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ANALISI DELLA BIODIVERSITÀ DELLA MEIOFAUNA COME MISURA DI SUCCESSO DI UN INTERVENTO DI RESTAURO DI *GONGOLARIA BARBATA* LUNGO LA RIVIERA DEL CONERO (MAR ADRIATICO CENTRALE).

ANALYSIS OF THE MEIOFAUNA BIODIVERSITY AS A MEASURE OF SUCCESS OF A *GONGOLARIA BARBATA* RESTORATION INTERVENTION ALONG THE CONERO RIVIERA (CENTRAL ADRIATIC SEA).

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Riassunto

L'incessante declino globale delle foreste di macroalghe brune lungo le coste rocciose del Mar Mediterraneo sta causando grandi squilibri negli ecosistemi associati, portando alla perdita della biodiversità e dei servizi ecosistemici forniti. Distribuendosi batimetricamente fra la superficie e la zona circalitorale superiore, queste foreste svolgono un ruolo fondamentale nei processi di sottrazione del carbonio, ossigenazione, ricircolo dei nutrienti, trasferimento di materia ed energia ai livelli superiori della rete trofica. Nel Mar Mediterraneo, in particolare, moltissimi studi per lo più condotti a livello locale hanno registrato la regressione delle popolazioni di *Cystoseira s.l.* La risposta della comunità scientifica si è tradotta nello sviluppo ed applicazione di diverse tecniche di restauro focalizzate sulla salvaguardia delle popolazioni di adulti in salute e sul miglioramento della fase di reclutamento. In questa tesi si è condotto, per un periodo di otto mesi, un esperimento di restauro di *Gongolaria barbata* nella località della Scalaccia Sud ad Ancona, inclusa nel SIC (Sito di Interesse Comunitario) "Costa fra Ancona e Portonovo". Per l'intervento di restauro sono state applicati sia approcci *in situ*, con il trasferimento di sassi naturali e strutture artificiali su cui sono state fatte attecchire nuove reclute nel sito donatore Scalaccia Nord, sia *ex situ,* con il trasferimento di reclute ottenute in acquari. Nonostante complessivamente il restauro della popolazione di *G. barbata* abbia avuto esito positivo, raggiungendo un incremento di oltre il 70% della copertura

di canopy nel sito ricevente, tuttavia con l'approccio *ex situ* si è verificata la perdita totale delle reclute ottenute in laboratorio dopo soli due mesi dal trapianto nel sito di restauro. I risultati di questo studio suggeriscono inoltre come fattori critici per il reclutamento *ex situ* la tempistica di raccolta delle strutture riproduttive (recettacoli) e di trapianto nel sito ricevente, oltre al controllo della formazione di biofilm sulle strutture artificiali.

Le analisi effettuate sulla meiofauna associata mostrano una risposta significativa in termini di abbondanza totale, e solo parzialmente in termini di struttura di comunità. In particolare, a otto mesi dall'inizio del restauro, soltanto alcuni gruppi risultano rispondere positivamente alla crescita di *G. barbata,* quali policheti, acari, chinoromidi e anfipodi, che corrispondono per la maggior parte a taxa rari.

La ricchezza di taxa sembra mostrare un trend di aumento nel tempo in prossimità del restauro, e arriva ad essere maggiore nei punti restaurati rispetto ai siti di controllo.

Globalmente, i risultati di questo studio suggeriscono che la scelta del sito (avvenuta tramite esperimenti precedenti) è fondamentale per il successo del restauro, tant'è vero che il trapianto ha resisto a molteplici tempeste avvenute nel periodo autunno-inverno. Inoltre, le variabili utilizzate per misurare il successo del restauro hanno dato risultati incoraggianti e suggeriscono la necessità di un programma di monitoraggio su più ampia scala temporale, per verificare il successo dell'intervento su lungo termine e la maturazione della popolazione di *G. barbata* fino all'auto-sostentamento nel tempo.

Index

1. **Introduction** ... 7

1.2 Ecological role of Gongolaria barbata in the Mediterranean ecosystems ... *7*

1.2 Distribution and regression of Cystoseira s.l. forests: main factors that induce degradation .. *12*

1.3 Solutions and approaches to contrast the degradation of macroalgal forests ... *15*

1.4 Afrimed project: purpose and application of techniques aiming at restoring degraded Cystoseira s.l. populations ... *20*

1. **Objectives** ... 24

3. **Material and methods** ... 25

3.1 Study area: SCI and ZPS in the Monte Conero Riviera and selection of the site for the restoration intervention. ... *25*

3.1.1 Geomorphological characteristics of sites ... 27

3.1.2 Biotic characteristics of the experimental site ... 28

3.2 Implementation of in situ and ex situ reproduction approaches. ... *30*

3.2.1 In situ recruitment approach ... 31

3.2.2 Ex situ recruitment approach ... 32

3.3 Restoration intervention and monitoring ... *34*

3.4 Environmental parameters monitored ... *37*

3.5 Response variables ... *38*

3.5.1 G. barbata vegetative phenology ... 38

3.5.2 Meiofauna ... 41

3.5.3 Data treatment and statistical analyses ... 45

4. **Results** ... 49

4.1 Ex situ recruitment ... *49*

4.2 In situ recruitment ... *52*

4.3 Temperature ... *56*

4.4 Algal coverage variation ... *57*

4.5 Meiofauna analysis abundance and diversity ... *58*

5. **Discussion** ... 68

5.1 Ex situ and in situ recruitment ... *69*

5.2 Meiofauna communities' response to the restoration intervention ... *76*

6. **Conclusions** ... 81

References ... 83

1. Introduction

1.2 Ecological role of *Gongolaria barbata* in the Mediterranean ecosystems

In temperate waters, among the canopy-forming species, brown algae belonging to the orders Fucales and Laminariales (Phaeophyceae) dominate globally at intertidal and subtidal depths (Orlando-Bonaca et al., 2021; Verdura et al, 2018). In the Mediterranean Sea are present species of the group *Cystoseira sensu latu* (*Cystoseira s.l.*), one belonging to the *Cytoseira* genus, reordered in 2019 and today including the genera *Gongolaria*, *Cystoseira,* and *Ericaria* (Molinari Novoa & Guiry, 2020). The species belonging to this group have high morphological plasticity that makes very difficult to distinguish them based on morphological characters (Verdura et al., 2018). Although they do not reach the dimensions of kelp (Laminariales) or other fucoids, *Cystoseyra s.l.* produce dense canopy with a maximum height of about 1 meter, forming the so-called brown algae forests, considered one of the most productive and important ecosystems along the Mediterranean coasts (Verdura et al., 2018). These macroalgae are defined as *habitat-forming species* because their vertical growth increases the three-dimensionality and therefore the complexity of the environment, favouring the co-existence of other taxa through the provision of space, food resources, shelter, and nursery areas for many species of fish, invertebrates, and algae (Orlando-Bonaca et al., 2021; Verdura et al., 2013;

Falace et al., 2018). These forests also play a crucial role in nutrient recycling and primary productivity, because branched canopies provide a great deal of secondary substrate for the settlement of macro- and microscopic algae, thus increasing the primary production and making available organic matter to the organisms through the entire trophic web.

Cystoseira s.l. species also play an important role in the adsorption of atmospheric carbon (ca 173 Tgc year 1 seized), they indeed act as carbon sinks (Bernal-Ibáñez et al., 2021), promoting the development of "blue carbon strategies" which investigate the potential of these plant ecosystems in the climate change mitigation effects (Bianchelli & Danovaro., 2020). The ecosystem services, which these algae provide, are comparable with those of land forests (Medrano et al., 2020).

Along the Conero Riviera, the study area of this thesis, the role of ecosystem engineer is played by *Gongolaria barbata* (endemic to the Mediterranean) (Fig.1) and *Cystoseira compressa*. These species are typical of the photophilic zone of the northern Adriatic Sea (Falace and Bressan., 2006). However, in the last decades, in this area, *G.barbata* is undergoing a strong regression. Its presence in the rest of the Mediterranean has always been sporadic due to the lack of suitable environmental conditions (Verdura et al., 2018, Tamburello et al., 2020).

G. barbata is a monoecious species, that has seasonal life cycles, characterized by maturation and release of gametes which occurs generally during the early summer period (Irving et al., 2009). The gametes are contained within fertile portions of the fronds called receptacles and, after the release, the zygotes take root on the free substrate with the formation of several small rhizoids (Irving et al., 2009). Within a few months the thallus develops by apical growth, it is formed by a single perennial cauloid from which depart minor lateral ramifications to form extended and three-dimensional fronds (Irving et al., 2009). The presence of aerocysts keeps the thallus erect. This species has a marked seasonal variation in the phenology of branches; this species experience a growth phase from winter until the late spring/early summer when reaching the reproductive stage with consequent fall of the branchlets (Falace and Bressan., 2006; Perkol-Finkel & Airoldi; 2010). This variation of habitus affects the abundance of epibionts, whose presence is greater with extensive branches (Falace and Bressan., 2006). The increasing frond complexity may also significantly influence the passage of currents and waves and, therefore, the rates of transport and deposition of suspended particles (Falace and Bressan, 2006).

Generally, the adult populations of *G. barbata* have a patchy distribution with patches of small size (usually few square meters), little spaced from each other (Verdura et al., 2018). In many areas, the disappearance of *G. barbata*, or other

Cystoseira species, is indicative of a strong environmental degradation condition (Orlando-Bonaca et al., 2021). In these cases, *Cystoseira s.l.* species are replaced by ones with lower structural complexity, such as turf-forming, filamentous, encrusting algae or other ephemeral seaweeds, mussel beds, or barren grounds (Perkol-Finkel & Airoldi., 2010). According to the Water Frameworks Directive (2000/60/EC) *Cystoseira s.l.* species are implemented as bioindicators of water quality and well-being of the ecosystem (Orfanidis et al., 2014; Bianchelli & Danovaro, 2016).

Figure 1. G. barbata after five months from the beginning of the restoration intervention.

The replacement of habitat-forming algae with turf-forming or encrusting algae seems to further inhibit recolonization by the former ones (Orlando-Bonaca et al., 2021; Maggi et al., 2018). Moreover, natural competition for the substrate between these species, with different vital strategies, is influenced also by anthropic factors that negatively affect the founding species. As consequence, the loss or regression of these underwater forests has particularly strong effects on the abundance, biomass, composition, structure, and biodiversity of the associated benthic assemblages, producing undesirable consequences at the ecosystem level (Bernal-Ibànez et al., 2021). As an example, results in Bianchelli (2016) report that shifts from Cystoseira-dominated forests to barren grounds provoke the reduction of available organic matter causing changes in abundance and composition of the meiofaunal assemblages. Since this benthic component has a key role in the energy and matter transfer to the higher trophic levels, the ecosystem' shift could have negative consequences on the entire ecosystem structure and functioning (Bianchelli & Danovaro, 2020). With the impairment of the overall ecosystem functioning, it can be argued that also the ecosystem services (for example, productivity, water quality, reduction of sediment load, ecotourism, etc.) are compromised.

Due to their distribution in the Mediterranean Sea, ecological characteristics, and sensitivity to anthropogenic stress, *G. barbata s.l.* species are included in

Annex II (List of marine species in danger or threatened in the Mediterranean) of the Barcelona Convention Protocol and defined as "strictly protected" by Annex I to the Berne Convention (Savonitto et al., 2021). According to the Habitat Directive (92/43/EEC) all *Cystoseira s.l.* species are considered as "of community interest" (Bianchelli & Danovaro, 2020). All *Cystoseira s.l.* species, except for *C. compressa*, are under conservation measures or interest (Tamburello et al., 2021).

1.2 Distribution and regression of *Cystoseira s.l.* forests: main factors that induce degradation

Canopy forming species are experiencing a global decline causing the loss of the most diverse and productive ecosystems in the Mediterranean Sea (Bianchelli & Danovaro, 2020), where macroalgal forests' decline is widely documented with high loss of the number of species reported since the last century: only 5 Fucales species of the 14 reported in the 1900s are now present in the Northern Mediterranean (Thibaut et al., 2005). Many studies report different information about the macroalgal forest distribution: some confirm wide fluctuation in canopy-forming species abundance, whereas others describe high temporal stability of the canopy of *Cystoseira* and *Sargassum* forests (Thibaut et al., 2016; Bianchelli & Danovaro, 2020 and citations therein;). This is because knowledge about canopy-forming species' distribution is largely lacking (Tamburello et al., 2021). Data about the distribution of *Cystoseira*-dominated forests are available only for 14% of the Mediterranean coastline, whereas data on their absence were available only for 2% of the basin. Moreover, data (most obtained from a monitoring activity within the EU Water Framework Directive on the western Mediterranean Sea) cover only 15 of 22 Mediterranean countries (Fabbrizzi et al., 2020). Overall, *Cystoseira* canopies are found along the Mediterranean coastline for 6,342.41 km out of a total coastal length of 46,000 km (Fabbrizzi et al., 2020). Recent data report, for this area, dramatic losses of 50 to 80% of Cystoseyra forests (Danovaro, unpublished data).

Local and global stressor as pollution, coastal urbanization, eutrophication, marine traffic, tourism activities, the introduction of alien species, in synergy with the climate change (causing ocean acidification and temperature rise), are directly or indirectly related to human activities and are identified as main drivers of the *Cystoseira s.l.* forests' regression trend (Gianni et al., 2013; Verdura et al., 2018). The introduction of substances (chemical pollutants, plastic waste, nutrients, and many others) causes changes in water characteristics and quality that affect highly sensitive macroalgal species. (Arévalo et al., 2007). Generally, nutrient enrichment should be kept under control because favour the proliferation and reproduction of opportunistic algal species. Instead, in

Tamburello et al. (2020) results suggest that, in oligotrophic systems, moderate nutrient enrichment can favour the resilience of canopy, increasing their competition ability. Moreover, new and emerging uses of marine resources (e.g., seabed mining, aquaculture) are also expected as additional sources of disturbance for marine coastal ecosystems (Wolff et al., 2018). So, anthropogenic stressors can cause strong changes in ecosystem characteristics, provoking fragmentation and deterioration of the habitats and increasing the intensity of natural threats to the macroalgal forest, such as grazing pressure (Tamburello et al., 2019). In this regard, sea urchins and herbivorous fish as goldline (*Sarpa salpa*) represent the most common grazer species and, in some cases, their proliferation is linked to overfishing of their predators, increase of temperature, or alien species that determine trophic web alteration (Medrano et al., 2020; Tamburello et al.,2018). Outbreaks of herbivorous can lead to overgrazing on macroalgal communities determining the formation of naked areas on hard bottoms, called “barren grounds”, dominated by algal turf and encrusting coralline algae (Bernal-Ibanez., 2021; Gianni et al., 2013). When an ecosystem shifts into an “alternate stable state”, presents a high resistance to return to its starting point (Bernal-Ibanez., 2021). Although macroalgal forests show a higher degree of resilience compared to coral reefs or mangroves, their natural recovery it’s really hard and slow (Tamburello et al., 2019; Gianni et al., 2013). This is because the success of the natural recovery is reduced by the natural low dispersal capacity of *Cystoseira s.l.* species, due to the rapid fertilization of its eggs and the sinking of zygotes (Orlando et al., 2021; Gianni et al., 2013). Moreover, studies report that the first development stages are strongly affected by the increase in sedimentation rates, water turbidity (affecting light intensity,) and altered substrate stability (Irving et al., 2009). Overall, these factors could be related to coastal urbanization and activities, as beach nourishment that altered hydrodynamic regime (Irving et al., 2009; Gianni et al; 2013). So, the combination of natural and anthropogenic stressors can lead to a real "regime shift" with consequences at all ecosystem levels, determining changes or loss of the ecosystem services provided.

