2. Phytobenthonic Population

Research unit

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Introduction

The Ridge of Santa Croce, characterised by a costal detritic substrate, has a rich sea bottom of residue of *Cladocora caespitosa*, tubes of serpulids, encrusting calcareous algae. After the laying of the artificial reefs, the macroalgal component has colonized the structures by integrating them, as found from the first observations during the first underwater samplings. Particularly, the soft thallus macroalgal colonization grows from the upper part of the artificial structures, where the macroalgae occupy almost the entire space available towards the lower part, while the calcareous algal colonization seems to move in the opposite direction. These calcareous algae, which characterized the sea bottom before the laying of the artificial reefs, result as the predominant structure and make a photosynthetically active layer which covers all the colonisable structures present on the site.
These encrusting calcareous algae generally occupy most of the primary substrate part available for the marine benthic community in the photic zone and although they grow very slowly, their presence and capability to deposit the calcium carbonate makes them very important in terms of production of organic and inorganic substance (Garrabou *et al*., 2000).

Objectives

In order to carry out a research which integrates the different aspects of the assessment of the biodiversity on the artificial reefs on the Ridge of Santa Croce, the experimental design is aimed to the study:

1. of the biological diversity through the qualitative analysis of the algal component which, being sessile, highlights the variations of chemical-physical parameters and can be considered a sensor-bioindicator. In particular, behaving as an integrator of the variations which take place in the surrounding environment, shows, through the population structure, the ecological conditions of the environment and it may be considered as a good

tool to assess the marine environment condition (according to the DIRECTIVE 2000/60/CE);

2. of encrusting calcareous algae, belonging to the Corallinales family which seem to be predominant on the sea bottom. They may be considered precious biological sensor-indicators able to supply useful information on the surrounding environment (Bressan & Babbini, 2003) due to their relatively long life-histories. Thus, the variation in the development of thalli (ecological approach) and their primary production (physiological approach, R.U. Talarico, Frisenda) may be a useful tool to assess the state of the environment and the potential primary productivity of the site itself.

1. Biological Diversity

From the very first observations carried out by means of Scuba diving, done starting from July 2004, it has been possible to identify a plentiful gradient of the algal component which grows from West to East.

Particularly, the pyramids located mainly in the Western part (D1, D4) show a rich animal component made of filterers (sea-squirts, sponges, mussels, oysters). The algal turf is present in patches and it is very reduced.

The central pyramids (D2, D5) present a similar animal component, but the vegetal component seems to be present as a continuous turf.

At the bottom of the D5 pyramid the *Cymodocea nodosa* was found only once, in summer 2004.

The pyramids in the East part (D3, D6) have an algal cover of some centimetres. Particularly the D3 pyramid differs from the others for the presence of a continuous algal turf about 4cm thick, probably due to the presence of a net, stretched over the structure in the autumn 2000, rich of thalli.

The calcareous algae are present on all the pyramids, colonizing both the cement layer and the mussels valves as well as the oysters present; on the antitrawling blocks, on the other hand, they greatly colonize the PVC part, while the macroalgae result being very scarce. Similarly, the situation noted on the TECNOREEF.

The wreck *"Quieto"* pontoon revealed, on the upper wall (the cabin's roof), a carpet of green algae measuring a couple of centimetres. The sedimentation does not represent an important environmental element, probably due to the current. The vertical walls seem to be colonized by red algae. Many calcareous algae cover the iron structure and the mussels valves.

The F.A.D. placed facing West, shows an important colonization from mussels, while only some filamentous red thalli and green algae.

The F.A.D placed facing East is so rich of mussels that in the central part the net is broken, the 0.5cm algal turf has colonized some areas on the upper part while the calcareous algae have colonized extensively the shells present.

Methodological Notes

Cataloguing and inventorying the species present may be a useful suggestion to study the biodiversity of the site. Subsequently, samplings were seasonally carried out during the whole year (summer 2004, autumn 2004, winter 2005, spring 2005) by scuba diving. The sampling of the algal material has been conducted through herborization and scratching of significant areas on the whole pyramid D3, considered the most interesting from the floristic point of view.

