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**THE EFFECT OF MARINE PROTECTED AREAS
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A CASE OF STUDY IN SOUTHERN SARDINIA
(CAPO CARBONARA MPA)**

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THE EFFECT OF MARINE PROTECTED AREAS ON THE BIOLOGICAL ENVIRONMENT.

A CASE OF STUDY IN SOUTHERN SARDINIA

(CAPO CARBONARA MPA)

ABSTRACT

The growing interest in environmental issues and the establishment of new protected areas on land and seaside correspond to the worldwide need to understand and preserve the natural development of the ecosystems.

The establishment of protected areas is fairly new for the marine habitat.

It is becoming increasingly clear that marine protected areas represent an effective biodiversity conservation tool. Nowadays, there is a widespread growing need to recognize ecosystem interactions and to improve the effectiveness of protected areas in order to understand biological interrelations and human impacts.

It was the lack of high quality and rigorous monitoring data concerning the marine protected areas (and not the lack of effects, which are almost certain) to bring us to perform the environmental monitoring described in this thesis.

In order to understand the biological interactions of a marine protected area in the Mediterranean sea, a study was carried out inside and around the Marine Protected Area of Capo Carbonara located off the southern Sardinia Island.

Samplings were performed to identify and quantify the benthonic fish stock and the composition of benthos in rocky shores at different depths and in different times of sampling.

The composition of fish assemblages was evaluated both by the non-destructive visual *census* with SCUBA technique and the destructive method of the trammel net fishing.

Fish assemblages in shallow rocky habitats (4–8 m deep) have been assessed between September 2004 and July 2005. Overall the list of target

species included 24 species from 8 families (*Labridae, Moronidae, Mullidae, Sciaenidae, Scorpaenidae, Serranidae, Sparidae, Sphiraenidae).*

The fish assemblages observed inside the protected area and outside, in the unprotected fished area were statistically different during all the sampling periods. Larger amount of fish was found in the protected than in the fished area, and differences were observed in total fish density, abundance, biomass: most fish species targeted by fisheries had a greater density (e.g. *Diplodus puntazzo, Diplodus sargus, Diplodus vulgaris*, *Epinephelus marginatus*, *Mullus surmuletus*, *Pagrus pagrus*, *Sciena umbra, Scorpaena porcus, Serranus scriba, Sphyraena Sphyraena* and *Synphodus tinca*) and/or size (e.g. *Dentex dentex, Diplodus puntazzo, Diplodus sargus*) within the protected area than in the fished areas outside.

In the internal protected areas the Shannon Weaver diversity index showed richer ichtiofauna than outside, near the boundaries.

Cluster analysis and MDS plot showed a general progressive increased similarity between protected and unprotected sites from the early census to the end, validating the spillover effects from inside to outside areas. These results indicate that reserve effects (protection) from fishing may have the potential to influence fish assemblages of outside areas.

The composition of benthos in hard substrate, was examined applying benthic biocoenosis *census* through photographic and SCUBA techniques.

Three years of sampling displayed the usefulness of the used techniques in a low budget study.

The Visual *census* outcomes for macro benthic biocoenosis assemblages (5-25 m deep), assessed from September 2004 to May 2006, revealed 325 species (4 Phyla of Thallophyta, 1 of Anthophyta and 10 Phyla of Invertebrata) in all the analyzed sites.

The analysis of the community structure revealed high biodiversity. In each sampling an ecological index classification as “Moderate” was recorded, both in the whole sanctuary and nearby the boundaries. In particular, some sites within the sanctuary showed a “Good” quality in different times and at different depths.

Moreover, ecological and environmental factors able to modify the benthic composition often risked to warp outputs e.g. the presence of an alien species, the *Caulerpa racemosa*. In particular the analysis of these green algae within the sites was necessary because of its influence on the environment and consequently on the statistical analysis outputs.

It is relevant that the recorded temporal scale of events can be useful for further analysis in the studied area and/or in other Marine Protected Areas.

The application of this protocol seems to be a functional tool to manage marine environments almost until a co-ordinated network between the Mediterranean Marine Protected Areas will be created.

ACKNOWLEDGEMENTS

First of all, I would like to thank my advisor Professor Dr. Angelo Cau for his guidance and support throughout my graduate studies. Without the numerous discussions and brainstorms with him, the results presented in this thesis would never have existed. I am grateful to Dr. Piero Addis, for his valuable comments and suggestions on my research. I thank Professor Dr. Anna Maria Deiana and Dr. Danila Cuccu for their help during my collaboration with the Department of Animal Biology and Ecology of Cagliari.

Last but not least, I would like to thank my wife and my little children for their constant love and support. It would be impossible for me to express my gratitude towards them in simple words. I dedicate this thesis to them.

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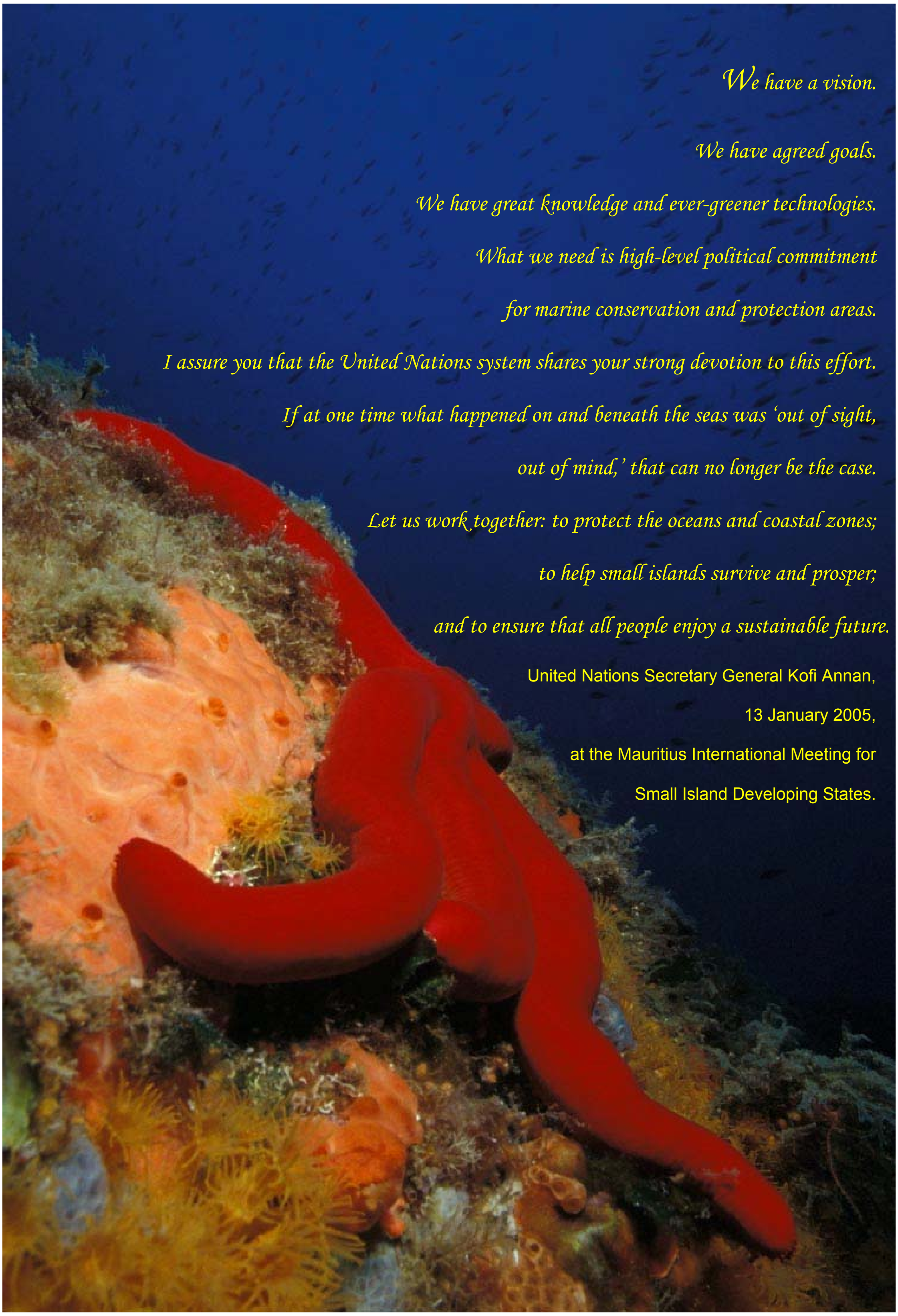
ACRONYMS

A1N	Sampling Sites (see figure 2.4)
A1S	Sampling Sites (see figure 2.4)
AC	Articulated Corallinacea
C1	Sampling Sites (see figure 4.1)
C2	Sampling Sites (see figure 4.1)
C3	Sampling Sites (see figure 4.1)
C4	Sampling Sites (see figure 4.1)
CBD	Convention on Biological Diversity
CoNISMa	Consorzio Nazionale Interuniversitario Scienze del Mare
DFA	Dark Filamentous Algae
DM	Decreto Ministeriale
EB	Encrusting Bryozoans
ECR	Encrusting calcified Rhodophytae
ECR	Encrusting Calcified Rhodophytae
EEI	Ecological Evaluation Index
ERS	Encrusting Red Sponges
ESC	Ecological State Class
ESG	Ecological State Group
GFA	Green filamentous algae
GFA	Green Filamentous Algae
GU	Gazzetta Ufficiale
ICRAM	Istituto Centrale per la ricerca Applicata al Mare
I1N	Sampling Sites (see figure 2.4)
I1S	Sampling Sites (see figure 2.4)
I2N	Sampling Sites (see figure 2.4)
I2S	Sampling Sites (see figure 2.4)
ISO	International Standards Organization
IUCN	International Union for the Conservation of Nature
MBBC	Macro Benthic Biocoenosis *Census*
MDS	Multi Dimensional Scaling
MDS	Massive Dark Sponges
MPA	Marine Protected Area
NGO	Non-Governmental Organisation
O1N	Sampling Sites (see figure 2.4)
O1S	Sampling Sites (see figure 2.4)
O2N	Sampling Sites (see figure 2.4)
O2S	Sampling Sites (see figure 2.4)
O3N	Sampling Sites (see figure 2.4)
O3S	Sampling Sites (see figure 2.4)
O4N	Sampling Sites (see figure 2.4)
O4S	Sampling Sites (see figure 2.4)
O5N	Sampling Sites (see figure 2.4)
O5S	Sampling Sites (see figure 2.4)
O6N	Sampling Sites (see figure 2.4)
O6S	Sampling Sites (see figure 2.4)
OECD	Organisation for Economic Co-operation and Development
P0A	Sampling Sites (see figure 4.1)
P0B	Sampling Sites (see figure 4.1)
P0C	Sampling Sites (see figure 4.1)
SBA	Soft Branched Algae
SCUBA	Self-Contained Underwater Breathing Apparatus
TNF	Trammel Net Fishing
TRB	Thin Ramified Bryozoans
TTS	Thin Tubular Sheet-like
UFVC	Underwater Fish Visual *Census*
WFD	Water Framework Directive

All the pictures were taken by the author himself.



We have a vision.

We have agreed goals.

We have great knowledge and ever-greener technologies.

What we need is high-level political commitment

for marine conservation and protection areas.

I assure you that the United Nations system shares your strong devotion to this effort.

If at one time what happened on and beneath the seas was 'out of sight,

out of mind,' that can no longer be the case.

Let us work together: to protect the oceans and coastal zones;

to help small islands survive and prosper;

and to ensure that all people enjoy a sustainable future.

United Nations Secretary General Kofi Annan,

13 January 2005,

at the Mauritius International Meeting for

Small Island Developing States.

1 INTRODUCTION

1.1 WHAT MARINE PROTECTED AREAS ARE

The marine and coastal areas are critical to the health and more in general to people and communities living nearby.

Healthy waters abound with life providing food, jobs and income. Shallow waters and their ecosystems sustain fisheries which can support regional incomes, coastal trades and traditional culture.

The biological diversity of marine systems is rapidly diminishing all over the world, endangered by over-fishing, loss of habitats due to destructive fishing techniques and inappropriate coastal development, pollution, invasions of alien species which change the natural ecological balance. The long-term threat of climate change, whose influence can already be observed in biologic invasion, changing currents and increasingly violent and destructive storms around the world, overrides all the above mentioned threats.

Marine Protected Areas, in their multitude of forms and sizes, are seen as one of the solutions to cope threats facing the coastal marine sphere. Marine Protected Areas are considered a sustainable development tool contributing to the long term incomes of nearby citizens, to their culture and prosperity.

These benefits include enhanced productivity from well managed coastal zones, shoals, beds of sea grass and seaweed, natural protection from wave erosion, increased recreational and tourist chances, as well as greater opportunities for education and research, especially about natural processes in ‘pristine’ regions (investing in the future through the long-term benefits of education).

The international community has recognised the potential role and benefits of Marine Protected Areas (MPAs). The main future goal must be to build representative networks of marine and coastal protected areas.

This is an actually global challenge that can only be realised through committed and effective cooperation.

MPAs are not considered anymore just a conservation tool, but a development and health tool.

The term MPA covers a wide range of different approaches for the management of coastal and marine areas. An MPA as defined by the IUCN is “any defined area within or adjacent to the marine environment, together with its overlying waters and associated flora, fauna, and historical and cultural features, which has been reserved by legislation or other effective means, including custom, with the effect that its marine and/or coastal biodiversity enjoys a higher level of protection than its surroundings.

In this definition, MPAs may include areas managed by governments, local communities, NGOs, development projects or any combination of these or other stakeholders including the private sector.

Many different terms have been used to describe MPAs, including ‘reserves’, ‘closed areas’, ‘no-take zones’, ‘sanctuaries’, ‘parks’ and ‘locally managed areas’. Much of the recent discussion has focused on ‘no-take MPAs’ intended to prohibit all extractive activities and thereby to sustain fisheries production in surrounding waters through “spillover” of fish stocks.

The success or sustainability of many MPAs comes from the collaborative planning with resource users to ensure managed access for each main stakeholder group, and from a community involvement in governance, so as to promote agreement and support.

Nowadays Mediterranean biodiversity is undergoing rapid alteration under the combined pressure of climate change and human impacts.

1.2 MARINE PROTECTED AREAS IN THE MEDITERRANEAN SEA.

Mediterranean marine biodiversity has received only a fraction of the attention accorded to its terrestrial counterpart, despite the great cultural and economic importance that the sea has been having for the Mediterranean countries (Bianchi N. and Morri C., 2000). At a rough estimate, more than 8.500 species of macroscopic marine organisms should live in the Mediterranean Sea, corresponding to somewhat between 4% and 18% of the world marine species. These data are even more relevant if we consider that the Mediterranean Sea is only 0.82% in surface area and 0.32% in volume compared with the world ocean. The high biodiversity of the Mediterranean Sea may be explained by historical reasons (its tradition of studies) dates older than almost any other sea), paleogeographic reasons (its tormented geological history through the last 5 my has been determining the occurrence of distinct biogeographic categories), and ecological reasons (its variety of climatic and hydrologic situations within a single basin). Figure 1.1 below, shows the 48 MPAs existing in 2003.

Nowadays, there is not a co-ordinated network between the MPAs in the Mediterranean sea, useful tool to implement strategies for the environment conservation and for the economy of the nearby citizens.

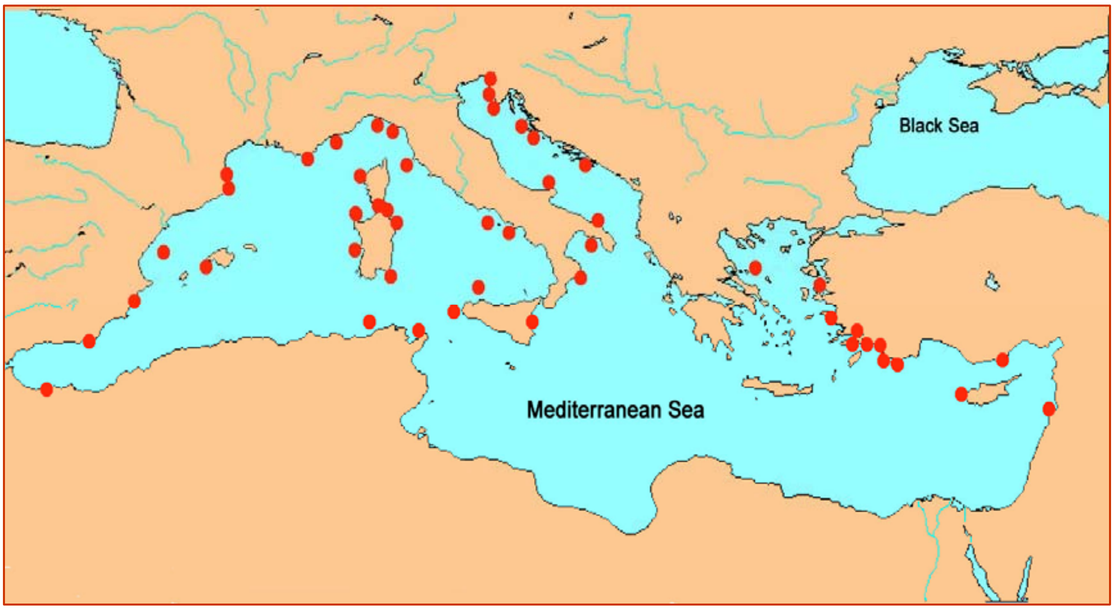


Figure 1.1: Marine Protected Areas in the Mediterranean Sea, December 2003 (Rais C., 2003), Modified.

1.3 STUDY DESIGN

The main objective of the present thesis is to understand the role and patterns of marine biodiversity within a Marine Protected Area of Sardinia; to demonstrate that MPAs increase biodiversity in comparison with non-protected marine areas, and to demonstrate the spillover effects that may occur.

The research assessed the evolution of marine environment and the MPAs influences inside and outside, by studying fish fauna in rocky shores and benthos in hard substrates.

For this purpose, a set of samplings was carried out within and outside the MPA.

The biological analysis were subdivided in two main groups:

1. The analysis of fish fauna using:
    a. visual census techniques and
    b. trammel net fishing
2. The analysis of flora using the macro benthic biocoenosis census.

Analysis of fish fauna was performed by divers using **SCUBA** (**S**elf-**C**ontained **U**nderwater **B**reathing **A**pparatus) techniques inside and just outside the sanctuary analyzing fish abundance, fish biomass and fish length data; trammel net fishing was a fishing technique performed with a boat and nets.

The analysis of flora was performed mapping benthic biocoenosis (all the interacting organisms living together) inside and just outside the MPA on hard substrates and estimating the composition with photographic *census* techniques.

The increased coverage of an alien green alga, the *Caulerpa racemosa* and its dangerous effects on the environment were the subject of an entire study carried in the analyzed area. This study is presented in the section 4.4 of this thesis.

The study was performed from 2004 to 2006.

1.4 THE MARINE PROTECTED AREA OF CAPO CARBONARA

The Marine Area of Capo Carbonara is located in the southern Tyrrenian sea, off the south-eastern coast of Sardinia.

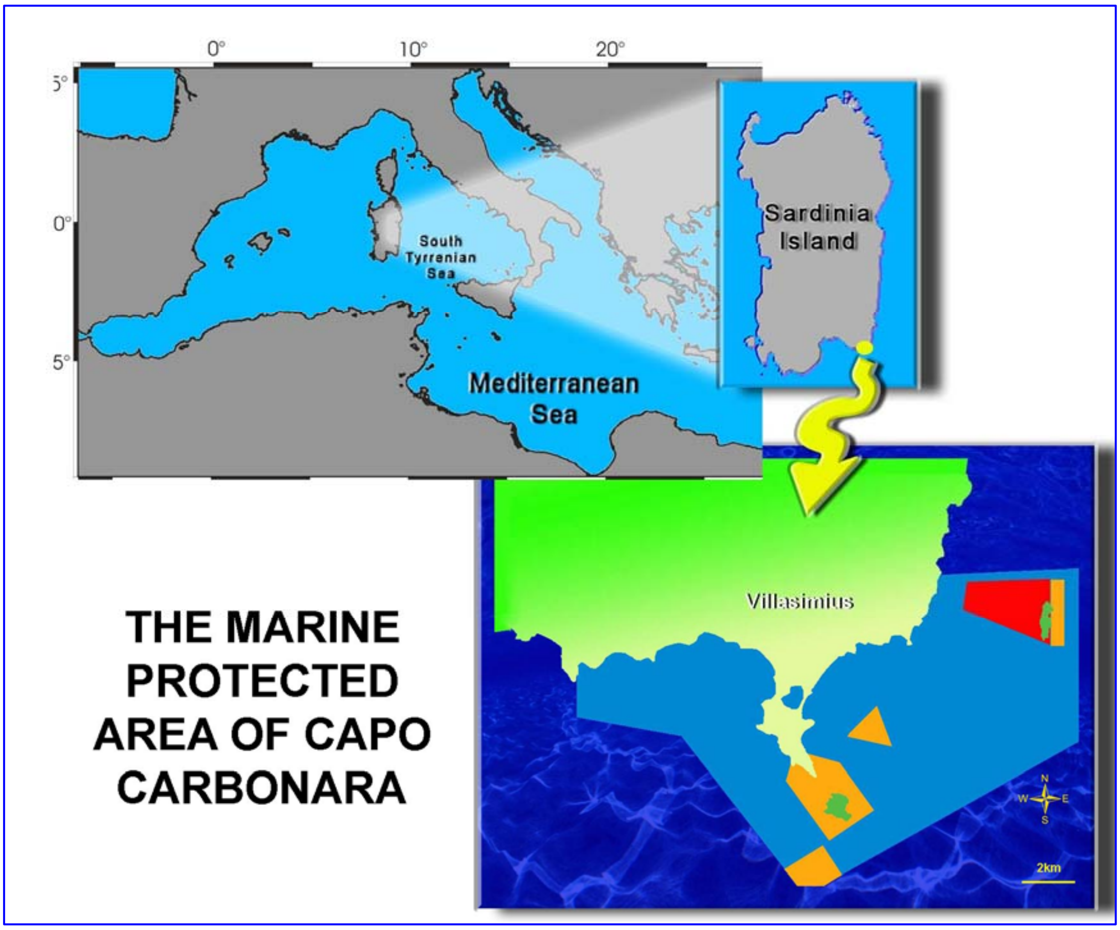
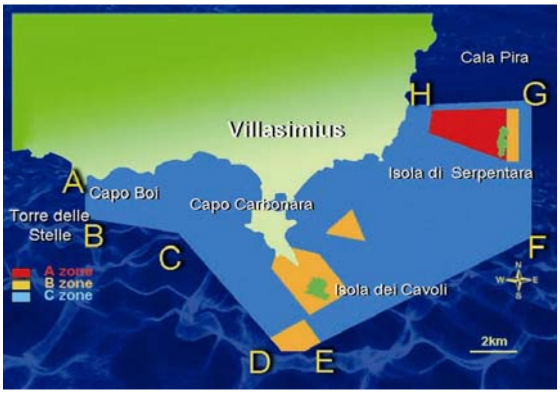


Figure 1.2: Location of the Marine Protected Area.

The MPA named as Capo Carbonara – Villasimius, has been instituted in 1988.

It measures 8857 hectares in surface and 32 km of coastlines which include granitic cliffs. The Area extends from the cape called "Capo Boi" to Cape "Punta Is Proceddus" including the island of Cavoli and Serpentara.



	Latitude	Longitude
A	39° 07' .54 N	009° 26' .47 E
B	39° 06' .54 N	009° 26' .47 E
C	39° 05' .62 N	009° 28' .49 E
D	39° 03' .37 N	009° 31' .40 E
E	39° 03' .37 N	009° 32' .05 E
F	39° 07' .40 N	009° 37' .19 E
G	39° 09' .50 N	009° 37' .19 E
H	39° 09' .13 N	009° 34' .13 E

MPA boundaries

Figure 1.3-1.4: Partition and view of the sanctuary from Capo Boi boundary.

CHRONOLOGICAL HISTORY OF THE MPA

- 1991 Identified as a "Area Marina di Reperimento" (National Low L.n.394–1991).
- 1998 Established with the original institutive Law (D.M. 15.09.1998).
- 1999 Modified by the D.M. of 03.08.1999 (G.U. n. 229 del 29.09.1999).
- In progress: Certification ISO 9001.

GENERAL RULES

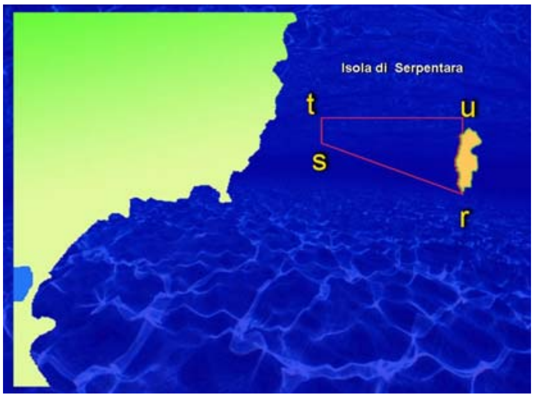
The regulations in force inside the entire MPA:

- Do not hunt, capture, pick, damage; in general, it is not allowed any activity provoking danger or disturb towards animal and vegetal species, including foreign species intake;
- Do not alter by any mean, directly or indirectly, the geophysical environment and the biochemical features of water; do not dump liquid and solid waste; do not introduce the inlet of any substances which could modify, even temporarily, the characteristics of the marine environment;
- Do not transport arms, explosive materials, destructive and rapture means, toxic and polluting substances.
- Do not practise any activity disturbing or hindering study programs and scientific research.

ADDITIONAL RULES

A ZONE: Primary Reserve, West Area of Isola di Serpentara.

Forbidden: removing, even partially, and damaging geological and mineral formations; navigation, admittance and stops for any ship and yacht; professional and sporting fishing; underwater fishing.



	Latitude	Longitude
r	39° 07' .83 N	009° 36' .43 E
r	39° 08' .78 N	009° 34' .76 E
t	39° 09' .18 N	009° 34' .76 E
u	39° 09' .18 N	009° 36' .46 E

Figure 1.5: A zone boundaries.

Allowed: admittance of personnel of the Administrator Authority for active service; previously licensed scientific researchers; diving previously licensed by Administrator Authority for scientific purposes and submerged sightseeing regulated by the same authority in limited areas and according to previously agreed upon routes.

B ZONE: General Reserve, East Area of Isola di Serpentara, Secca dei Berni, Isola dei Cavoli-Capo Carbonara, South Area of Isola dei Cavoli.

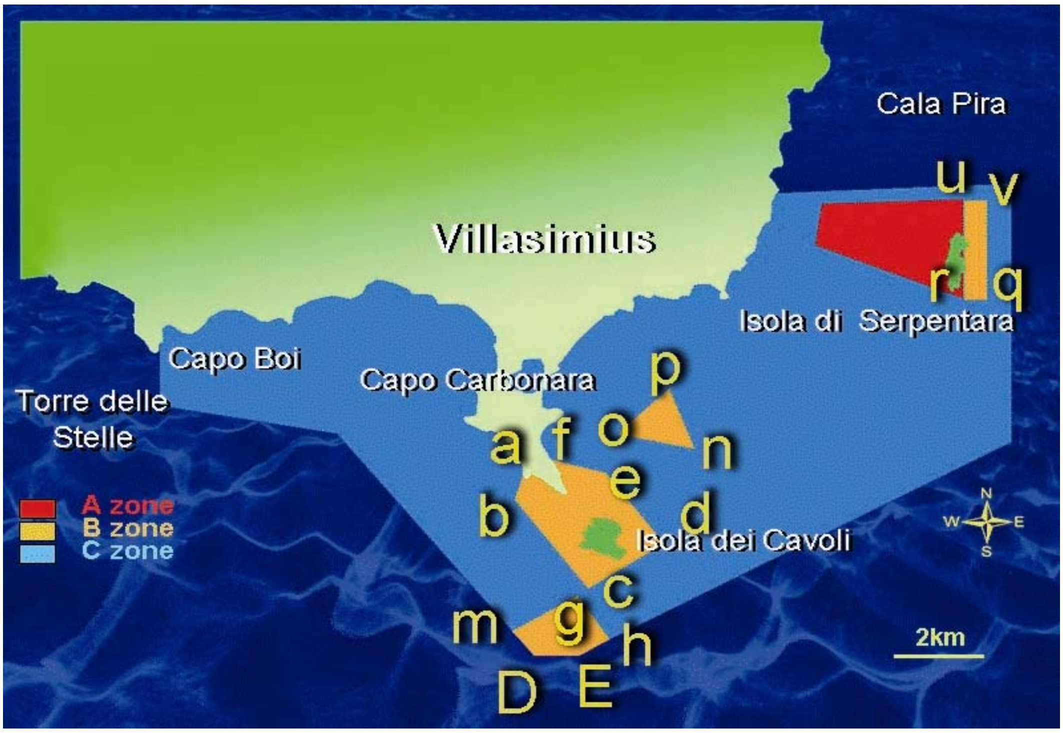


Figure 1.6 - B and C Zone boundaries.

Forbidden: free anchorage; free engine navigation; mooring; underwater fishing.

Allowed: crafts and reduced speed boats navigation (not more than 10 Knots) according to Administrator Authority; professional and sporting fishing previously licensed by the Administrator Authority; mooring to the structures set by the Administrator's Authority; diving, previously licensed.

C ZONE: Partial Reserve, all the other areas in the MPA.

Forbidden: free anchorage; mooring; underwater fishing;

Allowed: anchorage where equipped and signalled by the Administrator Authority; crafts and boats navigation; regulated mooring; professional fishing for fishers resident in the town of Villasimius and for those not resident but licensed by the Administrator Authority as for fishing strain and implements; diving with Administrator permission.

Underwater fishing is forbidden in all the MPA, without differences between Zone A-B-C.

It is also forbidden to anchor your boat in the area where the "*Posidonia oceanica*" grows.

East sector of Isola di Serpentara		**Secca dei Berni**	
r	39° 07' .83 N 009° 36' .43 E	n	39° 06' .47 N 009° 33' .31 E
q	39° 07' .83 N 009° 36' .84 E	o	39° 06' .70 N 009° 32' .25 E
v	39° 09' .33 N 009° 36' .84 E	p	39° 07' .29 N 009° 33' .06 E
u	39° 09' .18 N 009° 36' .46 E	u	39° 09' .18 N 009° 36' .46 E
Isola dei Cavoli - Capo Carbonara		**South sector of isola dei Cavoli**	
a	39° 06' .29 N 009° 30' .62 E	g	39° 04' .12 N 009° 31' .88 E
b	39° 05' .39 N 009° 30' .30 E	h	39° 03' .72 N 009° 32' .47 E
c	39° 04' .08 N 009° 31' .94 E	E	39° 03' .37 N 009° 32' .05 E
d	39° 04' .92 N 009° 33' .10 E	D	39° 03' .37 N 009° 31' .40 E
e	39° 05' .95 N 009° 31' .87 E	e	39° 05' .95 N 009° 31' .87 E
f	39° 06' .05 N 009° 31' .28 E	m	39° 03' .37 N 009° 31' .13 E

Table 1.1: Boundaries of the MPA.

1.5 INVESTIGATED SITES

1.5.1 CAPO CARBONARA

The MPA takes its name from the promontory of Capo Carbonara. It is located in the B zone and it is the last tongue of land in South East Sardinia. It is a watershed dividing the South East coast from the East coast.

Shoals start from 0 to 22 meters in the channel between the promontory and the Isola dei Cavoli. Here, shoals of juveniles *Diplodus vulgaris* can be seen.

Figures 1.7-1.8: Capo Carbonara and shoals of *Diplodus vulgaris.*

1.5.2 ISOLA DEI CAVOLI

Isola dei Cavoli is in the B zone of the sanctuary. It takes its name from the "Cavorus", which means crabs in Sardinian language.

Figures 1.9-1.10-1.11-1.12-1.13: Isola dei Cavoli, the lighthouse, a specimen of *Paramuricea clavata* and the Serranidae *Epinephelus marginatus.*

This is the most beautiful Island in the sanctuary, where waters are deep and shoals have depth from 0 to 50 metres and more. These waters are full of life and divers are present all year long.

1.5.3 ISOLA DI SERPENTARA

This is another gorgeous island entirely located in the no take zone (A zone). It is notable for its terrestrial endemic flora and fauna. Here flourishes the *Helicodiceros muscivorus* (L. fil.) Engler (figure 1.14).

Figures 1.14-1.15-1.16-1.17: A specimen of *Helicodiceros muscivorus*, eggs of sea-gull, a view of the Island and the Anthozoa *Parazoanthus axinellae*.

This Island is the sanctuary of birds, where their reproductive cycle can take place.

Waters, like the Isola dei Cavoli, are deep and shoals start from 0 to 50 metres.

1.5.4 PUNTA IS PROCEDDUS

Punta Is Proceddus, located in the northern boundary of the C Zone, is characterized by shallow shoals starting from 0 to 17 metres.

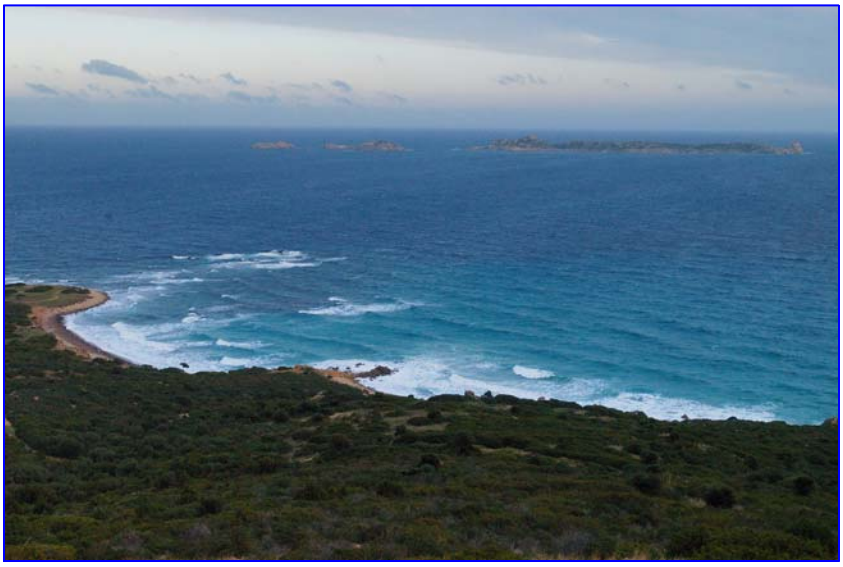


Figure 1.18: A view of Punta is Proceddus.

1.5.5 CALA PIRA

Cala Pira is a site situated outside the sanctuary (600 m North direction).

Figure 1.19: The beach of Cala Pira.

1.5.6 SOLANAS – CAPO BOI

Solanas is a place outside, located near the boundaries in the South West side of the MPA.

Granitic cliffs start on land and end at 25 meters of depth.

Figures 1.20-1.21: The tower of Capo Boi and the promontory of Torre delle Stelle, the Crustacean *Maja squinado*.

This site outside the sanctuary, but nearby the West boundary is rich in species of the Crustacean class, like the *Maja squinado*.

1.5.7 TORRE DELLE STELLE

In the last site, at Torre delle Stelle, there are lots of different environments; in fact in front of the beach of Genn'è Mari there is one of the most beautiful shoals starting from 10 to 40 meters. Here is present the unique specimen of Cnidaria *Gerardia savaglia* at 27 metres of depth.

Figures 1.22–1.23: The very rare species *Gerardia savaglia* and the world war II shipwreck Isonzo at 48 meters of depth.

In proximity three shipwrecks from 10 to 67 metres are substrata for different species of flora and fauna.

2 FISH FAUNA ANALYSIS

Many studies indicate that MPAs can improve fish stock inside and supply larval and adult fish biomass to adjacent areas (through spillover), helping therefore the enhancement of the surrounding fishery (Halpern and Warner, 2002; Halpern, 2003).

Pioneering works (Russ G. & Alcala A., 1996; Alcala A., 1999) in Apo Island (central Philippines) provided some early evidence that spillover of adult fish biomass from the reserve to fished areas occurs.

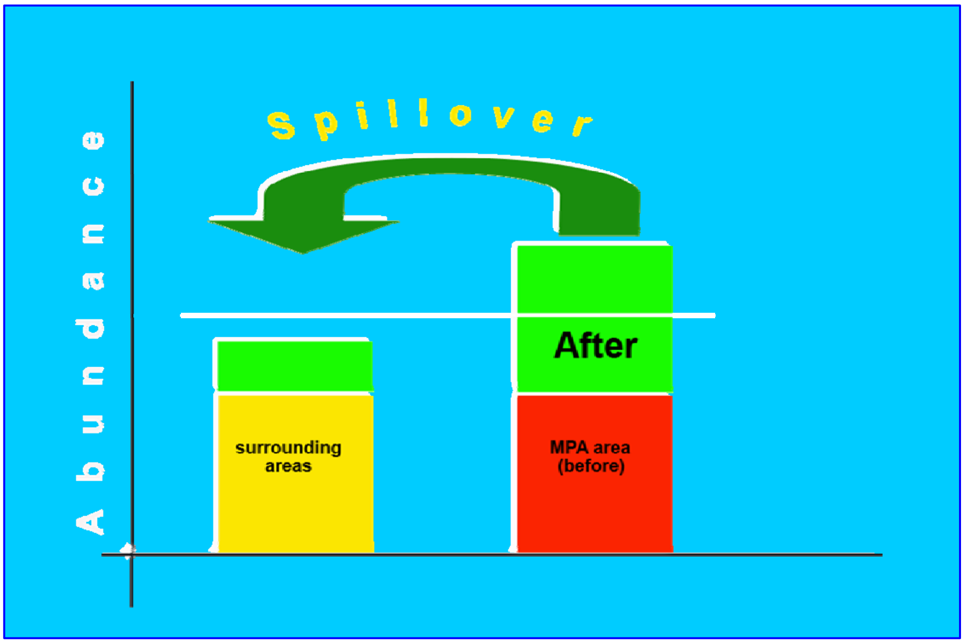


Figure 2.1: Spillover trend from MPA to surrounding areas.

A study by Rodwell *et al*., (2002) in the Mombasa Marine National Park shows, through simulation, that full protection leads to an increase in total fish biomass, and that movement of adult and larval fish from the MPA will increase total fishery catch in surrounding areas. Studies that measure actual yield enhancement from MPA are few (Roberts C. M. *et al.,* 1999), and fewer still deal with valuation of economic benefits from protection.

One of the best ways to demonstrate that the marine reserve helps to sustain a profitable coastal fishery, is to determine whether or not the fishery generates economic or resource rent (surplus value after all costs and normal returns have been accounted for).

The present study is at the beginning in the area of Capo Carbonara. There was only another study called “Afrodite” Project, a national project started in 2003 and ended in 2004, developed by the Environment Ministry, the ICRAM and CoNISMa; now it is the subject of many investigations in other MPAs as a way to evaluate their efficacy in enhancing the fishery in surrounding areas.

Many methods exist to measure resources along rocky shores and lagoon: none is perfect and all have advantages and disadvantages. They all share the characteristic of studying a section or subgroup of the population in question. For this reason, they require the use of sampling techniques, which can be divided into three categories:

- capture methods;
- mixed methods;
- non-capture methods.

Sampling techniques	Quality of data				Needs	
	Comprehensiveness	*Accuracy*	*Coverage*	*Bias linked to life cycle*	*Staff training*	Costs
Capture	Low*	Low to moderate	High	Yes	Low	Low
Mixed	Low	Moderate	Moderate	Yes	High	High
Non-capture	High	High	Low	No	High	Moderate

* except for explosives and poisons

Table 2.1: Summary of the different sampling methods.

Capture methods mainly involve recording information on fish captured in traps (and, more generally, with bait), in nets by trawling, and with lines. These methods are based on the analysis of the catch to study population's density.

Capture methods can be used at any time and at considerable depths. Moreover, they can be implemented on a wide scale and at low cost using scientifically unskilled staff. On the other hand, gear design and selectivity, the effect of baits, and the probability of certain species to be caught are all factors that have an effect on the comprehensiveness and the accuracy of these methods, which remain low to moderate. The one exception is fishing with explosives or poisons (e.g. rotenone), which gives comprehensive results, particularly in terms of species richness, but whose destructive effects are a major disadvantage, particularly for repeated samplings.

Mixed methods are somewhere between capture methods and non-capture methods. In particular, they include capture-tag-recapture methods, which are difficult to assess qualitatively or quantitatively.

On the other hand, mixed methods are very effective in determining age, growth, movement and behaviour in rocky shore fish populations.

Capture methods are often inadequate because of the “hyperdiversity” (referring not only to very high species diversity, but also to very high diversity of biotopes, ecological niches, behaviours, genomes and uses) and the wide range of reef and lagoon environments.

The most effective capture method is destructive and/or disruptive (e.g. dynamite, rotenone), but can also occasionally be useful for calibrating methods based on observation.

All destructive methods justify the development of true non-capture or ‘fishery independent’ methods like visual *census*, as listed below.

In this work, a capture method, the trammel net fishing, and a non-capture method, the visual *census*, were used mapping the state of fish fauna.

In the first year of sampling, the underwater video *census* technique was tested. It has some advantages: recorded events give the possibility to follow studies through the years, and to study marine geomorphology and environmental changes.

The greater disadvantage is that video images do not allow the perception of the third dimension as the human vision does. Again, sometimes it is difficult to understand the size of fishes because they are too far and at a different focal level; so, if a fish repeatedly goes in and out from the field of vision of the camera, it can be counted more than once.

For these reasons the underwater fish visual *census* is not a good method to study the state of fish fauna.

2.1 VISUAL *CENSUS*

This technique is especially useful for assessing pelagic or semi-pelagic fish stocks and reef fish populations (Harmelin-Vivien M. and Harmelin J., 1975). This method is also more comprehensive, more accurate and non-destructive. Underwater fish visual census (UFVC) was first used to measure fish and invertebrate abundances. It was then used to study the dynamics of exploited and unexploited populations, and the ecology and management of natural resources and MPAs environments (Vacchi M. *et al*., 1997).

The technique is ideally suited to monitor the abundance of reef fish as it allows the collection of community level data without the disturbance caused by more destructive sampling techniques.

Visual *census* encompasses many techniques used to quantify reef fish populations (Thresher R. E. & Gunn J. S., 1986). The more traditional belt transect method has been adopted to assess reef fish populations. This method has been widely used in the past and provides precision and accuracy similar to other methods (Samoilys M. & Carlos G., 1992).

The simplest form of belt transect method for visual *census* of fish populations involves an observer, equipped with SCUBA gear, estimating the abundance of fish within a given area (the belt transect). A multitude of factors, including fish mobility and habitat complexity, have been shown to affect the precision of the counting technique. Additional errors in abundance estimates are likely to be introduced through observer bias. Therefore, any program using more than one observer must ensure that differences in bias between observers are minimised, to allow comparisons of data collected by different observers.

2.2 VISUAL *CENSUS* TECHNIQUE

2.2 1 DATA COLLECTION

The following equipment is required for the collection of fish abundance data:

- small diving boat
- complete sets of scuba diving equipment
- underwater slate, pencil and data sheets
- reels with 25 metres of halyard (a line)
- hand held GPS

A minimum of three people are required for the collection of visual *census* data using this technique. One person conducts the surveys, while a second person lays a reel along the line of each transect. The third person remains in the boat as surface support.

2.2 2 SAMPLING PROCEDURE

The following section outlines the procedure for undertaking visual *census* surveys along belt transects.

The site is located from the surface using a GPS and/or past knowledge of the surrounding reef topography. The boat is anchored slightly away from the site so that divers entering the water do not swim across transects and do not disturb fish before the *census* begins.

Two divers enter the water. The first diver (observer) is equipped with a slate, pencil and data sheets, the second diver (reel layer) carries the reel.

The observer conducts the 25 metres by 5 metres surveys by swimming along the central line of the transects following the reef profile. The observer counts all fish viewed within 2 metres on either side from the central line.

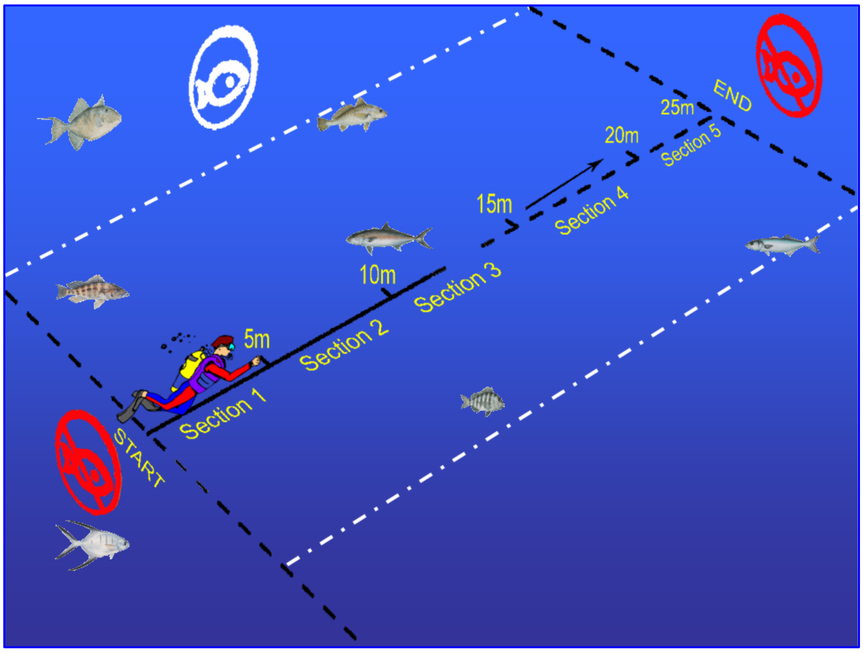


Figure 2.2: General scheme of visual *census* survey.

The reel layer follows the observer, laying the reel along the transect. The reel halyard is attached to a weight at the beginning of the transect.

During the scan of the section the most mobile target species are recorded, with progressively less mobile species recorded in subsequent counts. Fish entering the transect during, or after, the sampling of an area of transect are not included as they were not present during the initial count. Once the most mobile species have been counted the observer moves along the central line of the transect searching for the more cryptic and slower moving target species, being careful to include also individuals of the most mobile species which were obscured from view by the structure of the reef during the initial count of the area.

2.2.2.a TIMING OF *CENSUS*

Sampling is not performed in high activity periods as arly morning and late afternoon to reduce variability in fish densities (due to diurnal influences on behaviour). Sampling has been limited to the hours between 09.00 and 16.30 during winter months and between 08.30 and 17.00 during summer months.

2.2.2.b DATA RECORDING

In addition to abundance estimates of target species, a number of ambient parameters are recorded which describe the physical environment at the time of *census*. Before entering the water numerous parameters related to weather conditions and location are recorded on the data sheets, i.e:

1. Code: the code name of the site.
2. Transect: the number of the transect, where transect 1 is the first transect of a site encountered.
3. Date: the date of *census* in the format DD/MM/YY.
4. Observer: initials of the observer carrying out the *census*.
5. Cloud: measured as the fraction of the sky covered by cloud and expressed in quarters, e.g. 0/4 indicates a cloudless sky, 3/4 indicates that approximately three quarters of the sky are obscured by clouds.
6. Wind: wind direction.

7. Sea: sea state:
   - ☐ Calm: mirror-like to small ripples
   - ☐ Slight: large wavelets, crests breaking
   - ☐ Moderate: many white caps forming
   - ☐ Rough: large waves, 2-3m, white caps.
8. Sea: sea current
   - ☐ Absent: no current
   - ☐ Slight: no problems for divers
   - ☐ Moderate: diving is possible only in the current direction
   - ☐ High: diving is not possible.

Once in the water, the following data are recorded prior to commencing the survey of each transect.

