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Effects of *Paramuricea clavata* (Risso, 1826) (Anthozoa: Plexauridae) forests on settlement of benthic species: an experimental approach.

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“Alla mia cara amica Rossella,
i cui capelli biondi, sul fondo del mare, si muovono armoniosi,
morbidi, come le tenere gorgonie in corrente.
Conserva il gusto dell’andare per mare Rossella.
Che il mare è tenerezza; e silenzio,
cose delle quali le persone sensibili
hanno un assoluto bisogno!”

Armando Lombardi
La Maddalena, Agosto 2003

Al Mare

perché il tempo passa,
ma Tu non passi mai

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Chapter 1: Introduction

1.1 Overview on coralligenous assemblages

Coralligenous habitat is considered the second most important subtidal "hot spot" of species diversity in the Mediterranean Sea after the *Posidonia oceanica* meadows (Boudouresque 2004). Despite the wide use of this term, there is no real consensus among scientists studying Mediterranean benthic communities about what a coralligenous habitat is (Ballesteros 2006). The word "coralligenous" (*coralligène* in French), for the first time used by Marion 1883 to describe Marseilles hard bottoms, stems from a misunderstanding: during XVIII sec. it was easy to find in the bottom trawls, that fished between 30 and 70 m deep, red coral branches (*Corallium rubrum*) whence derives the name. Today we know that this species does not characterise this particular habitat, but it is only occasionally part of it.

Pérès and Picard (1964) defined coralligenous as the hard bottom "biocenose", developed in the circalittoral zone, characterised by encrusting red calcareous algae associated with a rich fauna in way to forming a high micro-spatial variability habitat (Fig. 1). They also make a distinction introducing the word pre-coralligenous: a zone that comprises sciaphilic erect algae with thallus weakly calcified therefore unable to produce a significant concretion, sometimes considered as an impoverished facies of the proper coralligenous. In the opinion of these two authors it is apparent that the coralligenous habitat is bound to two different phenomena: the first concerns light intensity, low enough to permit the colonization of sciaphilic algae and animals, the second refers to the ability of these organisms to form conspicuous limestone substrates.

Many authors in subsequent publications (Laubier 1966; Sarà & Pulitzer-Finali 1970; Ros et al. 1985; Gili & Ros 1985), claimed that defining coralligenous as a single biocenose is ambiguous and restrictive to describe this multidimensional environment. Therefore this habitat should be considered as a set of biocenose or a community puzzle (Cinelli & Colantoni 1993; Ballesteros 2006).

Thus, considering all the progress acquired in the knowledge of this field, we can define coralligenous habitat as a typical Mediterranean hard bottom of biogenic origin, mainly produced by the accumulation of calcareous encrusting algae, that can be colonized by other sciaphilic organisms, forming an intricate assemblage of species able to transform it in a multidimensional habitat with a high micro-spatial variability (Ballesteros 2006).

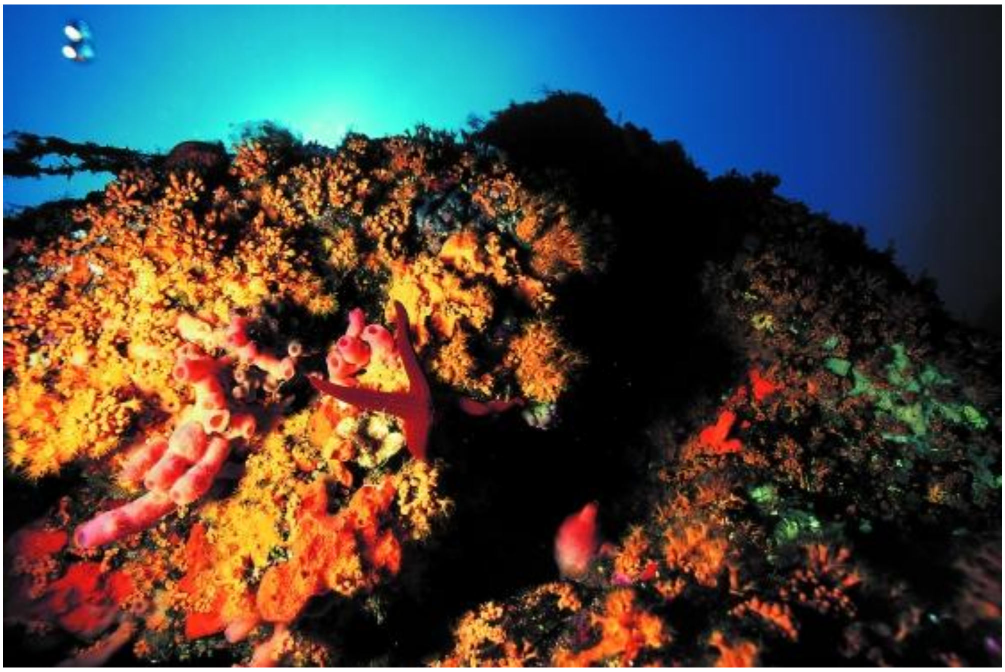


Fig. 1 - An example of the coralligenous system showing an explosion of colours and life represented by a high species variability on a small spatial scale (picture by Guido Villani).

1.2 Variables affecting coralligenous assemblages

Light seems to be the main environmental factor that determines the distribution of coralligenous assemblages (Ballesteros 1992; Marti et al. 2005) but inclination of substrata, hydrodynamism, nutrients, temperature and biologic interactions can also play a relevant role in structuring these benthic assemblages.

1.2.1 Light

Encrusting algae cannot withstand high levels of irradiance, but they need light as well to carry out photosynthesis process. Accordingly to Ballesteros (1992) the optimal amount of light for the development of these environments must range between 0.05% and 3% of the surface irradiance. In addition he find out that also quality of light should be taken into account: red algae in coralligenous system reach their deepest distribution linked with the ability of phycobilines to capture light in green window, the most abundant wavelength range in these habitats. We can find these ecological requirements at different depths in the Mediterranean Sea, in relation to local environmental conditions. The minimal depths recorded are around 10 - 15 m but on the other hand coralligenous framework can reach up to 100 - 120 m in clearest waters (Ballesteros 2006). Obviously light is in function of depth, sedimentation rate and topography of the area: some coralligenous assemblages can be found in shallower waters in places characterised by high turbidity or in dim-light areas or where the presence of other organisms can contribute to high variation of light on a small-spatial scale.

1.2.2 Inclination of substrata

Depending on the slope of the substrate, some areas in rocky walls or submarine landslides are shaded (cervices, holes, caves etc). Moreover inclination of substrata can acts on sedimentation rate (e.g. *Corallium rubrum* presents a negative geotropism that permits to the larvae to settle in places without sediments; Virgilio et al. 2006), hydrodynamism, disturbance factors and predation. In topographic condition of low light and in function of the prevailing algal builders the inner structure of coralligenous bottom can evolve in two main morphologies (Laborel 1987):

- Banks are mainly built over consolidate sediments on the continental shelf and can have a variable thickness that ranges from 50 cm to 3 - 4 m. They have a very cavernous structure and they have been compared to gruyere cheese.
- Rims usually develop in shallower waters than banks and cover vertical cliffs or the outer part of submarine caves. Their thickness ranges from 20 - 25 cm to 2 m and increases from shallow to deep waters.

1.2.3 Hydrodynamism and nutrients

Water movements can be divided in two main categories:

- Vertical movements (mainly due to wave action and winds along shore line and continental slope)
- Horizontal movements (geostrophic circulation and tidal currents)

In spite of the usual belief that waves are a superficial movement of the sea, the hydrodynamic force they generate can be occasionally important even at depths of 50 m (i.e. during storms). Their effect vary greatly in different areas, but it is connected with sedimentation rate and with the mixing of the nutrients in the water column. Since wave effect is stronger in shallow waters, the coralligenous framework is not strongly affected by their erosion in exception of superficial assemblages.

Regarding flowing currents that predominate in the depth range of coralligenous, they are very important because they influence oxygen and nutrients availability, sediment deposition and larval dispersal, an important factor that can influence the genetic variability and the range of distribution of a species.

Curiosity:

Gorgonians arrange their branches perpendicularly to the mainstream direction in order to filter larger volumes of water. Thus, looking at the orientation of the sea fans, it is possible to understand the direction of the principal current flow.

(by courtesy of Cycnus diving center)

1.2.4 Temperature

Although Pérès & Picard (1951) stated that coralligenous habitats show a relative stenothermy, there is evidence that also these systems, overall those shallower, display a marked seasonal variability, with warmer temperatures and the presence of thermocline in summer (20-23°C) and colder temperatures in winter (10-15°C) along all the water column (Ballesteros 2006).

In general most of coralligenous communities can tolerate the normal seasonal range of Mediterranean temperatures, but organisms usually found in deep waters are highly stenothermal: large-scale mortality events of benthic communities, like gorgonians, bryozoans or scleractinian corals thriving in coralligenous, have been related to abnormal stratification of water layers that leads to unusually long-lasting periods of high temperatures during summer (Cerrano et al. 2000; UNEP-MAP-RAC/SPA 2008).

1.2.5 Biologic interactions

The typical coralligenous structure with holes and crevices can sustain a complex epibionthic community dominated by suspension feeders (sponges, hydrozoans, anthozoans, bryozoans, serpulids, molluscs, tunicates). The smallest crevices of the coralligenous concretions can also host an extraordinarily rich endofauna, while many other epibionts organisms can cover the main macroalgae and other sessile invertebrates. In this multitude of organisms each one has a precise ecological significance and can be distinguished in four categories (slightly modified from Hong (1982) and Ballesteros (2006)):

- Builders: organisms which contribute to develop and consolidate the calcareous concretion. This category comprehends bryozoans (e.g. *Myriapora truncata*, *Pentapora fascialis)*, polychaetes (e.g. *Serpula vermicularis*), cnidarians (e.g. *Leptosammia pruvoti*), molluscs (e.g. *Vermetus* sp.), sponges (e.g. *Petrosia ficiformis*, *Chondrosia reniformis),* crustaceans and foraminiferans (e.g. *Miniacina miniacea*), but the main responsible of the growth of coralligenous concretions are red calcareous algae (Sartoretto et al. 1996). Their distribution depends on different local conditions, but *Mesophyllum* spp., *Lithopyllum* spp., *Neogoniolithon* spp. and *Peyssonnelia* spp. are identified as the most important for the coralligenous build up (Hong 1980; Ballesteros 1992). In the Mediterranean has been estimated the rate of production of carbonates by builders with a value of 103 g $CaCO^3$ m^{-2} yr^{-1} (Cebrian et al. 2000), production comparable to that estimated for tropical reef (Heiss 1995). In general the formation of encrusting coralline algae is very low: they may increase from 0.01 to 0.05 mm yr^{-1} depending on the building species and on depth (Vogel 1970). Ages obtained by radiocarbon dating of coralligenous frameworks situated at depths between 10 and 60 m in the northwestern Mediterranean Sea range from 640 ± 120 yr before the present (BP) to 7760 ± 80 yr BP (Sartoretto et al. 1996). From these data it is possible to understand why internal layers, mainly constituted by overlapping of dead algae's thalli, are considered to be sub-fossil.

- Vagile organisms: they inhabit holes and crevices and include different molluscs (e.g. *Octopus vulgaris*), crustaceans (e.g. *Palinurus elephas*), polychaetes (e.g. *Hermodice carunculata*), echinoderms (e.g. *Ophioderma longicaudum*), and fishes (e.g. *Epinephelus marginatus*, *Muraena helena)* (Fig. 2). Depending on size they can swarm everywhere from the surface to the cavities or even inside other organisms and thrive in the small patches of sediment usually retained by the framework.

Fig. 2 - Examples of settlers (*Muraena Helena*, *Palinurus elephas*, *Epinephelus marginatus*) that can find shelter in the coralligenous system. (pictures by Francesco di Meglio, Fabrizio Cecchini, D.K. Kersting).

- Epibiont organisms: they are represented by all sedentary species living on the calcareous substrate or on other organisms. A huge variety of frondose algae as *Halimeda tuna, Dictyota dicotoma, Halopteris felicina* grow above calcareous algae forming a veritable forests sometimes reaching up to 400% of cover (Ballesteros 1991) and host diverse animal assemblages. Type of sessile assemblages depends on currents and food availability: in places with oligotrophic waters, the dominant suspension feeders are sponges, bryozoans and hexacorals, but in richest zones gorgonians (*Paramuricea clavata*, *Eunicella cavolinii*, *Eunicella singularis*) can dominate the community leading to specific facies (Ballesteros 2006; Tunesi 2009).

- Eroders: organisms that erode calcareous concretions controlling with builders the growth balance of this framework. They are divided in:
    - grazers, represented by sea urchins, like *Sphaerechinus granularis* and *Echinus melus* whose algal stomach contents can represent 50% of the total (Sartoretto et al. 1996), and some gastropods;
    - macroborers including molluscs (e.g. *Lithophaga lithophaga*), sipunculids, polychaetes and several excavating sponges like *Cliona viridis* considered the most powerful destructive sponge of calcareous substrata (Rosell et al. 1999);
    - microborers, as cyanobacteria, green algae and fungi;

It is clear that coralligenous assemblages are formed by a vertical stratification of organisms. This leads to a considerable species diversity comparable to that of tropical coral reefs (Ros et al. 1985). Laubier (1966) considers it as a “mature” ecosystem with a set of communities that leads, as suggested by Pérès & Picard (1964), to a climax biocenosis. The intensity of colonization of the substrate is high and drives to a complex ecological network with intraspecific and interspecific interactions between organisms (Bavestrello et al. 1995).

Unlike other habitats where herbivory and carnivory processes are dominant, here the largest part of the living biomass in coralligenous assemblages consists of autotrophic organisms and suspension feeders. Space competition is strong and suggests that investment in production of allelochemicals plays an important role (see Fig. 3).

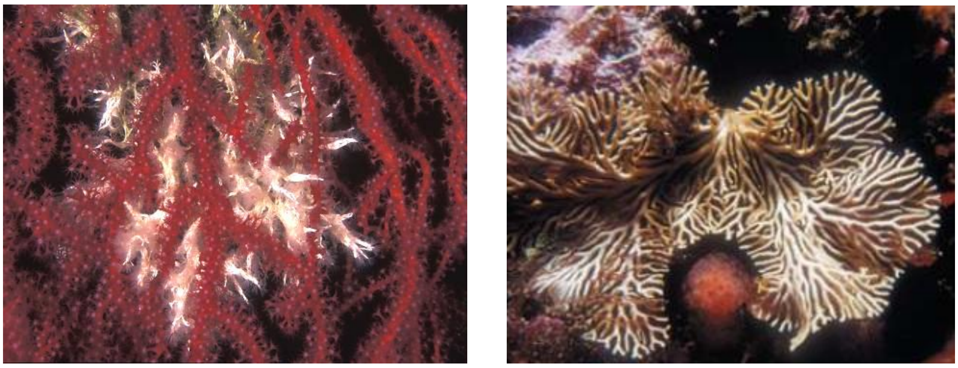


Fig. 3 - Examples of space competition: in the first case the polychaete *Filograna implexa* growing as epibiont of *Paramuricea clavata* (by courtesy of Egidio Trainito), in the second case the tunicate *Pseudodistoma cyrnusense* that inhibits growth of the bryozoan *Hornera frondiculata* with the production of allelochemicals (extracted from Ballesteros 2006).

Most of organisms have chemical defence that make them unpalatable or even toxic (Marti 2002) although this does not exclude the presence of predators like fishes, molluscs, echinoderms and vagile polychaetes. It is simple to find conditions of epibiosis, mutualism, commensalism and parasitism. In this so crowded environment, epibiosis is fundamental for the settlement of larvae. Some basibionts can tolerate different degrees of epibiosis and even almost complete overgrowth, whilst others have developed antifouling defences to avoid this phenomenon. Commensalism as well is one of the common relationships in these communities: the polychaete *Haplosyllis depressa chamaeleon* lives exclusively as a commensal of the sea fan *Paramuricea clavata*, crawling above the living colonies (Laubier 1966).

1.3 *Paramuricea clavata* (Risso, 1826) as a key species

This great environmental heterogeneity created by biotic and abiotic factors allows several different assemblages to coexist in a reduced space. One of the most beautiful and intriguing assemblage is that characterised by *Paramuricea clavata* (True 1970; Tunesi 2009). This organism, also called Mediterranean red gorgonian, is the largest and most colourful of the Mediterranean Sea: its colour varies from red to dark violet but in some places, especially in southern areas, they may have outer branches with yellow tones and the causes of this variation are still misunderstood. Unlike other gorgonians, their branches are thicken toward the end assuming a typical club-shape from which derives the scientific name.

Curiosity:

Parerythropodium coralloides, belonging to Alcyonacea, is commonly known as false red gorgonian. The common name comes from the remarkable similarity with *Paramuricea clavata* due to its red, pink or yellow polyps. These encrusting colonies generally settle on the branches of dead gorgonians occupying the hard skeleton which they lack.

(by courtesy of Egidio Trainito)

Paramuricea clavata is a colonial organism made up of very small polyps, equipped with eight tentacles (Octocorallia) with lateral pinnules, organized in a way to form broad fan that can exceed one meter in diameter or height. All the polyps belonging to a fan, so to a colony, are clones thus they have the same genetic. With its presence the red gorgonian can contribute to above 40% of total biomass of the community (True 1970; Gili & Ros 1985; Harmelin & Marinopoulos 1994) and brings significant structural complexity into the coralligenous habitat (Gili & Ballesteros 1991) creating characteristic facies (Tunesi 2009).

Its areal of distribution is limited to the western Mediterranean and in colder regions of the Adriatic Sea (Carpine & Grasshoff 1975). In some areas the population density can reach 55 colonies m^{-2} (Weinberg 1991; Coma et al. 1995; Linares et al. 2005) but the average densities vary depending on the local substrate and microhabitat: for example around the Portofino promontory densities goes from 9 to 28 colonies m^{-2} (Cerrano et al. 2000).

Red gorgonian is a sciaphilic species that grows at depths ranging from 5 to 110 m. It is abundant in steep rocky walls, but it also grows in horizontal or sub-horizontal surfaces if light levels are very low. Since it is a suspension feeder it prefers areas of high hydrodynamic activity. The colony size is directly dependent from water velocity thanks to the beneficial role of streams in carrying prey and increasing respiration, so in places like the Strait of Messina characterised by strong tidal currents, the growth rate is higher than in places with less powerful flows (Mistri & Ceccherelli 1994). Unlike stony corals and other gorgonian species, tissues of *Paramuricea clavata* do not host symbiotic zooxanthellae, and biomass accumulation is only due to heterotrophic nutrition: its diet mainly consists of plankton particles with size from 3.8 µm (nanoeucaryotes) to 700 µm (copepod eggs and nauplii can constitute the 80% of daily diet; Coma et al. 1994), but seems that an important role is played also by Particulate Organic Matter and Dissolved Organic Matter. In places where gorgonians are able to form wide forests, they play a significant role in transferring energy from planktonic system to benthic system (Mistri & Ceccherelli 1994).

Its polyps capture these particles in a passive way and digest them in the gastro vascular cavity. The food is available to the entire colony through the mesoglea (coenenchyme) that covers a horny skeleton quite flexible to the currents. Its main predators are polychaetes (e.g. *Hermodice carunculata*) and gastropods.

Curiosity:

Some species of Nudibranchs that feed on gorgonians, incorporate their stinging cells (cnidocytes) without digesting and storing them in cnidobags. These are located in expanded appendices called cerata.

In this way the properties of these stinging cells "borrowed" remain unchanged and can therefore be used as protection from predators. *Okenia mediterranea* and *Marionia blainvillea* (in the pictures below) are examples of nudibranchs that feed on *Paramuricea clavata*.

(www.seaslugforum.net)

(picture by Massimo Ponti)

Paramuricea clavata is gonochoric (with a sex ratio generally of 1:1) and an iteroparous species: reproduction is synchronous and happens every year. Spawning occurs at the end of May coinciding with increasing water temperature and correlated with the lunar cycle (from 3 to 6 days after the full moon or the new moon). Spawned eggs adhere, through the action of a mucous coating, to the outer surfaces of the female colonies. The final maturation of the planulae takes place among the polyps. Larvae have a limited mobility so they immediately settle on the surrounding substrate. Asexual reproduction by fragmentation is negligible and the maintenance of the population is based on sexual reproduction (Coma et al. 1995). The age of an individual can be measured in the basal cross section of the main axis where are present annual rings (like in trees): each of them is made up by two alter growth bands, light and dark, corresponding to different growth speeds in winter and summer. The growth rate of *Paramuricea clavata* is variable depending on the area, but it was estimated to be of about 3.0 (±0.3 SD) cm per year (Mistri & Ceccherelli 1994) so an individual of 1 m of high is about 33 years old.

This shows us that this species has a very slow growth rate and a long lifespan. This raises the question of its ability to cope with the increased recovery of strong disturbances (Coma et al. 1998a; Garrabou et al. 2009).

Curiosity:

The words “gorgon" is the Greek equivalent of "mermaid”. In Greek mythology the Gorgons were female entities that had snakes for hair: Medusa was one of them. Yet apart from some benign traditions, mermaids are not necessarily cute or romantic creatures, but can also be monstrous like the Gorgons. This is only a guess, but Gorgonian corals name could derive from the facts that sea fans branches in the current resembled to Gorgons hair. There is also a less fanciful explanation: the word *Gorgonia* in Latin simply refers to coral. So it is possible that sea fans are seen as being turned to stone as were any mortals who looked at a Gorgon.

(www.ancientsculpturegallery.com)

1.3.1 *Paramuricea clavata*'s facies

Paramuricea clavata can form facies characterised by a high density that reaches about up to 60 colonies m^{-2} (Linares et al. 2005). This assemblage is deeper than those characterised by the gorgonians *Eunicella singularis* and *E. cavolinii*, even if specimens of these species are commonly co-present with *Paramuricea clavata.* It is common in the western Mediterranean Sea and in the southern waters of the Adriatic basin and it could be found among 23 – 70 m depth (even if the species has a bathymetric range of 5 – 110 m depth) in sites moderately exposed to waves and currents (Tunesi 2009). Linares et al. (2008) curiously find out that differently from *E. Singularis*, *Paramuricea clavata* displayed a strong negative correlation between biomass and density of colonies indicating a negative density-dependence that suggests intra-specific competition among populations of this species. We can compare gorgonian facies with forest on land: as trees are forests fundamental unit, sea fans are the basic unit of the assemblage. Trees on land are arranged in a way that their leaf surface is better exposed to the sunlight to perform photosynthesis and with the same principle, under optimal conditions all gorgonian colonies have a parallel structure and their branches grow up in a way to form a web to retain planktonic particles carried by the current. Due to their big size and density sea fans can add heterogeneity to the habitat and form a sort of forest capable of create shelter for many necto-benthic organisms. Therefore gorgonians could represent the basic element of ecosystems that can in some cases modify and structure the landscape (Tunesi 2009; UNEP-MAP-RAC/SPA 2008a). After that, can we consider *Paramuricea clavata* as an ecosystem engineer (Jones et al. 1994)? Even if this concept is not simple to demonstrate it focuses on how organisms can affect the abiotic environment and how this feeds back to the biota. This framework can be explained synthetically by four cause/effect relationships linked together (Jones et al. 2010):

1. An engineer causes structural change;
2. Structural change causes abiotic change;
3. Structural and abiotic change cause biotic change;
4. Structural, abiotic and biotic change can have a feedback;

In general it is known that in some way all organisms affect, and are affected, by the environment in which they live. Hastings et al. (2007) introduced temporal and spatial scales in order to understand when the environment modification has to be explicitly considered: conceptually when the temporal and spatial scales of engineering effects differ from the temporal and spatial scales of direct organisms interactions we can talk about an ecosystem engineer species.

1.4 Disturbances factors affecting coralligenous habitats

Within coastal ecosystems hard bottom, characterized by high species diversity, have always played a very important role for humans needs. Beside this utilitarian aspects, these environments are dominated mostly by sessile organisms therefore are good indicators of water quality and environment changements. In addition many species that find shelter in coralligenous framework have an economic value (e.g. grouper *Epinepelus marginatus*, spiny lobster *Palinurus elephas*, red coral *Corallium rubrum*) and in recent years with the development of scuba diving, coralligenous high species diversity has become a tourist attraction making these assemblages an important economical resource. The knowledge of this environment is very important both for an environmentally friendly use and for its protection from its main degradation factors.

1.4.1 Human activities

Since ancient times mankind has been linked to the sea and to its resources. Over time this relationship has changed together with humans needs that, with their increasing, nowadays often lead to a situation of overexploitation or to a ill-use of this immense resource (Airoldi & Beck 2007). Fishing and diving are the main human activities involved in degradation of coralligenous assemblages (Ballesteros 2009):

Fishing – Trawling is the most destructive fishing method causing degradation of large areas of coralligenous (Boudouresque et al. 1990; Ballesteros 2009) (Fig. 4). Its damage consists in break down the coralligenous structure and negatively affect the photosynthetic production of encrusting and erect algae by increasing turbidity and sedimentation rates when used in the nearby of sedimentary bottoms (Palanques et al. 2001).

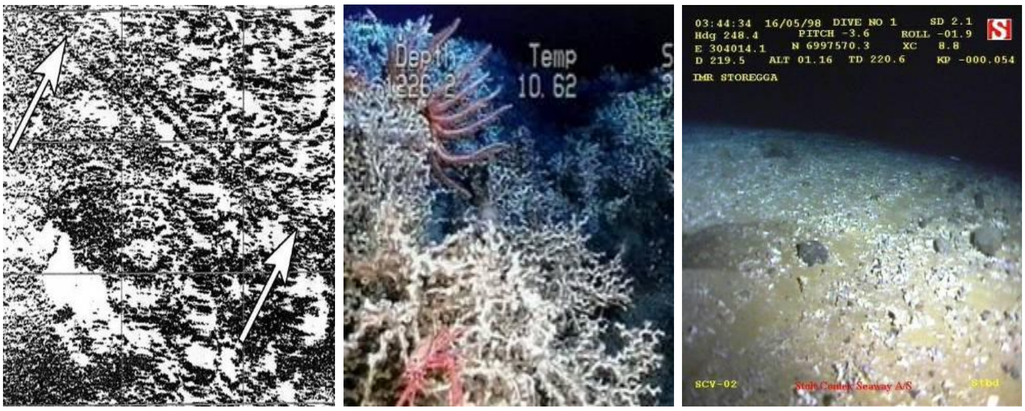


Fig. 4 - Sonar-gram from a side scan sonar showing a trawl track penetrating a deep sea coral area, usually ranging between 200 – 1000 m depth. In the other pictures it is shown how trawl fishing can turn a prosperous garden in a barren landscape. This is the same effect that this activity can have on Mediterranean coralligenous systems found in shallower waters (from www.imr.no).

