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Study of the effects of climate extremes on functioning of intertidal assemblages to design an early warning sensor network

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The most striking feature of Earth is the existence of life,
and the most striking feature of life is its diversity.
David Tilman

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List of papers

Published papers:

1. Fabbrizzi, E., Scardi, S., Ballesteros, E., Benedetti-Cecchi, L., Cebrian, E., Ceccherelli,, G., De Leo F., Deidun, A., Guarnieri, G., Falace, A., Fraissinet, S., **Giommi, C.**, Mačić, V., Passaron-Mangialajo, L., Mannino, A.M., Piazzi, L., Ramdani, M., Rilov, G., Rindi, L, Rizzo, L., Sarà, G., Souissi, J.B., Taskin, E., Fraschetti, S. (2020). Modelling Macroalgal Forest Distribution at Mediterranean Scale: Present Status, Drivers of Changes and Insights for Conservation and Management. *Frontiers in Marine Science*, 7:20. doi: 10.3389/fmars.2020.00020
2. Mancuso, F.P., Messina, C. M., Santulli, A., Laudicella, V.A., **Giommi, C.**, Sarà, G., & Airoldi, L. (2019). Influence of ambient temperature on the photosynthetic activity and phenolic content of the intertidal *Cystoseira compressa* along the Italian coast line. *Journal of Applied Phycology*, 31(5), 3069-3076.

In preparation:

3. **Giommi, C.**, Mangano, M.C., Isbell, F., & Sarà, G. (2020). Biodiversity and Ecosystem Functioning evidence from the Mediterranean area. (*in preparation*) Chapter 1
4. **Giommi, C.**, Lucchese, M., Helmuth, B., & Sarà, G. (2020). Differential response of intertidal algae in coping with a heat wave: a functional approach. (*in preparation*) Chapter 2
5. **Giommi, C**., Terzo, S.M.C., Mangano, M.C., Sarà, G. (2020). The role of an ecosystem engineer's density in modulating the response to a heat wave. (in preparation) Chapter 3

6. **Giommi, C**., Isbell, F., Mannino, A.M., Sarà, G. (2020). How temperature shapes algal diversity reveals early warning signals useful to predict climate change effects on tidal communities. (in preparation for *Ecology*) Chapter 4

Summary

In the current era of global change, marine systems are heavily impacted by human activities including overexploitation, eutrophication, pollution and species introductions, facing at the same time unique threats such as rising in air and seawater temperatures and ocean acidification. One consequence of these stressors is a documented increase in the frequency and magnitude of climate (or weather) extremes, such as severe droughts, period of heavy rainfall and heat waves. Recent prominent heat waves have attracted considerable scientific and public interest, but to date only a few studies have focused on the effects of extreme climate events on ecosystems even if there is a pressing need to understand the immediate and future consequences for ecological systems. Marine systems worldwide provide a variety of valuable ecosystem services including food production, waste assimilation, shoreline protection, climate regulation, and recreation. It is now widely known as the functions underlying these services are linked to biodiversity. Understanding the consequences of biodiversity loss in the world's oceans and how diversity contributes to the simultaneous maintenance of ecosystem processes and dynamics is the key to ensure an effective conservation and management.

With **chapter 1**, a systematic review and mapping exercise of the available evidence from literature allows collating evidence on the experimental biodiversity and ecosystem functioning (BEF) studies in a Mediterranean context (both terrestrial and aquatic systems), with the final aim to produce a knowledge baseline on this topic and highlight potential knowledge gaps and gluts. The applied technique strives to produce rigorous guidelines and has been proposed to reduce bias in ecological research.

The link between energy-individual-biodiversity and ecosystem functioning is often neglected, although the loss of biodiversity arises from an alteration of individual physiological, morphological and behavioural traits. Consequently, functional trait-based approaches have great potential to address a variety of ecological questions, including the impact of climate change on biodiversity and ecosystem service delivery. For this reason, **chapter 2**'s aim is to study the effect of a simulated heat wave on intertidal macroalgae, evaluating changes in the metabolic proxies of functioning (i.e. autotrophic oxygen production/consumption rate; photosynthetic efficiency) through time, at individual level.

Chapter 3 moves to the community level focusing on a mussel bed formed by the ecosystem engineer, the bivalve *Mytilaster minimus*. The aim of the chapter is to examine

the effect of a heat wave on the metabolic functioning of different levels of complexity along a mussel bed density gradient to assess if it plays a role in modulating community functioning. **Chapter 4**, through an in-field experiment, investigates how communities, on a natural gradient of diversity, respond under stressful conditions. The stressors are here represented by a heat wave combined with an anthropogenic stressor as the increased gasoline concentration in seawater due to the intensification of nautical traffic during the summer. Finally, **chapter 5** focuses on the development of low-cost miniaturized sensors interconnected in network, for a real-time identification of environmental variables of the fundamental niche of marine organisms.

This Ph.D. thesis is based on the study of functioning: from the lowest level of the ecological hierarchy, represented by the study of individual functional traits (chap. 2) the focus moves to community functioning, in particular studying the role of an ecosystem engineer (chap. 3). Finally, the functioning of microsystems as rocky pools (chap. 4) is investigated. For all the experiments (in-field and reproduced in mesocosms), a climatic driver such as heat wave (simulated or natural) is chosen. Furthermore, the habitat chosen for the study is the intertidal zone due to its intrinsic characteristics that make it a natural laboratory for the study of climate change. Finally, in the context of climate change, the development of sensors (chap. 5) is crucial and could be supporting research at all observation scales. The high anthropic pressure exerted on marine ecosystems has produced an increasing number of "new" environmental conditions at unpredictable speeds. Building up a network of bio-robotic systems for the high-resolution acquisition of environmental variables of the ecological niche will allow us to study the specific ability of each species, being it a commercially-important or an endangered one, to adapt to environmental change.

Chapter 1

General introduction: “Reviewing the relationship between ecosystem functioning and biodiversity”

Introduction

For a long time and in most fields of natural science, biodiversity was considered a simple by-product of static abiotic constraints. During the ‘80s, this one-directional perspective was considered as an incomplete view of biodiversity and researchers started to understand through a collective metabolic and growth activities of species, hundreds of tons of elements and compounds are moved between hydrosphere, atmosphere, and lithosphere (Loreau et al. 2002). These exchanges determine soil fertility, air, water quality, the habitability of ecosystem, biomes and ultimately the Earth itself (Lovelock 1979, Loreau et al. 2002). By the ‘90s, several theoretical and experimental studies were focused on the question of how the diversity of life forms could affect ecosystems and the so-called biodiversity and ecosystem functioning relationship (BEF) was formalized (Cardinale et al. 2012 and the references therein). Results from many studies suggested that the strength of biodiversity effects on ecosystem functioning ranges from weak and saturating relationships to strong and approximately linear relationships and three main classes of hypotheses were formulated to explain the role of biodiversity:

1. species are primarily singular (Figure 1: Linear). Each species gives its unique contribution to functioning, and therefore, an impact on functioning corresponds to the loss of every single species (e.g. keystone species is a particular case of this point);
2. species are primarily redundant. Loss of species is compensated for by other species with a similar function (Figure 1: Redundancy);
3. species impacts are context-dependent and therefore unpredictable. Every species gives a different contribution depending on the conditions (Figure 1: Idiosyncratic).

The strength of biodiversity effects partly depends on which species are lost (e.g. keystone species or ecosystem’s engineer) and on whether results from short- or long-term biodiversity experiments are considered, certainly biodiversity is one of the major determinants of ecosystem functioning (Tilman et al. 2014). Therefore, the understanding and the perception of ecosystem functioning seems to be different, encompassing a wide range of definitions and measures (Jax 2010), ending up with being a highly ambiguous term. For example, sometime the terms “ecosystem functioning” are used to refer to some overall function (performance) of the whole ecosystem (Naeem & Wright 2003, Hooper et al. 2005), and frequently used simply in place of ecosystem functions (processes and properties). Some authors explicitly stated that it is enough to take one of these processes and patterns as an indication of the functioning of the whole ecosystem (Boero & Bonsdorff 2007). Other argued that a single variable could not characterize the overall functioning of a given ecosystem (Bremner 2008). With the aim to facilitate the interpretation of the effects of change in biodiversity and clarify the meaning of these terms in the BEF debate, Gamfeldt et al. (2008) explicitly defined ecosystem functions as single processes that together constitute overall ecosystem functioning.

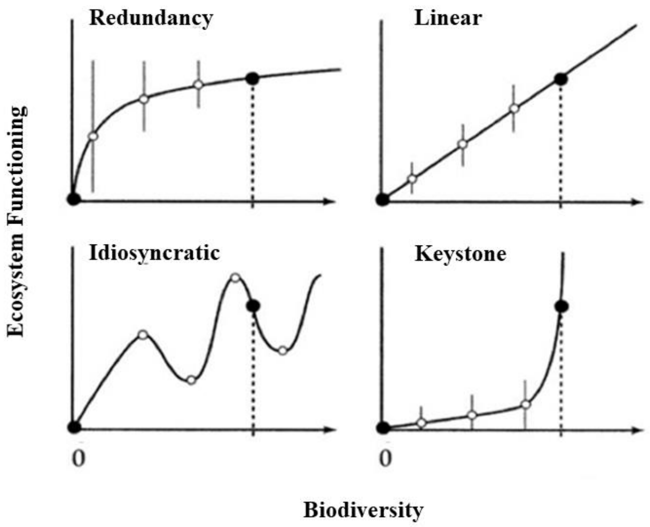

Figure 1. Graphical representations of relationships between BEF, modified from Loreau et al. 2002.

As the field of BEF developed, a related body of research began to form an agenda for the related research, built on the idea that ecosystems provide essential benefits to humanity (Cardinale et al. 2012). Thus, the concept of ecosystem service was coined for the first time in 1981 by Ehrlich & Ehrlich. Such a concept appeared within a broader circles of ecologists and economists only later in 1997 thanks to both a paper by Costanza et al. (1997) and the book *Nature's Services* by G. Daily that recognized the role of natural habitats in providing essential goods services to societies (Jax 2010, Cardinale et al. 2012). Later, the Millennium Ecosystem Assessment (MEA 2005) appraised, for the first time, the condition and trends in the world's ecosystems and the services they provide promoting a simple causal relationship conceptual framework: Biodiversity (B) - Ecosystem Functioning (EF) - Ecosystem Services (ES) - Human well-being (WB).

The rapid loss of species experienced since decades has inspired and fostered the number of studies that investigated the first part of this relationship, the links between biodiversity and ecosystem functioning (BEF; Gamfeldt et al. 2015; Clarke et al. 2017).

The second part of the relationship dealing with ecosystem service and well-being causal analysis (Naeem 2012) was that relatively newer and consequently the least explored. To strength and help in generalising the study of this latter aspect, scientists need wide and complete datasets from experiments occurring over and across different regions, avoiding biases due to study location (Clarke et al. 2017).

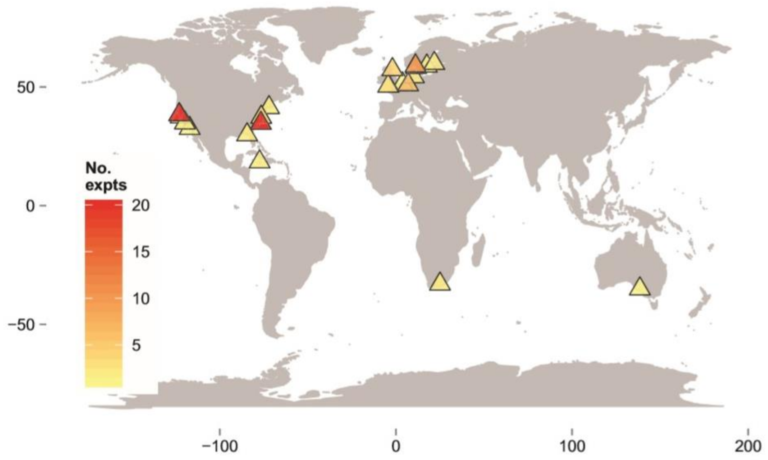

Figure 2. A global map showing the location of the experiments included in the meta-analysis by Gamfeldt et al. 2015.

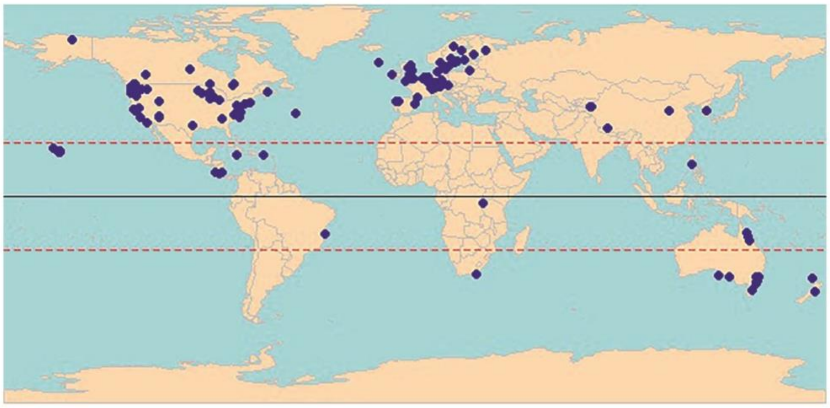

Figure 3. World map showing the locations (blue circles) of BEF experiments taken from four meta-analysis that explored different aspects of the BEF relationship as in Clarke et al 2017.

Simply looking at recent meta-analyses that have synthetized BEF outcomes (respectively Figure 2 and 3 in Gamfeldt at al. 2015, Clarke et al. 2017), a clear gap arises regarding the Mediterranean area, clearly poorly or not represented.

The major aim of this introductive chapter is to fill this gap through an evidence-based synthesis from the literature to create a BEF knowledge baseline in the Mediterranean area. Thus, here, by producing rigorous and comprehensive knowledge baselines, this chapter will attempt to reduce biases and informative gaps in this area of the ecological research.

Materials and Methods

To answer to the main question *“How many experimental studies testing for Biodiversity and Ecosystem Functioning relationship have been performed in the Mediterranean area?”* studies from the scientific literature, that looked at the Mediterranean area when dealing with Biodiversity-Ecosystem Functioning relationship, were retrieved.

A complex search string, resulting after the first step of the scoping phase, the inception meeting, allowed to select the more comprehensive and accurate set of keywords which was later run on Scopus, settled up on Title – Abstract - Keywords, by referring to the restricted timeframe from 1985 to 2018 (search ended in July 2018). Search string was as follows:

(“*diversity" OR "richness" OR "composition") AND ("ecosystem* function*" OR "ecosystem* process*" OR "communit* function*") AND ("experiment*") AND (Mediterranean)

One hundred and eleven (111) records were retrieved after the search carried out on the 13th July 2018. All records were screened *per* title and abstract or searched for the presence of the selected keywords. During that step, all records reporting on studies carried out of the selected geographic area (i.e. the Mediterranean Sea), or which were out-of-topic were removed (N = 40; Figure 4). The high number of removed records was likely due to the presence of the keyword “Mediterranean” which retrieve the Mediterranean climate that, apart than in the basin of study, could fall in areas such as Southern Australia and California, which were not in the target of in this work. After a full text reading (second screening stage), only records presenting experimental results were included in the following steps while not experimental (No exp) records, such as meta-analyses and reviews, or model-based studies were left out. A small number of works measuring an environmental-factor dependent EF measure were excluded (i.e. not strictly related to biodiversity, No biod: n = 3).

Seventhy-one (#71) records constituted the finale dataset and they were carefully analysed for evidence extraction over a specific coding and all the relevant outcomes have been crossed, synthesised and presented (Supplementary materials, Table 1).

These records were analysed looking at the temporal trend (number of records over a temporal scale), the spatial distribution (number of records per country), habitats (number of records per habitat category) and the experimental duration across the habitats. These studies were grouped also depending on the applied approach followed to study the biodiversity-functioning relationship, respectively: "pure BEF" studies that display biodiversity on the horizontal axis and a functioning measure is on the vertical axis, and "not-pure BEF".

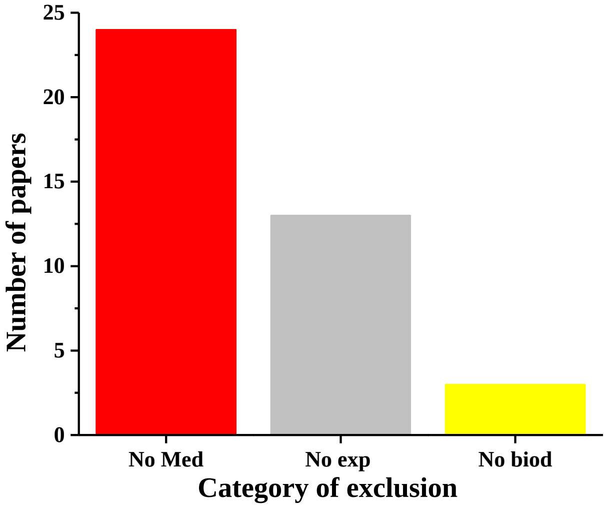

Figure 4. Category of exclusion. No Med: papers not belonging to the area of interest. No exp: meta-analyses and reviews papers, or model-based studies. No biod: works not included because environmental dependent EF measures.

Results and discussion

Looking at the temporal trend, as extrapolated by the retained 71 articles, from 1998 to 2008 just a few works had been issued, then a positive trend is shown with a peak of 13 works in 2017, testifying that BEF experimental studies are relatively recent (Figure 5).

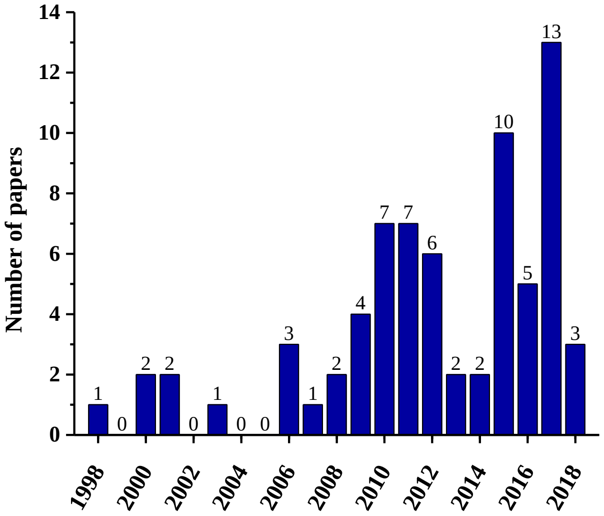

Figure 5. Temporal trends (number of records over a temporal scale).

Concerning the spatial distribution, the studies performed in Spain (N = 24), France (N = 21) and Italy (N = 9) represented a 76% of the total records, showing that the BEF relationship was more studied into the western basin of the Mediterranean (Figure 6). Most of the studies was carried out on terrestrial realm: 14 records dealt with forests and woodlands (most pines and oaks), 9 with grasslands, 10 with shrublands such as the Portuguese *montado*, the Spanish *dehasa,* and the French *garrigue* while the remaining focused on common gardens, Mediterranean *maquis*, and old-field set aside from cultivation. Only 16 studies were carried out in marine systems, mostly on seagrasses meadows, intertidal rocky shores and sublittoral soft bottoms, and just one study focused on a deep-sea reef. Six studies were carried out on freshwater riverine ecosystems (Figure 7).

An interesting “habitat-related” aspect regards the whole experiment duration. Freshwater experiments were at most 3-month long (Monroy et al. 2016), while in the marine environment, there are two experiments that lasted for a period of 1- and 2-years which are much longer than the duration of the others (Min and Max 10 days – 90 days duration) (Prado et al. 2010, Maggi et al. 2009). On the land, experiments lasted 3 to 7 years.

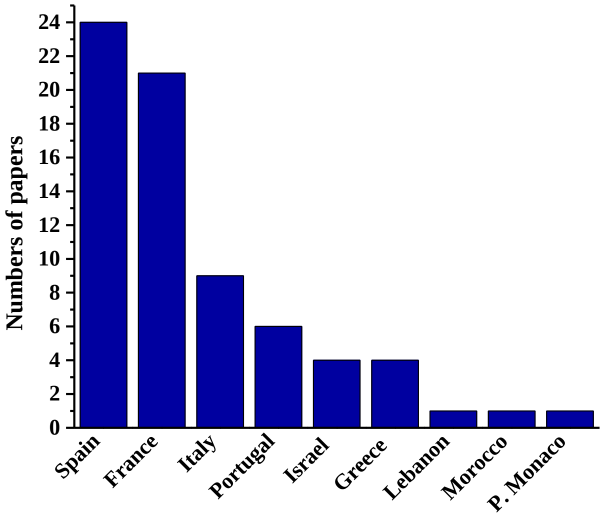

Figure 6. Spatial distribution of the study included in the analyses (number of records per country).

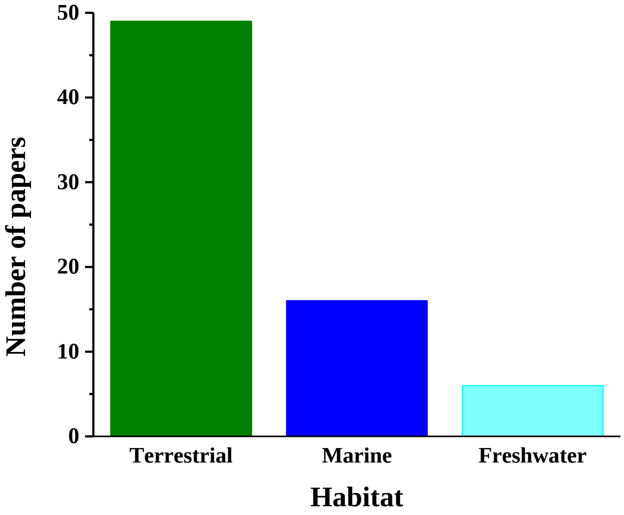

Figure 7. Habitat classification (number of records per habitat category).

The 85% of the experiments was manipulative (the manipulation regarded the biodiversity level and/or the level of treatment); 10 experiments were observational in which, for example, a restoration process of a natural system was monitored (Camacho et al. 2012), or, a natural gradient at the beginning and at the end of a drought period was measured (Karam et al 2011).

