UNIVERSITÀ DEGLI STUDI DI TRIESTE

XXXV CICLO DEL DOTTORATO DI RICERCA IN AMBIENTE E VITA

ASSESSING THE NATURAL CAPITAL ASSOCIATED TO *ERICARIA AMENTACEA* FORESTS

Settore scientifico-disciplinare: **BIO/07**

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ABSTRACT

In the Mediterranean Sea, brown macroalgae of the genus *Cystoseira sensu lato* (Fucales, Phaeophyceae, hereafter *Cystoseira s.l.*) form dense forests in subtidal and intertidal rocky habitats, playing a crucial role as ecosystem engineers by sustaining biodiversity and contributing to biogeochemical cycles. In the last four decades, these macroalgae have undergone a substantial and widespread regression, because of local and large-scale anthropogenic pressures and, more recently, global climatic stressors. This reduction in cover and biomass could lead to the loss of the associated biodiversity and consequently to the depletion of ecosystem functions.

Despite their importance for the functioning of the coastal ecosystems, baseline knowledge about *Cystoseira s.l.*, and in particular of *Ericaria amentacea*, is still scarce, limiting effective conservation and management strategies for this pivotal habitat former. In addition, nowadays there is a growing interest in the quantification of ecosystem relevance through biophysical and economic assessments of the natural capital, in order to raise awareness of the public about the protection of biodiversity and to clarify the strict relationship between ecosystems and human wellbeing.

In this doctoral dissertation, the theme of the estimate of the natural capital stocked in *E. amentacea* forests is addressed. The first part of the thesis aimed to increase the knowledge of this species, and to collect useful information for the assessment of natural capital through the emergy analysis. Spatial and temporal variations of biomass and key attributes of *E. amentacea* (e.g., biomass, total organic carbon and nutrient tissue contents) were studied in order to provide a large set of data that may help future research to improve our understanding of the contribution of this macroalgae to the functioning of coastal ecosystems. Also, the effects of full protection within Marine Protected Areas on *E. amentacea* stands have been assessed, to shed light on the effectiveness of full protection in enhancing the biomass of this species and the associated understory assemblages.

The high local scale variability of all the investigated functional trait variables found in the study areas suggests that quantifications of functional performances of these forests

over large spatial scales should rely on data from multiple sites, and that the information of key functional traits of *Cystoseira s.l.* species cannot be limited to sporadic, spatially limited assessments. Results also highlighted that the major role of these macroalgae can relates to carbon flows within coastal ecosystems rather than to carbon sequestration, so that they can be crucial for carbon supply towards neighboring areas and even deep systems. Such findings reinforce the need for adequate efforts to protect these marine forests and reverse their decline in the basin. However, current protection regimes within MPAs might be not sufficient, as several studies carried out in the Mediterranean Sea have pointed out and confirmed also from the analysis of *E. amentacea* stands performed in this study.

From a more practical point of view, increasing the baseline knowledge of *E. amentacea* has led to an accurate estimate of the natural capital stocked in this habitat. Indeed, in the second part of this elaborate, all the natural resources contained in *E. amentacea* canopies were evaluated. Results, expressed in both biophysical and monetary value, through the application of emergy analysis, filled the information gap about the natural capital values of these environments. Moreover, taking into account the macroalgae natural biomass turnover, allowed to obtain a more detailed analysis and to assess the real value of *E. amentacea* that equals the one of the most valuable ones at Mediterranean level, such as coralligenous assemblages and *Posidonia oceanica* forests. *E. amentacea* demonstrated then to be a value keeper and catalyst. This role is even more important in the intertidal, where conditions for life are extremely difficult.

The present work will contribute to increase the knowledge of key species traits and to achieve more accurate assessments of ecosystem goods, services, and the overall natural capital associated to brown macroalgae forests, which are essential to support policy makers in refining and implementing effective management strategies that could preserve the important ecological role of these species for the functioning of coastal ecosystems and for the provision of benefits to the human society.

INTRODUCTION

Ericaria amentacea as ecosystem engineer

Ericaria amentacea is a brown macroalga belonging to the group of fucoid algae *Cystoseira sensu lato* (Fucales). Endemic of the Mediterranean Sea, *E. amentacea* dominates the rocky shore where it plays a crucial role in the costal ecosystem. *E. amentacea* is a perennial macroalgae that naturally exhibits substantial phenological seasonal variations in its morphology/biomass and associated understory assemblages (Mannino et al., 2014). In fact, during the resting period (autumn/winter) only the perennial basal cauloid remains in most *Cystoseira s.l.* species. Later, in spring, new branches sprout and in summer the thalli consist of main axes covered by leaf-like structures. In late summer/autumn, the erect branches are shed and the cauloid persists during the following unfavorable, cold season. Such changes are reflected in the associated assemblages (Benedetti-Cecchi et al., 2003), resulting in an increase in photophilic algae in the understory during the resting season, alternating with an increase in abundance of vagile (e.g., polychaetes, crustaceans, holothurians) and epiphytic (e.g., hydroids) invertebrates during the growing season (Mancuso et al., 2021) (Figure 1).

Figure 1- *E.amentacea* forests (a) and its associated community (b).

Due to their own physical structure, they create highly complex three-dimensional habitats, providing secondary substrate that support a diverse associated community (Smale et al., 2018). Indeed, by simply developing in a suitable area, ecosystem engineers significantly affect other species by providing and maintaining habitats and microhabitats that would not exist otherwise. For example, kelp forests (Laminariales), in temperate oceans create a myriad of different habitats: their fronds modulate the impact of waves, shaping a whole new three-dimensional environment, holding nutrients, and creating refugia (Jones et al., 1994; Noisette & Hurd, 2018). Despite their smaller sizes, *Cystoseira s.l.* species play a similar role in the Mediterranean Sea, creating dense forests on intertidal and subtidal rocky reefs (Schiel & Foster, 2006). Fucalean brown algae form one of the most productive, diverse, and valuable marine habitat (Benedetti-Cecchi et al., 2001), delivering crucial ecosystem functions and services in coastal environments, by providing substrate for settlement of sessile organisms, food and shelter for a highly diverse biota, contributing to nutrient cycling and constituting a blue carbon sink (Ballesteros, 1990; Ballesteros et al., 1998; Cheminée et al., 2013; Pitacco et al., 2014; Mineur et al., 2015; Chiarore et al., 2019, Bianchelli & Danovaro., 2020).

Anthropogenic stressors are increasingly impacting natural environments, eroding the resilience of ecosystems, and leading to a reorganization of biodiversity at the global scale (Crain et al., 2008; Elmqvist et al., 2003; Folke et al., 2002; Millennium Ecosystem Assessment, 2005, Cebrian et al., 2021). Algal forests composed by the genera *Cystoseira*, *Ericaria*, and *Gongolaria*, except for *C. compressa*, likely represent the most endangered habitats in the Mediterranean Sea (Verlaque et al., 2019) and have undergone a major decline in the last decades (Thibaut et al., 2005; Blanfuné et al., 2016; Rindi et al., 2020). Above all, *E. amentacea* is considered one of the species most severely impacted by coastal development and sewage outfalls (Thibaut et al., 2014), and has been added to the Annex I of the Convention on the Conservation of European Wildlife and Natural Habitats, a section called “Strictly protected flora species” (Council of Europe, 1979; Thibaut et al., 2014), but current protection measures do not seem sufficient to stop its current decline (Thibaut et al., 2005). Because of their foundation role, the decline of *Cystoseira* species and their replacement by opportunistic or alien algae or by barren grounds can have negative cascading impacts on the biodiversity of rocky reef communities (Fabbrizzi et al., 2020; Bianchelli & Danovaro, 2020) and endangering the maintenance of essential ecosystem services (Ofrandis et al., 2021).

Evaluation of the natural capital: emergy analysis

Natural capital can be defined as the world's stock of natural assets, both renewable and non-renewable resources (e.g., geology, soil, air, water, and all living things), from which ecosystem functions and services arise. Ecological functions derive from the interaction of natural capital components, while ecosystem goods and services are the benefits that humans obtain, directly or indirectly, from ecosystem functions. Indeed, even when humans do not use some functions or even perceive their existence, our survival and our well-being depend on them (Paoli et al., 2022). Ecosystem services depend fundamentally on the structures and processes generated by living organisms and their interactions with, and processing of, abiotic materials. Natural capital is irreplaceable, and together with the manufactured one, generated the basis for human well-being (Vassallo et al., 2017). Over the last decades, the lack of understanding about the societal dependence upon natural ecosystems generated several environmental issues, which brought to the depletion and loss of the natural capital. Therefore, the sustainable exploitation of natural capital stocks is essential for ensuring a continuous provision of ecosystem services over time (de Groot et al., 2012) and integrated approaches capable of identifying and measuring the natural capital, combining ecological and socioeconomic aspects, are needed (Buonocore et al., 2020).

At the political level, natural capital accounting has been recognized by the United Nations, more recently through the definition of the Sustainable Development Goals (SDGs) of the 2030 Agenda. According to Vassallo et al. (2013) to obtain a comparable and an easily understanding value, the measure of natural capital must be both ecological and economic. In such way, benefits or costs associated with changes in ecosystem services could be incorporate in decisional process (Johnston and Russell, 2011). In the 1970s, the ecologist H.T. Odum realized that economic activities were not only shaped by economic rules but also by the constraints of an ecosystem (Amaral et al., 2016). From that time onwards, Odum started to explore a common evaluation framework of environmental accounting so that sustainability could be integrated into policymaking related to human activities and the emergy analysis arise. This method is able to assess the work done by nature to generate resources and provide ecosystem services (Brown et al., 2010). It expresses all resources on a common basis, the solar emergy Joules (sej), that is the energy needed to generate and maintain a process, and it provide a measure of the work done to provide a flow and represents the investment made by nature in space and time (Vassallo et al., 2017). The outcome of the analysis could be converted into monetary value, still representing the biosphere's investment. In this way the importance of natural capital and ecosystem functions could be better understood by policy makers and other stakeholders allowing an effective communication in socio-economic contexts (Turcato et al., 2015; Vassallo et al., 2021).

Thesis outline

Assessing the natural capital, and the ecosystem functions and services derived from it, is a key step in the achievement of a sustainable development and for the correct management of natural resources. Despite the importance of *Cystoseira s.l* for the costal ecosystem only De la Fuente et al., 2019 has evaluated the natural capital associated to this habitat in the Mediterranean Sea. In addition, little is still known on basic functional attributes (e.g. organic matter, organic carbon, and main nutrients) of *Cystoseira s.l.* species in the Mediterranean Sea, especially of *E. amentacea*. All these functional traits could change in space and time so, in order to have an accurate quantification of the natural capital associated to this habitat, a key step is to study and define spatio-temporal and intraspecific variations of this attributes.

Considering the key functional and ecological role of *E. amentacea*, and the abovementioned paucity of studies addressing this topic, the present doctoral dissertation aims at:

1. Increasing the base knowledge about *E. amentacea* functional traits underpinning the estimate of the associated natural capital (Part I)

2. Assessing the natural capital associated to *E. amentacea* forests considering spatio-temporal variation (Part II)

In Part I, the first chapter (Cannarozzi et al., 2023) aims to test spatio-temporal variations of *E. amentacea* and its associated assemblages and assessing the potential effects of full protection within Marine Protected Areas (MPA) in modifying patterns of variation in their biomass. Indeed, even if *Cystoseira s.l.* populations are under different conservation regimes, it is still far from being clearly ascertained whether current protection strategies within MPAs are effective for the conservation and management of these habitat formers and their understory assemblages.

In the second chapter (Cannarozzi et al., 2023/submitted), the intraspecific spatial variability of key functional traits variables of *E. amentacea* and other macroalgae belonging to the genus *Cystoseira s.l.* is studied. Despite their functional role, little is known on *Cystoseira s.l* contents of organic matter, organic carbon, and main nutrients (e.g., nitrogen and phosphorous), and very few attempts have been done to reduce this gap. Understanding whether environmental changes turn into significant spatial variability of biomass and contents of key elements in *Cystoseira s.l.*, and identifying relevant scales of variations, is essential for accurate quantifications of the contribution of *Cystoseira s.l.* stands to the overall functioning of coastal ecosystems in terms of matter and energy flows.

In Part II, (Cannarozzi et al., on process), the focus is to investigate and quantify the biophysical and monetary value of *E. amentacea* forests in order to fill the gap about natural capital information of transitional environments and better understand the role played by *Cystoseira s.l.* as good indicator of environmental quality. In general, understanding the value of the natural capital is the first step to be considered in ecosystem services analyses, where after the assessment of the natural resources it will be possible to ascribe a biophysical value also to functions generated by the considered habitats and, finally, to assess potential changes in the provisioning of ecosystem services that can be maintained at the current level only if the natural capital is preserved. Therefore, the evaluation of natural value of *E. amentacea* stands will allow to raise awareness of the public towards the preservation of this habitat-forming species, in order to protect biodiversity and support the policy makers in the process of creating new legal and management tools.

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PART I_ INCREASING THE BASE KNOWLEDGE ABOUT *E. AMENTACEA* FUNCTIONAL TRAITS UNDERPINNING THE ESTIMATE OF THE ASSOCIATED NATURAL CAPITAL

CHAPTER 1

Assessing the effect of full protection on the biomass of *Ericaria amentacea* stands and understory assemblages: evidence from two Mediterranean marine protected areas

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Abstract

Cystoseira sensu lato marine forests, which are among the most productive and diverse systems in rocky intertidal and subtidal habitats of the Mediterranean Sea, are experiencing a widespread decline throughout the basin due to increasing human pressures. Yet, whether Marine Protected Areas (MPAs) may represent effective tools for conservation of these important habitat formers and their associated assemblages is still unclear. Here, we compared the biomass of macroalgal stands of *Ericaria amentacea* (C. Agardh) Molinari & Guiry 2020 and their understory assemblages between the no-take zone and control sites in two Mediterranean MPAs. We did not find evidence supporting a significant effect of full protection in increasing the biomass of *E. amentacea* stands and associated assemblages, except for macroalgae of the understory in one of the investigated MPAs, raising concerns on the potential effectiveness of MPAs in mitigating human impacts on these marine forests. Such findings call for major efforts to implement long-term monitoring programs of protected *Cystoseira s.l.* forests in order to inform an adaptive management of conservation measures within MPAs and eventually to set active interventions of restoration.

Keywords: community ecology, conservation, *Cystoseira sensu lato*, human impact, macrobenthos, multivariate analysis

Introduction

Macroalgal forests are the largest and most productive components of coastal ecosystems worldwide [1]. In the Mediterranean Sea, brown algae such as *Cystoseira sensu lato* (including the genera *Cystoseira*, *Ericaria* and *Gongolaria*) are particularly important as habitat formers [2]. These fucoid algae dominate rocky intertidal and subtidal habitats, forming dense macroalgal stands that, due to their structure and high biomass, play a crucial role as ecosystem engineers by creating highly complex three-dimensional structures and providing secondary substrate, which in turn, support highly diverse understory and epiphytic assemblages [3]. Moreover, this complexity allows sustaining diversified trophic levels, enhancing primary and secondary productivity, along with many other ecosystem functions [4,5]. Canopies of *Cystoseira s.l.* are spatially organized into four distinct layers: an encrusting basal layer mainly consisting of calcareous algae, a turf layer made of sciaphilic algae, a mid-canopy layer of arborescent and laminar algae, and an upper-canopy layer formed by *Cystoseira s.l.* fronds and their epiphytes [6]. Due to their structural features, *Cystoseira s.l.* canopies are inhabited by a number of different species belonging to several phyla, among which other algae, crustaceans, molluscs, and polychaetes dominate [7,8]. In Mediterranean shallow rocky bottoms, these macroalgal stands also harbour a very high fish species richness compared to other habitat types, being important nurseries for many species of commercial interest [2,9], and the ecological importance of *Cystoseira s.l.* species is recognized in several protocols for the protection of marine biodiversity, such as the SPAMI Protocol of the Barcelona Convention [10]. Indeed, being able to highlight environmental degradation [11], *Cystoseira s.l.* are recognized as privileged descriptors of the ecological status of Mediterranean coastal marine communities [12].

Cystoseira s.l. forests are experiencing widespread decline due to the detrimental effects of different anthropogenic stressors such as pollution, eutrophication, overfishing and climate change [13-20] which, in densely populated coastal zones, could combine with direct physical disturbance from human trampling [21] and/or destructive fishing practices [22]. As a result of cumulative human pressure, *Cystoseira s.l.* canopies may be subjected to the reduction of cover and biomass, or local extinctions, being replaced by less structured and opportunistic algal species, like turf-forming algae, mussel beds or sea urchin barrens, leading to an overall simplification of the architectural and functional complexity of the habitat [23-25]. Protecting these canopy-forming species is, therefore, a priority for the management of coastal ecosystems, as their decline may trigger cascading effects leading to loss of biodiversity and ecosystem functions and services [26,27].

Marine Protected Areas (MPAs), and especially no-take zones, can be effective tools for conservation of macroalgal forests and the associated marine biodiversity. As first, MPAs could promote the persistence of large brown algae by ensuring their protection from different types of physical human disturbances (e.g., coastal development, destructive fishing) that can directly affect these species [28-30]. Moreover, the reduced level of cumulative human disturbance within their boundaries could confer to protected communities a greater resistance to environmental stress with respect to unprotected communities [31]. The role of MPAs in mitigating human pressure, nevertheless, may also rely on indirect effects of protection that, through the stabilization of community-wide regulative processes such as, for instance, top-down control of predator fish on grazers [32-34], may increase the overall abundance and stability of macroalgal stands and associated invertebrate assemblages [35,36]. However, evidence from several Mediterranean MPAs does not seem to support a consistent correlation among increased abundance of predator fish, reduced population size of the main grazers (i.e., sea urchins) and increased stands of canopy-forming brown algae in subtidal habitats [34], and idiosyncratic effects of protection on intertidal *Cystoseira s.l.* canopies were also found [29,37,38].

The proportion of *Cystoseira s.l.* populations under different conservation regimes (including MPAs, marine parks, Natura 2000 sites, etc.) in the NW Mediterranean Sea has largely increased in the recent years [25], but is still far from being clearly ascertained whether current protection strategies within MPAs are effective for the conservation and management of these habitat formers and their understory assemblages. In this study, we compared the canopy of *Cystoseira s.l.* between the no-take zone and control sites under less restrictive protection measures in two Mediterranean MPAs to test the effects of full protection on the biomass of canopies, and of the two main components of the associated understory assemblages, namely macroalgae and invertebrates.

Study area and sampling design

The experiment was carried out in two MPAs, the Portofino MPA (Ligurian Sea) and the Isole Ciclopi MPA (NW Ionian Sea) (Figure 1). The Portofino MPA (PFN) extends over 346 ha and was established in 1998, whereas the Isole Ciclopi MPA (CIC) is larger (623 ha) and older, dating back to 1989. The two MPAs have a single no-take, no-access zone (18 and 35 ha for PFN and CIC, respectively) enclosed within a buffer area comprising zones of general and partial protection, where fishing and recreational human activities (e.g., diving, boating) are allowed.

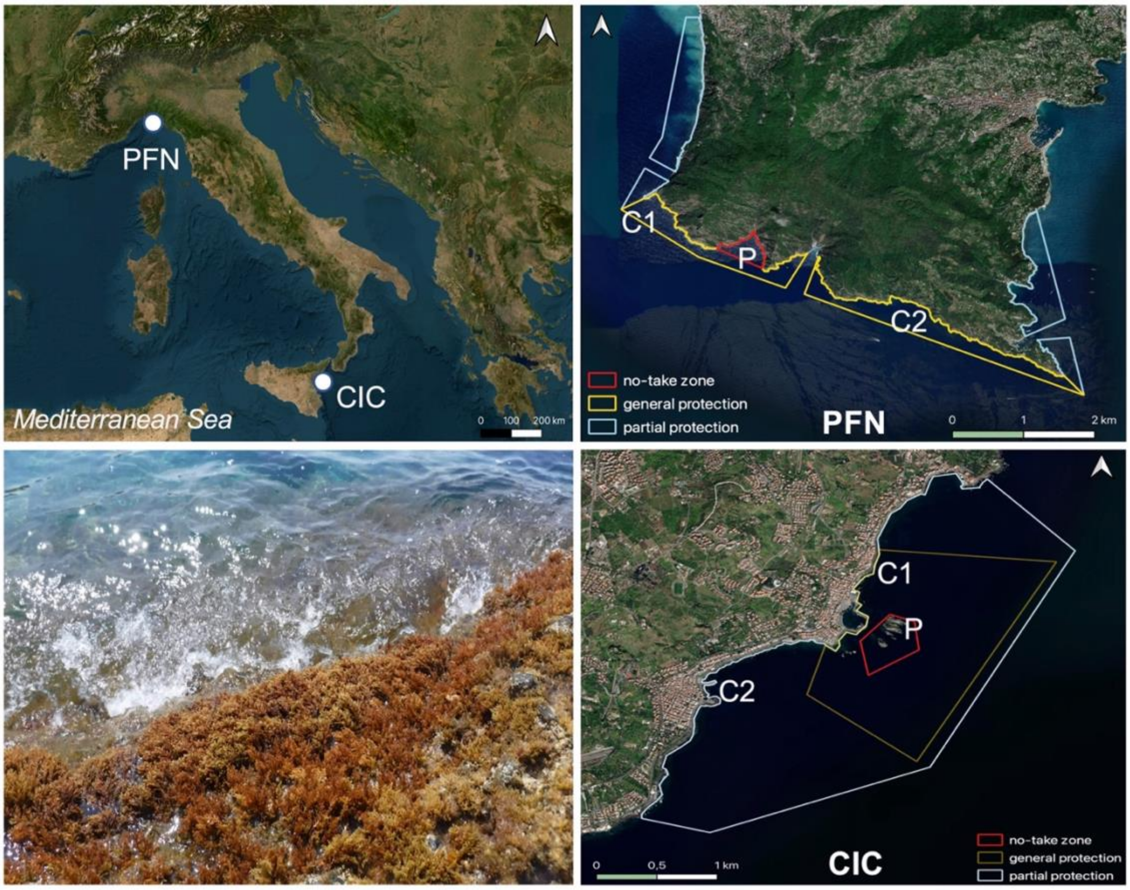

Figure 1. Study areas (the MPAs of Portofino, PFN, and Isole Ciclopi, CIC) and sampling sites. P = no-take, no-access area; C1 = control site 1; C2 = control site 2. Limits of zones under different protection regimes within the MPAs were also reported. Example of *E. amentacea* midlittoral fringe at PFN (bottom left corner).

In each MPA, *Ericaria amentacea* (C. Agardh) Molinari & Guiry 2020 canopies and associated assemblages of the intertidal fringe were sampled on sub horizontal rocky reefs at one site within the no-take, no-access zone (hereafter referred to as the protected site, P) and in two control sites (i.e., C1 and C2, collectively referred to as Cs) where most of human activities are allowed (Figure 1). Cs were characterized by environmental conditions comparable to those at P in terms of type and slope of rocky substrate, exposure to wave actions, currents and prevailing winds. In each site, five random samples of sessile assemblages were collected on two sampling occasions and, namely, October 2020 and July 2021. Sampling consisted of the complete removal of the *E. amentacea* thalli and the associated understory assemblage from the rocky substrate on a surface of approximately 160 cm^2 for each sampling unit. Samples were collected with the help of a PVC cylinder placed upon the canopy and an inox blade, which was slid under the cylinder in order to cut the *E. amentacea* thalli, to scrape off all the other organisms, and to keep everything in the cylinder. A plastic bag was placed on the top of the cylinder to prevent loss of scraped material and vagile species. Care was taken to collect all scraped organisms from the substrate. Samples were preserved in formaldehyde (4%) and sea water solution and brought back to the laboratory for subsequent processing.

Samples were sieved with 1 mm mesh and soaked in sea water for 24 hours to remove formaldehyde residues and were then sorted by separating organisms from inorganic debris and by detaching epiphytes (algae and invertebrates) from the *E. amentacea* thalli. Macroalgae (except *E. amentacea*) were grouped altogether, whereas invertebrates were classified into main taxonomic groups (phylum or class). The biomass of all groups of organisms was estimated as dry weight after desiccation at 80°C for 48 hours.