Another highly destructive gear, nowadays fortunately banded in the most of Mediterranean regions (AA.VV. 1988), is the so called "St. Andrew's Cross" used to collect red coral. With this instrument and its grating tools, up to 50 Kg of benthic fauna (mainly gorgonians) were wiped away in order to collect only 15 Kg of red corals (Ortiz et al. 1986). Regarding other fishing methods, they have an indirect effect on coralligenous system limited on target species. Luckily, since depth acts as a protective factor, no cascading effects through overfishing have so far been detected in coralligenous communities, as they have been in shallow rocky bottoms (Sala et al. 1998) although they may well exist. Regarding *Paramuricea clavata*, injuries caused by indiscriminate anchoring and fishing was considered the major cause of mortality in the Portofino promontory before the establishment of marine park (Bavestrello et al. 1997): lesions inflicted favour the development of aggregates of epibionts (mainly hydroids and bryozoans) which leads to greater mechanical stress, increasing resistance to currents and to a weakening of the entire colony with a lower number of reproductive, trophic and defensive polyps thus an alteration of the anatomo-physiological integrity (Wahle 1983).

Diving – due to its great variety of life and to its great visual appeal, amplified by the presence of *P. clavata* forests (Harmelin & Marinopoulos 1994), the coralligenous community is one of the most popular sites for recreational diving in the Mediterranean Sea (Boudouresque 2004). Divers impact mainly concerns large fragile invertebrates like bryozoans: studies on *Pentapora fascialis* are an example that put in evidence changes on density, dimension and position of these species in sites highly frequented. Remarkable is the fact that the impact was lower in areas where were present dense canopies of *Paramuricea clavata* that offered protection to this species (Sala et al. 1996). This does not exclude gorgonians vulnerability to this factor that increases by three times their mortality, due to toppling by divers and unintentional breaking (Coma et al. 2004; Harmelin & Marinopoulos 1994).

It is easy to deduce that human disturbances might lead the coralligenous community to be dominated by erosion-resistant species, such as encrusting and massive organisms, rather than erect, articulate and foliose species, affecting in this way all the coralligenous community (Garrabou et al. 1998).

1.4.2 Pollution

In polluted areas is frequent a decrease in species diversity that in coralligenous assemblages mainly affects bryozoans, crustaceans and echinoderms. Despite this statement, the density of sipunculids as well as the relative abundance of species with a wide ecological distribution sometimes is enhanced by pollution (Hong 1983). The abundances of the species responsible for coralligenous accretion decrease with the pollution gradient: specific causes are unknown although it is known that orthophosphate ions inhibit calcification (Simkiss 1964). To exacerbate this situation there is the increase of bioeroders in polluted areas (Hong 1980) that lead to affirm that pollution accelerates the destruction of coralligenous assemblages and inhibits building activity.

Another cause of deterioration of these communities is sedimentation rate, significantly increased in recent decades along coastal areas due to human activities (Airoldi 2003).

Besides increasing the water turbidity, hindering the metabolic functions linked with photosynthesis, the deposition of sediment on the bottom, its instability and its abrasive effect can be a source of stress to the benthic community, removing whole organisms or their parts and preventing the settlement of spores and larvae and subsequent recruitment (Taylor & Littler 1982). In France a case of sedimentation of polluted particulate matter caused catastrophic mortality of gorgonian populations that accumulated harmful substances in tissues and in their skeleton (Arnoux et al. 1992).

1.4.3 Invasive species

Since the beginning of the 20th century, the number of introduced species to the Mediterranean Sea has nearly doubled every 20 years and some of them have become invasive (Boudouresque & Verlaque 2002). Currently, the most dangerous alien species for the coralligenous community is the red alga *Womersleyella (Polysiphonia) setacea* able to form a dense carpet, 1–2 cm thick, over the encrusting algae (Ballesteros 2004). The establishment of this alga, persisting from year to year, has several adverse effects:

- decreases light availability reducing or even avoid photosynthesis and thus algae growth (Ballesteros 2006);
- increases sediment trapping (Airoldi et al. 1995);
- excludes other macroalgae species by overgrowth and pre-emption (Piazzi et al. 2002);
- inhibits recruitment of corallines and other species inhabiting the coralligenous community (Ballesteros et al. 1998);

These effects lead to a lower species diversity in sites invaded by *W. setacea* respect of that observed in non colonized sites (Piazzi et al. 2002).

Although mainly found in relatively shallow waters *Caulerpa taxifolia* is another worrying species since it has been recorded down to a depth of 99 m and in some places it has totally invaded the coralligenous community (Meinesz 1999). *C. racemosa* var. *cylindracea*, another invasive species that is quickly spreading in the Mediterranean Sea, is also able to grow in deep waters and the same is for *Asparagopsis taxiformis* and *Lophocladia lallemandii*, but no information about their impact on the coralligenous community is available (Ballesteros 2006).

1.4.4 Climatic anomalies

In the Mediterranean Sea, due to its conformation of semi-closed basin, the effects of global warming are amplified. In general climatic changes, significantly increased over the past 25 years, are considered the main cause for the major marine ecological disturbances, among with those of the benthic systems are the most worrying (Williams & Bunkley-Williams 2000).

Acidification - Atmospheric CO_2 concentration started to increase with the beginning of the industrial revolution and have been worsened due to increased human activities. This increase have two different impacts on marine environment:

- causes shifts in seawater carbonate chemistry, decreasing pH and leading to an acidification of waters (Caldeira & Wickett 2005).
- leads to a continuous warming of Earth's temperatures thus ocean temperatures (Solomon et al. 2007);

Acidification affects in particular marine organisms with a carbonate skeleton such as corals, foraminifera and coccolithophores among with coralline red algae are the most sensitive due to their skeletal composition of high-magnesian calcite, which is the most soluble form of $CaCO_3$ (Milliman 1974). This assertion let us affirm that the decline of coralline algae could have dramatic consequences for the ecosystems where they develop. Some studies verified that algae calcification is significantly reduced under elevated pCO_2 (Martin & Gattuso 2009), but while growth and calcification of coralline algae increases with increasing temperature in the normal range (Martin et al. 2006), temperature above the thermal optimum have a detrimental effect: necroses and death in the algal population with high pCO_2 was two- to threefold higher under long exposition to elevated temperatures (Martin & Gattuso 2009). Mortality events are attributed to long periods of high temperatures during the summer season. In the NW Mediterranean were reported mass mortalities for organisms belonging to five different invertebrate phyla (Ascidiae, Bryozoa, Cnidaria, Mollusca and Porifera) of benthic organisms living in coralligenous communities among with gorgonians (Cerrano et al. 2000; Garrabou et al. 2009; UNEP-MAP-RAC/SPA 2008b).

1.5 *Paramuricea clavata* and mass mortality events

The next disturbances factors we are going to examine, are strictly linked with gorgonians and with the mass mortality events that occurred in the NW Mediterranean on facies with *Paramuricea clavata* (Fig. 5).

Fig. 5 - A coralligenous landscape with colours turned off by a gorgonian mass mortality event during summer of 2008 (by courtesy of Egidio Trainito).

1.5.1 Thermal stress

Recent studies have identified a correlation between the North Atlantic Oscillation (particularly positive NAOs) and climate variability in the north-western Mediterranean Sea (Molinero et al. 2005) traduced in warmer winters and summers with heat waves: recordings of sea water temperature for the summer of 1999 in Liguria, presented a seasonal thermocline that went progressively down to a depth of 65 m (Cerrano et al. 2000). At the same time, the temperature in the water column above the thermocline rose up to 23-24°C and kept this value for about two months (Romano et al. 2000). Autonomous thermographs installed in Provence region (Perez et al. 2000) confirmed for the late summer of 1999 an average temperature 5 - 6 °C higher than that of the other years with no documented evidence also in Greece and Tunisia. Since that year, other intense mortalities were recorded, one during the summer of 2003, considered the warmest summer in the last thirty years (Marullo & Guarracino 2003; Harmelin 2004; Sparnocchia et al. 2006), and the others during summers of 2005 and 2006.

These heat waves events affected at least thirty invertebrate species over a huge area comprised by Italian, French and Spanish coasts. Among organisms damaged there were sponges, the red coral, bryozoans, molluscs, ascidians and the scleractinian corals shown repeated whitening. These climate anomalies hit in particular way gorgonians among with *Paramuricea clavata* is the most sensitive (Fava et al. 2009): the first sign of stress was an unusual mucus secretion followed by a progressive loss of pigmentation. The coenenchyme becomes greyish and starts to be affected by fungi and after that detached from the skeletal axis making it vulnerable to colonization (Fig. 6).

Fig. 6 - A particular of *Paramuricea clavata* showing necrotic branches and a bare area of the skeletal axis (picture by Massimo Ponti) and a colonization process by *Turbicellepora avicularis* as a consequence of necrosis (by courtesy of Egidio Trainito).

The typical assemblage of opportunistic species that overgrown on *P. clavata* is represented by the sponge *Pleraplysilla spinifera*, the hydroid *Garveia grisea*, the bivalve *Pteria hirundo* and the bryozoan *Turbicellepora avicularis*.

At the SEM analyses it is possible to see that on damaged portions, the epithelium lacks completely and spicules are disorganized. Moreover polyps are retracted and around them grows a dense concentration of protozoan ciliates that actively eat the polyp eliminating it completely (Cerrano et al. 2000).

1.5.2 Mucilage events

The proliferation of mucilaginous aggregates usually happens in the summer season and could have strong impacts on tourism, human health, fishing activities and on the ecosystem dynamics. The mucilaginous aggregates are mainly constituted by complex polysaccharides produced by phytoplankton and their formation is due by the synergism among different factors (Giani et al. 2005):

- increase in the micro algal concentration
- increase in the sea water temperature
- stasis of the hydrodynamic forces
- bacterial activity

Whatever the main mechanisms conducing this phenomenon, that are still poorly understood, these aggregates accumulating on the seabed have a harmful effect on the benthic population causing anoxia and suffocation (molluscs, coelenterates and crustacean), necrosis or even mass mortality of organisms with an erect position such as sponges, scleractinian and gorgonians (Rinaldi et al. 1995, UNEP-MAP-RAC/SPA 2008b).

A mucilage event pass through different stages, but the more dangerous for coralligenous assemblages starts with the formation of macro-aggregates suspended in the water that easily get entangled in projecting structures of erects organisms (Fig. 7). In the case of the Tyrrhenian sea was identified *Acinetospora crinita* (Phaeophyceae Ectocarpales) as the main component of the mucilaginous aggregates that affects *Paramuricea clavata* since this algae develops at greater depths respect *Nematochrysopsis marina* and *Chrysonephos lewisii* (Chrysophyceae) that affect mainly gorgonians of the genus *Eunicella* (Giuliani et al. 2005).

As observed by Mistri & Ceccherelli (1995), once the mucus flakes cover sea fans the polyps can't extrovert. Metabolic exchanges with the water are therefore inhibited and branches turn their colour into a green-brownish. At the end of the event, as a consequence of prolonged anoxic conditions, coenenchime traits which was covered by mucilage became necrotic and decayed leaving bare more or less extensive portions of the axial skeleton (Mistri & Ceccherelli 1995).

Fig. 7 – An obvious example showing mucillage aggregates entangled on sea fans branches (by courtesy of Egidio Trainito)

1.5.3 Pathogens

After the mass mortality event in the summer of 1999 many studies were conducted in order to identify possible triggers but only few studies were conducted to test the bacterial hypothesis (Martin et al. 2002; Bally & Garrabou 2007). Only during the last year was confirmed the presence of vibrios as an additional exacerbating factor enhanced by high temperatures (Vezzulli et al. 2010).

Due to high temperatures that lead to high respiratory demand (Previati et al. 2010), thermal stratification that involves a decrease in food and oxygen availability, *Paramuricea clavata* is exposed to a prolonged physiological stress. This situation place the organism under energetic constraints resulting in a dormancy phase or decreased activity that can leads to death at the end of summer (Coma et al. 2009). In these conditions, sea fans are more likely to be affected by bacterial disease: vibrios are normally associated with *Paramuricea clavata*, but their concentration and their virulence increase with the increasing of the temperature once passed the threshold of 22°C (Vezzulli et al. 2010). *Vibrio corallilyticus* was isolated by tissue samples of diseased colonies and all the Koch's postulates were satisfied proving the bacterial pathogenicity (Bally & Garrabou 2007). In the late summer of 2006 it was presented again another mass mortality event where the facies with *Paramuricea clavata* was hit down to 50 m (Cerrano & Bavestrello 2008). Sea fans death didn't correspond with the maximum of temperature, neither with the maximum density of *Vibrio* spp. both measured in midsummer, but has occurred later sustaining the hypothesis of the physiological weakening of gorgonians. Therefore, under a holistic view, temperatures and time are the main factors that affect negatively food and oxygen and positively *Vibrio* spp. activity and its concentrations in seawater (Vezzulli et al. 2010), but all the mechanisms involved in mass mortality events remain to be clarified.

1.6 Recovery after mass mortality events

In synthesis we can state that the occurrence of *Paramuricea clavata* mortality was linked with some main factors that can act in concomitance or individually:

- prolonged high sea temperatures
- low levels of phytoplankton biomass and nutrients
- presence of culturable *Vibrio* spp.
- presence of mucilage aggregates

among with temperature has a deep effect influencing all the others.

These events occur at the end of summer and involve a general decline in the coralligenous system, linked with gorgonians disease: affected colonies showed patchy or complete tissue loss exposing bare areas of the skeletal axis to exogenous factors.

In benthic communities, where competition for space is very strong, such bare branches constitute an immediately available free substratum on which overgrowing organisms can settle.

Regarding mucilage events, complete mortality was mostly observed in smaller colonies, probably because the damage leaves too little coenenchyme to supply enough energy for regeneration that should be rapid enough to exclude colonization (Mistri & Ceccherelli 1996). For the mass mortality directly linked with temperature instead, small colonies of *Paramuricea clavata* were less affected and recovered faster (Linares et al. 2005; Cerrano et al. 2005) probably for their lower metabolism, a high P/B ratio (production/biomass) (Mistri & Ceccherelli 1994), a less reproductive effort (Coma et al. 1995) and because they are more chemically defensive respect larger individuals (Dube et al. 2002).

The survivor and recovery of an organism is directly linked with the dimension and the perimeter of the damage. Marginal polyps can regenerate the tissue and grow over the pioneer epibiotic community (Mistri & Ceccherelli 1996; Cerrano et al. 2005) a behaviour described also for another Mediterranean sea fan *Eunicella singularis* (Theodor 1964) but if epibionts are strongly settle there is another strategy, first described for the other Mediterranean Sea fan *Eunicella cavolinii* (Bavestrello & Boero 1986), consisting in the auto amputation of the affected branches (Cerrano et al. 2005).

During these events, also the sex ratio can be easily altered. The male bias (3.3:1 instead of 1:1) observed in the Portofino promontory population is undoubtedly related to the 1999 mass mortality episode during which it was recorded a high level of female mortality (Cerrano et al. 2005). This imbalance is strongly linked with the increasing of the fertilization success, registered after the event, and if mass mortalities affect one sex more than another, the sex ratio could be an important parameter useful to study population resilience after die off (Cerrano et al. 2005).

Besides the alteration of sex-ratio, in the post-event period was registered a change in the population size structure with a decrease in the modal class of colonies size (Cupido et al. 2008). In the Ligurian population thanks to a successful sexual reproduction the density was soon restored (Cerrano et al. 2005) but in France for example, there was a delayed effect leading to a 70%

loss in *Paramuricea clavata* biomass linked with unsuccessful recruitment that did not offset mortality (Linares et al. 2005).

1.7 Our goal

Within habitats spatio-temporal changes (seasonality, type of substrate, etc.) and fluctuations of chemical and physical conditions (temperature, salinity, current regime, etc.) may occur. Besides being linked to these abiotic factors, assemblages are also influenced by biotic factors like predation, competition, facilitation etc. These factors can interact together in characterizing the distribution of organisms. It is important to understand the variability of populations in order to better define and interpret the causes of their changes.

In recent decades, in order to understand mechanisms and processes structuring benthic communities scientists started to use the experimental approach: this comprehends the study of natural systems through the *in situ* manipulation of one or more environmental variables, while other conditions are allowed to vary naturally. This represents an innovative method of study if compared to classical ecology, whose approach is purely descriptive. The results are more easily applicable than those obtained in laboratory tests, where all variables are strictly supervised by the researcher that alters quite significantly the natural environment.

For practical and economical reasons manipulative experiments are usually not conducted on a large spatial scale, and experimental sampling design often includes some randomly selected sites in order to extend the results to wide areas. Moreover the experimental approach is not yet widely used in underwater research, both for technical difficulties (weather, time of decompression, etc.) and high costs (diving equipment, boat rental, etc.), both for problems related to health and safety of the researchers.

Besides this kind of problems, in manipulative experiments the researcher must pay attention to find the appropriate control associated to the various artefacts due by handling, otherwise the experiment may give non accurate results.

Paramuricea clavata, with its wide fans protruding from the bottom, creates a three-dimensional habitat leading to the development of many different ecological niches in a small area thereby increasing assemblages complexity.

Their presence could have the same ecological role of other systems such coral reefs, *Posidonia oceanica* meadows or terrestrial forests that can modify the environment creating three-dimensional habitats modifying species diversity and the seascape.

Several disturbances threaten this species, but recent and more frequent catastrophic mass mortality events have been deeply affected the distribution and abundance of *Paramuricea clavata* forests in the last years. The possible effects of gorgonian forest loss are largely unknown. Our goal is to analyse the ecological role of these sea fan forests on the coralligenous benthic assemblages. In particular, what are their effects on the settlement and recruitment processes? The influence of *Paramuricea clavata* in the settlement and recruitment of benthic organisms was analysed by a field experiment carried out in two randomly selected places: Tavolara island and Portofino promontory. The experiment consisted in recreate the presence and absence of the gorgonian forest on recruitment panels, arranged in four plots per type, interspersed each other, and deployed at the same depth (Fig. 8).

This study is part of a research project conducted in collaboration among the universities of Bologna, Genoa and Polytechnic of Marche. As part of this project was carried out a similar experiment with the gorgonian *Eunicella cavolinii* (Koch, 1887).

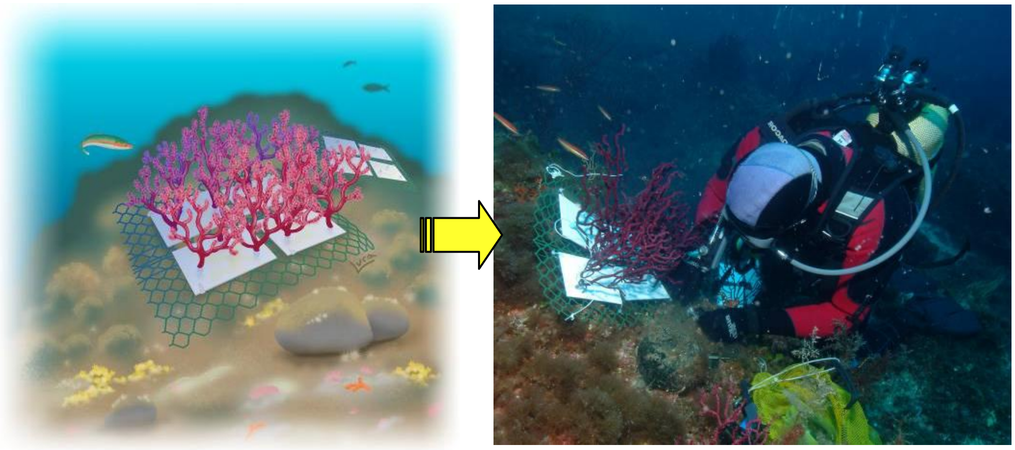
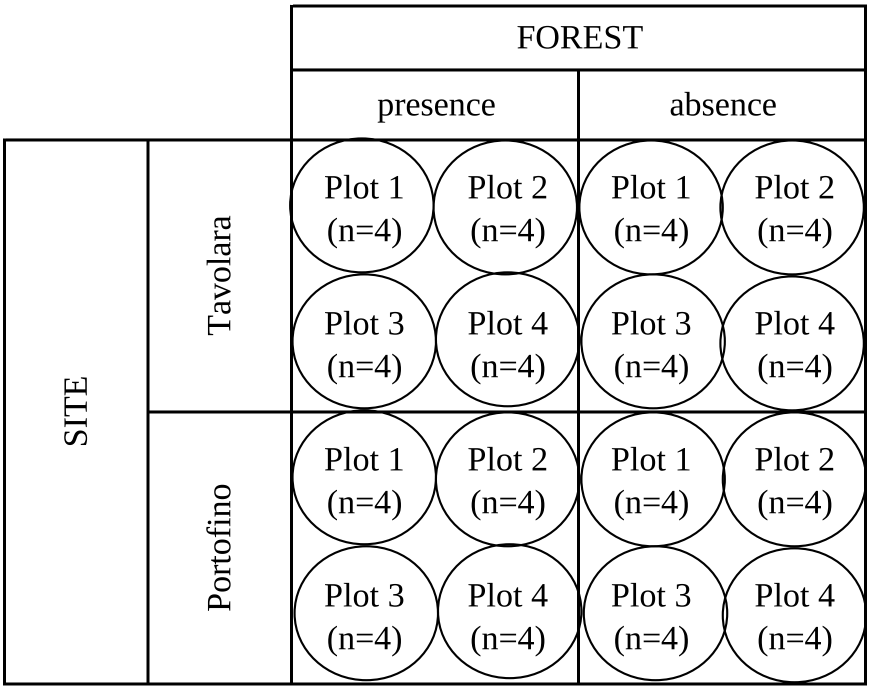


Fig. 8 - – On the left a drawing (by Luca Parisi) showing the project of this experiment while on the right a scientific diving operator at work occupied in the installation of the plots on the sea bottom (by courtesy of Egidio Trainito).

Chapter 2: Materials and Methods

2.1 Sampling design

Overall our experiment comprises two orthogonal factors (Site, Forest), a nested factor (Plot) and 4 replicates for every plot (see Fig. 9).



		FOREST	
		presence	absence
SITE	Tavolara	Plot 1 (n=4) Plot 2 (n=4) Plot 3 (n=4) Plot 4 (n=4)	Plot 1 (n=4) Plot 2 (n=4) Plot 3 (n=4) Plot 4 (n=4)
	Portofino	Plot 1 (n=4) Plot 2 (n=4) Plot 3 (n=4) Plot 4 (n=4)	Plot 1 (n=4) Plot 2 (n=4) Plot 3 (n=4) Plot 4 (n=4)

Fig. 9 – Sampling design.

Since conditions of marine environment are characterized by a high variability two locations (i.e. Tavolara and Portofino) have been chosen randomly in order to generalise the effect that gorgonian forests presence/absence could have independently of the site considered.

Forest is a fixed factor established by the scientist since it is the subject of this study. It is composed by two levels concretely represented by the presence and the absence of *Paramuricea clavata* on the artificial panels (Fig. 10). Plot is a random factor useful to evaluate possible heterogeneity at small distance within sites. It was represented by a 4 net with 4 recruitment panels. At each site, 4 plots with gorgonian forests (forested) and 4 without it (non-forested) were deployed.

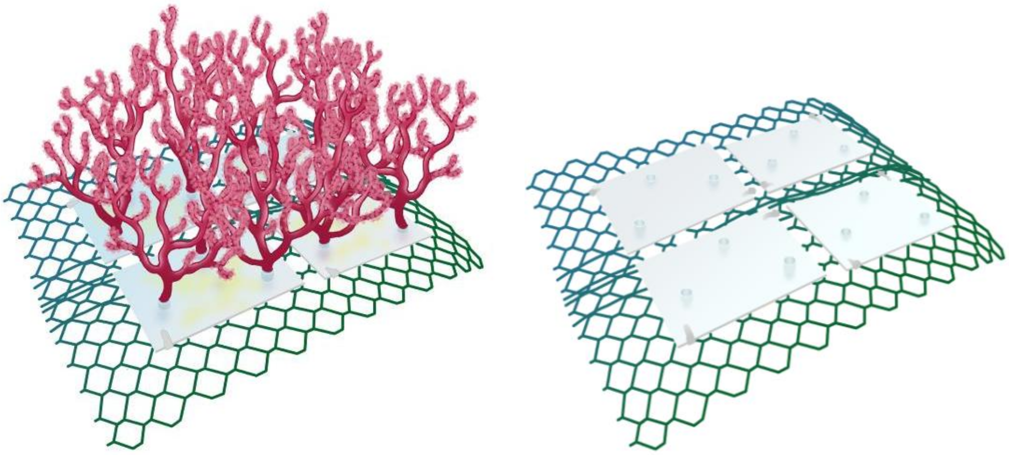


Fig. 10 – A schematic representation of the experiment plots showing how ideally it is recreated on a small scale a habitat with the presence of *Paramuricea clavata* and a habitat without it (drawing by Luca Parisi).

2.2 Risk assessment

Before the set-up of the experiment we did a general risk assessment analysing the main activities involved in the laboratory work and in the field work, possible hazards and actions to be taken to prevent or to deal with these problems (see Form 1 and Form 2 at the end of this chapter). Moreover we did a specific risk assessment with the software Deco planner analyzing dives profiles, air supply, mixtures to utilize (O_2 21% or EAN32), decompression and safety stops.

2.3 Experiment setup

Plots and all the necessary things were prepared in Ravenna's workshop using:

- Forex® PVC foam boards (15 x 10.5 cm)
- Eppendorf tubes (2 ml)
- plastic ties
- weights (75 gr)
- gardening net (45 x 30 cm)
- white line

As regards recruitment panels, Edgar (1991) and Smith & Rule (2002) have suggested that any artificial substratum should be deployed over periods of highest recruitment (i.e. Summer months). Many studies have used different artificial material types for studying benthic community patterns such as plastic (Scinto et al. 2009), acrylic (Bowden et al. 2006), Perspex (Turner & Todd 1993), ceramic (Maida et al. 1995), nylon, plastic bags and ropes (Rule & Smith 2005), polyvinylchloride (PVC) (Pech et al. 2002; Lapointe & Bourget 1999) obtaining results that met their expectations.

Every PVC board was drilled near two corners with holes for plastic ties and in the middle with three holes of suitable size for putting in Eppendorf tubes. In this way we can simulate a small forest with a density of 190 gorgonian m^{-2}. We tied four boards on a gardening net and everything was labeled in order to identify different replicates (boards) and plots (gardening nets) (Fig. 11). Two weights of 75 gr were tied on every plot to neutralize the buoyancy of the PVC boards. On every corner of the plot we tied up a white line to be used in the case if the sea bottom was too hard for steel nails.