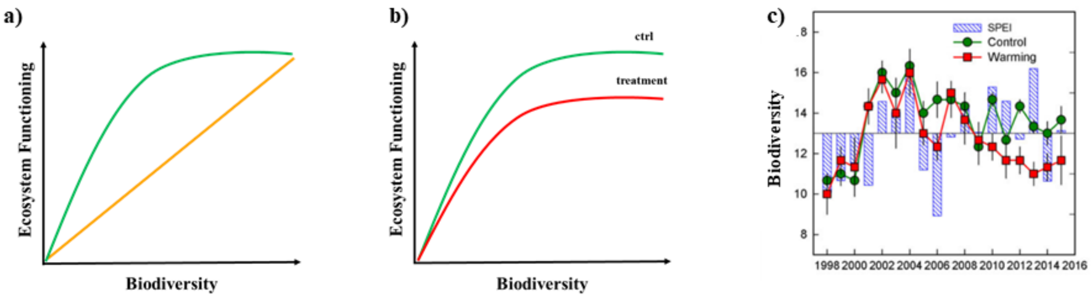

Figure 8. Scheme of the different approaches used to study the BEF relationship. a) pure BEF (N=28); b) pure BEF + other treatment (N=22); c) not-pure BEF (21), modified from Liu et al. 2017 – Ref. ID n. 5 in Table 1 Supplementary Materials.

Depending on the approach applied in studying the biodiversity-functioning relationship, studies were divided into “pure BEF” and “not-pure BEF”. Experiments belonging to the first group had the objective to study the effect of changing biodiversity on ecosystem functioning (Figure 8a). Thus, biodiversity was assigned to the x-axis while a measure of functioning was assigned to the y-axis. The biodiversity was manipulated i) by the simplest level of presence/absence of a species up to increasing levels of diversity (expressed mainly as species richness), or ii) studied along a natural gradient. In particular, when the function studied was the food web (e.g. top-down control), biodiversity was mostly manipulated as presence/absence (e.g. exclosure experiments), while when the function measured was a process such as primary production (e.g. wood or crop biomass) or decomposition, biodiversity was manipulated on number of species, on species identity, abundance, and density level.

The primarily studied ecosystem functions were: primary production, decomposition, nutrient cycling, food-web and metabolism; example of measures adopted to estimate each function are shown in Table 1.

Other studies looked for a driver effect that was added to the biodiversity-functioning relationship (N = 22; Figure 8b). Most drivers were climate-related. Indeed, while half studies simulated drought, other treatments were rainfall, heating or effect of fire and hypoxia. Moreover, other kinds of used stressors were pollutants, organic enrichment, habitat fragmentation or change in land use and biotic interaction such as grazing.

Table 1. Example of the different Ecosystem Functioning proxies found in the71 studies.

Decomposition	Decomposition rate Litter quality Litter mass or mixture mass loss Leaf litter degradation Fungal Biomass and sporulation Cellulolytic activity of the fungal isolates Soil heterotrophic microbial functional diversity Soil heterotrophic microbial activity (microbial catabolic profile)
Primary production	Biomass (harvesting, crop, wood) Coverage % Photosynthetic efficiency Seedling survival/vitality Water uptake capacity
Nutrient cycle	Nutrient fluxes N and C cycle Soil functions: Organic C; Total N and N fixation; (b-glucosidase, urease and acid phosphatase enzymes)
Metabolism	Oxygen consumption rate
Food Web	Top down control Spread of Non Indigenous Species (NIS)

Interestingly, in a group of 21 studies (“not-pure BEF”), the classical scheme of BEF was reversed (Figure 8c): a biodiversity metric was plotted on the y-axis against different intensities of disturbance on x-axis. As in the case of “pure-BEF”, also in this case drivers used in the experiments were mostly connected to climate change such as simulation of drought and/or heat, the effect of fire and hypoxia in marine sediments. Other drivers were connected to anthropogenic pressure such as habitat fragmentation or change in land use, pollutants, nutrient enrichment; few studies considered the effect of biotic interactions (e.g. large ungulates grazing).

Noticeably, 15 over 21 studies looked at diversity connected to a process or a functioning measure. For example, to assess if changes in nutrient supply caused by eutrophication induced alterations in primary producers leading to changes in herbivory pressure, Prado et al. (2010) presented an experiment of water column enrichment in *Posidonia oceanica* meadow. The response variables were the epiphyte species composition on the shoots, the plant and epiphyte quality (nutrient content) and quantity (biomass). In this case, even if biodiversity is on the y-axis, it was strongly connected to ecosystem processes such as primary production and quality of food availability for grazers (i.e. fish).

Moreover, in six experiments from this group, biodiversity was studied per se without considering it within a process or without associating it with another functioning measure. This could be the case study reported by Liu et al. (2017): these authors carried out a long-term experiment to study the effect of drought and nocturnal heating on community shrubland diversity and composition.

Concluding remarks

This exercise of evidence mapping allowed highlighting that biodiversity-ecosystem functioning experiments performed across the Mediterranean basin are few when compared to other areas (e.g. North America). Studies focused mostly on terrestrial systems and then such an analysis suggests that a set of new generation of experiments focused on marine realm is urgent. Not only expanding the number of case studies but also the scope and relevance of future experiments, we will increase our understanding about the consequences of changes in marine biodiversity.

The main outcome of this review is that most studies used biodiversity as a proxy of ecosystem functioning. Such a fact supports the idea that biodiversity is the major determinant of community and ecosystem dynamics and functioning (Tilman et al. 2014). As a main consequence, to use biodiversity as a measure of the overall functioning of a system according to what was posited by Gamfeldt et al. (2008) may be reasonable.

However, in habitats characterized by a high inherent variability - such as the intertidal habitat - mechanisms and relationships are performed on a temporal scale that could unlikely be captured by studies based on community structure and composition changes observations. Hence, even if it can be not easily feasible to synchronize the observation of a change to organism velocity of process and functioning, it could be the only way forward to do not lose details of needed information. Indeed, the first impact of a source of variation could appear not only through a change in species distribution but through the sublethal impact on an organism that could affect growth processes, reproduction

(Sarà et al. 2014). Changes in trait values due to environmental variations, disturbance and biotic interaction can be detected into organismal performance and these changes may influence processes at higher organizational levels. Consequently, functional trait-based approaches (Kearney & Portner 2009; Enquist et al. 2015) have great potential to address a variety of ecological questions, including the impact of global change on biodiversity and ecosystem service provision, ecological restoration or the assembly of biological communities (Carmona et al. 2016).

The climate change in its various expressions (e.g. constant temperature increase and intensity and frequency increase of extreme climate events) makes the understanding of this relationship more complex by also affecting the systems vulnerability and stability.

The BEF relationships become more complex under climate change scenarios that will increase the current variation rate, thus raising the unpredictability of processes on larger scales.

Moreover, it may worth to pay attention to the target EF variable, indeed many studies investigated only one response variable, and those that investigated several variables, almost without exception, simply juxtaposed the different results of averaged them (Jax 2010).

Ecosystem services are often regulated by multiple functions, which do not necessarily respond to changes in biodiversity in the same way (Cardinale 2012). Important frontiers to integrate EF and ES could be detailing the mechanistic link between EF-ES through a punctual and systematic measure of more function for each system.

Moreover, BEF research has focused mostly on small observational spatial scale to controlled experiments, which has made difficult to re-scale results to real ecosystems at larger scales where services (e.g. biomass, habitats or entire groups of organisms) from effects of biodiversity *per se* (Cardinale 2012).

On a final note, going back to the framework - driving the systematic review of evidence - and looking at the synthesis outcomes we can say that, starting from the Millennium Ecosystem Assessment, a lot has been done up to date. A knowledge gap raises on the second causal link (EF-ES) that prevents us connecting the Ecosystem Functioning with the Ecosystem Services.

A possible solution could be to define how many and which ecosystem variables have to be selected and measured to allow reasonable statements about the functioning of the whole ecosystem and base the decision of the rate of processes and the urgency with which it is necessary to know the answers. Only through a deeper analysis of EF it will be possible to quantify and link it to ecosystem services which is in fact a determinant link to society and take care of human health.

Based on the built knowledge baseline, the aim of this thesis was to study the effect of extreme climate events in a highly variable system such as the intertidal zone. It represents a good model habitat to study the small changes that occur under the action of a disturbance and that, by gradually approaching the system to a tipping point. Being able to forecast these small changes and to measure the speed at which the systems approach the tipping point is of great importance. In doing so, the metabolism was selected as a proxy of functioning (i.e. photosynthetic efficiency and oxygen consumption). I tried to select the more accurate scale of observation to capture the species and community response during the experiment. Starting from the lowest level of the ecological hierarchy, represented by the study of individual functional traits (chap. 2), then the focus moved to community functioning, in particular studying the role of an ecosystem engineer (chap. 3) in coping with heat wave. The functioning of microsystems as rocky pools (chat. 4) was investigated and a low-cost miniaturized sensor interconnected in network, useful for a real-time detection of environmental variables of the fundamental niche of marine organisms, was designed (chap. 5).

Author contribution. Chiara Giommi conceived the idea, led the writing of the ms., performed the systematic review, which was designed with Maria Cristina Mangano, Forest Isbell and Gianluca Sarà.

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Supplemetary materials

Table 1. A list of the 71 studies included in the analyses.

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Chapter 2

Differential response of intertidal algae in coping with a heat wave: a functional approach

Abstract

Since decades, natural systems have experienced radical changes, becoming weaker due to the anthropogenic impact and global climate change. This influences the biodiversity on which ecosystem functioning and ecosystem services depend.

Since the loss of biodiversity is caused by the alteration of the functional traits of an individual, here the functional traits of macroalgae through two techniques are studied: production and consumption of oxygen through photorespirometry; in vivo chlorophyll a fluorescence of photosystem II, derived by portable Pulse Amplitude Modulation (PAM) fluorometer. Furthermore, the effect of a heat wave, simulated but realistic, on the functional traits of intertidal rocky shore Mediterranean macroalgae has been evaluated. Throughout autumn 2018, in Western Sicily (Italy), five algal species (*Carpodesmia amentacea, Dictyopteris polypodioides, Jania rubens, Palisada tenerrima, Ulva rigida,*) and a turf community have been sampled. For each sample respirometric rates, in dark and light condition (mg/L O_2), and chlorophyll-a fluorescence (Fv/Fm) was estimated. The measurements were conducted in ambient condition (T0), after the 4-hour heat wave (T1), and after 24 hours from the heat wave end to assess any recovery time (T2).

Results showed that, at T0, turf community and *U. rigida* are the most productive, while *J. rubens* had the lowest production rates. Heat wave did not impair turf and *J. rubens*, while the effects of stress were evident on all other species. After 24 hours the impact of the climate driver was still present and the ability to recover is driven by species-specific features. Finally, the results showed that an integrated approach is necessary to hypothesize the role of species within the community and to predict winners and losers in a scenario of changing climate.

Keywords: Functional traits, Macroalgae, Heat wave, Metabolism, Photosynthetic efficiency, Recovery time.

Introduction

Starting from the twentieth century, natural systems had experienced dramatic modifications becoming more vulnerable due to the anthropogenic impact and global climate change. In this context, in 2001, the *Millennium Ecosystem Assessment* was commissioned by the United Nations to assess the human impact on the environment on a global scale. Main aim was to build on and integrate the existing scientific evidence and knowledge connecting ecosystem services explicitly to human well-being (Jax 2010). The MEA's conceptual framework promoted a simple causal relationship as follows: Biodiversity (B) → Ecosystem Functioning (EF) → Ecosystem Services (ES) → Human well- being, which highlights a strong deterministic linkage between biodiversity and human well-being. In particular, biodiversity is defined as the genetic, taxonomic, and functional diversity of life on Earth including temporal and spatial variability (Naeem et al. 2012) and it is now known to be a major determinant of community and ecosystem dynamics and functioning (Tilman et al. 2014, Isbell et al. 2017). From ecosystem functioning depends the ecosystem services i.e. "the benefits people obtain from ecosystem" (MEA, 2005). Since biodiversity drives and controls the whole network of connections, at a time when biodiversity is undergoing dramatic changes in distribution and abundance, it is increasingly necessary to protect it and to understand the role it plays in determining ecosystem functioning (the first link of the conceptual framework).

How and why biodiversity loss influences ecosystem functioning has been an intense area of ecological research over the last 30 years. Three major classes of biodiversity-functioning hypotheses were identified: the "singular hypothesis" implies that every species makes an unique contributions to ecosystem functioning, the "redundant hypothesis" implies that species are at least partially substitutable and the "idiosyncratic hypothesis" implies that a species makes different contributions to ecosystems depending on extrinsic and intrinsic factors (Naeem et al. 2002). Moreover, while different mechanisms can help us in explaining the causal biodiversity-ecosystem functioning (BEF) relationship (e.g. the portfolio effect and the niche complementarity), all theoretical models of BEF relationships include assumptions concerning differences in species' traits. Indeed, actually, if biodiversity were composed by identical species, there would be no theoretical mechanisms that produce any relationship between species richness and ecosystem processes. Therefore, the characteristic traits of species and the functions these species perform are crucial determinants for ecosystem processes (Scherer-Lorenzen 2005). A recent meta-analysis by Gamfeldt and co-workers (2015) presents 110 marine experiments from 42 studies that manipulated the species richness of organisms across a range of taxa and trophic levels and analysed the consequences for various ecosystem processes (categorised as production, consumption or biogeochemical fluxes). The authors underlined that marine biodiversity experiments have been largely carried out on species richness as a potential driver of functioning, while overlooking other aspects of biodiversity. Moving forward, they suggested to integrate functional traits and/or evolutionary differences in investigating the relationship between diversity and functioning (Gamfeldt et al. 2015).

Functional traits are defined as morpho-physio-phenological traits, which impact fitness indirectly via their effects on growth, reproduction and survival, the three components of individual performance (Violle et al. 2007). Changes in trait values due to environmental variations, disturbance and biotic interaction can be into organismal performance and these changes may influence processes at higher organizational levels. Consequently, functional trait-based approaches (Kearney and Portner 2009) have great potential to address a variety of ecological questions, including the impact of global change on biodiversity and ecosystem service provision, ecological restoration or the assembly of biological communities (Carmona et al. 2016).

Macroalgae are some of the most productive primary producers in the world (Mann 1973) and they are particularly important because of their roles as primary habitat provider, food sources, ecosystem engineers (Wernberg et al. 2012) and their large contribution to the global carbon sink (Queiros et al. 2019).

According to external morphology, internal anatomy and texture, rather than to phylogenetic and life-history affinities, macroalgae are ranked in seven functional groups: Microalgae, Filamentous algae, Foliose algae, Corticated foliose algae, Corticated Macrophytes, Leathery Macrophytes, Articulated Calcareous, Crustose algae (Littler & Littler 1980, Steneck & Dethier 1994). Algae belonging to the same functional group share the same anatomical features and therefore are described by the same ecological, physiological, and developmental characteristics (e.g. rate of mass-specific productivity, thallus longevity, canopy height) and it is expected that they show the same resistance to stress condition, increasing our ability to generalize. For instance, encrusting coralline algae are more adapted and resistant to high level of grazing environments, or turf forms are adapted to resist desiccation (Littler & Littler 1984).

Benthic marine macroalgae occur predominantly on rocky shorelines and shallow reef, indeed they are the most conspicuous organisms in the intertidal zone (Norton 1986). On this habitat, due to the rise and fall of the tide, algae and animal (mostly sessile or sedentary species) are usually exposed to both aquatic and terrestrial environments. The exposure to biophysical drivers of both marine and terrestrial climate makes intertidal shores a uniquely challenging environment supporting only those organisms with a high physiological adaptability. As a result, intertidal systems represent a natural laboratory to examine the relationships between abiotic stress, biotic interactions and ecological patterns in nature and may serve as an early warning observatory for studying the impact of climate change (Helmuth et al. 2006).

Climate change acts as an agent of disturbance on ecological systems. Rising in air and seawater temperature increases the frequency and magnitude of climate (or weather) extremes, such as severe droughts, period of heavy rainfall and heat waves (Meehl & Tebaldi 2004; Meehl et al. 2007). To date only few studies have focused on the effects of extreme climate events on ecosystems (Jentsch et al. 2011) and there is a pressing need to understand the immediate and future consequences for ecological systems.

Here, the effects of a simulated heat wave on macroalgae were examined, estimating changes in metabolic proxies of functioning (i.e. autotrophic oxygen production/consumption rate; photosynthetic efficiency) through time. The metabolic rate is the most fundamental biological rate, and it determines the rates of most other organic processes. Moreover, it is considered a unifying process in ecology because it links all hierarchic levels of ecological organization (Savage et al. 2004). In most aquatic animals, aerobic metabolic rate can be estimated indirectly from measurements of oxygen consumption rate (i.e. respirometry), and it has been used to answer fundamental questions related not only to physiology but also to ecology (Rosewarne et al. 2016). For example, it is an excellent method to study the actual extent to which thermal stress on animals causes an increase in oxygen consumption (McDonnell et al. 2016).

Respirometry is also used to estimate the metabolic activity of plants and algae (Falkowsky & Raven 2007), but differently from heterotrophic organisms, with autotrophic organisms, measurement of oxygen consumption (i.e. dark respiration) is complementary to the measurement of oxygen production (Richard et al 2011).

Furthermore, in this study, in addition to photorespirometric measurements, a direct estimate of photosynthetic efficiency such as *in vivo* chlorophyll-a fluorescence is derived by a pulse-amplitude-modulated (PAM) fluorometer. This is a non-destructive method to obtain real-time measures of the physiological status of the photosynthetic organism both in the field or in laboratory condition. The measurement of the photosynthetic responses in macroalgae is dominated by both the previous techniques (Tait et al. 2017).

Thus, the aim of the present study was to evaluate the effect of a simulated heat wave event during low tide on the metabolic rate of Mediterranean intertidal macroalgae. In order to estimate the resistance to the climatic driving forces, the measurement was recorded straight at the end of the thermal stress and repeated after 24 hours to evaluate the ability to recover. To achieve this, the five most abundant macroalgae of the autumnal community were studied: *Ulva rigida* C. Agardh a foliose algae of the Phylum Chlorophyta, *Palisada tenerrima* (Cremades) D. Serio, M. Cormaci, G. Furnari & F. Boisset a corticated algae of the Phylum Rhodophyta, *Jania rubens* (Linnaeus) J. V. Lamouroux an articulated calcareous algae of the Phylum Rhodophyta, *Carpodesmia amentacea* (C.Agardh) Orellana & Sansón a leathery large brown fucoid algae and *Dictyopteris polypodioides* (A.P. De Candolle) J.V. Lamouroux a sheet alga of the Order Fucales. Moreover, a multispecies assemblage of generally filamentous macroscopic algae defined as turf community was collected (Tebbet et al. 2019).

Material and Methods

Collection sites and acclimation

In order to represent the autumnal algal community of Southern Mediterranean rocky shores, throughout September and December 2018, during low tide, macroalgae were collected from four sites along the coast of Palermo (Sicily). The sites were North-West exposed and used as model of intertidal coasts: Punta Raisi (38.19085° N,13.11039° E); Isola delle Femmine (38.20048° N,13.24191° E), Barcarello (38.20913° N, 13.28193° W); Acapulco (38.19118° N, 13.35598° W).

Figure 1 - Sampling sites.

During the sampling, intact and in a good healthy state individuals were chosen in order to avoid wounds on the thallus that may exhibit oxygen-sensitive respiration affecting the respirometry (Bin Zere & Middelboe2005, Middelboe et al. 2006, Sand-Jensen et al. 2007).Samples were transferred to the laboratory and, after the removal of epiphytes and individuals of macrofauna, algae were stored in tanks filled with filter seawater and left to acclimatize for 24 hours.

Experimental design

At the end of the acclimation, the first measurements of the metabolic rates before the exposure to thermal stress (T0) were recorded. Respirometric measurements were carried out on twelve replicates for each species: six individual thalli were used to evaluate the oxygen production in light condition (illumination was provided overhead by a full spectrum LED grow light delivering a maximum PAR of 857 µmol photons m^{-2} s^{-1} for each thallus), six individual thalli were used to measure the dark respiration in custom-built chambers obscured to avoid photosynthesis. Respirometric rates (RR) (*O_2 rate*, mg O_2 l^{-1} h^{-1}DW-g^{-1}) were recorded for two hours.

Before the fluorometric measurements, the samples were dark adapted for thirty minutes to avoid non photochemical quenching (Mancuso et al. 2019). For each species, six replicates were measured and, within the replicate five measurements of maximum quantum yield (Fv/Fm) (see section: *In vivo* chlorophyll fluorescence) were randomly taken from the surface. The mean of the five values was used for the subsequent statistical analyses.

After the end of T0 measurements, replicates were divided into two groups: one exposed to increasing temperature and the other kept at ambient temperature and used as a control. To simulate the heat wave, replicates were placed under stands provided with infrared heat lamps of 100 watt (ZOOMED). Air exposure lasted four hours to simulate the low tide condition of maximum air exposure typical of a day with high atmospheric pressure. The temperature reached 38 °C corresponding to the higher air temperature recorded by Italian Institute of Environmental Research (ISPRA) temperature network (http://www.mareografico.it/). Temperature was constantly monitored through a thermometer placed near the algal surface.

Immediately after the end of the exposure, to evaluate the ability of the algae to cope with a heat wave, the second measurements of the metabolic rates (T1) were recorded following the same procedure carried out in T0. Then replicates were put singularly in becker filled with filtered sea water and kept for 24 hours at ambient temperature.

Finally, to evaluate the effect of the thermal stress through time and the ability of algae to recover, 24 hours after the end of the heat wave respirometric measurements and fluorometric measurements were repeated (T2).

Respirometric measurements

Single individuals were placed in glass respirometric chambers (320 ml) containing filtered (Whatman GF/C 0.45 µm) and sterilised (autoclaved) air-saturated seawater at salinity 38. Respirometry was carried out at 23 °C coincident with the seawater temperature recorded during the sampling. Temperatures were kept stable by means of a thermal bath and monitored throughout the recording period. To ensure the constant mixing of the water, each chamber was stirred with a magnet bar and an individual stirring device (Giomi et al. 2016). The concentration of dissolved oxygen (ppm or mg/L) was measured by means of 3 optical oxygen meters (Pyro Science Firesting O_2) through the use of specific software (Pyro Science) (Montalto et al. 2017).
At the end of the experiments, each individual was dried at 105 °C for 24 h and reweighed to determine the Dry Weight (DW, g). Respiration rates were reported as oxygen consumption/production per unit mass and expressed as mg O_2 l^{-1} h^{-1} g^{-1}dry tissue.
According to van Hoytema and co-workers, respirometric data were transformed in an estimation of photosynthetic efficiency: the Pg:R ratio, derived by dividing gross photosynthesis (Pg) by the dark respiration (R) where Pg is estimated by adding positive R rates to their corresponding Pn rates: ($Pg = Pn + |R|$).

In vivo chlorophyll fluorescence/ Flourometric measurements

Chlorophyll fluorescence (CF) is related to photosynthetic efficiency. Algae stress often results in reduced photosynthetic efficiency. Photons can be funneled into the PSII for photochemical use, dissipated as heat, or re-emitted as CF. To measure CF, a short photochemical quenching burst of light closes PSII reaction center. Photons from a subsequent burst of light will then be either dissipated as heat or re-emitted as CF, both of which are directly measured by an instrument. During the experiment, *in vivo* chlorophyll-a fluorescence of photosystem II (PSII) was assessed by a portable pulse amplitude modulation fluorometer (Junior-PAM, Waltz GmbH, Effeltrich, Germany).

