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Climate change threats to Mediterranean bioconstructors: trait-based approach for assessing coralligenous responses to extreme climatic events

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“The Sea is the immense reservoir of nature: from him, as it were, originated the globe; and who knows, perhaps it will also end with him”.

Jules Verne (1870)

Twenty Thousand Leagues Under the Seas

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Summary

Global warming and extreme climatic events are significantly affecting marine ecosystems structure and functioning, with Mediterranean coralligenous habitats being especially vulnerable due to the nature of their life history and functional traits. Throughout six different chapters, this PhD thesis investigated the impacts of multiple human-driven climatic stressors on the functioning of coralligenous and pre-coralligenous habitats. Scaling from the individual to the community levels, the integration of mechanistic, modelling and social science approaches allowed for addressing the potential effects of stressors on ecosystem functioning and services. The thesis addressed significant ecological questions related to a deeper understanding of climate change impacts on marine biodiversity, filling critical knowledge gaps, and providing insights for conservation strategies. Some of the main goals achieved include: i) the creation of a consistent ecological baseline for coralligenous community characterization and conservation status along the southwestern Italian coast, ii) a deeper understanding of the eco-physiological mechanisms underlying vulnerability to thermal stress and the role in carbon sequestration process of *Eunicella singularis* meadows; iii) the distribution and functional responses of *Astroides calycularis* reefs to extreme climatic events, highlighting the importance of stressor properties in species and population responses; and iv) the experimental identification of the thermal tolerance and niche overlap of the interacting species *A. calycularis* and the predator *Hermodice carunculata* (bearded fireworm), revealing a potential increase in predation pressure on the coral under warming scenarios. By combining scientific methodologies with stakeholder engagement, this work offered a comprehensive trait-based framework for preserving vulnerable Mediterranean coastal ecosystems.

General Introduction

Climate change and particularly global warming are profoundly affecting ocean dynamics and marine ecosystems, with significant impacts expected to persist (Hoegh-Guldberg & Bruno 2010). Alongside ocean acidification, global warming is a major driver, influencing all levels of ecological hierarchy, from species tolerance ranges and limits, to populations dynamics, communities composition and structure and ecosystem functioning (Doney et al. 2012, Poloczanska et al. 2016, Scheffers et al. 2016). Addressing climate change is a complex global challenge with far-reaching consequences for global biodiversity. Rising temperatures and thermal anomalies, whether occurring in isolation or in combination with other human-induced stressors (Garrabou1 et al. 2022, Hobday et al. 2016, Frölicher & Laufkötter 2018), pose a serious threat to marine ecosystems, directly impacting local biodiversity (Kružić & Popijač 2015, Smale et al. 2019), leading to mass mortality events among coastal benthic communities worldwide (Garrabou et al. 2009a). In recent years, the frequency of heat waves has significantly increased, rising from 30 % in 2012 to nearly 70 % in 2016 (Hobday et al. 2016, Smale et al. 2019). The interaction with other environmental stressors often exacerbates their effects on natural communities, resulting in synergistic relationship that lead to impacts that surpass initial expectations, posing substantial challenges to ecosystem stability and resilience (Gunderson et al. 2016, Jackson et al. 2021).

The Mediterranean Sea, due to its semi-enclosed nature, rising sea temperatures at a rate faster than the global average and biodiversity hotspot, is particularly vulnerable to climate change impacts. Among the most threatened habitats, coralligenous emerges as a critical habitat requiring immediate attention. Coralligenous habitats represent one of the most complex communities in terms of

biodiversity and connectivity within the Mediterranean Sea (Cocito 2004, Paoli et al. 2016, Bracchi et al. 2017). As "ecosystem engineers," coralligenous communities comprise both animals and calcareous encrusting algae capable of shaping and creating their own habitat (Crain & Bertness 2006, (Ingrosso et al. 2018, De Jode et al. 2019). The constituent organisms produce calcareous skeletons that persists on the substrate even after death, facilitating the settlement of other species and creating a distinctive stratified structure (Bianchi 2001). The complex architecture of coralligenous habitats results in a highly intricate, three-dimensional structure that confer highly heterogeneity degreeproviding numerous niches for a diverse assemblage of organisms, making them crucial for maintaining Mediterranean marine deeper biodiversity (Teagle et al. 2017, De Jode et al. 2019). These habitats are especially vulnerable to climate change due to the nature of their traits, slow growth rates, limited dispersal capabilities, and sensitivity to temperature changes and ocean acidification (Garrabou et al. 2009b, Teixidó et al. 2024). At the same time, coralligenous habitats provide valuable ecosystem services (ES) of remarkable ecological and economic value. Their heterogeneity due to the complex morphological structure creates biodiversity hotspots, offering refugia and food for a wide range of organisms (support and provisioning ES). Additionally, these habitats contribute to climate regulation through carbon sequestration (regulation ES) and enhance human well-being (cultural ES), maintaining essential ecosystem functions (Tribot et al. 2016, Tonin 2018, Thierry de Ville d'Avray et al. 2019).

The general aim of this PhD thesis was to investigate the impacts of different nature extreme climate events on Mediterranean coralligenous and pre-coralligenous communities integrating trait-based correlative and manipulative experimental approaches to mathematical modelling and socioecological methods. This multi-faceted approach allowed for a more holistic assessment of climate change impacts on vulnerable marine habitats, addressing current gaps in our understanding of the functioning of these complex habitats.

The thesis **Chapter 1** aimed to create a reference point for the characterization and assessment of the ecological conservation status of coralligenous communities in southwestern Italian coast, specifically in the Marine Protected Area (MPA) of Capo Gallo – Isola delle Femmine (Palermo, Sicily), utilizing non-destructive monitoring techniques and popular metrics (Piazzi et al. 2019). This chapter provided crucial understanding of the current state of these habitats and sets a baseline in their structure and biodiversity composition, setting the foundation for future assessments of their health. **Chapter 2** investigated the effects of long-term thermal anomalies on the functioning of the key-structuring species *Eunicella singularis* (here after white gorgonian), which represents an important habitat former and carbon sink in the corralligenous community (Coma et al. 2006, Gori et al. 2011). Correlative in-situ study (using benthic chambers and PAM techniques), together to analyses on

oxidative stress markers, allowed to assess the metabolic functioning responses of the gorgonian meadows to thermal stressor during the summer period. **Chapter 3** explored the distribution and niche overlap of the endemic orange coral *Astroides calycularis* and the native-invader polychaetae *Hermodice carunculata* (fireworm) by using Local ecological knowledge techniques through a semi-structured interview performed to diving centres along the Italian coasts. This approach combines scientific rigor with valuable insights from stakeholders who regularly interact with these habitats and benefits from the large spatial scale reached (Lucrezi et al. 2018). **Chapter 4** provided a mechanistic understanding of how *A. calycularis* and *H. carunculata* may cope with thermal stressors at eco-physiological level. Thermal Performance Curves (TPCs) of both species were experimentally obtained and thermal niche overlap was identified. **Chapter 5** investigated predator-prey interactions under changing climate conditions, focusing on the relationship between *Astroides calycularis* and the fireworm *Hermodice carunculata*. This chapter explored how changing temperatures may alter biotic interactions and allowed to identify the predation optimum temperature of the fireworm (Righi et al. 2020). **Chapter 6** assessed the effects ofs extreme climatic events (MHWs, heat spikes and the combining action of MHWs and storms – dropping salinity) on the early life stages of habitat-forming species. This chapter is crucial for understanding how the nature and different properties of interacting stressor may differentially influence organisms responses. Particularly, the dispersal stage of a vulnerable species, giving some insights on the effects at population level, and the long-term persistence and recovery of coralligenous habitats in the face of climate change (Carbonne et al. 2022).

This research represents a significant advancement in our understanding of coralligenous habitats under environmental changing conditions. By integrating multiple methodologies and addressing different interconnected ecological questions, from species eco-physiological responses to stressors, to biotic interactions – niche overlap and predation relationships – and community structure and sensitivity investigations, this work aims to fill critical knowledge gaps and provide a comprehensive assessment of climate change impacts on these vital and vulnerable native Mediterranean ecosystems.

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Chapter 1

Ecological and conservation status of coralligenous community in the MPA Capo Gallo- Isola delle Femmine: a baseline for future effects of anthropogenic disturbance

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Abstract

Bioconstructors organisms together with a large number of macrobenthic species provideimportant ecosystem services, creating high morphological complexity that may offer shelter and new habitats to a vast variety of different organisms. By doing so, they play an essential role in the correct functioning of the ecosystems. When these habitat formers are impacted by stressful environmental conditions (losing tridimensionality), the associated communities change in structure and composition. In order to be able to measure the effect of disturbance on natural habitat, it is mandatory to have a baseline of good ecological status, reachable just through accurate monitoring actions. The “STAR” protocol is one of the most used protocols for monitoring coralligenous since it proposes a non-destructive monitoring method by using photo sampling and in situ evaluations of main structuring species health status and gives the possibility to adapt monitoring to site-specific habitat properties. Three monitoring sites were selectedwithin the Marine Protected Area (MPA) of Capo Gallo – Isola delle Femmine in Palermo, including each one of them 3 plots. Photo-sampling consisted in 10 random shots within each plot for a total of 30 images per monitored site. Organisms within the photos were identified at the possible lowest taxonomic level and using the ImageJ software, the total area (cm2) of every identified taxa was estimated and converted to cover percentage. In addition, information on species richness (alpha and beta diversity), and the EQVSL (Ecological Quality Ratio Sensivity Level) were calculated. The data matrix of habitat ecological status indices was completed and analyzed on R Studio, resulting in an EQR' (Ecological Quality Ratio) of sufficient status at all three study sites within the Capo Gallo - Isola delle Femmine MPA.

Introduction

The Mediterranean basin represents one of the world's largest biodiversity hotspots, boasting more than 17,000 marine species, representing 7 percent of the world's marine biodiversity (Coll et al. 2010). These are huge numbers considering that the Mediterranean basin represents only 0.32 percent by volume of the world's oceans (Defant, 1961). Coastal habitats represent the most productive environments, hosting high biodiversity, but they are also some of the most impacted, leading to significant disruptions in the ecosystem services that nature provides (Teixidó et al. 2011, Pierdomenico et al. 2021, Santojanni et al. 2023). Biodiversity loss is estimated to have reached record numbers in recent years. Among the main causes of this decline undoubtedly climate change (CC) is one of the most important, but also the overexploitation of the marine resources, and the increased human expansion in coastal areas, degrading marine ecosystems (Seney et al. 2013), are reported as main source of disturbance within the basin (Claudet & Fraschetti 2010). In addition, future climate predictions at global scale show an extreme increase in surface water temperatures and an exponential increase in extreme weather events compared to today's events. Annual mean sea surface temperatures (SST) are estimated to increase by a range of +1.5°C to +3°C by 2100 (Adloff et al. 2015, Mariotti et al. 2015). In addition, an increase in marine heatwaves (MHW) frequency and severity is expected, phenomena closely related to massive mortality events (MME) of benthic communities in the last years (Bally & Garrabou. 2007, Frölicher & Laufkötter. 2018, Hobday et al. 2016), being organisms belonging to cnidaria and porifera taxa the most impacted, accounting for 85 percent of mortality reports (Bavestrello et al. 2014, Garrabou et al. 2019, 2022). The Mediterranean Sea has a variety of valuable habitats (most of them endemics) of special interest, such as seagrass beds, vermetid reefs, and coralligenous and maerl reefs. *Posidonia oceanica*, an endemic seagrass, is crucial for oxygen production, carbon sequestration, and safeguarding coastlines from erosion, while also supporting a rich diversity of marine life (Pergent-Martini et al. 2005). Vermetid reefs, which are biogenic structures formed by gastropod mollusks, serve as natural barriers against coastal erosion and create unique microhabitats. Particularly, coralligenous habitats (extending between 20 m and 100 m depths) presents high species richness, hosting about 10 percent of the Mediterranean biodiversity, offering shelter and food resources for numerous marine species, enhancing ecological stability (Ballesteros 2006). Collectively, these ecosystems deliver essential ecological services, supporting fisheries and tourism, and bolstering the resilience of Mediterranean coasts against the impacts of climate change and pollution (Zunino et al. 2020, Ferrigno et al. 2021). Coralligenous habitat is among the most structurally complex and fascinating in the Mediterranean Sea, representing

a biogenic formation structured mainly by calcareous algae (Rhodophyta) belonging to the subclass Corallinophycidae, which are the main engineers of coralligenous reefs. These algae, by depositing calcium carbonate, create three-dimensional structures that serve as substrates for a wide range of marine organisms. The coralline algae facilitation action allows the settlement of the secondary bioconstructors, including sponges, bryozoans, cnidarians, mollusks, crustaceans, and echinoderms (Garrabou & Harmelin 2002, Ballesteros 2006). Due to coralligenous rich biodiversity, high productivity, and crucial role in the carbon cycle, this habitat is considered as one of the most ecologically significant ecosystems in the Mediterranean Sea (Piazzi et al. 2012, Casas-Güell et al. 2015). These ecosystems provide refuges and nursery areas for a wide range of fish and invertebrates, thus contributing to the sustainability of fishery resources (Cheminée et al. 2021). In addition, coralligenous plays a key role in protecting coastlines from erosion, maintaining ecological balance, and ensuring the stability of marine ecosystems (Thierry de Ville d'Avray et al. 2019). Coralligenous habitats are highly sensitive to disturbance due to specific traits of some of their main structuring specie (mainly gorgonians), such as slow growth rates, high longevity and limited larval dispersal (Ballesteros 2006). For this reason, coralligenous habitats are included in the Habitats Directive 92/43/EEC, the SPA/BIO Protocol, the Barcelona Convention, and the Bern Convention as vulnerable habitats in need of preservation (Gennaro et al. 2020). Increasing temperatures pose a serious threat to them, since their key structuring species are living close to their thermal tolerance limits (Hoegh-Guldberg et al. 2007). Climate change and other anthropogenic impacts can trigger a range of suboptimal conditions for the survival of these habitats. Eutrophication and hypoxia can result in so-called 'dead zones' or areas with minimal oxygen, negatively impacting local biodiversity (Diaz & Rosenberg 2008, Breitburg et al. 2018). Ocean acidification significantly impacts the calcareous structures of coralligenous habitats, leading to alterations in growth and survival (Hoegh-Guldberg & Bruno 2010). Likewise, anthropogenic pressure from highly impacting fishing gears (Ferrigno et al. 2021), such as trawling, or unregulated and illegal fishing activities have severe impacts on benthic communities, including mechanical damage to the habitat structure, and increased turbidity. Also, recreational activities such as those of scuba-diving (Di Franco et al. 2009) can generate additional mechanical damage to the coralligenous community (Ballesteros 2006). Ultimately, biological invasions are known for altering and affecting local biodiversity, impairing species performance rates due to competition interactions and reducing beta diversity, leading to habitat homogenization (Piazzi & Balata 2008). For example, certain algae such as *Womersleyella setacea* or *Acrothamnion preissii* create fitted mats that can reduce the photosynthetic rates of underlying species (De Caralt & Cebrian. 2013, Evans et al. 2015). Due to its high density, *Caulerpa cylindracea* significantly alters the community structure in invaded areas, reducing both the diversity

and abundance of native macroalgae, particularly the bushy and encrusting species (Piazzi and Balata, 2008). This species is also considered a true "chemical engineer," as it creates compact, layered mats that can reach a thickness of 15 cm, trapping sediment and generating anoxic conditions beneath them (Klein & Verlaque 2008, Sinopoli et al. 2020).

To date, the importance of using biodiversity levels as an indicator of environmental health is widely recognized (Laurila-Pant et al. 2015), since high levels of biodiversity may correspond to high ecosystem functioning (Wu et al. 2023). Biodiversity may not only reflect the current state of an ecosystem but may also serve as a crucial indicator for predicting its future health and resilience. This underscores the need for continuing efforts to protect and enhance biodiversity as a key component of environmental conservation (Bianchi et al. 2022). The environmental restoration of coralligenous habitats is a complex and delicate endeavor aimed at restoring the health and functionality of these valuable ecosystems in the Mediterranean Sea (Ballesteros 2006). The restoration process faces significant challenges, including the slow growth of key structuring species, the complexity of ecological interactions, and ongoing environmental pressures such as climate change and ocean acidification (Pagès-Escolà et al. 2018). To address these challenges effectively, it is crucial to adopt an integrated approach that combines direct restoration efforts with sustainable management and preventive conservation. The success of these initiatives relies on the concerted efforts of scientists, managers, local communities, and policymakers, working together to ensure that these valuable biogenic structures continue to provide their essential ecological and economic benefits (Prada et al. 2019). For this reason, conservation strategies aiming to protect marine habitats with high levels of biodiversity are essential. Among the most used conservation actions, the establishment of Marine Protected Areas (MPAs) is the most popular. It aims to protect and limit anthropogenic disturbances (such as fishing, tourism, and diving) within a well-defined geographic area (Pomeroy et al. 2005). To date, a total of 27 MPAs have been established in Italian waters. MPAs must be supported by continuous monitoring and targeted studies to understand and improve their functioning, connectivity with other areas, and overall biodiversity conservation status (Francour et al. 2001). It is crucial to study, monitor, and learn about MPA to assess their health status accurately, interpret the findings, and develop appropriate guidelines and management measures (Di Iorio et al. 2021). These management tools have proven to be essential for the protection and conservation of coralligenous habitats in the Mediterranean. The effects of MPAs on coralligenous as a protective measure are multifaceted and include various ecological and management aspects (Bianchi et al. 2022). One of the greatest challenges in conservation ecology is developing economically viable and feasible sampling methods that are easy to implement and provide valuable insights for biodiversity conservation (Kipson et al. 2011). In the scientific literature, several monitoring techniques for

Mediterranean coralligenous habitats are discussed. The COARSE index, proposed by Gatti et al. (2012), was developed to monitor the status of coralligenous assemblages, initially during the expansion of the commercial port of Vado Ligure. It was later modified and tested along the French coasts in 2015. This index is based on the Rapid Visual Assessment (RVA) technique, carried out by divers to collect data on the ecological state of the coralligenous, considered a key indicator of marine biodiversity and seabed integrity by Directive 2008/56/EC. The ISLA index, proposed in 2017, measures the sensitivity of sessile organisms in coralligenous assemblages to stress and disturbance. One of the most widely used and cited protocols is the STAndaRdized coralligenous evaluation procedure (STAR), offering an integrated and standardized approach for assessing the ecological status of coralligenous walls (Piazzi et al. 2019, UNEP/MED 2024). The STAR protocol was developed with the goal of maximizing the amount of information collected by calculating most existing coralligenous quality indices while minimizing sampling effort (Piazzi et al., 2019 a, b). Various indexes for the calculation of the ecological status of the coralligenous community exist, being the ESCA (Ecological Status of Coralligenous Assemblages) among the most popular (Cecchi et al. 2014; Piazzi et al. 2017, Di Camillo 2023).

The reliability of monitoring and indices relies on their ability to establish a baseline or reference point with which to compare the ecological system's status over time. Without this foundational information, it is impossible to accurately assess the impacts that various disturbance sources may have on ecosystems. Baselines related to environmental conditions (long-term data series) and communities composition are essential key references for assessment of disturbance impact on conservation status of marine ecosystems (Battista et al. 2016). This information is also fundamental as reference points for stablishing recovery goals and measuring the success of ecological restoration actions.

In the specific case of the Capo Gallo - Isola delle Femmine MPA, no information regarding the composition or conservation status of the coralligenous habitat has ever been published in scientific literature. For this reason, the objective of this work was to define a baseline for the community composition and ecological conservation status of the coralligenous habitat within the MPA, providing preliminary information that can guide the development of appropriate management measures, and implementation of potential environmental restoration measures.

Materials and methods

Study area

The MPA of Capo Gallo-Isola delle Femmine is located on the northwest coast of Sicily (38°12'33.4" N 13°13'55.9" E) and covers an area of 2,173.00 ha, divided into zones with different levels of protection: A (maximum level), B (medium level), and C (minimum level). The area is characterized by rocky shores and vermetid reefs, surrounded by belts of *Cystoseira* spp. It features extensive meadows of *Posidonia oceanica*, and at depths of 25 to 70 meters, there are numerous reefs and platforms on vertical and sub-vertical rocky substrates with abundant coralligenous habitats.

Three different sites within the MPA were selected for monitoring activities. Sites spaced approximately 200 meters apart and were formed by vertical walls (90º-95º slope) that from 30 to 35 m depth hosted vast coralligenous communities (Figure 1).

Figure 1. Monitoring points of the coralligenous habitat within of the MPA of Capo Gallo - Isola delle Femmine, (1.Orlata B, 2. Parete Est, 3.Orlata A).

Monitoring design

Monitoring activities took place during spring period (April). Following the STAR protocol (Piazzi et al., 2019), for each site, three plots 2 m x 2 m were defined. Each plot was marked with four stakes, one at each corner, and a high-visibility colored line. For the photographic sampling, a Canon G7X Mark III housed in an ISOTTA case and equipped with an underwater flash was used. A rigid structure, made from PVC support tubes, was constructed in the shape of a truncated pyramid with a base of 40 cm x 50 cm (matching the size of the photographic frame) to support the camera and flash. A total of 10 photographs per plot were performed (30 photos per site). During the sampling operations, the health of colonies of the main structuring species was assessed for each plot by semi-quantitatively evaluating the percentage of necrotic tissue within the sample unit (ISPRA Manuals and Guidelines 191 2020). Additionally, the thickness of the limestone matrix and the structure of conspicuous megabenthic stands (expressed in cm^2) were assessed *in situ* using a penetrometer. Photographic sampling and measurements were performed by professional dive operators, who worked in pairs and conducted no more than two dives per day in accordance with ISPRA and

Environmental Agencies' safety regulations for diving activities (BPAS, ISPRA Manuals and Guidelines 94/2013).

Data analysis

Photographic samples were analyzed using ImageJ software. In accordance with the ISPRA Manual and Guidelines 191/2020 on the Monitoring and Assessment of the Ecological Status of Coralligenous Habitats, total area (cm^2) and percent cover (%) of the main structuring species were measured and estimated, respectively. Species within each photo frame were identified using macroalgae and macro/mega benthos identification manuals (ISPRA Manuals and Guidelines 191, 2020), and their occupied surface areas were estimated. A matrix of cover areas (cm^2) for individual species was created and then converted to percentage covers for each taxon present in each frame. From the photographic sample, the percent cover of each taxon, morphological group, specific richness, and the number of taxa/groups present were derived. These metrics were used to estimate the quality and abundance of taxa present, including Sensitivity Level (SL), EQRα (i.e. the mean number of the taxa/ groups per photographic sample), and EQR β (calculated with the average distance to centroid) (Table 1). These three parameters were then used to calculate the final Ecological Quality Ratio (EQR) value for ESCA index, as follows:

$$EQR = \frac{EQRi\,(SL) + EQRi\,(\alpha) + EQRi\,(\beta)}{3}$$

Where $EQRi\,(SL)$ is the Ecological Quality Ratio index (sensitivity level), $EQRi\,(\alpha)$ corresponds to the Ecological Quality Ratio index (alpha Biodiversity) and $EQRi\,(\beta)$ is the Ecological Quality Ratio index (beta Biodiversity). In accordance with the Water Framework Directive 2000/60/EC, five classes of ecological status were categorized based on EQR values: EQR ≥ 0.8 (High), 0.6 ≤ EQR < 0.8 (Good), 0.4 ≤ EQR < 0.6 (Moderate), 0.2 ≤ EQR < 0.4 (Poor), and EQR < 0.2 (Bad).