1.3 Solutions and approaches to contrast the degradation of macroalgal forests

Various strategies have been developed in recent decades to reduce macroalgal forests regression. Although, in 2009, an amendment of the Mediterranean Action Plan (Annex IV, SPA/BD Protocol - United Nations Environment Programme), adopted under the Barcelona Convention, recognized as a priority the conservation of all *Cystoseira s.l* species, excluding *C. compressa*, to date no specific measures have been taken for the protection of these habitat forming species (Tamburello et al., 2019). In the Mediterranean Sea, the establishment of marine protected areas, or the recognition of sites worthy of protection within the Natura 2000 network, are based on the presence of species such as *Posidonia oceanica*, while the presence of *Cystoseira s.l* is only accidental (Tamburello et al., 2019). In the last twenty years, however, the percentage of *Cystoseira* populations protected by MPA, national or regional Park, and Natura 2000 sites has highly increased up to 77.8 % (Tamburello et al., 2021). The definition of these areas works as a passive tool of protection, since the regulations partially or completely limit some human activities such as fishing, tourism, and urbanization. In this way, they favour the natural recovery of the macroalgal populations, however, the process is compromised due to the reduced capacity of dispersion of zygotes (Orlando-Bonaca et al., 2021). For this reason, when *Cystoseira s.l.* disappears from large geographical areas, the population can significantly recover only through active restoration strategies.

Ecological restoration refers to the process of managing or assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed to facilitate the achievement of conditions comparable to those preceding the impact, conserving biodiversity and promote the maintenance of ecosystem services (SER Primer, 2002). Restoration ecology in estuarine and marine environments is a relatively new field, its knowledge and application to date is much less than the actions implemented in terrestrial environment (Gianni et al., 2013). Unlike the restoration strategies of ecosystems made up of charismatic organisms such as corals, whose media impact is very strong, for ecosystems such as kelp or fucoid forests the restoration interventions weren't implemented globally but mainly in certain areas such as the Mediterranean, South and North America, Asia, especially in China, Japan and Korea (Gianni et al., 2013). A recent review reports the publication, considering success, partial success and failure, of only 26 articles on restoration interventions (Fraschetti et al., 2021).

The restoration of macroalgal populations is favoured mainly in areas where the historical presence of the species is documented, or where the environmental conditions are suitable for development. For the effectiveness of restoration, it's essential that the factors induced by regression are terminated (Medrano et al., 2020). In particular, in the Mediterranean Sea, this field is experiencing a phase of experimentation. Here the *Cystoseira s.l.* forests are subjected to three main restoration techniques (Gianni et al., 2013, Orlando-Bonaca et al., 2021, Verdura et al., 2018). The first approach, the most experienced one (Gianni et al., 2013), involves the transfer of adult and juveniles individuals from a natural population to a target site (De La Fuente et al., 2019; Verdura et al., 2018). The thalli can be attached on an artificial or natural substrate with metal hooks or by covering the basal portion with expanded polyurethane or epoxy resin (De La Fuente et al., 2019). According to various studies, the transplantation of individuals can lead to the collapse of the donor populations; to solve this problem, have been developed new non-destructive techniques that aim at improving the recruitment potential (Gianni et al., 2013). These techniques are less invasive, as they are based only on harvesting a small percentage (<5%) of the mature reproductive fronds from wild individuals (Verdura et al., 2018). Then, two possible approaches can be applied: *in situ* and *ex situ*. In the first, the mature reproductive fronds are transferred to the restoration site, placed inside small mesh bags and fixed directly on the new substratum to facilitate the rooting of the zygotes. In the *ex situ* treatment, reproductive branches are transferred to the laboratory and used for recruitment and growth (under controlled conditions) on artificial substrates, which are then transferred to the restoration site. Both treatments were implemented in a *G. barbata* restoration conducted by Verdura et al, in 2018, obtaining high recruitment with a similar trend between the two techniques. Five years after the start of the intervention, the values related to the size classes structure and the size of the restored population were comparable to those of the juvenile and adult reference populations, confirming the success of the restoration (Verdura et al., 2018). According to the authors, these treatments seem suitable for the restoration of other *Cystoseira s.l.* species with similar characteristics (Falace et al., 2018). In particular, *ex situ* approach is suitable for the restoration of species with reduced dispersion capacity (Falace et al., 2018; Verdura et al., 2018). For example, for *Cystoseira amantacea var stricta*, with a dispersion radius of less than 40 cm, the first *ex situ* treatment was developed in Falace et al. (2018). Knowing the species characteristics allows to develop species-specific cultivation protocols to maximize recruits and juvenile survival. The *ex situ* technique was also used in another recent restoration experiment of *G. barbata*, conducted by Orlando-Bonaca et al. (2021) in the Slovenian coasts. The study aimed at evaluating the survival rate and growth of juveniles during the first four months of transplantation, which are the most critical (Orlando-Bonaca et al., 2021). The study highlights the importance of developing a culture protocol aimed at making recruitment more effective and reducing the loss of individuals. Moreover, in this study, the growth of biofilm is prevented with antibiotics whereas the high mortality rates of recruits and juveniles related to predation are also minimized (Verdura et al., 2018). In Orlando-Bonaca et al. (2021) the structures transferred have been delimited by cages to limit the herbivories pression; other studies promote the use of nets, antifouling paints or direct mechanical remotion of grazer and organisms provoking fouling by hand (Gianni et al., 2013., Orlando-Bonaca et al., 2021).

Different studies promote the synergic application of passive and active strategies of restoration (Gianni et al., 2013; Medrano et al., 2020). A recent study conducted by Medrano et al. (2020) tested the difference in the success of active strategies of restoration of macroalgal forests (*Treptacantha elegans*) applied on barren grounds, inside and outside a no-take area of a marine reserve. After one year since the begin of the experiment, only the barren grounds restored inside the no-take area moved close to reference population' characteristics, confirming the effectiveness of a passive and active combined intervention (Medrano et al., 2020). The increase of the marine protected areas together with active restoration actions, appears to be the only possible way to favour the resilience of algal forest ecosystems (Gianni et al., 2013; Savonitto et al., 2020). A network of marine protected areas, indeed, would be able to spread the positive effects of protection actions even in surrounding areas.

1.4 Afrimed project: purpose and application of techniques aiming at restoring degraded *Cystoseira s.l.* populations

Afrimed is a European project on the restoration of the degraded macroalgal forests of *Cystoseira s.l.* species, that involves 11 partners and 8 countries (http://afrimed-project.eu/). Started on 16 January 2019, Afrimed has the aim to develop, implement and promote an effectively protocol to restore macroalgal forests in the Mediterranean Sea, in order to maximise the delivery of conservation, societal and economic benefits. The aim is to obtain concrete methods that can be replicated on a large spatial scale, coopering with private sector and public authorities. The project is organized in 7 work packages (WPs; Fig. 2):

1. *"identifying optimal locations to undertake restorative action, now and into the future, by bringing together existing knowledge of the status and distribution of macroalgal forests and the environmental and anthropogenic context of the regions in which they are found (WP1);*
2. *undertaking novel laboratory experiments in order to optimise restoration techniques in a range of environmental and anthropogenic contexts commonly found in the region. Exploring the response of Cystoseira species to future climatic conditions and modelling future distribution patterns in order to ensure restoration actions are relevant into the future (WP2);*
3. *passing from the lab to the field to refine restoration methods in "real-world" conditions (WP3);*
4. *identifying methods and contextual factors that improve the efficiency and success rate of restoration and the delivery of goods and services. Determining where the knowledge and lessons generated from the project can be applied to address policy needs and aspirations (WP4);*
5. *engaging with local stakeholders to promote and facilitate replicability and transferability of know-how and generate support from local groups and industries. Disseminating actions and results to increase the awareness of these important*

habitats and the benefits that can be obtained from restoring them (WP5)" (Afrimed project - http://afrimed-project.eu/).

Within AFRIMED, WP1-WP5 cover all the scientific aspects of the restoration, from mapping the populations to setting approaches and protocols, together with socio-economic aspects. WP6 is dedicated to the dissemination of the results and WP7 ensures the proper management of the project.

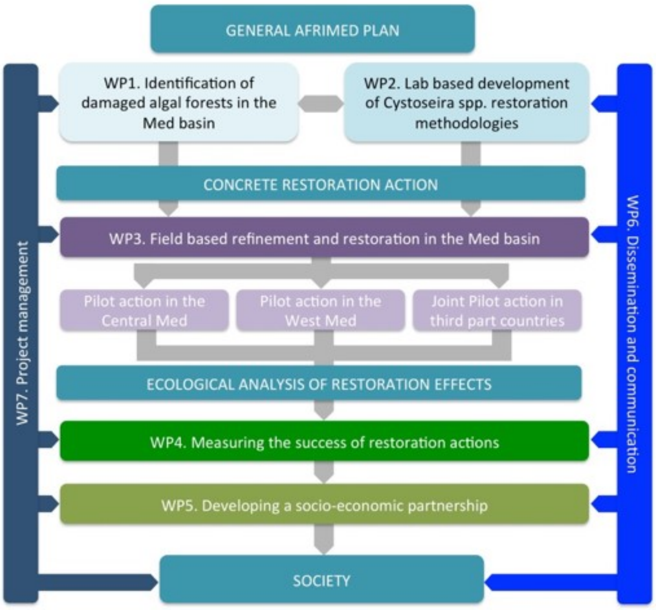

Figure 2. Illustration of the Afrimed Project strategy.

Within this project, this experimental thesis focuses on a restoration intervention of *G. barbata* along the "Monte Conero" Special Protection Area (SPA IT5320015) (Adriatic Sea). In this area *Cystoseira* forests have suffered particularly widespread and persistent loss (Perkol-Finkel & Airoldi, 2010), *G. barbata* shows high sensibility to local impact as trampling and swimming due to seasonally tourism and beach nourishment along with extreme storm events (Tamburello et al., 2021). Moreover, the geomorphological nature of the area characterized by marly sandstone cliffs combined to anthropic activities of rock mining (during 1939-1940) and beach nourishment (during 1997-2001) seem to has increased the natural trend of the cliffs and seabed to be eroded. This mechanism increases the abundance of the vertical substrates with respect to the horizontal substrates more suitable for the recruitment and juvenile survival (Perkol-Finkel & Airoldi., 2010).

In the present study we implement a restoration intervention at 15m^2 spatial scale, along the Monte Conero Riviera. In this area the *Cystoseira* populations results

1. Objectives

This thesis aims to assess the success of a *G. barbata* restoration intervention implemented along Conero Riviera (Marche, Italy), through:

1. comparison of the growth trend of the individuals recruited with *in situ* and *ex situ* approaches;

2. assessment of benthic biodiversity changes, using meiofauna as a proxy, associated with a *G. barbata* restoration intervention, in terms of total abundance, richness of taxa and taxonomic composition;

Benthic biodiversity variation and growth patterns are evaluated against the time variable and compared with those of juvenile and adult (reference ecosystem) populations of nearby sites. Results are based on data obtained from the analysis of samples taken in the field during an eight-month sampling period, from June 2021to January 2022.

This work is part of a wider European project called AFRIMED, which receives funds from the Executive Agency for Small and Medium Enterprise (EASME) and the European Maritime and Fisher Fund (EMFF) and has as its objective the restoration of degraded macroalgal forests dominated by *Cystoseira s.l.*.

3. Material and methods

3.1 Study area: SCI and ZPS in the Monte Conero Riviera and selection of the site for the restoration intervention.

The experiments carried out in this thesis were conducted in the coastal waters of Scalaccia site (Fig.3), located along the Conero Riviera (Marche, Italy), which has an extension of 15 kilometers between Ancona and Portonovo. The area is located within the Site of Community Importance (SCI IT5230005) of "Coast between Ancona and Portonovo", which in turn is included in the "Monte Conero" Special Protection Area (SPA IT5320015), in the Natura 2000 Network. These areas have been identified respectively based on the "Habitat" Directive 92/43/EEC and Directive 09/147/EC, and intended to ensure the conservation of species and habitats.

The mentioned above SCI covers 466 ha and is characterized by marly sandstone crag (Marche Region - The Natura 2000 Network in the Marche Region - www.ambiente.marche.it/Ambiente/Natura/Retenatura2000).

The Scalaccia site has been identified as the most suitable for a *G. barbata* restoration intervention, following the results of previous experiments, considering five different sites along the coast: "Piscinetta Del Passetto", "Scalaccia North", "Grotta Blu", "Scalaccia South" and "La Vela", in the framework of the EU project AFRIMED. The first two sites host adult and healthy populations of *G. barbata*, for the last three is reported the historical presence of the species with the confirmation of some individuals scattered in the site of La Vela (Perkol-Finkel and Airoldi, 2010). Moreover, they have been selected as subjected to different kinds of natural and anthropogenic stressors, such as urbanization, tourism, and a high level of hydrodynamism.

The previous experiment, which lasted one year, involved small-scale interventions at each selected site, with two approaches, based on *ex situ* and *in situ G. barbata* new individuals' recruitments. The results indicated that the *ex situ* approach has not been successful at any site (except for Piscinetta and Scalaccia), whereas the *in situ* approach resulted successful, particularly at donor sites, La Vela and Scalaccia South sites. La Vela, despite the very good results of *G. barbata* growth, was excluded as a site for a new restoration intervention because it's strongly exposed to hydrodynamism. In conclusion, in the previous experiment, considering both the algae growth rates and the associated biodiversity levels and seasonal variation (using meiofauna as a proxy), the Scalaccia site was selected for a restoration intervention at a spatial scale of about 15 m^2.

3.1.1 Geomorphological characteristics of sites

In the Scalaccia site, the Scalaccia North portion is an inlet in the rocky coast, whose geological conformation allows the occurrence of mitigated environmental conditions; the exposure to the North-East is reduced by rocks and rocky outcrops scattered in front of the shore, with some rocky corridors that allow a continuous exchange of the water masses with the rock pool. During the whole experiment, the monitored temperature reached maximum values of ca. 28°C in Summer, and minimum values of ca. 6°C during the winter season.

The protected environment of the rock pool, whose bottom reaches the maximum 1-2 m following a slight slope, allows the prosperity of *C. compressa* and, as already anticipated, the persistence of an adult population of *G. barbata*. The distribution of the species is patched, with dominance of *G. barbata* towards the shore.

Also Piscinetta (literally “small swimming pool”) is a rock pool and represents another donor site, particularly for the harvesting of the fronds and receptacles necessary to the *ex situ* treatment.

The Scalaccia South site, which can be reached by boat or swimming from the Scalaccia North donor site, is exposed to the northeast, whereas the submerged portion of the rock chosen for the experiment is parallel to the coast with southwest orientation. The irregularly shaped rocky surface has a gradual slope of about 30° and size ca.7x2 m respectively for width and height, to a total area of 14.06 m^2.

Figure 3. Map of the donor and restoration sites involved in the study.

3.1.2 Biotic characteristics of the experimental site

In addition to *C. compressa* and *G. barbata* there are others brown algae such as *Dyctyota dichotoma* and *Padina pavonica*, that are common species in the Mediterranean on rocky substrate. There are also green algae such as *Ulva lactuca* and *Codium fragile* and rodoficee belonging to the genus *Gracilaria*.

Seasonally occur algal blooms of *Ostreopsis ovata*, an epiphytic microalga that in great abundance forms aggregates and brownish mucilages covering all that is found on the seabed, including macroalgae like *Cystoseira* spp. In these sites, the flora biodiversity supports many animal species such as mussels (*Mytlilus galloprovincialis*), sea urchins (*Paracentrotus lividus*), hermit crabs, shrimps (*Palaemon spp.*), actinias, crabs (*Pachigrapsus marmoratus*) and many common fish species such as *Sarpa salpa*, *Oblada melanura*, *Salaria pavo* and many others. In recent years, however, there has been a strong proliferation of sea urchins, main competitors of macroalgae and mussels for the substrate, and an increase of formation of barren grounds, resulting in a reduction of the three-dimensionality of the environment.

The rocky surface chosen as a substrate for restoration, in particular, was dominated by the presence of *Ulva lactuca* during the preliminary survey conducted in May 2021.