1. Depth: recorded to the nearest metre at the start of each transect.
2. Start: the time at which the *census* begins for each transect, recorded in 24 hour notation e.g. 3.15 p.m. is recorded as 15.15.
3. Visibility: recorded when the observer first enters the water, prior to *census* and expressed in metres distance. This is recorded only once, unless it changes.

2.2.3 DATA MANAGEMENT

Due to the large volume of data collected during each survey trip, severe data management procedures must be followed to ensure safe and efficient storage of data.

The use of a laptop computer with data entry software is essential.

On the same day of the data collection, observe the following procedure:

Rinse data sheets in fresh water and then dry them.

Assign sample identification number to each transect.

Enter data onto laptop computer in the Access database (a user interface to Microsoft Access has been developed for this purpose).

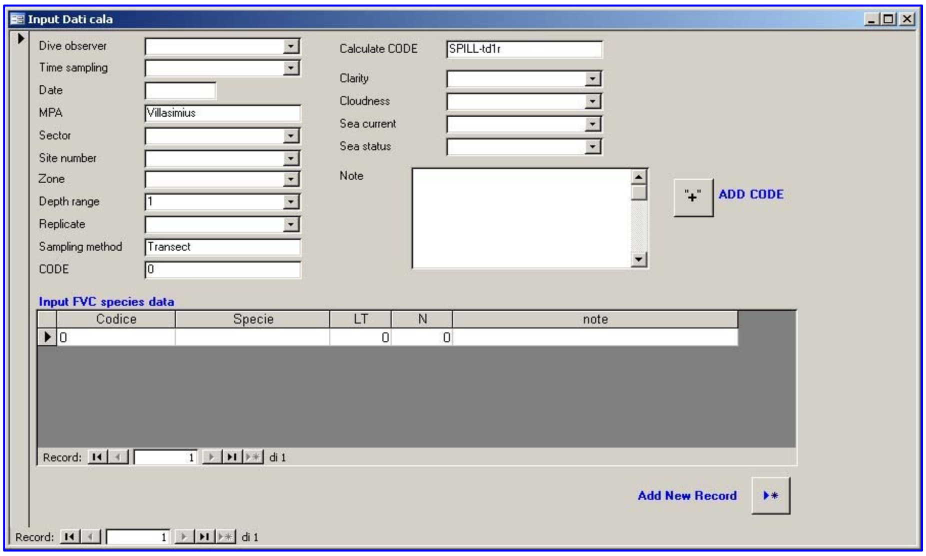


Figure 2.3: The input mask.

Fish species names are entered in the database.

In office, data are checked and added to the main database using the following procedure:

- Print raw data entered at sea and check against field data sheets. This checking procedure requires two personnel, one reads out the species and abundance data from the field sheets while the other checks these values against the print out of field entered data.
- Correct any error in the data and export to disk.
- Give disk to database manager for inclusion into the Access database.
- File field data sheets and data printout.

2.2.4 STATUS OF FISH STOCKS

A) Identifying and counting species

Identifying and counting species provide an estimate of species richness (e.g. the number of species), particularly for environmental inventories. This can be limited to a sector of the population for food and/or commercial purposes or it can be conducted from an ecological point of view. This is an important parameter to consider. Any appreciable attack on the environment, such as the destruction of habitat, usually brings about a decrease in species richness, which is an indicator of biodiversity (e.g. number of species, and their percentage in the population).

B) Counting individuals

Individuals are counted to estimate abundance (number of fish) and density (number of fish per unit surface area) (e.g. individuals per square metre). Abundance and density are factors that can be affected by fishing activities and so, in certain cases, are a reflection of fishing intensity.

This method tends to bring about an underassessment of density and biomass (Labrosse P. *et al*. 2002). There is not a level of underassessment but it can be minimized with a regular training.

As with abundance and density, mean fish sizes and biomasses are parameters that are affected by fishing activities, particularly with regards to the most heavily targeted species. For example, in the specific case of untouched or unexploited stock, the introduction of fishing activities will rapidly lead to a decrease in mean size and biomass for the largest and most long-lived species.

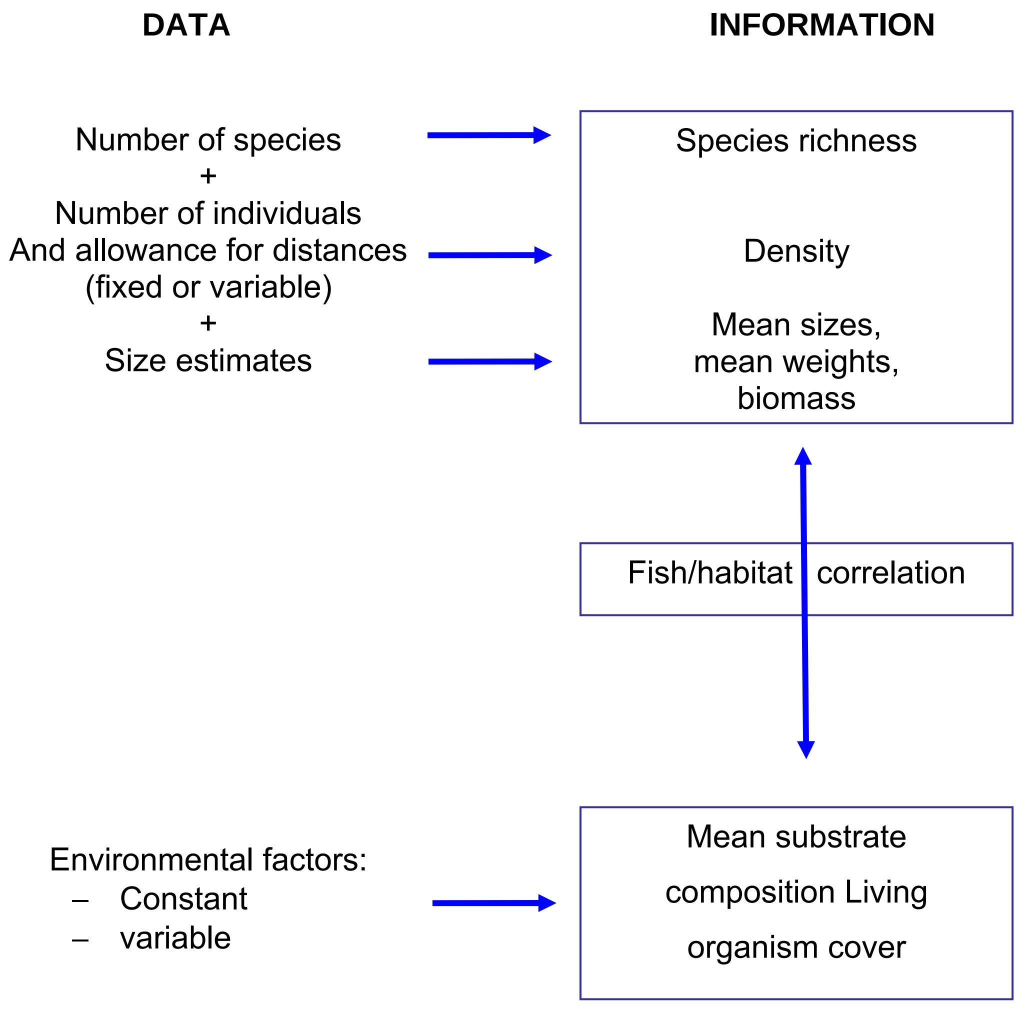


Table 2.2: Summary table

C) Statistical analysis

In order to gain an understanding of the community structure at and between sampling sites within the survey area, the following statistics indexes are applied to the data set for fish fauna.

LENGTH-WEIGHT RELATIONSHIP

Length-Weight relationships are important in fisheries science, notably to raise length-frequency samples to total catch, and to estimate biomass from underwater visual *census* length observations (Bohnsack J.A. and D.E. Harper, 1988).

LW is a mathematical formula for the weight of a fish in terms of its length; when only one of the two measures is known, the formula can be used to determine the other.

Typically given as:

$$W = a * L^b$$

Where W is the weight, **L** the total length, ***a*** and ***b*** are coefficients referred to the studied fish (http://www.fishbase.org).

The units of length and weight are centimetres and grams, respectively.

ECOLOGICAL INDEXES AND DATA COVERAGE

To analyse community structure, spatial and temporal variability are tested analysing ecological indexes and data coverage.

Biodiversity

Biodiversity, or species diversity, is the simplest measure of species richness; it describes the variety and richness of life on the survey area. This index makes no use of relative abundances. It is expressed as: Biodiversity richness: S

Species Richness

The Margalef richness index adjusts the number of species sampled in a reference area by the logarithm of the total number of individuals sampled, summed over species. The higher the Margalef index, the richer the diversity of the population.

The formula is:

Margalef richness: $d = \frac{S-1}{\ln N}$

Where S is the number of taxa and N is the number of individuals.

Shannon-Weaver index of diversity

The Shannon-Weaver index of diversity is simply the ecologist's definition of entropy expressed by:

Shannon-Weaver diversity: $H' = -\sum_{i=1}^{S} pi \log pi$

Where p_i is the fraction of individuals belonging to the i^{th} species. This is by far the most widely used diversity index.

The minus sign is used to get a positive result, since probabilities are always less than one, and the logs of numbers less than one are always negative.

This measurement takes into account species richness and proportion of each species within the local aquatic community. This diversity index measures the order (or the disorder) observed within a particular system. In ecological studies, this order is characterized by the number of individuals observed for each species in the sample.

Pielou Evenness Index

This evenness index, is a measure of how evenly distributed abundance is among the species that exist in a community. The Pielou index is defined between 0 and 1, where 1 represents a community with perfect evenness, and the index decreases to zero as the relative abundances of the species diverge from evenness. The Pielou index is calculated for each sample as:

Pielou index: $J = \frac{H}{\log S}$

Where H is the Shannon-Wiener Index for the sample and S is the number of taxa.

Bray - Curtis index

The Bray-Curtis index measures the degree of difference in community structure (especially community composition) between sites. This measure helps to evaluate the amount of dissimilarity between benthic invertebrate communities at different sites.

Bray - Curtis index $BC_{ij} = \frac{|n_{ik} - n_{jk}|}{(n_{ik} + n_{jk})}$

Simpson's Index of Diversity 1 - D

The index is a measure of the character of a community that takes into account both the abundance patterns and the taxonomic richness of the benthic invertebrate community. It is calculated from the proportion of individuals which belongs to each taxonomic group contribuiting to the total sample.

The value of this index ranges between 0 and 1, the greater the value, the greater the sample diversity. In this case, the index represents the probability that two individuals randomly selected from a sample will belong to different species.

Analysis of differences

Analysis of differences in fish assemblage structure was conducted using multivariate non-Metric Multidimensional Scaling ordination (MDS) and Bray-Curtis cluster analysis using the computer package PRIMER (Clarke K. R. and Warwick R. M., 1994). The Bray-Curtis similarity index was applied on square-root transformed data (to down-weight the influence of rare and extremely abundant species) generating a rank similarity matrix, which was then converted into an MDS ordination (Clarke K. R., 1993). To check on the adequacy of the low-dimensional approximations seen in cluster and MDS, the use of PRIMER v6.1.5 enabled clusters to be superimposed upon the MDS ordination (Clarke K. R. and Gorley R. N., 2006). One/two-way ANOSIM was used to investigate differences identified from MDS and cluster (Clarke K. R. and Warwick R. M., 1994). SIMPER analysis was used to ascertain the fish species that contributed most to the dissimilarity between sites and time.

2.2.5 SOURCES OF ERROR

No assessment method is perfect and Underwater Fish Visual censuses also include sources of error. Errors mainly come from one of this three sources: the observer, the fish behaviour, and the sampling method. Understanding these sources of error is vital for both minimising them, and taking them into account during analysis and interpretation of the results.

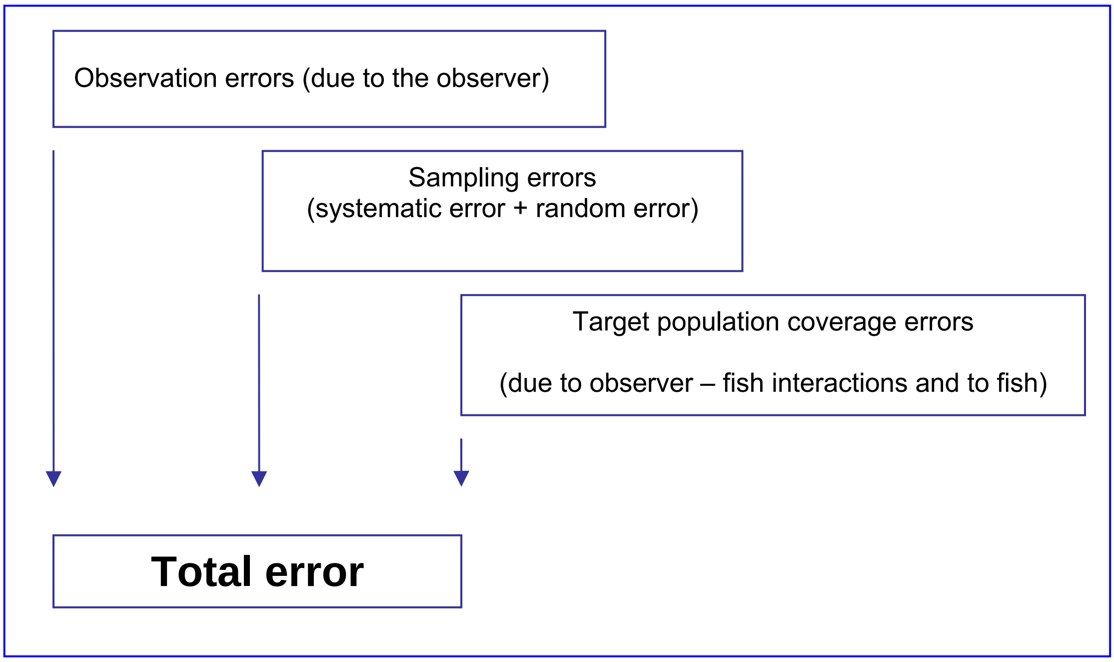


Table 2.3: Sources of error.

Sources of error due to the diver

Due to diving time restrictions and to the often shy nature of the animals surveyed, observers must be able to record informations as quickly as possible, and to rapidly identify and estimate sizes and distances with a reasonable level of accuracy. The slightest hesitation will result in a loss of data. Observers may pay more attention to one group of fish or to a part of the population that interests them more: this is a systematic error. Observers may also have a tendency to overestimate or underestimate sizes and/or distances. Moreover, there is always the risk of counting the same fish several times. Finally, for counts at variable distances, consideration must be given to the fact that fish detection ability tends to increase with size for almost all species, and with the size of the school for certain fish (number of individuals in a school).

Hesitation, inattention or paying too much attention to a certain area, are all increased when the conditions under which the *census* is being conducted worsen (e.g. strong currents, fatigue or cold) or when the quantity of information to be recorded is too great (too many species, too many fish). As a result, environmental, psychological, and physical conditions of observers' work should have as little influence as possible. This means that observers should master diving techniques and not to be subject to disturbances that reduce the acuity of their eyesight and/or their motor skills.

Sources of error due to observer–fish interaction

Such interactions mainly involve changes in fish behaviour due to the diver's presence. These changes vary and may result in either the fish fleeing away from, or being attracted to, the diver. For example, some species, such as those from the *Serranidae* genus, tend to be attracted to observers and follow them around. In contrast, *Sparus aurata* tends to keep away by remaining at the limit of visibility. Such behaviour also depends on the individuals' activity cycle (diurnal vs. nocturnal), age and location. The simultaneous trajectories of fish and observer can bring about either negative or positive biases in size estimation, depending on whether they are swimming in the same direction or in opposite directions with a visual angle that differs more than 90° from the transect.

The diver's movement as well as the method used, also has an influence on fish behaviour (e.g. the noise from air bubbles coming out of scuba diving equipment).

Regular visits to a site, particularly for monitoring, tend to decrease attraction or fleeing reactions, thus minimising these biases.

Sources of error due to fish

The main sources of error due to fish come from the distribution of species in time and space. This depends on different parameters associated with habitat, behaviour and activity cycles.

Certain species, which are sedentary during the day and that come out only at night, may not be detected by observers. Similarly those fish that come out of their hiding places only briefly, those that are highly mobile, or those which colonise certain biotopes during certain seasons can be missed. The probability of encountering species and thus being able to count them is influenced by their behaviour and their home range or territory. This is why it is difficult to make a comprehensive assessment of fish populations. It should also be noted that populations seen and sampled only account for a portion of all the species that live in the study area.

The various biological and ecological characteristics of fish influence measurements and estimates. Any interpretation of results must take into account that not all species are perceived (and therefore estimated) in the same way.

Sources of error due to sampling

Most sampling errors arise because results depend not only on the elements (all transects or stationary points) that make up the sample, but also on the method itself.

A sample is a limited subset of a population, from which the results obtained from the observed data are based. For technical, economic or simply logistical reasons (destruction of specimens, as when fish are caught by experimental fishing), it is normally not possible to collect data on the entire population. The study of a limited set makes it possible to increase both the number of measurements and their degree of accuracy.

Extrapolation of the findings obtained from sampling generally results in estimates for the entire population, which have a reasonable level of accuracy. If two samples composed of a given number of elements (set of transects or stationary points) are observed, the measurements calculated for each will be different, but they will result in comparable estimates of population parameters. The statistical population is defined as a set of entities on which statistical inferences and conclusions are based. Samples not taken according to a strict sampling plan (random or reasoned) will not be representative of the target fish population. The sample is considered to be equal to the statistical population.

The position of a transect and orientation can be considered sources of error associated with sampling. It is preferable a transect that covers an homogeneous environment, rather than one covering several different environments. Transitional areas between different biotopes should be therefore strictly avoided.

The accuracy of population estimates depends on the size of the sample (number of transects) and variability (differences between the measurements for each transect). This variability in individual measurements or random error (dispersion) must not be mistaken for systematic error (bias).

Four model situations are given:

Lack of accuracy can be linked to high bias and/or high dispersion.

Sampling-related bias can be reduced through a random selection of sample elements.

Errors in observation and representativity do not decrease when the size of the sample increases.

Dispersion depends on the population's heterogeneity. It is measured by variance.

When dispersion is high (i.e. there are significant differences between transects), better estimates will be gained by stratifying the population (e.g. by biotope).

How to limit sources of error

Firstly, new observers are trained, in situ, in the identification of the target species, and in the standard technique for visual *census* of belt transects. Secondly, experienced observers are continually standardised to minimise inter-observer bias.

Moreover, new observers and experienced observers must keep in mind that divers should have regular training to minimise errors caused by poor diving techniques.

The fish identification is the first step of training. The first key to identify fish is their shape, which is generally the same for almost all the species in a given family. The visual morphological characteristics that identify a species within a family are shape, colour of markings, and distinctive traits such as spots, lines or stripes, and their location on the body and/or fins. Behaviour and preferred biotopes are also useful for identification. Learning and retention of these features are necessary, though they are perhaps the most tedious part of training. The appearance of some fish changes over the life cycle; for example, many Perciformes like *Coris julis*, have different colours during their juvenile and adult phases and colours can differ according to sex.

Training in fish identification involves classroom - learning using available tools, and onsite exercises during dives. In order to avoid confusion over the use of common names, it is preferable to use each species' scientific name, which is always made up of a genus name (e. g. *Coris*, the genus) followed by a species name (e. g. *julis*); thus forming the name *Coris julis*).

A list of fish , which are of food and commercial interest, is given in.

If fish from a certain family cannot be precisely identified during a dive, the observer must rapidly note its main features (e. g. shape, colours, and markings) so as to be able to complete the identification through books afterwards. The use of simple sketches to illustrate and record specific marks on the body is invaluable.

Identification skills are further enhanced with underwater coaching where an experienced observer points out target species and highlights physical characteristics, habitat preferences and behavioural patterns that will aid in quick and accurate identification.

Difficulties in counting fish are mainly due to the limitations of the human eye, which can only count four objects at any one time.

Moreover, precise counting cannot be carried out on more than 10 to 20 individuals in a relatively sedentary school. Taking into account these limits, and in order to compensate for them, the most commonly used technique for counting schools is the so-called group-counting method. This consists of counting a shoal of 10 to 20 fish. This group becomes the basic counting unit and the observer judges how many groups there are in the entire area occupied by the school of fish. For large schools (more than 200 individuals), it may be useful to combine groups into super-groups, containing 5 to 10 base groups.

In more complex instances where a shoal of fish is made up of several different species (multispecies shoal), observers begin with the count of the most numerous species. The same applies to shoal with a range of sizes. During training, taking photographs is a good way to evaluate errors made, and to find out at what level they occurred.

Observers undertake annual standardisation exercises to maintain significantly close concordance in their counts. The procedure used for inter-observer standardisation is identical to that outlined above for the training of observers in the visual *census* technique.

2.3 SAMPLING DESIGN

Sampling sites were chosen in the pre-survey after eight months of diving (first eight months of the 2004).

Sampling time were scheduled as follows: Time 1: September – November 2004; Time 2: June – July 2005; Time 3: September – November 2005; Time 4: June – July 2005.

Six sectors of the reef fish communities were surveyed every six months (in autumn and spring) within and around the MPA (Cala Pira, Isola di Serpentara, Capo Carbonara, Isola dei Cavoli, Solanas and Torre delle Stelle sectors). The sampling sites are the places where the surrounding area was best represented (the analyzed site had the ecosystemic characteristic of the entire area).

Habitat was surveyed on each reef. It is described as the first stretch of continuous reef with a slope less than vertical. Similar habitats were selected to allow comparisons between sectors.

Figure 2.4: Sampling sites.

Transects were set within the sectors, along the middle of the reef slope (usually at a depth between two and eight metres). A total of eight replications in every transect were made. Each replication was 25 metres long and four metres wide (two per each side).

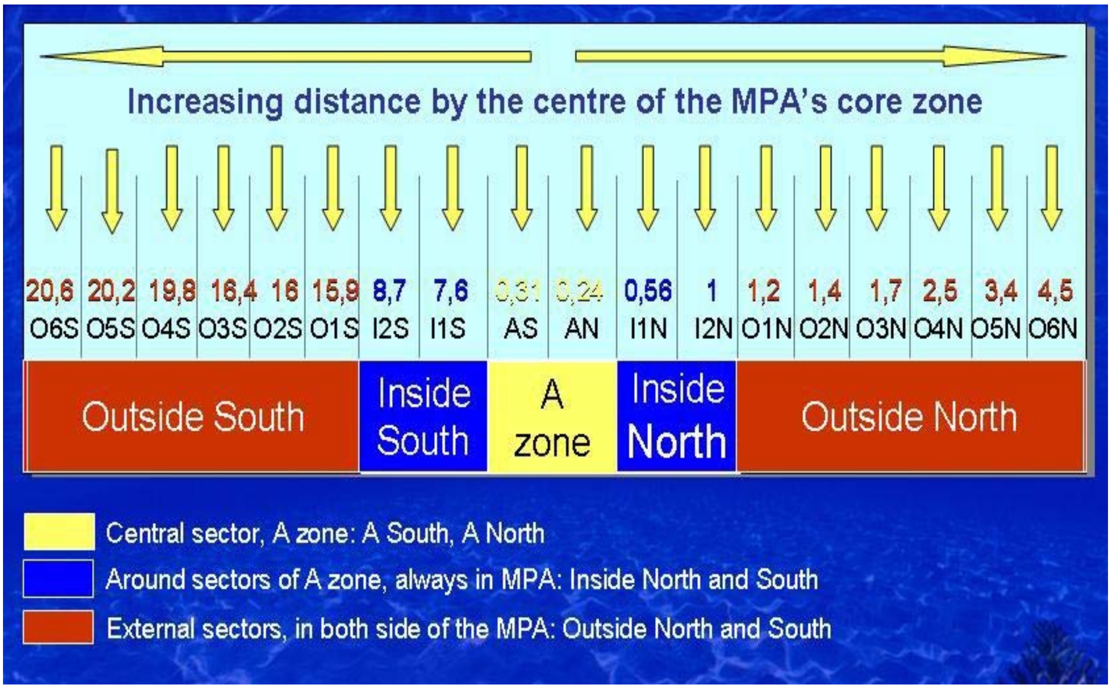


Figure 2.5: Fish visual *census*, distances by the center of the core zone.

The sampling sites are shown in figure 2.4: Yellow tags represent the sites in the central sectors, the core zone (A zone); Blue tags the sites inside the MPA (in B or C zones); Red tags the sites located out of the MPA border.

Transects are at increasing distance from the core zone.

The exact latitude and longitude of the sampling sites are reported in table 2.3.

ACRONYM	POSITION	ACRONYM	POSITION
A1N	N39 09.138 E9 36.171	O2S	N39 07.321 E9 26.269
A1S	N39 08.147 E9 36.187	O3N	N39 10.011 E9 34.236
I1N	N39 08.182 E9 36.373	O3S	N39 07.501 E9 26.087
I1S	N39 05.052 E9 32.361	O4N	N39 10.586 E9 34.776
I2N	N39 08.823 E9 33.997	O4S	N39 08.606 E9 24.188
I2S	N39 05.908 E9 31.004	O5N	N39 11.025 E9 34.692
O1N	N39 09.363 E9 34.051	O5S	N39 08.498 E9 24.107
O1S	N39 07.399 E9 26.392	O6N	N39 11.269 E9 34.101
O2N	N39 09.697 E9 34.131	O6S	N39 08.534 E9 23.906

Grid Lat/Lon ddd°mm.mmm'; Datum: WGS 84

Table 2.3: Latitude and longitude of the sampling sites.

To study fish fauna with visual *census* technique, a set of 24 targets species has been selected. All of them are usually object of fishing activities inside and outside the MPA.

TARGET *SPECIES*		
Coris julis	*Labrus merula*	*Scorpaena porcus*
Dentex dentex	*Labrus viridis*	*Scorpaena scrofa*
Dicentrarchus labrax	*Lithognathus mormyrus*	*Serranus cabrilla*
Diplodus puntazzo	*Mullus surmuletus*	*Serranus scriba*
Diplodus sargus	*Pagrus pagrus*	*Sparus aurata*
Diploids vulgaris	*Sarpa salpa*	*Sphyraena sphyraena*
Epinephelus costae	*Sciaena umbra*	*Symphodus tinca*
Epinephelus marginatus	*Scorpaena notata*	*Thalassoma pavo*

Table 2.4: Fish visual *census*; target species.

In four Times of sampling, a total of 432 replications were performed: 144 inside the MPA (48 in the A zone and 96 inside the sanctuary), and 288 outside.

DATI FVC : Tabella

Specie	LT	N	CODICE
Symphodus tinca	14,0	1	SPILL-t1A1Nd1r8
Symphodus tinca	15,0	1	SPILL-t1A1Nd1r8
Diplodus puntazzo	20,0	1	SPILL-t1A1Nd1r8
Diplodus puntazzo	14,0	1	SPILL-t1A1Nd1r8
Diplodus vulgaris	18,0	1	SPILL-t1A1Nd1r8
Diplodus vulgaris	20,0	1	SPILL-t1A1Nd1r8
Diplodus vulgaris	18,0	20	SPILL-t1A1Nd1r8
Serranus cabrilla	16,0	1	SPILL-t1A1Nd1r8
Serranus scriba	16,0	1	SPILL-t1A1Nd1r8
Serranus scriba	16,0	1	SPILL-t1A1Nd1r8
Serranus scriba	16,0	1	SPILL-t1A1Nd1r8
Serranus scriba	18,0	1	SPILL-t1A1Nd1r8
Serranus scriba	14,0	1	SPILL-t1A1Nd1r8
Serranus scriba	25,0	1	SPILL-t1A1Nd1r8
Serranus scriba	14,0	1	SPILL-t1A1Nd1r8
Sarpa salpa	18,0	18	SPILL-t1A1Nd1r8
Pagrus pagrus	25,0	1	SPILL-t1A1Nd1r8
Coris julis	6,0	1	SPILL-t1A1Sd1r1
Coris julis	12,0	1	SPILL-t1A1Sd1r1
Coris julis	15,0	1	SPILL-t1A1Sd1r1
Coris julis	15,0	1	SPILL-t1A1Sd1r1
Coris julis	18,0	1	SPILL-t1A1Sd1r1
Thalassoma pavo	12,0	1	SPILL-t1A1Sd1r1
Thalassoma pavo	6,0		SPILL-t1A1Sd1r1
Thalassoma pavo	12,0	1	SPILL-t1A1Sd1r1
Thalassoma pavo	16,0	1	SPILL-t1A1Sd1r1
Symphodus tinca	14,0	1	SPILL-t1A1Sd1r1
Symphodus tinca	16,0	1	SPILL-t1A1Sd1r1
Symphodus tinca	18,0	1	SPILL-t1A1Sd1r1
Diplodus sargus	15,0	1	SPILL-t1A1Sd1r1

Record: 1 di 4309

Figure 2.5: Example of recorded data.

A total of 6156 records were carried out and distinguished by species, size, amount and unequivocal sampling code.

2.4 RESULTS AND DISCUSSION

Preliminary snorkel and SCUBA surveys were used to create a list of target key species (in particular commercially important species) of fish found within all of the study sites.

The list of the 24 target species subdivided by taxonomic rank is scheduled in the appendix 5.

The 24 target species are listed by presence in the three sampling Zones.

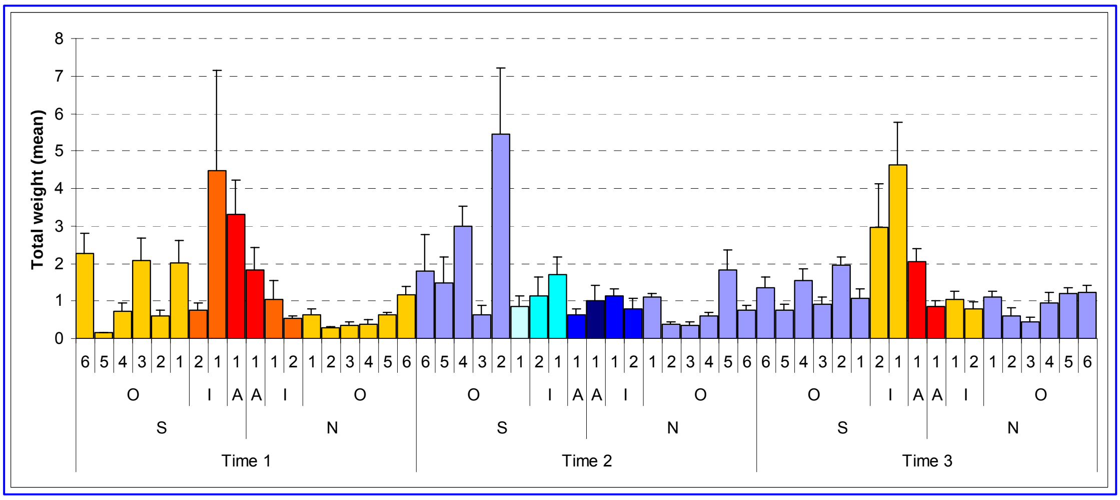
TARGET *SPECIES*	A ZONE	INSIDE	OUTSIDE
Coris julis	+	+	+
Dentex dentex		+	+
Dicentrarchus labrax	+		
Diplodus puntazzo	+	+	+
Diplodus sargus	+	+	+
Diplodus vulgaris	+	+	+
Epinephelus marginatus	+	+	+
Labrus merula	+		+
Labrus viridis	+		+
Mullus surmuletus	+	+	+
Pagrus pagrus	+	+	+
Sarpa salpa	+	+	
Sciaena umbra		+	+
Scorpaena notata	+	+	+
Scorpaena porcus		+	+
Scorpaena scrofa			+
Serranus cabrilla	+	+	+
Serranus scriba	+	+	+
Sparus aurata		+	+
Sphyraena sphyraena	+	+	
Spondyliosoma cantharus	+	+	+
Symphodus tinca	+	+	+
Thalassoma pavo	+	+	+

Table 2.5: The 24 target species occurred in the three sampling Sites.

In three years of sampling, a total of 8550 specimen were observed in the studied area.

Mean weight

The output of elaborated data from Length-weight relationships used for the studied target species was processed to obtain the mean weight of biomass for Sites in A zone (A) of the MPA, Inside the MPA (I) and Outside (O). These results showed high values of biomass in the inside zone in autumn season.



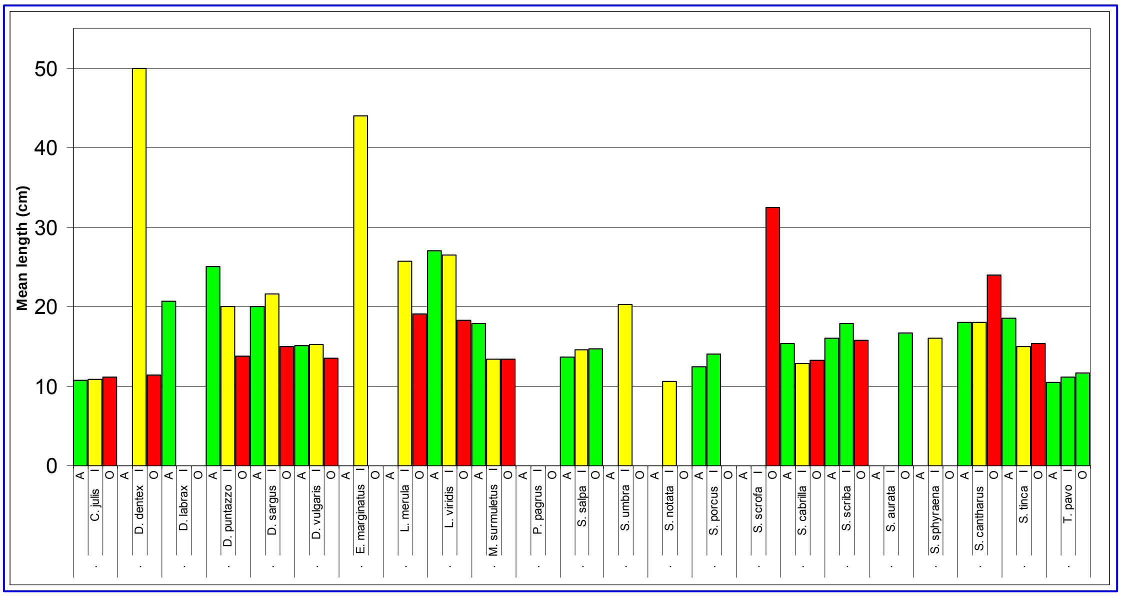
Graph 2.1: Total mean weight from the Core Zone (A, North and South), Inside Zone (I, North and South) to Outside of the boundaries (O, North and South); all weight are listed by Time of sampling (Time 1-2-3).

It is due to the fry. During this season, mean fish biomass was high in A Zone and spillover effect resulted from A Zone (Time 1) to Outside in the southern sampled sites in Time 2; what was more, a general increase in all the studied sites was observed from Time 1 to Time 3 indicating the effectiveness of the established MPA.

Mean weight of all the samplings scheduled by specimen are listed in appendix 6.

MEAN LENGTH

The processed data of species length were analyzed for every sampled site. High value of the mean length of the target fishes was obtained within the A zone. Predators as *Dentex dentex* and *Epinephelus margiantus* showed larger values within the inside zone (I) compared to the outside zone (O) and A zone inside the MPA.

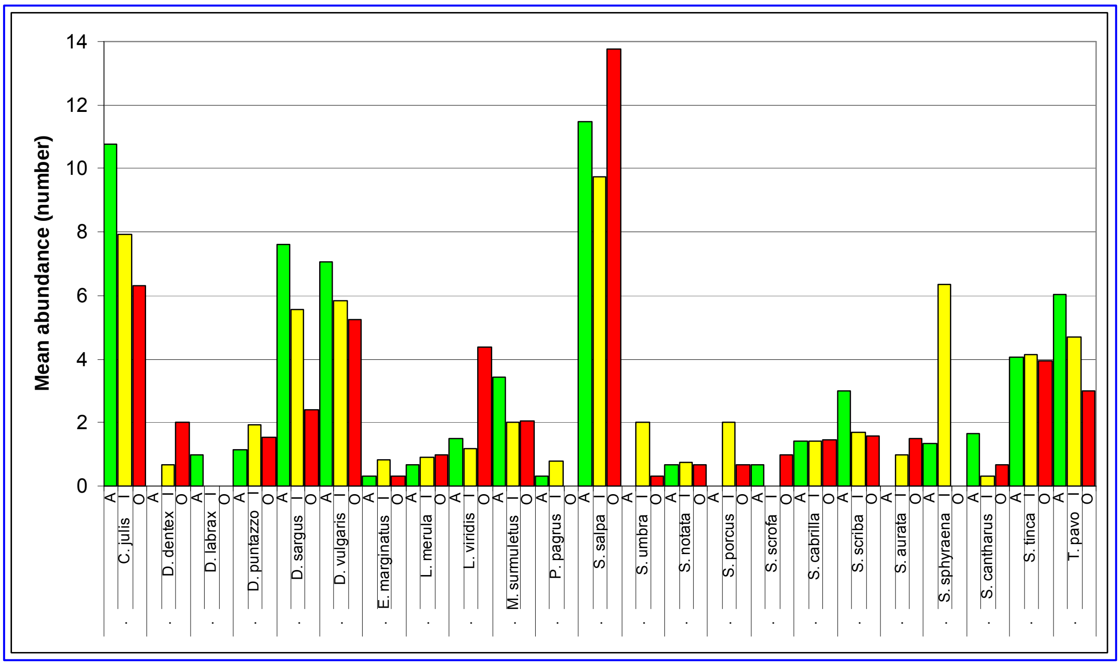


Graph 2.2: Mean length of the target species.

Mean length for the herbivore like *Sarpa salpa* (Sparidae) was similar in all the studied areas.

MEAN ABUNDANCE

In general Species' abundance was similar within the 3 studied zones. Some species as *Coris julis* were well represented in the inside zone (I).



Graph 2.3: Mean abundance of the species (in number).

Mean abundance of herbivore species like *Sarpa salpa* was high Outside (O) (13,6 species), inside (I) and in A zone (A).

C. Julis showed a mean value of 11 specimens within A zone, while its abundance decreased both in Inside and outside zones.

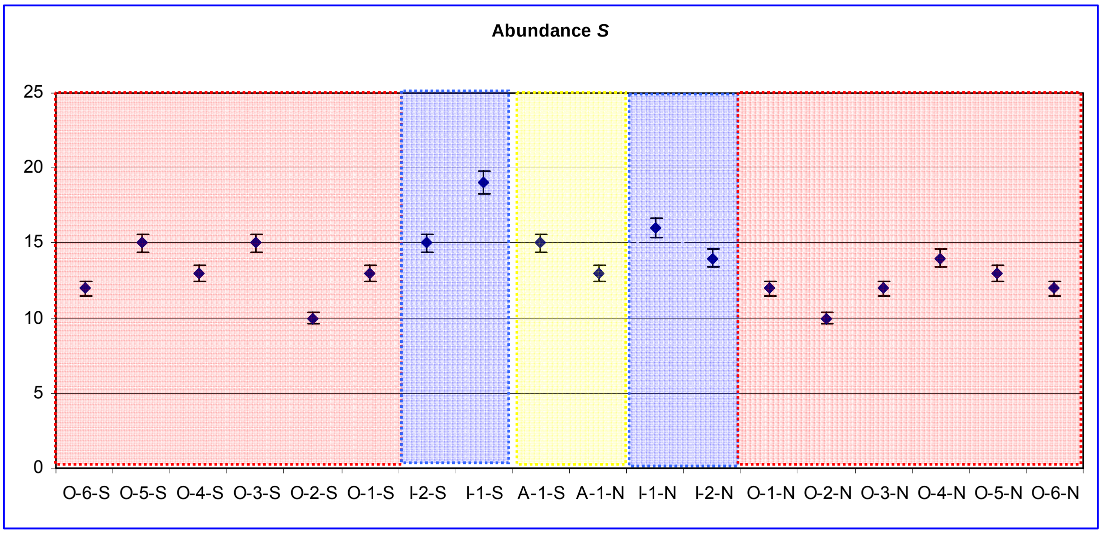
Mean abundance of *Dentex dentex* (2 specimen) was higher in the outside (O) zone than in the other sites.

High abundance of the species *Sphyraena Sphyraena* (6,5 specimen) was recorded in the inside (I) zone.

Mean abundance values of the species most targeted by fishers, belonging to the genera of *Diplodus* (mainly *D. sargus D. vulgaris)* and *Serranus (S.cabrilla, S.scriba*) resulted to be increasing in the MPA. This is a positive value due to the reserve effect as described by many authors (Bell J. D., 1983; Francour P., 1994; Harmelin M. *et al.*, 1995).

<u>ABUNDANCE</u>

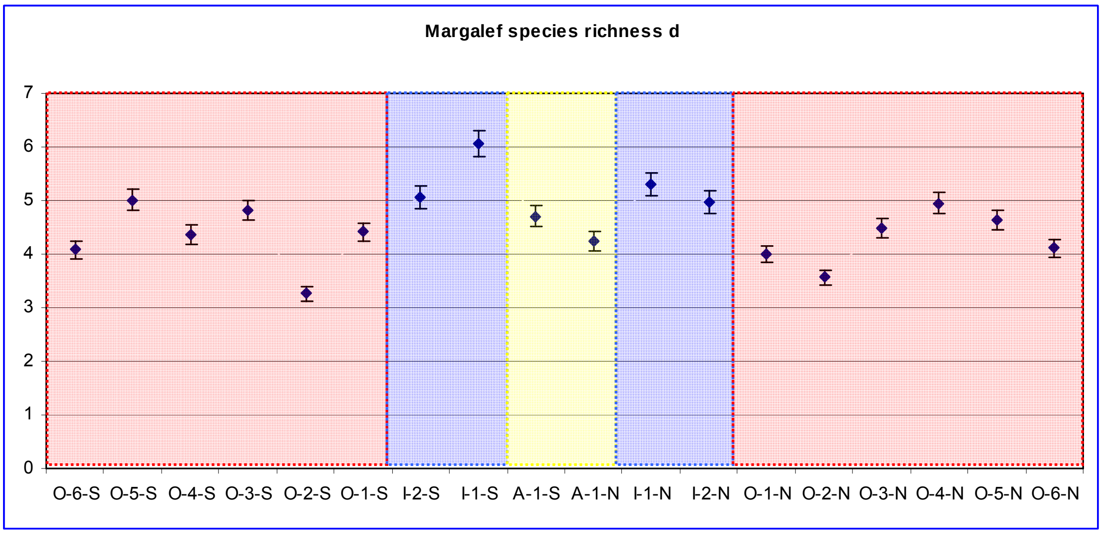
The analysis of Species' abundance along the different sites, point out high values for sites inside (see figure 2.4), (highest values can be observed in the site Inside (1S – Capo Boi). A regression in abundance is evident in either the Inside North (Isola di Serpentara, Punta Is Proceddus) and South sites (Capo Carbonara, Isola dei Cavoli) and the first two sites outside (O1N – O2N Cala Pira, O1S – O2S Capo Boi). A general increase of abundance can be observed outside from sites 3 to 6 (Outside North: Cala Pira and South: Capo Boi-Torre delle Stelle) suggesting a spillover outcome.



Graph 2.4: Abundance of species in the sampled areas.

SPECIES RICHNESS INDEX

Species richness indexes were higher in the inside North (I1N – Isola di Serpentara; I2N – Punta Is Proceddus) and South zone (Torre delle Stelle) of the MPA.

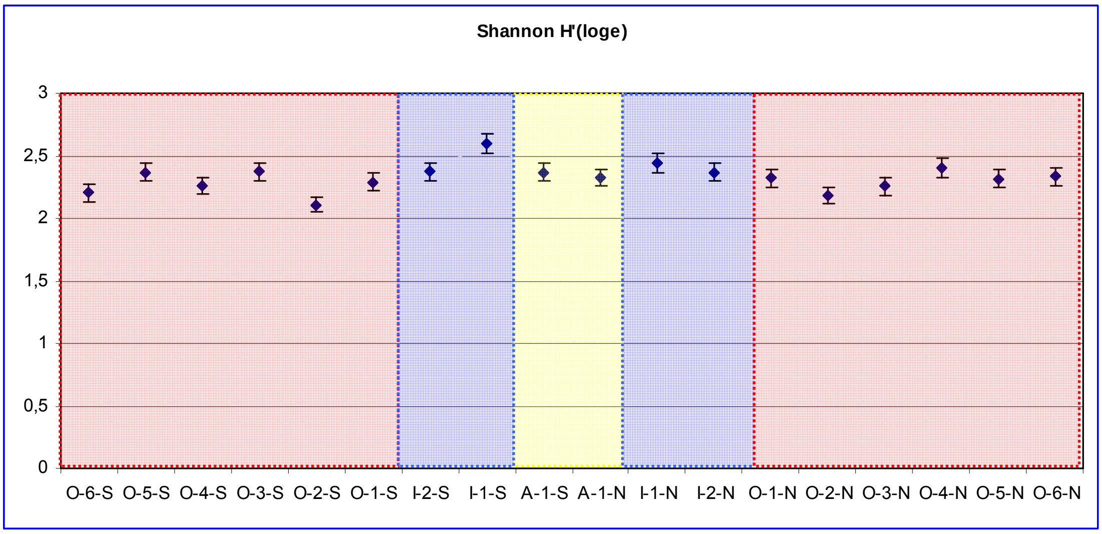


Graph 2.5: Trend of the diversity from the A Zone to Outside South and North.

Richness decreased in the boundaries but increased in the centre of the outside (O) areas (Outside North – Cala Pira and Outside South – Torre delle Stelle). This pattern could demonstrate the reserve effect because of the presence of fishing activities near the boundaries outside.

SHANNON-WEAVER DIVERSITY INDEX

Shannon Weaver takes into account the degree of evenness in species abundances.

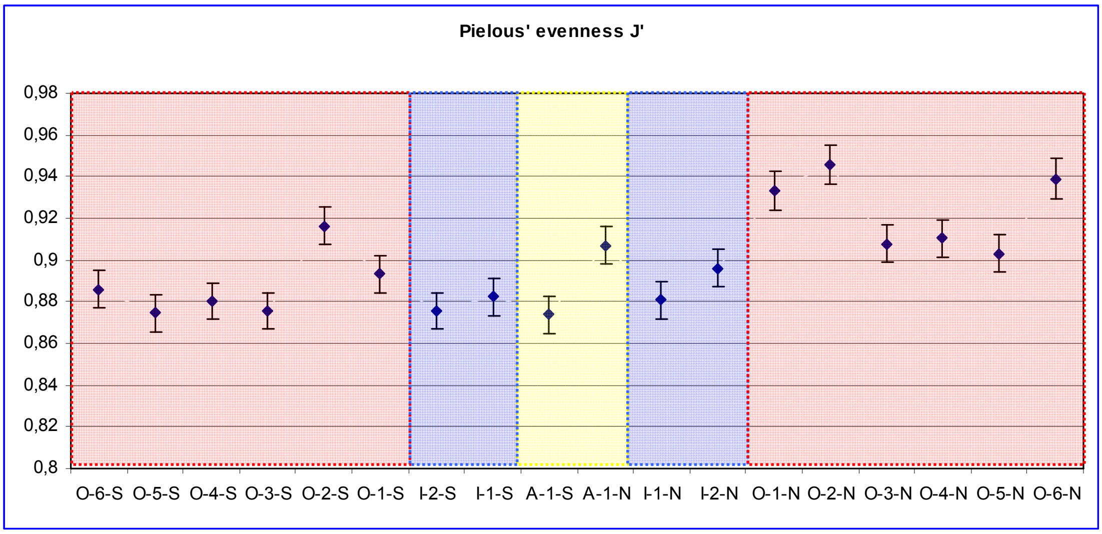


Graph 2.6: Diversity index from 2,1 to 2,6 in the studied areas.