In March and June 2005 the samples collected for the physiological investigation (R.U. Talarico, Frisenda) were saved in jars closed in black nylon bags to prevent the light, preserved in suitable portable fridges and taken rapidly to the Physiology Laboratory of the Biology Department.

The samples collected for the qualitative analysis have been fixed in 4% of formaldehyde in seawater, kept in the dark, stored in the Biology Department for further investigation. A stereo microscope Wild Heerbrug (mod. 376041 Plan 1x) has been used for the macroscopic determination, whereas an optical microscope Nikon Labophot has been used for the microscopic identification. For the taxonomical identification of the algae belonging to the Corallinales family, it was necessary to use the Scanning Electron Microscope (Leica Stereoscan 430i). The specialized literature used for the recognition of the species has different origin: English (Dixon *et al.*, 1977; AA.VV., 1980; Kapraun, 1980; Irvine, 1983; Hiscock, 1986; Burrows, 1991; Maggs, 1993; Desikachary *et al.*, 1998); French (Feldmann-Mazoyer, 1977; Coppejans, 1983; Cabioch *et al.*,1992; Coppejans, 1995); Spanish (Carillo *et al.*,1999) and Italian (Giaccone, 1973; Bressan *et al.*, 2003; Sartoni *et al.*, 2004).

Results

Floristic surveys

From the qualitative survey of samples 72 algal taxa have been found. They belong 75% to Rhodophyceae (54 taxa), 14% to Phaeophyceae (10 taxa) and 11% to Chlorophyceae (8 taxa) (Fig. 2.1).

The seasonal floristic spectrum reveals some fluctuations in its percentage values compared to those recorded for the annual spectrum (Fig. 2.2), which are found to be minimum for the Phaeophyceae, while for the Chlorophyceae and the Rhodophyceae the percentage variations are higher.

Analysing the numerical presence of the three algal groups (Fig. 2.3), it can be noticed that:
Rhodophyceae varied in the course of the year and showed higher values in November;
Phaephyceae are numerically lower in November, while we noticed an increase starting from February until May, where the presence recorded was higher;
Chlorophyceae showed some fluctuations peaking in presence in February.

Floristic list
For the identified species the phytogeographic element is indicated (Furnari *et al.*,1999) as well as the ecology of the species (Bouderesque, 1984). The classification used follows the indications present in Silva *et al.*,1996, the nomenclature has been updated according to Furnari *et al.*, 2003.

LIST
Rhodophyceae
Bangiophycidae
Porphyridiales
Porphyridiaceae
Chroodactylon ornatum (C. Agardh) Basson
C / -
Erythropeltidales
Erythrotrichiaceae
Erythrotrichia carnea (Dillwyn) J. Agardh
C / -
Florideophycidae
Gelidiales
Gelidiaceae
Gelidium pusillum (Stackhouse) Le Jolis
C/Infralittoral, subject to strong hydrodynamism, photophilous
Gracilariales
Gracilariaceae
Gracilaria bursa-pastoris (S.G. Gmelin) P.C. Silva
SC / Upper Infralittoral
Bonnemaisoniales
Bonnemaisoniaceae
Asparagopsis armata Harvey (solo tetrasporofito)
C / Infralittoral sciophilous

Cryptonemiales
Halymenaceae
Halymenia floresia (Clemente) C.Agardh
SC / Coralligenous concretion
Peyssonneliaceae
Peyssonnelia squamaria (S.G. Gmelin) Decaisne
M / Infralittoral usually relatively calm, sciophilous
Corallinales
Corallinaceae
Jania rubens (Linnaeus) J.V. Lamouroux var. ***rubens***
C / Infralittoral usually relatively calm, sciophilous
Hydrolithon boreale (Foslie) Chamberlain
C / Infralittoral
Hydrolithon cruciatum (Bressan) Chamberlain
- / Inter- Infralittoral
Hydrolithon farinosum (Lamouroux) Penrose & Chamberlain
C / Upper limit of the circalittoral
Lithophyllum incrustans Philippi
AB / Inter- Infralittoral
Lithothamnion minervae Basso
M / Circalittoral
Pneophyllum fragile Kützing
C / Inter- Infralittoral
Titanoderma pustulatum (Lamouroux) Näegeli ***var. canellatum***
- / Infralittoral
Gigartinales
Cystocloniaceae
Rhodophyllis divaricata (Stackhouse) Papenfuss
AB / Upper Infralittoral, intense hydrodynamism, sciophilous
Gigartinaceae
Chondracanthus acicularis (Roth) Fredericq
C / Inter- Infralittoral, portual
Rhodymeniales
Champiaceae
Chylocladia verticillata (Lightfoot) Bliding
At / Infralittoral, poor hydrodynamism, photophilous
Lomentariaceae
Lomentaria clavellosa Gaillon