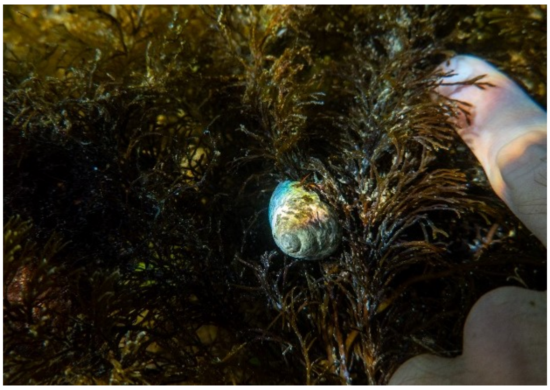

Figure 4. Some organisms found on the *G. barbata* in the Scalaccia South.

3.2 Implementation of *in situ* and *ex situ* reproduction approaches.

A dual approach has been applied for the restoration intervention, based on *in situ* and *ex situ* recruitment of *G. barbata*'s new individuals. In the first case, the recruitment of *G. barbata* provided two methods: recruitment on natural stones found in the donor sites and recruitment on artificial structures specially built and placed in donor site during the reproductive season. The *ex situ* approach, after collection of fertile receptacles from the donor populations, foresaw the recruitment on artificial structures in controlled environment, in the Aquarium facility at DiSVA.

For the construction of the structures (Fig.5-6) were used aluminium bars of 50 cm long, 2 cm wide and 4 mm thickness, flexed at the ends by determining the raising of the resulting central portion (42 cm). The holes were drilled on each bar, both in the raised section and at the ends, to allow the mounting of circular clay tiles (diameter of 7 cm and thickness 1.5 cm, surface intended for the recruitment of *G. barbata*) and anchoring to the rocky substrate, respectively. The circular tiles have been fixed to the bars through holes and steel bolts. The clay use for the tiles production is aimed to promote recruitment by providing a porous surface, better suited to simulate the natural substrate. In this work, the number of mounted tiles varies according to the type of technique; for structural units (bar and tiles positioned there) were assembled and secured by short bars placed transversely at both ends. The anchorage of the artificial structure to the seabed is guaranteed by self-tapping dowels (fischer), screwed with the use of a Nemo V2 HD underwater drill.

3.2.1 In situ recruitment approach

On 5th February 2021, 2 five-tiles structures (Fig.5) were placed at the donor site Scalaccia North by snorkelling. The transfer has been made after a survey to assess the maturation status of adult populations. The new recruits' growth was monitored by photographic images. The second method of *in situ* recruitment provided at the time of transplanting, the selection of 18 rocks showing *G. barbata* juveniles naturally recruited.

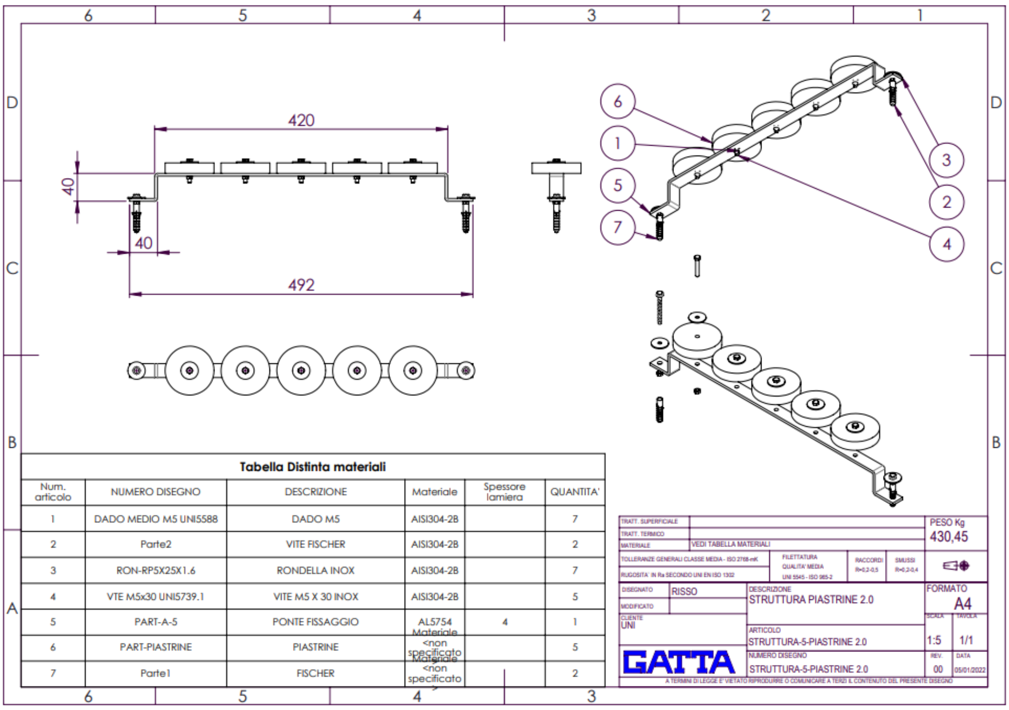

Figure 5. Artificial structure for macroalgal recruitment: five-tiles structure for *in situ* treatment.

3.2.2 *Ex situ* recruitment approach

For the *ex situ* recruitment approach, two 190 L tanks were prepared in the facility, each filled with 150 L of synthetic water. Four artificial structures (Fig.6) of nineteen tiles were distributed between the two tanks. Water circulation has been guaranteed by an Askoll Fluval pump sp2 (3600L/h). Each tank has been equipped with LED lights (GNC Silver Moon Marine), with a timer to reproduce 14h L: 10h D photoperiod, simulating the current season, and Teko tk 2000 chillers have been used to avoid temperature variations. During the whole experiment salinity (up to 35 psu), temperature (initially set to 16°C), light intensity and exposure time parameters have been checked and regulated.

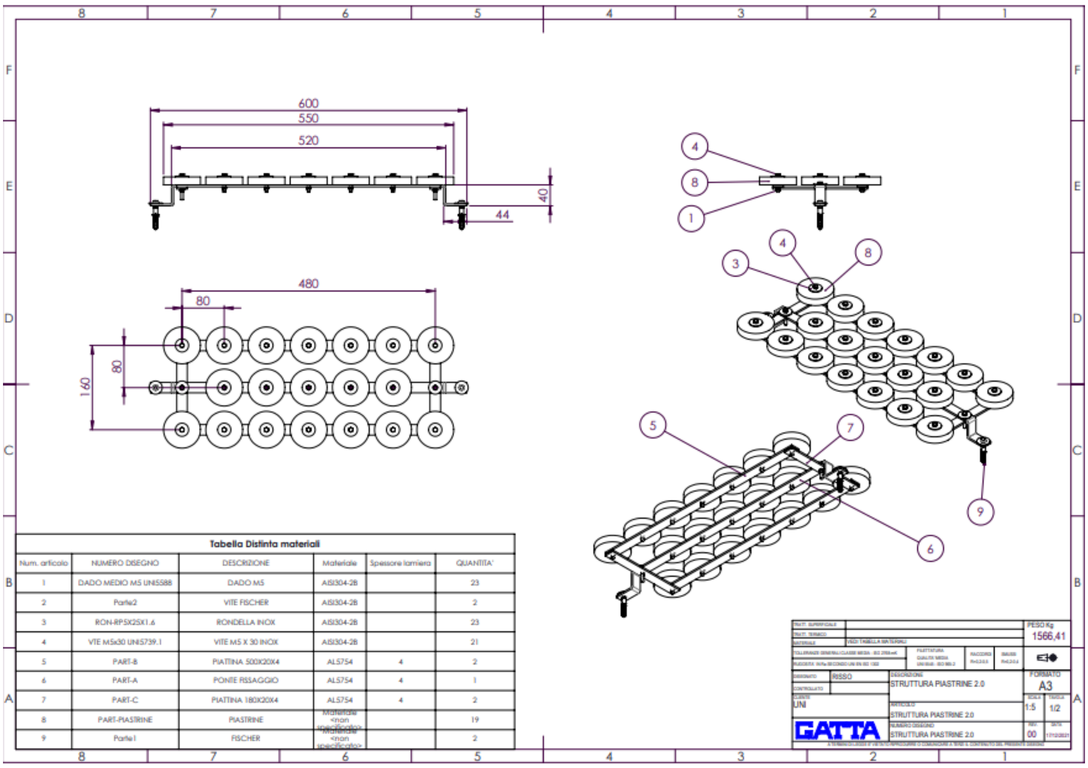

Figure 6. Artificial structure for macroalgal recruitment: nineteen-tiles structure for *ex situ* treatment.

For the experiment in tank 1, the mature fronds necessary for the recruitment were collected from donor sites (Scalaccia North and Piscinetta del Passetto) on 10th May 2021, following preliminary sampling to assess the degree of maturation of apical fronds. Receptacles, preserved in ziplock bags, were transferred to the laboratory (about 1.5 hours after the sampling), rinsed thoroughly to remove debris and visible biofouling, and finally they are stored for one night in the fridge, at 5 °C, to facilitate the release of gametes by thermal shock. The following day they were inserted in plastic mesh pockets, suspended over the structures just below the water surface (Fig. 7); the receptacles were removed after a week (on 17th June 2021).

The same procedure was used for tank 2, with the collection and removal of the apical fronds on 20th and 28th May 2021, respectively. In both tanks, the temperature was raised to 18 ºC in early June. In each tank on the bottom, in correspondence of the spaces between the tiles, have been placed six microscope slides, in order to be able to microscopically verify the presence of recruits and follow their growth.

After the receptacles' removal, for the first 3 days, measurements were carried out daily, then they were taken every two/three days and finally once a week. The last measurements were carried out with photographic monitoring, placing a ruler vertically on the surface of the slide. On June 14th (about one month after the beginning of the procedure), 450 ml/L of Von Stosch solution were added to each tank. The transfer to the restoration site took place in two times, each pair of transferred structures included a structure of tank 1 and one of tank 2.

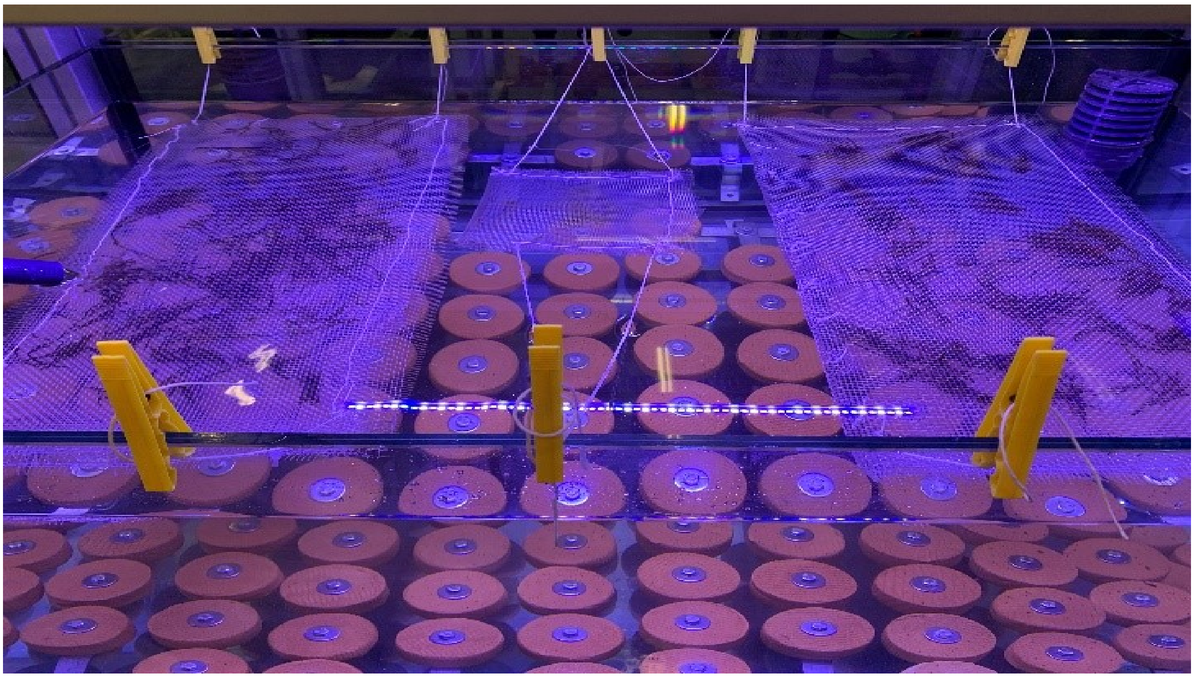

Figure 7. Suspended receptacles in *ex situ* treatment.

3.3 Restoration intervention and monitoring

On 1st June 21 began the restoration at the receiving site Scalaccia South; 18 rocks with natural recruits collected from the Scalaccia North, at a depth between 0.5 and 1.5 m, were placed on the rocky surface of the receiving site to form three plots (replicates, Fig.8). The substrate dominated by *Ulva spp.* has been cleaned to reduce competition in the early stages of development of new recruits. The rocks, of similar size, were fixed to the seabed with a two-component non-toxic epoxy marine glue (Veneziani subcoat). Near plot 3, a HOBO sensor was set to detect and record the temperature at regular intervals of one hour. During the activity were made photographs of plots to document the initial situation and, starting from the 7^{th} June 2021, were taken photos of the stones for the measurement of recruits' growth. For biodiversity analyses, three replicates were taken using the classic technique of scratching, suitable for sampling on hard substrates.

About two weeks later, on 16^{th} June, the artificial structures, positioned in the donor site, were transferred to the restoration site; they were placed and included within plots 1 and 2.

Also, on the same day were taken photos of the restoration, were measured the heights and a sample of meiofauna was collected. The transplant was completed with the transfer, between plots 1 and 2, of artificial structures with individuals obtained in the laboratory with *ex situ* approach (Fig.6). The positioning in the receiving site has been carried out in two times. Two of the 4 structures, belonging to tank 1 and one to tank 2, have been transferred on 22^{nd} June, while the last ones were moved on 8^{th} July, and placed near the structures deriving from the same tank.

All the transfer and anchoring operations of the structures were carried out using an underwater drill, by snorkelling. Due to the location of the site and the exposure to marine weather conditions, the monitoring activity has been carried out with greater intensity during the summer months, scheduling the sampling every two or three weeks; subsequently the monitoring has occurred with monthly frequency, from September 2021 to January 2022.

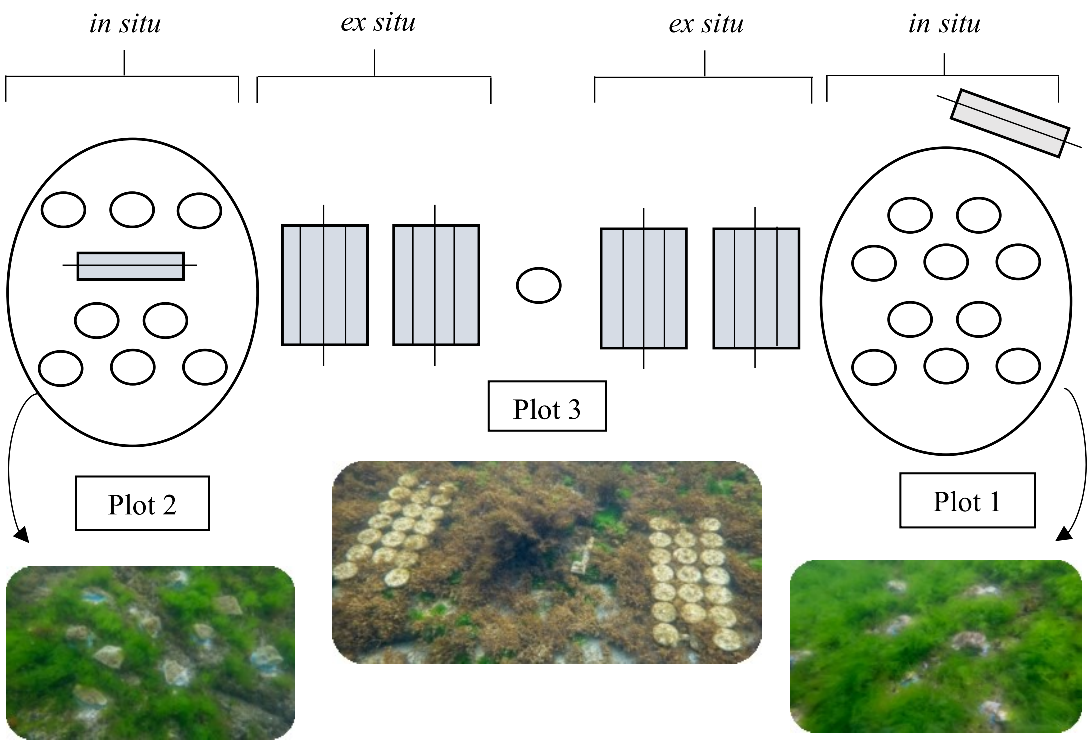

Figure 8. Restoration site. From the left to the right: plot 2 (*in situ*), structures (*ex situ*), plot 3 (*in situ*), structure (*ex situ*) and plot 1.