Table 1. Index of the sensitivity level (SL) of the main taxa considered in the ESCA index (Cecchi et al. 2014)

Taxa/group	**Mean % cover**	**SL**
Halimeda tuna	>5	6
Halimeda tuna	2.5-5	5
Flattened Rhodophyta with cortication	>0.1	5
Erect corticated terete algae	>1	5

Halimeda tuna	1-2.5	4
Flattened Rhodophyta with cortication	0.05-0.1	4
Erect corticated terete algae	0.5-1	4
Palmophyllum crissum	>0.2	4
Halimeda tuna	0.1-1	3
Flattened Rhodophyta with cortication	0.02-0.05	3
Erect corticated terete algae	0.1-0.5	3
Palmophyllum crissum	0.1-0.2	3
Zanardinia typus	>1	3
Halimeda tuna	0.01-0.1	2
Flattened Rhodophyta with cortication	0.01-0.02	2
Erect corticated terete algae	0.05-0.1	2
Palmophyllum crissum	0.05-0.1	2
Flabellia petiolate	>10	2
Zanardinia typus	0.05-1	2
***Peyssonnelia* spp.**	>10	2
Halimeda tuna	0-0.01	1
Flattened Rhodophyta with cortication	0-0.01	1
Erect corticated terete algae	0-0.05	1
Palmophyllum crissum	0-0.05	1
Flabellia petiolate	0-10	1
Zanardinia typus	0-0.05	1
***Peyssonnelia* spp.**	0-10	1
Encrusting Corallinales	>0	1
Algal turf (Filamentous uniseriate or pluriseriate algae)	20-40	-1
Introduced species (*Caulerpa cylindracea*)	0.01-0.05	-1
Pseudochlorodesmis furcellata	0.1-0.5	-1
Algal turf (Filamentous uniseriate or pluriseriate algae)	20-40	-2
Introduced species (*Caulerpa cylindracea*)	0.05-0.2	-2
Pseudochlorodesmis furcellata	0.5-1	-2
Algal turf (Filamentous uniseriate or pluriseriate algae)	40-60	-3

Introduced species (*Caulerpa cylindracea*)	>0.2	-3
Pseudochlorodesmis furcellata	>1	-3
Algal turf (Filamentous uniseriate or pluriseriate algae)	>60	-4

Data analysis

To analyse the data and determine if there were significant differences in species composition among monitoring sites, a Permutational Multivariate Analysis of Variance (PERMANOVA) was conducted. Factor “site” was fixed with 3 levels (Orlata A, Parete Est and Orlata B), while factor “plot” was random and nested in “site”. Additionally, PCoA (Principal Coordinates Analysis) was used to visualize the similarities and dissimilarities between samples; while SIMPER (Similarity Percentage Analysis) helped to identify which species contributed most to these differences. Additionally, the data were analysed to assess whether there were significant differences across the three levels of the "site" factor for each ecological measure (alpha biodiversity, beta biodiversity, and limestone matrix thickness). All statistical analyses were conducted using R software through the RStudio integrated development environment (version 4.4.1).

Results

Of the measurements taken in the field by the dive operators during the monitoring activities, the limestone matrix at the study sites had an average depth ranging between 2 cm and 3 cm. The main structuring species identified in the photographic frames were *Paramuricea clavata, Eunicella cavolini* and *Eunicella singularis*. Observations on necrotic tissue presence in the identified colonies revealed that just *P. clavata* showed low level of necrosis in the apical part of some branches (Table 2).

Table 2. Consistency of the limestone matrix in the plots analyzed and percentage of necrotic tissue in the main anthozoan species identified.

	Site 1			Site 2			Site 3		
	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6	Plot 7	Plot 8	Plot 9
Consistency of the matrix of calcareous algae									
thickness (cm)	2 ± 0.56	2 ± 0.31	2 ± 0.48	3 ± 1.01	3 ± 0.96	3 ± 0.44	2 ± 0.57	2 ± 0.90	2 ± 0.62
Percentage of presence of necrotic tissue									
Paramurice clavata	10%	10%	10%	10%	10%	10%	10%	10%	10%
Eunicella cavolini	0%	0%	0%	0%	0%	0%	0%	0%	0%
Eunicella singularis	0%	0%	0%	0%	0%	0%	0%	0%	0%

Table 3. Occurrences of species within the 3 sites of the MPA (Orlata A, Parete Est Orlata B).

Species	***Orlata A***	***Parete Est***	***Orlata B***
Algal felt	✓	✓	✓
Caulerpa cylindracea	✓	✓	✓
Rhodophyta limestone encrusting	✓	✓	✓
Peyssonnelia sp.	✓	✓	✓
Flabellia petiolata	✓	✓	✓
Ochrophyta encrustans	✓	✓	✓
Palmophyllum crassum	✓	✓	✓
Rhodophyta erect cylindrical	-	✓	-
Rhodophyta erette laminaria	✓	✓	-
Halimeda tuna	✓	✓	-
Hydrozoa	✓	✓	-
Encrusting sponges	✓	✓	✓
Encrusting bryozoans	✓	✓	✓
Encrusting ascidians	✓	✓	✓

Prostrate/hemispherical sponges	✓	✓	✓
Serpulids	✓	✓	✓
Parazoanthus axinellae	✓	✓	✓
Azooxanthellate scleractinians	✓	✓	✓
Branched bryozoans	✓	✓	✓
Arborescent/massive sponges	✓	✓	✓
Myriapora truncata	✓	✓	✓
Erect ascidians	-	✓	✓
Paramuricea clavata	✓	✓	✓
Alcyonum coralloides	✓	✓	✓
Pentapora fascialis	✓	✓	✓
Reteporella_grimaldii	✓	✓	✓
Adeonella calveti / Smittina cervicornis	✓	✓	✓

Alpha and beta diversity did not show significant differences among the three sites. Values for α-diversity were 6.80, 6.70, and 7.80 for *Orlata A*, *Parete Est* and *Orlata B*; while for β-diversity, the values obtained were 0.09, 0.07, and 0.18. For the calculation of EQR, we relied on the abundance values of the index of the sensitivity level SL (Table 1), where each taxa has assigned a sensitivity value ranging from 6 to – 4. This information was related to the abundance of each identified taxa for each photographed plot, and the Ecological Status Index was calculated by calculating the scores obtained for each site.

From the analyses for calculating the ESCA index, we obtained EQR values indicating "sufficient status", with values of 0.51, 0.51, and 0.49 for Orlata A, Parete Est and Orlata B respectively (Table 5).

Table 4. Ecological quality values sensitivity level (EQVSL), Ecological Quality Ratio (EQVrif) of Submerged Littoral, Ecological Quality Ratio alpha diversity (EQValpha), Ecological Quality Value of Rif (EQVrif), Ecological Quality Ratio of Algae (EQRa), Ecological Quality Ratio beta diversity (EQVbeta), Ecological Quality Ratio (EQRb) of Benthic Communities.

Area	***Site***	EQV_{SL}	$\boldsymbol{EQV_{rif}}$	$\boldsymbol{EQR_{SL}}$	EQV_{alpha}	$\boldsymbol{EQV_{rif}}$	$\boldsymbol{EQR_{a}}$	EQV_{beta}	$\boldsymbol{EQV_{rif}}$	$\boldsymbol{EQR_{b}}$
Isola delle femmine	Orlata A	358	**540**	0.66	6.8	**15**	0.45	0.085	**0.20**	0.43
	Parete Est	395	**540**	0.73	6.7	**15**	0.45	0.070	**0.20**	0.35
	Orlata B	417	**540**	0.77	7.8	**15**	0.52	0.183	**0.20**	0.92

Table 5. EQRSL Ecological Quality Ratio Sensivity Level, EQRa Ecological Quality Ratio alpha diversity, EQRb Ecological Quality Ratio beta diversity, ESCA Ecological Status of Coralligenous Assemblages. In the second table the status of coralligenous Marine Protected Area (MPA) of Capo Gallo – Isola delle Femmine (sufficient).

Site	***Area***	$\boldsymbol{EQR_{SL}}$	$\boldsymbol{EQR_{a}}$	$\boldsymbol{EQR_{b}}$	$\boldsymbol{EQR'}$	
Isola delle Femmine	Orlata A	0.66	0.45	0.43	0.51	**0.50**
	Parete Est	0.73	0.45	0.35	0.51	
	Orlata B	0.77	0.52	0.18	0.49	

The PCoA showed a clear differentiation between Orlata A and the other two sites was observed, indicating that Orlata A supports a significantly different ecological community. The overlap between Orlata B and Parete Est suggests that these two areas share a more similar species composition, though with some internal variability. The ellipse for Orlata A is rather compact but well separated, suggesting some consistency in the species present in the group, but with a distinct composition from the others. The other two groups (Orlata B and Parete Est) showed greater dispersion and some degree of overlap, implying similarities in species composition, but with higher internal variability.

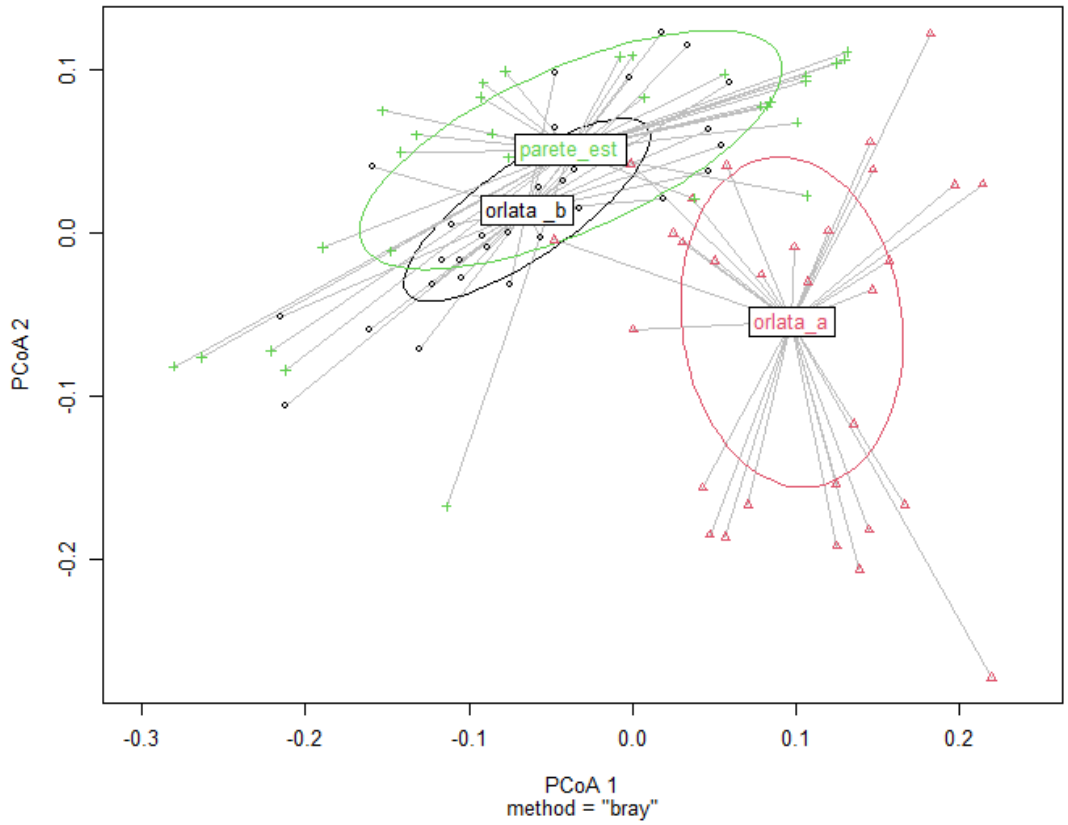

Figure 3. ***Principal Coordinates Analysis (PCoA) showing the variation in species composition across the studied sites.*** *The PCoA was performed based on a [distance/similarity metric, Bray-Curtis dissimilarity], with the first two principal coordinates (PCoA1 and PCoA2) explaining 37.17% and 21.23% of the total variance, respectively. Different symbols/colors represent studied sites and the proximity of points reflects the degree of similarity between samples. Orlata A site (red △), Orlata B site (black ◎),Parete est site (green +)*

From the SIMPER analysis it was possible to idenfy thatthe species that most contributed to the differences among sites were encrusting calcareous rhodophyta, *Paramuricea clavata*, algal felt, encrusting sponges, and *Peyssonnelia* sp. *Paramuricea clavata* was particularly abundant in Orlata A, making it one of the species with the highest contribution to the differentiation of the sites.

Discussions

The lack of baselines or references points in the scientific literature presents a critical challenge for ecological studies aimed to preserve or restore natural ecosystems (Brewer & Menzel 2009, Danovaro et al. 2021). Only through this process can we fully understand the direction and effects of biodiversity loss. The limited information on the conservation status of the coralligenous community within the Capo Gallo - Isola delle Femmine MPA posed a significant challenge for this study, as it was not possible to compare the current status with that of previous years. The photographic sampling approach is a simple yet effective method that allowed us to gain a

comprehensive view of the health status of the coralligenous community. The assessment and estimation of individual species facilitated the rapid analysis of a high volume of samples in a non-destructive manner, thereby minimizing damage to the sensitive coralligenous habitat (Balata et al. 2007b a).

The calcareous matrix layer of coralline algae represents a crucial component of the coralligenous, as it serves as the foundation upon which secondary bioconstructors develop (Ballesteros 2006, Orlando-Bonaca et al. 2017). Coralligenous algae, composed mainly of calcareous algae, are marine structures that develop slowly over time. According to Ballesteros (2006) the estimated growth rate for these formations is about 0.006-0.83 mm per year. This makes coralline algal structures some of the slowest growing species in marine ecosystems. Given their limited growth rate, any significant disturbance or impact on these encrusting organisms can lead to long-lasting and potentially irreversible consequences. Activities such as trawling, coastal construction, pollution, and increased sedimentation can severely compromise their integrity (Howard et al. 2023).

Obtained conservation status for Capo Gallo – Isola delle Femmine MPA was significantly lower with respect to other Italian MPAs monitored following the same approaches. For example, with respect to the assessments performed by Piazzi et al. (2021a) in different MPAs from Sardinia, our data revealed a significant lower values in species richness, similar to those observed in the anthropogenically impacted sites from the aforementioned study. However, without a historical monitoring series or a reference point for the Marine Protected Area of Capo Gallo - Isola delle Femmine, we can only hypothesize that the obtained data do not reflect a good conservation status, indicating a potential ongoing ecological disturbance. An interesting point is that no significant differences in the conservation status were observed between monitored sites within the protection zone B and A, suggesting a climate-driven effect and potentially excluding a direct anthropogenic impact, since zone A is the "no take zone" under strict surveillance and protection, aiming to minimize the negative influences of human activities. Additionally, the variations observed in the coralligenous assemblages could also be attributed to natural factors, such as biogeographical gradients, hydrodynamic conditions, and substrate morphology, as suggested in the literature (Casas-Güell et al. 2015, Piazzi et al. 2016, 2021b). These factors can create complex and differentiated habitats even in areas with similar ecological conditions, influencing species distribution and composition.

Coralligenous key structuring species exhibit some traits, such as slow growth or low dispersal ability, that make them more vulnerable to disturbance. The slow recovery or, in many cases, inability to recover at all from a stressful condition, underscores the critical need to protect these habitats. It is well known that invasive alien species pose a threat to biodiversity by causing habitat homogenization and, consequently, a decrease in beta diversity (Piazzi & Balata 2008). These invaded habitats tend

to simplify and lose their pre-invasion characteristics. In the sites surveyed by this study, the invasive alien species *Caulerpa cylindracea* was present at all three monitoring sites, significantly contributing to the lowering of the conservation status of the habitat. The scientific literature indicates that algal species such as *C. cylindracea* pose a serious threat to biodiversity by reducing both alpha and beta diversity (Piazzi & Balata 2008, Kersting et al. 2013), negatively affecting coralligenous communities by simplifying the habitat. Moreover, the presence and abundance of *Halimeda tuna* and *Flabellia petiolata* algae can serve as indicators of anthropogenic disturbance, as they are highly sensitive to sedimentation and the presence of invasive alien species (Cecchi et al. 2014). In this study, there was a low presence of *H. tuna*, a very important and sensitive species with an SL index (maximum value of 6), potentially contributing to the "sufficient" conservation status of the coralligenous community in the studied area.

The differences in species composition observed among sites within the MPA may be attributed to the varying levels of protection. The Orlata B and Parete Est sites were located in zone B, while the OrlataA site was in zone A. The species that most contributed to these differences included encrusting calcareous rhodophytes, *Paramuricea clavata*, algal felts, encrusting sponges, and *Peyssonnelia* sp. *Paramuricea clavata* was particularly abundant at Orlata A, making it one of the key species driving site differentiation. The stricter protection in zone A likely supports better preservation of upright structuring species like *Paramuricea clavata*. Even if Orlata A did not present the highest diversity index values, it counted with highly sensitive species, characterized of a good environmental and conservation status of the habitat.

In conclusion, the results of this study establish a baseline for assessing the health and conservation status of the coralligenous habitats within the Capo Gallo MPA - Isola delle Femmine. This research fills a critical knowledge gap by providing data on the condition of these habitats, for which information was previously lacking in the literature. From a management perspective, it is crucial to conduct efficient and continuous monitoring programs for identifying the main sources of disturbance, and assessing the conservation status and functioning of threatened key habitats. This may allow for timely interventions to limit further damage and may improve managers and policy makers capability to act prevention or mitigation actions to mitigate impacts on biodiversity (Pereira et al. 2010). Strategies for safeguarding and conserving coralligenous habitats include the establishment of MPAs to protect sensitive habitats and promote sustainable fishing and diving practices (Kipson et al. 2011, Fraschetti et al. 2013). The absence of references points for the conservation of key habitats presents a significant challenge for the management, practitioners and scientist involved in marine ecosystems protection actions. Establishing such a baseline requires a

coordinated effort, including continuous monitoring, scientific research, and the involvement of local communities, to ensure that management policies are grounded in reliable and up-to-date data.

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Chapter 2

Effect of natural thermal stressful conditions on *Eunicella singularis* meadows functioning: in-situ assessment of metabolic traits responses

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Abstract

Climate change is exerting a profound influence on marine ecosystems, resulting in structural alterations and significant biodiversity loss. One of the most concerning trends is the increase in sea temperatures and the heightened occurrence of extreme weather events, such as marine heatwaves (MHWs), which disproportionately affect vulnerable marine communities, particularly coralligenous and pre-coralligenous habitats within the Mediterranean Sea. These habitats are characterized by species with long life cycles, slow growth rates, and limited dispersal capabilities, making them particularly susceptible to environmental stressors. In light of these challenges, this study aimed to investigate the effects of climatic changes throughout the pre-summer and post-summer seasons on *Eunicella singularis* meadows, a prominent habitat-structuring gorgonian species found in Mediterranean coralligenous communities. By employing benthic chambers, field measurements were taken to assess key metabolic processes, including respiration rates of the colonies and the productivity of their symbiotic organisms. Additionally, laboratory analyses were conducted to examine oxidative stress responses in the coral, focusing on important stress indicators such as catalase and glutathione reductase. Finally, quantification of the total and organic suspended matter in the water column allowed us to assess the meadow role in ecosystem carbon sequestration process. This comprehensive approach provides valuable insights into how climate change impacts the functioning of these crucial habitats, contributing to our understanding of potential future responses of marine communities to ongoing environmental changes.

Introduction

Climate change (CC), along with different anthropogenic drivers, is causing significant alterations to the structure of natural habitats and their associated communities, thereby leading to biodiversity loss and changes in ecosystem functioning (Talukder et al. 2022, Weiskopf et al. 2020). The scientific literature has already shown how CC effects are acting at local and global scales and will continue to do so (Hoegh-Guldberg and Bruno 2010). Climate changes are developing at a disproportionate rate; consequently, their negative effects are becoming increasingly evident (Sarà et al. 2021). These changes will significantly alter the structure and function of marine ecosystems, with notable socioeconomic impacts associated to the ecosystem services that they provide. Temperature increase is undoubtedly one of the main sources of disturbance caused by CC, in addition to ocean acidification, eutrophication, and extreme storms (Gattuso et al. 2018). In fact, temperature is a crucial factor that regulates and maintains organism's homeostasis by directly influencing all biochemical reactions and metabolic rates of ectotherms, thereby determining species' thermal tolerance limits, phenology, and distribution patterns (Sokolova & Pörtner 2003, Sunday et al. 2012, Walther et al. 2002).Over the past decade, thermal anomalies such as marine heat waves (MHWs) have increased globally in frequency and intensity, with significant consequences for marine ecosystems, affecting all levels of the ecological hierarchy, from species reproductive cycles and distributions, to communities composition and structures, food web dynamics and nutrient cycling (Carbonne et al. 2022).

The Mediterranean Sea harbors numerous endemic ecosystems, such as coralligenous habitats and *Posidonia oceanica* seagrass beds, which may be highly threatened by climate change (Ballesteros 2006). Coralligenous habitats are formed by the accumulation of encrusting coralline algae along with other structuring benthic organisms found in shallow waters (Orlando-Bonaca et al. 2017). This habitat performs important and fundamental ecosystem services (E.S.): it is a biodiversity hotspot due to its morphological complexity, which creates refuge and feeding areas for a wide variety of organisms (supporting and provisioning E.S.); it contributes to the trapping of CO_2 (regulating E.S.); and it is also a source of human well-being (cultural E.S.) as it plays an essential role in the proper functioning of ecosystems (Thierry de Ville d'Avray et al. 2019, Tonin, 2018, Tribot et al., 2016, Zunino et al. 2020). Specifically, Mediterranean gorgonians are extremely important habitat-forming species providing biomass, structural complexity, and shelter for countless organisms (Ballesteros 2006; Gili & Coma 1998, Jones et al. 1994). Gorgonian forests thus play important ecosystem services, which are threatened by natural events such as climatic anomalies as well as by

human activities such as fishing and anchoring (Ponti et al. 2014, Sarda et al 2024). Thermal stress generates significant impacts on zooxanthellate corals; in fact, most of the carbon requirements of corals are met by photosynthetic products provided by their symbionts. As a result, coral bleaching leads to a significant reduction in metabolic resources, loss of energy reserves, slowed growth, alterations in the microbiome, and increased vulnerability to diseases (Johnson et al. 2015). Under stress conditions, zooxanthellae can be expelled or leave the host through various cellular processes related to oxidative stress. Normally, both the cnidarian host and zooxanthellae produce a full range of antioxidant enzymes to prevent damage caused by reactive oxygen species (ROS). However, under stressful situations, the imbalance between excessive ROS production and antioxidant defenses causes cellular damage, such as lipid peroxidation, protein oxidation, and DNA degradation, leading to the breakdown of the symbiotic relationship (Richier et al. 2005, 2008). Combining these factors can lead to increased coral mortality (Carbonne et al. 2024). In the Mediterranean basin, coral species with endosymbionts are less common than in tropical reef corals; however, the white gorgonian Eunicella singularis (Esper, 1794), one of the most common and abundant species in the western Mediterranean basin, harbors endosymbiotic zooxanthellae(Gori et al. 2011, 2012). The species has experienced massive mortality events in recent years (Coma et al. 2006, Linares et al. 2005) and it is estimated declined in its populations by 40 % over the last 20 to 30 years, being identified as a vulnerable species to climate change (Ferrier-Pagès et al. 2009). Upscaling these responses at ecosystem functioning level is fundamental to understanding the role of these animal forest in the current and future scenarios of climate change. Gorgonian meadows may play a key role in food web dynamics and nutrient cycling (Goldberg 2018). The total suspended organic matter (TSM) includes all components of particulate organic matter (POM) such as detritus, plankton and other organic particles. POM serves as a food source for many filter-feeding organisms, including gorgonians. As organisms consume POM, they assimilate carbon into their biomass, which is crucial for growth and energy storage. When these organisms die, their biomass can contribute to the sediment, effectively sequestering carbon in the benthic environment (Howard et al. 2023). This process can help mitigate climate change by storing carbon in the ocean.

The following study aims to investigate the effects of natural thermal stress on *E. singularis* meadows functioning by i) measuring in the field, through the use of benthic chambers, the eco-physiological response of the coral and its symbiont (oxygen production and consumption rates, and photosynthetic efficiency); ii) the expression of oxidative stress enzymes; iii) the role of the habitat-former in the C sequestration through the quantification of the total and organic suspended matter along the water column, before and after thermal stress.

Materials and methods

Study Area

The study area was the marine protected area of Capo Gallo and Isola delle Femmine (Zone A, 38.210188, 13.233185). The bathymetry at the site was approximately 20 meters deep. The area is characterized by a rocky platform rich in *Posidonia oceanica* meadows and *Eunicella singularis*. During a preliminary phase of the study, two sites with identical current exposure and similar chemical and physical characteristics were identified, differing only in the density of white gorgonian: one site with high density (HD) and one with low density (LD). The mean densities of *E. singularis* at the two sites were estimated using 1 m² quadrats. To investigate the coral's response to summer thermal conditions, a monitoring campaign was planned, consisting of two phases: the first pre-summer (June - July 2023, divided into two periods) and the second post-summer (November 2023 – December 2023).