Statistical analysis

Analysis of Variance (ANOVA) was used to test for differences in the total biomass of *E. amentacea*, associated macroalgae and invertebrates between the protected site P and Cs. The design for the analysis was the same for both MPAs, and included two factors: Time (Ti), random, with two levels (T1, October 2020, and T2, July 2021), and Site [Si], with three levels, one protected site and two controls, with $n = 5$ replicates. The design for the analysis was asymmetrical [39] as it involved a single protected site to be compared against two control sites. The Site term was, therefore, partitioned into two portions: the 1-d.f. fixed contrast of P versus Cs (P-vs-Cs) and the variability between Cs. This partitioning was applied to the other term in the analysis that includes the factor Site, that is the Ti × Si interaction. Finally, the residual variation was partitioned in two portions, Res P and Res Cs, which is the residual variability of observations at P and Cs respectively. The assumption of normality of the response variables was tested with the

Shapiro–Wilk test. Cochran's *C*-test [39] was used to test the assumption of homogeneity of variances prior to analysis and data were log(x+1) transformed to stabilize variance if required. For all response variables, the assumptions of normal distribution and variance homogeneity were met using raw data (or after data transformation), except macroalgae biomass at PFN. In this last case, non-normality and variance heterogeneity persisted after transformation. Although ANOVA is robust to slight deviations from non-normality, heterogeneous variances may increase the rate of Type I error and, thus, results of tests for this variable were interpreted using a more conservative significance level of $\alpha = 0.01$ [39].

Distance-based permutational multivariate analysis of variance (PERMANOVA) [40] was used to test for differences between P and Cs in the structure of invertebrate assemblages associated with *E. amentacea* canopies following the same design as for ANOVA. The analysis was based on Bray-Curtis dissimilarities of untransformed data and each term in the analysis was tested with 5000 random permutations.

A canonical analysis of principal coordinates (CAP) [41,42] was performed for the Ti × Si interaction term, to depict temporal and spatial patterns of variation in invertebrate assemblages. Invertebrate taxa that might be responsible for any group differences seen in the CAP plot were investigated by calculating the product moment correlations of original variables (taxa) with canonical axes [42]. Correlations of individual variables with the two canonical axes (r_1 and r_2) were represented as lines in a projection biplot. Taxa were included in the biplot only if exceeding an arbitrarily chosen value of correlation ($= \sqrt{r_1^2 + r_2^2} \geq 0.3$).

Results

Portofino MPA

The total biomass of *E. amentacea* did not differ between P and Cs at PFN (Table 1). Visual inspection of the graph in Figure 2a suggested higher biomass of *E. amentacea* in T2, although ANOVA did not detect significant temporal variations (Table 1).

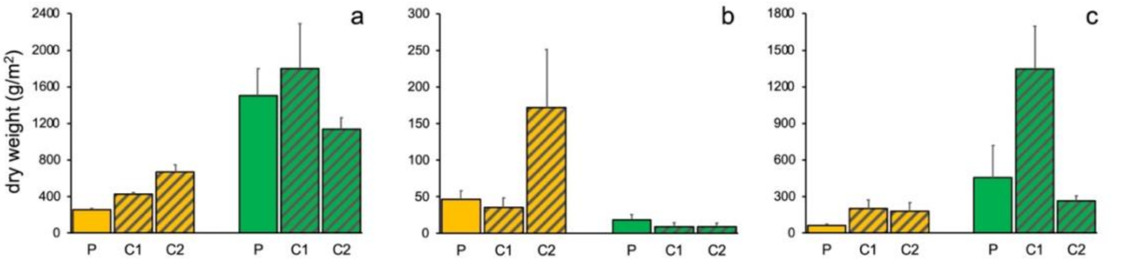

Figure 2. Mean ±SE ($n = 5$) total biomass (dry weight) of (a) *E. amentacea*, (b) macroalgae, and (c) invertebrates at the fully protected site (P) and the two control sites (C1 and C2, dashed bars) of PFN in each sampling time. Time 1 (T1) = orange bars, Time 2 (T2) = green bars. Note that scales on y-axis are different.

Table 1. Summary of asymmetrical ANOVA testing for differences between *P* and *Cs* in total biomass (dry weight) of *E. amentacea*, associated macroalgae, and invertebrates at PFN. The main effect of terms involved in higher-order interactions were not tested. Results of tests for the assumption of normality (Shapiro-Wilk test) and variance heterogeneity (Cochran's *C*-test) were also reported: NS = not significant, * = P < 0.05, *** = P < 0.001.

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		E. amentacea			Macroalgae			Invertebrates		
Source	d.f.	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Ti	1	1957.60	13.205	0.0681	9.64	2.543	0.2518	537.51	2.931	
Si	2	40.62	0.274	0.7849	3.34	0.880	0.5320	235.66	1.285	
P-v-Cs	1	27.4	0.182	0.7109	0.92	0.674	0.4199	95.94	0.523	0.5447
Cs	1	54.21	0.215	0.7238	5.75	1.001	0.4998	375.38	1.086	0.4869
Ti × Si	2	148.24	2.066	0.1487	3.79	2.764	0.0831	183.42	4.322	**0.0249**
Ti × P-v-Cs	1	43.90	0.612	0.4418	1.84	1.343	0.2579	21.01	0.061	0.8462
Ti × Cs	1	252.58	3.131	0.0959	5.74	2.867	0.1098	345.82	8.331	**0.0107**
Res	24	71.76			1.37			42.44		
Res P	8	56.94			0.11			44.30		
Res Cs	16	80.67			2.00			41.51		
Transformation			log(x+1)			log(x+1)			None	
Shapiro-Wilk test			$W = 0.967^{NS}$			$W = 0.913*$			$W = 0.969^{NS}$	
Cochran's *C*-test			$C = 0.443^{NS}$			$C = 0.753***$			$C = 0.330^{NS}$	

For macroalgae of the understory, values of total biomass were similar between times of sampling and sites, except for the high value recorded in T1 at C2 (Figure 2b), and no significant spatial and temporal variations were detected, nor did the biomass of macroalgae significantly differ between P and Cs (Table 1).

Significant spatio-temporal variations, instead, characterized the total biomass of invertebrates (significant Ti × Si interaction, Table 1), which showed a general increase

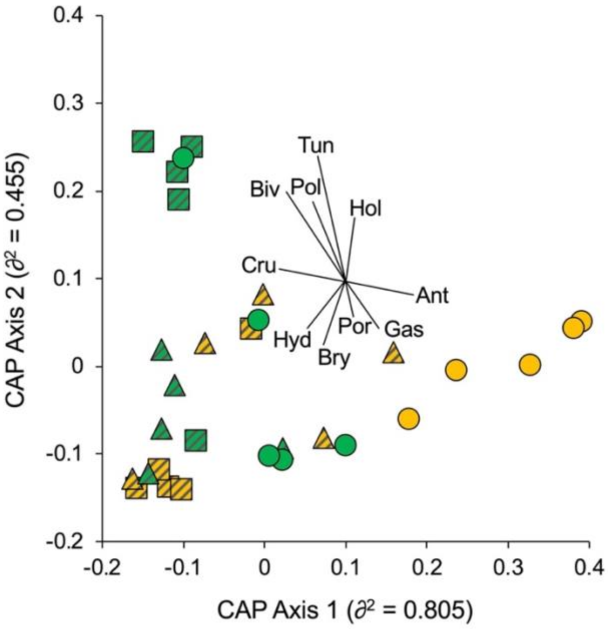

Figure 3. Canonical analysis of principal coordinates (CAP) for the factor Ti × Si based on the distance matrix among sampling units for total biomass of invertebrates at PFN. Squares (dashed) = control site 1 (C1), triangles (dashed) = control site 2 (C2), circles = no-take zone (P). Orange symbols = Time 1 (T1), green symbols = Time 2 (T2).

in T2, though not consistent among sites (Figure 2c). However, the biomass of this component of the understory assemblage did not differ between P versus Cs (Table 1). PERMANOVA did not detect significant differences between P and Cs in the multivariate structure of the understory invertebrate assemblages at PFN (Table 3). Assemblages significantly varied over time and among sites (significant Ti × Si interaction, Table 3). Patterns of variation were portrayed in the CAP plot (Figure 3), showing that the invertebrate assemblages at C1 were mostly characterized by bivalves (Biv), polychaetes (Pol), colonial tunicates (Tun) and holothurians (Hol) in T2, whereas the remaining taxa characterized the assemblages at this site in T1, and at P and C2 in all sampling times.

Isole Ciclopi MPA

No differences in the total biomass of *E. amentacea* between P and Cs were detected at CIC (Table 2). The total biomass of *E. amentacea* inconsistently varied among sites and between sampling times (significant Ti × Si interaction, Table 2). However, as for PFN, a general increase of biomass can be observed in T2 (Figure 4a).

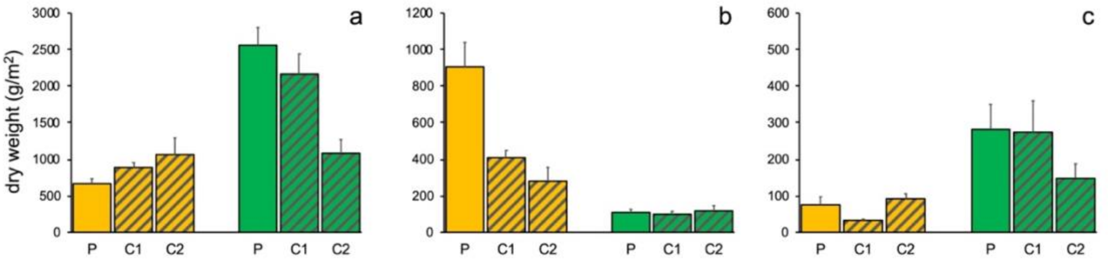

Figure 4. Mean ±SE ($n = 5$) total biomass (dry weight) of (a) *E. amentacea*, (b) macroalgae, and (c) invertebrates at the fully protected site (P) and the two control sites (C1 and C2, dashed bars) of CIC in each sampling time. Time 1 (T1) = orange bars, Time 2 (T2) = green bars. Note that scales on y-axis are different.

Table 2. Summary of asymmetrical ANOVA testing for differences between P and Cs in total biomass (dry weight) of *E. amentacea*, associated macroalgae, and invertebrates at CIC. The main effect of terms involved in higher-order interactions were not tested. Results of tests for the assumption of normality (Shapiro-Wilk test) and variance heterogeneity (Cochran's *C*-test) were also reported: NS = not significant.

		E. amentacea			Macroalgae			Invertebrates		
Source	d.f.	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Ti	1	2091.30	3.715		330.61	4.873		52.24	8.712	0.0982
Si	2	207.41	0.368		66.84	0.985		2.16	0.359	0.7356
P-v-Cs	1	161.37	0.287	0.6459	129.91	1.915		2.92	0.486	0.5577
Cs	1	253.46	0.519	0.6025	3.76	0.568		1.39	0.132	0.7784
Ti × Si	2	562.88	11.906	**0.0003**	67.85	12.517	**0.0002**	6.00	2.052	0.1505
Ti × P-v-Cs	1	637.72	1.307	0.4576	129.07	19.493	**0.0002**	1.40	0.480	0.4952
Ti × Cs	1	488.08	9.455	**0.0073**	6.62	2.571	0.1284	10.59	3.760	0.0703
Res	24	47.28			5.42			2.92		
Res P	8	38.60			11.11			3.14		
Res Cs	16	51.62			2.58			2.82		
Transformation			None			log(x+1)			log(x+1)	
Shapiro-Wilk test			$W = 0.955^{NS}$			$W = 0.989^{NS}$			$W = 0.981^{NS}$	
Cochran's *C*-test			$C = 0.329^{NS}$			$C = 0.452^{NS}$			$C = 0.306^{NS}$	

The total biomass of macroalgae associated with *E. amentacea* canopies significantly differed between P and Cs, although this pattern was not consistent between the two sampling times (significant Ti × P-v-Cs interaction, Table 2); at CIC, the biomass of macroalgae was significantly higher at P than at Cs in T1, whereas their total biomass was comparable among sites in T2, irrespective of protection level (Figure 4b).

No effects of full protection were detected for invertebrate biomass, and there were no statistically significant spatial and temporal variations (Table 2). A general increase in their biomass, nevertheless, seemed to characterize the understory assemblages in T2 (Figure 4c). The average multivariate structure of invertebrate assemblages significantly varied between times and among sites (significant Ti × Si interaction), but no significant differences imputable to full protection were detected (Table 3). The CAP plot for CIC (Figure 5) showed that invertebrate assemblages in T1 were dominated by hydroids (Hyd) at P and C2, whereas sponges (Por) and bryozoans (Bry) characterized C1. In T2, invertebrate assemblages were quite distinct, showing an increase in biomass of different sessile taxa (bivalves [Biv], tunicates [Tun], and anthozoans [Ant]) and of vagile organisms (crustaceans [Cru], holothurians [Hol], polychaetes [Pol]) in all sites (Figure 5).

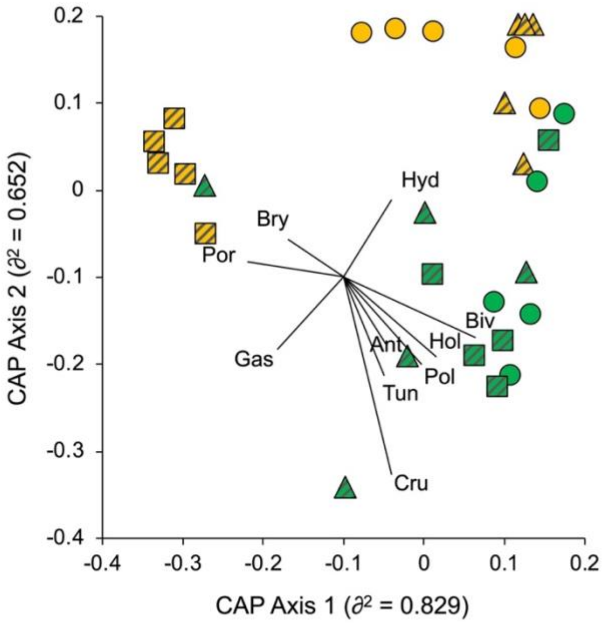

Figure 5. Canonical analysis of principal coordinates (CAP) for the factor Ti □ Si based on the distance matrix among sampling units for total biomass of invertebrates at CIC. Squares (dashed) = control site 1 (C1), triangles (dashed) = control site 2 (C2), circles = no-take zone (P). Orange symbols = Time 1 (T1), green symbols = Time 2 (T2).

Table 3. Results of asymmetrical PERMANOVA testing for differences between P and Cs in biomass of invertebrate assemblages associated to *E. amentacea* in both the investigated MPAs (PFN and CIC). The analysis is based on Bray–Curtis dissimilarities (untransformed data) and each test was performed using 5000 random permutations. Significant tests are given in bold. Denominators for tests (MS_{DEN}) were also indicated.

		PFN			CIC			
Source	d.f.	MS	Pseudo-*F*	*P*(perm)	MS	Pseudo-*F*	*P*(perm)	MS_{DEN}
Ti	1	6962.60	1.640		14908.00	2.444		
Si	2	6715.40	1.582		3396.40	0.557		
P-v-Cs	1	9595.80	2.261	*0.1694*	3382.10	0.554	*0.6978*	Ti × Si
Cs	1	3835.20	0.650	0.6415	3410.70	0.322	0.8175	Ti × Cs
Ti × Si	2	4244.40	2.605	**0.0168**	6101.00	6.551	**0.0002**	Res

Ti × P-v-Cs	1	2592.10	0.440	0.7678	1623.00	0.153	0.9332	Ti × Cs
Ti × Cs	1	5896.70	3.417	**0.0158**	10579.00	11.109	**0.0002**	Res Cs
Res	24	1629.50			931.34			
Res P	8	1437.25			889.38			
Res Cs	16	1725.60			952.29			

Discussion

Assessing the effectiveness of MPAs is crucial to ensure the achievement of conservation goals and to refine conservation strategies for an adaptive management of protected communities in the face of changing environmental conditions [43,44]. In most cases, assessments of the ecological effects of MPAs, and particularly of full protection in no-take zones, have focused on fish documenting increased assemblage diversity, abundance and biomass of protected populations with respect to unprotected areas [45-49]. Macrobenthic assemblages received comparatively less attention, except for commercial or charismatic invertebrate species [e.g., 50-52], and often responded to protection inconsistently [e.g., 29,37]. Moreover, if fish assemblages promptly respond to protection [e.g., 53], the effects of protection on macrobenthos can take much more time to become evident. Differently, these effects may occur through an increased temporal stability/resilience of assemblages rather than through changes in their average structure [36,54,55], or the effects can vary in time or concerning only some components of the assemblages [37].

E. amentacea (as all *Cystoseira s.l.* species) naturally exhibits substantial phenological variations in its morphology/biomass and associated understory [56,57]. In winter, only the perennial basal cauloids remain in most species. Later, in spring, new branches sprout and in summer thalli consist of main axes covered by many leaf-like structures. In late summer / autumn, the erect branches are shed and only the cauloid persists in a quiescent state during the following unfavorable, cold season. Such changes reflect on the associated assemblages [58], leading to the proliferation of photophilic algae in the understory during the resting season, alternating with an increase of vagile (e.g., polychaetes, crustaceans, holothurians) and epiphytic (e.g., hydroids) invertebrate abundance during the growing season [56,59]. These natural patterns clearly emerged in both study areas, with an overall average turnover in total biomass (including all assemblage components) between T1 and T2 equal to 1468 ± 346 g m^{-2} and 794 ± 335 g m^{-2}, at PFN and CIC respectively. Yet, for all the investigate variables, temporal variations were not consistent among sites, and changes in biomass of *E. amentacea* canopies, despite being quite evident on average, were not statistically significant. This is probably because *E. amentacea* biomass was not yet completely depleted in T1 (October 2021), as typically occurs during the resting period, thus smoothing out potential variations with respect to T2 (July 2022). Nevertheless, the interest here was not to confirm well-known cyclic patterns of change in *E. amentacea* but, rather, to understand whether the effects of protection on these fucoid algae and their associated assemblages, if any, were consistent through time or affected their temporal patterns of variation.

At PFN, our analysis did not detect significant differences between P and Cs in neither *E. amentacea* nor the associated macroalgae, nor in the structure of invertebrate assemblages of the understory, suggesting no effects of full protection on intertidal assemblages, at least concerning the biomass of the investigated components. It could be argued that control sites fell under a regime of general protection, and that this could have contributed to preserve the integrity of *E. amentacea* stands also at Cs. General and partial regimes of protection, nevertheless, allow several human activities (e.g., recreational uses, tourism), which are generally not strictly regulated, potentially leading to detrimental effects on marine communities [60-62]. Evidence from adjacent areas highlighted that in the last decades the effects of increasing cumulative human pressure have expanded, being likely responsible of detrimental changes in macrobenthos and plankton assemblages [63,64] despite the presence of conservation initiatives, and suggested the difficulty of local protection regimes to face widespread environmental degradation. However, it is worth noting that control sites at PFN, due to the local features of the whole coastline, are not easily accessible to human frequentation. Thus, the lack of significant effects of full protection were most likely due to the incidental reduction (or exclusion) of direct human impacts on *E. amentacea* stands at Cs to levels not significantly different from those experienced in the no-take, no-access zone P. Analogously to what occurred for PFN, no effect of full protection in modifying the biomass of *E. amentacea*, or the total and multivariate biomass of associated invertebrate assemblages, was detected at CIC. This MPA is located along a highly urbanized coastline, with high touristic frequentation during summer and beyond due to the favourable climate. In such an environmental context, the potential effects of protection on macrobenthic assemblages may be strongly reduced, or completely overcome, as a consequence of high anthropization [e.g., 36,63], and the abundance of *E. amentacea* could eventually correlate to gradients of human pressure rather than to the spatial arrangement of protection regimes [38]. In addition, CIC (but also PFN) is a relatively small MPAs, which make it more vulnerable to neighbouring indirect effects of coastal human activities (e.g., land and sea-based pollution, physical disturbance from human attendance). Interestingly, a significant effect of full protection was found for understory macroalgae at CIC, which had a higher biomass in T1 at P than at Cs. Control sites at CIC are easily accessible and, therefore, more exposed to direct effects of human frequentation, and especially to mechanical disturbance of human trampling with respect to P, where human access is forbidden. Since erect macroalgae are more vulnerable to trampling than turf algae [28], high trampling pressure could reduce the abundance of large, erect macroalgae favouring small, filamentous, turf-forming species, thus leading to an overall decrease of biomass in this component of the understory. We found no evidence of a significant decrease of biomass of *E. amentacea* at Cs probably because it is quite resistant to trampling [65], unlike other *Cystoseira s.l.* species [28].

MPAs are the main global strategy to conserve and restore marine biodiversity and associated ecosystem services [66,67]. However, they cannot provide an exhaustive solution to the complexity of human-driven environmental issues affecting marine ecosystems [68,69], often leading to contrasting outcomes depending on reserve features, social and environmental contexts, and ecology and biology of species involved [29,38,70]. Our findings do not support the hypothesis of a significant effect of no-take zones in increasing the biomass of *E. amentacea* stands and associated assemblages with respect to control areas under lower levels of protection, although further investigations to increase the spatial and temporal extents of these assessments are needed. As *Cystoseira s.l.* forests are declining from local to large scale [71,72], these results reinforce concerns that human pressures affecting these habitat formers cannot be effectively mitigated within MPA boundaries [25,61,73-75]. Managing human pressures at a wider spatial scale is crucial to ensure the effectiveness of local-scale conservation initiatives in enhancing the resilience of *Cystoseira s.l.* forests to present and future stressors [17,19,76,77]. Wide gaps in information on fucoid algae in the Mediterranean MPAs also limit our understanding of the actual role of protection in preserving the integrity of these habitat formers [25], which urgently call for major efforts in long-term monitoring of their distribution and ecological status in MPA networks at a basin scale. This would also allow identifying critical cases within MPAs requiring active interventions of restoration, especially when the decline of *Cystoseira s.l.* originate from transient environmental deterioration, and natural recovery may be hampered by hysteresis. Finally, future research needs to provide a deeper insight into the functioning of *Cystoseira s.l.* forests, and the ensuing good and services they provide, in order to optimize conservation investments taking into account their actual contribution to the overall natural capital of coastal ecosystems.

Author Contributions: L.C.: conceptualization, methodology, sampling and field works, sample processing, data curation, formal analysis, writing-original draft, writing-review and editing. S.B.: supervision, conceptualization, methodology, formal analysis, writing-original draft, writing-review and editing. G.A., V.A., M. C., A.P., C.P., I.R.: methodology, sampling and field works, sample processing, writing-review and editing. P.V.: writing-review and editing. A.F.: supervision, conceptualization, methodology, writing-original draft, writing-review and editing.

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CHAPTER 2

Spatial and Temporal Variations in Key Functional Traits of Mediterranean Fucoid Algae: Insights from *Ericaria amentacea* Intertidal Canopies

Submitted, Estuarine coastal and shelf science

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Abstract

Cystoseira sensu lato are fucoid algae that form dense stands on intertidal and subtidal rocky reefs sustaining species-rich associated assemblages. The increasing human pressure is causing the decline of these marine forests, raising wide concerns on the ecological consequences of their loss. Yet, little is known about functional trait variables of *Cystoseira s.l.* species, which are essential to a deeper understanding of their contribution to the functioning of coastal ecosystems. We analysed the intraspecific spatial variations of the total organic matter, C_{org}, N and P contents in *Ericaria amentacea* from intertidal rocky shores in the W Mediterranean Sea. We also explored potential interspecific variations by comparing the combined functional profile of trait variables between *E. amentacea* and *Cystoseira compressa*, an often cooccuring or vicariant species. Tissue contents of organic matter, C_{org}, N and P, were consistent among *E. amentacea* stands, supporting the hypothesis of a large-scale (100s km) spatial homogeneity of such key traits in this species. Overlapping functional profiles between *E. amentacea* and *C. compressa* also suggested putative interspecific trait congruencies. However, a small-scale (100s m) significant variability was found for all trait variables in *E. amentacea*, recommending caution in the use of local-scale trait values to generalize functional performances of *Cystoseira s.l.* forests over large areas. This study represents one of the first attempts to shed light on the variability of key functional attributes of *Cystoseira s.l.* species at a range of spatial scales, which could assist more reliable assessments of their functional features and improve their management and conservation.