IAct / Infralittoral sciophilous
Lomentaria verticillata Funk
M / -
Rhodymeniaceae
Rhodymenia pseudopalmata (J.V. Lamouroux) P. C. Silva
ABt / -
Ceramiales
Ceramiaceae
Aglaothamnion tenuissimum (Bonnemaison) Feldmann-Mazoyer var. ***mazoyerae*** G.Furnari, L'Hardy-Halos, Rueness & Serio
M / Poor hydrodynamism environment, sciophilous
Aglaothamnion tripinnatum (C.Agardh) Feldmann-Mazoyer
IA / Poor hydrodynamism Infralittoral, sciophilous
Anotrichium furcellatum (J.Agardh) Baldock
APct / Poor hydrodynamism Infralittoral, sciophilous
Antithamnion cruciatum (C.Agardh) Nägeli
IA / Infralittoral
Antithamnion heterocladum Funk
M / -
Antithamnion tenuissimum (Hauck) Schiffner
M / Corraligenous concretion
Ceramium comptum Børgesen
IA / -
Ceramium diaphanum (Lightfoot) Roth
SC / Upper Infralittoral
Ceramium flaccidum (Kützing) Ardissone
C / Infralittoral
Ceramium tenerrimum (G. Martens) Okamura
SC / Ubiquist
Compsothamnion thuyoides (J.E. Smith) Nägeli
ABt / Small port's, sciophilous
Lejolisia mediterranea Bornet
P / -
Monosporus pedicellatus (J.E.Smith) Solier var. ***pedicellatus***
AB / Poor hydrodynamism Infralittoral, sciophilous
Pleonosporium borreri (J.E. Smith) Nägeli
IA / Infralittoral, sciophilous
Pterothamnion crispum (Ducluzeau) Nägeli

SC / Poor hydrodynamism Infralittoral, sciophilous
Pterothamnion plumula (J.Ellis) Nägeli
SC / Infralittoral, Poor hydrodynamism Infralittoral, sciophilous
Seirospora sphaerospora Feldmann
M /Type of environment with poor hydrodynamism sciophilous
Spermothamnion strictum (C. Agardh) Ardissone
A / -

Dasyaceae
Dasya hutchinsiae Harvey
AB / Upper Infralittoral
Dasya ocellata (Grateloup) Harvey cfr.
IA / Infra-circalittoral, sciophilous
Dasya rigidula (Kützing) Ardissone
ABt / Inter-Infralittoral
Heterosiphonia crispella (C. Agardh) M. J. Wynne
IA / -

Delesseriaceae
Nitophyllum punctatum (Stackouse) Greville
IA / photphile Infralittoral, found in ports
Radicilingua reptans (Kylin) Papenfuss
AB / Infra-circalittoral, sciophilous
Radicilingua thysanorhizans (Holmes) Papenfuss
AB / environment with poor hydrodynamism sciophilous

Rhodomelaceae
Lophosiphonia reptabunda (Suhr) Kylin
IA /Upper infralittoral
Polysiphonia denudata (Dillwyn) Sprengel
SC / -
Polysiphonia elongata (Hudson) Sprengel
IA / / Corraligenous concretion
Polysiphonia furcellata (C.Agardh) Harvey
Apct / Inter-Infralittoral
Polysiphonia sanguinea (C. Agardh) Zanardini)
AB / -
Polysiphonia stricta (Dillwyn) Greville
CB / -
Polysiphonia subulata (Ducluzeau) P.& H. Crouan
AB / -