In-situ metabolic measurements

To obtain field metabolic measurements of *Eunicella singularis* colonies, ad-hoc benthic chambers were used to measure the respiration rates of coral colonies and the oxygen production of their symbionts (Figure 1). Each benthic chamber was constructed from a transparent polycarbonate cylinder, 25 cm high and 10 cm in diameter, with a total volume of 1.9 l. A transparent methacrylate cap with a printed manifold, created using a 3D printer, was attached to the top end of the cylinder to house the sensors. The sensors in the benthic chambers measured dissolved oxygen concentration, internal temperature, and light irradiance. To ensure proper recirculation within the chamber, a 5V recirculation pump connected to a self-contained battery pack was integrated. The chamber was also equipped with a blackout cover, allowing for the measurement of respiration and the production of photosynthetic organisms. Each individual colony was photographed from different angles using a Canon G7X Mark III and a YS-D3 flash with a reference unit before the installation of each benthic chamber. This was done to estimate the weight/volume occupied by the colony within each chamber. Measurements lasted 90 minutes for respiration, which was

Figure 1. Benthic chamber and environmental data logger station measuring on a colony of Eunicella singularis in the MPA of Capo Gallo - Isola delle Femmine.

assessed by darkening the chambers, and 90 minutes for oxygen production, which was measured by uncovering the benthic chambers.

Respiration rate (RR) and oxygen production.

Both metabolic rates were calculated according to the following formula:

$$RR = \frac{\beta_{[O2]} * t * Vol}{DW} \ (s*L/g)$$

where β[O2] is the slope of the measured oxygen concentration within the respirometric chamber (β[O2]), standardized per time unit (t, s) and the volume of the respirometric chamber (Vol, L), and calculated in relation to the organism body mass as dry weight (DW, g).

Relation Area Weight and Image Analysis.

To estimate the dry mass of the corals used in the RR measurements, 20 white gorgonian fragments of about 15 cm were collected during the sampling actions. Each fragment was assigned an ID, placed on graph paper, and then photographed. The weight of each fragment was measured using a precision microbalance in three conditions: wet, dry (105°C for 24 hours), and ashed free dry weight (450°C for 4 hours), to create an area-weight relationship. Photographs of each colony were imported into ImageJ software, which was used to estimate the occupied area in cm² of each sample and then the dry weight for each experimental colony was obtained and integrated in the metabolic rates calculations.

Symbiont photosynthetic activity.

At the end of the incubation period, 3 colony fragments per individual were sampled and immediately on board, the photosynthetic activity of each individual was assessed by measuring in vivo chlorophyll-*a* fluorescence of photosystem II (PSII) with a portable pulse amplitude modulation fluorometer (Junior-PAM, Waltz). Maximum quantum yield (F_v/F_m) was determined on 30-minute dark-adapted samples (Page et al., 2021) in order to oxidise the reaction centres of photosystem II. These values indicate the maximum capacity of the photosynthetic apparatus (Rasmusson et al., 2021). Then, the minimum (basal) fluorescence (F_o), and the maximum fluorescence (F_m) were measured after a saturation pulse (9000 mmol photon m^{-2} s^{-1}, 800 ms) of actinic light and estimated the maximum quantum yield as $F_v/F_m = (F_m - F_o)/F_m$ (Murchie & Lawson, 2013). F_v/F_m ratio represents the maximum potential yield and it is usually used as measure of stress that affects components of

photosystem II, such as the D1 protein (Beer et al., 2015). The maximum quantum yield of PSII (F_v/F_m), obtained using PAM fluorometry, can serve as an indicator of plant stress (Maxwell1 & Johnson, 2000; Beer Sven, 2015; Bhagooli et al., 2021; Rasmusson et al., 2021).

Antioxidant enzymes activity

At the end of the metabolic measurements, 3 colony fragments of 2 cm were collected and immediately stored in liquid nitrogen for oxidative stress analysis. The activity of the Glutathione Reductase (GR) and Catalase enzymes was quantified. For performing protein extraction *E. singularis* fragments were first crushed using a mortar and pestle (1 fragment = 1 sample) and placed into 5 mL microtubes. The samples were then homogenized in 1 mL of PBS (0.14 M NaCl, 0.003 M KCl, 0.01 M Na2HPO4, 0.002 M KH2PO4, pH 7.4). After homogenization, the samples were centrifuged at 4°C for 15 minutes at 10,000 × g. The resulting supernatant was transferred to 1.5 mL microtubes and immediately frozen at -80°C.

The enzymatic analysis of glutathione reductase was performed following the protocol of the MaRHE Marine Research and High Education Center. GR activity was measured using a spectrophotometer with an absorbance of 340 nm. This allows for the measurement of the oxidation reaction of NADPH to NADP+, which occurs concomitantly with glutathione reduction and is proportional to the decrease in absorbance over time. The catalase enzyme assay was performed according to the protocol of the MaRHE Marine Research and High Education Center. Catalase activity was assessed by spectrophotometric detection of absorbance at 240 nm, with double readings at different sample concentrations (20 micromoles and 40 micromoles).

Organic Suspended Matter quantification

For the estimation of the organic suspended matter, a total of 48 replicates of water were sampled at both HD and LD sites and at two different depths corresponding with the sea surface layer (1 m depth) and sea bottom level, where the gorgonian meadow was present (20 m). Water filtration was performed by using Whatman GF/F 47mm filters pre-treated by 90 minutes in the oven at 105°C. After filtering 2 liter per replicate, filters were frozen and stored at -20ºC. To estimate TSM, filters were then placed in an oven at 105°C for around 24 hours, until their weight was stabilized. The filters were weighed to obtain the TSM. To separate the organic from the inorganic fraction, the filters are treated in a muffle furnace at 450°C for an additional 4 hours and weighed again for ash (inorganic matter). The difference between the weight of the TSM and the weight of the ash allows for estimating the content of Organic Suspended Matter (OSM).

Data analysis

To assess the effects of climatic stressors in high and low density *Eunicella* meadows (factor "density") during the two monitored periods (pre and post-summer, factor "time") a two-way ANOVA was performed for respiration rate, gross primary production, and oxidative stress markers. Prior to conducting the ANOVA, the assumptions of normality and homoscedasticity were tested using the Shapiro-Wilk and Levene's test respectively. The analysis of OSM was equally performed applying a two-way ANOVA for "density" factor and "depth" (sea surface – 1m, and bottom – 20m). All statistical analyses were conducted using R software.

Results

Eunicella meadows metabolic performance (oxygen consumption and production and photosynthetic activity) was not significantly different between high and low density meadows, while some differences were observed between periods. Specifically, respiration rates did not show significant differences between both investigated periods (pre and post summer time, $F_{(1, 16)}= 1.658$, p-value = 0.1135, Figure 2a), even if slightly higher metabolism was observed during June-July with respect to autumn period.

Oxygen production from the gorgonian symbiont was higher during the pre-summer period followed by a decreased during the post-summer months, even if not statistically significant ($F_{(1, 16)}= 0.597$, p-value = 0.557, Figure 2b). Equally, photosynthetic efficiency (Fv/Fm) presented higher values before the warm period ($F_{(1, 16)}= 0.623$, p-value = 0.536, Figure 2c).

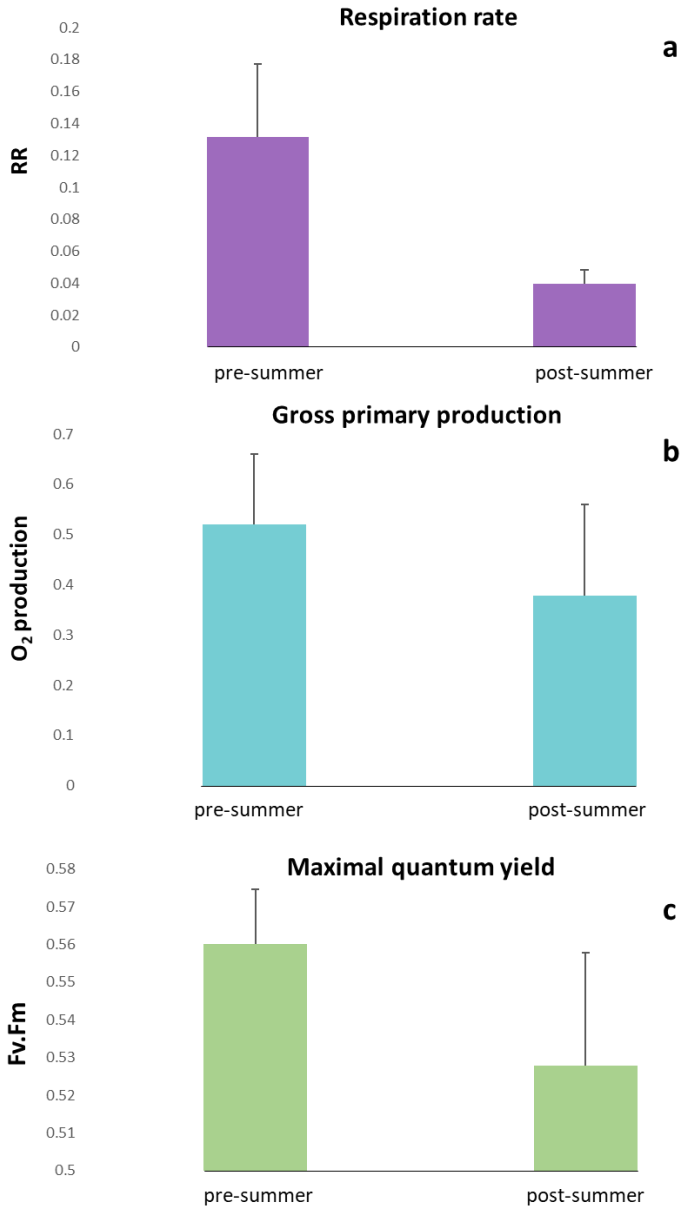

Figure 2. Respiration rate (a), photosynthetic activity (Fv/Fm) (b) and gross primary production (c) of Eunicella singularis during pre and post-summer periods. Data is reported as mean ± s.e.

Enzymatic assays did not showed significant differences between *Eunicella* meadows densities, while Glutathione reductase presented significant lower activity levels during the pre-summer period ($F_{(1, 20)}$= 5.520, p-value < 0.001, Figure 3a). Enzymatic activity of Catalase was not significantly different between periods, even if it seem to be slightly higher before the warmer season ($F_{(1, 20)}$= 1.081, p-value = 0.29, Figure 3b).

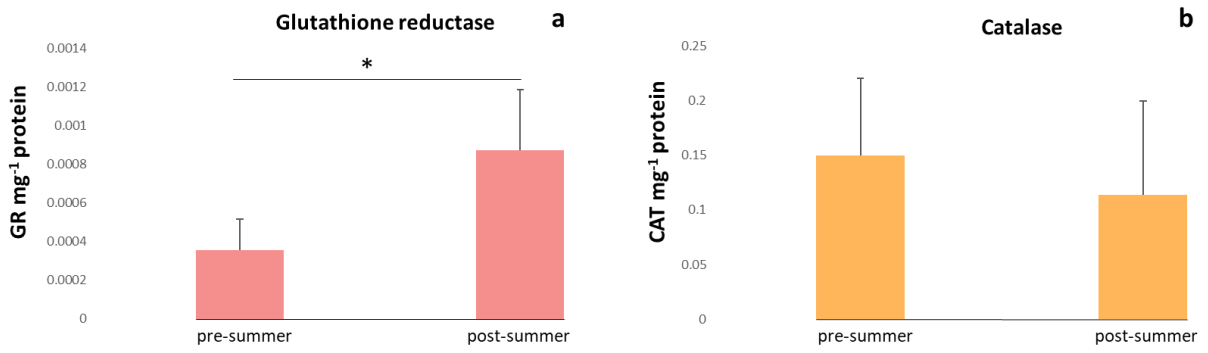

Figure 3. Glutathione reductase (a) and Catalase activity in Eunicella singularis during pre and post-summer periods. Data is reported as mean ± s.e.

Significant differences in OSM between sites and depths were observed. High-density meadow presented higher OSM values in the surface with respect to the bottom ($F_{(1, 1)}$= 5.855, p-value= 0.019); while low-density meadow did not presented significant differences in OSM between sea surface and bottom sampling areas ($F_{(1, 1)}$=, 1.654 p-value= 0.206, Figure 4).

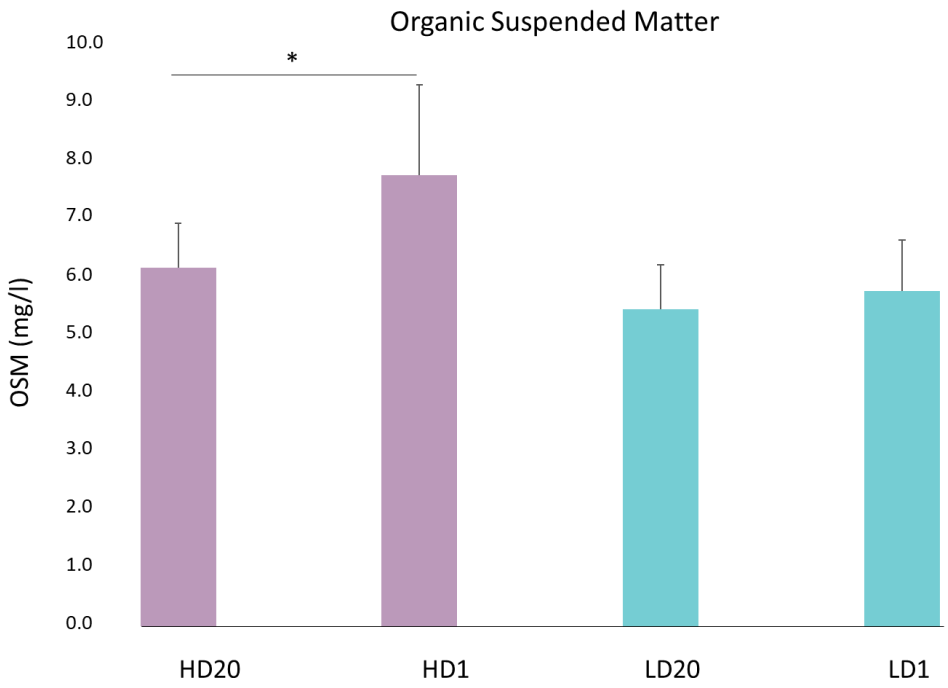

Figure 4. Organic suspended matter concentration at different meadows densities and depths, where HD1 and HD20 refer to water column in the high density meadows at 1 and 20 meters depth respectively; while LD1 and LD20 refer to water column in the high density meadows at 1 and 20 meters depth respectively. Data is reported as mean ± s.e.

Discussions

Integrated approaches based on metabolic measures, oxidative stress analysis, and water column biochemistry allowed us to comprehensively investigate and study the response of white gorgonian meadows to climatic stressors. White gorgonians have experienced a 40 percent decline over the past 20 to 30 years, potentially due to climate change effects (Coma et al., 2006; Linares et al., 2005). It may represent a serious loss of biodiversity since they are key habitat-forming species in the Mediterranean Sea (Coll et al. 2010). The data collected in the field revealed thermal anomalies during the summer of 2023, highlighting marine heatwaves (MHWs) and heat spikes during last August and September. The maximum temperatures recorded reached 25.6 ºC, exceeding the average for this period by more than 4 ºC (Figure 5).

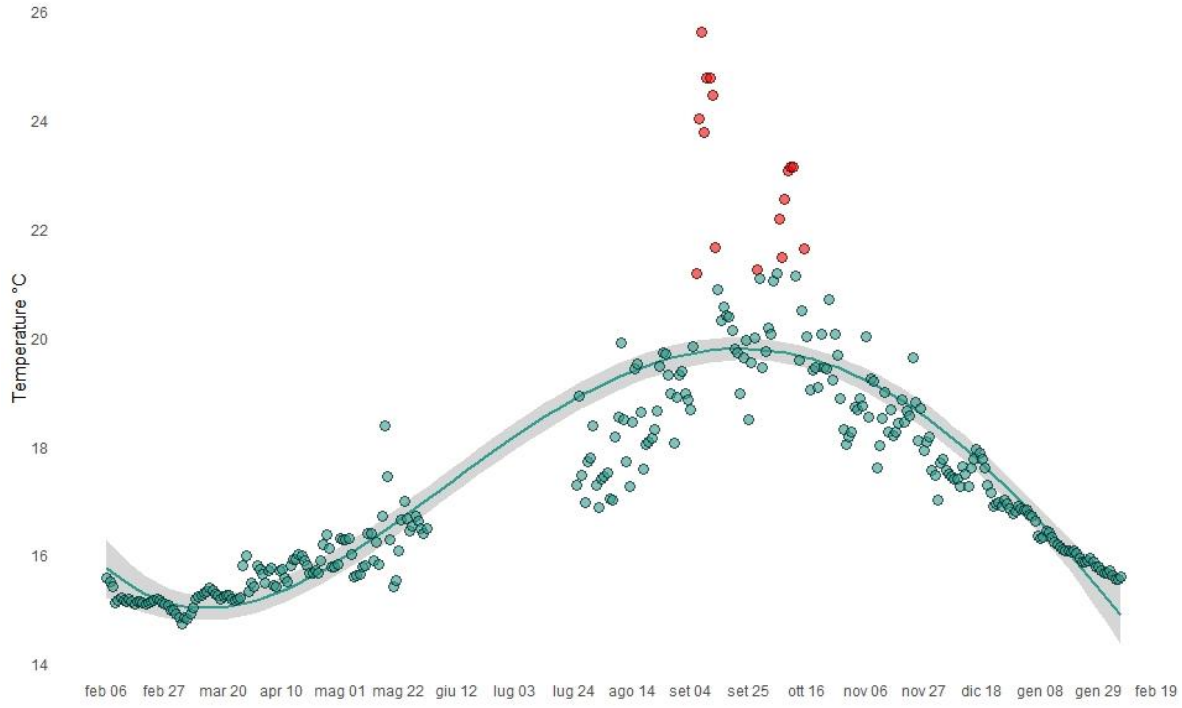

Figure 5. Thermal series at 20 m depth from June 2023 to February 2024 in the Capo Gallo - Isola delle Femmine MPA, Palermo. Red dots indicate days of thermal anomalies (sea surface temperatures exceeding the 95th percentile of our data).

During pre-summer months, the gorgonian meadows presented high respiration rates with respect to post-summer time. It is well-known that increases in temperature have direct effects on metabolism, which in turn influence species' performance (Sarà et al. 2013). Coral symbiont activity follow similar trend for oxygen production and photosynthetic activity (Fv/Fm), presenting higher values in June-July. These findings suggest that the coral and its symbionts were functioning

optimally prior to the summer heat, with enhanced metabolic activity and photosynthetic performance. Markers for oxidative stress confirm metabolic outcomes. The levels of glutathione reductase were lower during the pre-summer period, indicating a lower oxidative stress response when conditions were more favorable for the coral's metabolism. However, post-summer, glutathione reductase levels increased, suggesting that the coral experienced higher oxidative stress, likely due to environmental conditions such as elevated temperatures. Interestingly, catalase, another critical antioxidant enzyme that breaks down hydrogen peroxide, did not show significant differences between the two periods. This discrepancy between the two enzymes' responses is notable. While glutathione reductase was actively involved in counteracting oxidative stress post-summer, catalase might not have been triggered because the type or intensity of reactive oxygen species (ROS) generated, particularly hydrogen peroxide, may not have reached a threshold requiring its activation. Instead, the glutathione system (regulated by GR) appeared to be sufficient in managing the ROS during this time.

Although metabolic responses and oxidative stress markers indicated *Eunicella* meadows were exposed to stressful conditions, the overall health status of the corals during post-summer was good, with a low percentage of necrosis observed (Figure 6). Following the 2009 mortality events due to MHWs, the thermal tolerance of the species has been widely studied, though without conclusive results. Ferrier-Pagès et al. (2009) suggested that *E. singularis* shallow populations were more sensitive to temperature changes compared to deeper populations, and that temperature would not significantly affect their functioning until reaching 26 ºC. Ezzat et al. (2013) suggested that this threshold might actually be lower, with temperatures above 24 ºC potentially impair the functioning and energetic reserves of the species. While Pey et al. (2013) experimentally observed that seawater above 28 °C led to rapid and complete tissue necrosis for *Eunicella*. The interaction among environmental stressors in nature, often produces complex, non-linear effects on ecosystems, where the combined impact can exceed the sum of individual stressors, leading to unpredictable outcomes for species and ecological processes (Gunderson et al. 2016, Jackson et al. 2021, Orr et al. 2020). Additionally, to increasing temperature, marine communities face several stressors during the summer period. The period between June and July represents the time of planula release by *E. singularis* polyps. During this period, the polyps invest most of their energy in larval production, which can weaken organisms by

Figure 6. Colony of Eunicella singularis presenting signs of necrosis.

pumping a large part of their energy supply, leaving less energy available for somatic growth and other energetic processes (Ribes et al. 2007). In addition, the Mediterranean Sea is naturally oligotrophic, particularly during the summer period, which results in less food availability for suspension-feeding organisms such as *E. singularis*. Indeed, in the field, it has been suggested that some corals populations suffer from starvation during the whole summer (Coma et al. 2006) because thermocline prevents the upwelling of nutrients and the populations are thus more sensitive to stress (Coma et al. 2006).

Finally, our study highlights the ecological importance of *Eunicella singularis* meadows in carbon sequestration, underscoring their potential as key ecosystems in mitigating climate change. In particular, the ability of these meadows to filter and incorporate organic carbon from the water column is crucial for understanding their overall contribution to the marine carbon cycle. In addition to the function of absorbing CO_2 from the water through the photosynthesis of symbiotic algae (zooxanthellae), the assimilation of suspended organic carbon by *Eunicella* meadows may contributes to biomass formation, indirectly serving as an important nutrient source for other organisms within their habitat, thereby contributing to biodiversity and the overall health of the ecosystem. Additionally, the sequestered carbon could be stored long-term, providing an important function for climate change mitigation. Outputs suggested that coral density plays a crucial role in the effectiveness of carbon sequestration. In areas with high *Eunicella* density, the greater availability of surfaces for interaction with suspended organic matter may explain the observed increase in carbon absorption. The autotrophic contribution of symbiotic algae in *E. singularis*, together with its higher abundance, make this species more important in benthic-pelagic coupling processes (Coppari et al. 2019).

Outcomes raise questions about the future of these meadows in scenarios of environmental stress, such as warming waters, which could affect coral performances, population density and, consequently, their capacity providing ecosystem services related to carbon sequestration and habitat provision.

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Chapter 3

Using local ecological knowledge to assess the distribution and ecological status of endemic mediterranean coral *Astroides calycularis*

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Abstract

Continued rising temperatures are causing significant impacts on local and global scales, affecting both marine and terrestrial communities. Many studies claim that global warming is responsible for the increasingly frequent marine heat waves, responsible of massive mortality events of shallow benthic marine communities. In the present work we decided to investigate the spatial distribution and health status of the endemic Mediterranean coral *Astroides calycularis*, using a citizen engagement strategy such as Local Ecological Knolowledge. Furthermore, given that climate change is favoring the expansion of some thermophilic species native to the Mediterranean basin, such as *Hermodice carunculata*, this study allowed us to obtain the distribution of the corallivorous polychaete and possible interactions between *A. calycularis* and *H. carunculata*. Finally, we were able to obtain divers perceptions of thermal variations that have occurred over the past 3 years.