Keywords: *Cystoseira sensu lato*, rocky shores, algal forests, biomass, total organic carbon, restoration ecology, biodiversity conservation

Introduction

Intertidal and subtidal forests of brown macroalgae account for about 15% of the global net primary production in coastal ecosystems (Duarte et al., 2022), sustain composite food webs (Graham et al., 2004; Koenigs et al., 2015), confer high structural complexity to coastal habitats and provide nursery and spawning grounds for a number of marine species (Graham, 2008; Chemineé et al., 2013). In the Mediterranean Sea, the main representatives of these macroalgae belong to *Cystoseira sensu lato* species (including the genera *Cystoseira*, *Ericaria*, and *Gongolaria*, Molinari & Guiry (2020)), which colonize rocky bottoms from the intertidal to >50 m depth (Hereu et al., 2008) and whose known distribution in the basin extends over 30% of rocky coasts (Fabbrizzi et al., 2020). *Cystoseira s.l.* are fucoid algae forming dense stands on rocky reefs that, analogously to kelp in cold-temperate marine regions, act as habitat-formers allowing species-rich sessile assemblages to thrive in the understory or as epiphytes, providing feeding grounds and shelter to many vagile invertebrates and fishes within their canopies (Ballesteros et al., 1998; Chemineé et al., 2013; Mineur et al., 2015; Blanfuné et al., 2016; Mancuso et al., 2021).

Due to their ecological relevance, *Cystoseira s.l.* species have been included in several international protection agreements and have become conservation targets in Marine Protected Areas, although this does not seem to have prevented the decline of these important habitat-formers at a basin scale in the Mediterranean Sea (Fabbrizzi et al., 2020; Tamburello et al., 2022). Land-based pollution, increased sediment load, direct physical disturbance and coastal artificialization seriously threaten *Cystoseira s.l.* species, causing local extinctions and large-scale reduction of their populations (Benedetti-Cecchi et al., 2001; Strain et al., 2014; Mancuso et al., 2018; Bevilacqua et al., 2021). In subtidal reefs, *Cystoseira s.l.* populations may also be strongly affected by the indirect effects of overfishing that may lead to severe reduction of the abundance of predator fish of commercial interest (e.g., sparids) and release from predation the main herbivores (e.g., sea urchins) which, in turn, may exert excessive grazing pressure on these macroalgal canopies (Sala et al., 1998; Guidetti, 2006). In addition, the increasing frequency and intensity of thermal anomalies and extreme weather events probably exacerbate the effects of other human pressures by disrupting the reproductive cycles of *Cystoseira s.l.* species and, ultimately, erode the resilience potential of these marine forests (Bevilacqua et al., 2019; Peleg et al., 2020; Falace et al., 2021).

The ongoing reduction of *Cystoseira s.l.* stands across the basin, replaced by less complex algal turfs or barren grounds, has raised wide concerns about potential consequences for marine biodiversity and ecosystem functioning (Sales et al., 2011; Bianchelli & Danovaro, 2020; Peleg et al., 2020), fuelling intense efforts to restore lost populations (e.g., Verdura et al. 2018; De la Fuente et al., 2019; Clausing et al., 2022; Orlando-Bonaca et al., 2022). Despite the current emphasis on the potential depletion of ecosystem functions (e.g., carbon sequestration, nutrient cycling) associated with the decline of coastal marine forests (Krause-Jensen et al., 2018; Filbee-Dexter et al., 2022), little is known on basic functional attributes of *Cystoseira s.l.* species, such as their organic carbon and key nutrients (e.g., nitrogen and phosphorous) contents, and very few attempts have been carried out to fill this gap (e.g., Delgado et al., 1994; Ballesteros & Sant, 2022). Actually, the available data are too sparse and fragmented to allow conservative estimates of trait values for organic matter and contents of key chemical elements for most *Cystoseira s.l.* species. For example, one of the largest databases on biomass and biochemistry of aquatic organisms, provided by Brey et al. (2010), reports in total only 22 single data entries for carbon, nitrogen and phosphorous contents for the 15 *Cystoseira s.l.* species listed. A major issue in this respect is that possible spatial and temporal intra- and interspecific variations of such trait values remain largely unexplored, thus increasing the uncertainty about their potential use for functional assessments into wide ecological and geographic contexts.

Cystoseira s.l. canopies, especially in rocky intertidal habitats, experience a wide range of natural (e.g., air exposure, UV radiation, temperature fluctuations) and human-driven (e.g., nutrient enrichment, trampling) environmental stresses that may affect their metabolism and, thus, their organic components (AbouAisha et al., 1997; Milazzo et al., 2004; Nygard & Dring, 2008; Mancuso et al., 2019). As these perennial and semi-perennial brown macroalgae generally alternate between vegetative and dormant phases, nutrient uptake and energy contents may vary according to changes in phenology and/or seasonal fluctuations (Delgado et al.,1994; Celis-Pla et al., 2016). Understanding whether environmental changes turn into significant spatial and temporal variability in biomass, contents of carbon and nutrients (e.g., nitrogen and phosphorous) in *Cystoseira s.l.*, and identifying the relevant scales of variations, is essential for accurate quantifications of the contribution of *Cystoseira s.l.* stands to the overall functioning of coastal ecosystems in terms of matter and energy fluxes.

In this study, we analysed the intraspecific spatial, from local (1-2 km) to large-scale (100s of km), of key functional trait variables of *Ericaria amentacea* (C. Agardh) Molinari & Guiry canopies from intertidal rocky reefs in the W Mediterranean Sea, and specifically the contents of total organic matter, organic carbon (C_{org}), nitrogen (N), and phosphorous (P) per unit of dry mass. We also explored possible interspecific variations in these trait variables by comparing the combined functional trait profile of total C_{org}, N and P between *E. amentacea* and another often co-occurring *Cystoseira s.l.* species, *Cystoseira compressa* (Esper) Gerloff & Nizamuddin.

Materials and methods

Study area and sampling design

Patterns of spatial variations in contents of total organic matter, C_{org}, N and P per unit of dry mass were assessed by sampling *E. amentacea* populations at two locations, the Portofino Marine Protected Area (PFN, Ligurian Sea) and the Isole Ciclopi Marine Protected Area (CIC, South Ionian Sea) (Figure 1).

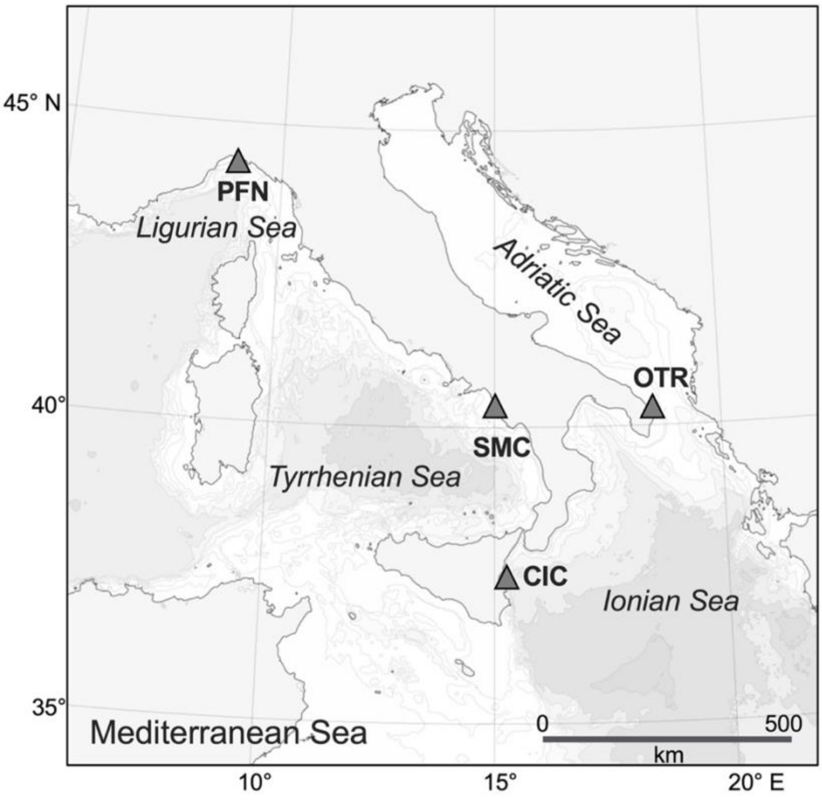

Figure 1. Study area and sampling locations. PFN: Portofino Marine Protected Area; CIC: Isole Ciclopi Marine Protected Area; SMC: Santa Maria di Castellabate Marine Protected Area; OTR: European Special Area of Conservation of Otranto. PFN and CIC were the two main study locations, where data on the biomass, total C_{org}, N and P contents of *E. amentacea* were collected on two times of sampling and in multiple sites within locations. Additional

data on total C_{org}, N and P contents were also collected in a single site and a single time of sampling at PFN and OTR for *C. compressa*, and at SMC for *E. amentacea*. See text and Table S1 in supplementary material for further details on sampling.

Sampling locations were selected within Marine Protected Areas (MPAs) to reduce as much as possible the potential influence of human activities on the investigated variables. At each location, samples were collected within *E. amentacea* stands (total substrate cover >80%) on sub-horizontal rocky reefs in the intertidal fringe (0-1 m depth) at three sites (1-2 km apart) which were characterized by comparable environmental conditions representative of each location in terms of type and slope of rocky substrate, wave exposure, currents and prevailing winds. At each site, five random replicate samples (a few meters apart) were collected.

Total organic matter content

For total organic matter contents, samples were collected following the sampling design described in section 2.1. Sampling consisted of the complete removal of the *E. amentacea* thalli from the rocky substrate on a surface of approximately 160 cm^2 for each replicate. Samples were collected using a PVC cylinder placed upon the canopy and an inox blade which was slid under the cylinder in order to cut off all *E. amentacea* thalli in the sampling unit. Samples were then preserved in formaldehyde (4%) and sea water solution and stored under cold and dark conditions, until biomass assessment. A first sampling was carried out in October 2020 (T1), when algae were in the dormant phase. The hypothesis was that the total organic matter content of *E. amentacea* may not vary even across distant (~1000 km) locations from different biogeographic regions (i.e., the NW Mediterranean, for PFN, and the Ionian regions for CIC; *sensu* Spalding et al. 2007) when the photosynthetic and metabolic activity of the *Cystoseira s.l.* species is strongly reduced, as occurs during its dormant phase (Sales and Ballesteros, 2012; Celis-Plá et al., 2014a; Kaleb et al., 2023). Sampling was then repeated in July 2021 (T2) to explore spatial patterns of variation in total organic matter content after *E. amentacea* experienced the intense regrowth phase typical of the vegetative period.

Total C_{org}, N, and P contents

A separate set of samples of *E. amentacea* thalli was collected at each site in each location for the analysis of total C_{org}, N, and P contents. However, to limit the damage on macroalgal stands, these samples were collected only in T2 (with the exception of PFN, where samples were taken also in T1 to obtain a more representative range of C_{org}, N, and P contents to use in the construction of the functional profile of *E. amentacea,* see section 2.6), with three replicates per each site. Each sample consisted of one 100 ml centrifuge tube filled with randomly-selected *E. amentacea* thalli (approximately 3-4 thalli per tube) cut off from the basal part to the apex. Samples were stored under cold and dark conditions and immediately brought back to the laboratory for further processing. Finally, in order to investigate C_{org}, N, and P contents of *E. amentacea* from other geographic areas and/or in other *Cystoseira s.l.* species, additional samples were collected in T2 following the sampling protocol described above and, specifically in the Santa Maria di Castellabate MPA (SMC, Tyrrhenian Sea, three samples of *E. amentacea* from one site), in the Portofino MPA (PFN, three samples of *C. compressa* from one site), and in the European Special Area of Conservation of Otranto (OTR, Southern Adriatic Sea, three samples of *C. compressa* from one site). The study areas are shown in Figure 1, whereas sampling details are summarized in the supplementary material (Table S1).

Sample processing

Samples of *E. amentacea* for the analysis of biomass were soaked in sea water for 24 hours to remove formaldehyde residues and were carefully cleaned from epiphytes. *E. amentacea* thalli were wiped up to remove the excess water and weighted altogether to determine the total wet mass (WM) per sample. All thalli in each sample were weighted again after drying at 80°C for 48 hours to obtain the total dry mass (DM). Samples were finally burnt in a muffle at 550° C for 4 hours, the ashes were then weighted, and the total organic matter was determined by subtraction as ash-free dry mass (AFDM). The total organic matter contents were expressed as AFDM per unit of DM ($g \cdot kg^{-1}$).

As soon as possible after field sampling, thalli of *E. amentacea* and *C. compressa* sampled for the analysis of total C_{org}, N and P contents were placed in seawater, carefully cleaned from epiphytes, wiped up and frozen at -20°C to avoid loss of organic compounds. Prior to analysis, samples were first dried in oven at 40°C to constant weight and then lyophilized to completely remove water under low temperature conditions in a freeze-dryer (FD-1A-50, Boyikang Laboratory Instruments Ltd.). Dried samples were finally homogenized by accurately cutting lyophilized algal tissues. Total C_{org}, N, and P were determined by acid digestion and chemical titration (UNI EN 13657:2004). Contents were expressed in grams per unit of DM (g · kg^{-1}). Limit of quantifications were 0.01 mg · g^{-1} for total N and P, and 0.1 mg · g^{-1} for total C_{org}.

Univariate statistical analysis

Analysis of Variance (ANOVA) was performed to test for spatial variations in total organic matter contents of *E. amentacea* samples separately for T1 and T2. The design for the analysis consisted of two factors: Location (Lo, random, with two levels, PFN and CIC), and Site (Si(Lo), random and nested in Lo, with three levels), with $n = 5$. The whole set of data on *E. amentacea* biomass (i.e., including samples from PFN and CIC collected in T1 and T2) was used to quantify weight-to-weight conversion factors of *E. amentacea*. Specifically, data were used to convert WM to DM and DM to AFDM. Conversion factors were estimated as the coefficients of a linear regression ($y = ax$) fitted to DM against WM and AFDM against DM, respectively. Pairwise Pearson's product-moment correlations (r) were also calculated.

ANOVA was performed for total C_{org}, N and P contents of *E. amentacea* in order to test for potential spatial variations of these variables. The design for the ANOVA included factors Location (Lo, random, with two levels, PFN and CIC) and Site (Si(Lo), random and nested in Lo, with three levels), with $n = 3$. For this analysis, only sample collected in T2 (which fell in the typical period of maximum growth of the algae) from PFN and CIC were used, as spatial replication in T1 and in other locations prevented any formal comparison. C_{org}, N, and P contents of *Cystoseira s.l.* from these additional sites were used for the functional analysis described in section 2.6.

The assumption of normality of all response variables was tested with the Shapiro–Wilk test and the Cochran's *C*-test was used to test the assumption of homogeneity of variances prior to analysis. In all cases the assumptions of normality and variance homogeneity were respected, except for data on P contents, which were forth root transformed to stabilize variances (Underwood, 1997).

Comparison of functional profiles

To compare the functional trait profile of total C_{org}, N and P between *E. amentacea* and *C. compressa* from single additional sites and *E. amentacea* from PFN and CIC, a functional hypervolume of total C_{org}, N, and P trait variables was constructed for *E. amentacea* following the approach proposed by Blonder et al. (2014). This procedure allows inferring the shape and volume of an *n*-dimensional real-valued continuous space that encloses a set of *m* observations through kernel density estimations (Blonder et al., 2014; 2017), and can be applied to model species distribution, traits and niche hyperspace. Here, we employed this approach to infer the combined functional range of total C_{org}, N, and P contents for *E. amentacea*. All samples from the two locations were used for the analysis ($m = 27$), and the obtained hypervolume was assumed to be representative of *E. amentacea* in the whole study region. The hypervolume was inferred based on three dimensions (i.e., total C_{org}, N, and P) using a Gaussian kernel density estimate with a bandwidth defined through the Silverman estimator, a quantile threshold of 0.95, and 1999 random replicate samples per point (Blonder et al., 2022). No normalization of data was applied prior to analysis as the three dimensions represented homogeneous variables (i.e., contents of chemical elements per unit of DM) expressed in the same units (i.e., $g \cdot kg^{-1}$). We used the hypervolume to (1) check if the functional range of total C_{org}, N, and P contents of *E. amentacea* can actually be representative of the whole study region by testing whether an independent set of samples of *E. amentacea* collected at SMC (Tyrrhenian Sea) fit the hypervolume. We also (2) checked for homogeneity of C_{org}, N, and P contents between *C. compressa* and *E. amentacea* by testing whether the set of samples of *C. compressa* from PFN and OTR fit the hypervolume of *E. amentacea*. This was done using the hypervolume inclusion test (Blonder et al., 2022), which determines if a set of points (representative of a set of samples) fall within a hypervolume with a priori fixed probability threshold. The probability threshold below which the points can be considered to be outside the hypervolume (and thus not congruent with the *n*-dimensional functional range of *E. amentacea*) was fixed to 0.5. All analyses were done in R version 4.2.2 (R Development Core Team 2022) using the packages “GAD” (Sandrini-Neto & Camargo, 2022), “vegan” (Oksanen et al., 2022) and “hypervolume” (Blonder et al., 2022).

Results

ANOVA detected significant variations in the total organic matter contents of *E. amentacea* in T1 at both the investigated spatial scales of sites (1000s m) and locations (100s km), although in the latter case at a borderline statistical significance (Table 1, Figure 2). In T2, while ANOVA detected again a significant variability at the scale of sites, no differences in the total organic matter contents were detected between the two locations (Table 2, Figure 2).

Table 1. Results of ANOVA testing for spatial variations in the total organic matter content per unit of dry mass of *E. amentacea* between PFN and CIC in T1 (October 2020). Results of tests for normality and variance homogeneity are also reported.

Source	d.f.	SS	MS	*F*	*P*
Lo	1	176050.0	176050.0	8.209	0.0457
Si(Lo)	4	85787.0	21447.0	20.469	0.0000
Res	24	25146.0	1047.7		

Data transformation None
Shapiro–Wilk test $W = 0.952^{NS}$
Cochran's *C*-test $C = 0.314^{NS}$

Table 2. Results of ANOVA testing for spatial variations in the total organic matter content per unit of dry mass of *E. amentacea* between PFN and CIC in T2 (July 2021). Results of tests for normality and variance homogeneity are also reported.

Source	d.f.	SS	MS	*F*	*P*
Lo	1	737.6	737.6	0.100	0.7676
Si(Lo)	4	29480.0	7369.9	3.029	0.0373
Res	24	58396.0	2433.2		

Data transformation None
Shapiro–Wilk test $W = 0.959^{NS}$
Cochran's *C*-test $C = 0.452^{NS}$

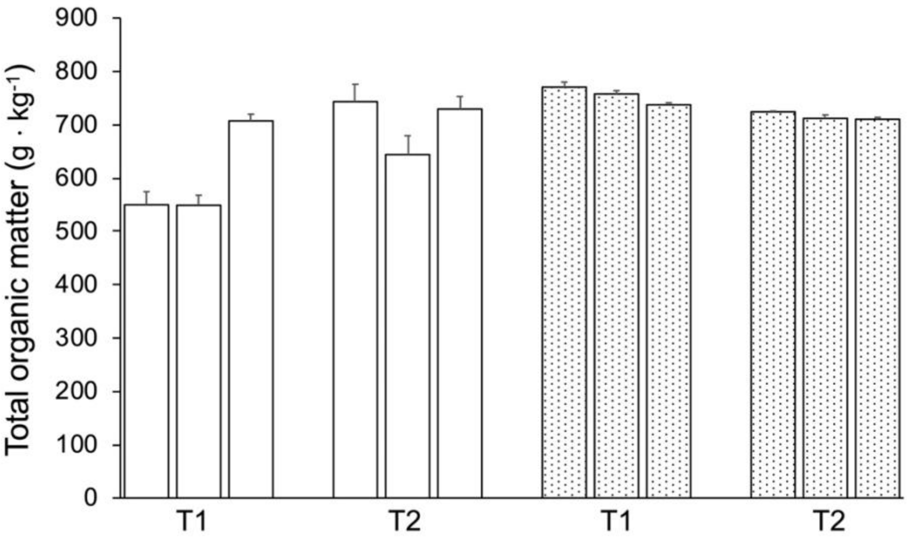

Figure 2. Mean (±SE, *n* = 5) content of total organic matter (expressed as grams of ash-free dry mass per kilogram of dry mass) of *E. amentacea* in site and times of sampling (T1, October 2020; T2, July 2021) at PFN (blank bars) and CIC (dotted bars).

Dry mass (DM) of *E. amentacea* was strongly correlated to its wet mass (WM) ($r = 0.94$, $P<0.001$) following a linear relationship (Figure 3), with an estimated weight-to weight conversion factor of 0.16 ± 0.01 (Table 3). Similarly, ash-free dry mass (AFDM) was in turn strongly correlated to DM ($r = 0.99$, $P<0.001$) following a linear relationship (Figure 3), with an estimated weight-to weight conversion factor of 0.71 ± 0.01 (Table 3).

Table 3. Weight-to-weight conversion factors of wet mass to dry mass (WM/DM) and dry mass to ash-free dry mass (DM/AFDM) estimated as coefficients of linear regression models (a summary of results of regression analysis is reported) fitted to the whole dataset (*n* = 60).

Conversion	Coefficient	SE	±95%CI	Adj. R^2	*P*
WM / DM	0.16	0.005	0.169–0.151	0.941	>0.001
DM / AFDM	0.71	0.006	0.726–0.701	0.977	>0.001

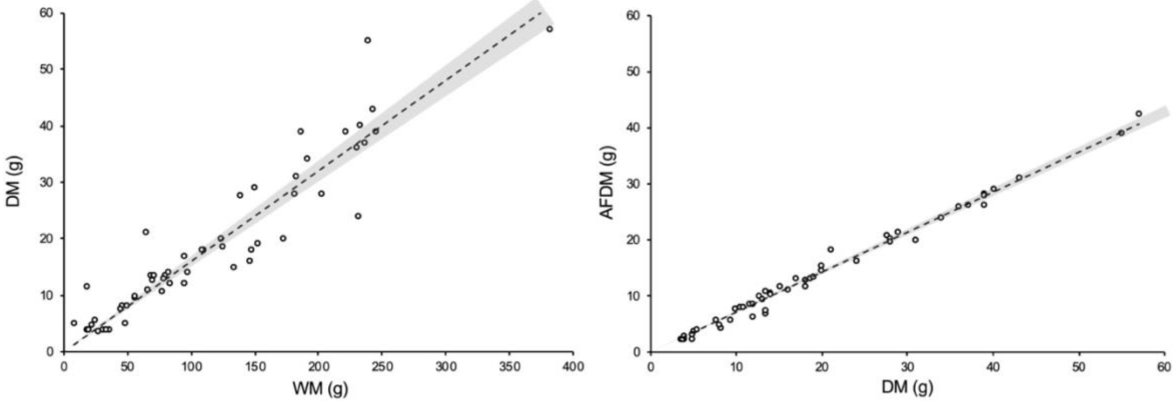

Figure 3. Linear models of dry mass (DM) against wet mass (WM) (left panel) and ash-free dry mass (AFDM) against DM (right panel) of *E. amentacea*. Shaded grey bands represent the ±95% CI of the linear model fitted to the data (*n* = 60). Results of linear regression analysis were reported in Table 3.

ANOVA detected a significant variability among sites within locations in total C_{org}, N and P contents of *E. amentacea*. However, differences among locations were not significant for all the considered variables (Table 4, Figure 4).

Table 4. Summary of ANOVAs testing for spatial variations in the total C_{org}, N and P contents of *E. amentacea* between PFN and CIC (T2 only). Results of tests for normality and variance homogeneity are also reported. ** = *P*<0.01; *** = *P*<0.001; NS = not significant.