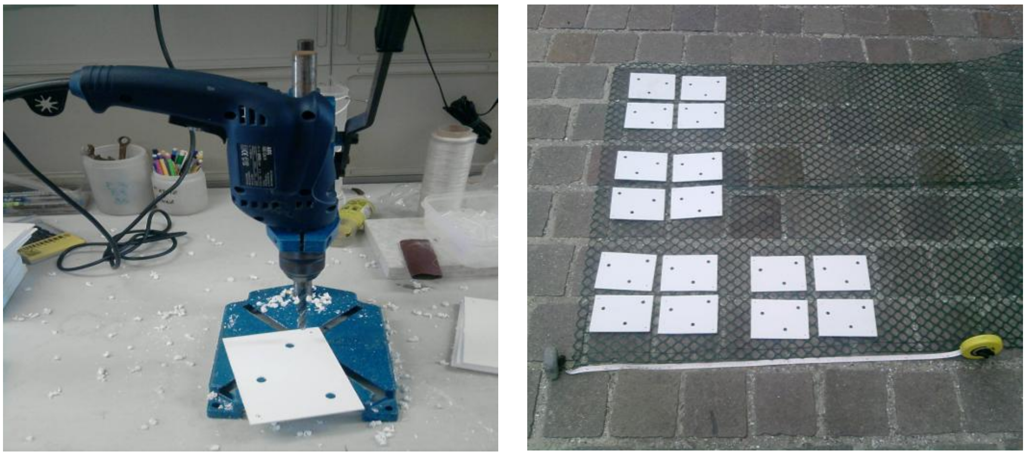


Fig. 11 – Images of the first stage of plots preparation (picture by Vincenzo Ventra).

Once arrived in the study area, the first step was to collect gorgonian fans. Thanks to SCUBA diving equipment we collected the terminal branches (about 20 cm long) of *Paramuricea clavata* colonies cutting them with shear in order to avoid further coenenchyme lacerations (Fig. 12). Colonies with epiphytic organisms or with naked skeletal parts were discarded.

Once on the surface we conducted graft operations on a support boat. For all the time required gorgonians were kept in running cold sea water avoiding air contact and stress linked with high temperatures. Whatever it have already been made a transplantation experiment on *Paramuricea clavata* showing a strong delicacy of this species respect other gorgonians, but confirming that this species can be used for this kind of experiments (Fava et al. 2009). Each branch was fixed in an Eppendorf tube with epoxy resin bicomponent (Subcoat by Veneziani): blue base and white hardener were mixed together by hand, using gloves, in a bucket with sea water of a temperature between 10 – 25°C.

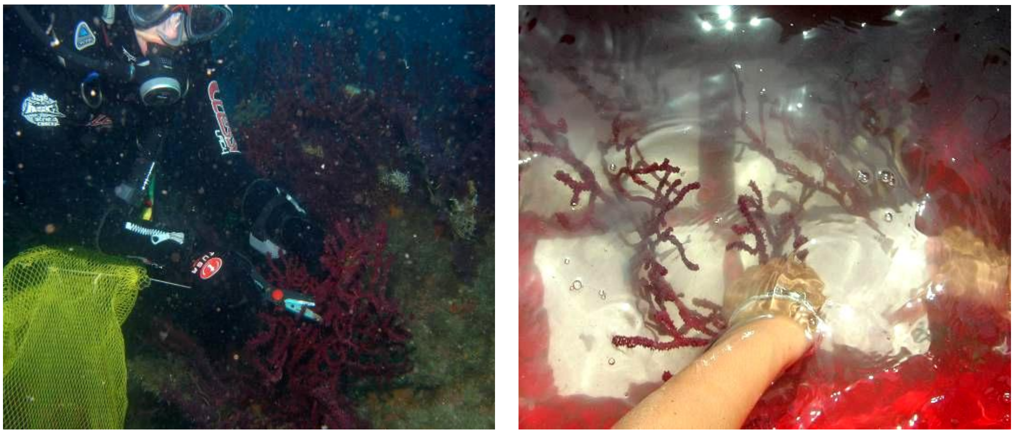


Fig. 12 - On the left a scientific diving operator cutting terminal branches of *Paramuricea clavata*. On the right grafts are mounted on the artificial panels (pictures by Massimo Ponti).

Once the resin had solidified the grafts were mounted on plots in large tanks full of seawater (Fig. 12). Epoxy resin was also put in Eppendorf tubes on non-forested plots in a way to verify possible negative chemical effects. During the second dive all the plots were fixed onto the coralligenous bottom using steel nails (10 cm long) and ropes if necessary, directing the fan of gorgonians perpendicular on the prevailing current direction indicated by the natural population. Plot were fixed randomly, but each one at the same depth and with the same inclination and orientation.

Study area

The locations considered for this study are located in Italy, more precisely in Sardinia and Liguria.

2.3.1 MPA Tavolara Punta Coda Cavallo, Sardinia

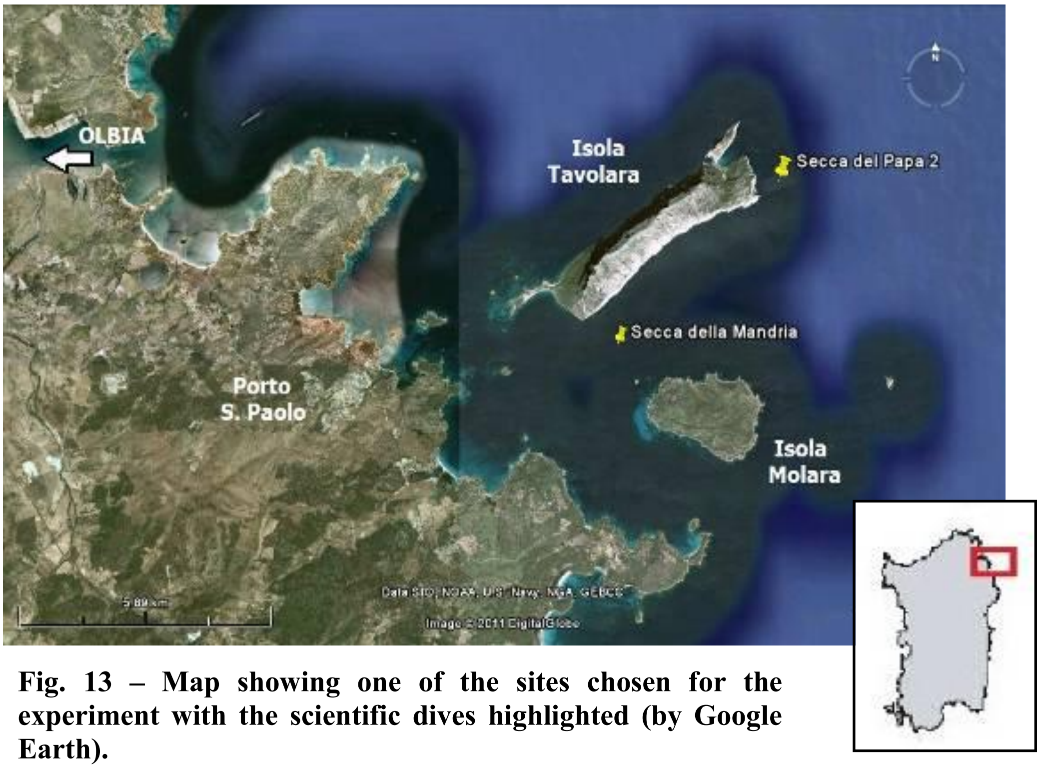


Fig. 13 – Map showing one of the sites chosen for the experiment with the scientific dives highlighted (by Google Earth).

Tavolara is a small island situated off the northeast coast of Sardinia near Olbia (Fig. 13).

According to ICRAM (2000) water temperatures for the Sardinian region range from 14°C (mean value registered from January to March) to 25°C as the maximum value registered for August.

Regarding salinity the highest values are usually reached along oriental coasts during spring months (about 38 psu) and then decreased to 37,5 psu during summer. For the O_2 values there is a particular situation since data recorded exceed 100% of saturation also during summer: this trend, usually verified in oligotrophic conditions, is due to the relevant role of oxygenation of the water column played by *Posidonia oceanica* meadows commonly widespread along the coasts of this region. Moreover in Sardinia waters are particularly clear (mean values of 20 m measured with Disco Secchi at 3000 m from coast) since the low intake of rivers and a general oligotrophic condition excluding particular points as large cities near the sea or the presence of ports. In order to synthesize trophic parameters ICRAM (2000) calculated TRIX index finding out a mean value of 2,68 corresponding to an elevated status: it is possible to state that the waters of the Sardinian region are characterized by low productivity, low trophic level, good clarity and no abnormal colouring due to algal blooms.

Fig. 14 – Tavolara island (picture by the author) and the map showing the different protection zones within the marine protected area (www.amptavolara.it).

Tavolara is 6 km long and 1 km wide, with the highest point reaching 565 m above the sea level. It is characterized by steep cliffs that fall down into the sea creating one of the most popular spots for scuba diving in the Mediterranean sea (Trainito 2007). Together with Molara and Molarotto islands and part of the coast, Tavolara falls within the marine protected area established on December 1997.

The entire area is divided in (see Fig. 14):

- two full protection zones (the south-eastern part of sea in front of Tavolara and the sea around Molarotto);

- four general protection zones (the sea around Molara and Molarotto, capo Ceraso, capo Coda Cavallo and the main part of sea around Tavolara);
- one partial protection zone which extends from Capo Ceraso to southern of San Teodoro;

Gorgonian fans were collected at the diving point "Secca della Mandria" (partial protection) at a depth of 40 m where gorgonians didn't show symptoms due to thermal stress. This is a shoal characterized by coralligenous system developed in the middle of a sandy plateau.

The experiment was set up at "Secca del Papa 2", the easternmost part of Tavolara island at about 37 m depth. In this place, surrounded by terrigenous sands and sciaphilic algae, the coralligenous habitat develops on a complex of limestone pinnacles that rise from the depth of 50 m up to 15 m providing an excellent habitat for *Paramuricea clavata*.

Set-up Tavolara date 8th June, 2010					
Activity	**Name place**	**Coordinates**	**Depth**	**Dive time**	**Orientation**
Collection	Secca della Mandria	40°52.956' N 9°42.171' E	36-40 m	60 min	not rated
settlement	Secca del Papa 2	40°54.918' N 9°44.858' E	36-38 m	60 min	90°N, with average inclination (visually estimated) of 30°

2.3.2 MPA Portofino, Liguria

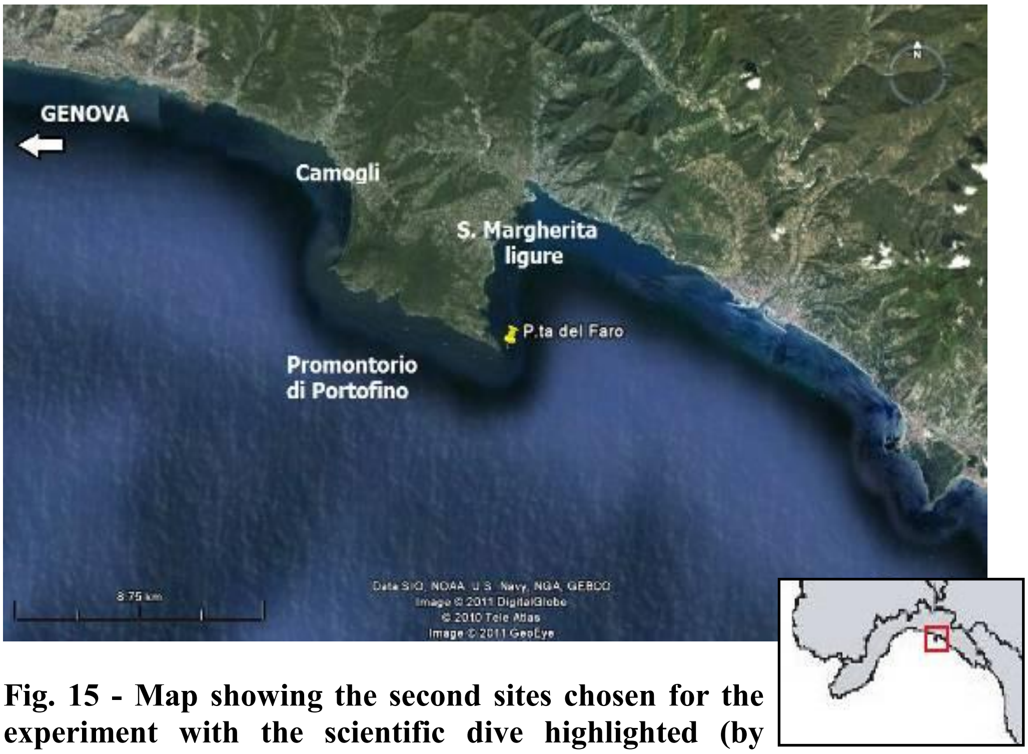


Fig. 15 - Map showing the second sites chosen for the experiment with the scientific dive highlighted (by Google Earth).

The Portofino promontory is situated eastwards of Genoa, on the southern coast of Liguria (Fig. 15). According to ICRAM (2000) temperatures in Liguria vary from a minimum < 14°C (value registered from January to April) and starts to increase in May reaching maximum values > 25°C in the summer. Salinity usually shows a gradient increasing from coast towards open sea. There is also a strong variability during the year due to the alternation of dry and rainy periods and sometimes leads to peaks of salinity even during winter. Regarding the O_2 it is possible to register maximum values around 8 mg/L during May concurrently with the highest algal activity. The minimum (6 mg/L) is registered in September when there the values for temperature and salinity are high. The annual trend never exceed the 100% of O_2 saturation confirming the nature of oligotrophic water and due to the prevalence of mineralization processes over those photosynthetic. Clarity of the water is influenced by rivers intake in the rainy season and by the high value of Chl a due to the increase of the algal activity registered from April to November that leads to minimum of 6 m and 10 m (Disco Secchi) measured at 500 m and 3000 m from the coast line.

The opinion of the sea quality of the Ligurian region based on TRIX value, according to ICRAM (2000), is the following: with a mean value of 4,31 coastal waters of this region fall in the good status category meaning a sea water moderately productive with an average trophic level, good clarity but they are occasionally involved in colouring and turbidity phenomena.

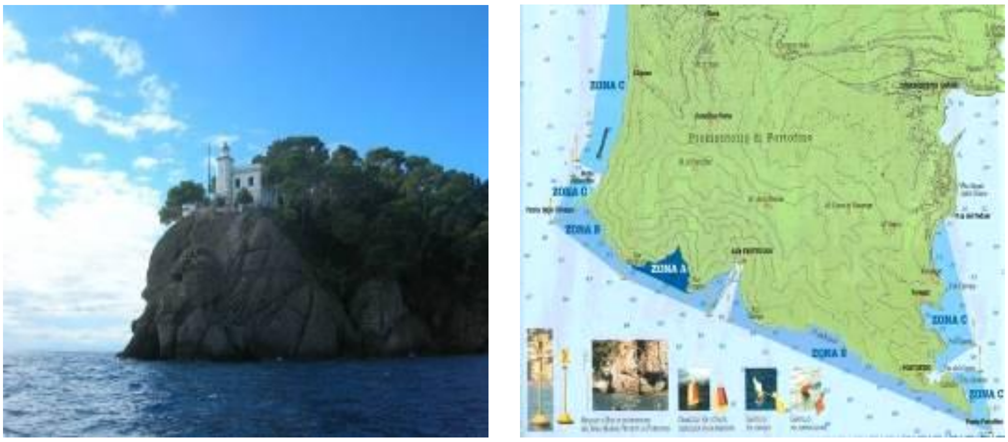


Fig. 16 - P.ta del Faro (picture by the author) and the map showing the different protection zones within the marine protected area (www.italiasub.it).

The Marine protected area of Portofino comprehends the whole promontory spreading from S. Margherita ligure to Camogli.

It was established in April 1999 in order to extend the protection offered from the Park also to the marine habitat. The marine protected area is divided in (see Fig. 16):

- one full protection zone (represented by Cala dell'Oro in the south western part of the promontory delimited by P.ta del Buco e P.ta Torretta);
- one general protection zone (the south coast extending from P.ta Chiappa to P.ta Portofino excluding a navigation lane in front of San Fruttuoso bay);
- two partial protection zone (that extend from Camogli to Porto Pidocchio and from Portofino to P.ta del Pedale);

The underwater cliffs of this promontory, full of gorges and cervices, have enhanced the formation of coralligenous habitat thus representing an area of great interest for biodiversity conservation in the Mediterranean.

All our work was carried out at the diving point “Punta del Faro” where a facies with *Paramuricea clavata* is well developed. Gorgonians branches were collected at a depth of about 35 m on the southern side exposed to the main littoral ligurian current flowing westwards. The plots instead were nailed on the eastern side of the promontory, steeper but characterized by a sedimentation rate of about 35g m^{-2} day^{-1} and considered a decantation area (Cerrano et al. 2005).

Set-up Portofino date 13rd June, 2010					
Activity	**Name place**	**Coordinates**	**Depth**	**Dive time**	**Orientation**
collection	P.ta del Faro	44°17.918' N 9°13.179' E	33 - 34 m	60 min	130°N
settlement	P.ta del Faro	44°17.918' N 9°13.179' E	32-33 m	60 min	130°N, with average inclination (visually estimated) of 45°

2.4 Data collection

After a period of about 4 months all the artificial panels were collected. The operation was carried out in order to collect grafted sea fans (used for measures of biomass), and every panel (subsequently analyzed in laboratory considering sessile organisms) in zip plastic bags, avoiding shaking and collecting the associated vagile fauna. All other non-degradable materials such as gardening nets, lines and steel nails if possible, were removed.

Once in the port, we started packaging operations. Gorgonians and tablets were shaken in a bowl and all the vagile fauna was sieved and stored in a labelled plastic jar with a buffered solution of 4% formaldehyde. Sea fan branches were cut away from the Eppendorf tubes and put back in the plastic bag with a label. They will be successively dried in an oven at 50°C, weighed and measured. Panels were photographed and put back in their bag with a buffered solution of 4% formaldehyde and stored in plastic boxes.

2.5 Laboratory Analysis

The identification of vagile fauna and measures of gorgonian biomass were conducted entirely in the Polytechnic University of Marche, Ancona's laboratories, while analyses on artificial panels were conducted in University of Bologna, Ravenna's laboratories.

We aimed to quantify all the species that have colonized the substrata in order to make a comparison between forested and non-forested plots. The method utilized was the frequency analyses: each tablet was carefully observed at the stereoscope using a reticulated frame composed by 400 squares prepared with Forex® PVC and fishing line.

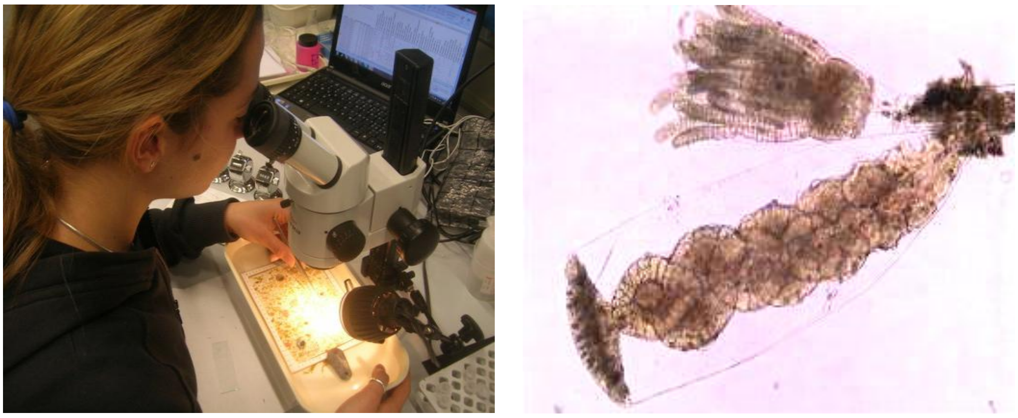


Fig. 17 - Laboratory analyses of the artificial panels at the stereoscope (by Vincenzo Ventra) and a microscope slide with a hydroids and its gonoteca (enlargement of 10x) (by the author).

In this way we obtain frequencies very accurate that can be assimilated to cover percentage data. Every organism found was photographed at the stereoscope and at the microscope (if it was possible to slide) (Fig. 17) and conserved for successively identification in a labelled Eppendorf tubes of 2 ml with alcohol 70% or in a buffered solution of 4% formaldehyde. The data were registered in Microsoft Excel set as follows (Table 1):

Table 1 - An example of the data-set utilized

Site	Forest	Plot	Tile	Side	Picture	# Sp. 1	# Sp. 2	# Sp. 3	# Sp. 4	# Sp. 5	# Sp. 6	# Sp. 7	# Sp. 8	...
Tavolara	N	1	A	UP	DSC_5402.JPG	76	0	0	5	0	0	2	104	...
Tavolara	N	1	B	UP	DSC_5408.JPG	194	0	0	8	0	0	20	68	...
Tavolara	N	1	C	UP	DSC_5378.JPG	139	0	0	15	0	0	2	44	...
Tavolara	N	1	D	UP	DSC_5396.JPG	164	1	2	9	0	0	13	102	...
Tavolara	Y	1	A	UP	DSC_5400.JPG	210	2	0	13	0	2	0	57	...
Tavolara	Y	1	B	UP	DSC_5382.JPG	89	0	0	1	0	0	3	12	...
…	…	…	…	…	…	...	...	...	...	...	...	...	...	...

2.6 Data Analysis

Frequencies counts on 400 sub-squares were transformed in percent cover data dividing them by 4 (Bianchi et al. 2004). The gorgonian forest effects were tested on different aspects with different statistical methods conducted with the software PRIMER 6 & PERMANOVA. The statistic was done on data registered from the analysis of 63 artificial panels since the replicate labelled Y4C was lost during collection operation in Portofino.

2.6.1 Analysis on assemblages structure

Differences in assemblage structures were analysed using principal coordinate analysis (PCO, i.e. metric multidimensional scaling) based on Bray-Curtis dissimilarities of square root-transformed data (Anderson 2003; Anderson & Willis 2003). Differences in community structures between gorgonian forest treatment (Fo: fixed factor with 2 levels: presence/absence), sites (Si: random factor with 2 levels: Tavolara/Portofino), and plots (Pl: random factor nested in Fo x Si with 4 levels) were assessed by permutational non-parametric multivariate analysis of variance (PERMANOVA; Anderson 2001, 2005). When less than 999 unique values in the permutation distribution were available, asymptotical Monte Carlo P-values were used instead of permutational P-values.

We consider P value < 0,01 very significant, < 0,05 significant and in the case of significant interaction among Site x Forest we did a post-hoc pair-wise test in order to investigate the forest effects at each site.

2.6.2 Analysis on single taxon abundance

Taxa contributing most to the differentiation of populations were identified by DistLM procedure and superimposing the corresponding correlation vectors to the PCO plot. Their mean value of abundance and its standard error were represented in histogram charts using Microsoft Excel.

Differences in abundances between gorgonian forest treatment, sites and plots were assessed by PERMANOVA (Anderson 2001, 2005) based on Euclidean distances of not transformed data. When less than 999 unique values in the permutation distribution were available, asymptotical Monte Carlo P-values were considered.

If the difference among plots was not significant (P > 0.25) we applied the pooling technique in order to eliminate this level and increase the power of the analyses. If the interaction among Site x Forest was significant, pair-wise test was performed to verify if gorgonian presence/absence affect species abundance in Tavolara and/or in Portofino.

2.6.3 Analysis on ecologic structure

In order to understand thoroughly which may be the effect of sea on the ecological structure of the assemblages, each taxon was assigned to ecological categories and pooled together (Table 2). Differences were studied using principal coordinate analysis (PCO, i.e. metric multidimensional scaling) based on Bray-Curtis dissimilarities of square root-transformed data (Anderson 2003; Anderson & Willis 2003) and ecologic groups that mainly contribute to the observed similarity patterns were selected by forward DistLM procedure and superimposed on the PCO graph. The variation on ecologic structure patterns within the different levels of this experiment were assessed by a PERMANOVA analysis. As seen before, if less than 999 permutation were available, were considered Monte Carlo P-values and in the case of significant interaction among Site x Forest a post-hoc test was performed in order to understand in which site differences were significant.

Table 2 – Ecologic groups

Phylum	Phaeophyta Rhodophyta Chlorophyta	Foraminifera	Porifera	Cnidaria	Annelida	Mollusca	Bryozoa	Chordata
Ecologic group	Erected algae	Forams	Encrusting sponge	Hydroid	Tube worm	Mollusc	Erect bryozoans	Social ascidiacean
	Encrusting algae			Colonial polyps			Stoloniferous bryozoans	Colonial ascidiacean
	Encrusting calcareous algae		Erect sponge	Solitary polyp	Calcareous tube worm		Encrusting bryozoans	Solitary ascidiacean

For each ecologic group the mean value of abundance and its standard error were calculated and represented in histogram charts. On each group was performed a univariate analysis using PERMANOVA on Euclidean distance matrix. The pooling technique was applied, if possible, as in the previous analyses and the significance of interaction among Site x Forest, was tested with a post-hoc test.

2.6.4 Analysis on higher Taxa group patterns

Differences among higher Taxa groups patterns were studied as in the previous analyses. The contribute of every group was defined with forward DistLM method and relative vectors have been superimposed on the PCO graph. Univariate analysis on each Phyla was performed in order to understand thoroughly principal variations that *Paramuricea clavata* forest could have on the assemblage dynamics.

2.6.5 Analysis on species diversity

Possible effects of Red mediterranean sea fan on coralligenous assemblages species diversity were analysed by calculating total abundances of species, species richness, Simpson diversity index and Hill's indices:

- Simpson diversity index (D)

  $$D = 1 - \frac{\sum_{i=1}^{S} n_i(n_i - 1)}{N(N-1)},$$

  where S is the number of species, N is the total % cover or total number of organisms and n_i is the % cover of a species or number of organisms of a species. D ranges from 1 to 0, with 1 representing infinite diversity and 0 representing absence of diversity.

  This index is often used to quantify the species diversity of a habitat since it consider the number of species present and their relative abundance. In a simple way it shows us the probability that two randomly selected individuals in the same habitat belonging to different species.

- Hill index of diversity (N_1)

  This index is linked with Shannon diversity index (H')

  $$H' = -\sum_{i=1}^{S}(p_i \log_2 p_i) \qquad p_i = \frac{n_i}{N}$$

  where S is the number of species and p_i the relative abundance of each species, calculated as the proportion of individuals of a given species (n_i) to the total number of individuals in the community (N), since it is calculated in the following way:

  $$N_1 = e^{H'}$$

  N_1 represent the equivalent number of species with equal abundance showing the same diversity of the sampled assemblages. As for Shannon index it gives a measure of both species numbers and the evenness of their abundance thus there is always the need to use another index to discompose the effect due to species richness (S) and evenness.

Differently to Shannon index, N_1 is sensitive to sample size and its value increase significantly with the increasing of the number of species considered. On the other hand, since these indices are related each other with a logarithmic function, H' is less fit for distinguishing differences in high diversity assemblages than N_1 (Soetaert & Heip 1990).

- Hill evenness index (N_{10})

  A whole range of Hill's diversity numbers exists and the general formula is showed below.

$$N_a = 1/\sqrt[(a-1)]{\sum p_i^a}$$

  where p_i is the relative abundance of each species (n_i/N). So to calculate N_{10} is necessary to replace a with 10. N_{10} represent the evenness component of the N_1 heterogeneity diversity.

On each index was performed a univariate analysis using PERMANOVA on Euclidean distance matrix similarly as we did with single taxon analyses.