Pterosiphonia pennata (C.Agardh) Sauvageau
SC / Inter-Infralittoral, High hydrodynamism, sciophilous

Phaeophyceae
Ectocarpales
Ectocarpaceae
Ectocarpus siliculosus (Dillwyn) Lyngbye
C / photophilous Infralittoral
Hincksia sandriana (Zanardini) P.C. Silva
SC / Poor hydrodynamism Infralittoral, sciophilous
Sphacelariales
Sphacelariaceae
Sphacelaria cirrosa (Roth) C.Agardh
SC / / photophilous Infralittoral
Sphacelaria plumula Zanardini
AB / Corraligenous concretion
Sphacelaria tribuloides Meneghini
C / Infralittoral, photophilous, thermophile
Stypocaulaceae
Stypocaulon scoparium (Linnaeus) Kützing
SC / Poor hydrodynamism Infralittoral, photophilous
Dictyotales
Dictyotaceae
Dictyota dichotoma (Hudson) J.V. Lamouroux var. ***dichotoma***
C / Poor hydrodynamism Infralittoral, photophilous
Cutleriales
Cutleriaceae
Cutleria multifida (J.E. Smith) Greville
SC / Port environment Infralittoral, photophilous
Cutleria chilosa (Falkenberg) P. C. Silva
M / -
Zanardinia typus (Nardo) G.Furnari **comb.nov.**
AP / Poor hydrodynamism Infralittoral, sciophilous

Chlorophyceae
Cladophorales
Cladophoraceae
Cladophora coelotrix Kützing
IA / -

Cladophora dalmatica Kützing
IA / Intertidal
Cladophora echinus (Biasoletto) Kützing
IA / Poor hydrodynamism Infralittoral, photophilous
Cladophora laetivirens (Dillwyn) Kützing
Sc/ Intertidal
Cladophora prolifera (Roth) Kützing
IA / Infralittoral, sciophilous

Valoniaceae

Valonia utricularis (Roth) C.Agardh
P / Infralittoral, high hydrodynamism, sciophilous

Bryopsidales

Bryopsidaceae

Bryopsis plumosa (Hudson) C.Agardh
AP / / Port environment Infralittoral, photophilous

Codiaceae

Codium vermilara (Olivi) Delle Chiaje
At / Poor hydrodynamism Infralittoral, sciophilous

Legend of Phitogeofraphic Elements (FURNARI *et al*., 1999)
C= Cosmopolitan (C: Cosmopolitan – SC: Sub-cosmopolitan)
A= Atlantic (A: Atlantic – AB:Boreo-Atlantic – ABt: Boreo-tropical Atlantic – At: tropical Atlantic)
IA= Indo-Atlantic (IA: Indo-Atlantic – IAtc: Indo-Atlantic cold temperature – IAt: Indo-Atlantic tropicals)
M= Mediterranean
AP= Atlantic-Pacific (AP: Atlantic-Pacific, APtc: Atlantic-Pacific cold temperatures)
P= Pan-tropical
CBA= Circumboreo-austral (CBA:Circumboreo-austral, CB: Circumboreal)

Reproduction

Of the 72 algal species found in the present study, 34% have been found in reproductive stage at least once (Fig. 2.4).

The highest concentration of the species in reproduction has been recorded in May, according to the seasonal characteristics of the red algae life cycle. A decrease of species found reproducing was recorded in the other months, with an absolute minimum in February. However, it has been noticed a seasonal trend, with an autumn-winter vegetative stage and a spring-summer reproductive stage.

Spatial distribution

The pyramid analysed was divided in three bathymetrical levels during the samplings:

A: horizontal surface of the top cube, place at 8 metres deep;

B: vertical surface of the top cube, positioned at 8-10 metres deep;

C: horizontal and vertical surface of the 4 lower cubes which make the base for the artificial pyramid reef, placed at 10-12 metres deep.

