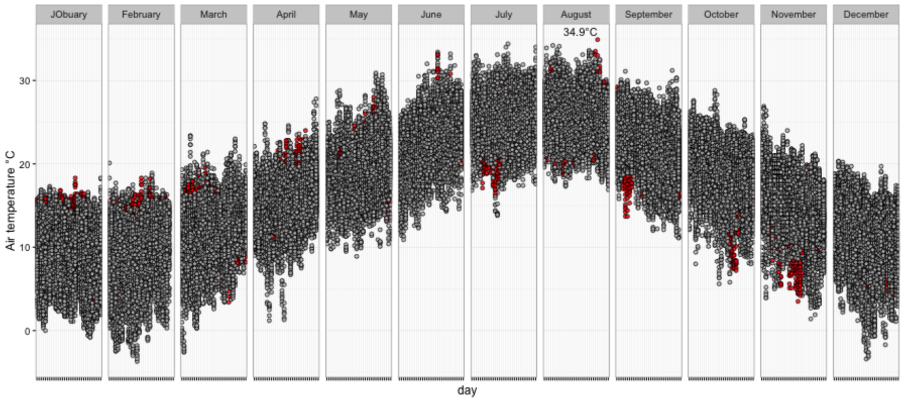

Figure S5 Oscillation of the air temperature oscillation in the last ten years in Santo Stefano site (Ob). Red dot highlights the year when maximum temperature recorded**.**

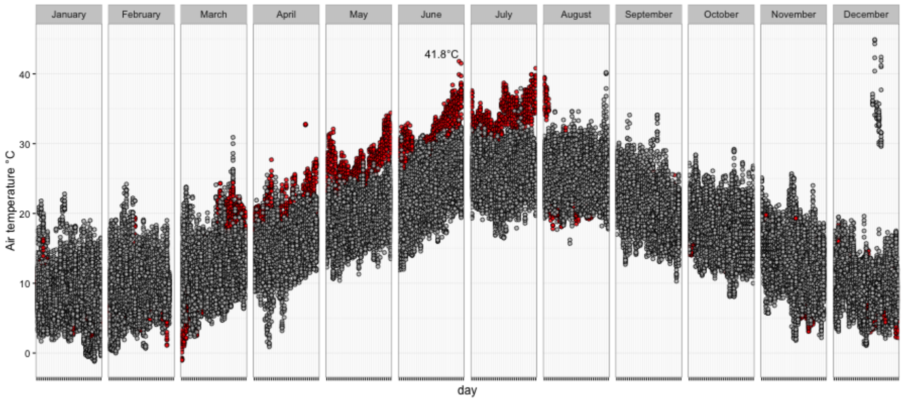

Figure S6 Oscillation of the air temperature oscillation in the last ten years in Punta Aderci site (Or). Red dot highlights the year when maximum temperature recorded**.**

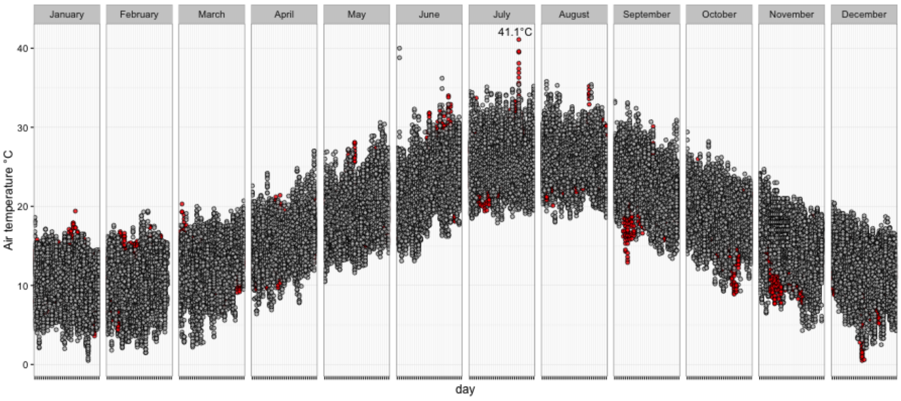

Figure S7 Oscillation of the air temperature oscillation in the last ten years in Santa Cesarea Terme site (Ot). Red dot highlights the year when maximum temperature recorded**.**

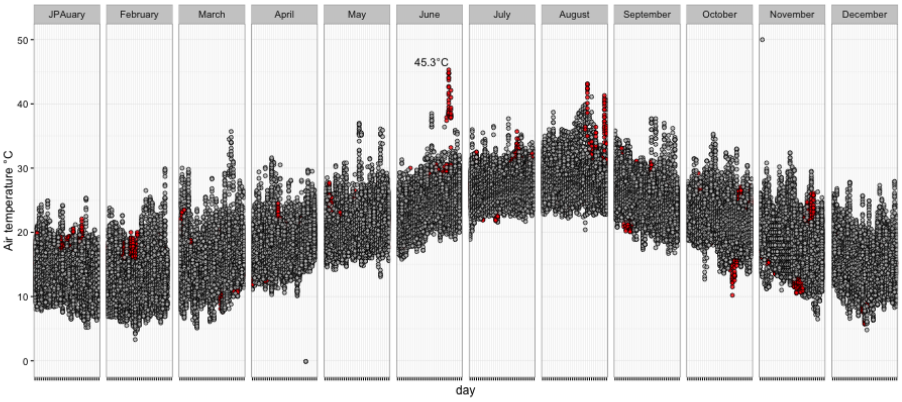

Figure S8 Oscillation of the air temperature oscillation in the last ten years in Altavilla site (PA). Red dot highlights the year when maximum temperature recorded**.**

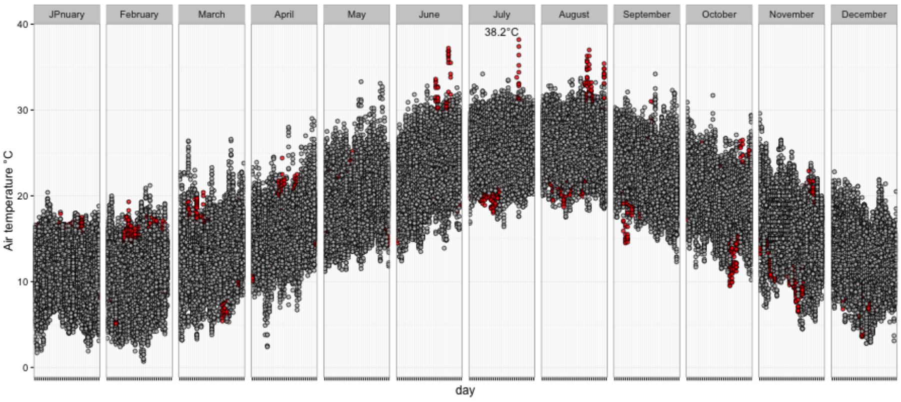

Figure S9 Oscillation of the air temperature oscillation in the last ten years in Faracchio site (Pn). Red dot highlights the year when maximum temperature recorded**.**

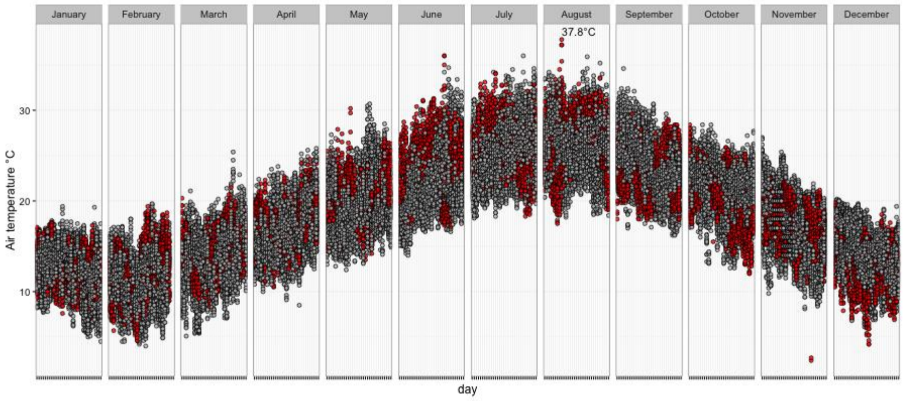

Figure S10 Oscillation of the air temperature oscillation in the last ten years in Portopalo di Capo passero site (Pp). Red dot highlights the year when maximum temperature recorded**.**

Chapter 4

Diversity and temporal dynamics of the epiphytic bacterial communities associated with the canopy-forming seaweed *Cystoseira compressa* (Esper) Gerloff & Nizamuddin[2]

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Abstract

Canopy-forming seaweed species of the genus *Cystoseira* form diverse and productive habitats along temperate rocky coasts of the Mediterranean Sea. Despite numerous studies on the rich macrofauna and flora associated with *Cystoseira* spp., there is little knowledge about the epiphytic bacteria. We analyzed bacterial populations associated with canopies of *Cystoseira compressa*, over an annual vegetative cycle (May-October), and their relationships with the bacterial populations in the surrounding seawater, at intertidal rocky shores in Vasto (Chieti - Italy). The bacterial diversity was assessed using Illumina Miseq sequences of V1-V3 hypervariable regions of 16S rRNA gene. *C. compressa* bacterial community was dominated by sequences of *Proteobacteria* and *Bacteroidetes*, *Verrucomicrobia*, *Actinobacteria* and *Cyanobacteria* especially of the *Rhodobacteriaceae*, *Flavobacteriaceae*, *Sapropiraceae*, *Verrucomicrobiaceae* and *Phyllobacteriaceae* families. Seawater libraries were also dominated by *Proteobacteria* and *Bacteroidetes* sequences, especially of the *Candidatus Pelagibacter* (SAR11) and *Rhodobacteriaceae* families, but were shown to be clearly distinct from *C. compressa* libraries with only few species in common between the two habitats. We observed a clear successional pattern in the epiphytic bacteria of *C. compressa* over time. These variations were characterized by gradual addition of OTUs (*Verrucomicrobia*, *Actinobacteria* and SR1) to the community over a growing

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[2] Published: Mancuso, F. P., Sofie, D., Willems, A., Airoldi, L., and De, O. (2016). Diversity and temporal dynamics of the epiphytic bacterial communities associated with the canopy- forming seaweed *Cystoseira compressa* (Esper) Gerloff & Nizamuddin. *Front. Microbiol.* doi:10.3389/fmicb.2016.00476.

season, indicative of a temporal gradient, rather than a radical reorganization of the bacterial community. Moreover, we also found an increase in abundance over time of *Rhodobacteraceae,* comprising six potential pathogenic genera, *Ruegeria*, *Nautella, Aquimarina*, *Loktanella*, *Saprospira* and *Phaeobacter* which seemed to be associated to aged thalli of *C. compressa*. These bacteria could have the potential to affect the health and ecology of the algae, suggesting the hypothesis of a possible, but still unexplored, role of the microbial communities in contributing to the extensive ongoing declines of populations of *Cystoseira* spp. in the Mediterranean Sea.

Keywords: Epiphytic bacteria communities, High throughput sequencing, 16S rRNA gene, canopy-forming seaweeds, Fucales, *Cystoseira compressa*, Mediterranean Sea.

Introduction

Canopy seaweeds of the genus *Cystoseira* C. Agardh (Fucales, Phaeophyceae) are among the most important habitat-forming species in the Mediterranean Sea. With the majority of its species endemic to the Mediterranean Sea (Ribera et al., 1992; Gomez-Garreta, 2002; Draisma et al., 2010), *Cystoseira*-dominated vegetations provide food and protection for rich associated communities, comprising other algae, invertebrates and fish (Mineur et al., 2015). In addition *Cystoseira* stands significantly enhance the structural complexity and productivity of coastal communities from the infralittoral down to the upper circalittoral zone (Giaccone et al., 1994; Bulleri et al., 2002; Falace and Bressan, 2006; Ballesteros et al., 2009).

During the last decades several *Cystoseira* species have retracted their ranges considerably to the point where several species have been reported to be locally lost (Soltan et al., 2001; Thibaut et al., 2005; Serio et al., 2006; Mangialajo et al., 2007, 2008; Perkol-Finkel and Airoldi, 2010; Thibaut et al., 2015). The loss of *Cystoseira* canopies leads to structurally less complex communities most often dominated by low-lying, turf-forming species (Benedetti-Cecchi et al., 2001; Connell et al., 2014) or sea urchin barrens (Agnetta et al., 2015). These shifts are attributable to the interactive effects of different local and global stressors (Asnaghi et al., 2013; Strain et al., 2014, 2015).

The ecological responses of seaweeds to most abiotic and biotic stressors are perceived and transmitted through the algal surface, which represents a highly active interface between the seaweed and the environment. The surface is involved in exchange processes such as the uptake and release of nutrients, waste products and secondary metabolites. Bacteria, which typically form biofilms on the algal surface, are hypothesized to affect the interactions between the seaweeds and the environment by modifying the properties of the external surfaces (Wahl et al., 2012). Bacteria interact with seaweeds, thereby modulating the health, performance and resilience of their hosts. Biofilms can reduce the access of their hosts to light, gases and nutrients and alter the interaction with other fouling epibionts, consumers and pathogens (Goecke et al., 2010; Wahl et al., 2012;). The tight relationship between seaweeds and microbiota renders these associations functionally equivalent to a single entity, or a holobiont (Egan et al., 2013). Although a growing number of papers focus on the bacterial communities associated with different seaweeds (e.g. Bengtsson et al., 2012; Wahl et al., 2012; Hollants et al., 2013; Miranda et al., 2013; Campbell et al., 2015), the underlying mechanisms of these associations remain largely unknown.

Recent investigations present a major stride toward documenting the phylogenetic composition of associated bacterial communities and their spatio-temporal dynamics (e.g. Staufenberger et al., 2008; Burke et al., 2011; Bengtsson et al., 2010;). Most studies concur that algal-associated bacterial communities are distinct from the surrounding environment and largely host-specific (Lachnit et al., 2009). Nevertheless, bacterial communities display considerable temporal and spatial variation (Campbell et al., 2015; Fuhrman et al., 2015). There is growing evidence that the communities of surface bacteria are highly influenced by the physiology of the host. Bengtsson et al. (2010) demonstrated that assembly and dynamics of the biofilm is correlated with the growth cycle of *Laminaria.* More recently, observations that microbial communities were more strongly associated with host condition (healthy versus stressed) in the brown alga *Ecklonia radiata* than with geographical location or environmental variables, highlights that host traits may be a critical determinant of the associated microbial community structure (Marzinelli et al., 2015). Despite these reports, the functional relationships with the host species remain largely an open question. Understanding the dynamics of epiphytic bacteria would allow to explore potentially overlooked mechanisms behind algal responses to environmental or anthropogenic stressors.

We characterized the composition and dynamics of epibiotic bacteria of the canopy-forming seaweed *Cystoseira compressa* (Esper) Gerloff & Nizamuddin along an intertidal rocky promontory in the southern Adriatic Sea. We used next generation sequencing Illumina Miseq of 16S rRNA gene libraries to characterize the diversity (richness, evenness and community composition) of bacterial communities and describe their successional changes over a vegetative growth season. We also tested whether bacterial communities associated to

C. compressa were distinct from those found in the surrounding seawater, to explore potential specificities towards the *C. compressa* host.

Material and methods

Study area and species

Cystoseira compressa and associated microbial communities were sampled along the rocky shore at Punta Aderci promontory, Vasto, Italy (42°10'50.3" N, 14°41'15.0" E) in the central Adriatic Sea (Figure 1 A). This promontory, situated in the central sector of Abruzzo coast, is characterized by clay–sand–conglomerate lithotypes (Miccadei et al., 2011), moderate exposure to wave action and an average tidal amplitude of ≈ 30 cm. We sampled populations of *C. compressa* at the sublitoral fringe (-10 cm to + 10 cm relative to Mean Low Water Level; MLWL). Seawater temperature ranges from a minimum of 8 °C in winter to 27.5 °C in summer (data from the "Istituto Superiore per la Protezione e Ricerca Ambientale", ISPRA, period 2000-2013, www.mareografico.it). The underwater rocky substrate is dominated by patches of mussels (*Mytilus galloprovincialis),* ephemeral algae (*Ulva rigida)* and perennial stands of *C. compressa* (Figure 1 B-C). *C. compressa* is the only canopy-forming alga in this habitat. Like other species of *Cystoseira, C. compressa* exhibit pronounced seasonal variations in vegetative growth (Gomez-Garreta, 2002; Falace et al., 2004;). At the study location, new branches develop from a perennial basis in May, providing a fresh substrate for colonizing bacteria. In July, thalli reach their maximum height and physiological activity (Figure 1 B) while in late August *C. compressa* loses most upright branches (Figure 1 C). The basal cauloid persists in a quiescent state during the cold winter season.

Sampling

Bacterial communities were collected from submerged thalli of *C. compressa* and the surrounding seawater six times from May to October 2014 during the vegetative growth season (Table S1). Each time, epiphytic bacteria were collected from 3 randomly selected thalli of *C. compressa*. Sterile cotton swabs on wooden sticks (Aptaca) were used to rub approximately 12 cm^2 of surface from the perennial base to the tip of primary branches. Swabs were immediately placed in sterile 1.5 ml Eppendorf tubes. Thalli that were overgrown with epiphytic seaweeds or animals were avoided. To compare the bacterial communities that grow on *C. compressa* with those present in the surrounding environment, two samples (n=2) of seawater (500 ml) were randomly collected each time using black polyethylene bottles (Kartell). Seawater samples were filtered in the field with an electric vacuum pump, connected to a portable electric generator, first onto 3.0 μm pore size cellulose acetate filters (Millipore), to remove most eukaryotes, and then onto 0.2 μm pore size cellulose nitrate filters (Sartorius) to retain the bacteria. Samples were transported on ice to the lab and stored at -80 °C until DNA extraction.