High evenness degree was registered in all the MPA, with higher values in the inside (I1S – Capo Carbonara; A1N Isola di Serpentara) sites.

PIELOU EVENNESS INDEX

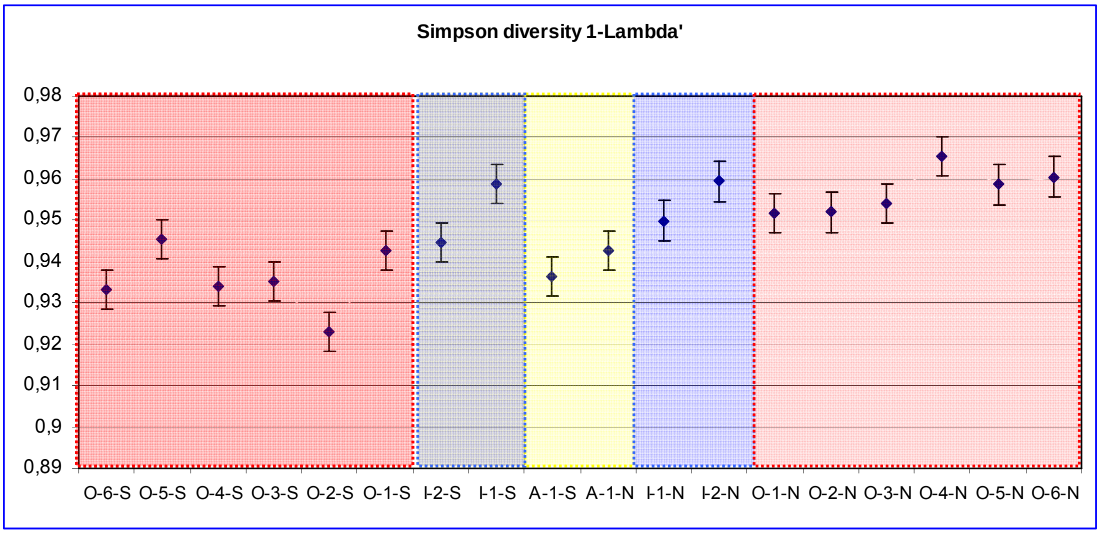
The distribution of the abundance among the species in the community is nearly 1 only in the site outside 02N (Cala Pira); in the other sites evenness is always high and it ranges from 0,87 (A1S – Capo Carbonara) to 0,93 (O6N – Cala Pira).



Graph 2.7: Evenness is nearly one in the site 02N (Punta is Proceddus).

SIMPSON'S INDEX OF DIVERSITY

Analysis of Simpson indexes highlighted higher diversity within the A zone, Inside (Punta Is Proceddus – Isola di Serpentara); Outside in northern zones (Cala Pira) and Inside (Capo Carbonara and Isola dei Cavoli).



Graph 2.8: High values of Simpson diversity in all the sites and higher from the centre to the Northern sites.

It must be pointed out that northern sites are near Cala Pira where the topology of the shore does not present any refuge for boats, or divers, moreover navigation is difficult within the zone because of the presence of many shoals at shallower waters.

UNIVARIATE AND MULTIVARIATE ANALYSIS

Analysis of similarity (ANOSIM) randomization test indicated significant global R value (R= 0,046), but not significant P value (P>3%), confirming weak differences between sites at **Time 1**.

Analyzing similarity by group, sites Outside in the North were similar to those in Outside South (R=0,087; P=17,5%).

The average dissimilarity of all pairwise coefficients with sites inside the sanctuary and outside in the North was 34,84. *Coris julis* contributed with 7,29 and a further 5,57 came from *Diplodus vulgaris*, representing 20,92% and 16%, respectively, of the overall value of 34,84, with cumulative values 20,92% and 36,92%.

Analysis of average dissimilarity between sites inside the sanctuary and outside in the South was 35,58. Of this, 11,00 was contributed by *Sarpa Salpa*, 5,17 by *Coris julis* and a further 4,35 by *Diplodus vulgaris,* corresponding to 30,92%, 14,52% and 12,22%, respectively, of the overall value of 35,58, with cumulative values 30,92%, 45,44% and 57,66%.

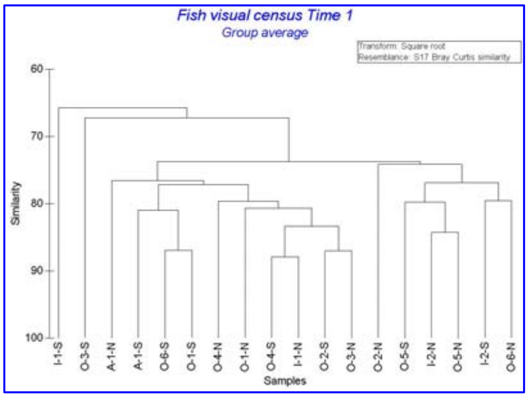
At Time 2, analysis of similarity (ANOSIM) randomization test indicated significant global R value (R= 0,323), and significant P value (P=0,1%) demonstrating that the composition of the fish biomass varied significantly between inside and outside sites.

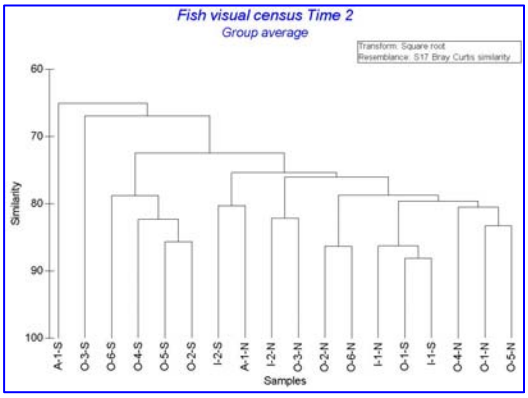
The average dissimilarity of all pairwise coefficients with sites inside the sanctuary and outside in the North was 26,70, composed of a 3,35 contributed by *Coris julis*, 3,12 by *Thalassoma pavo* and 3,09 by *Sarpa salpa*, with a contribution of 12,56%, 11,68% and 11,57%, respectively, to the overall value of 26,70, with cumulative values 12,56%, 24,24% and 35,81%.

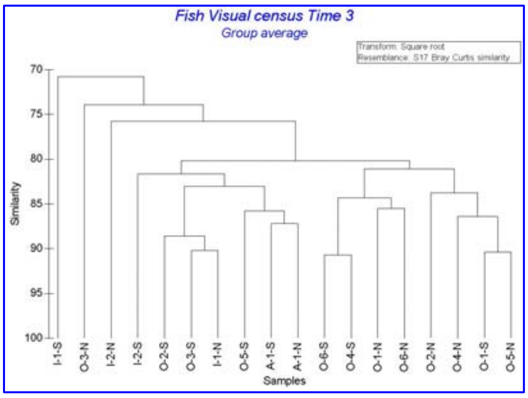
Analysis of average dissimilarity with sites inside the sanctuary and outside in the South was 28,24, with a 5,56 contributed by *Sarpa Salpa*, 3,45 by *Diplodus vulgaris*, 2,82 by *Diplodus sargus,* 19,68%, 12,22% and 9,97%, respectively, of the overall value of 28,24, with cumulative values 19,68%, 31,90% and 41,87%.

At Time 3, analysis of similarity (ANOSIM) randomization test indicated significant global R value (R= 0,145), and significant P value (P=1,5%) confirming that the composition of the fish biomass varied significantly between inside and outside sites.

The average dissimilarity of all pairwise coefficients with sites inside the sanctuary and outside in the North was 24,67 and







Cluster analysis showing changes in similarity from Time 1 to Time 3.
Analysis compared data from the sites inside and outside the sanctuary.
From Time 1 to Time 3, a general increase of similarity occurred.

Graphs 2.9-2.10-2.11: Cluster analysis.

1,40 of the dissimilarity was contributed by *Sarpa salpa*, 1,67 by *Thalassoma pavo* and 1,31 by *Diplodus vulgaris*, with a contribution of 16,32%, 10,76% and 8,73%, respectively, to the overall value of 24,67, with cumulative values 16,32%, 27,08% and 35,81%.

Analysis of average dissimilarity with sites inside the sanctuary and outside in the South was 19,65, with *Sarpa Salpa* contributing with a 2,68, 2,25 by *Thalassoma pavo*, 1,77 by *Symphodus tinca,* 13,63%, 11,46% and 9,01% respectively, of the overall value of 19,65, with cumulative values 13,63%, 25,09% and 34,10%.

The results demonstrate the evidence of reserve effect. Inside the sanctuary and at the three times of census, the carnivores and herbivores species of fish fauna confirm the good health of the food chain through:

1. The abundance; high values were recorded in 16 of 24 target species (*Coris julis, Diplodus puntazzo, Diplodus sargus, Diplodus vulgaris, Epinephelus marginatus, Labrus merula, Labrus viridis, Mullus surmuletus, Pagrus pagrus, Sciaena umbra, Scorpaena notata, Scorpaena porcus, Serranus scriba, Sphyraena sphyraena, Symphodus tinca, Thalassoma pavo*)
2. The length; more considerable for 20 of 24 species: *Coris julis, Dentex dentex, Dicentrarchus labrax, Diplodus puntazzo, Diplodus sargus, Diplodus vulgaris, Epinephelus marginatus, Labrus merula, Labrus viridis, Mullus surmuletus,, Sarpa salpa, Sciaena umbra, Scorpaena notata, Scorpaena porcus, Scorpaena scrofa, Serranus cabrilla, Serranus scriba, Sphyraena sphyraena, Spondyliosoma cantharus, Symphodus tinca).*

3. The total biomass, increased from time 1 to time 3 in all the sampled sites.
4. The diversity; it was higher in the A zone than Inside.
5. Species diversity
6. Richness; significantly high in the A zone and Inside.

The general increase in all parameters outside far from the boundaries can be read as a spillover effect from the sanctuary. It is confirmed by the abundance of herbivores outside such as inside and by the presence of hunter adult's species and hunted species. Besides the significant presence of different species in the outside sites is attributed to the sum of spillover effect to the natural protection of the substrata's orography.

The evidence of spillover effect from the MPA to the outside sites comes from:

1. The increase of biomass from Time 1 to Time 2.
2. The abundance, species richness and evenness in the sites outside, far from the boundaries.
3. The ANOSIM; a general increase of similarity occurred from Time 1 to Time 3 in all the analyzed sites.

Sites inside resulted more productive than other studied sites outside. This justifies fishing sport activities and underwater recreational activity in these areas.

These patterns are broadly in accord with those detected by the more numerous studies of reef habitats outside the Mediterranean Sea (Garcia Charton J.A. *et al*., 2000).

Outside near the boundaries of the sanctuary a general decrease of all recorded parameters was observed, indicating fishing activities.

What is more evident is the composition of fish biomass varying significantly between the inside and outside zones of the sanctuary.

In addition fish assemblages differed during different Times of sampling.

Time 1 was characterized by similarity between sites inside and outside whereas Time 2 and 3 by dissimilarity of the fish assemblages.

All recorded data point out a positive trend of the sanctuary's efficiency in the medium term (5-15 years); other effects can be seen in long terms (Garcia Charton J.A. *et al*., 2000).

3 TRAMMEL NET FISHING

Trammel net fishing is the other technique used in this work to study the state of fish fauna.

Figure 3.1: Fisherman dropping nets.

A trammel net (trammel means to hinder or entrap) generally consists of parallel layers of net suspended to a common lead line (Murphy B. R. and Willis D. W., 1996).

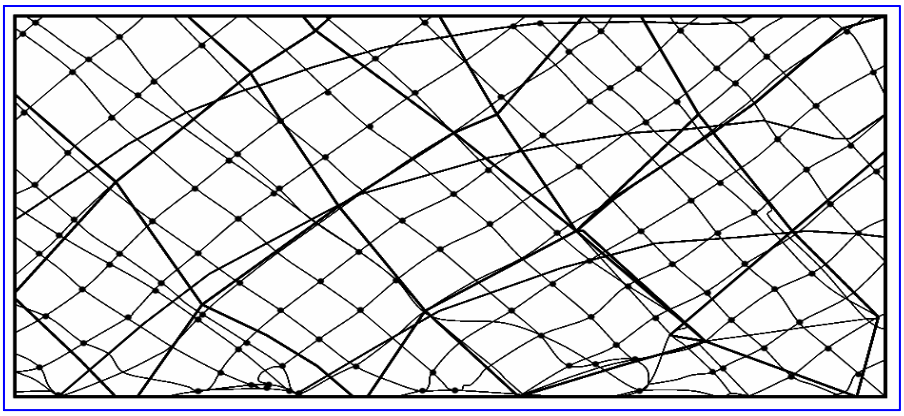


Figure 3.2: The large meshed guard nets and the inner smaller meshed web size

A slack, small mesh, inner panel of net is sandwiched between two outer layers of net, which are tight and have a larger mesh (three times the size of the centre net's mesh fig 35). The inner panel may be made of twisted or monofilament nylon, while the outer panels are generally made of twisted nylon filament.

Trammel nets are held vertically in the water by weights on the bottom (lead line), and floats on the top (float line). According to their design, these nets may be used to fish near the surface, midwater or for bottom fishing, either in inland or marine waters. Trammel nets are used to catch demersal, benthic and pelagic species.

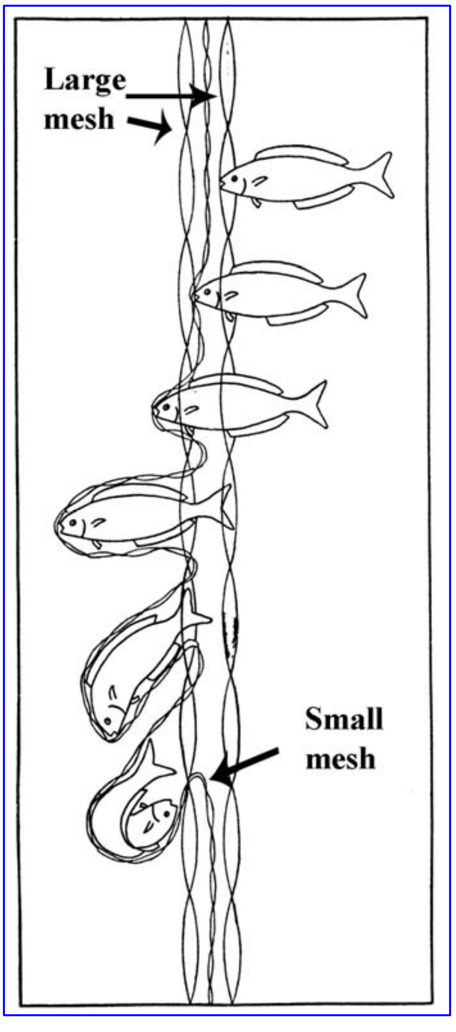


Figure 3.3: Net trapping.

Trammel nets entangle fish in three different ways. The fish may become wedged, held by the mesh around the body; gilled, caught by the gills; and tangled, held by teeth, spines or other protrusions without necessarily penetrating the mesh.

In addition, trammel nets entangle fish in bags or pockets of netting. This occurs when fish swim through one of the outer panels, hit the inner panel, and carry through to the other outer panel, creating a bag or pocket which traps the fish itself.

3. 1 FISHING TECHNIQUES

When the fishermen reach a favorable fishing ground or bank, they set their nets. The best fishing times are early morning and evening, when the fish seem to be more active. Therefore, the fishermen endeavour to have their nets set at the proper time every twelve hours. Nets are put out in gangs. At both ends of the gang, attached to the cork line, then down to the lead line, there are ropes presenting heavy anchors or weights at the bottom extremity (fig 3.4).

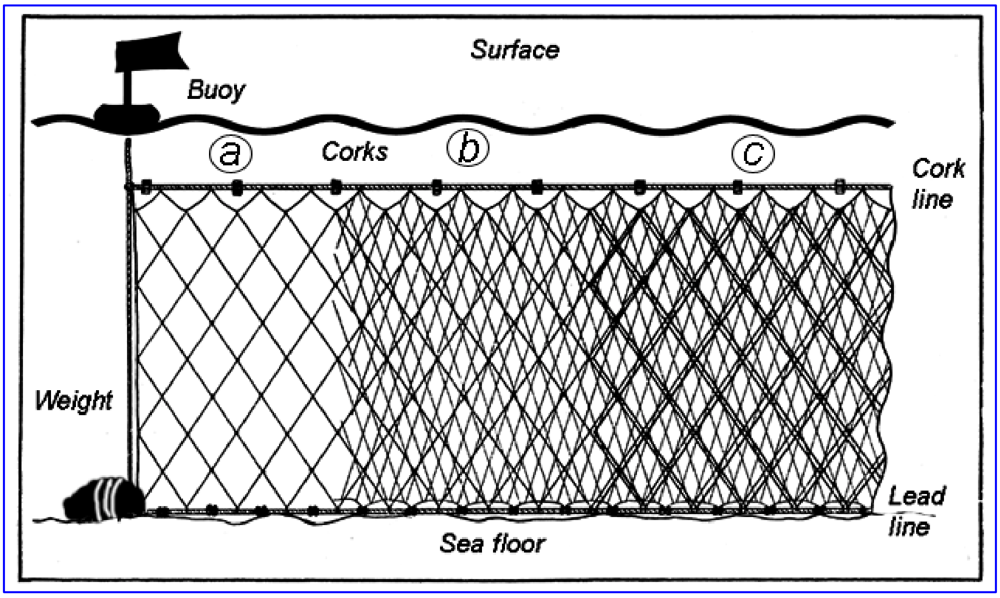


Figure 3.4: Fishing trammel net: (a) one guard mesh; (b) guard mesh and the smaller inner webbing, (c) both guard meshes and the inner net in place.

When setting a gang of nets, one end is put out with an anchor and a buoy attached; then the nets are thrown overboard gradually.

Figure 3.5: Lifting net operation.

Usually the nets are left in place for 12 to 24 hours before they are inspected for fish.

In taking up the nets, a fisherman takes hold of the buoy and lifts that end of the net and the anchor on board; then, while he hauls in the cork line, another man brings in the lead line, while a third picks the fish from the net.

3 1.1 STATUS OF FISH STOCKS

The study of the status of fish stocks with trammel net fishing took advantage of the same sampling method used for visual *census* in both sampling sites within the survey area. The same statistics indexes were applied to the acquired data.

3.2 MATERIALS AND METHODS

3 2.1 SAMPLING TIME

Sampling Times were planned as follow: from September to November 2004 (Time 1); and from September to November 2005 (Time 2). Only two samplings were planned within the investigation, due to the high costs of this technique. It was planned to compare data acquired with visual *census* technique.

3 2.2 SAMPLING DESIGN

The investigation was carried out using a local fishing boat equipped with trammel nets of 800 m in length (10 pieces of 80 m), using a mesh of eight and ten mm (diagonal stretched mesh size). In every sampling a series of 18 hauls was made at a sampling depth between 10 and 30 m on hard substrates; sampling sites can be seen in figure 3.6.

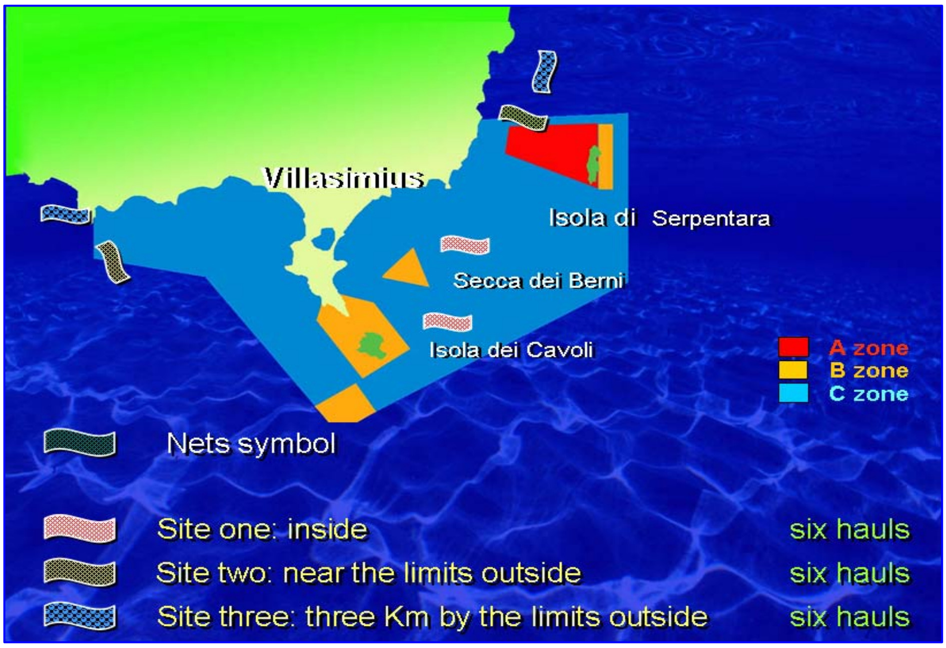


Figure 3.6: Trammel net fishing Sampling design.

Trammel net fishing and Visual *census* used the same set of Target Species (table 2.5) to allow a comparison between the two techniques.

Fishermen and researchers went fishing at night or in the early morning.

All organisms caught were classified at the lower taxonomic category; specimens were measured (standard length in cm) and weighed (in grams) in laboratory.

3.3 RESULTS AND DISCUSSION

A total of 24 target species were identified during the investigation by trammel net. They are listed by presence in the three sampling zones: inside the sanctuary (C Zone); near the boundaries (always in C Zone); and outside.

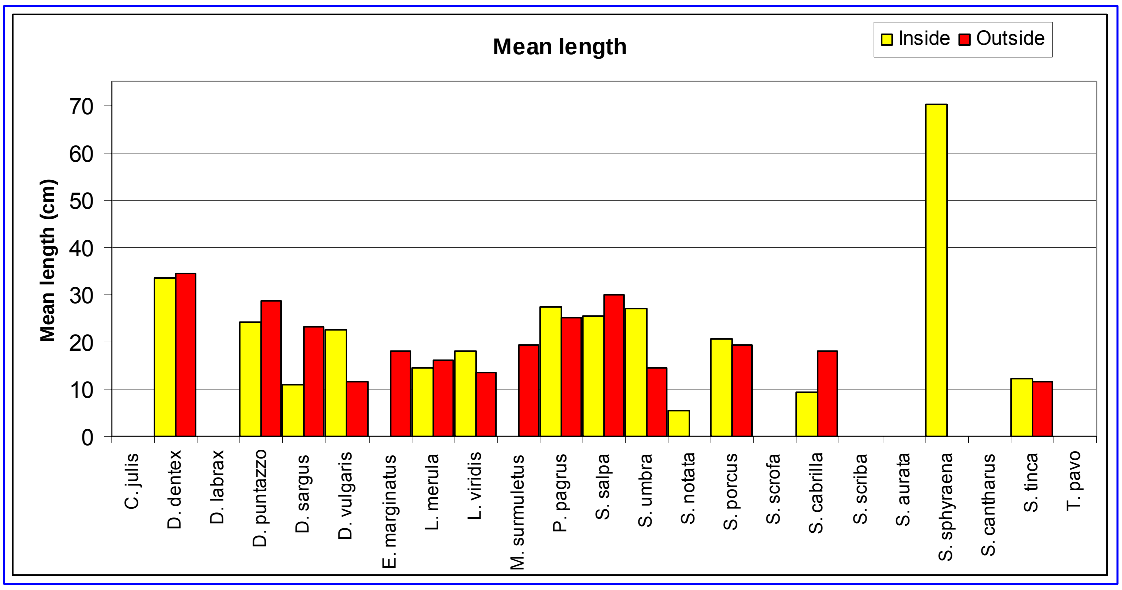
Species	Inside	Boundaries	Outside
Coris julis	+	+	+
Dentex dentex		+	+
Dicentrarchus labrax	+		
Diplodus puntazzo	+	+	+
Diplodus sargus	+	+	+
Diplodus vulgaris	+	+	+
Epinephelus marginatus	+	+	
Labrus merula	+	+	+
Labrus viridis	+	+	+
Mullus surmuletus	+	+	+
Pagrus pagrus	+		
Sarpa salpa	+	+	+
Sciaena umbra		+	
Scorpaena notata	+	+	+
Scorpaena porcus		+	+
Scorpaena scrofa			+
Seriola dumerili	+		
Serranus cabrilla	+	+	+
Serranus scriba	+	+	+
Sparus aurata		+	+
Sphyraena sphyraena	+	+	
Spondyliosoma cantharus	+	+	+
Symphodus tinca	+	+	+
Thalassoma pavo	+	+	+

Table 3.1: Trammel net fishing: species occurred in the three sampling zones.

Mean abundance

Values of mean abundance of the catches were greater for *Diplodus vulgaris* and *Scorpaena scrofa* inside (I) the sanctuary; *Pagrus pagrus* and *Dentex dentex* were most frequent outside. Only *Sphyraena sphyraena* was totally absent outside.

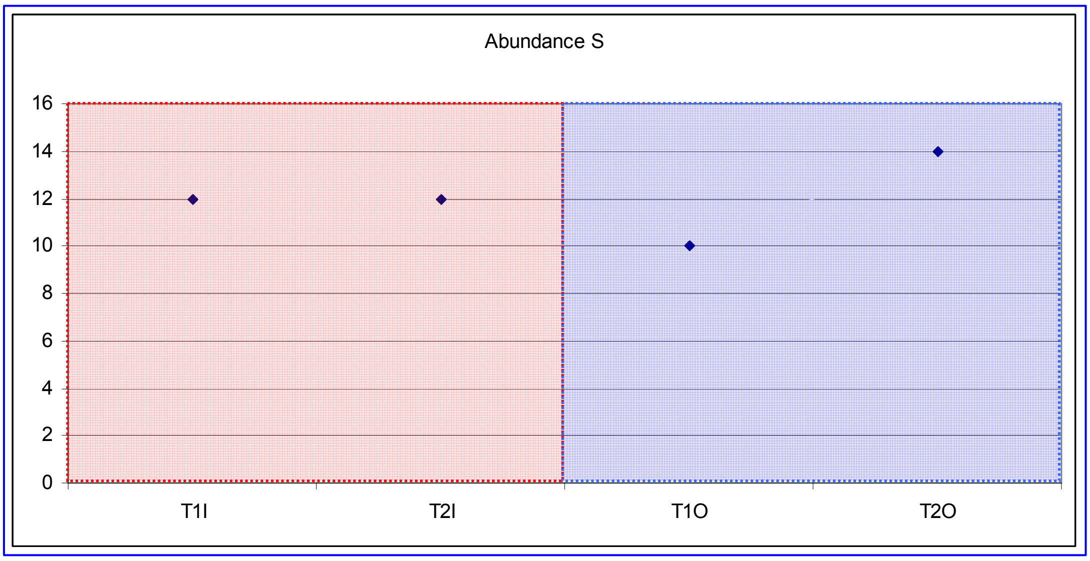
Mean length was nearly the same for almost the species.



Graph 3.1: Mean lenght of the species.

Abundance

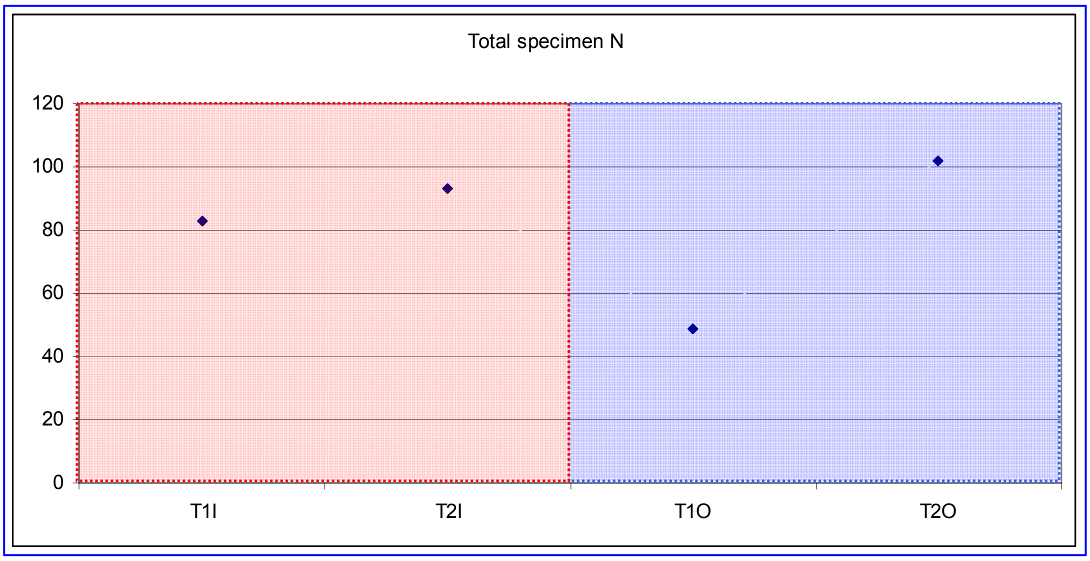
Inside the sanctuary species' abundance was the same (12) at the two Times of sampling. Outside, species number increased from Time 1 to Time 2 (from 10 to 14 species).



Graph 3.2: Species increase from Time 1 to Time 2 outside the sanctuary.

Species Richness index

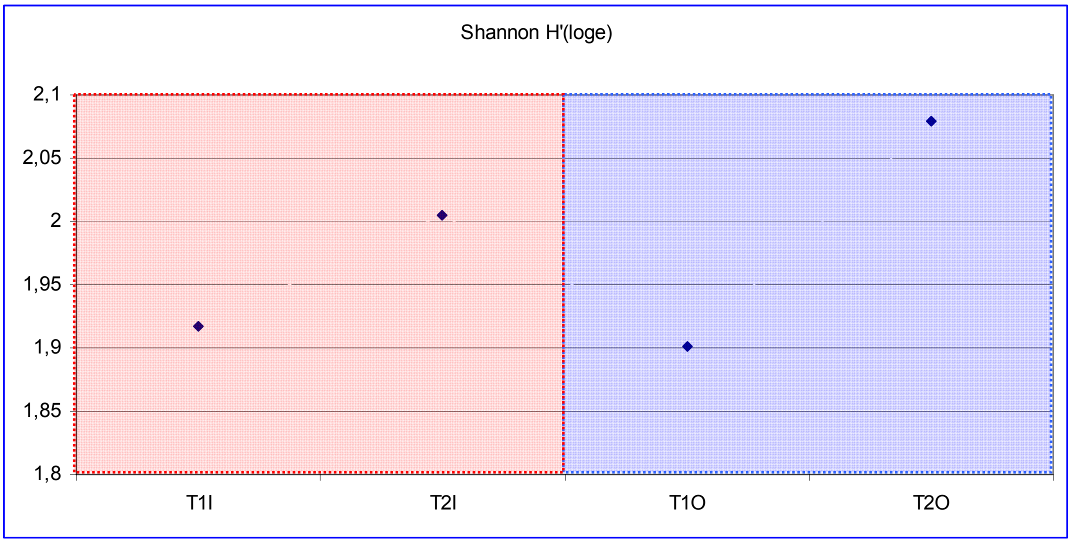
Values of richness index improve from Time 1 to Time 2 Inside (I, 83 T1 – 93 T2) and Outside (O, 49 T1 – 102 T2).



Graph 3.3: Specimen increase from Time 1 to Time 2 both inside and outside.

Shannon-Weaver diversity Index

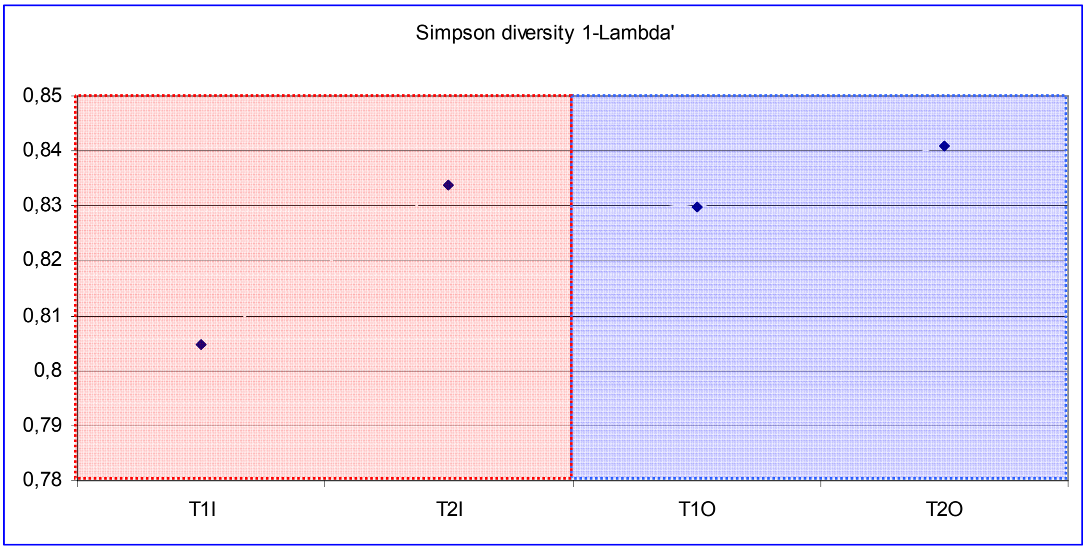
In all the sampled areas the values of Shannon Waver Diversity Index increased from Time 1 to Time 2. Higher values were observed in Time 2 outside (O).



Graph 3.4: Diversity increases from Time 1 to Time 2 both inside and outside.

Simpson's Index of Diversity

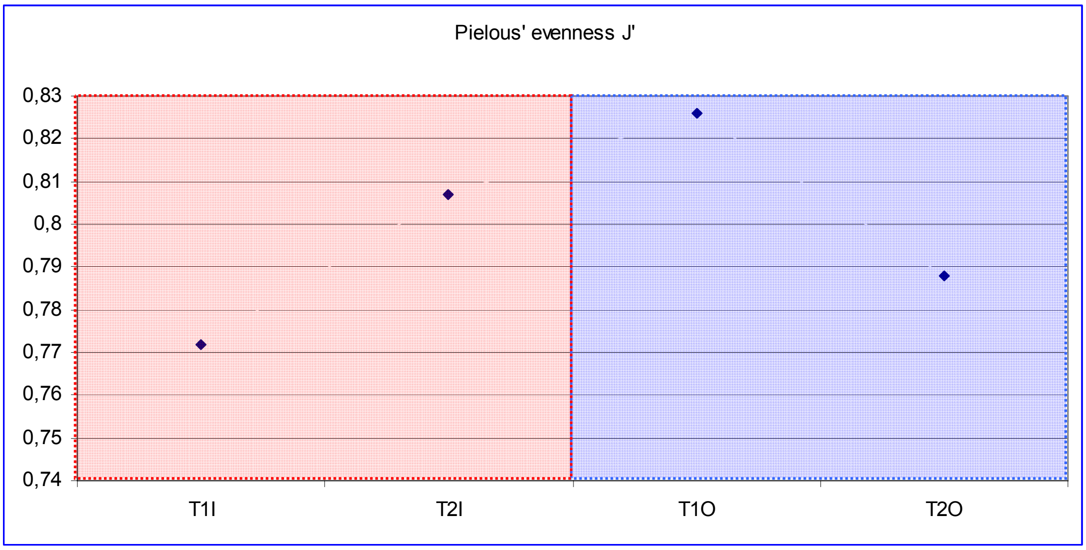
The index of diversity showed higher values from Time 1 to Time 2 both in the inside (I) and in the outside (O) zones.



Graph 3.5: Index of diversity increases from Time 1 to Time 2 both inside and outside.

Pielou Evenness Index

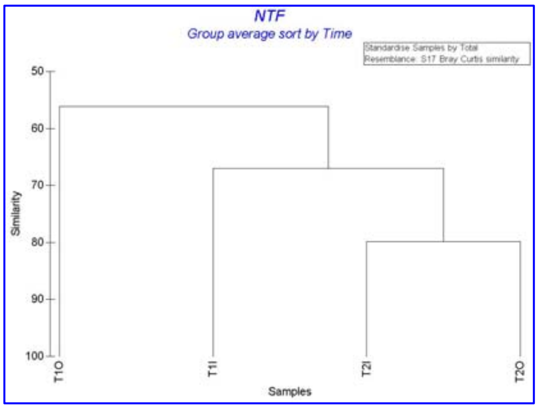
Evenness' values ranged between 0,7 and 0,8 Inside (I); between 0,8 and 0,7 Outside (O).

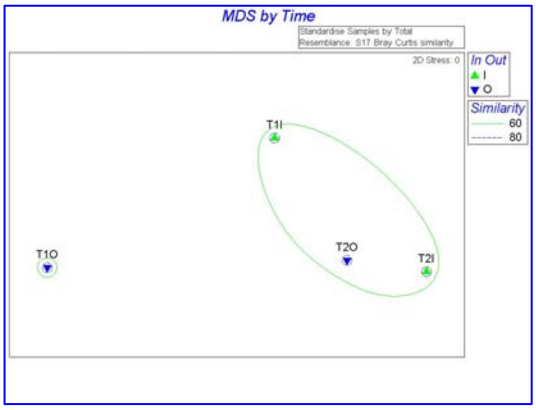


Graph 3.6: Evenness increases from Time 1 to Time 2 Inside and decreases Outside.

UNIVARIATE AND MULTIVARIATE ANALYSIS

Analysis of similarity (ANOSIM) by sites Inside and Outside the sanctuary indicated a significant global R value (R= -0,25), but not a significant P value (P>3%) confirming weak differences between sites as the cluster analysis shows and the multidimensional scaling plotted below confirms.





Graphs 3.7-3.8: Cluster analysis' and Multidimensional analysis sorted by Time.

Analyzing similarity by groups inside/outside, the average dissimilarity of all pairwise coefficients with sites inside the sanctuary and outside was 35,05. A 5,35 was contributed by *Pagrus pagrus,* 5,29 by *Scorpaena scrofa*, 5,25 by *Dentex dentex* and a further 4,57 by *Diplodus vulgaris*, corresponding to 15,25%, 15,10%, 14,40 and 13,03%, respectively, of the overall value of 35,05, with cumulative values of 15,25%, 30,35, 44,75 and 57,79%.

Trammel net fishing confirmed the reserve effect and the spillover of adult biomass from the sanctuary.

Furthermore, the effect of the elapsing time was very evident: all the studied sites demonstrated a positive trend of biomass, catches and total length from

Time 1 to Time 2 even if the evenness to find relevant biodiversity was low in the two sampled Times.

Increase of fish stock are already visible, as expected (Ward T.J. *et al.*, 2001). Commercial fish stocks, species, their length and average are well represented outside the sanctuary.

It is already evident that some changes occurred in the medium term (between 5-15 years) inside and outside the sanctuary, but others may occur in the long term (decades) (Ward T.J. *et al.*, 2001).

4 MACRO BENTHIC BIOCOENOSIS *CENSUS*

The macro benthic biocoenosis, the group of interacting organisms that live in benthic habitat (sessile organisms, that are attached to hard substrata or to objects on or near the substratum) and form a self-regulating ecological community, was studied to determine its composition and ecological parameters.

The high cost of monitoring marine communities, especially remote sites, together with the time limitations imposed by SCUBA, require a collection of statistically valid data as quick as possible. Therefore series of samples were carried out with the technique of Underwater Photographic *census*. Samples were carried out inside and in the surrounding area of Capo Carbonara's MPA, to evaluate the benthic structure in rocky shores and hard substrates.

Photographic census technique can be used underwater for either a quick qualitative assessment of a subtidal habitat, or a detailed quantitative analysis. Photographic sampling generally requires less diving time for a given number of samples. When conditions are less than optimal, underwater photography may be an effective tool. Moreover, divers do not need to be skilled in the enumeration and identification of species. The assessment and identification of species may be performed in the laboratory, where references may be more accessible, facilitating more detailed surveys. Photography also provides a permanent record that enables retrospective analyses and facilitates inter-worker calibration (Bullimore B. & Hiscock K., 2001).

4.1 PHOTOGRAPHIC TECHNIQUE

Photographic surveys can be laborious; the complete procedure consists of two-stages: (a) taking the photograph underwater and (b) subsequent laboratory analysis. Photographic equipment and processing are also expensive. The technique depends completely on underwater fragile equipment that can be occasionally ruined by flooding. As already mentioned, time and funding are two of the major constraints under which ecologists must work.

Furthermore, the usefulness of photography is reduced in turbid or poorly lit waters or in areas that are overshadowed by other structures. Photographs of poor quality can lead to insufficient or incomplete datasets. Photo coverage of large areas is problematic. If a photo is taken from a long distance, the resolution and water clarity may not be sufficient to identify organisms. An alternative consists in taking a series of overlapping photos and creating a photo-mosaic (Rogers *et al.,* 1994). Under optimal conditions, it is possible to make a repeatable and accurate mosaic.

Photography may be efficient in counts of cryptic species, especially in studies of substrates with a complex structure and with species exhibiting encrusting and hemispherical morphologies (Rogers & Miller 2000).

4.1.1 DATA COLLECTION

The following equipment is required to collect the macro benthic data:

- small diving boat
- hand held GPS
- complete sets of scuba diving equipment
- underwater photo camera
- desktops and notebooks
- high performance film scanner

A minimum of three people are required for the collection data: one takes the pictures, the second dives with the photographer for safety purpose. The third person remains in the boat as surface support.

4 1.2 SAMPLING PROCEDURE

The following section outlines the procedure for macro benthic biocoenosis *census*.

The site is located from the surface using a GPS and/or past knowledge of the surrounding reef topography. The boat is anchored near the site.

Two divers enter the water. The first diver (photographer) equipped with the camera goes to the site with the second diver.

4.1.3 DATA ANALYSIS

Once that slides are taken from the photo-shop, an identification number is assigned. After the digitalization, pictures are analyzed with appropriate software. Then all the data are recorded onto a computer in the Access database (a user interface to Microsoft Access has been developed for this purpose).

The community in hard and vertical substrate was studied using different metrics in order to gain an understanding between time and sampling sites within the survey area. The same statistics indexes already used for fish visual *census* and trammel net fishing were applied to the recorded data: species number, abundance, Shannon-Weaver diversity index, Pielou evenness index, Bray-Curtis similarity index (Multi Dimensional Scaling plot of similarity among sampling sites). Furthermore for macro benthic biocoenosis *census,* the collected data were analyzed to estimate quality assessment of marine environment using the Ecological Evaluation index (EEI), an original biotic index developed by Orfanidis (Orfanidis S. *et al*., 2001).

The use of bio indicators and indexes for the evaluation and assessment of the environment is becoming a prevalent procedure to analyse the various and often complex components of an ecosystem (Casazza G. *et al.*, 2002).

Bio indicators can be handled as information tools, as they represent, when properly selected, an objective system of information and evaluation. The methodology of their determination and their use can be uniformly specified and agreed upon. Indicators are also key tools for linking to policy objectives and targets, to report complexity in simple ways that policy makers can understand.

It is important to understand and share the definition of indicator and index. According to the Organization for Economic Cooperation and Development (OECD) an indicator is defined as a parameter, or a value derived from parameter/parameters, which provides informations about the state of a phenomenon/environment/area (Anon, 1993). The number of indicators must be limited to support their specific purpose's use.

New legislation for water protection, including protection of marine waters, have been adopted both in Italy, with the "Italian Water Directive" 1999-2000, and in Europe with the "European water framework directive" (Panayotis P. *et al.,* 2004).

The aim of these directives is to establish a common ecological status based on quality elements (indicators/indexes) from the different matrices: water, sediment and biota.

The present work was based on the new concept of environmental knowledge and protection elaborated by Orfanidis and Panayotis.

To study the quality of the coastal waters, chemical, biological and ecological parameters must be measured. Chemical parameters are commonly and widely measured, so that they do not need implementation and are not important for the topic of this thesis. This work takes into account the outputs of ecological and biological parameters.

Recent innovative legislation on water protection requires integrated analysis of the different environments.

In this work the study on the various components of the ecosystems has pointed out the importance of the ecological and biological parameters not considered before.

Sea floor creates different habitats that are influenced by chemical and physical factors, like wave strength, light gradient, water temperature; their status depends on depth, local pressure, variation in substrate composition which ranges from soft (gravel, sand, pebbles, detritus, and mud) to hard (rocks, wrecks, docks) and creates a further diversity in the submerged environment. Each of these habitats, with its own specific characteristics, is characterized by different biocoenosis.

This work takes in consideration the biocoenosis of macrophytes on the hard and vertical substrates using low budget monitoring.

The biological parameters, used in accordance to European Water Framework Directive for Water Policy (EEC, 2000), are measured through a new parameter, the Ecological Evaluation Index, i.e. EEI, (Orfanidis S. *et al.*, 2003), not commonly measured yet, in institutional monitoring programs of coastal waters in Sardinian seas.

EEI quantifies shifts in structure and function of transitional and coastal waters in different spatial and temporal scales by using nonlinear and linear relationships.

This study can be best carried out gaining informations with the non destructive photographic method. The remote probability of loss in taxonomic information, does not correspond to a loss of ecological quality information; identification to genera level is as informative as identification to functional form group when it comes to the ecological assessment of a marine ecosystem (Orfanidis S. *et al.*, 2003; Panayotis P. *et al.,* 2004).

Functional form groups of macroalgae consist of algae grouped together for the purposes of studying community structure (Littler M. & Littler D.,1980). Many authors have suggested a clear link between macroalgal form and function, and argued that predictable patterns of growth forms emerge under definite levels of environmental stress or disturbance (Phillips J., *et al.,* 1997). Functional groups are used to illustrate the large ecological forces that change the distribution, abundance and diversity of macroalgal communities (Hay M.,1994).

Macroalgal functional groups can be specifically related to levels of disturbance. Morphological, physiological and ecological adaptations can be related to the level of disturbance encountered (Phillips J. C. *et al*., 1997).

4.1.4 THE ECOLOGICAL STATUS AND ECOLOGICAL EVALUATION INDEX

Transitional and coastal waters are being severely threatened by anthropogenic pressure and climate change-induced sea level rise (Crooks S. & Turner R. K., 1999). For the management of these ecosystems it is critical to identify the impacts indicating the intensity of anthropogenic stress or ecological status.

The concept of ecological quality status was elaborated during the early 90's, to be used in a new frame of European water policy. For the purposes of the Directive "ecological water quality is an overall expression of the structure and function of the biological community taking into account natural physiographic, geographical and climatic factors as well as physical and chemical conditions, including those resulting from human activities" (EEC, 1994).

In ecology, a hypothetical community can be in pristine, uncorrupted and in its primitive state, or in degraded state stressed by a series of external events as impacts on natural elements: anthropization, pollution, alien species, climate change etc.

Cumulative evidences indicate that impacts are best investigated at population or community level (Lobban C. & Harrison P., 1994; Crowe T. *et al.*, 2000) and this requires an approach that integrates an ecological assessment into the more traditional chemical and physical evaluation (Gibson G. *et al.*, 2000).

However, the diagnosis of the ecological status is often a difficult task because of spatial and temporal variability in community features as a result of changes in physical and chemical conditions (Orfanidis S. *et al.*, 2001). One possibility is to study communities from a functional point of view (groups of functionally similar species). At a functional level, communities appear to be much more temporally stable and predictable than when examined at the species level (Steneck R. & Walting L., 1982; Steneck R. & Dethier M., 1994). For example, anthropogenic stress shifts the community structure towards dominance of opportunistic species (Borowitzka M., 1972; Regier H. & Cowell E., 1972).

Marine benthic macrophytes (macrophytobenthos) are mentioned in the WFD as a "quality element" for the classification of marine coastal areas. They include two fundamentally different groups of plants: the seaweeds (macroscopic algae) and the sea grasses (vascular plants). These macrophytes form the structural base for some of the most productive ecosystems in the world (Mann K. H., 1973; McRoy C. & Lloyd D., 1981), including rocky and soft bottom intertidal and subtidal zones, coral reefs, lagoons and salt marshes.