As ecosystem reference we considered the adult natural population present in Scalaccia North, for which we obtained data from previous experiments and monitoring activities.

3.4 Environmental parameters monitored

At the restoration site, near plot 3, a HOBO sensor (Fig.9) was placed for the measurement of temperature. The sensor was tied using two clamps to a tube previously fixed to the rock; for the operation was used a self-tapping screw applied by an underwater drill. The temperature was measured at regular intervals of one hour.

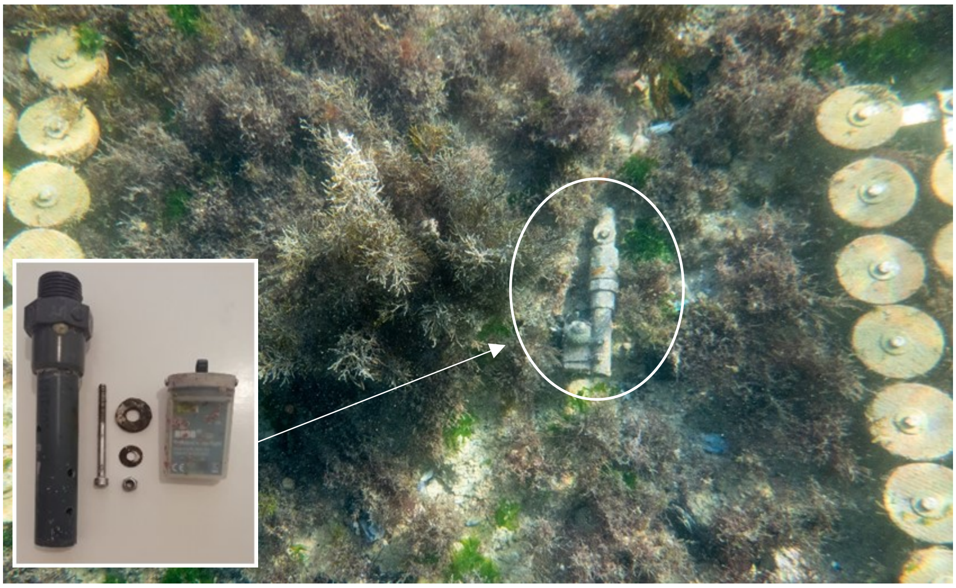

Figure 9. HOBO sensor (temperature detector) placed near plot 3.

3.5 Response variables

3.5.1 G. barbata vegetative phenology

The growth of *G. barbata* has been monitored through three parameters: height of the algal thalli, percentage of surface area covered by the canopy and the average number of individuals (the latter only for *ex situ* treatment, during the recruitment phase at laboratory).

At laboratory, to assess the growth of the *G. barbata's* recruits, obtained with *ex situ* approach, six slides were placed inside each bottom tank. The slides were observed under a binocular microscope to assess the presence and measure the growth of juvenile thalli. The growth, for the first three days, was measured daily. Subsequently, the measurements were taken using an aluminium ruler of about 30 cm from the base of the thalli to the apex, at intervals of two or three days (Fig.10). At sea, after the restoration intervention, six random measures were taken for each structure. During *ex situ* recruitment, also the number of individuals per tank was calculated. Measurements were taken at the same time as the height assessment, therefore on the same slides and at the same time. The values were taken by three different operators to obtain a reliable average; this was standardized on the surface of the microscopic slide (75x25 mm), excluding the opaque surface used to handle the microscopic slide (20x25 mm).

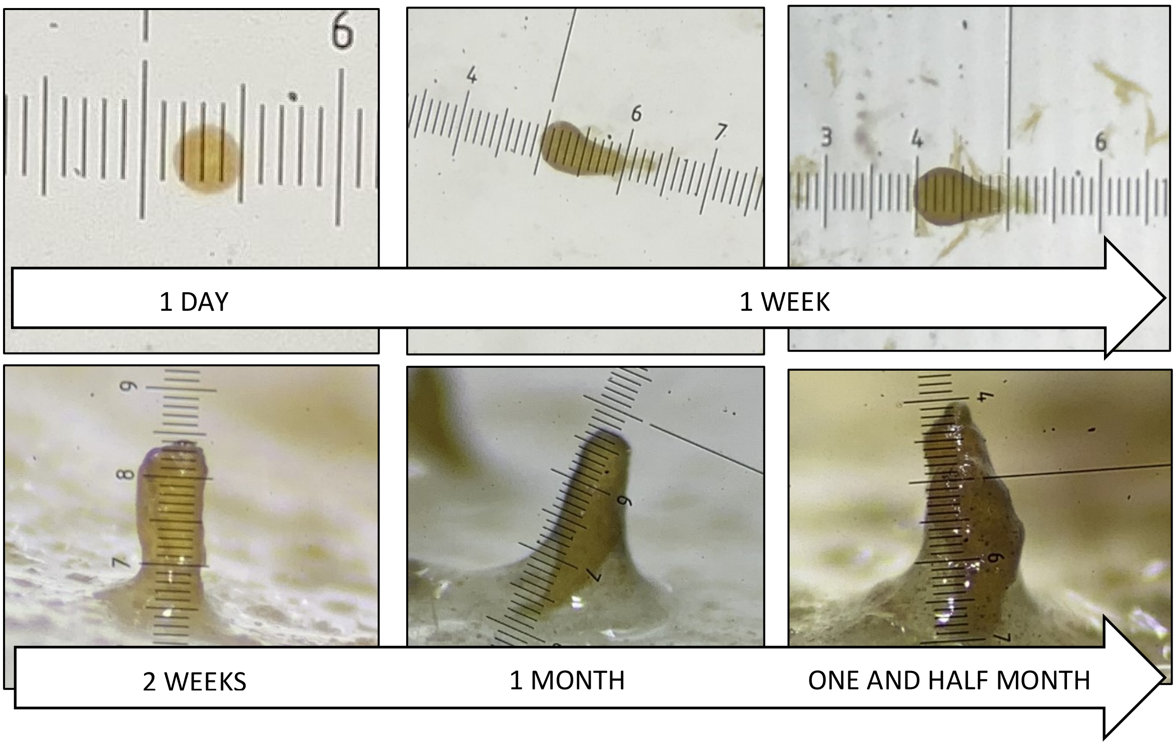

Figure 10. Development of *ex situ* recruits.

For *in situ* approach, all the measurements were acquired during snorkelling survey. From June to December 2021, algal thalli heights were measured in six and eight sampling times, respectively for structures and rocks. Until August, sampling was conducted every week, subsequently with monthly frequency. The measurement was carried out by placing a metal ruler, of about 30 cm, on the surface of stones/tiles and by analyzing the related photographs with the ImageJ program. As the growth progressed, the heights were taken directly by the operators through the ruler, from the base of the thalli up to the apices (Fig.11).

Figure 11. Height measurement with the ruler.

Four and two measurements were taken for each stone and tile respectively; in total, twenty measurements were taken at each sampling time for the 2 *in situ* structures and 76 for the natural rocks.

At each sampling time pictures (both for *in situ* and *ex situ* recruited algae) were taken also to assess the percentage of surface covered by the canopy of algal fronds. Surface analysis was carried out using the ImageJ program. The obtained values have been standardized on the surface of the square (50x50 cm) used to make the photographs (Fig.12).

Figure 12. Plot 1 picture for surface analysis.

During the sampling times on October (20/10/21) and January (31/01/2021) the presence of receptacles was detected on some individuals. After the harvesting and the transfer in the laboratory, the analysis at microscope showed the presence of gametes.

3.5.2 Meiofauna

Meiofauna were analysed in terms of total abundance, richness of taxa, community structure and taxonomic composition.

Meiofauna samples were collected with means of a modified manual corer, allowing the scratching of a rocky substrate (Danovaro & Fraschetti, 2002). The instrument used consists of a cylinder (length 15 cm; internal diameter 9 cm), made of transparent plexiglass, in which a metal scratcher is inserted through a side hole. During the operation, the cylinder was placed above the substrate, which is scraped using the scraper. The basal circumference, clinging to the rock, was covered with a polystyrene ring to improve the adhesion of the instrument to the irregular surface to be sampled (Fig.13).

Figure 13. Sampling operation with "scratching" technique.

The water and suspended sediment were collected inside a bag previously fixed to the upper end of the corer, with an elastic band. To avoid the loss of the sample at the end of the scraping, the core was overturned and closed at the free end with the appropriate cap. The bag containing the sample was removed from the corer, closed with elastic band and placed in a jar.

In the early stage of restoration the samples were taken in 3 replicates, randomly over the entire surface of the restoration site. Subsequently, since the *G. barbata's* individuals resulting from the *ex situ* recruitment began to decline and disappear, we collected samples under the growing *G. barbata* (*in situ* recruited) and in naked substrate (were *ex situ*-recruited *G. barbata* did not grow), as a reference.

After the transplant of all the rocks and structures, the samples were collected bimonthly.

The samples collected were immediately transferred to the laboratory for the extraction, after storage at -20 °C. Meiofauna were extracted by centrifugation in a density gradient, in a solution of Ludox HS40 (Heip et al., 1985; Danovaro, 2010), after been filtered through a 500 and 20 µm mesh for the separation of macrofauna and smaller particles.

The organisms retained on the 500 µm filter were stored in 50 ml centrifuge tubes with 70% ethanol solution, whereas the rest of the sample was collected in a beaker placed under the filter. The retained sample fraction was subjected to a second filtration with a 20 µm mesh for the meiofauna collection. The material remained on the filter was transferred to a 50 ml centrifuge tube and resuspended with Ludox 100% (sediment ratio: Ludox = 1:3; Ludox density=1.31 g cm^{-3}). After ultrasound treatment (three times for one minute with thirty seconds intervals), the samples were balanced and centrifugated for 10 minutes at 3000 rpm. This operation has been repeated three times for each sample to obtain an extraction efficiency of more than 90% (Danovaro, 2010). At the end of each centrifugation, the supernatant was collected on a 20 μm filter and, after being rinsed with tap water to remove Ludox residues, was placed in a tube with 70% ethanol and stained with Rosa Bengal (Fig.14). After the centrifugations, the remaining material was checked under a stereomicroscope to control for any eventual remaining organisms. The labelled samples were sealed with parafilm and stored in the refrigerator. The coloration took a few days at the end of which it was possible to carry out the sorting.

Figure 14. Samples preserved in 70% ethanol and coloured through Rosa Bengal.

The counting and identification of meiofaunal organisms (Fig.15) were performed at the stereomicroscope using a Delfuss cuvette and tap water. Magnifications of 16-40X were used, but when higher magnification was necessary, organisms were isolated and mounted on a slide to be observed under the optical microscope at 40-100X (Heip et al., 1985).

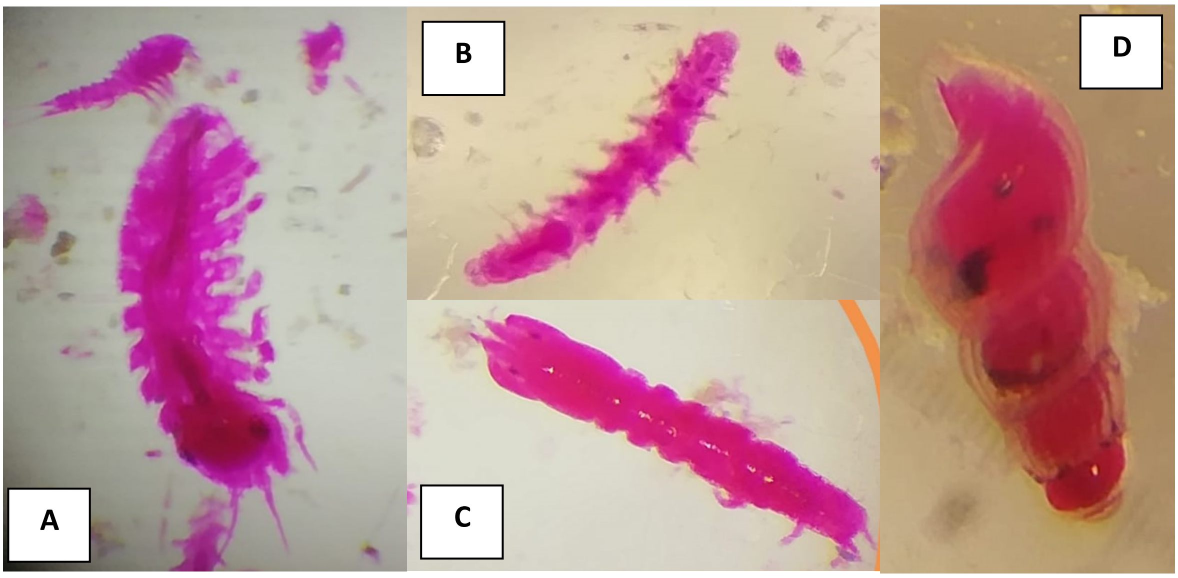

Figure 15. Some organisms found in the samples: isopods (A), polychaetes (B), tanaidaceous (C) and gastropods (D).

3.5.3 Data treatment and statistical analyses

Since 01/06/21 was set up the restoration at the receiving site Scalaccia South. Two approaches were implemented in the experiment: *in situ* and *ex situ*. For the *in situ* approach two methods have been used: recruitment on natural stones and recruitment on artificial structures (5 tiles) collected or placed in the donor site. The *ex situ* approach has four replicates consisting of artificial structures (19 tiles). Monitoring and sampling were carried out over a period of 8 months, from June to January 2022.

The variables considered over time are density of *G. barbata* individuals (only *ex situ* treatment), height of algae, percentage of algal coverage and meiofauna abundance, richness of taxa and taxonomic composition.

For the variation of the total number of *G. barbata* individuals, the evaluation was made on 13 and 10 times, respectively for the structures of tank 1 and 2 of the *ex situ* treatment.

The growth of individuals in *ex situ* treatment during laboratory culture was monitored through measurements of 17 and 12 times, for replicates of tank 1 and tank 2 respectively. For tank 1, twenty measurements were recorded for the first eight times and thirty for the remaining. For tank 2, ten additional heights were measured at each time in addition to the total number of the previous time, starting from ten measurements at T0 up to a maximum of sixty values. Following the transfer to the restoration site, the heights for the two treatments were taken simultaneously eight times; overall they were acquired for each time (unless the loss of individuals) 96 total measurements for *in situ* treatment replicates (four measurements per stone and two for each plate) and 24 measurements in *ex situ* replicates.

For the variation of the algal coverage percentage, data of 9 sampling were included in the study, at least four photographs were taken at each time.