Characterization of the bacterial community

Microbial DNA was extracted from the swabs using a protocol from Zwart et al. (1998). Briefly, the tip of the swab was placed into a 2ml tube with 0.5 g of zirconium beads (0.1 mm diameter) to lyse the cells, 0.5 ml 1X TE buffer (10 mM Tris, pH 8) and 0.5 ml buffered phenol (pH 7 to 8) were added to the tubes containing the swab tips and the tubes were vigorously shaken (30Hz) on a Bead-beater (Retsch) three times for two minutes with intermittent cooling on ice. The tubes were then centrifuged for 5 min at 10,000rpm in a cooled centrifuge (4°C) and the upper (aqueous) phase was transferred to a new tube and extracted with phenol-chloroform- isoamylalcohol (25:24:1). The DNA was then precipitated by adding 1/10 volume of 3 M sodium acetate (pH 5) and 2 volumes of 96% (v/v) ethanol and incubating overnight at -20°C. Subsequently, the DNA was washed with ethanol 70% (v/v) and dissolved in 1xTE buffer. The V1-V3 region of the bacterial 16S rRNA gene was amplified using forward pA (AGAGTTTGATCCTGGCTCAG 8–27) (Edwards et al., 1989) and reverse BKL1 1 (GTATTACCGCGGCTGCTGGCA 536– 516) (Cleenwerck et al., 2007) primers. PCR reaction mixes were made using the Faststart High Fidelity PCR system (Roche). The PCR mix consisted of: nuclease-free water; reaction buffer 1x; 0.8 mM of each dNTP; 0.5µM of each primer; 0.02 U *Taq* (FastStart Taq DNA Polymerase); approximately 30 ng template DNA. PCR conditions were: 94°C for 5 min, 30 cycles of 94°C for 30 s, 50°C for 30s, 72°C for 30 s, and final elongation at 72°C for 7 min. Libraries for Illumina MiSeq v3 (2 x 300 bp) were constructed using the NexteraXT DNA sample preparation kit with a dual indexing strategy consisting of two 8-base indices. Amplicons obtained from the first

PCR were cleaned using Ampure beads. Then we performed a second PCR, with 12 cycles, to attach the adaptors and the indexes on the amplicons obtained previously. After a further clean up with Ampure beads and equimolar pooling the samples were sent for sequencing.

The microbial amplicon sequences were processed using the UPARSE pipeline (Edgar, 2013), implemented in the USEARCH package version 8.0.1623 (Edgar, 2010), unless stated otherwise. Paired-end reads with a minimum length overlap of 60bp were merged, discarding reads with a length shorter or longer than 450 and 530, respectively. Moreover, no gaps were allowed in the alignment of the overlapping region. The reads were quality-filtered by imposing a maximum expected error of 0.5. Samples were pooled and truncated using the *trim.seqs* function in Mothur (Schloss et al., 2009). After dereplication, singletons were discarded, and sequences were binned into OTUs with a minimum identity of 97%. Chimeric sequences were detected with the UCHIME algorithm (Edgar et al., 2011) using the RDP gold database (training database v9) as a reference. Taxonomy assignment was performed in QIIME 1.9.0 using the Greengenes 16S rRNA gene dataset (13_8_99 release) (DeSantis et al., 2006) with RDP classifier method (Wang et al., 2007) and a confidence value of the 0.8. The sequences were classified from phylum to genus level. After classification chloroplast and mitochondrial sequences were removed from the dataset. Moreover, samples with a library size smaller than 1000 sequences were removed, because samples below this level can suffer from quality issues (Navas-Molina et al., 2013). Finally, to correct for possible contamination during the lab work, OTUs detected in the negative control were removed from the data set. For phylogenetic tree reconstruction, sequences were aligned with Clustal Ω with default parameters for nucleotide alignment. The phylogenetic tree was reconstructed using the gamma model of sequence evolution (options "-nt -gamma -no2nd -fastest -spr 4") in FastTree2 (Price et al., 2010; Hildebrand et al., 2014). Statistical analyses were performed in R software 3.1.2 (R Core Team, 2015) using the "*phyloseq"* R-package (McMurdie and Holmes, 2013).

Microbial composition was described from phylum to genus level. First, the relative abundance of each OTU within each sample was calculated, then the OTUs were sorted in descending order according to their relative abundance, and the most abundant ones, comprising at least 90% of the community, were retained. A phylogenetic tree was built and used to show differences between the bacterial communities of *C. compressa* and surrounding seawater. Moreover, the log2 fold change times based on the OTUs abundance data was calculated to show which OTU contributed more in the differences between habitats.

The original output files of each sample have been submitted to the NCBI sequence read archive under the accession SRX1563424. Sequences of all 3820 OTUs (97% clustering) have been submitted to GenBank under the accession numbers KU688205 - KU692024.

Analysis of spatial and temporal variations

We characterized the alpha and beta diversity of the bacterial communities collected from two habitats, *C. compressa* and surrounding seawater, over the six sampling times. To estimate alpha diversity, data sets were rarefied at the number of sequences of the sample with the least sequencing depth. Data were rarefied using the "*rarefy_even_depth*" function in the "*phyloseq*" library (we defined a random number seed to 33, R environment). OTU richness and the Chao1 index were calculated using the "*estimated_diversity*" index in the "*phyloseq*" library, while Shannon-Wiener index was estimated using the "*diversity*" function in the "*vegan*" R-package (Oksanen et al., 2015). Pielou's evenness was calculated as H/ln(S), where S and H are the estimated OTU richness and Shannon-Wiener diversity, respectively. For each habitat and each sampling time, we calculated mean values and standards errors for each of these metrics. Differences in alpha diversity parameters between habitats (2 levels, fixed factor) and sampling times (6 levels, random factor, orthogonal to habitat) were statistically tested by performing univariate permutational analyses of variance (PERMANOVA) with PERMANOVA+ (Anderson et al., 2008) for PRIMER v.6 (Clarke and Gorley, 2006). The analysis was based on a Euclidian distance matrix with type III of sum of squares, 9999 permutations, and unrestricted permutation of raw data. PERMANOVA was chosen for univariate analyses because it allows for two-factor designs, considers an interaction term and does not assume a normal distribution of errors.

Spatial and temporal variations of the bacterial communities structure were displayed by unconstrained ordination plots using the principal coordinate analysis (PCoA), based on a Bray-Curtis distance matrix calculated from the square-root transformed OTU abundance data. Differences between habitats and sampling times of the bacterial communities were statistically tested by using a multivariate PERMANOVA. The PERMANOVA analysis was based on a Bray-Curtis similarity matrix with type III of sum of squares, 9999 permutations and unrestricted permutation of raw data. SIMPER analysis was performed in PRIMER v.6 (Clarke and Gorley, 2006) to identify those OTUs that most characterized the epiphytic bacteria community composition of *C. compressa* at each time or that mostly contributed to the differences observed. Cut-off value was restricted to 60%. To explore how different OTUs contributed to the diversity patterns, bubble plots of the abundances of the main correlated OTUs were plotted on the PCoA graph.

Results

Targeting the hypervariable V1-V3 region of the 16S rRNA, a total of 15,799,968 paired-end raw reads were obtained using the Illumina Miseq v.3 platform. After quality filtering and discarding singletons, chimeras and chloroplast and mitochondrial sequences, our dataset contained 1,289,599 sequences with an average length of 483 ± 5 bp. The average number of reads was 44,469 per library (SD = 18,999; min= 8,727; max= 75,903) while the total OTU richness was 3820 at the 97% OTU definition (see Table S1 and S2 for more details). Classification of OTUs against the Greengenes database resulted in 56.9% and 16.2% of the OTUs being classified at family and genus levels, respectively. Classification success increased from 71.9% to 100% with higher taxonomic levels (Figure S1). Rarefaction curves showed saturation for most of the samples, indicative of a good coverage of diversity (Figure 2).

Bacterial diversity of C. compressa and surrounding seawater.

Cystoseira-associated bacterial diversity was significantly higher compared to the surrounding seawater at all sampling times (Figure 3 A, B, Table S3). Likewise, the Shannon index was always higher in bacterial communities associated to *Cystoseira* than in the surrounding seawater (Figure 3 C). Bacterial evenness was generally high both on *Cystoseira* and in the surrounding seawater, with Pielou's evenness index slightly higher on *Cystoseira* samples (Figure 3 D).

Phylogenetic characterization identified 33 phyla of Bacteria: 13 of these (*Proteobacteria, Bacteroidetes, Verrucomicrobia, Actinobacteria,* SR1, OD1*, Thermi,* GN02, *Chloroflexi*, *Planctomycetes*, TM7, *Fusobacteria*, *Cyanobacteria*), together with the OTUs that could not be classified at phylum level, comprised more than 90% of the diversity in the dataset. *Proteobacteria, Bacteroidetes, Actinobacteria*, *Verrucomicrobia* and *Cyanobacteria* were by far the most abundant taxa, accounting for 69.7%, 9.7%, 2.9%, 2.7% and 2.2% of the diversity, respectively (Figure 4 A and Table S4). At the family level, most sequences of the epiphytic bacteria on *C. compressa* were classified as *Rhodobacteraceae* (34.7%), *Flavobacteriaceae* (6.6%), *Saprospiraceae* and *Verrucomicrobiaceae* (5.2% each), while the seawater samples mainly comprised representatives of *Pelagibacteraceae* (40.2%) and *Rhodobacteraceae* (27.6%). About 15-16% of the OTUs, however, remained unclassified at family level in both habitats (Figure 4 B and Table S5). At genus level only the 28.6% of the OTUs were classified. *C. compressa* harbored *Loktanella* (8.8%), *Pseudoruegeria* (3.6%) (family *Rhodobacteraceae*) and *Haloferula* (2.6%) (family *Verrucomicrobiaceae*), while seawater samples showed an important presence of *Oceanibulbus* (5.1%) (family *Rhodobacteraceae*) and *Erythrobacter* (1.2%) (family *Erythrobacteraceae*). However the high percentage of unclassified OTUs at genus level does not allow providing detailed information of the two habitats at that level (Figure 4 C). Of the 13 phyla mentioned above, *Chloroflexi*, and TM7 were exclusively found associated to *C. compressa*. Overall, *C. compressa* hosted a much greater number of exclusive OTUs (121) than seawater (19) (Figure 5).

Successional changes in epibacterial diversity on C. compressa and surrounding seawater

The PERMANOVA revealed significant differences of bacterial community between *C. compressa* and surrounding seawater in all terms (Table S6; Habitats, pseudo-$F_{(df=1,17)}$ = 16.459 $p<0.05$; Date, pseudo-$F_{(df=5,17)}$ = 2.0629 $p<0.05$; Habitats x Date, pseudo-$F_{(df=5,17)}$ = 1.9768 $p<0.05$). The PCoA ordination displayed these differences (Figure 6). The proportion of variance accounted for by the first two axes was 70.4%. This high value makes us confident that our interpretation of the first pair of axes extracts most relevant information from the data. The first axis accounted for the major part of the variance (61.9%) and highlights the big differences between seawater on the one hand and the thallus surface on the other hand (Figure 6). The second axis accounted the 8.5% of the total variation and reflects the time series. This axis revealed a clear successional pattern of the epiphytic bacterial community of *C. compressa* from May to October that was not observed in the seawater samples (Figure 6). The successional pattern in *C. compressa* was also reflected in a continuous increase of OTUs richness that conversely was not observed in the surrounding seawater (Figure 7). Of the 3227 OTUs, a subset of 400 represented 90% of the diversity of the epiphytic bacteria on *C. compressa*. Of these, 173 were present in all samples. SIMPER analysis revealed a high number of OTUs contributing both to the similarity between samples at the same time point as well as to differences between sampling times. Between May (t1) and October (t6) 102 OTUs contributed to 66.9% of dissimilarity (Table S7 A). Of these, 32 OTUs showed higher Pearson correlation (>0.6) in their abundance over time with some OTUs that tend to decrease or increase from May to October (Figure 8 A). In October we observed an increase of OTUs belonging to *Rhodobacteraceae*. In particular the genera *Ruegeria, Nautella, Aquimarina, Loktanella, Saprospira* and *Phaeobacter* increased in abundance with the natural degradation of the thalli of *C. compressa* (Figure S2).

With respect to the seawater, of a total of 1085 OTUs, 100 represented 98% of the bacterial diversity. SIMPER analysis revealed that 27 OTUs contributed to the 32.6% of dissimilarity between May (t1) and October (t6) (Table S7 B). Of these 11 OTUs decreased in their abundance from May to October (cor. >0.6) (Figure 8 B). Two of these were identified at genus level as *Octadecabacter* (family *Rhodobacteraceae*) and *Flavobacterium* (family *Flavobacteriaceae*) (Table S7 B). Finally, of the higher correlated OTUs found in the two habitats from May to October there were not shared taxa.

Discussion

We describe for the first time the bacterial communities of the canopy-forming alga *Cystoseira compressa* and surrounding seawater using next generation sequencing data. The most abundant groups of bacteria in both habitats belonged to *Proteobacteria* and *Bacteroidetes*. Consistent with other studies (Staufenberger et al., 2008; Lachnit et al., 2009; Burke et al., 2011; Bengtsson et al., 2010), we found a clear difference between the bacterial communities of *C. compressa* and the surrounding seawater. The bacterial community of seawater remains more stable compared to that on *C. compressa*, that showed a clear successional pattern associated to ageing thalli. These variations were characterized by gradual addition of OTUs (*Verrucomicrobia*, *Actinobacteria* and SR1) to the epiphytic community, suggesting a clear successional trend. We also found an increase in abundance of potential microbial pathogens associated to older thalli of *C. compressa*.

Even though biofilm-forming bacteria need to be recruited from the surrounding environment, the large differences between seaweed-associated bacteria and those of the surrounding water column are indicative of a selection process whereby the seaweed, the bacteria or a combination of both have the capacity to modulate the recruitment of the biofilm. Our results support the idea of the presence of generalist epiphytes common to all or many macroalgae (Egan et al., 2013). *Alphaproteobacteri*a, *Bacteroidetes*, *Cyanobacteria, Verrucomicrobia* were abundantly found on other brown algae such as *Fucus vesiculosus* (Lachnit et al., 2011), *Saccharina latissima* (Staufenberger et al., 2008) as well as green algae (Burke et al., 2011). Of the four most abundant OTUs detected in this study two were identified as *Loktanella* and *Pseudoruegeria*. Different species of *Loktanella* have been found on *Fucus vesiculosus* (Lachnit et al., 2011; Stratil et al., 2013), *Ulva australis* (Burke et al., 2011) and other macroalgal species (Egan et al., 2013; Hollants et al., 2013; Miranda et al., 2013). The presence of these genera can be related to their capacity to utilize organic carbon sources released from the seaweeds (Bengtsson et al., 2011). The latter provide substrate but also nutrients and trigger chemotactic behavior of bacteria that are highly adaptive and capable of rapid metabolization of algal exudates (Goecke et al., 2010; Wahl et al., 2012).

During spring (~ May), when new branches of *C. compressa* provide a fresh substrate for colonizing bacteria, the epiphytic bacterial community was characterized by lower OTU richness, evenness and Shannon index. The low evenness was due a low number of OTUs and the dominance of 8 OTUs mainly belonging to the *Proteobacteria* that make up nearly 50% of the sequences in spring. We hypothesize that the lower OTU richness found in spring is a consequence of a natural colonization process of the microbial biofilm. In July, thalli of *C. compressa* at the study site reach their maximum dimension and physiological activity. Even though not directly observed in *C. compressa* species, the increase of seawater temperature induces a high photosynthetic activity and concomitant exudation rates of carbohydrates (Abdullah and Fredriksen, 2004; Wada et al., 2007) that can be beneficial for heterotrophic bacteria (Bengtsson et al., 2011, 2012). Hence, in July the growth of the epiphytic bacteria on *C. compressa* leads to an increase of OTUs richness and higher evenness values indicative of the presence of a well-structured community. The shift of the epiphytic community from May to July is also reflected by the increase of reads belonging to *Cyanobacteria*. This aspect was also observed on *Fucus vesiculosus* (Lachnit et al., 2011). In August, when *C. compressa* sheds the majority of upright annual axes, the epiphytic community of *C. compressa* seems to undergo important changes. In fact we observed a drastic decrease to half of OTU richness compared to July. However, we did not observe the same reduction on the evenness values. In September-October the OTUs richness and evenness seems to recover to levels observed in July. The higher OTUs richness may result from the decrease of the seaweed's physiological activity and antimicrobial activity whereby the quiescent status of the alga would explain the increase in abundances of *Rhodobacteraceae* and in particular of different genera such as *Ruegeria*, *Nautella*, *Aquimarina*, *Loktanella*, *Saprospira* and *Phaeobacter* as already observed in bleached parts of the red seaweed *Delisea pulchra* (Fernandes et al., 2011, 2012; Case et al., 2011; Zozaya-Valdes et al., 2015).

Extensive loss of *Cystoseira* species, including *C. compressa*, has been reported in recent years, which has been attributed to the interacting effects of local and global stressors (Perkol-Finkel and Airoldi, 2010; Strain et al., 2015). The exact mechanisms behind these losses have not been fully understood yet, and ongoing experiments have led to the hypothesis of a possible, but up till now unexplored, role of the microbial communities. In fact, the tight interaction between bacteria and their host suggests that the epiphytic microbial community can play an important role in the resilience capability of their host. Moreover, the metabolic capability of bacteria to grow and divide very rapidly may result in bacteria responding faster to external stressors compared their host. In this perspective bacteria should be a potential first indicator of environmental or anthropogenic stressors. Our results provide an important base-knowledge as first step to analyze the possible mechanisms by which *Cystoseira* interacts with surface bacteria. In fact, understanding the temporal dynamics of epiphytic bacteria under natural conditions can help to identify possible modifications of the biofilm due to external factors of stress. Then experiments should be performed to explore the response of the holobiont under the combined effects of local and global stressors known to be major causes of the loss of *C. compressa*. Particular consideration should be given to those taxa, such as *Ruegeria*, *Nautella*, *Aquimarina*, *Loktanella*, *Saprospira* and *Phaeobacter*, that tend to be more present during the natural degradation of *C. compressa*, to observe if stressors can directly increase the abundance of these taxa or alternatively affect the antimicrobial activities of the seaweed with consequent rise of deleterious taxa.