The variable measured in this study is the maximum quantum yield (Fv/Fm) which represents the maximum photochemical primary efficiency of PSII in a sample adapted to the dark, and is the most widely used value as a reliable indicator of the photochemical activity of the photosynthetic system (Bussotti et al. 2012). Before each measurement

(T0, T1, T2) thalli were dark adapted for 30 min and the minimum (basal) fluorescence (Fo) was measured. Then the maximum fluorescence (Fm) was measured applying a saturation pulse (9000 µmol quanta m^{-2} s^{-1}, 800 ms) of actinic light and maximal quantum yield was calculated as Fv/ Fm=Fm−Fo/Fm. This parameter is a valuable indicator of physiological stress (Murchie & Lawson 2013), widely used to monitor temperature-induced changes of photosynthetic activity and tends to decrease with increasing temperatures (Eggert 2012; Pereira et al. 2015).

Statistical Analysis. Differences in respirometric rates and *in vivo* chlorophyll fluorescence were tested separately. To study the effect of heat wave on algae a General Linear Model (GLM) approach was used. Usually, close range variables as the measurement of *in vivo* chlorophyll fluorescence are strongly skewed and heteroscedastic (too far from normal distribution) and, for [0, 1] range, in particular, a refined approach as beta-GLM is appropriate. Beta distribution does not belong to the Natural exponential class but, with the right parametrisation, it can be set as GLM-type model. In this specific case, given repeated measure, a beta-GLM with random slope was used. Just to have a coherent approach, a "standard" GLM with random slope was used to model respirometry measurements**.**

Results

Table 1 reports a significant negative effect of heat wave along time and a significant negative estimate of treatment group with respect to control. Table 2 reports on the interaction between groups and the measure at different steps, in which the direct effect of heat wave appears spread over them. The "heat wave step" (T1) had the strongest significant negative impact on PAM fluorimetric measurements. The estimate of the recovery step appears negative (Tab. 1-2) and then algae did not appear to be able to recover to their previous condition. Indeed, when the heat wave step was considered as a baseline, a non-significant estimate of recovery was found (not shown).

Table 1. Estimates of the Beta – GLM Model for PAM measurements (p < 0.001 - ***; p < 0.01 - ** p < 0.05 - *; p > 0.05 - ns).

Coefficients:	Estimate	Std. Error	z value	Pr(<|z|)	
(Intercept)	0.895	0.300	2.99	0.003	**
Time 1	-0.853	0.159	-5.36	0.000	***
Time 2	-0.855	0.159	-5.40	0.000	***
Treatments	-0.450	0.127	-3.55	0.000	***
dispersion:	9.304	1.258			
Random: species					
	Variance	StdDev			
(Intercept)	0.424	0.651			
AIC: -88.5					

Table 2. Estimates of the Beta – GLM Model for PAM measurements considering interactions (p < 0.001 - ***; p < 0.01 - ** p < 0.05 - *; p > 0.05 - ns).

Coefficients:	Estimate	Std. Error	z value	Pr(<|z|)	
(Intercept)	0.641	0.314	2.05	0.040	*
Time 1	-0.422	0.213	-1.98	0.047	*
Time 2	-0.564	0.211	-2.67	0.008	**
Treatments	0.054	0.221	0.24	0.808	
Time 1: Treatments	-0.871	0.305	-2.86	0.004	**
Time 2: Treatments	-0.585	0.302	-1.94	0.053	.
dispersion:	10.09	1.369			
Random: species					
	Variance	StdDev			
(Intercept)	0.438	0.662			
AIC: -92.5					

In contrast, the photorespirometry results did not show any significant evident difference (Table 3).

As suggested by the positive correlation (r = 0.34) between the measurements obtained by means of the two techniques, also respirometry measurements could show the heat wave effect in a deeper analysis. When turf community was excluded from the model, the analysis allowed to investigate the individual responses. Indeed, the estimates of heat wave step and the treatment group was significant in their negative impact on respirometry measure (Table 4). The small standard deviation of random effects in all tables, with respect to their own scale of measurement, suggested small structural differences among species, represented in graphical (Figure 2) analysis.

Table 3. Estimates of the GLM Model for photorespirometry measurements considering interactions (p < 0.001 - ***; p < 0.01 - ** p < 0.05 - *; p > 0.05 - ns).

Coefficients:	Estimate	Std. Error	z value	Pr(<|z|)	
(Intercept)	1.499	0.414	3.62	0.000	***
Time 1	-0.191	0.320	-0.60	0.550	
Time 2	0.493	0.320	1.54	0.123	
Treatments	-0.191	0.261	-0.73	0.46568	
Number of observation:	total=108	species=6			
Random effect variance(s): **Group=species**					
	Variance	StdDev			
(Intercept)	0.617	0.786			
Residual variance	1.357 (std. err.: 0.095)				
Log-likehood:	-192.106				
AIC: 396.2					

Table 4. Estimates of the GLM Model for photorespirometry measurements excluding turf ($p < 0.001$ - ***; $p < 0.01$ - ** $p < 0.05$ - *; $p > 0.05$ - ns).

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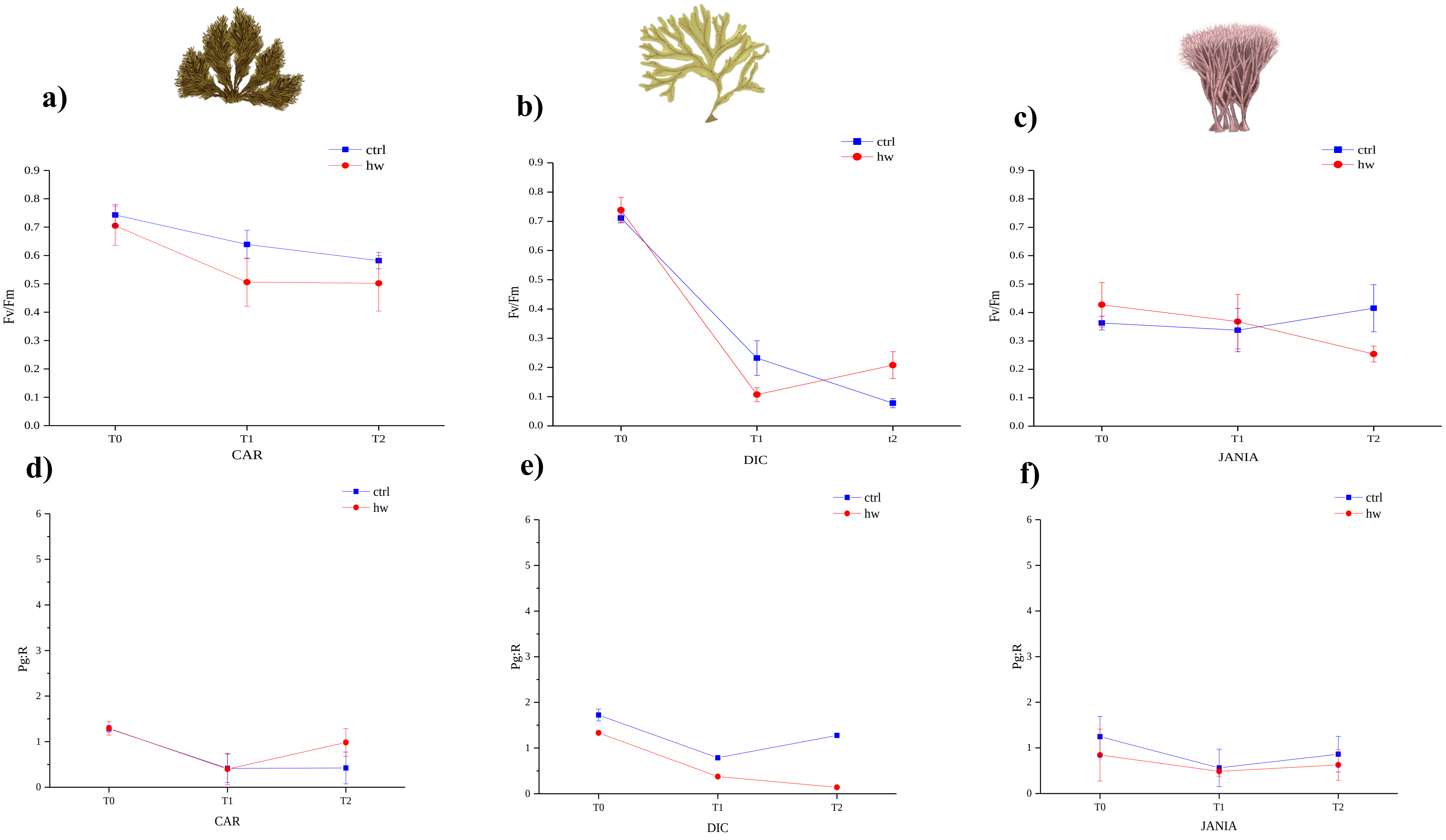
Coefficients:	Estimate	Std. Error	z value	Pr(<|z|)	
(Intercept)	1.720	0.360	4.78	0.000	***
Time 1	-0.597	0.255	-2.35	0.019	*
Time 2	0.039	0.255	0.15	0.877	
Treatments	-0.563	0.208	-2.71	0.007	**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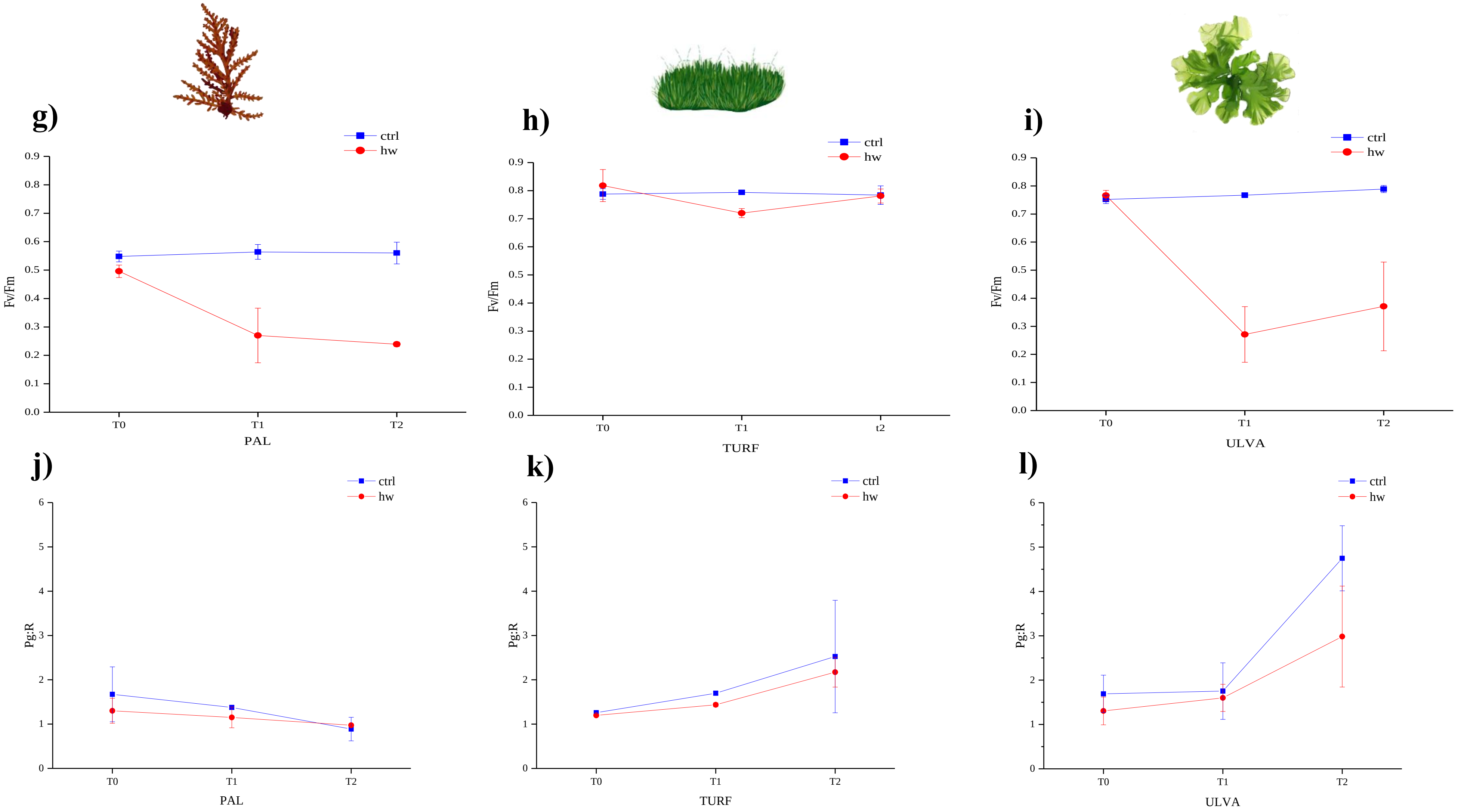

Figure 2 –Maximum quantum yield (Fv/Fm) and Photosynthetic efficiency (Pg:R) of turf community and the five macroalgal species across the experimental time (T0, T1 and T2) relative to control treatment (ctrl), in blue, and heat wave (hw) treatment, in red: *C. amentacea* (CAR) (a,d); *D. polypodioides* (DIC) (b,e); *J. rubens* (JAN) (c,f); *P. tenerrima* (PAL) (g, j); turf community (TURF) (h,k) and *U. rigida* (ULV) (i,l).

Discussion and Conclusion

Rocky intertidal shore conditions are extreme for the marine organisms, bringing them to be adapted to sudden changes of temperature and to resist the desiccation due to low tides. Coastal habitats are impaired by the combination of local anthropic impact on the coasts and global climate change, affecting species vulnerability and causing habitat loss (Strain et al. 2015).

Temperature is the pivotal driver of change within this system and to study how the constant rising of extreme climate events, such as heat wave, affect biodiversity loss is now crucial. Responses to extremes are species-specific because temperature influences directly physiology, individual performance and fitness and functional traits. Population vulnerability to steep increase of temperature depends on spatial and temporal distribution of the stressor, on the environmental and body temperature relationship and on the relationship between the body temperature and the performance of the organism (Harley 2008). By studying the individual functional traits of the species, it is possible to make some predictions about biodiversity loss, the ensuing alterations of the ecosystem functioning and the potential loss of ecosystem services essential for human well-being.

This study focused on the more abundant rocky intertidal macroalgae, of the autumnal community, as they are the major primary producers of the coastal environment and are usually recognized as key species within the communities of temperate marine systems. Macroalgae are vulnerable to disturbance and their fundamental role in structuring communities makes them a privileged candidate to test the long-term consequences of species loss on the function of ecosystems (Tait et al. 2011). Measurements of photosynthetic output provide informative metrics of the health and resilience of macroalgae (Bruno et al. 2005, Wernberg et al. 2010) and are routinely used to understand the effects of various stressors over small (physiological) and larger (ecological) scales (Tait et al. 2017). In this study to evaluate the effect of an extreme climate event such as a heat wave on the photosynthetic responses of macroalgae, functional traits are measured through two techniques (photorespirometry and variable chlorophyll a fluorescence) throughout three times: in ambient condition before the treatment exposure (T0), after four hours of treatment (T1), and after 24 hours from the treatment end to assess any recovery time (T2).

Consistent with the belonging morpho-functional group, algae with the higher photosynthetic efficiency were foliose species *Ulva rigida* and turf community formed of filamentous and foliose green algae. As described by Steneck e Dethier in 1994, the filamentous algal group comprises of small-sized thalli formed of one file of unidirectional growth cells, whereas the foliose show a thallus made of one layer of bidirectional growth cells. These two low-structural complexity groups maximize the exposure of photosystems to the light and, as Orfanidis and co-workers defined in 2001, they constitute the highest productivity groups. Conversely, the species with the lowest photosynthetic efficiency found was *Jania rubens*, a coralline-articulated algae, whose thallus is mostly calcified.

At T1, after four hours of emersion, results showed a strong effect on all the algae. It is known that high temperatures negatively act on efficiency and growth of *U. rigida* and *P. tenerrima* (Graba-Landry et al. 2018). In the case of *U. rigida* it is also possible that the effect of experimental treatments has been amplified by individually exposing foliose thalli that, although monolayer, in nature present a three-dimensional morphology, given their overlapping laminae, which likely reduce their vulnerability to disturbance.

In particular, *D. polypodioides* has shown to suffer from both emersion at ambient temperature and emersion at 38 °C. The algal impairment during emersion can be explained with its zonation. It is a typical infralitoral algae, but it is found in the lower horizons of the intertidal habitat and in rocky pools. This result is consistent with other studies, where it is shown that the optimal temperature for the growth and survival of the spores is at 23 °C, and temperatures higher than 28 °C and lower than 18 °C are damaging for this genus (Photen et al. 1983).

C. amentacea is a canopy algae foundation species of the rocky intertidal Mediterranean that plays a key role in monitoring structural complexity, productivity, nutrients cycle and biodiversity of this habitat. Strain and co-workers conducted a study in 2015 to assess the vulnerability of a *Carpodesmia* species to a combination of local anthropic stressors, such as water quality (amount of sediment and organic load), with global stressors due to climate change (increase in temperature). Their results show that *Carpodesmia* is more impacted by the combination of several *stresses* when one of them is a chronic thermal increase by 3 °C, raising the temperature up to 27 °C. Moreover, a recent study carried out in North Adriatic with a *Cystoseira* species showed that early and extreme episodes of warming could play a decisive role in triggering the survival of subsequent life stages (correct timing of zygotes releasing, growth of germlings, and also the vegetative phase of adults) (Bevilacqua et al. 2019). This is consistent with the results of this study and makes hypothesize that in a scenario where extreme climate episodes are increasing in intensity and frequency, the fate of this species is strongly threatened.

Based on descriptive analysis, *J. rubens* and turf community have showed a higher ability to cope with the thermal stress. In a one-year study aimed to characterize the photosynthesis and dark respiration of an incrusting coralline algae in response to climate change, by evaluating the effects of different acidification scenarios and of thermal increase by 3 °C, authors concluded that dark respiration is not altered by a thermal increment during the hot seasons and is positively affected during the cold ones. Moreover, photosynthesis showed benefits from a thermal increase during the winter (Martin et al. 2013). Consistently with these results, it can be hypothesized that coralline algae like *J. rubens* are not impaired by an increase in temperature. So, also in this scenario, although the amplitude of thermal increase due to the heat wave simulation is very different, *J. rubens* does not seem to change its performance and is still able to buffer the negative effects of environmental change. The resistance of turf morphology to stress is consistent with the characteristics of this aggregation that occurs to adapt to long emersion time by protecting its internal filaments. Also, turf is a simple assemblage generally formed of opportunistic species, characterized by short biological cycles and broad ecological valency. Turf colonization is typically of the early succession stages and in intertidal habitat represent the alternative state that may result from a catastrophic transition from the canopy-dominated state (Rindi et al. 2017).

Overall the species are not able to restore their initial conditions and the effect of the thermal stress is still present. However, some species show a higher ability to recover.

Turf and *J. rubens,* as discussed for T1, have not strongly changed their performance following a heat wave so the results after 24 hours cannot consider as a real recovery time. In particular, referring to the respirometric data, when turf community rates is not included in the statistical analysis, the effect of the treatment at T1 and T2 becomes significant, showing that the community might have a buffer role with respect to the single species in coping with a climate extreme.

In a study from 2017, conducted in the Korean rocky intertidal zone, the capacity of algae community to recover after events of total removal of the thalli was tested. The re-colonization of the substratum and the speed of this process have been used as response variables. A species belonging to the genus *Ulva* treated in that study was proved to be the fastest in colonization and to reach the highest coverage levels. Its success, also, does not seem to be changed by seasons (Kim et al. 2017). This could explain the trend of recovery of *U. rigida* described in this study. Finally, *P. tenerrima* and the brown algae *D. polypodioides* and *C. amentacea*, that under stress behaved similarly, have reacted differently during recovery time. The species *C. amentacea* after 24 hours didn't restore the performance but seemed to show a positive trend, whereas *P. tenerrima* and *D. polypodioides* still showed a strong effect of the heat wave.

From the numerous studies conducted by modern ecology, by now it is certain how biodiversity loss plays a key role in altering the ecosystemic functioning, but much uncertainty regarding the role of climate change still remains (Pires et al. 2018). In order to predict the responses to a climate extreme, in this study, the role of temperature in the alteration of functional traits has been investigated.

Photosynthetic measurements have proved suitable tools for assessing the consequences of stressor on macroalgae. *In vivo* chlorophyll a fluorescence and respirometry are complementary techniques for measuring photosynthesis and provide crucial information for the integration of physiology and ecology to understand the driver of change in macroalgae. However, without the simultaneous use of respirometry, variable fluorescence methods is unable to determine the net carbon balance but only provide an estimate of the response of the photosystems (Tait et al. 2017). Moreover, it might be worth to consider that photosynthetic rates based on individuals can be overestimated because don’t take in account mechanisms such as the self and neighborhood shading effects (Richards et al. 2011) resulting in responses of natural communities that can be quite different from those of isolated plants (Binzer et al. 2006, Tait et al. 2014).

Overall, the results of this study have shown that macroalgae species undergo a heat wave by modifying the expression of their functional traits but have species-specific responses, reminding us that the action of a stressor does not address its response in one single direction only (Connell et al. 2017).

In a future scenario with increase in temperature, in an extreme environment such as the intertidal, liable to tidal excursions, not all the species examined will have the characteristics to persist. Consequently, it can be imagined an intertidal dominated by algae forming turf and by articulated coralline species such as *J. rubens*, that would replace structuring species such as *C. amentacea*. This would entail an associated remarkable loss of biodiversity and stability of the system. A loss of species, with an ensuing alteration of biodiversity in a community of primary producers, would imply a reduction in the productivity of the ecosystem, decreasing the biomass, the consumption of resources and the efficiency of biogeochemical cycles in which the quality and quantity of ecosystemic services supporting human well-being are embedded.

Implication for BEF

Characteristic traits of species and the functions these species perform are crucial determinants for ecosystem processes. Consequently, in this study, through a functional trait-based approach, the impact of a climate extreme event such as a heat wave has been investigated. In doing so, the measurement of the photosynthetic efficiency of five intertidal macroalgal species and a turf community, through the two most used techniques (photorespirometry and PAM fluorometry) have been recorded.