Introduction

Climate change (CC) is having rapid and significant effects at local and global scales, with major consequences for ecosystems and human well-being (Hoegh-Guldberg & Bruno 2010). CC represents the largest and most pressing issue in marine and terrestrial ecosystems, as it acts at huge spatial and temporal scales thereby going on to alter the local levels of biodiversity, the structure and function of ecosystems, and the goods and services they provide (Ben-Hasan & Christensen 2019). Extreme climatic events such as heat temperature spikes and marine heatwaves (MHWs), phenomenon closely related to CC, are significantly increasing in frequency and duration in the last years (Hobday et al., 2018, Hobday et al. 2016, Hobday et al. 2018, Smale et al. 2019). The oceans absorb most of the heat generated by greenhouse gas emissions (about 93%), causing an increase in sea water surface temperature that is projected to be between 1.1ºC and 6.4ºC over the next 100 years (IPCC AR6 Sixth Assessment Report, 2021). Temperature, is an important factor that plays a key role in this context of change, as all biological processes are temperature-dependent (Brown, J et al. 2004, Kooijman 2010). Changes in temperature can influence organisms functional and life history traits, such as those related to the tolerance to abiotic factors, reproduction or growth processes, impacting significantly on the individuals fitness and ultimately in the population dynamics, species dispersal and distribution (Doney et al. 2012a, Poloczanska et al. 2016, Scheffers et al. 2016). These changes will also affect marine communities structure and the related biotic interactions, either strengthening or weakening existing interactions, and eliminating or creating new ones (Doney et al. 2012b). When organisms are unable to counteract the effects of changing thermal conditions, mortality events occur. These events are now widespread globally, particularly affecting shallow marine benthic communities (Barroso et al. 2016a, 2017, Díaz et al. 2019, Garrabou et al. 2019a). In the Mediterranean basin, the frequency of high-temperature summers and MHWs has risen exponentially, leading to massive mortality events among Porifera, Anthozoa and Bivalvia taxa (Galli et al. 2017, Kersting et al. 2013, Kružić & Popijač 2015; Cerrano et al. 2005). Most of the impacted species are habitat formers acting as refugia, nursery, feeding or living areas for a huge quantity of marine organisms, representing in most cases important biodiversity hotspots (Hoegh-Guldberg et al. 2007, Jones et al. 1994). At the same time, continued global environmental changes and rising seawater temperatures are also responsible for a demographic increased and latitudinal shift in the distribution of some warm-water affinity species (Encarnação et al. 2019, Righi et al. 2020a). These phenomena, in addition to facilitating the expansion of alien species, promotes the population growth and expansion of range-expanding native species (Righi et al. 2020b) . These species share a set of traits such as a generalist diet, high reproductive rates, a wide

range of tolerance to environmental conditions, and high levels of plasticity, which allow them to take advantage of changing climatic conditions at the expense of more vulnerable species, becoming in most cases “invasive” in their own native ecosystem. In this context, the varying responses of different marine organisms to climate change lead to “winners” and “losers” within this shifting environment.

Investigating how organisms respond to environmental conditions, as well as their distribution and interactions, is crucial to better understand the fate of marine communities in the current and future context of CC. The reliance on specialized and costly logistics for obtaining monitoring data poses particular challenges for marine research. This is especially true in the conservation context, where good data with sufficient spatio-temporal resolution are rarely available (Morrison et al. 2013). In this sense, engaging marine citizenship has become crucial to reach conservation goals (Anadóòn et al. 2009) In the last years, Marine Citizen Science (MCS) has arisen as a valuable methodological tool in the world of social and socio-ecological research (Sandahl & Tøttrup 2020 Wagner & Davis, 2003), providing essential information on different ecological processes and contributing to high-quality scientific research (Edgar et al. 2008; Hermoso et al. 2021). Particularly, collaborations between scuba-divers and scientists in MSC have demonstrated that valuable information can be obtained through participants' observations and their local ecological knowledge. Research covering extensive spatiotemporal scales (Edgar et al. 2014), the sightseeing of cryptic and rare species (Bonney et al., 2009), and the distribution of invasive species (Righi et al. 2020b) have all significantly benefited from MCS. Currently, that the consequences of global warming and the extreme climatic events are becoming more evident and rapid every day, expert divers represent an important resource as historical memory of the ecological status of marine habitats, acting as underwater observers of the variability of habitats over time and changing environmental conditions (Cattaneo-Vietti & Mojetta 2021; Hermoso et al. 2021).

Here, through guided interviews to scuba-divers using LEK techniques, we aimed to collect high temporal and spatial resolution information on I) the distribution of a vulnerable endemic habitat-former species, the shallow orange coral *Astroides calycularis* along the Italian coasts; II) the conservation status of its populations; III) its potential interaction with the polychaete *Hermodice carunculata*, a range-expanding predator in the Med; and IV) the scuba divers’ perception on the involved species and the temperature changes in the water column in the last years.

Material and methods

Structured interviews were conducted during autumn 2022 to diving centers staff from all coastal Italian regions (15 regions). From a total of 100 Italian diving centers contacted, 31 decided to actively collaborate in the study. Diving centers were identified through their website or social media (e.g., Facebook, Twitter, Instagram). Surveys were conducted in Italian by phone and Skype calls, depending on the availability of interviewees. The questionnaire (Supplementary Material S1) included 22 questions organized into 4 different sections:

I) *Astroides calycularis* distribution and health status: reporting the presence of orange coral (*Astroides calycularis*), including the depths at which it was observed. Divers were asked to assess the health of coral colonies at the dive sites they frequently visit, with particular attention to signs of coral necrosis. Necrosis was defined as tissue loss, exposure of the skeleton, loss of coloration (due to partial or total tissue loss), and colonization by saprophytic microorganisms, following the criteria by Gomez-Gras et al. (2019). To facilitate accurate identification of species and assessment of coral health, photographs were provided to support the questionnaire. These visual aids helped divers accurately recognize coral conditions, enabling them to provide reliable data on the health of *Astroides calycularis* at the dive sites.

II) *Hermodice carunculata* distribution and interaction with the orange coral: to explore the presence of the bearded fireworm (*Hermodice carunculata*) at various dive sites, as well as its cohabitation with the orange coral (*Astroides calycularis*). The focus of these interviews was to determine whether divers had observed interactions between the two species, particularly the possibility of predation by the fireworm on the coral. To ensure the accuracy of the information provided, divers were asked to share photographs that captured real interactions between *H. carunculata* and *A. calycularis*. These images served as visual evidence to support the divers' observations and provide concrete data on the species interactions in their natural environment. This approach aimed to provide a more reliable assessment of the potential predatory behavior of *Hermodice carunculata* towards *Astroides calycularis*, offering valuable insights into their ecological relationship and the possible impacts on coral communities at these diving sites.

III) Divers' perception: perception on both species when diving, as well as perception of changes in water temperature and thermocline over the last 3 years were addressed. The study also examined different perceptions of changes in water temperature and the thermocline over the past three years. The questions aimed to assess the divers' knowledge and understanding of thermocline dynamics and water temperatures, as well as their ability to observe these changes. The questionnaire included specific queries about the depth of the thermocline and any noticeable shifts in its position or characteristics. This approach sought to gather diverse observations and insights on potential

changes in thermal layers, providing valuable information on how these environmental factors may be evolving at the dive sites.

IV) Divers' personal information: experience of the interviewed diver (years of work experience in diving, number of dives per year), their familiarity with the diving sites, and personal details such as age and education level.

Responses were collected through categorical multiple-choice questions, dichotomous (yes/no) questions, and open-ended formats. Open-ended responses were later categorized for analysis. Divers were also encouraged to provide any additional information they believed would help, support or clarify their responses. This approach aimed to obtain a thorough understanding of the divers' backgrounds and observations, enriching the data with valuable insights to corroborate the findings.

Data analysis

For the analysis of the fireworm substrate preference we applied a Chi-squared test to evaluate the distribution (binary response variable) of the species among the four substrate types: sand, rock, *Posidonia oceanica* meadows and cave. Data was collected by asking interviewees to rate the water temperature as colder or warmer over the last 3 years. For the correlation between years of experience and perception of temperature increases and, mean annual number of dives and temperature perception Generalised Linear Models (GLMs) were performed. Statistical analyses were carried out using the free statistical software R, version 4.4.1. (R Core Package) Species distribution and co-occurrence maps were generated using QGIS software that allowed us to spatially visualize the collected data and identify distribution patterns and areas of overlap between *Hermodice carunculata* and *Astroides calycularis*.

Results

Characteristics of the respondents

A total of 31 scuba-divers were interviewed, most of them were over 35 years old and had extensive experience in the sector (61.3% of them had between 11 and 30 years of experience and 35.5% worked in the scuba diving industry more than 30 years). All of them worked daily in contact with the sea, with 43% of them performing more than 200 dives per year, and 23.3 % between 100 and 200 dives per year. In addition, high percentage of respondents had a higher education degree (46.6 % had bachelor's degree and 43.3% had a high school diploma) while 10% had secondary school.

Species habitat distribution and spatial overlap

A total of 136 dive sites were identified along the Italian coasts. Among those, there were 77 sites where interviewees reported the presence of the orange coral colonies presenting healthy and necrotic colonies (Figure 1 and 2 respectively).

Figure 4. Distribution map of Astroides calycularis observations along the Italian coasts. The size of the bubbles indicates the number of reports per site.

While the fireworm was observed in 33 sites, the majority of sightings were in the southern part of the Italian coasts, with few reports in Sardinia and the Elba Island (Figure 3).

Figure 5. Distribution map of Astroides calycularis in necrosis along the Italian coasts. The size of the bubbles indicates the number of reports per site.

A spatial overlap between the coral and the stinging polychaete of 29.4% along the Italian coasts was observed, being the 75% of the polychaete reports and the 78% of *A. calycularis* colonies observations reported in the first 20 meters of the water column. While the habitat of the orange coral was completely shallow rocky walls, the polychaete presented a significant difference in the substrate where it was present (61% rocky habitats, 12.9% caves, 9.6% sandy bottoms and 6.4% *Posidonia oceanica* meadows), showing a preference for a rocky substrate ($X^2_{df=3}$= 27.71, p-value = $4.17 \cdot 10^{-6}$). Co-presence between the two species was reported 40 times at 16 dive sites (11% of the total diving sites, Figure 4). Those reports referred to potential interaction between the species, where both were physically present together and the coral colonies showed signs of necrosis in the 100% of the reports. The distribution of these injured colonies was along the coasts of Sicily, Calabria, Apulia, Campania, Latium, Tuscany and Liguria.

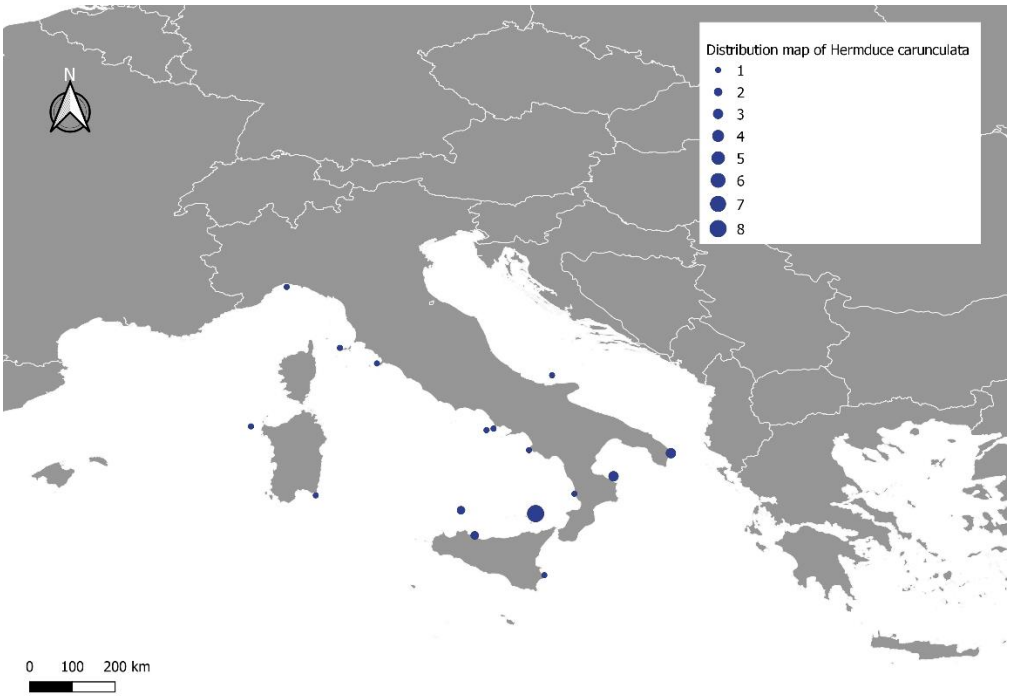

Figure 6. Distribution map of the polychaete Hermodice carunculata along the Italian coasts. The size of the bubbles indicates the number of reports per site.

Divers perception

From the results obtained from the semi-structured interviews, it was observed that 31% of divers was quite pleasant to dive in the presence of the coral colonies, while 68.4% said it was very pleasant. Divers expressed their perception of the presence of *H. carunculata*, with 57% responding that it did not bother at all, 9% that it bothered little, 14% that it bothered quite a bit, and 19% that it bothered a lot. Regarding diver's knowledge on thermocline, 100% of respondents known well what it was, and 48.3% of them reported that did not notice any changes in the thermocline depth in the last years, while 51.7% of the interviewees perceived a lowering of the thermocline depth. In addition, more than a half of respondents (51.6% of divers) perceived a water temperature rise in the past 3 years, while 45.1% did not notice any change, In addition, more than half of the respondents (51.6% of the divers) perceived an increase in water temperature in the past 3 years, while 45.1% did not notice any change. Those differences were not related to divers experience (GLM, z=-0.437, p-value=0.662) or divers' diving frequency (GLM, z=0.837, p-value=0.403).

Discussions

The use of LEK methods provided us with valuable insights into the distribution, realized niche overlap, and interaction of two key Mediterranean species experiencing opposite effects of climate change. These findings are closely related to the temperature changes that respondents have perceived along the Italian coasts in recent years. Experienced divers have noticed an increase in

temperature and a lowering of the thermocline, regardless of the area of Italy where they work. This is significant because Italy spans almost the entire latitudinal range of the Mediterranean Sea, giving us a unique overview of the central Mediterranean. Divers perception on Mediterranean warming agreed with the increasing temperature trends reported in the scientific literature (Lacoue-Labarthe et al. 2016; Mariotti et al. 2015), and may pose a risk for marine benthic species living close to their thermal tolerance limits, and an advantage to thermophilic organisms.

Reports from divers interviews allowed to define current species distribution range along the Italian coasts. The defined range expansion for the orange coral was wider that previously reported in literature, with more than 24 sightings reported over Pontine island (the currently known northern limit of the coral populations, (Musco et al. 2016). Divers knowledge also allowed to confirm the presence of the species in the Tremiti Islands and Venice lagoon, as was hypothesised from non-scientific reports on the species presence in this area of the central-western Adriatic (Musco et al. 2016), confirming the potential northward oriented latitudinal expansion of *A. calycularis*.

Many corals live near their thermal tolerance limits which implies the need for suitable chemical and physical environmental conditions (Hoegh-Guldberg & Brown 2007). Particularly, it has been experimentally observed that *A. calycularis* presents an upper thermal limit of 27.8 ºC (Tantillo et al. 2021), temperature easily exceeded during the summer period in the Mediterranean (Copernicus 2021). In this context, thermal fluctuations may have a detrimental effect on orange coral performance and population dynamics. In the literature there are few reports of mortality events of this habitat-former, located in the southern coast of Italy (Ischia and Pelagie islands) after summer MHWs in 2009 and 2023 respectively (Bisanti et al. 2022a; Gambi et al. 2010a). The present study expands upon the limited reports on *A. calycularis* mortality, reporting sightings of necrotic colonies along the coasts of northern Ionian, and Ligurian Sea, even if the higher number of reports concentrated in the Southern Tyrrhenian shallowed coasts. The recent increase in frequency, intensity and duration of MHWs in the Med (Garrabou et al. 2019), may significantly impair the habitat former performance and ability to offer ecosystem services related to biodiversity maintenance and enhancement (Micheli & Halpern 2005, Terrón-Sigler et al. 2014).

The fireworm present wide distribution, with higher abundances in the southern Tyrrhenian Sea as reported by (Righi et al., 2020b), confirming the distribution shift from east to west and the demographic increase of the species within the Mediterranean over time (Righi et al. 2020b). In addition, respondents answers allowed to identified 3 new presence reports of the species in the Gulf of Genoa Portofino MPA and Gallinara Island, and Elba Island, indicating an active expansion of the fireworm distribution in the last 4 years over the Italian northern coasts. *H. carunculata* is a species inhabiting multiple habitats, from coral reefs to algal and phanerogams meadows, to caves and

anthropogenic structures (Schulze et al. 2017). More than a half of the species sightings were performed associated to rocky substrate, suggesting a potential spatial expansion of the polychaete along rocky shorelines were *A. calycularis* colonies are frequently present. Our data confirmed what was reported by (Righi et al. 2020b) regarding the preference for rocky substrates by the fireworm. This may have greater significance as fireworm predation on orange coral has been verified (Bosch-Belmar et al., 2024), thus, threatening the habitat-former conservation status.

Distribution overlap and cohabitation between both, the predator and the prey, were reported by divers several times along the Italian coasts. *H. carunculata* is an omminovorous species, preferentially preying on corals (Souza et al. 2007; Wolf et al. 2014a) and may represent a threat to *A. calycularis* colonies, particularly during warmer months when water temperatures exceed the upper thermal tolerance limit of the coral and the fireworm presents high metabolism and predatory activity (Bosch-Belmar et al. 2024; Tantillo et al. 2021).

As reported by respondents, *A. calycularis* also represents an important cultural ES, attributing it high aesthetic value. A recent study has highlighted the importance of maintaining high levels of conservation of endemic Mediterranean habitats, such as coralligenous reefs and *Posidonia oceanica* meadows (Motta Zanin et al. 2024). This is because the willingness of cultural ecosystem service users to economically invest in the locations where these habitats are found depends on their conservation status (Zunino et al. 2020). In this sense, the conservation of this habitat-former would be important not only from an ecological perspective (since it is a biodiversity hotspot) but also from an economic point of view, as its presence and good state of conservation support local economies. On the other hand, despite what might be expected, divers generally do not have a negative perception of the stinging polychaete. They report that it does not significantly bother them during dives. In fact, the literature only highlights the negative impact of the species on artisanal fishing activities in southern Sicily (Tiralongo et al. 2023), remaining many knowledge gaps to be addressed.

The results of this study provide essential information and insights for Mediterranean biodiversity management and conservation plans. The effect of ocean warming on the distribution of potentially invasive predator and the conservation of a vulnerable habitat-forming species has been observed at large spatial scale. Thanks to LEK approach, the co-presence of both, predator and prey, was confirmed in the field, over a huge geographical distribution, raising an alarm about the significant negative impact that predation pressure could have on a species already stressed by climate change-driven environmental factors. Further studies on species interaction and the cascading effects this might have on the community structure supported by the coral and the overall local biodiversity are necessary.

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Chapter 4

Thermal performance and niche overlap of interacting species in the coralligenous habitat: the case study of the coral *Astroides calycularis* and the fireworm *Hermodice carunculata*

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Abstract

Global warming and particularly extreme climatic events (such as marine heatwaves) are significantly affecting marine ecosystems and the services they provide. In recent years, shallow benthic communities have experienced mass mortality events with the populations of the corals among those most affected *Astroides calycularis* among those most affected in the Mediterranean Sea. On the contrary, some species are taking advantage of rising temperature, such as the corallivorous polychaete *Hermodice carunculata*, which is considered a 'native invader' in the basin, since current climatic conditions may facilitate its distribution and increase its reproduction rate. To investigate the thermal tolerance of both species, thermal performance curves (TPC) were created through a mechanistic and modelling approaches by measuring the individual respiration rate (trait used as a proxy of metabolism). at 12 different temperatures (from 11°C to 33°C)".. Thermal optimum and thresholds were identified for both species, presenting mismatched thermal optima (19.8ºC and 25.5ºC for the coral and fireworm respectively). While A. *calycularis* presented a thermal niche typical of a Mediterranean species, the fireworm showed a thermophilus TPC. Main outcomes showed that the coral is currently living close to its maximum critical temperature (27.8ºC), at which the polychaete presents maximal metabolic performance. Outcomes confirm that increasing temperature represent a threat to *A. calycularis* functioning. In addition, the presence of *H. carunculata* may exacerbate the temperature effect, generating a multiple stressor condition that may impair the orange coral performance. Knowledge on species thermal tolerance is fundamental to

better understanding species interactions and communities' responses to climate change, becoming a useful basal information in the context of ecosystem conservation and management.

Introduction

The Mediterranean basin is the world's second-largest biodiversity hotspot, renowned for its rich abundance of endemic species (Durrieu de Madron et al. 2011). Due to its semi-enclosed morphology, the Mediterranean basin tends to warm at nearly three times the rate of the open oceans, being one of the most impacted regions by increasing temperature (Vargas-Yáñez et al. 2009, Lejeusne et al. 2010, Marbà et al. 2015). By 2050, it is predicted that 15-37% of species in the Mediterranean Sea will face serious impacts and potential extinction related to global warming. The vulnerability and sensitivity of species to climate change are directly related to their biological and ecological traits, which determine their ability to adapt to environmental changes (Fortini & Schubert 2017). Temperature is a crucial factor in this context of change, as all biological rates are influenced by it (Brown et al. 2004, Kooijman 2010). Temperature plays a direct role in biochemical reactions and metabolic rates of ectotherms, influencing their thermal tolerance limits, performance, phenology, and distribution patterns (Sokolova & Pörtner 2003, Sunday et al., 2012, Walther et al. 2002). The effects on these processes will alter community composition, the strength of biotic interactions and ultimately, shape the ecosystem structure and functioning (Doney et al. 2012). One of the most prominent and significant impacts of rising water temperature is the occurrence of marine benthic communities massive mortalities, often triggered by the increasing frequency and severity of extreme climatic events, such as marine heatwaves (MHW) ((Dadon, 2005,Smale & Wernberg, 2013, Crisci et al. 2011).

Astroides calycularis (Pallas, 1766) is a Mediterranean endemic colonial coral acting as habitat structuring species in the coralligenous community. Its bathymetric range spans from shallow waters to depths of 50 meters (Ocaña et al. 2000), with its highest abundance typically observed around 15 meters. *A. calycularis* has a crucial ecological role as habitat for numerous macrofaunal groups, acting as biodiversity hotspot of over 80 species of macroinvertebrates, including crustaceans, annelids, and mollusks (Terrón-Sigler et al. 2014a). This species has been identified as highly vulnerable to climate change since several mass mortality events affecting its populations have been reported after intense MHWs in the last years ((Bisanti et al. 2024;Bisanti et al., 2022 Gambi et al. 2018, Gambi et al. 2010).

Continued global environmental changes and rising seawater temperatures not only pose a serious threat to benthic organisms (Hughes et al. 2003) but also contribute to several species latitudinal shift, reshaping the distribution of species that prefer warmer waters (Encarnação et al. 2019; Righi et al. 2020). These climatic changes have provided an opportunity for invasive and thermophilic native Mediterranean species to expand their distribution range. The thermophilic polychaete *Hermodice carunculata* (Pallas, 1766), commonly known as the bearded fireworm, is considered a "native invader" benefiting from current climatic conditions that favor its geographical expansion and reproductive success within the warmer Mediterranean sea (Righi et al. 2020). Even if it has been observed preying on non-coral species such as anemones, sea cucumbers, jellyfish, and starfish (Righi et al. 2020), *H. carunculata* is classified as a corallivorous predator, feeding primarily on both soft and hard corals (Souza et al. 2007; Wolf et al. 2014) and the decaying tissues of weakened colonies (Wolf et al., 2014). Moreover, in the present thesis, predation pressure of the polychaete on *A. calycularis* has been confirmed and quantified (chapter 4, Bosch-Belmar et al. 2024). This pressure is exacerbated at high temperatures, when the coral's performance is compromised due to environmental conditions exceeding its upper thermal tolerance threshold.