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Figures

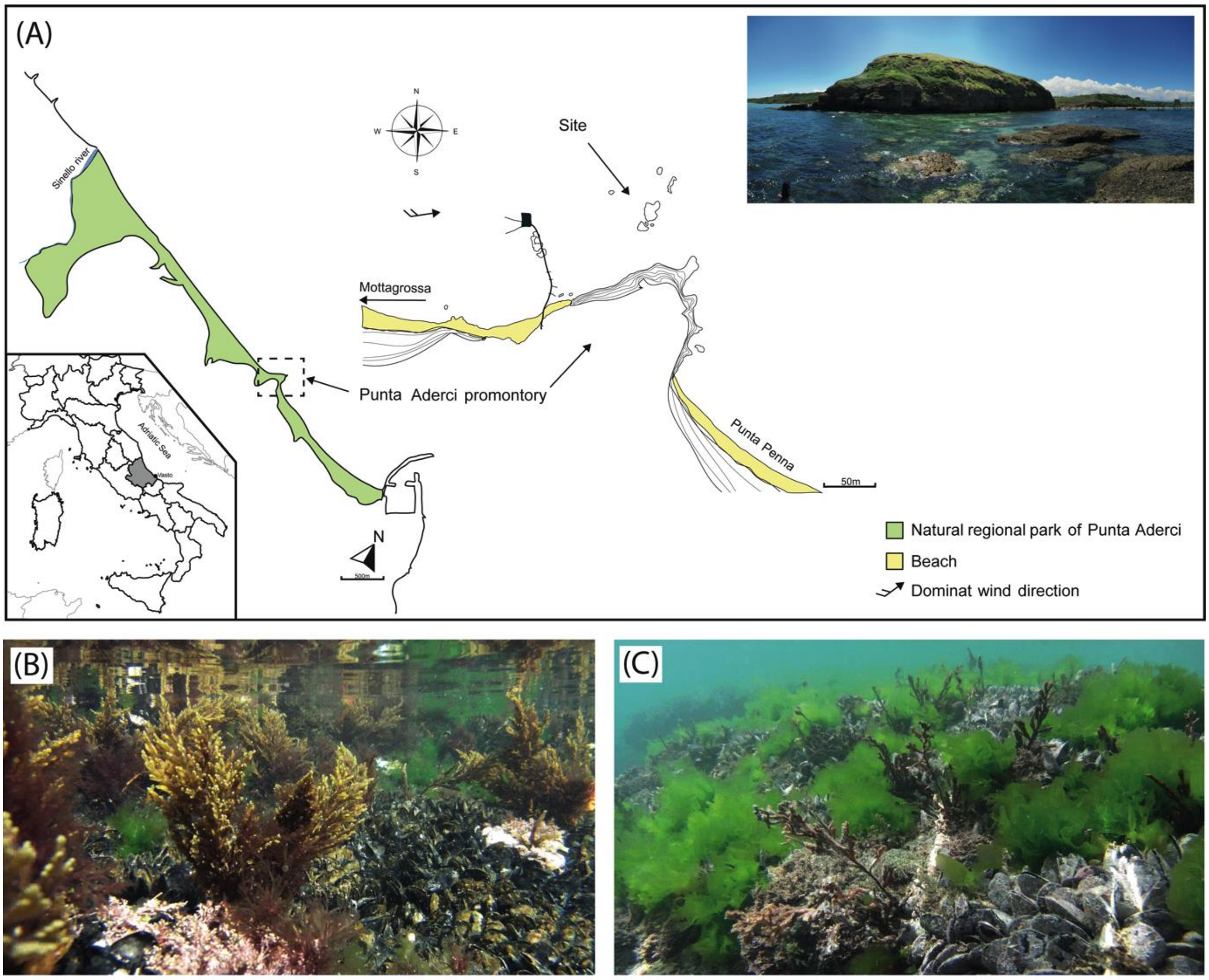

Figure 1: Main physiographic characteristics of the coastal area and sampling site of the Punta Aderci promontory (A). Underwater assemblage characterized by *Mytilus galloprovincialis*, *Ulva rigida* and *Cystoseira compressa* during summer (B) and late summer (C).

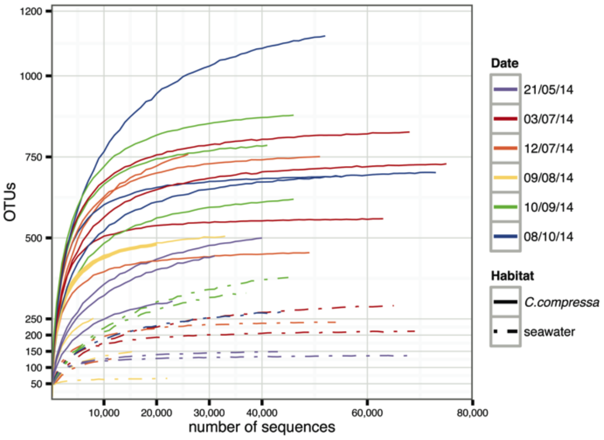

Figure 2: Rarefaction curves generated for each sample. Solid and dot-dash lines refer to *Cystoseira compressa* and seawater samples, respectively. Colors represent different time points.

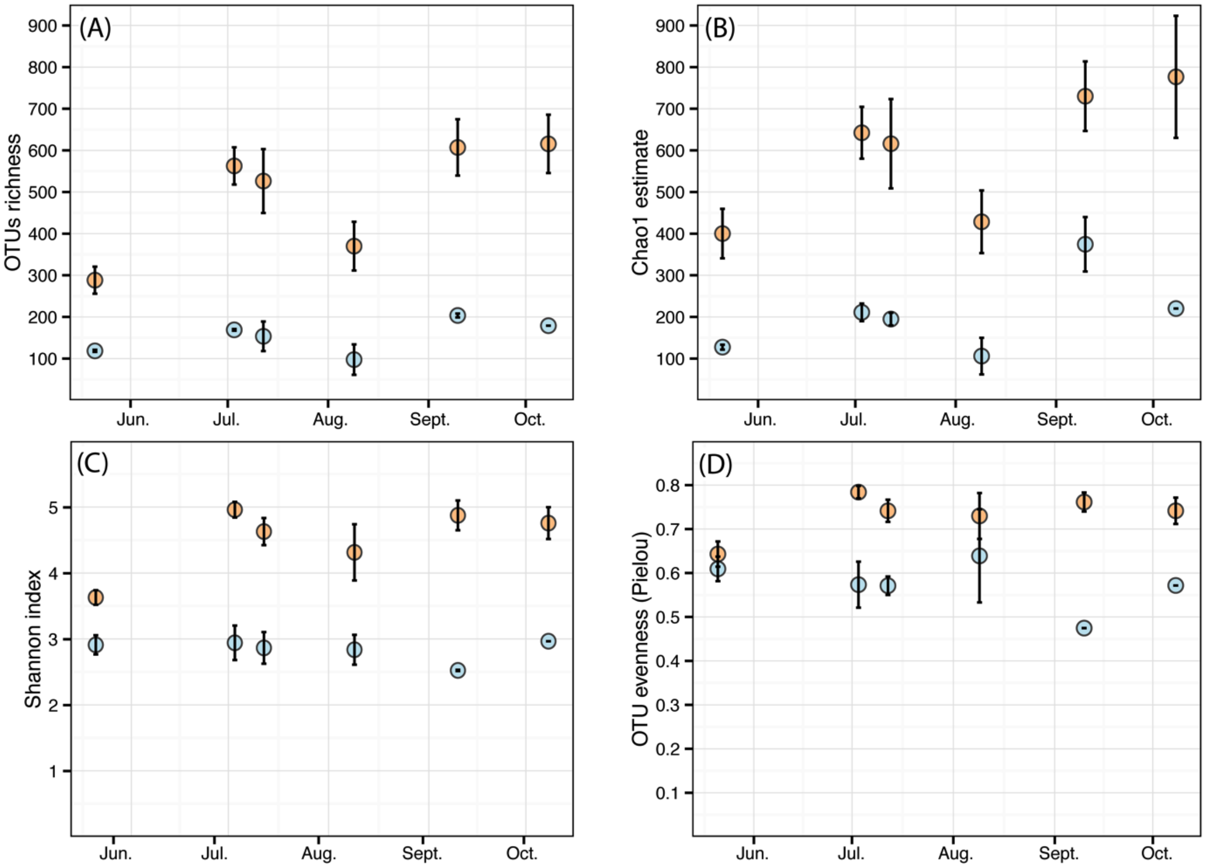

Figure 3: Alpha diversity measures of the microbial communities associated to *C. compressa* (orange) and surrounding seawater (blue) across seasons. Observed OTUs (**A**), Chao1 species richness estimates (**B**), Shannon diversity H' (**C**) and Pielou's evenness index (**D**). Values are means ± standard error (n=3-2).

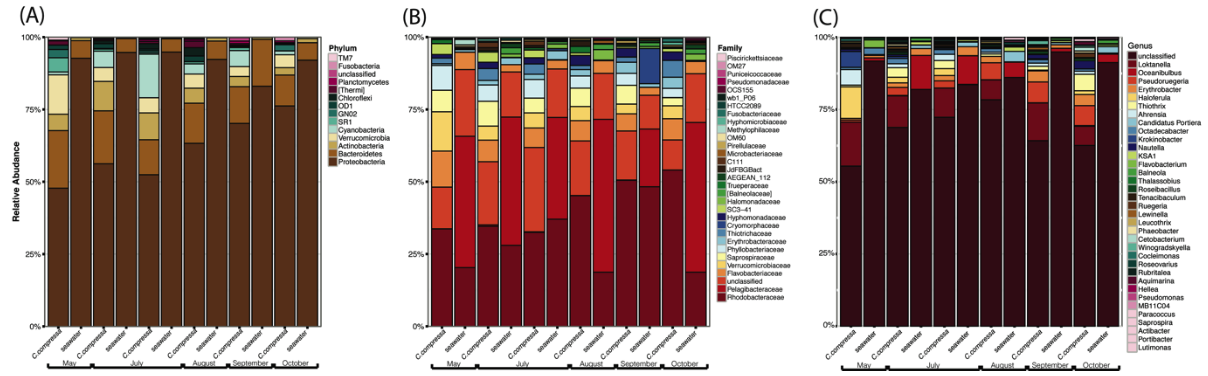

Figure 4: Pattern of the bacterial communities of *C. compressa* and surrounding seawater across seasons. Data reported are the relative abundance of the top 300 OTUs accounting for the 92% of the data set at phylum (A), family (B) and genus (C) levels.

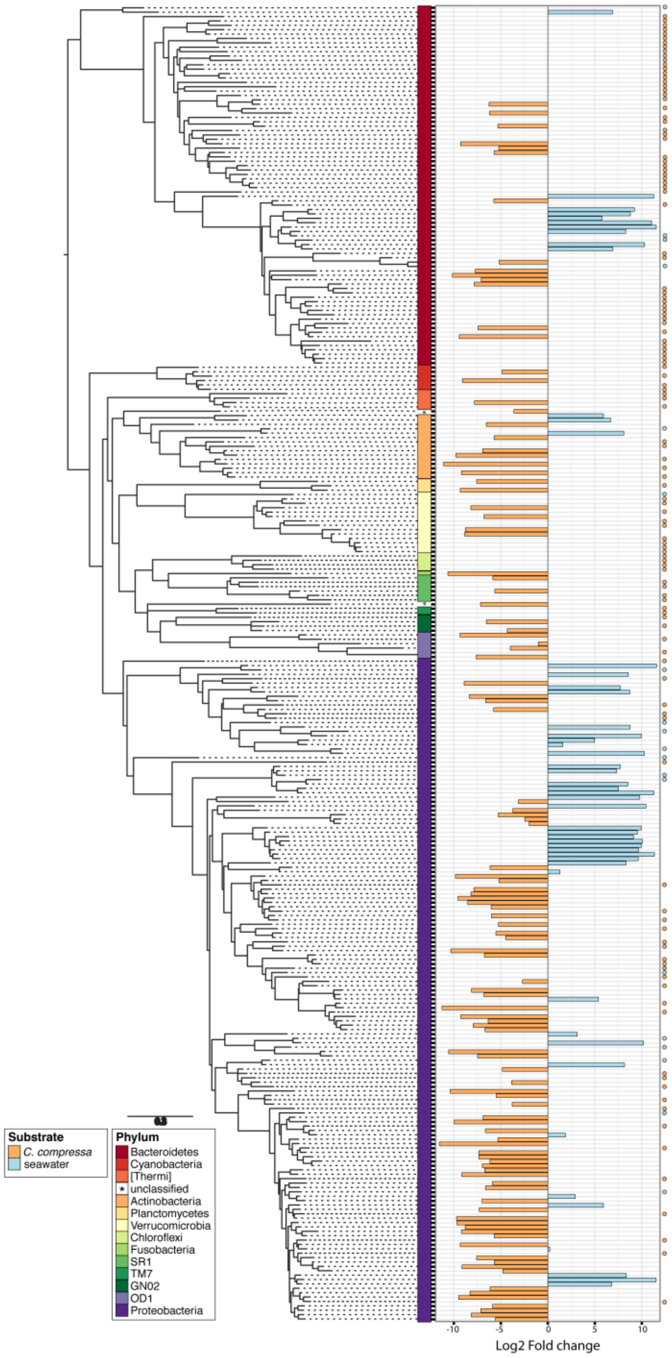

Figure 5: Phylogenetic tree of the dominant OTUs (*n* = 300) in bacterial communities associated with *C. compressa* and the surrounding seawater. The color strips denote phylum-level classification. Bar plot shows the Log2 fold change times based on the OTUs abundance on *C. compressa* (orange) and in seawater (blue). Dot points show exclusive species for each habitat.

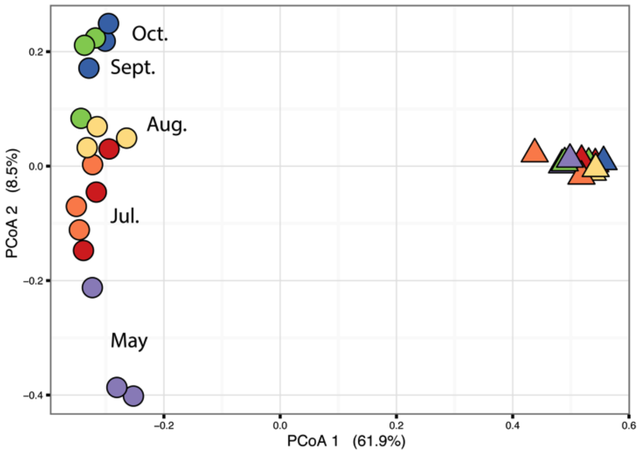

Figure 6: Principal coordinate analysis plot (PCoA) based on a Bray-Curtis distance matrix calculated from the square-root transformed OTU abundance data of the bacterial community of *C. compressa* and surrounding seawater across times. Violet, red, orange, yellow, green and blue points represent the following sample times respectively: 21-05-14, 03-07-14, 12-07-14, 09-08-14, 10-09-14 and 08-10-14.

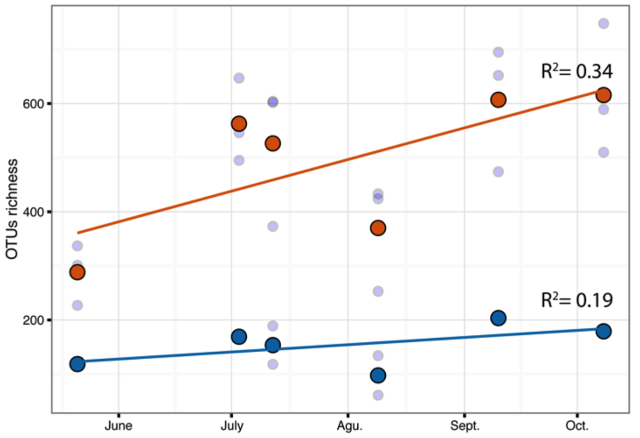

Figure 7: Correlation of OTUs richness over time for *C.compressa* (orange dots) and seawater (blue dots). Dots and lines are mean values and tendency respectively. Values of Pearson correlation (R^2) are shown inside the plot.

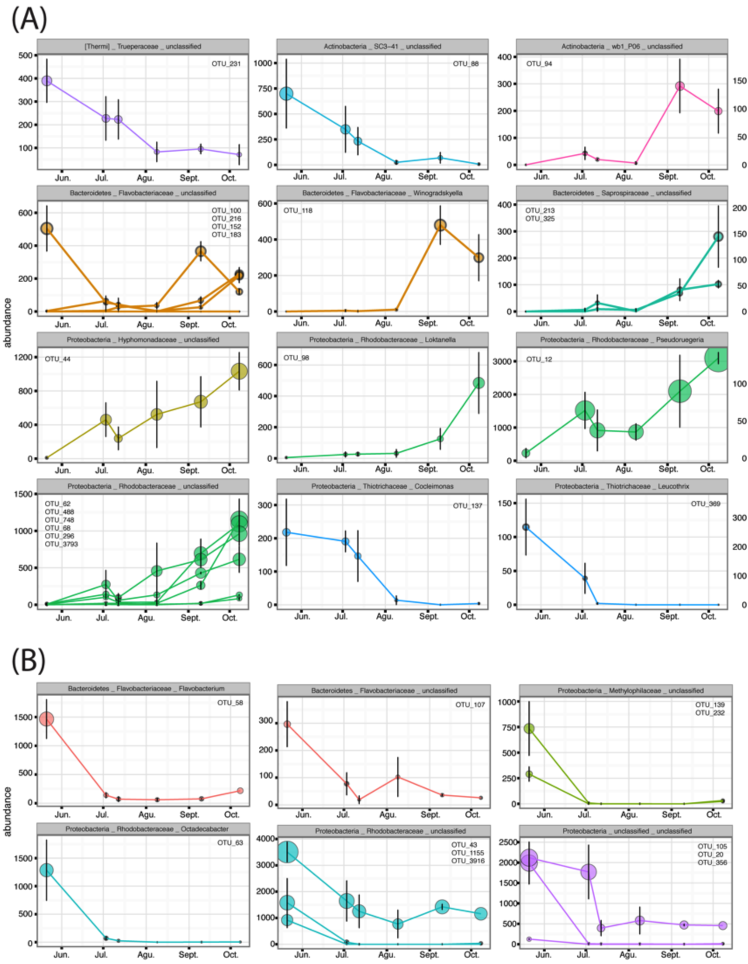

Figure 8: OTUs with higher Pearson correlation (>0.6) in their abundance over time, contributing to the differences in the epiphytic bacteria of *C. compressa* (A) and surrounding seawater (B) between time 1 and 6 (May-October). The OTUs are grouped by phylum, family and genus. OTUs number are reported inside each graph.

Supplementary materials

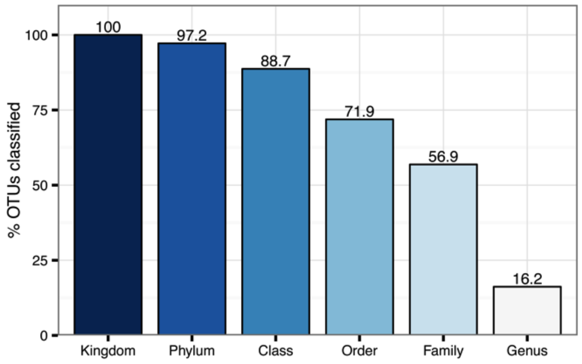

Figure S1. Total percentage of OTUs classified at each taxonomic level.