The variation of meiofauna, in terms of abundance, community structure and richness of taxa, associated with restoration has been evaluated considering two factors: time and type of treatment (*in situ* and *ex situ*). Evaluation of the associated meiofauna over time was based on data from samples obtained on six time: T0-T1-T2-T3-T4-T5. The evaluation of the restoration success relative to the type of treatment was based on the 3 sampling times: T3-T4-T5. At each time, samples were collected for each treatment, and for each sample three replicates were taken. The samples were taken every two months, unless adverse weather and sea conditions. Subsequently, the results were compared with the data of the natural populations, juvenile and adult, of Scalaccia North.

For algal growth, during the *ex situ* recruitment, the experimental design considered 2 factors as source of variability: Tank, fixed with 2 levels (Tank 1 and Tank 2), and Time, fixed with 11 levels. Once at sea, the *ex situ* recruited individuals growth was assessed by an experimental design considering only Time as source of variance.

For algal growth during the *in situ* recruitment, the experimental design considered 2 factors as source of variability: Substrate, fixed with 2 levels (Rock and Structure) and Time, fixed with 9 levels.

For the analysis of the associated biodiversity, we applied two experimental designs. The first one design consider 1 factor as source of variability: Treatment (fixed, 3 levels: beginning, *G. barbata* growing and no-growing), with 3 replicates. The second experimental design consider 2 factors as source of variability: Treatment (fixed, 2 levels: *G. barbata* growing and no-growing), and Time (fixed, 3 levels), with 3 replicates.

The experimental designs were applied to permutational analyses of variance (PERMANOVA), in uni- (for algal growth, total meio- and macrofaunal abundance) and multivariate context (for taxonomic composition of the meio- and macrofaunal assemblages) and based on Euclidean distance and Bray-Curtis similarity matrices, respectively. Analyses on meiofaunal taxonomic composition were repeated considering the whole assemblages and only the rare taxa. When significant differences were observed, pair wise tests were performed to establish between which levels significant differences were present. To visualize differences in the taxonomic composition, shade plots were also produced.

All statistical analyzes were done with the PRIMER7 software package.

4. Results

4.1 *Ex situ* recruitment

In the *ex situ* recruitment, the trend of algal growth was different between the two tanks. During all the sampling times, recruit's density was higher for tank 2 (Fig.16) than tank 1, respectively 283.4 ± 61.2 ind*cm^{-2} and 81.4 ± 19.5 ind*cm^{-2}. The first days the zygotes released were observable only under microscopic slide. After the receptacle removal (T3, Fig.16) the average zygotes density was 5.8 ± 3.0 ind*cm^{-2} and 315 ± 22.8 ind*cm^{-2}, respectively for tank 1 and 2.

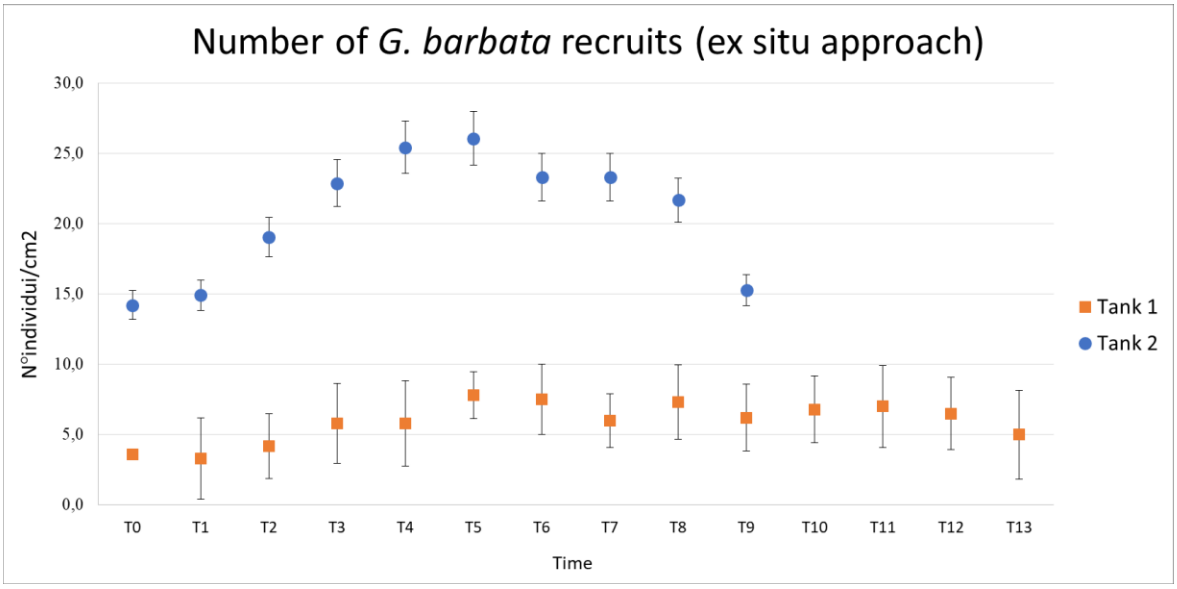

Figure 16. Recruitment trend of ex situ treatment during the cultivation.

Since this sampling time tank 1 showed an initial little increase (T5-T6, Fig.16) and a subsequent phase of stasis, while tank 2 density highly increased (T4-T5,

Fig.16), reaching a peak and then decreased. In both tanks, the maximum peak of density was reached at the fifth sampling time, 7.8 ± 1.7 ind*cm^{-2} e 26.1 ± 1.9 ind*cm^{-2}, respectively in tank 1 and 2. At the outplanting time (T9-T13, Fig.16), the density of recruits was 68 ± 5 ind*cm^{-2} and 210 ± 15.3 ind*cm^{-2}, in tank 1 and for tank 2, respectively.

In term of growth, an opposite trend was observed, with tank 2 individuals showing significantly lower height values during the culture phase, on almost all times (Fig. 17 A; PERMANOVA, $p<0.05$). In Tank 1, the growth showed a constant and significant increase during the first 30 days, and then a stasis phase (Fig. 17 A; PERMANOVA, $p<0.05$). In tank 2, the growth showed a constant and significant increase with a stasis phase from day 20 to day 30 (PERMANOVA, $p<0.05$). At the outplanting time the heights were 600-900 µm.

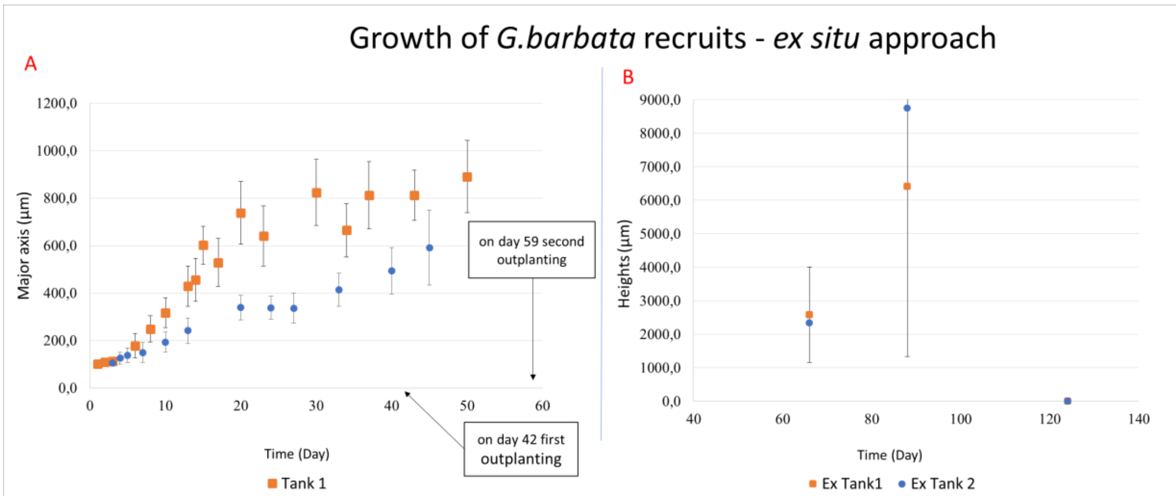

Figure 17. Growth trend of ex situ treatment: cultivation in laboratory (A) and growth in field (B).

The *pair wise* analysis report that the differences between the recruits belonging to tank1 and 2 are significant in most of the sample times (Tank X Time), except for the T0, which show p-value > 0.05. Inside each tank *pair wise* analysis report, in response to the factor Time, significant increase in heights between each time.

Table 1. PERMANOVA analysis conducted on the average height data of the *ex situ* recruits, in relation to the "Time" and "Tank" factors. Source = variability source, df = degree of freedom, MS = means of square, F = statistic F, P = P value (the same for all the next tables).

Source	df	MS	F	P
Tank	1	2,58E+06	202,2	0,001
Time	11	2,54E+06	199	0,001
Tank X Time	11	2,17E+05	17	0,001
Residuals	846	12765		

The maximum heights were reached at sea on day 88: 6416.7 ± 5076.3 µm and 8750.0 ± 10027.5 µm, respectively for algae deriving form tank 1 and tank 2 (Fig.12B). In field, significant growth was observed between T0 and T1 (PERMANOVA, $p<0.05$) (Table 2), but on day 124 (September 10th) we weren't able to detect the presence of the thalli. Since this sampling time, there wasn't anymore *G. barbata* individuals on *ex situ* structures (Fig.12B).

Table 2. PERMANOVA analysis conducted on the recruits' average height data collected during outplanting phase, values in relation to the time factor.

Source	df	MS	F	P
Time	1	11,2	22,9	0,001
Residuals	35	0,5		
Total	36			

Since the total disappearance of *G. barbata* individuals, was decided to use the area covered by *ex situ* structures as a comparison area for the *in situ* recruitment treatment, where the *G. barbata* has grown.

4.2 In situ **recruitment**

The figure 18 shows the variation of average heights of *in situ*-recruited individuals used for the restoration intervention, compared to juveniles of a previous outplanting pilot intervention at the Scalaccia South and adults from donor populations of the Scalaccia North and Piscinetta.

Graph A (Fig.18) shows the growth of the two approaches used for the *in situ* recruitment: natural rocks reached heights similar to structures during the entire experimental period. At the beginning of the restoration intervention, in June (T0, Fig.18A) the rocks' recruits measured 2.0 ± 0.6 cm, while on the structures, introduced at the end of June and beginning of July, they measured 4.6 ± 0.2 cm (T2, Fig.18A). The maximum heights were observed on 31th January 2022, in the last sampling time *G.barbata* thalli reached 24.1 ± 0.9 cm and 26.3 ± 0.7 cm, on rocks and structures respectively (T6, Fig. 18A).

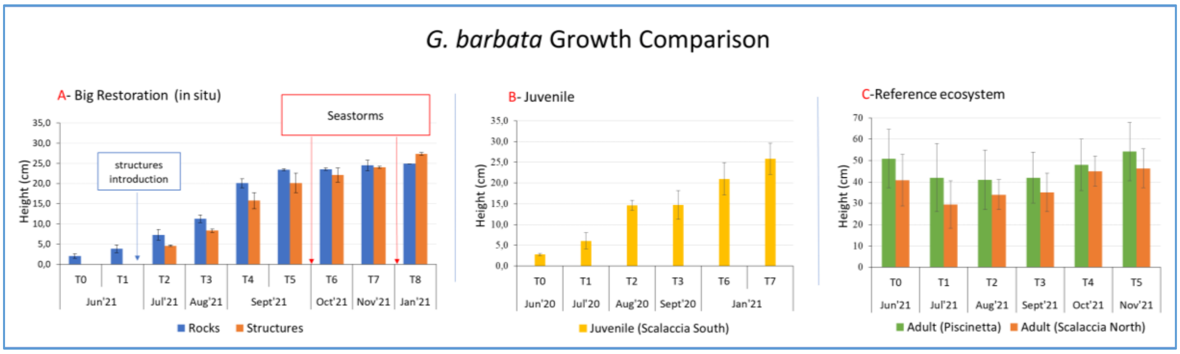

Figure 18. G. barbata trend of average heights of the restored population (A), juvenile population (B) and ecosystems reference (C).

The PERMANOVA analysis, conducted on the heights of the algae obtained by *in situ* approach, showed a significant effect of the factors source "Substrate" ("Rock"/ "Structure"), "Time", and "Substrate X Time" (Table 3).

Table 3. PERMANOVA analysis conducted on the average height data collected during outplanting phase, values in relation to the time factor.

Source	df	MS	F	P
Substrate	1	174,6	8,2	0,004
Time	8	6677,5	312,1	0,001
Substrate X Time	6	61,6	2,9	0,009
Residuals	760	21,4		

The *pair wise* analysis showed significant growth on the rocks for most of the sampling times, except between the last 3 times (T5-T6-T7), characterized by a reduction in growth rates (Fig.18). The same analysis showed significant growth on the structures for most of the sampling times, except between T5-T6, T5-T7, T6-T7 and T7-T8.

The same test reveals that the algal heights were higher on rocks than on substrates at the beginning of the outplanting, until T5 (*pair wise*, $p<0.05$) (Table 4).

Table 4. Pair ways analysis conducted on *in situ* treatment to test factor “Substrate X Time”.

	t	P	t	P
	T2		T3	
Rock VS Structure	2,9	0,008	0,8	0,388
	T3		T7	
Rock VS Structure	3,0	0,002	0,2	0,833
	T4		T8	
Rock VS Structure	2,4	0,016	1,6	0,106
	T5			
Rock VS Structure	2,1	0,046		

Also for the previous juveniles and donor populations, the peaks of height were reported in autumn-winter, on T4 and T5 sampling times for juvenile (Fig.18B) and adults (Fig.18C), respectively.

In the restoration site, the growth showed a similar trend to the juvenile population, with the same range of size, reaching the maximum of 25.8 ± 3.7 cm. Instead, in the same sampling period, in the reference ecosystems, the growth showed a range size two times higher than the restored population, reaching 54.2 ± 13.7 cm and 46.3 ± 9.1 cm, at Scalaccia North and Piscinetta, respectively (Fig.18C).

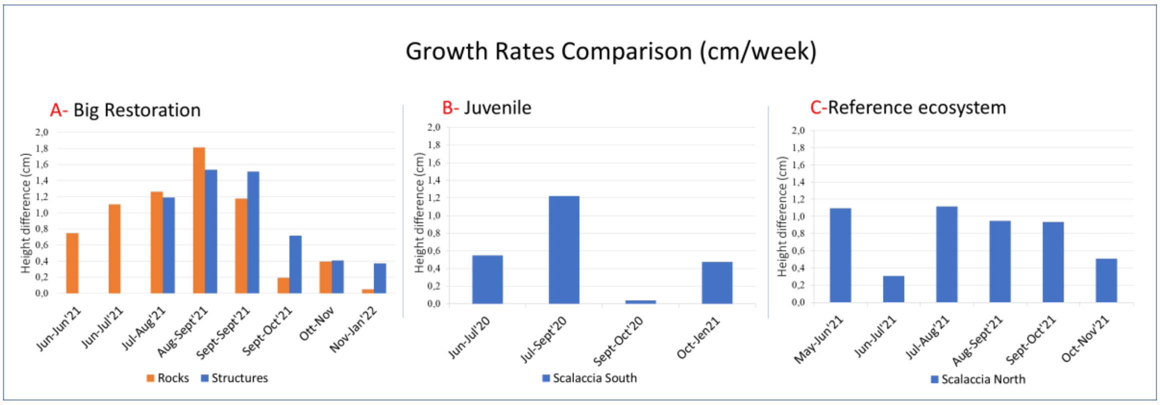

Figure 19. G. barbata growth trend in restored population (A), juvenile population (B) and ecosystem reference (C).

In the figure 15, is reported the growth rates (cm/week) comparison between the restored population (Scalaccia South), juvenile (Scalaccia South) of the previous outplanting pilot intervention and adult donor (Scalaccia North) population. Highest growth rates, 1.8 cm/week, were reached by restored individuals in August-September 2021. In the same period juveniles reported a peak of 1.2 cm/week (June-September 2020; Fig.19B), whereas adults showed 0.9 cm/week as highest growth rate (Fig.19C). Adult population showed the minimum growth rate during early summer (June-July 2020; Fig.19C), while the juvenile and restored population showed the minimum growth rates in autumn-winter (Fig.19A-B).