The three major taxonomic groups of seaweeds, Chlorophyceae, Phaeophyceae and Rhodophyceae, although representing distinct evolutionary lines, show similar ranges of morphologies. This similarity of form seems likely to be adaptive, conferring fitness to phyletically diverse organisms growing in a common habitat. The recognition of the importance of morphology has led to ecological classifications of seaweeds based on thallus morphology (thallus is the vegetative body of the algae composed of filaments or plates of cells, not differentiated into organs such as stems and leaves), longevity and life history (Feldmann J., 1951; Chapman V. & Chapman D., 1976; Russel G., 1977). More recently, Littler M. & Littler D. (1980) have proposed a functional-form model. This model was tested and verified experimentally: the functional characteristics of plants, such as photosynthesis, nutrient uptake, and grazer susceptibility, are related to morphology and surface area: volume ratios (Littler M., 1980; Littler & Arnold K., 1982; Littler M. & Littler D., 1984). Seaweeds and Sea grasses comprise two evolutionary and physiologically different groups (Larkum A. *et al*., 1989; Hemminga M. & Duarte C., 2000; Lobban C. & Harrison P., 1994) but have often been examined together because of morphological-functional similarities and the apparent overlap in habitats.

Because marine benthic macrophytes are mainly sessile organisms (sessile organisms are those which are not able to move about, usually permanently attached or fixed to a solid substrate of some kind, such as a rock, or the hull of a ship), they respond directly to the abiotic and biotic aquatic environment, and in consequence represent sensitive indicators of its changes. It is well documented that elevated concentrations of nitrogen and phosphorus in the water column do not necessarily indicate highly eutrophic conditions, neither low concentrations necessarily indicate absence of eutrophication (Cloern J., 2001). The motivation is that nutrient concentrations in the water column are related to nutrient load as well as to other biological and chemical processes. A reliable signal of increasing eutrophication is the replacement of late successional, perennial seaweeds, like *Cystoseira spp.* by opportunistic species like *Ulva spp*. and *Enteromorpha spp*. (Harlin M., 1995; Schramm W. & Nienhuis P., 1996; Schramm W., 1999). Several examples of impacts on marine phytobenthic communities are shown in Appendix 1.

Marine benthic macrophytes, particularly sea grasses, provide also substrate, habitat and protection for plants and animals, including economically relevant species (Pollard D., 1984). Since the canopy of leaves reduces wave energy and currents (Fonseca M. & Calahan J., 1992), they also significantly affect sediment stability (Fonseca M., 1996) and the retention of particles (Bulthuis D., *et al*., 1984; Dauby P. *et al*., 1995).

The appliance of the WFD obligates all the members of European Community first to evaluate the ecological status and then to identify restoration targets of their transitional and coastal waters. In 2004 the directive had not been applied yet: on October 18, 2005 the European Commission announced to have sent final warnings (reasoned opinions) to Italy, Spain and Greece for non-completely respecting of the EU Water Framework Directive (E.I.S., 2005).

In the studied area marine benthic macrophytes are the best biologic indicators; they are organized in two ecological state groups ESG I, representing the pristine, and ESG II for the degraded state.

Five classes of quality (Table 4.1) are foreseen, the high class reflecting pristine, undisturbed conditions of the ecosystem.

Mean abundance (%) of ESG II			
> 60	BAD	LOW	MODERATE
>30 - 60	LOW	MODERATE	GOOD
0 - 30	MODERATE	GOOD	HIGH
	0 - 30	>30 – 60	> 60
	Mean abundance (%) of ESG I		

Table 4.1: Matrix for the evaluation of the Ecological State Class (Panayotidis P., 2004) proposed by Orfanidis *et al.*, (2001), treaty with the classification induced in Directive 2000/60/EC.

ESG I includes seaweed species with a thick or calcareous thallus, low growth rates and long life cycles (late successional), whereas the ESG II includes sheet-like and filamentous seaweed species with high growth rates and short life cycles (opportunistic).

All Sea grasses are included in the first group, whereas Cyanophyceae and species with a coarsely branched thallus are included in the second group. Table 4.2 shows the list of the seaweed found in the MPA subdivided in genera and classified into ESGs.

Genera	**ESG**	***Genera***	**ESG**
Acetabularia	I	*Bryopsis*	II
Amphiroa	I	*Caulerpa*	II
Anadyomene	I	*Ceramium*	II
Corallina	I	*Chaetomorpha*	II
Cystoseira	I	*Champia*	II
Flabellia	I	*Cladophora*	II
Halimeda	I	*Cladophoropsis*	II
Haliptilon	I	*Codium*	II
Halymenia	I	*Colpomenia*	II
Hildenbrandia	I	*Dasycladus*	II
Jania	I	*Dictyopteris*	II
Kallymenia	I	*Dictyota*	II
Lithophyllum	I	*Dilophus*	II
Lithothamnion	I	*Enteromorpha*	II
Meredithia	I	*Gelidiella*	II
Neogoniolithon	I	*Gelidium*	II
Nithophyllum	I	*Halopteris*	II
Padina	I	*Hypnea*	II
Peyssonnelia	I	*Laurencia*	II
Posidonia	I	*Polysiphonia*	II
Sargassum	I	*Rivularia*	II
Taonia	I	*Ulva*	II
Vidalia	I	*Valonia*	II
Zanardinia	I		
Zonaria	I		

Table 4.2: Classification of Mediterranean seaweed genera subdivided in two Ecological State Groups (ESG I and II).

Spatial and temporal changes of benthic macrophytic communities are analyzed by seasonal sampling of ecologically uniform non-overlapping quadrats of the studied area.

Sampling followed a non-aligned block design, in which a sample is located randomly within a representative site. The absolute abundance (%) of each ESG is estimated as coverage (%) in each sample.

To evaluate the ecological status, the mean absolute abundance (%) of ESGs I and II sampled is non-linearly related to five different Ecological Categories or ESCs (Table 4.1). The ESCs are linearly related to the EEI (Table 4.3). The surface area is multiplied by its EEI and then divided by the sum of surface areas. The area values are then summed to estimate the spatial scale weighted EEI and the equivalent ESC (Table 4.3).

Numerical value of ecological categories	Ecological Evaluation Index (EEI)
High = 10	[≤10 - >8] = High
Good = 8	[≤8 - >6] = Good
Moderate = 6	[≤6 - >4] = Moderate
Low = 4	[≤4 - >2] = Low
Bad = 2	[2] = Bad

Table 4.3: A numerical scoring system for the evaluation of ecological status of transitional and coastal waters developed by Orfanidis.

In this way the environment in the studied area was evaluated and assessed using bio indicators and indexes.

4.2 MATERIALS AND METHODS

4.2.1 TIME SAMPLING

Sampling sites were chosen during a pre-survey investigation lasted approximately eight months (2004).

Samplings were scheduled in four sampling Times: Time 1 - September 2004; Time 2 - May 2005; Time 3 - September 2005; Time 4 - May 2006.

4.2.2 SAMPLING DESIGN

Three benthic habitats were investigated in the fully protected area (P0A P0B and P0C) from Time 1 to Time 4.

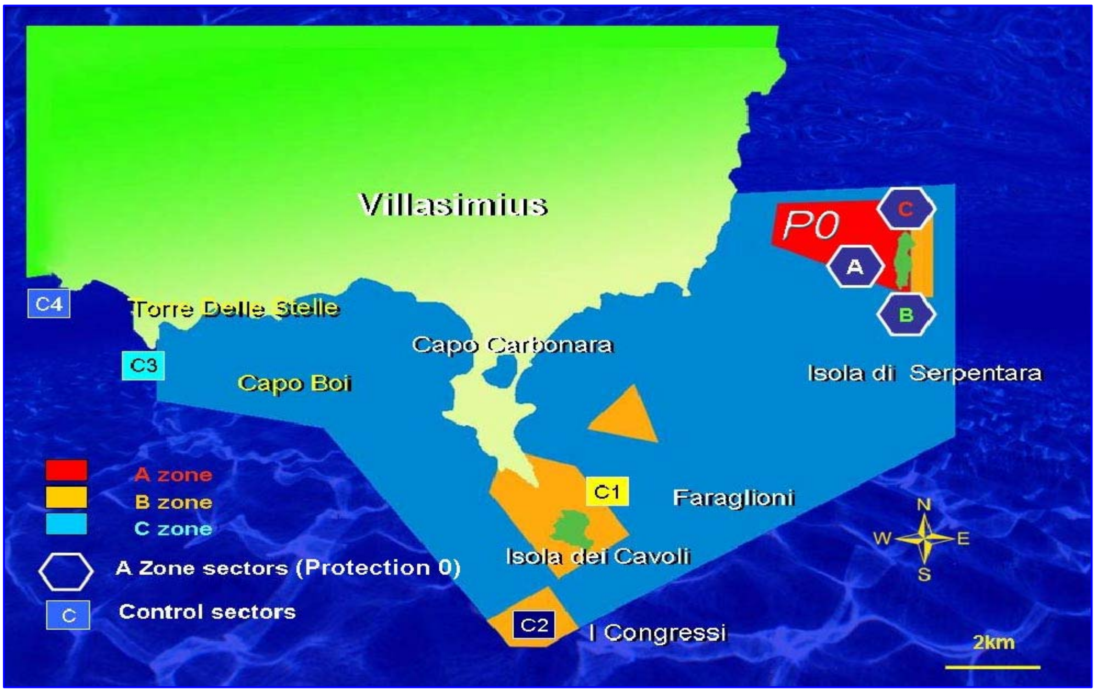


Figure 4.1: Sampled sites: P0 in A Zone, C1 and C2 in B Zone, C3 Outside near the Boundaries and C4 Outside.

LOCATION	ZONE	NAME	ACRONYM	POSITION
INSIDE	A	Protection	P0A	N39 08.069 E9 36.234
			P0B	N39 08.008 E9 36.298
			P0C	N39 09.141 E9 36.229
	B	Control	C1	N39 05.055 E9 32.406
			C2	N39 05.146 E9 31.497
	C		C3	N39 07.317 E9 26.348
OUTSIDE	OUT		C4	N39 08.428 E9 24.237
Datum: WGS 84 Grid Lat/Lon ddd°mm.mmm'				

Table 4.4: Position of the sampled area.

Two areas were investigate as control sites inside the MPA, I Faraglioni, (Control 1) and I Congressi (Control 2) from Time 2 to Time 4; two areas were investigated as control outside, one near the boundaries at Capo Boi (Control 3) and another at Torre delle Stelle, 6 Km far from the MPA (Control 4), from Time 2 To Time 4.

All these controls were useful to compare several habitats in different places located from south-eastern to southern side of Sardinia. This position is significant to study variation in coastal zones.

To compare different environments in the same studied area, three stations were carried out from shallow to deeper waters as listed below:

1. Stations at 5 metres; at this depth macro benthic biocoenosis in hard vertical/sub-vertical substrata are subject to daily fluctuations (day/night, sun, shadows), wind, waves and current depending by natural climate change; moreover, coverage of hard substrata depends on the community of invertebrate herbivores.
2. Stations at 15 metres; transitional water: link between shallow and deeper waters.
3. Stations at 25 metres; the deeper station.

The last depth corresponds to substrata near the bottom now or formerly covered by the sea grass *Posidonia oceanica.*

Biotopes of *P. oceanica* are the most productive in Mediterranean Sea.

These sites are influenced by bottom currents rich in sediments and nutrients.

A) IN FIELD PROCEDURES

This method uses cameras like Nikon f100 or Nikon f90 in their own underwater aluminium case.

The camera is equipped with a macro lens, the Nikon 105 mm, that gives elevated quality to the recorded pictures with a plane optical port.

An handmade quadrat frame of 16 x 23 cm is fixed to the case (see Fig.4.2 below); the area enclosed in the quadrat is registered by the film.

Figure 4.2: Researcher (myself) using the camera equiped with frame and flashes to record the studied substrata.

Two underwater flashes (Isotta 33 or Ikelite substrobe 100s or Nikon sb 105 or 103) working together, wired to the camera, are electronically connected to the camera.

Camera is set in manual with Time at 1/125 second and aperture variable from 22 to 32 depending on the subject photographed; flashes are set in manual from 20 to 30 cm by the subject.

Underwater pictures of the benthic assemblages are taken with the random number table at three different levels of depth: 5, 15 and 25 metres.

B) PICTURES MANAGEMENT

In laboratory, slides were scanned with the scanner (Nikon super Coolscan 4000) at a resolution of 4000 dot per inch, downloaded to a computer and placed in files with date, location and project. Each image was successively analysed with the software Adobe Photoshop®.

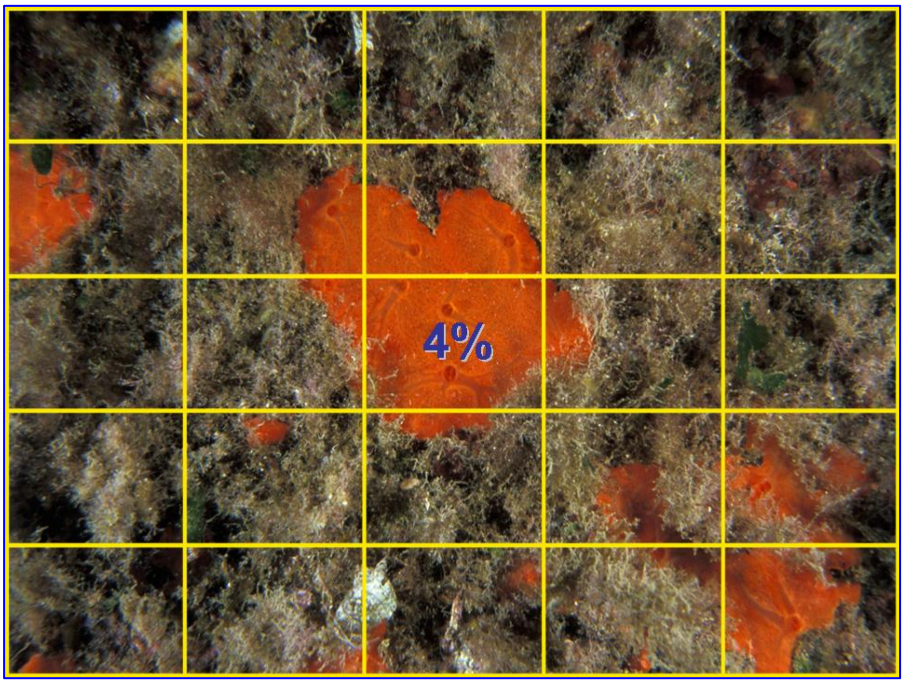


Figure 4.3: The grid subdividing the picture in 25 rectangles, each corresponding to 4 % of the whole image.

Each image was rotated so that framer material was evenly distributed on all margins, then all the pictures were cropped evenly and analyzed with software to estimate total abundance. Colours might be correct to lighten dark areas or adjust colour to aid in identification.

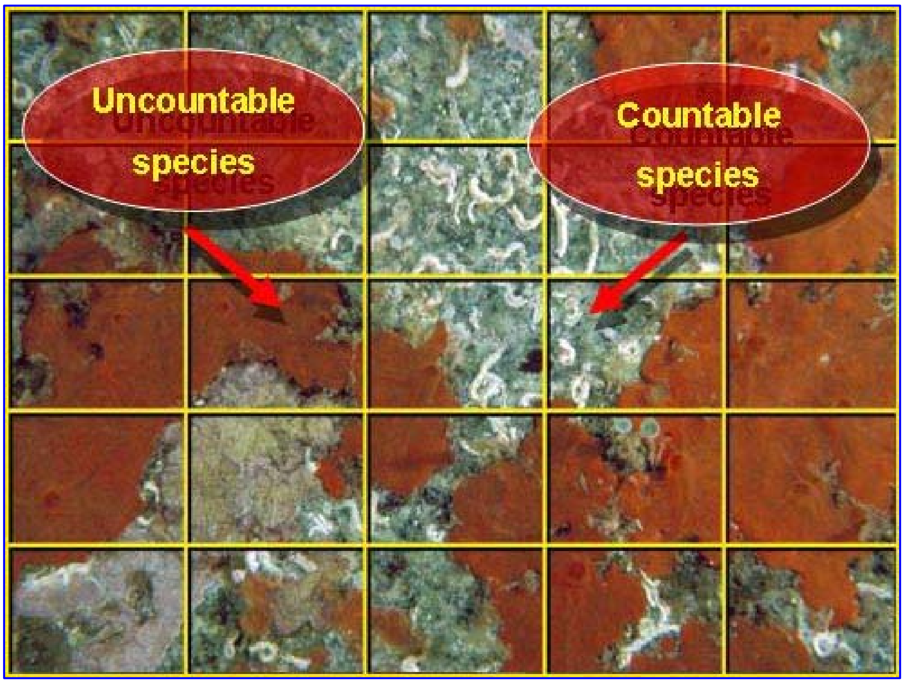


Figure 4.4: ***Spirastrella cuntactrix*****, an uncountable species and countable species like Polychaeta.**

A regular grid of 25 cells was superimposed on the original image to analyze it, expressing total coverage in percent, every cell corresponding to 4% of the entire image. This method makes easier the species identification and the abundance estimation (Fraschetti S. *et al.*, 2001).

For the abundance estimation, in particular, two count methods were used. Countable species were expressed as total number, while uncountable species were expressed as their percent coverage in each cell.

The systematic organization was at the species level whenever it was possible; otherwise it was at genera or family level. Some difficult systematic organizations of algae were collected in classes of reference defined by an acronym as listed below:

ACRONYMS LIST	
AC	Articulated Corallinacea
DFA	Dark Filamentous Algae
EB	Encrusting Bryozoans
ECR	Encrusting Calcified Rhodophytes
ERS	Encrusting Red Sponges
GFA	Green Filamentous Algae
MDS	Massive Dark Sponges
SBA	Soft Branched Algae
TRB	Thin Ramified Bryozoans
TTS	Thin Tubular Sheet-like

Table 4.5: **list of the acronyms used for the classification.**

All data obtained analyzing the slides were recorded in a Macro-benthic database using Microsoft Access®.

Records are distinguished by:

- □ Code: Project (SPILL), Time (T1, T2, T3), Protection/Control (P0, C1, C2, C3, C4); Site (A, B, C), Depths (d1, d2, d3) and replicate (r1,r2 etc.)
- □ Species: Species, genera, family or acronym
- □ Percentage of coverage
- □ Number of species

This table of the registered data was linked by species to a taxonomic list that provided the distribution of data by taxonomic rank.

REGISTRED DATA : Tabella

Codice	Specie	%	N
SPILL-T1P0Bd1r03	Polychaeta		105
SPILL-T1P0Bd1r03	Dasycladus vermicularis	1	
SPILL-T1P0Bd1r03	Cianobatteri	1	
SPILL-T1P0Bd1r03	Serpulorbis arenarius		4
SPILL-T1P0Bd1r03	EB - Encrusting Bryozoans	1	
SPILL-T1P0Bd1r04	Mucillagine	84	
SPILL-T1P0Bd1r04	EB - Encrusting Bryozoans	7	
SPILL-T1P0Bd1r04	Dasycladus vermicularis	1	
SPILL-T1P0Bd1r04	ECR - Encrusting Calcifed Rodophyt	1	
SPILL-T1P0Bd1r04	Polychaeta		100
SPILL-T1P0Bd1r04	Padina pavonica	1	
SPILL-T1P0Bd1r04	AC - Corallinaceae ramificate sottili	1	
SPILL-T1P0Bd1r05	Mucillagine	75	
SPILL-T1P0Bd1r05	Cianobatteri	1	
SPILL-T1P0Bd1r05	Padina pavonica	1	
SPILL-T1P0Bd1r05	Polychaeta		70
SPILL-T1P0Bd1r05	ECR - Encrusting Calcifed Rodophyt	1	
SPILL-T1P0Bd1r06	Mucillagine	61	
SPILL-T1P0Bd1r06	EB - Encrusting Bryozoans	16	
SPILL-T1P0Bd1r06	Cianobatteri	2	
SPILL-T1P0Bd1r06	Caulerpa racemosa	1	
SPILL-T1P0Bd1r06	AC - Corallinaceae ramificate sottili	1	
SPILL-T1P0Bd1r06	ECR - Encrusting Calcifed Rodophyt	2	
SPILL-T1P0Bd1r06	Padina pavonica	4	
SPILL-T1P0Bd1r06	Polychaeta		70
SPILL-T1P0Bd1r07	Mucillagine	63	
SPILL-T1P0Bd1r07	EB - Encrusting Bryozoans	8	

Record: 1 di 3763

Figure 4.5: Table of the registered data.

The taxonomic rank of the found species is listed in appendix 3 and 4.

CLASSIFICATION : Tabella

Phylum	Class	Subclass	Order	Family	Genera	Specie
RHODOPHYTA	RHODOPHYCEAE	FLORIDEOPHYCIDAE	CRYPTONEMIALES	CORALLINACEAE		AC - Corallinaceae ramifica
PORIFERA	DEMOSPONGIAE	TETRACTINOMORPHA	AXINELLIDA	AXINELLIDAE	Acanthella	Acanthella acuta
ARTHROPODA	CRUSTACEA	MALACOSTRACA	DECAPODA	MAJADAE	Acanthonyx	Acanthonyx lunulatus
CHLOROPHYTA	CHLOROPHYCEAE		DASYCLADALES	DASYCLADACEAE	Acetabularia	Acetabularia acetabulum
COELENTERATA	ANTHOZOA	HEXACORALLIA	ACTINARIA	ACTINIDAE	Actinia	Actinia equina
BRYOZOA	GYMNOLAEMATA		CHEILOSTOMIDA	AETEIDAE	Aetea	Aetea truncata
PORIFERA	DEMOSPONGIAE	CERACTINOMORPHA	POECILOSCLERIDA	AGELASIDAE	Agelas	Agelas oroides
COELENTERATA	HYDROZOA		HYDROIDEA	PLUMULARIIDAE	Aglaophenia	Aglaophenia sp.
PHAEOPHYTA	PHAEOPHYCAE	ISOGENERATAE	CUTLERIALES	CUTLERIACEAE	Cutleria	Aglaozonia parvula
COELENTERATA	ANTHOZOA	HEXACORALLIA	ACTINARIA	AIPTASIIDAE	Aiptasia	Aiptasia mutabilis
RHODOPHYTA	RHODOPHYCEAE	FLORIDEOPHYCIDAE	CRYPTONEMIALES	CORALLINACEAE	Amphiroa	Amphiroa rigida
RHODOPHYTA	RHODOPHYCEAE	FLORIDEOPHYCIDAE	CRYPTONEMIALES	CORALLINACEAE	Amphiroa	Amphiroa rubra
RHODOPHYTA	RHODOPHYCEAE	FLORIDEOPHYCIDAE	CRYPTONEMIALES	CORALLINACEAE	Amphiroa	Amphiroa sp.
CHLOROPHYTA	CHLOROPHYCEAE		CLADOPHORALES	ANADYOMENACEAI	Anadyomene	Anadyomene stellata
COELENTERATA	ANTHOZOA	HEXACORALLIA	ACTINARIA	ACTINIDAE	Anemonia	Anemonia sp.
COELENTERATA	ANTHOZOA	HEXACORALLIA	ACTINARIA	ACTINIDAE	Anemonia	Anemonia sulcata
ARTHROPODA	CRUSTACEA	MALACOSTRACA	DECAPODA			Anomura
ECHINODERMATA	CRINOIDEA		COMATULIDA	ANTEDONIDAE	Antedon	Antedon mediterranea
COELENTERATA	ANTHOZOA					Anthozoa
CHORDATA	ASCIDIACEA		ENTEROGONA	POLYCLINIDAE	Aplidium	Aplidium sp.
PORIFERA	DEMOSPONGIAE	CERACTINOMORPHA	VERONGIIDA	APLYSINIDAE	Aplysina	Aplysina aerophoba
ECHINODERMATA	ECHINOIDEA	REGULARIA	DIADEMATOIDA	ARBACIIDAE	Arbacia	Arbacia lixula
MOLLUSCA	BIVALVIA	PTERIOMORPHIA	ARCOIDEA	ARCIDAE	Arca	Arca noae
CHORDATA	ASCIDIACEA		PHLEBOBRANCHIATA	ASCIDIIAE	Ascidia	Ascidia mentula
CHORDATA	ASCIDIACEA		PHLEBOBRANCHIATA	ASCIDIIAE	Ascidia	Ascidia sp.
CHORDATA	ASCIDIACEA					Ascidiacea
ECHINODERMATA	ASTEROIDEA		SPINULOSA	ASTERINIDAE	Asterina	Asterina gibbosa
ECHINODERMATA	ASTEROIDEA					Asteroidea
COELENTERATA	ANTHOZOA	HEXACORALLIA	MADREPORARIA	DENDROPHYLLIDAE	Astroides	Astroides calycularis
PORIFERA	DEMOSPONGIAE	CERACTINOMORPHA	HALICHONDRIDA	AXINELLIDAE	Axinella	Axinella polypoides
PORIFERA	DEMOSPONGIAE	CERACTINOMORPHA	HALICHONDRIDA	AXINELLIDAE	Axinella	Axinella sp.
ARTHROPODA	MAXILLOPODA	MALACOSTRACA	THORACICA	BALANIDAE		Balanidae

Record: 1 di 325

Figure 4.6: Table of the registered species subdivided by taxonomic rank.

4.3 RESULTS AND DISCUSSION

In southern Sardinia the algal taxonomic literature, composition and distribution (i.e. data archives based on images) is quite scant. For these reasons the species identified during the investigation were archived in an interactive Access database showing all the acquired informations.

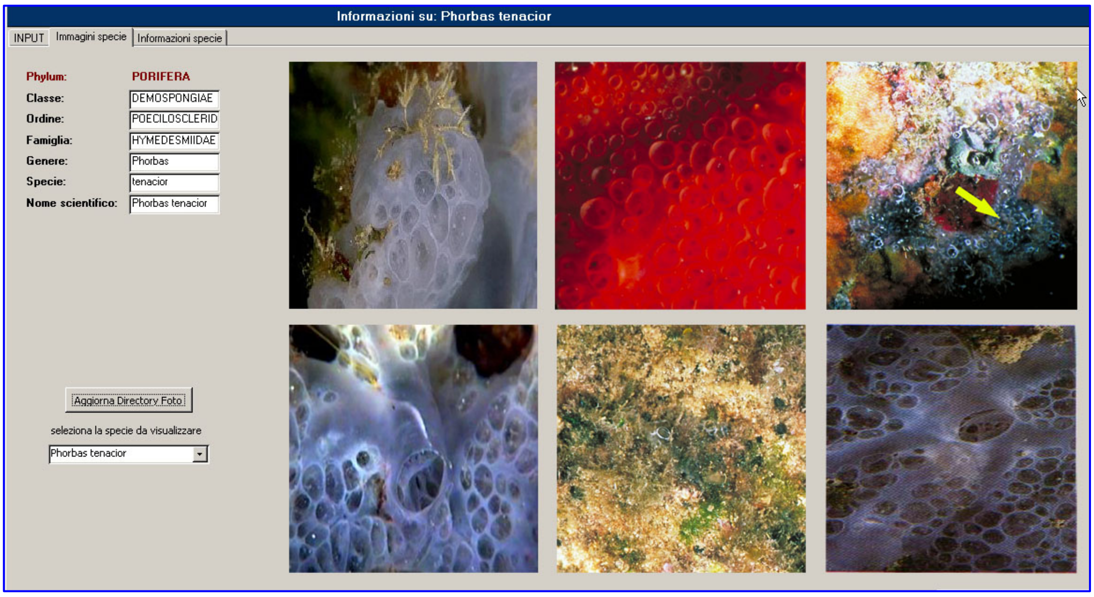


Figure 4.7: Table of the interactive database.

In three Times of *census* a total of 640 slides were captured and analyzed: 350 in A zone (P0A-P0B-P0C) and 290 in Control sites (B zone and Outside). The macro benthic database, obtained analyzing the slides, includes more than 5000 records distinguished by species and sampling code.

A total of 325 species were recorded (as reported in appendix 3 and 4) and subdivided in 15 Phyla:

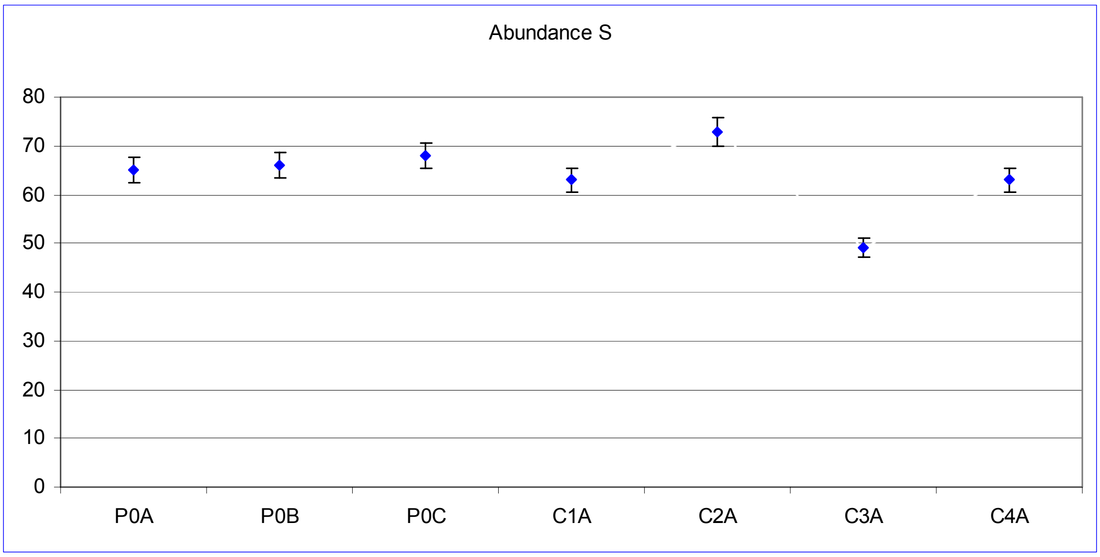
AGGREGATION	NUMBER OF PHYLA	PHYLA
TALLOPHYTA	4 PHYLA	PHAEOPHYTA RHODOPHYTA CHLOROPHYTA CYANOPHYCOTA
ANTHOPHYTA	1 PHYLUM	SPERMATOPHYTA
INVERTEBRATA	10 PHYLA	PROTOZOA PORIFERA COELENTERATA PLATHELMINTHES BRYOZOA ECHINODERMATA CHORDATA MOLLUSCA ANNELIDA ARTHROPODA

Table 4.6: Recorded Phyla.

4.3.1 ANALYSIS OF THE COMMUNITY STRUCTURE

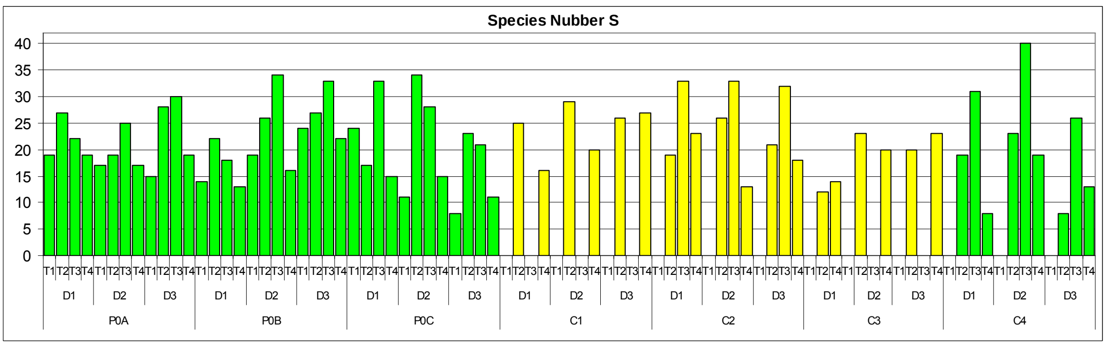
Abundance

The abundance of species (expressed as specimen/m^2) remarked high values in all the studied sites.



Graph 4.1: Abundance of species in the studied sites: Protection A, B and C and Control 1, 2, 3 and 4.

Control 2A and P0C sites were the richest in species, but also in all the other sites a good abundance level was registered.

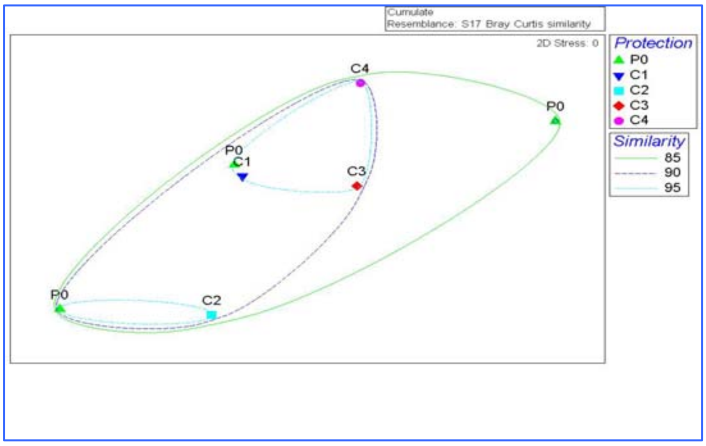


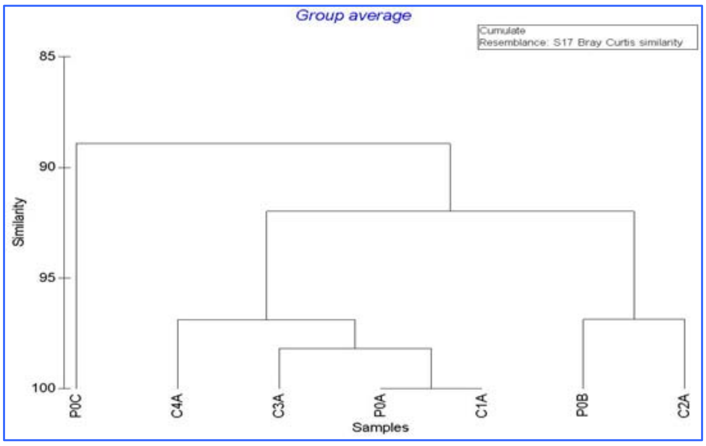
Graph 4.2: Number of species subdivided by Time and depth.

In the Control 3 site, placed outside the sanctuary, there were lower values than in the Control 1, 2 and 4 sites.

Species number differed significantly according to Time and Zone because of seasonality, but Time 2 and Time 3 had high density values at every depth and Site.

Multi Dimensional Scaling analysis reflects this trend; in fact all the Sites are within the 85% range of similarity. It can be seen in the cluster analysis, too. P0C presents lower similarity than other sites.

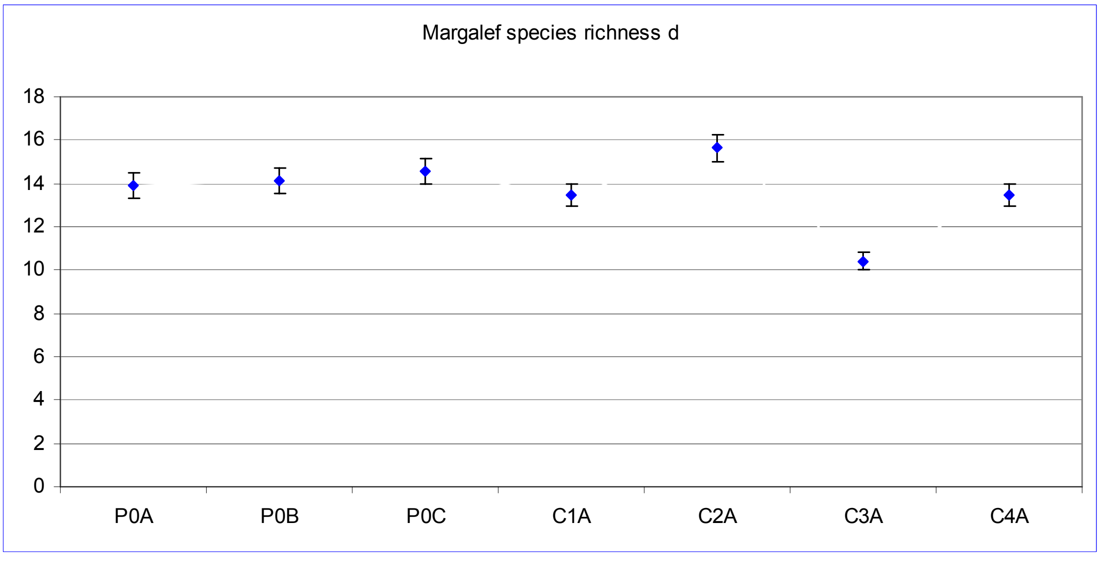




Graphs 4.3 – 4.4: Multi dimensional scaling and cluster analysis of the sampled sites.

Species Richness index

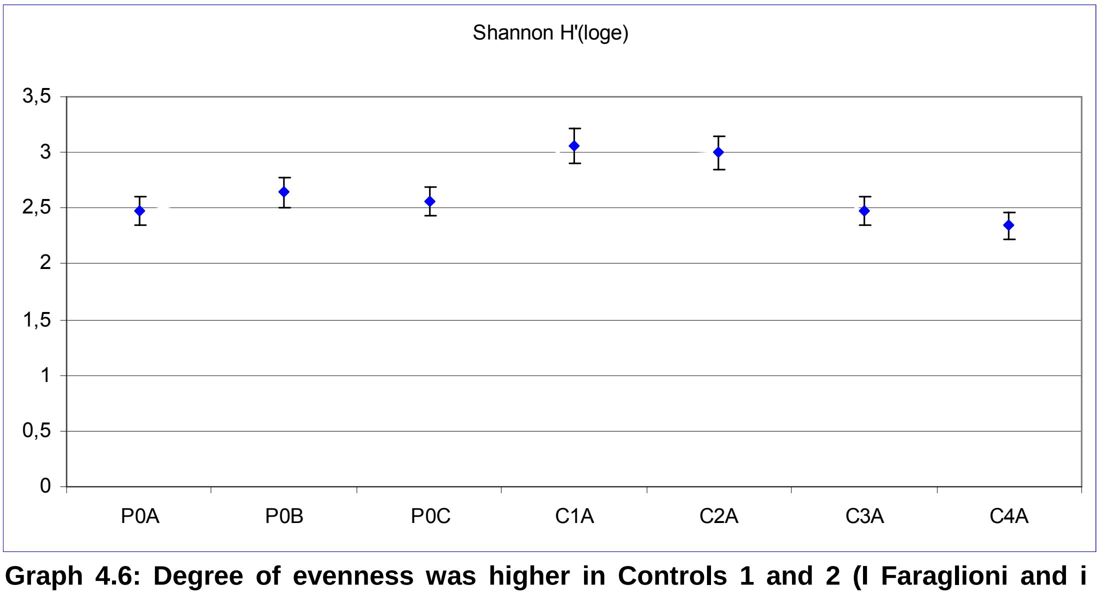
We obtained high values of Margalef index per Time and Zone. Higher values were calculated in P0C and C2A.



Graph 4.5: Only in Control 3, placed outside near the boundaries (Capo Boi), richness in species was lower than in the other sites.

Shannon-Weaver diversity Index

Shannon Weaver takes into account the degree of evenness in species abundances. Higher evenness degree was observed in Controls 1 and 2.

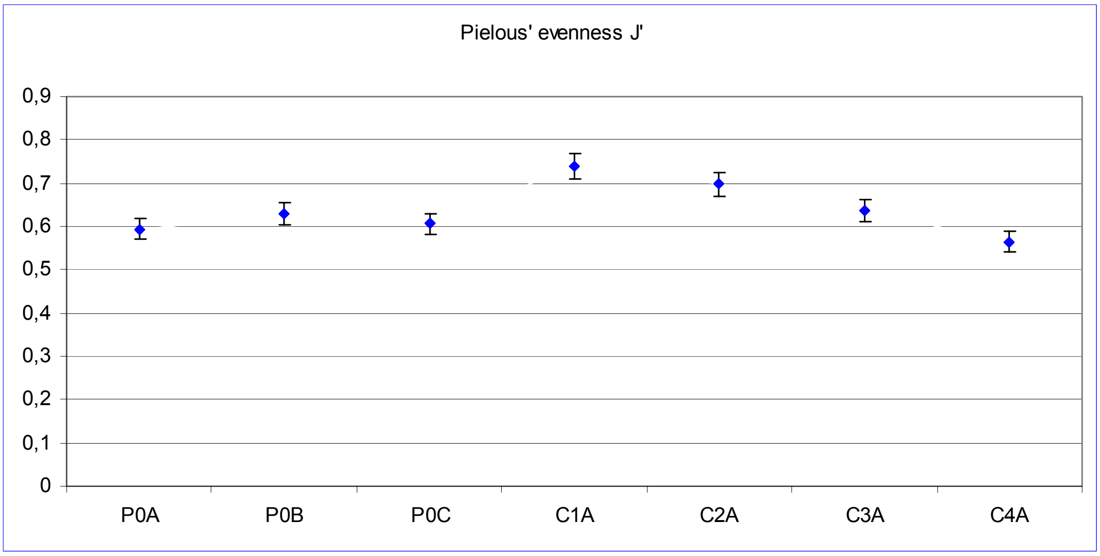


Graph 4.6: Degree of evenness was higher in Controls 1 and 2 (I Faraglioni and i Congressi – Isola dei Cavoli) than in other Studied sites.

Pielou Evenness Index

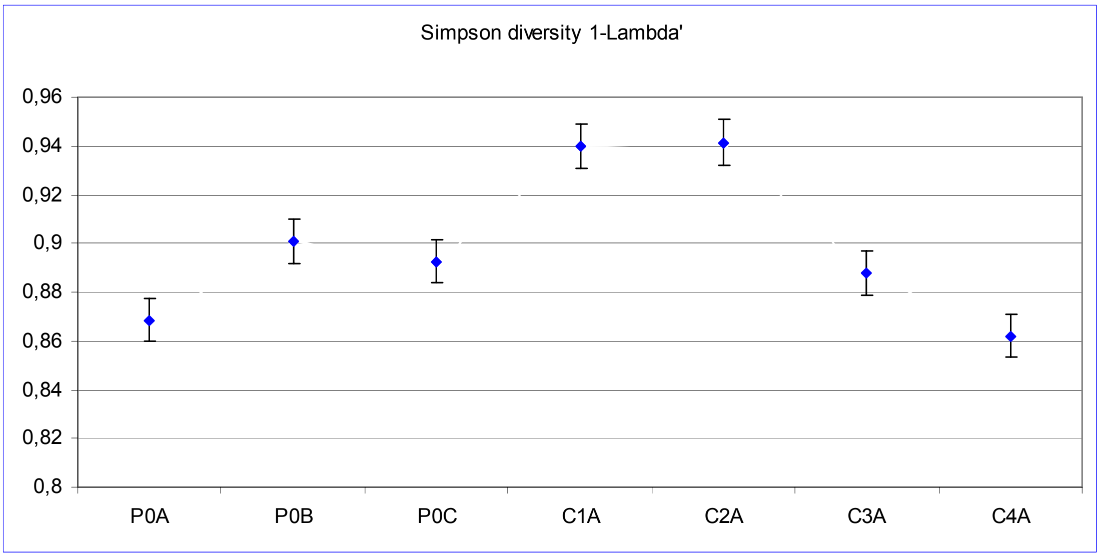
Evenness indexes were measured between 0,56 (Control 4A) and 0,74 (Control 1A).

All species were not equally abundant, but relative abundance did not diverge from evenness in all the sampled sites.



Graph 4.7: Evenness had higher values in controls C1 (I Faraglioni) and C2 (I Congressi) than in protected sites and in other controls.

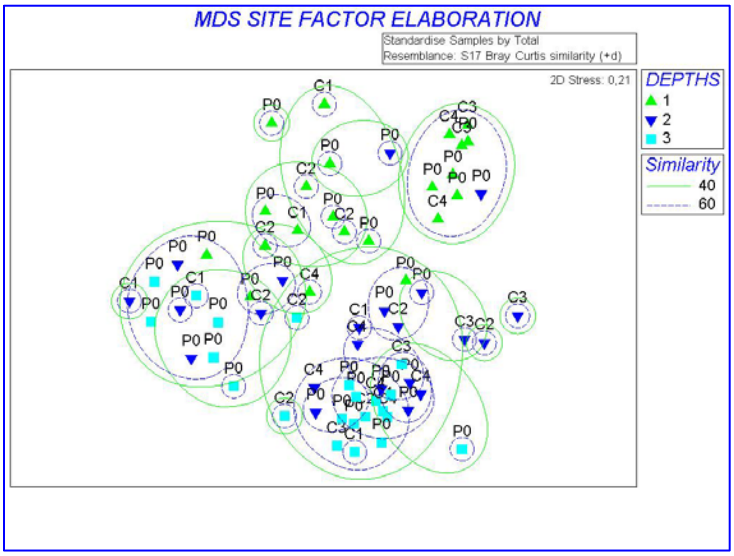
Diversity values resulted to be higher in Control 1 and Control 2. In these two sites, the probability that two individuals, randomly selected from a sample, belonged to different species was high.



Graph 4.8: higher Diversity in Control 1 and 2, lower in the other sites.

MDS Site Factor

The analysis of Multidimensional scaling plots of the macrobenthos assemblages highlighted similarity among sites at the same depth.



Graph 4.9: Multidimensional Scaling by site factor.

SIMPER ANALYSIS

Analysis of similarity (ANOSIM) randomization test indicated significant global R value (R= 0,252), and significant P value (P = 0,3%) confirming diversity between protected and control sites.

Protected P0A Site

The average dissimilarity of all pairwise coefficients with site P0A inside the A zone, was 37,71. Of this average similarity, 20,92 was contributed by *GFA,* 3,26 by *AC, 2,76* By *DFA,* 2,12 by *Caulerpa racemosa*, 1,85 by *Dasycladus vermicularis,* 1,43 by *Cystoseyra spp.* 1,35 by *Dictyota linearis* and 1,22 by ECR, with a percentage contribution of 55,48%, 8,64%, 7,32%, 5,63%, 4,90%, 3,78%, 3,59% and 3,25%, respectively, of the overall value of 37,71, with cumulated values of 55,48%, 64,12%, 71,44%, 77,07%, 81,96%, 85,74%, 89,33% and 92,58 %, respectively.

The Green filamentous algae was the Species/Category which mainly contributed to the differences in P0A site. Other relevant species or category were: Articulated Corallinacea, Dark filamentous algae, *Caulerpa racemosa*, *Dasycladus vermicularis*, species of *Cystoseira*, *Dictyota linearis* and Encrusting calcified Rhodophytae.

Protected P0B Site

The average dissimilarity of all pairwise coefficients was 32,02 for P0B. the main contributor to the overall average similarity was Cystoseira spp., with 6,66, followed by DFA, 5,38, *Caulerpa racemosa* 4,59, GFA 4,17, *Dasycladus vermicularis* 2,28, ECR 1,84, AC, Porifera1,73, *Spirastrella cunctatrix* 0,97, *Dictyota dicothoma* 0,82, and finally *Hildenbrandia rubr*a 0,90. The contribution percentage was 20,79%, 16,79%, 14,33%, 13,02%, 7,13%, 5,76%, 5,40%, 3,04%, 2,57% and 2,32%, respectively, of the overall value of 32,02, with cumulative percentages of 20,79%, 37,57%, 51,90%, 64,92%, 72,05%, 77,81%, 83,21%, 86,25%, 88,81%, and 91,13 %, respectively.

The species which mainly influenced the differences within P0B site were Cystoseira, Dark filamentous algae, *Caulerpa racemosa*, Green filamentous algae *Dasycladus vermicularis*, Encrusting calcified Rhodophytae, Articulated Corallinacea, *Spirastrella cunctatrix*, *Dictyota dichotoma* and *Hildenbrandia rubra*.