2.7 Practical considerations at underwater work

Before to discuss about results obtained, it is useful to understand briefly principal mechanisms and problems that have been faced during the implementation of the work.

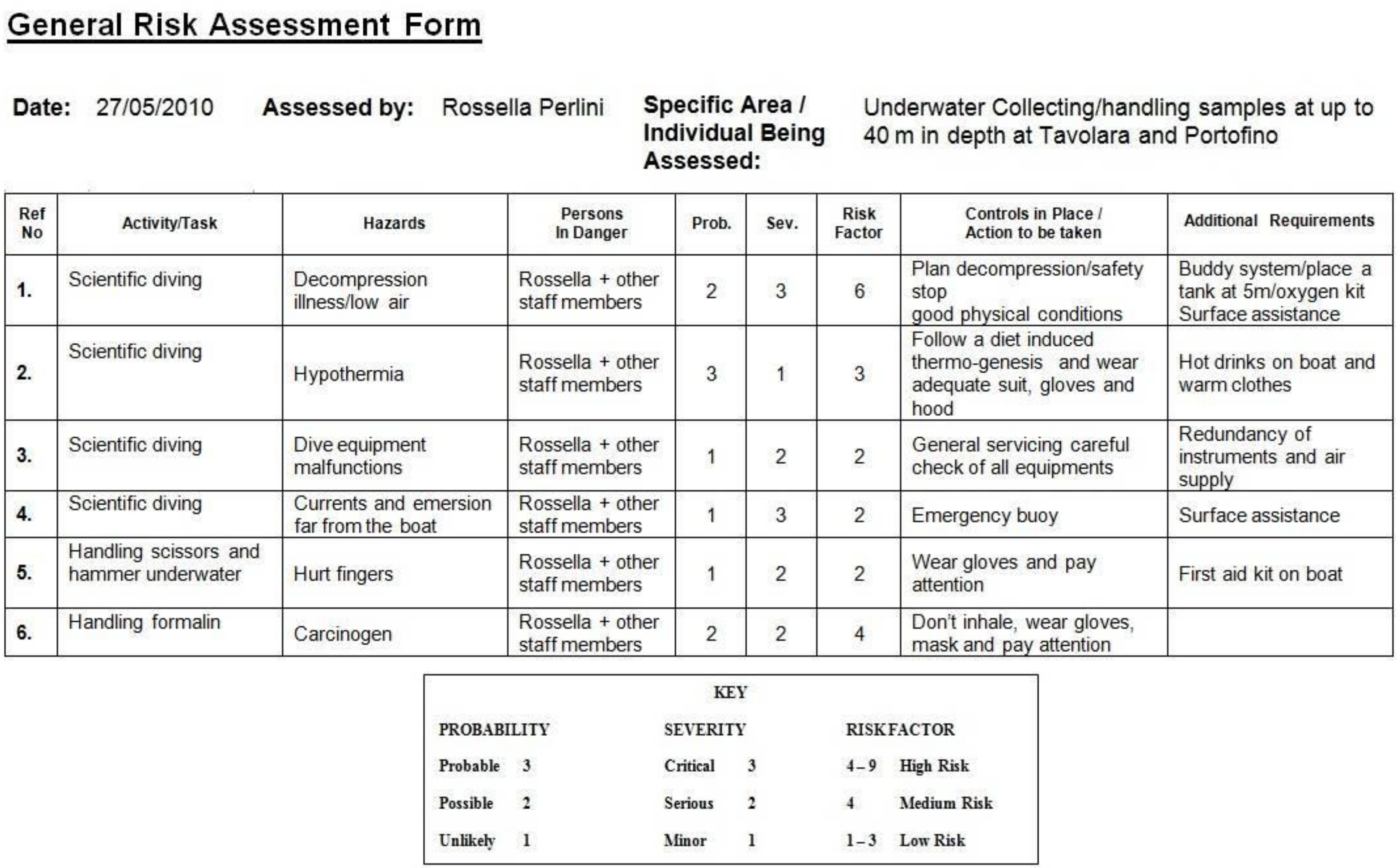
As regards field work logistic organization before and after the dives, have a planning of all the operations and work with a buddy was of fundamental importance (Fig. 18): preparation before the dives with the visualization of the various tasks to be performed and the dry run of operations allowed to refine the technique, be in tune with proper buddy and be prepared in advance to resolve possible problems, especially because at that depths the bottom time and the operator efficiency are relatively reduced.

The main problem faced underwater was nail down the plots onto the substrate that was often composed by a layer of few centimetres of calcareous coralline algae above a granitic base difficult to penetrate. In this case we looked for thicker coralligenous in the nearby using nails and lines or even tying the rope on rocks or on sea fan already presents. Another problem was related to grafts that in some cases have not been well established in Eppendorf tubes: in the case of *Eunicella cavolinii* the trouble was caused by the synergy between the small thickness of the branch and the few glue applied, in the case of *Paramuricea clavata* instead, once the glue solidified the entire graft slid away from the tube walls. To face this problem it is advisable to sanding the Eppendorf in order to make walls rough and enhance grip.

Fig. 18 – Buddy work during collection operations was extremely important (picture by Massimo Ponti).

Obviously all the operation were conducted in safe weather condition and in case of rough sea we waited for days before plunge. Apart from that in Portofino we had to conduct sensitive operations, such as collecting tablets and gorgonians paying attention to the vagile fauna, in the current on a wall with high slope. In this conditions of concentration and focalization on the work to do one of the 64 replicates just collected is gone missed.

Form 1 – Risk assessment form for field work



General Risk Assessment Form

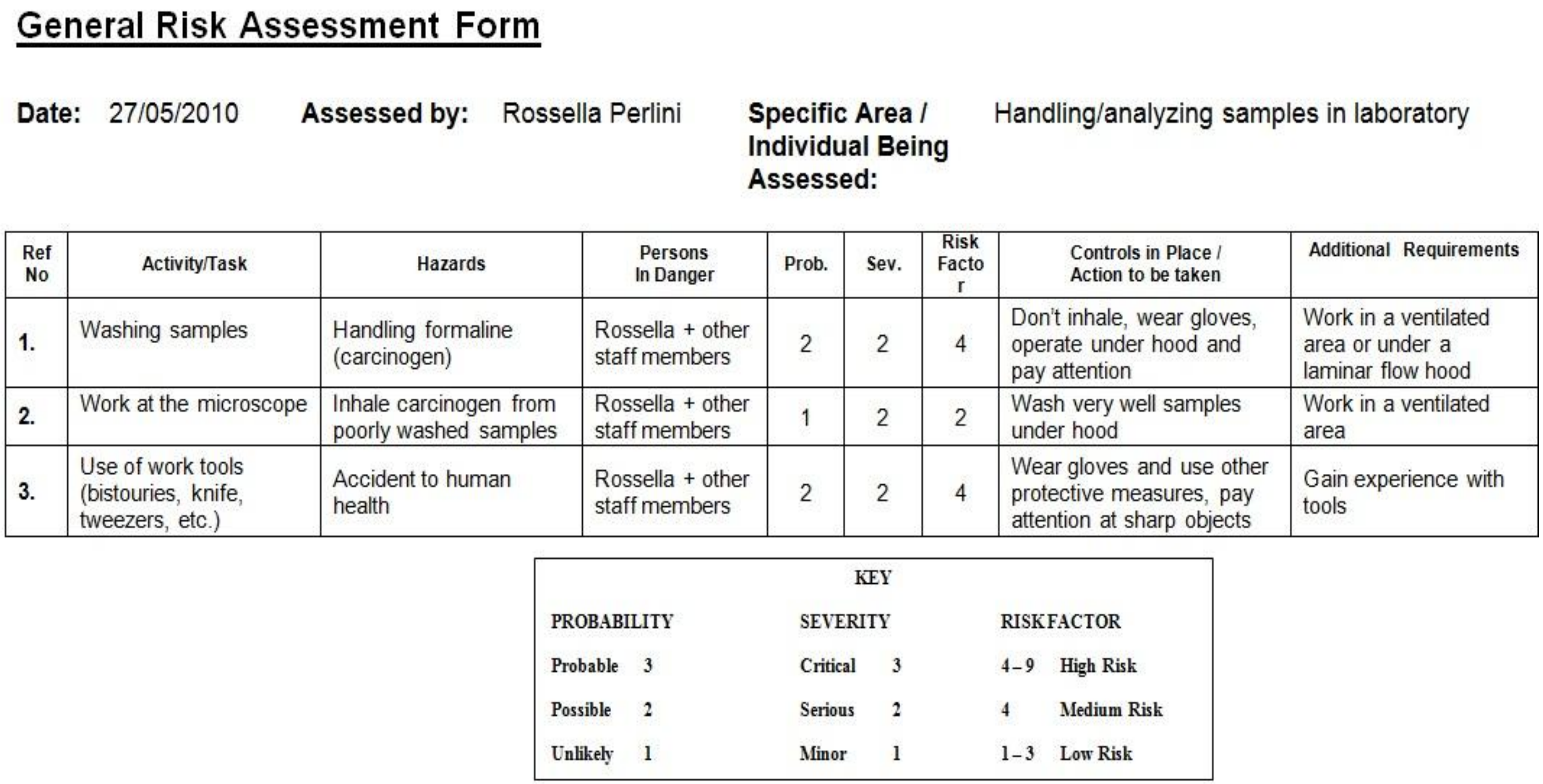
Date: 27/05/2010 **Assessed by:** Rossella Perlini **Specific Area / Individual Being Assessed:** Underwater Collecting/handling samples at up to 40 m in depth at Tavolara and Portofino

Ref No	Activity/Task	Hazards	Persons In Danger	Prob.	Sev.	Risk Factor	Controls in Place / Action to be taken	Additional Requirements
1.	Scientific diving	Decompression illness/low air	Rossella + other staff members	2	3	6	Plan decompression/safety stop good physical conditions	Buddy system/place a tank at 5m/oxygen kit Surface assistance
2.	Scientific diving	Hypothermia	Rossella + other staff members	3	1	3	Follow a diet induced thermo-genesis and wear adequate suit, gloves and hood	Hot drinks on boat and warm clothes
3.	Scientific diving	Dive equipment malfunctions	Rossella + other staff members	1	2	2	General servicing careful check of all equipments	Redundancy of instruments and air supply
4.	Scientific diving	Currents and emersion far from the boat	Rossella + other staff members	1	3	2	Emergency buoy	Surface assistance
5.	Handling scissors and hammer underwater	Hurt fingers	Rossella + other staff members	1	2	2	Wear gloves and pay attention	First aid kit on boat
6.	Handling formalin	Carcinogen	Rossella + other staff members	2	2	4	Don't inhale, wear gloves, mask and pay attention	

KEY

PROBABILITY		SEVERITY		RISK FACTOR	
Probable	3	Critical	3	4 – 9	High Risk
Possible	2	Serious	2	4	Medium Risk
Unlikely	1	Minor	1	1 – 3	Low Risk

Form 2 - Risk assessment form for laboratory work



General Risk Assessment Form

Date: 27/05/2010 **Assessed by:** Rossella Perlini **Specific Area / Individual Being Assessed:** Handling/analyzing samples in laboratory

Ref No	Activity/Task	Hazards	Persons In Danger	Prob.	Sev.	Risk Factor	Controls in Place / Action to be taken	Additional Requirements
1.	Washing samples	Handling formaline (carcinogen)	Rossella + other staff members	2	2	4	Don't inhale, wear gloves, operate under hood and pay attention	Work in a ventilated area or under a laminar flow hood
2.	Work at the microscope	Inhale carcinogen from poorly washed samples	Rossella + other staff members	1	2	2	Wash very well samples under hood	Work in a ventilated area
3.	Use of work tools (bistouries, knife, tweezers, etc.)	Accident to human health	Rossella + other staff members	2	2	4	Wear gloves and use other protective measures, pay attention at sharp objects	Gain experience with tools

KEY

PROBABILITY		SEVERITY		RISKFACTOR	
Probable	3	Critical	3	4 – 9	High Risk
Possible	2	Serious	2	4	Medium Risk
Unlikely	1	Minor	1	1 – 3	Low Risk

Chapter 3: Results

In this experiment have been reproduced, with the use of artificial recruitment panels, habitat with the presence of gorgonian forests and habitat without them, in order to study the *Paramuricea clavata* effect on the epibenthic assemblages and understand what are the main mechanisms that lead to the high diversity and variability that distinguishes coralligenous habitats (Boudouresque 2004).

The forested plots have a density of 190 sea fans per m^{-2}, with a mean height and width per colony respectively of 17.92 cm and 9.54 cm and a mean branches area of 77.02 cm^2 per every panel. The average biomass reproduced on the experimental panels has been estimated in 825 g DM m^{-2}, corresponding to 90.75 g AFDM m^{-2} (according to conversion factor in Coma et al. 1998b). This biomass is of the same order of magnitude of the natural populations with high density.

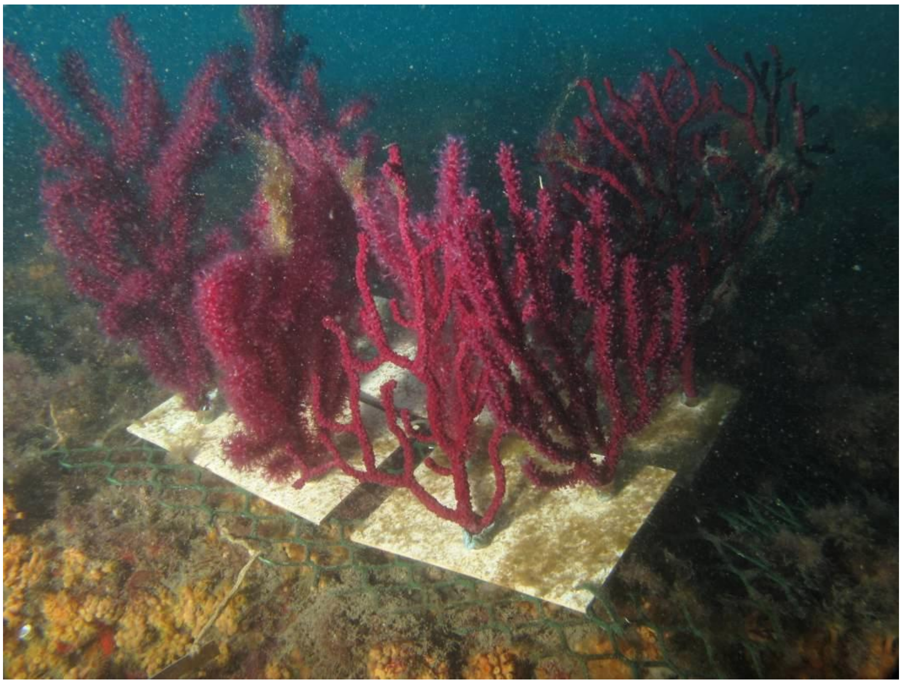


Fig. 19 – A forested plot (picture by Carlo Cerrano)

Measures carried out at the end of the experiment confirmed that the gorgonian biomass on the panels was distributed homogeneously among plots. This ensures that no effects have been introduced due to different densities of gorgonians and that the differences observed between the plots are due to natural variability. Overall 160 different taxa among with 79 algae belonging to Rhodophyta, Phaeophyta and Chlorophyta, 3 taxa of Foraminifera, 4 taxa of Porifera, 18 Cnidaria, 20 Annelida, 1 Mollusca, 32 Bryozoa and 3 taxa of Chordata were found (see Fig. 20).

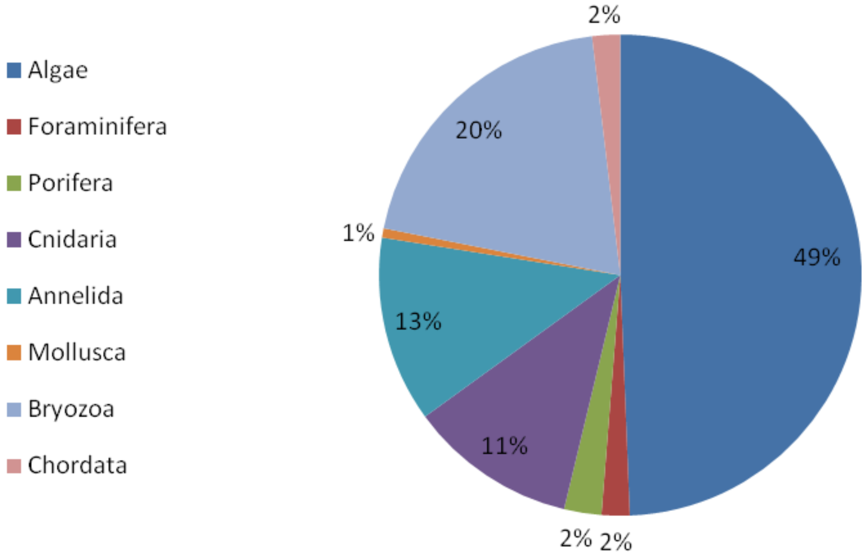


Fig. 20 – Percentage of taxa belonging to major taxonomic groups

3.1.1 Results on assemblages structure patterns

Epibenthic assemblages colonising the panels were well differentiated between sites but also for the presence/absence of gorgonian forest, as showed by the PCO ordination plot (Fig. 21).

The first two axes of the PCO explained 42.7% and 11.3% of the variability of epibenthic assemblages, respectively. The PERMANOVA test pointed out a significant interaction between Site and Forest (Table 3). As confirmed by the post-hoc pair-wise test, the presence of gorgonian forest affected the assemblages in both sites.

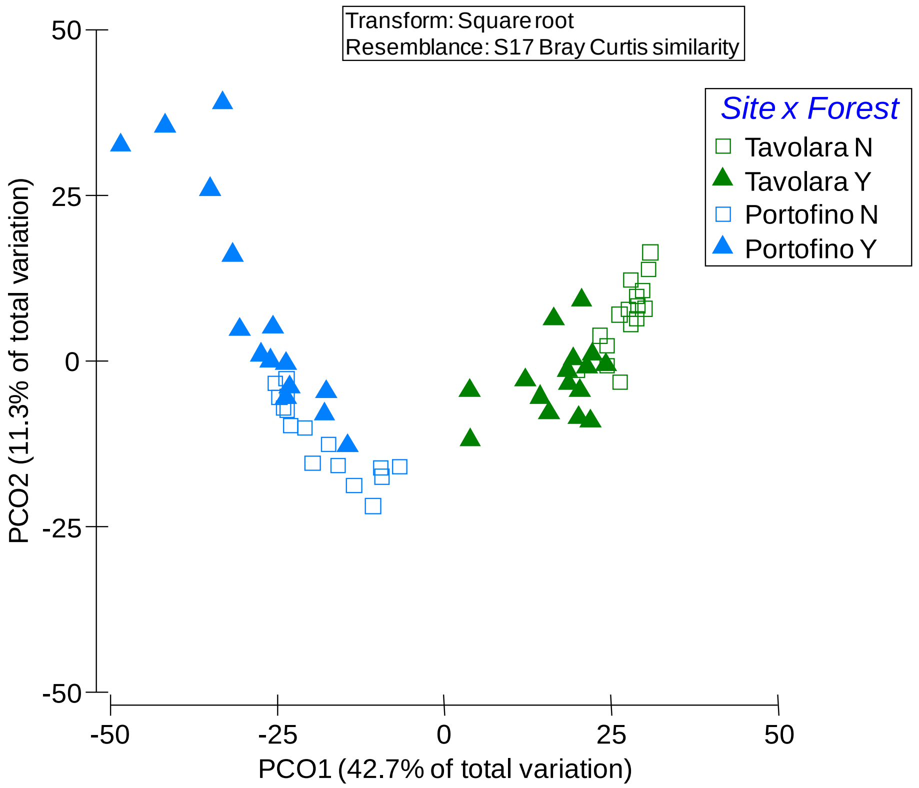


Fig. 21 – PCO ordination plot showing similarities among assemblages found in each recruitment panel, grouped by plot (1..4), site (Tavolara vs Portofino) and presence of the gorgonian forest (Y/N).

Table 3 – PERMANOVA results

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
Si	1	33061	33061	22.823	0.0001	9936	0.0001
Fo	1	5345	5345	1.128	0.5127	6	0.4160
SixFo	1	4738	4738	3.271	0.0039	9923	0.0011
Pl(SixFo)	12	17425	1452	2.754	0.0001	9742	0.0001
Res	47	24779	527				
Total	62	84386					

Taxa that mainly contribute to the observed similarity patterns were selected by forward DistLM procedure and reported in the following table (Table 4).

Vectors superimposed on the PCO plot graphically represented the relationships between the abundance of single taxa and patterns of similarity of the epibenthic assemblages colonising the panels (Fig. 22).

Table 4 – DistLM results that represent almost the 85% of variability

Variable	R^2	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
Anomia ephippium	0.2882	24318.0	24.696	0.001	0.28818	0.28818	61
Josephella marenzelleri	0.3885	8464.8	9.842	0.001	0.10031	0.38849	60
Chlorophyta sp. 1	0.4829	7966.5	10.771	0.001	9.44E-02	0.48290	59
Phaeophyta sp. 1	0.5490	5579.7	8.504	0.001	6.61E-02	0.54902	58
Chlorophyta sp. 2	0.5827	2839.6	4.596	0.001	3.37E-02	0.58267	57
Bryozoa sp.2	0.6136	2612.7	4.488	0.001	3.10E-02	0.61363	56
Crambe crambe	0.6384	2089.1	3.765	0.001	2.48E-02	0.63839	55
Ceramium sp.	0.6604	1857.0	3.499	0.001	2.20E-02	0.66040	54
Chlorophyta sp. 6	0.6806	1704.2	3.351	0.001	2.02E-02	0.68059	53
Clytia linearis	0.6994	1590.6	3.261	0.001	1.88E-02	0.69944	52
Rhodophyta sp. 6	0.7170	1478.3	3.157	0.001	1.75E-02	0.71696	51
Chlorophyta sp. 4	0.7332	1369.1	3.040	0.001	1.62E-02	0.73318	50
Hydrozoa sp. 1	0.7485	1292.9	2.985	0.001	1.53E-02	0.74850	49
Foraminifera sp. 2	0.7622	1153.4	2.759	0.001	1.37E-02	0.76217	48
Savignyella lafontii	0.7741	1009.8	2.490	0.001	1.20E-02	0.77414	47
Janua (Dexiospira) marioni	0.7861	1008.3	2.569	0.001	1.19E-02	0.78609	46
Rhodophyta sp. 8	0.7982	1023.5	2.705	0.002	1.21E-02	0.79822	45
Chlorophyta sp. 7	0.8086	873.9	2.380	0.001	1.04E-02	0.80857	44
Bryozoa sp.5	0.8182	810.9	2.273	0.001	9.61E-03	0.81818	43
Vinearia endoumensis	0.8275	781.8	2.255	0.003	9.26E-03	0.82745	42
Chlorophyta sp. 8	0.8363	749.5	2.225	0.001	8.88E-03	0.83633	41
Rhodophyta sp. 1	0.8451	741.83	2.270	0.002	8.79E-03	0.84512	40

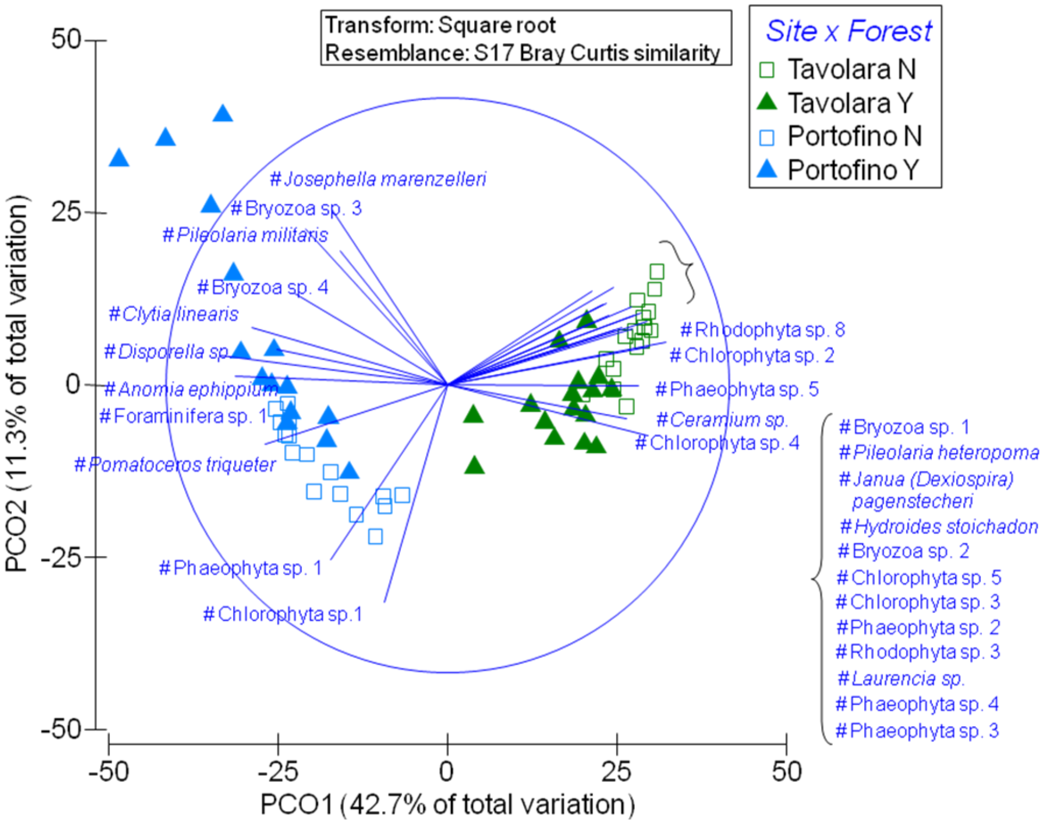


Fig. 22 - Correlation vectors of the taxa that more contribute to the similarity patterns, superimposed on the PCO ordination plot.

3.1.2 Possible effects of gorgonian forest on single taxon abundances

The possible effects of the gorgonian forest on the abundances of selected taxa were investigated. Taxa were selected based on their abundance, frequency and correlation with the observed pattern of similarities among assemblages. In general, algae represent the more abundant species.