As shown in Fig. 2.5 the red algae are mainly present on level A, decreasing on level B and increasing once again on level C. A similar trend is recorded for the green algae, while the brown algae show a decrease from the most superficial level to the deepest. The growth on the vertical surfaces seemed critical for the Rhodophyceae and the Chlorophyceae, this trend confirms the green and red algae affinity for the colonization of the horizontal surfaces.

Phytogeographic Elements

The algal flora has been divided in different groups, known as phytogeographic elements.

The groups taken into consideration, together with the percentages compared to the floristic list of the Ridge of Santa Croce, are:

C (37.1%): cosmopolitan species, whose distribution areas extend on the major part of seas.

A (21.4%): Atlantic species, whose elements come from the Atlantic Ocean,

IA (20.0%): Indo-Atlantic species, whose distribution area expands to the Indian and Atlantic Ocean.

M (11.4%): Mediterranean species, whose elements are limited to the Mediterranean (endemisms),

AP (5.7%): Pacific-Atlantic species, whose elements are present both along the North-American coasts, and along the Northern Pacific coasts.

P (2.9%): pantropical species, whose elements are found in the area between the two tropics.

CBA (1.4%): circumboreal-austral species, whose elements are spread in the tropics of the boreal hemisphere.

The Cosmopolitan species have been found to be predominant (37.1%), followed by the Atlantic and Indo-Atlantic species; the Mediterranean species have been found to be present with a lower percentage compared to the previous ones, but higher compared to the Atlantic-Pacific, Pantropical and Circumboreal-Australis related species, the latter being present in a low percentage (one species only).

Discussion and Conclusions

The Atlantic component, including the Atlantic and Atlantic-Pacific related species, make 27.1% of the taxa found until today (*Lithophyllum incrustans*, *Rhodophyllis divaricata*, *Chylocladia verticillata*, *Rhodymenia pseudopalmata*, *Anotrichium furcellatum*, *Compsothamnion thuyoides*, *Monosporus pedicellatus*, *Spermothamnion strictum*, *Dasya hutchinsiae*, *Dasya rigidula*, *radicilingua reptans*, *Radicilingua thysanorhizans*, *Polysiphonia furcellata*, *Polysiphonia sanguinea*, *Polysiphonia subulata*, *Sphacedlaria plumula*, *Zanardinia typus*, *Bryopsis plumose*, *Codium vermilara*) confirming the mild-cold characteristics of the site under study. 11.4% is constituted by the Mediterranean component, whose result agrees with those of the latest studies (Falace 2001 in Falace *et al*., 2005) which record a value of 10.7% for the Gulf of Trieste. The Mediterranean related species are given by *Peyssonnelia squamaria*, *Lithothamnion minervae*, *Lomentaria verticillata*, *Aglaothamnion tenuissimum*, *Antithamnion heterocladum*, *Antithamnion tenuissimum*, *Seirospora sphaerospora* and *Cutleria chilosa*. Particularly, *L.minervae*, species typical of the *mäerl* in the Mediterranean, is broadly present on the sea bottom of the Ridge of Santa Croce showing that this bottom offers environmental and phytocoenosis conditions comparable to those of other biogeographical sectors of the Mediterranean (Bressan & Babbini, 2003). The physical complexity of the *mäerl* habitat and its long persistence in time (species that form the *mäerl* grow very slowly, taking centuries or millenniums to accumulate on the sea bottom) makes these substrates suitable for the life of a great variety of animals and vegetals. Furthermore, the facies of the *mäerl* belonging to the Coastal Detritic biocoenosis are not common nor well characterised in the Italian seas: compared to the Atlantic *mäerl* they show a significant biological diversity which increases naturalistic value; they are, therefore, subject to control measures and considered amongst the benthonic habitats worthy safeguarding from the Habitat Directive of the European Union, in order to evaluate the extension and the biodiversity, as well as classified as remarkable in the SPAMI list (Barcelona Agreement, 10/06/95).

The red algae belonging to the Ceramiales family, characterised by opportunistic species which occupy niches that are found to be empty or half empty (Giaccone & Di Martino, 1997), revealed to be qualitatively and quantitatively prevailing, constituting 45.9% of the total species collected. This presence is justified also by the strong sedimentation which in some periods of the year characterizes the site.