		C_{org}		N		P	
Source	d.f.	MS	*F*	MS	*F*	MS	*F*
Lo	1	15129.0	5.413^{NS}	0.2402	0.168^{NS}	0.0025	0.547^{NS}
Si(Lo)	4	2795.0	10.062^{***}	1.4255	7.842^{**}	0.0032	7.548^{**}
Res	12	277.8		0.1818		0.0004	
Data transformation		None		None		√√ root	
Shapiro–Wilk test		$W = 0.963^{NS}$		$W = 0.913^{NS}$		$W = 0.902^{NS}$	
Cochran's *C*-test		$C = 0.352^{NS}$		$C = 0.275^{NS}$		$C = 0.297^{NS}$	

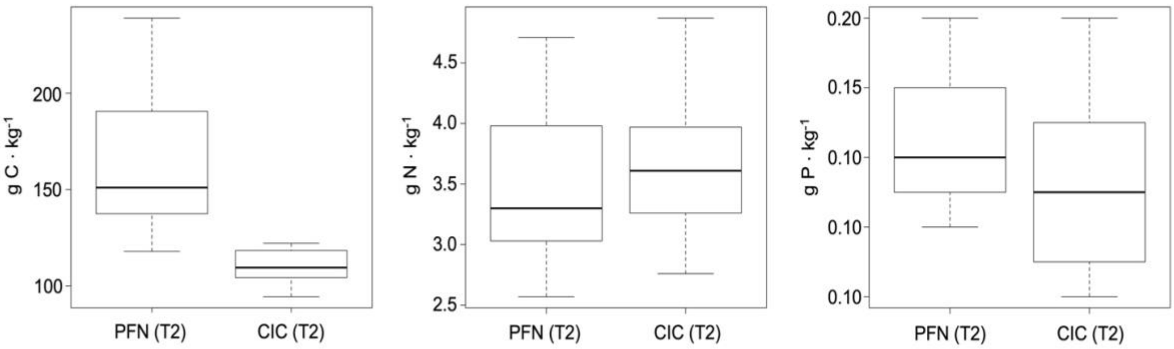

Figure 4. Box plots of total C_{org}, N and P values in *E. amentacea* at PFN and CIC (T2 only), expressed as grams per kilograms of dry mass. The black central line indicates the median, boxes indicate the 2nd and the 3nd quartile, and whiskers correspond to the 1nd and 4nd quartile.

Average values of C_{org}, N and P contents of *E. amentacea* from PFN and CIC were quite similar to those of the *E. amentacea* samples from SMC and the *C. compressa* samples from OTR, while *C. compressa* samples from PFN seemed to have lower C_{org}, N and P contents than the other locations (Table 5).

The three independent samples of *E. amentacea* from SMC fell within the hypervolume with an average probability of inclusion of 73%, indicating that the tested samples were highly compatible with the functional space of C_{org}, N and P contents inferred based on pooed data of *E. amentacea* from PFN and CIC. The same occurred for samples of *C. compressa* from PFN and OTR, which showed, respectively, an average probability of 75% and 70% to belong to the inferred hypervolume. See Figure 5 for a graphical comparison of the hypervolume of *E. amentacea* and test samples.

Table 5. Mean (±SE) total C_{org}, N and P contents (g · kg^{-1} of dry mass) of *E. amentacea* and *C. compressa* in the study locations. T1 = October 2020, T2 = July 2021.

Location (Time)	Species	C_{org}	N	P	Total no. of samples
PFN-CIC (T1-T2)	*E. amentacea*	134.42 (±14.71)	4.02 (±0.40)	0.11 (±0.01)	27
PFN (T2)	*C. compressa*	90.33 (±9.69)	2.88 (±0.27)	0.04 (±0.02)	3
SMC (T2)	*E. amentacea*	135.13 (±15.91)	3.22 (±0.54)	0.13 (±0.02)	3
OTR (T2)	*C. compressa*	148.37 (±19.50)	3.63 (±0.37)	0.07 (±0.02)	3

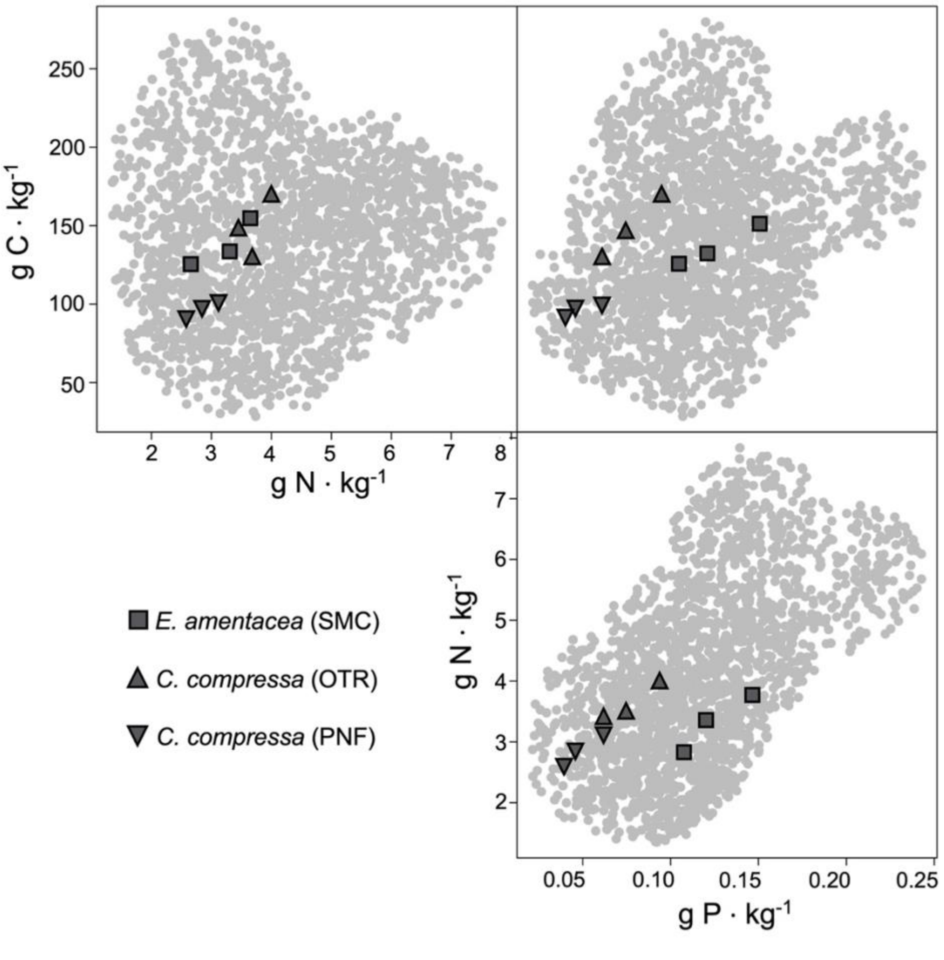

Figure 5. Hypervolume of *E. amentacea* in the three dimensions corresponding to the total C_{org}, N and P contents. The hypervolume is shown as 2-d projections for all combinations of the three functional trait axes. In each panel, the projection is represented by a cloud of (light grey) points which were randomly sampled (i.e., they represent a stochastic description of the hypervolume) from the hypervolume inferred based on real data on total C_{org}, N and P contents of *E. amentacea* at PFN and CIC (m = 27). Dark symbols in each panel indicate real values of samples of *E. amentacea* from SMC, and *C. compressa* from PFN and OTR.

Discussion

Variations in environmental conditions, especially nutrient concentrations, irradiance and seawater temperature, may strongly affect the physiology, nutrient uptake, and photosynthetic performance of macroalgae determining, ultimately, their biomass production and composition (e.g., Delgado et al., 1994; Nygård & Dring, 2008; Celis-Plá et al., 2015; 2016; Mancuso et al., 2019; Gennaro et al., 2019; Falace et al., 2021; Sanchez de Pedro et al., 2023). Responses to such variations and, more generally, the functional trait variables like organic matter, C_{org}, N and P contents, can nevertheless be species-specific or group-specific, depending on differences in life strategies, morphology and phenology among macroalgae (e.g., Stengel et al., 2014; Celis-Plá et al., 2015; Mauffrey et al., 2020). Previous studies on *Cystoseira s.l.* species, for example, found no significant correlations between tissue nutrients and dissolved nutrients in the ambient water (e.g., *Ericaria mediterranea* (Sauvageau) Molinari & Guiry; Delgado et al., 1994) whereas, species experimentally exposed to nutrient enrichment, showed either a transient effect of increasing nutrient contents (e.g., *Ericaria brachycarpa* (J.Agardh) Molinari & Guiry; Gennaro et al., 2019) or a lower nutrient uptake with respect to turf-forming algae (e.g., *Ericaria selaginoides* (Linnaeus) Molinari & Guiry; Stengel et al., 2014).

The two investigated locations were at the extremes of a broad latitudinal gradient and were characterized by different levels of coastal urbanization and associated human pressures (Cannarozzi et al., 2023). However, temporal patterns of nutrient concentrations during the period of study were similar (Teruzzi et al., 2021, see Figure S1 in supplementary material) and differences in average values of C_{org}, N and P contents were not statistically significant among *E. amentacea* stands from the two study locations, supporting the hypothesis of a large-scale (100s km) spatial homogeneity of such key functional trait values in this species despite potential effects of changes in environmental conditions. In contrast, a significant variability was detected at the scale of sites within locations (1000s m) for all trait variables, suggesting that smaller-scale variations in environmental conditions (e.g., habitat patchiness, local nutrient load) can exceed average large-scale effects on biomass production and composition, so that the amount of nutrients in macroalgal tissues might be strongly dependent on local environmental features and the historical nutrient regimes of sites (Gennaro et al., 2019). *Cystoseira s.l.* species generally undergo significant seasonal changes in nutrients and organic carbon in response to phenology during their annual cycle (Delgado et al., 1994; Celis-Plá et al., 2014b; 2016). Interestingly, contents of total organic matter differed between PFN and CIC in T1, but not in T2. This finding could seem counterintuitive, as differences in organic matter, if any, are expected to emerge in the vegetative-growing phase rather than during the resting period. Indeed, this result can be explained by the different onset and duration of the vegetative phase of *E. amentacea* between the two locations, as a consequence of the different biogeographic position and, therefore, in seasonality of irradiance and sea water temperature, which are among the main factors affecting algal productivity along with nutrient availability. In autumn, *E. amentacea* in Sicily could experience a vegetative resumption accompanied by fructification, while in Liguria it sheds its fronds, and the reproductive period concludes by the end of August. Most probably, environmental conditions in T1, although falling in autumn, were still suitable to maintain a relatively consistent productivity of *E. amentacea* in the South Ionian Sea, where CIC is located, but not at PFN, ~ 700 km north in the NW Mediterranean. The comparison of C_{org}, N and P cumulative profiles between *E. amentacea* and *C. compressa* indicated potential interspecific overlapping for tissue contents of these key trait variables, although further assessments are certainly required to confirm this preliminary result. The two species have a similar morphotype (i.e., caespitose thallus), size and phenology (Falace et al., 2005), which could be reflected in similar responses in terms of nutrient uptake and organic matter content. Moreover, different *Cystoseira s.l.* species may exhibit comparable tissue contents of nutrients and organic carbon when sharing the same habitat and/or geographic location (Ballesteros & Sant, 2022). *E. amentacea* and *C. compressa* seemed to have lower tissue contents of C_{org}, N and P compared to other *Cystoseira s.l.* species (Brey et al., 2010; Ballesteros & Sant, 2022). It is worth noting here that we focused on populations inhabiting the midlittoral rocky fringe, while most species in other studies typically occurred (or were sampled) in subtidal rocky habitats. Lower tissue contents of nutrients and organic carbon can therefore be due to differences in phenology, reservoir allocation, structural compounds (e.g., cellulose, alginates) among species inhabiting different habitats, or to gradients of decreasing nutrient availability with decreasing depth (Ballesteros 1989; Sant & Ballesteros, 2020; 2021).

As brown canopy-forming macroalgae are crucial for biomass production and energy flows along marine trophic nets (Umanzor et al., 2019; Duarte et al., 2022; Pessarodona et al., 2022), baseline information on key functional traits of *E. amentacea* and other *Cystoseira s.l.* species is essential for a deeper understanding of their contribution to the functioning of Mediterranean coastal ecosystems. Although our assessment is clearly not exhaustive in terms of spatial and temporal extent, it provides some cues on the potential role of *E. amentacea* in coastal carbon cycling. A rough estimate of biomass turnover (dry weight) of *E. amentacea* from previous works in the investigated locations accounts for 1059.3 ± 566.7 g m^{-2} y^{-1} (Cannarozzi et al., 2023), and our estimates of C_{org} content averaged 134.3 ± 15.0 g C kg^{-1} dry weight, resulting in a mean total organic carbon fixation of 1.419 t C ha^{-1} y^{-1}.

The magnitude of carbon fixation rates per unit area in *E. amentacea* could therefore be comparable to those of the Mediterranean seagrass *Posidonia oceanica* (L.) Delile, whose most recent estimates equal to 1.302 t C ha^{-1} y^{-1}, including leaves, sheaths and rhizomes fixation (Pergent-Martini et al., 2021). The overall contribution of *Cystoseira s.l.* forests to the total carbon budget of Mediterranean coastal areas is probably paltry by comparison with *P. oceanica* seagrass beds, which extend over a larger bottom surface than *Cystoseira s.l.* in the basin. It should also be noted that *Cystoseira s.l.* forests could not be considered long-term carbon sinks, as they primarily grow on rocky bottoms and unlike seagrasses, which allow carbon deposition in large structures made by the intricate complex of roots and dead rhizomes buried in soft sediments (i.e., mattes), lack substantial organic accretions that can underlay significant carbon sequestration (Duarte et al. 2013). However, macroalgae export about 43% of their production as both particulate and dissolved organic carbon (Krause-Jensen et al, 2016), and the net transport of carbon towards deep systems can double that of seagrasses (8.5 mol C m^{-2} for macroalgae in contrast to 4.4 mol C m^{-2} for seagrass; Barrón & Duarte 2015). In this view, *Cystoseira s.l.* stands can represent important carbon donors to neighbouring systems, where this carbon can be sequestered in sediments and deep waters over a long time-scale (Hill et al., 2015).

From a more practical perspective, our findings allow some pragmatic considerations to account for in applied ecological research on *E. amentacea* and other *Cystoseira s.l.*

species. Since organic carbon, nitrogen and phosphorous contents can largely vary at a very local scale, extrapolating functional properties or goods and services of these macroalgal stands (e.g., their natural capital) over large geographic areas could significantly under- or overestimate the ensuing quantifications, if based on data restricted to single, small spatial extents. This high small-scale variability could also have important implications for conservation and active restoration strategies involving *Cystoseira s.l.* species. For example, restoration interventions on *Cystoseira s.l.* stands are often restricted to relatively small areas (Verdura eta al., 2019; De la Fuente et al., 2019), which could match the scale at which key functional traits are more variable. Given that restoration costs for *Cystoseira s.l.* ranges between 57,000 and 7,000,000 €/ha, depending on the restoration techniques, logistics, and environmental context (Cebrian et al., 2021 and references therein), preliminary assessments on key functional traits of neighbouring and/or resident populations could help identify sites that could maximize the return of economic investments in terms of functional performance of restored *Cystoseira s.l.* stands.

The baseline knowledge of productivity, biomass, key functional traits, and especially their spatial and temporal variations from local to large scale, is still very limited for *E. amentacea* and, more generally, for many *Cystoseira s.l.* species, hindering reliable quantifications of uptake, fixation, export and storage of carbon and key elements of intertidal and subtidal stands of Mediterranean fucoid algae. This study is one of the first attempts to fill these gaps, which could contribute to refine assessments of the functional role of *Cystoseira s.l.* forests and to improve their management and conservation.

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PART II_ASSESSING THE NATURAL CAPITAL ASSOCIATED TO *E. AMENTACEA* FORESTS CONSIDERING SPATIO-TEMPORAL VARIATION

CHAPTER 1

Ericaria amentacea natural capital: the hidden treasure of Mediterranean infralittoral environments

On process

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Abstract

Natural capital accounting significantly advanced in the last decades aiming at the disentanglement of the relationships between natural capital and decision making and pushed by international institutions and programs.

In Italy, the Ministry of the Environment and Energy Security proposed, in 2014, a research program, titled "Environmental Accounting in Italian Marine Protected Areas" (EAMPA). The EAMPA framework, able to assess, in both ecological and economic terms, the natural capital and the ecosystem services in Marine Protected Areas (MPAs), did not include intertidal environments. Intertidal environments are fragile transitional zones exposed to a strong variability of environmental conditions. In rocky intertidal habitats, brown fucoid algae of the genus *Cystoseira sensu lato* C. Agardh (Fucales, Phaeophyceae) proliferate. *Cystoseira s. l.* are ecosystem engineers able to create three-dimensional forests. Among these algae, *Ericaria amentacea* (C. Agardh, Molinari & Guiry 2020) is recognized as a sensitive species suffering a widespread decline along the Italian rocky coasts and in the whole Mediterranean Sea. Here, the *E. amentacea* ecosystem value has been assessed in two Italian MPAs, also considering two different sampling times: 1) when only the perennial part of the algae, the cauloid, persists (dormant period) and 2) when both perennial and deciduous parts are presents (vegetative period).

Results, expressed as both biophysical and monetary value, through the application of emergy analysis, filled the information gap about the natural capital values of these environments. As far as spatial variability, we did not detect significant variations between the two MPAs (>1000 km apart), suggesting that the natural capital and functions value of *E. amentacea* might be comparable at a a regional scale and beyond.

In contrast, temporal variations were significant indicating that, when *E. amentacea* is flourishing, natural capital concentrates inside the forest impoverishing the immediately upper zone, thus highlighting the role of *E. amentacea* as a value keeper and catalyst. This role is even more important in the intertidal, where conditions for life are extremely difficult. Moreover, integrating the separate contributions of the perennial portion of the algal thalli and the deciduous fronds allowed to obtain a more comprehensive assessment of the actual value of *E. amentacea*, which equals the one of the most valuable marine ecosystems of the Mediterranean Sea, such as coralligenous outcrops and *Posidonia oceanica* meadows.

Keywords: system analysis, natural capital stock-flows, ecosystem functions, ecosystem services, ecological economics, habitat value, coastal habitats

Introduction

The term “capital” usually refers to a stock able to generate flows (Ekins et al., 2003). Natural capital is defined as the entirety of natural assets, both renewable and non-renewable (e.g., geology, soil, air, water, living organisms) (Natural Capital committee, 2021). As a consequence, when natural capital is concerned, flows match with ecosystem functions (useful for nature) and services (providing benefits to humans) (Natural Capital committee, 2021). The concept of natural capital is then crucially anthropocentric since it highlights how nature supports human well-being and it is therefore central for sustainable development (Bateman and Mace, 2020). That is why, unraveling the relationship between decision making and natural capital accounting, several innovative advances emerged in the last decades. For instance, international bodies such as the World Bank and the United Nations Environment Programme (UNEP) proposed metrics to assess both physically and monetary natural capital value (Bateman and Mace, 2020). At European level the natural capital accounting is recognized within the EU Biodiversity Strategy to 2020 (EC, 2011) as well as in the EU's Seventh Environment Action Programme (7th EAP) (EU, 2014), while key institutions (DG Environment, Eurostat and the European Environment Agency) led ecosystem accounting initiatives (Capriolo et al., 2020; Vigerstol and Aukema, 2011; Nedkov and Burkhard, 2012; Zulian et al., 2013; Burkhard and Maes, 2017; La Notte et al., 2017).

In Italy, the Ministry of the Environment and Energy Security (formerly Ministry for the Environment and the Safeguard of the Territory and the Sea) implemented a research programme, titled "Environmental Accounting in Italian Marine Protected Areas" (EAMPA). EAMPA aimed at the development of a framework to assess, with a multidisciplinary (ecological and economic) approach, the natural capital stocked and the ecosystem services provided in Marine Protected Areas (MPAs) (Franzese et al., 2017; Picone et al., 2017; Vassallo et al., 2017, Paoli et al., 2018; Paoli et al., 2020; Buonocore et al., 2020, 2021; Rigo et al., 2021; Dapueto et al., 2022; Bordoni et al., 2023). The framework was applied to coastal ecosystems within the Italian MPAs, but intertidal environments were not included in the framework. Intertidal environments are those transitional zones between the high and low tides where terrestrial and marine realms meet. This zone is particularly fragile being exposed to a strong variability of environmental conditions (Helmuth et al., 2006; Blanchette et al., 2008) and it is largely sensitive to anthropogenic pressures, leading to habitat degradation (Verlaque and Tine, 1979; Benedetti-cecchi et al., 2001; Soltan et al., 2001; Mancuso et al., 2018). Particularly relevant in the littoral Mediterranean fringe are brown fucoid algae of the genus *Cystoseira sensu lato* C.Agardh (Fucales, Phaeophyceae) among which *Cystoseira sensu stricto*, *Ericaria* and *Gongolaria*. *Cystoseira s.l.* dominate rocky intertidal and subtidal habitats, forming dense macroalgal forests that, due to their three-dimensional structures and intrinsic complexity (Tamburello et al., 2022), play a crucial role as ecosystem engineers (De La Fuente et., 2019; Rendina et al., 2023) establishing and maintaining a biodiverse associated community (Cannarozzi et al., 2023; Cheminée et al., 2013; Mancuso et al., 2023). Some species of *Cystoseira s.l.* are considered water and ecosystem quality indicators by the Water Framework Directive (WFD, 2000/60/EC). Indeed, their capability to describe the level of the ecosystem quality allows using them for the CARLIT index (CARtography of LITtoral and upper-sublittoral benthic communities) (Ballesteros et al., 2007; Mangialajo et al., 2007).

These macroalgae have been identified as priority species by the Barcelona Convention, protected by the Bern Convention and classified as endangered by international organizations such as RAC/SPA, MedPan and IUCN. Specifically, *E. amentacea* (C. Agardh, Molinari & Guiry 2020) is one of the most sensitive species of *Cystoseria s.l.* genus and its loss and degradation caused by human direct and indirect impacts are reported along the Mediterranean coasts (Benedetti-Cecchi et al. 2001; Thibaut et al., 2005; Mangialajo et al., 2007; Mangialajo et al., 2008; Strain et al., 2014; Thibaut et al., 2014; Mineur et al., 2015; Falace et al., 2018; Mancuso et al., 2018; Blanfuné et al., 2019; De La Fuente et al., 2019).

E. amentacea have a caespitose thallus on whose basal structure (cauloid) grow ramifications that in the maturity season (between April and July) reach 40-50 cm in height (Gómez-Garreta et al., 2002; Mangialajo et al., 2008; Giaccone et al., 2009; Mannino et al., 2009; Cormaci et al., 2012; Taşkin et al, 2012). Like other species of *Cystoseria s.l.*, *E. amentacea* has a seasonal cycle of growth: every year, in spring (between April and June), new branches develop reaching the full development during summer. In autumn (between August and October), the branches are shed, and only the cauloid persists (Gómez-Garreta et al. 2002; Mangialajo et al., 2008; Giaccone et al., 2009). From the natural capital perspective, the cauloid represents the natural capital while the branches are annual flows.

The aim of this study is to investigate and quantify the biophysical and monetary value of *E. amentacea* forests in order to fill the gap about natural capital information of

Mediterranean infralittoral forests and to better understand the role played by *Cystoseira s.l.* as good indicator of environmental quality. At this purpose both natural capital and annual flows components were assessed. *E. amentacea* survey was carried out in two sites of the west of Mediterranean Sea (Portofino MPA -NW Mediterranean- and Ciclopi Islands MPA -NW Ionian Sea-) considering different timeframes and taking into account the annual cycle of the algae.

Materials e methods

Study area

The analysis was conducted in two sites located in two different Italian regions: Portofino MPA (Liguria, NW of Italy) and Ciclopi Islands is MPA (Sicily, SW of Italy) (Figure 1). Two MPAs were chosen to avoid most human disturbances.

Both MPA are divided in three zones (A, B and C zone) in which human activities (e.g., diving, fishing, recreational boating) are regulated and subjected to different protection levels, from higher in A zone to lower in C zone (Venturini et al., 2016; Paoli et al., 2017).