4.3 Temperature

In the figure 20 is reported the height trend of the *in situ* treatment in relation to the temperature variation since the 1th June 2021 to the 31th January 2022. *G. barbata* growth seems to be inversely related to the temperature, with lower eights during the summer characterized by higher temperature (maximum peak on 14th August).

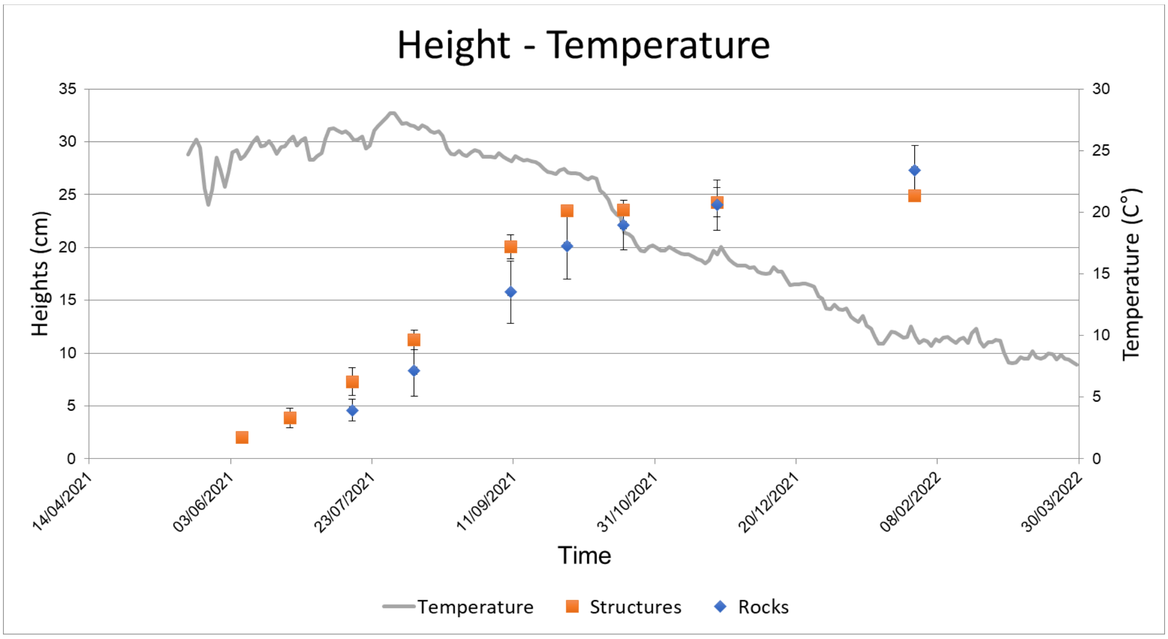

Figure 20. Height trend of the *in situ* treatment in relation to the temperature variation during the eight months sampling activity.

4.4 Algal coverage variation

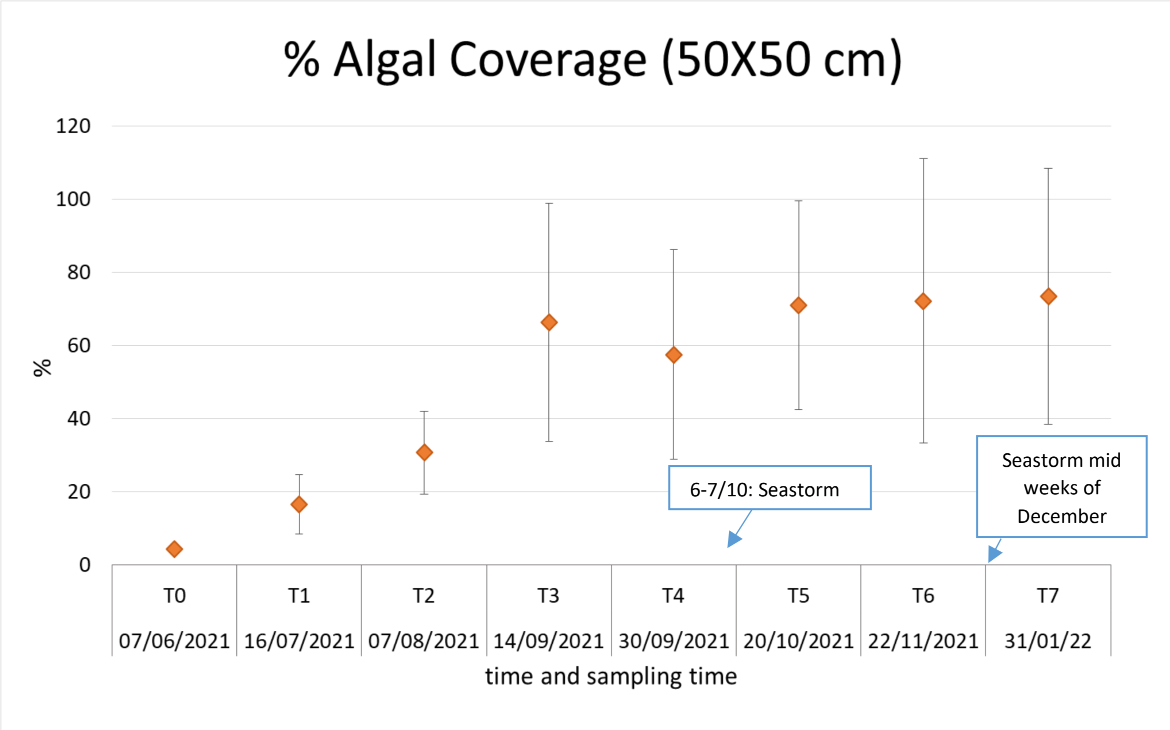

Figure 21. Percentage change in the restoration algal coverage compared to a reference area of 2500 cm^2.

At the first sampling time (T0, Fig.21), after only a week since the beginning of the restoration intervention, the algae cover only 4% of the area (2500 cm^2) used as reference. The percentage of coverage increase from June to mid-September with percentage values of 17, 31 and 66%, respectively for the T1, T2 and T3. After T3, a stasis phase was observed until T6 (58 - 73%).

4.5 Meiofauna analysis abundance and diversity

Figure 22 shows the total meiofaunal abundance variation under the *G. barbata* restored population, and also compared to the juveniles of a previous outplanting pilot intervention at the Scalaccia South and adults natural population of Scalaccia North. At the beginning of the restoration intervention (T0; Fig.22A), the meiofauna abundance was 102.75 ± 30.15 ind*10 cm^{-2} and after two weeks, in T1 sampling time, was reached the maximum abundance (445.01 ± 163.24 ind*10 cm^{-2}).

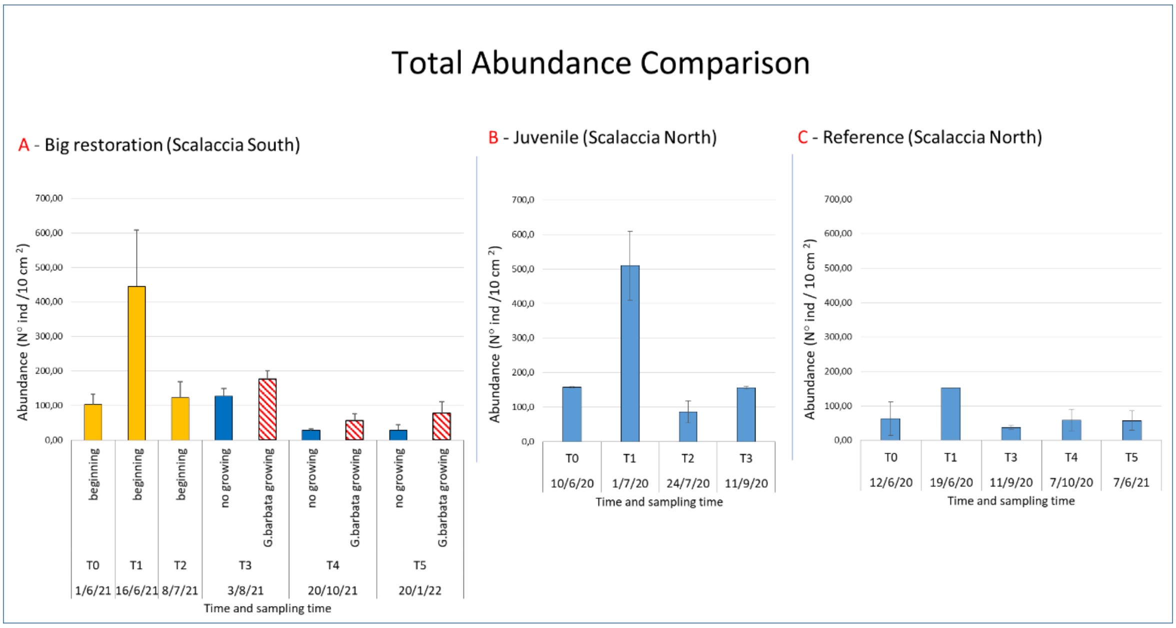

Figure 22. Total average abundance comparison between restoration experiment (A), juvenile natural population (B) and ecosystem reference (C).

On the following sampling times, T2-T3-T4, the abundances associated to the *G. barbata* presence decreased with the lowest value (55.85 ± 19.95 ind*10 cm^{-2}) detected on T4, ca. four months after the beginning (Fig.22A). On T3-T4 and

T5 sampling times the total abundances under *G. barbata* growing resulted higher compared to those where *G. barbata* did not grow, even if the differences were not significant (PERMANOVA > 0.05) (Tab. 3).

Significant differences are reported also by the PERMANOVA analysis for the second experimental design conducted on the meiofauna total abundance related to the factor "Treatment " ("beginning", "no growing" and "*G.barbata* growing" levels) (Table 4), in particular *pair wise* analysis show significant difference only between "beginning" and "no growing" levels.

Table 4. PERMANOVA results about meiofauna total abundance. Source= variability source, df=degree of freedom, MS=means of square, F= statistic F, P= P value (also for all the next tables).

Source	df	MS	F	P
Treatment 1	2	63644	3,3	0,029
Residuals	24	19189		
Total	26			

The PERMANOVA analysis conducted for the second experimental design on the total abundance showed a significant effect of the factors "Treatment" (*G.barbata* growing/ no growing) and "Time", but not for "Treatment x Time" (Table 5).

The *pair wise* analysis within level "no growing" showed significant differences between T3-T4 and T3-T5, while within level "growing" differences were between T3-T4.

Table 5. PERMANOVA results obtained for the second experimental design on the meiofauna total abundance.

Source	df	MS	F	P
Treatment	1	7782,1	5,5	0,031
Time	2	21786	15,4	0,001
Treatment X Time	2	219,8	0,2	0,848
Residuals	12	1415,5		

Under juveniles the trend was similar, with the maximum value (509.46 ± 99.36 ind*10 cm^{-2}) detected on T1 and the minimum (86.61 ± 31.34 ind*10 cm^{-2}) on T2. In the remaining time, T0 (at the beginning of the sampling activity) and T3 (three months later), were found abundances of 157.3 ± 1.2 ind*10 cm^{-2} and 156.03 ± 4.36 ind*10 cm^{-2}, respectively. In the reference ecosystems, the abundances were lower compared to those of restored and juvenile populations, with maximum and minimum peaks reached, respectively, on T1 (152.32 ± 0.57 ind*10 cm^{-2}) and T3 (37.36 ± 5.61 ind*10 cm^{-2}) sampling times (Fig.22C). On the other sampling times, the abundances were 63.35 ± 48.76, 59.10 ± 31.65 and 57.58 ± 28.79 ind*10 cm^{-2}, on T0, T4 and T5 respectively.

In figure 18 is reported the richness of taxa for all the samples, including the comparison of the restored population of Scalaccia South with juveniles and adults' populations of Scalaccia North. Under the restoration experiment the higher taxa richness, 18 taxa, was reached in the T1, two weeks after the restoration beginning, when the number of taxa was 12 (T0, Fig.23). In the restored population follow a reduction in T2, with only 11 taxa (Fig.23), the richness increased. Observing the subsequent three sampling times (T3-T4-T5), the graph (Fig.23A) showed higher richness of taxa under "no growing" than under "growing *G. barbata*", with the opposite pattern on T5. In the T3, are reported 14 and 13 taxa, on T4 11 and 10 and in T5 12 and 14, respectively for "no growing" and "*G. barbata* growing" samples.

Maximum peaks in juveniles and reference ecosystem populations were 15 (T1, Fig.23B) and 16 (T4, Fig.23C) taxa.

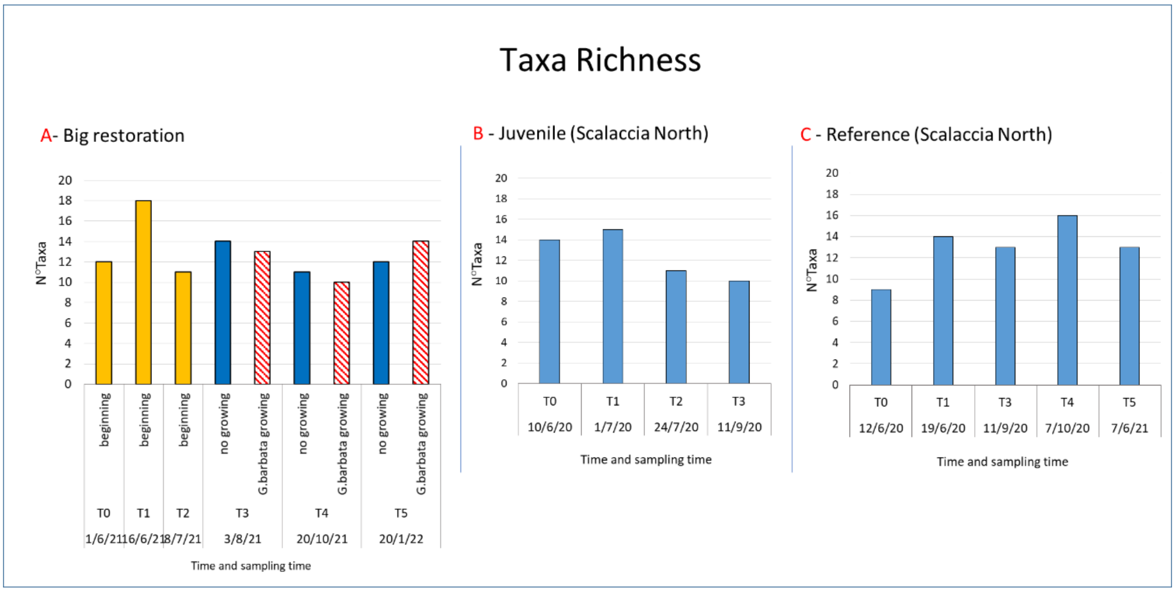

Figure 23. Taxa richness comparison between the restored population in the Scalaccia South (A), juvenile (B) and adult (C) natural populations of Scalaccia North.

Under juveniles population the minimum was reached on T2 (11 taxa), a month and half after the outplanting, while in the others sampling times the number of taxa was 14 and 10 respectively for T0 and T3 (three months later). Reference ecosystems' samples showed the minimum richness of taxa on the T0, while in

T1, one week after, reached 14 taxa and in T3 and T5, respectively three months and one year later, reached 13 taxa.