Researchers can effectively harness the functional traits of organisms to comprehend their metabolic and physiological adaptability in response to environmental changing conditions (Bosch-Belmar et al. 2022, Sarà et al. 2013). Among the approaches leveraging functional traits, the study of thermal performance curves (TPCs) proves invaluable. TPCs can elucidate how changes in temperature influence physiological and fitness-related aspects crucial for organismal survival, development, and reproduction (Sinclair et al. 2016, Kingsolver and Buckley 2020). This approach allows for the identification of the optimal temperature range for the species' survival, as well as the critical tolerance thresholds for both low and high temperatures (Angilletta et al. 2002, Sokolova et al. 2012, Bosch-Belmar et al., 2022). The information obtained from TPCs is essential since it not only defines the target species' response to different environmental conditions, but also helps establishing its fundamental thermal niche (i.e. the full range of temperatures within which a species can survive, grow, and reproduce in the absence of biotic interactions). This concept represents the species' physiological tolerance to temperature, determining the thermal conditions that allow it to maintain its basic biological functions. *Astroides calycularis* and *Hermodice carunculata* are two species that seems to respond oppositely to temperature, with overlapping distributions, potentially allowing for the stablishment of a predator-prey interaction. In response to the lack of information in the scientific literature, the main objective of this work was to i) experimentally construct the thermal tolerance curve for both species to understand their responses to changing temperature conditions and

their critical thermal thresholds, ii) observe if those curves representing the species thermal fundamental niche overlap, and how their optimum thermal range and critical thresholds may interact.

Materials and methods

Organism sampling

The sampling sites were located along the Palermo coast, specifically in the areas of Capo Gallo and Isola delle Femmine MPA (38°12'13.9"N, 13°15'58.7"E). These areas feature a rocky littoral platform surrounded by an infralittoral belt of *Cystoseira amentacea* var. stricta, predominantly found on the lower part of the platform's outer margin. Samplings were conducted on days characterized by calm marine weather conditions with low swell. Before conducting the thermal tolerance experiments, a preliminary sampling of a limited number of coral colonies and polychaetes individuals was performed to test their acclimation and maintenance in mesocosms conditions. The organisms were housed for 15 days in two 40 l aquariums under a 9 h photoperiod at 19 ºC and a salinity of 38 psu. Coral polyps were primarily fed with *Artemia salina* nauplii, while polychaetes were fed with fresh mussels and frozen mollusks. Following successful maintenance, sampling for the study was performed. A total of 120 colonies of *Astroides calycularis* and 113 stinging polychaetes were collected. Organisms were immediately transferred to 2 l jars equipped with an oxygenator and filled with seawater, and were immediately transported to the Ecology laboratory to minimize sampling stress as much as possible.

Experimental setting and organisms' maintenance in mesocosm

Once in the laboratory, coral colonies were immediately placed in PVC tanks filled with sea water collected during sampling. Each tank was equipped with a Resun ACO 01 aerator to ensure adequate oxygenation. At this stage, colony cleaning and removal of epiphytes were performed before transferring the colonies to the acclimation aquaria. Subsequently, the *Astroides calycularis* colonies and stinging polychaetes were transferred to 2 and 10 60 l – aquaria, respectively. These tanks were pre-set with integrated filters and sensors to maintain optimal and stable chemical and physical water conditions, including the temperature and salinity recorded during the sampling (19°C and 38 psu respectively). The aquaria were housed in a temperature-controlled room maintained at 19°C, with a 9:15h photoperiod of light/darkness conditions. All organisms underwent a 7 days acclimation period, with the same feeding regime followed during the maintenance test.

Measurements of metabolic rates

Respiration rate (RR) measured at different temperatures was used as proxy of metabolism. A total of 72 *A. calycularis* colonies and 108 polychaetes were used and randomly divided among the 12 selected experimental temperatures levels, ranging from 11ºC to 3 ºC (n= 6 for the coral and n=9 for the fireworm). The temperature range selection was based on the real temperatures recorded in the site where the species occurrence was reported by the GBIF online database (https://www.gbif.org/). To reach experimental temperatures, thermal ramps were performed by increasing/decreasing the water temperature 1ºC every hour, using heaters or coolers (Bosch-Belmar et al 2022, Schröer et al 2011). When the expected temperature was reached, the organisms were acclimated at the experimental temperature during 48 h before measurements started. Next, coral colonies and fireworms were individually placed in 300 ml and 580 ml respirometric chambers respectively. The chambers were filled with filtered seawater (0.45µm, Whatman GF/C), to ensure that the recorded O_2 decrease within the chambers was directly related to the species respiration process. The chambers were immersed in 2 freshwater baths maintained at the selected experimental temperatures through the use of thermal heaters (GRANT optima TXF150). To ensure constant mixing of the water within the chambers, a magnetic bar and an individual stirring device were associated to each chamber. Measurements were carried out using 4-channel Pyroscience FireStingO2 systems, with a reading frequency of 1s. At the end of each measurement, the volume of water contained within the respirometric chambers was measured using a graduated cylinder. Finally, the experimental organisms were weight for fresh weight and immediately frozen, so that further biometric analysis (dry weight and ash free dry weigh) could be performed. Respiration rate (RR) was calculated according to the following formula:

$$RR = \frac{\beta_{[O2]} * t * Vol}{DW} \ (s*L/g)$$

where $\beta_{[O2]}$ is the slope of the measured oxygen concentration within the respirometric chamber ($\beta_{[O2]}$), standardized per time unit (t, s) and the volume of the respirometric chamber (Vol, L), and calculated in relation to the organism body mass as dry weight (DW, g).

Thermal Performance Curve (TPC) and tolerance thresholds

Based on the individual RR obtained experimentally at each temperature treatment level, the thermal performance curves (TPCs) for both species were constructed. According to the pipeline provided by (Padfield et al. 2021), a total of 23 different non-linear least-squares thermal performance models were launched and compared using the "rTPC" package in R software. The best fitting model

was selected based on the lowest Akaike's information criterion (AIC) score. Key parameters identified by the selected model included the optimum temperature (T_{opt}) at which species performance is maximized; and the lower and upper critical thresholds (CT_{min} and CT_{max}) defining the species thermal tolerance range.

Astroides calycularis lethal temperature identification

Observational experiments of survival under different temperature conditions were also conducted on *A. calycularis* colonies to experimentally identify the lethal temperature for the species. Treatment temperatures were 27ºC (control), 28ºC, 29ºC, 30ºC, 31ºC, 32ºC and were selected according to values associated with summer MHWs occurring at sites where *A. calycularis* is normally present. The Kaplan Meier method is known for analyzing data related to "time-to-event" or time until the occurrence of a given event. Specifically, it was applied to estimate the 50% lethal temperature (LT50), which represents the temperature at which 50% mortality of experimental organisms is reached. A total of 48 colonies (8 per treatment) were used. The experiment was conducted in a constant temperature environment where 12 different tanks (2 per temperature level) were set up equipped with skimmers to keep the water clean and circulating heaters (GRANT optima TXF150) that allowed the temperature to be reached and maintained for each treatment over time. Monitoring consisted in daily observations and assessments of the health status of the experimental colonies. The presence of necrotic tissue was chosen as the response variable for the experiment. This variable has been widely used in the literature for coralligenous species health status assessment, both in field observations and in laboratory experiments as a proxy for partial and/or total mortality following disturbance (Pagès-Escolà et al. 2018; Gómez-Gras et al. 2019). Following previous studies (Gómez-Gras et al. 2019), we considered necrotic tissue all areas with obvious signs of irreversible damage, which may include the following characteristics:

- the loss of tissue;
- the denudation/exposure of the skeleton;
- loss of coloration derived from loss or partial loss of living tissue;
- colonization by saprophytic microorganisms.

Specimens showing no signs of damage were scored with absent necrosis (0%), while those showing tissue necrosis were scored as 100%. The experiment lasted 20 days; this time was set as a function of the mean duration of local MHWs and data available in the literature. Two indicators were calculated with the obtained data: the time in days required for the first signs of necrosis to appear in the colonies; the time in days required to reach LT50.

Results

Metabolic rates

Across the temperature range tested for *A. calycularis*, oxygen consumption rates changed from 0.3 mg $h^{-1}g^{-1}$DW (estimated at 23ºC) to 4.2 mg $h^{-1}g^{-1}$DW (estimated at 31ºC) (Fig. 1). At the warmest temperatures, a sharp increase in the coral respiration rate was measured from 27ºC (1.6 mg $h^{-1}g^{-1}$DW) to 33ºC (1.1 mg $h^{-1}g^{-1}$DW). Particularly, at 31 ºC and 33 ºC tentacles contraction was observed in all experimental individuals. This response may be related to the transition from the optimal to the *pejus* temperature range (Sokolova et al., 2012), or even to a direct switch to the *pessimum* range, as has been observed for some marine organisms at high temperatures (Jost et al., 2012). Consequently, data from 27 ºC to 33 ºC were considered uninformative for the TPC model and associated with an increasing likelihood of metabolic failure and/or ongoing mortality of the colonies.

Instead, the fireworm oxygen consumption rate rangedfrom 0.12 mg $h^{-1}g^{-1}$DW (estimated at 15ºC) to 0.32 mg $h^{-1}g^{-1}$DW (estimated at 25ºC) (Figure 1). As observed for the coral, metabolic response of the fireworm sharply increased at 31ºC and 33ºC. This could be attributed to thermal stress and confirms the observed behavior of the experimental individuals that presented reduced and absence of movement at 31ºC and 33ºC respectively.

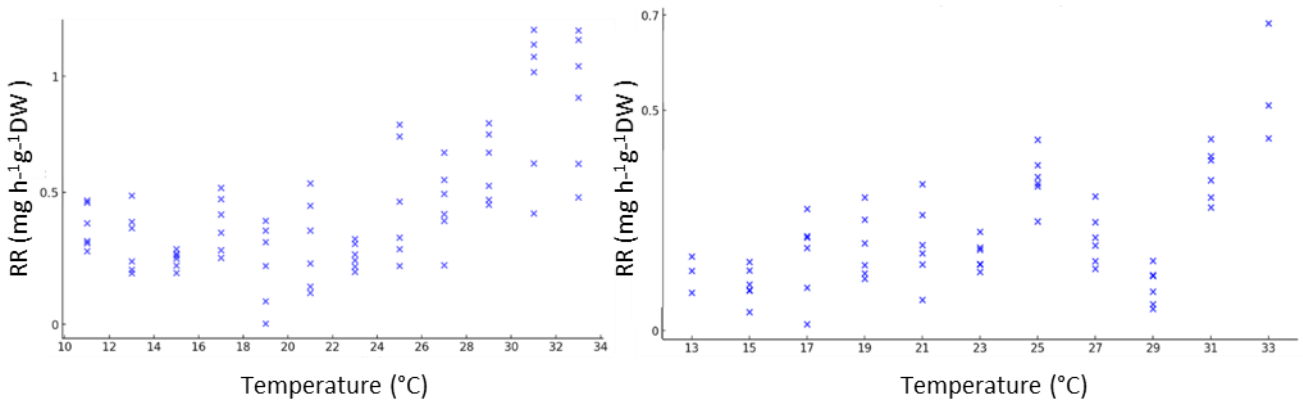

Figure 1. Respiration rate of A. calycularis (left) and H. carunculata (right) at different experimental temperatures ranging from 11 ºC to 33 ºC.

Thermal Performance Curves

From the complete set of non-linear models applied, the Sharpe-Schoolhigh was the best model fitting *A. calycularis* respiration rate data (lowest AIC value). The TPC of the coral exhibited a classic bell shape, typical of a temperate species. Species optimum temperature (temperature of maximum performance) was recorded at 19°C (1.2 mg $h^{-1}g^{-1}$DW), and the CT_{max} was identified at 27.8ºC; while the CT_{min} was not reached (Figure 2). The TPC of *H. carunculata* was obtained through the Pawar model fitting, presenting a left-skewed curve, typical of thermophilus species. The species presented a T_{opt} of 25.5°C, RR of 0.33 mg $h^{-1}g^{-1}$DW) and a CT_{max} of 31.5°C (RR of 0.1 mg $h^{-1}g^{-1}$DW) (Figure 2).

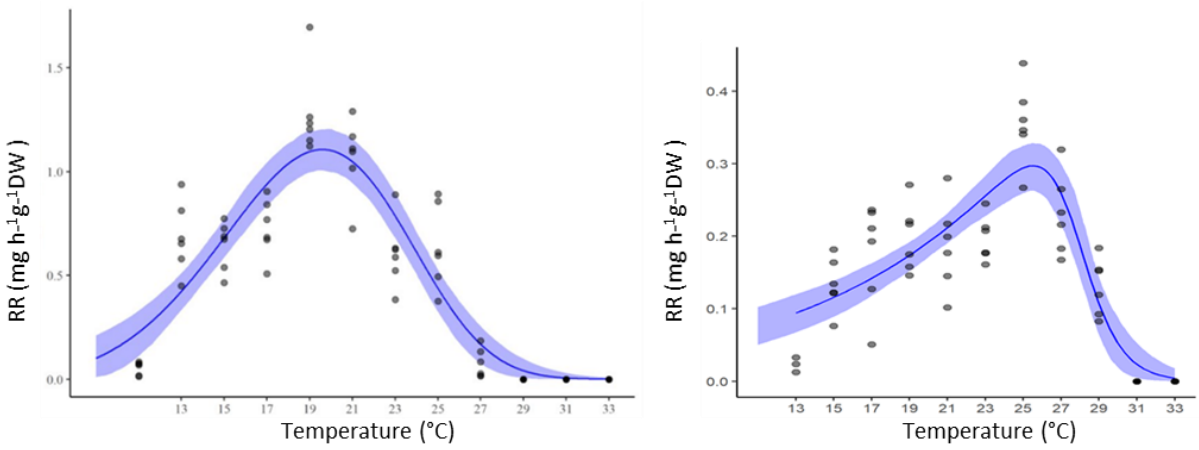

Figure 2 Thermal performance curve of Astroides calycularis (a) and Hermodice carunculata (b) obtained from the Sharpe-Schoolhigh and Pawar models respectively. Rrespiration rate at different temperatures.

Kaplan Meier survival analysis

Statistical analysis showed significant differences between the different treated groups (p-value = 3.4783-12, $p<0.001$). Any of the colonies exposed to 27°C treatment showed signs of tissue necrosis throughout the experiment. Post-hoc pairwise test identified 2 different groups of temperatures at which mortality was recorded (Table 1).

Table 1. Pairwise comparisons among the different temperatures tested.

	27	28	29	30	31 (ºC)
28	1.00000	-	-	-	-
29	0.93716	1.00000	-	-	-
30	0.00017	0.00245	0.00753	-	-
31	0.00026	0.00045	0.00026	0.09848	-
32	0.00064	0.00110	0.00064	0.12424	1.00000

The first group that included 28°C and 29°C experimental temperatures did not reach LT50 for the entire duration of the experiment, showing low mortality in the first week and a stable plateau phase until the end of treatment (Figure 3). In the 28°C experimental group, the first signs of necrosis were observed after 7 days of treatment; while in the 29°C group, the first signs of mortality were observed after 4 days. The second group (30°C, 31°C, 32°C) showed the first signs of necrosis after 24 hours (30°C and 31°C), with LT50 being reached after 4 days from the start of the experiment; while for the individuals exposed to 32°C, the first signs of tissue degradation were observed within the first 24 hours but reached LT50 on the second day of treatment (Fig 3).

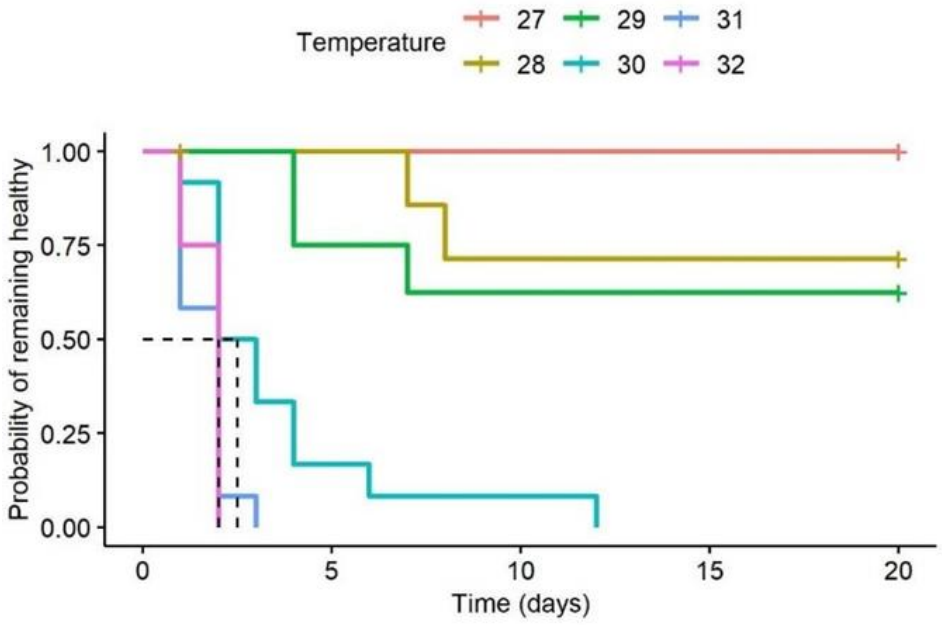

Figure 1. Survival analysis of A.calycularis colonies at 6 different temperatures levels (27°C, 28°C, 29°C, 30°C, 31°C, 32°C) over time. Method used was Kaplan Meier. Dashed black lines refer to LT50 for treatment at 32ºC and 31º (first), and for treatment at 30ºC (second line)

Discussions

Mechanistic approaches, as the one used in the present work, allow to obtain fundamental information on species direct responses to environmental change. Target species are considered as "winners and losers" (fireworm and orange coral respectively) in the current and future context of

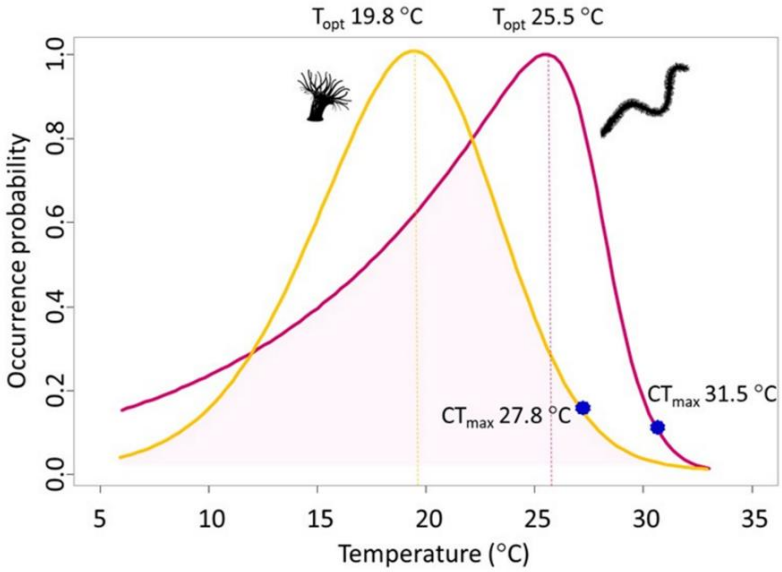

Figure 4. Overlap of the Thermal Performance Curves of both, A. calycularis (orange) and H. carunculate (red). Topt (thermal optimum), CTmax (maximum critical temperature).

climate change (Kearney & Porter 2009). Identified thermal optima and upper thermal thresholds from constructed TPCs may confirm the opposite response that both species exhibit to temperature. The coral showed a response typical of a Mediterranean species, with a CT_{max} around 27 ºC, a warm temperature easily reached during summer period (Copernicus 2021). Instead, the fireworm presented higher thermal optimum, indicating that it is a warm-water affinity species, taking advantage of increasing temperature trends. Even though the species have different optimal temperatures and critical thresholds, their fundamental thermal niches overlaped (Fig. 8), raising questions about how the species interaction will change, whether prey-predator relationships will be strengthened or weakened with rising temperatures, and how it will directly affect species distribution and indirectly community composition and functioning.

Species ecological niche is recognized as the basis for modelling species distributions (Kearney & Porter, 2009). The wide thermal range of *H. carunculata*, high optimum and upper thermal threshold may allow us to hypothesize that such an adaptable and tolerant species will continue to expand latitudinally and longitudinally within the Mediterranean Sea. Similar predictions have been done by (Righi, 2021; Righi et al., 2022) and Simonini et al. (2020), observing the "invasion" path of the species over the last 50 years from the east to the western Mediterranean coasts.

On the contrary, the upper thermal threshold of *Astroides calycularis* (27.8 ºC) may confirm the species vulnerability to increasing temperatures. Mass mortality events of the coral populations have been reported after the most severe MHWs in the last years. Summer periods with minimum and maximum temperatures ranging between 27.6 ºC and 29.4 ºC respectively were recorded during 2020 along the Pelagie Islands (Bisanti et al., 2022); while in 2009 in the coasts of Campania, temperatures above 28 ºC were recorded for several consecutive days during the warmer months of the year (M. Gambi et al., 2018). In addition, personal field observations of *A. calycularis* mortalities have been reported along the coast of Palermo after 2022 and 2023 MHWs. Results from Kaplan Meier analyses confirmed the negative effect of high temperatures on the coral survival. Signs of necrotic tissue were observed after 8 consecutive days with temperatures of 28 ºC. A temperature that in recent years was easily reached and sometimes exceeded during summer MHWs. Coral mortality may have a cascading effect through the ecological hierarchy, provoking local biodiversity loss of all related species to the habitat former, altering community structure and functioning.

Spatial overlap between both, the fireworm and the coral has been observed in the field, but also through the LEK approach presented in the chapter 3 from the present PhD thesis. The two species distributed over equal bathymetries (shallow waters) (Ocaña et al. 2000, Rosa et al. 2023), and their prey-predator interaction has recently been addressed in the scientific literature yet (Bosch-Belmar et al. 2024, studied present in the chapter 5 of the thesis), confirming the effective predation pressure of the fireworm on *A. calycularis* populations and hypothesing a reinforment of this interaction with global warming conditions.

In addition, the two thermal tolerance curves provide us with information about the CT_{max} of the coral, which is close to the optimal temperature of the fireworm, meaning that increasing temperature and the presence of the corallivorous polychaetae may act as potential interacting stressors threatening *A. calycularis* populations (Gunderson et al. 2016). Furthermore, it is already known how high water temperatures trigger the proliferation of bacteria and pathogens (Bally & Garrabou 2007), beyond those present on the spicules of the fireworm (Sussman & Siegal 2003). In fact, the fireworm is also known to be a reservoir and vector for several pathogens, including the bacterium *Vibrio shiloi*, which is responsible for bleaching colonial corals such as *Oculina patagonica* (Sussman & Siegal, 2003).

In conclusion, the effect of these thermal and biotic stressors will be able to have a known effect on different levels of the ecological hierarchy, leading to a potential loss of biodiversity and ecosystem functioning, since the colonies of *A. calycularis* is an habitat former hosting more than 80 species (Terrón-Sigler et al. 2014b). The present work allowed us to provide missing information on

the response to changing climatic conditions of the two Mediterranean species that have been the focus of attention in recent years. The data from this work may be guidelines on proper management and biodiversity protection measures at the Mediterranean and local scales of habitat former organisms such as *Astroides calycularis*. Data on thermal tolerances and upper thermal limits are essential for modeling species distributions and assessing potential climate impacts under future scenarios, such as those outlined by the IPCC. By combining thermal tolerance data with climate projections from IPCC scenarios, we can simulate species distribution patterns based on their thermal niches. This approach allows us to predict potential shifts and changes in where species are likely to thrive as environmental conditions evolve over time (Bosch-Belmar et al. 2022, Marchessaux et al. 2023).

Further research and in-depth studies on the functional traits of species, the properties of ecological communities, and the complex web of biotic interactions are essential to gain a more comprehensive understanding of the effects of rising temperatures and the biological interactions driven by climate change. Investigating these factors in greater detail will allow us to better grasp how species and ecosystems respond to climate-induced shifts, shedding light on the intricate dynamics at play and enabling more effective strategies to address the challenges posed by global warming.

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Chapter 5

Impacts of increasing temperature due to global warming on key habitat-forming species in the Mediterranean Sea: unveiling negative biotic interactions.