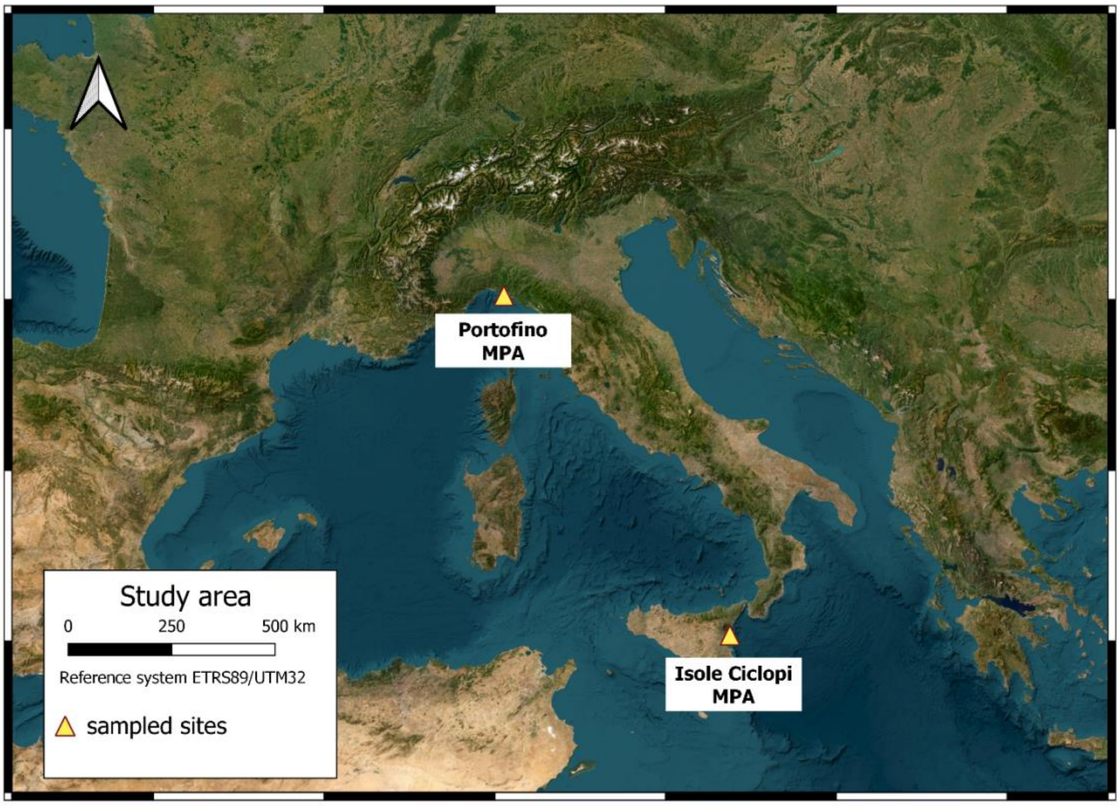

Figure 1: study sites

The weather of the two sites is that typical of the Mediterranean Sea but with some differences between sites. Portofino territory is characterized by hot summers, with an average temperature of 24°C, mild winters with an average temperature of 11°C, with an annual rainfall equal to 1300 mm/y (Brandolini et al., 2006; Faccini et al., 2008; Sacchini et al., 2012). The Sicily Island is subject to winds more than the Ligurian region and characterized either by the Mediterranean climate in the south and by continental and rainy one in the north central part (Brandimarte et al., 2011). For Ciclopi Islands, temperature range from 27°C in summer and 10°C in wet winter, with an annual lower rainfall of 680 mm/y (Colonese et al., 2011).

The Portofino MPA extends over 346 ha and was established in 1998. The MPA includes three little coastal municipalities and it is recognised for its high natural value and emerged and submerged landscape as well as for the rich biodiversity (Paoli et al., 2017). Portofino MPA is included in the European Natura 2000 Network as Site of Community Importance (SCI IT1332674: Fondali monte di Portofino) and since 2005 it is a SPAMI (Specially Protected Area of Mediterranean Interest) according to the decision of the RAC/SPA Office (Venturini et al., 2016; Paoli et al., 2017).

The surroundings of Portofino MPA maintain the features of a tourist resort with an economy mainly tied on tertiary sector (Paoli et al., 2017). The MPA of Portofino is surrounded by ports and marinas: in the western part those of Genoa, the county seat, with more than 5000 recreational boats that can reach the MPA in a couple of hours, while in the eastern part those of different resorts for a total of 2000 recreational boats (Venturini et al., 2021).

The MPA of Ciclopi Islands covers a surface of 623 ha and was established in 1989. The coasts of Ciclopi Islands MPA in the north are characterized by four islets of few square metres featured by vertical slopes and small pebble beaches: Faraglione di Mezzo and Faraglione Piccolo (Sciandrello et al., 2017). Instead in the west and south, the coasts are flat with sand dune systems. This MPA includes three marinas, of which the largest, in summer, hosts about 380 boats. The activities allowed, for licensed users, within both the MPAs are: sunbathing, diving, professional fishing, recreational fishing, and recreational boating (Costanzo et al., 2021).

Community assestement

In both areas, the intertidal fringe with dense *E. amentacea* canopies were sampled twice considering the annual cycle of the algae: October 2020 (when only the cauloid remains) and July 2021 (when branches reach their maximum development).

Fifteen random samples of *E. amentacea* forests and of the associated community were taken in each site on sub-horizontal rocky reefs, for a total of thirty samples. Sampling consisted of the complete removal of the *E. amentacea* thalli and the scratching of the associated understory assemblage from the rocky substrate on a surface of 160 cm^2 for each sampling unit, as previously used in other studies (Cannarozzi et al., 2023). Sampling was operated placing upon the canopy two PVC cylinders and a spatula, which was slid under the cylinder in order to cut the *E. amentacea* thalli, scratching all associated community. *E. amentacea* was then preserved in a solution of 4% formaldehyde in sea water and brought to the laboratory for subsequent processing. In laboratory, samples were then sieved with 1 mm mesh and soaked in sea water for 24 hours to remove formaldehyde residues. Samples were then sorted by separating the organisms from the inorganic debris and by detaching from *E. amentacea* thalli the epibionts (i.e., other algae and invertebrates). Algae (except for *E. amentacea*) were grouped altogether, whereas invertebrates were classified considering the main taxonomic groups (phylum or subphylum) and trophic habits (i.e., predators, suspenders, grazers, detritivorous) (hereinafter groups). *E. amentacea* and its associated community were then dried at 80 °C for 48 h, weighed, then combusted respectively at 500°C for 4 h (*E. amentacea* and other algae) and 550 °C for 1 h (macrozoobenthos). Finally, the samples were reweighed to measure total inorganic content to obtain, as difference, the ash free dry weight and the amount of carbon contained in the organisms (gC/m^2) by means of conversion factors (Brey, 1990). Using biomasses, calculated in carbon grams, the trophic network was modelled (through Matlab® software) and then the trophic levels (TL) of groups were obtained. Indeed, the main trophic and taxonomic groups composing the ecosystem were identified and described in Operational Taxonomic Units (OTUs) for both macroalgae and infauna. Then, a database of the biomass per unit area associated with each trophic group was created. The assessment of the biomass stocked in the OTUs is the basic information for the calculation of the primary production required, in space and time, to generate the natural capital stocked within the habitat (De la Fuente et al., 2019).

Natural capital evaluation

The emergy method (Odum, 1988, 1996) has been recently used to estimate the value of natural capital (Franzese et al., 2017; Picone et al., 2017; Paoli et al., 2018; De La Fuente et al. 2019; Buonocore et al., 2021; Rigo et al., 2021; Bordoni et al., 2023). Emergy measures natural capital by the calculation of natural resources used (directly and indirectly) to build up and maintain the biomass of all the organisms within the considered habitat (Buonocore et al., 2020). For this reason, this method is defined a “donor-side” approach (Ulgiati et al., 2011; Vassallo et al., 2017). Emergy is a thermodynamic methodology, in which all inputs supporting a system are expressed in terms of solar emergy Joule (sej), that equals the total amount of solar available energy, directly or indirectly, used to generate and maintain a product or a service (Odum, 1996). The solar emergy required to generate one unit of a product or a service is referred to as Unit Emergy Value (UEV, sej J^{-1}/sej g^{-1}). The emergy content of a system or a flow can be translated into currency equivalents values using the “emergy-to-money ratio” (EMR) (Lou and Ulgiati, 2013). EMR represents the average amount of emergy needed to generate one unit of money in the national economy (Odum, 1996). This conversion translates the values of biosphere’s investment in equivalents of a specific currency. This conversion allows to clarify the importance of natural capital to policy makers and other stakeholders granting an effective communication in socio-economic contexts (Turcato et al., 2015; Vassallo et al., 2021). Monetary equivalents of biophysical values, such as the natural capital value of the midlittoral zone, are ex- pressed in emergy-euros (em €). Therefore, the value of natural capital stocked in *E. amentacea* habitat was assessed through emergy analysis, following the procedure described by Vassallo et al. (2017) and Paoli et al. (2018; 2022).

First, a system diagram of the *E. amentacea* habitat is depicted by: 1) using the symbology proposed by Odum (1996), 2) considering all information gathered about system inputs and 3) identifying the components that characterize the system itself. According to Odum’s simbology the boundaries of the studied system are represented by a main rectangle; along the left side and the top of the rectangle the inputs are reported. Within the rectangle the components are arranged and represented according to the role they play: the producers as bullets and consumers as hexagons. All these flows converge in the heat sink, indicating the heat losses that occur with each energy transformation.

The evaluation of the biomass stocked and the assessment of trophic level (TL) of different groups are the basic information for the calculation of the primary production (PP). PP is the primary production required, in space and time, to start and keep the trophic web that generate the natural capital stocked in the considered system (Vassallo et al., 2017; Paoli et al., 2018). PP was calculated using the following equations (Pauly and Christensen, 1995; Vassallo et al., 2017):

$$PP = Ba + Be \qquad \text{eq.1}$$

in which Ba is the biomass of primary producers and Be the heterotrophic biomass necessary to sustain consumers. Be is calculated as follows

$$Be = \sum B_i * 7^{(LT_i-1)} \qquad i = 1, 2, 3, \ldots n \qquad \text{eq.2}$$

where B_i the biomass of each group of heterotrophs.

The second step is the assessment, of all natural inputs that support the ecosystem. Inputs value in traditional units is calculated according to the formulas reported in Table 1, while the emergy value of all inputs is obtained by multiplying the value in traditional units of each item by the corresponding UEVs (Table 2).

Table 1: calculation formulas for natural capital emergy inputs of marine habitats

Inputs	Formula	Unit	References
Carbon	Benthic biomass	g	Vassallo et al., 2017
Nitrogen	Benthic biomass ·7/41	g	Vassallo et al., 2017
Phosphorus	Benthic biomass /41	g	Vassallo et al., 2017
Solar radiation	annual solar radiation per unit area area · (1-albedo) · area · time for stocks formation	J	Vassallo et al., 2017
Rain (chemical energy)	annual rainfall · Gibbs free energy · water density · area · time for stocks formation	J	Odum, 1996
Wind	air density · drag coeff. · (wind speed · geostrophic wind velocity)^3 · area · seconds per year · time for stocks formation	J	Odum, 1996
Geothermal heat	area · geothermal flux · time for stocks formation	J	Odum, 2000
Current (kinetic)	1⁄2 · height water evaporated per year · (current velocity)^2 · supporting time · water sea density	J	Campbell et al., 2005
Current (geopotential)	1/2 · height of water evaporated on average in the Mediterranean due to currents^2 · water sea density · gravity · supporting time	J	Odum, 1996
Tide	1⁄2 · tides per year · (height)^2 · density sea water · supporting time ·gravity	J	Odum, 1996

Table 2: employed UEVs

Inputs	UEVs	Unit of measure	Reference

Carbon	1.02E+08	sej/g	Campbell and Tilley, 2014
Nitrogen	7.40E+09	sej/g	Odum, 1996
Phosphorus	2.86E+10	sej/g	Odum, 1996
Sun	1.00E+00	sej/J	By definition
Rain	2.93E+04	sej/J	Odum, 1996
Wind	2.41E+03	sej/J	Odum, 1996
Kinetic current	1.77E+07	sej/J	Odum, 1996
Geothermal heat	5.53E+04	sej/J	Brown and Ulgiati, 2010
Tides	2.71E+04	sej/J	Brown and Ulgiati, 2010
Geopotential current	3.80E+04	sej/J	Campbell et al., 2005

The overall system natural capital value is obtained from the sum of the emergy values of inputs through the following algorithm:

Maximum (carbon, nitrogen, phosphorus) + Maximum (solar radiation, rain, wind, currents) + tides + geothermal heat eq.3

Carbon, nitrogen and phosphorus as well as sun, rain, wind and currents are considered as co-products groups (nutrients and environmental resources respectively), i.e., resulting from the same processes occurring at the level of the biosphere. For this reason, only the biggest of each group is included in calculation (Odum, 1996; Vassallo et al., 2017; Paoli et al., 2018; De La Fuente et al., 2019). In order to convert the biophysical value into monetary terms, results obtained (sej) could be divided for the Emergy Money Ratio (EMR), here 9,60E+11 sej/ € taken from literature Pereira et al. (2013).

Results

Community assesetement

Through laboratory activity autotrophs were split in *E. amentacea* and other algae, while heterotrophs considered: Porifera suspenders, Cnidaria suspenders, Mollusca suspenders, Mollusca grazer, Crustacea grazer, Crustacea predators, Crustacea detritivorous, Crustacea suspenders, Annelida detritivorous, Annelida predators, Annelida suspenders, Nematoda mix, Echinodermata suspenders, Echinodermata detritivorous, Bryozoa suspenders, Platyhelminthes predators and Tunicata suspenders. All these organisms and their biomass, expressed in gC/m^2, are shown in the Table 3. At each site, in both seasons, Mollusca suspenders had the greatest biomass.

Furthermore, trophic levels are reported in Table 4.

Table 3: *biomass values for taxonomic functional groups of E. amentacea habitat: susp=suspender, graz= grazer, pred= predator, det=detritivorous, mix=omnivorous*

	Site	**Portofino October 2020**	**Portofino July 2021**	**Isole Ciclopi October 2020**	**Isole Ciclopi July 2021**
	Groups	Biomass (gC/m^2)			
Autotrophs	*E. amentacea*	1.56E+02	5.14E+02	3.04E+02	6.74E+02
	Other algae	2.48E+01	3.59E+00	1.56E+02	3.18E+01
Heterotrophs	Porifera susp	0.00E+00	0.00E+00	6.15E-01	0.00E+00
	Cnidaria susp	5.11E-02	2.18E-01	9.61E-02	9.81E-02
	Mollusca susp	4.24E+01	2.24E+02	1.68E+01	4.10E+01
	Mollusca graz	2.16E-01	7.61E-01	8.36E-01	7.55E-01
	Crustacea graz	5.35E-01	1.50E+00	2.67E+00	2.03E+01
	Crustacea pred	1.54E-01	4.32E-01	6.79E-03	9.96E-01
	Crustacea det	2.98E-01	8.39E-01	1.62E+00	1.24E+01
	Crustacea susp	1.31E+01	3.68E+01	1.55E+00	1.18E+01
	Annelida det	3.53E-02	2.96E-02	7.06E-02	1.27E-01
	Annelida pred	1.48E-01	1.24E-01	2.96E-01	5.32E-01
	Annelida susp	1.29E-01	1.08E-01	2.58E-01	4.64E-01
	Nematoda mix	7.01E-02	2.27E-03	0.00E+00	0.00E+00
	Echinodermata susp	0.00E+00	0.00E+00	1.75E-01	4.01E-01
	Echinodermata det	5.71E-02	4.84E-02	0.00E+00	0.00E+00
	Bryozoa susp	4.37E-01	2.65E-01	5.24E-01	3.02E-01
	Platyhelminthes pred	1.52E-02	0.00E+00	0.00E+00	0.00E+00
	Tunicata susp	5.22E-02	0.00E+00	1.53E-02	1.38E-01

Table 4: *trophic levels for taxonomic functional groups of E. amentacea habitat : susp=suspender, graz= grazer, pred= predator, det=detritivorous, mix=omnivorous*

	Site	**Portofino October 2020**	**Portofino July 2021**	**Ciclopi October 2020**	**Ciclopi July 2021**
	Groups	TL			
Autotrophs	Autotrophs	1.00	1.00	1.00	1.00
Heterotrophs	Porifera susp	2.00	2.00	2.00	-
	Cnidaria susp	2.00	2.00	2.00	2.00
	Mollusca susp	-	-	2.00	2.00
	Mollusca graz	2.00	2.00	2.00	2.00
	Crustacea graz	3.41	3.31	2.00	2.00
	Crustacea pred	2.00	2.00	3.42	3.38
	Crustacea det	2.00	2.00	2.00	2.00
	Crustacea susp	2.00	2.00	2.00	2.00
	Annelida det	3.31	3.33	2.00	2.00
	Annelida pred	2.00	2.00	3.52	3.34
	Annelida susp	2.00	2.00	2.00	2.00
	Nematoda mix	2.44	2.28	-	-
	Echinodermata susp	2.36	2.25	2.00	2.00
	Echinodermata det	2.00	2.00	-	-
	Bryozoa susp	1.00	1.00	2.00	2.00
	Platyhelminthes pred	2.00	2.00	-	-

	Tunicata susp	2.00	2.00	2.00	2.00

Natural capital evaluation

The functioning of the system *E. amentacea* forests is shown in Figure 2. Ecosystem functions generated by natural capital are depicted as outputs.

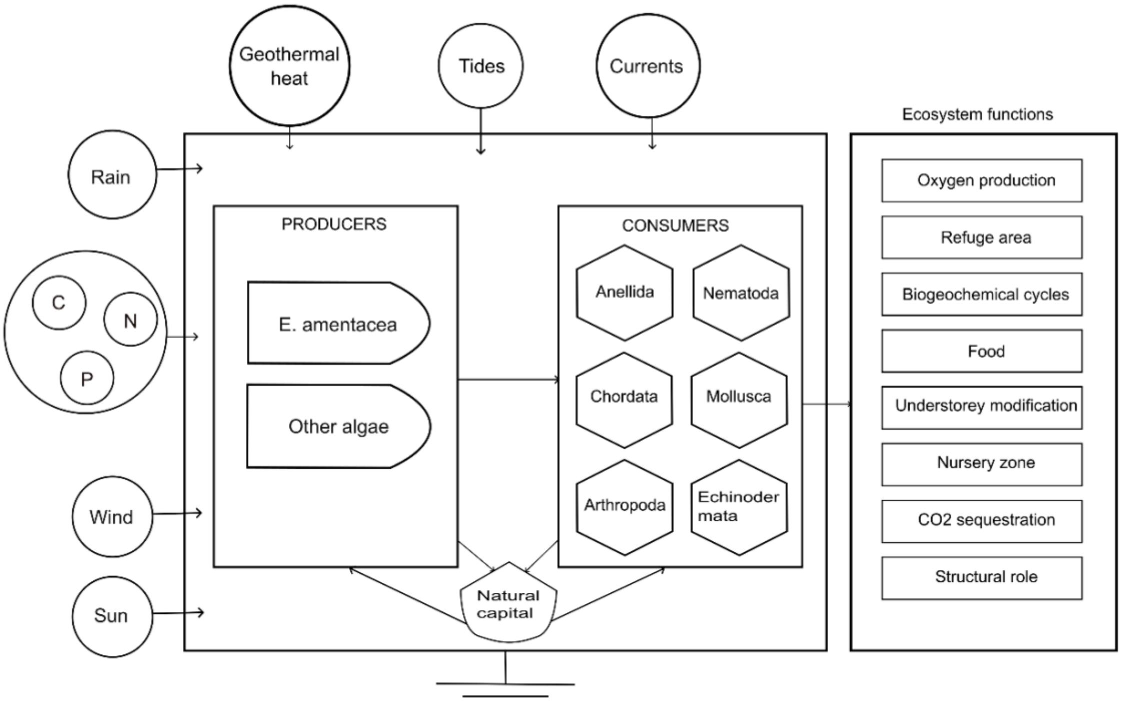

Figure 2: *Emergy diagram of* E. amentacea *habitat*

In Table 5 results about natural resources (inputs) associated to *E. amentacea* ecosystem in common units are represented, splitting autotrophs (auto) and heterotrophs (heter).

***Table 5:** E. amentacea habitat input values in common units for emergy calculation*

Input (per m^2)	Portofino October 2020		Portofino July 2020		Ciclopi Islands October 2020		Ciclopi Islands July 2021	
	Auto	*Heter*	*Auto*	*Heter*	*Auto*	*Heter*	*Auto*	*Heter*
Carbon (g)	1.81E+02	4.18E+02	5.17E+02	1.88E+03	4.59E+02	1.93E+02	7.06E+02	6.95E+02
Nitrogen (g)	3.08E+01	7.14E+01	8.84E+01	3.20E+02	7.84E+01	3.30E+01	1.20E+02	1.19E+02
Phosphorus (g)	4.40E+00	1.02E+01	1.26E+01	4.57E+01	1.12E+01	4.71E+00	1.72E+01	1.69E+01
Sun (J)	8.31E+08	1.11E+09	2.38E+09	4.98E+09	2.44E+09	5.93E+08	3.75E+09	2.13E+09
Rain (J)	2.85E+06	3.81E+06	7.72E+06	1.61E+07	5.70E+06	1.38E+06	1.55E+07	8.79E+06
Wind (J)	1.95E+07	1.92E+07	5.00E+07	5.00E+07	3.04E+07	7.36E+06	5.84E+07	3.31E+07
Kinetic current (J)	1.23E+01	1.64E+01	3.52E+01	7.36E+01	4.87E+00	1.18E+00	7.48E+00	4.25E+00
Geopotential current (J)	4.67E+03	6.24E+03	1.34E+04	2.80E+04	1.19E+04	2.88E+03	1.83E+04	1.04E+04
Geothermal heat (J)	9.10E+05	1.22E+06	2.61E+06	5.45E+06	3.09E+06	7.49E+05	4.74E+06	2.69E+06
Tides (J)	3.19E+05	4.26E+05	9.14E+05	1.91E+06	5.62E+05	2.36E+01	8.51E+05	8.28E+01

The inputs converted in emergy units are shown in Table 6.

Table 6: emergy values of different E.amentacea habitat inputs

Input (sej/m^2)	Portofino October 2020		Portofino July 2021		Ciclopi Islands October 2020		Ciclopi Islands July 2021	
	Auto	*Heter*	*Auto*	*Heter*	*Auto*	*Heter*	*Auto*	*Heter*
Carbon	1.85E+10	4.28E+10	5.29E+10	1.92E+11	4.70E+10	1.97E+10	7.22E+10	7.10E+10
Nitrogen	2.28E+11	5.29E+11	6.54E+11	2.37E+12	5.81E+11	2.44E+11	8.92E+11	8.78E+11
Phosphorus	1.26E+11	2.92E+11	3.61E+11	1.31E+12	3.21E+11	1.35E+11	4.93E+11	4.85E+11
Sun	8.31E+08	1.11E+09	2.38E+09	4.98E+09	2.44E+09	5.93E+08	3.75E+09	2.13E+09
Rain	8.35E+10	1.12E+11	2.26E+11	4.73E+11	1.67E+11	4.05E+10	4.53E+11	2.58E+11
Wind	4.70E+10	4.62E+10	1.21E+11	1.21E+11	7.33E+10	1.78E+10	1.41E+11	8.00E+10
Geopotential current	1.77E+08	2.37E+08	5.09E+08	1.06E+09	4.52E+08	1.10E+08	6.94E+08	3.94E+08
Kinetic current	2.17E+08	2.90E+08	6.22E+08	1.30E+09	8.61E+07	2.09E+07	1.32E+08	7.51E+07
Geothermal heat	5.03E+10	6.73E+10	1.44E+11	3.02E+11	1.71E+11	4.14E+10	2.62E+11	1.49E+11
Tides	8.63E+09	1.15E+10	2.48E+10	5.18E+10	1.52E+10	6.38E+05	2.31E+10	2.24E+06
Total	1.09E+12		4.24E+12		1.26E+12		2.91E+12	

The most important inputs, for both autotrophs and heterotrophs components, were found nitrogen among nutrients co-products group (66.46% of the total natural capital value on average) and rain within environmental resources co-products group (19.08% on average) (Figure 3).