Overall, results suggest a main role played by temperature to drive the photosynthetic response of macroalgal species impairing the performance of the target species at the end of the increased temperature and after 24 hours. The frequency, intensity and variability of extreme events is predicted to increase as a consequence of climate change. These results add information on the relation between climate change variables and biotic responses suggesting that the former will translate into ecological impacts threatening the biodiversity. Biodiversity loss is known to cause general reduction in ecosystem function on average (Stachowicz et al. 2007) and macroalgal loss will have crucial effects on BEF relationship, especially in the intertidal zone where they are the most conspicuous organisms.

- Macroalgae are the most productive marine macrophytes at the global scale, playing an important role within the blue carbon schemes that support ocean carbon sequestration globally.

- They critical contribute to the ecological function of many ecosystems through their diverse roles as primary habitat providers; food provisioning for grazers, thus influencing food web; protection from physical agents such as heat, desiccation, snow, restricting the variability in humidity and temperature for organisms that find refuge living under the canopy or inside the turf; protection from dislodgement due to wave action; providers of shade.
- When ecosystem engineer or foundation species (e.g. *Carpodesmia amentacea* in littoral frindge) they control the structural complexity, and the biodiversity in rocky reefs.
- Macroalgae prevent coastal erosion and, in some environment, they mediate sediment trasport.
- They contribute to the maintenance of biogeochemical cycles and water oxygenation.
- They represent food supply for the future

In conclusion, when making predictions about the effect of macroalgal biodiversity loss due to climate change it is needed to consider the consequences for other species, for ecosystem functioning of the marine system and ecosystem services based on these functions (e.g. water quality regulation and ecotourism).

Author contribution. Chiara Giommi conceived the idea, led the writing of the ms., performed the experimental design and carried out, with Marta Lucchese, all experiments and lab analyses. Chiara Giommi and Marta Lucchese drafted the first versions of this ms. and with Gianluca Sarà and Brian Helmuth, have written the final version. Marta Lucchese drew algae in Figure 2. Brian Helmuth and Gianluca Sarà provided funds, instrumentations and lab facilities.

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Chapter 3

The role of an ecosystem engineer’s in driving its own response to a heat wave and in modulating the community functioning

Abstract

It is widely accepted that biodiversity promotes ecosystem functioning through different mechanisms. The strength of biodiversity effects on functioning could depend on the role that species play in a system (e.g. keystone species or ecosystem engineer) rather than the number of species in the system. Bivalve *Mytilaster minimus* is an ecosystem engineer that forms dense mussel beds, increasing spatial complexity and hosting biodiversity hotspots across the intertidal Mediterranean rocky shores. To date, the linkage between extreme climatic events and ecological responses remains uncertain and more studies are still needed. The goals of this study were to assess the effects of a heat wave on the metabolic functioning of a mussel bed, of the population *M. minimus* and of the species assemblages associated to the bivalve and to test whether responses were driven by the density of the mussel beds. To do so, in autumn 2018, in Southern Italy, along 3 different levels of density (low, medium, high), for each component, randomly 6 quadrats 10 x 10 cm (N=54) were scraped. Replicates were divided in two groups, one exposed to increasing temperature (T=38 °C) and the other maintained at environmental temperature. The heterotrophic oxygen consumption (mg O_2 $l^{-1}h^{-1}g\text{-}DW^{-1}$) as a proxy of ecosystem functioning was adopted.

The metabolic rates of the ecosystem engineer species *M. minimus*, in both treated and controlled conditions, did not differ from the metabolic rates of the mussel beds; this would indicate that *M. minimus* plays a key role in driving the functioning. Metabolic rates of *M. minimus* and of the whole mussel bed community decreased with the increasing of the density of the mussel. Conversely, when the metabolic rate of the macrofaunal community was considered no pattern was depicted.

In addition, the thermal stress didn’t affect neither the mussel bed community, nor the population of *M. minimus* (ecosystem engineer species) nor the species assemblages associated to the bivalve (macrofaunal community). However, at medium density, both the metabolic rates of the mussel taken individually and those of the mussel bed under stressful condition differed from those in controlled conditions, indicating the presence of mechanisms responsible for this response. Finally, it has been hypothesized a balance between facilitation and intraspecific competition that varies according to density and environmental conditions could explain that pattern.

Keywords: Ecosystem Engineer, Density, Community Functioning, Metabolism, Heat wave, Climate Change.

Introduction

Natural systems are the result of a diversified and complex relational network in which human beings take part. The anthropogenic impact on environment causes changes in the ecological systems. The latter respond interactively to change and produce an environmental change – a phenomenon known as global change.

Global change causes direct effects on the ecological systems such as eutrophication, habitat degradation (Pimm et al. 1995; Chapin et al. 2000; Barnosky et al. 2011) and biodiversity loss. Indirect effects ensue, such as the alteration of biogeochemical cycles and of the dynamics whereby ecosystem components exchange energy and matter, actually changing the resulting ecosystem functioning.

The interest on ecosystem functioning has increased at the beginning of the 90ies because of new awareness about the impossibility to fully understand the flows of matter and energy without considering the vector and processor of these flows, i.e. the biotic component. How biodiversity, understood in its broader sense of biological diversity on all levels of organization, can affect the processes and functioning of ecosystem has been studied ever since (Tilman et al. 2014).

In communities in which a particular species plays a key role, its loss causes a considerable change in terms of functioning. This is true with keystone species, capable of controlling the dominance of other species, or ecosystem engineers (habitat-former) species, that can modify the resource availability through an increase in the structural complexity of the habitat (Lawton et al. 1994). In this case, the collapse of functioning is caused by the disappearance of a species that modeled the community, thus it is not the number of species *per se* that affects functioning, but species identity, functional differences among them and their interactions.

The ecological role of a species in natural systems is the product of an expression of its functional traits. A trait is a morphological, physiological, phenological or behavioral characteristic that can be measured individually, without needing to refer to the environment or other levels of organization (Violle et al. 2007). Species traits also include effect traits, that can affect the features of an ecosystem, and response traits, that is, a set of traits that allow the organism to persist in the community despite an environmental change. A trait can belong to both categories, for example water retention can modify the hydrological-reological features of the environment and, at the same time, provide a defense against the essication in case of temperature increase.

Diaz et al. (2013) related Effect traits with Specific Effect Function (SEF) and Response traits with Specific Response Function (SRF). A Specific Effect Function is the ability of a species to influence the ecosystem properties, and it is the outcome of one Effect trait or of the interaction of several Effect traits. A Specific Response Function is defined as the ability of a species to maintain or increase its number in response to an environmental change, biotic, such as competition or predation, or abiotic, such as pollution or temperature increase. The contribution of a species to the ecosystem functioning, thus, depends on the combination of these two functions. Particularly, if the species has high influence on the ecosystem features (SEF) and low resistance to disturbance (SRF), and is therefore more vulnerable, the species persistence could be at risk and the ecosystem functioning could be easily changed.

Alongside the functional traits, the species abundance in the system and the number of relations that they establish with the other components contribute to determine their importance. According to the *Mass ratio hypothesis* (Grime 1998; Garnier et al. 2004) a species affects an ecosystem feature proportionally to its (bio)mass. Then, the loss of an abundant species that plays a key role in an ecosystem can hugely condition its functioning. This is the case with the relationship between diversity and functioning in the presence of an engineer species.

The ecosystem engineers are abundant species with many facilitating relationships within the community. These species can: (i) have a positive impact on specific richness and abundance; (ii) play a critical role in structuring the community; (iii) determine its productivity (Hector et al. 1999; Jenkis et al. 1999).

Diversity of communities structured by ecosystem engineers appears to be density-dependent (Begin et al. 2004; Christie et al. 2009). Bertness (1989) observed how a *Semibalanus balanoides* population succeded in buffering thermal stress perceived during the tidal excursions with increasing intensity as its density increased. In the case of an ecosystem engineer the functional traits involved of its resistance to stress, by increasing its persistence in the system, maintain the ecosystem characteristic for which it is responsible.

In a global change scenario, thus, it is important to enquire into how foundation species respond to environmental change drivers. Moreover, to know the responses of the community to stress along the habitat-former density gradient could provide a predictive and management tool in a context of conservation.

The population of an ecosystem engineer and the community to which it belongs represent two different levels of ecological organization, with different emerging properties. Hence, their responses to environmental change will be different. Metabolic ecology is able to provide a tool that makes the measure of their resistance to stress homogenous and comparable (Humphries & McCann 2014). Indeed, metabolism is the biological processing of energy and matter and through the metabolic rate almost all biological activities are determined (Brown et al. 2014), helping in connecting all the levels of ecological organizations (Savage et al. 2004). To measure the metabolic rate of a population and a community to environmental change, thus, provides a comparable value of their stress when faced with an external disturbance.

Mussels work as ecosystem engineers and tend to aggregate in structures called mussel beds in the intertidal habitat. During low tide or under stressful conditions, such as those generated by a heat wave, intertidal habitat undergoes irradiation and desiccation that limit the physiological performance of the organisms living in it (Raffaelli & Hawkins 2012). Mussel beds, with an increase in structural complexity, create microhabitats capable of maintaining the values of internal humidity more or less constant, giving the macrofaunal organisms not only a shelter from biotic stressor, such as predation, but also from abiotic stressor, such as desiccation and heat wave. These microhabitats facilitate the persistence of a variety of small invertebrates (Arribas et al. 2013), such as amphipods, isopods and gastropods (Chapman et al. 2005). Their shells provide substrata for sessile organisms, such as algae, Ascidiacea and cirripedes crustaceans or semi-vagile animals such as limpets. The spaces and crevices among mussels are colonized by vagile organisms, such as crabs, errant polychaetes, amphipods e bivalves (Seed & Suchanek 1992). Moreover, infaunal organisms such as polychaetes and sipunculids settle in the sediment, waste products and shell fragments within the mussel bed.

The ecological role of mussel beds in mitigating thermal stress can be attributed to several individual effect traits, such as the mussel morphology, the ensuing capacity to retain water and humidity and to population traits such as the gregarism. To measure how the metabolic rate of the *M. minimus* population and of the community changes in response to a thermal disturbance along the density gradient of the mussel bed would allow observing the mitigating effect both on mussel population, at an intraspecific level, and on the entire community, at an interspecific level, and to quantify a population's contribution to the resistance of the community. The resistance, that is, the capacity of a system to counter an external disturbance, is one of the three aspects that contribute to describe the stability of an ecosystem; hence to measure it gives an indication about the functioning of the ecosystem itself (Scherer-Lorenzen 2005).

Previous observation showed that biodiversity associated increased with the increasing in the density of the mussel beds. As it is ecologically consolidated that the ecosystem functioning is diversity-dependent, if diversity proved to be ecosystem engineer-dependent density, it could be hypothesized that, when a community is structured by an ecosystem engineer, ecosystem functioning is not only affected by the presence of the ecosystem engineer *per se* but is also modulated by its density and that it can decrease when the density is not such as to sustain sufficient levels of associated biodiversity. The aims of this study is to investigate the role of a intertidal ecosystem engineer, the bivalve *Mytilaster minimus*, in driving the functioning of the community, to understand how the ecosystem engineer and the entire community respond to a heat wave and if the ecosystem engineer is able to mitigate the thermal stress based on its density.

Material and Methods

Sampling and acclimation

The study area was located along the shores of Altavilla Milicia (Palermo) (Figure 1).

Figure 1 – Satellite photograph of the sampling area.

The sampling site was characterized by an intertidal rocky platform covered by an extended mussel bed where three different density levels of bivalve *M. minimus* bed were identify. In autumn 2018, at low tide, for each density level, twelve quadrants 10x10 cm were randomly collected.

Density level was standardized on percentage coverage (Figure 2): High density: 100% of coverage; Medium density: 56% of coverage; Low density: 28% of coverage.

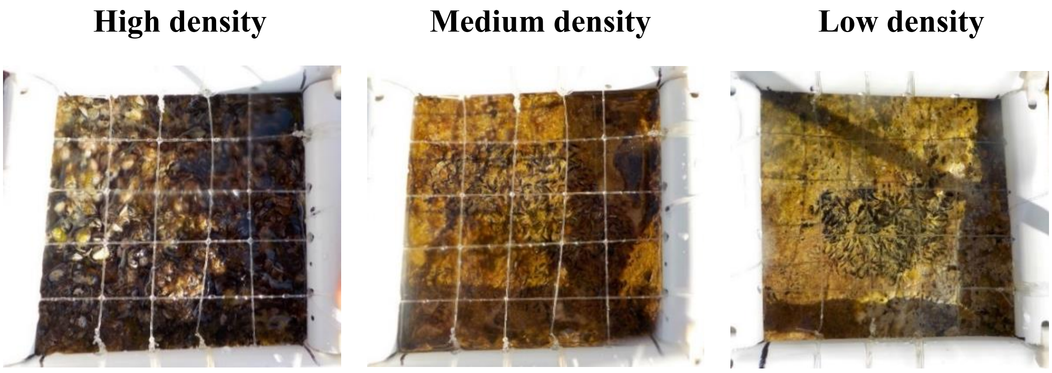

Figure 2- Photographs of different density levels during sampling.

Samples were brought back to the laboratory and prepared for the experiment. Here 18 samples (six for each density level) were split separating the ecosystem engineer population from the macrofaunal community. Samples were washed with sea water to remove the sediment and sieved (0.50 mm). Individuals of *M. minimus* of each sample were collected and used as the replicate for the population level, while the animals remaining in the sieve, i.e. invertebrates larger than 0.50 mm, along with sediment (granulometry > 0.50 mm), were isolated and used as replicates of macrofaunal community associated to the mussel bed. Whenever present on the sieve, macroalgae were removed from the sample, and epiphytes on *M. minimus* valves were also removed, though avoiding valve damaging. Thus, from 18 quadrants, 36 experimental replicates were obtained. Finally, other 18 quadrants were used as replicates of the entire mussel bed community for the different density levels. All the replicates (N = 54) were acclimated for 24 hours at 23 °C, corresponding to seawater temperature at the time of sampling and the tidal cycle was kept.

Experimental design

At the end of acclimation, replicates were divided into two groups: one exposed to emersion at increasing temperature and the other kept in emersion at ambient temperature and used as a control. To simulate the heat wave, replicates were placed under stands provided with infrared lamps of 100 watt (ZOOMED). Air exposure lasted four hours to simulate the low tide condition of maximum air exposure typical of a day with high atmospheric pressure. The temperature reached 38°C corresponding to the highest air temperature recorded by Italian Institute of Environmental Research (ISPRA) temperature network (http://www.mareografico.it/) in Palermo. Temperature was constantly monitored through a thermometer placed near the specimens.

At the end of the heat wave, to measure consumption rates, replicates were placed in sealed glass respirometric chambers (320 ml for macrofaunal community; 3000 ml for *M. minimus* population and the community mussel bed) containing filtered (Whatman GF/C 0.45 µm) and air-saturated seawater at salinity 38. Respirometry was carried out at 23 °C coincident with the seawater temperature recorded during the sampling. Temperatures were kept stable by means of a thermal bath and monitored throughout the recording period. To ensure the constant mixing of the water, each chamber was stirred with a magnet bar and an individual stirring device (Giomi et al. 2016). Also, to avoid organism dispersion inside the chamber, replicated of the mussel bed community and *M. minimus* population were placed in a Petri plate and those of macrofaunal community were placed inside a mesh (0.50 mm). All the measurements were performed in dark, so that the values were not altered by the photosynthetic oxygen production by the algae that had not been accidentally removed.

The concentration of dissolved oxygen (ppm or mg/L) was measured by means of 3 optical oxygen meters (Pyro Science Firesting O_2) through the use of specific software (Pyro Science) (Montalto et al. 2017). Measurements was performed *in continuum* for six hours. At the end of the experiments, individuals di *M. minimus* in both the population and the community replicates were counted and for the 10% of the total number of each replicate, the valve length was measured. Then, each replicate was dried at 105 °C for 24 h and reweighed to determine the Dry Weight (DW, g). Respiration rates were reported as oxygen consumption per unit mass and expressed as mg O_2 l^{-1} h^{-1} g^{-1}dry tissue. The value of the metabolic difference between treated and controlled samples was considered as the ability to cope with the heat wave thus acting as a proxy of community functioning.

Statistical analysis

Differences in metabolic rates were analysed by means of a three-factors analysis of variance (ANOVA). Ecological complexity (the population of *M. minimus*, the species assemblages associated to the bivalve population, the whole mussel bed), density (three levels: Low, Medium, High) and treatment (two levels: heat wave, control) were considered fixed factors. Before performing ANOVA, the assumption of homogeneity of variance was tested *a priori* by means of the Cochran's test. Data, where needed, were transformed (log x+1). Student-Newman-Keulls (SNK) test allowed the appropriate means comparison (Underwood 1997). ANOVA was carried out using the GMAV software (version 5.0).

Results

The comparisons between all heat wave (HW) and control treatments (CTRL) was significant (*p*<0.01, Table 1). As shown in Table 1, differences between levels of complexity (the population of *M. minimus* hereafter "Pop", the species assemblages associated to the bivalve population hereafter "Macro" and the whole mussel bed hereafter "Com") resulted also significant (*p*<0.001), as well as the differences among density levels (High hereafter "H", Medium hereafter "M", Low hereafter "L") (*p*<0.01).

Table 1 – ANOVA of results among different levels of complexity, density and treatment.

Source	**df**	**MS**	**F**	***p***
Complexity (COMP)	2	11.21	26.53	0
Density (DENS)	2	3.44	8.15	0.001
Treatment (TREAT)	1	3.16	7.47	0.009
COMP * DENS	4	1.7	4.02	0.008
COMP * TREAT	2	0.42	1	0.379
DENS * TREAT	2	2.3	5.45	0.009
COMP * DENS * TREAT	4	0.71	1.68	0.177
Residuals	36	0.42		

Oxygen consumption at varying mussel density and complexity.

Oxygen consumption varies significantly between high density, medium and low density levels ($p<0.01$); the two latter did not show any difference between them (Table 2). Oxygen consumption increased with density (Figure 3).

Table 2 – SNK test results among density levels.

Density	**Low**	**Medium**	**High**
Low		0.24867	0.00115
Medium	0.24867		0.00917
High	0.00115	0.00917	

Regarding the ecological level of complexity, significant differences among levels resulted from ANOVA (Table 3), with respective mean oxygen consumption levels for mussel population, macrofaunal community and whole community of 2.66 mg l^{-1} h^{-1} g^{-1} ± 3.08, 0.40 mg l^{-1} h^{-1} g^{-1} ± 0.43 and 0.81 mg l^{-1} h^{-1} g^{-1} ± 0.72.

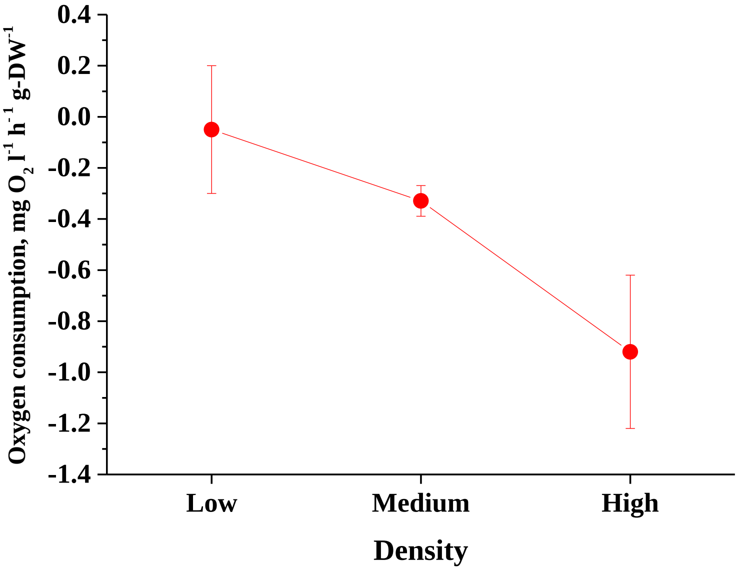

Figure 3 – Oxygen consumption at varying mussel density.

Table 3 – SNK test results among complexity levels.

Complexity	**Pop**	**Macro**	**Com**
Pop		0.00013	0.00055
Macro	0.00013		0.00171
Com	0.00055	0.00171	

Analyzing in detail among density levels, mussel population resulted significantly different from whole community only for low density levels, while no significant differences emerged between high density population and the three community density levels. Regarding the effect of density on oxygen consumption among structural complexity levels, oxygen consumption of population varies significantly as density varies, decreasing as the latter increases, consistently with the trend shown in Figure 4. Oxygen consumption rate of community varies significantly just between levels of high and low density. The macrofaunal community does not show any difference in oxygen consumption among the different density levels

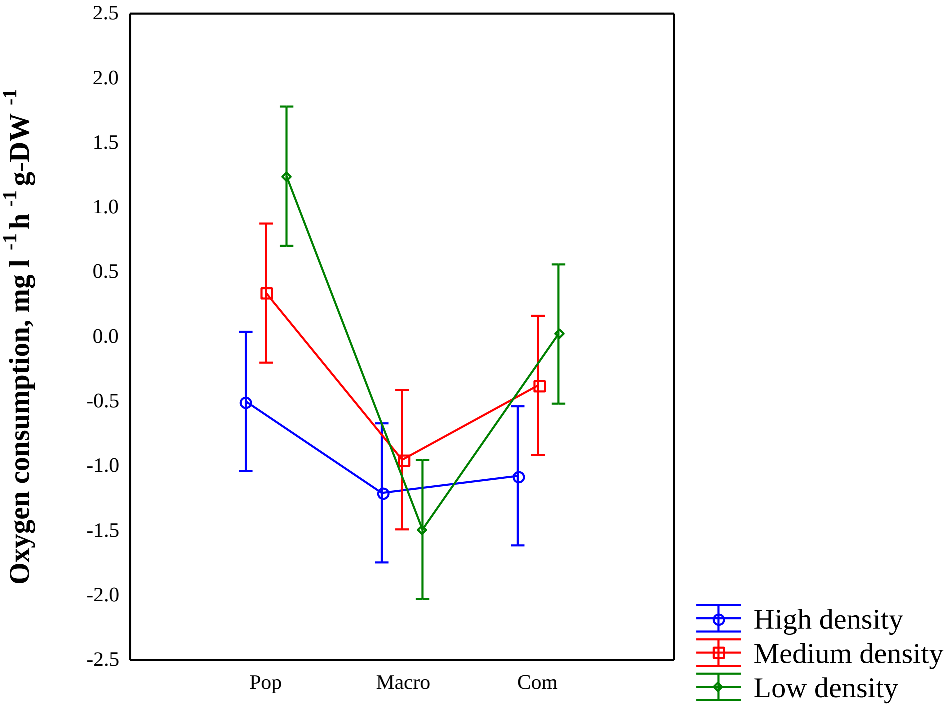

Figure 4 – Oxygen consumption relative to the interaction of different levels of complexity and density (Pop=the population of *M. minimus*, Macro=the species assemblages associated to the bivalve population and Com=the whole mussel bed).