Protected P0C Site

The average dissimilarity of all pairwise coefficients with site P0C inside the A zone, was 45,39. Of this average similarity, 19,53 was contributed by *GFA,* 7,71 by *DFA, 5,17 By AC,* 4,88 by *Caulerpa racemosa*, 2,19 by *Dictyota dichotoma,* 1,32 by ECR and 0,65 by *Peyssonnellia sp.*, with a percentage contribution of 43,02%, 16,99%, 11,38%, 10,74%, 4,82%, 2,90% and 1,43%, respectively, to the overall value of 45,39, and cumulative percentages of 43,02%, 60,01%, 71,40%, 82,14%, 86,96%, 89,86%, and 91,29%, respectively.

The Species which mainly influenced differences within P0C site were: Green filamentous algae, Dark filamentous algae, Articulated Corallinacea, *Dictyota dichotoma*, *Caulerpa racemosa*, *Dictyota dichotoma*, Encrusting calcified Rhodophytae, and *Peyssonnellia sp.*

Control 1 Site

The average dissimilarity of all pairwise coefficients with site Control 1 inside the B zone, was 26,90. Of this average similarity, 9,90 was contributed by *GFA,* 2,56 by ECR, 2,50 by *DFA, 1,88 By Hildenbrandia rubra,* 1,68 by *Spirastrella cunctatrix*, 1,57 by *Dasycladus vermicularis,* 1,53 by *Serpulidae* genera and 1,20 by *Parazoanthus axinellae*, 065 by *AC* and 0,51 by *Acetabularia acetabulum*, with a percentage contribution of 37,78%, 9,78%, 9,52%, 7,17%, 6,42%, 6,00%, 5,82%, 4,57%, 2,49% and 1,49%, respectively, of the overall value of 45,39, and cumulative percentages of 37,78%, 47,56%, 57,08%, 64,25%, 70,67%, 76,68%, 82,50%, 87,07%, 89,55% and 91,29%, respectively.

Species which mainly influenced differences within C1 site were *Green Filamentous Algae*, *Encrusting Calcified Rhodophytes*, *Dark Filamentous Algae*, *Hildenbrandia rubra*, *Spirastrella cunctatrix*, *Dasycladus vermicularis*, *Serpulidae*, *Parazoanthus axinellae*, *Articulated Corallinaceae* and *Acetabularia acetabulum.*

Control 2 Site

The average dissimilarity of all pairwise coefficients with site Control 2 inside the B zone, was 31,80. Of this average similarity, 7,76 was contributed by *AC,* 7,15 by *GFA*, 5,53 by *DFA,* 2,93 by ECR, 2,10 by *Hildenbrandia rubra,* 1,68 by *Cystoseira spp.*, 0,89 by *Dasycladus vermicularis and* 0,80 by *Padina pavonica* and with a percentage contribution of 24,42%, 22,47%, 17,39%, 9,22%, 6,61%, 5,29%, 2,79% and 2,52%, respectively, of the overall value of 31,80, and cumulative percentages of 37,78%, 47,56%, 57,08%, 64,25%, 70,67%, 76,68%, 82,50%, 87,07%, 89,55% and 91,29%, respectively.

Species which mainly influenced differences within C2 site were *Articulated Corallinaceae, Green Filamentous Algae*, *Dark Filamentous Algae*, *Encrusting Calcified Rhodophytae*, *Hildenbrandia rubra*, *Cystoseira spp.*, *Dasycladus vermicularis* and *Padina pavonica.*

Control 3 Site

The average dissimilarity of all pairwise coefficients with site Control 3 outside near the boundaries, was 15,68. Of this average similarity, 4,47 was contributed by *AC,* 2,89 by *Dictyotales*, 2,01 by ECR*,* 1,86 by *DFA*, 0,92 by *Halopteris scoparia,* 0,86 by *Dictyota linearis*, 0,57 by *Padina pavonica* and 0, 45 by *Peyssonnellia sp*; their percentage contribution was 28,48%, 18,40%, 12,81%, 11,84%, 5,87%, 5,47%, 3,64%, 3,25% and 2,84%, respectively, of the overall value of 15,68, and the cumulative percentages were 28,48%, 46,89%, 59,70%, 71,54%, 77,41%, 82,89%, 86,53%, 89,77% and 92,61%, respectively.

Species mainly influencing differences within C3 site were *Articulated Corallinaceae, Dictyotales, Encrusting Calcified Rodophitae, Dark Filamentous Algae*, *Halopteris scoparia, Dictyota linearis, Padina pavonica, Dictyota dichotoma and Peyssonnellia sp.*

Control 4 Site

The average dissimilarity of all pairwise coefficients with site C4 outside, was 35,04. Of this average similarity, 13,94 was contributed by *DFA,* 7,55 by *AC*, 5,13 by *Caulerpa racemosa,* 2,22 by, *ECR*, 2,17 by *GFA* and 0, 97 by *Cystoseira sp*; the percentage contribution was 39,80%, 21,54%, 14,65%, 6,34%, 6,19% and 2,78%, respectively, of the overall value of 35,04, and cumulative percentages were 39,80%, 61,34%, 75,99%, 82,33%, 88,52%, and 91,30%, respectively.

Species mainly influencing differences within C4 site were *Dark Filamentous Algae*, *Articulated Corallinaceae, Caulerpa racemosa, Encrusting Calcified Rhodophytae, Green filamentous algae* and *Cystoseira.*

The high concentration of species in the algae assemblages, as observed in the protected sites P0A and P0C and in the Controls C3 and C4, seems to be related to a deterioration of the community which is stressed by external events like: 1) damage of sublittoral stands with trawling techniques in the past or recently; 2) impacts of climate change; 3) presence of alien species (*C. racemosa*), 4) eutrophication pointed out by dominance of opportunistic species like *Green* or *Dark filamentous algae*.

Sites like Control 1 and 2, Protected P0B are in pristine and uncorrupted primitive state; in these sites there were still important assemblages of autochthones algae.

Analysis pointed out a better quality in the benthic assemblages of shallow subtidal waters (5m of depth). It was less evident in deeper habitat (15m-25m of depth). The same result was recorded in North-East Sardinia at the MPA of Tavolara - Punta Coda Cavallo (Ceccherelli G. *et al*., 2005).

In shallow waters this finding is probably due to indirect effects of an increase of consumers in the protected site (Micheli F. *et al.*, 2005), while the lack of direct impacts at deeper depths is indicative of very similar assemblages. The cover of encrusting algae was significantly higher at the subtidal sites suggesting a possible higher grazing pressure. One of the possible causes of the inconsistent results obtained between habitats is that trophic cascade effects could have a different influence at different heights on the shore. The evaluation of the interconnection among benthic habitats through trophic links is also highlighted to provide an estimate of the vulnerability to protection of various habitats.

The diversity and structure of the benthic assemblages were significantly influenced by the physical exposure of the coastal line; protection was not found to be a significant source of variation for assemblages investigated. In contrast, assemblages seemed to be more dependent on the geographical location within the MPA. Moreover, results indicate significant differences in structure assemblages among locations.

The no entry zone still needs to be protected; the acquired data validate the necessity to create in these waters the no entry zone. It is influenced by the presence of the alien species *C. racemosa* and by important seasonal currents.

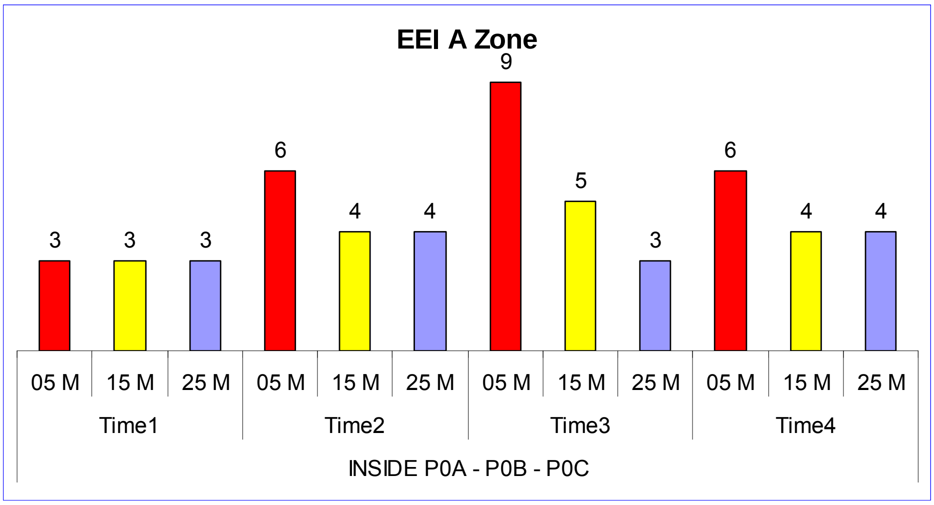
The partial reserve is more productive than other studied sites inside and outside. Biodiversity richness of flora and fauna justifies sport activities and underwater recreational activity.

This analysis highlights two zones both in B zone, the Isola dei Cavoli and I Congressi as the best analyzed places.

4.3.2 ECOLOGICAL EVALUATION INDEX

EEI IN PROTECTED SITES

Ecological State Class in Protected Sites is Low at Time 1 (EEI=3).

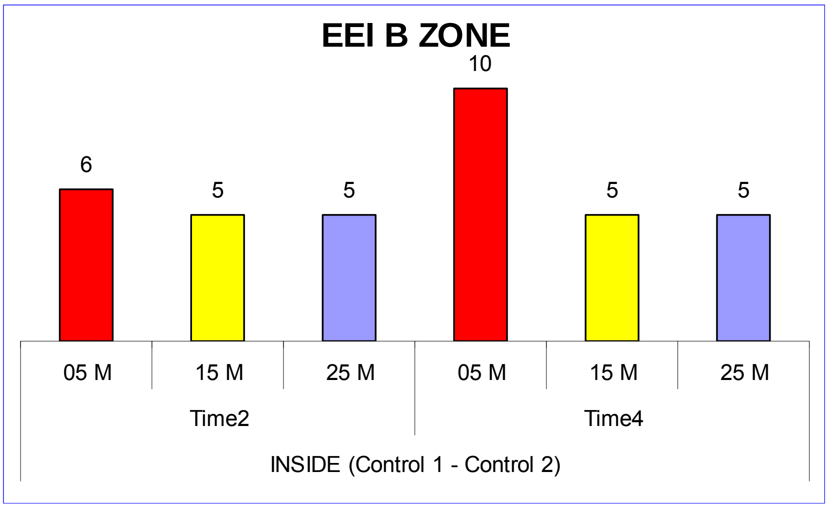


Graph 4.10: Ecological evaluation index for the A zone in the Isola di Serpentara subdivided by Time and depth.

At Time 2, the EEI improves from Low to Moderate at all the depths. At Time 3, the EEI for shallow waters sites become Good (EEI=9). Improvements can be observed also at the depth of 15 m. Time 4 and Time 2 show the same pattern.

EEI CONTROL 1 – CONTROL 2

Controls 1 and 2 (all inside the sanctuary, in B Zone) are characterized by moderate values of EEI at Time 2.

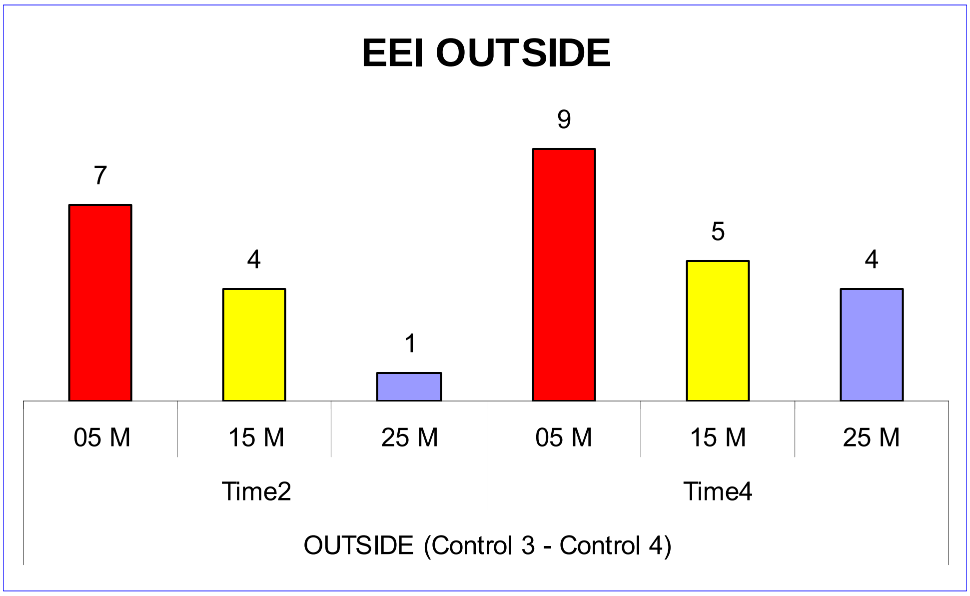


Graph 4.11: Ecological evaluation index for the B zone in the controls of the Isola dei Cavoli (I Faraglioni and the rocky islet I Congressi) subdivided by Time and depth.

At Time 4, EEI in shallow waters was 10, the best result for these sites. We observed similar values for deeper sites.

EEI CONTROL 3 – CONTROL 4

Control 3 (outside the sanctuary, near the boundaries) and Control 4 are characterized by a progressive increase of EEI in all the sampled depths.

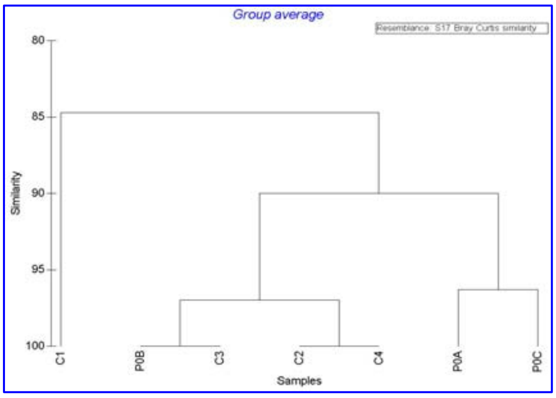


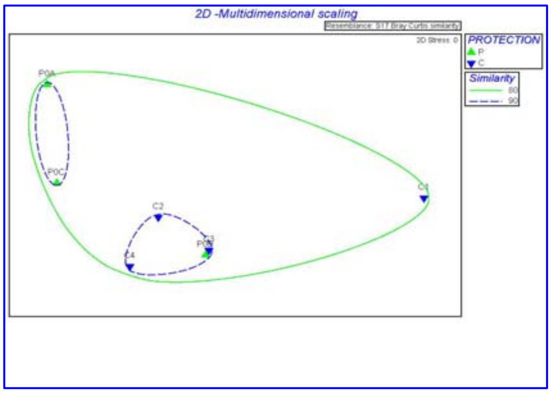
Graph 4.12: Ecological evaluation index for the C zone in Capo Boi and Torre delle Stelle subdivided by Time and depth.

The EEI of the 0,5m depth is Good at Time 1 and become High at Time 4. At the other depths, EEI values improve for 15 metres (from 4 to 5) and from Bad to Low (EEI 1-4) at 25 metres.

EEI - OVERALL

Cluster analysis shows high similarity (near 95%) for P0B and Control 3, Control 2 and Control 4, P0A and P0C.





Graphs 4.13 4.14: Cluster analysis and Multidimensional scaling for the Ecological evaluation index in the analyzed sites.

This is confirmed by the Multi Dimensional Scaling plot. Moreover Control 1 is far from the similarity of other Sites. The Similarity is grouped for C2-C4-P0B and P0C-P0A.

Simper analysis underlines that Control 1 and 2 sites are characterized by high values of *Cystoseira sp*. and *Green filamentous algae*, of the ESG I and ESG II. Significant values of average abundance were recorded for *Caulerpa racemosa*.

Analysis of similarity (ANOSIM) randomization test indicated significant global R value (R= 0,259), but not significant level for P (P>3%) confirming scarce differences between protected sites.

The average similarity of all pairwise coefficients was 52,37. The highest contribution came from *Cystoseira sp*, 11,46, followed by GFA, 10,02, DFA, 9,18 and *Caulerpa racemosa,* 4,39. Percentages were 21,88%, 19,13%, 17,53% and 8,38%, respectively, of the overall value, while cumulative percentages were 21,82%, 41,02%, 58,55% and 66,93%.

Analysis of the studied sites underlined general positive trend from Time 1 to Time 3 in every analyzed site. Ecological evaluation index was more influenced by the presence of the alien species *Caulerpa racemosa* in Protected Sites.

Moreover, it was influenced by the depth factor: all the sites at deeper depth had considerable concentrations of opportunistic Sheet like and Filamentous Algae with high growth rates of the ecological state group II.

Shallow waters generally included algae species with a thick or calcareous thallus, low growth rates and long life cycles (late successional) of the ecological state group I.

4.4 *CAULERPA RACEMOSA*

The green algae *Caulerpa racemosa* was frequently found in the studied area. It is considered as an “alien species”, probably introduced in the Mediterranean Sea with ballast water or attached to ships hulls (Flagella M. and Abdulla A., 2005).

The introduction of invasive marine species into new environments by ships and via other vectors has been identified as one of the four greatest threats to the world’s oceans. The other three are the land-based sources of marine pollution, the overexploitation of living marine resources and the physical alteration/destruction of marine habitat (Streftaris N. *et al*., 2005,).

Shipping moves over 80% of the world’s commodities and transfers internationally approximately 3 to 5 billion tonnes of ballast water each year.

Figure 4.8: Ship on the roadstead in the Gulf of Cagliari.

A similar volume may also be transferred domestically within countries and regions each year. Ballast water is essential to the safe and efficient operation of modern shipping, providing balance and stability to unladen ships. However, it may also represent a serious ecological, economic and health threat.

It is estimated that at least 7,000 different species are being carried in ships ballast tanks around the world. The vast majority of marine species carried in ballast water do not survive the journey, as the ballasting and deballasting cycle and the environment inside ballast tanks can be quite hostile to organism survival. Even those species that survive the voyage and are discharged have weak chances of surviving in the new environmental conditions, including predation by and/or competition from native species. However, when all factors are favourable, an introduced species can survive and establish a reproductive population in the host environment; it may even become invasive, outcompete native species and multiply into pest proportions, threatening biodiversity, fisheries and aquaculture. Some introduced species severely deplete native populations or deprive them of food. Others form colonies which can smother the existing fauna.

4.4.1 *CAULERPA RACEMOSA*

Caulerpa racemosa (Forsskal) J. Agardh is a green algae that presents the following characteristics: wide spreading, with long, coarse branching stolons, becoming very densely entangled in old colonies, which often become 1-2 m in diameter; stout descending rhizoid-bearing branches common; erect foliar branches often much crowded on the stolons, sometimes more remote, one to several centimetres tall, simple or very sparingly forked, covered with short clavate to spherical stalked branchlets; stolon being 0.3 - 0.8 mm in diameter and usually less than 10 cm long, though occasionally some reach 20 cm or more, the erect part is cylindrical, 1 - 10 cm long and 0.3 - 0.8 mm in diameter, unbranched or occasionally so and during the greatest part of the vegetative period they are devoid of vesicles and thus are similar in appearance to the stolon; assimilators of mature plants carry 1 - 5 pairs of pyriform vesicles measuring about 1.0-1.2 mm in diameter.

The invasive variety of *Caulerpa racemosa* (Forsskal) J. Agardh currently spreading in the Mediterranean Sea was first discovered in the early 1990s near Tripoli Harbour in Libya (Nizamuddin M., 1991). It spreads rapidly and it can be now found off the coasts of 11 countries overlooking the Mediterranean Sea (Tunisia, Libya, Egypt, Cyprus, Turkey, Greece, Malta, Croatia, Italy, France and Spain), and it has also reached the Canary Islands in the Atlantic Ocean (Verlaque M. *et al*., 2004). Recent work has shown that this invasive variety is *C. racemosa* var. *cylindracea* introduced from south-western Australia (Verlaque M. *et al.,* 2003).

It is well represented all over the Sardinia South Coast zone from shallow waters to 50 m of depth.

Caulerpa racemosa can spread by fragmentation (Smith C. and Walters L.,1999; Piazzi L., Ceccherelli G., 2001), and sexual reproduction (Panayotidis P. and Zuljevic´ A., 2001), and its spherical branchlets (ramuli) can act as propagules (Renoncourt L. and Meinesz A., 2002). Long-range dispersal of the alga seems to be a result of human activities (e.g. disturbance by anchors, fishing). *C. racemosa* can inhabit a wide range of subtidal substrata (sand, mud, rocks, dead matte of sea grass, from 0 to 50 m depth), and has the potential to expand its range around the entire coastline of the Mediterranean Sea.

C. racemosa modifies density and diversity of the benthic communities (Argyrou M. *et al.,* 1999, Piazzi L. *et al*., 2001, Dumay O. *et al.,* 2002).

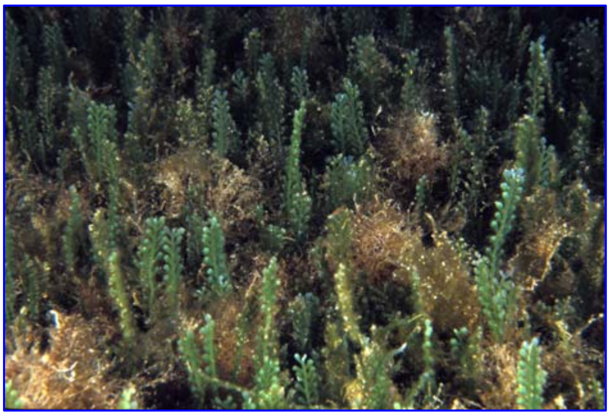
This chapter considers data of *C. racemosa* within sites from 5 to 25 metres occurred during Macro benthic *census*.

Figure 4.9: Stolon and erect part of *Caulerpa racemosa* on hard substrate.

In the studied areas, *C. racemosa* generally covers vertical and sub-vertical hard substrate. The meadow of *C. racemosa* is a dense network of overlapping stolons, resembling a green web on the sea floor.

C. racemosa has large cumulative stolon lengths and numerous rhizoidal pillars that must play a significant role in the uptake of the nutrients from the substratum (Williams S., 1984), and could interfere with the nutrient acquisition by other benthic algae.

Development of stolons can also have negative impact on other species holdfast to substrate. In the picture above *C. racemosa* is covering the zooids of the Anthozoa *Eunicella Cavolini.*



Caulerpa racemosa **in soft bottoms (Control 1) at 41 m of depth.**
Eunicella cavolini **enveloped with** ***Caulerpa racemosa*****, Green filamentous algae and fishing lines at Torre delle Stelle in Control 4.**

Figures 4.10 - 4.11: ***Caulerpa racemosa***

4.4.2 MATERIALS AND METHODS

Assemblages of *Caulerpa racemosa* were studied by analysing pictures of the macro benthic biocoenosis *census.* Time and Sampling sites were the same of the benthic biocoenosis census.

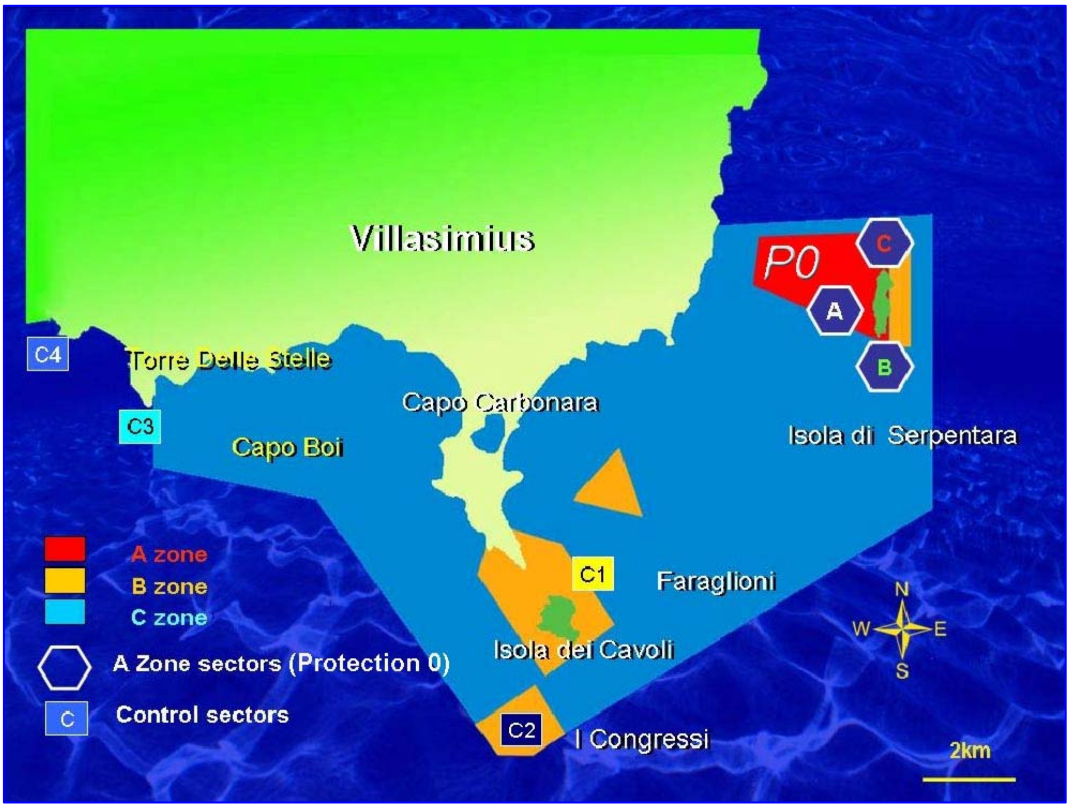
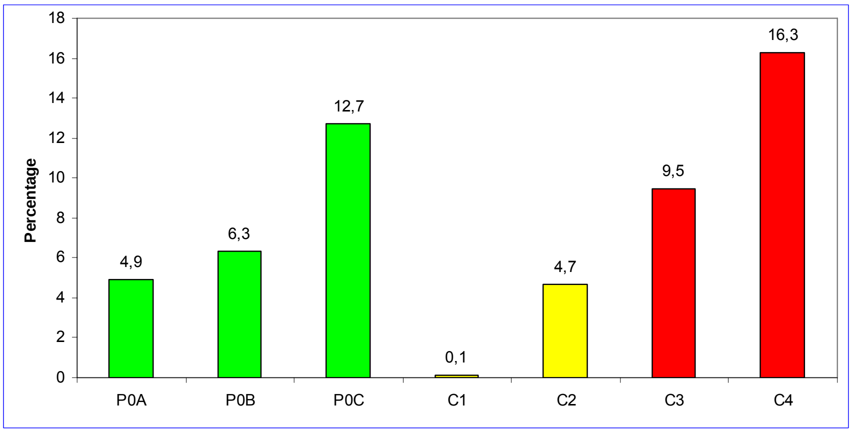


Figure 4.12: The studied area: the same sites examined for macro benthic analysis.

4.4.3 RESULTS AND DISCUSSION

C. racemosa was well represented in every sampled site. It was found in all strata of depth.



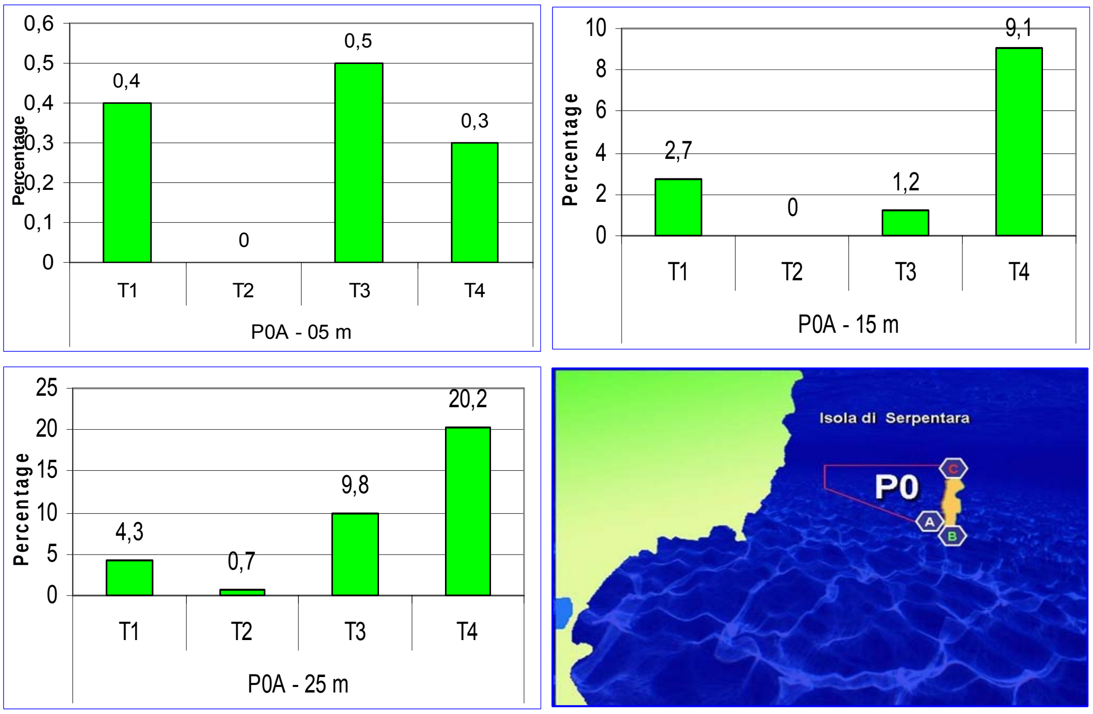
Graph 4.15: Percentage values of *Caulerpa racemosa* in protected and control sites.

Despite of its spreading was previously signalled only during warm seasons in other studies (Ruitton S. *et al.*, 2005), in the present investigation it was found all over the year, and in several sites it was well represented also in winter.

PROTECTED P0A

In site P0 high values of percentage can be observed in Time 1 and 3 (September 2004-September 2005) at five meters of depth.

This trend was different at 15 m where a higher percentage of *C. racemosa* was recorded in T4 (May 2006).

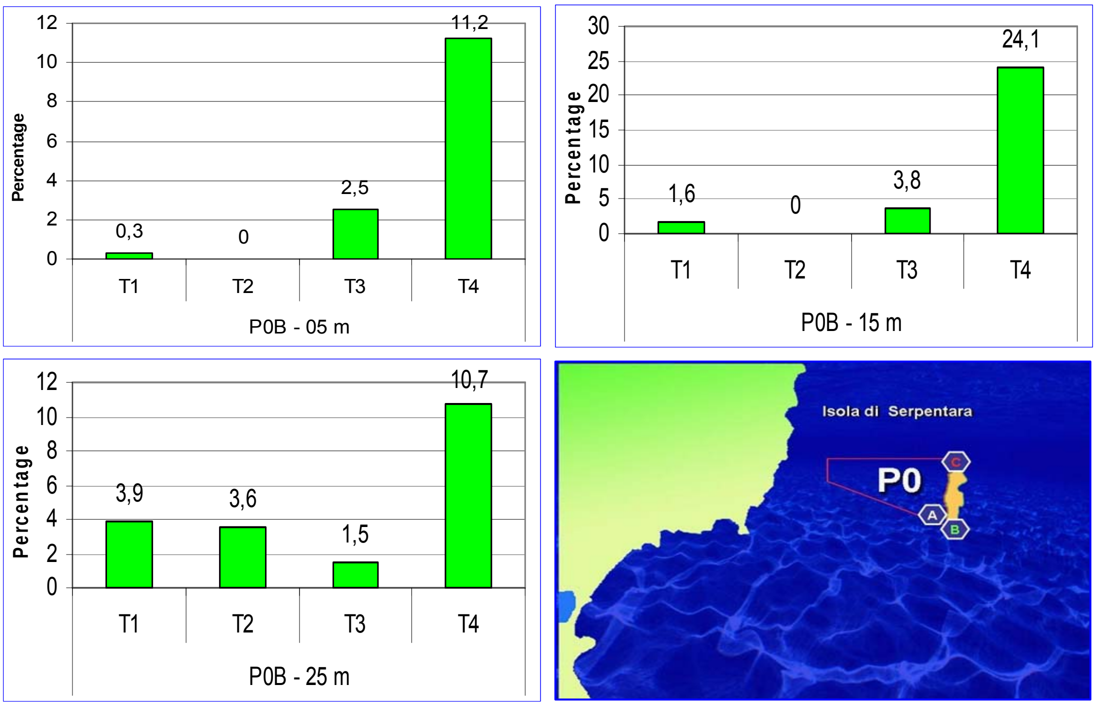


Graphs 4.16-4.17- 4.18: Recorded values of *Caulerpa racemosa* in Site A of the A Zone (Isola di Serpentara), subdivided by depth.

An increased density was recorded at 25 m of depth from T2 to T4.

PROTECTED P0B

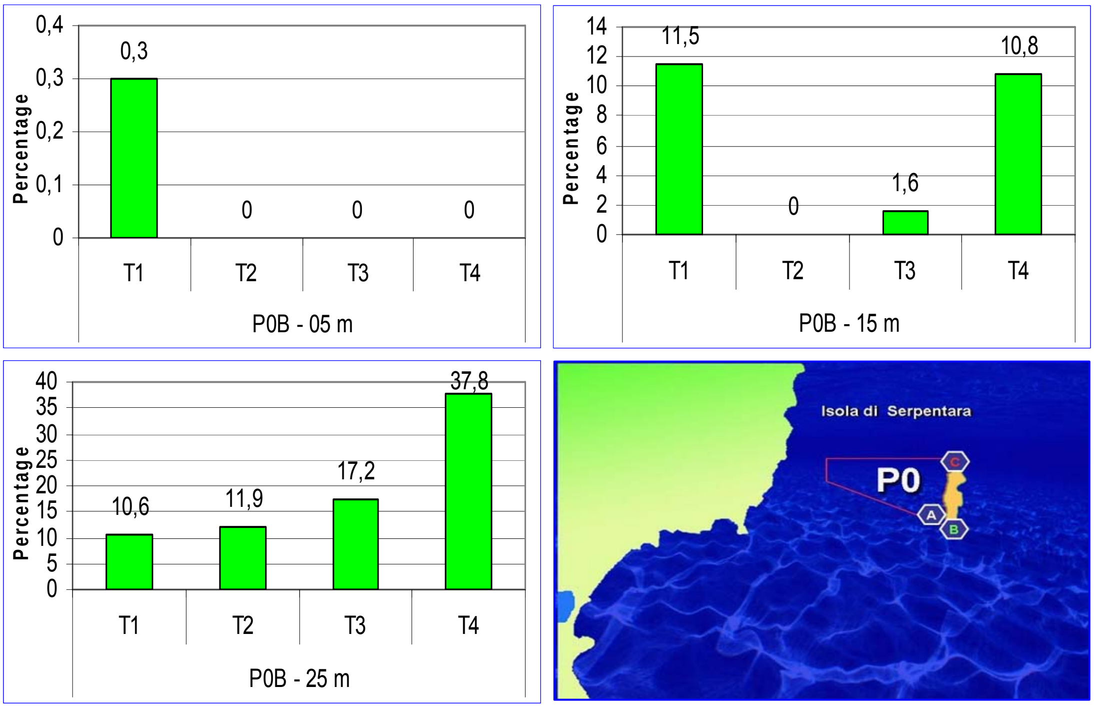
Considerable high values can be observed in T4 for all sites. Highest values were recorded at 15 m of depth.



Graph 4.19- 4.20.- 4.21: Recorded values of *Caulerpa racemosa* in in Site B of the A Zone (Isola di Serpentara), subdivided by depth.

PROTECTED P0C

C. racemosa was recorded at depth of 05 m only in T1 (September 2004);



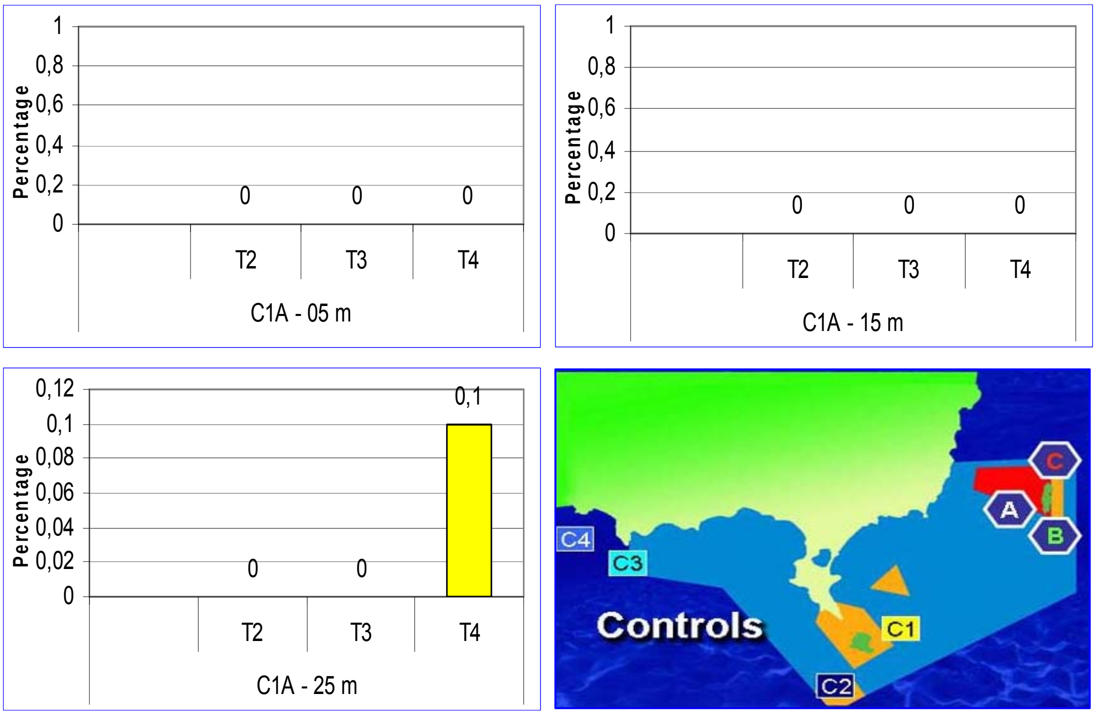
Graphs 4.22- 4.23- 4.24: Recorded values of *Caulerpa racemosa* in in Site C of the A Zone (Isola di Serpentara), subdivided by depth.

At 15 m there is an uncertain trend: percentage of coverage decreases in autumn and increases in winter-spring season.

At 25 m there is a remarkable increasing trend in the percentage of covering from T1 to T4.

CONTROL C1A

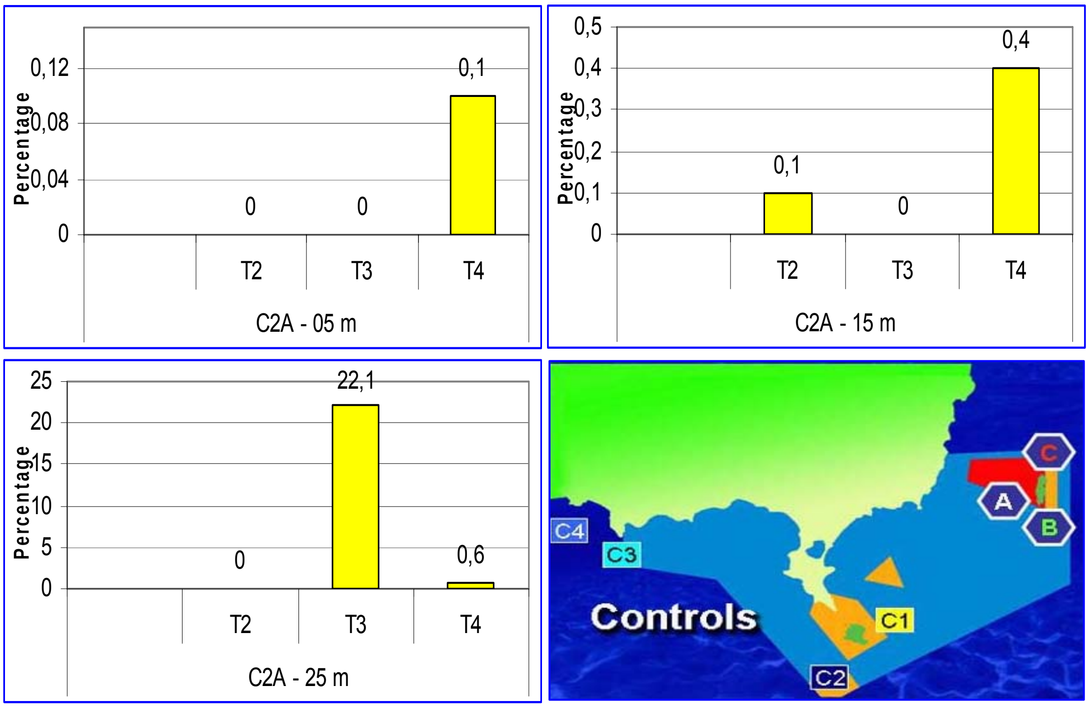
Control 1 is an exclusive sampled site: *C. racemosa* was found only at 25m of depth, at low percentage of coverage (0.1%) and in winter-spring season, even if its presence was recorded in soft bottom at 41 m of depth (Figure 4.11).



Graphs 4.25 - 4.26 - 4.27: Recorded values of *Caulerpa racemosa* in in Control 1 of the B Zone of the Isola dei Cavoli (I Faraglioni), subdivided by depth.

CONTROL C2A

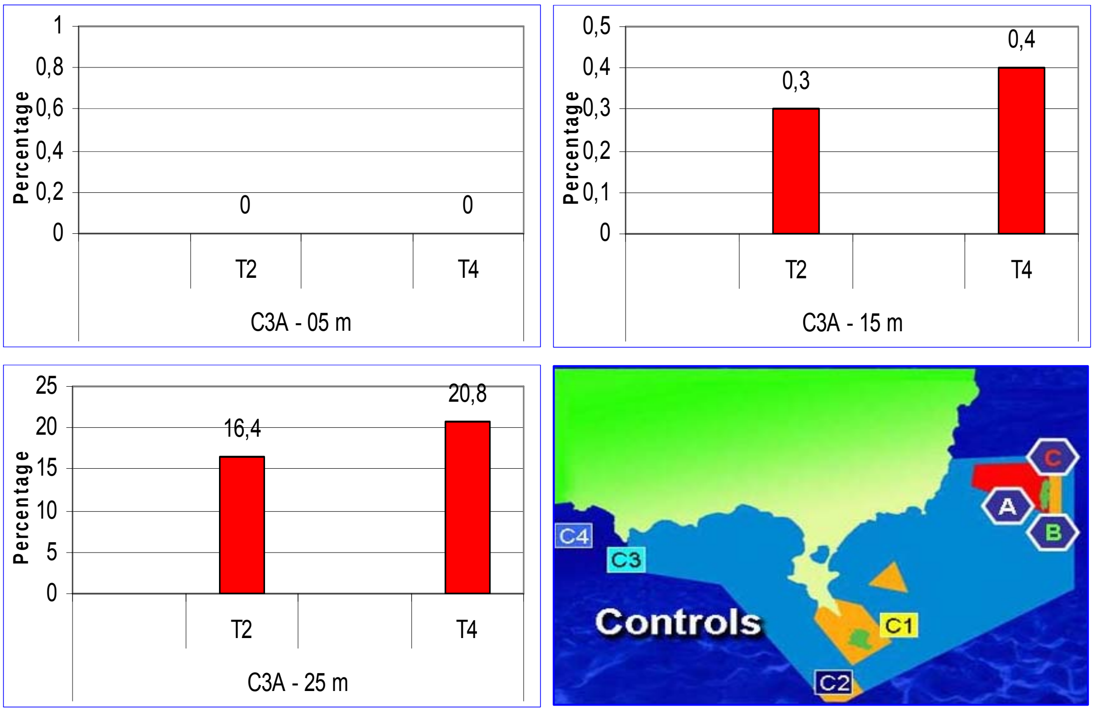
C. racemosa was present at low percentage (0,1%) at T2 at a depth of 15 m, at T3 at a depth of 25 m, and at T4 at all depths, but with relative high values only at 15 m of depth.



Graphs 4.28-4.29-4.30: Recorded values of *Caulerpa racemosa* in in Control 2 of the B Zone of the Isola dei Cavoli (I Congressi), subdivided by depth.

CONTROL C3A

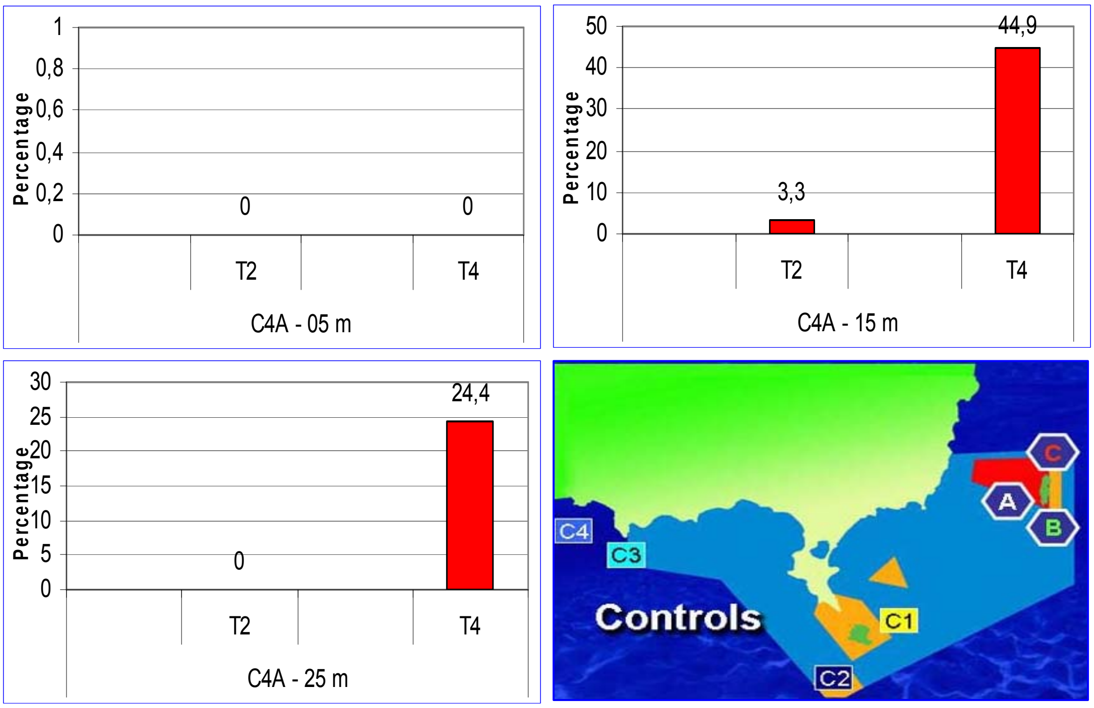
Species was not found at 5 metres of depth. In the others strata coverage increase from T2 to T4. Higher values were recorded at 25 m of depth.



Graphs 4.31-4.32-4.33: Recorded values of *Caulerpa racemosa* in Control 3 Outside near the boundary at Capo Boi (Solanas), subdivided by depth.