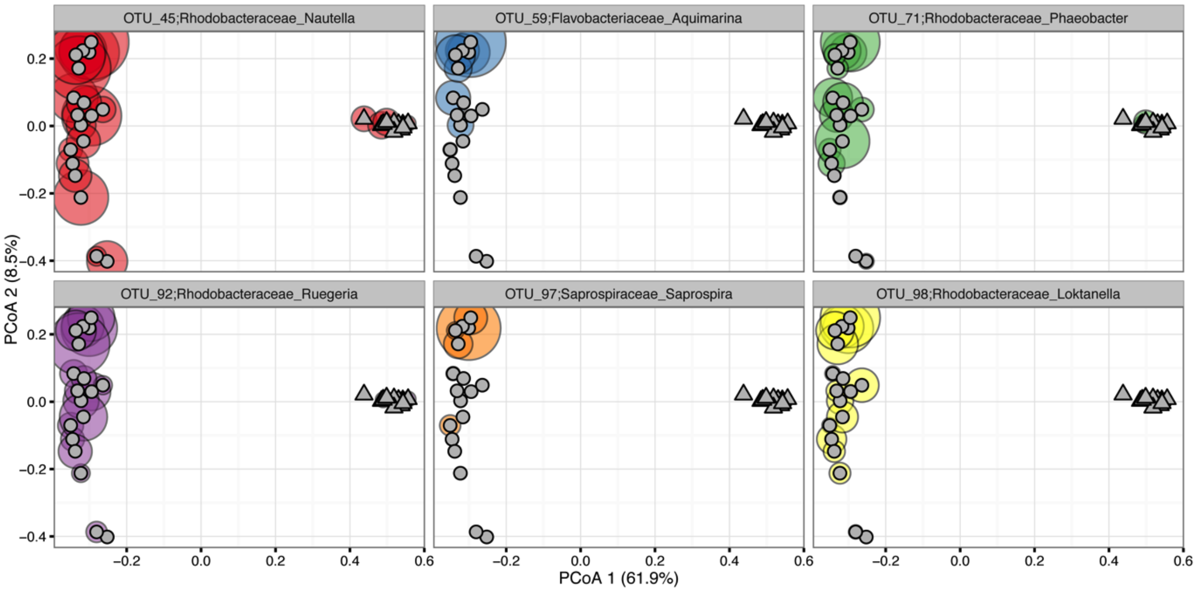

Figure S2: Bubble charts of the abundances of the six genera (*Nautella, Aquimarina, Phaeobacter, Ruegeria, Saprospira* and *Loktanella*) that increase across time on the thalli of *C. compressa* and surrounding seawater.

Table S1. Details of the number of reads per sample retained after different quality filtering steps. Sample 73 was omitted from the analyses due to the low number of reads.

Substrate	ID	Date	Merged	Filtered	removed	removed chlorophast, mithocondria, less than
C.compressa	8	21-05-2014	119,823	44,637	33,250	31,672
C.compressa	9	21-05-2014	112,486	40,601	33,422	23,533
C.compressa	10	21-05-2014	172,598	76,296	64,945	40,367
seawater	12	21-05-2014	164,537	58,083	44,742	43,263

seawater	13	21-05-2014	204,201	86,643	70,293	68,432
C.compressa	2	03-07-2014	213,826	87,441	73,397	63,373
C.compressa	4	03.07.2014	306,453	87,680	73,808	68,352

Resemblance: D1 Euclidean distance

C.compressa	14	03-07-2014	234,800	90,948	80,918	75,903
seawater	15	03-07-2014	271,811	116,017	69,345	65,393
seawater	16	03-07-2014	198,640	83,520	76,064	69,724
C.compressa	17	12-07-2014	105,541	37,160	30,435	26,479
C.compressa	18	12-07-2014	202,423	85,283	56,808	49,302
C.compressa	19	12-07-2014	169,705	66,655	57,166	51,435
seawater	31	12-07-2014	83,195	18,172	14,074	12,157
seawater	32	12-07-2014	152,240	66,933	58,861	54,799
C.compressa	23	09-08-2014	75,813	28,960	23,538	20,605
C.compressa	24	09-08-2014	115,872	44,243	35,506	33,153
C.compressa	25	09-08-2014	85,123	20,557	8,878	8,727
seawater	29	09-08-2014	83,669	29,721	26,816	22,393
seawater	30	09-08-2014	67,786	29,403	17,081	16,156
C.compressa	47	10-09-2014	156,830	57,344	43,053	41,304
C.compressa	48	10-09-2014	156,693	57,094	49,485	46,930
C.compressa	49	10-09-2014	190,610	68,437	53,770	46,105
seawater	59	10-09-2014	156,220	64,291	51,538	45,470
seawater	60	10-09-2014	168,793	53,840	41,601	37,125
C.compressa	61	08-10-2014	207,731	79,571	65,721	55,338
C.compressa	62	08-10-2014	258,970	105,155	81,269	73,531
C.compressa	63	08-10-2014	234,040	89,127	62,008	52,993
seawater	73	08-10-2014	60,354	343	113	0
seawater	74	08-10-2014	185,930	63,595	47,635	45,585
negative sample	75		10,525	2,875	2,075	0
	Tot.		**4,927,238**	**1,840,625**	**1,447,615**	**1,289,599**

Table S2. Summary statistics of the number of reads of *C. compressa* and surrounding seawater: number of samples, taxa and total, mean, standard deviation, minimum and maximum number of reads.

Substrate	**n° samples**	**taxa**	**Total seqs.**	**mean**	**min**	**max**	**sd**
all samples	29	3820	1,289.559	44.469	8.727	75.903	18.999
C. compressa	18	3227	809.102	44.950	8.727	75.903	18.717
Seawater	11	1085	480.497	43.682	12.157	69.724	20.348

Table S3. PERMANOVA results of alpha diversity indices.

Sums of squares type: Type III (partial)
Fixed effects sum to zero for mixed terms
Permutation method: Unrestricted permutation of raw data
Number of permutations: 9999

Factors			
Name	Abbrev.	Type	Levels
Habitat	Ha	Fixed	2
Date	Da	Random	6

PERMANOVA table of results **OTU_richness**

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Ha	1	7.63E+05	7.63E+05	67.886	0.0002	9806
Da	5	1.66E+05	33150	4.1378	0.0113	9943
HaxDa	5	56654	11331	1.4143	0.2605	9966
Res	17	1.36E+05	8011.5			
Total	28	1.23E+06				

PERMANOVA table of results **Shannon**

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Ha	1	18.628	18.628	50.953	0.0017	9844
Da	5	1.334	0.2668	1.7747	0.1734	9955
HaxDa	5	1.8567	0.37134	2.47	0.067	9962
Res	17	2.5557	0.15034			
Total	28	26.172				

PERMANOVA table of results **evenness**

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Ha	1	0.16833	0.16833	18.028	0.0116	9760
Da	5	1.73E-02	3.46E-03	0.90747	0.5027	9968
HaxDa	5	4.74E-02	9.48E-03	2.4873	0.069	9947
Res	17	6.48E-02	3.81E-03			
Total	28	0.30574				

PERMANOVA table of results **Chao1**

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Ha	1	1.01E+06	1.01E+06	104.84	0.0001	9824
Da	5	3.17E+05	63401	3.2481	0.0308	9940
HaxDa	5	46963	9392.5	0.48118	0.787	9954
Res	17	3.32E+05	19520			

Total	28	1.84E+06

Table S4. Relative abundance of the top 300 OTU at the phylum level on *C. compressa* and surrounding seawater. Values are percentages. Mean= overall mean per phylum.

	May		**July**				**August**		**September**		**October**		
	21/05/14		03/07/14		12/07/14		09/08/14		10/09/14		08/10/14		
	C. compressa	seawater	*C. compressa*	Seawater	*C. compressa*	seawater	*C. compressa*	seawater	*C. compressa*	seawater	*C. compressa*	seawater	**mean**
Proteobacteria	44.8	89.6	44.2	91.5	43.6	92.1	55.7	89.5	55.4	80.8	60.3	88.4	69.7
Bacteroidetes	18.7	5.9	14.4	4.6	10.1	4.5	12.2	6.1	10.0	15.8	8.5	5.8	9.7
Actinobacteria	5.3	1.0	8.0	0.4	7.7	0.3	4.6	1.1	2.8	0.6	2.0	1.4	2.9
Verrucomicrobia	12.7	0.1	3.8	0.1	4.3	0.1	4.3	0.2	2.7	0.1	3.5	0.5	2.7
Cyanobacteria	1.0	0.0	4.4	0.0	12.5	0.1	3.1	0.0	4.4	0.0	1.0	0.0	2.2
SR1	4.6	0.0	0.6	0.0	0.4	0.0	0.6	0.0	0.8	0.0	0.0	0.0	0.6
GN02	2.7	0.0	0.2	0.0	0.6	0.0	0.0	0.0	0.3	0.0	1.7	0.0	0.5
OD1	1.4	0.1	1.3	0.0	0.5	0.0	1.7	0.0	0.2	0.0	0.2	0.0	0.5
Chloroflexi	0.1	0.0	0.8	0.0	1.6	0.0	2.5	0.0	0.2	0.0	0.2	0.0	0.4
[Thermi]	1.3	0.0	0.4	0.0	0.6	0.0	2.0	0.0	0.4	0.0	0.2	0.0	0.4
Planctomycetes	0.2	0.0	0.3	0.0	0.5	0.0	0.5	0.0	0.7	0.0	0.5	0.0	0.2
unclassified	0.0	0.0	0.2	0.0	0.2	0.0	0.3	0.0	0.9	0.0	0.0	0.0	0.1
Fusobacteria	0.0	0.0	0.1	0.0	0.3	0.0	0.2	0.0	0.1	0.0	1.0	0.0	0.1
TM7	1.0	0.0	0.1	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1

Table S5. Relative abundance of the top 300 OTU at the family level on *C. compressa* and surrounding seawater. Values are percentages. Mean= overall mean per family per habitat.

	May		**July**				**August**		**September**		**October**			
	21/05/14		03/07/14		12/07/14		09/08/14		10/09/14		08/10/14		**mean**	**mean**
	C. compressa	seawater	*C. compressa*	seawater	*C. compressa*	seawater	*C. compressa*	seawater	*C. compressa*	seawater	*C. compressa*	seawater	*C. compressa*	seawater
Rhodobacteraceae	31.6	19.6	27.2	27.0	27.0	36.0	39.8	18.2	39.9	47.0	42.7	18.0	34.7	27.6
Pelagibacteraceae	0.0	43.9	0.4	42.8	0.2	34.1	0.1	51.3	0.1	19.4	0.0	49.7	0.1	40.2
unclassified	13.5	22.3	17.2	15.0	24.2	16.3	16.6	15.4	13.3	11.4	8.2	16.2	15.5	16.1

Flavobacteriaceae	11.7	5.8	5.9	2.5	5.6	2.9	6.2	4.2	4.7	2.7	5.8	4.1	6.6	3.7
Verrucomicrobiaceae	12.7	0.0	3.8	0.0	4.3	0.0	4.3	0.0	2.7	0.0	3.5	0.0	5.2	0.0
Saprospiraceae	7.0	0.0	6.8	0.0	4.2	0.1	5.6	0.0	5.1	0.0	2.3	0.0	5.2	0.0
Phyllobacteriaceae	8.0	0.1	4.4	0.0	5.4	0.0	3.7	0.0	3.2	0.0	2.5	0.0	4.6	0.0
Erythrobacteraceae	0.5	0.8	1.3	2.3	1.5	3.0	1.9	0.1	3.0	1.2	3.1	0.3	1.9	1.3
Thiotrichaceae	1.9	0.0	3.2	0.0	2.9	0.0	1.3	0.0	1.4	0.0	4.6	0.0	2.5	0.0
Cryomorphaceae	0.0	0.0	0.0	0.1	0.0	0.7	0.0	0.1	0.0	11.8	0.0	0.0	0.0	2.1
Hyphomonadaceae	1.1	0.0	1.7	0.1	1.8	0.0	3.0	0.0	2.4	0.0	2.3	0.0	2.1	0.0
SC3-41	3.5	0.0	2.8	0.0	2.2	0.0	0.8	0.0	0.5	0.0	0.9	0.0	1.8	0.0
Halomonadaceae	0.0	0.3	0.0	1.1	0.0	1.6	0.0	3.5	0.0	0.7	0.0	2.1	0.0	1.6
[Balneolaceae]	0.0	0.1	0.0	2.1	0.0	0.8	0.0	1.8	0.0	1.3	0.0	1.7	0.0	1.3
Trueperaceae	1.3	0.0	0.4	0.0	0.6	0.0	2.0	0.0	0.4	0.0	0.2	0.0	0.8	0.0

Table S6. Results of the PERMANOVA analysis, testing for significant differences in bacterial composition as a function of habitat (Ha) and sampling time (Da).

Resemblance: S17 Bray Curtis similarity (+d)
Sums of squares type: Type III (partial)
Fixed effects sum to zero for mixed terms
Permutation method: Unrestricted permutation of raw data
Number of permutations: 9999

Factors

Name	Abbrev.	Type	Levels
Habitat	Ha	Fixed	2
Date	Da	Random	6

PERMANOVA table of results **Beta diversity of *C. compressa* and surrounding seawater**

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms

Ha	1	39261	39261	16.459	**0.0006**	9911
Da	5	12608	2521.6	2.0629	**0.0104**	9904
HaxDa	5	12082	2416.4	1.9768	**0.0165**	9890
Res	17	20780	1222.4			
Total	28	89291				

Table S7. Results of the SIMPER analysis of the epiphytic bacteria of *C. compressa* (A) and surrounding seawater (B). OTUs contributed to the differences between time 1 (May) and time 6 (October).

(A) SIMPER analysis epiphytic bacteria of *C. compressa*

Groups t1 & t6

Average dissimilarity = 66.94

	Group t1	Group t6										
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%	Kingdom	Phylum	Class	Order	Family	Genus
OTU_8	26.17	72.2	1.14	1.03	1.7	1.7	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified
OTU_12	12.96	55.61	1.09	3.75	1.63	3.34	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Pseudoruegeria
OTU_29	12.16	51.23	0.98	6.17	1.47	4.8	Bacteria	Proteobacteria	Gammaproteobacteria	Thiotrichales	Thiotrichaceae	Thiothrix
OTU_18	38.24	2.79	0.89	2.14	1.33	6.13	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Krokinobacter
OTU_22	31.73	0	0.82	1.47	1.23	7.36	Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Haloferula
OTU_62	1.32	33.49	0.82	4.9	1.22	8.58	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified
OTU_488	1.55	33.03	0.8	9.24	1.19	9.77	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified
OTU_44	3.08	31.79	0.72	6.28	1.08	10.86	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Hyphomonadaceae	unclassified
OTU_748	3.59	30.76	0.69	6.22	1.03	11.88	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified
OTU_17	36.84	10.49	0.66	1.59	0.99	12.87	Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Haloferula
OTU_11	51.47	24.63	0.65	0.88	0.97	13.84	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified
OTU_4	66.69	45.96	0.64	1.3	0.96	14.8	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Loktanella
OTU_111	11.19	36.24	0.64	2.61	0.96	15.76	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Erythrobacteraceae	Erythrobacter
OTU_56	25.6	1.33	0.63	1.49	0.94	16.71	Bacteria	SR1	unclassified	unclassified	unclassified	unclassified
OTU_92	1.92	24.99	0.59	3.4	0.88	17.59	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Ruegeria
OTU_45	13.56	36.7	0.59	1.96	0.87	18.46	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Nautella
OTU_88	23.98	1.56	0.58	1.82	0.86	19.32	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	SC3-41	unclassified
OTU_100	22	0	0.56	3.85	0.84	20.16	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	unclassified
OTU_68	2.63	24.3	0.55	3.94	0.82	20.98	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified
OTU_91	20.77	0	0.53	1.29	0.8	21.78	Bacteria	GN02	BD1-5	unclassified	unclassified	unclassified

OTU_54	0.91	20.45	0.53	0.91	0.79	22.57	Bacteria	GN02	unclassified	unclassified	unclassified	unclassified
OTU_138	2.92	22.89	0.51	2.19	0.77	23.34	Bacteria	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Cetobacterium
OTU_64	19.67	0	0.51	1.86	0.76	24.1	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Saprospiraceae	unclassified
OTU_59	0	20.28	0.51	1.35	0.75	24.85	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Aquimarina
OTU_98	1.96	21.03	0.48	2.82	0.72	25.57	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Loktanella
OTU_25	27.18	8.78	0.46	2.18	0.69	26.27	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	unclassified
OTU_6	4.43	22.82	0.46	2.63	0.69	26.96	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Erythrobacteraceae	Erythrobacter
OTU_1371	10.01	27.53	0.46	1.46	0.69	27.65	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified
OTU_93	0	17.71	0.44	1.26	0.66	28.3	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	unclassified
OTU_97	0	16.31	0.43	1.19	0.64	28.94	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Saprospiraceae	Saprospira
OTU_15	37.2	21.15	0.42	1.33	0.63	29.57	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	Ahrensia
OTU_21	22.31	26.54	0.42	1.22	0.63	30.2	Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	unclassified
OTU_118	0	16.42	0.41	3.16	0.61	30.82	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Winogradskyella
OTU_213	0	16.09	0.4	3.66	0.6	31.42	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Saprospiraceae	unclassified
OTU_112	3.16	18.86	0.39	2.13	0.59	32.01	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified
OTU_189	0	15.37	0.38	2.93	0.57	32.58	Bacteria	Proteobacteria	Alphaproteobacteria	Kiloniellales	unclassified	unclassified
OTU_216	0	14.94	0.38	5.39	0.57	33.15	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	unclassified
OTU_80	14.5	0	0.38	1.32	0.56	33.72	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	unclassified
OTU_173	15.69	0.75	0.37	1.4	0.56	34.27	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	unclassified
OTU_42	17.43	5.51	0.36	1.5	0.53	34.81	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Saprospiraceae	unclassified
OTU_40	9.2	22.68	0.35	1.62	0.52	35.32	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified
OTU_140	13.37	0	0.35	1.38	0.52	35.84	Bacteria	TM7	unclassified	unclassified	unclassified	unclassified
OTU_401	1.8	15.24	0.34	3	0.51	36.35	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified
OTU_291	13.06	0	0.34	1.44	0.51	36.86	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Tenacibaculum
OTU_14	10.06	20.43	0.34	1.67	0.51	37.37	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified
OTU_152	1.28	14.61	0.34	5.42	0.5	37.87	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	unclassified
OTU_94	0.33	13.56	0.33	3.18	0.5	38.37	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	wb1_P06	unclassified
OTU_137	14.05	1.47	0.33	2.17	0.49	38.86	Bacteria	Proteobacteria	Gammaproteobacteria	Thiotrichales	Thiotrichaceae	Cocleimonas
OTU_210	0	12.68	0.33	2.05	0.49	39.34	Bacteria	Proteobacteria	Alphaproteobacteria	unclassified	unclassified	unclassified