Asparagopsis armata is noteworthy to report, species introduced in the Mediterranean from Australia and from New Zealand, naturalised on the European

coasts since 1925, showing a very broad diffusion as it is listed amongst the species of the Mediterranean vegetation. Only the tetrasporophyte was found.
Amongst the Chlorophyceae *Bryopsis plumosa* was found in significant quantities in June 2005 at all bathymetric levels. This species is found in very little lit environments and with a relative calm hydrodynamic.
Compared to the "biological shapes", it was noticed how the epibiontic thalli resulted to be predominant in the upper surface part (the horizontal surface of the pyramids is occupied by a turf more or less interrupted of green algae, with red algae as epiphytes), epilithic (calcareous algae in particular) in the deepest part.
The "statistical ecological groups", valid for the Western Mediterranean, Northern sector, present in this study refer essentially to the photophilous and sciophilous Infralittoral, even if for some species some certain information is missing.
It can, however, be noted that the component of the Infralittoral is important, constituted by photophilic species belonging to the Chlorophyceae and Phaeophyceae class, found on the upper part of the artificial reef, and by sciophilous species belonging mainly to the Rhodopyceae class and collected on the entire structure.
It must be borne in mind however, that the "soft" component of the macrophytobenthos feels the effects of the incidence of the climatic factors (seasonality) greatly, making discontinuous and immature every type of cover and recovering of the artificial reefs, even though the floristic wealth becomes interesting if compared with that on another protected area, but in-shore, the Natural Marine Reserve of Miramare, where the species found during a whole year long of samplings were 116 (Di Pascoli, 2003).

2. Corallinales

2.1 Cultures

Methodological notes

In order to evaluate the productivity (effective and potential) in natural environment (Ridge of Santa Croce) a first experimental design has started using epilithic algae collected in an in-shore location (Sistiana, Gulf of Trieste) with hydrological characteristics (important supply of fresh water) similar to the off-shore site (Ridge).
For the realisation of cultures in "non natural" environment, two different methodologies have been followed: spontaneous spore germination and induced spore germination.

The spontaneous spore germination was carried out placing some collectors (Bressan & Comelli, 1975), each with 10 slides (Menzel-Glaser 76x26mm), in a tank of the Trieste Aquarium, where encrusting calcareous algae were already present on the walls. The aquarium is equipped with an open-cycle system directly connected to the sea (T=15∞C, L:D=11:13; PAR incident=10mmol $m^{-2}s^{-1}$) (steam lights Hg80W). Every ten days, some slides were taken and brought (in the dark and at 10∞C) to the laboratory for the physiological analysis.

The induced spore germination was carried out directly in the laboratory, preparing a tailor-made tank in a thermostatic culture cell, at the Biology Department of the University of Trieste. It is a main 200 litre tank, for the collecting and distributing of the water, connected to four tanks (10 litres each) cascade placed (Fig. 2.6). The water flux is supplied by a tank pump Askoll Pratiko. The conditions of the cell were: T=15∞C, L:D=10:14; PAR from 26mmol $m^{-2}s^{-1}$ to 4mmol $m^{-2}s^{-1}$, according to the distance of the light source (Osram L36W/965 Biolux 2300 Im, Osram L36W/77 Fluora 1400 Im, Osram L36W/76 Natura de Luxe 1800 Im), with bundles perpendicular to the tanks. The encrusting calcareous algae used for the first induced spore germination, started the 24 January 2005, taken together with their substrate from 2 metres deep (T=6∞C) during a scuba dive, were placed in the tanks. Following the thermic "shock" of the samples, and within a few hours, the spores were expelled inseminating, cascading, the slides (30x26mm), previously treated (1/3HCI solution at 37%, 2/3 95% alcohol) and placed on the bottom of the tanks. In order to verify the influence of the culture medium on the species examined, we chose to isolate some samples and to keep them both in filtered sea water and in an enriched medium by nutrients (Erdschreiber modified) (Stein, 1973; Starr, Zeikus, 1993). Subsequently, after the deploying of the protocol (Di Pascoli *et al.,* 2005), other three induced inseminations were conducted using calcareous algae collected from the bottom of the Ridge of Santa Croce nearby the pyramids (25 May 2005 – 26 July 2005 – 11 November 2005). The samples were collected at 12-13 metres deep.