CONTROL C4A

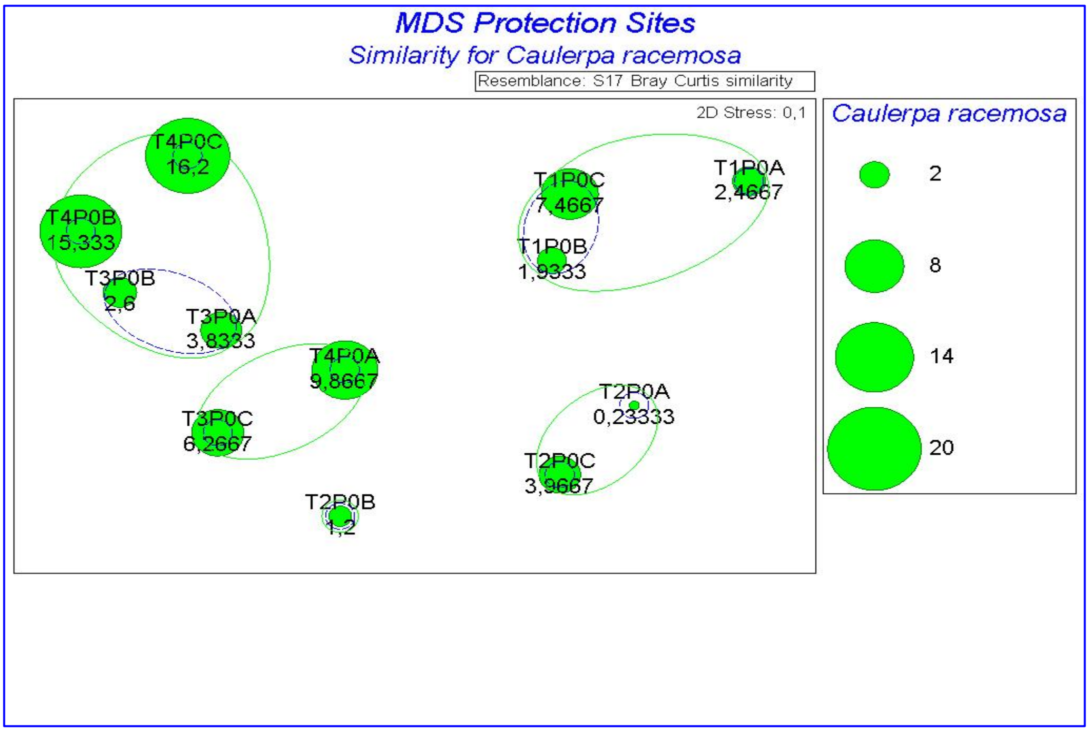
Control site 4 represents the conditions far away from the sanctuary boundaries. Here *C. racemosa* showed high percentage of coverage at 15 m (44%) and 25 m (24,4%) of depth. *Caulerpa racemosa* was not found at 5 m of depth.



Graphs 4.34-4.35-4.36: Recorded values of *Caulerpa racemosa* in Control 4 Outside the sanctuary at Torre delle Stelle, subdivided by depth.

ANALYSIS BETWEEN PROTECTED SITES AND CONTROLS

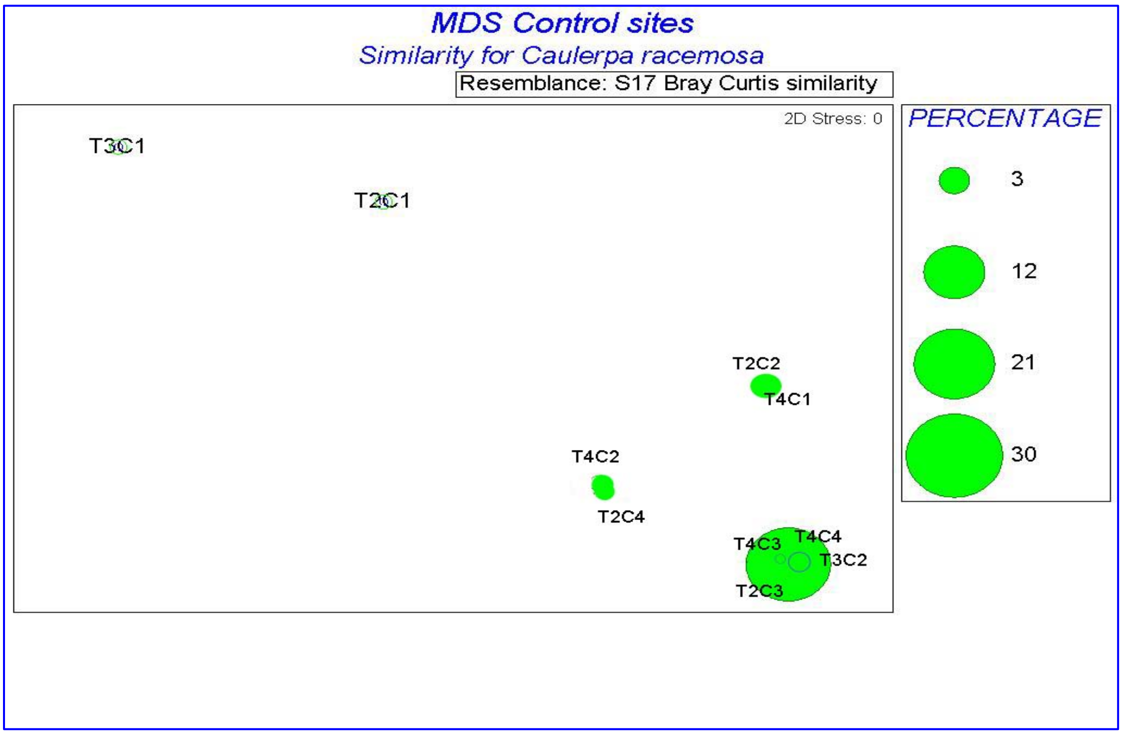
Comparing all the percentages of this species in the sampled areas, coverage increases from P0A to P0C and from C1 to C4 (Graph 4.15).



Graph 4.37: Recorded values of *Caulerpa racemosa* showed in multidimensional scaling for protected sites in A Zone (Isola di Serpentara).

Multidimensional scaling analysis for the protected area of *C. racemosa* showed similarity near the 60% in T3 P0A, T3 P0B. Higher values were observed in T4 P0B (15, 33%) and in T4 P0C.

In the Control sites a significant diversity was observed in Control 1 and Control 2, while similarity of about 60% and 80% was observed for the other control sites.



Graph 4.38: Recorded values of *Caulerpa racemosa* showed in multidimensional scaling for control sites.

Encrusting and erect algae of the ESG I (table 4.2), decreased in invaded areas, while opportunistic filamentous species (ESG II), increased their abundance. Macro algae of the ESG I could be damaged by the overgrowth of this invasive seaweed, as stolons may stop or extremely reduce the availability of light irradiance. Moreover, species reproducing sexually could be damaged by the lack of substrate available for spore settlement because of pre-emption of substrate by *C. racemosa* (Piazzi L. *et al*., 2001).

Previous studies confirmed that the difference between substrata covered or uncovered by *C. racemosa* is linked to the sediments concerned redox values, indicating that under the mats of *C. racemosa* a reduced environment occurs (Piazzi L. *et al*., 2001). This finding suggests that effects of *C. racemosa* and sediment can have greater implications on the system. In fact, besides influencing invaded macro algae, the relevant decrease of redox potential of sediment could also lead to a drastic reduction in diversity of infaunal assemblages.

Sediment constitutes a relevant component in assemblages dominated by *C. racemosa*, suggesting that it could be considered as a structural constituent of *Caulerpa* populations and of filamentous species.

Trapped sediment could play a role in the competitive mechanisms of *C. racemosa*, based on overgrowth and pre-emption of substrate, in the same way described for mats constituted by introduced turf-forming species (Piazzi L. *et al.*, 2007).

5 CONCLUSION AND PROSPECTIVES

The main objective of the present thesis was to record ichtyofauna and macro benthos within the MPA of south Sardinia where previous study had not been conclusive, and to evaluate the effectiveness of protective measures on the marine environment, through the study of spatial and temporal changes of the communities aforementioned.

During three years of sampling a great quantity of data was collected in the MPA of Capo Carbonara – Villasimius chosen for the study.

Fished species and their relative abundances were estimated inside the sanctuary and compared to outside areas.

Spillover and reserve effects were validated with fish visual *census* and trammel net surveys; these two scientific techniques were chosen to minimize the errors intrinsic in each technique. The analysed 24 target species of fish, subdivided in 8 families (*Labridae, Moronidae, Mullidae, Sciaenidae, Scorpaenidae, Serranidae, Sparidae, Sphiraenidae),* observed between Inside protected and outside unprotected fished area, were statistically different: more fish were found in the protected than in fished area, and difference was observed in total fish density, abundance, biomass: most target fish fisheries had a greater density (e.g. *Diplodus puntazzo, Diplodus sargus, Diplodus vulgaris*, *Epinephelus marginatus*, *Mullus surmuletus*, *Pagrus pagrus*, *Sciena umbra, Scorpaena porcus, Serranus scriba, Sphyraena Sphyraena* and *Synphodus tinca*) and/or size (e.g. *Dentex dentex, Diplodus puntazzo, Diplodus sargus*) within the protected area than in fished areas outside.

Analysis of several indexes showed richer ichtiofauna in the inside areas than near the boundaries outside, but an increasing similarity was also observed. This trend was confirmed by the cluster analysis and MDS: plot showed a general progressive increased similarity between protected and unprotected sites from the early time of census to the end, validating the spillover effects from the inside to the outside areas.

These results indicate that reserve effects (protection) from fishing may have the potential to influence fish assemblages of outside areas.

We hypothesize that the establishment of the MPA can contribute to the local fishery with the increasing of yields. It must be pointed out that local professional fishermen are allowed to practise fishing within the MPA (zone B – C) according to a “local fishery permission”. Positive effects on fishing are also expected in the boundaries zone as the spillover confirmed.

Benefits outside the sanctuary are already evident and they will become more prominent in the long term.

To improve the knowledge of mechanisms operating in MPAs further samplings are needed on a wider scale and considering protected and unprotected localities, including multiscaling prospective both in space (analysing several places) and time (more than 3 years), including physical factors (like orientation, depth, wave and current exposition), which operate in the environment.

More than 600 pictures of benthic biocoenosis permitted to schedule benthic assemblages on a large scale of the MPA. They were analysed according to stratified design in space, time and population structure.

A total of 325 species were recorded and subdivided in 15 Phyla: 4 of Tallophyta (Phaeophyta, Rhodophyta, Chlorophyta, Cyanophycota); 1 of Anthophyta (Spermatophyta); 10 of Invertebrata. They represent the snapshoot of the “state of the art” of the MPA.

The methodology used, i.e. underwater photography, although showed a loss in taxonomic information, did not entail a corresponding loss of ecological quality informations.

Macro benthic biocoenosis *census* highlights a “Moderate” ecological state in all the sanctuary and nearby the boundaries during all samplings.

All sites in which high values of the alien species *Caulerpa racemosa* were recorded, presented a low value of EEI, confirming the assumption that *C. racemosa* colonisation enhances the competitiveness of filamentous species of the ESG II changing the pristine ecological status.

Data analysis demonstrates the effectiveness of evaluation indexes for the study of the ecological state within and nearby the MPA.

Difference in benthos assemblages was recorded considering studied transect at the three different depths of 5, 15 and 25 meters. Data are consistent with the natural increase of inorganic compounds from shallow to deeper waters in the Mediterranean Sea.

It must be pointed out that where values of benthic biocoenosis were high also indexes of fish fauna biodiversity and biomass were proportionally high.

This was evident in the zones inside the sanctuary nearby the Isola dei Cavoli – Capo Carbonara and I Faraglioni.

In Sardinia the growing consensus for the coastal zones and living marine resources requires more severe protections. The crisis facing many marine ecosystems is increasingly attracting public attention. However, choosing the best methods to maintain or restore the health of marine ecosystems and the coastal zone is a difficult task for politicians, resources managers and a source of disagreement among user groups, scientist, the conservation community and local communities.

In the last ten years a growing body of literature documented the effectiveness of marine reserves to preserve habitats, fostering the recovery of overexploited species, and maintaining marine communities. For these reasons there is a rising demand for ecosystem-based management approaches to marine management, that considers the systems as a whole rather than as separable pieces of an interlocking puzzle. Incorporation of MPAs into a broader plan for coastal and ocean management offers an opportunity to revise current fragmented management approaches and to provide more inclusive representation of stakeholders concerned the health of marine ecosystems.

To maintain public support for marine reserves and protected areas, it is necessary to implement management plans, the role of regulatory authority and funds for enforcement, research and monitoring. Upgraded monitoring programs will ensure a robust data collection for local and regional application.

Finally, we think that results from monitoring programs should be also integrated with research programs for the evaluation of reserve performance and for the future design of more effective plans.

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APPENDIX 1: Anthropogenic stress on marine benthic macrophytic communities.

Anthropogenic stress	Benthic macrophytes	Impact
Eutrophication	Seaweeds	Dominance of opportunistic species, seaweed blooms, decline of diversity
	Seagrasses	Large scale and regional decline of meadows, dominance of fleshy seaweeds
Organic matter, Siltation	Seaweeds	Light reduction and alteration of hard substrate affects community structure
	Seagrasses	Decline of meadows through reduction of light and accumulation of organic matter in sediment
Heavy metals	Seaweeds	Inhibition of reproduction and development, changes in community structure
	Seagrasses	No direct effect has been observed
Oil splits	Seaweeds	Short term growth reduction in intertidal species
	Seagrasses	No direct effect has been documented
Global warming	Seaweeds	Changes in distribution patterns are expected
	Seagrasses	Changes in distribution patterns are expected
Increase of salinity	Seaweeds	Further expansion in estuarine ecosystems
	Seagrasses	Genera displacement, e.g. Cymodocea instead of Ruppia
Trawling	Seaweeds	Damage of sublittoral stands
Fishing	Seagrasses	Fragmentation - decline of meadows

Anthropogenic stress on marine benthic macrophytic communities (Orfanidis S. *et al*., 2001).

APPENDIX 2: Functional characteristics and growth strategies of marine benthic macrophytes.

Ecological State Group	Functional form group	External morphology	Internal anatomy	Productivity	Longevity (Succession)	Growth Strategies (sensu Grime)	Genera
II	A. Sheet-Group	Thin tubular and sheet like (foliose)	Uncorticated one-several cells thick,	High	Annuals (Opportunistic)	Ruderal	Ulva, Enteromorpha, Scytosiphon (erect phase), Dictyota
II	B. Filamentous-Group	Delicately branched (filamentous)	Uniseriate, multiseriate or lightly corticated	High	Annuals (Opportunistic)	Ruderal	Cyanophyceae, Chaetomorpha, Cladophora, Polysiphonia, Ceramium, Spyridia
II	C. Coarsely Branched Group	Coarsely branched upright	Corticated	Species specific	Annuals (Mid-successional)	Stress-tolerant- or Stress-tolerant-Competitors	Acanthophora, - Ruderal Caulerpa, Chordaria, Gracilaria, Laurencia, Liagora
I	D. Thick Leathery-Group	Thick blades and branches	Differentiated, heavily corticated thick walled	Low	Perennials (Late-successional)	Competitors	Cystoseira, Chondrus, Fucus, Laminaria, Padina, Sargassum, Udotea
I	E. Jointed Calcareous Group	Articulated - calcareous, upright	Calcified genicula, flexible intergenicula	Low	Perennials (Late-successional)	Competitors	Amphiroa, Corralina, Galaxaura, Halimeda, Jania
I	F. Crustose-Group	Epilithic, prostrate, encrusting	Calcified or uncalcified parallel cell rows	Low	Perennials (Late-successional)	Competitors	Hydrolithon, Lithothamnion, Peyssonnelia, Porolithon
I	G. Seagrasses	Highly differentiated from foliose to cylindrical (Leafs, rhizomes, roots, flowers, fruits)	Hghly differentiated (epidermis, mesophyll, vascular system)	Low	Perennials	Stress-tolerant (Pioneers to late successional)	Cymodocea, Posidonia, -Ruppia

Functional characteristics and growth strategies of marine benthic macrophytes. (Orfanidis S., *et al.,* 2001).

APPENDIX 3: Check list of studied Macro Phytobenthos in the MPA

Class	Order	Family	Genera	Species
CHLOROPHYCEAE	BRYOPSIDALES	CAULERPACEAE	*Caulerpa*	*Caulerpa racemosa*
		CODIACEAE	*Codium*	*Codium vermilara*
				Codium bursa
				Codium effusum
				Codium adherens
			Flabellia	*Flabellia petiolata*
			Halimeda	*Halimeda tuna*
	CLADOPHORALES	ANADYOMENACEAE	*Anadyomene*	*Anadyomene stellata*
		CLADOPHORACEAE	*Chaetomorpha*	*Chaetomorpha sp.*
			Cladophora	*Cladophora sp.*
	DASYCLADALES	DASYCLADACEAE	*Acetabularia*	*Acetabularia acetabulum*
			Dasycladus	*Dasycladus sp.*
				Dasycladus vermicularis
	SIPHONOCLADALES	SIPHONOCLADACEAE	*Cladophoropsis*	*Cladophoropsis modonensis*
		VALONIACEAE	*Valonia*	*Valonia macrophysa*
				Valonia utricularis
	TETRASPORALES	PALMELLOSPIDACEAE	*Palmophyllum*	*Palmophyllum crassum*
	ULOTHRICALES	ULVACEAE	*Enteromorpha*	*Enteromorpha sp.*
			Ulva	*Ulva sp.*

Check list of studied Macro Phytobenthos in the MPA: Phylum CHLOROPHYTA.

Class	Order	Family	Genera	Species
CYANOPHYCEAE	NOSTOCALES	RIVULARIACEAE	*Rivularia*	*Rivularia atra*
				Rivularia sp.

Check list of studied Macro Phytobenthos in the MPA: Phylum CYANOPHYCOTA.

Class	Subclass	Order	Family	Genera	Species
PHAEOPHYCAE		SPOROCHNALES	SPOROCHNACEAE	*Sporochnus*	*Sporochnus pedunculatus*
	CYCLOSPOREAE	FUCALES	CYSTOSEIRACEAE	*Cystoseira*	*Cystoseira amentacea*
					Cystoseira barbata
					Cystoseira compressa
					Cystoseira corniculata
					Cystoseira sp.
			SARGASSACEAE	*Sargassum*	*Sargassum sp.*
	HETEROGENERATAE	SCYTOSIPHONALES	SCYTOSIPHONACEAE	*Colpomenia*	*Colpomenia sinuosa*
	ISOGENERATAE	CUTLERIALES	CUTLERIACEAE	*Cutleria*	*Aglaozonia parvula*
					Cutleria adspersa
					Cutleria multifida
				Zanardinia	*Zanardinia prototypus*
					Zanardinia sp.
		DICTYOTALES	DICTYOTACEAE	*Taonia*	*Taonia atomaria*
				Zonaria	*Zonaria flava*
				Lobophora	*Lobophora variegata*
				Dictyopteris	*Dictyopteris membranacea*
				Dictyota	*Dictyota dichotoma*
				Dictyota	*Dictyota dichotoma v. intricata*
				Dictyota	*Dictyota linearis*
				Dictyota	*Dictyota spiralis*
				Dilophus	*Dilophus fasciola*
				Dilophus	*Dilophus spiralis*
				Padina	*Padina pavonica*
		ECTOCARPALES	RALFSIACEAE	*Ralfsia*	*Ralfsia verrucosa*
		SPHACELARIALES	SPHACELARIACEAE	*Cladostephus*	*Cladostephus verticillatus*
					Stypocaulaceae
				Halopteris	*Halopteris fillicina*
				Halopteris	*Halopteris scoparia*
				Stypocaulon	*Stypocaulon scoparium*

Check list of studied Macro Phytobenthos in the MPA: Phylum PHAEOPHYTA.

Check list of studied Macro Phytobenthos in the MPA: Phylum RHODOPHYTA.

Class	Subclass	Order	Family	Genera	*Species*
			BRYOPSIDACEES	*Bryopsis*	*Bryopsis sp.*
				Champia	*Champia parvula*
				Champia	*Champia sp.*
				Chrysymenia	*Chrysymenia sp.*
			CHAMPIACEAE	*Chrysymenia*	*Chrysymenia ventricosa*
				Chylocladia	*Chylocladia verticillata*
				Gastroclonium	*Gastroclonium sp.*
		BRYOPSIDALES		*Botryocladia*	*Botryocladia boergesenii*
				Botryocladia	*Botryocladia botryoides*
			RHODYMENIACEAE	*Botryocladia*	*Botryocladia sp.*
				Rhodymenia	*Rhodymenia ardissonei*
RHODO-PHYCEAE				*Rhodymenia*	*Rhodymenia pseudopalmata*
				Rhodymenia	*Rhodymenia sp.*
				Ceramium	*Ceramium sp.*
			CERAMIACEAE	*Polysiphonia*	*Polysiphonia opaca*
				Wrangleria	*Wrangelia penicillata*
			DELESSERIACEAE	*Nithophyllum*	*Nithophyllum sp.*
		CERAMIALES		*Chondrophycus*	*Chondrophycus sp.*
				Laurencia	*Laurencia complex*
	FLORIDEOPHYCIDAE			*Laurencia*	*Laurencia sp.*
			RHODOMELACEAE	*Osmundea*	*Osmundea sp.*
				Rithyphloea	*Rithyphloea tinctoria*
				Vidalia	*Vidalia volubilis*
				Lithophyllum	*Lithophyllum sp.*
RHODO-PHYCEAE				*Lithophyllum*	*Lithophyllum sp.*
		CORALLINALES	CORALLINACEAE	*Lithophyllum*	*Lithophyllum stictaeforme*
				Mesophyllum	*Mesophyllum lichenoides*
	FLORIDEOPHYCIDAE	CORALLINALES		*Neogoniolithon*	*Neogoniolithon marmillatum*
				Neogoniolithon	*Neogoniolithon sp.*
				Tenarea	*Tenarea tortuosa*
					AC - Corallinaceae ramificate sottili
				Amphiroa	*Amphiroa rigida*
			CORALLINACEAE	*Amphiroa*	*Amphiroa rubra*
				Amphiroa	*Amphiroa sp.*
				Corallina	*Corallina elongata*
				Corallina	*Corallina sp.*
				Haliptilon	*Haliptilon virgatum*
				Jania	*Jania rubens*
				Lithothamnion	*Lithothamnion sonderi*
				Lithothamnion	*Lithothamnion sp.*
			DUMONTIACEAE		*Dumontiaceae*
			HALYMENIACEAE	*Halymenia*	*Halymenia floresia*
			HILDENBRANDIACEAE	*Hildenbrandia*	*Hildenbrandia*

Class	Subclass	Order	Family	Genera	Species
					rubra
				Hildenbrandia	*Hildenbrandia sp.*
			KALLYMENACEAE	*Kallymenia*	*Kallymenia reniformis*
				Meredithia	*Meredithia microphylla*
			PEYSONNELIACEAE	*Peyssonnelia*	*Peyssonnellia sp.*
			GELIDIACEAE	*Gelidiella*	*Gelidiella sp.*
				Gelidium	*Gelidium latifolium*
				Gelidium	*Gelidium sp.*
				Pterocladia	*Pterocladia sp.*
				Pterocladiella	*Pterocladiella sp.*
		GIGARTINALES	HYPNEACEAE	*Hypnea*	*Hypnea furnariana*
				Hypnea	*Hypnea musciformis*
				Hypnea	*Hypnea sp.*
			PHYLLOPHORACEAE	*Phyllophora*	*Phyllophora crispa*
				Phyllophora	*Phyllophora sp.*
RHODO-PHYCEAE				*Schottera*	*Schottera nicaeensis*
				Schottera	*Schottera sp.*
	FLORIDEOPHYCIDAE		SPHAEROCOCCACEAE	*Sphaerococcus*	*Sphaerococcus coronopifolius*
				Sphaerococcus	*Sphaerococcus sp.*
		NEMALIALES	BONNEMAISONIACEAE	*Falkenbergia*	*Falkenbergia sp.*
			GALAXAURACEAE	*Sciania*	*Scinaia furcellata*
				Tricleocarpa	*Tricleocarpa fragilis*
			HELMINTHOCLADIACEAE	*Nemalion*	*Nemalion helmintoides*
			LIAGORACEAE	*Liagora*	*Liagora viscida*

Check list of studied Macro Phytobenthos in the MPA: Phylum RHODOPHYTA.

Phylum **SPERMATOPHYTA**

Class	Subclass	Ordine	Family	Genera	Species
MONOCOTYLEDONAE	HELOBIAE		POTAMOGETONACEAE	*Posidonia*	*Posidonia oceanica*

Check list of studied Macro Phytobenthos in the MPA: Phylum SPERMATOPHYTA.

APPENDIX 4. Check list of studied Macro Zoobenthos in the MPA

Kingdom ANIMALIA

Phylum	Class	Order	Family	Genera	Species
ANELLIDA	POLYCHAETA	SABELLIDA	SABELLIDAE	*Sabella*	*Sabella spallanzanii*
				Protula	*Protula tubularia*
			SERPULIDAE	*Serpula*	*Serpula vermicularis*

Check list of studied Macro Zoobenthos in the MPA: Phylum ANELLIDA.

Class	Subclass	Order	Family	Genera	Species
CRUSTACEA	CIRRIPEDIA	THORACICA	BALANIDAE	*Balanidae*	*perforatus*
			CHTHAMALIDAE	Chtamalus	*Chtamalus sp.*
	MALACOSTRACA	DECAPODA	MAJADAE	Acanthonyx	*Acanthonyx lunulatus*
			PAGURIDAE	Pagurus	*Pagurus sp.*
			PORCELLANIDAE	Maja	*Maja verrucosa*
			PORTUNIDAE	Carcinus	*Carcinus sp.*

Check list of studied Macro Zoobenthos in the MPA: Phylum ARTHROPODA.

Class	Order	Family	Genera	Species
GYMNOLAEMATA	CHEILOSTOMATIDA	CABEREIDAE	*Caberea*	*Caberea boryi*
		FLUSTRIDAE	*Carbasea*	*Carbasea papyrea*
		MICROPORIDAE	*Calpensia*	*Calpensia nobilis*
	CHEILOSTOMIDA	AETEIDAE	*Aetea*	*Aetea truncata*
		BUGULIDAE	*Bugula*	*Bugula sp.*
		CELLEPORIDAE		*Celleporidae*
			Cellepora	*Cellepora pumicosa*
		ESCHARELLIDAE	*Schizobrachiella*	*Schizobrachiella sanguinea*
		HIPPOPORINIDAE	*Pentapora*	*Pentapora fascialis*
			Pentapora	*Pentapora similis*
		MARGARETTIDAE	*Margaretta*	*Margaretta cereoides*
		MYRIAPORIDAE	*Myriapora*	*Myriapora truncata*
		RETEPORIDAE	*Sertella*	*Sertella septentrionalis*
			Sertella	*Sertella sp.*
		SAVIGNYELLIDAE	*Savignyella*	*Savignyella sp.*
		SCHIZOPORELLIDAE	*Schizomavella*	*Schizomavella linearis*
			Schizomavella	*Schizomavella sp.*
		SCRUPOCELLARIIDAE	*Scrupocellaria*	*Scrupocellaria sp.*
		SMITTINIDAE	*Smittina*	*Porella cervicornis*
	CTENOSTOMIDA	VESICULARIIDAE	*Zoobothryon*	*Zoobothryon sp.*
			Zoobothryon	*Zoobothryon verticillatum*
STENOLAEMATA	CYCLOSTOMATA	CRISIIDAE	*Crisia*	*Crisia sp.*
		DIATOSPORIDAE	*Diastopora*	*Diastopora sp.*
		HORNERIDAE	*Hornera*	*Hornera frondiculata*
			Hornera	*Hornera sp.*
		LICHENOPORIDAE	*Lichenopora*	*Lichenopora sp.*

Check list of studied Macro Zoobenthos in the MPA: Phylum BRYOZOA.

Class	Order	Family	Genera	Species
ASCIDIACEA	APLOUSOBRANCHIA	DIDEMNIDAE	*Didemnidae*	*Didemnidae sp.*
			Diplosoma	*Diplosoma listerianum*
		POLYCITORIDAE	*Clavelina*	*Clavelina lepadiformis*
			Cystodytes	*Cystodytes dellechiajei*
	ENTEROGONA	ASCIDIIAE	*Phallusia*	*Phallusia fumigata*
		POLYCLINIDAE	*Aplidium*	*Aplidium sp.*
	PHLEBOBRANCHIATA	ASCIDIIAE	*Ascidia*	*Ascidia mentula*
			Ascidia	*Ascidia sp.*
		CIONIDAE	*Ciona*	*Ciona intestinalis*
	STOLIDOBRANCHIA	MOLGULIDAE	*Molgula*	*Molgula occulta*
		PYURIDAE	*Halocynthia*	*Halocynthia papillosa*
			Microcosmus	*Microcosmus sp.*
		STYELIDAE	*Botryllus*	*Botryllus schloesseri*
OSTEICHTHYES	ANGUILLIFORMES	MURAENIDAE	*Muraena*	*Muraena helena*
	PERCIFORMES	TRIPTERYGIIDAE	*Tripterygion*	*Tripterygion trypteronotus*

Check list of studied Macro Zoobenthos in the MPA: Phylum CHORDATA.

Class	Subclass	Order	Family	Genera	Species
ANTHOZOA	HEXACORALLIA	ACTINARIA	ACTINIIDAE	*Actinia*	*Actinia equina*
				Anemonia	*Anemonia sp.*
					Anemonia sulcata
				Paranemonia	*Paranemonia cinerea*
			AIPTASIIDAE	*Aiptasia*	*Aiptasia mutabilis*
			SAGARTIIDAE	*Cereus*	*Cereus pedunculatus*
		CERIANTHARIA	ANTHIPATHARIA	*Cerianthus*	*Cerianthus sp.*
		MADREPORARIA	DENDROPHYLLIDAE	*Astroides*	*Astroides calycularis*
				Balanophyllia	*Balanophyllia europea*
				Leptosammia	*Leptosammia pruvoti*
			FAVIDAE	*Cladocora*	*Cladocora caespitosa*
		SCLERACTINIA	CARYOPHYLLIIDAE	*Caryophyllia*	*Caryophyllia sp.*
		ZOANTHARIA	EPIZOANTHIDAE	*Epizoanthus*	*Epizoanthus sp.*
		ZOANTHARIA	PARAZOANTHIDAE	*Parazoanthus*	*Parazoanthus axinellae*
	OCTOCORALLIA	ALCYONACEA	GORGONIIDAE	*Eunicella*	*Eunicella cavolinii*
					Eunicella sp.
	OCTOCORALLIA	STOLONIFERA	CLAVULARIIDAE	*Clavularia*	*Clavularia sp.*
		STOLONIFERA	CORNULARIDAE	*Cornularia*	*Cornularia cornucopie*
HYDROZOA		HYDROIDA	SERTULARIDAE	*Sertularella*	*Sertularella gaudichaudi*
				Sertularella	*Sertularella sp.*
			CORINIDAE	*Corynactis*	*Corynactis viridis*
			PLUMULARIIDAE	*Aglaophenia*	*Aglaophenia sp.*
				Plumularia	*Plumularia sp.*
	ATHECATAE	CAPITATA	TUBULARIIDAE	*Tubularia*	*Tubularia crocea*
		FILIFERA	EUDENDRIIDAE	*Eudendrium*	*Eudendrium racemosum*
					Eudendrium sp.
	THECATAE	CONICA	SERTULARIIDAE	*Dynamena*	*Dynamena cavolinii*

Check list of studied Macro Zoobenthos in the MPA: Phylum COELENTERATA.

Class	Order	Family	Genera	Species
ASTEROIDEA	FORCIPULATIDA	ASTERINIDAE	*Coscinasterias*	*Coscinasterias tenuispina*
	SPINULOSA		*Asterina*	*Asterina gibbosa*
CRINOIDEA	COMATULIDA	ANTEDONIDAE	*Antedon*	*Antedon mediterranea*
ECHINOIDEA	ECHINOIDA	ECHINIDAE	*Paracentrotus*	*Paracentrotus lividus*
	DIADEMATOIDA	ARBACIIDAE	*Arbacia*	*Arbacia lixula*
HOLOTHUROIDEA	ASPHIDOCHIROTIDA	HOLOTHURIIDAE	*Holothuria*	*Holothuria sp.*
STELLEROIDEA	SPINULOSIDA	ECHINASTERIDAE	*Echinaster*	*Echinaster sepositus*
	VALVATIDA	OPHIDIASTERIDAE	*Hacelia*	*Hacelia attenuata*
STELLEROIDEA			*Ophidiaster*	*Ophidiaster ophidianus*

Check list of studied Macro Zoobenthos in the MPA: Phylum ECHINODERMATA.

Class	Order	Family	Genera	Species
ECHIURIDEA	BONELLIIDA	BONELLIIDAE	*Bonellia*	*Bonellia viridis*

Check list of studied Macro Zoobenthos in the MPA: Phylum ECHIURA.

Class	Subclass	Order	Family	Genera	Species
BIVALVIA	HETERODONTA	MYOIDA	GASTROCHAENIDAE	*Gastrochaena*	*Gastrochaena dubia*
		VENEROIDA	CARDITIDAE	*Cardita*	*Cardita calyculata*
	PTERIOMORPHIA	ARCOIDEA	ARCIDAE	*Arca*	*Arca noae*
		MYTILOIDA	MYTILIDAE	*Lithophaga*	*Lithophaga lithophaga*
				Mytilaster	*Mytilaster sp.*
				Mytilus	*Mytilus galloprovincialis*
	PTERIOMORPHIA	OSTREOIDA	OSTREIDAE	*Ostrea*	*Ostrea edulis*
GASTROPODA	OPISTHOBRANCHIA	NEOGASTROPODA	MURICIDAE	*Murex*	*Murex sp.*
		NUDIBRANCHIA	CHROMODORIDIDAE	*Hypselodoris*	*Hypselodoris tricolor*
			DISCODORIDIDAE	*Discodoris*	*Discodoris atromaculata*
				Peltodoris	*Peltodoris atromaculata*
			FAVORINIDAE	*Dondice*	*Dondice banyulensis*
			FLABELLINIDAE	*Flabellina*	*Flabellina affinis*
				Flabellina	*Flabellina sp.*
		SACOGLOSSA	ELYSIIDAE	*Thuridilla*	*Thuridilla hopei*
	PROSOBRANCHIA	ARCHAEO GASTROPODA	FISSURELLIDAE	*Fissurella*	*Fissurella sp.*
			PATELLIDAE	*Patella*	*Patella sp.*
			TROCHIDAE	*Monodonta*	*Monodonta sp.*
			TROCHIDAE	*Monodonta*	*Monodonta turbinata*
		MESOGASTROPODA	LITTORINIDAE		*Littorinidae*
				Littorina	*Littorina sp.*
			RISSOIDAE		*Rissoidae*
			TURRITELLIDAE		*Turritellidae*
				Turritella	*Turritella sp.*
			VERMETIDAE		
				Serpulorbis	*Serpulorbis*

Class	Subclass	Order	Family	Genera	Species
					arenarius
				Vermetus	*Vermetus triqueter*
			COLUMBELLIDAE	*Columbella*	*Columbella rustica*
				Columbella	*Columbella sp.*
			CONIDAE		
			MURICIDAE	*Murex*	*Hexaplex trunculus*
GASTROPODA				*Ocinebrina*	*Ocinebrina edwardsii*
				Stramonita	*Stramonita haemastoma*
				Thais	*Thais haemostoma*
POLY-PLACOPHORA		CHITONIDA	CHITONIDAE	*Chiton*	*Chiton olivaceus*
				Chiton	*Chiton sp.*

Check list of studied Macro Zoobenthos in the MPA: Phylum MOLLUSCA.

Class	Order	Family	Genera	Species
TURBELLARIA	POLYCLADIDA	PROSTHECEREIDA	*Prostheceraeus*	*Prostheceraeus giesbrechtii*
				Prostheceraeus sp.

Check list of studied Macro Zoobenthos in the MPA: Phylum PLATHELMINTHES.

Check list of studied Macro Zoobenthos in the MPA: Phylum PORIFERA.

Class	Subclass	Order	Family	Genera	Species
CALCAREA	CALCARONEA	LEUCOSOLENIDA	GRANTIIDAE	*Leuconia*	*Leuconia aspera*
					Leuconia sp.
			SYCETTIDAE	*Sycon*	*Sycon raphanus*
		LITHONIDA	PETROBIONIDAE	*Petrobiona*	*Petrobiona massiliana*
DEMO SPONGIAE	CERACTINOMORPHA	DICTYOCERATIDA	DYSIDEIDAE	*Dysidea*	*Dysidea sp.*
			SPONGIDAE	*Cacospongia*	*Cacospongia sp.*
				Hippospongia	*Hippospongia communis*
				Hippospongia	*Hippospongia sp.*
				Ircinia	*Ircinia sp.*
					Ircinia variabilis
				Sarcotragus	*Sarcotragus spinosulus*
				Spongia	*Spongia officinalis*
		HALICHONDRIDA	AXINELLIDAE	*Axinella*	*Axinella polypoides*
					Axinella sp.
			HYMENIACIDONIDAE	*Hemimycale*	*Hemimycale columella*
		HAPLOSCLERIDA	HALICLONIDAE	*Dendroxea*	*Dendroxea lenis*
				Haliclona	*Haliclona sp.*
		PECILOSCLERIDAE	MICROCIONIDAE	*Clathria*	*Clathria coralloides*
		PETROSIIDA	PETROSIIDAE	*Petrosia*	*Petrosia ficiformis*
		POECILOSCLERIDA	AGELASIDAE	*Agelas*	*Agelas oroides*
			HYMEDESMIIDAE	*Phorbas*	*Phorbas fictitius*
					Phorbas paupertas

Class	Subclass	Order	Family	Genera	Species
					Phorbas sp.
					Phorbas tenacior
	CERACTINOMORPHA	POECILOSCLERIDA	MYXILLIDAE	*Crambe*	*Crambe crambe*
			PHORHIOSPONGIIDAE	*Batzella*	*Batzella inops*
		VERONGIIDA	APLYSINIDAE	*Aplysina*	*Aplysina aerophoba*
	HOMOSCLEROMORPHA	HOMOSCLEROPHO RIDA	PLAKINIDAE	*Oscarella*	*Oscarella lobularis*
	TETRACTINOMORPHA	ASTROPHORIDA	GEODIIDAE	*Geodia*	*Geodia cydonium*
		AXINELLIDA	AXINELLIDAE	*Acanthella*	*Acanthella acuta*
		CHONDROSIDA	CHONDRILLIIDAE	*Chondrosia*	*Chondrosia reniformis*
			CHONDROSIIDAE	*Chondrilla*	*Chondrilla nucula*
		HADROMERIDA	CLIONIDAE	*Cliona*	*Cliona celata*
					Cliona copiosa
					Cliona nigricans
					Cliona rhodensis
					Cliona schmidti
					Cliona sp.
					Cliona vastifica
					Cliona viridis
			SPIRASTRELLIDAE	*Spirastrella*	*Spirastrella cunctatrix*
			SUBERITIDAE	*Terpios*	*Terpios fugax*
HYALOSP ONGIAE	CALCINEA	CLATHRINIDA	CLATHRINIDAE	*Clathrina*	*Clathrina clatrus*
					Clathrina coriacea
					Clathrina sp.

Check list of studied Macro Zoobenthos in the MPA: Phylum PORIFERA.

Kingdom Protozoa

Class	Order	Family	Genera	Species
GRANULORETICULOSEA	FORAMINIFERIDA	HOMOTREMIDAE	*Miniacina*	*Miniacina miniacea*

Check list of studied Macro Zoobenthos in the MPA: Phylum PROTOZOA.

APPENDIX 5: Check list of studied fish species in Capo Carbonara MPA.

Phylum Chordata

Subphylum Vertebrata

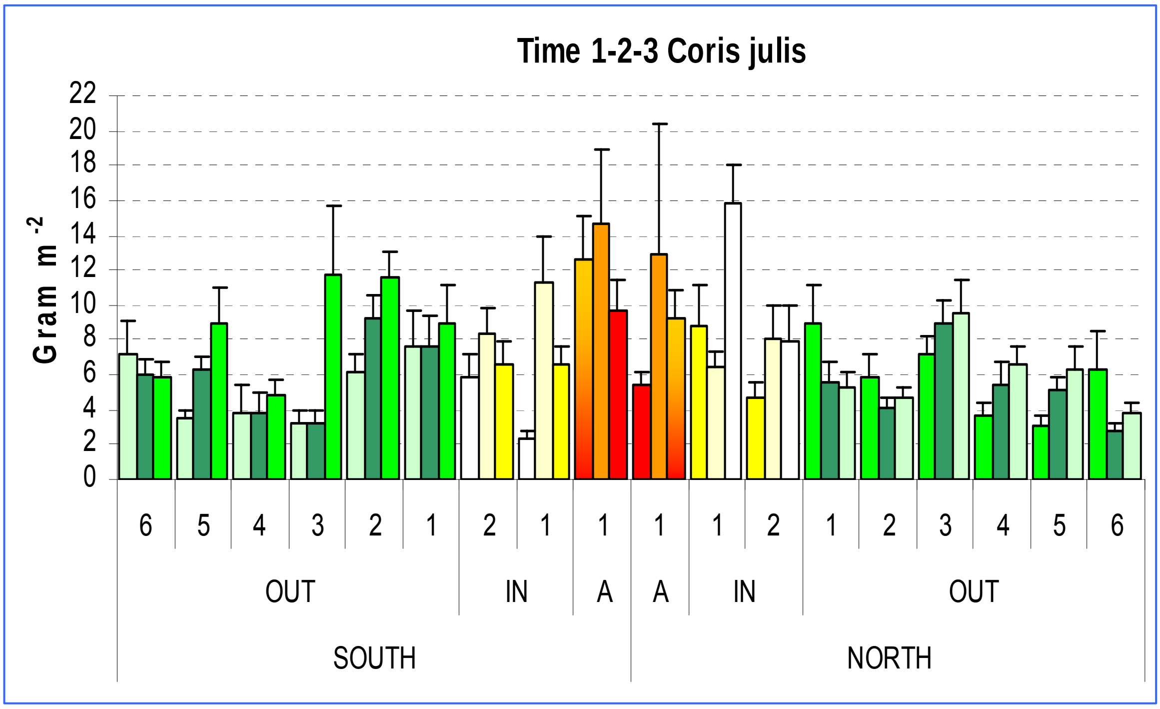
Class Osteichthyes

Subclass Actinopterigi

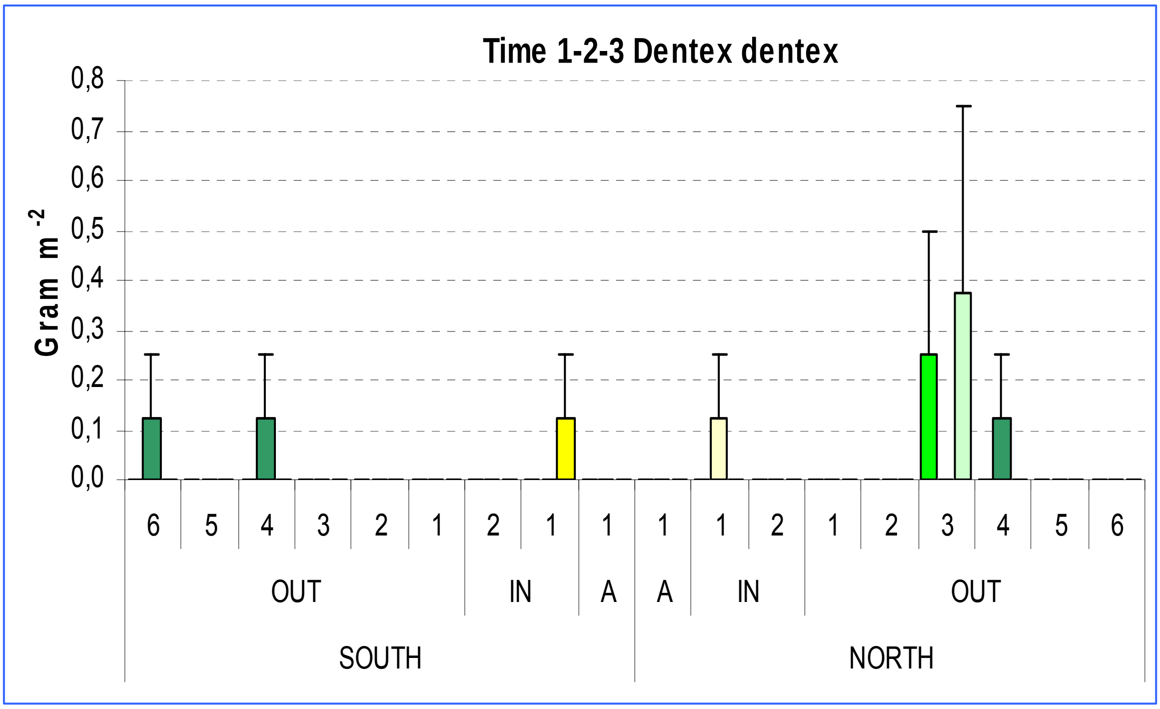
ORDER	FAMILY	GENERA	SPECIES
Perciformes	Labridae	*Coris*	*Coris julis*
		Labrus	*Labrus merula*
			Labrus viridis
		Symphodus	*Symphodus tinca*
		Thalassoma	*Thalassoma pavo*
	Moronidae	*Dicentrarchus*	*Dicentrarchus labrax*
	Mullidae	*Mullus*	*Mullus surmuletus*
	Sciaenidae	*Sciaena*	*Sciaena umbra*
	Serranidae	*Epinephelus*	*Epinephelus costae*
			Epinephelus marginatus
		Serranus	*Serranus cabrilla*
			Serranus scriba
	Sparidae	*Dentex*	*Dentex dentex*
		Diplodus	*Diplodus puntazzo*
			Diplodus sargus
			Diplodus vulgaris
		Lithognathus	*Lithognathus mormyrus*
		Pagrus	*Pagrus pagrus*
		Sarpa	*Sarpa salpa*
		Sparus	*Sparus aurata*
	Sphyraenidae	*Sphyraena*	*Sphyraena sphyraena*
Scorpaeniformes	Scorpaenidae	*Scorpaena*	*Scorpaena notata*
			Scorpaena porcus
			Scorpaena scrofa

Check list of studied fish species in Capo Carbonara MPA: Subclass Actinopterigi

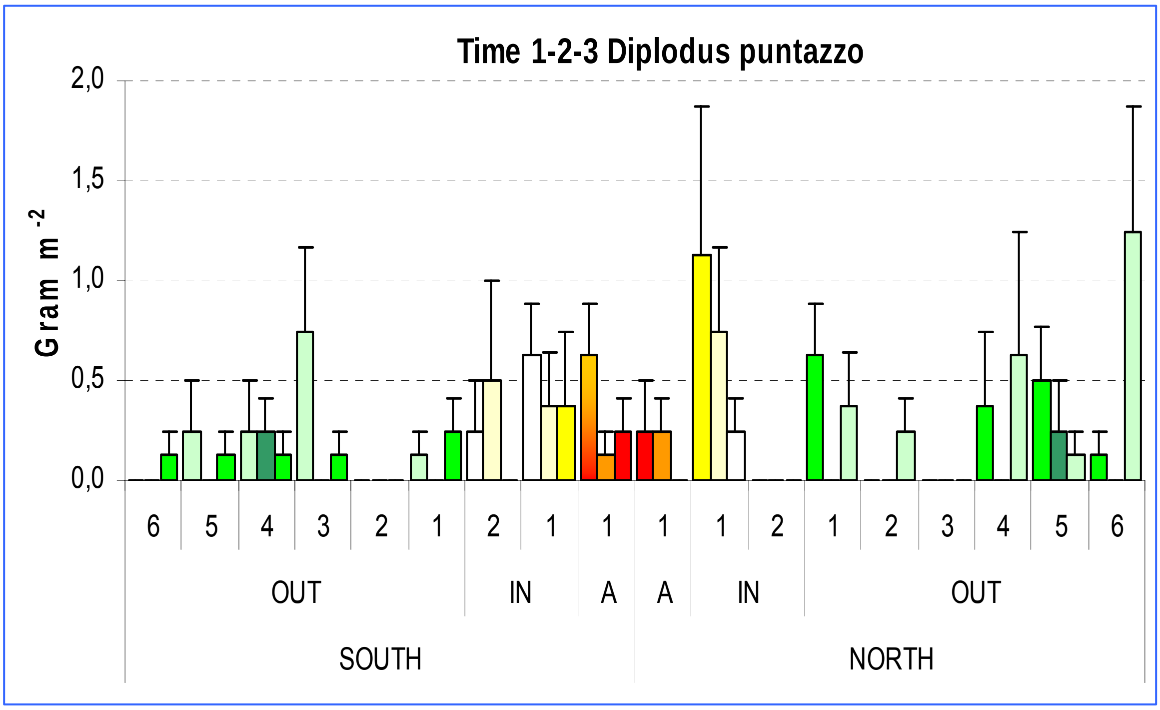
APPENDIX 6: Fish visual *census* biomass per specimen (mean)



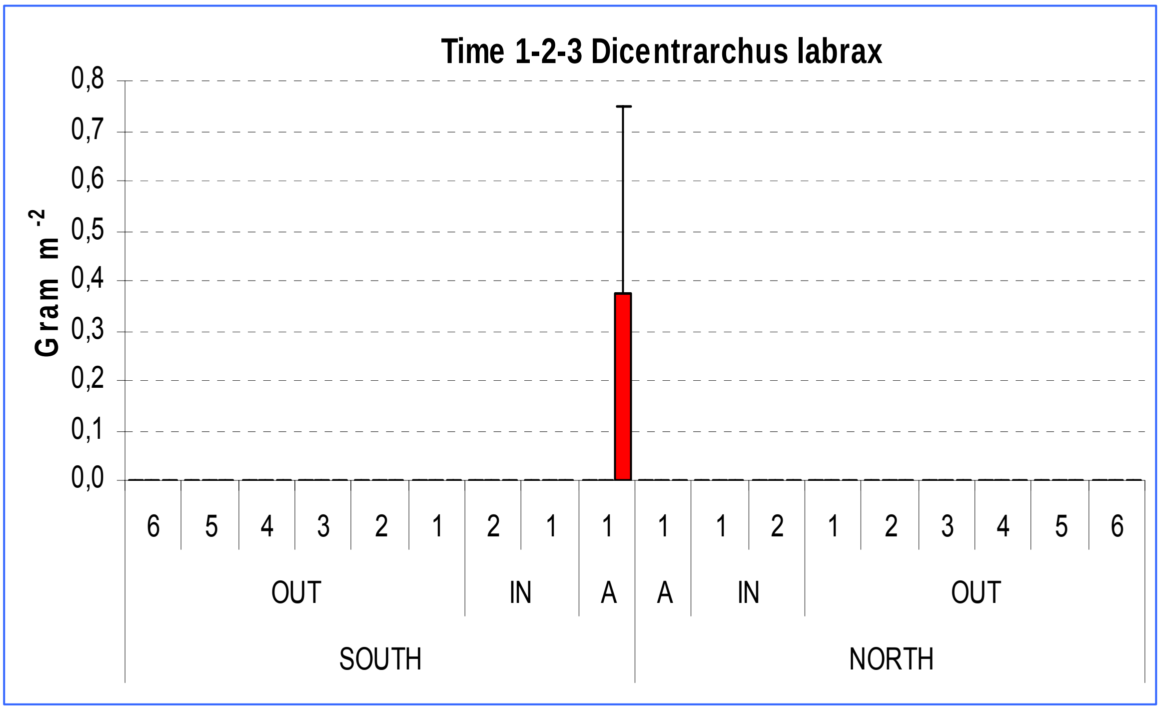
Biomass per specimen (mean) for *Coris julis*.