Effect of the heat wave on oxygen consumption at varying complexity levels.

Oxygen consumption between levels of treatment (HW; CTRL) was an estimate of the stress experienced under heat wave. To test the influence of density in mitigating the thermal stress, the combinations of density levels and treatment levels were analyzed.

Among the complexity levels, only the ecosystem engineer's population showed significantly differences between the treatment levels (Table 4).

Table 4 – SNK test results among complexity levels and treatment levels.

Complexity/Treatment	Pop CTRL	Pop HW	Macro CTRL	Macro HW	Com CTRL	Com HW
Pop CTRL		0.00982	0.00130	0.01179	0.16205	0.39426
Pop HW	0.00982		0.00013	0.00013	0.00043	0.00281
Macro CTRL	0.00130	0.00013		0.32646	0.05420	0.00835
Macro HW	0.01179	0.00013	0.32646		0.16611	0.05269
Com CTRL	0.16205	0.00043	0.05420	0.16611		0.32038
Com HW	0.39426	0.00281	0.00835	0.05269	0.32038	

Table 5 – SNK test results among density levels and treatment levels.

Density/Treatment	High CTRL	High HW	Medium CTRL	Medium HW	Low CTRL	Low HW
High CTRL		0.81831	0.94484	0.00158	0.02284	0.02351
High HW	0.81831		0.95133	0.00189	0.01528	0.02295
Medium CTRL	0.94484	0.95133		0.00184	0.03463	0.03037
Medium HW	0.00158	0.00189	0.00184		0.34049	0.24085
Low CTRL	0.02284	0.01528	0.03463	0.34049		0.81998
Low HW	0.02351	0.02295	0.03037	0.24085	0.81998	

High and low density levels were not significantly different. However, there were significant differences between treatments at low density, showing higher oxygen consumption value in HW treatment (Figure 5) (Table 5).

Also, a significant difference between treated and control populations was found only at medium density level (*p*=0.0020) (Figure 5), which explains the difference found between control and heat wave treatment within the population complexity level (*p*=0.00982) in Table 4.

Both mussel bed and macrofaunal community didn't show significant differences between levels of treatment and density levels.

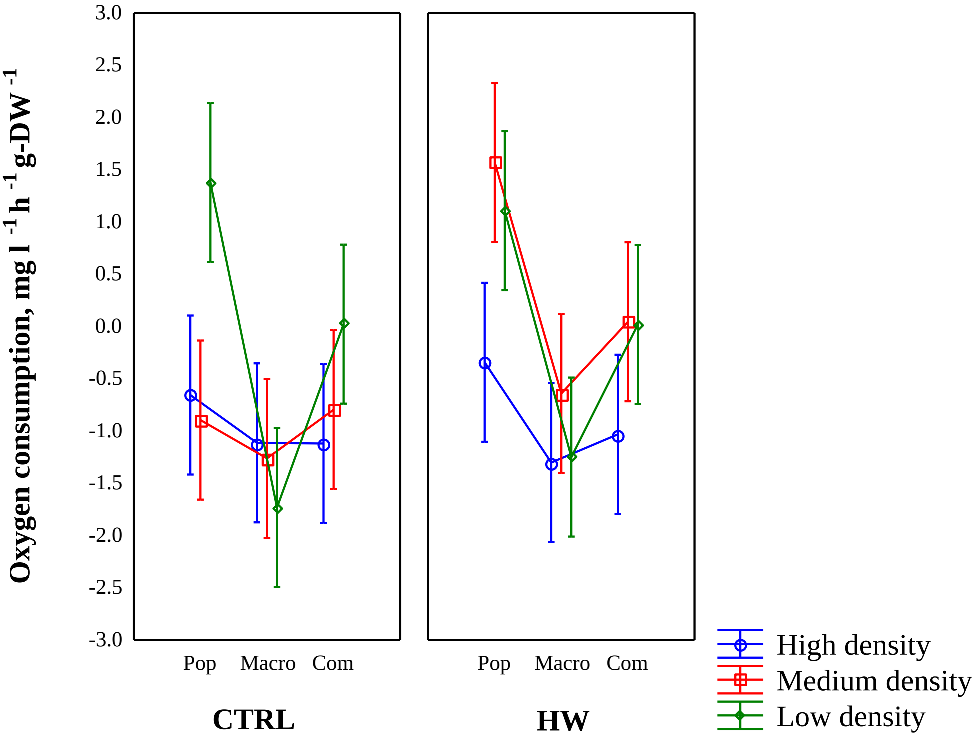

Figure 5 – Oxygen consumption relative to the interaction of different levels of complexity, density and treatment (Pop=the population of *M. minimus*, Macro=the species assemblages associated to the bivalve population and Com=the whole mussel bed).

Discussion

Climate change affects the ecosystem functioning dramatically. It is therefore important to know how natural systems basically function and their response to change. In the last years, ecological studies focused on how a community responds to environmental stressors and often aimed to measure the stability of different natural systems (Isbell et al 2015a; Ghedini et al. 2015). The understanding of natural responses and data, though, depend on the functioning measurements that are used, the stressor considered and the kind of ecosystem under scrutiny. Each community is structured in a different way and how depends on its features and from them the responses to stressors change. Comparing different study cases on disturbance and functioning, it has also been observed that different components of stability can give different responses to the same disturbance (Pires et al. 2018). The focus on individual responses is recognized as a way to bypass the issue that natural interactions variability raises for ecology. To study how the individual responds to disturbance and how its functional traits change according to this may allow to predict its consequences on higher organizational levels, where individuals are still the core. However, it is needed to consider that individual functional traits might also not manifest themselves in the community to an extent that allows to measure them individually, and in some cases, they can have an interaction-dependent manifestation. While the importance of functional traits is unquestioned, it is needed to collaterally analyse the community response as a whole and to consider the structure of a community, the role of structural components and consequently functional components. In doing so, a metabolic proxy of functioning such as the oxygen consumption, unifying all hierarchic levels of ecological organization allows to compare the different complexity level as done in the present study. Here, indeed, an ecosystem engineer, the bivalve mussel *Mytilaster minimus* showed specific and variable responses as a function of the degree of aggregation. Such a result could be in line with the Grime's *Mass ratio hypothesis* (MRH) theory which suggested that the traits of the dominant species in contributing to productivity (biomass) can play a key role in regulating the ecosystem functions. Our mussel bed functioning community was not different from that of the population suggesting that, independently from the density, the community metabolism was lower than the metabolism of the population. In the interaction between species there is, even when an ecosystem engineer is present, a community effect that reduces the overall metabolic rate. This can be ascribed to the species-specific features of the interacting species (*sensu* Dehling et al. 2014). Even though the community metabolic rate is lower than that of the population, the difference has proved statistically significant only in case of low density (Figure 4).

Consistently with the MRH, the role of the most abundant species in affecting ecosystem functioning decreased its importance with species density and consequently with the biomass but up to a certain threshold. At higher densities (high and medium level), the mussel population influenced the community functioning showing a high SEF. Thus, the mussel population, characterized by a high SRF and SEF, crucially contributed to the community functioning affecting its resistance.

The relationships between the environment and the biotic components changed not only the energy actually available for the individuals, but also the way by which biota uses and allocates it to different purposes.

In this respect, a clear decreasing trend of metabolic rates with an increase in density emerged according to a pattern that is quite common in ecology (Figure 3). The explanation is to be sought in the density-dependent mechanisms that develop as the number of individuals per surface unit increases. At high density, there is a reduction of fitness due to a decrease of per-capita resources, which results from increasingly stronger competitive relationships (Antonovich & Levin 1980, Violle et al. 2010). Resource reduction is the result of a decreasing energy availability for growth and reproduction (Sinclair et al. 1985, Jenkins et al. 1999). This would explain the size/density negative relationship recorded in the population. The difference between rates of assimilated energy and rates of metabolic cost returns the energy *surplus* that can be spent in reproduction. Metabolic rate decrease, thus, represents an adjustment aimed at maximizing the available energy budget.

Ghedini et al. (2017) investigated the variation of ingestion and metabolic rates upon varying density and they showed that both scalarly decreased with density. Although both had a negative relationship with density, the decline in food intake was greater than the decline in metabolic rate with density, decreasing the energy *surplus*. Since in previous studies (Reichle 1968) an increase in metabolic rate had been observed as the size decreased, Ghedini et al. (2017) studied the density/size interaction in determining the resulting metabolic rate. Thus, despite the size reduction with density increase, metabolism did not show any clear increase, as it is more affected by density factor rather than size factor.

In our case study, the influence of size in driving metabolic rate has been excluded, because there was no difference between the size mean values along the density gradient. The trend, thus, reflected a pure density-dependent effect without the interaction of other density-correlated factors. Such a trend was tested both for the population and the community mussel bed, confirming that also in this case the variation of metabolic rate was driven by the density of the habitat former.

Since all the densities have undergone the pulsing increasing temperature event as an expression of a heat wave, our results allowed to analyze whether and how stress influenced that density-dependent trend.

When both the population and the community underwent a heat wave, the metabolic rate measured at medium exceeded that of low density, altering the trend observed under control conditions. In contrast, in the case of the population, the variation of medium density metabolic rate was such that it was significantly different from the control thereby suggesting a heat wave effect. As the trend was similar both at community and at mussel population level and showed up only for a specific density level, once again the major results of the study emerge: (i) the role of the mussel population in affecting the community and (ii) the strong influence of density in driving the functioning and responses.

Density-dependent mechanisms help also in this case to explain the response pattern and we can invoke the negative relationship between density and individual fitness. Such a relationship is really well-studied across the current ecological literature, as increasing the number of individuals per unit of space, the resource availability declines as observed for example in Bertness & Leonard (1997), and Griffin et al. (2008). Nonetheless, there may be strong benefits in living aggregates and at high density, particularly under biotic or abiotic stressful conditions. Bertness' study (1989) is an example of how density-dependent mechanisms allow the buffering action of increasing temperature detrimental conditions. An example using soft bottom infaunal organisms showed that bivalves on one side experienced poor density-dependent mortality (Peterson 1991) while on other side could benefit of the protection against predators thanks to the add value derived from the aggregation (Peterson & Black 1993). As a main consequence, we derive that there are both costs and benefits in living at high density and the net balance depends - at the end - on the degree of environmental stress. Accordingly, the Stress Gradient Hypothesis by Bertness and Callaway (1994) assumed that the importance of the role of facilitation or competition mechanisms is not invariant along an environmental stress gradient, and the facilitation can be greater at high stress levels. Density-dependent facilitation and competition effects could then represent a *continuum* and their net effect depends on the intensity of effects exerted by environmental change. Unfortunately, disturbance gradient was not manipulated in our case study, and then the net effect was determined by a organismal density gradient.

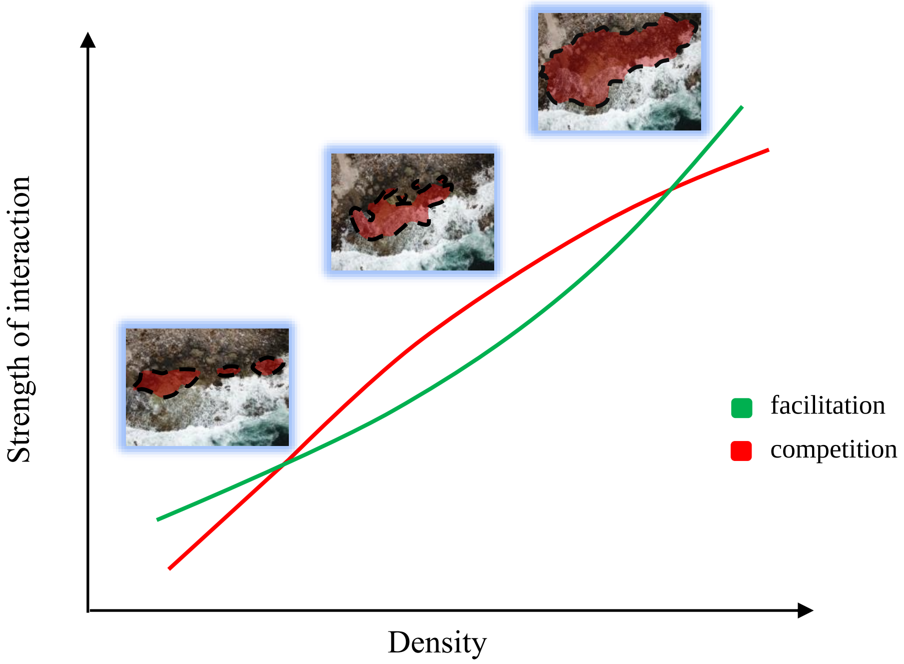

Figure 6 – Potential trends of competition and facilitation interactions along density gradient. At high density competition (red curve) is weaker than facilitation (green curve). At low density both facilitation and competition reach their lowest values, but competition is weaker than facilitation. Competition is stronger than facilitation within the intersection point.

We hypothesized that at high density stress buffering mechanisms such as water retention and defence against essication exceed the intraspecific competition effect, as showed by the mussel population responses, At low density, when resource competition was weaker, perturbations had less or even no impact (Owen-Smith 1990). Finally, medium density showed an imbalance toward competition. At that density level, biotic disturbance due to competition had an additional effect than abiotic disturbance; consequently, metabolic rates increased indicating a thermal stress. We summarized in Figure 6 such a response: at low and high density levels, the net effect is positive; within the intersection points the role of competition exceeds that of facilitation, biotic stress adds to abiotic stress and the net effect is negative.

The increase of the metabolic rate at medium density under stressful condition, was also found in the community – showing the role of the ecosystem engineer population in driving the community. Moreover, community was not effectively stressed at medium density. This could indicate that, although the habitat-former plays a key role in shaping the community functioning, how it does it in terms of direction of responses and ability to resist depended on the magnitude of interspecific relationships drove by density and assemblage type.

Thermal stress didn’t affect macrofaunal component. Macrofaunal individuals were separated from mussel population and so, actually, were acclimatized outside their habitat and such an experimental operation maybe generated an artefact. Thus, what we measured was an overall stressful effect involving both treated and no-treated (i.e. control) animal, generating a confounding effect.

In conclusion, along an intraspecific density gradient, density-dependent dynamics develop. They can favor or penalize each species through a suite of density-driven mechanisms involving environmental condition change, availability of resources, biotic and abiotic disturbances. Natural density found in a gregarious species seems the optimal condition to buffer the effects of environmental stochasticity and this depends on specific-context conditions (Hansen et al. 2019). Climate change add a further complication to this frame as climate driving forces directly alter both abiotic and biotic conditions inducing modifications of the density of a species. Such a result can support the recent ecological theory about ecosystem functioning and possible modifications due to the climate change (Isbell et al. 2015b, Smale et al. 2019). In this context, biodiversity has a central role as promoter and driver of ecosystem functioning (Solan et al 2012; O’ Connor and Byrnes 2013): when a foundation species becomes lost, the functioning is compromised as showed also by these results. Improving the ecological theory with new insights about how any species affects such relationships through the expression of functional traits is crucial to increase our understanding about the role of density and how it changes under stressful conditions as those generate possibly by climate changes. We need to increase the understanding on factors promoting stability and certainly density is the most prominent among them. And, consequently, we think to have further improved the knowledge about how (and why) processes such as competition and facilitation vary proportionally with changes in density. However, when the ability of the ecosystem engineer to respond to a disturbance is in the picture, although at the low and high density the effect can be negligible, a stressful effect as that generated by an external climate-driven event, such as our simulated heat wave, was able to affect the population of the mussel at the medium density impairing the proportionality of trends.

The main conclusion of this study may be that the analysis of a single process develops along the density gradient is not sufficient and it is not able to provide an adequate tool to predict the persistence over time of a foundational species and its associated biodiversity pool. Instead, we should increase the use of the magnifying glass through the experimental studies in order to get major details about the interactions among different processes at each level of environmental disturbance. In so doing, we will be able to develop a realistic management of ecosystems when assessing the effects of environmental change. Thus, with in mind management and conservation purposes, we suggest to investigate how much large is the net effect of density level/environmental conditions interaction on the persistence of a species in order to be able to predict the effects when both factors vary. Through a high temporal frequency monitoring of environmental variables such as temperature (e.g., through sensors deployment) and mapping of the foundation species (e.g., drone aerial imagery), we will be able to better understand to what extent the density-disturbance interaction may have negative effects, and therefore assess the functioning of these systems. In this way, bypassing the knowledge of biodiversity and studying only density, it will be possible to get information about the functioning, by creating a new generation of DEF (Density Ecosystem Functioning) experiments and monitoring frameworks. While to control the effects of a disturbance in the short term (especially if of climatic origin) is not feasible, the knowledge of the interaction between disturbance and density will enhance manager’s capacity of intervention at short term scale on the density factor by designing compensating measures potentiating the conservation action. This would indirectly ensure the maintenance of high levels of biodiversity and of the functioning depending on it, such as the capacity to cope with climate extreme events (viz. a heat wave). Such an understanding could be of great importance in a scenario of changing climate that will be characterized by an increase in the frequency, intensity and variability of extreme events, determining unexpected novel conditions.

Author contribution. Chiara Giommi, Stella Terzo and Gianluca Sarà conceived the idea; Chiara Giommi and Stella Terzo led the writing of the ms. and drafted the first versions of this ms., performed the experimental design and carried out all experiments and lab analyses. Gianluca Sarà provided funds, instrumentations and lab facilities.

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Chapter 4

How temperature shapes algal diversity reveals early warning signals useful to predict climate change effects on tidal communities

Ready to submit to *Ecology*

Abstract

Biodiversity-Ecosystem Function experimental studies focusing on marine systems are few and produce contrasting outcomes. There is an increasing need to provide experimental proofs on how communities respond under stressful conditions and how small changes lead to tipping point pushing a system to a new state. The intertidal habitat, in particular rocky pools, are a model habitat to study the approaching to tipping points of transitions due to climate change. In these systems, the thermal variance of the water is a function of the distance from the low tide mark and determine communities belonging to two different levels of algal diversity.

In summer 2017, in the Western Sicily, 12 rock pools were randomly chosen and the temperature (through thermo-loggers) and the dissolved oxygen concentration were measured for 24 hours at minute resolution a threshold at which a large shift occurs (DO, through portable field oxy-probes). Then a manipulative experiment to investigate the effects of a climatic event (heat wave) in combination with an anthropogenic stress (increased gasoline concentration) was carried out. Deploying the same instruments and measures during the experiment, the community metabolism (oxygen consumption, ppm) was used as a proxy of functioning considering each pool as a natural respirometric chamber. At the end of the treatments, biodiversity was measured at each pool on 3 scraped quadrats (10 x 10 cm), the obtained values have been related to community metabolism.

The study highlighted a heat wave effect on high and low diversity pools, with low diversity pools more impaired by the climatic stressor. The chemical stressor, instead, did not show any significant effect at daily scale. These results confirmed that under the action of a disturbance, the functioning – as expressed by oxygen consumption – can be altered over time and, interestingly, high richness increases the ability to restore control conditions.

Keywords: Intertidal rocky pools, Tipping point, Climate extremes, Community Metabolism, Macroalgae.

Introduction

Community of organisms is anything but static, being continuously shaped by abiotic and biotic disturbance (Paine et al. 1998). Anthropogenic pressure on coastal ecosystems is vast and diverse, simultaneous impacts such as pollution, eutrophication, and fishing pressure nowadays add up and interact with the effects of climate change (e.g., global warming, acidification, and sea-level rise) (Halpern et al. 2008). These driving factors influence environmental change over time. Even if Nature is usually assumed to respond to a gradual change in a smooth way, however, studies on lakes, coral reefs, oceans, forests and arid lands showed that smooth changes are interrupted by sudden drastic switches to a contrasting state (Scheffer et al. 2001). Indeed, such catastrophic shifts occur typically quite unannounced and unpredicted and early warning signals of approaching catastrophic changes are difficult to obtain. Ecological phase shifts are generally assumed to occur when environmental control variables, by gradually approaching the system's tipping point, exceed some threshold (Connell & Ghedini 2015), but the actual mechanisms driving these assemblage-level responses are still quite unknown (Harley et al. 2017). For this, intertidal zones offer a great opportunity to study mechanisms dealing with the biodiversity assembling in a context of natural disturbances. Indeed, rocky intertidal habitats globally represent a rare example of natural system that encompasses a wide range of magnitude and scale to the biophysical gradients (e.g. thermal stress, desiccation risk, wave action stress; Gaines and Denny 1993). Invertebrates and algae inhabiting this zone are ectotherms that regularly contend with the terrestrial environment during each low tide. For all these reasons, these habitats provide a unique perspective on the relationships between both aquatic and terrestrial climatic regimes (Judge et al. 2018) and have, since long time, served as a test bed to increase our understanding about the possible causal linkages among abiotic stresses, biotic interactions and ecological patterns in nature (Helmuth et al. 2006).

Inside these habitats, tidal pools - those low natural depressions dug in the rock where water remains trapped at low tides allowing the inhabitants to remain submerged for the entire tidal cycle - represent important physical patches forming a sort of particular wild natural micro/mesocosms useful to study these ecological dynamics (*sensu* Watson et al. 2012). Nevertheless, the biotic communities of tidal pools are less studied than those of the emergent rocky substrata and can be regarded as intermediate habitats between the subtidal and the emergent rocky shores (Metaxas & Scheibling 1993, Underwood 1981). While organisms living on the rock at low tide undergo the heating in air, organisms living in tidal pools undergo the heating in water but with larger fluctuations than those experienced in the subtidal zones, just few meters below. Temperature can vary daily in water also by up to 15 °C, depending upon the height of the pool and the position on the shore along the intertidal gradient; such factors determine the extent of isolation from the tide. Thus, distance from the outer rim, wave exposure, the degree of shading and the volume of the pool (Metaxas & Scheibling 1993, Benedetti-Cecchi & Cinelli 1996) are essential factors affecting the heating dynamics inside the water mass in the pools. Although, they form phenomenal habitats where to study the ecological responses of organisms living on the "water edge", studies on tidal pools were scant and limited in only describing benthic assemblages using only a bunch of pools per time on one shore, to record only presence or absence of the flora and fauna (e.g. Preston & Moore 1988, Brattstrom 1990). Not much research used tidal pools to test ecological models and theories exploiting the add value of having well-defined boundaries, facility of manipulation and manageable sizes. Also, biodiversity may change structure and composition as a function of the distance from the low tide mark, and it provides a step-forward in the study on how tipping points manifest. Indeed, while it is known that experiments dealing with the ecosystem functioning in marine habitats are few and produce contrasting outcomes, there is an increasing pressing call to provide experimental proofs (possibly manipulative) on how communities are able to respond under stressful conditions and how stressors can push the system beyond the threshold. Moreover, the experimental manipulation and the climate projection was often mismatched and next generation of experiments with realistic manipulation of temperature are recommended (Korell et al. 2019). Here we used a natural gradient of temperature increase to scale up responses along the ecological hierarchy and to predict the effects of future global warming at ecosystem level (Watson et al. 2012). Our approach is similar to other field-based experiments exploiting natural gradients of disturbance to mimic future forecasted (COP Paris climate conference; Hulme 2016) conditions of disturbance (e.g. natural upwelling areas or CO_2 seeps; Hall-Spencer et al. 2008; Barry et al. 2010).