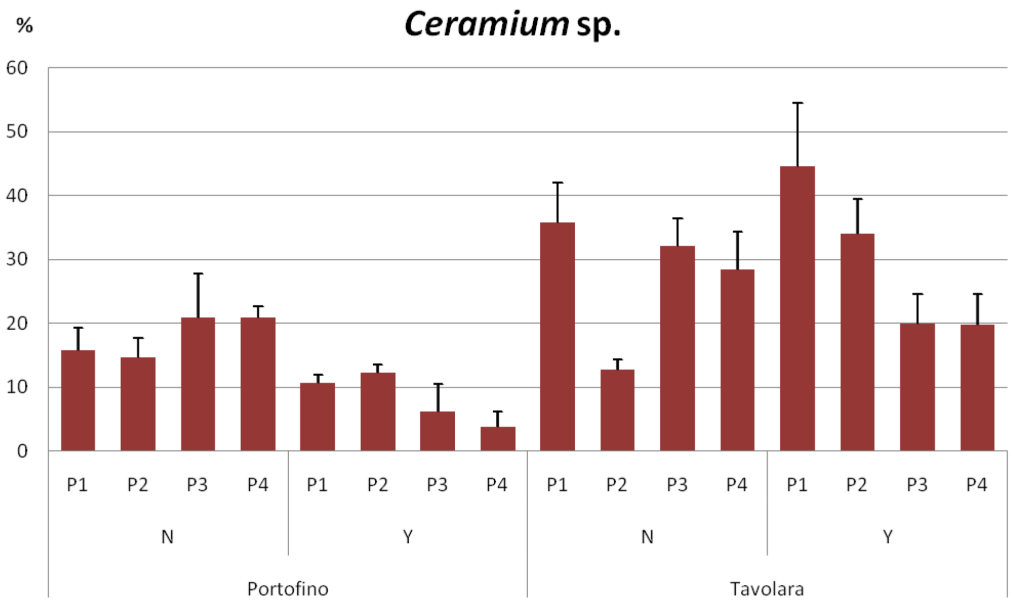


Fig. 23 - Mean percent cover of *Ceramium* sp. (±se).

Ceramium sp. is the most frequent and one of the more abundant. The histogram point out heterogeneity among different plots, even in this species (Fig. 23) and shows an evident difference among the two study area considered, it was confirmed by the analysis whose results are showed in the table below (Table 5).

Table 5 – PERMANOVA results for *Ceramium* sp.

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
Si	1	3667.5	3667.50	13.5030	0.0045	9857	0.0030
Fo	1	224.1	224.10	0.3850	0.4962	6	0.6506
SixFo	1	582.0	582.02	2.1429	0.1736	9833	0.1681
Pl(SixFo)	12	3267.3	272.28	2.9094	0.0032	9935	0.0051
Res	47	4398.6	93.59				
Total	62	11959.0					

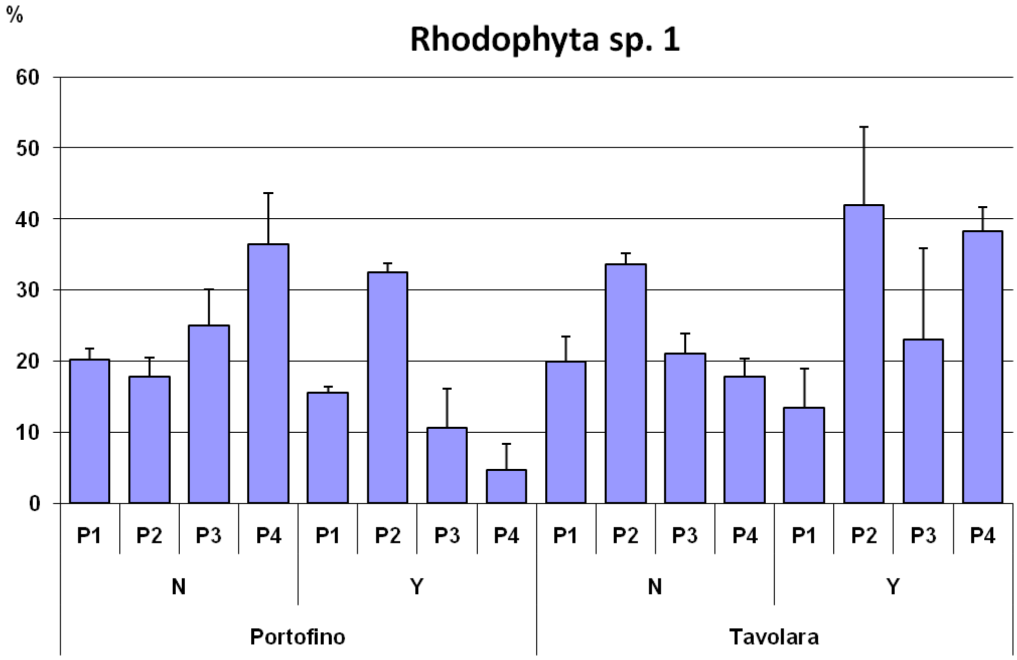


Fig. 24 - Mean percent cover of Rhodophyta sp. 1 (±se).

Rhodophyta sp. 1 is the second most abundant species found both in Tavolara and Portofino (Fig. 24). Also in this case the univariate analysis highlighted heterogeneity among different plots and there is no evidence that this species could be affected in some way by *Paramuricea clavata* (Table 6).

Table 6 – PERMANOVA results for Rhodophyta sp.1

Source	df	SS	MS	Pseudo-F	P(perm)	Perms	P(MC)
Si	1	521.02	521.02	1.2191	0.2835	9821	0.2839
Fo	1	33.10	33.10	0.0366	0.5055	6	0.8856
SixFo	1	903.81	903.81	2.1148	0.1701	9822	0.1708
Pl(SixFo)	12	5142.30	428.53	3.4629	0.0012	9942	0.0015
Res	47	5816.20	123.75				
Total	62	12230.00					

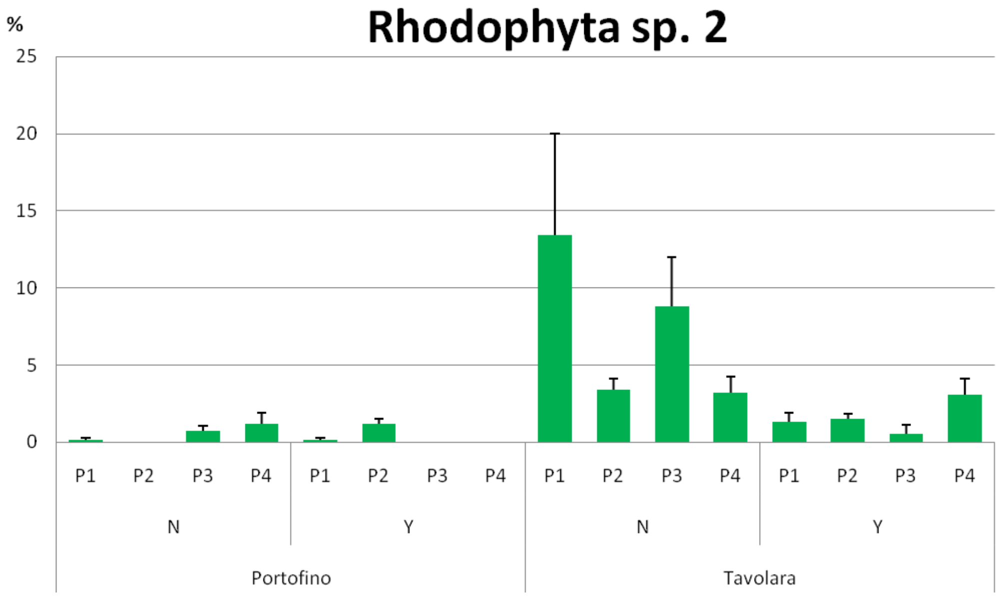


Fig. 25 - Mean percent cover of Rhodophyta sp. 2 (±se).

Rhodophyta sp. 2 was among the more frequent species and the analyses pointed out, besides heterogeneity among plots, that this species was significantly affected by *Paramuricea clavata* at one site (Tavolara) with a negative effect on its abundances in forested plots (Fig. 25 and Table 7).

Table 7 – PERMANOVA results for Rhodophyta sp.2

Source	Df	SS	MS	Pseudo-F	P(perm)	Perms	P(MC)
Si	1	248.82	248.82	9.6555	0.0072	9858	0.0080
Fo	1	130.96	130.96	1.1435	0.3374	10	0.4790
SixFo	1	114.52	114.52	4.4441	0.0426	9866	0.0552
Pl(SixFo)	12	309.74	25.81	1.7517	0.0406	9921	0.0868
Res	47	692.56	14.74				
Total	62	1501.10					

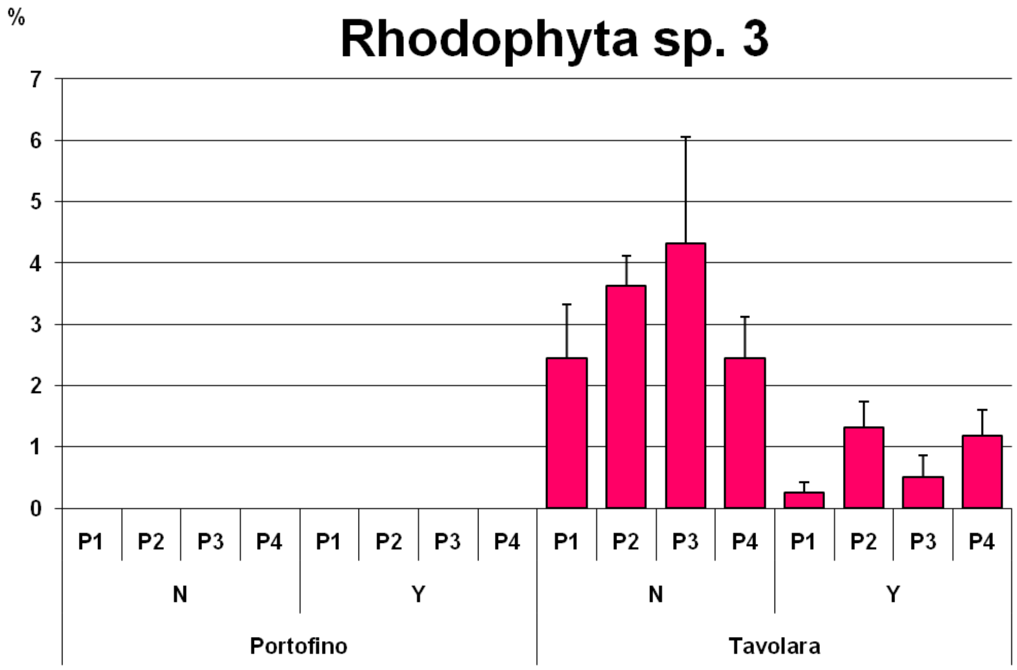


Fig. 26 - Mean percent cover of Rodophyta sp. 3 sp. 2 (±se).

The analyses carried out on the abundance of Rhodophyta sp. 3 pointed out that it was significantly affected by *Paramuricea clavata* at Tavolara site (Table 8) with an inhibition of its abundance in forested plots (Fig. 26).

Table 8 – PERMANOVA results for Rhodophyta sp. 3

Source	Df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
Si	1	63.185	63.1850	55.9570	0.0003	9517	0.0001
Fo	1	22.394	22.3940	1.0000	0.4946	2	0.5025
SixFo	1	22.394	22.3940	19.8320	0.0014	9584	0.0007
Pl(SixFo)	12	13.543	1.1286	0.8797	0.6070	9923	0.5708
Res	47	60.297	1.2829				
Total	62	183.040					

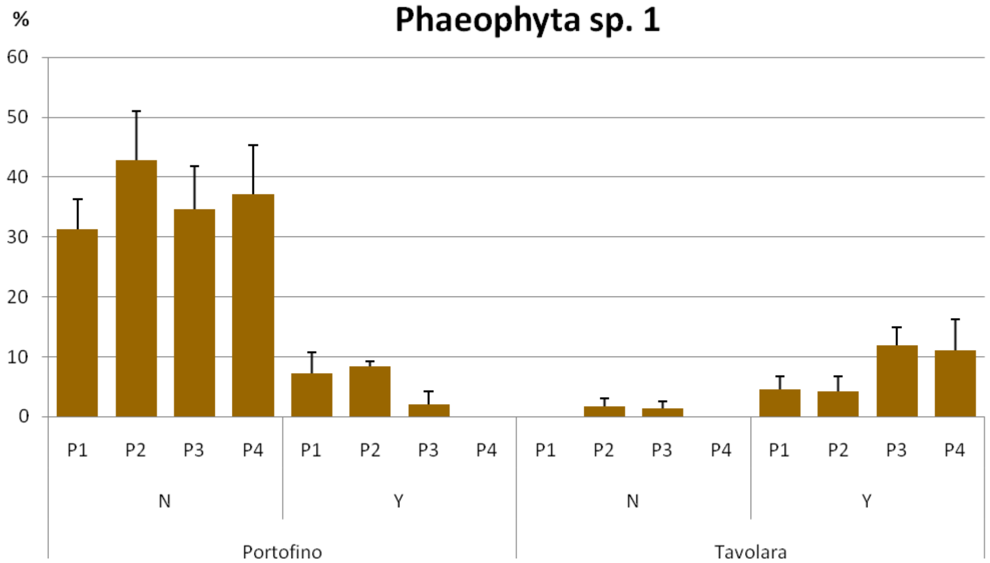


Fig. 27 - Mean percent cover of Phaeophyta sp. 1 (±se).

Phaeophyta sp.1 is one of the few among all the taxa found in this study that highlight significant differences among forested and non-forested panels in both experiment sites (Table 9). Nevertheless, the forest seems to have an opposite effect at different sites (Fig. 27).

Table 9 – PERMANOVA results for Phaeophyta sp. 1

Source	Df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
Si	1	4063.50	4063.50	71.942	0.0004	9798	0.0001
Fo	1	2421.50	2421.50	0.403	0.5039	6	0.6427
SixFo	1	6012.50	6012.50	106.450	0.0003	9778	0.0001
Pl(SixFo)	12	677.11	56.43	0.789	0.6581	9942	0.6519
Res	47	3362.20	71.54				
Total	62	16662.00					

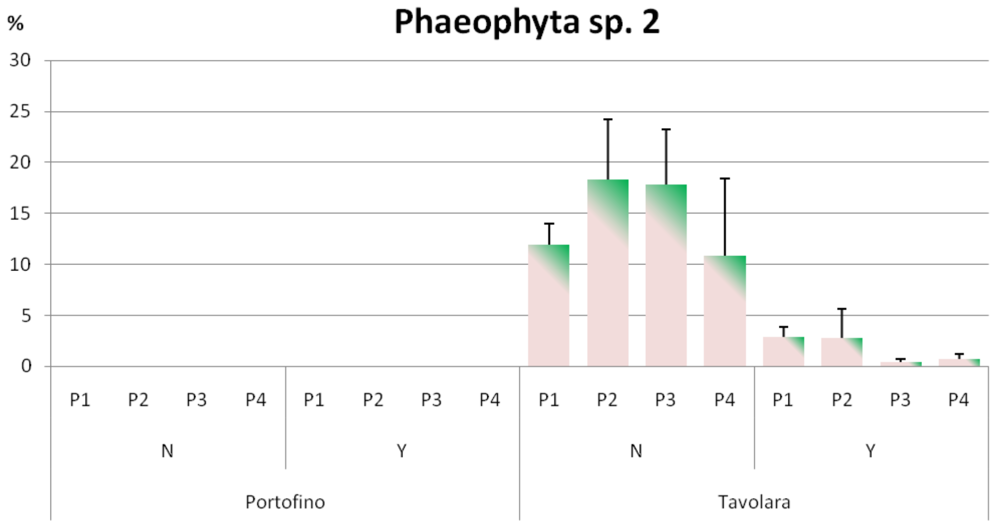


Fig. 28 - Mean percent cover of Phaeophyta sp. 2 (±se).

Phaeophyta sp. 2 was found only on artificial panels deployed at Tavolara (Fig. 28). This taxon revealed a strong inhibition due to the presence of *Paramuricea clavata* (Table 10).

Table 10 – PERMANOVA results for Phaeophyta sp. 2

Source	Df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
Si	1	1056.70	1056.70	61.98	0.0003	9597	0.0001
Fo	1	660.61	660.61	1.00	0.4993	2	0.4973
SixFo	1	660.61	660.61	38.75	0.0002	9724	0.0002
Pl(SixFo)	12	203.78	16.98	0.49	0.9152	9929	0.9124
Res	47	1635.60	34.80				
Total	62	4249.70					

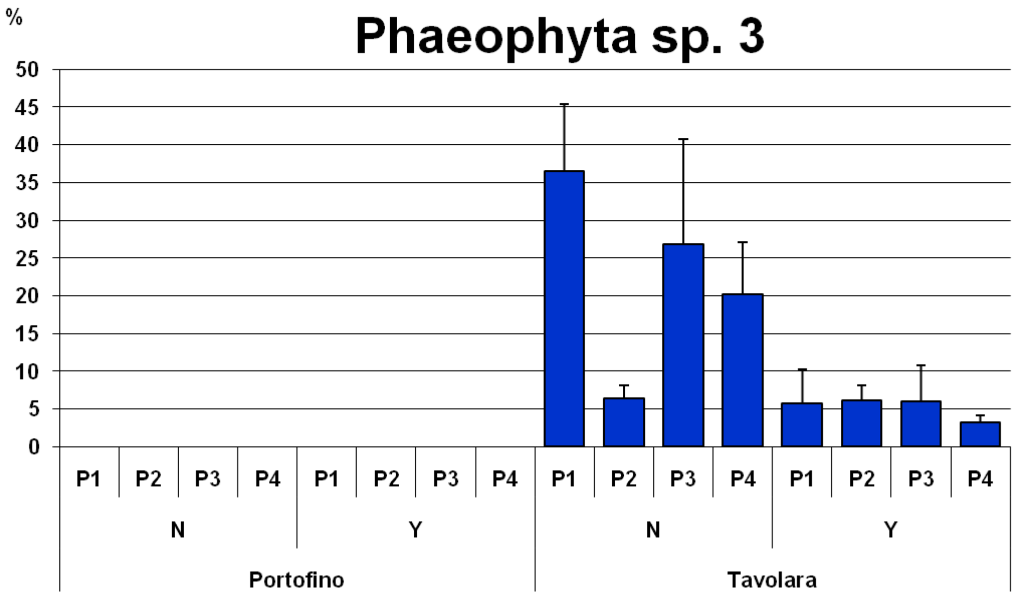


Fig. 29 - Mean percent cover of Phaeophyta sp. 3 (±se).

Phaeophyta sp. 3 was recorded only on Tavolara panels (Fig. 29). Despite significant heterogeneity among plots, the analysis confirmed the sensitivity of this species to the gorgonian forest that inhibited its abundance (Table 11).

Table 11 – PERMANOVA results for Phaeophyta sp. 3

Source	Df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
Si	1	45965	45965.0	1.02E+01	0.0003	9956	0.0001
Fo	1	11217	11217.0	5.76E-01	0.5019	6	0.7827
SixFo	1	19475	19475.0	4.32E+00	0.0032	9934	0.0010
Pl(SixFo)	12	54200	4516.7	3.03E+00	0.0001	9835	0.0001
Res	47	70144	1492.4				
Total	62	199550					

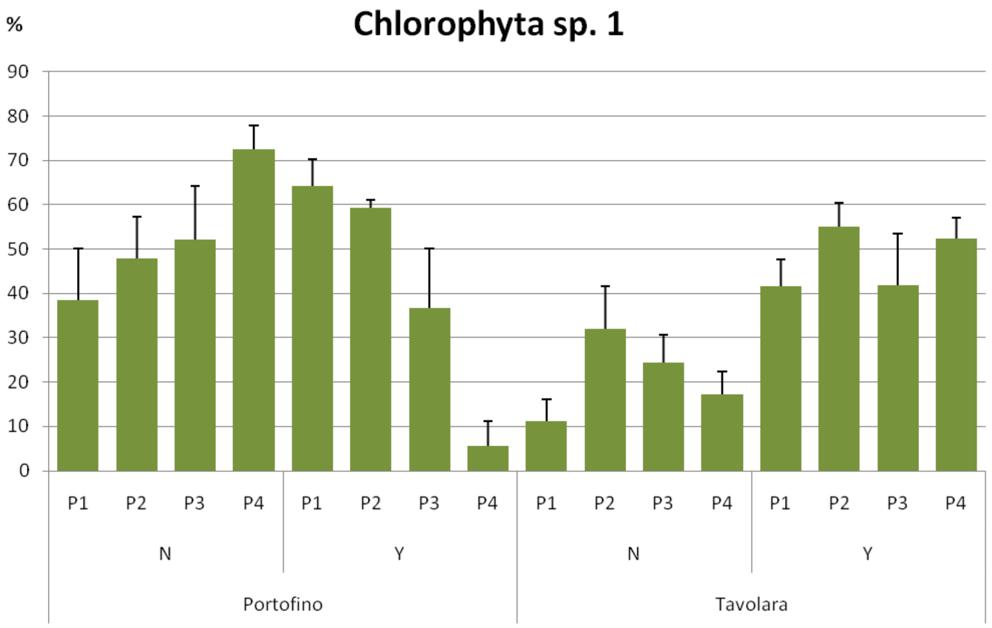


Fig. 30 - Mean percent cover of Chlorophyta sp. 1 (±se).

Chlorophyta sp. 1 is the most abundant species and its presence is found on panels of both sites. The chart (Fig. 30) shows heterogeneity among different plots, moreover within the site Tavolara there is an evident effect of gorgonian forest that enhance the abundance of this species (Table 12), this results was confirmed by the pair-wise test.

Table 12 – PERMANOVA results for Chlorophyta sp. 1

Source	Df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
Si	1	2499.2	2499.20	2.67450	0.1285	9821	0.1276
Fo	1	908.2	908.16	0.16215	0.6611	6	0.7605
SixFo	1	5600.9	5600.90	5.99360	0.0233	9852	0.0329
Pl(SixFo)	12	11244.0	937.03	3.53160	0.0014	9924	0.0010
Res	47	12470.0	265.33				
Total	62	33024.0					

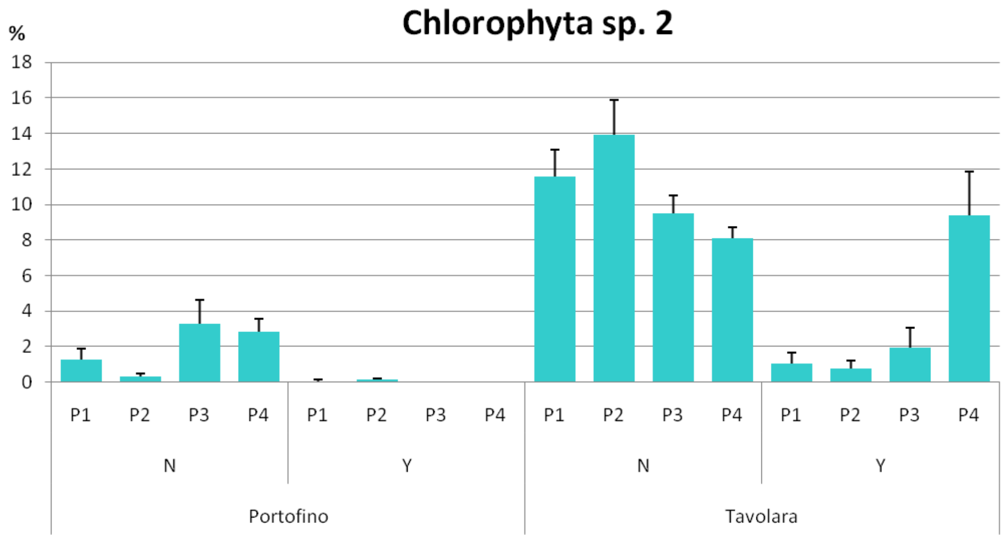


Fig. 31 - Mean percent cover of Chlorophyta sp. 2 (±se).

Chlorophyta sp. 2 is another taxon that showed significant effects at both study sites (Table 13). Despite the heterogeneity among plots, *Paramuricea clavata* seems to inhibit the recruitment of Chlorophyta sp. 2, even if with different intensity between sites (Fig. 31).

Table 13 – PERMANOVA results for Chlorophyta sp. 2

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
Si	1	573.10	573.10	22.898	0.0007	9841	0.0004
Fo	1	344.39	344.39	2.778	0.3281	6	0.3386
SixFo	1	123.98	123.98	4.954	0.0497	9810	0.0448
Pl(SixFo)	12	301.27	25.11	5.411	0.0002	9928	0.0001
Res	47	218.06	4.64				
Total	62	1566.20					

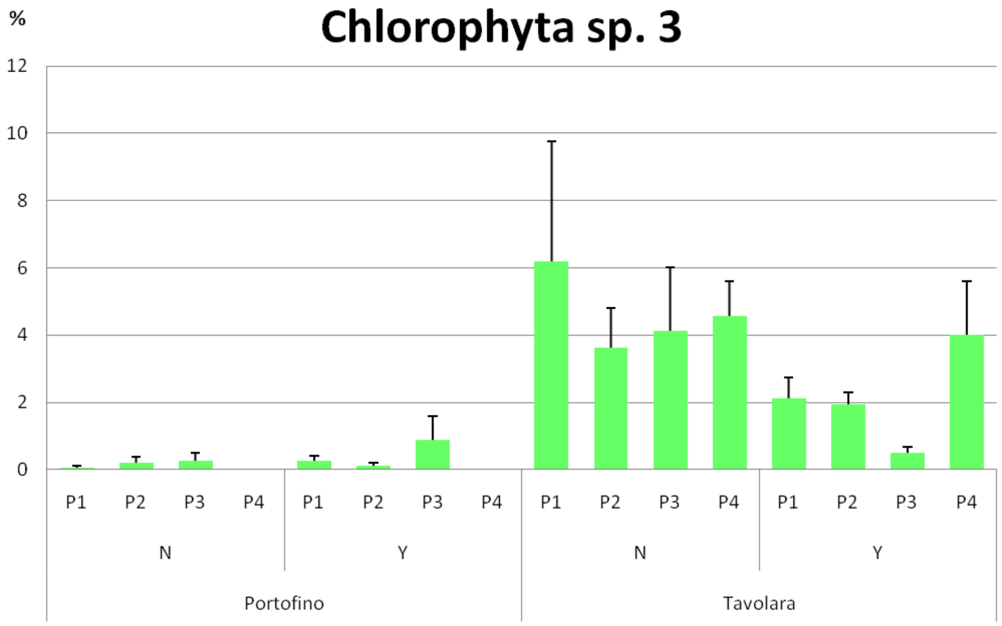


Fig. 32 - Mean percent cover of Chlorophyta sp. 3 (±se).

Chlorophyta sp. 3, was quite homogeneous among plots and showed an evident effect due to the forest at one site, Tavolara, where the taxon was more abundant (Table 14). *Paramuricea clavata* interfere negatively with the presence of this alga that grows much better on non-forested plots (Fig. 32).