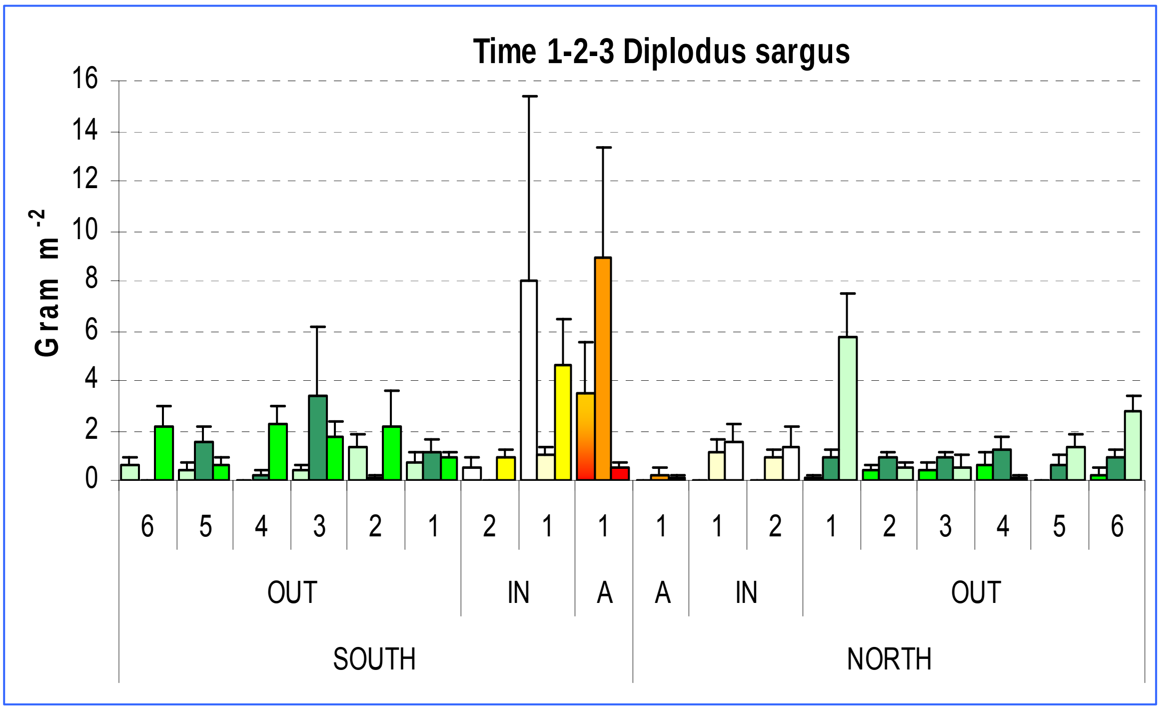
Biomass per specimen (mean) for *Dentex dentex*.



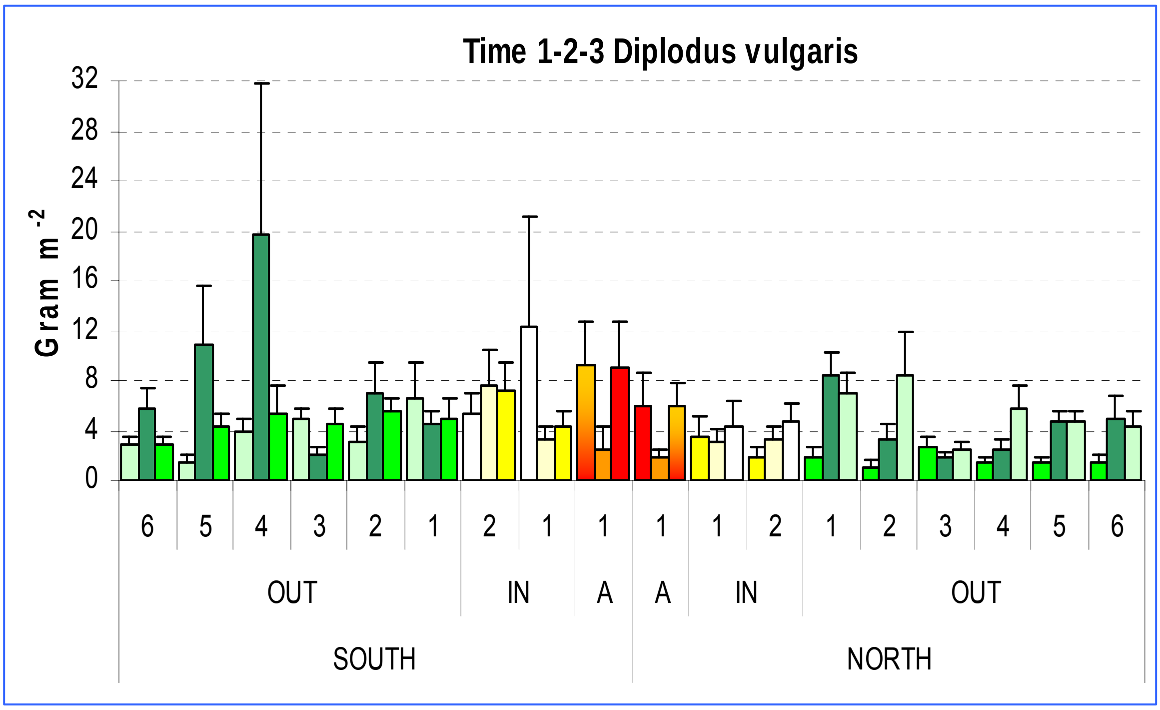
Biomass per specimen (mean) for *Diplodus puntazzo*.



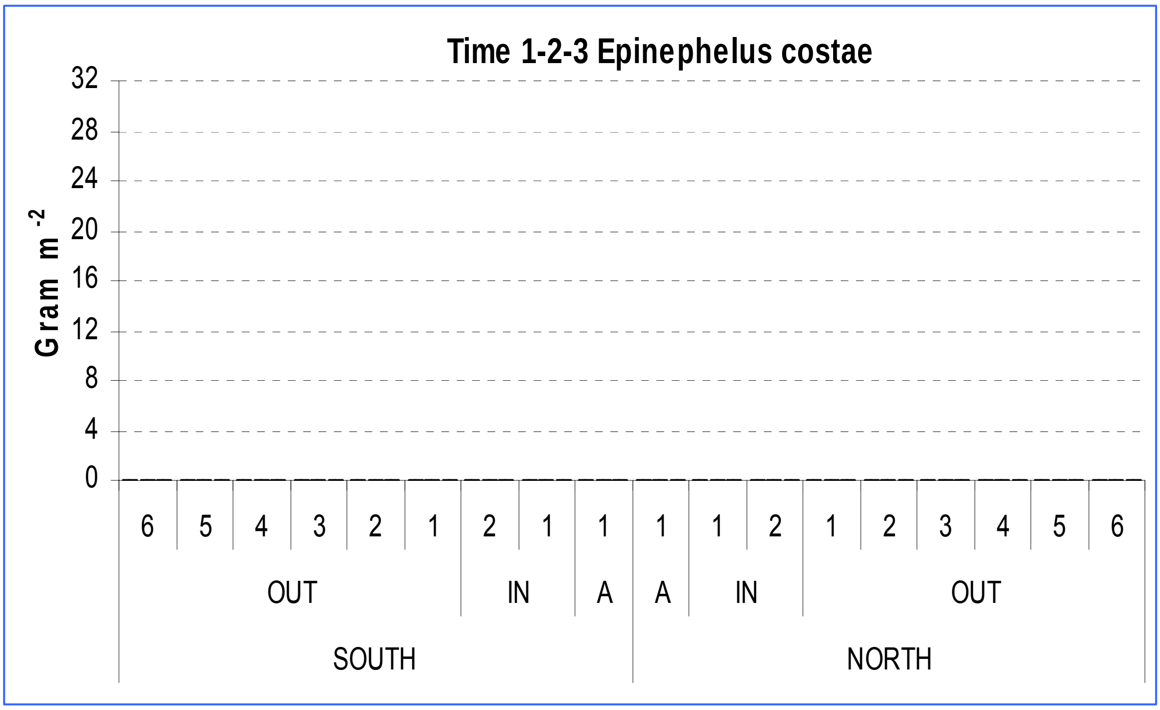
Biomass per specimen (mean) for *Dicentrarchus labrax*.



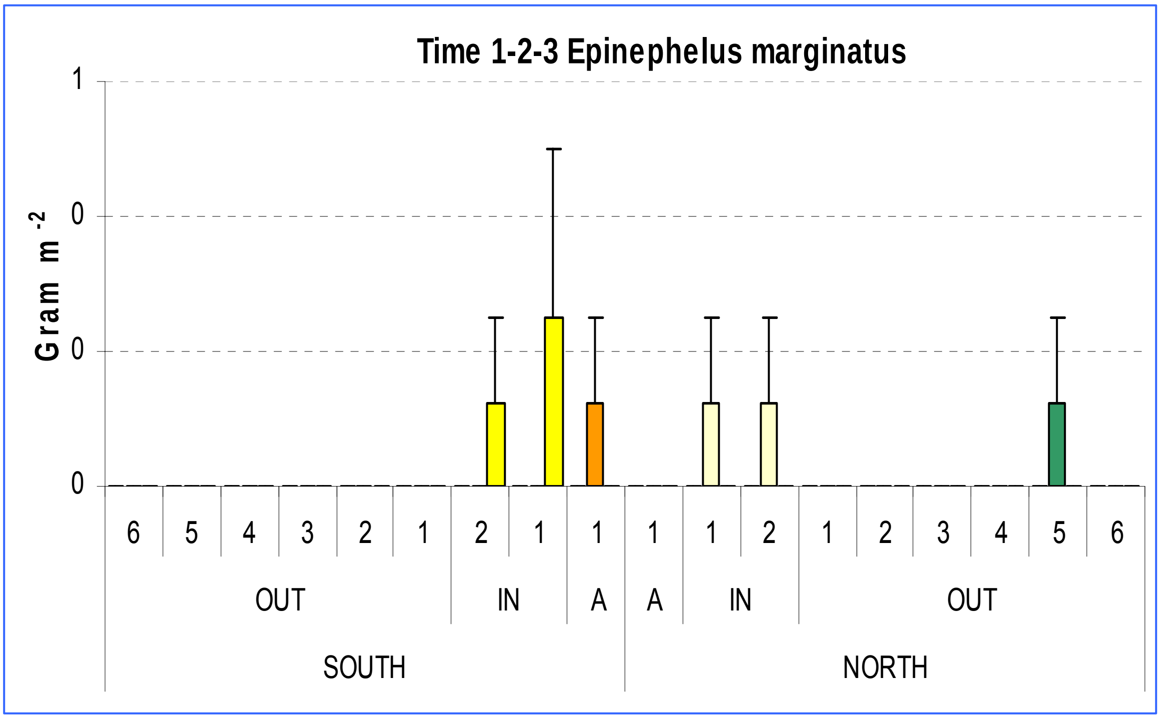
Biomass per specimen (mean) for *Diplodus sargus*.



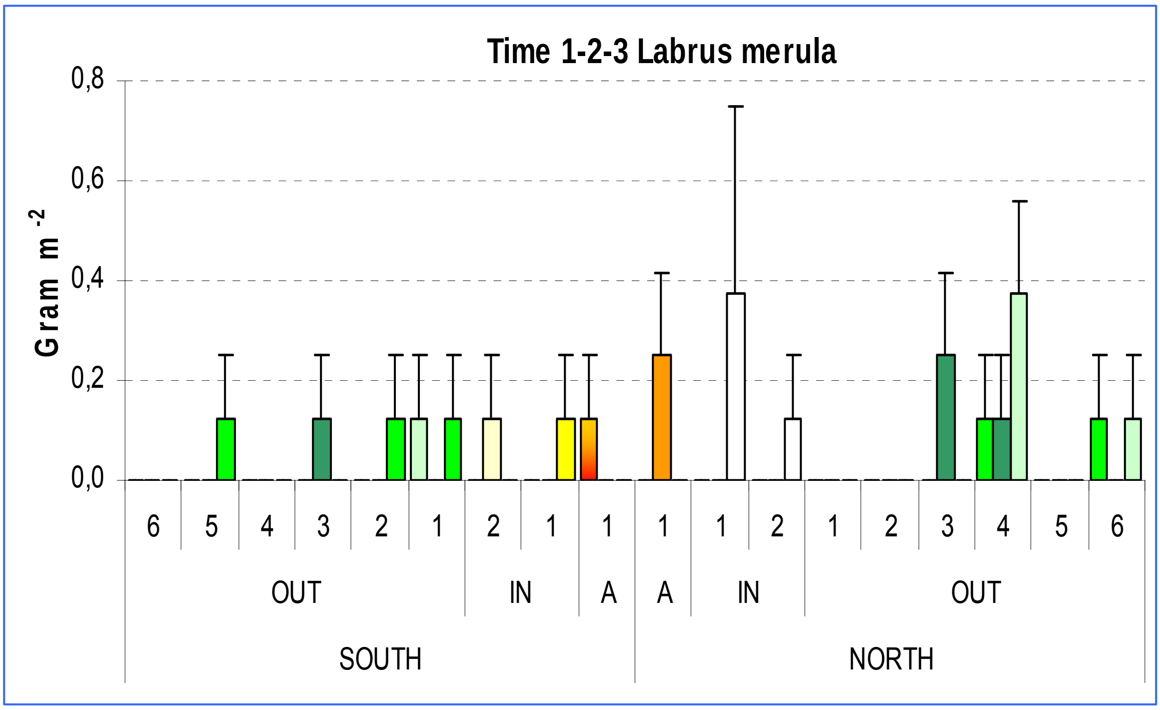
Biomass per specimen (mean) for D*iplodus vulgaris*.



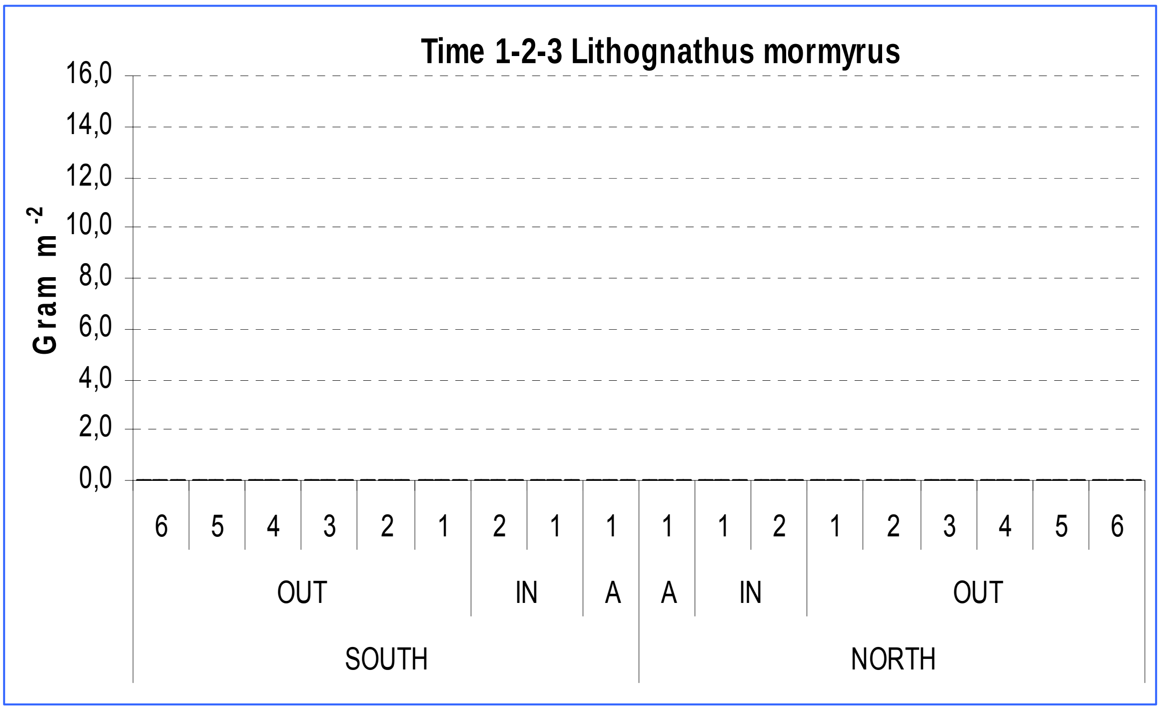
Biomass per specimen (mean) for *Epinephelus costae*.



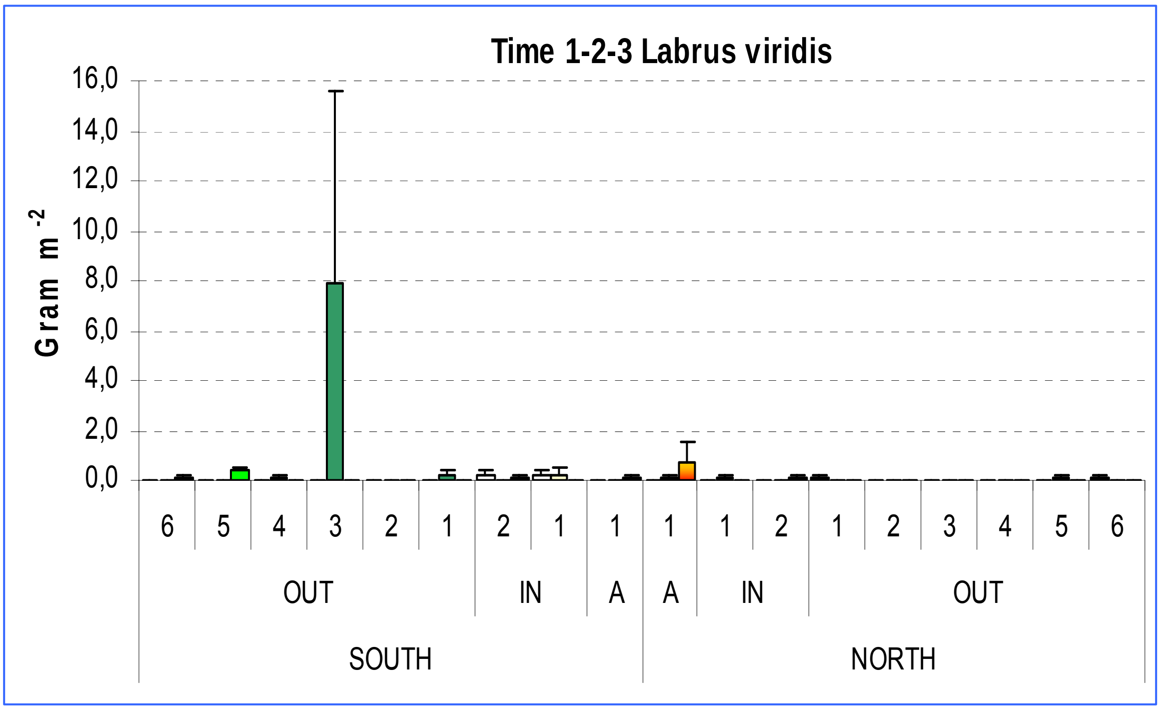
Biomass per specimen (mean) for *Epinephelus marginatus.*



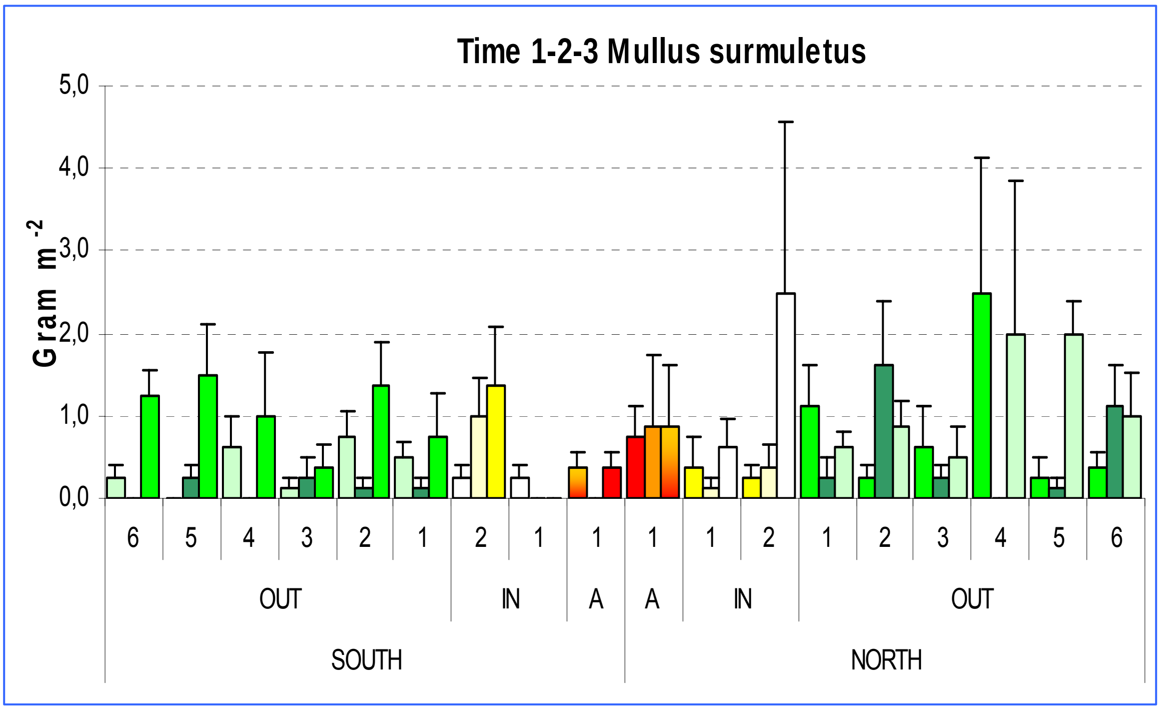
Biomass per specimen (mean) for *Labrus merula.*



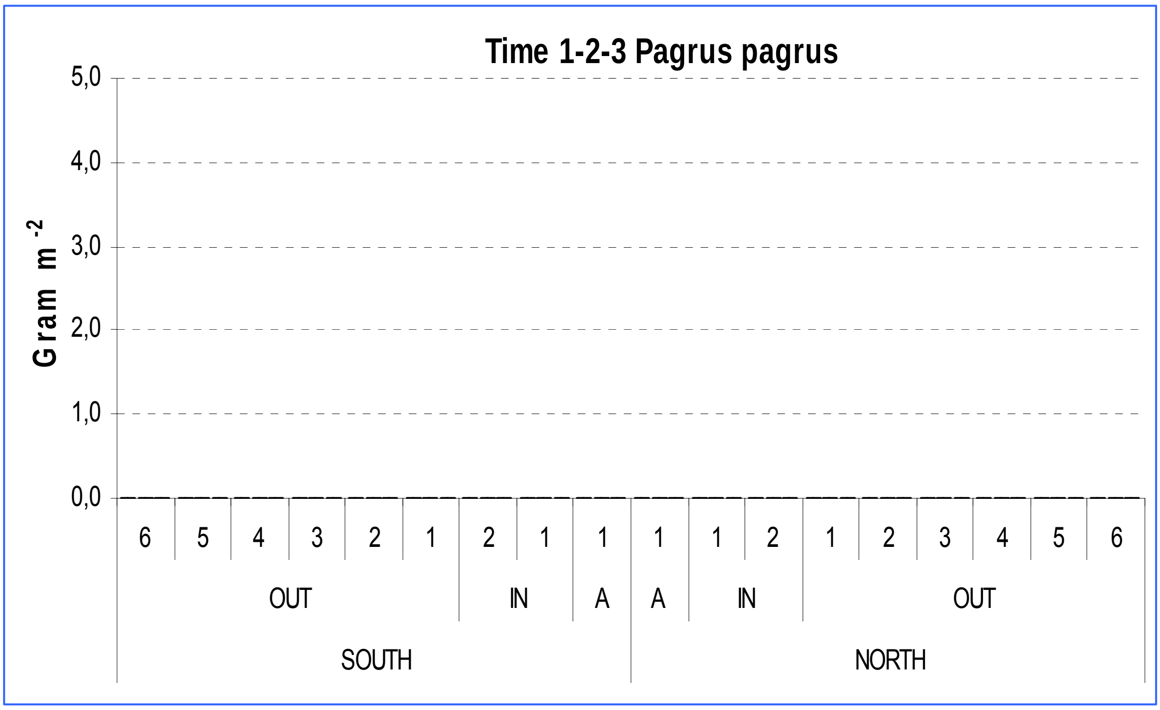
Biomass per specimen (mean) for *Lithognathus mormyrus.*



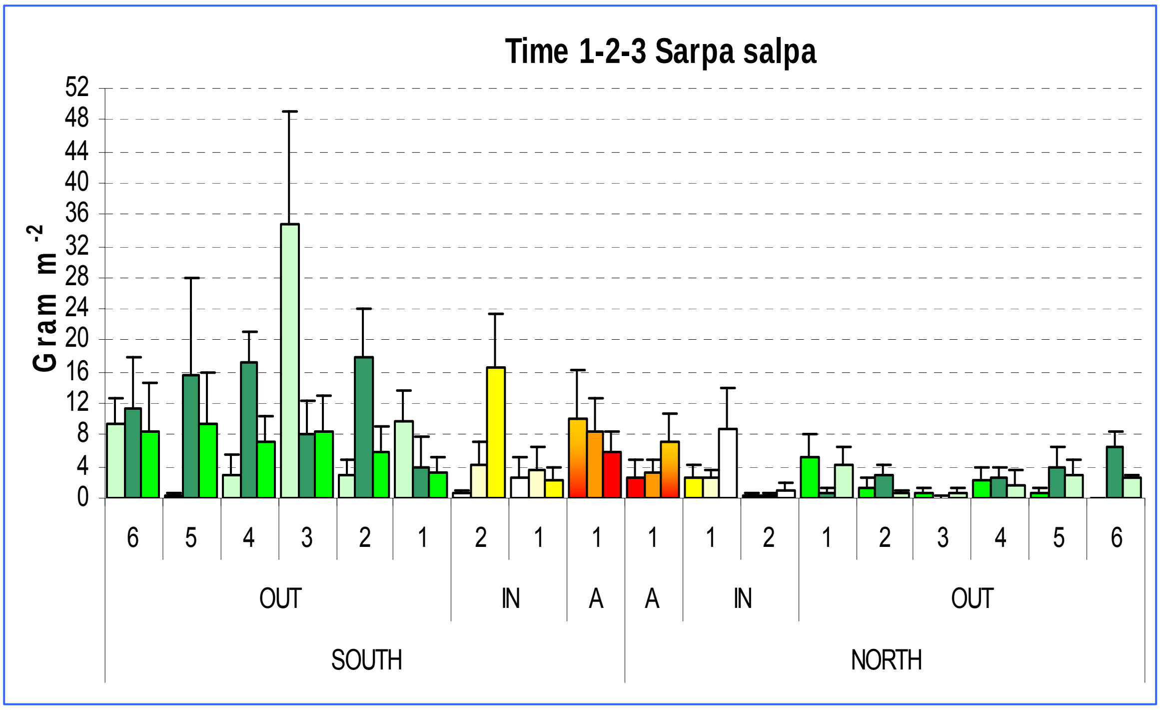
Biomass per specimen (mean) for *Labrus viridis.*



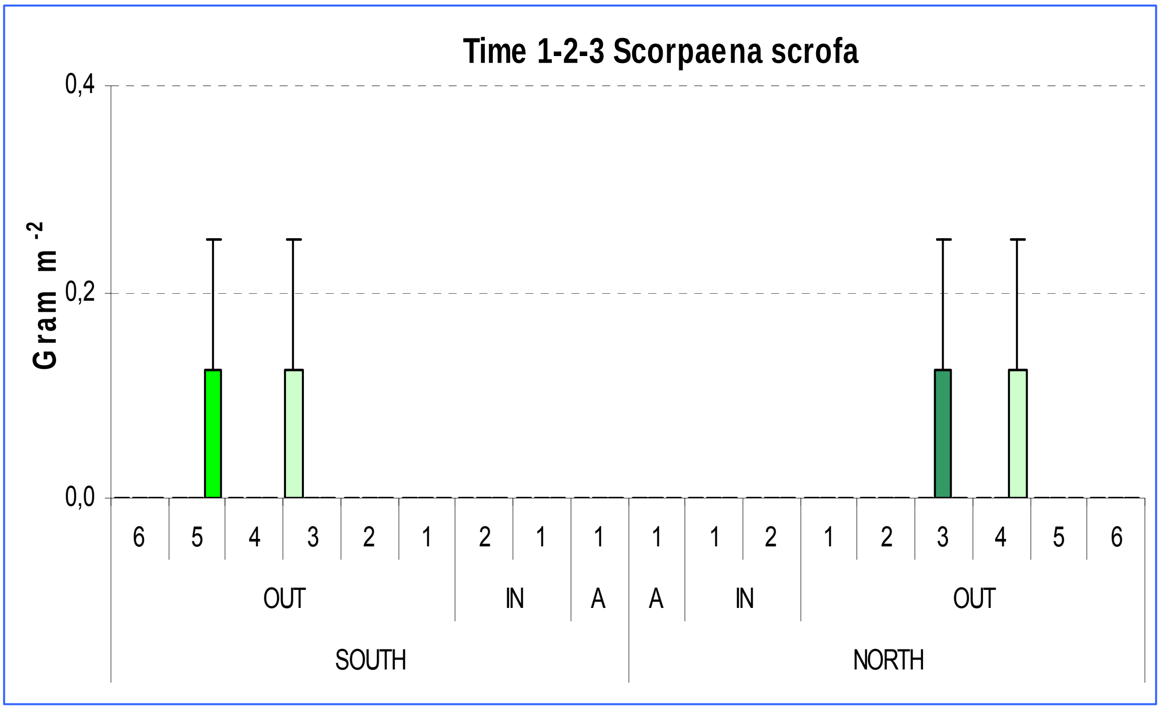
Biomass per specimen (mean) for *Mullus surmuletus.*



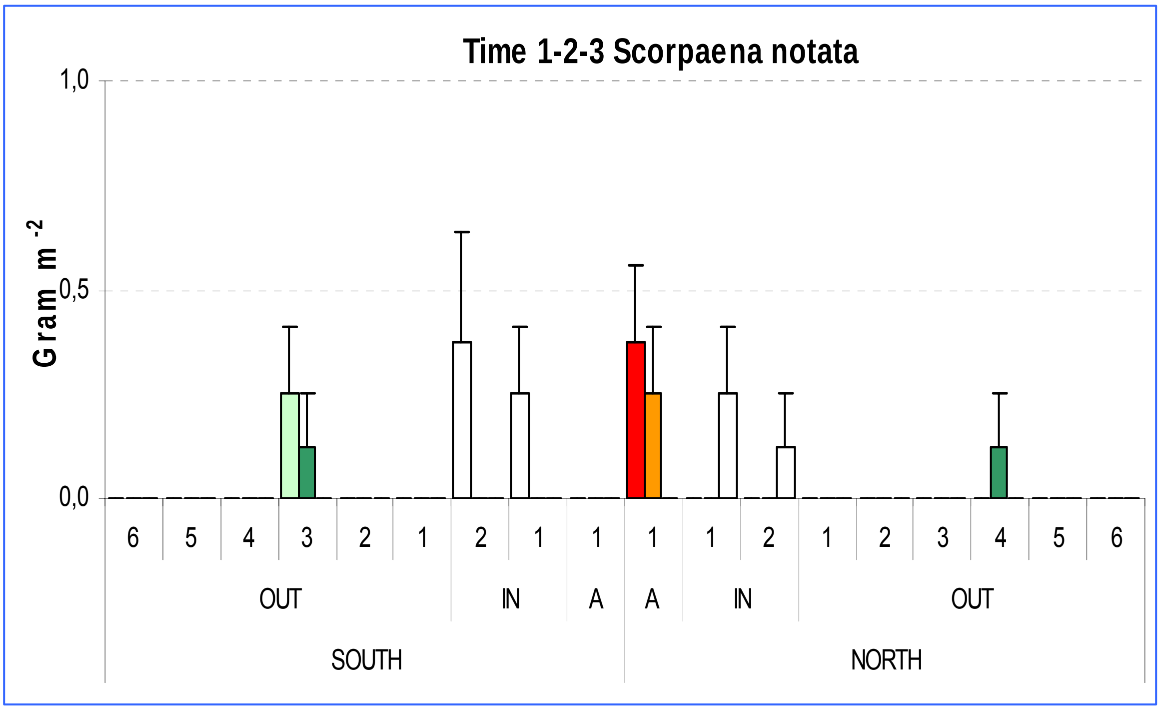
Biomass per specimen (mean) for *Pagrus pagrus.*



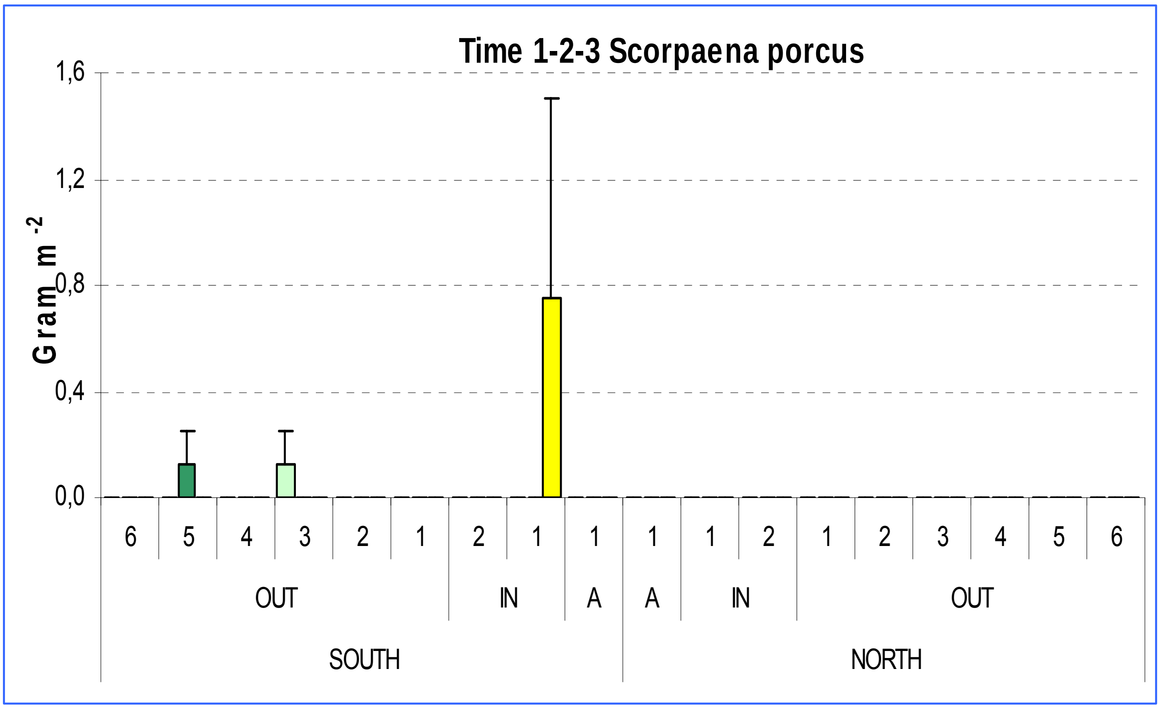
Biomass per specimen (mean) for *Sarpa salpa.*



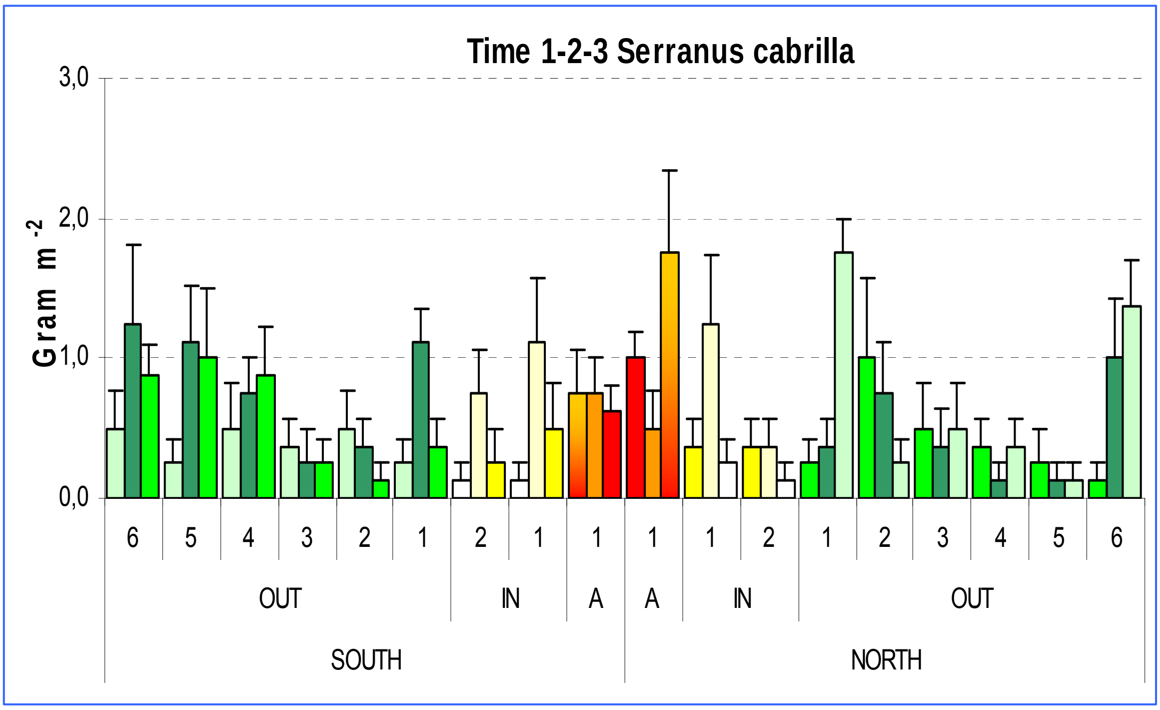
Biomass per specimen (mean) for *Scorpaena scrofa.*



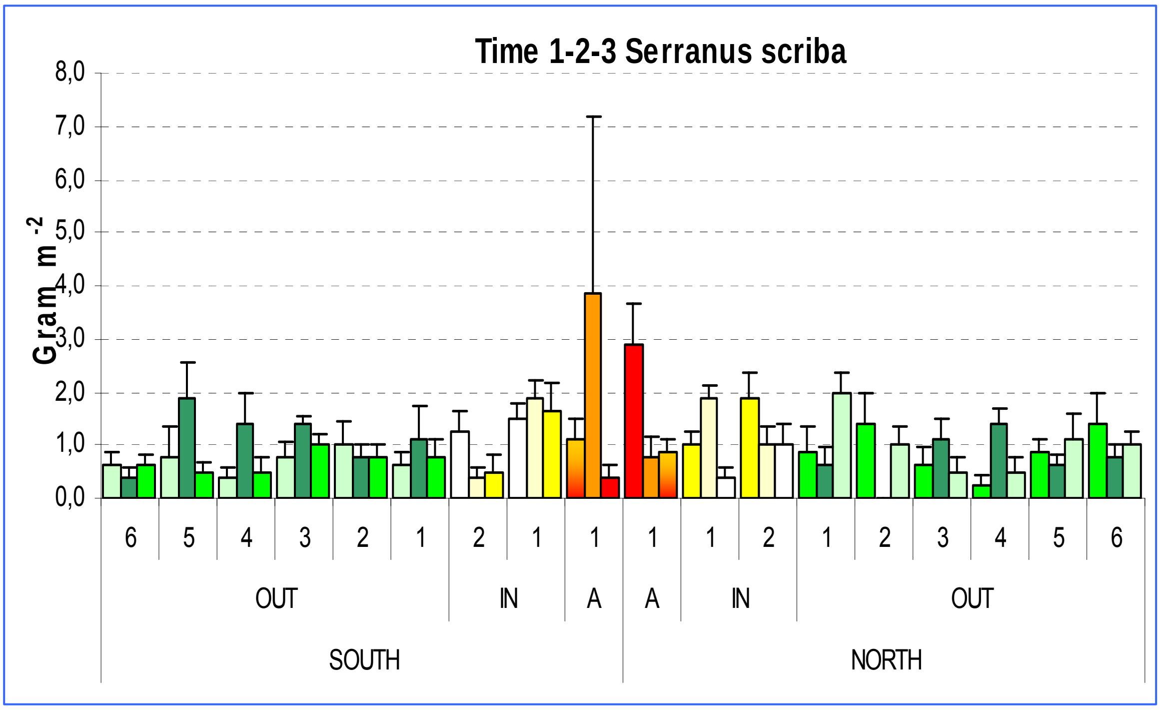
Biomass per specimen (mean) for *Scorpaena notata.*



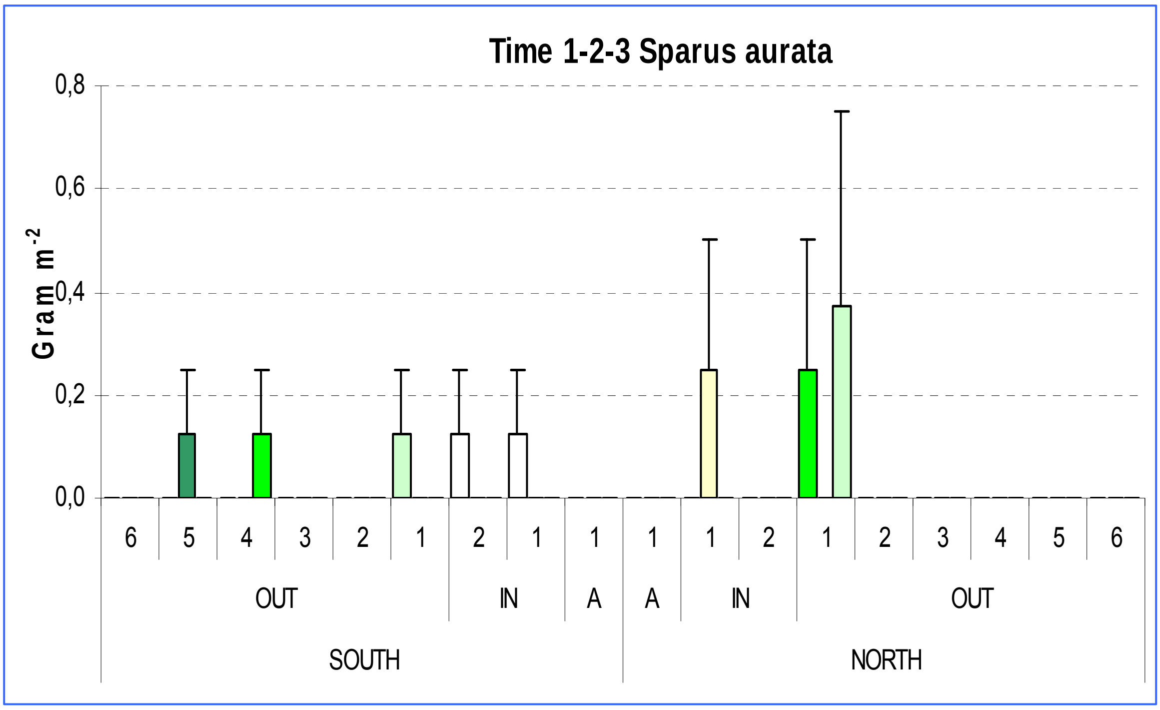
Biomass per specimen (mean) for *Scorpaena porcus.*