Accordingly, the main aim of this paper was to investigate the daily thermal variances of small water masses inside the pools to assess i) if gradual changes (on daily basis) in temperature could justify two different communities (i.e. less and more diverse communities) and ii) the appearance of tipping points beyond which a large shift occurs. Specifically, a pool system as for all the above-mentioned characteristics, was tested as an ultimate model disentangle critical points (i.e. when the state changes), reducing the failure likelihood encountered by other authors working in other habitats (Schröder et al. 2005). This because to gradually manipulate a stressful condition - in the wild - to fix exactly when a system changes is hard to do. A system characterized by two different communities contextually in the same site, may reduce the error deriving from the necessary use of artefacts to gradually reach the tipping points. The proposed experimental setup may increase the realism in reading the ecological functioning, in habitats that are undergoing highly variable environmental stressful pressure. Another aim was to determine how the initial contrasting levels of high or low diversity affected the resistance to an extreme climatic event - as a heat wave - in combination with an anthropogenic stress - as the increase of gasoline concentration (selected as potential proxy of the intensification of nautical traffic during summer).

Material & Methods

Site

The experiment was done in 12 littoral small pools on an intertidal rocky platform, located along the shores of Trapani, northwest of Sicily (38°6’27.32’’ N, 12° 42’26.36’’ E). The site was characterized by the presence of vermetid reef, a biogenic construction that with its broad horizontal extension, represents an amplification of the intertidal zone, providing an elevated potential of pool replicability. In summer 2017, along a stretch of coast of 100 m, as a function of the distance between the pools and the low tide mark two different levels of macroalgal diversity (Figure 1) were identified (High and Low diversity). Within each level, six pools, homogeneous in size, were randomly chosen (N = 12).

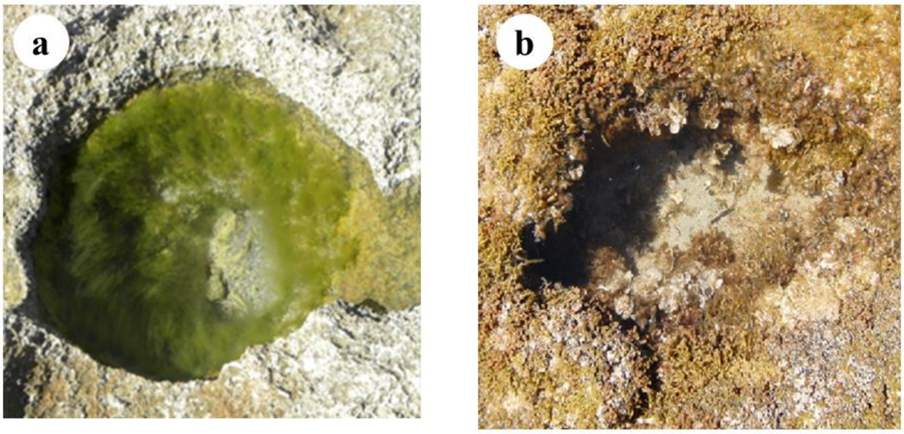

Figure 1. a) An example of a Low diversity pool, b) an example of a High diversity pool.

Geomorphological characteristics of pools and environmental variables

For each pool the distance (m) and the height (cm) from the fringe of the vermetid reef (i.e. the outer margin of the reef) was manually measured. In order to calculate the volume (l) of the pools, the perimeter, the major axis, the minor axis, and the depth (obtained as the average of ten measurements) were measured (cm). The volume was obtained by means of the elliptical cone formula $V = 1/3\ \pi abh$ (h = mean depth of the pool; a = semimajor axis; b = semiminor axis) and used for the treatments (see Experiment Paragraph).

To know the pristine condition of each pool, in terms of thermal and oxic patterns, temperature and dissolved oxygen (DO) concentration were measured inside the pools, for 24 hours at minute resolution. Temperature data loggers (model: iButton G1, prec. ± 1°C, res. ± 0.5°C, http://www.alphamach.com) were used to monitor temperature and dissolved oxygen loggers (HOBO U26 Dissolved Oxygen Data Logger; https://www.onsetcomp.com/products/data-loggers/u26-001) to monitor DO.

Experiments

Three pools for each level of diversity were selected and exposed to a simulated heat wave (HW) in water during light hours. Water inside the pools was heated by means of one or two, according to the pool volume, aquarium submersible heaters (AQUANET 100W) to reach 40 °C. The heat wave treatment lasted for four hours during low tide. Temperature was constantly monitored through a thermometer placed inside the pool.

The other six pools, three for each level of diversity, were exposed to a chemical stressor (Ga) adding 2 ml/L of gasoline to water, according to El-Shoubaky & Mohammad (2016), during low tide.

During the chemical and thermal stressors, in each pool, the temperature and the DO were measured for 24 h at minute resolution. The oxygen consumption (ppm) was used as proxy of metabolic functioning.

Sampling and Laboratory analysis

At the end of the treatments, water was removed from the pools and to characterize macroalgal biodiversity, 3 quadrats (10 x 10 cm) were collected by scraping. Samples were stored at -20 °C until laboratory analysis. In laboratory a qualitative analysis of macroalgal species was carried out. All the algal taxa were identified at species level, with the aid of a stereo microscope (ZEISS Axio Zoom V16) according to Irvine (1983), Fletcher (1987), Round et al. (1990), Burrows (1991), Maggs & Hommersand (1993), Irvine & Chamberlain (1994), Cormaci et al. (2012, 2014, 2017), Furnari et al. (2001) and Giaccone et al. (2003). The list of algae was reported, and the species richness was also calculated for each pool.

Statistical Analysis

Macroalgal biodiversity. To test differences in the macroalgal species assemblages found in the high diversity and low diversity pools, univariate and multivariate distance-based permutational nonparametric analyses of variance (PERMANOVA; Anderson 2001; McArdle & Anderson, 2001) were performed on the number of species (Species Richness, SR) and on the macroalgal community composition (presence/absence transformed data) including the diversity level (2 levels: High and Low) as fixed factor and pools as random factor (6 levels) nested in the diversity level. PERMANOVAs were based on Bray–Curtis similarity matrices using 9999 random permutations of the appropriate units (Anderson 2001).

SIMPER analysis, based on the Bray–Curtis similarity matrix and calculated from the presence/absence transformed data, was performed to determine macroalgal species that contributed most to the average similarity among pools in each diversity level and the species most responsible for the dissimilarities observed between high diversity and low diversity pools. The same Bray–Curtis similarity matrix was also applied to produce a non-metric, multidimensional scaling two-dimensional plot (MDS).

Experiments. At first, a descriptive analysis has been conducted to explain pools characteristics and find possible relationships between variables. To test the effect of geomorphological characteristics of pools (height on the fringe, distance from the fringe) on biodiversity (expressed as Species Richness, SR) Poisson-GLMs were used. Mean daily temperature recorded in the water inside the pool was also investigated and included later into the model as a possible influential variable. Furthermore, in order to find a possible threshold of temperature, which classify pools in high and low biodiversity, a regression tree technique was adopted.

Last point of the experiment dealt with the study of the effect of benzene and heat wave on pools, in particular, on the daily mean oxygen concentration. A GLMM approach was used to take into account repeated measures in modeling the response.

Results

Geomorphological characteristics of pools and environmental variables

The experimental pools included six high diversity pools and six low diversity pools, and their geomorphological characteristics are reported in Table 1. Under pristine conditions, in high diversity pools, values of DO ranged from 8.13 ± 2.72 ppm to 10.51 ± 5.16 ppm, and in low diversity pools ranged from 8.27 ± 6.65 ppm to 11.00 ± 6.24 ppm. Temperature showed value ranging from 26.31 ± 3.85 °C to 29.39 ± 4.52 °C in high diversity pools and from 27.19 ± 4.32 °C to 29.53 ± 3.58 °C in low diversity pools.

Table 1. Level of diversity (High; Low) and geomorphological characteristics of expermental pools: Perimeter, Major axis, Minor axis, Depth, Distance of the pool from the fringe (Dist. Fringe), Height of the pool from the fringe (Height of pool).

Pool	Level of diversity	Perimeter (cm)	Major axis (cm)	Minor axis (cm)	Depth (cm)	Dist. Fringe (m)	Height of pool (cm)
1	Low	237	73	73	19.25	14.57	11
2	High	247	88	64	18.14	13.68	13
3	High	200	72	72	20.59	13.88	8
4	High	204	77	66	12.75	12.7	3
5	Low	140	48	43	13.53	12.84	15
6	Low	281	93	68	17.05	16.7	12
7	High	95	67	50	16.65	10.5	6
8	High	195	74	44	13.85	9.75	15
9	High	180	58	58	13.65	13.1	6
10	Low	230	75	40	14.3	14	20
11	Low	137	41	41	13	13.89	21
12	Low	204	70	56	15	14.8	6

Macroalgal biodiversity

The number of macroalgal species found in the high diversity pools (ranging from 16 to 33 species), as expected was significantly higher (PERMANOVA, $p < 0.001$; Table 2) than that found in low diversity pools (from 3 to 10 species) (Figure 2). No significant differences were observed among pools in each diversity level (PERMANOVA, $p > 0.05$).

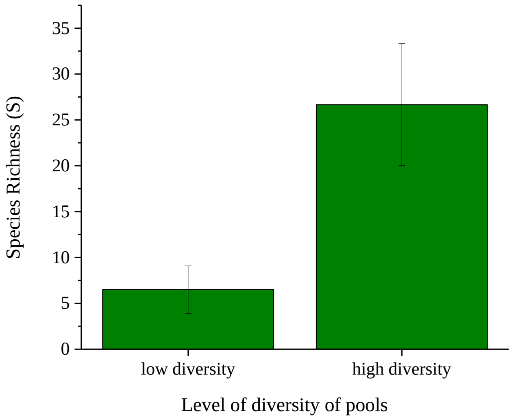

Figure 2. Macroalgal Species Richness (S) in high and low diversity pools.

Comparing the macroalgal community composition in the pools of each diversity level (high and low) no significant differences were observed (PERMANOVA, $p > 0.05$). Significant differences, instead, were observed between high diversity and low diversity pools (PERMANOVA, $p < 0.01$; Table 2). A SIMPER analysis (Table 3) evidenced that the high dissimilarity found between the two diversity levels of the pools (83.5 %), showed also by MDS analysis (Fig. 3), was explained by some species (e. g. *Cladophora prolifera*, *Jania rubens*, *Halopteris filicina*, *Spyridia filamentosa*, *Chondria tenuissima*) frequently present in the high diversity pool and completely absent in the low diversity pools. These species also provided the most important contribution to the average similarity among the high diversity pools together with *Chaetomorpha linum*. The latter which was present also in the low diversity pools contributed, together with *Enteromorpha intestinalis (var. intestinalis)* to their similarity (SIMPER results in Table 3).

Figure 3. Multi-dimensional scaling (MDS) analysis performed using macroalgal species assemblages (presence/absence transformed date) in high and low diversity pools

Table 2. Results of PERMANOVA analysis carried out to ascertains the differences between high and low diversity pools and among pools in each diversity level (high and low), (p < 0.001 - ***; p < 0.01 - ** p < 0.05 - *; p > 0.05 - ns).

PERMANOVA	**Main test**					
Variable	**Source**	**df**	**MS**	**Pseudo-F**	**P(MC)**	
Richness	Diversity level	1	8382.5	23.1	0.000	***
	Pool	9	367.7	8.9	0.084	**
	Residual	1	41.3			
	Total	11				
Community composition	Diversity level	1	9280.1	3.7	0.007	***
	Pool	9	2547.4	1.9	0.212	*
	Residual	1	1371.9			
	Total	11				

Table 3. Output of the SIMPER analyses carried out on macroalgal species assemblages. Macroalgal species included in this table were responsible of 50% cumulative similarity among pools in each diversity level (high and low) and of 50% cumulative dissimilarity between high and low diversity pools.

Species	Contrib%	Cum%
Low diversity pools (Average Similarity = 26.7%)		
Enteromorpha intestinalis var. intestinalis	37.37	37.37
Chaetomorpha linum	18.00	55.37
High diversity pools (Average Similarity = 36.0%)		
Chaetomorpha linum	10.58	10.58
Cladophora prolifera	6.91	17.49
Jania rubens	6.91	24.41
Halopteris filicina	6.43	30.84
Spyridia filamentosa	4.75	35.59
Ulva scandinavica	4.75	40.35
Dictyota dichotoma	4.63	44.98
Chondria tenuissima	4.41	49.39
Chylocladia pelagosae	4.41	53.80
Low vs High pools (Average dissimilarity = 83.5%)		
Cladophora prolifera	3.05	3.05
Jania rubens	3.05	6.11
Halopteris filicina	2.82	8.93
Spyridia filamentosa	2.70	11.63
Chondria tenuissima	2.57	14.20
Chylocladia pelagosae	2.57	16.78
Cladophora rupestris	2.55	19.33
Dictyota dichotoma	2.43	21.76
Padina pavonica	2.32	24.08
Ceramium comptum	2.16	26.24
Polysiphonia neglecta	2.14	28.38
Caulerpa cylindracea	2.03	30.41
Ulva scandinavica	1.93	32.34
Enteromorpha compressa	1.93	34.27

Laurencia glandulifera	1.92	36.19
Cladophora albida	1.82	38.01
Chondrophycus papillosus	1.78	39.79
Lithophyllum incrustans	1.78	41.57
Cladophora aegagropila	1.76	43.33
Cladophora laetevirens	1.72	45.05
Polysiphonia tripinnata	1.69	46.73
Ceramium codii	1.67	48.41
Sphacelaria sp.	1.67	5008

To investigate the relationship between the main geomorphological variable correlation have been calculated (Figure 4, 5, 6). A positive correlation coefficient (r = 0.54) was found between distance from the fringe and daily mean temperature measured in control condition and a positive correlation coefficient, r = 0.43, between height on the fringe and the daily mean temperature was estimated. Moreover, a negative correlation coefficient of r = -0.65 was evident for temperature and biodiversity.

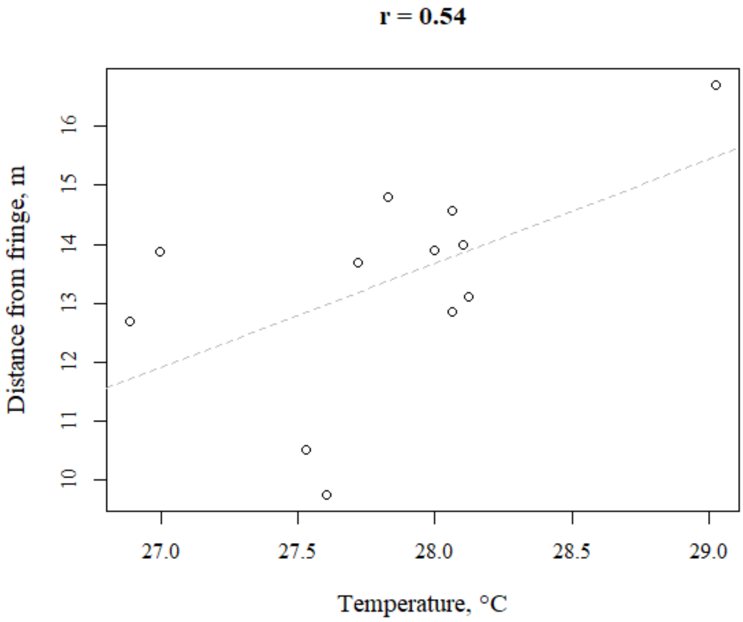

Figure 4. Correlation between Temperature and Distance from the fringe.

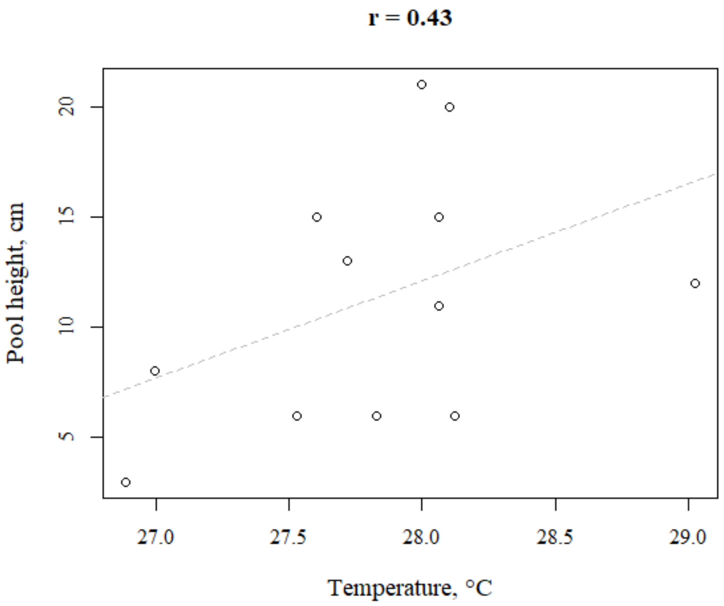

Figure 5. Correlation between Temperature and Pool height.

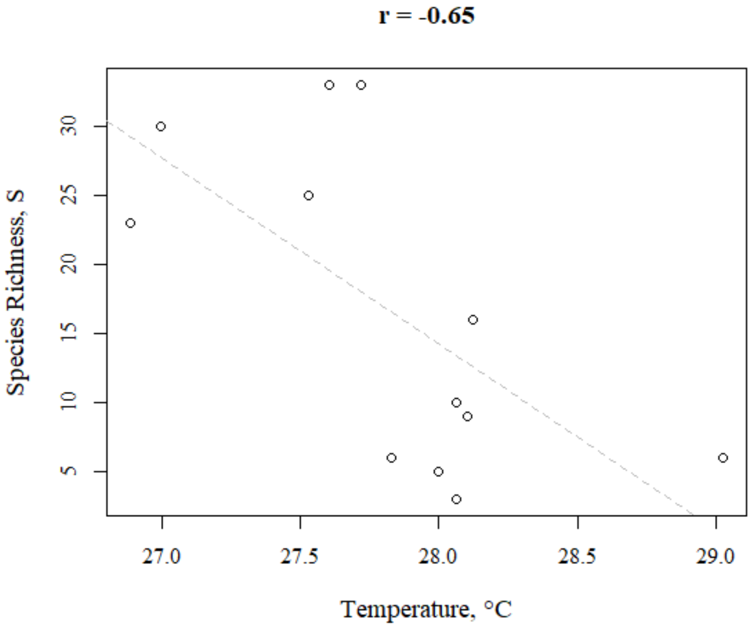

Figure 6. Correlation between Temperature and Species Richness.

To test the effect of geomorphology on biodiversity (as expressed by the number of species as a proxy) Poisson-GLMs was used. The most relevant results (table 4) showed that number of species was negatively affected mostly by distance from the fringe (-0.22, p = 0.000) and slightly by height of the fringe (-0.03, p = 0.000). Further, temperature showed a negative effect on biodiversity (-0.69, p = 0.000) and as a main consequence it was added to the original model (table 5). Thus, the AIC lowered from 113 to 98 and this suggested a higher predictability of this latter model.

Table 4. Estimates of the Poisson – GLM Model of the effect of geomorphologic variables on biodiversity, (p < 0.001 - ***; p < 0.01 - ** p < 0.05 - *; p > 0.05 - ns).

Coefficients:	**Estimate**	**Std. Error**	**z value**	**Pr(<|z|)**	
(Intercept)	5.948	0.497	11.965	0.000	***
Distance	-0.218	0.038	-5.748	0.000	***
Height	-0.029	0.013	-2.155	0.031	*
AIC: 112.97					

Table 5. Estimates of the Poisson – GLM Model of the effect of Distance from the fringe and temperature on biodiversity, (p < 0.001 - ***; p < 0.01 - ** p < 0.05 - *; p > 0.05 - ns).

Coefficients:	**Estimate**	**Std. Error**	**z value**	**Pr(<|z|)**	
(Intercept)	24.184	4.123	5.865	0.000	***
Daily mean temp	-0.69539	0.15268	-4.555	0.000	***
Distance	-0.16276	0.04129	-3.942	0.000	***
AIC: 98.079					

In order to find a possible threshold of temperature to classify pools in high and low biodiversity, a regression tree technique was used. Such an analysis allowed us to identify the threshold of 27.8 °C of mean daily temperature (Figure 7).

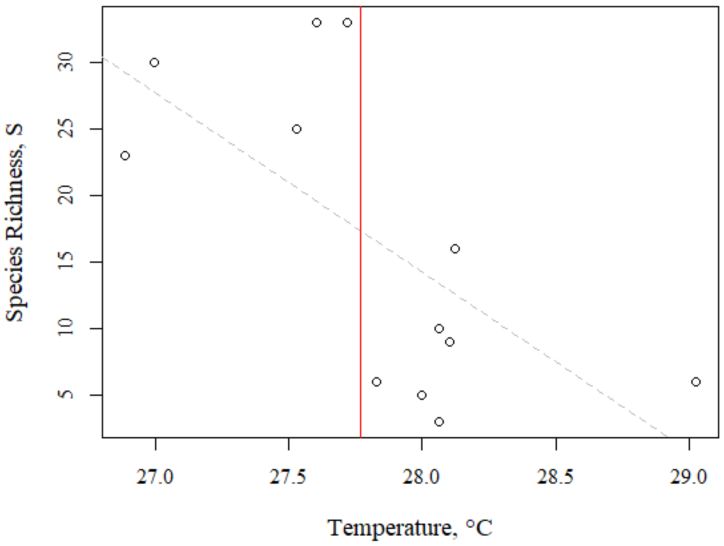

Figure 7. Correlation between Temperature and Species Richness. The red line denotes the threshold of 27.77 °C found through regression tree (please note that the assignment to the high diversity pool's group of the pool number 9 was biased at the beginning of the study).

Lastly, the effect of benzene and heat wave on communities of algae, using the daily mean oxygen concentration as response variable was studied. Benzene had not a significant effect on daily mean oxygen concentration (Tab. 6), while in contrast, heat wave showed a significant negative effect (-1.332, p = 0.0014). Level of biodiversity, classified in "high" and "low", was included into the model as explanatory variable and then when considering "high" level as baseline, "low" level showed a negative not significant estimate (-0.641; p = 0.06) which was slightly above the significance threshold (5%) suggesting a possible effect of biodiversity level on daily mean oxygen concentration.