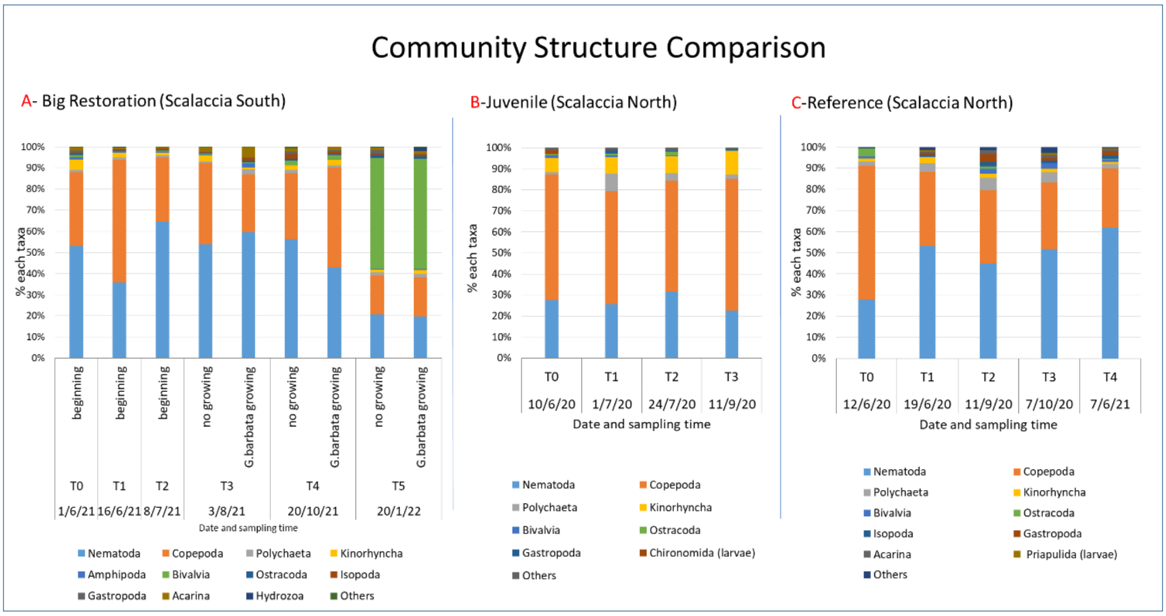

Figure 24. Community structure comparison between the restored population in the Scalaccia South (A), juvenile (B) and adult (C) populations of Scalaccia North.

For all the populations, the community structure analysis reported the predominance of nematodes and copepods, with variable percentage fractions. In general the others groups, common in all samples, were Polychaeta, Kinorynchа, Bivalvia, Ostracoda and Gastropoda. In the restored population, Nematoda represents the taxon with the highest abundance in the T0 (53.1 %) - T2 (64.5%) - T3 (“no growing” = 53.9%, “*G.barbata* growing” = 59.7%) sampling times, while in the T1 end T4, are the copepods that show higher abundance with 57.7% and 47.5% (“*G. barbata* growing” sample), respectively. Finally, on T5, after seven months since the beginning of the restoration, there is dominance of bivalves, with “no growing” and “*G. barbata* growing” samples, respectively, of 52.3% and 51.9%.

The second taxon with highest abundance was Copepoda, representing between ca. 18% and 57% of the assemblages. For the first four sampling times the third most present group were Kinorhyncha except on T3, in “*G. barbata* growing” sample, where is replaced by mites (4.8 %) and in the T4, in “no growing” sample, where is replaced by bivalves (2.4%). In the T5 bivalves were the third most present group, representing more of the 50 % of the organisms.

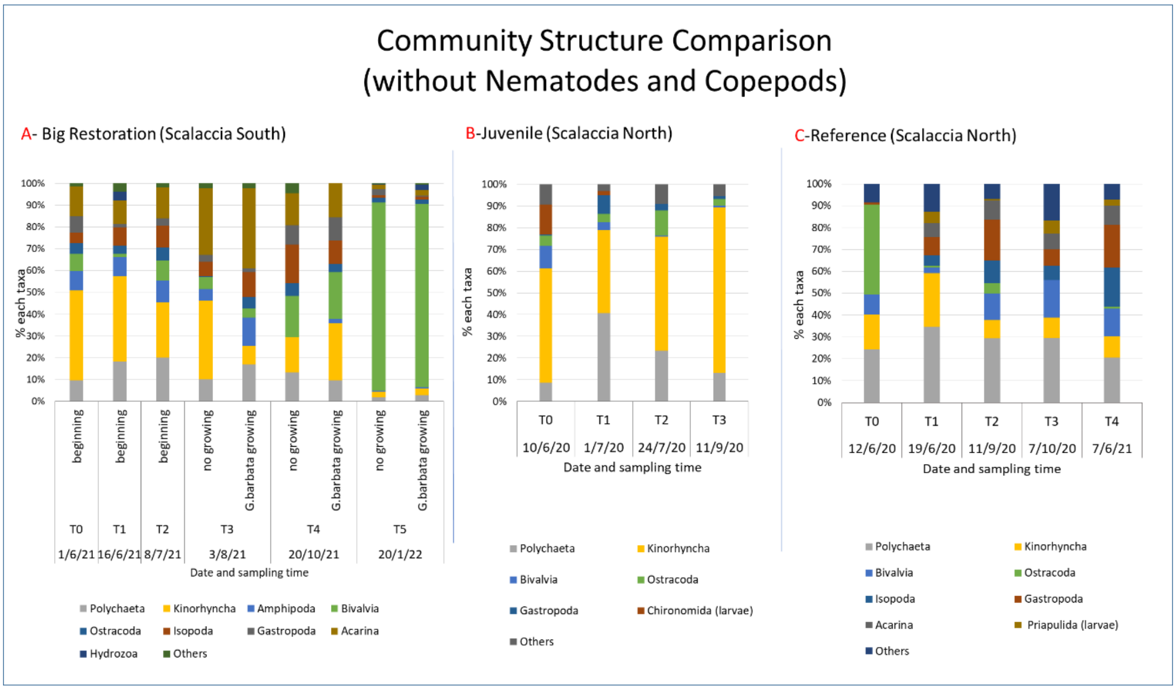

Figure 25. Community structure comparison (without nematodes and copepods) between the restored population in the Scalaccia South (A), juvenile (B) and adult (C) populations of Scalaccia North.

Under juvenile population (Fig. 24B) the analysis showed that, on all sampling times, more of 50% of the assemblage was composed by copepods, followed by nematodes (abundance between 22.6-31.5%). Lastly, also under juveniles population, the third most abundant group was represented by Kynoryncha (6.8-11.2%), except in the T1 where is replaced by polychaetes (polychaetes= 8.4% vs kinorhynchs= 7.9%). In the reference ecosystems, the three most abundant groups were nematodes (44.8-62%), copepods (28.1-35.3%) and polychaetes (2.1-6.3%). The only exception was on the T0, were the most abundant were copepods (63.4%) followed by nematodes (27.8%).

In the restoration, the contribution of rare taxa (representing < 1% each) is 0.2%-0.6%, and in particular is 0.2-0.6% in "no growing" and 0.3% in "*G.barbata* growing" samples (Fig.24A).

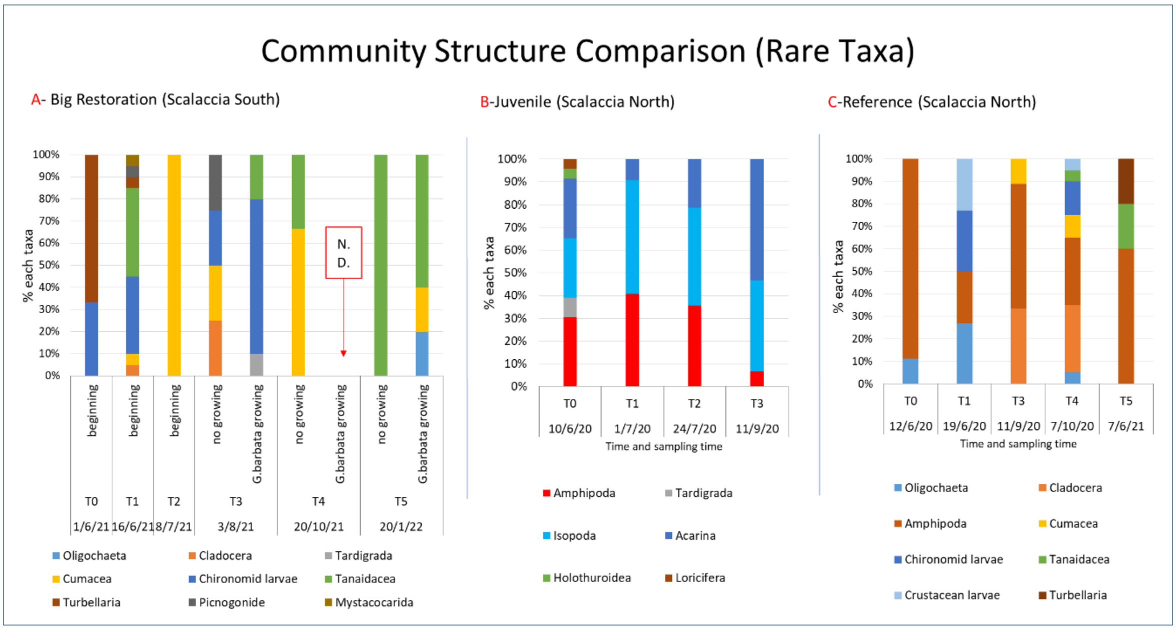

Figure 26. Taxa rare comparison between restored population (A), juvenile population (B) and reference ecosystem (C).

In the restoration site were identified 9 rare taxa: Oligochaeta, Cladocera, Tardigrada, Cumacea, larvae of Chironomidae, Tanaidacea, Turbellaria, Picnogonidae and Mystacocarida (Fig.26A). The last two were present in a really low percentage, and are completely absent in the samples collected for the other two populations. In the restoration site, the rare taxa present with higher abundance are chironomids larvae (0.1-0.2%), cumaceous (0.1-0.4%), and tanaidaceous (0.1-0.2%). In the "no growing sample" are found tanaidaceous (<0.1%), cumaceous (<0,1%), picnogonids (<0.1%), chironomids larvae (<0.1%) and cladocera (<0.1%), while in the "*G. barbata* growing" are present tanaidaceous (0.1-0.2%), cumaceous (<0.1%), chironomid larvae (<0.2%) and tardigrades (<0.05%). Many of the taxa present in the big restoration samples are in common to those of the reference ecosystems, except amphipods that in the restoration samples results among the main taxa. In reference ecosystems' samples, amphipods show abundance between 0.2-0.7%, and represent the most abundant rare taxon (Fig.26C). In juvenile population samples, the rare taxa included Amphipoda, Tardigrada, Isopoda, Acarina, Holoturoidea and Priapulida (larvae). Amphipods, isopods and mites represent the three groups with higher abundance, respectively, 0.1-0.5%, 0.3-0.6% and 0.3-0.4% (Fig.26C).

The PERMANOVA analysis conducted on the overall taxonomic composition for the first experimental design did not show significant differences.

The PERMANOVA analysis conducted on the overall taxonomic composition, for the second experimental design considering the "Treatment", "Time" and, "Treatment X Time" factors, are significant only in response to "Time" factor (Table 6).

Table 6. PERMANOVA results about meiofauna taxonomic composition.

Source	df	MS	F	P
Treatment	1	1484	1,6	0,172
Time	2	9086,8	9,7	0,001
Treatment2xTime	2	949,1	1	0,367
Residues	12	935,9		

In addition, *pair wise* analysis conducted for the second experimental design on the levels of the "Treatment" factor, were significant between T3-T4 and T3-T5 for both.

The same PERMANOVA results were obtained excluding the two most abundant groups: nematodes and copepods (Table 7). In this case, the repetition of *pair wise* analysis on the "no growing" detected significance only between T3-T5, while for the "*G. barbata* growing" the results were significant in all the times comparisons.

Table 7. PERMANOVA results about meiofauna taxonomic composition (second experimental design) (excluding namatodes and copepods).

Source	df	MS	F	P
Treatment	1	1884,5	1,3	0,25
Time	2	10819	7,6	0,001
Treatment X Time	2	1521,2	1,1	0,364
Residuals	12	1422		

Only some groups change significantly (PERMANOVA; $p<0.05$), in particular, applying the second experimental design, polychaetes, amphipods, chironomids and mites were significantly more abundant in *"G. barbata growing"* treatment (Fig. 27). Moreover, oligochetes and tardigrade that appear occasional were present only in *"G. barbata growing"* treatment (Fig. 27).

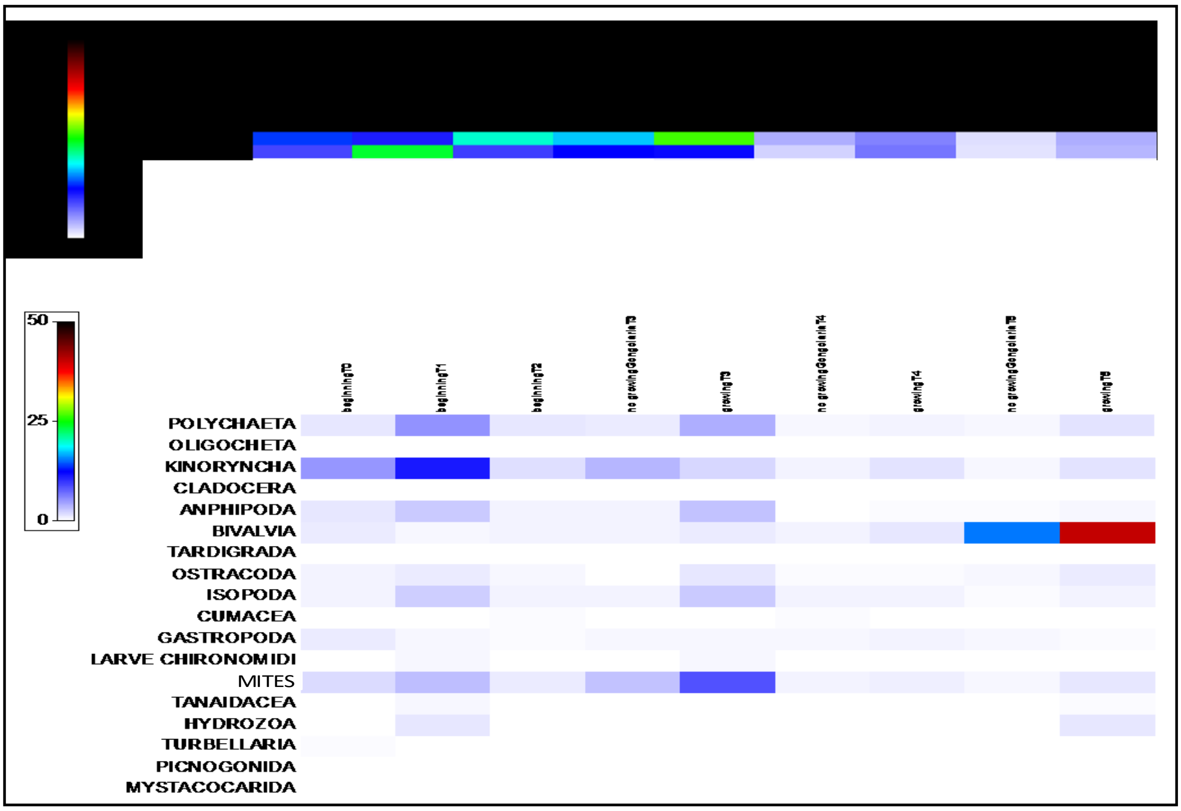

Figure 27. Shade plot analysis of the meiofauna taxonomic composition.

5. Discussion

Marine ecosystems are subjected to multiple stressors that led to habitats fragmentation, degradation and extinction processes. The ecosystems' equilibrium is threatened by local and global stressors (such as pollution, overfishing, resources' overexploitation, aquaculture, eutrophication, acidification, temperature rise, and many others), which are directly or indirectly related to human activity (Gianni et al., 2013, Fabbrizzi et al., 2020). Organisms strictly associated to the sea bottom, such as coral reefs, seagrasses, and macroalgal forests characterized by reduced mobility and dispersion capacity are particularly vulnerable to these threats. Acting as ecosystem engineers their presence increase the complexity of the bottom, furnishing constant protection and nutrient availability, favouring the attraction of many species and the forming biodiversity hot spots (Gianni et al, 2013, Orlando-Bonaca et al., 2019, Fabbrizzi et al., 2020).