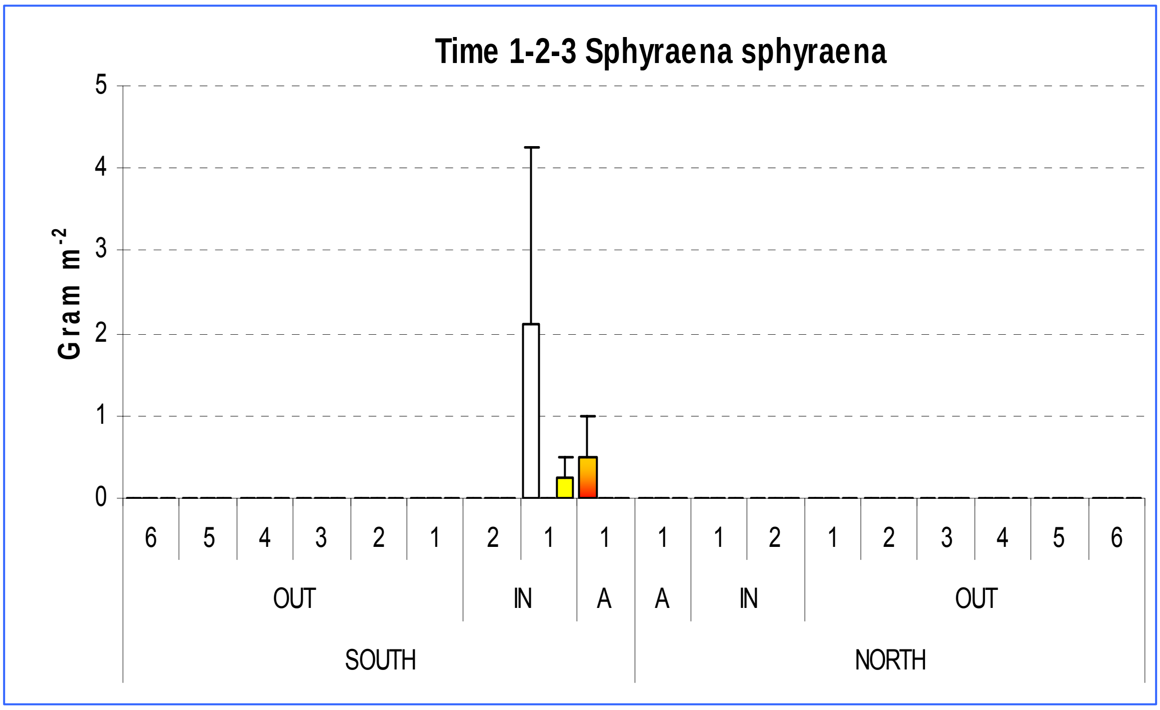
Biomass per specimen (mean) for *Serranus cabrilla.*



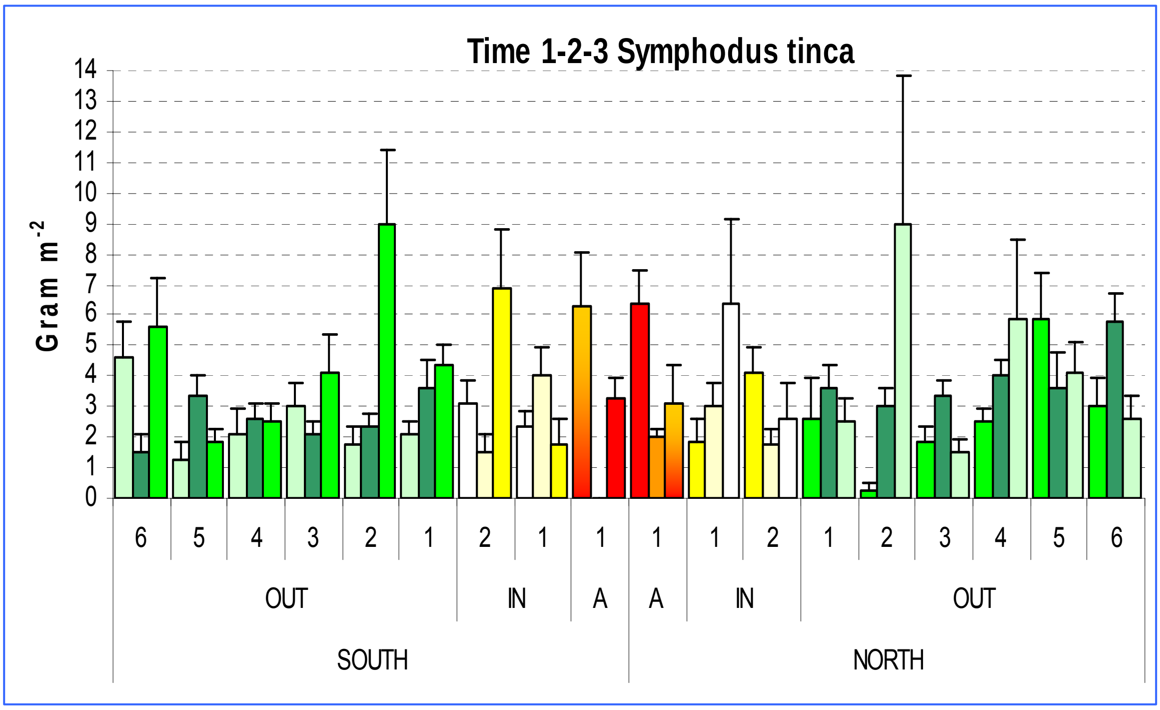
Biomass per specimen (mean) for *Serranus scriba.*



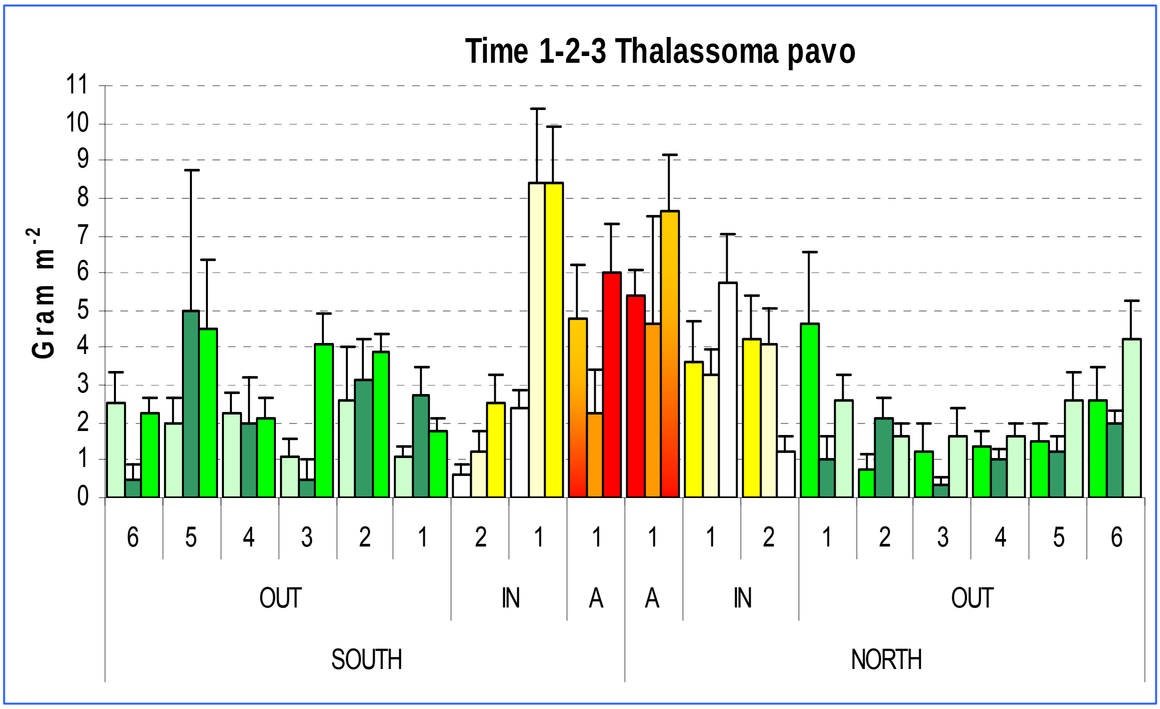
Biomass per specimen (mean) for *Sparus aurata.*



Biomass per specimen (mean) for *Sphyraena sphyraena.*

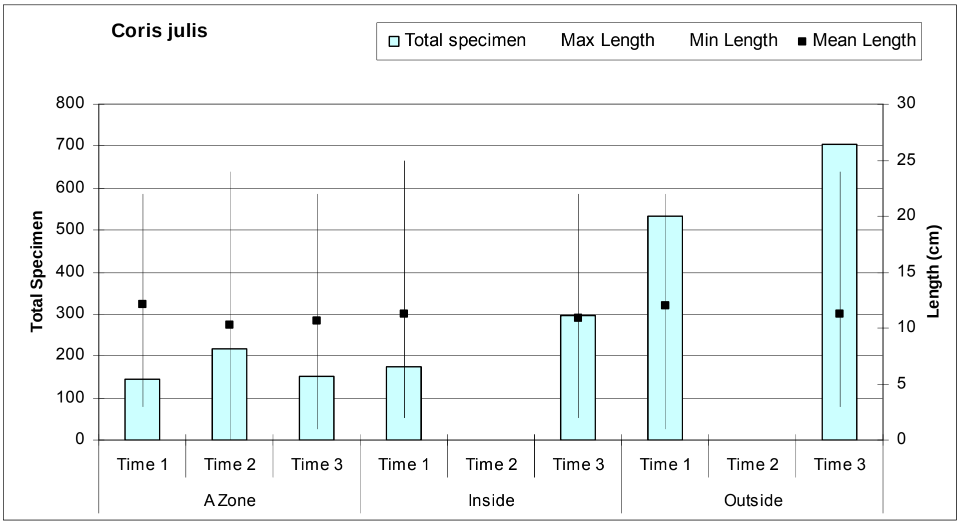


Biomass per specimen (mean) for *Symphodus tinca.*

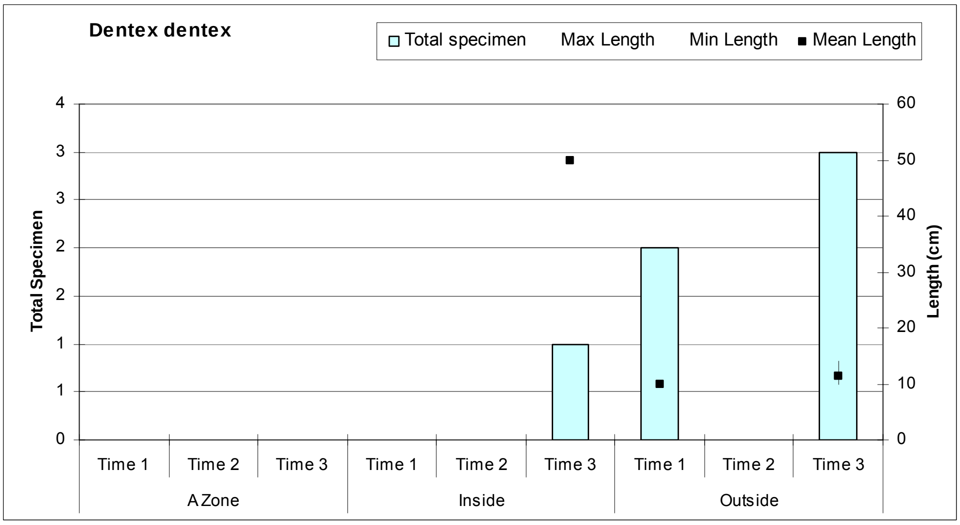


Biomass per specimen (mean) for *Talassoma pavo.*

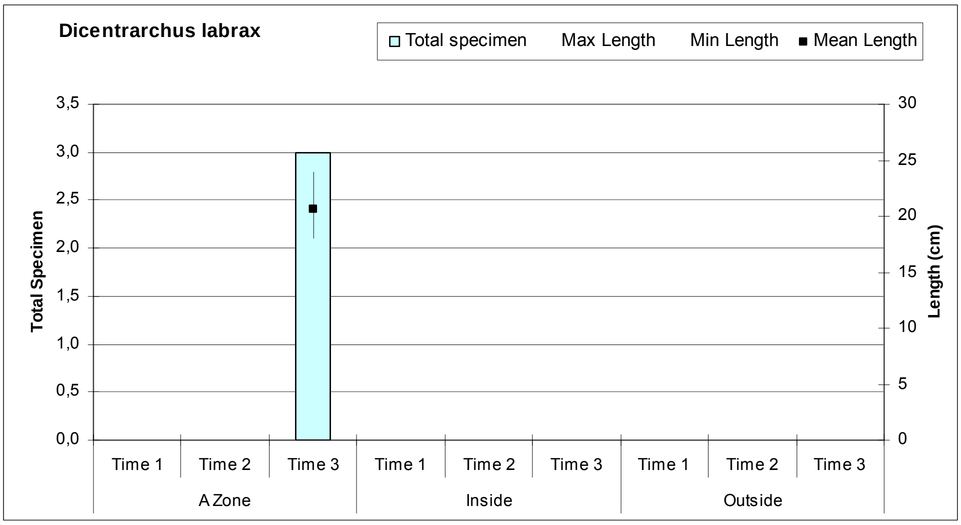
APPENDIX 7: Graphs of the Visual *census* species.



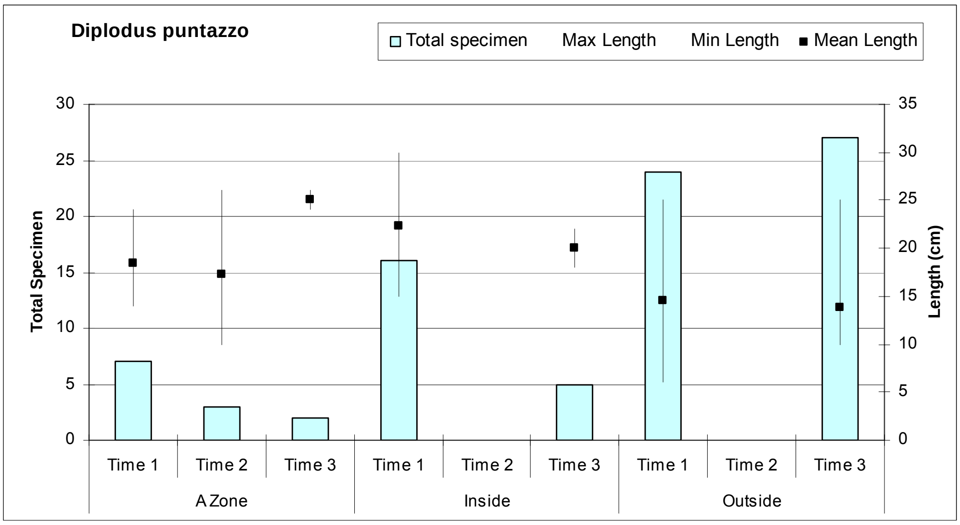
Coris julis: total specimen, mean and min/max length.



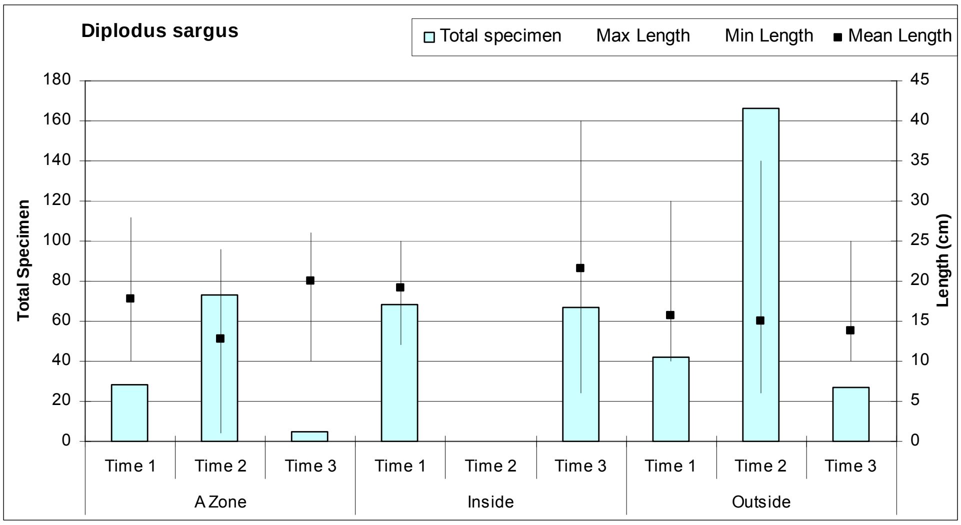
Dentex dentex: total specimen, mean and min/max length.



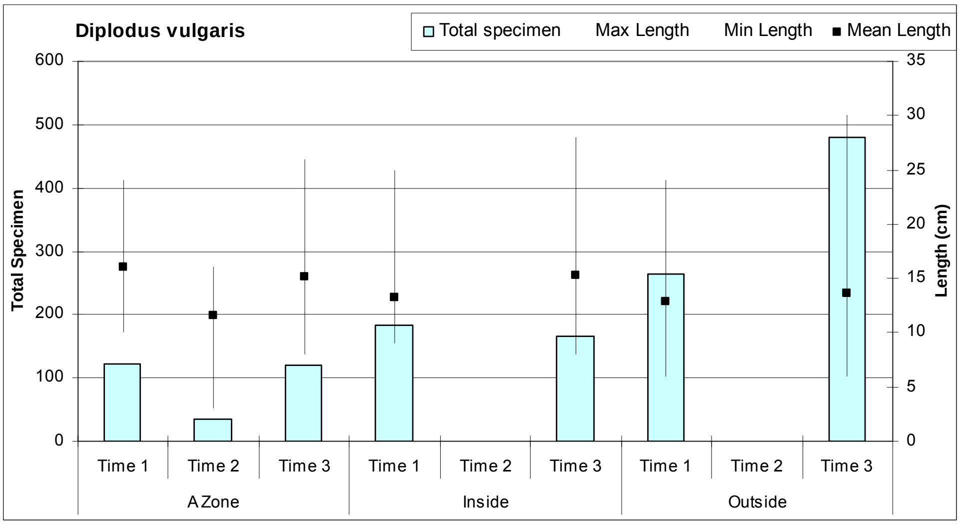
Dicentrarchus labrax: total specimen, mean and min/max length.



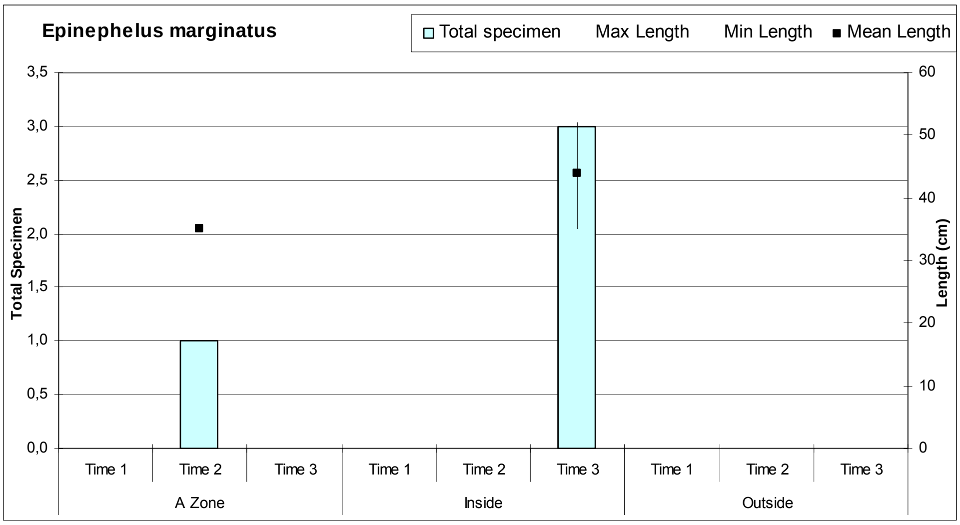
Diplodus puntazzo: total specimen, mean and min/max length



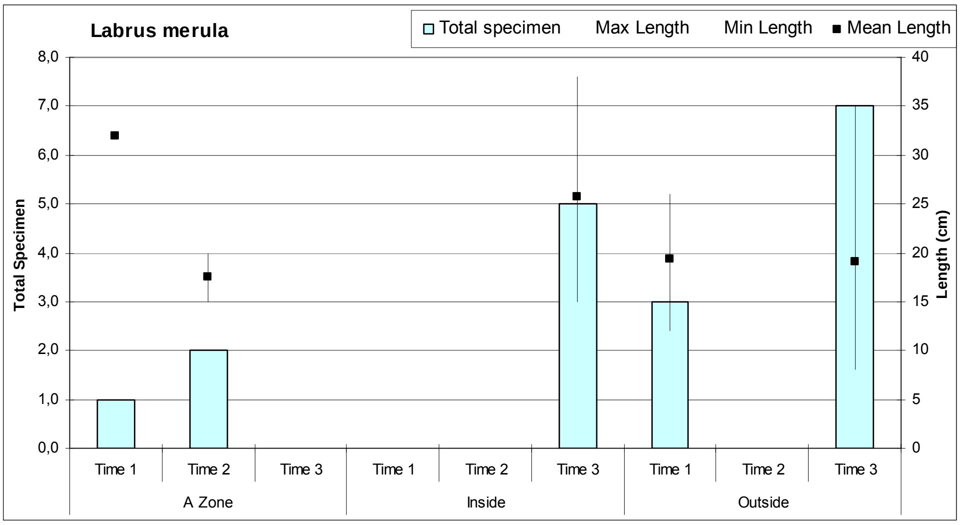
Diplodus sargus: total specimen, mean and min/max length.



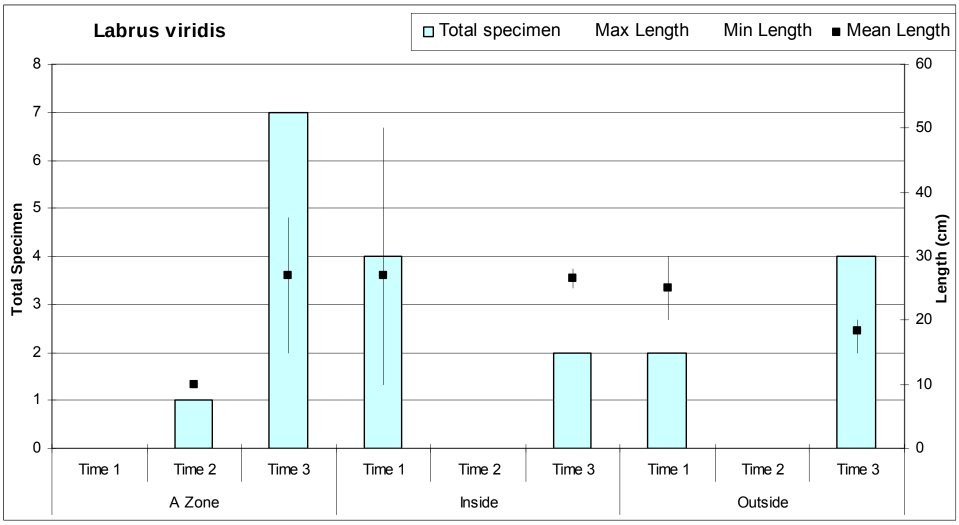
Diplodus vulgaris: total specimen, mean and min/max length.



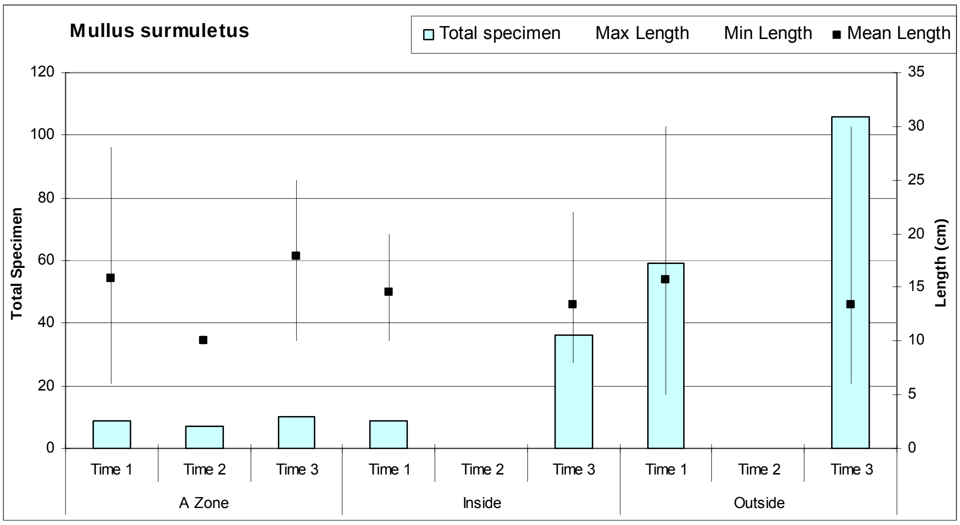
Epinephelus marginatus: total specimen, mean and min/max length.



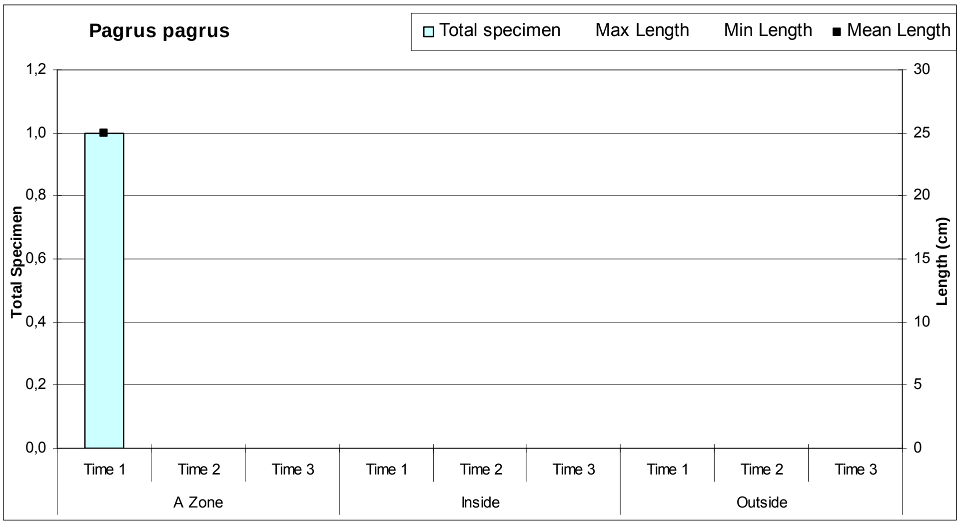
Labrus merula: total specimen, mean and min/max length.



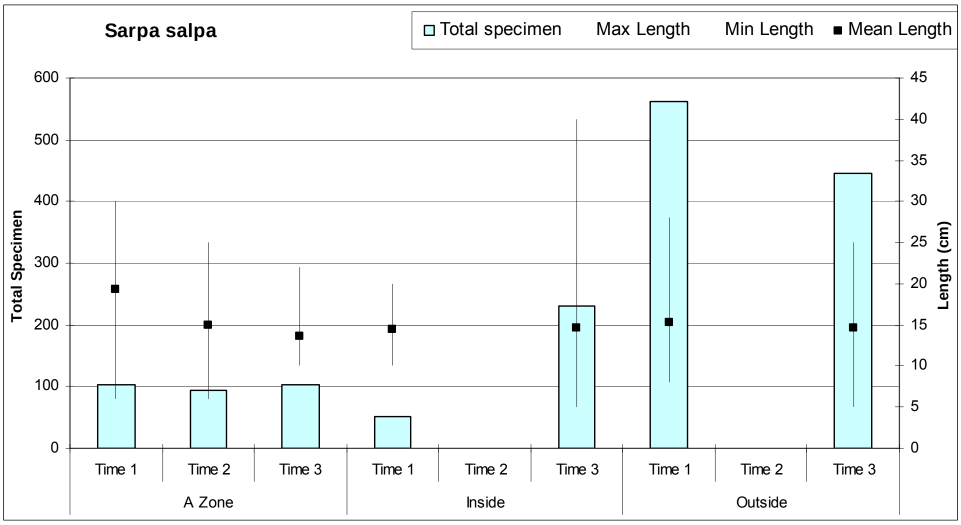
Labrus viridis: total specimen, mean and min/max length.



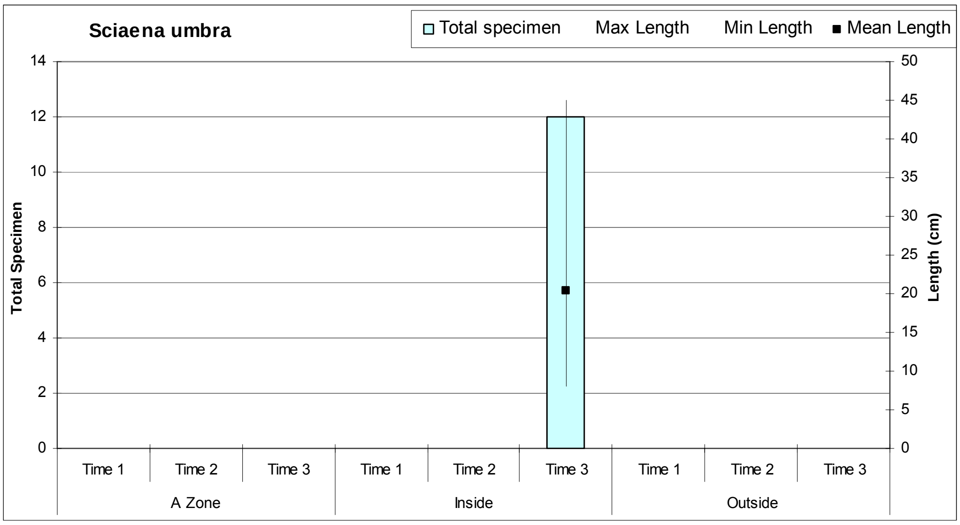
Mullus surmuletus: total specimen, mean and min/max length.



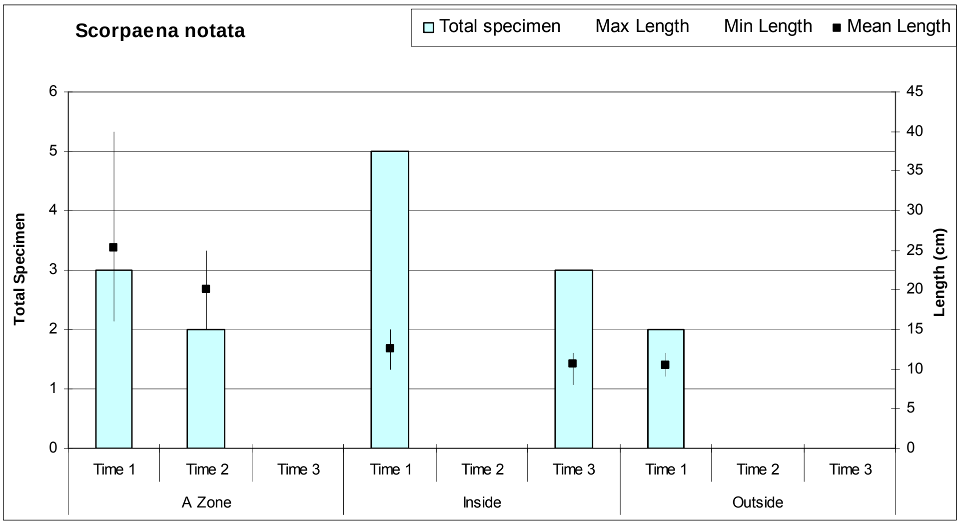
Pagrus pagrus: total specimen, mean and min/max length.



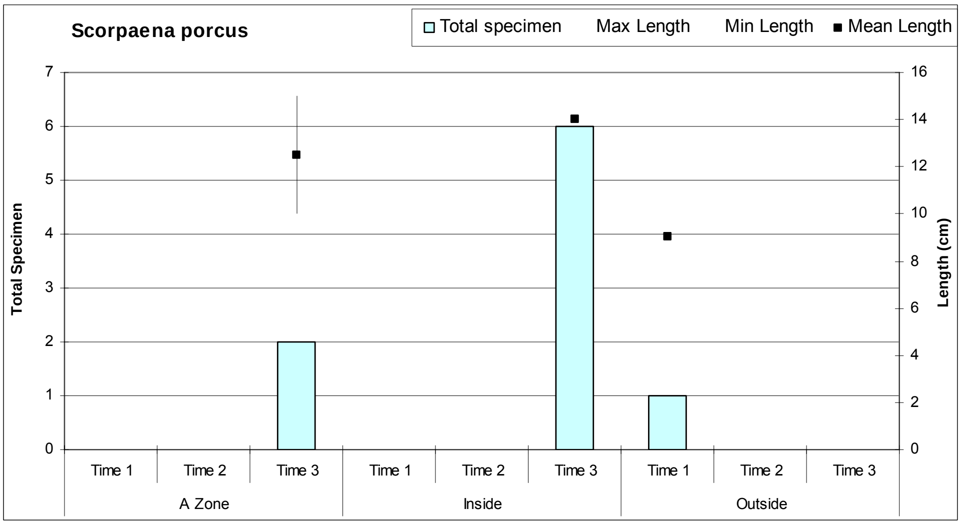
Sarpa salpa: total specimen, mean and min/max length.



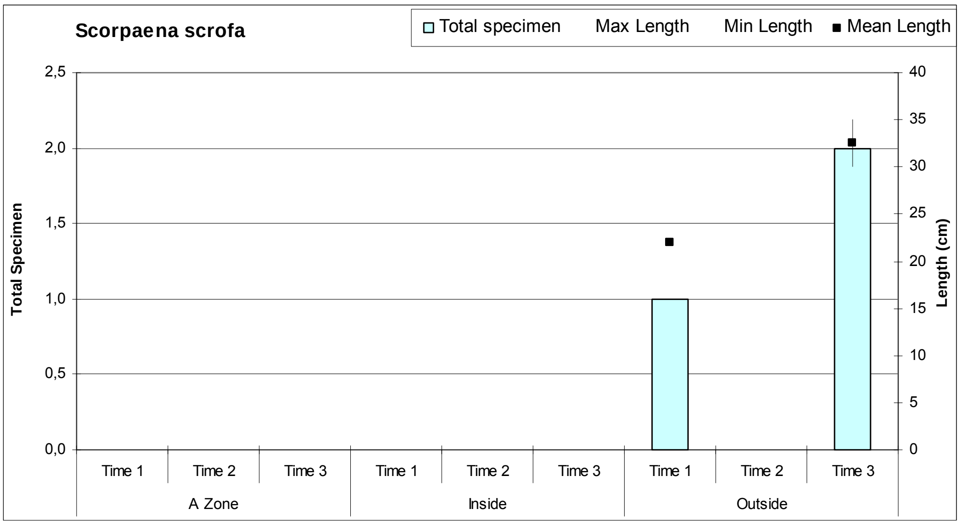
Sciaena umbra: total specimen, mean and min/max length.



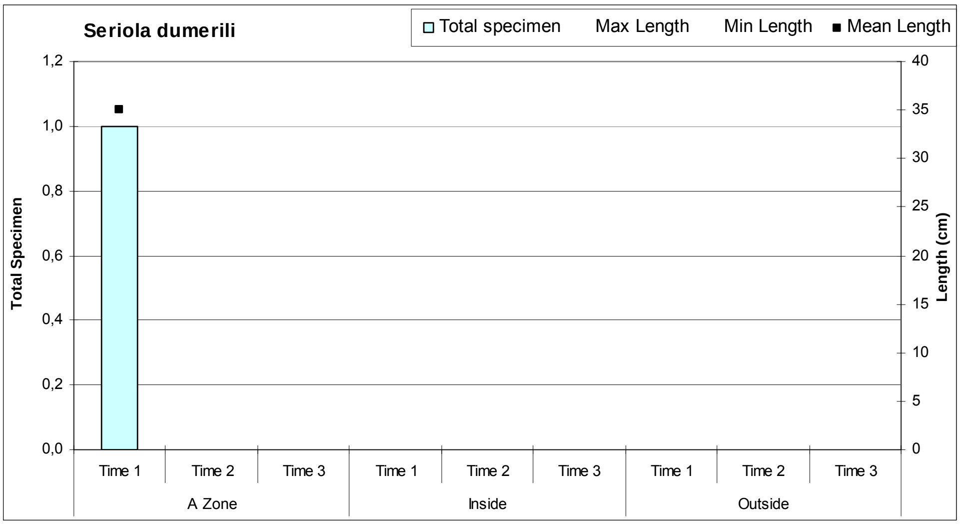
Scorpaena notata: total specimen, mean and min/max length.



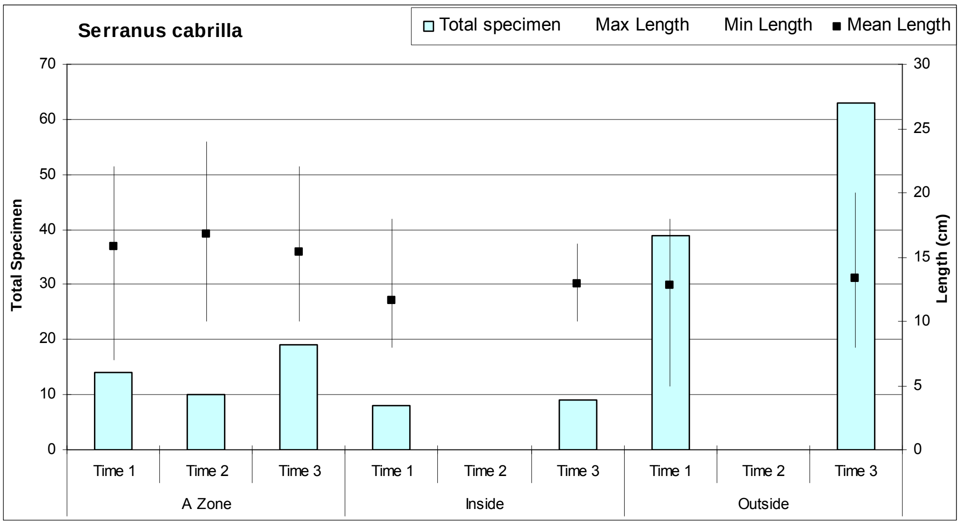
Scorpaena porcus: total specimen, mean and min/max length.



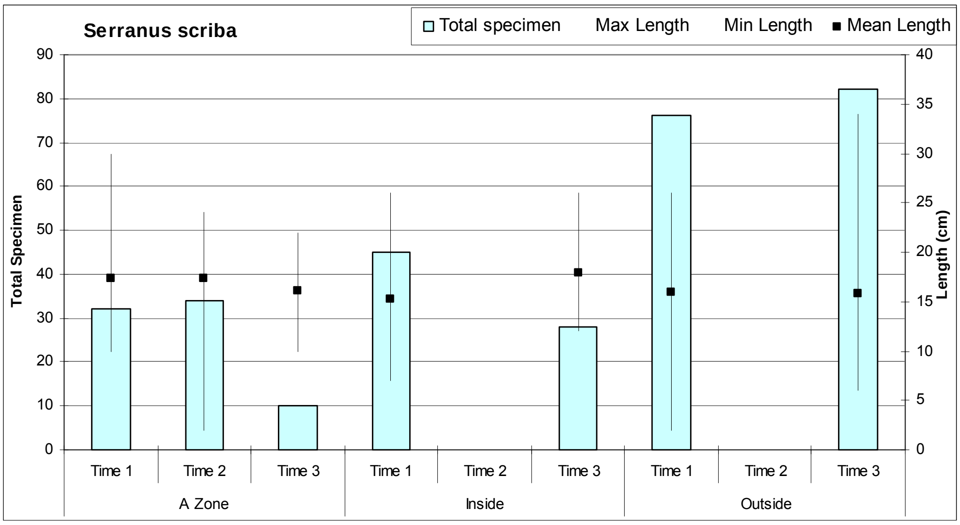
Scorpaena scrofa: total specimen, mean and min/max length.



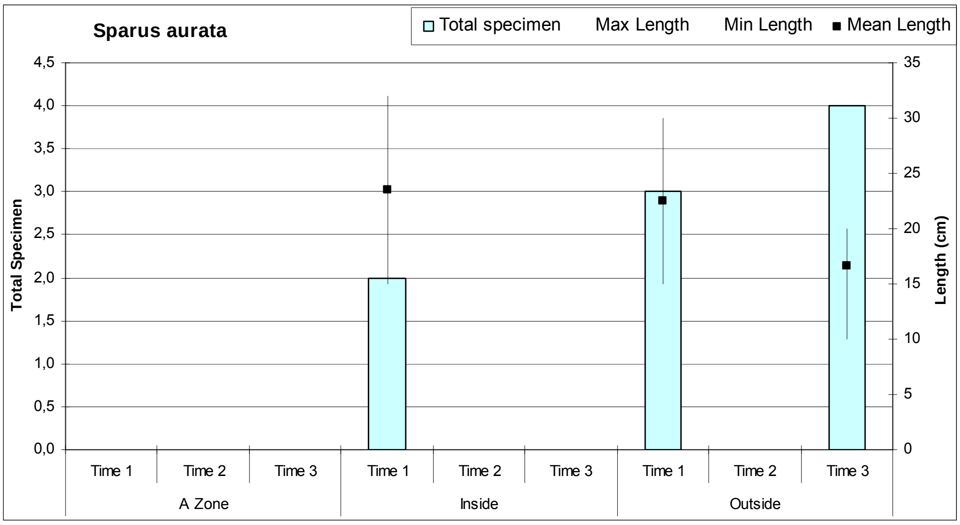
Seriola dumerilli: total specimen, mean and min/max length.



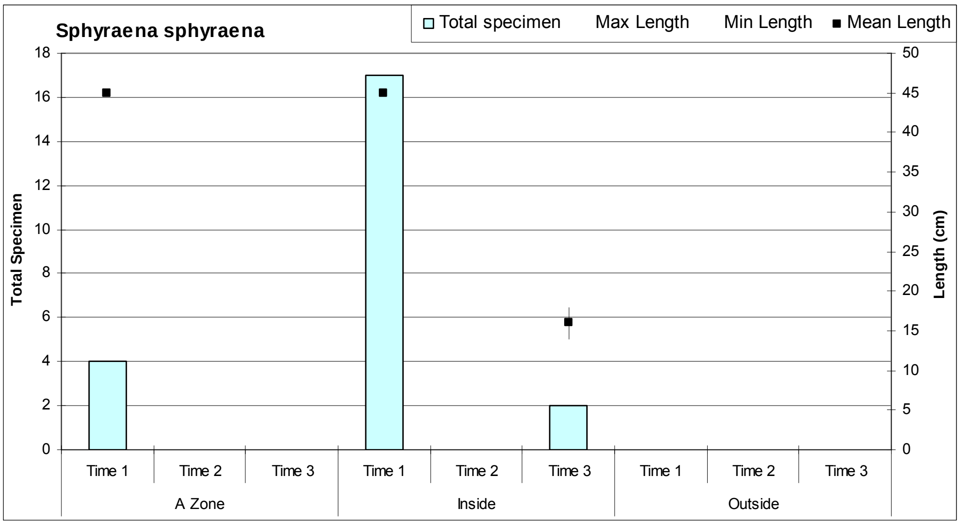
Serranus cabrilla: total specimen, mean and min/max length.



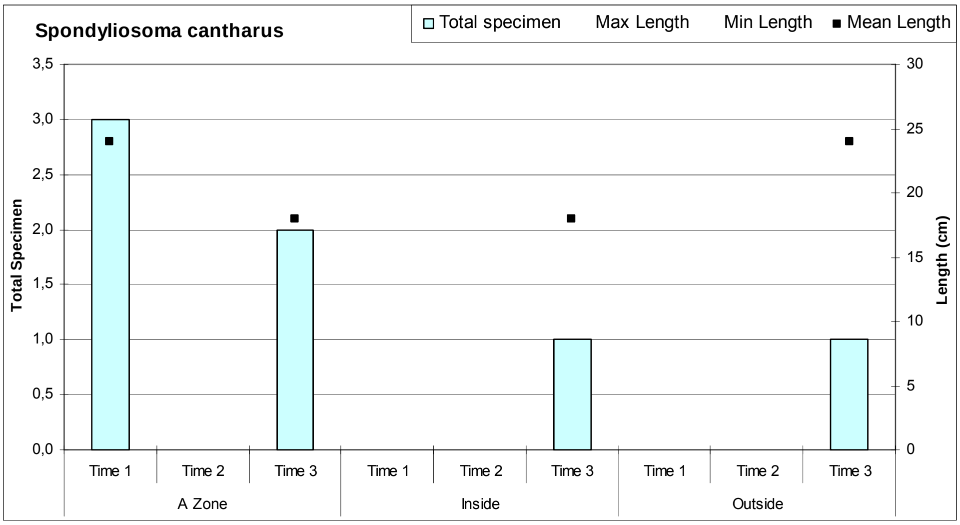
Serranus sciba: total specimen, mean and min/max length.



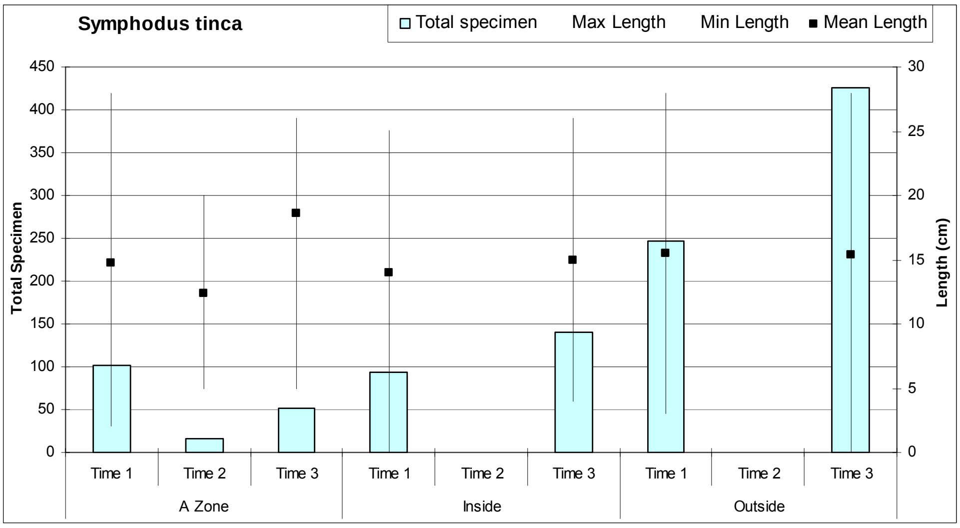
Sparus aurata: total specimen, mean and min/max length.



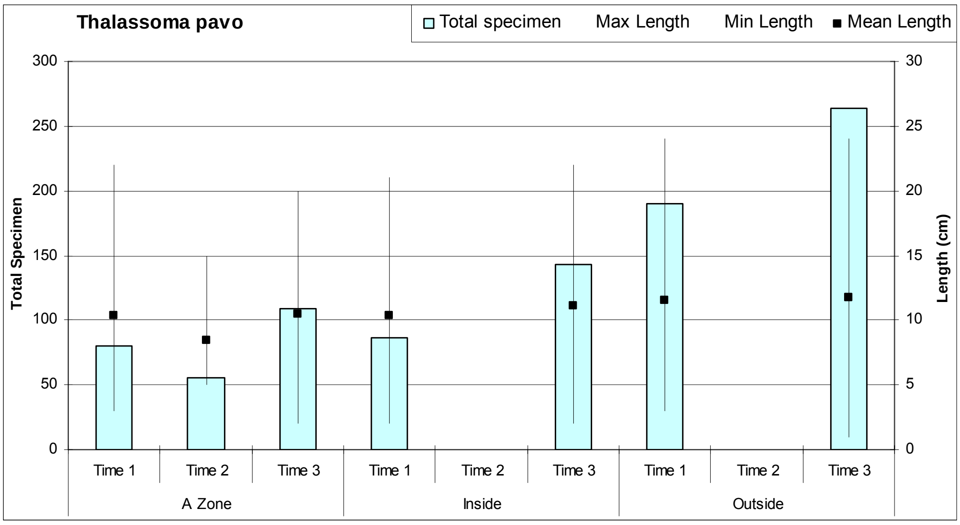
Sphyraena sphyraena: total specimen, mean and min/max length.



Spondyliosoma cantharus: total specimen, mean and min/max length.



Symphodus tinca: total specimen, mean and min/max length.



Thalassoma pavo: total specimen, mean and min/max length.