OTU_3443	0	12.85	0.32	4.99	0.48	39.83	Bacteria	Proteobacteria	Alphaproteobacteria	unclassified	unclassified	unclassified
OTU_231	19.47	7.02	0.32	1.94	0.48	40.31	Bacteria	[Thermi]	Deinococci	Deinococcales	Trueperaceae	unclassified
OTU_71	1.38	14.24	0.32	1.19	0.48	40.78	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Phaeobacter
OTU_193	4.45	16.81	0.32	2.24	0.48	41.26	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified
OTU_175	0.47	12.82	0.31	8.45	0.47	41.73	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Saprospiraceae	unclassified
OTU_82	2.58	14.93	0.31	1.98	0.47	42.2	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified
OTU_211	0	12.07	0.31	3.27	0.46	42.66	Bacteria	Planctomycetes	Planctomycetia	Pirellulales	Pirellulaceae	unclassified
OTU_951	1.99	13.59	0.3	2.52	0.45	43.11	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	unclassified
OTU_156	0	12.25	0.3	0.67	0.44	43.55	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	unclassified
OTU_163	0	11.25	0.29	1.23	0.44	43.99	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	unclassified
OTU_1	0.33	11.97	0.29	3.72	0.44	44.42	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified
OTU_966	3.63	15.24	0.29	2.47	0.43	44.86	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Octadecabacter
OTU_103	10.72	20.9	0.29	1.52	0.43	45.29	Bacteria	Proteobacteria	Alphaproteobacteria	unclassified	unclassified	unclassified
OTU_296	0.33	11.5	0.28	8.2	0.42	45.71	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified
OTU_72	15.97	4.94	0.28	1.66	0.42	46.14	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Saprospiraceae	unclassified
OTU_269	0.82	11.77	0.28	3.09	0.42	46.56	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Actibacter
OTU_442	1.29	12.37	0.28	2.49	0.42	46.97	Bacteria	Proteobacteria	Gammaproteobacteria	Thiotrichales	Thiotrichaceae	Leucothrix
OTU_183	0	10.91	0.28	10.27	0.41	47.38	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	unclassified
OTU_171	10.82	0	0.27	2.57	0.41	47.79	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Tenacibaculum
OTU_1991	0	10.89	0.27	1.48	0.41	48.2	Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Haloferula
OTU_136	11.44	0.67	0.27	3.72	0.41	48.61	Bacteria	GN02	BD1-5	unclassified	unclassified	unclassified
OTU_3916	1.28	11.86	0.27	4.04	0.4	49.01	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified
OTU_69	10.51	0	0.26	0.76	0.39	49.4	Bacteria	OD1	ZB2	unclassified	unclassified	unclassified
OTU_165	7.29	14.75	0.26	1.81	0.39	49.79	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	unclassified
OTU_275	0	10.32	0.26	3.09	0.39	50.18	Bacteria	Proteobacteria	unclassified	unclassified	unclassified	unclassified
OTU_369	10.38	0	0.26	4.5	0.39	50.57	Bacteria	Proteobacteria	Gammaproteobacteria	Thiotrichales	Thiotrichaceae	Leucothrix
OTU_204	6.75	16.78	0.26	1.8	0.39	50.96	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified
OTU_3797	0.33	10.63	0.26	1.36	0.39	51.34	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified
OTU_325	0	10.06	0.26	6.37	0.38	51.73	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Saprospiraceae	unclassified
OTU_23	11.91	18.74	0.26	1.84	0.38	52.11	Bacteria	Cyanobacteria	unclassified	unclassified	unclassified	unclassified

OTU_215	5.95	15.84	0.25	1.75	0.38	52.49	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified
OTU_132	11.96	14.89	0.25	1.29	0.38	52.86	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Hyphomonadaceae	unclassified
OTU_247	9.78	0	0.25	1.03	0.37	53.24	Bacteria	SR1	unclassified	unclassified	unclassified	unclassified
OTU_108	0.82	10.66	0.25	2.67	0.37	53.61	Bacteria	Proteobacteria	Alphaproteobacteria	Kiloniellales	unclassified	unclassified
OTU_129	12.74	4.6	0.25	1.63	0.37	53.98	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified
OTU_180	0	9.2	0.25	0.74	0.37	54.34	Bacteria	Bacteroidetes	unclassified	unclassified	unclassified	unclassified
OTU_214	1.28	11.14	0.24	1.72	0.36	54.71	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified
OTU_131	9.62	0	0.24	0.7	0.36	55.07	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Saprospiraceae	unclassified
OTU_264	0	9.6	0.24	1.39	0.36	55.43	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Tenacibaculum
OTU_16	18.69	12.31	0.24	1.26	0.36	55.79	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	unclassified	unclassified
OTU_66	4.25	12.67	0.24	1.05	0.36	56.15	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	unclassified
OTU_245	0	9.35	0.24	3.4	0.36	56.51	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Roseovarius
OTU_337	3.15	12.34	0.24	1.73	0.35	56.86	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified
OTU_447	0	9.18	0.24	1.64	0.35	57.22	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	unclassified	unclassified
OTU_328	0	9.26	0.24	5.18	0.35	57.57	Bacteria	Proteobacteria	Alphaproteobacteria	unclassified	unclassified	unclassified
OTU_3	1.33	10.58	0.23	4.12	0.35	57.92	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Oceanibulbus
OTU_90	0	9.3	0.23	4.12	0.35	58.27	Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	unclassified
OTU_2296	9.15	0	0.23	3.97	0.35	58.61	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Saprospiraceae	unclassified
OTU_153	5.16	14.27	0.23	1.53	0.35	58.96	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	unclassified
OTU_143	8.75	0	0.23	1.03	0.35	59.31	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Saprospiraceae	unclassified
OTU_147	0	9.32	0.23	1.03	0.34	59.65	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	unclassified
OTU_3793	0.33	9.03	0.22	4.13	0.33	59.98	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified
OTU_149	1	9.83	0.22	1.55	0.33	60.31	Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	unclassified

(B) SIMPER analysis bacteria in the surrounding seawater

Groups t1 & t6

Average dissimilarity = 32.59

	Group t1	Group t6										
OTU	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%	Kingdom	Phylum	Class	Order	Family	Genus
OTU_105	44.2	3.74	1.56	10.4	4.8	4.8	Bacteria	Proteobacteria	Alphaproteobacteria	unclassified	unclassified	unclassified
OTU_43	37.83	0	1.44	2.81	4.42	9.23	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified
OTU_63	34.99	2	1.27	4.42	3.89	13.11	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Octadecabacter
OTU_5	24.41	55.67	1.24	2.69	3.81	16.92	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Pelagibacteraceae	unclassified
OTU_1	44.77	69.43	0.97	3.97	2.98	19.9	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified
OTU_1155	59.13	34.03	0.97	13.49	2.98	22.88	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified
OTU_20	45.88	21.38	0.95	9.29	2.91	25.79	Bacteria	Proteobacteria	Alphaproteobacteria	unclassified	unclassified	unclassified
OTU_3916	29.9	5.92	0.92	5.48	2.83	28.62	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified
OTU_58	38.01	14.76	0.9	5.99	2.75	31.37	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium
OTU_139	26.65	4.8	0.84	4.65	2.58	33.94	Bacteria	Proteobacteria	Betaproteobacteria	Methylophilales	Methylophilaceae	unclassified
OTU_78	1	19.67	0.73	5.4	2.25	36.19	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomounclassifieddaceae	Candidatus Portiera
OTU_220	4.44	22.49	0.69	4.12	2.12	38.32	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	AEGEAN_112	unclassified
OTU_50	6.2	22.49	0.65	2.43	1.99	40.31	Bacteria	Bacteroidetes	[Rhodothermi]	[Rhodothermales]	[Balneolaceae]	Balneola
OTU_2	141.11	125.03	0.59	1.08	1.83	42.13	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Pelagibacteraceae	unclassified
OTU_188	5.96	20.27	0.58	1.48	1.77	43.9	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Pelagibacteraceae	unclassified
OTU_707	1.22	15.65	0.56	102.06	1.72	45.62	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Pelagibacteraceae	unclassified
OTU_75	3.05	16.06	0.52	3.04	1.58	47.21	Bacteria	Bacteroidetes	[Rhodothermi]	[Rhodothermales]	[Balneolaceae]	KSA1
OTU_1037	0	12.96	0.51	9.06	1.56	48.76	Bacteria	Proteobacteria	Deltaproteobacteria	Sva0853	unclassified	unclassified
OTU_3	24.29	35.74	0.47	1.07	1.44	50.2	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Oceanibulbus
OTU_107	17.04	5.1	0.46	5.48	1.41	51.61	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	unclassified
OTU_99	9	20.15	0.44	4.89	1.35	52.96	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	unclassified
OTU_604	0	11.14	0.44	9.06	1.34	54.29	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified
OTU_232	16.91	5.66	0.43	6.19	1.33	55.62	Bacteria	Proteobacteria	Betaproteobacteria	Methylophilales	Methylophilaceae	unclassified
OTU_542	0	10.95	0.43	9.06	1.31	56.94	Bacteria	SBR1093	A712011	unclassified	unclassified	unclassified
OTU_698	0	10.58	0.41	9.06	1.27	58.21	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	unclassified	unclassified

OTU_258	13.92	3.16	0.4	1.2	1.23	59.43	Bacteria	Proteobacteria	Gammaproteobacteria	unclassified	unclassified	unclassified
OTU_356	11.17	1	0.4	5.43	1.23	60.66	Bacteria	Proteobacteria	Gammaproteobacteria	unclassified	unclassified	unclassified

Chapter 5

Bacterial diversity changes to simulated local and global stressors on the intertidal canopy-forming alga *Cystoseira compressa* (Esper) Gerloff & Nizamuddin.

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Abstract

In Mediterranean Sea canopy-forming seaweeds of the genus *Cystoseira* are retracting considerably to the point where several species have been reported as locally lost in many areas. The causes of this regression are often attributable to a compound of different environmental and anthropogenic stressors, which effects and interaction are not fully understood. We suggest that epiphytic bacteria can be involved in mechanisms behind the response of seaweeds. Then, we carried out a field manipulative experiment to investigate the effects of nutrient enrichment, typically a local anthropogenic stressor, and a heat wave event caused by the global climate change on intertidal *Cystoseira compressa* during low tide. We found that the abrupt increase of air temperature caused by a heat wave affected the photosynthetic activity of the intertidal *C. compressa*. The effect of the heat wave was still evident after 3 hours of recovery but after 24 hours *C. compressa* recovered its photosynthetic activity. We didn't observed significant effects caused by the nutrient enrichment or by the interaction of the two stressors. Moreover, we found that the heat wave altered the structure of the epiphytic bacteria of *C. compressa*. Thalli exposed to the heat wave presented an increase of OTUs assigned to genera *Ruegeria, Nautella, Aquimarina, Loktanella* whose have previously been shown with the natural degradation of the thalli of *C. compressa,* or implied in causing disease or damage to macroalgae. As observed for the photosynthetic activity, these differences were not maintained in across time suggesting that the microbial community has the ability to recover. Moreover, there were not differences

induced by the nutrients enrichment or by the interaction of the two stressors on the epibacterial community of *C. compressa.*

Our results support the idea that understanding how *C. compressa* and epiphytic bacteria interact and how the holobiont respond to stressors could play an important role in understanding and determining the persistence of these important canopy-forming seaweeds.

Keywords: Photosynthetic activity, maximum quantum yield, epiphytic bacteria communities, High throughput sequencing, 16S rRNA gene, canopy-forming seaweeds, Fucales, *Cystoseira compressa*, seawater.

Introduction

Strong declines of marine canopy-forming seaweeds in the Mediterranean Sea have been reported over the last decades (Benedetti-Cecchi et al., 2001; Thibaut et al., 2005, 2015; Airoldi and Beck, 2007; Connell et al., 2008; Mangialajo et al., 2008; Perkol-Finkel and Airoldi, 2010). The causes of this regression are often attributable to the interaction of multiple stressors (Airoldi, 2003; Halpern et al., 2007; Wahl M., 2011; Strain et al., 2014). Declines in of canopy-forming seaweeds cause serious concern because these vegetations enhance the structural complexity and productivity of coastal communities significantly, providing food and protection for rich associated communities, comprising other algae, invertebrates and fish (Dayton, 1985; Chapman, 1995; Mineur et al., 2015). Lost canopies tend to be replaced by less complex communities most often dominated by low-lying, turf-forming species (Benedetti-Cecchi et al., 2001; Perkol-Finkel and Airoldi, 2010; Smale and Wernberg, 2013; Connell et al., 2014) or sea urchin barrens (Feehan et al., 2012; Agnetta et al., 2015) causing a general homogenization of the habitat and a loss of important ecosystem services (Mineur et al., 2015). Development of management strategies to preserve and restore these habitats requires identification of these stressors as well as their interactions. A recent meta-analysis has shown that nutrient enrichment interacts synergistically with a variety of other stressors, such as heavy metals, presence of competitors, low light and increasing temperature, leading to amplified negative effects on canopy-forming algae (Strain et al., 2014). In a context of global climate change interactions between nutrient enrichment and increase of temperature is highly relevant. Improving water quality by reducing nutrient concentration would increase the resilience on both juvenile and adult thalli of *Cystoseira* exposed to elevated temperatures (Strain et al., 2015). The dynamics of these interactions are, however, not fully understood and it is difficult to explain the mechanisms behind the response of the seaweeds. A possible explanation could involve a role of associated bacteria. Epiphytic bacteria growing on seaweed surfaces create a tight relationship with their host, making them equivalent to a single entity, or a holobiont (Egan et al., 2013). Bacteria may interact with seaweeds in symbiotic, pathological and opportunistic ways, modulating the health, performance and resilience of their hosts. Biofilms can reduce the access of their hosts to light, gases, nutrients and modulate the interaction with other fouling epibionts, consumers and pathogens (Goecke et al., 2010; Wahl et al., 2012). In recent years, mass mortality events of different habitat-forming organisms such as corals, seagrasses and seaweeds have been increasingly associated with pathogenic microorganisms (Rosenberg and Ben-Haim, 2002; Goecke et al., 2010; Egan et al., 2013). Such events are supposed to increase with changes in the marine environment induced by anthropogenic stressors (pollutants, urbanization etc.) and exacerbated by the climate change (Harvell et al., 2002; Lafferty et al., 2004; Campbell et al., 2011). Within the Rhodobacteraceae and Flavobacteriaceae different clades contains many known or putative pathogens, such as Nautella italica *R11*, Phaeobacter gallaeciensis LSS9 or *Saprospirae* sp., that cause disease of a wide range of marine organisms such as sea urchin, corals, sponges, oysters and algae (Webster et al., 2002; Pantos et al., 2003; Maloy et al., 2007; Becker et al., 2009; Bengtsson et al., 2011; Case et al., 2011; Zozaya-Valdes et al., 2015). We have recently described the natural seasonal variation in epiphytic bacteria of *C. compressa* over a growing season (chapter 4; Mancuso et al., 2016). Proteobacteria, Bacteroidetes, Verrucomicrobia, Actinobacteria and Cyanobacteria were identified as the dominant phyla associated with *C. compressa*. The results show also an increase in abundance over time of *Rhodobacteraceae* and in particular of six potentially pathogenic genera, *Ruegeria*, *Nautella*, *Aquimarina*, *Loktanella*, *Saprospira* and *Phaeobacter* correlated with aging of annual axes over the growing season.

Aimed to analyze the possible mechanisms by which *C. compressa* and their associated bacteria interact each other to external stressors. We carried out a field manipulative experiment in order to investigate the interaction of nutrient enrichment, typically a local anthropogenic stressor, and a heat wave event caused by the global climate change. We focused on the intertidal species *C. compressa* from the central Adriatic Sea. This region suffered from extensive losses of *Cystoseira* species, including *C. compressa*, in recent years.

The decline in *Cystoseira*-dominated vegetations has been attributed to interacting effects of local and global stressors (Perkol-Finkel and Airoldi, 2010; Strain et al., 2015).

We hypothesize that nutrient enrichment and extreme air temperature caused by a heat wave event can cause a decline of the photosynthetic activates of *C. compressa*. As a consequence we expect to observe an increase in the abundance of that bacteria usually observed with the natural degradation of the seaweed.

Material and methods

Study area and species

The experiment was conducted on *Cystoseira compressa* and associated microbial communities in the intertidal rocky shore at Punta Aderci promontory, Vasto, Italy (42°10'50.3" N, 14°41'15.0" E) in the central Adriatic Sea (Figure 1 A). This promontory, situated in the central sector of the Abruzzo coast, is characterized by clay–sand–conglomerate lithotypes (Miccadei et al., 2011), moderate exposure to wave action and an average tidal amplitude of ≈ 30 cm (Figure S1). Seawater temperature range from a minimum of 8 °C in winter to 27.5 °C in summer (Figure S2 A). Air temperature follows seasonal variation with the lowest mean values in winter of 6.5 °C and highest mean values in summer of 28°C (Figure S2 B). Extreme events, may push temperature well beyond 40°C as witnessed during heat waves in June and July 2005 (Figure S2 B). The underwater rocky substrate at the Punta Aderci promontory is dominated by patches of mussels (*Mytilus galloprovincialis)*, ephemeral algae (*Ulva rigida)* and perennial stands of *C. compressa*, the only canopy-forming alga in this habitat.

Experimental design

To test the interaction effect between nutrient enrichment and heat wave events on the intertidal *Cystoseira compressa* vegetations and associated epiphytic bacteria we conducted a 6 months field experiment (May – October 2014). Therefore, we randomly selected 32 thalli of *C. compressa*, ~50 cm apart, and assigned them either to control or nutrient enriched treatments (n = 16). We manipulated nutrients by the addition of 300 g of coated fertilizer (Osmocote Exact, 5-6 months release; N15-P9-K12). Nutrients were supplied in nylon mesh bags (1 mm mesh size) attached close to the thalli of *C. compressa* (5 cm). Mesh bags were replaced every 30 days providing a continuous nutrient supply of the treatment. To test the effectiveness of the nutrient manipulation we analyzed the nutrient content of the algal tissue for each treatment (n = 5). Carbon (C) and nitrogen (N) content were assessed with a ThermoFisher organic elemental analyzer (Flash 2000). The effect of nutrients (2 fixed levels = ambient and increased) on the C:N rate was tested using a 1-way ANOVA.