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Abstract

Marine heat waves affect marine ecosystems and the provision of services. Shallow benthic communities in the Mediterranean Sea have experienced mass mortality events affecting populations of some habitat formers such as the coral *Astroides calycularis*. Some species are instead taking advantage of rising temperature, such as the corallivorous polychaete *Hermodice carunculata*, a 'native invader' in the Basin. In this context, the investigation of coupled thermal tolerance may be of particular interest to predict the distribution of both "interactors" to get insight on future biodiversity loss rates due to exacerbation of biotic interactions related to climate change. Here we investigated the thermal tolerance of both species, to trace the thermal performance curves by measuring the individual respiration rate (as a proxy of metabolic machinery functioning) under 12 different temperatures (11°-33°C). Thermal optima and thresholds were identified and while the coral seems to currently living close the edge of maximum critical temperature, polychaete seems to be under maximal performance (i.e. spikes due to heat waves may be easily accommodated within the range of optimum temperature). Thus, increasing temperature seems realistically a threat to the coral with the risk that polychaete interaction further impair the coral performance. We derive that heat waves will impair the habitat former performance and will simplify the habitat complexity, while the distribution of habitat former interactors will be larger with possible further negative effects on ecosystem functioning.

Introduction

Competition and predation stand as pivotal forces shaping ecosystems, exerting considerable influence over community structure encompassing factors like species biomass, composition, and taxonomic as well as functional biodiversity (Freestone et al., 2020; López & Freestone, 2021). Biotic interactions, when intensified or prompted by new species introductions, can act as sources of disturbance. Such biological relationships, when heightened, contribute to novel interactions. Consequently, this may transpire when new species arrive on the scene. In this context, stressors often compound and interact, ultimately molding the responses of organisms and communities to the effects of multiple disturbances. The outcome of these interactions largely hinges on the magnitude and the relative timing of each stressor (Gunderson et al., 2016b; Jackson et al., 2021a). For instance, the interplay between climatic stressors and the dispersal capability of certain species can profoundly influence the organization of native communities. This synergy can pave the way for the emergence of novel ecological communities, characterized by unique arrangements and ecological interactions. When multiple stressors converge, their effects produce non-linear outcomes that may either be more pronounced (synergistic) or milder (antagonistic) than the combined effects of individual stressors (Darling & Côté, 2008; Gunderson et al., 2016b). The potential for interactive effects hinges on the identity and quantity of stressors involved, as well as the trophic position and life-history stage of the organisms affected (Lyons et al., 2016).

At higher levels of the ecological hierarchy, such as populations and communities, biotic interactions can serve as direct stressors (e.g., ecological stressors like competition, predation, and density effects;Power 1997, Darling and Côté 2008), or they can indirectly disrupt the availability of resources or local environmental conditions (Bruder et al., 2017). Present endeavors to forecast the ramifications of global change predominantly center around the ecological prerequisites of individual species (Kordas et al., 2015). However, an increasing awareness underscores the alteration of biological interactions due to stressors associated with global climate change (Bennett et al., 2015; Bulleri et al., 2016). Given that interactions are inherently dynamic, an imperative exists to

comprehend the mechanisms governing the dynamics between prey and predators in the face of unexpected alterations induced by abiotic factors, especially those linked to climate change, such as rising temperatures (Van Der Putten et al., 2004). Rising temperatures and recent marine heatwaves in the Mediterranean Sea have begun to significantly alter the composition and structure of communities (Garrabou et al., 2009; Gómez-Gras et al., 2019a). Habitat formers, particularly those found in shallow rocky environments like the vulnerable endemic coral *Astroides calycularis*, have faced severe consequences due to thermal stressors over the past few decades. These stressors have led to substantial mass mortalities in populations, particularly during the more frequent thermal anomalies that occur in the warmer months (Gambi et al., 2010b, 2018; Garrabou et al., 2022a). Despite the evident negative effect of climate change on the coral performance, there is no scientific evidence regarding the conservation of the species. Because stressors seldom act in isolation, various disturbances, including local factors like biotic interactions, may interact synergistically, impeding species performance and community functions in habitats that are already strained and vulnerable. Against the backdrop of ongoing climate change, particularly in the Mediterranean Sea, rising temperatures have facilitated the expansion and higher densities of non-native and thermophilic species. Among these is the stinging polychaete *Hermodice carunculata*, which is regarded as a "native invader" and is extending its distribution range (Righi et al., 2020c). Given its omnivorous behavior and habitat overlap with *A. calycularis*, this invasive species poses a tangible threat to shallow benthic communities, especially those that are already disrupted by climatic stressors.

In response to these concerns, we conducted manipulative mesocosm experiments to delve into the predatory behavior of the fireworm, *H. carunculata*, toward one of the Mediterranean Sea's most crucial shallow structuring species: the orange coral *A. calycularis*. Additionally, we explored the coral's metabolic response to this biotic stressor in the context of climate change. Our objective was to ascertain whether the heightened frequency of interaction between the fireworm and the coral, within the context of climate change, could impact the habitat former's performance, particularly in terms of metabolic responses and predation pressure. To address this, we undertook three distinct interaction experiments, each under escalating temperatures: i) The first experiment sought to determine whether *H. carunculata* exhibited a preference for feeding on *A. calycularis*. We conducted prey preference experiments involving two different Mediterranean coral species (*A. calycularis* and *Cladocora caespitosa*, the latter being a known prey for fireworms). ii) Once predation on *A. calycularis* was confirmed, a second experiment was designed to investigate the fireworm's predation rate on the coral colonies at varying environmental temperatures, thereby constructing a performance curve and identifying optimal and upper thermal predation thresholds for the polychaetae. iii) Lastly,

we conducted short-term interaction experiments between *H. carunculata* and *A. calycularis*, assessing the metabolic response of the threatened coral by measuring its respiration rate as a proxy for coral metabolism at two different temperatures (16 ºC and 27 ºC). This comprehensive study offers crucial insights into the role of climate change stressors on marine benthic communities and provides a mechanistic framework for predicting the impact of biotic interactions in driving such events, including mass mortalities.

Material and methods

Organisms' sampling and maintenance

To perform all experimental trials a total of 105 colonies of *A.calycularis,* 6 colonies of *Cladocora caespitosa* and 102 individuals of the fireworm *Hermodice carunculata* were collected along the coast of Palermo (Italy) (38°11′12.786″N; 13°21′41.1336″E) with sampling effort limited as much as possible to reduce disturbance to the natural populations while maintaining the robustness of the experimental design. Moreover, all colonies were sampled to have a similar size (when possible) to be easy to manipulate, comparable and suitable for mesocosm experiments. Once in the laboratory all colonies were carefully inspected and when possible, all epibiotic organisms were manually removed by forceps. Coral colonies and fireworms were divided and kept separately in three 60L tanks (3 for *A. calycularis* and *C. caespitosa* and 3 for *H. carunculata* individuals) filled with filtered seawater for 10 days, to allow correct acclimation and limit the potential stress deriving from sampling and manipulation. All aquariums were equipped with a skimmer and filtering system, water pumps, individual heater and temperature controller systems. HOBO temperature (Onset Inc., USA) loggers were employed to record temperatures throughout the trials and set at minute data recording. Corals were feed with *Artemia salina* nauplii every 2 days, while fresh mussels were offered to polychaetes every 5 days.

Measured fireworm behavioural traits

Preliminary observation period of the interaction between *H. carunculata* and *A. calycularis* was performed and according to what was observed predator behavior was categorised in terms of its structure and consequences, identifying three different behavioural traits to be measured in all three experiments: i) "exploring time": mean time that the predator employed moving around the experimental aquaria immediately after the trial started (measuring units: minutes); ii) "interaction time": mean time that the predator physically contacted the prey without feeding on it (measuring

units: minutes); and iii) “predation rate”: mean number of preyed calyxes for each prey species per day. Measuring duration varied with the measured trait: “exploring time” was continuously observed by an operator during the first 2 hours after the start of the prey-predator interaction; while “interaction time” and “predation rate” were measure at the beginning of the interaction (as “exploring time” variable) and at 5 different posterior times (once per day during 5 days between 3 and 5 pm, as that is the daily time during which the fireworm shows its maximum activity, Wolf and Nugues 2013).

Prey preference trials

Hermodice carunculata is a known corallivorous predator (Wolf et al., 2014b), which has been observed actively predating on the Mediterranean scleractinians *Cladocora caespitosa* (Simonini et al., 2018) and *Astroides calycularis* (authors field personal observation). In this experiment, we tested the predator’s preference towards two different corals to investigate behavioral traits of predators in major detail. After laboratory acclimation period (previously described), 12 individuals of *H. carunculata* (13.02 ± 1.35 g, mean ± s.e.) were fed to satiation and then left starving for 10 days prior to predation experiments (Simonini et al., 2018). Six colonies of *A. calycularis* and six of *C. caespitosa* were sampled and carefully inspected in the laboratory to eliminate any other potential prey. Colonies from both species were put in pairs (1:1 *A. calycularis*-*C.caespitosa* colonies) and acclimated in 6 different aquaria of 3L during 24 h prior the exposure to the fireworm. Colonies of the two species were placed in opposite sides of the aquarium. After this period, one specimen of *H. carunculata* (11.6 ± 1.81 g, mean ± se) was put in the center of each experimental aquaria (at the same distance from both colonies of the two colonial corals) and the 3 beforementioned behavioural traits (exploring time, interaction time and predation rate) were measured in each experimental unit over time (once per day during 5 days) (Masselli et al. 2020, Simonini et al. 2018). After each experiment, the polychaetes that did not consume the prey were provided with a control food (pieces of unfrozen mussel) to verify that they were not satiated and unwilling to consume any food.

Modeling predation curve over temperature

Once predation of *H. carunculata* on *A. calycularis* was experimentally tested, we investigated the predation response of the fireworm under different natural Mediterranean temperature conditions. Seven different temperatures ranging from 10 to 31 ºC were decided (10, 15, 20, 24, 27, 29 and 31 ºC) based on minimum and maximum temperatures observed in the last 5 years in the areas where the species are present. Six different coral colonies of similar size (between 18 and 20 polyps each) and 6 polychaetes (12.4 ± 1.21 g, mean ± s.e.), were independently used at each

temperature (for interactions) and 3 coral colonies per temperature were used as controls for behavior and morphological traits observations (63 coral colonies and 42 fireworms in total). Organisms were individually kept in 3L aquaria and temperature was increased/decreased at 1 ºC h^{-1} rate (Bosch-Belmar et al., 2021; Marchessaux et al., 2022). After reaching treatment temperature they were acclimated during 5 days at those conditions that were kept stable by means of a circulated thermal bath (Grant Optima TX150) and additionally monitored throughout the recording period by HOBO Pendant® loggers. In addition to traits measured for predators some behavior and morphological prey traits were assessed at the highest tested temperatures in this specific experiment: tentacle contraction (measured as 0/1 when prey was contacted by the predator) and the presence of necrotic tissue (as the number of calyxes presenting necrose at 27, 29 and 31 ºC) to assist us in discriminating between the effect of predation and high temperatures on the scleractinian. After acclimation, one fireworm specimen was put within each treatment coral aquaria and observations on predation were performed as for the prey preference experiment.

Prey metabolic response to predator contact

A first step consisted in experimentally studying the effect of contact between corals and fireworms to test whether coral metabolic change was significant under fireworm exposure under increasing temperatures. After acclimation period, two experimental sets were created according to temperature factor [T16 ºC and T27 ºC, n = 18 coral colonies (containing between 18-20 polyps) and 12 fireworms; 12.1 ± 1.5 g, mean ± se] by increasing/decreasing 1 ºC h^{-1} from each sampling temperature. Once each experimental temperatures were reached, 18 coral colonies per each temperature treatment were randomly selected and individually kept in 18 small aquaria (2L) at the reached experimental conditions for 5 days to allowed correct thermal acclimation (Simonini et al., 2018). Twelve of these aquariums were used for the exposure trials (contact with *H. carunculata*), while 6 of them were used as controls (without interaction with the fireworm). After 24h, one random selected *H. carunculata* specimen was moved to each "interaction" aquaria for 6h period according to Simonini et al. 2017, 2018. Beforehand, four video recording cameras (Go Pro version Hero 8 Black) were employed to record the whole steps of interaction in each tank (Martin & Bateson, 1993). Movies were analyzed by an operator to measure exploring time, interaction time and predation rate of both inter-players during each interaction trial.

Respirometric measurements. This second step consisted in measuring metabolic response as expressed by the oxygen consumption (RR, mg O_2 h^{-1} g^{-1} DW) as described below to estimate if the predation by fireworms was recorded at metabolic level by corals, as a proxy of predation risk. In

doing so, the 18 coral colonies from each treatment temperature trial were gently moved to respirometric chambers (volume = 310 ml) containing filtered (Whatman GF/C 0.45 µm) air-saturated seawater of salinity 38, which is the commonest salinity usually found in the South Tyrrhenian Sea subtidal range at the depth where both organisms co-occurred. To ensure the constant mixing of the water, each chamber was stirred with a magnet bar and an individual stirring device (Bosch-Belmar et al., 2016). Respirometric chambers were further randomly divided into three temperature-controlled water baths and the concentration of dissolved oxygen was measured simultaneously by means of 6 optical oxygen meters (model: Firesting O2; PyroScience Inc. Germany), each equipped with four optodes (24 optodes in total). Four respirometric chambers were used as control in that they were filled only with filtered seawater. Oxygen concentration measurements were performed continuously for 1 hour and a half, and data useful to estimate the consumption were extracted from the central hour to avoid edge effects. Temperatures in chambers were kept stable by means of a circulated thermal bath (Optima TX150, Grant Inc., UK) and additionally monitored throughout the recording period by HOBO. Respiration rate (RR, mg O_2 h^{-1} g^{-1} DW) was calculated according to Sarà et al. (2013). After the measurements, colonies were kept undisturbed in the respirometric chambers with aerated water for 1 hour (to increase the available recovery time for the treated organisms) and then, each replicate was dried at 90 °C for 24 h and the dry weight (DW, g) was determined (Goffredo et al., 2011) relationship. The same procedure was followed for *H. carunculata* individuals.

Data Analysis

To test if the fireworm predated preferentially on the two offered coral species a GLM with zero inflated Poisson family distribution was used, as our dataset contained many zeros. Moreover, "colony id" has been added to the model as random variable in order to remove or reduce a source of variance linked with a single colony, and to handle also with non-independence condition among polyps belonging to the same colony. Data regarding *H. carunculata* predation rate on *A. calycularis* were fitted through a non-linear model by using nlsLM and rTPC packages in R (Padfield & O'Sullivan 2020). Accordingly, a total of 21 non-linear least squares performance models were launched and compared, and this allowed us to identify the Briere (Briere et al. 1999) as the "best" fitting model for predation data (i.e. presenting the lowest AICc score). After checking for data normality and homoscedasticity respiration rate data from the "prey metabolic response to predator contact" experiment was analyzed through a Generalized Linear Model (GLM) with "time of interaction" as weighted factor. After each model was run, a check was made on the residuals to ensure that no particular trends were present and that they were normally distributed.

Results

Prey preference trial

Initially, fireworms showed an exploring behavior (18 ± 6.3 min, mean ± s.e.) moving around the aquarium. Once the prey was encountered, the predators passed over them with a "prey inspection" behaviour (measured as "interaction time") and "walk around" during 14 ± 7.7 min, mean ± s.e.), but no predation was observed during the day of initial interaction. When predation attacks were recorded during the subsequently observation days, fireworm behaviour consisted in everting the pharynx and preyed on corals through suction movements exerted by the pharyngeal musculature. Colonies of both species were partially consumed during the 5 observation days, firstly leaving bared the edge of coenosarc and then the complete calyx. Statistical analysis showed not significant difference between the predation rate (mean number of predated polyps per day) of the fireworm *H. carunculata* on both coral species (t= 1.458, p > 0.05), therefore, *H. carunculata* showed higher preference to prey on *A. calycularis* during the first exposure days exclusively feeding on the orange coral. Overall, higher predation rate was observed on *A. calycularis* than to *C. cespitosa*, being the total number of consumed polyps and the percentage of consumed prey during the 5 days of observations 17 and 9, and 12.86 ± 5.25 and 3.48 ± 1.42 respectively (Fig. 1). This potential preference may be due to the calyx morphology of *A. calycularis* polyps which present a more accessible structure for the feeding strategy employed by *H. carunculata*.

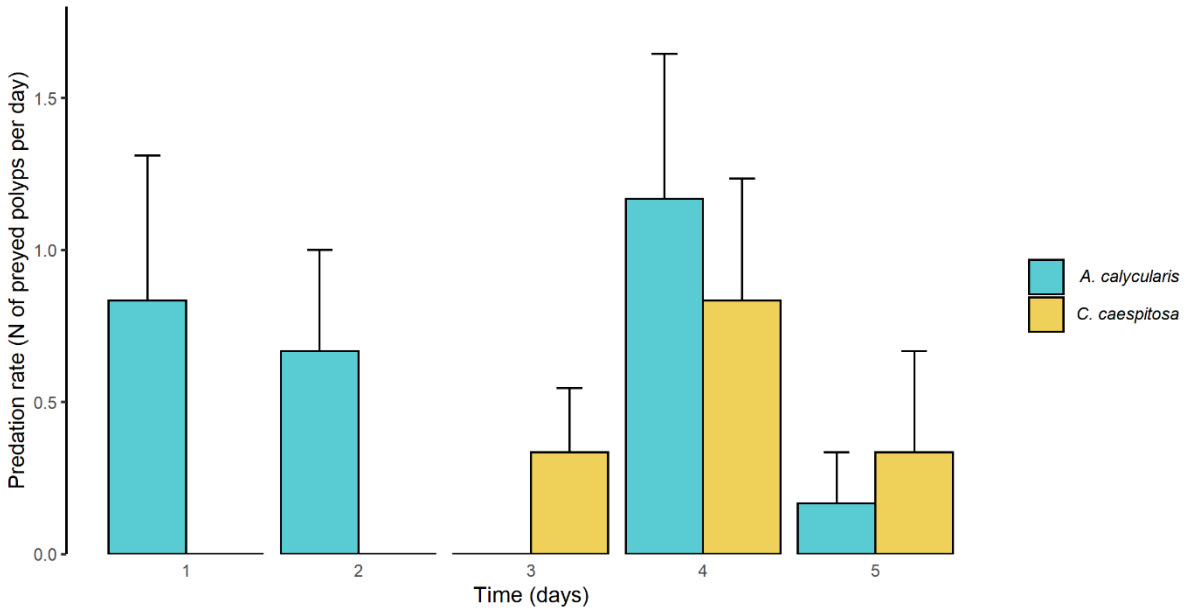

Figure 1 Hermodice carunculata predation rate (mean number of preyed polyps per day) on A. calycularis and C. caespitosa colonies over time (5 days of observations).

Modeling predation curve over temperature

Significant differences in *H. carunculata* interaction time and predation rate on *A. calycularis* were observed at different temperatures and over time ($p < 0.05$ for both). The highest interaction time between the fireworm and the coral was recorded at 24 and 27 ºC (21.7 ± 2.9 min, 28.3 ± 1.1 min respectively, mean ± s.e., Fig. 2a), but in agreement with the previous experiments, no predation was observed in the day of starting interaction. The highest predation rate over the tested temperature range was observed at 24 and 27 ºC, especially in the second and fourth day of experiment (Fig. 2b).

Modeling approach showed that Brier model was identified as the best fitting model for predation data (Appendix S1: Figure S1), showing an optimum (the highest predatory performance) at 25.6 ºC and a critical upper threshold (temperature where predatory performance drastically decreases) of 30.9 ºC (Fig. 3).

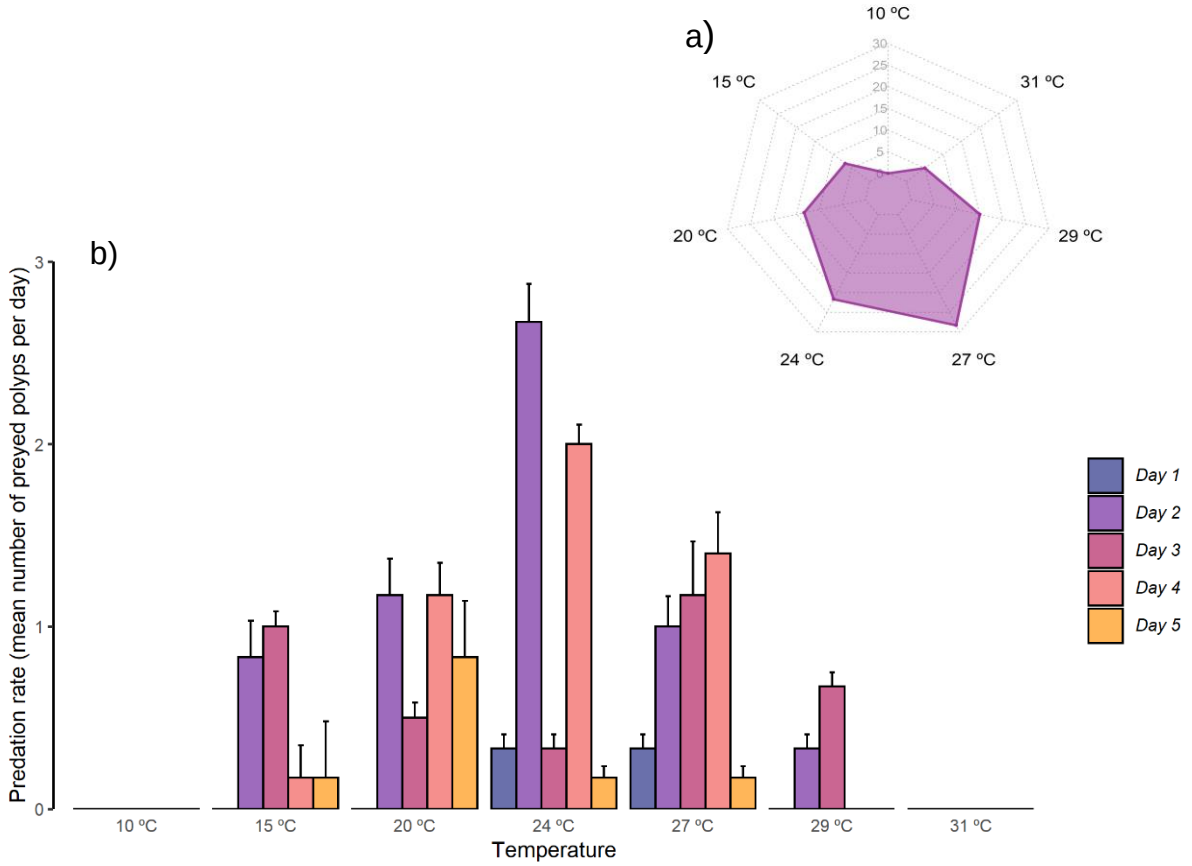

Figure 2. Measured behavioral traits on H. carunculata and A. calycularis interactions over temperature: a) "interaction time" between fireworm and orange coral at different temperatures. Light grey numbers in the grid refer to interaction minutes (ranging from 0 to 30 min); b) "predation rate" (mean number of preyed polyps per day) at different temperatures and over time (5 observation days).

Behavior corroborated the model predictions, in that we observed reduced mobility in the fireworm individuals at 29 ºC and the absence of movement and predation at 31 ºC (Fig. 3). Experimental corals (those exposed to *H. carunculata* and controls) showed tentacle contraction at 27 ºC, 29 ºC and 31 ºC, and necrotic tissue was observed at the highest temperature, highlighting the negative effect of high temperatures on the coral performance.

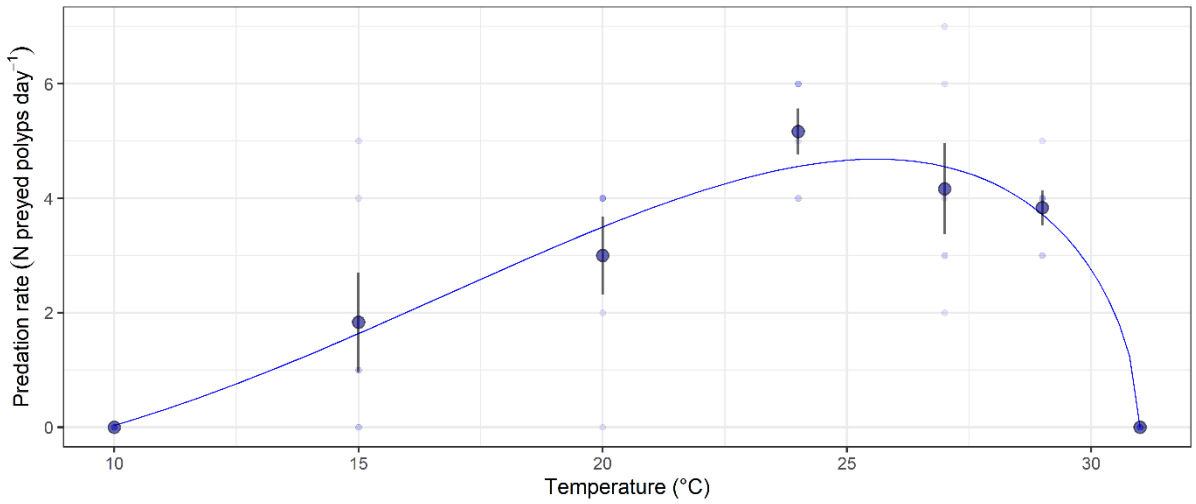

Figure 3. Predation curve of Hermodice carunculata, presenting the maximum predation performance (optimum) at 25.9 ºC and the upper critical threshold at 30.9 ºC.