When we consider the interactions between treatments and levels of biodiversity, our analysis (Tab. 7) showed a negative and significant estimate of interaction between heat wave effect and low level of biodiversity (-2.23, p = 0.0007). Lastly, a small variance of random effect suggests small structural differences among pools in how they undergo the experiment and for other variables not considered in the model.

Table 6. Estimates of the GLM Model of the effect of HW and Ga treatment on the pools, (p < 0.001 - ***; p < 0.01 - ** p < 0.05 - *; p > 0.05 - ns).

Coefficients:	**Estimate**	**Std. Error**	**z value**	**Pr(<|z|)**	
(Intercept)	9.393	0.295	31.80	<2e-16	***
TRTben	-0.140	0.418	-0.34	0.737	
TRThw	-1.332	0.041	-3.19	0.001	**
BIOcllow	-0.641	0.341	-1.88	0.060	.
AIC: 71.5					

Random effect variance(s):

Group=Pool

	Variance	StdDev
(Intercept)	0.0002	0.01462

Table 7. Estimates of the GLM Model of the effect of HW and Ga treatment on the different level of High and Low pools, (p < 0.001 - ***; p < 0.01 - ** p < 0.05 - *; p > 0.05 - ns).

Coefficients:	**Estimate**	**Std. Error**	**z value**	**Pr(<|z|)**	
(Intercept)	9.1090	0.2822	32.28	<2e-16	***
TRTben	-0.1027	0.4626	-0.22	0.824	
TRThw	-0.2352	0.4626	-0.51	0.611	**
BIOcllow	-0.0739	0.3990	-0.19	0.853	.
TRTben:BIOcllow	-0.0386	0.6585	-0.06	0.953	
TRThw:BIOcllow	-2-2302	0.6585	-3.39	0.000	***
AIC: 66.1					

Random effect variance(s):

Group=Pool

	Variance	StdDev
(Intercept)	0.0694	0.2635

Discussion

The main aim of this study was to provide a step-forward on how tipping points manifest. Our experimental setup appeared to be appropriate to study how gradual changes in temperature could justify two different communities. Rock pools are specialized habitats within the intertidal zones worldwide, but since they are not aerially exposed, they are not usually included in the studies of zonation. Thus, only in few studies, researchers thoroughly described the distribution patterns of their biota or they related these to the fluctuating physico-chemical conditions to which the pools are usually subjected (Huggett & Griffiths 1986). Here, instead, we investigated the effect of how temperature shapes diversity; to this purpose, the intertidal pool system was a useful case study thanks to the elevated degree of their replicability. Our analyses showed a very small variance of random effect, suggesting small structural geomorphological differences among pools. In addition, the revealed not significant differences among pools *per* each level of diversity (PERMANOVA, $p > 0.05$) suggested a high degree of homogeneity among them.

Not recently, Morris & Taylor (1983) followed in details changes in temperature, pH, salinity, oxygen concentration and carbon dioxide content in pools. They showed that the most fluctuating variables were temperature and dissolved oxygen and the amplitude of the fluctuations was related to the heights of the pools on the shore (Huggett & Griffith 1986). The temperature variability within the pools could be explained better ($r = 0.54$) when the distance from the outer rim of the intertidal platform (i.e. the infralittoral fringe) was considered while the height on the shore ($r = 0.43$) had less importance: the farer the pool, the higher the mean daily temperature.

Due to its natural gradient, intertidal zones offer a great opportunity to study mechanisms underlying the biodiversity switching due to disturbance. Macroalgae species richness in the pool was affected by distance from the fringe (-0.22, $p = 0.000$) and slightly by the height of the fringe (-0.03, $p = 0.000$) but as expected, the strongest negative effect on biodiversity was caused by temperature. That temperature plays a determinant role in driving plant or animal's physiological performance and survival (Somero 2010; Sinclair et al. 2016) is universally accepted. Present results go towards that line.

According to Scheffer et al. (2001), the state of an ecosystem may respond in different ways to an external condition (i.e. external variables, which do influence the system but are not, in turn, affected by it; Schröder et al 2015). When the condition is a temperature increase, the system may be quite inert over certain ranges of conditions, responding more strongly when conditions approach a certain critical level (i.e. tipping point) (Figure 8).

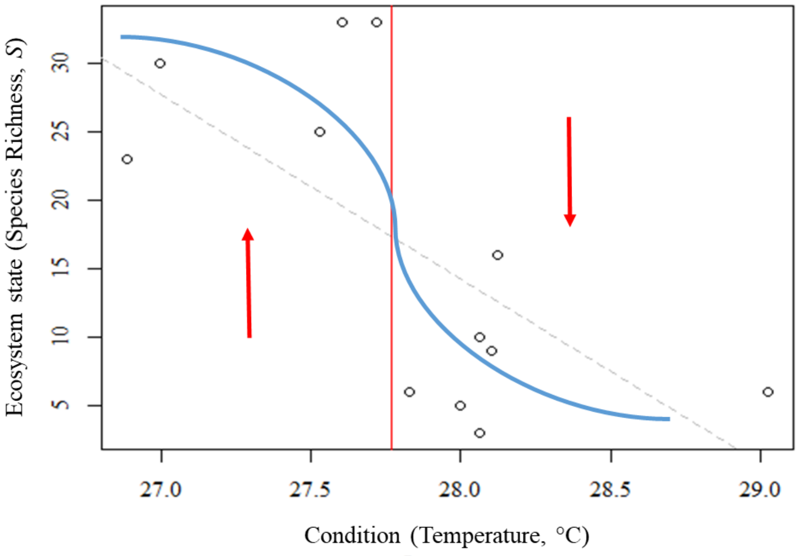

Figure 8. The possible way (in blue) in which an ecosystem equilibrium state (here as expressed by species richness) can vary with condition (here as expressed by temperature). In red the threshold found in this study (27.8°C), with arrows showing the direction of change. Modified by Scheffer et al. 2001 Figure 1b.

Thus, the system feedback that perpetuates the high or low diversity state seems to be the temperature in that High diversity pools had mean daily temperature 27.4 ± 0.66 °C and Low diversity pools had mean daily temperature 28.9 ± 0.56°C). Such differences created a non-linear dynamics. Accordingly, this study reveals that the critical level that pushes the splitting of pools into two different groups with two different levels of diversity was 27.8°C. Given the concern that increasing human pressure can lead to pronounced shifts in many natural and social systems (Lenton & Williams 2013, Brook et al. 2013, Worm & Lotze 2006), such a result is crucial for the understanding of the community response to anthropogenic disturbance. Indeed, seen that community transitions are difficult to slow, stop and reverse once they started, the identification of the tipping point may increase the ability to recognize the early warning signals of community shift. These thresholds are notoriously difficult to predict because communities "launch" few signals when approaching to tipping points (Connell & Ghedini 2015). According to the IPCC scenarios (RCPs 4.5 and 8.5), the temperature at these latitudes will change in the future of about 1.5°C. Such a value is just pretty the same to the difference between the mean daily temperature of high and low diversity pools (see Fig. 7). As a main consequence, such an information may be crucial in assisting us when we need to predict the effects of climate change on biodiversity modifications and in particular how benthic assemblages of shallow subtidal habitats may change under the predicted COP scenarios (Paris Climate Conference; Hulme 2016).

In this study, SIMPER analysis also revealed high dissimilarity (83.5 %) between the two levels of macroalgal diversity. Such results are in agreement with other studies showing a zonation along the intertidal gradient. Here, stress tolerant green algae (e.g. *Enteromorpha* and *Chaetomorpha*) dominate higher on the shore while other green algae (*Cladophora*), red algae (*Spyridia*, *Chondria*), brown algae (*Halopteris*), and corallines (*Jania*) were most abundant lower on the shore (Dethier 1982, 1984, Sze 1982, Kooistra et al. 1989). Interestingly, *Chaetomorpha linum* a filamentous green alga provided a contribution to the average similarity among the high diversity pools but also among the low diversity pools showing how much species can differ in the width of their performance curves (Enquist et al. 2015). Such a result may show how in a future increasing temperature scenario , the intertidal zones could be dominated by ephemeral green r-strategy algae that would replace more structured communities such as those reported in high diversity pools resulting in a remarkable loss of biodiversity and stability of the system.

Several prominent marine heat waves (prolonged periods of anomalously high sea surface temperatures; Hobday et al. 2016) have had severe impacts on marine ecosystems in recent (Oliver et al. 2018). Given the expected intensification in extreme temperature events due to anthropogenic climate change and the potential for profound ecological and socioeconomic impacts the last aim of this chapter was to determine how the initial contrasting levels of high or low diversity affected the resistance to a heat wave in combination with an anthropogenic stress - as expressed by the increase of gasoline concentration (selected as potential proxy of the intensification of nautical traffic during summer). According to numerous studies, heat waves appear to be events having large and massive influence on the metabolic functioning of organisms trapped in the pools but an increase in biodiversity corresponds to an increase in ecosystem resistance. The effect of the heat wave was lower in high diversity pool showing a stabilizing effect of diversity in communities subjected to climatic disturbance events. The chemical stressor, instead, did not show any significant effect probably because the temporal scale of observation was too short (daily scale) or maybe because this kind of stressor at such concentration represents a threat for communities only if is repeated in time simulating the effect of a chronic disturbance instead of a pulse one. In conclusion, present study showed that increasing temperature has a large effect on biodiversity in terms of composition and species richness. These results allowed also individuating the thermal threshold tracing the pace of biodiversity change and the state change. Chemical stressors did not apparently play a significant role in affecting the metabolism of species trapped in tidal pools, while the effect of heat wave was remarkable. In the Anthropocene, when global climate change is one of the most pervasive human transformations of the Earth, present results corroborate once time more that climate change can exert a driver so large to address ecological functions and affecting human socio-economic needs (Rosenzweig et al. 2007).

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Supplementary materials

Table 1. List of the algae recorded in each pool.

Pool	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Acrochaethium crassipes							+					
Alsidium helminthochorton		+						+				
Amphiroa sp.				+								
Calothrix fuscovinacea			+									
Calothrix sp.											+	
Caulerpa cylindracea		+		+					+			
Ceramium ciliatum var. ciliatum		+										
Ceramium ciliatum var. robustum		+										
Ceramium circinatum			+									
Ceramium codii			+				+	+				
Ceramium comptum				+			+		+			
Ceramium gracillimum								+				
Ceramium gracillimum var. bissoides				+								
Ceramium tenuissimum		+										
Chaetomorpha linum		+	+	+	+	+	+	+	+	+		+
Chaetomorpha mediterranea		+									+	
Chondria cerulescens								+				

Chondria mairei							+	+				
Chondria tenuissima		+	+	+					+			
Chondrophycus papillosus				+			+	+				
Chondrophycus thuyoides								+				
Chylocladia pelagosae		+	+	+					+			
Cladophora aegagropila					+	+		+				+
Cladophora albida								+		+	+	
Cladophora coelothrix					+			+				
Cladophora feredayi	+											
Cladophora laetevirens		+	+				+					
Cladophora liniformis	+	+				+	+					+
Cladophora nigrescens				+								
Cladophora pellucida			+					+				
Cladophora plantula										+		
Cladophora prolifera		+	+				+	+	+			
Cladophora pseudopellucida			+									
Cladophora rupestris		+	+				+		+			
Cladophora sericea										+		
Cladophora socialis								+				
Corallina officinalis		+		+				+				

Dictyota dichotoma		+		+			+		+			
Dictyota implexa		+										
Dictyota mediterranea							+					
Dilophus fasciola								+				
Ectocarpus siliculosus var. adriaticus								+				
Enteromorpha compressa			+		+	+	+		+			+
Enteromorpha intestinalis				+	+					+	+	
Enteromorpha intestinalis var. intestinalis	+	+					+	+				
Flabellia petiolata			+									
Gelidiella lubrica							+					
Gelidiella tenuissima				+								
Gelidium crinale		+	+			+		+				+
Gelidium melanoideum			+									
Gelidium pusillum									+			
Gymnogongrus pusillus				+								
Halopteris filicina		+	+	+			+	+				
Herposiphonia tenella			+									
Hydrolithon boreale				+								
Hydrolithon cruciatum		+										
Hydrolithon farinosum			+		+			+				

Jania longifurca			+						
Jania rubens	+	+				+	+	+	
Laurencia glandulifera	+	+						+	
Laurencia majuscola				+					
Lithophillum incrustans	+		+			+			
Mesophyllum lichenoides			+				+		
Microcoleus chthonoplastes						+			
Neogoniolithon brassica-florida	+						+		
Oligosiphonia sp.	+								
Padina pavonica	+	+	+			+			
Peyssonnelia atropurpurea							+		
Peyssonnelia dubyi						+			
Peyssonnelia orientalis	+								
Peyssonnelia polymorpha							+		
Peyssonnelia rubra	+								
Peyssonnelia stoechas							+		
Phaeophila dendroides				+					
Phaeophila sp.									+
Pheophyllum fragile			+						
Plocanium cartilagineum		+							

Pneophyllum confervicolum		+									
Polyisiphonia neglecta		+						+			
Polyisiphonia opaca		+									
Polyisiphonia pulvinata		+									
Polyisiphonia tripinnata	+							+		+	
Polysiphonia neglecta				+		+	+				
Polysiphonia pulvinata					+		+		+		+
Rytiphloea tinctoria	+						+				
Schizothrix calcicola		+					+				
Sphacelaria cirrosa			+								
Sphacelaria sp.	+	+				+					
Sphyridia filamentosa		+	+			+		+			
Titanoderma cystoseirae	+										
Ulva rigida							+				
Ulva rotundata	+										
Ulva scandinavica		+	+	+		+		+	+		
Ulvacaea plantula									+		
Ulvella lens							+				

Chapter 5

A sensor network as a tool of early detection of climate change

Introduction

Global climate change is acknowledged as the major cause of economic deterioration of most marine ecosystems (Halpern et al. 2008). High anthropic pressure has produced an increasing number of “new” developing environmental conditions at unpredictable speeds. To predict the biological responses upon a variation of environmental variables is critical to ensure the management and conservation of biodiversity and the resources upon which they depend. A lack of reliable predictions decreases the response capacity of the managers who just apply retroactive management measures, that is to say, only in conditions of full-blown overexploitation or a resource loss, they turn to mitigation actions. Formulating proactive measures implies an *early detection* of environmental conditions at a relevant scale for the organisms and deep knowledge of their tolerance limits. The action of a disturbance such as the climate change causes small yet continuous changes that gradually push the system to a tipping point provoking a radical change and transforming the system in a new alternative stable one, which oftentimes is irreversible (Harley et al. 2017). Being able to predict the small changes and measure the speed whereby systems get close to this no-return point is extremely important nowadays.

It is thus necessary to offer low-cost monitoring systems with the highest space-time resolution (mirroring the paces of biological functioning, time scales lower than one hour and space scale lower than decameter) to acquire environmental variables of the fundamental ecologic niche of marine organisms. The development of innovative technological systems that build an early warning network (proactive responses) is viewed as one of the most powerful ways to anticipate the climate change effects and it could be extremely helpful for researchers by expanding the scope and relevance of future experiments. A new generation of experiments is needed. Experiments that aim to understand how the space-time scale, realistic local extinction scenarios and other aspects of environmental changes (especially temperature, pollution, extreme climate events) affect the relationship between biodiversity and the various dimensions of the ecosystem functioning (Tomimatsu et al. 2013). Finding appropriate tools to explicitly connect these experiments to the ecosystem services is just as necessary (Cardinale et al. 2012).

Intertidal habitat, i.e. the region between the high and low tide mark, acts as a key testbed to explore the climate change effects on the organism’s distribution and abundance. Environmental conditions in this habitat are among the hardest on Earth: often the waves exert forces equivalent to those produced by wind speed of over 1000 km h^{-1} on earth, and the organisms are exposed to extremely fluctuating levels of temperature, salinity and pH (Zhou et al. 2018). In addition, intertidal organisms (e.g. algae, mussels, snails) are exposed to the terrestrial environment each day at low tide for 4-6 hours on average, a period that also depends on the sea conditions. These very features make the intertidal habitat, already a model system to study climate change, extremely appropriate to test in the marine environment tools that have been conceived for terrestrial habitat.

State of the art

The naturalists have been knowing that their presence can affect animal behavior (Schneirla 1950). Direct observation implies several difficulties as it requires long term study and the work of many researchers. Moreover, the observer's presence can alter the behavior of the species under scrutiny or its interactions with other species, and the observations remain biased by the observer's physical limitations. The field of biotelemetry developed from the need to locate the animals and observe and record their habits despite their ability to move fast, over large areas, at night, under water or in inclement weather conditions (Cooke et al. 2004). Locating animals in space has progressed from manual tracking of animal-borne radio-or acoustic signals to automated depth and geomagnetic loggers and satellite-based positioning systems that remove the observer's effect and provide precise worldwide locations in a short time (Brown et al. 2013). The data can also be recorded and memorized in a device placed in the animal (biologging) and can be downloaded when and if the logger is recovered (Cooke et al. 2016). This kind of information, however, indicates where the animal has been and how long it has stayed there, but the behavioral context is missing, highlighting the need to remotely measure the animal behavior and still maintain a high level of details in data recording. To meet such requests, in the last twenty years, the accelerometers have become the most common tool in the fields of human and animal biotelemetry (Bao & Intille 2004; Altun et al. 2010). The potential of accelerometers lies in the fact that they are cheap and compact tools that provide a means of measuring activity by recording high-resolution time acceleration (Barajas et al. 2017).

This technology was used to examine bioenergetic and a variety of aspects of ecology, physics, animal behavior and physiology (Nathan et al. 2012; Payne et al. 2014). When attached to an animal, the acceleration biologgers record and store acceleration in up to three axes, that includes dynamic acceleration (animal movement) and static acceleration (gravity). These data are helpful to remotely quantify the animal behavior and activity-related energy expenditure (Brown et al. 2013) giving very detailed information but showing some limitation in the recovery of the tool (Metcalfe et al. 2015). Accelerometers have been also used in experiments to measure the behavior and the energy budget. They have been tested mostly in terrestrial systems but recently researchers have applied this technology in marine organisms such as penguins (Yoda et al. 2001), salmons (Wilson et al. 2006), sharks (Whitney et al. 2007), sturgeons (Thiem et al. 2015). So far, most studies on fish have focused on behavioral models and only few energy budget estimations have been performed. Some studies (Qasem et al. 2012, Wright et al. 2014), however, suggest that body acceleration could be correlated to the oxygen consumption rate in animals, including fish.

Although the accelerometers application fields have increased strongly over the years, including water systems also, in the mentioned examples the information from the accelerometer is recorded and stored and, due to the difficulty inherent in transmitting data wireless through water, is never transmitted in real time to the final user nor it includes more sensors connected in a network. The last two features, as explained in the introduction, are crucial to study climate change which requires systems capable of reading fast environmental changes and, just as fast, informing the researchers and/or the managers.

Research objectives

Starting from an ecological question – the need to monitor environmental variables such as the wave energy at fine spatial and temporal scales - here, through developing a combined project, a product conceived by a multidisciplinary team of professionals in a different sector has been adjusted to the marine system. Indeed, this project has been developed in collaboration with Turingsense EU LAB s.r.l. an innovative start-up based in Forlì and founded in 2015 as a R&D department of another Californian start-up. Turingsense EU Lab is a leader start-up in movement analysis and its activities focus on the usage of wearable sensors.

Miniaturized sensors have lead to a fast use of wearable technologies worldwide to improve one's own performance, especially in training. The player and the athlete, need tools capable of indicating immediately and precisely information about one's own training. The sport performance results require to be stored and recovered instantaneously to monitor and allow to share sports data. Also the study of climate change needs tools for instantaneously, continuously and at high resolution monitoring abiotic variables on natural systems. Thus, inspired by a strong common need, in this thesis, a tool to analyze movement has been applied to a marine case of study.

Feasibility study

PIVOT is the first system, unique in its kind in the world, for tennis training. The kit comprises a hub wireless connected to the computer and satellite sensors (from 1 to 8 inertial units) that transfer data to a hub via Bluetooth (BL4 Bluetooth low energy). The sensors do not have any data logging and transmit data in real time. Each inertial unit (Figure 1) is composed by 3 sensors integrated on the same motherboard: an accelerometer, i.e. a device that converts acceleration into an electric signal, a gyroscope used to measure angular rate, which is how quickly an object turns, and a magnetometer to measure the magnetic field.

Figure 1. Inertial unit.

The major difficulty in using PIVOT in a marine intertidal application is no doubt the presence of water, indeed wireless signal are transmitted very differently in water and air. To rule out the need to file data and ensure real time functioning, specific lab experiments were conducted so as to test Bluetooth **transmission** of the inertial units to the hub. After making a rudimentary cover, the inertial units have been switched on and immersed in water and have been tested at different depths and frequencies (Table 1, Figure 2). The distance between the inertial units was always less than 2 m.

Table 1. Tests Details.

Depth	**Number of inertial units tested at the same time**	**Frequency**	**Trasmission outcome**
5 cm	1	50 Hz	ok
5 cm	5	50 Hz	ok
15 cm	1	50 Hz	no
15 cm	1	2 Hz	ok
15 cm	5	2 Hz	ok
15 cm	5	1 Hz	ok
25 cm	1	50 Hz	no
25 cm	1	2 Hz	ok
25 cm	5	2 Hz	ok
25 cm	5	1 Hz	ok
40 cm	5	2 Hz	ok
40 cm	5	1 Hz	ok

The sensors were also left immersed to understand if humidity could change their functioning and tests were repeated after 24 hours with the same outcomes.

The striking finding of being able to transmit via Bluetooth up to 40 cm of depth and at low frequencies allowed us to carry on with the study of other aspects such as **energy consumption** and the **waterproof cover**.

Figure 2. Photograph of the experiments to test transmission.

The size of a self-contained logger is mostly determined by battery size. PIVOT’s motherboard weighs 3.6 g and is connected to a battery. The weight of the motherboard with the battery is approx. 8.9 g and they are placed in a small black plastic box, so that the total weight is 15.2 g. In early tests to make the sensor as light as possible, instead of a battery, a microUSB cable connection to a power bank (see Figure 2) was tried out.

Last step was to make the **waterproof cover** for the inertial units. 3D printing was chosen, and the early trial were made with PLA (polylactic acid) filaments. This material was chosen because it is an inexpensive material and is considered an environmental-friendly plastic, created from the manufacturing of a variety of vegetal products, including corn, potatoes or sugar beets. It is also one of the most used materials in 3D printing, as it can be printed at low temperature and does not required a heated bed. The material turned out non impermeable, as during the experiments the water went through the cover after a few-hours immersion. Another possibly negative aspect was that this plastic, given the low melting temperature, is unfit to long exposure to sun rays and risks to deform and soften. The second material that was used was silicon. This turned out to be totally impermeable, but its density and rigidity did not allow neither to push the button to switch on the inertial unit nor to see the LED color that showed the quality of the signal. The most appropriate material was photopolymer resin, as it is impermeable even after days of immersion, malleable and transparent, thus allowing to see through it.