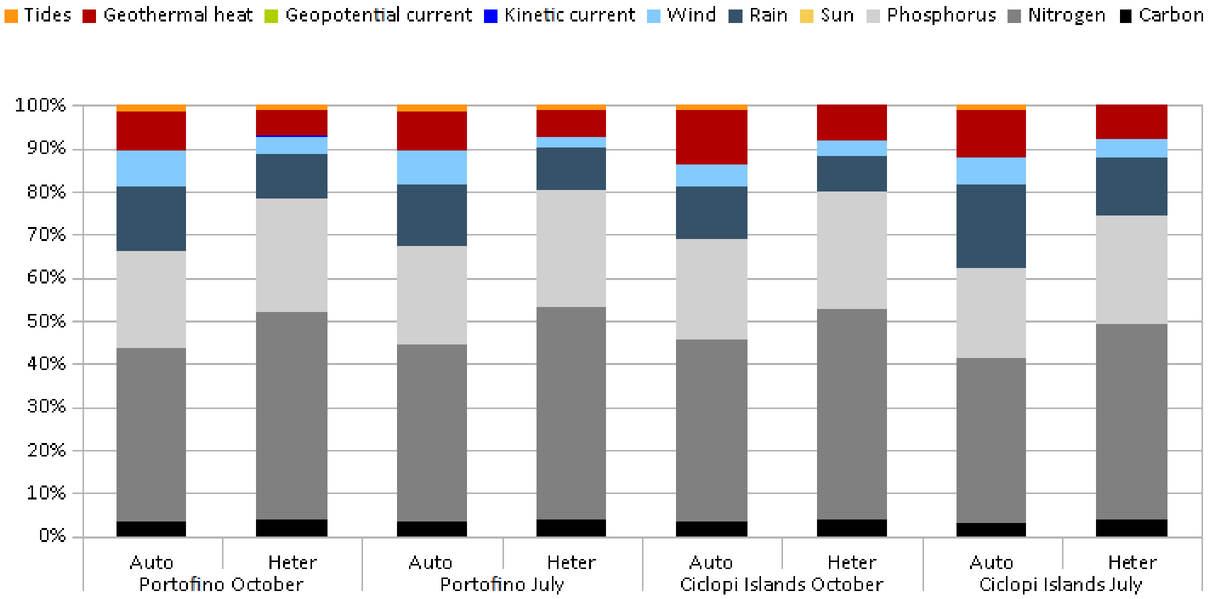

Figure 3: *Percentage contribution of different emergy inputs to total budget*

Figure 4 shows the percentage contribution of autotrophs (*E. amentacea* and other algae) and heterotrophs to the natural capital of the ecosystem. On average, heterotrophs give the main contribution (52.99%) even if while in Portofino heterotrophs contribute in both times more than autotrophs (the contribution of heterotrophs was 2.5 times higher than autotrophs on average), in Ciclopi Islands the opposite occurred, with the average contribution of autotrophs doubling that of heterotrophs. Considering autotrophs, *E. amentacea* always provides the major percentage contribution, especially in July 2021.

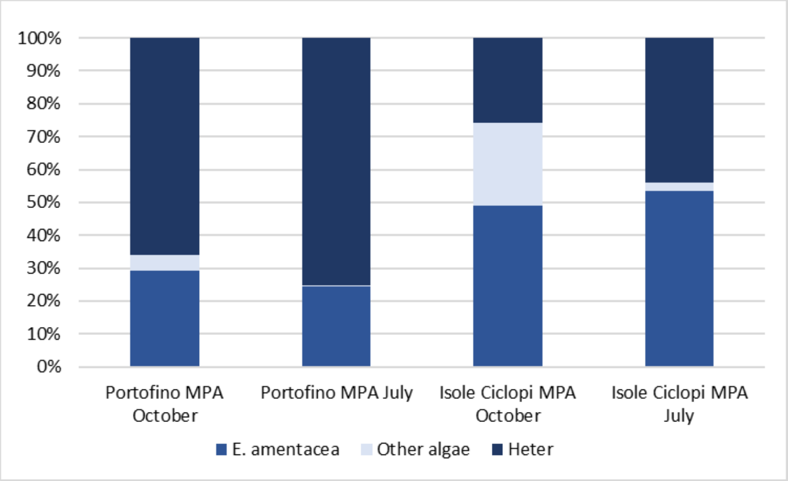

Figure 4: *Percentage contribution of autotrophs and heterotrophs to total budget; autotrophs are split in* E. amentacea *and other macroalgae contribution*

At last, the emergy values were transformed into the monetary values, as shown in Figure 5. For both sites *E. amentacea* has a major value (3.03 em€/m^2 in Ciclopi Islands MPA and 4.41em€/m^2 in Portofino MPA) in July. The value in Portofino is 3.89 times greater in July 2021 than in October 2020, while in Ciclopi Islands it is 2.31 times greater.

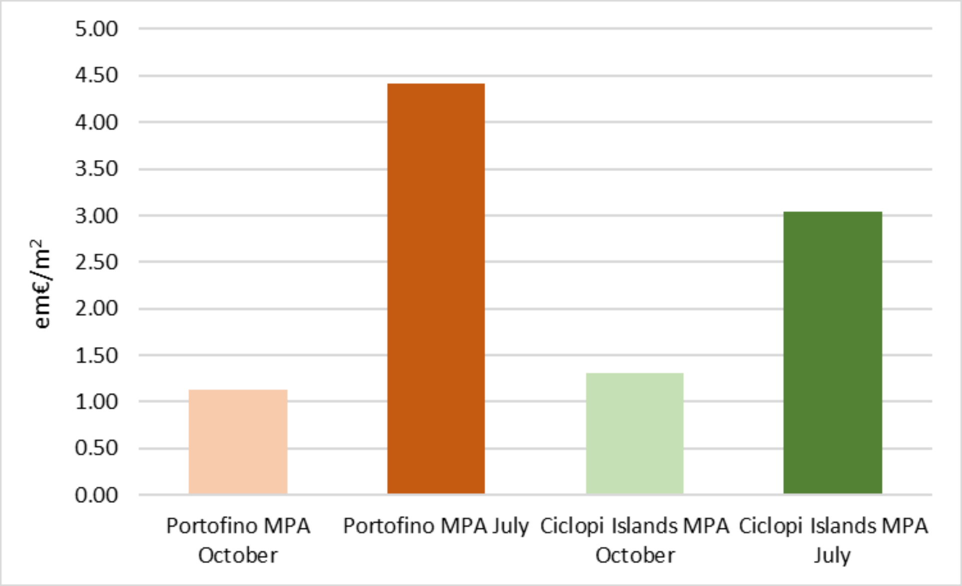

Figure 5: *Value of* E. amentacea *expressed in monetary equivalents*

Discussion

Natural capital of *E. amentacea* stands were compared at spatial (between the two sites: Portofino MPA and Ciclopi Islands MPA) and interannual (between the two sampling times) level through statistical analysis, considering the autotrophic and heterotrophic components together.

Spatially, the statistical analysis (PERMANOVA) did not highlight significant difference between Portofino MPA and Ciclopi Islands MPA (Table 7). Therefore, it is possible to assert that *E. amentacea* natural capital is not affected by differences existing in environmental features among the considered sites. This result can be explained considering that, even if the two sites differed in terms of climate conditions, they anyway belong to the Mediterranean basin and sets the stage to investigate if the natural capital values assessed can be representative of the entire region. Moreover, even if the relative distribution of autotrophs and heterotrophs in Portofino and Ciclopi Islands seems to be opposite, with a greater contribution of autotrophs to the Ciclopi Islands budget, this difference was not statistically significant, supporting the hypothesis of a low variability of the natural capital of *E. amentacea* at a basin scale.

Table 7: *Results of PERMANOVA testing for differences at spatial level*

Spatial comparison					
	Df	SumOfSquare	R2	F	P
Time	1	8.646362 E+24	0.03856915	2.326751	0.158
Residual	58	2.155318 E+26	0.96143085		
Total	59	2.241782 E+26	1.00000000		

Given this spatial homogeneity, temporal variations were evaluated by investigating autotrophic component, heterotrophic component and total natural capital in the two times for the two MPAs PERMANOVA results (Table 8) reported that *E. amentacea* ecosystem varies significantly between October 2020 and July 2021.

Table 8: *Results of PERMANOVA testing for differences at temporal level*

Total natural capital					
	Df	SumOfSquare	R2	F	P
Time	1	8.675911 E+25	0.290694	23.77007	**0.001**
Residual	58	2.116959 E+26	0.709306		
Total	59	2.984550 E+26	1.000000		
Autotrophic natural capital					
	Df	SumOfSquare	R2	F	P
Time	1	7.095321 E+24	0.3032218	25.24026	**0.001**
Residual	58	1.630445 E+25	0.6967782		
Total	59	2.339977 E+25	1.0000000		
Heterotrophic natural capital					
	Df	SumOfSquare	R2	F	P
Time	1	4.419307 E+25	0.1791875	12.66169	**0.001**
Residual	58	2.024373 E+26	0.8208125		
Total	59	2.466303 E+26	1.0000000		

This difference can be explained considering the annual life cycle of *E. amentacea* (Gómez-Garreta et al. 2002; Mangialajo et al., 2008; Giaccone et al., 2009). In fact, *E. amentacea* has a persistent cauloid that remains during the year, and deciduous branches that each spring are generated and each autumn are shed (Gómez-Garreta et al. 2002; Mangialajo et al., 2008; Giaccone et al., 2009). The greatest value found for July 2021 can be then ascribed to the newly and flourishing generated branches. Branches not only increase the algae biomass but also create a complex, three-dimensional environment that affects the habitat determining a modification of microclimate (e.g., humidity and temperature) (Bulleri et al., 2002; Falace et al., 2005; Riccato et al., 2009; Agnetta et al., 2015). Moreover, branches create a canopy that provide food, shelter and nursery grounds (Bulleri et al., 2002; Riccato et al., 2009; Agnetta et al., 2015; Falace et al., 2018; Chiarore et al., 2019; De La Fuente et al., 2019). All these features are those ecosystem functions that make *E. amentacea* ecosystem a biodiversity hotspot (Riccato et al., 2009) generating a cascade effects that catalyse the colonization by heterotrophs and enchance the natural capital. This role played by *E. amentacea* is even more relevant in a hostile zone, like the intertidal zone, which is characterised by deep variations in climate condition.

In Cannarozzi et al. (2023), biomass interannual variations were tested and found to be not significant, hypothesizing that the first time of sampling (October 2020) could have occurred in a period when branches start to fall and animals to move away, but still the *E. amentacea* ecosystem keeps, even partially, its vigor, thus resulting quite similar in terms of biomass to the second time of sampling (July 2021), typical of the vegetative period. However, the natural capital is a system measure, considering not only the biomass magnitude but also the quality and complexity of the trophic network established in the ecosystem itself. This is why natural capital is able to detect differences that the sole biomass measure fails to capture.

Furthermore, in the context of natural capital theory, the cauloid can be associated to the stock (natural capital) while the branches can be related to the generated functions (flows). This can be interpreted like a bank account where cauloid represents the savings and branches the interests. Consequently, in this research the value of cauloid natural capital and branches functions were estimated separately considering the assessment in October 2020 as a natural capital value proxy (on average 1.22 em€/m^2 in this study) and the value obtained in July 2021 as a natural capital and flows evaluation (on average 3.72 em€/m^2 in this study). The branches value can be then obtained as difference between the two sampling times: on average, 2.50 em€/m^2 in this study. In these terms, when *E. amentacea* is removed or lost due, for instance, to an anthropogenic impact or a strong marine weather event, a double loss can be envisaged: natural capital (cauloid) and annual functions (branches). Unfortunately, no data are available about *E. amentacea* cauloid age but considering the information about other species of *Cystoseira s.l.* a lifespan of 5-7 years can be considered (Giaccone et al., 2009; Iveša et al., 2022).

This means that considering the values here obtained the total loss can be calculated according to this formula:

Lost value = cauloid value + (branches value* algae lifespan)

Considering results here obtained and a 6 years cauloid age, this means that the value lost equals 16.22 em€/m^2. These remarks make the *E. amentacea* value similar or greater than those ascribed to ecosystems traditionally considered and proved to be the most valuable, like *Posidonia oceanica* meadows and coralligenous outcrops (Paoli et al., 2018; Rigo et al., 2021; Bordoni et al., 2023).

Moreover, since branches sprout in spring and are shed in autumn, the annual renewal of canopy makes *E. amentacea* fundamental for the entire coastal budget: fallen branches provide available organic matter for other ecosystems and become a source of nutrients for neighbouring zones. Therefore, the removal of *E. amentacea* would cause the loss of easily accessible nutrients potentially altering the equilibrium and the health of outer zones.

Finally, the results of this study were compared with the results of De La Fuente et al. (2019), a natural capital study of the habitat located above *E. amentacea* fringe. De la Fuente et al. (2019) sampling was carried out in July 2008 in the Ligurian Riviera: the same geographical zone and sampling period here considered. De la Fuente et al. (2019) results reported a decrease of natural capital value of *E. amentacea* as the continuity and prosperity of the algal belt increases (1.18 em€/m^2 when the belt was not continuous and 0.89 em€/m^2 when the belt was continuous). The current study, instead, shows that *E. amentacea* belt value increases when the ecosystem is more flourishing. Therefore, it can be assumed that when the *E. amentacea* ecosystem becomes more developed, given the microclimate conditions more suitable for life that it creates, the natural capital is attracted and concentrated, depleting the above layer.

Conclusions

The *E. amentacea* ecosystem value has been assessed in two MPAs located in the north and in the south of the Italian peninsula and on two different times (corresponding to the presence of only the perennial part of the algae and to the presence of perennial and deciduous part).

Results, expressed in both biophysical and monetary value through the application of emergy analysis showed no significant spatial variations between the two investigated sites, setting the stage to suggest the low variability of the natural capital and functions value of *E. amentacea* at a regional (100s km) or even larger spatial scale. Temporal variations, on the contrary, were significant, providing the approximate magnitude of changes in the natural capital (cauloid) and functions (branches) during the life cycle of the algae.

When *E. amentacea* forests are flourishing, the value is concentrated within the fringe belt impoverishing the immediately upper zone: *E. amentacea* plays as a value keeper and catalyst. This role is even more important in the intertidal, where conditions for life are extremely difficult and make the value of *E. amentacea* rising to the level of those communities considered as the most valuable ones.

Such findings provided a more detailed analysis of the natural capital of *E. amentacea* forests and contributed to fill the gap about this ecosystem in national protocols for environmental accounting of coastal habitats, allowing the inclusion of its value in international management policies and highlighting the need for the preservation of the habitat itself, but also of the environmental conditions that enable its persistence.

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CONCLUSION

This research has focused on the assessment of the natural capital associated to *E. amentacea* stands. On one hand, it has contributed to expand the baseline knowledge on this species, and more generally, on *Cystoseira s.l.*, which is essential to successfully conserve and manage these pivotal habitat-formers. On the other hand, it has clarified some methodological aspects that may be conducive to more reliable quantifications of the natural capital associated to these macroalgal stands, and to highlight their contribution to the functioning of costal ecosystems.

E. amentacea generally experience significant seasonal changes in nutrients and organic carbon in response to change in phenology during their annual cycle. Interestingly, I found that variations of these traits are particularly important at small-scale (1000s m), reflecting the role of local environmental conditions in influencing these key functional attributes, that can exceed average large-scale effects on biomass production and composition. From a more practical perspective, these results stressed the need for an accurate quantification of carbon and nutrient tissue contents, that should be carried out in replicate sites, to avoid erroneous estimations of the functional performances of *Cystoseira s.l.* (e.g., carbon storage, nutrient cycling, biomass production) when extrapolating them over large geographic areas. Results on interspecific comparisons, seems to suggest a potential similarity among species in these key traits, at least considering *E. amentacea* and *Cystoseira compressa*. Understanding whether this congruence in traits can be common for co-occurring species that share morphological features can facilitate the quantification of functional performances, or of the natural capital, of *Cystoseira s.l.* which rarely form monospecific forests.

For the first time, to my knowledge, this study provided a large set of data on biomass, carbon, and nutrient contents of *E. amentacea* that includes quantifications at multiple spatial scales, along with reliable weight-to-weight conversion factors for *E. amentacea* biomass. This information may help future research to improve our understanding of the contribution of this macroalgae to the functioning of the costal ecosystems.

Understanding the real value of this *Cystoseira s.l.* forests is fundamental for their management. Therefore, the *E. amentacea* ecosystem value has been assessed in two Italian MPAs, also considering two different sampling times: 1) when only the perennial part of the algae, the cauloid, persists (cold seasons) and 2) when both perennial and deciduous parts are presents (warm seasons). Results, showed that not significant spatial differences in the two investigated sites, making possible to assert the stability of natural capital and functions value of *E. amentacea* all along the Mediterranean basin. On the contrary, temporal differences were found, highlighting the amplitude of changes in natural capital and function during the life cycle of the macroalgae. Indeed, thanks to the high biomass turnover, the real value of this macroalgal stands could be defined by two key elements: the perennial basal cauloid and a deciduous part (branches) that are generated every year. The branches not only increase the algae biomass but also create a complex three-dimensional habitat, making *E. amentacea* a biodiversity hotspot and make the natural capital increase. Furthermore, since branches born in spring and dye in autumn, the annual renewal of canopy makes *E. amentacea* fundamental for the entire basin budget: fallen branches provide available organic matter for other ecosystems and become a source of nutrients for neighboring zones. Indeed, the cauloid can be associated to the stock (natural capital) while the branches can be related to the generated functions (flows). In this contest, the loss of *E. amentacea* canopies could cause the loss of easily accessible nutrients potentially altering the equilibrium and the health of outer zones.

Intertidal and subtidal *Cystoseira s.l.* forests are declining in the Mediterranean Sea under the effects of multiple and interacting pressures, including climate change, raising wide concerns on their future fate. The assessment of the effects of full protection on *E. amentacea* stands in the two Marine protected Areas outlined a substantial lack of effectiveness of protection on their biomass and the understory assemblages, highlighting those human pressures cannot be effectively mitigated within reserves, and calling for more intense efforts tailored to protect Mediterranean rocky reefs and the associated macroalgal stands. Baseline knowledge of key species traits and accurate assessments of the good, services, and the overall natural capital associated to marine *Cystoseira s.l.* forests, are essential to support policy makers in refining and implementing effective management strategies that could preserve the important ecological role of these species for the functioning of coastal ecosystems and for the provision of benefits to the human society. This study represented a further step in this direction.

SIDE PROJECT_ASSESSING AN ALIEN SPECIES IMPACT TROUGHT THE ESTIMATE OF THE NATURAL CAPITAL

Donor-side and user-side evaluation of the Atlantic blue crab invasion on a Mediterranean lagoon

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Abstract

The recent invasion of *Callinectes sapidus* in the Lesina Lagoon has raised great concern about its potential impacts on the ecosystem and on local fisheries. The effects of the blue crab presence on the receiving ecosystem were evaluated from both a donor-side perspective, through the application of emergy analysis, and a user-side perspective, by means of interviews to the local fishermen. While emergy analysis showed that *C. sapidus* brings to an increase of both natural capital and ecosystem functions values, results from interviews highlighted that the major problem caused by the presence of the blue crab in the lagoon concerned the local economy. As the first quantitative assessment of the ecological and economic impact of *C. sapidus* in invaded habitats, the present investigation provided original and useful information for a comprehensive risk assessment of the species in European waters and in Mediterranean Sea.

Keywords

Invasive alien species, Lagoon ecosystem services, Biophysical assessment, *Nanozostera noltii,* Natural capital, Maximum empower principle

Introduction

Non-indigenous species (NIS) are species that were introduced outside of their previous or present natural range by human activities (Rotter et al., 2020). In many cases these are able to survive and reproduce in the new environment (CBD, 1993). According to the IUCN (2018), in Europe there are over 12,000 NIS, 15 % of which are invasive. Whenever NIS colonize permanently the new environment, if they aggressively spread in other areas with potential threats to the local biodiversity and/or economic damage, they become invasive species (Molnar et al., 2008). Invasive Alien Species (IAS) can alter the equilibrium between biotic and abiotic components of an ecosystem bringing different effects and changing the ecosystem stability. They can compete with indigenous species, increase diffusion of new diseases, and bring negative effect on the economy of colonized areas. For these reasons, IAS are considered one of the most direct drivers of biodiversity loss and depletion of ecosystem services (Corriero et al., 2016; EEA, 2012). The Mediterranean Sea is among the marine regions most exposed to bio- invasion (Cerri et al., 2020) with recent estimates of >1000 NIS in the basin, 10 % of which being invasive (Zenetos and Galanidi, 2020). Especially crustaceans are emblematic of this ongoing process, ac- counting for about 1/5 of Mediterranean NIS, and in particular bra- chyurans (Brockerhoff and McLay, 2011). An emblematic example is the Atlantic blue crab *Callinectes sapidus* (Rathbun, 1986), which is rapidly expanding in the Mediterranean basin, and especially in transitional water systems, finding optimal conditions for their settlement also due to climate change (Mancinelli et al., 2013). *C. sapidus,* is a decapod crustacean belonging to the Portunidae family that inhabits estuaries, lagoons and shallow waters up to 90 m depth. Original from the west coast of the Atlantic Ocean, the blue crab was introduced in Europe at the beginning of the 20th century through ballast water (Nehring, 2011). Thanks to its physiological characteristics, the Atlantic blue crab can tolerate a wide range of temperature and salinity, and it can colonize different environments (Dulčic etal.,2011). In the native habitats, *C. sapidus* is considered a keystone species, because it plays an integral ecological and economic role. For all these reasons, *C. sapidus* has long been recognized to influence the structure and function of benthic food webs, either as a keystone species regulating carbon flows by reducing prey abundance and inducing trophic cascades. As a consequence, as in the native areas (Williams, 1984; Kampouris et al., 2019), the blue crab could represent an essential food and income resource (Seafood Watch Consulting Researcher, 2013; Cilenti et al., 2015).

Nonetheless, in the areas of recent colonization, *C. sapidus* is considered a threat. In the Mediterranean Sea, negative interactions were recorded with endemic biodiversity and local economy (Olenin et al., 2014; Kampouris et al., 2019) even if scarce information is available on the ecological impact of the species in invaded habitats to date.

The Lesina lagoon (SE Italy, Adriatic Sea) is known as an invaded system in Mediterranean where the abundant presence of the blue crab is reported (Cilenti et al., 2015, 2016; Renzi et al., 2020). This is a lagoon ecosystem largely exploited by humans: fishing and aquaculture activities represent a significant income source for the local economy (Breber et al., 2009). In this study Lesina lagoon was taken into consideration in order to: assess the direct impact of Atlantic blue crab on the local benthic community (with a focus on *Nanozostera noltii* seagrass bed), estimate the effect of the invasion on the natural capital and on the ecosystem functions' provisioning through emergy analysis, evaluate potential outcomes on the local economy. Emergy method is a “donor side” approach that provides a biophysical and monetary evaluation of natural capital and supporting flows. In recent years, the concepts of natural capital, ecosystem functions and ecosystem services become widely known: the great merit of ecosystem services theory is the ability of highlighting the relationships between natural realm and human economy (Sukhdev et al., 2010). Natural capital comprises all natural resources required for the economy and the human development. Ecological functions arise from natural capital components' interaction while ecosystem goods (e.g., energy) and services (e.g., ability to purify water) are the benefits that humans obtain, directly or indirectly, from ecosystem functions. Even when humans do not use some functions or even perceive their existence, our survival and our well-being depend on them. Natural capital value should be then maintained precautionary constant since the modification (in particular the depletion or the compromising) of a function can generate consequences affecting good or services that humans use (Paoli et al., 2022).