Prey metabolic response to predator contact

Recorded contact time between the fireworms and the experimental coral colonies was slightly lower at low temperature treatment, even if there were not significant differences between 16 and 27 ºC. Increasing temperature and contact with the stinging polychaete affected the metabolic response of the coral ($t = 6.95$, $p < 0.05$; $n = 12$). Respiration rate was significantly different at the two temperatures and when the fireworm was present (Fig. 4). At lower temperature (16 ºC), the coral presented low respiration rate values, that has more than significantly doubled when it was exposed to the fireworm. The higher temperature led to increased coral oxygen consumption rate, while the combined effects of high temperature and fireworm contact caused a significant increasing rate of oxygen uptake respect to separate effect of either factor, likely presenting a synergistic effect (Fig. 4). Once the contact took place, coral polyps showed tentacle contraction, behaviour that was maintained during the whole experiment and after the end of the interaction. On the contrary,

fireworms did not showed significant differences in respiration rate between temperatures, highlighting the potential polychaete ability to cope with changing temperature conditions.

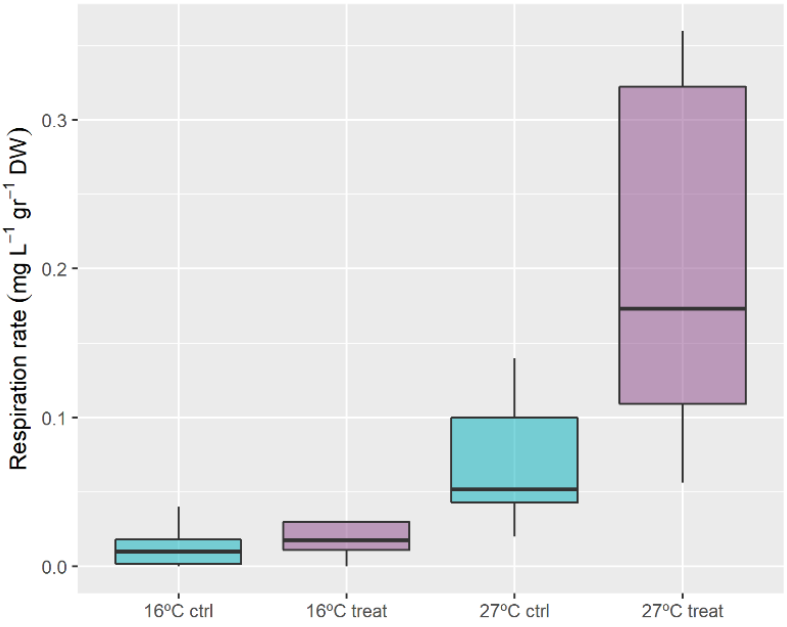

Figure 4. Metabolic response (respiration rate, mg L-1 gr-1 DW) of treated (fireworm interaction; purple bars) and control (no-interaction; green bars) A. calycularis experimental colonies at two different temperature treatments (16º C and 27 ºC).

Discussions

The interplay between temperature-dependent predator-prey interactions assumes significance in shaping ecosystem stability as temperatures rise. The advent of rapid temperature fluctuations and extreme events, such as heatwaves, introduces perturbations that challenge the intricate equilibrium between predators and prey. These disruptions hold the potential to induce unpredictable oscillations in population sizes, consequently heightening the risk of biodiversity loss. The loss of biodiversity can result in the disappearance of biotic interactions associated with local extinct species, along with the ecological services they provide, leading to direct consequences for human well-being (Valiente-Banuet et al. 2015).

Within this context, this study brings to the forefront the role of *Hermodice carunculata*, an endemic benthic infralittoral predator prevalent in the Mediterranean Sea. The predator-prey narrative intertwines with the impending challenges posed by future temperature alterations. While the adaptability of *H. carunculata* grants it a remarkable presence, its implications extend to the well-

being of its prey. The predator demonstrates a propensity not only to predate upon *A. calycularis* colonies but also to elicit a stress-induced metabolic response upon physical contact with the coral. Geographically, the ranges of these two species overlap, particularly along the Mediterranean's southern coasts, where the coral plays a pivotal role in shaping shallow infralittoral rocky habitats, hosting more than 80 different species of invertebrates (Terrón-Sigler et al., 2016). The interaction between abiotic (increasing temperatures) and biotic (predation risk by the polychaete) stressors may result in synergistic effects impairing with consequences at population level. Decreasing local populations of this habitat-forming species may suppose significant biodiversity loss and ecosystem services.

Meanwhile, *H. carunculata* has demonstrated a notable surge in both population density and distribution over the past decade (Righi et al., 2020c; Terrón-Sigler et al., 2016). The consequences of fireworm predation extend beyond immediate impact, encompassing direct targeting of coral polyps and larvae and, consequently, the compromise of coral fitness. This disruption holds the potential to trigger far-reaching consequences at the population level (Schulze et al., 2017b), orchestrated through the intricate channels of non-consumptive predation. Non-consumptive predation, a nuanced force, operates as an underlying architect of biotic interactions, sculpting the contours of prey morphology, physiology, behavior, growth, and reproduction (Truong et al., 2022). In alignment with this insight, our findings underscore that even a mere physical encounter with the fireworm prompts the retraction of coral polyp tentacles, a response that persists beyond the interaction itself. Brief exposure to the stinging polychaete manifests in a palpable decline in coral performance, evidenced by metabolic alterations and potential diminished feeding efficiency. This phenomenon highlights *Hermodice carunculata*'s opportunistic feeding behavior and adept predatory strategies, particularly evident in its interactions with cnidarians and starfish (Barroso et al., 2016b; Kudenov et al., 2017; Simonini et al., 2018). This observation concurs with the proposition that Mediterranean fireworm populations may sustain their resilience despite fluctuations in prey community composition (Simonini et al., 2018). Furthermore, this predator exhibits a notable capacity to navigate anthropogenic environmental changes, capitalizing on rising temperatures that fuel both poleward expansion and heightened population densities (Righi et al., 2020c).

Temperature, a central driver of biological phenomena, addresses the change in the dynamics of predator-prey interactions. As temperatures rise, the intricate relationship undergoes a transformation, reshaping the equilibrium of populations and inducing sweeping alterations in the delicate interplay of ecosystems and food webs (Bjorn et al., 2010). Elevated temperatures trigger an

upsurge in metabolism, prompting organisms to augment their food consumption to meet the heightened demands of increased energy expenditure.

The predation response of *H. carunculata* to temperature fluctuations unveils a skewed curve, characterized by a negative skewness. The predator's performance diminishes at lower temperatures, culminating in an apex at approximately 25.6 ºC, where the predation rate on the coral reaches its zenith. However, as temperatures transcend this threshold, a cascade of decline ensues, marked by a decline in mobility and reduced pursuit of prey at 29 ºC. Feeding ceases altogether at temperatures ranging between 29 and 31 ºC. Remarkably, this aligns with model predictions, pinpointing the upper critical temperature for feeding at an exacting 30.9 ºC. The variability in consumption rate with temperature hinges on the evolutionary response of both predator and prey to temperature alterations (Dell et al., 2014).

Thus, our results can be seen within the context of a phenological mismatch, particularly considering current and future ocean warming conditions (Renner & Zohner, 2018). Indeed, this polychaeta which responds to the mechanistic rules of temperature controlling biotic interactions, when living over certain thermal thresholds will have a negative impact on its ability to prey as predators and prey respond differently to temperature changes. It could result in a phenological mismatches, where peak prey availability no longer coincides with peak predator demand. This finally could have effects of predator reproduction and on its overall population dynamics. Thus introducing mechanisms into the prey-predator relationship makes visible the double side of the coin. On one hand, the predator's inclination towards optimal feeding may disrupt prey populations, already stressed due to abiotic conditions (high temperature), casting a shadow of non-consumptive predator impact up to a certain threshold (in this case, 25.6°C). On the other hand, as this threshold is approached and surpassed, a decline in predation pressure is anticipated, carrying implications for biodiversity loss on both fronts. This underscores the duality inherent in this complex relationship. Nevertheless, we need to increase our effort in the modelling area for instance adopting ensemble of ecological niche models based on key functional traits and downscaled climate change projections to predict the magnitude of interactive effects among stressors of different nature and properties. This advancement would enhance our mechanistic understanding of ecosystems, particularly the nature of the links that connect biotic interactions within the ambit of increasing temperatures, providing greater realism to the model predictions assisting risk assessment and management strategies. The outcome, in turn, offers a habitat-dependent framework to enrich our comprehension of the mechanisms that underlie community structure and functioning (Enquist et al., 2015) in the face of human-induced environmental changes. This understanding provides us elements to effectively anticipate and mitigate potential implications for local biodiversity and would efficiently assist

management and conservation plans for vulnerable species and habitats to current and future changing climatic conditions.

In conclusion, this study highlights the significance of temperature-dependent predator-prey interactions, particularly the role of a native-invader in shaping ecosystem stability amid rising temperatures. The emergence of rapid temperature fluctuations and extreme events may introduce perturbations that challenge the equilibrium of the ecosystem. The multiple stressor conditions, resulting from increasing temperatures and the intensification of negative biotic interactions, pose a significant threat to endemic key habitat forming species in the Mediterranean Sea. Such understanding is crucial for anticipating and mitigating potential implications for local biodiversity, informing management and conservation actions for vulnerable species and habitats in the current and future changing climatic scenarios.

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Chapter 6

Extreme climatic events effects on the early life stage of a Mediterranean habitat forming coral species

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Abstract

Marine ecosystems are undergoing rapid changes, including rising temperatures and more frequent extreme climatic events. Currently, the consequences of these phenomena heavily impact the structure and functioning of marine communities, especially benthic ones. Studying how key species respond to environmental change has become essential, as local biodiversity relies on their ecological conservation status. While the effects of climatic and anthropogenic stressors on many of these crucial habitat-formers have been extensively studied, there is limited information on their dispersive early-life stages, which are fundamental to population survival. The performance response of the endemic coral *Astroides calycularis* to various realistic stressful conditions (heat spikes, marine heatwaves, and marine heatwaves followed by a drop in salinity) was explored by measuring life history traits (mortality) and dispersal traits (larval duration and settlement rate) over time. The results indicate that rising temperatures and decreasing salinity significantly impair the species' performance, leading to accelerated metabolism, quicker settlement rates, and increased mortality. Such findings are crucial for understanding and predicting species distribution and population dynamics under current and future environmental change scenarios. They are also vital for conserving Mediterranean biodiversity associated with this endemic habitat-former.

Introduction

Variations in environmental temperature, combined with other stressors such as desalination, influence population, species, and community-level processes through effects on metabolic rates (O'Connor et al. 2007). There is substantial evidence of a universal temperature dependence linking individual metabolism to community-wide productivity, leading to predictable rates of population growth and regional diversity patterns (Allen et al. 2002, Cowen & Sponaugle 2009, Hoegh-Guldberg and Bruno 2010, Howard et al. 2024 McCauley et al. 2015). Temperature impacts metabolism, implying an inverse relationship between temperature and life-stage duration (Duarte 2007). For marine animals whose offspring develop in the water column as part of the meroplankton, the larval stage duration determines the period the larvae are subject to current movements, affecting the distance from the location where they are spawned. Additional stressors, such as extreme temperature events followed by salinity changes due to rainfall-induced desalination, can exacerbate these effects. Therefore, the influence of temperature and other environmental stressors on larval behavior and duration suggests a mechanistic link between oceanic conditions and biogeographic patterns mediated by larval dispersal and survival. Marine ecosystems are undergoing rapid changes, including rising temperatures and more frequent extreme climatic events (Cheng et al. 2019, Doney et al. 2011, Garrabou et al. 2022). The Mediterranean Sea is not an exception to global patterns of human-caused temperature variations, which are exacerbated by its semi-enclosed configuration, increasing the likelihood of thermal anomaly occurrences (Marbà et al. 2015). Indeed, it has experienced an increase in frequency, duration, and intensity of marine heat waves in the last 30 years (Collins et al. 2019, Garrabou et al. 2019, 2022). Model predictions for future ocean temperature based on the RCP8.5 scenario (Kwiatkowski et al. 2020) forecast a water temperature increase between 1.5°C and 3.0°C (compared to the current temperature) by the end of the 21st century, and long-lasting MHW, 4 times more intense and 42 times more severe than today's events (Diffenbaugh et al. 2007, Darmaraki et al. 2019). Extreme climatic phenomena generate stressful conditions for all marine communities that sometimes are not able to cope with it, dealing to reduce functioning and even local extinctions (Parmesan 2006). Benthic invertebrates represent the most impacted group by these thermal anomalies experiencing several mass mortality events in the last years (Garrabou et al. 2019). They usually represent key structuring habitats in local ecosystems, and the decrease or disappearance of

their populations can deal with ecosystem structure weakness, functioning alteration and biodiversity loss (García et al. 2018). In addition to heat waves and their increase in duration and intensity, the frequency of heavy rainfall, severe storms, or extreme flooding events after anomalous heat events have also increased, causing local lowering of water salinity along coastlines, a phenomenon called desalinization (Harley et al. 2006, Li et al. 2009). Salinity and temperature represent the most important factors affecting all vital processes in the marine environment (Brown et al. 2004, Ding et al. 2022). Changes in temperature can influence all biological processes including reproduction, the onset of spawning and embryonic and gonad development of benthic marine species and may therefore alter phenological processes (Birchenough et al. 2015). For instance, the thermal tolerances of numerous marine species experience a shift in distribution (Sunday et al. 2012) and a higher risk of mortality for those living close to their upper thermal thresholds (Gómez-Gras et al. 2019, 2022). Equally, it has been observed that decreasing salinity influences osmoregulatory patterns, reducing organismal performance (Li et al. 2009, Torres et al. 2021). The responses of organisms to disturbance would depend on their tolerance ranges and thresholds and their ability to withstand those stressful conditions, but also on the inherent features of the stressor and the possibility of other stressors taking over (Kéfi et al. 2019, Kingsolver & Woods 2016). To date, most of the research conducted on the topic has mainly focused on the effect of a single stressor, even if it is known that in nature, environmental change, especially that related to climate change, is multifactorial, changing over time and space with different consequences depending on the type of stressor interaction and the level of ecological hierarchy they are impacting (Gunderson et al. 2016, Orr et al. 2020, Jackson et al. 2021).

Many marine invertebrates exhibit a biphasic life cycle, including benthic adults and planktonic larvae, which inhabit different environments and can react differently to anticipated changes in climatological patterns (Gallego et al. 2017). So far, scientific literature has focused mainly on inquiring adult stages for responses to increase temperature, neglecting the juvenile and larvae stages even if they represent one of the most important steps of the life cycle of organisms, as they often are the most sensitive phases (Graham et al. 2017). In addition, for many marine species, dispersal occurs during the pelagic larval phase playing a key role in population dynamics. The changing environmental conditions interact with larvae traits such as larval stage duration and settlement time or larval survival (Bani et al. 2019, Watson et al. 2012), conditioning the fate of the species. Therefore, to predict the response of marine populations to multiple stressor conditions, one must understand its direct effects on ocean circulation (Van Gennip et al. 2017), as well as its indirect effect through the plastic response of dispersal and phenological traits (Reitzel et al. 2004).

The endemic coral *Astroides calycularis* (Pallas, 1766) is an important Mediterranean habitat-forming species that hosts more than 80 different species of marine vertebrates and invertebrates (Terrón-Sigler et al. 2014). It represents one of the most affected tridimensional habitats by changing environmental conditions in the Mediterranean Sea (Bisanti et al. 2022, Gambi et al. 2018). It has been observed that high temperatures negatively affect their populations, that interacting with other stressors of biotic nature can cause further impairment of the species performance (Bosch-Belmar et al. 2024). However, few studies reported the effect of environmental disturbance on its dispersal early-life stage (Carbonne et al. 2022). Due to the ecological importance of the species for the conservation of Mediterranean marine biodiversity and its evident high degree of vulnerability to changing environmental conditions, here we investigated the effect of different realistic (i.e., as really observed in nature) single stressors (exposure to heat-spike and MHW) and interacting stressors (MHW and salinity dropping simulating conditions after a storm) on the larval performance of *Astroides calycularis.* Demographic traits (mortality) and dispersal traits (larval stage duration and settlement rate) were monitored daily and measured during the days of the experiment, while metabolic trait (respiration rate) was measured immediately after the end of stressor exposure.

Materials and Methods

Study site and sampling procedure

Coral *Astroides calycularis*' larvae were collected along the Sicilian West Coast, in particular on the shores of Castellammare del Golfo (lat: 38.080972, long: 12.811639), a coastal area characterized by the presence of different brooks that maintain coastal salinity at 35 ± 0.21. The sampling was carried out during the second week of June, coincident with the peak of the coral reproductive cycle (maximum larvae release) and favorable weather conditions (minor current and wave action, and sampling temperature of 25 ± 0.43 ºC). Following the completion of all sampling operations, the collected larvae were transferred to the laboratory and randomly divided in two 5 liter cylindric aquariums equipped with oxygenators to maintain optimal oxygen levels and ensure continuous water flow and movement.

Experimental setup and treatments

After a 24-hour habituation period, approximately 300 larvae were randomly assigned to three experimental treatments and three control groups (n = 12 larvae per treatment). The larvae were placed in different cylindric aquariums with a capacity of 2 liters each, equipped with oxygenators to

maintain optimal oxygen levels and ensure continuous subtle water flow and movement. Single and multiple stressor treatments consisted of the following:

- One day heat spike (28°C for 24 hours);
- Marine Heatwave (28°C for 5 days);
- MHW (28°C for 5 days) along with an acute salinity dropping to 33 for additional 24h;
- controls (25ºC, natural spawning temperature, and natural salinity of 35 for all control treatments).

The experimental temperatures were reached increasing/decreasing 1ºC h^{-1} from each sampling temperature (Bosch-Belmar et al., 2024), and salinity treatment was performed by decreasing two salinity units of the natural salinity conditions over a 6 h time (Gavlik & Specker, 2004). After exposure time to each treatment, two different but correlated experimental sets were created: i) one related to the metabolic response of the larvae measured as the respiration rate; ii) the second one aimed to measure the larval stage duration, larval settlement rate and the mortality rate after the treatments exposure.

Larvae metabolic measurements

To investigate the metabolic response of the coral dispersal stage to stressor treatments, the respiration rate was used as a proxy of metabolism. Twelve larvae from each treatment were randomly selected and individually placed in a respirometric chamber (0.86 ml) filled with filtered sea water (Whatman GF/C 0.45 µm). All measurements were performed in continuum for 3 h and a recording frequency of 1 s through optical oxygen fibers (Firesting O2, PyroScience). The temperature was kept stable by using a heated circulating bath (Grant Optima TX150). At the end of the measurements, the wet and dry weights of the experimental larvae were measured by analytical balance (Mettler Toledo d = 0.0001g). Individual respiration rate (RR, mg O_2 h^{-1} g^{-1} DW) was calculated on the basis of the slope of the measured oxygen concentration in the respirometric chamber standardized per time unit, volume of the respirometric chamber, and dry weight of the larvae (Sarà et al., 2013).

Larvae dispersal ability

The experimental setup to investigate dispersal traits (larval stage duration and settlement) and mortality rate included three 50-l thermostatic baths containing 72 individual cups of 0.5 liters each (12 cups per treatment level). Each cup contained a Cembonit tile (1 cm x 2 cm) to facilitate larvae settlement opportunities (Agostini et al., 2018). The tiles were previously immersed in the field for 50 days to allow biofilm formation (Mallon et al., 2023). Three larvae of *A. calycularis* were placed in each cup (n = 36 larvae per treatment). The measured response variables included: i) larval

stage duration (days), ii) larval settlement rate (number of settled larvae per day of observation), and iii) mortality rate (number of dead larvae per day of observation). The experiment lasted 15 days, and the larvae status was observed every 48 hours for each aquarium.

Statistical analysis

To assess the effect of stressor treatments on larvae metabolism, data was first transformed due to the non-assumptions of normality and then an analysis of variance (ANOVA) was applied. The post hoc Tukey's Honest Significant Differences (HSD) test was used to deeper explore differences among treatments. The linear mixed model (LMM) was used to evaluate the effects of treatments on the settlement and mortality rates in the early life stage of *A. calycularis*. A hierarchical linear model was used since data were compiled from repeated measurements of the same pool of larvae over time. The models were implemented using the *lmer* function from the R package *lme4* (Bates et al., 2015). The fixed factors in the models included settlement, mortality and time; aquarium was designated as a random effect. All statistical analyzes were performed using R software.

Results

In general, increased temperature led to increased metabolism of experimental larvae (F_{value} = 21.28, df = 5, $p<0.001$). The larvae that participated in the treatments characterized by higher intensity and duration of the stressors (ie MHW and MHW + salinity drop) showed a higher respiratory rate compared to controls ($p<0.05$, Figure 1); while the larvae exposed to the heat spike treatment did not present significant differences in oxygen consumption compared to the control individuals ($p>0.05$, Fig. 1).

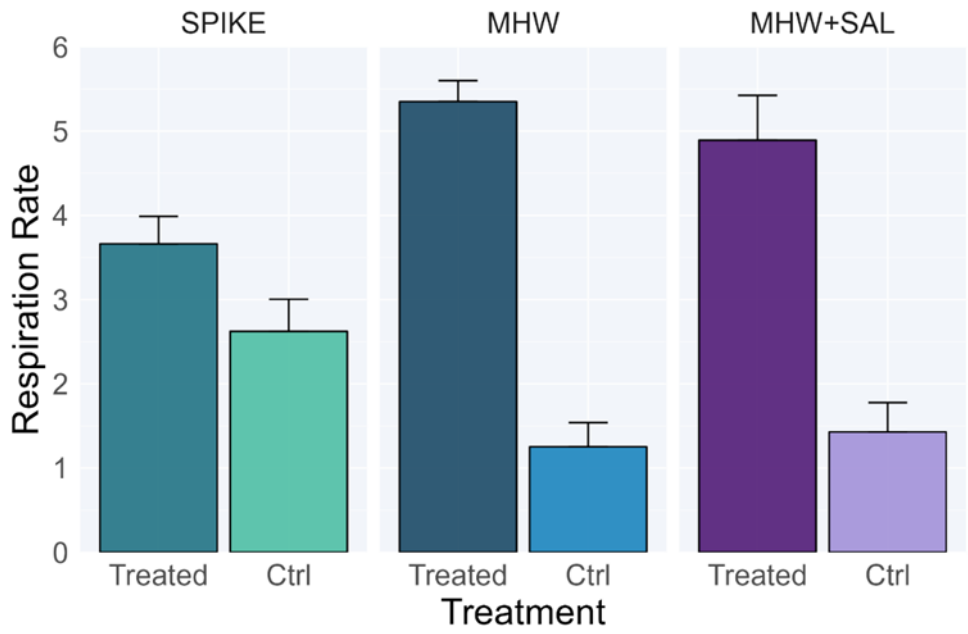

Figure 1. Astroides calycularis larvae respiration rate (mg O_2 h^{-1} g^{-1} DW) after exposure to the different treatments "SPIKE": heat spike at 28°C for 24 h; "MHW": Marine heat wave, 28°C for 5 consecutive days; "MHW + SAL": Marine heat wave followed by a dropping in salinity.