Table 14 –PERMANOVA results for Chlorophyta sp.3

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
Si	1	156.91	156.910	45.3580	0.0001	9779	0.0001
Fo	1	20.67	20.672	0.7390	0.6702	9	0.5557
SixFo	1	27.97	27.973	8.0860	0.0118	9808	0.0177
Pl(SixFo)	12	41.41	3.451	0.5989	0.8724	9929	0.8316
Res	47	270.80	5.762				
Total	62	518.85					

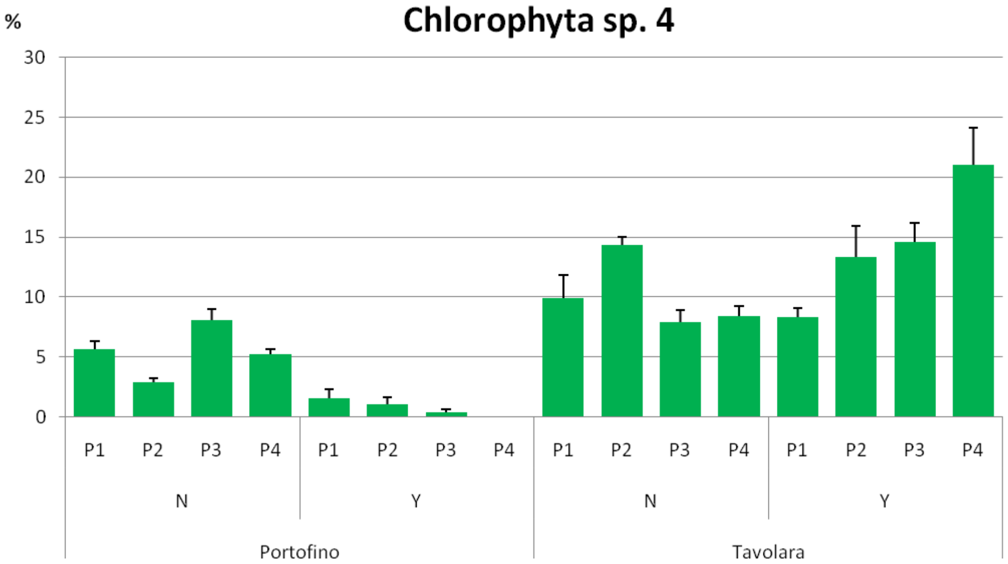


Fig. 33 - Mean percent cover of Chlorophyta sp. 4 (±se).

Chlorophyta sp. 4 was more abundant within Tavolara, where no differences between forested and non-forested plots were detected. In contrast its abundance was reduced by the presence of gorgonian forest in the other site (Fig. 33 and Table 15).

Table 15 – PERMANOVA result for Chlorophyta sp. 4

Source	df	SS	MS	Pseudo-F	P(perm)	Perms	P(MC)
Si	1	1302.80	1302.80	32.0710	0.0001	9839	0.0002
Fo	1	0.92	0.92	0.0030	0.6671	6	0.9673
SixFo	1	309.72	309.72	7.6243	0.0158	9837	0.0169
Pl(SixFo)	12	489.01	40.75	5.6894	0.0001	9938	0.0001
Res	47	336.64	7.16				
Total	62	2413.80					

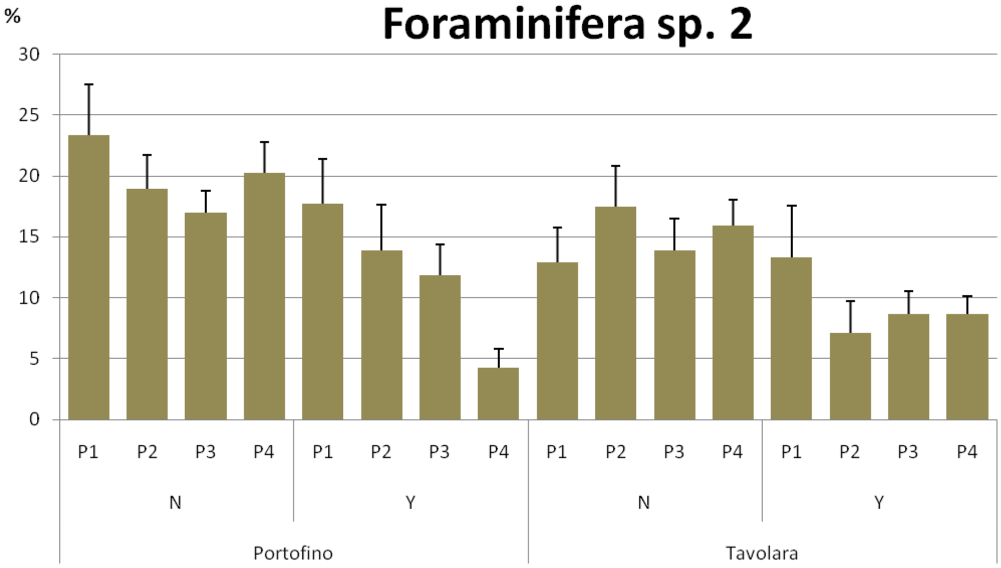


Fig. 34 - Mean percent cover of Foraminifera sp. 2 (±se).

Forams were among the most frequent species, but as it possible to see in the chart of Foraminifera sp. 2 there is no evidence of effects determined by gorgonian (Fig. 34). The analyses pointed out a marked inequity among sites and heterogeneity among plots (Table 16).

Table 16 – PERMANOVA results for Foraminifera sp. 2

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
Si	1	210.42	210.42	4.644	0.0513	9828	0.0531
Fo	1	725.74	725.74	34.171	0.5036	6	0.1082
SixFo	1	21.24	21.24	0.469	0.5365	9861	0.5055
Pl(SixFo)	12	544.28	45.36	1.351	0.2178	9922	0.2230
Res	47	1577.50	33.57				
Total	62	3063.90					

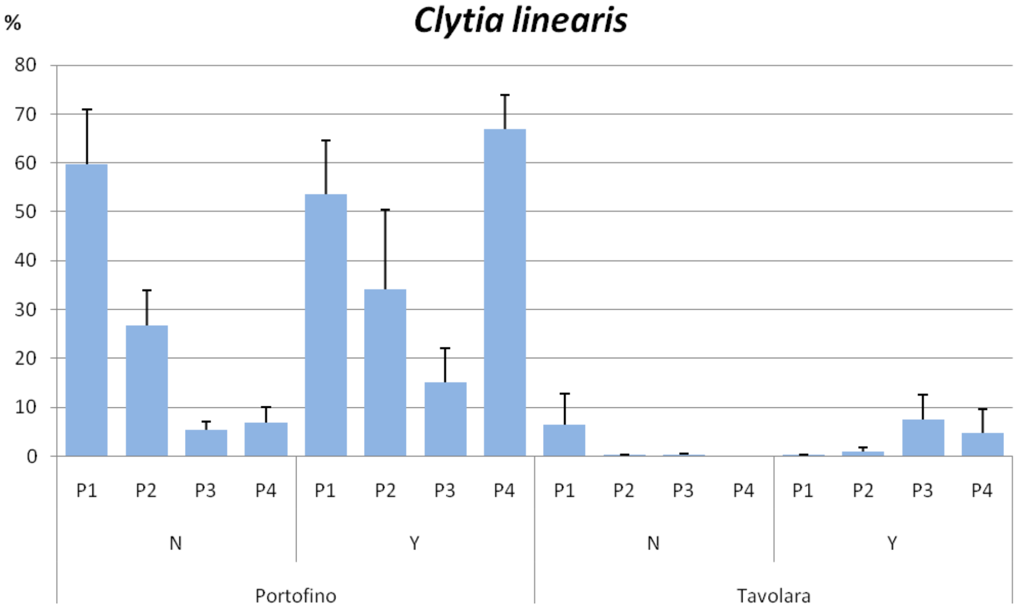


Fig. 35 - Mean percent cover of *Clytia linearis* (±se).

Clytia linearis was clearly more abundant in one site (Fig. 35). Its abundance was very heterogeneous among plots and resulted positively affected by the experimental gorgonian forest at the site where it was most abundant, as confirmed by statistical tests (Table 17).

Table 17 - PERMANOVA results for *Clytia linearis*

Source	Df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
Si	1	45965	45965.0	10.2030	0.0003	9938	0.0001
Fo	1	11217	11217.0	0.5760	0.4960	6	0.7914
SixFo	1	19475	19475.0	4.3228	0.0041	9947	0.0014
Pl(SixFo)	12	54200	4516.7	3.0264	0.0001	9815	0.0001
Res	47	70144	1492.4				
Total	62	199550					

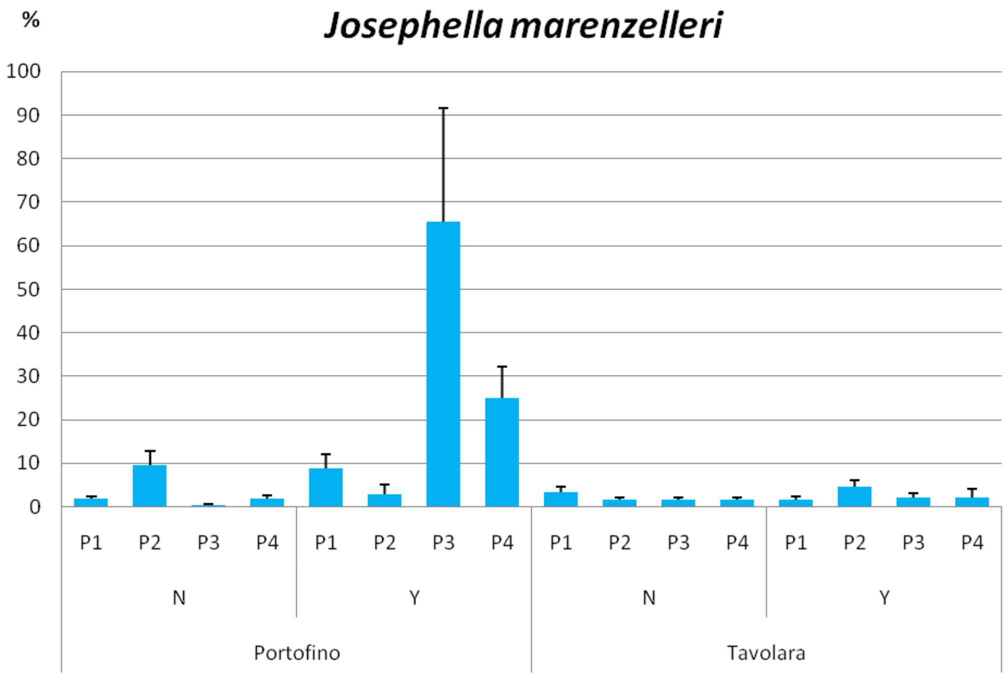


Fig. 36 - Mean percent cover of *Josephella marenzelleri* (±se).

The serpulid polychaete *Josephella marenzelleri* was very frequent, even if not abundant (Fig. 36). Its abundance was not affected by *Paramuricea clavata* forest. PERMANOVA highlighted a significant heterogeneity among different plots (Table 18).

Table 18 – PERMANOVA results for *Josephella marenzelleri*

Source	Df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
Si	1	145.50	145.50	2.8490	0.0804	9856	0.1142
Fo	1	126.75	126.75	1.1068	0.5066	6	0.4910
SixFo	1	114.52	114.52	2.2424	0.1420	9924	0.1611
Pl(SixFo)	12	614.66	51.22	4.2973	0.0002	9907	0.0006
Res	47	560.22	11.92				
Total	62	1552.10					

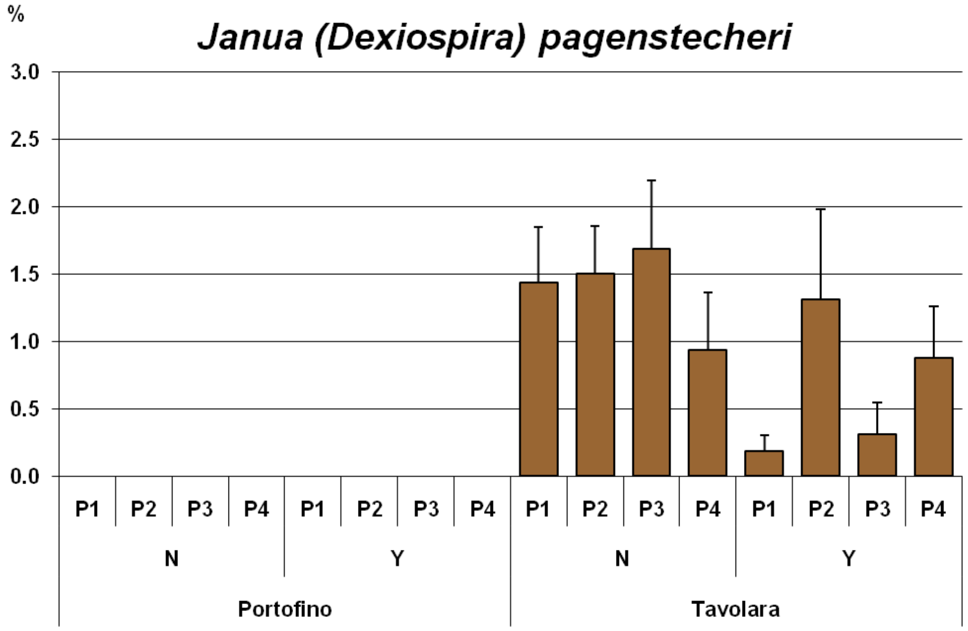


Fig. 37 - Mean percent cover of *Janua (Dexiospira) pagenstecheri* (±se).

The serpulid polychaete *Janua* (*Dexiospira*) *pagenstecheri* was present only at Tavolara, where showed a heterogeneity among plots with a P value greater than 0.25, so the pooling technique was applied in order to improve the other tests. After that, a significant interaction Site x Forest (Table 19) allowed to recognise that the presence of sea fan forest inhibits this algae growth (Fig. 37).

Table 19 – PERMANOVA results for *Janua (Dexiospira) pagenstecheri* after the pooling.

Source	Df	SS	MS	Pseudo-F	P(perm)	Perms	P(MC)
Si	1	16.7370	16.7370	46.2820	0.0001	9832	0.0001
Fo	1	2.0325	2.0325	1.0000	0.5040	2	0.5038
SixFo	1	2.0325	2.0325	5.6205	0.0204	9838	0.0182
Res	59	21.3360	0.3616				
Total	62	42.2140					

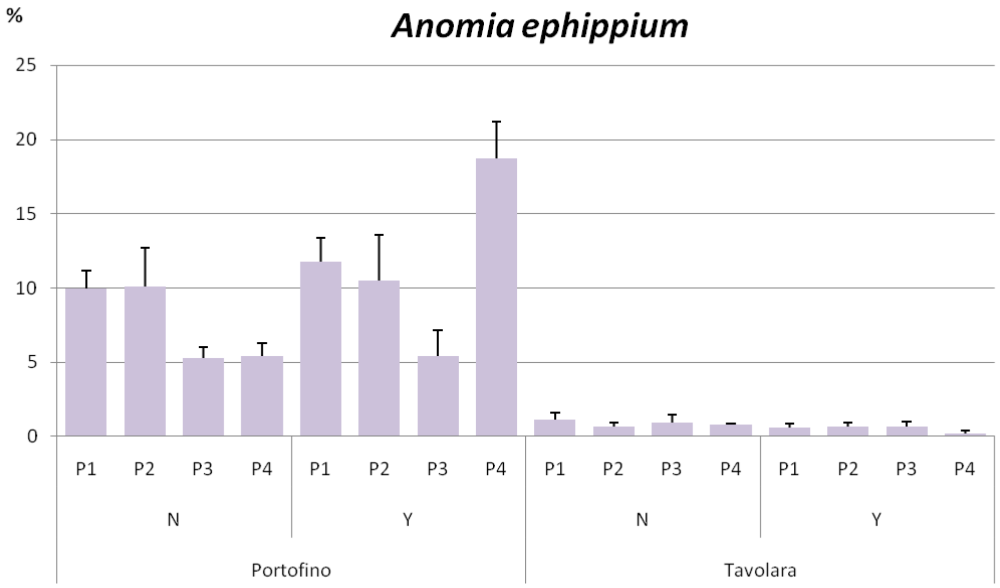


Fig. 38 - Mean percent cover of *Anomia ephippium* (±se).

This was the only mollusc found and its presence was quite frequent (Fig. 38). The graph and the statistic showed a strong evidence of difference among plots and sites (Table 20).

Table 20 – PERMANOVA results for *Anomia ephippium*

Source	Df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
Si	1	1256.40	1256.40	38.2980	0.0002	9820	0.0002
Fo	1	49.73	49.73	0.6824	0.4988	6	0.5592
SixFo	1	72.87	72.87	2.2214	0.1568	9878	0.1632
Pl(SixFo)	12	394.82	32.90	4.4774	0.0001	9932	0.0002
Res	47	345.38	7.35				
Total	62	2014.80					

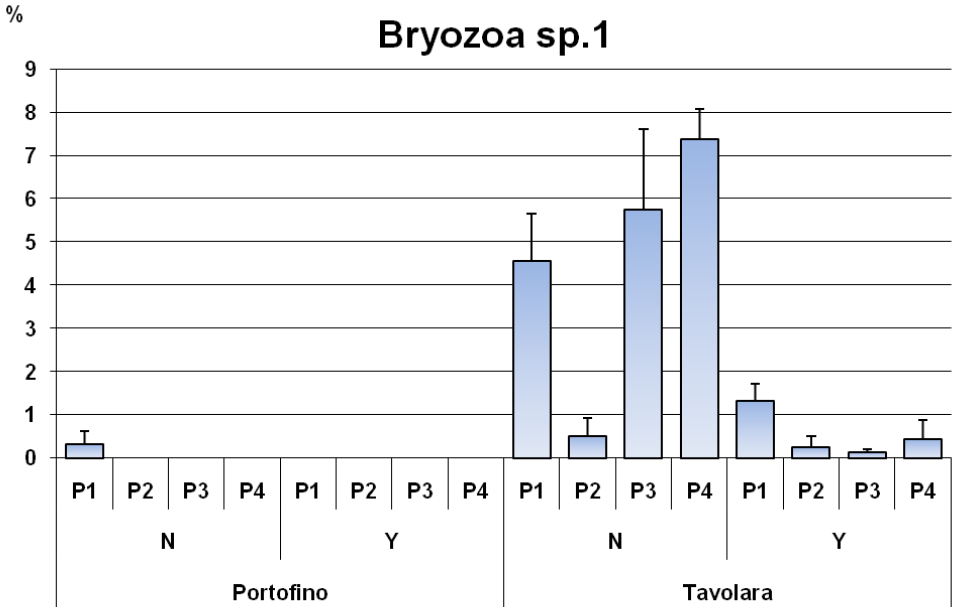


Fig. 39 - Mean percent cover of Bryozoa sp. 1 (±se).

Bryozoa sp. 1 was more abundant at Tavolara site and it was significantly affected by the gorgonian presence since its presence was major in absence of the forest (Fig. 39), as confirmed by PERMANOVA showed below (Table 21).

Table 21 – PERMANOVA results for Bryozoa sp. 1

Source	Df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
Si	1	97.959	97.959	11.0170	0.0059	9735	0.0054
Fo	1	65.667	65.667	1.0809	0.3292	10	0.4884
SixFo	1	60.750	60.750	6.8323	0.0221	9822	0.0233
Pl(SixFo)	12	107.040	8.920	6.0052	0.0001	9927	0.0001
Res	4.70E+01	69.813	1.485				
Total	62	404.210					

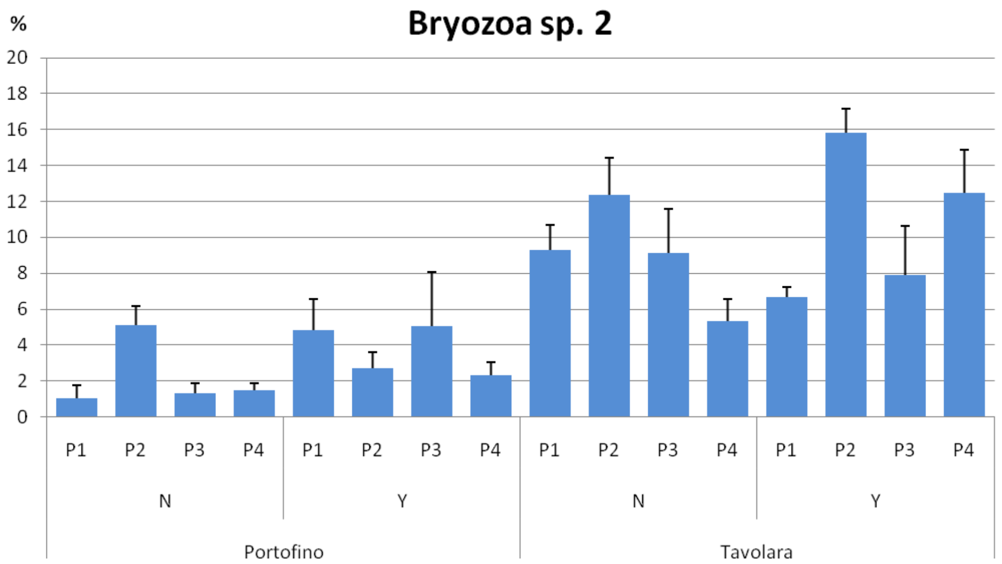


Fig. 40 - Mean percent cover of Bryozoa sp. 2 (±se).

Bryozoa sp. 2 is one of the bryozoans more frequent on the panels. The analysis pointed out differences among plots and sites (Table 22), and there was evidence of an effect due to the gorgonian forest (Fig. 40).

Table 22 – PERMAMOVA results of Bryozoa sp. 2

Source	Df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
Si	1	743.630	743.630	23.506	0.0005	9837	0.0003
Fo	1	39.163	39.163	219.180	0.1644	6	**0.0427**
SixFo	1	0.179	0.179	0.006	0.9398	9836	0.9445
Pl(SixFo)	12	380.560	31.714	2.820	0.0078	9943	0.0064
Res	47	528.590	11.247				
Total	62	1693.400					

In the following table (Table 23) it is possible to see all the results of the univariate analyses conducted on the selected taxa.

Table 23 - Summary of PERMANOVA test on the abundances of selected taxa.

Taxa	Level				Pairwise test			
					Tavolara		Portofino	
	Si	Fo	SixFo	PI(SixFo)	P	Effect	P	Effect
Chlorophyta sp. 1	0.1285	0.7605	**0.0233**	**0.0014**	**0.0263**	Y > N	0.4902	
Phaeophyta sp. 1	**0.0004**	0.6427	**0.0003**	0.6581	**0.0278**	Y > N	**0.0144**	N > Y
Chlorophyta sp. 2	**0.0007**	0.3386	**0.0497**	**0.0002**	**0.0226**	N > Y	**0.0299**	N > Y
Clytia linearis	**0.0003**	0.7914	**0.0041**	**0.0001**	**0.0278**	Y > N	0.0597	
Rhodophyta sp. 2	**0.0072**	0.479	**0.0426**	**0.0406**	**0.0311**	N > Y		
Phaeophyta sp. 2	**0.0003**	0.4973	**0.0002**	0.9152	**0.0274**	N > Y		
Phaeophyta sp. 3	0.0003	0.7827	**0.0032**	0.0001	**0.0014**	N >> Y	0.0659	
Bryozoa sp.1	0.0059	0.4884	**0.0221**	0.0001	**0.0342**	N > Y	0.3576	
Rhodophyta sp. 3	0.0003	0.5025	**0.0014**	0.607	**0.0035**	N > Y		
Janua (Dexiospira) pagenstecheri	0.0003	0.5038	**0.042**	0.4245	**0.0215**	N > Y		
Chlorophyta sp. 3	**0.0001**	0.5557	**0.0118**	0.8724	**0.0305**	N > Y	0.3914	
Chlorophyta sp. 4	**0.0001**	0.9673	**0.0158**	**0.0001**	0.2658		**0.0135**	N > Y
Hydroides stoichadon	**0.0022**	0.4824	**0.0165**	**0.0326**	0.0585		0.4059	
Phaeophyta sp. 4	**0.0006**	0.4635	**0.0297**	**0.0002**	0.0656		0.2859	
Bryozoa sp.2	**0.0005**	**0.0427**	0.9398	**0.0078**				
Rhodophyta sp. 4	**0.0045**	0.6506	0.1736	**0.0032**				
Phaeophyta sp. 5	**0.0007**	0.1643	0.8289	**0.0001**				
Entalophoroecia sp.	**0.0436**	0.5057	0.1263	**0.0139**				
Chlorophyta sp. 5	**0.0003**	0.4956	0.0695	**0.0009**				
Rhodophyta sp. 5	**0.0118**	0.3541	0.1872	**0.0459**				
Foraminifera sp. 1	**0.0009**	0.7476	0.2902	**0.0001**				
Anomia ephippium	**0.0002**	0.5592	0.1568	**0.0001**				
Bryozoa sp. 3	**0.0128**	0.4902	0.063	**0.0058**				
Josephella marenzelleri	**0.0804**	0.491	0.142	**0.0002**				
Savignyella lafontii	0.5085	0.4607	0.359	**0.0001**				
Phaeophyta sp. 6	0.5104	0.0609	0.8466	**0.003**				
Rhodophyta sp. 6	0.2035	0.6512	0.1078	**0.0005**				
Rhodophyta sp. 1	0.2835	0.8856	0.1701	**0.0012**				
Rhodophyta sp. 7	**0.0147**	0.5521	0.5115	0.4476				
Neodexiospira pseudocorrugata	**0.0007**	0.4315	0.059	0.2307				
Pileoloaria militaris	**0.0406**	0.5251	0.1283	0.0005				
Bryozoa sp. 4	**0.0025**	0.4729	0.0618	0.2179				
Disporella sp.	**0.0003**	0.5349	0.4255	0.9016				
Pomatoceros triqueter	**0.0001**	0.9448	0.838	0.9491				
Pileolaria heteropoma	**0.0007**	0.501	0.0771	0.4782				
Laurencia sp.	**0.0356**	0.099	0.8541	0.9265				
Rhodophyta sp. 8	**0.0004**	0.4854	0.8066	0.3453				
Foraminifera sp. 2	0.0513	0.1082	0.5365	0.2178				
Chlorophyta sp. 6	0.3534	0.2797	0.362	0.1403				
Foraminifera sp. 3	0.1807	0.0893	0.7833	0.0791				
Terebellidae sp.	0.1344	0.4986	0.1436	0.7866				

3.1.3 Effects of gorgonian forest on ecological structure patterns

In order to assess the possible effects of the gorgonian forest on the ecological structure of the assemblages, the taxa were grouped in ecological categories. Ecological groups correlation vectors were plotted on the PCO graph and represent the relationships between the abundance relative to ecological groups and their patterns of similarity of the assemblages colonising the panels (Fig. 41). The PERMANOVA analysis highlighted a strong heterogeneity among plots, moreover the interaction Site x Forest resulted significant (Table 24). Post-hoc pair-wise test detected significant difference in the ecological structure of the assemblages between forest and non-forest treatment only at one site (Tavolara).