In response to the ongoing decline of brown algal forests along the Mediterranean rocky shore, and the ecosystem services they provide (biodiversity's enhancement and trophic webs sustainment, protection of the coast from hydrodynamism and erosion processes, retention of resuspended sediments, and consequent increase in water quality), restoration is considered an effective strategy to favour and accelerate the recovery of these ecosystems, compromised by the biological characteristics of low dispersal capacity typical of the most of the species locally present (Fraschetti et al., 2021, Orlando-Bonaca et al., 2019, Verdura et al., 2018). In particular, active restoration techniques focused on the enhancement of recruitment proved to be the most suitable, avoiding the danger of damage and exhaustion of natural healthy populations still present (Gianni et al., 2013). According to the five steps procedure promoted in the recent work of Cebrian et al. 2021 adopted in the AFRIMED strategy, to plan an intervention, is necessary a deep knowledge of the characteristic of the species and sites involved to a right evaluation of the best techniques to implement. The observation and data reported in this work show the results of an experimental restoration intervention of *G. barbata* along the Conero Riviera where the survival of macroalgal populations belonging to this species, to date, is confined primarily in rock pools. Here the geomorphological characteristics, and so the spatial isolation, favour the mitigation of hydrodynamism and temperature anomalies effects occurring in the Adriatic basin.

5.1 Ex situ and *in situ* recruitment

According to previous studies, to contrast the obstacles due to the biological characteristics of *G. barbata* and taking into account the environmental conditions of the study area, this work have been focused on the enhancement of the recruitment process, comparing two approaches (Mangialajo et al., 2012). Indeed, the *in situ* and *ex situ* approaches have been compared to assess which could be the more suitable for the Conero Riviera area. In this regard, previous thesis works have been identified the specific site, more suitable to the *G. barbata* growth along the whole Riviera, considering the main factors that can influence the *G. barbata* growth, such as urbanization, hydrodynamic regime, substrate instability, which were also identified, together with sedimentation rate, as the main threats for this species (Strain et al., 2015). The two approaches have given very different results, with the complete stop of the growth for the thalli obtained by *ex situ* treatment on the artificial structures. To estimate the success of the two approaches, we analysed the results of the recruitment, growth trend and the percentage variation of the area covered by *G. barbata,* providing the first comparison with natural populations present in the donor sites and population restored in a preliminary outplanting intervention.

After the analysis conducted on the heights data during the whole *ex situ* experiment, we have identified the outplanting phase as the critical stage, despite the statistical analysis reported a significant growth between the two first sampling times in the field. In particular, the growth has continued for the first month reaching average heights of about 1 mm (higher for recruits developed in the tank 2), but then the thalli have disappeared. Conversely, taking into account the two times of outplanting, we have seen that after ca. one month the average height of the algae transferred earlier (one structure from each tank) reached the order of centimeters (1.5 ± 0.1 cm), while the others at the same time were not detected except for only one individual.

These results have suggested an important role of the outplanting timing and recruits' wellness at this time. In our study, the cultivation phase resulted successfully based on the observation and analysis of the recruitment and height trend. The results showed significant differences between the two tanks along time, despite both cultures being carried out under the same laboratory conditions. Indeed, tank 1 showed lower density compared to tank 2, but reached higher heights, this effect could be related to the natural variability in propagule release among the individuals (Savonitto et al., 2021), but also to the different timing of the start of culture experiments and outplanting in the restoration site. Indeed, the receptacles grown up in tank 2 were collected two weeks (20th May 2020) after those in tank 1. Receptacles used in tank 2 resulted more mature but also more covered by epiphytes (predominance of diatoms), probably belonging to species characterized by the same reproduction time. Their proliferation in the tank during the release of *G. barbata's* recruitment phase may have induced competition and consequent energy loss destined for growth (Savonitto et al., 2021). Comparing our results with previous studies, we obtained, after 1 month, eights between 0.6-0.7 mm, which result higher respect to those obtained by Verdura. (2018) for the same species (0.2-0.4 mm), but lower compared to those of Savonitto (2021), who reported average heights of 1.38 ± 0.13 mm. These observations have suggested the need of antibacterial solution addition to preserve the good state of the culture, by reducing the formation of biofilm and the consequent suffocation effect on the recruits, as previously reported (Savonitto et al., 2021).

The recruits obtained with the *in situ* approach were still present at the last sampling, determining, for the first eight months, the success of the first phase of the restoration intervention. Only few individuals were lost during the monitoring after the restoration intervention, and mostly due to sea storms. Of the 19 stones and the 2 structures transferred to the site, none of them was lost, despite the occurrence of two seastorm events in early October and mid-December 2021 respectively. The *G. barbata* individuals recruited with the *in situ* approach reached heights comparable to the size range of the juvenile population present in the same area (restored in a preliminary intervention), confirmed by similar growth rates. Similar results were achieved using both natural rocks and artificial structures; indeed, statistical analysis reported differences between the growth of individuals on natural (rocks) and artificial substrate (five-tiles structures) only at the beginning of the monitoring.

Growth rates from September to January 2022 confirmed the slowdown in the growth, observed in the trend of heights during the autumn-winter months. The minimum values were detected in September-October'21, in alignment with the data reported for the juvenile population, and at November-January'22. The stasis phase observed in autumn could be related to the natural biology of the species and the mechanical stress induced by the hydrodynamics condition occurring in the autumn-winter months, as reported in previous studies (Strain et al., 2015, Verdura et al., 2018, and preliminary thesis work).

Conversely, the highest growth rates reached in summer (in particular from June to August) by the juvenile of the restored population compared to those of the adult, could be explained by the different life stage of the individuals. The juvenile individuals, in fact, recruited in the late spring, are characterized by higher growth rates (despite the reaching of lower eights) than those reported for the maintenance of the canopy, observed during the monitoring of the adults at Scalaccia North. This effect could be also further favoured by the characteristics of the restoration intervention, carried out in areas where adults are not present, thus avoiding the competition for lights, which tipically occurs in well-developed canopies, where new recruits and juveniles are overhung by adult branches (Tamburello et al., 2019, Irving et al., 2009).

The analysis of the surface covered by *G. barbata* during the whole experiment reported an increase of ca. 70 % in algal covering in the restoration site. Since the beginning of the experiment, the growth has led the percentage of covering from 4 % (June 2021) to about 75% (January 2022). Similarly to the growth rates, this analysis reported a rapid increase (to ca. 60%) in the first four sampling times, and a subsequent stabilization in the last three sampling times (ca. 71-73%), confirming the good response of the *G. barbata* individuals to the outplanting in the restoration site.

Overall, our results confirmed the suitability of the selected site for a wider restoration intervention, as hypothesized by previous experiments, and the most efficiency of the *in situ* recruitment approach, both using natural rocks and artificial structures.

As expected, a first comparison between the data collected during the last year on the adult population, present in the donor site (Scalaccia North), and those reported in our experiment, reveals important differences in eights range size and growth rates. Although the good results of the growing trend in the restored population, the adult population reached a size range two times bigger than the restored population (for both methods of the *in situ* approach). However, it is necessary to consider that the reference population is formed by plurennial individuals and that they are located within a rock pool, therefore subject to mitigated conditions compared to the population restored at Scalaccia South.

In this regard, longer monitoring activities are necessary to assess if the restored population will reach the size and canopy cover similar to the donor populations. Moreover, in this study we did not consider other environmental stressors that could affect the individuals' growth differently in specific sites, as example in donor and restores sites, living in different conditions (i.e., rock pool and Scalaccia South site). As example, high sedimentation rates and hydrodynamism can cause the reduction of the light available due to the resuspension phenomenon, influencing the growth rates of the restored population (Irving et al., 2009). This effect is present with different entity along the whole Conero Riviera, probably affecting also the adults' population. Other variables, such as difference in temperature (due to the water surface rise), could influence the development in adult populations in different sites of the Mediterranean Sea (Bevilacqua et al., 2019).

As already mentioned, and according to previous studies, the correct evaluation of the success of a restoration intervention of macroalgal species requires monitoring on a wider period. Indeed, the indicators of the good state of a restored population, as density, structure and distribution require a long time to be representative of a restored population (Verdura et al., 2018, Savonitto et al., 2021).

5.2 Meiofauna communities' response to the restoration intervention

Meiofauna represent the most abundant group of the benthos, which include small organisms (20-500µm), protists and multicellular metazoans (Zeppilli et al., 2015, Bianchelli & Danovaro, 2016). Due to their high abundance and diversity, widespread distribution, rapid generation time, and fast metabolic rates, these organisms show rapid responses to environmental changes, presenting properties of good bioindicators (Zeppilli et al., 2015). Feeding on benthic prokaryotes, microalgae, detritus, and fresh phytodetritus and other meiobenthic organisms, they act as vital contributors of ecosystem function, including nutrients' cycling and energy and matter transfer to higher trophic levels (Bianchelli et al., 2016, Zeppilli et al. 2015, Schratzberger & Ingels, 2015). Moreover, meiofauna promote microbial activity and favour organic matter degradation. Since previous studies confirm the meiofaunal high sensibility to the *Cystoseira* forests loss (Bianchelli & Danovaro., 2020), in this study we used this group as proxy of the success of the restoration intervention. To do this, we analysed changes in terms of total meiofaunal abundance, community structure, and richness of taxa in the restoration site.

The first variable examined in this study was the total abundance of organisms detected in the six sampling times, from June to January 2022. In the last three sampling times, we analysed also samples collected under the structures used in the *ex situ* approach, allowing the comparison between points in which *G. barbata* has grown or not. In the first eight months, meiofaunal total abundance shows significant variability along time, with the highest abundance reported for the first three times (beginning period). Taking into account the significant differences reported between the two treatments (*G. barbata growing/no growing)* in the restoration site, we can hypotisize that the mere presence of *G. barbata* promotes an increase in the number of organisms (only significantly for the factor Treatment).

Although the different conditions present in the restoration and donor sites, the meiofauna abundance trend along time, detected for the restored population, is similar to those reported for the natural juveniles and adults' population present in the Scalaccia North. In addition, the restored and juvenile populations showed an anomalous peak of abundance on the second sampling time (T1), with the achievement of the highest abundances (almost three times the abundance detected in the other sampling times); these peaks occurred during June/early July in correspondence with the season characterized by the greater density of the meiofauna organisms (Danovaro et al., 2002). This increase was detected also in the adult population, with a lower entity (about the double abundance respect the other sampling times). Based on these observations we could consider this increase an effect of the seasonal fluctuation of the meiofauna, that probably results mitigated in adult population due to the effect of a develop canopy that favour the maintenance of highest abundance in all the sampling times, furnishing protection, and constant nourishment. This theory could be supported by the study of Hicks et al. (1989) that attribute the relative stability of meiofauna assemblages to the unlimited nutritional resources offered by macroalgal coverage. Moreover, we can't exclude an effect of attraction on organisms, linked to the development of new individuals, both new juveniles or restored ones.

Observing the taxonomic composition and relative abundance of each taxon, in all samples, in all sampling times, and in all populations, nematodes and copepods (mostly harpacticoid copepods) were the most dominant, as also reported in previous studies for hard bottom substrates (Danovaro et al., 2002). The only exception is detected on T5 sampling times characterized by the dominance of bivalves (> 50 %), probably related to their seasonal fluctuation and recruitment period. The bivalves abundance results higher in the *G.barbata growing* samples, together with the higher abundance of amphipods and polychaetes. This could be related to the properties of the macroalgal as optimal recruitment substrate for these groups and several other species (Danovaro et al., 2002). Other main groups identified in the samples are Ostracoda, Isopoda, Gastropoda, and Hydrozoa, which seem to have higher relative abundances in *G. barbata growing* samples in all the three comparison times (even if not significant).

The α - biodiversity assessed in the restored, juveniles and adults' populations results similar, but the relative abundance of each group caused important differences in terms of most abundant and rare taxa. As example, amphipods resulted principal taxa only in the restored population.

However, the overall taxonomic composition (with or without nematodes and copepods) did not vary significantly among the beginning phase, the *growing* and the *no growing* samples. Despite these results, the variability of the taxonomic composition for the restored population resulted significantly only for the factor time, suggesting a response of meiofaunal assemblage composition to the algal growth. Over the above mentioned only the other two taxa, chironomid and mites, show significantly higher abundance in *G. barbata growing* samples in the first eight months from the start of the experiment. The higher presence and fast response of these four groups is probably related to their higher mobility compared to organisms such as bivalves or ostracods. In addition, the sporadic presence of tardigrade and oligochaetes were observed only in *G. barbata growing* samples.

Is necessary taking into account that all the observations and evaluations on the data included in the study are subjected to a high variability due to sea conditions during the sampling activities. In particular, we can observe reduction during the winter, typical of the seasonal fluctuation of the meiofauna, but we suggest that the abundance reduction and different taxonomic composition could be related also to the occurrence of two seastorm events, respectively in October and December 2021. As reported for the experiment conducted by Danovaro et al. (2002) conducted in the Monte Conero sites, the meiofauna assemblages differ between shallow and deeper stations and the replicates taken in the more shallow station (as the site involved in this study) have higher variability than the others, probably due to the high hydrodinamysm, typical of the area.

Overall, our results suggest that a longer monitoring activities are required in order to assess the response of meiofaunal assemblages, in terms of abundance and biodiversity, to the algae' population growth. Moreover, also other benthic components should be monitored, as macrofauna, since they could have a faster and differential response when compared to meiofauna.

6. Conclusions

The results obtained in this study report the success, in the first eight months, of the *G. barbata* intervention implemented in the Scalaccia South on 1^{th} June 2021. The monitoring activities conducted on both approaches applied (*in situ* and *ex situ* recruitments*)*, show higher suitability of the *in situ* treatment for the restoration of this species, considering the complete failure of the *ex situ* treatment. We analysed also the possible causes of the *ex situ* treatment failure, assuming the important role of the timing in the receptacle harvesting and outplanting process.

In terms of meiofaunal response to the restoration intervention, total abundance and community structure show significant differences only considering the factor Treatment and Time. Despite these results, we can observe a general trend of higher abundance (even if not significant for the Treatment X Time factor) in the samples collected under the *G. barbata* grown individuals. In addition, we report that only some groups (amphipods, polychaete, mites and chironomid) respond positively to the restoration in the first eight months since the beginning of the experiment.

Despite the positive and encouraging results obtained in this study, to corroborate the success of this restoration intervention is necessary to implement a monitoring activity over a longer time period. This is because the development of macroalgal habitat-forming populations requires a long time to achieve a mature status and the capacity to self-sustaining through natural recruitment. A longer monitoring needs to be settled, with future samplings focused also on the evaluation of the occurrence of natural recruitment, to establish the possibility of the restored population to survive and extend its coverage. This is expected to enhance the three-dimensional characteristics of the habitat, thus favouring the enhancement of the biodiversity and associated ecosystem services, on a broad time scale.

High expectations are reported for the future of this restoration intervention, personal observations conducted during the sampling in October 2021 and January 2022 report the presence of receptacles indicative of reaching reproductive status. This unexpected presence is in line with the observations reported in the recent study of Bevilaqua et al. (2019), which demonstrated the reproductive status of *Cystoseira s.l.* in early February, three months earlier than the typical reproductive period. This indicates a high possibility to obtain natural recruitment in the next spring.

Restoration interventions, such as the one conducted in this study, can be the key to achieve a higher level of knowledge on the dynamics and forces playing in these systems, allowing in the future the implementation of large-scale restoration projects with the hope of restoring these habitats at a basin scale, since they represent a common good of all living species belonging to the blue planet.

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