The identification and the counting of the thalli collected after the induced insemination were then carried out by means of an optical microscope (Nikon Labophot) equipped with a camera (Nikon FX-35A; Nikon Microflex HFX). The thalli area was calculated on photographs, converted into digital images, using appropriate software (ImagePro Plus 4.5). From the comparison amongst the images of the different samples (Lee, 1977;Morcom *et al*.,1988; Haddad *et al.,* 1994) it was possible to evaluate the growth rate in time. The photographs, useful for the calculation of the areas of adult thalli present on the slides, were made possible using a digital camera Nikon Coolpix 4500 suitably mounted on the stereo microscope.

Results

Hydrolithon boreale (Foslie-Chamberlain) (Fig. 2.7) thalli were developed from the spontaneous insemination, but the activity of the autotrophic (diatoms and blue-green algae) and heterotrophic component present in the habitat as well as the low number of cells developed did not allow a correct attribution of O_2 (produced and used) to the calcareous alga. After the first induced spore germination in the laboratory, it was possible to determine, from the very first hours, that the predominant species was *Pneophyllum fragile* Kützing (Fig. 2.8), while *Hydrolithon boreale* was present with a lower percentage. After two days from the insemination 6266 individuals were counted.

The growth of *H.boreale* was favoured in the enriched medium culture, where though the number of diatoms and blue-green algae resulted high. The samples placed not filtered in sea water were of lower dimensions and came in some teratologic shapes. *P fragile* grew uniformly in all culture media.

The spontaneous insemination does not result valid for the aim of the study due to the strong presence of the autotrophic and heterotrophic component, while in the induced insemination the use of the three media (enriched sea water, filtered sea water, sea water) leads to different results.

The thalli inseminated in January were monitored and preserved in sea water first in the culture tanks and eventually in Petri capsules for 5 months and undergone physiological analysis (R.U. Talarico, Frisenda). The thalli present on the slides grew so as to occupy 27.3 mm^2 (Fig. 2.9). The constant growth recorded confirms that the culture conditions do not interfere with the metabolism of the algae. Furthermore, the development of conceptacles, structures destined to contain reproductive organs, which was observed from May (after 4 months from insemination) shows that the algae reached the sexual maturity and confirms once again the validity of the methodology used for the studying in non natural environment of the Corallinales present on the Ridge of Santa Croce.

The growth of *H. boreale* and *P.fragile* thalli (Fig. 2.10) show a similar trend, even though the first grows less than the second. We noticed how the dimensions doubled going from the first to the second month of experimentation.

In the induced inseminations starting from 25 May 2005, 26 July 2005 and 11 November 2006 (3790, 6361 and 3371 thalli present after one week from the insemination respectively) *Hydrolithon boreale*, *H. farinosum and Pneophylllum* were found to be the predominant species confirming the resemblance between the two sites taken into examination for the deploying of the protocol (Sistiana and the Ridge of Santa Croce).