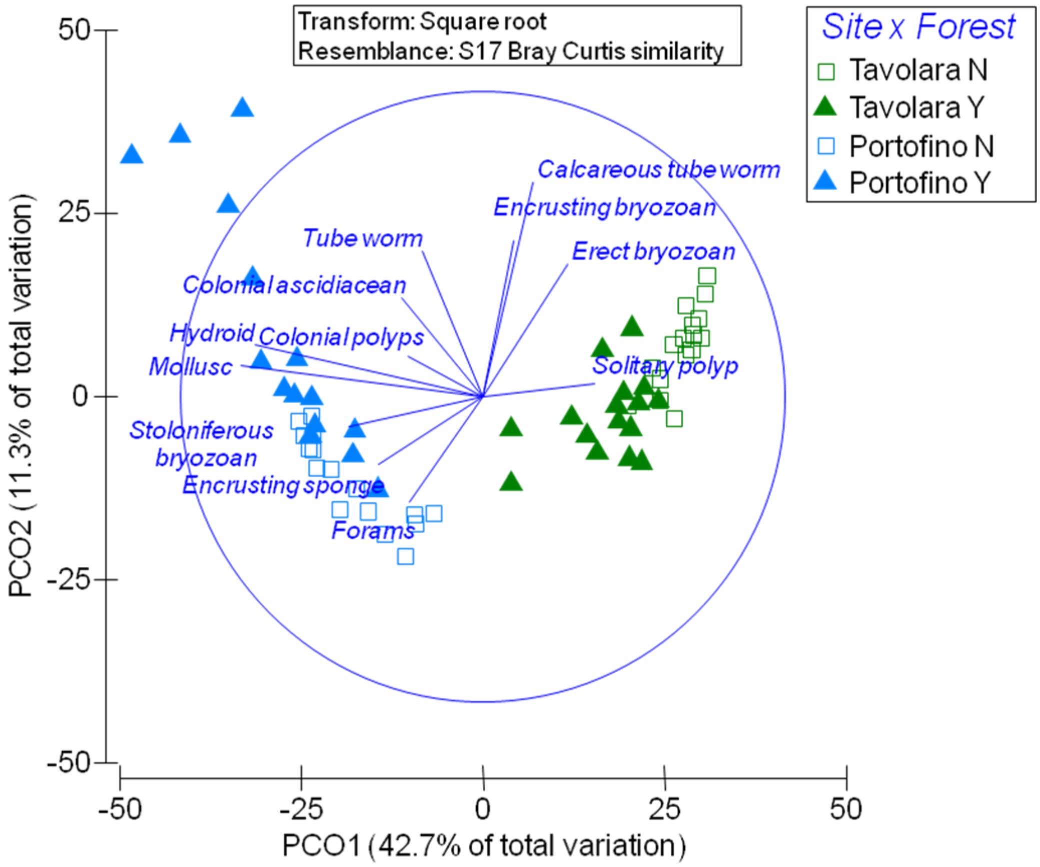


Fig. 41 - Correlation vectors of the ecological groups that more contribute to the similarity patterns, superimposed on the PCO ordination plot.

Table 24 – PERMANOVA results

Source	Df	SS	MS	Pseudo-F	P(perm)	Perms	P(MC)
Si	1	8040.5	8040.50	17.7740	0.0002	9949	0.0001
Fo	1	1269.4	1269.40	0.6903	0.4948	6	0.6418
SixFo	1	1838.9	1838.90	4.0649	0.0177	9940	0.0112
Pl(SixFo)	12	5443.3	453.61	3.4442	0.0001	9865	0.0001
Res	47	6190.1	131.70				
Total	62	22101.0					

The groups that mainly contribute to the differentiation of the ecological structure of the assemblages were identified by forward DistLM procedure and are reported in the table below (Table 25).

Table 25 - DistLM results that represent almost the 66% of variability

Variable	R^2	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
Erected algae	0.32007	27009.0	28.715	0.001	0.32007	0.32007	61
Mollusc	0.41722	8198.4	10.003	0.001	0.09715	0.41722	60
Encrusting algae	0.49499	6562.0	9.0849	0.001	0.07776	0.49499	59
Encrusting bryozoan	0.52743	2737.7	3.9818	0.001	0.03244	0.52743	58
Calcareous tube worm	0.55655	2457.1	3.7427	0.001	0.02912	0.55655	57
Stoloniferous bryozoan	0.57767	1782.7	2.8012	0.001	0.02113	0.57767	56
Forams	0.59853	1760.1	2.8574	0.001	0.02086	0.59853	55
Encrusting Calcareous algae	0.61707	1564.3	2.6141	0.002	0.01854	0.61707	54
Hydroid	0.63239	1292.8	2.2087	0.009	0.01532	0.63239	53
Colonial ascidiacean	0.64759	1282.5	2.2426	0.002	0.01520	0.64759	52
Erect bryozoan	0.66034	1076.4	1.9153	0.022	0.01276	0.66034	51

3.1.4 Effects of gorgonian forest on single ecological groups

Histograms for main ecological groups are showed in the following pages while for results of the statistical test were summarised in Table 33.

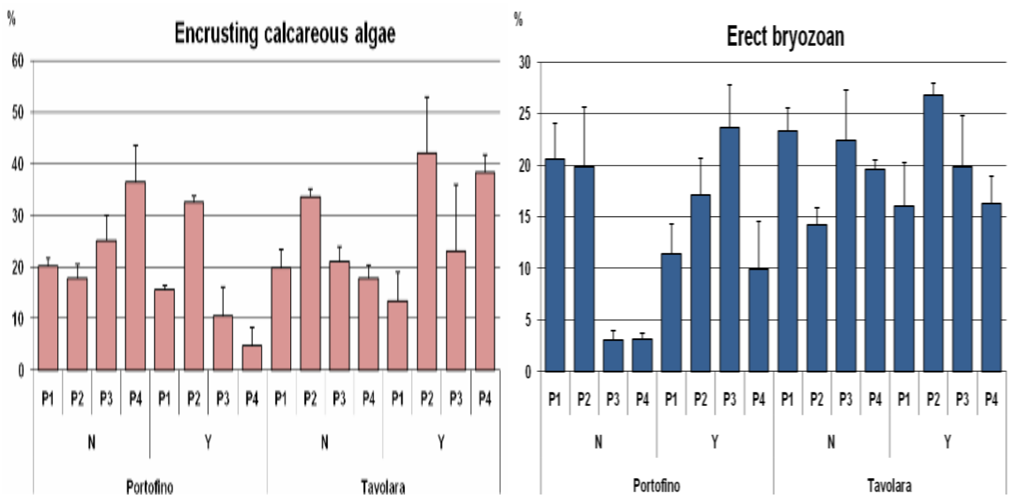


Fig. 42 – Mean percent cover of Encrusting calcareous algae and Erected bryozoan (±se).

Table 26 – PERMANOVA results for Encrusting calcareous algae

Source	Df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
Si	1	521.02	521.02	1.22E+00	0.2884	9853	0.3005
Fo	1	33.10	33.10	3.66E-02	0.5001	6	0.8846
SixFo	1	903.81	903.81	2.11E+00	0.1688	9839	0.1701
Pl(SixFo)	12	5142.30	428.53	3.46E+00	0.0009	9936	0.0015
Res	47	5816.20	123.75				
Total	62	12230.00					

Table 27 – PERMANOVA results for Erect bryozoan

Source	Df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
Si	1	608.17	608.17	34'686.0	0.0944	9829	0.0899
Fo	1	55409.00	55409.00	0.9	0.4967	6	0.5252
SixFo	1	63021.00	63021.00	0.4	0.5577	9792	0.5656
Pl(SixFo)	12	2110.00	175.83	38'645.0	0.0005	9934	0.0007
Res	47	2138.40	45498.00				
Total	62	4976.90					

Overall, the presence of gorgonian forest did not affect the total percent cover of encrusting calcareous algae (Table 26) and erected bryozoans (Table 27). PERMANOVA showed a high heterogeneity among plots for both groups, as it is possible to see in the charts (Fig. 42), and no differences between sites.

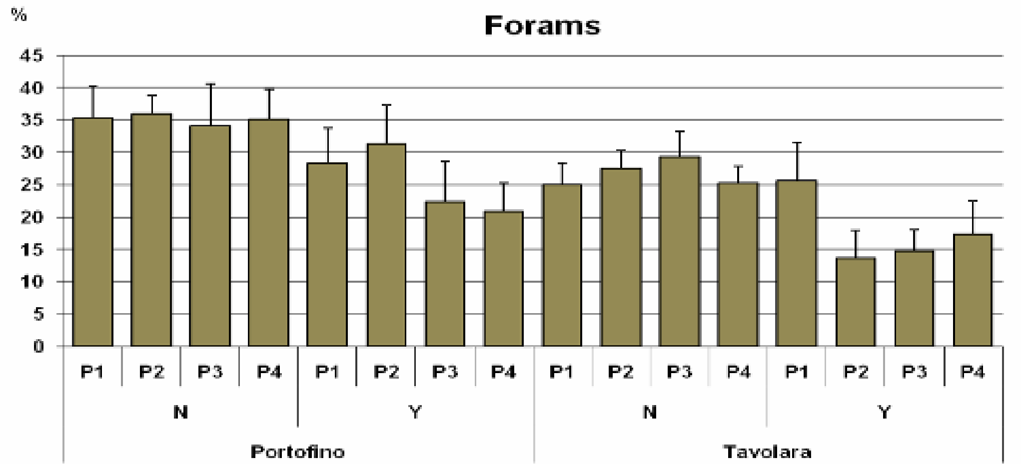


Fig. 43 – Mean percent cover of Forams (±se).

As regards the forams group, statistic revealed both a clear effect of the gorgonian forest and a significant difference among sites (Table 28). Despite forams were more abundant at one site, as it is possible to see in the charts, the presence of *Paramuricea clavata* forest has the same effect of inhibition of the forams abundance in both sites (Fig. 43).

Table 28 – PERMANOVA results for Forams

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
Si	1	1023.40	1023.40	18.07	0.0021	9811	0.0011
Fo	1	1317.80	1317.80	1'565.10	0.3312	6	**0.0154**
SixFo	1	0.84	0.84	0.01	0.8990	9829	0.9012
Pl(SixFo)	12	678.32	56.53	0.65	0.7863	9945	0.7934
Res	47	4070.40	86.61				
Total	62	7133.80					

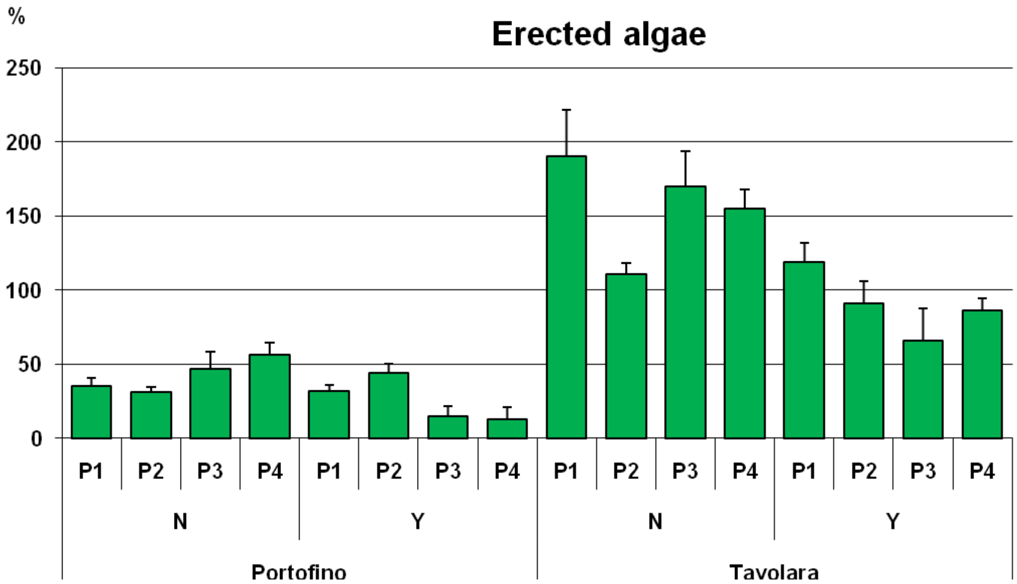


Fig. 44 - Mean percent cover of Erected algae (±se).

The overal abundace of erected algae showed relevant differences among plots and a significant interaction among Site and Forest (Table 29) due to a negative effect of sea fan forests on growth of erected algae at one site (Tavolara Fig. 44).

Table 29 – PERMANOVA results for Erected algae

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
Si	1	1.26E+09	1.26E+09	64'595	0.0003	9822	0.0001
Fo	1	2.66E+04	2.66E+04	27'635	0.3277	7	0.3521
SixFo	1	9.61E+03	9.61E+03	49'347	0.0492	9830	0.0461
Pl(SixFo)	12	2.34E+04	1.95E+03	2'501	0.0131	9938	0.0139
Res	47	3.67E+04	7.80E+02				
Total	62	2.21E+09					

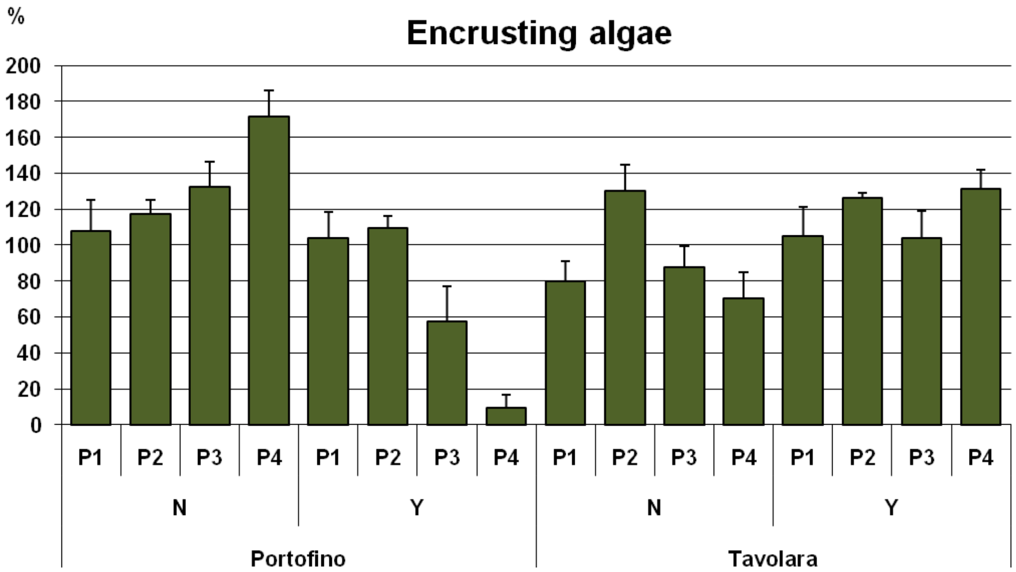


Fig. 45 – Mean percent cover of Encrusting algae (±se).

Encrusting (non-calcareous) algae showed heterogeneity among plots and it underwent an inhibitory effect at one site (Portofino Table 30 and Fig. 45).

Table 30 – PERMANOVA results for Encrusting algae

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
Si	1	166	165.82	46.7	0.8353	9864	0.8368
Fo	1	5567	5567.00	0.2	0.5022	6	0.7428
SixFo	1	29428	29428.00	8'295.0	0.0134	9848	0.0152
Pl(SixFo)	12	42703	3558.50	51'083.0	0.0002	9930	0.0001
Res	47	32741	696.62				
Total	62	1063900000					

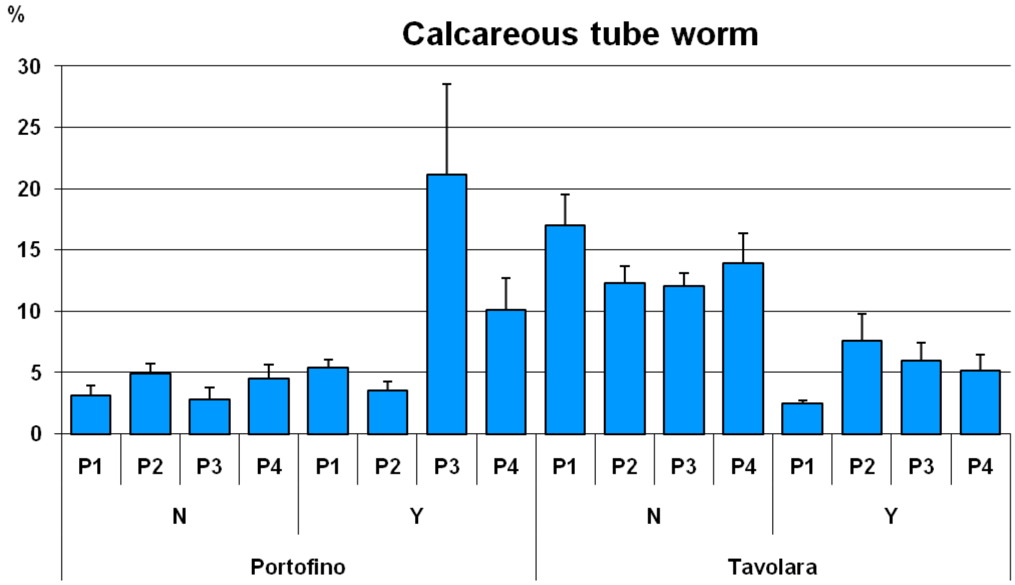


Fig. 46 – Mean percent cover of Calcareous tube worm (±se).

The growth of calcareous tube worms was heterogeneous among plots but shows a sensitivity to gorgonian within Tavolara (Table 31): and the forest significantly inhibit their presence (Fig. 46).

Table 31 – PERMANOVA results for Calcareous tube worm

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
Si	1	107.14	107.14	14'737.0	0.2582	9870	0.2470
Fo	1	21716.00	21716.00	25.5	0.6785	6	0.8997
SixFo	1	851.29	851.29	11'709.0	0.0031	9840	0.0061
Pl(SixFo)	12	874.75	72896.00	33'077.0	0.0018	9918	0.0017
Res	47	1035.80	22038.00				
Total	62	2907.70					

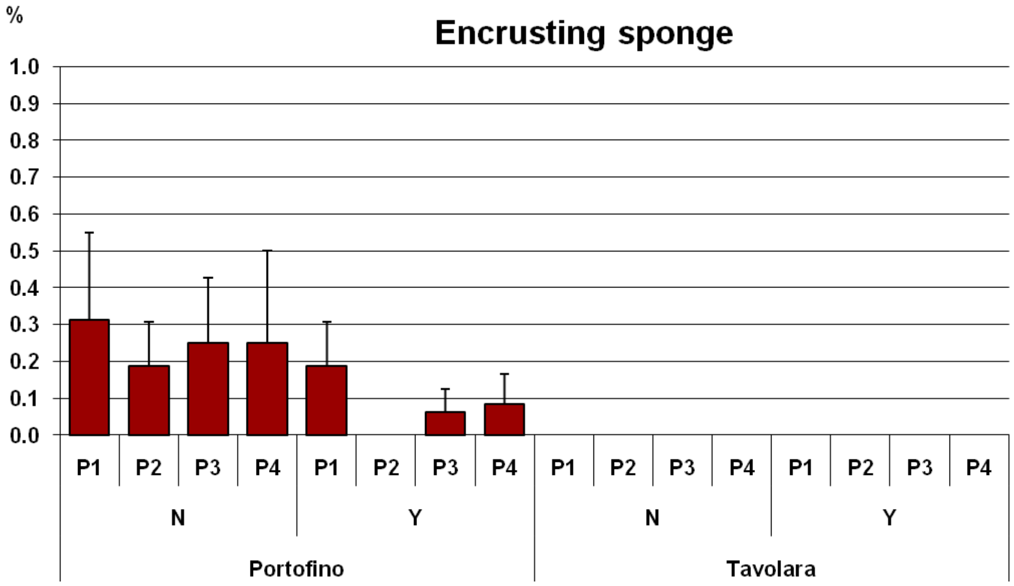


Fig. 47 – Mean percent cover of Encrusting sponge (±se).

Encrusting sponges was found only in Portofino where there significant differences among plots (Table 32) and the growth was negatively influenced by *Paramuricea clavata* presence (Fig. 47).

Table 32 – PERMANOVA results for Encrusting sponge

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P (MC)
Si	1	4.35E-01	4.35E-01	49	0.0003	7935	0.0001
Fo	1	1.09E-01	1.09E-01	1	0.6614	3	0.4892
SixFo	1	1.09E-01	1.09E-01	12	0.0064	7827	0.0035
Pl(SixFo)	12	1.04E-01	8.68E-03	1.83E-01	0.9990	9846	0.9983
Res	47	2.23E+00	4.74E-02				
Total	62	3.00E+00					

Table 33 – Summary of PERMANOVA test on the abundances of ecologic groups.

Ecologic group	Level				Pairwise test			
					Tavolara		Portofino	
	Si	**Fo**	**SixFo**	**Pl(SixFo)**	**P**	**Effect**	**P**	**Effect**
Erected algae	**0.0003**	0.3521	**0.049**	**0.0131**	**0.0178**	N > Y	0.1280	
Encrusting algae	0.8353	0.7428	**0.013**	**0.0002**	0.1502		**0.0057**	N >> Y
Calcareous tube worm	0.2582	0.8997	**0.003**	**0.0018**	**0.0276**	N > Y	0.1764	
Encrusting sponge	**0.0003**	0.4892	**0.006**	0.999			**0.0123**	N > Y
Forams	**0.0021**	**0.0154**	0.899	0.7863				
Hydroid	**0.001**	0.4133	0.408	**0.0001**				
Colonial polyps	**0.0586**	0.4901	0.192	**0.0249**				
Stoloniferous bryozoan	**0.0052**	0.5428	0.164	**0.0446**				
Mollusc	**0.0001**	0.5646	0.156	**0.0004**				
Erected bryozoan	0.0944	0.5252	0.558	**0.0005**				
Encrusting bryozoan	0.1807	0.0661	0.771	**0.0103**				
Colonial ascidiacean	0.2115	0.4924	0.277	**0.0274**				
Encrusting calcareous algae	0.2884	0.8846	0.169	**0.0009**				
Solitary polyp	**0.0102**	0.5074	0.498	0.2443				
Social ascidiacean	0.4880	0.4988	0.487	0.5185				
Tube worm	0.3298	0.3132	0.333	0.6983				
Erected sponge	0.9843	0.6012	0.107	0.4595				
Solitary ascidiacean	0.4919	0.5053	0.491	0.5190				

3.1.5 Effects of gorgonian forest at Phylum taxonomic level

Among Phyla encountered in this study in general were found significant differences among sites and heterogeneity among plots (Table 35), reflecting the results obtained for the analyses of species abundance and for the ecological groups.

Here below are showed DistLM results of the main phyla that contribute to explain the major difference encountered in the structure patterns (Table 34).

Table 34 – DistLM results that represent almost the 60% of variability

Variable	R^2	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
Rhodophyta	0.31566	26637.0	28.1370	0.001	3.16E-01	0.31566	61
Mollusca	0.39360	6576.5	7.7111	0.001	7.79E-02	0.39360	60
Phaeophyta	0.46417	5955.1	7.7704	0.001	7.06E-02	0.46417	59
Chlorophyta	0.50341	3311.3	4.5830	0.001	3.92E-02	0.50341	58
Annelida	0.53571	2726.3	3.9663	0.001	3.23E-02	0.53571	57
Chordata	0.55766	1852.4	2.7790	0.001	2.20E-02	0.55766	56
Foraminifera	0.57948	1841.2	2.8537	0.001	2.18E-02	0.57948	55
Cnidaria	0.59782	1547.1	2.4616	0.003	1.83E-02	0.59782	54
Bryozoa	0.61083	1097.9	1.7719	0.031	1.30E-02	0.61083	53

Vectors of main sensitive Phyla were plotted on the PCO graph showed below (Fig. 48) and results with P values of the univariate analysis are reported in the Table 35.

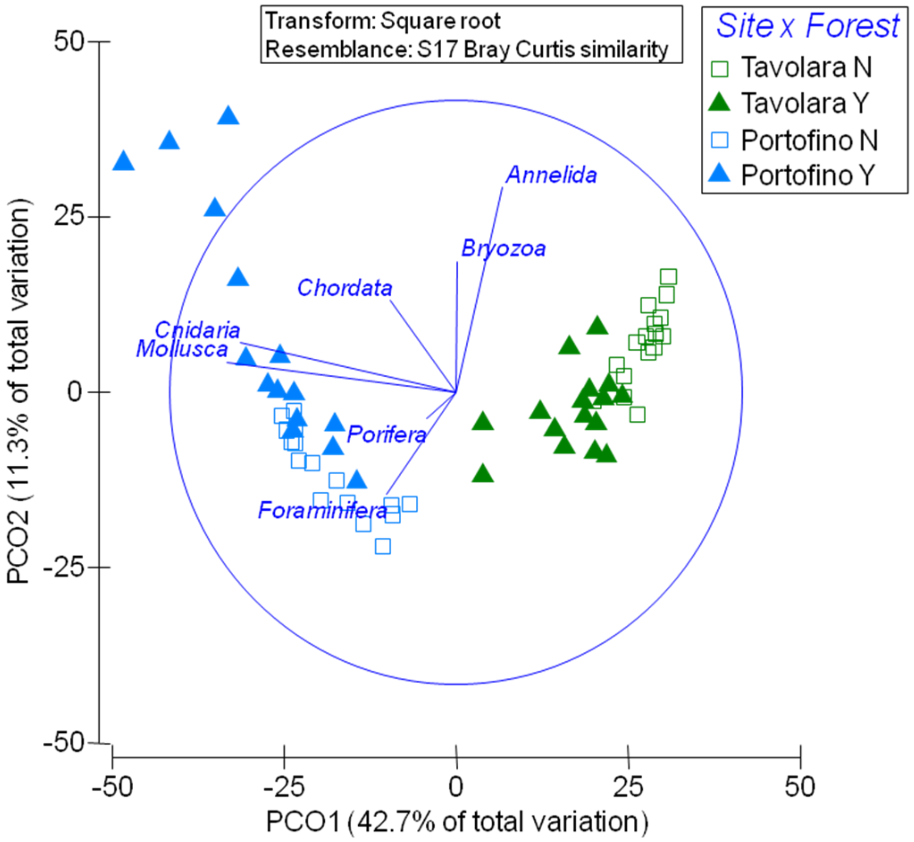


Fig. 48 – Correlation vectors of the Phyla that more contribute to the similarity patterns, superimposed on the PCO ordination plot.

Table 35 – Summary of PERMANOVA test on the abundances of Phyla

Phylum	Level				Pairwise test			
					Tavolara		Portofino	
	Si	Fo	SixFo	Pl(SixFo)	P	Effect	P	Effect
Rhodophyta	**0.0006**	0.4946	0.4903	**0.0001**				
Paeophyta	0.7311	0.5044	**0.0431**	**0.0018**	0.6567	0.6924	**0.049**	N >> Y
Chlorophyta	**0.0004**	0.3303	0.5777	**0.0007**				
Foraminifera	**0.0018**	**0.0154**	0.9075	0.7867				
Porifera	0.1395	0.3332	0.4095	0.6986				
Cnidaria	**0.0012**	0.5036	0.3953	**0.0001**				
Annelida	0.2569	0.6613	**0.0022**	**0.002**	**0.0013**	N >> Y	0.1822	
Mollusca	**0.0012**	0.5036	0.3953	**0.0001**				
Bryozoa	0.6136	0.3358	0.9459	**0.0001**				
Chordata	0.4351	0.4964	0.5112	0.064				

According to the univariate analysis, Phaeophyta and Annelida are the two phyla that were influenced by synergism between gorgonian presence/absence and environmental conditions of the site (see charts below), while Foraminifera highlighted sensitivity to the factor Forest independently from environmental condition (see correspondent graph in the paragraph 3.1.4).