After two months of nutrient manipulation we simulated a heat wave event during low tide (emersion phase). Before the low tide the 16 thalli (8 controls and 8 nutrient enriched) of *C. compressa* were collected and placed in a plastic box containing natural seawater and placed in proximity of the site. Then for each treatment we randomly selected 4 thalli that we subjected to the artificial heat wave. The heat wave treatment was applied simulating the maximum air temperature (42 °C) recorded in summer 2005 from the Ortona ISPRA station (Figure S2). The air exposure time was 3 hours, the maximum air temperature were reached after 1 hour and constantly maintained for an additional 2 hours. To do so we used controlled-temperature units constituted of a Plexiglas boxes (25 x 25 cm) in which four electrical heat traces were mounted (Figure 1 B-C). Power was supplied by a portable electric generator. After the heat wave simulation the thalli were re-attached to the site using epoxy putty (Subcoat S, Veneziani). The air temperature was monitored by digital thermometer with external probe (±0.1 °C).

Photosynthetic activity of **C. compressa**

During the experiment in vivo chlorophyll-a fluorescence of PSII of *C. compressa* was measured using an underwater pulse amplitude modulated fluorometer, (DIVING PAM, Walz, Germany). We measured maximum quantum yield of fluorescence (Fv/Fm), an indicator of quantum efficiency (Maxwell and Johnson, 2000), on 30 min dark-adapted samples. The effects of the nutrient concentration (2 fixed levels = ambient and increased) and temperature (2 levels = ambient and increased) on the photosynthetic stress of *C. compressa* were tested using 2-way ANOVAs. For simplifying the analysis, three separate 2-way ANOVAs were performed for the end of air exposure, after 3h and 24h of recovery (Figure 1 D).

Sampling and characterization of the bacterial community

Epiphytic bacteria on *C. compressa* were sampled over the course of the entire experiment. From each treatment we collected three replicates of the microbial biofilm by rubbing approximately 12 cm^2 of surface of *C. compressa* using sterile cotton swabs on wooden sticks (Aptaca). Swabs were immediately transferred to sterile 1.5 ml Eppendorf tubes. Thalli overgrown with epiphytic seaweeds or animals were avoided. Samples were transported on ice to the lab and stored at -80 °C until DNA extraction.

Microbial DNA extraction and bioinformatic analysis were performed as described in Mancuso et al (2016) with follow modification. Before the analyses we filtered OTUs for which the variance across all samples was very low using an arbitrary variance threshold of 1000. Microbial community data were compared between nutrient conditions and presence of absence of heat wave using PERMANOVA (Anderson, 2001) in the PERMANOVA+ add-on for PRIMER v6 (Anderson et al., 2008). The analysis was based on Bray–Curtis distance of square root-transformed OTUs abundance. P-values were calculated using 9999 random permutations and unrestricted permutation of raw data and Type III sums of squares. To visualize multivariate patterns in microbial assemblages we used non-metric Multi Dimensional Scaling (nMDS) based on Bray-Curtis distance matrix calculated from the square-root transformed OTU abundance data. SIMPER analysis (Clarke, 1993) was performed to identify those OTUs that most contributed to the differences between the experimental treatments. Cut-off value was restricted to 50%.

Results

Photosynthetic performance of C. compressa

Algal tissue nutrients analysis revealed that the C:N ratio of *C. compressa* was significantly lower in the high compared to low nutrient treatments (**Nu**: $F_{(df=\ 1,8)}= 8.792$, $P<0.01$, Table 1A, Figure S3 A). Differences in the nutrient treatment was also visible on the photosynthetic activity of *C. compressa*, where thalli exposed to high nutrients showed significantly higher Fv/Fm values compared to natural conditions (**Nu**: $F_{(df=\ 1,8)}= 21.43$, $P<0.05$, Table 1B, Figure S3 B). However, this difference was not significant before the heat wave simulation, although mean Fv/Fm value was higher in thalli exposed to high nutrients (**Nu**: $F_{(df=1,4)}$= 1.918, P>0.05, Table 1C, Figure S3 C).

At the end of the heat wave, a significant decline in the photosynthetic activity for both nutrient conditions was observed (**Hw**: $F_{(df=1,28)}$= 46.2, P<0.05, Figure 2 A, Table 2), while no differences were detected for nutrients treatment (**Nu**: $F_{(df=1,28)}$= 0.07, P>0.05, Figure 2 A, Table 2). Three hours after the heat wave simulation, thalli did not recover as can be judged from the lower Fv/Fm values (**Hw**: $F_{(df=1,28)}$= 84.994; P<0.001, Figure 2 B, Table 1), while still no differences were detected for nutrients treatment (**Nu**: $F_{(df=1,28)}$= 0.098; P>0.05, Figure 2 B, Table 2). After a 24h of recovery phase, there were no detectable effects of the heat wave or of nutrients on maximum photosynthetic yield (**Hw**: $F_{(df=1,28)}$= 3.692, P>0.05; **Nu**: $F_{(df=1,28)}$= 0.202, P>0.05, Figure 2 C, Table 2), indicating that a full recovery of *C. compressa* was achieved. During all experiment no significant interactions were found between nutrients and heat wave treatments (Table 2 A-B-C, Figure 3 A-B), although interaction plots showed a beginning of antagonistic interaction after 24 hours of recovery (Figure 3 C).

Epiphytic bacteria

Targeting the hypervariable V1-V3 region of the 16S rRNA, a total of 29,986,579 paired-end raw reads were obtained using the Illumina Miseq v.3 platform. After quality filtering and discarding singletons, chimeras and chloroplast and mitochondrial sequences, our dataset contained 1,922,395 sequences with an average length of 483 ± 5 bp. The average number of reads was 33,145 per library (SD = 19,225; min= 3,401; max= 84,872) while the total OTU richness was 3,074 at the 97% OTU definition (Table S1). Classification of OTUs against the Greengenes database resulted in 26.8% and 3.5% of the OTUs being classified at family and genus levels, respectively, while were maximum with higher taxonomic levels (Figure S4). Rarefaction curves showed saturation for most of the samples, indicative of a good coverage of diversity (Figure 4).

The results of PERMANOVA analysis showed that before the heat wave simulation there were no differences between the epibacterial communities of thalli of *C. compressa* exposed to high or low nutrients (**Nu**, pseudo-$F_{(df=1,4)}$= 1.3585, P>0.05, Table 3 A). At the end of the heat wave the microbial community structure differed between *C. compressa* exposed or not to the heat wave treatment (**Hw**, pseudo-$F_{(df=1,6)}$= 3.7568, P<0.05, Table 3 B, Figure 5), but not between nutrient conditions or the interaction between the two stressors (**Nu**, pseudo-$F_{(df=1,6)}$= 1.6136, P>0.05; **NuxHw**, pseudo-$F_{(df=1,6)}$= 1.5799, P>0.05, Table 3 C). After the heat wave simulation there were no detectable effects of the heat wave or of nutrients indicating a recovery of the epiphytic bacteria of *C. compressa* (**Hw**: $F_{(df=1,6)}$= 1.001, P>0.05; **Nu**: $F_{(df=1,6)}$= 1.095, P>0.05, **NuxHw**, pseudo-$F_{(df=1,6)}$= 1.0837, P>0.05, Figure 2 C, Table 2).

A SIMPER analysis selected 74 OTUs contributing to 50% of differences in the bacterial community between thalli of *C. compressa* exposed or not to the heat wave. The OTUs that were more abundant in thalli exposed to the heat wave belonged at the family-level mostly to the taxa Rhodobacteraceae and Flavobacteriaceae (Figure 6 Table S2). In particular, the genera *Loktanella*, *Nautella*, *Pseudoruegeria* and *Ruegeria* of the Rhodobacteraceae family and the genera *Aquimarina*, *Krokinobacter*, *Winogradskyella* and *Tenacibaculum* of Flavobacteriaceae family increased in abundance with thalli of *C. compressa* exposed to the heat wave compared to that left at ambient temperature (Figure 7-8).

Discussion

We investigate for the first time the effects of nutrient enrichment and an extreme event (air heat wave) caused by the global climate-change on the intertidal *C. compressa* and its associated epiphytic bacteria. We found that the heat wave caused a clear decline of the photosynthetic activity of the intertidal *C. compressa*. The effect of the heat wave was still evident after 3 hours of recovery, while, after 24 hours *C. compressa* recovered its photosynthetic activity. Despite high levels of nutrients reflected a slightly increase on the photosynthetic of *C. compressa*. We didn't observed significant effects caused by the nutrient enrichment. This could be due to the possible lack of efficacy of nutrient manipulation before the heat wave simulation, which could also explain why we didn't found an interaction effect of the two stressors as already observed by Strain et al. (2014). As regards the epiphytic bacteria of *C. compressa*, we found significant differences in the bacterial community structures between thalli exposed to the heat wave compared to that left at ambient temperature. Thalli exposed to the heat wave presented an increase of OTUs assigned to genera *Ruegeria, Nautella, Aquimarina, Loktanella* whose have previously been shown with the natural degradation of the thalli of *C. compressa* (Mancuso et al., 2016), or implied in causing disease or damage to macroalgae (Case et al., 2011). However, these differences were not maintained in across time suggesting, as observed for the photosynthetic activity, that the microbial community has the ability to recover. Moreover, there were not differences induced by the nutrients enrichment or by the interaction of the two stressors on the epibacterial community of *C. compressa*. These lacks of effects could be attributed to the same reasons explained above.

Microbial community can change over multiple timescales and in response to different biological and non-biological forces. The response of bacteria to changing condition can occur rapidly in terms of hours or between day and night, depending on their photosynthetic characteristics, interaction with predators, or response to daytime production. Unpredictable short-term environmental stressors such as storm, heat wave could play an important role on the bacteria dynamic alternating not only the structure of the microbial community but also processes such as competition, grazing, sloppy feeding, viral infection and cross feeding (Fuhrman et al., 2015). Our results confirm that an extreme event of heat wave can alter in few hours the structure of the epiphytic bacteria of *C. compressa*. Despite these variations are likely to be caused by the heat shock they should also backed up by a decrease of the physiological condition of *C. compressa*. Therefore our results could confirm the evidence that host traits are important in influencing the structure of host-associated microbial communities (Marzinelli et al., 2015). Algal defense mechanisms, such as furanones, can change in relation to the photosynthetic condition of seaweeds, which in turn changes over time. Seasonal variations of *C. compressa* associated bacterial community were already observed. When *C. compressa* is in quiescent status there was an increase in abundances of Rhodobacteraceae and in particular of different pathogens genera such as *Ruegeria*, *Nautella*, *Aquimarina*, *Loktanella*, *Saprospira* and *Phaeobacter* (Mancuso et al., 2016). Our result showed that the abrupt increase of temperature caused by and heat wave event facilitate the growth of these deleterious bacteria that could accelerate the process of degradation of *C. compressa.*

Despite in this study was not observed an interaction between stressors. In July 2015 we repeated the experiment on the same population and consistent with what already observed by (Strain et al., 2014) we found a synergistic negative effect between nutrient enrichment and air heat wave on the photosynthetic activity of *C. compressa* (data not showed). Processing data of the epiphytic bacteria are still ongoing and the work will be complete in 2016. This evidence brings to hypothesize that the type of interaction between stressors could be time depending.

Our results support the idea that understanding how *C. compressa* and epiphytic bacteria interact and how the holobiont respond to stressors could play an important role in understanding and determining the persistence of these important canopy-forming seaweeds. Future, experiment should be design taking into account also the response of algal defense (e.g. release of furanones). Moreover, because there are evidences that climate-change will increase the odds for more extreme weather events such as hurricanes, heavier rainfalls and flooding, increased conditions for wildfires and dangerous heat waves (Hegerl et al., 2011; Coumou and Rahmstorf, 2012). Experimental designs should be taken into account the effect of repeated extreme events and the temporal variability of their effects.

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Tables and figures

Table 1 ANOVA results showing the effects of nutrients treatment (low vs. high) into the tissue (A) and on photosynthetic stress (Fv/Fm) of *C. compressa*, one moth (B) and just before (C) the heat wave simulation.

(A)		**Algal tissue nutrients C:N**				**(B)**			**Fv/Fm 07-14**		
	Df	Sum Sq	Mean Sq	F value	Pr(>F)		Df	Sum Sq	Mean Sq	F value	Pr(>F)
Nu	1	40.72	40.72	8.792	0.018**	Nu	1	0.001456	0.001456	21.43	0.00169***
Residuals	8	37.05	4.63			Residuals	8	0.0005434	0.0000679		
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(C)		**Fv/Fm before experiment**									
	Df	Sum Sq	Mean Sq	F value	Pr(>F)						
Nu	1	0.00452	0.004523	1.918	0.188						
Residuals	14	0.03302	0.002358								

Signif. codes: *p<0.1; **p<0.05; ***p<0.01											

Table 2 ANOVA results of photosynthetic stress (Fv/Fm) in response to the heat wave (Hw) and increased of nutrients (Nu), at the end of air exposure (hw), after 3 hours (3h.rc) and 24 hours (24h.rc) of recovery.

		hw				**3h.rc**				**24h.rc**			
	D	**Sum**	**Mean**	**F**	**Pr(>F**	**Sum**	**Mean**	**F**	**Pr(>F**	**Sum**	**Mean**	**F**	**Pr(>F**
Nu	1	0.001	0.001	0.070	0.793	0.001	0.001	0.098	0.756	0.001	0.001	0.202	0.657
Hw	1	0.322	0.322	46.200	**0.000**	0.476	0.476	84.994	**0.000**	0.015	0.015	3.692	0.065
Nu:Hw	1	0.000	0.000	0.050	0.825	0.016	0.016	2.865	0.102	0.002	0.002	0.620	0.438
Residual	28	0.195	0.007			0.1567	0.0056			0.1114	0.00398		

Signif. codes: 0'***' 0.001'**' 0.01'*' 0.05'.' 0.1' ' 1													

Table 3 Results of PERMANOVA analysis, testing for significant differences in bacterial composition as a function of nutrients (Nu) and heat wave (Hw) treatments before (A) at the end (B) and after the heat wave simulation (C).

Factors			
Name	Abbrev.	Type	Levels
Heat wave	Hw	Fixed	2
Nutrients	Nu	Fixed	2

(A) Before experiment

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Nu	1	1901.7	1901.7	1.3585	0.3053	10
Res	4	5599.4	1399.8			
Total	5	7501.1				

(B) At the of heat wave simulation

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Hw	1	3647.3	3647.3	3.7568	**0.0043**	5789
Nu	1	1566.6	1566.6	1.6136	0.1949	5813
HwxNu	1	1533.8	1533.8	1.5799	0.1977	5816
Res	6	5825.1	970.86			
Total	9	11597				

(C) Three weeks after heat wave simulation

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Hw	1	994.5	994.5	1.0017	0.4672	8840
Nu	1	1087.7	1087.7	1.0956	0.3195	8896
HwxNu	1	1075.9	1075.9	1.0837	0.3399	8860
Res	8	7942.6	992.82			
Total	11	11101				

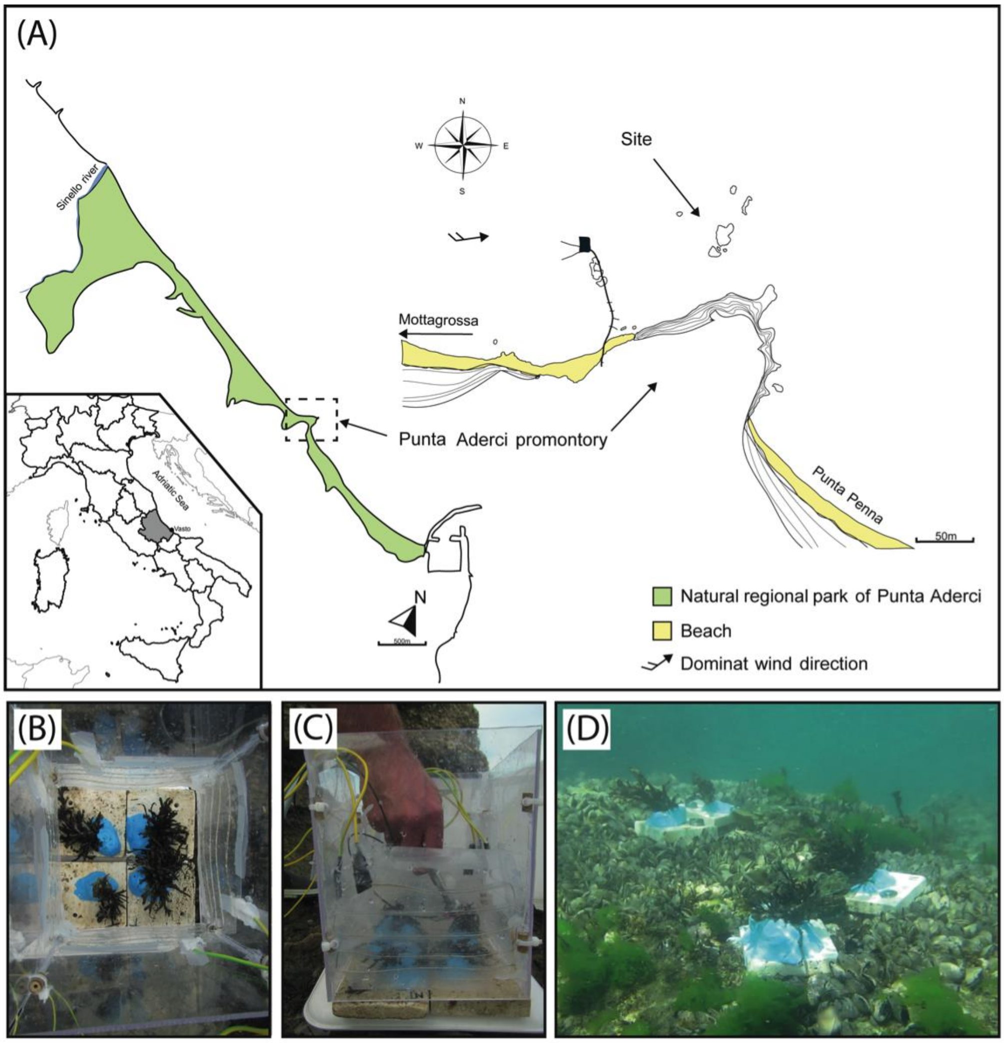

Figure 1 Main physiographic characteristics of the coastal area and experimental site of the Punta Aderci promontory (A). Upper (B) and lateral (C) views of the controlled-temperature unit used to simulate the heat wave. Thalli of *C. compressa* were replaced into the field to follow the recovery (D).