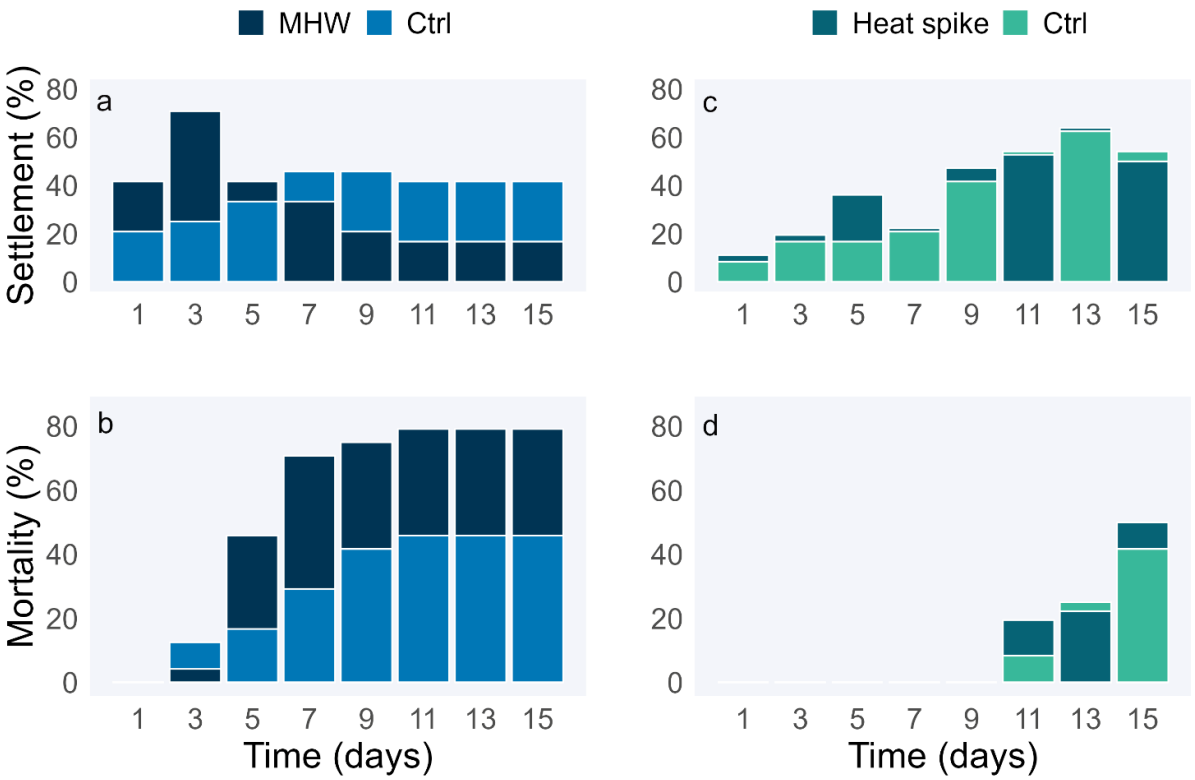

Figure 2. Percentage of settlement and mortality of A. calycularis larvae after exposure to 'MHW': Marine heat wave, 28 ° C for 5 consecutive days (a, b) and 'Heat spike': heat spike at 28 ° C for 24 h, treatments and their controls (c, d). Values are mean ± SE.

Treatment and time showed a significant effect on the settlement of larvae exposed to MHW (β = -0.167, SE = 0.033, t = -5.052, $p < 0.001$). Larvae exposed to MHW conditions showed higher settlement rates compared to the control group (70% and 41.6% settled larvae, respectively), but also a higher mortality rate over time (β = 0.100, SE = 0.036, t = 2.754, $p<0.01$, Fig. 2a, b); while individuals exposed to heat spikes did not show significant differences with respect to controls either in settlement (β= -0.025, SE= 0.021, t= -1.173, $p> 0.05$) nor mortality rates (β= 0.025, SE= 0.014, t= 1.778, $p> 0.05$). However, the settlement timing was different between treatments. Larvae exposed to MHW presented a faster settlement with respect to controls and heat spike-treated larvae. After 2 days from the starting of observations, the highest settlement rate (71% of larvae) was observed with respect to the control group, which presented slower but increasing settlement over time (Figure 2a). Heat spike-treated individuals and controls showed the highest settlement rate after 13 days from the end of the exposure, presenting 61% and 62% of experimental larvae settled, respectively (Figure 2c). Larvae exposed to the MHW treatment experienced the first mortalities after 2 days from the beginning of the observations, presenting a mortality peak after 10 days of observations with more than 70% of dead larvae, while controls showed much lower mortality rates over time with 42% of larvae dead at the end of the experiment (Figure 2b). Note that a large percentage of larvae settled from the MHW treatment died after settlement, which was the major cause of mortality rate measured in this group. The rate of swimming larvae in the water column at the end of the experiment was significantly higher in the control groups (30%) than in the MHW treatment (4%). Experimental larvae belonged to heat spike treatment showed a similar pattern of mortality rate for treated and control groups (50% and 42%, respectively) with the first recorded mortalities starting after 10 days from the beginning of the observations (Figure 2d).

Discussions

The increase in frequency of climatic disturbances, along with an increase in their duration and intensity, leads to a series of impacts that affect the entire ecological hierarchy, from the individual to the whole ecosystem (Bellard et al. 2012, Sarà et al. 2021). The effects at population level deserve particular attention, since they can alter population's dispersion and connectivity patterns (Doney et al. 2012), jeopardizing the distributional spatial directions of the species. This is particularly of concern when the affected species is an ecological engineer or a habitat-former, whose mass mortality due to environmental change involves high risk for local biodiversity. The outcome of the present study showed a significant effect of current thermal and salinity changes caused by humans on the metabolism and survival of the early life stage of a threatened marine structuring

species. Not only the intensity, but also the duration of the stressor played a key role in the responses of coral larvae. Temperature significantly influences all physiological processes (e.g., photosynthesis and metabolism), consequently regulating individual performance (Seibel & Drazen 2007). Augmented temperatures increase basal metabolic rates, and the energy demand can exceed the metabolic capacity of the species to counteract the stressor action (Kooijman 2010, Lemoine & Burkepile 2012). Although increasing temperatures address increasing respiration rate, larvae exposed to heat spike treatment were able to compensate for the high temperature effect at the metabolic level. Instead, those exposed to MHW showed significantly higher metabolic rates than controls and heat spike treatment, potentially high energy expense (Angilletta et al. 2002). MHWs do not occur in isolation but interact, often synergistically, with other stressors, such as elevated turbidity (Tait et al., 2021), decreased salinity (Sen Gupta et al., 2020) and anoxia (Gruber et al., 2021), suggesting that effects on individuals may be more complex than simple temperature reaction norms. Here, multiple stressors (MHW + salinity drop) impaired the functioning of the larvae energetic machinery (Sokolova et al. 2012), provoking further acceleration of their metabolism. The joint action of both stressors may present a potential synergistic effect on larvae' metabolism by increasing the oxygen consumption rate to maintain aerobic metabolism. However, if metabolic demands increase and individuals cannot meet the elevated energy requirements, reduced performance (eg, growth and reproduction) and ultimately decreased fitness will occur. This observation is corroborated by the results obtained from larvae exposed to marine heatwave conditions followed by a salinity drop: within just 24 hours of exposure, nearly 100% of the larvae in the settlement experiment perished.

The observed variation in settlement rate and timing, as well as mortality, reflected the complex responses of the life cycle of the organism to environmental variability. The escalation in metabolic rate, coupled with the early and pronounced settlement rate of larvae exposed to MHW treatment, may lead to the larvae's faster utilization of their energy reserves to counterbalance the stressor's impact on metabolism and enhance their chances of survival through premature settlement (Serrano et al. 2018). This "last-ditch effort" to optimize their fitness was accompanied by a heightened larval mortality rate, even after settlement. Indeed, this pattern was not observed in the heat-spray-exposed group, which mirrored the control responses. As a result, larvae can navigate for fewer distances as they might need to settle earlier, recruiting closer to their native population, consequently reducing their potential for long-distance dispersal and connectivity (Graham 2012), with unpredictable long-term genetic effects (Van der Ven et al. 2016). This finding is central for evaluating the role of temperature dependency and the joint synergistic effects of other stressors on an important ecological factor as the planktonic larval duration. Indeed, most marine organisms rely

on planktonic phases for dispersal, which facilitates the connection between disjoint adult populations through planktonic propagules. Planktonic larval duration constrains the potential dispersal distances of fish and invertebrates, thereby affecting the neighborhood size of marine populations and the potential connectivity among disjoint populations (Duarte 2007). Accordingly, our experimental species, *A. calycularis*, as many other coral species spread across all oceans, has a reproductive cycle that ends with the release of swimming larvae during the summer period (Casado-Amezua et al. 2013, Pellón & Badalamenti 2016). Thus, thanks to recent research (Gouvêa et al. 2015, Paxton et al. 2015, McRae et al. 2021), we can also presume that temperature changes with other multiple stressor affect timing of reproductive adult events. Frequency and intensity of spawning represents a major physiological energetic bottleneck (Kooijman 2010) for adults, as they must allocate and invest a substantial amount of energy into the production of larval stages (Leuzinger et al. 2012). Consequently, if adults are simultaneously faced with environmental stressors, such as local thermal anomalies, and larvae are subsequently compromised to some extent, as observed in this study, these combined effects involving adults and their larvae, can affect propagation magnitude across entire biogeographical sectors of the species' distribution. Studies have already shown how denser populations tend to produce more larvae per surface unit (Hartmann et al. 2017). This allows us to hypothesize that under the CC scenario in which the former endemic habitat-former populations will face extreme thermal anomalies, in addition to the observed consequences on larvae performance, there could be a decrease in the quality or the quantity of gametes. This is called maternal effect (Kooijman, 2010). The effect of other stressors related to climate change on the performance of this species has been recently investigated, reporting remarkable results on additional environmental conditions that could threaten the well-being of orange coral populations. For instance, Carbonne et al., (2022) experimentallyreported that the warm temperature and low pH had a negative impact on the survival, settlement, and growth of recruits of *A. calycularis*. On the other hand, abrupt changes in salinity as a source of disturbance are underestimated as a detrimental factor in marine habitats, though some studies have reported negative effects of decreased salinity on different marine invertebrates performance (Chui & Ang 2017, Podbielski et al. 2022, Torres et al. 2021). For example, Chui & Ang (2017) refer to the salinity decrement as an environmental condition leading to unsuccessful settlement of coral larvae. In conclusion, our findings highlight how current and future extreme climatic events, related to increasing temperatures and salinity drops, significantly impair the performance of the early life stages of an endemic Mediterranean habitat former. Rising temperatures may accelerate metabolism and lead to earlier and faster settlement of larvae, thereby reducing dispersal ability and increasing population mortality. The early life stages of the species are unlikely to withstand the combined effects of rising temperatures and salinity changes. Predicting the

success of organisms in their habitats, particularly during early life stages, is crucial for understanding the impact of environmental changes on species distributions and population dynamics (Mokany & Ferrier 2011). This is especially important for species that serve as foundational habitat components, representing biodiversity hotspots. Given the fundamental role of such species in coastal marine ecosystems and the increasing evidence of negative effects from various environmental stressors related to climate change and anthropogenic disturbances on coral performance, understanding their response to changing environmental conditions is pivotal. This information can inform the management of marine protected areas (Balbar & Metaxas 2019) and consequently, our ability to predict local drivers affecting larval dispersal is crucial for addressing conservation science.

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Concluding remarks

This dissertation aimed to provide a comprehensive examination of the effects of human-driven climatic stressor on Mediterranean coralligenous and pre-coralligenous communities structure and functioning. The integration of mechanistic, modelling and social science approaches allowed to address different functional aspects of this endemic Mediterranean habitat, scaling from the individual to the community levels, to infer about the potential effects of stressors on ecosystem functioning and services. Our research aligns with the growing body of evidence demonstrating that climate change is manifesting through increasingly severe extreme weather phenomena, such as intense heat waves, prolonged droughts, and extreme rainfall (Clarke et al., 2022). These events generate significant impacts on marine biodiversity and its ecosystems (Smale et al., 2019).

First achieved goal has been the creation of a consistent baseline for coralligenous community characterization and conservation status in the southwestern Italian coast (MPA Capo Gallo – Isola delle Femmine), pointing out climatic stressors as potential main source of disturbance for the habitat (chapter 1). Such study allowed the identification of two of the most vulnerable habitat-forming species from these communities, the white gorgonian *Eunicella singularis* meadows and *Astroides calycularis* reefs. It marked the starting point for functional studies, both correlative and manipulative, conducted in the field and laboratory.

In situ metabolic measurements and oxidative stress analyses revealed the effect of natural thermal stressors on the functioning of white gorgonians (chapter 2). Our findings align with those of previous studies (Coma et al., 2006; Gori et al., 2011) in demonstrating the vulnerability of *E. singularis* to thermal stress. However, our work extends beyond by providing a more detailed understanding of the eco-physiological mechanisms underlying the species vulnerability. Additionally, its role as a carbon sink was confirmed, highlighting the importance of meadow density in performing this vital ecosystem function.

The vulnerability of *A. calycularis* to extreme climatic events was experimentally demonstrated in both the dispersive larvae and adult stages (chapters 4 and 6). The nature of the stressors involved proved crucial in the species' response, leading not only to a decline in performance but also to population-level impacts, with a reduction in the larvae time spent in the water column and low settlement and survival rates. These results were further confirmed by the study on the thermal tolerance of the adult stage, identifying 27.8°C as the upper thermal critical threshold for the

species. As shown in the literature, this temperature is easily reached during summer-autumn periods in the Mediterranean Sea (Copernicus 2021), posing a serious threat to this habitat.

The social science approach provided valuable information regarding the distribution and conservation status of the species along the Italian coasts. Additionally, the overlap distribution of *A.caycularis* and the stinging corallivorous polychaete *Hermodice carunculata* (a Mediterranean native invader) was verified (chapter 3). The method has proven to be highly efficient in obtaining information on a broad spatial scale, minimizing sampling effort (Sandahl & Tøttrup, 2020; Wagner & Davis, 2003).

Manipulative laboratory experiments confirmed both the effect of temperature on the thermal tolerance of the fireworm, which proved to be a thermophilic species with an optimal performance temperature at 25.5°C, and the actual predation of the polychaete on the coral (chapter 4 and 5). This information highlighted the combined effect posed by increasing temperature and predation risk to the habitat-former. The overlap between the critical upper thermal limit of *A. calycularis* and the temperature of maximum metabolic and predatory performance of *Hermodice carunculta* suggested a potential increased predation pressure under warming scenarios (Bosch-Belmar et al., 2024), creating a multiple stressor condition for the coral, significantly threatening its conservation and the fate of its associated biodiversity.

In conclusion, outcomes from this PhD thesis contribute significantly to our understanding of how Mediterranean coralligenous habitats are responding to climate change and have important implications for the conservation and management of these habitats. By providing a comprehensive assessment of these impacts across multiple ecological levels, from baseline community composition to eco-physiological responses and species interactions, this work fills critical knowledge gaps and offers valuable insights for developing effective conservation strategies. Moreover, our work highlights the value of engaging local stakeholders in monitoring and conservation efforts. As we face the ongoing challenges of global environmental change, such integrated, interdisciplinary research will be crucial in our efforts to protect and preserve these invaluable marine ecosystems for future generations.

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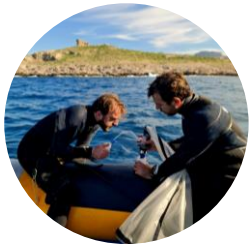
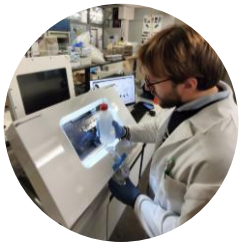
10. Acknowledgements



Giungere al termine di questo percorso di dottorato rappresenta per me un traguardo importante e, allo stesso tempo, un'occasione per riflettere sul lungo cammino che ho intrapreso. Questo lavoro non sarebbe stato possibile senza il contributo, il sostegno e la vicinanza di molte persone che, in modi diversi, hanno lasciato un segno profondo in questa esperienza. Vorrei dedicare questo spazio a ringraziare coloro che hanno reso possibile questo risultato.

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una fuente de inspiración, y llevaré siempre conmigo todo lo que he aprendido de ti, no solo en el ámbito académico, sino también en el personal. Este logro también es mérito tuyo, y te estaré siempre agradecido por todo lo que has hecho.

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Supplementary material

I) Distribuzione e stato di salute del corallo arancione (*Astroides calycularis*):

1) Quali siti d'immersione frequenti solitamente? (sito e comune)
   a) _____
   b) _____
   c) _____
   d) _____
   e) _____

2) Nei siti d' immersioni da te frequentati hai avvistato questi organismi (Coralli arancioni)? (foto 1)
   - ☐ Si
   - ☐ No

2.1) Se sì, dove e a quale profondità?

_____

_____

3) Ne hai mai visti bianchi? (foto 2)

□ Si
□ No

3.1) Se sì, dove e a quale profondità?

_____

_____

II) Distribuzione *Hermodice carunculata e interazione con il corallo arancione*:

4) Durante le tue immersioni hai avvistato il Vermocane (*Hermodice carunculata)* (foto 3)?
□ Si
□ No

4.1) Se sì, dove e a quale profondità?

_____

_____

4.2) Se l'hai avvistato, in quale di questi ambienti?
□ fondale sabbioso
□ prateria di *Posidonia oceanica*
□ parete rocciosa
□ grotta

4.3) La presenza del vermocane durante l'immersione ti dà fastidio?
□ Per niente
□ Poco
□ Abbastanza
□ Molto

5) Hai mai visto il vermocane ed il corallo arancione insieme? (foto 4)
   - □ Si
   - □ No

5.1) Se sì, dove e a quale profondità?

_____

_____

6) Hai delle foto di questi organismi che vorreste condividere? La finalità della raccolta delle foto è quella di avere una validazione visuale del dato di avvistamento. Le foto da te fornite devono essere firmate, e non verranno utilizzate senza il permesso dell'autore.
   - □ Si
   - □ No

III) Percezione del subacqueo sulla temperature e su entrambe le specie durante l'immersione

7) Hai notato dei cambiamenti nella temperatura dell'acqua negli ultimi 3 anni rispetto ad anni precedenti? (riferito alle immersioni realizzate tra giugno e settembre)
   - □ Si, negli ultimi anni è più calda
   - □ Si, negli ultimi anni è più fredda
   - □ No, non ho notato nessun cambiamento

8) Sai cosa sia il termoclino?
   - □ Si
   - □ No

8.1) Se sì, negli ultimi 3 anni hai notato cambiamenti nella profondità del termoclino?
   - □ Si
   - □ No

8.2) In caso affermativo, saresti in grado di descrivere questi cambiamenti?

_____
_____

- Da quanti anni fai immersione? _____
- Quante immersioni fai in media ogni anno? _____
- Quando ti immergi, in media quante persone hai intorno? _____
- Come *diving* center, in media quanti subacquei portate in immersione ogni stagione? _____

IV) Informazioni personali dei subacquei:

Quanti anni hai?

- 18-25
- 26-35
- 36-50
- > 50

- Qual è il tuo livello d'istruzione?
- Scuola Elementare
- Scuola Media
- Diploma
- Laurea triennale
- Laurea magistrale
- Post laurea

- 10) Ci sono altre informazioni relative al questionario che vorresti condividere con noi?: _____ _____ _____ _____
- Vorresti condividere altre informazioni sulle tue immersioni come, ad esempio, date e profili termici?
- Si
- No

- Saresti disposto ad essere contattato per ulteriori informazioni?
  - Si (e-mail e/o numero di telefono: _____
  - No

List of papers

Published papers:

1. Bosch-Belmar M., **Tantillo M. F**., and Sarà G. (2024). Impacts of increasing temperature due to global warming on key habitat-forming species in the Mediterranean Sea: Unveiling negative biotic interactions. Global Ecology and Conservation.

2. Schubert Nadine, Tuya F., Peña V., Horta P.A., P.A Salazar P.A, Neves P., Ribeiro C., Otero-Ferrer F., Espino F., Schoenrock K., Ragazzola F., Olivé I., Giaccone T., Nannini M., Mangano M. C, Sará G., Mancuso F. P., **Tantillo M. F**., Bosch-Belmar M., Martin S., Le Gall L., Santos R., Silva J. (2024). "Pink power"- the importance of coralline algal beds in the oceanic carbon cycle. Nature Communications 15: 8282.

3. Gjoni, V., Marchessaux G, Glazier D. S., J. S. Wesner, Bosch-Belmar M., Mancuso F. P., **Tantillo M. F**., Marsiglia N., and Sarà G. (2024). Metabolic scaling of an invasive mussel depends on temperature and chemical cues from an invasive predator. Biology letters 20: 20240066.

4. Giacoletti Antonio, Bosch-Belmar Mar, Mangano Maria Cristina., **Tantillo Mario Francesco**, Gianluca Sarà., Giacomo Milisenda (2024) Predicting the effect of fouling organisms and climate change on integrated shellfish aquaculture. Marine Pollution Bulletin 201: 116167.

5. Piazzolla Daniele, Scanu Sergio, Mancuso Francesco Paolo, Bosch-Belmar Mar, Bonamano Simone, Madonia Alice, Scagnoli Elena, **Tantillo Mario Francesco**, Russi Martina, Savini Alessandra, Fersini Giorgio, Sarà Gianluca, Coppini Giovanni, Marcelli Marco, Piermattei Viviana (2024) An integrated approach for the benthic habitat mapping based on innovative surveying technologies and ecosystem functioning measurements. Scientific reports 14: 5888.

6. Marchessaux Guillaume, Gjoni Vojsava, **Tantillo Mario Francesco**, Bejean Théo, Sarà Gianluca (2024). The expansion of the Atlantic–Mediterranean ghost crab *Ocypode cursor* (Linnaeus, 1758): Distribution, environmental niches and future perspectives. Aquatic Conservation: Marine and Freshwater Ecosystems 34: 4119.

Under review/ submitted/ in preparation:

1. **Tantillo Mario Francesco**, Bosch-Belmar Mar and Sarà Gianluca. How extreme climatic events threaten the larval settlement and survival of the endemic Mediterranean coral *Astroides calycularis* (Pallas1766). **Under review** to Science of the Total Environment (Chapter 6)

2. Bosch-Belmar Mar, Mancuso Francesco Paolo, **Tantillo Mario Francesco**, Russi, Martina, Piermattei Viviana, Piazzolla Daniele, Madonia Alice, Marcelli Marco, Sarà, Gianluca. Metabolic traits and thresholds to inform marine ecological restoration. **Under review** in Journal of Applied Ecology.

3. Mancuso Francesco Paolo, Bosch-Belmar Mar, **Tantillo Mario Francesco**, Russi Martina, Piermattei Viviana, Marcelli Marco, Sarà, Gianluca. Endemic Mediterranean seagrasses poised to survive climate change challenges. **Under review** in Ecological Applications.

4. **Tantillo Mario Francesco**, Mar Bosch-Belmar and Gianluca Sarà. Ecological and conservation status of coralligenous community in the MPA Capo Gallo- Isola delle Femmine: a baseline for future effects of anthropogenic disturbance. **In prep.** (Chapter 1).

5. **Tantillo Mario Francesco**, Bosch-Belmar Mar, Mancuso Francesco Paolo, Seveso Davide, Madaschi Andrea, Galli Paolo and Sarà Gianluca. Effect of changing environmental conditions on metabolic performance and oxidative stress markers of *Eunicella singularis*. **In prep** (Chapter 2).

6. **Tantillo Mario Francesco**, Bosch-Belmar Mar, Mangano Maria Cristina, and Sarà Gianluca. Using Local Ecological Knowledge to assess the distribution and ecological status of the endemic Mediterranean coral *Astroides calycularis* (Pallas 1766). **In prep** (Chapter 3).

7. **Tantillo Mario Francesco**, Bosch-Belmar Mar, and Sarà Gianluca. Concurrent effect of increasing temperature and biotic interactions on habitat complexity and biodiversity: the case study of the coral *Astroides calycularis* and the fireworm *Hermodice carunculata*. **In prep** (Chapter 4).