In order to evaluate the growth in the natural environment the slides were placed on suitable supporters (collectors), taken to the Ridge of Santa Croce and positioned at three different depths (8 metres: upper part of the pyramid chosen for the experimentation, 10 metres: horizontal surface of the first cubes which form the base of the pyramid, 12 metres: bottom nearby the pyramid). Three slides for each sea level were collected at regular intervals (November 2005, February 2006, April 2006), during the scuba dives, placed in black containers, surfaced and then put in a portable fridge to be taken to the laboratory. They underwent physiological analyses (R.U. Talarico, Frisenda), and the development of the area was then calculated for the three lapses of time studied (after two, five and seven months of permanence in the field).
In November it was possible to isolate on the slides, other than the thalli born in culture, some thalli born after the deployment of the collectors (Fig. 2.11). The thalli born in the field showed a greater growth, in terms of surface, at 12 metres deep, while the thalli born in the laboratory the greater growth was recorded at the intermediate level of the pyramid, at 10 metres deep. In the following months it was possible to notice how the sea level where the calcareous algae grew most seemed to be higher, at 8 metres, where the average growth of each thallus resulted greater than 50 mm^2 in April. Considering each single period (Fig. 2.12), a greater average increase of the surface at 8 and 12 metres in February was noticed, whereas in April, even though keeping this trend, the average surface for each thallus present a lower sea level, 12 metres, resulted lower to that of February.
Comparing the three periods, it is clear that there was a lack of homogeneity in the growth rates, due to different factors which characterised the three months of collecting, both from the temperature point of view (17°C in November, about 7 °C in February and April), and the sea transparency (a layer with a significant concentration of suspended particles at 10 metres deep). Finally, the thallus present at 8 metres, especially in the 2006 collection, revealed some epiphyte algae, easily eliminable which colonized both the slides and the plastic supporters, while at lower depths, both at 10 and at 12 metres, the epiphytes were barnacles and bryozoans, who compete with the calcareous algae for the colonization of the substrate.

2.2 Cover survey

Methodological notes

From the first observations conducted during the samplings and the scuba dives, it was possible to notice how the encrusting calcareous algae were present on the major part of the artificial reefs.

To estimate the contribution of this algal component to cover the whole surface, a new monitoring technique was used (Di Pascoli *et al.*,2006). The need, thus, to obtain significant results through a quick procedure, in accordance with the timing of the scuba dives, led to the deploying of the methodology, on the basis of new information technologies (Tribollet *et al.*,2001; Miller *et al.*,2003; Hily *et al.*,2004; Preskitt *et al.*, 2004), aimed to evaluate rapidly the algal covering, oriented to a ready eco-physiological interpretation (energetic flux) in terms of monitoring. The attention was focused on the parameters essential to the latest needs of environmental management, such as: promptness reply; methodological reliability; experimental repeatability, and, last but not least, economical management.
This method, not destructive, was first tested during some samplings conducted while apnoea and scuba dives, during two different surveys (Susterisc, 2005; Poloniato, 2005) which required the usage of: a Nikon Coolpix 4200 digital camera; Nikon WP-CP2 diving suit; a spacer holding a stainless steel frame (20x20 cm); eventually, it was placed on one pyramid present on the Ridge of Santa Croce. The size of the least representative area of each sample-collection were defined experimentally and made in a greater into a 400cm^2 square. The area, as well as the distance between the camera and the sample, was kept constant for every survey, by means of a tailor-made stainless steel support. This support had the task to give the camera the necessary stability in order to obtain at the same time: constancy in the sampling replicas and clear and well defined images. The distance set from the object enabled us to include in the framing: both the surveying area, and the square frame useful as a calibration unit. Every photo-sample was analysed quantitatively and the covering area was calculated by means of a graphic elaboration software (Image-Pro Plus 4.5®), able to measure the surfaces of the defined areas. The areas occupied by the algae were redesigned accurately using a graphic pen (400-V2 Trust), after calibrating the programme with the suitable unit of measurement.

Results

The advantage and innovation from the usage of modern technologies (digital underwater images and software elaborations) has enabled us to calculate the surface occupied with extremely accuracy. The covering has appeared from the very first observations mainly present on the lower cubes, at the base of the pyramid. From the analysis of 70 photo replicas, conducted while dives, it has been revealed that 55.4% of the vertical surfaces of the lower cubes is occupied by algae, while the upper part is occupied by 49.7%, confirming the values expected from the initial observations.

Conclusions

The calcareous algae are a predominant component on the artificial reefs and the methodological approach used to evaluate the recovering and the production from the Corallinales has highlighted their real contribution. The culture study and the following development on the Ridge of Santa Croce has enabled us to evaluate the increase of biomass of this algal component in relation to the photosynthetic activity, highlighting a different adaptation to the three sea levels taken into consideration.

The recovering, by means of a non-destructive methodology, deployed for this experimentation, has confirmed the importance, in terms of presence, of Corallinales, evidenced by the floristic survey and by the collection of elements typical for environments of particular interest, such as the mäerl.