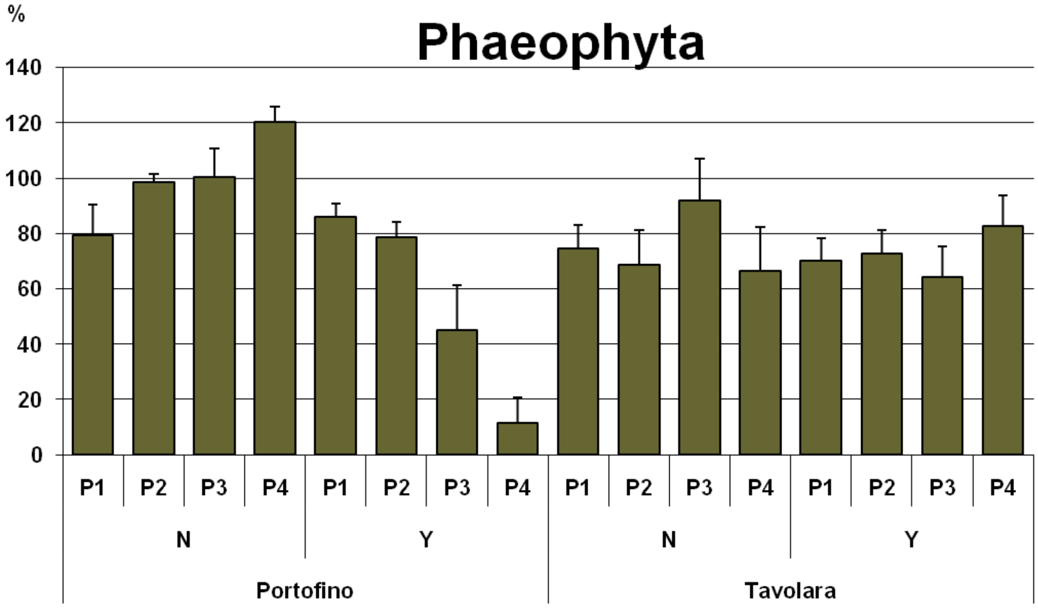


Fig. 49 - Mean percent cover of Phaeophyta (±se).

Phaeophyta distribution patterns are showed in the chart that highlighs heterogeneity among plots confirmed also by statistical analysis (Table 36). At Portofino, *Paramuricea clavata* forests seems to hamper their growth (Fig. 49).

Table 36 – PERMANOVA results for Phaeophyta

Source	df	SS	MS	Pseudo-F	P(perm)	Perms	P(MC)
Si	1	202.13	202.13	0.13789	0.7311	9830	0.7199
Fo	1	8738.40	8738.40	1.29650	0.5044	6	0.4594
SixFo	1	6739.90	6739.90	4.59790	0.0431	9842	0.0559
Pl(SixFo)	12	17637.00	1469.80	3.33890	0.0018	9939	0.0016
Res	47	20689.00	440.20				
Total	62	52191.00					

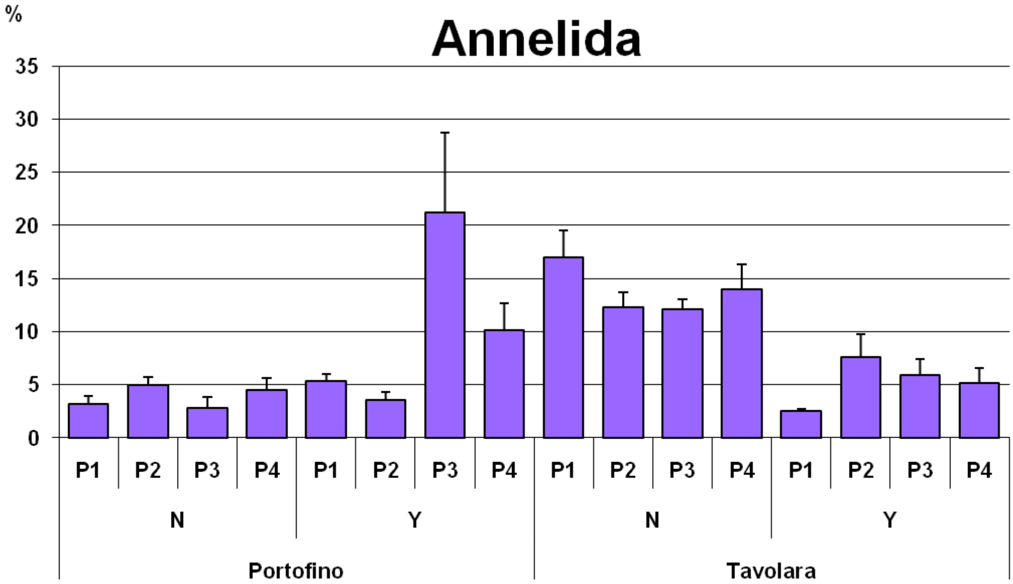


Fig. 50 - Mean percent cover of Annelida (±se).

Statistic performed on Annelida highlighted heterogeneity among plots (Table 37), moreover organisms of this Phylum showed sensitivity to the experiment at Tavolara where they reach higher densities on the panels without the *Paramuricea clavata* forest (Fig. 50).

Table 37 – PERMANOVA results for Annelida

Source	df	SS	MS	Pseudo-F	P(perm)	Perms	P(MC)
Si	1	105.66	105.66	1.4351	0.2569	9833	0.2569
Fo	1	20.49	20.49	0.0239	0.6613	6	0.9030
SixFo	1	855.50	855.50	11.6200	0.0022	9822	0.0055
Pl(SixFo)	12	885.85	73.82	3.2904	0.0020	9925	0.0021
Res	47	1054.50	22.44				
Total	62	2938.70					

3.1.6 Effects on gorgonian forest on species diversity

A careful analysis was performed on diversity indices calculated on the percent cover data. The first graph (Fig. 51) reports variation among the total percent cover: a difference between sites is clearly visible (Table 38).

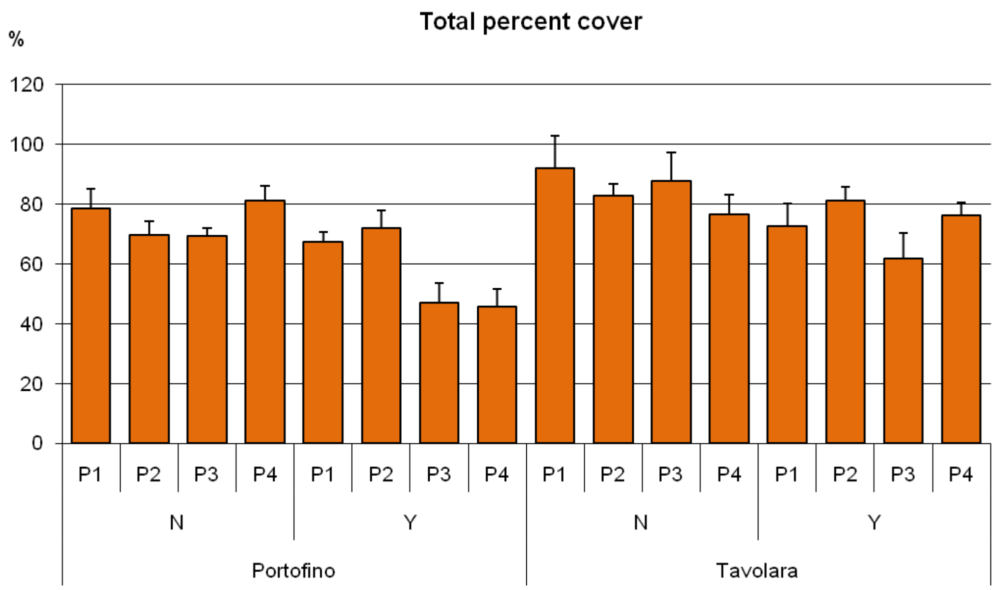


Fig. 51 – Total percent cover (±se).

Table 38 – PERMANOVA results for total percent cover

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
Si	1	39180	39180.0	7.6030	0.0215	9826	0.0190
Fo	1	51731	51731.0	35.6470	0.4928	6	0.1021
SixFo	1	1451	1451.2	0.2816	0.6020	9814	0.6118
Pl(SixFo)	12	61954	5162.9	1.9768	0.0514	9939	0.0482
Res	47	122750	2611.8				
Total	62	269750					

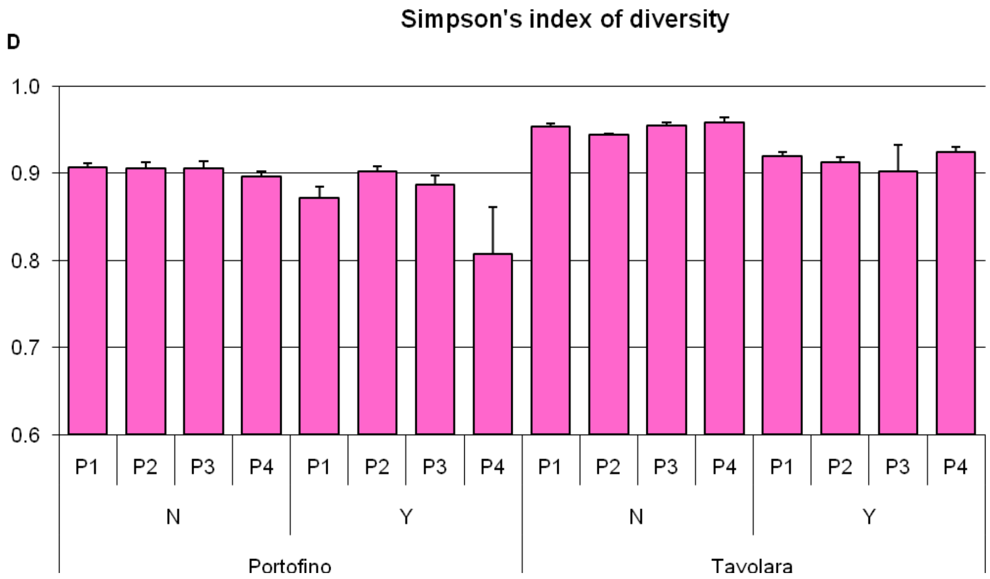


Fig. 52 – Simpson diversity index (±se).

The statistic analysis on Simpson's index shows high heterogeneity among plots and strong difference between sites. Moreover, there was a significant effect of the *Paramuricea clavata* forest presence that reduced the overall diversity expressed by this index (Table 39).

Table 39 – PERMANOVA results for Simpson's index

Source	Df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
Si	1	3.67E-02	3.67E-02	23.001	0.0001	9828	0.0006
Fo	1	2.16E-02	2.16E-02	3894.900	0.1596	6	**0.0100**
SixFo	1	5.55E-06	5.55E-06	0.003	0.9615	9869	0.9523
Pl(SixFo)	12	1.92E-02	1.60E-03	2.075	0.0325	9934	0.0333
Res	47	3.62E-02	7.71E-04				
Total	62	1.08E-01					

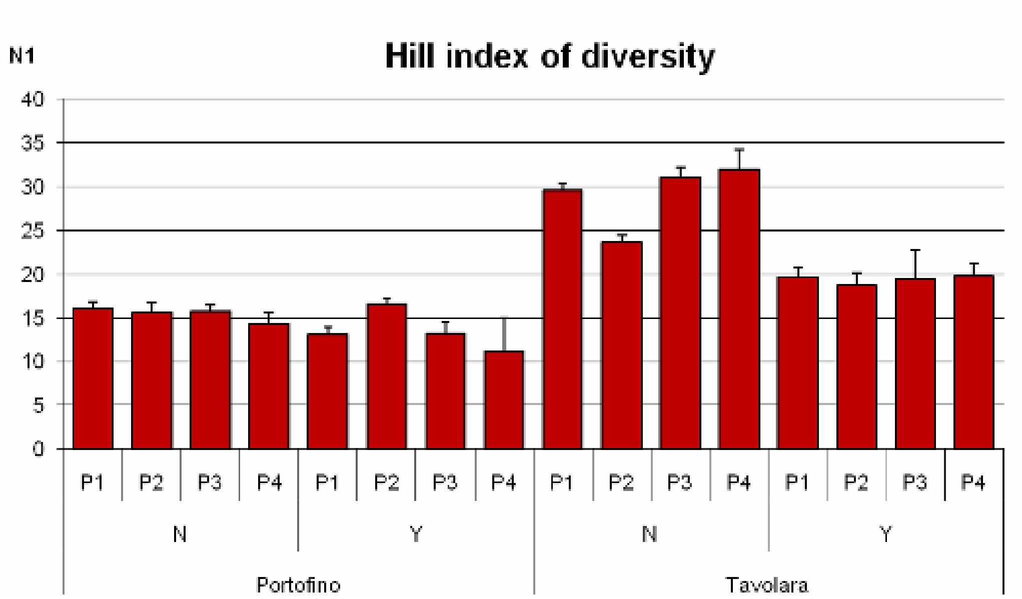


Fig. 53 – Hill diversity index (±se).

The species heterogeneity, expressed by the Hill's N_1 index, was significantly lowered by the presence of gorgonians at Tavolara (Table 40 and Fig. 53).

Table 40 – PERMANOVA results for N_1 index

Source	df	SS	MS	Pseudo-F	P(perm)	Perms	P(MC)
Si	1	1487.80	1487.80	78.4120	0.0001	9842	0.0001
Fo	1	524.67	524.67	2.2444	0.3311	8	0.3789
SixFo	1	233.77	233.77	12.3210	0.0075	9833	0.0050
Pl(SixFo)	12	228.10	19.01	1.9245	0.0505	9917	0.0556
Res	47	464.22	9.88				
Total	62	2923.60					

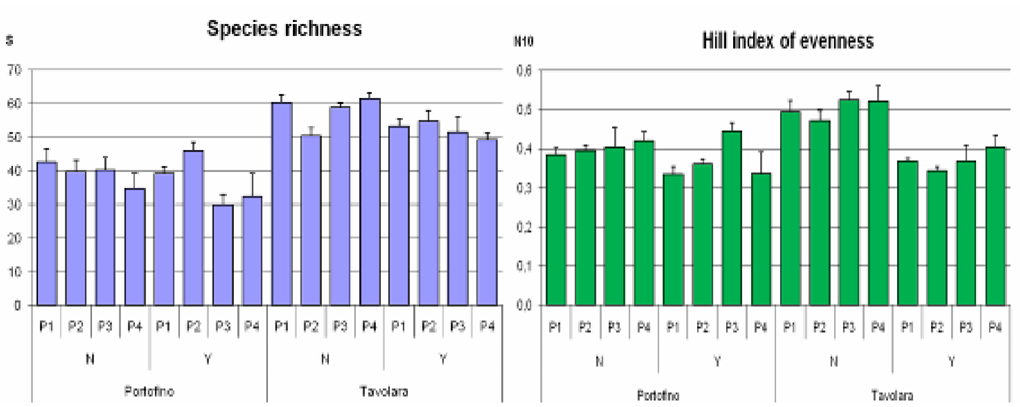


Fig. 54 – Species richness and Hill evenness index (±se).

Species richness (Fig. 54 on the left) showed significant difference among plots and between sites it was not affected by gorgonian forests (Table 41), while N_{10} (Fig. 54 on the right) has the same pattern of N_1 index showing significant interaction Site x Forest due to a reduction of the mean evenness in forested panels at Tavolara (Table 42).

Table 41 – PERMANOVA results for Species richness

Source	df	SS	MS	Pseudo-F	P(perm)	Perms	P(MC)
Si	1	4474.30	4474.30	49.0020	0.0002	9819	0.0001
Fo	1	253.39	253.39	6.2825	0.3316	6	0.2505
SixFo	1	40.33	40.33	0.4417	0.5182	9831	0.5268
Pl(SixFo)	12	1098.00	91.50	2.2747	0.0251	9937	0.0212
Res	47	1890.70	40.23				
Total	62	7672.90					

Table 42 – PERMANOVA results for N_{10}

Source	df	SS	MS	Pseudo-F	P(perm)	Perms	P(MC)
Si	1	4.26E-02	4.26E-02	10.4960	0.010	9838	0.0069
Fo	1	1.06E-01	1.06E-01	2.6844	0.332	7	0.3558
SixFo	1	3.94E-02	3.94E-02	9.7189	0.006	9857	0.0086
Pl(SixFo)	12	4.87E-02	4.06E-03	1.3111	0.251	9937	0.2465
Res	47	1.46E-01	3.10E-03				
Total	62	3.82E-01					

A summarizing table with results obtained by the univariate analysis on diversity indices is shown below (Table 43).

Table 43 – Summary of PERMANOVA test on diversity indices

Index	**Level**				**Pairwise test**			
					Tavolara		**Portofino**	
	Si	**Fo**	**SixFo**	**Pl(SixFo)**	**P**	**Effect**	**P**	**Effect**
S	**0.0002**	0.2505	0.5182	**0.0001**				
N	**0.0215**	0.1021	0.6020	**0.0018**				
D	**0.0001**	**0.0100**	0.9615	**0.0325**				
N1	**0.0001**	0.3789	**0.0075**	**0.0001**	**0.0025**	N >> Y	0.1238	
N10	**0.0100**	0.3558	**0.0064**	**0.0020**	**0.0007**	N >> Y	0.2733	

As it is possible to see difference among plots and between sites is confirmed for every index calculated. Simpson's index highlight a very significant effect of the factor Forest and in this case of Hill's indices differences are pointed out only at Tavolara.

Chapter 4: Discussion

The first result that catches the eye, observing the recruitment panels, is the separation among sites in terms of assemblages structure: this difference is strongly highlighted by the PCO ordination plot (see Fig. 21) and by the analyses carried out both on assemblages and abundances of single taxa. This discrepancy is quite visible also from the species diversity indices that shows higher values at Tavolara. Since the experimental plots were placed to similar depth with the same orientation and inclination, to avoid further addiction of variability, this difference is probably due to different environmental conditions of the chosen sites:

- while Tavolara is an island quite away from the coast, in a sparsely populated area with little fluvial input, Portofino is a promontory surrounded by small towns and several rivers;
- according to TRIX values the quality of the water of sites considered belong to two different categories: elevated status and good status (ICRAM 2000);
- in Portofino clarity of the water is influenced by rivers intake and by algal activity that in some periods of the year leads to colouring and turbidity phenomena (ICRAM 2000), moreover the site chosen for the experiment is characterized by a high sedimentation rate (Cerrano et al. 2005). Sardinia waters instead are particularly clear and dominated by a general oligotrophic condition (ICRAM 2000);

Despite the large differences between sites, the gorgonian forest significantly affected the recruited assemblages at both sites, even if in a different way in terms of both intensity and direction. This can be understood by analyzing the effects of the gorgonian forest on the recruitment of individual species and single ecological or taxonomic groups.

The taxonomic identification of species requires specialist skills and long time. An optimal level of identification was reached for polychaetes and cnidarians, while for many other taxa the analyses by specialists are still ongoing. This unfortunately limits, at least in part, the interpretability of results.

On overall, the presence of *Paramuricea clavata* reduced the settlement and recruitment of several algae, and this was more evident at Tavolara site, where in general these taxa were more abundant. The shadow caused by the gorgonian might reduce the availability of light and therefore the growth of some algae. The effect of shading might be greater in places where the waters are on average more clear, as occurs at Tavolara. Conversely, at Portofino the shadowing effect of gorgonian forest is less visible and could be masked by the high turbidity of the water.

The recruitment of Chlorophyta sp. 2 was negatively affected by the gorgonian forest at both sites, but with different intensity. Phaeophyta sp. 1 showed contrasting results, it was significantly reduced by the presence of gorgonian at Portofino and relatively favoured at Tavolara. As an exception, Chlorophyta sp. 1 seems to be facilitated by gorgonian presence at Tavolara.

The hydrozoans *Clytia linearis* was more abundant at Portofino, but despite this, its presence was facilitated on forested vs. non-forested panels at Tavolara. It is known that hydroids are influenced by environmental abiotic factors such as irradiance, sedimentation and water flow (Gili & Huges 1995). Abundances patterns of this species could support the shadowing hypothesis: in Portofino with high turbidity of the water *Clytia linearis* could grow better than in Tavolara, but the significant result is obtained right there, where the clarity of the water is higher and the gorgonian forest effect is not masked by turbidity. Moreover since in Tavolara water flow is higher, *Clytia linearis* can be facilitated by the gorgonian forest effect of mitigating current.

The serpulid polychaete *Janua* (*Dexiospira*) *pagenstecheri* was found only at Tavolara, where its abundance was lowered by the presence of the gorgonian forest. Similar results were obtained analysing the sum of all calcareous tube worms and all the Annelida. Analogue result was also found in a similar experiment carried out by Scinto et al. (2009): after four months it was evident a higher serpulids settlement outside gorgonian forest. This distribution pattern could be due to alimentary competition since both gorgonian and most of the sessile polychaetes found are suspension feeders.

Anomia ephippium is the unique mollusc founded on the panels examined. Its presence seems not to be affected by gorgonian, but mostly by differences between sites: in Portofino, where the concentration of suspended particles are high (Cerrano et al. 2005), this species could find nutritional condition more favourable.

The effect of sea fan forests on forams, if evaluated on the singular species, seems to be not significant, but all together, this group is clearly negative affected: abundances are lower where gorgonian are present. To explain this pattern we can make two main different hypotheses related to habitat preferences or food competition. In the first case we know that benthic foraminifera prefer different types of bottoms (rocky, sandy or algal substrate) thus their presence could be simply connected with those of algae, more abundant on non-forested panels. On the other hand, some benthic foraminifera have small chamberlets which contain symbiotic algae that provide them energy (Boudagher-Fadel 2008) and the explanation for their successful recruitment outside gorgonian forest could be related to the hypothesis of shadowing, formulated for algae. Another motivation could be associated to sediments: even if it is known that gorgonian three-dimensional structure modifies and reduces water flow enhancing sedimentation on a micro scale (Gili & Coma 1998), we can hypothesize that forams presence is higher on non-forested plots where the higher abundance of algae can act as sediment trap, as observed during laboratory analyses. There could be also alimentary competition, since gorgonian particles retention may prevail on that of forams. Anyhow, knowing exactly which species they are could help to find the reasons for their distribution patterns.

Even if with different abundances between sites, Bryozoa sp. 2 was the only taxa unequivocally favoured by the presence of *Paramuricea clavata*. This result could be in agreement with that found by Scinto et al. (2009).

We found opposite results as regards the group of encrusting sponges, since their presence is enhanced outside gorgonian forests.

The Simpson index is considered one of the most meaningful and robust diversity measures, despite it is heavily weighted towards the most abundance species in the sample and is less sensitive to species richness. Its analysis confirmed the presence of differences in the species diversity among plots and between sites respectively due to natural high variability of the coralligenous system and to obviously different types of environment in the sites considered.

Moreover, according to this index, species diversity was significantly reduced by the presence of *Paramuricea clavata* forest on the experimental panels at both sites. This seems in contrast with what we expected, but this result may be influenced by the large algal component on non-forested panels, as also observed by Scinto et al. (2009).

The other diversity indices showed significant effect of the gorgonian forests only in one site (Tavolara). The strong difference among sites is determined by species richness that has higher values at Tavolara. The reduction of species diversity (Hill's N_1 index) due to the presence of gorgonians appeared related to a worst evenness (Hill's N_{10} index) rather than to less species richness (S).

In light of these results, can we consider *Paramuricea clavata* as an ecosystem engineer (for definition see paragraph 1.3.1)?

Among the characteristics of the engineering species described by Hastings et al. (2007), *Paramuricea clavata* present the following:

- It is a long-living organisms producing three-dimensional hard skeletons, such gorgonians characterised by a lifespan that can exceed a full century (Roark et al. 2006). It can play an important role as habitat-modifiers creating elevated and complex tertiary structures that can alter physical condition of the surrounding habitat. In this way they control local microclimate creating a sort of "buffer zone" where environmental conditions change less and slower respect to the surrounding ambient (Cerrano et al. 2010). With our experiment, we have substantially modified the habitat structure, altering physical conditions and adding three-dimensionality and complexity at the environment.
- Several organisms can find shelter from predators and stress in this patch with peculiar physico-chemical features that can increase their fitness. *Paramuricea clavata* reduces water flow stabilizing "understorey" habitat for a diversified assemblage (Gili & Coma 1998), moreover seems that this species could promote or disadvantage settlement and recruitment of coralline algae, sponges and erected bryozoans (Scinto et al. 2009). Apart from all the fauna that can be found on the red sea fan (Fig. 55) or within its facies, our results

highlight that *Paramuricea clavata* acts on species abundances patterns inhibiting the growth of some species (i.e. algal component) and facilitating the presence of others.

Fig. 55 - *Pseudosimnia carnea* and *Astrospartus mediterraneus* on *Paramuricea clavata* branches (by courtesy of Egidio Trainito).

- The patches with *Paramuricea clavata* can be more or less connected with others (engineered vs. unengineered patches) influencing in this way genetic variability of associated populations (Hastings et al. 2007).
- Sea fans forests modify the benthic trophic status and the ecosystem functioning since they play a significant role in the energy transfer processes between planktonic and benthic communities (Mistri & Ceccherelli 1994). Our study pointed out how the possible "umbrella" effect, of *P. clavata* on irradiance, can shift an autotrophic system based on light and algal production into a heterotrophic system, based on filter feeders and nourishment carried by the water column.
- A loss or a reduction of *P. clavata* colonies alter significantly the seascape of rocky bottoms (Cerrano & Bavestrello 2008) and could lead to a shift from a hard coralline dominance to a fleshy green algae (Scinto et al. 2009) reducing β species diversity and affecting the functioning of the entire community (Sala & Knowlton 2006).

For all these reasons we can consider gorgonian forests as a habitat forming species, definition usually used for habitats formed by kelp forests, seagrasses or macroalgae canopies, sponges or bivalve reefs (Bruno & Bertness 2001).

Could be useful repeat this experiment in more than two sites in order to identify which are the environment factors that acts in synergy with gorgonian forests and to evaluate thoroughly results found until now. Prolong the time of the panels exposition to the sea environment could let us understand better the system shifts due to gorgonian forests and which is their effect on species succession mechanisms. Further studies should be useful in order to understand if *Paramuricea clavata* forests can acts as genetic corridors and influence genetic patterns of the coralligenous assemblages. Moreover, it would be curious to implement new experiments in order to assess whether the effect is determined by the presence of gorgonian as modifier of the physic environmental conditions or if there is some biological implication such as the release of chemical substances that influence the surrounding assemblages.

Paramuricea clavata forests can significantly modify system mechanisms on a small scale and could have a stabilizing effect on the coralligenous assemblages. Which are their effects on a wider scale? Can this species influence the underwater landscape ecology? Ultimately, which changes might be the result from its loss?

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