The assessment of natural capital must be precise and reliable, and, for this reason, it requires scientifically valid environmental accounting methods (Vassallo et al., 2007). In particular, emergy analysis allows to quantify the value and the changes in natural capital stock and in ecosystem functions it generates, providing results easily understandable by policy makers and other stakeholders. In this study, a three-steps approach was developed: (1) realization of a field experiment with a controlled introduction of blue crabs to evaluate the effect of the invasion on the benthic community; (2) estimate of natural capital value and ecosystem functions supporting the lagoon system through emergy analysis application on results from the experiment; (3) questionnaires administration to fishermen working in the lagoon to understand the impact of the Atlantic blue crab on local economy.

Materials and methods

Study area: Lesina Lagoon

Lesina Lagoon (N 41.88◦, E 15.45◦) is one of the largest wetlands of the central and southern Italy, situated on the southern Adriatic coast in the Apulia region. Lesina lagoon is a semi-closed system influenced by both fresh and marine waters. The lagoon covers a 51.36 km2 area with a maximum depth of about 1.5 m. The catchment area is about 600 km2. The lagoon is characterized by shallow waters and limited exchanges with the sea, as many other Mediterranean lagoons, characterized, if compared with Atlantic systems, by a less influence of tides and a lower freshwater input (Breber et al., 2002; Manzo et al., 2016). Due to the limited tidally-driven exchanges between the lagoon and sea, the hydrology of the Lagoon is strongly influenced by precipitation, evaporation and freshwater inputs, which explain also the heterogeneous spatial distributions of several environmental variables (Roselli et al., 2009). The lagoon's main hydrological features, temperature and salinity, follow a seasonal trend, with minimum values in winter and maximum ones in summer. Temperatures range from 3 to 32 ◦C, salinity from 5 to 38 ppt and an average annual precipitation equal to 400–700 mm (Vignes et al., 2010; Roselli et al., 2009). Lesina lagoon is characterized by an east–west salinity increasing gradient that is the main driver of the distribution patterns of seagrass species and macrobenthic assemblages (Manzo et al., 2016). Lesina lagoon is an important habitat for various plants and animal species, and it plays a major role in the local economy in terms of fisheries and tourism (Ferrarin et al., 2014). The seagrass *Nanozostera noltii* covers most of the Lagoon bed. Seagrasses, in recent years, are suffering from a drastic decline due to the rise of the water turbidity and other anthropogenic causes. In the lagoon there are also numerous species of fish caught by small artisanal fisheries and reared through extensive aquaculture practiced for many years, exploiting the migratory movements of the euryhaline marine species between marine and lagoon environments. Lagoon management is, therefore, recognized as the main instrument to preserve the ecological features and prevent the depletion of valuable aquatic resources and degradation of sensitive habitats (Manzo et al., 2016). For these reasons Lesina lagoon, which is part of the Gargano National Park, is designated as both Special Protection Area (SPA-IT9110037) and Site of Community Importance (SC- IT9110015), following the implementation of the Birds and Habitats Directive (2009/147/EC, 92/43/EEC).

However, due to its physical characteristics the lagoon of Lesina, like the other semi closed environment sites such as harbour and brackish water, is more prone to invasion than pristine sites. Indeed, first record of *Callinectes sapidus* in this ecosystem is reported by Florio et al. (2008) in 2007, where five exemplars of blue crab were caught by fyke nets in June, July and October 2007. Moreover, the first record of ovigerous *C. sapidus* was reported in 2015 by Cilenti et al. (2015) where mature female were caught in four sites near the channels during the breeding migration to more saline waters.

Field experiment

The effect of the introduction of *C. sapidus* on the macrobenthic community of the lagoon was tested carrying out a manipulative field experiment (Fig. 1a). According to the procedure following by Garbary et al. (2014) Four square mesocosms (3 × 3 m) were built within *N. noltii* meadows (Fig. 1b). The mesocosms were made with rigid plastic nets (mesh size of 1.5 cm), attached to long stakes, and firmly buried into the soft substrate. Two mesocosms were assigned as control (without the crab) and two as treatment, where three exemplars of blue crab each were introduced (Fig. 1c, Table 1). The experiment started on the end of July 2018 and ended after 30 days. To analyze the community associated to *N. noltii* and to estimate the impact of the presence of the blue crab on the seagrass bed, both the autotrophic and heterotrophic components have been sampled. Specifically, for macrozoobenthos assemblage three sediment samples per each mesocosm (12 sediment samples in total), were collected in August 2018 using an Ekmann Grab with a surface of 225 cm2. Once the samples were taken, they were rinsed directly in the lagoon with a sieve with a 1 mm mesh size and stored in a fixing solution

(4 % formalin), to preserve macroinvertebrates and the seagrass for the subsequent analysis.

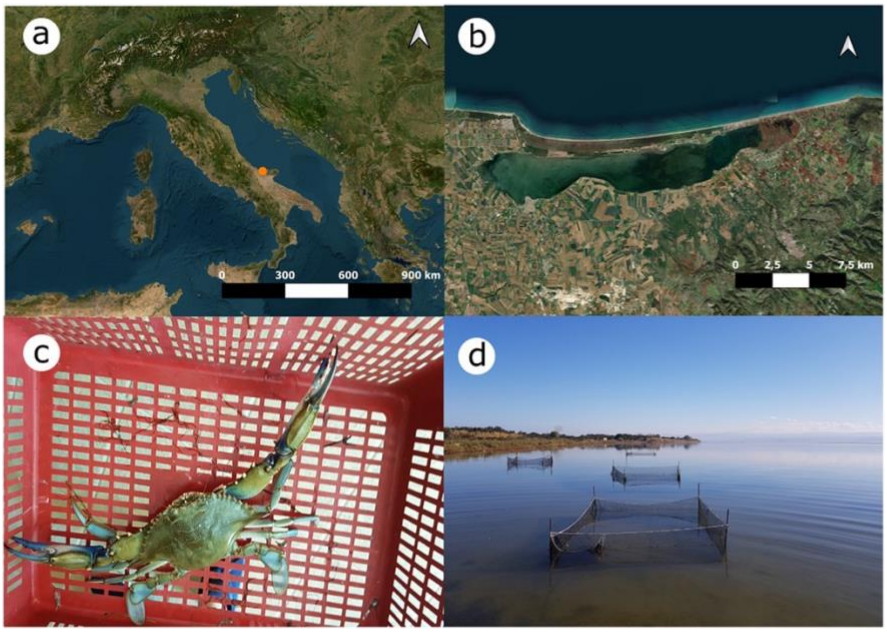

Fig. 1. a) Study site and position of the four mesocosms; b) Lesina lagoon.; c) a specimen of C. sapidus used in the experiment; d) detail of the four square mesocosms built on *Nanozostera noltii* meadows in the Lesina lagoon.

Table 1. *Callinectes sapidus* body measure, experimental mesocosm 1 and 2. Table shows the gender, carapace width (CW), carapace length (CL) and carapace weight (CWW).

gender	CW (mm)	CL (mm)	CWW (g)
F	175.00	70.00	223.20
F	160.00	68.00	196.00
F	155.00	70.00	177.00
M	151.00	60.00	170.80
M	115.00	55.00	99.80
M	115.00	55.00	75.00

Laboratory analysis: community assessment

The total number of shoots of *N. noltii* in the samples and their biomass were quantified to assess the eelgrass bed status. In the laboratory the orthotropic rhizomes, with live leaves attached, were rinsed and separated from dead leaves and the macrozoobenthos, and successively counted to obtain the total number of shoots per sample. This led to an evaluation of the *N. noltii* density in each mesocosm. According to Buia (2003), the biomass of the orthotropic rhizomes was estimate as dry weight by weighting the rhizomes, earlier wrapped in foil, and placed in an oven at 60–80 ◦C for at least 24 h until it reaches a constant weight. For the analysis of macrozoobenthic assemblages, samples were sorted separating all benthic organisms from the sediment and from the seagrass leaves. Then, each organism was identified at family level, counted and preserved in a labelled Eppendorf with a solution of 70 % alcohol. Finally, the biomass of macrobenthos, aggregated at class/ phylum level, was estimated as wet weight.

Data analysis

Data about orthotropic rhizomes, macrozoobenthic biomass and density of shoots of *N. noltii* and benthic fauna were used as response variables to assess the impact caused by the presence of the *C. sapidus*. To verify if the impacted ecosystem is different from the original one, a nested analysis of variance (ANOVA) was performed, using the software R to test for differences in: 1) biomass and abundance of main invertebrate taxa (i.e., Mollusca, Polychaeta, and Crustacea), 2) the total abundance and biomass of macrozoobenthos, and 3) the number and biomass of orthotropic rhizomes. In all cases, the design for the analysis included two factors: Treatment (Tr, two levels, presence and absence of C. sapidus, fixed) and Mesocosm (Me, (Tr) two levels, control and experimental, random, nested in Tr).

Natural capital and ecosystem functions assessment

The investigated lagoon system is characterized by a stock of natural resources that constitutes the natural capital and provides functions and ecological services, required to support life and generate well-being. To assess the value of natural capital and ecosystem functions of the investigated system and to assess the effect of *C. sapidus* introduction, the emergy analysis was applied. This method, introduced by H.T Odum (1988, 1996), represents a “donor side” approach to measure natural capital and ecosystem functions value. The estimate is realized by assessing the costs of capital and functions production and maintenance in terms of biophysical flows used to support their generation and functioning (Ulgiati et al., 2011). In this method, all inputs supporting the system are accounted for in terms of their solar emergy, defined as the total amount of solar energy, directly or indirectly, required to make a given product or to support a process, a territory or a population (Paoli et al., 2018), and measured as solar equivalent Joules (sej) (Odum, 1996). Therefore, the more energy is embodied in generating natural resources and ecosystem functions, the greater is their value (Odum, 1988, 1996), since higher was the cost bearded by the nature to maintain them.

The conversion of inputs flows to solar energy is done through a conversion factor named Unit Emergy Value (UEV): the equivalent solar energy needed to obtain a unit of a certain product. UEV unit of measure is sej/unit (sej/J1, sej/g1, sej/€1). UEVs represent a measure of the environmental support provided to a system: the higher the UEV of a product corresponds the greater the environmental cost to produce it (Brown and Ulgiati, 1997; Franzese et al., 2009). When the UEV is expressed in sej^{-1} is named transformity. The transformity is a quality and efficiency indicator, indeed high transformities characterize com- plex processes and high-quality products. The outcomes of an emergy assessment in sej can be translated into currency equivalents values using the emergy-to-money ratio (EMR) (Lou and Ulgiati, 2013). EMR represents the average amount of emergy needed to generate one unit of money in the national economy (Odum, 1996). In this way the importance of natural capital and ecosystem functions could be better understood by policy makers and other stakeholders allowing an effective communication in socio-economic contexts (Turcato et al., 2015; Vassallo et al., 2021). To assess the value of natural capital and ecosystem functions of Lesina lagoon, emergy analysis was applied following the procedure described by Vassallo et al. (2017) and Paoli et al. (2018, 2022).

The biophysical value of resources stock stored in living structures of the system is a proxy of natural capital, whereas the ecosystem functions are measured as annual flow of resources supporting the system (Paoli et al., 2018; Vassallo et al., 2021). Natural capital evaluation is based on the identification of the main benthic trophic groups composing the food web of the studied ecosystem (namely lagoon ecosystem in this study). Once trophic groups were identified, their biomasses were assessed. The benthic biomasses data were obtained by laboratory analysis and transformed in gC/m2 (the amount of carbon contained in the organ- isms). Biomasses originate through food network, starting from photosynthesis: all inputs required to initiate and keep going the photosynthetic process must be then assessed (i.e., nutrients and natural resources). The amount of nutrients (carbon, nitrogen and phosphorus) stored in the primary biomass together with the flows of natural resources (i.e., solar radiation, wind, chemical energy of rain, geothermal flow and freshwater) involved in the process of primary biomass accumulation were calculated by means of the formulas expressed in Table 2. Similarly, the flows of nutrients and natural resources exploited on a yearly base by the biomass itself were accounted to estimate the ecosystem functions (Paoli et al., 2018). Formulas for functions assessment are similar but referred to a yearly basis. For the sake of clarity, the results of the experimental mesocosms are reported both considering and excluding the contribution due to the blue crab. This means that in the first case the community performances are assessed considering the biomass of the crab while in the second this biomass is removed from calculations. Finally, the assessment of the ecosystem functions made also possible the assessment of the system's self-sufficiency (Paoli et al., 2018). This is made comparing the intensity of flows consumed by the system with the flows generated by the system itself: accordingly, a system is defined in surplus when available flows are greater than exploited (time of stock formation is lower than one year) and in deficit when the system is expected to entirely exploit the internal flows and it gets missing resources from other, external systems.

Table 2. Formulas for the calculation of nutrients and natural resources flows that sup- port the natural capital generation.

Items	Formula	Unit	References
Carbon	Benthic biomass	g	This work
Nitrogen	Benthic biomass ·7/41	g	This work
Phosphorus	Benthic biomass /41	g	This work
Solar radiation	annual solar radiation per unit area area · (1-albedo) · area · time for stocks formation	J	This work
Rain (chemical energy)	annual rainfall · Gibbs free energy · water density · area · time for stocks formation	J	Odum, 1996
Wind	air density · drag coeff. · (wind speed · geostrophic wind velocity)^3 · area · seconds per year · time for stocks formation	J	Odum, 1996
Geothermal heat	area · geothermal flux · time for stocks formation	J	Odum, 2000
Fresh water	annual fresh water · Gibbs free energy · water density · area · time for stocks formation	J	Odum et al. (2000)

All these inputs have been then converted into emergy units by using specific UEVs (Odum, 1996), reported in Table 3 and finally added up to obtain the total emergy value of the system.

Table 3. UEVs employed for emergy analysis based on 15.20E+24 sej/year emergy baseline (Brown and Ulgiati, 2010).

Items	UEV	References

Carbon	1.02E+08 sej/g	Campbell et al. (2014)
Nitrogen	7.40E+09 sej/g	Odum (1996)
Phosphorus	2.86E+10 sej/g	Odum (1996)
Solar radiation	1.00E+00 sej/J	Odum (1996)
Rain (chemical energy)	2.93E+04 sej/J	Odum (1996)
Wind	2.41E+03 sej/J	Odum (1996)
Geothermal heat	5.53E+04 sej/J	Odum (1996)
Fresh water	7.81E+04 sej/J	Odum et al. (2000)

The obtained biophysical values have been then converted into monetary terms applying the EMR, here corresponding to 9.60E+11 sej/ € (Pereira et al., 2013). Monetary equivalents of biophysical values have been expressed in emergy-euros (em€).

Impact of Callinectes sapidus on local economy

In recent decades, community participation has acquired a crucial role in the context of environmental management. This means that, apart from the results gathered from the analytical studies, social and cultural values, economic realities and political factors are also important (Gutrich et al., 2005). For this reason, human perception is a key factor that needs to be considered during the decision-making process (Petrosillo et al., 2010). To assess the damage caused by the presence of the Atlantic blue crab on local economy, data were collected by means of questionnaires administered to a sample of 20 among the 70 fishermen working in Lesina lagoon, through face-to-face interviews from April to August 2018. Each interview lasted between 20 and 30 min and each question was read to interviewees by interviewers who recorded the answer. Questions were structured to collect data on the fisherman's perception and awareness about the damaged caused by the presence of *C. sapidus* in the lagoon ecosystem, and to estimate the economic loss due to blue crab invasion. The questionnaires were articulated in three main sections:

1. Characterization of fishing activity in Lesina lagoon: hours and workdays, main species withdrawn in the lagoon;

2. Fishermen's knowledge about *C. sapidus*: where is it from, how it reached the lagoon and economic value;

3. Damage caused by *C. sapidus*: damage on ecosystem and on human activities, decrease of hours and days of work after the invasion.

Results

Community assessment

ANOVA did not detect a significant effect of the presence of *Callinectes sapidus* on the biomass of *Nanozostera noltii* beds (Table 4), although inspection of the graph in Fig. 2 suggests a reduction of biomass in treated mesocosms. In contrast, ANOVA detected a significant decline in the shoot's density of seagrass in mesocosms where *C. sapidus* was added with respect to controls (Table 4).

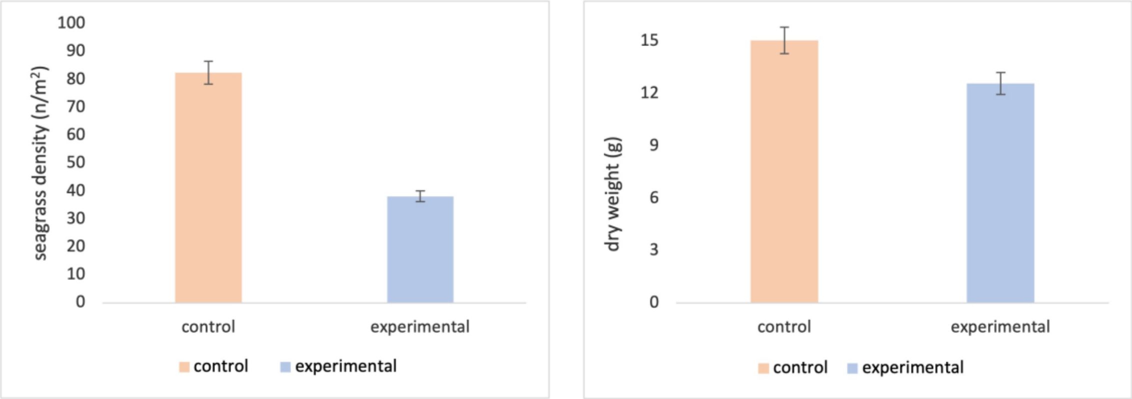

Fig. 2. *Nanozostera noltii* study. Data of abundance and biomass of orthotropic rhizomes were used to produce measures of eelgrass bed decline.

Table 4 .Nested ANOVA results for both abundance and biomass data of orthotropic rhizomes in all the mesocosms.

	Density (n/m²)					**Biomass (g/m²)**				
	Df	SS	MS	F	P	Df	SS	MS	F	P
Tr	1	5896.000	5896.000	13.196	0.006 **	1	18.330	18.250	0.290	0.605
Me(Tr)	2	1822.000	911.000	2.038	0.193	2	36.800	18.380	0.292	0.755
Residuals	8	3575.000	447.000			8	504.00	63.010		

*p < 0,001 ***

Macrobenthic assemblages (abundance and biomass of the main taxa founded are reported in Appendix A) were dominated by Polychaeta that are the 56 % of the associated community, in particular Nereididae, followed by Mollusca (25 %), mainly belonging to Semelidae family (20 %), meanwhile Crustacea represent only a 1 % of the macrobenthic assemblage (Fig. 3). ANOVA shows that there were no significant dif- ferences between the control and the treated mesocosms (with the blue crab) 1) in the abundance and biomass of the main macrozoobenthic groups and 2) the abundance and biomass of the whole macrozoobenthic community (Table 5).

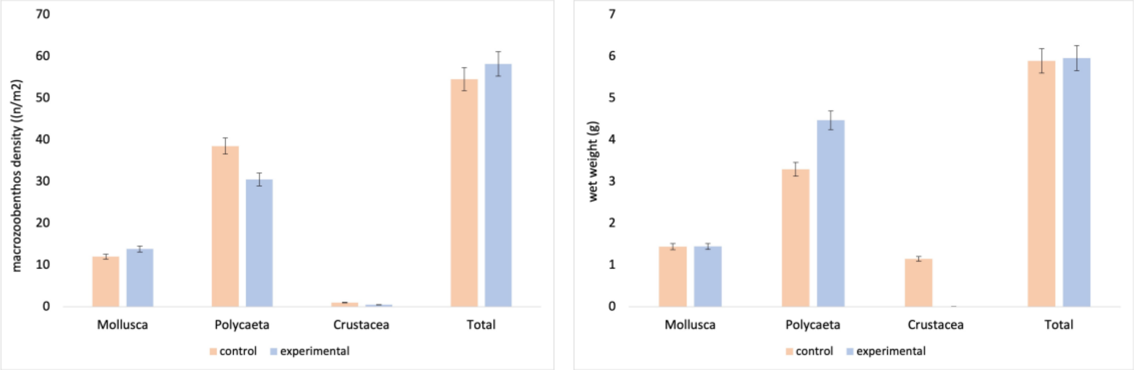

Fig. 3. *Nanozostera noltii* study. Data of abundance and biomass of orthotropic rhizomes were used to produce measures of eelgrass bed decline.

Table 5. ANOVA results on abundance and biomass of macrozoobenthic community. The design for the analysis included two factors: Treatment (Tr, two levels, presence and absence of *C. sapidus*, fixed) and Mesocosm (Me, two levels, random, nested in Tr). Table shows degree of freedom (Df), sum of squares (SS), mean of squares (MS), f- value (F) and p-value (P).

	Abundance (n/m^2)					**Biomass (g/m^2)**				
	Df	SS	MS	F	P	Df	SS	MS	F	P
Mollusca										
Tr	1	10.100	10.100	0.200	0.700	1	4.100	4.100	1.900	0.200
mesocosmM(Tr)	2	206.800	103.400	1.900	0.200	2	13.900	6.900	3.100	0.100
Residuals	8	428.000	53.500			8	13.900	2.200		
Polychaeta										
Tr	1	192.000	192.000	1.300	0.300	1	0.000	0.000	0.000	1.000
Me(Tr)	2	40.300	20.200	0.100	0.900	2	0.200	0.100	0.100	0.600
Residuals	8	1210.700	151.300			8	1.300	0.200		
Crustacea										
Tr	1	0.750	0.800	0.500	0.500	1	3.900	3.900	0.900	0.300
Me(Tr)	2	2.800	1.400	0.900	0.400	2	7.900	3.900	1.000	0.400
Residuals	8	12.700	1.600			8	3.900	3.900		
Total										
Tr	1	40.300	40.300	0.200	0.700	1	0.000	0.000	0.000	0.900
Me(Tr)	2	365.700	182.800	0.800	0.500	2	14.100	7.000	1.400	0.300
Residuals	8	1826.700	228.330			8	40.700	5.100		

Natural capital and ecosystem functions assessment

The emergy system diagrams of the Lesina lagoon before and after the *Callinectes sapidus* introduction are reported in Fig. 4. They show the external sources feeding the system (depicted as circles around the main box), the internal processes (within the main box) and the provided services (as output).

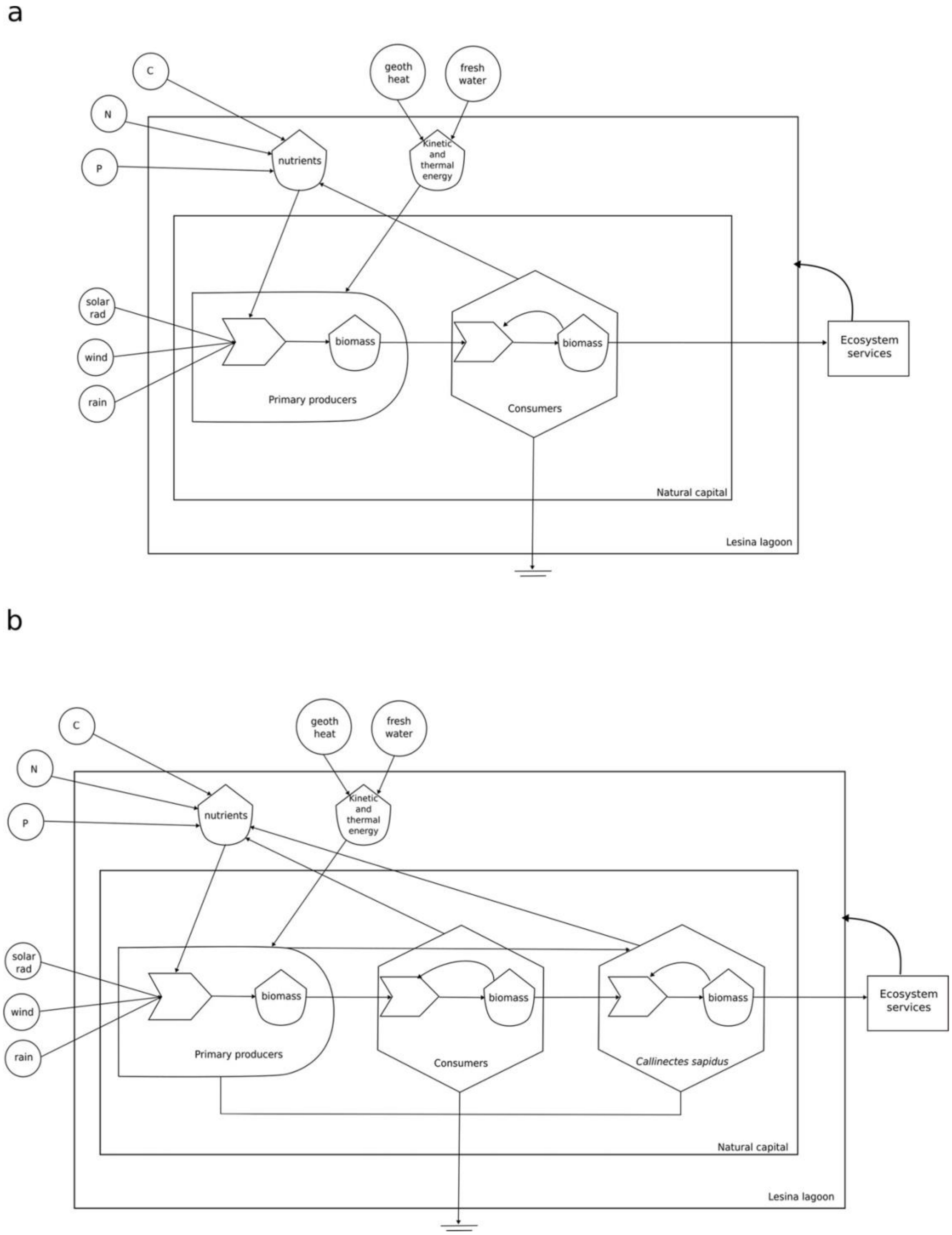

Fig. 4. System diagram of Lesina lagoon; respectively without (a) and with (b) the presence of the blue crab.