Case study

Globally, there are few, if any, natural systems that compared in magnitude and scale to the biophysical gradients (e.g. thermal stress, desiccation risk, wave action stress) that occur in rocky intertidal habitats (Gaines and Denny 1993, Helmuth et al. 2006). Wave action is an integral part of the daily life of assemblages of algae and invertebrates of intertidal rocky shores. Energy from waves contribute to keep organisms moist, to deliver oxygen (Jørgensen et al. 1985) and food (Sebens et al. 2016), to remove metabolic wastes, and to inhibit predators.

The formidable forces of moving and braking water can pose substantial challenges and hazards to intertidal organisms. Biological adaptations to minimize the risks of life in wave-swept conditions pervade the design features of many species. Shell shape, body size, and the relative flexibility of the stalks and thallus of attached plants, algae, and animals, for instance, are all related to contending with the stresses imposed by waves. Although the organisms are dependent upon the sea or the ocean, many of the organisms occupying rocky intertidal shores experience stress which already meets or exceeds their lethal tolerance limits (Stillman and Somero 1996).

The regime of wave action varies in space and time and is influenced by physical and biological components of the intertidal environments such as the physiological and morphological characteristics of the organisms and the properties of the substratum to which the organisms are attached. The risk of damage, dislodgement or death by a given wave force varies among species that differ in shape, flexibility, or internal structure, among other factors.

Climate events such as global warming, storminess and precipitation, acting as agents of disturbance, can drastically modulate the effects of abiotic factors such as wave action (Sanford 1999, Hughes 2000). The influence of these factors on intertidal assemblages is expected to increase according to the environmental scenarios predicted by several models of climate change (Elsner et al. 2008). In particular, there are indications that the frequency, intensity and variability of extreme events such as storms (Muller & Stone

2001), heat wave (Hobday et al. 2018), droughts and floods (Easterling et al. 2000a-b) will increase as a consequence of climate change.

The case of study proposed in this project concerns the deploy of PIVOT to monitor the wave energy in intertidal rocky shore and the evaluation of the dislodgement risk for a canopy forming macroalgae such as *Carpodesmia amentacea* (C. Agardh) Orellana & Sansón due to the increasing of extreme climate event.

Crashing intertidal waves can produce water velocities exceeding 8 m/s and accelerations of up to 400 m/s^2 (Denny 1985). Numerous studies have established that exposure to wave action can affect all aspects of the life history of a marine organism (Carrington & Denny 1994). Animals and algae show different strategies to adapt to this turbulent environment and to face the dislodgement risk. Sacoglossan gastropods produce an adhesive mucous tether to maintain attachment to substratum or food plant while feeding or resting (Wong & Sigwart 2019). Mussel beds self-organize into spatial patterns consisting of bands parallel to the shore to reduce losses from dislodgement and predation, because of the adherence of mussels to one another (Sherratt & Mackenzie 2016). Moreover, biotic interaction can affect dislodgement resistance. The grazing pressure exerted by limpets on the stipe and in the holdfast of kelps may impact the dislodgement and fracture rates (Hereward et al. 2018). Fucoid growing on mussel shell showed a lower dislodgement resistance than those growing on the rock indicating that mussels provide an unstable substrate for mature fucoids and highlighting a negative effect of mussel-modified habitat on fucoids (Wangkulangkul et al. 2016).

Macroalgae critical contribute to the ecological function of many ecosystem through their diverse role as primary habitat providers, food sources and ecosystem engineer (Thomsen et al 2010). They provide shade and restrict the variability in humidity and temperature on the shore (Bulleri & Benedetti Cecchi 2008), filter swimming larvae or drifting adults and provide a refuge from predation by large mobile predators (Coull & Wells 1983). These habitat-mediated effects imply that changes in algal habitat structure should influence the diversity of niches available to rocky shore infauna (Thrush et al. 2011). In the Mediterranean Sea, brown algae belonging to the *Carpodesmia* genus play a valuable role as foundation species and they are the most important canopy-forming widespread in this biogeographic region (Rodríguez-Prieto et al. 2013) (Figure 3).

In addition to its ecological relevance, this species was chosen as a case study because one of the larger algae of the intertidal, thus adequate to attach the sensor and because it is exclusive to a high hydrodynamic environment (i.e. fringe), a necessary condition for the measurement of wave energy recorded on a sessile organism. *Carpodesmia amentacea* is exposed to multiple disturbances that cause a decline in its abundance in many coastal areas (De la Fuente et al 2019).

The main pressures affecting the valuable ecosystems formed by *C. amentacea* are sedimentation, low water quality, anthropization, overgrazing and wave action. Indeed, the dislodgment of entire plant along with its substrate often occurs as a result of storm (Garden & Smith 2015). When hydrodynamic forces exceed attachment or tissue strength - a threshold response that implies a relationship between wave forces and dislodgement, results mortalities and biodiversity loss (de Bettignes et al 2015).

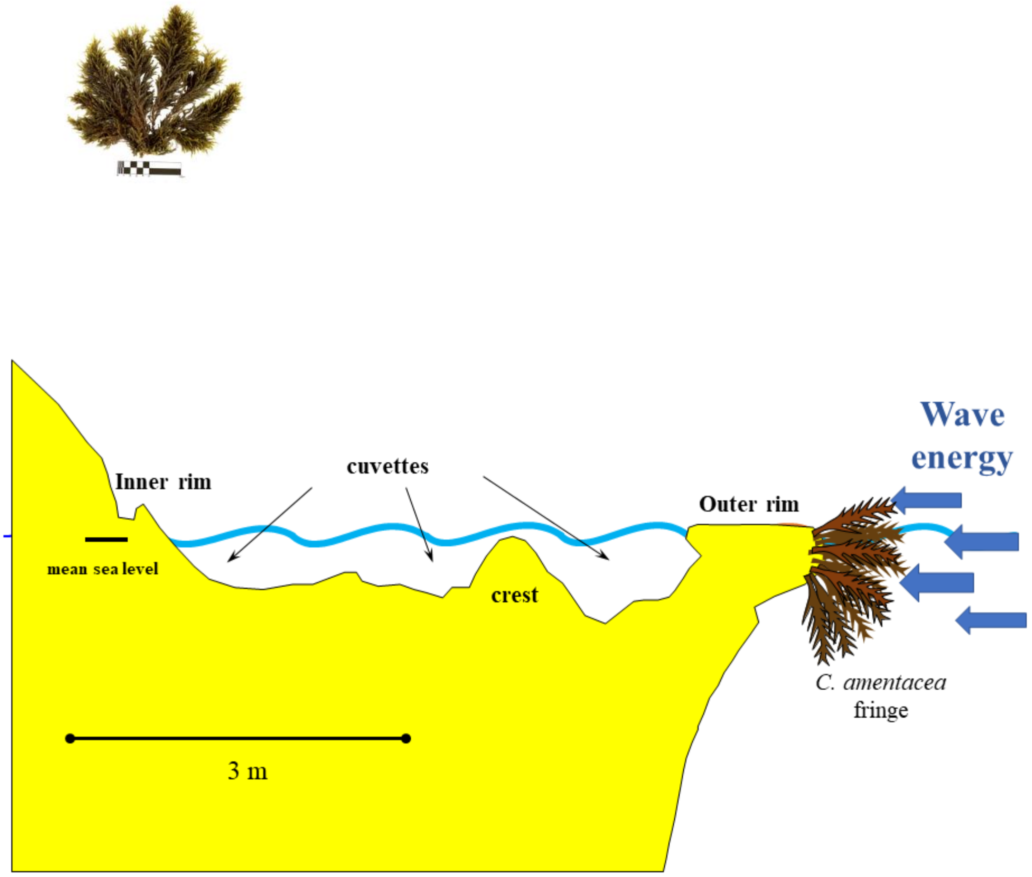

Figure 3. On the left a individual of *Carpodesmia amentacea*. On the right, the scheme of the intertidal biogenic habitats vermetid reefs. The space included between the inner and the outer rim is the cuvette which extension represents the reef width and may host several submerged pools and crevices. Below the

outer margin, the vermetid reef is fringed by a belt made by the canopy-forming algae *C. amentacea*, which grow above the wave-cut notch.

Thus, macroalgal biodiversity loss may imply the loss of crucial functions as refuge, biogeochemical cycles maintenance, food provisioning, water oxygenation and protection from physical agents from which, related ecosystem services of this habitat arise: water quality regulation and ecotourism (De La Fuente et al. 2019a).

In particular canopy mortality due to wave dislodgement, may represent an important feedback that increases resilience of a turf-dominated state and prevents reestablishment of canopy (Burek & Scheibling 2018) resulting in the persistence of a lower complexity system.

Long term monitoring at individual scale through a network of sensors for a real time identification of wave action is particularly interesting to provide a useful tool for predicting disturbance patterns of intertidal communities (Carrington Bell & Denny 1994). Moreover, knowing the frequency and magnitude of extreme climate event such as storm surge allow to predict effect of future climate change on macroalgal community and ecosystem functions based on them.

Once measured the force required to break the organism off, these sensors could inform early warning systems where the alert thresholds will be set within the tolerance limits of the target species (in this case *C. amentacea*). Thus, it will be possible to link to each different level of wave energy an amount or percentage cover of dislodged thalli.

This information could be also valuable in nondestructive restoration techniques, such as the recent ex situ outplanting of midlittoral *C. amentacea* (De La Fuente et al. 2019b) to identify the more suitable receiving site. Indeed, the possible dislodgement by wave action represents one of the main threats in the days after the attachment of early life stages.

Moreover, intertidal algae are exposed to a highly variable photic regime because of crashing waves. Wing & Patterson (1993) calculated that the enhanced light-flash utilization-efficiency wrought by wave-induced light-flashes may contribute to significant gains in primary productivity. Thus, monitoring waves pattern could be useful to evaluate the productivity of intertidal systems and understand the effect resulting from the alteration of the pattern due to climate change.

In this case of study (Figure 4) PIVOT inertial units, sealed in waterproof cover, are secured via two stainless steel annealed locking wires connected to the thalli of *C.*

amentacea. On each thallus are attached two sensors: the first on the apical frond to measure wave energy in the most exposed portion of the organism and the second close to the holdfast. During field test sensors are connected by thin cables to the power banks. Thalli chosen for the measurement are at 20-40 cm of distance from each other and transmitting via Bluetooth to the hub. The hub is deployed within a 2 m distance and in a location upper of the high tide mark to be repaired from seawater. The hub is alimented by a battery and connected Wi-Fi to a computer and/or to a cloud.

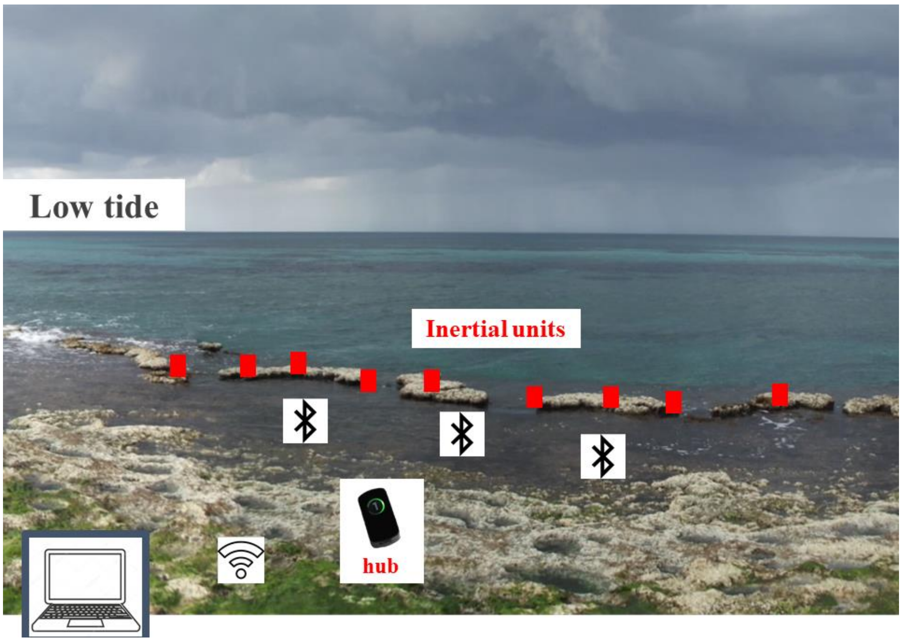

Figure 4. Diagram of the deployment of the sensors network in intertidal rocky shore.

Future development

The network of sensors will allow a field monitoring of movement at the scale of organisms, over a wide-spatial (e.g. wide stretches of coast) and high temporal frequency. Sensors need to be relatively inexpensive to be affordable by public authority, managing authority (Marine Protected Area - MPA), and research group interested in the early detection of climate change. Low cost is important also because due to the high energy habitat and turbulence of water some devices may be lost.

Another crucial aspect regards the alimentation of the inertial units. To enhance battery longevity, a primary limitation to miniaturization, the sensors could be designed to log data continuously but transmit data only when sensors are exposed to air at low tide and a strong network link can be made.

The sensor test ran on algae so far, could be applied to vagile or sedentary animals too, like crabs or snails. Once described the individual's pattern of movement of the target species, every alteration from the studied behavior could be read as a proxy of functioning and a measure of stress. For example, monitoring on the field the alteration of movement under a heat wave could inform on how these animals cope with climate change. In this case, it is needed to consider that existing sensors are small enough to be used to study many, although not all, intertidal organisms. Indeed, in the Mediterranean coast, intertidal species are generally of small dimension, therefore the sensor needs to be as light and small as possible to avoid being itself a stress for the animal. Real-time data transmission requires considerable battery power, in direct conflict with the need to miniaturize sensors. Differently than in the feasibility study, where sensors were connected to a battery through USB cables, a valid alternative is energy harvesting. Indeed, energy could be derived from ambient energy (e.g., solar power, thermal energy, wind energy, or kinetic energy), and captured, and stored.

Conclusions

In conclusion, the project developed in this thesis has brought an important advancement for the future applications of a network system that inform in real time for monitoring and detection of environmental variables, proving that Bluetooth technology could be a useful tool also in marine systems. The results obtained could also open up to the integration of other sensors into the motherboard such as pressure sensors, thermometers, and oximeters, allow to study important consequences of climate change such as sea-level rise, thermal extremes and hypoxia and/or anoxia episodes.

The development of data sensing tools that are accurate and most importantly relevant to the species of experimental interest is now more important than ever. This instrumentation must be combined with laboratory and field experiments that holistically evaluate the state of the species (Judge et al. 2018). Once these approaches are taken, they may potentially be scaled up to regional assessments of species performance and distribution. Advanced sensor techniques will allow for more accurate ecological forecasting that enhances the understanding of what to expect in the future. There are many unique challenges to monitoring intertidal organisms, however, the rapid pace of technological improvement is making these challenges easier to overcome, and thus promises new insights from this model system.

The idea and case study developed are protected by copyright. PIVOT is a brand of Turingsense Inc. (California). All rights reserved.

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Chapter 6

Final considerations

Anthropogenically induced global climate change has resulted in both increased average temperature and increased environmental variability at most locations globally (Easterling et al. 2000, Doney et al. 2012) and it is acknowledged as the major cause of economic deterioration of most marine ecosystems (Halpern et al. 2008). In particular, there are indications that the frequency, intensity, and variability of extreme events such as storms, heat wave, droughts and floods will increase as a consequence of climate change (Hobday et al. 2018). These effects, which are predicted to accelerate in the future, have resulted in species needing to acclimate to warmer and more variable climates at unprecedented rates, placing an ever-growing number of species at risk of extinction (Somero 2010, Urban 2015). Biodiversity is one of the major determinants of ecosystem functioning (Tilman et al. 2014) and its loss may have at least as great an impact on ecosystem functioning as other anthropogenic drivers of environmental change (resources, disturbance, or herbivory; Tilman et al. 2012)

The action of a disturbance such as the climate change causes small yet continuous changes that gradually push the system to a tipping point provoking a radical change and transforming the system in a new equilibrium, which oftentimes is irreversible (Harley et al. 2017). Being able to predict the small changes and measure the speed whereby systems get close to this no-return point is extremely important nowadays.

Nevertheless, to date, only a few studies focused on the effects of extreme climate events on ecosystems. Typically, their approach was observational dealing with long-term datasets to explain unforeseen events that occurred during data collection or experimental dealing with treatments designed to mimic field-relevant extreme conditions (Sorte et al. 2010). In addition, often the experimental manipulation and the climate projection were mismatched resulting in a nonrealistic treatment (Korell et al. 2019). Most community-level studies have been carried out in terrestrial systems with only few experimental studies focusing on the marine one (Gamfeldt et al. 2015).

This thesis opens with an evidence-based synthesis from the literature to create a BEF experiment knowledge baseline in the Mediterranean area and to reduce biases and informative gaps in this area of ecological research. Starting from this understanding, I focused on the experimental investigation of the effects of climate extreme on the functioning of intertidal assemblages.

Realism had particular importance in this dissertation. First, the choice of the habitat: rocky intertidal habitat (i.e. the region between the high and low tide mark) largely because of the steep gradient in thermal and desiccation stresses that occurs during low tide, has long served as a natural laboratory for examining relationships between abiotic stresses, biotic interactions, and ecological patterns in nature (Helmuth et al. 2006). Second, the selection of experiment treatments: both the air heat waves carried out in chap. 2 and 3 are simulated but realistic indeed corresponding to temperature experienced by organisms in Palermo (i.e. highest air temperature recorded by the Italian Institute of Environmental Research (ISPRA) temperature network). Lastly, similarly to other field-based experiments, in chap. 4 a natural gradient of disturbance was exploited to mimic future forecasted (COP Paris climate conference; Hulme 2016) conditions of disturbance.

Predicting the impacts of climate change on marine systems is an ongoing challenge especially due to the rapidity of environmental changes. Another aim of this dissertation is to improve the velocity on which scientists are able to capture not only fluctuation of environmental variables, but also responses of organisms to these increasingly rapid fluctuations. In chapter 5, through developing a combined project in collaboration with the start-up Turingsense EU LAB s.r.l., a miniaturized wearable sensor was modified to monitor the wave action at fine spatial and temporal scales. Wave action is an integral part of the daily life of assemblages of algae and invertebrates of intertidal rocky shores. And even if energy from waves contributes to keeping organisms moist, to deliver oxygen and food, to remove metabolic wastes, and to inhibit predators, it represents one of the main risks of damage, dislodgement or death among species. The project developed in this thesis has brought an important advancement for the future applications of a network system that inform in real-time for monitoring and detection of environmental variables, proving that Bluetooth technology could be a useful tool also in marine systems. The results obtained could also open up to the integration of other sensors into the motherboard such as pressure sensors, thermometers, and oximeters, allow to study important consequences of climate change such as sea-level rise, thermal extremes and hypoxia and/or anoxia episodes. Moreover, the development of this innovative technological system could help in building an early warning network (proactive responses) to anticipate the climate change effects and it could be extremely helpful for researchers by expanding the scope and relevance of future experiments.

Unfortunately, a disturbance is considered to have occurred when it causes visible changes in some structural property of interest; for example, biomass, species density or community structure (Connell & Ghedini 2015). And if there is no observable change in community structure, we conclude that the disturbance had no effect. This kind of approach is unbalanced toward the study of resilience rather than the resistance. Instead, the response to a disturbance (e.g. heat wave) is the result of many interactions, and as seen in chapter 3, it could be tuned by the density level of an ecosystem engineer and the ensuing balance between facilitation and competition. To improve our ability to detect environmental change, we need to better understand to what extent the density-disturbance interaction may have negative effects, and therefore assess the functioning of these systems. High temporal frequency monitoring of environmental variables such as temperature (e.g., through sensors deployment) and mapping of the foundation species (e.g., drone aerial imagery), could promote a new generation of Density Ecosystem Functioning (DEF) experiments and monitoring framework that could enhance the manager's capacity of intervention at short term scale on the density factor by designing compensating measures potentiating the conservation action.

Another unifying aspect within the framework of this dissertation is the utilization of an ecosystem function measure as response variable and the attempt to scale this up as a measure of the overall functioning (*sensu* Gamfeldt et al. 2008). While in chapter 2 photosynthetic responses of different species of macroalgae to a heat wave were evaluated trough two metabolic proxy of functioning such as the oxygen consumption/production and the photosynthetic efficiency, responses of community (chapter 3) and microsystems such as intertidal pools (chapter 4) were measured through the oxygen consumption. Some of the most fundamental metabolic functions, in terms of both cell function and environmental importance, involve the production and consumption of oxygen (Wilson et al. 2014). Oxygenic photosynthesis and aerobic respiration are basic measures of ecosystem function because the production, respiration, cycling, and overall availability of carbon affect everything from the number of trophic levels, to the types of organisms present (Odum 1956). Moreover, the metabolic rate is the most fundamental biological rate, it determines the rates of most other organic processes, and it is considered a unifying process in ecology because it links all hierarchic levels of ecological organization (Savage et al. 2004).

Laboratory and field experiments in combination with the use of natural gradient to mimic predicted future scenario and the deployment of network of sensors that inform in real time are the powerful tools to improve our understanding of the effect of climate chance on natural system and decelerate the pace of current biodiversity loss.

Suggestions that come out from this study are:

- Increase the frequency and resolution of the measure of environmental change trough sensors and monitoring (as proposed in chapters 3 and 5) to increase our awareness of global climate change.
- Increase the realism of the experiment favoring the study of natural gradient, the field experiments, or setting the adequate intensity of factors in mesocosm experiments.
- Always evaluate a measure of functioning during the experiments to be able to connect to at least one of the macro processes such as primary production and carbon fixation, decomposition, and nutrient cycle.

In conclusion, from results of this study emerge that extreme climate events such as heat wave overall strongly affect the natural system, but the responses and the recovery time can be different among species (chapter 2). Moreover, live together in a community represents an advantage, a refuge showing a buffer effect. The ability to cope with the stressor is regulated both on the structure of the community (e.g. presence of an ecosystem engineer) and on the composition (species richness).

Starting from the Millennium Ecosystem Assessment (MEA 2005) a lot of research focused on the evaluation of the role that biodiversity plays in modulating the ecosystem functioning, anyway researchers still need to strengthen the second causal relationship proposed in the framework (see chapter 1 p. 3). Only through a punctual measure of the ecosystem functioning the scientific community will have a consistent baseline that will allow the assessment of ecosystem services on which society relies and from which the human wellbeing depends.

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