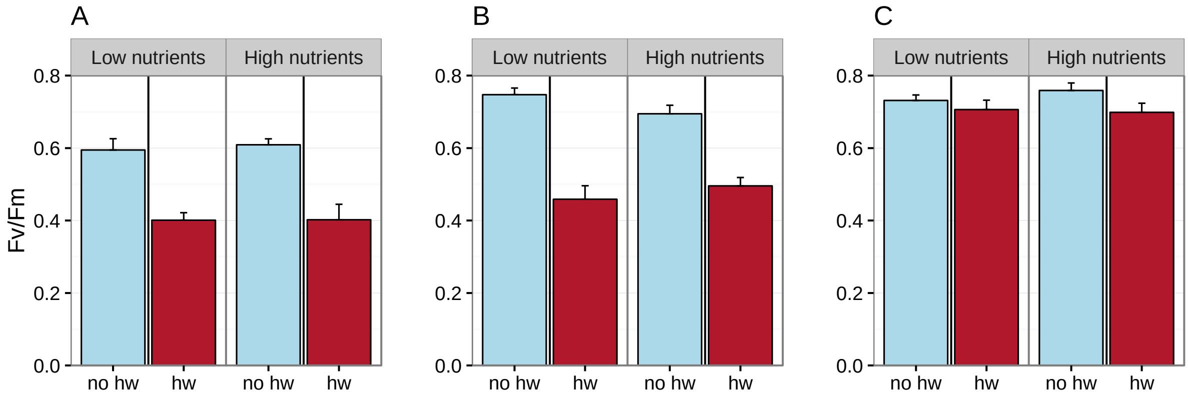

Figure 2 Photosynthetic stress (Fv/Fm) in response to different nutrient levels (low vs. high) and absence (no hw) or presence (hw) of the heat wave event at the end of the experiment (A) and after short (B) and long recovery (C). Data are average values (n= 4, ± 1 SE).

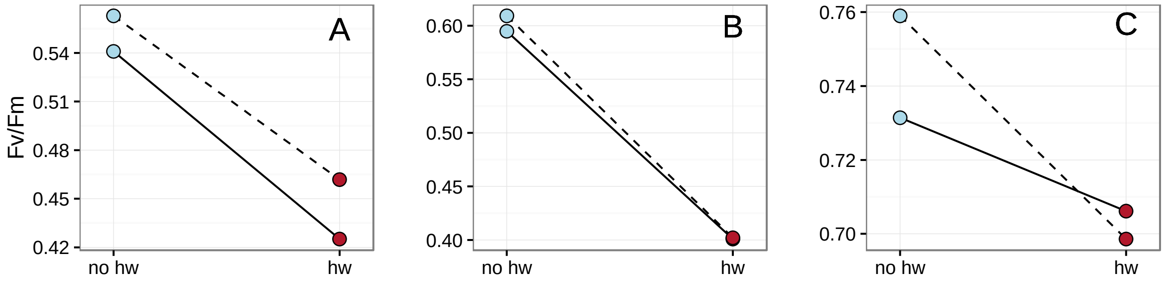

Figure 3 Interaction plots of photosynthetic stress (Fv/Fm) in response to low (solid lines) and high (dash lines) nutrient levels and absence (no hw) or presence (hw) of the heat wave event at the end of the experiment (A) and after short (B) and long recovery (C). Data are average values.

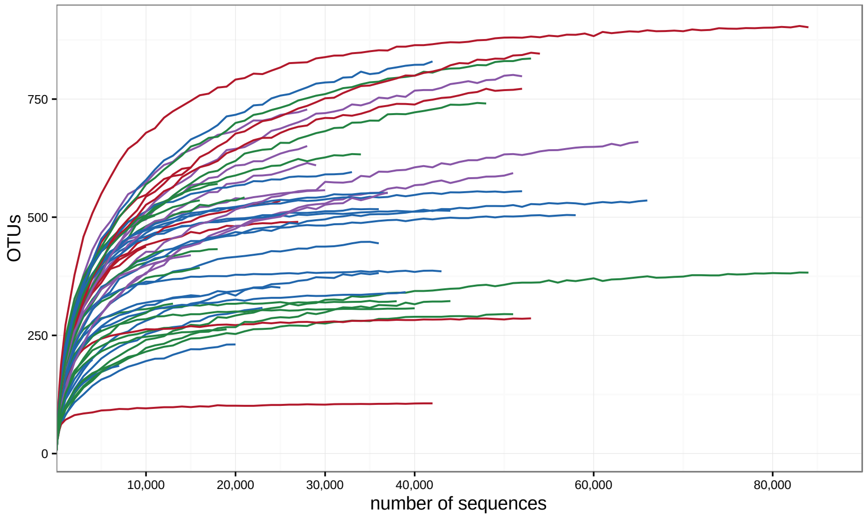

Figure 4 Rarefaction curves generated for each sample of *C. compressa*. **Blue lines**=Low nutrients and no heat wave; **green lines**= high nutrients and no heat wave; **red lines**= low nutrients and heat wave; **violet lines**= high nutrients and heat wave.

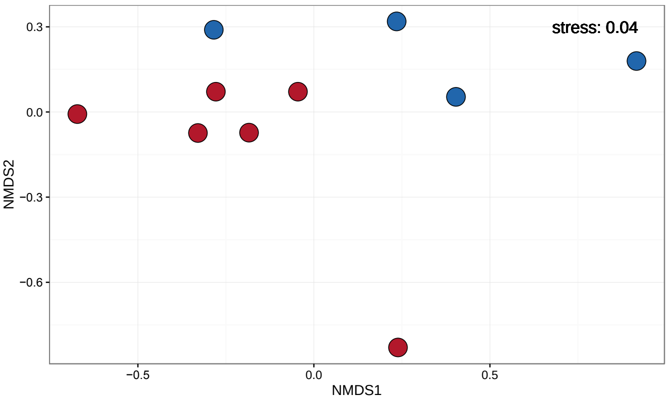

Figure 5 Non-metric Multi Dimensional Scaling plot (nMDS) of the epiphytic bacteria of *C. compressa* exposed (red points) or not (blue points) to the heat wave. nMDS based on a Bray-Curtis distance matrix calculated from square-root transformed OTU abundance data.

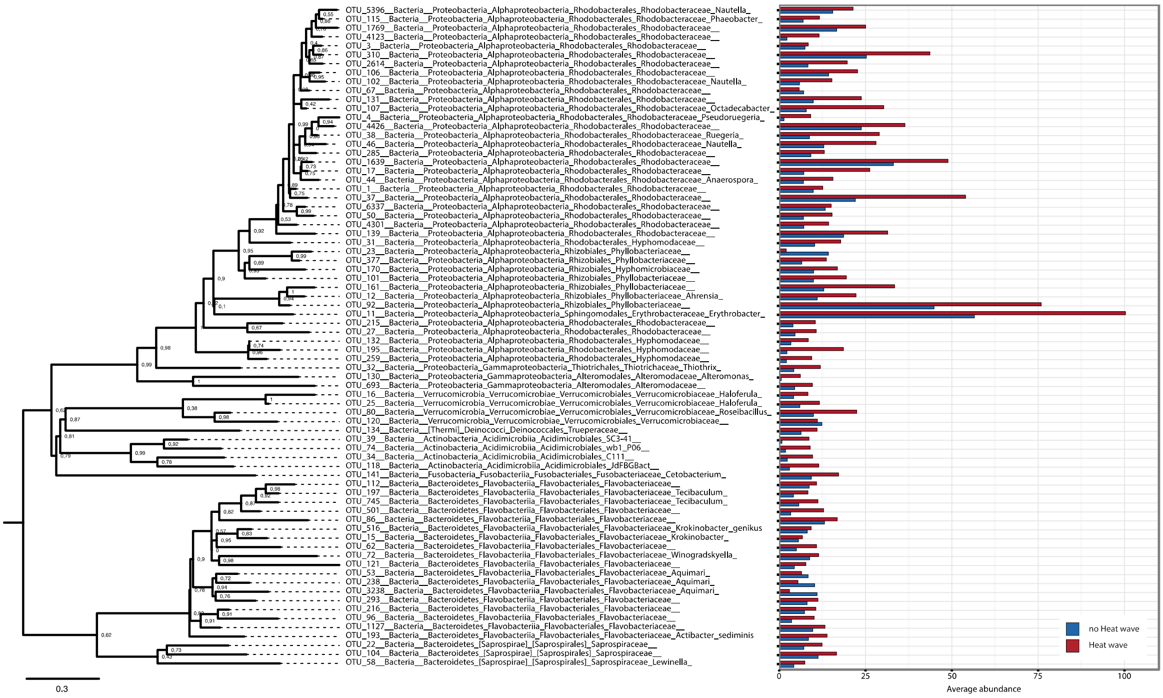

Figure 6 Phylogenetic tree of the 74 OTUs contributed to the 50% of the dissimilarity in bacterial community structure of natural thalli *C. compressa* versus thalli exposed to the heat wave. Mean abundance (square root-transformed) on natural (blue bars) and stressed (grey bars) *C. compressa* is shown next to each OTU.

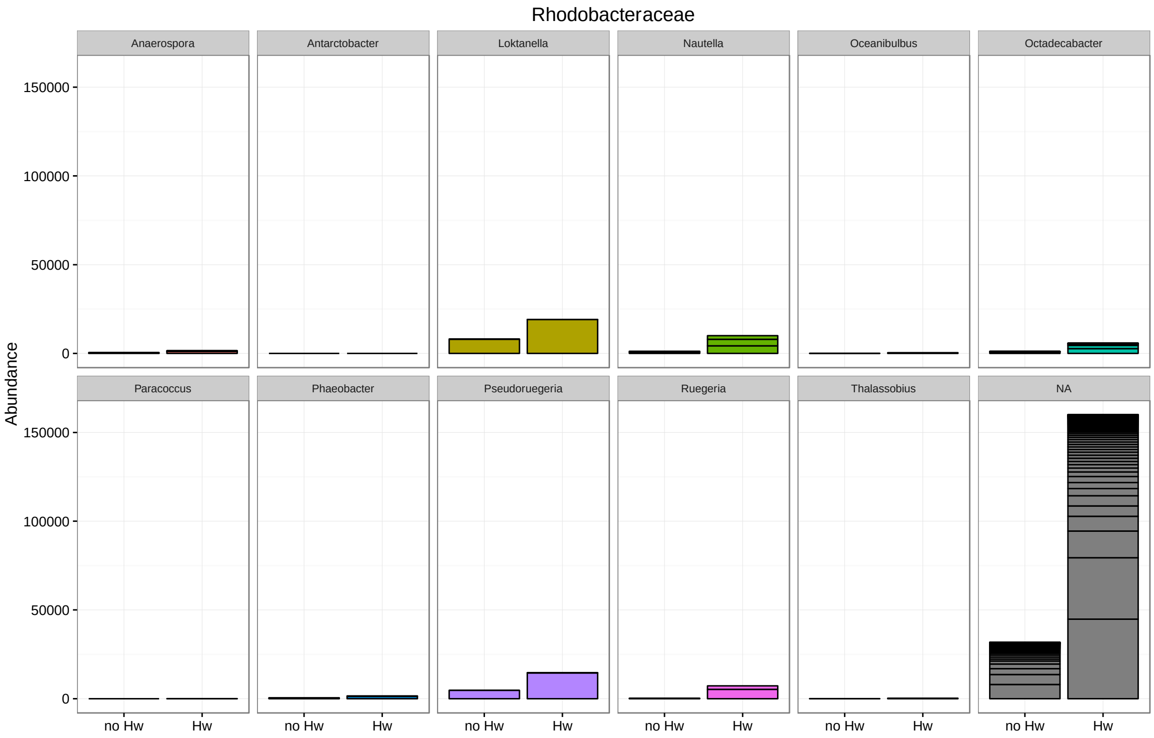

Figure 7 Difference in abundances of genera belonging to the Rhodobacteraceae family caused to the heat wave. **no Hw**= ambient temperature; **Hw**= heat wave.

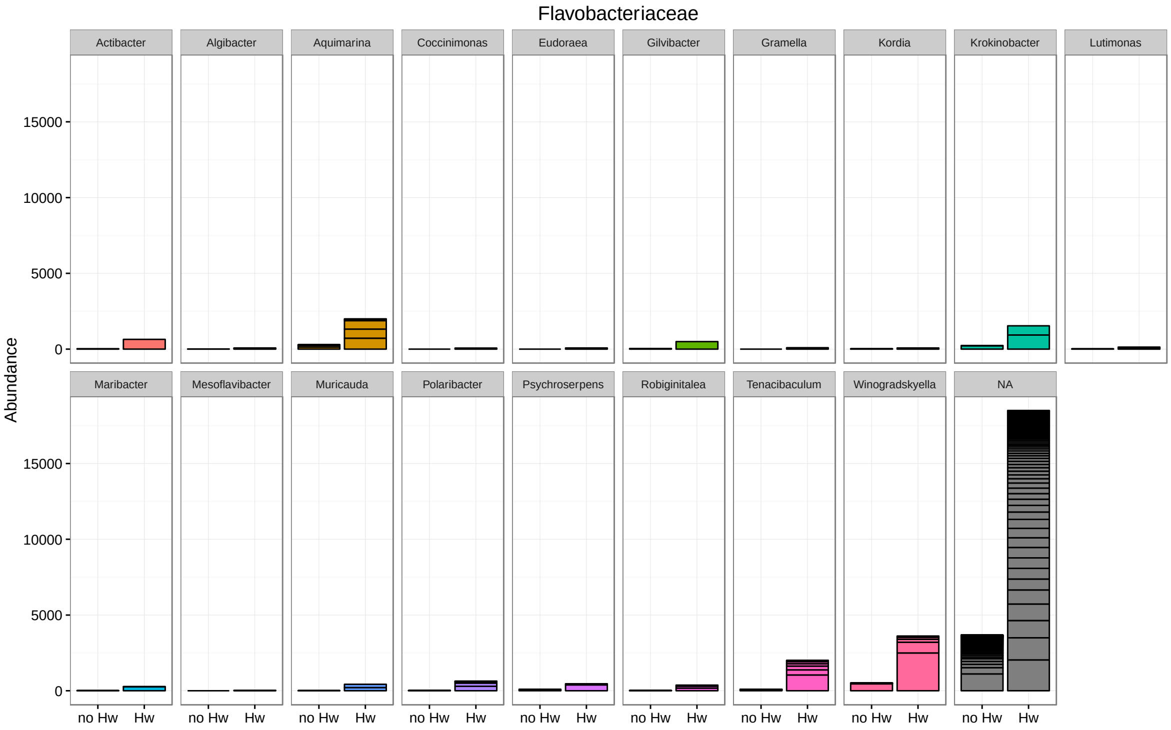

Figure 8 Difference in abundances of genera belonging to the Flavobacteriaceae family caused to the heat wave. **no Hw**= ambient temperature; **Hw**= heat wave.

Supplementary materials

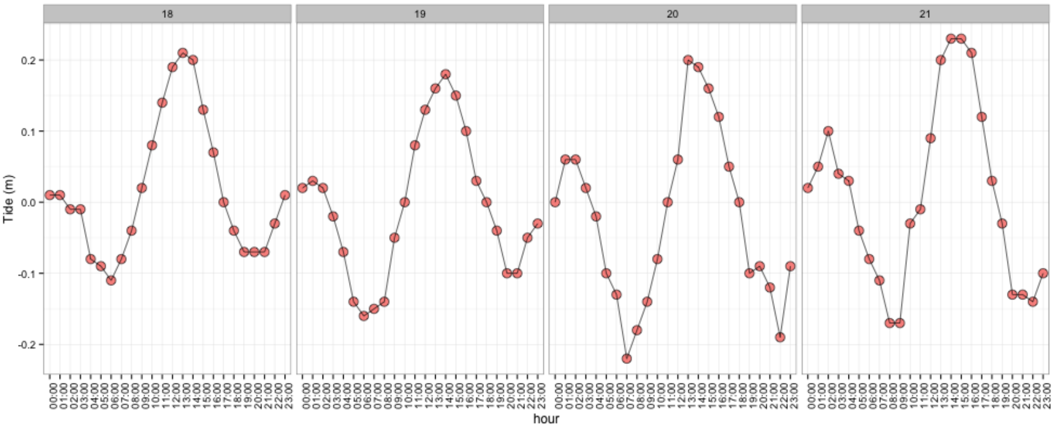

Figure S1 Example of tide excursion from measured at a buoy of Ortona in August 2014. In 24h there is a presence of two low tide exposing the *Cystoseira compressa* stands. Data was obtained from ISPRA ("Istituto Superiore per la Protezione e Ricerca Ambientale").

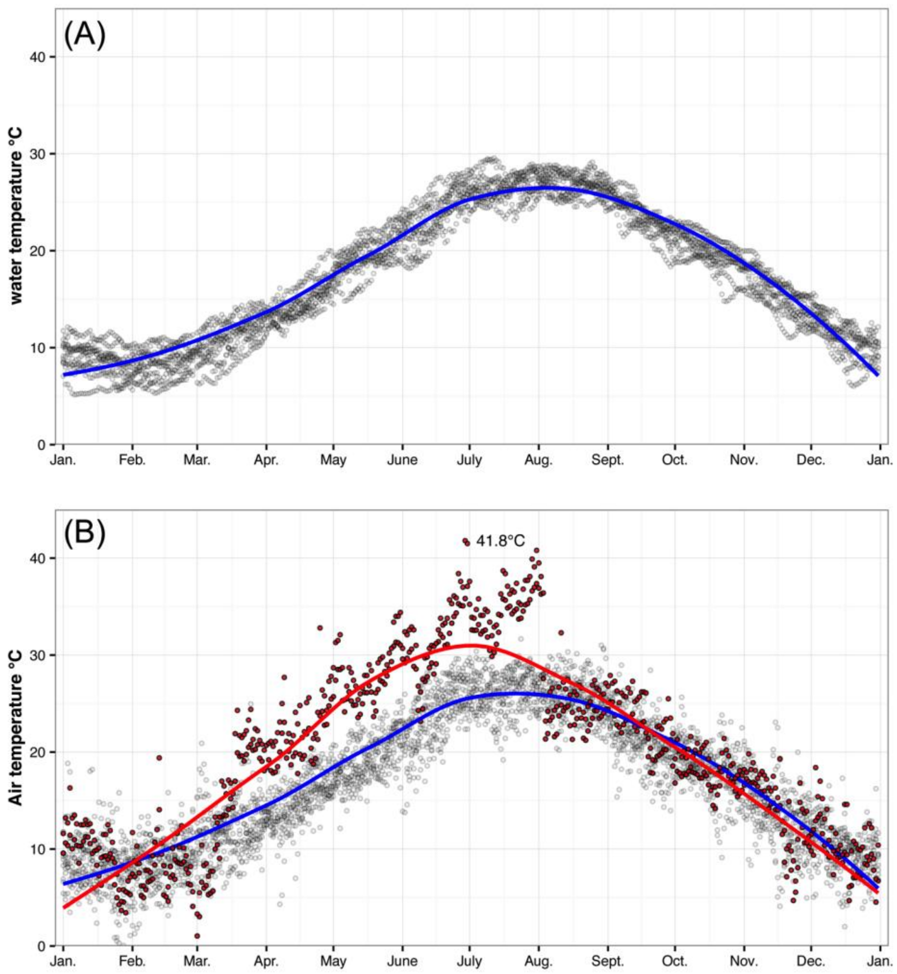

Figure S2 Monthly variability of water (A) and air (B) temperature measured from Ortona buoy between 2000 and 2013. Grey points are average daily temperature of each year. Blue lines are the average temperature oscillation of across year. Red points and line highlights maximum and average air temperature of the temperature anomaly occurred in 2005 respectively. Data obtained from ISPRA ("Istituto Superiore per la Protezione e Ricerca Ambientale"), values from 2009, 2010 and 2011 were excluded due to the lack of information.