The emergy associated to flows of energy and matter identified in the diagrams in 3 is presented in Tables 6 and 7.

Table 6 . Conversion in emergy units of the flows required to create the natural capital stored in the Lesina lagoon. UEVs are reported in Table 3.

Input	**Quantity**				**Emergy (sej)**		
	Unit of measure	Control	Experimental (considering crab)	Experimental (excluding crab)	Control	Experimental (considering crab)	Experimental (excluding crab)
Carbon	g/m^2	2.24E+01	4.60E+02	1.26E+01	2.29E+09	4.71E+10	1.29E+09
Nitrogen	g/m^2	3.83E+00	7.86E+01	2.15E+00	2.83E+10	5.82E+11	1.59E+10
Phosphorus	g/m^2	5.47E-01	1.12E+01	3.08E-01	1.57E+10	3.22E+11	8.81E+09
Solar radiation	J/m^2	6.30E+08	1.56E+09	6.10E+08	6.30E+08	1.56E+09	6.10E+08
Rain	J/m^2	7.33E+05	1.82E+06	1.39E+06	2.15E+10	5.33E+10	4.08E+10
Wind	J/m^2	9.92E+06	2.46E+07	9.62E+06	2.39E+10	5.95E+10	2.32E+10
Geothermal heat	J/m^2	6.25E+06	1.55E+07	6.26E+05	3.57E+10	8.87E+10	3.46E+10
Fresh water	J/m^2	6.46E+05	1.60E+06	6.06E+06	4.88E+11	1.21E+12	4.73E+11
Total					**5.76E+11**	**1.94E+12**	**5.64E+11**

Table 7. Conversion in emergy units of the functions flows yearly exploited in the Lesina lagoon.

Input	**Quantity**				**Emergy (sej/year)**		
	Unit of measure	Control	Experimental (considering crab)	Experimental (excluding crab)	Control	Experimental (considering crab)	Experimental (excluding crab)
Carbon	g/year/m^2	2.55E+01	8.73E+02	8.90E+00	2.61E+09	8.93E+10	9.10E+08
Nitrogen	g/year/m^2	4.35E+00	1.49E+02	1.52E+00	3.22E+10	1.10E+12	1.12E+10
Phosphorus	g/year/m^2	6.22E-01	2.13E+01	2.17E-01	1.78E+10	6.10E+11	6.21E+09
Solar radiation	J/year/m^2	1.38E+09	1.85E+09	1.38E+09	1.38E+09	1.85E+09	1.38E+09
Rain	J/year/m^2	1.61E+06	2.15E+06	3.16E+06	4.72E+10	6.30E+10	9.26E+10
Wind	J/year/m^2	1.93E+06	2.57E+06	1.93E+06	4.65E+09	6.20E+09	4.65E+09

Geothermal heat	J/year/m^2	1.42E+06	1.89E+06	1.42E+06	7.85E+10	1.05E+11	7.85E+10
Fresh water	J/year/m^2	1.37E+07	1.83E+07	1.37E+07	1.07E+12	1.43E+12	1.07E+12
Total					**1.23E+12**	**2.70E+12**	**1.25E+12**

Freshwater contribution always plays a major role in the lagoon ecosystem in terms of both natural capital and yearly functions flows that were respectively: 84.72 % and 87.17 % in the control mesocosm; 62.41 % and 52.96 % in the experimental mesocosms including crab; 83.82 % and 85.58 % in the experimental ones excluding crab. In control ecosystem, the second main contribution is due to geothermal heat (6.20 % for the natural capital and 6.38 % for the functions flows). When the experimental system is considered, the second main contribution is represented by nitrogen if the crab is included in calculations (29.96 % for natural capital and 40.84 % for functions flows) and by rain if the crab is excluded (7.23 % for natural capital and 7.41 % for functions flows).

Both natural capital and functions flows showed an increase (equal to about three times and two times respectively) after the crab invasion. On the contrary, when the experimental system is analyzed excluding the blue crab biomass, natural capital and flows decreased. Moreover, Table 8 shows the results about the system's self-sufficiency including and excluding the blue crab: in the first condition the lagoon system reported a deficit condition and the carrying capacity of the ecosystem was exceeded by about 33 %, thus exploiting resources from outside; on the contrary, excluding the blue crab presence the system was able to maintain itself and the quantity of resources exploited did not exceed the carrying capacity of the ecosystem.

Table8. Results about the lagoon system's self-sufficiency.

	Unit of measure	**Control**	**Experimental (considering crab)**	**Experimental (excluding crab)**
Surface exceeding the ecosystem capacity	m^2	0.00	0.33	0.00
Total surface needed to maintain the lagoon system	m^2	1.00	1.33	1.00
Lagoon system condition	-	surplus	deficit	surplus

The results obtained through the emergy evaluation and the corresponding conversion into monetary terms are reported in Table 9.

Table 9. Natural capital and flows values expressed in biophysical (sej/m) and monetary terms (em€/m^2) before and after the blue crab insertion.

	Control	Experimenta II		Unit of measure
		Considering crab	Excluding crab	
Natural capital stock	5.76E+11	1.94E+12	5.64E+11	sej/m^2
	6.00E-01	2.02E+00	5.88E-01	em€/ m^2
Functions flows	1.28E+12	2.70E+12	1.25E+12	sej/m^2 year
	1.33E+00	2.81E+00	1.31E+00	em€/m^2 year

Indirect impact of Callinectes sapidus on local economy

Questionnaires given to local fishermen for the assessment of indirect damage caused by *C. sapidus* in the Lesina lagoon reported that all fishermen consistently catch eel (*Anguilla anguilla*) and sea bream (*Sparus aurata*), followed by the big-scale sand smelt (*Atherina boyeri*) (95 % of fishermen) and the sea bass (*Dicentrarchus labrax*) (75 % of fishermen) (Fig. 5). Instead, blue crab is caught by only the 25 % of the anglers. The time spent working by the anglers decreased by about 30 % after the invasion of *C. sapidus*. Questions about the knowledge of the blue crab and its origin highlighted that only six fishermen, over the interviewed twenty, know where the blue crab is from and how it reached Lesina lagoon. Nonetheless, many of them declared to be aware of the possible damage caused by *C. sapidus* to the lagoon ecosystem. Indeed, thirteen over twenty anglers claimed that, after the invasion of the blue crab, other fish species declined because of the aggressive behavior of the crab. In addition, for the 75 % of the fishermen the major damage imposed by blue crab is on the fishing equipment. Moreover, 45 % of the fishermen said that, after the invasion, the catches decreased too. Using the data obtained from the questionnaires and ascribing sample results to entire fishermen population operating in the lagoon, it was possible to estimate the invasion of *C. sapidus* in Lesina lagoon led to an income decrease of about the 30 %, that could be express in a total economic loss equal to about 200,000 € per year (Fig. 6).

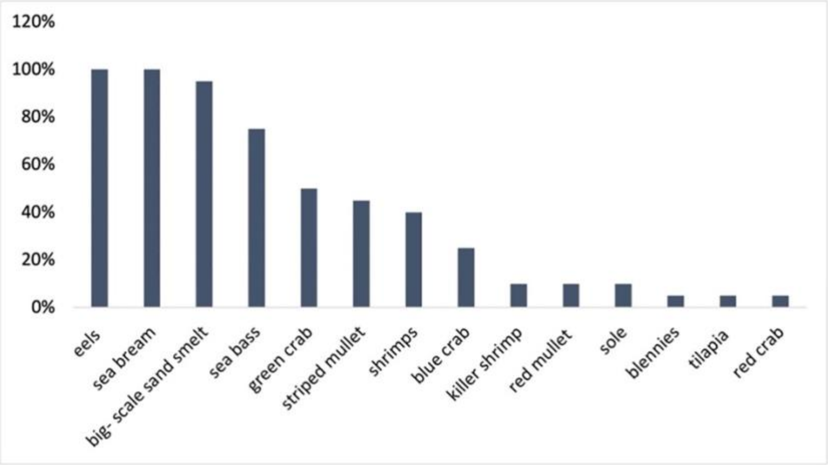

Fig. 6. Main fish species, expressed as percentage, fished in Lesina lagoon.

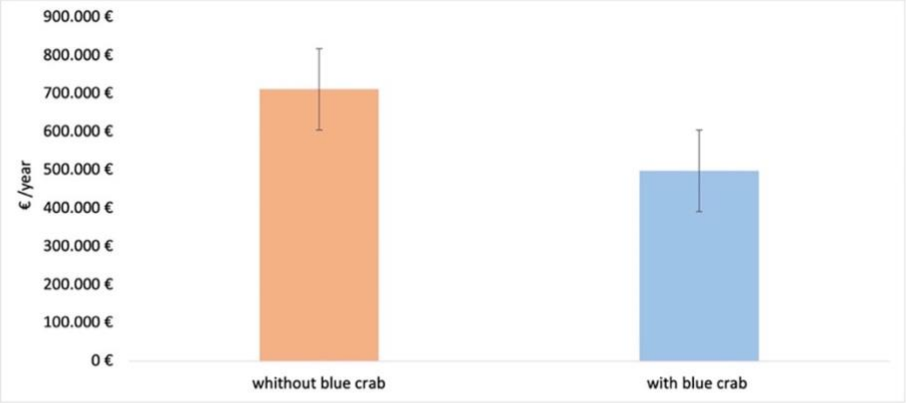

Figure 6. Yearly economic impact derived from the presence of the blue crab in Lesina lagoon.

Discussion

Community assessment

ANOVA results showed that there was a significant change regarding the abundance of the *Nanozostera noltii* seagrass rhizome in the experimental cages (with the blue crab). This is because the crab, with its sifting feeding strategy focused on the consumption of epiphytic in- vertebrates on seagrass leaves, could have a potential role in the decline of the seagrass (Kampouris et al., 2019). On the long term this behavior might have negative effects on the system functioning and on the *Callinectes sapidus* population itself due to the habitat loss. Seagrass meadows represent a potential nursery and feeding area for *C. sapidus*, offering a suitable habitat especially for the early blue crab life stages (postlarvae stages) (Read, 2011; Bilkovic et al., 2021). Further research should be performed after longer period.

The benthos compartment is a major sub-system within the marine system and whose structure and composition represent a reliable indicator of the biological and environmental status (Fabiano et al., 2004; Paoli et al., 2016). In particular, the presence of bivalves of the Semelidae family in the experimental mesocosms highlights a good environ- mental status: these species are totally sedentary and then more sensitive to disturbances (Cilenti et al., 2002). The results of the experimental manipulation, aiming to assess the ecological impact derived from the invasion of the blue crab, showed that there are no significant differences in the whole macrozoobenthic community between the control mesocosms and the experimental one, regarding both abundance and biomass data. This was confirmed by the analysis of variance (ANOVA) where biomass and abundance of the main invertebrate groups (i.e., Mollusca, Polychaeta and Crustacea) were used as response variable dependent by the presence of the blue crab. This is somehow unexpected because the *C. sapidus* invasion is usually assumed to have impact on the abundance of organisms in the receiving system even if a general lack of ecological information may be the cause of an incorrect interpretation (Mancinelli et al., 2017a). Furthermore, a possible future improvement of the assessment might consider the biomass of detrital material and to carry out the field experiment on multiple seasons in order to assess variability that could be due to the *C. sapidus* feeding on *Zostera* seeds as already demonstrated (Fishman and Orth, 1996).

Natural capital and environmental flows assessment

The European Union, with a dedicated action under the EU Biodiversity Strategy to 2020 (COM/2011/0244), calls Member States to assess the state of ecosystems and their services to estimate their eco- nomic value while promoting the integration of such values into national accounting systems by 2020. It is, therefore, urgent to define and apply metrics and assessment frameworks capable of assessing natural capital stocks and environmental flows (Vassallo et al., 2007). For these reasons, in this study, a biophysical approach for the assessment of the natural capital and the ecosystem functions of the system was applied to quantify the effects due to the blue crab colonization of a Mediterranean coastal system. Both natural capital and ecosystem functions increased in the mesocosms impacted by the introduction of *C sapidus*. This is caused by the biomass increase due to the blue crab and to its complexity (in terms of energy requirement) characterized by a higher trophic level than other benthic organisms. In fact, if the experimental system is analyzed: 1) natural capital is more than three times greater when the contribution of the crab is considered in comparison with values excluding crab 2) functions value is more than two times greater when the contribution of the crab is considered in comparison with values excluding crab 3) both stock and flows values become very similar to the control system when the crab biomass is excluded from calculations. Instead, both considering the control system and the experimental system without the blue crab it is evident a natural capital and flows decrease, highlighting the burden suffered by the system to support the invasion. In other terms, the introduction of a non-indigenous invasive species asks for a toll to the receiving system but plays a pivotal role increasing the system's ability to convey, store and exchange energy. This might be read as a manifestation of the maximum empower principle theorized by Odum in its early research (Odum and Pinkerton, 1955; Odum, 1996). This is an interpretation of the general functioning of self-organizing systems (especially biological systems) far from the equilibrium. Formerly the principle was proposed by Lotka in the early 1920s (maximum power principle - Lotka, 1922), and it states that self- organizing systems capture and use energy to develop network designs that maximize the energy flux through a system that is compatible with its environment, and the systems prevailing on the long run are those that maximize power (Odum and Pinkerton, 1955; Odum, 1988, 1995, 1996; Cai et al., 2004; Hall, 2004; DeLong, 2008). Lotka (1922, 1925) defined the maximum power principle as the fourth law of thermodynamics that constrains and guides the self-organization of open systems (Odum, 1995).

From this perspective, the introduction of a non-indigenous species is expected to be successful (invasive) if it can maximize the flow of energy maintaining the system. It is the case of the blue crab in the Lesina lagoon, that had triggered an increase of the natural capital and ecosystem functions. The experimental mesocosms are young ecosystems: ecosystems evolve through time following a strategy of development (succession). If, according to the “fourth law” the maximum empower principle is assumed to drive succession, self-organization will evolve into structures more and more able to utilize energy in the new condition. Holling (1986) depicted the ecosystems developmental behavior drawing a lazy eight figure eight characterized by four primary ecological states: 1) resources exploitation 2) resources conservation 3) creative destruction of stored resources 4) recharge of available resource energy and material to build another ecosystem and start a new cycle. Odum (1999) showed that these four stages produce a pattern of re- sources cycling. Succession is then a pulsing than a linear process. When a pulsing cycle has been established, it occurs in all levels of nature hierarchical organization: if a system is maximizing empower this means that its subsystems are maximizing resources use alike. A level of larger organization is then maintained by the pulsing of smaller scale sub- systems. A sudden variation in energy sources intensity (like blue crab introduction) is then reflected at all hierarchical levels, even if, prob- ably, with less strength at smaller scale (Campbell, 2000). The analyzed system, namely experimental mesocosm, perfectly fits this pattern, with greater changes shown at higher level than at lower ones. Currently it is not possible to assess the development stage of the system and other potential modifications could happen. This evaluation would require an indefinite, probably very long, period of time and will be the object of future researches. As final appraisal it can be highlighted that the increased emergy budget due to the invasion resulted in a deficit condition of the system, in fact, the natural ecosystem functions maintained in the lagoon system require resources not locally available and yielded in external systems (Paoli et al., 2018). This might be dependent on the very high density of blue crab forced in the mesocosms, but it provides an indication of the limits of the system to support a new, energy demanding species whose development must cope with local resources availability.

Effect on the local fisheries

Questionnaires survey is nowadays a powerful tool for investigating the distribution and for gathering key information about stakeholder's knowledge and perception. According with results, after the invasion of the blue crab, the economy of the lagoon seemed to suffer a great damage. Many fishermen declared that the fish available to be caught in the lagoon decreased of about 45 % after the invasion of the crab. Such findings confirmed what occurred in other areas of the Mediterranean Sea where blue crab populations have reached high abundances and has led to considerable negative effects on fishing activities (Mancinelli et al., 2017a). This could be correlated to the predatory behavior of the blue crab actively feeding on economically important fish species, like the Mediterranean seabream and seabass and flatfish, even when they are trapped in the net, prawns or mussel in farming areas (Kampouris et al., 2019). A small fraction of fishermen also claimed that, after the invasion of the blue crab, the hours of work per day increased of about 30 % to fix the damaged equipment. With its strong claws, *C. sapidus* can easily cut the mesh of deployed nets, highlighting that the nets used in the lagoon are not suitable for the blue crab in comparison to the effectiveness of the wire traps used in native counties (Glamuzina et al., 2021). Also, the blue crab fishing has not developed in the lagoon probably because of the low demand and low price (about 3 €/kg) of this species in the local market. On the contrary the blue crab in its native country is considered a valuable seafood and supports an important fishery (Mancinelli et al., 2017b). Indeed, it represents the most important fishery in Chesapeake Bay, providing livelihood and incomes for more fishermen that any other species (EBFM report, Green, 2012). The analysis of questionnaires suggested that there is not enough knowledge about the origin of the *C. sapidus* and its potential as seafood. Management actions tailored to promote the exploitation of the blue crab and improving fishing tools will be therefore essential to increase the resilience of the socio-economic system to the invasion and to con- trol *C. sapidus* populations in the lagoon.

Conclusion and future directions

In its native geographic range, *Callinectes sapidus* is considered a valuable seafood, providing livelihood and more incomes for fishermen that any other species (Mancinelli et al., 2017b). On the contrary in the areas of recent invasion it is consider a treat and a problem to be solved. In this study, the statistical results, where data about the abundance and biomass of the macrozoobenthic assemblage and the orthotropic rhizomes were used as response variables to evaluate the ecological impact derived from the presence of *C. sapidus* (considered as explanatory variables), revealed no significant differences between the control and experimental mesocosms for the macrozoobenthic assemblage, but highlighted a significant change in the abundance of the seagrass rhizomes in the experimental mesocosms. Emergy analysis, which allowed to assess the value of natural capital and functions flows that characterized the investigated system, showed that both obtained values grew of about three times and two times respectively after the invasion of the blue crab in the Lesina lagoon. However, with *C. sapidus* the system resulted in deficit, that means that the primary productivity needed for maintaining this organism is yielded out form the studying system boundaries (Paoli et al., 2018). On the contrary, the questionnaires administered to the local fishermen highlighted that in Lesina lagoon *C.*

sapidus is considered a problem because, with its aggressive behavior can harm other fish species of commercial value and damage the fishing equipment bringing to huge economic losses.

This work highlights the major problem caused by the presence of the blue crab in the lagoon regarding the local economy. Results of the emergy analysis show that the blue crab brings to an increase of the overall value of ecosystem. This suggests the ability of the crab to be turned into a profitable resource. Consequently, the current invasion of the blue crab could offer the possibility of identifying new policies aiming to control the crab population increase and to revert it into profits for local fishermen. For example, in his work Cilenti et al. (2015) proposed a targeted fishery of the mature female during the breeding migration to more saline water. In addition, the correct engagement of stakeholders is imperative to reach that aim. Indeed, for ensuring long- term partnership and provide valuable assets to bridge the gap between science, policy and stakeholders the increase of knowledge is essential (Rölfer et al., 2022). Moreover, promoting *C. sapidus* as valuable seafood and providing fishermen with appropriate equipment, could be the first step for identifying successful policies and management strategies.

CRediT authorship contribution statement

Laura Cannarozzi: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. Chiara Paoli: Conceptuali- zation, Methodology, Software, Formal analysis, Writing – review & editing, Supervision. Paolo Vassallo: Conceptualization, Methodology, Software, Formal analysis, Writing – review & editing, Supervision. Lucrezia Cilenti: Conceptualization, Methodology, Software, Re- sources, Validation, Writing – review & editing, Supervision, Funding acquisition. Stanislao Bevilacqua: Formal analysis, Writing – review & editing, Visualization. Nicola Lago: Writing – review & editing. Tommaso Scirocco: Investigation. Ilaria Rigo: Conceptualization, Methodology, Software, Formal analysis, Visualization, Writing – original draft, Writing – review & editing, Supervision.

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Appendix

Table A.1. Composition of the macrozoobenthic community. Abundance and biomass (wet weight): data are reported as average values of the samples from each mesocosm.

	Abundance (n/m^2)				**Biomass (g/m^2)**			
	average		standard deviation		average		standard deviation	
	control	experimental	control	experimental	control	experimental	control	experimental
Mollusca	**4..00E+00**	**4.61E+00**	**4.95E+00**	**4.63E+00**	**1.10E+00**	**1.49E+00**	**9.71E-01**	**1.59E+00**
Militidae	6..67E-01	5.17E+00	1.21E+00	4.79E+00	3.93E-01	2.17E+00	6.66E-01	2.06E+00
Semelidae	8..83E+00	6.67E+00	6.05E+00	5.85E+00	1.44E+00	9.56E-01	1.06E+00	1.30E+00
Cardiidae	2..50E+00	2.00E+00	1.05E+00	1.41E+00	1.46E+00	1.34E+00	8.54E-01	1.33E+00
Polychaeta	**9.63E+00**	**7.63E+00**	**1.24E+01**	**8.93E+00**	**3.61E-01**	**3.61E-01**	**5.99E-01**	**5.30E-01**
Nereidi	2.63E+01	1.97E+01	1.04E+01	5.82E+00	1.31E+00	1.22E+00	4.47E-01	2.71E-01
Harmothoe	0.00E+00	1.67E-01	0.00E+00	4.08E-01	0.00E+00	6.00E-03	0.00E+00	1.47E-02
Spionidae	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
others	1.22E+01	1.07E+01	5.56E+00	3.33E+00	1.35E-01	2.22E-01	5.17E-02	8.28E-02
Arthropoda	**8.00E-01**	**2.73E+00**	**2.09E+00**	**5.90E+00**	**2.31E-01**	**6.37E-03**	**1.24E+00**	**1.23E-02**
Idoteidae	6.67E-01	0.00E+00	1.21E+00	0.00E+00	1.58E-02	0.00E+00	2.80E-02	0.00E+00
Gnathidae	1.67E-01	5.00E-01	4.08E-01	8.37E-01	2.00E-03	1.00E-02	4.90E-03	1.82E-02
Gammaridae	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
Decapoda	1.67E-01	0.00E+00	4.08E-01	0.00E+00	1.13E+00	0.00E+00	2.77E+00	0.00E+00
Chironomidae	3.00E+00	1.32E+01	4.00E+00	6.15E+00	4.17E-03	2.18E-02	5.23E-03	9.68E-03

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