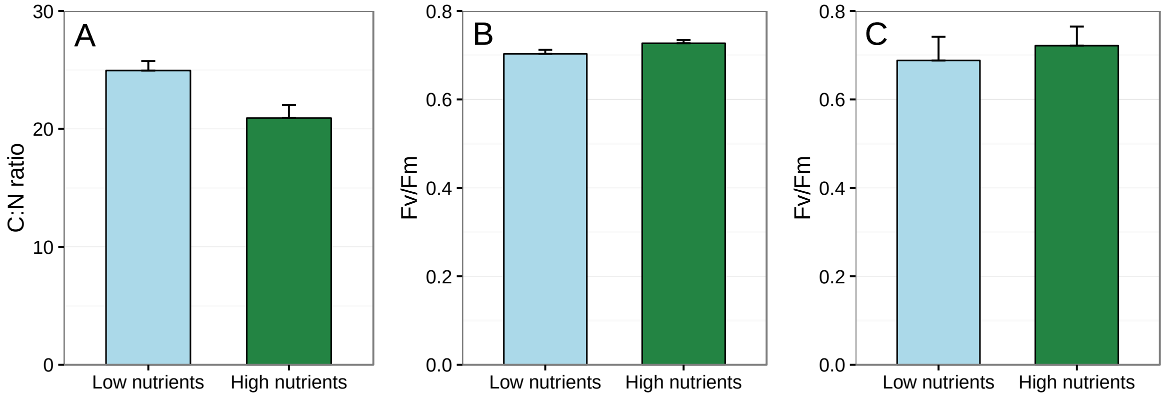

Figure S3 Effects of nutrients treatment (low vs. high) into the tissue (A) and on photosynthetic stress (Fv/Fm) of *C. compressa*, one moth (B) and just before (C) the heat wave simulation. Data are average values (n= 5-8, ± 1 SE).

Table S1 Summary statistics of the number of reads of *C. compressa*: number of samples, taxa, total reads, mean, minimum, maximum and standard deviation of number of reads.

	n°samples	**taxa**	**Total seqs.**	**mean**	**min**	**max**	**sd**
C.compressa	58	3,074	1,922,395	33,145	3,401	84,872	19,255

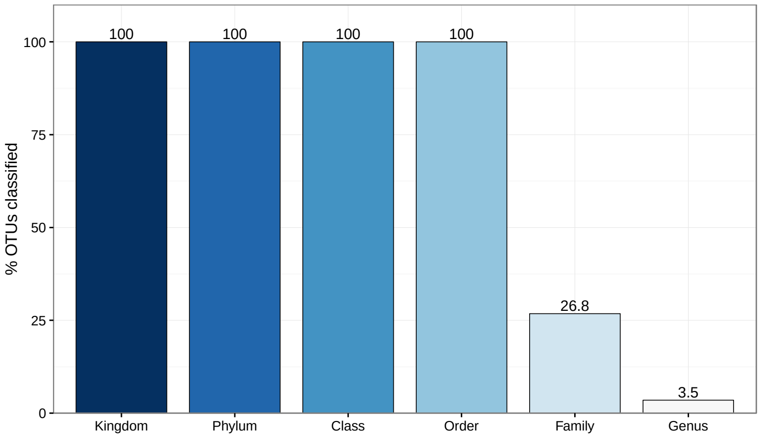

Figure S4 Total percentage of OTUs classified at each taxonomic level.

Table S2 Result of the SIMPER analysis. 74 OTUs contributed to the difference between thalli of *C. compressa* exposed or not to the heat wave.

Groups - Hw & + Hw

Average dissimilarity = 52.54

	Group - Hw	**Group + Hw**				
OTUs	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
OTU_2614	56.53	100.29	1.58	1.3	3.01	3.01
OTU_3	44.87	75.89	1.19	0.94	2.27	5.28
OTU_17	22.08	53.99	0.95	1.6	1.8	7.08
OTU_37	23.78	36.44	0.74	2.22	1.42	8.5
OTU_50	7.83	30.29	0.74	1.5	1.41	9.9
OTU_1	8.78	28.98	0.68	1.01	1.3	11.2
OTU_130	2.27	18.64	0.6	0.81	1.15	12.35
OTU_11	25.27	43.65	0.59	1.2	1.13	13.48
OTU_5396	12.95	33.45	0.58	1.86	1.11	14.59
OTU_38	7.16	26.24	0.57	1.36	1.09	15.67
OTU_4	33.08	48.88	0.56	1.54	1.06	16.74
OTU_58	13.11	16.82	0.47	1.95	0.9	17.63
OTU_67	18.68	31.46	0.46	1.84	0.88	18.51
OTU_46	12.92	28.05	0.45	1.32	0.85	19.36
OTU_310	11.02	22.26	0.43	2.39	0.81	20.18
OTU_31	9.91	23.81	0.41	1.71	0.79	20.97
OTU_23	14.33	22.71	0.41	1.74	0.78	21.75
OTU_27	15.55	21.41	0.4	1.17	0.76	22.51
OTU_72	9.93	22.43	0.39	1.77	0.75	23.26
OTU_39	9.76	13.27	0.33	1.32	0.63	23.89
OTU_216	3.33	12.9	0.33	0.78	0.63	24.52
OTU_377	8.35	19.7	0.33	1.56	0.62	25.14
OTU_106	14.28	2.03	0.32	0.7	0.61	25.75
OTU_132	11.27	16.6	0.32	1.36	0.61	26.36
OTU_115	9.91	19.44	0.32	2.15	0.6	26.97
OTU_107	7.02	15.33	0.32	2.26	0.6	27.57
OTU_12	16.67	25.04	0.31	1.24	0.59	28.16
OTU_285	7.05	15.59	0.31	2.12	0.59	28.75
OTU_293	4.27	11.93	0.31	0.79	0.59	29.34
OTU_195	7.16	12.43	0.3	1.2	0.58	29.92
OTU_131	7.14	14.35	0.3	1.76	0.58	30.5
OTU_1639	13.33	15.07	0.3	1.69	0.57	31.06
OTU_102	10.28	17.81	0.3	1.17	0.57	31.63
OTU_1769	9.98	16.89	0.29	1.1	0.56	32.19
OTU_62	12.38	11.09	0.29	1.37	0.54	32.73
OTU_34	7.33	10.64	0.28	1.34	0.54	33.27
OTU_170	5.89	15.26	0.28	1.17	0.53	33.8
OTU_112	9.36	17.23	0.27	1.23	0.52	34.32

OTU_693	3.38	8.44	0.27	0.63	0.52	34.83
OTU_501	1.84	8.98	0.27	1.07	0.51	35.34
OTU_197	3.01	11.5	0.26	2.16	0.5	35.84
OTU_4426	9.17	13.08	0.26	1.59	0.5	36.34
OTU_161	2.26	11.6	0.26	1.35	0.5	36.84
OTU_6337	1.4	9.14	0.26	0.88	0.5	37.34
OTU_4123	6.56	13.66	0.26	2.61	0.49	37.83
OTU_118	8.11	11.21	0.26	1.43	0.49	38.32
OTU_139	4.62	10.73	0.26	1.12	0.49	38.81
OTU_25	10.27	5.47	0.26	1.4	0.49	39.3
OTU_104	8.12	9.3	0.25	1.67	0.47	39.77
OTU_134	8.86	11.41	0.25	1.41	0.47	40.24
OTU_16	11.01	3.02	0.25	1.31	0.47	40.71
OTU_32	8.47	13.89	0.24	1.34	0.46	41.16
OTU_4301	3.98	10.5	0.23	1.42	0.44	41.61
OTU_96	5.68	11.26	0.23	1.36	0.44	42.05
OTU_22	5.58	6.72	0.23	0.88	0.44	42.49
OTU_516	0.79	8.64	0.23	1.5	0.43	42.92
OTU_44	9.89	12.62	0.22	2.02	0.43	43.35
OTU_193	2.08	9.44	0.22	1.71	0.42	43.78
OTU_238	4.49	9.66	0.22	1.2	0.42	44.2
OTU_121	5.98	11.68	0.22	1.72	0.41	44.61
OTU_3238	0.6	6.13	0.22	0.51	0.41	45.02
OTU_15	6.37	10.97	0.21	1.01	0.41	45.43
OTU_745	2.36	9.69	0.21	1.72	0.41	45.84
OTU_74	3.61	10.13	0.21	1.84	0.4	46.24
OTU_80	8.4	6.5	0.21	1.19	0.4	46.63
OTU_86	8.67	10.81	0.21	1	0.39	47.03
OTU_92	7.5	8.46	0.21	1.38	0.39	47.42
OTU_141	4.97	10.82	0.2	1.42	0.39	47.81
OTU_215	6.94	11.7	0.2	1.57	0.39	48.19
OTU_120	4.39	7.72	0.2	0.78	0.39	48.58
OTU_53	4.19	8.34	0.2	0.79	0.38	48.96
OTU_1127	4.15	8.35	0.2	1.2	0.38	49.34
OTU_259	4.27	7.44	0.2	0.96	0.38	49.72
OTU_101	7.11	5.8	0.2	1.07	0.37	50.09

General conclusions

Canopy-forming seaweeds of retracting due to the combined effects of multiple pressures both from human activities and climate instabilities (Steneck et al., 2002; Airoldi, 2003; Airoldi and Beck, 2007; Halpern et al., 2007; Wahl M., 2011; Ban et al., 2014; Strain et al., 2014). Declines of canopy-forming seaweeds cause serious concern because these vegetation enhance the structural complexity and productivity of coastal communities, providing food and protection for other algae, invertebrates and fish (Dayton, 1985; Chapman, 1995; Mineur et al., 2015). Understanding the state and the dynamics of canopy-forming seaweeds under multiple local anthropogenic stressors and extreme events caused by climate change is a crucial step to identify possible management scenarios to limit the loss and preserve these important habitats. This thesis was conceptually divided in three main parts: 1) identify the causes driving the loss of canopy-forming seaweeds at global level; 2) describing the status of the main intertidal canopy-forming algae around the Italian coast; 3) test experimentally the effects of selected stressors and identify factors potentially enhancing resilience.

The first chapter provided quantitative evidence, based on meta-analysis, of factors driving the shift of canopy-forming seaweeds to turf-forming algae. Although there have been meta-analyses conducted on the nature and type of interactions between local anthropogenic and other stressors on algal communities (Crain et al., 2008; Wahl M., 2011), these studies have not specifically considered the effects on both canopy-forming algae and turf-forming algae. Other reviews on canopy-forming algae and turf-forming algae have largely been based on a qualitative rather than quantitative assessment of the literature (Dayton, 1985; Chapman, 1995; Airoldi, 2003). The results of the first chapter provide important information for management actions to protect and restore the status of canopy-forming seaweeds. In fact, the management of nutrient levels, rather than fishing, heavy metal pollution or high sediment loads, would provide the greatest opportunity for preventing the shift from canopy to mat-forming algae, particularly in enclosed bays or estuaries because of the higher prevalence of synergistic interactions between nutrient enrichment with other local and global stressors, and as such it should be prioritized (Strain et al., 2014).

The second chapter focused on the status of intertidal canopy-forming *Cystoseira* species along the Italian cost and explored which environmental and anthropogenic factors can explain their status. Particular attention was placed on the status of *C. compressa* that is the most common intertidal species around the Italian coast. I focused on the intertidal rocky shore because species living on it are facing selective pressures from both environmental and anthropogenic factors. The loss of *Cystoseira* species around in Mediterranean Sea have been already documented in numerous location (Fraschetti et al., 2001; Perkol-Finkel and Airoldi, 2010). Several factors have been suggested to drive these loss, including urbanization and eutrophication (Thibaut et al., 2005; Mangialajo et al., 2008; Sales and Ballesteros, 2009; Villalonga, 2010; Mineur et al., 2015), increase in water turbidity and sedimentation (Airoldi, 2003; Perkol-Finkel and Airoldi, 2010; Strain et al., 2015), over-grazing (Agnetta et al 2015) and climate change (Asnaghi et al., 2013). The results highlight a depleted status of *Cystoseira* species around the Italian coast. Population density, used as proxy of urbanization, explained most of the variations of the status of *C. compressa*. The increasing of coastal human population combined with the effect of global climate change suggest that conditions for the *Cystoseira* species will be worst in future years. Our results also support the conclusions of the first chapter (Strain et al., 2014) which are that management actions, aiming to preserve or restore *Cystoseira* habitats, should focused on the impacts related to coastal urbanization.

The third, forth and fifth chapters were dedicated to experimentally test the effects of selected stressors. Based on the results from the first 2 chapters, I tested the effect of two stressors: nutrient enrichment, caused by the local human activities, and heat-wave events, caused by climate change. The effects of heat-waves have been already investigated in different marine ecosystems, such as seagrasses (Winters et al., 2011; Thomson et al., 2015), corals (Garrabou et al., 2009) invertebrate and macroalgae (Wernberg et al., 2012; Smale and Wernberg, 2013) and fish (Wernberg et al., 2012). Marine heat waves can cause mass mortality, altering the ecosystem structure or changing species distribution causing a contraction or loss of important foundation species (Wernberg et al., 2012; Smale and Wernberg, 2013; Thomson et al., 2015) (e.g. canopy-forming seaweeds or reef-building corals) with consequent reduction of habitat complexity and loss of important ecosystem services (Worm et al., 2006; Smale et al., 2013). In contrast, the effect of an air heat-wave event, as abrupt increase of air temperature, has been investigated mainly on terrestrial habitats (Chen et al., 2011; Sunday et al., 2012) but it received little attention in coastal marine systems (Bell, 1993; Helmuth et al., 2002). I found that extreme heat wave drastically reduce the photosynthetic activity *C. compressa*. The effect was significant at all the localities and persisted also after three hours of recovery. The fact that after 3 hours of recovery thalli of *C. compressa* showed even higher levels of impact and no resilience capability, leads to hypothesize that repeated heat wave events would increase the vulnerability of this species. This should be crucial in *C. compressa* populations already exposed to several factors of stress, and more efforts should be spent to understand the long-term effects of these extreme events particularly in populations subjected to other stressors, to better predict the future persistence of these important habitat. Another important results of this experiment are the potential role of local species diversity and thermal history of *C. compressa*. Biodiversity is now known to be a major determinant, perhaps the major determinant, of community and ecosystem dynamics and functioning (Tilman et al., 2014). There is a mounting evidences that biodiversity increase stability and the resistance of communities to climate events (Loreau and de Mazancourt, 2013; Isbell et al., 2015). Our results show that species diversity was significantly related to lower impacts of the heat wave on *C. compressa.* Preserving more diverse assemblages could favour the long-term sustainability of *C. compressa* population in the face of environmental and anthropogenic changes as well as variation caused by climate change. Another aspect playing an important role on the performance of an organism subjected to heat exposure event is the thermal history of a species. The thermal history sums the evolutionary history and more important the thermal events recently experienced by individuals, including short-term acclimation to environmental variations (Giomi et al., 2016). I found that recent thermal history play an important role on the algal response, with thalli of *C. compressa* living close to their thermal limits seem to be most impacted.

The fourth chapter I explored the possible overlooked role of the epiphytic bacteria growing on *C. compressa.* Bacteria interact with seaweeds in symbiotic, pathological and opportunistic ways, modulating the health, performance and resilience of their hosts. Biofilms can reduce the access of their hosts to light, gases, nutrients and modulate the interaction with other fouling epibionts, consumers and pathogens (Wahl et al., 2012; Goecke et al., 2010). In recent years, mass mortality events of different habitat-forming organisms such as corals, seagrasses and seaweeds have been increasingly associated with pathogenic microorganisms (Rosenberg and Ben-Haim, 2002; Goecke et al., 2010; Egan et al., 2013). To better understand the response of canopy seaweeds to multiple stressors we need to understand the response of the biological association (seaweed and bacteria) as whole or holobiont entity (Egan et al., 2013). I characterised for the first time the epiphytic bacteria associated to the surface of of *C. compressa* using Illumina Miseq sequences of V1-V3 hypervariable regions of 16S rRNA gene, and investigated their seasonal variations and their relationships with the bacterial populations in the surrounding seawater. Consistent with other studies (Burke et al., 2011; Bengtsson et al., 2010; Lachnit et al., 2009; Staufenberger et al., 2008), I found a clear difference between the bacterial communities of *C. compressa* and the surrounding seawater. The bacterial community of seawater remains more stable compared to that on *C. compressa*, that showed a clear successional pattern, interestingly characterized by an increase in abundance of potential microbial pathogens associated to older thalli of *C. compressa*. These results provide an important base-knowledge because, understanding the temporal dynamics of epiphytic bacteria under natural conditions can help to identify possible modifications of the biofilm due to external factors of stress.

In the fifth chapter, I analysed experimentally in the field the interacting effects of nutrient enrichment and heat-wave events on *C. compressa* population, and explored whether any resulting changes in the photosynthetic activity of *C. compressa* were associated to changes in the epiphytic bacterial communities. Consistent with results obtained in chapter 3, the heat-wave caused marked declines of the photosynthetic activity of the intertidal *C. compressa*. These effects persisted for at least 3 hours, while recovery generally occurred after 24 hours. The heat-wave altered the structure of the epiphytic bacteria of *C. compressa*.

Thalli exposed to the heat-wave presented an increase of OTUs assigned to genera *Ruegeria, Nautella, Aquimarina, Loktanella* whose have previously been shown with the natural degradation of the thalli of *C. compressa* (Mancuso et al., 2016), or implied in causing disease or damage to macroalgae (Case et al., 2011). As observed for the photosynthetic activity, these differences decreased over time, suggesting that the microbial community has the ability to recover. Differently from previous work, this experiment did not detect significant effects related to nutrient enrichment, suggesting that the effects of nutrients could be context dependent. These results open new questions concerning the mechanisms by which the epibacterial community could influence the responses and future persistence of these important canopy-forming seaweeds.

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